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Mapping Lichen Diversity as a First Step for Air Quality Assessment

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Abstract. The evaluation of air quality is an important topic. It is well known that lichens have a set of characteristics that make them well suited for biomonitoring purposes. Sampling lichen diversity is not as expensive as chemical analysis, allowing a dense sampling grid and reducing the costs. Lichen diversity can be used to identify more disturbed areas, resulting from pollution, land use or ecological variables. In recent years, in order to enable extended use of lichens and to reduce ambiguities, i.e., variations due to unwanted environmental variables, efforts have been made to develop a feasible protocol for lichen sampling for biomonitoring purposes. This work aims at providing the information needed *a priori* for an air quality assessment study, in the form of a map showing areas where lichen diversity and abundance is lower. This study was done by sampling foliose and fruticulose lichen diversity and frequency, in a region in southwest Portugal (Sines) with large industrial facilities. A long-term study has been underway in the same area since the 1970s using lichens as bioindicators to evaluate air quality. In this work, we used a standard protocol to determine a lichen diversity value (LDV), to be used as an indicator of environmental quality. In order to reduce uncertainty concerning the type of disturbance affecting lichens, sampling sites were restricted using well-defined criteria. Whenever possible, sampling site variables were quantified. This method allowed us to reduce the many sources of variability affecting lichen diversity.

Key words: air quality, biomonitoring, environmental variables, lichen diversity.

1. Introduction

It is well established that lichens are sensitive to a wide range of habitat changes, most of them man-driven. This sensitivity is due to particular physiological characteristics of lichens, and allows them to be used as indicators and monitors of habitat changes, providing an integrated measure of all disturbances occurring in their environment.

Biomonitoring with lichens can be done in three ways: using variations in diversity and/or abundance, using variations in physiological parameters, or using

lichens as accumulators of pollutants (Branquinho, 2001). Numerous works using lichen diversity have been done worldwide, assuming that sensitive species decline in polluted areas, and tolerant species remain (Martin and Coughtrey, 1982; Galun and Ronen, 1988; Showman, 1988; Wetmore, 1988; Nimis *et al*., 1991, Vokou *et al*., 1999; Geebelen and Hoffmann, 2001; Giordani *et al.*, 2002; Loppi *et al*., 2002; Piritsos and Loppi, 2003). The importance of lichen biodiversity studies was highlighted by Cislaghi and Nimis (1997), who showed a good agreement between lung-cancer mortality and lichen diversity in NE Italy.

Maps of lichen biodiversity or abundance enable identification of areas with different levels of disturbance. However, the direct causes of such disturbances are difficult to assess only from biodiversity data. When we need to identify the stresses related to decreases in lichen biodiversity, further analysis of the abiotic variables affecting lichen communities is needed. Therefore, in environmental pollution studies, further chemical analysis is advisable in order to identify the pollutants (Nimis *et al*., 2002a). Besides pollution, lichen biodiversity is also sensitive to abiotic variables related to macro- and micro-climatic variations. Regarding macroclimatic variations, we can consider changes in temperature, precipitation, geomorphology, and soil chemistry (Brunialti and Giordani, 2003). These macroclimatic variations cannot be avoided when studying lichen biodiversity for biomonitoring purposes, particularly, if the area studied is large enough to enclose different climatic areas. These variations must, however, be considered when comparing lichen biodiversity data from different geographical areas or even within the same area but with significantly different abiotic variables (Giordani *et al*., 2001; Brunalti and Giordani, 2003).

