AIR BIOMONITORING BY TRANSPLANTED LICHENS IN THE NEGEV DESERT, ISRAEL

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Abstract

Thalli of the lichen Ramalina maciformis (Del.) Bory were collected in the Negev Desert in August 1997 and transplanted with their substrate, flintstones, to 24 bio-monitoring sites in the Negev Desert. An assessment of the impact of anthropogenic activities was made by measurements of the concentration of mineral elements in the lichen and by an examination of its physiological status.

After a transplantation period of nine months, the lichens were retrieved in April 1998 and the concentration of 22 mineral elements in the thallus was determined by ICP-AES. In addition we examined the following parameters determining the status of the lichen: 1. Electric conductivity indicative of cell membrane integrity; 2. Spectral reflectance response of the thallus expressed as normalized difference vegetation index (NDVI) indicative of greenness and health of the thallus; 3. Production of ethylene indicative of stress; 4. Chlorophyll a fluorescence as a means to monitor aspects of photosystem II (PSII) activities in the lichen.

Preliminary results show that lichens exposed to air contaminants at a site of toxic waste deposition contain high concentrations of Ca, Cu, Pb and Mn and significant low K concentrations due to leakage of this element from injured cell membranes. Conductivity measurements performed to test the integrity of cell membranes corroborated this assumption.

NDVI values indicating damage to chlorophyll were relative low in lichens retrieved from sites near Beer Sheba. The stress-ethylene production was the highest in one site near Beer Sheba. The maximum quantum yield of PSII expressed as fluorescence ratio Fv/Fm was low in two sites in the Ramat Hovav Industrial Area.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Vast areas in the Negev Desert, especially near the Ramat Hovav Industrial Area, about 10-15 km south of the town of Beer Sheba, are exposed to environmental contamination. In the absence of instrumental monitoring, we believe that the capability of lichens to monitor air pollutants should be utilized in full. Our experience with desert lichens in Israel was limited to analyses of the elemental content of *in situ* thalli [1] or of detached and decomposing lichen material [2,3]. The use of transplants of an epilithic fruticose desert lichen to monitor airborne pollutants in the desert is innovative and leads to the development of a biotechnological procedure to be used for environmental quality research projects all over the world.

2. METHODS

In August 1997 we collected flintstones carrying the fruticose lichen *Ramalina maciformis* (Del.) Bory on a hill near Tellalim in the Negev Desert, and transferred them to 24 biomonitoring sites in the region. A few sites were selected to act as biomonitoring stations in the Ramat Hovav Industrial Area where large amounts of chemicals from all parts of the country, including organic compounds and heavy metals, are disposed. This area includes also evaporating ponds (Fig. 1).

In April 1998 the stones carrying the lichens were retrieved and transferred to the laboratory at Tel Aviv University. The lichens were detached from the substrate and rinsed immediately for 10-20 s with double-distilled water. This procedure was repeated three times to eliminate dust. Then the thalli were air-dried and divided into samples. The possible loss of ions such as K and Mg known to occur upon the rinsing of desiccated thalli [4] was thus minimized.

2.1. Determination of the concentration of elements in lichen thalli

For the measurement of the concentrations of Ca, Ti, Cu, Mg, Fe, Si, Ni, Cd, Pb, Zn, Ag, V, Cr, Mn, K, Na, Ba, Sr, B, S, P and Al in *R. maciformis*, subsamples of 1-2 g of rinsed and air-dried thalli were further dried at 105°C for 24 h. Subsamples of 250 mg were wet-ashed in 10 ml of concentrated pure HNO_3 (Merck) in test tubes of 50 ml in a heating block for a duration of 8 h at temperatures of 80, 100 and 120°C. The elemental concentration was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using the Spectroflame ICP® (Spectro, Kleve, Germany).

