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Effects of sulphur, fluoride and heavy metal pollution on the chlorophyll fluorescence of Scots pine (*Pinus sylvestris* L.) needles

Abstract: The photochemical activity of photosystem II (PSII) was measured by chlorophyll *a* fluorescence intensity of Scots pine needles, from trees growing in various forest experimental areas exposed to air and soil pollution. Fluorescence techniques was used as a no-invasive method of detection injuries of the photosynthetic apparatus. Chlorophyll fluorescence parameters such as PSII photochemical efficiency (F_v/F_m), chlorophyll fluorescence decrease ratio (Rf_d) and size of the acceptor pools available to PSII (S_m) indicated seasonal changes in the activity of the photosynthetic apparatus of pine needles. Significant differences in the variable fluorescence parameter ratios F_v/F_m and Rf_d between the studied sites were found in middle-winter dormancy period in January and also in samples collected in August. Winter inactivation of photosynthesis results in a reduction of the photochemical efficiency F_v/F_m and Rf_d . Presented results demonstrate that the effect of industrial pollution on Scots pine needles is detectable by of measurements of fluorescence parameters. They showed that Scots pine trees indicated physiological adaptation to environmental pollution and this can be monitored by the fluorescence signals earlier than by other methods.

Additional key words: chlorophyll *a* fluorescence, pollution, Scots pine, seasonal changes, stress adaptation

Abbreviations: F_o = initial fluorescence (all reaction centers of PSII are open); F_m = maximum fluorescence (all reaction centers of PSII are closed); S_m = size of the acceptor pools available to PS II; F_v = variable fluorescence (i.e. $F_v = F_m - F_o$); F_s = steady state chlorophyll fluorescence; Rf_d = fluorescence decrease ratio (vitality index); PSII = photosystem II

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Introduction

Very wide geographical range of the natural occurrence of Scots pine causes large variability genetic of many features of this species (Giertych 1993). Wood productivity of coniferous trees is directly related to the rate of photosynthetic carbon assimilation which, in turn, is determined by complex interplay between the plant photosynthetic apparatus and the environment. Among the stress factors that cause decline in coniferous forests. SO_2 , heavy metals and fluor are most important on most all areas in Europe. When plants are exposed to environmental pollution above

the level normal physiologically acceptable range, photosynthesis gets inactivated (Bolhar-Nordenkamp et al. 1989, Miszalski and Mydlarz 1990). The PSII complex is the most susceptible to sulphur dioxide and nitrogen dioxide action (Smith and Lytle 1997) out of the various components of the photosynthetic apparatus. Chlorophyll *a* fluorescence is a process competing with photosynthetic electron transport and can be employed to study the potential photosynthetic capacity and to detect damages to the photosynthetic apparatus (Pukacki at el. 1983, Pukacki 1991, Lichtenthaler 1996, Matysiak and Lorenc-Plucińska 2000). Therefore, fluorescence measurements can be used as a tool

for early diagnosis of stress (Pukacki and Modrzyński 1998). At physiological conditions about 85% of absorbed light energy will be consumed from excited chlorophyll *a* via photosynthetic quantum conversion, whereas due to de-excitation by heat emission about 14% and due to red + far red chlorophyll fluorescence about 1% are dissipated. However under stress, the photosynthetic quantum conversion declines, and correspondingly heat emission and chlorophyll fluorescence increase considerably.

In the present work, the experiments were made in Scots pine populations of long-term air and soil pollution effect on PSII activity, (F_v/F_m ratio) and vitality index (Rf_d) (Lichtenthaler and Rinderle 1988a, Lichtenthaler 1996). This index ($Rf_d = (F_m - F_s)/F_s$), is the photochemical quenching coefficient and is correlated to the potential photosynthetic net CO₂ assimilation (Babani et al. 1996, Krupa and Moniak 1996). The ratio S_m/F_m , is the fluorescence parameter indicating size of the acceptor pools available to maximal fluorescence yield.

In the present paper were used populations of Scots pine which represent north and central Europe. These populations show different in shoot and needles growth (Fober 1997).

