International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 6, p. 130-138, 2017

RESEARCH PAPER OPEN ACCESS

Combined effect of salinity and copper on the rates of polyphénols, flavonoids, and proline accumulation in the leaves of the *Atriplex canescens* (Pursh) Nutt

Ghamnia Youcef*[1], Reguieg Yssaad Houcine Abdelhakim[1], Belkhoudja Moulay[2]

[1]*Department of Biology, University of Abdelhamid Ibn Badis, Mostaganem, Algeria*

[2]*Department of Biology, University of Ahmed Benbella, Es-Senia, Oran, Algeria*

Key words: *Atriplex canescens* (Pursh) Nutt, Salinity and copper, Polyphenols, Flavonoids, Proline.

http://dx.doi.org/10.12692/ijb/11.6.130-138 Article published on December 12, 2017

Abstract

Atriplex is a halophyte plant that accumulates high levels of salt in these tissues. It is also considered as a accumulative plant of heavy metals in the soil. Copper is considered to be a toxic metal when it is at high concentrations. This study investigated the effect of different concentrations of copper (0, 2000, 2500, and 3000 ppm) combined with 0.5 and 3% salt (NaCl) during one month of stress on the rates of polyphenols, flavonoids, and proline in the leaves of the *Atriplex canescens* Pursh Nutt. The analysis of the antioxidants at the leaf level of the *Atriplex canescens* is marked by an increase in the rates of polyphenols and flavonoids under the action of copper combined with 0.5 and 3% NaCl. The higher levels of polyphenols and flavonoids (178 mg. g^{-1} dry weight and 173 mg. g^{-1} dry weights respectively) are obtained at the dose of 3000 ppm copper combined with 3% NaCl. Plants exposed to different concentrations of copper combined with 0.5% NaCl accumulated significant amounts of proline in their leaves compared to controls, the highest levels of which are recorded in plant leaves which receive 2500 and 3000 ppm copper combined with 3% NaCl (447.8 and 546 µg .g^{-1} dry weight respectively) . Finally, it is noted that the *Atriplex canescens* has mechanisms that allow it to tolerate excess copper combined with NaCl.

* **Corresponding Author:** Ghamnia Youcef ✉ youcef_ghamnia@yahoo.fr

Introduction

Soil salinity affects plant growth and development through toxic stress (Okcu *et al*., 2005) and oxidative (Seckin *et al*., 2009). According to Marouf and Reynaud (2007), oxidative stress by salt or heavy metals refers to the set of physiological and metabolic disturbances caused by biotic or abiotic agents in plants. The problem of contaminated soils is now very worrying for emerging countries. Copper is a trace element for plants (Thomas *et al.,* 1998), It plays an important role in its action on the strengthening of the metabolism of plant proteins and the assimilation of CO_2 and in the synthesis of ATP (Lewis *et al*., 2001). The use of copper-based fungicides in vineyards, orchards and other crops susceptible to fungal diseases (including potato, hops and tomato) are the main source of copper soil pollution (Tiller and Merry, 1981), Copper contamination is often accompanied by poor soil physical structure (compaction, settlement, etc.), low water retention, nutrient poverty, soil acidity of drought, and severe erosion (Simmons *et al.,* 2008). In copper-contaminated soil, dehydrogenases and catalases involved in soil respiration are inhibited (Ratnikov *et al.,* 2005). To protect these effects, the development of effective techniques to decontaminate polluted sites has become indispensable. Phytoremediation is defined as a group of technologies that uses plants to reduce, remove, degrade, or immobilize contaminants in soils (Ghosh and Singh, 2005). Phytoextraction is the most widely used method of phytoremediation that exploits the properties of certain plants to accumulate large quantities of heavy metals (Rufus *et al*., 1997; Salt *et al*., 1998). Halophytes are naturally salt tolerant plants, used for ecological, food, and antioxidant production and other bioactive substances (Belkhodja and Bidai, 2004; Glenn *et al*., 2013). These plants are of particular interest in understanding the tolerance mechanisms as well as improving yields. *Atriplex* are naturally tolerant species expressing a high accumulation of salt in their aerial parts and are interesting for the fixation and depollution of heavy metals in soils (Walker *et al*., 2014). The accumulation of antioxidant molecules is one of the main adaptive mechanisms to stress (Zhang *et al.,* 1999), according to Sudhaker *et al. (*2001) antioxidants (polyphenols and flavonoids) accumulate more during salt stress. Proline is an amino acid often considered as a biomarker of stress (Szabados and Savouré, 2009), osmoprotectant, when it accumulates at the cellular level improving stress tolerance (Delauney and Verma, 1993; Nanjo *et al*., 1999). Our work aims are to evaluate the combined effect of copper and salinity on the rates of polyphenols, flavonoids and the accumulation of proline in the leaves of the *Atriplex canescens* Pursh Nutt.

