Research Article

Lead Induced Oxidative Stress and Antioxidant Defense Response in Durum Wheat (*Triticum durum* Desf.)

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ABSTRACT

Early effects of different concentrations of lead (50, 100 and 150µM) in *durum wheat* were assessed by measuring antioxidant enzymes activities (CAT, APX and GPX), lipid peroxidation, glutathione reduced and photosynthetic pigments levels during a kinetico f 0, 24, 48 and 72 hours. Our results show the vulnerability of the root system face the metallic stress compared to that of the leaves. However, Pb^{2+} induced an oxidative stress after 24 h of treatment, coincided with the stimulation of lipid peroxidation and the decrease of photosynthetic pigments. Our data also revealed the importance of catalase, and gaïacol peroxidase which appeared to be the keys enzymes to cope with Pb toxicity.

Keywords: Oxidative stress, antioxidant enzyme, lipid peroxidation, Pb, response.

INTRODUCTION

Worldwide, metal industry and agricultural use of metal-containing fertilizers and pesticides have contributed significantly to metal pollution. Resulting concentrations of toxic metals in the environment often exceed those from natural sources[1].

Heavy metals at extremely micro concentrations affect different cellular components, thereby interfering with the normal metabolic functions. If toxic metals accumulate in crop plants, the uptake of potentially hazardous elements via the food chain tremendously increases in humans[2].

A better understanding of metal-induced molecular, cellular and physiological responses in plants will therefore contribute to the development or adjustment of strategies to alleviate metal-associated risks for human health.

Plants growing on metal-enriched soils suffer from decreased growth and performance, both restricting crop yield. At the molecular level, oxidative stress is widely studied as a key sign of plant stress. This process is commonly described as an imbalance between reactive oxygen species (ROS) and antioxidants in favor of the former[3]. Recently summarized the intense relationship between metal toxicity, redox homeostasis and antioxidant capacity in plants.

Depending on the chemical properties and behavior of metals in biological systems, their toxicity is attributed to either one of the following mechanisms: (ii) interference with functional sites in proteins; (i) displacement of essential elements, thereby disturbing enzymatic functions; or (iii) enhanced ROS production[3]. Redox-active metals such as Cu and Fe directly induce ROS production through Fenton and Haber-Weiss reactions[4].

Non-redox-active metals (Cd, Pb and Zn) however only enhance ROS production via indirect mechanisms such as inhibiting enzymes functioning in the cellular antioxidative defense network[5].

Lead (Pb) is one of the major widespread toxic metals found in the environment with potential danger to human health and to ecosystems.

Plants can uptake Pb from soil solution by roots but, the largest proportion of Pb^{2+} is accumulated in roots in an insoluble form[6].

Lead can cause a broad range of physiological and biochemical dysfunctions on the plant growth, water status, mineral nutrition or nitrate assimilatio[7,8].

Although Pb transport from plant roots to shoots is usually very low[9], photosynthesis is especially affected by lead exposure[10].In particular, Pb2+ ions decrease chlorophylls and carotenoids contents, photosynthetic rate and Co_2assimilation.

This metal is known to interfere with morphological, physiological and biochemical functioning of exposed plants and can induce a broad range of noxious effects such as, decrease in seed germination, root elongation, and plant biomass and inhibition of chlorophyll biosynthesis. Inside the cell, Pb can affect respiration and enzyme reactions[11].

The presence of Pb in plants can also induce the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which can disrupt the redox status of cells and cause lipid peroxidation[12]. According to[11], Pb-induced toxicity to plant is closely related to its form in the soil solution.

To minimize this, cells are equipped with enzymatic and non-enzymatic mechanisms to eliminate or reduce their damaging effects.

These protective mechanisms include antioxidant enzymes such as catalase, peroxidase like ascorbate (APX) and guaiacol peroxidase (GPX) and other antioxidant compounds such as glutathione, carotenoids, etc.[13]

The present study aims to investigate the effects of different concentrations of lead on *wheat* plants, following exposure after 24, 48 and 72 hoursby studies effects of Pb and lead-induced oxidative stress on photosynthetic pigments and total protein contents, lipid peroxidation and glutathione reduced levels and antioxidant enzymes catalase, ascorbate peroxidase and gaïacol peroxidase.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The uniform seeds of wheat (*Triticum durum* Desf. cv Cirta) were surface sterilized with 5% NaOCl for 15 min, rinsed with distilled water, imbibed for 24 h in room temperature, and then transferred to the gauze on the tap of plastic aerated pots containing ½ Hoagland nutrient medium (pH 6.2). The plants were grown in a self-regulating culture room and the light/dark period was 16/8 h, temperature 25/20 °C, and relative humidity 75%.

