32
Absorption and Adsorption of Heavy Metals by Microalgae

Drora Kaplan
Department of Environmental Hydrology & Microbiology, Zuckerberg Institute for Water Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer, Israel

Abstract
Heavy metals are released into waterways due to various anthropogenic activities. Their treatment is of special importance, since heavy metals are nonbiodegradable and persist in the environment posing a threat to the biota due to their toxic nature. In this chapter, the current methods that have been used to remove and/or detoxify heavy metals in aquatic environments are reviewed with specific emphasis on microalgae. A literature survey indicates microalgae have developed a wide spectrum of absorption (extracellular) and adsorption (intracellular) mechanisms for coping with heavy metal toxicity. The potential of prokaryotic and eukaryotic microalgae living cells or their dead cell biomass in comparison to currently available physicochemical processes aimed at removing toxic heavy metals is discussed. Also addressed are the mandatory requirements to bring this heavy metal removal potential to an applicable, commercial stage.

Keywords heavy metals; microalgae; bioremediation; absorption; adsorption; metal speciation

32.1 INTRODUCTION

Metals are elements that occur naturally in rocks in relatively low concentrations. They have useful properties and are important components in our daily life. Metals and metalloids comprise about 75% of the known elements. Only H, B, C, N, P, O, S, halogens, and noble gases are not included in this category. Based on chemical and physical properties (the chemical approach), metals have been classified as light, heavy, and metalloids (semi-metals). The term **heavy metals** is widely used and refers to metals and metalloids with an atomic density greater than 5 g cm^{-3}. Sometimes the term **toxic heavy metals** is used to emphasize the impact of these elements on the environment and more specifically on their effect on the biota (the biological approach). Since **heavy metals** exert toxic effects on living organisms, they are termed **toxic heavy metals**. Some of the heavy metals, such as copper, nickel, and zinc, are, at very low concentrations, essential for life (also termed microelements or trace elements) because they play important roles in metabolic processes taking place in living cells (Gadd, 1993). However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Other heavy metals such as cadmium, lead, and mercury are nonessential and are known to cause severe damage in organisms even at very low concentrations. Metals in the environment occur in different chemical forms (metal speciation): as ions dissolved in water, as vapors, or as salts or minerals in rocks, sand, and soils. They can also be bound

Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition. Edited by Amos Richmond and Qiang Hu.

in organic or inorganic molecules or attached to particles in the air (Raspor, 1991; Wedepohl, 1991). The chemical form of a metal in the environment is constantly changing due to a wide spectrum of dynamic biochemical processes. The latter are influenced by biotic (interactions with living organisms, e.g., microorganisms, plants, and animals) and abiotic factors (e.g., temperature, pH, organic matter, and ionic strength) (Hafeburg & Kohe, 2007). Metal speciation (chemical forms) is determining metals solubility, mobility, availability, and toxicity. It is generally accepted that for most metals the free ion is the species' most toxic to aquatic life (Sunda & Guillard, 1976; Anderson & Morel, 1978). Some organic forms such as methyl-mercury are taken up very efficiently by living organisms. It is more toxic than other mercury species (George, 1991). A wide range of anthropogenic activities contribute to the discharge of heavy metals to the environment, for example, intensive agriculture, metallurgy, energy production, and microelectronic and sewage sludge. Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, their toxicity poses major environmental and health problems and requires a constant search for efficient, cost-effective technologies for detoxification of metal-contaminated sites.

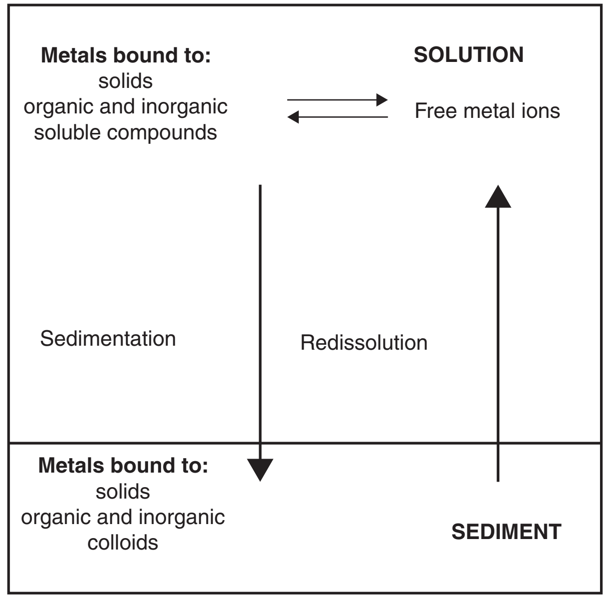


Figure 32.1. Schematic representation of possible metallic forms in an aquatic ecosystem. (From Kaplan, D. (2004). Reproduced with permission of John Wiley & Sons.)

32.2 HEAVY METALS IN THE AQUATIC SYSTEM

In the aquatic environment metallic elements may be present in a number of different chemical forms (species), distributed between the sediment and the solution (Florence & Batley, 1980; Foster & Wittman, 1981; Raspor, 1991). Metals may be present in the sediment as insoluble inorganic complexes, as suspended particles, or in association with organic colloids. In solution, metals may be present as free metal ions, and as organic and inorganic complexes. The equilibrium among all these metal species is interchangeable and depends on such environmental factors as temperature, pH, and alkalinity as well as on the biota thriving in the water (Canterford & Canterford, 1980; Peterson, 1982). A scheme illustrating the possible interactions among the various metal forms in an aquatic environment is presented in Figure 32.1. Biological availability of trace metals either as required nutrient (e.g., iron and zinc) or as toxicant (e.g., cadmium and lead) is dependent on its chemical form (Barber, 1973). Since in most cases the free metal ions, which are the bioavailable forms, are the most toxic species (Sunda & Guillard, 1976; Anderson & Morel, 1978; Gachter et al., 1978; Hudson, 1998), any process that accelerates their transformation into bound forms results in a reduction of toxicity to the biota (Butler et al., 1980; Canterford & Canterford, 1980; Peterson, 1982).

