INSTM Bulletin
Marine and Freshwater Sciences

Vol. 49, 2024, 78–88

Research Article

Cellular Compartmentalization and Accumulation of Aluminium in the Halophyte *Arthrocnemum indicum*

Dhouha BELHADJ SGHAIER *[1]

University of Carthage. National Institute of Marine Sciences and Technology. Group of Aquatic Bacteriology and Biotechnology (GB2A), Tunisia.

***Correspondence**: dhouhasghaier@hotmail.fr

Received: 18/02/2024; **Accepted**: 25/05/2024; **Published**: 19/07/2024

Abstract: It has been demonstrated that halophytes had the potential to accumulate heavy metals and therefore had the remediation potential of soils affected by metals. In this context, *Arthrocnemum indicum* was supplied with increased doses of Aluminium (Al) (200, 500, 800 µM) alone or added to NaCl (200 mM). Results demonstrated that Al was found mainly in the cell wall in the shoots and the roots.

The addition of the salt displaced the localization of Al in the root sand, it was found bound to intercellular and proteic components. These results suggest that the subcellular distribution of Al plays an important role in avoidance of metal toxicity.

Keywords: Aluminium; NaCl; heavy metals; halophytes, detoxification.

1. Introduction

Wetland systems are known to have great economic value and ecological importance on a global scale, encompassing a wide range of ecosystem services (Woodward and Wui, 2001). Within wetlands, salt marshes are providers of varied and unique ecological and economic services on a global scale, ranging from wildlife conservation and coastal defense to water purification (Woodward and Wui, 2001). Usually located in transitional marine systems, the marsh ecosystem is generally occupied by flora adapted to stressful environments. Halophytes are the typical colonizers of salt marshes due to their high tolerance to several abiotic stresses, such as elevated temperature (Bita and Gernts, 2013), high salinity (Metoui-Ben Mahmoud et al., 2024) and heavy metal pollution (Sarwar et al., 2017; Sghaier et al., 2022), presenting morphological and physiological adaptations that allow them to inhabit these unfavorable environments. Several studies have shown that halophytic plants are more adopted to cope with abiotic stresses, including heavy metals (HM) (Sarwar et al., 2017; Sghaier et al., 2022). In this context, several researchers have compared the tolerance to heavy metals (HM) and their accumulation between certain halophytes and the known hyperaccumulative plants generally used in the remediation of soils contaminated by metals (Zaier et al., 2010;

Amari et al., 2014; Sghaier, 2023). All these experiments have demonstrated the superiority of halophytes to tolerate and accumulate these pollutants. *Arthrocnemum indicum* (Willd.) Moq., belongs to the Amaranthaceae family, it is a shrub species that grows optimally in saline and harsh conditions (Nisar et al. 2021). Plants can regulate their metabolism in response to HM and protect themselves to some extent against their toxicity. Understanding plant-metal interactions can help reduce the risks associated with the introduction of heavy metals into the food chain and solve safety problems in the environment (Fourati et al., 2016).

As consequence, studies have been conducted to improve knowledge on the tolerance mechanism of plants confronted with a high accumulation of trace metals without major metabolic alterations (Revathi and Subhashree, 2013). Plants have developed very complex systems to control the absorption, accumulation and detoxification of heavy metals (Leitenmaier and Küpper, 2013).

Commonly, these mechanisms varied from exclusion, inclusion and accumulation (Mnasri et al., 2015). Various mechanisms that govern metal tolerance in plant cells (Mnasri et al., 2015, Fourati et al., 2016) are the selective exclusion of the metal during absorption, the excretion of the metal, the retention of the metal in the roots, the specific tolerance of the enzymatic systems, the immobilization by means of the cell wall and extracellular carbohydrates, the complexation by binding of low molecular weight peptides phytochelatins (PCs) or by ligands such as organic acids and amino acids, and finally by compartmentalization (Carrier et al., 2003). Hence, heavy metals can be stored/accumulated either in the cell walls, cytoplasm, or in cellular vacuoles (Carrier et al., 2003; Fourati et al., 2016; Sghaier et al., 2020). Tolerance can also be achieved in some plants that hyperaccumulate metals by transporting metals from the roots to the shoots, conserving a low concentration of metals in the roots (Kramer et al., 1997). Understanding how plants are able to specifically accumulate HM is fundamental to select the species that could be used for Phytomanagement (Montargés-Pelletier et al., 2008). However, the mechanisms that manage this character remain ambiguous. The objective of this work was to study compartmentalization and subcellular localization of Aluminium (Al) in halophytes plants to design sustainable strategies for the management and safety of the environment or ecosystems.

