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# **Effect of different concentrations of ZnSO<sub>4</sub> on *Pistacia atlantica* plantlets growth under semi-controlled conditions**

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**Abstract.** The effect of ZnSO<sub>4</sub> stress on some morphological, physiological and biochemical parameters of *Pistacia atlantica* plantlets was studied under semi-controlled conditions. The plants were grown in pots and irrigated with distilled water for 30 days. Then, the plantlets were treated with 0, 0.01, 0.1 and 1 mmol of ZnSO<sub>4</sub> for 18 days. The application of these concentrations caused stress on the young *Pistacia atlantica* plantlets by reducing the growth of morphological parameters. The amount of free proline in shoots and roots grows significantly with the concentration in the medium of ZnSO<sub>4</sub> for all the treatments. On the other hand, no significant difference in relative water content was noted. These results are indicating that *P. atlantica* can be used in phytoremediation because of its rusticity and resistance to stress caused by Zinc.

**Keywords.** Water – Soil – Heavy metals – Phytoremediation – *Pistacia atlantica*.

**Effet des différentes concentrations de ZnSO<sub>4</sub> sur la croissance de semis de Pistacia atlantica Desf.), cultivés en conditions semi-contrôlées**

**Résumé.** L'effet du stress causé par ZnSO<sub>4</sub> sur quelques paramètres morphologiques, physiologiques et biochimiques des plantules de *Pistacia atlantica* a été étudié sous conditions semi contrôlées. Les plantules ont été cultivées dans des pots et irriguées avec de l'eau distillée durant 30 jours. Ensuite, les plantules ont été traitées à 0- 0,01- 0,1 et 1 mmol de ZnSO<sub>4</sub> pendant 18 jours. Il a été établi que l'application de ces concentrations de ZnSO<sub>4</sub> cause un stress non significatif aux plantules de pistachier qui s'exprime par une faible réduction de la croissance au niveau des caractères morphologiques. Après 18 jours de stress, le taux de proline chez *P. atlantica* est très élevé, dans les parties aérienne et racinaire et croît significativement en fonction de la concentration du milieu en ZnSO<sub>4</sub> pour tous les traitements. Par ailleurs, une différence non significative pour la teneur en eau a été enregistrée. Les résultats obtenus au terme de cette étude suggèrent que *P. atlantica* pourrait être utilisé en phytoremédiation, du fait de sa rusticité et de sa résistance au stress causé par le Zinc.

**Mots-clés.** Eau – Sol – Métaux lourds – Phytoremédiation – *Pistacia atlantica*.

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## **I – Introduction**

Phytoextraction is related to plants that are able of absorbing heavy metals through their rooting system to the aerial parts (Zheng *et al.*, 2011). *Pistacia atlantica*, which has a huge rooting system and is adapted to degraded environments with various climates. The tree forms mycorrhizal associations with many species of fungi which play a role in the restoration of polluted soils by heavy metals, by reducing their amounts (Fortas and Dib, 2003). This work aims to study the mechanism of tolerance of *Pistacia atlantica*, under metal stress, in order to use this tree in phytoremediation.

## II – Material and methods

The seeds are collected from fruits of *P. atlantica*, at the maturation stage in Messaad region. They were stored in the refrigerator until their use. The seeds of *P. atlantica* may need stratification for better and uniform germination (Belhadj, 2007). After stratification, sprouted seeds are sown directly in a peat-sand sterilized mixture. Growth assay is performed under semi-controlled conditions. The seedlings are irrigated daily, once a day with 20 ml (per pot), firstly with distilled water for 30 days and then with  $\text{ZnSO}_4$  solutions at different concentrations (0.01, 0.1, 1 mmol and control). Forty pots were used for each test.

Morphological (stem height (cm), number of nodes, total number of leaves, number of compound leaves, root length (cm), number of secondary roots, shoot and root biomass ratio of the aerial/root part), physiological [water content, calculated by the formula of Heller (1998)] and biochemical parameters [proline contents in the aerial and the root parts of the seedlings, as determined by the method described by Troll and Lindsley (1955)] were measured. All data were subjected to statistical analysis using Excel software. The analysis of variance (ANOVA) and Newman-Keuls test were performed.

