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The Effect of Air Pollution on the Integrity of Chlorophyll, Spectral Reflectance Response, and on Concentrations of Nickel, Vanadium, and Sulfur in the Lichen *Ramalina duriaei* (De Not.) Bagl.

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The following study was designed to determine the environmental impact of pollutants emitted by combustion of heavy fuel oil in Ashdod, in Southwestern Israel. For this purpose we measured concentrations of total S, V, and Ni in the local epiphytic fruticose lichen *Ramalina duriaei,* which grows in the peripheral region of the town, and compared these results with those obtained in thalli collected 100 km away, in the HaZorea Forest, northeastern Israel. We also transplanted thalli from the HaZorea Forest to the Ashdod region for a 10-month period. At the end of the experiment we measured the elemental content in all samples. In addition we measured chlorophyll degradation expressed as changes in the 435 nm/415 nm OD ratio, and changes in the spectral response of the thalli. In several sites in the Ashdod region we found high concentrations of S, V, and Ni in transplanted thalli, which correlated with the NDVI values. These findings agree with other measurements of SO_2 and V in the Ashdod area. We suggest that a high V/Ni ratio in lichens is a tracer for air pollution caused by the combustion of heavy fuel oil. © 1997 Academic Press

INTRODUCTION

Ashdod is a small town (110,300 inhabitants) in the southwestern part of Israel, which was entirely planned and constructed in 1956. The town is polluted by two major sources of air emissions, the Eshkol heavy oil-fueled power plant and the oil refineries, in addition to other industrial sources. According to the report of the Ashdod–Yavneh Regional Association for Environmental Protection (Anonymous, 1994) the consumption of heavy fuel oil in the Eshkol Power Plant in 1993 was 1,092,716 tons and the calculated SO_2 emitted by this source was 48,121 tons. According to the same report, the consumption of heavy fuel in the oil refineries in 1993 was 223,047 tons and the calculated SO_2 emitted from the refineries was 6693 tons. The mean annual concentration of SO_x in the air in the town of Ashdod in 1993 was 5–6 ppb in the center and 2–3 ppb in the peripheral areas (Anonymous, 1994). The concentration of V in local particulate aerosols was monitored in Ashdod in 1993 and found to violate the local standard 153 times in that year (Anonymous, 1994). According to this reference, the Eshkol Power Plant is one of the major sources of V pollution.

The application of lichens to the monitoring of atmospheric deposition, particularly of SO_2, is well established (e.g., Hawksworth and Rose, 1970; Showman, 1975; Nimis *et al.*, 1990; Von Arb *et al.*, 1990; Diamantopoulos *et al.*, 1992; Bråkenhielm and Qinghong, 1995; Gauslaa, 1995). SO_2 and acid rain may determine the presence/absence and the overall condition of different lichen species. Experiments involving the exposure of lichens to SO_2 fumigation (e.g., Rao and LeBlanc, 1965; Showman, 1972; Henriksson and Pearson, 1981; Balaguer and Manrique, 1991) or to simulated acid rain (e.g., Scott and Hutchinson, 1987; Holopainen and Kauppi, 1989; Hallingbäck and Kellner, 1992; Sanz *et al.*, 1992) have contributed a great deal to our knowledge of the effects of S-containing compounds, both in gas form or in solution, on the physiology of the lichen thallus and on the integrity of the photobiont chlorophyll.

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Lichens have proven to function as biomonitors of airborne heavy metals and other elements as well. Studies on the uptake, accumulation, retention, localization, release, tolerance, and toxicity of metals in lichens have been thoroughly reviewed (e.g., James, 1973; Nieboer *et al.*, 1978; Brown and Beckett, 1984; Puckett, 1988; Richardson, 1988, 1992; Nash, 1989; Brown, 1991; Garty, 1992, 1993).

The present study aimed to investigate the impact of industrial activity, with an emphasis on heavy fuel oil combustion, on air quality in selected sites in the Ashdod area as reflected by the physiology of the only fruticose (shrub-like) epiphytic indigenous lichen which still survives in the marginal regions of the town. Another purpose of our study was the detection of pollutants emitted by local anthropogenic activity and the determination of their impact on thalli of the same lichen species collected 100 km away from this area and transplanted to biomonitoring sites in the Ashdod region.

In the present study we wanted to examine potential alterations of two physiological parameters in lichen thalli exposed to air pollutants under field conditions. One of these parameters was the integrity of the photobiont chlorophyll. This parameter has been previously studied in lichens. In addition, we applied spectroscopic measurements to study the reflectance response of lichen thalli exposed to air pollution in the study area. Satterwhite and coworkers (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) of the rocks. Quantitative field surveys in the semiarid region of eastern Australia showed that microphytic crusts (lichens, mosses, and blue–green algae) contributed up to 27% of the ground cover (O'Neill, 1994). A record was made of the spectral reflectance of crusts on two types of soil and under different moisture conditions at wavelengths between 0.4 and 2.5 μm. The author showed that the presence of microphytic crusts on both types of soil decreased reflectance throughout the spectrum. Karnieli and coworkers (1996) showed that the levels of spectral reflectance of wet limestone covered with endolithic lichens are lower than those of dry limestone. The spectral response of wet lichen-covered limestone is similar to that of higher plants. Digital measurements of spectral reflectance are usually taken for the assessment of stress in vegetation. A linear transformation of reflectance data has been developed as vegetation indices (VIs), commonly used in the past decades for such assessments. 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 region of maximum chlorophyll absorption, and the near infrared band (NIR), 700–1100 nm, which corresponds to the maximum reflectance of incident light by living vegetation. The basic VI, called 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):

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

The normal values range from −1.0 to +1.0. The most important feature of the index is that the surfaces of healthier vegetation have higher index values than surfaces of stressed vegetation since the former have a strong "greenness" signal. Therefore, NDVI has been empirically correlated with photosynthetic activity, fractional vegetation cover, green leaf biomass or leaf area, productivity, and carbon in standing biomass. As such, it is appropriate for various applications such as image classification and detection of the seasonal yield and cover.

