

Transplanted Lichens in and around the Mount Carmel National Park and the Haifa Bay Industrial Region in Israel: Physiological and Chemical Responses

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This study investigated the impact of air pollution on the spectral reflectance of the epiphytic lichen *Ramalina lacera*, indicated by the normalized difference vegetation index (NDVI), on the integrity of chlorophyll, indicated by the ratio OD435 nm/OD415 nm, and on the integrity of cell membranes, indicated by electric conductivity. Data relating to physiological parameters of injury were integrated with data concerning the detrimental deposition of mineral elements. The transplanted lichen, originating in a relatively unpolluted site in Israel, was placed in 17 sites on and around the Carmel Mountain and in 2 sites in an industrial region in the Haifa Bay, northwest Israel, for a period of 10 months. The accumulated amounts of Ca, Ti, Cu, Mg, Fe, Si, Ni, Zn, V, Cr, Mn, Cl⁻, K, F⁻, Na, Ba, Sr, B, S, P, Al, PO₄³⁻, SO₄²⁻, and NO₃⁻ were related to alterations in spectral reflectance and injury caused to chlorophyll and cell membranes. At the end of the period of exposure, the retrieved transplants from the Haifa Bay exhibited low NDVI values and low OD435 nm/OD415 nm ratios, indicating chlorophyll degradation, and high electric conductivity values, indicating damaged cell membranes. NDVI values correlated positively with OD435 nm/OD415 nm ratios and negatively with accumulated amounts of Ba, Cu, Ni, S, SO₄²⁻, V, and Zn. OD435 nm/OD415 nm ratios correlated negatively with amounts of Ba, Cu, Ni, NO₃⁻, SO₄²⁻, and V. Values obtained for electric conductivity correlated positively with amounts of B, Ba, Cl⁻, Cr, Cu, Na, Ni, NO₃⁻, S, and SO₄²⁻. Both elemental and ion content and the physiological status of the *R. lacera* transplants indicated that the greater part of the biomonitoring sites on and around the Carmel Mountain were slightly polluted

or unpolluted, whereas the Haifa Bay region was rather polluted. The greater part of the Haifa Bay pollution derives from the combustion of heavy fuel oil. © 2001 Academic Press

Key Words: air pollution; lichen; *Ramalina lacera*; chlorophyll; cell membranes.

INTRODUCTION

The capability to accumulate airborne chemical elements led to the application of lichens as biomonitors of air quality. The majority of the investigations of the accumulation of air pollutants by lichens focused on pollutants produced by smelters, power plants, and busy roads in urban and rural sites (Nash and Wirth, 1988; Richardson, 1992). These studies of elemental accumulation in lichens around pollution sources yielded tables of elemental content of lichens near industrial/urban or other contaminated sites, apart from unpolluted background regions (Nieboer *et al.*, 1978; Nash, 1989, 1996; Garty, 1993; Nash and Gries, 1995). Several studies detected reduced levels of elemental content, relative to sites subject to industrial and/or vehicular activity, in biosphere reserves (Markert and Zhang, 1991; Markert and Wtorova, 1992; Wtorova and Markert, 1995) and national parks (Frenzel *et al.*, 1990; Sawicka-Kapusta and Rakowska, 1993; Bennett, 1995).

The present investigation was designed to assess the elemental and ion content of lichen thalli collected in an unpolluted site and then transplanted to both a national park and an industrial region. The Mount Carmel National Park was elected as its Mediterranean forests, nature reserves, and recreation areas were threatened by anthropogenic

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activities such as the power station located to the southwest. The Haifa Bay industrial area, which is the center of the greater part of the heavy industry of the country, was chosen as its potential impact threatened the northwestern edges of the park. The industrial activities in the Haifa Bay include oil refineries, a power station, a steel smelter, and manufacturers of food, fertilizers, chlor-alkali and other chemical products, glass and ceramics, metal and metal plating, wood and paper, plastic, concrete and cement, electronic components, and car tires. The main pollutants emitted by these industries are SO₂, NO_x, O₃, CO₂, metal-containing particles, dust originating from the cement industry, volatile organic compounds, fertilizer dust, and fumes deriving from traffic.

Different kinds of Mediterranean trees in the Haifa Bay meet the requirements of substrates for *Ramalina lacera* and for other fruticose lichens, yet carry for the greater part the resistant foliose lichen *Xanthoria parietina*. A study of the apparent elimination of fruticose lichens and additional sensitive lichens had the greatest relevance to the output of industrial contaminants.

Three parameters of physiological status were related to the elemental and ion content of lichen thalli. In addition to measurements of the spectral reflectance response, we assessed the integrity of both cell membranes and chlorophyll in the lichen *Ramalina lacera* (With.) J. R. Laund.

Measurements of spectroscopic features of plants have led to the construction of different vegetation indices (VI). Most indices are based on a combination of the ratio of two portions of the electromagnetic spectrum: the red band (R) at 600–700 nm, which corresponds to the region of maximum chlorophyll absorption, and the near-infrared band (NIR) at 700–1100 nm, which corresponds to the maximum reflectance of incident light by the living vegetation. The basic vegetation index, the Simple Ratio, is a ratio of the digital value of these two bands. The most widely used VI is known as the normalized difference vegetation index (NDVI): $NDVI = (NIR - R) / (NIR + R)$ (Rouse *et al.*, 1974). The values yielded by the above-mentioned equation range from -1.0 to $+1.0$. The most important feature of NDVI is that healthy vegetation surfaces have high index values, as the former have a stronger “greenness” signal. As NDVI correlates empirically with photosynthetic activity, fractional vegetation cover, green leaf biomass or leaf area, primary productivity, and carbon in standing biomass, it is suitable for various applications, such as image classification and detection of seasonal or annual change in crop yields or veg-

etation cover (Tucker, 1979; Curran, 1980; Holben *et al.*, 1980; Asrar *et al.*, 1984; Sellers, 1985; Prince, 1990). Satterwhite and co-workers (1985) studied the impact of lichens on the reflectance spectra of granitic rock surfaces and detected an interference of lichens in the visible and near-infrared reflectance spectra (400–1100 nm). According to Karnieli and co-workers (1996), the level of spectral reflectance of wet limestone, covered with endolithic lichens, is lower than that of dry limestone. The spectral response of wet lichen-covered stones is similar to that of higher plants.

Alterations in the spectral reflectance of lichen thalli were related in the present study to two physiological parameters: the ratio chlorophyll *a*/phaeophytin *a* in the algal cells as an indication of the status of the photobiont chlorophyll (Ronen and Galun, 1984) and electrolyte leakage as an indication of the status of cell membranes. The accumulation of certain air pollutants in the lichen thallus is assumed to correlate with low NDVI values, low OD435 nm/OD415 nm ratios, and high electric conductivity values. High NDVI values should correlate with high OD435 nm/OD415 nm ratios, as both parameters refer to the status of the photobiont chlorophyll. Reduced amounts of mineral elements and ions in the national park, relative to the industrial region, were supposed to agree with little alteration in physiological parameters.

The present study aimed further at a comparison of the relative sensitivity of three different methodologies to detect the most suitable means to assess the vitality of lichens in a study area with different levels of air pollution. A decrease of vitality of lichen transplants would provide early warning signs indicating change in the function of additional plants in the ecosystem.

MATERIALS AND METHODS

Collection of Lichen Material, Site Description, and Transplantation

The fruticose lichen *R. lacera*, previously (Garty *et al.*, 1993) *Ramalina duriaei* (De Not.) Bagl., growing on twigs of carob trees (*Ceratonia siliqua* L.), was collected with its substrate in the HaZorea Forest, Ramoth Menashe, northeast Israel, in July 1996. The HaZorea Forest is affirmed to be “clean” with respect to air pollution (Garty and Fuchs, 1982, Garty *et al.*, 1985, 1997a–d, 1998a,b, Silberstein *et al.*, 1996). Approximately 500 lichen-covered twigs were gathered in HaZorea and transplanted to 19 different monitoring sites in the Haifa Bay and in the

