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Aluminium toxicity in *Avena sativa* cv. Kassandra and a comparison with the toxicity caused by some other metals

By

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With 7 Figures

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Summary

KARATAGLIS S. 1987. Aluminium toxicity in *Avena sativa* cv. kassandra and a comparison with the toxicity caused by some other metals. – Phyton (Austria) 27 (1): 1–14, with 7 figures. – English with German summary.

The toxic effect of Al found in acid and neutral medium as well as that of Cu, Cd and Mn on seedlings of *Avena sativa* cv. kassandra, a cultivated greek species, was studied.

The toxic action of all metals strongly affects the root length as their concentration increases. On the contrary the increase of shoot is less affected in all cases. At high concentrations, shoots were produced even though there was no sign of a root. Since toxic metals have to pass through the root before they could influence meristematic and other growth activity in the shoot, this is understandable.

It is also a fact that the toxic effect of Al on the root is greater when the metal is located in acid medium (pH = 4.2) rather than in neutral (pH = 7.0). Mn when in neutral medium (pH = 7.0) favours the shoot and root growth and does not manifest

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any toxic phenomena. It was finally observed that Al low concentrations stimulates plant growth and the activity of esterase enzymes. However, in higher concentrations (32 and 40 ppm Al) inhibition of the enzymatic action of esterases and that of the root growth is almost complete, while the shoot growth remains primitive.

The various results of the toxic metal effect are discussed based on the analysis of those factors responsible for root inhibition and the activity of esterase enzymes.

Zusammenfassung

KARATAGLIS S. 1987. Giftwirkung von Aluminium auf *Avena sativa* cv. kassandra und ein Vergleich mit durch andere Metalle verursachten Giftwirkungen. – Phyton (Austria) 27 (1): 1–14, mit 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurden die Giftwirkungen von Al bei saurer und neutraler Reaktion sowie von Cu, Cd und Mn auf Keimpflanzen von Avena sativa cv. kassandra, eine griechische Kultursorte, untersucht. Alle Metalle beeinträchtigten das Wurzelwachstum entsprechend ihrer Konzentration, das Sproßwachstum wurde durchwegs weniger stark beeinflußt. Bei hohen Konzentrationen wurden Sprosse gebildet, selbst wenn keine Wurzeln erkennbar waren. Dies ist verständlich, weil die giftigen Metalle die Wurzel passieren müssen, ehe sie Meristeme und andere Wachstumsaktivitäten beeinflussen.

Die Wirkung von Al ist in saurer Lösung (pH 4,2) größer als in neutraler. Mn in neutraler Lösung fördert das Sproß- und Wurzelwachstum in allen Konzentrationen und zeigt keinerlei Giftwirkungen. Al in geringen Konzentrationen fördert das Wachstum und auch die Aktivität von Esterasen. In hoher Konzentration (32 und 40 ppm Al) wird die Esterase-Aktivität und das Wurzelwachstum gehemmt, während der Sproß normal wächst. Die Ergebnisse werden hinsichtlich der für die Wurzelhemmung und der Esterase-Aktivität maßgeblichen Faktoren diskutiert.

(Editor transl.)

Introduction

A number of researchers have recently focused their attention on the effect of heavy metals upon plant growth. Low concentrations of different metals are considered necessary for the normal growth of plants, their presence, however, in higher concentrations hinders plant development (ANTONOVICS & al. 1971, FOY 1977, 1982 a, b, 1983, ALAN & ADAMS 1979, KARATAGLIS 1982, MCNEILLY 1982, ANIOL 1983).

The presence of toxic metal concentrations in the soil causes a disturbance in the vegetation of the area and a limited number of plant species is observed. The most obvious inhibitory effects are produced on the root system which is prevented from developing naturally (BRADSHAW 1970, WONG & BRADSHAW 1982, SAPRA & al. 1982). Plants growing in soils rich with toxic metals are often incapable of taking up water since their roots are short, extending only on the surface, and therefore in no position to take advantage of the underlying water.

Aluminium is a very common metal which although not a heavy metal could on chemical grounds have similar effects. In fact root inhibition by aluminium has been recorded (RORISON 1960, CLYMO 1962, CLARKSON 1965, 1969), and it was shown to be due to an effect on root mitosis (CLARKSON 1965).

The solubility of Al is very different from other metals. In soil it is available below pH 4.5 (MAGISTAD & al. 1943). So it could preserve no effects at high pH. Yet, heavy metals which become insoluble at high pH can still exert toxic effects.

