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Response of Epiphytic Microflora from *Pinus sylvestris* Needles to Alkaline Deposition

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Plant surfaces are colonized by various microorganisms including mycelial fungi, yeasts, bacteria and algae. One of the major functions of this normal epiphytic flora is protecting the plant from invasion by pathogens (Blakeman and Fokkema 1982). Since the epiphytic microbial communities are in direct contact with the air they are also exposed to anthropogenic pollutants. The detrimental effect of heavy metals (Bewley and Campbell 1980), artificial acidification (Helander and Rantio-Lehtimäki 1990; Ranta 1990), and ozone and sulphur dioxide (Fenn *et al.* 1989) on the phyllosphere microflora of trees have been reported. Recently, Dowding and Richardson (1990) have highlighted the impact of air pollution in Europe using epiphytic yeasts as a biological monitor.

Alkaline aerosols are introduced into the environment from industrial sources such as fuel combustion, clay products manufacture, cement and concrete processing, and iron and steel manufacture (Anttila 1990). In this paper we report on the influence of alkaline deposition on the epiphytic microflora of Scots pine (*Pinus sylvestris* L.) needles.

MATERIALS AND METHODS

The Koverhar iron and steel works was established in 1961 and is located on the Baltic sea cost in the southern part of Finland. In 1989 the reported emissions from the point source were 1416 tons SO_2, 490 tons NO_x and 2026 tons dust annually (data quoted from the factory 1990). According to Fritze (1991) the dust contains Fe, Zn, and Al, and is highly alkaline in nature. This is reflected in the raised pH of forest soil (from pH 4.1 to 6.6) and bark (from pH 3.7 to 4.8) of the Scots pines (*Pinus sylvestris* L.) from the affected area. Additionally the epiphytic lichen species diversity has been shown to be reduced due to the pollution (Fritze 1991). Eight study sites were established; four in the affected area, and four in the control area (see Fritze 1991). Two of the control and polluted sites were located upwind or downwind, respectively, from the point source. All the study sites were situated in a dry *Calluna*-type (Cajander 1949) Scots pine forest.

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At each study site five Scots pines were chosen (mean age 40 to 60 years) from which twigs, at a height of between 4 to 5 m, were removed and stored in sterile glass bottles. Samples were collected on five occasions: 13.6, 27.6, 17.7, 4.9, and 18.9 1990.

In the laboratory needles, representing the growth of 1988, were aseptically cut from the twigs. Nine individual needles were washed in 5 ml sterile 1/4 strength Ringer solution (Merck no. 15525) adjusted to 0.5 % (weight/vol) with Tween 80. Five replicates (trees) were used for one study site. After overnight incubation on a rotary shaker (210 rpm) at room temperature a dilution series of the washing solution was plated on 2 % malt agar (0.05 g chloramphenicol l^{-1}) to determine the number of fungal colony forming units (CFU) and on a nutrient agar (containing per liter tap water: meat extract 1,26 g, yeast extract 1.26, agar 12 g, 0.05 g cycloheximide) to determine the number of bacterial CFU.

The fungal plates were counted after 4 days incubation at 15 °C. Using plates having more than 10 CFU, 100 fungal colonies from the affected and the control areas, respectively, were isolated in pure culture for identification to the genus level. The bacterial CFU was counted after 7 days incubation at 15 °C and after 10 days the fungal CFU counts were repeated.

