ANNALS OF
BOTANY
Founded 1887

REVIEW: PART OF A SPECIAL ISSUE ON HALOPHYTES AND SALINE ADAPTATIONS

How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas?

Stanley Lutts[1],* and Isabelle Lefèvre[1,2]

[1]*Groupe de Recherche en Physiologie végétale (GRPV) – Earth and Life Institute – Agronomy (ELI-A),
Université catholique de Louvain, 4–5 (Bte 7.07.13) Place Croix du Sud, 1348 Louvain-la-Neuve, France and [2]Institute of
Plant Molecular Biology, Biology Centre CAS, Branišovská 31, 37005 České Budějovice, Czech Republic
* For correspondence. E-mail: stanley.lutts@uclouvain.be*

● **Background** Many areas throughout the world are simultaneously contaminated by high concentrations of soluble salts and by high concentrations of heavy metals that constitute a serious threat to human health. The use of plants to extract or stabilize pollutants is an interesting alternative to classical expensive decontamination procedures. However, suitable plant species still need to be identified for reclamation of substrates presenting a high electrical conductivity.
● **Scope** Halophytic plant species are able to cope with several abiotic constraints occurring simultaneously in their natural environment. This review considers their putative interest for remediation of polluted soil in relation to their ability to sequester absorbed toxic ions in trichomes or vacuoles, to perform efficient osmotic adjustment and to limit the deleterious impact of oxidative stress. These physiological adaptations are considered in relation to the impact of salt on heavy metal bioavailabilty in two types of ecosystem: (1) salt marshes and mangroves, and (2) mine tailings in semi-arid areas.
● **Conclusions** Numerous halophytes exhibit a high level of heavy metal accumulation and external NaCl may directly influence heavy metal speciation and absorption rate. Maintenance of biomass production and plant water status makes some halophytes promising candidates for further management of heavy-metal-polluted areas in both saline and non-saline environments.

Key words: Antioxidants, glycinebetaine, halophytes, metal distribution, metallothioneins, mucilage, osmoprotectants, phytoextraction, phytochelatins, phytoremediation, phytostabilization, ROS scavenging, salt marsh species.

INTRODUCTION

Heavy metals are present in soil as natural components of the earth's crust. With the noticeable exception of in metalliferous soils, these trace elements are available at very low concentrations and are therefore harmless to living organisms. In recent decades, however, anthropogenic activities have been increasingly associated with the accumulation of heavy metals in the environment. Industrial activities such as mining, smelting, burning of fossil fuels and dumping of municipal sewage sludge but also agricultural practices related to long-term use of excessive fertilizers, fungicides, pesticides and sewage sludge have led to a dramatic increase in toxic heavy metals in numerous areas. Pollution with Cu, Cd, Pb and Zn have frequently been reported (Ernst and Nelissen, 2000; D'Amore *et al.*, 2005; European Environmental Agency, 2007). These pollutants constitute a serious threat to environmental stability but also to human health through contamination of the food chain. In Europe, polluting activities are estimated to affect about 3 million sites (Sirguey and Ouvrad, 2013). In numerous cases, polluted areas are contaminated by several heavy metals and even organic pollutants. While the organic pollutants may be degraded or converted to harmless compounds through microbial activity in the rhizosphere (Batty and Dolan, 2013), heavy metals are not degraded and thus persist for long periods in soil.

Most plant species are sensitive to the harmful effects of heavy metals. These toxic compounds severely inhibit growth (Lombi *et al.*, 2001), affect plant water status (Barceló and Poschenrieder, 1990), drastically compromise photosynthesis (Greger and Ögren, 1991) and induce oversynthesis of reactive oxygen species (ROS) responsible for numerous physiological disorders that ultimately lead to plant death (Pilon-Smits *et al.*, 2000). Several plant species are able to colonize metalliferous soils, and most of them exclude heavy metals, except hyperaccumulating species known to accumulate more than 1 % Zn or Mn, 0·1 % Ni, Cu, Co or Pb and 0·01 % Cd (on a dry weight basis) in the shoots (Baker and Brooks, 1989; Cobett, 2003). These plant species are usually considered as 'hyperaccumulators' for a single element, despite the fact that several metals are commonly present in polluted substrates. These hyperaccumulating plants also exhibit a shoot concentration higher than root concentration for the considered heavy metal, thus demonstrating their ability not only to absorb but also to translocate heavy metals (Vázquez *et al.*, 1994; Yang *et al.*, 2004). Hyperaccumulation nevertheless appears as a rare, although fascinating adaptation in the plant kingdom and the ecological significance of hyperaccumulation evolution appears to be, at least partly, related to the selection of resistance to biotic constraints (Poschenrieder *et al.*, 2006).

Plants are crucial components of the environmental management of polluted areas. Vegetation cover helps to reduce water and wind erosion and contribute to re-establish biodiversity and ecosystem stability in such areas. In some cases, plants may be introduced in these ecosystems with the final aim to stabilize the heavy metals (phytostabilization) or to remove them from contaminated soil (phytoextraction). Efficient phytomanagement of heavy-metal-contaminated soils requires the identification and selection of adequate plant species. While most hyperaccumulating species produce low amount of biomass, suitable plant species for phytomanagement should exhibit a deep root system, high shoot biomass production and a perennial growing habit. Moreover, these plants must be able to cope with low levels of fertility and poor soil structure, which may induce mineral deficiencies and compromise water uptake. Promising plants for phytomanagement should therefore possess numerous traits allowing them to cope with multiple stress factors.

In some areas, high concentrations of bioavailable heavy metals are associated with high concentrations of soluble salts. This occurs, in particular, in coastal areas impacted by urban activities and in semi-arid regions where mining contributes to the release of both heavy metals and soluble salts (US Environmental Protection Agency, 2000). For phytomanagement of those areas, an adapted plant material should be identified: in addition to heavy metal resistance, those plants must withstand the ion and osmotic stresses derived from the

high NaCl burdens of these habitats (Wali *et al.*, 2014). Halophytic plant species are now receiving increasing attention as putative candidates for rehabilitation of these specific heavy-metal-contaminated sites (Supplementary Data Table S1). Indeed, in contrast to salt-sensitive glycophytes, halophytes maintain growing capacity at high salt doses and remain alive on substrates characterized by a high electrical conductivity. Low NaCl doses frequently stimulate plant growth (Colmer and Flowers, 2008; Ruan *et al.*, 2010; Rozema and Schat, 2013). Salt stress itself is a complex environmental constraint that includes both a water stress component related to the low external osmotic pressure of soil solution and an ionic component related to the accumulation of toxic ions and, to a lesser extent, to stress-induced deficiencies in essential elements. In their natural environment, halophytic species are also exposed to other abiotic stresses such as high temperatures or high light intensities (Ruan *et al.*, 2010; Walker *et al.*, 2014). Although most physiological studies reported in the literature have been performed with NaCl, soil salinity may also be due to soluble ions other than Na^+ and Cl^-. Halophytes are thus expected to be able to cope with multiple stress factors. Moreover, numerous physiological targets directly affected by heavy metals are also affected by salinity and it may be hypothesized that plants naturally resistant to salinity may exhibit promising adaptive properties to sustain the external presence or even accumulation of heavy metals in their tissues (Fig. 1).
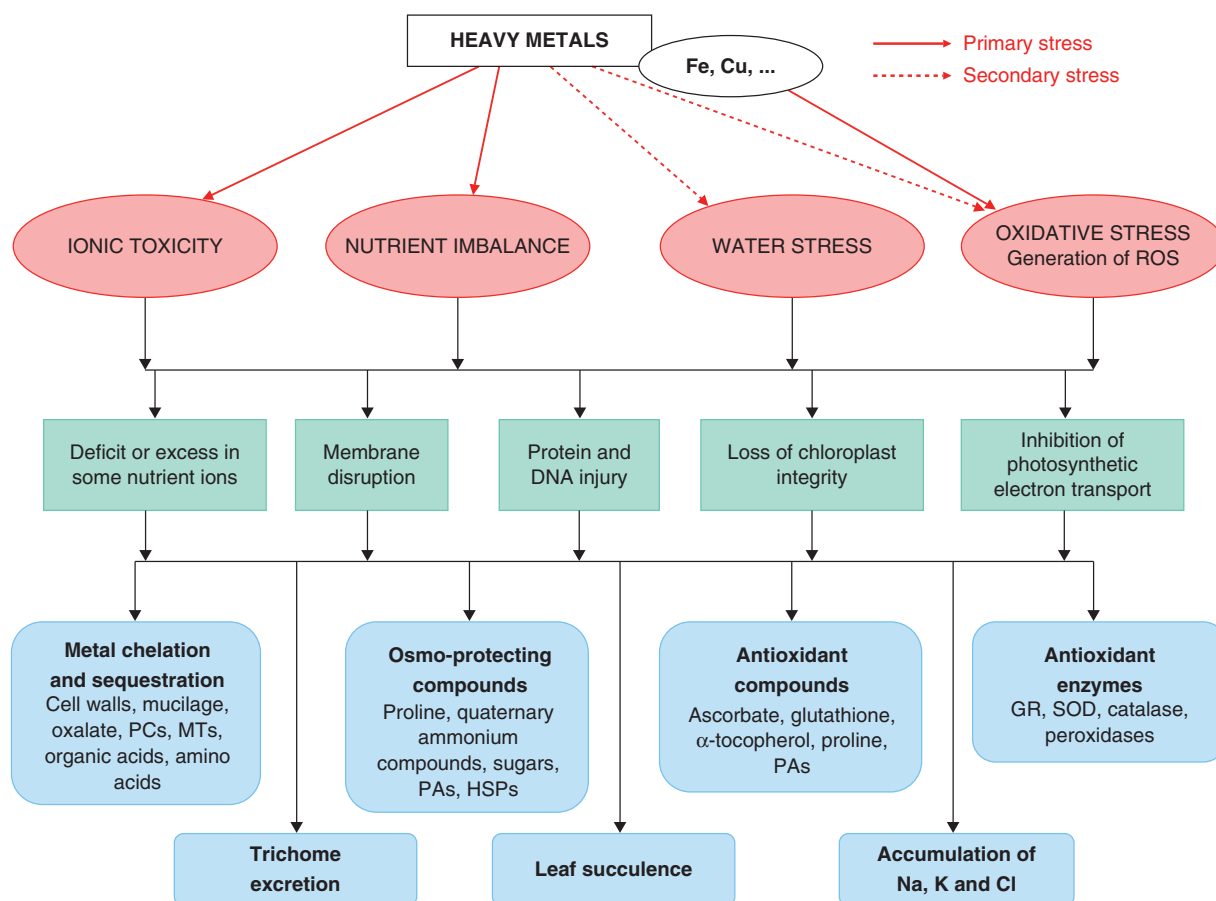

Fig. 1. Schematic representation of heavy metal toxicity in plants and the mechanisms of resistance set up by halophytes.

SALT IMPACT ON HEAVY METAL SPECIATION AND BIOAVAILABILITY

Absorption of heavy metals by plants depends on their bioavailability, which is the fraction of the total contaminant in the interstitial water and soil particles that is available to an organism. Heavy metal bioavailability in soils depends on a multitude of factors other than their concentration and may proceed differently in the bulk soil and in the rhizosphere. Soil composition, soil acidity and redox potential have a strong impact on the bioavailability of most heavy metals (Lombi *et al*., 2001). Biotic factors related to root activity (Marschner 1995) and to root-associated microorganisms (Carvalho *et al*., 2006) also have a direct influence on heavy metal bioavailability.

Salinity influences heavy metal speciation, although this parameter has often been neglected. Several studies indicated that salinity increases metal mobility, due to the complexation of salt-derived anions with heavy metals and to the competition between salt-derived cations with heavy metal ions for sorption sites on the solid phase (Hatje *et al*., 2003; Acosta *et al*., 2011). Such an effect has been mainly studied with Cd; according to McLauglin *et al*. (1994), salinity frequently increased Cd accumulation in potato tubers, even if the soil contains only low amounts of cadmium. Similar observations have been reported for sunflower (Li *et al*., 1994) and wheat (Mühlingh and Laüchli, 2003).

Experiments performed with water and sediment from estuaries also indicated that the kinetics of metal adsorption and desorption on suspended particulate matter are dependent on the metal and on the salinity of the water, except for Mn (Hatje *et al*., 2003). The general effect of salinity is to move the adsorption edge of metals to higher pH, with the greatest change being found for Cd. This general decrease of metal sorption on soil particulate matter may be related to chlorocomplexation, at least for metals such as Cd and Zn, and to competitive effects with divalent cations such as Ca^{2+} and Mg^{2+} (Hatje *et al*., 2003). The effect of salt on the increase in metal mobility in soil was observed with different types and amount of electrolytes (Acosta *et al*., 2011). This increase in metal mobility depends on the total amount of heavy metals present and the type of salt inducing the salinization. The effect of the chloride salts NaCl, $CaCl_2$ and $MgCl_2$ and the sulfate salt Na_2SO_4 on the mobility of Cu, Cd, Pb and Zn indicated that the main mechanisms regulating metal mobility were (1) competition with Ca and or Mg for sorption sites, (2) complexation with chlorides and (3) complexation with sulfates (Acosta *et al*., 2011). The main mechanism regulating Cd, Pb and Zn mobility was competition with Ca^{2+} for sorption sites, while for Cu it was the formation of $CuSO_4$. The double charge of Ca^{2+} and Mg^{2+} ions induced higher metal mobilization than the single charge of Na^+ ions. The higher ability of Ca^{2+} compared with Mg^{2+} to increase metal mobilization is related to the differences in size and atomic mass of these cations, Ca^{2+} being heavier and larger than Mg^{2+}. In soil solution, Cd forms complexes with chloride ions, leading to the formation of $CdCl_n^{2n}$ ionic species (Bingham *et al*., 1983) (Fig. 2). According to Bingham *et al*. (1984), Cd present in saline soils is mainly present as free Cd^{2+} ions and $CdCl^+$ complex, although $CdCl_2$ may also be observed together with low amounts of anionic species ($CdCl_3^-$ and $CdCl_4^{2-}$). Chlorocomplexes have high mobility in soils and are less efficiently retained by clay particles and organic matter than the free bivalent ion (Garcia-Miragaya and Page, 1976). In contrast, sulfate salinity had only a minor impact on Cd bioavailability, as Cd does not easily form complexes with SO_4^{2-} and these complexes are poorly available for the plant (Bingham *et al*., 1986).

The extent of $CdCl^+$ absorption by plants remains an open question. While Cd^{2+} may be easily absorbed by Ca^{2+} non-selective transporters, the size of the $CdCl^+$ ion makes such absorption much less efficient. According to Smolders *et al*. (1998), $CdCl^+$ absorption is only 10–13 % of Cd^{2+} absorption under classical soil pH conditions. The effect of NaCl on Cd absorption independently of Cd bioavailability may be studied in nutrient solution. Osmotic stress induced at the root level may somewhat compromise root selectivity and induce damage to root cell membranes, especially in glycophytes: resulting damage may thus contribute to non-selective entry of $CdCl^+$ (Helal *et al*., 1999; Stevens *et al*., 2003). In the halophyte *Atriplex halimus*, Lefèvre *et al*. (2009b) demonstrated that NaCl decreases rather than increases Cd accumulation, therefore suggesting that this species is able to maintain root integrity under osmotic stress and that transporters remain able to discriminate against the $CdCl^+$ complex.

HALOPHYTE RESPONSES TO CO-OCCURRENCE OF SALT AND HEAVY METALS IN THE ENVIRONMENT

Wetland halophytes

Estuaries are often situated close to highly populated and industrial areas. As a consequence, tidal flooding may provide large amounts of pollutants and salt marsh ecosystems thus frequently act as important sinks for heavy metals. Salt marshes may be regarded as crucial sites for phytostabilization of heavy metals, especially if plant species accumulate toxic ions in below-ground tissue (Doyle and Otte, 1997). In contrast, accumulation in stems and leaves implies a recycling of heavy metals through litter deposit (Weis and Weis, 2004). In this case, differences in decomposition rates between plant species and tissues play an important role and have been reported among halophytic species. Reboreda and Caçador (2007) demonstrated that areas colonized by *Halimione portulacoides* are potential sources of Cu, Cd and Pb whereas colonization by *Spartina maritima* contributes to the stabilization of heavy metals in salt marsh sediment.

