HEAVY METAL TOLERANCE IN *AGROPYRON REPENS* (L.) P. BAUV. POPULATIONS FROM THE LEGNICA COPPER SMELTER AREA, LOWER SILESIA

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ABSTRACT

The copper smelter "Legnica" is one of the oldest plants in Lower Silesia. Among the few weed species spontaneously migrating to the area around the emitter there is couch grass (*Agropyron repens* (L.) P. Bauv.). The purpose of this study was to analyse whether the local couch grass populations, growing at various distances from the smelter, differ in tolerance to heavy metals occurring in this area. The populations were tested for tolerance to five metals (Cu, Zn, Pb, Cd, Ni) using the root elongation method. The highest tolerance to Pb developed in two populations localized nearest the smelter. Similarly, all populations of couch grass from the vicinity of the smelter show a high tolerance to copper, particularly the plants from the most contaminated site. The IT for the latter population is almost 150%, even at the highest dose of Cu. For Zn a nearing IT as for Cu was obtained. Comparing the shape of IT curves for Cd, special emphasis is put on the fact that a fixed tolerance to cadmium occurs only in the population localized closest to the emitter. The analysis of Ni-tolerance curves, of which the content in local soil is minimal, does not confirm the thesis on possibility of development of co-tolerance in the surveyed populations. It appeared that stress conditions existing near the smelter do not inhibit seed production in couch grass, but prevent a successful course of their germination on polluted soil. The improvement of soil even by 50% (addition of unpolluted soil) does not improve the poor process of germination in couch grass growing nearest to the smelter. Of importance is the fact that the highest number of seeds germinated on their own, polluted soil. The need of metals' content for plant germination in populations most distant from the smelter is evidenced by an almost 30% reduction of germination ability of local seeds after addition of unpolluted soil. Another significant observation was the fact that, in spite of a poor germination of seeds on unpolluted soil, the further development of seedlings in populations more distant from the smelter was more intense and faster than on their native polluted soil. Thus, a certain amount of metals may stimulate the germination processes of seeds in tolerant populations, but on the other hand, it may be a hindrance to subsequent juvenile phases. During observations of development of rhizomes, taken from three polluted couch grass populations cultivated on unpolluted soil, in all of them a very slow development of rhizome buds was recorded. However, the slowest rate of appearance of buds was found in the population taken nearest the emitter which, in greenhouse conditions and at lack of metals in soil, showed a markedly poor tendency toward vegetative reproduction. All the contaminated populations display a high activity of peroxidase in leaves and roots, frequently more than twice as high than in control populations. The activity of catalase is also markedly higher, but only in two of the most contaminated populations. The recorded for the first time populations of couch grass tolerant to metals make them useful for recultivation purposes.

KEY WORDS: *Agropyron repens*, tolerance to heavy metals, seed germination, vegetative growth, enzyme activity.

INTRODUCTION

Investigations on various aspects of resistance and tolerance of higher plants to various heavy metals are performed already over half a century. The most interesting localities for such investigations are metalliferous areas, colonized by typical species of metallophytes (for reviews Antonovics et al. 1971, Ernst 1974, Brooks et al. 1985, Baker 1987, Shaw 1990, Farago 1994, Ross 1994). Contrary to the natural and evolutionary old phenomena connected with hyperaccumulation of elements, investigations are performed in areas where, because of anthropogenic reasons (industrial emissions, dumps of waste with an abundance of heavy metals), mechanisms of heavy-metal tolerance are developing in a relatively short time (for reviews Bradshaw, McNeilly 1981, Lepp 1981, Martin, Coughtrey 1982, Baker, Walker 1989, Ernst et al. 1992, Macnair 1993). The spontaneous appearance of such tolerance mechanisms is observed above all in grasses, of which various species – since Bradshaws (1952) first observations – became almost classical study objects for ecologists and physiologist (Cox, Hutchinson 1980, 1981, Symeonidis et al. 1985, Brown, Brinkmann 1992, Frenckell-Insam, Hutchinson 1993a, 1993b, Patra et al. 1994, Archambault, Winterhalder 1995, Ye et al. 1997). Examples of experimentally confirmed metal resistance in Europe are the tolerant populations of broadly distributed grasses *Agrostis capillaris* (=*Agrostis tenuis*), *A. stolonifera, Anthoxanthum odoratum, Deschampsia caespitosa, Festuca rubra* (Ernst 1993), as well as, the recently recorded tolerant populations of *Festuca ovina* (Brown, Brinkmann 1992) and the thoroughly investigated at metal contaminated sites *Typha latifolia* (Ye et al. 1997a, 1997b). For respective populations of the above mentioned species the tolerance, multiple tolerance and co-tolerance (cross tolerance) are described. The phenomenon of tolerance has, so far, not been described in couch grass, which, in its whole cosmopolitic range, shows a considerable phenotypic plasticity and genotypic variation (Taylor, Aarssen 1998), thus, theoretically, it might also get resistant to the toxic activity of metals in a similar way as it gets resistant to many herbicides (Werner, Rioux 1997).

