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Effect of heavy metals (copper, zinc, cadmium and lead) on the accumulation of proline and soluble sugars in *Atriplex canescens* (Pursh) Nutt

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Abstract

The *Atriplex canescens* is well adapted to extreme environmental conditions and present properties accumulator of heavy metals. Our study is to investigate the effect accumulator of heavy metals (copper, zinc, cadmium and lead) by the *Atriplex canescens*s stressed after 60 days of the sowing. It applying five different doses to the plant *Atriplex canescens* for two weeks for: Zn, Cd, Pb (0, 2500, 5000, 7500, and 10000 ppm) and for copper (0, 2000, 2500, 3000 and 3500 ppm). The contents of proline and the soluble sugars were analyzed by spectrophotometry. The results obtained show an increase in the content of proline and soluble sugars according to the increasing concentration of heavy metals to the levels of the leaves and the roots. The contents of proline and the soluble sugars in leaves are widely superior to those of the roots. The highest contents inproline and soluble sugars in the leaves (74.45 mg. g^{-1} dry weight of the proline and 23.82 mg. g^{-1} dry weight of soluble sugars) are obtained in the dose of 10000 ppm of (zinc, cadmium) respectively. On the other hand, the highest contents proline and soluble sugars in roots are obtained in the metallic dose of 10000 ppm for the cadmium and the zinc respectively (46mg. g^{-1} dry weight of the proline and 10.26 mg. g^{-1} dry weight of soluble sugars). The obtained results are in favour of the implication of *Atriplex canescens* in a project of phytoremediation to clean up the contaminated soils.

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Introduction

The environmental pollution became in a few decades one of the major problems which conditions the future of our planet. Among main pollutants, there are heavy, persistent metals which accumulate in the soil by contaminating the environment. The phytoremediation is one of the biological methods based on the use of plants to remedy soils contaminated by heavy metals (Cunningham and Berti, 1993; Raskin *et al.,* 1994). In stressful conditions, plants can react by implementing mechanisms, among others, physiological (Kylin and Quatrano,1975; Parida and Das,2005) and biochemical (Brugnoli and Lauteri, 1991). So, the synthesis of organic compounds would contribute to the osmoprotection (Rathinasabapathi *et al.,* 2000) or osmotic regulation (El- Shintinawy and Hassanein, 2001).

Several species belonging to the genus Atriplex are well adapted to extreme environmental conditions and can present properties accumulator of heavy metals (Martinez *et al.,* 2003). *Atriplex canescens* (Pursh) Nutt is a halophyte being a part of the family of chenopodiaceae, come from North America (Mulas and Mulas, 2004).

The osmotic adjustment is one of the adaptive mechanisms main thing of halophytes, which contains the accumulation of molecules in answer to a stress (Zhang and *al.,* 1999) thanks to the induction of genes involved in the synthesis of amino acids as the proline (Szabados and Savouré, 2009), and also in the synthesis of soluble sugars (Zerrad *et al.*, 2006; Bouchoukh,2009; Azzouz, 2011). The proline is an amino acid often considered as a biomarker of stress (Szabados and Savouré, 2009; Djerroudi-Zidane*etal.,* 2010). According to Mile *et al.* (2002), the accumulation of proline is one of adaptive strategies activated by the plant in front of constraints of the environment. According to another point of view, the accumulation of the proline is not a reaction of adaptation to the stress, but rather the sign of a metabolic disturbance (Cheikh M'hamed *et al., 2007).* The soluble sugars play a central role in the structure, the metabolism and the operation of the plants. They are more involved in many mechanisms of response to stress, biotic or abiotic (Ramel, 2009).

In this framework is registered our work which aims to assess the effect of the heavy metals copper, zinc, lead and cadmium on the contents proline and the soluble sugars of *Atriplex canescens* (Pursh) Nutt.

Materials and methods

Plant material

The plant material having been the object of the present study concerns the seeds of the *Atriplex canescens* (Pursh) Nutt which come from the region of El Bayedh are shelled, disinfected to the hypochlorite of sodium to 5 % during a few minutes and rinse in the distilled water.

Methods of culture

The Seeds are germinated in alveoli filled with compost up to the seedling stage in a green house. Then, seedlings are transplanted in cylinders (height 50 cm and diameter 20 cm), completely papered with a coat of gravels to assure the drainage, filled with a mixture sand / compost (2V / V).A watering every three days is operated to the nutrient solution of Hoagland (1938) to 30% of the capacity of retention of the substrate.

