



Growth and physiological responses of cereals species under lead stress

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Abstract

Cereals are the most important staple foods for mankind worldwide and represent the main constituent of animal feed. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. A study was conducted to determine the effect of different concentrations of lead on morphological parameters (root length), the concentrations of chlorophyll in plant leaves provide information about the physiological state of plants and were determined using a spectrophotometer. Seed were grown under laboratory conditions at 0, 0.15, 0.3 and 0.6 g/l of metal ions of lead. The experiment was evaluated in Petri dishes over a period of 14 days. All results, when compared to control, showed Pb adversely affecting the morphological and physiological parameters of the test plants. All cereal species showed very higher decrease ($p < 0.001$) in radicle length to increased level of Pb (CH_3COO)₂. However, a significant decrease of radicle number for all plants was observed at concentrations 0.6 g/l of metal. The increase in lead concentration also caused a decline in the net rate of chlorophyll total in *Triticoseale wittmack*. Among the 4 studied plants, the most sensitive to Pb exposure were *Triticoseale wittmack*.

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Introduction

Cereal grains were the first agricultural attempts by early man, and people still enjoy them today depending on where they live and what grows there well. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crops; they are therefore staple food crops. In their natural form, they are a rich source of vitamins, minerals, carbohydrates, fats, oils and protein (Sarwar *et al.*, 2013). The increasing demand for food safety stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins. Food safety issues and potential health risks make as one of the most serious environmental concerns (Gebregziabher and Tesfaye, 2014).

Heavy metal pollution has become a worldwide concern due to the increasing levels of pollution and its obvious impacts on human health. These are of great concern from the public health point of view, and being environmental pollutants it can occur naturally in the environment and can come from industrial (e.g. mining, metallurgical, incineration, pesticide etc.) or agricultural sources (e.g. pesticide and fertilizers use). These contaminants are highly toxic and may accumulate in seafood, whose consumption can represent an important route of human exposure to these harmful substances and ultimately threaten human health (Antizar-Ladislao, 2008). In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang and Shi, 2001). Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis (Chang *et al.*, 1996; Wang and Shi, 2001; Beyersmann and Hartwig, 2008).

Therefore, it is important to assess heavy metal pollutant concentrations in crops to ensure safe food production and prevent environmental and public health risks.

The main objective to the following research is to study cereals cultivars responses to lead acetate stress during growth and determining cultivars for optimal tolerance.

Materials and methods

Plant growth and lead treatment

Four species of cereals (*Triticum durum*, *Triticum aestivum*, *Hordeum vulgare* and *Triticoseale wittmack*) were used in the experiment to study the effects of lead (Pb). Experiment was performed in February 2017 in a plant biology laboratory, University of Tebessa, Algeria. Prior to germination, seeds were surface-sterilized with 10% (v/v) sodium hypochlorite for 10 min and rinsed several times with distilled water. Next, 10 seeds were placed in petri dishes (90-mm diameter) on filter paper and were treated separately with solutions containing 0.15, 0.3 and 0.6 g Pb L⁻¹, supplied as lead acetate Pb (CH₃COO)₂. Control treatments were supplied with nutrient solution. The experiment was completely randomized and consisted of four treatments replicated three times.

Studied parameters

The parameters studied during this work are:

Radicle length

Maximum length of seed roots was determined as the longest root length, on average, of the sample of ten seedlings (Simmons *et al.*, 1995).

Number of radicle

This parameter is obtained by counting the total number of roots for each treatment and dividing it by the total number of grains (germinated or not) (Harrièche, 2004).

Chlorophyll content

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 the samples were extracted in triplicate to minimize experimental errors. Chlorophyll contents were measured by using absorbance recorded at 647 nm and 663 nm for maximum absorption of chlorophyll-a and chlorophyll-b respectively.

The extinction coefficients were determined by a UV-Vis spectrophotometer. Pigment contents were calculated in $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight by applying the absorption coefficient equations described by Lichtenthaler (1987).

Statistical analysis

In all experiments, three replicates were performed for each sample, and each treatment was repeated three times. Data presented here are mean values and standard deviation ($\pm\text{SD}$). Two-way analysis of variance (ANOVA) was carried out using post hoc

multiple comparison from the SNK test to determine the difference between the levels of Pb-stress in each studied parameter (a significance level of 0.05 was used for all statistical tests).

