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THE INFLUENCE OF ZINC ON SEED GERMINATION AND GROWTH IN THE FIRST ONTOGENETIC STAGES IN THE SPECIES *CUCUMIS MELO* L.

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Key words: : *Cucumis melo* L., germination, growth of seedlings, zinc

Abstract. The paper presents the results of a study regarding the influence of treatment with zinc in different concentrations (50 mg/l, 100 mg/l, 200 mg /l, 300mg/l, 400 mg /l, 500 mg/l, 600 mg /l) on seed germination and growth in early ontogenetic stages of *Cucumis melo* L. We analyzed the following indicators: the percentage of germinated seeds; the length of root, the length of the hypocotyl and the length of the seedling; the number of the laterale roots; the tolerance index and the seedling vigor index. The results underline the specific variations of analysed indicators, depending on the concentrations used for the treatments of seeds. The concentrations used for treatment do not influence negatively the seed germination, but affected the seedling growth (especially the root elongation), the formation and growth process of lateral roots and the seedling vigour index. The delay effect of growth process is very pronounced in the case of high concentration.

Introduction

The heavy metals come from natural sources and from anthropogenic sources. In certain concentration, the heavy metals become pollutants with toxic potentially; they persist a long time in the environment because of their reduced mobility and can transferred along food chains. Environmental pollution with heavy metals presents a risk for plants, animals and for man. Some heavy metals (zinc, copper, etc) are important for the life of plants; other heavy metals (lead, cadmium, etc) do not have a biological role.

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At low concentration, zinc is a essential element for the life of plants. It is involved in many biochemical processes (in the metabolism of the carbohydrates, proteins, lipides; in the synthesis of the acid nucleic; stimulates the tryptophan synthesis – a precursor of auxin) and physiological processes (in the process of photosynthesis; in the process of germination, in the maintain the turgor of tissues, etc)(Caramete et al., 1974; Băjescu et Chiriac, 1984; Davidescu et al., 1988; Burzo I., et al., 1999; Rout and Das, 2003; Tsonev and Lidon 2012); it is involved in the maintaining the structural integrity and in the control of biomembranes permeability, in the cell protection against damage caused by reactive oxygen species (Cackmak, 2000). It is the only metal represented in the six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases) (Jackson et al., 1990; Broadley et al., 2007). In high concentrations it can be toxic (Rout and Das, 2003; Malecka et al., 2012).

Zinc behavior in soil and its availability for plants varies depending on the soil conditions, plant species, climate and on the agricultural practices (Dvorak et al., 2003).

The normal content of zinc in plants varies between 10 – 150 ppm (Mulligan et al., 2001) or between 25ppm - 150ppm (Malik et al., 2011); in concentrations of 400 ppm it becomes toxic (Mulligan et al., 2001). Depending on the tolerance of plant to zinc excess, the toxic level can be lower (Băjescu and Chiriac, 1984). According to Chaney and Marschner quoted by Broadley et al., (2007) and Malecka et al., (2012), symptoms of toxicity were usually visible when the concentration of zinc in the leaf was higher than 300mg/kg dry material, even though some cultivated plants present symptoms of toxicity in concentrations in the leaf lower than 100mg/kg dry material.

As manifestations of zinc toxicity, there are mentioned: the phenomenon of chlorosis, delay/inhibition of plant growth, reduction of chlorophyll synthesis, reducing the agricultural production (Woolhouse, 1983; Rout and Das, 2003; Broadley et al., 2007, Krusdar 2004; Yadav, 2010); deficiences of some elements (Băjescu and Chiriac, 1984; Yadav, 2010, Vassilev et al., 2011); reducing the rate of the photosynthesis and the transpiration (Krusdar et al. 2004; Vasiliev et el. 2011).

The toxicity of zinc varies greatly depending on many factors: plant species, plant age, metal concentration, time of exposure, interaction with other ions in the environment, nutrients composition of the growing environment, etc.

Zinc toxicity is generally rare on lands unchanged by human activity (except lands with geological anomalies) (Băjescu and Chiriac 1984). The toxicity was reported on acid soils (Woolhouse 1983; Băjescu and Chiriac 1984); on polluted soils with waste coming from the human activity (mines, industry, agriculture –

overuse of fertilizers and pesticides that contain zinc; burning fossil fuels, etc.) (Broadley et al., 2007; Yadav, 2010, Vassilev et al., 2011).