Application of stress

After 60 days, the plant was stressed by heavy metals (Zn, Cd and Pb) at doses of 0,2500, 5000, 7500 and 10000 ppm and for the copper 0,2000, 2500,3000 and 3500 ppm.

After two weeks of the stress, plants are taken, leaves, and roots separated, and dried for 24 hours at 80°C. Then, the dry samples are crushed is deposited in closed vials using plasma plug.

The parameters analyzed

Content of soluble sugars

The content of soluble sugars is determined by the method of Dubois *et al.* (1965)by the measure of the absorbance at 490 nm.

Content of proline

The content of proline is determined by the method of Bates *et al.* (1973). The contents are expressed $mg.g^{-1}$ of dry weight after reading of the D.O to 520 nmon a spectrophotometer JENWAY 6505UV/Vis.

Statistical analyzes

The results obtained are treated statistically using the STATBOX software. Version 6.4, an analysis of the variance calling to the test of Newman-Keuls P = 5%.

Results

Soluble sugars

Soluble sugar content of the leaves and roots of stressed plants to copper

The results obtained show an important increase in the soluble sugars content in the leaves and roots of plants subjected to copper stress compared to control plant (Fig. 1).

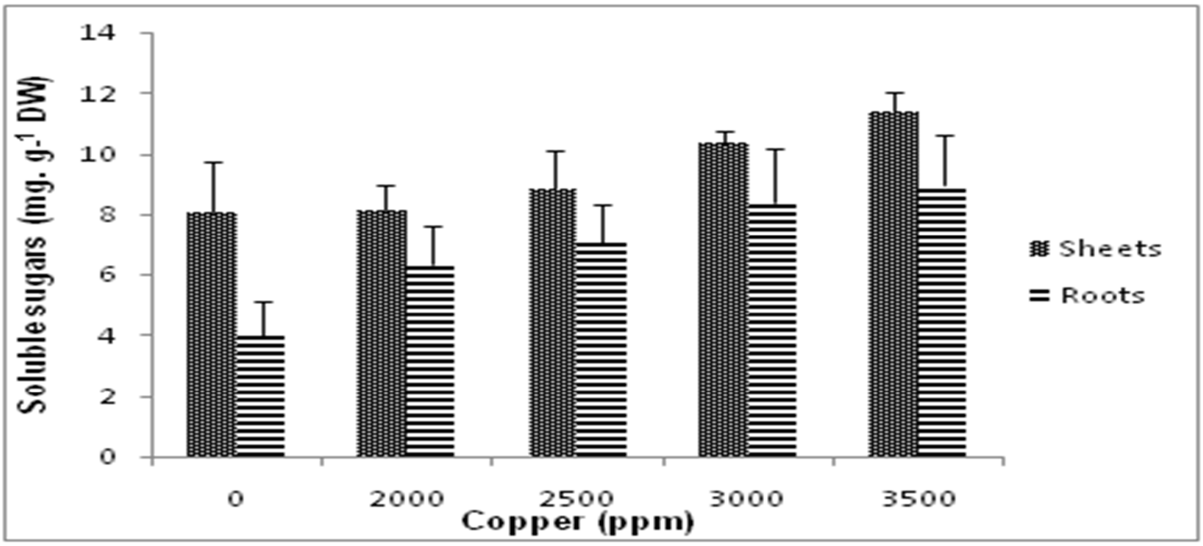

Fig. 1. Soluble sugars content ($mg.g^{-1}$ dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to copper.

The contents obtained for the leaves of plants treated to 2000 ppm copper increase slightly compared to that of the leaves of the control plants. The enrichment of the solutions at 3000 and 3500 ppm of copper causes a more important accumulation. On the other hand, the soluble sugar content of plants stressed to copper increases proportionally with the applied doses.

The statistical analysis reveals that the accumulation of soluble sugars is significant in leaves and roots under the effect of all the treatments to copper.

Soluble sugar content of the leaves and roots of stressed plants to zinc

The results of Figure 2 show that the soluble sugars content at leaves witnesses is widely superior to that of roots witnesses, which represents a 49.62% increase in soluble sugars content than that recorded in the roots.