32.3 MICROALGAL RESPONSE TO HEAVY METALS STRESS

Microalgae are very sensitive to changes in their environment. In response to trace levels in the concentration to various organic and inorganic pollutants including heavy metals, changes take place in their overall metabolism. Microalgae are therefore often used as biological sensors for detecting potential toxic effects of heavy metals (Torres et al., 2000 and references therein; Chouteau et al., 2004; Durrieu et al., 2011). Microalgal biosensors harboring various algal species, chlorophyta, cyanobacteria, and diatoms, are described in a recent review and the advantages of such biosensors, in comparison to physical and chemical analyses, are discussed (Brayner et al., 2011). Toxic effects may be caused by a number of mechanisms: (a) the blocking of functional groups of biologically important molecules (e.g., enzymes and transport systems for essential nutrients and ions); (b) the displacement and/or substitution of essential metal ions from biomolecules and functional cellular units; and (c) induction of cellular generation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radical. High levels of ROS can oxidize proteins, lipids, and nucleic acids. This may result in modification and inactivation of enzymes as well as disruption of cellular and organellar membrane integrity (Mallick & Rai, 1992; Rai et al., 1994; Halliwell & Gutteridge, 1999; Sharma & Dietz, 2009). Clearly, toxic metals have the potential to interfere with a wide spectrum of activities in living organisms. Almost every aspect of their metabolism, growth, and differentiation may be affected. Some prokaryotic and eukaryotic organisms have developed means for rendering toxic heavy metals to innocuous forms. They possess a variety of mechanisms to maintain metal homeostasis and prevent poisoning. Heavy metal resistance in microalgae as well as in other microorganisms may result from the ability to prevent uptake (**avoidance**). This is achieved by adsorption of toxic metal ions to cell-associated materials and/or cell wall components (Kaplan et al., 1987a; Xue et al., 1988; Das et al., 2008; De Philippis & Micheletti, 2009; Naja & Volesky, 2011) or secretion of metal-binding organic compounds to the surrounding environment (McKnight & Morel, 1979; Van der Berg et al., 1979; Levy et al., 2008). In both cases, specificity is rather low and any metal cation may interact with negatively charged residues of the organic compounds to form complexes (see Fig. 32.2 for more details). Metal resistance may also result from the ability to cope with high amounts of heavy metals inside tissues (**tolerance**), an active process that involves the uptake (**absorption**) and accumulation of the metal ions inside the cell. Heavy metals enter microalgae cells via micronutrient transporters (Sunda & Huntsman, 1998

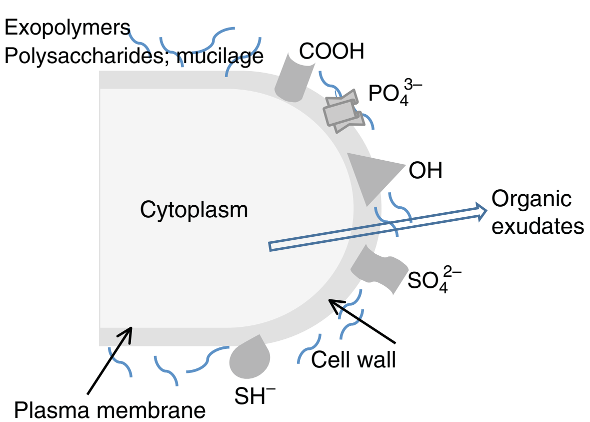
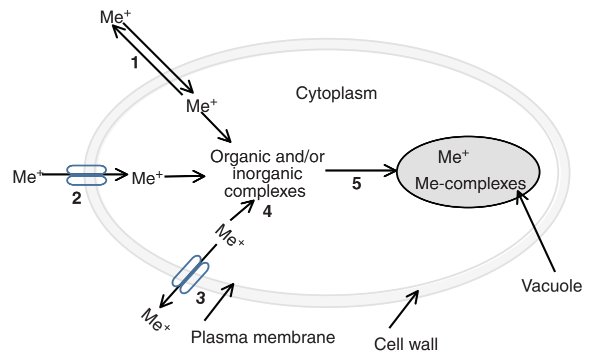


Figure 32.2. Possible absorption sites contributing to metal excluding (avoidance) mechanisms.



1 – Diffusion (inactive process); 2 – active uptake via ion transporters; 3 – active efflux of free metal ions; 4 – Formation of complexes of the free metal ions with various organic and/or inorganic compounds in the cytoplasm; 5 – transport to the vacuole and stored either as free metal ion or as complexes.

Figure 32.3. Metal ions uptake and detoxification processes (absorption) occurring inside living cells.

and references therein; Kramer et al., 2007; Arunakumara & Zhang, 2008). Once in the cell, heavy metal detoxification may be achieved by binding to specific intracellular compounds and/or transport of the metals to specific cellular compartments (Nagano et al., 1984; Heuillit et al., 1986; Vymazal, 1987; Perales-Vela et al., 2006; Arunakumara & Zhang, 2008). A schematic illustration is presented in Figure 32.3. A common mechanism for intracellular metal detoxification in living organisms is the formation of metal-binding peptides or proteins such as metallothioneins (Hamer, 1986; Grennan, 2011; Hassinen et al., 2011) and phytochelatins (Kondo et al., 1984; Gekeler et al., 1988; Kaplan et al., 1995; Hirata et al., 2005; Perales-Vela et al., 2006; Huang et al., 2009). For more details concerning the various types of metal-binding proteins and peptides in prokaryotic and eukaryotic microalgae, see reviews by Robinson (1989) and Lefebvre & Edwards (2010). Organic compounds such as malate, citrate, and polyphosphate were also reported as intracellular chelating agents (De Filippis & Pallaghy, 1994; Gasic & Korban, 2006; Perales-Vela et al., 2006).

Biosorption of heavy metals by microalgae is generally a biphasic process (Roy et al., 1993; Das et al., 2008). The first phase is adsorption by extracellular cell-associated materials, for example, polysaccharides and mucilage (Kaplan, 1988; Xue et al., 1988; De Philippis et al., 2011 and references therein; Mishra et al., 2011), and cell wall components, for example, carboxy and hydroxy groups, as well as sulfate and phosphate (Crist et al., 1981, 1999; Volesky, 1990; Eccles, 1999; Das et al., 2008; Naja & Volesky, 2011). This is a non-metabolic, rapid process, occurring in both living and nonliving cells. It is dependent on a number of parameters: pH (Hassett et al., 1981; Lau et al., 1999; Das et al., 2008; Gadd, 2009; Naja & Volesky, 2011), heavy metal species (Fourest & Volesky, 1997; Radway et al., 2001; Gadd, 2009; Naja & Volesky, 2011), type of algae (Kaplan et al., 1987a; Donmez et al., 1999; Chong et al., 2000; De Philippis et al., 2007), and biomass concentration (Donmez et al., 1999; Naja & Volesky, 2011). The second phase is absorption and accumulation inside the cell. This is a slow process involving active transport through the cell membrane into the interior and binding to proteins and other intracellular components. It is a metabolic-dependent mechanism that is inhibited by low temperatures, absence of an energy source, metabolic inhibitors, and uncouplers and occurs only in living cells (Wilde & Benemann, 1993). Understanding the mechanisms that convey metal resistance in various prokaryotic and eukaryotic microalgae can provide strategies for their removal from the environment.

