Chemical Composition of Needles and Cambial Activity of Stems of Scots Pine Trees Affected by Air Pollutants in Polish Forests[1]

Wojciech Dmuchowski,[2] Ewa U. Kurczynska,[2] Wieslaw Wloch[3]

Abstract

*The impact of environmental pollution is defined for the chemical composition of Scots pine (*Pinus sylvestris *L.) needles and cambial activity in the tree stems in Polish forests. The research investigated 20-year-old trees growing in two areas in significantly different levels of pollution. The highly polluted area was located near the Warsaw steelworks, while the area with relatively low level of pollution (control area) was in the Kampinos National Park, 10.5 km away from the steelworks. The research revealed significant differences in chemical composition of needles between the two areas. The increased content of almost all of the studied elements was observed in all of the typical pollutants such as sulfur, chlorine, and heavy metals; and in the nutrients such as nitrogen, potassium, magnesium, calcium, and sodium. Differences were also observed in relations between particular elements and accumulation of nutrient elements (ANE). The changes in chemical composition of needles affected by environmental pollution were accompanied by a reduction of cambial activity. This reduction was not expressed as a decrease in the number of cells in cambial zone, but as a decreased number of cells on the phloem side, both at the tree-top level and at breast height. These differences were particularly visible in the second half of the vegetative season.*

Introduction

Air pollution is considered one of the main causes of forest decline in central Europe (ECE 1984, Molski and Dmuchowski 1986, Nihlgard 1985, Schutt and Cowling 1985). According to the results of national forest health monitoring, more than 50 percent of trees in Poland represent the highest defoliation classes (Malachowska and Wawrzoniak 1995). Among the European countries, only the forest condition in the Czech Republic is worse than in Poland (United Nations Economic Commission for Europe 1997).

The negative influence of air pollutants on tree growth has been well established (Smith 1990). Air pollution affects various physiological and biochemical processes (Biggs and Davis 1981, Burton and Morgan 1983, Bytnerowicz and Grulke 1993), as well as morphology and anatomy of shoot and root system of trees (Heale and Ormrod 1982, Kozlowski and others 1991, Kurczynska and others 1996, Smith and Davies 1978). Air pollutants reduce shoot growth of seedlings, and height growth of older trees may be reduced as well (Kozlowski and others 1991). The leaf area of trees exposed to air pollutants may be reduced because of inhibition of leaf formation, arrested leaf expansion, and accelerated leaf abscission (Kozlowski and others 1991). Air pollutants also reduce diameter growth of seedlings and mature trees (Thompson 1981). However, influence of pollution on cambial activity and differentiation of its derivatives, processes which regulate tree growth and development (Zajaczkowski and Wodzicki 1978), have not yet been adequately investigated.

Recently published results showed that in the polluted environment physiological polarity of pine stems was affected. This resulted in changed polar transport of auxin at the cellular level of the cambial zone (Wodzicki and others 1990a). Polar transport of auxin, in turn, is responsible for regulation of cambial activity and differentiation of its derivatives (Larson 1994).

This study investigated the effects of industrial/urban air pollution on chemical composition of needles as well as cambial activity and differentiation of its derivatives, especially on the phloem side, of Scots pine (*Pinus sylvestris* L.) trees in the polluted and control forest sites near Warsaw, Poland.

[1] An abbreviated version of this paper was presented at the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems, February 5-9, 1996, Riverside, California.

[2] Plant Chemist, Plant Physiologist, respectively, Botanical Garden of Polish Academy of Sciences, ul. Prawdziwka 2, 02-973 Warsaw, Poland.

[3] Anatomist, Department of Biophysics and Cell Biology, Silesian University, ul. Jagiellonska 28, 40-032 Katowice, Poland.

Materials and Methods

Investigations were carried out at two sampling areas along an air pollution gradient (Molski and Dmuchowski 1990) during one growing season in 1992. These sampling areas were the Kampinos National Park (a control area located upwind, 10 km away from the Warsaw Steelworks) and a site in the vicinity of the Warsaw Steelworks (a polluted area located upwind, 0.2 km away from the Warsaw Steelworks). Sampling areas were established in relatively uniform and representative portions of the stands.

