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Local wind monitoring matched with lichen *Pseudevernia furfuracea* (L.) Zopf transplantation technique to assess the environmental impact of a biomass power plant

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Abstract: The potential environmental impact of a biomass power plant (BPP) was evaluated by transplanting thalli of the lichen *Pseudevernia furfuracea* (L.) Zopf in the surrounding area for 3 months. Four meteorological stations were placed at four sites to monitor local winds. At the end of the period, thalli were retrieved and electrical conductivity percentage (EC%), lipid peroxidation, lichen vitality, photosynthetic efficiency, chlorophylls, xanthophylls+carotenoids, OD435/OD415, and concentrations of some elements frequently emitted by BPPs were measured. A univariate-multivariate analysis revealed clusters of sites with dissimilar mean values of ecophysiological parameters. They were merged, when not significantly differing, and classified as different homogeneous ecophysiological zones, one of which was considered an internal control for pigments, revealing a "complete recovery gradient" for the photobiont. The parameter "potential number of times the winds passing through the BPP zone reach each site" was calculated. It showed a significant negative correlation with spatial variation of pigment amounts. As regards the mycobiont, both EC% and lipid peroxidation resulted in the formation of two zones with different degrees of increase compared to the lichen origin area (lack of complete recovery). Most of the elements emitted by traffic and BPP correlated with EC% and thiobarbituric acid reactive substances, except Ti, which was negatively correlated with OD435/OD415.

Key words: Biomass power plant, local wind monitoring, multivariate-univariate techniques, internal control, pigment levels, mycobiont damage

1. Introduction

Electrical energy production is one of the human activities requiring the highest fuel consumption and it also results in very strong emissions of pollutants and greenhouse gases. In light of projections of global liquid gas and oil depletion (IEA, 2012), many countries have invested in alternative renewable energy sources. Biomass combustion is one of the most appealing due to its carbon neutral balance and its ability to follow the demand (Price, 2011). It is predicted that more than 3500 biomass power plants will be operational worldwide in 2020 (Siebertz, 2013). Nevertheless, there are many doubts about the environmental compatibility of this type of energy production, since the carbon balance is not neutral when it is evaluated using a life-cycle assessment. The low energy density of biomass makes it necessary to burn much more biomass than fossil fuels to produce the same electrical power, resulting in higher CO_2 emissions (Nordin, 1994). Moreover, depending on the burning conditions and raw materials, biomasses can generate high amounts of hazardous atmospheric pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), and organic and inorganic acids (UNI-EN, 2011). Many of these pollutants negatively affect plants. Phthalates hinder morphometric parameters, increase malondialdehyde (MDA) levels, and decrease pigment amounts in higher plants. PAHs impair photosynthetic efficiency in lichens, probably due to phosphorylation of the antenna complex and subsequent detachment from photosystem II (Kummerova et al., 2005), or they can depress plant root fresh weight after photoactivation (Ren et al., 1996). Manganese promotes chlorophyll degradation in epiphytic lichens (Hauck et al., 2003), nickel increases the susceptibility to atmospheric pollution of species like *Usnea amblyoclada* (Müll. Arg.) Zahlbr. and *Ramalina celastri* (Sprengel) Krog & Swinscow. (Rodriguez et al., 2007), arsenic affects both pigment levels and chlorophyll fluorescence in *Pyxine cocoes* (Sw.) Nyl. (Baipai et al., 2012), and chromium exposure of the lichen *Ramalina farinacea* (L.) Ach. reduces the chlorophyll a/chlorophyll b ratio and cell viability (Unal et al., 2010). In lichens, acids can promote conversion of chlorophyll to pheophytin by removing a magnesium atom and inhibition

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of both RuBisCo activity and the electron transport system (Chen et al., 1992).

Evaluation of temporal and spatial changes in the physiological status of lichens due to atmospheric pollution by means of the transplant technique is a very effective method for biomonitoring of anthropogenic impacts. However, many experimental designs have not carefully evaluated the dimension of the impact in a study area or effectively associated the impact with the main source(s) of pollution in the study area. These unsatisfactory results can be due to both the lack of an internal control to prevent the confounding effect of important local factors (e.g., mesoclimate) and the qualitative/poor use of winds in tracing the environmental diffusion of pollutants.

The aims of the present work were:

1) to perform wind monitoring: a) at the local scale to compare the data (means of measurements made at four sites within the study area) with those of the nearest meteorological station located outside (several tens of kilometers) the study area (regional monitoring network), and b) near the biomass power plant (BPP) to calculate the potential number of times winds passing through the zone where the power plant is located reach each exposure site of transplants (PNTWRS) located in the four geographical sectors;

2) to correlate the spatial variation of lichen ecophysiological parameters with the distance of each site from the BPP, PNTWRS, traffic rate, and concentrations of elements in thalli selected for their representation in BPP emissions;

3) to use a univariate-multivariate approach to detect clusters of sites differing in values of ecophysiological parameters, as well as an internal control;

4) to make comparisons between the central tendency values of the clusters revealed by the univariate-multivariate approach including the internal control, the whole study area (their general mean), and the lichen origin area (the external control);

5) to test for statistically significant differences in mean values of distance from the BPP and PNTWRS between clusters of sites significantly differing in mean values of ecophysiological parameters, i.e. (logically) considering distance from the BPP and PNTWRS as factors contributing to spatial zonation of the physiological status of lichens.

2. Materials and methods

2.1. Study area

The study area (Figure 1) extends over ca. 27 km^2. The main anthropogenic sources of atmospheric pollutants are a BPP with a nominal power of 13.3 MW (Actelios – gruppo Falck), which burns wood from manufacturing wastes and thinning of the Sila forests and expired olive pomace, and an extended road network including the A3 motorway, state highways SS19 and 19bis, provincial road 234, the small town of Settimo di Montalto, and the two districts of Quattromiglia and Commenda, including part of the town of Rende. The area is located in the middle-upper valley of the Crati River with the east and west edges having a mean altitude of 249 m a.s.l. and 235 m a.s.l., respectively. The middle belt, where 4/5 of the study area is located, has a mean altitude of 183 m a.s.l., with a SD of 30.65, a CV of 16.73%, and 2/3 of all values ranging from 145 to 195 m a.s.l. The annual values of climatic parameters are rainfall 1237 mm, mean temperature 16.4 °C, and mean relative humidity (RH) 65.1% (Arpacal, 2015).

2.2. Lichen transplant

Due to very poor lichen colonization of the study area, thalli of *Pseudevernia furfuracea* (L.) Zopf, including their phorophytes, were collected at La Fossiata (identified as lichen origin area = LOA) in the Sila National Park (Calabria Region; rainfall 1644 mm, mean temperature 10.1 °C, and mean RH 76.8%) and transplanted at 32 sites (Figure 1) in the study area. Since thalli could be removed or damaged as well as affected by wind-born soil particles they were exposed at a height of 3 m above the ground. The transplantation lasted 3 months (20 February 2013 to 20 May 2013).

2.3. Wind monitoring within the study area

Local wind monitoring was performed with four meteorological stations (Vantage Vue – Davis) at four of the 32 sites, one for each geographical sector of the study area.