Statistical analysis using the Newman-Keuls test at P = 5% shows a significant effect very high (P = 0.00) of zinc treatment on the accumulation of soluble sugars in the stressed leaves were in comparison with the leaves witnesses. Thus, the soluble sugars in all treatments are highly significant (P = 0.00) for the roots.

Soluble sugar content of the leaves and roots of stressed plants to cadmium

The results of Figure 3 show that the rate of soluble sugars in the aerial and root parts increases proportionally with the increase in the concentration of cadmium.

For the same concentrations of cadmium, the accumulation of sugars at the level of leaves is higher than that registered in roots. Soluble sugars distribute in a way balanced in leaves to stressful doses 2500, 5000, 7500 and 10000 ppm of cadmium respectively. On the other hand, the soluble sugars content the higher in roots is obtained for the dose of 5000ppm of cadmium.

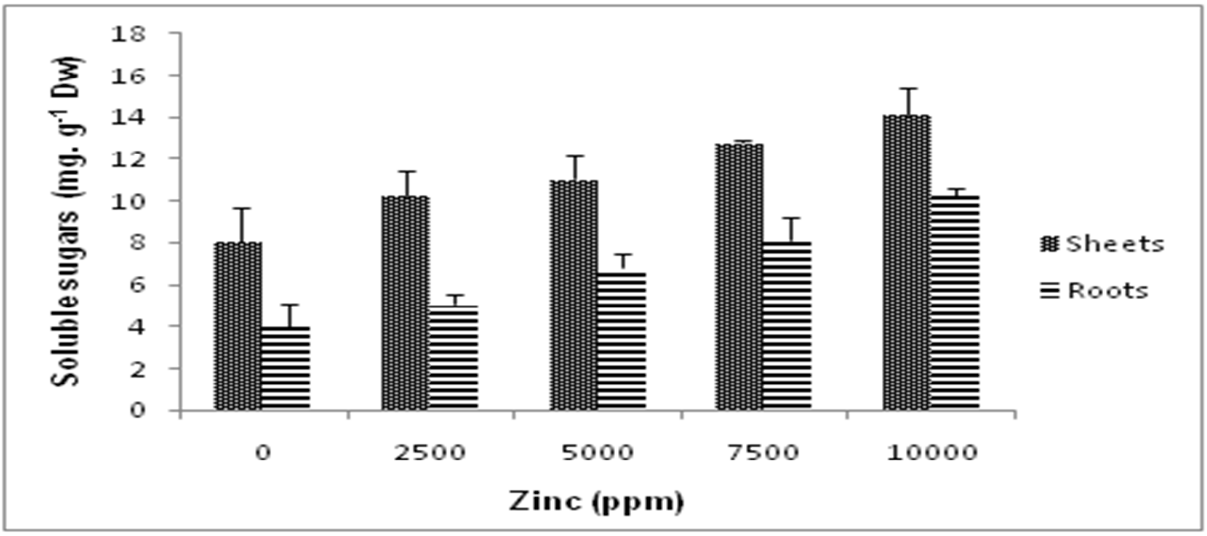

Fig. 2. Soluble sugars content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to zinc.

The analysis of the variance reveals a very highly significant difference for the leaves of the stressed plants compared to the leaves of the control plants. On the other hand, there is a significant difference in the roots of stressed plants compared to the roots of the control plants.

