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Variability between and within *Agrostis tenuis* Populations Regarding Heavy Metal Tolerance

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Summary

Samples of *Agrostis tenuis* SIBTH. tolerant populations from the mine of Parys Mountain in North Wales as well as from normal areas were studied. This study concerned the determination of their tolerance to various concentrations of Cu, Zn and Pb by evaluating their effect on the rooting of tillers. The following were noticed: Populations growing on soils containing toxic concentrations of metals showed a respective tolerance to these metals. This was a great variability as to the index of tolerance among populations collected from various sites of the mine. Also we noticed variability as to the index of tolerance among individuals of the same populations. Finally, there were some indications that the population collected from the edges of the mine was probably affected genetically by a gene flow.

Zusammenfassung

Die Veränderlichkeit der Schwermetalltoleranz zwischen und innerhalb von *Agrostis tenuis*-Populationen

Anhand der Bewurzelung von Ausläufern wurde die Toleranz von *Agrostis tenuis* SIBTH. gegenüber Cu, Zn und Pb in der Umgebung der Mine Parys Mountain (North Wales) untersucht. Pflanzen, die auf Böden mit toxisch wirkenden Konzentrationen dieser Metalle gewachsen waren, erwiesen sich gegenüber den Metallen resistent. Der Toleranzindex der an verschiedenen Plätzen gesammelten Populationen aber auch innerhalb der einzelnen Populationen erwies sich als sehr variabel. Es werden auch genetische Veränderungen (Genfluß) vermutet. (Editor transl.)

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Introduction

Some of the heavy metals (e. g. Cu, Zn) are essential nutrients for plant growth while others (e. g. Pb, Hg) are non essential although occasionally they may be found in plant tissues. However, even the metals of the first category can be very toxic when present in the root medium in large concentrations. Different species differ in their tolerance to heavy metal toxicity. Moreover, a marked variability may exist among populations and individuals of the same species. This variability has been attributed (GREGORY and BRADSHAW, 1965; McNEILLY & BRADSHAW 1968, GOODMAN & ROBERTS 1971, BRADSHAW 1971, WU *et al.* 1975) to partial and gradual adaptation of populations which happened to grow on soils rich in heavy metals e. g. mine soils (WILD 1970, BRADSHAW 1971).

RUSSEL & MORRIS (1970) has reported a higher tolerance to copper in a population of *Ectocarpus* ssp. growing on the hull of ships as compared to other populations of the same alga. The difference was attributed to the presence of copper compounds used in the paint of the hull. A similar cause was reported by HODGSON (1969) in *Enteromorpha* ssp. with mercury.

Some lichens are so common in soils around copper workings that they have used as ecological indicators for metalliferous veins (RUNE 1953). WILDE (1968) noted that a species of lichens *Lecanora* is found on natural rocky outcrops in Rodesia with its thallus actually growing over exposed malachite without any apparent signs of damage.

Several species of mosses were shown to be highly tolerant to copper (PERSSON 1948, 1956, NOGUCHI 1956, NOGUCHI & FURUTA 1956, ERNST 1965, 1968). These species have been described as "copper mosses"

Certain cormophytic species, beside the thallophytic organism, have been found tolerant to some toxic metals. Ferns for example, are known to grow on serpentine soils high in nickel and chromium (KRUCKEBERG 1964). WILD (1968) recorded the ferns *Pallaea calomelanos* and *Chelianthes hirta* on copper and occasionally nickel soils in Rodesia. Other notable cases of tolerance have been found in some groups of higher plants as Cyperaceae, Compositae, Orchidaceae, Ranunculaceae, e. t. c. (ANTONOVICS *et al.* 1971).

The largest number of tolerant species are found in the largest groups, i. e. the Leguminosae and the Graminae. This by occupying a correspondingly large range of habitats, offer the greatest possibilities for the evolution of tolerant species.

One of the most tolerant plants in the Graminae family, is *Agrostis tenuis*. It is found in most old British mines.

In the work reported in this paper an evaluation was made of the tolerance to various heavy metals of *Agrostis tenuis* populations which were collected at random from the site between the edges and the centre of Parys Mountain. Also, an attempt was made to learn whether there is a variability between and within populations of this species regarding tolerance to Cu, Zn and Pb.

