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Influence of the Soil Ca on the Tolerance of *Festuca rubra* Populations Against Toxic Metals

By

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With 4 figures

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Summary

KARATAGLIS S. S. 1981. Influence of the soil Ca on the tolerance of *Festuca rubra* populations against toxic metals. — Phyton (Austria) 21 (1): 103–113, 4 figures. — English with German summary.

Festuca rubra populations from toxic or non-toxic areas were studied. Their tolerance against the soil content in toxic metals and in combination with the Ca content was also correlated.

It was demonstrated that the *Festuca rubra* populations developed in an environment with high concentrations of toxic metals and with high concentrations of Ca at the same time, showed very little or almost no tolerance against these metals. On the contrary, populations from other mines with normal Ca concentrations in their soil indicated increased tolerance against the toxic metals found in it. This behaviour expressed by the *Festuca rubra* populations of the Ecton mine is probably due to the high Ca concentration found in the soil in the form of $CaCO_3$. $CaCO_3$ along with the heavy metals has the ability to form undissolved or not easily dissolved carbonate salts. As a result there are no free ions of toxic metals in the immediate environment of the root and consequently the plants cannot be selected against these metals.

Zusammenfassung

KARATAGLIS S. S. 1981. Der Einfluß des Boden-Ca auf die Toleranz von *Festuca rubra*-Populationen gegenüber toxischen Metallen. — Phyton (Austria) 21 (1): 103–113, 4 Abbildungen. — Englisch mit deutscher Zusammenfassung.

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Es wurde die Toleranz von *Festuca rubra*-Populationen von durch toxische Metalle belasteten und von nichttoxischen Böden gegenüber Cu, Zn und Pb verglichen und mit dem Ca-Gehalt der Böden in Beziehung gesetzt. *Festuca rubra* von Böden mit hohem Gehalt an toxischen Metallen und gleichzeitigem hohen Ca-Gehalt zeigten nur geringe oder fast keine Toleranz gegenüber diesen Metallen. Pflanzen von Böden mit normalem Ca-Gehalt hingegen zeigten eine mit dem Schwermetallgehalt des Bodens ansteigende Toleranz. Dieses Verhalten wird damit erklärt, daß bei hohem Gehalt von Ca in Form von $CaCO_3$ in Gegenwart von Schwermetallen unlösliche oder schwerlösliche carbonatische Salze entstehen. Dadurch finden sich in der Rhizosphäre keine freien Ionen toxischer Metalle und es kann in weiterer Folge auch keine Selektion der Pflanzen gegenüber diesen Metallen stattfinden.

(Editor transl.)

Introduction

It is well known that apart from other factors the distribution and cover of plants are determined by the nature of soil as well. Soil supplies plants with the necessary salts. Plants can also receive elements which do not play such an important role in their development as others do, but which must necessarily be abundant in the soil.

Most of the heavy metals found in mine waste are poisonous and consequently comprise a factor inhibitory to the evolution of certain plant species (FRIEDBERG 1974, BREMNER 1974). Despite the unfavourable conditions occurring in mine areas, it is possible for a number of plant species to develop satisfactorily as tolerant ecotypes against these toxic metals (TURESSON 1922, GREGOR 1944, BRADSHAW 1952, HESLOP-HARRISON 1964).

Presence of toxic metals in the soil remains to be one of the most prominent ecological factors affecting plant cover and distribution of a place. The study, however, of the amount of toxic metals contained in the soil does not yield the real image of the soil toxicity degree which is ultimately determined by the amount of the toxic metal found in the disposition of the plant. ALLOWAY & DAVIES (1971) discovered that the more the total soil content in heavy metals, the higher is the amount available to plants. Despite all this, however, there are different factors capable of influencing the quantity being available in heavy metals such as soil pH, concentratoins of other nutritive salts phosphoric in particular, content in humo, content in H_2O, presence of antagonistic ions etc.

From all these factors, the least known is perhaps the one referring of ions of heavy toxic metals on the development of plant organisms. It was toward this direction we have piloted our interest. *Festuca rubra* populations collected from toxic and non-toxic areas were used as experimental materials. There has been an attempt to correlate their tolerance with the content of the toxic metal in the soil and in connection with the Ca presence.

