

Heavy Metal Bioaccumulation and Toxicity with Special Reference to Microalgae

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Abstract The bioaccumulation and toxicity of heavy metals were reviewed with special reference to microalgae, the key component of the food web in aquatic ecosystems. Heavy metals enter algal cells either by means of active transport or by endocytosis through chelating proteins and affect various physiological and biochemical processes of the algae. The toxicity primarily results from their binding to the sulphhydryl group in proteins or disrupting protein structure or displacing essential elements. Metals can break the oxidative balance of the algae, inducing antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and ascorbate peroxidase (APX). The amount of oxidized proteins and lipids in the algal cells thus indicates the severity of the stress. Algal tolerance to heavy metal is highly dependent upon the defense response against the probable oxidative damages. Production of binding factors and proteins, exclusion of metals from cells by ion-selective transporters and excretion or compartmentalization have been suggested with regard to reducing heavy metal toxicity. However, a comprehensive description on the mechanisms underlying metal toxicity of microalgae and gaining tolerance is yet to be elaborated.

Key words bioaccumulation; heavy metal; microalgae; toxicity

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1 Introduction

With the rapid industrial development, various wastes containing different metal ions are directly or indirectly discharged into the environment, bringing about serious environmental pollution, and threatening marine life (Bishop, 2002; Wang, 2002). Heavy metal disperses through the various trophic levels of an ecosystem, depending on the bioaccumulation characteristics of the metal of concern. Bioaccumulation occurs when a portion of a metal is retained by an organism. The term bioaccumulation describes an active process in which taking up metal is metabolically controlled. However, heavy metal bioavailability, accumulation and toxicity in aquatic biota depend essentially on many environmental variables (Pawlik-Skowrońska and Skowroński, 2001; Pawlik-Skowrońska, 2002).

Taking up metals is basically considered as a two-step process (Goyal *et al.*, 2003; Ferraz *et al.*, 2004). Complexation, ion exchange, adsorption, inorganic microprecipitation, oxidation and/or reduction have been proposed to explain the uptake process (Liu *et al.*, 2002). Metal ions are adsorbed first to the surface of cells by the interactions between the metal ions and metal-functional

groups such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thiol, *etc.*, present in the cell wall and then they penetrate the cell membrane and enter the cells (Wang and Chen, 2006). When the extracellular concentration of metal ions is higher than that of intracellular, metal ions can penetrate into the cell across the cell wall, and in fact, several possible mechanisms have been suggested to underline their transport (Van Ho *et al.*, 2002; Zalups and Ahmad, 2003). Molecular mimicry is one of such mechanisms whereby metal ions either compete for binding to multivalent ion carriers or, after binding to low molecular weight thiols (such as cysteine), enter the cell by active transport. In another type of mechanism, metal ions bound to chelating proteins (such as metallothioneins) may enter the cell by endocytosis (Van Ho *et al.*, 2002; Zalups and Ahmad, 2003). Metal ions can also enter the cells if the cell wall is disrupted by natural or artificial force (Wang and Chen, 2006). After entering, the metal ions are compartmentalized into different subcellular organelles. Vijver *et al.* (2004) summarized the accumulation strategies in which essential and non-essential metal ions may undergo different processes (Wang and Chen, 2006). However, it seems that the intracellular accumulation is an energy-driven process dependent on active metabolism. Despite the fact that many parameters play roles in the process of accumulation (Bajguz, 2000), it is clear

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that different species of algae accumulate heavy metal ions to various degrees (Jordanova *et al.*, 1999).

2 Heavy Metal Toxicity to Microalgae

Though metal toxicity to algae has been thoroughly studied in the past couple of decades (Tripathi *et al.*, 2000), a comprehensive description about the mechanism underlining the toxicity is yet to be elaborated. Toxicity of a metal seems to be related to cell surface interactions or to intracellular accumulation (Morlon *et al.*, 2005). In the case of algae, toxicity primarily results from metal binding to sulphhydryl groups of proteins or the disruption of protein structure or displacement of an essential element (De Filippis and Pallaghy, 1994). However, it seems that it is related, at least in part, to the oxidative stress induced in living systems (Pinto *et al.*, 2003). Metals can break the oxidative balance of the algae, inducing antioxidant enzymes such as SOD, GPX, and APX. The amount of oxidized proteins and lipids in the algal cells thus indicates the severity of the stress (Okamoto *et al.*, 1996). Oxidative damage could be caused either by means of increasing the cellular concentration of reactive oxygen species (ROS) (Macfarlane and Burchett, 2001) or by reducing the cellular antioxidant capacity (Sies, 1999).