MATERIALS AND METHODS

Site Description

The principal features of the sites selected for this study are shown in Table 1. This table also presents the distance of the sites from the two major sources of emission of SO_2 in the city of Ashdod: the Eshkol Power Plant and the oil refineries.

Experimental Design and Sampling

The present study utilized the fruticose lichen *Ramalina duriaei* (De Not.) Bagl. which grows on trunks and branches of carob trees (*Ceratonia siliqua* L.) in the HaZorea Forest, Esdraelon Valley, northeastern Israel, and on trunks and branches of *Acacia albida* Delile trees in the nature reserve of the town of Ashdod.

Collection and Preparation of Lichen Material

In March 1993 about 350 carob twigs covered with *R. duriaei* thalli were detached from trees in the HaZorea Forest and transferred to 11 sites in the region of Ashdod (Table 1). The lichen-covered twigs were suspended on local trees with PVC cords. Simultaneously, twigs carrying thalli of *R. duriaei* were taken back to the original carob trees to be resuspended as control specimens. On the day of transplantation/resuspension, fresh *in situ* thalli of the lichen were collected both in the HaZorea Forest

TABLE 1
Description of the Biomonitoring Sites

Site no.	Site name	Remarks	Distance from the Eshkol Power Plant, Ashdod (km)	Distance from the oil refineries, Ashdod (km)
1	Ashdod, Nature Reserve (center)	Near an industrial area, northern Ashdod (urban–industrial)	3.8	2.0
2	Ashdod, Nature Reserve (east)	About 200 m west of the Tel Aviv–Ashdod highway, northern Ashdod (suburban)	4.2	2.2
3	Near the motorway entering Ashdod	About 50 m north of the main motorway entering the town (urban–industrial)	2.5	2.2
4	Ashdod, 1.4 km east of the Eshkol Power Plant	Near a dusty road among sand dunes (urban–industrial)	1.4	1.2
5	Sede Uziyahu	Close to the local cemetery (rural)	9.7	10.0
6	Kevutzat Yavneh	Close to the local cemetery (rural)	7.1	5.2
7	Sand dunes, south of Ashdod	Out of the town (suburban)	7.0	6.9
8	Nitzanim Sand Dunes	(rural)	12.2	13.5
9	Hafetz Hayyim	2 km west of the kibbutz (rural)	14.3	12.6
10	Nir Galim	West part of the village, near the interior road (rural)	3.2	2.5
11	HaZorea Forest	Carob tree plantation, 1 km south of the kibbutz (rural)	100 km from the city of Ashdod	
12	Ashdod, near the oil refineries	In an urban–industrial area	2.2	—

and in the *A. albida* nature reserve near Ashdod to be compared with the transplanted and the resuspended specimens.

In January 1994, batches of *in situ* thalli collected in the HaZorea Forest and in the *A. albida* nature reserve near Ashdod, resuspended thalli from the HaZorea Forest and transplanted thalli retrieved from the Ashdod region, were transferred to the laboratory at the Tel Aviv University. The thalli were detached from the substrate, rinsed for a few seconds in doubly distilled water, and kept for 48 hr in the laboratory in a dry state and in ambient light before the investigation was initiated. The thalli were divided into samples of 25–30 g each. The samples were divided into subsamples either for measurement of physiological parameters or for the determination of elemental concentration.

Analysis of the Integrity of the Photobiont Chlorophyll in the Lichen Thallus

The method developed by Ronen and Galun (1984) was used to measure integrity of the photobiont chlorophyll. We used subsamples of 20 mg each. The chlorophyll was extracted overnight in the dark in 5 ml dimethyl sulfoxide (DMSO, Merck, analytical grade). The ratio of chlorophyll *a* to phaeophytin *a* (OD 435 nm/OD 415 nm) was determined using a Novaspec II spectrophotometer (Pharmacia LKB).

Measurement of the Spectral Reflectance Response of the Lichen Thallus

Digital measurements of the spectral reflectance were taken to assess the biological stress of *R. duriaei* thalli. The spectral characteristics of the thalli were measured by a Li-Cor LI 1800 field spectrometer. The instrument was fixed to a 2-nm wavelength spectral resolution, increments between 400 and 1100 nm, and a 15° field of view (FOV). The lichen samples were put in petri dishes on a black-coated board to minimize external reflectance or back scatter. The spectrometer was placed about 1 m above the sample, at nadir. The illumination source, a 1000 W quartz halogen lamp, was positioned at a zenith angle of 45°, approximately 1 m away from the sample. The reflectance was determined by dividing the spectrum of radiance of each sample by the down welling irradiation as measured by a halon panel spectrum. The time intervals between measurements were made as short as possible in order to avoid the effect of light oscillation. Each spectrum was then calculated as an average of four spectra, as the petri dishes were rotated at 90° between each scan to avoid roughness and shadowing effects. Laboratory measurements by a field spectrometer are more likely to have a random noise particularly below 500 nm and above 1000 nm. The presence of the random noise in these portions of the spectra was minimized using two median filters in sequence consisting of moving window of five and three points. We calculated the NDVI values of each spectra using Eq. (1) (see Introduction).

Concentrations of Total Sulfur, Nickel, and Vanadium in the Lichen

The concentrations of total S, Ni, and V in the lichen thallus were determined in the following manner: thalli of the lichen were washed gently and then dried first for 48 hr at room temperature and oven dried for 24 hr at 90°C. Twelve oven-dried samples of 0.35 g each were inserted into a glass tube. Two milliliters of concentrated HNO_3 (65%) was added to each tube. The tubes were kept for 3 days at room temperature. Then the tubes were kept incubated at 60°C for 5 hr and cooled. The volumes were filled up to 50 ml with doubly distilled water. The determination of the elements in the acidic solution was performed by the use of inductively coupled plasma atomic emission spectrometry (ICP-AES) using the Spectroflame ICP (Spectro, Kleve, Germany).

The data obtained in regard to the physiological parameters and the results of the chemical analysis of the elemental content of the entire range of transplanted and resuspended thalli, in addition to the data of the *in situ* thalli, were evaluated by the Duncan's multiple range test using first a one-way ANOVA. The difference between treatments were considered to be significant at $P < 0.05$. A Pearson correlation test was used to obtain the correlation coefficients of elemental concentrations in the lichen thalli and the physiological parameters examined in this study.