Variations in lichen biodiversity may be due to changes in microclimatic conditions, particularly light, water and nutrients. These alterations may be driven by local sources of disturbance, such as roads or farms, different land uses or habitat fragmentation (Jonsson and Jonsell, 1999; Sillett and Goslin, 1999; Moen and Jonsson, 2003). Biodiversity of corticolous lichens (lichens growing on bark) may also change due to characteristics such as tree age or tree species and correspondent bark pH (Kuusinen, 1996; Herk, 2001), or even to tree health status (Hauck and Runge, 2002). Therefore, in atmospheric pollution biomonitoring studies using lichens, microclimatic variations that are able to influence biodiversity data must be reduced or, ideally, completely removed. Designing a restrictive sampling protocol can minimize expected sources of variations. Although some care must be taken when interpreting lichen biodiversity data, it is a time- and money-saving strategy, as it allows surveys covering large areas and identifies more disturbed sites with some accuracy. Subsequently, chemical analysis, in general more expensive than biodiversity studies, can be focused on those disturbed sites only (Nimis and Purvis, 2002b). Several lichen biodiversity studies indicate that foliose and fruticulose lichen growth forms are more sensitive to air pollution than crustose ones (Owczarek *et al*., 1999; Branquinho, 1997, 2001), and it has been recommended that some crustose species should be withdraw from analyses in air pollution studies

because their optimum is in the immediate vicinity of the pollution source (Tretiach and Ganis, 1999). In fact, some studies only use these groups to interpret the impact of atmospheric pollution on lichens (Showman, 1997; Carvalho *et al*., 2002a). The greater surface exposure of foliose and fruticulose lichens is most probably what affects their sensitivity to atmospheric pollution.

The objectives of this work were to provide the information needed *a priori* for an air quality assessment study. For this purpose, we used foliose and fruticulose epiphytic lichens as biomonitors, with determinations of lichen diversity and frequency, to infer how disturbed or altered our study area was. We built a map where the sites where chemical analysis effort should be concentrated. It is also a goal of this work to determine if the sampling design adopted for lichen biodiversity assessment was adequate to evaluate the impact of atmospheric pollution rather than microclimatic variations.

2. Methods

2.1. SITE DESCRIPTION

The study area is located on the coast of SW Portugal, covering approximately 1500 km^2 (*c*. 50 × 30 km)(Figure 1). This area has small elevations to the east (*Serra de Grândola*, 341 m and *Serra do Cercal*, 325 m) that lie parallel to the coast, in north-south direction. The annual average temperature is 15.0–17.5 °C, the average annual precipitation 400–800 mm and annual insolation 2900–3000 h (averages from years 1931 to 1960).The prevailing winds blow from the N–NW.

The most important features of this area are the large industrial facilities established around Sines since the late 1970's, with a coal power plant, an oil refinery, a chemical plant and, recently, an industrial landfill. The sea harbor allows the arrival of raw materials for the industrial plants such as coal, oil, gas, and chemicals. There are two main motorways and one railway.

2.2. DATA COLLECTION

A total of 73 points were sampled for lichen biodiversity (Figure 1). Approximate site locations were pre-selected in a square grid of 4 km. The choice of the exact location of the sample was based on the following criteria: i. a large area, covered with *Quercus suber*; ii. the same type of management; iii. the highest possible site of the sampling point; and iv. avoidance of sites with local disturbances, particularly paved roads or other facilities like farms or small factories. All these conditions were intended to minimize local microclimatic alterations due to local disturbance sources.

All *relevés* were done on *Q. suber* trees. This is a west Mediterranean species, with silvicultural use that includes the harvesting of the outer bark (cork), every 9–12 yr in Portugal (Oliveira, 2002). The epiphytic lichen communities of *Q. suber* have been studied by several authors (Jones, 1980; Jones, 1985; Fos *et al*., 1994;

