Photosynthesis, Chlorophyll Integrity, and Spectral Reflectance in Lichens Exposed to Air Pollution

J. Garty,* O. Tamir, I. Hassid, A. Eshel, Y. Cohen, A. Karnieli, and L. Orlovsky

ABSTRACT

The major objective of the present study was to identify the relationship of physiologial parameters of the photosynthetic system with the elemental content of the lichen *Ramalina lacera* (With.) J.R. Laund. Thalli of *R. lacera* were collected in an unpolluted site and transplanted in a national park and an industrial region in Israel for 8 mo. Analyses of photosynthetic activity, chlorophyll integrity, spectral reflectance, and amount of 11 metals were performed after this period of exposure. The normalized difference vegetation index (NDVI), indicative of the spectral reflectance response of the thallus, correlated with photosynthetic rate and chlorophyll and K content and correlated inversely with amounts of Ba, Cr, Cu, and Ni. The NDVI appears to enable the detection of early signs of pollutant-induced stress before changes in other physiological parameters become apparent. Elevated amounts of Cr, Cu, Fe, Mn, Ni, and Zn in lichens transplanted to an industrial area and the correlation of Mn and Ni, Mn and V, Ni and V, Fe and Mn, Fe and V, and Fe and Zn point for the greater part to metal processing in a steel smelter. Correlations of Cr and Ni, Cu and Ni, Zn and Cu, Cu and Mn, and Zn and Ni could be related to metal processing in the industrial area but indicate also vehicular activity as a possible originator.

Use of the term *bioindicator* is related to the response of organisms to different levels of pollution. It refers to the capability of the organism to indicate the presence and amount of atmospheric pollutants (Sloof et al., 1988; Nimis et al., 1993). Bioindicators may be categorized into three main groups: (i) a scale of indicator species noting the presence of each species, (ii) true indicators—individual species exhibiting damage proportional to dose, and (iii) accumulators of potentially toxic materials (Grodziński and Yorks, 1981).

Bioindicators and bioaccumulators that provide quantitative information on levels of pollution and allow the identification of change in pollution in the course of time are defined as *biomonitors* (Manning and Feder, 1980; Martin and Coughtrey, 1982; Sloof et al., 1988; Nimis et al., 1993). Sloof et al. (1988) specified 11 criteria to be met by monitors of metals, of which the following are of primary importance: (i) abundant occurrence in the area of interest, independent of local conditions; (ii) availability for sampling at all seasons; (iii) tolerance of pollutants at relevant levels; and (iv) known response to monitored quantity.

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Published in J. Environ. Qual. 30:884–893 (2001).

The capability of lichens to accumulate different airborne chemical elements, including heavy metals, is a distinctive feature documented by James (1973), Nieboer et al. (1978), Richardson (1992), Garty (1993), Nash (1996), and Jacquiot and Daillant (1997).

Physiological reactions studied in this context of air pollution and lichens are: rate of respiration (Baddeley et al., 1972), decrease of ATP content (Kardish et al., 1987), IAA content (Epstein et al., 1986), production of stress-ethylene (Garty et al., 1997d), electrolyte leakage (Garty et al., 1997a,b; Silberstein et al., 1996; Tarhanen et al., 1996, 1999), and malondialdehyde (MDA) content (Egger et al., 1994; González and Pignata, 1994; Cuny, 1999). The above-mentioned physiological parameters, displaying change upon exposure to chemical pollutants, were found to indicate stress in the lichen thallus as a whole.

The present study investigated the effect of anthropogenic activity on air quality in selected sites in northwest Israel as reflected by the elemental content of lichens (i). Additional parameters of air pollution were chlorophyll content and integrity of algal symbionts (ii), chlorophyll *a* fluorescence (iii), net photosynthetic (NP) rate (iv), and spectral reflectance response (v).

The present study investigated the accumulation of airborne elements in thalli of the epiphytic fruticose (shrub-like) lichen, *Ramalina lacera* (With.) J.R. Laund., which abounds in Israel. This accumulation was brought about by transplantation in a study area with different levels of air pollution, thus establishing a biomonitoring network.

An effective method to estimate the effect of air pollution on lichens is the determination of the integrity of the photobiont chlorophyll. Ronen and Galun (1984) extracted photosynthetic pigments by immersion of lichen thalli in dimethyl sulfoxide (DMSO). They suggested that the ratio of optical density at wavelengths of 435 nm and 415 nm is a reliable parameter for an estimation of chlorophyll degradation. The advantages of DMSO as a solvent for the extraction of photosynthetic lichen pigments are that the extraction is simple, rapid, and complete, and that the extract is easily stored in the cold without degradation.

The measurement of modulated chlorophyll *a* fluorescence is a noninvasive method to analyze photosynthetic parameters in lichens (e.g., Lange et al., 1989; Hovenden and Seppelt, 1995; Kappen et al., 1998). These studies dealt with a wide range of physiological conditions prevailing in unpolluted areas. The present study applied

Abbreviations: DMSO, dimethyl sulfoxide; NDVI, normalized difference vegetation index; NP, net photosynthesis; Pchl, net CO_2 fixation rate per milligram chlorophyll; PSII, Photosystem II; Pw, net CO_2 fixation rate per gram dry weight.

the pulse amplitude methodology (PAM) to measure chlorophyll fluorescence and monitor the status of Photosystem II (PSII).

The effect of pollutants on the photosynthetic rate was studied extensively and related mainly to the effect of gaseous SO_2, other gases (e.g., Fields, 1988; Nash and Gries, 1991, 1995; Hyvärinen et al., 1993; Gries, 1996), and different metals (Puckett, 1976; Beckett and Brown, 1983) under controlled conditions. Comparative analyses of elemental content and rate of photosynthesis of lichens under field conditions were performed to a lesser extent.

An additional physiological parameter applied to assess the vitality of algal cells in the lichen was the spectral reflectance of the thallus. This parameter was used mainly for higher plants and to a lesser extent for cryptogams.

In the last three decades, since the launch of the first remote sensing satellite, considerable effort was made to study the state and dynamics of vegetation by means of vegetation indices (VIs) (Bannari et al., 1995; Gamon and Qiu, 1999). Different VIs were developed based on combinations of two or more spectral bands assuming that multiband analysis would provide more information. The multispectral and multitemporal nature of satellite imagery facilitates the investigation of the vegetation component, based on its typical spectral reflectance, which is apparent for the greater part in the red (600–700 nm) and near infrared (NIR) (700–1100 nm) bands of the electromagnetic spectrum (Tucker, 1979; Sellers, 1985).

