THE INFLUENCE OF CERTAIN HEAVY METALS ON SEEDS GERMINATION OF *AGERATUM HOUSTONIANUM* MILL.

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Abstract

The paper presents the results of a study regarding the influence of treatment with lead nitrate and zinc sulfate on the seeds germination of *Ageratum houstonianum* .We analyzed the following indicators: the percentage of germinated seeds in different intervals (24 - 96 hours); the length of root and hypocotyl (at 96 hours). The results underline the specific variations of the analysed indicators, depending on the nature of metals and their concentrations used for the seed treatments. The treatments with lead nitrate (with concentration of 100m Λl; 300 mg⁄ l and 500 mg⁄ l) and zinc sulfate (with concentration of 400mg/l; 600mg Λl şi 800 mg⁄ l) delay the seeds germination and the growth of the root and hypocotyl. After 96 hours the beginning of the experiment, the percentage of germinated seeds has value between 82 % and 75,33 %, in the case of lead treatment and between 80,66 % and 66 %, in the case of zinc treatment. In the control variants, the percentage of germinated seeds has a value of 86,66 %. The delay effect on the hypocotyl and root length growth is very pronounced in the case of variant treated with high lead nitrate and zinc sulphate concentrations.

Key words: lead, zinc, germination, *Ageratum houstonianum*

This paper continues our research regarding the effect of the treatment with heavy metals done in the previous years for different species (*Medicago sativa, Raphanus sativus, Festuca partensis, Lens culinaris, Pisum sativum*) (Stratu A., et al., 2008; 2009; 2010; Olteanu Z., et al., 2008).

Among the heavy metals, we chose for the study the lead and the zinc. According to Dou Z. X., (1988) quoted by Jiang W., et al., (2000), Pb does not play any physiological part for the plants, however, in small quantities it can stimulate growth in some plants. According to other authors (Burzo I., et al., 1999; Jiang W., et al., 2000; Matei S., et al., 2001; Tomulescu I. M., et al., 2004; Kiran Y., Şahin A., 2005) the toxic effects are associated with a delay in germination, disruption of mitosis, the inhibition of the enzymatic activity, the growth of the root in length, the induction of chlorosis, the reduction of the intensity of photosynthesis and respiration. Pb inhibits the synthesis of chlorophyll pigments and the activity of the enzymes in Calvin's cycle (Haider S., et al., 2006).

Zn is a component of enzymes: carboanhydrase, alcohol dehydrogenase, alkaline phosphatase, hexokinase; it stimulates the activity of certain enzymes (peroxidase, catalase, enolase, etc.), the synthesis of tryptophan – a precursor of auxin; it positively influences the process of photosynthesis, it regulates the metabolism of carbohydrates and proteins (Bouma D., 1983; Davidescu D., et al., 1988; Burzo I., et al., 1999); it plays a role in the stability of tertiary and quaternary structures, of the cytoplasm of ribosomes; it is involved in reducing nitrates (Davidescu D., et al., 1988). According to Aydinalp C., Marinova S. (2009), the zinc (in concentration of 5ppm, 10ppm, 20ppm, 40ppm) have positive effects on the growth of the seedlings in alfalfa. Sharma S., et al., (2010) noticed the fact that the zinc in concentration of 25mM increased seed germination, the growth in length of the root and of the hypocotyl, the chlorophyll content and the fresh weight. Although Zn is a microelement with important functions in the life of plants, it can be toxic in high concentrations; according to Herrero E.M., et al., (2003) and Ataci O., et al., (2005), (the authors quoted by Sharma S., et al., 2010) decreases the percentage of germinated seeds .

This paper has as a purpose to investigate the effect of the treatment with lead and zinc on the germination of the seeds of *Ageratum houstonianum* Mill. *Ageratum houstonianum* Mill (Asteraceae family) is a decorative species of small size, annual, frequently cultivated in the urban green areas.

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MATERIAL AND METHOD

As a biological material, we used seeds of *Ageratum houstonianum* Mill. Seven experimental variants have been created: a control variant (with distilled water) (the variant marked C) and six variants of heavy metal treatments. The heavy metals used were: zinc and lead.

