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# PHYSIOLOGICAL AND ANTIOXIDANT RESPONSES OF *ASPLENIUM SCOLOPENDRIUM* TO ELEVATED AMOUNTS OF LEAD IN SOIL

***Oana Alexandra Drăghiceanu, Liliana Cristina Soare, Monica Popescu***

***Key words**: pollution, heavy metals, ferns*

## INTRODUCTION

Widely spread and easy to extract and process, lead was among the first metals used by humans. It can be found in the Earth's crust in concentrations up to 15 ppm, and its main feature is persistence. According to Skerfving and Bergdahl (2015) "Inorganic lead is the most often studied toxic agent", this being due to the increasing anthropogenic pressure on the environment.

In addition to mining and processing activities, the main sources of pollution are: using Pb batteries, leaded petrol, paints, waste incineration, production and distribution of electricity, effluents from sewage plants. Lead pollution is also due to pesticides (lead arsenate), fertilizers (triple super $PO^{4-}$ containing 11.1 µg Pb $g^{-1}$, Tilemsi RP with 29,2 µg Pb $g^{-1}$, etc.) and the amendments applied to the soil (compost 1 has 7.5 µg Pb $g^{-1}$) (Raven and Loeppert, 1997; Shreene, 2010).

Considered by Environmental Protection Agency as the most common heavy metal in the environment, Pb occurs mainly in the upper layers of the soil in inorganic state (PbS associated with oxides of Mg, Fe and Al hydroxides) (Ahmad et al., 2015). Soil texture is an important factor in determining the Pb concentration: in fine texture soils the amount of Pb can reach as much as the 3,889 mg $kg^{-1}$, and in coarse soils the concentration is lower (530 mg $kg^{-1}$) (Shreene, 2010).

Due to the strong bond to the soil particles, lead is considered the most immobile heavy metal in the soil. Soil characteristics influence the availability of Pb, namely macroporosity favours its mobility and transport (Karathanasis, 2000), the high pH leads to the dissolution of humic acid and the dissolution of Pb in soil, and at neutral pH it increases Pb solubility due to the formation of organic compounds due to the growth in the dissolved organic material (Shreene, 2010).

Lead has no biological function, it cannot be broken down into less toxic forms, easily enters the food chain and affects plant, animal and human health. It has been found that, in plants, lead influences physiological processes: photosynthesis, absorption of elements resulting in chlorosis and slowing down growth (Sengar et al., 2008; Roberts et al., 2014). Photosynthesis is directly affected by interference of lead with the reactions caused by light or indirectly, by lead action on the synthesis of carbonates (Carelli et al., 1994). Pb interacts with the enzymatic -SH groups, inhibiting the activity of many enzymes (Sengar et al, 2008) and causes cell damage by the oxidative stress caused by the production of species of free radicals: ($O^{2-}$, OH, $H_2O_2$), which reacts very quickly with DNA, lipids and proteins (Zou et al, 2010). The aim of the research was to evaluate the physiological response and the defensive potential of the species *Aspleniumscolopendrium* exposed to different concentrations of Pb added to the soil as acetate.

## MATERIALS AND METHODS

Mature samples of *Asplenium scolopendrium* collected in the Vâlsan Valley were planted in pots with soil to which lead was added. The experimental variants were: 0,V1=250, V2=500, V3=1,000 and V4=500 mg $Kg^{-1}$ $Pb^{+2.}$ The plants were maintained in this experimental system for 3 months, and every 3 days were watered with 200 ml of distilled water. Air temperature and atmospheric humidity were measured (August: 26°C±2, September and October: 20°C±2) and (39% in August, other months 47%). At regular intervals the pots were moved to ensure uniform conditions. For each experimental variant minimum 3 specimens with 4-5 mature leaves were used. The experiment had 3 repetitions.

The evaluation of the physiological response of the species *Asplenium scolopendrium* was done by determining the intensity of photosynthesis and the amount of assimilating pigments: chlorophyll *a* (Chl *a*) and *b* (Chl *b*), carotenoids. To highlight the changes in the antioxidant activity determined by counteracting oxidative stress produced by the heavy metal Pb, catalase activity was determined (CAT).

To determine the intensity of photosynthesis the $CO_2$ analyzer S151 was used, and the results were expressed in $CO_2$ in µmol $CO_2$ $m^2s^{-1}$. The amount of assimilatory pigments was determined from an acetone extract, using a spectrophotometric method by means of Holm's formulae. Catalase activity was determined by titration, and the results were expressed in ml $KMnO_4$ $g^{-1}$ s.p.

## RESULTS AND DISCUSSIONS

For the five different experimental variants, after one month (Figure 1), 2 months (Figure 2) and 3 months (Fig. 3) from the start of the experiment determinations of the intensity of photosynthesis were used.