Vegetation indices were confirmed to correlate with other parameters of vegetation such as green biomass (Tucker, 1979), chlorophyll concentration (Buschmann and Nagel, 1993; Peñuelas and Filella, 1998), leaf area index (Asrar et al., 1984), foliar loss and damage (Vogelmann, 1990), photosynthetic activity (Sellers, 1985), and carbon fluxes (Tucker et al., 1986). Indices were found to be applicable to different image analyses such as crop classification (Ehrlich and Lambin, 1996) and green coverage (Elvidge and Chen, 1995). Indices are also suitable for analyses of change vectors in multitemporal space applied to multitemporal local area imagery obtained by the Advanced Very High Resolution Radiometer (AVHRR) (Lambin and Strahler, 1994).

The most widely used vegetation index for ecological applications is the normalized difference vegetation index (NDVI), formulated as (Rouse et al., 1974):

$$\mathrm{NDVI} = (\rho\mathrm{NIR} - \rho\mathrm{R})/(\rho\mathrm{NIR} + \rho\mathrm{R}) \qquad [1]$$

This index, as well as its less popular modifications, is based on the difference between the maximum absorption of radiation in the red due to chlorophyll pigments and the maximum reflection of radiation in the near infrared (NIR) due to leaf cellular structure, and the fact that soil spectra, lacking these mechanisms, do not show a dramatic spectral difference.

The main objective of the present study was a comparison of the sensitivity of four (ii–v) of the above-mentioned methodologies in order to detect the most suitable method to assess the vitality of a lichen biomonitor in an area with different levels of pollution.

METHODS

Study Area, Lichen Sampling, and Transplanting

The study was conducted in the Haifa Bay and the Mount Carmel region, northwest Israel. Mount Carmel rises at the edge of the Haifa Bay (Fig. 1). This mountainous area is covered for a large part by Mediterranean forests, nature reserves, and recreation areas. The Haifa Bay industrial area that centers the greater part of the heavy industry of the country was chosen as its potential effect threatened the northwestern edges of the park. The industrial activities in the Haifa Bay include oil refineries, a power station, a steel smelter, manufacturers of food and 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 SO_2, NO_x, O_3, CO_2, metal-containing particles, dust emitted by the cement industry, volatile organic compounds, fertilizer dust, and fumes, deriving from traffic and activity in garages. There are no regular measurements of pollutants by automatic monitors in the greater part of the study area.

The lichen *Ramalina lacera* growing on twigs of carob trees (*Ceratonia siliqua* L.) was collected with its substrate in the HaZorea Forest, Ramoth Menashe, northeast Israel, in April 1998. This area is affirmed to be "clean" with respect to air pollution (Garty et al., 1997a). About 500 lichen-carrying twigs of carob trees, 30 to 60 cm long, were pruned at 3 to 4 m above ground and transferred immediately to 19 sites in the Haifa Bay–Mount Carmel region. Climatic conditions throughout the study area are very similar (Table 1). A description of these sites is given in Fig. 1. The lichen-covered twigs were suspended by polyvinyl chloride (PVC) cords 2 to 3 m above ground level on local trees. Concurrently, some of the twigs were retransferred to the original carob trees in HaZorea (Site 9) as controls. At the end of the exposure period, in November 1998, the lichen material was retrieved and transported to the laboratory.

Determination of Elemental Content of Lichen Thalli

The thalli were detached from the twigs and rinsed immediately for 15 s 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 in order to minimize the loss of water-soluble elements (i.e., K, Mg [Buck and Brown, 1979], and Na) known to occur upon the rinsing of desiccated thalli. It was assumed that a rapid, repetitive procedure would not remove particles enclosed by near-surface hyphae, such as Cu.

Eleven elements were investigated; some of them (Ba, Cu, K, Ni, V and Zn) were found to correlate with one or more of the applied physiological parameters. Heavy metals such as Cr, Fe, Hg, Mn, and Pb were investigated, being rather toxic and/or linked with industrial activity. The samples were further divided into subsamples, either for measurement of physiological parameters or for determination of elemental content. For the determination of elemental content, subsamples of 1 to 2 g of rinsed and air-dried thalli were pulverized in a mortar under liquid nitrogen. The powder produced in this manner was dried for 24 h at 105°C. Subsamples of 250 mg were digested in 10 mL of concentrated analytical HNO_3 (Merck, Darmstadt, Germany), in test tubes of 50 mL, in a heating block for 8 h at a temperature of 120°C. The elemental content was determined by inductively coupled plasma–

atomic emission spectrometry (ICP–AES) by Spectroflame ICP (Spectro, Kleve, Germany). 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.

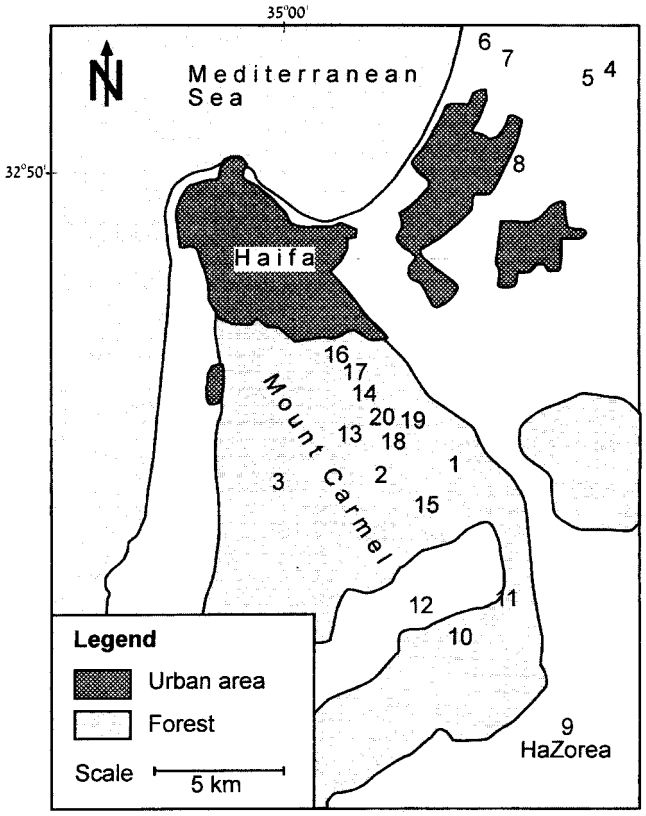

Fig. 1. The study area. Description of the biomonitoring sites:

