

**TESTING CRITERIA FOR ZINC TOLERANCE AND
HIPERACCUMULATION COMPARISON IN *PHASEOLUS
VULGARIS* PLANTS**

**METODE DE TESTARE A POTENTIALULUI DE
HIPERACUMULARE SI TOLERANTA A ZINCULUI LA
PLANTELE DE *PHASEOLUS VULGARIS***

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In this study is tested the variability and heavy metal (zinc) hiperaccumulation potential in Phaseolus vulgaris plants, as a modality for the reduction of the danger represented by zinc, through the reduction of the heavy metal mobility and biodisponibility in soil through phototherapy. There were effectuated studies on the plants treated with increasing Zn concentrations, confronted with visual symptoms, biomass production, and heavy metal concentration in plant's organs. Tolerance for increased zinc concentrations is quantified with the help of three parameters as are biomass (aerial parts and roots), main root length, and the influence of the zinc quantity from treatment solution on the accumulation of some micro and macroelements in plant.

Key words: zinc, *Phaseolus vulgaris*, biomonitoring, soil, hiperaccumulation potential

Introduction

Plants in there developing process were adapted to certain conditions of life, there spreading on Earth is in the first place determinate by there requests on external factors and by the degree of adaptation at environmental conditions. Plants are not growing anywhere, there are grouped in ecological communities well define (forests, swamps *etc.*) determinate by climatically and edaphically factors, interns and externs factors, the last ones having as results the regrouping of the plants. If in the course of growing and developing, plants have some bad conditions, it will suffer changes on biochemical structure and even that can dun pests and diseases [12]. Plants are managing to adapt on the changed environment because of the:

- Changes on enzymatic system;
- biosyntheses of anticorps;
- biosyntheses of activators and inhibitors;
- Accumulation of active osmotic substances.[1]

Morphological and anatomical adaptability of environmental factors was observed and studied on time. Heavy metal used on industry and agriculture is

generated in industrial processes and as burning result it represents an extreme danger in the next cases: it can maintain much time on the environment; it can be transported at distance from the source; it can lay on alive tissues where it goes to food, water and beddable air; has a large spectrum of toxic action.[5,6]

From contaminated soil, these substances reach to fruits, vegetables, and the green that is eat be animals and then on food chain by the humans. Heavy metals have direct impact on the vegetation. Because of the fact that it doesn't act selective, heavy metals reach on plants from fields without exception, affecting all flora and animals from that area. [2,10]. The permanence of heavy metals in vegetal products (levees, vegetal *etc.*) that in many parts can be explained by the norms of using pesticides and demonstration that some plants has the capacity to absorb and keep for a while in tissues the chemical toxic compounds. Heavy metals, once reach the environment it disperse and participate to different transformations through oxidative, reduction reactions. The processes are with energy consume from the sun. [4] Plants emerge energy at herbivore animals, that are eaten than be the carnivores animals. Each steep in the alimentary chain went to rests that are also food for the organism that decompose.[16,11]. Absorption mechanisms of metals in bioaccumulation plants and the base of specificity for absorbed metal by plants non accumulative and the one accumulative:

- absorption bigger of the metal in roots together with bigger rates of translocation of the metal in roots and aerial parts;
- accumulation of metals in roots, involving the allocation of roots biomass to the regions rich in heavy metals and a roots system more developed as the aerial part of the plant, that favors reaching of the metal and there absorption. [1,3,13]

Next to chemical, biological and physical processes is necessary the study of concentration contamination effect at all biological levels. [8]

Zinc has an essential role on plants nutrition, is involved in protein metabolism, the synthesis of ribonucleic acids, glucoses, vitamins, C, P and on chlorophyll forming; in the activity of different enzymatic systems, zinc is a compound of the dehydrogenates, pretenses and peptidases; in the synthesis of phitohormones of growth; influencing germination maintaining tissue turgescence, resistance to cold and diseases; in animal and plant nutrition. In 1869, Paulin evident the action favorable to cultures of *Aspergillus niger* and in 1909 Sommer and Lipman shows that this oligoelement is very important in superior plant nutrition. Zinc is involved in metals enzymes and even biosynthesis of DNA și RNA and of proteins rich in S , at plants microorganisms and animals. Zinc is essential for gases changes and breathable gases. Many enzymes are activated by zinc, and has a role of cofactor in enzymatic systems.[7] High levels of zinc in soil can determine excessive accumulations of zinc at plants with different negative effects on the absorption and using some elements in nutrition [14,15]. In rapport with other heavy metals, zinc is less toxic for plants in the next order about toxicity: **Cu > Ni > Co > Zn > Mn**. Toxicity of zinc is on acid soils, where is favorite the accessibility of zinc. The critical level of zinc in soil where it became

