TESTING CRITERIA FOR ZINC TOLERANCE AND HIPPER-ACCUMULATION IN SOYBEAN (*GLYCINE MAX*) PLANTS

Monica Butnariu[1], Veronica Sărățeanu[1], Cristina Adriana Dehelean[2]
[1]University of Agricultural Sciences and Veterinary Medicine of Banat, Calea Aradului, No. 119, 300645 Timisoara, Romania
[2]Farmacy Faculty, Victor Babes University of Medicine and Farmacy, Eftimie Murgu Place No. 2, 300041 Timisoara, Romania

Abstract

In this study is tested the variability and heavy metal (zinc) hipper-accumulation potential in Glycine max plants, as a modality for the reduction of the danger represented by zinc, through the reduction of the heavy metal mobility and bioavailability in soil through phytoterapy. 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: *soybean, bioindicator, zinc, hipper-accumulation*

Introduction

Plants are grouped in vegetation communities well defined (forests, steppe, marshes *etc.*) determined by climatic and soil factors. In case of the appearance of some unfavourable conditions during plants' life there are appearing disturbances under the influence of biochemical and physiologic resistance potential (Gus *et* Rusu 2002).

Plants are succeeding in their adapting process to changed environmental conditions due to:

- modifying of enzymatic system;
- antibodies biosynthesis;
- biosynthesis of some activators or inhibitors;
- accumulation of osmotic active substances (Askoy *et al.* 2000).

Morphologic and anatomic adaptation of the plants to environmental factors is noticed and studied for a long time. Heavy metals used in industry and agriculture, or are resulting on the background of industrial processes and as a result of burning, are representing a great danger because of the next causes:

- they are maintaining in the environment for a long time period;
- they are transported long distances from the sources;
- they are depositing in the tissues of living organisms from where they get into the food, water, inspired air;
- they have a wide spectre of the toxic action (Butnariu *et al.*, 2003, 2004).

These substances are getting in fruits, vegetables, grass grazed by the animals from the contaminated sol and then they are getting in the animal organisms and in humans. Heavy metals have direct impact on the vegetation carpet. Because of the fact that the heavy metals aren't acting selective they are getting in all the plats from field, affecting an important part of the flora and fauna (Assunção *et al.* 2003, Goian *et* Ianos 1995).

Heavy metals persistence in the vegetal products (vegetables, leaves *etc.*) is explained especially through the passing over the doses of the fertilizers and pesticides. This fact demonstrates some abilities of the plants concerning the absorption and keeping the toxic chemical compounds for a long time in their tissues. The heavy metals are dispersing and participate to different transformations through oxidation, reduction *etc.* reactions once arrived in environment. These processes are developed with high energy consumption provided by the sun (Bowman *et al.* 2003). The plants are providing energy to the herbivores that are feed for the carnivores. Every level in this trophic chain is producing residues that are food for decomposers (Gstoettner *et* Fisher 1995).

The metal absorption mechanisms of the bioaccumulating plants and the specificity base concerning the absorbed metal are based on two mechanisms that are explaining partially the accentuated absorption of the metals by the bioaccumulating plants in comparison with the non-bioaccumulating species:

- increased absorption of the metal in roots correlated with high metal translocation rates from the root to the aerial parts;
- metal accumulation in roots, implicating preferential allocation of the root biomass to areas rich in metals and a root system more developed in comparison with the dry mass of the aerial parts, this fact favouring the improved absorption of the metals (Askoy *et al.* 2000, Baker *et* Whiting 2003, Kupper *et al.* 2000).

Near to the knowledge of the chemical, biological and physical processes is necessary to study the effect of the contaminants concentration at all levels in biological hierarchy (Dumitru *et al.* 1995).

Zinc has an important role in plant nutrition, being implicated in protein metabolism, ribonucleic acids synthesis, glucoses, B, C, P vitamins synthesis and in chlorophyll formation. Also, this metal is implicated in the activity of different enzymatic systems, is a component in hydrogenase, proteinase, and peptidase, in growing phytohormones synthesis is influencing in a favourable way the germination, tissues turgescence maintaining, resistance for low temperatures and cryptogamic diseases, in animal and plant nutrition. In 1869 Paulin is evidencing zinc favourable action on some *Aspergillus niger* cultures, and in 1909 Sommer and Lipman show that this oligoelement is indispensable in superior plant nutrition. Zinc is implicated in many metalo-enzymes and even in DNA and RNA biosynthesis and in proteins rich in sulphur thus in plants, microorganisms and animals. Zinc is essential for gaseous respiratory exchanges. Many enzymes are activated by zinc, this having the cofactor role in these enzymatic systems (Butnariu *et* Goian, 2005). Increased zinc contents from soil can determinate excessive zinc accumulation in plants with different negative effects on the absorption and use of other nutrients (Lacatusu *et al.* 1992, 1994).

