

Chapter 9

PHYTOREMEDIATION OF MULTIPLY METAL-CONTAMINATED ENVIRONMENTS: SYNERGISTIC AND COMPETITIVE EFFECTS BETWEEN HEAVY METALS DURING UPTAKE AND TRANSPORT

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ABSTRACT

Phytoremediation is a promising alternative to conventional metal treatment methods; however, most phytoremediation studies separately consider the removal of each individual metal, which may not fully reflect the situation present in real world contamination sites. Metal-contaminated environments seldom contain a single species of metal, and are instead host to several types of toxic metals and other contaminants. Consequently, the synergistic and antagonistic effects displayed between essential and non-essential metals, as well as these between metallic and non-metallic contaminants, are an important factor in determining the bioremediative efficiencies of plant species. The present chapter outlines the uptake, transport and sequestration mechanisms relevant to heavy metal accumulation, considers the potential competitive and cooperative interactions that occur between metals during these processes, details the current literature regarding bioremediation in multiply metal-contaminated environments and offers insights into the biochemical interactions underlying the trends observed for the beneficial and detrimental effects displayed between the accumulations of certain metals. We also illustrate the potential of metal remediation by aquatic macrophytes, a group

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known for the effective remediation of multiple metals, which possess life histories that render them particularly conducive to studies investigating the impact of multiple metals on metal uptake.

Keywords: Phytoremediation, Synergistic, Antagonistic, Transport, Heavy metal

1. INTRODUCTION

Industrial, agricultural and domestic processes all contribute strongly to the release of metal compounds into the environment, often in forms more available to biological systems than the metallic compounds naturally present in soils and sediments. Unlike many other types of contaminants, metals do not degrade naturally over time, and their capacity to accumulate progressively through the food chain renders them particularly dangerous to apex predators, including humans. Both essential and non-essential metals display toxic effects, primarily involving developmental defects and various neoplasms, above a threshold concentration. As such, it is imperative to control the extent of metal release into the environment, and to reduce the amount that is already present in natural soil and freshwater sources.

While effective methods, such as chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery, have been developed for the removal of metals from industrial wastes, the costs associated with these processes prevent their application over large areas of metal-contaminated soil or water (Zahoor and Rehman 2009). In addition, such methods frequently rely on mechanisms that are impossible to implement in natural environments without significantly damaging the local ecosystem. Consequently, several alternative approaches have been proposed for metal remediation in natural environments, and phytoremediation in particular has attracted much attention as a cost-effective means of metal removal in such locales (Yao et al. 2012). Phytoremediation, the sorption, reduction or sequestration of metals by dead or living plant tissues, allows the removal of contaminants without leaving a lasting impact on the environment, which renders this method ideal for metal remediation in metal-contaminated soils and freshwater sources, either by itself or in tandem with conventional metal removal techniques (Ali et al. 2013).

Metal removal characteristics of plants are well-described in the literature. Terrestrial plants and free-floating macrophytes are exposed to metals primarily by their root surfaces, while root, stem and leaf tissues of emergent and submerged aquatic plants are all in contact with the metal-contaminated environment, which figures heavily into the accumulation trends displayed by soil- and waterborne plants (Figueira et al. 2012; Verbruggen et al. 2013). In addition, while all plant material is expected to display some amount of metal uptake, certain plants are known to preferentially sequester certain metals (such as the zinc hyperaccumulator *Arabidopsis halleri* or the chromium hyperaccumulator *Leersia hexandra*) and may accumulate metal concentrations ~1000 times that of the environment in their tissues (Mishra et al. 2008; Liang et al. 2009; Liu et al. 2011). While the plant species is an important determiner of remediation capacity, metal accumulation also depends on the length of exposure, the metal of interest, its concentration, environmental parameters (*e.g.* temperature, salinity and pH) and the presence synergistic or antagonistic interactions with other metals in

the environment. Depending on the valence, concentration and uptake mechanisms of “competing” metals, their presence may assist in, hinder, or be altogether irrelevant to the remediation characteristics of the metal of interest; and since polluted areas are seldom contaminated with only a single type of metal, interactions between multiple metal species are inevitable in most real-world applications of phytoremediation.

Given the importance of metal-metal interactions in the uptake and transport of metals, the present chapter will be devoted to the mechanisms by which metal entry and transportation occur in plants, and the changes that occur in accumulation behavior when multiple metals compete or cooperate within these pathways.

