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# Monitoring Epiphytic Lichen Biodiversity to Detect Environmental Quality and Air Pollution: the Case Study of Roccamonfina Park (Campania Region - Italy)

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## 1. Introduction

Biomonitoring of air pollution, i.e. monitoring environmental pollution through the use of living organisms (Nimis & Skert, 1999), may be based either on the tendency of some organism to accumulate pollutants in their tissues (bioaccumulation) or on the changes that occur in the composition of animal and plant communities after exposure to pollutants (bioindication). Compared to instrumental monitoring, the use of biomonitors allows to measure as a whole the global effect that abiotic and biotic factors exert on biota, what is not possible by just analyzing the concentrations of single selected pollutants in the environment.

Biomonitoring provides useful information about the global conditions affecting the environment over a given area. Of course it should not be considered as a substitute of instrumental monitoring, but rather, a necessary complement of it. It may also be suited to screen areas subjected to any risk of contamination, so helping to plan landscape policies and to set land nets of air quality (ANPA, 2001). Biomonitoring of air pollutants can be passive or active. Passive methods observe organisms growing naturally within the area of interest. Active methods detect the presence of air pollutants by placing test organisms of known response and genotype into the study area (Szczepaniak & Biziuk, 2003).

Biomonitoring may be obtained by using organisms either as bioindicators or as bioaccumulators. Bioindicators are defined as organisms that allow to identify human-generated environmental pollutants and to determine their level on a scale of qualitative determination (Conti & Cecchetti, 2001). A good bioindicator should present:

- high sensitivity to environmental pollutants;
- low mobility in space;
- long living cycle;
- wide distribution over the studied area;
- high genetic evenness.

Bioaccumulators are defined as organisms that reflect the chemical content of atmosphere and can so be used for the quantitative determination of contaminants (Conti & Cecchetti, 2001). A good bioaccumulator should present:

- high tolerance to environmental pollutants;

- wide distribution over the studied area;
- low mobility in space;
- long living cycle;
- high capability to accumulate pollutants.

Due to their morphological and physiological characters, lichens are doubtless among the most suited organisms available for the studies on atmospheric quality, either as bioindicators or as bioaccumulators. Lichens in effect are long-lived, slow-growing organisms, that show a good constancy of morphology over time and do not shed parts during growth. In addition, since these no-rooted organisms are not provided with protective structures in their tissues, such as cuticle or stomata, they absorb passively during their entire life cycle any element present in the atmosphere, either by rain or by particulate deposition (Costa et al., 2002). Moreover, the lichen surface, structure, and roughness facilitate the interception and retention of particles (Sloof & Wolterbeek, 1993). Thus, metal absorption by lichens depends not only on intercellular absorption (exchange process and/or intercellular accumulation) but also on entrapment of particles that contain metals.

Lichens are particularly sensitive to environmental stresses, especially with regard to pollution, eutrophication and climate change (ANPA, 2001), since the metabolism of these organisms is directly dependent on gas exchange. These organisms respond rapidly to atmospheric changes, particularly if determined by anthropogenic factors. Thus, biodiversity of epiphytic lichens may be kept as a good indicator of air pollution (Nimis et al., 1989; Piervittori, 1999; Giordani et al., 2002; Loppi et al., 2002).

## 1.1 The Lichens

### 1.1.1 What are lichens?

More than two thousands years ago, Theophrastus, the father of botany, coined the term "lichen" to denote the product of the actions of some unknown organism on tree barks (Ozenda & Clauzade, 1970). Up to nineteenth century, lichens were considered as individually recognizable organisms (Ozenda & Clauzade, 1970). Lichens were recognized to be composed by two different organisms in 1869 (Schwender, 1869), but it was not clear what was the kind of association between the two biological partners. Today, the common view is that they represent one of the most interesting case of symbiosis, since they are constituted by the association of a fungus, called *mycobiont* (Ascomycetes, rarely Basidiomycetes), and an unicellular alga (Chlorophyceae or Cyanobacteriae), called *photobiont*. Yet the question of whether it should be considered as a symbiosis or an extremely evolved parasitism is controversial (Ahmadjian, 1993).

The coexistence between the two partners attains a very high degree of morphological constancy and physiological equilibrium, so that the product of this association, the so called "lichenic *thallus*" (Fig. 1), may assume the rank of an unitary organism. This is also due to its capability to produce metabolites that neither fungus nor alga would be able to produce when living alone. This kind of association is commonly treated as a symbiosis. The benefit to the fungus is in that it obtains organic matter produced by algae through photosynthesis; whereas the algal partner, thanks to the protection offered by fungal mycelium, acquires the ability to survive in dry substrates where normally, it could not live alone (Nimis & Skert, 1999). However, the Lichens, do not reproduce sexually as a whole organism: the fungus maintains its gamic reproduction, whereas the alga just propagates by scission and loses its ability to produce zoospores (Hawksworth, 1988; Ahmadjian 1993). Thus, some author consider quite problematic to define these associations as a valid

taxonomic category, but rather tend to consider them as a kind of “lichenized fungi” (Tehler, 1996). In fact, the typical asexual strategy of lichens is that of *fragmentation*: in the most simple cases, a single fragment may grow into a new lichen; as well as lichens may produce specialized microscopic particles composed of algal cells enveloped by fungal *ifae* (*soredia*) that produce a new individual. In other cases, small peaces of thallus including the photosynthetic partner (*isidia*) may reproduce the whole organism. Lichen fragments, soredia and isidia can be transported at great distances by wind and water.