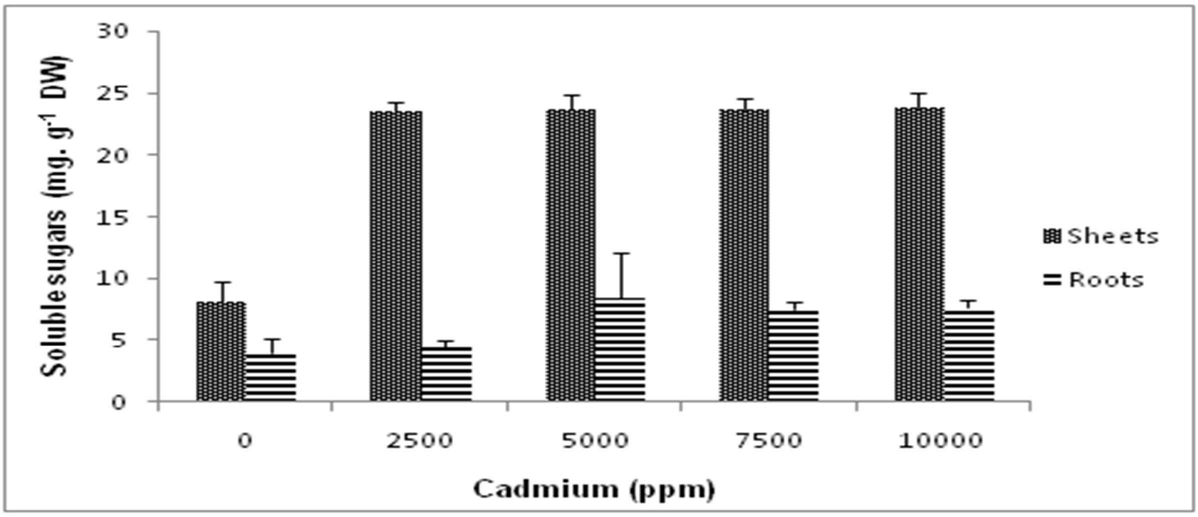

Fig. 3. Soluble sugars content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to cadmium.

Soluble sugar content of the leaves and roots of stressed plants to lead

The results found (Fig. 4) for lead stressed plants at different concentrations (2500, 5000, 7500 and 10000 ppm) reveal an increase in soluble sugars in the leaves and roots of *Atriplex canescens*. The contents obtained soluble sugars are higher in the leaves of the plant that to roots.

Statistical analysis using the test of Newman-Keuls to p = 5% reveals that the contents insoluble sugars accumulated in leaves and roots of *Atriplex canescens* are highly significant (p = 0.00) compared to the leaves and roots of the control plants.

The proline

Proline content of the leaves and roots of stressed plants to copper

The results of the figure 5 show that the accumulation of the proline is more important in the leaves than in the roots of *Atriplex canescens*.

The accumulation of the proline in leaves and roots increases gradually with increasing doses of copper.

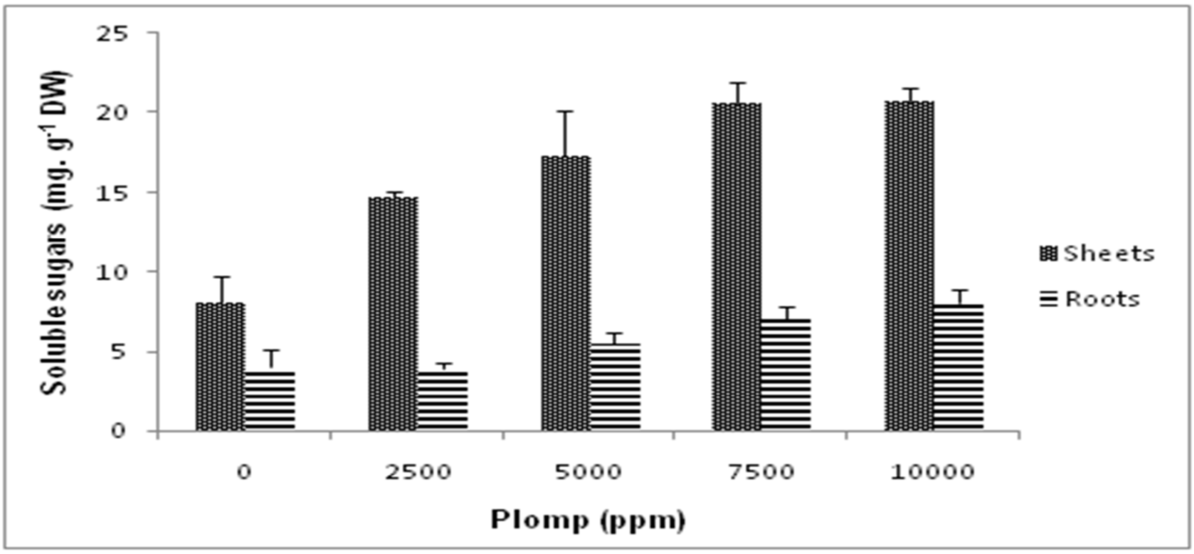

Fig. 4. Soluble sugars content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to lead.