## III – Results and discussion

The effect of  $\text{ZnSO}_4$  was not significant on the growth of the seedlings, for the duration of our test, despite a clear reduction in stem height, number of nodes, number of leaves, root length and number of secondary roots, as well as biomass of both aerial and root parts (Fig. 1). Similar results were obtained by Benhassaine *et al.* (2011) for the length of the stems and the roots, on of *P. atlantica* salt stressed seedlings.

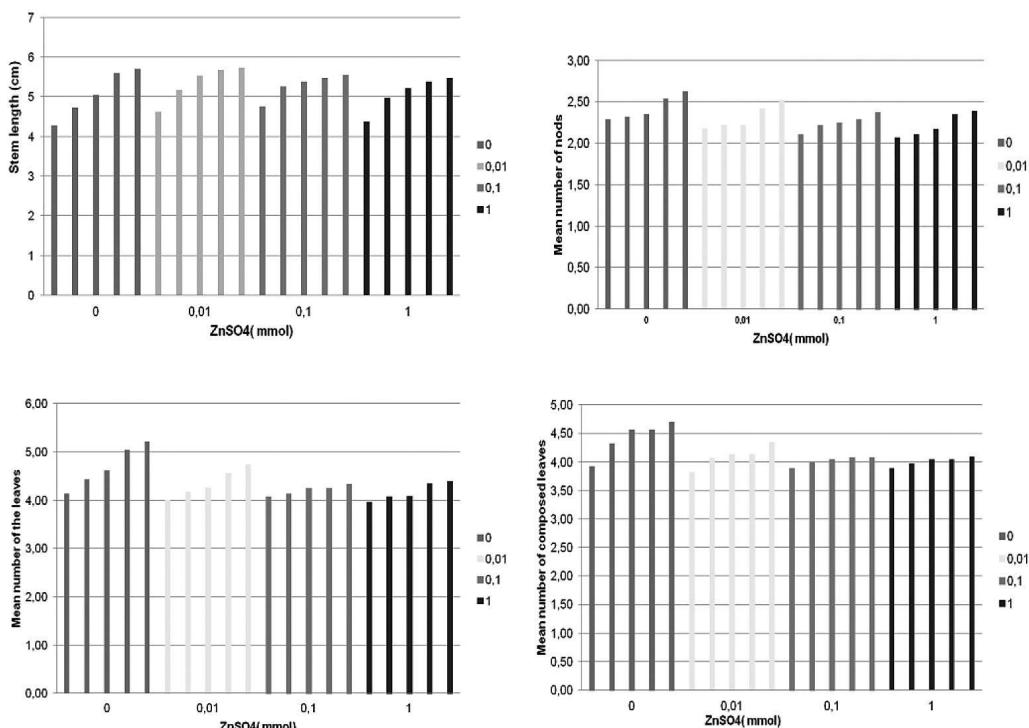


Fig. 1. Evolution of morphological characters of *P. atlantica* plantlets cultivated in different concentrations of  $\text{ZnSO}_4$ .

Plants exposed to  $ZnSO_4$  shows a insignificant variation in water content (Fig. 2 and Table 1) which means a good tissue hydration despite the stresses status. According to Brunet *et al.* (2008), low variations in the water content for *Lathyrus sativus* plants treated with lead were observed. However, the applied stress levels induced a very highly significant increase ( $P < 0.001$ ) in proline contents for the shoot and root portions of the seedlings (Fig. 3 and Table 2). The more the concentration of  $ZnSO_4$  increases, the more the proline contents become marked. This result is in line with those of Monneveux and Nemmar (1986). The highest levels of proline are recorded in the aerial parts. This is consistent with the work of Benhassaini *et al.* (2011).