Aluminium does not occur at high levels in all soils. In some it can be very low (FOY 1982 a, b, 1983). In others, it can reach very high levels, especially laterite soils produced by intense weather, in which the soil may consist almost entirely of aluminium oxide. These soils are the major sources of the world supply of bauxite.

Bauxite mining now occurs at a very high rate, several thousand hectares are probably mined every year, and it has become very important to understand the toxicity of Al, and to be able to put it in the context of the well known toxicities of other metals which can cause severe land restoration problems (BRADSHAW & CHADWICK, 1980).

This paper studies the toxic action of Cu, Cd, Mn and Al on the growth of *Avena sativa* cv. kassandra seedlings, developed in water cultures, as well as the consequences of different Al concentrations on the activity of esterase enzymes.

Materials and Methods

Avena sativa L. cv. kassandra seeds were grown in water solution 0.5 g/l $Ca(NO_3)_2 . 4H_2O$, in 300 ml plastic beakers. Cu^{2+}, Cd^{2+}, Mn^{2+}, and Al^{3+} ions were added in the form of Copper sulphate, Cadmium sulphate, Manganese sulphate and Aluminium chloride (anhydrous) at five different concentrations. Each of these concentrations was twice its precedent. A layer of black alkathene beads (2 mm in diameter) 3 to 5 beads in depth was formed on the surface of the solution and 25 seeds of *Avena* were placed on the surface at each metal ion concentration. The experiment was carried out in triplicate at each metal ion concentration. Controls were grown under the same conditions but in the absence of metal ions. The pH of the growth solutions containing metal ions was adjusted to pH 7.0. In the case of Al, plants were also grown at pH 4.2 to ensure retention of Al^{3+} ions in solution. The pH of the Al^{3+} solution was checked daily and maintained at pH 4.2 ± 0.1. Every other day all solutions were changed so as to maintain metal concentrations at steady levels and to allow aeration of the roots.

The plastic culture beakers were maintained in a growth room at 23 ± 1° C and 85% humidity, with a photoperiod of 16 hours light and 8 hours dark. After 14–16 days of growth the length of the shoot and that of the longest root was measured in all metal concentrations.

Some of these randomly chosen plants were used for the determination of the amount of metal contained. Samples were dried in an oven at 80–90° C to a constant weight. 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 tube was heated on an aluminium block for three hours at 50° C or until all plant material was digested. The tubes were left overnight at room temperature and the following day were heated on the aluminium block for one hour at 50° C. The temperature was then increased to 120° C until digestion was complete in about three 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 Variant Atomic Absorption Spectrophotometer, which was calibrated using standard solutions for Cu, Cd, Mn and Al.

In order to measure the dry weight of the plants we allowed them to dry at room temperature for a week and then placed them in the oven at 80–90° C to a constant weight. After weighing 10–15 individuals from every concentration we determined the average weight of the plants for each metal.

Finally wishing to examine to what extent Al influences the acitivity of esterase enzymes we conducted the following experiment. Fifty seeds were grown in Al concentrations of 0, 8, 16, 24, 32 and 40 ppm in water solution always containing a steady quantity of 0.5 g/l $Ca(NO_3)_2.4H_2O$. The procedure followed this time was similar to the previous one with the only exception of the pH solution which was maintained at 4.2. We checked the pH daily and changed the solution every other day. Fifteen days later the leaf and shoot of the plants were homogenised following the same technique used for gel homogenetion and staining described in previous papers (KARATAGLIS 1975 a, b, 1977 and SYMEONIDIS & al. 1979). These homogenised plant parts were subsequently electrophorized on polyacrilamide gel using the method of isoelectric focusing with carrier ampholite pH 4.0–6.5.

Results

The effect of Cu on growth

The shoot height in the two lowest concentrations displayed almost no difference compared to the control but above 0.5 ppm concentration plants appeared to be relatively shortened. The root length is strongly decreased at 0.125 ppm Cu with a decrease in the initial length of 58.5%, while at higher concentrations (0.5 ppm) root tips are hardly discernible (Fig. 1).

The first symptoms of chlorosis begin to show at 0.25 ppm concentration, gradually becoming more intense as the Cu concentration increases.

The dry weight of the plants decreases with increasing concentrations of Cu in the growth medium (Fig. 5).

