



Original Article

## Lichens as bio indicators of atmospheric pollution in Porto, Portugal

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### Abstract

The emission of air pollutants mainly from anthropogenic sources has led to the degradation of air quality. These pollutants determine the occurrence or worsening of respiratory disorders. Biomonitoring provides information on the quality of the environment or its modifications, having been used as an alternative to monitoring of chemical pollutants. The variation of the biodiversity of lichens can be used as a warning to check if other biological systems are being affected by atmospheric pollution. Lichens have been used as bioindicators, since they have differential sensitivity to air pollution. The purpose of this study was to assess the diversity and abundance of lichens in different zones of the Porto (North Portugal) with respect to the rates of atmospheric pollutants. Three zones were selected for sampling, represented by urban, suburban and rural zones. LDV was calculated for each zone. The average concentrations of CO, CO<sub>2</sub>, SO<sub>2</sub>, NO<sub>2</sub>, O<sub>3</sub> and PM10 were also measured. The most sensitive lichens were present in the area with the highest LDV. In places where there were higher concentrations of pollutants, namely NO<sub>2</sub> and SO<sub>2</sub> a lower LDV. This study suggests that lichens can be used as prevention systems to protect public health, in particular diseases related to air pollution and as a potential alternative or complement to expensive chemical monitoring equipment.

**Key-words:** lichens, bio indicators, atmospheric pollution, environmental health.

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## Introduction

Atmospheric pollutants have attracted great environmental concern over the last few decades because of evidence that they are associated with respiratory and cardiovascular diseases in human (Godinho *et al.*, 2008). Emissions from traffic today are the main cause of poor air quality in cities. NO was the main polluting gas emitted, oxidized by ozone to yield NO<sub>2</sub>. However, vehicles emit a cocktail of other pollutants including CO, CO<sub>2</sub>, volatile organic compounds, polycyclic aromatic hydrocarbons, particulates and metals (Larsen *et al.*, 2007). Secondary pollutants include ozone and aerosols (Larsen *et al.*, 2007). The Porto District is characterized by urban, suburban and rural zones. The urban zone has heavy vehicle traffic and industrial activities, but in the peripheral suburban and rural zones, these factors decrease by descending order, respectively. From an ecotoxicological perspective, contaminants are all chemical compounds that are fundamentally released into the environment as a result of anthropogenic activities, which cause harms to living organisms (Conti and Cecchetti, 2001). Lichen are recognized as being very sensitive to atmospheric pollution (Gombert *et al.*, 2004). Lichens respond to factors that influence human and environmental health (Larsen *et al.*, 2007). Lichen provides a sample of a complex mixture that humans and

biota have been exposed to in quite a long time. This will be of critical importance for health studies, since one of the most difficult tasks is to relate the low pollution levels with long-term chronic effects on health. However, Cislighi and Nimis (1997) report a high degree of correlation between lung cancer and the biodiversity of lichens as a result of atmospheric pollution. These high correlation levels have been found for the more common atmospheric pollutants, such as SO<sub>2</sub>, NO<sub>2</sub>, dusts and SO<sub>4</sub><sup>2-</sup>. Lichens are considered the result of a symbiotic association of a fungus and an algae. The fungus is usually *Ascomycetes*, although on rare occasions it may be either a *Basidiomycetes* or a *Phycomycetes* (Conti and Cecchetti, 2001). The algae is a *Cyanobacteriae* or a *Chlorophyceae* (Conti and Cecchetti, 2001). First studies with bioindicators date back to the 1960s. As far back as 1866, a study was published on epiphyte lichens used as bioindicators (Conti and Cecchetti, 2001). Today, epiphytic lichens have been widely used as bioindicators of the effects of atmospheric pollutants. Bioindicators are organisms which can be used for identification and quantification of environmental damages (Conti and Cecchetti, 2001). Biomonitors are organisms often used for the quantification of contaminants (Conti and Cecchetti, 2001). Lichens may be used as bioindicators by mapping all species present

in a specific area, based on the number, frequency and tolerance of the lichens present in the area under study, and, these are good predictor, to a good level of approximation, the degrees of eight atmospheric pollutants measured using automatic control stations (Conti and Cochiti, 2001). Lichens are an excellent bio indicator of air quality because they: (i) have a wide geographical distribution (except in marine zones); (ii) are abundant, sessile, as well being representative of the collection area; (iii) are available all year round, [are perennial]; (iv) have uniform morphology over time; (v) lack waxy cuticle and stomata and readily absorb gases and dissolved substances in the air through their surface (Loppi *et al.*, 2002). It is also a quick and inexpensive method that provides results on which predictions for human health can be based (Loppi *et al.*, 2002).

