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Estimation of the Toxicity of Different Metals, Using as Criterion the Degree of Root Elongation in *Triticum aestivum* Seedlings

By

S. KARATAGLIS *)

With 2 Figures

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Summary

KARATAGLIS S. 1987. Estimation of the toxicity of different metals, using as criterion the degree of root elongation in *Triticum aestivum* seedlings. – Phyton (Austria) 26 (2): 209–217, with 2 figures. – English with German summary.

The toxicity of ten different metals was studied using as criterion the degree of root growth inhibition of the seedlings of a cultivated greek wheat variety (*Triticum aestivum* cv. Vergina).

The following order of metal toxity was revealed: copper > chromium > nickel > zinc > lead > cadmium > aluminium > iron. It was also observed that all Mg and Mn concentrations used (pH = 7) did but a little affect the root growth of the seedlings which displayed no symptoms of chlorosis. Such a kind of results are of value in predicting the toxicity of heavy metals in plant growth on contaminated soils.

In most metals and up to a certain point the amount of metal taken up by the plant is analogous with the concentrations of solutions used in the experiment.

Zusammenfassung

KARATAGLIS S. 1987. Bewertung der Giftigkeit verschiedener Metalle anhand der Wurzelverlängerung von *Triticum aestivum*-Sämlingen. – Phyton (Austria) 26 (2): 209–217, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Toxizität von zehn verschiedenen Metallen wurde anhand der Hemmung des Wurzelwachstums von Sämlingen einer griechischen Kultursorte des Weizens (*Triticum aestivum* cv. Vergina) untersucht. Es wurde folgende Reihenfolge der Toxizität

*) Dr. S. KARATAGLIS, Associate professor, Botanical Institute, University of Thessaloniki, Thessaloniki 540 06, Greece.

festgestellt: Cu > Cr > Ni > Zn > Pb > Cd > Al > Re. Es wurde ferner festgestellt, daß Mg und Mn bei allen untersuchten Konzentrationen (pH = 7) das Wurzelwachstum nur wenig beeinflußten, Chlorose wurde nicht beobachtet. Derartige Ergebnisse sind für die Vorhersage der Giftwirkung von Schwermetallen auf das Pflanzenwachstum auf contaminierten Böden von Bedeutung. Bei den meisten Metallen steigt deren Aufnahme durch die Pflanze entsprechend der dargebotenen Konzentration an.

1. Introduction

A number of recently published papers report on the consequences of different toxic metals on native or cultivated Gramineae (ADAMS & PEARSON 1967, ANTONOVICS & al. 1971, FOY 1973, 1976, 1983 a, 1983 b, BRADSHAW & CHADWICK 1980, BRADSHAW & MCNEILLY 1981, WONG & BRADSHAW 1982, TAYLOR & FOY 1985). A good deal of these metals in concentrations sometimes lower, sometimes higher, is considered necessary to the normal development of plants. There is however a certain concentration point, beyond which toxic metals are capable of producing several symptoms such as depression of plant growth, stunting and thickening of the root system, chlorosis and necrosis of leaves, reducing fresh weight, spots on the leaves etc.

Many of the heavy metals in soil occur as inorganic components or they are bound to organic matter, clay or hydrous oxide of Fe, Mn and Al (HODGSON 1963, ALLOWAY 1968, JENNE 1968, FOY et al. 1978). Because of this precipitation and sorption of most metals by soils, only Zn, Cu, Pb, Cd and Ni toxicities have occurred frequently. Some other elements may be toxic in solution cultures but are not phytotoxic in soils, even at very high levels (e. g. Cr, Ag, Sn, Ga and Ge) (FOY & al. 1978). Toxis metals (Cu, Zn, Pb, Ni, Cd, etc.) are especially interesting in that they not only cause phytotoxicity, but also because they are taken up by plants from the soil to be subsequently transported to the food web (BRANDE & al. 1975. MAHAFFEY & al. 1975, WEAVER & al. 1976. CHADWICK 1976). The problem becomes even more serious when the cultivated plants are of mass production and consumption.

