Polish Journal of Environmental Studies Vol. 11, No. 5 (2002), 483-492

Effect of Polluted Soil and Fertilisation on Growth and Physiology of Silver Birch *(Betula pendula* Roth.) Seedlings

K. Bojarczuk, P. Karolewski, J. Oleksyn, B. Kieliszewska-Rokicka, R. Żytkowiak, M. G. Tjoelker[1]

Institute of Dendrology, Polish Academy of Sciences, 62-035 Kórnik, e-mail: bojark@man.poznan.pl
[1] Department of Forest Science, Texas A&M University, 2135 TAMU, College Station, TX 77843-2135, USA, e-mail: m-tjoelker@tamu.edu

Received: 26 March, 2002
Accepted: 17 April, 2002

Abstract

One-year-old seedlings of silver birch *(Betula pendula* Roth.) were grown in pots filled with a soil substrate that originated from an area polluted by a phosphate fertiliser factory and characterised by a high soil Al level and low Ca/Al ratio or with a substrate from an area regarded as free from toxic pollution. In addition the effect of fertilisation with a mixture of nutrients was evaluated. Birch seedlings grew slowest in the unfertilised polluted substrate. In the unfertilised polluted substrate seedlings were characterised by high biomass allocation to roots (60% vs. 30 to 40% in control or fertilised substrate), lower diversity of ectomycorrhizae and the lowest rate of root and substrate microbial respiration. Roots of seedlings grown in the polluted soil were characterised by a significantly higher level of phenolic compounds. Fertilisation of plants grown in the polluted soil accelerated their growth, and lowered RWR (g root g^{-1} plant) and increased biomass allocated to foliage. Our results indicate that elimination of air pollution does not decrease the toxic effect of a polluted soil. Fertilisation may improve the condition of seedlings growing in polluted soil, however it was not able to eliminate entirely the adverse effect of soil pollution.

Keywords: Soil pollution, plant development, mycorrhizae, phenolic compounds, photosynthesis, root respiration, microbial respiration, Ca/Al ratio

Introduction

Environmental pollution decreases the growth rate of trees and shrubs, and may even result in the decline of whole forest stands. To a large extent this may be due to soil pollution, which has a negative effect on the development of root systems, especially in long-lived organisms, such as trees. Acid precipitation - caused by emissions of sulphur dioxide and nitrogen oxides - is the major reason for considerable acidification of soils in many areas of Poland [29]. In recent years in Poland and other eastern and central European countries, an increase in acidity of rainwater and wet deposition is observed as a result of a decrease in dust alkaline components through introduction of more efficient dust removal and insufficient desulphurization in flue gases of power and heating plants [32, 37].

Toxic ions of aluminium, zinc, copper, lead, cadmium and other metals are often accumulated in the degraded soils of industrial regions. Toxic ions inhibit root development and at higher concentrations lead to root necrosis or death [3, 33, 47]. Aluminium is widespread in the

Correspondence to: Dr. K. Bojarczuk

Earth's crust, and its availability to plants increases with decreasing pH of the soil [4, 17, 51]. The biological activity of aluminium (its solubility and exchangeability) is related not only to the acidity of soil solutions, but also to the concentration of ions of calcium, magnesium and phosphorus, as well as the composition of the organic fraction of the soil [8, 33]. Excessive concentrations of aluminium ions in the soil not only inhibit the uptake of Ca, Mg, P and some other nutrients by plants from the soil [1, 15], but also block their transport inside plants and utilisation in metabolic processes [5, 13, 42]. This results in an unfavourable decrease in the Ca/Al ratio and Mg content of plants [18, 38]. Consequently, plant growth and development is inhibited due to adverse effect of high Al concentration on important physiological processes, such as photosynthesis [40] and respiration [21, 30].

Little information is available on the mechanisms of tree defence against the influence of industrial pollution. Many studies show that deciduous trees are less sensitive to environmental pollution than conifers [6, 57]. Nevertheless, it has been found that some broad-leaved trees, such as birch (formerly regarded as relatively tolerant), exhibit growth decline in industrial regions. The toxic influence of metal ions on plants can be most effectively limited by reducing industrial air pollution. However, even if imissions are greatly reduced, the toxic compounds accumulated in the soil may exert a negative impact on root system development for many years [29].

The sensitivity of plants to pollution depends largely on their general condition. Fertilisation (for example, with nitrogen and phosphorus compounds) of birch trees growing in poor soils in industrial sites has greatly improved their condition [12]. A positive role in the protection of plants against the effects of toxic metals may be played by soil microorganisms, mycorrhizal fungi and fungi antagonistic to pathogens [9]. In degraded soils, microbial activity decreases and the composition of the soil microflora changes [46]. High concentrations of toxic metal ions (e.g. aluminium) are usually negatively correlated with mycorrhizal colonisation [44, 54].

In our previous studies we found significant differences in growth, survival, CO_2 exchange and pollutant accumulation in trees grown in the vicinity of a phosphate fertiliser factory (PFF) and in control area [35, 36, 40]. However, one limitation of these studies was related to potential site and soil fertility differences among sites. The aim of this study was to analyse the influence of polluted soil from a control and industrial site near the PFF on the physiology and growth of birch seedlings. Identification of factors limiting the growth and development of plants in polluted areas may contribute to a better understanding of the mechanisms of plant sensitivity to toxic compounds and may help to formulate guidelines for reforestation of degraded areas.

Material and Methods

One-year-old silver birch seedlings of the comparable size (root length 10 cm, shoot length 12 cm, diameter of root collar 2.0-2.5 mm) were selected for the experiment. Seedlings were obtained from the Agricultural University - Arboretum Zielonka nursery. In the middle of May the seedlings were planted in pots (17 cm diameter, 13 cm height) filled with an unpolluted soil substrate from the experimental forest Zwierzyniec (52°15' N and 17°04' E) or with a polluted soil collected 2 km from the Poznan Phosphate Fertiliser Factory in Lubon, PFF (52°15'10" N and 16°50'31" E). Fertiliser production began there in 1917 and since then the emission of toxic pollutants (mainly SO_2, NO_X, HF) gradually increased through the 1980s. In the 1990s the amount of emitted pollutants decreased considerably due to the termination of sulphuric acid and granulated superphosphate production [23].

The substrate was collected from the top 20 cm of soil after removal of litter. Four substrate treatments were introduced:

(1) unpolluted soil without fertilisation (C_{-FERT});
(2) unpolluted soil with fertilisation (C_{+FERT});
(3) polluted soil without fertilisation (P_{-FERT});
(4) polluted soil with fertilisation (P_{+FERT})-

Birch seedlings in C_{+FERT} and P_{+FERT} were fertilised twice a week with a nutrient solution ($N\text{-}NH_4$ - 2.3%, $N\text{-}NO_3$ - 0.7%, K - 2.0%, Cu - 70 mg I^{-1}, Fe - 400 mg I^{-1}, Mn - 150 mg I^{-1}, Mo - 20 mg I^{-1}, Zn - 150 mg I^{-1}) at a concentration of 15 ml nutrient solution in 11 of water. In addition to fertilisation, all pots were watered to excess. The experiment was established in a greenhouse in two randomised complete blocks with three replications of 12 plants each for each of four soil treatments.

