THE INFLUENCE OF LEAD ON SEED GERMINATION AND SEEDLINGS GROWTH OF OCMUM BASILICUM L. AND SALVIA COCCINEA BUCHOZ EX ETL. SPECIES

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Abstract. The paper presents the results of a study regarding the influence of treatment with lead in different concentrations (100 mg/l, 200 mg/l, 300mg/l, 400 mg/l) on seed germination and growth in early ontogenetic stages of Salvia coccinea Buchoz ex Etl. and Ocimum basilicum L. The following indicators were analyzed: the percentage of germinated seeds, the mean germination time, the length of the root, the length of the hypocotyl, the length of the seedling, the tolerance index. The results underline the specific variations of analysed indicators, depending on the concentrations used for the treatments of seeds and the test species. The concentrations used for treatment did not change significantly the seed germination, but they influenced negatively the seedling growth (especially the root elongation). The delay effect on the growth process was very pronounced at higher concentrations. Ocimum basilicum compared to Salvia coccinea presented a higher sensitivity to the lead treatment.

Keywords: cultivated species, germination indices, lead, toxicity.

Introduction

Lead is found in the Earth’s crust as minerals: galena, cerrusite, etc. The anthropic sources of environmental pollution with lead are represented by: the activities of extraction of lead and zinc, industrial activities (metallurgic industry, lead based dyes production, etc.), fossil fuel burning, solid waste burning, emission from cars, etc. (Zaharia, 1999; Mulligan et al., 2001; Sharma and Dubay, 2005).

Lead is considered a non-essential element for the life of plants (Woolhouse, 1983) and a polluting agent for the environment (Sharma and Dubay, 2005). From the solution of the soil, the lead enters the plants, is accumulated in the root and can be partially translocated into the aerial organs (Małkowski et al., 2005; Patra, 2004).

The effects of lead on plants depend on several factors: the concentration of metal, type of salts, type of soil, species (Patra, 2004; Ghani, 2010). In low concentrations it can stimulate the growth in some plants (Dou, 1988 quoted by Liu et al., 1994 and Jiang et al., 2000) and some metabolic processes (Patra, 2004). As side effects of lead excess in plants, we mention: alteration of membrane permeability, modifications of the activity of some enzymes (Woolhouse, 1983; Kaur et al., 2010, Maleka et al., 2012, Stancheva et al., 2014); reduction of cellular division, (Chakravarty and Srivastava, 1992; Liu et al., 1994; Kiran and Şahin, 2005); reduction of germination, reduction/inhibition of seedling/plant growth (Lane et al., 1978, Liu et al., 1994; Kiran and Şahin, 2005; John et al., 2009); reduction of content of chlorophyll pigments (John et al., 2009; Ghani, 2010); inhibition of synthesis of chlorophyll pigments and the activity of the Calvin cycle enzymes (Haider et al., 2006); chlorosis, reduction of photosynthetic rate (Woolhouse, 1983).

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Ocimum basilicum L. and Salvia coccinea Buchoz ex Etl. are annual species, cultivated, of the Lamiaceae family. Ocimum basilicum is an aromatic species, with special medicinal importance and also it is largely used as a spice. Salvia coccinea is a decorative species for its flowers. It presents flowering in stages, of long duration, being cultivated in urban green spaces (Preda, 1989) and in private gardens.

Research done on the species Ocimum basilicum showed the fact that the exposure to different heavy metals induces the following effects: unfavourable influence on germination and seedling growth – treatment with lead (Galeș et al., 2008; Pădurariu et al., 2010), accumulation in the root of some heavy metals such as chrome (Bishehkolaie et al., 2011), cadmium, lead and zinc (Stancheva, 2014), reduction of gas exchanges, alteration of antioxidant enzyme activity and content of some substances with antioxidant role (Stancheva, 2014). The species Salvia has the capacity to germinate in the presence of some moderate and high concentrations of zinc in substrate, but the process of growth of the root and hypocotyls is diminished (at moderate concentrations) or inhibited (at high concentrations) (Stratu et al., 2014).

This paper is aimed at the investigation of the effect of the lead treatment of different concentrations on the germination and growth in the first ontogenetic stages in the species Ocimum basilicum L. and Salvia coccinea Buchoz ex Etl.

