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Influence of emissions from a copper smelter on the content of copper, zinc and magnesium ions and nitrogen and protein in the leaves of alders*

Abstract

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The greatest number of alders trees planted in the zone of emission from a copper smelter, had undamaged or only slightly injured leaves. On average there was 200 times more Cu and 3 times more Zn than in the control leaves. A little more Mg was accumulated in the leaves of alders growing in emission zone than in control ones. Nitrogen and proteins did not differentiate significantly the control trees from those growing in the emission zone. Cu and Zn are accumulated for the winter rest in shoots of trees from where they are mobilized and transferred to developing leaves.

Additional key words:
Copper, Zinc, emissions, Winter rest, Alders protein.

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INTRODUCTION

One of the best methods of determining the degree of environmental pollution by industrial emissions of metals depends on the determination of their content in plant tissues grown in the zone of emission. This method permits simultaneously to establish the range of intraspecific variability in tolerance of plants to the element analysed and to establish the critical values above which occur disturbances in the production of protein, which leads in consequence to the inhibition of plant growth.

The content of copper, zinc and magnesium in an organism has a profound influence on the biological activity of plants. Copper and zinc are microelements the divalent ions of which have a capacity to enter some enzymes forming with them

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the metaloproteids that are biologically active, and the removal of the metals from them inactivates the enzymes.

The very important oxidases in the process of detoxifying plant organisms, such as phenol-, ascorbic acid-, and cytochrome oxidases as well as laccase, are cuproproteids. On the other hand carbonic anhydrase and leucine aminopeptidase are enzymes containing zinc.

Magnesium is a macroelement absorbed in an environment free of pollution in quantities several hundred times greater than copper or zinc. The relative proportions of the elements under discussion in normal dry weight of plant tissues are as follows, Cu – 6 ppm, Zn – 20 ppm, and Mg – 2000 ppm (Stout 1961). Magnesium is a constant, important component of chlorophyll, mitochondria and some metaloporphirins. It is also an activator of very many enzymes of which the most important one is ribulosebiphosphate carboxylase (RuBP).

It is a characteristic feature of all the above mentioned metals and particularly of copper and zinc that when they occur in excess they are possibly inhibitors of enzyme activity.

MATERIALS AND METHODS

Under investigation were alder leaves from 3 populations. Within each population there were progenies of individual trees planted separately. In the first year of life all progenies were raised in a polythene tent. In the second year all healthy seedlings were divided into two groups, one of which was still cultivated in the tent and the other was planted in the open nursery. Both groups received the same soil preparation and they were similarly watered. Besides black alder, we have tested also one sample of mixed progeny of 5 speckled alder trees (N-05-525), and a progeny of one tree that was a natural hybrid *A. incana* × *A. glutinosa* (N-05-518).

For each progeny a leaf sample was collected from 10 trees that grew for 2 years in a zone of emission of heavy metals and SO_2 from a copper smelter, as well as samples collected from the same trees two years earler, before they were planted in the experimental area, and when they grew in an environment free of pollution from industrial sources. The latter samples have been treated as controls in Table 1 where they are given in brackets.

The results of biochemical analysis have been evaluated by a *t*- test in order to establish the significance of differences between the analysed control leaves and those which grew in the zone of emission from the copper smelter. The significance of differences between progenies as well as between populations (provenances) have been determined by an *F* test.

The content of metals has been determined using Perkin – Elmer (USA) atomic absorption spectrophotometer. For the purpose two methods of wet burning were employed. For the determination of zinc and magnesium the collected leaves have been washed in distilled water, dried and 250 mg of dry weight has been burned in a solution of sulphuric acid and hydrogen peroxide with selenium added as a catalyst. The burning process was run at a temperature of 340°C. The solution after burning has been quantitatively transferred to a 100 ml volumetric flask. For each progeny 3 separate samples were burned.

In order to determine the content of Cu^{2+} 5 g of dry matter has been burned in a mixture of the following acids $HNO_3+H_2SO_4+HClO_4$ in deionized water. In the final part of the digestion process a 20% solution of ammonium persulfate was added. After digestion the solution has been transferred to a 50 ml volumetric flask, supplementing to volume with deionized water. It is one of the best methods of burning, however it requires great care in view of the possibility of perchloric acid explosion (Pinta 1977).

The protein and nitrogen content have been calculated as a percentage of dry weight and was determined by the method of Kjeldahl (Grewling 1976).

RESULTS

Results of all chemical analyses and statistical evaluations are presented in Table 1.