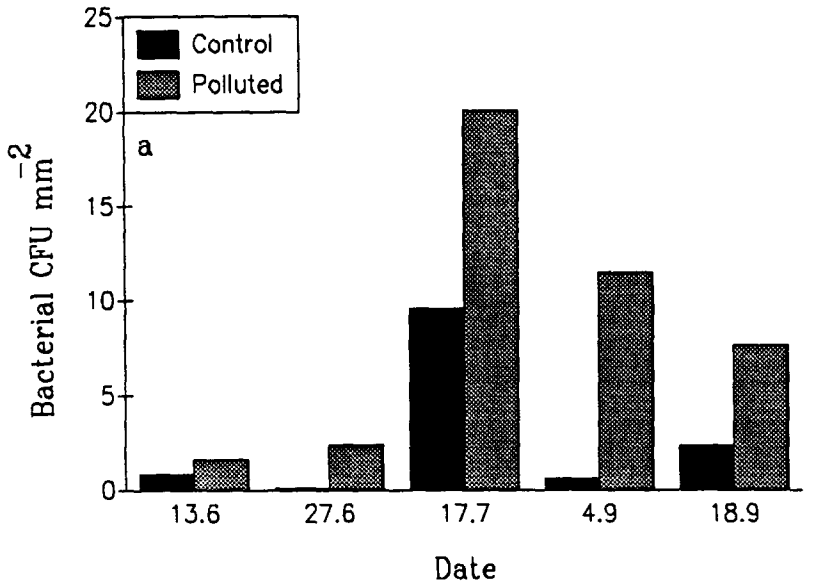
The CFU of the bacteria and fungi are expressed per area of needles used. For this the needle length and width was measured with a caliber rule after the experiment.

Nested analyses of variance was used to test the data for differences between the affected and control area and for the significance of wind direction. Natural logarithm transformations were performed on all data, except to one bacterial data (18.9) which needed a sin transformation, to improve the normality of the bacterial or fungal CFU data within one collecting date. As an error term for the highest level area x wind direction was used.

RESULTS AND DISCUSSION

On all sampling dates bacterial numbers were higher on the pine needles from the affected area as compared to the control needles (Fig. 1a). The difference between areas was significant for two sampling dates (Table 1). The prevailing wind direction influenced the data. The difference between the polluted and control trees was much more pronounced at the downwind study sites, where significant differences were detected on three sampling dates (Table 1).

Compared to the bacterial colonization, fungal colonization showed an opposing trend (Fig. 1b). Except for one occasion, more fungal colonies were isolated from needles of the control area as from the affected area.



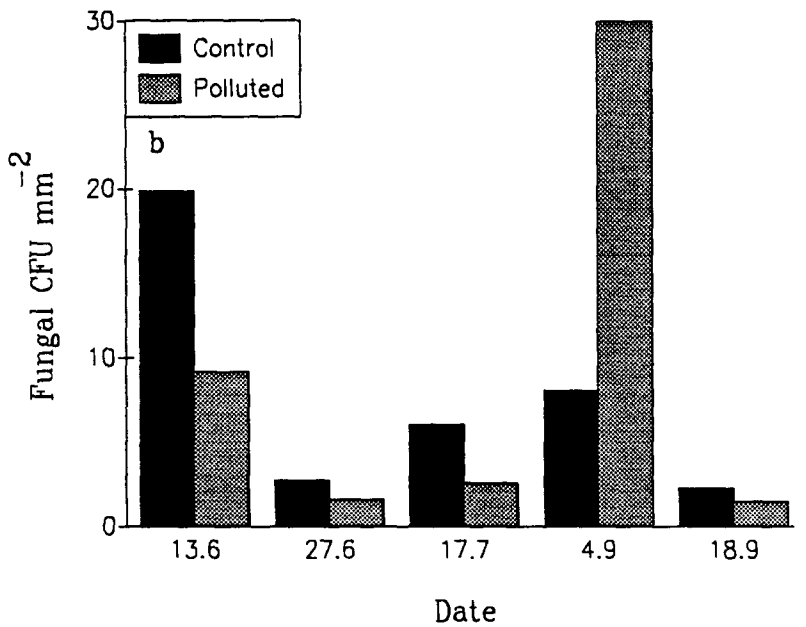


Figure 1. Bacterial (a) and fungal (b) colony forming unit (CFU) of Scots pine (*Pinus sylvestris*) needles from alkaline deposition affected and control areas on five sampling dates

Table 1. Analysis of variance for the effects of alkaline deposition and wind direction on the numbers of bacteria isolated from pine needles on the five sampling dates