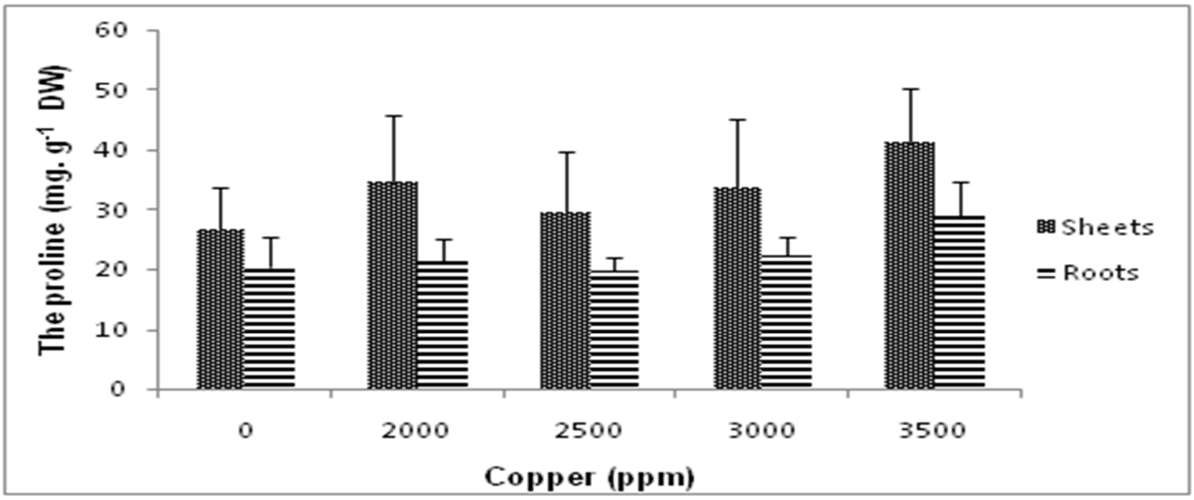

Fig. 5. Proline content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to copper.

The highest proline content in leaves and roots is obtained at a dose of 3500 ppm copper.

In the roots, the light decrease of the proline was noted for the stressful dose of 2500 ppm of copper compared with the control.

The statistical analysis shows a not significant effect on the accumulation of the proline in leaves and roots of the plant for all the doses applied of copper.

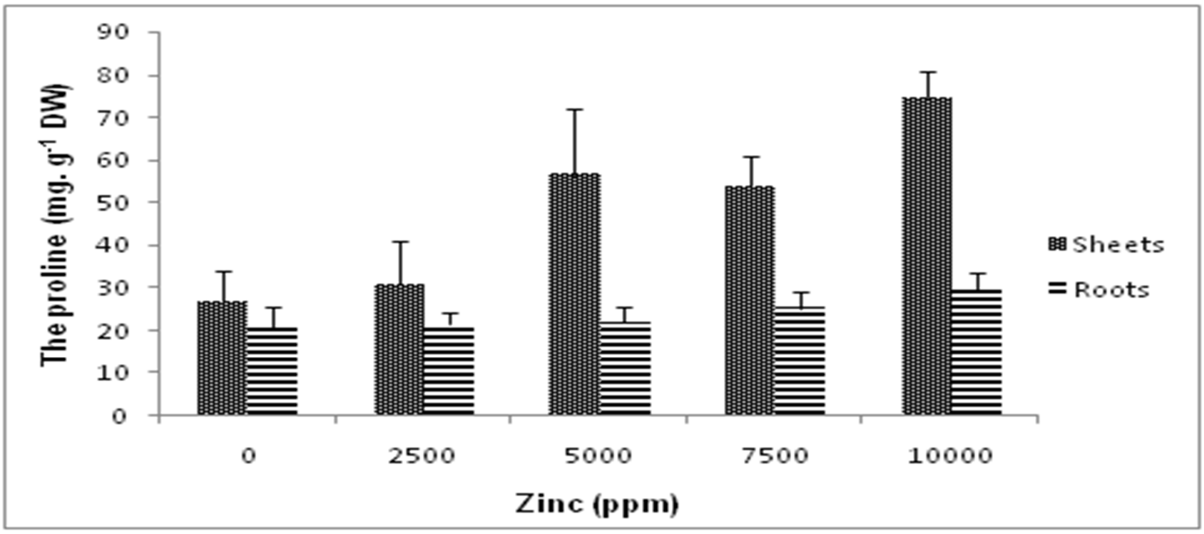

Fig. 6. Proline content (mg.g-1 dry weight) of the leaves and roots of the *Atriplex canescens* (Pursh) Nutt stressed to zinc.