Materials and Methods

Adult populations of *Agrostis tenuis* SIBTH. from three sites having toxic levels of copper, zinc and lead in the soil, and one population from the boundary was collected from the Parys Mountain mine. Another adult population from uncontaminated soil was also collected for comparison.

Material was collected randomly from different sites far distant from one another in order to avoid sampling populations with clones of the same genotype. Plants after collection were grown in plastic trays and kept in a glasshouse for more than two months. Healthy tillers from these plants were tested by allowing them to root in solutions containing heavy metals (JOWETT 1964, GREGORY & BRADSHAW 1965, MCNEILLY 1968). Tillers were placed in a culture solution with and without metal in a continuously illuminated cabinet for 10 days, at a temperature of 23°—25° C. An index of tolerance was derived from the longest root growth in toxic solutions expressed as a percentage of growth in the control solution as follows:

$$\text{Index of Tolerance} = \frac{\text{mean length of longest root in solution with metal}}{\text{mean length of longest root in solution without metal}} \times 100$$

Copper and zinc were added as sulphates, and lead as nitrate salts. All solutions contained 0.5 g/l of hydrated calcium nitrate and were changed every 2 days. The levels of the three metals used were 0.25 ppm for Cu, 3.75 ppm for Zn and 6 ppm for Pb.

Tolerance indices for the populations are shown as histograms in Fig. 1. Each figure represents three replicants for each population. In all the cases 10 clones from each population were used in the test.

The levels of contaminants in the soils have been measured by a Unicam atomic absorption SP 90. After three measurements the mean of contaminants and the corresponding indices of tolerance for all populations were illustrated in Table 1.

Table 1

Indices of tolerance of *Agrostis tenuis* populations related to the levels of metal in original soil

Population	Index of tolerance (%) to			Metal content in the soil (ppm)		
	Cu	Zn	Pb	Cu	Zn	Pb
1	4.60	7.93	8.79	70	78	56
2	18.52	12.15	10.67	110	310	400
3	27.40	34.85	15.25	900	870	1800
4	50.48	32.73	35.31	1300	1000	4500
5	68.57	34.00	51.26	2350	6300	2550

Results and Discussion

All populations from contaminated soil showed a high level of copper or zinc or lead tolerance, while the populations from uncontaminated soil did not (Fig. 1). The individual tolerance of the pasture population to the three metals is low. Population 2 showed a higher tolerance than 1 as expected because the latter was at first collected as a control population. If we compare the populations 2 and 1 as to 5, we can see that the boundary population shows an index of tolerance equal to the 27% of the respective index of 5 as regards Cu, while 1 represents the 7% of it. The respective percentages for Zn are 35% for the boundary and 23% for the pasture. Thus we can describe population 2 as rather tolerant to Cu. This may be due to the fact that it came from places situated on the boundary of an area, where the environmental factors are obviously different from those of an open surface not adjacent to toxic sites (GADGIL 1969).

Population 3 occurs already inside the mine. The concentration of the soil in heavy metals is higher than that of the respective population occuring on the boundary. As a consequence, this population is expected to display a higher tolerance than that of 2. In fact, as far as Cu and Zn are concerned, the index of tolerance of population 3 is higher than the respective one of population 2 and markedly higher than that of the pasture population. As regards the lead, the difference is small and may be regarded as negligible. The fact these two populations differed markedly in their index of tolerance although they were located close to each other may be attributed to differences in the heavy metal content of the soils of this two locations or to gene flow. By examining the soil analysis, we notice that the area on which population 3 was growing contained larger quantities of toxic metals than those of the population 2, which shows almost the same quantities as those of the population 1. Consequently, population 3 evidences, as a matter of fact, because of its higher concentration in toxic metals in the soil a higher index of tolerance than 1 and 2 respectively. This suggests that population 3 is adapted to higher levels of toxic metals. Population 2, which is located on the boundary, should physiologically react as a pasture population and consequently show no difference from that of 1. Experimental data, however, showed that population 2 differed considerably from 1. This may be explained if we assume that there is a gene flow from the mine to the surrounding area (McNEILLY & BRADSHAW 1968; JAIN & BRADSHAW 1966).

A considerable increase of the index of tolerance for Cu as well as for Pb was reported regarding populations 4 and 5, which were collected from sites near the main road of the mine. The index of tolerance regarding Zn remained at the same level for all the investigated populations which were situated inside the mine. These populations, located approximately in the centre of the whole area of the mine, are both the most adapted and the most selected ones, which managed to survive on these adverse and toxic sites containing large amounts of toxic metals (BRADSHAW 1970).

