

Chapter 18

Role of Aquatic Macrophytes in Biogeochemical Cycling of Heavy Metals, Relevance to Soil-Sediment Continuum Detoxification and Ecosystem Health

Przemysław Malec, Beata Mysliwa-Kurdziel, M.N.V. Prasad, Andrzej Waloszek, and Kazimierz Strzałka

18.1 Introduction

Soil (*per se*) is the abiotic component of life-supporting system and is considered to be the soul of life. A clean soil is an essential prerequisite for protecting the health of all biota, including humans, which is a prime concern. In recent years, dependency on heavy metals has increased manifolds particularly in a variety of industries such as plastic, textiles, microelectronics, battery industry, wood preservatives, smelting, river dredging, mining (spoils and tailings), and metallurgy. Also, heavy metals are components of, e.g., coal combustion products, urban refuses, sewage sludges, automobile exhausts, and some of fertilizers (Kabata-Pendias 2001). Under these circumstances, the potential of wetland plants in environmental protection has been widely recognized all over the world. In particular, the biodiversity of wetlands and the naturally operating principles of biogeochemical cycles have unequivocally demonstrated their significance in cleansing heavy metal contaminated water (Fig. 18.1). Water plants offer efficient and environmentally friendly solutions for clearing contaminated water, sediments, brownfields, and wastewater. In the last 5 years, the publications on various aspects of wetland science have sharply increased [data gleaned from <http://www.sciencedirect.com> until 2010 (Fig. 18.2)].

P. Malec • B. Mysliwa-Kurdziel • A. Waloszek • K. Strzałka (✉)
Faculty of Biochemistry, Department of Plant Physiology and Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30–387 Kraków, Poland
e-mail: kazimierzstrzalka@gmail.com

M.N.V. Prasad
Department of Plant Sciences, University of Hyderabad, Prof. C.R. Rao Road, Gachhibowli, Central University P.O., Hyderabad 500 046, India

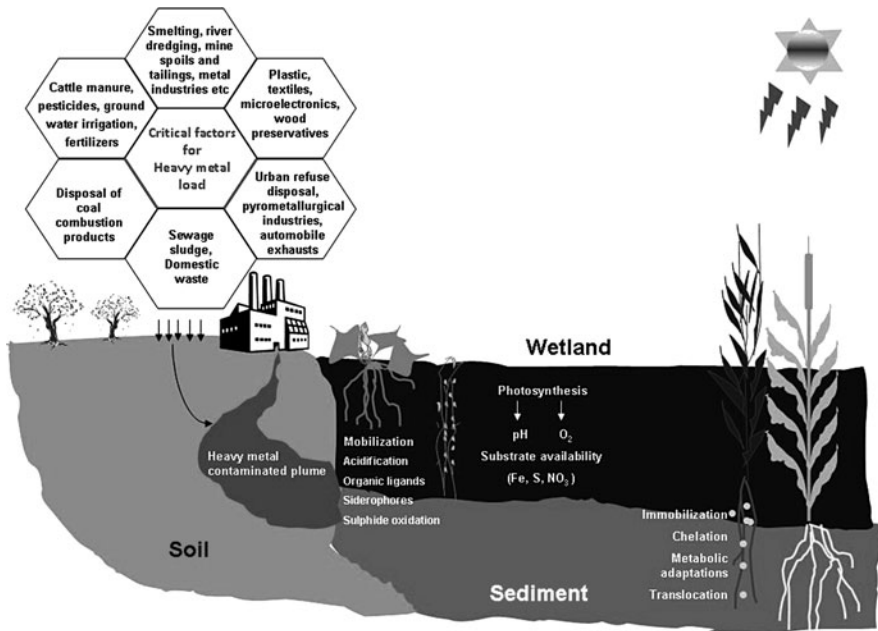


Fig. 18.1 Biogeochemistry of trace metals and their dynamics in wetland including the main detoxification strategies

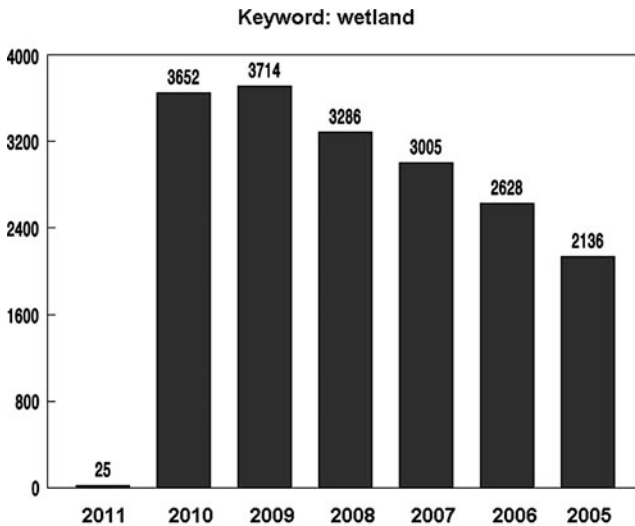


Fig. 18.2 There has been a steep rise in publications on “wetlands” during the last 5 years for their significance to ecosystem services (data source: <http://www.sciencedirect.com>; database status recorded on January 31st, 2011)

Many members of the angiospermous families, namely, *Cyperaceae*, *Potamogetonaceae*, *Ranunculaceae*, *Typhaceae*, *Haloragaceae*, *Hydrocharitaceae*, *Najadaceae*, *Juncaceae*, *Pontederiaceae*, *Zosterophyllaceae*, *Lemnaceae*, and *Typhaceae*, have aquatic and semiaquatic habitats. Members of these families exhibit great diversity, inhabit freshwater bodies, and complete their life cycle there. Aquatic plant lifestyles may vary greatly; for example, they may be (a) free floating, such as duckweed, (b) totally submersed, such as naiad, (c) bottom rooted and floating, such as waterlily, (d) emergent and rooted, such as quillwort, (e) totally emergent, such as cattails, and (f) streambank and wet area plants, such as alders (Jackson 1998).

Natural wetlands, especially floodplains, serve as a unique ecosystem with a wide variety of functions. One of the most important functions is their ability to act as a buffering zone to extremes associated with the discharge of rivers. The soils of floodplains also play a crucial role in the biogeochemical cycling of toxic heavy metals and nutrients including the treatment of contaminants transported by flood and storm water. These functions lead to the formation of contaminated hot spots at selected locations in floodplain areas as a result of the deposition of heavy metal contaminated sediment. Wetland soils and sediments often function as pollutant sinks (Du Laing et al. 2009; Rinklebe et al. 2007) and these require an adequate management strategy. Understanding the main biogeochemical processes is essential in this context.

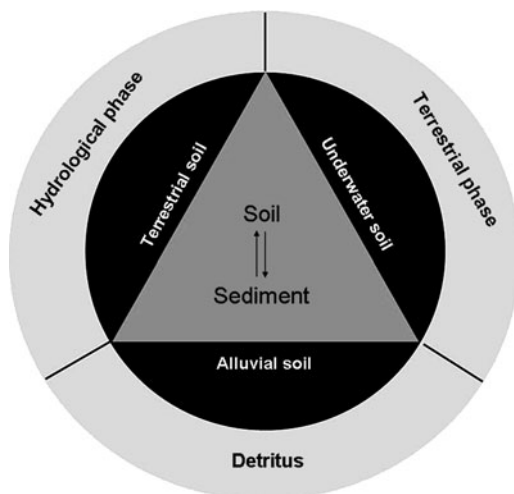
Constructed wetlands are artificial ecosystems dedicated to detoxification and the removal of contamination dissolved in the water phase. The following components that are involved in pollutant removal constitute a typical constructed wetland: substrates (soil/sediment), water, plants, and associated microbial populations. Constructed wetlands could be either aerobic (surface flow) or anaerobic (subsurface). In aerobic wetlands, plants are placed in shallow, slightly permeable soil (as e.g., clay or mine spoil). Anaerobic wetlands consist of vegetation planted in a deep, permeable mixture of substrate such as soil, peatmoss, compost, sawdust, straw/manure, hay bales, and gravel (Kosolapov et al. 2004; Gutknecht et al. 2006; Knox et al. 2006).

The determination of the chemical species of compounds present in aquatic ecosystems, both in solution and in bottom sediments, and the bioavailability of the various species and their forms of occurrence play a crucial role in assessing threats to the environment. The toxicity of elements, as well as the presence or absence of synergism and antagonism with respect to other elements, would play an important role in planning remediation strategies (Namieśnik and Rabajczyk 2010).

18.2 Soil-Sediment Continuum

Soils and sediments are connected or interlinked by a hydrological phase and these are functionally similar and share a number of common features. In fact, there is no clear borderline between soils and sediments, because both are interlinked by

Fig. 18.3 Soils and sediments are connected and interlinked by hydrological phase and these are functionally similar and share a number of common features



hydrological and terrestrial phases, which can be explained by (a) underwater soils, (b) terrestrial soils, (c) and alluvial soils (Fig. 18.3).