Therefore plants widely grown and consumed, developing in toxic or even problematic areas, demand a better and more detailed knowledge of the effect of different toxic metals on primary plant functions as well as of the possible mechanisms plant employ to overcome unfavourable soil toxic conditions. Nevertheless, with regard to the toxic metal effect on vegetation, development and uptake by cultivated plants our information is limited since different species and varieties of the same species show dissimilarities in their behaviour to the same toxic metal.

We have been familiar with the direct and most typical consequences different concentrations of toxic metals bring upon shoot and mainly root growth (BRADSHAW 1952, CLYMO 1962, MCGRATH et al. 1980, SAPRA et al. 1982, WONG & BRADSHAW 1982, BAKER & al. 1983, TAYLOR & FOY 1985). This root behaviour is frequently adopted as a medium in comparing the toxic action of various metal concentrations in many tolerance tests. Based on this characteristic behaviour we studied the toxic effect of ten different metals on the root and shoot growth of a greek cultivated variety of wheat (*Triticum aestivum* cv. Vergina). The degree of uptake of toxic metals by all young plants has also been examined.

II. Materials and Methods

Water solution 0.5 g/l $Ca(NO_3)_2 \cdot 4H_2O$, as suggested by WILKINS (1957), JOWETT (1958) and MCNEILLY & BRADSHAW (1968), was used as a medium for the plants to grow in, to which one of following metals Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Cr^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+} and Al^{3+} was added. These metals were used in five different concentrations where each one was twice its precedent (Fig. 1). Copper sulphate, zinc sulphate, lead nintrate, nickel nitrate, cadmium sulphate, chromio sulphate, manganese sulphate, magnesio nitrate, ferrous sulphate and aluminium chloride anhydrous, were the compounds employed in each of the above cases.

A three to five layer of black alkathene beads, 2 mm in diameter, was floated on the surface of the solution in plastic beakers with 300 ml capacity. Twenty wheat seeds from the cultivated variety *Triticum aestivum* cv. Vergina (Γ-84856) were put in each of the metal concentrations. The experiment was carried out in triplicate at each metal ion concentration. The pH of the growth solution containing metal ions was adjusted to 7.0 except for Al, which was adjusted to 4.2 ± 0.1 to ensure retention of Al^{3+}ions in solution. The pH of the Al^{3+}solution was measured daily, while every other day all solutions were replaced in order to keep the metal concentrations steady and to allow aeration of the roots.

The plastic beakers were placed in a growth chamber at $23 \pm 1°$ C and 85% humidity, with a photoperiod of 16 hours light and 8 hours dark. 16 days later the length of the longest root, that of each shoot in different concentrations as well as metal uptake by the plant, were measured.

Plant material was dried for two days at 90° C approximately. 0.5 g of dry material was placed in the bottom of a digestion tube, to which was added 10 ml of concentrated Analar nitric acid. The tubes were left overnight to predigest, then placed on an aluminium block for 1 hour at 50° C, then increased the temperature to 120° C until digestion was complete (all the brown fumes had cleared and the digestion liquid was clear) in about 3 hours time. The tubes were left to cool and their contents made up to 50 ml with double distilled water. Analysis was carried out using a Varian Atomic Absorption Spectrophotometer, using standard solutions.

III. Results and Discussion

The growth of seeds during experimentation ranged between 80–90 in low concentrations and became even less in higher ones. This fluctuation should probably be ascribed to the different degree of toxicity expressed by the metals.

With reference to root system growth the consequences of the toxis activity on the root system were immediate and most characteristic resulting in root inhibition (CLARKSON 1965, 1969, MATSUMOTO & al. 1976, 1977, KARATAGLIS 1982, SAPRA & al. 1982. TAYLOR & FOY 1985). In higher concentrations root growth was completely inhibited (MCNEILLY 1982) whereas the degree of toxicity exerted on root growth by different metals varied considerably. In fact, there were instances where apart from the shortening of the root, one also observed the root system usually becoming malformed with short, curved side roots. In contrast to the root, the shoot length did not exhibit any pronounced decrease.

