INFLUENCE OF TOXIC METAL IONS ON PHENOLS IN NEEDLES AND ROOTS, AND ON ROOT RESPIRATION OF SCOTS PINE SEEDLINGS*

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ABSTRACT

Nitrates of aluminum, cadmium, manganese and lead cause changes in the content of phenolic compounds (o-dPh and TPh) in needles and roots, and in the rate of dark respiration (DR) of roots of one-year-old seedlings of Scots pine (*Pinus sylvestris* L.). The changes depend on the cation, the salt concentration used, and the analyzed plant part. The observed changes in the levels of phenolic compounds in needles and roots, and the rate of respiration in roots, indicate the following rank in toxicity of the studied metal cations: Mn < Al < Pb < Cd.

KEY WORDS: phenolic compounds, Scots pine, *Pinus sylvestris* L., environmental pollution, heavy metals, toxic cations

INTRODUCTION

Phenolic compounds influence not only the course of several important processes such as seed germination, plant growth and flowering (Harborne 1964, Gliński et al. 1986, Niemann and Van Genderen 1980), but they also participate in the mechanism of plant tolerance to such stress factors as viral infection, various wounds to tissues and water deficit (Rubin and Arcichowska 1971, Hoque 1982, Czech-Kozłowska and Krzywański 1984). This type of defence mechanisms are primarily associated with an intensified lignification process which leads to a separation of healthy tissues from necrotic ones or from pathogens (Vance et al. 1976, Glazener 1982).

In contrast to the influence of the above mentioned stress factors, the action of gaseous air pollutants of the oxidative type (O_3, NO_2, PAN) is associated with unfavourable changes in the phenolic compounds. It is suggested that under their influence phenols are oxidized to much more toxic compounds – the quinones, which in turn form complexes with some amino acids and proteins, in the form of toxic macromolecular polymers (Howell 1970, Howell and Kremer 1973). This occurs all the more rapidly the more sensitive are the plants to the respective gases (Tingey et al. 1976). Excessive accumulation of phenols may be an unfavourable phenomenon since it inhibits, among others, ATP synthesis, oxidative phosphorylation, activity of enzymes with – SH groups and CO_2 assimilation (after Howell and Kremer 1973). An increase of phenols was also observed following exposition to gases of the acidifying type, HF (Yee-Meiler 1974, 1977) and SO_2 (Karolewski 1990).

On the other hand, very little is know about the action of toxic metal cations on the level and changes in phenolic substances. These substances are known to participate in stimulation of respiration processes in plants (Tomaszewski 1961, Rubin and Arcichowska 1971). This may be of importance for the acquisition of additional energy needed for the regeneration of injured tissues. Thus an attempt was made to determine the influence of some metal cations both on the changes in the level of phenolic compounds and on the intensity of respiration.

MATERIAL AND METHODS

For the study one-year-old seedlings of Scots pine (*Pinus sylvestris* L.) were used, obtained from seeds from plus trees growing in a commercial stand in the Wałeck-Myślibórz Lake District. In early May 1992 the seedlings were transferred from a nursery into a greenhouse and after detailed root washing they were placed for 2 days into plastic, 8-liter containers with distilled water. After this time the water was replaced with a nutrient solution produced under the commercial name „Nowokont", diluting it with distilled water so that in the final use its mineral composition (in mg x dcm^{-3} of solution) was as follows: nitrogen (N) – 140, phosphorus (P_2O_5) – 25, potassium (K_2O) – 110, calcium (CaO) – 55, magnesium (MgO) – 20, sulphur (SO_4) – 5, iron (Fe) – 0.145, manganese (Mn) – 0.02, boron (B) – 0.015, zinc (Zn) – 0.01, copper (Cu) – 0.01 and molybdenum (Mo) – 0.005. The solution was brought to an initial pH 4.2 by adding sufficient conc. sulphuric acid while monitoring with a pH-meter. Every 7 days the solution was changed to a fresh one with the same composition and pH. During the experiment the pH of the solution was not adjusted. Daily a 0.5 h aeration of the

Abbreviations used: o-dPh – ortho-diphenols, TPh – total phenols, DR – dark respiration, PAR – photosynthetically active radiation.