They were grown in adapted 'Hoagland solution' (Hoagland and Arnon 1950), continuously aerated containing: KNO_3 3mM, Ca $(NO_3)_2$ 1 mM, KH_2PO_4 2 mM, $MgSO_4$ 0.5mM, Fe-Ethylene diamine tetra acetic acid (EDTA) 32.9µM, and micronutrients: H_3BO_4 30 µM, $MnSO_4$ 5 µM, $CuSO_4$ 1 µM, $ZnSO_4$ 1 µM, and $(NH_4)6Mo_7O$ 1 µM for 10 days.

After growing for 15 days (3-leaf stage), plantlets were subjected to 50, 100 or 150mM PbCl2. After 24, 48 and 72 h of root métallique stress, the shoot samples were collected, washed for 2 min by distilled water, immediately preserved to assay for various biochemical estimations.

Chlorophyll Assay

Chlorophyll was extracted in 80% acetone. Absorbance was measured at 663 and 645 nm by a spectrophotometer. Extinction coefficients and equations reported by[14] were used to calculate the amounts of Chl a, Chl b, Chl a+b and carotenoids. Measurements were done in triplicate.

Determination of Lipid Peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS), and product of lipid peroxidation[15]. Samples (0.2 g) are ground in 3 mL of trichloroacetic acid (0.1%, w/v). The homogenate was centrifuged at 10,000 ×g for 10 min and 1 mL of the supernatant fraction was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min, chilled on ice, and then centrifuged at 10,000 ×g for 5 min.

The absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

Antioxidant Enzymes Activity Analysis

Fresh leaves were homogenized in 50 Mm potassium phosphate buffer pH 7.6, the homogenized samples were centrifuged at 12000 ×g for 20 min and the supernatant was used as crude enzyme extract in CAT, APX and GPX[16].

Catalase (CAT) activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2[17].

The reaction mixture 3 ml contained 50 mM phosphate buffer pH 7.2, 15 mM H_2O_2 and 100 µl of crude enzyme extract. The activity was calculated using the extinction coefficient 39400 $M^{-1}cm^{-1}$.

Ascorbate peroxidase (APX) activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µl of crude enzyme extract18. The activity was calculated using the extinction coefficient 2800$M^{-1}cm^{-1}$.

For Guaïacol peroxidase (GPX) activity, the reaction mixture consisted of 50 mM potassium phosphate, 9 mM guaïacol buffer pH 7.2, 50 µ H_2O_2 and 100 µl of crude enzyme extract. The enzyme activity was measured by monitoring the increase in absorbance at 470 nm extinction coefficient of 2470 M^{-1} per cm during polymerization of guaïacol[19].

RESULTS AND DISCUSSION

Lead effects on photosynthetic pigments were presented on Figure 1. Data show that pigment composition was not affected until 48 h. At 72 h, we noted a significant reduction in total chlorophyll (Figure 1c), which reached 28% after treatment with 150 mM of lead. This reduction only affects chlorophyll b (Figure 1b) while an insensitivity of chlorophyll a (Figure 1a) was noted. The different Pb concentrations used have no effect on carotenoid content (Figure 1d) during the treatment period.

Our study on the effect of Pb on the different photoreceptor pigments showed, in our case, a total chlorophyll reduction after treatment for 72 h.

This reduction is more pronounced at high concentrations (100 and 150 mM) of Pb. This reduction only affects chlorophyll b while the chlorophyll a and carotenoids showed no changes in their levels compared to the control. In our case, total chlorophyll shows a decrease at

the end of the treatment, it may be due to the low pass of the metal ion to the aerial parts during the first days of treatment.

Degradation of chlorophylls is a well-known aspect of lead toxicity[8]. Lead toxicity on photosynthetic pigments is associated to its availability to inhibit their biosynthesis pathways[20].