Materials and methods

Plant materials

The experiments were performed on one-year-old needles, of three populations Scots pine trees growing at the following experimental sites: (1) under pollution in the vicinity of a mineral fertilizer factory (Luboń), (2) close (3.5 km) to the copper foundry of (Głogów) and (3) control, relatively free of pollution, (Kórnik). In the three sites the same set of twenty Scots pine provenances are growing. The phosphate fertilizer plant in Luboń was established in 1917 and in the early 1980s it had a more or less continuous increase in fertilizer production and pollution emission. Now the annual emission rates have declined: SO₂ from 2608 t in 1980 to 80 t in 1996, fluoride from 169 t in 1980 to 1.15 t in 1996. Soil chemical properties were presented by Reich et al. (1994). The copper smelter in Głogów had an emission of SO₂ in 1985, of 57,000 t y⁻¹ and more than 3,000 t y⁻¹ of dusts (Oleksyn et al. 1988). Soil at the polluted site from 0 to 20 cm in depth had a higher concentration of heavy metals: Cu, Pb and Cd (Zwoliński 1996). The site characteristics of soil contamination and air pollution imissions were also presented by Prus-Głowacki et al. (1999) and Oleksyn et al. 1999). Data on the origin of the populations used in this study are as follows: No 3 – Serebryanskoe (Russia) – 58°46', No 8 – Rychtal (Poland) 51°08' and No 16 – Zahorie (Slovakia) –

48°46'. Other details are available in Oleksyn and Białobok (1986) and Pukacki (1999).

Fluorescence measurements

Intact leaf fluorescence parameters were measured in laboratory on detached shoots using the Plant Efficiency Analyzer (PEA), Hansatech Ltd., King's Lynn, Norfolk, England). Fluorescence was measured after 30 min dark adaptation in constant temperature (20°C, ±1.0°C). Before the measurements the samples were placed in dark plastic box on wet paper to prevent desiccation. After dark adaptation the fluorescence was measured during 2 min exposure to a 1340 mol m⁻² s⁻¹ flash of actinic red light. Illumination was provided by an array of 6 light emitting diodes ($\lambda_{max}=650$ nm), focused on the sample surface to provide a homogeneously illuminated spot of the about 4 mm in diameter. Measurements were made in the morning. For each sample it was measured and calculated fluorescence parameters: F_o , F_m , F_s , S_m , F_v , F_v/F_m , Rf_d and S_m/F_m .

Statistical comparisons were made by analysis of variance, and the means were compared with the Duncan's multiple range test.

Results and discussion

As a criterion stress injury and/or stress adaptation of Scots pine trees biophysical methods of measuring chlorophyll *a* fluorescence kinetics were used, namely chlorophyll fluorescence ratios Rf_d values (vitality index) and ratio F_v/F_m , which is the photochemical conversion efficiency of PSII. Typical chlorophyll-fluorescence induction kinetics (Kautsky effect) of dark adapted Scots pine needles are given on Figures 1 and 2. Chlorophyll fluorescence shows the typical induction curve with intermediate points (J, I) and maximum fluorescence F_m . Maximum fluorescence depends on the saturation of the reaction centers by the additional light (closed reaction centers). In the period till F_m is attained electron transfer is not working optimally. The time for reaching the steady state depends on the conditions, species and adaptation of plants. For Scots pine needles F_s was found between the 100 and 120s.

Fig. 2 shows the kinetics of chlorophyll fluorescence on a logarithmic time scale. The first rise from O to J occurs within 0.1 ms. The second intermediate step to point I in about 2.0 ms. The maximum fluorescence F_m was observed after 170 to 300 ms. This was found at different levels of photochemical efficiency of PSII needles in winter period in the Scots pine populations from polluted sites and in the control.