Characteristics of wind blowing in the area where the BPP is located were estimated by placing one meteorological station as near as possible to the power station and, at the same time, without danger of vandalism (site 13). This station is distant ca. 1 km from BPP, with no relief place between them, and the difference in altitudes is extremely low (less than 25 m). This means that both of them experience winds very similar in provenience direction, frequency, and velocity (MEASNET, 2009). Wind parameters were recorded once every 15 min by a Weather Link data logger and were reversed on Excel calculation sheets once a month to perform further mathematical and statistical calculations. To avoid bias due to soil and building interference (topographic roughness) with recording activity the meteorological stations were set up according to the international standards provided by the AASC (1985) and EPA (1987).

2.4. Physiological parameters

2.4.1. Integrity of cell membranes

Integrity of plasma membranes was evaluated according to Marques et al. (2005).

Thalli were first rinsed in deionized water until stability of conductivity (Garty et al., 1993). They were subsequently

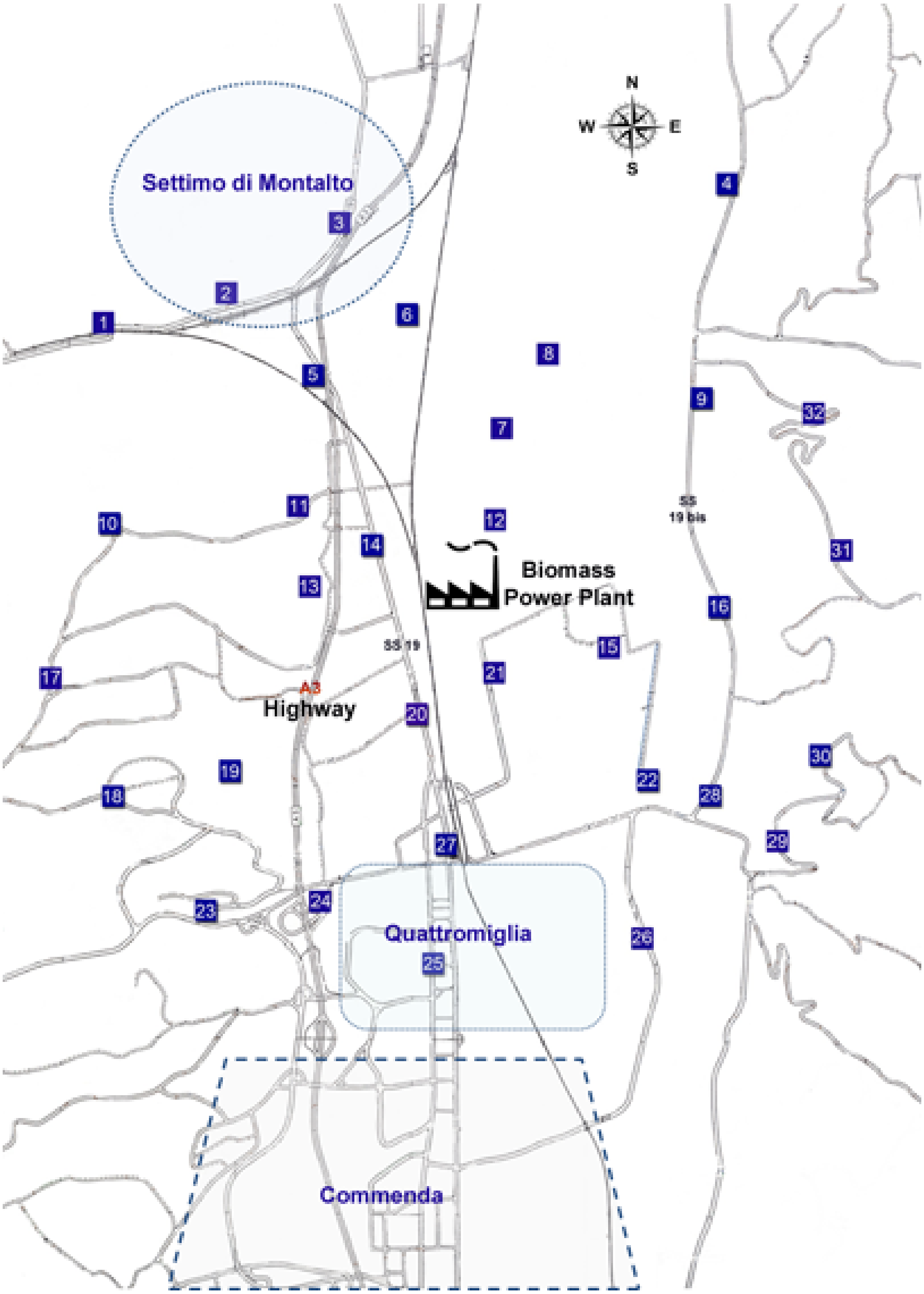

Figure 1. Spatial distribution of thalli exposure sites and location of the biomass power plant and road network within the study area. Wind monitoring stations were set up at sites 2, 13, 31, and 24.

shaken for 1 h in 50 mL of deionized water (whose conductivity was measured before and after shaking) and then boiled (10 min) to rupture. Conductivity was measured after cooling. The degree of membrane damage was estimated as the ratio between the conductivities measured before and after boiling and expressed as percentage (EC%) after subtracting contribution to conductivity due to deionized water.

2.4.2. Membrane lipid peroxidation

Lipid peroxidation was measured according to the experimental procedure of Huang et al. (2004). First, 50 mg of thalli was rinsed in distilled water and homogenized in 0.1% trichloroacetic acid, and subsequently a small amount (1.5 mL) of homogenate was centrifuged. An aliquot of supernatant (0.5 mL) was added to glass tubes and made to react with 0.6% thiobarbituric acid in 10% trichloroacetic acid at 95 °C for 30 min. After cooling solutions were centrifuged and the concentrations of thiobarbituric acid reactive substances (TBARS, µmol/g dry weight) were calculated by measuring absorbance (Lambda 4 spectrophotometer, PerkinElmer) of supernatant at 532 nm using the extinction coefficient of the thiobarbituric acid–MDA complex (155 mM^{-1} cm^{-1}).

2.4.3. Lichen viability

Thalli (15 mg) were incubated at 25 °C in the dark with 0.005% Triton X-100 phosphate buffer and 0.6% 2,3,5-triphenyltetrazolium chloride (TTC), a mitochondrial dehydrogenases substrate that is converted in triphenyl formazan (TPF) (Bačkor and Fahselt, 2005). After removing the surfactant two subsequent extractions were performed respectively with dimethyl sulphoxide and n-hexane, whose fraction was separated by centrifugation and measured at 492 nm (Lambda 4 spectrophotometer, PerkinElmer). Lichen viability was expressed as absorbance units at 492 (Lin et al., 2001), a value directly related to the amount of produced formazan.

2.4.4. Pigments and pheophytization quotient

Lichen thalli were previously rinsed several time with $CaCO_3$ saturated acetone to remove lichenic substances that could promote chlorophyll pheophytization. Lichen materials (60 mg) were extracted in dimethyl sulfoxide and polyvinylpolypyrrolidone under green light, at room temperature, for a total of 24 h. After centrifugation the absorbance of the supernatant was measured at 665, 649, and 480 nm (Lambda 4 spectrophotometer, PerkinElmer) and concentrations of pigments (chlorophyll a, chlorophyll b, and carotenoids) were calculated according to the Wellburn equations (1994). The chlorophyll conversion in pheophytin was expressed as OD435/OD415, where OD = optical densities, 435 and 415 = nm (Ronen and Galun, 1984).