TABLE 1
Description of the Biomonitoring Sites

Site No.	Site name	Description	Remarks
1.	HaZorea Forest	Carob Tree plantation, 1 km south of kibbutz	Rural, unpolluted
2.	Jabotinski Park, 1.5 km north of Binyamina		Suburban, about 500 m north of a limestone quarry
3.	Ramat HaNadiv, Zichron Yaacov	On the Mountain	Rural, a memorial garden
4.	Mevo Elyakim	Mount Carmel National Park	About 300 m east of car parking area
5.	Oren HaSela parking site	Mount Carmel National Park	About 400 m west of recreation area
6.	Keren HaCarmel road Junction	Mount Carmel National Park	Close to monastery
7.	Keren HaCarmel	Mount Carmel National Park	
8.	Oranei HaPisgah	Mount Carmel National Park	About 300 m west of a recreation area
9.	About 400 m SW from Damun Junction	Mount Carmel National Park	
10.	Beit Oren	Mount Carmel National Park	About 200 m east of Beit Oren Junction
11.	Kedumim ancient quarry	Mount Carmel National Park	About 150 m east of recreation area
12.	Parking area near artificial lake	Mount Carmel National Park	Frequent bus arrivals
13.	Rakit recreation area	Mount Carmel National Park	About 250 m north of recreation area
14.	Yearoth HaCarmel Hotel	Mount Carmel National Park	About 300 m north of hotel
15.	Nahal Oren mouth		About 150 m south of Nahal Oren road
16.	Carmel Park office area	Mount Carmel National Park	
17.	Antenna of communication facility	Mount Carmel National Park	Unpopulated area
18.	Hai Bar	Mount Carmel National Park	Close to zoological park
19.	Haifa Bay oil refineries	(Industrial)	About 600 m north east of refineries
20.	Haifa Power Station, Haifa Bay	(Industrial)	1.5 km east of power station

Mount Carmel region, 25 or 26 twigs per site. The lichen-covered twigs were secured with PVC cords and suspended 2 to 3 m above ground level on local trees. Concurrently, 26 additional twigs were resuspended on the original carob trees as control specimens. The biomonitoring sites are detailed in Table 1 and in Figs. 1 and 2. Cognizant of factors other than pollutants, such as local climate, especially in urban regions, we transplanted the lichen material in open space, distinctly removed from built sites and major motorways, including the two sites in the Haifa Bay.

Early Treatment of the Transplanted Lichen Material

In May 1997 the resuspended and transplanted twigs were retrieved and rinsed immediately with double-distilled water at a temperature of 20°C to eliminate dust, leaf debris, insects, etc. The rinsing procedure was repeated three times, for 5 s each, to minimize the loss of water-soluble elements, i.e., Na and K. It was assumed that a rapid, repetitive procedure would not remove particles, such as Pb and Cu, enclosed by near-surface hyphae.

Measurement of the Spectral Reflectance of the Lichen Thallus

The spectral characteristics of the lichen were measured in the laboratory by a LiCor LI 1800 field spectrometer. All laboratory measurements were performed under constant irradiance from a fixed angle. The instrument was fixed to a 2-nm-wavelength spectral resolution, with increments between 400 and 1100 nm and a 15° field of view. The lichen samples were placed in petri dishes on a black-coated board to minimize the external reflectance or backscatter. The spectrometer was installed 1 m above the sample. A 1000-W quartz halogen lamp was positioned at a zenith angle of 45°, approximately 1 m from the sample. The incident radiation was measured as the radiation reflected by a halon reflectance panel. The reflectance was calculated as the ratio of the incident radiation to the radiation reflected by the lichen. The petri dishes were rotated by 90° between each scan to avoid roughness and shadowing effects. Each spectrum was calculated as an average of four spectra. The reflectance in the R spectral region was determined as the average of reflectance values between 650 and 700 nm. In the same manner, the reflectance in the NIR spectral

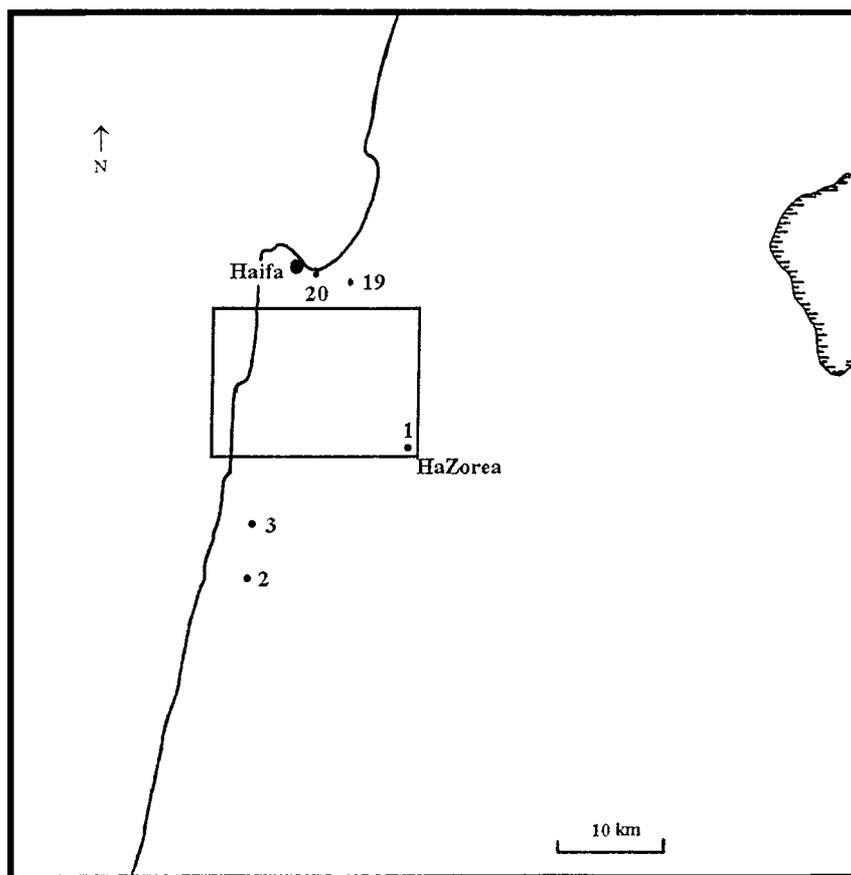


FIG. 1. The northern part of Israel, indicating HaZorea, the Haifa Bay (sites 19 and 20), site 2 (Jabotinski Park), and site 3 (Ramat HaNadiv).

region was determined as the average of reflectance values between 800 and 1000 nm. The NDVI values of each spectrum were calculated by the equation presented in the Introduction.

Assessment of Cell Membrane Integrity in Lichen Thalli

Subsamples of 1 g were used to assess the integrity of cell membranes. The samples were immersed in 100 ml of double-distilled water for 60 min. The electric conductivity of the water, which expresses electrolyte leakage (Pearson and Rodgers, 1982; Garty *et al.*, 1993, 1998b), was measured by an electric conductivity meter (TH-2400; El-Hamma Instruments).

Analysis of the Integrity of the Photobiont Chlorophyll

Samples of 20 mg were used to measure the integrity of the photobiont chlorophyll in the lichen. The

chlorophyll was extracted overnight in the dark in 3 ml of dimethyl sulfoxide (DMSO; Merck, analytical grade), and the ratio of chlorophyll *a*/phaeophytin *a* (OD435 nm/OD415 nm) was determined according to Ronen and Galun (1984) by the use of a Novaspec II spectrophotometer (Pharmacia LKB, Biochrom Ltd., Cambridge, UK).

Determination of the Elemental and Ion Content of the Lichen Thalli

The amounts of Ca, Ti, Cu, Mg, Fe, Si, Ni, Zn, V, Cr, Mn, K, Na, Ba, Sr, B, S, and Al in the lichen thalli were determined in the following manner: subsamples of 1–2 g of rinsed and air-dried thalli were further dried at 105°C for 24 h. Subsamples of 250 mg were wet-ashed in 10 ml of concentrated (65%) analytical pure HNO₃ (Merck) in test tubes of 50 ml in a heating block for a duration of 8 h at temperatures beginning with 80°C and increasing to 100 and 120 °C. The elemental content was determined by the Inductively Coupled Plasma-Atomic

