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Phytoremediation of Heavy Metal-Contaminated Saline Soils Using Halophytes: Current Progress and Future Perspectives

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Abstract: Soil salinity is a destructive environmental stressor that greatly reduces plant growth and productivity. In recent years, large tracts of farmland in arid and semiarid regions have been simultaneously affected by salinity and heavy metal pollution, arousing widespread environmental concern. Phytoremediation, defined as the use of plants to remove pollutants from the environment and/or to render them harmless, is a low cost, environmentally friendly, and effective method for the decontamination of soils polluted by heavy metals. Halophytes, which can survive and reproduce in high salt environments, are potentially ideal candidates for phytoremediation of heavy metal-contaminated saline soils. In this review, we discuss the current progress on the use of halophytes, their tolerance mechanisms to salt and heavy metal toxicity, and their potential for phytoremediation in heavy metal-contaminated saline soils. The relative mechanisms are discussed and the future perspectives are proposed.

Keywords: Saline soil; Heavy metal; Halophyte; Phytoremediation; Tolerance mechanism

1. Introduction

Saline soils are generally considered to have an electrical conductivity (EC) >4 dS m^{-1} (approximately 40 mM NaCl), measured from a saturated soil paste extracted at 25 □ with an exchangeable sodium of 15% (Shrivastava and Kumar 2015). Due to climate change and the rapid development of irrigated agriculture, soil salinization is becoming an increasingly serious concern worldwide, posing a threat to both environmental and agricultural sustainability (Bui 2013; Singh 2015). It is estimated that approximately 20% of agricultural land and 50% of cropland in the world are affected by this problem, and this continues to increase (Li et al. 2014a; Manousaki and Kalogerakis 2011b; Van Oosten and Maggio 2015).

With rapid worldwide industrial and agricultural development, heavy metal contamination of soil has become a serious environmental concern, particularly in China (Li et al. 2014c; Liu et al. 2013). Heavy metal pollution of soil has deleterious effects on microorganisms and plants, and poses a potential health risk to animals and humans through routes of exposure, such as direct ingestion, contact with contaminated soil, and the food chain (Chen et al. 2014; Liu et al. 2015a; Soodan et al. 2014). In recent years, one particular tract of farmland in arid and semiarid regions has been affected by both salinity and heavy metal pollution (Wang et al. 2013a) and remediation of heavy metal-contaminated saline soils to reduce the risk to human health is a worldwide environmental goal.

Phytoremediation, defined as the use of plants to remove pollutants from the environment and/or to render them harmless, has attracted widespread attention in recent years as it is a cost-effective and environmentally friendly technology (Liu et al. 2013; Lombi et al. 2001; Salt et al. 1998). However, most of the known phytoremediators are glycophytes, which cannot be used to remediate heavy metals from saline soils as they cannot survive the combination of salt and heavy metal pollution long enough to be effective (Wang et al. 2013a). Halophytes are plants that can survive and reproduce in saline environments with salt concentrations of 200 mM NaCl or more (Flowers and Colmer 2008), and are ideal alternative phytoremediators of heavy metal-contaminated saline soils (Manousaki and Kalogerakis 2011a,b; Wang et al. 2013a). Compared with glycophytes, halophytes have some additional advantages for phytoremediation of heavy metal-contaminated soils, such as higher tolerance to heavy metals and greater amounts of heavy metal uptake (Flowers et al. 2010; Taamalli et al. 2014; Wang et al. 2013a). Moreover, salinity can increase heavy metal mobility in soils and promote the translocation of heavy metals from roots to shoots (Acosta et al. 2011; Manousaki et al. 2008).

In recent years, researchers have undertaken numerous studies on phytoremediation of heavy metal-contaminated saline soils using halophytes, (Christofilopoulos et al. 2016; Korzeniowska and Stanislawska-Glubiak 2015; Manousaki et al. 2014; Santos et al. 2015a; Santos et al. 2015b). However, to date, only a handful of studies have reviewed either the functional biology of halophytes or the potential for halophytes to phytoremediate heavy metal-contaminated saline soils (Lutts and Lefèvre 2015; Manousaki and Kalogerakis 2011a; Manousaki and Kalogerakis 2011b; Van Oosten and Maggio 2015; Wang et al. 2013a). Reviews focusing on the recent advances in phytoremediation of heavy metal-contaminated saline soils using halophytes remain rare. In this manuscript, we summarize the current progress on the use of halophytes, the tolerance mechanisms of halophytes to salt and heavy metal toxicity, and the phytoremediation potential of halophytes in heavy metal-contaminated saline soils. We also discuss the relative mechanisms and propose future directions for progress.

2. Comprehensive summary of the current use of halophytes

Halophytes account for approximately 1% of global flora and 2% of terrestrial plant species, and can tolerate salt concentrations that are lethal to 99% of other plant species (Anjum et al. 2014; Flowers and Colmer 2008). Approximately 430 halophyte species have been recorded in China, a substantial number of which are considered to have economic potential (Zhao et al. 2002). In a recent report, halophytes were suggested as possible alternatives to glycophytes to help ease the pressure on demand for good quality land, water for conventional cropping systems, and the utilization of land degraded by salinity (Panta et al. 2014).

2.1 Halophytes as animal feed and human food

Halophytes are used as forage and animal feed, replacing traditional ingredients in domestic animal diets in arid and semiarid regions, although there are restrictions on the use of some species due to their high salt content and anti-nutritional compounds (El Shaer 2010; Panta et al. 2014). Moreover, the use of halophytes as animal feed largely depends on their biomass production, nutritional value, and voluntary feed intake (Norman et al. 2013). *Atriplex lentiformis* was reported to be a valuable fodder plant for ruminants (Glenn et al. 2013). Similarly, *Atriplex amnicola*, a halophyte with high biomass productivity, has significant potential as a safe forage crop in metal-contaminated soils (Eissa et al. 2016). *Suaeda glauca* was reported as a very promising feedstock for lambs in Northeast China due to its comparable herbage yield and crude protein content (Sun et al. 2012).

Halophytes have been used as human food for a long time, especially in areas where saline water and soils occur (Panta et al. 2014). The perennial halophyte nipa *Distichlis palmeri* shows potential for development as a perennial grain and forage crop in saline and flooded soils (Pearlstein et al. 2012). Quinoa (*Chenopodium quinoa*) is a well-known halophytic crop that produces high-quality protein seeds with essential amino acids and vitamins (Fita et al. 2015). The salt grass *Distichlis palmeri* has a higher fiber content than wheat and has been used as a food crop in many countries in South America (Panta et al. 2014). In addition, the leaves of some halophytes, such as *Atriplex triangularis*, *Salicornia bigelovii* and *Diplotaxis tenuifolia* can be used as leafy vegetables (de Vos et al. 2013; Panta et al. 2014; Ventura et al. 2011; Zerai et al. 2010).

