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The influence of lead acetate and actinomycetes on germination and growth of vetch plant (*Vicia sativa* L.)

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ABSTRACT

The influence of different lead acetate concentrations (10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 2 x 10^{-2} M) both with and without presence of actinomycetes on germination and initial growth (root, leaf length and stem height) of vetch plant (*Vicia sativa* L.) was rated. Different lead acetate concentrations and actinomycetes (*Streptomyces* sp.) of 5, 7 and 9 breed's types were used to treat vetch plant seeds. It was perceived that high lead acetate concentration of 2 x 10^{-2} M has inhibitory effect on the studied parameters. Probes with the highest concentrations of lead with the presence of actinomycetes as much as 60% more seeds germinated compared to the probes without actinomycetes. Low lead acetate concentrations have stimulating effect on these processes, as applied in the presence of microorganisms, and in probes without inoculation.

Key words: Leaves, Root, Seedlings, Stem, Toxicity.

INTRODUCTION

Heavy metals contamination is a serious problem (Šmejkalová *et al*. 2003), whereby they enter into food chain through plants. The amount of heavy metals absorbed by plants depends on the environmental concentrations and specific species and plant organs (Filipović-Trajkovic *et al*. 2012). Their transport in above-ground plant parts depends on the growth and development stage (Liu *et al*. 2005). The lead excess in plant cells can cause negative changes in the biochemical-physiological processes: photosynthesis, transpiration, respiration, and enzymes inhibition (Van Asshe and Clijasters, 1990). Inhibitory lead effect is manifested in the process of seed germination and during the vegetation period (Shafiq *et al*. 2008, Kumar and Jayaraman, 2014). Actinomycetes can decrease the lead toxic effects as saprophytic microorganism that influence the biopolymers activities, such as lignin, cellulose, hemicellulose, pectin (Vijayakumar *et al*. 2007). Approximately 60% of biologically active compounds used in agriculture are derived from the genus Streptomyces (Ilic *et al*. 2007). Antibiotic and vitamins-producing actinomycetes are beneficial for the plants physiological processes (Berg *et al*. 2001; Kumar *et al*. 2010), and extracellular enzymes.

The aim of this research is examination under controlled conditions the lead acetate influence on the vetch plant seed germination and young plants growth and in the presence of actinomycetes.

MATERIALS AND METHODS

The experimental intoxication with different lead acetate concentrations (10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M i 2 x 10^{-2} M) and actinomycetes (*Streptomyces* sp.) were used to treat vetch plant seeds.

Hundred vetch plant seeds (*Vicia sativa* L.) were lined up in boxes on dry filter paper (Fig. 1). The seeds were treated with 20 ml of a lead acetate with different concentrations and were placed in thermostat at 22ºC for germination and control sample with 20 ml of distilled water. Series of probes were set with lead-acetate and 3 ml added suspension of *Streptomyces sp*. with 4,8 x 10^{10} cells ml^{-1} of 5, 7 and 9 breed's types, according to the Andjelkovic *et al*. (2014). Actinomycetes cultures were multiplied in a medium according Krasiljnikov (1965) and 48 h latter the germinated seeds were counted. Boxes were returned to thermostat for two more days and left in laboratory at the room temperature. Length of the root, stem height and leaves length was measured. Measurements were conducted on 7^{th}, 14^{th}, 21^{st} and 28^{th} day of germination. Stem height and leaves length were measured in all four terms, but root length was measured on the first and the last term.

The results were processed using STATISTICS 10.0 program. The significance of the difference between the investigated probes was determined upon the varriance analysis, i.e. LSD test.

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Fig 1: The vetch plant seeds.

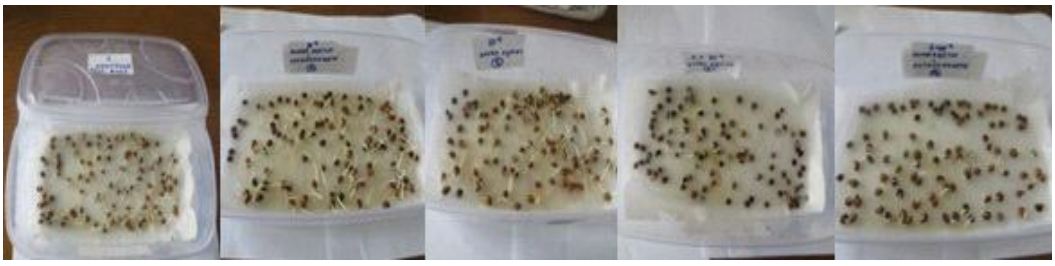

Fig 2: Plant's organs changes at the first measurement.