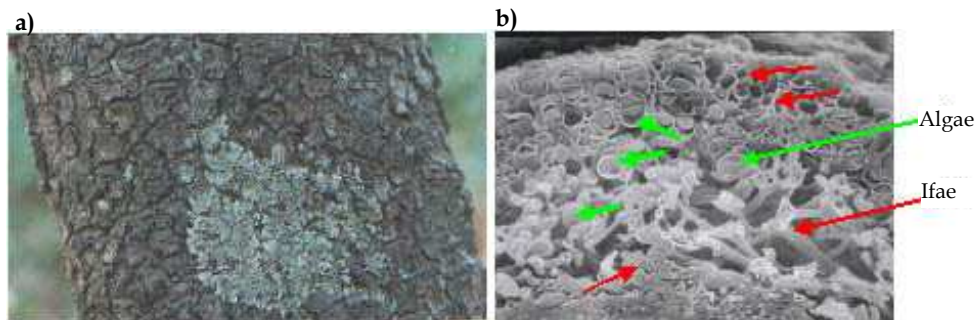


Fig. 1. Lichen thallus and its section.

a) Thallus *in situ* of *Physcia biziana* (A.Massal.) Zahlbr. var. *leptophylla* Vezda. b) Cross section SEM (1700X) of the thallus

### 1.1.2 Lichen character

Anatomically, fungal *ifae* constitute the most conspicuous part of a thallus. In the most simple early-evolved ones, the so called *homeomerous* (Fig. 2a) lichens, fungal ifae and algal cells are just assembled in a homogeneous and undifferentiated interlacement. A more complex structure is found in *heteromerous* thalli (Fig. 2b), the most widespread lichens, where different layers are recognizable: an *upper cortex*, a *medulla* layer and a *lower cortex*, constituted by fungal pseudo-tissues; and a photobiont layer housed between the upper cortex and the medulla.

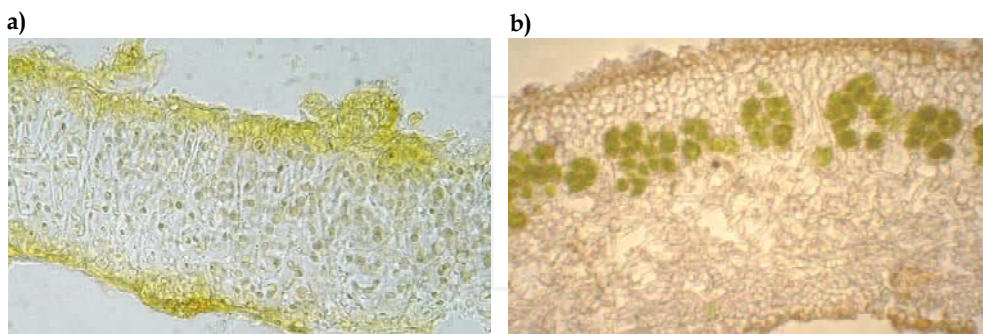


Fig. 2. Thallus structure.

TEM sections of lichen thalli. a) homeomerous; b) heteromerous

Most of the lichens belong to one of the three main morphological categories: the *crustose* type (Fig. 3a), strictly adhering to their substratum and not provided of a lower cortex; *foliose* type (Fig. 3b), with a kind of leaf appearance, provided with both upper and lower cortex,

more or less attached to its substrate; and *fruticose* type (Fig. 3c), generally branched with variable shapes (sections from circular to flat) and structure (pendulous strands or hollow upright stalks).

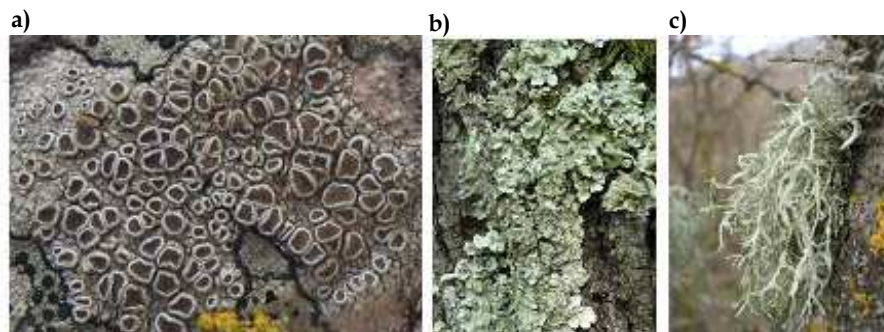


Fig. 3. Main lichen morphological types.

Lichen growth types. a) crustose (*Lecanora chlorotera* Nyl.); b) foliose (*Flavoparmelia caperata* (L.) Hale); c) fruticose (*Ramalina farinacea* (L.) Ach.)

Depending on their particular morpho-physiological attributes, lichens may colonize the most variable substrates, such as soil (*terrícolas* types Fig. 4a), rocks (*saxícolas* types Fig. 4b), tree barks (*epífitas* types Fig. 4c). The vast majority of lichens are adapted to tolerate a wide range of changing environments; but there are also some species that are strictly confined to particular habitats. Homeomerous lichens are generally less tolerant to changing environments, and are the first to disappear if even few variables are subject to change. Heteromerous, on the contrary, and particularly crustose lichens, tend to show a wider adaptation and are found in a greater range of different environments, going from Antarctic to equatorial deserts.

The ability of lichens to tolerate such hard environmental conditions is probably due to their capacity to switch quickly from an active to a latent living state, through the rapid dehydration of the thallus. For this reason, lichens are considered to be *poikilohydric* organisms, that is they may survive to extreme low levels of water content (Nash, 1996).