Measurements of chlorophyll *a* fluorescence showed a decrease in F_v/F_m value from November to January (Fig. 3). During the autumn the F_v/F_m ratio

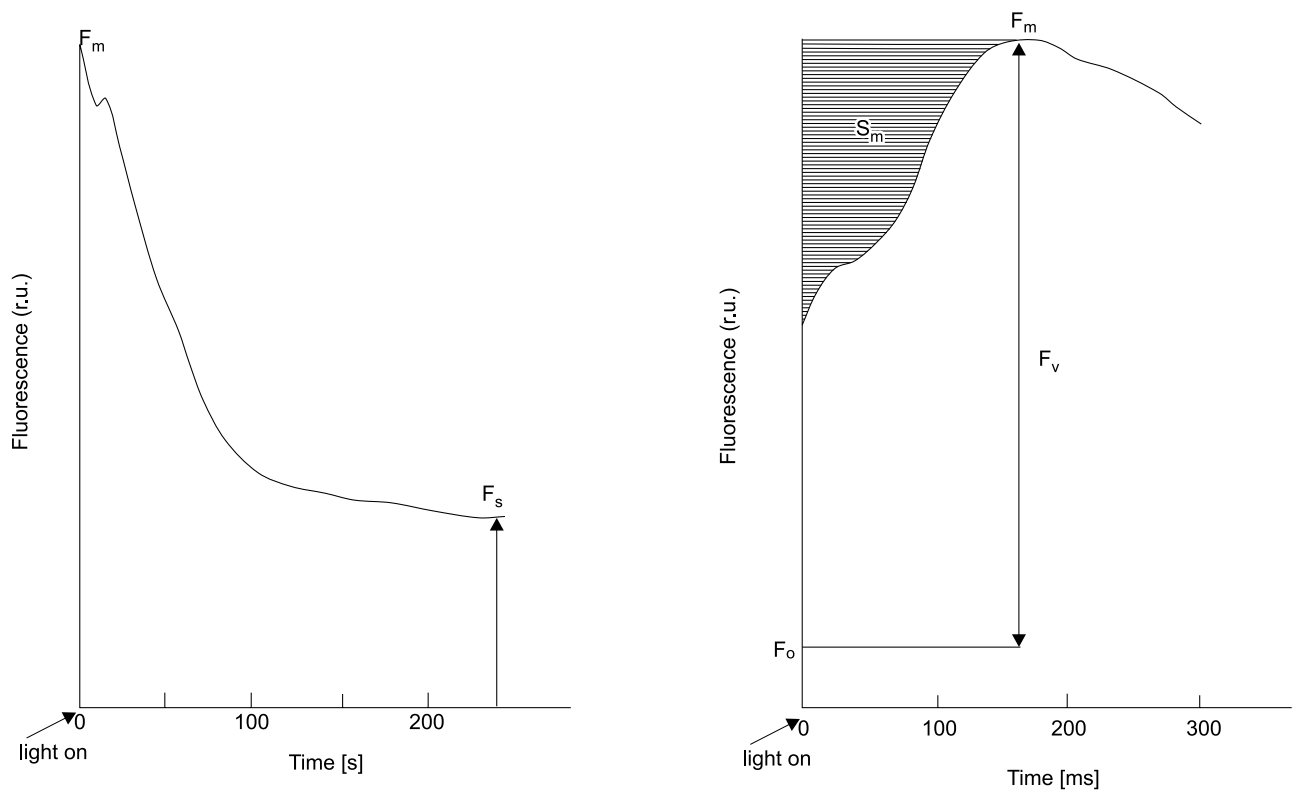


Fig. 1. Kautsky kinetics of chlorophyll *a* fluorescence in of Scots pine of one-year-old needles after 30 min dark adaptation

