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REVIEW

Cell composition and metal tolerance in cyanobacteria

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This review examines interactions between cyanobacteria and metals with an emphasis on metal tolerance in these organisms. Aspects of metal toxicity and accumulation in various cyanobacteria species as related to cell composition will also be reviewed.

Keywords: cyanobacteria, metals, tolerance

Introduction

Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes that are widely distributed in freshwater, marine and terrestrial environments (Fogg *et al.* 1973). Cyanobacteria range in size from less than 1 to greater than 100 μm in diameter (Castenholz & Waterbury 1989). They are found as unicellular, colonial, filamentous or branched filamentous forms. The ecological importance of cyanobacteria has been recognized as initial colonizers of arid land, primary producers of organic matter and their ability to fix nitrogen (Carr & Whitton 1973, Fay 1983).

Several researchers have reported on the high metal binding ability of bacterial, fungal and algal cells (Beveridge & Murray 1976, Hoyle & Beveridge 1983, 1984, Wood & Wang 1983, Beveridge 1986, 1989, Greene *et al.* 1986, Kiff & Little 1986, Towsley *et al.* 1986, Trevor *et al.* 1986, Greene & Darnall 1990, Slawson *et al.* 1992). A significant contribution to metal sorption has been attributed to these organisms since they are abundant in natural environments (Beveridge 1984, 1986, Greene & Darnall 1990, McHardy & George 1990). In bacteria heavy metals accumulate in the cell wall and may even contribute to fossilization of microorganisms since some metals inhibit autolytic enzymes responsible for wall degradation (Ferris *et al.* 1988). In this way, heavy metals can remain immobilized in soil for prolonged periods.

Living or non-living microbial cells can reversibly bind significant quantities of metal ions from aqueous solutions, and various functional groups such as carboxyl, amino, phosphoryl, sulfhydryl and hydroxyl, which are found on cell wall components, and proteins and lipids are implicated (Siegel & Siegel 1973, Christ *et al.* 1981, Greene *et al.* 1986, Singh *et al.* 1989b, Greene & Darnall 1990). The use of non-living microbial cells for biosorption has been suggested to be advantageous for selective removal and recovery of metal contaminants from water since dead organisms are not affected by conditions that would normally be detrimental to living organisms. However, it is noted that non-living algal cells do not possess the metabolic activities of living systems, e.g. the ability to volatilize, precipitate and accumulate metals intracellularly (Olson & Brinckman 1987).

Cyanobacteria are probably the largest, most diverse and most widely distributed group of photosynthetic prokaryotes (Stanier & Cohen-Bazire, 1977), and are often abundant in metal-contaminated freshwater habitats (Say & Whitton 1980, Whitton 1980, Whitton *et al.* 1981, Whitton &

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Shehata 1982). The ability of cyanobacteria to accumulate heavy metals and radionuclides from their external environment has been demonstrated in several studies (Horikoshi *et al.* 1979, Stratton & Corke 1979a, b, c, Baxter & Jensen 1980, Laube *et al.* 1980, Massalski *et al.* 1981, Jensen *et al.* 1982, Wang & Wood 1984, Pettersson *et al.* 1985a, Fischer 1985, Verma & Singh 1990, Avery *et al.* 1991).

It is the intent of this review to summarize information about cyanobacteria cell composition and probable sites of metal deposition, and, in particular, review data from studies on cyanobacteria and heavy metals.

Cell composition of cyanobacteria

Cell envelope

The cell envelope of cyanobacteria consists of two membrane bilayers (the outer and cytoplasmic membrane) and between them, in the periplasmic space, a peptidoglycan layer (Jensen 1985). Many species have additional layers external to the outer membrane which have been called cell wall layer, sheath, capsule or slime (Drews 1973, Golecki 1977, Stanier & Cohen-Bazire 1977, Drews & Weckesser 1982). Differences in terminology can be attributed to the various techniques used in cell envelope preparation which may influence their delicate structure (Drews & Weckesser 1982). Spinae, fimbriae and S layers also have been described in some species (Dick & Stewart 1980, Perkins *et al.* 1981, Smarda 1988, Schultze-Lam *et al.* 1992). The cyanobacteria fine structure indicates a Gram-negative cell organization (Allen 1968; Edwards *et al.* 1968, Butler & Allsopp 1972). However, it has been shown that the peptidoglycan layer is thicker in some cyanobacteria than the Gram-negative bacteria. For example, in the unicellular cyanobacteria *Synechocystis* sp. the peptidoglycan layer is 12 nm, 10 nm in Synechococcus sp. and in the filamentous forms such as *Oscillatoria princeps*, 200 nm, and 8 nm in *Fischerella* sp. (Halfen & Castenholz 1971, Jurgens *et al.* 1983, 1985, Woitzik *et al.* 1988, Pritzer *et al.* 1989). Also, the degree of cross-linkage in *Synechocytis* sp. (56%) and *Synechococcus* sp. (57%), and the presence of a polysaccharide covalently linked to muramic acid 6-phosphate of peptidoglycan via phosphodiester bound found in *Synechocystis* sp., *Synechococcus* sp. and *Microcystis* sp. are properties comparable to those of Gram-positive bacteria (Jurgens *et al.* 1983, 1989, Jurgens & Weckesser 1986, Woitzik *et al.* 1988). *Synechocystis* sp. PCC 6714 also has been found to react positively in the Gram-reaction (Jurgens *et al.* 1985). It has been proposed that cyanobacteria might have developed a unique cell wall organization combining structural elements typical of both Gram-negative and Gram-positive bacteria (Jurgens *et al.* 1983, Jurgens & Weckesser 1986). However, many studies published so far are restricted to a few strains only and representatives of other genera will have to be studied to prove the generality of this proposal.

Cyanobacteria strains studied to date contain the constituents of A1γ-type peptidoglycan, glucosamine, muramic acid, glutamic acid, alanine and diaminopimelic acid (Drews & Weckesser 1982, Jurgens *et al.* 1983, Jurgens & Weckesser 1986). The chemotype A1γ peptidoglycan of cyanobacteria is the same as found in Gram-negative and Gram-positive bacteria where the carboxylate groups are believed to be the important sites for metal deposition (Beveridge & Murray 1980; Beveridge & Koval 1981). Amidation of some carboxyl groups in the *Synechocystis* sp. peptidoglycan has been reported but it is not known whether glutamic or diaminopimelic acids are amidated (Jurgens *et al.* 1983).

The purified cytoplasmic membrane of the unicellular cyanobacteria *Anacystis nidulans* is composed of lipids, proteins, carotenoids and negligible amounts of chlorophyll *a* (Omata & Murata 1983). The cytoplasmic membrane of *Synechocystis* sp. was similar to *Anacystis nidulans* in its carotenoid content and in the presence of at least 40 polypeptide bands (Omata & Murata 1984). A carotenoid-binding protein was purified from the cytoplasmic membrane of *Synechocystis* sp. (Bullerjahn & Sherman 1986) and an aa_3-type cytochrome oxidase protein was identified in *A. nidulans* (Molitor *et al.* 1987).