2.2. Assessment of the integrity of cell membranes in the lichen thallus

Previous reports indicated the injury caused to cell membranes as corresponding to an increase of electrical conductivity of water due to electrolyte leakage, upon exposure of lichen thalli to chemicals under laboratory conditions [5-7]. For an assessment of the integrity of cell membranes, batches of lichen thalli collected in April 1998 were divided into subsamples of 1 g and immersed in 100 ml of double distilled water for 60 min. The electrical conductivity of the water was measured by an electric conductivity meter (TH-2400, EL-Hamma Instruments).

2.3. Measurement of the spectral reflectance response of lichen thalli

A non-invasive method to assess the impact of air pollutants on lichens is the evaluation of change in the normalized difference vegetation index (NDVI). Digital measurements of spectral reflectance were used at first 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 chlorophyll 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 [8], is a ratio of the digital values of these two bands. The most widely used VI is known as the normalized difference vegetation index. NDVI = (NIR-R) / (NIR+R). 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.

The spectral characteristics of R. maciformis collected at the end of the exposure period were measured digitally in the laboratory by a Li-Cor® LI 1800 field spectrometer (Li-Cor, Lincoln, NE, USA). For details see Ref. [9].

2.4. Measurement of the production of ethylene in R. maciformis

The stress-indicative feature of ethylene is used in several studies of lichens in Israel [9-11] and in Finland [12-15]. Desert lichens were not proved yet to produce ethylene. In several cases lichens were found to produce large amounts of **ethylene** following exposure to acidic chemicals [13], including heavy metal solutions under low pH conditions [16]. For a measurement of the production of ethylene in *R. maciformis*, subsamples of 1 g each, of thalli retrieved in April 1998, were soaked in 20 ml of 5 mM $FeCl_2$, pH 3.5. The submersion of these subsamples in a solution of iron salt at a low pH was used to maximize the potential production of ethylene [9]. After the soaking procedure, all subsamples 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 were withdrawn with a syringe and 1 ml was injected into a gas chromatograph Varian 3350 equipped with an activated alumna 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 150°C.

2.5. Measurement of chlorophyll fluorescence

A non-invasive method to analyze photosynthetic parameters in lichens is the measurement of chlorophyll a fluorescence [17-21]. The studies referred to, dealt with a wide range of physiological conditions including those prevailing under low temperatures. Much less attention was paid to the assessment of changes in vivo of chlorophyll fluorescence in lichen thalli exposed to pollutants. An application of novel methods to measure chlorophyll fluorescence as a non-invasive means to monitor aspects of photosystem II (PSII) activities in lichens exposed to SO2 fumigation was reported by Gries and coworkers [22]. To characterize the status of PSII in fumigated lichens the authors used a PAM (pulse amplitude modulated) fluorometer, and detected changes in the rapid induction kinetics of chlorophyll fluorescence upon fumigation, which indicated the inhibition of PSII by SO2. A decrease in PSII photochemical reactions measured as a change of the ratio: variable fluorescence/ maximal fluorescence (Fv/Fm) was detected also in dark adapted lichens exposed to ozone [23] and to heavy metals [24,25]. On the other hand, a study of chlorophyll a fluorescence in lichen thalli from a polluted region in Spain revealed that the ratio Fv/Fm in these thalli was about the same as for control samples [26].

Fluorescence measurements of thalli from the Negev were performed in the following manner: the thalli were first immersed in distilled water for five min to revive full photosynthetic activity. A pulse amplitude modulation fluorometer (the Diving PAM, Walz, Effeltrich, Germany) was applied to measure the potential quantum yield of PSII of the lichens. The thalli were dark-adapted for ten min by so-called dark leaf clips. Then the shutter of one clip at a time was opened and a 0.7 s long saturating flash of white light was administered by the instrument through an optical fibre. The potential quantum yield of PSII was calculated as Fv/Fm as Fv is the variable fluorescence defined as maximal fluorescence (Fm, during the saturating flash) less the minimal fluorescence (Fo, as sampled in darkness just before the saturating flash).