Zinc used were as sulfate solutions in a concentration of 400 mg/l (the variant marked V1-z); 600 mg/l (the variant marked V2-z) and 800mg /l (the variant marked V3-z). Lead used were as nitrate solutions in a concentration of 100mg/l (the variant marked V1-p), 300mg / l (the variant marked V2-p) and 500 mg/l (the variant marked V3-p). In selecting the concentrations of heavy metals solutions we started from the critical concentration of lead and zinc in soil (100mg/l for lead; 400 mg/l for zinc) (accordind to Alloway B. J. 1990; Beckett P. H. T., Davis R.D., 1979, quoted by www.cprm.gov.br/). The seeds were placed to germinate in Petri dishes, on a filter paper humidified with distilled water (a control variant) and heavy metals solutions (a treatment variants), in laboratory conditions. For each variant, three replicas were used, each with 50 seeds.

The following indicators have been analyzed: the percentage of germinated seeds in different intervals (24 - 96 hours); the length of the root and hypocotyl (at 96 hours after the beginning of the experiment). All the results presented in tables were expressed as mean value ± SD.

The data obtained from the length of the root and of hypocotyl were interpreted statistically. It was used the unifactorial Anova test and in order to test the difference between averages the Tukey test was used (Zamfirescu Şt. and Zamfirescu O., 2008; Microsoft Excel program).

RESULTS AND DISCUSSIONS

The germination of seeds. At *Ageratum houstonianum* germination is epigenous. 24 hours after assembling the experiment, we did not notice germinated seeds. 48 hours after assembling the experiment, the percentage of germination has values between 61.32 – 63.32 % in the case of treatment with lead and between 7.33 – 40% in the case of the treatment with zinc. For the control variant, the percentage of germination registered an average value of 68.66 % (*tab. 1, 2*).

During the period analysed, the percentage of germinated seeds grows progressively; at the end of the experiment, it has values ranging between 75.33 % - 82% for the variants treated with zinc sulphate and between 80, 66 % - 66 % for the variants treated with nitrate of lead. For the control variant, the percentage of germinated seeds presented an average value superior (86.66%) to the variants of treatment (*tab. 1, 2*).

For the variants of treatment with heavy metals, the percentage of germination decreases with the increase of concentration of the solution used for the treatment. This fact makes us state that the treatment with zinc and lead in the concentrations used presents a tendency of delay of germination for the species studied. The lead of concentrations 100 mg/l, 300 mg/l and 500 mg/l decreases the percentage of germination by 5.38 %, 8.46 % and 13.08 % respectively. The zinc of concentrations 400 mg/l, 600 mg/l şi 800 mg/l decreases the percentage of germination by 6.93 %, 20.78 % and 23.85 % respectively.

96 hours after assembling the experiment, the *length of the root* and of the hypocotyls in the control presented average values higher than the variants of treatment, situation valid for both metals used for the treatment (*tab. 3, 4*). We mention the fact that in the case of the treatment with sulphate of zinc of concentration 800 mg / l we have not done measurements regarding the length of the hypocotyls axis because for this concentration, the zinc inhibits the growth in length of the hypocotyls. The delay effect of growth in length of the root and hypocotyls, it is more prominent in the case of the treatment with zinc (*tab. 3, 4*).

The statistics of Anova unifactorial test (F calculated: 140.06 - length of root, treatment with lead; 474.51 - length of root, treatment with zinc; 23.62 - length of hypocotyl, treatment with lead; 252.15 - length of hypocotyl, treatment with zinc) was higher than the critical value (2.28 for length of root and hypocotyl, treatment with lead and length of root, treatment with zinc; 3.10 for length of hypocotyl, treatment with zinc); $p<0.001$. This fact indicates that the heavy metals solutions have a significant influence (unfavourably) on the growth in length of the root and of the hypocotyl. The results of the Tukey test indicates that: the control differs significantly of all the variants of treatment with heavy metals – for length of the root; control differs significant of the variants of treatment: V2-p, V3-p, V1-z şi V2-z - for length of the hypocotyl.

In the literature in the field (Lerda D., 1992; Goldbold D. L., Kettner C, 1991; Sharifah B. A., Hishashi O., 1992 - the authors quoted by Kabir M., et al., 2008) it is shown the fact that the delay of the growth in length of the root in the case of the treatment with heavy metals would be due to the reduction of the mitotic division in the meristematic zone of the root.