Materials and methods

The biological material, was represented by seeds of Ocimum basilicum L. (Genovese variety) and Salvia coccinea Buchoz ex Etl. obtained from S. C. Unisem S. A. Iași. The treatments were represented by five experimental variants: a control variant (with distilled water) and four variants with lead solutions. Lead was used as nitrate solutions in concentrations of 100 mg/l, 200 mg/l, 300mg/l, 400 mg/l. In selecting the concentrations used for the experiment we started from the critical concentration of lead in soil (100 mg/l) (according to Alloway, 1990; Beckett and Davis 1979, quoted by www.cprm.gov.br).

The seeds were placed to germinate in Petri dishes, on a filter paper humidified with distilled water (a control variant) and nitrate of lead solutions (treatment variants).

The plates were kept in a growth chamber (Snijders Scientific type), at 12:12h photoperiod, 22°C/24°C, and 60% relative humidity. The initial volume of distilled water or nitrate of lead solutions (at placing the seeds) was of 4 ml. During the experiment, germination substrate was wetted with distilled water (control variant) and nitrate of lead solutions (treatment variants). For each variant, three replicates (each with 25 seeds) were used. The total duration of the experiment was of 10 days after mounting. The number of germinated seeds was recorded each 24 h.

The following indicators were analyzed: the percentage of germinated seeds; the mean germination time; the length of the root; the length of the hypocotyls, the length of the seedling; the tolerance index. The mean germination time was calculated by the formula described by Ellis and Roberts (1981) (Moradi et al., 2008). The tolerance index of heavy metals (TI) was calculated by the formula described by Iqbal and Rahmati (1992) (Ahmad et al., 2012).

The measurements (the length of the root, the length of the hypocotyls, the length of the seedling) were performed on 30 seedlings for each experimental variant. The results presented in figures are expressed as mean value ± standard error. The data obtained from
the germination indices (n=3), and the morphological indicators (n=30) were statistically interpreted. The unifactorial Anova test (using Microsoft Excel software) followed by the Tukey test (α = 0.05) was used in order to test the differences between means (Zamfirescu and Zamfirescu 2008).

Results and discussions

At the end of the experiment, the percentage of germination registered average values between 89.33 % and 97.33% for Ocimum basilicum seeds and between 72 % and 84 % for Salvia coccinea seeds (Fig. 1). Comparing with the control, in the treatment variants, the percentage of germination was reduced (with the exceptions of 100mg/l and 400mg/l treatments at Salvia coccinea), with a more pronounced effect in Ocimum basilicum. From a statistic point of view, the lead in the concentrations used did not determine significant changes (p > 0.05) of the germination percentage at the two species tested (Fig. 1).

![Figure 1. The percentage of the germinated seeds](image)

The mean germination time (MGT) registered values between 3.16 – 3.81 in Ocimum basilicum and between 4.48 – 4.65 in Salvia coccinea. Comparing with the control, the MGT of the 400mg/l treatment variant was longer. The situation was encountered at both species tested, but from a statistic point of view, the increase of MGT value was significant only for Ocimum basilicum (p < 0.05) (Fig. 2.).

The reduction of the germination in the case of the treatment with lead of different concentrations was reported also by other authors: Chakravarti and Srivastava (1992), for the species Helianthus annuus, at concentrations of 0.01M, 0.025 M; 0.05M, 0.1M, 0.25M (lead in form of chloride); Shaukat et al., (1999) at the species Pennisetum americanum after 8 days of exposure to concentrations of 200 ppm and 400ppm (lead in form of nitrate). In Ageratum houstonianum, the treatment with lead (in form of nitrate), in concentration of 100mg/l, 300mgl, and 500mg/l reduced the seed germination by 5.38%, 8.46% and 13.08% respectively, after 96 hours from assembling the experiment (Stratu and Costică, 2013).

In some cases, the lead in low concentration stimulates the germination in some medicinal species in the family Hyacinthaceae (Street et al., 2007): in Bowiea volubilis (in
concentration of 0.5mg/l) and in *Merwilla natalensis* (in concentrations of 0.5mg/l and 2mg/l)

Figure 2. The mean germination time (MGT) (*- represent significat differences at p < 0.05)

The results obtained regarding the influence of the lead on the indicators of germination are due to the morphological and structural particularities of the seeds in the test species taken into the study, but also to another factors. It is considered that the seed coat can represent: a barrier between the embryo and the environment around it (Araújo and Monteiro, 2005); the first barrier for the lead ions absorbed by the germinated seeds (Sereghin and Ivanov, 2001).

