Impact of lead on the amount of chlorophyll and carotenoids in the leaves of *Triticum durum* and *T. aestivum, Hordeum vulgare* and *Avena sativa*

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**Abstract**

Lead is one of the most dangerous pollutants to both the environment and humans. It causes structural changes in photosynthetic apparatus and reduced biosynthesis of chlorophyll pigments inhibits carbon metabolism. The aim of our study was to determine the dynamics of photosynthetic pigments in leaves of wheat (*Triticum durum* and *T. aestivum*), barley (*Hordeum vulgare*) and oats (*Avena sativa*) at different lead acetate, Pb(CH₃COO)₂ levels: 0, 0.15, 0.30 and 0.60 g/L. The results of this research indicate that these concentrations significantly affected chlorophyll content of *H. vulgare* and *A. sativa* as compared to *T. durum* and *T. aestivum*. Analysis of variance showed that lead concentration and interaction between cereal species had a significant effect on all chlorophyll characteristics at 0.1% probability and on carotenoids contents at 1% significance. Lead acetate in 0.3 and 0.6 g/L concentrations had a highly significant effect on chlorophyll *a, b* and carotenoids in *H. vulgare* seedlings, its carotenoids contents increased from 0.002 mg/g FW at 0 g/L to 0.107 mg/g FW at 0.6 g/L, whereas its chlorophyll content decreased under heavy metal stress, corresponding to the concentration of the metal ion. Carotenoids of *A. sativa* were not affected compared to Chl *a* and Chl *b*, while higher concentrations significantly increased chlorophyll contents of the seedlings from 1.384 mg/g FW of total chlorophyll at 0 g/L to 1.838 mg/g FW at 0.6 g/L. The increased amount of carotenoids was indicative of the formation of free radicals in plants under heavy metal stress, while decreased levels of chlorophyll content were an indication of reduction in the growth of the plants leading to decrease in the yield. It is suggested that chlorophyll content can be adopted as a very useful *in vivo* indicator of heavy metal toxicity.

**Keywords** species response; pigments; contaminated soils; oxidative damage; stress factor.

**Introduction**

Heavy metals, for example lead (Pb), occur naturally on the earth’s surface and are released during the weathering process. However, human activities such as disposal of industrial and domestic waste water, car emissions, Pb acid batteries wastes, paints and treated woods and the use of different organic and mineral composts are the primary sources of Pb contamination (Srivastava et al., 2015). It poses an immense threat to all organisms (Shulman, 2017; Kabir et al., 2018; Kozak & Brygadyrenko, 2018). Lead-contaminated soils cause the deterioration of soil richness and efficiency of CO₂ as a result of stomatal closing (Sharma & Dubey, 2005). Furthermore, it causes accumulation of a large number of ROS, which disrupt the ultrastructure of cellular organelles especially the cell membranes (Shahid et al., 2015). The later reduction of molecular oxygen to H₂O yields the intermediates O₂⁻, HO• and H₂O₂, which are potentially toxic, because they are causing reactions from other chemicals, compared to O₂ (Schützendübel et al., 2002). Despite heavy metal toxicity, several plants are able to keep out, compartmentalize, accumulate or hyperaccumulate heavy metals and can also develop a wide range of adaptive strategies (Ahmad et al., 2011). Among the studied plants (Souahi, 2021), there were species that responded to stress conditions to a lower degree; there were also species that significantly reacted to stress conditions.

The objective of the research presented here was the dynamics of photosynthetic pigments of crops after the plants’ exposure to Pb(CH₃COO)₂.

**Materials and methods**

Four species of cereals (*Triticum durum* Desf. cv. WAHA and *T. aestivum* L. cv. HDR1, *Hordeum vulgare* L. cv. RIHANE and *Avena sativa* L. cv. AVONE), obtained from the Algerian Interprofessional Cereals Office (OAI) of Tebessa, were used in the experiment on the effects of lead. Healthy and homogenous seeds were soaked for 10 minutes in 10% (v/v)
solution of sodium hypochlorite, after rinsing three times in distilled water. After testing the seeds’ germination on filter paper in Petri dishes, the seedlings were transplanted to pots, filled with a mixture of sand/compost for cultivation to a solution containing control, 0.15, 0.30 and 0.60 g/L, supplied as lead acetate every 48 h, with four plants per replicate cultivated in a greenhouse. After 8 weeks of the stress, leaves of each variant were taken to measure photosynthetic pigments.

Pigments were extracted by grinding 0.1 g freshly sampled leaves in 80% acetone at room temperature for 72 h in the dark according to Arnon (1949). Photosynthetic pigments of all samples were extracted in triplicate to minimize experimental errors. Immediately afterward, absorbance at 467, 436, and 663 nm was measured on a Biomate 5 spectrophotometer to calculate chlorophyll \( a \), chlorophyll \( b \), and carotenoids (xanthophylls + carotenes) using the formulas indicated by Lichtenthaler (1987).

\[
\text{Chl } a (\mu\text{g/mL}) = (12.7 \times \text{DO}_{647}) - (4.68 \times \text{DO}_{663}),
\]

\[
\text{Chl } b (\mu\text{g/mL}) = (22.9 \times \text{DO}_{647}) - (4.68 \times \text{DO}_{663}),
\]

\[
\text{Carotenoids (\mu g/mL)} = (5 \times \text{DO}_{470}) - (2.846 \times \text{DO}_{663}) - (14.876 \times \text{DO}_{647}).
\]

Tubes were protected from light throughout the process. The data for different photosynthetic pigments were subjected to two-way analysis of variance. The experiment was arranged in two factor (stresses and lead acetate application) factorial arrangements (4 × 4 × 3) with three replications. Mean data along with standard errors are presented in the Figures and compared at 5% significance level using post-hoc tests (Bonferroni correction).