Growth Analysis

Destructive harvests were conducted at intervals of about seven days. We harvested plants seventeen times. At each harvest we randomly chose four plants from each treatment (i.e. two plants per block) for an analysis of seedling growth dynamics. At harvest, the medium was washed from roots with distilled water, and individual plants were separated into leaves, stem, and root. All parts were oven-dried (65°C) and dry masses were determined. These data were used to determine root (RWR), foliage (LWR) and stem (StWR) mass fractions.

The area of leaves, area and length of roots were measured using image analysis systems WhinSeedle and WinRhizo (Regent Instruments Inc., Quebec, Canada).

Estimation of Dehydrogenase Activity in Substrate

Three samples of the substrate in each treatment were collected three times (June 8, July 20, and September 23) for measurements of the respiratory activity of soil microorganisms. Dehydrogenase activity (DHA) was measured in 10 g soil samples by the tetrazolic method described by Thalmann [53] and modified by Rossel [43]. Soil samples were incubated in 5 ml of 0.5 M Tris buffer, pH 8.0, containing 1% 2,3,5-triphenyltetrazolium chloride (TTC), as an electron acceptor, for 24 hours at 30°C in darkness. Suitable reference samples (1/soil + Tris buffer, 2/ TTC + Tris buffer) were analysed to eliminate the influence of non-enzymatic absorbance. The 2,3,5-triphenyltetrazolium formazan (TTF) resulting from the

enzymatic reaction was extracted with 25 ml of 96% ethanol and measured spectrophotometrically at 480 nm. Enzyme activity was expressed as nmol of TTF g^{-1} dry soil 24 h^{-1}.

Characterisation of Ectomycorrhizal Morphotypes

At the end of the experiment (in early October) roots were characterised according to numbers and morphological types. Ectomycorrhizal morphotypes were distinguished on the basis of shape, colour and surface texture, and different morphotypes were analysed separately using previously described methods [26].

Gas Exchange Measurements

In the period of intensive growth of birch seedlings (August), the rates of light-saturated net photosynthesis (A_{max}) in leaves and dark respiration (RS) of fine roots (<2 mm in diameter) were measured. Gas exchange was measured with portable photosynthesis systems (LCA-3, Analytical Development Corporation, Hoddesdon, England) used in the differential mode. The system was calibrated against known CO_2 standards. Net photosynthesis was measured according to a previously described protocol [39] on intact leaves under field conditions using the Parkinson leaf chamber PLC-B. Measurements of net photosynthesis were taken on a sunny day between 900 and 1300 h [photosynthetic photon flux density (PPFD) 1321 ± 39 µmol $m^{-2}s^{-1}$]. During the measurements, air temperature was 27.6°C (± 0.4°C), ambient relative humidity average 64%, and ambient CO_2 concentration averaged 402 (± 0.2) mol mol^{-1}. Measurements of A_{max} were taken on five randomly chosen plants per treatment (one leaf per plant).

Dark respiration rates were measured on detached fine roots (<2 mm) between 900 and 1300 h using LCA-3 systems and the Parkinson leaf chamber PLC-C. Evaluation of RS rates recorded after ca. 5 to 7 minutes when A CO_2 reached a constant level. During the measurements, air temperature was 22°C (± 0.1°C), and ambient CO_2 concentration averaged 398 (± 1.3) mol mol^{-1}. Measurements of RS were obtained from the same plant used for A_{max} measurements (five plants per treatment).

Total Phenols

The content of total soluble phenols (TPh) was determined in 0.25 g of foliage and 0.1 g in roots after double extraction for 15 and 10 min in boiling 95 and 80% ethanol, respectively. The analyses were performed using the spectrophotometric method described by Johnson and Shaal [20] and modified by Singleton and Rossi [48] using the Folin-Ciocalteu phenol reagent. All samples were taken at the same time as the gas exchange measurements. The content of total phenols was expressed in µmol of chlorogenic acid per gram of dry mass. TPh was measured on foliage or roots of three randomly chosen plants per treatment.

Substrate, Foliage and Root Element Concentrations

At the end of the experiment (October) we measured concentrations of soluble forms of macro- and micronutrients and pollutants in the substrate ($N\text{-}NH_4$, $N\text{-}NO_3$, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Cd, and Al). Macronutrients were analysed in an extract of 0.03 N acetic acid, and micronutrients and pollutants in a modified Lindsey extract. After extraction, $N\text{-}NH_4$ and $N\text{-}NO_3$ were assayed with ion-selective-electrodes; K, Ca and Na with flame photometry; chlorides and sulphates nephelometrically; Mg, Fe, Mn, Zn, Cu and Cd with atomic absorption spectroscopy (AAS); and Al calorimetrically with aluminon. At the end of the experiment (in October) concentrations of selected elements were also measured in roots and leaves: total N by the micro-Kjeldahl method; K, Ca, S, Mg, Fe, Mn, Zn, Cd and Al by the same methods as described for the substrate. Data for substrate are averaged for each treatment from two combined samples and those for foliage from three randomly chosen plants per treatment.

Statistical Analyses

For all variables, statistical differences among treatments and sampling dates were analysed using analysis of variance (GLM procedures). Relationships between the sampling day and studied traits were made using correlation and regression analyses. For presentation, both correlation and regression are used but we do not assume that direct causal relations are involved. All statistical analyses were conducted with JMP software (version 3.2.2, SAS Institute, Cary, NC, USA).

Results

Substrate had great effects on dry mass growth and proportional biomass partitioning of silver birch seedlings. At the end of the experiment plants grown on polluted substrate (P_{-FERT}) showed significantly lower root, aboveground and total plant mass than those grown on the control substrate C_{-FERT} (Fig. 1 A, B, C all $p < 0.0001$). Fertilisation significantly improved seedling growth on both substrates (Fig. 1). However, fertilisation did not eliminate differences in growth among seedlings cultivated on polluted vs. control substrate (Fig. 1 and 2). The only exception was root dry mass that did not differ among C_{+FERT} and P_{+FERT} treatments at the end of the experiment (Fig. 1A; $p = 0.81$). These results suggest that other limitations besides substrate fertility constrain plant growth in polluted soils.