Several processes take place near the sediment–water interface (Lerman 1978). There is considerable debate on the distinction between soils and sediments (Blum 2005). Sediment pollution is caused by natural and anthropogenic influence. According to Gross (1978), human activities have significantly altered sediment characteristics. Industrial effluents and urban wastes are sources of pollutants in waters and sediments. Heavy metals in floodplain soils and sediment remediation including detoxification mechanisms involving plants are gaining considerable global attention (Rinklebe et al. 2007). The metal detoxification in plants is complex and usually involves a combination of several mechanisms, both limiting the metal circulation within plant organisms and preventing damages caused by a metal-induced oxidative stress (see below).

18.3 Role of Macrophytes in Trace Metal Dynamics in Wetland Sediments

The influence of aquatic plants and their metabolism may alter the distribution of trace metals between the solid and aqueous phases. There is little knowledge on how the combined defects of wetland plants influence the biogeochemistry of wetland sediments and thereby the trace metal dynamics (Namieśnik and Rabajczyk 2010). To survive in water-saturated sediments, aquatic macrophytes

have developed specialized adaptations. To support root respiration under anoxic conditions, they can effectively transfer oxygen from the surface to the roots (Armstrong 1979; Dacey 1980). A remarkable fraction of this oxygen may penetrate the rhizosphere, where it can take part in reoxidation of reduced sediment components, such as Fe^{2+} and Mn^{2+} (Mendelssohn et al. 1995). This process, called *Radial Oxygen Loss* (ROL), may lead to the increase in metal mobility in wetland ecosystems. Aquatic macrophytes, translocating the oxygen into the rhizosphere, increase the redox potential and thus also decrease the pH and increase the release of metals (see: Yang and Ye 2009 and references therein). It was shown that several species of aquatic macrophytes, including *Carex rostrata*, *Phragmites australis*, *Typha angustifolia*, and *T. latifolia*, can tolerate a very low pH and have been found growing under field conditions in pH as low as 2–4.4 (Nixdorf et al. 2002). Recently, the presence of low-pH-tolerant aquatic macrophytes has been shown to alter the mobility of Cu in constructed wetlands dedicated for treating acid mine drainage (Nyquist and Greger 2009).

Physical transport processes and biogeochemical reactions driven by aquatic plants may result in extensive sulfur cycling (see e.g., Choi et al. 2006). The oxidation of sulfides in sediments results in the production of oxidized sulfur (i.e., SO_4^{2-} and S^0), which may stimulate the release of metals to the water column (Simpson et al. 1998). Also, in freshwater wetlands, the degradation of organic sulfur from ant residue may lead to an increase in sulfate concentrations in the water column (Lefroy et al. 1994; Wind and Conrad 1995). Elevated sulfate concentrations in sediments were studied by Urban et al. (1994). In the presence of organic carbon produced by plants and in the absence of other electron acceptors, organic carbon is degraded, resulting in the formation of sulfides (Urban et al. 1994). In the presence of metals, sulfides can form metal sulfide precipitates in the sediments and may control the metal concentration in interstitial water (Boulegue et al. 1982; Emerson et al. 1983; Huerta-Diaz et al. 1998). For example, the reaction of sulfide with iron compounds has been shown to lead to the precipitation of iron monosulfide (FeS) and pyrite (FeS_2) in sediments (Berner 1984; Howarth and Jørgensen 1984). It was found that the acid-volatile sulfide (AVS) constituting the most labile fraction of sediment sulfide plays a key role in the determination of metal bioavailability in sediments (Di Toro et al. 1990).

Additionally, plants affect the biogeochemical dynamics of wetland sediments via evapotranspiration-induced advection, which increases the loading of dissolved constituents into the rhizosphere. The release of organic carbon into the sediments by aquatic plants stimulates many biotic and abiotic reactions. The chemical degradation of organic carbon results in the increase in reductive properties of sediments (Du Laing et al. 2009).

In particular, organic mercurial compounds from contaminated treatment wetlands are taken up by aquatic plants, passed to the leaves, and volatilized into the atmosphere at comparatively low concentrations (Du Laing et al. 2009).

18.4 Mechanisms of Tolerance to Metals in Aquatic Macrophytes

As sessile organisms, plants must deal with environmental limitations to survive. Plants growing in metal-polluted habitats have developed complex mechanisms to tolerate elevated concentrations of metals and to control cell homeostasis in potentially harmful environment. In the case of wetland plants, metal tolerance is particularly important as they are usually exposed to both overlying water and/or sediment and they take up nutrients and other minerals from both environments (Hinman and Klaine 1992). In general, aquatic macrophytes share the following common features: (a) the root system is reduced or completely degenerated, (b) the cuticle is very thin; therefore, shoots are able to absorb metals directly from water, and (c) the vascular system is reduced and the transpiration stream is limited or lacking (Basiouny et al. 1977). Aquatic plants play an important role in the uptake, storage, and recycling of metals. The uptake of elements depends on their chemical forms available in the environment as well as on the morphology and physiology of macrophytes. Free-floating species (e.g., *Eichhornia*, *Lemna*, *Pistia*, and *Spirodela*) absorb metal ions through both the roots and leaves. In contrast, rootless species (e.g., *Ceratophyllum demersum*) absorb basically through leaves (Chandra and Kulshreshtha 2004). Therefore, the location and capacity of particular tolerance mechanisms may be different than those identified in terrestrial plants. However, in contrast to most studied terrestrial plants (reviewed by Hall 2002), the biochemical and molecular backgrounds of the tolerance of aquatic macrophytes to metals are far less known (Table 18.1).

18.4.1 Metal Immobilization

Immobilization is one of the most common mechanisms of plant adaptation to elevated concentrations of heavy metals. The avoidance of metal stress may be achieved by the binding of metal ions in the apoplast which prevents their internalization. Metals may be deposited in cell walls as metalloorganic complexes formed with ligands present in this compartment (e.g., polysaccharides and organic acids). For example, the binding of Pb in cell walls has been demonstrated in the free-floating macrophyte *Lemna minor*. In this case, elevated concentrations of (1,3)-P-glucan (callose) have been found to be accumulated in the cell walls (predominantly in root tips) of plants treated with $\text{Pb}(\text{NO}_3)_2$ (Samardakiewicz et al. 1996). Subsequent X-ray microanalysis demonstrated that the accumulation of callose correlated with the lead deposition in cell walls. In contrast, lead has not been found in the ground cytoplasm, pointing to cell wall deposition as an effective mechanism of metal avoidance (Samardakiewicz and Woźny 2000). The immobilization of metals in the cell walls of the photosynthetic tissues of aquatic plants

Table 18.1 Representative recent articles on biochemical mechanisms of heavy metal tolerance and detoxification in aquatic macrophytes

Physiological process	Plant species	Metal	Mechanism identified	References	
Immobilization	<i>Lemna minor</i>	Pb	Cell wall deposition	Samardakiewicz et al. (1996)	
	<i>Spirodela intermedia</i> , <i>Lemna minor</i> , <i>Pistia stratiotes</i> , <i>Potamogeton lucens</i> , <i>Salvinia herzogii</i> , <i>Eichhornia crassipes</i>	Cd, Ni, Cu, Zn, Pb, Cr	Sorption by (non-living) biomass, ion exchange	Schneider and Rubio (1999) and Miretzky et al. (2006)	
	<i>Ludwigia stolonifera</i> , <i>Salvinia herzogii</i> , <i>Pistia stratiotes</i>	Cd, Ni, Cd, Cr	Sorption by living biomass; ion exchange	Elifantz and Tel-Or (2002) and Suñe et al. (2007)	
	<i>Hydrilla verticillata</i>	Cu	Cell wall deposition	Xue et al. (2010)	
	<i>Fontinalis dalecarlica</i>	Cd	Cell wall deposition	Bleuel et al. (2005)	
	Chelation	<i>Pistia stratiotes</i>	Cd	GSH, phytochelatins	Sanità di Toppi et al. (2007)
<i>Fontinalis antipyretica</i> , <i>Fontinalis dalecarlica</i>		Cd	GSH	Bleuel et al. (2005)	
<i>Elodea canadensis</i>		Ni Pb Cu	Non-protein thiols	Maleva et al. (2009) Dogan et al. (2009) Malec et al. (2009a)	
<i>Lemna trisulca</i>		Cd	Non-protein thiols	Malec et al. (2010)	
<i>Egeria densa</i>		Cd	Cd-binding, metallothionein-like protein	Malec et al. (2009b)	
<i>Azolla filiculoides</i>		Cd, Cu, Ni, Zn	Expression of type 2 metallothionein	Schor-Fumbarov et al. (2005)	
Metabolic adaptation		<i>Eichhornia crassipes</i>	Cd	AsA, APX, DHAR, MDHAR, SOD, GRD	Sanità di Toppi et al. (2007)
		<i>Elodea canadensis</i>	Ni	CAT, GRD	Maleva et al. (2009)
		<i>Potamogeton crispus</i>	Ag Cd	AsA, POD PAO, DAO	Xu et al. (2010) Yang et al. (2010)
		<i>Potamogeton pusillus</i>	Cu	GPX, GRD, POD	Monferrán et al. (2009)
	<i>Fontinalis antipyretica</i>	Cd, Cu, Pb, Zn	SOD, CAT, GRD, APX	Dazy et al. (2009)	
	<i>Alternanthera philoxeroides</i>	Cd	POD, SOD, CAT	Ding et al. (2007)	
	<i>Lemna minor</i> , <i>Spirodela polyrrhiza</i>	Cu	SOD, CAT	Kanoun-Boulé et al. (2009)	
	<i>Lemna minor</i>	Cd, Zn, Al	SOD, CAT, APX	Tkalec et al. (2008) and Radić et al. (2010)	
	<i>Azolla filiculoides</i>	Pb	V-H + -ATPase	Oren-Benaroya et al. (2004)	
	Translocation	<i>Potamogeton pectinatus</i> , <i>Potamogeton crispus</i>	As, Cd, Cu, Pb, Zn	Acropetal translocation	Peter et al. (1979) and Wolterbeek and van der Meer (2002)
<i>Elodea canadensis</i>		Cd	Acropetal translocation	Fritioff and Greger (2007)	
<i>Hydrilla verticillata</i>		Cu	Acropetal and basipetal translocation	Xue et al. (2010)	
<i>Baumea juncea</i> , <i>Baumea articulata</i> <i>Schoenoplectus</i> , <i>Juncus subsecundus</i>		Cd	Acropetal translocation	Zhang et al. (2010)	

