THE INFLUENCE OF SOME HEAVY METALS ON *MEDICAGO SATIVA* SEED GERMINATION AND SEEDLING GROWTH

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Abstract The paper presents the results of a study concerning the influence of high concentrations of lead (Pb), nickel (Ni), and cadmium (Cd) on some physiological, biochemical and morphological indicators in the dynamics of the process of seed germination and in the incipient phases of seedling growth of *Medicago sativa*.

The results underline the specific variations of the analysed indicators (water and dry matter content, total mineral elements content, activities of some oxydoreductase enzymes, content of assimilating pigments, lengths of radicle), determined by the nature of metals and their concentrations used for the treatment of the studied seeds.

Regardless of the concentration, Ni, Pb, and Cd determine the intensification of the activity of superoxidedismutase, an indicator enzime of the stress state. Ni, in concentrations of 50ppm and 300ppm, determines the deminishing of the asimilating pigments content, the inhibation of the seeds germination as well as the growth of the length of radicle.

INTRODUCTION

Among the heavy metals, Pb, Cd and Ni, in very small quantities, have a role in the plants nutrition while present in large quantities they have toxic effects. From the phytotoxic effects of these heavy metals presented by the writings in the field, we quote: the delay of the germination process (treatments with: Cd in beans, Pb in corn and barley); the modification of the assimilating pigments content (treatments with Cd in beans and corn, Pb in corn and salad); the disturbance of the photosynthesis and respiration processes; the modification of some oxidoreductases activity (catalase, superoxidedismutase, peroxidase, glutation reductase); the reduction of the roots growth rate (treatments with Cd in pine) [1, 5, 9, 10].

The paper presents the results of the research concerning the influence of high concentrations of Pb, Ni and Cd on some physiological, biochemical and morphological indicators in the dynamics of the process of seed germination and during the first phases of seedling growth for *Medicago sativa.*

MATERIAL AND METHODS

The vegetal material is represented by seeds of *Medicago sativa.* The analyzed experimental variants are in number of 7, that is: 1 – witness and 6 variants of treatment with heavy metals. The heavy metals were administered in acetate form, in the following concentrations: Pb (50ppm - C1; 200ppm – C2); Ni (50ppm- C1; 300ppm- C2); Cd (0,5ppm – C1; 1ppm - C2). The treatment time was 3 hours.

After the application of the treatment, the seeds were set for germination into Petri plates, on a sub layer of filter paper, in laboratory conditions.

At intervals of 6 - 10 days, according to the energy and germinative faculty, there were performed analyses of the biochemical and physiological indicators.

The content of <u>dry matter</u> was determined by the gravimetric method which consists, in principle, in the indicator evaluation by maintaining the biological material for a determined amount of time, in the drying oven, at a temperature of 105^{o}C.

The catalase activity was determined by spectrophotometric method which consists, in principle, in the evaluation of the peroxide consumption in a determined time interval [2].

The method for batching the peroxidase activity is based on the measurement of the colour intensity of the o-dianisidine oxidation product by oxygenated water under peroxidase action [7].

The activity of superoxiddismutase is determined through the capacity of this enzyme to inhibit the reduction of the tetrazolium salt (nitro blue tetrazolium - NBT) by the superoxid radicals generated in the reaction environment by riboflavine photoreduction [6].

The batching of the asimilating pigments is performed trough the spectrophotometric method that consists, in principle, in the asimilating pigments extraction into acetone, in several stages, operation followed by their spectrophotometric batching, at appropriate wave lengths for each pigment.

The content of total mineral elements was determined through the dry calcination method at the temperature of 450^{o}C.

The average radicle length was achieved at 10 days from mounting the experiment, by measurement with the rule.

RESULTS AND DISCUSSIONS

The alfalfa, a valuable fodder plant, is cited in the literature in the field as being a bioaccumulating heavy metals species [4, 8].

The results of the performed determinations are presented in the table I. Specific variations of the analyzed indicators determined by the nature and concentrations of the metals used for the seeds treatment are increased.