Table 1

The percentage of germinated seeds (% germinated seeds) – treatment with lead

The variant/The concentration of the solutions	24 hours	48 hours	72 hours	96 hours	% relative to control
C (0)	0	68.66 ± 7.02	82.66 ± 5.03	86.66 ± 1.44	100
V1-p (100 mg/l)	0	63.32 ± 6.11	78.66 ± 1.15	82 ± 4.61	94.62
V2-p (300 mg/l)	0	63.32 ± 6.11	76.66 ± 3.05	79.33 ± 3.05	91.54
V3-p (500 mg/l)	0	61.32 ± 4.16	71.33 ± 4.61	75.33 ± 8.32	86.92

(10^{-4}M and 10^{-5}M) inhibit the growth of the root in *Zea mays* and *Allium cepa*. According to Jiang W.,

Table 2

The percentage of germinated seeds (%germinated seeds) – treatment with zinc

The variant/The concentration of the solutions	24 hours	48 hours	72 hours	96 hours	% relative to control
C (0)	0	68.66 ± 7.02	82.66 ± 5.03	86.66 ± 1.44	100
V1-z (400 mg/l)	0	40 ± 2	76 ± 5.29	80.66 ± 4.16	93.07
V2-z (600 mg/l)	0	8.66 ± 4.16	52.66 ± 3.05	68.66 ± 4.16	79.22
V3-z (800 mg/l)	0	7.33 ± 0.67	46.66 ± 4.16	66 ± 5.29	76.15

et al., (2000), the concentration of 10^{-3}M $Pb(NO_3)_2$

Table 3

The lenght of the root and of the hypocotyl (mm) – treatment with lead

The variant/The concentration of the solutions	The length of the root	The length of the hypocotyl
C (0)	11.9 ± 2.48	5.79 ± 1
V1-p (100 mg/l)	7.76 ± 1.9	5.56 ± 0.77
V2-p (300 mg/l)	4.03 ± 1.27	5 ± 0.64
V3-p (500 mg/l)	3.83 ± 0.98	4.26 ± 0.52

Table 4

The lenght of the root and of the hypocotyl (mm) – treatment with zinc

The variant/The concentration of the solutions	The length of the root	The length of the hypocotyl
C (0)	11.9 ± 2.48	5.79 ± 1
V1-z (400 mg/l)	2.16 ± 0.37	3.03 ± 0.66
V2-z (600 mg/l)	1.36 ± 0.39	1.43 ± 0.50
V3-z (800 mg/l)	1.26 ± 0.44	-

In the opinion of some authors (Muhammad S., et al., 2008), the delay of the growth in length of the seedlings would be due to the fact that the heavy metals affect the activity of the enzymes that hydrolyze the substances of reserve in the cotyledons and endosperms.

The results obtained by us confirm some of the data in the literature of specialty. The studies performed by Dou Z. X., (1998), Jiang W. S., Liu D. H., (1999) (authors quoted by Kiran Y., and Şahin A., (2005), show that low Pb concentrations mildly inhibits the root growth in seedlings of *Brassica juncea;* this plant has the important ability to take Pb from the solution and to accumulate it in its root.

Andro A. R., et al., (2010) noticed the fact that the treatment with nitrate of lead of different concentration (100 mg/l; 300 mg/l, 500 mg/l) delays the growth in length of the root and of the hypocotyls axis in *Mentha piperita L.*

Stratu A., et al., (2010) noticed the fact that the treatment with zinc sulphate, in a concentration of 0,0003 % and 0,03 % delays the

growth of the root in *Lens culinaris* (72 hours after the beginning of the experiment) and *Pisum sativum* (Ialomiţa 1 variety) (96 hours after the beginning of the experiment).

CONCLUSIONS

The treatments with lead nitrate and zinc sulfate in the concentrations used delay the seeds germination and the growth of the root and hypocotyl. The delay effect of germination is more prominent in the case of high concentrations (500mg/l for lead and 800 mg/l for zinc).

The delay effect of growth in length of the root and hypocotyls, it is more prominent in the case of the treatment with zinc.

REFERENCES

Aydinalp, C., Marinova, S., 2009 - *The effects of heavy metals on seed germination and plant growth of alfalfa plant (Medicago sativa)*, Bulgarian Journal of Agricultural Science, vol. 15 (4), p. 347-350.

Andro, A. R., Pădurariu, C., Zamfirache, M. M., Ivănescu, L., Galeş, R., 2010 - *Lead effects upon the germination and development oh Mentha piperita L., seedlings,* Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii, t. 26 (1), p. 25 - 28.

