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REVIEW ARTICLE

Presence of fungi in Scots pine needles found to correlate with air quality as measured by bioindicators in northern Spain

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Summary

Atmospheric pollution has increased worldwide during the last decades affecting forest ecosystems. Thermal power plants give off high levels of contaminants, which can damage forest health. Bioindicators can be helpful tools used for evaluating environment changes by giving an accurate measure of the extent of pollution. The focus of this study was to analyse fungal mycoflora in *Pinus sylvestris* stands near a thermal power plant and the possible correlation between the presence of the fungi and the air quality as measured by lichens and mosses. Fungi associated with pine needles were selected as subjects of this study because of their phytopathological importance and association with the pine trees' decline. Samples for this study were selected from eight plots in close proximity to a thermal power station in northern Spain. Symptoms of decline were previously observed in all the plots before sampling was performed. Lichens were used as bioindicators of environmental quality in two ways, first using the Index of Atmospheric Purity and second through categorization of lichen species based on their sensitivity. In addition, botanical quality was measured regarding the presence or absence of mosses. With two seasonal samplings (autumn and spring) and the use of four types of pine needle plant material (green needles, dried needles, half–green, half-dried needles and fallen needles), both endophytes and epiphytes present in the needles were isolated and observed. Thirty fungal species in total were identified of the 1095 isolates obtained. Furthermore, significant differences in fungal isolates were observed between seasons and among the different plant material. Results showed that both environmental air quality and botanical quality were negatively correlated with the relative isolation frequency of fungi. The higher number of isolates was attributed to a possible infection produced by fungi, which could be a leading factor in the trees' decline.

1 Introduction

Over the last few decades, industrial activities in many countries have increased air pollution affecting forests and its biodiversity. In these cases, wind patterns have had a great influence because wind acts as a spreading agent of atmospheric pollution (Fernández-Salegui et al. 2002). Bioindicators are useful tools for measuring such environmental changes owing to pollution. They provide an accurate indication of the degree and strength of the pollution's environmental impact and its potential damage to other organisms including human beings (Ederra 1997).

Many studies have demonstrated the effectiveness of using flora organisms such as lichens and mosses as environmental impact indicators. Nylander (1866), a pioneer in lichen research, observed that in 'Jardins du Luxemburg' in Paris lichens had completely disappeared as a result of pollution. Hawksworth and Rose (1970) provided a list of epiphytic lichens whose presence or absence in a zone was related to the concentration of sulphur dioxide in the air. Thus, due to their physiology, lichens have been used as bioindicators or biomonitors in various studies in different countries (e.g. Loppi and Pirintsos 2003; Jeran et al. 2007; Mayer et al. 2009). Like lichens, mosses, which are sensitive to atmospheric pollution, especially SO_2, normally disappear or become scarce with, in the end, only a few species represented in polluted areas. This confirms the effectiveness of mosses as indicators of atmospheric pollution (Siebert et al. 1996; Jeran et al. 2003). Furthermore, they can also be used as biomonitors of heavy metal contamination (Pesch and Schroeder 2006; Nguyen-Viet et al. 2007; Cao et al. 2008), fresh water pollution (Lopez et al. 1997) and other factors, such as environmental stability and botanic quality (Guerra et al. 1989; García et al. 1999).

Fungi are the most frequent cause of forest disease; therefore, fungi associated with pine needles can be used to determine the forest condition. The relationship between fungi, plants and the environment is intricate, and changes in any of these factors could reduce an ecosystem's health (Hansen and Lewis 1997). Pollution is one of the environmental factors that negatively affect the forest stands, producing indirect effects on the health of the area's flora (Montoya and López 1997). Among fungi, both endophytes and epiphytes are associated with needles. They can be pathogenic, saprophytic or live in mutualism with the host; however, the behaviour of one species may change when exposed to an alteration in the environment (Sieber 2007; Botella and Diez 2011). Some endophytes are potentially pathogenic, but disease or decline is only caused in combination with other detrimental factors (Sieber 2007). Many authors have previously studied the effect of pollutants on endophytes (Helander et al. 1993, 1994; Asai et al. 1998) as well as the effect of healthy tissue and symptomatic tissue from different parts of the tree on fungal communities present (Hata and Futai 1995; Bettucci and Alonso 1997; Müller et al. 2001; Ragazzi et al. 2003; Santamaria and Diez 2005; Zamora et al. 2008).

Salemaa et al. (2004) observed that bryophytes, lichens and vascular plants have different capacities to accumulate pollutant and grow in polluted environments. Notwithstanding, it is necessary to study all the life forms when one evaluates

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forest ecosystems. The relationship between several organisms and their interaction with atmospheric pollution are indeed difficult to interpret. Thus, the main objectives of present study were (i): study the composition and frequency of fungi associated with needles in *P. sylvestris* stands near a thermal power plant; (ii): evaluate the air quality in the power plant surroundings using the index of atmospheric purity (IAP) with lichens and the botanical quality (BQ) with bryophytes; and (III): study a possible correlation between the frequency of fungal isolates and the air quality using botanical quality evaluation methods.