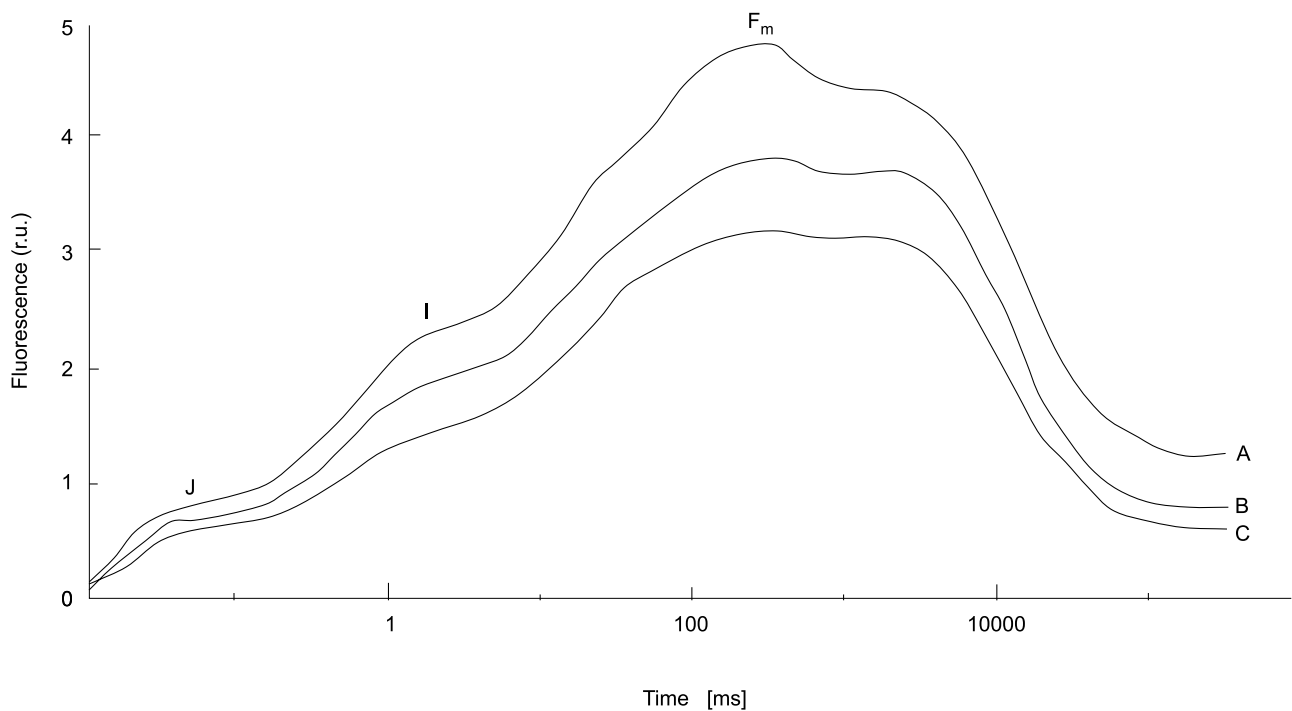


Fig. 2. Induction kinetics of chlorophyll *a* fluorescence after 30 min dark adapted Scots pine of one-year-old needles. The intermediate steps between F_o and F_m are indicated as (J and I). Curve A represents needles from trees in Kórnik, curve B needles at the polluted site Głogów, and curve C, at the polluted site Luboń