The greatest growth differences among treatments were found in proportional biomass partitioning and in root growth and morphology. Seedlings grown on polluted substrate showed significantly higher RWR and lower LWR through the course of the growing season (Fig. 3). From mid July to the end of August plants on C_{+FERT} substrate allocated ca. 30% of biomass above ground (Fig. 3). At the same time, plants grown on C_{+FERT} and P_{+FERT} allocated 40% and those on P_{-FERT}

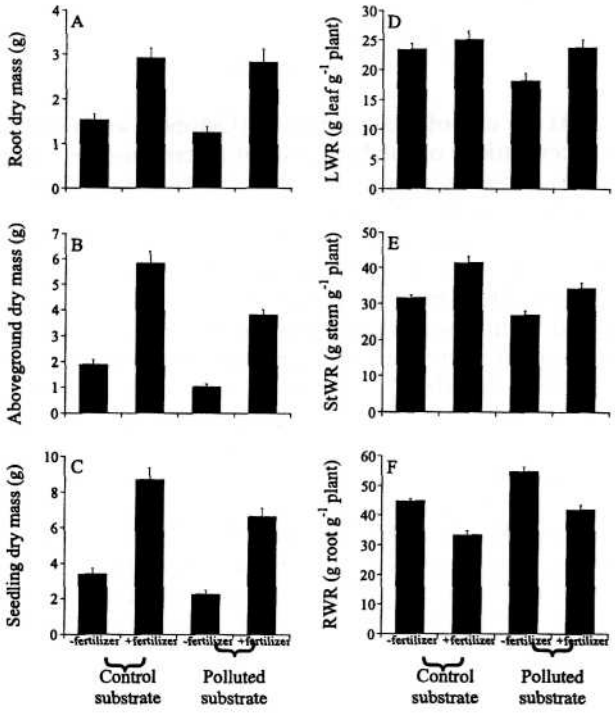


Fig. 1. Effect of substrate type and fertilisation on growth parameters (+SE) and proportional biomass allocation of silver birch *{Betulapendula)* seedlings grown on four substrates: C_{-FERT} - unfertilised control; C_{+FERT} - fertilised control; P_{-FERT} - unfer tilised polluted; P_{+FERT} - fertilised polluted. Measurements were taken at the end of the experiment after 150 days of growth.

> 50% to roots (Fig. 3). In all treatments except P_{-FERT} the highest values of LWR (between 30 and 40%) were observed in late July and early August (Fig. 3). The LWR of plants in P_{-FERT} treatment was much lower (ca. 20%) and reached its highest value already in late June.

Significant differences among treatments were found in root length and projected root area (Fig. 4; treatment, seedling age and the interaction of treatment x age were significant at $p < 0.009$). The steepest and almost linear increase of root area was observed for both P_{-FERT} and P_{+FERT} treatments (Fig. 4). Noticeable increase in root

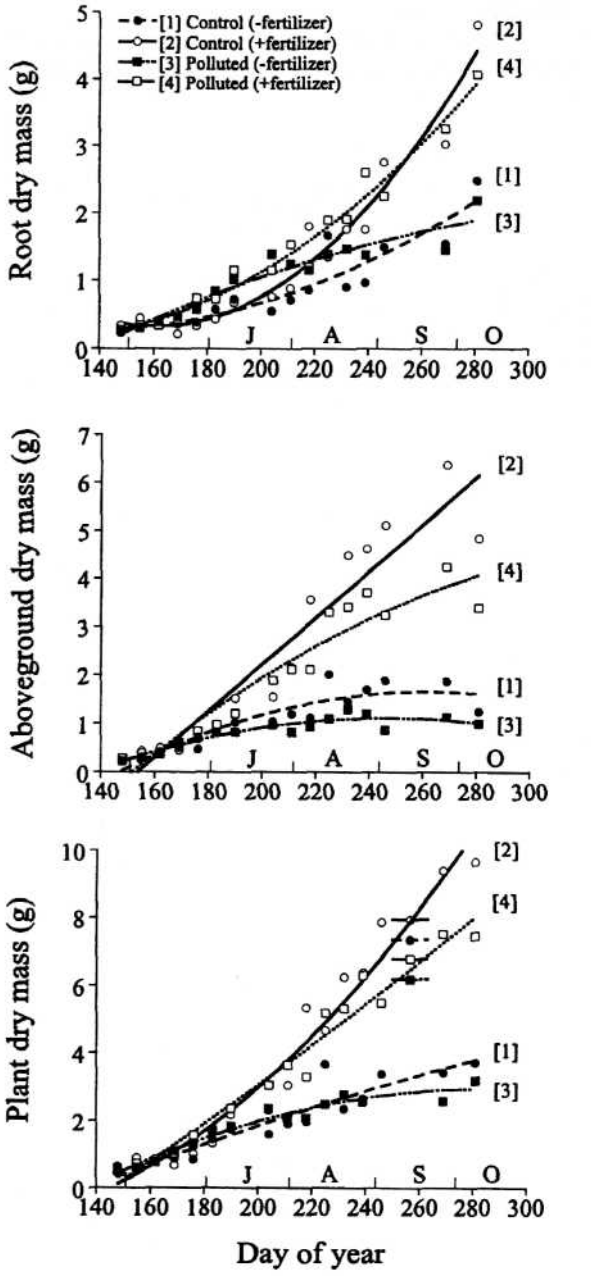


Fig. 2. Seasonal variation of root, aboveground and total mass silver birch *{Betula pendula)* seedlings grown on four substrates. Symbols as in Fig. 1.

Table 1. Enzymatic activity of dehydrogenase in the soil substrates: C_{-FERT} - unfertilized control substrate; C_{+FERT} - fertilized control substrate; P_{-FERT} - unfertilized poluted substrate; P_{+FERT} - fertilized polluted substrate. Data are the means of three ana lyses ± standard deviation.

Treatment	Enzymatic activity (nmol TTF·g^{-1} d.m.·24 h^{-1})		
	8th Jun	20th July	23rd September
C_{-FERT}	9.28 ± 0.82	24.16 ± 2.13	29.93 ± 3.45
C_{+FERT}	11.93 ± 0.63	29.48 ± 8.19	32.72 ± 8.38
P_{-FERT}	2.42 ± 0.25	8.30 ± 1.63	8.90 ± 0.67
P_{+FERT}	2.64 ± 0.31	13.07 ± 3.18	8. 86 ± 0.75

length and area of seedlings in C_{-FERT} and C_{+FERT} treatment began in mid-June and did not differ between these treatments until early August (Fig. 4).

There were distinct differences in seasonal changes of leaf arearroot area ratio among treatments (Fig. 4). The highest values of that ratio were observed in early July in the C_{+FERT} treatment, when leaf area was > 15 fold higher than that of roots. The seasonal pattern of leaf area:root area ratio was very similar in seedlings grown in C_{-FERT} and P_{+FERT} treatment, whereas for plants in P_{-FERT} it

