Effect of Aluminium on the Development of Poplar (*Populus tremula* L. × *P. alba* L.) *in vitro* and *in vivo*

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Abstract

Adventitious bud cultures were established by using buds of selected poplar clones (*Populus tremula* L. × *P. alba* L.) as initial explants. The Murashige and Skoog medium (1/2 and 1/4 MS) was used for multiplication and rooting of shoots. To the media, aluminium was added in the form of sulphate, at a concentration 50−70 mg Al dm^{-3}. The culture was continued *in vitro* for more than 12 months. The cultures developed on media with aluminium (Al+) were more tolerant to aluminium in the medium during multiplication than those developed on media without aluminium (Al−). Rooted poplar cuttings obtained from cultures on media with aluminium (Al+) grew better in soil from an area polluted by a phosphate fertilizer factory (Luboń) than those from media without aluminium (Al−). This soil was characterized by a high Al level, low Ca/Al ratio and low pH, as compared to the control soil, from an area regarded as free from toxic pollution.

Keywords: *Populus tremula* × *P. alba*, *in vitro*, *in vivo* culture, development, aluminium, soil pollution

Introduction

Industrial and agricultural pollution leads to permanent and increasing acidification of soils, which accumulate toxic ions such as aluminium, copper, lead, zinc or cadmium. The ions hinder the development of plant roots and shoots, and at higher concentrations may lead to plant death [8, 18]. The mechanisms of defence against industrial pollution are still poorly understood. In general, broad-leaf trees are regarded as less sensitive to environmental pollution than conifers [9, 23]. However, the decline of some broad-leaf species was observed in experimental plots around some factories. This applies, for example, to silver birch (*Betula pendula* Roth.), which was earlier classified as relatively tolerant [9, 26]. The trees originating from different populations may vary in sensitivity to industrial pollution [25, 28]. Plant tolerance to toxic metal ions is conditioned by several mechanisms which are controlled by different genes but operate simultaneously and complement one another [1, 2, 16].

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Tissue culture has been used in research on plant responses to toxic metal ions [13, 14, 33]. This method has enabled selection of plants tolerant to some metal ions: aluminium, copper, manganese or nickel [3, 17, 30, 32].

The main objective of this study was to assess the influence of aluminium on development of shoots and roots of poplar cultured *in vitro* and *in vivo*. This can broaden our knowledge of plant sensitivity to toxic ions and enable production of plants with a greater tolerance of industrial pollution.

Material and Methods

From a poplar clone (*Populus tremula* L. × *P. alba* L.), apical and lateral buds were collected as explants from June through September. Tissue culture was conducted with the use of a modified ½ or ¼ strength Murashige and Skoog medium [24], supplemented with hormones (BAP 0.25−0.5 mg·dm^{-3} and NAA 0.1 mg·dm^{-3}), and solidified with Bactoagar. Except for control cultures, aluminium ions were added to the medium as aluminium sulphate (50−75 mg Al dm^{-3}). The pH of the medium was stabilized at the level 4.5. Every 4−5 weeks, the cultures were

Table 1. Effect of aluminium on the development of poplar cultures derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–). Concentration of aluminium sulphate 50 and 75 mg Al dm^{-3}. Mean values marked with the same letters are not significantly different at P<0.05

Treatment (mg Al dm^{-3})	No. of shoots	Shoot length (cm)	Degree of chlorosis (scale 0-3)	Degree of browning (scale 0-3)
Al– cultures, control	7.0 b	3.6 c	1.3 bc	1.3 ab
Al+ cultures, control	8.4 b	4.4 d	0.5 a	1.1 a
Al– cultures, 50	4.3 a	1.4 a	1.7 cd	2.2 c
Al+ cultures, 50	6.8 b	3.0 c	0.9 b	1.2 ab
Al– cultures, 75	3.9 a	1.0 a	1.9 d	2.5 c
Al+ cultures, 75	6.9 b	2.2 b	0.9 b	1.7 b

passaged to fresh agar media with the same composition as during multiplication. Shoots of about 2 cm in length were rooted in perlite saturated with the same but liquid medium. Poplar culture on media with aluminium (Al+) and without it (Al–), was conducted continuously and after at least 12 months their development was controlled. The assessment included the following parameters: number and length of shoots as well as their quality, i.e. degree of chlorosis and browning on a scale of 0-3 (0 = lack of chlorosis/browning; 1 = very slight, 1–20% of chlorosis/browning; 2 = moderate, with 21-60% of chlorosis/browning; 3 = substantial, with 61-100% of chlorosis/browning).

