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Letter to Editor

Effect of Polluted Substrate on Growth and Health of Silver Birch (*Betula pendula Roth.*)

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Abstract

From May till October 1999, 1-year-old birch seedlings were grown in a greenhouse in a substrate (forest soil under a birch tree + perlite, 1:1), without and with added aluminium chloride (40 and 160 mg Al dm^{-3}). The added aluminium chloride inhibited the growth of seedlings, especially of their roots. At the end of the experiment the substrate treated with aluminium chloride contained more Al and Cl than the control. In comparison with control plants, the treated plants did not differ in assimilation of Ca, Mg and K ions, but their leaves and roots contained more Al. Disease symptoms on leaves of treated seedlings were similar to those observed on birch trees growing in a contaminated area (near a phosphate fertilizer factory in Luboń). From collected birch leaves, symptomatic and asymptomatic, 18 species of fungi were isolated. From leaves of treated seedlings and of trees growing in the polluted area some fungal species were isolated more often than from control seedlings. Those species included: *Aureobasidium pullulans, Hormonema dematioides, Alternaria alternata*, and *Cladosporium herbarum*.

Keywords: *Betula pendula,* development, aluminium chloride, soil pollution, fungi

Introduction

Among broad-leaved trees, birch is regarded as tolerant to effects of industrial pollution, including aluminium ions [1-3]. However, birch decline was often observed on experimental plots located close to industrial regions [4, 5].

As a result of industrial development and intensive farming, soil is becoming more and more acidic [6, 7]. In degraded soil, toxic metal ions, like aluminium, copper, lead, zinc, or cadmium, accumulate. The high concentration of those ions in the soil causes not only morphological changes in roots but also necroses or even death of roots [8-10]. Such changes have a negative effect on root system development, often leading to the decline of whole forest stands [11-13].

Aluminium is one of the most common elements in the Earth's crust. Its availability to plants increases with decreasing soil pH [1, 14, 15]. A high concentration of soluble (i. e. assimilable) aluminium in the substrate results in an inhibition of the uptake of many nutrients by plants [16-18] and their use in metabolic processes [7, 33, 36, 37]. An unfavourable decrease in Ca/Al ratio is observed and the Mg content of plants also declines [16, 19]. Those changes may cause premature yellowing of leaves, inhibition of plant growth and development, and disturbances in physiological processes [20-23].

Industrial pollution affects to a large extent not only the occurrence but also development of fungi [24]. Particularly active in appearance of pathological changes become the weakness pathogens which can include endophytes [25]. A positive relationship between the frequency of endophyte occurrence and tree vitality was described [26, 27]. The influence of pollution on the endophytes may vary and can depend on many factors. Some authors have reported that acid rain treatments can reduce the number of isolated endophytes but have no effect on species com-

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position of endophytes assemblages [28, 29]. To our knowledge there is very little information available in the literature on the effect of pollutants in birch leaf fungi.

The aim of this study was to assess the influence of polluted soil and aluminium ions in the substrate on growth and health of silver birch (*Betula pendula* Roth.). During the assessment, it was important to determine the type and location of disease symptoms and to identify the fungi associated with them, in relation to the level of soil pollution and asymptomatic leaves.

Material and Methods

Effect of Aluminium on Seedling Growth

One-year-old seedlings were planted in 2-litre pots (14 cm in diameter, the substrate weighing about 1.25 kg). The substrate (pH 4.5) was composed of a mixture (1:1) of perlite with soil collected under a birch tree in the "Zwierzyniec" experimental forest near Kórnik. Some birch seedlings were treated with a solution of aluminium chloride (40 or 160 mg Al dm^{-3}) once a week. All seedlings in the experiment were also fertilized weekly with a commercial mixture of nutrients ($N\text{-}NH_4$ 2.3%, $N\text{-}NO_3$ 0.7%, K 2%, Cu 70 mg l^{-1}, Fe 400 mg l^{-1}, Mn 150 mg l^{-1}, Mo 20 mg l^{-1}, Zn 150 mg l^{-1}) at a concentration of 15 ml per 1 l of water. The experiment was established in a greenhouse in a randomized block design, in 2 blocks with 3 replications of 12 plants each. To analyze the growth dynamics of plants treated with aluminium chloride and of control plants, 4 plants were taken from each variant of the experiment (i. e. two plants per block) every 7 or 14 days. At harvest, the medium was washed from roots using distilled water, and individual plants were separated into leaves, stem and roots. All parts were oven-dried (65°C) and dry masses were determined. The area of leaves, area and length of roots were measured using image analysis system WhinSeedle and WinRhizo (Regent Instruments Inc., Quebec, Canada).

