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Comparison of five physiological parameters to assess the vitality of the lichen *Ramalina lacera* exposed to air pollution

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To determine the environmental impact of industrial activity, we examined and compared the following parameters of physiological integrity in the epiphytic fruticose lichen *Ramalina lacera*. (1) Potential quantum yield of PSII expressed as the chlorophyll fluorescence ratio F_v/F_m. (2) Spectral reflectance expressed as values of normalized difference vegetation index (NDVI). (3) Production of ethylene. (4) Electrolyte leakage brought about by degrading cell membranes in terms of electric conductivity. (5) The ratio A_{435nm}/A_{415nm} indicating the disintegration of chlorophyll. The amounts of Ba, Cu, K, Ni, S, V and Zn contained in the lichen thallus were measured to quantify the degree of pollution. Some of the lichen-carrying twigs collected at a relatively unpolluted control site were resuspended on the original trees as controls. Other lichens were transplanted to 19 biomonitoring sites. Transplanted lichens in polluted sites contained higher amounts of Ba, Ni, S, V and Zn than lichens in the control site and in most of the rural sites upon an exposure period of 8 months. Statistical analysis revealed negative correlations between F_v/F_m ratios and Ba contents of transplants. High Ba, Cu, Ni and Zn contents correlated negatively with NDVI values. NDVI values correlated with A_{435nm}/A_{415nm}, F_v/F_m and K. The ratio A_{435nm}/A_{415nm} correlated positively with K and negatively with Ba, Cu and Zn. The production of ethylene correlated positively with Cu and Ba and negatively with A_{435nm}/A_{415nm}, F_v/F_m and NDVI. Electric conductivity values correlated positively with Ba, Cu, Na, S, V and Zn and negatively with NDVI. Both elemental content and physiological alterations in transplants of *R. lacera* point to a high degree of contamination in the Haifa Bay region, which is polluted by fuel-oil combustion apart from other industrial activities. The present work suggests that in our specific study area, the most sensitive parameter to assess the vitality of the lichen thallus is electric conductivity whereas ethylene production is less sensitive.

Introduction

Due to their different sensitivity to air pollution, lichens are potentially useful for air monitoring purposes (Richardson 1992, Gries 1996). However, progress in this direction can only be achieved if sensitive and reliable methodologies are developed to assess pollution-induced stress-effects in these organisms. The measurement of modulated chlorophyll (Chl) *a* fluorescence in lichens is one of several methodologies used to assess air pollution. This parameter has been successfully applied to stress detection in vascular plants (Clement and van Hasselt 1996, Epron 1997).

The measurement of modulated Chl *a* fluorescence is a non-invasive method to analyze photosynthetic parameters in lichens (Lange et al. 1989, Demmig-Adams et al. 1990, Hovenden and Seppelt 1995, Leisner et al. 1996, 1997, Sundberg et al. 1997, Kappen et al. 1998). The above-mentioned studies deal with a wide range of physiological conditions including those prevailing under low temperatures. Less attention has been paid to the assessment of changes in in vivo Chl fluorescence in lichen thalli exposed to pollutants. An application of the pulse amplitude methodology (PAM) to measure Chl fluorescence as a non-invasive means to monitor the status of photosystem II (PSII) in lichens exposed to gaseous sulfur dioxide (SO_2) was reported by Gries et al. (1995). The authors detected changes in the

Abbreviations – nductively coupled plasma-atomic emission spectrometry; NDVI, normalized difference vegetation index; PAM, pulse amplitude modulated; PSII, photosystem II

rapid induction kinetics of Chl fluorescence upon fumigation, which indicated the inhibition of PSII by SO_2. In a study of the effects of SO_2 fumigation on photosynthetic carbon dioxide (CO_2) gas exchange, Chl *a* fluorescence emmission and antioxidant enzymes in lichens, Deltoro et al. (1999) investigated two lichen species. Net photosynthetic rates were more adversely affected in *Evernia prunastri* than in *Ramalina farinacea*, both exposed to SO_2. The authors found, in addition, that processes dependent on thylakoid membrane integrity such as PSII-mediated electron flow and nonphotochemical quenching were reduced by exposure to SO_2 in *E. prunastri*.

In a study of the effects of ozone on macrolichens (Scheidegger and Schroeter 1995), 7 lichen species were ozone-fumigated over 80 days. Chl fluorescence measurements using a PAM-101 fluorescence-measuring system, revealed a significant reduction of the variable fluorescence/maximal fluorescence (F_v/F_m) ratio upon ozone fumigation in 5 of the investigated species, indicating severe stress on PSII.

An additional study of the impact of a chemical pollutant on Chl fluorescence in dark-adapted lichens revealed an impairment of PSII photochemical reactions, measured as a decrease in F_v/F_m as a result of Pb uptake, particularly in cyanobiont lichens (Branquinho et al. 1997b). On the other hand, a study of Chl *a* fluorescence in lichen thalli, from a polluted region in Spain, revealed a F_v/F_m ratio that was about the same as for control samples (Calatayud et al. 1996).

An additional non-invasive method to assess the impact of air pollutants in lichens is the evaluation of changes in the normalized difference vegetation index (NDVI). Digital measurements of spectral reflectance were first used to assess stress in higher plants. A linear transformation of reflectance data led to the development of vegetation indices (VIs). Most VIs are based on a combination of the ratio of two portions of the electromagnetic spectrum: the red band (R), 600–700 nm, which corresponds to the maximum Chl absorption and the near infrared band (NIR), 700–1100 nm, which corresponds to the maximum reflectance of the incident light by the living vegetation. The basic VI (= simple ratio), first introduced by Jordan (1969), is a ratio of the values of these two bands. The most widely used VI is known as the normalized difference vegetation index: NDVI. The resulting values range from -1.0 to $+1.0$. The most important feature of NDVI is that healthy vegetation surfaces have higher index values than stressed vegetation surfaces, since the former have a stronger 'greenness' signal.

