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Lead Phytotoxicity Induced Changes in Biochemical Markers in Germination and Seedlings in Durum Wheat *Triticum durum* Desf. Cv.Vitron and Gta

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Abstract: Among the heavy metals most commonly found on land, lead is a strongly represented pollutant in soil and sediments. It is easily absorbed and accumulated in different parts of plants. On the macroscopic scale, lead causes unfavorable effects on plants. First and in the event of excess, it may exert toxicity affecting several stages of germination development in leaf formation and root elongation in the early stages of development. The effect of lead on two varieties of durum wheat *Triticum durum* cv Gta and Vitron have been analyzed on germination under laboratory conditions. The objective is to determine the influence of lead on (i) germinal parameters and (ii) the stress physiological markers. The experiment was set up in a completely randomized design with 3 replications of 20 seeds per variety, including 4 concentrations of $Pb(NO_3)_2$ (0, 320, 430 and 660 ppm) on the germination. Average ambient temperature was 22°C, the humidity was 32% and photoperiod light/dark was 16/8 h. The obtained results showed a real sensitivity of germination to lead. Indeed, it was noted a total absence of germination of the two varieties in all seeds treated with the highest concentration. The treatment of seeds by the increasing doses of lead decreased the germinative faculty considerably, the content of pigments and disrupted the cellular metabolism, the proteins, the proline and glutathione levels. These results indicated that lead stress caused a decrease in germinal parameters and significant impairment of cell biochemical markers. Such effect was inversely proportional to the doses used. It was also noted that the Gta variety was less sensitive to lead toxicity than Vitron.

Keywords: Lead, Germination, Wheat, Pigment, Protein, Proline, Glutathione

Introduction

Heavy metals enlist a relatively series of elements with the specific density over 5 cm^3 (Kozlowski *et al*., 2003) and the relative atomic mass above 40 (Raskin *et al*., 1994). These metallic elements are toxic and naturally found in the environment at low concentrations (Siddiqui *et al*., 2014).

Lead is very widespread in Algeria, it is found in many industrial and agricultural factories, such as mining, fertilizers, pesticides and also the manufacture of automobile batteries, in addition to big number of cars realizing huge amounts of lead into the environment. Besides, Algeria is amongst the country using lead in gasoline to improve octane combustion, where its level in oil is in the order of 0.45 g L^{-1} (Semmadi, 1989) Moreover, it has been shown that the level of atmospheric Pb near the highways is around 140 kg Pb/day (Semmadi, 1989). Soil contamination by heavy metals is a natural phenomenon which has become widespread as a result of agricultural and industrial practices of anthropogenic activities. Lead is easily absorbed and accumulated in different parts of the plants including root, shoot, fruit and grain (Sharma and Shanker, 2005) and causes a serious disorder in plant and animal communities by being inserted into the food

chain and exerting high toxicity (Adriano, 2001; Alkorta *et al*., 2004; Behanzin *et al*., 2015). At the cellular level, it can affect respiration, photosynthesis, water and mineral absorption, (Nagajyoti *et al*., 2010), causes oxidative stress and disrupt the activity of various enzymes which are very important for the cellular metabolism (Singh *et al*., 1997; Viehweger, 2014).

At the macroscopic level, low concentrations of lead inhibited germination strongly (Mishra and Chaudhuri, 1998; Tomulescu *et al*., 2004). Also, at higher doses, it inhibited the germination of beans completely (Wierzbicka and Obidzinka, 1998). Lead also reduces significantly the development of seedlings and sprouts (Mishra and Chaudhuri, 1998). In Algeria, since the government has subsidized the cultivation of cereals as that of wheat, some farmers are growing this plant near lead pollution sources, ignoring however, its risk to human health.

The objective of this work is to investigate the lead toxicity on germination, which is one of the fundamental stages in the plant life and crop production. However, two cultivars of durum wheat *Triticum durum* cv. Gta and Vitron have been grown in the laboratory conditions. Thus, germination parameters and the stress physiological markers were evaluated.