3. RESULTS

Table I presents the concentration of elements in thalli of *R. maciformis* retrieved from seven biomonitoring sites. Lichens in the site contaminated by toxic waste contain large amounts of Ca, Cu, Pb and Mn and considerable smaller amounts of K, which indicates a leakage of this essential mineral nutrient from the lichen cells. These findings match the high conductivity values observed in the toxic waste site (Table II) indicating injury caused to cell membranes. Table II shows damage to cell membranes in an additional site in the Ramat Hovav Industrial Area, the gravel area. On the other hand, low electric conductivity values were obtained for the control site and for additional sites at a distance from the industrial region.

Table III shows relative low NDVI values for lichens retrieved in and near Beer Sheba. The other samples exhibited fair or high NDVI values.

Low values were obtained for ethylene production in most of the sites, including the control site (Table IV). Higher values were obtained only for the site near the telecommunication antenna.

Table V shows that the maximum quantum yield of PSII expressed as fluorescence ratio Fv/Fm was very high in the control site as well as in a few sites at a distance from the Ramat Hovav Industrial Area. Relative low Fv/Fm ratios were obtained for the toxic waste site, near the old Turkish railway and near the telecommunication antenna.

4. PLANS FOR FUTURE WORK

In December 1998 we plan to initiate a second round of transplantation of *R.. maciformis* thalli in the biomonitoring sites to be selected according to the results to be obtained in October-November 1998. In April-May of 1999 we will test the physiological status of the lichens as well.

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TABLE I. MEAN VALUES OF MINERAL ELEMENT CONCENTRATION ($\mu g\ g^{-1}$ dry weight) IN THALLI OF *RAMALINA MACIFORMIS* COLLECTED WITH SUBSTRATE IN CONTROL SITE, AUGUST 1997, TRANSPLANTED TO BIOMONITORING SITES, RETRIEVED APRIL 1998. NUMBER OF REPLICATES WAS 10.

Site No.	2	3	4	7	10	19	23
Site name	Red Sculpture R. Hovav	Gravel area R. Hovav	Yona Efrat site	Toxic waste R. Hovav	IDF antenna south of Beer Sheva	Eshel HaNassi School	Mishol Girit, Beer Sheva
Ca	74350	76110	67030	95070	74170	60490	61860
Ti	15.8	22.6	19.5	21.2	21.5	17.1	17.8
Cu	4.9	5.6	3.9	20.4	4.2	3.9	4.3
Mg	543	683	484	694	579	458	435
Fe	640	862	642	793	758	658	561
Si	82.9	109.0	77.2	97.1	91.9	58.2	64.4
Ni	1.3	1.5	1.5	1.6	1.2	1.5	1.3
Cd	0.9	1.2	0.9	0.9	0.9	0.9	0.9
Pb	7.8	8.7	6.9	99.8	8.8	7.3	7.1
Zn	22.9	22.8	23.7	35.0	21.3	63.6	41.5
Ag	0.9	1.7	0.9	0.9	0.7	0.9	0.9
V	7.0	6.0	5.1	3.6	4.4	4.1	4.2
Cr	2.2	3.0	1.9	3.6	2.2	2.3	2.5
Mn	21.1	27.3	24.1	45.9	24.1	21.6	21.3
K	1596	1691	1482	1047	1638	1470	1359
Na	325	390	266	569	215	197	444
Ba	9.5	11.4	9.1	11.1	10.8	9.5	10.4
Sr	105	95.9	93.6	118	90.2	64.7	73.7
B	6.7	6.0	14.4	16.0	6.2	14.8	14.8
S	2531	2561	2356	2624	2270	2368	2211
P	569	561	565	516	530	570	568
Al	601	777	590	690	686	587	519

TABLE I (continued)