Results

The results of a different analysis of the variance (ANOVA) indicated clearly that the treatments operated by the various concentrations of lead, exerted a very highly significant effect on the parameters studied of the different species.

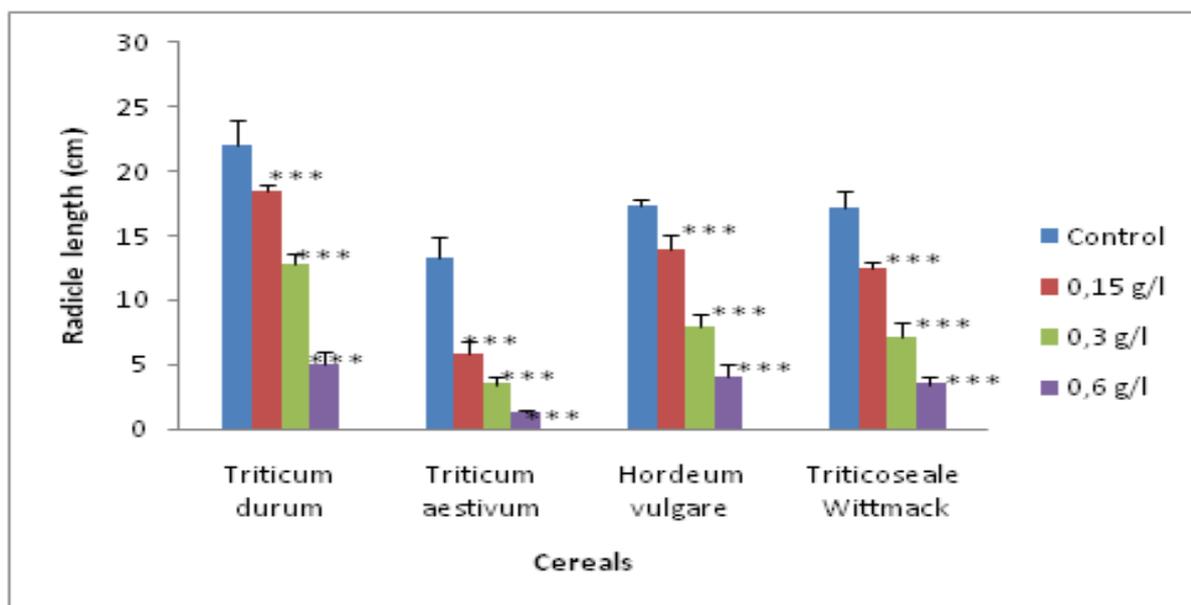


Fig. 1. Effect of lead on radicle length of cereals. Results are the mean of three replicates \pm SD. *Asterisks indicate significant differences between the treatments and the control of the same plant species (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Radicle length

The effects of metal stress on radicle length have been showed in Figure 1. Comparison of radicle length means in different metal levels showed that when metal level increase, seedlings radicle length decrease. In fact, when Pb was absent (0 $\text{g}\cdot\text{l}^{-1}$), radicle length was almost 22 cm in control seeds (*Triticum durum*) as well as in *Triticum aestivum*, *Hordeum vulgare* and *Triticoseale wittmack* seeds. With the presence of Pb (CH_3COO)₂ (0.15 to 0.6 $\text{g}\cdot\text{l}^{-1}$), Pb seems to have an inhibitor action and the length of the radicle is being shortened in depending on the concentration of Pb (CH_3COO)₂. The most reduction in radicle length related to 0.6 $\text{g}\cdot\text{l}^{-1}$ (5, 1.25, 4 and 3,5 cm respectively in *Triticum durum*, *Triticum*

aestivum, *Hordeum vulgare* and *Triticoseale wittmack* plants). But, for all lead levels, reduction was significantly higher in *Triticum aestivum* plant than in other cereals.

Number of radicle

Exposing root vegetables to different levels of Pb resulted in reductions of number as shown in Figure 2. A retarded development in Pb-treated plants compared to the controls was observed. In the presence of 0.6 $\text{g}\cdot\text{l}^{-1}$, significant reduction was found in number of radicle for the four species of cereals ($P < 0.05$), and a marked decrease in root number was observed at 0.15, 0.3 g Pb L⁻¹ in *Triticum durum* Chlorophyll content.

Total chlorophyll content in leaves did not change significantly after treatment of wheat and barley plants with lead acetate (figure 3). In *Triticoseale wittmack* exposed to 0.15, 0.3 and 0.6 g Pb L⁻¹ total chlorophyll contents were markedly reduced from the 14 day of experiment.