Materials and Methods

The plant material studied consists of two varieties of durum wheat *Triticum durum* Desf cv. Gta and Vitron. 20 seeds per variety are germinated in 8.5 cm diameter petri dishes on blotting paper (Whatman) after a 24 h pre-imbibition to facilitate germination. The test was carried out under standard laboratory conditions, according to a completely randomized 3-repeat procedure and 4 concentrations of $Pb(NO_3)_2$ added to the germination medium (0, 330, 430 and 660 ppm). Treatment with solutions containing lead was made at a rate of 25 mL per concentration. The average ambient temperature is 22°C, the humidity is 32% and the light/dark photoperiod is 16/08 h.

The Germination Capacity

The germinative capacity is determined by the ratio of the number of germinated seeds to the total number of seeds germinated multiplied by 100 (Shirafew and Baker, 1996).

Dosage Pigments

After extraction, by the uses of Dimethyl Sulfoxide (DMSO) as solvent without maceration of the leaves (Hiscox and Israelsam, 1979), the chlorophylls and carotenoids pigments are determined by reading their absorbance at wavelengths between 400 and 700 nm and then the contents of chlorophylls (a) and (b) µg/g of fresh weight (fw) were obtained, according to the equations of Arnon and Inney (1949).

Proteins Assay

Total proteins were determined by the method of Bradford (1976). A sample of 500 mg of fresh leaves was ground and homogenized in 1 mL of phosphate buffer (pH = 7.0) and then centrifuged at 5000 t/min for 10 min. Then 50 mL of previously prepared Trichloroacetic Acid (TCA) was added and a second centrifugation was carried out at 8000 rpm for 15 min. The debris was dissolved in 1 mL of 0.1 N NaOH and 5 mL of Bradford reagent was added. The Optical Density (OD) of the samples is read at 595 nm and then the content of the proteins is determined using a calibration curve containing bovine serum albumin.

Dosage of Proline

To determine the proline content, a sample of 500 mg of fresh plant tissue was ground in 3% (w/v) aqueous sulfosalicylic acid and then centrifuged at 5000 rpm. The proline of the supernatant was estimated by the ninhydrin reagent (Bates *et al*., 1973). Reading of the absorbance of the fraction with toluene aspirated from the liquid phase was read in a spectrophotometer at 520 nm.

Determination of Glutathione

The glutathione content is determined by the method of Weckbeker and Cory (1988). Thus, 250 mg sample of each concentration was cold milled in a mortar with 2ml of buffer solution (pH = 6.5) placed in the centrifuge at a 5000 t/min for 15 minutes. The supernatant was recovered by filtration. Then 0.8 mL of the supernatant was taken and 0.2 mL of Sulfosalicylic Acid (SSA) 0.25% solution was added, stirred and then it left for 15 min in an ice bath and immediately centrifuged at 1000 g/min for 5 min.

The assay: The reaction medium consists of 0.5 mL of the supernatant (enzyme extract) 1 mL Tris-EDTA buffer (0.02 M EDTA) and 0.25 mL of DTNB. The whole mixture is vortexed and left for 5 min at room temperature to the stabilization of the color that develops instantaneously.

The glutathione content was determined pat reading (OD) at 412 nm. The calibration of the spectrophotometer is carried out by a solution containing 0.5 mL distilled water, 1 mL Tris-EDTA and 0.25 mL DTNB. The concentration of glutathione is obtained by the formula:

$$GSH = (OD \times 1 \times 1.525)/(13.1 \times 0.8 \times 0.5 \text{ mg protein})$$

Or:

- GSH: Glutathione concentration in ($nM.mg^{-1}$ of protein)
- OD: Optical density at 412 nm

- 1: Total volume of the solutions used in the deproteinization (0.8+0.2 mL supernatant SSA)
- 1.525: Total volume of the solutions used in the assay of GSH (0.5 mL supernatant, 1ml Tris-EDTA +0.25 mL DTNB)
- 13,100: Absorbance coefficient of the group (SH) at 412 nm
- 0.5: Volume of the float found in 1.525 mL
- 0.8: Volume of the homogenate found in 1 mL

It is noted that the GSH concentration is measured by the addition of 1 mg of proteins. That is why this dosage must be accompanied by protein assay.