2. METAL UPTAKE, TRANSPORT AND SEQUESTRATION MECHANISMS IN PLANTS

2.1. General Trends in Metal Accumulation in Plants

Plants can be grouped under four categories according to their accumulation behavior: non-specialists (or “ordinary plants”), bioindicators, excluders and hyperaccumulators (van der Ent et al. 2013). The majority of plant species can be categorized as non-specialists with regards to survival in metal-contaminated environments, and are capable of tolerating small amounts of metals, but do not possess the specialized mechanisms necessary for alleviating the increased stress associated with high metal concentrations. Bioindicators are hardy plants that tolerate contaminants to a greater degree than non-specialists, and the metal concentrations in their tissues often reflect the extent of metal contamination in the environment, which renders them important for the monitoring of metal pollution. In contrast, excluders resist metal contamination by preventing metal ions from entering their metabolism, though they also experience toxic effects at higher doses of metals, against which their contingency mechanisms begin to falter. Finally, hyperaccumulators store much higher concentrations of metals within their tissues, potentially utilize these metals as a form of defense mechanism, and experience little to no toxic effects in return, sometimes relying on metals to such an extent that concentrations that would be fatal to non-specialists, excluders and bioindicators may be necessary for the survival of a hyperaccumulator species (Rascio and Navari-Izzo 2011; van der Ent et al. 2013). It should be noted that a plant may display different accumulation trends for different contaminants, e.g. by hyperaccumulating only a select number of pollutants and displaying no such capacity for others (Antiochia et al. 2007). For a plant to be classified as a hyperaccumulator of a given metal, it must be able to tolerate concentrations above a set threshold for that metal; this threshold concentration is $100 \mu\text{g g}^{-1}$ for Cd, Se and Tl, $300 \mu\text{g g}^{-1}$ for Cu, Co and Cr, $1000 \mu\text{g g}^{-1}$ for Ni, As and Pb, $3000 \mu\text{g g}^{-1}$ for Zn and $10000 \mu\text{g g}^{-1}$ for Mn (Baker 1981; McGrath et al. 2000; van der Ent et al. 2013).

Following (or sometimes concurrent with) uptake, five broad types of mechanisms can facilitate the remediation of air, water or soil-borne contaminants: phytoextraction, phytovolatilization, rhizofiltration, phytodegradation and phytostabilization (Figure 1) (Cummings 2009; Valida et al. 2010; Dordio and Carvalho 2011). Of these, phytodegradation and phytovolatilization are less applicable to metals, given that metals cannot be broken down

into nontoxic materials and do not usually form volatile compounds in biological systems. Phytostabilization and rhizofiltration involve the modification and sequestration of toxic metals at the uptake site, while phytoextraction entails both the uptake of metal ions from the surrounding soil or water, and their transport from the roots to the other parts of the plant.

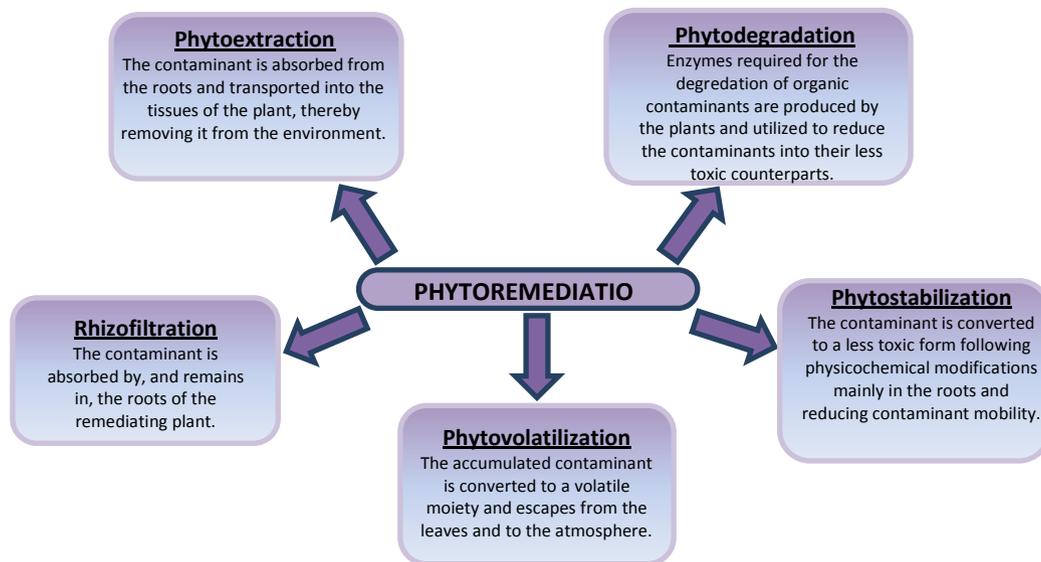


Figure 1. Mechanisms by which plants facilitate the removal of pollutants from the environment.

Metal uptake in terrestrial plants and free-floating macrophytes occurs primarily through the roots, as metals and their compounds are usually non-volatile, and solid particles that are deposited on above-ground tissues are not easily recovered by plants. As metal cations are readily available in water, however, all tissues of submerged macrophytes can take part in metal uptake. In both aquatic and terrestrial plants, uptake begins with the transitory association of metal ions to the plant surface by adsorption, followed by proton pump-, membrane transporter- or ion channel-mediated internalization (Hall 2002). In addition, metal ions or complexes may directly penetrate cell membranes without the aid of a transporter protein, though chelating mechanisms exist to prevent the undesired metal entry in this manner (Simkiss 1983; Macfie and Welbourn 2000).

Plant symbionts may intimately interact with the metabolism of their hosts and, by extension, play a role in metal uptake by plants. Rhizobacteria (PGPR) are a group of bacterial symbionts that enhance nutrient uptake and contribute to plant defense systems, and their presence is also known to enhance the mobilization of metal ions by acidification, redox reactions and the production of chelating agents, such as siderophores. Rhizobacteria can also enhance the dissolution of metal ions from bulk particles, which is a prerequisite of metal uptake (Evangelou et al. 2007). Arbuscular mycorrhizal fungi (AMF) may also assist in the entry of metals into plant roots; however, there is some evidence that these fungi may also function as a filter for metals, thereby preventing metal uptake by plant tissues (Hildebrandt et al. 2007; Zhuang et al. 2007). Little is known about the AMF-mediated pathways that regulate metal uptake by plant roots; however, the metallothionein gene of *Gigaspora margarita* has been found to be up regulated upon Cu introduction, while GintZnT1 gene

expression in *Glomus intraradices* is reported to be increased under Zn overexposure, and *GintABC1* in response to Cd and Cu (Lanfranco et al. 2002; Gonzalez-Guerrero et al. 2005; Clemens 2006a). As such, metallothioneins, phytochelatins and metal transporter genes of symbiotic fungi also appear to be important for the metal uptake of their hosts.

