Phytotoxicity of Lead on *Lolium perenne* L and *Lactuca sativa* var. *capitata* L: Effects of Germination and Growth

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ABSTRACT

Lead is one of the most important pollutants in the environment. Plants absorb lead when it is present in the environment. *Lolium perenne* L and *Lactuca sativa* var. *capitata* L are species used for toxicity tests. The experiments were performed with different concentrations of lead nitrate (0.05 g/L; 0.1g/L; 0.5 g/L; 1 g/L).

Test targets refer to seeds germination, the growth of seedlings, fresh biomass weight, dry biomass weight and physiological parameter (dry weight/fresh weight; the quantity of dry mass from 100 g fresh weight) at seven and fourteen days.

Lead toxicity inhibits germination of seeds and reduces the growth of seedlings. Lead can decrease germination percent, root length and strain length. Seedlings growth inhibition is not necessarily correlated with biomass reduction.

Keywords: *germination, lead nitrate, physiological parameter, seedlings.*

INTRODUCTION

Release of heavy metals in the environment is a serious problem. Metals are not biodegradable because of their persistence in soil for a long period of time, about 150-5000 years (Adriano, 2001; Saxena *et al.*, 1999). Bioaccumulation of metals may result in an increase of pollutants in the ecosystem, with long-term effect on the ecosystem (Jelea *et al.* 2007).

Lead is one of the most important pollutants in the environment. Metal's toxicity affects the soil (when it exceeds 1-50 character ppm). The metal is passively taken over by the root system of the plant (Mihali *et al.*, 2013; Shparyk and Parpan, 2004). Roots absorb significant quantities of lead from soil. The absorption of lead increases with the increase of soil pH (from 3.0 to 8.5).

For most of the species, lead is absorbed and accumulated for 95% in roots and the rest is translocated to aerial parts of the plant (Clemens *et al.*, 2002; Clemens, 2006; Wierzbicka *et al.*, 2007). The lead quantity which is translocated to the aerial parts of the plant is reduced. Lead is deposited in the intercellular spaces, cell walls and vacuoles. Small deposits are visible in the endoplasmic reticulum, dictyosome and dictyosome derived veyicles (Sharma and Dubey, 2005). Lead accumulation in plants depends on the concentration of exogenous lead. This accumulator potential can be used in phytoremediation process. Heavy metals can be removed from polluted soil by using the phytoextraction method which involves the accumulation of pollutants in plants (Zayed *et al.*, 1998).

The metal can cause numerous physiological effects to plants, among which we mention the effects on growth (Lamhamdi *et al.*, 2013; Sengar *et al.*, 2008; Seregin and Ivanov, 2001; Sethy and Ghosh, 2013; Sharma and Dubey, 2005), the production of chlorophyll, the content of water and protein (Pourrut *et al.*, 2011).

The test targeted seeds germination, the growth of seedlings, fresh weight, dry weight and physiological ratios (dry weight/fresh weight; the quantity of dry mass from 100 g fresh weight) of *Lolium perenne* and *Lactuca sativa*.

MATERIALS AND METHODS

The experiences have been carried out on two standard species. The seeds of the *Lolium perenne* L (*Mara*) are a Greenmaster product. *Lactuca sativa* var. *capitata* L (*May King*) is a Garden seed line product from Hungary.

Germination vessels were Linhardt. Blotting paper was used as germination layer. It has a good capillary growth of water and a pH between 6.0 and 7.5. For each experiment were used 50 seeds. The conditions of the experiment were the following: temperature of 20°C, relative air humidity of 38.30% and photoperiod from 10 hours to 12 hours (OECD, 2003).

The phytotoxicity test followed the effects of different lead nitrate concentrations (0.05 g/L – V1; 0.1g/L – V2; 0.5 g/L – V3; 1 g/L – V4) The standard evaluation of ecotoxicity is presented in the following procedures: SR ISO 11269-1: 1993 and ISO 11269-2: 2005.

The counting of germinated seeds has been done twice. The first one has been done for calculating the germination energy (according SR/99) and the second for calculating the

germination capacity (Bam *et al.*, 2006). The germination energy, the germination capacity and the speed of germination were determined (Bam *et al.*, 2006).

Later, it was appreciated the growth of vegetative organs (root and strain) after seven days and after fourteen days. After fourteen days, roots and strains were separated. It was calculated the germination index, the relative growth rate of the roots and toxicity index (Oros, 2011).

Initially, it was determined the fresh weight of vegetative organs. Dry weight was determined after the plant material was held for three days at 60°C.

Different mathematical methods were used in this study. In some cases, results were calculated as a percentage and in other cases the data obtained has been processed statistically using the Student's T test. The aberrant values have been eliminated according to the Chauvenet criterion (Snedecor and Cochran 1978; Weber, 1980). Statistic consideration has been considered from $p < 0.05$.

