EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 2 Number 9 (September-2014) pp. 292-304

www.ijcrar.com

Phytoextraction of Heavy Metals and Ions from Tannery Effluent Using *Suaeda monoica* Forsk. with Reference to Morphology and Anatomical Characters

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KEYWORDS

Phytoextraction; heavy metals; *Suaeda monoica*; tannery effluent; mesophyll volume

A B S T R A C T

Morphology and anatomical adaptations to heavy metals and ions in the salt tolerant population were very specific. They include restricted toxic ion uptake, production of organic osmolytes, succulence in stem, and development of vesicular hairs on the leaves. The present work deals with the morphological and anatomical adaptations in phytoextraction of heavy metals and ions from tannery effluent using *Suaeda monoica* Forsk. Plants are treated with tannery effluent and with optimal concentrations of salt. Plant samples are harvested for experimental purpose at an intervals of 25, 50, 75, 100 and 125 days. Our results indicated that no morphological injury symptoms in *Suaeda monoica* were observed throughout the phytoextraction period. Increased growth, biomass accumulation and significant changes in leaf diameter, mesophyll volume and mesophyll thickness were observed in *Suaeda monoica* treated with tannery effluent when compared to salt and control. The results were justifying that this species has a potential candidate for phytoextraction of heavy metal and ions from the tannery effluent contaminated soil.

Introduction

Heavy metal pollution due to industrial effluents is gaining worldwide attention (Mishra *et al*., 2008). Contamination of agricultural soil by heavy metals has become a serious environmental concern due to their mostly negative impact on crop growth and ecosystems (Ruan and Teixeria da Silva, 2011; Lokhande and Suprasanna, 2012; Zhang *et al*., 2013).

The tannery industry is of particular concern in this regard due to the indiscriminate discharge of metal-rich effluents, toxic sludge and noxious gases into adjacent environmental compartments causing considerable environmental damage (Tariq *et al*., 2006). More than 250 chemicals (inorganic and organic) are employed in the tannery industry in excess of 300 kg of chemicals per ton of hide treated (Buljan *et* *al*., 2000). The examined tannery effluents contained excess quantities of environmentally unwanted heavy metals (Ali *et al*., 2013).

Salinity is a major adverse environmental constraint to plant productivity, limiting the utilization of about 800 million ha of agricultural land globally (Dendooven *et al*., 2010; Li *et al*., 2011). As estimated, 80,000,000 ha of cultivated land are affected by soil salinity, which corresponds to 5% of all cultivated land (Askaril *et al*., 2006). High levels of soil salinity can cause water deficit, ion toxicity, and nutrient deficiency leading to molecular damage and even plant death (Maggio *et al.,* 2010). Many halophytes often have high metal tolerance that is strongly linked to traits for salt tolerance. Some halophytes are also considered as hyper-accumulator of certain metals (Pastor and Hernandaz, 2012). Several halophytic plant species have been tried in the past for their possible use in reclamation of salt-affected soils (Ravindran *et al*., 2007; Rabhi *et al*., 2010; Koyro *et al*., 2011; de Souza *et al*., 2012 and Ayyappan *et al*., 2013) and heavy metals (Bonanno and Giudice, 2010; Bonanno, 2011; Duarte *et al*., 2013).

The objective of the present study is to introduce *Suaeda monoica* Forsk. as a high biomass yield halophyte for phytoextraction of tannery effluent contaminated soil to assess the feasibility of heavy metal and salt bioaccumulation from tannery effluent as an alternative to other leaching techniques.

Experimental

Selection of species

Fast growing salt marsh halophyte *Suaeda monoica* Forsk. was selected for bioaccumulation of heavy metals and salts. *Suaeda monoica* is a pure halophyte, similar to *Suaeda maritima* in appearance growing in hyper saline soils. Compared to *Suaeda maritima* its distribution is limited.

Experimental site

The experimental site was located at Anichampalayam village, Villupuram District (11° 55' N and 79° 32' E) of Tamil Nadu, India. The field experiments were conducted from January 2012 – June 2012. The experimental area received an average annual rainfall of 135.6 cm spread over the year. Temperature ranged from a summer maximum of 33.26 ºC to a winter 29.68 °C.