Three trees were selected from each sampling area for anatomical investigation, and six trees were selected for chemical analysis of needles (18-20-year-old; 8-9 m height) of Scots pine (*Pinus sylvestris* L.) with approximately the same diameter at breast height (30-32).

Foliar mineral composition of Scots pine (N, P, K, Mg, Ca, Mn, S, Cl, and heavy metals) was determined as an indicator of health condition and contamination of the experimental trees. The samples of needles were taken from the same six trees and at the same time as in the anatomical study. Needles from the previous years' growth from the second and third whorls were collected (Dmuchowski and Bytnerowicz 1995).

Concentration of chemical elements were determined by using the following analytical procedures: metals and phosphorus were assessed by spectrophotometry atomic absorption method (Perkin-Elmer 1990, Morton and Roberts 1991) on a Perkin-Elmer 1100 fitted with a Flows Analysis System (FIAS-2000) and Graphite Furnace (HGA-700).[4] Sulfur (total) was assessed by infrared absorption method by using LECO SC-132 Sulfur System (LECO Corporation 1987) and chloride by potentiometric titration using selective chloride ion electrode (LaCroix and others 1970).

Content of elements in needles was presented as the sum of the accumulation of nutrient elements (ANE). To calculate ANE, all parameters of the contents of elements in needles were converted into gram-equivalent values. The basis of the conversion was the atomic mass of elements and the valency of their ions (Ostrowska and others 1988).

For anatomical analysis, samples included current-year wood, cambium, and current-year phloem (once in the winter season of dormancy, and April 30, May 8, June 5, July 3 and 31, August 28, and September 25) from tree-top level and from breast height of each investigated tree. Samples were fixed, dehydrated, and embedded in Epon (Kurczynska and others 1996). Samples were cut into a series of cross sections (3 m thick) with an ultramicrotome. The sections were attached to microscope slides and stained with leucofuchsin (PAS-reaction) and toluidine blue, mounted in Euparal, and investigated under light microscope. To determine the number of wood, cambium, and phloem cells, 20 radial rows were counted on the cross section from each tree. Data were analyzed by Student's t-test ($p<0.05$) and compared between sampling areas.

The soil at the two sites is of the same origin and represents the same type podsolic (Dmuchowski and others [In press]). The differences between soil conditions are caused by over 30 years of pollutant emission from the Warsaw area. On both sites the percentage of exchangeable cations in the sorption complex in the organic level of soils was the same but differences were identified in the mineral levels of soils. In the soil from the Kampinos area hydrogen was the dominant cation, which is typical for coniferous forests, while in the soil from the Warsaw area, calcium was dominant. In the organic layer of the soil from the Warsaw area, 2 times more iron was measured, 30 times more zinc, 6 times more copper, 20 times more cadmium, and 3 times more chromium than in the Kampinos area.

[4] Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

Results and Discussion

For both areas the chemical analysis of pine needles did not reveal a deficiency of any of 15 analyzed elements (*table 1*). Certain macroelements like magnesium, potassium, and phosphorus showed deficiencies in damaged trees (Huttl 1985, Rehfuess 1983, Tomlison 1987, Zotll and Huttl 1989).

The chemical composition of pine needles at the two locations was much different, particularly in regards to elements recognized as typical pollutants (*fig. 1*). For instance, 2-year-old needles from the Warsaw area contained eight times more lead than the needles from the Kampinos National Park. The levels of lead, zinc, and chromium defined as toxic for the Scots pine were exceeded. The sulfur content in plants in Central Europe reflects the impact of air pollution with sulfur dioxide. Both investigated areas differed considerably in this point. The high sulfur levels in the needles from the steelworks are evidence of a detrimental impact of air pollution with sulfur dioxide on the Scots pine (*fig. 2*). In the Kampinos National Park the impact of pollution was not so strong. At present there are no areas in Poland excluded from the impact of air pollution by sulfur dioxide.

Differences were also observed also in the contents of elements performing basic physiological functions (*fig. 1*). The needles from the Warsaw area contained more magnesium, calcium, potassium, and sodium, but less nitrogen and manganese than the needles from the Kampinos National Park. The level of phosphorus was the same.