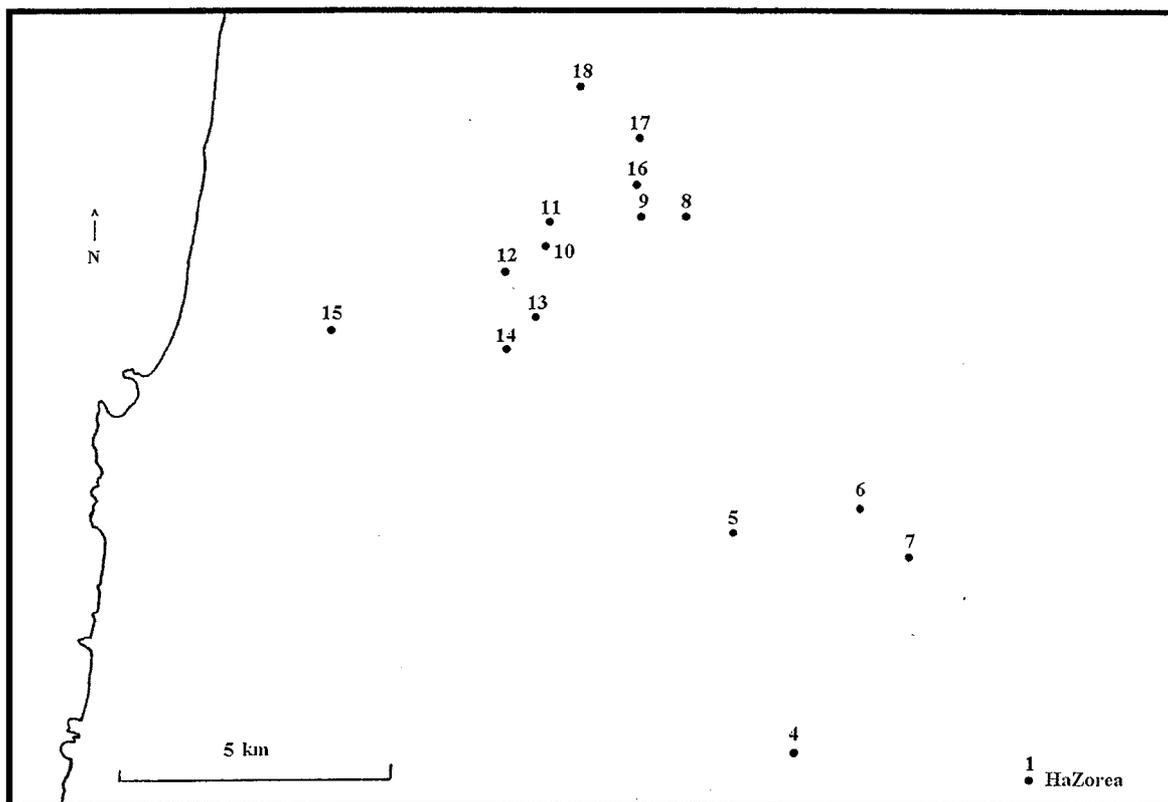


FIG. 2. Biomonitoring sites in the Carmel mountain region. 1, HaZorea Forest; 4, Mevo Elyakim; 5, Oren HaSela parking site; 6, Keren HaCarmel road junction; 7, Keren HaCarmel; 8, Oranei HaPisgah; 9, Damun Junction; 10, Beit Oren; 11, Kedumim quarry; 12, artificial lake; 13, Rakit; 14, Yearoth HaCarmel Hotel; 15, Nahal Oren mouth; 16, Carmel Park office; 17, antenna; 18, Hai Bar.

Emission Spectrometer (ICP-AES) Spectroflame ICP (Spectro, Kleve, Germany). The auxiliary gas used for S determinations was Ar, 99.9995%. The ions of F^- , Cl^- , PO_4^{3-} , NO_3^- , and SO_4^{2-} were extracted by an H_2O treatment. The amount of these ions in the thallus was determined by a DX-300 ion chromatograph (Dionex Corp., Sunnyvale, CA) using an AS₄-SC analytical column and a guard column, an anion micromembrane suppressor, and sodium carbonate/bicarbonate as eluent. The measurements were calibrated by the special standards of Dionex Corp. The reference material used to check the accuracy of our measurements was the IAEA-336 supplied by the International Atomic Energy Agency, Vienna, Austria. The reference sheet was updated in June 1999.

Statistical Evaluation of the Data

The results of the chemical and physiological analyses were evaluated by a one-way ANOVA and by a least significant difference (LSD) test to determine the significance of differences between biomonitoring sites. The difference was considered to be signifi-

cant in the case of $P < 0.05$. A Pearson correlation test was used to obtain correlation coefficients of the physiological parameters and the elemental and ion content of the lichen.

RESULTS

Changes in the Spectral Reflectance Response of Lichen Thalli

Table 2 presents the NDVI values for thalli collected in July 1996 in HaZorea, resuspended in the same site or transplanted to the Haifa Bay and the Mt. Carmel regions and retrieved in May 1997. Three sites (3, 7, and 18) on the Carmel Mountain, apart from the resuspended thalli in site 1, reached high NDVI values, whereas the other sites reached lower values, especially site 19, located near the oil refineries in the Haifa Bay.

The Integrity of the Photobiont Chlorophyll and Cell Membranes in the Lichen Thallus

The OD 435 nm/OD 415 nm ratios for thalli retrieved in May 1997 are presented in Table 2. High

TABLE 2

Mean \pm SD of NDVI, 435 nm/415 nm Ratio Expressing Chlorophyll Status, and Electric Conductivity of Water Expressing Electrolyte Leakage in Thalli of *Ramalina lacera* Collected in the HaZorea Forest in July 1996 and Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region and Retrieved in May 1997

Site No.	<i>n</i>	NDVI <i>X</i> \pm SD	<i>n</i>	OD435 nm/OD415 nm <i>X</i> \pm SD	<i>n</i>	Electric conductivity (mSm ⁻¹) <i>X</i> \pm SD
1	4	0.380 \pm 0.002 b*	12	1.43 \pm 0.02 ab	12	4.28 \pm 0.25 c
2	4	0.355 \pm 0.004 e	12	1.41 \pm 0.02 abcd	12	3.71 \pm 0.12 d
3	4	0.386 \pm 0.002 a	12	1.42 \pm 0.03 abc	4	4.35 \pm 0.38 c
5	4	0.361 \pm 0.002 d	12	1.42 \pm 0.02 abc	12	3.70 \pm 0.44 d
6	4	0.363 \pm 0.003 cd	12	1.40 \pm 0.02 bcd	12	4.98 \pm 0.36 b
7	4	0.378 \pm 0.007 b	12	1.42 \pm 0.03 abc	12	2.99 \pm 0.24 f
8	4	0.368 \pm 0.006 c	12	1.40 \pm 0.04 bcd	12	3.33 \pm 0.14 e
9	4	0.350 \pm 0.002 fg	12	1.44 \pm 0.02 a	5	5.06 \pm 0.32 b
10	4	0.349 \pm 0.003 gh	12	1.42 \pm 0.02 abc	12	2.45 \pm 0.15 g
12	4	0.343 \pm 0.002 i	12	1.34 \pm 0.06 e	12	3.74 \pm 0.22 d
13	4	0.330 \pm 0.002 j	12	1.41 \pm 0.02 abcd	12	2.99 \pm 0.26 f
14	4	0.354 \pm 0.003 ef	12	1.38 \pm 0.05 d	12	2.14 \pm 0.28 h
15	4	0.345 \pm 0.004 hi	12	1.39 \pm 0.07 cd	12	3.57 \pm 0.21 d
16	4	0.365 \pm 0.004 cd	12	1.43 \pm 0.02 ab	12	3.64 \pm 0.32 d
18	4	0.380 \pm 0.002 b	12	1.42 \pm 0.03 ab	12	3.22 \pm 0.13 ef
19	4	0.227 \pm 0.004 k	12	1.26 \pm 0.11 f	6	5.42 \pm 0.36 a
ANOVA						
<i>F</i> Ratio		458.55		11.83		108.56
<i>F</i> probability		0.00		0.00		0.00

Note. Sites 5–18 belong to the Mount Carmel National Park. *n*, number of replicates; *X*, mean value; SD, standard deviation.

* Values in each column followed by the same letter do not differ significantly at *P* < 0.05 using one-way ANOVA and LSD test.

ratios indicate a high level of chlorophyll integrity. The table shows that the ratio was significantly lower for thalli retrieved at site 12 near the parking area at the artificial lake and near the oil refineries (site 19) in the Haifa Bay. Table 2 shows high conductivity values for samples retrieved in the vicinity of the oil refineries (site 19) and at two road junctions on the Carmel mountain (sites 6 and 9). Lower values for electric conductivity at the other sites indicated that the cell membranes in these sites were not or little injured.

The Elemental Content of the Lichen

A comparative analysis of the elemental and ion content of *R. lacera* transplants suggested that the study area is not uniform and should be divided into different subareas. The first part was represented by site 1, HaZorea, from which the transplant material was taken and transferred to the other biomonitoring stations. Lichens from this site contained relatively low levels of anthropogenic elements, such as Ni, Zn, V, and Cr (Table 5) and Cu, Pb, and S (Table 6). The levels of Ca (Table 3) and F⁻ (Table 7) were, however, relatively high.

The second part, represented by site 2 was located in a small local park and was influenced by the activity of a limestone quarry. In this site the transplants contained high levels of Mg (Table 4) and F⁻ (Table 7).

The third part of the study area displayed a great extent of variation among sites. This part included site 3 and sites 4–18, which belong to the Mount Carmel National Park. Some of these sites displayed a significant increase of certain elements. Samples retrieved from site 17, located at a distance of 3 km from the industrial area in the Haifa Bay, contained high levels of Ti (Table 3), K (Table 4), Ni, Zn, and V (Table 5), and F⁻ and Cl⁻ (Table 7). Lichens from site 9 contained relatively large amounts of Zn (Table 5).

The most polluted sites, based on measurements of elemental content of *R. lacera* transplants, were those located in the Haifa Bay area. Levels of Ba (Table 4), Ni, Zn, V, and Cr (Table 5), and Cu, S, and SO₄²⁻ (Table 6) were relatively high in the vicinity of the oil refineries (site 19). The above-mentioned elements in addition to Na, Mg, Sr, B (Table 4), Mn and Fe (Table 5), and Cl⁻ and NO₃⁻ (Table 7) reached high levels in the vicinity of the Haifa Power Plant (site 20) (Tables 3–7).