At the two species tested we noticed the fact that the seeds formed after the imbibition, a mucilage of opaque consistency. Mucilages are non-homogenous polysaccharides and presents high hydrating capacity. They form a gelatinous barrier at the level of the seeds (Windsor et al., 2001); it is considered that the mucilage facilitates the germination (Western et al., 2000). We consider that, because of the high capacity to hydrate, they maintain the humidity and help the germination in the conditions of the presence in the substrate of some high concentrations of lead. According to Gupte et al., (2012), the mucilage in the seeds of *Ocimum basilicum* have the capacity to absorb ions of copper in aqueous solutions.

The research done by Lane and Martin, (1977) in *Raphanus sativus* showed the fact that the seed testa prevents the contamination of the embryo with lead until the moment when it is broken (during the imbibition). After the testa is broken, the lead is absorbed very rapidly in tissues. An important exception was noticed in the meristematic area of the radicula and hypocotyl, probably in order to reduce the accumulation of lead in the meristematic tissue.

*The length of the root, hypocotyls, and seedlings* in the variants of treatment recorded average values lower than in the control (with the exceptions of the hypocotyls: in the 100mg/l treatment on *Ocimum basilicum* and the 200mg/l treatment on *Salvia coccinea*), values that decrease with the increase in the metal concentration. This fact indicates that the treatment exhibited an unfavourable influence on the seedlings organs elongation (Figs. 3; 4; 5). The lead in concentration of 200mg/l - 400mg/l had a stronger inhibitory effect on root elongation in *Ocimum basilicum* (by 57.48% - 85.36 % compared with the control) than in *Salvia coccinea* (by 48.2% - 58.7 % compared with the control).
Figure 3. The length of the root (LR) (* - represent significant differences at p < 0.05)

Figure 4. The length of the hypocotyl (LH) (* - represent significant differences at p < 0.05)

Figure 5. The length of the seedling (LS) (* - represent significant differences at p < 0.05)
The negative influence of lead on the growth in length of the root and of the seedlings was significant \((p < 0.05)\) in both species, for all the concentrations used for the treatment. In the case of the hypocotyl, the growth in length was reduced significantly \((p < 0.05)\) only in *Ocimum basilicum*, at concentrations of 300mg/l and 400mg/l.

Between the concentrations used for the treatment and the morphological parameters analysed some strong negative and significant correlations \((p < 0.05)\) or moderate negative and insignificant correlations (in the case of the length of the hypocotyl in *Salvia coccinea*) were set (Table 1).

Table 1. Pearson’s correlation coefficients \((r)\) between the metal concentration and the morphological parameters \((* - r \text{ significant at } \alpha = 0.05)\)

<table>
<thead>
<tr>
<th>Species</th>
<th>The length of the root</th>
<th>The length of the hypocotyl</th>
<th>The length of the seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ocimum basilicum</em></td>
<td>-0.9763*</td>
<td>-0.8971*</td>
<td>-0.9861*</td>
</tr>
<tr>
<td><em>Salvia coccinea</em></td>
<td>-0.9212*</td>
<td>-0.5599</td>
<td>-0.9484*</td>
</tr>
</tbody>
</table>

Also, the macroscopic observations underlined the fact that the area at the top of the primary root and in some cases also from the top of the lateral roots presented some brown colour. This coloration (a possible toxicity symptom) was noticed in both species taken into the study, for all the concentrations used. The brown colour of the root in the case of the treatment with lead was reported by Woźni et al., (1982) in *Lupinus luteus* L. cv. Jantar and by Liu et al., (1994) in *Allium cepa*.

