CHAPTER 2

LITERATURE REVIEW

Ankistrodesmus convolutus (isolate 101)

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2.0 Literature Review

2.1 Heavy Metals in the Environment

2.1.1 Definition Of Heavy Metal

According to Rainbow (1993), the 'trace metal' term may be used synonymously with 'heavy metal' but alternatively the former may be restricted to heavy metals which are essential in living systems only. Trace metals then refer to the essential metals chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), tin (Sn), vanadium (V), zinc (Zn), and the non essential metals cadmium (Cd), gold (Au), lead (Pb), mercury (Hg) and silver (Ag). In order to avoid the confusion in nomenclature, it is best to simply state the specific metal ions concerned (Niedoer *et al.*, 1980).

Rainbow (1993) reviewed the different definitions of heavy metals. According to chemists, heavy metals are metal ions "heavier" than most other metal ions (except Mo). These included the second and third row transition metal ions (i.e: the metal ions from yttrium (Y) to Cd and lanthanum (La) to Hg) and the representative metal ion, tin and lead. Based on this definition none of the heavy metals are essential for life (Figure 3). However, there are no specific chemical or physical properties that link these metal ions together. According to biologists, heavy

Transition metals

1A	2A	3B	4B	5B	6B	7B	8B			1B	2B	3A	4A	5A	6A	7A	8A
1 H																	2 He
3 *Li*	*4* *Be*											5 B	6 C	7 N	8 O	9 F	10 He
11 *Na*	*12* *Mg*											*13* *AL*	14 Si	15 P	16 S,	17 Cl	18 AR
19 *K*	*20* *Ca*	*21* *Sc*	*22* *Ti*	*23* *V*	*24* *Cr*	*25* *Mn*	26 *Fe*	*27* *Co*	*28* *Ni*	*29* *Cu*	*30* *Zn*	*31* *Ga*	32 Ge	33 As	34 Se	35 Br	36 Kr
37 *Rb*	*38* *Sr*	*39* *Y*	*40* *Zr*	*41* *Nb*	*42* *Mo*	*43* *Tc*	*44* *Ru*	*45* *Rh*	*46* *Pd*	*47* *Ag*	*48* *Cd*	*49* *In*	50 *Sn*	51 *Sb*	52 Te	53 I	54 Xe
55 *Cs*	*56* *Ba*	*57* *La**	*72* *Hf*	*73* *Ta*	*74* *W*	*75* *Re*	*76* *Os*	*77* *Ir*	*78* *Pt*	*79* *Au*	*80* *Hg*	*81* *Tl*	*82* *Pb*	*83* *BI*	*84* *PO*	85 At	86 Rn
87 *Fr*	*88* *Ra*	*89* *Ac!*	*104* *Rf*	*105* *Ha*	*106* *Unh*	*107* *Uns*		*109* *Une*									

* Lanthanide Series	*58* *Ce*	*59* *Pr*	*60* *Nd*	*61* *Pm*	*62* *Sm*	*63* *Eu*	*64* *Gd*	*65* *Tb*	*66* *Dy*	*67* *Ho*	*68* *Er*	*69* *Tm*	*70* *Yb*	*71* *Lu*
! Actinide Series	*90* *Th*	*91* *Pa*	*92* *U*	*93* *Np*	*94* *Pu*	*95* *Am*	*96* *Cm*	*97* *Bk*	*98* *Cf*	*99* *Es*	*100* *Fm*	*101* *Md*	*102* *No*	*103* *Lr*

Figure 3 : The periodic table of the elements. The metalic elements are in italics
Source : Borovik (1989)

metals include virtually any transition metal ions. Several of the metal ions such as Ni, Cu and Zn are essential for life, but have harmful effects if present in excess. The toxic actions of heavy metals dependent on their chemical properties.

2.1.2 Mechanisms Of Metal Ion Toxicity

Ochiai (1987) pointed out five mechanisms of metal ion toxicity : (i) displacing essential metal ions from biomolecules and other biologically functional units, (ii) blocking essential functional groups of biomolecules, including enzymes and polynucleotides, (iii) modifying the active conformation of biomolecules, especially enzymes and polynucleotides, (iv) disrupting the integrity of biomolecules, and (v) modifying some other biologically active agent. The chemical forms or speciation of the metal ions are important factors affecting the mechanisms of metal toxicity (Hughes & Poole, 1989).

The basis for the five mechanisms, especially the first three mechanisms, is the ability of metal ions to bind strongly to oxygen, nitrogen and sulfur atoms. These atoms are abundant in biological systems and can serve as ligands to all essential metal ions. In many cases, the metal ion at the active site can be displaced by a different metal ion, forming a derivative with altered or no biological activity. The binding of heavy metals to proteins and nucleic acids may alter their protein and thus, affects the oxidative phosphorylation and membrane permeability. In addition, toxic metal ions can coordinate to essential functional groups of proteins which can render the protein inactive. Tables 5 and 6 show the concentrations of heavy metals which are lethal to microalgae.