Statistical Analysis

All the results of this work were statistically treated by the analysis of variance with 2 criteria of classification to $\alpha = 5\%$, $\alpha = 1\%$ and $\alpha = 0.1\%$ by the use of Minitab reference manual (1998).

Results

Germination Capacity

The effect of lead on germination of the two wheat varieties Gta and Vitron were studied. The obtained results (Fig. 1) show that in control groups the seeds were viable and had a good faculty. Indeed, the fifth day, all seeds had reached a maximum germination (>95%). However, we noticed that lead exerted a negative and significant action on germination process and its success ($p<0.01$) (Table 1). There was a total absence of germination in all seeds of both varieties treated with the highest concentrations of 660 ppm.

Chlorophyll and Carotenoids Content

The results showed that total chlorophyll content in leaves of seedlings in control plants have reached the maximum level in Vitron cultivar. The content varies depending on the variety, the Pb concentration and the type of pigment. Indeed, seed treatment with lead appears to stimulate slightly the total synthesis of the total chlorophyll in the Gta variety (Fig. 2a) and inhibit it in the Vitron variety. Furthermore, concerning the carotenoids content (Fig. 2b), Pb decreased it in the Vitron variety and increased it in the Gta, especially in the seeds treated with (430 ppm). In the control groups, it was noticed that the first variety had more carotenoids than Gta one (Table 1).

Protein Content

The effect of Pb on protein content is presented in Fig. 3. Protein content was high in the Vitron variety (0.50µg/g.fw) than its counterpart of Gta (0.30µg/g.fw). Contrary, it is reported that in the latter variety, the seed treatment by lead decreased to a very highly significant protein synthesis in young seedlings (Table 1), especially those treated with 330 ppm. Conversely, for Vitron, Pb appears to exert no significant effect on this parameter.

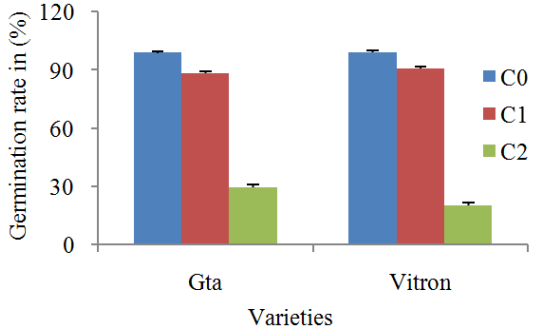

Fig. 1. Effect of lead on wheat germination capacity. C_0: 0 ppm control without treatment. C_1: 320 ppm, C_2: 430 ppm, C_3: 660 ppm for 1 week