Materials and methods

Plant material

The seeds of *Atriplex canescens* Pursh Nutt collected during the period of December 2016 in the region of El-Bayed in southwestern Algeria are shelled, disinfected with sodium hypochlorite (2%) for a few minutes, and then rinsed with distilled water. After germination in alveoli, the seedlings are replanted in plastic pots (16 cm in diameter and 13.8 cm high) filled by 2280 g of mixture of sand and soil (two volumes/one volume), a watering every three days is carried out at the so nutrient solution (Hogland, 1938) at 80% of the substrate retention capacity, seven treatments were selected with 4 repetitions, or 28 pots in total.

Applying stress

Stress is applied to plants aged 3 months using different saline and metal solutions for one month (twice a week). Control plants are watered twice a week by the nutrient solution.

At the end of the stress, the plants are removed, leaves and roots separated, and stewed for 48 hours at 80 ° C. Dry samples are crushed and deposited in glass vials.

The parameters analyzed

Dosage of polyphenols

The determination of total polyphenols is performed with the colorimetric reagent Folin-Cio calteu according to the method quoted by (Wong *et al.,* 2006).

Determination of flavonoids

The method of aluminum trichloride ($ALCL_3$) cited by (Djeridane *et al.,* 2006) is used to quantify flavonoids in leaf extracts.

Determination of proline

Proline is determined according to the technique used by (Troll and Lindesly, 1955), simplified and developed by **(**Dreier and Goring, 1974**).**

The principle is the quantification of the reaction proline-ninhydrin by spectrophotometric measure. Proline is paired with the ninhydrin forming a colorful complex.

Statistical analysis

The results obtained are processed statistically using the Stat box software, Version 6.4.

Results

The rates of polyphenols in the leaves of the stressed plants by copper combined with 0.5 % NaCl

The results showed an increase in the rates of polyphenols in the leaves of the stressed plants at 2000, 2500, and 3000 ppm combined with 0.5% NaCl (159, 178 and 178 mg.g-1 dry weight, respectively) compared with the leaves of the control plants 31 mg.g-1 dry weight (Fig. 1).

The analysis of the results indicates a significant effect of copper combined with 0.5% NaCl on the rate of polyphenols (P = 0.05).

Table 1. The experimental device.

Concentration	Lot_1	Lot_2	Lot_3	Lot_4	Lot_5	Lot_6	Lot_7
NaCl (%)	0	0 ,5	0 ,5	0 ,5	3	3	3
Copper (ppm)	0	2000	2500	3000	2000	2500	3000

The rates of polyphenols in the leaves of the stressed plants by copper combined with 3 % NaCl

The results obtained in the leaves of the plants of *Atriplex canescens* Pursh Nutt show that the leaves of the stressed plants are richer in polyphenols compared to the leaves of the control plants, this results is an increase in the rates of Polyphenols in the leaves of plants stressed at 2000, 2500 and 3000 ppm of copper combined with 3% NaCl (157, 167 and 178 mg-1 g dry peas, respectively) compared with control plants (31 mg-1 G dry peas) (Fig. 2)

Analysis of the variance revealed a highly significant effect of copper combined with 3% NaCl (P = 0.05).