RESULTS AND DISCUSSION

Lolium perenne L seeds germinated in the third day (Tab. 1). The germination energy was calculated after three days. *Lolium perenne* L had the following values: 56% (control); 78% (treatment V1); 70% (treatment V3) and 62% (treatments V2 and V4).

Germination speed of seeds had the following values: 58.33% (control); 82.97% (treatment V1); 70.45% (treatment V2); 76.08% (treatment V3); 72.09% (treatment V4). Germination capacity was determined in the seventh day (Tab. 1). In

Tab. 1. Germination energy and germination capacity of *Lolium perenne* L

Days	M		V1		V2		V3		V4	
	No. germ. seed	%	No. germ. seed	%	No. germ. seed	%	No. germ. Seed.	%	No. germ. seed.	%
1 day	-	-	-	-	-	-	-	-	-	-
2 days	-	-	-	-	-	-	-	-	-	-
3 days	28	56	39	78	31	62	35	70	31	62
4 days	35	70	40	80	40	80	43	86	37	74
5 days	44	88	47	94	44	88	46	92	40	80
6 days	46	92	47	94	44	88	46	92	42	84
7 days	48	96	47	94	44	88	46	92	43	86

Note: M = control; V1 = treatment 0.05 g/L; V2 = treatment 0.1g/L; V3 = treatment 0.5 g/L; V4 = treatment 1 g/L.

case of *Lolium perenne*, germination capacity was between 96% (control) and 86% (treatment V4).

The seed germination of *Lactuca sativa* var. *capitata* L was performed immediately (Tab. 2). In the first day, the germinated percent was between 56% (control) and 24% (treatment V4). The germination energy for *Lactuca sativa* var. *capitata* L had the following values: 94% (control); 90% (treatments V1); 84% (treatment V2); 76% (treatment V3) and 78% (treatment V4).

Germination capacity for *Lactuca sativa* var. *capitata* was between 98% (control) and 76% (treatment V3). The growth of lead nitrate concentration from the solution modified the germination capacity of the seeds (Tab. 2).

Decrease germination percentage may be due to metal interference with amylolytic enzymes and proteases (Sengar *et al.* 2008).

The germination speed of seeds of *Lactuca sativa* had the following values: 95.91% (control); 95.74% (treatment V1); 97.67% (treatment V2); 100% (treatment V3); 95.12% (treatment V4).

Table 3 presents average values and standard errors, calculated after the measurement of vegetative organs (root and strain) of *Lolium perenne*. The roots of control seedlings had 22.93 ± 1.36 mm. In case of experimental versions V1 and V2, after seven days of treatment with lead nitrate, average values reached 28.54 ± 2.74 mm (+ 11.38%, $p < 0.001$) and 33.80 ± 3.00 mm (+ 47.50%, $p < 0.05$).

Tab. 2. Germination energy and germination capacity of *Lactuca sativa* var. *capitata* L

Days	M		V1		V2		V3		V4	
	No. germ. seed.	%	No. germ. seed.	%	No. germ. seed.	%	No. germ. seed.	%	No. germ. seed.	%
1 day	28	56	27	54	15	30	11	22	12	24
2 days	40	80	42	84	34	68	20	40	29	58
3 days	47	94	45	90	42	84	38	76	39	78
4 days	49	98	47	95	43	86	38	76	41	82
5 days	49	98	47	95	43	86	38	76	41	82
6 days	49	98	47	95	43	86	38	76	41	82
7 days	49	98	47	95	43	86	38	76	41	82

Explication at table 1.

Tab. 3. The influence of lead nitrate treatment, after seven days and fourteen days, on *Lolium perenne* L seedlings

Experimental versions	Roots length (mm)				Strains length (mm)			
	X	Es	D%	p	X	Es	D%	p
After seven days								
M	22.93	+ 1.36			38.10	+ 2.20		
V1	28.54	+ 2.74	+ 11.38	<0.001	52.38	+ 1.92	+ 37.48	< 0.05
V2	33.80	+ 3.00	+ 47.50	<0.05	54.12	+ 1.32	+ 42.04	NS
V3	20.06	+ 2.55	- 12.51	<0.05	51.46	+ 1.57	+ 35.06	NS
V4	7.03	+ 1.40	- 69.34	NS	22.65	+ 1.27	- 40.56	NS
After fourteen days								
M	55.06	+ 3.23			75.08	+ 2.62		
V1	55.46	+ 2.23	+ 0.74	NS	90.73	+ 1.79	+ 37.48	<0.05
V2	52.04	+ 2.64	- 5.46	NS	88.61	+ 2.27	+ 18.02	NS
V3	17.80	+ 1.00	- 67.66	NS	67.39	+ 1.86	- 10.24	NS
V4	2.08	+ 0.51	- 96.22	NS	28.64	+ 2.89	- 61.85	NS

N

ote: The table includes the average $\pm$ the standard error ($X \pm ES$), percentage differences compared to control (D%) and statistic significance considered from $p < 0.05$. The statistically non-significant values are marked with NS.