This "worst weed of the world" (Holm et al. 1977) performs also in the vicinity of copper smelters in Lower Silesia an important role in the secondary succession – as a pioneer of vegetation entering the most contaminated areas near the emission sources. Our and other authors observations (Brej 1983, Fabiszewski et al. 1983, 1986, Rebele et al. 1993) performed in the vicinity of the copper smelter "Legnica" showed that couch grass, gifted with a considerable strenght of vegetative reproduction and "competition", enters frequently the barren, highly polluted soil in the nearness of the smelter. Together with *Artemisia vulgaris, Convolvulus arvensis, Cirsium arvense, Tanacetum vulgare, Festuca rubra, Agrostis stolonifera* and from among shrubs *Sambucus nigra*, couch grass is an important component of the spontaneous green belt around the smelter. Many years observations have shown also distinct interpopulation differences in *Agropyron repens* in the smelter area, according to distance and wind direction. Those differences concerned different individual- and population traits of this weed, as well as its reactions to differentiated doses of metals contained in soil and fly ash, indicating the existence of different couch grass "biotypes" in the discussed area. The differentiation of growth (height and habit) of clones and the general plasticity of the species were an inspiration to perform closer investigations on existence or lack tolerance in *A. repens*. An additional stimulus was the tolerance to copper found in *Convolvulus arvensis*, common in the emission zone of the copper smelter (Fabiszewski 1983). Anyhow, the half-a-century existence of the copper smelter "Legnica" and its constantly high emissions of fly ash (reduced distinctly only in the nineties) is a theoretically sufficient period for development of tolerance in some species, particularly in grasses.

The main purpose of the present paper was to investigate, whether the separated for many years and growing in various distances from the smelter local populations of *A. repens* differ in tolerance to heavy metals, and whether we deal with multiple tolerance or also with co-tolerance. As couch grass is a rhizomic perennial plant of clonal growth, it was interesting to find out, whether the possible tolerance to heavy metals, investigated means of classic method using the tolerance indices of roots, is also reflected in reproduction of this grass, thus, above all in vitality of seeds and vegetative reproduction of clones. The existence of tolerant populations was also checked by testing the activity of some enzymes.

A. repens is a widespread and troublesome weed, but initiating the secondary succession on anthropogenic territories. The ascertainment of its resistance to heavy metals may give practical indications how to gain plant material for recultivation of waste land containing high concentrations of heavy metals.

MATERIALS AND METHODS

Populations of *Agropyron repens* were collected from several localities in the "Legnica" copper smelter area. During observations performed for many years three populations, localized near the smelter and highly exposed to pollution, were distinguished. In the zone of dominating winds and nearest the smelter (800-5000 m) the populations A and B occur (Figs 1, 2), whereas on the lee side the population C is localized. As control populations the localities K_1 and K_2 were chosen, situated beyond the emission zone, in opposite directions of the smelter at distances between 12 and 15 km (Fig. 3). The distinct differences between polluted and unpolluted populations were confirmed by results of chemical analyses of soil and plants (Tables 1, 2).

Tolerance tests

Clonal materials of *Agropyron repens* from all the investigated sites were cultivated for two years in an experimental

Fig. 1. The Legnica copper smelter in Lower Silesia, Poland. Photo by F. Rebele.

Fig. 2. Population of *Agropyron repens* in site B.

garden on standard soil, to obtain seeds and new rhizomes. After two vegetational seasons a sufficient number of seeds was gained for experiments with seed germination and index of root tolerance.The populations were tested in respect to Cu, Zn, Pb, Cd and Ni tolerance, using root elongation methods (Wilkins 1978, Bradshaw, McNeilly 1981). Seeds were germinated in dishes on 1/10 strength of a normal Hoaglands solution. Used were various metals in various ranges of concentration: Cu: 0.5-6 mgl^{-1}, Zn: 1-12 mgl^{-1}, Pb: 0.5-6 mgl^{-1}, Cd: 0.05-2.5 mgl^{-1}, Ni: 0.1-1,2 mgl^{-1}. Only lead was used in form of $Pb(NO_3)_2$, whereas the remaining metals were used in form of sulphate salts. The concentrations of metals in the experiment were used in result of earlier experiments showing the toxicity of the examined elements for couch grass in field and laboratory conditions. For every metal and every concentration five replications with 50 seed each were used. The controls consisted of seeds germinated only on culture medium (without toxic metals). The experiment was carried out in three replications in constant light (15000 lux) and temperature (21˚C). Twelve days after sowing the root lenghts of all germinated seeds were measured and means calculated for each treatment. The index of metal tolerance was obtained by the ratio:

$$IT(\%) = \frac{\text{mean longest root length in toxic solution}}{\text{mean longest root length in control solution}} = x\ 100\%$$

A minimum of 16 roots was used for each concentration of metal. The statistical analysis of results concerning the tolerance indices was carried out on the basis of a one or two-way analysis of variance. Means were tested by the Students Test. Significant differences refer to p<0,05.

Seed and rhizome experiments using soil

The same clonal material from the experimental garden was used for experiments with seed germination, development of seedlings and growth of couch grass rhizomes. For these experiments soil was used from polluted sites (A, B, C) and from the control site (K_2). Germination and growth of seedlings was carried out on polluted substrates (e.g., population A on soil A... etc.) and on a mixture 50:50 of the respective polluted soil with control soil. The third experiment consisted in seed germination exclusively on control soil (K_2). Five replications with 20 seeds each were applied. All experiments were carried out in a greenhouse, using adequate plastic pots for seed germination, development of seedlings and growth of