2.4.5. Photosynthetic efficiency

Before performing the parameter measurement the thalli were hydrated and adapted at room temperature for 1 h. A lichen sample was first held in the dark for 10 min and then exposed to a saturating light flash (2400 µmol s^{-1} m^{-2}). Fluorescence measurements were made following both treatments and were indicated respectively as Fo (minimal fluorescence) and Fm (maximum fluorescence). The difference between Fm and Fo was indicated as Fv (variable fluorescence). The photosynthetic efficiency (an estimate of the amount of solar energy converted in chemical energy) was expressed as Fv/Fm (Jensen, 2002).

2.4.6. Element concentrations in transplanted thalli

Lichen thalli (100 mg) were first crushed in liquid nitrogen and then mineralized in a microwave oven after addition of 12 mL of ultrapure HNO_3. After digestion and dilution with distilled water the samples were analyzed by ICP-MS (Elan DRC PerkinElmer SCIEX) at the Laboratory of Mass Spectroscopy of the Department of Biology, Ecology, and Earth Sciences (University of Calabria) and expressed on a dry weight basis (µg/g dry weight).

The accuracy and repeatability of analytical determinations were checked by using lichen reference material (BCR-482). The investigated elements were Sn, Sb, V, Co, Ni, Mo, Cd, Al, Mn, Ti, Cr, Cu, Pb, Zn, and As, selected according to the EPA (2015).

2.4.7. Statistical analyses

To quantitatively relate the spatial variation of ecophysiological parameters measured in exposed thalli to the main local point source of pollution (the biomass power plant), we calculated the parameter "potential number of times the winds passing through the zone where the BPP is located reach the exposure sites" (PNTWRS) in the four geographical sectors during the experimental period.

For each geographical sector (NW, SW, SE, NE) we measured the distance of the exposure sites from the BPP, the frequency with which the wind passing through the zone where the BPP was located entered each geographical sector (having located the BPP in the middle of the study area, that is at the crossing of the lines delimiting the four geographical sectors), and the wind velocities.

First we calculated the time (minutes) in which the wind coming from the BPP zone reaches the sites in each geographical sector (TWRS) by dividing the distances from the BPP by the mean wind velocity in each geographical sector. Then we calculated the time the winds passing through the BPP zone blows in each geographical sector (TWBGS) by multiplying the frequency with which the winds entered each geographical sector by the total thalli exposure time expressed in minutes. PNTWRS was calculated by dividing TWBGS by TWRS.

A Bray–Curtis ordination method was applied to the sites × ecophysiological parameters dataset. This technique was used to search for a reference condition for the study area (McCune and Grace, 2002), i.e. a "local control". The Sorensen index was selected as the distance measure. The end points were chosen by means of the variance-regression method to avoid bias effects due to outliers. A multiresponse permutation procedure using the Sorensen index as the distance measure was performed on the results of the Bray–Curtis analysis to test the null hypothesis of no differences in ecophysiological parameters between clusters resulting from the ordination. The analyses were performed with PC-ORD 4 software. One-way analysis of variance with a post hoc multiple comparison (Tukey test) was performed to test the following null hypothesis: no differences between central tendency values of ecophysiological parameters measured in the lichen origin area and in each cluster resulting from the ordination analysis of the sites × ecophysiological parameters dataset. This approach made it possible to detect an "internal control", made by merging two clusters not significantly different from each other and from the LOA (lichen origin area). Student's t-test was performed to test the null hypothesis: no differences between central tendency values of ecophysiological parameters measured in the LOA and in the whole exposure area. A nonparametric correlation analysis (Spearman) was performed to test the following null hypotheses: a) no association between spatial variations of pairs of element concentrations, b) no association between variation of traffic rate and variation of ecophysiological parameters, c) no association between variation of distance of each station from BPP and variation of ecophysiological parameters, d) no association between variation of PNTWRS and variation of ecophysiological parameters, e) no association between variation of element concentrations and variation of ecophysiological parameters in exposed thalli.

Due to the occasional lack of linearity or semilinearity in covariance of environmental factors and biological responses, we also tested the possible association between the distance of each site from the BPP and the PNTWRS and ecophysiological parameters by means of one-way ANOVA, calculating their means in relation to the different "homogeneous ecophysiological" zones (factor of ANOVA) made of single clusters or by merging clusters (not significantly different) revealed by the univariate-multivariate analysis of the sites × ecophysiological parameters dataset. Normality and homoscedasticity conditions were tested and verified before performing central tendency values evaluation tests. Univariate analyses were performed with MINITAB-Release 13.2 software.

3. Results

Figures 2–6 and Table 1 respectively show the frequencies and velocities of wind detected at each of the four meteorological stations located in the study area and at the nearest station forming part of the regional monitoring network located 50 km away (Fitterizzi station). When the dominant winds are considered for the whole study area (mean of the four meteorological stations) the result (W = 64.57%) is very similar to that of the Fitterizzi station (W = 65.63%). However, when the frequencies recorded in the zone where the BPP is located are taken into account, the strongest component is from the SE (30.059%) so that the dominant wind blows from the S (63.21%). The highest wind velocities were measured at site 13. Moreover, the velocities measured at the Fitterizzi station are, on average, seven times higher than those measured at the meteorological stations located within the study area. Table 2 shows the central tendency values (with related standard deviation and coefficient of variation) of PNTWRS calculated for each of the four geographical sectors of the monitoring area. To quantitatively test the effect of the dominant wind (S = 63.21%) in the zone where the BPP is located on the potential diffusion of substances emitted by the power plant we performed a one-way ANOVA of the mean PNTWRS values with the factor "geographical sectors" (levels: NW, SW, SE, NE). The results are statistically significant (F = 10.56, P < 0.0005, Tukey test: NE > SE, SW and NW > SW) and support the hypothesis that the northern part of the study area is reached by the winds passing through the zone where the BPP plant is located more frequently than the southern part. Thus, it is probably more often affected by the substances emitted by the power plant.

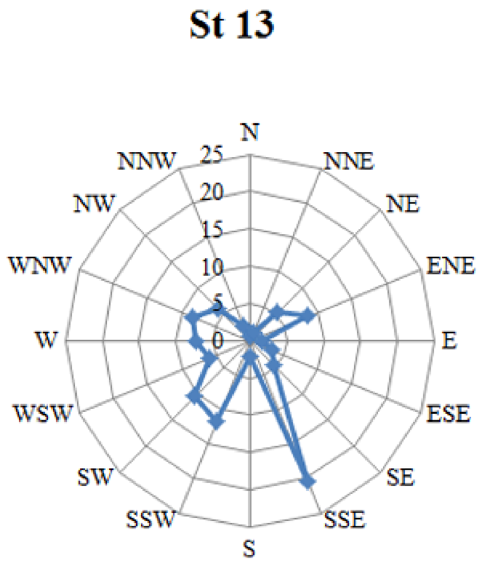

Figure 2. Wind rose showing the frequencies relative to 16 geographical sectors for station 13.