Lichens and microphytic communities were found to produce a signal which is almost similar to that of higher vegetation, especially under complete or almost complete water saturation: their in vivo NDVI can be as high as 0.30 units (Karnieli et al. 1996). A few studies were performed to assess changes in reflectance response in lichens. For instance, Gouaux and Vincent (1990) compared thalli of *Peltigera canina* from polluted versus unpolluted urban 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. Similarly, Cox et al. (1991) found that lichens exposed to Cu concentrations $> 20\ \mu g\ g^{-1}$ exhibited a significant shift of 2–3% in the spectral response.

An alternative method to estimate the air-pollution impact on lichens is by determination of the integrity of the photobiont Chl (Ronen and Galun 1984). In the present work, we investigated the assumption that the ratio Chl *a*/phaeophytin *a* in algal cells, indicative of the status of the photobiont Chl, correlates with the spectral reflectance and with the F_v/F_m ratio in thalli exposed to pollutants under field conditions. The accumulation of certain air pollutants in the lichen thallus is assumed to coincide with low NDVI values, low F_v/F_m ratios and a low A_{435nm}/A_{415nm} ratio. These 3 parameters are exclusively related to the photobiont.

Previous studies have shown that injury caused to cell membranes upon exposure of lichen thalli to chemicals under laboratory conditions is correlated with an increase of electric conductivity of water due to electrolyte leakage (Puckett 1976, Tarhanen et al. 1997). In the present work, we investigated electrolyte leakage as a physiological parameter related to the accumulation of certain air pollutants.

The production of ethylene in lichens was found to depend on temperature (Epstein et al. 1986), light (Ott 1993), and thallus water content (Ott and Schieleit 1994). According to Schieleit (1996), the ethylene-producing activity exists in axenic cultures of the mycobiont as well as in the photobiont and is obviously a metabolic product of the lichen thallus (Schieleit and Ott 1996, 1997). Certain lichens produce elevated amounts of ethylene upon exposure to chemicals, especially $FeCl_2$, under laboratory conditions (Lurie and Garty 1991, Garty et al. 1995a,b, Kauppi et al. 1998). In the present work, an attempt was made to evaluate fluctuations in the production of ethylene, indicative of environmental stress, by transplanted lichens in biomonitoring sites.

The main objective of the present work is to compare the sensitivity of 5 different methodologies in order to detect the most suitable method to assess the vitality of a lichen used as a biomonitor in a study area with different levels of air pollution.

Materials and methods

Lichen material

The fruticose lichen *Ramalina lacera* (With.) J.R. Laund. previously known as *Ramalina duriaei* (De Not.) Bagl. growing on twigs of carob trees (*Ceratonia siliqua* L.) was collected adherent to its substrate in the HaZorea Forest, Ramoth Menashe, NE Israel, in April 1997. This forest is considered to be 'clean' with respect to air pollution (Garty et al. 1988). About 600 lichen-covered twigs were transplanted to 19 different monitoring sites in the Haifa Bay and in the Mount Carmel region (Table 1, Figs. 1 and 2). The greater part of the heavy industry of north-western Israel is located in the Haifa Bay. The Mount Carmel area rises at its

Table 1. Description of biomonitoring sites.

No.	Site name	Description	Remarks
1	HaZorea Forest	Carob tree plantation	About 1 km south of the kibbutz HaZorea
2	Jabotinski Park		About 500 m north of a quarry, 1.5 km north of Binyamina
3	Ramat Hanadiv, Zikhron Yaacov	Memorial garden	
4	Mevo Elyakim		About 300 m east of car parking area
5	Oren Hasela parking site		About 400 m west of recreation area
6	Keren HaCarmel road-junction		
7	Keren HaCarmel		
8	Oranei HaPisgah		About 300 m west of recreation area
9	Damun junction		About 400 m south-west of road-junction
10	Beit Oren		About 200 m east of Beit Oren road-junction
11	Kedumim ancient quarry		About 150 m east of recreation area
12	Parking area near artificial lake		
13	Rakit recreation area		About 250 m north of recreation area
14	Yearoth HaCarmel Hotel		About 300 m north of hotel
15	Nahal Oren mouth		About 150 m south of Nahal Oren road
16	Carmel Park office area		
17	Antenna of communication facilities		
18	Hai-Bar		Close to zoological park
19	Haifa Bay oil refineries	Industrial facilities	About 600 m north-east of refineries
20	Haifa Power Station, Haifa Bay	Industrial facilities	1.5 km east of power station

edge. This mountainous area is covered for a large part by Mediterranean forests, nature reserves and parks. Most of the sites selected for lichen-transplantation belong to the Mount Carmel National Park. A certain part of the mountain range is built up and belongs to the city of Haifa. It is important to note that no data exist on concentrations of atmospheric pollutants in most of the study area.

Concurrently, lichen-carrying twigs were resuspended on the original carob trees as control specimens. We did not transplant additional twigs to another clean area in order to assess the impact of the transplant effect, because we found previously that resuspended thalli in HaZorea did not exhibit stress due to resuspension in comparison with in situ thalli picked at the same time (Garty et al. 1993).