TABLE 3

Mean \pm SD of Ca, Ti, Al, and Si in *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

Site No.	Ca ($X \pm SD$)	Ti ($X \pm SD$)	Ca/Ti	Al ($X \pm SD$)	Al/Ti	Si ($X \pm SD$)	Al/Si
1	34,310 \pm 3522 a*	31.50 \pm 4.70 fgghi	1089	1088 \pm 156 cde	35	147 \pm 21 abcd	7.4
2	19380 \pm 1808 c	26.90 \pm 5.86 hij	720	774 \pm 101 gh	29	81 \pm 24 efg	9.5
3	9140 \pm 907 h	24.20 \pm 3.33 j	378	730 \pm 66 h	30	48 \pm 19 g	15.2
4	14000 \pm 2874 ef	32.70 \pm 6.55 efgh	428	905 \pm 167 fg	28	51 \pm 15 g	17.7
5	29540 \pm 5605 b	29.20 \pm 5.77 ghij	1012	1132 \pm 248 cde	39	111 \pm 35 de	10.2
6	18240 \pm 1387 c	39.10 \pm 7.95 cde	466	1312 \pm 185 ab	33	177 \pm 44 a	7.4
7	11300 \pm 2409 gh	27.80 \pm 4.13 hij	406	984 \pm 124 ef	35	108 \pm 44 def	9.1
8	11890 \pm 1356 fg	29.20 \pm 3.99 ghij	407	877 \pm 106 fgh	30	117 \pm 26 cde	7.5
9	15560 \pm 1052 de	35.70 \pm 3.89 defg	436	1102 \pm 96 cde	31	136 \pm 77 bcd	8.1
10	17890 \pm 1643 c	47.60 \pm 4.97 a	376	1082 \pm 116 cde	23	61 \pm 15 g	17.7
11	17490 \pm 4352 cd	39.80 \pm 17.71 bcd	439	1028 \pm 397 def	26	86 \pm 55 efg	11.9
12	19340 \pm 3594 c	49.30 \pm 14.25 a	392	1348 \pm 311 a	27	130 \pm 82 bcd	10.4
13	14630 \pm 1754 e	39.40 \pm 6.31 bcde	447	1125 \pm 139 cde	28	131 \pm 103 bcd	8.6
14	14600 \pm 2305 e	25.00 \pm 4.03 ij	584	796 \pm 117 gh	32	47 \pm 13 g	16.9
15	15350 \pm 2153 de	35.50 \pm 4.09 defg	432	1069 \pm 83 cde	30	71 \pm 9 fg	15.0
16	15020 \pm 2852 e	43.30 \pm 7.12 abc	347	1206 \pm 146 abc	28	151 \pm 53 abc	7.9
17	14405 \pm 1946 e	45.90 \pm 8.84 ab	314	1198 \pm 207 abc	26	81 \pm 27 efg	14.8
18	14760 \pm 2425 e	38.10 \pm 9.61 cdef	387	1162 \pm 260 bcd	30	159 \pm 40 ab	7.3
19	19780 \pm 1877 c	38.30 \pm 7.85 cde	516	1083 \pm 134 cde	28	71 \pm 13 fg	15.2
20	19400 \pm 1174 c	40.70 \pm 5.52 bcd	477	1177 \pm 79 bcd	29	69 \pm 3 fg	17.0
ANOVA							
F ratio	49.39	9.47		8.72		8.21	
F probability	0.00	0.00		0.00		0.00	

Note. Sites 4–18 belong to the Mount Carmel National Park. Number of replicates was 10 in all cases. X, mean values given as $\mu\text{g g}^{-1}$ on d. wt. basis; SD, standard deviation.

* Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by one-way ANOVA and LSD test.

The Correlation of the Physiological Parameters with the Values Indicating the Elemental Content of the Lichen *Thallus*

The Pearson correlation tests revealed that NDVI values correlated positively with OD435 nm/OD415 nm ratios and negatively with amounts of Ba, Cu, Ni, S, SO_4^{2-} , and V. Table 8 indicates a positive correlation of electric conductivity values and amounts of B, Ba, Cl^- , Cr, Cu, Na, Ni, NO_3^- , S, and SO_4^{2-} . Many pairs of chemical elements and ions detected in *R. lacera* reached high correlation coefficients. All of them correlated positively. The correlation coefficients of selected pairs are presented in Table 9.

DISCUSSION

The transplantation method was demonstrated to be very useful, as it provided information on the impact of anthropogenic activity in a region which

lacks regular automatic measurement of airborne chemical pollutants. In this study, lichens were collected in their natural relatively unpolluted habitat and transferred to different sites, including some which were without local fruticose lichens. The results show that certain elements and ions accumulated in the lichen transplants derive from geochemical sources (Al, Ti, Ca, Si, Fe, Mn, K, P, PO_4^{3-}). Additional elements trace oil combustion (Ni, V, S) and vehicular activity (Pb). The comparative analysis of alterations in the physiological parameters applied to assess the vitality of lichens exposed to varying levels of pollution revealed a different degree of sensitivity for each of the parameters.

The Chemical Composition of the Transplants

An attempt to differentiate the impact of anthropogenic pollutants from the influence of soil dust and salt spray was based on ratios of Al/Ti, Ca/Ti, and Al/Si, confirmed to be reliable tracers of geochemical

TABLE 4

Mean \pm SD of K, Na, Mg, Ba, Sr, and B in *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

Site No.	K ($X \pm SD$)	Na ($X \pm SD$)	Mg ($X \pm SD$)	Ba ($X \pm SD$)	Sr ($X \pm SD$)	B ($X \pm SD$)
1	2740 \pm 237 cd	437 \pm 46 d	941 \pm 68 fgh	17.18 \pm 2.04 b	33.97 \pm 2.10 a	9.70 \pm 1.70 defg
2	3030 \pm 330 a	346 \pm 18 gh	2032 \pm 143 a	11.36 \pm 1.36 efghi	23.48 \pm 2.17 def	12.04 \pm 6.17 bc
3	2850 \pm 242 bc	757 \pm 72 b	991 \pm 61 efg	9.34 \pm 1.35 i	17.42 \pm 4.67 i	10.26 \pm 2.63 cdef
4	3050 \pm 375 ab	595 \pm 96 c	942 \pm 104 fgh	11.92 \pm 2.11 defg	17.47 \pm 1.44 i	9.60 \pm 1.31 defg
5	2420 \pm 322 ef	422 \pm 54 de	986 \pm 165 efg	15.11 \pm 3.82 bc	27.25 \pm 5.43 bc	10.34 \pm 2.39 cde
6	2840 \pm 171 bc	705 \pm 93 b	1097 \pm 87 d	11.52 \pm 1.84 efgh	18.66 \pm 1.76 ghi	13.93 \pm 5.19 b
7	2610 \pm 191 de	354 \pm 46 fg	876 \pm 86 hij	9.92 \pm 1.41 fghi	13.69 \pm 2.04 j	8.56 \pm 0.99 efgh
8	2690 \pm 296 cd	428 \pm 51 de	816 \pm 77 jk	9.74 \pm 1.41 hi	21.10 \pm 3.22 fgh	10.67 \pm 2.77 cde
9	3040 \pm 217 ab	751 \pm 39 b	1035 \pm 41 de	13.08 \pm 2.08 cde	25.96 \pm 4.81 bcd	10.37 \pm 1.41 cde
10	2140 \pm 171 g	222 \pm 15 i	680 \pm 37 l	9.85 \pm 0.63 ghi	18.90 \pm 1.79 ghi	9.10 \pm 1.88 defgh
11	2430 \pm 258 ef	292 \pm 19 h	689 \pm 100 l	10.84 \pm 3.68 fghi	18.88 \pm 2.93 gh	8.03 \pm 1.60 fgh
12	3080 \pm 329 a	358 \pm 62 fg	997 \pm 142 ef	16.17 \pm 4.91 b	24.55 \pm 5.80 cde	10.35 \pm 2.26 cde
13	2870 \pm 343 abc	382 \pm 40 efg	850 \pm 60 ij	12.01 \pm 2.52 def	17.24 \pm 1.89 i	7.74 \pm 1.54 gh
14	2590 \pm 242 de	187 \pm 13 i	741 \pm 47 kl	9.71 \pm 2.45 hi	18.91 \pm 6.21 ghi	7.26 \pm 1.62 h
15	2420 \pm 103 ef	457 \pm 39 d	912 \pm 32 ghi	11.91 \pm 0.56 defg	19.51 \pm 1.65 ghi	10.92 \pm 1.76 cd
16	2700 \pm 226 cd	331 \pm 29 gh	875 \pm 106 hij	13.94 \pm 2.71 cd	21.30 \pm 6.74 efgh	9.72 \pm 2.64 defg
17	3030 \pm 216 ab	589 \pm 45 c	966 \pm 62 efg	11.59 \pm 1.73 efgh	18.23 \pm 2.43 hi	8.52 \pm 1.63 efgh
18	2730 \pm 231 cd	406 \pm 37 def	874 \pm 74 hij	13.94 \pm 3.79 cd	21.72 \pm 5.31 efg	8.69 \pm 1.79 defgh
19	2290 \pm 197 fg	561 \pm 91 c	1331 \pm 108 c	20.83 \pm 1.64 a	26.36 \pm 2.35 bcd	12.10 \pm 1.86 bc
20	2780 \pm 123 cd	1306 \pm 150 a	1417 \pm 55 b	20.90 \pm 1.66 a	28.69 \pm 1.09 b	17.83 \pm 0.93 a
ANOVA						
F ratio	11.69	162.54	113.23	19.96	17.00	8.87
F probability	0.00	0.00	0.00	0.00	0.00	0.00

Note. Sites 4–18 belong to the Mount Carmel National Park. Number of replicates was 10 in all cases. X, mean values given as $\mu\text{g g}^{-1}$ on d. wt. basis; SD, standard deviation.

* Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by one-way ANOVA and LSD test.

origin, and the correlation of Na and Cl, which is related to marine aerosols. Many lichens display a linear relation of Al and Ti content (Nash, 1989). Our results, which show a rather uniform Al/Ti ratio in all of the 20 sites, implied that much particulate matter entrapped by the thallus is soil derived. The high Ca content of the resuspended material from site 1 and the high Ca/Ti ratio obtained for this site are probably derived from the upper calcareous soil layer, blown by the wind in the HaZorea Forest.

High correlation coefficients obtained for Na and Cl could trace the marine aerosol as a common source. An attempt to determine the dependence of the Na and Cl content on distance from the sea did not detect significant correlations of these factors, neither for Na ($r = -0.22$; $P = 0.34$) nor for Cl^- ($r = -0.30$, $P = 0.19$). These findings led to the assumption that the presence of NaCl derived at least in part from the deposition of dust over the study area. An analysis of the chemical composition of aerosols settling in Israel, following dust storms, revealed that the Na content of dust was

$4.9 \pm 1.35 \text{ mg g}^{-1}$ and that of Cl^- was $2.04 \pm 1.41 \text{ mg g}^{-1}$ (Ganor *et al.*, 1991). According to Foner and Ganor (1992) halite (NaCl) was the major mineral present in aerosols sampled in Tel Aviv.

Relatively high V and Ni contents of lichens in sites 19 and 20 in the Haifa Bay indicate the presence of industrial plants powered by heavy fuel combustion. Indeed, V and Ni are well-known tracers of oil refineries and power plants (Ganor *et al.*, 1988). The relatively high Ni, V, and Zn contents of samples from site 17, which is close to the Haifa Bay, trace the pollution originating in this industrial area. Indications of wind directions provided by the Meteorological Service, Beth Dagan, Israel (personal communication, 1998) point to the probability of the transfer of pollutants from the Haifa Bay industrial region to site 17 on the Carmel Mountain. Of the total sum of the winds blown on the Carmel Mountain in the period September–November 1996, 26% arrived from the Haifa Bay. A similar trend of wind directions (28.5%) was recorded in December 1996–February 1997, whereas 14% of the total amount of

TABLE 5

Mean \pm SD of Ni, Zn, V, Cr, Mn, and Fe in *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

Site No.	Ni ($X \pm SD$)	Zn ($X \pm SD$)	V ($X \pm SD$)	Cr ($X \pm SD$)	Mn ($X \pm SD$)	Fe ($X \pm SD$)
1	3.98 \pm 1.18 defg*	44.00 \pm 11.30 bc	4.49 \pm 0.85 hi	4.08 \pm 0.45 b	23.70 \pm 2.00 bcd	1032 \pm 151 defg
2	4.50 \pm 0.90 cd	42.50 \pm 10.55 bcd	5.51 \pm 0.63 efg	3.03 \pm 0.40 def	22.50 \pm 1.51 def	863 \pm 117 ij
3	3.43 \pm 0.98 fgh	34.50 \pm 3.41 d	4.01 \pm 0.42 i	2.39 \pm 0.36 g	18.10 \pm 0.88 i	726 \pm 57 jk
4	3.31 \pm 0.91 gh	34.20 \pm 5.25 d	4.51 \pm 0.63 hi	3.15 \pm 0.46 de	20.30 \pm 2.75 gh	884 \pm 178 hi
5	4.54 \pm 1.02 cd	42.30 \pm 13.00 bcd	5.06 \pm 0.92 fgh	4.00 \pm 0.86 b	26.40 \pm 4.27 a	1095 \pm 227 cdef
6	3.90 \pm 0.97 defg	43.40 \pm 7.29 bcd	5.92 \pm 1.14 de	3.87 \pm 0.59 b	25.30 \pm 1.83 ab	1238 \pm 145 ab
7	4.10 \pm 0.75 defg	36.70 \pm 4.72 cd	4.73 \pm 0.45 ghi	2.93 \pm 0.42 def	23.40 \pm 2.01 cde	949 \pm 107 ghi
8	2.87 \pm 0.60 h	40.60 \pm 6.35 cd	4.30 \pm 0.63 hi	3.16 \pm 0.37 de	19.80 \pm 1.87 hi	863 \pm 102 ij
9	4.77 \pm 0.65 cd	61.60 \pm 20.61 a	6.86 \pm 0.58 c	3.97 \pm 0.32 b	23.90 \pm 1.73 bcd	1057 \pm 114 defg
10	4.33 \pm 0.93 cde	39.20 \pm 3.85 cd	6.51 \pm 0.58 cd	2.69 \pm 0.65 efg	20.50 \pm 1.08 gh	977 \pm 111 fghi
11	3.98 \pm 1.46 defg	43.90 \pm 14.25 bc	6.40 \pm 2.01 cd	2.72 \pm 0.85 efg	19.50 \pm 3.31 hi	890 \pm 324 hi
12	5.21 \pm 0.88 c	39.00 \pm 5.68 cd	7.01 \pm 1.62 c	3.34 \pm 0.88 cd	25.20 \pm 3.55 abc	1143 \pm 253 bcde
13	4.31 \pm 1.22 def	43.90 \pm 8.81 bc	6.28 \pm 0.62 cde	3.03 \pm 0.50 def	21.20 \pm 1.99 fgh	1007 \pm 116 efgh
14	3.48 \pm 0.90 efgh	50.80 \pm 24.56 b	4.30 \pm 0.70 hi	2.62 \pm 0.40 fg	19.40 \pm 1.26 hi	715 \pm 102 k
15	4.23 \pm 1.14 def	38.90 \pm 7.28 cd	5.50 \pm 0.73 efg	3.20 \pm 0.28 de	21.80 \pm 1.32 efg	1014 \pm 55 defgh
16	4.32 \pm 0.69 cdef	41.80 \pm 4.47 bcd	5.83 \pm 0.64 def	3.97 \pm 0.47 b	23.80 \pm 1.81 bcd	1200 \pm 127 bc
17	8.54 \pm 1.26 b	60.70 \pm 9.51 a	12.48 \pm 0.91 a	3.88 \pm 1.26 b	21.80 \pm 1.03 efg	1126 \pm 180 bcde
18	3.90 \pm 0.90 defg	42.00 \pm 11.78 bcd	6.25 \pm 1.40 cde	3.74 \pm 0.65 bc	22.90 \pm 1.97 def	1149 \pm 226 bcd
19	8.87 \pm 1.23 b	63.30 \pm 5.83 a	11.31 \pm 0.98 b	4.80 \pm 0.41 a	22.40 \pm 1.65 def	1143 \pm 120 bcde
20	10.57 \pm 1.16 a	66.80 \pm 3.19 a	12.23 \pm 0.62 a	4.79 \pm 0.37 a	26.70 \pm 0.82 a	1376 \pm 60 a
ANOVA						
F ratio	40.22	8.46	73.79	13.41	12.88	11.33
F probability	0.00	0.00	0.00	0.00	0.00	0.00

Note. Sites 4–18 belong to the Mount Carmel National Park. Number of replicates was 10 in all cases. X, mean values given as $\mu\text{g g}^{-1}$ on d. wt. basis; SD, standard deviation.

* Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by one-way ANOVA and LSD test.

winds, blown in the period March–May 1997, arrived from the Haifa Bay. We could not detect a gradient for V, Ni, or Zn contents of *R. lacera* transplants, originating in the southwest, where another power station, the Oroth Rabin Power Station near Hadera, is located at a distance of 32 km from site 17. As different metals, e.g., V (Juichang *et al.*, 1995) decrease exponentially with an increase of distance from power plants, it is reasonable to assume that the source of V, Ni, and probably Zn in site 17 and in the Haifa University area is the Haifa Bay and not remote sources, e.g., the above-mentioned power station. The small amounts of Pb in *R. lacera* probably reflect the introduction of unleaded gasoline in Israel in September 1990. The total number of motor vehicles in Israel was increased progressively: 370,895 in 1973 (Statistical Abstract of Israel, 1975) and 1,542,870 in 1996 (Statistical Abstract of Israel, 1997). The Pb content of *R. lacera* collected either in situ or upon resuspension in HaZorea (Table 10) did not follow the same trend in the same period. Samples from the Haifa Bay contained more Ba than

samples from the Mount Carmel National Park. Ba is locally used in the manufacturing of glass and ceramics. According to Monaci and Bargagli (1997), Ba has many applications in the automotive industries, including the production of rubber, lubricating oil additives, and fuel synthesis, and was found in gasoline, unleaded gasoline and diesel oil. The presence of Ba in the emission of diesel- and unleaded-gasoline-powered vehicles was reported also by Que Hee (1994) in the United States. Crude oil used in the oil refineries in the Haifa Bay contains Ba, and no measures were taken to remove this element from the final product.

For a comparison of values of elemental content of *R. lacera* obtained in the present study and values obtained for other sites in the world, it is preferable to review data of other species of *Ramalina*. As statistical baseline values for chemical elements in this genus are still missing, we recommend a comparison of our data with data of elemental/ion content for other *Ramalina* spp. studied in different countries. Table 11 presents a few studies on this topic. For

TABLE 6

Mean \pm SD of Cu, Pb, Li, S, and SO_4^{2-} in *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

Site No.	Cu ($X \pm \text{SD}$)	Pb ($X \pm \text{SD}$)	Li ($X \pm \text{SD}$)	S ($X \pm \text{SD}$)	SO_4^{2-} ($X \pm \text{SD}$)	$\text{SO}_4^{2-}/\text{S}$ ($X \pm \text{SD}$)
1	4.80 \pm 0.33 fg*	n.d.**	0.67 \pm 0.12 ab	3263 \pm 262 cd	804 \pm 498 cd	0.24
2	4.72 \pm 0.58 gh	n.d.	0.60 \pm 0.41 abcd	2940 \pm 203 fg	561 \pm 32 efg	0.19
3	3.93 \pm 0.43 ij	n.d.	0.38 \pm 0.08 efg	2369 \pm 142 l	433 \pm 71 g	0.18
4	5.29 \pm 0.71 ef	n.d.	0.61 \pm 0.10 abcd	2527 \pm 148 kl	445 \pm 69 g	0.17
5	5.34 \pm 0.67 e	n.d.	0.63 \pm 0.16 abc	3404 \pm 341 c	968 \pm 324 c	0.28
6	5.52 \pm 0.40 de	21.70 \pm 3.65	0.55 \pm 0.38 bcde	3194 \pm 127 de	965 \pm 120 c	0.30
7	4.55 \pm 0.47 gh	21.00 \pm 3.78	0.54 \pm 0.08 bcde	2749 \pm 245 hij	679 \pm 56 def	0.24
8	4.69 \pm 0.36 gh	21.00 \pm 3.16	0.49 \pm 0.13 cdef	2472 \pm 233 l	585 \pm 67 efg	0.23
9	5.50 \pm 0.21 de	n.d.	0.45 \pm 0.08 def	2820 \pm 101 ghij	653 \pm 134 def	0.23
10	4.68 \pm 0.43 gh	n.d.	0.73 \pm 0.28 a	2544 \pm 136 kl	540 \pm 88 fg	0.21
11	4.69 \pm 0.59 gh	n.d.	0.54 \pm 0.22 bcde	2696 \pm 141 ijk	540 \pm 81 fg	0.20
12	4.56 \pm 0.60 gh	n.d.	0.77 \pm 0.25 a	2929 \pm 402 fgh	735 \pm 299 de	0.25
13	4.29 \pm 0.52 hi	n.d.	0.53 \pm 0.14 bcde	2681 \pm 160 jk	573 \pm 128 efg	0.21
14	3.61 \pm 0.38 j	n.d.	0.22 \pm 0.10 g	2485 \pm 123 l	431 \pm 90 g	0.17
15	4.31 \pm 0.33 ghi	n.d.	0.40 \pm 0.12 ef	2687 \pm 265 ijk	603 \pm 63 efg	0.22
16	5.87 \pm 1.11 cd	21.06 \pm 2.37	0.34 \pm 0.25 fg	2873 \pm 210 fghi	700 \pm 118 def	0.24
17	6.34 \pm 0.39 c	22.00 \pm 4.83	0.67 \pm 0.20 ab	3012 \pm 131 ef	685 \pm 78 def	0.22
18	5.35 \pm 0.36 c	n.d.	0.38 \pm 0.13 efg	3037 \pm 173 ef	671 \pm 95 def	0.22
19	10.49 \pm 1.04 a	24.10 \pm 3.21	0.68 \pm 0.13 ab	3767 \pm 256 b	1970 \pm 347 b	0.52
20	9.93 \pm 0.65 b	26.20 \pm 7.57	0.63 \pm 0.07 abc	4327 \pm 134 a	2820 \pm 391 a	0.65
ANOVA						
F ratio	94.69	—	5.32	50.97	78.80	
F probability	0.00	—	0.00	0.00	0.00	

Note. Sites 4–18 belong to the Mount Carmel National Park. Number of replicates was 10 in all cases. X, mean values given as $\mu\text{g g}^{-1}$ on d. wt. basis; SD, standard deviation.

* Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by one-way ANOVA and LSD test.

** Under the detection limit.

additional data obtained for other lichen genera, see reviews of Nash (1989, 1996), Garty (1993), Nash and Gries (1995), and Bennett (2000).

Comparison of Physiological Parameters Applied to Assess *Thallus* Vitality and Elemental Content

Previous comparative analyses of physiological status and elemental content of transplanted lichens in polluted sites provided important information on the impact of airborne chemicals on lichen vitality. Some of these studies revealed a causal link between impact of air pollutants and chlorophyll degradation (e.g., Benlap and Harper, 1990; Boonpragob and Nash, 1991; Bartok *et al.*, 1992; Gonzalez and Pignata, 1994, 1997, 1999; Levin and Pignata, 1995; Silberstein *et al.*, 1996; Gonzalez *et al.*, 1996, 1998; Cañas *et al.*, 1997; Cañas and Pignata, 1998; Carreras *et al.*, 1998; Riga-Karandinos and Karandinos, 1998). Our findings relating to the chlorophyll integrity of *R. lacera*, which was unaffected by dust emitted from the limestone quarry in site 2, suggested

that the dust pollution in this area had a less detrimental effect on algal pigments than that reported for lichens in the vicinity of a limestone quarry in Greece. According to Zaharapoulou *et al.*, (1993) a severe decrease of chlorophyll content and high phaeophytization occurred in *Physcia adscendens* along the load gradient of dust near this quarry.

Additional studies performed in the 1990s focused on the linkage between the content of airborne elements accumulated in lichen transplants and the degradation of cell membranes (e.g., Rope and Pearson, 1990; Boonpragob and Nash, 1990; Garty *et al.*, 1997a,b, 1998a,b). Most of these studies indicated a severe leakage of K from thalli displaying a high degree of electric conductivity due to cell membrane impairment.

The application of the NDVI ratio, on the other hand, was much less frequently reported with regard to lichens and airborne pollutants. Studies performed by Garty *et al.* (1997a–d) indicated that low NDVI values coincided with high levels of S, Ni, V, SO_4^{2-} , Pb, Mn, Al, Cr, Fe, and Ti.