Most studies published have shown that the outer membrane of cyanobacteria includes lipopolysaccharides (LPS), proteins, lipids and carotenoids (Jurgens *et al.* 1985, 1989, Murata *et al.* 1981, Woitzik, *et al.* 1988). LPS were identified in a number of different cyanobacteria, including unicellular strains such as *Anacystis nidulans* (Weise *et al.* 1970, Golecki 1977; Katz *et al.* 1977), *Aphanothece halophytica* (Jones & Yopp 1979), *Synechococcus* sp. (Schmidt *et al.* 1980a, Schrader *et al.* 1981, Woitzik *et al.* 1988), *Synechocystis* sp. (Schmidt *et al.* 1980b, Jurgens *et al.* 1985, Jurgens & Weckesser 1985b), *Microcystis aeruginosa* (Raziuddin *et al.* 1983, Martin *et al.* 1989), *Microcystis* sp. (Jurgens *et al.* 1989), *Agmenellum quadruplicatum* (Buttke & Ingram 1975) and *Gloeobacter violaceus* (Schneider & Jurgens 1991), and also filamentous forms, e.g. from *Anabaena variabilis* (Weckesser *et al.* 1974),

Phormidium (Mikheyskaya *et al*. 1977), *Schizothrix calciola* (Keleti *et al*. 1979) and *Fischerella* sp. (Pritzer *et al*. 1989). Those studies showed similarities between LPS from cyanobacteria and Gram-negative bacteria, but considerable diversity in their chemical composition and biological characteristics, even between strains, has been reported. For example, 2-keto-3-deoxyoctonate, although considered a specific marker for LPS, was absent in *Anabaena variabilis*, *Anabaena flos-aquae*, *Synechocystis* sp., *Schizothrix calciola*, *Microcystis aeruginosa* and *Synechococcus* strains (Weckesser *et al*. 1974, Wang & Hill 1977, Keleti *et al*. 1979, Schmidt *et al*. 1980a, b, Schrader *et al*. 1981, Martin *et al*. 1989), and was found in small or negligible quantities in *Agmenellum quadruplicatum*, *Anacystis nidulans*, *Phormidium* sp. and *Synechococcus* strains (Weise *et al*. 1970, Buttke & Ingram 1975, Katz *et al*. 1977, Mikheyskaya *et al*. 1977, Schmidt *et al*. 1980a, Resch & Gibson 1983). Also, cyanobacteria such as *Anabaena variabilis, Schizothrix calcicola, Synechococcus* sp. and *Synechocystis* sp. have been reported to contain low amounts of phosphate in lipid A (Weckesser *et al*. 1974, Keleti *et al*. 1979, Schmidt *et al*. 1980a, b, Jurgens & Weckesser 1985b). No phosphate was detected in the LPS of *Anacystis nidulans* and *Microcystis aeruginosa* strains (Weise 1970, Katz *et al*. 1977, Martin *et al*. 1989). In contrast, a significant amount of phosphate was detected in the LPS of *Agmenellum quadruplicatum*, *Gloeobacter violaceus* and *Microcystis aeruginosa* strains (Buttke & Ingram 1975, Raziuddin *et al*. 1983, Martin *et al*. 1989).

The LPS of bacteria has been reported to be anionic and located in the outer surface. The phosphate groups within the lipid A and core oligosaccharide (2-keto-3-deoxyoctonate and various hepatoses) are believed to provide the negative charge (Beveridge 1981, Beveridge & Koval 1981, Hoyle & Beveridge 1983, Lugtenberg & van Alphen 1983). The phosphoryl groups are reported to be the primary sites for metal interaction (Couglin *et al*. 1983, Ferris & Beveridge 1984, 1986a,b). The LPS of several cyanobacteria such as *Anabaena variabilis*, *Anacystis nidulans*, *Synechocystis* sp. and *Synechococcus* sp. have been reported to be exposed on the outer surface (Weckesser *et al*. 1974, Katz *et al*. 1977, Schmidt *et al*. 1980a, b). However, most the mentioned studies showed that the LPS of cyanobacteria were neutral or exhibited a low negative charge since they did not contain acidic constituents (low or no phosphorus, 2-keto-3-deoxyoctonate and other acidic sugar derivates). It is not known which polymers of the outer membrane provide the charge.

No LPS could be found in *Anabaena flos-aquae* and in the sheath-forming *Chlorogloeopsis* sp. (Wang & Hill 1977, Schrader *et al*. 1982b). It has been shown that sheathed cyanobacteria LPS can be detected in cell wall fractions. However, it is difficult to isolate LPS using whole cells (Pritzer *et al*. 1989, Schneider & Jurgens 1991).

Proteins of different molecular weights have been found in the outer membrane of *Anacystis nidulans* (Golecki 1977, Murata *et al*. 1981, Resch & Gibson 1983), *Synechocistys* sp. PCC 6714 (Omata & Murata 1984, Jurgens *et al*. 1985), *Synechococcus leopoliensis* and *Synechococcus* sp. (Resch & Gibson 1983), *Synechococcus* sp. (Woitzik *et al*. 1988), *Microcystis* sp. (Jurgens *et al*. 1989), *Fischerella* sp. (Pritzer *et al*. 1989), and *Gloeobacter violaceus* (Schneider & Jurgens 1991). Some of these studies found that the proteins exhibit different properties than those from porins of Gram-negative bacteria (Resch & Gibson 1983, Jurgens *et al*. 1985, 1989, Woitzik *et al*. 1988, Schneider & Jurgens 1991). However, a pore forming protein was detected in outer membrane extracts of *Anabaena variabilis* and proved to be cation selective, probably due to an excess of negative charges in or near the pore (Benz & Bohme 1985). In the cell wall of *Aphanothece halophytica*, *Oscillatoria limnetica* and *Phormidium* sp. a glycoprotein was identified which might be involved in the gliding mechanisms (Simon 1981).

Carotenoids as constituents of the outer membrane were also detected in various unicellular cyanobacteria strains such as *Anacystis nidulans* (Resch & Gibson 1983), *Synechococcus* sp. (Resch & Gibson 1983, Woitzik *et al*. 1988), *Synechocystis* sp. (Omata & Murata 1984, Jurgens & Weckesser 1985b; Jurgens & Mantele 1991) and *Microcystis* sp. (Jurgens *et al*. 1989). An acidic carotenoid was isolated from the outer membrane and was absent in the cytoplasmic membrane (Omata & Murata 1984). Polar head groups were detected in the carotenoids and were attached to neutral sugars in carotenoid glycosides and/or hydroxy groups in xanthophylls (Jurgens & Mantele 1991). Carotenoids in the outer membrane of cyanobacteria have been analyzed in only a few strains. The function of the carotenoids in the outer membrane of cyanobacteria is also unknown.

Lipids have been isolated from the outer membrane of unicellular cyanobacteria such as *Anacystis nidulans* (Murata *et al*. 1981), *Synechocystis* sp. (Jurgens & Weckesser 1985b, Jurgens *et al*. 1985), *Synechococcus* sp. (Woitzik *et al*. 1988) and *Microcystis* sp. (Jurgens *et al*. 1989). In *Synechococcus* sp. (Woitzik *et al*. 1988) and *Synechocystis* sp. (Jurgens

& Weckesser 1985b), five and one unidentified strongly polar lipids were found, respectively.