2.2. Metal Transport Mechanisms

Due to the detrimental effects of many metals, plants generally lack the capacity for their specific uptake and transport, and instead possess mechanisms that exclude them from their tissues. However, despite the lack of specialized mechanisms for the uptake of elements with no metabolic functions, many non-essential and even severely toxic metals are readily recovered by plant tissues, as similarities in valence states, hydrodynamic radii or other chemical properties may allow a non-essential metal to utilize the mechanisms evolved for the transport of other metals (some toxic effects of non-essential metals are also caused by this similarity, which permits them to replace cofactors in enzymes and therefore disrupt enzymatic activity). Cd, for example, has been reported to utilize the metabolic pathways that function for the transport of Zn (Clemens 2006b), and the divalent cation-transporting protein IRT1 may transport Cd^{+2} in addition to essential ions such as Fe^{+2} , Mn^{+2} or Co^{+2} (Cohen et al. 1998). Likewise, chromate (CrO_4^{-2}) anions can utilize pathways intended for sulfate transport, while arsenate and selenium are sufficiently similar to phosphate and sulfur to be actively recovered by plants. This lack of specificity may allow essential and non-essential metals to compete over a shared transport pathway, and competitive interactions may also exist between groups of chemically similar essential metals (Jadia and Fulekar 2009). Consequently, the concentrations of both essential and non-essential metals in the environment contribute considerably to the efficiency of phytoremediation efforts.

A great variety of transporter proteins function in the transfer of metal ions within and between cells and tissues (Table 1). These include metal transporting ATPases, the natural resistance associated macrophage protein (NRAMP) family, the cation diffusion facilitator (CDF) family, the ZRT/IRT (Zn regulated transporter/iron regulated transporter) like protein (ZIP) family, the Ca^{2+} -sensitive cross complementor 1 (CCC1) family, the yellow-stripe 1-like (YSL) subfamily, the iron-regulated protein (IREG) family and the copper transporter (COPT) family. Other membrane proteins involved in the transport of transition metals are multidrug resistance-associated proteins (MRP), the ABC transporters of the mitochondria (ATM), the cation exchanger (CAX) family, three subfamilies of ATP-binding cassette (ABC) transporters and the pleiotropic drug resistance (PDR) transporters. In addition, phytochelatins, metallothioneins and certain organic acids, amino acids and phosphate derivatives are known to display metal-binding functions. AtFRD3 (Ferric Reductase Defective 3) and AtZIF1 (Zinc Induced Facilitator 1) are other proteins suspected to be heavily involved in the transport of metals (Guerinot 2000; Williams et al. 2000; Rogers and Guerinot 2002; Green and Rogers 2004; Lee et al. 2005; Kramer et al. 2007; Manara 2012).

Table 1. Proteins and other organic materials involved in metal uptake, transfer or sequestration

Metabolite name	Classification	Metals	Mechanism*	Reference
Type 2 metallothionein	MT	Cd, Cu, Zn	3	Hildebrandt et al. 2007
PCS (phytochelatin synthase)	PC	Cd, As, Hg	3	Hildebrandt et al. 2007
HSP90	Heat shock	Cd, Cu, Zn		Hildebrandt et al. 2007
GST	GSH	Cd, Cu, Zn	3	Hildebrandt et al. 2007
ABC-type transporter protein	Vacuole transport protein	Cd, Mg	2	Lu et al. 1997
Anthocyanins	Secondary metabolite	Mn, Cd, Zn, Co, Ni, Mg	3	Pilon-Smits and Pilon 2002
IRT1	ZIP family	Cd, Zn, Fe	2	Meagher 2000
Zn transporter	ZIP family	Fe, Mn, Zn	2	Hildebrandt et al. 2007
AtHMA4	P-type ATPase	Zn, Cd	2	Yang et al. 2005
CDF family proteins	CE family	Zn, Co, Cd	2	Yang et al. 2005
OsNramp1, 2, 3	Nramp	Cd, Mn, Co, Zn	2	Belouchi et al. 1997
Glomalin	Glycoprotein	Cu, Cd, Pb	4	Gonzalez-Chavez et al. 2004
EDTA (HEDTA, DTPA, CDTA, EGTA etc.)	Aminopolycarboxylic acid	Cd, Ni, Pb, Zn, Cu	1	Evangelou et al. 2007
Phytosiderophores	Siderophore	Fe, Zn, Cu, Mn	1	Yang et al. 2005
TgMTP1, COT1, ZRC1	Vacuolar metal ion transporter (CE family)	Ni, Cd, Co, Zn	4	Persans et al. 2001
ACC deaminase	Deaminase	Cd, Co, Cu, Ni, Pb, Zn	1	Grichko et al. 2000
YCF1 (transgenics)	Recombinant protein	Pb, Cd	4	Kramer 2005
HMA4 (transgenics)	Recombinant protein	Zn, Cd	2	Kramer 2005

Mechanisms are divided into four parts; 1) Uptake from environment or extracellular matrix, 2) Transportation systems, 3) Chelation mechanisms and 4) Segregation or sequestration in vesicular structures.