2.2 Halophytes as oilseed and energy crops

Some species of seed-bearing halophytic plants are potential sources of edible oil. *Salicornia bigelovii*, a well-known oilseed halophyte, is highly salt-tolerant and can produce up to 30% seed oil (Glenn et al. 1991; Troyo-Diéguez et al. 1994; Ventura et al. 2015). The seeds of halophytes, particularly *Suaeda fruticose,* could be used as a source of oil for human consumption (Weber et al. 2007). In addition, halophytes include many oilseed-producing species, including *Batis maritima* (Marcone 2003), *Crambe abyssinica* (Mandal et al. 2002), *Kosteletzkya virginica* (He et al. 2003), *Crithmum maritimum*, and *Zygophyllum album* (Zarrouk et al. 2003).

Several studies have indicated that halophytes have the potential to produce biomass and renewable energy in saline environments (Abideen et al. 2011; Panta et al. 2014; Qadir et al. 2008). Species such as *Halopyrum mucronatum*, *Desmostachya bipinnata*, *Phragmites karka*, *Typha domingensis*, and *Panicum turgidum* were shown to be suitable for bioethanol production (Abideen et al. 2011). In addition, *Salicornia fruticosa*, *Cressa cretica*, *Arthrocnemum macrostachyum*, *Alhagi maurorum*, *Halogeton glomeratus*, *Kosteletzkya virginica*, and *Atriplex rosea* are promising biodiesel candidates (Abideen et al. 2015).

2.3 Halophytes for phytodesalination and phytoremediation purposes

Phytodesalination aims to improve crop production using halophytes or salt-tolerant plants, and has shown promising results for different types of remediation programs, such as agricultural soil restoration, road runoff, and wastewater desalination (Jesus et al. 2015; Rabhi et al. 2010; Shelef et al. 2012; Suaire et al. 2016). It has been reported that *Suaeda maritima* and *Sesuvium portulacastrum* accumulated greater amounts of salts in their tissues and enabled a greater reduction of salts in the soil (Ravindran et al. 2007). *Atriplex halimus* and *Zygophyllum fabago* showed higher accumulation of Na and Cl, which may be effective in the long-term desalination of mine tailings (Parraga-Aguado et al. 2014). Phytodesalination using

Echinochloa stagnina has proved to be an effective alternative method to restore saline clay soil, which is cost effective and beneficial for crop production (Ado et al. 2016). The use of *Atriplex nummularia* to remediate saline and sodic soils was recommended because of its considerable ability to extract salts and produce high biomass yield (de Souza et al. 2014). However, a more robust evaluation of the suitability of different halophyte species for the practical purposes of land desalination is still needed (Panta et al. 2014).

Halophytes may tolerate heavy metals and xenobiotics, offering greater potential for phytoremediation (Anjum et al. 2014). The high resistance of halophytes to heavy metals is strongly linked to their salt tolerance characteristics (Wang et al. 2013a). Moreover, it has been proposed that heavy metal-tolerant plants and halophytes share a number of traits in common (Shevyakova et al. 2003; Van Oosten and Maggio 2015). Therefore, it has been suggested that halophytes are naturally better adapted to environmental stresses, including heavy metals, than glycophytes. (Anjum et al. 2014; Manousaki and Kalogerakis 2011a; Manousaki and Kalogerakis 2011b; Wang et al. 2013a). Some halophytes, such as *Atriplex halimus* (Manousaki and Kalogerakis 2009), *Spartina alterniflora* (Nalla et al. 2012), *Sesuvium portulacastrum* (Ayyappan et al. 2016) and *Tamarix africana* (Santos et al. 2015a) have proved to be well-suited for phytoextraction or phytostabilization of heavy metal-contaminated saline soils.

2.4 Halophytes for other commercial purposes

Halophytes have a range of other commercial purpose besides food and fuel. *Sesuvium portulacastrum* can be used in traditional medicine as a remedy for fever, kidney disorders, scurvy, and HIV (Lokhande et al. 2013). It was suggested that *Salicornia herbacea* has antioxidant and whitening effects on the skin and is an effective skin rejuvenating agent (Sung et al. 2009). Moreover, extracts from *Salicornia europaea*, *Glehnia littoralis*, and *Suaeda fruticosa* were shown to have anti-proliferation and toxic effects on human colon cancer cells (Buhmann and Papenbrock 2013). Halophytes can also be used as insecticides (Rele et al. 2003; Saïdana et al. 2008), food additives, and cosmetics (Buhmann and Papenbrock 2013).

3. Mechanisms of halophyte tolerance to salt and heavy metal stress

There is a wide range of morphological, physiological, and biochemical mechanisms in halophytic plants, which vary widely in their degree of salt tolerance (Manousaki and Kalogerakis 2011b). Generally, halophytes potentially have high tolerance to heavy metals that is strongly linked to characteristics for salt tolerance (Wang et al. 2013a). Therefore, it may be hypothesized that halophytic plants may have adaptive properties to tolerate the external presence or accumulation of heavy metals within their tissues (Lutts and Lefèvre 2015). In general, the resistance mechanisms of plants to metal and salt stress are mainly through inter- and intracellular compartments (Mühling and Läuchli 2003).

3.1 Mechanisms of halophyte tolerance to salt stress

Halophytes have developed a diversity of tolerance mechanisms to cope with salt stress. Although some specific strategies are well-known, such as osmotic adjustment, ion balance, and antioxidant resistance mechanisms (Anjum et al. 2014), in general, tolerance mechanisms are complex and not well understood.

Halophytes use osmotic adjustment to meet the challenge of low external water potential, although species differ in succulence and in the solutes accumulated (Flowers and Colmer 2008). In general, osmotic adjustment in halophytes can be achieved by the ion accumulation/compartmentalization or exclusion, and biosynthesis of compatible solutes (Hartzendorf and Rolletschek 2001; Parida and Das 2005). Many halophytes accumulate inorganic ions to a concentration equal to or greater than that of the surrounding root solution to maintain an osmotic gradient necessary for the uptake of water from the soil (Bradley and Morris 1991). Moreover, halophytes usually adopt the strategy of sequestration/accumulation of inorganic ions in vacuoles to decrease cell water potential, control energy consumption, and to protect salt-sensitive enzymes in the cytoplasm (Anjum et al. 2014; Moghaieb et al. 2004). Apse et al. (1999) reported that the removal of sodium from the cytoplasm or compartmentalization in the vacuoles is achieved by a salt-inducible enzyme Na^+/H^+ antiporter. In addition, some halophytes exclude salt from their cells either by the roots or as secretions and export through salt-excreting organs (Manousaki and Kalogerakis 2011b).