Table 3 shows the range of concentrations of the elements measured in the transplants at the end of the exposure period. Nickel, lead, and cadmium do not show any enrichment. The same applies to chromium, copper, zinc, and arsenic, although high or extremely high concentrations were detected in lichens at some sites. All the other elements show widespread enrichment in the study area, which is slight for vanadium, cobalt, tin, and antimony and moderate for aluminum, titanium, and manganese. Mycobiont parameters do not correlate

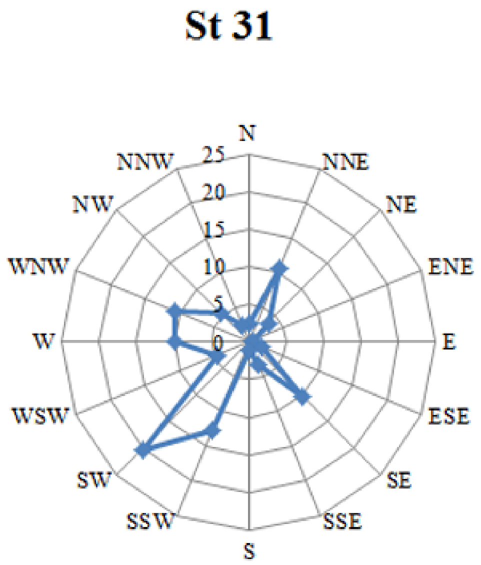
St 31

Figure 3. Wind rose showing the frequencies relative to 16 geographical sectors for station 31.

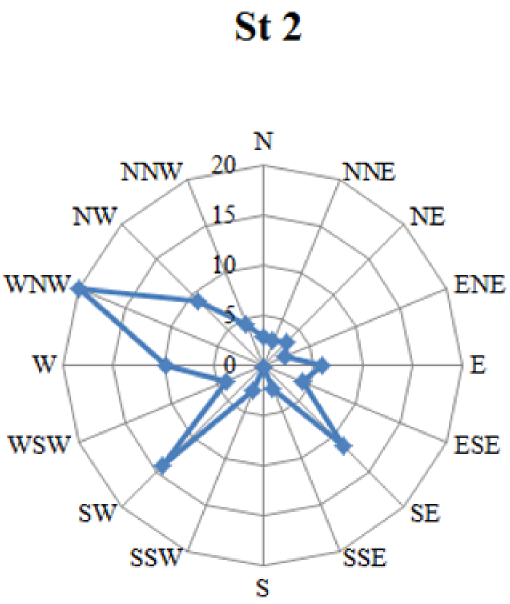
St 2

Figure 4. Wind rose showing the frequencies relative to 16 geographical sectors for station 2.

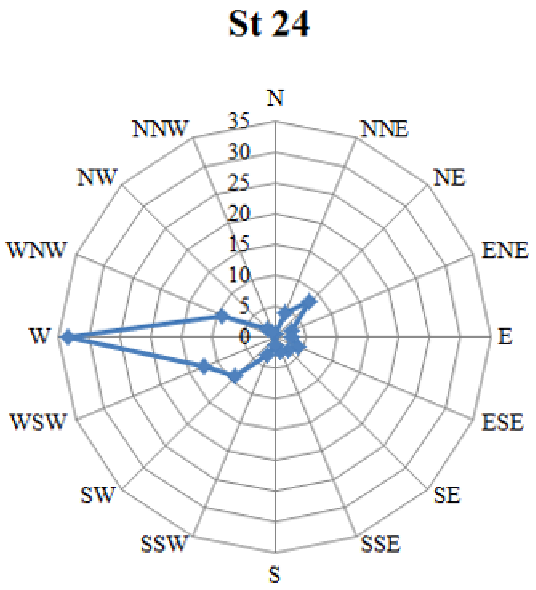
St 24

Figure 5. Wind rose showing the frequencies relative to 16 geographical sectors for station 24.

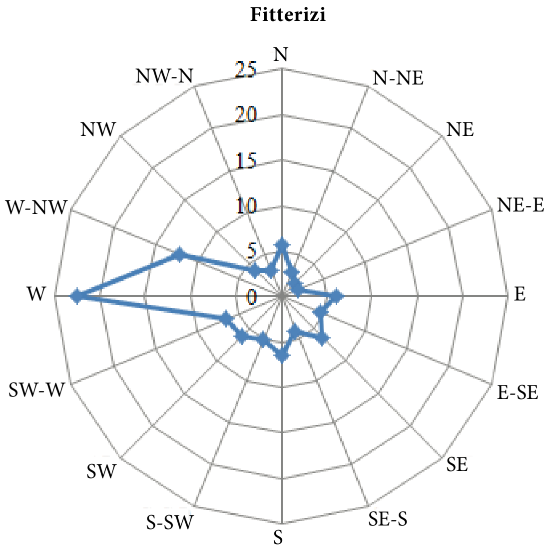
Fitterizi

Figure 6. Wind rose showing the frequencies relative to 16 geographical sectors for Fitterizzi station.

with PNTWRS, whereas three of the five photobiont parameters do (Table 4). On the other hand, only EC% and TBARS levels show a direct significant correlation with the spatial variation of bioaccumulated elements in exposed thalli (Table 5). Figure 7 shows the ordination diagram resulting from the Bray–Curtis analysis of the sites × ecophysiological parameters dataset, in which 90.61% of the variation is associated with axis 1, along which six clusters (designated A to F) are detectable.

Table 6 reports the ecophysiological parameters expressed as single values and related means of each cluster resulting from the multivariate analyses, the mean of the whole study area (all 32 sites), replicates, and mean of the lichen origin area. It is evident that the segregation of both the single values and the means of clusters along axis 1 is mainly due to an increase in the values of chlorophyll a, chlorophyll b, xanthophylls+carotenoids, and pheophytization coefficient from cluster E to cluster C. The results of the multiresponse permutation procedure ($T = -13.178$, $P = 0.00000000$, chance-corrected within-group agreement, $A = 0.72169000$) reject with an extremely high probability the null hypothesis of no differences

Table 1. Lower limit (LL), upper limit (UL), 1st quartile (1st Q), 3rd quartile (3rd Q), and median (Med) of wind velocities (m/s) at the four meteorological stations located within the study area and multiyear data series of the nearest meteorological station (locality Fitterizzi) of the regional monitoring network located outside the study area (50 km away).

	13	31	2	24	Fitterizzi
UL	5.318	3.902	3.890	3.718	44.4
3rd Q	3.368	2.180	2.207	2.426	11.2
Med	2.497	1.505	1.559	2.001	8.15
1st Q	1.783	1.126	1.103	1.549	6
LL	1.094	0.362	0.542	0.183	1.4

Table 2. Central tendency values (means), standard deviation, and coefficient of variation of the parameter PNTWRS calculated for each of the 4 geographical sectors of the study area.

Geographical sectors	NW	NE	SW	SE
Mean	2888	5155	1068	2120
Standard deviation	1176	4963	459	1188
Coefficient of variation (%)	40.70	96.27	42.97	56.03

Table 3. Lower limit (LL), upper limit (UL), 1st quartile (1st Q), 3rd quartile (3rd Q), and median (Med) of the element concentrations (µg/g dry weight) measured in transplanted thalli at the end of the exposure period.