The biological response of microorganisms to essential and toxic elements is shown in Figure 4. When the essential elements are supplied within the normal range, there is optimal biological response, as indicated by the plateau. A further increase in the concentration of the essential elements results in the impairment of biological function. The response of microorganisms to toxic elements however shows progressive decline in biological activity with increasing in concentration (Wan Azlina, 1993).Upitis *et al.* (1989), reported the optimal levels of nutrients requested by *Chlorella* sp., *Spirulina platensis*, *Dunaliella salina* and *Cyanidium caldarium*) (Table 7). Table 8 shows the amounts of mineral which nutrients result in insufficient, normal and above normal growth of *Chlorella*.

2.2 Sources of Heavy Metal Pollution

In general, there are three classes of pollution sources, differing from one another by the mode in which they enable the pollutants to enter waterways: contaminated sediment, non-point (diffuse) sources and point sources (Doust *et al.*, 1994).

Table 5 : EC$_{50}$ values of heavy metals on microalgae

Species	**Metal Tested**	**Observation**
Cyclotella moneghiniana (GW)	Hg	EC$_{50}$: 0.11
	Cu	0.53
	Cd	0.90
	Zn	0.71
	Pb	3.25
	Cr	2.86
Cyclotella moneghiniana (CT)	Hg	EC$_{50}$: 0.16
	Cu	0.74
	Cd	0.59
	Zn	0.87
	Pb	1.54
	Cr	2.68
Skeletonema costatum	Hg	EC$_{50}$: 0.44
	Cu	1.45
	Cd	1.15
	Zn	1.98
	Pb	3.11
	Cr	2.83
Navicula confervacea	Hg	EC$_{50}$: 0.21
	Cu	0.97
	Cd	0.21
	Zn	0.59
	Pb	1.49
	Cr	2.95
Nitzschia obtusa	Hg	EC$_{50}$: 0.46
	Cu	0.64
	Cd	1.34
	Zn	0.93
	Pb	2.47
	Cr	2.38
Nitzschia palea	Hg	EC$_{50}$: 1.26
	Cu	0.52
	Cd	1.91
	Zn	0.60
	Pb	3.25
	Cr	2.73-

Source : Gowrinathan & Rao (1989)

Table 6: Effects of heavy metals on photosynthesis and respiration in microalgae

Species	Metal	Heavy metal concentration (mgL^{-1}) causing 100% inhibition	
		Photosynthesis	**Respiration**
C. moneghiniana (GW)	Hg	5.0	1.0
	Cu	>10.0	10.0
	Cd	>10	>10
	Zn	>10	>10
	Pb	>10	>10
	Cr	40	20
C. moneghiniana (CT)	Hg	>5.0	5.0
	Cu	10.0	10.0
	Cd	>10.0	>10.0
	Zn	>10.0	>10.0
	Pb	>40.0	>40.0
	Cr	40.0	40
S.costatum	Hg	1.0	5.0
	Cu	10.0	>10.0
	Cd	>10.0	>10.0
	Zn	>10.0	10.0
	Pb	40.0	40.0
	Cr	>40.0	30.0
N.confervacea	Hg	>5.0	5.0
	Cu	10.0	10.0
	Cd	10.0	10.0
	Zn	10.0	10.0
	Pb	>20.0	>20.0
	Cr	40.0	40
N.obtusa	Hg	5.0	5.0
	Cu	10.0	10.0
	Cd	10.0	10.0
	Zn	10.0	10.0
	Pb	40.0	40.0
	Cr	40.0	>40.0
N.palea	Hg	>5.0	5.0
	Cu	>10.0	>10.0
	Cd	>10.0	10.0
	Zn	10.0	1.0
	Pb	>40.0	40.0
	Cr	40.0	40.0

Source : Gowrinathan & Rao (1989)

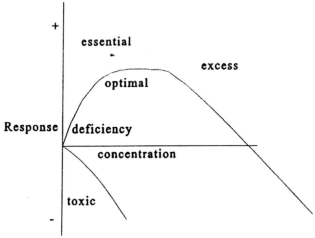

Figure 4 : Biological response of living system essential and toxic elements
Source : Wan Azlina (1993)

Table 7 : Range of optimal nutrient levels required by microalgae as shown in *Chlorella, Spirulina, Dunaliella, Cyanidium*