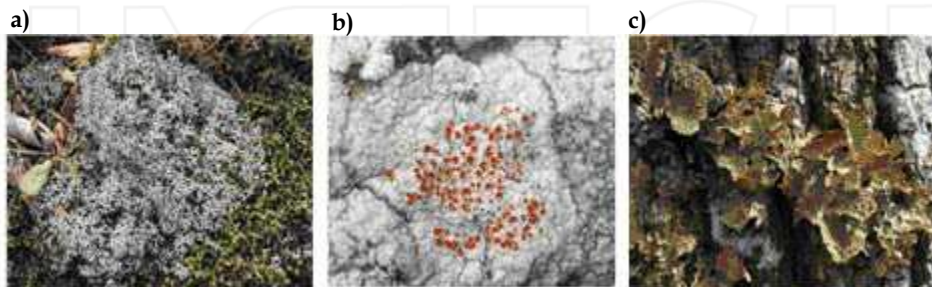


Fig. 4. Main lichen substrates.

Lichens growing on their natural substrates. a) soil (*Cladonia rangiformis* Hoffm., *terrícolas* types); b) rocks (*Caloplaca erythrocarpa* (Pers.) Zwackh, *saxícolas* types); c) tree barks (*Lobaria pulmonaria* (L.) Hoffm., *epífitas* types)

## 1.2 Techniques of biomonitoring

Several techniques were proposed to detect air quality by sampling the communities of epiphytic lichens (Barkman, 1963; De Sloover & Leblanc, 1968; Hawksworth & Rose, 1970, Ferry et al., 1973, Nash & Wirth, 1988; Richardson, 1992; Cislighi & Nimis, 1997; Purvis, 2000, Van Dobben et al., 2001). A project aimed to develop an objective and reproducible model of bioindication, suited to different air pollutants, was launched during the 80s in Switzerland (Liebendöerfer et al., 1988; Herzig et al., 1987; Herzig & Uregh, 1991), giving rise to the Index of Air Purity (IAP), adopted with just small differences by several countries. In Italy, an index of lichen biodiversity (IBL) was proposed by Nimis (ANPA, 2001) as a way to provide an indirect evaluation of air quality. According to this method, the frequency of occurrence of epiphytic lichen species within a sampling grid provides information on the long-term effects of air pollutants, eutrophication and anthropogenic factors on sensitive organism (Asta et al., 2002).

## 1.3 Aims of the study

In this study, lichen distribution was examined in a district of Campania region to monitor the evolution of air quality at landscape scale. A new methodological approach was tried by overlapping the results of biomonitoring samplings to land cover maps, in order to highlight the relations between air pollution and land use patterns (Pinho et al., 2004; Paoli et al., 2006; Pinho et al., 2008).

The main objectives of this work were the following: i) to evaluate air quality on the studied area with the aid of IBL index, and reporting geo-referenced data on a thematic map; ii) to relate lichen distribution and biodiversity to land use spatial patterns; iii) to put the basis for a comparative analysis focussed on changes induced by the present socio-economic evolution of the plain, from agriculture to industrial and tertiary; iv) to provide a reproducible protocol for monitoring air quality, identifying clusters of lichen species linked to particular land use models and formulating previsions about environmental quality on areas characterized by similar dynamics.

## 2. Materials and methods

### 2.1 Study area

This study was conducted in the large area (84 km<sup>2</sup>) of Roccamonfina volcano (Campania Region, South Italy, Long. 13°58'18"; Lat. 41°17'43") (Fig.5). This area was interested by active volcanism from 630,000 to 50,000 years ago. This complex is a kind of *stratovolcano* that was subjected to the collapse of the crater area, generating a caldera. Its volcanic apparatus is somehow similar to that of Vesuvius, particularly as for constitution, insulation and morphology (De Rita & Giordano, 1996). Moreover, the contiguous area of the regional park are, at present, experiencing a rapid process of industrial reconversion.

Annual rains over the area range from 916 to 1046 mm (Fig.6), with a predominant autumn-winter distribution and a dry summer period ranging from 1 to 3 months. Mean temperatures get their maximum value during the months of July-August and their minimum in January. According to the phytoclimatic classification of Italy (Nimis & Martellos, 2008), the area belongs to the humid sub-Mediterranean belt.

The survey area can be divided in two main districts: (a) Roccamonfina's regional park characterized by woodland of *Castanea sativa* Mill., with small scattered villages; (b) the

southern part of the study area, with small urban agglomerations, industrial and commercial zones localized on the lower part of the volcanic hill and on the plain.

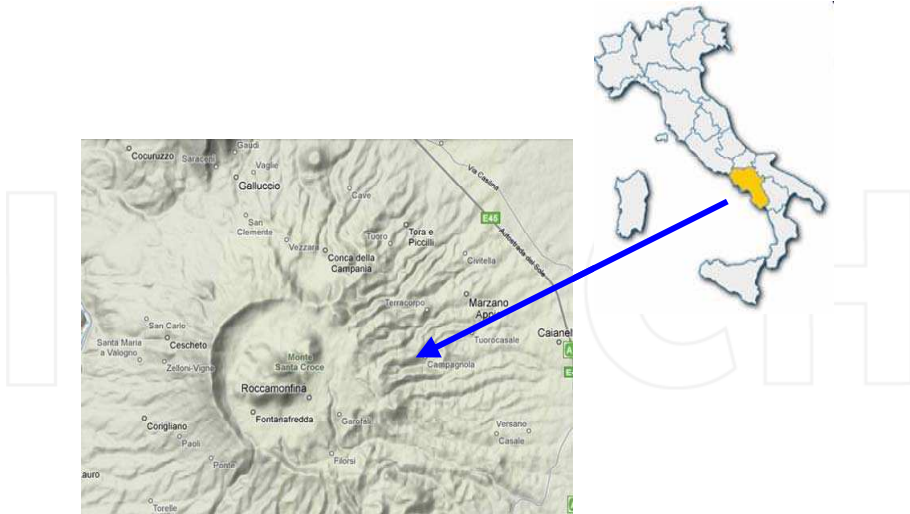


Fig. 5. Location of the study area.