has also been observed. In particular, a significant biosorption of Cd has been detected in aquatic moss *Fontinalis dalecarlica* (Bleuel et al. 2005). The submerged macrophyte *Hydrilla verticillata* has been found to accumulate Cu of up to 30 830 mg kg⁻¹ dry weight. In this organism, most of the Cu has been deposited in the cell walls of shoots (Xue et al. 2010). Interestingly, a metal-binding capacity has also been demonstrated in the dead, dried biomass of aquatic plants. The sorption of Cd²⁺, Ni²⁺, Cu²⁺, Zn²⁺, and Pb²⁺ by the biomass of three macrophytes (*Spirodela intermedia*, *Lemna minor*, and *Pistia stratiotes*) has been investigated. In all these plant species, effective metal sorption was observed. The *Lemna minor* biomass presented the highest mean removal percentage and *Pistia stratiotes* the lowest for all metals tested. It has been shown that this sorption is based on an ion exchange between monovalent metals (K⁺ and Na⁺) as counterions present in the macrophyte biomass and heavy metal ions and protons taken up from water. No significant differences were observed in the amounts of metal exchange when multimetal or individual metal solutions were used (Miretzky et al. 2006). Other authors have demonstrated that the dried biomass of *Potamogeton lucens*, *Salvinia herzogii*, and *Eichhornia crassipes* were excellent biosorbents for Cr²⁺, Cd²⁺, Ni²⁺, Cu²⁺, Zn²⁺, and Pb²⁺. The sorption mechanism of these biomaterials was found to proceed mainly by ion exchange reactions between metal ions and cationic weak exchanger groups present on the plant surface (Schneider and Rubio 1999). The exchange of bound heavy metal ions against the discharge of light metal ions such as Ca²⁺, Mg²⁺, K⁺, and Na⁺ has been proposed as playing a role in Cd and Ni biosorption by the living biomass of roots, floating roots, and the leaves of *Ludwigia stolonifera* (Elifantz and Tel-Or 2002) and also for Cd and Cr uptake by *Salvinia herzogii* and *Pistia stratiotes* (Suñe et al. 2007).

18.4.2 Chelation

In plants, the synthesis of thiol-containing, cation-chelating compounds, such as glutathione (GSH), phytochelatins, and metallothioneins, is a frequent response to heavy metals transported into the symplast (Cobbett and Goldsbrough 2002). Other compounds such as amino acids, organic acids, and phenols have also been identified as heavy metal chelators (Rausser 1999). Chelating agents are found to limit the circulation of toxicants within the plant organism, forming stable complexes with metal ions (Prasad 1995). For example, in *Pistia stratiotes* and *Eichhornia crassipes* exposed to Cd, glutathione levels remarkably increased. In a species more responsive to Cd exposure – *P. stratiotes* – this increase in glutathione concentration correlated with abundant phytochelatin synthesis both in roots and in leaves (Sanità di Toppi et al. 2007). Also, Cd²⁺ exposure in the mosses *Fontinalis antipyretica* and *F. dalecarlica* increased the glutathione pool. Analytical electron microscopy provided evidence that GSH may be an agent for intracellular Cd²⁺ detoxification in these bryophytes (Bruns et al. 2001). A significant increase in

nonprotein thiols has been observed in *Lemna trisulca* fronds treated with low doses of Cd (Malec et al. 2010). In photosynthetic tissues of *Elodea canadensis*, the induction of nonprotein –SH groups was accompanied with an accumulation of Pb (Dogan et al. 2009) and Ni (Maleva et al. 2009) and the synthesis of low-molecular-weight peptides was observed in the presence of elevated Cu concentrations in the medium (Malec et al. 2009a). In the shoots of a closely related plant, *Egeria densa*, cadmium induced both an increase in the nonprotein thiol concentration and the synthesis of Cd-binding polypeptide, containing aromatic amino acid residues and sharing biochemical properties with metallothioneins (Malec et al. 2009b). A cDNA encoding a type 2 metallothionein, isolated from the wetland fern *Azolla filiculoides*, was termed AzMT2. The expression of AzMT2 was enhanced by the addition of Cd²⁺, Ni²⁺, Cu²⁺, and Zn²⁺ to the growth medium. The increase in the transcript level of AzMT2 was proportional to the metal content in the plant. The more moderate response of AzMT2 to Zn and Cu ions, which are essential micronutrients, in comparison to toxic Cd, suggests a role for AzMT2 in metal homeostasis (Schor-Fumbarov et al. 2005).

18.4.3 Metabolic Adaptations

Heavy metal uptake is known as a factor inducing oxidative stress in plants (Mittler 2002). In response to ROS production induced by metals, aquatic plants increase the activity of enzymes involved in the scavenging of ROS, as e.g., superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Similarly, metal stress was shown to stimulate the activity of the enzymes involved in biosynthesis and/or modification of cellular reducing agents such as ascorbate (AsA) (ascorbate peroxidase – APX, dehydroascorbate reductase – DHAR, monodehydroascorbate reductase – MDHAR), glutathione (glutathione peroxidase – GPX, glutathione reductase – GRD), and polyamines (PAs) (polyamine oxidase – PAO, diamine oxidase – DAO). In the aquatic fern *Azolla filiculoides*, the accumulation of lead in vacuoles has been found to be accompanied with the enhanced activity of tonoplast V-H⁺-ATPase (Oren-Benaroya et al. 2004). Also, the ability to accumulate proline, being a result of enhanced 1-pyrroline-5-carboxylate-pyrroline-5-carboxylate synthetase (P5CS), as observed in salt-tolerant *Najas* sp., has been suggested to play a role in the general resistance to metal stress in aquatic macrophytes (Rout and Shaw 1998). There is an increasing number of studies confirming the role of antioxidants and the enzymes involved in their metabolism as well as other enzymes in the tolerance of aquatic plants to stress caused by heavy metals. Selected examples of the recent relevant literature are given in Table 18.1.

18.4.4 Translocation

In terrestrial plants, metal ions taken up by the root system could be effectively translocated to other organs via the xylem (Pich and Scholz 1996). Metal translocation may be responsible for a different distribution and final deposition of toxicants within the plant organism (Fritioff and Greger 2007). Accordingly, the root systems of aquatic plants play an important role in the uptake of elements (Jackson 1998), but mobility may be dependent on both the particular metal and the macrophyte species. Acropetal translocation of several elements (As, Cd, Cu, Pb, and Zn) has been observed in two *Potamogeton* species: *P. pectinatus* and *P. crispus* (Peter et al. 1979; Wolterbeek and van der Meer 2002). An apoplastic route has been proposed for Cd translocation in *Elodea canadensis* (Fritioff and Greger 2007). On the other hand, no translocation of Cu from root to other parts was found in *Potamogeton natans* (Fritioff and Greger 2006). Similarly, in *Phragmites australis* treated with Cr, Hg, Mn, or Zn, metal concentrations in plant organs decreased in the order root > rhizome > leaf > stem. All four organs showed significant differences in the concentration of these elements, which suggests low metal mobility (Bonanno and Lo Giudice 2010). Recently, it has been shown that both xylem-based acropetal and phloem-based basipetal Cu translocations may function in *Hydrilla verticillata*. However, the copper bioaccumulation factors of *Hydrilla* shoots were significantly higher than those of roots, suggesting a dominant role for acropetal translocation in this plant (Xue et al. 2010). Also, a remarkable translocation of Cd has been recently observed in four emergent wetland plants (*Baumea juncea*, *Baumea articulata*, *Schoenoplectus validus*, and *Juncus subsecundus*) (Zhang et al. 2010). However, detailed mechanisms of the transport of metals in the vascular systems of aquatic plants remain unknown.