	Sn	Sb	V	Co	Ni	Mo	Cd	Al	Mn	Ti	Cr	Cu	Pb	Zn	As
UL	1.25	1.23	5.67	0.73	2.93	0.55	0.28	2713	94	157	65	134	12	2707	111
3rd Q	0.72	0.40	4.05	0.62	2.61	0.31	0.22	1944	71	111	3.67	27	5.94	79	0.47
Med	0.48	0.26	3.60	0.56	2.44	0.24	0.18	1774	65	97	3.15	8.38	4.78	40	0.37
1st Q	0.31	0.17	3.35	0.51	2.18	0.19	0.15	1555	56	85	2.79	5.82	4.31	33	0.30
LL	0.23	0.09	2.62	0.41	1.68	0.16	0.11	1130	40	37	2.29	4.82	3.44	29	0.20

in ecophysiological parameters between clusters and suggests that the spatial variation of at least one factor, rather than a stochastic process, must promote cluster segregation. The mycobiont parameters do not show any significant differences between the clusters resulting from the multivariate analyses within the study area, while the mean values of TBARS of both the whole study area and clusters B, D, and E are significantly higher than that of the lichen origin area (Tables 7a and 7b). For the photobiont parameters, the whole study area means of photosynthetic efficiency and OD435/OD415 are respectively lower and higher than the means calculated for the lichen origin area (Table 7b). Several significant differences were found when the clusters resulting from the multivariate analyses were compared with the lichen origin area (LOA). The means of all ecophysiological parameters are always higher in cluster C than in clusters B, D, and E and sometimes higher than in the other clusters (cluster F: chlorophyll a; cluster A: chlorophylls a and b and xanthophylls+carotenoids; lichen origin area: xanthophylls+carotenoids and OD435/OD415). Clusters A and F never show significant differences from each other and always have significantly higher values than clusters D and E, which in turn never differ significantly from each other. Cluster B never differs from cluster A but does differ from cluster F for chlorophyll a and xanthophylls+carotenoids; moreover, it always has higher values of ecophysiological parameters than cluster E but not higher than those of cluster D. The LOA has higher values of chlorophyll a, chlorophyll b, and xanthophylls+carotenoids than clusters D and E, as well as higher values of chlorophyll a and xanthophylls+carotenoids than cluster B, whereas its

Table 4. Results of nonparametric (Spearman) correlation analysis of the spatial variation of PNTWRS and ecophysiological parameters measured in thalli of *P. furfuracea* at the end of the exposure period. Only significant correlations are shown. r = Spearman's correlation, P = probability level.

Ecophysiological parameters	r	P
Chlorophyll a	–0.361	0.042
Chlorophyll b	–0.417	0.018
OD435/OD415	–0.355	0.046

OD435/OD415 value is lower than those of clusters A, B, C, and F. Tables 7c and 7d show the results of the one-way ANOVA and t-test performed on the mean values of PNTWRS and distance from BPP respectively using the photobiont homogeneous physiological status zones and mycobiont homogeneous physiological status zones as factors. Only PNTWRS gives significant results.

4. Discussion

Meteorological data made available by regional environmental agencies are usually based on multiyear series recorded by single stations located in specific and relatively broad geographical zones. While their predictive value can be considered appropriate for the surrounding areas, the more the distance increases from the monitoring site the less effective is the extrapolation. When atmospheric pollution biomonitoring studies are carried out in a study area covering several tens of square kilometers, the use of wind directions and frequencies detected on such a large spatial scale can be misleading due to the high local diversification of these parameters produced by the interaction among moving air masses, terrain characteristics (topography, degree of woodland cover, urbanization), and local changes in temperature and pressure (Wagner and Mathur, 2013).

Table 5. Results of nonparametric (Spearman) correlation analysis of the spatial variation of element concentrations and ecophysiological parameters measured in the exposed thalli of *P. furfuracea* at the end of the exposure period. Only significant correlations are shown. r = Spearman's correlation, P = probability level.

Correlations	r	P
EC%-Sb	0.438	0.012
EC%-Co	0.467	0.007
EC%-Cu	0.475	0.006
TBARS-Mn	0.481	0.005
TBARS-Sn	0.390	0.027
TBARS-Sb	0.400	0.023
TBARS-Cr	0.589	<0.0005
TBARS-Co	0.413	0.019
TBARS-Ni	0.391	0.027
TBARS-Cu	0.653	<0.0005
TBARS-Mo	0.362	0.042
TBARS-Pb	0.567	0.001
TBARS-Zn	0.474	0.006
TBARS-As	0.544	0.001
OD435-OD415-Ti	–0.386	0.029

Our use of four meteorological stations within a 27 km^2 area (one station per 6.75 km^2) gave the following results: a) both the most frequent wind (W = 64.57%) and the others blowing from the other geographical sectors were comparable between the study area and the nearest station of the regional meteorological monitoring network located at Fitterizzi, about 50 km north of the study area (Arpacal, 2015); b) the station located near the biomass power plant (13) recorded the highest wind component from the SE, unlike the other stations (including the Fitterizzi

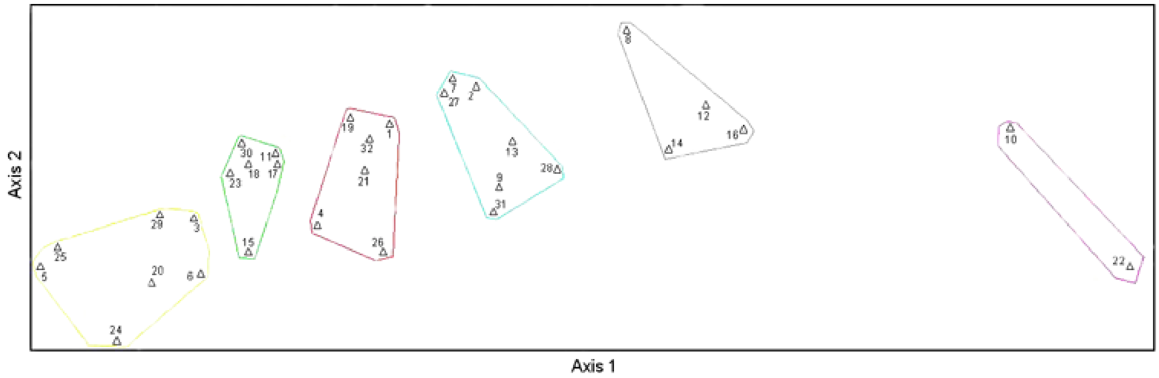

Figure 7. Ordination diagram resulting from the Bray–Curtis analysis of the sites × ecophysiological parameters dataset.

Table 6. Values of the ecophysiological parameters measured at each of the 32 sites and in the lichen origin area (LOA) and their means calculated for each of the six clusters revealed by multivariate analysis of the sites × ecophysiological parameters dataset, whole study area (WSA), and LOA.

