Effect of Lead Nitrate on Morphological and Biochemical Parameters of *Atriplex halimus* L.

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ABSTRACT

Soil pollution is one of the main environmental stresses due to the effect of various contaminations by heavy metals like lead. The aim of this study is to examine the response of a halophile species *Atriplex halimus* L. under lead stress. This plant is watered from the germination stage with different concentrations of lead nitrate $Pb(NO_3)_2$ 1000, 3000, 5000 and 7000 ppm for 100 days. The response of plants is evaluated by an analysis of the growth mass parameters (fresh and dry weight) and biochemical parameters (chlorophyllian pigments, proline and total proteins). The results reveal a growth inhibition of the aerial and root parts by $Pb(NO_3)_2$ treatment compared to control. On the other hand, the biochemical response indicates that the amount of chlorophyll pigments reduced by the effect of different treatment of lead. In contrast, proline and total proteins contents increased in leaves and roots of *Atriplex halimus* L. as a response to stress.

تأثير نترات الرصاص على الخواص المورفولوجية والبيوكيماوية *Atriplex halimus* L.

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الكلمات الدالة

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المُستلخص

يعد تلوث التربة أحد الضغوط البيئية الرئيسية بسبب تأثير الملوثات المختلفة بواسطة المعادن الثقيلة مثل الرصاص. الهدف من هذه الدراسة هو اختبار استجابة نوع محب للملح *Atriplex halimus* L. تحت ضغط الرصاص. تم سقي هده النبتة ابتداءا من مرحلة الإنتاش بتراكيز مختلفة من نترات الرصاص (5000 , 3000 , 1000 $Pb(NO_3)_2$ و 7000ppm لمدة 100 يوم. استجابة النباتات تم تقييمها من خلال تحليل خواص كتلة النمو النباتية (الوزن الطازج والجاف) والخواص البيوكيماوية (أصباغ اليخضور ,البرولين والبروتينات الكلية). تكشف النتائج عن تثبيط نمو الأجزاء الهوائية والجذرية تحت تراكيز نترات الرصاص مقارنةً بالشاهد. من ناحية أخرى ، تشير الاستجابة البيوكيماوية إلى أن كمية أصباغ اليخضور تنخفض تحت تأثير مختلف تراكيز الرصاص. في المقابل ، زادت محتويات البرولين والبروتينات الكلية في أوراق وجذور *Atriplex halimus* L. كرد فعل على الإجهاد.

Introduction

Pollution of soils by toxic metal, especially lead, is a constraint of the environment and plants. Lead is a well-known metal in the environment (Hamid, et al. 2010) with a very large distribution in soil and water (Pourrut, et al. 2011). In addition, it can induce a disturbance in photosynthesis (Hamid, et al. 2010) and reduce production (Moyo and Chimbira, 2009). It has been observed that plants react by synthesizing and accumulating organic compounds such as proline and glycine betaine under biotic (Bano and fatima, 2009) and abiotic constraints (Achour, et al. 2015; Nareshkumar, et al. 2015; Rahim Guealia, et al. 2017). Manousaki and Kalogerakis (2011) report that halophytes can grow and accumulate metals in soils, particulary when affected by salinity, while many metal accumulating glycophytes can't tolerate it. Thus, several studies indicate that halophytes express a resistance capacity to metallic stress (Sharma, et al. 2010), such as the genus Atriplex show plant-heavy metals relationship (Kachout, et al. 2010; Lotmani, et al. 2011). One of the properties possessed by this species is its tolerance to salinity, which reflects in halophilic character (Manousaki and Kalogerakis, 2011). To contribute to the phytoremediation of soils affected by heavy metals, we were interested in examining the behavior of 100 day old *Atriplex halimus* L. plants under a lead diet. The choice of this species is justified not only by is halophilic character but also by various animal food interests (Ben Salem and Smith, 2008), ecological as well in soil protection against desertification due to their highly developed root system and its ability to accumulate salts in its foliar system (Belkhodja and Bidai, 2004).

This work is based on an analysis of growth parameters such as fresh and dry weight, and biochemical parameters such as chlorophylls, proline and total protein in leaf and root parts of young plants of *Atriplex halimus* L. subjected to $Pb(NO_3)_2$ treatment.

Material And Methods

Vegetal material and applied stress

This study is carried out on a halophyte of the genus Atriplex. Seeds of *Atriplex halimus* L. were sown in a controlled greenhouse in a mixture of peat and sand. Seeds in each pot were watered from the first day with distilled water (control) and four concentrations of $Pb(NO_3)_2$ (1000, 3000, 5000 and 7000 ppm) at the rate of five repetitions per treatment. Watering was done three times a week for 100 days using alternating distilled water, nutrient solution and lead treatment.

