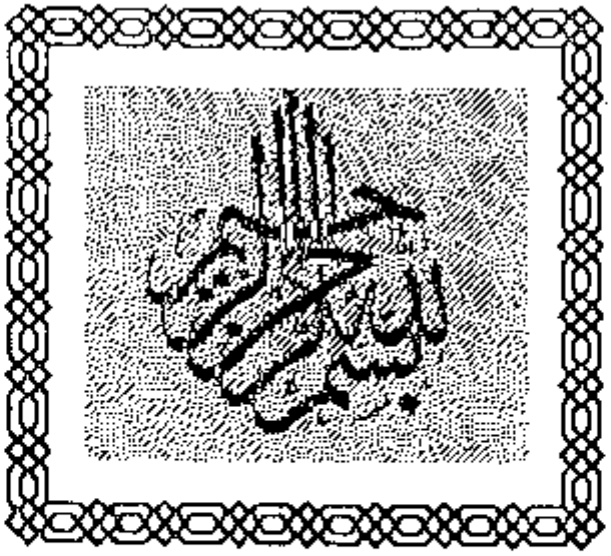

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صدق الله العظيم

الآية ٢١ سورة الحديد
القرآن الكريم

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To the memory of my father

CONTENTS

INTRODUCTION

INTRODUCTION

In recent years considerable interest has been devoted to problems concerning pollution of the environment by heavy metals. Variations in trace element composition beyond the normal physiological range of indigenous organisms must result in some physiological adaptation, selection of genetic mutants or ecological succession of species. Moreover, pertubations induced by heavy metal accumulation in water bodies could lead to elimination of more susceptible phytoplanktonic species resulting in changed productivity and degradation of water body.

The continuous anthropogenic input of heavy metals into the aquatic environment constitutes a potential threat to natural ecosystems because of direct toxic action on aquatic organisms.

Many metals are bioaccumulated, and some are biomagnified in food chains and thereby become a risk to "top predators", including humans. (Rachlin et al., 1982).

The phytoplankton is a complex assemblage of interacting organisms, often including species which are sensitive, resistant or intermediate in their tolerance to pollutants. These degrees of tolerance may provide a yardstick for identifying the intensity and potential for ecological damage caused by anthropogenic pollutants discharged to surface waters (Carolina et al., 1995).

Algae are more and more considered as important in a large variety of environmental issues, like lake putrefaction or sequestering of

toxic, essential or precious metals. They can provide an alternative food source (Wolterbeek et al., 1995), or counteract the greenhouse effect. (Millamena et al., 1990; Ramelow et al., 1991; Sunda et al., 1991; Swinbanks, 1991; Talbot et al., 1991; Crist et al., 1992). In many of these issues, metal ions play essential roles. Algae may be used in determining general water quality and growth - limiting nutrients, in metal toxicity tests (Payne, 1976; Vymazal, 1990; Kusk and Nyholm, 1991; Rai et al., 1991), and in the removal of metals and radionuclides from contaminated waste waters (Garnham et al., 1992 b).

Algae are widely investigated for their ability to accumulate metals removing them from sediments (cf. Wnorowski, 1991).

The algal species present in polluted sites could be useful bioindicators if species occurrences were associated with specific concentrations of metals, or if they were specifically linked with a particular polluting metal. In this context, the algal assay has become a valuable tool for the detection of environmental disturbances and for assessment of the trophic state of water bodies, (Kallqvist, 1984; Cairns and Pratt, 1989).

HISTORICAL REVIEW

HEAVY METALS

Reddy and Prasad (1990) stated that, from a biological point of view, heavy metals can be divided into two categories: essential and non-essential. However, essential heavy metals have also been reported to be toxic at high concentrations. For example, some

heavy metals including copper, zinc, nickel and chromium, are essential for growth at very low concentration but toxic at slightly higher levels (Gadd and Griffiths, 1978; Reed and Gadd, 1989).

Heavy metals have been increasing in the environment from industrial waters, agricultural runoff and mining activities. Many of these metals have a direct influence on various physiological and biochemical processes including growth, photosynthesis and respiration. They may influence chlorophyll content and various enzyme activities and cause degeneration of chloroplast and mitochondria (Reddy and Prasad, 1990). The overall toxic reaction caused by heavy metals is depressed growth due to depressed photosynthesis, direct inhibition of growth processes and decreased nutrient uptake and transport (Greger and Lindberg 1987; Fernandes and Henriques 1991; Greger and Ögren 1991; Greger et al., 1991; Greger and Bertell, 1992).The reduction of photosynthetic rate of algal cells in the presence of toxicants may be due to the inhibition of synthesis of chlorophyll *a* (Rosko and Rachlin, 1977, Wong and Chang, 1988).