The Lichen Diversity Value (LDV) method proved to be applicable for assessing lichen diversity. This technique is based on the fact that epiphytic lichen diversity is greatly and steadily diminished with the increase of air pollution and environmental stress (Svoboda *et al.*, 2010). LDV and Index atmospheric purity (IAP) has been mainly applied to assess and monitor environmental alteration especially in relation to the effect of atmospheric pollution in several European countries, such as Italy (Gombert *et al.*, 2003), Portugal (Pinho *et al.*, 2008), France (Gombert *et al.*, 2004), London (Larsen *et*

*al.*, 2007) and Slovenia (Jeran *et al.*, 2002). LDV method, with peculiar high degree of objectiveness and repeatability, represents a fundamental statistical approach for routine investigations of lichen diversity in environmental monitoring.

This study aims to find out and understand how lichens (abundance and diversity) could be affected by atmospheric pollution in Porto, emphasizing the importance for their reliable utilization to environmental conservation and public health protection.

## **Material and Methods**

### **Study area**

The Porto District is part of the Northern Region of Portugal (figure 1), spreading their municipalities by the subregion of Grande Porto, Ave and Tâmega. It represents the core of the traditional province of Douro Litoral and has one of the largest metropolitan areas in Europe with a total population of about 1,7 million inhabitants, being the largest in Portugal.

Three areas in subregion of Grande Porto were selected and studied: urban zone (Pavilhão Rosa Mota in Porto city), characterized by intense surrounding vehicle traffic; suburban zone (Parque da Cidade da Maia at 10 Km to Porto city) and rural zone (Village Valinhas Sto Tirso at 30 Km to Porto city).

### Sampling procedure of lichens (abundance and diversity)

Then a reconnaissance of the study area, was carried out in order to verify the frequency/distribution of suitable trees on which it was possible to observe lichens. The trees with: (i) damaged or decorticated parts; (ii) knots; (iii) seepage tracks and (iv) parts where the bryophyte cover was higher than 25% weren't sampled. Trees with similar size, free-standing, circumference larger than 70 cm and conservation state were used. The sampling were made in three trees *Quercus robur* in each local sample, with one grid of five quadrat segments 10 x 10 cm squares each, being attached vertically to the trunk so that the lower edge of each segment is 1 m above the highest point of the ground (Asta *et al.*, 2002). Sampling was conducted sequentially in four cardinal points of the tree trunk (North, South, East, West) (figure 2).

All lichen species present within each quadrat segment are recorded and the frequency of occurrence of each species in the 5 squares of each quadrat segment noted.

#### Calculation of the LDV

For the calculation of LDV the frequencies of all lichen species found on each tree. Were summed For each tree there are four Sums of Frequencies (SF<sub>N</sub>, SF<sub>E</sub>, SF<sub>S</sub>, SF<sub>W</sub>). Next, for each cardinal point, the arithmetic Mean of the Sums of Frequencies (MSF) (Asta *et al.*, 2002):

$$MSF_N = (SF_{1N} + SF_{2N} + SF_{3N} + SF_{4N} + \dots + SF_{nN}) / n$$

Where:

MSF – Mean of the sums of frequencies of all the sampled trees

SF – Sum of frequencies of all lichen species found at one cardinal point

N, E, S, W –North, East, South, West

n - Number of trees sampled.

The LDV is the sum of the MSFs of each cardinal point (Asta *et al.*, 2002):

$$LDV = (MSF_N + MSF_E + MSF_S + MSF_W)$$

### Sampling procedure of atmospheric air pollutants

The determination of atmospheric air pollutants was made by direct-reading equipment, namely: IAQ - CALC 8760 (CO and CO<sub>2</sub>); Gasman- Crowcon (O<sub>3</sub> and NO<sub>2</sub>); Tetra - Crowcon (SO<sub>2</sub>) and Aerosol Monitor - 8520 Model DustTrak (PM10). The equipment was placed at a height of approximately 1,5 m above the ground. The equipments were subject to 10 minutes stabilization process and measurements were performed in 10 minutes (monitors at 1- min intervals). All samplings were made at the same time the sampling of lichens were carried out.

### Results and Discussion

(16) lichen species were found. The most frequent species were *Favoparmelia caperata*, *Parmotrema chinense* e *Punctelia subrudecta* (table 1).