Site no.	Site name	Remarks
1	Mizpor David Eisen	on the Carmel Mountain (rural)
2	Har Alon recreation area	on the Carmel Mountain (rural)
3	Kibbutz Beit Oren	on the Carmel Mountain (rural)
4	Kibbutz Afek, Haifa Bay	(rural)
5	Ein Afek, Haifa Bay	nature reserve (rural)
6	Electro Chemical industries, Haifa Bay	(industrial, including a chlor-alkali plant)
7	steel smelter, Haifa Bay	close to main motorway (industrial)
8	Kfar Bialik Village, Haifa Bay	(rural)
9	near Hazorea Forest, Ramoth Menashe (source of lichen material)	carob tree plantation 1 km south of the kibbutz (rural)
10	Mevo Elyakim	on the edge of the Carmel Mountain (rural), about 300 m east of recreation area
11	Derech Nof HaCarmel	on the Carmel Mountain (rural), a forest trail
12	Oren HaSela recreation area	on the Carmel Mountain (rural)
13	Mizpor Yad LaBanim	on the Carmel Mountain (rural), in the forest
14	antenna of telecommunication facility	on the Carmel Mountain (rural)
15	Nahal Chik	on the Carmel Mountain (rural), a forest trail
16	close to the University of Haifa	on the Carmel Mountain (rural)
17	Mizpor HaMifratz	on the Carmel Mountain (rural), observation point
18	HaRakafoth recreation area	on the Carmel Mountain (rural)
19	Maayan HaNesharim	on the Carmel Mountain (rural), recreation area
20	Givat HaHaganna recreation area	on the Carmel Mountain (rural)

Measurement of Chlorophyll Content

Five air-dried samples from each of the sites were ground to produce subsamples of 0.1 g. The total chlorophyll ($a + b$) content was determined according to Moran (1982) using 15 mL of dimethyl sulfoxide (DMSO; Merck, analytical grade) as an extraction solvent (Ronen and Galun, 1984).

Analysis of the Integrity of the Photobiont Chlorophyll

Samples of 20 mg were used to measure the integrity of the photobiont chlorophyll. The chlorophyll was extracted overnight in the dark in 3 mL of DMSO, and the ratio of chlorophyll *a* to phaeophytin *a* (OD435 nm/OD415 nm) was determined according to Ronen and Galun (1984) by means of a Novaspec II spectrophotometer (Pharmacia LKB, Biochrom Ltd., Cambridge, UK).

Measurement of Chlorophyll Fluorescence

Based on a time series of measurements, thalli were immersed in distilled water for five min to revive full photosynthetic activity. Before measurement the thalli were dark-adapted for 10 min by means of "dark leaf" clips. The potential quantum yield of PSII was measured using a pulse amplitude modulated fluorometer (Diving PAM, Walz, Effeltrich, Germany). The results were expressed as an F_v to F_m ratio. F_v/F_m is the potential (or optimal) quantum yield of electron transfer through Photosystem II. It is calculated as the maximal fluorescence (F_m) less the minimal fluorescence (F_o), divided by F_m, of a dark-adapted plant $(F_m - F_o)/F_m = F_v/F_m$. These values, which have no units, are frequently used for studying effects of "stress" on the photosynthetic apparatus (Schreiber and Bilger, 1993).

Measurement of the Net Photosynthetic Rate

Air-dried thalli, 6 g for each site, were wetted in 100 mL of double distilled water for 30 min and put in a humid chamber for 8 h to revive full photosynthetic activity. Net CO_2 uptake in the light or release in the dark were measured using a modified infrared gas analyzer (IRGA; Li-6400, Li-Cor, Lincoln, NE). The standard leaf chamber was replaced by a three-way manifold that connected the measuring head to a chamber constructed of a transparent plexiglass tube of 5 cm diameter and 20 cm length sealed at two ends with rubber stoppers. Light was supplied by a high-pressure Na vapor lamp situated 1 m above the chamber at a photosynthetic active radiation (PAR) intensity of 300 μmol photons m^{-2} s^{-1}. A sample of ca. 5 g fresh wt. thallus was inserted and the chamber was sealed. As a steady rate of CO_2 exchange was established, measurements were taken at intervals of 10 s for a duration of 3 min in the light and 2 min in the dark.

Measurement of Spectral Reflectance Response and Calculation of Normalized Difference Vegetation Index

The spectral chracteristics of the lichen were measured digitally in the laboratory by a Li-Cor LI 1800 field spectrometer. All laboratory measurements were performed under constant irradiance with light approaching from a fixed angle. Thalli from different biomonitoring sites appear to have a similar water content (13.72 ± 0.38%) if rinsed and air-dried in the

Table 1. Climatological data comparing several parameters in the study area in the period of the experiment (April–November 1998) (Beth-Dagan, Meteorological Service of Israel, personal communication, 1999).

	Mean daily temperature					
	Mean minimum	Mean maximum	Mean temperature	Mean relative humidity	Precipitation	Number of rainy days
	°C			%	mm	
				Haifa Bay		
Apr.	15.0	24.7	19.9	61	63	4
May	17.7	27.4	22.6	65	10	4
June	20.8	28.9	24.9	67	0	0
July	23.4	31.7	27.6	65	0	0
Aug.	25.4	32.7	29.1	68	0	0
Sept.	23.5	30.4	27.0	64	7	3
Oct.	19.3	28.5	23.9	60	1	1
Nov.	16.3	26.4	21.4	65	13	4
				Mount Carmel		
Apr.	14.8	23.9	19.4	64	67	3
May	16.1	24.7	20.4	73	11	4
June	18.3	25.2	21.8	78	0	0
July	20.3	27.2	23.8	78	0	0
Aug.	22.3	29.0	25.7	81	0	0
Sept.	21.1	28.8	25.0	72	8	3
Oct.	19.1	26.6	22.9	67	4	1
Nov.	16.4	23.0	19.7	72	30	4

laboratory for 2 d. The instrument was fixed to a 2 nm wavelength of spectral resolution, the scanning width was between 400 and 1100, and the field of view (FOV) was 15°.

The 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 sun-simulating halogen lamp was positioned at a zenith angle of 45°, at a distance of 1 m from the sample. The incident radiation was measured by dividing the spectrum radiance of each sample by the downwelling irradiation as measured by a halon reflectance panel. Each individual spectrum was rotated at 90° between each scan to avoid roughness and shadowing effects. Each average spectrum was calculated as the mean of four spectra. The reflectance in the red spectral region was determined as the average of reflectance values between 650 and 700 nm. In the same manner, the reflectance in the near infrared (NIR) spectral region was determined as the average of reflectance values between 800 and 1000 nm. The NDVI values for each spectrum were calculated by Eq. [1].