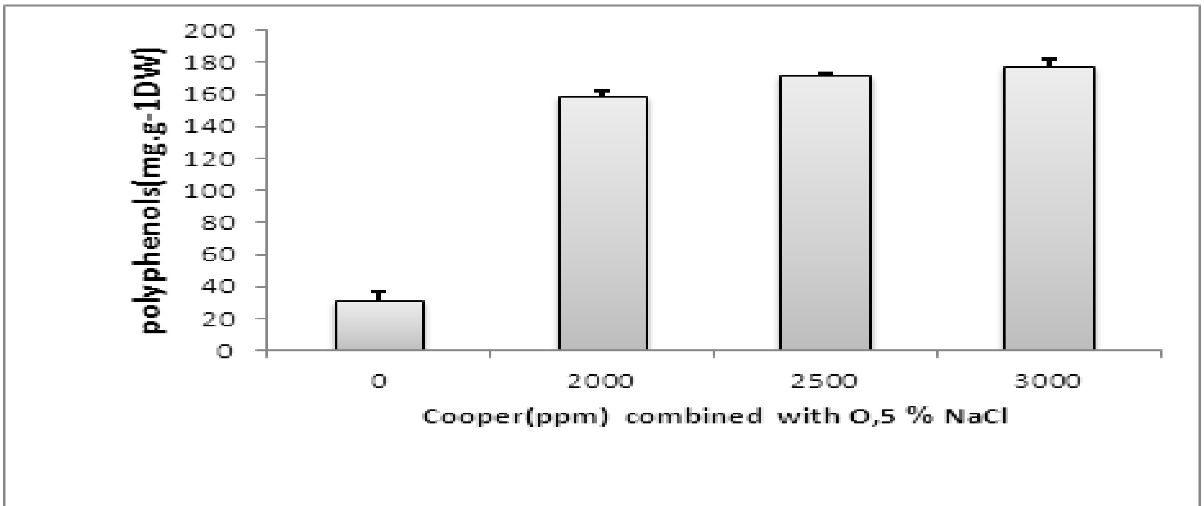

Fig. 1. Polyphenols (mg. g^{-1} dry weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 0.5% NaCl.

The rates of flavonoids in the leaves of the stressed plants by copper combined with 0.5 % NaCl

The results obtained under treatments 0, 2000, and 2500 ppm of copper combined with 0.5% NaCl showed no significant variation in flavonoids content in plant leaves (Fig. 3). On the other hand for leaves of plants stressed at 3000 ppm of copper combined with 0.5% NaCl the results show an increase in the flavonoids content, with a rate of 163 mg. g^{-1} dry weight.

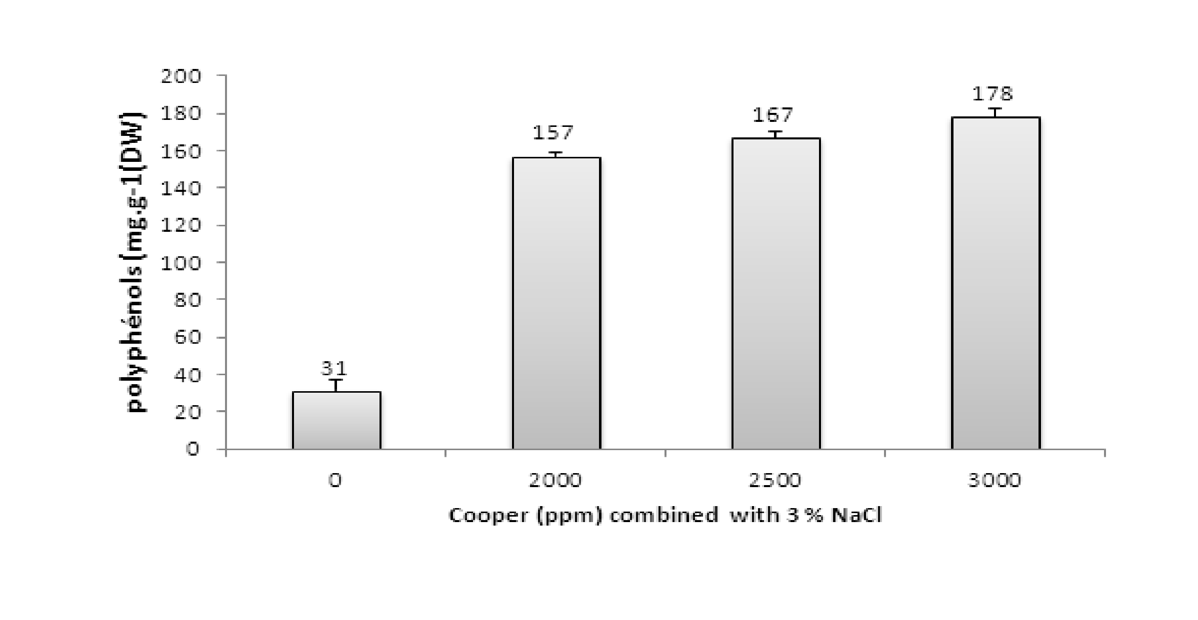

Fig. 2. Polyphenols (mg. g^{-1} dry weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 3% NaCl.