***Table 1** — Concentration of macronutrients in Scots pine needles and normal and deficiency levels of these elements in plants.*

Element	Kampinos	Warsaw	Level normal	Level deficiency	Reference
N (percent)	1.32-1.66	1.83-1.44	1.3-1.6	<1.2-1.3	Wehrmann 1963; Gussone 1964, Fiedler and others 1969, cit. Smidt 1988.
P (percent)	0.11-0.18	0.12-0.18	0.11-0.17	<0.11	Gussone 1964, Fiedler and others 1969, cit. Smidt 1988.
K (percent)	0.31-0.50	0.44-0.66	0.35-0.66	<0.30	Zottl 1964; Heinzdorf 1967, cit. Smidt 1988.
Ca (percent)	0.15-0.33	0.32-0.58	0.23-0.50	<0.05-0.10	Gussone 1964, cit. Smidt 1988: Kraus 1965.
Mg (mg/kg)	753-910	1207-1518	500-1300	<500	Wehrmann 1963; Fiedler and others 1969, cit. Smidt 1988.
Mn (mg/kg)	538-916	76-139	175-810 [1](40-2000)	[1](20)	Wehrmann 1963; Fiedler and others 1969, Materna 1962; Bergman1982; Knabe 1985; Isermann 1986.

[1] *Picea abies*

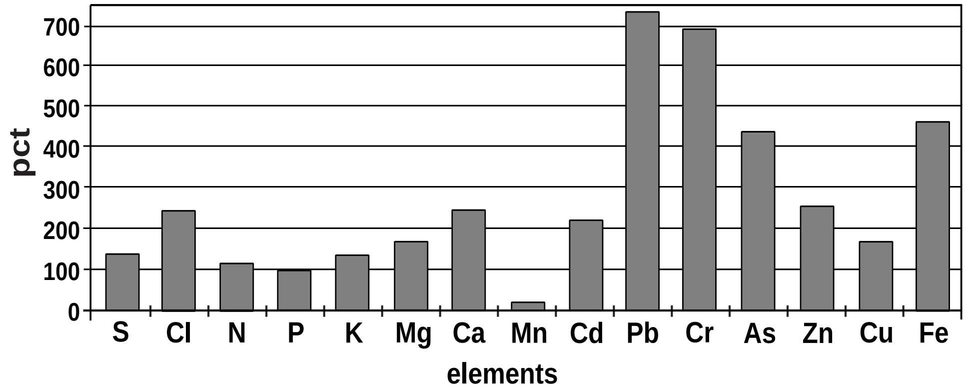
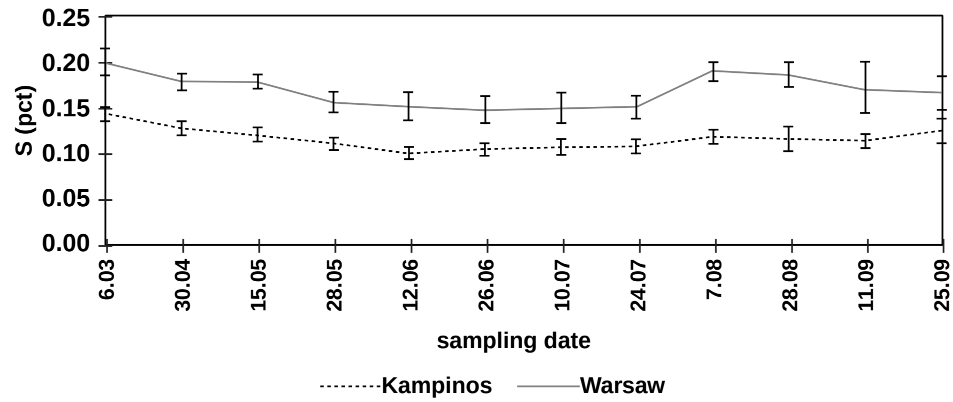


Figure 1 — Chemical composition of needles in the polluted area shown as percent of the concentrations in the control areas.

Figure 2 — Total sulfur content in previous-year needles.