RESULTS

The Integrity of the Photobiont Chlorophyll in the Lichen Thallus

The OD ratios at 435 nm/415 nm for *in situ* thalli sampled in January 1994 are presented in Table 2. High ratios obtained for *in situ* thalli indicate a high level of integrity of the chlorophyll. This finding agrees with previous studies showing values of 1.45 ± 0.03 (Ronen and Galun, 1984) and 1.42 ± 0.03 (Garty *et al.*, 1993). Table 3 shows that the ratio was significantly lower for thalli retrieved from three sites in the Ashdod region (sites 3, 4, and 6) after exposure for 10 months. Similar low values have been obtained for *R. duriaei* thalli, kept in acidified solutions, 0.56 ± 0.03 (Ronen and Galun, 1984) and 1.03 (Garty *et al.*, 1992); or transplanted to a polluted site in Tel Aviv, 1.03 ± 0.22 (Garty *et al.*, 1985); and in the Haifa Bay, Israel, 1.11 ± 0.14 (Garty *et al.*, 1993).

Changes in the Spectral Reflectance Response of the Lichen Thalli

Figure 1 shows typical spectra of *in situ* thalli collected in the HaZorea Forest and in the nature reserve, Ashdod, in January 1994. These spectra are very similar to those of healthy higher plants: a significant dip between 600 to 700 nm which corresponds to the region of chlorophyll absorption; a sharp increase of the spectral reflectance around 700 nm (denoted as the "red edge"); and a relative high and continuous near infrared (NIF) reflectance between 700 and 1100 nm. The NIR plateau is dominated by the refractive index differences between the various internal leaf components e.g., cell walls,

TABLE 2

Chlorophyll Integrity Expressed as OD 435 nm/OD 415 nm ratio and NDVI Reflecting Changes in the Spectral Reflectance in the Thallus, in *in Situ* Thalli of *Ramalina duriaei* Collected in January 1994 in the Nature Reserve, Ashdod, and in the HaZorea Forest

	Chlorophyll degradation (OD 435 nm/OD 415 nm)			NDVI		
	n	*X* ± SD	Duncan's test	*n*	*X* ± SD	Duncan's test
Ashdod, Nature Reserve (center)	12	1.47 ± 0.02	a*	4	0.26 ± 0.006	b
Ashdod, Nature Reserve (east)	14	1.43 ± 0.03	b	4	0.26 ± 0.00	b
HaZorea Forest	13	1.47 ± 0.02	a	4	0.28 ± 0.001	a
ANOVA						
F ratio		12.8154			7.8000	
F probability		0.0001			0.0108	

Note. N, number of replicates; *X*, mean values; SD, standard deviations. *Values of each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by Duncan's multiple range test.

TABLE 3

Chlorophyll Integrity Expressed as OD 435 nm/OD 415 nm Ratio, and NDVI Reflecting Changes in the Spectral Reflectance in the Thallus, in Thalli of *Ramalina duriaei* Collected with Their Substrate in the HaZorea Forest in March 1993 and Resuspended at the Same Site or Transplanted to the Ashdod Area and Retrieved in January 1994

Site no.	Site name	Chlorophyll degradation (OD 435 nm/OD 415 nm) *n*	*X* ± SD	Duncan's test	NDVI *n*	*X* ± SD	Duncan's test
1	Ashdod, Nature Reserve (center)	15	1.43 ± 0.02	ab*	4	0.25 ± 0.00	a
2	Ashdod, Nature Reserve (east)	15	1.33 ± 0.16	b	4	0.22 ± 0.00	c
3	Near the motorway entering Ashdod	15	0.94 ± 0.26	d	4	0.09 ± 0.00	g
4	Ashdod, 1.4 km east of the Eshkol Power Plant	15	1.09 ± 0.21	c	4	0.11 ± 0.005	f
5	Sede Uziyahu	13	1.42 ± 0.04	ab	4	0.23 ± 0.00	b
6	Kevutzat Yavneh	15	0.86 ± 0.22	d	4	0.09 ± 0.00	g
7	Sand dunes, south of Ashdod	14	1.44 ± 0.02	ab	4	0.22 ± 0.00	c
8	Nitzanim Sand Dunes	12	1.42 ± 0.03	ab	4	0.21 ± 0.006	d
9	Hafetz Hayyim	14	1.42 ± 0.05	ab	4	0.20 ± 0.00	e
10	Nir Galim	14	1.44 ± 0.08	ab	4	0.21 ± 0.005	cd
11	HaZorea Forest	12	1.42 ± 0.02	ab	4	0.25 ± 0.00	a
12	Ashdod, near the oil refineries	14	1.45 ± 0.01	a	4	0.24 ± 0.005	a
ANOVA							
F ratio			39.3039			1654.2168	
F probability			0.0000			0.0000	

Note. N, number of replicates; *X,* mean values; SD, standard deviations. *Values of each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by Duncan's multiple range test.

airspaces, chloroplasts, and water contents. Table 2 shows the respective NDVI values which range from 0.28 units (for the HaZorea Forest) to 0.26 units (for the two other groups of the *in situ* thalli).

Figure 2 presents the spectra of thalli of *R. duriaei* collected in the HaZorea Forest in March 1993 and resuspended in the same site or transplanted to the Ashdod region, and retrieved in January 1994. Here the dip in the red region is much less pronounced, the red edge is more gentle and the NIR plateau much lower. Thalli from sites 3, 4 and 6 represent an extreme situation where the spectra are presented by an almost straight line, raising gradually. The latter shape is characteristic of dead plants or plants under severe stress. Table 3 indicates lower NDVI values than those of the *in situ* thalli. In most of the cases the values range between 0.21 and 0.25 units except for thalli from sites 3, 4 and 6 where the NDVI values are as low as 0.9–0.11 units.