Root growth as regards the successive concentrations of Pb, Cd, Al, Cu and Ni (Fig. 1) was gradually decreased. The arisen differences in the length of root did not exceed the ration 1 : 2 when we compared the first metal concentration with the control. On the contrary, in the case of Zn, Cr and Fe (Fig. 1) the size of the roots abruptly shrinked while in higher concentrations inhibition was entire. The differences in the root length between plants of the first concentration and the control were greater than the 1 : 3 ratio. The complete inhibition of the root and the feable development of the shoot are indicative of the presence of some crucial metal concentration which prevents plants from growing (MCNEILLY 1982). These different concentrations produced by different metals denote the different degree of metal toxicity. The absence of root does not exclude the presence of weak shoots a fact accounted for by the existence of the endosperm.

The average height of the seedlings directly dependent upon metal concentration was decreased but not as much as that of the root (Fig. 1). In Pb or Al concentrations, however, the length of the shoot plants did not present great dissimilarities; nonetheless their shoots (at the higher concentrations) were thinner and consequently their dry weight was restricted. In high concentrations (e. g. Zn, Cr, Fe) shoots would grow even though there was no presence of roots. However, shoot growth has never been observed to be inhibited in the extent that root growth was. This is understandable since the toxic metals have to pass through the root before they could influence meristematic and other growth activity in the shoot.

Despite the short duration of the experiment (16 days only), the plants which developed in some of the metals and according to the concentrations used showed symptoms of chlorosis. There are evidences that most metals produce a similar kind of metabolism. A leaf necrosis appears which is specific to a particular metal (GEMMEL 1977) but there is also a general chlorosis of the younger leaves common to all metals. It has been suggested that the origin of these symptoms lies in the roots. So chlorosis is probably a secondary characteristic.

