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Cadmium Accumulation in *Atriplex Nummularia* L. (Old Man Saltbush) Grown on Contaminated non Saline and Saline Environment

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Abstract

In this study, the salt bush *Atriplex nummularia* L., which is a C4 perennial shrub with an excellent tolerance to drought and salinity, is investigated with the main aim to assess its phytoremediation potential for Cd removal from contaminated soils. In the present study, the halophyte used has the ability to excrete the excess NaCl under salt effect through salt glands and trichomes covering their leaf surface. Therefore, it may be suggested that Cd salts have been excreted through trichomes and salt glands to leaf surface then washed out mainly by dew. On the other hand, the halophytes Atriplex, plants used in the present study have salt glands. In this respect, many halophyte species are no more able to accumulate high amount of Na in trichomes covering the leaf surface but also other elements may accumulate when present in excess. Thus, halophytes are potentially ideal elements for phytoextraction or phytostabilization prone to be polluted by heavy metals contaminating soils and especially heavy metals affected by soil salinity.

Keywords: Cadmium; salt glands; phytoextraction; *Atriplex nummularia;* salinity

Introduction

Phytoremediation is an emerging cleanup technology that uses plants for environmental restoration. The use of metal-accumulating plants to clean soil and water contaminated with toxic metals is the most rapidly developing component of this alternative technology, as it offers the benefits of operating in situ, it is a low cost and an environmentally sustainable method as the soil can be reclaimed without a concomitant decrease in soil fertility. Nevertheless, the success of phytoextraction depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts of biomass using established crop production and management practices (Begonia and *al*., 1998; Clemens and *al*., 2002).

However, hyperaccumulators are small plants with a shallow root system and slow growth and difficult to manage therefore their use is rather limited (Garbisu and Alkorta, 2001; Raskin and *al*., 1997; Salt and *al*., 1998).

The main purpose of this work was to assess potential of phytoremediation in the cadmium removal from contaminated soils using the salt tolerance of *Atriplex nummularia* L. (old man saltbush). In order to achieve that goal the accumulation of Cd via root uptake at different saline conditions is investigated as there is notable evidence that salinity is a key factor in the translocation of metals from roots to the aerial parts of the plant (Otte, 1991) so studying of the metal accumulation in salty conditions can be very useful in phytoremediation purposes. In addition, Cd excretion through salt glands on the surface of the leaves is investigated as a probable detoxification mechanism of the plant. There is considerable evidence from previous findings that species of the genus *Atriplex* appear to have been adapted to many different saline soil types hence it is probably not surprising that their salt glands secrete with minimal selectivity a variety of different ions on their leaf surface and that these are representative of the ionic composition of the root environment (Storey et Thomson, 1994; Hagemeyer et Waisel, 1988). Furthermore, the effect of salinity on the metal uptake and the changes in physiological parameters such as biomass production, and chlorophyll content in reference to metal and salt toxicity were also examined , we have examined the possibility of whether or not these plants excrete the metals together with salt, as this could be a unique advantage for phytoremediation applications. The aim of this study was to investigate the possibility of using *Atriplex nummularia* halophytic plant as phytoremediation as well as its efficiency under Algerian conditions.

I. Material and Methods

To investigate the possibility of using a halophytic plant as phytoremediation a pot experiment was carried out in greenhouse at the Department of Biology, Khemis-Miliana University, Algeria. The plants of *Atriplex nummularia* Lindl were collected from the area of Tadmait in the province of Djelfa, Algeria. *Atriplex nummularia* plants (60-days-old) were placed individually into plastic pots filled with a mix made by 2 parts of the same amount of organic substrate and a part of quartz sand in a 2:1 ratio. Irrigation was with one-half strength Fortal solution and with excess volume of tap water on alternated days for acclimatization, during 20 days. At the beginning of the experimental phase plants were divided into 6 experimental groups with 7 plants per each group-treatment, with a total of 42 pots. The experimental design is presented in Table 1.Treatments were designed as shown in Table1.

The experiment was conducted for a period of 5 weeks and it took place in an open air area with protection against rain. Cadmium at concentration of 20 ppm of dry weight of soil was added as aqueous solution of CdCl2 in one dose at the beginning of the experiment. Plants were watered four times per week with approximately 100 ml of tap water or salty water which was prepared from edible NaCl salt and tap water in concentration of 2 or 6g/l according to experimental conditions. Pots were watered to keep moisture content approximately at 60% of water holding capacity.