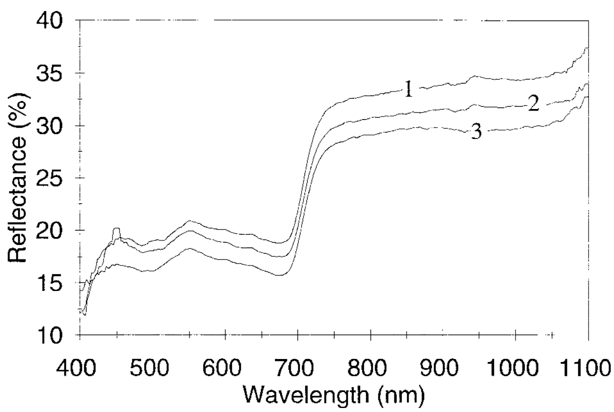

FIG. 1. Average spectral reflectance of *in situ* thalli of *R. duriaei* collected in the *Acacia albida* nature reserve near Ashdod and in the HaZorea Forest in January 1994. 1, Nature Reserve, center; 2, Nature Reserve, east; 3, HaZorea Forest.

The Concentration of Total S, Ni, and V in the Lichen Thallus

The concentrations of total S, Ni, and V in *in situ* thalli of *R. duriaei* collected in March 1993 and in January 1994 are presented in Tables 4 and 5, respectively. The concentrations of total S, Ni, and V in *in situ* thalli sampled in the nature reserve, Ashdod, were higher than those in thalli collected at the same time in the HaZorea Forest.

The concentrations of S, Ni, and V in *R. duriaei* collected in the HaZorea Forest in March 1993, resuspended in the same site or transplanted in the Ashdod region and retrieved in January 1994, are presented in Table 6. Thalli retrieved from sites 3, 6, and 12 contain the highest concentration of S. The highest concentration of Ni was detected in thalli retrieved from sites 4 and 6. The highest concentra-

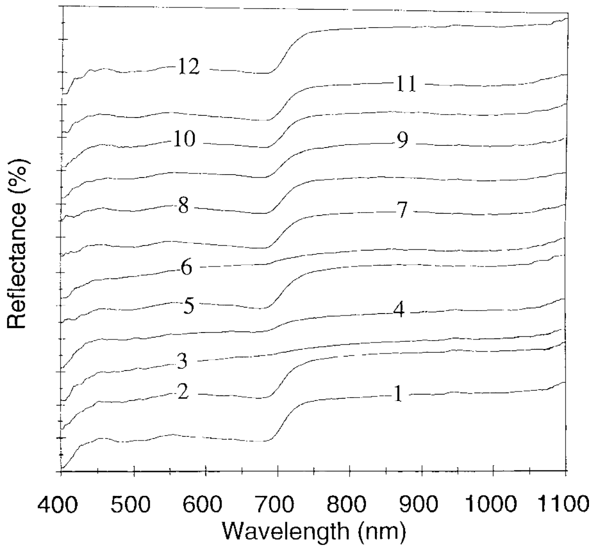

FIG. 2. Average spectral reflectance of *in situ* thalli of *R. duriaei* collected in the HaZorea Forest in March 1993, resuspended at the same site or transplanted to the Ashdod region and retrieved in January 1994. All curves have been offset vertically for clarity. Site numbers are as in Table 1.

tion of V was found in thalli exposed in sites 1, 4, and 6. A high concentration of V was also detected in thalli retrieved from sites 3, 10, and 12.

Table 6 also presents the ratio of the mean concentration of the elements V/Ni measured in *in situ*, resuspended and transplanted thalli of *R. duriaei*. The V/Ni ratio for the *in situ* thalli collected in sites 1 and 2, for transplanted thalli retrieved from sites 1, 2, 3, 4, and 12, which are mostly suburban or urban–industrial, in addition to two of the rural sites (6 and 10), is higher than the ratio obtained for the other sites (sites 5, 7, 8, 9, and 11).

The Correlation of the Physiological Parameters with the Values Indicating the Concentration of Elements in the Lichen Thallus

High correlation coefficients ($r > 0.50$; $P < 0.05$) were obtained in comparing parameters in resuspended or transplanted thalli of *R. duriaei* retrieved in January 1994 (Table 7). Table 7 indicates that the values for S, Ni, and V correlate inversely with the OD 435 nm/OD 415 nm ratio and with the NDVI. Direct correlations were obtained for the three elements. The OD 435 nm/OD 415 nm ratio correlated directly with the NDVI.

DISCUSSION

The hypothesis investigated in this study is that industrial activity, especially that involving the combustion of heavy fuel oil, has a considerable impact on air quality. Two different analyses of physiological alterations in the lichen thallus, which corresponded with concentration of three mineral elements in the *R. duriaei* lichen material, support our hypothesis. The impact of deteriorated air quality is reflected by two types of response by the lichen. Both types of response point to effects on the photobiont, i.e., the unicellular green alga *Trebouxia*, the autotroph partner in the *R. duriaei* thallus. The degradation of the photobiont chlorophyll was expressed as the sharp decrease in the OD ratio. This occurred not only in thalli exposed at a distance of 1.2–2.5 km from the two major sources of heavy fuel oil combustion in the study area, but also in a rural site (site 6) located of 5.2 km from one of those sources and 7.1 km from the other. A significant decrease in the OD ratio indicating a serious degradation of chlorophyll, was observed in previous studies following the ex-

TABLE 4

Concentrations of Total S, Ni, and V in *in Situ* Thalli of *Ramalina duriaei* Collected in March 1993 in the Nature Reserve, Ashdod, and in the HaZorea Forest

Site name:	Ashdod, Nature Reserve (center)				Ashdod, Nature Reserve (east)				HaZorea Forest				ANOVA		
	1				2				11						
Site no.	n	$X \pm$ SD	Duncan's test	CV	n	$X \pm$ SD	Duncan's test	CV	n	$X \pm$ SD	Duncan's test	CV	F ratio	F prob.	Mean CV
S	10	1950 ± 169	b*	0.08	10	2289 ± 111	a	0.04	10	1724 ± 219	c	0.12	27.209	0.0000	0.08
Ni	10	14.3 ± 2.9	a	0.20	10	15.3 ± 2.5	a	0.16	9	9.9 ± 1.4	b	0.14	12.912	0.0001	0.16
V	10	23.1 ± 3.7	b	0.16	10	31.7 ± 3.8	a	0.11	10	6.4 ± 1.3	c	0.20	164.108	0.0000	0.15
V/Ni[a]		1.61				2.07				0.64					

Note. N, number of replicates; X, mean values given as μg g^{-1} on dry weight basis; SD, standard deviation; CV, coefficient of variation (SD/mean). *Values of each horizontal line followed by the same letter do not differ significantly at $P < 0.05$ by Duncan's multiple range test.

