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Piotr Karolewski, Jerzy Siepak, Hanna Gramowska

Response of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Douglas fir (*Pseudotsuga menziesii*) needles to environment pollution with flourine compounds

Abstract: The objective of the study was to determine differences in the response of trees of three species: *Pinus sylvestris*, *Picea abies* and *Pseudotsuga taxifolia* to environment pollution with fluorine compounds. The amounts of free and complexed fluorine (F_A) and total fluorine (F_B) were determined in the needles of trees of the three species growing in a polluted area and in an area considered free from pollution. The results of this study showed that Douglas fir is a greater sensitivity to fluorine compounds than Norway spruce and Scots pine, despite its high resistance to fluorine absorption. Estimation of the degree of environment pollution on the basis visible injury and the content of fluorine compounds in needles is discussed. The results were supplemented with observations of lily of the valley (*Convallaria maialis*) – a plant particularly sensitive to injury by fluorine compounds.

Additional key words: *Convallaria majalis*, sensitivity, injury

Address: P. Karolewski, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland, e-mail: pkarolew@rose.man.pl, J. Siepak and H. Gramowska, Department of Water and Soil Analysis, Faculty of Chemistry, A. Mickiewicz University, Drzymały 24, 60-613 Poznań, Poland e-mail: zawig@amu.edu.pl

Introduction

Among environment pollutants of anthropogenic origin, fluorine compounds belong to the most harmful group. At comparable concentrations in the soil, water or air, they are much more toxic for vegetation than sulphur dioxide, nitrogen oxides or ozone (Kluczyński 1989). The same author refers to a number of studies that demonstrate that fluorine compounds easily penetrate plants via roots and leaves. Contrary to other harmful compounds, fluorine remains permanently bound in the stems of the plants and even after cessation of emission it persists in young plants in the next growing season (Garber 1962; Keller 1974). Accumulation of fluorine compounds in leaves and needles is typically greater than in stems, trunks or roots (Piskornik and Godzik 1970; Jamrich 1972; Mucha et al. 1977; De Cormis 1978).

In June 1999 we observed necrotic lesions in the needles *of Pinus sylvestris*, *Picea abies* and *Pseudotsuga menziesii* growing adjacent to the Poznań Phosphate Fertilisers Plant (PZNF) in Luboń. Such symptoms are typical of the injury caused by fluorine compounds (Horntvedt and Robak 1975; Weinstein 1977; Kluczyński 1989). This observation was a surprise as for the last few years such necrotic injury has not been observed in the area, owing to significant reductions in emissions of toxic substances to the atmosphere (Table 1). The occurrence of necrotic lesions in the needles of the three coniferous species in June 1999, was most probably related not only to the emission of fluorine compounds but also an extensive drought in

Table 1. Average emission rates from the Poznań Phosphate Fertiliser Factory in Luboń: sulphur dioxide (SO_2), fluorine compounds (F) and nitrogen oxides NO_x (expressed as N_2O_5) in t yr^{-1} (B. Generalczyk – personal communication)

Year	Emission		
	F	SO_2	NO_x
1990	6.30	220	64
1991	2.30	127	105
1992	1.10	156	138
1993	0.91	116	82
1994	0.96	105	68
1995	1.14	115	94
1996	1.15	80	82
1997	1.11	3	30
1998	1.14	0	19

Table 2. Average emission rates of fluorine compounds from the Poznań Phosphate Fertiliser Factory in Luboń in kg $month^{-1}$ in 1999 (B. Generalczyk – personal communication)

I	II	III	IV	V	VI	VII	VIII
106.3	165.0	132.6	86.6	0.0	87.0	165.8	68.9

the region. Drought and high temperature are known to enhance the sensitivity of plants to fluorine, see references in Kluczyński (1989). Moreover, immediately prior to the observation of injury, the plant had ceased emission of harmful compounds to the atmosphere for a 1.5-month period (Table 2). A sudden recurrence of emissions after this period could produce such a harmful effect on the trees. It should be emphasised that a decrease in emissions to the atmosphere in the last few years cannot be treated as representative of the degree of environment pollution. In fact, the total amount of fluorine compounds emitted annually to the atmosphere has not changed for the last few years. A recent decline in demand for fertilisers has reduced production to 7–10 days a month at this plant. It is known however, that the effect of an equivalent dose of a toxic substance is greater the shorter the period of exposure and the higher its concentration (Zahn 1975; Umbach et al. 1983).

The objective of the study was to explore the possibility of estimating fluorine pollution on the basis of the status of needles of the three species *Pinus sylvestris*, *Picea abies* and *Pseudotsuga menziesii*. An attempt was made at establishing a correlation between the status of the needles and the content of fluorine in them. Moreover, we examined the correlations between the amount of fluorine in the needles showing different degrees of injury (from no visible injury to necrosis) and the concentrations of different forms of this element.