Collection of the tannery effluent

The effluent samples were collected from the tannery industry situated at Chrompett near Chennai in clean plastic cans and stored at 4^{0}C for the analysis. The effluent was directly collected from the outlet of the industry.

Plant collection

Suaeda monoica plants were collected from the Pichavaram mangrove forest located between Vellar and Coleroon estuaries (latitude 11^{0}22’ N- 11^{0}30’ and longitude 79^{0}45’ E- 79^{0}52’) in Cuddalore District of Tamil Nadu, South India.

Design of the experiment

Red soil and sand (3:1 ratio) free from pebbles and stones were filled in polythene bags. The seedlings with similar size were transplanted from the nursery bed and planted at the polythene bags.

The experiment comprised of the following three sets of treatments with five replicates and average values are reported.

1. Control- Without any treatment.
2. Salt treatment- Halophytes were treated with optimum level of NaCl for 4 times with a gap of 12 days.
3. Effluent treatment- Halophytes were treated with 250 ml of 75% tannery effluent 4 for times with a gap of 12 days.

The experiment was took place in an open-air area with natural light, temperature, and humidity, to keep the plants under conditions as similar as possible to those the field. With the use of a plastic cover, care was taken no to let the plants to rained on, in order to avoid having any secreted heavy metals and ions washed away. Plants were watered every 2-3 days, depending on the evaporative demand, with approximately 200 ml of tap water. Care was taken not to prevent leaching of heavy metals and ions/salts from the polythene bags. Physical and chemical characteristic of tannery effluent, soil and halophytes are determined before planting and harvesting. Plant samples are harvested for experimental purpose at an intervals of 25, 50, 75, 100 and 125 days.

Physico-Chemical characteristic of experimental site

Analysis of physico-chemical properties of characteristics of the tannery effluent is shown in table 1. Physico-chemical characteristics of soil treated with salt and tannery effluent are given in the table 2.

Analysis of growth characteristics

The total length of the seedlings and fresh weight were measured immediately after removing the seedlings from the experimental field. Leaf area was calculated by using the Li-Cor 3100 leaf area meter (Li-Cor Inc., USA). The dry weight of the seedlings was determined after they had been dried for 80 °C for 24 h.

Leaf anatomy and thickness

Leaf bits of 0.5 × 1.5 cm were cut from the middle intervenal region of upper Canopy from each experimental field. Five fully expanded leaves were sampled for each experimental field. The cross sections of leaves were prepared by using a rotary microtome and observed in a calibrated microscope.

Mesophyll volume

Mesophyll volume was measured by precalibrated ocular micrometer and thickness in mm was multiplied by 100 to calculate the mesophyll volume in cm^3/dm^2 of leaf area following the method of Patterson *et al* (1978).

Statistical analysis

The experimental data were processed statistically by adapting the technique of analysis the variance of Standard Deviation (Snedecor and Cochran, 1967).

Result and Discussion

Observations on morphology, growth, and anatomical parameters were made at 25 days interval after 25th, 50th, 75th, 100th and 125th days of transplanting *Suaeda monoica* plants in the tannery effluent and salt treated soil. The data gathered from periodical observations were processed and statistically analysed and the results are presented in the form of tables and plates.

Morphology and Growth

Morphology and growth characteristics of *Suaeda monoica* plants grown in tannery effluent, salt treated soil and control seedlings are given in plates 1 and 2. During 125 days of cultivation, the maximum increase in growth characteristics was observed in *Suaeda monoica* cultivated in tannery effluent and salt treated soil when compared to control. However, highest increase was noticed at 100 days cultivation period and there after only marginal increase was observed.

Shoot length, total number of leaves and leaf area of *Suaeda monoica* plants cultivated in tannery effluent and salt treated soil and control seedlings are presented in the table 3. Tannery effluent treated soil exhibited maximum shoot length (123.78%) when compared to salt treated soil (109.80%) and control (40%). Similarly highest total number of leaves (290%) and leaf area (375%) was noticed in tannery effluent treated plants when compared to salt treated plants and control after 125 days of cultivation.

Table 4 shows the fresh weight and dry weight *of Suaeda monoica* plants treated under tannery effluent and salt. Maximum increase in fresh weight (290%) was observed in tannery effluent treated soil when compared to salt treated soil (220%) and control (60%). Like fresh weight dry weight was also increased significantly in tannery effluent (279.9%) treated soil when compared to salt treated soil and control after 125 days of cultivation.