toxic is different from a soil to another, determinate by accessibility of zinc and plants tolerance at the excess [9, 21]. Zinc, unlike copper is easier to absorbed by the root systems and translocated in aerial parts of the plant determinates visual toxicity symptoms. It are generally unspecified the same as to the Fe, Mn and P deficiency. [17, 23] Concentrations in plants which zinc toxicity is present is between 300 and 500 ppm. In rapport with zinc plant tolerance the toxic level can be reduced. [6,22] Plants can be adapted at higher concentrations of zinc in tissues, concentrations that can get to over twenty times more than the normal level, without the plants being affected. [19,20]

Materials and Methods

Experiment accomplish *Phaseolus vulgaris* was tested like “sentinel species” for heavy metal nickel, because is well know that nettle is a plant with selectivity for this metal. [34]. The experiment evaluates soil quality using an active biomonitoring method, using *Phaseolus vulgaris* plants treated with zinc chloride, at different concentration, chosen to not pollute soil use [33]. *Phaseolus vulgaris* plants were planted in 18 pots (six pots for every concentration of wetting solution) witch contains 800 g soil and were treated with a solution containing metallic ion Zn^{2+} with different concentration, and six pots were wet with these solutions. This experience was during May – Julie 2006, plants have moderate needs for heat. Plants growing and development is done at the same temperature and light conditions. After seeding, plants were daily treated with 250 ml solution containing Zn^{2+} . After a few days from emergence nettle plants have started their development, having almost the same dimensions and color, indifferent by Zn^{2+} concentration of solution used to wet the plants.[35] Verifying that soil quality is influenced by zinc chloride introduction, this being the premise that soils with bad quality induces a significant reduction in root length comparing with a good soil quality. For this purpose it was measured the roots length of every plant and the average roots length for every level for every treatment. They have compared the average roots length for every concentration used with the untreated variant. Results are evaluated using standards. [25,30].

Heavy metal water soluble in nitromuriatic acid from soil extraction. For heavy metal determination we weighed approximately 1.5 g from soil sample with an 0.0001g exaction in a 100 ml reaction vessel. It wetting approximately 0.5- 1.0 ml water and it addition under mixture, 10 ml hydrochloric acid, then 5 ml nitric acid drop by drop if is necessary for reducing foaming. For 16 ours is left at room temperature for easy oxidation of organic part of soil, after this time it boils till drying. Nitromuriatic acid extraction must be realized under a well-ventilated hoot. Is important to add boiling moderator granules, into control and the other samples, especially to avoid violent boiling and solution loss. After cooling reaction vessels, in soil samples add distillate water, filtering by filter paper and there are react with distillate water at 50 ml. Solutions obtained are prepared for determine zinc, iron, copper and molybdenum. For soil samples that contain more than 20% (m/m)

organic carbon it must be treated with an extra quantity of nitric acid. Nitromuriatic acid is not totally dissolvent for the most of soils; the efficiency of extraction is different from a metal to another and is influenced by matrix compound. Metals extracted in nitromuriatic acid can't be considered total fractions, but also can't be consider bioaccessible fractions, because the extraction process is to power for representing a biological process [25, 31].

Vegetal material preparation. Plants samples analyses for microelements and heavy metal determination is based to spectrometry measurement of atomic absorption at one element concentration in nitromuriatic acid extract. The plants were identified in the department of botany. Equipment used was compound for atomic absorption spectrophotometer-VARIAN SpectrAA 1100 and hydrure system VARIAN DGA 77. Mineralization: 5 grams mortared plants and sifting are introduced in a porcelain capsule. It adds 15 ml acids mixture (HNO_3 : HClO_4 : H_2SO_4 2:1:0.2), and then is boiled till evaporation. Operation repeating till the residue has white-yellow color. The capsule let cooling at room temperature than there are react with hydrochloric acid (HCl 0.5 n) at 50 ml [32].