32.4 CURRENT TECHNIQUES FOR METALS DETOXIFICATION

Industrial activities, such as mining, electroplating, and the production of electronics, fertilizers, pesticides, and pharmaceutics, significantly increase the contamination of the environment by toxic heavy metals. Unlike organic compounds, heavy metals cannot be degraded and tend to accumulate in living organisms. Because of their toxicity to living organisms, heavy metals pose a serious threat to the health of the environment. A number of physicochemical approaches are presently available for metal detoxification and removal from polluted environments. Yet, in recent years, with the increase in awareness and more stringent regulation, there is increasing interest in applying biological approaches for this purpose.

32.4.1 Physicochemical approaches

The most common physicochemical methods available for remediation of metal-contaminated waters are precipitation by adjusting the pH, membrane filtration, ion-exchange, flocculation, and/or adsorption by organic compounds. These methods often lack the specificity required for treating target metals. They are also inefficient and expensive, especially in cases where metal concentration in the wastewater is low. In addition, high cost and complicated operation often limit their use in large-scale *in situ* operations. For a detailed description of the common physicochemical methods currently available, see recent reviews by Rao et al. (2010) and Fu & Wang (2011).

32.4.2 Biological approaches

Biological approaches are based on the use of naturally occurring processes. Many microorganisms take part in the biogeochemical cycling of toxic heavy metals. Microalgae and other microorganisms play a significant role in the transformation of heavy metal ions in the environment (Gadd, 2004; Hafeburg & Kohe, 2007). Organic compounds released from growing cells, as well as biodegradation products of various origins, may serve as complexing agents for metal ions, thereby decreasing metal toxicity (McKnight & Morel, 1979; Kaplan et al., 1987a; Gasic & Korban, 2006; Worns et al., 2006). The binding of metal ions to cell wall components of microalgae was also reported (Kaplan et al., 1987b; Kaplan, 1988; Xue et al., 1988; Mehta & Gaur, 2005; Gupta & Rastogi, 2008). Various metabolic processes such as photosynthesis, respiration, and nutrient uptake take place during the growth of microalgae. All influence the equilibrium between free metal ions and the bound forms, as well as that between sedimentation and redissolution in the aquatic environment. Microalgae thriving in metal-contaminated sites also possess intracellular mechanisms that enable them to cope with the toxic effects of metals (Perales-Vela et al., 2006; Seth et al., 2011). Such species may be used for *in situ* bioremediation of large water bodies contaminated with low concentrations of metal ions (for more detailed comparisons between physicochemical and biological approaches for metal detoxification see Wilde & Benemann, 1993; Eccles, 1999; Volesky, 2001; Gadd, 2009; Naja & Volesky, 2011).

32.5 POTENTIAL APPLICATIONS OF MICROALGAE IN HEAVY METAL BIOREMEDIATION

The remarkable increase in the number of publications (research papers and reviews) dealing with metal removal, transformation, and detoxification, using biological processes since 1990, including those which describe the use of microalgae, clearly indicates the interest and potential of the biological approach (Gadd & White, 1993; Kratochvil & Volesky, 1998; Eccles, 1999; Gadd, 2001; Volesky, 2001; Perales-Vela et al., 2006; Das et al., 2008; Doshi et al., 2008; Gadd, 2009; Chojnacka, 2010; De Philippis et al., 2011 and references therein; Macek & Mackova, 2011; Mishra et al., 2011). To date, it is generally accepted that technologies based on naturally occurring biological processes have a number of advantages over presently available physicochemical techniques for remediation of sites contaminated with toxic heavy metals. These advantages have been discussed in detail, in a number of publications (Wilde & Benemann, 1993; Eccles, 1999; Gadd, 2001; Volesky, 2001;

Ahalya et al., 2003; Das et al., 2008; Macek & Mackova, 2011).The potential of many organisms (algae, bacteria, cyanobacteria, fungi, and plants) as well as dead biomass derived from them for metal bioremediation was examined (Gadd & White, 1993; Wilde & Benemann, 1993; Volesky & Holan, 1995; Kratochvil & Volesky, 1998; Donmez et al., 1999; Hirata et al., 2005; Kawakami et al., 2006; Fu & Wang, 2011; Naja & Volesky, 2011).