Cucumis melo (melon) is a annual herbaceous species, known before our era (Pârvu, 2004). The fruit is appreciated for its flavor and for its succulence; it is used in nutrition and in human traditional medicine (the fruit has some properties: aperitif, refreshing, diuretics, laxatives, tissues regeneration; the seeds has expectorant properties) (Pârvu, 2004; Bojor and Perianu, 2005).

Growing and development *Cucumis melo* species on land polluted with heavy metals due to the presence in the vicinity of industrial units or due to excessive application of pesticides and/or fertilizers containing zinc can cause toxicities of this plant.

Under controlled conditions of laboratory, a method often used to detect the toxicity of heavy metals toxicity is to investigate of the effect of aqueous solutions of their salts on seed germination and seedlings growth in different test species.

The influence of some metals (aluminium) and heavy metals (cadmium and crom) on seed germination, plantlet growth and development of *Cucumis melo* species were presented by Symeonidis et. al., (2004), Venkateshwarlu, (2010), Akinci and Akinci (2010).

This paper wants to investigate the effect of the zinc treatment of different concentrations on the germination and growth in the first ontogenetic stages in the species *Cucumis melo* L. (Cucurbitaceae family).

1. Materials and Methods

As a biological material, we used *Cucumis melo* L. seeds (Roxana cultivar) obtained from seed retailers (S. C. Unisem S. A). Eight experimental variants have been created: a control variant (with distilled water) and seven variants of zinc treatments.

Zinc was used as sulphate solutions ($Zn\ SO_4 \times 7\ H_2O$). At experiment we used following zinc concentrations: 50 mg/l; 100 mg/l; 200 mg/l; 300mg/l; 400 mg/l; 500mg/l; 600 mg/l. In selecting concentrations used for experiment we considered the zinc critical concentration in soil (400 mg / l) (according to Alloway, 1990; Beckett and Davis 1979, quoted by www.cprm.gov.br/).

The seeds were disinfected for 10 minutes with 2 % hydrogen peroxide and then washed several times with distilled water.

The seeds were placed to germinate in Petri dishes on a filter paper humidified with distilled water (in control variant) and with zinc sulphate solutions (in treatment variants). The plates were kept at room temperature (23/20^{o}C), a photoperiod corresponding to the month of May, 2014.

The initial volume of distilled water or sulphate of zinc solutions (at placing the seeds) was of 5 ml. During the experiment, germination substrate was wetted with distilled water (in control variant) and with zinc sulphate solutions (in treatment variants). For each variant, three replicas were used (each with 15 seeds).

The following indicators have been analyzed:

- the percentage of germinated seeds at eight day after the beginning of the experiment, that it was calculated by following formula: (number of germinated seeds/total number of seeds) x 100;
- the length of the root, the length of the hypocotyls, the length of the seedling and the number of lateral roots (at ten day after the beginning of the experiment);
- the tolerance index; the seedling vigor index.

The tolerance index of heavy metals (TI) was calculated by formula described by Hakmaoui et al., (2007): (root length under metal treatment/ root length in control) x 100. The seedling vigor index (SVI) was calculated by formula described by Moradi et al., (2008): [germination percentage x seedlings length (cm)].

For each experimental variant, the measurements were performed at 30 seedlings. All the results presented in figures were expressed as mean values ± standard error (for germination indices $n = 3$; for the length of the root, the length of the hypocotyls, the length of the seedling and the number of laterale roots indices $n = 30$). The data obtained from germinated seeds percentage, from morphological indicators (length of the root, length of the hypocotyls, length of the seedling; the number of lateral roots) were statistically interpreted. It was used the unifactorial Anova test; in order to test the difference between averages, it was used the Tukey test ($\alpha = 0.05$) (Zamfirescu and Zamfirescu 2008; Microsoft Excel program).

2. Results and discutions

The seed is the only stage in the life cycle of plants well protected against the stress caused by various factors; soon after imbibition and subsequent growth process they become sensitive to stress (Li et al., 2005).

2.1 Effect of zinc on the germination

In *Cucumis melo* germination is epigenous. Eight days after the beginning of the experiment, the percentage of germination in the control registered an average value of 73.33%, and in the variants of treatment, average values between 73.33 % and 86. 88% (Fig. 1). A tendency to stimulate the germination with values between 6.05 % and 18.18 % was recorded in the case of the concentration between 50mg/l and 400mg/l; the effect of stimulation was more obvious in the case of concentrations of 100mg/l and 200mg/l.