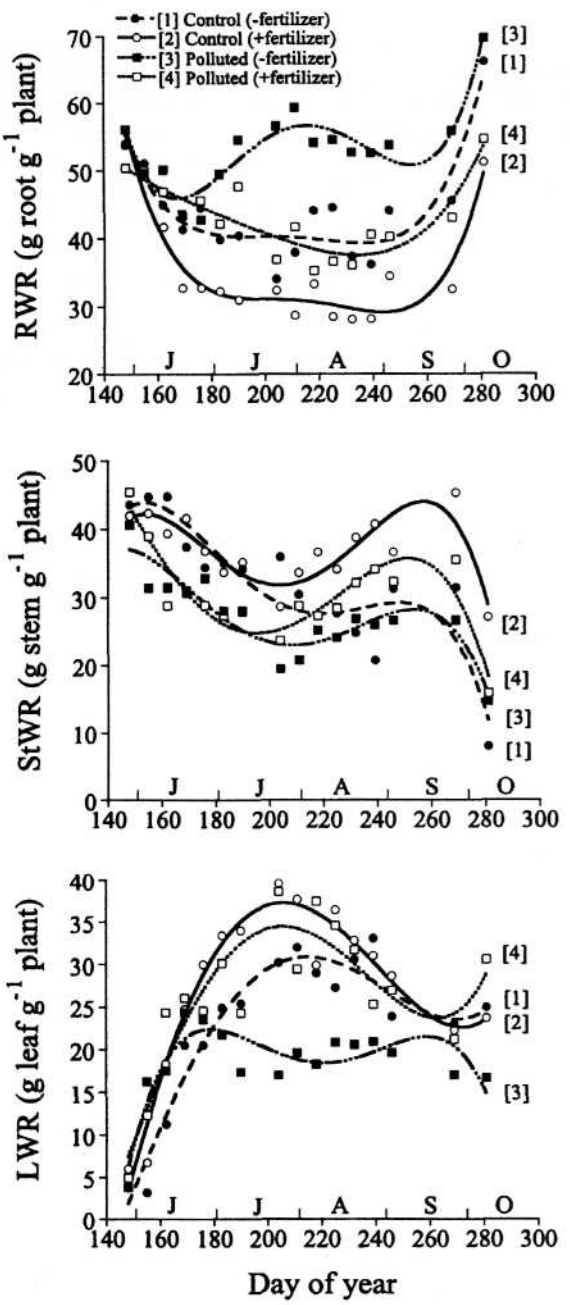


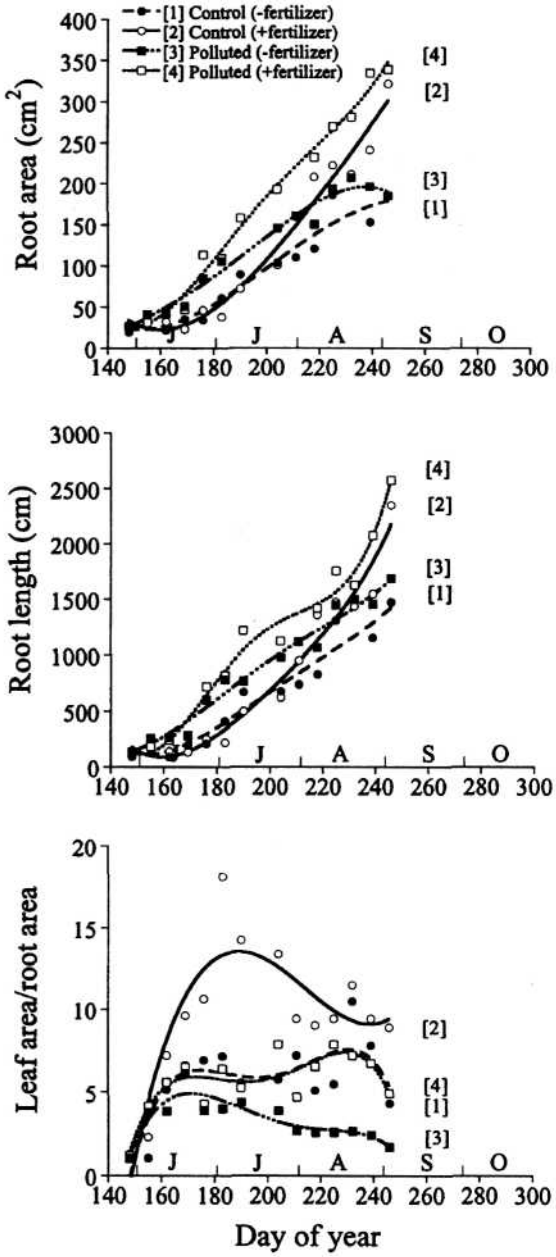
Fig. 3. Effect of substrate type and fertilisation on seasonal differences in proportional biomass allocation to roots (RWR), stem (StWR) and foliage (LWR) of silver birch *(Betula pendula)* seedlings. Symbols as in Fig. 1.

steadily declined from 5 in mid-June to 1 in early September (Fig. 4).

The highest respiratory activity of soil microorganisms was recorded in the unpolluted substrate with fertilization (C_{+FERT}) and the lowest in the polluted substrates, especially in samples collected in July and September (Table 1). In the unpolluted substrate (C_{+FERT} and C_{-FERT}) the activity of dehydrogenase was markedly increased by fertilisation on all three sampling dates, whereas in the polluted substrate (P_{-FERT} and P_{+FERT}) microbial respiration increased only in July, i.e. in the period of the most intensive growth.

At the end of the experiment, the state of mycorrhizae on birch seedlings was assessed in all treatments. Considerable differences in both the numbers and diversity of mycorrhizae between treatments were detected. Seedlings grown in control substrates (C_{-FERT} and C_{+FERT}) were the most strongly colonised by mycorrhizal fungi (about 100%). Only a handful of non-mycorrhizal roots were observed in those plants. Eight morphotypes of mycorrhizae were distinguished (Table 2). Morphotypes II and V were found on roots of birch seedlings in all treat-

Fig. 4. Effect of substrate type and fertilisation on root area,



length and leaf area to root area ratio of silver birch *(Betula pendula)* seedlings. Symbols as in Fig. 1.

Table 2. Description of ectomycorrhizal (EM) morphotypes distinguished on roots of *Betula pendula* seedlings. Substrate: 1 — control without fertilization; 2 — control + fertilization; 3 — polluted without fertilization; 4 — polluted + fertilization.

Type EM	Morphological features	Substrate
I	Light brown; branching monopodial pinnate or unbranched; smooth and shiny; emanating hyphae and rhizomorphs not observed	1, 3
II	Light brown to brown; mostly unbranched, occasionally irregular branching; tips bent; matt; occasionally white emanating hyphae	1, 2, 3, 4
III	Light brown to brown; thin branching irregular; tips bent; smooth and shiny; abundant white, woolly emanating hyphae	1, 2, 3, 4
IV	Light silver to grey; long, thin; mostly unbranched; bent; occasionally white emanating hyphae; white sclerotia present	1
V	Coal black; mostly unbranched; tips straight; grainy and shiny; black hyphae stiffly radiating from mantle (*Cenococcum*)	1, 2, 3, 4
VI	Black; mostly unbranched; tips straight; black, woolly emanating hyphae	1
VII	Brown; thin; mostly unbranched; straight; smooth and matt; no emanating hyphae	1
VIII	Brown; thick; short; monopodial pinnate; tips straight; matt; few short emanating hyphae	1

merits (polluted and unpolluted, fertilised and unfertilised). Unfertilised plants were characterised by a greater diversity of mycorrhizae than fertilised plants. On unfertilised seedlings grown in the unpolluted substrate (C_{-FERT}) all eight morphotypes were found, whereas in the other treatments only 3-4 morphotypes were present (Table 2).