Poplar cultures were kept in 300-ml jars at 22–23°C in a growth chamber illuminated with mercury-discharge lamps (7500 mW/m^2) for 16 hours a day. All *in vitro* experiments were established in a randomized block design, with 4 replicates of 6 jars each. While testing differences between combinations, the Tuckey test was used at the significance level of 0.01 or 0.05.

In late May, poplar cuttings cultured and rooted in a medium with Al (Al+) and without it (Al–) were moved to a greenhouse and planted in 14-cm pots (about 1.5 l in volume) filled with a relatively unpolluted soil (Z) from the Zwierzyniec Forest (52°15'N and 17°04'E) or a polluted soil (L) collected 2 km from the Poznań Phosphate Fertilizer Factory in Luboń (52°15'10"N and 16°50'31"E). Fertilizer production began there in 1917 and since then the emission of toxic pollutants

Table 2. Concentration of macro- and micronutrients (soluble forms, mg · dm^{-3}) in control (Z) and polluted (L) substrates.

Nutrients and Polutans	Control substrate (Z)	Polluted substrate (L)
Ca	254	153
Mg	65	69
Na	161	227
Cl	58	64
S-SO_4	61	101
Fe	43	43
Zn	1.9	2.6
Mn	5.7	4.2
Cu	0.72	0.28
Pb	1.2	0.46
Cd	0.03	0.02
Al	38.2	92.7
pH	5.1	4.5

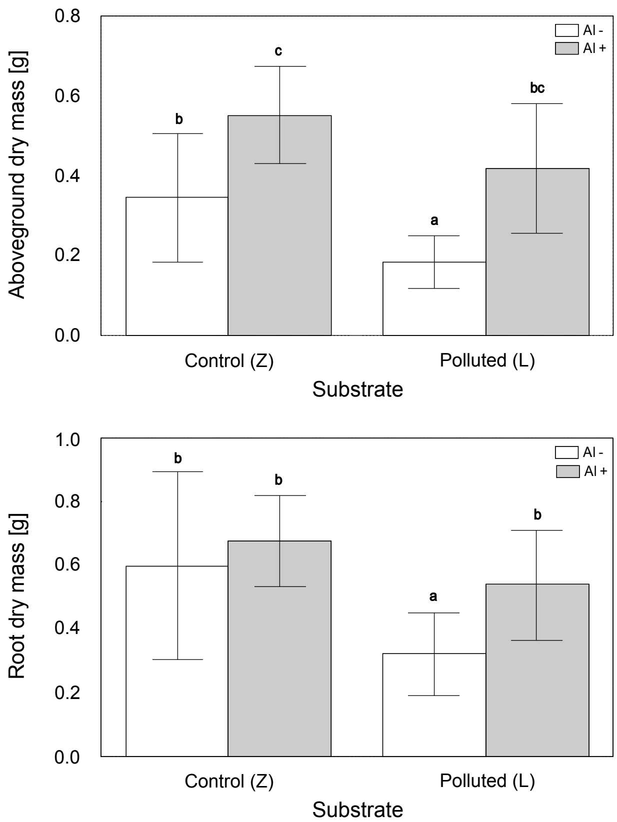

Fig. 1. Development of poplar cuttings, derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–), after planting in a control substrate (Z) or a polluted substrate (L). (Experiment I, 1998).