In order to identify the presence of possible ionic equilibrium disturbances in the trees affected by environmental pollution, the ionic equilibrium indicators were calculated: ratio of calcium to magnesium (*fig. 3*) and ANE total of all sample elements (*fig. 4*). The calculation revealed certain differences, but these were not statistically significant. It seems that anatomical changes in stems of Scots pine in the Warsaw area are not caused by mineral deficiencies, but perhaps by toxic effects of environmental pollutants. Concentration of sulfur in needles was at toxic levels. Concentrations of heavy metals were always higher than normal levels in plants, but below toxic levels. Elevated concentrations of sulfur and heavy metals can cause negative effects on trees, especially under synergistic influences of other pollutants (*table 2*).

Figure 3 — Ratio Ca/Mg in previous-year needles.

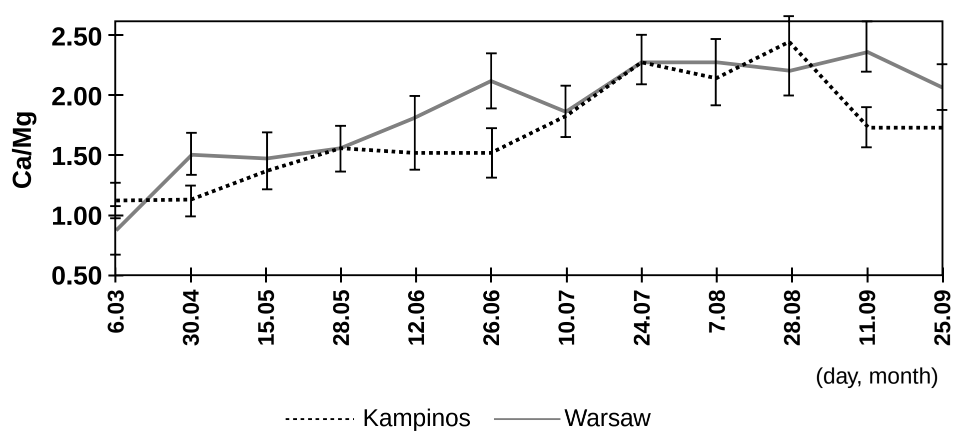


Figure 4 — Accumulation nutrients elements (ANE) in previous-year needles.

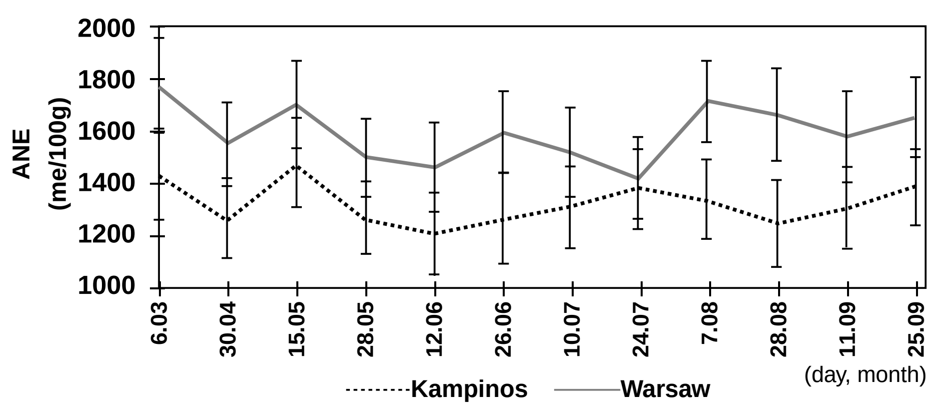


***Table 2** — Concentration of macronutrients in Scots pine needles and normal and toxic levels of these elements in plants.*