The concentrations used for the treatment do not influence significantly the seed germination ($p > 0.05$). The seeds of *Cucumis melo* are able to germinate in the presence of some moderate and high concentrations of zinc in substrate. We consider that the results were due to structural peculiarities of seeds and other factors. In the species *Cucumis melo* the cells from the seed supermidermal layers of the seed coat present the tickened and lignified walls (Zanoschi and Toma, 1985).

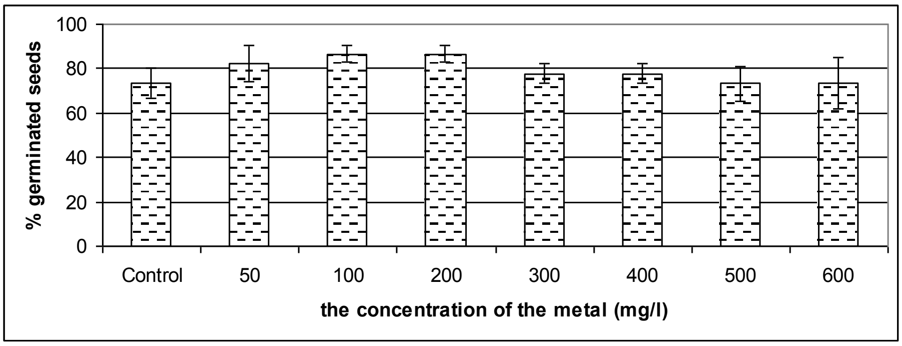

Fig.1. The percentage of germinated seeds at eight day after the beginning of the experiment

According to Seregin and Kozhevnikova, (2005), seed germination is the most resistant process to heavy metals; this resistance was due to weak penetration through the seed coat of heavy metals. The seed coat can be a barrier between the embryo and the environment in the immediate vecinity (Araùjo and Monteiro, 2005); it protects the embryo against the heavy metals toxicity, isolated embryo were more sensitive to heavy metals than seeds intact (Li et al., 2005).

Research carried out by Seregin and Kozhevnikova (2005) indicated the following aspects: seed coat is not a universal barrier that limits the penetration of heavy metals inside the seed. The effects of heavy metals on germination are based on their ability to reach the tissues of the embryo passing through the physiological barriers represented mainly by the seed coat. This ability is determined by several factors: the structure of the seed coat that varies depending on the species; the diameter of the hydrated ions and other physical and chemical properties of the metal ions.

Similar effects relating to the germination were recorded in the species *Salvia coccinea* in the case of using some identical concentrations of zinc for the treatment of seeds (Stratu et al., 2014). The effects of stimulation of the germination in the case of treatment with zinc in lower concentrations were reported in other cultivated species: *Cicer arietinum* (after 72 hours) in concentrations of 10 mM and 25 mM (Sharma et al., 2010); *Cicer arietinum, Macrotyloma uniflorum, Vigna radiata, Vigna unguiculata* in the case of concentrations of 10, 20, 50 ppm (Shivakumar and Thippeswamy, 2012).

In some cultivated species the treatment with zinc reduces the seed germination: in *Phaseolus vulgaris* (in concentration of 30 ppm and 70 ppm) (Çavuşoğlu et al., 2009); in *Lycopersicon esculentum* (in concentration of 200ppm, 300ppm, 400ppm, 500ppm, 600ppm) (Ashagre et al., 2013). In *Cicer arietinum* var. pusa-256 the treatment with solutions of zinc sulphate in concentration of 75 mM and 100 mM reduces significantly the germination of the seeds, after 72 hours (Sharma et al., 2010).

2.2 The effect of zinc on seedlings growth

Ten days after having started the experiment, the length of the root, the length of the hypocotyl and the length of the seedling in the variants of treatment, presented average values lower than in the control, values decreasing with the increase of the concentration of the metal (Fig. 2). The applied treatment had a negative influence on the seedlings growth. The delay of the growth in length comparing with the control was more pronounced in the case of the root (by 25.75 % - 85.75 %) than the hypocotyl (by 5.05 % - 72.71 %). The rate of reduction of the growth compared to the control exceeds the value of 50 % in concentrations between 200mg/l and 600mg/l in the case of the root and in concentration between 300mg/ and 600mg/l in the case of the hypocotyl and the seedling (Fig. 3).