The treatment with a concentration of 0.5 g/L lead nitrate and with 1 g/L lead nitrate determined a low growth of roots. Percentage differences compared to control are lower with 12.51% ($p < 0.05$) or 69.34%.

Regarding strain percentage differences compared to control indicated an increase of 37.48% and 42.04% in the experimental versions V1 and V2. After treating the seedlings for seven days with a concentration of 1 g/L lead nitrate, it was observed an insignificant decrease of strain with 40.56% compared to control. Reduction of the seedling is determined mainly by low growth of the root, because of the reduced transport of nutrients to the seedling.

The results obtained after fourteen days of treatment with lead nitrate show a low growth of

the roots with an increased metal concentration in solution. The declines compared to control were between 5.46% (treatment V2) and 96.22% (treatment V4).

Roots are sensitive to the stress of the metal ions from solution when roots come in contact with it. Reduced root growth was due to reduction of meristematic cells, present in this region. In addition to that, the absorption of nutrients is reduced and oxidative stress is produced (John *et al.*, 2009).

Experiments performed on *Lactuca sativa* var. *capitata* L for seven days with lead nitrate showed the inhibition of roots in experimental versions V3 and V4 (Tab. 4). Percentage differences compared to control were - 32.22% (treatment V3) and - 86.54% (treatment V4).

Tab. 4. The influence of lead nitrate treatment, after seven days and fourteen days, on *Lactuca sativa* var. *capitata* L seedlings

Experimental versions	Roots length (mm)				Strains length (mm)			
After seven days								
	X	Es	D%	p	X	Es	D%	p
M	9.06	+ 0.49			14.54	+ 0.60		
V1	13.88	+ 0.71	+ 53.20	NS	14.47	+ 0.56	- 0.49	NS
V2	9.71	+ 0.37	+ 7.17	NS	11.62	+ 0.72	- 20.09	< 0.001
V3	5.96	+ 0.38	- 32.22	NS	14.50	+ 0.77	- 0.28	NS
V4	1.22	+ 0.08	- 86.54	NS	7.27	+ 0.30	- 50.00	NS
After fourteen days								
	X	Es	D%	p	X	Es	D%	p
M	17.48	+ 0.74			20.27	+ 0.37		
V1	18.60	+ 0.79	+ 6.40	NS	19.95	+ 0.57	- 1.58	NS
V2	14.35	+ 0.70	- 17.91	< 0.001	17.74	+ 0.89	- 12.49	< 0.05
V3	7.03	+ 0.34	- 59.79	NS	15.00	+ 0.89	- 26.00	NS
V4	3.14	+ 0.34	- 82.04	NS	8.17	+ 0.38	- 59.70	NS

Explication at table 3.

Tab. 5. Germination index (GI), relative growth rate of the roots (Er) and toxicity index (TI) on *Lolium perenne* L and *Lactuca sativa* var. *capitata* L

Exp. versions	GI (%)		Er (%)		TI (%)	
	<i>Lolium perenne</i>	<i>Lactuca sativa</i>	<i>Lolium perenne</i>	<i>Lactuca sativa</i>	<i>Lolium perenne</i>	<i>Lactuca sativa</i>
V1	97.91	95.91	100.72	106.40	98.61	102.04
V2	91.66	87.75	94.51	82.09	86.62	72.03
V3	95.83	77.55	32.32	40.21	30.97	31.18
V4	89.58	83.67	3.77	17.96	3.37	15.02

This inhibition is stronger for the roots, which may be correlated to their higher lead content (Liu *et al.* 2008). Reduction of roots produces the reduction of strains (- 50.00%, treatment V4).

Many scientific studies (Belimov *et al.*, 2003; Capelo *et al.* 2012; Han *et al.*, 2007) have shown inhibition of seedling growth under lead stress. Seedling growth reduction from lead exposure may be attributed to nutrient metabolic disturbances and disturbed photosynthesis.

Table 4 presents the results obtained after seven days and fourteen days of treatment with lead nitrate on *Lactuca sativa* var. *capitata* L. Literature data indicates the inhibition of root growth (Ekmekçi *et al.*, 2009) by inhibiting cell division or by decreasing the elongation of the smooth zone (Fiskesjo, 1997).