Copper. The content of this metal increased after two years of growth of the alders in the zone of emission to 1073 µg/g dw, which is about 200 times more than in the control material (5.2 µg/g dw). The extreme values were observed in progeny N-05-514, from the Karczma Borowa population, in which the plants grown in control conditions had 3.0 µg Cu^{+2}/g dw wheras in the polluted zone the value was 1502 µg Cu/g dw, that is 500 times higher. It needs to be mentioned here that the mean content of copper in the dry weight of the control alder leaves corresponds almost ideally to the content of this element normally reported for plants (Stout 1961). However individual progenies differ from each other in their ability to accumulate the element, from 443 µg in progeny N-05-487 to 1502 µg in progeny N-05-514. Differences between populations in the quantities of accumulated copper in leaves collected from the trees belonging to these populations are also significant ($F=27^{**}$).

In the population sample for speckled alder (N-05-525) we have found more copper accumulating in control conditions than in black alder (6.0 µg and 5.2 µg/g dw respectively), but half as much when the same trees were grown in the polluted area (504 µg and 1073 µg/g dw respectively).

The progeny of the hybrid tree (N-05-518) was similar in its ability to accumulate copper to black alder, having 1037 µg Cu^{2+}/g dw in the polluted environment.

Zinc. This metal is accumulated by control trees of the studied alder species at a level of 73 µg Zn^{2+} for black alder and 80 µg/g dw in speckled alder. These values indicate a high average accumulation of zinc ions by alders, about four times higher than the value normally considered as essential for the growth of other plant species (Stout 1961).

In the zone of industrial emission of zinc from the copper smelter the accumulation of this element is on the average three times higher than for the control trees

Table 1

Average content of Cu, Zn, Mg (μg/g dw) and % of N and protein in the leaves of alders in the emission zone of a copper smelter. Control results in brackets

Population and family		Cu	Zn	Mg	N	Prot.
Babki	N-05-483	1111 (5.0)	217 (46)	2760 (2620)	3.1 (3.0)	19 (19)
	N-05-484	1198 (3.0)	279 (38)	3067 (2860)	2.8 (3.3)	18 (21)
	N-05-485	731 (4.2)	155 (52)	2360 (3040)	3.5 (2.7)	22 (17)
	N-05-486	1021 (3.0)	205 (58)	3000 (2320)	2.7 (3.1)	17 (19)
	N-05-487	443 (4.7)	131 (46)	2213 (1840)	3.3 (3.4)	21 (21)
	N-05-488	1264 (2.2)	201 (56)	2920 (2480)	2.9 (3.0)	18 (18)
	N-05-489	934 (4.0)	240 (70)	3200 (2580)	3.2 (2.9)	20 (18)
Czeszewo	N-05-490	1133 (5.5)	221 (68)	3227 (3000)	3.9 (3.6)	18 (22)
	N-05-491	1129 (5.7)	247 (98)	2600 (2440)	3.1 (3.1)	19 (19)
	N-05-492	965 (4.0)	261 (54)	3067 (1960)	3.3 (3.4)	20 (21)
	N-05-493	1100 (5.5)	223 (94)	2253 (2780)	3.0 (3.1)	19 (19)
	N-05-494	1171 (5.7)	288 (82)	2920 (2660)	3.0 (3.3)	19 (21)
	N-05-495	1056 (5.5)	213 (70)	2853 (2600)	2.9 (2.6)	18 (16)
	N-05-496	981 (4.0)	271 (72)	3093 (3240)	3.0 (2.9)	19 (18)
	N-05-497	944 (4.7)	196 (64)	2813 (2740)	3.2 (3.4)	20 (21)
	N-05-498	1106 (5.5)	259 (78)	3000 (2540)	2.8 (3.2)	17 (20)
	N-05-499	1041 (4.2)	224 (46)	3187 (3260)	3.0 (3.4)	18 (21)
	N-05-500	948 (5.5)	229 (56)	2787 (2940)	3.1 (3.2)	19 (20)
	N-05-501	867 –	429 (54)	2907 (3120)	3.0 (2.9)	19 (18)
Karczma Borowa	N-05-503	1321 (4.7)	271 (74)	3600 (2680)	3.0 (3.0)	19 (18)
	N-05-504	1310 (4.7)	253 (94)	2747 (2640)	2.9 (2.7)	18 (17)
	N-05-505	1121 (8.5)	249 (88)	3067 (2940)	2.8 (3.5)	18 (22)
	N-05-507	1367 (1.0)	276 (86)	2867 (3340)	3.2 (2.9)	20 (18)
	N-05-508	1202 (2.2)	297 (142)	2947 (2660)	2.9 (3.3)	18 (21)
	N-05-509	1081 (5.0)	223 (144)	2907 (2830)	3.1 (3.2)	19 (20)
	N-05-510	929 (4.7)	223 (90)	3067 (3320)	3.1 (2.3)	19 (14)
	N-05-511	1129 (11.2)	209 (54)	3213 (2900)	3.1 (3.3)	19 (20)
	N-05-512	877 (17.0)	229 (86)	2587 (2660)	3.1 (2.5)	19 (16)
	N-05-513	1089 (7.7)	241 (96)	2840 (3120)	2.9 (2.9)	18 (18)
	N-05-514	1502 (3.0)	283 (52)	3107 (2560)	2.9 (3.1)	18 (20)
	N-05-515	1237 (8.0)	371 (68)	3733 (2800)	3.7 (2.4)	23 (15)
	N-05-516	1015 (7.2)	315 (62)	3053 (2700)	3.8 (2.3)	18 (15)
	N-05-517	1096 (4.2)	191 (66)	3120 (3080)	3.0 (2.2)	18 (14)
Max.		1502 (17.0)	429 (144)	3733 (3340)	3.8 (3.6)	23 (22)
Min.		443 (1.0)	131 (38)	2213 (1840)	2.7 (2.2)	17 (14)
Grand. mean:		1073 (5.2)	246 (73)	2942 (2765)	3.1 (3.0)	19 (19)
T-test		31***	17***	2.5*	NS	NS
F-test betw. family		2.3**	NS	NS	2.4**	2.4**
F-test betw. populations		27*	NS	NS	NS	NS
	N-05-518	1037 (4.7)	259 (78)	2947 (2640)	3.2 (3.0)	19 (19)
	N-05-525	504 (6.0)	175 (80)	2467 (2540)	3.1 (2.7)	19 (17)