Total chlorophyll content was decreased by 25,18 % ($P < 0.05$), 56,83 % ($P < 0.001$) and 50,11 % ($P < 0.001$) on 0.15, 0.3 and 0.6 g Pb L⁻¹ respectively.

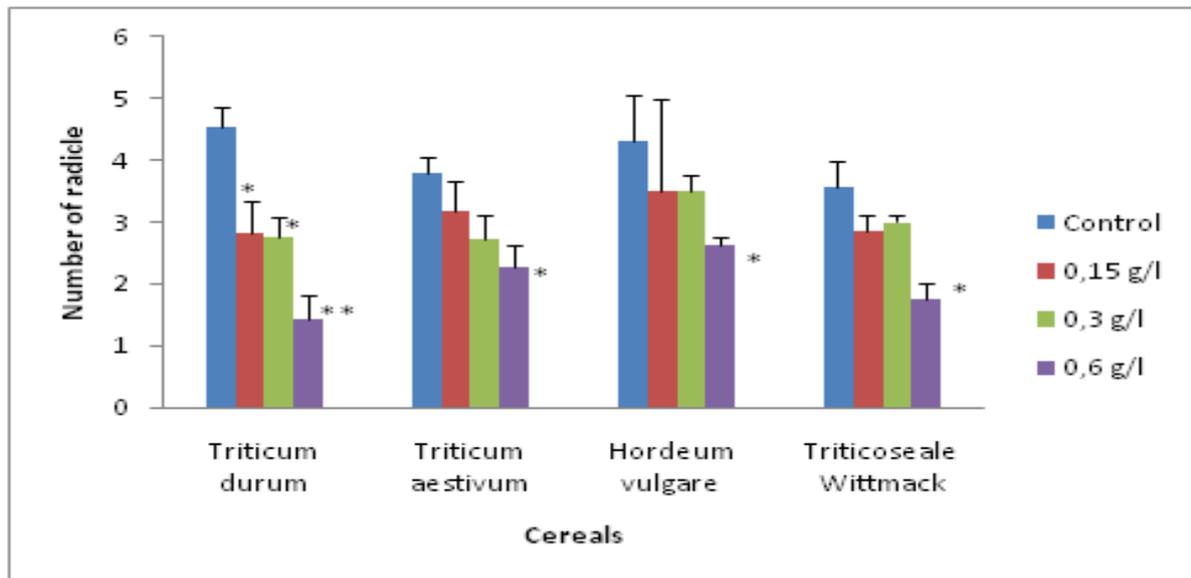


Fig. 2. Effect of lead on radicle number of cereals. Results are the mean of three replicates \pm SD.*Asterisks indicate significant differences between the treatments and the control of the same plant species (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Discussion

Toxic effects of cereals have been widely described by many workers (Souahi *et al.*, 2014; Tegegne, 2015). At low concentrations, lead inhibits the growth of roots and aerial plant parts (Islam *et al.*, 2007; Kopittke *et al.*, 2007). This inhibition is stronger for the root, which maybe correlated to its higher lead content (Liu *et al.*, 2008). Lead toxicity may also cause swollen, bent, short and stubby roots that show an increased number of secondary roots per unit root length (Kopittke *et al.*, 2007). Arias *et al.* (2010) reported significantly inhibited root elongation in Mesquite (*Prosopis* sp.). In several plant species, including *Triticum aestivum* (Dey *et al.*, 2007; Kaur *et al.*, 2013), *Z. mays* L. (Kozhevnikova *et al.*, 2009), *Pisum sativum* (Malecka *et al.*, 2009), and *Sedum alfredii* (Gupta *et al.*, 2010), a decrease in the length and in root dry mass under Pb toxicity have been reported (Mun-zuroglu and Geckil, 2002).

However, the effect of low concentrations is not clearly established, and the observed growth inhibition is not necessarily correlated to a reduction in biomass (Kosobrukhov *et al.*, 2004; Yan *et al.*, 2010).