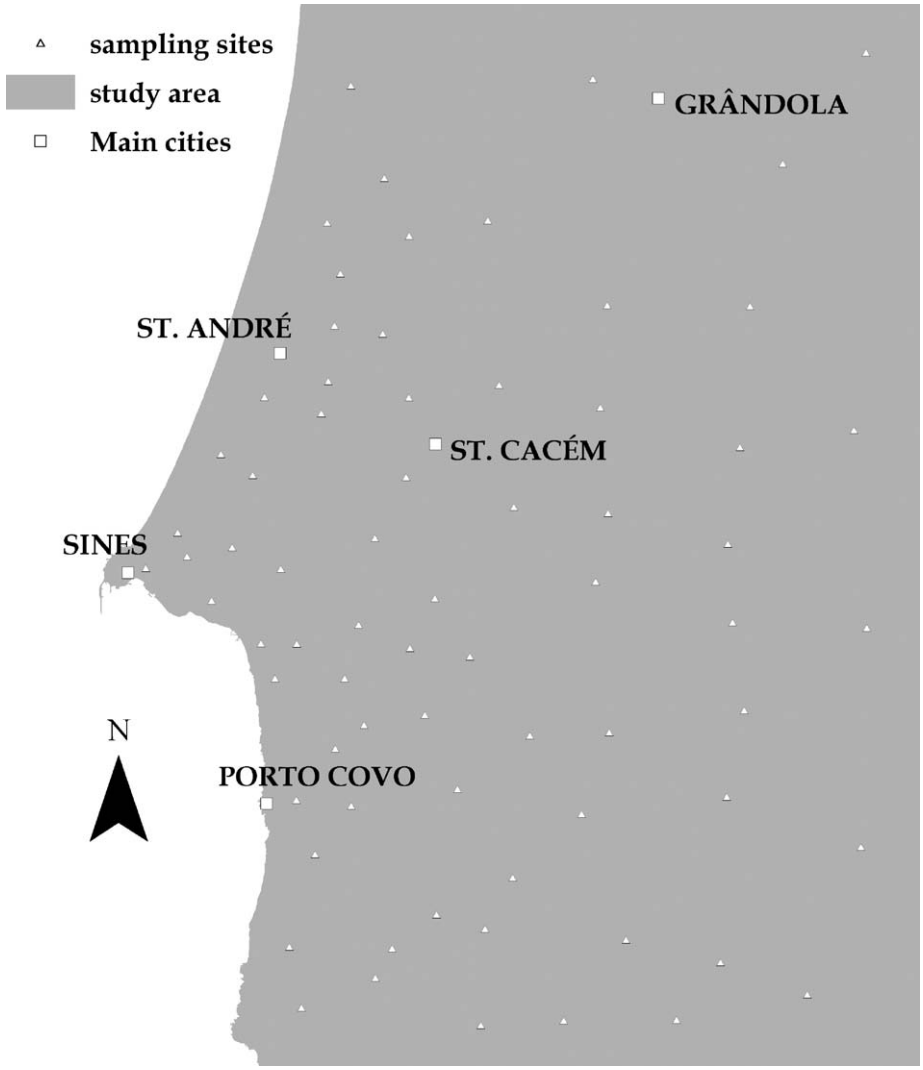

Figure 1. Study area, location of the main cities and industrial facilities and sampling points. The gray lines refer to the UTM grid.

Fos, 1998; Carvalho *et al*., 2002a). In the present work, only this tree species was chosen, to avoid microclimatic variability.

At each site, 10 trees were surveyed for lichen biodiversity (rarely less, minimum 6); a total of 689 trees were sampled. The sampled trees fulfilled certain prerequisites: a. trunk more than 35 cm in diameter, b. trunk inclination less than 75 ° (15 ° deviation from vertical), and c. apparently healthy. The sampling protocol was adapted from the protocol by Asta *et al.* (2002) only for foliose and fruticulose lichen. On each tree trunk, a 10 by 50 cm grid with five 10 by 10 cm divisions was used and placed between 120 and 200 cm above the ground, in the

four main orientations (N, S, E and W). Because both harvested and un-harvested trees were sampled, the grid was always placed on virgin cork. For each orientation, species occurring inside the grid were identified and the number of squares (1–5) in which they occurred was recorded as their frequency. The following measurements were recorded for each tree: i. sampling height (cm), ii. perimeter at breast height (cm), iii. inclination (°) (90° = vertical), and iv. harvesting status, i.e., whether that tree had been previously harvested for cork (0/1). At each sampling site, further information was also recorded: i. UTM position, ii. site exposure, N-W-E-S (transformed to 1, 2, 3, 4 respectively for statistical analysis), iii. site location (flat land, hill, plateau) (transformed to 1, 2, 3 respectively for statistical analysis), iv. distance from the sea (m), v. distance from the Sines industrial area (m) and vi. altitude (m).