Furthermore, normality and homogeneity of all the data were analysed using the statistical program XLstat 7.5.2 (Addinsoft, Paris, France).

Results

Analysis of variance in Table 1 showed that heavy metal concentration and interaction between cereals species had significant effect on all chlorophyll characteristics at 0.1% significance level.

Table 1

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Chlorophyll ( a )</th>
<th>Chlorophyll ( b )</th>
<th>Carotenoids</th>
<th>S.O.V</th>
<th>Chlorophyll ( a )</th>
<th>Chlorophyll ( b )</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal species</td>
<td>9</td>
<td>380.10**</td>
<td>36.77**</td>
<td>Concentration (B)</td>
<td>3</td>
<td>1.769</td>
<td>9.051***</td>
</tr>
<tr>
<td>A x B</td>
<td>9</td>
<td>10.290***</td>
<td>16.485**</td>
<td></td>
<td>3</td>
<td>1.769</td>
<td>9.051***</td>
</tr>
</tbody>
</table>

Note: * - P < 0.05; ** - P < 0.01; *** - P < 0.001.

During the experiment, from the obtained data it was evident that the samples in the control group (without the addition of Pb(CH_3COO)_2) to Hordeum vulgare had higher concentration of chlorophyll \( a \) (Fig. 1), chlorophyll \( b \) (Fig. 2) and chlorophyll \( t \) (Fig. 3) compared with other experimental samples. It should be noted that concentration of carotenoids in chloroplasts of barley (Fig. 4) that grew in the substrate concentrations of Pb(CH_3COO)_2: 0.15, 0.30 and 0.60 g/L significantly increased as early as in the first week of the experiment. It was experimentally determined that the amount of carotenoids in the samples that had been exposed in the substrates to the concentrations of 0.15, 0.30 and 0.60 g of PbL increased with the decrease in the amounts of chlorophyll \( a \) and chlorophyll \( b \).

The amount of chlorophyll \( a \) decreased by 20.1% and 23.3% respectively after exposure to 0.30 and 0.60 g/L, whereas chlorophyll \( b \) decreased by 76.7%, 53.4% and 55.7%, and the amount of chlorophyll \( t \) decreased by 25.1%, 33.4% et 36.3%, compared with the results of the control samples. The dose of 0.15 g/L increased the amount of carotenoids to 0.158 mg/g FW, 0.3 g/L to 0.164 mg/g FW and 0.6 g/L to 0.181 mg/g FW (P < 0.01). Chlorophyll content was significantly affected by Pb treatment, especially in Avena sativa. It is interesting to note that the contents of chlorophyll \( a \), \( b \) and carotenoid pigments increased progressively with increasing concentration of Pb dose. There was a two-fold increase in Pb-treated seedlings compared with the control.

The amount of chlorophyll \( a \) increased to 0.984 mg/g FW after exposure to the dose 0.15 g/L, to 1.007 mg/g FW after 0.30 g/L and to 1.020 mg/g FW after 0.60 g/L (P < 0.001) as compared with the control.

The dose of 0.30 g/L increased the amount of chlorophyll \( b \) by nearly 26.9% and the dose of 0.60 g/L (P < 0.001) caused 34.4% increase, while chlorophyll \( t \) increased to 1.653 mg/g FW after influence of 0.15 g/L, to 1.822 mg/g FW after 0.30 g/L, and to 1.883 mg/g FW after 0.60 g/L.

Discussion

Our study of the natural senescence of the barley plants demonstrated that the prolonged action of high concentrations of Pb(CH_3COO)_2 affected the photosynthetic apparatus of the plants. This led to the initiation of the chloroplast degradation process and, as a consequence, destruction of chlorophyll \( a \) and chlorophyll \( b \) with increasing synthesis of carotenoids. Protection mechanisms required until the chloroplasts were completely destroyed. These processes can be considered as responses to the ROS.
Fig. 4. Effect of different lead concentration on carotenoids in the leaves of species of cereals (x ± SD, n = 3); P < 0.05 with Bonferroni correction

Carotenoids are available in all photosynthetic organisms and are very important constituents of the thylakoid membrane in chloroplasts (Parida et al., 2008). There are evidences that carotenoids serve as a defence in important constituents of the thylakoid membrane in chloroplasts (Parida et al., 2008). Stomatal conductance of lead-stressed plants was reported to reduce by 40–50% as compared with control. Reduction in leaf area, vascular bundles and total chlorophyll contents, and reduced CO2 influx because of stomatal closure are the vital reasons for the shortened photosynthesis under lead stress (Romanowska et al., 2006). Wearysko-Chmielewska & Chwiel (2005) reported that lead stress harmed the ultrastructure of chloroplasts due to strong affinity for nitrogenous and sulfuric ligands of protein. Qufei & Fashui (2009) stated that accumulation of lead in leaves damaged the secondary structure of photosystem II in duckweed ( Spirodicta polysiphonita (L.) Schleid ) and reduced the assimilation and transfer of energy among different enzymes. It has been reported to change activities of photosystem I as well as photosystem II in peas ( Pisum sativum L. ). It reduced the rate of electron transport during Hill chemical reaction and inhibited cyclic as well as non-cyclic photophosphorylation (Romanowska et al., 2008).

References