2.3. Sequestration of Accumulated Metals

Once within plant tissues, metals are eliminated under three principal mechanisms: They can be neutralized by the cell membrane, sequestered within the cell following internalization,

or retained outside the cell (Basile et al. 2012). Within each category, several specific pathways exist for the minimization of detrimental effects following metal uptake (Figure 2). In cell membrane-mediated neutralization, a negatively charged, membrane-bound residue fixes the metal group to the cell membrane and prevents its entry into the cell, which prevents the metal from interacting with intracellular materials. Keeping the metal ions outside the cytoplasm entails both the blockage of metal ion entry, which involves decreases in membrane permeability and transporter expressions, and the expulsion of intracellular metal ions by specialized transporters. Metal ions that are already present within the cell can also be deposited within apoplasts by the action of membrane proteins, or rendered harmless by metal-binding moieties such as metallothioneins, organic acids, amino acids and phytochelatin. Once bound, the metal-ligand complex is subsequently deposited within a vacuole. Tonoplast transporters are also effective in sequestering metal ions within vacuoles (Manara 2012).

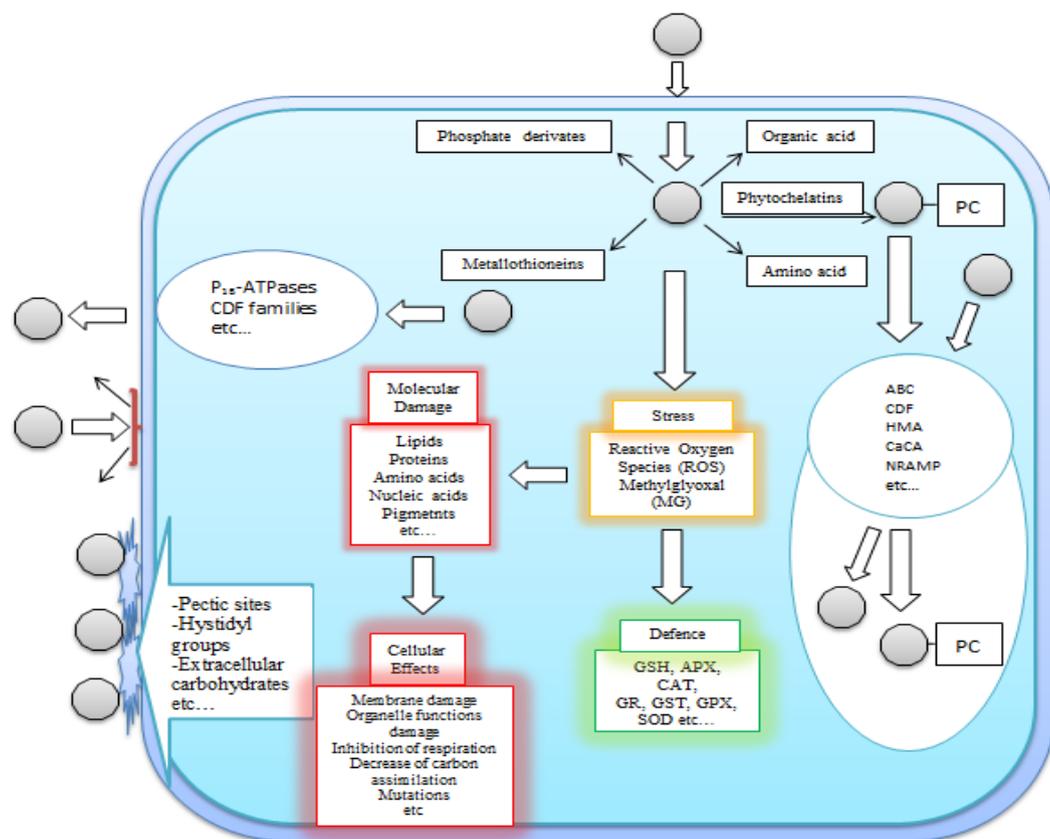


Figure 2. Response mechanisms for the presence of excess metal ions within the cell (Hossain et al. 2012; Manara 2012; Zitka et al. 2013).

While some metals are essential for living organisms, other metals participate in no known metabolic activity and are not necessary to sustain life. Non-essential metals, as well as excess amounts of essential metals, are exported outside the cell or sequestered within a vacuole or metal-ligand complex (Peng and Gong 2014). However, if these compensatory mechanisms are insufficient to counteract the excess metal concentrations, these metals cause

intracellular damage under three principal mechanisms, (a) by interacting with thiol, histidyl and carboxyl groups present in peptides, (b) by stimulating the formation of reactive oxygen species (ROS) and (c) by displacing essential metals as cofactors and therefore interfering with protein function (Schutzendubel and Polle 2002). ROS are regularly produced as a result of many intracellular reactions, but the compensatory mechanisms are normally able to alleviate the potential damage that would be caused by these molecules. Metal ions are known to stimulate the formation of free radicals and ROS such as singlet oxygen ($^1\text{O}_2$), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot\text{OH}$) (Sharma and Dietz 2009). The creation of additional ROS presence by metal ions may therefore overwhelm the response mechanisms in place. These radicals then react with cellular components to create various types of cellular damage, such as lipid peroxidation, protein oxidation, enzyme inactivation and DNA damage (Hossain et al. 2012). These effects, in turn, result in physiological or metabolic damage to the cell.