2 Materials and methods

2.1 Study area

Samples were collected from eight plots 4 km away from the Velilla thermal power plant in northern Spain (Fig. 1). At that location, coal and anthracite are the principal thermal power plant fuels. The mean values of emissions per year were as follows: CO_2 2147 $*10^6$ kg/year; N_2O, 23 215 kg/year; NO_x/NO_2, $1124*10^4$ kg/year; SO_x/SO_2, $1663*10^4$ kg/year; As, 111.3 kg/year; Cd, 12.7 kg/year; Cr, 133 kg/year; Ni, 336 kg/year; Pb, 467 kg/year (PRTR-Spain 2012). Four of the plots (5, 6, 7 and 8) were located in the north-east, which, according to previous studies carried out by the Velilla thermal power plant itself (Ibarra 1993; Tecmena 1996), was one of the dominant wind directions causing the atmospheric dispersion of SO_2. Foliar damage (i.e., chlorosis), symptoms of decline (i.e., defoliation and discoloration) and high sulphur content in pine needles had previously been reported (Tecmena 1996) in that location. The other four plots (1, 2, 3 and 4) were situated outside the area under the maximum influence of the pollution. In all plots, middle-aged Scots pine (*P. sylvestris*) stands were dominant (around 30–40 years old). They were closed-canopy forests, monospecific stands with diameter at breast height around 20 cm and mean tree height of 10–15 m. The sites had a southern exposure and the following climate features: average annual temperature of 10°C, total annual precipitation above 900 mm and average relative humidity of 75% (Allue-Andrade 1990; Ninyerola et al. 2005; AEMET 2012). Other characteristics of the sampling plots (i.e., altitude, location, coordinates and edaphic types according to FAO soil classification) are given in Table 1.

2.2 Study of fungal populations

Different tree samples in the autumn and spring seasons were collected. A mean daily temperature of 7.5°C was recorded during the spring sampling (April) and a temperature of 5.0°C during the autumn sampling (November). Samples were taken from 32 trees (four per plot), which were then observed and categorized as green needles, dried needles, half-green, half-dried needles and, lastly, fallen needles. Green, dried and half-green, half-dried needles were taken from the lower part of the canopy of the tree by using pole pruning shears (approximately 5 m high). Fallen needles were collected at ground level close to the sampled tree. A total of 256 moist chambers with Petri dishes and paper towels were prepared to incubate the samples at room temperature (24 ± 2°C) under diffused daylight until there was production of fruiting bodies. The surface of the samples was not sterilized to allow the growth of endophytes and fungal epiphytes. Fungal structures were analysed and later identified according to their morphological characteristics by using species keys (Sutton 1980; Von Arx 1981; Goidànich 1990; Haulin 1990; Watanabe 1994; Butin 1995; Allen et al. 1996; Kiffer and Morelet 1997).

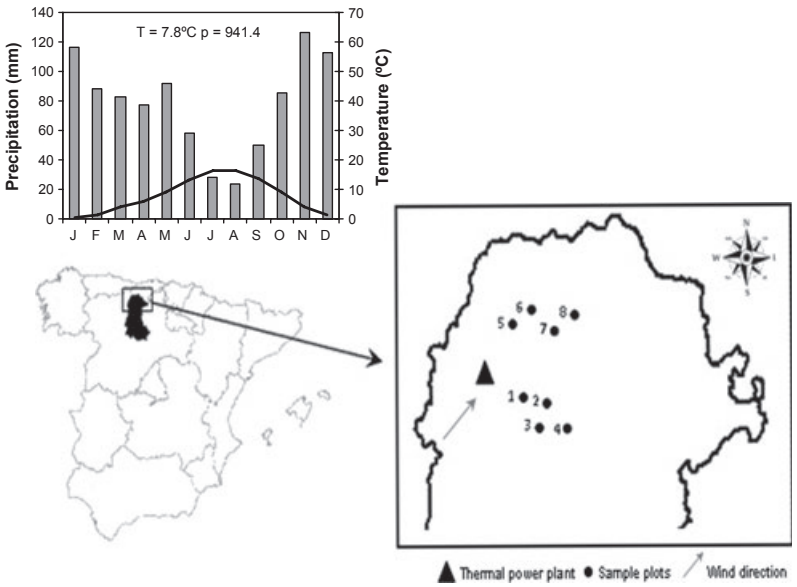

Fig. 1. Geographical position of the study plots, thermal power station and wind direction and climograph of the study area.

Table 1. Sampling plots localization and characterization. Number of plot; Name, village the plot is located in; XUTM and YUTM coordinates of the centre of the plot; Altitude from sea level in meters; Soil type according to FAO soil classification in "Mapa de suelos de Castilla y León (Forteza and Tejero 1987)."