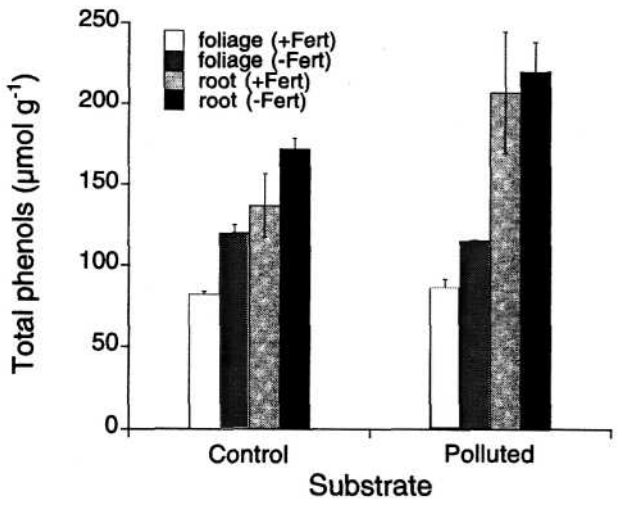


Fig. 5. Total concentration of soluble phenolic compounds (TPh, ± SE) in roots and leaves of silver birch *{Betula pendula)* seedlings grown in unpolluted (Control) and polluted soil with fertilisation (+Fert) or without fertilisation (-Fert).

In the case of soil pollution, roots are more strongly affected by toxic compounds in the substrate than are other parts of the plant. This was confirmed by comparing the concentrations of free phenolic compounds in birch seedlings grown in the control and polluted substrates. In roots of birch grown in the polluted substrate, the level of total phenols (TPh) was significantly higher than in birch grown in the unpolluted substrate, but in leaves no significant effect of the polluted soil on TPh level was observed (Fig. 5). The influence of soil pollution on TPh level was statistically significant in roots and not significant in leaves, irrespective of whether fertilisation was applied. In contrast to the effect of pollution, fertilisation caused a decrease in TPh level in both roots and leaves (Fig. 5, Table 3).

Table 3. Analysis of variance of total phenol (TPh) concentration in different organs of *Betula pendula* seedlings grown on control and polluted substrate. See Figure 5 for TPh values.

Source of variation	DF	Sum of squares	F ratio	P > F
Organ (O)	1	41591.7	49.57	<0.0001
Substrate (S)	1	5189.1	6.18	0.024
O x S	1	5277.7	6.29	0.023
Fertilisation (F)	1	4962.3	5.91	0.027
F x O	1	128.3	0.15	0.70
S x F	1	369.0	0.44	0.52
O x S x F	1	62.4	0.07	0.79
Error	16	13425.9		
Total	23			

The rate of light-saturated net photosynthesis (A_{max}) was significantly affected by source of substrate ($p = 0.02$) but only marginally by fertilisation ($p = 0.13$). On average A_{max} of plants grown on the control substrate was higher by 31% than those grown on the polluted substrate (210 vs. 160 nmol $g^{-1}s^{-1}$, respectively; Fig. 6).

In contrast to the response of A_{max}, both source of substrate ($p = 0.01$) and fertilisation ($p < 0.0001$) significantly affected the rate of root respiration (RS; Fig. 6). On average, root RS of plants grown on the polluted substrate was 29% lower than those grown on the control substrate (8 vs. 6 nmol $g^{-1}s^{-1}$, respectively). Much higher differences in root RS can be attributed to fertilisation. Root RS of fertilised plants was higher by almost 3-fold in comparison with rates of unfertilised plants (Fig. 6).

Birch seedlings grew better in the soil substrate from the control area, especially if fertilisation was applied (C_{+FERT})- The unpolluted substrate (C_{-FERT} and C_{+FERT}) contained higher concentrations of assimilable nutrients ($N\text{-}NO_3$, K, Ca, Mg, Fe, Mn) than the polluted substrate (P_{-FERT} and P_{+FERT})- Aluminium concentration in the polluted substrate was three times higher at the beginning and two times higher at the end of the experiment than in the unpolluted substrate (Table 4). The Ca/Al

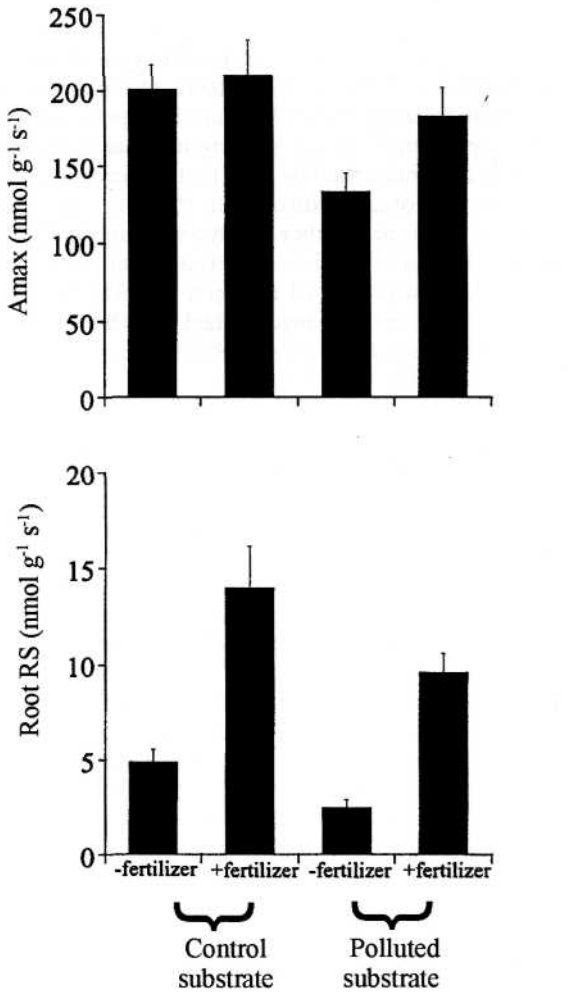


Fig. 6. Mass-based light-saturated net photosynthesis and root respiration rates (±SE) of silver birch *{Betula pendula)* seedlings grown on different substrates.

ratio was higher in the unpolluted soil than in the polluted soil (Table 4). Leaves and roots of birch seedlings grown in polluted substrates contained more aluminium than those grown in unpolluted substrates (Table 5).

Table 4. Concentration of macro- and micronutrients and pollutants in polluted and unpolluted substrates (assimilable forms in mg per 1000 g of soil dry weight). The analysis was performed at the end of the experiment (October).
Substrates: C_{-FERT} - unfertilized control substrate; C_{+FERT} - fertilized control substrate; P_{-FERT} - unfertilized polluted substrate; P_{+FERT} - fertilized polluted substrate.