The study was conducted in a volcanic area of Campania region (Italy)

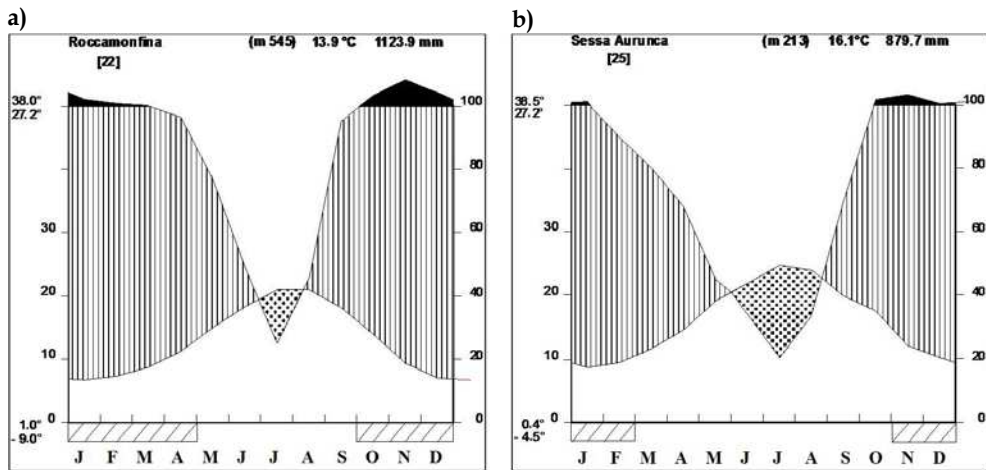


Fig. 6. Thermopluviometric diagrams.

Two stations differing for the relative importance of the dry summer period are represented in the figure. a) Roccamonfina; b) Sessa Aurunca. Data are averages of about 50 years (1951-99)

This area was selected since it includes both regions characterized by a high index of naturalness and districts more disturbed by anthropic influence, with a gradient of environmental pollution decreasing from urban settlements to the agro-forestry areas.

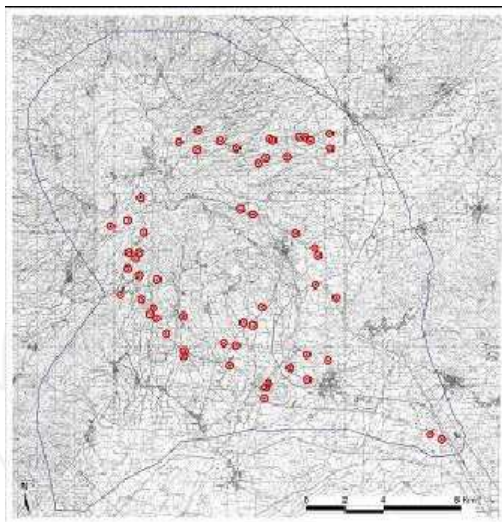


Fig. 7. Topographic map of the study area. The red circles represent the location of the 56 sampling stations

## 2.2 Lichen sampling

Location of sampling stations and trees follows the guidelines by ANPA (2001). Fifty-six random sampling stations (1 km<sup>2</sup> each) were selected (Fig. 7), each divided in 4 square areas of 250 x 250 m. Relevés of epiphytic lichen vegetation were carried out on chestnut (*Castanea sativa* Miller.) and oak trees (*Quercus pubescens* Willd.). The selected trees were provided with similar bark properties (subacid, mesotrophic to oligotrophic barks with similar water storing capacity). The trees had to satisfy the following requirements (Nimis, 1999b; Asta et al., 2002): (a) free-standing well-lit tree; (b) inclination of the trunk not exceeding 10°; (c) circumference larger than 60 cm; (d) absence of evident factors of disturbance or pathologies. Damaged or decorticated parts of the trunk, parts with knots, parts corresponding to seepage tracks after rain, parts with more than 25% cover of bryophytes, were excluded from the samplings.

The sampling grid consisted of four vertical ladders of 10x50 cm, each divided in five 10x10 cm unit areas; each of the four ladders was applied to one of the four cardinal points, with the base at 100 cm from the ground (Fig. 8). To exclude from sampling any unsuited part of the trunk, a shift from verticality up to 20° clockwise was allowed when positioning individual ladders (Castello & Skert, 2005).

In each station, about 3-12 trees were sampled, for a total of 89 chestnut and 119 oak trees. The species represented in each unit of the grids were listed, and a frequency value ranging from 0 to 5 was obtained for each species in each ladder, depending on the number of unit in which the species was found. This values were summed for each of the four cardinal points and averaged for the number of trees in the station, obtaining four indices of lichen biodiversity each referred to one of the four cardinal points. The sum of these four indices was kept as the lichen biodiversity indicator for the station (Castello & Skert, 2005).