[a] Ratio of mean values of V and Ni.

TABLE 5

Concentrations of Total S, Ni, and V in *in Situ* Thalli of *Ramalina duriaei* Collected in January 1994 in the Nature Reserve, Ashdod, and in the HaZorea Forest

	Site name: Ashdod, Nature Reserve (center)				Ashdod, Nature Reserve (east)				HaZorea Forest						
	1				2				11				ANOVA		
Site no.	*n*	*X* ± SD	Duncan's test	CV	*n*	*X* ± SD	Duncan's test	CV	*n*	*X* ± SD	Duncan's test	CV	*F* ratio	*F* prob.	Mean CV
S	10	2298 ± 320	b*	0.13	9	2514 ± 103	a	0.04	8	1958 ± 114	c	0.05	14.464	0.0001	0.07
Ni	10	15.3 ± 2.1	a	0.13	9	14.5 ± 3.4	a	0.23	9	10.3 ± 3.8	b	0.36	6.907	0.0041	0.24
V	10	30.0 ± 6.0	b	0.20	9	28.0 ± 2.0	a	0.07	8	5.5 ± 1.2	b	0.21	102.902	0.0000	0.16
V/Ni[a]		1.96				1.93				0.53					

Note. N, number of replicates; *X*, mean values given as $\mu g\ g^{-1}$ on dry weight basis; SD, standard deviation; CV, coefficient of variation (SD/mean). *Values of each horizontal line followed by the same letter do not differ significantly at $P < 0.05$ by Duncan's multiple range test.

[a] Ratio of mean values of V and Ni.

posure of *R. duriaei* in rural, suburban, and urban sites in central Israel (Garty *et al.*, 1985, 1988; Kardish *et al.*, 1987) and in the vicinity of a steel smelter in the northwestern part of Israel (Garty *et al.*, 1993). In comparison, Boonpragob and Nash (1991) used a 433 nm/415 nm OD ratio to estimate the impact of the Los Angeles urban environment on chlorophyll integrity of transplants of *Ramalina menziesii* and detected an increase of phaeophytins. Further comparative data are provided by Gonzalez and Pignata (1994) who detected a decrease of the ratio chlorophyll *a*/phaeophytin *a* in transplanted thalli of *Punctelia subrudecta* upon exposure to contaminated urban air in sites in Cordoba, Argentina.

TABLE 6

Concentrations of Total S, Ni, and V in Thalli of *Ramalina duriaei* Collected with Their Substrate in the HaZorea Forest in March 1993 and Resuspended at the Same Site or Transplanted to the Ashdod Area and Retrieved in January 1994

		S				Ni				V				
Site no.	Site name	*n*	*X* ± SD	Duncan's test	CV	*n*	*X* ± SD	Duncan's test	CV	*n*	*X* ± SD	Duncan's test	CV	V/Ni[a]
1	Ashdod, Nature Reserve (center)	10	2343 ± 186	d*	0.07	10	15.6 ± 3.4	de	0.21	10	28.8 ± 6.5	b	0.22	1.84
2	Ashdod, Nature Reserve (east)	10	2325 ± 145	d	0.06	10	7.3 ± 1.3	g	0.17	10	11.9 ± 1.4	d	0.11	1.63
3	Near the motorway entering Ashdod	10	3135 ± 420	ab	0.13	10	19.1 ± 4.6	bc	0.24	10	23.1 ± 7.4	c	0.32	1.20
4	Ashdod, 1.4 km east of the Eshkol Power Plant	10	2598 ± 386	c	0.14	10	22.2 ± 2.8	a	0.12	10	34.4 ± 6.8	a	0.19	1.54
5	Sede Uziyahu	10	2391 ± 251	cd	0.10	10	11.5 ± 2.1	f	0.18	10	11.4 ± 1.2	d	0.10	0.99
6	Kevutzat Yavneh	10	3290 ± 320	a	0.09	10	19.8 ± 3.8	ab	0.19	10	34.3 ± 8.0	a	0.23	1.73
7	Sand dunes, south of Ashdod	10	2153 ± 151	de	0.07	10	9.3 ± 1.7	fg	0.18	10	9.1 ± 1.2	de	0.13	0.97
8	Nitzanim Sand Dunes	10	1912 ± 129	e	0.06	10	7.9 ± 2.4	g	0.30	10	4.5 ± 0.9	f	0.20	0.56
9	Hafetz Hayyim	10	2149 ± 146	de	0.06	10	11.4 ± 1.4	f	0.12	10	7.0 ± 0.9	ef	0.12	0.61
10	Nir Galim	10	2643 ± 177	c	0.06	10	17.0 ± 3.2	cd	0.18	10	22.3 ± 3.6	c	0.16	1.31
11	HaZorea Forest	10	1945 ± 245	e	0.12	10	11.0 ± 1.7	f	0.15	10	5.8 ± 2.1	ef	0.36	0.52
12	Ashdod, near the oil refineries	10	2973 ± 422	b	0.14	10	14.1 ± 2.3	e	0.16	10	21.6 ± 3.8	c	0.17	1.53
ANOVA														
F ratio			27.885				31.742				58.093			
F probability			0.000				0.000				0.000			
Mean CV					0.09				0.18				0.19	

Note. N, number of replicates; *X*, mean values given as $\mu g\ g^{-1}$ on dry weight basis; SD, standard deviation; CV, coefficient of variation (SD/mean). *Values of each vertical column followed by the same letter do not differ significantly at $P < 0.05$ by Duncan's multiple range test.

[a] Ratio between mean values of V and Ni.