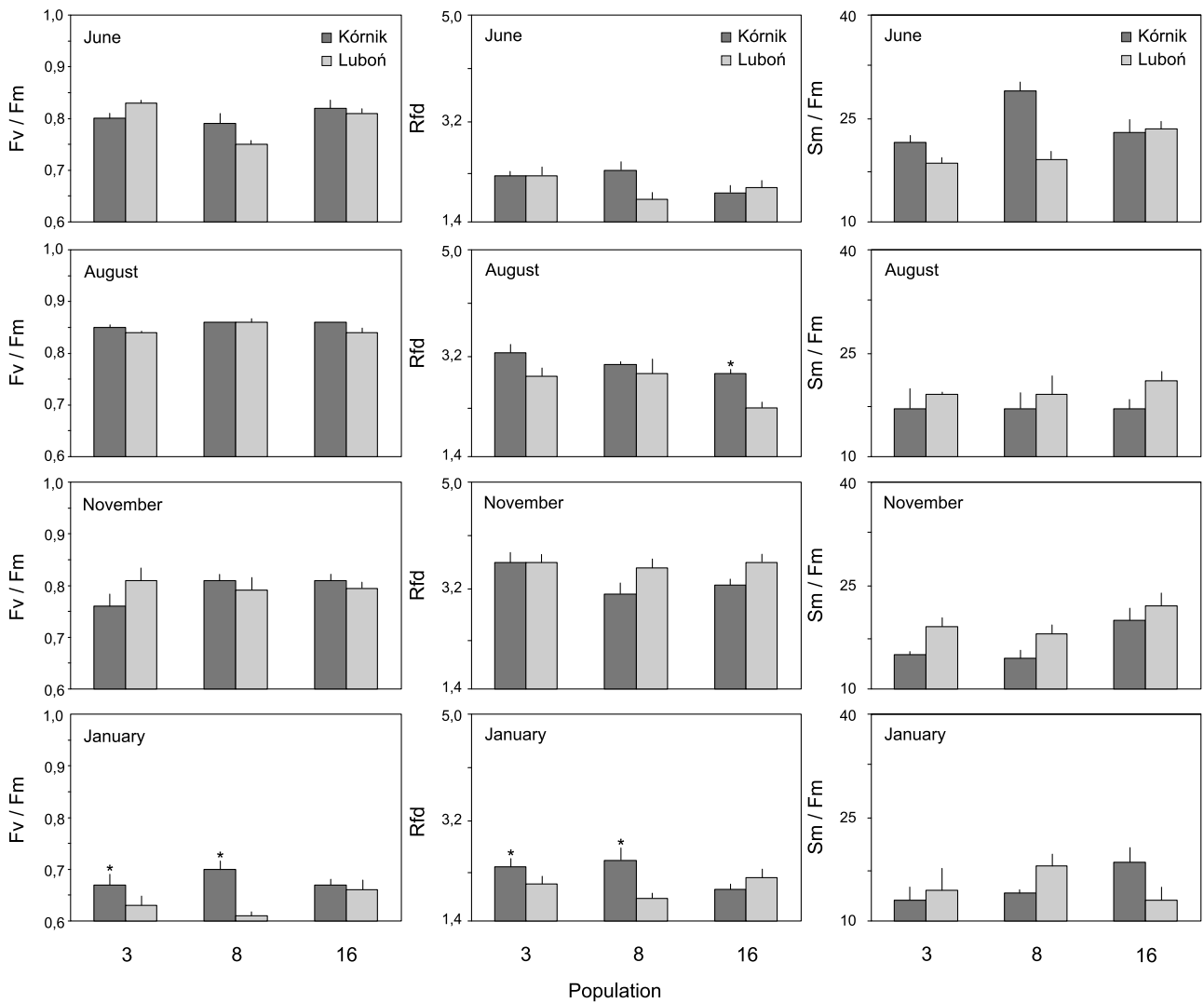


Fig. 3. Seasonal changes of chlorophyll *a* fluorescence parameters: F_v/F_m , R_{fd} and S_m/F_m in one-year-old needles of Scots pine populations in the polluted (Luboń) and control site (Kórnik). Population: No 3 Serebryanskoe (Russia), No 8 Rychtal (Poland) and No 16 Zahorie (Slovakia). Ten readings were made on each plant and values shown are the mean for three trees. Vertical bars indicate the standard deviations. Significance level: * ($p < 0.05$) from control values according to ANOVA

Table 1. Chlorophyll *a* fluorescence parameters of one-year-old Scots pine needles collected in August in control (Kórnik) and polluted site (Głogów). Population: No 3 Serebryanskoe (Russia), No 8 Rychtal (Poland) and No 16 Zahorie (Slovakia). Standard deviation (\pm SD) values are shown in parentheses. Significance level: *, ** ($p < 0.05$ and $p < 0.01$), from control values according to ANOVA

Population No.	Site	Fluorescence parameters		
		F_v/F_m	R_{fd}	S_m/F_m
3	Kórnik	0.845 (0.004)	3.1 (0.1)*	19.7 (0.2)
	Głogów	0.854 (0.001)	3.6 (0.2)	19.6 (1.0)
8	Kórnik	0.839 (0.010)*	2.9 (0.2)	19.5 (1.0)
	Głogów	0.855 (0.004)	2.8 (0.1)	21.7 (0.8)
16	Kórnik	0.854 (0.004)	2.9 (0.2)*	17.9 (1.0)*
	Głogów	0.855 (0.004)	2.7 (0.2)	23.1 (0.7)
Mean	Kórnik	0.846 (0.006)*	2.9 (0.08)	19.0 (0.8)**
	Głogów	0.854 (0.001)	3.0 (0.30)	21.4 (1.0)