Thalli were collected during field sampling and brought to laboratory for any dubious identification. Taxonomic determination was done referring to Nimis (1987; 1992; 1993a;



1993b), Ozenda & Clauzade (1970), Clauzade & Roux (1985) and Poelt (1969.). We also took in account lichen floras of the nearby areas of Sannio e Daunia, Vesuvius, Matese and Partenio mounts (Garofalo et al. 1998-1999; Aprile et al. 2001; 2002-2003a; 2002-2003b) . Nomenclature follows Nimis & Martellos (2008). Ecological indices, expressing the level of biological tolerance of the species regarding environmental features, were calculated referring to the criteria of ITALIC system (<http://dibiobs.univ.trieste.it>). The ecological attributes included response to pH, light levels, water availability, geographical distribution and evenness. In addition, we determined two indices related to the degree of human impact on the atmosphere, the indices of *eutrophycation* and *poleophoby*. The former is related to the frequency of lichens tolerating (or escaping) nitrogen compounds dispersed as dust in the atmosphere. The second is related to the frequency at which lichens tolerating (or escaping) urban environment are found, so accounting for the general degree of human disturbance over an area. Finally, indices related to lichen morphology, photobiont association and reproductive type were calculated.



Fig. 8. Example of the sampling grid on a chestnut tree. The sampling grids were positioned on each tree at 1 m from ground level in the four cardinal directions. See text for details

### 2.3 Map analysis

The surveys were geo-referenced using GPS stations (GPS MAP 60 CS) in three-dimensional mode with the static method and calculation of the average position. Thus, in addition to the floristic and ecological data, all lichen species positions were also stored in a Data Base Management System and processed with a pattern analysis to obtain information about their arrangement in space. Subsequently, the continuous grid “Air Quality map”, was obtained in Ilwis 3.3/3.5 environment, by data point interpolation, constrained by barriers, using the Moving Average Algorithm with the weight function “Inverse Distance” (Fig. 9):

$$W = \frac{1}{d^n} - 1 \quad (1)$$

where  $W$  is the weight,  $n$  is the weight exponential and  $d$  represents the relative distance of point to output pixel, given by:

$$d = \frac{D}{D_0} \quad (2)$$

in which  $D$  = Euclidean distance of point to output pixel and  $D_0$  = limiting distance.

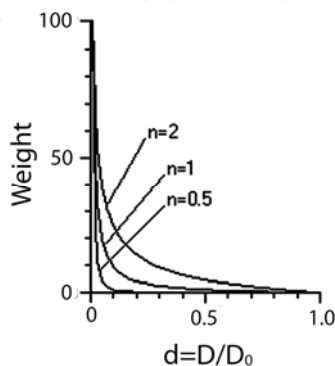


Fig. 9. Curves of the Inverse Distance Weight (IDW).

The selected function for the interpolation is quite sensible to sudden changes of the point data value, across the space. So, for any unsampled location, it is possible to predict a value based on the assumption that things that are close to one another are more alike than those that are farther apart. Using a barrier during the interpolation operation, the estimated cell value is calculated inside the space limited by the barrier

The air quality map was then overlapped to a land-use map, elaborated from Corine 2000 Land Cover map and field data. Dem, slope and aspect maps from morphological data were used in the cross-tabulation raster operations to produce a multi-thematic geo-database.

#### 2.4 Statistical analysis

Lichen data were analyzed with Principal Component Analysis (PCA) and with cluster analysis with group average method (UPGMA), based on continuous values of the frequency of individual species and using the Euclidean distance. Two dendrograms were obtained that identified the main clusters of lichen species and locations. The clusters of species were used for identifying lichen indicator assemblages of species, whereas the clusters of locations were interfaced in GIS with land use patterns. Once obtained the main clusters of the stations, for each group the average values assumed by the main indicator indices were calculated and the corresponding graphic spectra were produced.

The results of cluster analysis were also overlapped with Corine Land Cover data, in order to define the features of land use in the main groups of station. An index of "naturalness" was calculated for each cluster as the ratio of the total surface falling into class level 3 (natural vegetation) against the sum of classes 1 and 2 (settlements and agricultural areas). Pearson correlation analysis was then applied to detect any common trend among the index

of naturalness obtained by land use analysis, the IBL index obtained by lichen species frequencies and all the other indices obtained by lichen indicator attributes such as eutrophication, poleophoby, etc.

### 3. Results

#### 3.1 Lichen flora

A total of 48 epiphytic lichen species (Fig. 10) were identified on the 208 trees examined in the study area, 59 % of which were foliose, 31 % crustose and 10 % fruticose. Most of the lichens found were typical of the vegetation unit of *Xanthorion parietinae* and *Parmelion caperatae*, characterized by the presence of relatively common species and so not expressing a high biodiversity value. No endangered species were found over the area.

The ecological indicators of this lichen flora were mainly falling in the middle of the ranges for most of the selected criteria of bioindication. Species richness, on the contrary, was found to change considerably among the locations, providing a relatively higher indicator value.

Most of the species were typical of humid-sub humid Mediterranean climate, though a considerable number of species resulted to be relatively unusual for this climatic belt. Among these, *Caloplaca herbidella*, *Anaptychia ciliaris*, *Ochrolechia balcanica*. All these species are usually found in cooler environments, such as temperate and boreal arctic areas.