Identification of lichen species was mainly done in the field. When this was not possible, species of the same genus were merged as *genus* spp. for lichen frequency calculation. The species included in these groups (*Usnea* spp., *Parmelia* spp. and *Ramalina* spp.), accounted for less than 2% of total lichen frequencies. All species are coded with the British Lichen Society recording code numbers (Coppins, 2002) (Table I).

2.3. STATISTICAL ANALYSIS AND GEOGRAPHICAL DATA TREATMENT

The lichen frequency data from the diversity *relevés* was organized as in Asta *et al.* (2002) in order to obtain a lichen diversity value (LDV). The final LDV of the site resulted from the sum of the averages of lichen frequencies from each orientation. The resulting LDV was interpolated using ordinary Kriging. The geographical data was processed using the software Idrisi32 (Clark Labs, 2000) and GeoMS (CMRP, 2000). In order to understand the influence of abiotic variables on lichen diversity and frequency, all data were subjected to principal component analysis (PCA). Statistical analyses were performed with Statistica 5.1 (StatSoft, 1996).

3. Results and Discussion

3.1. MAPPING THE DISTURBED SITES

This work set out to use lichen biodiversity data to provide the information needed for an air quality assessment study in SW Portugal, and to limit chemical analysis effort to certain sites. For this purpose, we use foliose and fruticulose epiphytic lichens sampled at 73 points covering an area of 30 × 50 km (Figure 1). Before the late 1970s, when the Sines industrial area was built, there were no main industrial sources of atmospheric pollution in the region. Interestingly, lichen biodiversity was sampled in the late 1970s and early 1980s, and the results may be considered as a reference or background study in the region (Jones *et al*., 1981; Jones, 1983). An update of that lichen biodiversity study took place in 1998 (Carvalho *et al*., 2002a).

Table I. List of lichen species identified within the 73 sampling points in SW Portugal (Sines), together with the respective British Lichen Society recording code numbers

BLS code numbers	Species name	BLS code numbers	Species name
491	*Diploicia canescens* (Dickson) Massal.	1235	Ramalina fastigiata (Pers.) Ach. 1810
511	*Evernia prunastri* (L.) Ach. 1810	1236	*Ramalina fraxinea* (L.) Ach. 1810
582	*Hypogymnia physodes* (L.) Nyl. 1896	1458	*Usnea ceratina* Ach. 1810
983	*Parmelia glabratula* (Lamy) Nyl. 1883	1470	*Usnea rubicunda* Stirton (1881)
985	*Parmelia borreri* (Sm.) Turner	1530	*Xanthoria parietina* (L.) Th.Fr. 1860
987	*Parmelia caperata* (L.) Ach. 1803	1531	*Xanthoria polycarpa* (Hoffm.) Rieber 1891
995	*Parmelia exasperata* De Not. 1847	1631	*Physcia tenella* (Scop.) DC. 1805
1008	*Parmotrema chinense* (Osbeck) Hale & Ahti 1986	2032	*Parmelia*
1012	*Parmelia reticulata* Taylor 1836	3669	*Parmelia hypoleucina* Steiner
1018	*Parmelia soredians* Nyl. (1872)	3670	*Parmotrema stuppeum* (Taylor) Hale 1974
1020	*Parmelia subaurifera* Nyl. 1873	3673	*Parmelia austrosinensis* Zahlbr.
1021	*Parmelia subrudecta* Nyl. 1888	3771	*Phaeophyscia hirsuta* (Mereschk.) Moberg 1978
1022	*Parmelia sulcata* Taylor 1836	3936	*Ramalina pusilla* Le Prev. Ex Duby
1024	*Parmelia tiliacea* (Hoffm.) Ach. 1803	3951	*Ramalina lusitanica* H. Magn.
1107	*Phaeophyscia orbicularis* (Necker) Moberg 1977	3957	*Ramalina obtusata* (Arnold) Bitter 1901
1112	*Physcia adscendens* (Fr.) Oliv. 1882	3958	*Ramalina canariensis* Steiner (1904)
1122	*Physcia tribacia* (Ach.) Nyl. (1874)	3963	*Ramalina duriaei* (de Not.) Bagl. (1879)
1123	*Physcia tribacioides* Nyl. (1874)	4342	*Usnea subscabrosa* Nyl. Ex Motyka (1937)
1126	*Physconia enteroxantha* (Nyl.) Poelt 1966	4478	*Ramalina implectens* Nyl.
1127	*Physconia grisea* (Lam.) Poelt1965	5367	*Ramalina*
1231	*Ramalina calicaris* (L.) Fr. (1824)	5464	*Usnea*
1234	*Ramalina farinacea* (L.) Ach. 1810		