Microalgae are very abundant in the natural environment and are well adapted to a wide range of habitats, for example, fresh- and seawater, domestic and industrial effluents, salt marshes, and constructed wetlands. They have a remarkable ability to take up and accumulate heavy metals from their surrounding environment (De Filippis & Pallaghy, 1994; Han et al., 2006, 2007; Perales-Vela et al., 2006; Rodríguez-Zavala et al., 2007; Arunakumara & Zhang, 2008; Doshi et al., 2008; Levy et al., 2008; De Philippis & Micheletti, 2009; Mishra et al., 2011; De Philippis et al., 2011). Their ability to sequester various metal ions such as copper, cadmium, nickel, gold, and chromium is well documented (Darnall et al., 1986; Harris & Ramelow, 1990; Asku et al., 1992; Cho et al., 1994; Chong et al., 2000; Wong et al., 2000; Tam et al., 2001; Han et al., 2006, 2007; Foster et al., 2008; Vogel et al., 2010; Kumar et al., 2012). Therefore, attempts were made to use microalgae, living cells or their dead biomass, for removing heavy metals from contaminated waters (Gadd, 1990; Volesky, 1990; Wilde & Benemann, 1993; Rehman & Shakoori, 2001; Mehta & Gaur, 2005; Doshi et al., 2006; Arief et al., 2008; Monteiro et al., 2010; Ribeiro et al., 2010; Durrieu et al., 2011; Pereira et al., 2011). The use of living cells is most efficient for removal of metal ions from large water bodies containing low concentrations (ppb range) of metal ions. Thus, living prokaryotic and eukaryotic microalgae can be used as a complementary treatment step, following physicochemical processes which are applied in sites containing high metal concentrations. Resistant microalgal species isolated from metal-contaminated sites have a higher capacity for accumulating heavy metals compared with species isolated from noncontaminated sites (Trollope & Evans, 1976; Wong & Pak, 1992; Wong et al., 2000; Malik, 2004; Kalinowska & Pawlik-Skowronska, 2010 and references therein). During algal growth, metals are removed from the surrounding environment and accumulated in the cells by both nonmetabolic-dependent processes (adsorption) and metabolic-dependent ones (absorption) (Perez-Rama et al., 2002; Malik, 2004; Kadukova & Vircıkova, 2005; Mehta & Gaur, 2005; Perales-Vela et al., 2006; Topperwien et al., 2007; Lavoie et al., 2009). Biosorption of heavy metals by living immobilized prokaryotic and eukaryotic microalgae cells, using various immobilizing material, is an additional option. Generally immobilized cells are more efficient in the removal of heavy metals compared to free living cells (Malik, 2004). In addition, by using immobilized cells harvesting of the algal biomass is more efficient (Malik, 2002 and references therein; Bayramoğlu et al., 2006; Saeed & Iqbal, 2006; Anjana et al., 2007; Khattar et al., 2007; Moreno-Garrido, 2008; De-Bashan & Bashan, 2010). Provided adequate environmental conditions for supporting microalgae growth, such as light, temperature, and pH, are present, the use of living microalgal biomass offers an efficient, simple, and cost-effective method. Microalgae in consortium with other microorganisms, such as microbial mats, are also capable of removing metals and metalloids as well as other recalcitrant organic compounds from contaminated sites (Bender et al., 2000 and references therein; Bender & Phillips, 2004; Munoz et al., 2006; Munoz & Guieysse, 2006; Loutseti et al., 2009; Kumar & Gaur, 2011; Kumar et al., 2012). Microalgal biomass has been successfully used as sorbing material (Corder & Reeves, 1994; Donmez et al., 1999; Klimmek et al., 2001; Paperi et al., 2006; Gupta & Rastogi, 2008, 2009; Aneja et al., 2010; Colica et al., 2010). The vast majority of the studies were conducted with synthetic solutions containing single metal ion and only limited information is available on biosorption by active (living cells) or inactive (nonliving cells) prokaryotic or eukaryotic biomass exposed to a mixture of several metals simultaneously (Mendoza-Cozatl et al., 2006; Munoz et al., 2006; Topperwien et al., 2007; Monteiro et al., 2011). In a few studies, the effect of dissolved organic matter on metal speciation and detoxification is also addressed (Lamelas & Slaveykova, 2007; Lamelas et al., 2009). Commercial algal biomass AlgaSORB® was produced based on the basic and applied work of Darnall (1991). Moreover, metals adsorbed on cell wall surfaces of algal biomass can be recovered and the sorbing material can be restored for reuse (Kotrba & Ruml, 2000 and references therein; Volesky, 2001 and references therein; Gong et al., 2005; Paperi et al., 2006; Gupta & Rastogi, 2008, 2009; Das, 2010). Removal of metals from sites contaminated with high concentrations of metals can be achieved using nonviable biomass as biosorbents (Kotrba & Ruml, 2000; Singh et al., 2007; Loutseti et al., 2009). Yet, it should be noted that biomass obtained from different algal species differ largely in their binding capacity for various heavy metals (Donmez et al., 1999; Chong et al., 2000; Wong et al., 2000; Klimmek et al., 2001; Radway et al., 2001; Wilke et al., 2006; Micheletti et al., 2008; Mishra et al., 2011). The metal-binding capacity of biosorbents depends on the cell wall composition of the organism it is derived from and on the chemical composition of the metal ion solution to be treated. Therefore, in order to choose the most adequate biosorbent for metal decontamination of a specific site, it is essential to know which metals are present there and the concentration of each. Selection of the appropriate biomass is actually dictated by the metals to be removed, and the correct choice is essential for achieving efficient bioremediation.

In conclusion, many species of prokaryotic and eukaryotic microalgae, as well as their inactive cell biomass, can be used for bioremediation of metal-polluted sites. In order to bring this potential to the applicable stage on a commercial basis, more information on metal detoxification efficiency upon exposure of microalgal biomass to various metal-contaminated effluents is required. Such effluents usually contain a mixture of inorganic and organic compounds which might affect metal speciation and their availability and therefore influence the efficiency of the detoxification processes. So far most experiments were made in laboratory-scale reactors thus treatment of large volumes of contaminated sites requires upscaling. To achieve this target, interdisciplinary cooperation among professionals from various fields, for example, biologists, chemists, engineers, and environmentalists, would be fruitful.

REFERENCES

Ahalya, N., Ramachandra, T.V. & Kanamadi, R.D. (2003) Biosorption of heavy metals. *Res. J. Chem. Environ*. 7(4): 71–79.

Anderson, M. & Morel, F.M. (1978) Copper sensitivity of *Gonyaulax tamarenis*. *Limnol. Oceanogr*. 23: 283–295.

Aneja, R.V., Chaudhary, G., Ahluwalia, S.S. & Goyal, D. (2010) Biosorption of Pb^{2+} and Zn^{2+} by non-living biomass of *Spirulina* sp. *Indian J. Microbiol*. 50(4): 438–442.

Anjana, K., Kaushik, A., Kiran, B. & Nisha, R. (2007) Biosorption of Cr(VI) by immobilized biomass of two indigenous strains of cyanobacteria isolated from metal contaminated soil. *J. Hazard. Mater*. 148: 383–386.

Arief, V.O., Trilestari, K., Sunarso, J., Indraswati, N. & Ismadji, S. (2008) Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies. *Clean* 36(12): 937–962.

Arunakumara, K.K.I.U. & Zhang, X. (2008) Heavy metal bioaccumulation and toxicity with special reference to microalgae. *J. Ocean Univ. Chin*. 7: 60–64

Asku, Z., Sag, Y. & Kustal, T. (1992) The biosorption of copper by *C. vulgaris* and *Z. ramigera*. *Environ. Technol*. 13: 579–586.

Barber, R.T. (1973) Organic ligands and phytoplankton growth in nutrient-rich seawater. In: *Trace Metals and Metal Organic Interactions in Natural Waters* (ed. P. Singer), pp. 321–338. Ann Arbor Science, Ann Arbor, MI.

Bayramoğlu, G., Tuzun, I., Celik, G., Yilmaz, M. & Arica, M.Y. (2006) Biosorption of mercury(II), cadmium(II) and lead(II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads. *Int. J. Miner. Process*. 81: 35–43

Bender, J., Duff, M.C., Phillips, P. & Hill, M. (2000) Bioremediation and bioreduction of dissolved U(VI) by microbial mat consortium supported on silica gel particles. *Environ. Sci. Technol*. 34: 3235–3241.