Researches examining the effects of heavy metals on uni-or multicellular algae have reported that, often cells respond to toxic levels of metals by increasing cell size (Fisher et al., 1981; Stauber and Florence, 1987; Bolaños et al., 1992). Shehata and Badr (1980) observed that certain metals increase the growth rates of particular algae, whereas they have no effect on others. Some unicellular algae have been reported to respond to toxic levels of metals by depressing

cell division rates (Fisher et al., 1981). It has been suggested that metals inhibit cell division by binding reactive thiols on the tubulin molecule, which is important in spindle formation during mitosis (Onfelt, 1983). Copper may prevent the production of methionine which is necessary for cell division (Davies, 1976) or may bind to SH-groups and interfere with a number of metabolic pathways, essential for maintaining normal cell division rates.

Sicko-Goad and Stoermer (1979) found that the phosphate nutrient status of algal cells may mitigate the deleterious effects of heavy metals. In experiments with cultures of the pennate diatom, *Diatoma tenue* var. *elongatum* they found that the primary effects of lead treatment, when coupled with phosphate uptake, were swelling and reduction in number of mitochondria and incorporation of lead into polyphosphate bodies.The primary effect of copper treatment was the reduction in number of mitochondria without incorporation of copper into the polyphosphate bodies.

Heavy or trace metals are often found as deposits in various cellular organelles, such as mitochondria (Stuve and Gatle, 1970; Silverberg, 1976), chloroplasts (Fujita et al., 1977), nuclei (Choie and Richter, 1972; Skaar et al., 1973; Moore and Goyer, 1974), and in cell walls. Evidence of movement from the wall or plasma membrane area to the vacuole or cytoplasm is often reported (Gerrard et al., 1974; Murray and Kidby, 1975; Brown and Smith, 1976, 1979; Beveridge and Murray, 1980). On the contrary Sicko-Goad (1982) found no evidence of such deposits in any organelles or

cellular compartments in three algal species (*Melosira granulata*, *Fragilaria capucina*, and *Anacystis cyanea*), though photosynthetic membranes and mitochondria were most sensitive to metals, especially copper. There were changes in the vacuole relative volume in both diatoms when chloroplast relative volumes were calculated as a cytoplasmic percentage rather than a cellular percentage. There was an indication that the main effects observed in the diatoms were a result of membrane leakage, perhaps at the tonoplast, resulting in a greater water content and subsequent size increase of the vacuole. The ultrastructural responses of *Anacystis* are consistent with the physiological responses as reported by Sicko-Goad(1982). The decrease in surface area of thylakoid membranes observed with both lead and copper treatments can be correlated with the reduction in photosynthesis reported as a result of exposure to these metals (Steeman-Nielsen and Wium-Anderson, 1971; Bazzaz and Govindjee, 1974; Thomas et al., 1977; Gupta and Arora, 1978). Copper treatment also resulted in significant differences in the number of cyanophycin granules and poly-B hydroxybutyrate granules.

However many of the reported ultrastructural changes in cultured algal cells resulting from heavy metal exposure are based on experiments lasting for several days or weeks, at concentrations that are uncommon in aquatic environments (Sicko-Goad, 1982).

COPPER

Copper is found in aquatic environments in different chemical states (Kamp-Nielsen, 1972): ionic Cu^{+} or Cu^{++}; organic complexes Cu-peptides, Cu-humus.

It is an essential element for all organisms, being a constituent of enzymes which catalyse oxidative reactions in a variety of metabolic pathways. Lustigman (1986) and Stauber and Florence (1987) stated that copper is an essential micronutrient for growth, metabolism, and enzyme activities of various algae, cyanobacteria, and other organisms. At high concentrations Cu is toxic to most organisms, an attribute which has led to its use in a variety of fungicides and marine antifouling compounds. It acts as an algicide or an algistatic agent (Gupta and Arora, 1978).

Copper toxicity is directly related to its free ion (hydrated) (Sunda and Guillard, 1976), but inorganic complexes are also believed to be toxic, although to a lesser extent (Florence, 1982).

Abalde et al (1995) reported that copper exposure induces changes in cellular volume and is toxic to the marine microalga *Dunaliella tertiolecta*, leading to depressed photosynthesis and cell division rates.

Copper inhibition of growth rate is dependent on cell density (Stauber and Florence, 1987), leading to decreased heavy metal toxicity when the cell density increases (Vasseur and Pandard, 1988; Rai et al., 1991). Also, Steemann-Nielsen and Kamp-Nielsen (1970) demonstrated the influence of cell concentration on the effects of Cu

on growth rate. This is due to the binding of Cu by the cell walls and slime envelopes. At a Cu concentration where no growth of algae can take place, the algae are by no means killed and after being transferred to Cu free medium they start to grow again. The influence of Cu depends on the division stage of the algae. If the initial steps of cell division have taken place, the cell continues to divide.