Materials and methods

Plant material

The study examined the needles of three coniferous species of trees: Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), Norway spruce (*Piecea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.). Additionally the observations of lily of the valley/May lily (*Convallaria majalis* L.), a plant particularly senstive to fluorine, were made. The content of fluorine was determined in one-year-old needles collected from 5-year-old trees of each species, growing at a distance of 2 km SE from the fertiliser plant (52°15' 20"N and 16°50' 31"E). The reference materials were needles from the trees of same species and the same age growing in the Forest Range Zwierzyniec, near Kórnik (52°14' 36"N and 17°05' 00"E), about 15 km from the fertiliser plant and considered free of acute pollution. The determinations were also performed for the leaves of lily of the valley. Each species was represented by three trees/plants growing in each of the sites (n=3 repetitions). The needles were collected from 3 shoots from each tree and analysed as pooled samples. All the shoots were not shaded and grew in a similar position in the crown of the trees in the polluted and control areas. Directly after cutting the shoots, in June 1999, the needles or leaves collected from the polluted area were cut into fragments without visible injury (NI) – and with visible necrotic injury (I). The needles and leaves from the control area (C) and those from the polluted area were separately oven-dried for 3 days at 65°C, ground, and subjected to analysis for the content of fluorine compounds (ppm F = mg F kg^{-1} dry mass).

Equipment

Measurements were performed on an Orion ionometer (USA) employing an ion-selective fluoride electrode and a calomel electrode as a reference.

Reagents

The necessary reagents were magnesium oxide purified for analysis andused for dry mineralisation and a buffer solution TISAB for potentiometric determinations.

Methods

The contents of free and complexed flourine (F_A) and total fluorine (F_B) were determined. The samples for analysis were dried and ground. The amount of F_A was determined by direct extraction using TISAB buffer and potentiometric determination with an ion-selective fluoride electrode. For determinations of F_B the samples were ashed in a muffle furnace at 500°C, and then extracted in the TISAB buffer and further analyzed as described above.

Results and discussion

Environment pollution by fluoride compounds was suggested by the observed needle injury to the coniferous trees. It is known that conifers are more sensitive than deciduous trees to many toxic compounds, including fluorine (Białobok et al. 1984; Balsberg-Påhlsson 1989). Significant species differences in tolerance to fluorine compounds have also been described for conifers (Rohmeder and Schönborn 1965; Wentzel 1968; Bossavy 1970). A possible explanation of this phenomenon is that species differ in absorption of fluorine compounds, as has been noted among coniferous species (De Cormis 1970, 1978). The amount of fluorine accumulated in the needles or leaves is not always related to fluorine toxicity. Kluczyński (1989) cites many studies noting that the absorption of fluorine is higher in resistant than sensitive tree species. However, most often a lack of correlation between the tree sensitivity and the content of fluorine in needles or leaves is reported, since plant sensitivity may depend upon both resistance to absorption and tolerance of the already absorbed toxic substance (Weinstein 1977). An additional reason for the ambiguity in the observations is intraspecific variation a dependence on the origin of the population (Oleksyn et al. 1997), internal factors (age and stage of physiological development of plants and their organs) (Horntvedt 1971; Keller 1976; Giertych et al. 1999a,b), and needle structure (Giertych et al. 1997), as well as the effect of environmental factors (temperature, humidity, irradiance, etc.) (Horntvedt and Robak 1975). Our observations indicate differences in the degree of injury among the three study species. The needles of Douglas fir were much more injured than those of the spruce and pine. In the trees growing in the polluted area, needles of Douglas fir without injury (chloroses or necroses) could not be found, in contrast to the other two species. The portions of individual needles of Douglas fir without (NI) and with (I) visible injury both contained lower amounts of fluorine (F_A and F_B) than needles of the spruce and pine trees (Fig. 1). In agreement with prior studies, this observation suggests that the amount of accumulated fluorine need not be positively correlated with the sensitivity of a given species as measured by the degree of visible injury.