Plot	Name	XUTM	YUTM	Altitude	Soil type
1	Muñeca	352.528	4.741.302	1286	Humic Cambisol
2	Muñeca	352.576	4.741.251	1262	Humic Cambisol
3	Muñeca	352.552	4.741.158	1265	Humic Cambisol
4	Muñeca	352.588	4.741.106	1284	Humic Cambisol
5	Valcovero	355.926	4.746.250	1445	Lithosol
6	Valcovero	355.723	4.746.325	1418	Lithosol
7	Cristo de la Sierra	355.575	4.746.352	1412	Lithosol
8	Cristo de la Sierra	355.451	4.746.373	1390	Lithosol

The SAS/STAT® software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used to evaluate the effect of the plots, plant materials (green, dried, half-green, half-dried and fallen needles), and sampling season (autumn and spring) and their interactions. An analysis of variance was performed using the relative isolation frequencies (RIF) as the dependent variable (Santamaria and Diez 2005). The RIF was calculated with the following formula:

$$\mathrm{RIF} = \frac{n_{ijk}}{N_{ijK}},$$

where n_{ijk} represents the number of isolates recorded in the plot i, sampling time j and sampling of plant material k, and where N_{ijk} is the number of samples examined in the plot i, sampling time j and sampling of plant material k. When tested by the Kolmogorov–Smirnov method, these data ($n > 50$) were normally distributed. A Tukey–Kramer test for multiple comparisons was used when significant differences were found in the ANOVA table.

2.3 Methods to evaluate environmental quality using lichens

Two methods were employed to evaluate environmental quality: a quantitative method (IAP) and a qualitative method (species sensitivity). The IAP, improved by Nimis (1990), was calculated depending on the presence or absence of lichens in a 10 cm^2-squared grid placed 120 cm high in each of the five trees that showed the highest lichenic cover inside each plot. This value (IAP_{Plot}) represented the frequency of the species in the inventory:

$$\mathrm{IAP}_{\mathrm{plot}} = \sum f_{\mathrm{t/n}},$$

where f_t = frequency of lichens; n= number of tree sampled.

All inventories were made by the same person to reduce margin of error (Kinnunen et al. 2003). The general IAP of each plot was calculated by averaging the value of five trees. IAP categories were calculated as previously described (Wirth 1995). The lower values corresponded with areas experiencing less environmental quality, while the higher values were linked to better environmental quality (Calvo and Sanz 2000; Jeran et al. 2002; Scerbo et al. 2002; Fernández-Salegui and Terrón 2003).

The qualitative method included analysing and identifying the species present at each plot. Firstly, categorization of species sensitivity was carried out (Hawksworth and Rose 1970; references therein), and secondly, an evaluation of lichen's morphology was completed. Qualitative values were designated depending on the species pollutant sensitivity: sensitive, intermediate and tolerant (Calatayud and Sanz 2000). Afterwards, lichen morphology was analysed (Ederra 1997) according to the following sensitivity talus order: crustaceous < foliaceous < fruticulosus. An analysis of variance was carried out to evaluate the effect of plots, biotype (crustaceous, foliaceous and fruticulosus), sensitivity (sensitive, medium and tolerant) and their interactions on number of species (the dependent variable). When significant differences were found in both ANOVA tables, a Tukey–Kramer test for multiple comparisons was used by means of the GLM procedure of SAS ($p = 0.05$). These data showed a normal distribution when tested by a Shapiro–Wilk test ($n < 50$).

2.4 Botanical quality evaluation with mosses

The methodology used in this evaluation was developed by Guerra et al. (1989) and relates several indices to calculate one botanical quality coefficient to classify zones with different vegetation conservation. Botanical quality refers to the land quality degree from a botanical point of view which is related to its natural conservation rate. The following indexes were applied: Floristic diversity coefficient (FLDC) of a location indicates the floristic diversity of every location with respect to the overall area.

$$\mathrm{FLDC} = \frac{\text{Number of species of the location}}{\text{Total number of species of the overall area}} \times 100$$

Rarity specific coefficient (RSPC) of every species refers to the abundance of one species with respect to the total.

$$\text{RSPC} = \frac{\text{Number of locations} - \text{Number of locations where the species is present}}{\text{Number of locations}} \times 100$$

Specific originality coefficient (SPOC) of a location establishes a relation among the abundance of every species and the total number of species.

$$\text{SPOC} = \frac{\sum \text{RSPC}}{\text{Number of species}}$$

These indexes were used to calculate the Botanical quality coefficient (BQ) by the following formula:

$$\text{BQ} = \text{FLDC} + \text{SPOC} + \text{NB},$$

where NB= number of habitats occupied by bryophytes.

In accordance with the resulting BQ, several groups and levels were established and then used to evaluate bryophytic flora, moss conservation and its degradation state (García et al. 1999).

2.5 Correlation analyses

The IAP, the botanical quality (BQ), relative frequency of isolates (RIF) and the abundance of tolerant, intermediate, sensitive, crustaceous, foliaceous and fruticulosus species were all compared to explore possible relationships between the methods. As some variables were not normally distributed, a nonparametric Spearman's correlation test ($p = 0.05$) was used to find correlations among the variables studied. These analyses were performed by Corr procedure from SAS.