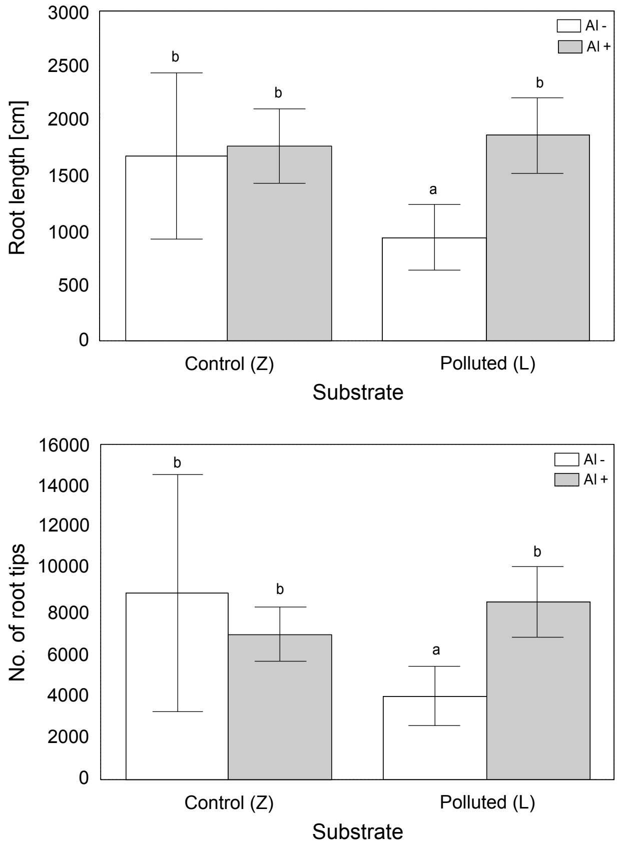

Fig 2. Development of root system of poplar cuttings, derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–), after planting in a control substrate (Z) or a polluted substrate (L). (Experiment I, 1998).

(mainly SO_2, NO_x, HF) gradually increased till the late 1980s. In the 1990s the amount of emitted pollutants decreased considerably due to the termination of sulphuric acid and granulated superphosphate production [21].

The potted plants were fertilized weekly with a commercial mixture of nutrients (pH 4.5). Biomass increments were measured weekly from June till September. The measurements included: fresh and dry weight of the whole plant, roots, shoot, leaves, secondary growth, and fallen leaves. The proportional biomass allocation to foliage (LWR) and roots (RWR) were calculated. With the use of a scanner, leaf area was measured, as well as area and length of roots in three classes of thickness, and numbers of root tips and branching points were assessed. At the beginning and end of the greenhouse experiment (late May and early October), also concentrations of available forms of micro- and macronutrients (Ca, Mg, Na, Cl, S-SO_4, Fe, Mn, Zn, Cu, Cd, Pb, Al) were measured in the soil with methods described in an earlier paper [8].

In the greenhouse experiments, poplar cuttings were planted in 2 blocks with 4 replications of 12 plants each. All presented results were analyzed statistically by IMP 3.1.6.2 software (SAS Institute, Cary, NC, USA).

Results

After more than 12 months of growth in media with aluminium (Al+) and without it (Al–), poplar cultures were transferred to a similar medium with aluminium sulphate at a concentration of 50 or 75 mg Al dm^{-3} (Table 1), to assess their sensitivity to aluminium ions. The Al+ cultures proved to be more tolerant to the toxic aluminium ions, as they produced stronger and more numerous shoots than Al– cultures. The greater tolerance of Al+ plants was also reflected in their markedly better quality (i.e. lower degree of chlorosis and browning), as compared to Al– plants.