The results of our study showed that 43 different lichen species, 27 of them foliose and 16 fruticulose, were identified in the area (Table I). These lichen species had already been reported in previous lichen studies in the area (Jones *et al.*, 1983; Carvalho *et al.*, 2002a). These previous works show a greater total number of foliose and fruticulose species (more than 65), but in these works lichens were sampled from two different phorophytes, one of them, *Olea europaea*, known to be very rich in epiphytic species.

Several methods for sampling lichen biodiversity have been adapted to measure the impact of atmospheric pollution, land use, land management or forest management (Kricke and Loppi, 2002). One of the most common approaches in biomonitoring with lichens is by means of indexes of atmospheric purity (IAP). For their calculation, most of these indexes require a toxitolerance factor for each species, which is determined on a somewhat subjective basis (Nimis *et al.*, 1991). Furthermore, there is evidence that the tolerance of a given species to air pollution may differ according to general climatic conditions (Nimis *et al.*, 1991). In other works, the number of lichen species was shown to be a reliable and objective measure of the impact of pollution on lichen flora (Jones, 1981, Herzig *et al.*, 1989; Nimis, 1990; Branquinho, 1997; Carvalho *et al.*, 2002a). Recently, Italy, Switzerland, and Germany have established national guidelines for monitoring environmental changes with lichens (Asta *et al.*, 2002). They found that the best relation between lichen biodiversity and environmental changes was obtained by calculating a LDV based on frequency measurements. In this work we used this LDV to build a map of the more disturbed sites in the area (Figure 2).

The spatial interpolation of the obtained LDV values is shown in the map depicted in Figure 2. With this map, it is possible to locate the most disturbed zones in the studied area. The areas with greatest disturbance were located parallel to the coast on a strip of approximately 10–12 km. The area near the industrial area of Sines is particularly disturbed, most probably related to the industrial area, as is the area to the south of Sines. We can explain the disturbed areas south of Sines as a consequence of the prevailing winds, blowing from N to the S, transporting air pollution from Sines to the southern areas.

Given that the map allows delimitation of the most disturbed and thus relevant areas for further studies, it is possible to concentrate atmospheric pollution analysis on these sites, saving time and money. On the other hand, in the areas with high LDV, chemical analysis of atmospheric pollutants should be used sparingly, only to evaluate background levels of atmospheric pollution.