*Flavoparmelia caperata* and *Parmotrema chinense* were to be found in all zones. *Flavoparmelia caperata* is the one

most abundant species found in Portugal (Godinho *et al.*, 2008). *Punctelia subrudecta* was only found in urban and suburban zones and it may be due to the fact that it was strongly associated to atmospheric pollution and also humidity (González and Pignata, 1997). In Loppi *et al.* (2002) study, the genus with highest number of species in “natural zone” was *Parmelia*. In another study, a great impact was observed during unusual atmospheric conditions in west of London in two of the most abundant lichen species in Europe *Parmelia sulcata* and *Hypogymnia physodes* (Purvis *et al.*, 2008). In our study both lichen species were found, however *Parmelia sulcata* seems to be more sensitive, because it was just found in rural and suburban zones. In order of sensitivity to air pollutants, lichens are classified by the following descending order: fruticulose, foliaceous and crustose, respectively. Most lichens found in our study was foliaceous, and only two lichens fruticulose, namely *Cladonia spp.* and *Evernia brunasti*. Apparently the area sampled less polluted was the suburban zone, followed by the rural zone, where the presence of *Cladonia spp.* was just observed. The urban zone is the potentially more polluted zone, where only foliaceous lichens.

LDV can be taken as estimates of environmental quality: high values correspond to good situations while low values indicate poor quality (Loppi *et al.*,

2002). The LDV for each zone is shown in table 2.

in table 3, where the LDV interpretation scale is shown, all zones were classified with Low LDV. Nevertheless the urban zone had still the least LDV (25). The highest LDV in suburban zone (39), very close to rural zone (36). However on sublevel index LDV, suburban and rural zone were classified with Low to moderate LDV, while just rural zone was classified with Low. When compared to other studies, LDV of District of Porto, on different zones were similar. LDV among forested areas in Italy are high (75) compared to urban areas (19) (Giordani, 2007). In London gradient of lichens diversity increase with the distance from the city centre, suggesting traffic influence (Larsen *et al.*, 2007).

Pollutants discharged to the atmosphere are not constant in space and time. Low LDV in suburban and rural areas may also be influenced by winds, which may bring urban pollution from the city center.

High concentrations of atmospheric pollutants seems to show to limit epiphytic lichen abundance. Table 4 shows the average concentration of atmospheric pollutants in each zone, and its apparent influence on LDV.

Lichens have been used to biomonitor several pollutant levels of air quality, particularly sulphur, nitrogen, fluoride, oxygen, metals, radionuclide, dioxins and

other organic compounds. However, information on the pollutants involved and their working mechanisms is scarce, (Dobben *et al.*, 2001). Regarding CO and CO<sub>2</sub>, the results in all zones were similar. The NO<sub>2</sub> was very high in urban zone. In Urban zone low LDV could be associated with high NO<sub>2</sub> concentrations, probably due to the dust from traffic vehicle intensity or emitted from industrial origin. NO<sub>2</sub> derived from traffic emissions, limited lichen diversity (Larsen *et al.*, 2007). An impact on the health of *Parmelia sulcata* and *Hypogymnia physodes* was recorded in west London following a period of high exhaust emissions coupled with unusual weather conditions, suggesting that nitrogen and particles were responsible (Larsen *et al.*, 2007). In our study most lichen species were absent in areas with highest peak NO<sub>2</sub> and SO<sub>2</sub> concentrations. As concentrations of SO<sub>2</sub> decreased in urban areas, nitrogen played an increasing role in changing lichen communities (Pinho *et al.*, 2008). Because of their structure, lichens depend mainly on atmospheric deposition for their nutrition, especially for their nitrogen supply (Gombert *et al.*, 2003). The different forms of nitrogen can be supplied for by major sources: mainly ammonia in rural environments and mainly nitrogen oxides in urban environments (Gombert *et al.*, 2003). Nitrophytic lichens seem to increase in more industrial and urban areas, potentially because

dust is considered one of the main causes for the rise in bark pH of *Quercus* trees (Pinho *et al.*, 2008), that is known to influence sulphur speciation, which determines toxicity and may influence speciation and bioavailability of other potentially toxic elements (Larsen *et al.*, 2007). In our study, nitrophytic lichens genus, such as *Physcia* was just found in rural zone, where NO<sub>2</sub> concentration was zero. However, Gombert *et al.* (2003) found a significant positive correlation between traffic index and the total nitrogen concentration of *Physcia adscendens*. Results of study of Gombert *et al.* (2003) showed that nitrogen concentrations of *P. adscendens* depend on road traffic and road vicinity. In Gombert *et al.*, (2003) study, a relationship between nitrogen oxides emitted by traffic and lichen nitrogen concentrations was assumed. High diversity in the neighborhood of the pollution sources seems to be mainly due to the occurrence of a high number of nitrophytic species, e.g genus *Physcia* (table 1). In rural areas, the main effects of nitrogen deposition on lichens can result in changes of the communities, such as a great occurrence of nitrophytic species, often associated with a rise of bark pH and a decrease in biodiversity (Gombert *et al.*, 2003).