Element	Scots pine		Level		Species	Reference
	Kampinos	Warsaw	normal	toxic		
S (percent)	0.10-0.14	0.15-0.20	0.06-0.09	>.12	*Pinus sylvestris*	Huttunen and others 1985; Dmuchowski and Bytnerowicz 1995.
Cd (mg/kg)	0.18-0.36	0.39-0.60	0.01-0.3		plants	Allen and others 1974;
			0.05-0.2	>5	plants	Kabata-Pendias and Pendias 1993;
				4.8	*Picea* sp.	Burton and Morgan 1983.
Pb (mg/kg)	1.9-2.4	11.9-17.6	<10	>30	plants	Kabata-Pendias and Pendias 1993;
				19	*Picea* sitch.	Burton and Morgan 1983.
As (mg/kg)	0.05-0.12	0.29-0.54		>1	plants	Macnicol and Becket 1985;
				>5	plants	Kabata-Pendias and Pendias 1993.
Cu (mg/kg)	3.3-5.1	5.8-8.9	2-20	>20	plants	Kabata-Pendias and Pendias 1993;
			2.5-25		plants	Hewitt and Smith 1974;
			25		leaved trees	Linzon and others 1979.
Zn (mg/kg)	31-42	68-104	10-100	>100	plants	Kabata-Pendias and Pendias 1993; Karweta 1976;
			226		*Picea* sitch.	Burton and Morgan 1983;
			250		leaved trees	Linzon and others 1979.
Cr (percent)	0.30-0.98	2.47-4.72	0.1-1.0	>1	plants	Kabata-Pendias and Pendias 1993
Cl	0.02-0.04	0.04-0.08	0.02-0.10		*Picea abies*	Keller 1972

During the growing season, cambial activity and the number of cells on the xylem and phloem side were higher at the tree-top level than at breast height, independent on the sampling area. We found no statistically significant differences in the number of cells in the cambial zone between trees grown in control and polluted areas (*fig. 5*), and in the number of xylem cells between trees grown in these two areas (*fig. 6*). The number of phloem cells was significantly different between trees grown in control and polluted area (*fig. 7*). The decrease in the number of phloem cells was especially visible in the second part of the growing season. It is possible that changes in the phloem are very important cause of tree decline in the polluted environment. For instance, in normally growing trees the maximal mass of functioning phloem appears in the second part of the growing season, when the most intensive transport of sugars to the stem and roots occurs. Decreasing the number of cells participating in that transport could lead to the disturbance in translocation of assimilates to different parts of trees and cause limited growth.

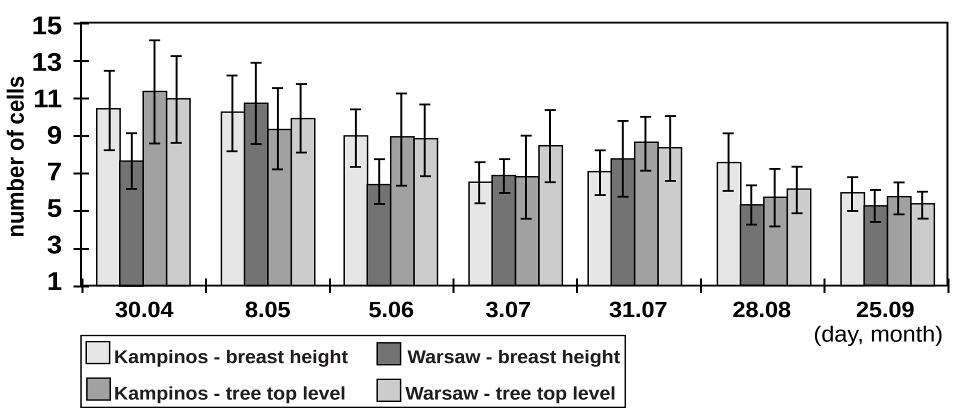


Figure 5 — Number of cells in the cambial zone during growing season. Each bar represents the average value (± SD) from three trees.

Figure 6 — Number of wood cells during growing season. Each bar represents the average value (± SD) from three trees.