To maintain the ionic balance within vacuoles, the cytoplasm accumulates compatible solutes that do not interfere with normal biochemical reactions (Parida and Das 2005). These compatible solutes include proline (Pro) (Pagter et al. 2009; Singh et al. 2000), soluble carbohydrates (Touchette 2006), amino acids (Mansour 2000), and glycine betaine (GB) (Moghaieb et al. 2004). Salinity stress can increase the production of reactive oxygen species (ROS), which cause oxidative damage to lipids and disturb the cellular metabolism (Anjum et al. 2014; Anjum et al. 2012; Apel and Hirt 2004). The levels of antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), and superoxide dismutase (SOD) increase under salt stress in plants and are correlated with salt tolerance (Anjum et al. 2014; Benavídes et al. 2000; Parida and Das 2005). High salinity induces oxidative stress in *Spartina densiflora* and its salt tolerance appears to be related to the implementation of a specific antioxidant response (Canalejo et al. 2014). In addition, excess salt concentration can trigger an increase in levels of plant hormone abscisic acid (ABA) to alleviate the inhibitory effect of NaCl on photosynthesis, growth, and translocation of assimilates (Gómez-Cadenas et al. 1998; Popova et al. 1995).

3.2 Mechanisms of halophyte tolerance to heavy metal stress

Halophytes usually have high tolerance to heavy metals compared with common plants (Manousaki and Kalogerakis 2011a). Tolerance of halophytes to salt and heavy metals may, at least in part, possess common physiological mechanisms (Manousaki and Kalogerakis 2011b). Since heavy metals induce both secondary water stress and oxidative stress in plants, and the capability of halophytic plants to synthesize organic compatible solutes may allow them to tolerate heavy metals (Manousaki and Kalogerakis 2011b; Nedjimi and Daoud 2009). Sharma and Dietz (2006) showed that Pro, produced under osmotic stress, has a significant beneficial function in tolerance to metal stress via metal binding, antioxidant defense, and signaling. Moreover, antioxidant synthesis in halophytes improves resistance against oxidative stress caused by heavy metals. Halophytes tend to have higher levels of SOD, CAT, and POD activity, and lower levels of damage to their lipid membranes from ROS under heavy metal stress (Wang et al. 2013a). In addition, some halophytes can tolerate heavy metal toxicity via excretory mechanisms. It was shown that glandular tissues of halophytes not only excrete Na^+ and Cl^-, but also remove heavy metal ions from photosynthetically active tissues onto the leaf surface (Kadukova et al. 2008; Manousaki et al. 2008).

In general, plants respond to heavy metal toxicity via several molecular mechanisms (Figure 1). These include (1) metal ions binding to cell walls, (2) metals chelating within cytosol, (3) ROS defense mechanisms, and (4) metal sequestration in vacuoles (Ali et al. 2013; Gargouri et al. 2013; Kushwaha et al. 2016). The cell wall is an important site for metal storage in plants as it provides a large number of metal-binding sites (Maestri et al. 2010). The cell wall is rich in pectic and histidyl groups and plays a key role in the immobilization of metal ions, which alleviates metal toxicity to plant cells (DalCorso et al. 2013). The toxic effects of metal ions in cytosol can be eliminated by specific high affinity ligands, such as phytochelatins (PCs) (Liu et al. 2015b; Zhang et al. 2010), soluble protein (Luo et al. 2011), metallothioneins (MTs) (Clements 2001), and Pro (Sytar et al. 2013; Wali et al. 2016). Plant cells possess a range of antioxidant mechanisms to deal with metal ion-induced ROS, including activating scavenging enzymes, such as CAT, SOD, APX, and glutathione peroxidase (GPX). These enzymes act in concert, reducing the superoxide ion (O_2^-) to hydrogen peroxide (H_2O_2), and H_2O_2 to water using reactions involving

APX or GPX as electron donors (Canalejo et al. 2014; DalCorso et al. 2013). Storage and concomitant detoxification of excess heavy metals generally occurs within the vacuole (Martinoia et al. 2007). The main families involved in vacuole metal sequestration are ABC (ATP Binding Cassette), CDF (Cation Diffusion Facilitators), P1B-type ATPases, CAX, and NRAMP (natural resistance-associated macrophage) transporters (DalCorso et al. 2013; Kushwaha et al. 2016).

4. Phytoremediation of heavy metals in saline soils using halophytes

Several approaches have been used to remediate heavy metal-contaminated saline soils; however, the remediation effects were limited due to the complexity of the combined pollution, immature technology, and high costs (Tahmasbian and Safari Sinegani 2016; Wang et al. 2013a). Phytoremediation has been highlighted as an alternative technique to traditional methodologies as it provides a cost-effective, long-lasting, and aesthetic solution for remediation of contaminated sites (Liu et al. 2011; Salt et al. 1995; Sinegani et al. 2015). It was proposed that selecting target halophytes that can accumulate moderate amounts of heavy metals and produce high biomass yields may be a convenient and efficient means for phytoremediation heavy metal contaminated saline soils (Wang et al. 2013a).

4.1 Categories of phytoremediation

There are five different phytoremediation strategies, each having a different mechanism for remediating metal-contaminated environments: (1) phytoextraction, in which pollutant-accumulating plants are used to remove metal or organic pollutants from soil by concentrating them in harvestable plant parts; (2) phytostabilization, in which the plants completely immobilize metals through accumulation in the roots or precipitation within the rhizosphere and/or by changing their chemistry; (3) phytovolatilization, in which plants absorb elemental forms of metals from the soil, biologically convert them to gaseous species within the plant and subsequently released them into the atmosphere; (4) phytofiltration, in which the plant roots (rhizofiltration) or seedlings (blastofiltration) absorb or adsorb metals from water and aqueous waste streams; and (5) phytodegradation, in which pollutants are degraded into insoluble or nontoxic compounds through metabolic processes and/or interaction with microorganisms in the rhizosphere (Anjum et al. 2014; Padmavathiamma et al. 2014; Salt et al. 1995; Salt et al. 1998).

Of the five strategies, phytoextraction and phytostabilization have been highlighted as alternative techniques for the remediation of heavy metal-contaminated soils as they offer high remediation efficiency and feasibility (Alkorta et al. 2010; Garbisu and Alkorta 2001; Kumar et al. 1995; Manousaki and Kalogerakis 2011b). Phytoextraction appears to be the most promising technique and has received increasing attention (Sinegani et al. 2015; Tahmasbian and Safari Sinegani 2014; Tahmasbian and Safari Sinegani 2016). In general, hyperaccumulators and high-biomass plants are two ideal candidates for phytoextraction (Hernández-Allica et al. 2008). The term ‘‘hyperaccumulator’’ was first used by Brooks et al. (1977) to describe plants that contain greater than 0.1% nickel (Ni) in their dried leaves (Brooks et al. 1977). Currently, a “hyperaccumulator” is defined as a plant with leaves that contain a metallic element at a concentration exceeding a specified threshold, when growing in natural conditions (i.e., not in experimental cultivation) (Pollard et al. 2014). The proposed nominal threshold criteria (all in units of µg metal per g of dry leaf tissue) for hyperaccumulators are: 100 for Cd, Se, and Tl; 300 for Co, Cr, and Cu; 1000 for As, Ni, and Pb; 3000 for Zn; and 10,000 for Mn (Van der Ent et al. 2013). To date, approximately 500 species of plants are known hyperaccumulators of heavy metals and metalloids (Pollard et al. 2014).