In November 1997, 8 months after the beginning of the experiment, resuspended thalli from HaZorea and transplanted thalli from the Haifa Bay-Mount Carmel region, were retrieved and transferred to the laboratory at the University of Tel Aviv. The thalli were detached from the twigs and immediately rinsed for 15 s with double-distilled water, at 20°C, to eliminate dust, leaf debris, insects, etc. The rinsing procedure was repeated 3 times, 5 s each, in order to minimize the loss of water-soluble elements, i.e. K, Mg (Buck and Brown 1979) and Na, known to occur upon rinsing of desiccated thalli. It was assumed that a rapid, repetitive procedure would not remove particles enclosed by near-surface hyphae, like Pb and Cu. The samples were further divided into subsamples, either for measurement of physiological parameters or for determination of elemental content.

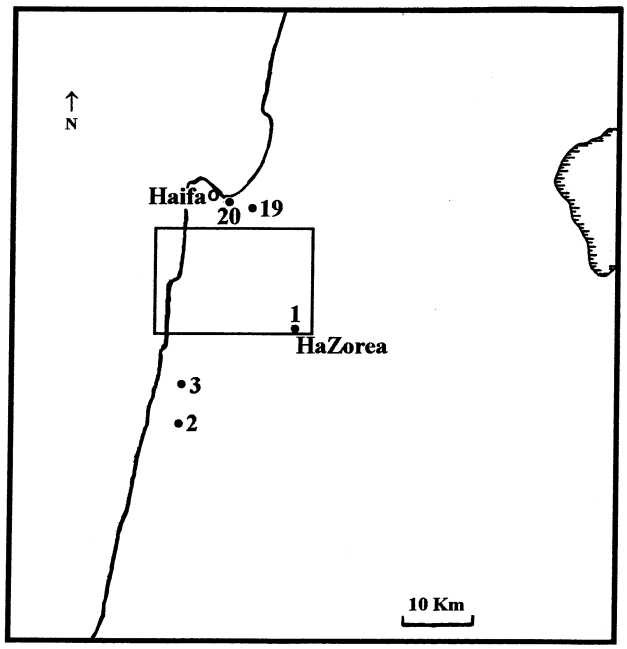

Fig. 1. The northern part of Israel indicating HaZorea, the Haifa Bay (sites 19 and 20), site 2 and site 3.

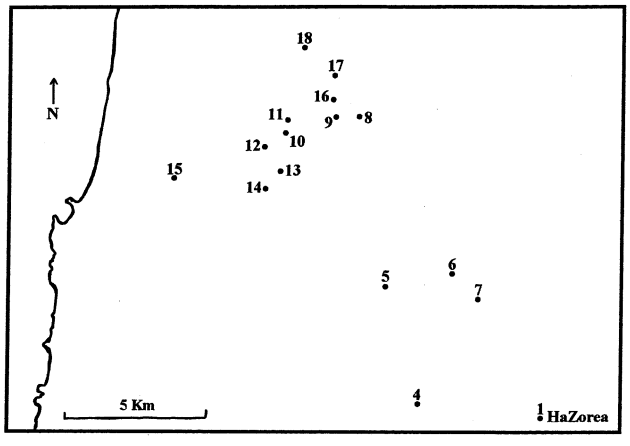

Fig. 2. Biomonitoring sites in the Mount Carmel region. For description of sites see Table 1.

Measurement of Chl fluorescence

Prior to measurements, the thalli were immersed in distilled water for 5 min to allow photosynthetic activity to be fully regained. Subsequently, the thalli were dark-adapted for 10 min by means of 'dark leaf' clips. Finally, the shutter of one clip at a time was opened and a 0.7 s, saturating flash of white light (12000 μmol m^{-2} s^{-1}, which was found to be saturating for F_m) was administered by a pulse-modulated fluorometer (Diving PAM, Walz, Effeltrich, Germany) through an optical fiber. The potential quantum yield of PSII was calculated as F_v/F_m, where F_v is the variable fluorescence defined as maximal fluorescence (F_m) during the saturating flash minus minimal fluorescence (F_o), as sampled in virtual darkness (0.1 μmol m^{-2} s^{-1} from the modulated red measuring light) just before the saturating flash.

Measurement of the spectral reflectance response and calculation of NDVI

The spectral characteristics of the lichen were measured digitally in the laboratory by a Li-Cor® LI 1800 field spectrometer (Li-Cor, Lincoln NE, USA). All laboratory measurements were performed under constant irradiance and with light approaching from a fixed angle. The water content of the samples was assessed and found to be $14 \pm 4\%$ for all rinsed and air-dried samples, kept in the laboratory for 2 days. The instrument was fixed to a 2 nm wavelength of spectral resolution, the scanning width was between 400 and 1100 nm 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 thus calculated as the mean of 4 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 NIR spectral 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 $NDVI = (NIR - R)/(NIR + R)$.

Analysis of photobiont Chl

Samples of 20 mg were used to measure the integrity of the photobiont Chl. The Chl was extracted overnight at room temperature in the dark in 3 ml of dimethyl sulfoxide (DMSO, Merck, analytical grade), and the ratio of Chl *a* to phaeophytin *a* (A_{435nm}/A_{415nm}) was determined according to Ronen and Galun (1984) by means of a Novaspec II spectrophotometer (Pharmacia LKB, Biochrom Ltd., Cambridge, UK).