RESULTS AND DISCUSSION

Obtained results indicate lead acetate inhibitory activity (Table 1). The negative correlation between lead acetate concentrations and number of germinated seeds was perceived. In the presence of maximum lead acetate concentration 2 x 10^{-2} M 32 seeds germinated, ie 32% compared to the control. At the low lead acetate concentration (10^{-5} M), 90% of the seeds germinated. Ilić *et al*. (2015) obtained similar results indicated the toxic effect of higher concentrations, while smaller concentrations have stimulatory effect. In probes with actinomycetes the toxic effects of lead decreased and the number of the germinated vetch seeds increased. Probes with the highest concentrations of lead with the presence of actinomycetes as much as 60% more seeds germinated compared to the same concentration without actinomycetes. The largest leaf length (4.20 cm) was recorded at the first measurement in the probe with

Table 1: Germination of vetch (*Vicia sativa* L.) in the presence of different concentrations of Pb acetate and actinomycetes.

Variants	Number of germinated seeds	The number of plants that grew until the end of experiment
1.	100	89
2.	90	80
3.	85	80
4.	81	69
5.	78	63
6.	32	29
7.	97	92
8.	93	78
9.	92	80
10.	95	89
11.	90	78
12.	68	65

Table 2: Leaves length and stem height of vetch plant (*Vicia sativa* L.) in the presence of different concentrations of Pb acetate and actinomycetes in four measurement.

Variants / Measuring	Leaves length (cm)				Stem height (cm)			
	I	II	III	IV	I	II	III	IV
1.	4,00^{a}	5.00^{a}	7.00$^{a, b}$	0.38^{c}	6,90^{c,d}	18.39^{a}	21.38^{b}	19.92^{b}
2.	3,20^{b}	4.40$^{a, b}$	5.50^{c}	0.52^{a}	8,36^{a}	12.50$^{f, g}$	17.06^{i}	17.26^{e}
3.	2,61^{d,e}	4.20$^{a, b, c}$	6.80^{b}	0.46^{a}	5,94^{e}	14.40^{d}	18.63^{e}	17.75^{d}
4.	3,00^{b,c}	4.30$^{a, b}$	5.20$^{c, d}$	0.40^{c}	4,90$^{f, g}$	15.10^{c}	18.57^{e}	15.06^{h}
5.	2,43^{e,f}	3.30$^{d, e}$	5.40$^{c, d}$	0.44^{a}	4,59$^{f, g}$	13.50^{e}	18.31^{f}	14.60^{ij}
6.	1,82^{h}	3.00$^{e, f}$	4.00^{e}	0.26^{g}	3,84^{h}	8.53^{h}	11.87^{k}	14.47^{i}
7.	4,20^{a}	4.80$^{a, b}$	7.80^{a}	0.32$^{e, f}$	7,12^{c}	17.68^{b}	21.66^{a}	20.80^{a}
8.	4,20^{a}	4.67$^{a ,b}$	7.70$^{a, b}$	0.35$^{d, e}$	7,12^{c}	17.23^{b}	17.69^{g}	18.41^{c}
9.	2,90^{c}	4.00$^{b, c, d}$	7.40$^{a, b}$	0.30^{f}	6,50^{d}	14.60$^{c, d}$	20.81^{c}	17.25^{e}
10.	2,21$^{f, g}$	4.30$^{a, b}$	4.90$^{c, d, e}$	0.37$^{c, d}$	4,47^{g}	15.10^{c}	20.21^{d}	17.00^{e}
11.	2,03^{g,h}	3.30$^{d, e}$	5.20$^{c, d}$	0.22^{h}	5,00^{f}	13.50^{e}	17.47^{h}	15.59^{g}
12.	2,31$^{f, g}$	3.40$^{c, d, e}$	4.50$^{d, e}$	0.47^{a}	5,90^{e}	12.40^{g}	13.42^{j}	16.70^{f}

* The same letters in superscript indicate high homology, ie the absence of statistically significant differences ($p < 0.05$).

Table 3: Root length of vetch plant (*Vicia sativa* L.) in the presence of different concentrations of Pb acetate and actinomycetes.