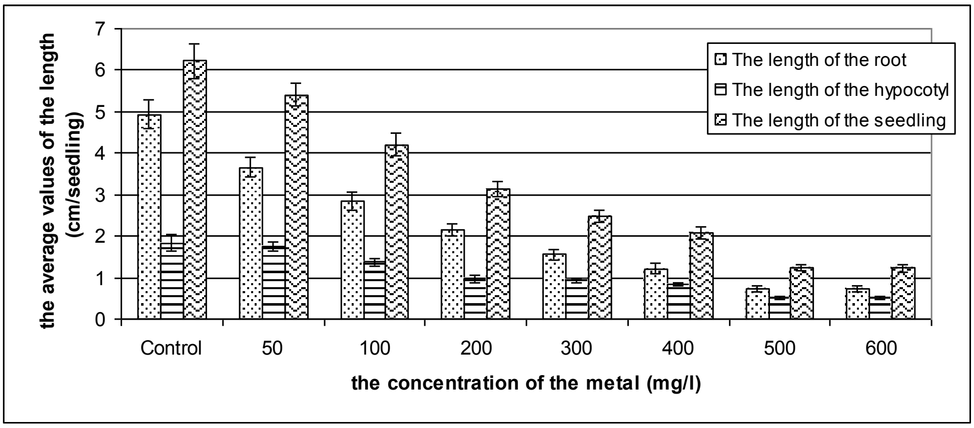

Fig. 2 The values of the morphological indicators at ten days after the beginning of the experiment

From the statistic point of view, the negative influence is significant ($p < 0.05$). The results of the Tukey Test indicate the fact that: the control differs significantly from all the variants (except for the concentration of 50mg/l) for the length of the root, the hypocotyl and the seedling.

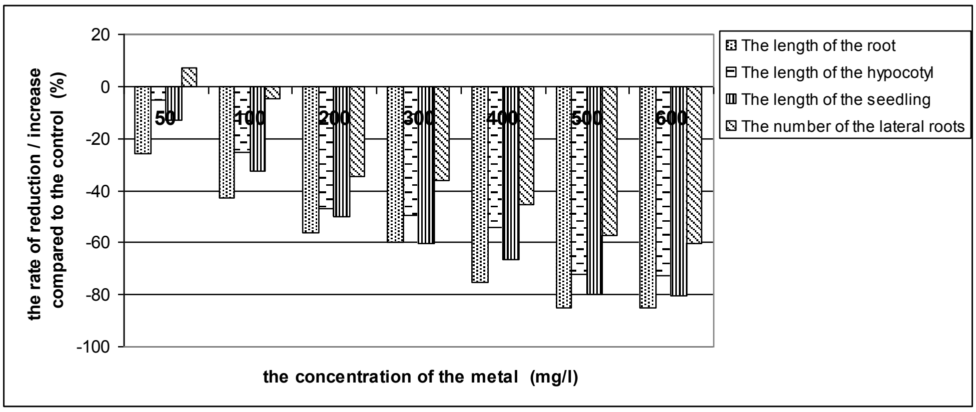

Fig. 3. The rate of reduction compared to the control of the morphological indicators

In the literature it is mentioned that the root growth is more sensitive to heavy metals toxicity than the shoot growth (Araujo and Monteiro, 2005); the root is the first organ to come into contact with heavy metal ions in the substrate and is likely the first organ which will present the toxicity symptoms of the heavy metals (Meyer et al., 2010).

Our results obtained in this paper confirm some data in the available literature referring to the effect of zinc on the growth of the seedlings/plants. In the species *Salvia coccinea* the following effects were observed seven days after the beginning of the experiment: significant delay of the root growth at concentrations between 100 mg/l and 300mg/l; the inhibitory effect at concentrations between 400mg/l and 800mg/l (Stratu et al., 2014).

An inhibiting effect for the growth of the root/hypocotyl caused by high concentrations of zinc sulphate (40 mM) were noticed by Nag et al, (1989) in the seedlings of *Vigna radiata.* The delay of growth in length for the root and the root/ the hypocotyls was also reported by other authors: Çavuşoğlu K. et al., (2009) in the species *Phaseolus vulgaris*; Sharma et al., (2010) in the species *Cicer arietinum*; Ashagre et al., (2013) in the species *Lycopersicon esculentum* cultivar Roma VF. In *Arabidopsis thaliana*, the seedlings growth was inhibited in lower concentration of zinc in solution (Li et al., 2005). Also, the treatment with zinc in concentration of 100 – 400 μg/g (soil dry mass) determined the significant decrease in root and shoot growth parameters at different stages of development in *Artemisia annua* plants (Khudsar et al., 2004).