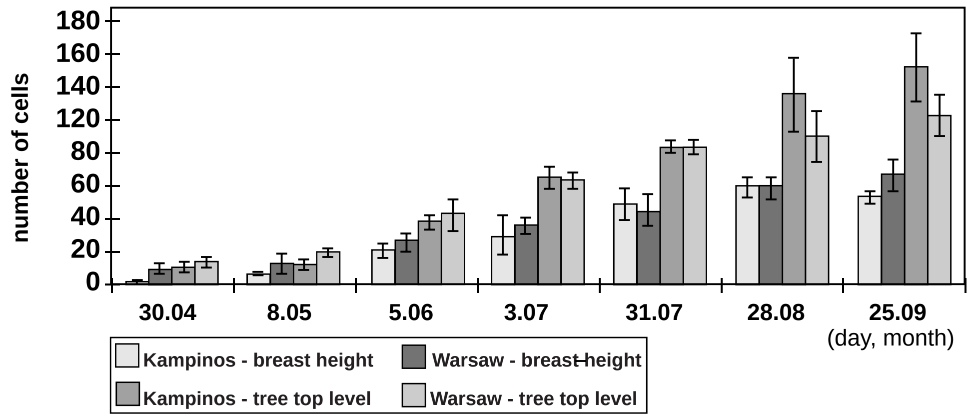
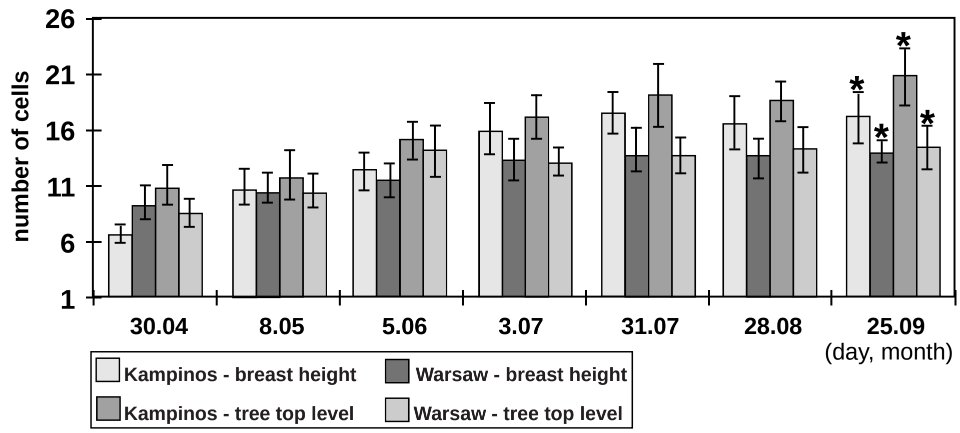


Figure 7 — Number of phloem cells during growing season. Each bar represents the average value (± SD) from three trees. Bars marked by * are significantly different in area category.



Conclusions

The Scots pine trees growing in the more polluted Warsaw Steelworks environment differed considerably from the trees growing in the relatively unpolluted Kampinos National Park environment. In the polluted environment, the needles contained much higher concentrations of elements recognized as typical pollutants: sulfur and heavy metals. And the levels of sulfur, lead, zinc, and chromium in the needles of trees from the Warsaw area can be defined as toxic for the Scots pine.

The differences in the content of elements performing basic physiological functions were of minor importance. No deficiency of any element was identified. The differences in chemical composition of needles were accompanied by differences in ionic equilibrium. However, these differences were not statistically significant.

Tree stem tissues such as cambium, phloem, and wood at the tree-top level were more sensitive to pollutants than tissues in older parts of trees (breast height). The limited production of phloem (which was statistically significant) and xylem cells of the trees growing in the polluted environment may have an essential impact on the disturbances in transport of water to tree-crowns and transport of assimilates from crowns to roots. This fact may cause the limitation of growth and development of the trees growing in the polluted environment and may lead to their mortality

The growth limitation of the trees from the Warsaw area seems to be caused to a larger extent by environmental pollution from sulfur, heavy metals, and other toxic agents (which were not examined, such as nitric oxide) rather than by the disturbances in ionic equilibrium in trees.

Acknowledgments

This work was supported by the program "Effects of Atmospheric Deposition and Climate Change on Forest Ecosystems in Central and Eastern Europe and the United States," sponsored by the USDA International Cooperation and Development (ICD). We thank Laurie Dunn for technical editing of this manuscript.

References

Allen, S.E.; Grishaw, H.M.; Parkinson, J.A.; Quarmby, C. 1974. **Chemical analysis of ecological materials.** Oxford: Blackwell Scientific Publication; 565 p.

Biggs, A.R.; Davis, D.D. 1981. **Sulfur dioxide injury, sulfur content, and stomatal conductance of birch foliage.** Canadian Journal of Forest Research 11 69-72.

Burton, K.W.; Morgan, E. 1983. **The influence of heavy metals upon the growth of sitka spruce in South Wales forest. I.** Upper critical and foliar concentrations. Plant and Soil 73: 327-336.