The statistical analysis shows a highly significant effect on the flavonoids content in the leaves of the stressed plants at 3000 ppm of copper combined with 0.5% NaCl ,compared to the stressed plants at 2000, 2500 ppm and 0 ppm of the copper combined with 0.5% d e NaCl).

The rates of flavonoids in the leaves of the stressed plants by copper combined with 3 % NaCl

The results show that the flavonoids levels in the leaves of the stressed plants increase proportionately with the increase in combined doses of copper and salt (Fig. 4).The rate of flavonoids in plant leaves is 124 mg. g^{-1} dry weights for the control at 130 and 141 mg. g^{-1} dry weight respectively for the doses of 2000 and 2500ppm of copper combined with 3% NaCl. On the other hand the highest rate is recorded at the dose of 3000 ppm copper combined with 3% NaCl (173 mg g^{-1} dry weight).

The statistical analysis showed a highly significant effect in the leaves of the stressed plants the dose of 3000 ppm copper combined with 3% NaCl.

However, the effect is significant for the doses of 2000 and 3000 ppm of copper combined with 3% NaCl compared with the leaves of the control plants.

Proline content in leaves and roots of plants stressed by copper combined with 0.5 % NaCl

The results obtained (Fig. 5) show that the accumulation of proline in stressed plants increases with the intensity of the stress applied; this accumulation is higher in the leaves than in the roots, which represents a 45% increase in proline content than that recorded in the roots.

The highest proline content is recorded at the dose of 3000 ppm copper combined with 0.5 % NaCl (375.9 μg^{-1} dry weight). On the other hand, the highest proline content is recorded in the roots at the dose of 2500 ppm copper combined with 0.5 %NaCl.

The leaves and roots of the control plants represent the lowest proline content.

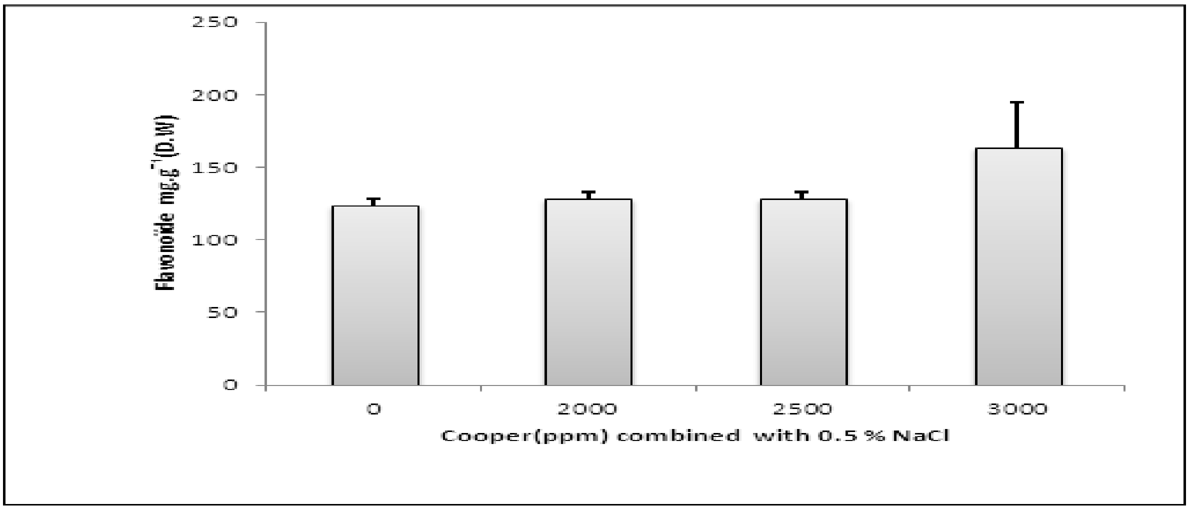

Fig. 3. Flavonoids content (mg. g-1 dry weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 0.5% NaCl.