Element	Biomass	Nutrient solution (mgL^{-1})
	$g\ kg^{-1}$ DW	
N	70 - 90	400 - 850
P	8 - 10	60 - 120
K	6 - 8	50 - 110
Mg	4 - 5	25 - 50
S	4 - 7	35 - 70
	$mg\ kg^{-1}$ DW	
Fe	300 - 400	3 - 5
Mn	30 - 50	0.1 - 0.2
B	1 - 1.3	0.05 - 0.1
Zn	15 - 30	0.02 - 0.04
Cu	3 - 5	0.01 - 0.02
Mo	0.4 - 0.6	0.01 - 0.02

Source : Upitis *et al.* (1989)

Table 8 : Status of mineral nutrition of in *Chlorella*

	N	P	K	Mg	S	Fe	Mn	Cu	Zn	Mo
Level	$g\ Kg^{-1}$ DW					mgL^{-1}				
Deficiency	< 60	<5	<3	<2	<3	<150	<20	<2	<15	<0.1
Normal	75-100	8-12	6-10	3-6	4-6	200-800	30-80	4-15	20-30	0.4-0.6
Abundant	-	13-16	11-16	6-10	6-12	900-1500	100-350	15-25	35-40	1-4
Normal Proportions	8-10	1	0.6-0.8	0.4-0.6	0.5-0.6	80-100	8-10	1	2-3	0.2-0.4

Source: Upitis *et al.* (1989)

2.2.1 *Contaminated Sediment*

Hydrophobic contaminants enter into an aqueous environment and the aquatic biota is continuously exposed to toxic substances. The pollutants adsorb and accumulate on sediment particles and suspended particulates (Tatem, 1986).

2.2.2 *Non-Point Sources*

Non-point sources are diffuse inputs of contaminants from diverse points of origin and cannot be traced to any single discharge. Large areas may be polluted by heavy metals contained in runoff, subsurface flow, or deposition from the atmosphere. For example, runoff of chemicals into surface water and seepage into the ground from croplands, livestock feedlots, logged forests, streets, lawns, septic tanks, construction sites, parking lots and roadways can cause serious pollution (Todd, 1994).

2.2.3 *Point Sources*

A point source refers to the direct discharge of industrial or municipal effluents at specific locations through pipes, ditches, or sewers into bodies of surface water. The sources include factories, sewage treatment plants (which remove some but not all pollutants), active and abandoned underground mines. Offshore sources which are from urban areas are fairly easy to identify, monitor and regulate. In 'More Developed

Countries' (MDC) many industrial discharges are strictly controlled whereas in 'Less Developed Countries' (LDC) such discharges are largely uncontrolled (Todd, 1994).

2.3 Monitoring Heavy Metal Pollution

In any attempt to quantify environmental variables and characteristics of aquatic ecosystems, it is important to monitor the physical, chemical and biological changes that occur as a result of anthropogenic activities (Dixit *et al.*, 1992)

Concurrent with the escalating growth of chemical engineering, there have been advances in the technology of water pollution analysis using direct chemical assay techniques (Doust *et al.*, 1994). However, these are severely limited in the monitoring of suspected pollutants in water. An enormous amount of data are needed and multiple sampling must be undertaken to eliminate variations in metal concentration with time, seasons, freshwater run-off, currents, tides and other factors (Phillip, 1977; Root, 1990). The use of this analysis is also expensive and laborious (Phillip, 1977).

Other limitations include the restrictions in the analytical methods, and chemical detection levels (Hertz, 1991). Many of the hazardous organic compounds produce adverse biological effects only at concentrations below existing analytical detection capabilities (Cairns & Van der Schalie, 1980). Some chemicals cannot be effectively isolated and therefore may pass undetected by chemical techniques because their concentrations drop below detection levels (Cairns & Van der Schalie, 1980). Very little is known about the behavior of these chemicals in complex natural ecosystems and many toxicants may act differently in combination than they do individually (Yasuno & Whitton, 1986; Crowder, 1991). The analyses of chemicals in sediments are also subject to some error, according to local variations in sedimentation rates of particulate material and in the amounts of organic material present (Phillip, 1977). According to John *et al.* (1990), sediment toxicity tests often rely on equilibration of chemicals in a sediment-water mixture, or employ benthic organisms whose survival, growth, or reproductive success may be dependent not only on the presence of toxicants, but also on physical characteristics of the sediment (sand, silt, organic content).

The hazardous chemicals entering aquatic environments may be taken up by organisms, and thus, the initial indication of large-scale impending environmental problems is often detected through immediate impacts on the biota. The level of pollutants detected in the tissues of organisms is the only direct measure of the proportion of the total toxicant delivery to biota, and therefore indicates the fraction that is likely to enter and affect the aquatic ecosystem (Phillips, 1978).

The analysis of biota is based on the premise that the most accurate information regarding the integrity of an ecosystem can be obtained by monitoring the organisms that actually live within the area of interest (Cairns & van der Schalie, 1980). The response of biota to pollution stress can be observed at the ecosystem, community, population, individual and suborganisma levels of organisation (Cairns & Niederleher, 1987; Chaphakar, 1991). Sublethal levels of contaminants that have accumulated in the tissues of organisms can be measured to indicate the extent of pollution that has been integrated over a period of time. Table 9 shows some of the examples used to monitor heavy metal pollution.