Bender, J. & Phillips, P. (2004) Microbial mats for multiple applications in aquaculture and bioremediation. *Bioresour. Technol*. 94: 229–238.

Brayner, R., Couté, A., Livage, J., Perrette, C. & Sicard, C. (2011) Micro-algal biosensors. *Anal. Bioanal. Chem*. 401: 581–597.

Butler, M., Hasken, A.E.J. & Young, M.M. (1980) Copper tolerance in the green algae *Chlorella vulgaris*. *Plant Cell Environ*. 3: 119–126.

Canterford, G.S. & Canterford, D.R. (1980) Toxicity of heavy metals to the marine diatom *Ditylum Brightwellii* (west) Grunow: correlation between toxicity and metal speciation. *J. Mar. Biol. Assoc. UK* 60: 227–242.

Cho, D., Lee, S., Park, S. & Chung, A. (1994) Studies on the biosorption of heavy metals onto *Chlorella vulgaris*. *J. Environ. Sci. Health A* 29: 389–409.

Chojnacka, K. (2010) Biosorption and bioaccumulation – the prospects for practical applications. *Environ. Int*. 36: 299–307.

Chong, A.M.Y., Wong, Y.S. & Tam, N.F.Y. (2000) Performance of different microalgal species in removing nickel and zinc from industrial wastewater. *Chemosphere* 41: 251–257.

Chouteau, C., Dzyadevych, S., Chovelon, J-M. & Durrieu, C. (2004) Development of novel conductometric biosensors based on immobilized whole cell *Chlorella vulgaris* microalgae. *Biosens. Bioelectron*. 19: 1089–1096.

Colica, G., Mecarozzi, P. & De Phillippis, R. (2010) Treatment of Cr(VI)-containing wastewaters with exopolysaccharide-producing cyanobacteria in pilot flow through and batch systems. *Appl. Microbiol. Biotechnol*. 87: 1953–1961.

Corder, S.L. & Reeves, M. (1994) Biosorption of nickel in complex aqueous waste streams by cyano-bacteria. *Appl. Biochem. Biotechnol*. 45/46: 847–857.

Crist, R.H., Martin, J.R. & Crist, D.R. (1999) Interaction of metal ions with acid sites of biosorbents peat moss and *Vaucheria* and model substances alginic and humic acids. *Environ. Sci. Technol*. 33: 2252–2256.

Crist, R.H., Oberholser, K., Shank, N. & Nguyen, M. (1981) Nature of binding between metallic ions and algal cell wells. *Environ. Sci. Technol*. 15: 1212–1217.

Darnall, D.W. (1991) Removal and recovery of heavy metal ions from wastewaters using a new biosorbent; AlgaSORB. In: *Innovative Hazardous Waste Treatment Series. Biological Process* (eds H.M. Freeman & P.R. Sferra), pp. 65–72. Technomic Publishing Company, Lancaster, PA.

Darnall, D.W., Green, B., Henzel, M.T., Hosea, J.M., McPerson, R.A., Sneddon, J. & Alexander, M.D. (1986) Selective recovery of gold and other metal ions from an algal biomass. *Environ. Sci. Technol*. 20: 206–208.

Das, N., Vimala, R. & Karthika, P. (2008) Biosorption of heavy metals – an overview. *Indian J. Biotechnol*. 7: 159–169.

Das, N. (2010) Recovery of precious metals through biosorption – a review. *Hydrometallurgy* 103: 180–189.

De-Bashan, L.E. & Bashan, Y. (2010) Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresour. Technol*. 101: 1611–1627.

De Filippis, L.F. & Pallaghy, C.K. (1994) Heavy metals: sources and biological effects. In: *Advances in Limnology Series: Algae and Water Pollution* (eds L.C. Rai, J.P. Gaur & C.J. Soeder), pp. 31–77. Eschweizerbartsche Press, Stuttgart.

De Philippis, R., Colica, G. & Mecarozzi, P. (2011) Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. *Appl. Microbiol. Biotechnol*. 92: 697–708.

De Philippis, R. & Micheletti, E. (2009) Heavy metal removal with exopolysaccharide-producing cyanobacteria. In: *Heavy Metals in the Environment* (eds L.K. Wang, J.P. Chen, Y.-T. Hung & N.K. Shammas), pp. 89–122. CRC Press, Boca Raton, FL.

De Philippis, R., Paperi, R. & Sili, C. (2007) Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria. *Biodegradation* 18: 181–187.

Donmez, G.C., Akzu, Z., Ozturk, A. & Kutsal, T. (1999) A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochem*. 34: 885–892.

Doshi, H., Ray, A., Kothari, I.L. & Gami, B, (2006) Spectroscopic and SEM studies on bioaccumulation of pollutants by algae. *Curr. Microbiol*. 53:148–157.

Doshi, H, Ray, A., Seth, C.S. & Kothari, I.L. (2008) Bioaccumulation of heavy metals by green algae. *Curr. Microbiol*. 56: 246–255.

Durrieu, C., Guedri, H., Fremion, F. & Volatier, L. (2011) Unicellular algae used as biosensors for chemical detection in Mediterranean lagoon and coastal waters. *Res. Microbiol*. 162: 908–914.

Eccles, H. (1999) Treatment of metal-contaminated wastes: why select a biological process? *Trends Biotechnol*. 17: 462–465.

Florence, T.M. & Batley, G.E. (1980) Chemical speciation in natural waters. *CRC Crit. Rev. Anal. Chem*. 9: 219–296.

Foster, S., Tomson, D. & Maher, W. (2008) Uptake and metabolism of arsenate by anexic cultures of the microalgae *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. *Mar. Chem*. 108: 172–183.

Foster, U. & Wittman, G.T.W. (1981) *Metal Pollution in the Aquatic Environment*. Springer-Verlag, New York.

Fourest, E. & Volesky, B. (1997) Alginate properties and heavy metal biosorption by marine algae. *Biochem. Biotechnol*. 67: 215–226.

Fu, F. & Wang, Q. (2011) Removal of heavy metal ions from wastewaters: a review. *J. Environ. Manage*. 92: 407–441.

Gachter, R.J.S.D., Davis, J.S. & Mares, A. (1978) Regulation of copper availability to phytoplankton by macromolecules in the lake water. *Environ. Sci. Technol*. 12: 1416–1422.

Gadd, G.M. (1990) Biosorption. *Chem. Ind*. 2: 421–426.

Gadd, G.M. (1993) Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiol. Rev*. 11: 297–316.

Gadd, G.M. (2001) Microbial metal transformations. *J. Microbiol*. 39: 83–88.