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solutions was employed pumping air from the surrounding. The seedlings grew at a temperature of 20-25°C and a relative air humidity of 70-90%. Natural illumination was used with artificial supplementation on cloudy days from mercury-incandescent and glow-lamps, so that the light intensity varied from 80 to 120 Wm^{-2} (density of photon beam 400-600 M μm^{-2} s^{-1} PAR).

After a period of about 2 months the seedlings were exposed to the action of salt solutions with 4 metals in the form of nitrates: aluminum – $Al(NO_3)_3$, manganese – $Mn(NO_3)_2$, lead – $Pb(NO_3)_2$ and cadmium – $Cd(NO_3)_2$. For this purpose the roots have been well washed with distilled water and placed in 0.25-liter containers with solutions of the studied salts. For each of the salts a range of concentrations was used: 1.0, 2.5, 5.0, 10.0 and 20.0 mM dcm^3, and distilled water was used as the control. Each variant of the experiment was represented by 4 seedlings (replicates). The plants were held in the salt solutions for 4 days (July 9th to 13th).

Both during the growth of seedlings in containers with the nutrient medium and in the salt solutions light was prevented from reaching the roots.

After that period the roots were washed with distilled water, dried with filter paper, cut off and the intensity of their dark respiration was measured. For this purpose an LCA-2, ADC (Analytical Development Corporation, Hoddesdon, England) analyzer was used. It acts in a differential measurement system. The measurements of respiration were made for all the variants of metal concentration in the solutions and the results are presented per units of dry weight in nM CO_2 g^{-1} dry wt. s^{-1}. For this purpose a special conversion curve was developed from fresh to dry weight. Root respiration rate was measured and calculated using methods described by Oleksyn and al. (1992). Roots and needles of the same seedlings have been used to determine the content of phenols. In this case however, the level of these compounds in the needles and roots of seedlings exposed to the action of various salt solutions has been conducted on all variants, except for salt concentration 1 mM dcm^{-3}.

The content of phenols was determined per 1.0 g needle and per 0.5 g root samples after double extraction for 15 and 10 min in boiling ethanol, at 95% and 80% concentrations, respectively. The analyses were performed by spectrophotometric methods, differentiating into ortho-diphenols (o-dPh) and total sum of phenols (TPh). For the determination of o-dPh the method described by Johnson and Schaal (1957) was applied using Arnow's reagent, and for the determination of TPh using Folin-Ciocalteu's phenol reagent basing on the method proposed by Johnson and Schaal (1957) and Swain and Hillis (1959). The content of phenols has been expressed in µM of chlorogenic acid g^{-1} fresh wt. In the figures the content of o-dPh and TPh is presented as percentage deviations from the water control.

The results have been verified statistically using variance analysis and grouping means that do not differ significantly from each other at 95% confidence level.

RESULTS AND DISCUSSION

The action of nitrates of aluminum, manganese, lead and cadmium caused differences in the levels of o-dPh and TPh,

TABLE 1. Content of ortho-diphenols (o-dPh) and total phenols (TPh) in needles and roots of one-year-old Scots pine seedlings subjected to the action of nitrates of aluminum (Al), manganese (Mn), lead (Pb) and cadmium (Cd) for a period of 4 days expressed as µM of chlorogenic acid g^{-1} fresh weight and the intensity of root dark respiration (DR) expressed as nM CO_2 g^{-1} dry weight s^{-1}