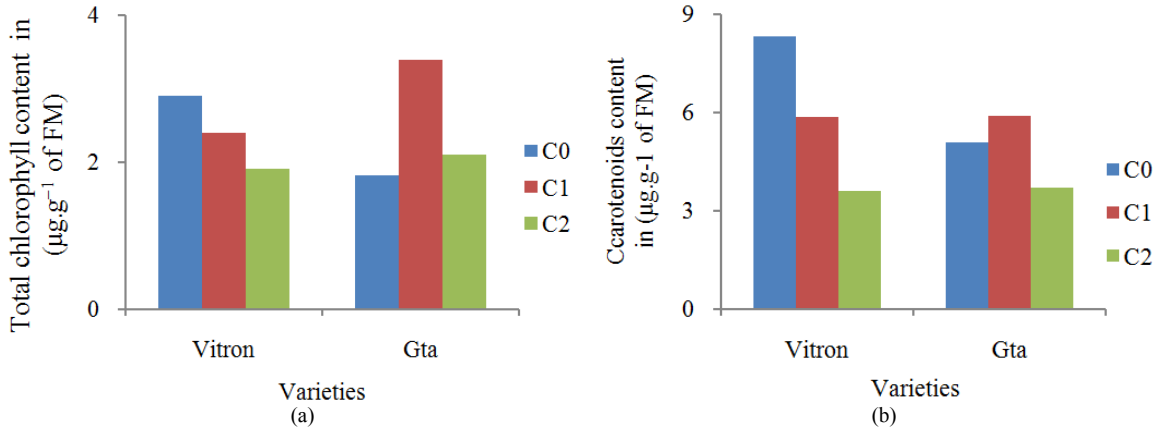

Fig. 2. Effect of lead on wheat pigment content during seedlings. C_0: 0 ppm control without treatment. C_1: 320 ppm, C_2: 430 ppm, C_3: 660 ppm for 1week. (a) Chlorophylls; (b) Carotenoids

Table. 1. Values of the F observed (Fobs) of the variance analysis 2 classification criteria of the studied parameters

S var	d.d.l	GC en (%)	Total Chl	Carotenoids	Proteins	Proline	Glutathione
Con	2	8960.13***	22.22***	96.49***	15.66***	0.91 ns	3.95*
Var	1	24.93***	0.10 ns	32.51***	1.70 ns	0.63 ns	0.07 ns
Interaction	2	55.28***	24.48***	38.27***	8.78**	1.0 ns	2.52 ns
Error	18						
Total	23						

*S var: Source of variation; d.d.l: Degree of freedom; GC: Germination Capacity; (*p<0.05: Significant, **p<0.01: High significant, ***p<0.001: Very high significant)*

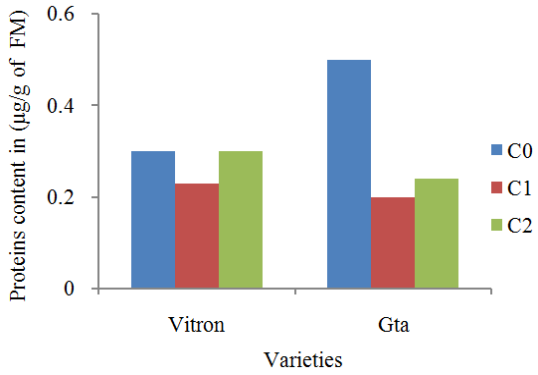

Fig. 3. Effect of lead on protein content of wheat seedlings. C_0: 0 ppm control without treatment. C_1: 320 ppm, C_2: 430 ppm, C_3: 660 ppm for 1 week

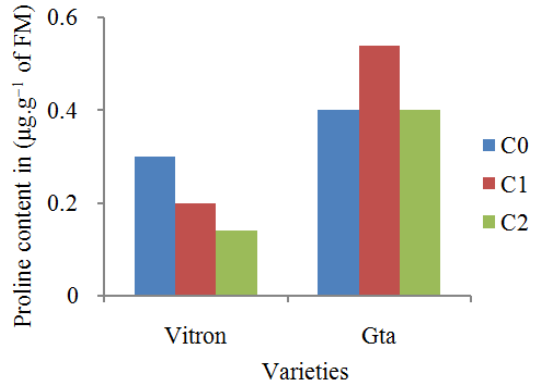

Fig. 4. Effect of lead on proline content in wheat seedlings. C_0: 0 ppm control without treatment. C_1: 320 ppm, C_2: 430 ppm, C_3: 660 ppm for 1 week

Proline Content

The results related to lead effect on proline content (Fig. 4) showed that the Gta variety contains more free amino acid, the proline than the Vitron variety (0.44 against 0.3 µg/g.fw), respectively. Contrary, in lead treated seeds, a slight stimulation but not significant (Table 1) of proline synthesis in the Gta variety (0.54 µg/g.fw) was noted and caused by 330 ppm concentration, accompanied with a reduction of proline in the Vitron variety. The effect was inversely proportional to the applied doses, respectively 0.2 and 0.14 µg/g.fw for the concentration of 330 and 430 ppm.