18.5 Effect of Metals on Photosynthesis in Aquatic Macrophytes

Photosynthesis, which is the basis of all food chains up to human life, is the site of direct or indirect action of heavy metals (for a review, see Myśliwa-Kurdziel et al. 2002, 2004; Mallick and Rai 2002; DalCorso et al. 2008). As to the direct effects of heavy metals on photosynthesis, damage to the reaction centers of photosystems (PS I and PS II), the water splitting complex, the LHClI antenna complex, cyt b_6/f , and other components of the photosynthetic transport chain have been observed. Heavy metals can also decrease photosynthetic efficiency through the inhibition of chlorophyll biosynthesis (for a review, see Myśliwa-Kurdziel and Strzałka 2002b), pigment degradation, metal-induced changes in chloroplast structure as well as water uptake imbalance, stomatal closure, and many others. A decline in the photosynthetic efficiency of plants exposed to different heavy metals consequently

results in lower biomass production, plant growth inhibition, and can also be the reason for plant death.

The photosynthetic activity of macrophytes treated by heavy metals has long attracted attention, first because of a great need for sensitive environmental bioindicators, and next, because of the potential use of water plants in phytoremediation (for a review see Ayeni et al. 2010). In both cases, the avoidance of damage to the photosynthetic apparatus by heavy metals as well as easy, fast, and unequivocal detection of injury is the main interest of research conducted all over the world. The best candidates for phytoremediation are plants, which are able to survive and perform photosynthesis together with efficient metal accumulation. Although research on the impact of heavy metals on photosynthesis in metal-polluted plants is conducted by many groups, a comparison of the results obtained in different laboratories is often difficult due to wide variations in experimental conditions, which may involve the observation of whole plants, leaves, or isolated organelles, as well as due to different parameters measured and different techniques applied. Metal concentration and the way it is sourced result in further differences between experimental systems. Selected examples of plant species as well as the methods used in investigations of the influence of heavy metals on photosynthetic efficiency are given in Table 18.2.

There is a growing interest in using the technique of chlorophyll fluorescence measurement in the detection and investigation of the effects of heavy metals on plant physiology (Krause and Weis 1991; Joshi and Mohanty 2004). Among others, it is widely used to study macrophytes. The main advantage of this method is that it is rapid, simple, and noninvasive. Most importantly, recently developed portable instruments enable in situ measurements, and thus the monitoring of metal-induced plant injury becomes possible in real time. Fluorescence originates from chlorophyll in photosynthetic tissues exposed to light and it is widely accepted that the fluorescence emission in vivo amounts to approximately 0.6–3% of the absorbed light (Krause and Weis 1991). Chlorophyll fluorescence is a process competitive to photosynthetic reactions, and therefore, its analysis gives an insight into the physiological state of the photosynthetic apparatus. If dark-adapted leaf is illuminated, one can observe the Kautsky effect, i.e., a rapid increase in fluorescence emission from the minimum (the F_0 level), to the maximum (the F_m value), which is followed by a decrease of fluorescence accompanying the onset of the electron transport activity. The fluorescence F_v/F_m ratio ($F_v = F_m - F_0$), which gives information about the efficiency of the photochemical conversion of absorbed light energy in PS II reaction centers, is the parameter mostly used in studies of aquatic macrophytes. However, in some cases the F_v/F_0 ratio is shown to be more effective than the F_v/F_m ratio in monitoring the development of stress (see table). Appenroth et al. (2001) have investigated the toxic effects of Cr on the photosynthetic activity of *Spirodella polyrhiza* and shown that transient chlorophyll in vivo measurements, namely the O-J-I-P test, enables an indication of a decrease in the number of active reaction centers and damage to the oxygen evolving complex as the main targets of Cr. Several water plant species, known as hyperaccumulators, have the ability to accumulate nonessential metals and, up to a certain metal concentration, apparently

Table 18.2 Representative recent publications about the influence of heavy metal on photosynthesis in water plants

Plant species	Metals	What has been measured	Main observations/conclusions	References
<i>Salvinia natans</i>	Cr	Fluorescence ratio F_v/F_m The activity of ribulose-1,5-biphosphatecarboxylase-oxygenase (Rubisco) Transsthylakoidal pH gradient Activities of antioxidant enzymes CO ₂ assimilation and photosynthetic pigments Cu uptake	"Salvinia possess efficient antioxidant machinery that curtails oxidative stress caused by Cr-rich waste water and protects photosynthetic machinery from damage"	Dhir et al. (2009)
<i>Salvinia minima</i>	Cu 1–3 mg/L		Salvinia is a good remediator to remove high Cu concentration	Al-Hamdani and Blair (2004)
<i>Callitriche cophocarpa</i>	Cr (VI)	Chlorophyll fluorescence: F_v/F_m	Callitriche is a good candidate for phytoextraction of Cr	Augustynowicz et al. (2010)
<i>Eichhornia crassipes</i>	Cr (III); Cr(VI) 1 and 10 mM	Photosynthetic pigments Chlorophyll fluorescence: F_v/F_m ; F_v/F_0	Cr ³⁺ was less toxic than Cr ⁶⁺ and, in some cases, even increased photosynthesis and chlorophyll content	Paiva et al. (2009)
		Net photosynthetic rate (Pn), stomatal conductance (gs) and substomatal CO ₂ concentration (Ci) photosynthetic pigments	F_v/F_0 ratio was more effective than F_v/F_m ratio in monitoring the development of Cr ⁶⁺ -induced stress	
<i>Eichhornia crassipes</i>	Different metals-studies of polluted river (Brais) – seasonal changes	Chlorophyll α fluorescence parameters: F_0 , F_m , F_v/F_m , non-photochemical dissipation (qN and NPQ) Transmission electron microscopy (TEM)	Stress conditions in water hyacinth along the Paraíba do Sul River (PSR) in southeastern Brazil in 2005–2006 was monitored Even if F_v/F_m ranged between 0.77 and 0.81, which indicated that high maximum quantum yield was maintained and the plants performed normal photosynthesis, some ultrastructural changes in chloroplasts were observed Membrane integrity was maintained, which suggest an adaptation mechanism to the environmental conditions	Lage-Pinto et al. (2008)
<i>Wolffia globosa</i>	Cd, Cr	Biomass production	<i>W. globosa</i> is a good Cd accumulator, but moderate Cr accumulator	Boonyapookana et al. (2002)
		Chlorophyll content	<i>W. globosa</i> was indicated as potential bioremediator of contaminated aquatic environment	

<i>Spirodela polyrrhiza</i>	Cr	Oxygen evolution Chlorophyll fluorescence O-J-I-P test	Indicated the main sites of action of metal	Appenroth et al. (2001)
<i>Spirodela polyrrhiza</i> <i>Lemma minor</i>	Ni	Electron microscopy for investigation of chloroplast structure Growth rate	Ni treatment resulted in changes of chloroplasts structure to chloro-amyloplasts and amylo-chloroplasts, but not to gerontoplasts The contents of the chlorophylls <i>a</i> and <i>b</i> decreased strongly; carotenoids level remained approximately constant The observed accumulation of starch without stimulation of the photosynthetic activity indicated less efficient export of carbohydrates out of the plastids <i>Spirodela</i> appeared more sensitive to nickel than <i>Lemma</i> ; however, both species were sensitive, which make them not particularly suitable for phytoremediation but suitable for ecotoxicological testing instead	Appenroth et al. (2010)
<i>Jussiaea rapens</i>	Cd	Chlorophyll fluorescence parameters: F_v/F_m , qP, ETR (electron transport rate)	Cd induced the decrease of the photosynthetic activity mainly due to the damage of Photosystem II Chlorophyll <i>a</i> , <i>b</i> and total chlorophyll content decreased, but carotenoid content increased	Li et al. (2008)

do not show the symptoms of toxic damage. For example, *Salvinia* sp., a fast growing free-floating aquatic plant, known for its high productivity of biomass, has been shown to accumulate different heavy metals, namely, Cr, Cu, Cd, As, and Pb, and the extent of metal uptake was higher for its higher concentration in water (Nichols et al. 2000; Al-Hamdani and Blair 2004; Hoffmann et al. 2004; Dhir et al. 2009). In the course of the accumulation, the decrease in the content of photosynthetic pigments, both chlorophylls and carotenoids, a lowered CO₂ assimilation, and an inhibition of plant growth were observed (e.g., Nichols et al. 2000; Al-Hamdani and Blair 2004). However, Dhir et al. (2009) showed an increase in the photosynthetic activity of PS I and PS II as well as transthylakoidal pH gradient and a decrease in the activity of ribulose-1,5-biphosphatecarboxylase-oxygenase (Rubisco) when measured for thylakoids isolated from Cr-treated *Salvinia natans*. They also showed only a slight decrease in chlorophyll content and no effect on the in vivo chlorophyll fluorescence parameter (F_v/F_m). From an analysis of cellular antioxidants, they concluded that *Salvinia* has an efficient antioxidant machinery that curtails oxidative stress caused by Cr in wastewater and in this way protects the photosynthetic apparatus from damage (Dhir et al. 2009).