4.2 Phytostabilization of heavy metals in saline soils using halophytes

Belowground plant tissues are generally the major sink area for most metals, which accounts for more than 90% of the total phytoaccumulated metal (Anjum et al. 2014). Moreover, several halophytes can simultaneously accumulate multiple metals in their roots. Accumulation of Cu, Pb, Ni, and Zn in three annual species, *Atriplex hortensis* var. *purpurea*, *Atriplex hortensis* var. *rubra,* and *Atriplex rosea* was investigated by Kachout et al. (2012). They found that accumulation was higher in roots than shoots for all of the heavy metals examined. However, the three plants with the highest bioconcentration factors (BCFs) and lowest translocation factors (TFs) have the greatest potential for phytostabilization (Kachout et al. 2012).

Latest findings show that *Atriplex lentiformis* and *Atriplex undulata* are able to minimize heavy metal accumulation in their aerial parts and could be used for phytostabilization of heavy metals (Eissa 2015). Cambrollé et al. (2008) reported that *Spartina densiflora* and *Spartina maritima* accumulated heavy metals in both below- and aboveground tissues, and could be highly effective for the phytostabilization of soils.

Both *Scirpus maritimus* and *Juncus maritimus* exhibited potential to phytostabilize Cd (Almeida et al. 2006). In particular, *J. maritimus* showed potential for phytoextraction (or phytostabilization) of Cd, Cu, and Zn in estuarine environments (Almeida et al. 2004). *Atriplex halimus* subsp. *schweinfurthii*, a newly found Cd hyperaccumulator, retains most of the Cd in its roots and has the potential to phytostabilize Cd-contaminated saline soils (Nedjimi and Daoud 2009). Similarly, Cd was preferentially accumulated in belowground tissues of *Juncus maritimus* and *Phragmites australis*, which can effectively retain and phytostabilize this metal in salt marshes and estuarine environments (Nunes da Silva et al. 2014). Noticeably, *Salicornia ramosissima* can be effectively used for phytoaccumulation and phytostabilization as it has considerable bioaccumulation potential and is able to bioaccumulate Cd in the roots, acting as a sink for this metal (Pedro et al. 2013).

Suaeda fruticose accumulates large amounts of Cd^{2+} and Cu^{2+} in its tissues, especially in roots, suggesting it could be used for decontaminating saline soils polluted by Cd^{2+} and Cu^{2+} (Bankaji et al. 2015). *Spartina alterniflora* is a promising candidate for phytostabilization of Cu-contaminated salt marsh, as it can survive and sequestrate most of the Cu in its underground parts (Chai et al. 2014).

The belowground biomass of *Juncus maritimus* can sequester mercury (Hg), which makes it suitable for phytostabilization (Hg complexation in the rhizosediment) and phytoaccumulation (Hg sequestration in the belowground biomass) of Hg-contaminated saline soils (Marques et al. 2011). *Triglochin maritima* showed higher Hg retention in the belowground organs, whereas *Sarcocornia perennis* showed more translocation to the aboveground tissues (Castro et al. 2009). *Atriplex atacamensis* is a promising candidate for the phytoremediation of Arsenic (As)-contaminated soil, as it accumulated up to 400 µg/g DW AS in the shoots and 3500 µg/g DW in the roots (Vromman et al. 2011).

4.3 Phytoextraction of heavy metals in saline soils using halophytes

Atriplex halimus can accumulate about 830 mg kg^{-1} Cd and 440 mg kg^{-1} Zn in its aerial tissues, which produces up to 5 Mg dry matter ha^{-1} yr^{-1} and may, therefore, be an effective species for phytoextraction (Lutts et al. 2004). Similarly, Manousaki and Kalogerakis (2009) found that metal accumulation in the shoots of *A. halimus* was relatively low. However, its high biomass production and rapid growth suggests this species is suitable for phytoextraction (Manousaki and Kalogerakis 2009). *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* were both essential for phytoextraction of Cd from salt-contaminated soils, although *S. portulacastrum* was significantly more tolerant to Cd than *M. crystallinum* (Ghnaya et al. 2005). *Tamarix smyrnensis* is neither a Pb nor a Cd hyperaccumulator; however, its metal accumulation levels in shoots and high biomass production suggests that it could be used for phytoextraction (Manousaki et al. 2009).

Portulaca oleracea has been shown as a promising hyperaccumulator for the phytoextraction of Cr(VI), as the total Cr concentrations exceeded 4600 mg kg^{-1} in roots and 1400 mg kg^{-1} in stems (Alyazouri et al. 2013). *Sesuvium portulacastrum* was an efficient accumulator of Cr, Cd, Cu, Zn, Na, and Chl, mainly through its leaves, which indicates that this species could be used for phytoextraction of fields contaminated with multiple pollutants (Ayyappan et al. 2016). Further species of halophytes suitable for phytoextraction of heavy metal-contaminated soils are summarized in Table 1 (Debez at al. 2011).

4.4 Phytovolatilization and phytoexcretion of heavy metals in saline soils using halophytes

Phytovolatilization is the process in which plants take up contaminants and release them into the atmosphere through transpiration (Van Oosten and Maggio 2015). Phytovolatilization is unique to a select group of heavy metals and metalloids including mercury (Hg) and selenium (Se) that have relatively high volatility (Wang et al. 2012). Although phytovolatilization of Hg and Se has been reported in glycophyte (Shrestha et al. 2006; Kushwaha et al. 2016; Wang et al. 2012), studies on the use of halophytes for phytovolatilization are still scarce. Some relevant studies are summarized in Table 2.

Phytoexcretion is a novel phytoremediation technology for sites contaminated with heavy metals. Manousaki et al. (2008) showed that *Tamarix smyrnensis* (salt cedar) can excrete a significant amount of metals onto the leaf surface, which is a possible detoxification mechanism and also a good phytoextraction technique. Some halophytes in the families Poaceae, Tamaricaceae, Chenopodiaceae, and Frankeniaceae have salt-excreting organs that can remove heavy metals from their leaves (Table 2) (Manousaki and Kalogerakis 2011b).

4.5 Factors affecting phytoremediation efficiency

Factors that influence the efficiency of the phytoremediation process are shown in

Figure 2.