TABLE 7

Pearson Correlation Coefficients[a] between Physiological Parameters and Si, Ni, and V in Resuspended and Transplanted Thalli of *Ramalina duriaei* Retrieved from the Biomonitoring Sites in January 1995

Parameters	*r*	Parameters	*r*
OD 435 nm/OD 415 nm, Ni	−0.70*	NDVI, Ni	−0.71*
OD 435 nm/OD 415 nm, S	−0.73**	NDVI, S	−0.66*
OD 435 nm/OD 415 nm, V	−0.64*	NDVI, V	−0.59*
OD 435 nm/OD 415 nm, NDVI	0.95***	Ni, V	0.91***
		Ni, S	0.57*
		V, S	0.57*

[a] According to Pearson correlation test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

The correlations of S, Ni, and V obtained in the thalli of *R. duriaei* in the present study suggest a common source of emission of these elements, S, Ni, and V were described as indicators of pollution emitted by power plants in Israel (Foner and Ganor, 1986; Ganor *et al.*, 1988; Ganor and Brenner, 1991). The correlation of S, Ni, and V obtained in the present study corresponds with the correlation coefficients in total suspended matter obtained in Tel Aviv, around a heavy fuel oil power plant, (Foner and Ganor (1986). These authors found the correlation coefficients for Ni and V to be 0.996, for Ni and S to be 0.526, and for S and V to be 0.479.

In order to test the contribution of atmospheric deposition to the concentrations of S, Ni, and V in *in situ*, resuspended or transplanted thalli of *R. duriaei* we referred to data of another study of Ganor and co-workers (1991) of the chemical composition of aerosols settling in Israel upon dust storms. The authors found that the settling particles contain the three elements tested by us in the present study. Their concentrations were as follows: 2400 ± 1850 $\mu g\ g^{-1}$ of S, 53.1 ± 14.3 $\mu g\ g^{-1}$ of Ni, and 97 ± 31.6 $\mu g\ g^{-1}$ of V. We applied these data by dividing the values for the mean concentrations of S, Ni, and V in the dust by the mean concentration of the same mineral elements detected in our *in situ*, resuspended on transplanted lichen thalli. The results are presented in Table 8. The ratio of the concentration of the elements in dust to the concentration of the element in the lichen, obtained for V and Ni in the rural sites 5, 7, 8, 9, and 11 and in the eastern part of the nature reserve in Ashdod (site 2), is much higher for V, or higher for Ni, than the ratio of these two metals obtained for the other sites, including sites 6 and 10 which are rural by definition. The ratio of S in dust/S in lichen was lower than 1.0 for thalli either resuspended or transplanted and retrieved from sites 3, 4, 6, 10, and 12 whereas the ratio for the other sites was 1.0–1.2.

Great differences between the ratio in "clean" rural sites and that of the other sites may reflect the contribution of anthropogenic aerosols to the mineral–element concentration in the lichen thallus. The pollution in the less-clean sites is from the Eshkol Power Plant, the oil refineries, industrial and vehicular activity, and from domestic heating.

As to the contribution of the motorways to the elemental composition in *R. duriaei* growing/transplanted in the study area, we refer to Ward and Sampson (1989). The authors examined the elemental content of the moss *Hypnum jutlandicum* and surface soil samples as a function of distance from the traffic flow on the M25 motorway near London, England. The authors detected an exponential decrease of the amounts of V and Ni. According to Ward (1989) the elements Ni and V are found in metal plating and welded metals, whereas V is found also in lubricating oil. However, such a decrease of the V content as mentioned above could not be observed in the lichens (*Cladonia convoluta*) transplanted for 3 months in the vicinity of a main road 27 km south of Budapest, Hungary, by Tuba and Csintalan (1993) in spite of the influence of an

TABLE 8

Ratios of the Mean Concentration Values of S, Ni, and V in Dust (Ganor *et al.*, 1991) and the Mean Concentration Values of These Elements in *Ramalina duriaei*

	S	Ni	V
In situ, March 1993			
Ashdod, Nature Reserve (center)	1.2	3.7	4.2
Ashdod, Nature Reserve (east)	1.0	3.5	3.0
HaZorea Forest	1.4	5.4	15.1
In situ, January 1994			
Ashdod, Nature Reserve (center)	1.0	3.5	3.2
Ashdod, Nature Reserve (east)	0.9	3.6	3.5
HaZorea Forest	1.2	5.1	18.0
Resuspended or transplanted, January 1994			
Ashdod, Nature Reserve (center)	1.0	3.4	3.4
Ashdod, Nature Reserve (east)	1.0	7.3	8.1
Ashdod, near the chemical plant	0.8	2.8	4.2
Ashdod, 1.6 km east of E.P.P.	0.9	2.4	2.8
Sede Uziyahu	1.0	4.6	8.5
Kevutzat Yavneh	0.7	2.7	2.8
Sand dunes, south of Ashdod	1.1	5.7	10.6
Nitzanim Sand Dunes	1.2	6.7	21.5
Hafetz Hahyyim	1.1	4.6	13.8
Nir Galim	0.9	3.1	4.3
HaZorea Forest	1.2	4.8	16.7
Ashdod, near the oil refineries	0.8	3.8	4.5

oil-fueled power station located at a distance of approximately 2 km and an oil refinery located at a distance of approximately 6 km.

The calculation of the coefficients of variation (SD/mean) for element concentration in the thalli of *R. duriaei* yields in general higher values for Ni and V than for S (Tables 4–6). It has been assumed (Garty *et al.*, 1977; Garty, 1985; Garty and Ammann, 1987) that coefficients of variation indicate the nature of the elements accumulated and entrapped by lichens. As suggested in the above-mentioned studies, particles were indeed entrapped in the thallus of *Caloplaca aurantia* (Garty *et al.*, 1979) in accordance with their particulate nature. In this work, we suggest that the higher CVs for Ni and V reflect the particulate nature of these metals, which makes for a relative low rate of dispersion. The lower CVs obtained for S represent the greater rate of S dispersion in the air. Similarly, Loppi and co-workers (1994) used the epiphytic lichen *Parmelia caperata* as a biomonitor of trace metals in the Pistoia area (Central Northern Italy) and found differences between the CVs of the elements tested in their study. The authors suggested that the low CV obtained for Cu as well as in several other studies (Olmez *et al.*, 1985; Bargagli *et al.*, 1987a,b; Gasparo *et al.*, 1989) suggests that it is dispersed in small particles.