Analyzed parameters

- Fresh weight (FW)

Both aerial and root parts of the plant were separated and weighed rapidly to prevent water loss.

- Dry weight (DW)

Fresh aerial and root parts were wrapped in perforated aluminum foil to allow water evaporation, put in the oven at 80 ^{o}C. The weight was measured after 48 hours.

- Chlorophylls

The pigment extraction was carried out according to the method of Lichtenthaler (1987). 100 mg of fresh foliar sample were kept in 10 ml 95 % acetone and the tube was allowed to keep in dark at 4 ^{o}C for 48 hours. The optical density (OD) was taken at 663 and 647 nm. The chlorophyll contents a and b was calculated by the following relations:

Chlorophyll a (µg / ml) = (12.25 OD 663) - (2.79 OD 647)
Chlorophyll b (µg / ml) = (7.15 OD 647) – (5.10 OD 663)

- Proline

The extraction was carried out according to the method of Nguyen and Paquin (1971). 100 mg of dry material was extracted using 1.25 ml 95 % ethanol, followed by three rinses in 1.25 ml of 70% ethanol. The final solution was collected in a test tube.

The assay carried out by the technique of Bergman and Loxley (1970) and was performed by taking 2.5 ml of the supernatant, to which 1 ml of chloroform and 1.5 ml of distilled water was added. After stirring, the solution was kept at rest for 24 hours in the cold for good separation. The optical density was noticed using spectrophotometer at 515 nm. A standard curve was established using proline solutions, from which the proline contents was calculated and expressed in µg.g -1 DM (dry matter).

- Total proteins

The total proteins extraction was carried out according to the method of Bradford (1976), using 100 mg of fresh cold milled plant material in 1 ml of 0.1 M phosphate buffer (pH 7) where the extraction solution was compounded of 17.41 g of K2HPO4, 1 ml of Triton x100 and 2.29 g of EDTA. After centrifugation at 3000 rpm for 10 min, 100 µl of supernatant was mixed with 5 ml of Bradford reagent and then incubated for 5 min.

The optical density was measured using spectrophotometer at 595 nm. The total proteins content was expressed in µg. ml-1 and converted into µg.g-^{1} FM (fresh matter). A standard curve was established using a Serum Albumin Beef solution for calculation.

Statistical analysis

The results were analyzed statistically using the Statistica 7.1 software. Analysis of variance (ANOVA) was used to test that the means of measured outcomes. Fisher's Least Significant Differences (LSD) was used to compare pairs of mean at α=0.05. Pearson's correlation coefficient was used to assess the strength of the relationships between the studied parameters. The letters in the figures identify the homogeneous groups of the Analyzed parameters of the independent organs according to the LSD test at p <0.01, α <0.05.

Results

Multivariate variance analysis (ANOVA) revealed a significant effect of lead on all parameters studied in *Atriplex halimus* L. aged of 100 days (table 1).

The statistical significance of the results is represented for all the parameters by * and ** when it is respectively a significant difference and a highly significant difference at p < 0.05.

Table 1 : Pearson correlation matrix between lead (Pb), aerial fresh weight (AFW), root fresh weight (RFW), aerial dry weight (ADW), root dry weight (RDW), chlorophyll a (Ch a), chlorophyll b (Ch b), leaf proteins (Lprot), root proteins (Rprot), leaf proline (Lprol) and root proline (Rprol)

	AFW	RFW	ADW	ADW	Chl a	Chl b	Lprot	Rprot	Lprol	Rprol	*Pb
AFW	1,00										
RFW	0,96**	1,00									
ADW	0,93**	0,93**	1,00								
ADW	0,92**	0,84**	0,88**	1,00							
Chl a	0,87**	0,81**	0,85**	0,88**	1,00						
Chl b	0,58**	0,55**	0,40*	0,55**	0,64**	1,00					
Lprot	-0,83**	-0,81**	-0,8**	-0,86**	-0,75**	-0,47*	1,00				
Rprot	-0,15	-0,09	-0,17	-0,18	-0,24	-0,35	0,35	1,00			
Lprol	-0,13	-0,01	-0,16	-0,31	-0,21	-0,24	0,46*	0,55**	1,00		
Rprol	-0,84**	-0,80**	-0,85**	-0,79**	-0,74**	-0,57**	0,79**	0,48*	0,40*	1,00	
*Pb	-0,90**	-0,88**	-0,90**	-0,88**	-0,92**	-0,50**	0,80**	0,20	0,06	0,72**	1,00

* Significant difference at p < ٠,٠٥. ** Highly significant difference at p < ٠,٠٥

Fresh weight

Pb(NO3)2 has a highly significant effect on the fresh weight of the aerial and roots parts ($p < 0.05$). Our results show a negative correlation between lead and both aerial and root parts respectively (r = -0.90*, r = -0.88**).