Assessment of the integrity of cell membranes

Batches of lichen thalli, collected in November 1997, were divided into subsamples of 1 g and immersed in 100 ml of double-distilled water for 60 min. The electric conductivity of the water was measured by an electric conductivity meter (TH-2400, El-Hamma Instruments, Mevo Hama, Israel). The pH of the solution used for the assessment of cell-membrane integrity was measured by a pH meter Delta 340, Mettler, UK. The instrument was calibrated by pH 4.0 and 7.0 standards (Merck).

Measurement of ethylene production

For this analysis, we used subsamples of 1 g of resuspended thalli collected in HaZorea and of transplanted thalli from the Haifa Bay-Mount Carmel region. Each subsample consisted of a few thalli with only one damaged surface each and not of thalli fragments, to avoid and/or minimize the production of stress-ethylene as a result of wounding. The samples were soaked in 20 ml of either double-distilled water, pH 5.6 or 5 m*M* $FeCl_2$, pH 3.5, for 30 min. The submersion of the second group of subsamples in a solution of iron salt at a low pH was performed to create unfavorable conditions and obtain a noticeable production of ethylene, to indicate stress brought about by the absorption and accumulation of air pollutants. After the soaking procedure, the samples were wiped gently with filter paper to remove excess moisture and then placed in sealed 50 ml Erlenmeyer flasks. After 3 h, 4 ml of the gas in each flask was withdrawn with a syringe and 1 ml was injected into a gas chromatograph Varian 3350 equipped with an activated alumina column and a flame ionization detector. The carrier gas was N_2, injected at a flow rate of 30 ml min^{-1}, the injector temperature was 110°C, the column temperature was 110°C and the detector temperature was 150°C.

Determination of elemental content

For a determination of elemental content of Ba, Cu, K, Ni, S, V and Zn, subsamples of 1 g of rinsed and air-dried thalli were pulverized with liquid nitrogen to make a powder. This powder was dried for 24 h at 105°C. Subsamples of 250 mg were digested in 10 ml concentrated pure HNO_3 (Merck) 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).

Statistical analysis

Least significant difference (LSD) tests and a one-way analysis of variance (ANOVA) were applied to determine the significance ($P < 0.05$) of the difference between various biomonitoring sites. A Pearson correlation test was used to obtain correlation coefficients for elemental content and physiological parameters.

Table 2. Potential quantum yield of PSII expressed as fluorescence ratio F_v/F_m, A_{435nm}/A_{415nm} ratio expressing Chl integrity and NDVI in thalli of *Ramalina lacera* collected in HaZorea Forest in April 1997, resuspended in the same site or transplanted in Haifa Bay and Mount Carmel region, and retrieved in November 1997. Means ± SD (n) = number of replicates. Values in each column followed by a common letter do not differ significantly at $P<0.05$ by one-way ANOVA and LSD test.

Site no.	n	F_v/F_m	n	A_{435nm}/A_{415nm}	n	NDVI
1	(18)	0.69 ± 0.04a	(12)	1.40 ± 0.09ab	(4)	0.334 ± 0.002f
2	(18)	0.65 ± 0.07abcde	(12)	1.40 ± 0.03b	(4)	0.329 ± 0.002fg
3	(18)	0.68 ± 0.03ab	(12)	1.41 ± 0.03ab	(4)	0.332 ± 0.005f
4	(19)	0.63 ± 0.09bcde	(12)	1.44 ± 0.02a	(4)	0.354 ± 0.005d
5	(18)	0.66 ± 0.05abcde	(12)	1.38 ± 0.07abc	(4)	0.361 ± 0.004c
6	(18)	0.69 ± 0.02a	(12)	1.43 ± 0.03a	(4)	0.342 ± 0.003e
7	(19)	0.67 ± 0.11abcd	(12)	1.39 ± 0.06abc	(4)	0.377 ± 0.001a
8	(18)	0.68 ± 0.03abc	(12)	1.35 ± 0.06bc	(4)	0.323 ± 0.006g
9	(18)	0.70 ± 0.02a	(12)	1.38 ± 0.07abc	(4)	0.357 ± 0.007cd
10	(18)	0.62 ± 0.06de	(12)	1.37 ± 0.02abc	(4)	0.342 ± 0.005e
11	(18)	0.63 ± 0.05cde	(12)	1.38 ± 0.06abc	(4)	0.353 ± 0.003d
12	(18)	0.52 ± 0.16g	(12)	1.21 ± 0.16d	(4)	0.301 ± 0.001h
13	(18)	0.66 ± 0.04abcde	(12)	1.31 ± 0.07c	(4)	0.383 ± 0.003a
14	(18)	0.68 ± 0.04abc	(12)	1.36 ± 0.06abc	(4)	0.368 ± 0.003b
15	(18)	0.65 ± 0.08abcde	(12)	1.35 ± 0.09bc	(4)	0.324 ± 0.003g
16	(17)	0.70 ± 0.03a	(12)	1.36 ± 0.06abc	(4)	0.361 ± 0.006c
17	(18)	0.68 ± 0.03abc	(12)	1.40 ± 0.02ab	(4)	0.364 ± 0.003bc
18	(18)	0.61 ± 0.04ef	(12)	1.40 ± 0.02ab	(4)	0.334 ± 0.003f
19	(18)	0.56 ± 0.14fg	(12)	1.12 ± 0.24e	(4)	0.225 ± 0.007I
20	(18)	0.55 ± 0.19g	(12)	1.01 ± 0.22f	(4)	0.158 ± 0.007j
ANOVA						
F ratio		7.00		15.09		568.50
F probability		0.00		0.00		0.00

Results

Table 2 shows the potential quantum yield of PSII in terms of the fluorescence ratio F_v/F_m, Chl integrity in terms of the ratio A_{435nm}/A_{415nm} and spectral reflectance response in terms of NDVI values for thalli of *R. lacera* of the Haifa Bay-Mount Carmel region (April-November 1997).