decreased from 0.83 to 0.62 for population No 8 (Rychtal). The decrease indicated blockage in quantum yield for PSII electron transport. These findings are in agreement with the results of course of winter of lowered F_v/F_m ratio of *Fagus sylvatica* cortex (Larcher and Nagele 1992). Highest differences for the value of the F_v/F_m ratio about 17% were found in January in the Polish population no 8 (Rychtal). The lowering of F_v/F_m is mostly due to a decrease of F_m rather than due to variation in F_o (data not shown). However, all values of the F_v/F_m ratio in population no 8 (Rychtal) from the control site in this season were higher than values from the polluted area (Luboń) (Fig. 3). In contrast to the changes in F_v/F_m , the photochemical efficiency of size of the acceptor pools available to PS II (S_m/F_m), were general lower in samples from control area (Fig. 3). Differences increased in the winter period when the effectiveness of photosynthesis in chloroplasts was significantly lower. The Rf_d ratio increased from June to November and then decreased up to January. However in August the Rf_d ratio was slightly higher in all populations of Scots pine, from the control area Kórnik than from Luboń. Trees of population no 16 (Zahorie) from the polluted site Głogów exhibited decreased values of the ratio Rf_d comparing to the control site. However Rf_d parameter for population Serebryanskoe 3 was higher in polluted than in control site (Tab. 1). The differences in fluorescence parameters between populations was not significant. In early winter as temperature decreased, the temperature induced conformational changes in the PSII reaction centers, resulting in a modification of lipid protein interaction which is believed to play a crucial role in the maintenance of the molecular organisation of the photosystem. The modified lipid composition of thylakoid membranes can change the stability of the photosynthetic apparatus (Vogg et al. 1998) and the Rf_d ratios could be useful indicators of photosynthesis inhibition in forest trees under air and soil pollution. The decrease of Rf_d ratio in needles from the polluted areas could be explained as photoinhibitory effect on the Calvin cycle by changes in the environmental conditions and physiological status of plants (Krupa and Moniak 1993, Krupa and Baszyński 1995, Krupa et al. 1993). If the electron transfer is slowed, the number of closed reaction centers is higher, which means that F_s increases relatively to F_m . For well stress adapted trees the Rf_d value is >2.3 , and trees of lower vitality indicate Rf_d -value <2.0 (Lichtenthaler and Rinderle 1988b, Pukacki et al. 1996, Pukacki and Modrzyński 1998).

In conclusion, chlorophyll *a* fluorescence can be used as a fast and simple method indicating adaptive or recoverable changes in the photosynthetic activity of Scots pine. The long-term effects of fluoride, SO_2 ,

and NO_x result in changes of fluorescence parameters F_v/F_m and Rf_d . The clear and significant differences, between control and polluted sites occurred when samples were collected during the winter. The results also indicate that Scots pine trees of northern provenance are in slight better physiological condition than provenance of central Europe.

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Wpływ skażenia środowiska siarką, fluorem i metalami ciężkimi na fluorescencji chlorofilu w igłach sosny zwyczajnej (*Pinus sylvestris* L.)

Streszczenie

Badano fotochemiczną aktywność fotosystemu II (PS II) igieł sosny zwyczajnej mierząc fluorescencję chlorofilu *a* igieł trzech populacji rosnących pod wpływem skażenia gleby i powietrza. Analizowano następujące parametry fluorescencji chlorofilu *a*: F_v/F_m , Rfd i S_m/F_m . Stwierdzono ich sezonowa zmienność. Istotne różnice statystyczne pomiędzy stanowiskami wzrostu badanych populacji wystąpiły dla parametru F_v/F_m i Rfd w okresie spoczynku zimowego

w styczniu. Zaburzenia fotosyntezy zimą prowadzą do redukcji wydajności fotochemicznej wyrażonej stosunkiem F_v/F_m i Rfd .

Uzyskane wyniki wskazują iż kondycję drzew iglastych rosnących w skażonym środowisku można ocenić przez pomiar fluorescencji chlorofilu *a* w igłach. Przedstawione badania wspierają hipotezę zakładającą, że sosna zwyczajna należy do drzew o wyraźnej fizjologicznej zdolności adaptacji do skażonego środowiska.