3 Results

3.1 Study of fungal populations

A total of 30 fungal species (both endophytes and epiphytes) were identified of 1095 isolates obtained. Table 2 shows isolation frequencies obtained from every species, plot, sampling time and quality of plant material. *Alternaria complex* was the most common fungal species and it appeared in all sampling materials in both sampling seasons and in all study sites. Moreover, *Cladosporium herbarum, Cladosporium* sp., *Epicoccum nigrum, Leptostroma pinastri, Lophodermium pinastri, Naemacyclus niveus, Penicillum* sp., *Sclerophoma pithyophila* and *Stachybotrys* sp. were also very common. *Cytospora* sp., *Fusarium roseum, L. pinastri, L.ophodermium pinastri, N. niveus, S. pithyophila, Sphaeropsis sapinea* and *Verticillium* sp. were also found in some samplings and are well known for their pathogenicity. The RIF of these species was analysed separately and named as RIF pathogenic. For statistical analysis, three variables were considered: plot, sampling season, sampling plant material and the interaction site-sampling season. Because three-way interaction did not turn out to be significant, the data were analysed altogether. The model showed significance when $\alpha < 0.05$ was considered. ANOVA (Table 3) revealed that the relative frequency of isolation (RIF) was different among sampling plots ($p = 0.0044$), sampling season ($p < 0.0001$), the different types of plant material ($p < 0.0001$) and the interaction plot*sampling material ($p = 0.0292$). The interaction among the three variables plot (P), season (S) and sampling material (SM) did not result significant. Once ANOVA was calculated for the relative frequency of isolations, the Tukey–Kramer test was applied to variables that demonstrated significant differences. Between the seasons, it was observed that RIF mean values from autumn were higher than the values from spring. Furthermore, the RIF from fallen needles and green needles were very similar to each other but lower than the ones obtained from dried needles and half-green, half-dried needles, which did not show significant differences between them either.

3.2 Environmental quality evaluation according to lichens

The lichen species identified in each plot, their biotype and sensitivity are shown in Table 4. A total of 23 species of lichens were identified. *Pleurococus viridis, Cetraria clorophylla, Cetraria sepincola, Coelocaulon aculeatum*, *Evernia prunastri, Hypogymnia physodes*, *Hypogymnia tubulosa*, *Lecanora carpinea*, *Lecanora chlarotera*, *Lecanora varia*, *Pseudevernia furfuracea* and *Usnea hirta* were the most frequent. Crustacean biotype belonged to 30.4% of the species, whereas 43% and 26% were foliaceous and fruticulosus, respectively. Furthermore, 17.39% of the species found were tolerant types, while 47.8% of them were intermediate and 4.3% sensitive. IAP values obtained were between 18 and 25, and there was no instance of an IAP value of 0, which would correspond to the state of lichenic desert. After the results, the study area was divided into three environmental quality sites according to IAP values: polluted, 0–10; (not represented in our study); moderately polluted, 10–20 (plots 5, 6, 7); and not strongly polluted, 20–30 (plots 1, 2, 3, 4, 8). Results from ANOVA, evaluating the plot, biotype and sensitivity, revealed that the number of lichenic species was significantly different among biotypes ($p < 0.0001$), sensitivity types ($p < 0.0001$) and for the interaction biotype*sensitivity ($p < 0.0001$).

Table 2. Fungal species distribution and isolation frequencies. The isolation frequencies for each species are the percentages relative to the total number of samples from each season (autumn and spring) and each sampling plant material (G, green needles; D, dried needles; HG-HF, half green-half dried needles; F, fallen needles).