However, in our case, according to the dose and period of lead treatment, it is very unlikely that this phenomenon could be due to biosynthesis inhibition. It could be due to the stimulation of chlorophyllase as demonstrated by[21].

However, pigment contents were inversely correlated with lipid peroxidation. This correlation indicates that Pb action on photosynthetic pigments can be mediated by ROS. Chl b is known to be more sensitive to lead than Chl a[22].

The accumulation of malondialdehyde was followed during the period of treatment with Pb in the roots and leaves of *durum wheat*. The Figure 2a shows that at leaf level, the amount of MDA showed no changes compared to controls throughout the treatment for the three metal treatment (50, 100 and 150 mM). The root level (Figure 2b), MDA accumulation varies depending on dose and time of treatment.

Indeed, concentrations of 50 and 100 mM of Pb are capable to increasing the production of MDA from about 30% compared to the control after 48 hours of treatment, while a concentration of 150 mM this increases production 43 %.

For a longer exposure (72 h), membrane lipid peroxidation remains more pronounced in the presence of 100 and 150 mM metal. Indeed, this stimulation was 71% compared to the control in the roots treated with 100 mM and reached 100% for a 150 mM to Pb treatment.

The results indicate that Pb is a stress-inducing agent oxidant causing significant disruption of cellular metabolism. We began by determining the level of membrane lipoperoxides mainly represented by malondialdehyde.

Our results showed that the Pb causes an accumulation of MDA only in the durum wheat roots treated with 50, 100 and 150 mM of the metal, so that the sheets do not show any change in their MDA content relative to control plants. This may signal onset of oxidative stress at the root of the seedlings.

According to these results, many conclusions can be drawn. Lead strongly modulated the antioxidant enzymes. Such an increase or decrease in the activity of these enzymes has been reported with a variety of heavy metals[7,5].

In leaves, various antioxidant enzymes exhibited different responses during lead exposure. No significant change was observed before 24 h of exposure.

The kinetic analysis of the effects of different doses of Pb on the level of catalase activity in *durum wheat* shows the maintenance of activity equivalent to that of control in the aerial part of the plant (Figure 3a). In the root part, we noted an increase in the activity of this enzyme after 24 h of treatment irrespective of the dose of Pb (Figure 3b). CAT activity increased throughout Pb treatment and reached a maximum value after 48 h of lead treatment (30 % of control).

The abundant data available highlights that the antioxidant response depends on plant species, metal concentration and treatment duration.

Detoxification of this molecule may be considered by several enzymes: Catalase (CAT) localized in peroxisomes and mitochondria, the guaiacol peroxidase or GPX localized in the cytoplasm and cell walls and enzymes ascorbate glutathione cycle (APX). This cycle is located in several cellular compartments such as chloroplasts, cytosol, peroxisomes and plasma membranes[23,24].

In durum wheat, Pb has no effect on the activity of leaf catalase. The lack of response of this enzyme in the leaves may be due to a non-involvement of catalase in the response to stress caused by the lead and that the removal of hydrogen peroxide is then made by means of other enzymatic pathways or enzyme no. In the roots, we noted a sensitivity of catalase to stress caused by Pb which is in the form of a stimulation of its activity particularly at 72 h of treatment.

In the leaves, we noted an unchanged activity compared to the control of ascorbate peroxidase in 24, 48 and 72 hours of treatment whatever the dose of Pb in the nutrient solution (Figure 4a). In roots decreased 21% compared to the control of the activity of ascorbate peroxidase at 48 h of treatment (Figure 4b).

In the leaves, our results showed the non-involvement of the enzyme ascorbate peroxidase in H_2O_2 detoxification process. Therefore, the absence of catalase activity, guaiacol peroxidase and ascorbate peroxidase in wheat leaves treated with Pb leaves suggest that the amount of hydrogen peroxide measured in the leaves is not sufficient to cause a modification of the antioxidant activities or late response of these enzymes.

Peroxidase guaiacol allows the removal of hydrogen peroxide induces the polymerization of guaïacol in tétragaïacol.

This enzyme shows an unchanged level of activity compared to the control at the leaf system (Figure 5a). However, at the root portion (Figure 5b), we noted a significant stimulation whatever the dose used in the nutrient solution at 48 and 72 h of treatment.