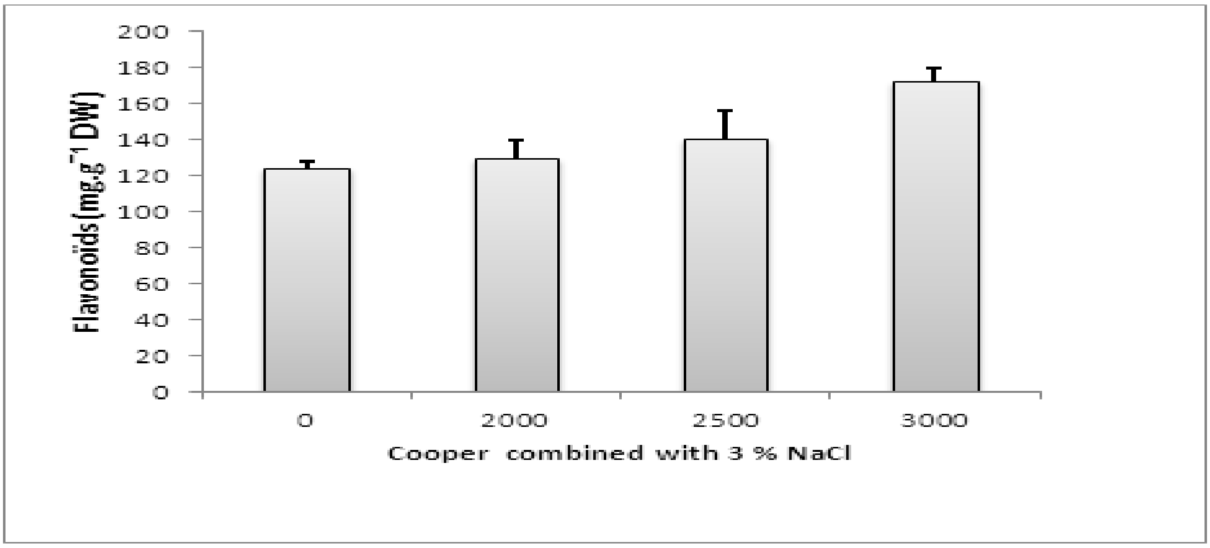

Fig. 4. Flavonoids content (mg. g-1 dry weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 3% NaCl.

The statistical analysis reveals a highly significant effect of proline content in the leaves and roots of stressed plants compared to controls.

Proline content in leaves and roots of plants stressed by copper combined with 3 % NaCl

It is noted that the proline content in the leaves and roots of the plants increases proportionately with the doses of the applied copper (Fig. 6), the highest proline content is recorded in plant leaves at a dose of 3000 ppm copper combined with 3% NaCl (546 µg.g-1 dry weight). The lowest proline content was observed in leaves of unstressed plants (218.4 µg.g-1 dry weight), In the roots of stressed plants, a higher level of proline was obtained at a dose of 3000 ppm copper combined with 3% NaCl (356.0 µg. g-1 dry).

The statistical study shows that the accumulation of proline is highly significant in the leaves and roots of the stressed plants.

Discussion

Polyphenols and flavonoids are higher in the leaves of stressed plants; these results are consistent with those Lotmani and Mesnoua, (2011) that showed an increase in the capacity of the antioxidants of the

Ariplex halimus to cope with the stress of copper. Anshula and Gurpreet, (2013) confirm the toxic effect of copper that increases the activity of antioxidants in the plant Chickpea (*Cicer arietinum* L). The synthesis of polyphenols increases proportionately with the increase in the salt content of barley, artichoke and red pigment (Ali and Abbes, 2003; Ksouri *et al.*, 2007; Rezazadah *et al.*, 2012). According to Rezazadeh *et al.* (2012), the flavonoids levels in the leaves of the artichoke (*Cymara scolymus* L) Increase with the increase in saline concentrations, and in the leaves of okra (*Abelmoschus esculentus* L.) (Bendkhel and Denden (2012).