The chemical composition of sheaths and slimes have been reported for *Clorogleopsis* sp. (Schrader *et al.* 1982a), *Anabaena cylindrica* (Dunn & Wolk 1970, Cardemil & Wolk 1979), *Anacystis nidulans* (Sangar & Dugan 1972), *Nostoc* sp. (Mehta & Vaidya 1978), *Gloeobacter violaceous* (Schneider & Jurgens 1991), *Crococcus minutus* (Adhikary *et al.* 1986), *Gloeothece* sp. (Tease & Walker 1987, Weckesser *et al.* 1987), *Calothrix* sp. (Weckesser *et al.* 1988), *Microcystis flos-aquae* (Plude *et al.* 1991), *Microcystis aeruginosa* (Nakagawa *et al.* 1987), *Fischerella* sp. (Pritzer *et al.* 1989), *Phormidium* sp. and *Anabaenopsis circularis* (Bar-Or & Shilo 1987). Those studies showed the slime layers or sheaths of cyanobacteria consist mainly of polysaccharides composed of at least one uronic acid and several neutral sugars, sometimes in combination with protein. Sulfate as a constituent of the sheath fraction has been reported in *Gloeobacter violaceous*, *Gloeothece* sp., *Fischerella* sp. and *Phormidium* sp. (Tease & Walker 1987, 1991, Bar-Or & Shilo 1987, Pritzer *et al.* 1989, Schneider & Jurgens 1991). Phosphate was found in the sheath of *Chroococcus minutus*, *Gloeobacter violaceous* and *Fischerella* sp. (Adhikary *et al.* 1986, Pritzer *et al.* 1989, Schneider & Jurgens 1991). The occurrence of several negatively-charged components such as uronic acid, sulfate and phosphate may provide various binding sites for cations.

The filamentous cyanobacteria *Phormidium* sp. and *Anabaenopsis circularis* were found to produce polyanionic macromolecules (flocculants) which differ in their biochemical compositions. In *Phormidium* the anionic density was relatively high and was attributed to carboxyl uronic acid residues and sulfate groups, while in *Anabaenopsis circularis* the ionic density was lower and was derived from carboxyl keto acid residues (Bar-Or & Shilo 1987).

Slime from different species of the colonial *Microcystis* can interact strongly with cations (Nakagawa *et al.* 1987, Doers & Parker 1988, Parker 1982) and appears to be involved in oxidative precipitation of manganese nodules in certain lakes (Richardson *et al.* 1988). The sheaths of *Lyngbya aestuarii* and *Scytonema myochrous* were involved in the formation of $CaCO_3$ (Pentecost & Bauld 1988) as was the S layer of *Synechococcus* GL 24 (Schutze-Lam *et al.* 1992). In *Microcystis flos-aquae* slime galacturonic acid was predominant and it was suggested that charge attraction to carboxyl groups contributed to cation binding (Plude *et al.* 1991). In addition, sugars with certain configurations of hydroxyl groups (axial, equatorial and axial in hexoses) can complex with some metals (Angyal 1972). Isolated sheaths of two filamentous cyanobacteria, *Calothrix parietina* and *Calothrix scopulorum*, composed of 50% (of sheath dry weight) neutral sugar, 5% amino acids and small amounts of glucosamine and galacturonic acid, showed binding of heavy metals, such as iron, zinc, copper, nickel, manganese, molybdenum and cobalt, up to at least 0.7% of sheath dry weight (Weckesser *et al.* 1988). Isolated sheath of *Gloeothece* sp. also showed heavy metal binding capacity (Tease & Walker 1987).

Cell inclusions

A number of intracellular inclusions have been observed in cyanobacteria species. Some cell inclusions such as DNA, ribosomes, thylakoids with associated phycobilisomes, polyglucose bodies, lipid inclusions, carboxysomes, cyanophycin granules and polyphosphate granules occur regularly in all cyanobacterial cells under normal growth conditions. Cyanobacteria also contain inclusions that are not found on a regular basis in all cells and they are common to only certain isolates. Over 30 inclusions have been described in this group (Jensen 1985). Some of the inclusions, mainly those of regular occurrence, have been widely studied and much is known about their structure and function (Lang 1968, Wolk 1973, Stanier & Cohen-Bazire 1977, Allen 1984, Jensen 1985).

In addition to chromosomal DNA, plasmids are found in many cyanobacteria (Asato & Ginoza 1973, Simon 1978, Lau *et al.* 1980, Reaston *et al.* 1980, Lambert & Carr 1982), but the role of the plasmids in the cells are not known. Some studies have been suggested that the extrachromosomal genes in cyanobacteria could be involved in conferring resistance to heavy metals and antibiotics (Olafson *et al.* 1979, Singh & Pandey 1982), and in the production of gas vacuoles (Walsby 1977).

Several studies using energy-dispersive X-ray analysis have shown that heavy metals were sequestered into polyphosphate granules of cyanobacteria (Crang & Jensen 1975, Baxter & Jensen 1980, Jensen *et al.* 1982, Rachlin *et al.* 1984, Pettersson *et al.* 1985a). Polyphosphate granules are composed of polyphosphate, the elements potassium, manganese and calcium, and probably lipid and protein (Baxter & Jensen 1980, Jensen *et al.* 1982). Polyphosphate, which has a negative surface charge, as well as proteins and lipids provide binding sites for heavy metals.

Differentiated cells

Several species of filamentous cyanobacteria have the ability to differentiate vegetative cells into two other types of cells, the heterocyst and akinete. Heterocysts are specialized cells which provide an environment with a reduced oxygen concentration suitable for nitrogenase activity (Wolk 1975, Haselkorn 1978, Rippka *et al*. 1979). They are produced under diazotrophic growth conditions and approximately 10% of the total cellular population develop into heterocysts (Rippka 1988). During differentiation, structural and biochemical changes occur and this is accompanied by genetic rearrangements (Wildon & Mercer 1963, Lang & Fay 1971, Winkenbach & Wolk 1973, Fleming & Haselkorn 1974, Tel-Or & Stewart 1977, Giddings & Staehelin 1978, Haselkorn 1986, Buikema & Haselkorn 1991). The most prominent change in the cellular structure is the presence of a complex, multi-layered envelope layer outside the cell wall. The outer envelope is composed of an unknown 'fibrous' outer layer, a central highly branched polysaccharide 'homogeneous' layer and a 'laminated' glycolipid inner layer (Lang & Fay 1971, Wilcox *et al*. 1973, Cardemil & Wolk 1976, Haselkorn 1978). The inner layer is composed entirely of glycolipids uniquely found in heterocysts (Walsby & Nichols 1969, Wolk & Simon 1969, Winkenbach *et al*. 1972, Lambein & Wolk 1973, Lorch & Wolk 1974, Davey & Lambein 1992a,b). It is believed the laminated layer is impermeable to water, ions, neutral hydrophilic solutes and dissolved gases (Haselkorn 1978, Murry & Wolk 1989, Ernst *et al*. 1992). An active exchange of metabolities is believed to occur between the heterocysts and the vegetative cells possibly via thin channel-like structures, called microplasmodesmata (Wildon & Mercy 1963, Lang & Fay 1971, Wilcox *et al*. 1973, Giddings & Staehelin 1978). It has been demonstrated that heterocysts are dependent upon vegetative cells for carbon compounds (Wolk 1968) since they lack both photosystem II and ribulose biphosphate carboxylase (Bradley & Carr 1971, Winkenbach & Wolk 1973). Vegetative cells are dependent upon nitrogen fixed in heterocysts (Meeks *et al*. 1978).