Materials and Methods

1. Collection of plant material

Festuca rubra plants used in this study were collected from the old, deserted copper mine of Ecton. Eleven populations selected from different positions of the Ecton mine, and one from the Trelogan Zn/Pb mine were totally placed under study. The vegetation these regions presented was scattered and consisted of groups of plants which in some cases ran apart from each other at relatively great distances. *Festuca rubra* populations

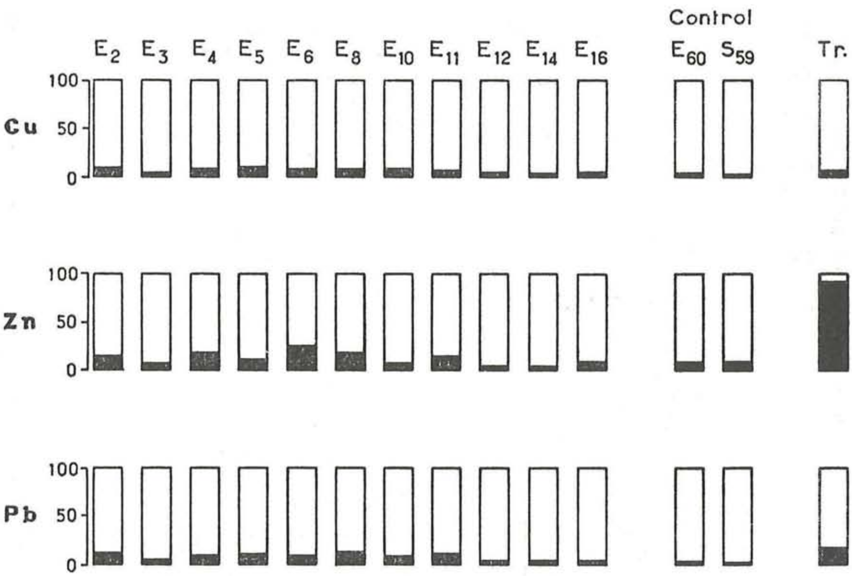

Fig. 1. The root length of *Festuca rubra* populations grown in various toxic solutions, expressed as a percentage of their growth

were also selected from non-toxic regions so as to make the comparison of the above populations between them and with the pasture one feasible (Ecton and Sefton Park). Ten different genotypes were collected from each population and were later replanted in plastic beakers containing normal soil (John Innes soil). These materials were kept in a green-house for 8—10 weeks until their development was fulfilled and they became ready for checking. The temperature of the green-house was almost steady ranging between 25°—30° C, whereas the photoperiod was about 16 hours per day and reinforced by artificial light during the winter or on dark days.

2. Copper, Zinc and Lead tolerance test

The toxic action of heavy metals against plants produces several effects. Yet, the most evident and characteristic effect is the suspension of the root growth in relatively low concentrations of toxic metals and the complete stoppage of growing in higher concentrations. It was therefore necessary to determine the degree of tolerance by measuring the root growth in aqueous solutions with or without toxic metals. The following ratio pictures the index of tolerance (I. T.):

$$IT = \frac{\text{Mean length of longest root in solution with metal}}{\text{Mean length of longest root in solution without metal}} \times 100$$