In the leaves of the seedlings, the GPX activity was unchanged during treatment regardless of the dose of the metal. The response of this enzyme differs depending on the plant species[25-28]. Other metals are able to modify its activity, such as copper and nickel[29].

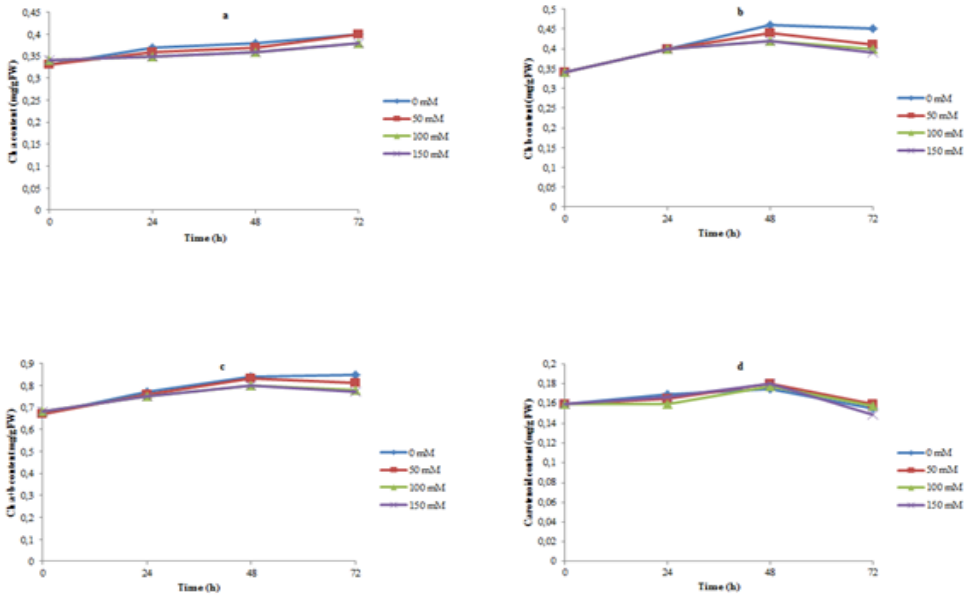

Figure 1: Effects of different concentration(s) of lead on the content of photoreceptor pigments in leaves of wheat. The average values and the errors standards were determined from 3 individual measurements.

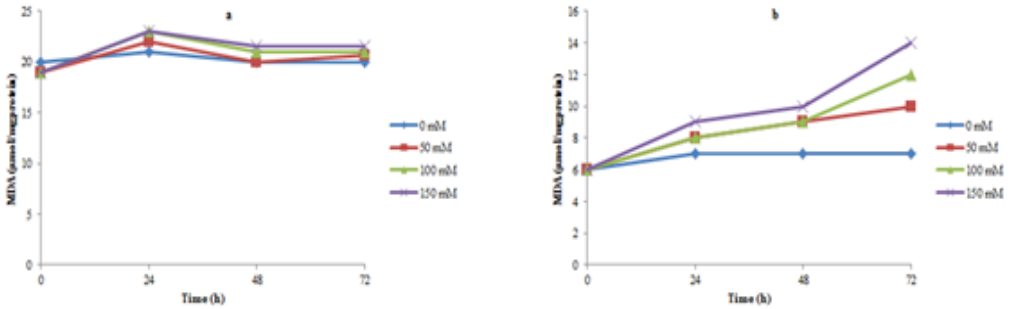

Figure 2: Effects of lead on the content of malondialdehyde (MDA) in leaves (a) and roots (b) of wheat. The average values and the errors standards were determined from 3 individual measurements.