4.5.1 Characteristics of phytoremediation plant species

Different species have different metal accumulation and translocation capacities and phytoremediation efficiencies. For example, both monocotyledons and dicotyledons accumulate Cu in their roots, whereas monocotyledons mainly accumulate Pb in their roots. *Aster tripolium*, *Atriplex verucifera*, *Salicornia europaea,* and *Chenopodium album* were all shown to be suitable for Pb phytoremediation, while the phytoavailability of Pb and its interactions with plants were widely dependent on the type of plant (Fitzgerald et al. 2003; Khodaverdiloo and Taghlidabad 2013). *Triglochin striata* is a potential phytostabilizer, as it retains metals in its belowground structures, mainly roots and rhizomes. Conversely, *Phragmites australis* is a potential phytoextractor, as it stores accumulated metal in aboveground tissues. Moreover, *Juncus maritimus* and *Spartina patens* retain metal in both above- and belowground structures (Almeida et al. 2011).

4.5.2 Characteristics of the medium

It is well-known that soil properties can affect metal mobility and phytoavailabiliy, and thus can influence plant metal uptake (Liu et al. 2011, 2013). Soil pH is a major factor influencing the bioavailability of metals in the soil for plant uptake. Generally, the concentrations of heavy metals in soil solution can be increased by decreasing the soil pH. The Cation exchange capacity (CEC) increases with increasing clay content in the soil, while the availability of metal ions decreases (Sheoran et al. 2016). Gabrijel et al. (2009) suggested that the soil organic reactive surfaces may reduce trace element bioavailability and phytoaccumulation, while salinity could increase Cd accumulation in aboveground tissues (Gabrijel et al. 2009).

4.5.3 The root zone

The rhizosphere of plants may influence the mobility of metals as it accumulates more metals than the surrounding sediment. *Halimione portulacoides* caused a marked increase in dissolved-phase metal concentrations (Almeida et al. 2008). Different root apexes of *S. salse* affected the uptake of Cd and regulation of Ca transporters or channels in root cell plasma membranes. For example, the Cd^{2+} influx was greatest in the rhizosphere, near the root tip. These may provide a theoretical basis for improving the phytoremediation of Cd contamination (Liu et al. 2012).

Root exudates may play a significant role in determining the efficiency of metal phytoremediation. First, the root exudates can provide nutrients for rhizosphere microorganisms. Second, they can also affect solubility, mobilization, and phytoavailability of heavy metals by improving the solubility of heavy metal ions (Badri and Vivanco 2009; Koo et al. 2010). High molecular weight (HMW) fractions are important components of root exudates. The HMW fluorescent substances can affect the chemical forms and mobility of Cu, and affect the phytoremediation process, enhancing Cu(II) accumulation in plants (Pan et al. 2011).

Studies have also shown that plant growth-promoting rhizobacteria (PGPR) play a key role in plant health and nutrition. A number of mechanisms, including the synthesis of siderophores, the production of phytohormones, and bacteria connected to plant-growth-promoting (PGP) activity, can alleviate various stresses. Therefore, isolation and utilization of PGPR could prove to be beneficial for mitigating the saline-alkaline stress to plants growing in polluted environments. In addition, the addition of the cadmium resistant microbial consortia increased *Juncus maritimus* metal phytostabilization capacity. On the other hand, in *Phragmites australis*, microbial consortia amendment promoted metal phytoextraction (Teixeira et al. 2014).

4.5.4 Chemical characteristics of contaminants

Plants can benefit from the microbial activity of the phytoremediation process. However, metals above certain levels are known to be toxic to microorganisms. *Juncus maritimus* and *Phragmites australis* displayed important differences in terms of bacterial community structure, although they presented similar microbial abundances and showed a similar response to both Cu and Pb, even at intermediate metal levels. Changes in the microbial community structure could indirectly affect phytoremediation (Mucha et al. 2013).

In addition, the influences of different concentrations (10–2000 μM) of heavy metals (Cu, Mn, Ni, and Zn) on germination patterns and seedling size were analyzed in two halophyte species *Atriplex halimus* and *Salicornia ramosissima*. All of the metals tested affected the final germination percentage in *A. halimus*, but only Ni reduced germination in *S. ramosissima*. This study showed that metal concentrations affected the seedling development and that metals might limit plant colonization, influencing phytoremediation (Márquez-García et al. 2013). Heavy metals in sediments colonized by *Halimione portulacoides* and *Spartina maritima* were different. Previous studies have shown that Cu, Zn, and Pb varied with the depth of the soil and the colonizing species. In the rhizosphere of *S. maritima*, there were higher concentrations of Cu, Zn, and Pb than in the sediments surrounding the roots of *H. portulacoides* (Reboreda and Cacador 2007).

4.5.5 Environmental conditions

The reducible fractions of heavy metals in *S. alterniflora*, presented the highest percentages, occurring in the order Zn < Cd < Pb < Cu. Additionally, *S. alterniflora* improved the conversion of metals from the immobilized to the mobilized fraction, directly increasing the accumulation of heavy metals and their bioavailability and mobility. (Chai et al. 2014; Fitzgerald et al. 2003). Fe^{3+} and $Mn^{3+}/^{4+}$ oxides provide important bindings sites for heavy metals under oxic conditions. Similarly, sulphide provides vital bindings sites for Cu and Pb under anoxic conditions. Environmental conditions affect the mobility of heavy metals in saltmarsh sediments that are influenced by biogeochemical processes. *Spartina alterniflora* may increase the seasonal fluctuation of heavy metal bioavailability in saltmarsh ecosystems (Wang et al. 2013b).

4.5.6 Addition of chelating agents

Generally, most metal ions adsorbed on the soil were difficult to extract due to their high metal binding capabilities. Chelating agents play an important role in the removal of toxic metals through washing. Typical chelating agents include ethylenediaminetetraacetic acid (EDTA) and ethylenediaminedisuccinic acid (EDDS) (Peters 1999). The application of advanced biodegradable and non-toxic organic chelators could improve metal extraction from contaminated soils as organic solvents (Ullmann et al. 2013). Three types of chelating agents (EDTA, nitrilotriacetic acid, and acetic acid) could be out-competed by soil colloids in attracting cations, and when soils with a rich clay fraction retained cations, the remediation was more efficient. This influences the capacity of metal adsorption and pH in the soil (Mosekiemang and Dikinya 2012).

Vetiveria zizanioides was first used for the phytostabilization of Pb. However, the application of EDTA and EDDS can release Pb from bound fractions into the labile pool and facilitate Pb uptake by this species. The antioxidant enzyme activities were lower compared with those of the chelates, which suggested that chelating agents may reduce the activities of antioxidant enzymes in *Vetiveria zizanioides* (Andra et al. 2011).