At the end of the experiments (October) concentrations of soluble forms of macro- and micronutrients and pollutants in the substrate were measured. Concentrations of selected elements were also measured in roots and leaves with methods described in an earlier paper [16].

Results of the greenhouse experiment were analyzed statistically with Statistica 98 software, and significance of differences between variants were assessed with Tukey test, at the level of 0.05 and 0.01.

Effect of Aluminium on Birch Health and Occurrence of Fungi in Leaves

Disease symptoms were observed on:

(1) birch seedlings grown in the greenhouse, in the substrate containing various concentrations of aluminium chloride (0, 40 or 160 mg Al dm^{-3}); and

(2) birch trees growing on a plot with a high aluminium content of the soil (Table 1), at a distance of 2 km from Phosphate Fertilizer Factory in Luboń.

In the greenhouse experiment, seedlings growing in the substrate without added aluminium chloride were used as the control. In the second experiment, the control was composed of trees growing in the experimental forest near Kórnik, 30 km away from the phosphate factory.

A total of 505 small fragments of the same size were cut away from leaves with disease symptoms and from visually healthy leaves (from trees growing on the polluted and unpolluted sites and from seedlings in the greenhouse). The fragments were sterilized by soaking for 30 sec in 4% calcium hypochloride and 5 sec in 0.5% sublimate. Next, they were washed three times in sterile distilled water and after air drying they were placed on malt extract agar (Merck, pH 5.5).

Results

Effect of Aluminium on Seedling Growth

Birch seedlings grown in a greenhouse and treated with aluminium chloride were characterized by poor growth of both under- and aboveground parts. The slowest growth of shoots and roots was observed in seedlings treated with the higher dose of aluminium (160 mg Al dm^{-3}). Inhibition of plant development was observed as early as in week 8 of the experiment, while in the seedlings treated with the lower dose (40 mg Al dm^{-3}), similar changes were observed 4 weeks later (Fig. 1). Aluminium chloride present in the substrate caused a reduction of root biomass (decrease in dry weight of roots), as compared to the control, as well as a re-

Table 1. Concentration of macro- and micronutrients (assimilable forms in mg per 1000 g of soil dry weight) in the control soil (from Zwierzyniec) and polluted soil (Luboń).

Nutriens, pollutants, pH	Control soil	Polluted soil
K	173	107
Ca	393	68
Mg	51	42
$S\text{-}S0_4$	70	12
Fe	87.1	46.1
Zn	3.4	1.6
Mn	34.0	5.5
Cu	0.78	0.42
Cd	0.072	0.004
Al	25.9	110.1
pH	5.8	4.2

duction in area and length of the root system (Fig. 2). The limited growth of the root system in seedlings grown in the substrate containing aluminium chloride resulted also in a reduced biomass of aboveground parts (dry weight of leaves and all aboveground parts) and smaller leaf area, in comparison with the control (Fig. 3). At the end of the experiment (7 October) the seedlings grown in the substrate with the highest concentration of aluminium ions (160 mg Al dm^{-3}) lost all leaves (Fig. 3) and 50% of them died.

A chemical analysis of the substrate, performed at the end of the experiment, confirmed that the substrate treated with aluminium chloride contained much more aluminium and chloride than the control (Table 2). Plants grown in the substrate with aluminium chloride contained also more aluminium in leaves and roots than birch seedlings grown in the control (Table 3). No significant differences in assimilability of Ca, Mg, and K ions were recorded between plants from different variants of the experiment (Table 3).

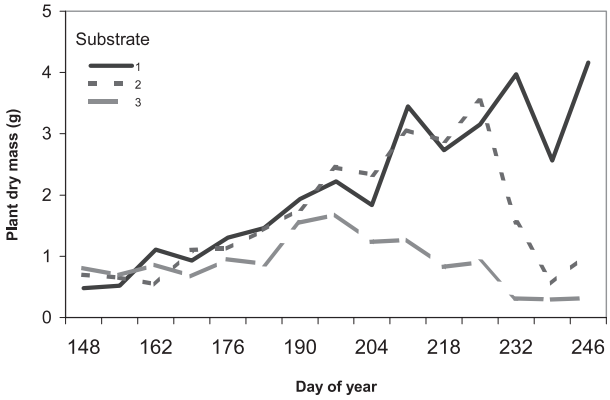

Fig. 1. Effect of aluminium chloride on growth (dry weight) of birch seedlings during the growing season (May-October).
1 = control substrate, without Al
2 = treated with 40 mg Al dm^{-3}
3 = treated with 160 mg Al dm^{-3}

Table 2. Concentration of macro- and micronutrients (assimilable forms in mg per 1000 g of soil dry weight) in the control substrate (soil from Zwierzyniec + perlite, 1: 1) and the substrate treated with aluminium chloride, at the and of the experiment (in greenhouse).