3.2. EVALUATING THE INTRA-SITE INFLUENCE ON LDV

Besides pollution, lichen biodiversity is also sensitive to other abiotic variables, related to intra-site variations. Therefore, in an atmospheric pollution biomonitoring study using lichens, intra-site variations that may influence biodiversity data should be reduced or, ideally, completely removed. Designing a restrictive

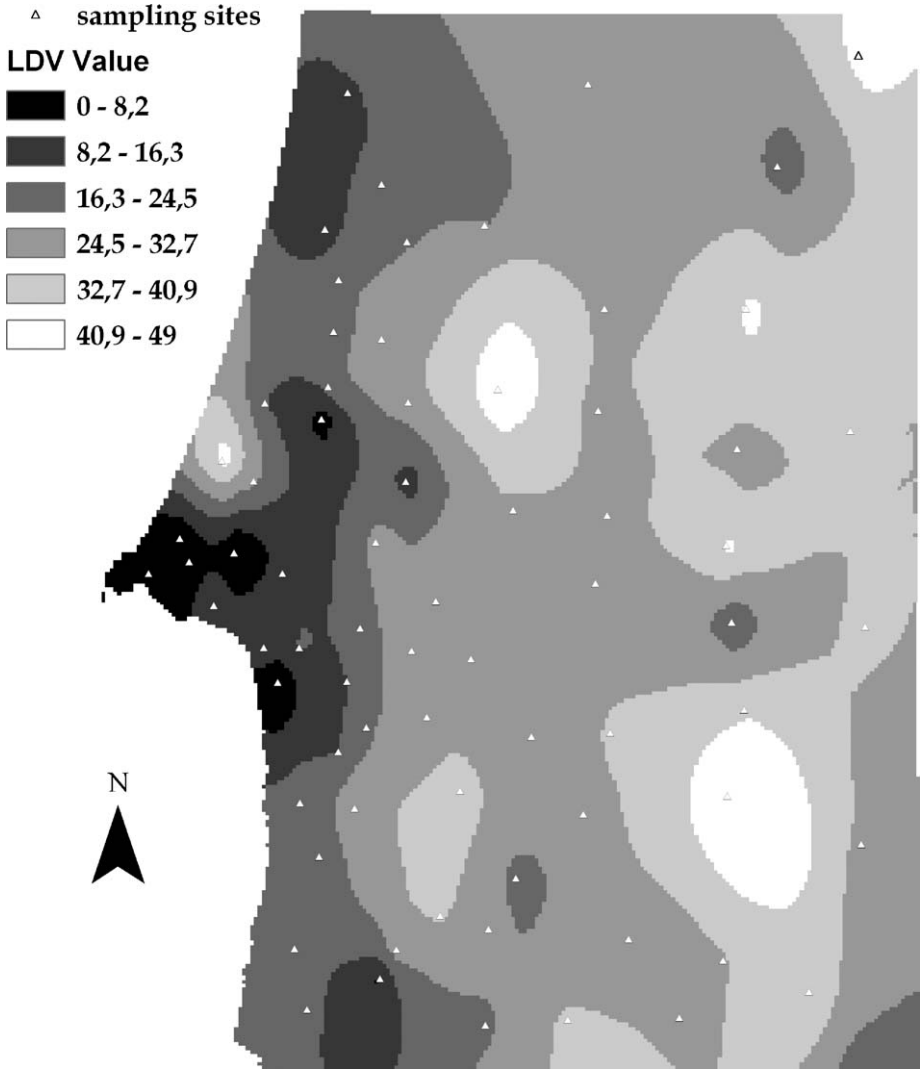

Figure 2. Plot of Lichen Diversity Value (LDV), represented in six classes. The darker areas represent low LDV, grey represents intermediate and light-grey high LDV. White triangles mark sampling points.

sampling protocol can minimize expected sources of variations. In this section, we assess whether our sampling design was successful in minimizing the influence of intra-site variations on LDV.