		Autumn (AU)				Total	Spring (SP)					
Fungi	Plot[1]	G	D	HG-HD	F	AU	G	D	HG-HD	F	Total SP	Total
Alternaria complex (Fr.) Keissler	1,2,3,4,5,6,7,8	53.1	31.3	59.4	6.3	37.5	96.9	84.4	90.6	18.8	72.7	55.1
Lophodermium pinastri (Schrad.) Chev	1,2,3,4,5,6,7,8	6.3	78.1	34.4	93.8	53.1	6.3	46.9	18.8	96.9	42.2	47.7
Cladosporium herbarum (Pers) Link ex. S.F.	1,2,3,4,5,6,7,8	37.5	43.8	68.8	34.4	46.1	28.1	46.9	37.5	31.3	35.9	41.0
Sclerophoma pithophylla (Cda.) Höhn	1,2,3,4,5,6,7,8	56.3	53.1	81.3	34.4	56.3	18.8	18.8	21.9	15.6	18.8	37.5
Naemacyclus niveus (Pers. ex Fr.) Fuck. ex Sacc	1,2,3,4,5,6,7,8	6.3	43.8	12.5	71.9	33.6	6.3	31.3	12.5	71.9	30.5	32.0
Cylindrocarpon sp. Wollenw	1,2,3,4,5,6,7,8	50.0	34.4	53.1	12.5	37.5	18.8	28.1	15.6	25.0	21.9	29.7
Leptostroma pinastri (Desm.)	1,2,3,4,5,6,7,8	31.3	53.1	40.6	31.3	39.1	12.5	25.0	9.4	28.1	18.8	28.9
Stachybotrys sp. Corda	1,2,3,4,5,6,7,8	25.0	15.6	25.0	6.3	18.0	43.8	34.4	53.1	6.3	34.4	26.2
Cladosporium sp. Link Fr.	1,2,3,4,5,6,7,8	21.9	28.1	25.0	21.9	24.2	21.9	18.8	28.1	28.1	24.2	24.2
Epicoccum nigrum Link ex Fr.	1,2,3,4,5,6,7,8	28.1	31.3	34.4	3.1	24.2	25.0	25.0	37.5	9.4	24.2	24.2
Cytospora sp. Ehrenb. ex Fr.	1,2,3,4,5,6,7,8	12.5	18.8	34.4	28.1	23.4	0.0	0.0	9.4	3.1	3.1	13.3
Penicillum sp. Link ex Fr.	1,2,3,4,5,6,7,8	0.0	0.0	0.0	0.0	0.0	37.5	31.3	28.1	9.4	26.6	13.3
Unidentified Anamorphic fungi 1	1,2,3,4,5,6,7,8	6.3	12.5	9.4	9.4	9.4	3.1	15.6	9.4	15.6	10.9	10.2
Unidentified Anamorphic fungi 8	1,2,4,5,6,7,8	9.4	12.5	21.9	15.6	14.8	0.0	0.0	3.1	0.0	0.8	7.8
Helmintosporum sp. Link ex Fr.	4,5,6,7,8	6.3	15.6	15.6	6.3	10.9	0.0	0.0	3.1	0.0	0.8	5.9
Sphaeropsis sapinea (Fr.) Dyko and Sutton	1,2,3,5,6,7,8	12.5	15.6	3.1	0.0	7.8	0.0	0.0	0.0	0.0	0.0	3.9
Unidentified Anamorphic fungi 7	2,3,5,7,8	6.3	6.3	6.3	12.5	7.8	0.0	0.0	0.0	0.0	0.0	3.9
Unidentified Anamorphic fungi 5	1,2,4,5,6,8	3.1	3.1	9.4	3.1	4.7	3.1	0.0	3.1	0.0	1.6	3.1
Chaetomium cochliodes Palliser	1,2,5,6,7,8	0.0	0.0	0.0	0.0	0.0	12.5	0.0	9.4	0.0	5.5	2.7
Unidentified Anamorphic fungi 4	1,3,4,5,8	3.1	0.0	9.4	3.1	3.9	0.0	6.3	0.0	0.0	1.6	2.7
Unidentified Anamorphic fungi 6	2,4,6,7,8	3.1	3.1	6.3	6.3	4.7	0.0	0.0	3.1	0.0	0.8	2.7
Cylindrocladium sp. Morgan	1,2,3,6,8	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1	12.5	4.7	2.3
Trichotecium roseum (Persoon) Link ex S.F. Gray	2,3,6,7	3.1	0.0	6.3	3.1	3.1	0.0	3.1	0.0	0.0	0.8	2.0
Unidentified Anamorphic fungi 3	1,6,7,8	3.1	9.4	3.1	0.0	3.9	0.0	0.0	0.0	0.0	0.0	2.0
Gonatobotrys sp. Corda	2,5,7,8	0.0	0.0	12.5	0.0	3.1	0.0	0.0	0.0	0.0	0.0	1.6
Nectria sp. 5.6	0.0	0.0	0.0	0.0	0.0	0.0	9.4	0.0	0.0	2.3	1.2	
Stachylidium sp. Link. ex S.F. Gray	2,6,7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.4	0.0	2.3	1.2
Fusarium roseum Link emend. Snyd and Hans	6.8	6.3	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.8
Unidentified Anamorphic fungi 2	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1	1.6	0.8
Verticillum sp. Nees. ex Link	1	3.1	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.4

[1]Plots where fungi were isolated from.

Table 3. ANOVA table for the relative fungal isolation frequencies.

Source	df	MS	*F*-value	p-value
Model	42	0.0855	3.79	0.0009
Plot	7	0.0967	4.28	0.0044
Season	1	0.6858	30.36	<0.0001
Sampling material	3	0.3729	16.51	<0.0001
P*S	7	0.0221	0.98	0.4710
S*SM	21	0.0337	1.49	0.1826
P*SM	3	0.0824	3.65	0.0292

df, degrees of freedom; MS, mean squares; P, plot; S, season; SM, sampling material.

3.3 Botanical quality evaluation according to mosses

A total of 19 moss species were identified. Plots 1 and 2 had the highest species richness, while plots 5, 6 and 7 had the lowest. Table 5 shows a list of the mosses identified at each plot and the RSPC of each moss species. The following species

Table 4. Relationship of lichen species found in each plot, biotype that they presented and species sensitivity obtained from bibliographic reference (Calatayud and Sanz 2000).