Regarding the behaviour of the populations, the genotypic differences existing among them, were investigated (Fig. 1) and found from the results of utilizing different single clones of the same population in the experiment that individual clones of a population may vary markedly (Figs. 2a, b, c). For instance, the index of tolerance to Cu regarding the population 1 varied from 0 and 10 and only 10% between 10 and 20.

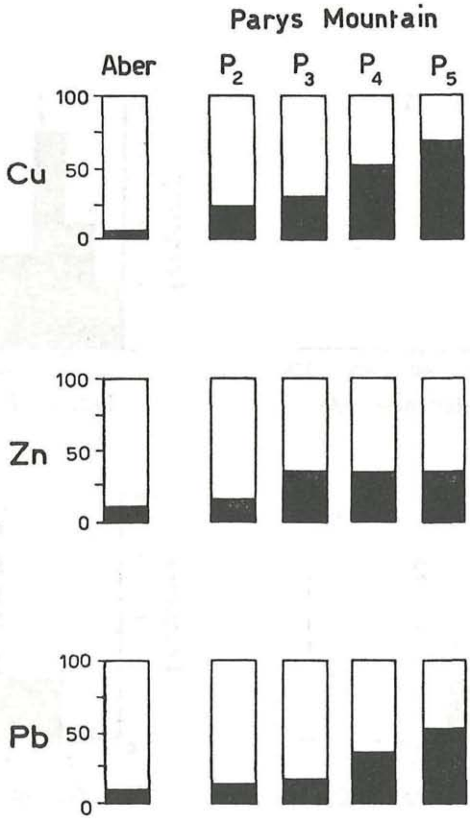

Fig. 1. Root length of *Agrostis tenuis* populations in various toxic solutions, expressed as a percentage of their growth. The populations were collected from Parys Mountain (2—5) and Aber as control

Among the boundary population the index of tolerance varied between 0 and 50, whereas 45% of the individuals showed an index of tolerance between 0 and 10. In the case of 3 the index rises up to 60, while the majority of the individuals showed an index between 20 and 30. Among the populations 4 and 5 no individual showed an index of tolerance lower than 20, the index being shifted up to 100. Consequently we can ascertain a marked variation in metal tolerance between genotype in mine populations. On the contrary,