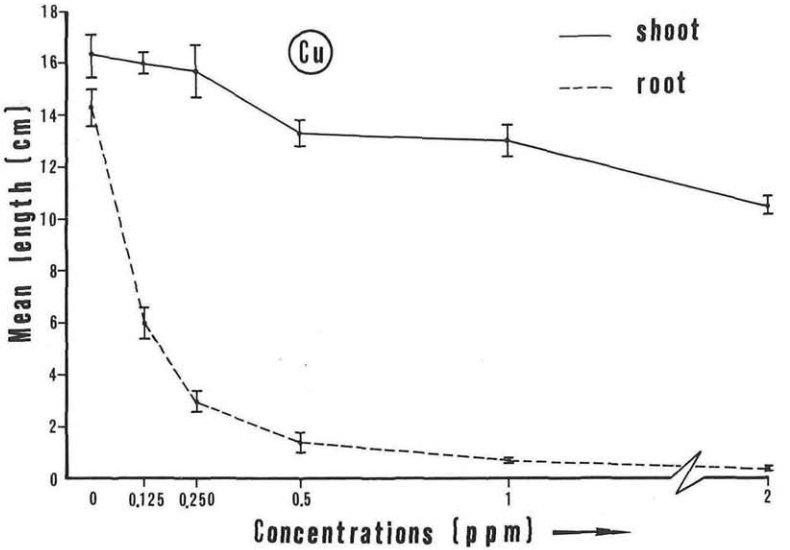

Fig. 1. The effect of increasing concentrations (ppm) of copper on the growth of shoots (——) and roots (– – – –) of *Avena sativa* cv. kassandra (mean ± s. d.)

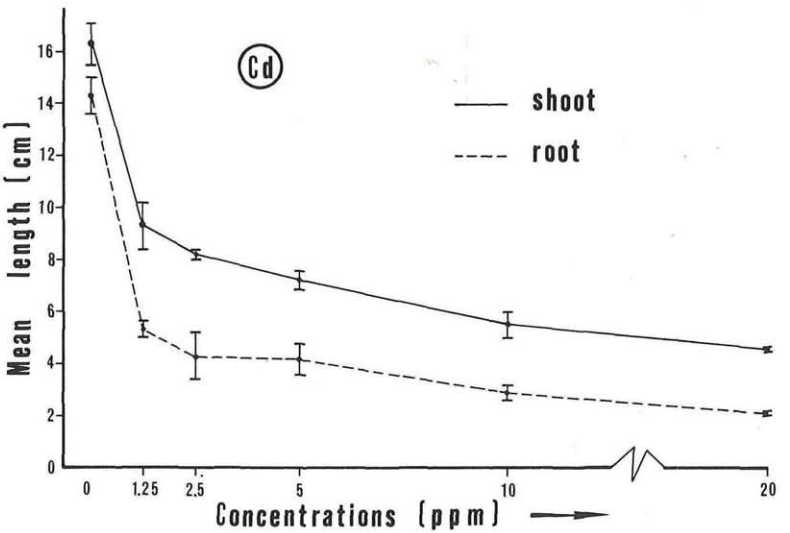

Fig. 2. The effect of increasing concentrations (ppm) of cadmium on the growth of shoots (——) and roots (– – – –) of *Avena sativa* cv. kassandra (mean ± s. d.)

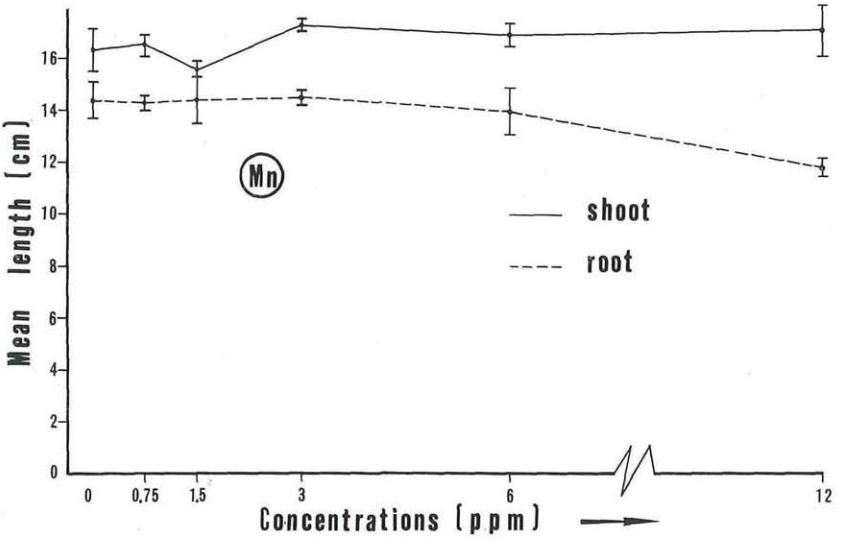

Fig. 3. The effect of increasing concentrations (ppm) of manganese on the growth of shoots (——) and roots (– – – –) of *Avena sativa* cv. kassandra (mean ± s. d.)