Salt marsh halophytes interact with microorganisms of the rhizosphere by supplying organic substances. *Spartina maritima* colonization increased sulfate-reducing bacteria activity and plant behaviour may explain variations in metal sulfides (Otero and Macias, 2002). Extracellular activity is a useful index of microbial activity and bacteria metabolize humic compounds, reducing their molecular weight and increasing the mobility and solubility of these heavy metal carriers. Reboreda and Caçador (2008) clearly demonstrated that halophyte roots directly influence the extracellular enzymatic activity (EEA) of hydrolytic enzymes such as peroxidase, phenol oxidase,
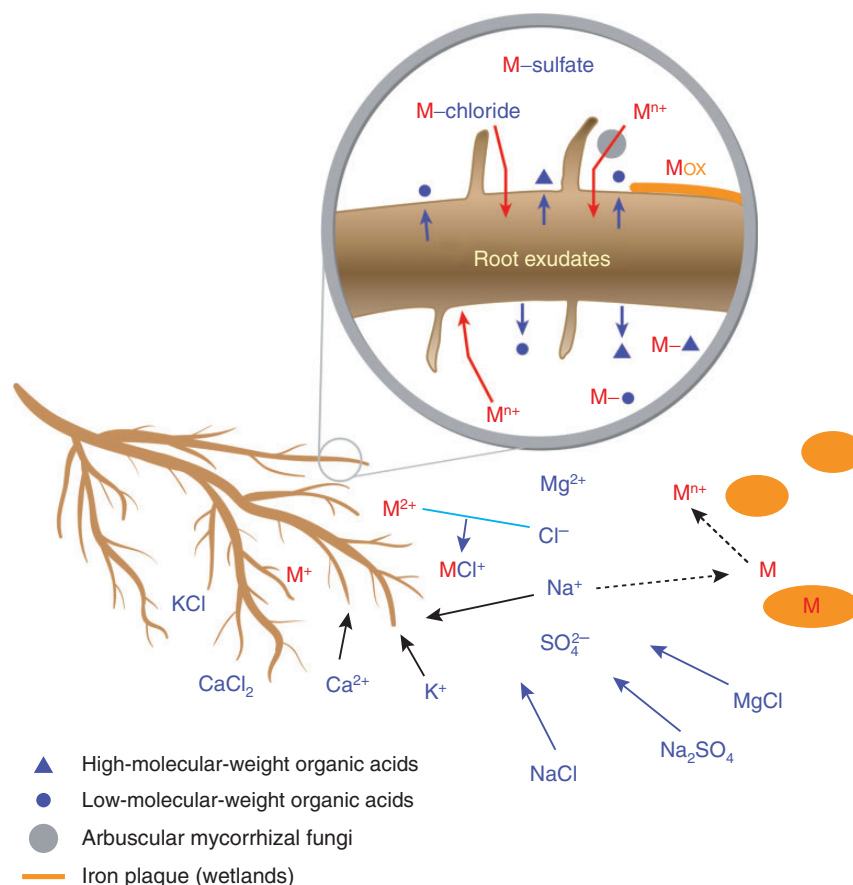
FIG. 2. Schematic representation of the different forms of metal (M) uptake by plants. In soil solution, heavy metals may be present under free form. They can also form complexes with chloride or sulphate. Salinity usually improves heavy metal bioavailability by substituting Na to metals adsorbed on soil particles. Roots can release high- and low-molecular weight organic acids which may form complexes with heavy metals. Arbuscular mycorrhizal fungi may interact to some extent with heavy metal absorption by plants. Some metals may also precipitate at the root surface to form oxyhydroxides. This is especially so in wetland plant species forming an iron plaque at the root surface.

β-glucosidase, *N*-acetyl-β-glucosaminidase and acid phosphatase. This microbial EEA is a key factor not only affecting organic matter biogeochemical recycling, but also heavy metal speciation. These enzyme activities exhibit a seasonal variation that is directly related to the plant cycle and this variation is responsible for periods of organic metal-bound decrease (Duarte *et al.* 2008).

Terrestrial halophytes

Even in highly polluted soils, heavy metal ions are present in such concentration that their effect on the osmotic potential of soil solution remains negligible. However, secondary water stress related to physiological drought has been reported in numerous studies devoted to the physiological impact of pollution by heavy metals and metalloids: Cd and Zn toxicities reduce transpiration, water potential, leaf relative water content and stomatal conductance (Varga *et al.*, 1999; Drazkiewicz and Baszyński, 2005; Vaillant *et al.*, 2005). Under severe Cd and Zn toxicities, accumulation of amorphous deposits in the vessels probably derived from cell walls may account for a lower water flow rate (Barceló *et al.*, 1988; Barceló and Poschenrieder, 1990). Whether a decrease in transpiration is a consequence of

water deficiency stress or a result of the deleterious impact of metal toxicity on stomatal guard cells remains unclear. The application of 50 µM Cd may affect leaf conductance independently of abscisic acid (ABA) synthesis (Perfus-Barbeoch *et al.*, 2002). According to Ghelis *et al.* (2000) and Perfus-Barbeoch *et al.* (2002), Cd^{2+} ions interfere with voltage-dependent Ca^{2+} channels, which, in turn, lead to early stomatal closure.

Xerohalophytes display an excellent ability to perform in environments characterized by low water availability. Some of these species may be considered as pioneer species for environmental protection, and may be used not only for phytoremediation but also for sand dune fixation, desert greenification and landscaping. Resistance to water stress is expected to contribute somewhat to resistance to heavy metals. Resistance of Syrian beancaper (*Zygophyllum fabago*) is directly related to the capacity of this species to maintain water status compatible with plant metabolism and growth (Lefèvre *et al.*, 2005, 2010*a*). Lefèvre *et al.* (2014) reported that Zn toxicity had no impact on stomatal density while Cd treatment increased it slightly after 28 d of exposure to 10 µM Cd. In both cases, however, water use efficiency drastically increased in response to heavy metals and efficient osmotic adjustment allowed the plants to reduce Ψ_w. A similar ability to regulate plant water status in

heavy-metal-stressed plants was recorded for the xerohalophyte *Atriplex halimus*. This shrub is distributed around the Mediterranean (Walker *et al.*, 2014) and may be exposed to heavy metal toxicity in dry areas (Manousaki and Kalogerakis, 2009; Rabier *et al.*, 2014). Laffont-Schwob *et al.* (2011) demonstrated that it forms endomycorrhizaea on heavy-metal-polluted soils, which may help the plant to cope with toxic ions. Lefèvre *et al.* (2010*b*) demonstrated that in this species that a polyethylene glycol (PEG)-resistant cell line displays a higher resistance to heavy metals than a PEG-sensitive cell line, confirming that water or osmotic stress resistance is an important component of Cd and Zn tolerance, independently of morphological adaptation and stomatal regulation. According to these authors, the PEG-resistant cell line regulates secondary oxidative stress in a more efficient way than the PEG-sensitive one. *Atriplex hortensis* and *Atriplex rosea* also exhibit a high level of resistance to Cu, Pb, Ni and Zn (Sai Kachout *et al.*, 2012). The closely related Chilian halophyte *Atriplex atacamensis* displays an unusual ability to overcome As toxicity and is still able to remain alive and grow at 1000 μM arsenate. This species originates from the Atacama Desert, one of the driest areas in the world. Vromman *et al.* (2011) demonstrated that As-treated plants are able to efficiently close their stomata in order to limit water losses and to accumulate glycinebetaine as an efficient osmoprotectant.

Sesuvium portulacastrum grows luxuriantly at 100–400 mM NaCl concentrations in arid and semi-arid regions (Lokhande *et al.*, 2013). This herbaceous perennial halophyte shows tolerance against several toxic heavy metals, especially Cd and Pb (Ghnaya *et al.*, 2005, 2007*a*). According to Ghnaya *et al.* (2013), the high citric acid content in the xylem sap and shoots of *S. portulacastrum* could explain its high potential to translocate and accumulate Pb, and build-up of citrate in the leaves may be involved in metal chelation allowing subsequent sequestration in the vacuole. *Sesuvium portulacastrum* is also able to display significant As accumulation (up to 155 μg g^{-1} d. wt) when exposed to 1000 μM As(V) and has therefore been recommended as a potential candidate for application in As removal and re-vegetation projects in As-contaminated sites (Lokhande *et al.*, 2011).

Although copper is an essential element for all living organisms, Cu excess is highly toxic for plants. The common ice plant (*Mesembryanthemum crystallinum*) is able adapt to high external Cu doses. Thomas *et al.* (1998) suggested that in this facultative halophyte, environmental stresses such as NaCl and heavy metals overlap to some extent. Shevyakova *et al.* (2003) demonstrated in this species that Cd-treated plants activate the peroxidase system, therefore decreasing the damaging effect of ROS (see 'Biochemical basis of heavy metal tolerance in halophytes'). Ghnaya *et al.* (2007*a*) demonstrated that K$^+$ uptake is impaired in roots of common ice plants exposed to Cd^{2+} but is compensated for by an increase in the K$^+$-use efficiency.

HEAVY METAL ABSORPTION IN HALOPHYTES

Uptake of metals by plants depends on various factors such as the plant species, the age and growth stage of the plant, seasonal variations, the existence of an iron plaque on the roots, metal speciation and bioavailability in the environment,

and metal characteristics (Caçador *et al.*, 2000). Although numerous data are available for the molecular basis of Na$^+$ uptake and accumulation in halophytes, and for maintenance of K$^+$ selectivity (reviewed by Flowers and Colmer, 2008), modalities of heavy metal absorption remain poorly documented in these species. In glycophytic plant species, numerous data are available on metal transporters belonging to various families, such as the copper transporter (CTR), cation diffusion facilitator (CDF), zinc–iron permease (ZIP), cation exchanger (CAX), natural resistance-associated macrophage protein (NRAMP), non-selective cation channel (NSCC) and heavy metal ATPases (HM-ATPase) (Migeon *et al.*, 2010; Migocka *et al.*, 2011; Kiyomo *et al.*, 2012). Data are also available regarding hyper-accumulators (Weber *et al.*, 2004; Oomen *et al.*, 2009; Wei *et al.*, 2009). Surprisingly, no exhaustive data are available yet, to the best of our knowledge, on specificities of halophytes in terms of regulation of the corresponding gene.

Forms of metal uptake and root exudates

Among the above factors, oxidation state and coordination environment of specific metals directly affect their absorption, translocation and detoxification in root tissues (Salt *et al.*, 2002) (Fig. 2). Whether metals are taken up predominantly under their free form or bound to inorganic or organic complexes is not clearly defined, even in glycophytes.

Different forms ('species') of one metal can have different rates of uptake. For example, many bacteria can methylate the metalloid arsenic in methylarsonic acid (MMAA) and dimethylarsinic acid (DMAA). As a consequence, uptake of arsenic by *Spartina alterniflora* and *Spartina patens* varied with the species of arsenic, with DMAA having the lowest availability, followed by MMAA, As(V) and As(III) having the highest bioavailability. Inorganic arsenicals were mainly accumulated in the roots while DMAA was readily translocated to the shoot (Carbonell-Barrachina *et al.*, 1998).

Plants can release soluble organic substances from their roots, including low-molecular-weight (LMW) organic acids, high-molecular-weight (HMW) polysaccharides and other organic substances that may form complexes with heavy metals (Bertin *et al.*, 2003) (Fig. 2). These compounds have been demonstrated to affect the solubility, mobilization and phytoavailability of heavy metals (Boularbah *et al.*, 1996; Bertin *et al.*, 2003). As demonstrated by Mucha *et al.* (2005) in the halophyte *Juncus maritimus*, some root exudates are efficient complexing agents increasing metal availability. This is mainly the case for oxalate and malonate complexing metals (Pb, Cr, Cu, Zn, Ni and Cd), and these agents are able to increase metal bioavailability in polluted estuarine environments. Oxalic acid is the most efficient for solubilization of poorly available metals such as Cr and Ni. The extent of exudation may vary depending on sediment properties.

Addition of citric acid as an organic acid chelator in the root environment enhanced Cd uptake in *Halimione portulacoides*, while it decreased Ni uptake (Duarte *et al.*, 2007). Pan *et al.* (2011, 2012) identified two protein-like fluorescence peaks quenched by Cu^{2+} or Pb^{2+} in the excitation emission matrix (EEM) spectrum of *Salicornia europaea* root exudates investigated by EEM fluorescence spectroscopy. These results

indicated that the fluorescent substances are strong Cu^{2+} and Pb^{2+} complexing organic ligands. For Cd uptake by *Suaeda salsa*, its linkage to proteins and LMW SH-containing compounds appeared to be an important process for its uptake, as root treatment with *N*-ethylmaleimide, a thiol blocker, strongly inhibited Cd^{2+} influx to roots (Li *et al.*, 2012).

Both organic and inorganic complexes can increase metal uptake by plants. Complexes of metals with hydrophilic ligands could enter the xylem vessels through an apoplastic pathway, at the tip of the roots where the Casparian bands are not developed or at breaks in the endodermis. Another hypothesis suggests that the depletion of free metal ions near the site of root uptake leads to the dissociation of metal complexes in this area, resulting in an enhanced diffusional flux and enhanced metal uptake (Degryse *et al.*, 2006). As a result, dissolved metal complexes can increase metal uptake, the magnitude of this increase depending both on the concentration and on the lability of the complexes.

Release of soluble organic substances from plant roots is a well-known process and may consume more than 20 % of photosynthates. Phytosiderophores are involved in Fe uptake, mainly in Graminaceae, and play a key role in plant nutrition on substrates with high pH, where Fe is marginally available. Data concerning the interaction between phytosiderophores and heavy metals are contradictory. Indeed, phytosiderophores are capable of solubilizing Fe from poorly soluble oxides but also other metals such as Mn, Cu and Zn. An increase of heavy-metal complexation by phytosiderophores followed by subsequent absorption was reported under Fe deficiencies (Chaignon *et al.*, 2002). No data are available concerning the impact of NaCl on this process.

Arbuscular mycorrhizal fungi (AMF; Fig. 2) associated with plant roots may interfere with heavy metal absorption (Liao *et al.*, 2003). AMF were reported to enhance nutrient supply and improve water use efficiency and photosynthesis in salt marsh plant species, but the rate of AMF colonization drastically decreased at moderate salinities (Caravaca *et al.*, 2005). In the halophytes *Arthrocnemum macrostachyum* and *Sarcornia fruticosa* growing in contaminated areas, reduced plant uptake of toxic metals, particularly Pb, is a beneficial impact of AMF on plant development (Carrasco *et al.*, 2006). A different result was obtained for *Aster tripolium*, where AMF clearly increased Cd and Cu accumulation in this species (Carvalho *et al.*, 2006).

In wetlands, anoxic conditions may favour a reduced form of heavy metals through the modification of redox potential. According to Weis and Weis (2004), such forms decrease bio-availability of metals compared with terrestrial systems with oxidized soils. These reducing conditions also limit the depth to which plant roots can penetrate and therefore restrict the uptake of contaminants (Williams, 2002). However, different forms of metals have different availability and different rate of uptake by plants. For instance, ferrous Fe is more available as it can be transported across the root plasma membrane through Fe(II) transporters in dictyoledons and non-graminaceous monocotyledons (Briat and Lobréaux, 1997). Solubility of Mn becomes relevant in soil with low pH or waterlogged soils, through its reduction from Mn(III/IV) to Mn(II) (Hernandez-Soriano *et al.*, 2012). In wetland plant rhizosphere, oxidation of ferrous Fe to its ferric form leads to the precipitation of Fe oxyhydroxides

and the formation of 'Fe plaque' on the roots, consisting mainly of Fe/Mn (hydr)oxides (Doyle and Otte, 1997; Cambrollé *et al.*, 2008). This Fe plaque has a large capacity to adsorb metals, allowing metal retention on the root surface. Species such as *Spartina densiflora* have a higher ability than others to retain metal around their roots through greater formation of plaques (Cambrollé *et al.*, 2008). Manganese plaque resulting from precipitation of Mn at the root surface has also been reported (Sundby *et al.*, 1998). However, whether the presence of the plaque reduces or increases the uptake of metal by plants still remains of debate. Different metals, environmental conditions or physiological properties may account for these different results (Weis and Weis, 2004).

Heavy metal movement in the roots

According to Li *et al.* (2012), Cd uptake by halophytic species such as *Suaeda salsa* is regulated by Ca transporters or channels in root cell plasma membranes. This has been shown through the use of verapamil, a voltage-dependent Ca channel blocker, and La^{3+}, a voltage-independent Ca channel blocker, both of which inhibited Cd influx into roots. These findings support the hypothesis of similar metal uptake and transport characteristics between halophytes and glycophytes, but this needs to be tested by complementary approaches involving the precise identification and characterization of metal transporters in halophytes.

Likewise, in non-halophytic plants, localization of metal absorption occurred mainly in the root tip just behind the root apex (Li *et al.*, 2012). The rate of Cd^{2+} fluxes reached a maximum value at 100–200 μm from root apex in *Suaeda salsa* (Li *et al.*, 2012). However, metal concentration along the roots is not constant. Caetano *et al.* (2008) identified different preferential layers of metal accumulation in *Spartina maritima* and *Sarcocornia fruticosa* that were not related to metal concentration in the surrounding environment.