Zinc is less phytotoxic n report with the other heavy metals, being situated in the next place concerning toxicity **Cu > Ni > Co > Zn > Mn**. Zinc toxicity is mentioned especially on soils with acid reaction. The critic level of the accessible zinc from soil, which determinates the toxicity, is different in report with the soil features that are determining zinc accessibility and the plants toleration for zinc excess (Frey *et al.* 2000, Gus *et* Rusu 2002). Zinc is easily absorbed by the roots in comparison with copper, being translocated in the aerial part of the plants, and determining visible toxicity systems. They are usually non-specific and are similar with iron, manganese or phosphorus deficiency (Parker 1995, Zhao *et al.* 2000).

Concentrations in plants that are manifesting zinc toxicity are situated usually between 300 and 500 ppm. The toxic level can be more reduced in report with zinc plant tolerance (Butnariu *et al.* 2003, Maciuca 2004). Plants can be adapted to increased concentrations of zinc in tissues in concentrations that can pass twenty times the normal level in plant without visible toxicity symptoms (Parvu, 1997, 2003).

Experimental

Experiment accomplish *Glycine max* was tested like "sentinel species" for heavy metal zinc, because is well known that soybean is a plant with selectivity for this metal (SR ISO 11259-1 February 1999). The experiment evaluates soil quality using an active biomonitoring method, using *Glycine max* plants treated with zinc chloride, at different concentration, chosen to not pollute the soil used in experience (SR ISO 11047 July 1999).

Soybean plants were planted in 18 pots (six pots for every concentration of watering solution) which are containing 800 g soil and these were wetted with a solution containing metallic ion Zn^{2+} with different concentration. This experience was realised during May – July 2006, because the soybean plants have moderate needs for heat. Plants growing and development take place in constant temperature and lightning conditions. After seeding, plants were daily wetted with 250 ml solution containing Zn^{2+}. After few days from germination soybean plants have started their development, having almost the same dimensions and colour, indifferent by Zn^{2+} concentration of solution used to wet the plants (SR ISO 11259-2 March 1999). There we have verified if the soil quality is influenced by zinc chloride introduction, this being the premise that soil with bad quality induces a significant reduction in root length in comparison with a good qualitative soil. For this purpose they have measured the roots length of the plants and the average roots length for every treatment variant and then they have compared the average roots length for every concentration used with the untreated variant. Results are evaluated using standards (Azot Kjeldahl - SR EN 25553 / 2000; Cobalt, nickel, copper, zinc, cadmium, lead - SR ISO 8288 / 2001).

Heavy metal water soluble in nitromuriatic acid from soil extract. For heavy metal determination there was weighted about 1.5 g from soil sample with a 0.0001 g exact in a 100 ml reaction pots. It was wetted with approximately 0.5- 1.0 ml water and there was added during stirring 10 ml of hydrochloric acid, then was added 5 ml nitric acid drop by drop to reduce the foaming. The mixture obtained is left for 16 hours at room temperature for easy oxidation of the organic fraction of the soil. After this time the solution mixture is boiled until drying. Nitromuriatic acid extraction must be realized under a well-ventilated hoot. To avoid violent boiling and solution loss is important to add boiling moderator granules in samples. Then the reaction pots are cooling because in soil samples is added distillate water, and then is filtered with filter paper and then is mixed with 50 ml distillate water. Solutions obtained are prepared to determinate zinc, iron, copper and molybdenum. The soil samples that are containing more than 20% (m/m) organic carbon must to be treated with an extra quantity of nitric acid. Nitromuriatic acid is not dissolving totally the most of soils; the efficiency of extraction is different from a metal to other being influenced by the matrix compounds. Metals extracted in nitromuriatic acid can't be considered total fractions or bioaccessible fractions, because the extraction process is to represent a biological process (Azot Kjeldahl - SR EN 25553 / 2000; ISO 11455).