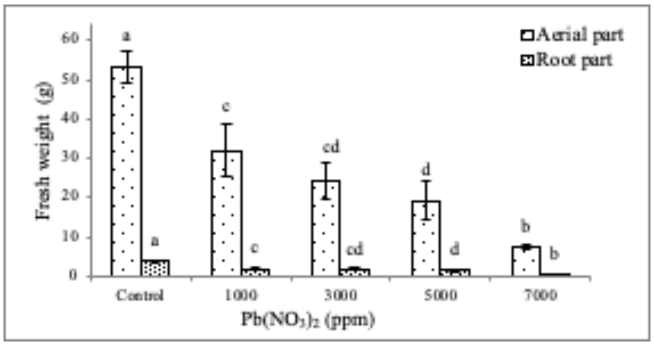

Figure 1. Variation in fresh weight (g) of aerial and root parts of *Atriplex halimus* L. aged of 100 days under $Pb(NO_3)_2$ stress

Figure 1 illustrates a remarkable reduction of fresh weight (FW) of the aerial part passing from 53.47 g to 7.31 g and the root part varying from 4 g to 0.14 g for control and stressed plants. In addition, the homogeneous groups analysis established has allowed us to distinguish significant differences in aerial and roots fresh weight between control plants and plants stressed by $Pb(NO_3)_2$.

Dry weight

ANOVA variance analysis reveals a highly significant effect of $Pb(NO_3)_2$ on plants dry weight (DW). Pearson's Correlation Coefficient respectively shows a negative correlation between lead and DW in both aerial and root parts, respectively (r = -0.90** and r = -0.88**).

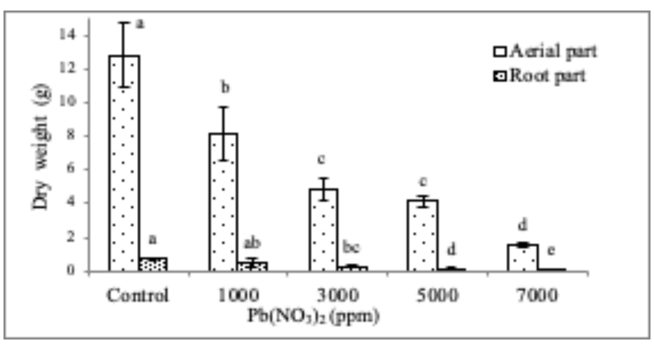

Figure 2.Variation of dry weight (g) of aerial and root parts of Atriplex halimus L. aged of 100 days under $Pb(NO_3)_2$ stress

The histogram of figure 2 characterizes a decrease in dry weight in both aerial and root parts of stressed plants by this metal compared to control plants. Indeed, the DW undergo a reduction of the fluctuating values for the aerial part of the control plants towards those plants stressed at 7000 ppm (12.86 g against 1.54 g) and 0.75 g up to 0.04 g respectively for the root part of the control plants and stressed plants at the same concentration of $Pb(NO_3)_2$. The LSD test indicates a significant difference for the aerial part on the decline between control plants and stressed plants. The root part recorded a significant effect between control and stressed plants at 3000, 5000 and 7000 ppm of $Pb(NO_3)_2$.

Chlorophylls

The statistical results show a highly significant effect of lead on photosynthetic pigments. A strong negative correlation is signified between lead and chlorophyll a expressed by r = -0.92**. Chlorophyll reveals a negative correlation with pollutant metal expressed by r = -0.50**. Figure 3 shows that the content of chlorophyll a are obviously higher in the control plants leaves and those of the plants subjected to 1000 ppm $Pb(NO_3)_2$ (110.22 for 106.13 µg . g-1 FW). These pigments undergo a significant leaf drop as soon as the concentration of the metal increases. On the other hand, the chlorophyll b content is about three times lower in the leaves compared with chlorophyll a for both control and the 1000 ppm treatment (110.22 against 40.33 µg . g-1 FW for the controls and 106.13 against 35.35 µg . g-1 FW).

When the concentration of lead in the middle increases, chlorophylls a and chlorophyll b decrease without a significant effect under all treatments; results indicate also that the chlorophyll b content varies practically in the half compared to chlorophyll a. On the other hand, the lead effect does not seem to influence the chlorophyll b since the contents of this pigment are very close to those recorded in the leaves of the control plants.