Statistical Analyses

The results of the chemical and physiological analyses were evaluated by a one-way analysis of variance (ANOVA), thus evaluating the effects of the sampling site on the bioaccumulation and the physiological status of the photobiont. The data are expressed as F ratios and F probability values. As we had no prior knowledge of the investigated parameters, we applied in the next phase a Student–Newman–Keuls (SNK) test (Hochberg and Tamhane, 1987) for most physiological parameters and for Ba, Cr, Fe, K, Mn, Ni, and V. The number of replicates for each site was similar. This test pairs means using the studentized range distribution. In the case of equal sample sizes, it also compares pairs of means within homogenous subsets, using a stepwise procedure. Means are ordered from high to low and extreme differences are tested first. For F_v/F_m (n = 9–10), Cu (n = 4–10), Pb (n = 3–10), and Zn (n = 8–10), we applied the Tukey test (Hochberg and Tamhane, 1987). This test uses the studentized range statistic to perform pairwise comparisons between groups and sets the experimental error rate as the error rate for the collection of all pairwise comparison.

The correlations between parameters were tested by the Pearson's correlation test. Where necessary, log transformations were applied before this test.

RESULTS

Elemental Content of Resuspended and Transplanted Thalli

Tables 2 and 3 present the content of 10 elements in thalli of *R. lacera* retrieved in November 1998 from 19 biomonitoring sites in the Haifa Bay–Mount Carmel region and the control site in HaZorea. The degree of air pollution in each site was estimated by dividing the mean amount of nine of the elements in transplanted thalli in 19 biomonitoring sites by the mean amount of elements in thalli at the control site. Table 4 presents these ratios and indicates the following: transplanted thalli of *R. lacera* accumulated Cu, Fe, Mn, V, and Zn; Site 7 (mean ratio = 5.80), Site 6 (mean ratio = 2.09), and Site 4 (mean ratio = 1.83) were subject to the heaviest elemental contamination in the Haifa Bay; Sites 5, 12, and 16 were moderately polluted, whereas the other sites were relatively clean.

An attempt to detect mercury in the transplanted material revealed that this toxic heavy metal could not be detected in any of the samples, except for those of Site 6, located near the chlor-alkali plant in the Haifa Bay. These samples (n = 10) contained 18.32 ± 7.38 mg kg^{-1} Hg.

Changes in the Photosynthetic Activity of the Lichen Material

The values of chlorophyll content, photosynthetic rate, and potential quantum yield of PSII are presented in Table 5. The lowest chlorophyll content was detected in lichens from Sites 6 and 8 in the Haifa Bay. Photosynthetic rates, expressed as CO_2 fixation by photobiont cells per g dry wt. of the thallus, were extremely low in Sites 6 and 8, whereas the highest values were obtained for Sites 18 and 19. However, the net CO_2 fixation per mg chlorophyll in industrial sites in the Haifa Bay was similar to that of the greater part of the Carmel Mountain sites.

Table 2. The Ba, Cr, Fe, K, and Mn content of thalli of lichen collected with their substrate near the HaZorea Forest in April 1998 and resuspended at the same site or transplanted to 19 sites in the Haifa Bay–Mount Carmel region. All lichen thalli were retrieved in November 1998. The elemental contents are given as mg kg^{-1} dry weight. The number of replicates for these metals was 10 for each site. X = mean values; SD = standard deviations. Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by the SNK test.

Site no.	Ba	Cr	Fe	K	Mn
	$X \pm$ SD	$X \pm$ SD	$X \pm$ SD	$X \pm$ SD	$X \pm$ SD
1	10.7 ± 2.6ef	3.8 ± 1.0c	856 ± 198ef	2230 ± 218bc	22.6 ± 1.5ef
2	10.2 ± 2.0ef	2.3 ± 0.4d	801 ± 76ef	2194 ± 105bc	22.9 ± 1.3ef
3	12.4 ± 3.1def	2.8 ± 0.5cd	993 ± 161cdef	2211 ± 84bc	24.3 ± 3.0def
4	17.3 ± 3.0b	3.6 ± 0.6c	1293 ± 152b	1776 ± 116f	34.8 ± 2.1b
5	13.6 ± 1.8cde	3.2 ± 0.7cd	1226 ± 153bc	2046 ± 123cde	27.7 ± 1.7cd
6	15.4 ± 1.9bcd	3.6 ± 0.6cd	1327 ± 173b	674 ± 167h	32.5 ± 3.0b
7	23.2 ± 2.9a	11.0 ± 1.2a	5010 ± 504a	1329 ± 153g	90.8 ± 6.9a
8	11.7 ± 1.9def	2.5 ± 0.8cd	1012 ± 138cdef	1874 ± 167ef	25.5 ± 2.1de
9	11.7 ± 2.5def	3.0 ± 0.5cd	801 ± 69ef	2239 ± 196bc	22.0 ± 1.4ef
10	9.5 ± 2.8ef	5.0 ± 1.6b	764 ± 80f	2030 ± 137cde	20.6 ± 1.1f
11	8.6 ± 2.2f	2.7 ± 0.5cd	803 ± 99ef	2157 ± 220ef	21.3 ± 1.5f
12	10.3 ± 1.3ef	5.5 ± 1.4b	922 ± 94ef	1808 ± 192ef	23.0 ± 2.1ef
13	13.5 ± 3.2cde	2.9 ± 0.5cd	960 ± 99def	2104 ± 81def	25.9 ± 1.0de
14	12.1 ± 1.9def	3.5 ± 0.6cd	1049 ± 119cde	1903 ± 164cde	25.8 ± 1.7de
15	11.7 ± 1.6def	5.3 ± 2.1b	899 ± 142ef	2089 ± 284ef	24.0 ± 1.9def
16	13.3 ± 1.1cde	3.7 ± 0.5c	1063 ± 98cde	2093 ± 150cde	27.3 ± 1.4cd
17	16.1 ± 5.8bc	3.4 ± 0.7cd	1182 ± 330bcd	2234 ± 152bcd	29.5 ± 6.3c
18	9.8 ± 3.0ef	2.3 ± 0.7d	893 ± 260ef	2317 ± 188ef	23.4 ± 2.9ef
19	12.4 ± 3.5def	2.7 ± 0.5cd	911 ± 122ef	2462 ± 89ef	25.9 ± 2.3de
20	11.1 ± 1.9ef	3.0 ± 0.3cd	916 ± 67ef	2091 ± 194ef	24.1 ± 1.3def
Analysis of variance					
F ratio	15.15	44.89	239.64	55.77	291.78
F probability	0.00	0.00	0.00	0.00	0.00

The potential quantum yield of PSII in terms of fluorescence ratio F_v/F_m (Table 5) in Sites 6 and 8 in the Haifa Bay was significantly lower than in the control site (Site 9) and in most of the biomonitoring sites.