TABLE 7

Mean \pm SD of P, PO₄³⁻, NO₃⁻, F⁻, and Cl⁻ in *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

Site No.	P (X \pm SD)	PO ₄ ³⁻ (X \pm SD)	NO ₃ ⁻ (X \pm SD)	F ⁻ (X \pm SD)	Cl ⁻ (X \pm SD)
1	1828 \pm 215 ab*	2530 \pm 386 ab	83 \pm 15	315 \pm 58 a	314 \pm 55 def
2	1856 \pm 316 ab	2810 \pm 491 a	n.d.**	308 \pm 44 b	220 \pm 24 ghi
3	1821 \pm 221 abc	2550 \pm 440 ab	87 \pm 23	246 \pm 28 b	534 \pm 79 b
4	1590 \pm 385 cdef	2380 \pm 815 abcd	66 \pm 16	204 \pm 8 cde	422 \pm 89 c
5	1538 \pm 305 defg	2000 \pm 938 cdef	67 \pm 15	210 \pm 48 bcde	330 \pm 59 de
6	1637 \pm 147 bcde	2230 \pm 510 bcdef	90 \pm 20	203 \pm 58 cde	548 \pm 118 b
7	1683 \pm 273 bcde	2200 \pm 733 bcdef	55 \pm 8	212 \pm 10 bcd	273 \pm 52 efg
8	1298 \pm 252 hi	1880 \pm 541 efg	n.d.	177 \pm 46 defg	278 \pm 22 efg
9	1861 \pm 219 ab	2280 \pm 579 bcde	53 \pm 16	146 \pm 46 g	550 \pm 95 b
10	1098 \pm 122 i	1490 \pm 303 g	n.d.	193 \pm 71 def	194 \pm 20 hi
11	1347 \pm 267 gh	2000 \pm 416 cdef	50 \pm 16	209 \pm 33 bcde	242 \pm 35 fgghi
12	1970 \pm 213 a	2350 \pm 613 abcde	67 \pm 23	173 \pm 43 efg	332 \pm 66 de
13	1743 \pm 330 abcd	2190 \pm 654 bcdef	n.d.	103 \pm 44 h	267 \pm 63 efgh
14	1629 \pm 296 bcde	2460 \pm 560 abc	n.d.	198 \pm 35 de	177 \pm 37 i
15	1374 \pm 134 fgh	1790 \pm 197 fg	84 \pm 19	236 \pm 32 bc	365 \pm 63 cd
16	1388 \pm 294 fgh	1910 \pm 644 defg	52 \pm 12	152 \pm 57 g	213 \pm 40 ghi
17	1553 \pm 500 defg	2150 \pm 276 bcdef	241 \pm 38	243 \pm 48 b	575 \pm 72 b
18	1383 \pm 205 fgh	1490 \pm 401 g	41 \pm 3	156 \pm 36 fg	275 \pm 40 efg
19	1501 \pm 184 efgh	2190 \pm 300 bcdef	117 \pm 26	204 \pm 16 cde	391 \pm 101 cd
20	1753 \pm 110 bcd	2470 \pm 400 abc	842 \pm 107	193 \pm 14 def	1520 \pm 270 a
ANOVA					
F ratio	7.36	3.99	—	14.14	110.44
F probability	0.00	0.00	—	0.00	0.00

Note. Sites 4–18 belong to the Mount Carmel National Park. Number of replicates was 10 in all cases. X, mean values given as $\mu\text{g g}^{-1}$ on d. wt. basis; SD, standard deviation.

* Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by one-way ANOVA and LSD test.

** Under the detection limit.

The present findings, showing a decrease of NDVI values in lichens from the Haifa Bay, may be compared with the field study of Gouaux and Vincent (1990), who performed a comparative analysis of polluted versus unpolluted areas by means of infrared color photography. The change of spectral response, indicated by the visible and infrared bands and by a decrease of the vegetation index (IR/R), was attributed to the action of pollutants. The inverse correlation of NDVI and Cu detected in the present investigation is in accordance with findings of Cox and co-workers (1991), who found that lichens exposed to Cu concentrations of $> 20 \mu\text{g g}^{-1}$ exhibited a significant shift of 2–3% of the spectral response.

The negative correlations obtained for NDVI and S, NDVI and Ni, and NDVI and V raised the questions whether these correlations may be attributed to the effect of atmospheric levels of SO₂ and whether Ni and V affect the algal chlorophyll in *R. lacera*. The latter possibility, which links NDVI, chlorophyll integrity, and two trace elements, should be established in controlled experiments. An adverse

effect on the lichen photobiont may be the result of the acid deposition recently recorded in the study area (Shamay *et al.*, 1990; Singer *et al.*, 1993, 1996), which is likely to co-occur with high levels of ambient Ni and V in the proximity of the two industrial sites in the study area. Of the two principal sources of SO₂ in the Haifa Bay, the Haifa Power Station in 1996 emitted 0.77 ton h⁻¹ (35% of the total SO₂ emission in the region), and the oil refineries emitted 0.71 ton h⁻¹ (32% of the total SO₂ emission), as reported by the Haifa District Association of Municipalities for the Environment (1997). The SO₂ emission by the two above-mentioned sources is reflected to a large extent by the high SO₄²⁻/S ratio in sites 19 and 20 (Table 6).

The increase of visible reflectance of the thallus due to the degradation of chlorophyll in the algal partner is a rather spectacular effect of air pollution. This spectral effect is adequately quantified by the NDVI equation. A further study of the linkage between alterations of NDVI values under controlled conditions, e.g., by application of Ba, Pb, and Zn to

TABLE 8

Pearson Correlation Coefficients of Ion and Elemental Content, NDVI, Electric Conductivity, and OD435 nm/OD415 nm Ratio in Thalli of *Ramalina lacera* Collected in the HaZorea Forest in July 1996, Resuspended in the Same Site or Transplanted in the Haifa Bay and the Mount Carmel Region, and Retrieved in May 1997

	NDVI	Electric conductivity	OD435 nm/OD 415 nm	Ba	Cu	Ni	S	SO ₄ ²⁻	V	Zn	NO ₃ ⁻	Cl ⁻	B	Cr
NDVI														
Electric conductivity	N.S.													
OD435 nm/OD 415 nm	0.89***	N.S.												
Ba	-0.60*	0.56*	-0.57*											
Cu	-0.81***	0.62*	-0.68***	0.80***										
Ni	-0.91***	0.52*	-0.79***	0.70***	0.87***									
S	-0.52*	0.60*	N.S.	0.88***	0.85***	0.80***								
SO ₄ ²⁻	-0.79***	0.63**	-0.74***	0.82***	0.91***	0.84***	0.92***							
V	-0.90***	N.S.	-0.74***	0.59**	0.81***	0.96***	0.68***	0.71***						
Zn	-0.82***	N.S.	N.S.	0.57**	0.74***	0.82***	0.66***	0.68***	0.82***					
NO ₃ ⁻	N.S.	0.75***	-0.55*	0.56**	0.67***	0.78***	0.73***	0.84***	0.67***	0.60**				
Cl ⁻	N.S.	0.80***	N.S.	0.51*	0.64***	0.71***	0.67***	0.78***	0.61***	0.58**	0.94***			
B	N.S.	0.75***	N.S.	0.57**	0.65***	0.56*	0.73***	0.79***	N.S.	N.S.	0.73***	0.79***		
Cr	N.S.	0.68***	N.S.	0.86***	0.83***	0.70***	0.88***	0.76***	0.64***	0.68***	0.52*	0.53*	0.59**	
Na	N.S.	0.84***	N.S.	0.47*	0.61***	0.60**	0.60**	0.70***	0.51*	0.52*	0.81***	0.95***	0.79***	0.54*

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.005$.

TABLE 9

Pearson Correlation Coefficients of Selected Pairs of Elements of Geochemical Origin in Thalli of *Ramalina lacera*

	Al	Fe	Mn	Si	Ti	K	P
Al							
Fe	0.87***						
Mn	0.70***	0.82***					
Si	0.62***	0.50*	0.53*				
Ti	0.80***	0.65***	N.S.	N.S.			
K	N.S.	N.S.	N.S.	N.S.	N.S.		
P	N.S.	N.S.	N.S.	N.S.	N.S.	0.71***	
PO ₄ ³⁻	N.S.	N.S.	N.S.	N.S.	N.S.	0.57**	0.84***

Note. Mean values obtained from Tables 3-7.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.005$.

lichen thalli, will contribute to a better understanding of early warning signals exhibited by sensitive lichens in contaminated environments. It is not surprising that the greater part of the ions detected in *R. lacera* transplants, which exhibited an inverse correlation with NDVI values in the present study, showed the same inverse correlation with the OD435 nm/OD415 nm ratio. The latter parameter is well established for lichens (Garty *et al.*, 1993, 1997a-c, 1998a; Silberstein *et al.*, 1996). However, the inverse correlation of NDVI with the SO₄²⁻ and NO₃⁻ contents of *R. lacera* needs additional investigation, as it seems to reflect the presence of acid rain.

The impairment of cell membranes in the transplanted lichen thalli in the study area, especially in the Haifa Bay, could be the result of the presence of either SO₂, indicated by the correlation of electric conductivity values and the concentration of S and SO₄²⁻, or any of the other ions, such as B, Ba, Cl, Cr, Cu, Na, Ni, and NO₃⁻, found to correlate positively with electric conductivity values. The effect of Cu on lichen membrane integrity was tested by measurements indicating the loss of cations (Branquinho *et al.*, 1997). The intracellular content of K in *Ramalina fastigiata* showed a maximal decrease of 44% relative to the control, which correlated significantly with increasing Cu concentrations. A significant decrease of K in *R. lacera*, as detected in the present study, coincided indeed with relatively high Cu contents of lichens retrieved in the vicinity of the oil refineries.