Akinetes are produced at the end of the growth exponential phase and are present only in some heterocystous cyanobacteria (Herdman 1988). The information available to date suggests they are involved in the reproduction and perennation of cyanobacteria (Nichols & Adams 1982, Herdman 1987). The akinetes are most easily recognized by light microscopy due to the present of numerous refractive cyanophycin granules (Miller & Lang 1968, Jensen & Clark 1969, Sutherland *et al*. 1979). They are usually larger than vegetative cells and their thick cell wall is surrounded by a thick outer envelope (Wildon & Mercer 1963).

Several studies developed with heterocystous cyanobacteria have reported that heavy metals decrease nitrogenase activity, produce multiple heterocysts, increase or decrease heterocyst frequency and cause pronounced damage (Stratton & Corke 1979a, Delmotte 1980, Massalski *et al*. 1981, Pettersson *et al*. 1985b, Rai & Raizada 1987, Dubey & Rai 1990). However, ultrastructural obseration revealed that heterocyst cells are more tolerant to heavy metals than vegetative cells (Pettersson *et al*. 1985b, Singh & Singh 1992b).

Metal uptake and accumulation

The uptake of heavy metals by microorganisms generally comprises two phases: binding of cations to the negatively-charged groups on the cell surface (passive) and the subsequent metabolism-dependent, intracellular uptake (active). The passive process is very rapid and occurs a short time after the microorganisms come into contact with the metal; the active process is slow (Khummongkol *et al*. 1982, Les & Walker 1984, Campbell & Smith 1986).

Adsorption followed by metabolism-dependent intracellular cation uptake has been described for cadmium in *Anacystis nidulans* and *Chrococcus paris*, copper in *Anacystis nidulans*, *Chrococcus paris*, *Nostoc calcicola* and *Nostoc muscorum*, zinc in *Anacystis nidulans* and *Chrococcus paris*, nickel in *Anabaena cylindrica*, mercury in *Nostoc calcicola*, lead in *Nostoc muscorum*, and chromium in *Anabaena doliolum* (Shehata & Whitton 1982, Les & Walker 1984, Schecher & Driscol 1985, Singh 1985, Singh & Yadava 1985, Campbell & Smith 1986, Verma & Singh 1991, Rai *et al*. 1992, Pandey & Singh 1993). Intracellular uptake can also be a result of permeation and diffusion due to increased membrane permeability (Gadd 1988). Passive accumulation of manganese, cobalt, zinc, silver, tin, cesium, mercury, neptunium, plutonium and americium has been demonstrated in *Synechococcus* sp. with concentration factors ranging from zero for cesium and neptunium to 10^6 for tin, mercury and lead, the order being $Pu \simeq Hg = Sn \geqslant Am > Ag > Zn > Co > Mn > Cs \simeq Np$ (Fischer 1985). The aluminum intracellular accumulation in *Anabaena cylindrica* also was suggested to occur via passive diffusion (Pettersson *et al*. 1986).

Some representative values of cyanobacteria metal accumulation are summarized in Table 1.

Many metals, such as copper, zinc, iron, nickel, manganese and cobalt, are essential for cyanobacterial growth and metabolism in low concentrations. However, at high concentrations they become toxic or lethal, as do the non-essential metals, such as lead, chromium and cadmium. For example, copper can be used as an algicide in water reservoirs and drainage systems (Verma & Singh, 1991). The effects of heavy metals in different species of cyanobacteria are summarized in Table 2 and the toxicity order of some metals is also presented in Table 3.

The uptake rate of a heavy metal is dependent on its speciation which is a key factor in determining its toxicity. Environmental variables such as pH, redox potential, salinity, alkalinity, temperature, available nutrients, metal concentration, cell density, extracellular metabolites and organic acids can affect metal toxicity (Gadd & Griffiths 1978, Rai *et al.* 1981, Reed & Gadd 1990).

One important influence on the physicochemical state of a metal is pH. An increase in toxicity under

Table 1. Some examples of metal accumulation by cyanobacteria

Organism	Element	Uptake	References
Unicellular			
Anacystis nidulans	Cd	3.7 nmol μg^{-1} protein	Singh & Yadava (1985)
Chroococcus paris	Cd	53 mg g^{-1} dry weight	Les & Walker (1984)
	Cu	120 mg g^{-1} dry weight	Les & Walker (1984)
	Zn	65 mg g^{-1} dry weight	Les & Walker (1984)
Synechococcus nic[7]	Ni	11 $\mu g\, g^{-1}$ dry weight	Wang & Wood (1984)
Synechococcus elongatus	U	158 $\mu g\, g^{-1}$ dry weight	Horikoshi *et al.* (1979)
Synechococcus sp.	U	1764 $\mu g\, g^{-1}$ dry weight	Sakaguchi *et al.* (1978)
Synechocystis PCC6803	Cs	510 nmol $(10^9)^{-1}$	Avery *et al.* (1991)
Filamentous			
Anabaena 7120	Cu	7 $\mu g\, mg^{-1}$ cells	Massalski *et al.* (1981)
	Cd	70 $\mu g\, mg^{-1}$ cells	Massalski *et al.* (1981)
Anabaena cylindrica	Al	33.1 mg g^{-1} dry weight	Pettersson *et al.* (1985b)
Nostoc calcicola	Cu	96.69 nmol mg^{-1} protein	Verma & Singh (1990)
Nostoc calcicola[a]	Cu	242.15 nmol mg^{-1} protein	Singh *et al.* (1989b)
Nostoc muscorum	Ni	8.41 μmol mg^{-1} dry weight	Singh *et al.* (1992)
Oscillatoria UTEX1270	Ni	19 $\mu g\, g^{-1}$ dry weight	Wang & Wood (1984)
Oscillatoria sp.	Cu	2.35 $\mu g\, g^{-1}$ dry weight	Ray & White (1976)
	Zn	505 $\mu g\, g^{-1}$ dry weight	Ray & White (1976)
	Cd	0.98 $\mu g\, g^{-1}$ dry weight	Ray & White (1976)
	Pb	568 $\mu g\, g^{-1}$ dry weight	Ray & White (1976)
Plectonema terebrans	Fe	4.3 mg g^{-1} dry weight	Raghukumar *et al.* (1989)

[a]Immobilized cells.

Table 2. Effect of heavy metals on cyanobacteria

Metal	Species	Concentration	Effect	References
Mercury	*Anabaena inaequalis*	39.88 nM	complete inhibition of growth	Stratton *et al.* (1979)
		> 0.498 μM	inhibition of nitrogenase activity and CO_2 fixation	Stratton *et al.* (1979)
	Anabaena flos-aquae	0.2 μM	50% inhibition of growth	Sharma & Bisen (1992)
		9 μM	50% inhibition of O_2 evolution	Sharma & Bisen (1992)
		22 μM	50% inhibition of CO_2 fixation and H^+ uptake activity	Sharma & Bisen (1992)
		25 μM	50% inhibition of carbonic anhydrase activity	Sharma & Bisen (1992)

Continued . . .