Nutrients, pollutants pH	Control substrate	Substrate treated with 40 mg Al dm^{-3}	Substrate treated with 160 mg Al dm^{-3}
Ca	395	428	428
Mg	121	104	123
Na	237	208	269
Cl	52	182	409
$S\text{-}S0_4$	30	9	15
Fe	28	26	27
Zn	6	4	3
Mn	13	11	12
Cu	0.72	0.52	0.48
Al	26.9	43.6	52.3
pH	6.1	5.5	5.1

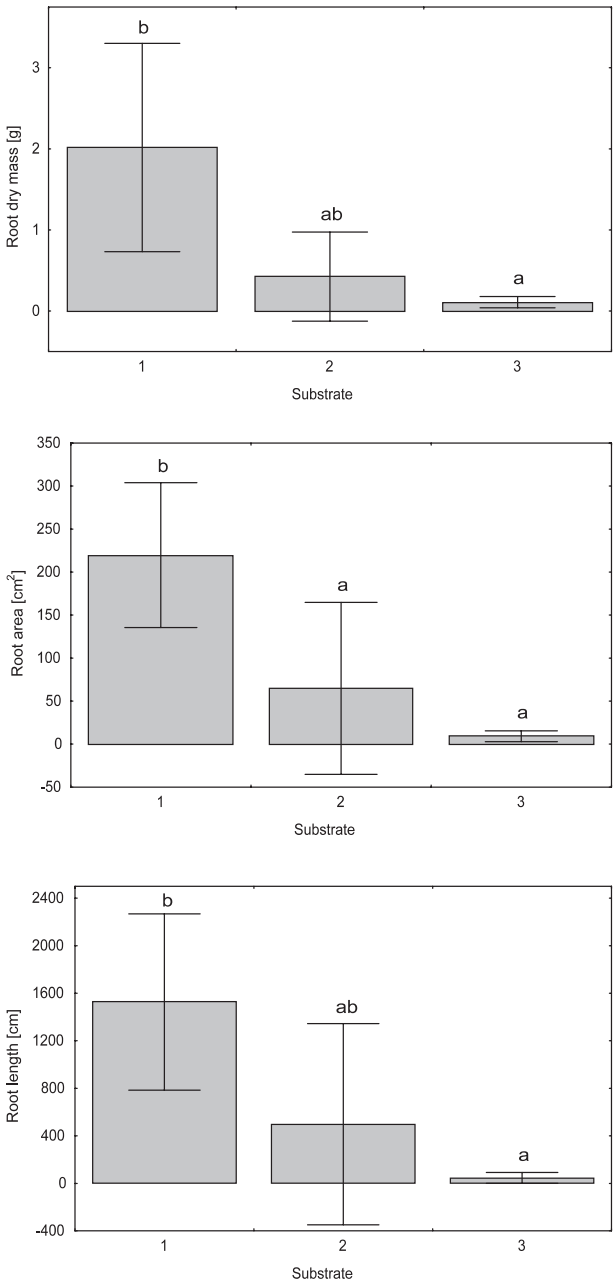

Fig. 2. Root system development in seedlings grown in the substrate treated with aluminium chloride. Values marked with the same letters are not significantly different from one another (P=0.05).
1 = control substrate, without Al
2 = treated with 40 mg Al dm^{-3}
3 = treated with 160 mg Al dm^{-3}

Disease Symptoms on Leaves

In the greenhouse experiment, macroscopic changes on leaves were observed. On plants grown in the control substrate in greenhouse conditions, the changes were similar to those observed on leaves of trees growing on the unpolluted plot (small yellow spots on leaves in the lower part of the stem). On leaves of seedlings grown on media treated with aluminium chloride and of trees growing on the polluted plot, yellow and/or brown discoloration of areas between veins (40 and 160 mg Al dm^{-3}) or of whole leaf blades (160 mg Al dm^{-3}) and browning of leaf margins were observed (Table 4).