Table.1. Experimental design

Experimental Treatment ("code name")	Cd concentration [ppm]	Salt concentration [g/l]
0/0	0	0
0/2	0	2
0/6	0	6
Cd/0	20	0
Cd/2	20	2
Cd/6	20	6

Once per week the heights of plants were measured in order to observe and quantifiably evaluate the health status of the plants. Finally with the expiration of the 5 weeks period, 0.2 g of fresh leaves were taken from each plant for the measurement of chlorophyll content, washed with deionised water. Chlorophyll content was measured according to the method of (Harborne, 1984). Measurement of chlorophyll content was done by direct determination of the absorbance at wavelengths 648 and 664 nm using spectrophotometer.

The above ground parts of the plants were cut washed with tap water and twice with deionised water and dried 48 hours at 65°C. The roots were carefully taken out of the soil, washed with tap water and twice with deionised water and dried at 65°C for 48 hours.

II. Resultats and disscussion

II.1 Cadmium accumulation by *Atriplex nummularia* at different salinities

The data from these experiments suggest that increasing salinity increases the cadmium uptake by *A. nummularia* (Table 1). Cd accumulation in the roots increases with increasing salinity reaching the amount of 0.57 ppm from the treatment with 6g/l (Cd/6) salinity.

Correspondingly the accumulation in the shoots increases with increasing salinity reaching the amount of 1.07 ppm from the treatment with 6g/l (Cd/6) salinity but the toxic level of Cd in leaves of plants was not exceeded which is 5-30 ppm dry weight (Orcutt et Nilsen, 2000).

Table.2. Cadmium concentration (ppm) in individual parts of *Atriplex nummularia* treated with 20 ppm Cd of dry weight of soil at different soil salinities. Values shown are means (n=3) with minimum and maximum values.

Treatment	Cd concentration in plant tissue (ppm)	
	Shoots	Roots
0/0	0.31 ± 0.11^{a}	0.48 ± 0.10^{a}
0/2	0.46 ± 0.29^{a}	0.26 ± 0.18^{a}
0/6	0.13 ± 0.01^{a}	0.23 ± 0.12^{a}
Cd/0	0.59 ± 0.12^{a}	0.57 ± 0.11^{b}
Cd/2	0.71 ± 0.44^{b}	0.48 ± 0.17^{a}
Cd/6	1.07 ± 0.38^{c}	0.57 ± 0.17^{b}

Also it is observed (Table 1) that salinity affects significantly the translocation of Cd from the roots to the aerial parts of the plant and this is also visible from the leaf/root ratio which increases with increasing salt concentration in soil from 1 .03 for plants treated in non saline environment (Cd /0) to 1.48 for the treatment with 2g/l (Cd/2) salinity and finally reaching the amount of 1.88 from the plants treated with 6g/l (Cd/6) salinity.

Conclusively this study shows that with increasing salinity, the concentration of Cd in *Atriplex nummularia* increases, and there is a direct gradient increment in metal concentrations from roots to the aerial parts of the plants. These results are in general agreement with other researchers which found increased concentrations of metals in most plant parts under saline conditions and it was speculated that this may be related to higher mobility of metals in the sediment and/or higher water uptake, (due to increased transpiration), leading to a higher flux of metals into the plant (Otte, 1991; Fitzgerald and *al.*, 2003).

II.2. Release of Cd by the leaves of *Atriplex nummularia*

One of the mechanisms which help plant to cope with the excess of salt is to excrete it through salt glands onto their leaf surface (Manousaki and *al*., 2008). Studies with *Tamarix aphylla* have shown that the salt glands secrete a variety of ions other than sodium and chloride and the divalent cations were the major components of the secretory product of the Tamarix trees (Storey et Thomson, 1994). Our study supports that *A. nummularia* excrete cadmium which is associated with salt crystals on the adaxial leaf surface confirming the fact that the roots of this plant have a low selectivity to the uptake of ions from the soil.

Moreover cadmium release rises with increasing salinity when there is more salt excretion (Fig. 1). Concentration of Cd excreted by plants grown in saline environment with 6g/l (Cd/6) salinity is 6.2 times higher than plants grown in non saline environment.