For the greenhouse experiments, poplar shoots cultured *in vitro* for at least 12 months in media with aluminium (Al+) or without it (Al–) were rooted in perlite, and next transferred to a greenhouse and planted in a control soil (Z) and polluted soil (L). Although the level of pollution by the factory at Luboń had recently decreased (21), the soil from that area was characterized by a 2.5 times higher Al concentration, a lower Ca/Al ratio, and a lower pH than the control soil (Table 2). Poplar cuttings from Al– cultures grew slower in the polluted soil, forming a lower biomass of both aboveground parts and roots, than those planted in the control soil (Fig. 1). Moreover, the Al– plants growing in the polluted soil

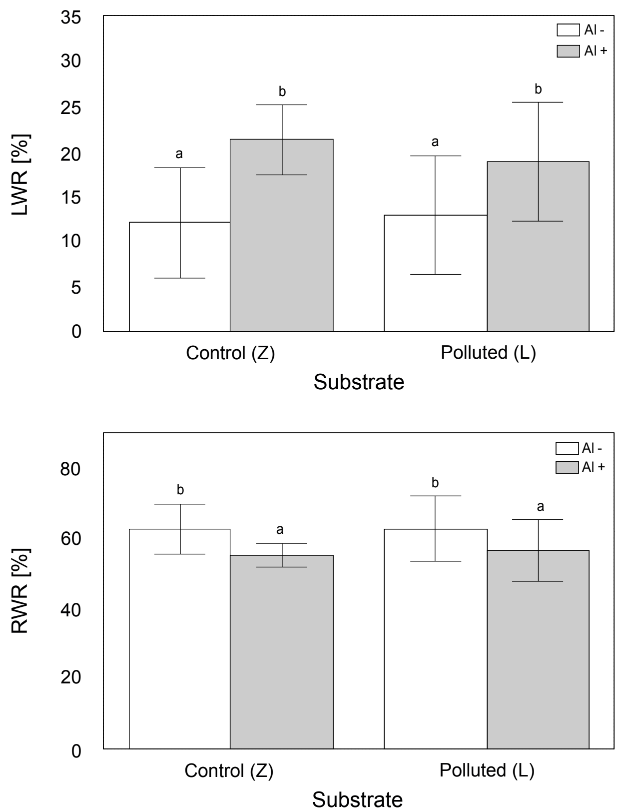

Fig.3. Effect of substrate type on proportional biomass allocation to foliage (LWR) and roots (RWR) of poplar cuttings, derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–), after planting in a control substrate (Z) or a polluted substrate (L). (Experiment I, 1998).

were distinguished by a poorer development of roots (smaller length and number of root tips) than those from the control soil (Fig. 2).