Photosynthesis inhibition is a well-known symptom of lead toxicity (Xiong *et al.*, 2006; Hu *et al.*, 2007; Liu *et al.*, 2008; Piotrowska *et al.*, 2009; Singh *et al.*, 2010; Cencki *et al.*, 2010). This inhibition is believed to result from the following indirect effects of lead rather than from a direct effect: distorted chloroplast ultra structure from the affinity lead has for protein N and Sligands (Elzbieta and Mirosława, 2005; Islam *et al.*, 2007), decreased ferredoxin NADP⁺-reductase and delta-aminolevulinic acid dehydratase (ALAD) activity at the origin of chlorophyll synthesis inhibition (Gupta *et al.*, 2009; Cencki *et al.*, 2010), inhibition of plastoquinone and carotenoid synthesis (Kosobrukhov *et al.*, 2004; Chen *et al.*, 2007; Liu *et al.*, 2008; Cencki *et al.*, 2010), obstruction of the

electron transport system (Qufei *et al.*, 2009), impaired uptake of essential elements such as Mn and Fe (Chatterjee *et al.*, 2004; Gopal and Rizvi, 2008) and substitution of divalent cations by

lead (Gupta *et al.*, 2009; Cenkeci *et al.*, 2010), inhibition of Calvin cycle enzymatic catalysis (Mishra *et al.*, 2006; Liu *et al.*, 2008), and increased chlorophyllase activity (Liu *et al.*, 2008).

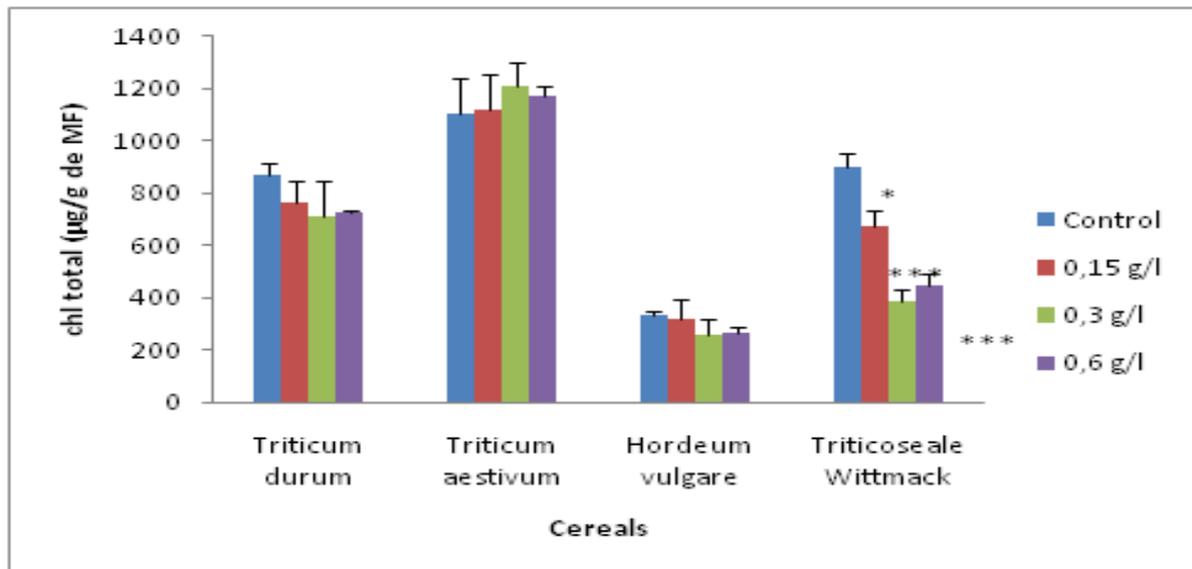


Fig. 3. Effect of lead on the concentrations of chlorophyll total in cereals leaves. Results are the mean of three replicates \pm SD. *Asterisks indicate significant differences between the treatments and the control of the same plant species (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Moreover, the effect of lead toxicity varies with plant species, i.e., hyper accumulators naturally tolerate more lead toxicity than do sensitive plants (Arshad *et al.*, 2008).

Conclusion

The increasing metal pollution of agricultural soils makes necessary studies about the response of crop plants to different levels of contamination in order to evaluate their potential use in phytoremediation.

It is obvious from our results that lead treatment even at low concentrations induces large disturbances in profound metabolic changes (e.g. in photosynthetic capacity), and finally in a strong inhibition of plant growth.

In the assayed conditions, *Triticoseale wittmack* was the most sensitive to the applied Pb concentrations. Future experiments will be aimed at searching for the mechanisms responsible for the improved protection of *Triticoseale wittmack* against the deleterious effects of lead.

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