Species	Biotype[1]	Sensitivity[2]	Plots[3]
Algae			
Pleurococcus viridis Agardh.	C	T	1,2,3,4,5,6,7,8
Lichens			
Calophaca ferruginea (Huds.) Th. Fr.	C	I	5,6,7,8
Cetraria clorophylla (Willd.) Vainio	Fl	I	1,2,3,4,5,8,7,8
Cetraria islandica (L.) Ach.	Fr	U	1,2,4
Cetraria sepincola (Ehrh.) Ach.	Fl	U	1,2,3,4,5,6,7,8
Cladonia sp. Hill ex Browne	Fr	D	1,2,3,4
Coelocaulon aculeatum (Schaer.) Link	Fr	U	1,2,4,5,6,7
Evernia prunastri (L.) Ach	Fr	I	1,2,3,4,5,6,7,8
Hypogymnia farinacea Zopf	Fl	I	2,3,4
Hypogymnia physodes (L.) Nyl	Fl	T	1,2,3,4,5,6,7,8
Hypogymnia tubulosa (Schaer.) Havaas	Fl	T	1,2,3,4,5,6,7,8
Lecanora carpinea (L.) Vainio	C	I	1,2,3,4,5,6,7,8
Lecanora chlarotera Nyl.	C	I	1,3,4,5,6,8
Lecanora varia (Hpffm.) Ach	C	U	1,2,3,6,7,8
Parmelia glabra. (Schaer.) Nyl.	Fl	U	1,2,3,4
Parmelia subauriphera Nyl.	Fl	I	5,6,7,8
Parmelia sulcata Taylor	Fl	I	1,2,4
Parmeliopsis ambigua (Wulfen) Nyl.	Fl	I	5,6,7
Platismatia glauca (L.) Club.and C. Club.	Fl	I	3,4
Pseudevernia furfuracea (L.) Zopf	Fr	I	1,2,3,4,5,6,7,8
Rinodina corticola (Arnold.) Arnold	C	U	5,8
Scoliciosporum umbrinum (Ach.) Arnold	C	T	6,7,8
Usnea hirta (L.) Wigg.	Fr	S	1,2,3,4,5,6,7

[1]C, Crustaceous; Fl, Foliaceous; Fr, Fruticulosus.
[2]T, Tolerant; I, Intermediate; U, Unknown; D, Depend on species; S, Sensitive.
[3]Plots where lichens were found.

Table 5. Mosses species found in each plot.

Species	Plots[1]	RSPC
Aulacomnium androgynurr (Hedw.) Schwagr.	1,2,3,7,8	73.68
Brachythecium salebrosum (F. Weber and D. Mohr) Schimp	5,6,7,8	78.95
Ceratodon purpureus (Hedw.) Brid.	1,2,3,4,5,6,8	63.16
Dicranoweisia cirrata (Hedw.) Milde	2,3,4	84.21
Dicranum scoparium Hedw.	1,2,3,4	78.95
Didymodon vinealis (Brid.) Zander	2,4	89.47
Grimmia decipiens (Schultz) Lindb.	2,3	89.47
Grimmia pulvinata (Hedw.) Sm.	1	94.74
Hedwigia stellata Hedenas	5,6	89.47
Hypnum cupressiforme Hedw.	1,2,4,5,6,8	68.42
Pohlia elongata Hedw.	6,7,8	84.21
Polytrichum piliferum Hedw.	5,6,7,8	78.95
Racomitrium canescens (Hedw.) Brid.	1	94.74
Racomitrium elongatum Frisvoll	1,2,3,4,5,8	68.42
Racomitrium heterostichum (Hedw.) Brid.	7,8	89.47
Rhytidiadelphus triquetrus (Hedw.) Warnst.	1,2,3,4	78.95
Scleropodium purum (Hedw.) Limpr.	1,2,3,4	78.95
Syntrichia ruralis (Hedw.) F. Weber and D. Mohr	3	94.74
Tortula subulata Hedw.	1, 7	89.47

RSPC, rarity specific coefficient.
[1]Plots where mosses were found.

Aulacomnium androgynum, *Ceratodon purpureus*, *Hypnum cupressiforme* and *Racomitrium elongatum* had the highest number of representation. Four groups of decreasing botanical quality were made according to the quartiles of the results: group 1 (3rd quartile (Q_3); plots 1 and 2), group 2 (2nd quartile (Q_2); plots 3 and 4), group 3 (1st quartile (Q_1); plots 5 and 6) and group 4 (plots 7 and 8).