Proline content of the leaves and roots of stressed plants to zinc

The obtained results (Fig.6)*shows that the proline content in the leaves and roots of Atriplex canescens* increases proportionally with the doses of zinc applied to the plant. We record in the stressed plants to the zinc, the higher content of the proline in leaves compared to the roots.

The statistical analysis shows that the accumulation of the proline under the effect of zinc is highly significant in the leaves, whereas it is significant in the roots of the plant.

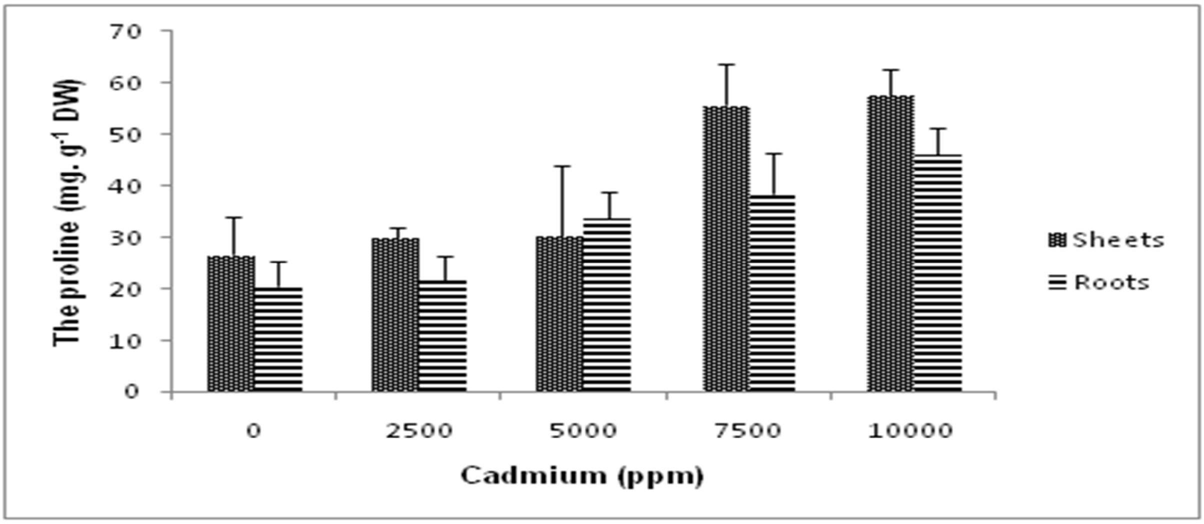

Fig. 7. Proline content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to cadmium.

Proline content of the leaves and roots of stressed plants to cadmium

The results obtained (Fig. 7) reveal that the cadmium stress applied to the plant leads to an increase in the proline level in the leaves at 2500, 5000, 7500 and 10000 ppm of cadmium compared to the control. The accumulation of proline at the level of roots increases progressively with the increasing doses of cadmium. The rate of the proline at the dose of 5000 ppm of cadmium is higher in roots compared to the leaves of the plant.