Site No.	5	8	13	14	17	20	25
Site name	Power Station Isr. Elec. Co.	Old Turkish Railway	Tel Sheva	Kibbutz Revivim	Mashabbe Sade	Kibbutz Sede Boker (cemetery)	Control Site
Ca	75603	70858	60978	64357	71086	63861	58921
Ti	16.3	17.1	18.5	15.6	26.2	15.6	32.2
Cu	5.2	4.8	4.0	3.7	8.8	3.9	3.8
Mg	421	390	475	453	517	394	345
Fe	541	575	628	583	539	557	554
Si	13.7	37.9	47.5	18.1	16.5	48.5	17.3
Ni	3.4	3.1	3.3	3.1	2.9	1.1	1.1
Cd	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Pb	8.2	16.4	8.4	9.1	8.2	6.9	8.2
Zn	25.8	21.0	19.1	18.6	23.3	19.7	13.9
Ag	0.8	0.8	0.8	0.8	0.8	0.8	0.8
V	4.5	4.8	3.3	3.5	3.8	3.1	4.0
Cr	1.7	2.6	2.6	2.8	2.1	2.1	1.6
Mn	23.4	26.3	22.8	23.7	28.0	19.0	19.8
K	1400	1345	1376	1443	1340	1370	1422
Na	271	244	400	259	233	192	151
Ba	8.8	10.2	9.5	9.3	12.1	10.4	8.5
Sr	86.0	92.4	69.4	77.1	85.9	81.7	70.7
B	4.0	7.0	13.0	12.0	10.0	19.4	6.0
S	2157	2264	2142	2045	2040	1990	1716
P	486	535	469	503	543	556	479
Al	507	517	573	541	481	542	542

TABLE II. VALUES OF ELECTRICAL CONDUCTIVITY (mSm^{-1}) IN THALLI OF *RAMALINA MACIFORMIS* COLLECTED IN CONTROL SITE, AUGUST 1997, TRANSPLANTED TO BIOMONITORING SITES, RETRIEVED APRIL 1998.

(n = number of replicates; X = mean values; SD = standard deviation).
Values in the vertical column followed by the same letter do not differ significantly at $p<0.05$ using one-way ANOVA and SNK test.

Site number and name	n	X ± SD	
1 Ramat Beka	12	2.85± 0.27	b
2 Red Sculpture, Ramat Hovav	12	2.54± 0.31	cd
3 Ramat Hovav (gravel area)	12	2.96± 0.43	b
4 Yona Efrat site	12	2.06± 0.16	fghi
5 Power Station, Israel Electric Corporation	12	2.15± 0.18	fg
6 Local council, Ramat Hovav	6	2.11± 0.20	fgh
7 Toxic waste site, Ramat Hovav	12	3.34± 0.30	a
8 Old Turkish railway, adjacent to toxic waste site	6	1.94± 0.20	ghi
9 Telecommunication antenna, a few km south of Beer Sheva	12	1.59± 0.12	jk
10 IDF antenna, a few km south of Beer Sheva	12	1.94± 0.21	ghi
11 Pumping site, Ramat Hovav	12	2.66± 0.25	c
12 Evaporation ponds, Ramat Hovav	12	1.98± 0.16	ghi
13 Tel Sheva, near Beer Sheva	12	2.47± 0.15	cd
14 Kibbutz Revivim (zoological garden)	12	2.51± 0.20	cd
15 Mitzpeh Revivim, east	12	1.97± 0.12	ghi
16 Mitzpeh Revivim, west	12	2.38± 0.21	de
17 Mashabbe Sade Kibbutz	12	2.23± 0.13	ef
18 Meitar (Efrati's house)	12	1.80± 0.08	ij
19 Eshel HaNassi School	12	1.62± 0.16	jk
20 Kibbutz Sede Boker (cemetery)	12	1.47± 0.11	k
21 Pistachio orchard, Sede Boker Kibbutz	12	1.87± 0.11	hi
22 Motorola antenna	12	1.96± 0.21	ghi
23 Mishol Girit, Beer Sheva	12	2.43± 0.13	cde
24 Shekhunat Vered, Beer Sheva	12	2.08± 0.20	fgh
25 Control site	12	1.22± 0.15	l
ANOVA			
F ratio		66.987	
F probability		0.000	

TABLE III. NDVI IN THALLI OF *RAMALINA MACIFORMIS* COLLECTED IN CONTROL SITE, AUGUST 1997, TRANSPLANTED TO BIOMONITORING SITES, RETRIEVED APRIL 1998.