3.4 Correlation between indexes

A correlation matrix was performed to compare the variables of the plots (n = 8) among them (Table 6). The IAP had a strong positive correlation with the BQ measured by mosses ($r = 0.9639$, p = 0.0001) and a negative correlation with the total RIF ($r = -0.7229$, p = 0.0427), whereas at the same time, the BQ was also negatively correlated with the total RIF ($r = -0.810$, p = 0.0149). Botanical quality was also negatively correlated with spring RIF ($r = -0.738$, p = 0.0366) and with RIF from dried ($r = -0.7305$, p = 0.0396) and half-green, half-dried samples ($r = -0.755$, p = 0.0305). The abundance of tolerant lichens was positively correlated with the abundance of crustaceous species ($r = 0.963$, p = 0.0001), total RIF ($r = 0.740$, p = 0.0360), autumn RIF ($r = 0.933$, p = 0.0007) and with dried ($r = 0.750$, p = 0.0321) and half-green, half-dried RIF ($r = 0.854$, p = 0.007) and negatively correlated with the abundance of fruticulosus species ($r = -0.871$, p = 0.0048). Nevertheless, pathogenic RIF did not show a correlation with any air quality indexes but was positively correlated with abundance of intermediate species ($r = 0.716$, p = 0.0456). The abundance of crustaceous lichens had a negative correlation with the abundance of both foliaceous ($r = -0.783$, p = 0.0215) and fruticulosus species ($r = -0.861$, p = 0.0061) and a positive correlation with autumn RIF ($r = 0.855$, p = 0.0068), dried needles RIF ($r = 0.704$, p = 0.050) and half-green, half-dried needles RIF ($r = 0.783$, p = 0.0215). In addition, the frequency of fruticulosus lichens was negatively correlated with autumn RIF ($r = -0.816$, p = 0.0135) and with half-green, half-dried RIF ($r = -0.719$, p = 0.0442). Among RIF, some correlations were also shown. The total RIF was positively correlated with autumn (p = 0.0024), spring (p = 0.0039), dried needles (p = 0.0075) and with half-green, half-dried needles RIF ($p < 0.0001$). Furthermore, both autumn and spring RIF were positively correlated with dried and half-green, half-dried needles RIF. Finally, a high correlation was also found between dried and half-green, half-dried needles RIF ($r = 0.8675$, p = 0.005).

4 Discussion

The number of fungal taxa obtained in the study was in accordance with other previous surveys on *P. sylvestris* (Zamora et al. 2008) and other hosts (Collado et al. 2000; Danti et al. 2002; Santamaria and Diez 2005; Botella et al. 2010; Botella and Diez 2011). Many of them were dominant species that failed to show site preference, these included *Alternaria complex*, *C. herbarum, Cladosporium* sp.*, E. nigrum, L. pinastri, Lophodermium pinastri, N. niveus, Penicillum* sp.*, S. pithyophila* and *Stachybotrys* sp. It is widely known that these species have a cosmopolitan behaviour (Zamora et al. 2008) but some are associated with pathogenicity: *Cytospora* sp.*, F. roseum, L. pinastri, Lophodermium pinastri, N. niveus, S. pithyophila, S. sapinea* and *Verticillium* sp.. Also found were some secondary colonizers whose frequent presence can be related to the disturbances the plant suffers. The decline observed in the stands of the present study may perhaps be produced by fungal infections. The highest RIF were found in dried tissues (dried and half-green, half-dried needle samples) and were significantly different from healthy tissue (green needles), which was previously observed in other surveys (Hata and Futai 1995; Bettucci and Alonso 1997; Santamaria and Diez 2005; Zamora et al. 2008).

Plots with the lowest IAP values (plots 5, 6 and 7) were supposed to have had higher pollutant presence as already observed (Levin and Pignata 1995; Jeran et al. 2002; Scerbo et al. 2002; Mayer et al. 2009). They were located downwind of the thermal power plant and inside an area of maximum influence; it is known that the direction of this dominant wind is related to the presence of pollution in lichens. Previous studies obtained in different countries (Case 1980; Nimis 1990; Calvo and Sanz 2000) also observed that areas under the influence of dominant wind patterns usually have the lower IAP values. Through IAP calculation, an analysis of lichens permitted the evaluation of air quality with regard to the presence of different environmental contaminants (Conti and Cecchetti 2001). In the present study, the ANOVA table of lichen taxa and their sensitivity suggested that the number of certain species present in each plot was related to their sensitivity. Fernández-Salegui and Terrón (2003) found that the most sensitive species disappear with the first environmental changes, while tolerant ones continue that would explain the positive correlation of the abundance of tolerant with crustaceous lichens, which is the less sensitive biotype, and the negative correlation of tolerant with fruticulosus lichens (most sensitive) (Ederra 1997; Jeran et al. 2007). In our study, the more crustaceous species the plots have, the less foliaceous and fruticulosus species were present. Nevertheless, not all bibliographic references coincide about sensitivity (Blasco et al. 2008).

Our results showed that BQ was highly positively correlated to IAP values, suggesting that low air quality areas presented the less botanical quality. It has been widely stated by other authors (Rao 1982; Nimis et al. 1994; Markert et al. 1996a,b; Jeran et al. 2003) that the composition of moss flora is strongly related to atmospheric pollution; as the concentration of pollutant increases in a giving area, the number of species present in that area decreases. For instance, Zechmeister and Hohenwallner (2006) observed that SO_2 had a negative influence on the presence of bryophytes in the town of Linz (Austria).