In the control site (site 1) and in most of the biomonitoring sites, F_v/F_m ratios were high. The F_v/F_m ratios were low in sites 12, 19 and 20. Table 2 shows that the ratio A_{435nm}/A_{415nm} for thalli from the control site was higher than for thalli from sites 12 and 13 on Mount Carmel, and much higher than for thalli from sites 19 and 20 in the Haifa Bay. Table 2 shows also that the NDVI values were higher for lichens from the control site (site 1) than for lichen from sites 8, 12 and 15 and much higher than for lichens from sites 19 and 20.

Table 3 shows that sites 2, 6, 19 and 20 reached high values for electric conductivity and for the pH of the solution used for the assessment of membrane integrity.

Table 3. Electric conductivity of water expressing electrolyte leakage (mS m^{-1}) and pH of water used for this analysis in samples of *Ramalina lacera* collected in HaZorea Forest in April 1997, resuspended in the same site or transplanted in Haifa Bay and Mount Carmel region, and retrieved in November 1997. Means ± SD. Values in each column followed by a common letter do not differ significantly at $P<0.05$ by one-way ANOVA and LSD test. (n) = number of replicates.

Site no.	n	Electric conductivity (mS m^{-1})	n	pH of water used for electric conductivity measurement
1	(12)	2.52 ± 0.18ef	(12)	5.69 ± 0.06d
2	(12)	3.93 ± 0.38ab	(12)	5.99 ± 0.04a
3	(12)	2.80 ± 0.55d	(12)	5.57 ± 0.04f
4	(12)	2.43 ± 0.32efg	(12)	5.77 ± 0.08c
5	(12)	2.58 ± 0.11e	(12)	5.70 ± 0.08d
6	(12)	3.86 ± 0.50b	(12)	6.00 ± 0.04a
7	(12)	1.80 ± 0.15m	(12)	5.47 ± 0.05g
8	(12)	1.87 ± 0.17klm	(12)	5.48 ± 0.08g
9	(12)	2.22 ± 0.19hij	(12)	5.40 ± 0.07hi
10	(12)	2.30 ± 0.21ghi	(12)	5.38 ± 0.07i
11	(12)	2.04 ± 0.26jkl	(12)	5.42 ± 0.10ghi
12	(12)	2.37 ± 0.14fgh	(12)	5.57 ± 0.07f
13	(12)	2.04 ± 0.07jkl	(12)	5.46 ± 0.12gh
14	(12)	1.84 ± 0.20lm	(12)	5.37 ± 0.07i
15	(12)	2.08 ± 0.13jk	(12)	5.46 ± 0.06gh
16	(12)	1.90 ± 0.14klm	(12)	5.40 ± 0.09i
17	(12)	2.12 ± 0.11ij	(12)	5.47 ± 0.11g
18	(12)	1.82 ± 0.09m	(12)	5.58 ± 0.05f
19	(12)	3.46 ± 0.30c	(6)	5.97 ± 0.08ab
20	(6)	4.12 ± 0.13a	(6)	5.92 ± 0.09b
ANOVA				
F ratio		90.21		95.90
F probability		0.00		0.00

Table 4. Mean production of ethylene (nl g^{-1} h^{-1}) ± SD produced by thalli of *Ramalina lacera* collected in HaZorea Forest in April 1997, resuspended in the same site or transplanted in the Haifa Bay and Mount Carmel region, retrieved in November 1997 and soaked either in water (H_2O) pH 5.6, or in 5 m*M* $FeCl_2$, pH 3.5, for 30 min. Values in the vertical column followed by a common letter do not differ significantly at $P<0.05$ using one-way ANOVA and LSD test. (n) = number of replicates. NS = not significant. * No material left.

Site no.	n	H_2O treatment	n	$FeCl_2$ treatment
1	(10)	0.16 ± 0.10 NS	(10)	4.02 ± 0.57cde
2	(9)	0.52 ± 0.83 NS	(8)	3.76 ± 0.57def
3	(10)	0.02 ± 0.08 NS	(9)	3.66 ± 0.42efg
4	(10)	0.23 ± 0.13 NS	(10)	3.82 ± 0.35cdef
5	(9)	0.30 ± 0.11 NS	(10)	4.27 ± 0.46c
6	(10)	0.16 ± 0.13 NS	(9)	3.45 ± 0.21fg
7	(9)	0.41 ± 0.51 NS	(10)	2.66 ± 0.55i
8	(10)	0.25 ± 0.15 NS	(10)	3.37 ± 0.55fgh
9	(10)	0.08 ± 0.09 NS	(10)	2.90 ± 0.33hi
10	(10)	0.14 ± 0.10 NS	(10)	3.54 ± 0.50efg
11	(10)	0.12 ± 0.11 NS	(10)	3.35 ± 0.48fgh
12	(10)	0.20 ± 0.13 NS	(9)	5.60 ± 0.54b
13	(10)	0.17 ± 0.09 NS	(9)	4.17 ± 0.68cd
14	(10)	0.29 ± 0.29 NS	(10)	4.17 ± 0.63cd
15	(10)	0.15 ± 0.10 NS	(10)	3.95 ± 0.36cde
16	(10)	0.13 ± 0.19 NS	(10)	3.18 ± 0.46gh
17	(9)	0.29 ± 0.06 NS	(10)	3.60 ± 0.68efg
18	(9)	0.48 ± 1.01 NS	(9)	2.91 ± 0.29hi
19	(9)	0.17 ± 0.12 NS	(9)	6.66 ± 1.17a
20*		– – – –		– – – –
ANOVA				
F ratio		1.48		27.13
F probability		0.10		0.00