3. EFFECT OF MULTIPLE METALS ON METAL UPTAKE AND TRANSPORT

3.1. Mechanisms Underlying Synergistic and Antagonistic Effects between Metals

Non-essential metal uptake and transport mechanisms generally utilize pathways normally involved in the absorption of essential metals, and mechanisms for their exclusion or sequestration are often shared between different types of metal species (Pence et al. 2000; Williams et al. 2000). Consequently, the presence of an essential or non-essential metal may alter the uptake, transport and sequestration of other metals. These interactions may include direct effects, such as competition over specific binding sites or co-uptake by transport proteins, as well as more indirect mechanisms in which the presence of one metal activates defensive processes that protect the plant from other metals (or, conversely, trigger the enhanced uptake of another metal). In addition, remediative efforts using live plants are obviously futile if a contaminant in the environment is fatal to the intended remediative agent, even if the plant in question may effectively sequester the remaining pollutants (a chromium hyperaccumulator, for example, cannot necessarily be used in locales contaminated with both chromium and arsenic, unless it possess sufficient resistance to arsenic in addition to chromium). As such, the ability of a plant to remediate a contaminated environment depends on the environmental conditions present in the surrounding medium, including not only the metallic nutrients and their uptake mechanisms, but also the precise composition of the metal mixture present in the environment. These metal-metal interactions can be classified under three closely related categories:

3.1.1. Binding-Mediated Effects (Competition and Co-uptake)

While non-essential metals are often considered in their capacity to compete with essential metals, shared use of identical uptake and transport mechanisms may also force multiple non-essential metal species to compete over a limited number of binding sites. Conversely, metals utilizing these mechanisms will tend to co-accumulate if a competitive

environment is absent. Major classes of metal-binding proteins, such as phytochelatins and metallothioneins, are not specific to a single metal (but may heavily favor complexes with a specific metal, e.g. for phytochelatins and Cd) and the adsorption-mediated initiation of metal uptake depends on the surface chemistry of the plant and the metal ion, allowing metals with similar valence states to potentially substitute for each other (Zenk 1996). As such, metals displaying similar affinities to common binding sites present on plant surfaces or in tissues and cells may show similar trends in accumulation, and potentially exclude each other in higher concentrations. This dose-dependent effect potentially contributes to the complex, dose- and tissue-specific interplay of antagonistic and synergistic interactions observed in some studies (Liu et al. 2008).

3.1.2. Compensatory Mechanism-Mediated Effects

The presence of excess metal concentrations triggers compensatory processes that prevent the entry of metals into plant tissues, allowing the plant to survive in environments that would otherwise be fatal (Steffens 1990; Maksymiec 2007). These mechanisms, which rely on the above-mentioned binding and chelation effects, are often general and may sequester a large variety of metals, allowing the plant to exclude or co-accumulate multiple metal species that are present in the environment, even if the defensive mechanism in question was activated by a single species of metal. These pathways may also alter the manner in which the uptakes of essential elements are maintained, which disrupts cellular homeostasis and contributes to the tissue damage created by heavy metal presence (Hall 2002). Element depletion may also activate scavenging pathways that are utilized by metals to facilitate tissue or cell entry, and therefore enhance metal remediation. As such, the lack of an essential metal, such as Fe, may trigger the upregulation of membrane transporters that non-specifically uptake other metals, such as Cd (Cohen et al. 1998; Thomine et al. 2000).

3.1.3. Toxicity-Based Effects

Non-essential metals, as well as excess amounts of essential metals interfere with the function of enzymes, and indirectly facilitate the creation of reactive oxygen species responsible for many forms of cellular damage. In addition, environments contaminated with metals often feature other forms of pollution, and species intended for use as live phytoremediation agents must be sufficiently resistant to any contaminant that is present at the site of interest, and should preferably accumulate all such pollutants. However, combinations of metals may be more toxic than when administered individually, and the presence of a severely detrimental element may hamper remediative efforts with a plant species that displays effective uptake of other metals. As such, the additive or synergistic toxicity of metals should be taken into account when real-world applications of phytoremediation are considered. The reverse (and unlikely) case, of decreased metal toxicity due to competitive effects, and should not be considered significant for live plants intended for use in bioremediation, and may be undesirable as the competition may also decrease the amount of metal accumulated by the plant, thereby lowering remediation efficiency.

3.2. Specific Examples of Synergy and Antagonism between Metal Pairs

A large number of synergistic and antagonistic interactions between metals are described in the literature, some of which are presented in Table 2. The interactions of important and widespread pollutants, such as Cd, Zn and Pb, are relatively well-characterized; however, the nature of these interactions may vary significantly between individual studies (Chaoui et al. 1997; Grispen et al. 2006). These discrepancies are in line with both the complexity of multi-metal interactions, and the variable nature of multi-metal uptake mechanisms; as transporters that assist in the transfer of two or more metals may create synergistic or antagonistic interactions depending on the availability of each metal, and the impact of metal toxicity on plant metabolism may affect the uptake of other metals. Plant species, metal concentrations and environmental parameters are therefore expected to significantly alter the outcome of uptake in multi-metal environments. Such concentration-dependent interactions have been noted between Cd and Zn, which are known to share transport proteins, as well as between Pb and Zn, and Cd and Pb (Lombi et al. 2001; Zhao et al. 2002; Grispen et al. 2006; Angelova et al. 2008).