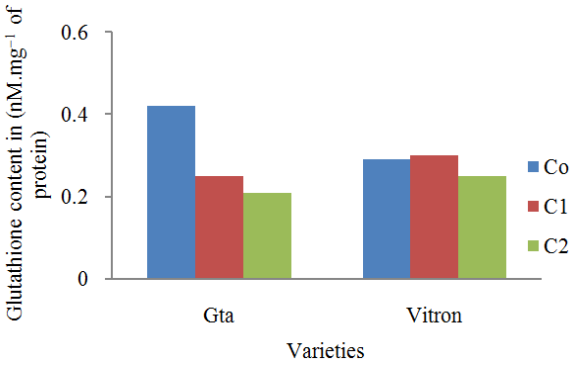

Fig. 5. Effect of lead on glutathione content in wheat seedlings. C_0: 0 ppm control without treatment. C_1: 320 ppm, C_2: 430 ppm, C_3: 660 ppm for 1week.

Glutathione Content (GSH)

The observation in (Fig. 5) shows that the Gta variety has more glutathione than Vitron (almost one and a half) in the controls (0.42 nM GSH.mg^{-1} protein against 0.29 nM GSH.mg^{-1} protein). On the other hand, seed treatment by different concentrations of lead nitrate $(PbNO_3)_2$ induced a significant decrease in the glutathione content ($p<0.05$), (Table 1) for all levels in both varieties.

Discussion

This study shows the depressive effect of lead on the germination phenomenon of durum wheat *Triticum durum*, cv. Gta and Vitron. These results are similar to other works cited in the literature and found by Chatterjee *et al*. (2004) who observed the same effect of high lead levels on rice seeds. Accordingly, the work of Seregin and Ivanov (2001) on *Phaseolus vulgaris* and *Pisum sativum* seeds has proved that these species are now considered to be susceptible to heavy metals, which do not germinate even in very low concentrations. The same effects were observed on the germination of *Brassica perkinensis* (Xiong, 1997), carrot and radish (Chen *et al*., 2002). All these studies have shown that lead is able to reduce germination, coleoptiles growth and the number of sprouts per seed cultivated plant species.

Furthermore, in regard to the lead effect on the leaf pigments, identical results have been found by researchers like Padjama *et al*. (1990; Moreno-Casseles *et al*., 2000;

Kosobrukhov *et al*., 2004; Qureshi *et al*., 2005; Chen *et al*., 2007; Gopal and Rizvi, 2008) who noted a disturbance in pigments contained in the leaves of young plants grown from seeds treated with heavy metals. In addition, chlorophyll (b) appears to be more sensitive than chlorophyll (a) (Wozny *et al*., 1995; Vodnik *et al*., 1999). However, these effects vary among plant species. The reduction mechanisms of synthesized pigments can be explained by the inactivation of pigment synthesis by inhibition of the enzyme system which is implicated as δ aminolevulinate dehydratase or ALAD and protochlorophyllide reductase (Padjama *et al*., 1990; Van Assche and Clijsters, 1990) or by substituting for divalent ions associated with metallo-enzymes. The supply of Mg^{2+}, Fe^{2+} and Zn^{2+} (Van Assche and Clijsters, 1990; Krupa and Bazynski 1995), which is the basis of the synthesis of chlorophyll and Zn^{2+} ion which can be replaced by (Pb^{2+}) or ii), an indirect way, by inducing a deficiency of these divalent ions. An identical decrease in chlorophyll content induced by metal stress was reported earlier in plants such as *Picea abies, Zea mays, Quercus palustrus, Acer rubrum* and *Helianthus annus*.

However, concerning carotenoids, it was found that the Vitron variety contains more carotenoids than the Gta variety in the control groups. By contrast, in the Pb treated seeds, it was noted a relatively high concentration in the Gta compared to its control. This increase in concentration is probably a positive reaction to counteract stress and reduce its effect. It is known that this class of pigments plays a key role in eliminating free radicals, which allow the removal of lipid peroxides (Palozza and Krinsky, 1999), or by the physical deactivations of singlet oxygen, especially in the case of lycopene (Sandquiest *et al*., 1994).