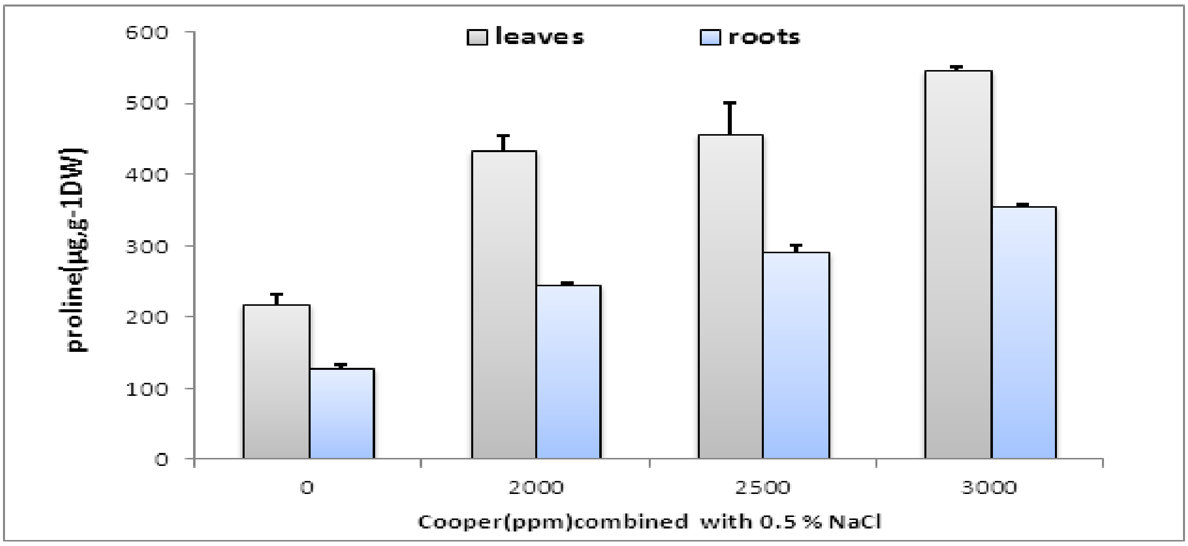

Fig. 5. Proline content (µg g^{-1} dry weight) in the leaves and roots of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 0.5% NaCl.

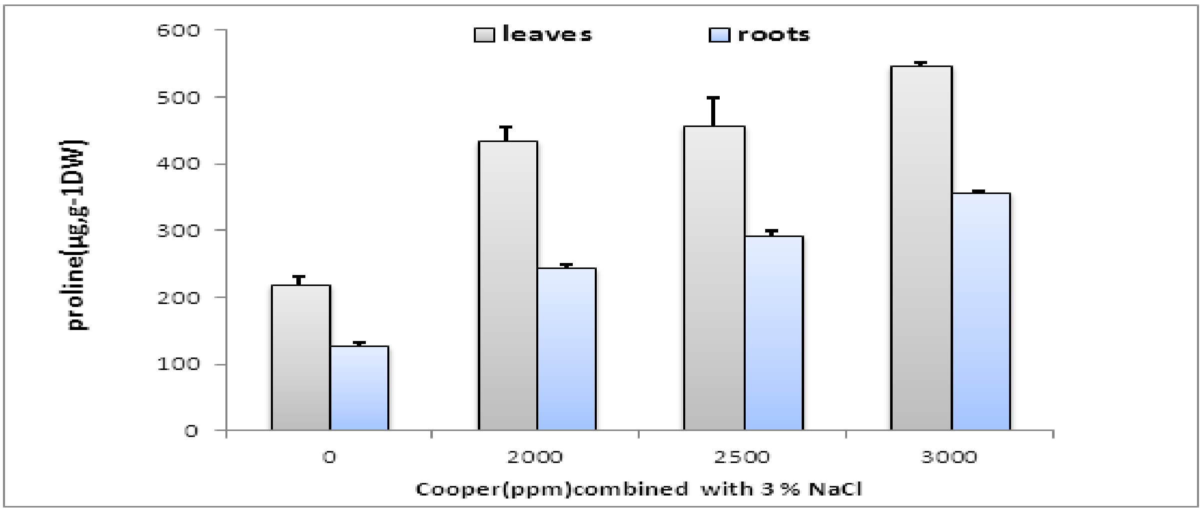

Fig. 6. Proline content (µg g^{-1} dry weight) of the leaves and roots of the *Atriplex canescens* (Pursh) Nutt stressed with copper combined with 3% NaCl.

The accumulation of proline in the leaves and roots of *Atriplex canescens* plants increased with increased doses of copper combined with NaCl. This accumulation is more important in the leaves of stressed plants at 2500 and 3000 ppm of copper combined with 3% NaCl.

These results are consistent with those of researchers such as(Sharma and Dietz, 2006; Lei *et al.*, 2007) which show the relationship of the toxic effect of metals, such as Mn, Zn, Pb, Co, Cu with the increase of proline in leaves and roots of plants stressed by these metals.