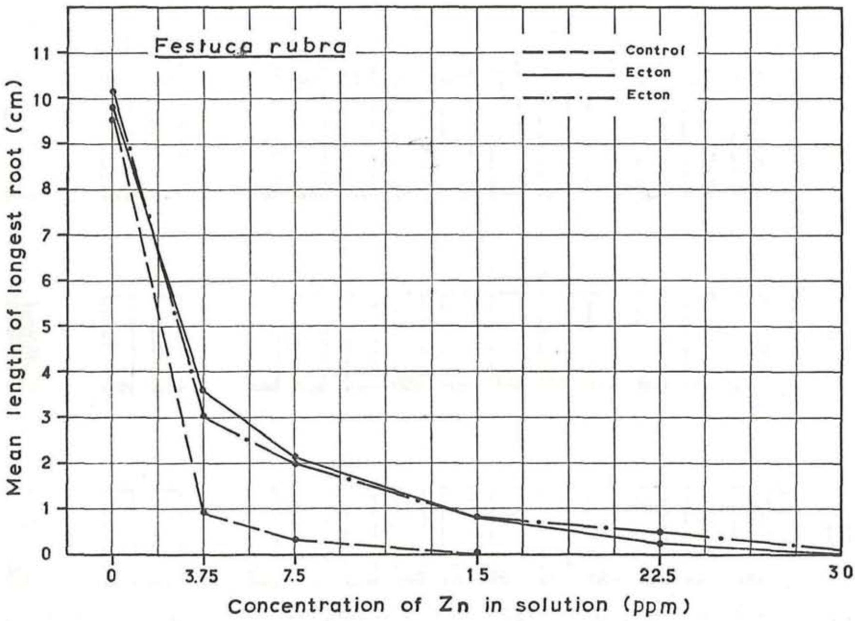

Fig. 2. The effect of zinc concentrations on the length of the longest *Festuca rubra* root coming from Ecton compared with a non-tolerant genotype

This method of checking the tolerance is the most rapid and characteristic one (Gregory & Bradshaw 1965, McNeilly & Bradshaw, 1968). A detailed description of this method was given in previous papers (Karataglis 1976, 1978a, b).

3. Analysis of soil for the determination of total metals

The soil analysis is of a particular interest because it is through it that we can achieve a qualitative and quantitative estimation of the edaphic factors that are dominant in mine areas. It is therefore possible to comparatively estimate the action of selective forces the soil exerts on the distribution of species around the mine regions where toxic metals are usually found in high concentrations.

The soil about to be analysed was collected from the plant rhizosphere at a depth of 6—10 cm from the ground. The samples were first dried at a temperature of 25° C until they gained a steady weight and they were then passed through a 2 mm ∅ sieve and finally ground. Heavy metals were extracted by boiling 1 g of soil with 8 ml of 4/1 (v/v) dense nitric/perchloric acid mixture. The samples were kept in a room temperature overnight to predigest. The following day they were heated for half an hour at 50° C and for 3 hours at 120°—130° C. After the freezing of the beakers, 20—30 ml of twice deionized water were being added in each beaker. The content was

Table 1

Distribution of copper, zinc, lead and calcium in the original soil from the different sites (Metal content in ppm)

Populations	Cu	Zn	Pb	Ca
a) Ecton				
E_2	3.600	1.100	2.700	42.200
E_3	2.300	980	2.800	47.200
E_4	8.800	1.800	3.500	48.000
E_5	5.000	1.100	3.800	47.000
E_6	2.600	2.800	2.300	70.800
E_8	2.100	2.050	2.900	47.000
E_{10}	10.600	1.070	8.500	68.800
E_{11}	13.800	1.450	12.500	69.000
E_{12}	13.700	1.400	8.400	72.000
E_{14}	2.200	1.150	3.000	47.000
E_{16}	8.200	1.250	3.200	68.000
b) Control				
E_{60}	70	57	60	1.100
S_{59}	60	65	65	900
c) Trelogan				
Tr.	60	16.800	1.870	850

filtered in 100 ml volumetric flasks with the help of Whatman 42 filtered paper. The final volume of the filter reached 100 ml.

The determination of the heavy metals was measured by using a Unicam Sp 90 atomic absorption spectrophotometer with separate hollow cathode lamps for each element at the appropriate wavelength.

The analysis of the soil samples is given in Table 1.

Results

Two randomly picked *Festuca rubra* genotypes and a control one were taken from the Ecton and Trelogan mines with the purpose of finding their inhibitory action on root growth in five different metal concentrations, while the $Ca(NO_3)_2$ (0.5 g/l) concentration was kept stable (Figs. 2, 3, 4).

Figures 2, 3 and 4 demonstrate that the non-tolerant *Festuca rubra* genotype and the genotypes coming from toxic regions presented the same root length everytime all of the genotypes grew in $Ca(NO_3)_2$ solution and in the absence of heavy toxic metals (Cu, Zn, Pb). An addition of 0,25 ppm Cu or 3,75 ppm Zn or 6,0 ppm Pb into the $Ca(NO_3)_2$ solution resulted in exerting an important inhibitory action on the root growth of the normal individuals (Figs. 2, 3, 4). To be more specific, the inhibition exerted on root growth reached about 90%, equal to each of the studied heavy metals (Figs. 2, 3, 4).