Troposphere ozone (O<sub>3</sub>) is not directly emitted in significant quantities by human activities, resulting mainly from the interaction between solar radiation, oxygen

and precursor pollutants, particularly NO<sub>x</sub>, but also CO. O<sub>3</sub> concentration was similar in urban and rural zones. Long-term influences of gaseous pollutants (particularly globally rising background ozone concentrations) on lichen communities and succession under changing climatic conditions are unknown. In urban areas or close to roads, studies have shown the positive correlation between traffic density and levels of different primary pollutants (Gombert *et al.*, 2003). In recent decades, urban pollution has been modified with decreasing sulfur dioxide and increased NO<sub>x</sub> mainly emitted by road traffic or emerging with secondary process (O<sub>3</sub>) (Gombert *et al.*, 2003).

Sensitivity to SO<sub>2</sub> and other atmospheric pollutants in general varies according to species (Conti and Cecchetti, 2001). However, during the last decades, SO<sub>2</sub> has been the main pollutant affecting the distribution of epiphytic lichens in urban and industrial areas (Giordani, 2007). Several works have reported good correlations between atmospheric SO<sub>2</sub> deposition and total S concentration in lichens (Pinho *et al.*, 2008). *Hypogymnia physodes* is particularly resistant to SO<sub>2</sub>, it has been used in the area surrounding a fertilizer plant, where sulphur levels of 3000 ppm had been found (Conti and Cecchetti, 2001). *Physcia aipolia* is sensitive to SO<sub>2</sub> (Gombert *et al.*, 2004). In this study *Hypogymnia physodes* in zones with high SO<sub>2</sub> concentration. To the contrary,

the genus *Physcia* was only found in rural zone, where SO<sub>2</sub> concentration is low. SO<sub>2</sub> can be transported far away from this emission (Jeran *et al.*, 2002) it can explain the value obtained in suburban zone. Laboratory exposure lichens to SO<sub>2</sub> cause relevant membrane damage to lichen cells, which may cause a reduction in protein biosynthesis, or there may be negative effects on the nutritional interchange between symbionts (Conti and Cecchetti, 2001). Sulphur accumulation in lichens seems to come from vehicle traffic when compared with the influence of SO<sub>2</sub> from industry (Conti and Cecchetti, 2001).

Particulate matter may be important in affecting lichen distribution in and around urban areas, because most atmospheric pollutants can be deposited there. In rural zone the PM<sub>10</sub> concentration is the highest. Low LDV in our results could be associated with this. There could also be a relationship with higher LDV in suburban zone and PM<sub>10</sub> concentration, where more low.

Giordani (2007) affirms that the relationship between LDV and environmental variable changes under different ecological conditions: atmospheric pollutants are the main limiting factor, but synergistic effects could be happen. In urban environments, in addition to SO<sub>2</sub>, the simultaneous occurrence of the phytotoxic gaseous pollutants can expected be to result in combined effects. Loppi *et al.* (2002) reported that synergistic

effects are observed when concentrations are below or at the threshold for individual injury response. This effects can explain the absence or scarcity of certain sensitive lichen species. Other factors, including humidity, light, and temperature may also play a role. Besides, bark and soil chemistry can also influence lichen quantity and community composition (Purvis *et al.*, 2008). In this study this parameter was not evaluated.

## Conclusion

This study showed a relationship between the diversity of lichens and the concentration of chemical pollutants found in Porto District. In places where there were higher concentrations of pollutants, namely NO<sub>2</sub> and SO<sub>2</sub> a lower LDV. Lichens can be used to provide a warning signal before severe damages occur on ecosystem and health. This study suggests that lichens can be used as prevention systems to protect public health, and diseases specifically related to air pollution, and as a potential alternative or complement to expensive chemical monitoring equipments.

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**Table 1.** Lichen species found in each zone.