Table 6 shows that the NDVI values for lichens from Sites 19 and 20 on Mount Carmel were higher than for lichens from most of the other sites, and much higher than for lichens from Sites 6 through 8 in the Haifa Bay. Table 6 shows, in addition, that the ratio OD435 nm/OD415 nm was much higher for thalli from most of the sites than for thalli from Sites 6 and 7 in the Haifa Bay.

Physiological Status of the Photobiont of *Ramalina lacera* Relative to the Elemental Content of the Thallus

Table 7 shows that chlorophyll content correlated inversely with Ba content. The photosynthetic rate correlated with chlorophyll content, NDVI, and K, and correlated inversely with amounts of Ba, Cu, Ni, V, and Zn. The NDVI correlated with K and correlated inversely with Ba, Cr, Cu, and Ni. The K content of the lichen correlated inversely with the Ni content. Signifi-

Table 3. The Ni, V, Cu, Pb, and Zn content of thalli of lichen collected with their substrate near the HaZorea Forest in April 1998 and resuspended at the same site or transplanted to 19 sites in the Haifa Bay–Mount Carmel region. All lichen thalli were retrieved in November 1998. The elemental contents are given as mg kg^{-1} dry weight. The number of replicates for Ni and V was 10 for each site. For Cu, Pb, and Zn, n = no. of replicates. X = mean values; SD = standard deviations. Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by the SNK test for Ni and V and by the Tukey test for Cu, Pb, and Zn.

Site no.	Ni	V		Cu		Pb		Zn
	$X \pm$ SD	$X \pm$ SD	n	$X \pm$ SD	n	$X \pm$ SD	n	$X \pm$ SD
1	4.0 ± 0.9cdef	3.9 ± 0.9efgh	9	6.2 ± 1.2d	4	11.0 ± 0.8b	10	20.0 ± 2.5e
2	2.5 ± 0.6h	3.4 ± 0.4gh	10	4.5 ± 0.3d	7	12.3 ± 2.9b	10	18.7 ± 3.3e
3	3.8 ± 0.7defg	4.6 ± 0.9defg	10	4.6 ± 0.5d	–	n.d.	8	42.7 ± 18.5cd
4	5.1 ± 0.7cd	7.5 ± 0.6c	10	5.6 ± 0.4d	3	11.7 ± 1.2b	9	28.8 ± 4.4cde
5	4.5 ± 0.9cdef	7.1 ± 1.1c	10	6.4 ± 1.4d	5	13.8 ± 2.4b	10	32.3 ± 14.9cde
6	8.7 ± 0.6b	16.1 ± 1.2b	10	11.3 ± 1.7bc	5	13.4 ± 2.3b	10	81.9 ± 16.5b
7	18.5 ± 1.9a	21.7 ± 2.7a	10	45.9 ± 5.3a	10	60.9 ± 7.1a	10	336.5 ± 33.3a
8	3.9 ± 0.7cdef	5.2 ± 0.5d	10	12.2 ± 8.3b	–	n.d.	10	18.6 ± 2.3e
9	4.4 ± 0.6cdef	3.4 ± 0.5gh	10	5.7 ± 0.5d	–	n.d.	10	19.9 ± 2.9e
10	4.9 ± 0.9cd	3.1 ± 0.4h	10	4.9 ± 0.4d	–	n.d.	10	19.5 ± 2.8e
11	3.5 ± 0.7efgh	3.7 ± 0.5fgh	10	4.1 ± 0.4d	3	10.9 ± 0.9b	10	26.8 ± 3.4cde
12	5.4 ± 1.1c	4.2 ± 0.5defg	10	5.2 ± 0.3d	–	n.d.	10	45.7 ± 29.8c
13	4.2 ± 0.7cdef	5.1 ± 0.4de	10	4.8 ± 0.3d	–	n.d.	10	19.5 ± 1.7e
14	5.2 ± 0.9cd	7.2 ± 0.5c	10	5.6 ± 0.4d	4	14.8 ± 2.2b	10	23.4 ± 2.8de
15	5.2 ± 1.2cd	4.1 ± 0.7defg	4	6.5 ± 0.9d	5	11.8 ± 2.0b	10	20.0 ± 7.8e
16	4.7 ± 0.8cde	6.5 ± 0.4c	10	7.5 ± 1.0cd	8	14.5 ± 3.5b	10	25.6 ± 5.3de
17	4.1 ± 1.4cdef	5.3 ± 1.7d	10	6.3 ± 1.7d	9	13.2 ± 2.9b	10	19.8 ± 4.9e
18	3.2 ± 1.4fgh	4.9 ± 1.6def	9	5.2 ± 1.0d	–	n.d.	10	16.2 ± 5.4e
19	2.6 ± 1.0gh	3.9 ± 0.5defg	10	4.7 ± 0.4d	5	13.5 ± 2.4b	10	16.8 ± 2.7e
20	4.8 ± 1.1cde	6.5 ± 0.8c	10	5.1 ± 0.4d	–	n.d.	10	29.9 ± 6.6cde
Analysis of variance								
F ratio	15.15	44.89		144.40		130.31		325.08
F probability	0.00	0.00		0.00		0.00		0.00

Table 4. Ratios between mean amounts of nine elements detected in lichen transplanted to the Haifa Bay–Mount Carmel region and the mean amount of the same elements as determined in the lichen thalli resuspended near HaZorea (site 9). Ratios were obtained by using mean values of Tables 2 and 3.