Various mechanisms such as production of heavy metal binding factors and proteins (metallothionein, GSH and phytochelatin conjugates), exclusion of toxic heavy metals from cells by ion-selective metal transporters and excretion or compartmentalization have been proposed for reducing heavy metal toxicity to organisms (Hu *et al.*, 2001; Gharieb and Gadd, 2004). Algal tolerance to heavy metal, however, seems to be dependent highly on the defense responses against the possible oxidative damages (Pinto *et al.*, 2003), exudation capacity of chelating compounds, active efflux of metal ions by primary ATPase pumps and reduced uptake (Gaur and Rai, 2001). The earlier reported metal-binding proteins in plants were generally assumed to be metallothioneins (Vijver *et al.*, 2004), the small gene-encoded, Cys-rich polypeptides which play a key role among metal-binding ligands recognized so far (Cobbett and Goldsbrough, 2002; Gaur and Rai, 2001). Some believe that many plant species including some algae exhibit another mechanism in responding to heavy metal toxicity, *i.e.* production of proline (Pro) (Mehta and Gaur, 1999). The accumulation of Pro in stressed plants is associated with the damage of membranes and proteins (Verma, 1999). Siripornadulsil *et al.* (2002) proposed that Pro reduces heavy metal stress by detoxification of free radicals produced in response to metal poisoning. The mechanisms by which Pro reduces free radical damage include physical quenching of oxygen singlets and chemical reaction with hydroxyl radicals (Alia *et al.*, 2001).

Once entering the cell, the heavy metal ions may either be detoxified or adversely affect cell processes such as photosynthesis and cell division (Stauber and Davies,

2000). Many heavy metal ions have a direct influence on various physiological and biochemical processes of microalgae. As the growth reflects the metabolism of the cell, it has been used as a key indicator of the toxicity of heavy metal ions in microorganisms (Carr *et al.*, 1998) and it depends on the proper functioning of various metabolic processes, such as photosynthesis, respiration and nutrient uptake, *etc.* (Tripathi and Gaur, 2006). Growth inhibition in microalgae is related to the amount of heavy metal ions bound to the algal cell surface, in some cases, to the amount of intracellular heavy metal ions (Franklin *et al.*, 2000, 2002a; Ma *et al.*, 2003) and to the chemical nature of the heavy metal ions (Tripathi and Gaur, 2006). However, for zinc, growth inhibition may not be related to the intracellular metal concentration, but to extracellular zinc (Wilde *et al.*, 2006). In fact, the possible mode of toxic action of zinc is related to the cell membrane, where it may disrupt the uptake of calcium which is necessary for the Ca-ATPase activity in cell division (Stauber and Florence, 1990).

Heavy metal ions can cause plasma membrane depolarization and acidification of the cytoplasm (Cardozo *et al.*, 2002; Conner and Schimid, 2003). In fact, membrane injury is one important effect of heavy metal ions that may lead to the disruption of cellular homeostasis. A chain of metabolic events, beginning with the respiration and photosynthesis and continuing with uptake and assimilation of nutrients, dilution of intracellular level of the heavy metal ions, *etc.* seems to play an important role in balancing the cellular homeostasis, regardless of whether they are strongly or weakly correlated with the algal growth (Tripathi and Gaur, 2006). Membrane injuries seem to be common in cyanobacterial response to metal toxicity (Rangsayatorn *et al.*, 2002). Heavy metal ions (such as Pb^{2+}) are capable of binding to thylakoid membrane resulting in the alteration of the ultrastructure of thylakoids, which would eventually deteriorate the routine functions of thylakoids (Heng *et al.*, 2004). There are some reports on the destruction of the chloroplast by heavy metal ions at higher concentrations (Lamaia *et al.*, 2005). In fact, it is well known that Cd^{2+} disorganizes chloroplast causing the damage of photosynthetic pigments (Leborans and Novillo, 1996). As a consequence of this, the photosynthetic activity could severely be affected, causing the growth inhibition or complete death of the cells. Biosynthesis of phycocyanin and carotenoid could also be affected by the heavy metal ions (Atri and Rai, 2003). In addition, heavy metal ions could interrupt routine metabolic processes by competing for the protein binding sites, activate enzymes and various biological reactive groups, causing poor or no growth. The presence of heavy metal ions in the growth medium could induce the activity of the peroxidase that is involved in the degradation of indole acetic acid (IAA), a hormone widely known for its ability of stimulating plant growth and multiplication. Some heavy metal ions may inhibit enzymes in the cytoplasm such as esterase and b-D-galactosidase

(Franklin *et al.*, 2001). Taking into account of all the previous reports, it could be suggested here that heavy metal ions can inhibit the growth of microalgae in different ways, which depend on the species, the metal types and the condition in the growing media.