There are many reports on the potential use of *Eichhornia crassipes* (water hyacinth), a floating macrophyte hyperaccumulator species native to South America, for the monitoring of heavy metals in aquatic environments (e.g., Lage-Pinto et al. 2008) as well as for the phytoremediation process (Lu et al. 2004; Paiva et al. 2009). Paiva et al. (2009) have shown that, with a decrease in leaf gas exchange, chlorophyll fluorescence parameters and photosynthetic pigment content in water hyacinth were more sensitive to Cr(VI) than Cr(III). In some cases, Cr(III) even increased photosynthesis and chlorophyll content. The sensitivity and effectiveness of the applied methods and examined parameters in the monitoring of Cr(VI)-induced stress development are also discussed in that work. The indirect influence of metals on photosynthetic activity via alterations of thylakoid stacking was observed in some experiments (Paiva et al. 2009; Lage-Pinto et al. 2008).

Callitriche cophocarpa is a new plant that has recently been recommended as a promising species for Cr(VI) bioremediation (Augustynowicz et al. 2010). The fluorescence F_v/F_m ratio was not affected in plants treated with 50 and 100 μ M Cr (VI), although in the latter a fast recovery of the F_v/F_m ratio after an initial decrease was observed. Photosynthetic activity was completely inhibited after 8 days of treatment with 700 μ M Cr(VI).

In many experiments, the inhibition of photosynthetic activity was accompanied by a decrease in chlorophyll and carotenoid content, and chlorophyll *a* seemed to be more sensitive than chlorophyll *b* (Vajpayee et al. 2000; Paiva et al. 2009; Augustynowicz et al. 2010). It has also been reported that photosynthesis in aquatic plants was also affected when Mg²⁺ in chlorophyll molecules was substituted by heavy metals, such as mercury, copper, cadmium, nickel, zinc, and lead (Küpper et al. 1996, 1998). Such substitution was observed for chlorophyll in the LHCII complex under low light intensity (Küpper et al. 2002).

It should be emphasized that photosynthetic activity or the lack of it can itself change the bioavailability of heavy metals in a water environment. There are

well-known diurnal changes in CO_2 and carbonate concentrations in water, correlating with changes in pH in the course of photosynthesis. When photosynthesis operates, CO_2 and bicarbonate are consumed and water alkalinity increases. The opposite process is observed at night, when all organisms respire. Most heavy metals (e.g., Cu, Zn, Pb, and Cd) are more soluble in low pH, so the toxicity of these metals decreases during periods of light. Additionally, extremely active photosynthesis in calcium-rich water leads to marl formation and insoluble forms of heavy metal coprecipitation, but also to phosphate coprecipitation (Otsuki and Wetzel 1972), which can profoundly change nutritive and toxicological conditions for the long period. On the other hand, the main external product of photosynthesis is oxygen, so photosynthesis makes the environment more oxidative, which can change the redox state of redox-active metals such as Fe or Mn and, in consequence, their toxicity. Wurts and Durborow (1992) provide a compact review of the interdependences of pH, CO_2 , alkalinity, hardness, and gas exchange in aquatic environment. In the longer timescale, heavy metal bioavailability in water can change with periods of winter and summer stagnation and spring and autumn circulation (for review, see e.g., Lithner et al. 2000).

18.6 Polymetallic Contamination

Sources of monometallic environmental contamination are rare and have a predominantly local character, where there are mainly plants using specific metal salts for technological processes like tanneries (Cr), some wood impregnation plants (Cu, As, and Cr), and the car battery industry (Pb). Most pollution, however, is emitted from polymetallic sources, among which the mining and smelting industries have the greatest impact. So heavy metal effects on water plants should be investigated not only for particular elements but also for combinations of some metals and for groups of elements, which are geochemically and/or technologically connected. The most important groups are the “calamine” or Zn–Pb-industry group, where the main toxic elements are Zn, Pb, and Cd, often with significant additions of Tl, Ag, and other (Tremel et al. 1997; Wierzbicka et al. 2004; Aravind and Prasad 2004), and the group connected with copper ores, which also contains Zn, Pb, Cr, Mn, As, Sb, and traces of other metals (e.g., Chojnacka et al. 2005). The bioavailability of particular elements for plants in water ecosystems is very complicated and depends on many abiotic and biotic factors, often interrelated (see e.g., Weis and Weis 2004; Butler 2006). Mathematical and statistical models, which can be used for theoretical analyses of this phenomenon, were described by Samanta (2010) and Ince et al. (1999), respectively. The main metals, for example, whose excess can toxically act on plants in water contaminated by Pb–Zn industry, are zinc and cadmium, but not lead, which in natural conditions is usually cumulated mainly in sediments, because of its low solubility. Particular elements can act additively, synergically, or antagonistically, and the reaction of plants can depend on concentrations, concentration ratios, plant species, and environmental conditions.

In aquatic plants, one of the most comprehensive investigations of a two-metal effect is the protection of *Ceratophyllum demersum* (Coontail) by zinc ions during acute cadmium stress, as described in a set of papers by Aravind, Prasad, and coworkers. Part of it is summarized in a review paper of Aravind and Prasad (2005a). They show the ability of Zn^{2+} to alleviate the toxic effect of Cd^{2+} on the chloroplast membrane structure, pigment biosynthesis, activity of photosystems, photosynthetic electron transport and net photosynthesis rate (Aravind and Prasad 2004), energy transfer in photosystem II (Malec et al. 2008), as well as on the carbonic anhydrase conformation (Aravind and Prasad 2005b). Plants treated with both Cd and Zn ions showed significantly lower Cd and higher Zn uptake than those treated with the same Cd concentration only. Cd-induced oxidative stress symptoms, such as lipid peroxidation, lipoxygenase activity, and electrical conductivity, were efficiently reduced by Zn addition. On the other hand, the activity of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase showed a very high increase in Cd + Zn-treated plants as compared to Cd- or Zn-only treated ones. Interestingly, plants treated with only Zn concentrations showed neither high Zn uptake nor increase of antioxidant enzyme activity (Aravind and Prasad 2003).

The content of antioxidants, such as thiols, ascorbate, and glutathione, was decreased by Cd treatment, but Zn supplementation restored its level by enhancing the activity of enzymes of the ascorbate–glutathione cycle. This phenomenon has not been observed under only Zn treatment (Aravind and Prasad 2005c). In plants subjected to Cd stress and supplemented with zinc, a higher antioxidative status resulted in a significantly lower level of reactive oxygen species, such as superoxide anion-radical, hydroxyl radical, and H_2O_2 . Also, in these plants, protein and DNA damages were remarkably lower compared to Cd-only treated material (Aravind et al. 2009). The heavy metal stress-induced formation of glutathione was significantly increased by Zn supplementation unlike the organic acids-mediated chelation mechanism, which seemed to be insensitive to Zn (Aravind and Prasad 2005d).

In the same *Ceratophyllum demersum*, Bunluesin et al. (2007) have observed a decrease in Cd accumulation in solutions with a high Zn/Cd ratio, whereas Zn accumulation decreased in the presence of Cd. A full comparison of these results with these obtained by Aravind and coworkers (Aravind and Prasad 2003, 2004, 2005a, b, c, d; Aravind et al. 2009) is impossible, however, because of different conditions of plant growth and treatment procedures. Similar results concerning Zn and Cd uptake by *Eichhornia crassipes* (water hyacinth) were obtained by Hasan et al. (2007). These authors observed that the accumulation of both Zn and Cd with an admixture was lower than with solutions of Cd or Zn only.

The set of Aravind and coworker publications (Aravind and Prasad 2003, 2004, 2005a, b, c, d; Aravind et al. 2009) is a unique analysis of different aspects of two heavy metal interactions in water plant. Summarizing these data, it seems that the decrease in cadmium toxicity by zinc is caused by an interplay of the following mechanisms: (a) cadmium's toxicity can be induced by Cd substitution of Zn in Zn-containing active sites. A Zn excess counteracts this effect; (b) a high

concentration of both metals seems to act synergistically to the activation of some plant defense systems against heavy metal action, e.g., the antioxidative system. In the presence of both Cd and Zn, these systems could be more efficient than in plants under Cd stress only because of lower metabolic disturbance; (c) in some cases (as in the system investigated by Aravind and coworkers), the intensive accumulation of Zn results in the increase of Zn/Cd ratio and, in consequence, in reduction of the toxic action of cadmium.

Recently, a short study on the oxidative stress caused by the acute excess of Cu and/or Zn in *Spirodela polyrhiza* was published (Upadhyay and Panda 2010). These authors observed that the equimolar addition of Zn to Cu at high concentration (100 μM) caused a significant decrease in Cu-induced oxidative stress symptoms, namely in the concentrations of MDA, H_2O_2 , and superoxide as well as a decline in the lipoxygenase activity. Activities of antioxidative enzymes (CAT, POD, and AsA POD) were strongly elevated in these plants. Interestingly, this effect correlated with an increase in both photosynthetic pigment and biomass production. Contrary to this, Zn alone caused a slight increase in oxidative stress symptoms. The induction of antioxidant system was observable only at high Zn concentrations (50–100 μM). With medium and low equimolar concentrations (10–50 μM) of both metals, a very complicated interplay was observed. These results apparently show that Cu and Zn in equimolar concentrations act synergistically in lower and antagonistically in higher concentrations. The pattern of action of the same pair of ions on *Lemna minor* (Dirilgen and Inel 1994) was different – at low concentrations (0.1–0.5 ppm) they act antagonistically to plant growth rate and dry to fresh weight ratio, whereas at higher concentrations (up to 2 ppm) they operated independently.