2. Materials and Methods

2.1. Plant Sampling

Young plants were obtained by cutting propagation taken from mother plants and placed for rooting in plastic pots (for more details see Sghaier et al., 2015) (Figure 1). During rooting, the cuttings were irrigated with non-saline tap water then by nutrient solution (Hewitt, 1953) enriched with iron and micronutrients. After this acclimation period (15 days), plants were divided into eight groups of three plants that were supplied for 3 months with aluminum chloride ($AlCl_3$) and supplemented or not with NaCl (200 mM).

Control plants were regularly irrigated with the same nutritive solution and the remaining groups were subjected to Hewitt solution added with, (a) Al 200 µM; (b) Al 500 µM; c) Al 800 µM; (d) Al 200 µM + NaCl 200 mM; (e) Al 500 µM + NaCl 200 mM; (f) Al 800 µM + NaCl 200 mM. After 4 months of the start of the experiments, plants were harvested and divided into shoots and roots and rinsed three times in cold distilled water and blotted with filter paper (for more details see Sghaier et al., 2016).

Figure 1. *Arhrocnemum indicum* 3 months old grown in pots

2.2. Metals Extraction Procedure

A sequential extraction was carried out in order to evaluate the metal content in the cellular components of *A. indicum* (Farago and Pitt, 1977). Different parts of plant materials dried in the oven 70°C for 7 days) (leaves and roots; 1 g dry weight; n = 3) were treated individually. The first extraction agent used was 80% ethanol (a.p., Merck, 10 ml) for 24 h (for more details see sghaier et al. 2016). In the following step, the residue was placed in a solution of pronase E (from Streptomyces griseus, Merck) added to 0.03 g of chloramphenicol (P98%, TLC). Later, the same residue was added to 10 ml of a pectinase solution (1% P5146, Sigma; pH 4, temperature 25°C.) and stirred for 24 h.

The fourth extraction step consisted in adding 10 ml of NaOH solution (0.5 M) (a.p. P98%, Sigma) to the residue, and after that, a continuous final stirring with 100 ml of 5% hydrochloride (prepared from 37% hydrochloride per year, Merck) was carried out for 12 h at 25°C. The final stage consists of an acidic digestion of the vegetable residue (the digestion was treated in Teflon bombs) with HNO_3/$HClO_4$ (7.1, v,v) (HNO_3 65% by weight, Merck; $HClO_4$ 70% by weight ACS-ISO, Panreac) then dried in an oven at 110°C for 3 h. After cooling, all the extracts / fractions (ethanolic, aqueous, protein, pectic, polysaccharides, lignins and cellulosics) were filtered and diluted with 10 ml of a 0.01 M HNO_3 solution.

By this method, the different types of proteins cannot be determined, which implies that its exact location in the cell will not be defined. The metals bound to the cell wall have thus been designated by their constituents, which are pectic, polysaccharide, lignin and cellulose fractions. The metals linked to certain amino acids, chlorophyll, the compounds of low weight (all extracted with ethanol) and those extracted in the aqueous fraction are considered as soluble metal fractions (Farago and Pitt, 1977). The total elements present in each extracted fraction were determined by inductively coupled plasma atomic emission spectroscopy [ICP-AES; Horiba Jobin-Yvon, Horiba Jobin-Yvon, France, Ultima model].

2.3. Statistical Analysis

The Al contents in different subcellular fractions (cell wall, protein and soluble fractions) of the roots and leaves of *A. indicum* were expressed in micrograms of metal per gram of plant matter on a dry weight (µg/g). The experiments were repeated in triplicate for statistical analysis. A unidirectional analysis of the variance (ANOVA) or when the assumptions of the parametric tests were not satisfactory, the Kruskal-Wallis test was used to compare the average accumulation of Al in the different fractions. Depending on the type of test (parametric or non-parametric), a Bonferroni test or multiple pairwise comparisons were performed when significant differences were found (α = 0.05 significance level). The analysis was performed with SPSS v. 22.0 for Windows.

3. Results

Aluminum was predominantly bound to Cellulose and Polysaccharide fractions in the leaves (Figure 2), ranging from 25.47 % to 34.10 % and from 24.39 % to 32.27

%, respectively followed by the lignin fraction with low concentration than former ranging from 21.34 % to 32.27 %.