Table 2. Continued.

Metal	Species	Concentration	Effect	References
	Nostoc calcicola	0.10 μM	50% inhibition of growth; 23.8% inhibition of nucleic acid; loss of photosynthetic pigments (phycocyanin > chlorophyll *a* > carotenoids)	Singh & Singh (1992b)
		0.20 μM	50% inhibition of O_2 evolution	Singh & Singh (1987a)
		0.25 μM	50% inhibition of $^{14}CO_2$ uptake	Singh & Singh (1987a)
		0.25 μM	complete inhibition of growth	Singh & Singh (1992a)
		0.25 μM	complete inhibition of growth, O_2 evolution and CO_2 incorporation	Singh & Singh (1992b)
		1.25 μM	about 50% efflux of intracellular electrolytes; 50% efflux of phycocyanin	Singh & Singh (1992b)
		10 μM	50% inhibition of GS activity; 44% inhibition of nitrogenase activity	Singh *et al.* (1987)
		15 μM	98.1% inhibition of NH_4^+ uptake; 55.8% inhibition of GS activity	Singh & Singh (1992a)
	Plectonema boryanum	0.498 mM	reduction in the number of lipid inclusions and in the volume of the intrathylakoidal spaces; production of extraneous membrane whorls	Rachlin *et al.* (1982)
	Cylindrospermum IU942	0.46 μM	inhibition of growth, chlorophyll *a* fluorescence and Hill activity	Singh *et al.* (1989a)
	Spirulina platensis	6 μM	50% inhibition of Hill activity	Murthy *et al.* (1989)
Methyl mercury	*Nostoc calcicola*	0.10 μM	complete inhibition of growth	Singh & Singh (1992a)
		10μM	98.1% inhibition of NH_4^+ uptake; 70.1% inhibition of GS activity	Singh & Singh (1992a)
Cadmium	*Anabaena inaequalis*	0.444 μM	elongation of filaments; yellowing of the vegetative cells; development of empty apical cell; increase in heterocyst frequency	Stratton & Corke (1979a)
		0.533 μM	complete inhibition of growth	Stratton & Corke (1979a)
		35.59 μM	complete inhibition of CO_2 fixation	Stratton & Corke (1979a)
		0.178 mM	complete inhibition of nitrogenase activity	Stratton & Corke (1979a)
	Anabaena flos-aquae	0.118 μM	50% inhibition of growth; reduction in the surface area of thylakoids and volume of the polyphosphate bodies	Rachlin *et al.* (1984)
		0.4 μM	50% inhibition of growth	Sharma & Bisen (1992)
		1.18 μM	reduction in the surface area of thylakoids, volume of polyphosphate bodies, volume of cell wall layers, intrathylakoidal spaces and polyhedral bodies sizes; increase in number and volume of lipid inclusions and in the number of membrane limited crystalline inclusions	Rachlin *et al.* (1984)
		11.83 μM	reduction in cell size, volume of the cell wall layers, surface area of thylakoids, polyhedral bodies size, volume of the polyphoshate bodies, and number and volume of cyanophycin granules	Rachlin *et al.* (1984)

Continued . . .

Table 2. Continued.

Metal	Species	Concentration	Effect	References
		30 μM	50% inhibition of O_2 evolution	Sharma & Bisen (1992)
		38 μM	50% inhibition of CO_2 fixation	Sharma & Bisen (1992)
		45 μM	50% inhibition of H^+ uptake activity	Sharma & Bisen (1992)
		64 μM	50% inhibition of carbonic anhydrase activity	Sharma & Bisen (1992)
		118.33 μM	reduction in the volume of the polyphosphate bodies, surface area of thylakoids, polyhedral bodies size and in the number of membrane limited crystalline inclusions; decrease of Mg^{2+} and Ca^{2+} in the polyphosphate bodies	Rachlin *et al.* (1984)
	Anabaena cylindrica	17.79 μM	inhibition of growth and nitrogenase activity; cell lysis; chlorosis; broken filaments; cellular malformation; increase in heterocyst frequency	Delmotte (1980)
	Anabaena variabilis	0.11 μM	50% inhibition of growth	Rachlin *et al.* (1984)
	Anabaena 7120	0.1 mM	distorted cell showing high electron-dense with corrugated appearance; distorted heterocysts, half empty	Massalski *et al.* (1981)
		1 mM	complete inhibition of growth	Laube *et al.* (1980)
	Nostoc UAM208	2.31 μM	50% inhibition of nitrogenase activity	Fernandez-Pinas *et al.* (1991)
		4.89 μM	50% inhibition of growth	Fernandez-Pinas *et al.* (1991)
	Nostoc linckia	0.444 μM	34.6% inhibition of O_2 evolution; 41.9% inhibition of $^{14}CO_2$ uptake; 47.7% inhibition of ATP content	Husaini *et al.* (1991)
	Anacystis nidulans	5 μM	complete inhibition of O_2 evolution; 81.5% inhibition of Hill activity	Singh & Singh (1987b)
		53.38 μM	50% inhibition of PO_4^{3-} uptake	Singh & Yadava (1984)
		88.97 μM	complete inhibition of NH_4^+ uptake	Singh & Yadava (1984)
		0.178 mM	inhibition of NO_3^- uptake	Singh & Yadava (1983)
	Chroococcus paris	8.89 μM	inhibition of growth	Les & Walker (1984)
	Plectonema boryanum	0.889 μM	increase in the surface area of the thylakoids and in the volume of the polyphosphate bodies; reduction in the number of lipid inclusions; production of extraneous membrane whorls	Rachlin *et al.* (1982)
Nickel	*Anabaena inaequalis*	> 2.13 μM	complete inhibition of growth	Stratton & Corke (1979b)
		0.170 mM	complete inhibition of CO_2 fixation	Stratton & Corke (1979b)
		0.341 mM	complete inhibition of nitrogenase activity	Stratton & Corke (1979b)
	Anabaena flos-aquae	10.2 μM	85% inhibition of growth	Spencer & Greene (1981)
	Anabaena cylindrica	10.2 μM	18% inhibition of growth	Spencer & Greene (1981)

Continued . . .

Table 2. Continued.