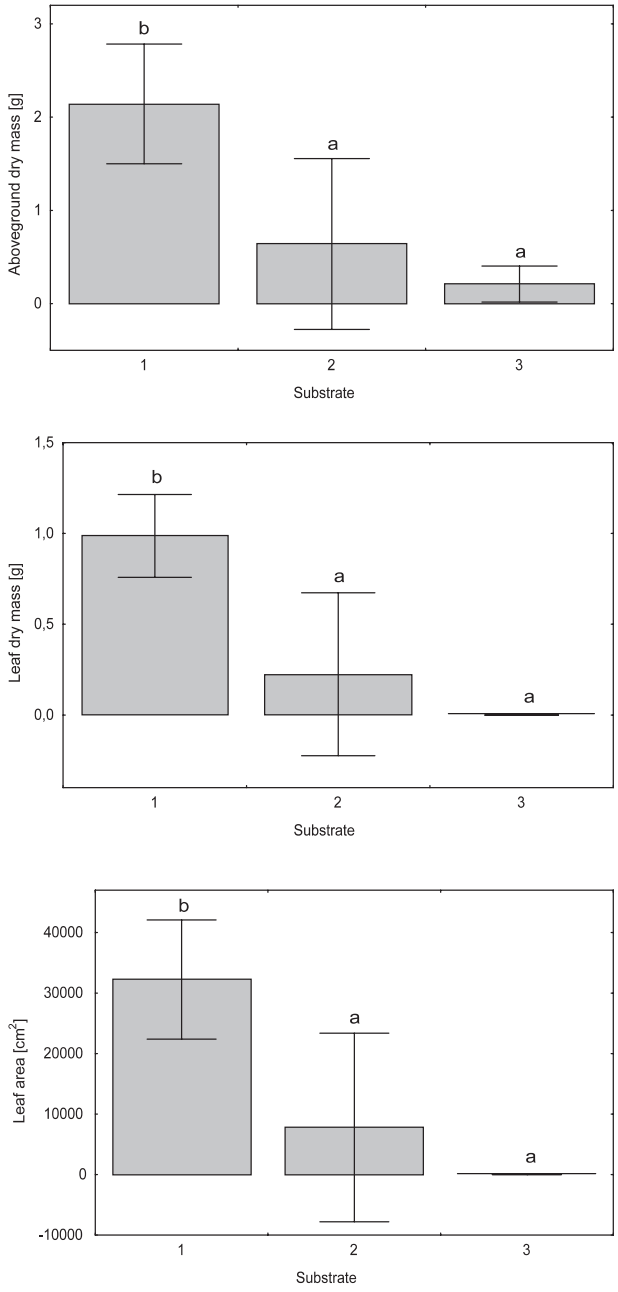

Fig. 3. Development of aboveground parts of birch seedlings grown in the substrate treated with aluminium chloride. Values marked with the same letters are not significantly different from one another (P=0.05).
1 = control substrate, without Al
2 = treated with 40 mg Al dm^{-3}
3 = treated with 160 mg Al dm^{-3}

Occurrence of Fungi

The 505 leaves sampled yielded a total of 310 fungal isolates. The greater number of isolates obtained from plants compared to control was obtained in the case of the polluted plot in Luboń (85%, control: 51.8%) and seedlings treated with 40 mg Al dm^{-3} (61.8%, control: 45.5%). The smaller number of isolates (27.3%) compared to control (45.5%), was obtained from leaves collected from seedlings grown in the substrate with the 160 mg Al dm^3 (Table 5).

The fungi isolated from leaves of birch trees represented 16 species (certain isolated fungi were classified only by genus). The greatest diversity of fungi (14 species) was found in leaves of trees from control plot (Zwierzyniec). Among the fungi isolated from leaves of trees growing on the polluted plot (Luboń), only 9 species were identified. The following species were absent in comparison with control plot: *Asteroma* sp., *Aureobasidium* sp., *Discossia* sp., *Fusicladium* sp., *Melanconium betulinum, M. stromaticum* and *Penicillium* sp. On the other hand, the fungi *Cladosporium macrocarpum* and *Ulocladium consortiale* were not found in leaves collected from birch trees grow-

Table 3. Concentration of macro- and micronutrients in leaves and roots of birch seedlings after 5 months of cultivation in a greenhouse: Ca, K and Mg (% in dry matter), Mn and Al (ppm in dry matter).