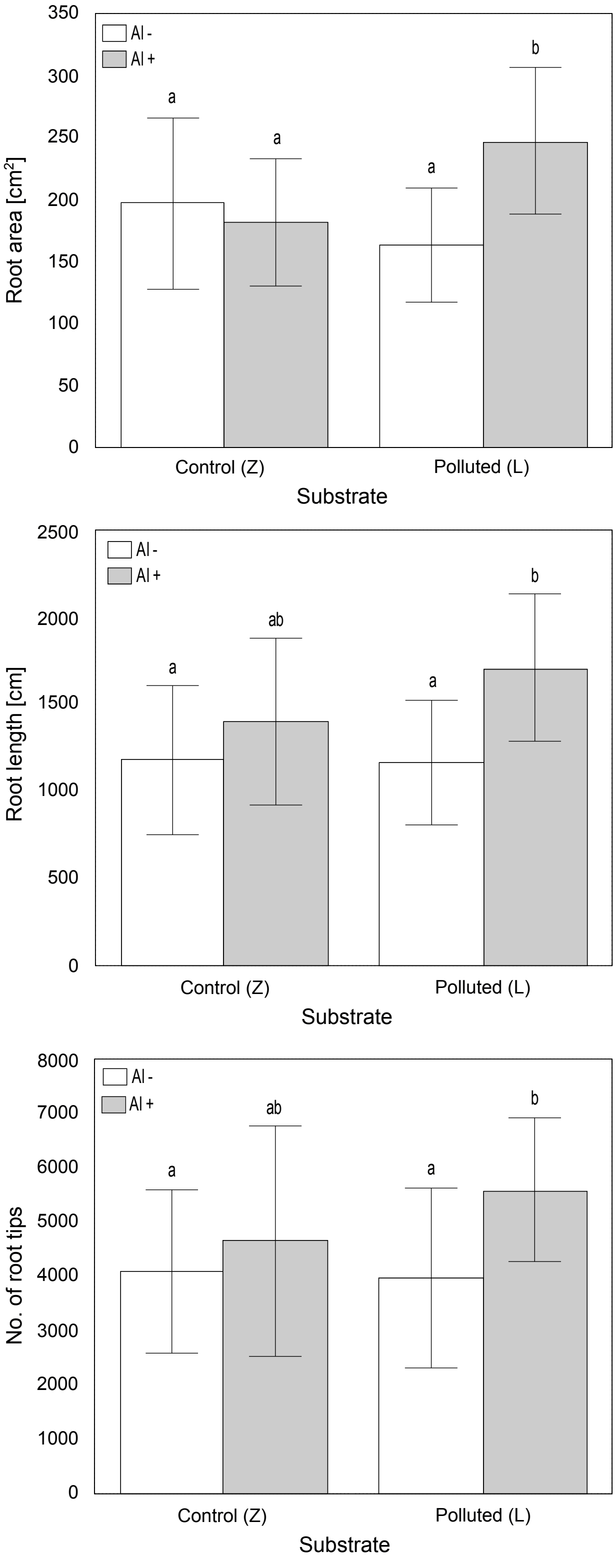

Fig. 4. Effect of substrate type on the development of the root system of poplar cuttings, derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (AL-), after planting in a control substrate (Z) or a polluted substrate (L). (Experiment II, 1999.)

Poplar cuttings from Al+ cultures grew very well in the unpolluted soil (Z), formed a greater biomass of above-ground parts (Fig. 1), and reached a higher proportional biomass allocation to foliage (LWR) than those from Al– cultures (Fig. 3). Also in the polluted soil, Al+ plants were characterized by a better growth of aboveground parts and roots than Al– plants (Fig. 1). Cuttings from Al+ cultures showed significantly higher LWR and lower RWR (Fig 3). It is noteworthy that the development of roots of Al+ cuttings was very good in the polluted soil. They were distinguished by greater root length, area, and number of root tips than Al– cuttings. Similar results were obtained in two years of experiments (Figs. 2 and 4).

Discussion

Plants vary in sensitivity to toxic compounds in the soil. Many studies showed that the sensitivity depends on many factors, such as physicochemical properties of the soil, concentration of organic matter and nutrients, but primarily on soil pH [19, 22, 29]. Aluminium is widespread in the Earth's crust and its availability to plants increases with decreasing pH of the soil [7, 9, 10]. The biological activity of this element (its solubility and exchangeability) is related not only to the acidity of the soil solution but also to the presence of calcium or magnesium ions [12, 15, 31].

Poplar cuttings from *in vitro* cultures on media without aluminium (Al–) were characterized by poor growth of aboveground parts and roots in the polluted soil from Luboń (Figs. 1 and 2). That soil had a lower pH, a higher concentration of Al ions, and a lower Ca/Al ratio than the control soil (Table 2). The toxic Al ions present in the substrate can damage root cells, which results in limiting the transport of many nutrients and blocking their participation in important metabolic processes, such as photosynthesis and respiration [20, 27, 29]. Research on birch seedlings (*Betula pendula* Roth.) has shown that high concentrations of aluminium in the substrate decreased uptake of calcium, potassium, phosphorus, iron, and zinc, and consequently the growth of roots and shoots was limited [8, 18].

The majority of *in vitro* studies on plant tolerance to toxic ions (such as aluminium, copper, cadmium, and zinc), concerned herbaceous plants [11, 17, 30, 32]. Also callus of *Acer rubrum*, from trees growing in a polluted area, was cultured *in vitro* and proved to be more tolerant to the activity of toxic metal ions (copper, nickel, cobalt) than callus from trees growing in an unpolluted area [33]. As a result of repeated passaging of poplar and birch cultures to media containing aluminium, the produced plants were characterized by a better regeneration under stress conditions, i.e. in media with the addition of toxic ions of aluminium and copper [4, 5, 6]. Microcuttings, i.e. shoots of poplar and birch propagated *in vitro* on media with aluminium were also more tolerant to aluminium in the substrate during rooting than shoots cultured earlier without aluminium [4, 5].