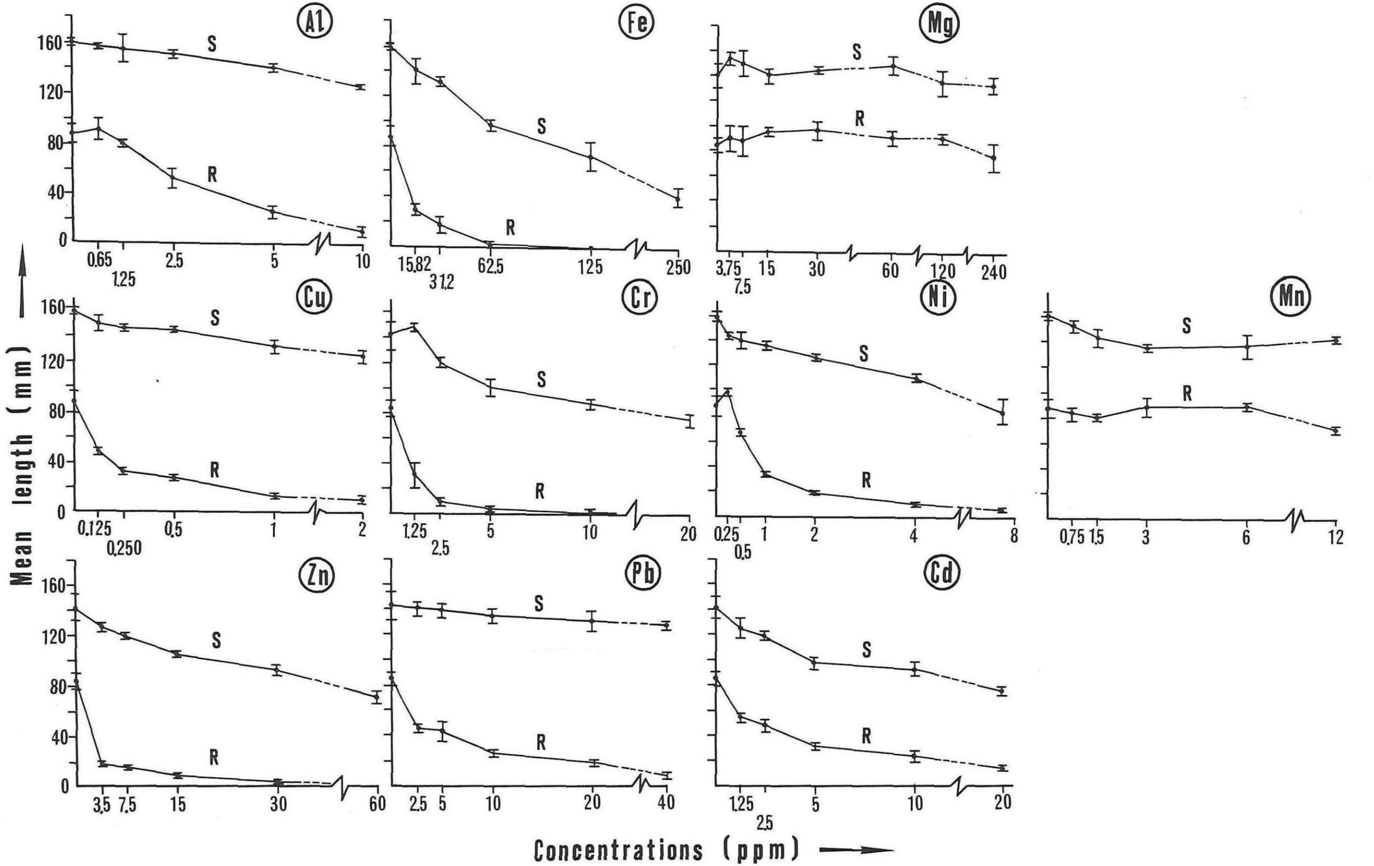

Fig. 1. The effect of increasing concentrations (p. p. m.) of various toxic metals on the growth of roots (R) and shoots (S) of *Triticum aestivum* cv. Vergina seedlings (mean ± s. d.).

One should point out that in all Mn and Mg concentrations the length of roots and the height of shoots were but little influenced (Fig. 1). Chlorosis symptoms were not observed in any of the above concentrations. The bright green colour of the plants during experimentation suggested that these metals were not toxic, at least in the concentrations and the pH (= 7) we have used.

Based on the results of this research work and on root inhibition as a criterion for the evaluation of metal toxicity we have concluded the following:

$$Cu > Cr > Ni > Zn > Pb \simeq Cd > Al > Fe$$

This evaluation sequence is generally in agreement with the results of papers produced by researchers who studied the toxic action of metals on root inhibition. JOWETT (1958), for example, using *Agrostis tenuis* and *Agrostis stolonifera* as material came up with Cu > Ni > Zn > Pb. CRAIG (1978) using *Zea mays* (SR 52) gave: Cu > Ni > Pb > Zn. HOGAN & RAUSER (1979) studying *Agrostis gigantea* resulted in Cu > Ni > Co > Zn. NEIBOER & RICHARDSON (1980) for barley gave the following toxicity sequence: Hg > Pb > Cu > Cd > Cr > Ni > Zn, whereas WONG & BRADSHAW (1982) for *Lolium perenne* gave the order of toxicity: Cu > Ni > Mn > Pb > Cd > Zn > Al > Hg > Cr > Fe, questioning the inexplicably high Mn toxicity.

From the above mentioned it is made clear that the mechanism of toxicity of different metals, in terms of root growth inhibition, varies according to the species we use. This probably happens because these metals can readily be complexed by substances within the plant, such as proteins, with other functions, or some of these differences between species relate to specific ecological adaptations (MARTIN 1968, WONG & BRADSHAW 1982).