Metal	Conc. mM	Needles		Roots		
		o-dPh	TPh	o-dPh	TPh	DR
Al	0	16.5 ab	29.4 ab	25.2 ab	27.8 a	24.4 a
	1	–	–	–	–	35.4 b
	2.5	16.0 ab	27.3 ab	21.0 ab	24.4 a	30.2 ab
	5	13.7 a	27.7 ab	16.4 ab	22.4 a	29.6 ab
	10	18.0 ab	34.3 b	15.5 a	23.7 a	22.3 a
	20	19.2 b	26.2 a	25.7 b	31.9 a	29.1 ab
Mn	0	16.5 b	29.4 a	25.2 b	27.8 a	24.4 a
	1	–	–	–	–	27.0 a
	2.5	18.3 bc	30.9 a	22.4 b	28.0 a	30.1 a
	5	14.0 a	34.6 a	21.2 ab	29.4 a	25.7 a
	10	17.0 b	32.9 a	15.7 a	26.9 a	26.6 a
	20	19.8 c	36.5 a	19.2 ab	24.7 a	30.5 a
Pb	0	16.5 a	29.4 a	25.2 b	27.8 b	24.4 ab
	1	–	–	–	–	30.6 b
	2.5	17.4 ab	32.4 a	17.9 a	21.8 ab	21.8 ab
	5	14.6 a	52.2 b	14.7 a	15.6 a	18.0 a
	10	20.9 b	37.7 ab	17.7 a	21.8 ab	24.2 ab
	20	20.9 b	39.1 ab	15.1 a	17.4 a	17.9 a
Cd	0	16.5 a	29.4 a	25.2 b	27.8 b	24.4 a
	1	–	–	–	–	28.7 a
	2.5	18.3 abc	39.7 a	16.2 a	16.0 a	20.8 a
	5	17.5 ab	38.4 a	15.9 a	13.1 a	24.0 a
	10	21.6 bc	45.0 a	12.4 a	17.4 a	21.0 a
	20	22.8 c	133.4 b	11.5 a	14.3 a	21.4 a

The small letters indicate groups not significantly differentiated within a confidence level of 95%.

depending on the cation, the salt concentration used, and the analyzed plant part (Table 1).

Evaluating changes in needles in the levels of o-dPh (Fig. 1) and TPh (Fig. 2), an increase in the content of these substances was observed parallel with increase in salt concentration. The pattern and direction of these changes was similar regardless of the cation used. On the other hand, comparing the quantitative changes in the level of the two studied phenolic parameters, differences were noted depending on the cation used. This reaction was more intense for cadmium than for lead, and least for manganese and aluminum. This is in agreement with the observations of other authors who studied the effect of toxic metal cations on disturbances in the intensity of various physiological processes and biochemical reactions. The particular toxicity of Cd and Pb ions, compared to the ions of other metals, has been indicated among others by Carlson et al. (1975) and by Chaney and Strickland (1984).

Changes in the content of phenols in roots (Figs. 3 and 4) which occur as a result of the action of nitrates of various cations, were characterized by a lowering of the level of these substances, thus opposite to the reaction in the needles. How-