It is notable that antagonistic effects are more frequently noted in phytoremediation research, while synergistic effects are comparatively more pronounced in studies that concern the toxicity of metals. This situation may be interpreted as a result of the differences in the models and metal concentrations utilized in these two types of research. In toxicity studies, near-lethal doses are usually applied, resulting in rapid, synergistic lethality. In remediation studies, hardier plants and tolerable metal concentrations are used, potentially bringing competitive interactions to the fray.

4. NON-METAL CONTRIBUTORS TO METAL REMEDIATION

While the present chapter underlines the effects of metal contaminants on the uptake profiles of each other; non-metal contaminants, chelating agents, symbiotic organisms and stress factors may display supportive or detrimental effects similar to these recorded for mixtures of metals (Table 3). The inclusion of EDTA or other chelating agents, for example, are known to solubilize metals and better facilitate their uptake from the soil, and the deliberate introduction of these materials has been suggested as a means to improve remediation efficiency (Meers et al. 2005; Evangelou et al. 2007). However, these materials may also allow the metals present on the surface to leach through to deeper layers (Wu et al. 2004). Other environments, such as these provided by rhizospheres, instead serve to decrease metal uptake (Meagher and Heaton 2005). Non-metal contaminants, such as organic hydrocarbons, may also affect metal uptake, and usually create synergistically toxic effects (e.g. for PCP and Cu in *Lolium perenne* and *Raphanus sativus*, or for nitrilotriacetate and Cd, Cu and Zn in *L. perenne* and *Lactuca sativa*) (Kulli et al. 1999; Lin et al. 2006).

Table 2. Synergistic and antagonistic interactions between metal accumulations and toxicities in higher plants

Organism(s)	Metal/Metalloid	Nature of effect*	Reference
<i>Glycine max</i>	As/Cd, As/Pb, As/Cd/Pb	S (As/Cd, As/Cd/Pb)/A (As/Pb)	Luan et al. 2008
<i>Brassica spp.</i>	Cu/Zn	A (Zn influenced by Cu but not vice-versa)	Ebbs and Kochian 1997
<i>Pelargonium hortorum</i>	Cu/Zn	A	Orrono et al. 2012
<i>Pisum sativum</i>	Cd/Mn	A	Hernandez et al. 1998
<i>Brassica napus</i>	Cd/Zn	S	Grispen et al. 2006
<i>Brassica napus</i>	Cd/Pb, Cd/Zn, Pb/Zn	S/A (dependent on tissue and treatment)	Angelova et al. 2008
Higher plants	Cd/Fe	S/A (S at low doses, A otherwise)	Siedlecka and Krupa 1999
<i>Amaranthus spp.</i>	Fe/Ni	A	Shevyakova et al. 2011
<i>Allium fistulosum</i>	Hg/Se	A	Afton and Caruso 2009
<i>Glycine max</i>	Hg/Se	A	Yathavakilla and Caruso 2007
<i>Brassica juncea</i>	Hg/Se	A	Mounicou et al. 2006
<i>Beta vulgaris</i>	Cd/Mn	A	Singh and Agrawal 2007
<i>Phaseolus vulgaris</i>	Cd/Zn	A	Chaoui et al. 1997
Submerged aquatic plants	Cd, Cu, Hg, Pb in mixtures	S	Jana and Choudhuri 1984
<i>Triticum aestivum</i>	Cd/As	S	Liu et al. 2007
<i>Oryza sativa</i>	Cd/Cu	S (Cd influenced by Cu but not vice-versa)	Huang et al. 2009
<i>Lemna minor</i>	Cd/Pb, Cd/Zn, Pb/Zn	A	Mohan and Hosetti 1997
<i>Vetiveria zizanioides</i>	Cd/Zn	S	Xu et al. 2009
<i>Lactuca sativa</i>	Fe/Cd	A	Thys et al. 1991

* S denotes a synergistic relationship between metal(loid) ions; A denotes an antagonistic relationship.

5. AQUATIC MACROPHYTES: AN IDEAL GROUP FOR MULTIPLE METAL CONTAMINATION STUDIES?

5.1. Macrophyte Biology and Remediation Potential

Macrophytes, aquatic higher plants, are the dominant plants in the shores of flowing or still freshwater sources, and may be submergent, emergent or free-floating. Emergent macrophytes grow near the shore and break the water surface, though their roots and part of their stem are below the water. Submerged macrophytes, in contrast, are wholly below the

water, except possibly for their flowers, while free-floating macrophytes are largely above the water surface; they may be loosely attached to the substrate by their roots or be altogether rootless. Macrophytes are ecologically important due to their release of oxygen into the freshwater ecosystem, as well as their status as a principal food source for herbivorous fish. In addition, a thick covering of these plants serve as a refuge to invertebrates and small fish, and protect these animals from predation.