Location site no.	Element										
	Ba	Cr	Cu	Fe	K	Mn	Ni	V	Zn	Σ	*X*
1/9	0.92	1.26	1.09	1.07	1.00	1.03	0.92	1.13	1.00	9.42	1.05
2/9	0.87	0.76	0.79	1.00	0.98	1.04	0.57	0.99	0.94	7.93	0.88
3/9	1.06	0.93	0.81	1.24	0.99	1.11	0.87	1.35	2.15	10.51	1.17
4/9	1.48	1.21	5.00	1.61	0.79	1.59	1.18	2.19	1.45	16.50	1.83
5/9	1.16	1.07	0.98	1.53	0.91	1.26	1.04	2.09	1.62	11.66	1.30
6/9	1.32	1.20	1.99	1.66	0.30	1.48	1.99	4.72	4.11	18.75	2.09
7/9	1.98	3.67	8.09	6.25	0.59	4.14	4.24	6.36	16.87	52.20	5.80
8/9	1.00	0.84	2.15	1.26	0.84	1.16	0.90	1.54	0.93	10.63	1.18
10/9	0.81	1.69	0.86	0.95	0.91	0.94	1.13	0.90	0.98	9.17	1.02
11/9	0.73	0.91	0.72	1.00	0.96	0.97	0.79	1.08	1.34	8.51	0.94
12/9	0.88	1.84	0.92	1.15	0.81	1.05	1.24	1.24	2.29	11.41	1.27
13/9	1.16	0.97	0.85	1.20	0.94	1.18	0.97	1.50	0.98	9.74	1.08
14/9	1.04	1.16	0.99	1.31	0.85	1.17	1.19	2.11	1.18	11.00	1.22
15/9	1.00	1.77	1.15	1.12	0.93	1.10	1.19	1.21	1.00	10.48	1.16
16/9	1.14	1.24	1.32	1.33	0.93	1.24	1.07	1.90	1.28	11.46	1.27
17/9	1.38	1.15	1.10	1.48	1.00	1.34	0.95	1.55	0.99	10.94	1.22
18/9	0.84	0.76	0.92	1.11	1.03	1.06	0.74	1.43	0.81	8.71	0.98
19/9	1.06	0.92	0.83	1.14	1.10	1.18	0.61	1.15	0.84	8.82	0.98
20/9	0.95	1.02	0.89	1.14	0.93	1.10	1.11	1.92	1.50	10.56	1.17
Σ	20.77	24.35	31.44	28.56	16.80	25.15	22.71	36.36	42.26		
X	1.09	1.28	1.65	1.50	0.88	1.32	1.20	1.91	2.22		

cant correlations were obtained for pairs of the greater part of the metals.

For a comparative analysis of the sensitivity of methodologies applied to assess the vitality of *R. lacera*, we listed the number of sites in which each of the physiological parameters exhibited a significant deviation from the "normal" status. For this purpose we counted the values not followed by the letter *a* according to either SNK or Tukey tests. Both the OD435 nm/OD415 nm ratio expressing chlorophyll integrity and the F_v/F_m ratio expressing the potential quantum yield of photosynthesis exhibited a deviation from the "normal" status in two sites only (6 and 7 and 6 and 8, respectively). Chlorophyll ($a + b$) content exhibited a significant deviation in eight sites (3, 6, 8, 10, 13, 15, 16, 17). The net CO_2 fixation rate

Table 6. Values of the normalized difference vegetation index (NDVI) and the OD435 nm/OD415 nm ratio in thalli of lichen collected near the HaZorea Forest in April 1998, resuspended in the same site or transplanted in the Haifa Bay–Mount Carmel region, and retrieved in November 1998. The number of replicates for NDVI was four for each site and for OD435 nm/OD415 nm was 12 for each site. *X* = mean values; SD = standard deviations. Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by the SNK test.

Site no.	NDVI	OD435 nm/OD415 nm
	X ± SD	*X* ± SD
1	0.340 ± 0.011de	1.37 ± 0.04a
2	0.348 ± 0.012cd	1.34 ± 0.11a
3	0.324 ± 0.006ef	1.40 ± 0.02a
4	0.267 ± 0.015ij	1.31 ± 0.11a
5	0.287 ± 0.010hi	1.40 ± 0.02a
6	0.111 ± 0.007l	0.90 ± 0.28b
7	0.131 ± 0.004k	0.57 ± 0.10c
8	0.269 ± 0.009ij	1.36 ± 0.05a
9	0.338 ± 0.005de	1.39 ± 0.05a
10	0.309 ± 0.021fg	1.40 ± 0.04a
11	0.349 ± 0.022cd	1.37 ± 0.05a
12	0.255 ± 0.016j	1.39 ± 0.02a
13	0.323 ± 0.004ef	1.34 ± 0.04a
14	0.283 ± 0.018hi	1.30 ± 0.06a
15	0.282 ± 0.007hi	1.38 ± 0.04a
16	0.300 ± 0.005gh	1.31 ± 0.06a
17	0.312 ± 0.012fg	1.38 ± 0.04a
18	0.387 ± 0.011a	1.40 ± 0.04a
19	0.376 ± 0.008ab	1.36 ± 0.02a
20	0.366 ± 0.009bc	1.34 ± 0.07a
Analysis of variance		
F ratio	145.48	69.78
F probability	0.00	0.00

Table 5. Values of net CO_2 fixation rate per g dry wt. (Pw), per mg chlorophyll (Pchl), chlorophyll content (mg g^{-1} dry wt.), and potential quantum yield expressed as F_v/F_m in thalli of lichen collected near the Nazorea Forest in April 1998, resuspended in the same site or transplanted in the Haifa Bay–Mount Carmel region, and retrieved in November 1998. The number of replicates for Pw, Pchl, and chlorophyll content was five for each site. For F_v/F_m, *n* = the number of replicates. *X* = mean values; SD = standard deviations. Values in each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by the SNK test for Pw, Pchl, and chlorophyll content and by the Tukey test for F_v/F_m.