(n = number of replicates; X = mean values; SD = standard deviation).
Values in the vertical column followed by the same letter do not differ significantly at $p<0.05$ using one-way ANOVA and SNK test.

Site number and name		n	X ± SD	
1	Ramat Beka	4	0.332 ± 0.004	gh
2	Red Sculpture, Ramat Hovav	4	0.341 ± 0.002	defg
3	Ramat Hovav (gravel area)	4	0.354 ± 0.006	bc
4	Yona Efrat site	4	0.332 ± 0.006	gh
5	Power Station, Israel Electric Corporation	4	0.337 ± 0.007	efg
6	Local council office, Ramat Hovav	4	0.347 ± 0.003	cde
7	Toxic waste site, Ramat Hovav	4	0.320 ± 0.005	i
8	Old Turkish railway, adjacent to toxic waste site	4	0.348 ± 0.006	cde
9	Telecommunication antenna, a few km south of Beer Sheva	4	0.373 ± 0.004	a
10	IDF antenna, a few km south of Beer Sheva	4	0.339 ± 0.003	efg
11	Pumping site, Ramat Hovav	4	0.334 ± 0.006	fg
12	Evaporation ponds, Ramat Hovav	4	0.305 ± 0.005	j
13	Tel Sheva, near Beer Sheva	4	0.292 ± 0.004	l
14	Kibbutz Revivim (zoological garden)	4	0.360 ± 0.005	b
15	Mitzpeh Revivim, east	4	0.351 ± 0.006	bcd
16	Mitzpeh Revivim, west	4	0.375 ± 0.006	a
17	Mashabbe Sade Kibbutz	4	0.352 ± 0.004	bcd
18	Meitar (Efrati's house)	4	0.296 ± 0.003	jkl
19	Eshel HaNassi School	4	0.344 ± 0.008	cdef
20	Kibbutz Sede Boker (cemetery)	4	0.355 ± 0.007	bc
21	Pistachio orchard, Sede Boker Kibbutz	4	0.296 ± 0.010	jkl
22	Motorola antenna	4	0.340 ± 0.007	defg
23	Mishol Girit, Beer Sheva	4	0.293 ± 0.005	kl
24	Shekhunat Vered, Beer Sheva	4	0.303 ± 0.004	jk
25	Control site	4	0.323 ± 0.003	hi
	ANOVA			
	F ratio		80.554	
	F probability		0.000	

TABLE IV. THE PRODUCTION OF ETHYLENE (nl g^{-1} h^{-1}) BY THALLI OF *RAMALINA MACIFORMIS* COLLECTED IN THE CONTROL SITE IN AUGUST 1997, TRANSPLANTED TO BIOMONITORING SITES, RETRIEVED APRIL 1998 AND SOAKED IN 5 mM $FeCl_2$ AT pH 3.5.