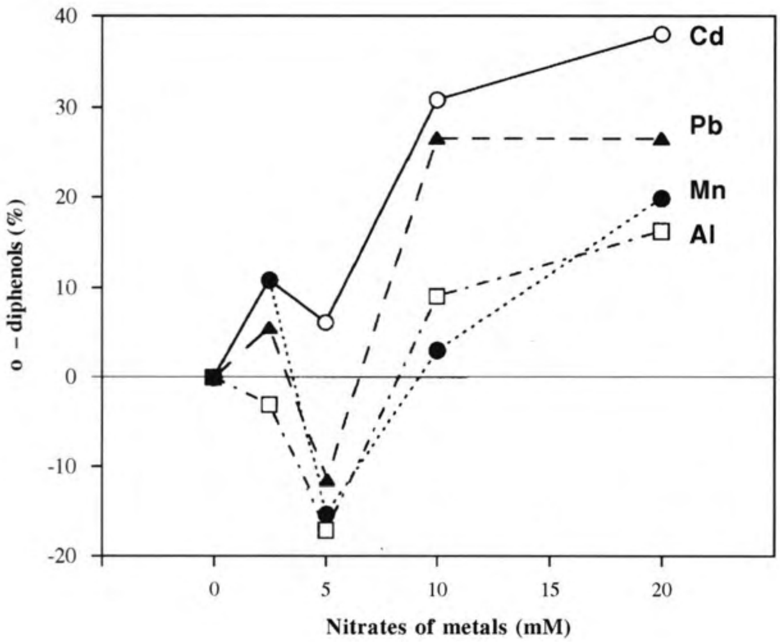

Fig. 1. Changes in the content of ortho-diphenols (o-dPh) in needles of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of aluminum (□), cadmium (○), manganese (●), and lead (▲) for a period of 4 days expressed as percentage deviations from the water control

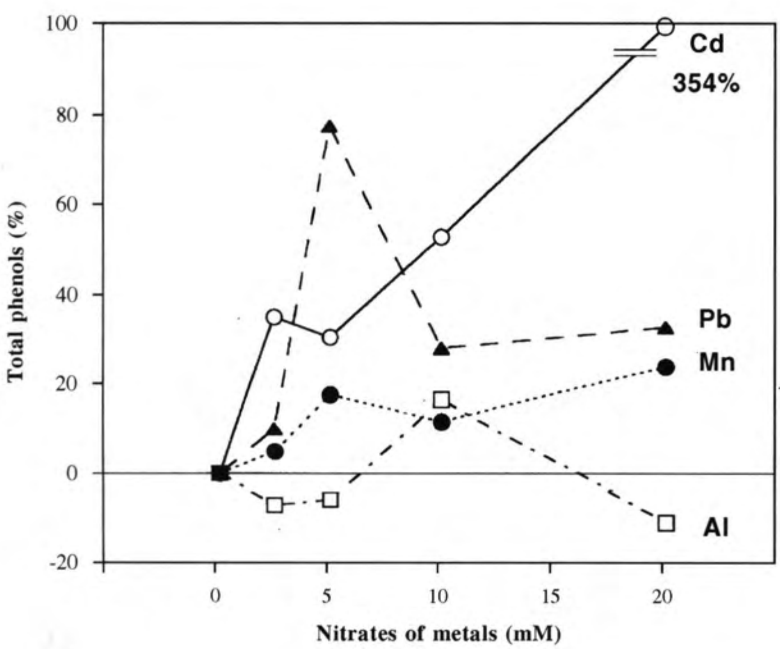

Fig. 2. Changes in the content of total phenols (TPh) in needles of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of aluminum (□), cadmium (○), manganese (●), and lead (▲) for a period of 4 days expressed as percentage deviations from the water control