Statistical analysis revealed no significant effect of $Pb(NO_3)_2$ treatment on Chlorophyll a for plants subjected to 1000 ppm compared to control; the concentration increase of Pb(NO3)2in the substrate causes a significant decrease of this pigment compared to the control.

For chlorophyll b, the results show no significant effect between all the studied plants.

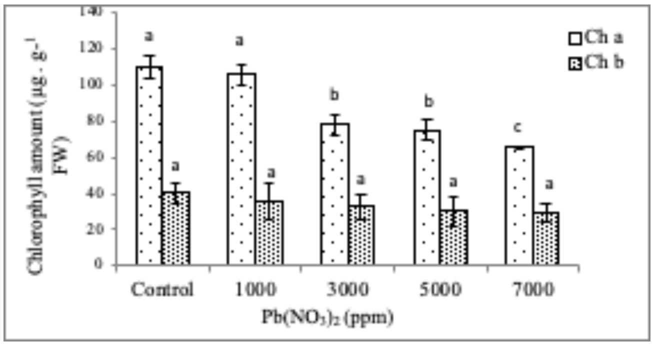

Figure 3. Variation of chlorophyll a and chlorophyll b amount (µg . g-1 FW) of *Atriplex halimus* L. leaves aged of 100 days under $Pb(NO_3)_2$ stress

Proline

$Pb(NO_3)_2$ acts in a highly significant way on the accumulation of proline. The results in table 1 indicate a positive correlation between the factor "treatment" and the factor "root proline" (r = 0.72**).

The results illustrated in figure 4 show that proline levels increase in the leaves and roots under the $Pb(NO_3)_2$ effect as the environment becomes more concentrated up to 5000 ppm. Beyond this, proline evaluate as well in the leaves as in the roots under 7000 ppm treatment; under this treatment, this amino acid is practically distributed in both organs in the same level (631.42 for 655.71 µg . g-1 DW). The LSD test shows a significant difference in root proline compared to control plants; while for leaves, the significant difference compared to control plants is recorded only in plants watered by 5000 and 7000 ppm of $Pb(NO_3)_2$.

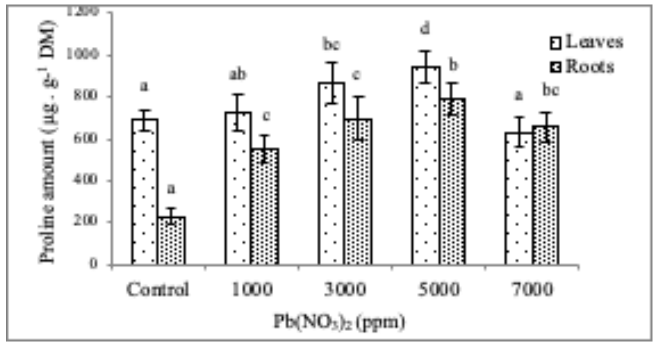

Figure 4. Variation in proline amount (µg . g-1 DM) of *Atriplex halimus* L. leaves and roots aged of 100 days under $Pb(NO_3)_2$ stress

Total proteins

The ANOVA test shows a significant effect of lead on total proteins. The statistical study indicates a positive correlation between the factor "treatment" and the accumulation of total proteins in leaves (r = 0.80**). Figure 5 records a foliar accumulation of total proteins in all plants exposed to Pb(NO3)2 compared to unstressed plants. At the root level, the protein content remains stable up to 3000 ppm; at 5000 ppm, total proteins increase significantly compared to the control and previous treatments. Beyond that a rapid reduction of total proteins expressed under 7000 ppm treatment to reach values close to those recorded in the root proteins of control plants. Homogeneous groups record a significant difference for leaves between control plants and those watered by 1000, 3000 and 5000 ppm of $Pb(NO_3)_2$ The significant difference for roots occurred only in plants treated by 5000 ppm compared to control plants.