Leaf Anatomy and Thickness

Since leaf is a heterogeneous assemblage of tissues, a part of which are photosynthetically active, the anatomy and the structure of the leaf may affect its photosynthetic performance. The leaf thickness was reduced maximum in control plants, when compared to tannery effluent and salt treated *Suaeda monoica* plants (Plate 3 and table 5). Spongy and Palisade parenchyma cell size were enlarged maximum in tannery effluent cultivated plants than salt treated soil cultivated plants and control. The length and breadth of parenchyma cells were increased in tannery effluent cultivated plants, when compared to control plants. The maximum Mesophyll thickness (152.60µ) and Mesophyll volume ($15.26 cm^3/dm^2$) was observed in tannery effluent treated soil when compared to salt treated soil and control after 125 days of cultivation. Increase in leaf thickness might be due to deposition of metals and salts and also depends upon the nature of their succulence.

Morphology and Growth

Heavy metals (trace elements) are beneficial for plant growth and physiology, after excessive uptake by plants, these elements may participate in some physiological and biochemical reactions that can destroy normal growth of the plant by disturbing absorption, translocation, or synthesis processes. They may combine with some huge molecule, such as nucleic acid, protein, and enzyme, or may substitute of metabolic activities. Therefore, the growth and procreation of the plant is prohibited and leads to death (Wei and Zhou, 2008).

After 125 days of cultivation of halophytes under tannery effluent and salt treated soil, exhibited the maximum growth and it is also evident from the present study that growth and development was found to be minimum in the absence of tannery effluent (control). These results are in accordance with Ghnaya *et al* (2005, 2007) who suggested high potentials of *Sesuvium portulacastrum* to accumulate cadmium in shoots without growth retardation. The ability to tolerate both Cd^{2+} and Pb^{2+} accumulation in the shoots without deleterious effects on growth suggests an efficient protection of the cellular biochemical machinery against free metal ions (Cd^{2+} and Pb^{2+}) and could be of crucial interest for phytomanagement of polluted areas which are frequently contaminated by several heavy metals. Eid and Eisa (2010) tested the effect of artificial pollution with 25 mg kg^{-1} soil of multiple Zn, Cu and Ni on *Sporobolus virginicus* and *Spartina patens* grown for 8 weeks. They reported that no growth inhibition on shoot biomass was occurred.

Nirmal Kumar *et al* (2011) shown that *Avicennia marina* possesses the capacity to take up selected heavy metals via its roots and storing and its leaves without any sign of injury. As it grows more aged, its capability of accumulating heavy metals is also increasing much fold. This suggests the potential of *Avicennia marina* as a phytoremediation species for many mangrove ecosystems. Cambrolle *et al* (2012) indicated that growth parameters were virtually unaffected by leaf tissue concentrations as high as 1500 mg Zn kg^{-1} dry mass, demonstrating the strong capability of *Halimione portulacoides* to protect itself against toxic Zn concentrations and this salt-marsh shrub may represent a valuable tool in the restoration of Zn-polluted areas.

Duarte *et al* (2012) identified the most abundant salt marsh halophytic species of Tagus estuary *Halimione portulacoides,* considered as suitable for Cr(VI) phytoremediation processes by phytoextraction. Chai *et al* (2013) observed that, growth of *Spartina alterniflora,* a salt marsh halophyte was not inhibited under Cu stresses (50, 200, 800 mg kg^{-1}) with no chlorotic and brown points on leaves and could be considered to be a promising candidate for phytoremediation of copper contaminated areas.

It has been postulated that halophytes species recruit non-selective salt-sensitive mechanisms to sequester toxic ions in the vacuole and/or salt glands/trichomes (Lutts *et al*., 2004). Metal deposit in the cell walls as a result of binding to pectic compounds could be also considered as an important mechanism for metal detoxification in halophyte species, as demonstrated in *Halimione portulacoides* (Sousa *et al*., 2008). Both sequestrations in cell walls and in foliar trichomes enable halophyte to avoid toxic accumulation of heavy metals in the cytoplasm of mesophyll cells (Reboreda and Cacador, 2007; Sousa *et al*., 2008).