The Cu content in the plants increases as its concentration in the solution increases (Fig. 6).

The effect of Cd on growth

The length of shoot and root show a remarkable decrease compared with the control, a 43% reduction in shoot and a 63% reduction in root length at 1.25 ppm Cd. In subsequent concentrations further shortening of the shoot and root is relatively small (Fig. 2).

Symptoms of chlorosis occur at 1.25 ppm Cd and become more intense as the concentration of Cd increases. In addition the root and seeds obtain a reddish colour.

The dry weight of the plants grown at 1.25 ppm drops to 80% of that of the control and those grown at 20 ppm to 74% of the control (Fig. 5). There is a characteristic increase of Cd contained in the plants as it also increases in the solution of the first three concentrations. After that any increase of the Cd content in the plants is smaller (Fig. 6).

The effect of Mn on growth

The shoot has almost the same length in all concentrations of Mn while the root appears a little shortened only in those plants grown at 12 ppm (Fig. 3). In all concentrations of Mn, plants developed a bright green colour and as a result no signs of chlorosis were visible.

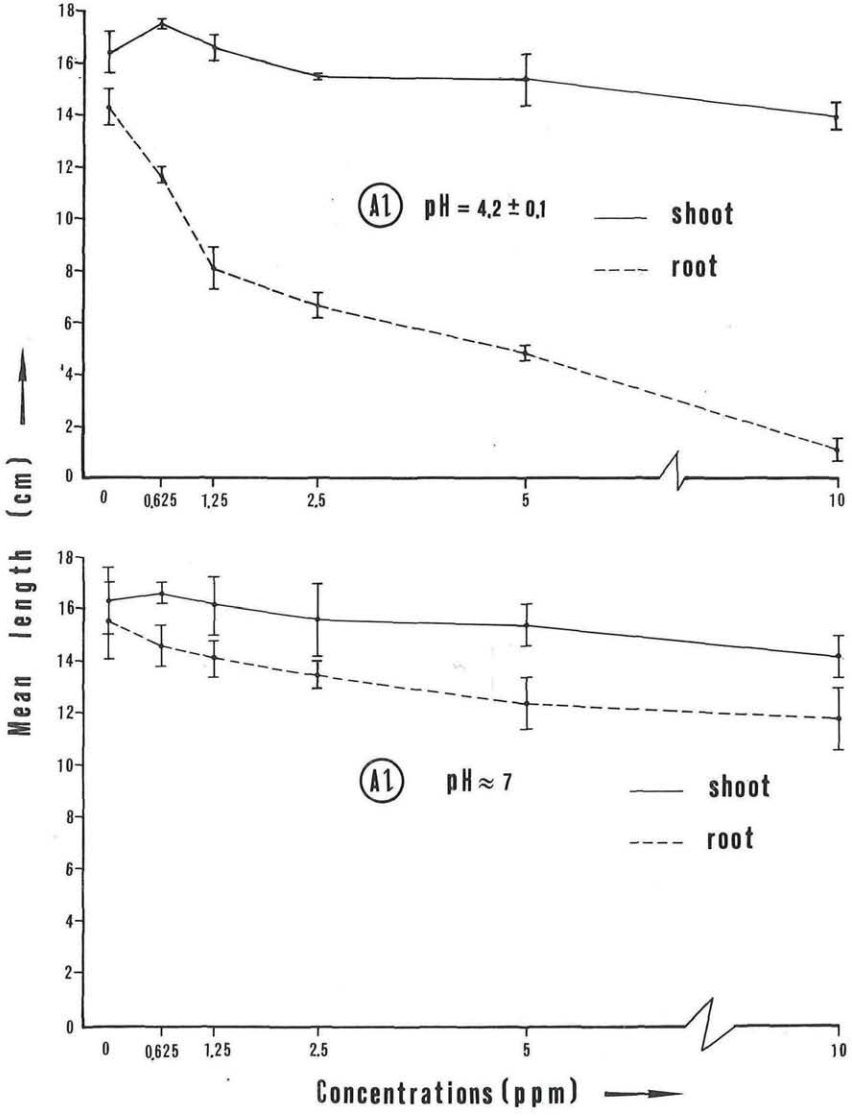

Fig. 4. The effect of increasing concentrations (ppm) of aluminium and pH on the growth of shoots (——) and roots (– – – –) of *Avena sativa* cv. kassandra (mean ± s. d.)