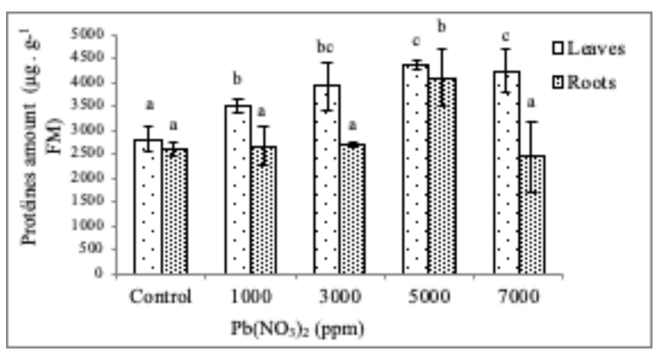

Figure 5. Variation in level of protein (µg . g-1 FM) of Atriplex halimus L. leaves and roots aged of 100 days under $Pb(NO_3)_2$ stress

Discussion

The effect of lead on plants was studied by many authors (Pourrut, 2008; Hu, et al. 2012; Hussain, et al. 2013; Murugalakshmikumari and Ramasubramanian, 2014). The results showed a decrease in fresh and dry weight of all plants treated with $Pb(NO_3)_2$, which was already reported by Patra, et al. (2004). Fresh weight results are in agreement with those registered in tomato (Akinci, et al. 2010). Murugalakshmikumari and Ramasubramanian (2014) reported that under lead acetate, the roots and the aerial part fresh weight decrease in okra plants. On the other hand, the drop in dry weight of root and aerial parts recorded under $Pb(NO_3)_2$ treatment is reported in several species such as tomato (Akinci, et al. 2010), tobacco (Alkhatib, et al. 2011), sunflower (Azad, et al. 2011) and groundnuts (Nareshkumar, et al. 2015). This reduction could be due to the decrease in the foliar photosynthesis rate under effect of heavy metal according to Rodrıguez – Serrano, et al. (2009). Photosynthesis is one of the process that is most affected by abiotic stress (Hasegawa, et al. 2000). Cenkci, et al. (2010) thus Alkhatib, et al. (2011) reported that lead cause inhibition of photosynthesis. Other works reported that replacement of essential ions such as iron and manganese by lead can cause this inhibition (Chatterjee, et al. 2004). The drop of chlorophylls a and chlorophyll b contents was recorded in this work in *Atriplex halimus* L. under increasing lead concentration, could be due according to the inhibition of the Calvin cycle enzymes by lead (Liu, et al. (2008). It was reported in tomato (Akinci, et al. 2010), radish (El-Beltagi and Mohamed, 2010), Chenopodium album L. (Hu, et al. 2012), wheat seedlings (Kaur, et al. 2012) and wheat (Janmohammadi, et al. 2013). Saeideh and Rashid (2014) observed a decrease in chlorophyll a and an increase in chlorophyll b in green soybeans. Under low lead concentrations, Sarvari, et al. (2002) reported an increase in chlorophylls in corn and cucumber.

In general, there is a strong relationship between tolerance and proline accumulation in higher plants (Hadjadj, et al. 2011). Indeed, free proline accumulates in response to stress in many species (Djerroudi-Zidane, et al. 2010; Achour, et al. 2015). According to Ashraf and Fooland (2007), glycine betaine and proline are the most accumulated osmolytes in various species in response to environmental stress such as drought, salinity and heavy metals. There is competition between this amino acid and chlorophyll on glutamate, which is commun precursor (Grennan, 2006). The applied lead treatment increase the proline levels in the leaves and roots, which is statistically explained by a positive correlation between lead and accumulated proline in roots. This accumulation allows protection of the cell membrane and participates in osmotic adjustment (Hassani, et al. 2008). According to Hadjadj, et al. (2011), the level of tolerance is related to the amount of accumulated proline. Similar results were found in beans (Zengin and Munzuroglu, 2005) and in black soy bean seedlings (Singh, et al. 2012). Similarly, it was reported in the leaves and roots of sunflower seedling (Azad, et al. 2011), sugar beet (Naderi, et al. 2013) and groundnuts (Nareshkumar, et al. 2015) under stress situations.

In addition, lead has also a high affinity for proteins that possess thiol groups or metal cofactors (metallo-enzymes) (Pourrut, 2008). The increase level of in soluble proteins content under the stress of heavy metals may be related to the synthesis of stress proteins (Mishra, et al. 2006). Our results showed an accumulation of foliar and root proteins under lead treatment. In contrast, lead has reduced proteins content in several species such as wheat (Janmohammadi, et al. 2013), corn (Hussain, et al. 2013) and okra (Murugalakshmikumari and Ramasubramanian, 2014).

Conclusion

The enrichment of the external environment of *Atriplex halimus* L. with $Pb(NO_3)_2$ leads to inhibition of growth and photosynthesis. Thus, an osmotic response is triggered by the accumulation of proline and soluble proteins at the foliar and root level under the effect of this metal. These results indicate some tolerance to lead which could be a possible strategy to undertake for phytoremediation of soils polluted by heavy metals.

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