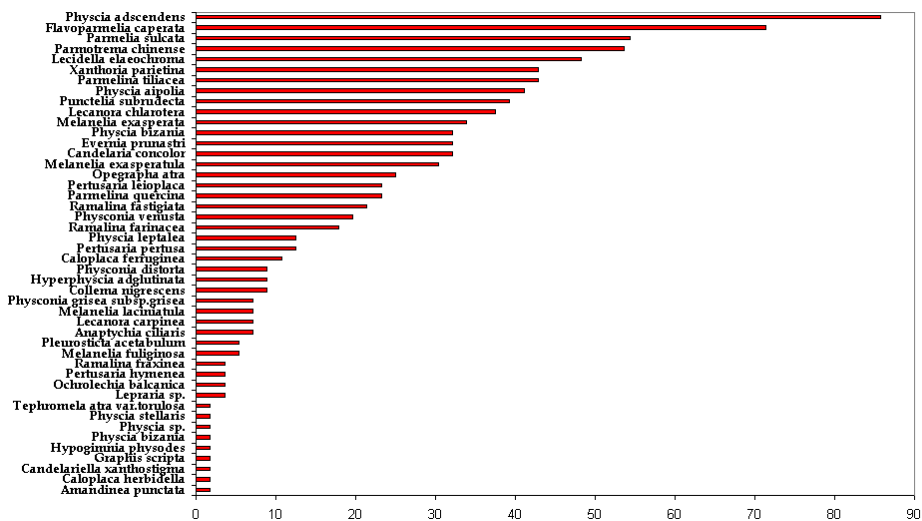


Fig. 10. List of the lichen species found on the study area.

The red bars represent % frequencies of the lichen species

Concerning poleophoby, most of the species resulted to be distributed in natural- semi natural habitats, or also in slightly disturbed environments. Concerning eutrophication, the species indicated wide ranges of adaptation, though indicating in the average values not very high levels of contamination.

The index of biodiversity value (IBL) ranged between 3 and 103 on the studied station. Lower IBL values were found in the proximities of the main roads and urban settlements, whereas the higher values were found in woodland and natural areas.

### 3.2 GIS analysis

A quantitative analysis of Corine land use classes revealed that deciduous forests and agromosaics occupy most of the study area (Fig. 11). Broadleaved forests are characterized mainly by chestnut groves that dominate on the slopes of the volcano. Oak woods are found close to the plain areas either as forest stands or along the borders of agricultural fields. Thus, this landscape appears highly fragmented, with land properties separated by vast forest corridors, which represent an important factor of ecological continuity. The area occupied by settlements is mainly distributed at the lower altitudes, with a scattered distribution, but ecologically relevant due to the dense net of road and highways that interconnect them.

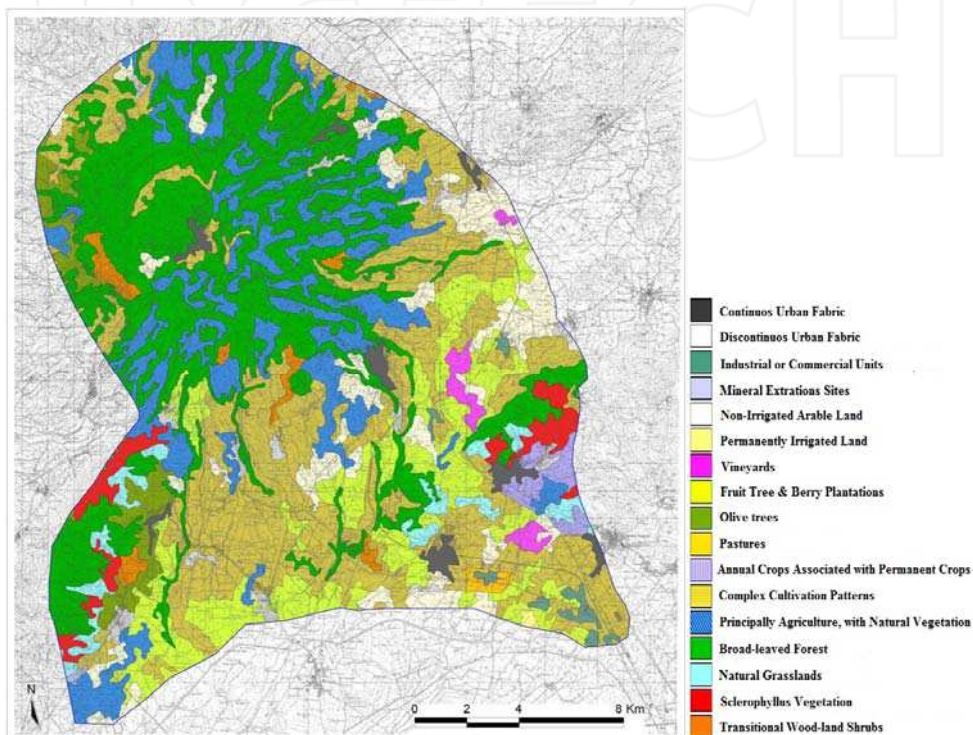


Fig. 11. Corine land cover map

Using point interpolation tools of ILWIS 3.5, all the floristic data, based on the values assumed by IBL index over the sampled area, were spatialized in GIS environment and after a "slicing" operation, a map of air quality was produced (Fig. 12). Six classes of IBL were identified by quantitative analysis. Following, the map obtained was overlapped with the DEM of the area, the slope map and the Land Cover map. Low IBL values were found in the plains near the highway exits, where vehicular transit is quite intense and cars and camions are often subjected to stop for variable time. Low-intermediate values of IBL were generally found at low altitude in all the areas subjected to industrialization, whereas higher IBL values were found in all the areas covered by vegetation, and particularly at higher altitudes, where deciduous forests substitute the agricultural fields.