In the present study *Suaeda monoica* cultivated in tannery effluent and salt treated soil stimulated the leaf production and increased the number of leaves throughout the study period when compared to control plants. Along with increase in the leaf number, there was increase in the leaf area. The increase in leaf area might be due to increase in the volume of mesophyll cells with the increase in the water content of the leaves and greater accumulation of heavy metals in the mesophyll tissue with the consequent increase in the leaf thickness.

In the present study it is observed that presence of salts increased the growth and biomass in tannery effluent treated plants. This is in accordance with other studies that NaCl stimulated the shoot and root growth in *Atriplex patula* (Ungar, 1996), *Sonneratia alba* (Akatsu *et al.,* 1996), *Atriplex nummularia* (Jordan *et al.,* 2002) and *Suaeda salsa* (Zhang *et al.,* 2005). Jenci and Natarajan (2009) also observed that shoot length, fresh weight, dry weight, and leaf area increased in *Excoecaria agallocha* up to 300 mM NaCl concentrations compared to non-saline controls.

Plate.1 Growth characteristics of *Suaeda monoica* cultivated in control, salt and tannery effluent treated soil after 125 days of cultivation

Plate.2 Leaf characteristics of *Suaeda monoica* cultivated in control, salt and tannery effluent treated soil after 125 days of cultivation

Plate.3 Anatomy of leaf characteristics of *Suaeda monoica* cultivated in control, salt and tannery effluent treated soil after 125 days of cultivation