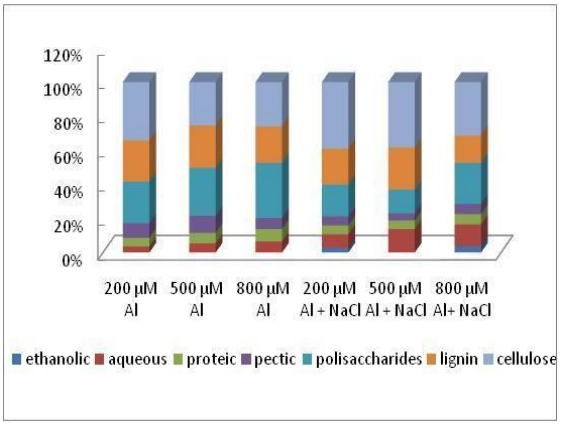

Figure 2. Al concentrations in the leaves (average %; n = 3) in different fractions, Regarding the fractions, from bottom to top (ethanolic, aqueous, proteic, pectic, polysaccharidic, ligninic, and cellulosic).

In the roots, Al was mainly bound to cellulose and lignin (Figure 3), with concentrations ranging from 29.89 % to 39.7 % and from 20.24 % to 26.71 %, respectively.

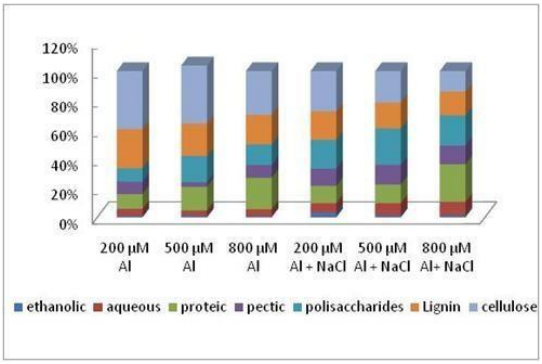

Figure 3. Al concentrations in the roots (average %; n = 3) in different fractions, Regarding the fractions, from bottom to top (ethanolic, aqueous, proteic, pectic, polysaccharidic, ligninic, and cellulosic).

Deeper, the three fractions in which metal compartmentalization can be grouped in this work (cell wall, proteic and intracellular/soluble fractions, Figures 4, 5), 84.79 ± 5.01% of Al in the leaves was accumulated in the cell wall and with very low values in the soluble fraction (with absence of ethanolic fraction) (3.66 ± 1.2%) and in the proteic fraction (5.73 ± 0.92%) (Figure 4).

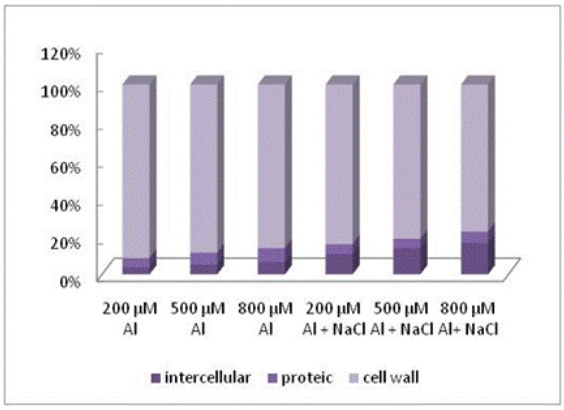

Figure 4. Al concentration in the leaves (average %; n = 3) located intracellularly (ethanolic + aqueous fraction), on the proteic fraction, and the cell wall (pectic + polysaccharidic + ligninic + cellulosic fractions).

The same predominance of Al in the cell wall components (78.3 ± 0.4%) was observed in the roots, with a residual presence of Al bound to proteins (17.5 ± 3.2%), and 5.2 ± 0.9% bound to soluble components (Figures 5).

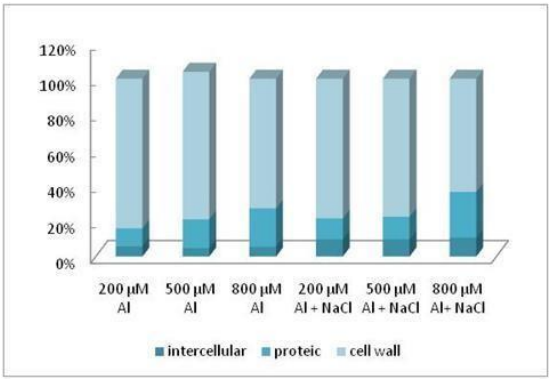

Figure 5. Al concentration in the roots (average %; n = 3) located intracellularly (ethanolic + aqueous fraction), on the proteic fraction, and the cell wall (pectic + polysaccharidic + ligninic + cellulosic fractions).