As far as we know, the results presented above are the only detailed investigations of effects of two metal interactions in aquatic plants. Other information on this topic is very fragmentary. For example, Charles et al. (2006) analyzed the toxicity of copper and uranium to *Lemna aequinoctialis*, by characterizing concentrations inhibiting growth for 50% and concentrations inducing minimum detectable toxic effects for Cu, U, and the combination of both. They concluded that these metals applied in combination are less toxic than individually.

The uptake and organ distribution of the four heavy metals Zn, Cu, Cd, and Pb in individual and mixed solutions in *Potamogeton natans* were analyzed by Fritioff and Greger (2006). The uptake of each metal into roots, shoots, and leaves was the same in both individual and mixed metal solutions, with the exception of Cu and Cd. Cu applied in mixed solutions had double the concentration in leaves. The accumulation of Cd in roots decreased more than half when Cd was applied in mixed solutions.

Recently, Yan et al. (2010) have published a study on the competitive effect of Cu^{2+} and Zn^{2+} on the biosorption of Pb^{2+} by *Myriophyllum spicatum*. Along with a detailed analysis of Pb biosorption equilibria in different conditions and an FT-IR analysis of Pb^{2+} binding in the biological matrix, they stated that the amount of Pb^{2+} sorbed is significantly decreased in the presence of copper and zinc ions, the former having the stronger effect.

Finally, it is necessary to point out that some aquatic plants in the presence of high Fe^{2+} concentration in substrate can form an iron oxyhydroxide root plaque as an effect of oxidation of ferrous to ferric ions by oxygen that is radially excreted from roots (ROL – see above). This plaque was described as the place where Mn and Zn ions could be immobilized (St-Cyr and Campbell 1996), or a buffer for Ca, Cu, Mn, Zn, and P (Jiang et al. 2009 and references therein). The role of this plaque in root-sediment (soil) ion exchange seems to be different for particular ions, plant species, and environmental conditions.

18.7 Conclusion

The data presented above indicate that aquatic macrophytes are organisms that may affect the cycling of heavy metals in wetland ecosystems.

Water plants possess a capacity to absorb and bioconcentrate heavy metals both from soil/sediment and/or from the surrounding water column via different mechanisms. These include cell wall binding, ion exchange-based sorption, active transport, chelation, translocation, and deposition in different organs. These processes lead eventually to the immobilization of metals in the plant biomass. Moreover, the presence of metabolic adaptations, including scavengers of ROS, antioxidants and their modifying enzymes, and specific ion transporters, allows many species of aquatic plant to survive and grow in elevated concentrations of metals. Additionally, the nonliving biomass of some wetland plants could still bind metals from a water environment. Therefore, wetland sediments are usually considered as sinks for metals. In their anoxic zone, they may contain high concentrations of reduced metals (Weis and Weis 2004).

On the other hand, aquatic macrophytes may influence many physical and chemical processes in their environment. In particular, photosynthetic activity may alter the bioavailability of heavy metals in wetland ecosystems. The Radial Oxygen Loss (ROL) resulting in partial oxygenation of the rhizosphere may lead to profound changes in metal mobility in rhizosphere soils (Yang and Ye 2009).

Finally, competitive or synergistic effects of the elements available to aquatic macrophytes in a particular ecosystem may greatly influence the circulation and deposition of heavy metals in the environment.

Metals are important components of the biosphere. Many metals are micro-nutrients (e.g., Cu, Fe, Mn, Ni, and Zn) as they are involved in metabolic processes as the cofactors of enzymes in plants. At elevated concentrations, derived from e.g., anthropogenic sources, metals became toxicants causing hazardous pollution to ecosystems on a global scale (Mohan and Hosetti 1999). The use of aquatic macrophytes in the phytoremediation of metal-polluted areas is currently attracting considerable interest (Prasad 2003, 2004). Therefore, a detailed knowledge of the molecular mechanisms of the uptake, tolerance, and transport of heavy metals through aquatic plants is essential both for an understanding of the functioning of wetland ecosystems and for the development of specific phytoremediation technologies.

Acknowledgments This paper was prepared in the frames of European Regional Development Fund: the Polish Innovation Economy Operational Program (contract No. POIG.02.01.00-12-167/08, project Małopolska Centre of Biotechnology) and was financially supported by the Statutory Funds of the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University.

References

- Al-Hamdani SH, Blair SL (2004) Influence of copper on selected physiological responses in *Salvinia minima* and its potential use in copper remediation. *Am Fern J* 94:47–56
- Appenroth KJ, Krech K, Keresztes Á, Fischer W, Koloczec H (2010) Effects of nickel on the chloroplasts of the duckweeds *Spirodela polyrhiza* and *Lemna minor* and their possible use in biomonitoring and phytoremediation. *Chemosphere* 78:216–223
- Appenroth KJ, Stöckel J, Srivastava A, Strasser RJ (2001) Multiple effects of chromate on the photosynthetic apparatus of *Spirodela polyrhiza* as probed by OJIP chlorophyll *a* fluorescence measurements. *Environ Pollut* 115:49–64
- Aravind P, Prasad MNV (2003) Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: a free floating freshwater macrophyte. *Plant Physiol Biochem* 41:391–397
- Aravind P, Prasad MNV (2004) Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. *Plant Sci* 166:1321–1327
- Aravind P, Prasad MNV (2005a) Cadmium–zinc interactions in a hydroponic system using *Ceratophyllum demersum* L.: adaptive ecophysiology, biochemistry and molecular toxicology. *Braz J Plant Physiol* 17:3–20
- Aravind P, Prasad MNV (2005b) Zinc mediated protection to the conformation of carbonic anhydrase in cadmium exposed *Ceratophyllum demersum* L. *Plant Sci* 169:245–254
- Aravind P, Prasad MNV (2005c) Modulation of cadmium-induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate–glutathione cycle and glutathione metabolism. *Plant Physiol Biochem* 43:107–116
- Aravind P, Prasad MNV (2005d) Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids. *Chemosphere* 61:1720–1733
- Aravind P, Prasad MNV, Malec P, Waloszek A, Strzałka K (2009) Zinc protects *Ceratophyllum demersum* L. (free-floating hydrophyte) against reactive oxygen species induced by cadmium. *J Trace Elem Med Biol* 23:50–60
- Armstrong W (1979) Aeration in higher plants. *Adv Bot Res* 7:226–332
- Augustynowicz J, Grosicki M, Hanus-Fajerska E, Lekka M, Waloszek A, Kołoczec H (2010) Chromium(VI) bioremediation by aquatic macrophyte *Callitriche cophocarpa* Sendtn. *Chemosphere* 79:1077–1083
- Ayeni OO, Ndakidemi PA, Snyman RG, Odendaal JP (2010) Chemical, biological and physiological indicators of metal pollution in wetlands. *Sci Res Essays* 5:1938–1949
- Basiouny FM, Haller WT, Garrard LA (1977) Evidence for root Fe nutrition in *Hydrilla verticillata* Royle. *Plant Soil* 48:621–627
- Berner RA (1984) Sedimentary pyrite formation: an update. *Geochim Cosmochim Acta* 48:605–615
- Bleuel C, Wesenberg D, Sutter K, Miersch J, Braha B, Bärlocher F (2005) The use of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals: 3. Cd²⁺ accumulation capacities and biochemical stress response of two *Fontinalis* species. *Sci Total Environ* 345:13–21