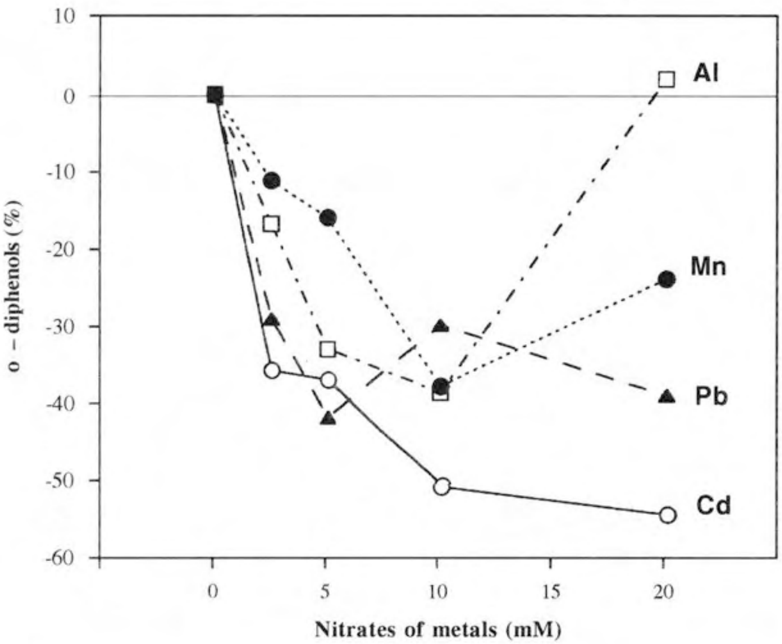

Fig. 3. Changes in the content of ortho-diphenols (o-dPh) in roots of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of aluminum (□), cadmium (o), manganese (●), and lead (▲) for a period of 4 days expressed as percentage deviations from the water control

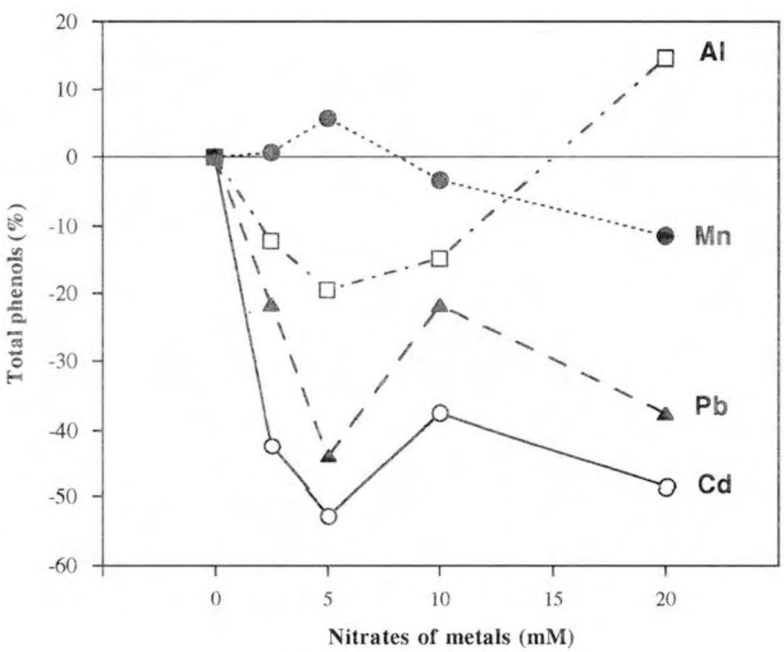

Fig. 4. Changes in the content of total phenols (TPh) in roots of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of aluminum (□), cadmium (o), manganese (●), and lead (▲) for a period of 4 days expressed as percentage deviations from the water control

ever also here the reaction was more intense following treatment with nitrates of cadmium and lead than with those of aluminum and manganese. As some authors suggest, roots are more sensitive to the action of toxic metals than other organs (Borkowska 1988, Gabara 1992, Tukiendorf 1992). This is understandable since, in this type of experimental designs, roots are the first plant organs to encounter the toxic metal ions and, at the same time, they constitute the first barrier which can function defensively against them (Wierzbicka 1992).

The relations observed in this experiment between changes in the levels of phenols in needles and roots of pine seedlings, which occur under the influence of nitrates of toxic metals are similar to those observed in earlier experiments concerning levels of proline as a result of the action of sulphites (Karolewski and Shevyakova 1990). The rapid decline in the level of this imino acid in the roots was associated with its discharge outside. Thus here again the most likely explanation for the decline in phenols is the loss of metabolites through outflow resulting from injuries to cell membranes. This would be also suggested by the studies of Kamp-Nielsen (1971) and Chen et al. (1991), who demonstrated the destructive action of toxic metal cations of the plasmatic membranes. This has caused their increased permeability and in effect an excessive outflow of various type of compounds.