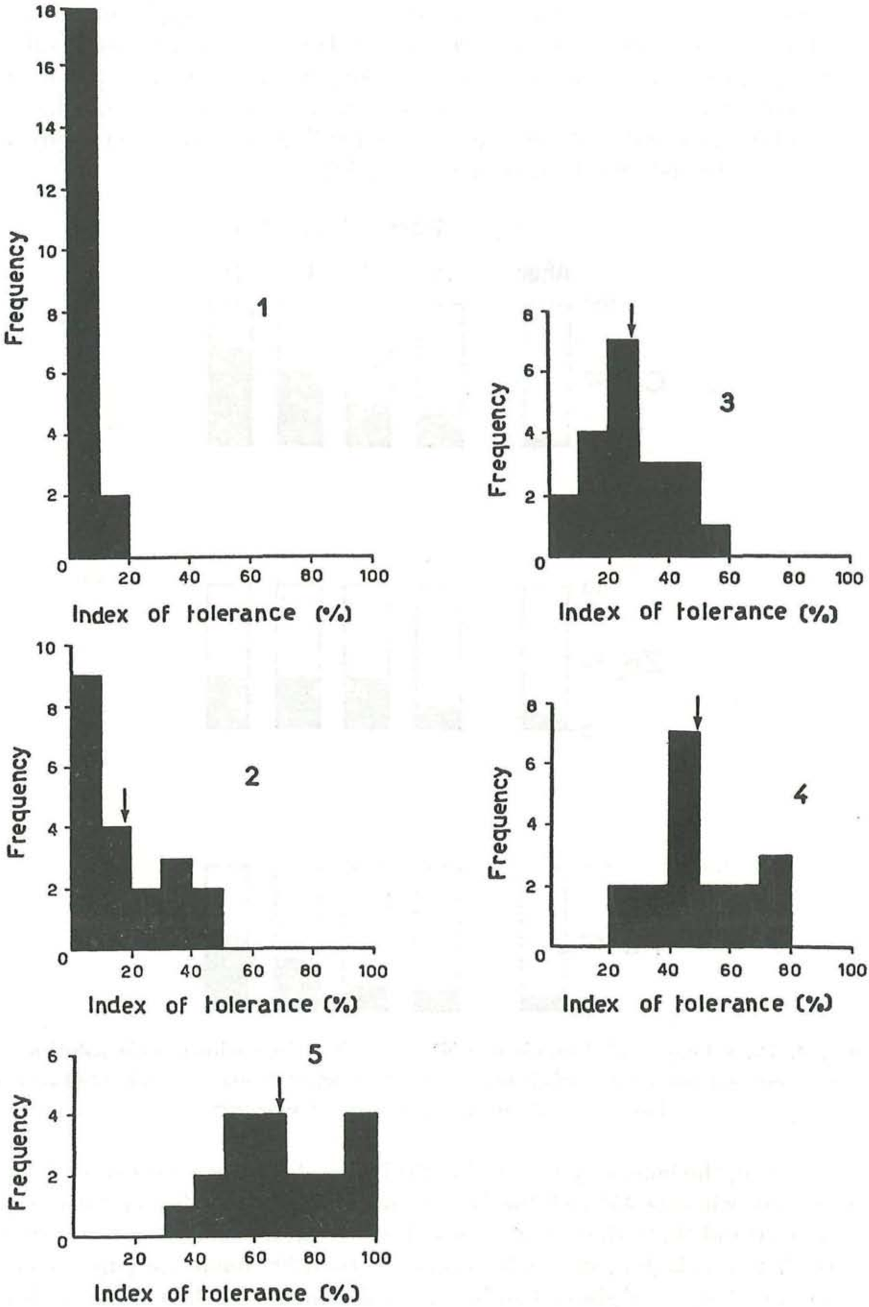

Fig. 2. (a) Distribution of copper tolerance in populations of *Agrostis tenuis*. Means are indicated by arrows

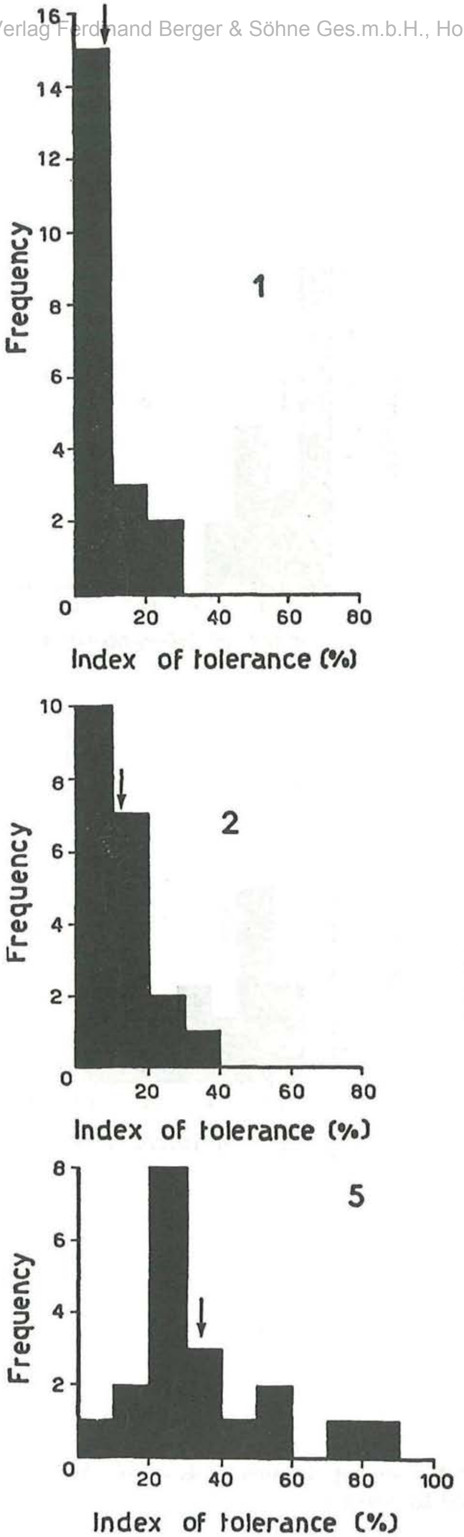

Fig. 2. (b) Distribution of zink tolerance in populations of *A. tenuis*. Means indicated by arrows