The dry weight of the plants grown in Mn did not decrease but increased reaching 6% between the control and last concentration (12 ppm) (Fig. 5).

The Mn contained in the plants increased along with its concentration in the solution (Fig. 6). Plants grown at 12 ppm contained 5000 ppm.

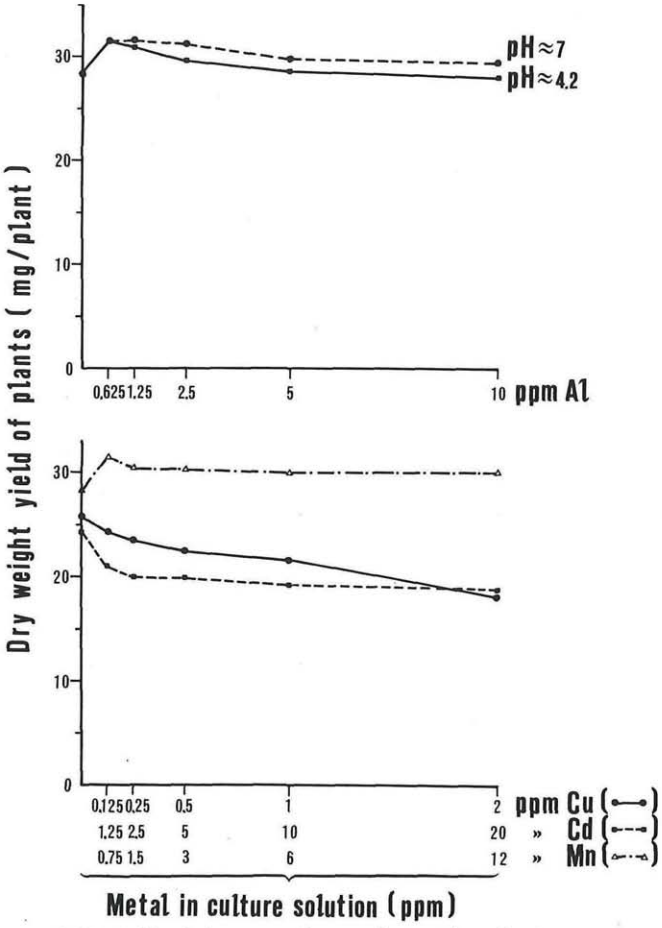

Fig. 5. Dry weight yield of *Avena sativa* cv. kassandra plants grown on different toxic metal concentrations (ppm)

The effect of Al on growth

The shoot height of the plants grown at 0.625 and 1.25 ppm Al and at pH 4.2 increased slightly in contrast to the root which showed a marked decrease in length. The height of the shoot of plants grown at pH 7.0 remained relatively unchanged up to the last concentration. There was a

somewhat small shortening in the root over the same concentration range at pH 7.0 (Fig. 4). The tips of the plants grown at 0.625 and 1.25 ppm and pH 4.2 began to turn slightly yellow. In subsequent concentrations the symptoms gradually became more intense. In the case of the solution with a pH value of 7.0 however, such symptoms were not observed.

As regards the dry weight our observations were as follows: When the pH of the growth solution was 4.2, the dry weight increased about 8% between the control and the first concentration (0.625 ppm) and decreased 3% between the control and the last (10 ppm). When the pH was 7.0 there was a 7.5% increase in the dry weight of the former case and 2% of the latter (Fig. 5).

In the case of the Al content in the plants we observe that it is quite intense in the first three concentrations and becomes moderate in the rest (Fig. 6).