In Figure 1 are plotted the results obtained after determining the rate of photosynthesis in the first month of influence. It was found that the values were between µmol $CO_2$ $m^2s^{-1}$. Similar values were determined for the control and the first three tested concentrations of Pb (V1, V2, V3).

The significance of the difference between media, analyzed using Duncan test, shows that photosynthesis values recorded for M, V1, V2 and V3 do not differ significantly ($p > 0.05$). A significant inhibition of photosynthesis was observed in the variant V4, compared to the control and to the other concentrations tested.

The significant difference between V4 version and the other variants was also maintained after 2, and respectively 3 months after the initiation of the experiment, while between variants M, V1, V2, V3 there were still no significant differences.

Singh et al. (1997) believes that photosynthesis is one of the most sensitive physiological processes, and the effect of Pb on it is varied.

Inhibition of photosynthesis under the influence of Pb is mentioned in various researches. Thus, inhibition of photosynthesis in maize, soybeans and sunflower under the influence of $PbCl_2$ was reported by Bazzaz et al (1974, 1975). Romanowska et al. (2006) reports reduction in photosynthetic activity in *Pisum sativum* following exposure to Pb.

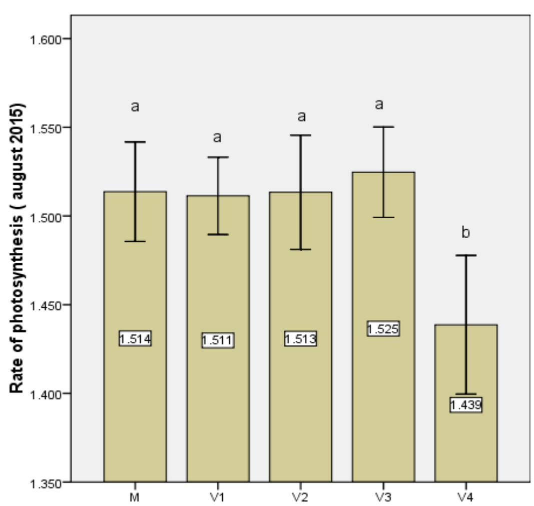


Figure 1. Influence of lead on the intensity of photosynthesis (µmol $CO_2$ $m^2s^{-1}$) in *Asplenium scolopendrium* after one month's influence

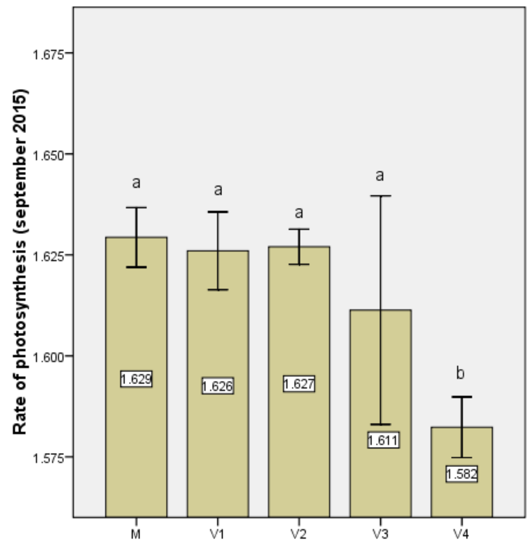


Figure 2. Influence of lead on the intensity of photosynthesis (µmol $CO_2$ $m^2s^{-1}$) in *Asplenium scolopendrium* after 2 months' influence

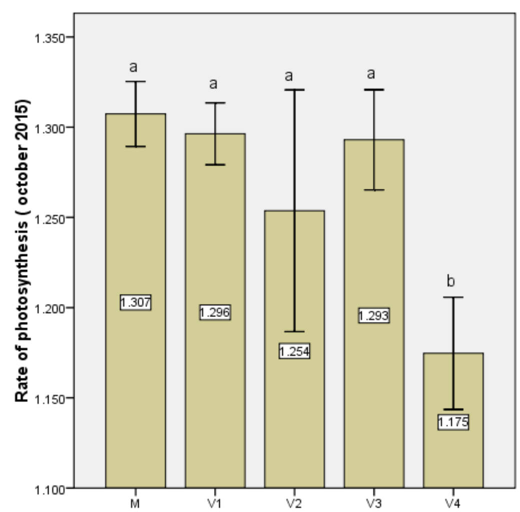


Figure 3. Influence of lead on the intensity of photosynthesis (µmol $CO_2$ $m^2s^{-1}$) in *Asplenium scolopendrium* after 3 months' influence

The fact should be noted that the endoderm of the root acts as a barrier limiting Pb translocation from root to stalk and leaves (Sobotik et al. 1998; Sharma and Dubey, 2005).