Variants / Measuring	I	IV
1.	5,20[b]	9.80[b]
2.	4,65[b, c]	8.78[e]
3.	4,50[b, c]	9.28[c]
4.	2,94[d]	5.09[h]
5.	1,11[e]	2.16[j]
6.	0,59[f]	0.67[l]
7.	6,00[a]	10.26[a]
8.	6,00[a]	9.18[d]
9.	4,59[b, c]	8.43[f]
10.	4,30[c]	7.70[g]
11.	1,10[e]	2.84[i]
12.	0,57[f]	1.17[k]

* The same letters in superscript indicate high homology, ie the absence of statistically significant differences ($p < 0.05$).

actinomycetes, while the lowest value of this parameter was in probe with lead acetate concentration of 2 x 10^{-2} (1.82 cm). Based on the results of Fisher's test, the statistically significant difference in the negative sense was observed in relation to the control and all the probes with lead acetate. There is no statistically significant difference between the control and probes only with actinomycetes and they make a homogeneous group.

The maximum stem height (8.36 cm) was recorded in the probe with lead acetate concentrations of 10^{-5}, and the lowest (3.84 cm) in the probe with lead acetate with a concentration of 2 x 10^{-2} (Table 2). The negative impact of lead acetate manifested in the most probes for the root length, although stimulatory effects of lower concentrations evinced in the presence of actinomycetes. The negative effects manifested in the probes with the highest concentration of lead acetate, both with and without the actinomycetes (Fig. 2).

The results conducted by Dalal and Bairgi (1985) indicated the inhibitory impact of lead. They record decrease of the seed germination, root and seedlings growth of different varieties of *Corchorus* L., with different concentrations of lead, especially at a concentration of 20 mgl^{-1}. One of the primary effects of the toxic lead influence on plants is the root growth inhibition due to inhibition of cell division (Eun *et al*. 2000). Khan *et al*. (2017) stated the transfer ratios of Cr and Zn in plants without Pb treatment were lower as compared to those in plants treated with varying levels of Pb.

At the second measurement the maximum leaves length was recorded in the control probe. Statistically significant difference was observed in relation to the control in probes with lead acetate 10^{-2}, 10^{-3}, 2 x 10^{-2}, and probes with actinomycetes with the following concentrations of lead acetate: 10^{-2}, 10^{-4}, 2 x 10^{-2}. The minimum leaves length was recorded in probes with the highest lead acetate concentration (2 x 10^{-2}). Stem height was highest in the control probe (18:39 cm). All of the probes had statistically significant lower stem height related to the control. Jaffer *et al*. (1999); Morzeck and Funicelli (1982) and other authors indicated the lead toxicity in the plant germination and growth processes.

The maximum leaves length at the third measurement is recorded in the probe with actinomycetes inoculation (7.80 cm). The probes with actinomycetes and lead acetate concetrations of 10^{-4} and 10^{-5} did not show statistically differences. The minimum value of the parameter examined within this measurement is registered in the probe with lead acetate of 2 x 10^{-2} (4,00 cm). Stem height had the largest value in the probe with actinomycetes inoculation (21.66 cm). It was significantly higher in the relation to the control, while in all other probes the lower value was recorded in relation to probes with distilled water. The minimum value for the stem height was detected in the probe with lead acetate concentration of 2 x 10^{-2} (11,87 cm). Examining the impact of cadmium and lead on *Thespesia populnea* L., Kabir *et al*. (2008) suggested the negative impact of cadmium and lead on the seed germination and seedling growth. Also, Sêdzik *et al* (2015) recorded toxic effects of lead to the seed germination, root, seedlings growth and dry biomass compared to the control.

At the final measurement the maximum stem height was recorded in the probe with actinomycetes inoculation (20.80 cm). In relation to the control this had statistically significant difference in the positive manner. Stem height in other probes was statistically significantly lower than the control. Such trend has been registered with the root length (Table 3). Similar results obtained Shafiq *et al*. (2008) examining the influence of lead and cadmium on *Leucaena leucocephala* L.

In conclusion, actinomycetes reduce the harmful effects of lead since the moment of germination. Actinomycetes chelating role is unknown, so their influence on lead acetate mitigating role on adverse effects is reflected by the secretion of bio-stimulating substances that diffuse into vetch plant seeds.

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