The high values obtained for electric conductivity, which coincide with a low K content in lichens from site 19 in the Haifa Bay, are comparable with the findings of Tarhanen and co-workers (1996), who studied the effect of industrial emissions on membrane permeability in lichens. K leakage was detected

as the most sensitive indicator of membrane disturbance. The authors obtained positive correlations for K leakage, decrease of distance from pollution sources, and modeled atmospheric SO₂ concentrations. Tarhanen *et al.* (1997) showed the presence of connective factors between K leakage, impairment of membranes, and the concentration of atmospheric ozone. The measurement of atmospheric ozone in our study area is limited to the Haifa Bay. A possible linkage between K leakage, membrane disintegration, and the concentration of ozone over the Haifa Bay seems to merit additional investigation: recently it was found that the concentrations of ozone above the Haifa Bay exhibited a significant increase of 38% in 1996, in comparison with levels in 1992-1995, and reached the maximal value of 216 µg m⁻³ in an automatic monitoring station located in the immediate vicinity of our biomonitoring site. According to the Haifa District Association of Municipalities for the Environment (1997) the increased ozone concentration in the Haifa Bay and in the adjacent study area is produced by an increase of the total number of motor vehicles in these years.

The greater part of the Mount Carmel National Park in Israel is found to be either unpolluted or slightly polluted by anthropogenic activity. The lack of a gradient for the V, Ni, and Zn content of transplants from southwest (Oroth Rabin Power Plant near Hadera) to northeast (Mount Carmel) suggests that this power plant exerts only little or no influence on the air quality in the National Park. High levels of contaminants accumulated in transplants from the Haifa Bay and damage caused to the photobiont cells and the lichen cell membranes in the same samples indicate the impact of combustion of heavy fuel oil in this part of the study area. Based on our findings, we recommend the use of *R. lacera*

TABLE 10
Amounts of Pb in *Ramalina lacera*, *in situ* or Resuspended in HaZorea, 1974–1997

Date	<i>In situ</i>	Resuspended
January 1974	8.0 ^a	—
May–December 1978	—	12.0 ^a
February 1979	14.0 ^a	—
February 1979–March 1980	—	13.0 ^a
July 1980–July 1981	—	10.0 ± 4.0 ^b
July 1981–July 1982	—	38.5 ± 12.0 ^c
December 1981	22.6 ± 7.1 ^d	—
December 1981–December 1982	—	20.6 ± 6.8 ^d
January 1983–January 1984	—	20.5 ± 13.2 ^e
July 1984	21.8 ± 6.7 ^f	—
July 1984–July 1985	—	31.4 ± 14.7 ^f
July 1985	22.1 ± 13.9 ^f	—
June 1992	20.0 ± 8.0 ^g	—
June 1992–March 1993	—	17.9 ± 2.4 ^g
March 1993	24.7 ± 6.9 ^h	—
June 1993	23.4 ± 3.8 ^h	—
March–June 1993	—	22.5 ± 5.8 ^h
March 1993–January 1994	—	12.0 ± 9.0 ⁱ
February 1994	5.0 ± 3.0 ^j	—
November 1994	15.0 ± 2.0 ^j	—
February–November 1994	—	10.0 ± 3.0 ^j
July 1996–May 1997	—	0.0–20.0 ^k

Note. Pb values presented as $\mu\text{g g}^{-1}$ on dry weight basis.

^aGarty and Fuchs, 1982.

^bGarty *et al.*, 1985.

^cGarty, 1987.

^dGarty, 1988.

^eJ. Garty and J. Hagemeyer, unpublished.

^fGarty *et al.*, 1988.

^gJ. Garty, unpublished.

^hGarty *et al.*, 1997a.

ⁱGarty *et al.*, 1997b.

^jGarty *et al.*, 1997c.

^kWithin the detection limit.

transplants in the Haifa Bay area and suggest that emphasis be placed on the examination of heavy metal content and the correlations of OD435 nm/OD415 nm ratios, NDVI values, and values for electric conductivity.

Relative Sensitivity of Physiological Parameters

For a comparison of the sensitivity of the three methodologies applied in the present study, we listed the sites which displayed a significant deviation from the “normal” status for each of the physiological parameters. The OD435 nm/OD415 nm ratio indicated stress in two sites (12 and 19). Values followed by letters e and f did not overlap with values followed by letters a, b, c, and d (Table 2). Overlapping OD435 nm/OD415 nm values for the greater part of the sites (see values followed by let-

ters a, ab, abc, abcd, bcd, cd, and d; Table 2) showed that this parameter did not discriminate very well between sites and correlated with six elements/ions only (Table 6). Thus, it was suggested that the parameter of chlorophyll degradation is not sensitive enough to assess the vitality of lichen transplants. The review of Fields (1988) supported this assumption, as fumigation with high concentrations of certain gaseous pollutants was required to induce chlorophyll degradation.

The NDVI parameter distinguished between four groups of sites with no coincident values (a, b, j, k), whereas the other sites showed different degrees of overlapping (values followed by letters ef, fg, gh, hi, etc.). The NDVI parameter correlated with seven element/ions. This parameter thus appeared to be more sensitive than the OD435 nm/OD415 nm ratio.

TABLE 11
Selected *Ramalina* Spp. Studied as Biomonitors of Airborne Elements/Ions

Species	Element/ion	Location	Reference
<i>Ramalina siliquosa</i>	F ⁻	Wales, UK	Perkins <i>et al.</i> , 1980; Perkins, 1992
<i>R. subfarinacea</i>	F ⁻	Wales, UK	Perkins <i>et al.</i> , 1980; Perkins, 1992
<i>R. fastigiata</i>	F ⁻	Wales, UK	Perkins <i>et al.</i> , 1980; Perkins, 1992
<i>R. farinacea</i>	F ⁻	Wales, UK	Perkins <i>et al.</i> , 1980; Perkins, 1992
<i>R. farinacea</i>	Pb	France	Deruelle, 1984
<i>R. farinacea</i>	S, Ca, Cu, Cd,Pb, Mn, Zn, Fe	The Plain of Megalopolis, Greece	Riga-Karandinos and Karandinos, 1998
<i>R. maciformis</i>	Mn, Cu, ZnPb, Cr, Ni	Negev Desert, Israel	Garty, 1985
<i>R. maciformis</i>	Fe, Zn, Mn,Cu, Pb	Negev Desert, Israel	Garty <i>et al.</i> , 1995
<i>R. maciformis</i>	K, Na, Mg, Ca	Negev Desert, Israel	Garty <i>et al.</i> , 1996
<i>R. lacera</i>	Pb	Algeria	Semadi and Deruelle, 1996
<i>R. stenospora</i>	Th, Cs, Se, Hg, Cr, Ir, Ce, Sc, Rb, Fe, Zn, Co, Ta, Eu, K, Sb	Louisiana, USA	Thompson <i>et al.</i> , 1987
<i>R. stenospora</i>	V, Mn, Al	Louisiana, USA	Mueller <i>et al.</i> , 1987
<i>R. stenospora</i>	Al, Cu, Fe, Zn,Pb	Louisiana, USA	Walther <i>et al.</i> , 1990
<i>R. menziesii</i>	Cd, Cu, Pb, Zn, Mg, K, Co, P, Na, Mn, Fe, Si,Ba, Sr, Ca	California, USA	Boonpragob and Nash, 1990
<i>R. menziesii</i>	Fe, Mg	California, USA	Knops <i>et al.</i> , 1991
<i>R. ecklonii</i>	S	Cordoba, Argentine	Levin and Pignata, 1995; Gonzalez <i>et al.</i> , 1996; Gonzalez and Pignata, 1999

The electric conductivity parameter, which indicates the integrity of cell membranes, divided the sites into eight different groups with only one case of overlapping and correlated with 10 elements/ions. Thus, this physiological response to environmental stress was suggested to be the most sensitive parameter of the three. Fields (1988) indicated the following order of sensitivity of lichen physiological response to fumigation: N₂ fixation > K⁺ efflux/total electrolyte leakage > photosynthesis, respiration > pigment status. In the present study, the order of sensitivity of physiological response under field conditions was electrolyte leakage > NDVI > chlorophyll degradation.

CONCLUSIONS

The present study illustrates the accuracy of the information obtained by biomonitoring methods concerning the deposition of mineral elements, apart from the ability of these methods to assess environmental pollution in extended regions. *R. lacera* was found to be an outstanding accumulator, as the elements tend to concentrate in noticeable amounts in this lichen. Its function as an environmental sensor

is clearly displayed, as physiological damage coincided with the accumulation of trace elements, including heavy metals. The methodology used in this study was shown to be effective and low cost, as a complementary method to automatic systems of measurement. The current study reaffirms the global applicability of biomonitoring methods.

Findings obtained by biomonitors such as lichens have to be compared with the elemental composition of bulk deposition. An increasing number of studies correlated lichen elemental content with atmospheric metal burdens obtained by particulate trappers, rain gauges, and snow-sample analyses. Comparative analyses of elemental content and atmospheric particulates provide an additional indication of the presence of entrapped metal-containing particles and increase the validity of lichens as environmental biomonitors.

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