The results obtained confirm some data in the available literature regarding the influence of the ions of lead on the process of incipient growth. In *Lupinus luteus* L. cv. Jantar the lead (in form of lead chloride) reduced the growth of the root and the hypocotyl of the seedlings (Woźni et al., 1982). In *Allium cepa* the roots reduced their growth after 48 hours of treatment with lead nitrate in concentration of \(10^{-3} – 10^{-4}\) M; the cell division was affected (Liu et al., 1994). In *Zea mays*, concentration of the lead (in form of nitrate) of 10, 20, 30 mg/kg soil, reduced significantly the growth of the seedling root, the stem being the least affected (Ghani, 2010). In *Lens culinaris*, the lead (in form of lead chloride), in concentration of 0.125 mM, 0.250 mM, 0.500 mM, and 1mM reduced the growth in length with the growth of the metal concentration (Kiran and Şahin, 2005). In *Ageratum houstonianum* the treatment with lead (in form of nitrate) reduced significantly the growth in length of the root (concentration 100 mg/l - 500 mg/l) and of the hypocotyls (concentration 300 mg/l and 500 mg/l), after 96 hours following the assembling of the experiment (Stratu and Costică, 2013).

The effect of delay/inhibition of the root and seedling growth in the considered species is probably due to the metabolic and physiological disorders caused by high concentrations of lead used in the treatment. In the pea, the lead ions in the growth substrate induced oxidative stress at the level of the root (Maleka et al., 2012), which can affect the growth process. According to Lane et al. (1978), the lead can influence the growth through changes in the rate of cell division and/or modifications of the process of cell elongation. The effect of growth inhibition would be caused by the lead interference with the auxin that regulates the cell elongation. The inorganic lead salts (chlorides and nitrates) reduce the
mitotic activity and inhibit the root growth (Patra, 2004). The cytotoxic effects of the lead are quoted by several authors: Chakravarty and Srivastava (1992), in *Helinathus annuus*; Liu et al. (1994), in *Allium cepa*; Kiran and Şahin (2005), in *Lens culinaris*.

In both test species, the delay of the growth in length comparing with the control was more pronounced in the case of the root than the hypocotyls. Similar results were obtained also by other authors (Woźny et al., 1982; Shaukat et al., 1999; Stratu and Costică, 2013).

According to Sereghin and Ivanov (2001), at the surface of the root, the lead attaches to the groups of carboxyl in the molecule of the uronic acids, components of the mucilage; this fact limits the entering of the lead ions inside the root and forms a protecting barrier for the radicular system. At cell level, the lead interacts with the functional groups of proteins, polyssacharides, nucleic acids, etc. In the root, the lead is transported to the level of the endodermis, especially through apoplast; the endodermis of the root would act as a partial barrier in the diffusion of the lead from the cortex to the central cylinder, respectively from the root toward the stem (Lane and Martin, 1977), thus limiting the lead transport to other organs of the plant.

**The tolerance index** presented a gradually decreased value proportional with the increase of the metal concentration. For the concentration of 100mg/l, TI presented similar values for the two species (Fig. 6), which indicates a high tolerance and, respectively, low-moderate toxicity. For concentrations of 200mg/l and 400mg/l, *Ocimum basilicum* presented a much lower tolerance (14.64 % - 42.52%) comparing with *Salvia coccinea* (41.3-51.8%) (Fig. 6). This fact underlines that the lead in the concentrations mentioned presents a high degree of toxicity in both species, but *Ocimum basilicum* is much more sensitive. A low tolerance to high concentrations of lead was also reported for *Albizia lebbeck* and *Leucaena leucocephala* (Iqbal and Shazia, 2004). It is considered that the plants tolerance to lead is associated with: their ability to chelate the lead at the level of the cell walls (by the carboxyl groups forming the carbohydrates); synthesis of osmolytes and the activation of a defensive antioxidant system (Sharma and Dubay, 2005).

![Figure 6. The tolerance index (TI)](image)

The results obtained underlined the fact that lead in the concentrations used had a stronger effect on the growth processes (especially in the root) than on the germination.
This aspect was also noticed by other authors: Iqbal and Shazia (2004) in the seedlings of *Albizia lebbeck* and *Leucaena leucocephala* – treatment with lead; Li et al., (2005), in seedlings of *Arabidopsis thaliana* for some heavy metals (Hg$^{2+}$, Pb$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$).

**Conclusions**

The lead in the concentrations used presented the following effects: it did not influence significantly the germination percentage in the two species; in high concentration, it determined a significant increase of the mean germination time in the *Ocimum basilicum*; it reduced the root and hypocotyl elongation. The root growth was more affected than the growth of the hypocotyl. The tolerance index was gradually reduced with the increase of the concentration of the metal in the solution.

*Ocimum basilicum* compared to *Salvia coccinea* presented a higher sensitivity towards the used lead concentrations.

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