- Blum WEH (2005) Do we need a journal of soils and sediments (Part 2)? *J Soils Sediments* 5:195–196
- Bonanno G, Lo Giudice R (2010) Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. *Ecol Indic* 10:639–645
- Boonyapookana B, Upatham E, Kruatrachue M, Pokethitiyook P, Singhakaew S (2002) Phytoaccumulation and phytotoxicity of cadmium and chromium in duckweed *Wolffia globosa*. *Int J Phytoremediation* 4:87–100
- Boulegue J, Lord CJ, Church TM (1982) Sulfur speciation and associated trace metals (Fe, Cu) in the pore waters of Great Marsh, Delaware. *Geochim Cosmochim Acta* 46:453–464
- Bruns I, Sutter K, Menge S, Neumann D, Krauss GJ (2001) Cadmium lets increase the glutathione pool in bryophytes. *J Plant Physiol* 158:79–89
- Bunluesin S, Pokethitiyook P, Lanza GR, Tyson JF, Kruatrachue M, Xing B, Upatham S (2007) Influences of cadmium and zinc interaction and humic acid on metal accumulation in *Ceratophyllum demersum*. *Water Air Soil Pollut* 180:225–235
- Butler TW (2006) Geochemical and biological controls on trace metal transport in an acid mine impacted watershed. *Environ Geochem Health* 28:231–241
- Chandra P, Kulshreshtha K (2004) Chromium accumulation and toxicity in aquatic vascular plants. *Bot Rev* 70:313–327
- Charles AL, Markich SJ, Ralph P (2006) Toxicity of uranium and copper individually, and in combination, to a tropical freshwater macrophyte (*Lemna aequinoctialis*). *Chemosphere* 62:1224–1233
- Choi JH, Park SS, Jaffe PR (2006) The effect of emergent macrophytes on the dynamics of sulfur species and trace metals in wetland sediments. *Environ Pollut* 140:286–293
- Chojnacka K, Chojnacki A, Górecka H, Górecki H (2005) Bioavailability of heavy metals from polluted soils to plants. *Sci Total Environ* 337:175–182
- Cobbett C, Goldsbrough P (2002) Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol* 53:159–182
- Dacey JW (1980) Internal winds in the water-lilies: adaptation for life in anaerobic sediments. *Science* 210:1017–1019
- DalCorso G, Farinati S, Maistri S, Furini A (2008) How plants cope with cadmium: staking all on metabolism and gene expression. *J Integr Plant Biol* 50:1268–1280
- Dazy M, Masfaraud JF, Férard JF (2009) Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. *Chemosphere* 75:297–302
- Dhir B, Sharmila P, PardhaSaradhi P, Abdul Nasim S (2009) Physiological and antioxidant responses of *Salvinia natans* exposed to chromium-rich wastewater. *Ecotoxicol Environ Saf* 72:1790–1797
- Ding B, Shi G, Xu Y, Hu J, Xu Q (2007) Physiological responses of *Alternanthera philoxeroides* (Mart.) Griseb leaves to cadmium stress. *Environ Pollut* 147:800–803
- Dirilgen N, Inel Y (1994) Effects of zinc and copper on growth and metal accumulation in duckweed, *Lemna minor*. *Bull Environ Contam Toxicol* 53:442–449
- Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM, Redmond MS (1990) Toxicity of cadmium in sediments: the role of acid volatile sulfide. *Environ Toxicol Chem* 9:1487–1502
- Dogan M, Saygideger SD, Colak U (2009) Effect of lead toxicity on aquatic macrophyte *Elodea canadensis* Michx. *Bull Environ Contam Toxicol* 83:249–254
- Du Laing G, Rinklebe J, Vandecasteele B, Tack FMG (2009) Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review. *Sci Total Environ* 407:3972–3985
- Elifantz H, Tel-Or E (2002) Heavy metal biosorption by plant biomass of the macrophyte. *Water Air Soil Pollut* 141:207–218
- Emerson S, Jacobs L, Tebo B (1983) The behavior of trace metals in marine anoxic waters: solubilities at the oxygen-hydrogen sulphide interface. In: Wong CS, Boyle E, Bruland KW, Burton JD, Goldberg ED (eds) *Trace metals in sea water*. Plenum Press, New York, pp 579–608

- Fritioff A, Greger M (2006) Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans*. *Chemosphere* 63:220–227
- Fritioff A, Greger M (2007) Fate of cadmium in *Elodea canadensis*. *Chemosphere* 67:365–375
- Gross MG (1978) Effects of waste disposal operations in estuaries and the coastal ocean. *Annu Rev Earth Planet Sci* 6:127–143
- Gutknecht JLM, Goodman RM, Balsler TC (2006) Linking soil process and microbial ecology in freshwater wetland ecosystems. *Plant Soil* 289:17–34
- Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J Exp Bot* 53:1–11
- Hasan SH, Talat M, Rai S (2007) Sorption of cadmium and zinc from aqueous solutions by water hyacinth (*Eichhornia crassipes*). *Bioresour Technol* 98:918–928
- Hinman ML, Klaine SJ (1992) Uptake and translocation of selected organic pesticides by the rooted aquatic plant *Hydrilla verticillata* Royle. *Environ Sci Technol* 26:609–613
- Hoffmann T, Kutter C, Santamaria JM (2004) Capacity of *Salvinia minima* Baker to tolerate and accumulate As and Pb. *Eng Life Sci* 4:61–65
- Howarth RW, Jørgensen BB (1984) Formation of ^{35}S -labelled elemental sulfur and pyrite in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during short-term $^{35}\text{S}\text{SO}_4$ reduction measurements. *Geochim Cosmochim Acta* 48:1807–1818
- Huerta-Diaz MA, Tessier A, Carignan R (1998) Geochemistry of trace metals associated with reduced sulfur in freshwater sediments. *Appl Geochem* 13:213–233
- Ince NH, Dirilgen N, Apikyan IG, Tezcanli G, Ustun B (1999) Assessment of toxic interactions of heavy metals in binary mixtures: a statistical approach. *Arch Environ Contam Toxicol* 36:365–372
- Jackson LJ (1998) Paradigms of metal accumulation in rooted aquatic vascular plants. *Sci Total Environ* 219:223–231
- Jiang FY, Chen X, Luo AC (2009) Iron plaque formation on wetland plants and its influence on phosphorus, calcium and metal uptake. *Aquat Ecol* 43:879–890
- Joshi MK, Mohanty P (2004) Chlorophyll *a* fluorescence as a probe of heavy metal ion toxicity in plants. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence, a signature of photosynthesis*. Springer, New York
- Kabata-Pendias A (2001) *Trace elements in the soil and plants*. CRC Press, Boca Raton
- Kanoun-Boulé M, Vicente JF, Nabais C, Prasad MNV, Freitas H (2009) Ecophysiological tolerance of duckweeds exposed to copper. *Aquat Toxicol* 91:1–9
- Knox AS, Paller MH, Nelson EA, Specht WL, Halverson NV, Gladden JB (2006) Metal distribution and stability in constructed wetland sediment. *J Environ Qual* 35:1948–1959
- Kosolapov DB, Kuschik P, Vainshtein MB, Vatsourina AV, Wiebner A, Kasterner M, Miler RA (2004) Microbial processes of heavy metal removal from carbon deficient effluents in constructed wetlands. *Eng Life Sci* 4:403–411
- Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annu Rev Plant Physiol Plant Mol Biol* 42:313–349
- Küpper H, Küpper F, Spiller M (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *J Exp Bot* 47:259–266
- Küpper M, Küpper F, Spiller M (1998) In situ detection of heavy metal substituted chlorophylls in water plants. *Photosynth Res* 58:123–133
- Kupper H, Setlik I, Spiller M, Kupper FC, Prasil O (2002) Heavy metal-induced inhibition of photosynthesis: targets of in vivo heavy metal chlorophyll formation. *J Phycol* 38:429–441
- Lage-Pinto F, Oliveira JG, Da Cunha M, Souza CMM, Rezende CE, Azevedo RA, Vitória AP (2008) Chlorophyll *a* fluorescence and ultrastructural changes in chloroplast of water hyacinth as indicators of environmental stress. *Environ Exp Bot* 64:307–313
- Lefroy RDB, Chaitep W, Blair GJ (1994) Release of sulfur from rice residues under flooded and non-flooded soil conditions. *Aust J Agric Res* 45:657–667
- Lerman A (1978) Chemical exchange across sediment–water interface. *Annu Rev Earth Planet Sci* 6:281–306