The dynamics of the inhibition and germination show small and fluctuating differences in the course of its occurrence, exhibiting a greater sensibility to concentrations of 50 ppm and respectively 300 ppm in the case of the Ni element. These concentrations have an inhibiting effect on the seeds germination and they induce the decrease of the water content by comparison with the witness. The results of some experiments presented in the literature in the field prove the ability of the roots and stems of *Medicago sativa* to accumulate ions of Ni from watery solutions [3].

In many cases, the presence of heavy metals in the environment and their transfer into the plant determine oxidative disturbances in the tissues and protection mechanisms derangements as a result of the concentration growth of the reactive oxygen species (oxygen radicals, superoxid ions, hydroxyl ions, peroxyl ions, alcoxyl ions etc.) which are potential toxic for the living cell. Most cells have the ability to remove the reactive oxygen species through specific mechanisms that detect and maintain them at the lowest possible level. In these processes the antioxidative enzymes (superoxiddismutase, catalase, different peroxidases) as well as antioxidants (ascorbate, tocopherol and gluthathione) are involved [9].

The results of our experiments show that the activity of the superoxiddismutase, determined in the treated seeds is superior to the activity of the enzyme from the witness sample in the variants with Pb and Cd. In the case of Ni the enzyme activity is strongly inhibited both after six and ten days from performing of the tests (fig. 1).

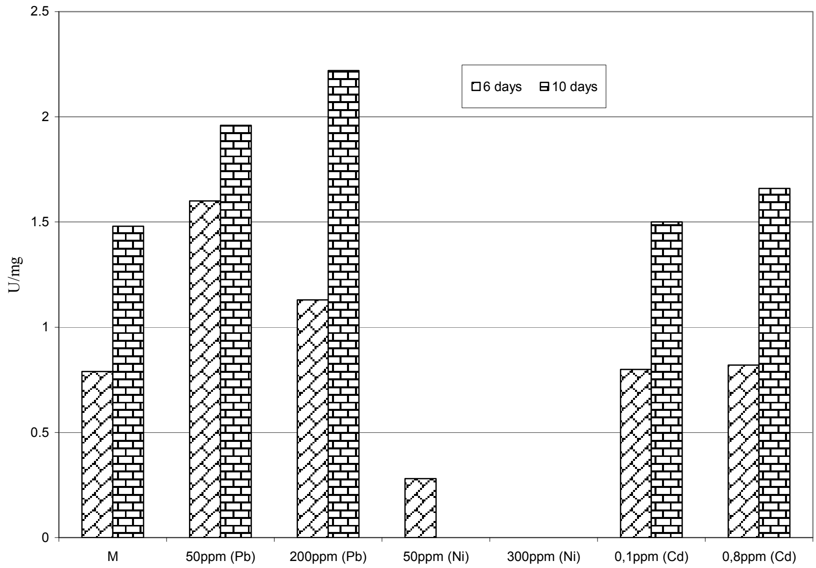

Figure 1 – The dynamics of the activity of superoxidedismutase in seeds of *Medicago sativa* treated with salts of Pb, Ni and Cd in various concentrations

Considering the activity of superoxidedismutase on experimental variants, we find values superior to the witness in the seeds treated with Pb in both studied stages and with Cd after ten days from the treatment of the seeds as well as values comparable with those of the witness in the variants of treatment with Cd analyzed after six days. In the lead case, after ten days from the treatment, the superoxiddismutase activity is obviously accentuated, doubling its value in the case of the superior concentration (C2). This behaviour is probably owed to the need to adapt to the presence of the toxic, the display of the answer after ten days from applying the treatment determining the activation of the enzymatic system responsible for the removal of the superoxid ions. The resulting hydrogen peroxide is removed by the catalase action.

After six days from the treatments performing we find the diminishing of the catalase activity in all of the studied experimental variants, more accentuated in the case of Ni, followed by Cd and then by Pb (fig. 2). In addition, the catalase activity in the seeds treated with salts of the mentioned elements, in lower concentration, is superior to the seeds treated with superior concentrations. This behaviour is similar to that found at the analysis of the results regarding the superoxiddismutase activity.