The accumulation of Proline was reported in several plants, sunflower under the effect of cadmium (Balestrasse *et al.*, 2005), in *Hydrilla verticillata* (L.f.) Royle under the combined action of lead and cadmium (Singh *et al.*, 2012). According to Belkhodja and Bidai (2004), the accumulation of proline increased significantly with the increase in salinity in the seeds of *Atriplex halimus* at the germination stage. Zid and Grignon, (1991) has shown that proline is a substance that accumulates in tissues subject to abiotic stress and is a means of resistance and tolerance to salinity

Conclusion

The response of *Atriplex canescens* to saline and metallic stresses resulted in an increase in antioxidants (polyphenols, flavonoids) and the accumulation of proline, which shows a relationship between the biosynthesis of proline and the rise in rates of polyphenols and flavonoids, this increase is more important in the leaves of plants stressed by 2500 and 3000 of copper combined with 3% NaCl. To this end, it is noted that the *Atriplex canescens* has mechanisms that allow it to tolerate excess copper combined with NaCl, up to 3000 ppm of copper combined with 3% NaCl.

References

Anshula S, Gurpreet S. 2013. Studies on the effect of Cu (II) ions on the antioxidant enzymes in chickpea (*Cicer arietinum* L) cultivars. Singh Journal of Stress Physiology & Biochemistry **9(1),** 5-13.

Ali R M, Abes HM. 2003. Reponses of Salt stressed barely seedlings to phenylurea. Plant Soil Environ **49(4),** 158-162.

Balestrasse KB, Gallego SM, Benavides MP, Tomaro ML. 2005. Polyamines and proline are affected by cadmium stress in nodules and roots of soybean plants. Plant and Soils **270(1),** 343-353. https://doi.org/10.1007/s11104-004-17920.

Belkhodja M, Bidai Y. 2004. Réponse de la germination des graines d' *Atriplex halimus* L sous stress salin. Revue Sécheresse **15(4),** 331-335.

Bendkhil B, Denden M. 2012. Effect of salt stress on growth anthocyanins membrane permeability and chlorophyll fluorescence of okra (*Abelmoschus esculentus* L) seedlings. American Journal of Plant physiology **7(4),** 174-183. http://scialert.net/abstract/?doi=ajpp.2012.174.183

Delauney AJ, Verma DPS. 1993. Proline biosynthesis and osmoregulation in plants. Plant Journal **4(2),** 215-223.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2006. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. Food Chemistry **97(4)**, 654-660. https://doi.org/10.1016/j.foodchem.2005.04.028.

Drier W, Goring M. 1974**.** Der einfluss hoher Salzkonzentrationen auf physiologische parameter von maiswurzeln. From the journal Wiss Z Humboldt Univ Berlin **23,** 641-646.

Ghosh M, Singh SP. 2005 .A Review on Phytoremediation of Heavy Metals and Utilization of It's by Products. Asian Journal on Energy and Environment **6(04),** 214-231

Glenn EP, Anday T, Chaturvedi R, Martinez-Garcia R, Pearlstein S, Soliz D, Stephen GN, Felger RS. 2013. "Three halophytes for saline-water agriculture: An oilseed, a forage and a grain crop". Environmental and Experimental Botany **92**, 110-121. http://dx.doi.org/10.1016/j.envexpbot.2012.05.002.

Lewis S, Dokin ME, Depledge MH. 2001. Hsp 70 expression in *Enteromorpha intestenalis* (Chlorophyta) exposed to environmental stressos. Aquat Toxicology **51(3),** 277-291. https://doi.org/10.1016/S0166-445X(00)00119-3.

Lei Y, Korpelainen H, Li C. 2007. Physiological and biochemical responses to high Mn concentrations in two contrasting *Populus cathayana* populations. Chemosphere **68(4),** 686-694. https://doi.org/10.1016/j.chemosphere.2007.01.066.

Lotmani B, Mesnoua M. 2011. Effects of copper stress on antioxidative enzymes, chlorophyll and protein content in *Atriplex halimus*. African Journal of Biotechnology, **10(50),** 10143-10148. https://doi.org/10.5897/AJB10.1804.

Marouf A, Reynaud. 2007. La boanique de A à Z 1662 définition Edition Dunod www.eyrolles.com/Droit/Livre/la-botanique-de-a-a-z-9782100506385.

Nanjo T, Kobayashi M, Yoshiba Y, Sanada Y, Wada Y, Tsukaya K, Kakubari W, Keishiro W Y, Hirokazu T, Yoshitaka K, Kazuko YS, Kazuo S. 1999. Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. The Plant Journal. **18(2),** 185-193. https://doi.org/10.1046/j.1365-313X.1999.00438.x.