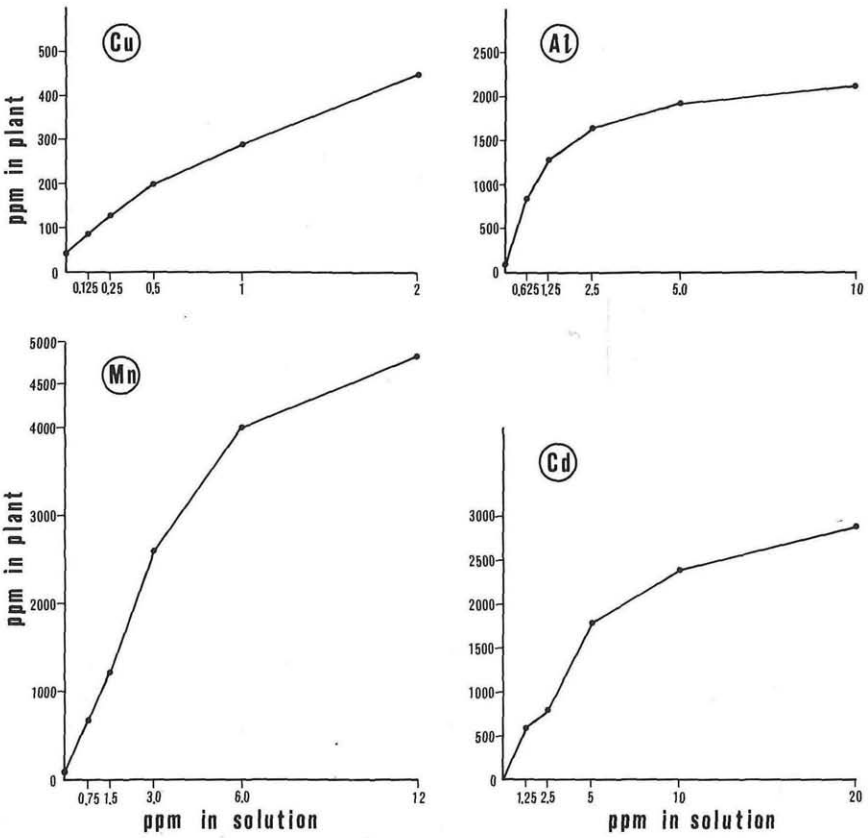

Fig. 6. Metal concentration (ppm) in *Avena sativa* cv. kassandra plants grown in different toxic metal concentrations

Observations on the electrophoretic patterns of esterases

The previous growth experiments show that as Al concentration increased, plants displayed a decreased in their shoot and root length (Fig. 4). In concentrations of 24 ppm the roots are hardly visible, whereas in those of 32 and 40 ppm there are none; therefore, only the upper parts (leaf and shoot) of the plants could be homogenised for electrophoretic analysis.

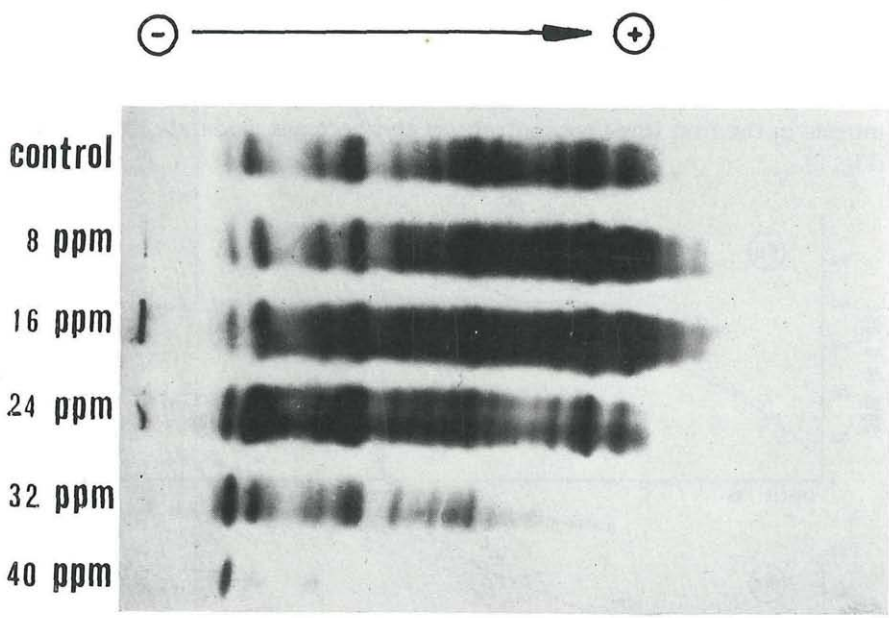

Fig. 7. Photograph of polyacrylamide gel of the esterase enzymes of *Avena sativa* cv. kassandra grown at different Al concentrations and at pH = 4.2