Scanning electron microscopy (SEM) X-ray microanalysis of heavy metals in transverse sections of roots revealed gradients in root tissues specific to the heavy metal in question. In *Avicennia marina*, in response to Zn treatment, relative Zn concentration decreased from the epidermis through the parenchyma of the cortex, to reach a low relative concentration in the endodermal layer, while an increase was observed in xylem tissue. In response to Cu treatment, relative Cu concentration remained similar from the epidermis through parenchyma to the endodermal cell wall. Subsequent significant decreases were observed within endodermal layer and xylem, with very low concentrations in the phloem. In response to Pb, highest relative Pb concentration was located in the epidermis and a significant decrease was observed within the cortical parenchyma, with small amounts of Pb reaching the vascular tissue (MacFarlane and Burchett, 2000).

After absorption, Cd is rapidly transported to vascular tissues by the symplastic pathway, where it may be translocated to more distal positions in root zones (Li *et al.*, 2012). The correlation between bypass flow, quantified by the fluorescent dye 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt, and Cd accumulation in leaves of *Zygophyllum fabago* suggests that it contributes to Cd translocation through the transpiration stream

(Lefèvre *et al.*, 2009*a*). However, heavy metal translocation from roots to shoots is restricted in most halophytes, as roots accumulate larger amount of metals than shoots (Caetano *et al.*, 2008; Sousa *et al.*, 2008; Han *et al.*, 2012*a*, *b*). Limiting ion movement in root tissues may be performed through different mechanisms. Accumulation in cell walls is one important means of metal immobilization. For instance, in *Halimione portulacoides*, on average 65 % for various studied heavy metals was stored in the root cell wall (Sousa *et al.*, 2008). Castro *et al.* (2009) observed a high proportion of insoluble Hg, varying from 72·7 to 99 %, in roots of different salt marsh species. These authors reported a higher ability of monocotyledonous species to retain Hg in root than dicotyledonous species. In *Avicenia marina*, the root epidermis provided a major barrier to the transport of Pb only, while the endoderm provided a barrier to the movement of numerous heavy metals such as Zn and Cu into the stele (MacFarlane and Burchett, 2000). Studies have also shown that some heavy metals may be transported from roots to stems and leaves bound to organic acid. In *Salsola kali*, X-ray absorption spectroscopy showed that in roots, Cd was preferentially bound to O ligands, while in stems and leaves Cd was most likely to be bound to O and S groups (de la Rosa *et al.*, 2004). The authors suggested that in roots Cd may be bound to O groups from small organic acids involved in the transport of metals. It may also be bound to carboxyl and hydroxyl groups of cell wall.

Some proteins of the P_{1B}-ATPase family (heavy metal ATPases; HMAs) play a key role in root to shoot translocation of trace elements, especially Cd. AtHMA4 is expressed at the plasma membrane of specific root cells and is directly involved in Zn and Cd xylem loading while AtHMA2 is involved in Zn transport (Migeon *et al.*, 2010). Xu *et al.* (2010) demonstrated that low salt supply increased Cd accumulation in leaves of *Arabidopsis thaliana* and *Solanum nigrum* and that NaCl elevates the expression of *HMA4*. Future studies focusing on HMA regulation in halophytes should provide valuable information.

HEAVY METAL TRANSPORT AND ACCUMULATION IN HALOPHYTES

Heavy metal accumulation is the ultimate consequence of heavy metal absorption and translocation from root to shoot. It is therefore directly influenced by the transpiration rate and shoot relative growth rate. In halophytes, moderate NaCl doses stimulate plant growth and it could thus be hypothesized that growth stimulation will contribute to dilute heavy metal content, leading to a lower concentration in NaCl-treated halophytes, especially if salinity itself reduces heavy metal absorption. However, quantitative analysis does not support this hypothesis and in several cases salinity not only reduces heavy metal absorption and increases plant growth, but also improves physiological tolerance to accumulated heavy metals. Han *et al.* (2013*a*) demonstrated that NaCl may reduce the concentration of aminocyclopropane carboxylic acid, the immediate precursor of ethylene, and thus delay senescence in Cd-treated plants of *Kosteletzkya virginica*, while conversely it increased the concentration of cytokinins (zeatin and zeatin riboside), which may act as anti-senescing phytohormones, and contributed to the maintenance of photosynthesis, even in Cd-accumulating leaves.

Salt influence on transfer coefficients

The bioaccumulation factor (BF) is defined as the ratio of the metal concentration in plant tissues to that in the soil. This parameter may thus be used to assess the efficiency of metal accumulation in plants. It differs widely according to the heavy metal in question and commonly decreases with increasing external concentration of pollutant. The transfer factor (TF) is defined as the ratio of metal concentration in shoot to that in roots (Sousa *et al.*, 2008). Most plant species, with the noticeable exception of hyperaccumulators, exhibit a TF factor lower than 1·0. Some halophytes, such as *Arthrocnemum macrostachyum*, accumulate Cd at high concentration with a BF far exceeding the critical value of 1 but always exhibit a TF value lower than 1. As previously noticed for BF, TF values also decrease with high external concentration of pollutant (Redondo-Gómez *et al.*, 2010).

The major chemical form of heavy metal translocation in halophytes remains unknown. In tumbleweed (*Salsola kali*), X-ray absorption spectroscopy studies have demonstrated that Cd is mainly bound to oxygen in the roots, and to oxygen and sulfur groups in the shoots, suggesting that small organic acids may be partly involved in Cd transport (de la Rosa *et al.*, 2004). The involvement of organic acids in heavy metal translocation may, however, differ strongly depending on the element considered: Duarte *et al.* (2007) demonstrated that citric acid improves Cd translocation but drastically decreases Ni translocation in the halophyte *Halimonie vulgaris*. Ghnaya *et al.* (2013) demonstrated that the halophyte *Sesuvium portulacastrum* translocated higher amounts of lead from the root to the shoot compared with the glycophyte *Brassica juncea* in relation to a higher accumulation of citric acid in the xylem sap. In contrast, a minor relationship was observed between Pb concentration and fumaric, malic or α-cetoglutaric acid in the xylem. In *Atriplex halimus*, stem oxalate concentration clearly increased in response to Cd exposure, but such an increase mainly concerns the insoluble form (Lutts *et al.*, 2004). In some halophytes Cd precipitation may occur in the form of oxalate to prevent Cd from reaching photosynthetically active leaves (Lefèvre, 2007).

The presence of chloride not only influences heavy metal mobility in the soil but also within the plant. Wali *et al.* (2014) observed that the presence of salt facilitated the translocation of Cd to the shoot through enhancement of Cd flow in the xylem. According to Gutknecht (1981), transport of Hg^{2+} through lipid bilayers is markedly enhanced by chlorocomplexation: at pH 7·0 and Cl^- concentration ranging from 10 to 100 mM, only the dichloride complex of mercury ($HgCl_2$) crosses the membrane at a significant range. Girault *et al.* (1998) demonstrated that the addition of NaCl into medium containing Cd–lipid complexes promotes the release of bound Cd from lipids and results in formation of soluble Cd–Cl complexes. Ozkutlu *et al.* (2007) indicated that Cd is easily re-translocated from the source into the sink organs, most probably via phloem loading. These authors observed that NaCl sprayed on flag leaves previously immersed in Cd-containing solution clearly increased Cd transport and accumulation to the grain and concluded that

remobilization of Cd is promoted in the presence of Cl through the formation of mobile $CdCl_n^{2-n}$ complexes. In contrast, no effect of NaCl on Zn mobilization was recorded, which may be due to the fact that Zn is not complexed to the same extent by Cl.

Mucilage

Mucilage consists of a mixture of polysaccharides found in various organs (e.g. roots, rhizomes, seed endosperm) of several plant species. It assumes numerous functions in plants, including a contribution to long-distance water transport in mangroves and buffering leaf water status against environmental fluctuations, thus enabling leaves to maintain low water potential when water deficit develops (Zimmermann et al., 1994). Negatively charged mucopolysaccharides are major constituents of mucilage. Ghanem et al. (2010) reported that a significant proportion of Na^+ accumulated in halophytic species may bind to mucilage. It been hypothesized that a similar retention also occurs for heavy metals accumulated in the form of divalent cations within shoots but also root tissues (Morel et al., 1986; Javed et al., 2013). Pectic polysaccharide extracted from plants resistant to water stress may also accumulate high amounts of arsenic (Fox et al., 2012), chromium (Lakshmanraj et al., 2009) and aluminium (Miyasaka and Hawes, 2001). Gorai et al. (2014) have demonstrated that mucilage hydration is increased as a result of preliminary osmotic stress in the desert shrub *Henophyton deserti*. While mucilage content may sometimes decrease in salt-treated glycophytes (Badi and Sorooskzadeh, 2011), it increased in halophytes and a higher neutral monosaccharide composition may directly affect ion binding capacities (Ghanem et al., 2010).

Trichome excretions

Cadmium excretion by trichomes has been reported, even in a glycophyte species such as tobacco, and NaCl treatment increased the size of the trichome cells (Choi et al., 2001, 2004). Sarret et al. (2006) reported that Zn exposure increased trichome density in young leaves but not in mature leaves of tobacco. Similarly, Cu induced the expression of genes coding for sequestering metallothionein (MT) in *Arabidopsis* trichomes (Guo et al., 2003). Glandular trichomes of the hyperaccumulating species *A. halleri* accumulate heavy metals but do not excrete them at the leaf surface.

Numerous halophytes possess leaf trichomes and salt glands that are able to remove large amounts of salt from the photosynthetically active tissues. In several cases, ion excretion is not specific for Na^+ or Cl^- ions and high amounts of divalent cations, especially Ca^{2+}, were also found in those structures (Storey and Thomson, 1994). Heavy metal excretion through salt glands was frequently considered as a possible detoxification mechanism. MacFarlane and Burchett (1999) reported that Zn excretion in glandular trichomes of *Avicennia marina* may be as high as $0·35 \, \mu g \, cm^{-2}$ per month. Weis and Weis (2004) reported that heavy metal excretion accounts for more than $260 \, g$ Pb and Cu $ha^{-1} \, yr^{-1}$ in *Spartina alterniflora*. Manousaki et al. (2008) demonstrated that soil salinity results in an increased excretion of Cd on the *Tamarix smyrnensis* leaf surface.

In the xerohalophyte *Atriplex halimus*, trichomes contain a stalk cell, embedded in epidermal cells, and a bladder cell exhibiting a huge vacuole and a reduced cytoplasm with a few organelles (Smaoui et al., 2011). Heavy metals can be transported via endocytosis vesicles and by Golgi and endoplasmic reticulum. According to Lefèvre et al. (2009b), more than 30 % of the accumulated Cd was found within the trichomes at the leaf surface, but external salinity reduces the proportion of excreted Cd.

The real contribution of trichome excretion to heavy metal resistance, however, remains uncertain and it elucidation requires a comprehensive approach. Moreover, pollutant accumulation on the leaf surface constitutes a major drawback for phytomanagement as it implies a risk of metal recycling to the soil surface through rain leaching.

Leaf succulence

A significant proportion of halophytes do not possess salt glands or trichomes. In those species, leaf succulence may help to dilute the content of accumulated toxic ions in photosynthetic leaves and maintain plant water content through avoidance of low leaf water potential related to the accumulation of compatible solutes and ions. Leaf succulence is related to specific anatomical and physiological properties leading to a low specific leaf area. Salt-induced increases in leaf succulence have been reported in numerous halophytes (Munns and Tester, 2008; de Vos et al., 2013; Katschnig et al., 2013). Succulent shoots containing expanded cells may provide enough space to accumulate toxic ions. The common ice plant (*Mesembryanthemum crystallinum*) increased stem succulence, providing a long-term reservoir of water storage under high salinity (Thomas and Bohnert, 1993; Agarie et al., 2007). *Sesuvium portulacastrum*, another heavy-metal-resistant halophyte, is able to increase leaf succulence when exposed to ion toxicities (Wang et al., 2012). Although modification of leaf traits is mainly induced by the osmotic component of salt stress (Munns and Tester, 2008), Wang et al. (2012) demonstrated that Na^+ is more effective than K^+ in increasing leaf succulence and that NaCl toxicity is attributable largely to the effect of Cl^-. Heavy metals in free or chelated forms may accumulate in vacuoles, and leaf succulence increases vacuole storage capacity. Hence, with expansion of the vacuolar volume, salinity may improve the efficiency of sequestration strategies in succulent halophytes.

Histological distribution

Heavy metal accumulation in compartments exhibiting only a low metabolic activity, such as cell walls or vacuoles, may be regarded as an efficient tolerance mechanism diminishing metal concentration in the cytoplasm (Carrier et al., 2003). The proportion of metals retained in the cell walls varies depending on the metal in question but also according to cell-wall composition and properties.

In the halophyte *Halimione portulacoides*, more than 65 % of the absorbed heavy metals accumulated in the root cell wall and almost 50 % of those accumulated in the leaves are also

retained by cell-wall polymers (Sousa *et al.*, 2008). According to these authors, the lowest Cu and Zn content was measured in the cellulosic fraction while a significant proportion may be linked to the proteic fraction. Using sequential extraction procedures, Reboredo (2012) supported these assumptions with the observation that in response to Zn treatment, carbohydrates of the cell walls (cellulose, hemicellulose and pectins) in stems and leaves of *H. portulacoides* were the preferential Zn binding sites with approx. 40 % of the total Zn concentration, while this fraction corresponded to 30 % of the total Zn concentration in roots. Between one-third and one-quarter of the total Zn concentration in all organs was associated with the proteic fraction. Moreover, Reboredo (2012) highlighted a direct relationship between Zn increase in roots and an increase of the proteic fraction involved in Zn binding. A similar relationship was observed for carbohydrates in the leaves. In *Avicennia marina*, a higher concentration of heavy metal was also observed in cell-wall matrices than in the cytoplasmic fraction (MacFarlane and Burchett, 1999, 2000). In *Sesuvium portulacastrum*, Walli *et al.* (2014) reported that an increasing proportion of accumulated Cd in NaCl-treated plants was bound to chloride, pectates and proteins. These authors suggested that compartmentalization and chelation processes are involved in the positive impact of salt on the Cd tolerance of this species.

It is a well established that the distribution of elements in plant tissue is highly inhomogeneous. The use of sequential extraction methods gives an indication of this distribution but does not appear to be sufficient as the redistribution of elements during the process cannot be excluded. Hence, this type of approach needs to be validated based on more reliable methodologies.

Application of X-ray fluorescence analytical techniques to analyse multi-elemental distribution in plant tissues down to the cell level has demonstrated its accuracy in determining ion composition at the micrometre scale (Nečemer *et al.*, 2008) (Fig. 3). These techniques have been used to study the spatial distribution of heavy metals mainly in hyperaccumulating species. They have revealed strategies of metal detoxification and highlighted the effect of heavy metals on the distribution of essential elements in tissues. The hyperaccumulating species *Noccaea (Thlaspi) praecox* preferentially accumulated heavy metals in the epidermis and in vascular bundle collenchyma (Vogel-Mikuš *et al.*, 2008). The chemical form of heavy metal in the environment also influences its distribution in tissues, as demonstrated with $CdCl_2$ and $CdSO_4$ supplementation on Cd distribution in *N. praecox* (Koren *et al.*, 2013). In this species, a reallocation of essential mesophyll cations (Fe, Mn and Zn) was observed with increasing Cd concentrations in the environment (Pongrac *et al.*, 2010). These changes in elemental distribution may be a consequence of interference by heavy metals with the homeostasis of essential ions, but may also imply dynamic reallocation between tissues and sub-cellular compartments. To the best of our knowledge, only one study has been performed on the precise localization of metal ions in tissues of halophytic species. The succulent perennial facultative halophyte *Zygophyllum fabago* was able to induce protection of the photosynthetically active tissues and maintenance of cell turgor in leaves in response to Cd or Zn treatments through the accumulation of Cd or Zn ions in less metabolically active tissues and through the reallocation of some essential elements such as

K (Lefèvre *et al.*, 2014). X-ray microanalysis of elements in halophytes grown in saline environments indicated different strategies of Na exclusion from photosynthetically active chlorophyll tissues in *Bassia indica*, *Atriplex prostrata*, *Spartina maritima*, *Limonium angustifolium* and *Atriplex spongiosa* (Storey *et al.*, 1983; Pongrac *et al.*, 2013). It may be hypothesized that some mechanism involved in salt ion compartmentation in tissues and cells should also be partly involved in metal ion distribution. Investigation of the tissue and cellular distribution pattern of heavy metals in leaves would contribute significantly to our understanding of plant tolerance mechanisms. Despite their low limit of detection, other techniques such as SEM X-ray microanalysis allow line scans of some ion profiles on transverse sections of plant tissue. In leaves from *Avicennia marina* seedlings treated with Zn, SEM X-ray microanalysis revealed a decreasing Zn gradient from xylem tissue through photosynthetic mesophyll to hypodermal (water) tissue, with a subsequent increase in concentration in the glandular tissue on the adaxial surface (MacFarlane and Burchett, 1999, 2000). A similar gradient was observed across leaf tissue in response to Cu treatment, but there was no increase in Cu concentration in glandular tissue (MacFarlane and Burchett, 2000).The authors observed that the Zn gradient was similar to that observed for Na and K in *A. marina* leaf tissue, reinforcing the hypothesis that the various adaptations halophytes have developed to cope with hypersaline environments may, at least partly, be transposed to heavy metals.