Table 4 shows that water-treated thalli from all biomonitoring sites produced small undifferential amounts of ethylene, thus making it impossible to distinguish between sites. $FeCl_2$-treated thalli, on the other hand, produced higher ethylene concentrations. When treated with $FeCl_2$, transplanted thalli from site 19 in the Haifa Bay and from site 12 near the car parking area on Mount Carmel produced more ethylene than thalli from other biomonitoring sites.

The amounts of 7 mineral elements detected in *R. lacera* collected in HaZorea in April 1997, resuspended in the same site or transplanted to the Haifa Bay and the Mount Carmel region and retrieved in November 1997, are presented in Table 5. Lichen transplants from most of the sites which belong to the Mount Carmel National Park exhibit pollutant levels which closely resemble those of the control site at HaZorea (site 1). Two sites (19 and 20) in the Haifa Bay differ distinctly from the other sites. Lichen transplants in

Table 5. The content of Cu, Zn, Ba, K, S, Ni and V given in μg (g DW)$^{-1}$ ± SD in thalli of *Ramalina lacera* collected in the HaZorea Forest in April 1997, resuspended in the same site or transplanted in the Haifa Bay and Mount Carmel region and retrieved in November 1997. (n) = 10. Values in each vertical column followed by a common letter do not differ significantly at $P<0.05$ by one-way ANOVA and LSD test.

Site no.	Cu	Zn	Ba	K	S	Ni	V
1	5.2 ± 0.4cde	32 ± 4def	14.1 ± 2.6cd	2130 ± 184h	2562 ± 126gh	2.8 ± 1.3efg	4.1 ± 0.8hij
2	5.0 ± 0.4defgh	25 ± 4g	13.6 ± 1.8cde	2195 ± 115gh	2559 ± 105gh	1.8 ± 0.7hij	4.5 ± 0.6fgh
3	5.5 ± 0.3cd	30 ± 5ef	12.5 ± 1.4defgh	2294 ± 98fg	2730 ± 95ef	2.1 ± 1.3ghi	4.8 ± 0.5efg
4	5.1 ± 0.5def	32 ± 3def	12.0 ± 1.3efgh	2257 ± 157gh	2562 ± 131gh	2.6 ± 1.2fgh	3.9 ± 0.7ijk
5	5.0 ± 0.2defgh	38 ± 4b	13.0 ± 1.6cdefg	2603 ± 166c	3266 ± 181a	2.1 ± 1.0fgh	5.6 ± 0.7cd
6	5.8 ± 0.6c	40 ± 3b	14.1 ± 0.9cd	2638 ± 153bc	2921 ± 129cd	3.2 ± 1.2ef	7.4 ± 0.5b
7	5.2 ± 0.3cde	37 ± 3bc	12.2 ± 1.5defgh	2882 ± 207a	2649 ± 178fg	1.9 ± 1.0hi	4.2 ± 0.8ghi
8	4.8 ± 0.5efgh	29 ± 3f	11.2 ± 1.1gh	2429 ± 156de	2168 ± 82j	1.3 ± 0.7ij	3.7 ± 0.8ijkl
9	5.4 ± 0.5cde	38 ± 4b	12.6 ± 0.8defgh	2905 ± 136a	2810 ± 105de	5.1 ± 1.1bc	5.4 ± 1.1cde
10	4.4 ± 0.2hi	29 ± 3f	11.9 ± 1.7efgh	2238 ± 176gh	2469 ± 106h	2.5 ± 1.1fgh	3.7 ± 0.7ijkl
11	4.5 ± 0.6ghi	30 ± 4ef	11.3 ± 1.4fgh	2170 ± 143gh	2513 ± 164h	3.6 ± 1.0de	5.1 ± 1.1def
12	4.5 ± 0.2ghi	31 ± 4def	11.8 ± 1.8fgh	2438 ± 137de	2324 ± 56i	2.1 ± 0.9ghi	3.6 ± 0.3jkl
13	3.9 ± 0.4i	34 ± 4de	11.0 ± 1.9h	2635 ± 126bc	2712 ± 133ef	1.9 ± 0.9hi	2.9 ± 0.5m
14	5.1 ± 1.4defg	24 ± 2g	11.2 ± 1.1fgh	2746 ± 113b	2325 ± 1381	1.9 ± 0.6hij	3.4 ± 0.3klm
15	4.8 ± 1.5efgh	23 ± 4g	11.5 ± 1.8fgh	2555 ± 137cd	2194 ± 113j	1.5 ± 0.6ij	3.6 ± 0.3jkl
16	4.5 ± 0.5fghi	29 ± 3f	10.6 ± 1.4h	2451 ± 196de	2242 ± 111ij	1.0 ± 0.0j	3.2 ± 0.6lm
17	5.2 ± 0.3de	34 ± 3cd	13.2 ± 2.6cdef	2651 ± 160bc	2712 ± 80ef	6.0 ± 1.9ab	8.1 ± 0.6a
18	4.4 ± 0.7hi	29 ± 5f	14.9 ± 7.0bc	2420 ± 155ef	2342 ± 185i	4.4 ± 1.0cd	4.6 ± 0.9fgh
19	11.1 ± 1.6a	50 ± 5a	19.2 ± 1.9a	1873 ± 111i	3024 ± 151bc	4.9 ± 0.6c	5.8 ± 0.6c
20	7.0 ± 0.6b	47 ± 6a	16.5 ± 1.6b	1662 ± 134j	3103 ± 173b	6.3 ± 0.6a	6.8 ± 0.8b
ANOVA							
F ratio	43.85	32.87	8.66	44.05	54.16	24.38	42.81
F probability	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 6. Pearson correlation coefficients between pairs of elements and physiological parameters in *Ramalina lacera* collected in the HaZorea Forest in April 1997, resuspended in the same site or transplanted in the Haifa Bay and Mount Carmel region and retrieved in November 1997. Mean values for each site were used for the calculation of the correlation coefficients. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$, NS = not significant.