Microelements and heavy metals from plants determination. From plant samples it was determined heavy metal and microelements (iron, magnesium, calcium) content using an atomic absorption spectrophotometer. At every sample set there was a control sample for the reactive we used. For every determinate element it marks a calibration curve, after it determine the proper element from analyzed samples. Determination method of microelements at atomic absorption spectrophotometer is respecting ISO 11466 [27, 28, 29].

Phosphorus content determination. Sample preparation: A part of vegetal ashes analytical weighed is wet with distillate water then add 40 ml HCl , it puts into a water bath and after 30 minutes it filters. The obtained solution is reacting at 100 ml. It takes 15 ml from solution, it put into a balloon of 100 ml adding 2.5 ml NH_4OH , 1 ml $(\text{NH}_4)_2\text{MoO}_4$ and 0.25 ml SnCl_2 . It let rest until appears a blue coloration. Work mode: for determine phosphorus it marks a standard curve like in the next example: 0.1199 g KH_2PO_4 is react to 250 ml. From the solution it takes 10 ml witch react to 250 ml. From obtained solution it takes in 100 ml balloons 1, 2, 3...9ml treated like analyzed sample with molybdenum chloride and stannous chloride, after 15 minutes it colors. The solution from balloons will have concentrations between 0.01 and 0.09 mg P_2O_5 at 100 ml. Results: there are reading samples extinction, and from standard curve is read concentration levels of samples. All determine values are obtained by comparing to 100 g drying substance. [26]

Results and Discussions

The experiment evaluate soil quality using an active method of biomonitoring of zinc effect on *Phaseolus vulgaris* plant's growth and development into a soil after the next characteristics: reaction is slightly acid to neutral, $\text{pH} = 6.6$; the total of changeable bases $\text{SB} = 20.6\text{me}/100\text{g sol}$; total

capacity of cationic exchange T = 21.4 me/100g sol; changeable acidity SH =0.8 me/100g sol; saturation degree in bases V = 96.3.

Table 1.

Physic and chemical composition of the soil from the experiment

CaCO ₃ %	Sand (2.0-0.2 mm)%	sand (0.2- 0.02 mm)%	sand(0.02- 0.002 mm)%	chemical clay,0.002 mm%	phisycal clay, 0.01 mm%	Humus %	P ppm	P ppm determ.	K ppm	N total %
-	0.7	42.5	22.3	24.5	47.2	4.03	84.6	79.02	127	0.56

Table 2.

Heavy metal content of the soil from the experiment

No.crt.	Cd ppm	Cu ppm	Zn ppm	Ni ppm	Pb ppm	Co ppm	Cr ppm
sample1	0	18.555	60.7	28.845	18.86	7.72	0.8688

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Table 3.

Characteristics of *Phaseolus vulgaris* plants tested as vegetal bioindicators for zinc

Concentration g/l	Nr. plants	Volume of the solution ml	Length, cm			Final number of levees	Biomass quantity, mg	Length of root, mm	Elongation rate mm/day
			1 day	40 days	90 days				
water	1	90 x 250	1.2	5	5	5	4.90	3.2	0.00352
	2		1.3	5.5	5.8	5	4.89	3.2	
	3		1.2	4	4	5	4.72	3.1	
	4		1.8	7	7.2	7	4.78	3.2	
1g/l Zn ²⁺	1		1.4	3	5.9	5	5.09	5.4	0.04783
	2		2.0	5.5	7.1	8	5.10	4.4	
	3		1.7	4	5.2	7	5.88	4.5	
	4		1.9	5	5.7	7	5.15	4.5	
	5		1.5	4.5	5.4	7	5.75	5.4	
	5		1.5	4	5.3	7	5.81	4.5	
2g/l Zn ²⁺	1		1.4	10.1	15.9	9	5.81	5.7	0.0145
	1		1.0	10.5	15.1	9	7.10	5.5	
	3	1.8	10.3	16.9	9	5.07	5.7		
	4	3.0	17	17.4	10	5.95	5.5		
	5	1.7	10.1	17.3	10	5.10	5.5		
	5	1.1	9	15.5	9	5.11	5.5		