At the cellular level, metal transporters are important for metal storage or sequestration into vacuoles as well as distribution to organelles. Some heavy metals may enter plant cells through ZIP transporters and NSCC. Vacuoles are primary storage sites for toxic Cd, which is sequestered as Cd–phytochelatin (PC) complexes transported by ABC transporters. The protein systems excluding heavy metals from the cell cytosol localize also to the plasma membrane and tonoplast and are energized by ATP or by electrochemical gradients generated by H^+-ATPase or by V-ATPase and pyrophosphatase (PPase). Cadmium is excluded from the cytosol through an energy-dependent system as a free ion as well as complexed forms (Migocka *et al.*, 2011). Numerous data are available for CDF, zinc–iron ZIP, NRAMP and HMA transporters in terms of both cellular location and heavy metal selectivity (Migeon *et al.*, 2010). Several of these transporters have been studied using transgenic plants or a yeast heterologous system (Nagata *et al.*, 2010; Kiyomo *et al.*, 2012). Nevertheless, information of salt effects on these transporters and on the specificities of their regulation in halophytic plant species are lacking and are urgently needed.

Plants employ efficient strategies to limit the translocation of non-essential heavy metals such as Cd or Pb to developing embryos in maturing seeds (Ernst and Nelissen, 2000). In maturing seeds of angiosperms, assimilates are unloaded from the phloem symplastically through the chalaza and then into the apoplast via transfer cells. While non-essential elements are excluded, elements such as Zn or Cu may penetrate reproductive structures. Tauris *et al.* (2009) showed that genes coding for Zn transporters are expressed in the transfer cells and that Zn may accumulate in their vacuole for temporary storage. Han *et al.* (2013b) demonstrated that in some halophytes, NaCl not only reduces Zn accumulation to the seeds in Zn-contaminated
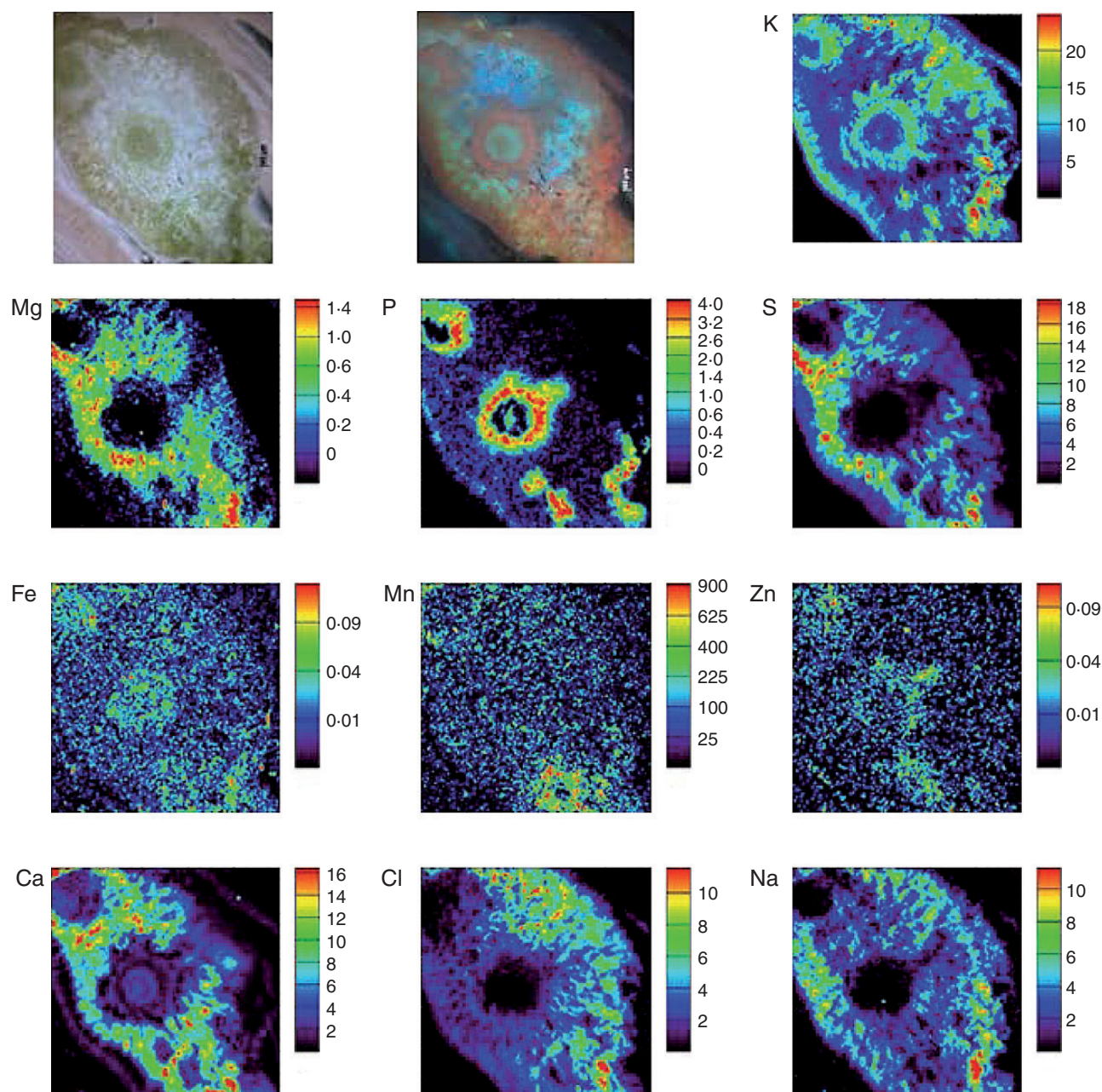
FIG. 3. Photographs of a representative leaf cross-section of control plants of *Zygophyllum fabago* as viewed by light microscopy (upper left), and by ultraviolet (UV) light source excitation (upper middle). The associated quantitative elemental maps are generated using the GEOPIXE II software package after micro-PIXE analysis. Units for Ca and Cl are percentage by weight. Units for Mg, P, Fe, Mn and Zn are square-root percentage by weight. The scan size for all images is $1000 \times 1000\,\mu m$. Details regarding growth conditions, sampling and methods preparation are presented in Lefèvre *et al*. (2014).

substrate but also modifies Zn distribution through an improvement in the efficiency of chalazal retention.

BIOCHEMICAL BASIS OF HEAVY METAL TOLERANCE IN HALOPHYTES

Oxidative stress

The production of ROS is an unavoidable consequence of aerobic metabolism and is strongly increased in response to mineral toxicities. ROS can be extremely reactive and oxidize biological molecules such as DNA, carbohydrates, proteins and lipids. The first step in O_2 reduction produces the relatively short-lived and not readily diffusible hydroperoxyl ($HO_2^{\bullet-}$) and superoxide ($O_2^{\bullet-}$) radicals. These oxygen radicals are highly reactive, and can oxidize specific amino acids such as histidine, methionine and tryptophan. They can cause lipid peroxidation in a cellular environment, thereby weakening cell membranes. Further reduction of O_2 generates hydrogen peroxide (H_2O_2), a relatively long-lived molecule that can diffuse some distance from its site of production. The biological toxicity of H_2O_2 through oxidation of SH-groups has long been known and is

enhanced by the presence of metal catalysis through the Haber–Weiss or Fenton reaction. One of the most toxic ROS is the hydroxyl radical (OH$^{\bullet}$), which has a high affinity for most biological molecules near its site of production. It is the prime cause of oxidative damage to proteins and nucleic acids and in lipid peroxidation (Demidchik, 2014). It is also involved in oxidative stress signalling and programmed cell death (PCD) (Demidchik *et al.*, 2010; Demidchick, 2014). Involvement of this hydroxyl radical in the activation of the Ca^{2+} and K^{+} plasma membrane channel has recently been evidenced, leading to Ca^{2+} influx and K^{+} efflux as an early response to oxidative stress resulting notably from transition metals (Zepeda-Jazo *et al.*, 2011; Rodrigo-Moreno *et al.*, 2013*a*; Demidchick, 2014). As halophytes display a strong need to regulate Na and K concentration inside their cells (Flowers *et al.*, 2010), regulation of their channels in response to the hydroxyl radical should be considered. Recent studies have shown the importance of K reallocation in cells of photosynthetically active tissues in *Zygophyllum fabago* in response to heavy metal toxicities (Lefèvre *et al.*, 2014).

As previously reported in this review, anoxic conditions may in some cases favour the accumulation of reduced forms of transition metals such as Cu, Mn and Fe. In the presence of H$_2$O$_2$, these metals may catalyse the production of hydroxyl radicals through the Fenton reaction both in the apoplast and in the cytosol (Rodrigo-Moreno *et al.*, 2013*b*). While Cu and Fe are essential components of the plant antioxidant defence system as part of superoxide dismutase (SOD) (see below), when they are absorbed in excess they may lead to metabolic disturbances such as loss of chloroplast integrity, alteration of plastid membrane composition and inhibition of photosynthetic electron transport. The disruption of electron transport itself may hasten further ROS overproduction. Mn is not a highly reactive Fenton metal ion, and it becomes oxidized only when bound to specific ligands, such as phenolic compounds (Shabala *et al.*, 2014). Cadmium and zinc are not involved in the Haber–Weiss reaction and do not directly generate synthesis of ROS. However, secondary oxidative stress is an important component of Cd and Zn toxicities. Plants contain antioxidants such as glutathione (GSH), ascorbate and α-tocopherol, in addition to a wide range of other less predominant compounds with antioxidant activities. Enzymatic defences include catalase and peroxidase enzymes that remove H$_2$O$_2$ and SODs that catalyse the disproportionation of superoxide radicals to H$_2$O$_2$ and O$_2$.

Halophytes are constitutively well equipped to cope with oxidative stress as ROS are also overproduced when plants are exposed to high NaCl doses, as reviewed by Ozgur *et al.* (2013) and Bose *et al.* (2014). As reported by these authors, several studies showed that halophytes have higher constitutive antioxidant defence activity compared with glycophytes, which may be useful in the context of heavy metal toxicities given that some adaptations to these stresses overlap with those related to salinity. Wang *et al.* (2014) reported that *Salicornia brachiata* can up-regulate catalase and SOD activities in response to heavy metals such as Cd and Ni, and in response to metalloids such as As. Lefèvre *et al.* (2010*b*) compared the response with Cd in a PEG-resistant and a PEG-sensitive cell line of *Atriplex halimus*: the greater ability of the PEG-resistant cell line to cope with 100 μM CdCl$_2$ is mainly due to an increase in

endogenous antioxidants (GSH and ascorbic acid), a high constitutive SOD (EC 1.15.1.1) activity and an efficient Cd-induced stimulation of glutathione reductase (EC 1.6.4.1). A low GSSG/GSH ratio is necessary to achieve optimal metabolism. Pilon-Smits *et al.* (2000) reported that chloroplastic glutathione reductase assumes crucial functions in heavy metal tolerance. Cadmium was found to up-regulate the expression of GSH reductase (GR) coding genes and both transcriptional and post-translational impacts should be considered (Rodríguez-Serrano *et al.*, 2006). Although salinity induces oxidative stress in glycophytic species, it has been reported to reduce Cd-induced oxidative damages in some wetland halophytes: an obvious increase in α-tocopherol and in ascorbic acid in response to NaCl was reported to reduce oxidative damage resulting from Cd stress (Han *et al.*, 2013*a*). Salinity also increased GSH synthesis, which is of primary importance for subsequent response to heavy metals as GSH is the precursor of PCs (see below) involved in vacuolar sequestration of toxic heavy metals. Heavy metal-induced oversynthesis of PCs may thus compromise resistance to oxidative stress as a consequence of a depletion of the GSH pool in glycophytes, while halophytes may be able to increase GSH concentration in a more efficient way. In *Sesuvium portulacastrum*, *in vivo* tissue fluorescence imaging confirmed that NaCl drastically reduced the concentration of H$_2$O$_2$ in both leaves and stems of Cd-treated plants (Wali *et al.*, 2014).

Cadmium increased the expression of *CAT1*, *GST* and *Prx Q* genes coding for enzymes involved in antioxidant activities (Cong *et al.*, 2013). Wu *et al.* (2013) observed that only *CAT* gene expression levels significantly increased in response to Zn, Pb or the combination of both metals in shoots of *Suaeda salsa*, while activities of the antioxidant enzymes SOD, glutathione peroxidase (GPx) and catalase (CAT) increased in response to Zn and Zn + Pb. It appears that SOD activity is highly efficient in protecting halophytes from extreme environmental changes and that CAT expression/activity is higher in halophytes than in glycophytes (Jithesh *et al.*, 2006*a*, *b*; Bose *et al.*, 2014).

PCs and MTs

PC are LMW polypeptides with the general structure (γGlu-Cys)$_n$Gly, where *n* varies from 2 to 11. These compounds are enzymatically synthesized from glutathion by phytochelatin synthase (PCS) and contribute to metal detoxification by transporting the metal to the vacuoles. The precise role of PCs in heavy metal resistance remains of debate. Although metallophytes rarely use this expensive strategy to cope with high doses of heavy metals, it has been demonstrated that the ability to synthesize PCs and the presence of constitutive and functional PCS are ancestral characters (Petraglia *et al.*, 2014) that may help the plant to minimize damage under conditions of excess metal supply in non-metallophytes (Tennstedt *et al.*, 2009). In black mangrove *Avicennia germinans*, which exhibit a high level of tolerance in polluted environments, evidence was provided that high concentrations of Cu^{2+} and Cd^{2+} induce overexpression of *AvPCS* coding gene within a few hours (Gonzalez-Mendoza *et al.*, 2007). Although the overexpression remains transient, it appears sufficient to

trigger an efficient protecting mechanism during longer-term exposure.

MTs are cystein-rich metal binding proteins that occur in almost all organisms, including plants, animals and micro-organisms. Plant MTs are divided into four types according to the location and distribution of cystein residues (Cobbett and Goldsbrough, 2002). As plant MTs efficiently bind metals, they are thought to be involved in metal homeostasis or tolerance mechanisms. In *Bruguiera gymnorhiza*, a gene coding for type 2 MT (MT2) was clearly up-regulated by Zn, Cu and Pb (Huang and Wang, 2009). Similar findings were reported in *Avicennia marina*, and transgenic *Escherichia oli* LB21 overexpressing a gene coding for the fusion protein GST-AmMT2 showed increased tolerance to Zn, Cu, Pb and Cd exposure (Huang and Wang, 2010). MT2 tends to be present mainly in leaves while MT1 is observed mainly in roots. Hence, it may be hypothesized that overexpression of the MT2 encoding gene in halophytes may assume positive functions in these plant species. In halophytes, however, different elements have different impact on expression of genes coding for MT2. In *Prosopis juliflora*, *PjMT-2* was significantly up-regulated by zinc while its expression was unchanged under Cu and Cd stress treatments (Usha *et al*., 2009). Likewise, *SbMT-2* expression from *Salicornia brachiata* was up-regulated by Zn and Cu but remained unaffected in the presence of Cd (Chaturvedi *et al*., 2012). In this species, sequestration of Zn is four-fold higher compared with Cu, suggesting that the impact of a given heavy metal on gene expression is not directly related to sequestering ability of the protein. Chaturvedi *et al*. (2012) also reported that *SbMT-2* may be induced in response to salinity, heat and drought but is down-regulated in response to cold stress.

Osmoprotectants

When exposed to high NaCl concentrations in the soil solution, halophytes encounter osmotic stress. To maintain water uptake, numerous organic compounds accumulate to reduce internal osmotic potential and thus maintain favourable water gradients (Flowers and Colmer, 2008). In addition to their impact on plant water status, these compounds also display protecting functions for cellular structures and enzymes. It is thus not surprising that heavy metals induce their synthesis, even in the absence of NaCl. Conversely, the ability of halophytes to quickly synthesize these protecting compounds in response to ion toxicity may, at least partly, explain their ability to cope with toxic heavy metals.