Cluster	Sites	EC%	MDA	A492	Fv/Fm	Chl a	Chl b	Xan+Car	OD435/ OD415
A	1	28	25	0.98	0.627	848	244	417	1.26
	4	34	47	1.08	0.594	898	280	445	1.30
	19	20	12	1.27	0.683	845	257	437	1.25
	21	27	33	1.66	0.395	854	260	434	1.31
	26	27	59	0.93	0.498	852	260	451	1.32
	32	31	28	1.47	0.655	849	256	430	1.26
	Mean	27.8	34	1.23	0.575	858	259	436	1.28
	CV%	16.92	49.14	23.57	18.94	2.33	4.49	2.55	2.34
B	2	28	21.	1.20	0.563	780	235	397	1.25
	7	33	19	1.40	0.381	805	230	400	1.30
	9	36	64	0.99	0.421	779	246	405	1.24
	13	20	36	1.35	0.559	764	231	394	1.26
	27	18	19	1.31	0.392	811	241	389	1.29
	28	30	49	1.39	0.628	752	230	378	1.29
	31	23	72	1.30	0.590	780	251	406	1.24
	Mean	27	40	1.28	0.505	782	238	395	1.27
	CV%	27.63	51.98	11.81	22.09	2.92	3.84	2.70	2.11
C	3	17	28	0.90	0.663	999	281	470	1.28
	5	20	24	1.09	0.531	1150	309	522	1.36
	6	15	42	1.43	0.296	1012	278	474	1.27
	20	31	44	1.04	0.679	1048	304	492	1.32
	24	21	49	1.33	0.572	1070	322	518	1.33
	25	26	23	1.44	0.570	1123	330	501	1.33
	29	20	23	0.83	0.408	1015	301	476	1.33
	Mean	21	33	1.15	0.531	1060	304	493	1.32
	CV%	25.38	33.88	21.73	25.83	5.50	6.36	4.31	0.90
D	8	39	19	1.33	0.680	689	218	360	1.18
	12	20	36	0.83	0.717	649	199	356	1.20
	14	27	48	0.46	0.684	676	220	357	1.17
	16	48	52	1.21	0.223	641	206	342	1.16
	Mean	33	39	0.96	0.576	664	211	354	1.18
	CV%	37.16	38.24	41.12	40.97	3.39	4.73	2.26	0.994
E	10	31	37	1.24	0.448	520	166	274	1.13
	22	23	59	1.16	0.574	458	158	263	1.13
	Mean	27	48	1.19	0.511	489	162	268	1.13
	CV%	20.96	32.5	4.71	17.43	8.95	3.49	2.89	0

Table 6. (Continued).

F	11	23	16	1.45	0.655	923	263	449	1.29
	15	26	42	1.37	0.589	972	277	459	1.31
	17	20	22	0.69	0.655	933	270	432	1.33
	18	19	19	1.24	0.697	949	275	444	1.33
	23	28	22	0.89	0.185	940	284	474	1.29
	30	13	6.3	1.38	0.666	920	280	452	1.23
	Mean	21	21	1.17	0.575	939	275	452	1.30
	CV%	28.16	57.50	27.73	38.03	2.06	1.89	3.49	3.19
	Mean WSA	26	34	1.18	0.546	853	255	422	1.27
	CV%	28.71	47.22	22.39	25.64	19.25	15.89	14.72	4.92
LOA	LOA1	13	6.8	1.13	0.642	1255	357	528	1.18
	LOA2	17	7.66	1.39	0.651	854	239	383	1.17
	LOA3	33	7.18	1.02	0.612	925	268	413	1.20
	LOA4	18	5.26	0.97	0.643	789	238	385	1.17
	LOA5	18	8.21	0.98	0.712	899	265	424	1.17
	Mean	20	7.02	1.10	0.652	944	273	427	1.18
	CV%	38.68	15.92	15.94	5.62	19.17	17.84	13.92	1.10

station), where the wind from the south was dominant (63.21%); c) wind speeds (strongest blasts) were quite modest in the study area (classifiable as breezes according to the Beaufort scale), with the highest values measured at the station close to the biomass power plant, while wind velocities (strongest blasts) measured 50 km north of the study area were much stronger (3- to 4-fold). This suggests that atmospheric emissions from the power plant are not greatly dispersed by wind (a conclusion drawn from the Fitterizzi station data), which promotes their diffusion mainly through the northern sector of the study area. Our interpretation is that the urban area of Rende, located south of the BPP (dominant wind SW = 42.19%, station 24) behaves as a "heat island" (Arnfield, 2003), both attracting and redirecting winds to the north due to the south-north orientation of the town. This confirms the inadequacy of using wind data measured at a single station located outside of and far from the study area to support interpretations of spatial variation of contamination/physiological stress data. When the wind parameters are used in a quantitative way, they both support their descriptive use and are effective in associating the spatial ecophysiological changes of the lichen transplants with the biomass power plant. The parameter PNTWRS clearly indicates that the monitoring sites located in the northern sector of the study area are much more frequently (2.7 times more) reached by winds passing through the biomass power plant area than sites located in the southern sector, consistent with the dominant wind direction. Variations of the ecophysiological parameters within the exposure area did not correlate with the distance of the monitoring sites from the biomass power plant. Such a correlation has not often been detected in biomonitoring activities and we think that the reachability of each exposure site by contaminants emitted by local point sources is a function not only of distance but also of the frequency and velocities of winds (that carry the contaminants) and their interaction with characteristics of the terrain/mesoclimate. The parameter that we calculated, taking into consideration wind frequency and velocity as well as distance, showed better covariance with the spatial ecophysiological variations of the transplants; in fact, chlorophyll a, chlorophyll b, and OD435/OD415 all showed a significant negative correlation with PNTWRS (Table 4). The values of the coefficients were low-moderate (Fowler et al., 1998). However, due to the lack of variables such as terrain, topography and "interactions between airflows and mesoclimate" in our parameter, all of which play an important role in affecting local wind behavior (Yang et al., 2014), such a correlation can be considered satisfactory. To our knowledge, no previous work has used such a parameter in relation to the ecophysiological status of lichen transplants to associate it indirectly to the atmospheric emissions from an anthropogenic point source of pollutants.

Table 7. Results of (a) one-way ANOVA of ecophysiological parameters with the factor "Groups" (including the lichen origin area - LOA - external control), i.e. those revealed by multivariate analysis of the sites × ecophysiological parameters dataset; (b) Student's t-test performed to compare the means of the ecophysiological parameters of the whole study area (WSA) and the LOA. SSC = Statistically significant comparisons – Tukey test. (c) One-way ANOVA with "Homogeneous Pigment Amounts Zones" as random factor to test the null hypothesis of no differences in PNTWRS and distance of each station from the BPP, (d) Student's t-test with Mycobiont Impaired Status Zone (MISZ) and Mycobiont Transition Status Zone (MTSZ) as levels of the factor Mycobiont Physiological Status Zones to test the null hypothesis of no differences in PNTWRS and distance of each station from the BPP.

(a) One-way ANOVA			
Parameter	F	P	SSC
EC%	2.27	0.063	---
Fv/Fm	0.64	0.694	---
TBARS	3.73	0.007	B, D, E > LOA
A492	0.80	0.576	---
Chl a	25.52	<0.0005	A > D, E B > E C > A, B, D, E, F F > B, D, E LOA > B, D, E
Chl b	17.61	<0.0005	A > D, E B > E C > A, B, D, E F > D, E LOA > D, E
Xan + Car	29.33	<0.0005	A > D, E B > D, E C > A, B, D, E, LOA F > B, D, E LOA > B, D, E
OD435/OD415	30.03	<0.0005	A > D, E, LOA B > D, E, LOA C > D, E, B, LOA F > D, E, LOA
(b) Student's t			
Parameter	t	P	Comparisons
EC%	1.54	0.168	=
TBARS	9.31	<0.0005	WSA > LOA
A492	0.87	0.415	=
Fv/Fm	−3.52	0.002	WSA < LOA
Chl a	−1.06	0.337	=
Chl b	−0.79	0.472	=
Xan + Car	−0.17	0.874	=
OD435/OD415	6.98	<0.0005	LOA < WSA
(c) One-way ANOVA			
Parameter	F	P	
PNTWRS	2.98	0.049	
Distance of each site from BPP	2.04	0.132	
(d) Student's t			
Parameter	t	P	Comparisons
PNTWRS	2.30	0.030	MISZ > MTZ
Distance of each site from BPP	−2.04	0.053	---