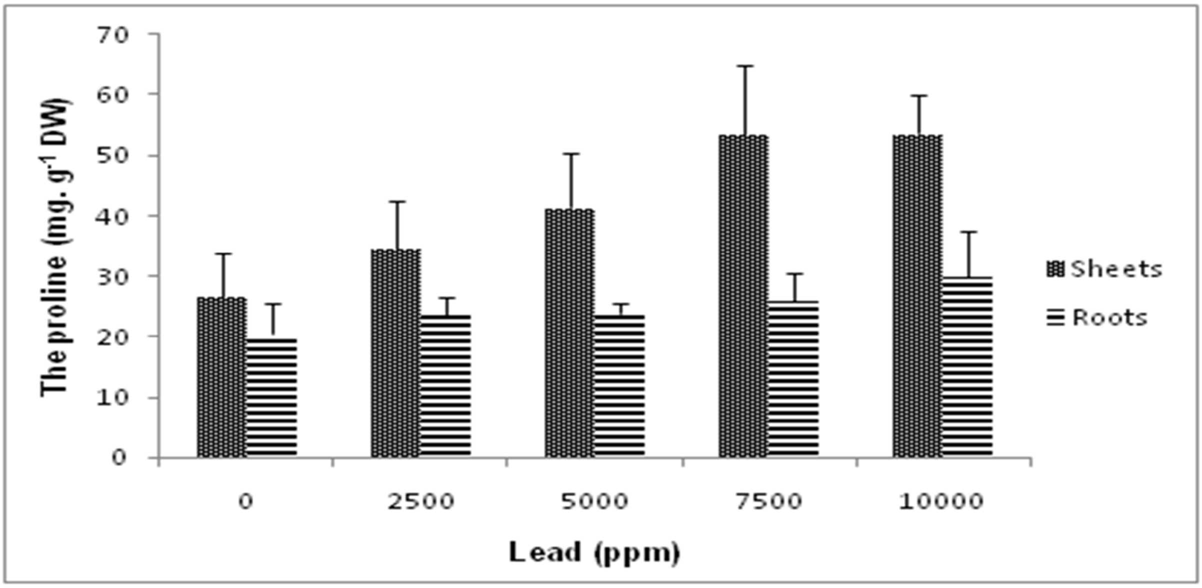

Fig. 8. Proline content (mg.g-1 dry weight) of the leaves and roots of *Atriplex canescens* (Pursh) Nutt stressed to lead.

The statistical study shows a highly significant effect of the cadmium on the accumulation of the proline in leaves and roots compared to the control plant.

Proline content of the leaves and roots of plants stressed plants to lead

The rate of proline in the leaves and roots of *Atriplex canescens* increases progressively with the increasing doses of lead applied to the plant. The proline content is more important in the leaves than in the roots of the plant (Fig. 8).

The statistical analysis shows that the accumulation of the proline is significant in the leaves with all treatments of lead. On the other hand, she is not significant in the roots of the plant.

Discussion

The main results obtained show that: The contents of soluble sugars are high at the level of leaves and roots of *Atriplex canescens* under metallic stress compared with the plant witness. These results are consistent with those of Hajihashemi *et al.*(2006), or the accumulation of soluble sugars observed at the foliar level is among the phenomena the most observed in stress response.

The important accumulation of soluble sugars in the stressed plant with heavy metals (copper, zinc, cadmium and lead) is a response to the metallic stress, unlike the not stressed plant or the soluble sugar content is low, because according to Bouzoubaa *et al.*(2001), The accumulation of soluble sugars could have an osmotic role preventing the dehydration of cells and maintaining the balance of osmotic strength to keep turgor and cytosolic volume as high as possible.

The proline content in leaves and roots to *Atriplex canescens* increases proportionally with the concentration of heavy metals. This accumulation is more important in the sheets than in the roots, which could explain its synthesis at the level of leaves and then its migration to the roots (Djerroudi-Zidane*et al.,* 2010).The results obtained on the important accumulation of proline in the stressed plant corroborate with those of Thiery*et al.* (2004); Djeddi (2006) then Hadjadj*et al.* (2011) which bind the quantities accumulated amounts of proline to stress tolerance.

Our results are consistent with those of Balestrasse *et al.* (2005), where he observed increase of the proline under the effect of the cadmium to the soybean. Similarly Singh *et al.* 2012 obtained under the combined action of lead and cadmium an increase of proline at *Hydrilla verticillata* (l.f.)Royle. Debtor (2012) reported that the contribution of lead acetate induced an important increase in proline in radish (*Raphanus sativus).*

*The accumulation of proline is reported in Artichoke (Cynara scolymus L.) (Karimiet al., 2012), wheat leaves (Janmohammadiet et al., 2013) and the aerial part of sugar beet (Naderiet al., 2013).*Such an increase of the proline was also observed under the effect of other metals such as Mn (Lei *et al.,* 2007), Zn, Pb, Co, Cu (Sharma and Dietz, 2006).

Conclusion

The acquired results confirmed that: The tolerance of *Atriplex canescens* to the stressful concentrations of copper, zinc, lead and cadmium metals.

The accumulation of copper, zinc, lead and cadmium metals is higher in the leaves than in the roots of *Atriplex canescens.*

The accumulation of soluble sugars and proline is very important in the stressed Atriplex compared to the unstressed plant.

The accumulation of soluble sugars and proline are higher in the leaves than in the roots of *Atriplex canescens.*

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