Proline is frequently reported to accumulate in cytosol, concomitantly with vacuolar Na^+ sequestration. Besides its osmotic function, it also protects quaternary structures of proteins and stabilizes the cell membrane through a direct interaction with phospholipids. Rastgoo and Alemzadeh (2011) reported that numerous distinct heavy metals (Cd, Co, Pb and Ag) increased the concentration of proline in *Aeluropus littoralis*. Shevyakova *et al*. (2003) considered that proline acts as an efficient ROS scavenger in the facultative halophyte *Mesembryanthemum crystallinum* exposed to Cd toxicity. In the Syrian beancaper (*Zygophyllum fabago*), proline but also soluble sugars accumulated as osmoprotectants, and the most Cd- and Zn-resistant plants presented a higher concentration

of soluble sugars than the sensitive plants (Lefèvre, 2007). Saiyood *et al*. (2012) estimated that a high level of resistance to a complex mixture of inorganic pollutants in *Suaeda maritima* was directly related to a high capacity to accumulate protecting proline and soluble sugars in the roots, stems and leaves. In *Atriplex halimus*, proline and soluble sugars also increased in leaves in response to Cd treatment (Lefèvre *et al*., 2009*b*). Other amino acids such as histidine, glutamine, alanine or asparagine appeared to be directly involved in metal tolerance through the formation of complexes (Sharma and Dietz, 2006). Cysteine is required for methionine and GSH/PC synthesis, and therefore is a central metabolite in antioxidant defence and metal sequestration. Liu *et al*. (2011) observed an increase in the branched-chain amino acids leucine, isoleucine and valine, and in glutamate, glutamine, tyrosine, arginine, phenylalanine and tryptophan in *Suaeda salsa* in response to Cd. However, it remained unclear whether this accumulation belonged to a strategy of metal stress tolerance or resulted from protein degradation. In the same species, Zn treatment induced an increase in numerous amino acids such as valine, isoleucine, leucine, threonine, asparagine and phenylalanine (Wu *et al*., 2013).

Heavy metals and NaCl may trigger synthesis of quaternary ammonium compounds such as glycinebetaine in different halophytic plant species. Accumulation of glycinebetaine maintains membrane integrity and protection of other cellular structures. It stabilizes macromolecule structure, and protects chloroplasts and photosystem II. It has long been known that halophytes have a superior ability to accumulate large amounts of these compounds (Ozgur *et al*., 2013; Bose *et al*., 2014). Lefèvre *et al*. (2009*b*) observed that Cd triggers glycinebetaine oversynthesis in leaves of *Atriplex halimus*. In *Kosteletzkya virginica*, NaCl and Cu have additional effects on synthesis of quaternary ammonium compounds (Han *et al*., 2012*b*). In this species, NaCl also delays Cd-induced senescence, as revealed by the maintenance of maximum quantum yield efficiency, effective quantum yield of photosystem II and electron transport rates, which appear as a direct consequence of glycinebetaine accumulation (Han *et al*., 2013*a*). The metallotolerant halophyte *Sesuvium portulacastrum* also accumulates *trans*-4-hydroxyprolinebetaine and 3,5,4-trihydroxy-6,7 dimethoxyflavoine 3-glucoside (Adrian-Romero *et al*., 1998). However, in *Suaeda salsa*, the concentration of glycinebetaine decreased in response to Cd treatment while an increase in choline was recorded (Liu *et al*., 2011). As betaine is synthesized from choline, the authors suggested that the elevated choline should be related to the depleted glycinebetaine.

Polyamines (PAs), small aliphatic amines assuming a myriad of biological functions during plant growth and development, are reported to be involved in plant response to most ion toxicities. They may act as protective molecules to repair or prevent stress-induced injuries through direct interaction with numerous cell structures and biochemical pathways, as recently reviewed by Lutts *et al*. (2013). In glycophytes, in response to ionic stresses, results have indicated that they can stabilize membranes or nucleic acids, act as free radical scavengers and/or as activators of antioxidant enzymes, can play a role in signalling through induction of nitric oxide (NO) and Ca^{2+} influx across the plasma membrane, and can regulate a variety of cation and K^+ channels in the context of ion homeostasis (Lutts *et al*., 2013; Pottosin *et al*., 2014; Pottosin and Shabala, 2014).

However, their beneficial role remains controversial as the catabolism of PAs generates H_2O_2 and other ROS in the apoplasm (Mohaptra *et al.*, 2009). PA signalling appears to be an essential component in salinity tolerance in halophytes (Bose *et al.*, 2014). However, studies looking at the response of PAs to heavy metals in halophytes are rare in the literature. In *Atriplex halimus*, the PAs putrescine, spermidine and spermine increased in response to Cd (Lefèvre *et al.*, 2009*b*) and a similar increase was noted in *Atriplex atacamensis* exposed to high doses of arsenate (Vromman *et al.*, 2011).

In response to Cd, *myo*-inositol-1-phosphate synthase (MIPS) transcripts are clearly up-regulated in leaves of *Suaeda salsa*. Given that MIPS is a pivotal enzyme that catalyses *myo*-inositol synthesis, metabolic products of which (such as *myo*-inositol hexakisphosphate) impact uptake and translocation of heavy metals, the metabolic pathway of *myo*-inositol and the detoxification function of its downstream products may be modified in response to Cd (Cong *et al.*, 2013). In *Mesembryanthemum crystallinum* exposed to Cu, heat shock protein 60 (HSP 60), which may participate in protein folding following exposure to denaturing conditions caused by stress, was recorded to increase ten-fold compared with controls (Thomas *et al.*, 1998).

THE USE OF HALOPHYTES IN PHYTOREMEDIATION

Heavy metal-contaminated soils are difficult to remediate with conventional strategies. Soil washing, vapour extraction and flushing cannot be applied on large surfaces for economic reasons. Similarly, the use of electrokinetics or thermal desorption is not justified on former mining areas and large industrial sites. Remediation techniques using different plant species have emerged over the last two decades. These phytotechnologies, collectively known as phytoremediation, are less invasive, less expensive, aesthetically pleasant and may contribute to restore soil structure and function. Phytoremediation is considered to cost 10–1000 times less than conventional civil engineering technologies (Barceló and Poschenrieder, 2003). It incorporates a range of phytotechnologies which differ according to the process by which plants remove, immobilize or degrade contaminants. Phytoextraction involves the use of pollutant-accumulating plants to remove metals from soil by concentrating them in the harvestable parts. Contaminated crop disposal options include pyrolysis, composting and compaction to reduce the volume of plant biomass, and incineration, ashing and liquid extraction as final disposal (Sas-Nowosiemska *et al.*, 2004). Phytodegradation mainly concerns organic pollutants and implies the use of plants and associated micro-organisms to degrade organic pollutants. Rhizofiltration is the use of plant roots to absorb and adsorb pollutants from water and contaminated waste streams. Removal of gaseous pollutants through the atmosphere is called phytovolatilization (Moreno *et al.*, 2005). Phytostabilization is a management strategy for stabilizing toxic contaminants: its purpose is to establish a vegetation cover that will reduce soil erosion, wind-blow of contaminated particles and water pollution by interception of incident precipitation. Vegetative stabilization improves the chemical and biological properties of the site through an increase in organic matter content, cation exchange capacity and biological activity.

As far as heavy metals are concerned, the original concept of phytoremediation preferentially focused on phytoextraction while phytostabilization received much less attention (Fig. 4). Successful phytoextraction requires soil cleaning to a level that complies with environmental regulations. Although numerous studies have investigated hyperaccumulating plant behaviour in laboratory conditions, field trials or commercial operations that demonstrate successful phytoextraction remain scarce (Conesa *et al.*, 2012). The most important parameter for efficient phytoextraction is the amount of pollutant removed by the plant per unit area. This parameter is maximized as a result of a good compromise between metal concentration and biomass production. Phytoextraction is expected to be a long-term process and suitable plants therefore must be perennials. Finally, heavy metal pollution is rarely limited to the upper soil layer and pollutants may also be present in deeper horizons of the profile: plants exhibiting a deep root system may thus be required for efficient phytoextraction. Moreover, the plants must be able to cope with polymetallic pollution, as this frequently occurs in polluted areas.

Selecting the plants suitable for restoration is one of the key factors in phytoremediation of heavy metal-contaminated sites. Biomass production is an important property for both phytoextraction and phytostabilization. Some perennial halophytes are able to produce up to 30 t d. wt ha^{-1} year^{-1} (Lutts *et al.*, 2004; Manousaki *et al.*, 2008). In salt-affected areas, halophytes may provide the only possibility for revegetation purposes. In a whole plant of *Tamaryx smyrnensis*, total Cd removal from soil increased from 9·4 µg in the absence of salt to 19·7 µg at 0·5 % NaCl (Manousaki *et al.*, 2008). Milić *et al.* (2012) showed that *Salicornia europaea*, *Suaeda maritima*, *Salsola soda* and *Halimonie portulacoides* accumulated high concentrations of several heavy metals in the above-ground organs and also that populations differ widely in bioaccumulation capacity. Hence, these authors assumed that selection can improve halophyte capacity for heavy metal removal through phytoextraction. In contrast, shoot accumulation of heavy metals is not suitable for phytostabilization because it constitutes a risk of pollutant accumulation at the surface through litter decomposition if plants are not harvested. Agoramoorthy *et al.* (2008) demonstrated that five halophytic species were efficient accumulators contributing to reduce soil and water contamination by heavy metals in mangrove ecosystems. Mazharia and Homaeeb (2012) reported that even an annual halophyte such as *Chenopodium botrys* may be included as a cleaning culture in agricultural schemes to remove heavy metals, and especially Cd, from area with low-level contamination: this plant may remove up to 180 g Cd ha^{-1}, which is six times more than Cd removal by the hyperaccumulator *Noccaea caerulescens*. A high level of Zn accumulation (335 mg Zn kg^{-1} shoot d. wt) was reported in *Leptochloa*, and Eisa and Eid (2011) showed that this species may efficiently contribute to reduce Zn concentration in moderately-contaminated soils. Positive results were also obtained for Ni with *Sporobolus virginicus* (34 mg Ni kg^{-1} shoot d. wt) and this species is also commonly considered as one of the only species able to accumulate Cu in aerial parts (Eid, 2011).

Halophytes may be of particular interest for stabilization of some mine tailings, which can be extremely saline with an

PHYTOSTABILIZATION　　　　　　　　　　　　　　　　　**PHYTOEXTRACTION**

Plant–AMF interactions

⬆ Root densities

⬆ Uptake of HM

⬆ Sequestration of HM in AMF cells

Chelation of HM in soil
through the release of
AMF exudates

Plant–AMF interactions

⬆ Root densities

⬆ Uptake of HM

⬆ Plant biomass
(growth-promoting substances)

⬆ HM translocation

⬆ HM concentration in leaves

Exudates from AMF
(i.e. glomalin)

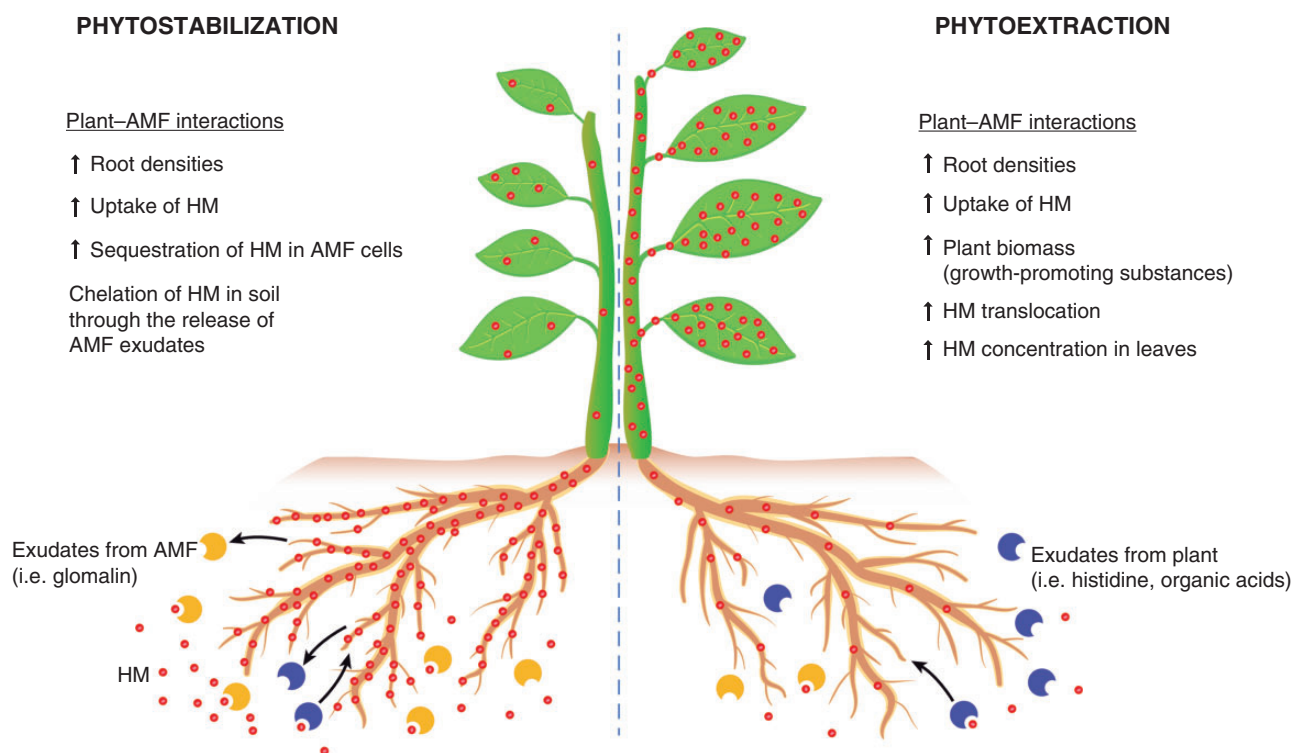Exudates from plant
(i.e. histidine, organic acids)

HM

Fig. 4. Halophytic plant species may be used for phytostabilization (left) or phytoextraction purposes (right) of heavy metals (HM). Phytostabilization contributes to reduce pollutant dispersion and may take advantage of exudates from arbuscular mycorrhizal fungi (AMF) reducing pollutant availability. Although heavy metal may be uptaken by the root system, translocation to the shoot part remains limited. By contrast, phytoextraction aims to extract pollutants from contaminated soil and to accumulate it in the aerial harvestable part of the plant. Root exudates increasing heavy metal bioavailability may thus help to accumulate pollutants within the plant tissues. In both cases, AMF may contribute to increase the process, but sequestration of pollutants in AMF is required to improve phytostabilization while the plant growth-promoting property of AMF is a major component of phytoextraction (Göhre and Paszkowski, 2006; Hildebrandt *et al*., 2007).

EC up to $22\,\mathrm{dS\,m^{-1}}$ (Mendez and Maier, 2008). In arid environments, *Atriplex lentiformis* (Torr.) S. Wats may cope with Pb/Zn mine tailings (Mendez *et al*., 2007) while *Atriplex canescens* is well adapted for phytoremediation of alkaline copper mine tailings (Sabey *et al*., 1990). Regarding phytostabilization of salt marsh environments, the use of species that are able to retain high amounts of metals in roots efficiently contributed to reduce their availability (Weis and Weis, 2004). In this way, monocotyledonous species such as *Triglochin maritima* and *Juncus maritimus* provide higher Hg stabilization than the dicotyledonous species *Sarcocornia perennis* and *Halimione portulacoides* (Castro *et al*., 2009).