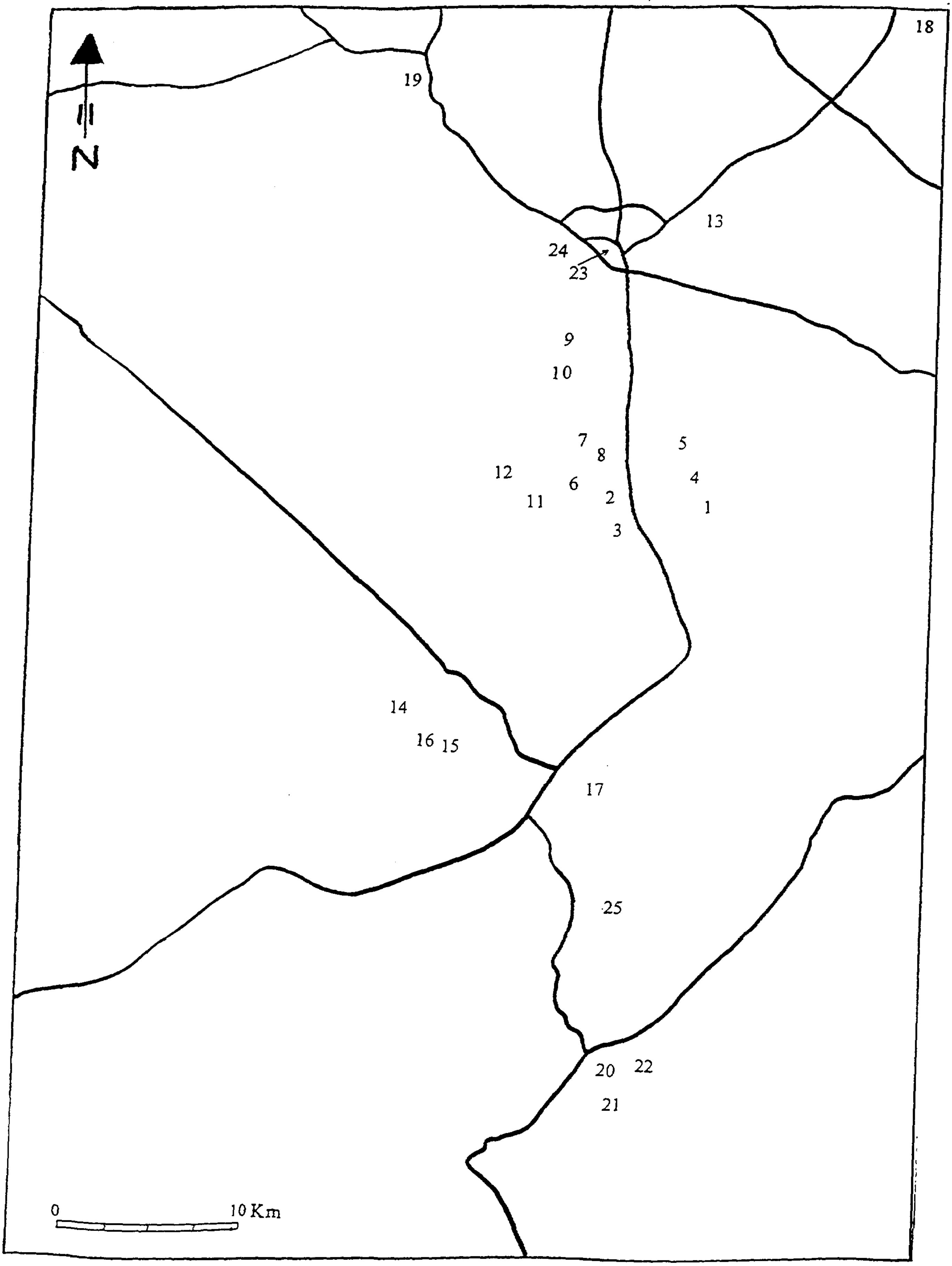
(n = number of replicates; X = mean values; SD = standard deviation).
Values in the vertical column followed by the same letter do not differ significantly at $p<0.05$ using one-way ANOVA and SNK test.