Bytnerowicz, A.; Grulke, N.E. 1993. **Physiological effects of air pollutants on western trees.** In: Olson, R.K.; Binkley, D.; Bohm, M. eds. The response of western forests to air pollution. Ecological Studies 97; New York: Springer-Verlag; 183-233.

Dmuchowski, W.; Bytnerowicz, A. 1995. **Monitoring environmental pollution in Poland by chemical analysis of Scots Pine (*Pinus sylvestris* L.) needles.** Environmental Pollution 87: 87- 104.

Dmuchowski, W.; Kurczynska, E.U.; Wloch, W.; Gworek, B. [In press]. **Wplyw emisji Huty Warszawa na sklad chemiczny igiel sosny zwyczajnej.** III Krajowe Sympozjum, Reakcje biologiczne drzew na zanieczyszczenia przemyslowe, Poznan-Kornik; 22-26.05.

ECE. 1984. **Effects of acidifying deposition and related pollutants on forest ecosystems.** Geneva: Executive Body for the Convention on Long-range Transboundary Air Pollution; Third Session; 1984 March 5-9.

Heale, E.L.; Ormrod, D.P. 1982. **Effects of nickel and copper on *Acer rubrum, Cornus stolonifera, Lonicera tatarica,* and *Pinus resinosa.*** Canadian Journal of Botany 60: 2674-2681.

Hewitt, E.J.; Smith, T.A. 1974. **Plant mineral nutrition.** London: The English University Press; 340 p.

Huttl, R.F. 1985. **Neurtige Waldschaden und Nahlrelementversorung von Fichtenbestand in Sudwestdeutschland.** Thesis, Freiburber Bodenkundliche Abhandlungen 16, University Freiburg, FRG.

Huttunen, S.; Laine, K.; Torvela, H. 1985. **Seasonal sulfur contents of pine needles as indices of air pollution.** Ann. Bot. Fennici, 22: 343-359.

Isermann, K. 1987. **Revitalisierung geschadigter Fichten - Altbestande durch Mineraldungung.** Allg. Forstzeitscrift 39.

Kabata-Pendias, A.; Pendias, H. 1993. **Biogeochemia pierwiastkow sladowych.** Warsaw: Wydawnictwo Naukowe PWN; 363 p.

Karweta, S. 1976. **Zmiany zawartosci cynky i olowiu w roslinach jako skutek zanieczyszczenia powietrza przez zaklad hutniczy metali niezelaznych.** Zeszyty Problemowe Postepow Nauk Rolniczych 179: 583-587.

Keller, T. 1972. **Uber die Uberwach tung der Immissionen von Kehrrichtverbrennungsanlagenmit Hilfe von Nadelanalysen auf Chloride.** Mitt. Forstl. Bundesvers. Anst. 97/II: 271-280.

Knabe, W. 1984. **Merkblatt zur Entnahme von Blatt- und Nadelproben fur chemische Analysen.** Allg Forstzeitschrift 33/34: 847-848.

Kozlowski, T.T.; Kramer, P.J.; Pallardy, S.G. 1991. **The physiological ecology of woody plants.** Harcourt Brace Jovanovich: Academic Press, Inc.

Kraus, H.H. 1965. **Undersuchungen uber die Melioration degradierter Sandboden im Nordostdeutschen Yiefland.** Arch. Forstwes. 14: 265-285.

Kurczynska, E.U.; Beltowski, M.; Wloch, W. 1996. **Morphological and anatomical changes of Scots pine dwarf shoots induced by air pollutants.** Environmental and Experimental Botany 36: 185-197.

LaCroix, R.L.; Keeney, L.M.; Walsh, L.M. 1970. **Potentiometric titration of chloride in plant tissue extracts using the chloride ion electrode.** Soil Science Plant Analysis 1: 1-6.

Larson, P.R. 1994. **The vascular cambium. Development and structure.** Timell, T.E. ed., Springer-Verlag.

Leco Corporation. 1987. **Application Bulletin.** St. Joseph, MI.

Linzon, S.N.; Chai, B.L.; Temple, P.J.; Pearson, R.G.; Smith, M.L. **1979 Lead contamination of urban soils and vegetation by emissions from secondary lead industries.** Journal of the Air Pollution Control Association 26: 650-655.

Macnicol, R.D.; Beckett, P.H.T. 1985. **Critical tissue concentrations of potentially toxic elements.** Plant Soil 85: 107-129.

