

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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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, $\text{Pb}(\text{CH}_3\text{COO})_2$ 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 carotenoid 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 1.883 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 lead to a great reduction in crop yield (Ashraf et al., 2015). Because lead is a harmful metal with a notable mobility from soil to plant, it has been investigated seriously, especially in terms of its effect on food quality and safety (Uzu et al., 2009). The most extreme acceptable weekly intake of Pb in human food is about 25 $\mu\text{g}/\text{kg}$ of human body weight (Fang et al., 2014). It is available in small amounts in almost all food crops and its concentration is considerably heightened when crops are grown in Pb-contaminated soils.

Furthermore, farmland heavy metal contamination often results in elevated Cd and Pb concentrations in crops, such as in wheat (*Triticum aestivum* L.) or rice (*Oryza sativa* L.) (Khan et al., 2008; Li et al., 2014; Zhao et al., 2015; Xing et al., 2016). As a result, both the contaminated soil itself and the on-site crops harvested may contain elevated heavy metal concentrations, leading to substantial negative effects on the health of local residents and those that consume these food products (Yeganeh et al., 2013; Chen et al., 2018; Kribek et al., 2019).

Lead has been reported to considerably impair biochemical and physiological processes in plants, such as plant ontogeny, cell membrane permeability, chlorophyll contents, photosynthesis, plant respiratory processes and cell division (Souahi et al., 2017; Zhou et al., 2018). Among different metabolic processes, photosynthesis is one of the most significant

physiological traits of plants. However, it has been reported to be negatively affected by various heavy metals (Shu et al., 2012). Previous studies (Heckathorn et al., 2004; Kambhampati et al., 2005) reported that plants exposed to Pb ions showed a relatively large decline in the total chlorophyll and that photosynthetic efficiency. It has been shown that plants exposed to lead ions exhibited a decrease in the photosynthetic rate because of distorted chloroplast, limited synthesis of chlorophyll, blocked electron transport, stopped activities of Calvin cycle enzymes, as well as deficiency of CO_2 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 H_2O yields the intermediates O_2^- , HO^\cdot and H_2O_2 , which are potentially toxic, because they are causing reactions from other chemicals, compared to O_2 (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 $\text{Pb}(\text{CH}_3\text{COO})_2$.

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 (OAIC) 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 647, 663, and 470 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).

Chl *a* ($\mu\text{g/mL}$) = $(12.7 \times \text{DO}_{663}) - (2.69 \times \text{DO}_{647})$,

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

Chl *t* ($\mu\text{g/mL}$) = (Chlorophyll *a* + Chlorophyll *b*),

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

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 ($4 \times 4 \times 3$) 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

Analysis of variance of the measured traits

S.O.V	Df	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>t</i>	Carotenoid
Cereal species (A)	3	405.29***	308.106***	529.25***	36.77***
Concentration (B)	3	3.737*	1.769	0.380	9.051***
A x B	9	10.290***	13.762***	16.485***	3.971**

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 $\text{Pb}(\text{CH}_3\text{COO})_2$) 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 $\text{Pb}(\text{CH}_3\text{COO})_2$: 0.15, 0.30 and 0.60 g of Pb/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 Pb/L 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% and 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 inclusion 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.

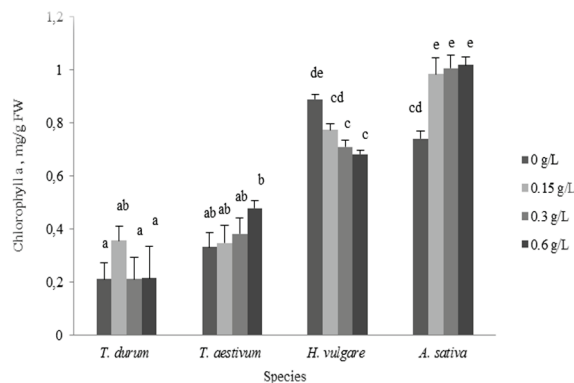


Fig. 1. Effect of different lead concentration on chlorophyll *a* in the leaves of species of cereals ($x \pm \text{SD}$, $n = 3$); $P < 0.05$ with Bonferroni correction

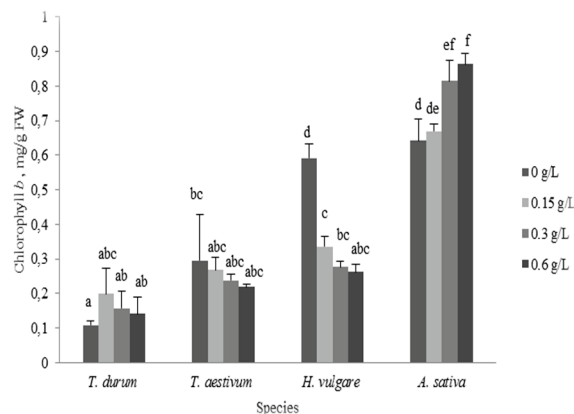


Fig. 2. Effect of different lead concentration on chlorophyll *b* in the leaves of species of cereals ($x \pm \text{SD}$, $n = 3$); $P < 0.05$ with Bonferroni correction

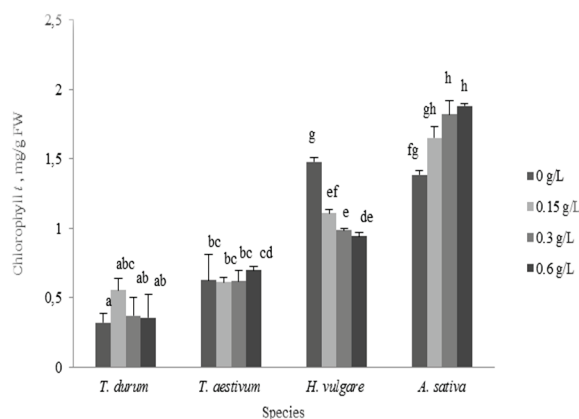


Fig. 3. Effect of different lead concentration on chlorophyll *t* in the leaves of species of cereals ($x \pm \text{SD}$, $n = 3$); $P < 0.05$ with Bonferroni correction

Discussion

Our study of the natural senescence of the barley plants demonstrated that the prolonged action of high concentrations of $\text{Pb}(\text{CH}_3\text{COO})_2$ 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 occurred until the chloroplasts were completely destroyed. These processes can be considered as responses to the ROS

generation in the cell under adverse abiotic factors in the first stage, including the mechanism of protection of plant chloroplasts.