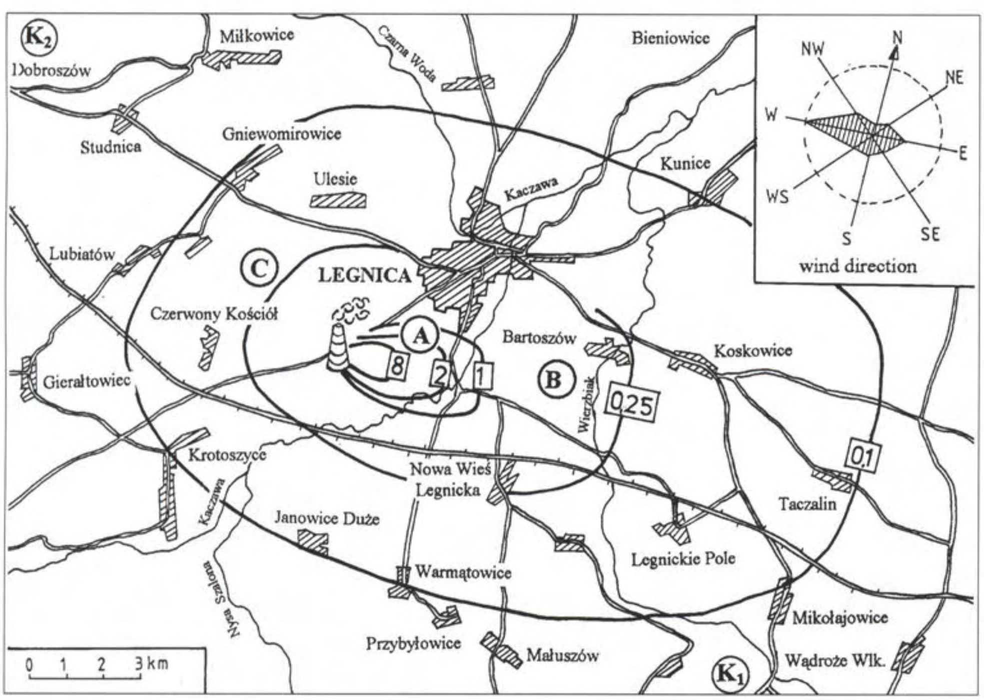

Fig. 3. Location of five *Agropyron repens* populations growing under different influence of copper smelter. Contaminated (A, B, C) and uncontaminated populations (K_1, K_2). Izolines showing theoretical copper precipitation (t/Cu/km^2/year), after Fabiszewski et al. (1983).

rhizomes. The experiments with seed germination and development of seedlings lasted two weeks. Couch grass rhizomes (with seven nodals) taken from populations A, B, C and K_2 were grown only on unpolluted soil (control K_2) for five weeks. Five replications were carried out using 10 rhizomes from each population. Before placing of rhizomes in soil their apical buds were cut, to avoid the known in couch grass apical dominance controlling the activity of axillary buds (Hakansson 1982, Robertson et al. 1989).

Chemical analysis

Couch grass leaves and rhizomes collected from five populations were throughly washed and dried and next wet-ashed in a mixture of perchloric/nitric acid, 4:1. Five heavy metals (Cu, Zn, Pb, Cd and Ni) were determined using the atomic absorption spectrophotometer (Varian 2A) according to the procedure by Allen (1989). Soil samples were taken from sites of occurrence of the five analysed *A. repens* populations (A, B, C, K_1, K_2) (Fig. 3). Air-dried soil samples were passed through a 2 mm sieve. Exchangeable and soluble metals were extracted with 1M ammonium acetate solution (pH 7) for 1 hour. Concentrations of metals were measured in the filtrate (three replications) using the AAS method (Varian 2A). Soil pH was determined by glass electrode on a saturated soil paste (Allen 1989).

Enzymatic analysis

For confirmation of fixed mechanisms of tolerance at biochemical level, the activity of catalase and peroxidase in leaves and roots of the five couch grass populations was determined. Live plants were carried to the laboratory during four hours, where the further chemical treatment was carried out. The extraction of both the enzymes from leaves and roots was performed according to the method by Maehly and Chance (1967). Used were roots and leaves from specially collected, living and well cleaned plant samples. Further procedures, including incubation of enzymes, were carried out according to the methodology by De Vos and Schat (1991) and Patra et al. (1994). The catalase activity was expressed in mmol H_2O_2 utilized gl^{-1} fresh weight min^{-1}. The peroxidase activity was expressed in absorbancy units g^{-1} f. wt. min^{-1}.

RESULTS AND DISCUSSION

Field situation

All the polluted and unpolluted *Agropyron repens* populations originated in result of spontaneous succession. It is possible that at least some of the populations date back to the establishment of the smelter. The influence of metals' emission, assessed according to existence of biological symptoms, was recorded at a distance of 15 km SE and 7 km NW from the smelter (Fabiszewski et al. 1983). Thus, the populations A, B and C were situated in the emission zone, whereas populations K_1 and K_2 were beyond the area of influence of the smelter. This is confirmed by results of recent soil analyses (Table 1) and concentrations of four heavy metals (Table 2) in vegetative parts (leaves, rhizomes) of couch grass. Between sites A, B and C differences – not always significant – were found in contamination levels of soil and plants. The highest contents of metals in soil, leaves and rhizomes occur in site A, situated in direction of dominating winds and nearest the smelter. This concerns particulary such strongly toxic metals as Pb and Cd. High contents of Cu, Pb and Cd in soil are reflected in the high accumulation of these elements in the vegetative parts of *A. repens*, particularly in

TABLE 1. Heavy metal concentration (mg/kg^{-1}) and pH in soil of different stands of *Agropyron repens* (n = 3)

Site	Cu	Zn	Pb	Cd	pH
A	2475 ± 342	159 ± 14	944 ± 110	3.5 ± 0.7	5.8
B	1940 ± 278	168 ± 19	890 ± 97	0.9 ± 0.1	5.6
C	1720 ± 189	108 ± 11	614 ± 49	0.9 ± 0.2	6.0
K_1	32 ± 7.00	27 ± 7.0	11 ± 3.0	0.3 ± 0.1	6.1
K_2	33 ± 3.0	29 ± 3.0	18 ± 6.0	0.3 ± 0.1	6.7