Site no.	Pw (μmol CO_2 h^{-1} g dry wt.$^{-1}$)	Pchl (μmol CO_2 h^{-1} mg Chl^{-1})	Chlorophyll ($a + b$) content (mg g^{-1} dry wt.)		F_v/F_m
	X ± SD	*X* ± SD	*X* ± SD	*n*	*X* ± SD
1	23.30 ± 2.00cde	10.17 ± 1.56cde	2.333 ± 0.414a	20	0.730 ± 0.025a
2	28.02 ± 2.13bc	12.48 ± 2.16abcde	2.304 ± 0.455a	20	0.731 ± 0.033a
3	19.45 ± 6.94ef	12.00 ± 3.16bcde	1.617 ± 0.360cd	19	0.703 ± 0.039a
4	–	–	–		–
5	29.75 ± 2.08ab	11.89 ± 0.67bcde	2.505 ± 0.166a	19	0.691 ± 0.086a
6	0.95 ± 0.32h	7.85 ± 3.89e	0.132 ± 0.039f	20	0.472 ± 0.157b
7	–	–	–		–
8	7.91 ± 1.38g	9.80 ± 2.43de	0.829 ± 0.160e	20	0.470 ± 0.200b
9	26.92 ± 4.95bcd	11.51 ± 2.13bcde	2.354 ± 0.308a	20	0.693 ± 0.043a
10	22.04 ± 3.48def	15.07 ± 4.17ab	1.542 ± 0.459cd	20	0.698 ± 0.052a
11	28.32 ± 1.98bc	11.78 ± 1.11bcde	2.424 ± 0.314a	20	0.719 ± 0.023a
12	20.07 ± 3.22ef	9.42 ± 2.28de	2.224 ± 0.570ab	20	0.704 ± 0.043a
13	15.60 ± 1.51f	12.05 ± 1.53bcde	1.301 ± 0.098d	20	0.710 ± 0.028a
14	20.94 ± 2.87ef	9.83 ± 1.00de	2.135 ± 0.276abc	20	0.709 ± 0.030a
15	19.02 ± 3.62ef	11.49 ± 1.22bcde	1.652 ± 0.237bcd	20	0.734 ± 0.019a
16	17.94 ± 1.29ef	10.75 ± 1.38bcde	1.694 ± 0.268bcd	20	0.705 ± 0.065a
17	20.42 ± 2.96ef	12.86 ± 1.86abcd	1.590 ± 0.116cd	20	0.661 ± 0.115a
18	34.63 ± 4.23a	16.16 ± 1.67a	2.151 ± 0.259abc	20	0.726 ± 0.022a
19	33.99 ± 5.72a	14.61 ± 1.32abc	2.359 ± 0.534a	20	0.706 ± 0.043a
20	23.41 ± 1.40cde	8.97 ± 1.04de	2.625 ± 0.167a	20	0.729 ± 0.029a
Analysis of variance					
F ratio	31.35	5.09	20.043		21.46
F probability	0.00	0.00	0.00		0.00

Table 7. Pearson's correlation coefficients between pairs of elements and physiological parameters in lichen retrieved in November 1998. NDVI = normalized difference vegetation index; Pw = net CO_2 fixation rate per g dry wt.; Pchl = net CO_2 fixation rate per mg chlorophyll. Underlined parameters = following log transformation.

	Correlation Coefficient		Correlation Coefficient
Chlorophyll content; Ba	−0.51*	Ba ; Mn	0.94**
Pw ; Pchl	0.67**	Cu ; Mn	0.47*
Pw ; NDVI	0.82**	Cu ; Ni	0.83**
Pw ; Ba	−0.47*	Cu ; Zn	0.79**
Pw ; Cu	−0.74**	Cr ; Ni	0.64**
Pw ; K	0.78**	Ba ; Ni	0.69**
Pw ; Ni	−0.73**	Ba ; V	0.80**
Pw ; chlorophyll content	0.98**	Fe ; K	−0.49*
Pchl ; V	−0.56*	Fe ; Mn	0.99**
Pchl ; Zn	−0.62**	Fe ; V	0.86**
NDVI ; Ba	−0.69**	Fe ; Zn	0.87**
NDVI ; Ni	−0.71**	Ni ; K	−0.73**
NDVI ; Cu	−0.78**	Mn ; Ni	0.81**
NDVI ; K	0.96**	Mn ; V	0.85**
NDVI ; Cr	−0.65**	Ni ; V	0.82**
Ba ; Fe	0.88**	Ni ; Zn	0.88**

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

per milligram chlorophyll (Pchl) exhibited a significant deviation in 13 sites (1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 20). The net CO_2 fixation rate per gram dry weight (Pw) exhibited a significant deviation in 15 sites (1, 2, 3, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20), whereas NDVI exhibited a significant deviation in 18 sites (all sites except 18 and 19).

DISCUSSION

The present study, which applied the lichen *Ramalina lacera* as a bioindicator of airborne chemical elements on a regional scale, differentiated among sites with reference to both elemental content and physiological status. The level of some of the heavy metals accumulated in *R. lacera* transplanted in the Haifa Bay is comparable with the levels mentioned in previous works dealing with lichens and pollutants in industrial areas that include power plants and/or oil refineries (Nash and Sommerfeld, 1981; Nimis et al., 1990, 1993; González and Pignata, 1994; Juichang et al., 1995; Haapala et al., 1996; Garty et al., 1997a–d).

The elevated amounts of Cr, Cu, Fe, Mn, Ni, and Zn in lichens at Sites 4, 6, and 7 in the Haifa Bay industrial area and the correlation of Mn and Ni, Mn and V, Ni and V, Fe and Mn, Fe and V, and Fe and Zn, point for the greater part to metal processing in the steel smelter. These metals are well-known pollutants acknowledged to be emitted by nickel smelters (Nieboer et al., 1972), steelworks, and iron foundries (Gailey and Lloyd, 1983; Gailey et al., 1985).

The elevated content of Pb in Site 7 is traceable to vehicular activity on the main road crossing the Haifa Bay from south to north, near the steel smelter. The correlation of Cr and Ni, Cu and Ni, Zn and Cu, Cu and Mn, and Zn and Ni could be related to metal processing in the Haifa Bay, but indicates also vehicular activity as a possible originator (Garty et al., 1977; Ormrod, 1984; Ward, 1989; Boonpragob and Nash, 1990).

High amounts of Ba in lichens from Sites 4, 6, and 7 in the Haifa Bay point to the combustion of heavy fuel oil in the Haifa Power Plant, in the Haifa Bay Oil Refineries, and in additional industrial enterprises. Crude oil used in the oil refineries in the Haifa Bay contains Ba.

The physiological implications of the enlarged elemental content in contaminated sites are indicated at large by diminished amounts of K that correlate inversely with other elements. In some cases, the K content of transplanted thalli at the end of the experiment was found to be lower than the K content of thalli resuspended in HaZorea (Site 9). Potassium appeared to have leaked out in those polluted sites (e.g., Sites 4, 6, 7, 8, 12, and 14) as a result of cell membrane degradation in varying degrees. Various studies showed that lichens exposed to general pollution produce leachates with high concentrations of K and a high electric conductivity (e.g., Alebic-Juretic and Arko-Pijevac, 1989). Negative correlations of K content with that of Ba, Cr, Cu, Fe, Mn, Ni, V, and Zn indicate that these elements are potential stressors if accumulated in excessive quantities in the lichen. The effect of heavy metal accumulation on the leakage of K is documented by Puckett (1976), Burton et al. (1981), Brown and Buck (1985), and Tarhanen et al. (1996, 1999). Yet, to the best of our knowledge, no data are available on the effect of Ba and V salts with reference to injury caused to cell membranes and the leakage of K.