Steemann-Nielsen et al. (1969) have described the effect of deleterious concentrations of copper ions on the rate of photosynthesis in the green alga *Chlorella pyrenoidosa*. It was shown that the effect, is for a large part indirect, being due to an inhibition of cell division. This causes an accumulation of photosynthetic products. The rate of photosynthesis decreases when the concentration of these products exceeds a certain value. In experiments of longer duration a direct influence of Cu on photosynthesis was also observed. Inhibition of photosynthesis was observed in several species of microalgae, for instance, in the diatom *Nitzschia closterium* (Stauber and Florence, 1987).

Many authors stated that *Ditylum brightwellii* is a Cu-sensitive diatom species blooming in estuaries and coastal waters (Canterford and Canterford, 1980; Rijstenbil and wijnholds, 1991; Rijstenbil et al., 1993). At 10^{-11} M Cu^{2+}, cell division was slightly inhibited, but growth ceased abruptly between $10^{-10.2}$ and $10^{-9.3}$ M Cu^{2+} (Brand et al., 1986). In addition, increasing Cu levels have affected the cell structure and integrity (Rijstenbil et al., 1994). An increase of cell volumes upon Cu stress has been reported also by

Fisher et al., (1981) and Rijstenbil and Wijnholds (1991).

Copper sulphate has been extensively used to control undesirable algal growth in freshwater lakes and reservoirs. Most algae are susceptible to 1-2 ppm (4-8μM) of copper sulphate (Fitzgerald et al., 1952; Bartsch, 1954; Palmer and Maloney, 1955; Maloney and Palmer, 1956; Fitzgerald, 1964). Some, however, tolerate much higher concentrations (Galloway and Krauss, 1959). George et al., (1965) reported that 0.5ppm (2.2μM) copper sulphate gives 91.8% and 1.1 ppm (4.4 μM) 96.2% reduction in algal growth. Greenfield (1942) has reported that photosynthesis in *Chlorella* was inhibited by copper concentrations higher than 0.1 μM. However, it was reported that the effect of Copper on photosynthesis and respiration of *Chlorella* is magnified when copper is applied under anaerobic conditions (Davies, 1965).

Many reports have demonstrated that concentrations of copper as low as 1 ppm can be toxic to various phytoplankton species (Mandelli, 1969; Steemann Nielsen et al., 1969; Steemann Nielsen and Kamp-Nielsen, 1970; Martin and Olander, 1971; Erickson, 1972).

Using algal cells, De Filippis (1979) demonstrated that the ability of metals to induce potassium leakage, a measure of damage to the permeability barrier of the cell, closely corresponded to their sulfhydryl reactivity. In higher plants, ATP-ases in the plasmalemma, enzymes involved in the permeability barrier of cells, may be primarily affected, since these enzymes are very sensitive to

various sulfhydryl reagents, including heavy metals such as copper (Beffagna et al., 1979; Vara and Serrano, 1982; Katz and Sussman, 1987; Gräf and Weiler, 1989; Kennedy and Gonsalves, 1989). Moreover, plasma membrane ATP-ase-related processes, viz. H^+-efflux and plasmalemma polarization, may be inhibited by copper in vivo, as reported for maize roots (Kennedy and Gonsalves, 1987). In this connection, Salin (1987) stated that many organo-chemicals (quinones, nitroaromatics, aromatic hydroxylamines) and transition metals (e.g. copper) are pro-oxidants, which catalyze and accelerate the formation of oxyradicals in plants. Due to this oxidative potential, copper is a very toxic metal. An excess of Cu-induced oxy-radicals disturbs the pro-vs antioxidant balance and enhances lipid peroxidation (membrane disintegration, failure of osmoregulation) which shortens the lifetime of cells (Lee and Hassan, 1985; De vos and Schat, 1991).

At low values, Cu stimulates phytoplankton growth (Brand et al., 1983; Verweij et al. 1992;), but a slight excess metals binds SH sites, while also oxidizes SH to disulfur (-SS-) bridges in proteinaceous bio-molecules, (Stauber and Florence, 1987, 1990; French and Evans, 1988; Rijstenbil and Poortvliet, 1992). With speciation models it was calculated that for most algae, ion activities of Cu^{2+} limit growth below 10^{-13}M, and become sublethal above 10^{-11}M (Brand et al., 1986; Verweij et al., 1992).

Shioi et al. (1978) demonstrated that copper strongly inhibited 2,6-dichloroindophenol (DCIP) photoreduction in the broken cells of the