(Tab. 1) being 246 μg Zn^{2+}/g dw for black alder and 175 μg/g dw for speckled alder. The hybrid alders, accumulate more zinc than black alders, and much more than speckled alders. It is notable that there is an opposite trend in the accumulation of metals by tissues of leaves from control conditions, where there were more of both Cu^{2+} and of Zn^{2+} accumulating in leaves of speckled alder, than in the hybrid ones and least in the black alders. Differentiation in the accumulation of zinc between progenies is substantial (min. 131, max. 429 μg/g dw) however statistically non--significant. Also not significant are the differences between populations, which is in contrast to the situation with magnesium.

Magnesium. This is a macroelement the content of which in the leaves of alders from the experimental area increased 6% relative to the content of this element in control material, this however is a value that is statistically significant (Tab. 1, $t=2.5$*). On the other hand the intra-and inter-population differences proved non--significant.

In the leaves of speckled alder, similarily as was the case with the other metals discussed, there was less magnesium than in the leaves of black alder and the hybrids.

Hybrids of *A. incana* × *A. glutinosa*, similarily as was the case for Zn and Cu, have had an intermediate concentration of magnesium in leaves relative to the parental species.

TRANSPORT AND ACCUMULATION OF COPPER, ZINC AND MAGNESIUM

It is not quite clear whether the metals entering plants through roots and leaves after the end of the growing season are transported in toto to roots, or whether they occur also in the stem and shoots and what is more important, are they transported to developing leaves at the beginning of the vegetative period. All mineral salts must move acropetally in the xylem as vegetation starts. If therefore in this tissue heavy metals are deposited bound with proteins, then as long as they are soluble they will be transported to developing leaves.

In order to identify this problem two year old shoots have been cut from alders growing in conditions of industrial emissions from a copper smelter and placed in water in a phytotron together with control shoots of the same species of alders growing in a zone free of population. The shoots were detached at the end of February. After 6 weeks the leaves have been already developed and had open blades. They were collected and after wet combustion by the method described earlier they were subjected to analysis for the content of Cu, Zn and Mg in an atomic absorption spectrophotometer. The results obtained are presented in Table 2.

Before the leaves opened a part of the shoots was partitioned, combusted and also subjected to analysis for the content of the studied elements. It was found that during winter rest in oneyear old and two-year old shoots of black and speckled alder and of their hybrid there are deposited metals however in quantities (Tab. 2) much smaller than in mature leaves at the end of the vegetative period. On the average in the winter shoots there were 239 μg Cu/g dw while in the autumn leaves the value was 1073 μg/g dw, which indicates than either a part of the copper was dispatched together with leaf fall or it was transported deeper into the trunk or roots. Compared to control shoots the content of Cu in shoots of alders growing in the zone of emission is more than 20 times greater. When leaves start to develop on such shoots some of the copper is being transported to them, so that at the time they are fully flushed they contain already 84 μg Cu/g dw, while the leaves of control shoots had only 6 μg Cu/g dw.

NITROGEN AND PROTEIN

Since both these parameters are strongly correlated, the concentration of protein being estimable from the content of nitrogen in plant tissues, they will be discussed jointly here.