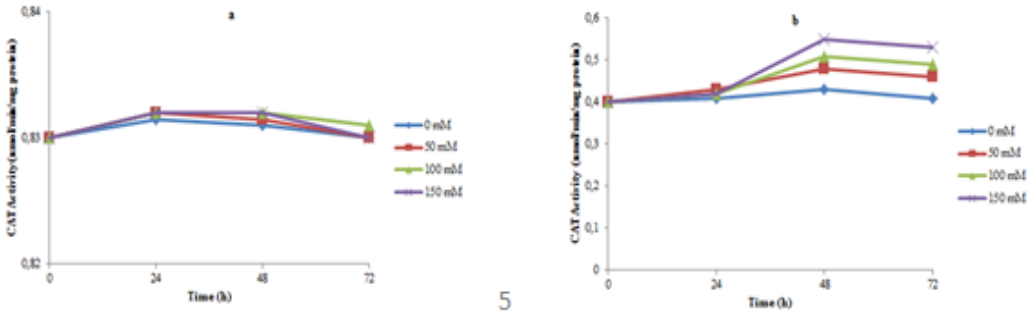

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Figure 3: Effects of different concentrations of lead on the catalase activity in leaves (a) and roots (b) of wheat. The average values and the errors standards were determined from 3 individual measurements.

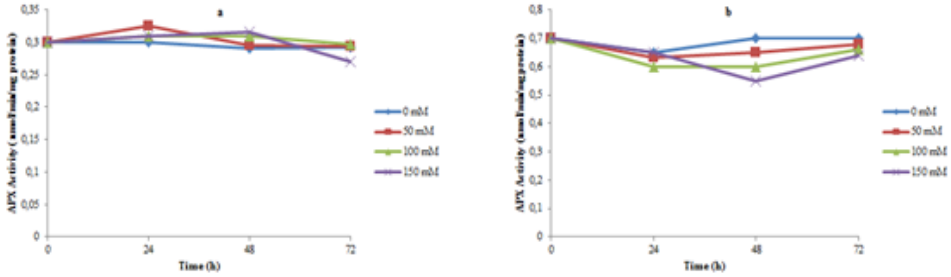

Figure 4: Effects of different concentrations of lead on the ascorbate peroxidase activity in leaves (a) and roots (b)of wheat. The average values and the errors standards were determined from 3 individual measurements.

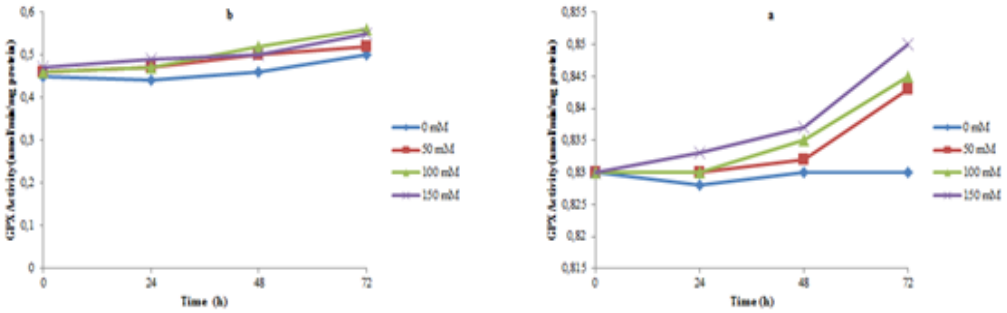

Figure 5: Effects of different concentrations of lead on the guaïacol peroxidase activity in leaves (a) and roots (b) of wheat. The average values and the errors standards were determined from 3 individual measurements.

CONCLUSION

In conclusion, our results demonstrate that lead uptake by roots was not linear along first day in *wheat* plants treated with different concentrations of lead.

This result is important because elevations of Pb uptake rate caused an oxidative stress in roots and induced lipid peroxidation.

On the effect of Pb on the different pigments photoreceptors, the results show a total chlorophyll reduction after 48 h of treatment.

This reduction is more pronounced at high concentrations (100 and 150 mM) of Pb and only affects chlorophyll b while the chlorophyll a and carotenoids showed no changes in their content compared to the control.

The amount of MDA showed no changes compared to controls throughout the treatment for the three metal treatments (50, 100 and 150 mM).

The root level, MDA accumulation varies depending on dose and time of treatment.

The kinetic analysis of the effects of different doses of Pb on the level of catalase, ascorbate peroxidase and guaïacol peroxidase activities shows the maintenance of activity equivalent to that of control in the leaves.

In the root part, our results also show that catalase and guaïacol peroxidase accumulation was strongly induced in presence of the height concentrations, after 48 h of treatment irrespective of the dose of Pb.

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