2.4 Characteristics of a Good Biological Indicator

Individual biomonitors respond differentially to different sources of bioavailable metals (solution, sediment-bound or incorporated in food). Therefore, it is necessary to make use of a suite of biomonitors covering all sources. The comparative use of different biomonitors might also allow identification of the particular source of a contaminant metal (Phillips & Rainbow, 1988; Phillips, 1990).

A biological indicator can be defined as an "organism which accumulates substances in its tissues in a way so as to reflect environmental levels of those

Table 9 : Some bioindicators used in monitoring heavy metal pollution

Group	**Example**	**Referance**
Aquatic mammals		
Seal:	accumulated heavy metal in the hair and skin.	Yedlier *et al.*, 1993*
Fin whales:	accumulated organic Hg in the kidneys and muscles.	Sanpera *et al.*, 1993*
Beavers:	accumulated Ni and Pn in the liver and kidneys.	Pankakoski *et al.*, 1993*
Birds	accumulated Cd and Pb in the egg shells.	Burger and Gochfeld, 1993
	accumulated heavy metal in the feathers of herring gulls.	Lewis *et al.*, 1993*
Fish	accumulated heavy metal in the livers.	Evans *et al.*, 1993*
Invertebrates		
Shrimp and blue crab:	It been used to study the accumulation of metals at various trophic levels.	Burger and Gochfeld, 1993
Rithrogena hageni (mayfly larva):	The most sensitive organism for detecting Zn contamination in the upper Arkansas River.	Nelson and Roline, 1993
Algae and aquatic plant	Very popular in monitoring environment contaminated by heavy metals.	Lange *et al.*, 1994
Blue green algae *Oscillatoria*:	Have been proved useful for monitoring metal pollution.	Ray *et al.*, 1976
Scenedesmus and *Selenastrum*:	Have been reported the most efficient (90%) accumulates Cu^{2+} and Cr^{2+} than *Chlorella*.	Brady *et al.*, 1994
Trees	Assessing heavy metals from atmospheric and soil.	Lange et al., 1994
Potamogeton richardsonii and *Equisetum fluviatile*:	Have been proved useful for monitoring metal pollution.	Ray *et al.*, 1976

substances or the extent to which the organism has been exposed to them" (Helawell, 1986).

Phillip (1977) and Ray *et al.* (1976) highlighted some characteristics of a good biological indicator. The organism used as a biological indicator should be abundant in the study region so that adequate tissue samples can be obtained for analysis. The organisms should be sedentary in order to be representative of the area of collection (locality). The organism should accumulate the pollutant without being killed by the levels encountered, in order to have lived sufficiently long for allowing sampling of more than one year. The organism should exhibit a high concentration factor for metals, allowing direct analysis without preconcentration. The organism should be easy to sample and hardy enough to survive in the laboratory studies on the uptake of the metals.

The organism should have a finite number of binding sites for metals and the actual metal concentration should depend upon the relative concentration of the elements in its environment (Ray *et al.*, 1976). As the site of action between metal and the organic ligand appears to involve nitrogen, sulfur or other electronegative functional groups, the degree of selectivity will be influenced by the chemical constituents of the organism and the stabilities of the complexes formed (Ray *et al.*, 1976). The organism surveyed should exhibit the same correlation between their metal contents and those in the surrounding water at all locations studied under all conditions.

The use of biological indicator organisms to define areas of trace metal pollution appears to be most attractive. Inexpensive and relatively simple analysis is required and the time-averaged value for the relative biological availability of metals at each site studied can be obtained (Phillip, 1977).

2.5 Microalgae: Good Biological Indicators

Algae possess many characteristics which fulfill the requirements of good biological indicators (McCormick *et al.*, 1994).

The algae are an ecologically important group in most aquatic ecosystems because of their nutritional needs and their position at the base of aquatic foodwebs. Algae are among the microorganisms that are first to be affected by pollution and they respond rapidly and predictably to a wide range of pollutants.

Algal indicators provide relatively unique information concerning ecosystem condition compared with commonly used animal indicators. They provide potentially useful early warning signals of deteriorating conditions and the possible causes. Algal assemblages provide one of the few benchmarks for establishing historical water quality conditions and for characterising the minimally impacted biological condition of many disturbed ecosystems.

Algal cells are able to discriminate between a wide variety of chemical compounds, especially in transporting and incorporating the elements such as N, P, Mn, Fe and Zn which are necessary for cell growth (James *et al.*, 1987). Species that exhibit high affinity for nutrients appear to be better at discriminating between the necessary nutrient and competing ions than those species exhibiting low nutrient affinity.