Vegetal material preparing. Plants samples are analysed for microelements and heavy metal content with spectrometry measurements of atomic absorption for one element concentration in nitromuriatic acid extract. Equipment used is atomic absorption spectrophotometer-VARIAN SpectrAA 1100 and hydrure system VARIAN DGA 77. Mineralization: 5 grams mortared plants and sifted are introduced in a porcelain capsule. There is added 15 ml acid mixture (HNO_3: $HClO_4$: H_2SO_4 2:1:0.2), and then is boiled until is evaporates Operation is repeated till the residue has white-yellow colour. The capsule is cooled at room temperature and then is added 50 ml hydrochloric acid (HCl 0.5 n) (ISO 11454/1994).

Microelements and heavy metals content in plants. From plant samples it was determined heavy metal and microelements (iron, magnesium, calcium) content using an atomic absorption spectrophotometer. For every sample replicate there was a control sample for the reactive used. For every element determined from samples was realised a calibration curve. Microelements determination method with atomic absorption spectrophotometer is respecting ISO 11466 (pH - SR ISO 10523/97; Magnesium - STAS 5574/77 and SR ISO 7980/97; iron - SR 13315/95).

Phosphorus content determining. Sample preparing. A part of vegetal ashes analytical weighted is wetted with distillate water then is added 40 ml HCl, and then is put into a vapour bath and after 30 minutes is filtered. The solution obtained is reacting at 100 ml. Then is taken 15 ml from the solution, this is put into a 100 ml balloon and then is added 2.5 ml NH_4OH, 1 ml $(NH_4)_2MoO_4$ and 0.25 ml $SnCl_2$. the mixture is let until appears a blue coloration. ***Work mode:*** to determinate phosphorus content there is realised a standard curve as in the next example: 0.1199 g KH_2PO_4 is reacting with 250 ml solution. From the solution is taken 10 ml which reacts with 250 ml solution. From the solution obtained are taken 1, 2, 3…9 ml in 100 ml balloons and are treated with molybdenum chloride and stannous chloride, and after 15 minutes they are coloured. The solution from balloons will have concentrations between 0.01 and 0.09 mg P_2O_5 in 100 ml. ***Results:*** there is determined samples extinction, and from standard curve are determined the concentration levels of the samples. All determined values are reported to 100 g dry matter (phosphates, total phosphorus - SR EN 1189 / 2000).

Results and Discussions

The experiment evaluates the soil quality (table 1, table 2) using an active biomonitoring method determining the zinc effect on the soybean plants growth and development in a soil with the next features:

- soil reaction is neutral with pH = 6.6;
- exchangeable bases sum is EB = 20.6 me/100g soil;
- total cationic exchange capacity T = 21.4 me/100g soil;
- exchange acidity EH = 0.8 me/100 g soil;
- bases saturation degree V = 96.3.

Table 1: Chemical and physical composition of the soil used in experience

Carbonate $CaCO_3$%	Coarse sand (2.0-0.2 mm)%	Fine sand (0.2-0.02 mm)%	Dust (0.02-0.002 mm) %	Clay 2, under 0.002 mm%	Physical clay, under 0.01 mm%	Humus %	P ppm	P ppm calcul.	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 used in experience

Cd ppm	Cu ppm	Zn ppm	Ni ppm	Pb ppm	Co ppm	Cr ppm
0	18.555	60.7	28.845	18.86	7.72	0.8688

This study tests the variability and hyper-accumulation of the zinc in soybean plants as a modality for the reduction of the danger represented by zinc, through the reduction of the mobility and bioavailability of the heavy metal through phytoremediation. There are realised studies on the plants grown and treated during growth with increased zinc concentrations being determined the visual symptoms, biomass yield and metal concentration in plant organs. The tolerance for increased zinc concentrations is quantified with the help of the three parameters as are biomass (root and aerial part), the length of the main root and the influence of the zinc on the soybean plants chemical composition.

Soybean plants are seeded in 25 May 2006 ant the experience lasts 90 days. The development characteristics of the plants are presented in table 3.