Gadd, G.M. (2004) Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122: 109–119.

Gadd, G.M. (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *J. Chem. Technol. Biotechnol*. 84: 13–28.

Gadd, G.M. & White, R.D. (1993) Microbial treatment of metal pollution – working biotechnology? *Trends Biotechnol*. 11: 353–359.

Gasic, K. & Korban, S.S. (2006) Heavy metal stress. In: *Physiology and Molecular Biology of Stress Tolerance in Plants* (eds K.V. Madhava Rao, A.S. Raghavendra & K. Janardhan Reddy), pp. 219–254. Springer, Dordrecht.

Gekeler, W., Grill, E., Winnacker, E.L. & Zenk, M.H. (1988) Algae sequester heavy metals via synthesis of phytochelatin complexes. *Arch. Microbiol*. 150: 197–202.

George, S.G. (1991) Cell biochemistry and transmembrane transport of some metals. In: *Metals and Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance* (ed. E. Merian), pp. 551–522. VCH Weinheim, New York.

Gong, R., Ding, Y., Liu, H. & Chen, Q. (2005) Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. *Chemosphere* 58: 125–130.

Grennan, A. (2011) Metallothioneins, a diverse protein family. *Plant Physiol*. 155: 1750–1751.

Gupta, V.K. & Rastogi, A. (2008) Biosorption of lead from aqueous solutions by green algae *Spirogyra* species: kinetics and equilibrium studies. *J. Hazard. Mater*. 152: 407–414.

Gupta, V.K. & Rastogi, A. (2009) Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions. *J. Hazard. Mater*. 163: 396–402.

Hafeburg, G. & Kohe, E. (2007) Microbes and metals: interactions in the environment. *J. Basic Microbiol*. 47: 453–467.

Halliwell, B. & Gutteridge, J.M.C. (1999) *Free Radicals in Biology and Medicine*, 3rd edn, 936 pp. Oxford University Press, New York.

Han, X., Wong, Y.S. & Tam, N.F.Y. (2006) Surface complexation mechanism and modeling in Cr(III) biosorption by a microalgal isolate, *Chlorella miniat*. *J. Colloid Interface Sci*. 303: 365–371.

Han, X., Wong, Y.S., Wong, M.H. & Tam, N.F.Y. (2007) Biosorption and bioreduction of Cr (VI) by a microalgal isolate, *Chlorella miniata*. *J. Hazard. Mater*. 146: 65–72.

Hamer, D.H. (1986) Metallothionein. *Annu. Rev. Biochem*. 55: 913–951.

Harris, P.O. & Ramelow, G.J. (1990) Binding of metal ions by particulate biomass derived from *Chlorella vulgaris* and *Scenedesmus quadricauda*. *Environ. Sci. Technol*. 24: 220–228.

Hassett, J.M., Jennett, J.C. & Smith, J.E. (1981) Microplate technique for determining accumulation of metals of algae. *Appl. Environ. Microbiol*. 41: 1097–1106.

Hassinen, V.H., Tervahauta, A.I., Schat, H. & Karenlampi, S.O. (2011) Plant metallothioneins – metal chelators with ROS scavenging activity? [review article]. *Plant Biol*. 13: 225–232.

Heuillit, E., Moreau, A., Haplern, S., Jeanne, N. & Puiseux-Dao, S. (1986) Cadmium binding to thiol-molecule in vacuoles of *Dunaliella bioculata* contaminated with CdCl2: electron probe microanalysis. *Biol. Cell* 58: 79–86.

Hirata, K., Tsuji, N. & Miyamoto, M. (2005) Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. *J. Biosci. Bioeng*. 100(6): 593–599.

Huang, Z., Li, L., Huang, G., Yan, Q., Shi, B. & Xu, X. (2009) Growth-inhibitory and metal-binding proteins in *Chlorella vulgaris* exposed to cadmium or zinc. *Aquat. Toxicol*. 91: 54–61

Hudson, R.J.M. (1998) Which aqueous species control the rates of trace metal uptake by aquatic biota? Observations and predictions of non-equilibrium effects. *Sci. Total Environ*. 219: 95–115.

Kadukova, J. & Vircıkova, E. (2005) Comparison of differences between copper bioaccumulation and biosorption. *Environ. Int*. 31: 227–232.

Kalinowska, R. & Pawlik-Skowronska, B. (2010) Response of two terrestrial green microalgae (Chlorophyta, Trebouxiophyceae) isolated from Cu-rich and unpolluted soils to copper stress. *Environ. Pollut*. 158: 2778–2785.

Kaplan, D. (1988) *Algal Polysaccharides as Natural Metal Chelators*. BARD & North Carolina Biotechnology Center, Beaufort, SC, pp. 51–55.

Kaplan, D., Abeliovich, A. & Ben-Yaakov, S. (1987b) The fate of heavy metals in wastewater stabilization ponds. *Water Res.* 21: 1189–1194.

Kaplan, D., Christiaen, D. & Arad (Malis), S. (1987a) Chelating properties of extracellular polysaccharides from *Chlorella* spp. *Appl. Environ. Microbiol*. 53: 2953–2956.

Kawakami, S.K., Gledhill, M. & Achterberg, E.P. (2006) Production of phytochelatins and glutathione by marine phytoplankton in response to metal stress. *J. Phycol*. 42: 975–989.

Khattar, J.I.S., Sarma, T.A. & Sharma, A. (2007) Optimization of chromium removal by the chromium resistant mutant of the cyanobacterium *Anacystis nidulans* in a continuous flow bioreactor. *J. Chem. Technol. Biotechnol*. 82: 652–657.

Klimmek, S., Stan, H.J., Wilke, A., Bunke, G. & Buchholz, R. (2001) Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. *Environ. Sci. Technol*. 35: 4283–4288.

Kondo, N., Imai, K., Isobe, M., Goto, T., Murasugi, A., Wada-Nakagawa, C. & Hayashi, Y. (1984) Cadistin A and B, major unit peptides comprising cadmium binding peptides induced in a fission yeast – separation, revision of structure and synthesis. *Tetrahedron Lett*. 25: 3869–3872.

Kotrba, P. & Ruml, T. (2000) Bioremediation of heavy metal pollution exploiting constituents metabolites and metabolic pathways of livings. A review. *Collect. Czech Chem. Commun*. 65: 1205–1247.

Kramer, U., Talke, I.N. & Hanikenne, M. (2007) Transition metal transport. *FEBS Lett*. 581: 2263–2272.

Kratochvil, D. & Volesky, B. (1998) Advances in the biosorption of heavy metals. *Trends Biotechnol*. 16: 291–300.