In the absence of salt, total accumulated Al in the leaves (Figure 2, 4) showed significant difference, the intracellular / soluble fraction presented significant differences ($F = 39.462$, $p < 0.001$), showing an augmentation at 500 and 800 µM Al. On the other hand, Al bound to proteins increased with increasing treatment doses ($F = 366.373$, $p < 0.001$). The pectic fraction show fluctuation with the lowest Al accumulation was revealed at the highest concentration of Al.

Similar to what was observed in the leaves, total Al concentration in the roots (Figures 3, 5) presented significant differences across treatments (F = 5.656, p = 0.046), with the highest accumulation being verified when 200 and 500 µM Al were supplied in the nutritive solution; a corresponding increase was observed in the Al bound to cell wall components (H = 6.489, p = 0.039).

With the addition of 200 µM of NaCl, the response of total Al accumulation in the leaves (Figures 2, 4) was different to that without salt, with significant differences between treatments precisely between the lowest and the highest doses (p > 0.05). On a closer inspection, some differences were found compared to the "salt-free" treatments, Al in ethanolic fraction was detected only in the combined treatment and the aqueous fraction presented elevated accumulation in the combined treatment. In addition, Al diminished in the pectic fraction and there was a displacement of the Al to lignin as a greater accumulation was revealed, with significant differences between the two acute concentrations, 200 µM and 800 µM (F = 14.286, p = 0.005).

In the roots (Figure 3, 5), Al bound to the components of the cell wall diminished, in particular those in celluloses (F = 9.871, p = 0.013) and lignin (F = 26.429, p = 0.001); and Al was displaced to proteic and intercellular fraction.
The pectic fraction showed a maximum accumulation from the 500 µM to the 800 µM treatment, (F = 28.1812, p = 0.001). Intracellular/soluble Al was more easily accumulated in the roots at higher concentrations in the presence of salt, (F = 13.915, p = 0.006). In the absence of salt, the increase in total Al corresponded to an increase in the cell wall components (F = 8.215, p = 0.019), specifically in the cellulose and lignin fractions (F = 6.906, p = 0.028). With salt added to the treatments, there was also a significant decrease in the accumulation of Al in cell wall components (F = 14.377, p = 0.005), despite the increase in Al bound to polysaccharides and in the pectic fraction (F = 89.366, p = 0.00003). Increasing Al concentration with NaCl has also favored significant changes in the soluble fractions (F = 13.915, p = 0.006), with the 800 µM treatment resulting in greater Al accumulation in the aqueous fraction (F = 8.370, p = 0.018).

4. Discussion

The exposure of plants to heavy metals induced an alteration of cellular mechanisms (Choppala et al., 2014) and gene expression (Chaffai and Koyama, 2011; Sarwar et al., 2017).
The mechanisms used to mitigate metal toxicityinvolved chelation by ligands and/or sequestration in the vacuole or cell wall. Subcellular compartmentalization of metals has been considered as a potential key element in the removal of trace elements of metals from active metabolic sites, limiting them to a limited area to reduce their toxicity (Fourati et al., 2016).
In this study, *A. indicum* accumulated Al in the leaves preferentially in the polysaccharide and the cellulose fractions of the cell wall (more than 84%); a small amount was accumulated in the protein fraction and also in the intracellular central part of the cells. In the roots, the metal ion was distributed to the cellulose and lignin fraction.

It has been shown that a large proportion of Al has been linked to the components of the cell wall of the leaves and roots which seem to function as the first barrier protecting the plant from toxicity (Sghaier et al., 2016). It is possible that Al forms a strong bond with cell wall components such as cellulose, polysaccharides, pectin and lignin (Sghaier et al., 2016). In fact, the cell wall provides a large number of metal binding sites (Sghaier et al., 2020). The cell wall is rich in pectic and histidyl groups and plays a key role in the immobilization of

metal ions. In the same context, *Tamarix gallica* an Al accumulator showed a similar subcellular localization of the metal (Sghaier et al., 2016).