In a previous study (Lucadamo et al., 2016) we showed that, among the elements displaying varying degrees of enrichment in exposed thalli, titanium and cobalt concentrations significantly correlated with PNTWRS while the pairs aluminum-titanium and vanadium-cobalt covaried in a statistically significant manner (Spearman rank correlations and cluster analysis). All of them are part of both the woody materials burned in the Actelios power plant and the fumes usually emitted from BPPs (CEN/TC, 2003). On the other hand, arsenic, chromium, and copper were associated with the BPP fumes (Lucadamo et al., 2016) due to their widespread use as wood preservatives (as copper chromium arsenate) in the chemically treated woods that BPPs burn (D.M. 5 February 1998; D.M. n. 186, 5 April 2006), while antimony, tin, and molybdenum showed a significant correlation with traffic rate, consistent with their environmental source from car mechanical wear. Our data clearly indicated that the spatial variation of many of the elements (except titanium) correlated with the spatial variation of the physiological stress of the mycobiont (EC% and TBARS), as often reported previously (Sujetoviene, 2014). On the whole, this result suggests that the mycobiont, due to its disproportionate representation in the lichen biomass with respect to the alga, is affected by most of the local anthropogenic sources of atmospheric pollution. Despite their low levels in exposed thalli, nickel and lead showed a significant correlation with TBARS levels. This finding is difficult to explain, although we suspect that it may be due, at least partially, to the covariance of the concentrations of these elements with those of strong promoters of cell membrane damage (Cr-Ni: $r = 0.501$, $P = 0.003$, Cr-Pb: $r = 0.695$, $P < 0.0005$, Cu-Ni: $r = 0.420$, $P = 0.017$, Cu-Pb: $r = 0.496$, $P = 0.004$). Titanium is the only element whose variation showed a significant negative correlation with the variation of a parameter of the photobiont (OD435/OD415). This element can be strongly bioaccumulated by lichens in the surroundings of industrial and urban areas (Nash and Gries, 1995). Some studies have shown that titanium has a beneficial effect on plants (Carvajal and Alcaraz, 1998), whereas others have reported negative effects such as iron and magnesium deficiencies (Bedrosian and Hanna, 1966) and depressed growth and chlorosis (Hara et al., 1976). Moreover, algal exposure to TiO_2 results in chlorophyll degradation due to its photocatalytic generation of oxidative species (Peller et al., 2007). Despite this result, the negative correlations detected between photobiont parameters (chlorophyll a, chlorophyll b, OD435/OD415) and winds passing through the BPP area suggest that at least some of the substances emitted by the power plant negatively affect the algal partner. Organic compounds are among the main components of BPP exhausts (Booth, 2014) and some of them are reported to be strongly accumulated in lichens (Muir et al., 1993) and to reduce biomass and pigment levels and promote chlorophyll degradation in plants (Zhang et al., 2015). Hence, they can be considered one of the possible candidates for the observed negative effects on the photobiont.

Comparison of the mean values of chlorophyll a, chlorophyll b, and xanthophylls+carotenoids between the LOA and the whole study area did not reveal any significant differences, while the combined multivariate-univariate analysis showed that there were four zones with different degrees of physiological status of the photobiont (chlorophyll a, chlorophyll b, xanthophylls+carotenoids, OD435/OD415) in the study area: a Photobiont Healthy Status Zone, consisting of clusters F and A (PHSZ), which did not differ from the LOA (except for OD435/OD415); a Photobiont Impaired Status Zone, made up of clusters D and E (PISZ), which were always significantly lower than A, F, and the LOA and 2/3 of whose sites were located north of the BPP (consistent with the results of the ANOVA of the PNTWRS mean values calculated for each geographical sector); a Photobiont Transition Zone (PTZ), consisting of cluster B (sometimes differing in pigment levels from the clusters of the Healthy and Impaired Zones, see Table 7); and a Photobiont Eutrophicated Zone (PEZ), made up of cluster C. This last cluster had the highest values of photobiont parameters, which were significantly different from those of clusters D and E (for all parameters), cluster F (chlorophyll a), cluster A (chlorophyll a, chlorophyll b, xanthophylls+carotenoids), and the LOA (xanthophylls+carotenoids and OD435/OD415). We consider the PHSZ to be an "internal control" for chlorophyll a, chlorophyll b, and xanthophylls+carotenoids since the two clusters that make it up show minimal differences in these parameters (6% on average), never differing significantly from each other or from the LOA. We have already successfully used an internal control to more accurately detect differences in photobiont parameters within a study area between clusters of sites showing different mean values of algal partner parameters (Lucadamo et al. 2015). A local control, i.e. internal to the study area, is much more suitable than the LOA (or an "external" cluster of sites with comparable environmental characteristics to the study area) to accurately detect the effects of local anthropogenic pollutant sources because of the absence of an important confounding factor like mesoclimate, given the significant effect of temperature and humidity on lichen ecophysiological status (Pirintsos et al., 2011). The PNTWRS spatial variation not only covaried with the spatial variation of chlorophyll a, chlorophyll b, and OD435/OD415 but was also associated with the formation of the "homogeneous pigment amounts" (HPA) zones. The latter were used in one-way ANOVA as "random" factor to test its effect on the variation of the mean values of PNTWRS and distance from the BPP. Indeed, once the differences between the HPA zones are not considered the result of a stochastic process these zones (PHSZ, PIZ, PTZ, and PEZ) can be viewed as a random sample of different levels of the factor HPA zones. The ANOVA gave a significant result only for PNTWRS (F = 2.98, P < 0.05) and not for distance from the BPP (F = 2.04, P > 0.05). This is probably due to the not very complex local topography, with reliefs located only in the extreme western and eastern parts of the study area, which consists mostly of relatively flat territory with modest slopes and escarpments. This suggests that, because of the paramount importance of winds in carrying pollutants through a study area, the distance of each site from an anthropogenic point source of atmospheric pollutants cannot always effectively associate environmental pollution with its main local source.

Given the inverse association between pigment levels and the frequency with which the winds passing through the power plant zone reach each site, the increase in pigment levels with respect to the internal control (PEZ) cannot be associated only with the lowest amounts/disappearance of substances emitted from the power plant (lowest value of PNTWRS).

We suspect that vehicular traffic can contribute to the enhancement of these parameters. Despite the lack of a significant correlation between the traffic rate and the photobiont parameters, five of the six sites in cluster C showed the highest traffic rates (3 = 1401 cars/h, 5 = 1375 cars/h, 20 = 1422 cars/h, 24 = 1594 cars/h, 25 = 1090 cars/h) and these levels can be classified as "intermediate" when compared to other Italian urban conditions (Crisafulli et al., 2010). Both reduced and oxidized forms of nitrogen are important components of car exhausts (Audi, 2001). In light of the above-mentioned traffic rates we can hypothesize that associated nitrogen emissions are not very strong, so that the local total nitrogen levels result in a fertilization phenomenon that increases pigment concentrations (Sujetoviene, 2015). The lack of a significant correlation between spatial variation of traffic rate and spatial variation of photobiont parameters is probably due to the overriding effect of the BPP contamination in the whole study area, so that the effect of car emissions can be appreciated only when they reach the highest levels within the study area. However, such an effect may contribute (in addition to the above-mentioned topography and mesoclimate variation) to a weakening of the correlation between PNTWRS and pigment levels.