		June	13	June	27	July	17	Sept	4	Sept	18
Sour	df	SS	F	SS	F	SS	F	SS	F	SS	F
area	1	52.4	2.26	389	48.2[a]	215	5.21	198	19.2[a]	0.60	1.47
wind	2	46.5	4.75[a]	16.1	2.71	82.5	13.2[c]	20.6	4.61[a]	0.82	1.42
tree	36	176		107		112		80.6		10.4	

Sour = source; [a] = $p < 0.05$; [c] = $p < 0.001$

An influence of the prevailing wind direction can again be seen (Table 2). The difference between the polluted and control trees was much more pronounced at the downwind study sites, where significantly higher fungal needle colonization of the control trees was measured on two of the sampling dates.

Table 2. Analysis of variance for the effects of alkaline deposition and wind direction on the numbers of fungi isolated from pine needles on the five sampling dates

		June	13	June	27	July	17	Sept	4	Sept	18
Sour	df	SS	F	SS	F	SS	F	SS	F	SS	F
area	1	25.2	1.73	4.71	1.24	2.84	0.68	12.0	60.4[c]	7.68	0.45
wind	2	29.2	10.1[c]	7.59	3.89	8.33	4.99[a]	0.39	0.25	33.8	16.5
tree	36	51.8		35.1		30.1		28.1		36.8	

Sour = source; [a] = $p < 0.05$; [c] = $p < 0.001$

Of the fungal genera *Aureobasidium* was the most frequently isolated and it appeared to be present in much greater numbers on the needles from the control area at all the sampling times (Table 3). Other fungal genera were rare and showed no value as bioindicators of alkaline deposition. Sterile hyphae were predominant on the needles of the affected area. Using the solution resulting from needle washing (as in this study) tends to select sporulating fungi, however, the predominance of *Aureobasidium* may also be due to the yeast-like growth habit of the fungus as each separate cell can form an individual colony.

This study thus shows a trend towards a denser bacterial and sparser fungal colonization of the phylloplane of pine needles collected from an area receiving alkaline deposition as when compared to control needles. The fungal genera most

frequently isolated at all study sites was *Aureobasidium* and therefore the reduction of the fungal CFU in the area receiving alkaline deposition is mainly due to the reaction of this genus. From studies involving artificial acidification *Aureobasidium pullulans* has been reported to be the most abundant fungus on birch leaves (Helander and Rantio-Lehtimäki 1990) and spruce needles (Ranta 1990), in both cases the fungal CFU was reported to decrease in response to the acidic treatment. Fenn *et al.* (1989) detected a decrease of *A. pullulans* on the leaves from Valencia orange (*Citrus sinensis* L.) following to the fumigation with SO_2. Metallic pollution from a smelter revealed a negative correlation between the numbers of *A. pullulans* isolated from Hawthorn leaves and the emitted heavy metals (Zn, Pb, and Cd) (Bewley and Campbell 1980) but *Sporobolomyces roseus* was, according to their results, a better bioindicator of metallic pollution.

Table 3. Percentage of fungal genera composition at five sampling dates

Date	13.6		27.6		17.7		4.9		18.9	
Genera	pol	con	pol	con	pol	con	pol	con	pol	con
	%		%		%		%		%	
Alternaria	0	0	1	0	1	0	0	0	0	0
Aureobasidium	88	100	47	94	76	99	94	100	72	84
Cladosporium	0	0	0	2	6	1	1	0	7	0
Epicoccum	0	0	1	0	0	0	0	0	0	0
Fusarium	0	0	0	0	1	0	0	0	0	0
Sterile hyphae	12	0	31	2	15	0	4	0	21	16
Penicillium	0	0	0	2	1	0	1	0	0	0
Torula	0	0	1	0	0	0	0	0	0	0
Yeasts	0	0	19	0	0	0	0	0	0	0

pol = polluted area, con = control area

The results suggest that the fungal CFU and the isolation frequency of *A. pullulans* from the phylloplane of a plant species where this fungus dominates can be used as a biomonitor for assessing air quality. High variation of the fungal CFU within one tree (Ranta 1990) and between trees (this study) call for a standardization of the method.

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