Particular attention has been paid to the relation between the origin of a population and accumulation of toxic pollutants (Huttunen 1978; Białobok et al. 1980; Geburek and Scholz 1992). Results of our

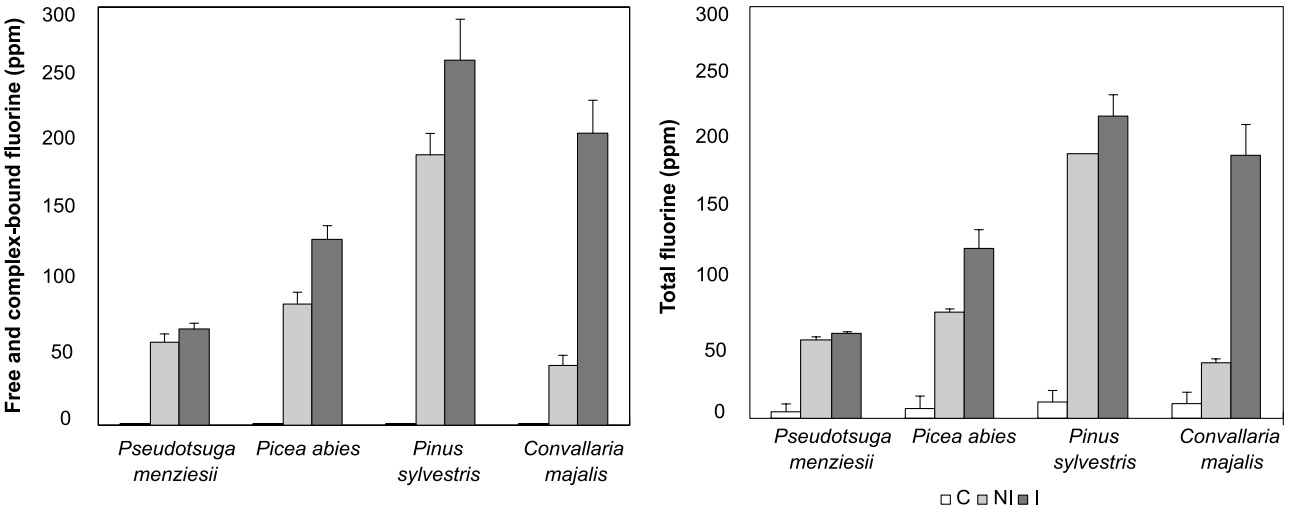

Fig. 1. Free and complex-bound fluorine contents (A), and total fluorine amount (B) in control (C), not injured (NI) and injured (I) needles of three coniferous species – Scots pine (*Pinus sylvestris*), Norway spruce (*Pice abies*), Douglas fir (*Pseudotsuga menziesii*), and in the leaves of May lily (*Convallaria majalis*)

Table 3. Significance of differences (p – values) between three coniferous species – Scots pine, Norway spruce and Douglas fir in fluorine content (F_A – free and complex-bound, F_B – total amount) of control (C), not injured (NI) and injured (I) needles

Fluorine	C	NI	I
F_A	0.9999	0.0006	0.0010
F_B	0.6527	0.0001	0.0001

study on the trees growing in the control area (and of unknown origin) lacked statistically significant differences in fluorine accumulation among the three species (Table 3). In the polluted area, the largest accumulation of fluorine forms (F_A and F_B) was determined in the needles of *Pinus sylvestris*, followed by *Picea abies* and *Pseudotsuga menziesii* (Fig. 1). The same ranking was observed in both the visibly uninjured (NI) and injured (I) portions of individual needles. These data suggest that Douglas fir is a species characterised by a greater resistance to absorption of fluorine via needles and roots, but simultaneously it is poorly resistant to the effect of the absorbed fluorides. The needles of the other two species accumulated much greater amounts of fluorine. Given that the needles of Scots pine and Norway spruce showed much less pronounced visible injury, the fluoride tolerance mechanism occurring in these species lies at the physiological-biochemical level.

The amount of fluoride ions (F_A) determined in the needles comparised, on average, 90.3% of the total fluorine content (F_B), and in particular tissues varied from 82 to 100%. The concentrations of fluorine in the needles in our study were relatively high. For example in *Picea abies* they were at a similar level as in needles of *Picea pungens* Egel., which also showed fluorine injury (Gramowska et al. 1998). The amount of fluorine (F_B) in the needles of *Pinus sylvestris* were much higher (195 ppm – NI and 220 ppm – I) than determined earlier (in 1994) in the needles of *Pinus nigra* (13.5 ppm – on average NI + I), growing in the same region (Giertych et al. 1999a,b). A still greater difference is noted when comparing these values with the level of fluorine in the needles of trees of the same species growing a short distance from the Fertiliser Plant, but on the eastern side where they are less exposed to the toxic emission plume (3–9 ppm; Zerbe et al. 1994). In comparison, the values obtained were much lower than the contents of fluorine in the other areas highly polluted by fluorine compounds. For instance, Maňkovská and Steinnes (1995) reported concentrations up to 310 ppm F in the needles of *Pinus sylvestris* and a Świeboda (1978) reported as much as 462 ppm F in the same species.