4.6 Enhancing strategies for phytoremediation

In some cases, the single phytoremediation technology may not be effective in removing pollutants from contaminated soils. Therefore, some improvements in methods are advocated, including chemical-assisted phytoremediation and bioaugmentation-assisted phytoremediation, to completely remove pollutants.

4.6.1 Chemical-assisted phytoextraction

Polyamines (PAs) are ubiquitously distributed in all living organisms, and their positive charges at physiological pH levels enable PAs to interact electrostatically with polycistronic macromolecules. Several mechanisms have been proposed to explain the protective nature of PAs, including scavenging free radicals, stabilizing membranes and cellular structures, and maintaining a cation-anion balance. Both mono- and dicotyledonous plants increase their accumulation of endogenous PAs under salt stress, and the pattern of PA metabolism in response to salinity appears to be dependent on the plant defense system and duration of exposure to salt stress. Exogenous PA application has been proposed as a convenient and effective approach for combating the salt tolerance of plants and ultimately improving crop productivity and phytoremediation in high salinity soils (Parvin et al. 2014).

The addition of EDTA resulted in a two-fold increase in Cd^{2+} concentration in shoots due to the improvement of Cd^{2+} absorption efficiency, which was concomitant with an increase in Cd^{2+} translocation to the shoots. This suggested that *Spartina kali* is a promising species for the phytoremediation of Cd^{2+} (Ben Rejeb et al. 2013). A combined application of EDTA, citric acid (CA), and dissolved organic matter (DOM) removed up to 91% Cu from the topsoil (Liu and Lin 2013). Both EDTA and CA were capable of increasing the metal uptake within *Helianthus annuus* (Turgut et al. 2004). *Parthenium hysterophorus* and *Zea mays* were used to test the influences of gibberellic acid (GA3), indole-3-acetic acid (IAA), and EDTA in phytoextraction of Pb. The results showed that EDTA increased the uptake of Pb, whereas GA3 and IAA increased both uptake and translocation. GA3 combined with EDTA improved Pb phytoextraction (Hadi et al. 2013).

The addition of carbon nanotubes (CNTs) to Cd accumulation in *S. alterniflora* alleviated higher Cd stress by restoring shoot growth, retrieving water content, and allowing the plant to reach full height. Furthermore, CNTs mitigated the deleterious effects of Cd stress by improving K^+ and Ca^{2+} content, while reducing Na^+/K^+ and Na^+/Ca^{2+} ratios, irrespective of the level of Cd stress. Furthermore, CNTs reduced the production of organic solutes under Cd stress, resulting in higher Cd accumulation in roots than shoots. Therefore, the combined application of CNTs and *S. alterniflora* to the phytoremediation of Cd pollution is potentially effective (Chai et al. 2013).

Similarly, liming materials may raise pH and ameliorate the problem of excessive acidity. A study of phytoremediation of metal-contaminated saline soil using *Sarcocornia fruticosa* showed that liming promoted plant growth and enhanced the capacity of the plants to stabilize metals in roots, thereby reducing soluble metal concentrations (González-Alcaraz et al. 2011).

Graphene nanocomposites show potential to detect and remove heavy metal ions, organic and other environmental pollutants (Chang and Wu 2013). Graphene, and its derivatives graphene oxide (GO) and reduced graphene oxide (RGO), have high surface areas and many functional groups (–OH,–COOH,–O) that are beneficial for the adsorption or preconcentration of heavy metal ions, such as Pb^{2+}, Cd^{2+}, and Cu^{2+}, which form complexes and remove heavy metal ions (Lü et al. 2013). However, researches on the application of graphene nanocomposites in energy and environmental studies are not complete, as their influences on the ecosystem and human living environments are still unclear.

Biochar has attracted much research attention recently. Optimized biochar can improve the metal phytoextraction ability of *Amaranthus tricolor*. Biochar changed soil biological activity partly due to alterations in soil pH (Lu et al. 2015). Cu^{2+}

removal by biochar was highly dependent on pyrolysis temperature and environmental pH, Biochar from *S. alterniflora* and water hyacinth also provided some benefits, including nutrient release, heavy metal immobilization, and improvement in soil particle aggregation (Ca) for soil amelioration (Li et al. 2015). *Alternanthera philoxeroides* combined with biochar was effective at removing heavy metals from contaminated water, and the adsorption mechanism was related to the precipitation and complexation of Pb^{2+} with free carboxyl/hydroxyl functional groups and the ion replacement between Pb^{2+} and alkaline earth cations (Yang et al. 2014).

4.6.2 Bioaugmentation-assisted phytoremediation

Phragmites australis enhanced Cd (phytoextraction) and reduced the bioavailability of contaminants in the rhizosphere (phytostabilization). However, *Phragmites australis* cannot be used for phytoremediation of soil contaminated with Cd and PCP. Therefore, it is important that supplementary methods should be developed, such as exploring the partnership of plants and microbes (Hechmi et al. 2014).

Microorganisms can influence the mobility of metals in the sediment either by accumulation or by producing substances, such as organic compounds or sulphides that directly change metal mobility. Autochthonous bioaugmentation could be a valuable strategy to enhance the ability of naturally present saltmarsh plants to remediate Cd in estuarine systems. In fact, when *Juncus maritimus* and *Phragmites australis* rhizospheres were inoculated with a microbial consortium containing microorganisms that were resistant to Cd, the total amount of Cd increased up to seven times in *Phragmites australis* stems and up to 48% in *Juncus maritimus* belowground structures. Bioaugmentation can contribute towards the use of a sustainable environmental resources to moderately recover impacted environments (da Silva et al. 2014).

Plants and microorganisms compete for nutrients. Therefore, the need for additional fertilizers must be determined for effective phytoremediation (Mucha et al. 2011). *Atriplex halimus* can evaluate metals from contaminated soil during phytoremediation. Different organic amendments affect the metal bioavailability and uptake of metals by plants. The application of manure increases soil organic matter content and pH, and reduces metal bioavailability in the soil. This resulted in a higher total metal accumulation in plant biomass and a greater amount of metal removed from the soil, which increased metal accumulation in shoots from 37 to 139 mg/pot of Cu, from 299 to 445 mg/pot of Zn, and from 1.8 to 3.7 mg/pot of Cd. Therefore, the combination of *A. halimus* with manure was effective for phytoremediation (Pérez-Esteban et al. 2013). Manure, gypsum, hydrogel, and peat all influenced soil characteristics by changing the nutrient availability and fertility that was suitable for plant growth in degraded soil (Zupanc et al. 2014).

Low molecular weight organic acids (LMWOAs) are important root exudates that are able to bind metals, forming complexes and changing their bioavailability. Previous studies showed that LMWOAs enhance metal uptake. In particular, acetic acid was an efficient enhancer of phytoextraction. Moreover, the addition of LMWOAs minimizes the environmental risk, not only facilitating the decomposition of organo-metal complexes in the short term, but also increasing the proportion of free ions and enhancing the uptake by plants (Duarte et al. 2011). Sun and Hong (2011) suggested that citric acid plays an important role in the stress response of *Leymus chinensis*. The application of 50 mg/L citric acid effectively relieved salt stress (Sun and Hong 2011).