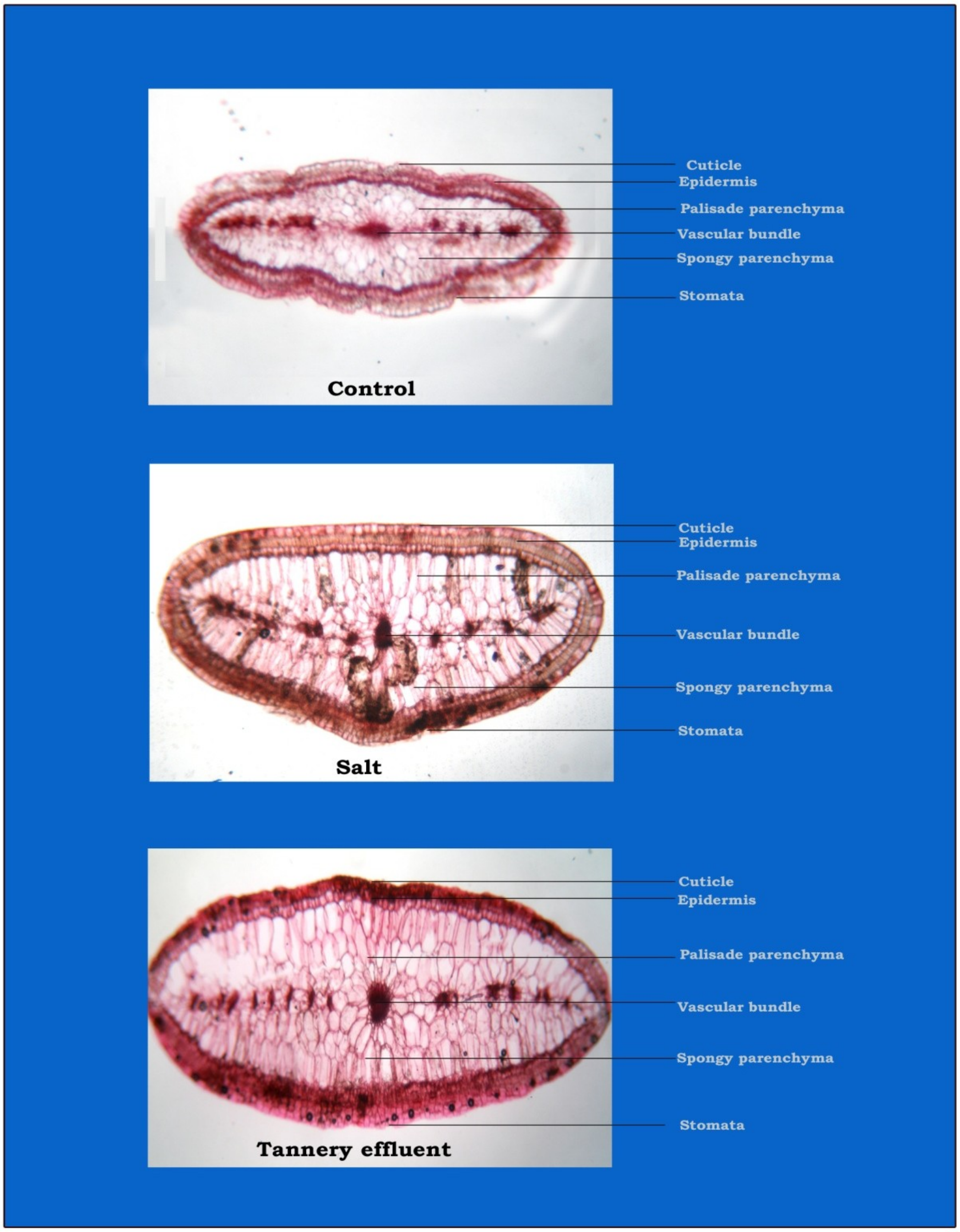

Table.1 Physico-chemical characteristics of Tannery effluent

S.No	Parameters	Raw Effluent	BIS LIMITS IS 2490-2009
1.	Colour	Brown	-
2.	Odour	Offensive	-
3.	Turbidity	Turbid	-
4.	pH	10.70	5.5-9.0
5.	Electrical Conductivity (dSm^{-1})	4.99	
6.	Total hardness	568.00	100
7.	Total dissolved solids (mg/l)	3432.00	2100
8.	Total suspended solids (mg/l)	1589.00	100
9.	Alkalinity	1350.00	NM
10.	Biological Oxygen Demand	699.00	30
11.	Sodium (meq/l)	89.20	NM
12.	Chloride (meq/l)	54.63	NM
13.	Potassium (meq/l)	8.72	NM
14.	Calcium (meq/l)	9.90	NM
15.	Magnesium (meq/l)	10.88	NM
16.	Chromium (mg/l)	142.40	2.0
17.	Cadmium (mg/l)	28.20	2.0
18.	Copper (mg/l)	78.96	NM
19.	Zinc (mg/l)	212.90	1.0

NM- Not mentioned

Table.2 Physio-chemical characteristics of salt and Tannery effluent treated soil

S.No	Parameters	Salt treated soil	Tannery effluent treated soil
1.	pH	7.70	8.30
2.	Electrical Conductivity(dSm^{-1})	4.48	5.25
3.	Sodium (meq/l)	45.88	48.00
4.	Potassium (meq/l)	6.82	7.16
5.	Chloride(meq/l)	39.00	43.00
6.	Calcium (meq/l)	7.94	8.17
7.	Magnesium (meq/l)	8.13	8.88
8.	Chromium (mg kg^{-1})	4.80	82.00
9.	Cadmium (mg kg^{-1})	5.00	23.88
10.	Copper (mg kg^{-1})	18.19	54.90
11.	Zinc (mg kg^{-1})	26.00	55.00

Table.3 Shoot length, Total no. of leaves and Leaf area of *Suaeda monoica* cultivated in tannery effluent and salt treated soil

S. No	Days	Shoot length (cm/plant)			Total no. of leaves (Total.no/plant)			Leaf area (cm^2/plant		
		Control	Salt	Effluent	Control	Salt	Effluent	Control	Salt	Effluent
1.	25	26.0±1.30	33.5±1.67	37.0±1.85	130±6.50	260±13.00	290±14.50	20.1±1.00	129.9±6.49	195.8±9.79
2.	50	27.3±1.36	40.5±2.02	45.8±2.29	153±7.65	325±16.25	406±20.30	26.1±1.30	209.9±10.40	335.1±16.70
3.	75	29.3±1.46	49.2±2.46	56.2±2.81	169±8.45	455±22.75	623±31.15	32.1±1.60	359.1±17.90	545.5±27.20
4.	100	33.8±1.69	63.6±3.18	74.3±3.71	184±9.20	676±33.80	1044±52.20	37.6±1.88	545.1±27.20	845.0±42.20
5.	125	36.4±1.82	70.3±3.58	82.8±4.14	197±9.85	754±37.70	1131±56.55	41.2±2.06	571.5±28.50	930.5±46.50