Nutrients	Plants from control substrate	Plants from substrate treated with 40 mg Al dm^{-3}	Plants from substrate treated with 160 mg Al dm^{-3}
Leaves			
K	2.41	2.14	1.94
Ca	1.3	1.2	1.2
Mg	0.70	0.74	0.71
Mn	215.0	250.0	255.0
Al	187.0	259.0	324.0
Roots			
K	0.33	0.31	0.34
Ca	1.04	0.93	1.20
Mg	0.24	0.23	0.24
Mn	91.0	165.0	195.0
Al	1041.0	1458.0	1689.0

Table 4. Disease symptoms on leaves used for isolation of fungi.

Collection site	Observed symptoms
Control plot (Z)	On lower leaves few small yellowish spots (symptomless leaves were collected for fungi isolation)
Polluted plot (L)	Yellowish discoloration of areas between veins, brown spots on leaves, leaf margins brown and wither
Control seedlings	On some lower leaves small yellowish spots, (symptomless leaves were collected for fungi isolation)
Seedlings treated with 40 mg Al dm^{-3}	Yellow discoloration between veins or on whole leaf blades, leaf margins brown and wither, leaf size smaller
Seedlings treated with 160 mg Al dm^{-3}	Yellow discolorations on whole leaf blades, leaf margins brown and wither, leaf size markedly smaller

Z – Zwierzyniec, L – Luboń

Table 5. Fungi isolated from leaves of birch trees growing on a control plot (Zwierzyniec – Z) and a polluted plot (Luboń – L) and of control and treated seedlings.

Fungal species	Frequency of occurrence (%)*				
	Trees		Seedlings		
	Control plot - Z	Polluted plot - L	Control	40 mg Al dm^{-3}	160 mg Al dm^{-3}
Alternaria alternata (Fr.) Keiss	1.2	27.6	8.0	17.6	11.7
Alternaria tenuissima (Kunze) Wilt.	2.4	6.4	14.0	5.9	5.8
Asteroma sp.	6.0	0	0	0	0
Aureobasidium pullulans (de Bary) Arnaud.	4.8	8.5	24.0	35.2	47.0
Aureobasidium sp.	1.2	0	0	0	0
Cladosporium cladosporioides (Fres.) de Vries	3.6	13.5	0	0	0
Cladosporium herbarum (Pers.) Link ex Gray	12.0	20.3	12.0	23.5	0
Cladosporium macrocarpum Preuss.	0	3.4	0	0	0
Discossia sp.	1.2	0	0	0	0
Discula betulina (West) v. Arx	30.1	3.4	36.0	0	0
Fusicladium sp.	4.8	0	0	0	0
Hormonema dematioides Lagerb. Et Melin	18.0	31.9	4.0	0	0
Melanconium betulinum Kunze et Schm.	10.8	0	0	0	0
Malanconium stromaticum Corda	1.2	0	0	0	0
Mucor heterosporus Fischer	0	0	0	2.9	5.8
Penicillium sp.	2.4	0	0	5.9	29.4
Trichothecium roseum Link	0	0	0	5.9	0
Ulocladium consortiale (Thűm.) Simmons	0	3.4	0	0	0
Non-sporulating fungi	0	17.0	0	0	0
Number of incubated leaf fragments	160	180	55	55	55
Number of isolates (%)	83 (51.8)	153 (85)	25 (45.5)	34 (61.8)	15 (27.3)

* Frequency of occurrence was defined as the percentage of isolates of individual species in relation to the total number of isolates for each both plot and for each treatment.

ing on control plot. The most common fungi (isolated in more that 20% of total number of isolates) included: *Alternaria alternata, Cladosporium herbarum* and *Hormonema dematioides* in leaves from the polluted plot, and *Discula betulina* in leaves from the control plot (Table 5).

The fungi isolated from leaves of birch seedlings represented 9 species; 6, 7 and 5 species from leaves of seedlings both non-treated and treated with 40 and 160 mg Al dm^{-3}, respectively. In leaves from the variant with the pollutant of 160 mg Al dm^{-3} *C. herbarum* was absent in comparison with both control and the dose of the 40 mg Al dm^{-3}. The most frequent (>20%) were *Aureobasidium pullulans* in both control and treated seedlings (40 and 160 mg Al dm^{-3}), *Discula betulina* in control seedlings, *Cladosporium herbarum* in seedlings treated with 40 mg Al dm^{-3}, and *Penicillium* sp. in seedlings treated with 160 mg Al dm^{-3} (Table 5).