Malachowska, J.; Wawrzoniak, J. 1995. **Stan uszkodzenia lasow w Polsce w 1994 r.** na podstawie badan monitoringowych. Warszawa, Poland: Biblioteka Monitoringu Srodowiska, Panstwowa Inspekcja Ochrony Srodowiska.

Materna, J. 1962. **Ernahrung der Waldbaume und Forstungung.** Tagungsberichte, Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin 45-52.

Molski, B.; Dmuchowski, W. 1990. **Distribution and movement of selected elements in Poland using pine needles analysis.** In: Grodzinski, W.; Cowling, E.B.; Breymeyer, A.I., eds. Ecological risks. Perspectives from Poland and the United States. Washington, D.C.: National Academy Press 215-231.

Molski, B.A.; Dmuchowski, W. 1986. **Effects of acidification on forests and natural vegetation, wild animals and insects.** In: Schneider, T., ed. Acidification and its policy implications. Elsevier-Amsterdam 29-51.

Morton, S.; Roberts, D. 1991. A**TI unicam, atomic absorption spectrometry - methods manual.** Issue 2, Unicam LTD; 398 p.

Nihlgard, B. 1985. **The ammonium hypothesis-an additional explanation to the forest dieback in Europe.** Ambio 14: 2-8.

Ostrowska, A.; Szczubialka, Z.; Gawlinski, S.; Paul, M. 1988. **Application of the ANE value and share of particular elements in this value for appraising the state of supplying pine trees (*Pinus sylvestris* L.) with nutrient elements.** Ann. Warsaw Agricult. Univ. - SGGW-AR, Forest and Wood Technology 37: 31-40.

Perkin Elmer. 1990. **Analytical methods for atomic absorption spectrophotometry.** Bodenseewerk, Pub. B353.

Rehfuess, K.E. 1983. **Walderkrankungen und Immissionen-eine Zwischenbilanz.** Allg. Forstl. Jahrb. 58: 201-224.

Schutt, P.; Cowling, E.B. 1985. **Waldsterben, a general decline of forest in Central Europe: symptoms, development and possible causes.** Plant Disease 69: 548-558.

Smidt, S. 1988. **Schadstoffe und Nadrstoffe in Blattorbanen, Naturliche Gehalte und Grenzwerte**. Institut fur Immissionsforschung und Forstchemie, Interner Bericht 3: 1-18.

Smith, H.J.; Davis, D.D. 1978. **Histological changes induced in Scots pine needles by sulfur dioxide.** Phytopathology 68: 1711-1716.

Smith, W.K. 1990. **Air pollution**. In: DeSato, R.S., ed. Air pollution and forests**.** New York: Springer-Verlag, Inc.; 9-23.

Tomlison, G.H. 1987. **Nutrient deficiencies and forest decline.** Canada: Pulp and Paper, 88: 43.

Thompson, M.A. 1981. **Tree rings and air pollution.** A case study of Pinus monophylla growing in east-central Nevada. Environmental Pollution; Ser. A 26: 251-266.

United Nations Economic Commission for Europe, 1994. **Forest foliar condition in Europe.** EC-UN/ECE-FBVA, Brussels, Geneva, Vienna .

Wehrmann, J. 1963. **Die Beurteilung der Stickstoffernahrung von Frichten - und Kiefernbestanden**. Allg. Forstzeitschr. 32/33: 502-504.

Wodzicki, T.J.; Wodzicki, A.B.; Rakowski, K.J.; Zakrzewski, J. 1990a. **Natural auxin mobility in stem cambial region of *Pinus sylvestris* L. under conditions of soil pollution with sulfuric acid and urea.** Folia Forestalia Polonica; Ser. A 32: 50-63.

Zajaczkowski, S.; Wodzicki, T.J. 1978. **Auxin and plant morphogenesis.** A model of regulation. Acta Soc. Bot. Pol. 47: 233-243.

Zottl, H.W.; Huttl, R.F. 1989. **Nutrient deficiencies and forest decline.** In: Bucher, J.B.; Bucher-Wallin, I. eds., Air pollution end forest decline. Vol. 1, EAFV, Birmensdorf; 189-193.