Besides the optimal choice of suitable plant species, amendments may also be used to modify heavy metal bioavailability in contaminated soil. The nature of the amendments selected is a direct function of the final aim of the phytoremediation strategy. In the case of phytoextraction, bioavailability is often a limiting factor, especially for some elements such as Pb. Synthetic chelators (EDTA: ethylenediamine tetra-acetic acid; DTPA: diethylenetrinitrilopentaacetic acid; NTA: nitriloacetic acid; PDA: pyridine-2,6-dicarboxylic acid; EDDS: ethylenediamine disuccinate) have been tested to enhance heavy metal solubility, pollutant absorption and translocation by the plants. Data concerning such 'assisted-phytoextraction' using halophytic species remain scarce. Ben Rejeb *et al*. (2013) recently demonstrated that the addition of EDTA ($1\,\mathrm{mmol\,kg^{-1}}$ d. wt soil) to an artificially Cd-contaminated soil resulted in a

two-fold increase in Cd concentration in shoots of *Salsola kali* as a consequence of improved Cd absorption and translocation to the shoots. According to these authors, salinity had no impact on the efficiency of the chelating agent to increase the rate of phytoextraction, suggesting that NaCl had no influence on the formation of EDTA–Cd complexes. Lead usually presents a low solubility in soil solution and is poorly available for plant uptake. Zaier *et al*. (2014) showed that EDTA significantly increased shoot Pb^{2+} in the halophyte *Sesuvium portulacastrum*. Besides its impact on Pb bioavailability for root absorption, EDTA also promoted Pb translocation from roots to shoots and clearly increased TF values. Jordan *et al*. (2002) found that the halophyte *Atriplex nummularia* and the glycophyte *Zea mays* extracted similar amounts of heavy metal from a contaminated mine-tailing soil in the absence of EDTA; however, when EDTA was added to the contaminated substrate, the halophyte removed significantly more Cu and Pb than the glycophyte. The root metal concentration was nevertheless higher for the former than for the latter, suggesting that the chelator increased translocation of metal from root to shoot in the halophytic species.

Regarding phytostabilization, the final aim is to reduce metal mobility in the substrate. Amendments will then improve the nature of the rooting medium, facilitate plant establishment, supply plant nutrients in a slow-release form and reduce metal leaching. Numerous organic and inorganic materials have been used to reduce solubility and bioavailability of heavy metals,

including liming material, phosphate, zeolite, and aluminosilicates such as bentonite or fly ash (Kumpiene *et al.*, 2008). To the best of our knowledge, this aspect has not been specifically studied in halophytes and the impact of salt on their properties also remains poorly studied.

Heavy metal-contaminated sites are also often concomitantly contaminated by organic pollutants. Some halophytes such as *Spartina alterniflora* were recently demonstrated to increase bioremediation of oil-contaminated salt marsh (Tate *et al.*, 2012). Nitrogen fertilizer application was demonstrated to enhance biodegradation of crude oil by some halophytes (Jackson and Pardue, 1999) and the ability to identify halophytic plant species exhibiting resistance to heavy metals and organic pollutants is of great interest for further phytomanagement of soil polluted by a complex mixture of compounds. Some authors also suggest that halophytes may be used for 'phytodesaliniszation', although this seems to be realistic only for soil of low salinity. Studies with *Suaeda salsa* indicated that a density of 15 plants m^{-2} could remove more than $3500 \, kg \, Na^+ \, ha^{-1}$ if the whole shoot is harvested at the end of the growing season (Zhao, 1991). Similar positive results were observed for *Suaeda fruticosa* (Chaudhri *et al.*, 1964). Field trials, however, are still required to determine clearly if sodium and heavy metals may be removed simultaneously from a salt- and heavy metal-contaminated substrate. The use of a mix of several halophytes presenting complementary properties in terms of pollutant removal is another option that needs to be tested under field conditions.

CONCLUSIONS

Through their ability to survive under harsh conditions, halophytes have developed various mechanisms of adaptation to saline environments, but also to other constraints. Salinity often occurs concomitantly with other environmental factors, such as aridity, flooding and pollution. While some tolerance mechanisms are stress-specific, others may help the plant to cope with a wide range of abiotic constraints. Properties involved in saline or drought tolerance may thus indirectly contribute to heavy metal tolerance, as heavy metals induce ionic toxicity, secondary water stress and an oxidative burst. As shown in Fig. 5, efficient regulation of stomatal conductance, osmotic adjustment and high water-use efficiency may help in the maintenance of plant water status. Stress-induced senescence is delayed in halophytes compared with glycophytes, and efficient management of oxidative stress helps to maintain integrity of the cellular structure. High trichome density and selective accumulation of toxic ions in older leaves may help halophytes to maintain cellular homeostasis and metabolic functions in photosynthetically active leaves. Not all these properties are necessarily present in
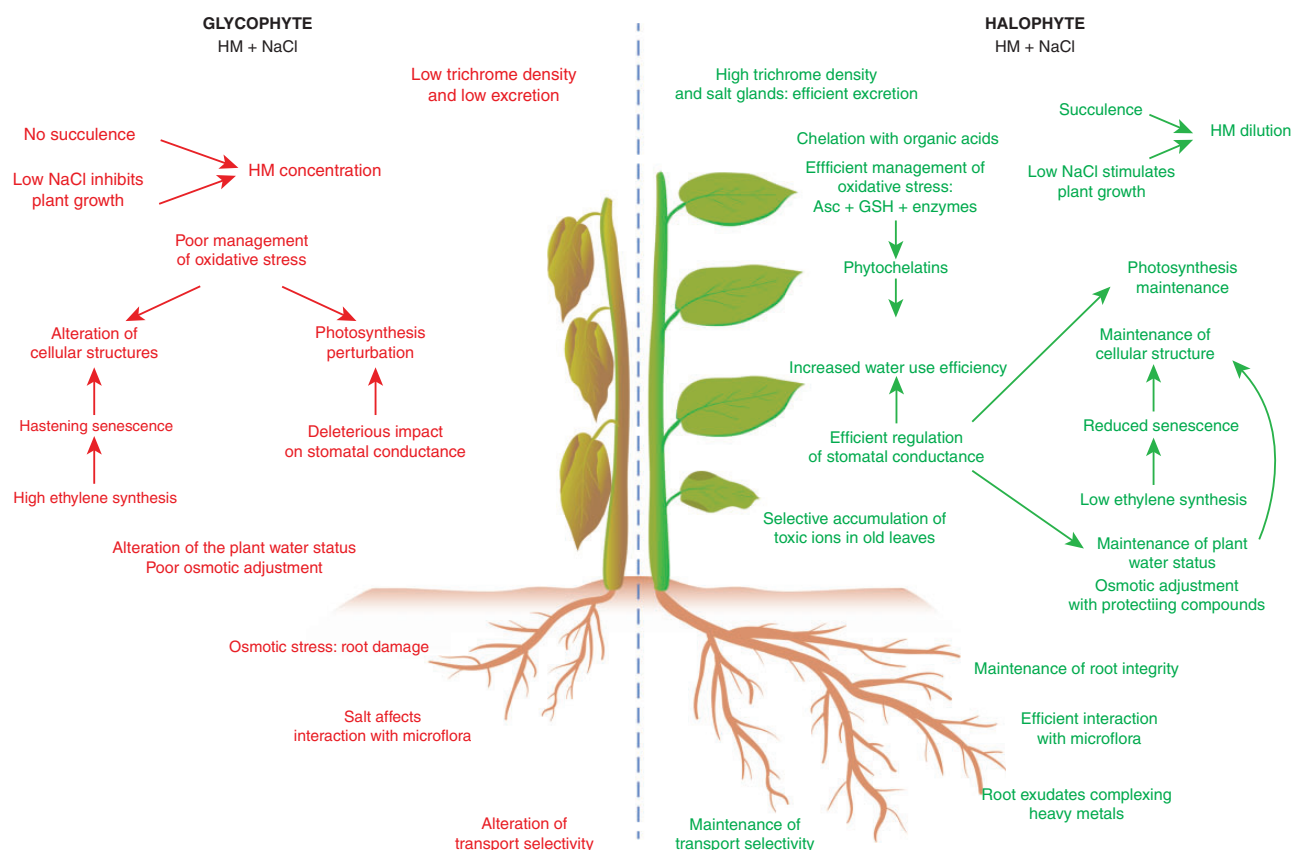

FIG. 5. Synoptic view of properties allowing halophytic plant species to cope with heavy metal toxicity (HM) (right) compared with glycophytes (left). Halophytes may accumulate toxic ions in older leaves, in trichomes and salt glands. They display efficient regulation of stomatal conductance and perform osmotic adjustment, helping to maintain the plant water status. Appropriate regulation of oxidative stress is performed through over-synthesis of endogenous antioxidants [ascorbate (Asc) and glutathione (GSH)] and activation of specific enzymes (SOD, ascorbate peroxidase, catalase, glutathione reductase and dehydroascorbate reductase). Low levels of ethylene synthesis may help to delay stress-induced senescence.

a given plant material but their occurrence may help some halophytes to cope with salinity, heavy metals and their combination. Given the large amount of biomass produced by some halophytic plant species, their deep-rooting system and their tolerance to abiotic constraints, perennial halophytes may be useful candidates for phytoremediation of heavy metals in marginal lands. Depending on the properties of the particular species, some halophytes may be of special interest for phytextraction or phytostabilization purposes.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of Table S1: list of halophyte species involved in heavy metal studies.

ACKNOWLEDGEMENTS

LITERATURE CITED

Acosta JA, Jansen B, Kalbitz K, Faz A, Martínez-Martínez S. 2011. Salinity increases mobility of heavy metals in soils. *Chemosphere* **85**: 1318–1324.

Adrian-Romero MS, Wilson J, Blunden G, Yang MH, Carabot-Cuervo A, Bashir AK. 1998. Betaines in coastal plants. *Biochemical Systematics and Ecology* **26**: 535–543.

Agoramoorthy G, Chen FA, Hsu MJ. 2008. Threat of heavy metal pollution in halophytic and mangrove plants of Tamil Nadu, India. *Environmental Pollution* **155**: 320–326.

Agarie S, Shimoda T, Shimizu Y, *et al.* 2007. Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *Journal of Experimental Botany* **58**: 1957–1967.

Badi HN, Sorooshzadeh A. 2011. Evaluating potential of borage (*Borago officinalis* L.) in bioremediation of soil. *African Journal of Biotechnology* **10**: 146–153.

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* **1**: 81–126.

Barceló J, Poschenrieder C. 1990. Plant water relations as affected by heavy metal stress: a review. *Journal of Plant Nutrition* **13**: 1–37.

Barceló J, Poschenrieder C. 2003. Phytoremediation: principles and perspectives. *Contribution to Science* **2**: 333–344.

Barceló J, Vásquez MD, Poschenrieder C. 1988. Cadmium-induced structural and ultrastructural changes in the vascular system of bush bean stems. *Botanica Acta* **101**: 254–261.

Batty LC, Dolan C. 2013. The potential use of phytoremediation for sites with mixed organic and inorganic contamination. *Critical Reviews in Environmental Science and Technology* **43**: 217–259.

Ben Rejeb K, Ghanya T, Zaier H, *et al.* 2013. Evaluation of the Cd^{2+} phytoextraction potential in the xerohalophyte *Salsola kali* L. and the impact of EDTA on this process. *Ecological Engineering* **60**: 309–315.

Bertin C, Yang X, Weston LA. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* **256**: 67–83.

Bingham FT, Strong JE, Sposito G. 1983. Influence of chloride salinity on cadmium uptake by Swiss chard. *Soil Science* **135**: 160–165.

Bingham FT, Sposito G, Strong JE. 1984. The effect of chloride on the availability of cadmium. *Journal of Environmental Quality* **13**: 71–74.

Bingham FT, Sposito G, Strong JE. 1986. The effect of sulfate on the availability of cadmium. *Soil Science* **141**: 172–177.

Bose J, Rodrigo-Moreno A, Shabala S. 2014. ROS homeostasis in halophytes in the context of salinity tolerance. *Journal of Experimental Botany* **65**: 1241–1257.

Boularbah A, Morel JL, Bitton G, Mench M. 1996. A direct solid-phase assay specific for heavy-metal toxicity. II. Assessment of heavy-metal immobilization in soils and bioavailability to plants. *Soil and Sediment Contamination* **5**: 395–404.

Briat JF, Lobréaux S. 1997. Iron transport and storage in plants. *Trends in Plant Science* **2**: 187–193.

Burke DJ, Weis JS, Weis P. 2000. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuarine, Coastal and Shelf Science* **51**: 153–159.

Caçador I, Vale C, Catarino F. 2000. Seasonal variation of Zn, Pb, Cu and Cd concentrations in the root-sediment system of *Spartina maritima* and *Halimione portulacoides* from Tagus estuary salt marshes. *Marine Environmental Research* **49**: 279–290.

Caçador I, Caetano M, Duarte B, Vale C. 2009. Stock and losses of trace metals from salt marsh plants. *Marine Environmental Research* **67**: 75–82.

Caetano M, Vale C, Cesario R, Fonseca N. 2008. Evidence for preferential depths of metal retention in roots of salt marsh plants. *Science of the Total Environment* **390**: 466–474.

Cambrollé J, Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME. 2008. Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment. *Marine Pollution Bulletin* **56**: 2037–2042.

Cambrollé J, Mancilla-Leytón JM, Muñoz-Vallés S, Luque T, Figueroa ME. 2012. Zinc tolerance and accumulation in the salt-marsh shrub *Halimione portulacoides*. *Chemosphere* **86**: 867–874.

Cambrollé J, Mateos-Naranjo E, Redondo-Gómez S, Luque T, Figueroa ME. 2011. The role of two *Spartina* species in phytostabilization and bioaccumulation of Co, Cr, and Ni in the Tinto-Odiel estuary (SW Spain). *Hydrobiologia* **671**, 95–103.

Caravaca F, Del Mar Alguacil M, Torres P, Roldán A. 2005. Microbial activities and arbuscular mycorrhizal fungi colonization in the rhizosphere of the salt marsh plant *Inula crithmoides* I. Along a spatial salinity gradient. *Wetlands* **25**: 350–355.

Carbonell-Barrachina MA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick WH Jr. 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. *Plant and Soil* **198**: 33–43.

Carrasco L, Caravaca F, Alvarez-Rogel J, Roldán A. 2006. Microbial processes in the rhizosphere soil of a heavy metals-contaminated Mediterranean salt marsh: a facilitating role of AM fungi. *Chemosphere* **64**: 104–111.

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.

Carvalho SM, Caçador I, Martins-Loução MA. 2006. Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. *Plant and Soil* **285**: 161–169.

Castro R, Pereira S, Lima A, *et al.* 2009. Accumulation, distribution and cellular partitionning of mercury in several halophytes of a contaminated salt marsh. *Chemosphere* **76**: 1348–1355.

Chaignon V, Di Malta D, Hinsinger P. 2002. Fe-deficiency increases Cu acquisition by wheat cropped in a Cu-contaminated vineyard soil. *New Phytologist* **154**: 121–130.

Chaturvedi AK, Mishra A, Tiwari V, Jha B. 2012. Cloning and transcript analysis of type 2 metallothionein gene (*SbMT-2*) from extreme halophyte *Salicornia brachiata* and its heterologous expression in *E. coli*. *Gene* **499**: 280–287.

Chaudhri II, Shah BH, Naqvi N, Mallick IA. 1964. Investigations on the role of *Suaeda fructicosa* Forsk. in the reclamation of saline and alkaline soils in west Pakistanian plains. *Plant and Soil* **21**: 1–7.

Choi YE, Harada E, Kim GH, Yoon ES, Sano H. 2004. Distribution of elements on tobacco trichomes and leaves under cadmium and sodium stresses. *Journal of Plant Biology* **47**: 75–82.

Choi YE, Harada E, Wada M, *et al.* 2001. Detoxification of cadmium in tobacco plants: formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta* **213**: 45–50.

Cobbett C. 2003. Heavy metals and plants – model systems and hyperaccumulators. *New Phytologist* **159**: 289–293.

Cobbett C, Goldsbrough PB. 2002. Phytochelatins and metallothionein: roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology* **53**: 159–182.

Colmer TD, Flowers TJ. 2008. Flooding tolerance in halophytes. *New Phytologist* **179**: 964–974.

Conesa HM, Evangelou MWH, Robinson BH, Schulin R. 2012. A critrical review of current state of phytotechnologies to remediate soils: still a promising tool? *Scientific World Journal* **2012**.

Cong M, Lv J, Liu X, Zhao J, Wu H. 2013. Gene expression responses in *Suaeda salsa* after cadmium exposure. *SpringerPlus* **2**: 232.

D'Amore JJ, Al-Abed SR, Schekel KG, Ryan JA. 2005. Methods for speciation of metals in soils: a review. *Journal of Environmental Quality* **34**: 1707–1745.

Degryse F, Smolders E, Merckx R. 2006. Labile Cd complexes increase Cd availability to plants. *Environmental Science and Technology* **40**: 830–836.

de la Rosa G, Peralta-Videa JR, Montes M, Parsons JG, Cano-Aguilera I, Gardea-Torresdey JL. 2004. Cadmium uptake and translocation in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species: ICP/OES and XAS studies. *Chemosphere* **55**: 1159–1168.

Demidchik V. 2014. Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Journal of Experimental Botany* **109**: 212–228.

Demidchik V, Cuin TA, Svistunenko D, et al. 2010. *Arabidopsis* root K^+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. *Journal of Cell Science* **123**: 1468–1479.