Discussion

In this study, added aluminium chloride in the substrate inhibited the growth of birch biomass. Higher was the concentration of aluminium ions, the faster the decline of seedling health, in particular aluminium chloride strongly inhibited root system development (root length and area). However, no disease symptoms could be seen on the roots. Some authors [30] emphasize that necroses are observed on roots, as they are the most exposed to the toxic effects of aluminium ions. Aboveground parts of treated birch seedlings were also characterized by poor growth. On leaves of those seedlings some disease symptoms were noticed: chlorosis, yellow discoloration between veins, browning of leaf margins and diminishing of leaves (similar symptoms were observed on leaves of birch trees growing near Phosphate Fertiliser Factory Luboń, excluding area reduction)**.** In the opinion of some authors the changes could result from a direct influence of aluminium or shortages of some nutrients, like Ca, Mg or K and water deficiency [16, 17, 31-33]. In this study, however, birch seedlings growing in the substrate containing various concentrations of aluminium chloride did not show any differences in assimilability of Ca, Mg, and K ions, but their leaves and roots contained more aluminium than those of control plants. It seems plausible that the deficiency of water, resulting from inhibition of root development and inclusion of aluminium in metabolic processes, induced yellowing and diminishing of leaves.

Pollution in the Luboń plot evidently reduced the number of fungal species, however in this case more isolates (85%) were obtained in comparison with control plot (51.8%). Treatments of seedlings with aluminium chloride 40 and 160 mg Al dm^{-3} had an effect rather on the number of isolates than on the number of fungal species. Decrease in the number of isolates obtained from birch leaves after 160 mg Al dm^{-3} treatment and increase in the one obtained from leaves treated with 40 mg Al dm^{-3} was found.

A total of 14 species of fungi were isolated from asymptomatic birch leaves (trees: 14 species, seedlings: 6 species), but with different frequency of occurrence. *Aureobasidium pullulans* and *Fusicladium* sp., considered endophytes, were also isolated from leaves of plants subjected to simulated acid rain (28). Some of the isolated fungi also are epiphytes, e. g. *Alternaria alternata* and *Cladosporium herbarum*, which penetrate the leaf interior already at the beginning of the process of aging [34].

The most frequently isolated fungal species in the case of asymptomatic leaves of both control trees and seedlings was *Discula betulina* (30.1% and 36.0%, respectively; Table 5). This species was much rarer in leaves of trees growing on the polluted plot and absent from leaves of seedlings treated with aluminium chloride. Symptoms similar for *D. betulina* were observed only on the leaves from which the fungus was isolated. It is noteworthy that this fungus is able to cause anthracnosis on birch leaves (mainly on *Betula pendula* and *B. pubescens*) [35].

The majority of the fungi identified in this study (e. g. *D. betulina, A. alternata, C. herbarum, A. pullulans*) dominated the population of fungi isolated from leaves of declining birch trees, growing on unpolluted forest site (moist coniferous forest, Lipinki Łużyckie Forest District, West Poland). The leaves characterized by symptoms similar to those observed on aluminium treated seedlings – 40 and 160 mg Al dm^{-3} (prematurely yellowing leaves with reduced area), but they had significantly lower N, Mg and K contents than control leaves [Przybył, unpublished data]. The primary factor responsible for birch decline was related to the water deficiency in trees, resulting from prolonged periods of drought recorded in Poland a few years previous to the birch decline. Ultrastructural changes in chloroplasts of leaves of declining trees were similar to those observed during natural yellowing [32]. Therefore, is difficult to answer the question what kind of disease symptoms correlate strictly with aluminium pollution. In this moment we can suggest that *D. betulina* causing birch leaf anthracnose can be sensitive to aluminium treatments.

In summary, it must be emphasized that:

1) the isolated fungi may not be directly responsible for the symptoms visible on leaves,

2) increase in the number of isolates was found in birches growing on Luboń polluted plot and in seedlings treated with 40 mg Al dm^{-3}, (decrease in the ones was found in leaves of seedlings treated with 160 mg Al dm^{-3}),

3) the pollution on the Luboń plot had a negative effect on the total number of fungal species,

4) frequency of occurrence of *D. betulina* on Luboń plot was lower, while the frequency of *A. alternata, C. herbarum* and *H. dematioides* was evidently higher compared with asymptomatic leaves,

5) *D. betulina* occurring in the asymptomatic birch leaves of seedlings was not isolated from the ones of seedlings treated with aluminium chloride (40 and 160 mg Al dm^{-3}),

6) frequency of occurrence of *A. pullulans* was higher in leaves of seedlings treated with aluminium chloride

(40 and 160 mg Al dm^{-3}), compared with asymptomatic leaves.

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