Also, plants avoid stress produced by heavy metals through various mechanisms of tolerance and resistance (Ekmekçi et al., 2009), one of these mechanisms being the retention of heavy metals at the root (Fernandes and Henriques, 1991). Ekmekçi et al., 2009, states that "plants avoid stress induced by Pb by binding it to the cell wall."

As can be seen in Fig. 4, chlorophyll *a* ranged between 1.216 (mg $g^{-1}$ s.p.) and 1.192 (mg $g^{-1}$ s.p.); in fig. 5 chlorophyll *b* varies between 0.794 (mg $g^{-1}$ s.p.) and 0.778 (mg $g^{-1}$ s.p.), and in Fig. 6, and for carotenoid pigments values ranged within [.387-0.406]; the amount of assimilating pigments was not significantly affected by treatment with Pb.

In the leaves of *Monochoria korsakowi* the content of chlorophyll *a* decreased by over 50% at concentrations of 20, 50 or 100 mg $Pb^{2+}$, while chlorophyll *b* did not drop significantly, and carotenoids were not influenced (Kim et al., 2008).

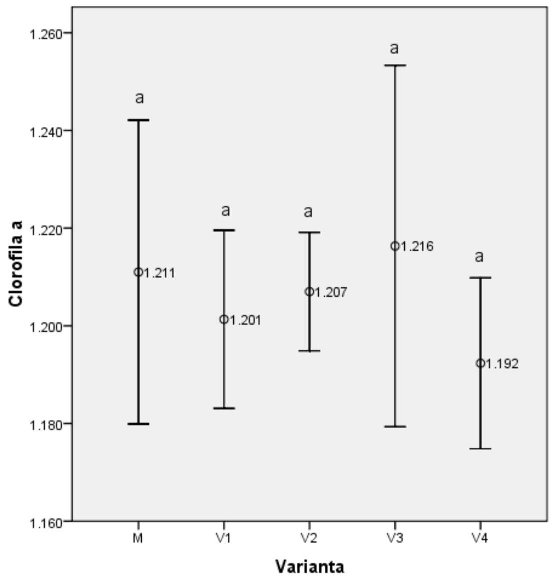


Figure 4. Influence of lead on the amount of chlorophyll *a* (mg $g^{-1}$ s.p.) in *Asplenium scolopendrium* after 3 months' influence

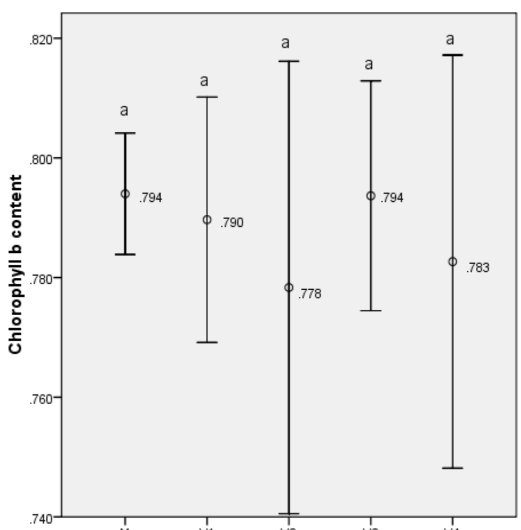


Figure 5. Influence of lead on the amount of chlorophyll *b* (mg $g^{-1}$ s.p.) in *Asplenium scolopendrium* after 3 months' influence

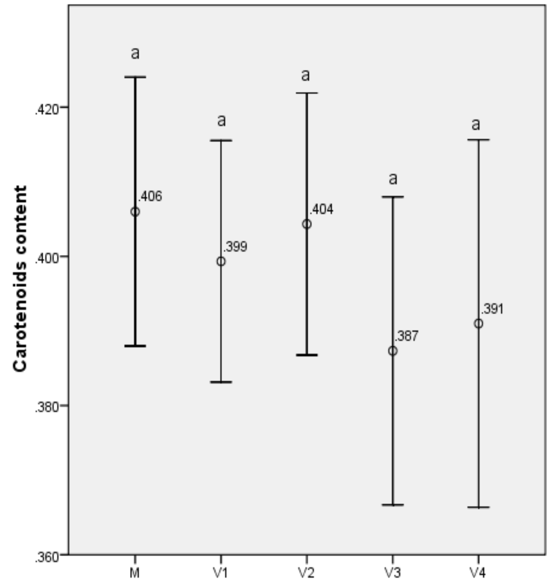


Figure 6. Influence of lead on the amount of carotenoid pigments in *Asplenium scolopendrium* after 3 months' influence