In addition, it has been demonstrated that the cell wall represents a central storage site for other metals such as Cd and Zn (Carrier et al., 2003; Reboredo, 2012). Reboredo (2012) supported the idea that the preferred binding sites were the carbohydrates of the cell walls (cellulose, hemicelluloses and pectins) and that this is the first barrier to block cell penetration.
In the leaves of *T. gallica,* As and Al were found mainly in the cell wall component (Sghaier et al., 2016). In addition, in the leaves *of Lactuca sativa*, 64% of the total Cd was bound to the cell walls (Ramos et al., 2002) and a similar proportion of Cd associated with the cell wall fraction has been reported in *Lupinus albus* (Zornoza et al., 2002). In *Halimione portulacoides*, more than 50% of the metals have been accumulated by the polymers of the cell wall (Reboredo 2012).

Overall, the compartmentalization of the Al showed a greater coherence between the roots and the leaves, most of the metals being bound to the cell wall (>85%) with absence or presence of NaCl. It has been reported that the decrease in the concentration of metals in the cytoplasm could be related to the accumulation of metals in the cell walls acting as a barrier against the harmful effects of metals (Mnasri et al., 2015). Aluminum forms such strong bonds with the cell wall that its quantity will generally remain unchanged with the addition of other metal cations (Krzesłowska, 2011).
The compartmentalization of Al in the cell walls is a very important mechanism responsible for the detoxification of Al, as has been observed in the main Al hyperaccumulator, C*amellia sinensis* (Gao et al., 2014), in the Chara corallina region (Tolra et al., 2011) and in cultured tobacco cells, where the absorption and distribution of Al showed that most of the Al (>90%) accumulated in the cell wall (Chang et al., 1999).The main percentage of metals was bound to the cell wall more than intracellular component may be of crucial importance as a detoxification mechanism in the leaves and roots of *A. indicum*.

More deeply, the compartmental model seems to be closely linked to the species studied and the metals in question. In *Sesuvium portulacastrum*, known as Ni-tolerant plants, an increase in Ni doses was accompanied by an increase in the percentage of Ni fixed to the cell wall while in *Cakile maritima*, a Ni-sensitive plant, the soluble fraction contained approximately more than 60% of the total Ni of the shoots for all the concentrations applied (Fourati et al., 2016).

In addition to the cell wall, the vacuole seemed to be another preferential site for Al. Similarly, Ni, Cd and As were found to bind to the soluble fraction (Psaras et al., 2000). Several studies have revealed that the metal can be located in the intracellular soluble/fraction, the central part of the cells which most of this area is occupied by the vacuole (Lombi et al., 2002). Heavy metals such as Ni, Cd and Zn are generally stored in the vacuoles of epidermal cells (Psaras et al., 2000; Küpper et al., 2002). The partitioning of Al resulted differently from the leaves and roots, but the importance of this location showed a slight decrease in the presence of salt. The compartmentalization of the Al in the roots was more uniformly distributed the protein and soluble fractions together containing 15 to 35% of the total Al (without ad with salt, respectively) (Figure 3 and 5). The fraction containing organelles contained less metals than that attached to the cell wall (Fourati et al., 2016; Sghaier et al., 2020). The inactivation of toxic metal ions by the synthesis of PCs followed by the formation of metal-phytochelatin complexes, is a general homeostasis mechanism in plants (Krzesłowska, 2011).

Depending on the acidic conditions that confer the stability of these complexes, the Al-phytochelatin complexes could be stored in vacuoles (Meharg and Hartley-Whitaker, 2002). PCs are synthesized from glutathione by PC-synthases and play a role in the distribution and accumulation of Al and certain other highly toxic metals such as Ag, Hg and Cd (Cobbett, 2000) thus eliminating these toxic elements from the cytosol (Verbruggen et al., 2009). GSH can also detoxify toxic metals by forming glutathione complexes (GSH)-HM and sequestering in vacuoles, which could be excluded from the apoplast (Krämer, 2010). Metallothioneins are other low molecular weight chelating protein molecules enriched with cysteine, responsible for the formation of complexes with toxic metals (MT-HM complexes) (Anjum et al., 2015).

The protein fraction has also been shown to be of great importance in the compartmentalization of Al in the roots. Transport and storage in the vacuole require increasing levels of sulfur-rich peptides, including PCs and organic acids (Sanita di Toppi and Gabbrielli, 1999).

In the present work, the Al accumulate in the pectic fraction of the leaves in small quantity (less than10%), regardless of the treatment applied, and its accumulation in the roots was of minor expression (<8% of the total Al, on average). Instead, cellulose and lignin were the preferred binding sites in the leaves, cellulose and polysaccharide fractions were the preferred binding locations in the roots. The binding of Al to the pectin of the cell wall may not always be an essential tolerance mechanism (Krzesłowska, 2011). At high external concentrations, the protein fraction acquired importance in the leaves, especially in the absence of salt. With the presence of NaCl in the nutrient solution, the Al bound to the protein fraction was detected only at the highest tested treatment. A similar behavior was observed in *Sesuvium portulacastrum*, at low dose, the insoluble fraction of the shoots presented only 29% while the soluble fraction sequestered 55% of the total cell Ni.