3 The Foreground of Such Research

Morlon *et al.* (2005) investigated the cellular growth and intracellular concentrations of selenite in the unicellular green alga *Chlamydomonas reinhardtii* and concluded that toxicity is mainly linked to intracellular accumulation of selenite. For all the experimental parameters (except lipid content), namely protein, carbohydrate, and photosynthetic-pigment levels, uptake and assimilation of nitrate, photosynthetic O₂ evolution and respiration and growth of *Scenedesmus* sp., were decreased by Cu and Zn (Tripathi and Gaur, 2006). They concluded that almost constant lipid level in *Scenedesmus* sp. under metal stress might be due to the tolerance of the lipid metabolism enzymes or to a simultaneous decrease (or increase) in the activities of enzymes involved in the production and consumption of lipids. Heng *et al.* (2004) reported that Cd²⁺ and Pb²⁺ could inhibit the growth of *Anabaena flos-aquae* by 50% at the respective concentrations of 0.15 and 1 µg mL⁻¹. The high toxicity of Cd²⁺ at very low concentration was further confirmed by Lasheen (1990), who reported a growth inhibition of algae even at 0.05 µg mL⁻¹. The dose-dependent manner of growth inhibition was proved also by Fargasova (1999) who studied the effects of Cd²⁺, Cu²⁺, Zn²⁺, Pb²⁺ and Fe²⁺ on the green alga *Scenedesmus quadricauda*. Fathi *et al.* (2000) further confirmed that the metals induced concentration-dependent reduction in the cell count of *Scenedesmus bijuga* and *Anabaena spiroides*. Siripornadulsil *et al.* (2002) observed that exposure of *Chlamydomonas* to lower toxic levels (50 to 100 mol L⁻¹) of Cd resulted in reduced growth rates and chlorophyll content per cell (70% less chlorophyll per cell) and increased lipid peroxidation and GSH oxidation. The mode of toxicity of copper has been investigated in several studies with microalgae (Wilde *et al.*, 2006). Wilde *et al.* (2006) reported that pH of the medium is an important factor in determining the metal toxicity to microalgae. Meanwhile El-Naggar *et al.* (1999) reported that low concentrations of heavy metal (Co²⁺) could stimulate growth of *Nostoc muscorum*. This was further confirmed by El-Enany and Issa (2000) who studied the growth of *Nostoc linckia* and *Nostoc rivularis* under low Cd²⁺ and Zn²⁺ concentrations. Substitution of heavy metals for Zn²⁺ in some metalloenzymes could be the possible reason for the growth promotion at low metal concentrations (El-Sheekh *et al.*, 2003).

4 Discussion and Conclusion

Heavy metal contamination of aquatic environment is a

matter of great concern. Heavy metals present in a bioavailable form may be bioaccumulated and thereby detrimentally affect organism health. Bioaccumulation of heavy metals by microalgae has often been discussed as microalgae occupy the top position of the food web in all aquatic ecosystems. Growth is widely used in assessing the toxicity of heavy metals to microalgae, because it is the metabolic process most frequently and apparently affected.

Most of the studies with microalgae (*Chlorella*, *Chlamydomonas*, *Scenedesmus* and *Pseudokirchneriella* sp.) have shown that the increase of metal toxicity with the increase of pH is a result of decreased competition between the metal ion and H⁺ at the cell surface (Franklin *et al.*, 2000; De Schampelaere *et al.*, 2003). However, some studies have shown that the increase of metal toxicity with the decrease of pH is due to the predominance of the free metal ion at low pH (Rai *et al.*, 1993). Morlon *et al.* (2005) stated that toxicity is primarily related to intracellular accumulation, while, Wilde *et al.* (2006) stated that zinc-induced inhibition is not related to the intracellular zinc concentration, but to extracellular zinc. Furthermore, Hassler and Wilkinson (2003) reported that in the case of zinc uptake in *Chlorella kesslerii*, neither zinc concentration in the solution nor surface-bound zinc was a good predictor of zinc uptake fluxes. Wilde *et al.* (2006) reported that copper has no effect on other cell functions such as photosynthesis, respiration, ATP production, electron transport and membrane ultrastructure, though it inhibits the cell division of *Chlorella* sp. However, Wong *et al.* (1994) reported copper-induced structural alterations in thylakoid membranes of *Chlorella* sp. and inhibition of photosynthesis. Although many plants and algae respond to heavy metal stress with increased steady state levels of Pro, Siripornadulsil *et al.* (2002) reported that *Chlamydomonas* does not accumulate Pro in response to Cd exposure. Their results are consistent with earlier investigations on wild-type *Chlamydomonas* strains, where carboxylate groups accounted for nearly half of all Cd binding sites (Adhiya *et al.*, 2002).

Toxicity studies are generally conducted under different laboratory and field conditions with various species. Thus the differences in major findings are unavoidable. Furthermore, most of the conclusions have been made on the basis of physiology. Despite the fact that many genes involved in the uptake or detoxification or tolerance to metal ions have been identified (Rosen, 2002), molecular genetic bases are still lacking. In fact, understanding the molecular and genetic base for these mechanisms will be an important aspect in developing bioremoval tools to clean the contaminated water bodies (Kuroda *et al.*, 2002; Bae *et al.*, 2003; Wang, 2005).

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