TABLE 2. Heavy metal concentration (mg/kg^{-1}) in leaves (L) and rhizomes (R) of *Agropyron repens* in five different populations (n = 5)

Site		Cu	Zn	Pb	Cd
A	L	1872 ± 170	167 Ě 17	427 ± 39	2.92 ± 0.4
	R	2375 ± 130	214 ± 21	460 ± 43	3.94 ± 0.3
B	L	1798 ± 98	193 ± 14	329 ± 17	1.03 ± 0.1
	R	2110 ± 104	278 ± 23	630 ± 28	1.05 ± 0.3
C	L	1700 ± 111	189 ± 27	280 ± 16	1.10 ± 0.2
	R	220 ± 39	114 ± 19	390 ± 22	1.80 ± 0.4
K_1	L	27 ± 7	30 ± 6	10 ± 1	trace
	R	29 ± 7	28 ± 8	14 ± 4	trace
K_2	L	16 ± 3	27 ± 6	13 ± 2	0
	R	30 ± 6	43 ± 9	17 ± 3	0

rhizomes. The content of some elements in rhizomes from sites A, B and C exceedes the level of these metals in soil. The concentration of Pb and Zn in leaves is also frequently higher than in soil. These results testify to the high cumulation abilities of couch grass, its leaves and rhizomes, but also to the longlasting – almost half a century – influence of contaminating factors upon the analysed populations. The contamination of soil and vegetation in the studied area is generally not drastic, as it does not reach the levels given for metallophytic areas (Bradshaw, McNeilly 1981, Ernst 1993), but as a typical industrial area it is rather heavily contaminated (Kabata-Pendias, Pendias 1992). The situation in field, the old age of the smelter and the presence of couch grass speak for the possibility of rise of tolerance mechanisms to heavy metals, known already in other species from contaminated areas. Chemical investigations of plants from control populations confirmed the relevancy of choice of these sites as unpolluted, reference populations.

Tolerance testing

Root tests are used since the beginning of studies on the phenomenon of tolerance to metals in various species and various areas (Wilkins 1978, Baker 1987). Because roots are more directly confronted with heavy metals in the environment than shoots, therefore they are considered as important plant organs in investigations, indicating the appearance of tolerance. Fig. 4 shows the lead tolerance indices for populations of *Agropyron repens* at various Pb concentrations. The roots of all the three contaminated populations (A, B, C) react to the presence of lead with a high degree of tolerance, in opposition to the control populations. The highest tolerance developed undoubtedly in populations A and B, in which already low concentrations of lead gave a stimulative effect. A lower degree of tolerance, though still more distinct than in the control, was recorded in population C, where the curve

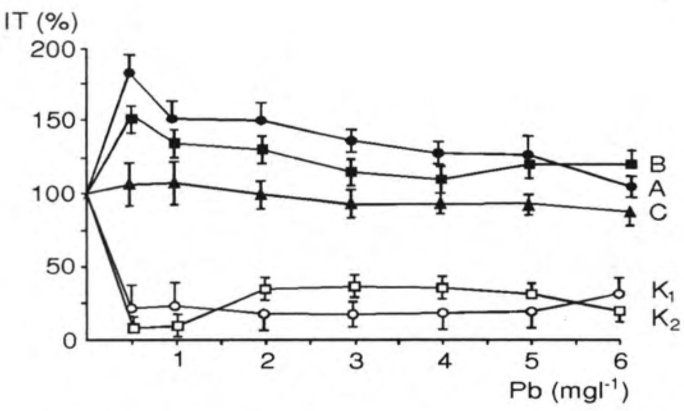

Fig. 4. Lead tolerance indices of five *Agropyron repens* populations. A-C from contaminated area, K_1, K_2 – uncontaminated area. Bars indicate S. E.

oscillates stably about 100% IT in spite of differentiated concentrations of Pb. A similar run of the curve of Pb tolerance was observed by Brown and Brinkmann (1982) in *Festuca ovina*, and earlier by Simon (1978). However, those investigations were performed in more metalliferous areas and the plants tolerated higher concentrations of lead. A similar IT for Cu and Zn is given in Figs 5 and 6, where the tolerant populations (A, B, C) display a considerable degree of variation in reaction. Their tolerance is, however, always higher than in the control. In spite of the similar run of curves for copper and zinc in tolerant populations, it should be noted that Cu toxicity is twice as high for couch grass roots than Zn toxicity. The applied doses of copper are in this case identical with these of lead and range from 0,5 to 6,0 mgl^{-1}. All populations from the environs of the smelter show a high degree of tolerance to copper, however, an extremely high tolerance is shown by population A comming from the most contaminated area. Even with the highest dose of Cu (6 mgl^{-1}) its index of tolerance is almost 150%.

The reaction to cadmium differs from that to the remaining metals. Decidedly tolerant to Cd is the population A, localized closest to the emitter. It shows a constant index of tolerance to all concentrations of Cd, with a slight growing tendency at 1,0-2,5 Cd mgl^{-1}. This population departs positively from the other ones. Population B appeared to be of averrage tolerance at all the cadmium doses used. Population C is least tolerant from among the three populations collected in the polluted area; at higher doses of Cd a marked inhibition in root growth was observed. As seen in Fig. 7, there is no tolerance in both the control populations – at all Cd doses, beginning from the lowest one. The comparison of IT curves showes clearly that a fixed tolerance to cadmium occurs only in population A, whereas the remaining ones should be recognized as intolerant to this element. This is probably connected with the low level of Cd found in soil of the area under study (Table 1).