Similarly, at a high concentration of Na^+ in the medium, the cation retention capacity of the cell wall could be saturated by Na^+ ion then Cd^{2+} would be less fixed by this cell compartment (Ayachi, et al., 2023).
The increase in the external concentration of Ni was accompanied by significant changes in the model of cellular accumulation of Ni in the shoots, resulting in an increase in the percentage of Ni cell wall fraction reaching 37% and a reduction in that of the soluble fraction from 55% to 47%. In *A. indicum*, at higher concentrations, the roots showed a shift from Al to lignin. The increase in the lignification of the cell wall and the subsequent metal deposits is another mechanism that has been described to protect plant cells from the toxic effects of high concentrations (Probst et al., 2009).

5. Conclusion

Compartmentalization is a key aspect of the elimination of trace metal elements outside key metabolic sites, contributing to the survival of plants in saline depressions contaminated with heavy metals. This study gives an overview of the different distributions and localizations of Al within *A. indicum*. The cell wall is considered as the first barrier that opposes the entry of toxic metals into the cell and can thus protect the cytoplasm by preventing this passage of the binding of metal ions. A better understanding of the sequestration of metals in plants could possibly contribute to the development of biorecovery techniques for the remediation of soils contaminated with heavy metals.

Authors contributions

Belhadj Sghaier D. carried out all the experiments, assured data analysis and prepared the manuscript.

Conflicts of Interest: The authors declare no conflict of interests.

References

1. Amari, T., Ghnaya, T., Debez, A., Taamali, M., Ben Youssef, N., Lucchini, G., Sacchi, G.A. & Abdelly, C. (2014). Comparative Ni tolerance and accumulation potentials between *Mesembryanthemum crystallinum* (halophyte) and *Brassica juncea*: metal accumulation, nutrient status and photosynthetic activity. *Journal of Plant Physiology,* 171(17), 1634-1644. https://doi.org/10.1016/j.jplph.2014.06.020
2. Anjum, N.A., Hasanuzzaman, M., Hossain, M.A., Thangavel, P., Roychoudhury, A., Gill, S.S., Rodrigo, M.A.M., Adam, V., Fujita, M., Kizek, R., Duarte, A.C., Pereira, E. & Ahmad, I. (2015). Jacks of metal/metalloid chelation trade in plants-an overview. *Frontiers in Plant Science,* 6. https://doi.org/10.3389/fpls.2015.00192
3. Ayachi, I., Ghabriche, R., Ben Zineb, A., Hanana, M., Abdelly, C. & Ghnaya, T. (2023). NaCl effect on Cd accumulation and cell compartmentalization in barley. *Environmental Science and Pollution Research,* 30, 49215-49225. https://doi.org/10.1007/s11356-023-25791-2
4. Bita, G. & Gernts, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science, 4* 273, 1-18. https://doi.org/10.3389/fpls.2013.00273
5. Carrier, P., Baryla, A. & Havaux, M. (2003). Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil. *Planta*, 216, 939-950. https://doi.org/10.1007/s00425-002-0947-6
6. Chaffai, R. & Koyama, H. (2011). Heavy metal tolerance in *Arabidopsis thaliana*. *Advances in Botanical Research,* 60, 1-49. https://doi.org/10.1016/B978-0-12-385851-1.00001-9
7. Chang, Y.C., Yamamoto, Y. & Matsumoto, H. (1999). Accumulation of aluminum in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminum and iron. *Plant, Cell & Environment,* 22(8), 1009-1017. https://doi.org/10.1046/j.1365-3040.1999.00467.x
8. Cobbett, C.S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology,* 123(53), 825-832. https://doi.org/10.1104/pp.123.3.825
9. Choppala, G., Saifullah, Bolan, N., Bibi, S., Iqbal, M., Rengel, Z., Kunhikrishnan, A. & Ok, Y.S. (2014). Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. *Critical reviews in plant sciences*, 33(5), 374-391. https://doi.org/10.1080/07352689.2014.903747
10. Farago, M. E. & Pitt, M. J. (1977). Plants which accumulate metals. Part III. A further investigation of two australian species which take up zinc. *Inorganica Chimica Acta*, 24, 211-214. https://doi.org/10.1016/S0020-1693(00)93876-3
11. Fourati, E., Wali, M., Vogel-Mikuš, K., Abdelly, C. & Ghnaya, T. (2016). Nickel tolerance, accumulation and subcellular distribution in the halophytes *Sesuvium portulacastrum and Cakile maritima*, *Plant Physiology & Biochemistry, 108, 295-303.* https://doi.org/10.1016/j.plaphy.2016.07.024
12. Gao, H. J., Zhao, Q., Zhang, X. C., Wan, X. C. & Mao, J. D. (2014). Localization