Unlike chlorophyll a, chlorophyll b, and xanthophylls+carotenoids, the mean Fv/Fm value of LOA differed significantly (P < 0.01) from that of the whole study area. Nevertheless, there were no significant differences among the clusters nor between them and the LOA. Clusters A, F, and D had mean Fv/Fm values typical of healthy lichens, except for two sites (Scheidegger and Schroeter, 1994), while about 74% of the sites in clusters B, C, and E had values lower than the mean values of LOA, with differences ranging from about 4% to 71%. However, these values were quite evenly spread within clusters B, C, and E, resulting in mean values not significantly different from each other and from the remaining clusters. This suggests that the spatial impairment of pigment concentrations differs from that of photosynthetic efficiency both in locations within the study area and in statistical significance, probably due to different contaminants or different interactions of contaminants affecting the two types of parameters resulting in a relatively modest effect on the chlorophyll fluorescence.

Interestingly, the OD435/OD415 value of the lichen origin area was unexpectedly low, lower than both the mean value of the whole study area and those of clusters A, F, B, and C but not different from the values of clusters D and E. We obtained a rather similar result in a previous study (Lucadamo et al., 2015): the pheophytization quotient of thalli from the origin area was comparable with that of transplants, showing a reduction of pigment levels (with lichens from the same LOA as in the present study). Due to the nature of the geological substrates of the Sila plateau (siliceous-acidic rocks), *P. furfuracea* (L.) Zopf thalli showed in both cases a slight enrichment in aluminum (above 1000 µg/g dry weight), an element that when hydroponically given to inbred lines of maize resulted in an activation of chlorophyllase (Mihailovic, 2008). This enzyme promotes the first step of chlorophyll a degradation by converting it into chlorophyllide a, while in the second step chlorophyllide a changes into pheophorbide a via magnesium-dechelatase activity (Tang et al., 2000). Pheophorbide a and pheophytin a have virtually the same absorption spectrum because the phytol chain, in which they differ, contributes minimally to the absorption process with respect to the chlorine component (Milenkovic et al., 2012). We hypothesize that the very modest (but statistically significant) decrease in the pheophytization quotient in the *P. furfuracea* (L.) Zopf thalli from the LOA may be due to the conversion of chlorophyll a into pheophorbide a as a consequence of the slight enrichment in aluminum.

When the mycobiont physiological parameters are taken into account, only the TBARS levels differed significantly between the LOA and both the whole study area and clusters B, D, and E (i.e. the same clusters that showed a different degree of impairment of the photobiont parameters). This means that: a) a local control for mycobiont parameters was not detected; b) there is a less distinct separation, in the study area, between the Mycobiont Impaired Status Zone (MISZ) (clusters B, D, E) and the remaining area; in fact, its clusters (A, F, C) did not differ from the clusters of the MISZ (or from each other) or from the LOA, so that it can be considered a Mycobiont Transition Status Zone (MTSZ) only in relation to the LOA. This suggests that the photobiont and mycobiont are affected by a different spatial development of physiological impairment, with the former showing a full-recovery zone (PHSZ or local control) and the latter a more widespread physiological stress due to the lack of a full-recovery zone. We reached a similar conclusion when we monitored the environmental impact of a cement plant (Lucadamo et al., 2015): the detection of a clear recovery gradient of photobiont parameters and an impairment of the mycobiont physiological status affecting the whole study area.

Although there was a significant correlation between some elements and EC%, the number of significant correlations with TBARS was much higher, suggesting that lipid peroxidation is the mechanism most frequently affected by the investigated elements in the mycobiont. Hence, only TBARS levels were effective in forming distinct zones of mycobiont physiological status. Moreover, the elements that covaried with peroxidation levels were associated with both the BPP and traffic rate, suggesting that both sources contribute to mycobiont impairment. This is probably the reason why covariance between distance from the BPP as well as PNTWRS and TBARS is lacking and why the t-test of significant differences between MISZ and MTSZ in both distance from the BPP and PNTWRS gave a positive result for PNTWRS ($t = 2.39$, $P = 0.030$) even though the TBARS levels of MISZ and MTSZ were comparable ($P > 0.05$).

On the whole, our data suggest that the fungus was exposed to all the atmospheric pollutants (due to its prevailing contribution to lichen biomass), so that the lack of an effect of some of them was probably substituted by the effect of the others, generating a stress continuum in the study area. In contrast, due to its inner location and very small contribution to lichen biomass, the photobiont was affected only by pollutants emitted (and subsequently dispersed by winds throughout the study area) in high concentrations by anthropogenic point sources and/or by those able to reach the inner part of the lichen (i.e. gases or liposoluble substances).

The results of the present work strongly support the idea that an effective improvement in impacts biomonitoring by lichen transplantation technique can be carried out by a strictly quantitative evaluation of local scale wind patterns and a careful interpretation of the different environmental response performances of lichen symbionts.

The analysis of local winds showed that the use of data from the nearest meteorological station located outside the study area (regional monitoring network) was inadequate to estimate both the real velocities of the winds blowing throughout the investigated area and the velocity, frequency, and direction of winds blowing in the zone where the BPP is located. Measurement of these last parameters, together with the transplant exposure time and distance of each site from the BPP, made it possible to calculate the parameter PNTWRS. Despite the confounding effect of topography, mesoclimate, and other anthropogenic pollution sources, this parameter was effective in indirectly associating the BPP emissions with spatial variation of pigment levels of the photobiont of the *Pseudevernia furfuracea* transplants. Notwithstanding the lack of significant differences between the mean chlorophyll a, chlorophyll b, and xanthophylls+carotenoids values of the whole study area and the LOA, a gradient of spatial change in pigment levels (chlorophyll a, chlorophyll b, xanthophylls+carotenoids, and OD435/OD415) was detected by means of a multivariate-univariate approach, with the formation of four distinct zones of the photobiont: a healthy status zone (internal control), an impaired status zone, a transition zone between the healthy one and impaired one, and a eutrophicated zone. This zonation was associated with different mean values of PNTWRS, although the eutrophicated zone must also be associated with a factor positively affecting the photobiont, probably the level of traffic. The efficacy of PNTWRS in relating the spatial change in pigment levels to the BPP was probably due to the relatively low complexity of the topography and terrain of the study area, parameters that could be included (together with mesoclimate characteristics) in the PNTWRS calculation to improve its performance. A decrease in photosynthetic efficiency was found only for the whole study area when compared to the LOA, supporting the development of another spatial impairment pattern of the photobiont probably due to different sources of contaminants affecting pigment concentrations and chlorophyll fluorescence or different interactions between contaminants. The mycobiont showed a more diffuse alteration of its physiological status than the photobiont. No unimpaired zone was detected, with two zones showing a different degree of impairment with respect to the LOA. Metals that were associated with the BPP and traffic correlated positively with membrane damage (EC%) and lipid peroxidation (TBARS), supporting the hypothesis that the mycobiont, because of its much higher representation in the lichen biomass than the algal partner, can be simultaneously affected by the main anthropogenic sources of pollutants. Consequently, it may be less suitable than the photobiont for accurate detection of the effect of a single important pollution source (especially when pollutant data are missing) due to the difficulty in finding a complete recovery zone (internal control) within the study area for the mycobiont parameters.

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