They have not differentiated the group of samples collected in the polluted environment from that of the control material. Also non-significant were any differences observed between populations (Tab. 1). However very large differences were observed between progenies in the production of nitrogen and protein. It is an interesting fact that there was an increase in the level of nitrogen and protein in the polluted area compared to the nonpolluted one.

Leaves of all alder systematic groups analysed here have on the average had the same level of protein, 19% of the leaf dry weight.

TREE MORTALITY

In the zone of emission, where there is a large concentration of copper and zinc there can be a substantial intraspecific differentiation in the reaction of plants to elements. It was found that some progenies have had substantially injured leaves, while others experienced little leaf injury. Such poisoning which damaged half the leaf blade and caused the dying of plants was clearly of random nature. This is well illustrated by (Tab. 3), representing a listing of analyses of the injured trees and of tree mortality.

Table 2

Content of Cu, Zn and Mg(μg/g dw) in shoots and leaves of alders grown in the emission zone Shoots were cut before leaf bursting and placed into distilled water. Control results in brackets

Species	Shoots			Leaves		
	Cu	Zn	Mg	Cu	Zn	Mg
Alnus glutinosa	262 (10)	175 (110)	125 (75)	83 (7)	123 (70)	496 (120)
Alnus incana	225 (10)	148 (80)	116 (72)	76 (6)	176 (80)	512 (110)
A. glutinosa × *A. incana*	230 (11)	248 (81)	176 (97)	92 (5)	172 (78)	560 (130)
Mean	239 (10)	190 (90)	139 (81)	84 (6)	157 (76)	522 (120)

In Table 3 use was made of the following scale of leaf injury: 0 – uninjured leaves, 1 – single necrotic spots on leaves, 2 – larger spots and drying of leaf margins, 3 – more than half of the leaf blade injured.

After conversion of percentage values to angular ones (arcus sinus transformation) the significance of interpopulational and interprogeny differences were evaluated for tree mortality and leaf injury by the industrial emissions.

In both the studied groups of alders, raised in the plastic tent (P) and in the nursery (N) there were no significant differences between progenies in the rate of tree mortality. This trait however significantly differentiated populations of alders raised in the plastic tent. The highest mortality was found in the population Babki where on the average 15% of trees died and the maximal value of 33% was found in progeny N-05-487. The other two populations have only had 6.4 and 6.7% of dead trees. Among progenies N-05-498, N-05-510, N-05-512 and N-05-517 dead trees were not found at all.

As regards leaf injury the studied populations did not differ at all regardless whether the nursery material was raised under plastic or in the open. Differences between the progenies were found only in the first degree of injury to alder leaves raised under plastic, while in the second degree of injury for those raised in the open.

Only one progeny, N-05-501 (P) has had all trees with undamaged leaves, and six progenies from the populations Karczma Borowa had more than 80% of trees without leaf injury. These were progenies N-05-501, N-05-503, N-05-504, N-05-511, N-05-513, N-05-514. It is interesting for future selection purposes that in these progenies there were no leaves at all injured to the 3rd level on our scale.

Table 3

Tree mortality and leaf injuries on an experimental area in the zone of SO_2 and heavy metal emission from a copper smelter. The scale of leaf damage used is definde in text

Population and family		Dead trees %		Trees % with damaged leaves							
				Plastic				Nursery			
				Damage intensity							
		P.	N.	0	1	2	3	0	1	2	3
Babki	N-05-483	30	–	66	27	5	2	–	–	–	–
	484	18	–	61	37	2	0	–	–	–	–
	485	6	–	73	25	0	2	–	–	–	–
	486	6	–	68	22	10	0	–	–	–	–
	487	33	–	70	20	5	5	–	–	–	–
	488	11	15	55	42	5	2	61	28	9	2
	489	1	2	48	47	5	0	47	44	7	2
Czeszewo	N-05-490	12	28	57	32	7	5	52	42	6	0
	491	5	16	54	42	2	2	56	33	9	2
	492	2	11	53	37	10	0	16	52	32	0
	493	10	10	49	27	22	2	28	55	17	0
	494	8	15	56	32	10	2	39	22	37	2
	495	12	–	64	27	9	0	–	–	–	–
	496	7	–	70	24	6	0	–	–	–	–
	497	6	–	59	32	7	2	–	–	–	–
	498	0	36	53	40	7	0	80	17	3	0
	499	7	11	63	25	10	2	44	47	7	2
	500	5	35	79	17	2	2	63	37	0	0
	501	4	–	100	0	0	0	–	–	–	–
Karczma Borowa	N-05-503	11	29	83	10	7	0	56	32	12	0
	504	1	64	88	10	2	0	66	30	0	4
	505	16	20	58	37	5	0	48	45	5	2
	507	12	29	63	30	7	0	66	27	7	0
	508	15	5	53	37	5	5	65	31	4	0
	509	4	42	79	17	2	2	84	16	0	0
	510	0	2	80	15	5	0	80	15	5	0
	511	4	47	83	17	0	0	83	17	0	0
	512	0	6	49	47	2	2	49	47	2	2
	513	3	16	90	10	0	0	90	10	0	0
	514	5	43	81	12	7	0	81	12	7	0
	515	11	13	61	30	7	2	61	30	7	2
	516	13	25	71	17	5	7	85	15	0	0
	517	0	–	58	35	7	0	–	–	–	–
Max.		33	64	100	47	22	7	90	55	37	4
Min.		0	2	48	0	0	0	16	12	0	0
Grand mean		8	23	66	27	6	1	61	31	8	1
F-test betw. family		NS	NS	3.1**	13**	NS	NS	3.8**	NS	2.5*	NS
F-test betw. pop.		10**	NS	NS	NS	NS	NS	112**	NS 9	NS	NS
N-05-518		3	31	78	22	0	0	63	20	17	0
N-05-525		2	–	66	27	7	0	–	–	–	–