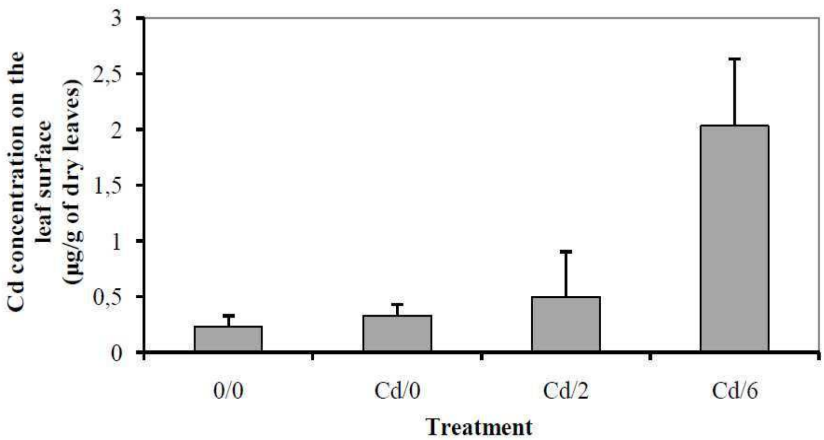

Figure.1. Cadmium excretion from leaf tissue of *Atriplex nummularia* treated with 20 ppm Cd of dry weight of soil at different soil salinities. Values shown are means (n=3) with minimum and maximum values.

The fact that the release of metals from the leaf tissue onto the leaf surface is a method for coping with up taken metals is supported from previous findings (Manousaki et *al*., 2008).

Furthermore, in the same findings it was suggested that the relationship of metal release with salt excretion proposes that there will be greater metal release at higher salinities, when there is more salt excretion (Weis, 2004). All these characteristics of *Atriplex nummularia* could be big advantage for phytoremediation study to improve and facilitate the cadmium removal from the soil.

Conclusion

Cadmium uptake by *Atriplex nummularia* increases with increasing salinity. Furthermore salinity affects significantly the translocation of Cd from the roots to the aerial parts of the plant. But the toxic level of Cd in leaves of plants is not exceeded suggesting that *A. nummularia* uses an excretion mechanism to excrete excess of metals on its leaf surface as a possible detoxification mechanism.

Moreover the excretion of the metal increases with increment of salinity and this confirms the fact that the salt glands of *A. nummularia* are not selective and the composition of the secreted salts is related to the composition of the root environment. This characteristic of salt tolerance of *Atriplex nummularia* L. (old man saltbush) plants can be viewed as a novel phytoremediation process for the remediation of sites contaminated with heavy metals that we have termed “phyto-excretion.”

References

Begonia G.B., Davis C.D., Begonia M.F.T., Gray C.N. 1998. “Growth responses of Indian Mustard [Brassica juncea (L.) Czern.] and its phytoextraction of lead from a contaminated soil”, Bulletin of Environmental Contamination Toxicology, 61, 38-43.

Clemens S., Palmgren M.G., Krämer U. 2002. “A long way ahead: understanding and engineering plant metal accumulation”, Trends in Plant Science, 7, 309-315.

Fitzgerald E., Caffrey J., Nesaratnam S., McLoughlin P. 2003. Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland‟, Environmental Pollution,Vol. 123, pp. 67-74.

Garbisu C., Alkorta I. 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment”, Bioresource Technology, 77, 229-236.

Hagemeyer J., Waisel Y. 1988. Excretion of ions (Cd2+, Li+, Na+ and Cl-) by *Tamarix aphylla*‟, Physiologia plantarum, Vol.73, pp. 541–546.

Harborne J.B. 1984. Chlorophylls. In:Phytochemical Methods, 2nd edn. Chapman and Hall, London, pp. 214–219.

Manousaki E., Kadukova J., Papadantonakis N., Kalogerakis N. 2008. Phytoextraction and phyto-excretion of Cd by *Tamarix smyrnensis* growing on contaminated non saline and saline soils. Environ Res106:326–332

Orcutt D.M., Nilsen E.T. 2000. The Physiology of Plants under Stress‟, Soil and Biotic Factors, John Wiley & Sons, NewYork, pp 481–517

Otte M.L. 1991. Contamination of coastal wetlands with heavy metals: factors affecting uptake of heavy metals by salt marsh plants. In: Rozema, J., Verkleij, J.A.C. (Eds.), Ecological Responses to Environmental Stresses. Kluwer Academic, pp 126–133

Raskin I., Smith R.D., Salt D.E. 1997. “Phytoremediation of metals: using plants to remove pollutants from the environment”, Current Opinion in Biotechnology, 8, 221-226.

Salt D.E., Smith R.D., Raskin I. 1998. “Phytoremediation” Annual Review of Plant Physiology and Plant Molecular Biology, 49, 643-668.

Storey R., Thomson W.W. 1994. An X-ray Microanalysis Study of the Salt Glands and Intracellular Calcium Crystals of Tamarix‟, Annals of Botany, Vol. 73, pp. 307-313.

Weis J., Weis P. 2004. Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration‟, Environmental International,Vol. 30, pp. 685-700.