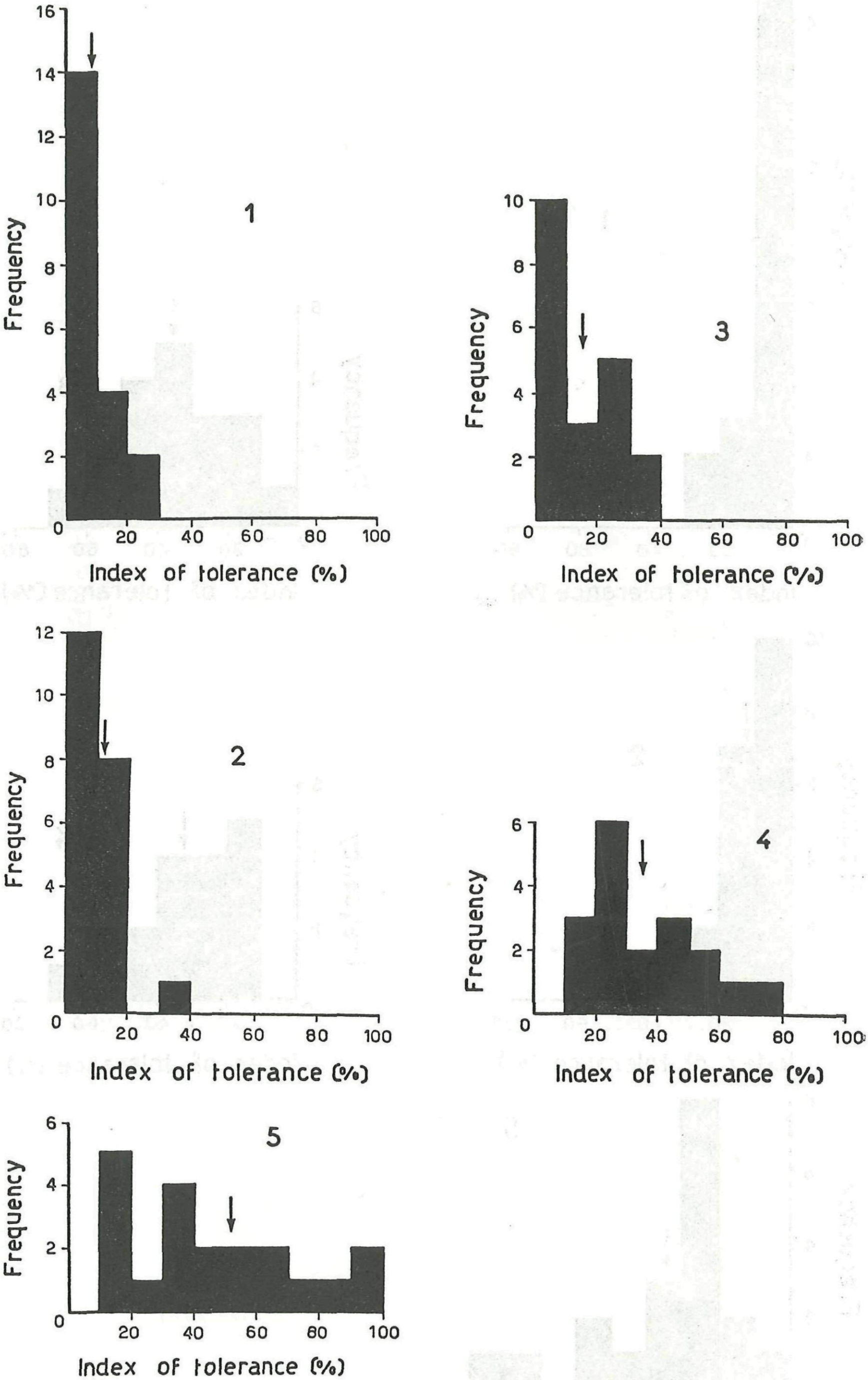

Fig. 2. (c) Distribution of lead tolerance in populations of *A. tenuis*. Means indicated by arrows

in a control population there appears to be a considerable uniformity.

From the aforesaid data follows that the selection in mines is perhaps not so intense for the toxic metals Cu, Zn, Pb, (McNeilly & Bradshaw 1968, Antonovics & Bradshaw 1970, Bradshaw 1970) as to leave a uniform high level of tolerance as it is the case in the control population.

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