of fluoride and aluminum in subcellular fractions of tea leaves and roots. *Journal of Agricultural and Food Chemistry,* 62(10), 2313-2319. https://doi.org/10.1021/jf4038437

13. Hewitt, E. J. (1953). Sand and water culture methods used in the study of plant nutrition. *Soil Science Society of America Journal*, 17, 301-301. https://doi.org/10.2136/sssaj1953.03615995001700030033x
14. Kramer, U., Smith, R.D., Wenzel, W.W., Raskin, I. & Salt, D.E. (1997). The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense Halacsy*. *Plant Physiology,* 115(4), 1641-1650. https://doi.org/10.1104/pp.115.4.1641
15. Krämer, U. (2010). Metal Hyperaccumulation in Plants. *Annual Review of Plant Biology*, 61, 517-534. https://doi.org/10.1146/annurev-arplant-042809-112156
16. Krzesłowska, M. (2011). The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. *Acta Physiologiae Plantarum,* 33, 35-51. https://doi.org/10.1007/s11738-010-0581-z
17. Küpper, H., Lombi, E., Zhao, F., Wieshammer, G. & McGrath, S.P. (2001). Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum, Alyssum bertolonii* and *Thlaspi goesingense*. *Journal of Experimental Botany*, 52(365), 2291-2300. https://doi.org/10.1093/jexbot/52.365.2291
18. Leitenmaier, B. & Küpper, H. (2013). Compartmentation and complexation of metals in hyperaccumulator plants. *Frontiers in Plant Science*, 4, 374. https://doi.org/10.3389/fpls.2013.00374
19. Lombi, E., Zhao, F.J., Fuhrmann, M., Ma, L.Q. & McGrath, S.P. (2002). Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. *New Phytologist*, 156(2), 195-203. https://doi.org/10.1046/j.1469-8137.2002.00512.x
20. Meharg, A.A. & Hartley-Whitaker, J. (2002). Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist,* 154(1), 29-43. https://doi.org/10.1046/j.1469-8137.2002.00363.x
21. Metoui-Ben Mahmoud, O., Hidri, R., Abdelly, C. & Debez, A. (2024). *Bacillus pumilus* isolated from sabkha rhizosphere ameliorates the behavior of the facultative halophyte *Hordeum marinum* when salt-challenged by improving nutrient uptake and soil health-related traits. *Plant Stress*, 11,100383. https://doi.org/10.1016/j.stress.2024.100383
22. Mnasri, M., Ghabriche, R., Fourati, E., Zaier, H., Sabally, K., Barrington, S., Lutts, S., Abdelly, C. & Ghnaya, T. (2015). Cd and Ni transport and accumulation in the halophyte *Sesuvium portulacastrum*: implication of organic acids in these processes. *Frontiers in Plant Science,* 6, 156. https://doi.org/10.3389/fpls.2015.00156
23. Montargès-Pelletier, E, Chardot V, Echevarria, G, Michot, L.J, Bauer, A. & Morel, J.L. (2008). Identification of nickel chelators in three hyperaccumulating plants: an X-ray spectroscopic study. Phytochemistry, 69(8), 1695-1709. https://doi.org/10.1016/j.phytochem.2008.02.009
24. Sarwar, N, Imran, M, Shaheen, M.R, Ishaque, W, Kamran, M.A, Matloob, A, Rehim, A. & Hussain, S. (2017). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, 171, 710-721. https://doi.org/10.1016/j.chemosphere.2016.12.116
25. Probst, A., Liu, H., Fanjul, M., Liao, B. & Hollande, E. (2009). Response of *Vicia faba* L. to metal toxicity on mine tailing substrate: geochemical and morphological changes in leaf and root.