The *Alnus incana×glutinosa* hybrid from progeny N-05-518 had 78% of trees with undamaged leaves and the remaining 22% of trees had only injuries in the 1st category. This is a more promising result than either for black alder or the speckled alder, where 66% of trees had undamaged leaves, 27% were damaged to the 1st degree and 7% to the 2nd. (Tab. 3).

DISCUSSION

An analysis of the copper and zinc content has shown that the alders are capable of accumulating exceptionally high quantities of these elements in their tissues. One would suspect an analytical error or some misunderstanding of reading techniques were it not for the fact that the values for control material gave almost an ideal agreement with the copper levels reported for plants in the literature.

In spite of having accumulated in the tissues great quantities of copper and zinc, at the limit of plant tolerance to these metals (Carroll and Loneragan 1969, Hodenberg and Finck 1975), attaining maximal values of 1502 μg/g dw and 429 μg/g dw for copper and zinc respectively, black, speckled and hybrid alders grew well in the first two years after outplanting in the zone of emission and they have even shown primarily only slight leaf injuries.

Considering the mechanisms of resistance to the metals in question one has to remember that the values of copper and zinc content reported refer to the dry weight of leaves and in the roots, on the basis of literature information there usually occur much larger quantities of accumulated copper, zinc and other metals. Weigel and Jäger (1980) have found in bean shoots 240 μg Zn/g dw and 15 μg Cu/g dw while in the roots there was simultaneously 1650 μg Cu and 200 μg Zn/g dw.

Levitt (1958) suggested there are two mechanisms of plant resistance to heavy metals. One of them is "avoidance" depending on the possibility of refraining from absorbing ions of heavy metals by the resistant plants. So far however no example is known referring to trees in which resistance would depend on this mechanism. The second mechanism of tolerance depends on the ability to form compounds which bind the metals or transport them away from parts of the organism which perform more important vital functions, both on the within cell and within plant level or else on the plants ability to form enzymes resistant to metals.

In the studied alder trees it was not found that the high levels of copper and zinc are having a negative influence on the production of proteins, which amounted to 19% of the tissue dry weight, both in the trees from the zone of emission and in the control ones.

Both the metals, copper and zinc, have a similar distribution in various subcellular fractions. In *Notofagus fusca* it was found to be 33.2% Cu and 23.2% Zn in chloroplasts, 7.5% Cu and 8.4% Zn in mitochondria, 5.3% Cu and 3.9% Zn in ribosomes and the supernatant contained 53.8% Cu and 64.5% Zn (Timperley et al. 1973). The soluble complex compound containing copper and zinc has had the nature of a bivalent anion, while nickel occurred primarily in the form of a cation

(Peterson 1969, Timperley et al. 1973). It is believed that most of copper occurs in cytosol in a form bound to metallothioneins proteins of low molecular weight. Some of them are obtained from plant and animal tissues, which are proteins rich in cystein binding ions of copper, zinc, cadmium and probably other metals (Bartolf et al. 1980, Kägi et al. 1980, Kägi and Nordberg 1979, Reuser 1984).

A different form of low molecular weight proteins was also identified which however does not have the nature of a metallothionein but which is also capable of binding copper. It has 30-50% less cystein than the metallothioneins, namely 11%, and its molecular weight is arround 1700 (Reuser 1984).