Excessive level of toxic elements usually caused reduction of physiological and biochemical processes in plant growth (Souahi et al., 2014; Souahi et al., 2016). Inhibition of the photosynthetic pigment biosynthesis is one of the primary effects on plants subjected to heavy metals, including Pb (Li et al., 2014; Hou et al., 2017). Previous authors showed that lead stress results in heavy reduction of chlorophyll (Sengar & Pandey, 1996; Haider et al., 2006; Akinci et al., 2010; Souahi et al., 2017). It has been reported that heavy metals disturb chlorophyll biosynthesis by stopping the key enzymes of photosynthesis or reducing the uptake of Mg ion which makes up the important part of the chlorophyll molecules (Pourrut et al., 2011). Chlorophyll breaks down into Mg, phytol and the primary cleavage product of the porphyrin rings, the reaction occurring in four steps. This reaction is catalysed by Mg-dechelataase, red chlorophyll catabolite reductase, chlorophyllase, oxygenase and pheophorbide; and after cleavage of porphyrin ring, the typical green colour of chlorophyll is lost (Harpaz-Saad et al., 2007). Though level of toxicity varies among plant species, it is generally related more to chlorophyll *b* than chlorophyll *a* (Xiong et al., 2006). 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). Weryszko-Chmielewska & Chwil (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 (*Spirodela polyrrhiza* (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).

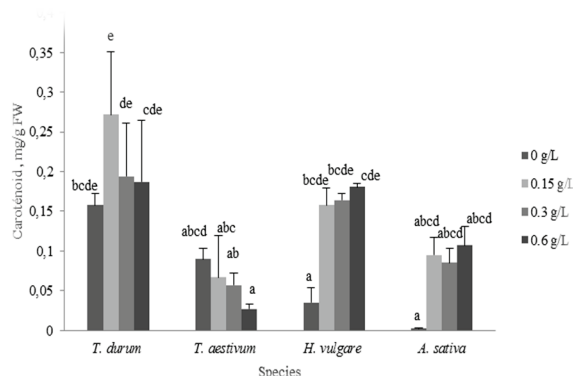


Fig. 4. Effect of different lead concentration on carotenoids in the leaves of species of cereals ($\bar{x} \pm SD$, $n = 3$); $P < 0.05$ with Bonferroni correction

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 chloroplasts and an antioxidative function under oxidative stress, protecting the organic matter (essentially chlorophyll molecule) from the destruction by light in the process of photooxidation and in the stress factors caused by unfavourable environmental factors (Green & Dunford, 1996; Merzlyak et al., 2008).

Plant species differing in Pb tolerance show varying behaviour of certain enzymes under Pb treatment. Igoshina & Kositsin (1990), while studying the effect of Pb on carboanhydrase activities in the tolerant and sensitive species of melic-grass (*Melica nutans*), observed that in a tolerant melic-grass population, Pb activated carboanhydrase activity whereas in the sensitive plants the activity of this enzyme remained unaffected. Azmat et al. (2009) reported that *Phaseolus mungo* and *Lens culinaris* plants undertake adaptive mechanisms aimed at protecting photosynthesis against the damaging effects of lead; foliar morphological modifications were induced by exposure to 1.2 mM Pb, which resulted in an increased

number of trichomes and stomata, thus allowing these species to maintain photosystem II efficiency and reduce water evaporation from the leaves during stress. Accordingly, a recent study (Chen et al., 2019) has found that reduced Chl *a* content accompanied by a significantly increased total phenolic content in leaves of *Kandelia obovata* under high concentrations of heavy metal (Zn) enhances the heavy metal tolerance.

Wheat germplasm provides a large range of genes and rich sources of genetic variation for improving tolerance to heavy metal, which can provide a solution for the current environmental contamination (Alybayeva et al., 2014; Rabnawaz et al., 2017; Ali et al., 2018). Subsequently, it is needed to screen the genetic potentiality of the genotypes under heavy metal stress to evaluate their effect on plant growth and productivity to identify tolerant genotypes (Alybayeva et al., 2016).

Conclusions

Lead has been used by humans since the antiquity because of some useful properties. Nonetheless it interferes with plants directly or indirectly, impairing their enzymatic activity and causing oxidative damage.

It is clear from our results that lead treatment even in low concentrations induces enormous influences on ion uptake by plants, which results in significant metabolic changes, and finally in a strong inhibition of plant growth. *Avena* and *Hordeum* show different susceptibilities to lead treatment and *A. sativa* appears far more resistant. Future experiments will be aimed at identifying mechanisms responsible for the improved protection of *A. sativa* against the harmful effects of lead.

However, several mechanisms behind the lead toxicity in plants are yet not understood well and need further studies. The identification of exact metabolic pathways adapted by plants under lead toxicity at molecular level in connection with plant nutrition is the key area for future research.

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