The described above phenomena of root reaction to various heavy metals should be included among the known from literature multiple tolerance, i.e., a combination of physiologically and genetically independent metal-specific tolerances (Ernst et al. 1992, Schat, Ten Bookum 1992, Frenckell-Insam, Hutchinson 1993a). Instead, co-tolerance occurs when defence mechanisms are released also to metals present in soil in smaller amounts.This phenomenon was analysed by many authors (e.g., Symeonidis et al. 1985, Schat, Vooijs 1997). In our case the influence of Ni may be involved, emitted in small amounts by the smelter "Legnica". Analysing Fig. 8 in

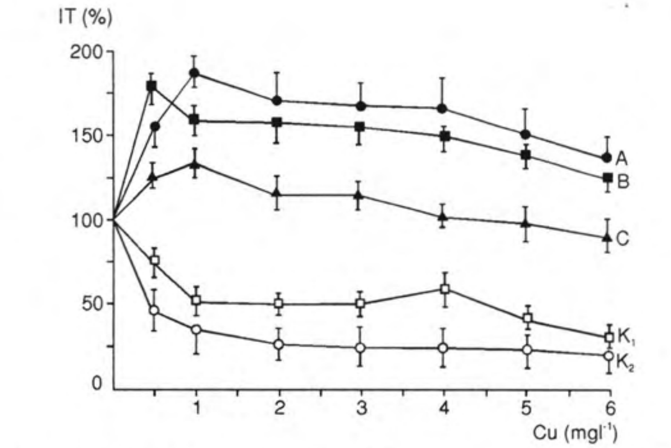

Fig. 5. Copper tolerance indices of five *Agropyron repens* populations. A-C from contaminated area, K_1, K_2 – uncontaminated area. Bars indicate S. E.

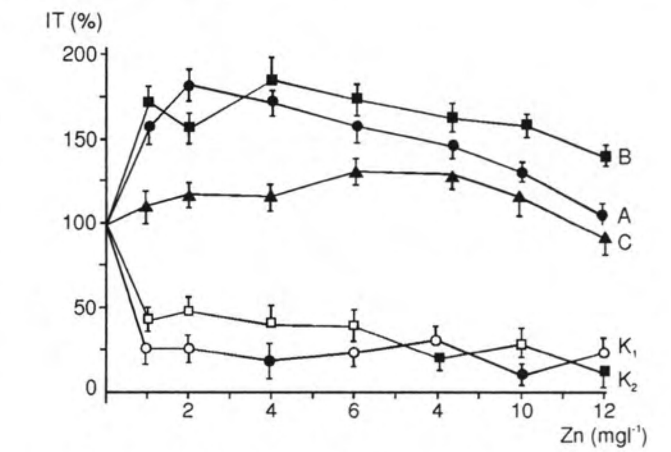

Fig. 6. Zinc tolerance indices of five *Agropyron repens* populations. A-C from contaminated area, K_1, K_2 – uncontaminated area. Bars indicate S. E.

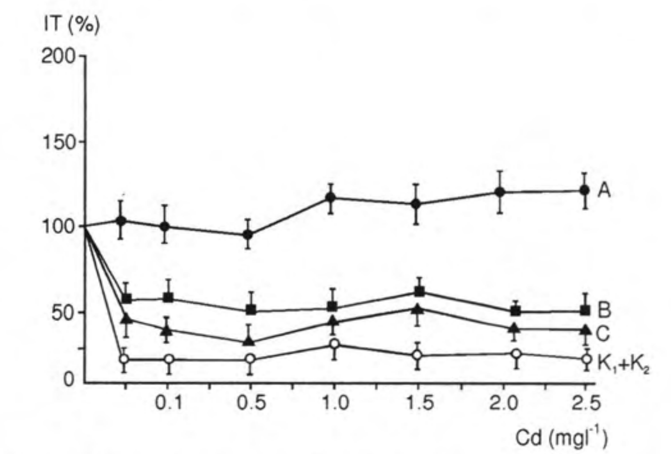

Fig. 7. Cadmium tolerance indices of five *Agropyron repens* populations. A-C from contaminated area, K_1, K_2 – uncontaminated area. Bars indicate S. E.

this respect, the thesis on development of tolerance to metals, insignificant as pollutants in the investigated area, should be rejected. From the run of IT curves it results that population A (localized closest to the smelter and showing the decidedly highest tolerance to the remaining metals) is at the same time not tolerant to nickel, as its IT values approximate those of the control population.

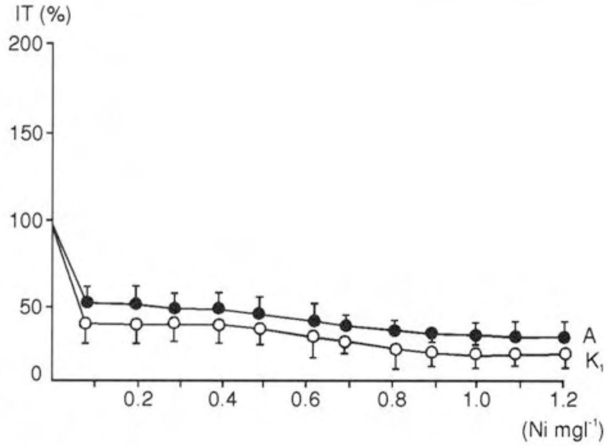

Fig. 8. Nickel tolerance indices of two *Agropyron repens* populations. A – contaminated, K_1 – uncontaminated area. Bars indicate S. E.

Germination of seeds, seedlings, development of rhizomes

Tolerance to heavy metals in plants is most often detected by means of root tests, imitating the processes taking place in field where various toxic metals affect the whole plant. Experiments with other plant organs are rarely carried out. There is also no evidence concerning the comparative reactions of whole in contaminated and uncontaminated populations. For instance, the studies by Lolkema et al. (1984) show that shoot reactions to copper in *Silene vulgaris* reveal an earlier inhibition of growth than root reactions. To increase our knowledge on couch grass reactions in conditions of contaminated soils, tests on seed germination and development of seedlings and rhizomes were performed.