As to the physiological parameters related exclusively to the photobiont cells in *R. lacera*, we should point to the high values for the OD435 nm/OD415 nm ratio obtained for resuspended thalli in HaZorea, indicating that the air quality prevailing in this site did not affect the photobiont chlorophyll. The present findings are in accordance with high OD435 nm/OD415 nm ratios previously obtained for *R. lacera* in HaZorea (Ronen and Galun, 1984; Kardish et al., 1987). The present study shows that the exposure of *R. lacera* in all sites of the Mount Carmel Park did not cause a significant decrease of the OD435 nm/OD415 nm ratio. On the other hand, the low ratios for OD435 nm/OD415 nm in Sites 6 and 7 in the Haifa Bay are in accordance with data relating to the disintegration of the photobiont chlorophyll in lichens exposed to pollutants under field conditions (Boonpragob and Nash, 1991; González and Pignata, 1994; Levin and Pignata, 1995; González et al., 1996). The inverse correlation between OD435 nm/OD415 nm ratio and heavy metal content, observed in the present study, is in accordance with previous studies showing a chlorophyll degradation upon exposure of lichens to heavy metal solutions under laboratory conditions (Puckett, 1976; Garty et al., 1992; Chettri et al., 1998).

The effect of heavy metals on changes in the potential quantum yield of photosynthesis under laboratory conditions was studied only recently. Branquinho and coworkers found that the uptake of Cu (Branquinho et al., 1997a) and Pb (Branquinho et al., 1997b) caused a decrease of the potential quantum yield. Using this criterion, *Usnea* spp. were found to be more sensitive to Cu than *Ramalina fastigiata*. The emission of pollutants other than heavy metals was previously found to de-

crease the potential quantum yield upon controlled fumigation experiments. The effect of SO_2 fumigation on chlorophyll fluorescence (Gries et al., 1995; Nash and Gries, 1995) revealed that the change in rapid induction kinetics of chlorophyll fluorescence after fumigation indicated an inhibition of Photosystem II by SO_2. Of seven lichen species fumigated with ozone, Scheidegger and Schroeter (1995) detected five species undergoing a significant reduction of the potential quantum yield, indicating severe stress on Photosystem II due to ozone.

To the best of our knowledge, only a few field studies applied the chlorophyll *a* fluorescence parameter to estimate the degree of stress by air pollutants in lichens. A study of the potential quantum yield in lichen thalli from a polluted region in Spain revealed that its values were about the same as for control samples (Calatayud et al., 1996). On the other hand, a study performed by Niewiadomska et al. (1998) on the effect of different levels of air pollution on the photosynthetic activity of lichens found significantly lowered fluorescence parameters in four lichen species from the more polluted sites. The authors concluded that measurements of chlorophyll reveal the very early signs of a decreased photosynthetic capacity caused by air pollution.

Different procedures were applied to calculate the rate of net photosynthesis (NP) in lichens. The most common procedure relates the fixation of CO_2 to the dry weight of the thallus (e.g., Lange et al., 1977; Kappen, 1985; Sanz et al., 1992; Tretiach and Carpanelli, 1992; Green et al., 1997). Other procedures relate CO_2 fixation to thallus surface (e.g., Leisner et al., 1997; Lange et al., 1999) or to the amount of chlorophyll (e.g., Wessels and Kappen, 1994; Zotz et al., 1998). Most of the studies using NP values, based on per-area or per-chlorophyll units, deal with lichens in nonpolluted areas. The present results show that the lowest NP values, calculated on a dry weight basis, obtained for lichens from Sites 6 and 8, appear to be linked to severe chlorophyll degradation.

The impairment of other components of the photosynthetic systems is indicated by low NP values, calculated per chlorophyll unit and low quantum yield, in Sites 6 and 8 in the Haifa Bay. These low values coincided with a high elemental content.

The inverse correlation obtained in the present study for NDVI values and Ba, Cr, Cu, and Ni accords to a certain extent with previous studies testing the possible linkage of elemental content with NDVI. These studies indicated an inverse correlation of NDVI and S following 100 d of exposure (Garty et al., 1997a); NDVI and Al, Cr, Fe, Ni, Sulfate S, Ti, and V following 10 mo of exposure (Garty et al., 1997b); NDVI and Ni, S, and V following 11 mo of exposure (Garty et al., 1997c); and NDVI and Mn, Ni, Pb, and Sulfate S following 10 mo of exposure (Garty et al., 1997d).

Our findings indicating a statistical linkage of pollutants and NDVI are comparable with results obtained by Gouaux and Vincent (1990), who analyzed thalli of *Peltigera canina* from polluted versus unpolluted areas by means of infrared color photography digitization and data processing. Changes in the spectral response indicated by the visible and infrared bands and by a decrease of the vegetation index (IR/R) were attributed to the harmful effects of pollutants. A controlled experiment conducted by Cox et al. (1991) revealed that lichens exposed to Cu concentrations >20 $\mu g\ g^{-1}$ exhibited a significant shift of 2 to 3% in the spectral reflectance response.

Based on the number of sites in which each of the physiological parameters exhibited a significant deviation from the "normal" status, it appears that in the specific area investigated in this study, which includes a large unpolluted part and another polluted part, the most sensitive parameters of lichen viability were NDVI and net CO_2 fixation rate per g dry wt. (Pw). The least sensitive were chlorophyll integrity, expressed as the OD435 nm/OD415 nm ratio, and F_v/F_m. This rank accords partly with Fields (1988), who listed the order of sensitivity of lichen physiological response to fumigation. According to this review, the parameter of the pigment status is not sensitive enough to assess the vitality of lichens exposed to pollutants, as fumigation with high concentrations of certain gaseous pollutants was required to induce chlorophyll degradation. Fields (1988) listed the order of sensitivity of lichen response to fumigation as follows: N_2 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: NDVI $>$ net CO_2 fixation (Pw, Pchl) $>$ F_v/F_m, chlorophyll content, and integrity.

As a nondestructive method, NDVI demonstrates the possibility to detect pollutant-induced stress in lichens by heavy metals and other substances. It appears that this parameter enables the detection of early signs of pollutant-induced stress before changes in other physiological parameters become measurable.

However, an assessment of the relative sensitivity of physiological parameters appears to be applicable to different study areas with varying levels of pollution.

ACKNOWLEDGMENTS

This work was supported by the Haifa Association for Environmental Protection and by the Israel Electric Corporation, Ltd. We thank Josepha Sole and Haya Lehr for their technical assistance and Rachel Garty-Spitz for her valued contribution to the manuscript.

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