The number of lateral roots/seedling showed lower values compared to the control with one exception (in concentration of 50mg/l) (Fig. 4). The significant reduction ($p < 0.05$) in the number of lateral roots was found at concentrations between 200mg/l and 600 mg/l; for this concentrations, the rate of reduction compared to the control showed values between 34.54 % and 60.46 % (Fig. 3).

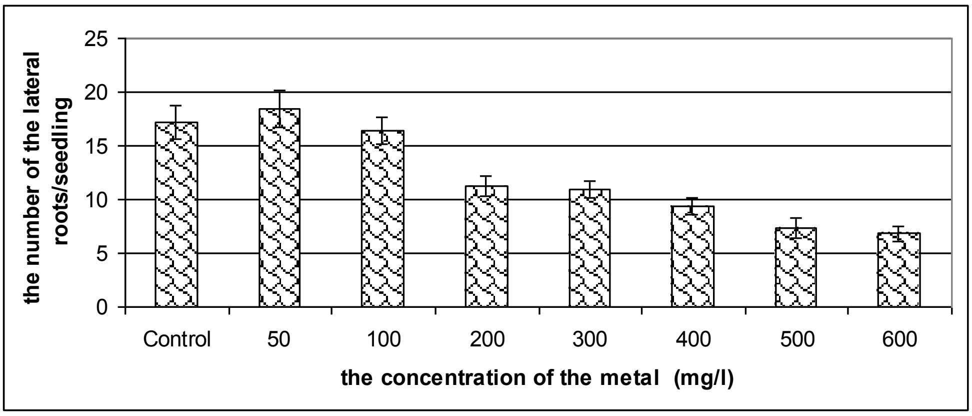

Fig. 4. The number of the lateral roots

Also, our macroscopic observations underlined the following aspects:

- in concentrations between 200mg/l and 600mg/l the root "avoids" the toxic conditions of stress, respectively the contact with the filter paper soaked with solution of zinc; seedlings and germinated seeds are placed so that the contact with the substrate is achieved by the cotyledonary leaves/seed coats (that cover partially or totally the cotyledons) and few lateral roots; this phenomenon is very pronounced for concentrations of 500mg/l and 600 mg/l; the area at the tip of the root is affected and presents a brown colour; it is affected the formation and growth process of the lateral roots;
- in concentrations of 500mg/l and 600mg/l, the area at the tip of the root is atrophied and the formation and growth process of the lateral roots is profoundly affected.

The effect of delay /inhibition of the root growth and the seedlings growth in *Cucumis melo* is probably due to the oxidative stress conditions created by the high concentration used in the treatment and the influence of zinc on the cell division. The stress condition can affect the functions of the root and the physiological processes of the seedling. Yadav (2010) indicates that the exposure of the plants to high levels of heavy metals has as primary response the generation of reactive oxygen species (ROS). According to Rout and Das (2003), the process of plants growth depends on the cell division. The cell division and the elongation are the stages by which it is achieved the cell growth (Burzo et al., 1999).

As effects of the exposure to the zinc excess, there are mentioned: the reduction of the biomass and inhibition of the cell division and elongation

(Khudsar et al., 2004); the inhibition of the plant growth, the browning of the root (Vassilev el al., 2011). In sugarcane the excess of zinc negatively influenced the root growth, the mitotic efficiency, has induced the appearance of chromosomal aberrations and oxidative stress (Jain et al., 2010). According to Liu et al., (1996), zinc in concentrations between 10^{-4} and 10^{-2} mol/L inhibited the root growth in *Allium cepa*, reduced the mitotic index and induced chromosomal aberrations. In many species the toxicity of the zinc is associated with the inhibition of the growth of the root; the process of cell elongation is especially affected (Woolhouse, 1983).

2.3 The effect of zinc on tolerance index and seedling vigour index

Tolerance index (TI). The tolerance index based on the root growth gives an estimate of the effect of the heavy metals toxicity on the short run (Hakmaoui et al., 2007). In the species taken into account, TI presented values between 83.38% and 16.61%. TI decreases progressively with the increase of zinc concentration in solution (Fig. 5). TI values lower than 50 % were recorded at concentrations between 200 mg/l and 600mg/l.