Observations in field indicated that stress conditions existing near the smelter do not reduce the process of seed production in *A. repens*. At the same time an extremely rare occurrence of seedlings in populations B and C and particularly in A was recorded, whereas they were easily observable in control populations. Explanation of this phenomenon is given in Fig. 9. The degree of soil contamination with heavy metals in the nearness of the smelter at site A is so high that seed germination in the native population is decidedly limited. Even the improvement of soil status by 50% (addition of unpolluted soil K_2) does not improve the germination status. The equally poor seed germination in population A on control soil evidences the low fertility of seeds, disturbance of generative processes or even embryo injuries. Similar remarks concerning seed germination on soil with heavy metals are given by Ernst et al. (1992) and Archambault, Winterhalder (1995). A much better course of germination was observed in populations B and C from less toxic habitats. Worthy of notice is the highest number of germinating seeds on their own, polluted soil. This evidences the stimulating effect of moderately contaminated soil, but also the fact, that both the populations developed the proper mechanisms of tolerance. The fact of indispensable presence of metals for plants of both the populations is confirmed by a ca. 30% decrease of seed germination ability after addition of unpolluted soil, as well as by the poor germination in variant with unpolluted soil. In consequence of the above described picture of seed germination measurements of dry weight of 10 two-week seedlings from each soil combination were performed (Fig. 10). The condition and size of seedlings from population A, growing on soil A, $A+K_2$ and unpolluted K_2, departs markedly from the remaining populations B and C. Of importance is the fact that the further development of seedlings in populations B and C, in spite of seed germination on unpolluted soil (Fig. 9), was more intense and progressed faster than on polluted soil. An addition of 50% of unpolluted soil (variants $B+K_2$ and $C+K_2$) already stimulated a better development and growth of seedlings. The comparison of results, presented in Figs 9 and 10, suggest that a specified amount of metals may appear necessary for seed-germination processes in tolerant couch grass populations, but it also may constitute a hindrance for further developmental processes at juvenile phases. Perhaps the phase in which *A. repens* uses the materials accumulated in the germ is a phase,

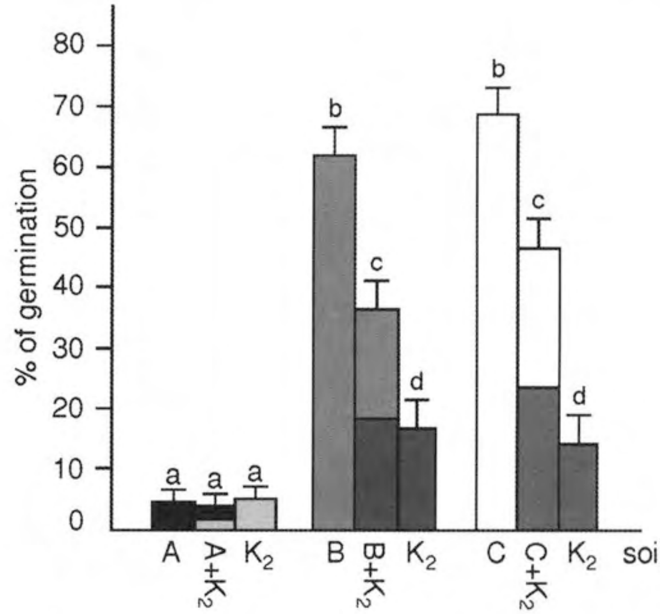

Fig. 9. Percent of seed germination of *Agropyron repens* collected from contaminated sites (A, B, C) and uncontaminated (K_2). $A+K_2$, $B+K_2$, $C+K_2$ indicate 50:50 contaminated-uncontaminated soil, K_2 – uncontaminated soil. S. D. at P>0,05, N=5.

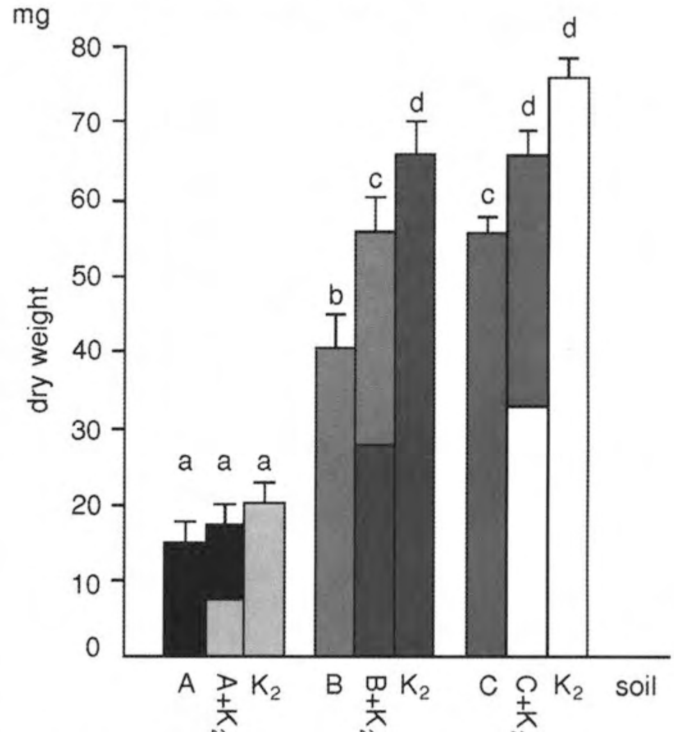

Fig. 10. Dry weight of 8 two weeks seedlings of *Agropyron repens* growing on contaminated soils (A, B, C). $A+K_2$, $B+K_2$, $C+K_2$ indicate 50:50 contaminated-uncontaminated soil, K_2 – uncontaminated soil. S. D. at P>0,05, N=5.