Genotypes coming from toxic areas presented an inhibition in their

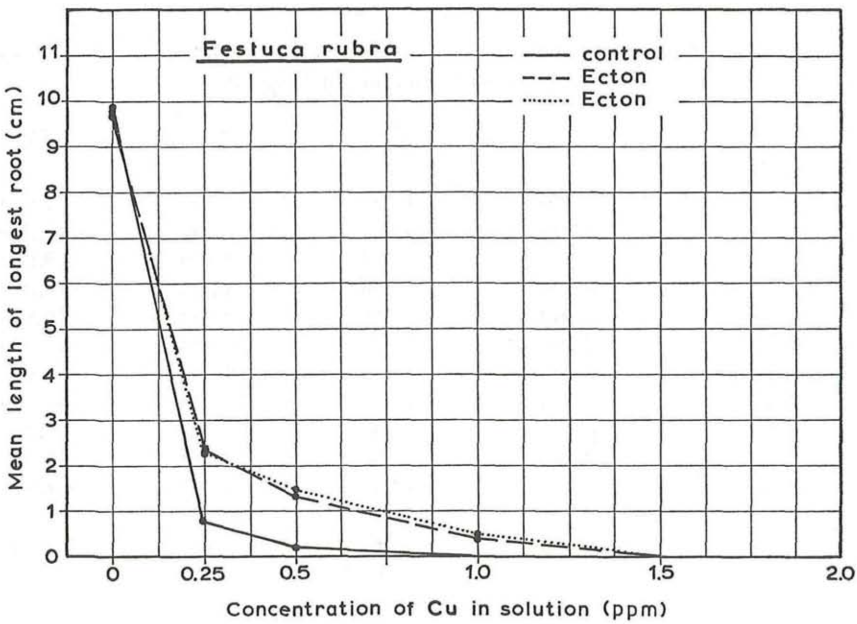

Fig. 3. The effect of copper concentrations on the length of the longest *Festuca rubra* root coming from Ecton compared with a non-tolerant genotype

increase against the three metals in connexion with the normal solution after the addition of the following concentrations (Cu = 0,25 ppm, Zn = 3,75 ppm and Pb = 6,0 ppm) of heavy metals, but their length was obviously longer than that of the normal population (see Figs. 2, 3, 4).

These differences in root length become more evident when individuals grew in a solution with double metal concentration in which individuals from the normal population practically presented no root (Figs. 2, 3, 4). We have chosen the concentrations of 0,25 ppm for Cu, 3,75 ppm for Zn and 6,0 ppm for Pb as the most appropriate to use in our experimental measurements. This selection was performed for two reasons: On one hand, because the difference in root length between normal and tolerant individuals was obvious in these concentrations and on the other hand, the non-tolerant ones had roots the length of which could be measured. This was not the case, however, in the concentration that immediately followed where the root length had almost been insignificant and the mistakes the measurements yielded much more.

The soil analyses of all the samples from Ecton and Trelogan showed characteristically that the quantities they contained in toxic metals were remarkable (see Tab. 1). The checking, however, of the Ca content indicated