Porto (Urban zone)	Maia (Suburban zone)	Sto Tirso (Rural zone)
<i>Punctelia subrudecta</i>	<i>Punctelia subrudecta</i>	<i>Parmotrema chinense</i>
<i>Hypogymnia physodes</i>	<i>Hypogymnia physodes</i>	<i>Flavoparmelia caperata</i>
<i>Parmotrema chinense</i>	<i>Parmotrema chinense</i>	<i>Parmelia sulcata</i>
<i>Flavoparmelia caperata</i>	<i>Flavoparmelia caperata</i>	<i>Usnea spp.</i>
<i>Leprocaulon microscopium</i>	<i>Parmelia tiliacea</i>	<i>Physcia spp</i>
	<i>Parmelia sulcata</i>	<i>Lepraria incana</i>
	<i>Pertusaria amara</i>	<i>Pertusaria amara</i>
	<i>Evernia brunasti</i>	<i>Parmelia Castanha</i>
	<i>Cladonia spp.</i>	<i>Cladonia spp.</i>

**Table 2.**LDV in each zone.

Porto (Urban zone)	Maia (Suburban zone)	Sto Tirso (Rural zone)
25	39	36

**Table 3.**LDV interpretation scale (Brodeková *et al.*, 2006).

Index LDV	Sublevel index LDV	LD Values	Zones
Very high	Very high	>81	
High	High to very high	71-80	
	High	61-70	
Moderate	Moderate to high	51-60	
	Moderate	41-50	
Low	Low to moderate	31-40	Suburban and Rural
	Low	21-30	Urban
Very low	Very low to low	11-20	
	Low	0-10	

**Table 4.**Average concentrations of atmospheric pollutants in each zone.

Zone	CO (ppm)	CO <sub>2</sub> (ppm)	NO <sub>2</sub> (ppm)	O <sub>3</sub> (ppm)	SO <sub>2</sub> (ppm)	PM10 (µg/m <sup>3</sup> )
Porto (Urban)	3,1	333	2,7	0,17	2	1
Maia (Suburban)	2,6	305	0	0,02	2	1
Sto Tirso (Rural)	3,1	294	0	0,25	1	7

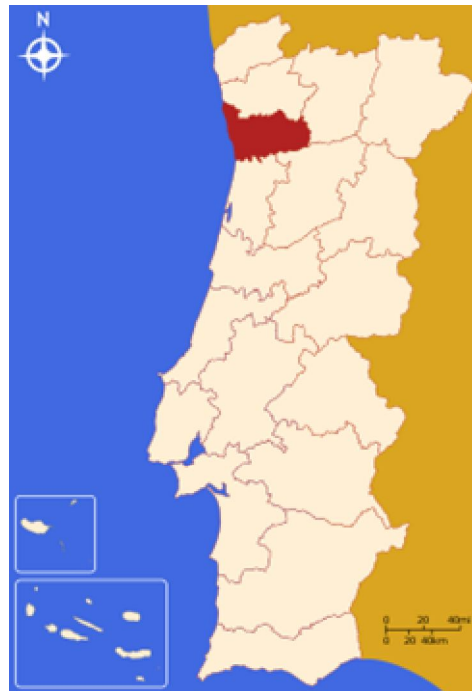


Fig 1. Location of the district of Porto

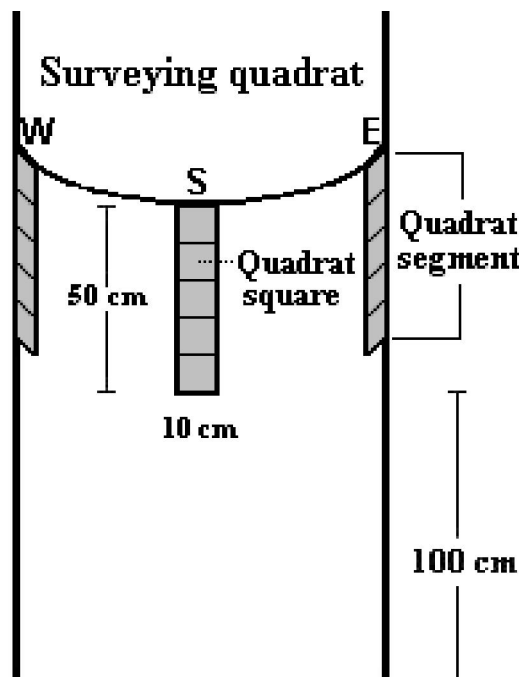


Fig 2. Tree sampling exemplification (Asta *et al.*, 2002)