Table.4 Fresh weight and Dry weight of *Suaeda monoica* cultivated in tannery effluent and salt treated soil

S. No	Days	Fresh weight (g / plant)			Dry weight (g / plant)		
		Control	Salt	Effluent	Control	Salt	Effluent
1.	25	11.6±0.58	22.0±1.10	27.0±1.35	4.6±0.23	9.2±0.46	10.9±0.54
2.	50	13.8±0.69	30.8±1.54	42.6±2.13	5.5±0.27	12.9±0.64	16.2±0.81
3.	75	15.6±0.75	41.8±2.09	63.4±3.17	6.7±0.33	17.5±0.87	25.6±1.28
4.	100	17.4±0.87	62.7±3.13	93.9±4.69	8.0±0.40	26.3±1.31	38.0±1.90
5.	125	18.5±0.92	70.4±3.52	105.3±5.26	8.8±0.44	29.5±1.47	41.5±2.07

Table.5 Mesophyll characteristics of *Suaeda monoica* cultivated in tannery effluent, salt treated soil and control soil. The values are means (± SD) of five replicates

Name of the species	Treatments	Mesophyll thickness (µ)	Mesophyll volume (cm^3 / dm^3)
Suaeda monoica	Control	43.8±2.19	4.38±0.21
	Salt	146.9±7.34	14.69±0.71
	Tannery Effluent	152.6±7.63	15.26±0.76

Recent studies also pointed out that the NaCl stimulated the fresh weight, dry weight and leaf area of *Suaeda altissima* (Meychik *et al*., 2013), *Suaeda monoica* (Ayyappan *et al*., 2013) and in *Mesembryanthemum crystallinum* (Abd El-Gawad and Shehata, 2014).

The growth and survival of plants at high salinities depend on their ability to cope with low water potentials and high concentrations of chloride (or sulphate) and sodium ions and the halophytes showed a range of growth responses to salinity. It would appear that the growth response at moderate salinities might be largely the consequence of an increased uptake of solutes that are required to induce cell expansion, since this maintain the pressure potential in plant tissues (Ajmal Khan *et al.,* 2000).

The reduction in control plants plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant height (Shanker *et al*., 2005).

Anatomical characteristics

In the present study, the maximum thickness in leaves was observed in *Suaeda monoica* was mainly due to deposition of metals and salts and also depends upon the nature of their succulence. *Suaeda monoica* is a succulent halophyte unlike glycophytes tend to accumulate sodium in the vacuole to higher levels than in the cytoplasm and as the volume of the vacuole is much greater than of the cytoplasm in fully expanded cells, the total sodium content of the root will approximate to the sodium content of the vacuole (Yeo and Flowers, 1986).

Therefore, halophytes have been suggested to be naturally better adopted to cope with environmental stresses, including heavy metals compared to salt-sensitive crop plants such as sunflower (*Helianthus annuus*), corn (*Zea mays*), pea (*Pisum sativum*) and mustard (*Brassica juncea*) commonly chosen for phytoextraction purposes (Jordan *et al*., 2002; Ghnaya *et al*., 2005, 2007). In addition, it has been speculated that salt-tolerant plants may also be able to accumulate metals (Jordan *et al*., 2002) and thus offer greater potential for phytoremediation research. Besides tolerance mechanisms allowing these plants to cope with internal accumulated ions such as sequestration of NaCl in vacuoles and production of compatible osmolytes in the cytoplasm, halophytes have a diversity of secondary mechanisms to handle excess salt. At the leaf level, some halophytes have salt glands, salt bladders, trichomes or succulent tissues to remove the excess of deleterious toxic ions from photosynthetically active tissues and regulate plant tissue ion concentration (Lefevre *et al*., 2009).

Once an HM has entered the cell, a plant uses various strategies to cope with its toxicity. Once such strategy consists of transporting the HM out of the cell or sequestrating it into the vacuole, thereby removing it from the cytosol or other cellular compartments where sensitive metabolic activities takes place (Dalcorso *et al*., 2010). Therefore, the central vacuole seems to be a suitable storage reservoir for excessively accumulated HMs. In fact, two vaculor proton pumps, a vacuolar proton-ATPase (V-ATPase) and vaculor protonpyrophosphate (V-Ppase), energize vacuolar uptake of most solutes.

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