Metal	Species	Concentration	Effect	References
	Nostoc muscorum	4.2 μM	53% inhibition of growth; 39% inhibition of $^{14}CO_2$ uptake; 17% inhibition of nitrogenase activity	Rai & Raizada (1985)
		1 μM	4-fold increase nitrogenase activity	Asthana *et al.* (1990)
		5 μM	3-fold increase RUBPcase activity	Asthana *et al.* (1990)
		10 μM	69% inhibition of RUBPcase activity, decrease in $^{14}CO_2$ uptake	Asthana *et al.* (1990)
		15 μM	complete inhibition of growth	Asthana *et al.* (1990)
		17.03 μM	about 50% inhibition of growth; 17% inhibition of CO_2 fixation; 27% inhibition of nitrogenase activity; 75% and 80% loss of K^+ and Na^+, respectively	Rai & Raizada (1987)
		30 μM	complete inhibition of nitrogenase activity	Asthana *et al.* (1990)
		1000 μM	complete inhibition of O_2 evolution	Asthana *et al.* (1990)
	Cylindrospermum IU942	4.25 μM	inhibition of growth, Hill activity and chlorophyll *a* fluorescence	Singh *et al.* (1989a)
	Plectonema boryanum	1.70 mM	decrease in cell volume; increase in the surface area of the thylakoids; reduction in the number of lipid inclusions and in the volume of the intrathylakoidal spaces; production of extraneous membrane whorls	Rachlin *et al.* (1982)
Copper	*Anabaena* 7120	0.1 mM	cell lysis and cell distortion with corrugated appearance mostly darkly stained	Massalski *et al.* (1981)
		1 mM	complete inhibition of growth	Laube *et al.* (1980)
	Nostoc calcicola	5 μM	complete inhibition of O_2 evolution and Hill activity	Verma & Singh (1991)
		40 μM	50% inhibition of PSII; 95.4% inhibition of $^{14}CO_2$ fixation; 15.5% decrease in the PSI activity; 32.3% decrease in ATP content	Pandey *et al.* (1992)
		100 μM	50% inhibition of GS activity and 40% inhibition of nitrogenase activity	Singh *et al.* (1987)
	Cylidrospermum IU942	0.47 mM	inhibition of growth, Hill activity and chlorophyll *a* fluorescence	Singh *et al.* (1989a)
	Plectonema boryanum	1.57 mM	increase in cell volume; reduction in the number and volume of lipid inclusions, production of extraneous membrane whorls	Rachlin *et al.* (1982)
	Aphanizomenon flos-aquae	0.472 μM	73% inhibition of growth; 85% inhibition of nitrogenase activity; 75% inhibition of CO_2 fixation; 75% inhibition of chlorophyll *a*	Wurtsbaugh & Horne (1982)
	Chroococcus paris	3.15 μM	inhibition of growth	Les & Walker (1984)
	Anacystis nidulans	5 μM	complete inhibition of O_2 evolution and Hill activity	Singh & Singh 1987b
Zinc	*Anabaena variabilis*	52 μM	50% inhibition of growth	Rachlin *et al.* (1984)
	Plectonema boryanum	1.53 mM	reduction in the number of lipid inclusions and in the volume of the intrathylakoidal spaces; production of extraneous membrane whorls	Rachlin *et al.* (1982)
	Chroococcus paris	30.59 μM	inhibition of growth	Les & Walker (1984)
	Anacystis nidulans	5 μM	complete inhibition of O_2 evolution; 75.5% inhibition of Hill activity	Singh & Singh (1987b)
Manganese	*Plectonema boryanum*	1.82 mM	increase in the surface of the thylakoids; reduction in the number of lipid inclusions	Rachlin *et al.* (1982)

Continued . . .

Table 2. Continued.

Metal	Species	Concentration	Effect	References
Cobalt	*Plectonema boryanum*	170 mM	decrease in cell volume; reduction in the number of lipid inclusions and polyhedral bodies; reduction in the volume of the intrathylakoidal spaces; production of extraneous membrane whorls	Rachlin *et al.* (1982)
Silver	*Nostoc muscorum*	37.08 nM	50% inhibition of growth; 88% inhibition of CO_2 fixation; 30% inhibition of nitrogenase activity; 75% and 90% loss of K^+ and Na^+, respectively	Rai & Raizada (1987)
		0.026 μM	55% inhibition of growth; 88% inhibition of $^{14}CO_2$ uptake; 52% inhibition of nitrogenase activity	Rai & Raizada (1985)
Lead	*Anabaena flos-aquae*	5.6 μM	50% inhibition of growth	Rachlin *et al.* (1984)
	Anabaena 7120	1 mM	complete inhibition of growth	Laube *et al.* (1980)
	Plectonema boryanum	0.483 mM	increase in cell volume and surface area of the thylakoids; reduction in the number of lipid inclusions	Rachlin *et al.* (1982)
Chromium	*Anabaena doliolum*	7.69 mM	50% inhibition of growth; 57% inhibition of CO_2 fixation; 77% inhibition nitrogenase activity; 79.2% inhibition of nitrate reductase; 29.4% inhibition of GS activity; 36% decrease in heterocyst differentiation	Dubey & Rai (1987)
		.7.69 mM	52% inhibition of NO_3^- uptake; 54% inhibition of NH_4^+ uptake; 75% inhibition of nitrate reductase; 28.6% inhibition of GS activity	Rai & Dubey (1989)
		7.69 mM	37% inhibition of ATP content; 39.4% inhibition of O_2 evolution; 41% inhibition of CO_2 fixation; 33.4% inhibition of nitrogenase activity	Rai *et al.* (1992)
Tin	*Anabaena doliolum*	42.13 μM	50% inhibition of growth; 50% inhibition of CO_2 fixation; 69% inhibition of nitrogenase activity; 66.7% inhibition of nitrate reductase; 32.4% inhibition of GS activity; 42% decrease in heterocyst differentiation	Dubey & Rai (1987)
		42.13 μM	60% inhibition of NO_3^- uptake; 48% inhibition of NH_4^+ uptake; 70.8% inhibition of nitrate reductase; 25.7% inhibition of GS activity	Rai & Dubey (1989)
Aluminum	*Anabaena cylindrica*	190 μM	accumulation of cyanophycin granules; degradation of the thylakoid membranes; decrease in the intrathylakoid electron density and polysaccharide sheath	Pettersson *et al.* (1985b)
		370 μM	complete inhibition of growth; decrease in CO_2 fixation and chlorophyll *a*; total inhibition of nitrogenase activity	Pettersson *et al.* (1985b)
	Anacystis nidulans	0.741 mM	complete inhibition of growth	Lee *et al.* (1991)
Cesium	*Synechocystis* PCC6803	1 mM	inhibition of growth	Avery *et al.* (1991)

Table 3. Order of metal toxicity to cyanobacteria

Species	Order of metals	References
Anabaena inaequalis	Hg ≫ Ni ≥ Cd	Stratton & Corke (1979c)
Anabaena doliolum	Cr > Sn	Dubey & Rai (1987)
Nostoc muscorum	Ag > Ni	Rai & Raizada (1985)
Cylindrospermum IU942	Hg > Ni > Cu	Singh *et al.* (1989a)
Chrococcus paris	Cu > Cd > Zn	Les & Walker (1984)

acidic conditions has been reported for Cd^{2+} in *Nostoc calcicola* and *Anacystis nidulans*, Cu^{2+} in *Anacystis nidulans* and *Nostoc muscorum*, UO_2^{2+} in *Synechococcus elongatus*, Pb^{2+} in *Nostoc muscorum* and Al^{3+} in *Anabaena cylindrica*, and was suggested to be due to the increase in free metal ions available to the cyanobacteria (Horikoshi *et al.* 1979, Singh & Pandey 1981, Pettersson *et al.* 1985b, Schecher & Driscoll 1985, Singh 1985, Singh & Yadava 1985). Under acidic conditions metals tend to exist in the free ionic form, whereas under alkaline conditions they may precipitate as insoluble complexes or in an hydroxylated form which might have an altered activity (Gadd & Griffiths 1978, Babich & Stotzky 1983). However,acidic conditions can result in competition between free metals ions and H^+ for the same uptake sites, leading to a decrease in cellular heavy metal uptake and toxicity (Peterson *et al.* 1984). Maximal accumulation of Cs^+ in *Synechocystis* PCC6803 was reported to occur at pH 10 and was attributed to hyperpolarization of the membrane (Avery *et al.* 1991). Also, the optimal growth pH of 8.5 for *Anacystis nidulans* was favorable for maximum Cd^{2+} uptake (Singh & Yadava, 1985).