Rooted cuttings from *in vitro* cultures with aluminium (Al+) in the present study were distinguished by a better development of roots and aboveground parts *in vivo* in a polluted soil from Luboń than control cuttings cultured without aluminium (Al–) (Figs. 1–4).

In the presented study, *in vitro* culture resulted in production of poplar cuttings characterized with a reduced sensitivity to the toxic aluminium ions, both *in vitro* and in a soil from a polluted area. Selection of plants tolerant to toxic ions contained in the soil can enable more effective management of degraded habitats.

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Reference

1. ALI G., SRIVASTAVA P.S., IQBAL M. Morphogenic and biochemical responses of *Bacopa monniera* cultures to zinc toxicity. Plant Science. **143**, 187, **1999**.
2. ANIOŁ A., GUSTAFSON P. Genetics of tolerance in agronomic plants. In: J.Show, vol. 16. Heavy metals tolerance in plants - Evolutionary aspects. CRC Press, Boca Raton, Florida: 255, **1990**.
3. BARNABAS B., KOVACS G., HEGEDUS A., ERDEI S., HORVATH G. Regeneration of doubled haploid plants from *in vitro* selected microspores to improve aluminium tolerance in wheat. Journal of Plant physiology. **156**, 217, **2000**.
4. BOJARCZUK K. Effect of aluminium toxicity on the development of poplar (*Populus tremula* L. x *P. alba* L.) cultured *in vitro*. Acta Societatis Botanicorum Poloniae. **68**, 245, **1999**.
5. BOJARCZUK K. Effect of aluminium on *in vitro* rooting of birch (*Betula* p*endula* Roth.) and poplar (*Populus tremula* L.x *P.alba* L.) microcuttings. Acta Societatis Botanicorum Poloniae. **69**, 251, **2000**.
6. BOJARCZUK K. Effect of toxic metals on the development of poplar (*Populus tremula* L. x *P. alba* L.) cultured *in vitro*. Polish Journal of Environmental Studies. **13,** 2, **2004**.
7. BOJARCZUK K., OLEKSYN J. Rozwój jednorocznych sadzonek sosny (*Pinus sylvestris* L.) i brzozy (*Betula pendula* Roth.) w zanieczyszczonych podłożach inokulowanych grzybem *Trichoderma harzianum* Rifai. Arbororetum Kórnickie. **39**, 163, **1994**.
8. BOJARCZUK K., KAROLEWSKI P., OLEKSYN J., KIELISZEWSKA-ROKICKA B., ŻYRKOWIAK R., TJOELKER MG. Effect of polluted and fertilisation on growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Polish Journal of Environmental Studies. **11**, 483, **2002**.
9. BOUDOT J.P., BECQUER T., MERLET D., ROUILLER J. Aluminium toxicity in declining forests: a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France). Annales of Forest Science. **51**, 27, **1994**.
10. BOUDOT J.P., MAITAT O., MERLET D., ROUILLER J. Evidence for the redissolution of soil spodic horizons under the influence of acid deposition in the Vosges mountains (north-eastern France). Science of the Total Environment. **184**, 211, **1996**.
11. CHAKRAVARTY B., SRIVASTAVA S. Effect of cadmium and zinc interaction on metal uptake and regeneration of tolerant plants in linseed. Agriculture, Ecosystems and Environment. **61**, 45, **1997**.
12. 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* seedlings. Proceedings of CEC-IUFRO Symposium 1993 Hamstad, Sweden. 173, **1995**.
13. CONNER A.J., MEREDITH C.P. Stimulating the mineral environment of aluminium toxic soils in plant cell culture. Journal of Experimental Botany **36**, 870, **1985**.