Nutrients and Pollutants	Substrate			
	C_{-FERT}	C_{+FERT}	P_{-FERT}	P_{+FERT}
$N\text{-}NH_4$	5.4	1.3	4.1	1.4
$N\text{-}NO_3$	8.7	20.8	2.1	2.8
K	53.8	113.6	21.4	58.0
Ca	245	266	131	170
Mg	34.9	48.4	31.8	33.8
Na	194.9	179.4	160.1	158.8
Fe	57.1	53.8	30.4	35.9
Zn	2.68	3.36	2.07	3.45
Mn	17.5	17.5	4.9	6.9
Cu	0.38	0.5	0.24	0.44
Cd	0.01	0.007	0.02	0.01
Al	17.7	19.1	34.0	33.5
Ca/Al	13.8	13.9	3.8	5.1

Table 5. Concentration of macro- and micronutrients in birch seedlings after 5 months of culture in unpolluted and polluted substrates. N, K, Ca, Mg, Na (% d.m.); Fe, Zn, Mn, Cu, Cd and Al in ppm (wt./wt. dry mass). Substrates: C_{-FERT} - unfertilized control substrate; C_{+FERT} - fertilized control substrate; P_{-FERT} - unfertilized polluted substrate; P_{+FERT} - fertilized polluted substrate. Data are the means of three analyses ± standard deviation.

Element	Leaves				Roots			
	C_{-FERT}	C_{+FERT}	P_{-FERT}	P_{+FERT}	C_{-FERT}	C_{+FERT}	P_{-FERT}	P_{+FERT}
N	2.06±0.02	3.27±0.02	1.76±0.02	2.6±0.07	0.76±0.01	1.65±0.03	0.82±0.03	1.36±0.01
K	2.38±0.02	2.2±0.06	1.68±0.03	2.24±0.15	0.29±0.03	0.32±0.02	0.26±0.0	0.26±0.02
Ca	1.3±0.02	0.8±0.0	0.7±0.01	0.6±0.03	0.32±0.01	0.33±0.02	0.13±0.01	0.19±0.01
Mg	0.7±0.01	0.48±0.0	0.58±0.0	0.55±0.01	0.17±0.01	0.2±0.02	0.19±0.0	0.23±0.01
Na	0.077±0.01	0.066±0.01	0.073±0.0	0.14±0.03	0.237±0.01	0.226±0.01	0.276±0.01	0.346±0.01
Fe	204.3±10.32	158.7±14.67	97.3±3.87	100.4±9.61	584.3±3.14	553.3±18.71	502.2±4.46	505.3±6.43
Zn	254.9±10.3	197.3±4.42	219.3±2.71	175.9±5.89	151.0±4.60	135.8±22.06	141.7±3.86	163.0±24.90
Mn	379.0±1.76	364.0±0.95	366.0±6.52	359.0±3.99	151.0±11.25	136.0±34.21	142.0±5.47	163.0±0.86
Cu	11.6±0.32	12.1±0.36	10.4±0.79	10.9±1.21	17.2±1.03	17.9±4.1	11.6±0.32	17.4±0.15
Cd	0.74±0.20	0.82±0.15	0.52±0.09	0.11±0.05	1.99±0.41	1.14±0.4	0.62±0.09	0.11±0.01
Al	234.0±2.52	287.0±12.12	312.0±1.00	401.0±15.7	2559.0±43.09	2590.0±21.6	2849.0±47.26	2746.0±30.02

Discussion

Polluted soil substrate has profound effects on the growth and physiology of silver birch seedlings. Plants grown on polluted substrate were characterised by lower seedling dry mass and higher below ground biomass allocation accompanied by lower leaf to root area ratios (Figs. 1, 3 and 4). Our data indicated that growth and development of silver birch, which is generally regarded as a species less sensitive to industrial pollution among broad-leaved trees [6, 11, 56] could be significantly limited by soil contamination frequently observed in the vicinity of point sources of air pollutants.

On the basis of many studies it was found that the sensitivity of plants to the influence of toxic ions in the soil depends on soil type, and particularly on soil pH and concentration of nutrients [1, 16, 24, 41]. It was also shown that fertilisation of trees planted in degraded habitats may improve their general condition and lower their sensitivity to industrial pollution [18, 24]. The results of the fertilisation treatment in this study indicated that even when an optimal range of soil nutrient concentrations are supplied and air pollution is excluded, pollutants accumulated in soil can significantly affect even a less susceptible tree species such as silver birch.

Intensive growth, especially of aboveground parts, was observed in fertilised seedlings grown in the unpolluted soil (Figs. 1-4). Fertilisation also significantly accelerated plant growth in the polluted soil, and had a particularly favourable influence on the biomass partitioning reflected in lower RWR and increased LWR (Fig. 3).

In our experiment the slowest growth increase in biomass (dry mass of the whole plant and of aboveground parts) and the highest root weight ratios were observed in unfertilised seedlings grown in the polluted soil. The slow growth of the plants was most likely due to the low concentration of nutrients and the high concentration of aluminium, as compared with the unpolluted soil. The uptake of aluminium ions by plants may be blocked by increased concentrations of calcium and phosphorus ions, which compete for uptake sites at the root surface. This in effect reduces the toxicity of aluminium, especially at low soil concentrations [8, 10, 28, 49]. The polluted soil was characterised by low concentrations of calcium and potassium (Tab. 4). Toxic ions present in the substrate may adversely affect plants by damaging root cells, which leads to an inhibition of the transport of water and nutrients. Moreover, shortages of calcium and potassium ions contribute to reduced membrane permeability and to leakage of some cations from the cytosol [28, 31, 50]. The polluted substrate also had a lower Ca/Al ratio than the control substrate, which was probably a reason for the lower rate of plant growth and lower rates of photosynthesis and root respiration (Fig. 6).

Our results also indicate that slow plant growth in a polluted soil could be linked to lower microbial activity, as evidence from reduction of the respiratory activity of soil microorganisms. Many studies have shown that in degraded soils, microbial activity decreases and the composition of the soil microflora changes [14, 27, 45, 46, 55].

In fertilised soil an increased activity of soil microflora was recorded, especially in July, when the growth of seedlings was the most intensive. Fertilisation did not affect the number of formed mycorrhizae, but had a significant effect on diversity, particularly in the unpolluted soil. The majority of fungi forming ectomycorrhizal symbioses with trees are characterised by sensitivity to excessive concentrations of inorganic nitrogen in the soil, which may result in a reduced number of mycorrhizae [9, 12, 19, 44]. However, some species of mycorrhizal fungi tolerate higher concentrations of nitrogen [25, 34]. In this study the reduction of some mycorrhizal morphotypes on roots of fertilised seedlings was probably a result of the unfavourable influence of inorganic compounds introduced into the substrate. This is consistent with research indicating that strains of mycorrhizal fungi vary in sensitivity to fertilisation, which may be reflected in the absence of some mycorrhizal morphotypes on roots of fertilised seedlings [9, 12, 44]. Similar findings were observed in seedlings of *Q. rubra* grown in soil from an urban area, polluted with high levels of nitrogen and heavy metals, which had seven ectomycorrhizal morphotypes, whereas seedlings grown in an unpolluted soil developed 10 ectomycorrhizal morphotypes [2].

Concentrations of some secondary metabolites, such as phenolic compounds or enzymes, are often analysed as indicators of plant reactions to stress factors, such as soil pollution with toxic ions [7, 21, 52]. Toxic aluminium compounds in the substrate result in changes in the concentration of phenolic compounds in leaves and roots, and altered respiration rates [22]. In our study birch seedlings grown in the polluted soil contained significantly higher concentrations of total phenols in roots than seedlings grown in the unpolluted soil, but no significant effect of soil pollution on TPh levels in leaves was observed.