Environmental and experimental botany, 66(2), 297-308. https://doi.org/10.1016/j.envexpbot.2009.02.003

26. Psaras, G.K. & Manetas, Y. (2001). Nickel localization in seeds of the metal hyperaccumulator *Thlaspi pindicum Hausskn*. *Annals of Botany*, 88(3), 513-516. https://doi.org/10.1006/anbo.2001.1470
27. Ramos, I., Esteban, E., Lucena, J.J. & Gàrate, A. (2002). Cadmium uptake and subcellular distribution in plants of *Lactuca*sp. Cd-Mn interaction. *Plant Science,* 162(5), 761-767. https://doi.org/10.1016/S0168-9452(02)00017-1
28. Reboredo, F. (2012). Zinc compartmentation in *Halimione portulacoides* (L.) Aellen and some effects on leaf ultrastructure. *Environmental Science and Pollution Research*, 19, 2644-2657. https://doi.org/10.1007/s11356-012-0757-8
29. Revathi, S. & Venugopal, S. (2013). Physiological and biochemical mechanisms of heavy metal tolerance. *International Journal of Environmental Sciences,* 3(5), 1339-1354.
30. Sanità di Toppi, L. & Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environmental and Experimental Botany,* 41(2), 105-130. https://doi.org/10.1016/S0098-8472(98)00058-6
31. Sghaier, D.B., Pedro, S., Diniz, M.S., Duarte, B., Caçador, I. & Sleimi, N. (2016). Tissue localization and distribution of As and Al in the Halophyte *Tamarix gallica* under controlled conditions. *Frontiers in Marine Science*, 3, 274. https://doi.org/10.3389/fmars.2016.00274
32. Sghaier, D.B., Pedro, S., Duarte, B., Caçador, I. & Sleimi, N. (2022). Photosynthetic responses of two salt-tolerant plants, *Tamarix gallica and Arthrocnemum indicum* against arsenic stress: a case study. In: Srivastava PK, Singh R, Parihar P, Prasad SM (eds), *Arsenic in plants: uptake consequences and remediation techniques*. Wiley. https://doi.org/10.1002/9781119791461.ch7
33. Sghaier, D.B., Pedro, S., Duarte, B., Diniz, M.S., Caçador, I. & Sleimi, N. (2020). Arsenic Accumulation, Compartmentation, and Complexation in *Arthrocnemum indicum*. In: Hasanuzzaman M. (ed), Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives I. Springer. https://doi.org/10.1007/978-981-15-2156-0_25
34. Sghaier, D.B., Duarte, B., Bankaji, I., Caçador, I. & Sleimi, N. (2015). Growth, chlorophyll fluorescence and mineral nutrition in the halophyte *Tamarix gallica* cultivated in combined stress conditions: Arsenic and NaCl. *Journal of Photochemistry and Photobiology (B) Biology*, 149, 204-221.https://doi.org/10.1016/j.jphotobiol.2015.06.003
35. Sghaier, D.B. (2023). Comparative physiology of *Arthrocnemum indicum* and *Tamarix gallica* under aluminum alone or combined with NaCl. *Euro-Mediterranean Journal for Environmental Integration*, 8, 835-846. https://doi.org/10.1007/s41207-023-00408-9
36. Tolra, R., Vogel-Mikus, K., Hajiboland, R., Kump, P., Pongrac, P. & Kaulich, B. (2011). Localization of aluminium in tea (*Camellia sinensis* L.) leaves using low energy X-ray fluorescence spectro-microscopy. *Journal of Plant Research*, 124, 165-172. https://doi.org/10.1007/s10265-010-0344-3
37. Verbruggen, N., Hermans, C. & Schat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist,* 181(4), 759-776.

https://doi.org/10.1111/j.1469-8137.2008.02748.x

38. Woodward, R.T., & Wui, Y. (2001). The economic value of wetland services: a meta-analysis. *Ecological Economics*, 37(2), 257-270. https://doi.org/10.1016/S0921-8009(00)00276-7
39. Zaier, H., Mudarra, A., Kutscher, D., Fernandez de la Campa, M.R., Abdelly, C. & Sanz- Medel, A. (2010). Induced lead binding phytochelatins in *Brassica juncea* and *Sesuvium portulacastrum* investigated by orthogonal chromatography inductively coupled plasma-mass spectrometry and matrix assisted laser desorption ionisation-time of flight mass spectrometry. *Analytica Chimica Acta,* 671 (1-2), 48-54. https://doi.org/10.1016/j.aca.2010.04.054
40. Zornoza, P., Vazquez, S., Esteban, E., Fernandez-Pascual, M. & Carpena, R. (2002). Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. *Plant Physiology and Biochemistry*, 40(12), 1003-1009. https://doi.org/10.1016/S0981-9428(02)01464-X