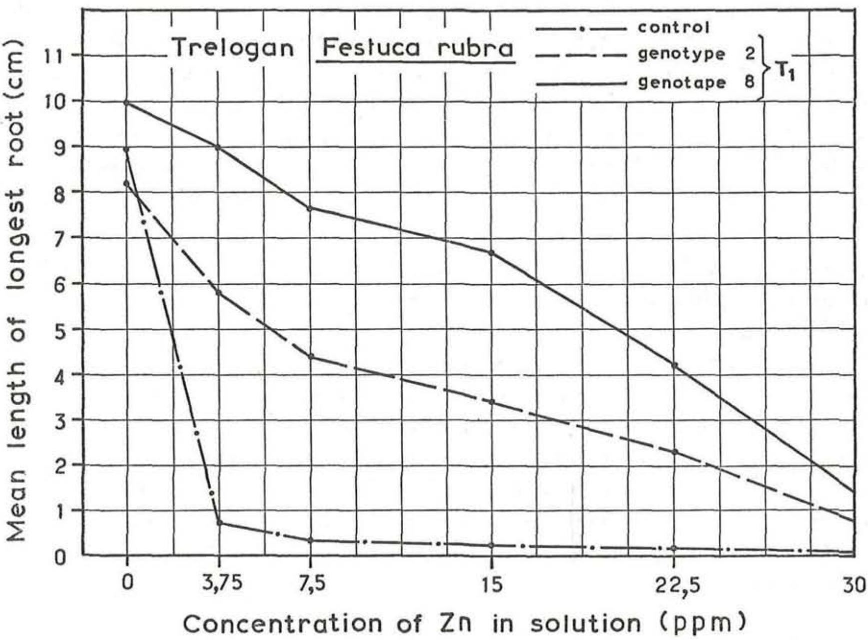

Fig. 4. The effect of zinc concentrations on the length of the longest *Festuca rubra* root coming from Trelogan compared with a non-tolerant genotype

that only the Ecton samples presented a very high concentration, whereas those from the Trelogan ranged within normal bounds. Analyses of the Ecton samples rendered quantities in Ca which were twenty times more than the corresponding ones of the toxic metals (Tab. 1).

Checking the results of the rooting test we have found out that none of the studied populations in the Ecton mine area presented and increased index of tolerance against Cu, Zn, or Pb. On the contrary, plants from the Trelogan mine yielded increased indices of tolerance against Zn and Pb found in the soil. The individuals of populations collected from non-toxic areas presented a uniform number of low indeces of tolerance against the three toxic metals (Fig. 1).

An exception to this behaviour expressed by the Ecton populations was population E_6 which showed a somewhat increased tolerance against Zn. This fact has led us to study the variability existing within the populations. Such a study contributed to our finding out that there were three from the 10 genotypes of this populations which presented an icreased index of tolerance against Zn thus resulting in yielding a rather increased mean value of the population.

It was confirmed by previous studies of the tolerance the individuals among populations had that the different genotypes of one and only population presented different tolerance against one or more metals. This checking enabled us to find out which of the ten genotypes of each population had the highest index of tolerance in the three metals. It is thus we have come to have the variability degree prevailing over the different populations. Table 2 indicates this inner variability in the individuals of several populations located in the Ecton biotops and in connexion with the Trelogan population and the control one.

The results of this study have pointed out the fact that there is no variability within the populations since most of them have very few individuals with an index of tolerance above 20%. For Cu no individual presented an index of tolerance higher than 20%. Only two individuals of the E_8 population presented an index of tolerance a little above 20%. As far as Zn is concerned some of the studied populations produced a very small number of individuals with an index of tolerance higher than 20%. Finally, the inner homogeneity of ten different genotypes of the Trelogan population against the three metals can be clearly detected. Although the dispersion of the individuals fluctuates between high levels of index of tolerance for Zn (from 60%—100%), however, this population seems to be quite homogeneous (Table 2).

Discussion

The plant cover of areas contaminated by heavy metals mainly consists of a certain characteristic range of species which have occasionally been studied by several researchers (see review by ANTONOVICS *et al.* 1971). A distinction between metallophytes and pseudometallophytes drawn by some scientists should be noted here (LAMBINON & AUQUIER 1963, SMITH & BRADSHAW 1979). The first are obligate metallophytes, that is plants found almost entirely on metalliferous spoils and are characterized as indices of metalliferous areas (RUNE 1953). The latter are facultative metallophytes, that is plants found both on metalliferous and normal soils (LAMBINON & AUQUIER 1963). However, and according to SMITH & BRADSHAW (1979) the plants of the first group can also occur on normal soils.

Towards the end of the 50's and the beginning of the following decade, it was stated by WILKINS (1957) and JOWETT (1964) that the toxic action of Pb was ameliorated by the addition of Ca ions to water-cultures. PROCTOR 1971 indicated that the presence of Ca in high concentrations on the soil and the added in the form of ions quantity of Ca in water cultures, ameliorates the toxic action of different toxic metals.