Algae are non-pathogenic and can be easily grown in ponds (Brady *et al.*, 1994). Preliminary comparisons suggest that algal indicators are a cost-effective monitoring tool (McCormick *et al.*, 1994). Algae are known to concentrate heavy metals from ambient waters and pass them on to higher tropic levels (Gowrinathan & Rao, 1990).

Both living and dead algal cells have the potential to accumulate metals. Table 10 lists the heavy metal content in algal cells of some species (Brady *et al.*, 1994). The use of algal cells to accumulate heavy metals such has the potential for economic recovery of metals or treatment of waste waters (Wan Azlina, 1993). According to Wan Azlina (1993) the rapidly developing field of paleolimnology and the utilisation of algal microfossils also offer a unique opportunity for biomonitoring

Table 10 : Heavy metal content in three microalgae species

Species	Initial Conc. (mgL^{-1})	Metal (mgg^{-1})			
		Cr^{3+}	Cr^{2+}	Cu^{3+}	Pb
Scenedesmus sp.	2.5			7.1	
	25			67.3	
	100	178.7	68.6	213.7	219.8
	10	27.1	3.3		20.6
Selenastrum sp.	2.5			8.2	
	25			83.9	
	100	204.1	72.9	237.5	246.8
	10	34.2	3.5		4.3
Chlorella sp.	2.5			7.1	
	25			67.6	
	100	176.5	101.4	174.1	179.1
	10	23.1	3.9		19.7

Source : Brady *et al.* (1994)

programs. As a result of cultural activity, diatom and chrysophyte communities have experienced many changes, including shifts in species composition and changes in species richness and diversity. Species changes can be quantified and correlated with environmental variables and used to reconstruct pre-industrial chemical, physical and biological conditions, and to identify changes that have natural variability. Algal microfossils can even identify changes that have occurred within the last few years, and thus they can be used as early warning indicators of changes in water quality. Paleolimnological approaches can be effectively integrated, in a cost-effective manner, into many long-term monitoring programmes.

2.6 Interaction Between Algae and Heavy Metals

According to Beveridge & Murray (1980), metal binding in microorganisms may involve at least a two-stage process. The metal ions will first interact with the reactive groups before being deposited in increased amounts. The metals accumulated may be greater than the stoichimetric amounts.

In another model suggested by Chaudhary *et al.* (1988), metal uptake in algae involves two processes. Firstly, the metals are adsorbed on the algal cells before diffusing slowly into the cells by a passive transport mechanism. Once inside the cell, the metal ions may then bind to low molecular weight polyphosphates (Higham *et al.*, 1986). Thus, the intracellular uptake of metals may be caused by permeation and diffusion resulting from increased membrane permeability (Gadd, 1988). The translocation of cations across the wall and membrane into the cell is slower than surface binding and it is a metabolism dependent process. The rate of intracellular uptake may be influenced by the physiological state of cells and the nature and composition of the growth medium. It is inhibited by low temperatures, the absence of an energy source and the presence of metabolic inhibitors or uncouplers.

The synthesis of intracellular traps such as metallothione (metal-binding protein) is one of the mechanisms to be induced in the presence of metals such as Cu, Zn, Cd and Hg (Gadd, 1988). These traps may not only function to regulate the intracellular concentrations of the more beneficial elements but also prevent harmful, heavy metals from reaching toxic levels (Wan Azlina, 1993).

The accumulation of heavy metals by cell walls of microalgae is often rapid, reversible and complete within 10 minute (min). In many instances, uptake can be described by Freundich or Langmuir sorption isotherms, exhibiting a linear equilibrium between the concentrations of metals in solution and that bound to cell surfaces or cell walls (Khummongkol *et al.*, 1982). Such uptake is mostly unaffected by metabolic poisons, modest variations in temperature and / or light - dark treatment (Gadd, 1988). Passive sorption can be affected by other cations, including H^+, Ca^{2+},

Mg^{2+}, Na^{+} and Mn^{2+}, and by other heavy metals (Sakaguchi *et al.*, 1979). In certain cases, the amount of heavy metal passively accumulated by dead cells or by cell wall preparations may be greater than that for living cells (Mang *et al.*, 1978) suggesting that additional binding sites have become available during such experimental treatments.

"Resistance" and "tolerance" are arbitrary terms and often used interchangeably (Reed *et al.*, 1989). "Resistance" is sometimes used in connection with a direct response to metal exposure, whereas "tolerance" may rely on intrinsic properties of the organism or the physical and chemical nature of the environment. The term "tolerance" is used in a broad sense to include specific mechanisms of resistance as well as indirect mechanisms (e.g., impermeability).