Tukendorf et al. (1984) have isolated from spinach roots protein fractions having a molecular weight of 9500 and 12500 and from leaves one fraction of MW 8500. These authors suggest that the copper protein complex arrises thanks to the earlier occurrence of a protein in the cells the synthesis of which is stimulated by an excess of copper. They have also found that only copper in the form ions is capable of interfering with the photochemical processes.

As was indicated earlier large quantities of copper are to be found in the roots of plants where Cu was accumulated and retained in a form strongly bound to proteins and deposited in cell membranes and in the intercellular spaces, as a result of which it does not have any greater influence of the Cu-metalloenzyme (Brams and Fiskell 1971, Ernst 1975, Hogan and Rauser 1981). Morisson et al. (1981) have extracted about 40% of copper bound in metallophilous plant tissues using water and a further 40% with 0.2 M hydrochloric acid. These authors have found that about 90% of the total bound metal is associated not with one but with several ligands and occurred primarily in the form of the fraction containing the polar component. The solubility in water of some low molecular weight protein fractions containing metallic ions and their relatively low molecular weight may be the cause of their rapid transportation and accumulation of substancial quantities of copper and zinc found in the young leaves growing on shoots formed in the zone of industrial metallic emissions. In spite of the fact that the transport was only from two-year old shoots and probably part of the metal was washed out into the water in which the shoots were held, the amount of copper in the leaves was 16 times greater than in the control leaves.

Some progenies of alders which have trees with undamaged leaves such as N-05-501 (Table 1) have simultaneously had a much lower content of copper in leaves (867 μg/g dw) and a much higher content of zinc (429 μg/g dw). This may be an indication that a hypothesis be adopted concerning the competitive relation of the two elements discussed as regards the protein carriers. It is also possible that this is dependent on the differences in the rates of absorption of the two elements by roots and leaves. The relative proportions of Cu and Zn in leaves would then depend on the relative size of the space occupied by the root system and the tree crown.

The hypothesis about the competitive absorption of Cu^{2+} in relation to Zn^{2+} was proposed by Bowen (1981) who observed that the absorption of copper by roots of barley and sugarcane was inhibited by zinc. Harrison et al. (1984) have supported this contention having found that also ions of Mn^{2+} compete with copper.

The investigations performed so far permit the adoption of the very probable conclusion that copper is absorbed as a copper ion and not bound into a complex. This is probably one of the reasons for the greater tolerance of these plants to copper in a polluted environment which similarily as alder can probably accumulate also large quantities of zinc.

The greater resistance of alders to emissions of copper, zinc and other metallic elements may be favourably influenced by the root nodules with bacteria of *Actinomycetes alni* that bind free nitrogen from air. On the one hand it has been established long ago (Bond and Hewitt 1967) that copper sulphate has had a significant influence on the binding of atmospheric nitrogen in the root nodules and on the growth of alder seedlings. On the other hand one can suspect that the excess of copper, as well as of zinc, lead and other heavy metals contained in the dusts emitted by a copper smelter may be bound in the nodules in the form of chelates with amino acids, proteins and organic acids. A suggestion exists that such an excess of natural chelates in peat and boggy soils will irreversibly bind copper and this is the reason for the lack of this element in such soils. The second observation which would support this hypothesis about the favourable influence of the root nodules on the resistance of the host is the fact that one of the most resistant trees to pollution from a copper smelter is *Robinia pseudacacia* L.

Zinc is accumulated in the cell sap primarily as zinc citrate and in this complex form it is to be found in vacuoles. Large accumulation of this element in a plant causes an inhibition of root elongation (Godbold et al. 1984). Resistance to zinc excess is however associated primarily with the cytoplasm and not with the vacuoles since it was found that an increased content of citrates in some plants is not automatically associated with an increased resistance to zinc, and besides in the meristematic tissues, which are not vacuolized, there is trice as much of the element accumulating in ecotypes of *Deschampsia caespitosa* tolerant to Zn than in sensitive ones (Godbold et al. 1984). In order to explain the tolerance of plants to zinc Woolhouse (1983) proposed a hypothesis in which he assumes that the mechanism depends primarily on aspects of internal compartmentation rather than on exclusion. To transfer a metal from the cell wall to the vacuole there must exist a precise transfer mechanism located between the tonoplast and the plasmalemma. According to numerous authors (Findenegg and Broda 1965, Joseph et al. 1971) zinc is absorbed in a passive manner. Also it does not appear that there exist among trees some enzymes developed in the evolutionary process which would have a greater tolerance to metals. Mathys (1975) has shown that enzymes extracted from more tolerant plants were inhibited in vitro conditions by Cu, Zn, Pb and other metals in the same way as enzymes of plants sensitive to metallic pollution. Thus Ernst (1976) assumes that "high specificity of metal tolerance in angiosperms is dependent on cells as they already exist, and not on fundamental change in their constituents". However the reaction of enzymes in vivo is quite different than in vitro and Mathys (1975) established that the amount of zinc which would completely inhibit several enzymes in plants sensitive to zinc would be even too small for the optimal action of these enzymes in plants tolerant to zinc and copper. Besides Cox and Hutchinson (1980) when studying the activity of acid phosphatase from roots of tolerant and sensitive herbaceous plants to Zn and Cu have found that the activity of the enzyme from tolerant plants was significantly less inhibited by individual metallic elements than in plants which are not tolerant, but only so if the plants were first subjected to the action of zinc or copper.