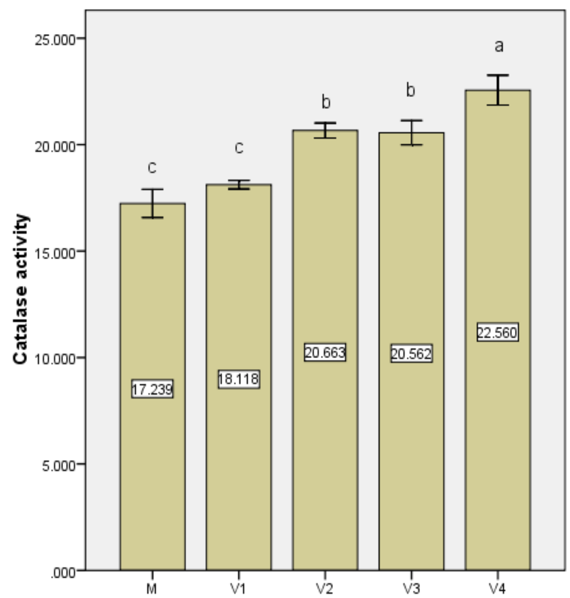


Figure 7. Influence of lead on catalase activity (ml $KMnO_4$ $g^{-1}$ s.p.) in *Asplenium scolopendrium* after 3 months' influence

In the experiment conducted by Roberts et al. (2014) on the *Azolla caroliniana-Anabaena* association it was observed that, at 1 mg Pb $L^{-1}$, the chlorophyll production was affected very little; chlorophyll *b* significantly decreased only in the Pb concentration of 5 mg Pb $L^{-1}$, and the carotenoids significantly decreased at concentrations of 5, 10, 20 mg Pb $L^{-1}$. In the case of antocyans there were no significant differences.

When comparing the results one should also take into account the following aspect: the toxic effects of Pb occur at much lower concentrations in hydroponic systems compared with the soil, because of the humic and inorganic substances that Pb is linked to, so that it is less available for plants (Carelli et al., 1994).

For wheat, regardless of concentration (0, 100, 500, 2000 mg Pb $kg^{-1}$), in the initial stages Pb stimulated the synthesis of chlorophyll, and in later stages it inhibited it, and affected the activity of catalase (Liu et al. , 2010).

Catalase activity (Fig. 7) intensified with increasing amounts of lead in the soil: the increase was 30.86% in the plants exposed to 1,500 mg $Kg^{-1}$ $Pb^{+2}$ compared to the control, which resulted in significant differences (the Duncan test).

CAT can reduce $H_2O_2$ into $H_2O$ and $O_2$, maintaining a low level of ROS (John and Scandalios, 1993).

The increase in catalase activity, due to increased concentrations of Pb, was reported in the literature by Naderi et al. (2013) for sugar beet, an also by Hosseini et al. (2007) for two varieties of *Brassica napus*.

## CONCLUSIONS

*Aspleniumscolopendrium* shows tolerance to concentrations of lead ranging between 250 and 1,500 mg $kg^{-1}$. After three months of influencing, the intensity of photosynthesis is inhibited significantly in the presence of a quantity of 1,500 mg $kg^{-1}$. Under the influence of oxidative stress caused by the increased concentration of lead an increased activity of catalase occurs. The results show that *Asplenium scolopendrium* possess effective antioxidant protection mechanisms against increased concentrations of lead in soil.


## ABSTRACT

The heavy metal lead is a persistent environmental pollutant. Our studies were carried out to evaluate physiological response and defensive potential of *Asplenium scolopendrium* exposed to different concentrations of lead added in soil as lead acetate. Plants were exposed to 0, 250, 500, 1000 and 1500 mg $Kg^{-1}$ $Pb^{+2}$ for 3 months. Plants possessed efficient enzymatic antioxidant defense mechanism that played important role in limiting stress. The physiological response of *Asplenium scolopendrium* was investigated by photosynthesis rate and assimilatory pigments (chlorophyll and carotenoids), while the antioxidant defense was evaluated by catalase activity. After 3 months of influencing, the photosynthesis rate is significantly inhibited by high concentration of lead (1500 mg $Kg^{-1}$ $Pb^{+2}$). Lower concentrations of lead not produced significant changes in the intensity of photosynthesis. Chlorophyll and carotenoid contents were not affected significantly by Pb treatment. The activity of catalase noted an increase with increasing amount of lead in the soil (an increase of 30.86% in V4 plants, in presence of 1500 mg $Kg^{-1}$ $Pb^{+2}$, compared to the control plants).


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**AUTHORS' ADDRESS**

DRĂGHICEANU OANA ALEXANDRA, SOARE LILIANA CRISTINA, POPESCU MONICA - Universitatea din Pitești, Facultatea de Științe, Centrul de Cercetări pentru Protecția Naturii, e-mail: oni_k_a@yahoo.com; soleil_cri@yahoo.com; monica_26_10@yahoo.com.