Okcu G, Kaya MD, Atak M. 2005. "Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.)". Turkish journal of agriculture and forestry **29(4),** 237-242.

Ratnikov AN, Sviridenko DG, Zhigareva TL, Popova GI. 2005. Effects of heavy metals and organic fertilizers on a biological activity of soddy-podzolic soil and yield of barley. International Workshop "Current developments in remediation of contaminated sites" 27 – 29 October 2005, IUNG, Pulawy, Poland.

Rezazadeh A, Ghasemnezhad A, Barani M, Telmadarrehei T. 2012. Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara scolymus* L.) leaves. Research Journal of Medicinal Plants **6,** 245-252. https://doi.org/10.3923/rjmp.2012.245.252

Rufus LC, Minnie M, Li YM, Sally LB, Eric P B, Scott-Angle J, Alan JMB. 1997. Phytoremediation of soil metals .Current Opinion in Biotechnology **8(3),** 279-284. https://doi.org/10.1016/S0958-1669(97)80004-3.

Salt DE, Smith, Raskin RD, Raskin I. 1998. Phytoremediation, Annual Review of Plant Physiology and Plant Molecular Biology **49**, 643-668. https://doi.org/10.1146/annurev.arplant.49.1.643.

Sharma SS, Dietz KJ. 2006. The significance of amino acids and amino-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany **57(4),** 711-726. https://doi.org/10.1093/jxb/erj073.

Seckin B, Sekmen AH, Türkan I. 2009. "An Enhancing Effect of Exogenous Mannitol on the Antioxidant Enzyme Activities in Roots of Wheat under Salt Stress". Journal of Plant Growth Regulation **28(1),** 12.

Simmons Jeffery A, William Currie S, Keith Eshleman N, Karen Kuers, Susan Monteleone, Tim Negley L, Bob Pohlad R, Carolyn Thomas L. 2008. Forest to reclaimed mine land use change leads to altered ecosystem structure and function. Ecological Applications **18(1),** 104-118. https://doi.org/10.1890/07-1117.1.

Singh A, Kumar CS, Agarwal A. 2012. Physiological study of combined heavy metal stress on *Hydrilla verticillata* (l.f.) Royle. International Journal of environmental sciences **2(4),** 2234-2242. https://doi.org/10.6088/ijes.00202030106.

Sudhakar C, Lakshmi A, Giridarakumar S. 2001. Changs in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Science **161(3),** 613-619. https://doi.org/10.1016/S0168-9452(01)00450-2.

Szabados L, Savouré A. 2009. Proline: a multifunctional amino acid. Review- Trends in Plant Science **15(2),** 89-97. https://doi.org/10.1016/j.tplants.2009.11.009.

Thomas JC, Malick FK, Endreszl C, Davies C, Elizabeth CD, Kent SM. 1998. Distinct responses to copper stress in the halophyte *Mesembryanthemum crystallinum*. Physiologia Plantarum **102(3),** 360-368. https://doi.org/10.1034/j.13993054.1998.1020304.x.

Tiller KG, Merry RH. 1981. Copper pollution of agricultural soils. In: J. F. Loneragan, A. D. Robson and R. D. Graham (Editors). Copper in Soils and Plants, Academic press, Sidney, 119-137.

Troll W, Lindsey JA. 1955. Photometric method for the determination of proline. Journal of biological Chemistry 1955, **215(2),** 655-660.

Walker DJ, Lutz S, Sanchez- Garcia M, Correa E. 2014. "*Atriplex halimus* L.: Its biology and uses". Journal of Arid Environments **101,** 111-121. https://doi.org/10.1016/j.jaridenv.2013.09.004.

Wong CC, Li H B, Cheng KW, Chen FC. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay .Food Chemistry **97(4),** 705-711. https://doi.org/10.1016/j.foodchem.2005.05.049

Zhang J, Nguyen HT, Blumo A. 1999.Genetic analysis of osmotic adjustment in crop plants. Journal of Experimental Botany **50(332),** 291-302. https://doi.org/10.1093/jxb/50.332.291

Zid E, Grignon C. 1991. Early selection tests of the plant resistance stress. Case of the saline and water stress. The plant improvement for the arid climatic adaptation. (Ed) Aupelf-Uref, John Libbey Euro text,. 101-108.