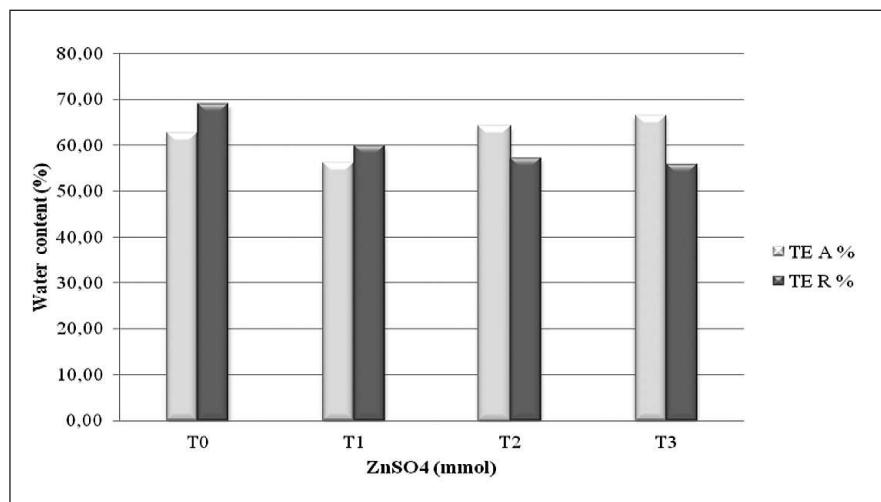


Fig. 2. Water content (TE) variation of the aerial parts (A) and root parts (R) of *P. atlantica* plantlets cultivated in different concentrations of  $ZnSO_4$  (T0,T1,T2,T3).

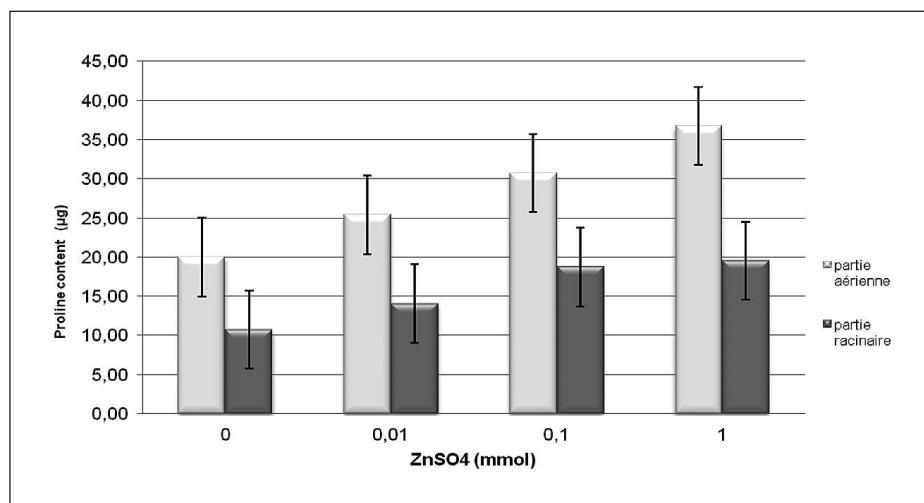


Fig. 3. Effect of  $ZnSO_4$  concentrations on the proline contents of *P. atlantica* plantlets.

**Table 1.** Newman-Keuls Test for the water content ( $\alpha < 5\%$ )

	ZnSO <sub>4</sub> (mmol)	0	0.01	0.1	1
Aerial part	Water content ± S.D.	62.75 ± 16.40	56.26 ± 10.39	63.95 ± 16.75	66.56 ± 18.55
	Homogenous groups	A	A	A	A
Root part	Proline content ± S.D.	65.88 ± 15.40	59.91 ± 11.49	57.23 ± 18.75	
	Homogenous groups	A	A	A	A

**Table 2.** Newman-Keuls test for the proline contents (µg) in the aerial and the root parts of *P. atlantica* plantlets cultivated in different concentrations of ZnSO<sub>4</sub>

ZnSO <sub>4</sub> (mmol)	Aerial part Mean (µg) ± S.D.	Root part Mean (µg) ± S.D.	Mean (µg) ± S.D.
0	20.000 ± 1.039 d	10.733 ± 0.306 c	15.367 ± 0.672
0.01	25.400 ± 4.000 c	14.067 ± 1.155 b	19.733 ± 2.577
0.1	30.733 ± 2.309 b	18.733 ± 0.306 a	24.733 ± 1.307
1	36.733 ± 3.055 a	19.533 ± 0.808 a	28.133 ± 1.932

## IV – Conclusions

Our results showed that *Pistacia atlantica* can withstand stressing status caused by zinc in soil solution. Thus this tree can be used in soil and water remediation in contaminated areas by this metal.

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