Fig. 2 supplies us with the results concerned with the uptake of metals by all plants (shoot and root). Although all metal concentrations used were low, uptake by plants was however considerable, with the only exception of Fe. We also observed that the uptake of some metals was carried out rather intensely in the first concentrations (e. g. Cr, Zn, Al, Ni) becoming less evident in the subsequent. On the contrary the uptake of certain other metals (e. g. Cu, Pb, Mn) continued to be analogous with the solution concentrations we used.

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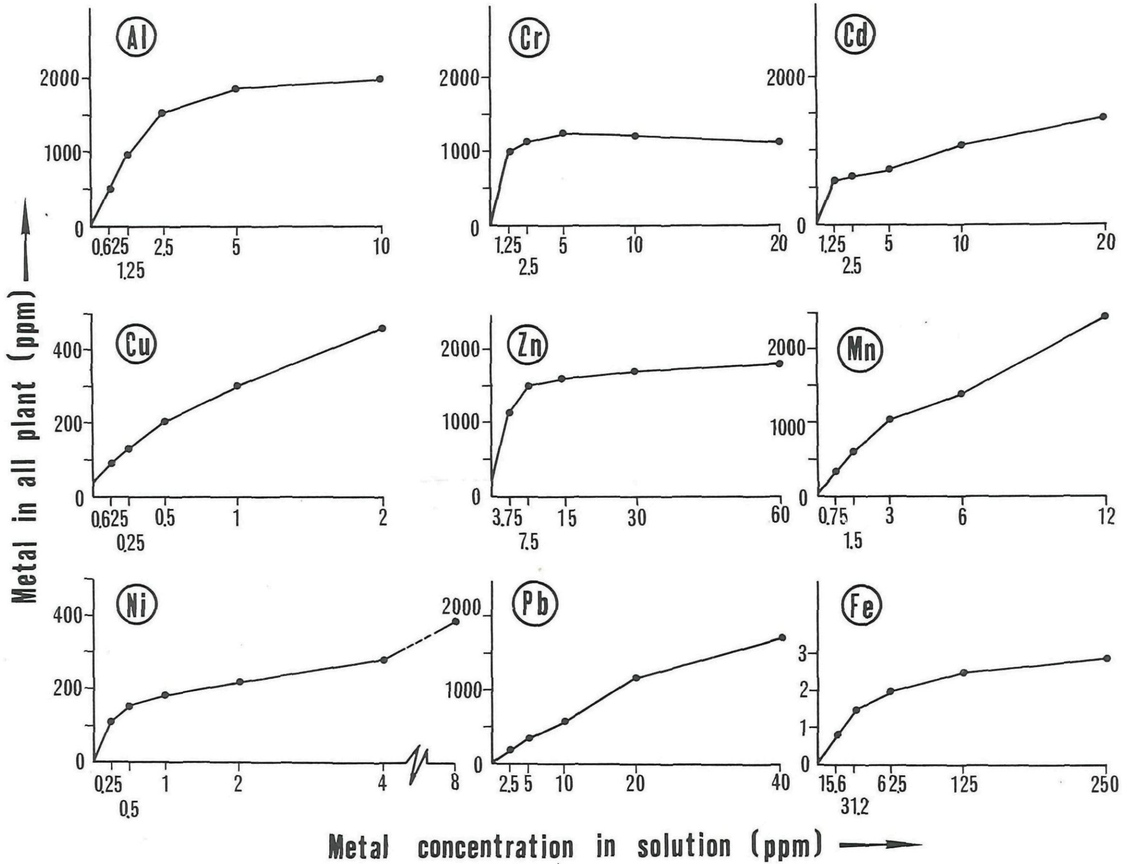

Fig. 2. Uptake (p. p. m.) by *Triticum aestivum* cv. Vergina seedlings, grown in different increasing toxic metal concentrations.

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