The presence of other cations can also affect heavy metal uptake and toxicity. A decrease in toxicity of several heavy metals has been described as a result of direct competition between different cations for the same uptake/binding site (Table 4). Also, cations can affect metal uptake due to adsorption of cation and metal on a common cell surface where one may increase the cell permeability to the second one or have different sites of binding to cellular ligands (Table 5). Calcium and magnesium salts also form complexes with toxic metals in hard and eutrophic waters which reduce their toxicity (Whitton 1970, Rai *et al.* 1981).

Reduction of metal toxicity in cyanobacteria has been attributed to the phosphate concentration in cells. The toxicity of aluminum and copper in *Anabaena cylindrica* and *Nostoc calcicola*, respectively, was suggested to be ameliorated by phosphorus-rich cells where both metals were accumulated in polyphosphate granules as a detoxifying mechanism (Pettersson *et al.* 1988, Verma *et al.* 1991, 1993). Organic ligands synthesized by the cyanobacteria or from other sources are also capable of binding heavy metals and consequently decrease toxicity (Clarke *et al.* 1987, Wurtsbaugh & Horne 1982).

The number of cyanobacterial cells can affect metal uptake. Increases in cyanobacterial cell densities did not increase the amount of cadmium absorbed per cell in *Anacystis nidulans*, uranium in *Synechococcus elongatus*, and copper and lead in *Nostoc muscorum* (Horikoshi *et al.* 1979, Schecher & Driscoll 1985, Singh & Yadava 1985). Decreases in uptake/toxicity of cadmium in dense cultures was attributed to lower amounts available per cell than in low cell density cultures where the increased distance between cells also contributed to more adsorption of cadmium (Singh & Yadava 1985). In *Nostoc muscorum*, high numbers of cells was found to form aggregates with the extracellular sheath. This decreased the total cell surface area exposed to solution and, as a consequence, metal uptake was moderate (Schecher & Driscoll 1985).

Mechanisms of metal tolerance

Studies have shown that cyanobacteria can survive and reproduce in metal-contaminated habitats. The genera *Oscillatoria*, *Phormidium*, *Plectonema* and *Schizothrix* were dominant in zinc-enriched water (Say & Whitton 1980, Whitton 1980), and isolates from such sites are often resistant to zinc (Shehata & Whitton 1981). A range of cyanobacteria, i.e. *Anabaena*, *Nostoc*, *Oscillatoria*, *Phormidium* and *Scytonema*, has been recovered from copper-rich soils (Whitton & Shehata 1982), and *Plectonema* is a frequent inhabitant near mine tailings containing high levels of zinc, cobalt, nickel and lead (Whitton *et al.* 1981).

In laboratory studies, selection of metal-tolerant cyanobacteria strains resulted in isolation of a zinc-tolerant strain of *Anacystis nidulans* (Shehata & Whitton 1982), a nickel-tolerant mutant of *Synechococcus* (Wood & Wang 1983) and a chromium-tolerant strain of Oscillatoria (Filip *et al.* 1979).

Studies carried out on heavy metal tolerances by microorganisms revealed that they may occur by several mechanisms, such as extracellular binding or precipitation, impermeability and exclusion, internal

Table 4. Antagonistic interaction amongst metal cations

Metal	Parameter	Species	References
Ca–Hg	NH_4^+ uptake and GS activity	*Nostoc calcicola*	Singh & Singh (1992a)
Mg–Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Cu–Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Cu–Hg	O_2 evolution		Singh & Singh (1987a)
Cu–Hg	GS and nitrogenase activity		Singh *et al.* (1987)
Cu–Hg	Hg accumulation		Pandey & Singh (1993)
Ni–Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Ni–Hg	$^{14}CO_2$ uptake		Singh & Singh (1987a)
Ni–Hg	GS and nitrogenase activity		Singh *et al.* (1987)
Cd–Hg	growth	*Anabaena inaequalis*	Stratton & Corke (1979c)
Ca–CH_3Hg	NH_4^+ uptake and GS activity	*Nostoc calcicola*	Singh & Singh (1992a)
Mg–CH_3Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Cu–CH_3Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Ni–CH_3Hg	NH_4^+ uptake and GS activity		Singh & Singh (1992a)
Ca–Cd	NO_3^- uptake	*Anacystis nidulans*	Singh & Yadava (1983)
Ca–Cd	NH_4^+ and PO_4^{3-} uptake		Singh & Yadava (1984)
Zn–Cd	NO_3^- uptake		Singh & Yadava (1983)
Zn–Cd	NH_4^+ and PO_4^{3-} uptake		Singh & Yadava (1984)
Ni–Cd	growth, $^{14}CO_2$ uptake and nitrogenase activity	*Anabaena inaequalis*	Stratton & Corke (1979c)
Ca–Ni	growth, $^{14}CO_3$ uptake and nitrogenase activity	*Nostoc muscorum*	Rai & Raizada (1985)
Ni–Cd–Hg	growth	*Anabaena inaequalis*	Stratton & Corke (1979c)
Ca–Ag	nitrogenase activity	*Nostoc muscorum*	Rai & Raizada (1985)
Ca–Cr	growth, $^{14}CO_2$, uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity	*Anabaena doliolum*	Dubey & Rai (1990)
Ca–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Mg–Cr	growth, $^{14}CO_2$ uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity		Dubey & Rai (1990)
Mg–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Mn–Cr	growth, $^{14}CO_2$ uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity		Dubey & Rai (1990)
Mn–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Ca–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity		Dubey & Rai (1990)
Ca–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Mg–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity		Dubey & Rai (1990)
Mg–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Mn–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, heterocyst differentiation and nitrogenase activity		Dubey & Rai (1990)
Mn–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Ca–Cu	Cu uptake	*Nostoc muscorum*	Schecher & Driscoll (1985)
Ca–Pb	Pb uptake		Schecher & Driscoll (1985)

detoxification and metal transformations (Reed & Gadd 1990). The first three mechanisms have been demonstrated in cyanobacteria and are discussed below.