Table 3. Non-metal materials reported to affect the uptake of metals

Materials	Description	Metal(loid)s	Reference
Anthocyanins	Vacuolar Pigment	Mn, Cd, Zn, Co, Ni, Mg	Pilon-Smits and Pilon 2002
Phytosiderophores	Siderophore	Fe, Zn, Cu, Mn	Yang et al. 2005
EDTA	Synthetic aminopolycarboxylic acid	Zn, Cu, Cd, Ni	Meers et al. 2005
Rhizosphere	Root environment	Hg, As	Meagher and Heaton 2005
Tartaric acid	Low mw org. acids	Cd, Pb, Cu, Zn	Ke et al. 2006
EDDS (ethylene diamine disuccinate)	Natural aminopolycarboxylic acid	Cu, cd, Zn, Pb	Yip et al. 2009
TCE (trichloroethylene)	Linear halogenated carbons	Hg	Zhang et al. 2013
TNT (trinitrotoluene)	Nitroaromatics	Cd, Pb	Lee et al. 2007
Dioxin	PCB (polychlorinatedbiphenyls)	Cd, Cu	Wu et al. 2012
Benzoapyrene	PAH (polycyclic aromatic hydrocarbons)	Cd, Cu, Pb	Sun et al. 2011

Macrophytes are moderately capable metal accumulators, and can deposit environmental toxins in their root, stem or leaf tissues (Axtell 2003; Miretzky et al. 2004). Their ability to readily remediate metals from an aquatic medium makes them model research subjects in the field of toxicology (Vardanyan and Ingole 2006). In addition to their remediative capacity, macrophytes are easy to harvest and culture, serve as bioindicator species for a variety of metal species (Garczarska and Ratajczak 2000), proliferate rapidly and serve as the initial link between aquatic toxins and higher steps of the food chain (Singh et al. 2006), which makes them preferred organisms in phytoremediation studies. For example *Lemna minor* is one of the most commonly utilized duckweed species in academic studies (figure 3). *L. minor* has been reported for the effective remediation of several metals, with high removal rates within 24 h of exposure to Pb and Cr (Hurd and Sternberg 2008; Üçüncü et al. 2013). In addition to *L. minor*, other macrophytes (e.g. *L. gibba*, *Microspora* spp.) have been successfully tested in remediation studies using aquatic media.



Figure 3. *Lemna minor* fronds in (a) wilderness and (b) laboratory culture.

5.2. Potential Uses of Macrophytes in Phytoremediation

In addition to land-based metal removal efforts, there is a considerable amount of research performed on the phytoremediation of contaminated freshwater ecosystems (Table 4). However, large-scale studies in natural environments are lacking in this area. Laboratory experiments are generally limited to the remediation of small volumes of water, which may not be sufficiently predictive of the *in situ* remediative potential of a plant species, as the success of phytoremediation efforts depends on a large number of environmental variables. As such, pilot studies in large bodies of water are necessary to evaluate whether aquatic plant-based treatment methods are sufficient for the remediation of contaminated

freshwater sources. Examples of such large-scale studies include the removal of wastewater metals in alga- or duckweed-containing pools (Sekomo et al. 2012), and the use of *L. minor* for the remediation of a eutrophic lake (Ansari and Khan 2008).

Tablo 4. Macrophyte species used for bioremediation studies, and their accumulation capacities

Family species	Heavy metals	Accumulation/removal rates (%)	References
Araceae <i>Lemna minor</i>	Cu Cr Pb	37.17%-51% Over 99% 89.96-96.78%	Üçüncü et al. 2013
Azollaceae <i>Azolla filiculoides</i>	Cd Cu	1623.20 g g ⁻¹ 6013.1 g g ⁻¹	Valderrama et al. 2013
Araceae <i>Lemna minor</i>	As	430 mg kg ⁻¹ DW	Favas et al. 2012
Azollaceae <i>Azolla caroliniana</i>		397 mg kg ⁻¹ DW	
Araceae <i>Lemna gibba</i> L.	Zn	4.23 to 25.88 mg g ⁻¹ DM	Khellaf and Zerdaoui 2012
Araceae <i>Spirodela polyrrhiza</i> L.	As	400–900 mg kg ⁻¹ DW	Zhang et al. 2011
Pontederiaceae <i>Eichhornia crassipes</i>	Hg, As	In roots 0.45±0.02 µg g ⁻¹ (Hg), 0.34±0.012 µg g ⁻¹ (As) In leaves 0.29±0.02 µg g ⁻¹ (Hg), 0.25±0.01 µg g ⁻¹ (As)	Mishra et al. 2008
Araceae <i>Lemna minor</i>		In root 0.38±0.03µg g ⁻¹ (Hg), 0.29±0.01µg g ⁻¹ (As) In leaves 0.25±0.01µg g ⁻¹ (Hg), 0.21±0.001µg g ⁻¹ (As)	
<i>Spirodela polyrrhiza</i>		In root 0.35±0.01µg g ⁻¹ (Hg), 0.26±0.01 µg g ⁻¹ (As) In leaves 0.23±0.02 µg g ⁻¹ (Hg), 0.16±0.01 µg g ⁻¹ (As)	
Pontederiaceae <i>Eichhornia crassipes</i>	As	600 mg As ha ⁻¹ d	Alvarado et al. 2008
Araceae <i>Lemna minor</i>		140 mg As ha ⁻¹ d	
Araceae <i>Lemna minor</i> L.	Cu	6,135 µg Cu g ⁻¹	
Araceae <i>Lemna minor</i> L.	Pb, Cd	1116 µg g ⁻¹ (Exposed to 50 µg Pb m L-1)	Saygıdeğer and Doğan 2004
Araceae <i>Lemna minor</i>	Pb, Ni	% 76(Pb), % 82(Ni)	Axtell 2003