In the results presented in Table 1, in contrast to copper where very significant differences in tolerance to metals were observed between progenies and populations, as regards to zinc such differences were not observed, even though progeny N-05-487 accumulated thrice as little zinc in leaves as progeny N-05-501. Significant and highly heritable differences in tolerance to copper and zinc have been found in *Agrostis tenuis* (Mc Neilly and Bradshaw 1968) and in *Anthoxanthum odoratum* (Gartside and Mc Neilly 1974). In order however to answer the question whether among the studied alders there exists any tolerance to Zn it would be necessary to perform an additional analysis of roots, since in that part of the plant most metals accumulate and there the greatest differences between progenies and populations are likely to occur.

As compared to copper, zinc is accumulated in relatively much lower quantities. Leaves of the studied control alders have had only three times less zinc than leaves from the zone of emission. In the case of copper the appropriate ratio amounted to 1 : 206.

Magnesium similarly as zinc was an element that in its absorption did not significantly differentiate the progenies nor the populations of the studied alders (Tab. 1). It was absorbed in larger quantities by trees which were under the influence of emissions (2942 μg/g dw of leaves) than by control trees (2765 μg/g dw), in both cases however the level seems higher than 2000 μg/g dw the level considered sufficient for most plants (Stout 1961). This is very useful for alders growing in stress conditions since magnesium plays many important functions the most important of which are : 1. Participation in the photosynthetic process as the central atom of the magnesium porphirins, 2. Activation of enzymes, 3. Influence on the structure of ribosomes. All the functions of magnesium mentioned above have a direct influence on the production of proteins, which it appears are produced by alders in surprisingly high quantities.

The high level of magnesium in the leaves of alders may have been influenced by the kind of soil on which the experimental area was established. It is known that sandy clays usually have more magnesium that sandy soils, in which this element is in short supply for plants. The experimental area was located on clayey sands and sandy clays.

Nitrogen and protein were produced by the alders growing in the polluted environment in quantities almost identical (3.1 μg N/g dw and 19 μg prot./g dw) as by trees growing in the control environment. It was found however that while there are no interpopulational differences in this trait, there are very significant differences between alders progenies, and this was so in both the groups of plants. This phenomenon was also observed in the case of other tolerant plants to polluted environments with heavy metals. Even very high accumulation of copper and zinc in the leaves has had no influence on the production of proteins in plants sensitive to metals. The protein formed in the leaves of alder is an important element in the enrichment of soils in nitrogen, and what is more important, decomposing leaves supply large quantities of protein ligands which can bind in the soil metals emitted by industry.

For the further selection of more tolerant alders to emissions of metals it is an important fact that there exist differences between progenies, among which progeny N-05-516 producing most proteins (3.8 μg N/g dw and 18 μg prot./g dw) had the ability to accumulate less than average quantities of copper and larger than average quantities of zinc and magnesium.

CONCLUSION

Studies on *Alnus glutinosa*, *A. incana* and the hybrid *A. incana* × *A. glutinosa* planted in an experimental area located in the zone of emission from a copper smelter have shown that:

1. In terms of mortality the alders after two years of growth under stress conditions had increased losses by only 8%, and the differences between the three studied populations proved highly significant.

2. In terms of the degree of leaf injury the greatest number of trees 66% had undamaged leaves, and a further 27% had only slightly injured leaves.

3. In terms of nitrogen (protein) production no negative influence of the emission on the alders was observed.

4. Very significant interprogeny and interpopulation differences were observed in the ability to accumulate Cu^{2+} in the leaves of alders. Speckled alders having on the average half as much copper in leaves as the black alder or the hybrid progeny. In the leaves from the emission zone there was on the average 200 times more copper than in leaves from the control trees.

5. The accumulation of Zn^{2+} in the leaves of alders did not differentiate the progenies nor the populations, it was established however that speckled alder accumulated less of the element than black alder. Trees from the emission zone have on the average had 3 times more Zn in the leaves of black alder and 2 times as much in speckled alder as the leaves from trees grown in the control conditions.