De Vos AC, Broekman R, de Almeida Guerra CC, van Rijsselberghe M, Rozema J. 2013. Developing and testing new halophyte crops: A case study of salt tolerance of two species of the Brassicaceae, *Diplotaxis tenuifolia* and *Cochlearia officinalis*. *Environmental and Experimental Botany* **92**: 154–164.

Doyle MO, Otte ML. 1997. Organism-induced accumulation of iron, zinc and arsenic in wetland soils. *Environmental Pollution* **96**: 1–11.

Drazkiewicz M, Baszyñki T. 2005. Growth parameters and photosynthetic pigments in leaf segments of *Zea mays* exposed to cadmium, as related to protection mechanisms. *Journal of Plant Physiology* **162**: 1013–1021.

Duarte B, Delgado M, Caçador I. 2007. The role of citric acid in cadmium and nickel uptake and translocation in *Halimonie portulacoides*. *Chemosphere* **69**: 836–840.

Duarte B, Reboreda R, Caçador I. 2008. Seasonal variation of extracellular enzymatic activity (EEA) and its influence on metal speciation in a polluted salt marsh. *Chemosphere* **73**: 1056–1063.

Eid MA. 2011. Halophytic plants for phytoremediation of heavy metals contaminated soil. *Journal of American Science* **7**: 377–382.

Eisa SS, Eid MA. 2011. Assessment of the phytoextraction potential of some fast growing halophytes and maize plants. *Australian Journal of Basic and Applied Science* **5**: 88–95.

Ernst WHO, Nelissen HJM. 2000. Life-cycle phases of a zinc- and cadmium-resistant ecotype of *Silene vulgaris* in risk assessment of polymetallic mine soils. *Environmental Pollution* **107**: 329–338.

European Environment Agency. 2007. Progress in management of contaminated sites (CSI015). *Copenhagen*: EEA.

Flowers TJ, Colmer TD. 2008. Salinity tolerance in halophytes. *New Phytologist* **179**: 945–96.

Flowers TJ, Galal HK, Bromham L. 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology* **37**: 604–612.

Fox DI, Pichler T, Yeh DH, Alcantar NA. 2012. Removing heavy metal in water: the interactions of cactus mucilage and arsenate (As(V)). *Environmental Science and Technology* **46**: 4553–4559.

Garcia-Miragaya J, Page AL. 1976. Influence of ionic strength and inorganic complex formation on the sorption of trace amounts of Cd by Montmorillonite. *Soil Science Society of America Journal* **40**: 658–663.

Ghanem ME, Han RM, Classen B, et al. 2010. Mucilage and polysaccharides in the halophyte plant species *Kosteletzkya virginica*: localization and composition in relation to salt stress. *Journal of Plant Physiology* **167**: 382–392.

Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel MH, Abdelly C. 2007a. Effects of Cd^{2+} on K^+, Ca^{2+} and N uptake in tow halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: consequences on growth. *Chemosphere* **67**: 72–79.

Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel MH, Abdelly C. 2007b. Cd-induced growth reduction in the halophyte *Sesuvium portulacastrum* is significantly improved by NaCl. *Journal of Plant Research* **120**: 309–316.

Ghnaya T, Nouairi I, Slama I, et al. 2005. Cadmium effects on growth and mineral nutrition of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*. *Journal of Plant Physiology* **162**: 1133–1140.

Ghnaya T, Zaier H, Baioui R, et al. 2013. Implications of organic acids in the long-distance transport and accumulation of lead in *Sesuvium portulacastrum* and *Brassica juncea*. *Chemosphere* **90**: 1449–1454.

Ghelis T, Dellis O, Jeannette E, Bardat F, Miginiac E, Sotta B. 2000. Abscisic acid plasmalemma perception triggers a calcium influx essential for RAB18 gene expression in *Arabidopsis thaliana* suspension cells. *FEBS Letters* **483**: 67–70.

Girault L, Boudou A, Dufourc EJ. 1998. ^{113}Cd-, ^{31}P-NMR and fluorescence polarization studies of cadmium(II) interactions with phospholipids in model membranes. *Biochimica et Biophysica Acta* **1414**: 140–154.

Gorai M, El Aloui W, Yang X, Neffati M. 2014. Towards understanding the ecological role of mucilage in seed germination of a desert shrub *Henophyton deserti*: interactive effects of temperature, salinity and osmotic stress. *Plant and Soil* **374**: 727–738.

Greger M, Ögren E. 1991. Direct and indirect effects of Cd^{2+} on photosynthesis in sugar beet (*Beta vulgaris*). *Physiologia Plantarum* **83**: 129–135.

Gonzalez-Mendoza D, Moreno AQ, Zapata-Perez O. 2007. Coordinated responses of phytochelatin synthase and metallothionein genes in black mangrove, *Avicennia germinans*, exposed to cadmium and copper. *Aquatic Toxicology* **83**: 306–314.

Göhre V, Paszkowski U. 2006. Contribution of the abuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* **223**: 1115–1122.

Guo Y, Bundithya W, Goldsbrough PB. 2003. Characterization of the metallothionein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytologist* **159**: 369–381.

Gutknecht J. 1981. Inorganic mercury (Hg^{2+}) transport through lipid bilayer membrane. *Journal of Membrane Biology* **61**: 61–66.

Han RM, Lefèvre I, Ruan CJ, Qin P, Lutts S. 2012a. NaCl differently interferes with Cd and Zn toxicities in the wetland halophyte species *Kosteletzkya virginica* (L.) Presl. *Plant Growth Regulation* **68**: 97–109.

Han RM, Lefèvre I, Ruan CJ, Beukelaers N, Qin P, Lutts S. 2012b. Effects of salinity on the response of the wetland halophyte *Kosteletzkya virginica* (L.) Presl. to copper toxicity. *Water, Air, and Soil Pollution* **223**: 1137–1150.

Han RM, Lefèvre I, Albacete A, et al. 2013a. Antioxidant enzyme activities and hormonal status in response to Cd stress in wetland halophyte *Kosteletzkya virginica* under saline conditions. *Physiologia Plantarum* **147**: 352–368.

Han RM, Quinet M, André E, et al. 2013b. Accumulation and distribution of Zn in the shoots and reproductive structures of the halophyte plant species *Kosteletzkya virginica* as a function of salinity. *Planta* **238**: 441–457.

Hatje V, Payne TE, Hill DM, McOrist G, Birch GF, Szymczak R. 2003. Kinetics of trace element uptake and release by particles in estuarine waters: effects of pH, salinity, and particle loading. *Environment International* **29**: 619–629.

Helal HM, Upenov A, Issa GJ. 1999. Growth and uptajke of Cd and Zn by *Leucanea leucocephala* in reclaimed soils as affected by NaCl salinity. *Journal of Plant Nutrition and Soil Science* **162**: 589–592.

Hempel M, Botté SE, Negrin VL, Chiarello MN, Marcovecchio JE. 2008. The role of the smooth cordgrass *Spartina alterniflora* and associated sediments in the heavy metal biogeochemical cycle within Bahía Blanca estuary salt marshes. *Journal of Soils and Sediments* **8**: 289–297.

Hernandez-Soriano MC, Degryse F, Lombi E, Smolders E. 2012. Mangenese toxicity in barley is controlled by solution manganese and soil manganese speciation. *Soil Science Society of America Journal* **76**: 399–407.

Hildebrandt U, Regvar M, Bothe H. 2007. Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* **68**: 139–146.

Huang GY, Wang YS. 2009. Expression analysis of type 2 metallothionein gene in mangrove species (*Bruguiera gymnorrhiza*) under heavy metal stress. *Chemosphere* **77**: 1026–1029.

Huang GY, Wang YS. 2010. Expression and characterization analysis of type 2 metallothiobnein from grey mangrove species (*Avicennia marina*) in response to metal stress. *Aquatic Toxicology* **99**: 86–92.

Jackson WA, Pardue JH. 1999. Potential for enhancement of biodegradation of crude oil in Louisiana salt marshes using nutrient amendments. *Water Air and Soil Pollution* **109**: 343–355.

Javed MT, Stoltz E, Lindberg S. Greger M. 2013. Changes in pH and organic acids in mucilage of *Eriophorum augustifolium* roots after exposure to

elevated concentrations of toxic elements. *Environmental Science and Pollution Research* **20**: 1876–1880.

Jithesh MN, Prashanth SR, Sivaprakash KR, Parida AK. 2006a. Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative, light and hyperosmotic stresses in the highly salt tolerant grey mangrove, *Avicennia marina* (Forsk.) Vierh. By mRNA analysis. *Plant Cell Report* **25**: 865–876.

Jithesh MN, Prashanth SR, Sivaprakash KR, Parida AK. 2006b. Antioxidative response mechnisms in halophytes: their role in stress defence. *Journal of Genetics* **85**: 237–254.

Jordan FL, Robin-Abbott M, Maier RM, Glenn EP. 2002. A comparison of chelator-facilitated metal uptake by a halophyte and a glycophyte. *Environmental Toxicology and Chemistry* **21**: 2698–2704.

Kadukova J, Kalogerakis N. 2007. Lead accumulation from non-saline and saline environment by *Tamarix smyrnensis* Bunge. *European Journal of Soil Biology* **43**: 216–223.

Katschnig D, Broekman R, Rozema J. 2013. Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology. *Environmental and Experimental Botany* **92**: 32–42.

Kiyono M, Oka Y, Sone Y, et al. 2012. Expression of the bacterial heavy metal transporter MerC fused with a plant SNARE, SYP121, in *Arabidopsis thaliana* increases cadmium accumulation and tolerance. *Planta* **235**: 841–850.

Koren S̆, Arčon I, Kump P, Nečemer M, Vogel-Mikuš K. 2013. Influence of CdCl$_2$ and CdSO$_4$ supplementation on Cd distribution and ligand environment in leaves of the Cd hyperaccumulator *Noccaea* (*Thlaspi*) *praecox*. *Plant and Soil* **370**: 125–148.

Kumpiene J, Lagerkvist A, Maurice C. 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. *Waste Management* **28**: 215–225.

Laffont-Schwob I, d'Enjoy-Weinkammerer G, Pricop A, Prudent P, Masotti V, Rabier J. 2011. Evalutation of a potential candidate for heavy metal phytostabilization in polluted sites of the Mediterranean littoral (SE Marseille): endomycorrhizal status, fitness biomarkers and metal content of *Atriplex halimus* spontaneous populations. *Ecological Questions* **14**: 89–90.

Lakshmanraj L, Gurusamy A, Gobinath MB, Chandramohan R. 2009. Studies on biosorption of hexavalent chromium from aqueous solutions by using boiled mucilaginous seeds of *Ocimum americanum*. *Journal of Hazardous Materials* **169**: 1141–1145.

Lefèvre I. 2007. *Investigation of three Mediterranean plant species suspected to accumulate and tolerate high cadmium and zinc levels*. PhD Thesis, Université catholique de Louvain, Louvain-la-Neuve, Belgium.

Lefèvre I, Correal E, Lutts S. 2005. Cadmium tolerance and accumulation in the noxious weed *Zygophyllum fabago*. *Canadian Journal of Botany* **83**: 1655–1662.

Lefèvre I, Correal E, Faz-Cano Á, Zanuzzi A, Lutts S. 2009a. Structural development, water status, pigment concentrations, and oxidative stress of *Zygophyllum fabago* seedlings in relation to cadmium distribution in the shoot organs. *International Journal of Plant Sciences* **170**: 226–236.

Lefèvre I, Marchal G, Meerts P, Corréal E, Lutts S. 2009b. Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. *Environmental and Experimental Botany* **65**: 142–152.

Lefèvre I, Correal E, Lutts S. 2010a. Impact of cadmium and zinc on growth and water status of *Zygophyllum fabago* in two contrasting metallicolous populations from SE Spain: comparison at the whole plant and tissue level. *Plant Biology* **12**: 883–894.

Lefèvre I, Marchal G, Ghanem ME, Correal E, Lutts S. 2010b. Cadmium has contrasting effects on polyethylene glycol – sensitive and resistant cell lines in the Mediterranean halophyte species *Atriplex halimus*. *Journal of Plant Physiology* **167**: 365–374.

Lefèvre I, Vogel-Mikuš K, Jeromel L, et al. 2014. Differential cadmium and zinc distribution in relation to their physiological impact in the leaves of the accumulating *Zygophyllum fabago* L. *Plant Cell Environment* **37**: 1299–1320.

Li YM, Chaney RL, Schneiter AA. 1994. Effect of soil chloride level on cadmium concentration in sunflower kernels. *Plant and Soil* **167**: 275–280.

Li L, Liu X, Peijnenburg WJGM, et al. 2012. Pathways of cadmium fluxes in the root of the halophyte *Suaeda salsa*. *Ecotoxicology and Environmental Safety* **75**: 1–7.

Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH. 2003. Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. *Chemosphere* **50**: 847–853.

Liu X, Yang C, Zhang L, et al. 2011. Metabolic profiling of cadmium-induced effects in one pioneer intertidal halophyte *Suaeda salsa* by NMR-based metabolomics. *Ecotoxicology* **20**: 1422–1431.

Lokhande VH, Gor BK, Desai NS, Nikam TD, Suprasanna P. 2013. *Sesuvium portulacastrum*, a plant for drought, salt stress, and fixation, food and phytoremediation. A review. *Agronomy and Sustainable Development* **33**: 329–348.

Lokhande VH, Srivastava S, Patade VY, et al. 2011. Investigation of arsenic accumulation and tolerance in *Sesuvium portulacastrum* (L.) L. *Chemosphere* **82**: 529–534.

Lombi E, Wenzel WW, Gobran GR, Adriano DC. 2001. Dependency of phytoavailability of metals on indigenous and induced rhizosphere processes: a review. In: GR Gobran, WW Wenzel, E Lombi, eds. *Trace elements in the rhizosphere*. Boca Raton, FL: CRC Press, 3–24.

Lutts S, Hausman JF, Quinet M, Lefèvre I. 2013. Polyamines and their roles in the alleviation of ion toxicities in plants. In: P Ahmad, MM Azooz, MNV Prasad, eds. *Ecophysiology and responses of plants under salt stress*. New York: Springer, Science + business media, 315–353.

Lutts S, Lefèvre I, Delpérée C, et al. 2004. Heavy metal accumulation by the halophyte species Mediterranean saltbush. *Journal of Environmental Quality* **33**: 1271–1279.

Mahon S, Carman KR 2008. The influence of salinity on the uptake, distribution, and excretion of metals by the smooth cordgrass, *Spartina alterniflora* (Loisel.), grown in sediment contaminated by multiple metals. *Estuaries and Costs* **31**: 1089–1097.

Manousaki E, Kalogerakis N. 2009. Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. *Environmental Science Pollution Research* **16**: 844–854.

Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N. 2008. Phytoextraction and phytoexcretion of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non-saline and saline soils. *Environmental Research* **106**: 326–332.

Marschner H. 1995. *Mineral nutrition of higher planrts*. San Diego: Academic Press.

Mateos-Naranjo E, Redondo-Gómez S, Cambrollé J, Figueroa ME. 2008a. Growth and photosynthetic responses to copper stress of an invasive cordgrass, *Spartina densiflora*. *Marine Environmental Research* **66**: 459–465.

Mateos-Naranjo E, Redondo-Gómez S, Cambrollé J, Luque T, Figueroa ME. 2008b. Growth and photosynthetic responses to zinc stress o fan invasive cordgrass, *Spartina densiflora*. *Plant Biology* **10**: 754–762.

Mazharia M, Homaeed M. 2012. Annual halophyte *Chenopidium botrys* can phytoextract cadmium from contaminated soils. *Journal of Basic and Applied Science Research* **2**: 1415–1422.

McFarlane GR, Burchett M. 1999. Zinc distribution and excretion in leaves of the grey mangrove *Avicennia marina* (Forsk.) Vierh. *Environmental and Experimental Botany* **41**: 167–175.

McFarlane GR, Burchett M. 2000. Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquatic Botany* **68**: 45–59.

McLaughlin MJ, Tiller KG, Beech T, Smart MK. 1994. Soil salinity causes elevated cadmium concentration in field-grown potato tubers. *Journal of Environmental Quality* **23**: 1013–1018.

Mendez MO, Maier RM. 2008. Phytoremediation of mine tailings in temperate and arid environments. *Review of Environmental Science and Biotechnology* **7**: 47–59.

Mendez MO, Glenn EP, Maier RM. 2007. Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. *Journal of Environmental Quality* **36**: 245–253.

Migeon A, Blaudez D, Wilkins O, et al. 2010. Genome-wide analysis of plant metal transporters, with an emphasis on poplar. *Cellular and Molecular Life Science* **67**: 3763–3784.

Migocka M, Papierniak A, Kosatka E, Klobus G. 2011. Comparative study of the active cadmium efflux systems operating at the plasma membrane and tonoplast of cucumber root cells. *Journal of Experimental Botany* **62**: 4903–49012.