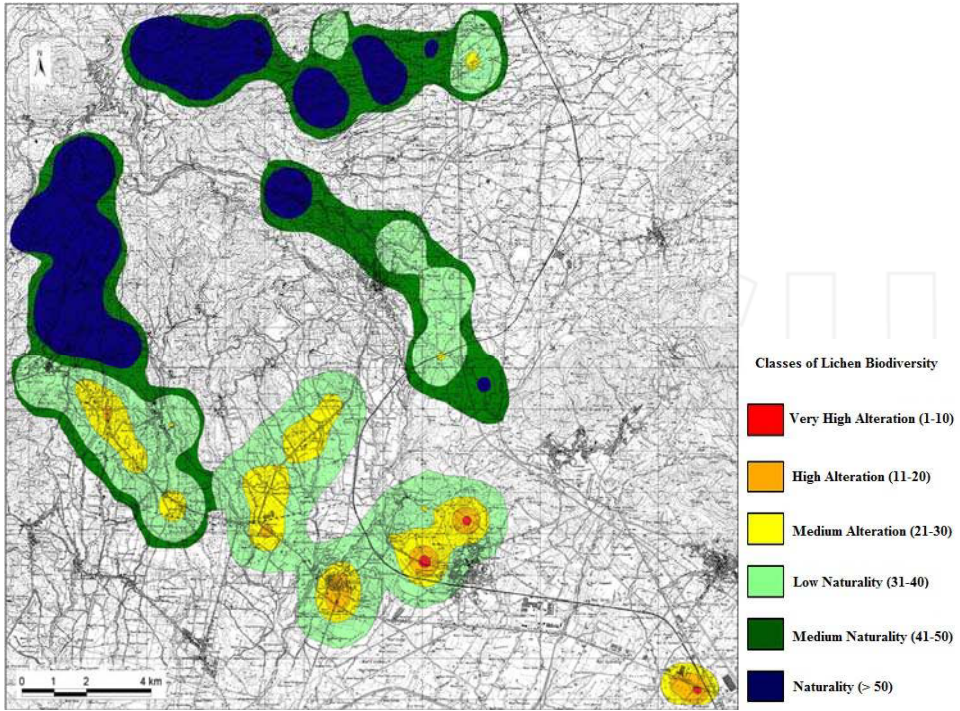


Fig. 12. Map of air quality

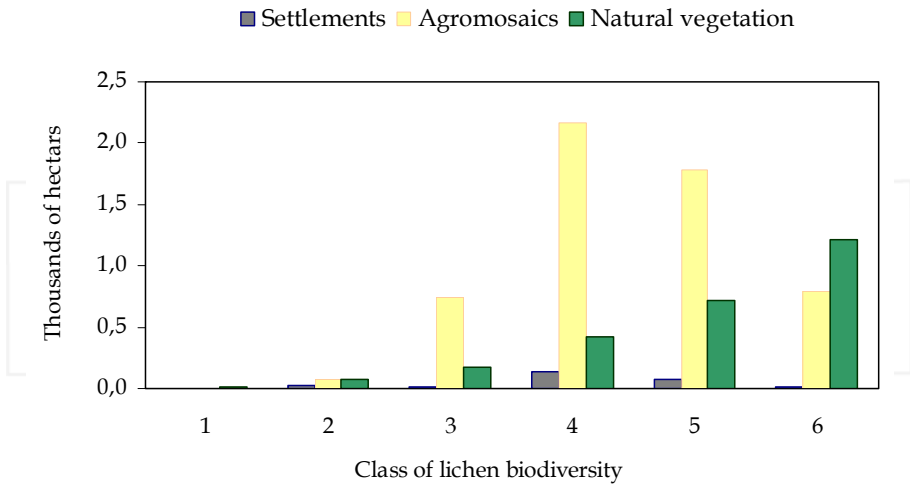


Fig. 13. Overlay of IBL belts with the map of land use cover.  
 1: Very high alteration; 2: High alteration; 3: Medium alteration; 4: Low naturality;  
 5: Medium naturality; 6: Naturality

The overlay between the six IBL belts and the map of land use confirms that maximum lichen biodiversity values are found in environments characterized by natural vegetation and agromosaics (Fig. 13).

### 3.3 Classification of the stations

Cluster analysis applied to sampling station revealed the presence of 4 main groups (Fig. 14), characterized by an increasing level of lichen biodiversity index. Cluster A included 28 stations mainly distributed in the most anthropized environment, mainly agricultural areas quite close to urban settlements and highways, at an average altitude of 130 m s.l.m.; IBL value in this cluster was 20.9, whereas the index of naturalness assumed the value of 0.22. Cluster B was composed by 6 stations distributed in environments quite similar to the previous ones, at a mean altitude of 206 m s.l.m., with IBL value of 44.3 and naturalness index of 0.17. Cluster C included 10 stations distributed in more natural environments, at a mean altitude of 395 m s.l.m., with IBL value of 61.4 and index of naturalness of 3.71. Cluster D included 12 stations in natural and agricultural areas, at a mean altitude of 411 m s.l.m., with IBL value of 76.3 and naturalness index of 1.94.