In the aquatic habitats, heavy metals may exist in dissolved or particulate forms. The dissolved forms include free ions and complex ions chelated with inorganic or organic ligands. While the particulate forms may exist as colloids, aggregates and precipitates. The relative proportion of each component may alter the overall metal toxicity (Reed *et al.*, 1989).

An irreversible increase in plasmalemma permeability (Reed *et al.*, 1983) of microalgae may lead to the loss of cell solutes such as potassium (K^{+}) or organic osmotic and changes in cell volume (Christensen *et al.*, 1979). In addition, physiological processes such as photosynthetic, respiration and nutrient uptake may also be affected. For example, electron transport and carbon fixation rate during photosynthesis may decrease (Shioi *et al.*, 1978; Davies *et al.*, 1980) while oxygen consumption during respiration may be inhibited (Rivkin, 1979). It may also disrupt nutrient uptake processes (Harrison, 1983) and inhibit enzyme activity due to the displacement of essential metal ions (Rebhun *et al.*, 1984). Protein synthesis is also inhibited as a result of metal toxicity (Kremer *et al.*, 1982). Other effects include abnormal morphology development (Say *et al.*, 1977) and ultrastructural changes such as mitochondrial swelling (Silverberg, 1976), granulation (Thomas *et al.*, 1980), multinuleation (Massalski *et al.*, 1981), alterations in vacuolar and chlolloplast size (Smith, 1983), the impairment of motility and loss of flagella in certain microalgae (Nakono, 1978), the degradation of photosynthetic pigment coupled with reductions in growth (Monahan, 1976). In extreme cases, the cells may die (Fennikoh *et al.*, 1978).

2.7 Mechanisms of Heavy Metal Tolerance in Algae

The ability of algae to survive and reproduce in metal-polluted habitats may either be inherited through their evolution in extreme environmental conditions (Brock, 1988) or through genetic adaptation over extended time periods by mutation, genetic exchange and selection. The resistance may be acquired through the extrachromosomal genetic elements called plasmids (Brock, 1988) or due to physiological changes resulting from metal exposure (Reed *et al.*, 1989).

2.7.1 Extracellular Binding and Precipitation

The extracellular organic ligands such as organic acids and polypeptides in blue-green algae can reduce metal toxicity. However, the mechanism is effective when cell density is high or when the concentrations of other organic compounds are high (Van den Berg *et al.*, 1979). This suggests that their significance may be limited by dilution in many natural environments (Lewis *et al.*, 1982) and may be important in certain natural waters, such as in places near sewage outfalls (Hershelman *et al.*, 1981). One example of blue green alga which produces extracellular organic acid is *Anabaena cylindrica* (Fogg *et al.*, 1955). A copper-tolerant isolate of *C. vulgaris* releases more extracellular organic compounds than Cu-sensitive isolate after prolonged exposure to the metal (Butler *et al.*, 1980).

Other specific iron-chelating siderophores are another group of extracellular organic products found in some blue green algae. In eukaryotic algae, complexing agents for other metals are also produced (Gadd, 1988). For example, strong Cu-complexing compounds are produced in *Anabaena* species (Clarke *et al.*, 1987).

The antagonistic interaction between heavy metals and Fe uptake in planktonic algae may affect the production of such compounds and thus modulate metal toxicity (Harrison *et al.*, 1983).

Extracellular metal crystallization may occur on the surfaces of some algae. For example, *Cyanidium caldarium* is able to recover and remove Cu, Ni, aluminum (Al) and Cr from acidic mine wastes by cell surface precipitation of metal-sulfide microcrystals (Wood *et al.*, 1983).

Some algae may secrete copious amounts of polymers such as polysaccharides with anionic properties to form a large extracellular aggregate (Daneil *et al.*, 1981). The components of algal cell walls , behave similarly to cation exchangers and are able to bind heavy metal cations. For example, in *Scenedesmus obliquus*, the cell wall behaves like a weakly acidic cation exchanger although some heavy metals (e.g., Cd) may accumulate as neutral complexes (Stary *et al.*, 1983). In *Vaucheria* sp. (Crist *et al.*, 1981), metals bind through ionic bonding and covalent bonding to cell surface groups including carboxyl, sulfate, amino and carboxyl groups while in red algae, metals bind to the cell wall carrageenan (Veroy *et al.* , 1980).

The cell wall of blue-green algae has a similar structure to those of Gram-negative eubacteria, where most metal deposition occurs at the polar regions of the consituent membranes, or within the peptidoglycan layer (Beveridge *et al.*, 1980).

This may lead to great amounts of metals being accumulated and thus, such uptake cannot be interpreted solely in terms of ion-exchange phenomena.