Our findings of high concentrations of S in *in situ* thalli of *R. duriaei* suggest high SO_2 concentrations in the air in the relevant sites. This suggestion is based on previous studies of exposure of lichen thalli to either SO_2 under field conditions or upon fumigation experiments there is an association between the concentration of S-containing gases in the air and S content accumulated in the lichen thallus (Malhorta and Khan, 1983; Takala *et al.*, 1985; Zakshek *et al.*, 1986; Garty *et al.*, 1988; Manninen *et al.*, 1991; Bruiteg, 1993).

The finding of V in lichens growing in Israel is reported for the first time here. Selected concentration values of V in lichens from different parts of the world are summarized in Table 9. A comparison of our data with those in other countries reveals that the background concentrations of V in Israel (site 11, Tables 3, 4, and 6) are in the same range or a little above those recorded for several rural sites throughout the world, including the Arctic region and the northern Taiga. Lichens collected in rural regions of the Arctic and the northern Taiga contain low concentrations of many mineral elements (Nash and Gries, 1995) and may therefore be utilized as a point of reference. The higher values of V obtained in sites 1, 3, 4, 6, 10, and 12 (Table 6) are lower or much lower than those obtained for lichens in different sites in the world adjacent to sites of heavy metal smelting or mining activities. On the other hand, these values resemble values obtained in lichens collected near petrochemical industries (Pakarinen *et al.*, 1983) or near power plants using heavy fuel oil (Nygård and Harju, 1983).

The condition of the thallus in regard to its content of V was described by Addison and Puckett (1980). In a study on the deposition of atmospheric pollutants as measured by the elemental content of lichen thalli (Al, K, S, Ti, and V) in the Athabasca oil sand area (northern Alberta, Canada), the authors reported on visible changes in the condition of the thallus apparently related to the concentrations of elements. According to a semiquantitative estimate, both S and V were significantly correlated ($P < 0.01$) with the condition of the lichen *Evernia mesomorpha*. The elemental content of S and V accounted for 22 and 6%, respectively, of the variability in the condition of the lichen. The definition of "thallus condition" in the above-mentioned study was based on a qualitative estimate of the conditions of the thallus on a scale from 1 (least) to 5 (most luxuriant) made for the samples collected for analysis.

A more specific parameter which indicates alterations of lichen physiology upon exposure to V was reported by LeSueur and Puckett (1980). The authors showed that low V concentrations (0.2 μg g^{-1}) induced a reduction of phosphatase activity in *Cladina rangiferina*. To the best of our knowledge, V was not reported to be detrimental to the photobiont chlorophyll in lichens but was found to decrease photosynthesis in chlorophyta in fresh water (Nalewjako *et al.*, 1995). The present study shows an inverse correlation between concentration of V and the decrease of the OD nm ratio and the NDVI in the thallus of *R. duriaei*. This points to a possible linkage of the accumulation of V with degradation of chlorophyll in the *Trebouxia* cells.

The use of lichens as biomonitors of atmospheric heavy metal deposition, including V, was discussed by Mowinckel and co-workers (1995). The authors evaluated the epiphytic lichen *Hypogymnia physodes* and bark from *Pinus sylvestris* as biomonitors. In areas where metal contribution from local wind-blown soil dust is small compared with the atmospheric deposition, bark and epiphytic lichens were found to be suitable biomonitors for Cu, Cr, Fe, and V. Based on the heavy deposition of V in the study area (Anonymous, 1994) and the V content in settling dust (Ganor *et al.*, 1991) we believe that the lichen *R. duriaei* reflects the presence of V in the air.

Indirect evidence shows that Ni may have an impact on the photobiont chlorophyll in lichens. This

TABLE 9
Selected V Concentrations Reported in Lichens

Species	Location	V ($\mu g\ g^{-1}$)	Reference	Remarks
Hypogymnia physodes	Kokkola, W. Finland	16–291	Laaksovirta and Olkkonen, 1977	Iron smelting and fertilizer plant
Lecanora conizaeoides	Copenhagen area, Denmark	19	Andersen *et al.*, 1978	Rural
		12 ± 1	Andersen *et al.*, 1978	Suburban
		14 ± 3	Andersen *et al.*, 1978	City center
Cladonia spp.	Arctic Canada	1 ± 1	Nieboer *et al.*, 1978	
Lecanora conizaeoides	Frederiksvaerk, Denmark	0.9–169	Pilegaard, 1978	Around a steelworks and an iron foundry
Hypogymnia physodes	Frederiksvaerk, Denmark	5.6 ± 0.2	Pilegaard, 1979	Before transplantation to the vicinity of the steelworks
Hypogymnia physodes	Frederiksvaerk, Denmark	4.7–10.7	Pilegaard, 1979	Following transplantation to the vicinity of the steelworks
Hypogymnia physodes	Rural sites, Denmark	1.7–7.7	Pilegaard *et al.*, 1979	
Lecanora conizaeoides	Rural sites, Denmark	2.2–7.1	Pilegaard *et al.*, 1979	
Cetraria cucullata	Northwest territories, Canada	1.78 ± 2.84	Puckett and Finegan, 1980	
Cetraria nivalis	Northwest territories, Canada	1.2 ± 2.4	Puckett and Finegan, 1980	
Cladina stellaris	Northwest territories, Canada	3.98 ± 8.52	Puckett and Finegan, 1980	
Cladina arbuscula	Athabasca oil sands, Northern Alberta, Canada	3–140	Addison and Puckett, 1980	Oil extraction complex
Hypogymnia physodes	In the vicinity of a power plant using heavy fuel oil, Kristiinankaupunki, southwestern Finland	1.6–57.4	Nygård and Harju, 1983	
Hypogymnia physodes	Fågelmosse, Skoldvik, Finland	32.6	Pakarinen *et al.*, 1983	A site affected by petrochemical industry
Hypogymnia physodes	South Finland	6.6 ± 1.4	Pakarinen *et al.*, 1983	
Cladonia spp.	South Finland	0.9–1.5	Pakarinen *et al.*, 1983	
Parmelia sulcata	De Kempen, The Netherlands	9.0–48.0	de Bruin and Hackenitz, 1986	In a region formerly polluted by zinc smelters
Ramalina stenospora	Calcasieu Parish, Louisiana	2.1–10.0	Mueller *et al.*, 1987	Around an industrial corridor
Parmelia sulcata	Theodore Roosevelt National Park, North Dakota	9.2–24	Gough *et al.*, 1988a	
Hypogymnia enteromorpha	Little Bald Hills, Redwood National Park, California	0.73–6.3	Gough *et al.*, 1988b	
Usnea spp.	Little Bald Hills, Redwood National park, California	0.21–1.4	Gough *et al.*, 1988b	
Flavoparmelia caperata	Ohio River Valley, Ohio	2.9–27.1	Showman and Hendricks, 1989	Collected in 1973
Parmotrema madagascaricum	Five sites in Venezuela	2.6 ± 0.2	Gordon *et al.*, 1991	High altitude, southface El Alvila National Park
		8.8 ± 3.0	Gordon *et al.*, 1991	Low altitude, southface (Caracas side) El Alvila National Park
Parmelia caperata	La Spezia (northern Italy)	1.66–5.81	Nimis *et al.*, 1993	
Parmelia sulcata	Malbun Village, Principality of Liechtenstein	3.9 ± 1.6	Herzig, 1993	Values defined as low by the author
Parmelia sulcata	Schaan Bofel, Switzerland	14.6 ± 6.4	Herzig, 1993	Agricultural area with influence of industry. Values defined as critically high by the author