In addition to accumulative potential, the toxicity of the contaminant on the remediative organism is an important aspect of bioremediation. In most phytoremediation studies, the detrimental effects that the contaminant may have had on the plant are also evaluated, and understanding the cellular or tissue stress responses that the remediative agent may produce in

response to the contaminant is important to determine how the remediation process occurs. By extension, the behaviors of the remediative agent can also be taken to be representative of the physiological and morphological responses displayed by contaminant-exposed organisms. These responses are often severe, and their details are frequently described in the literature. For example, in a study using *Chlorococcum hemicolum*, the presence of Ni was found to decrease total sugar, chlorophyll and carotenoid levels due to metal stress-related effects (Harish et al. 2008). In another study, the effects of sewer water Cu on the seeds of *L. minor* and *Raphanus sativus* were determined, and the first 8-16 days of the 64 day-long study were marked by ammonia-derived toxicity, as the higher pH was found to be detrimental for *Lemna* (Fjallborg 2003). In a third study, two-metal combinations of Cr, Pb and Cu were tested for toxic effects on *L. minor*, and high biomass inhibition was observed in every mixture containing Cu. (Üçüncü et al. 2013).

It is also notable that different sections of a plant are subject to different remediative capacities and characteristics; while some plants uptake metals using their roots; others accumulate metals in their leaves. Three aquatic macrophytes (*Eichhornia crassipes*, *L. minor* and *Spirodela polyrrhiza*) were shown to remediate As and Hg more effectively with their roots, compared to their leaves, in the wastewater of a coal mine. Likewise, in *Acacia victoria*, Pb accumulation was shown to be concentrated in the roots (Mahdavi et al. 2014). On the other hand, some species store metals in their shoots, such as *Schoenoplectus lacustris*, which was shown to be a shoot accumulator for Mn and Cd (Duman et al. 2007).

Consortiums are also important in this field of research, and may be more effective than phytoremediation efforts involving a single species. A study, utilizing the plants *Pistia stratiotes*, *Eichhornia crassipes*, *Hydrocotyleum bellatta*, *Lemna minor*, *Typha latifolia*, and *Scirpus acutus*, has demonstrated that mixtures of plants are more capable phytoremediators compared to individual plants, suggesting that a cooperative effect may exist when tissues from different plants are used (Farid et al. 2014).

In addition to their ecological utility, phytoremediation studies are also relevant to the fields of molecular biology and genetics, as the selection of metal-accumulating strains, or the insertion of genes that confer metal resistance, may result in the emergence of strains with enhanced bioremediation capacity.

5.3. Efficiency of Macrophytes for Metal-Metal Interaction Studies

Due to their small size, ease of procurement and rapid growth, macrophytes are popular models for toxicology research, and these features also render them desirable for use in multiple-metal studies. In addition, as water as a highly uniform medium and ensures the even distribution of metal ions, the use of macrophytes eliminates the problem of non-uniform metal concentrations that may be present in field studies with land plants, as well as the possibility of local metal depletion caused by metal uptake (Gerhardt et al. 2009). The existence of emergent, submergent and free-floating species also allows the testing of multiple metal uptake methods on closely related species, which presumably have similar mechanisms for metal transport, but may display different uptake properties due to different tissues being exposed to metals. Further, the ready availability of metals in aquatic environments makes macrophytes ideal for large-scale applications, and especially for commercial uses of phytoremediation, as these easy-to-grow plants can be procured at a low

costs and utilized for the removal of metals in large volumes of freshwater. Single metal studies incorporating many of the ecologically important metals have been performed on macrophytes (Table 4), and the analysis of changes in metal accumulation capacities following multiple metal concentrations should allow greater insight into the competitive and cooperative interactions that occur for metal uptake and transport in aquatic environments. While uptake mechanisms in aquatic and terrestrial environments bear close similarities, whether the synergies and antagonisms present in soil-borne metals are closely reflected in an aqueous environment is nonetheless another question that merits attention.

CONCLUSION

Metal uptake capacities in both terrestrial and aquatic plants are determined by a great variety of factors, including not only environmental conditions, but also the physiological state of the phytoremediation agent and other contaminants present in the vicinity. As metal contaminations are rarely found in isolation, future studies involving the real world applications of phytoremediation must consider the network of interactions behind the uptake, transport, chelation and sequestration of metals, metalloids, organic contaminants and other environmental pollutants. These interactions are often complex and may switch between synergy and antagonism depending on the concentrations of the interacting metals, as well as the presence of a third metal. As such, greater insight into the mechanisms underlying metal uptake and transport is required to predict the nature of metal-metal interactions in a given system. In addition to the synergistic or antagonistic effects caused by multiple metal presence, the effect of other chemicals, such as non-metal pollutants, chelating agents or local bacterial flora, will affect remediation behavior.

Macrophytes, due to their ease of maintenance, rapid growth and tendency to readily recover heavy metals from the surrounding water, are promising plants for the study of heavy metal uptake in both singly and multiply contaminated environments. Further, their heavy metal uptake characteristics are well-known in single-metal studies, allowing easier comparisons between single-metal and multi-metal comparison behaviors.

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