The amounts of heavy metals taken up by passive mechanisms and bound on the cell surfaces or cell walls of algae are often quite low, compared to those accumulated intracellularly through extraprotoplast sorption (Gadd *et al.*, 1978). In *Synedra ulna*, 20% of the Hg uptake is due to extraprotoplast sorption (Fujita *et al.*, 1975) and similar results were also reported for Cd uptake in *Eremosphaera viridis*. During short-term incubation (5 min), over 80% of accumulated metal is through extraprotoplast sorption in *Ankistrodesmus braunii* and *C.vulgaris* (Geisweid *et al.*, 1983). Similar accumulation pattern was also observed in *Mougeotia*, *Microspora* and *Hormidium*, for 50% of the metal accumulated (Francke *et al.*, 1980).

2.7.2 Impermeability and Exclusion

The "slow" phase of heavy metal accumulation is often regarded as being due to intra-protoplast uptake, in contrast to "rapid" physical binding or biosorption. The slow phase only can be reflected, in part at least, as active (metabolism-dependent) uptake (Khummongkol *et al.*, 1982). However, in certain cases, algicidal concentrations of heavy metals may induce passive metal accumulation within the protoplast, due to permeabilization of the cellular membrane systems and the exposure of further sites for metal binding (Gadd, 1988).

Active transport systems have been described for several heavy metals in microalgae. For example, a specific high affinity ($K_s = 17$ mol μm^{-3}) active uptake system which is capable of concentrating by a factor 2700 for Ni in *Anabaena cylindrica* and for Cd in *Anacystis nidulans* was observed (Singh *et al.*, 1983). In C. *pyrenoidosa* active uptake of Cd is completely inhibited in darkness or at 4 °C but not by a range of divalent cations (Hart *et al.*, 1980). In *Dunaliella salina*, Cd uptake is competitively inhibited by Mn. In *Thalassiosira weissflogii* a marine diatom, antagonism between Cd and Fe uptake was demonstrated (Harrison *et al.*, 1983).

The mechanism of tolerance in certain algae is attributed to the decreased internal accumulation of heavy metals. For example, the Cu-tolerant strains of *C. vulgaris* (Butler *et al.*, 1980; Foster, 1976) and *Ectocarpus siliculosus* accumulate lower amounts of Cu compared to the sensitive strains (Hall et al., 1989 and 1989b). Other strains of *E. siliculosus* and *Scenedesmus* develop tolerance of heavy metals through the physical exclusion mechanism (Hall, 1979). However, in many other algae, the development of metal tolerance is not coupled to a decrease in metal uptake. For example, some isolates of *Chlorella* and *Scenedesmus* from Cu-enriched lake take up greater amounts of metal ions than non-tolerant strains (Silverberg *et al.*, 1976; Stockes *et al.*, 1973 and Stokes, 1981).

2.7.3 Internal Detoxification

Metals are transported internally and localized within the protoplast sites through the mechanism of internal detoxification (Trevors, 1986). In several algae, internal localization may involve precipitation within specific intra-protoplast sites. For example, metal-tolerant strains of *Scenedesmus acutiformis* (Silverberg, 1976) accumulate Cu within vacuoles and the nucleus, and Pb in the nucleus (Silverberg *et al.*, 1977). Cd is localized in the nucleus of *Porphyra umbilicalis* (McLean *et al.*, 1977) and in the physodes for *Fucus vesiculosus* (Lignell *et al.*, 1982). Uranium (U) uptake in *Synechococcus elongatus* is due to the formation of dense, internal deposits (Horikoshi *et al.*, 1979). In *Anabaena cylindrica*, *Plectonema boryanum*, and certain eukaryotic microalgae, Al and Cd may be sequestered within polyphosphate bodies (Vymazal, 1987; Jensen *et al.*, 1982).

Cu is localised intracellularly in electron-dense spherical bodies which consist of polyphosphate granules and in electron-dense irregular bodies containing sulfur (S), calcium (Ca), and Cu ("Cu" granules). These bodies may maintain low cytoplasmic levels of Cu, as shown in the diatoms *Amphora* and *Navicula* for reducing Cu toxicity (Daniel *et al.*, 1981).

Another aspect of internal compartmentalization is the synthesis of metal-binding components which play important roles in the detoxification process. For example in *Synechococcus* sp., a cadmium-binding protein-aqeous component ($M_r \cong 8100$) was synthesized in response to Cd exposure (Olafson *et al.*, 1979). Several metal-binding components are found in the Cu-tolerant *Scenedesmus acutiformis* , Cd-tolerant *C. pyrenoidosa* and Cd resistant in *Dunaliella bioculata* (Hart *et al.*, 1980; Heuillet *et al.*, 1988). Recent studies show that the metal binding "proteins" from a wide range of algae including *C. fusca*, *S.acutiformis* and *Porphyridium cruentum* consist of phytochelatins (peptides with the general structure $(\gamma\text{-Glu-Cys})_n$ - Gly where n = 2 to 22), which bind heavy metal ions by thiolate coordination (Gekeler *et al.*, 1988).