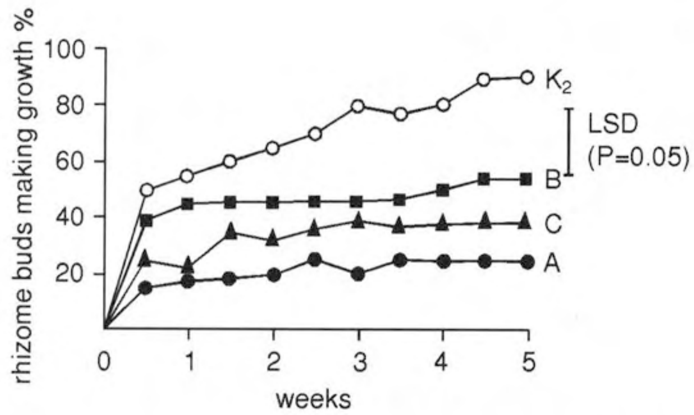

Fig. 11. Seven nodal rhizomes of *Agropyron repens* growing on uncontaminated soil (K_2). Rhizomes were taken from contaminated (A, B, C) and uncontaminated populations (K_2). N=5.

TABLE 3. In vivo catalase and peroxidase activity for five population of *Agropyron repens*. For each enzym activity in leaves or adventous roots means respectively are significant at P>0.05. N=4

			Catalase activity	Peroxidase activity
Tolerant populations	A	leaf	0.70 ± 0.07 a	5.03 ± 0.15 a
		root	0.57 ± 0.03 d	3.50 ± 0.30 c
	B	leaf	0.73 ± 0.02 a	4.90 ± 0.21 a
		root	0.63 ± 0.03 d	3.95 ± 0.30 c
	C	leaf	0.28 ± 0.4 c	3.95 ± 0.40 a
		root	0.39 ± 0.5 b	3.80 ± 0.17 c
Untolerant populations	K_1	leaf	0.17 ± 0.14 c	1.98 ± 0.006 b
		root	0.27 ± 0.00 b	1.87 ± 0.03 d
	K_2	leaf	0.14 ± 0.002 c	1.99 ± 0.002 b
		root	0.22 ± 0.02 b	1.73 ± 0.04 d

so to say, independent of soil. It may also be supposed that we deal here with not yet fixed tolerance abilities or with a differentiation of tolerance for the particular phases of plant development. Another confirmation of tolerance developed in quackgrass was the experiment with cultivation of seven-nodal rhizomes coming from the polluted populations and placed in pots with unpolluted soil K_2 (Fig. 11). During five-weeks' observations a generally slower development of rhizome buds was recorded in all populations from polluted areas. However, the slowest rate of appearance of buds occured in population A, which showed a markedly poor development with lack of metals in soil. In the discussed experiment population A has once more shown a highly developed tolerance to heavy metals. All the described experiments confirm the occurrence of tolerance mechanisms in the populations under study. The phenomena of tolerance developed during the 50-years exposure to heavy metals enable couch grass to persist in this area.

Enzyme activities

The high activity of various enzymes is regarded as a significant fact confirming the existence of fixed mechanisms of tolerance to heavy metals (Van Assche, Clijsters 1990, Ernst et al. 1992). The rise and the physiological and biochemical fixation of resistance to metals, as remarked by Baker (1987), decides whether the plant acquires this resistance through "avoidance" (external protection against influence of stress), or through "tolerance" (the plant experiences an internal stress and defends itself against it). According to Levitt (1980), tolerance to heavy metals is a physiological phenomenon enabling the plant to live and to perform all functions in presence of toxic concentrations of metals. Fixed tolerance mechanisms develop undoubtedly – as results from many studies (Ernst et al. 1992, Verkleij 1993) – mainly at the molecular and cell level. In earlier studies predominantly the content and activity of acid phosphatase in roots was analysed, to confirm the existence of biochemical mechanisms of tolerance (Woolhouse 1970, Cox, Thurman 1978). Conclusions of those studies appeared to be certain only for ascertainment of tolerance in serpentine plants (Johnson, Proctor 1984). Taking Patra et al. (1994) as an example, in the present paper as test for assessment of catalase and peroxidase activity both in leaves and roots was used. These enzymes are induced by numerous stress factors, among them heavy metals (Van Assche, Clijsters 1990). The results presented in Table 3 evidence explicitly the high activity of peroxidase, frequently more than twice as high, in all the polluted couch grass populations both in leaves and roots. The activity of catalase was found to be also significantly higher, but only in two of the *Agropyron repens* populations (A and B). Activity of the latter enzyme in the least polluted population C was indeed higher than in the control, but this difference was insignificant both for leaves and roots. As emphasized in earlier study results, population C showed also the poorest reactions in investigations of root index tolerance and other tests. This may indicate that the tolerance mechanisms of this *Agropyron repens* population were not fully fixed. Both the analysed enzymes perform similar vital functions intensifying activity under oxidative stress, a feature often associated with heavy metal tolerance (De Vos, Schat 1991, Patra et al. 1994). Anyhow, the high activity of peroxidase and the higher activity of catalase may evidence that tolerance mechanisms at biochemical level do exist in all the three populations of couch grass, however, they concern in the lowest degree the least polluted population C, in which the tolerance is probably still *in statu nascendi*. This problem can be solved in further biochemical investigations, e.g., the occurrence of specific peptides, called phytochelatins, in plants resistant to heavy metals, which probably play a key role in metal homeostasis (Verkleij 1993).