Several studies report that proximity to local sources of pollution, such as farms, roads and small factories, significantly change local lichen biodiversity (Fos, 1998; Herk, 2001; Carvalho *et al.*, 2002b), creating artifacts in large-scale studies. Our restrictive sampling protocol avoided local atmospheric pollution by only choosing sampling sites far from any source of local disturbance,

particularly farms, small factories, or paved roads, and by sampling in the highest place possible (in general more exposed to general wind rather than local wind). The results of the LDV variogram (data not shown) showed strong anisotropy in the N-S/E-W directions, probably related with the influence of the sea. The observed absence of nugget effect also points to the absence of local sources of disturbance. This means that the sampling grid was essentially adequate for the changes we observed, and that we were able to monitor regional rather than local LDV changes.

In order to test the influence of other environmental variables on lichen biodiversity data, a PCA with all *relevés* and variables was performed. The resulting plot can be seen in Figure 3. The low extracted variance (16% for the two first factors) can be explained by the high number of variables that are not well explained by the represented factors. Although the explained variance for the first two axes is rather low, our main objective was fulfilled because LDV and distance to industrial

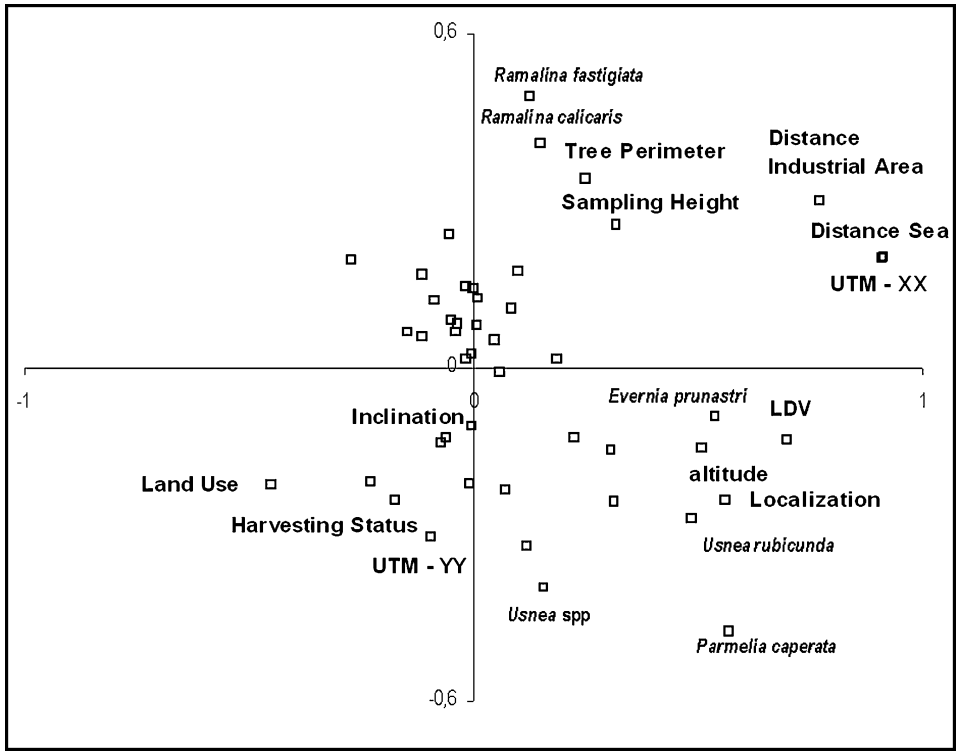

Figure 3. First and second factors of a principal component analysis based on the frequency of foliose and fruticulose lichen species identified on all *relevés*, together with site and tree features. All environmental variables are represented with their name in bold. The species with a relation larger than 0.4 with the axis appear with the respective name. The remaining species are represented only by white squares. The two factors explain 16% of data variance.

area are strongly related with the first axis and conclusions can be extracted from significantly represented variables.