Table 3: Features of *Glycine max* (soybean) plants tested as vegetal bioindicators for zinc and nickel

Concentr. g/l	Plant no.	Added solution volume, ml	height, cm			Final leaves no.	Biomass quantity, mg	Root length, 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	
	6		1.5	4	5.3	7	5.81	4.5	
2g/l Zn^{2+}	1		2.4	16.2	15.9	9	5.82	5.7	0.0145
	2		2.0	17.5	15.1	9	7.10	5.5	
	3		2.8	17.3	16.9	9	5.07	5.7	
	4		3.0	17	17.4	10	5.95	5.5	
	5		2.7	17.2	17.3	10	5.10	5.5	
	6		2.1	9	15.5	9	5.12	5.5	

Average elongation rate is calculated after Parker (1995) as is follows: *elongation rate (mm/day) = [(final length of the longest root – final length of the shortest root) average / (metal exposure time)]*.

The normal zinc amount in soil is 100 ppm (after *Romanian Official Monitor*, no. 303 from 5.11.1997). The initial zinc amount is 50.7 ppm and after the experimental determinations this amount has increased to 151 ppm, proportionally with the zinc concentration in the watering solution (table 4).

Soybean plants have accumulated the zinc ion in roots in amounts between 30 ppm in control plants and 120 ppm in the case of the variants wetted with 1g/l Zn^{2+} solution. In the same way vary the zinc amount from the plants stem this being between 10 ppm Zn^{2+} and 98 ppm Zn^{2+}, in leaves between 27 ppm Zn^{2+} and 95 ppm Zn^{2+}, and in flower between 25 ppm Zn^{2+} and 80 ppm Zn^{2+} (table 4).

Table 4: Zinc content in soybean root, stem, leaf, and flower and from soil

Solution concentration (g/l)	*Zinc content (ppm)*				
	Soil	*Root % d.m.*	*Stem % d.m.*	*Leaf % d.m.*	*Flower % d.m.*
Water	50.7	30	10	27	25
1	124	85	50	83	51
2	151	120	98	95	80

Absorption of increased zinc amounts is determining in a negative way the assimilation of other chemical elements in plant. From table 5 can be noticed that in plants have decreased the contents in iron, magnesium and copper in comparison with the variant without zinc in the watering solution. The iron amount from soybean plants has decreased from 22 mg in control plants to 12 mg in the case of the variants wetted with the greatest zinc concentration. The manganese amount has decreased from 29 mg to 7 mg in the same way as the iron, and the copper decreased from 20 mg to 5 mg. Also in the case of phosphorus it was registered the same decrease trend the valued determined decreasing from 1.4 mg to 0.02 mg (table 5).

Table 5: Different chemical elements content in soybean (*Glycine max*) plants

Treatment g/ l Zn	C (mg % d.m.)	N (mg % d.m.)	P (mg % d.m.)	Fe (mg % d.m.)	Mg (mg % d.m.)	Cu (mg % d.m.)
0 g/l	22.95	1.85	0.411	22	29	20
1 g/l	24.20	2.29	0.541	18	12	8
2 g/l	28.95	3.14	0. 87	12	7	5

Carbon, nitrogen and phosphorus amounts have increased with the increase of the zinc concentration in the watering solution (table 5).

The increased zinc amount leads to the accumulation of the metal in plants determining negative effects on the absorption and the use of other nutritive elements.

Conclusions

After the analysis of the data obtained in this experience we have noticed that the soybean plans have a great tolerance to zinc excess thus:

- zinc has a positive effect for plants development in all the cases of the plants treated with increased zinc amounts in the watering solution;

- soil quality is not influenced by the metal introduced in conformity with SR ISO 11259-1 from February 1999 – *Soil quality*. Determining the pollutants effect on the flora;
- Zn^{2+} heavy metal ion is accumulated in plants organs (roots, stem, leaves, and flowers) in report with the concentration increase in the watering solution, but a part remained in the soil from the pots;
- plants roots show increased amounts of heavy metal in all the cases tested in comparison with the aerial parts of the soybean plants;
- other chemical elements showed an decreased concentration during the increase of the concentration of the metallic ions in soybean plants, but in normal limits;
- excessive zinc concentrations in plants are associated with the decrease of the concentration in other chemical elements as are iron, manganese and copper;

Bioindicator plants species can open a wide research field concerning the heavy metals pollution, this research type being more accessible in comparison with instrumental monitoring, but there are still some aspects to be clarified being necessary the perfection of some coherent surveying methods for the environment. Taking in account the financial problems specific for Romania and other objective reasons related with the difficult approach of the instrumental surveying, biomonitoring is representing an interesting alternative or a completion.

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