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WPŁYW HUTY MIEDZI LEGNICA NA POWSTANIE U PERZU (*AGROPYRON REPENS*) ZJAWISKA TOLERANCJI NA METALE CIĘŻKIE

STRESZCZENIE

Huta Miedzi Legnica jest najstarszą z działających na Dolnym Śląsku hut, znaną ze swej uciążliwości dla środowiska. Jej 50-letnia działalność stworzyła zagrożenie dla większości okolicznych ekosystemów. Do najbardziej toksycznych dla roślin należą emitowane przez hutę tlenki siarki, a z metali – ołów, miedź i kadm. Wśród nielicznych gatunków chwastów spontanicznie migrujących w obszarze położonym wokół emitora, znajduje się perz właściwy *Agropyron repens*. Celem pracy było zbadanie, czy rosnące w różnych odległościach od huty lokalne populacje perzu różnią się tolerancją na występujące w tym terenie metale ciężkie. Populacje te były testowane na tolerancję pięciu metali (Cu, Zn, Pb, Cd, Ni) przy zastosowaniu testu wzrostu korzeni w kulturach wodnych. Korzenie trzech skażonych populacji reagowały na obecność ołowiu wysokim stopniem tolerancji w przeciwieństwie do populacji kontrolnych. Największa tolerancja na Pb wykształciła się w dwóch populacjach położonych najbliżej huty. Podobnie, wszystkie populacje perzu z okolic huty wykazują wysoki stopień tolerancji na miedź, a zwłaszcza rośliny pochodzące z rejonu najbardziej skażonego. Nawet przy największej dawce Cu, indeks tolerancji (IT) dla tej populacji wynosi blisko 150%. Zbliżony obraz indeksu tolerancji korzeni jak dla Cu, uzyskano dla Zn. Reakcja populacji na kadm jest odmienna od reakcji na pozostałe metale. Porównując przebieg krzywych obrazujących indeks tolerancji dla Cd należy podkreślić, że utrwaloną tolerancję na kadm wykazuje jedynie populacja zlokalizowana najbliżej emitora. Analiza wyników krzywych tolerancji na Ni, którego zawartość w miejscowych glebach jest znikoma, nie potwierdza tezy o możliwości wykształcenia się u badanych populacji zjawiska kotolerancji. Kolejnym problemem była odpowiedź na pytanie czy stwierdzona w testach korzeniowych tolerancja znajduje swe odbicie w reprodukcji perzu, a przede wszystkim w żywotności nasion i rozmnażaniu wegetatywnym. Okazało się, że stresowe warunki w pobliżu huty miedzi nie ograniczają produkcji nasion u badanych populacji perzu, natomiast uniemożliwiają pomyślny przebieg ich kiełkowania na skażonej glebie. Nawet polepszenie stanu takiej gleby o 50% (dodatek gleby nieskażonej) nie poprawia złego procesu kiełkowania u populacji rosnącej najbliżej huty. Znacznie lepszy przebieg kiełkowania obserwowano w populacjach perzu pochodzących z siedlisk dalej leżących od huty. Zwraca tu uwagę stwierdzenie, że najwięcej nasion skiełkowało na swym własnym, skażonym podłożu. Niezbędność zawartości metali dla kiełkowania roślin najbardziej odległych od huty potwierdza obniżenie aż o około 30% zdolności kiełkowania miejscowych nasion po dodaniu gleby nieskażonej. Inną istotną obserwacją było stwierdzenie, że mimo słabego kiełkowania nasion perzu na glebie nieskażonej, dalszy rozwój siewek w populacjach bardziej odległych od huty przebiegał intensywniej i szybciej niż na glebie skażonej. Wynika z tego, iż pewna obecność metali może okazać się stymulująca dla procesów kiełkowania nasion populacji tolerancyjnych perzu, ale z drugiej strony może ona stanowić barierę dla rozwoju kolejnych faz juwenilnych. W kolejnym doświadczeniu, w trakcie obserwacji rozwoju rozłogów pobranych z trzech skażonych populacji perzu i uprawianych na glebie nieskażonej, stwierdzono u wszystkich bardzo wolny rozwój pączków rozłogowych. Jednak najwolniejsze tempo pojawiania się pączków wystąpiło u populacji pobranej najbliżej emitora, która w warunkach szklarniowych, przy braku metali w glebie ma wyraźnie słabą reprodukcję wegetatywną. Utrwaloną tolerancję na metale u badanych populacji perzu potwierdzono badaniami aktywności enzymów. Wszystkie populacje skażone wykazują w liściach i korzeniach wysoką aktywność peroksydazy, często ponad 2-krotnie wyższą niż w kontroli. Również aktywność katalazy jest istotnie wyższa – ale tylko u dwóch najbardziej skażonych populacji. Na podstawie wszystkich eksperymentów można stwierdzić obecność w badanych populacjach perzu, w różnym stopniu wykształconych mechanizmów tolerancji na testowane metale, gromadzone od 50 lat w glebie. Stwierdzenie po raz pierwszy tolerancyjnych na metale populacji perzu stwarza możliwość ich wykorzystania w celach rekultywacyjnych. Uzyskane wyniki sugerują, że tolerancja u perzu istnieje na poziomie organów, jak też różnych faz rozwojowych roślin.

SŁOWA KLUCZOWE: *Agropyron repens*, skażenia przemysłowe, tolerancja na metale ciężkie, wzrost korzeni, kiełkowanie, aktywność enzymów.