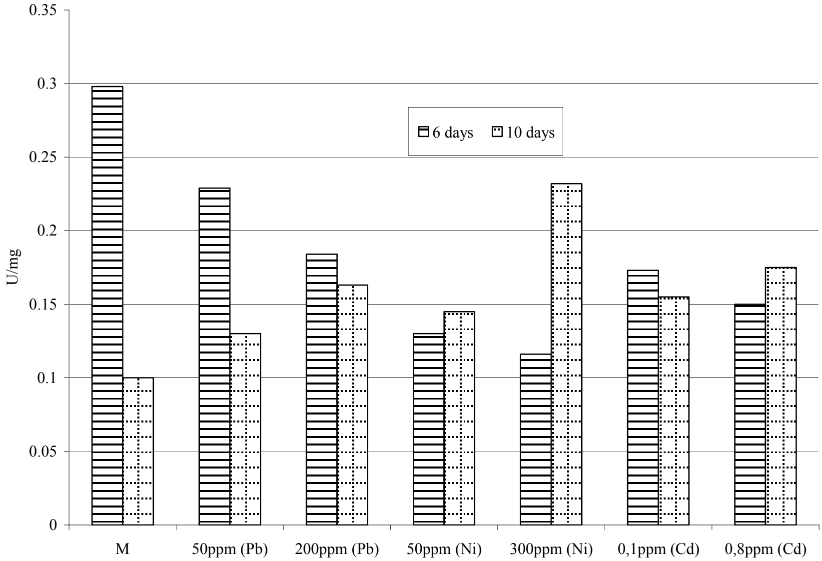

Figure 2 – The dynamics of the activity of catalase in seeds of *Medicago sativa* treated with salts of Pb, Ni and Cd in various concentrations

After ten days, the catalase presents, in the entire experimental variants, activities superior to the witness sample. The enzyme activities are higher in the variants with superior concentrations. We assume that the way in which the catalase activity varies can be explained by the fact that the seeds can easily take over small concentrations of mineral elements that, in higher concentrations constitute into stress factors determining the metabolic answer of activation of the protective enzymatic systems.

In all of the investigated experimental variants the peroxidase activity presents amplitudes inferior to the witness sample. This behaviour can be owed to the inhibiting effects of the salts of the metals the analyzed seeds have been treated with. After ten days from performing the treatments the obvious diminishing of the behaviour of the enzyme activity in accord with the witness sample is found (fig. 3).

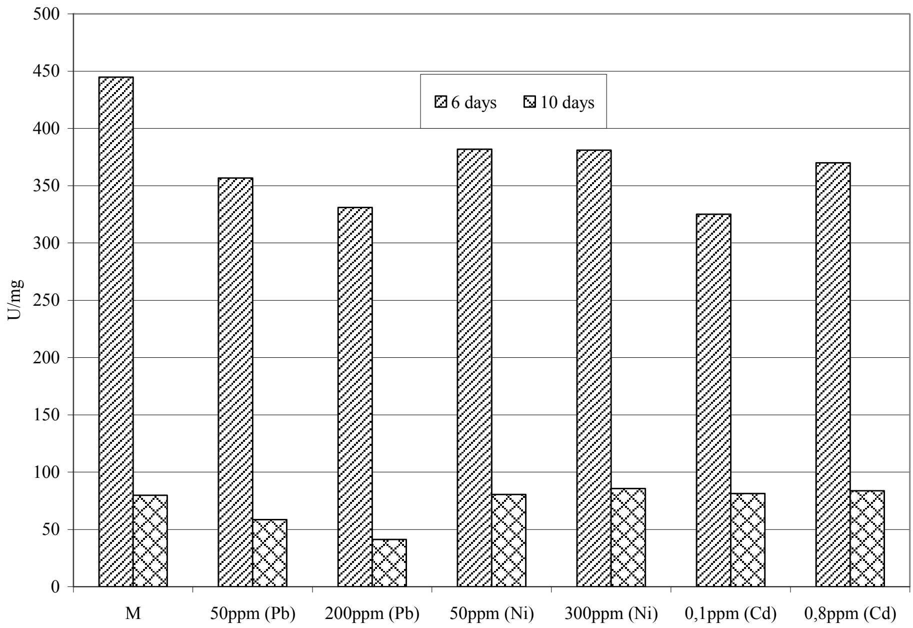

Figure 3 – The dynamics of the peroxidase in seeds of *Medicago sativa* treated with salts of Pb, Ni and Cd in various concentrations

The analysis of the growth of seedlings in their first stages (up to the age of 10 days) demonstrates the stimulating effect of Pb and Cd (in minimum concentrations) on the biosynthesis of the assimilating pigments and of the organic substance quantity (Table I).