Extracellular binding or precipitation

Little is known about metal deposition in the cyanobacteria cell envelope, since most metal-bind-

Table 5. Synergistic interaction amongst metal cations

Metal	Parameter	Species	References
Cd–Hg	$^{14}CO_2$ uptake and nitrogenase activity	*Anabaena inaequalis*	Stratton & Corke (1979c)
Cd–Hg	NO_3^- uptake	*Anacystis nidulans*	Singh & Yadava (1983)
Cd–Hg	NH_4^+ and PO_4^{3-} uptake		Singh & Yadava (1984)
Cd–Hg	$^{14}CO_2$ uptake	*Nostoc calcicola*	Singh & Singh (1987a)
Cd–Hg	GS and nitrogenase activity		Singh *et al.* (1987)
Ni–Hg	growth	*Anabaena inaequalis*	Stratton & Corke (1979c)
Ni–Hg	Hg accumulation	*Nostoc calcicola*	Pandey & Singh (1993)
Ni–Cd–Hg	$^{14}CO_2$ uptake and nitrogenase activity	*Anabaena inaequalis*	Stratton & Corke (1979c)
CH_3Hg–Hg	O_2 evolution	*Nostoc calcicola*	Singh & Singh (1987a)
CH_3Hg–Hg	GS and nitrogenase activity		Singh *et al.* (1987)
Ni–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity	*Anabaena doliolum*	Rai & Dubey (1989)
Ni–Cr	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)
Co–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Co–Cr	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)
Zn–Cr	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Zn–Cr	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)
Ni–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Ni–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)
Co–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Co–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)
Zn–Sn	growth, NO_3^- and NH_4^+ uptake, nitrate reductase and GS activity		Rai & Dubey (1989)
Zn–Sn	growth, $^{14}CO_2$ uptake, O_2 evolution, nitrogenase activity and heterocyst differentiation		Dubey & Rai (1990)

ing studies have been carried out using whole cells. The use of energy-dispersive X-ray analysis detected localized electron dense granules of lead in the cell wall of *Plectonema boryanum* exposed to lead (Rachlin *et al.* 1982). Two isolated sheaths of *Gloeothece* ATCC27152 grown with and without a source of combined nitrogen showed substantial adsorption of cadmium (Tease & Walker 1987). It was observed that sheath material from cells grown with $NaNO_3$ bound more cadmium than N_2-fixing cultures and the difference was attributed to variations in the chemical composition of the sheath. Isolated sheaths of *Calothrix parietina* and *Calothrix scopulorum* were found to bind heavy metals in the order Fe > Zn > Cu > Ni > Mn > Mo > Co (Weckesser *et al.* 1988). The sheaths of *Lyngbya aestuarii* and *Scytonema myochrous* have been shown to be a site for calcium carbonate deposition (Pentecost & Bauld 1988).

Some information is available regarding the ability of some cyanobacteria to produce extracellular secretions to protect themselves from toxic metals. The ability of extracellular organic material to reduce the toxicity of heavy metals was demonstrated by Fogg & Westlake (1955), showing that cultures of *Anabaena cylindrica* produced polypeptides which complexed cupric, zinc and ferric ions. Wang & Tischer (1973) demonstrated the presence of polypeptides in *Anabaena flos-aquae*. Murphy *et al.* (1976) showed the occurrence of hydroxamates when blooms of *Anabaena flos-aquae* were present in the water column. Cyanobacteria also respond to

iron limitation by secreting low molecular weight iron chelators known as siderophores which can bind other metal ions such as copper (Clarke *et al*. 1987).

Metal impermeability and exclusion

Decreased metal transport, impermeability or metal efflux systems have been observed in some cyanobacteria. The active transport of Ni^{2+} in *Anabaena cylindrica* is dependent on the membrane potential, is decreased in the dark, and is inhibited by metabolic uncouplers and electron transport inhibitors (Campbel & Smith 1986). Active Cd^{2+} uptake has also been reported in *Anacystis nidulans* but was competitively inhibited by Ca^{2+} and Zn^{2+} (Singh & Yadava 1983). In *Nostoc calcicola*, an energy-dependent Cu^{2+} efflux system was present in a resistant mutant which resulted in a net reduction in Cu^{2+} (Verma & Singh 1991).

In *Synechocystis* PCC6803 the toxicity of Cs^+ was suggested to be due to replacement of cellular K^+ by Cs^+. Release of Cs^+ into the medium was also observed. This was attributed to an increased internal osmotic pressure resulting from more Cs^+ being accumulated than was compensated for by release of K^+ (Avery *et al*. 1991).

An exclusion mechanism was suggested to explain reduced Cu^{2+} uptake by a tolerant strain of *Anabaena doliolum* (Rai *et al*. 1991). In a tolerant strain, isolated by repeatedly subculturing into medium containing increasingly higher levels of copper, only 32 and 40% Cu^{2+} was taken up compared with the wild-type. The acquired tolerance was attributed to a change in the cytoplasmic membrane permeability caused by increased lipid production.

Internal detoxification

Cyanobacteria can accumulate metals internally, with localization involving binding or precipitation at specific sites. *In situ* energy-dispersive X-ray analysis demonstrated the sequestering of cadmium, cobalt, copper, mercury, nickel, lead and zinc into polyphosphate bodies of *Plectonema boryanum* (Jensen *et al*. 1982). Polyphosphate bodies in *Anabaena cylindrica* and *Anacystis nidulans* also accumulated aluminum and titanium, respectively (Crang & Jensen 1975, Pettersson *et al*. 1985a). In *Anabaena flos-aquae*, cadmium was incorporated into both the cellular cytoplasm and polyphospahte bodies (Rachlin *et al*. 1984). These studies suggest that polyphosphate bodies are a means of binding cations in a non-toxic state within cells, and may serve as storage sites for metals and for detoxification if metals are present at toxic levels. Other examples include *Synechococcus elongatus*, where uranium uptake leads to formation of dense, internal deposits (Horikoshi *et al*. 1979). A *Synechococcus* sp. synthesized large quantities of an intracellular polymer that could bind nickel, the cell interior appearing highly granular (Wood & Wang 1983). A cellular detoxification mechanism was also suggested to explain the presence of extra intracellular membrane whorls in *Plectonema boryanum* exposed to nickel, cobalt, zinc, mercury, copper and cadmium (Rachlin *et al*. 1982).

Another aspect of internal compartmentalization is the synthesis of metal-binding components which may function in detoxification. A metal-binding metallothionein protein has been isolated from a *Synechococcus* sp. (Olafson *et al*. 1979). Historically, the definition of metallothionein is a cadmium–zinc and copper-containing sulfur-rich protein from equine renal cortex (Kagi & Vallee 1960). Currently, proteins possessing several features similar to those of the equine renal metallothionein are designated as metallothionein. These metallothioneins are characterized as a group of low molecular weight proteins, with a high metal and cysteine content, no aromatic amino acids or histidine, fixed distribution for cysteine residues and binding metals in metal–mercaptide complexes (Kagi *et al*. 1974; Kojima *et al*. 1976). Metallothioneins were described in equine kidney (Margoshes & Vallee 1957), and have been isolated and characterized from eukaryotic (Nordberg & Kojima 1979) and prokaryotic (Olafson *et al*. 1979, Higham & Sadler 1984, Sakamoto *et al*. 1989) organisms. Although their precise physiological role is still unknown, metallothioneins have been shown to be involved in heavy metal detoxification and/or homeostasis (Olafson 1981, 1982). Recently, in *Synechococcus* PCC 630 and PCC 7942, metallothionein was found to be a gene product and the gene (*smtA*) was characterized (Robinson *et al*. 1990, Huckle *et al*. 1993). Deletion of the *smt* locus was shown to reduce Zn^{2+}/Cd^{2+} tolerance (Turner *et al*. 1993).

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