In summary, results of our study indicate that toxic substances in the soil indirectly exert a significant negative influence on the growth and development of birch seedlings. This effect may be observed even after a considerable reduction in the emission of toxic-gases at a polluted site. Our results confirm earlier observations that in many cases a reduction in air pollution does not result in an immediate improvement of the environmental situation in regions where soils are degraded.

Acknowledgements

This study was supported by The Polish Committee for Scientific Research (KBN), grant No 5PO6M00512, 6PO6L04121.

Reference

1. ASP H., BENGTSSON B., JENSEN P. Growth and cation uptake in spruce *(Picea abies* Karst.) grown in sand culture with various aluminium contents. Plant and Soil. **3,** 127, **1988.**
2. BAXTER J.W, PICKETT S.T.A., CARREIRO M.M., DIGHTON J. Ectomycorrhizal diversity and community structure in oak forest stands exposed to contrasting an thropogenic impacts. Canadian Journal of Botany. **77,** 71, **1999.**

3. BOJARCZUK K. Effect of aluminium on in vitro rooting of berch *(Betula pendula* Roth.) and poplar *(Populus tremula* L. *P.alba* L.) microcuttings. Acta Societatis Botanicorum Poloniae. **69** (4), 251, **2000.**
4. BOJARCZUK K., OLEKSYN J. Rozwoj jednorocznych sadzonek sosny *(Pinus sylvestris* L.) i brzozy *(Betula pendula* Roth.) w zanieczyszczonych podfozach inokulowanych grzybem *Trichoderma harzianum* Rifai. Arboretum Kornickie. **39,**163, **1994.**
5. BORKOWSKA B. Toksycznosc glinu (Al). Wiadomosci Botaniczne. **32,** 167, **1988.**
6. BOUDOT J.P., BECQUER T., MERLET D., ROUILLER J. Aluminium toxicity in declining forest: a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France). Annales of Forest Scence. **51,** 27, **1994.**
7. BURZYNSKI M, BUCZEK J. Uptake and assimilation of ammonium ions by cucumber seedlings from solutions with different pH and addition of heavy metals. Acta Societatis Botanicorum Poloniae. **76,** 197, **1998.**
8. CLEGG S., GOBRAN G.R., HUTTL F.R., NILSSON L.O., JOHANSSON U.T. Effects of aluminium on growth and root reactions of phosphorus stressed *Betula pendula* seed lings. Procc. of CEC-IUFRO Symposium 1993 Hamstad, Sweden. 173, **1995.**
9. EGERTON-WARBURTON L.M., GRIFFIN B.J. Differen tial responses of *Pisolithus tinctorius* isolates to aluminium *in vitro.* Canadian Journal of Botany. **73,** 1229, **1995.**
10. ERICSSON T., GORANSSON A., GOBRAN G. Effects of aluminium on growth and nutrition in birch seedlings under magnesium- or calcium-limiting growth conditions. Zeitschrift fur Pflanzen und Bodenkunde. **161,** 653, **1998.**
11. ERNST W.H.O., VERKLEJ J.A.C., SCHAT H. Metal toler ant plants. Acta Botanica Neerlandica. **41,** 229, **1998.**
12. ESTIVALET D., PERRIN R, LE-TACON F., BOUCHARD D. Nutritional and microbiological aspects of decline in the Vosges forest area (France). Forest Ecology and Management. **37,** 233, **1990.**
13. FARID **J.** Aluminium effects on growth, nutrient net uptake and transport in 3 rice *(Oryza sativa)* cultivars with different sensitivity to aluminium. Physiolgia Plantarum. **83,** 441, **1991.**
14. GORANSSON A., ELDHUSET T.D. Effects of aluminium on growth and nutrient uptake of small *Picea abies* and *Pinus sylvestris* plants. Trees. **5,** 136, **1991.**
15. GORANSSON A., ELDHUSET T.D. Effects of aluminium ions on uptake of calcium, magnesium and nitrogen in *Be tula pendula* seedlings growing at high and nutrient supply rates. Water, Air and Soil Pollution. **83,** 351, **1995.**
16. GORANSSON A, ELDHUSET T.D. Is the Ca+K+Mg/Al ratio in the soil solution a predictive tool for estimating dam age? Water, Air and Soil Pollution. **1,** 57, **2001.**
17. GREGER M., TILLBERG J.E., JOHANSSON M. Alumin ium effects on *Scenedesmus obtusiusculatus* with different phosphorus status. I. Mineral uptake. Physiologia Plan tarum. **84,** 193, **1992.**
18. HENRIKSEN T.M., ELDHUSET T.D., STUANSE A.D., LANGERUD B.R.. Effects of aluminium and calcium on *Picea abies* seedlings. Scandinavian Journal of Forest Re search. 7, 63, **1992.**
19. JENTSCHE G., BRANDES B., HEINZEMANN J., MARSCHNER P., GODBOLD D.L. Sand culture of mycorrhizal plants. Zeitschrift fiir Pflanzen und Bodenkunde. **162,** 107, **1999**
20. JOHNSON G., SCHAAL L.A. Accumulation of phenolic substances and ascorbic acids in potato tubers tissue upon injury and their possible role in disease and resistance. American Potato Journal **24,** 200, **1957.**
21. KAROLEWSKI P., GIERTYCH M. Influence of toxic metals ions on phenols in needles and roots respiration of Scots Pine seedlings. Acta Societatis Botanicorum Poloniae. **63,** 29, **1994.**
22. KAROLEWSKI P., GIERTYCH M. Wptyw nawozenia wapniowego na poziom fenoli u sosny zwyczajnej *(Pinus syl vestris* L.) w warunkach skazenia zwiazkami glinu, siarki i fluoru. Arboretum Kornickie. **40,** 169, **1995.**
23. KAROLEWSKI P., SIEPAK J., GRAMOWSKA H. Re sponse of Scots pine *(Pinus sylvestris),* Norway spruce *(Picea abies)* and Douglas fir *(Pseudotsuga menziesii)* needles to environment pollution with fluorine compounds. Dendrobiology. **45,** 41, **2000.**
24. KELTJENS W.G., VAN LOENEN E. Effects of aluminium and mineral nutrition on growth and chemical composition of hydroponically grown seedlings of five different forest tree species. Plant and Soil. **119,** 39, **1989.**
25. KIELISZEWSKA-ROKICKA. Effect of nitrogen level on acid phosphatase activity of eight isolates of ectomycorrhizal fungus *Paxillus involutus* cultured in vitro. Plant and Soil. **139,** 229, **1992.**
26. KIELISZEWSKA-ROKICKA B., RUDAWSKA M, LESKI T. Ectomycorrhizae of young and mature Scots pine trees in industrial regions in Poland. Environmental Pollution. **98,** 315,**1997.**
27. KIELISZEWSKA-ROKICKA B. Phosphate status and acid phosphatase activity in soil and ectmycorrhizal in two mature stands of Scots pine *(Pinus sylvestris* L.) exposed two differ ent levels of anthropogenic pollution. Acta Societatis Botan icorum Poloniae. **68,** 3111, **1999.**
28. KINRAIDE T.B., RYAN P.R., KOCHIAN L.V. Al^{3+} - Ca^{2+} interactions in aluminium toxicity. II Evaluating the Ca^{2+} displacement hypothesis. Planta. **192,** 104, **1994.**
29. KOMISAREK J., KOCIALKOWSKI W.Z., RACHWAL L., SIENKIEWICZ A. Wptyw $CaCO_3$ na zawartosc roznych form Cu, Zn i Pb w glebach skazonych. PTPN Prace Komitetu Nauk Rolniczych i Nauk Lesnych. **59,** 53, **1990.**
30. LIMA M.L., COPELAND L. The effect of aluminium on respiration of wheat roots. Physiologia Plantarum. **90,** 51, **1994.**
31. LLUGANY M, POSCHENRIEDER C, BARCELO J. Monitoring aluminium induced inhibition of root elongation in for maize cultivars differing in tolerance to aluminium and proton-activity. Physiologia Plantarum. **93,** 263, **1995.**
32. MARQUARDT W., BRUGGEMANN E., AUEL R., HERRMANN H., MOLLER D. Trends of pollution in rain over East Germany caused by changing emissions. Tellus, Series B. **53,** 529, **2001.**
33. MOHR H.D. Extraction of easy soluble of Fe and other heavy metals from various substances by electro-ultrafiltration (EUF) and their relation to the heavy-metal contents of plants. Plant and Soil. **83,** 65, **1985.**
34. OHENOJA E. Mushrooms and mushroom yields in fer tilized forests. Ann Bot. Fennici. **15,** 38, **1978.**
35. OLEKSYN, J. Height growth of different European Scot pine *Pinus sylvestris* L. provenances in a heavily polluted and a control environment. Environ. Pollut. **55,** 289, **1988.**
36. OLEKSYN, J., BIALOBOK, S. Net photosynthesis, dark respiration and susceptibility to air pollution of 20 European provenances of Scots pine Pinus sylvestris L. Environ. Pollut. (Series A). **40,** 287, **1986.**