2.7.4 Metal Transformation

Chemical transformation of heavy metals through oxidation, reduction, methylation, and demethylation are another mechanism of resistance in microorganisms (Reed, 1989). For example, a Hg-resistant *Chlorella* volatilized more than twice as much

Hg^{2+} as a sensitive strain and most of this activity is stimulated by light; cell-free extracts are also capable of volatilization of Hg^{2+} and phenylmercuric acetate.

Methylation may be an important detoxification mechanism since the volatility, solubility, and the toxicity of a given element may be altered (Wood *et al.*, 1983). For example, certain heavy metals and metalloids such as As, Cd, Hg, Pb, selenium (Se), and Sn are involved in biomethylation reactions in various microorganisms, including algae. Other metals, such as antimony (Sb) and Sn are elements of methylated compounds (James, 1987). Detoxification strategy to convert arsenic to various organoarsenicals has been shown in certain marine algae. Marine algae also debutylate tributyltin into less toxic di- and monobutyltin compounds although it should be stressed that light-stimulated proteolysis also occurs. The production of methyltin derivatives by algae has also been encountered although it is possible that this is derived in part from epibiotic bacteria.

In aquatic habitats, disproportion reactions and transmethylations between inorganic and methylated metal forms, which complicate any interpretation of the relative roles of biotic and abiotic factors can occur readily. Methylcobalamin (vitamin B_{12}) , including CH_3I, $(CH_3)_3\ S^+I^-$, and $N^+CH_2COO^-$ has been proposed as a methylating agent by direct and indirect biological action (Wood *et al.*, 1983). Marine macroalgae may contain high concentrations of arseno sugars which may or may not be methylated. For example, in *Ecklonia radiata* decomposition products of arseno sugars are very important for the formation of arsenobetaine (trimethylarsoniumbetaine) which is a ubiquitous component of marine animal tissues.

Phytoplankton may reduce the toxicity of heavy metals by transforming them into less toxic forms. Reduction of Cr and Cu, either directly or through photoreduction or complexation (and detoxification) by absorbed organic carbon are examples of this type of effect (James, 1987).

2.8 Effects of Environment Factors on Heavy Metals Toxicity

The toxicity and biological availability of any heavy metal is dependent on its physicochemical state. One of the most important environmental variables is pH. Under acidic conditions, metals tend be more as toxic free or hydrated ions whereas in alkaline media (including sea water) they may precipitate as insoluble complexes (Sunda *et al.*, 1976). For example, in *C. vulgaris* an increase of pH reduces Zn and Hg toxicity. However, in *Stichococcus bacillaris,* low pH reduces the toxicity and this is attributed to a decrease in transplasmalemma electrical potential and driving force for metal accumulation (Skowronski, 1986). Hydrogen ions may compete with free metal ions for uptake sites and so a decrease in pH may lead to a decrease in heavy metal toxicity (Peterson *et al.*, 1984). The growth of algae in acidic, metal-enriched mine wastes is a further indication that low pH is not always associated with increased toxicity (Gadd *et al.*, 1978).

Heavy metal accumulation and toxicity can be affected by the presence of cations other than H^+ (Reed, 1989). For example, the presence of calcium and magnesium salts reduce the toxicity of Cu, Zn, Cd, and Hg. This may be presumably due to coprecipitation or complex formation and explain in part the decreased toxicity associated with an increase of water hardness in fresh water (Say *et al.*, 1977; Delmotte, 1980). However, direct competition between Ca^{2+} or Mg^{2+} and metal cations may also reduce heavy metal accumulation and toxicity . Nutrient concentration may affect heavy metal toxicity (Reed, 1989). For example phosphorus (as phosphate) decreases heavy metal toxicity in a wide range of microalgae from diverse groups due to precipitation. Conversely, under conditions of nutrient limitation, heavy metals may decrease the ability of algae to utilise the limiting nutrients (Cloutier *et al.*, 1980; Harrison *et al.*, 1983). The presence of organic substance of algal origin, or from other sources, is also likely to ameliorate the toxicity of heavy metals (Lewis *et al.*, 1982).

Biological factors may influence metal toxicity (Reed, 1989). For example, algal cell density, usually shows a negative correlation with metal toxicity due to an increase in cellular binding sites (Delcourt *et al.*, 1978). Actively growing cultures may accumulate more metals than stationary cultures (De Fillipis *et al.*, 1976). Older regions of macroalgal thalli often take up greater amounts of heavy metals than younger, faster-growing regions. However, it is unlikely that any biological indicator can accurately reflect the heavy metal content of all waters at all times. Indeed, according to Foster (1976) under some circumstances, the accumulation of certain heavy metals in algae from metal-pollution sites may be lower than that of algae from unpolluted waters.