Milić D, Luković J, Ninkov J, et al. 2012. Heavy metal content in halophytic plants from inland and maritime saline areas. *Central European Journal of Biology* **7**: 307–317.

Miyasaka SC, Hawes MC. 2001. Possible role of root border cells in detection and avoidance of aluminium toxicity. *Plant Physiology* **125**: 1978–1987.

Mohaptra S, Minocha R, Long S, Minocha SC. 2009. Putrescine overproduction negatively impacts the oxidative state of poplar cells in culture. *Plant Physiology and Biochemistry* **47**: 262–271.

Morel JL, Mench M, Guckert A. 1986. Measurements of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates from maize (*Zea mays* L.) roots. *Biology and Fertility of Soils* **2**: 29–34.

Moreno FN, Anderson CWN, Sytewart RB, Robinson BH. 2005. Mercury volatilization and phytoextraction from base-metal mine tailings. *Environmental Pollution* **136**: 341–352.

Mucha AP, Almeida CM, Bordalo AA, Vasconcelos MT. 2005. Exudation of organic acids by a marsh plant and implication on trace metal availability in the rhizosphere of estuarine sediments. *Estuarine and Coastal Shelf Science* **65**: 191–198.

Mühlingh KH, Läuchli A. 2003. Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance. *Plant and Soil* **253**: 219–231.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**: 651–681.

Nagata T, Morita H, Akizawa T, Pan-Hou H. 2010. Development of a transgenic tobacco plant for phytoremediation of methylmercury pollution. *Applied Microbiology Biotechnology* **87**: 781–786.

Nečemer M, Kump P, Ščančar J, et al. 2008. Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies. *Spectrochimica Acta – Part B Atomic Spectroscopy* **63**: 1240–1247.

Oomen RJ, Wu J, Lelievre F, et al. 2009. Functional characterization of NRAMP3 and NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **181**: 637–650.

Otero XL, Macias F. 2002. Variation with depth and season in metal sulfide in salt marsh soils. *Biogeochemistry* **61**: 247–268.

Ozgur R, Uzilday B, Sekmen AH, Turkan I. 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. *Functional Plant Biology* **40**: 832–847.

Ozkutlu F, Ozturk L, Erdem H, McLaughlin M, Cakmak I. 2007. Leaf-applied sodium chloride promotes cadmium accumulation in durum wheat grain. *Plant and Soil* **290**: 323–331.

Pan X, Yang J, Zhang D, Mu S. 2012. Lead complexation of root exudates of salt marsh plant *Salicornia europea* L. *Chemical Speciation and Bioavailability* **24**: 60–63.

Pan X, Yang J, Zhang D, Chen X, Mu S. 2011. Cu(II) complexation of high molecular weight (HMW) fluorescent substances in root exudates from a wetland halophyte (*Salicornia europaea* L.). *Journal of Bioscience and Bioengineering* **111**: 193–197.

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C. 2002. Heavy metal toxicity: cadmium penetrates through calcium channels and disturbs the plant water status. *Plant Journal* **32**: 539–548.

Petraglia A, De Benedictis M, Degoila F, et al. 2014. The capability to synthesize phytochelatins and the presence of constitutive and functional phytochelatin synthases are ancestral (plesiomorphic) characters for basal land plants. *Journal of Experimental Botany* **65**: 1153–1163.

Pilon-Smits EA, Zhu YL, Sears T, Terry N. 2000. Overexpression of glutathione reductase in *Brassica juncea*: effects on cadmium accumulation and tolerance. *Physiologia Plantarum* **110**: 455–460.

Pongrac P, Vogel-Mikuš K, Vavpetič P, et al. 2010. Cd induced redistribution of elements within leaves of the Cd/Zn hyperaccumulator *Thlaspi praecox* as revealed by micro-PIXE. *Nuclear Instrument and Methods in Physics Research B* **268**: 2205–2210.

Pongrac P, Vogel-Mikuš K, Regvar M, et al. 2013. On the distribution and evaluation of Na, Mg and Cl in leaves of selected halophytes. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* **306**: 144–149.

Poschenrieder C, Tolrà R, Barcelo J. 2006. Can meal defend plants against biotic stress? *Trends in Plant Science* **11**: 288–295.

Pottosin I, Shabala S. 2014. Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. *Frontiers in Plant Science* **5**: 154.

Pottosin I, Velarde-Buendia AM, Bose J, Fuglsang AT, Shabala S. 2014. Polyamines cause plasma membrane depolarization, activate Ca^{2+}-, and modulate H^+-ATPase pump activity in pea roots. *Journal of Experimental Botany* **65**: 2463–2472.

Rabier J, Laffont-Schwob P, Pricop A, et al. 2014. Heavy metal and arsenic resistance of the halophyte *Atriplex halimus* L. along a gradient of contamination in a French Mediterranean spray zone. *Water, Air and Soil Pollution* **225**: Article 1993.

Rastgoo L, Alemzadeh A. 2011. Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metal stress. *Australian Journal of Crop Science* **5**: 375–383.

Reboreda R, Caçador I. 2007. Halophyte vegetation influences in salt marsh retention capacity for heavy metals. *Environmental Pollution* **146**: 147–154.

Reboreda R, Caçador I. 2008. Enzymatic activity in the rhizosphere of *Spartina maritima*: potential contribution for phytoremediation of metals. *Marine Environmental Research* **65**: 77–84.

Reboredo. 2012. Zinc compartmentation in *Halimione portulacoides* (L.) Aellen and some effects on leaf ultrastructure. *Environmental Science and Pollution Research* **19**: 2644–2657.

Redondo-Gómez S, Mateos-Naranjo E, Andrades-Moreno L. 2010. Accumulation and tolerance charcateristics of cadmium in a halophytic Cd-hyperaccumulator *Arthrocnemum macrostachyum*. *Journal of Hazardous Materials* **184**: 299–307.

Redondo-Gómez S, Andrades-Moreno L, Mateos-Naranjo E, Parra R, Valera-Burgos J, Aroca R. 2011. Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass, *Spartina densiflora*. *Journal of Experimental Botany* **62**, 5521–5530

Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, et al. 2006. Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation *in vivo*. *Plant Cell and Environment* **29**: 1532–1544.

Rodrigo-Moreno A, Andrés-Colás N, Poschenrieder C, Gunsé B, Peñarrubia L, Shabala S. 2013a. Calcium- and potassium-permeable plasma membrane transporters are activated by copper in Arabidopsis root tips: linking copper transport with cytosolic hydroxyl radical production. *Plant, Cell and Environment* **36**: 844–855.

Rodrigo-Moreno A, Poschenrieder C, Shabala S. 2013b. Transition metal: a double edge sward in ROS generation and signaling. *Plant Signaling & Behaviour* **8**: e23425.

Rozema J, Schat H. 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany* **92**: 83–95.

Ruan CJ, Teixeira da Silva J, Mopper S, Qin P, Lutts S. 2010. Halophyte improvement for a salinized world. *Critical Review in Plant Sciences* **29**: 329–359.

Sabey BR, Pendleton RL, Webb BL. 1990. Effect of municipal sewage-sludge application on growth of two reclamation shrub species in copper mine soil. *Journal of Environmental Quality* **19**: 580–586.

Sai Kachout S, Ben Mansoura A, Leclerc JC, Mechergui R, Rejeb MN, Ouerghi Z. 2009. Effects of heavy metals on antioxidant activities of *Atriplex hortensis* and *A. Rosea*. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **9**: 444–457.

Sai Kachout SS, Ben Mansoura A, Mechergui R, Leclerc JC, Rejeb MN, Ouerghi Z. 2012. Accumulation of Cu, Pb, Ni and Zn in the halophyte plant *Atriplex* grown on polluted soil. *Journal of Science Food Agriculture* **92**: 336–342.

Saiyood S, Vangnai AS, Inthorn D, Thiravetyan P. 2012. Treatment of total dissolved solids from plastic industrial effluent by halophyte plants. *Water, Air and Soil Pollution* **223**: 4865–4873.

Salt DE, Prince RC, Pickering IJ. 2002. Chemical speciation of accumulated metals in plants: evidence from X-ray absorption spectroscopy. *Microchemical Journal* **71**: 255–259.

Sarret G, Harada E, Choi YE et al. 2006. Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. *Plant Physiology* **141**: 1021–1034.

Sas-Nowosiemska A, Kucharski R, Malkowski E, Pogrzevba M, Kuperberg KM, Kryński K. 2004. Phytoextraction crop disposal – an unsolved problem. *Environmental Pollution* **128**: 373–379.

Shabala S, Shabala L, Barcelo J, Poschenrieder C. 2014. Membrane transporters mediating root signalling and adaptive resposnes to oxygen deprivation and soil flooding. *Plant, Cell and Environment* **37**: 2216–2233.

Sharma SS, Dietz KJ. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany* **57**: 711–726.

Shevyakova NI, Netronina IA, Aronova EE, Kuznetsov VIV. 2003. Compartmentation of cadmium and iron in *Mesembryanthemum crystallinum* plants during the adaptation to cadmium stress. *Russian Journal of Plant Physiology* **179**: 57–64.

Sirguey C, Ouvrad S. 2013. Contaminated soils salinity: a threat for phytoextraction? *Chemosphere* **91**: 269–274.

Smaoui A, Barhoumi Z, Rabhi M, Abdelly C. 2011. Localization of potential ion transport pathways in vesicular trichome cells of *Atriplex halimus* L. *Protoplasma* **248**: 363–372.

Smolders E, Lembregts RM, McLaughlin MJ, Tiller KG. 1998. Effect of soil solution chloride on cadmium availability to Swiss chard. *Journal of Environmental Quality* **27**: 426–431.

Sousa AI, Caçador I, Lillebø AI, Pardal MA. 2008. Heavy metal accumulation in *Halimione portulacoides*: intra- and extra-cellular metal binding sites. *Chemosphere* **710**: 850–857.

Stevens DP, McLaughlin MJ, Heinrich T. 2003. Determining toxicity of lead and zinc runoff in soils: salinity effects on metal partitioning and phytotoxicity. *Environmental Toxicology and Chemistry* **22**: 3017–3024.

Storey R, Thomson WW. 1994. An X-ray microanalysis study of the salt glands and intracellular crystals of *Tamarix*. *Annals of Botany* **73**: 307–313.

Storey R, Pitman, MG, Stelzer R, Carter C. 1983. X-ray micro-analysis of cells and cell compartments of *Atriplex spongiosa*: I. Leaves. *Journal of Experimental Botany* **34**: 778–794.

Sunby B, Vale C, Caçador I, Catarino F. 1998. Metal-rich concretions on the roots of salt marsh plants: mechanism and rate of formation. *Limnology and Oceanography* **43**: 245–252.

Tate PT, Sik Shin W, Pardue JH, Jackson WA. 2012. Bioremediation of an experimental oil spill in a coastal Louisianna salt marsh. *Water, Air and Soil Pollution* **223**: 1115–1123.

Tauris B, Borg S, Gregersen PL, Holm PB. 2009. A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. *Journal of Experimental Botany* **60**: 1333–1347.

Tennstedt P, Peisker D, Böttcher C, Trampczynska A, Clemens S. 2009. Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiology* **149**: 938–948.

Thomas JC, Bohnert HJ. 1993. Salt stress perception and plant growth regulators in the halophyte *Mesembryanthemum crystallinum*. *Plant Physiology* **103**: 1291–1304.

Thomas JC, Malick FK, Endreszl C, Davies EC, Murray KS. 1998. Distinct response to copper stress in the halophyte *Mesembryanthemum crystallinum*. *Physiologia Plantarum* **102**: 310–317.

US Environmental Protection Agency (EAT). 2000. *Introduction of Toxic Metals, EPA/600/R-99/107.* Cincinnati, OH: National Risk Management Research Laboratory, Office of Research and Development.

Usha B, Venkataraman G, Parida A. 2009. Heavy metal and abiotic stress inducible metallothionein isoforms from *Prosopis juliflora* (SW) D.C. show differences in binding to heavy metals *in vitro*. *Molecular Genetics and Genomics* **281**: 99–108.

Vahedi A. 2013. The absorption and metabolism of heavy metals and mineral matters in the halophyte plant *Artemisia aucheri*. *International Journal of Biology* **5**: 63–70.

Vaillant N, Monnet F, Hitmi A, Sallanon H, Coudret A. 2005. Comparative study of responses in four *Datura* species to a zinc stress. *Chemosphere* **59**: 1005–1013.

Varga A, Garcinuñ-Martinez RM, Záray G, Fodor F. 1999. Investigations of effects of cadmium, lead, nickel and vanadium contamination on the uptake and transport processes in cucumber plants by TXRF spectrometry. Spectrochimica Acta Part B. **54**: 1455–1462.

Vázquez MD, Poschenrieder C, Barceló J, Baker AJM, Hatton P, Cope GH. 1994. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J and C Presl. *Botanica Acta* **107**: 243–250.

Vogel-Mikuš K, Simčič J, Pelicon P, *et al.* 2008. Comparison of essential and non-essential element distribution in leaves of the Cd/Zn hyperaccumulator *Thlaspi praecox* as revealed by micro-PIXE. *Plant, Cell and Environment* **31**: 1484–1496.

Vromman D, Flores-Bavestrello A, S ̆lejkovec Z, *et al.* 2011. Arsenic accumulation and distribution in relation to young seedling growth in *Atriplex atacamensis* Phil. *Science of the Total Environment* **412/413**: 286–295.

Walker DJ, Lutts S, Sánchez-García M, Correal E. 2014. *Atriplex halimus*: its biology and uses. *Journal of Arid Environments* **100/101**: 111–121.

Wali M, Ben Rjab K, Gunsé B, *et al.* 2014. How does NaCl improve tolerance to cadmium in the halophyte *Sesuvium portulacastrum*? *Chemosphere* **117**: 243–250.

Wang D, Wang H, Han B, *et al.* 2012. Sodium instead of potassium and chloride is an important macronutrient to improve leaf succulence and shoot development for halophyte *Sesuvium portulacastrum*. *Plant Physiology and Biochemistry* **51**: 53–62.

Wang HL, Tian CY, Jiang L, Wang L. 2014. Remediation of heavy metals contaminated saline soils: a halophyte choice. *Environmental Science and Technology* **48**: 21–22.

Weber M, Harada E, Vess C, Roepenack-Lahaye EV, Clemens S. 2004. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential hyperaccumulation factors. *Plant Journal* **37**: 269–281.

Wei W, Chai T, Zhang Y, Han L, Xu J, Guan Z. 2009. The *Thlaspi caerulescens* NRAMP homologue TcNRAMP3 is capable of divalent cation transport. *Molecular Biotechnology* **41**: 15–21.

Weis J, Weis P. 2004. Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environment International* **169**: 737–745.

Williams JB. 2002. Phytoremediation in wetland ecosystems: progress, problems and potentials. *Critical Review in Plant Sciences* **21**: 607–635.

Wu H, Liu X, Zhao J, Yu J. 2012. Toxicological responses in halophyte *Suaeda salsa* to mercury under environmentally relevant salinity. *Ecotoxicology and Environmental Safety* **85**: 64–71.

Wu H, Liu X, Zhao J, Yu J. 2013. Regulation of metabolites, gene expression, and antioxidant enzymes to environmentally relevant lead and zinc in the halophyte *Suaeda salsa*. *Journal of Plant Growth Regulation* **32**: 353–361.

Xu J, Yin H, Liu X, Li X. 2010. Salt affects plant Cd-stress responses by monitoring growth and Cd accumulation. *Planta* **231**: 449–459.

Yang WE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ. 2004. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance). *Plant and Soil* **259**: 181–189.

Zaier H, Ghnaya T, Lakhdar A, *et al.* 2010. Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: tolerance and accumulation. *Journal of Hazardous Materials* **183**: 609–615.

Zaier H, Ghnaya T, Ghabriche R, *et al.* 2014. EDTA-enhanced phytoremediation of lead-contaminated soil by the halophyte *Sesuvium portulacastrum*. *Environmental Science and Pollution Research* **21**: 7607–7615.

Zepeda-Jazo I, Velarde-Buendía AM, Enríquez-Figueroa R, *et al.* 2011. Polyamines interact with hydroxyl radicals in activating Ca^{2+} and K^+ transport across the root epidermal plasma membranes. *Plant Physiology* **157**: 2167–218.

Zhao KF. 1991. Desalinisation of saline soils by *Suaeda salsa*. *Plant and Soil* **135**: 303–309.

Zimmermann U, Zhu JJ, Meinzer FC, *et al.* 1994. High molecular weight organic compounds in the xylem sap of mangroves: implication for long distance water transport. *Botanica Acta* **107**: 218–229.