It is well-known that earthworms influence different soil enzyme activity, which is related to soil remediation (Tica et al. 2013). To some extent, earthworms trigger the degradation process of pollutants (Schaefer and Juliane 2007), as their activities not only increase the soil pH, macroporosity, and soil organic matter content, but also the metal concentrations in plants. For example, earthworm activity increased Pb and Cd accumulation in lettuce leaves by up to 46% (Leveque et al. 2014). Furthermore, the combined toxicological effects of Cd and Pb were influenced by the competing absorption of each metal and their bioavailability to the earthworm *Eisenia fetida* (Wu et al. 2012).

A variety of candidate salt tolerance genes have been identified in *Arabidopsis thaliana*. These genes encode Na^+ and K^+ transporters and are involved in the general stress or antioxidant response, or in compatible solute metabolism. *HKT1* family member of complexity and allelic variation can facilitate K^+/Na^+ homeostasis in response to salt stress (Hasegawa 2013). Moreover, extremophile species, such as *Thlaspi goesingense*, can be screened for transferable genes (Van Oosten and Maggio 2015). Genetic engineering provides an opportunity to transfer resistance genes to plants that have greater biomass and rapid growth, which can be used in phytoremediation (Rozema and Schat 2013).

5. Concluding remarks and perspectives

Halophytes have several additional advantages over glycophytes in the phytoremediation of heavy metals from saline soils. First, for some halophytes, salt is a key promoter in the translocation of heavy metals from roots to shoots. Second, salinity can improve heavy metal bioavailability in soils, especially for mobile heavy metals. Third, many halophytes can also accumulate large amounts of salt while extracting heavy metals from saline soils (Anjum et al. 2014).

Halophyte tolerance to salt and heavy metals may rely on common physiological mechanisms, including the adaptive strategies of: (1) osmotic adjustment through accumulation, exclusion, and compartmentalization; (2) the components of the antioxidant defense system; (3) cell walls and subcellular compartmentalization; (4) the cellular proteins and polypeptides-mediated, as well as metal chelation or detoxification; and (5) metal excretion and complexing lagans. This review summarizes some phytoremediation technologies that have been demonstrated for some species of haplotypes (Table 3).

In general, the adaptive traits of halophytes provide them with robust mechanisms to survive in toxic and extreme environments. This could be applied effectively to some crops with large biomass or/and rapid growth that have become a hotspot topic in recent research. In a broader context, using halophytes represents an important alternative to current technologies to avoid conflicts between requirements for more food, food quality, biofuels production, with the consequent desertification and salinization of agricultural land. Thus, in single or multiple-contaminated situations, combined remediation technologies should be applied to improve the efficiency of phytoremediation. This review also advocates that future research should consider the following aims: (1) provide a clear, detailed understanding of the relationship between simultaneous salt and metal tolerance; (2) use suitable halophytes that have economic value for the phytoremediation of heavy metals in saline-alkaline soil. Phytoremediation can restore polluted soil and provide some economic return. However, the long-term accumulation of heavy metals in roots over time may affect biomass production in later cropping seasons. Therefore, using Compost-Like-Output as a growth substrate will remediate heavy metal-contaminated saline soils for energy crops; (3) develop further the genetic engineering and biotechnological tools to allow the transfer the resistance genes into those plants with large biomass and rapid-growth, to circumvent the limitations of plant species under abiotic stress and to improve the potential applications of halophytes in many unexplored contexts; (4) biochar and graphene are popular in current researches. However, researches on their application to environmental pollution are not complete as some effects are still unknown; (5) the biodegradable enhancers, such as saponins and EDDS, should be further studied on their potential for phytoremediation on heavy metal-contaminated saline soils in future. Many biosurfactants can enhance the recovery of soil-bound metals. Cyclodextrin can simultaneously mobilize heavy metals and enhance the complexation and elution of organic and heavy metal; and (6) CO_2 can enhance plant biomass and is often used to help plants restore heavy metal in contaminated soils.

However, this may be a risk to food safety under the predictions of elevated atmospheric CO_2 in the future. This problem needs to be taken into consideration when halophytes are used in phytoremediation (Tian et al. 2014). Moreover, elevated CO_2 could increase the mobility of Cd and Zn due to the enhanced formation of DOM-metal complexes in the rhizosphere of *Sedum alfredii* (Li et al. 2014b).

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Tables

Table 1

Capacity for phytoextraction of heavy metals in different halophytes

Table 2

Phytovolatilization and phytoexcretion of heavy metals by halophytes

Table 3

Phytostabilization and phytoextraction of heavy metals by halophytes

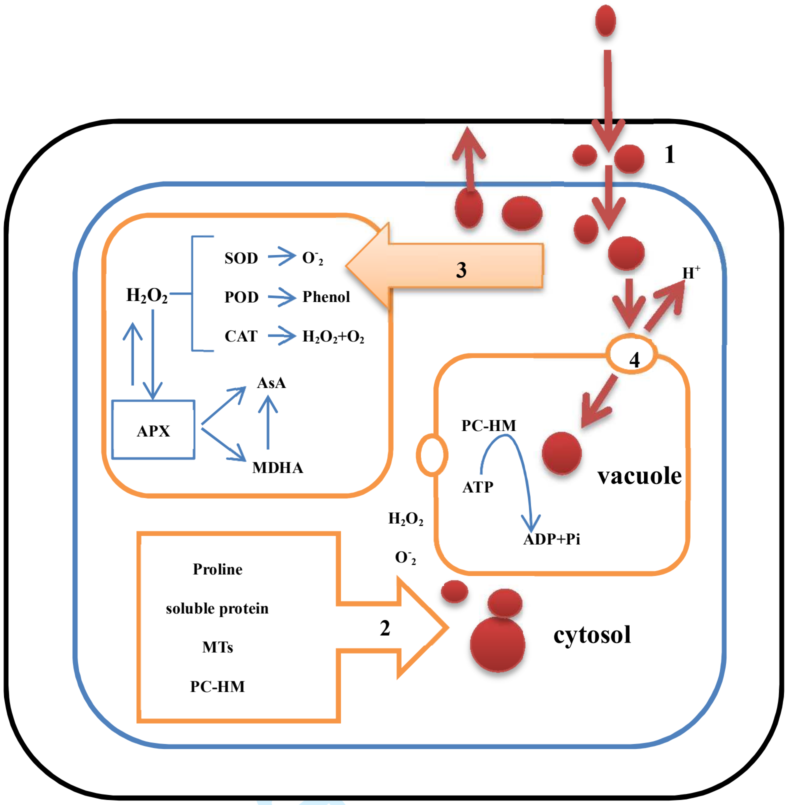
Figure Captions

Figure 1

Molecular mechanisms of heavy metal tolerance in halophytes (1 metal ions binding to cell wall; 2 metals chelated in cytosol; 3 Reactive Oxygen Species defense mechanisms; and 4 metal sequestration in vacuoles. Filled circles = metal ions)