Note. Values are given in $\mu g\ g^{-1}$ on dry weight basis.

metal was found to be associated with the algal zone in an experiment in which lichens were exposed to Ni under laboratory conditions (Flora and Nieboer, 1980; Goyal and Seaward, 1981; Richardson and Nieboer, 1983). The affinity of Ni for the photobiont cells in the lichen thallus may partly explain the damage induced in the chlorophyll of the *Trebouxia* cells in *R. duriaei* upon exposure to air polluted by Ni in the Ashdod area.

No data are available on the concentration of Ni, V, and S in the air of HaZorea Forest, from which the lichen material was collected to be transplanted to the Ashdod area. However, 20 years of measurements of the concentration of Ni in *R. duriaei, in situ* or resuspended (Table 10) reveal that this area is still uncontaminated by the emission of the power stations located in the region, as concentrations of Ni in *R. duriaei* collected or retrieved from the HaZorea Forest did not change over that period. These data provide a basis for the continuous use of *R. duriaei* thalli from the HaZorea Forest as transplanted biomonitors around power plants in the country.

Three groups of spectral reflectance curves are presented in Figs. 1 and 2: the *in situ* thalli group, the resuspended group, and the transplanted group. It may be seen that the spectra of the *in situ* thalli have characteristics similar to the characteristics of higher vegetation, e.g., a low dip in the red region, a sharp red edge, and a high NDVI plateau. These features are less pronounced in some of the transplanted thalli and are not observed in thalli retrieved from severely contaminated sites. An analysis of the spectra, such as the NDVI calculation (Tables 2 and 3), shows the significant differences between the groups. This type of spectroscopic measurement and analysis shows the advantage of remote sensing biomonitoring of air pollution. Although spectroscopic measurements are limited in their capability to determine the source of pollution, the method offers a fast and inexpensive technique for evaluating physiological status of lichens.

The application of the NDVI parameter in *R. duriaei* as a tool to assess stress induced by environmental contamination may be compared with the study of Cox and co-workers (1991) who found that exposure to Cu caused significant shifts in the spectral response of lichens forming carpets in northern Canada. The present study further supports the application of this nondestructive measure as a sensitive indicator of the impact of polluted air on the lichen photobiont in biomonitoring sites.

In conclusion, we believe that the contamination of the greater part of the region of Ashdod by SO_2, V, and Ni produced by anthropogenic activity is relatively high. The decrease of the OD nm ratio and the NDVI and the accumulation of S, Ni, and V in the thallus of *R. duriaei* reveal to us a potential burden of pollutant that can be expected to endanger this lichen, which is the only indigenous fruticose epiphytic lichen still growing in the nature reserve near Ashdod.

TABLE 10

Mean Concentrations (μg g^{-1} on Dry Weight Basis) ± SD of Ni in *in Situ* Thalli of *Ramalina duriaei* an in Thalli Collected at HaZorea Forest and Resuspended with Their Substrate on the Same Trees for 4–12 Months

Date of collection	Treatment	Ni	References
January 1974	F[a]	13.0	Garty and Fuchs, 1982
May–December 1978	R[b]	10.0	Garty and Fuchs, 1982
February 1979	F	10.0	Garty and Fuchs, 1982
February 1979–March 1980	R	9.0 ± 0.8	Garty, 1987
July 1981–July 1982	R	39.5 ± 16.2[c]	Garty, 1987
December 1981	F	20.1 ± 11.2	Garty, 1988
December 1981–December 1982	R	14.6 ± 8.0	Garty, 1988
January 1983	F	12.5 ± 3.9	Garty and Hagemeyer, 1988
January 1984	F	9.9 ± 4.8	Garty and Hagemeyer, 1988
January 1983–January 1984	R	12.3 ± 4.7	Garty and Hagemeyer, 1988
July 1984	F	22.1 ± 9.8	Garty *et al.*, 1988
July 1985	F	12.8 ± 3.9	Garty *et al.*, 1988
July 1984–July 1985	R	19.3 ± 4.8	Garty *et al.*, 1988
March 1993	F	9.9 ± 1.4	This study
January 1994	F	10.3 ± 3.8	This study
March 1993–January 1994	R	11.0 ± 1.7	This study

[a] Fresh *in situ* thalli.
[b] Resuspended thalli.
[c] Following extraordinary car activity in the forest in May and June 1982.

Based on our findings, we recommend the use of *R. duriaei* transplants in the Ashdod area and suggest that emphasis be placed on the examination of the V/Ni ratio and on the correlation of the OD ratio with the NDVI and the metal contents in the lichen thalli.

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