Kumar, D. & Gaur, J.P. (2011) Metal biosorption by two cyanobacterial mats in relation to pH, biomass concentration, pretreatment and reuse. *Bioresour. Technol*. 102: 2529–2535.

Kumar, D., Rai, J. & Gaur, J.P. (2012) Removal of metal ions by *Phormidium bigranulatum* (Cyanobacteria)-dominated mat in batch and continuous flow systems. *Bioresour. Technol*. 104: 202–207.

Lamelas, C., Pinheiro, J.P. & Slaveykova, V.T. (2009) Effect of humic acid on Cd(II), Cu(II), and Pb(II) uptake by freshwater algae: kinetic and cell wall speciation considerations. *Environ. Sci. Technol*. 43: 730–735.

Lamelas, C. & Slaveykova, V.T. (2007) Comparison of Cd9II), Cu(II) and Pb(II) biouptake by green algae in presence of humic acid. *Environ. Sci. Technol*. 41: 4172–4178.

Lau, P.S., Lee, H.Y., Tsang, C.C.K., Tam, N.F.Y. & Wong, Y.S. (1999) Effect of metal interference, pH, and temperature on Cu and Ni biosorption by *Chlorella vulgaris* and *Chlorella miniata*. *Environ. Technol*. 20: 953–961.

Lavoie, M., Le Faucheur, S., Fortin, C. & Campbell, P.G.C. (2009) Cadmium detoxification strategies in two phytoplankton species: metal binding by newly synthesized thiolated peptides and metal sequestration in granules. *Aquat. Toxicol*. 92: 65–75

Lefebvre, D.D. & Edwards, C. (2010) Decontaminating heavy metals from water using photosynthetic microbes.

In: *Emerging Environmental Technologies*, Vol. II (ed. V. Shah), pp. 57–73. Springer, Dordrecht.

Levy, J.L., Angel, B.M., Stauber, J.L., Poon, W.L., Simpson, S.L., Cheng, S.H. & Jolly, D.F. (2008) Uptake and internalisation of copper by three marine microalgae: comparison of copper-sensitive and copper-tolerant species. *Aquat. Toxicol*. 89: 82–93.

Loutseti, S., Danielidis, D.B., Economou-Amilli, A., Katsaros, Ch., Santas, R. & Santas, Ph. (2009) The application of a micro-algal/bacterial biofilter for the detoxification of copper and cadmium metal wastes. *Bioresour. Technol*. 100: 2099–2105.

Macek, T. & Mackova, M. (2011) Potential of biosorption technology. In: *Microbial Biosorption of Metals* (eds P. Kotrba, M. Mackova & T. Macek), pp. 7–17. Springer, Dordrecht.

Malik, A. (2004) Metal bioremediation through growing cells. *Environ. Int*. 30: 261–278.

Malik, N. (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals* 15: 377–390.

Mallick, N. & Rai, L.C. (1992) Metal induced inhibition of photosynthesis, photosynthetic electron transport chain and ATP content of *Anabaena doliolum* and *Chlorella vulgaris*: interaction with exogenous ATP. *Biomed. Environ. Sci*. 5: 241–250.

McKnight, D.M. & Morel, F.M.M. (1979) Release of weak and strong copper complexing agents by algae. *Limnol. Oceanogr*. 24: 823–837.

Mehta, S.K. & Gaur, J.P. (2005) Use of alga for removing heavy metal ions from wastewater: progress and prospects. *Crit. Rev. Biotechnol*. 25: 113–152.

Mendoza-Cózatl, D.G., Rangel-González, E. & Moreno-Sánchez, R. (2006) Simultaneous Cd^{2+}, Zn^{2+}, and Pb^{2+}, uptake and accumulation by photosynthetic *Euglena gracilis*. *Arch. Environ. Contam. Toxicol*. 51: 521–528.

Micheletti, A., Colica, G., Viti, C., Tamagnini, P. & De Philippis, R. (2008) Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. *J. Appl. Microbiol*. 105: 88–94.

Mishra, A., Kavita, K. & Jha, B. (2011) Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. *Carbohydr. Polym*. 83: 852–857.

Monteiro, C.M., Pasula, M.L. & Malcata, F.X. (2010) Cadmium removal by two strains of *Desmodesmus pleiomorphus* cells. *Water Air Soil Pollut*. 208: 17–27.

Monteiro, C.M., Pasula, M.L. & Malcata, F.X. (2011) Capacity of simultaneous removal of zinc and cadmium from contaminated media, by two microalgae isolated from a polluted site. *Environ. Chem. Lett*. 9: 511–517.

Moreno-Garrido, I. (2008) Microalgae immobilization: current techniques and uses. *Bioresour. Technol*. 99: 3949–3964.

Munoz, R., Alvarez, M.T., Munoz, A., Terrazas, E., Guieysse, B. & Mattiasson, B. (2006) Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. *Chemosphere* 63: 903–911.

Munoz, R. & Guieysse, B. (2006) Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water Res*. 40: 2799–2815.

Nagano, T., Miwa, M., Suketa, Y. & Okada, S. (1984) Isolation, physicochemical properties, and amino acid composition of a cadmium-binding protein from cadmium treated Chlorella ellipsoidea. *J. Inorg. Biochem*. 21: 61–71.

Naja, G. & Volesky B. (2011) The mechanism of metal cation and anion biosorption. In: *Microbial Biosorption of Metals* (eds P. Kotrba, M. Mackova & T. Macek), pp. 19–58. Springer, Dordrecht.

Paperi, R., Micheletti, E. & De Philippis, R. (2006) Optimization of copper sorbing–desorbing cycles with confined cultures of the exopolysaccharide-producing cyanobacterium Cyanospira capsulata. *J. Appl. Microbiol*. 101: 1351–1356.

Perales-Vela, H.V., Peña-Castro, J.M. & Cañizares-Villanueva R.O. (2006). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere* 64: 1–10.

Pereira, S., Micheletti, E., Zillel, A., Santos, A., Moradas-Ferreira, P., Tamagnini P. & De Philippis, R. (2011) Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? *Microbiology* 157: 451–458.

Perez-Rama, M., Alonso, J.A., Lopez, C.H. & Vaamonde, E.T. (2002) Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*. *Bioresour. Technol*. 84: 265–270.

Peterson, R. (1982) Influence of copper and zinc on the growth of a freshwater alga *Scenedesmus quadricauda*: the significance of chemical speciation. *Environ. Sci. Technol*. 16: 443–447.