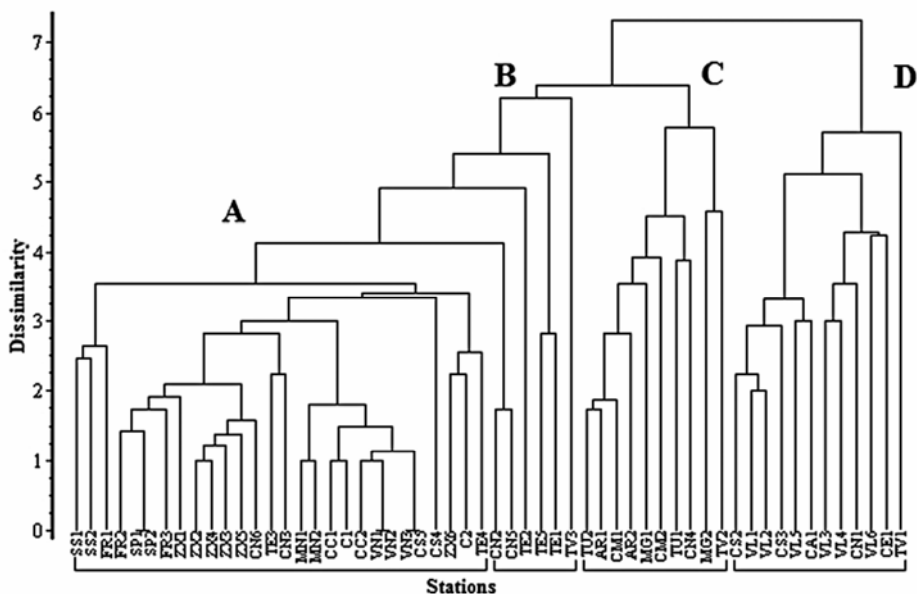


Fig. 14. Dendrogram of the sampling stations. Four clusters (A, B, C, D) resulting by multivariate analysis applied to the sampling stations. See text for details

### 3.4 Correlation analysis and ecological indices

The index of lichen biodiversity was directly correlated with altitude ( $R=0.96$ ,  $P<0.05$ ) and with the frequency of *common* species ( $R=0.97$ ,  $P<0.05$ ). It was also directly correlated with *crustose* ( $R=0.96$ ,  $P<0.05$ ) and *fruticose* ( $R=0.97$ ,  $P<0.05$ ) species, and inversely correlated with *foliose* ones ( $R=-0.97$ ,  $P<0.05$ ). The index of naturalness was inversely correlated with the

frequency of the species adapted to highly eutrophized environments ( $R=-0.98$ ,  $P<0.05$ ), and directly correlated with the presence of *extremely rare* species ( $R=0.96$ ,  $P<0.05$ ). The correlation between the IBL and the index of naturalness was relatively high ( $R=0.68$ ) but not significant.

Among the ecological indicators, the index of eutrophication and the index of poleophoby showed consistent trends with naturalness indicators expressed by land use patterns. Cluster C, the one provided with the highest naturalness value, presented the highest frequency of species relatively unadapted to eutrophication and to urban environment, and the lowest value of the species characterized by the opposite adaptation.

#### 4. Discussion

A good consistency was found between the values assumed by IBL index across space and the environmental quality presumed by the analysis of the Corine land cover maps. IBL resulted to be higher in natural and semi-natural areas, whereas it was very low around urban areas and settlements. Low values of IBL were also found in areas relatively close to natural environments but subjected during recent times to rapid changes toward industrialization. It was particularly interesting the data relative to agricultural areas. IBL assumed intermediate values in these areas, detecting a relatively lower value of air quality. In fact, air quality in agricultural areas is expected to decrease if compared to natural environments, due to the extensive use of chemicals for plant defence, particularly fungicides. It is noteworthy that agriculture in this area usually takes place in close interconnection with natural environments. Much of the areas classified as agromosaics belonged to the subcategories of complex systems, that often are bordered or intermixed with natural woods. So, this study also proved that IBL index is a sufficiently sensible tool to detect environmental patterns, even when these patterns are not strongly evident if analyzed at a large scale detail. In many cases, the patterns of IBL values fitted with the field-based land use pattern recognition in a considerably higher extent if compared with Corine land cover analysis. Thus, a bioindicator based recognition of environmental quality was proved to guaranty even higher definition and predictivity than common landscape geographic approach.

On a scale of higher detail, however, in some cases the analysis based on just the IBL value did not result to be the best suited to detect the global quality of environment. The trends of IBL index across the four main groups identified by cluster analysis, did not assume very high and significant correlation values with the selected indicator of naturalness. IBL resulted to be highly correlated with the massive presence of common lichen species, whereas it was not strongly correlated with presence of rare species. These last species, however, were strongly correlated with naturalness. The highest values of IBL were not found in cluster C, the one with highest naturalness value, but in cluster D, where chestnuts and mixed woods were strongly intermixed with agricultural lands. Moreover, the species negatively correlated to eutrophication and adaptation to urban environment were found in a higher proportion in cluster C compared to cluster D. We may hypothesize that in cluster D, the impact of mechanical interventions linked to agriculture and also to the mechanization of chestnut harvesting, could have negatively affected the most sensitive elements of lichen flora, but nevertheless the IBL was high because the most common lichen species grew in a higher number due to the best availability of growth substrates and for the best climate given the highest altitude. Thus, the quantitative approach of IBL analysis

should always be integrated with the study of the qualitative indicators expressed by the lichen flora in order to get a fine tuning of the previsions about air quality.

In conclusion, measuring lichen biodiversity through the IBL index allowed us to characterize the state of environment on the studied area. This method put the basis for a wider study on the dynamic evolution of a district interested by a process of industrial reconversion very close to a natural reserve. A good responsiveness of statistical clustering towards geo-statistical features of lands was found, confirming the reliability of IBL index as an indicator of environmental quality. The assemblages of lichen species were found to reflect not only environmental quality on a local scale, but also the more complex set of variables related to land use patterns at landscape level.

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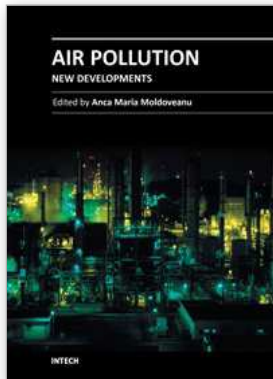
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