Land use and management in the region was previously reported as a possible influence on lichen biodiversity (Carvalho *et al*., 2002a). To avoid interference from land use and management, our restrictive sampling protocol required that the sampling always occurred in cork-oak woodlands. We avoided sampling in intensive agriculture areas, cattle breeding sites and abandoned sites. This way the sampled cork-oak woodlands presented low variability regarding management, with differences manly related to the development of the understory. In fact, when we tested the variable "land use" in our PCA (Figure 3), we verified that it is not very significantly influencing LDV or determining the distribution of lichen species. This way we accomplished one of our aims by reducing the land use influence on LDV thereby maximizing the influence of atmospheric pollution on LDV.

Other sources of changes in LDV, on a local scale, are tree features such as variations in tree health (Fos, 1998), tree species, tree inclination, cork harvesting status, and tree age. All sampled trees were in apparent good health (they showed no foliar damage). Tree species is a very important source of variance in biomonitoring studies, mainly due to specific bark characteristic, ion content and pH (Herk, 2001). Previous studies in this area showed that sampling on different tree species was an important variable influencing lichen diversity data. For this reason, we chose to sample lichens only from one tree species, cork oak, which is also the most abundant tree in the area. All tree features tested (tree inclination, perimeter, harvesting status and sampling height) did not contribute much to the data variance, showing that the sampling was sufficiently restricted to avoid the influence of intra-site variation.

3.3. MAJOR VARIABLES INFLUENCING LDV

Since our data was not influenced by microclimatic variations, we attempted to evaluate the major variables influencing LDV at the regional scale. For this purpose, we used the following regional variables in a PCA: distance to the industrial area, distance to the coast, latitude (UTM-YY), longitude (UTM-XX), altitude and site location (Figure 3).

The first axis significantly separated the variables distance to the Sea, LDV and distance to the industrial area, as well as site altitude and location (Figure 3). This shows that LDV increases with distance to the coast, with distance to the industrial area and with greater altitudes (Figure 3). The lichens that contribute the most to this enrichment in LDV are *Evernia prusnastri*, *Parmelia caperata* and *Usnea rubicunda*. Some of those lichens are in fact known to be sensitive to salinity or air pollution and have their optimum on the highest sites. To conclude, we may say that the most important environmental variables explaining LDV variation are distance to the coast and distance to industrial site, as well as altitude. Unfortunately, with this PCA it is not possible to separate the effect of the industrial area from the effect of proximity to the sea. Sea influence in this region has been studied and its

effect could be observed at up to 12 km distance (Figueira, 2002), agreeing with the spatial continuity observed in this work.

The main objective of this work was to provide a map that could identify the most disturbed areas. Because our final goal is to relate lichen biodiversity to air pollution, it would be desirable to remove the influence of all other variables. Some variables could be removed with a careful sampling protocol (such as those related with microclimatic alterations) and some others do not have a large influence on LDV (site location or latitude). The next step will be to study the possibility of distinguishing between air pollution and the influence of the sea. Further chemical analysis could provide us with a better understanding of the variables that drive changes in biodiversity.

4. Conclusions

The LDV map obtained allows us to limit and concentrate chemical sampling, by identifying areas with either great or little disturbance. The influence of microclimatic variations on the lichen biodiversity data is negligible, ensuring that any differences between sites were due not to the existence of local disturbance sources or to different tree characteristics, but rather to disturbance occurring on a regional scale, such as air pollution. The best explanation for the LDV variations obtained is given by distance to the sea and to Sines industrial area and altitude. Regarding the distance to the sea and to the industrial areas, because the two sources of disturbance are very close, it is not possible to distinguish them. Further pollution measurements could however clarify this question. There seems to be an effect of the wind in spreading pollution originating from Sines to the S, with more disturbed areas occurring in that direction. Because chemical analysis will be carried out on lichens from the same area, it will be possible to greatly improve the interpretation of the data and accurately relate each pollutant level to the observed LDV and even to the frequency of each individual species. This will greatly enhance the tuning of lichen diversity analysis as a first step for air quality assessment in the future.

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