With regard to the amino acid proline, the results are in almost identical to those found by Chen *et al*. (2002) which indicate that the proline content increases when treatment doses were low and decreases drastically when they were high. Note that proline is a good indicator of environmental stress. It is considered a non-specific agent of the plant defense system against the toxicity of heavy metals and plays several protecting roles (Sharma and Dubey, 2005) including the inhibition of lipid peroxidation (Mehta and Gaur, 1999), attachment of free radicals (Alia and Matysik, 2001) and also as a metal chelating agent (Fargo and Mullen, 1979). The explanation for the rise in proline content in seeds treated with high concentrations is the formation of a non-toxic complex (Proline-Pb) by chelating lead ions, which is considered a detoxification mechanism. Moreover, treatment of seeds with elevated levels of heavy metals induce changes at the molecular level that affect the essential physiological functions and metabolism is disrupted by blocking and inhibiting the enzymatic activity regulating synthesis reactions.

Concerning proteins and glutathione, it was noted that the Pb toxicity induced an increase of these markers in leaf mesophyll tissue of lead treated seeds. This is certainly due to an induction of these biochemical markers caused by the effect of exposure to metal stress (Mesmar and Jaber, 1991). The protein content was increased in plants of the variety Vitron treated with the highest dose and remained stable compared to control of other concentrations. These results are supported by Mohan and Hosetti (1997; Saxena *et al*., 2003; Mishra *et al*., 2006), which indicate that heavy metals induce an increases in the content of glutathione and protein with low doses. The action of lead on the protein content shows that high concentrations can reduce protein content. Their synthesis is slowed or even inhibited (Chen *et al*., 2002; Chatterjee *et al*., 2004). This reduction appears as a result of the consequences of the action of lead, such as decreased or stimulated protease activity and/or decreased free amino acid content (Xiong *et al*., 2006). Furthermore, lower concentrations seem, on the contrary to increase total protein content (Mishra *et al*., 2006). This protein accumulation may be the result of the synthesis by the plant defense system against this metal, or in the sequestration of metal by glutathione to neutralize the high concentrations of Pb (Rauser, 1999; Mourato *et al*., 2015).

In this context, the treatment of plants by lead has caused a rise of glutathione content in both varieties regardless of the concentration used. This is, because glutathione can contribute to the synthesis of phytochelatins, which appear to play an important role in the detoxification and tolerance of plants to lead (Gupta *et al*., 1995; Piechalak *et al*., 2002). These PCs have the main role of sequestering and/or chelating lead and some heavy metals in the cytoplasm (Barrameda-Medina *et al*., 2014) before transport and final isolation in the vacuoles so as not to interfere with cellular metabolism (Piechalak *et al*., 2002; Seregin *et al*., 2002; Małecka *et al*., 2008).

In general manner, the high concentrations of heavy metals induces changes at the molecular level that affect the essential physiological functions where the metabolism is damaged by blocking and inhibiting the enzymatic activity governing the synthesis reactions (Wolinska *et al*., 1980). However, there was also a slight stimulation caused by low lead concentration (Ernst *et al*., 1992). This stimulation is due to the activation of cell metabolism such as the uptake of O_2 in tobacco leaves (Prasad and Hagemeyer, 1999; Prassad *et al*., 2001) where an increase in the activity of PSII (photosynthetic activity) was observed (Baszynski and Tukendorf, 1991; Krupa and Bazynski, 1995)

Conclusion

The results obtained indicate that the treatment of wheat seeds by lead show that the germination

phenomenon has been altered, as well as the amino acid proline, the tripeptide glutathione, the proteins and also the photoreceptor pigments.

The comparison of these cultivars with the effect of the lead makes appear that the Gta variety was less sensitive than Vitron. The effect of lead on the parameters studied was inversely proportional to the doses used in the treatment.

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Author's Contributions

You can see the "Authors and Contribution Declaration for a Scientific Publication"

Ethics

The authors state that there is no conflict of interest and that is a research team task.

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