37. OLEKSYN, J., REICH, P.B. Habitat destruction and biodi versity in Poland. Conservation Biology. **8,** 943, **1994.**
38. OLEKSYN J, KAROLEWSKI P., GIERTYCH M.J., WERNER A, TJOELKER M.G., REICH P.B. Altered root growth and plant chemistry of *Pinus sylvestris* seedlings sub jected to aluminium in nutrient solution. Trees. **10,** 135, **1996.**
39. OLEKSYN, J., ZYTKOWIAK, R., REICH, P.B, TJOELKER, M.G, KAROLEWSKI, P. Ontogenetic pat tern of leaf CO_2 exchange, morphology and chemistry in *Betula pendula* trees. Trees. **14,** 271, **2000.**
40. REICH P.B, OLEKSYN J, TJOELKER M.G. Relationship of aluminium and calcium to net CO_2 exchange among di verse Scots pine provenances under pollution stress in Po land. Oecologia. **97,** 82, **1994.**
41. RENGEL Z. Role of calcium in aluminium toxicity New Phytologist. **121,** 499, **1992.**
42. RENGEL Z. Uptake of aluminium by plant cells. New Phytologist. **134,** 389, **1996.**
43. ROSSEL D, TARRADELLAS J, BITTON G., MOREL J.L. Use of enzymes in ecotoxicology: A case for dehyd rogenase and hydrolytic enzymes. In: Tarradellas J, Bitton G., Rossel. D. (eds.), CRC Lewis Publishers Inc., Boca Raton Fla, pp. 179-206. **1997.**
44. RUDAWSKA M., KIELISZEWSKA-ROKICKA B., LESKI T., OLEKSYN J. Mycorrhizal status of a Scots pine *(Pinus sylvestris* L,) plantation affected by pollution from a phos phate fertilizer plant. Water, Air and Soil Pollution. **85,** 1281, **1995.**
45. RUDAWSKA M., LESKI T. Aluminium tolerance of differ ent *Paxillus involutus* Fr. strains originating from polluted and non polluted sites. Acta Societatis Botanicorum Polon iae. **67,** 115, **1998.**
46. SCHONHAR S. Untersuchungen uber Feinwurzelschaden und Pilzbefall in Fichtenbestanden des Schwarzwaldes. Al lgemeine Forest und Jagdzeitung. **160,** 229, **1989.**
47. SILVA I.R, SMYTH T.J, RAPER D.C, CARTER T.E, RUFTY T.W. Different aluminium tolerance in soybean. An evaluation of the role of organic acids. Physiologia Plan tarum. **112,** 200, **2001.**
48. SINGLETON V.I, ROSSI J.A. Colorimetry of total phen olics with phosphomolybdicphosphotungistic acid reagent. American Journal. Enology Viti. **16,** 144, **1965.**
49. SMALLIDGE P.J, LEOPOLD D.J. Effects of watershed liming on *Picea rubens* seedling biomass and nutrient con centration. Water, Air, and Soil Pollution. **95,** 193, **1997.**
50. STEFANI A, ARDUINI I, OUNIS A. *Juglans acutus* germi nation and initial growth in the presence of heavy metals. Annales Botanici Fennici. **28,** 37, **1991.**
51. SLASKI J. Mechanizm tolerancyjnosci na toksyczne dziatanie jonow glinu u roslin wyzszych. Wiadomosci Botan iczne. **36,** 31, **1992.**
52. SLASKI J.J, ZHANG G, BASU U, STEPHENS J.L, TAYLOR G.J. Aluminium resistance wheat (Triticum aes tivum) is associated with rapid, Al-induced changes in activ ites of glucose-6-phosphate dehydrogenase and 6-phosphog luconate dehydrogenase in root apices. Physiologia Plan tarum. **98,** 477, **1996.**
53. THALMANN A, VON,. Zur Bestimmung der Dehyd rogenaseaktivitat im Boden mittels Triphenyltetrazolium chlorid (TTC). Landwirtschaftliche Forschung. **21,** 249, **1968.**
54. VILKKA L, AULA L, NUORTEVA P. Comparison of the levels of some metals in roots and needles *Pinus sylvestris* in urban and rural environment at two times in the growing season. Annales Botanici Fennici. **27,** 53, **1990.**
55. WILKINS D.A, HODSON H.J. The effects of aluminium and *Paxillus involutus* on the growth of Norway spruce *(Picea abies* L. Karst.) New Phytologist. **113,** 225, **1989.**
56. WINTERHALDER K, INNES J.L, OLEKSYN J. Land scape degradation by smelter emissions near Sudbury, Cana da and subsequent amelioration and restoration. IUFRO Task Force on Environmental Change. 87, **2000.**
57. WIT-RZEPKA M, RACHWAL L. Reakcje brzoz na zanie czyszczenia z hut miedzi Czesc I. Przeglad literatury. Arbor etum Kornickie. **34,** 171, **1989.**