The soils of the sites examined were calcareous and consequently rich in Ca (see also SMITH, Ph. D., 1973, pp. 29, Table IIb). NAVROT & RAVIKOVITCH (1969), however, have shown that calcareous soils are usually very

Table 2

Distribution of Cu, Zn and Pb tolerance in populations of *Festuca rubra* from Ecton (E_2-E_{16}), from Trelogan (Tr.) and Controls (C). The numbers show the frequency of individuals (on 30 that were measured) in the corresponding range of index of tolerance (IT)

Metal	IT	E_2	E_3	E_4	E_5	E_6	E_8	E_{10}	E_{11}	E_{12}	E_{14}	E_{16}	C	Tr.
Cu	0—10	19	30	28	18	25	24	23	27	30	30	28	30	29
	10—20	11	—	2	12	5	6	7	3	—	—	2	—	1
Zn	0—10	8	24	3	19	—	3	20	16	28	30	23	30	—
	10—20	16	6	17	11	14	16	10	12	2	—	7	—	—
	20—30	6	—	10	—	12	11	—	2	—	—	—	—	—
	30—40	—	—	—	—	4	—	—	—	—	—	—	—	—
	40—50	—	—	—	—	—	—	—	—	—	—	—	—	—
	50—60	—	—	—	—	—	—	—	—	—	—	—	—	—
	60—70	—	—	—	—	—	—	—	—	—	—	—	—	4
	70—80	—	—	—	—	—	—	—	—	—	—	—	—	3
	80—90	—	—	—	—	—	—	—	—	—	—	—	—	9
	90—100	—	—	—	—	—	—	—	—	—	—	—	—	14
Pb	0—10	16	29	19	17	20	25	20	18	29	29	30	30	21
	10—20	14	1	11	13	10	13	10	12	1	1	—	—	9
	20—30	—	—	—	—	—	2	—	—	—	—	—	—	—

poor in available quantities of heavy metals. This fact was attributed to the ability of $CaCO_3$ to engage heavy metals from the soil mainly as undissolved or not easily dissolved form of carbonate compounds. Taking NAVROT's & RAVIKOVITCH's (1969) observations into account one can assume that the $CaCO_3$ of soil covering the area placed under study, acts in such a way as to engage the toxic metals of Cu, Zn and Pb in the form not easily dissolved carbonate salts. As a consequence it is possible to have the formation of the following carbonate salts:

a) those of Copper, $(CuOH)_2\,CO_3$ or $Cu(CuOH)_2\,(CO_3)_2$
b) those of Zinc, $ZnCO_3$ or $2.\,ZnCO_3.3Zn\,(OH)_2$ and
c) those of Lead, $PbCO_3$

Any free metal ions existing in the rhizosphere are likely to be engaged as carbonate undissolved or not easily dissolved salts causing the absence of free toxic metal ions from the immediate environment of the root. It can thus be explained why the *Festuca rubra* plants collected from the Ecton mine and being able to grow in high concentrations of toxic metals such as Cu, Zn, Pb, express little and in some cases almost no tolerance against these toxic metals.

Contrary to the behaviour the plants from the Ecton mine demonstrate the populations of Trelogan present an increased index of tolerance against Zn and Pb as well. The soil analysis of the Trelogan mine gave low concentrations in Ca (ranging within normal bounds) and high concentration mainly in Zn. The quantities are less for Pb but are found on levels toxic to the plant organisms. Consequently, the low content in Ca observed on the Trelogan soil and the high concentrations of toxic metals brought forth the demonstration of an increased tolerance in plants against toxic metals found in the soil. A further comparison between the indices of tolerance against Pb of plants coming from the Ecton and Trelogan mines and in combination with the soil concentrations in Pb we may notice that although the soil content in Pb of the Trelogan mine was much lower than that of the Ecton the index of tolerance in the Trelogan plants was of those of the Ecton.

So we have come to believe that the presence or absence of Ca ions in the immediate environment of the root greatly affects the toxicity of heavy metals ameliorating or even erasing their toxic action.

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