14. DANTAS A.C.D., FORTES G.R.D., DA SILVA J.B., NEZI A.N., RODRIGUES A.C. Tolerance to aluminium in apple rootstocks somaclones in nutrient solution. Pesquisa Agropecuaria Brasileira. **36**, 615, **2001**.
15. ERICSSON T., GŐRANSSON A., GOBRAN G. Effects of aluminium on growth and nutrition in birch seedlings under magnesium- or calcium-limiting growth conditions. Zeitschrift für Pflanzen und Bodenkunde. **161**, 653, **1998**.
16. ERNST W.H.O., VERKLEJ J.A.C., SCHAT H. Metal tolerant plants. Acta Botanica Neerlandica. **41**, 229, **1998**.
17. GORI P., SCHIFF S., SANTANDREA G., BENNICI A. Response of *in vitro* cultures of *Nicotiana tabacum* L. to cooper stress and selection of plants from Cu-tolerant callus. Plant Cell Tissue Organ Culture. **53**, 161, **1998**.
18. GÖRANSSON A., ELDHUSET T.D. Effects of aluminium ions on uptake of calcium, magnesium and nitrogen in *Betula pendula* seedlings growing at high and nutrient supply rates. Water, Air and Soil Pollution. **83**, 351, **1995**.
19. GÖRANSSON A., ELDHUSET T.D. Is the Ca+K+Mg/Al ratio in the soil solution a predictive tool for estimating damage? Water, Air and Soil Pollution. **1**, 57, **2001**.
20. KAROLEWSKI P., GIERTYCH M.J. Influence of toxic metal ions on phenols in needles and roots, and on root respiration of scots pine seedlings. Acta Societatis Botanicorum Poloniae, **63**, 29, **1994**.
21. KAROLEWSKI P., SIEPAK J., GRAMOWSKA H. Response 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**.
22. 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 species. Plant and Soil. **119**, 39, **1989**.
23. MC LAUGHLIN D. A decade of forest tree monitoring in Canada: evidence of air pollution effects. Environmental Reviews. **3**, 135, **1998**.
24. MURASHIGE T., SKOOG F. A revised medium for rapid growth and bio-assays with Tobacco tissue cultures. Physiologiae Plantarum. **15**, 473, **1962**.
25. 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 subjected to aluminium in nutrient solution. Trees. **10**, 135, **1996**.
26. RACHWAŁ L., WIT-RZEPKA M.,. Reakcje brzóz na zanieczyszczenia z hut miedzi. Cz. II. Wyniki badań terenowych. Arboretum Kórnickie **34**, 185, **1989**.
27. REICH P.B. Altered root growth and plant chemistry of *Pinus sylvestris* seedlings subjected to aluminium in nutrient solution. Trees. **10**, 135, **1996**.
28. REICH P.B., OLEKSYN J., TJOELKER M.G. Relationship of aluminium and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Poland. Oecologia, **97**, 82, **1994**.

29. RENGEL Z. Uptake of aluminium by plant cells. New Phytologist. **134**, 389, **1996**.
30. ROUT G.R., SAMANTARY S., DAS P. *In vitro* selection and characterization of Ni-tolerant callus lines of *Setaria italica* L. Acta Physiologiae Plantarum. **20**, 269, **1998**.
31. SMALLIDGE P.J., LEOPOLD D.J. Effects of watershed liming on *Picea rubens* seedling biomass and nutrient concentration. Water, Air, and Soil Pollution. **95**, 193, **1997**.
32. VAN SINT JAN V., COSTA DE MACEDO C., KINET J-M., BAUHARMONT J. Selection of Al-resistant plants from a sensitive rice cultivar, using somaclonal variation, *in vitro* and hydroponic cultures. Euphytica. **97**, 303, **1997**.
33. WATMOUGH S.A., HUTCHINSON T.C. Metal resistance in red maple (*Acer rubrum*) callus cultures from mine and smelter sites in Canada. Canadian Journal of Forest Research. **27**, 693, **1997**.