6. Magnesium was observed and accumulated in the leaves of alders in the zone of heavy metal emission in larger quantities than in leaves of alders growing on the control area. However there were no significant differences between populations or progenies, in the ability to accumulate magnesium.

7. Cu and Zn are accumulated for the winter rest in shoots of trees from where they are mobilized and transferred to developing leaves. There is relatively much less magnesium in the shoots.

8. Nitrogen and proteins did not differentiate significantly the control trees from those growing in the emission zone of a copper smelter. There were however significant differences between progenies in the ability to produce proteins though these differences were not observable between populations.

SUMMARY

Studies on *Alnus glutinosa*, *A. incana* and *A. incana* × *A. glutinosa* in an area in the zone of emission from a copper smelter has shown that the alders after two years of growth under stress conditions have increased losses by only 8%, the differences between the three studied populations proved highly significant. In all 66% of the trees had undamaged leaves. No negative influence of the emission on the protein production was observed. Very significant interprogeny and interpopulation differences were observed in the ability to accumulate Cu^{++} in the leaves. In the leaves from the emission zone there was on the average 200 times more copper than in control leaves. Speckled alders having on the average half as much copper in leaves as the black alder or the hybrids.

The accumulation of Zn^{++} in the leaves of alders did not differentiate the progenies nor the populations. In the leaves from the emission zone there was on average 3 times more Zn than in control leaves. Cu and Zn are accumulated for the winter rest in shoots of trees from where they are mobilized and transferred to developing leaves.

Magnesium was absorbed and accumulated in larger quantities than in control leaves, however there were no significant differences between populations or progenies.

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Wpływ emisji z huty miedzi na zawartość jonów miedzi, cynku i magnezu oraz azotu i białka w liściach olsz

Streszczenie

Badania *Alnus glutinosa*, *A. incana* i *A. incana* × *A. glutinosa* w strefie emisji z huty miedzi wykazały, że po dwóch latach wzrostu w warunkach stresu, śmiertelność drzew zwiększyła się tylko o 8%, lecz różnice między populacjami były bardzo istotne. Sześćdziesiąt sześć procent

drzew miało liście nie uszkodzone. Nie stwierdzono też istotnego wpływu emisji na produkcję białka w liściach. Bardzo istotne różnice międzypopulacyjne i międzyrodzinowe stwierdzono w odniesieniu do cechy zdolności akumualcji miedzi w liściach. W liściach drzew ze strefy emisji było średnio 200 razy więcej miedzi niż w liściach kontrolnych. Olsza szara miała średnio o połowę mniejszą ilość miedzi w liściach niż olsza czarna i mieszaniec między tymi gatunkami. Akumulacja cynku w liściach olsz nie różniła się istotnie w badanych populacjach i rodzinach. W liściach ze strefy emisji było średnio trzykrotnie więcej Zn niż w liściach kontrolnych. Cu i Zn są na okres zimowego spoczynku akumulowane w pędach, skąd wiosną są uruchamiane i przenoszone do rozwijających się liści.

Magnez był absorbowany i akumulowany w liściach ze strefy emisji w większych ilościach niż w liściach kontrolnych, jednakże nie było istotnych różnic w akumulacji tego pierwiastka przez badane populacje i rodziny olsz.

ЛЕОН МЕЙНАРТОВИЧ

Влияние эмиссии с медеплавильного завода на содержание ионов меди, цинка, магния, а также азота и белка в листьях ольхи

Резюме

Исследования *Alnus glutinosa, A. incana, A. incana × glutinosa* в зоне эмиссии с медеплавильных заводов указывают на то, что после двух лет произрастания в условиях стресса, выпадение деревьев увеличилось лишь на 8%, но различия между популяциями были очень существенными. Шестьдесят шесть процентов деревьев имело неповрежденные листья. Не отмечено также существенного влияния эмиссий на содержание белков в листьях. Очень существенные различия между популяциями и родами были найдены в отношении аккумуляции меди. Содержание этого элемента в листьях деревьев произрастающих в зоне эмиссий было в среднем 200 раз большим нежели в контроле. Ольха серая характеризовалась в среднем на 50% меньшим содержанием меди в листьях нежели ольха черная и гибриды между этими видами.

Аккумуляция цинка в листьях ольхи не отличалась существенно в исследуемых популяциях и родах. В листьях из зоны эмиссии содержание было в среднем три раза большим нежели в контроле. Cu и Zn аккумулируются в период зимнего покоя в побегах, откуда весной они освобождаются и переносятся в развивающиеся листья.

Магний абсорбировался и аккумулировался в листьях из зоны эмиссии в больших количествах чем в контроле, однако не было существенных различий между исследуемыми родами и популяциями в аккумуляции этого элемента.