According to the data collected, *Pseudotsuga menziesii* is more sensitive to fluorine toxicity than *Pinus sylvestris* or *Picea abies*. The two latter species are characterised by a similar sensitivity to the toxic effect of fluorine. The majority of reports on the subject give similar results. According to Bolay and Bovay (1965), Rohmeder and Schönborn (1965), De Cormis (1970), Jamrich (1972) oraz Szalonek (1978), *Pinus sylvestris* and *Picea abies* are species relatively sensitive to the effect of fluorine compounds. The same authors also indicate a greater sensitivity of *Pseudotsuga menziesii* compared to *Picea abies* (Horntvedt and Robak 1975), and both species are less sensitive than *Pinus sylvestris* (Rohmeder and Schönborn 1965; Wentzel 1968; Bossavy 1970). *Pseudotsuga menziesii* exhibited the greatest sensitivity among 11 species of coniferous trees analysed by Treshowa and Packa (1970, after Horntvedt and Robak 1975) as reported by Weinstein (1977).

One of the most important problems in bioindicator studies is the identification of species which rapidly respond with diagnostic symptoms of exposure to even low concentrations of the toxic substance. In the same environmental conditions Douglas fir revealed more visible injury to its needles than Scots pine and Norway spruce. Our observations were made immediately after needle injury occurred near the fertilizer plant (within 2–3 days). This is important since

Table 4. Significance of differences (p – values) of fluorine contents (F_A – free and complex-bound, F_B – total amount) between control (C), not injured (NI) and injured (I) needles/leaves of three coniferous species – Scots pine, Norway spruce and Douglas fir and May lily

Fluorine	Treatment of needles/leaves	Douglas fir	Norway spruce	Scots pine	May lily
F_A	C and NI	0.0001	0.0006	0.0001	0.0013
	C and I	0.0001	0.0001	0.0011	0.0033
	NI and I	0.1013	0.0220	0.1407	0.0077
F_B	C and NI	0.0005	0.0001	0.0001	0.0090
	C and I	0.0004	0.0003	0.0002	0.0009
	NI and I	0.0796	0.0081	0.2051	0.0015

species from the genus *Pinus* largely retain needles even when severely injured, whereas species from the genus *Picea* lose their needles even when slightly injured. The greater injury of *Pseudotsuga* needles occurred despite the fact that the amount of the accumulated fluorine (F_A and F_B) in the needles of Scots pine and Norway spruce was much greater, both in visually injured and uninjured portions of individual needles. This observation is evidence for a greater sensitivity of Douglas fir to fluorine compounds, despite its high resistance to fluorine absorption.

Overall, the amount of fluorine accumulated in the visibly injured and uninjured portions of individual needles on the trees growing in the polluted area differed (Table 4). A significant difference was determined in Norway spruce, which may indicate the presence of metabolic mechanisms protecting against the tissue degradation. A statistically significant and even greater difference in the content of fluorine between the uninjured parts and those with necrosis was found for the lily of the valley. This finding suggests that a determination of the fluorine content in this herbaceous plant can be a very effective procedure for estimation of fluorine pollution in a given area, even before the appearance of visible symptoms. Determination of the fluorine content in the needles of Scots pine can also be a useful indicator of environment pollution with fluorine compounds since fluorine accumulation in this species is almost twice as high as in the needles of Douglas fir and Norway spruce. According to many authors, symptoms of injury to one-year old needles of Scots pine appear even at a very low content of fluorine (<50 ppm), and according to Horntvedt (1971) at a fluorine content as low as 10 ppm. In our region this is the main forest tree species, which permits widespread biomonitoring based on observations of this species.

The results permit another important conclusion. The significance differences in the amounts of fluorine in trees growing in the control conditions (C) and in the polluted area demonstrated that analysis of free and complexed fluorides is of a greater indicative value than that of the total content of fluorine.

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Reakcja igieł sosny zwyczajnej (*Pinus sylvestris*), świerka pospolitego (*Picea abies*) i daglezji zielonej (*Pseudotsuga menziesii*) na zanieczyszczenie środowiska przez związki fluoru

Streszczenie

Celem badań było określenie zróżnicowania w reakcji drzew sosny zwyczajnej, świerka pospolitego i daglezji zielonej, na wpływ związków fluoru. Analizowano zawartości fluoru wolnego i związanego kompleksowo (F_A) oraz całkowitego (F_B) w igłach drzew rosnących w terenie skażonym oraz uznanym za wolny od wpływu zanieczyszczeń. Wyniki tych badań wskazują na większą wrażliwość daglezji niż świerka i sosny mimo, że charakteryzuje się ona znaczną odpornością na pochłanianie fluoru.

W pracy dyskutowana jest możliwość wykorzystywania obserwacji widocznych objawów uszkodzeń i pomiarów zawartości fluorków, do oceny stopnia skażenia środowiska przez związki fluoru. W badaniach dodatkowo uwzględniono konwalię – jako roślinę wskaźnikową na ten typ zanieczyszczeń.