Radway, J.C., Wilde, E.W., Whitaker, M.J. & Weissman, J.C. (2001) Screening of algal strains for metal removal capabilities. *J. Appl. Phycol*. 13: 451–455.

Rai, P.K., Mallick, N. & Rai, L.C. (1994) Effect of Cu and Ni on growth, mineral uptake, photosynthesis and enzyme activities of *Chlorella vulgaris* at different pH values. *Biomed. Environ. Sci*. 7: 56–67.

Rao, K.S., Mohapatra, M., Anand, S. & Venkateswarlu, P. (2010) Review on cadmium removal from aqueous solutions. *Int. J. Eng. Sci. Technol.* 2(7): 81–103.

Raspor, B. (1991) Metals and metal compounds in waters. In: *Metals and Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance* (ed. E. Merian), pp. 233–256. VCH Weinheim, New York.

Rehman, A. & Shakoori, A.R. (2001) Heavy metal resistance *Chlorella* spp., isolated from tannery effluents, and their role in remediation of hexavalent chromium in industrial waste water. *Bull. Environ. Contam. Toxicol*. 66: 542–547.

Ribeiro, R.F.L., Magalhaes, S.M.S., Barbosa, F.A.R., Nascentes, C.C., Campos, L.C. & Moraes, D.C. (2010) Evaluation of the potential of microalgae *Microcystis novacekii* in the removal of Pb^{2+} from an aqueous medium. *J. Hazard. Mater*. 179: 947–953

Robinson, N.J. (1989) Algal metallothioneins: secondary metabolites and proteins. *J. Appl. Phycol*. 1: 5–18.

Rodríguez-Zavala, J.S., García-García, J.D., Ortiz-Cruz, M.A. & Moreno-Sánchez, R. (2007) Molecular mechanisms of resistance to heavy metals in the protist *Euglena gracilis*. *J. Environ. Sci. Health A* 42(10): 1365–1378.

Roy, D., Greenlaw, P.N. & Shane, B.S. (1993) Adsorption of heavy metals by green algae and ground rice hulls. *J. Environ. Sci. Health A* 28: 37–50.

Saeed, A. & Iqbal, M. (2006) Immobilization of blue green microalgae on loofa sponge to biosorb cadmium in repeated shake flask batch and continuous flow fixed bed column reactor system. *World J. Microb. Biotechnol*. 22: 775–782.

Seth, C.S., Remans, T., Keunen, E., Jozefczzak, M., Gielen, H., Opdenakker, K., Weyens, N., Vangronsveld, J. & Cuypers, A. (2011) Phytoextraction of toxic metals: a central role for glutathione. *Plant Cell Environ*. doi: 10.1111/j.1365-3040.2011.02338.x.

Sharma, S.S. & Dietz K.J. (2009) The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci*. 14: 43–50.

Singh, A., Mehta, S.K. & Gaur, J.P. (2007) Removal of heavy metals from aqueous solution by common freshwater filamentous algae. *World J. Microbiol. Biotechnol*. 23: 1115–1120.

Sunda, W.G. & Guillard, D.M. (1976) Relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res*. 34: 511–529.

Sunda, W.G. & Huntsman, S.A. (1998) Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Sci. Total Environ*. 219: 165–181.

Tam, N.F.Y., Wong, J.P.K. & Wong, Y.S. (2001) Repeated use of two *Chlorella* species, *C. vulgaris* and WW1 for cyclic nickel biosorption. *Environ. Pollut*. 114: 85–92.

Topperwien, S., Xue, H., Behra, R. & Sigg, L. (2007) Cadmium accumulation in *Scenedesmus vacuolatus* under freshwater conditions. *Environ. Sci. Technol*. 41: 5383–5388.

Torres, E., Cid, A., Herrero, C. & Abalde, J. (2000) Effect of cadmium on growth, ATP content, carbon fixation and ultrastructure in the marine diatom *Phaeodactylum tricornutum* Bohlin. *Water, Air, Soil Pollut*. 117: 1–14.

Trollope, P.R. & Evans, B. (1976) Concentration of copper, iron, lead, nickel and zinc in freshwater algal blooms. *Environ. Pollut*. 11: 109–116.

Van der Berg, C.M.G., Wong, P.T.S. & Chau, Y.K. (1979) Measurement of complexing materials excreted from algae and their ability to ameliorate copper toxicity. *J. Fish. Res. Board Can*. 36: 901–905.

Vogel, M., Gunther, A., Rossberg, A., Li, B., Bernhard, G. & Raff, J. (2010) Biosorption of U(VI) by the green algae Chlorella vulgaris in dependence of pH value and cell activity. *Sci. Total Environ*. 409: 384–395.

Volesky, B. (1990) *Biosorption of Heavy Metals*, 396 pp. CRC Press, Boca Raton, FL.

Volesky, B. (2001) Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy* 59: 203–216.

Volesky, B. & Holan, Z.R. (1995) Biosorption of heavy metals. *Biotechnol. Prog*. 11: 235–250.

Vymazal, J. (1987) Toxicity and accumulation of cadmium with respect to algae and cyanobacteria: a review. *Toxicity Assess*. 2: 387–415.

Wedepohl, K.H. (1991) The composition of the upper earth's crust and the natural cycles of selected metals. Metal in natural raw materials, natural resources. In: *Metals and Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance* (ed. E. Merian), pp. 3–17. VCH Weinheim, New York.

Wilde, E.W. & Benemann, J.R. (1993) Bioremoval of heavy metals by the use of microalgae. *Biotechnol. Adv*. 11: 781–812.

Wilke, A., Buchholz, R. & Bunke, G. (2006) Selective biosorption of heavy metals by algae. *Environ. Biotechnol*. 2(2): 47–56.

Wong, J.P.K., Wong, Y.S. & Tam, N.F.Y. (2000) Nickel biosorption by two *Chlorella* species, *C. vulgaris* (a commercial species) and *C. Miniata* (a local isolate). *Bioresour. Technol*. 73: 133–137.

Wong, M.H. & Pak, D.C.H. (1992) Removal of copper and nickel by free and immobilized microalgae. *Biomed. Environ. Sci*. 5: 99–108.

Worns, I., Simon, D.F., Hassler, C.S. & Wilkinson, K.J. (2006) Bioavailability of trace metals to aquatic microorganisms: importance of chemical, biological and physical processes on biouptake. *Biochimie* 88: 1721–1731.

Xue, H.B., Stumm, W. & Sigg, L. (1988) The binding of heavy metals to algae surfaces. *Water Res*. 22: 917–926.