Figure 2

Factors affecting phytoremediation efficiency



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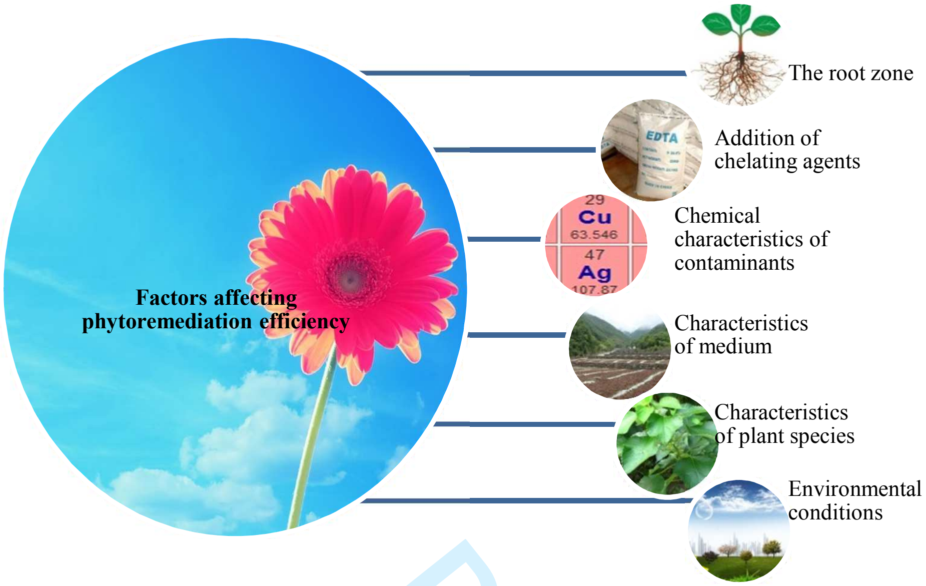

Figure 2 Factors affecting phytoremediation efficiency

Table 1

Capacity for phytoextraction of heavy metals in different halophytes

Halophytes	Heavy metals	Accumulation tissues	Reference
Atriplex halimus	Cd, Zn	Shoots	(Lutts et al. 2004)
Halimione portulacoides	Cd, Cu	Aboveground tissues	(Reboreda and Caçador 2007)
Tamarix aphylla *Armeria maritima* *Avicennia marina*	Cu, Zn, Cd	Salt glands, trichomes	(Hagemeyer and Waisel 1988; Lutts et al. 2004)
Sesuvium portulacastrum	Cd	Roots, shoots	(Ghnaya et al. 2007)

Table 2

Phytovolatilization and phytoexcretion of heavy metals by halophytes

Phytoremediation strategy	Halophytes	Heavy metals	Performance	Reference
Phytovolatilization	*Salicornia bigelovii*	Se	Se removal up to 251.6 ± 140.5 μg/m^{2}d	(Huang et al. 2013; Shrestha et al. 2006)
	Scirpus robustus *Typha latifolia*		Se removal up to 89% of addition for 3 weeks	
	Schoenoplectus californicus		Se removal up to 75% of addition for 3 weeks	
	Carex obnupta		Se removal up to 59% of addition for 3 weeks	
	Polypogon monspeliensis		Se removal up to 47% of addition for 3 weeks	
	Spartina alterniflora *Phragmites communis*	Hg	The conversion of ionic Hg into elemental Hg and subsequent volatilization from plants back to global atmosphere	(Windham et al. 2001)
Phytoexcretion	*Tamarix aphylla* *Atriplex halimus*	Cd, Zn Cu, Pb	Showed these heavy metals through glandular tissues, mainly from leaf tissues onto the leaf surface.	(Hagemeyer and Waisel 1988; Lefèvre et al. 2009)
	Tamarix smyrnensis	Cd, Pb	Uses salt excretion mechanism to excrete excess metals onto leaf surfaces.	(Manousaki et al. 2008)

Table 3

Phytostabilization and phytoextraction of heavy metals by halophytes

Phytoremediation strategy	Halophytes	Heavy metals	Performance	Reference
Phytostabilization	*Spartina maritima*	Cu, Pb	Retention and accumulation of heavy metals belowground	(Manousaki et al. 2008; Reboreda and Caçador 2007)
	Halimione portulacoides	Zn	Accumulation of metal in tissues	(Andrades-Moreno et al. 2013)
	Arthrocnemum macrostachyum	Cd	Accumulation of metal in roots	(Redondo-Gómez et al. 2010)
	Atriplex halimus subsp. *schweinfurthii*	Cd	Accumulation of metal in roots	(Nedjimi and Daoud 2009)
	Elsholtzia splendens	Cu	Accumulation of metal in roots	(Jiang et al. 2008)
	Commelina communis	Cu	Accumulation of metal in roots	(Wang and Zhong 2011)
	Spartina alterniflora	Cu	Accumulation of metal in roots	(Chai et al. 2014)
	Suaeda salsa	Pb, Zn	Accumulation of metals in roots	(Wu et al. 2013)
	Salicornia ramosissima	Cd	Accumulation of metal in roots	(Pedro et al. 2013)
	Salicornia brachiata	Cd, Ni, As	Accumulation metals in roots	(Xu et al. 2010)
	Phragmites australis	Cd	Accumulation of metal in roots	(Nunes da Silva et al. 2014)
	Sarcocornia perennis	Cd, Zn, Cu, Co	Accumulation of metal in roots	(Lefèvre et al. 2010)
Phytoextraction	*Halimione portulacoides*	Cu, Cd	Accumulation in aboveground	(Reboreda and Caçador 2007)

			plant tissue for subsequent removal	
	Moringa oleifera	Cd	Through leaf extraction	(Howladar 2014)
	Zygosaccharomyces rouxii	Cd, Zn, Cu, Pb	Extraction and exclusion of heavy metals	(Li et al. 2013)
	Tamarix smyrnensis Bunge	Cd, Pb	Accumulation in harvestable parts	(Kadukova et al. 2008; Manousaki et al. 2009)
	Atriplex halimus	Cd, Pb, Mg, Zn	Translocation to aerial parts	(Lutts et al. 2004; Manousaki and Kalogerakis 2009)
	Sesuvium portulacastrum *Mesembryanthemum crystallinum*	Cd	Accumulation was highest in roots, then transferred to shoots	(Ghnaya et al. 2005)