The analysis of results of all four metal ions used in the study indicate that there is a statistically significant correlation between the intensity of root respiration and the level of phenolic compounds. The correlation coefficients are r=0.611 for o-dPh and r=0.685 for TPh, and both are significant at the 99% level. This would suggest a possible participation of these compounds in reactions connected with the respiration processes. That would confirm the results of Gamborg et al. (1961) and of Tomaszewski (1961), who demonstrated the participation of phenols in respiratory processes. Besides, the latter author has found, that there exists a reduction-oxidation system in which phenols play the role of electron carriers, and phenolase acts as the terminal oxidase. That author obtained an increase of tissue respiration after treating them with some phenols. On the other hand, an increase and decrease in the intensity of this process has been obtained respectively following introduction of a substrate or inhibitor of phenolase.

Changes in the intensity of root respiration, analyzed separately for various metal cations, indicate a significant statistical differentiation only in the case of aluminum and lead nitrates (Table 1). There do however occur distinct differences in the course of changes in the intensity of root respiration under the influence of Al and Mn, as compared to those under Cd and Pb. In the first case the course of DR intensity

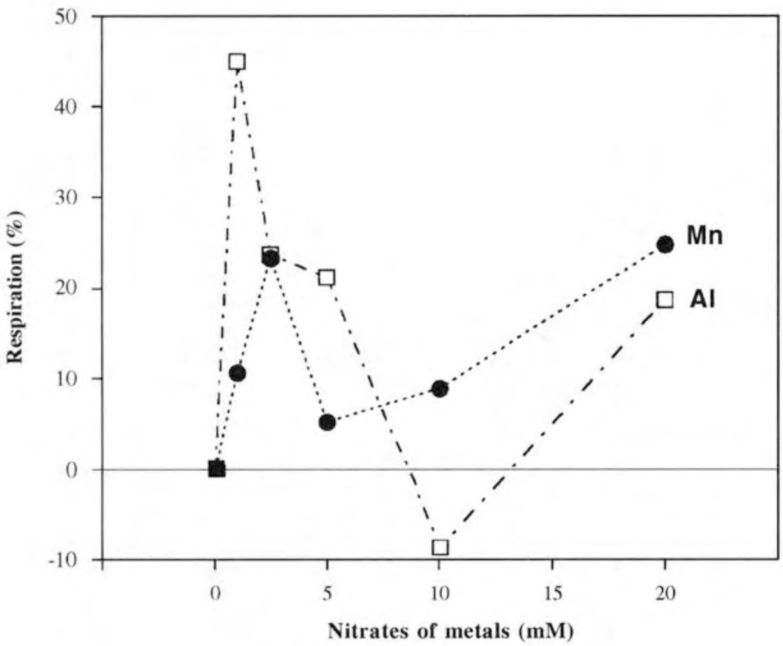

Fig. 5. Changes in dark respiration (DR) intensity in roots of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of aluminum (□), and manganese (●) for a period of 4 days, expressed as percentage deviations from the water control