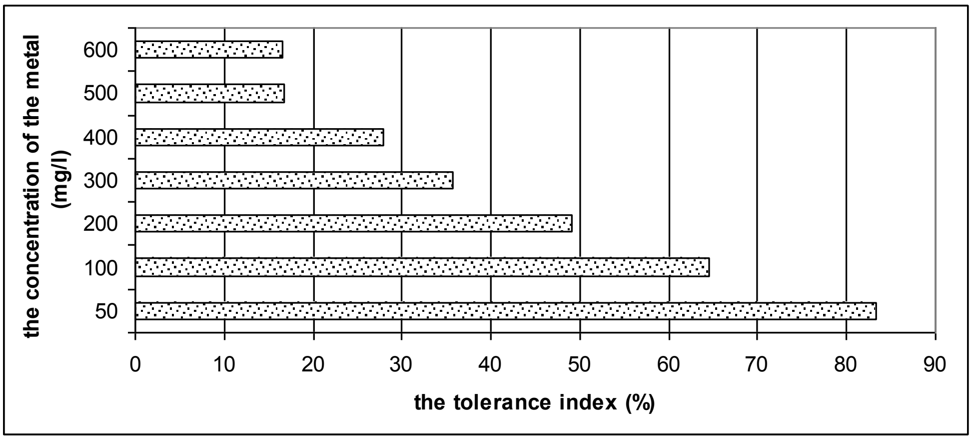

Fig. 5. The tolerance index

Cheng (2003) showed that the plants have their own mechanisms of heavy metals detoxification; these mechanisms are integrated and protect the plants against the negative effects of the heavy metals. Woolhouse (1983) describes four mechanisms involved in the plant tolerance to the stress caused by zinc: the immobilisation of zinc in cell walls; the compartment of zinc as soluble and insoluble complexes; the development of enzyme systems resistant to zinc.

The seedlings vigour index decreased progressively with the increase of zinc concentration in the solutions used in the treatment (Fig. 6). In concentrations between 200mg/l and 600mg/l the rate of reduction of the seedlings vigour index compared to the control exceeded the value of 40 %.

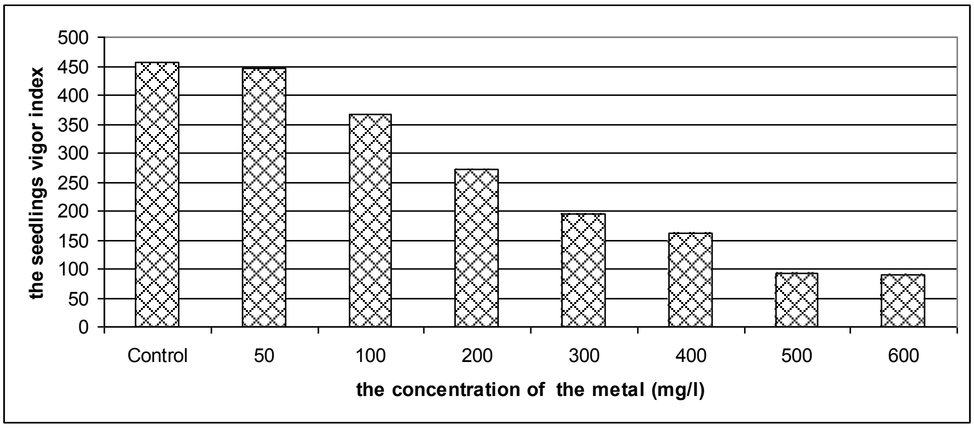

Fig. 6. The seedlings vigor index

The results obtained concerning the tolerance index and the seedlings vigour index are in agreement with those reported by Ashagre et al., (2013) in the species *Lycopersicon esculentum;* it was noticed the reduction of the tolerance index and the seedling vigour index with the increase of zinc concentration. The stress caused by heavy metals reduces the root vitality and affects the growth process (Cheng 2003).

The results obtained showed that the seedlings growth compared to the seed germination was more sensitive to the treatment with zinc in the concentrations used. This aspect was showed by other authors as well: Akinci and Akinci (2010) in *Cucumis melo* cv. Barada, in the case of the treatment with chromium; Li et al., (2005), in seedlings of *Arabidopsis thaliana* for some heavy metals (Hg $^{2+}$, Pb $^{2+}$, Cu $^{2+}$ and Zn $^{2+}$).

Conclusions

The seeds of *Cucumis* germinated in the presence of some moderate and high concentrations of zinc in substrate. The concentrations used for the treatment do not influence negatively the seed germination, but affect the seedlings growth (especially the root elongation), the formation and growth process of the lateral roots and the seedlings vigour.

The effect of delay of the growth process is very pronounced in the case of high concentrations.

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