Reducing the height of the plant, mainly due to reduced root growth, leads to the reduction of nutrients and water transport. The toxic effect of lead on plant growth is time and dose dependent.

Table 5 presents results about germination index, relative growth rate of the roots and toxicity index calculated after fourteen days. Increasing the concentration of lead decreases all parameters taken into account. Toxicity index combines germination index and relative growth rate of the roots. The treatment with 1 g/l lead nitrate showed the lowest toxicity index on *Lolium*

perenne namely 3.37. In *Lactuca sativa* toxicity index was 15.02.

In table 6 are presented the physiological parameters of *Lolium perenne*. The ratio dry weight/fresh weight varies from 0.960 (control) to 0.565 (treatment V2). In case of strains, ratio grows from 0.212 (control) to 0.320 (treatment V4). Recorded data relating to the ratio of dry and fresh weight does not provide accurate information because the number of germinated seeds is different.

One of the physiological parameter taken into account was the amount of dry weight of 100 g fresh weight. This parameter decreased from 96% (control) to 56.52% (treatment V2) in case of the root.

In case of dry weight quantity strain from 100 g, fresh weight increased from 21.27% (control) to 32.07% (treatment V4). In percentage terms, compared to control, it is established that the dry substance accumulation from the root is decreased with 36.61% (treatment V1) and 41.13% (treatment V2). The dry substance accumulation from strain is growing from 8.83% (treatment V2) to 50.77% (treatment V4).

Effects of concentration of lead nitrate are not clearly established. Seedlings growth inhibition is not necessarily correlated with biomass reduction.

Tab. 6. The mass of the plants and the physiological parameter values of *Lolium perenne* L and *Lactuca sativa*

Experimental versions	Vegetal mass (g)				Physiological parameter					
	Roots		Strains		Roots			Strains		
<i>Lolium perenne</i>	p	u	p	u	mu/mp	%Su	C%	mu/mp	%Su	C%
M	0.025	0.024	0.235	0.050	0.960	96.00	100	0.212	21.27	100
V1	0.023	0.014	0.248	0.063	0.608	60.86	63.39	0.254	25.40	119.41
V2	0.023	0.013	0.285	0.066	0.565	56.52	58.87	0.231	23.15	108.83
V3	0.025	0.024	0.198	0.052	0.960	96.00	100	0.262	26.26	123.46
V4	0.010	0.009	0.106	0.034	0.900	90.00	100	0.320	32.07	150.77
<i>Lactuca sativa</i>										
M	0.080	0.017	0.801	0.046	0.212	21.25	100	0.057	5.74	100
V1	0.029	0.013	0.393	0.035	0.448	44.82	210.91	0.089	8.90	155.05
V2	0.024	0.014	0.558	0.043	0.583	35.00	164.70	0.077	7.70	134.14
V3	0.009	0.005	0.172	0.017	0.625	55.55	261.41	0.098	9.88	170.73
V4	0.008	0.003	0.092	0.025	0.375	37.50	176.47	0.271	27.17	473.34

Note: p – fresh weight, u – dry weight, %Su – physiological parameter that expresses the amount of dry weight of 100 g fresh weight.

Similar results have been highlighted by other studies (Yan *et al.*, 2010).

In table 6 are also presented physiological parameter of *Lactuca sativa* var. *capitata* L. Dry weight/fresh weight report from the roots, grows from 0.212 (control) to 0.625 (treatment V3). In case of the strain, the ratio increases from 0.057 (control) to 0.271 (treatment V4). Wierzbicka (1998) reported the increase of dry weight of plant organs. The peak is due to the synthesis of the cell wall polysaccharides.

Physiological parameter that expresses the amount of dry weight of 100 g fresh weight increases from 21.25 % (control) to 37.50 % (treatment V4) for the root. In case of the strain, the ratio grows from 5.74% (control) to 27.17 g% (treatment V4). It is observed the increasing accumulation of dry substance in the roots (64.70% - treatment V2 to 161.41% - treatment V3) and strains (34.14% - treatment V2 to 373.34% - treatment V4).

Inhibiting the growth of vegetative organs is associated with accumulation of dry vegetal mass. Inhibiting the growth of seedling in parallel with the accumulation of dry substance might be due to the measures of protection against chemical aggression.

CONCLUSIONS

The stress produced by the metal in solution has resulted in decreased germination energy and germination capacity from both species. Effects of lead nitrate on vegetative organs depend on the concentration of the metal in the solution.

Increasing the concentration of lead decreases germination index, relative growth rate of the root and toxicity index.

Vegetative organs growth damage has resulted to decrease in fresh biomass of the plants. Decreasing the content of vegetal mass depends on the concentration of the metal in the solution.

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