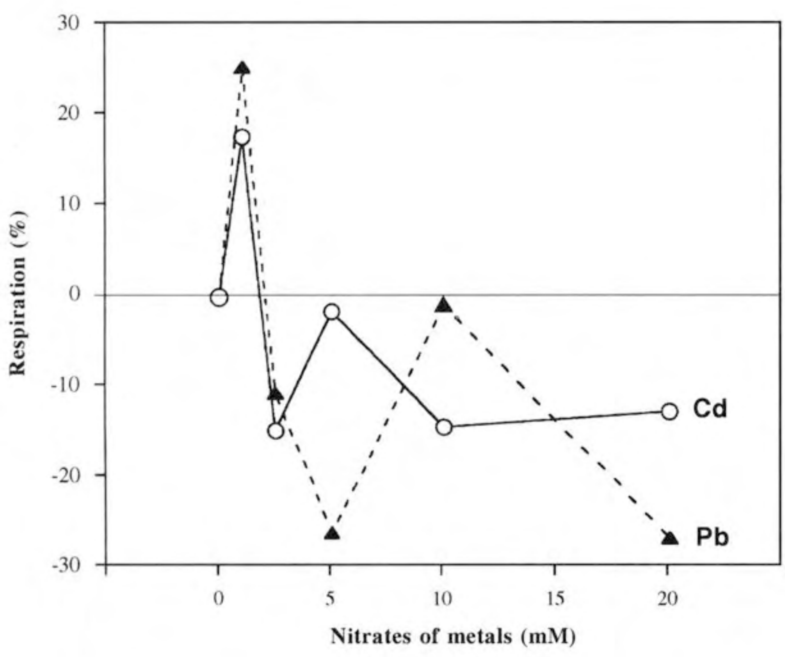

Fig. 6. Changes in dark respiration (DR) intensity in roots of one-year-old Scots pine seedlings subjected to the action of 2.5, 5.0, 10.0 and 20.0 mM solutions of nitrates of cadmium (o) and lead (▲) for a period of 4 days, expressed as percentage deviations from the water control

is characterized by the occurrence of only one wide peak, over a considerable range of salt concentrations (Fig. 5). On the other hand the action of Cd and Pb twice cause an increased CO_2 evolution (Fig. 6). The first peak occurs at the lowest level of salt concentration and most probably corresponds to an increased intensity of root respiration, as was observed under the influence of Al and Mn. This would indicate an increased defence reaction involving supply of additional energy, essential for reparation and regeneration processes. Among others Woźny et al. (1990), in a chapter of a review study concerning the influence of cadmium ions on plant respiration, quote a whole list of papers of various authors who conducted measurements of the activity of some respiratory enzymes, of the NAD/NADH ratio, of O_2 absorption and CO_2 emission in the dark, all of which indicate that the action of Cd at relatively low concentrations causes a stimulation of respiration and an inhibition only at higher concentrations. This type of reaction has already been described in the case of plants subjected to other abiotic stress agents, such as toxic gases (Pierre and Queiroz 1981). The decrease in respiration intensity that follows is a negative process. The inhibitive action of Al and Mn ions on the intensity of root respiration in maize, when the concentration of these ions was sufficiently increased, has been described by Pfeffer et al. (1986).

The second of the observed peaks (Fig. 6) can on the other hand be the result of carbon dioxide evolution as a product of degradation reactions in the root tissues. Such complex patterns in the level of CO_2 production as a response to the concentration or intensity of a toxic agent would explain the still existing controversies concerning the direction of changes in respiration intensity under the influence of abiotic stress agents (Karolewski 1989).

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WPŁYW JONÓW TOKSYCZNYCH METALI NA FENOLE W IGŁACH I KORZENIACH ORAZ NA ODDYCHANIE KORZENI SIEWEK SOSNY ZWYCZAJNEJ

STRESZCZENIE

Azotany glinu, kadmu, manganu i ołowiu wywołują zmiany zawartości związków fenolowych (o-dPh i TPh) w igłach i korzeniach oraz natężeniu oddychania ciemniowego (DR) korzeni, jednorocznych siewek sosny zwyczajnej (*Pinus sylvestris* L.). Zmiany te zależą od rodzaju kationu metalu, stężenia jego soli i badanego organu. Poziom o-dPh i TPh wzrasta w igłach, a obniża się w korzeniach badanych siewek. Zmiany natężenia oddychania korzeni są zróżnicowane i zależą od stopnia toksyczności kationu metalu. Obserwowane zmiany poziomu związków fenolowych w igłach i korzeniach oraz intensywności oddychania korzeni siewek sosny wskazują na następującą toksyczność badanych kationów metali: Mn < Al < Pb < Cd.

SŁOWA KLUCZOWE: fenole, sosna zwyczajna, *Pinus sylvestris* L., zanieczyszczenie środowiska, metale ciężkie, toksyczne kationy.