In our study, the total RIF was negatively correlated with IAP and BQ and positively correlated with the abundance of tolerant lichen species, which indicated that the places with lower air quality had higher numbers of fungal isolates and therefore poorest forest health conditions. Furthermore, dried and half-green, half-dried needles RIF, which had the highest RIF values, were also negatively correlated with botanical quality and showed a positive correlation with the abundance of tolerant and crustaceous lichens. Nevertheless, no correlation among pathogenic RIF and air quality was observed. This could be explained because some fungi, not grouped as pathogenic in the present study, could play a secondary role in the decline processes of forest trees due to being endophytic (Giordano et al. 2009). It was previously reported that some tree endophytes are mostly harmless colonizers of healthy plant tissues. In spite of their potential pathogenicity, disease or decline is only caused in combination with other inciting factors, otherwise they may remain latent (Sieber 2007). On the contrary, previous studies carried out under controlled conditions (Helander and Rantio-Lehtimäki 1990; Ranta 1990;

Table 6. Spearman's correlations for all variables.

	BQ	IAP	Tolerant abundance	Intermediate abundance	Sensitive abundance	Crustaceous abundance	Foliaceous abundance	Fruticulosus abundance	RIF Total	RIF Pathogenic	RIF Autumn	RIF Spring	RIF Green	RIF Dried	RIF HG_HD
BQ															
IAP	0.964														
Tolerant abundance	−0.570	−0.515													
Intermediate abundance	−0.552	−0.596	−0.275												
Sensitive abundance	−0.447	−0.465	0.221	0.527											
Crustaceous abundance	−0.647	−0.612	0.963	−0.198	0.218										
Foliaceous abundance	0.335	0.382	−0.683	0.420	−0.051	−0.783									
Fruticulosus abundance	0.677	0.620	−0.871	−0.026	−0.151	−0.861	0.385								
RIF Total	−0.810	−0.723	0.740	0.184	0.524	0.695	−0.431	−0.600							
RIF Pathogenic	−0.299	−0.352	−0.146	0.716	0.514	−0.259	0.548	−0.026	0.275						
RIF Autumn	−0.683	−0.600	0.933	−0.080	0.340	0.855	−0.506	−0.816	0.898	0.120					
RIF Spring	−0.738	−0.687	0.473	0.209	0.396	0.491	−0.491	−0.268	0.881	0.168	0.635				
RIF Green	−0.518	−0.354	0.202	0.304	0.420	0.285	−0.079	−0.252	0.446	−0.091	0.236	0.446			
RIF Dried	−0.731	−0.600	0.750	−0.012	0.058	0.704	−0.391	−0.700	0.850	0.048	0.880	0.707	0.285		
RIF HG_HD	−0.755	−0.679	0.854	0.043	0.424	0.783	−0.481	−0.719	0.970	0.229	0.964	0.790	0.333	0.867	
RIF Fallen	0.024	−0.115	−0.146	0.056	0.398	−0.078	−0.404	0.488	0.120	0.030	−0.108	0.395	−0.164	−0.205	0.006

Correlations were considered significant at $p < 0.05$ (in bold font).
Considered variables: BQ, botanical quality; IAP, index of atmospheric purity; tolerant, intermediate and sensitive abundance, abundance of lichen species; crustaceous, foliaceous and fruticulosus abundance of species of each biotype; RIF, relative isolation frequency (total, pathogenic, autumn, spring, type of needles: green; dried; hg-hd, half-green, half-dried; fallen).

Helander et al. 1993, 1994; Helander 1995; Asai et al. 1998) showed that endophytic fungi were affected by atmospheric pollution with a subsequent reduction in their presence, probably because of a reduction in spore germination and hyphal growth (Danti and Sieber 2004). However, some of these studies were conducted with symptomless material, while symptoms of decay were observed in all the plots of the present study before sampling.

Regarding pathogenic infections, there is not a homogeneous effect of air pollutants on plant diseases. Some pathogenic fungi that cause specific diseases are favoured by pollutants presence, and they produce a stimulatory effect increasing the disease incidence (Helander 1994; Magan et al. 1995). Conversely, the incidence of some diseases is decreased by the presence of pollutants, producing an inhibitory effect (Wani et al. 1997; Khan et al. 1998). Nevertheless, it is likely that forest pathogenic fungi behaviour vary with the gradients of pollutants as previously described (Khan and Khan 2011). Further studies are recommended to establish the relationships between air quality, effects of thermal power plants and pathogenic fungi in *P. sylvestris* stands.

5 Conclusions

Based on the results of the present study, it can be concluded that air quality measured by lichens, mosses and frequency of fungi associated with needles are negatively correlated. Thus, decline symptoms observed in the study stands (i.e. chlorosis, defoliation and discoloration) may be produced by fungal infections. Lichens, mosses and fungi methods of analysis require little time and inexpensive equipment; therefore, they are likely a useful tool in first step evaluation of forest ecosystem health. Analysis of fungi associated with needles can be combined with air pollution indicators such as lichens and mosses, which are already known as effective bioindicators. Nevertheless, further studies are recommended to establish the effects of atmospheric pollution in fungal communities in Scots pine (*P. sylvestris*) stands.

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