	F_v/F_m	A_{435nm}/A_{415nm}	NDVI	Conductivity	Ethylene ($FeCl_2$ treated)	pH leachate	Ba	Cu	K	Ni	S	V
F_v/F_m	•											
A_{435nm}/A_{415nm}	0.75***	•										
NDVI	0.70***	0.88***	•									
Conductivity	NS	NS	−0.65***	•								
Ethylene ($FeCl_2$ treated)	−0.73***	−0.86***	−0.72***	NS	•							
pH leachate	NS	NS	−0.57**	0.90***	NS	•						
Ba	−0.46*	−0.55*	−0.73***	0.66***	0.53*	0.73***	•					
Cu	NS	−0.59**	−0.68***	0.55*	0.63***	0.58**	0.85***	•				
K	0.60***	0.59**	0.78***	−0.58**	−0.50*	−0.54*	−0.57**	−0.49*	•			
Ni	NS	NS	−0.50*	NS	NS	NS	0.66***	0.47*	NS	•		
S	NS	NS	NS	0.60***	NS	0.55*	0.60***	0.52*	NS	0.52*	•	
V	NS	NS	NS	0.53*	NS	0.47*	0.57**	NS	NS	0.77***	0.67***	•
Zn	NS	−0.60***	−0.58**	0.53*	0.47*	0.53*	0.74***	0.74***	NS	0.64***	0.81***	0.61***

these sites contained relatively high amounts of S, Zn, Ba, Ni and V. In site 17 on Mount Carmel, which is close (3 km) to the industrial area in the Haifa Bay (6 km to the oil refineries and 7 km to the Haifa Power Station), the thalli contained relatively high amounts of Ni and V. In the industrial zone in the Haifa-Bay, the thalli contained lower amounts of K than resuspended thalli in the control site.

Table 6 shows that the F_v/F_m ratio correlated positively with the A_{435nm}/A_{415nm} ratio, NDVI values and K content, but negatively with concentrations of ethylene of $FeCl_2$-treated thalli and with Ba content. NDVI values correlated positively with ratios of A_{435nm}/A_{415nm} and with K content but negatively with electric-conductivity values, values for ethylene-production of $FeCl_2$-treated thalli and Ba, Cu, Ni and Zn content.

Discussion

The elemental content of the lichen transplants suggests that the study area may be divided into two, according to levels of air pollution: (1) very polluted sites (sites 19 and 20) and (2) relatively clean, or slightly polluted sites, i.e. the control site at HaZorea and most of the sites on Mount Carmel.

The results of the present work reveal definite links between chemical contamination and the lost vitality response of the photobiont in *R. lacera*, mainly in the transplants of the Haifa Bay. It also demonstrates the capability of 5 different methods to detect pollutant-induced stress in lichens by substances such as heavy metals and sulfur-containing compounds in industrial areas.

Loss of vitality in *R. lacera* transplants retrieved from site 12 near the parking area seems to be linked to factors other than the elements mentioned in the present work. Data on pollutants emitted from motor vehicles, mainly buses, in this site, are not available.

The decrease of F_v/F_m ratios, A_{435nm}/A_{415nm} ratios and NDVI values in the lichen *R. lacera* exposed to anthropogenic activity in the study area, is in accordance with studies on the impact of acid rain on lichens (Hallingbäck and Kellner 1992, Kytöviita and Crittenden 1994, Shevtsova and Neuvonen 1997). The occurrence of acid rain in the study area was already acknowledged. Shamay et al. (1990) presented reports of a few sites on Mount Carmel which were exposed to acid rain at pH 4.4–4.7, with high concentrations of SO_4^{2-}. In 65% of 40 rain events in the period 1989–1992, the rain water collected on Mount Carmel was acidic ($pH < 5.6$), and in nearly 40% of the events strongly acidic (pH 4.0–4.5) as reported by Singer et al. (1993).

The injury evident in photobiontic cells of thalli containing relatively high Cu levels in the Haifa Bay is in accordance with previous findings of Cu causing a degradation of Chl in lichens under laboratory conditions (Puckett 1976, Garty et al. 1992). Chl degradation in lichens was also found to coincide with relatively high Cu levels under field conditions (Garty et al. 1988, Chettri et al. 1998).