The results of this electrophoresis are shown in Fig. 7, where one can clearly see an increase in the activity of esterase enzymes in the first three concentrations (8, 16 and 24 ppm). More concretely, in the first two concentrations (8 and 16 ppm), apart from the intensity of the bands, two more occur in the area of rapid electrophoretic mobility (2 front zones of weak intensity) compared with the control. The additional bands will later disappear (third concentration at 24 ppm), while there is a concomitant decrease of intensity in bands of rapid and medium electrophoretic mobility. Only those of slow electrophoretic mobility continue to display increased intensity. In concentrations of 32 ppm the disappearance of bands with rapid electrophoretic mobility can be clearly seen. Bands of slow electrophoretic mobility do not differ from the control ones as regards their intensity. In the

last concentration of 40 ppm, however, a nearly complete inhibition in the esterase activity is observed. Of all bands only the first one from the area of slow electrophoretic mobility maintains its intensity, while a few of the slow and few of the medium electrophoretic mobility are slightly intense.

Discussion

Inhibition of the root length caused by the toxic action of several metals is perhaps the most characteristic expression of the degree of metal toxicity (WONG & BRADSHAW 1982, KARATAGLIS 1982, SAPRA & al. 1982). In fact, as shown in Figs. 1, 2, 4 (Al pH = 4.2) the root length decreases as metal concentration increases. According to many researchers this is due to the action of some mechanism conected with cell division. LEVAN (1945), for example, described several cytological irregularities on the meristematic cells of root tips caused by the effect of different metal salts. CLARKSON (1965, 1969) and MATSUMOTO & al. (1976, 1977) agree that this inhibitory action is exerted either on cell division causing inhibition in the DNA synthesis, or on cell extension resulting in the arrest of root lengthening.

The toxic action of metals not only influences the root growth but also the shoot with less evident results (Figs. 1, 2, 4). As a matter of fact in most instances the shoot was shorter and thinner compared to the control thus denoting to some extent the degree of the toxic metal effect. Dry weight is therefore affected by metal toxicity, it being closely connected with root length. However, as indicated in Figs. 1, 2, 3, 4, 5 only Cd and Cu are characterized by a decrease both in dry weight and in the size of shoot and root. Our observations are different as we examine Mn and Al. More precisely, low Al concentrations stimulate plant growth (CATE & SUKHAI 1964, HOWELER & CADAVID 1976, GUERRIER & al. 1977). Yet, it has been reported that Al high concentrations cause root inhibition (MCNEILLY 1982). It must also be stressed that the toxic action of Al is manifested in acid environment and not in neutral. As for Mn it has been concluded that there was an increase in the dry weight denoting the absence of Mn toxicity, at least in the pH and concentrations we have used. In addition we can consider it necessary for plant growth since in all its concentrations no phenomena of chlorosis were detected.

Examining the toxic action of several Al concentrations with respect to the behaviour of esterase enzymes, we notice that low concentrations increase the enzyme activity while there is a corresponding increase in the shoot (pH = 4.2). In high concentrations (32 and 40 ppm), however, its toxic action is remarkably intense resulting in the complete arrest of the enzyme's actions in the last concentration where the root has not developed at all and the shoot is very small (MCNEILLY 1982). The above concentrations seem to be toxic enough to influence directly or indirectly some metabolic process closely related with cell dividions (CLARKSON 1965, 1969). Additionally, they

inactivate those genetic positions responsible for the production of esterase enzymes in this way blocking a group of other substances which contribute to natural growth of plants (AFONOVA 1958, MAIER 1977). It is obvious that the presence of a toxic element in great quantities can interfere with plant metabolism thus causing inhibition in the action of certain enzymes (ERNST 1976, MAIER 1977, 1978 a), or stimulation of some others (MAIER 1978 b), or substitution of elements for basic functional positions (EPSTEIN 1969) or development of organic acids (BROOKES & al. 1981, THURMAN & RANKIN 1982) etc.

Unfortunately, the precise natural tolerance mechanisms of plant organisms against different toxic metals have not only been unknown but many a times they have been conflicting (MATHYS 1975, THURMAN and RANKIN 1982), since behaviour of several plants species and varieties of a single species differ considerably as regards their tolerance towards the same metals.

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