Phaseolus vulgaris plants had been planted in 25.05.2006 and the experiment was during 90 days. The characteristics of plants developing can be seen in the next table:

Elongation rate media has been calculated after Parker 's formula(1995):
Elongation rate (mm/day) = [(final length of the longest root – final length of the shortest length) media/ (time of Zn exposure)]. [18]

Medium normal quantity of zinc in soil is 100 ppm, and the initial quantity of zinc in 50.7 ppm, after testes the quantity grows proportionally with the zinc concentration in water solution at 151 ppm. *Phaseolus vulgaris* plants had accumulated Zn¹⁺ in roots in quantities between 30 ppm zinc at witness plants and 98 ppm zinc in plants treated with solution de zinc 1 g/l Zn¹⁺. In the same way varies the zinc quantities from aerial parts of *Phaseolus vulgaris* between 30 ppm 98 ppm zinc and levees between 17 ppm zinc 75 ppm zinc and flowers 15 ppm 50 ppm zinc.

Table 4.

Zinc content in root, stem, leaf and flower of *Phaseolus vulgaris* and from soil's plant

Plants species	Cconcentration from solution (g/l)	Zinc content (ppm)				
		Soil	Root % s.u.	Stem % s.u.	Leaf % s.u.	Flower % s.u.
<i>Phaseolus vulgaris</i>	Apã	50,7	30	30	17	15
	1	124	87	54	53	41
	1	141	98	98	75	50

The absorption increase quantity of zinc influenced the assimilation of chemical elements. At *Phaseolus vulgaris*, plants treated with solution that has different concentration of Zn¹⁺, it accumulation went to a Mg, Fe, and Cu concentration decrease on plants from the experiment so that the Fe quantity from *Phaseolus vulgaris* plants decrease from 22 mg in witness plants to 12 mg, at plants treated with high level of zinc, the concentration of magnesium decrease from 18 mg to 11 mg and copper quantity decreased from 10 mg to 7 mg and the same decrease can be seen at phosphorus 1.411 mg and 0.87 mg.

Table 5.

The content of chemical elements in *Phaseolus vulgaris* plants

Plants species	Treatment g/l Zn	C (mg % s.u.)	N (mg % s.u.)	P (mg % s.u.)	Fe (mg % s.u.)	Mg (mg % s.u.)	Cu (mg % s.u.)
<i>Phaseolus vulgaris</i>	0g/l	11.95	1.85	0.411	22	18	10
	1g/l	14.10	1.19	0.541	19	15	7
	1g/l	18.95	3.14	0.87	12	11	7

The quantities of C, N and P grow proportionally as the zinc concentration grow water solution. The high level of zinc in soil went to the accumulation of the metal in plants with negative effects on the absorption and on using the elements on nutrition. Zinc concentration had an important role on the accumulation of active substances on *Phaseolus vulgaris* plants.

Conclusions

The study reveals that *Phaseolus vulgaris* plants have a tolerance on zinc excess:

- Zinc has a positive effect on plants development;
- Soil quality is not influenced by the heavy metal;
- Zn^{1+} was proportionally accumulated with the increment in water in roots, aerial parts, leaves and flowers on experimental plants but remained in soil;
- Plants roots, in all tested cases had a heavy metal higher content reported with aerial parts;
- While metallic ions concentration was accumulated in big quantities on plants, others chemical elements had a smaller concentration, but in normal limits.
- Excessive concentration of zinc in plants are associated with the reduction of the others elements (Fe, Mn, Cu).

For these plants the light has influence on the entire vegetation period, but the most important period is on flowering, to favorite the accumulation of volatile oils. The content of volatile oils increase at light, but because the plants were growing in the same conditions of light, the variation of volatile oils quantity can be explained by the Zn concentration increase in water solution. Bioindicator plants are important in research about heavy metal pollution, this type of research been much more convenable than instrumental monitoring, but there are still many aspects to clarify there is necessary the research for many coherent methods of surveillance of environment and finding new measures for environmental protection and conservation of ecosystems biodiversity.

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