Our findings showing that low F_v/F_m ratios in sites 19 and 20 in the Haifa Bay coincided with relatively high amounts of Ba, Cu, Ni, S, V and Zn in the lichens are not sufficient

to indicate what element or combination of pollutants is responsible for the damaged PSII. We may refer to Branquinho et al. (1997a) who found that levels of intracellular Cu above ca. 4.0 μmol g^{-1} in *Ramalina fastigiata* were conclusively linked to a decline of the Chl fluorescence. The authors found that the fluorescence parameter F_v/F_m was applicable to investigations of the relative sensitivity of lichens to Cu uptake. *Usnea* spp. were indicated by their F_v/F_m ratio to be more sensitive to Cu than *R. fastigiata*. Branquinho et al. (1999) found also that a total inhibition of PSII photochemical reactions occurred in *R. fastigiata* under field conditions near a copper mine when intracellular concentrations exceeded a threshold of approximately 2.0 μmol g^{-1} Cu. As no samples of this lichen were found under field conditions beyond the Cu threshold, the authors suggest the fluorescence parameter F_v/F_m to be a good estimate of the survival capacity of *R. fastigiata* in the field.

The present work, in addition to its findings on the impact of chemicals on the photobiont in *R. lacera*, provides evidence of injury caused to cell membranes, as the electric conductivity was found to be high in sites 2 and 6 in Mount Carmel and in sites 19 and 20 of the Haifa Bay. The electric conductivity values obtained for site 1 (control) differed substantially from the values obtained for site 20 and coincided with differences of the Ba, Cu, Ni, S, V and Zn content. The very low K-content of thalli in sites 19 and 20 provides additional evidence of injury caused to cell membranes. High K concentrations in the leachates of these lichens support this assumption. Although wet-dry cycles could induce K loss from the lichen, the fact that a very low K content was observed only in the industrial area suggests that K leakage is related mainly to air pollution.

The negative correlation of K with Cu is comparable with previous studies (Puckett 1976, Goyal and Seaward 1982, Branquinho et al. 1997a). To our knowledge, the linkage of elevated Ba and the efflux of K was not studied in lichens.

The inverse correlation of ethylene concentrations and F_v/F_m, A_{435nm}/A_{415nm}, NDVI and K content indicates a multi-system injury. The stress detected in the lichen is probably irreversible. The direct correlation of ethylene production and the Ba, Cu and Zn content of the lichen is comparable with findings of previous studies showing that both Cu and Zn increase the production of ethylene significantly in *R. lacera* at a low pH under laboratory conditions (Lurie and Garty 1991, Garty et al. 1995a) and in *Cladina stellaris* (Kauppi et al. 1998).

The role of heavy metals in the production of ethylene in lichens is not clearly defined. It is possible that a high production of ethylene indicates an unspecific stress response of the thallus, and that an increased ethylene production could be the effect of a loss of vitality of either photobiont or mycobiont. On the other hand, the high ethylene production of *R. lacera* upon exposure to environmental pollution followed by exposure to $FeCl_2$ salt could be a synergistic effect of certain airborne metals and of other elements. The ethylene production of *R. lacera* should be considered in the light of a study (Bouzayen et al. 1991) which showed that Fe is an essential cofactor in the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene in a suspension-cultured tomato (*Lycopersicum esculentum*). The activity of the ethylene-forming enzyme (EFE) was almost completely abolished by the metal-chelating agent 1,10-phenanthroline, whereas the subsequent addition of 0.4 m*M* $FeSO_4$ reversed this inhibition immediately. A partial reversion with $CuSO_4$ and $ZnSO_4$ occurred, according to the above-mentioned study, as a consequence of the release of Fe ions from the 1,10-phenanthroline complex. It is uncertain whether the evolution of ethylene in lichens takes place by the conversion of ACC. Inhibitors of the ACC pathway did not inhibit the production of ethylene in *R. lacera* and the stimulation of ethylene-production upon exposure to Fe(II) was not affected by inhibitors of the ACC pathway (Lurie and Garty 1991). Contradictory findings were presented by Ott and Zwoch (1992) who reported that in the presence of ACC, lichens produced more than 10 times the amount of ethylene than untreated samples. The authors detected ACC in additional lichen-species and suggested that lichens have a biosynthetic pathway similar to that of higher plants. Schieleit and Ott (1994) proposed the existence of an ethylene-producing activity in axenic cultures of both mycobiont and photobiont and proved the presence of ACC in different lichen species and in axenic cultures of mycobionts and photobionts.

For a comparison of the sensitivity of the 5 methodologies applied in the present work, we listed the number of sites in which each of the physiological parameters exhibited a significant deviation from the 'normal' status. The F_v/F_m ratio, the A_{435nm}/A_{415nm} ratio and the NDVI parameter indicated stress (low values) in lichens from the same 3 sites (12, 19 and 20). The electric conductivity parameter indicated stress (high values) in 4 sites: 2, 6, 19 and 20, whereas ethylene ($FeCl_2$-treated thalli) indicated stress (high values) only in two sites (12 and 19).

An examination of the number of accumulated mineral elements (excluding K), which exhibited a significant correlation with each of the physiological parameters, revealed that the F_v/F_m ratio correlated with only one element (Ba), both the A_{435nm}/A_{415nm} ratio and ethylene concentrations correlated with 3 elements (Ba, Cu and Zn), the NDVI parameter correlated with 4 elements (Ba, Cu, Ni and Zn) whereas the electric conductivity correlated with 5 elements (Ba, Cu, S, V and Zn).

As the greater part of the study area belongs to an unpolluted national park, whereas another part is polluted, the assessment of the relative sensitivity of the physiological parameters appears to be applicable to different study areas with varying levels of pollution. In this specific study area, however, the most sensitive parameter of lichen vitality was the electric conductivity, and the least sensitive was ethylene production.

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