- Li M, Zhang LJ, Tao L, Li W (2008) Ecophysiological responses of *Jussiaea rapens* to cadmium exposure. *Aquat Bot* 88:347–352
- Lithner G, Borg H, Ek J, Fröberg E, Holm H, Johansson AM, Kärrhage P, Rosén G, Söderström M (2000) The turnover of metals in a eutrophic and an oligotrophic lake in Sweden. *AMBIO: J Human Environ* 29:217–229
- Lu X, Kruatrachue M, Pokethitiyook P, Homyok K (2004) Removal of cadmium and zinc by water hyacinth *Eichhornia crassipes*. *Sci Asia* 30:93–103
- Malec P, Maleva M, Prasad MNV, Strzałka K (2009a) Copper toxicity in leaves of *Elodea canadensis* Michx. *Bull Environ Contam Toxicol* 82:627–632
- Malec P, Maleva MG, Prasad MNV, Strzałka K (2009b) Identification and characterization of Cd-induced peptides in *Egeria densa* (water weed): putative role in Cd detoxification. *Aquat Toxicol* 95:213–221
- Malec P, Maleva MG, Prasad MNV, Strzałka K (2010) Responses of *Lemna trisulca* L. (Duckweed) exposed to low doses of cadmium: thiols, metal binding complexes, and photosynthetic pigments as sensitive biomarkers of ecotoxicity. *Protoplasma* 240:69–74
- Malec P, Waloszek A, Prasad MNV, Strzałka K (2008) Zinc reversal of cadmium-induced energy transfer changes in photosystem II of *Ceratophyllum demersum* L. as observed by whole-leaf 77 K fluorescence. *Plant Stress* 2:121–126
- Maleva MG, Nekrasova GF, Malec P, Prasad MNV, Strzałka K (2009) Ecophysiological tolerance of *Elodea canadensis* to nickel exposure. *Chemosphere* 77:392–398
- Mallick N, Rai LC (2002) Physiological responses of non-vascular plants to heavy metals. In: Prasad MNV, Strzałka K (eds) *Physiology and biochemistry of metal toxicity and tolerance in plants*. Kluwer, The Netherlands, pp 111–147
- Mendelssohn IA, Kleiss BA, Wakeley JS (1995) Factors controlling the formation of oxidized root channels a review. *Wetlands* 15:37–46
- Miretzky P, Saralegui A, Fernández-Cirelli A (2006) Simultaneous heavy metal removal mechanism by dead macrophytes. *Chemosphere* 62:247–254
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7:405–410
- Mohan BS, Hosetti BB (1999) Aquatic plants for toxicity assessment. *Environ Res* 81:259–274
- Monferrán MV, Agudo JS, Pignata ML, Wunderlin D (2009) Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte *Potamogeton pusillus*. *Environ Pollut* 157:2570–2576
- Myśliwa-Kurdziel B, Prasad MNV, Strzałka K (2002) Consequences of heavy metal exposure to the processes related to the light phase of photosynthesis. In: Prasad MNV, Strzałka K (eds) *Physiology and biochemistry of metal toxicity and tolerance in plants*. Kluwer, The Netherlands, pp 229–255
- Myśliwa-Kurdziel B, Strzałka K (2002) Influence of metals on the biosynthesis of photosynthetic pigments. In: Prasad MNV, Strzałka K (eds) *Physiology and biochemistry of metal toxicity and tolerance in plants*. Kluwer, The Netherlands, pp 201–228
- Myśliwa-Kurdziel B, Prasad MNV, Strzałka K (2004) Photosynthesis in heavy metal stressed plants. In: Prasad MNV (ed) *Heavy metal stress in plants from biomolecules to ecosystems*, 2nd edn. Springer, Berlin, pp 462–470, ISBN: 3-540-40131-8
- Namieśnik J, Rabajczyk A (2010) The speciation and physico-chemical forms of metals in surface waters and sediments. *Chem Spec Bioavailab* 22(1):1–21
- Nichols PB, Couch JD, Al-Hamdani SH (2000) Selected physiological responses of *Salvinia minima* to different chromium concentrations. *Aquat Bot* 68:313–319
- Nixdorf D, Lessman D, Steinberg CEW (2002) The importance of chemical buffering for pelagic and benthic colonization in acidic waters. *Water Air Soil Pollut* 3:27–46
- Nyquist J, Greger M (2009) A field study of constructed wetlands for preventing and treating acid mine drainage. *Ecol Eng* 35:630–642
- Oren-Benaroya R, Tzin V, Tel-Or E, Zamski E (2004) Lead accumulation in the aquatic fern *Azolla filiculoides*. *Plant Physiol Biochem* 42:639–645

- Otsuki A, Wetzel RG (1972) Coprecipitation of phosphate with carbonates in a marl lake. *Limnol Oceanogr* 17:763–766
- Paiva LB, Gonçalves de Oliveira J, Azevedo RA, Ribeiro DR, da Silva MG, Vitória AP (2009) Ecophysiological responses of water hyacinth exposed to Cr^{3+} and Cr^{6+} . *Environ Exp Bot* 65:403–409
- Peter R, Welsh H, Denny P (1979) Translocation of lead and copper in two submerged aquatic angiosperm species. *J Exp Bot* 30:339–345
- Pich A, Scholz G (1996) Translocation of copper and other micronutrients in tomato plants (*Lycopersicon esculentum* Mill.): nicotianamine-stimulated copper transport in the xylem. *J Exp Bot* 47:41–47
- Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. *Environ Exp Bot* 35:525–545
- Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. *Russ J Plant Physiol* 50:686–700
- Prasad MNV (2004) Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters soil-amending chelators and transgenic plants. In: Prasad MNV (ed) Heavy metal stress in plants from biomolecules to ecosystems, 2nd edn. Springer, Berlin, pp 345–391, ISBN: 3-540-40131-8
- Radić S, Babić M, Skobić D, Roje V, Pevalek-Kozlina B (2010) Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L. *Ecotoxicol Environ Saf* 73:336–342
- Rausser WE (1999) Structure and function of metal chelators produced by plants: the case for organic acids, aminoacids, phytin, and metallothioneins. *Cell Biochem Biophys* 31:19–48
- Rinklebe J, Franke Ch, Neue HU (2007) Aggregation of floodplain soils based on classification principles to predict concentrations of nutrients and pollutants. *Geoderma* 141:210–223
- Rout N, Shaw B (1998) Salinity tolerance in aquatic macrophytes: probable role of proline, the enzymes involved in its synthesis and C4 type of metabolism. *Plant Sci* 136:121–130
- Samanta GP (2010) A two-species competitive system under the influence of toxic substances. *Appl Math Comput* 216:291–299
- Samardakiewicz S, Strawiński P, Woźny A (1996) The influence of lead on callose formation in roots of *Lemna minor* L. *Biol Plantarum* 38:463–467
- Samardakiewicz S, Woźny A (2000) The distribution of lead in duckweed (*Lemna minor* L.) root tip. *Plant Soil* 226:107–111
- Sanità di Toppi L, Vurro E, Rossi L, Marabottini R, Musetti R, Careri M (2007) Different compensatory mechanisms in two metal-accumulating aquatic macrophytes exposed to acute cadmium stress in outdoor artificial lakes. *Chemosphere* 68:769–780
- Schneider IAH, Rubio J (1999) Sorption of heavy metal ions by the nonliving biomass of freshwater macrophytes. *Environ Sci Technol* 33:2213–2217
- Schor-Fumbarov T, Goldsbrough PB, Adam Z, Tel-Or E (2005) Characterization and expression of a metallothionein gene in the aquatic fern *Azolla filiculoides* under heavy metal stress. *Planta* 223:69–76
- Simpson SL, Apte SC, Batley GE (1998) Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. *Environ Sci Technol* 32:620–625
- St-Cyr L, Campbell PGC (1996) Metals (Fe, Mn, Zn) in the root plaque of submerged aquatic plants collected in situ: relations with metal concentrations in the adjacent sediments and in the root tissue. *Biogeochemistry* 33:45–76
- Suñe N, Sánchez G, Caffaratti S, Maine M (2007) Cadmium and chromium removal kinetics from solution by two aquatic macrophytes. *Environ Pollut* 145:467–473
- Tkalec M, Prebeg T, Roje V, Pevalek-Kozlina B, Ljubešić N (2008) Cadmium-induced responses in duckweed *Lemna minor* L. *Acta Physiol Plantarum* 30:881–890
- Tremel A, Masson P, Garraud H, Donard OFX, Baize D, Mench M (1997) Thallium in French agrosystems II. Concentration of thallium in field-grown rape and some other plant species. *Environ Pollut* 97:161–168

- Upadhyay RK, Panda SK (2010) Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed *Spirodela polyrhiza* L. *J Hazard Mater* 175:1081–1084
- Urban NR, Brezonik PL, Baker LA, Sherman LA (1994) Sulfate reduction and diffusion in sediments of Little Rock Lake, Wisconsin. *Limnol Oceanogr* 39:797–815
- Vajpayee P, Tripathi RD, Rai UN, Ali MB, Singh SN (2000) Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere* 41:1075–1082
- Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environ Int* 30:685–700
- Wierzbicka M, Szarek-Lukaszewska G, Grodzińska K (2004) Highly toxic thallium in plants from the vicinity of Olkusz (Poland). *Ecotoxicol Environ Saf* 59:84–88
- Wind T, Conrad R (1995) Sulfur compounds, potential turnover of sulfate and thiosulfate, and numbers of sulfate-reducing bacteria in planted and unplanted paddy soil. *FEMS Microbiol Ecol* 18:257–266
- Wolterbeek HT, van der Meer AJGM (2002) Transport rate of arsenic, cadmium, copper and zinc in *Potamogeton pectinatus* L.: radiotracer experiments with As-76 Cd-109, Cd-115, Cu-64 and Zn-65, Zn-69. *Sci Total Environ* 287:13–30
- Wurts WA, Durborow RM (1992) Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds; SRAC Publication No, 464. Southern Regional Aquaculture Center, Princeton
- Xu QS, Hu JZ, Xie KB, Yang HY, Du KH, Shi GX (2010) Accumulation and acute toxicity of silver in *Potamogeton crispus* L. *J Hazard Mater* 173:186–193
- Xue P, Li G, Liu W, Yan C (2010) Copper uptake and translocation in a submerged aquatic plant *Hydrilla verticillata* (L.f.) Royle. *Chemosphere* 81:1098–1103
- Yan Ch, Li G, Xue P, Wei Q, Li Q (2010) Competitive effect of Cu(II) and Zn(II) on the biosorption of lead(II) by *Myriophyllum spicatum*. *J Hazard Mater* 179(1–3):721–728
- Yang H, Shi G, Wang H, Xu Q (2010) Involvement of polyamines in adaptation of *Potamogeton crispus* L. to cadmium stress. *Aquat Toxicol* 100:282–288
- Yang J, Ye Z (2009) Metal accumulation and tolerance in wetland plants. *Front Biol China* 4:282–288
- Zhang Z, Rengel Z, Meney K (2010) Cadmium accumulation and translocation in four emergent wetland species. *Water Air Soil Pollut* 212:239–249