Bouma, D., 1983 - *Diagnosis of Mineral deficiencies using plant tests,* În Encyclopedia of Plant Physiology. New Series , vol. 15 A - Inorganic Plant nutrition (ed. by A. Lauchli, R. L. Bieleski). Springer- Berlin- Heidelberg, New York, Tokio, p. 120 -141.

Burzo, I., Toma, S., Crăciun, C., Voican, V., Dobrescu, A., Delian E., 1999 - *Fiziologia plantelor de cultura,* Vol. I, Intreprinderea Editorial – Poligrafica, Ştiinţa Chişinău.

Davidescu, D., Davidescu, V., Lăcătuşu, L., 1988 – *Microelementele în agricultură,* Ed. Academiei Române, Bucureşti

Haider, S., Kanwal, S., Uddin, F., Azmat, R., 2006 - *Phytotoxicity of Pb: II. Change in chlorophyll absorbtion spectrum due to toxic metal Pb stress on Phaseolus mungo and Lens culinaris,* Pakistan Journal of Biological Sciences, vol. 9 (11), p. 2062 - 2068.

Jiang, W., Liu, D., Hou, W., 2000 - *Hyperaccumulation of lead by roots, hypocotyls, and shoots of Brassica juncea,* Biologia plantarum, vol. 43 (4), p. 603 - 606.

Kabir, M., Iqbal, M. Z., Shafiq, M., Farooqi, S. R., 2008 - *Reduction in germination and seedling growth of Thespesia populnea L., caused by lead and cadmium treatments*, Pakistan Journal of Botany, vol. 40 (6), p. 2419 - 2426.

Kiran, Y., Şahin, A., 2005 - *The effects of the lead on the seed germination, root growth, and root tip cell mitotic divisons of Lens culinaris Medik*, G.U. Journal of Science, vol.18 (1), p.17 - 25 .

Matei, S., Matei, G. M., Lăcătuşu, R., 2001 – *Histomorphological modifications of maize, lettuce and soybean plants cultivate on heavy metals polluted soils*, Lucrările cele de-a XVI – a Conferinţe naţionale pentru ştiinţa solului, vol. 30 C, p. 188 -198.

Muhammad, S., Iqbal, M. Z., Mohammad, A., 2008 – *Effect of lead and cadmium on germination and seedling growth of Leucaena leucocephala,* J. Appl. Sci. Environ. Manage., vol. 12 (2), p. 61 – 66.

Olteanu, Z., Stratu, A., Murariu, A, Costică, N., 2008 - *The influence of some heavy metals on Medicago sativa seed germination and seedling growth*. An. Şt. Univ. "Al. I. Cuza" Iaşi, seria Genetică şi Biologie moleculară, vol. IX, p. 55 – 63.

Sharma, S., Sharma, P., Datta, S. P., Gupta, P., 2010 – *Morphological and biochemical response of Cicer arietinum L. var. pusa-256 towards an excess of zinc concentration*, Life Science Journal, vol. 7 (1), p. 95 – 98 (http://www.sciencepub.net)

Stratu, A., Frenţoaie, L., Murariu, A., 2008 - *The influence of some heavy metals on seed germination and seedling growth at Raphanus sativus* L., Lucrări ştiinţifice Univ. Agron. Med. Vet. "Ion Ionescu de la Brad"Iaşi, ser. Horticultură, vol. 51, p. 185 - 191

Stratu, A., Olteanu, Z., Murariu, A., Costică, N., 2009 - *The influence of some heavy metals on seed germination and seedling growth at Festuca pratensis.* An. Şt. Univ. "Al. I. Cuza" Iaşi, seria Biologie vegetală, vol. LV, fasc. 1, p. 82 - 86.

Stratu, A., Lupuleac, R., Costică, N., Murariu, A., 2010 - *The influence of certain heavy metals on seeds germination at Lens culinaris Medik and Pisum sativum L.*, Analele ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, tomul LVI, fasc. 2, s. II a. Biologie vegetală, p. 25 - 30

Tomulescu, I. M., Radoviciu, E. M., Merca, V. V., Tuduce, A. D., 2004 - *Effect of copper, zinc and lead and their combinations on the germination capacity of two cereals*. Journal of Agricultural Sciences, Debrecen, vol. 15, p. 39 - 42.

Zamfirescu, Şt. Zamfirescu, O., **2008** - *Elemente de statistică aplicate în ecologie*, Ed. Universităţii „Alexandru Ioan Cuza", Iaşi.

www.cprm.gov.br/pgagem/Manuscripts/jeliazkova.htm