Site number and name	n	X ± SD	
1 Ramat Beka	10	1.86± 0.53	de
2 Red Sculpture, Ramat Hovav	10	1.76± 0.63	de
3 Ramat Hovav (gravel area)	10	2.33± 0.54	cde
4 Yona Efrat site	10	2.30± 0.42	cde
5 Power Station, Israel Electric Corporation	9	2.52± 1.22	cde
6 Local council office, Ramat Hovav	-	- -	-
7 Toxic waste site, Ramat Hovav	10	2.73± 0.60	bcd
8 Old Turkish railway, adjacent to toxic waste site	-	- -	-
9 Telecommunication antenna, a few km south of Beer Sheva	10	4.52± 2.08	a
10 IDF antenna, a few km south of Beer Sheva	10	2.26± 1.00	cde
11 Pumping site, Ramat Hovav	10	3.01± 1.34	bc
12 Evaporation ponds, Ramat Hovav	10	2.83± 0.77	bcd
13 Tel Sheva, near Beer Sheva	10	1.36± 0.20	e
14 Kibbutz Revivim (zoological garden)	10	1.78± 0.40	de
15 Mitzpeh Revivim, east	10	2.20± 0.37	cde
16 Mitzpeh Revivim, west	10	3.07± 0.38	bc
17 Mashabbe Sade Kibbutz	10	1.34± 0.71	e
18 Meitar (Efrati's house)	10	1.35± 0.25	e
19 Eshel HaNassi School	10	3.55± 0.49	b
20 Kibbutz Sede Boker (cemetery)	9	2.05± 0.41	cde
21 Pistachio orchard, Sede Boker Kibbutz	10	1.56± 0.55	e
22 Motorola antenna	10	1.98± 0.39	cde
23 Mishol Girit, Beer Sheva	9	1.44± 0.41	e
24 Shekhunat Vered, Beer Sheva	10	1.57± 0.42	e
25 Control site	10	2.28± 0.59	cde
ANOVA			
F ratio		10.556	
F probability		0.000	

TABLE V. MAXIMUM QUANTUM YIELD OF PSII EXPRESSED AS FLUORESCENCE RATIO Fv/Fm IN THALLI OF *RAMALINA MACIFORMIS* COLLECTED IN CONTROL SITE, AUGUST 1997, TRANSPLANTED TO BIOMONITORING SITES, RETRIEVED APRIL 1998, DARK ADAPTED IN THE LABORATORY.

(n = number of replicates; X = mean values; SD = standard deviation).
Values in the vertical column followed by the same letter do not differ significantly at $p<0.05$ using one-way ANOVA and SNK test.

Site number and name	n	X ± SD	
1 Ramat Beka	20	0.606 ± 0.038	abc
2 Red Sculpture, Ramat Hovav	20	0.486 ± 0.106	efg
3 Ramat Hovav (gravel area)	20	0.616 ± 0.063	abc
4 Yona Efrat site	20	0.629 ± 0.043	ab
5 Power Station, Israel Electric Corporation	19	0.506 ± 0.064	def
6 Local council office, Ramat Hovav	20	0.542 ± 0.079	cde
7 Toxic waste site, Ramat Hovav	20	0.433 ± 0.061	gh
8 Old Turkish railway, adjacent to toxic waste site	20	0.442 ± 0.099	fgh
9 Telecommunication antenna, a few km south of Beer Sheva	18	0.415 ± 0.139	h
10 IDF antenna, a few km south of Beer Sheva	20	0.497 ± 0.101	defg
11 Pumping site, Ramat Hovav	20	0.571 ± 0.124	abcd
12 Evaporation ponds, Ramat Hovav	19	0.548 ± 0.075	bcde
13 Tel Sheva, near Beer Sheva	20	0.625 ± 0.085	ab
14 Kibbutz Revivim (zoological garden)	20	0.635 ± 0.032	a
15 Mitzpeh Revivim, east	20	0.652 ± 0.051	a
16 Mitzpeh Revivim, west	20	0.634 ± 0.082	a
17 Mashabbe Sade Kibbutz	20	0.549 ± 0.074	bcde
18 Meitar (Efrati's house)	20	0.627 ± 0.034	ab
19 Eshel HaNassi School	20	0.645 ± 0.062	a
20 Kibbutz Sede Boker (cemetery)	20	0.514 ± 0.148	de
21 Pistachio orchard, Sede Boker Kibbutz	20	0.593 ± 0.095	abc
22 Motorola antenna	20	0.618 ± 0.047	abc
23 Mishol Girit, Beer Sheva	20	0.597 ± 0.077	abc
24 Shekhunat Vered, Beer Sheva	20	0.643 ± 0.027	a
25 Control site	20	0.643 ± 0.027	a
ANOVA			
F ratio		15.817	
F probability		0.000	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