From among the forms of analyzed assimilated pigments there prevails in the quantitative aspect in all experimental variants, chlorophyll a, followed in decreasing order by chlorophyll b and by carotenoidic pigments. An aspect to be noticed is the fact that the treatments with Pb (50ppm) and Cd (0,1 and 0,8ppm) determined the increase of the content of a and b chlorophyll and of total assimilating pigments, in comparison with the witness, while the treatments with Ni (50ppm and 300ppm) have opposite effects.

The concentrations of Pb and Ni used in treating seeds determine a slowing down of the growth in length of the radicle, an effect that is more pronounced in the higher concentrations case (Pb - 200ppm and Ni – 300ppm).

Hernandez and co-workers (2002) cite as effects of the treatment with Pb in concentration of 200 ppm in *Medicago sativa*: the increase of biomass, of the content of proteins and of asimilating pigments.

In 1994 Mehra and Farago (cited by Tomulescu and co-workers, 2004) find that the treatments with lead have toxic effects, various and contradictory. It was shown that the lead ions arriving in the plant affect the enzymes activity, disturb the electrolytic equilibrium, inhibit the cellular division and negatively influence the germination process and the growth of plants.

Our results confirm some of the literature data in the field regarding the positive or negative effects of the three heavy metals used in the experimental variants studied.

CONCLUSIONS

The seeds of *Medicago sativa* have a higher sensitivity to concentrations of 50 ppm and respectively 300 ppm Ni.

The antioxidant enzymatic systems have a protective effect; they are metabolically interconnected acting synergically. The superoxiddismutase, catalase and peroxidase present, generally, comparable variations, their activities diminishing from the lowest to the highest concentration in the first interval of time investigated, and a display that is inversed in the second interval. The general tendency is of intensification in the activity of the studied enzymes after six days from performing of the treatments.

The concentrations of heavy metals used in the treatment of seeds have a stimulating (Pb or Cd) or inhibiting (Ni) effect on the biosynthesis of chlorophyll a and b.

Table I. The influence of Pb, Ni and Cd on the germination and growth of the seedlings of *Medicago sativa*

Period of time	Physiological, biochemical and morphological indicators	Control	Pb (ppm)		Ni (ppm)		Cd (ppm)	
			50	200	50	300	0,5	1
The 6th day of germination	water content (%)	70,46	79,58	74,12	61,30	56,50	85,87	84,67
	dry matter (%)	29,54	20,42	25,88	38,70	43,50	14,13	15,33
	catalase (U/mg protein)	0,298	0,229	0,184	0,130	0,116	0,173	0,150
	peroxidase (UP/mg protein /minute)	444,73	356,74	331,10	381,96	381,0	325	370
	superoxiddismutase (mg/protein)	0,79	1,60	1,13	0,28	0	0,80	0,82
The 10th day of germination	water content(%)	87,83	88,56	85,09	83,86	73,30	87,75	86,86
	dry matter (%)	12,17	11,44	14,91	16,14	26,70	12,25	13,16
	total mineral elements (%)	5,17	6,23	5,85	5,66	8,25	4,60	4,24
	organic substance (%)	7,00	5,21	9,06	10,48	18,45	7,65	8,92
	catalase (U/mg protein)	0,100	0,130	0,163	0,145	0,232	0,155	0,175
	peroxidase (UP/mg protein/minute)	79,85	58,53	41,31	80,55	85,63	81,24	83,60
	superoxid dismutase (mg/ protein)	1,48	1,96	2,22	0	0	1,50	1,66
	chlorophyll a (mg/g fresh material)	0,547	0,705	0,549	0,425	0,396	0,825	0,786
	chlorophyll b (mg/g fresh material	0,048	0,066	0,042	0,011	0,033	0,303	0,111
	carotenoidic pigments (mg/g fresh material)	0,024	0,028	0,024	0,010	0,010	0,068	0,005
	total pigments (mg /g fresh material)	0,619	0,799	0,615	0,446	0,439	1,196	0,902
	the medium length of the radicle (cm)	2	1,2	1	1,5	0,8	-	-

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