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Effects of copper (I) oxide on growth and biochemical compositions of two marine microalgae

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Abstract: *In copper based antifouling (AF) paints Cu (I) oxide was largely used as booster biocide. In this study effect of Cu (I) oxide on two marine microalgae, Tetraselmis suecica and Dunaliella tertiolecta was demonstrated. EC_{50} (96 hr) concentrations estimated for T. suecica and D. tertiolecta were 1.3 mg l^{-1} and 1.34 mg l^{-1}, respectively. Copper (I) oxide induced changes in growth, chlorophyll, carbohydrate and protein contents were observed in T. suecica and D. tertiolecta. At low concentration of 0.0625 mg l^{-1}, 3-26% and 1-16% growth stimulation was observed in T. suecica and T. tertiolecta respectively. Increasing Cu (I) oxide concentrations proportionately decreased the carbohydrate and protein contents. This study clearly indicates the toxicity of excessive Cu (I) oxide on growth and biochemical compositions of T. suecica and D. tertiolecta.*

Key words: *Copper (I) oxide, Macroalgae, Growth performance, Biochemicals, Antifouling paints.*

Introduction

The toxic chemicals used in the AF paints cause severe environmental pollution. After the ban on toxic organotin (TBT, TPT, etc.) compounds, usage of Cu based AF paints have largely increased (Valkirs *et al*., 2003), especially in the form of Cu (I) oxide.

Heavy metals released from AF coatings also tend to cause change in the growth, biochemical metabolism and reproductive potential of the marine organisms. Among the metals, Cu is having an essential role in the algal metabolism (Thompson *et al*., 1987; Lage *et al*., 1996). However, in higher concentrations Cu tends to damage the cell wall membrane function causing reduction in potassium ion concentration inside the cells (Overnell, 1975; Khobot'yev *et al.,* 1976; De Filippis, 1976). Copper is also universally used as biocide as it is lethal to microorganism at higher concentrations (Rhie and Lee, 1999). Copper concentrations less than 1 µg l^{-1} retards the growth of fresh and marine microalgal species (Steeman-Nielsen and Winn-Anderson, 1971; Davey *et al*., 1973). Excessive Cu is accumulated on the cell wall and then absorbed into the cell, and affects the enzymes (SH-groups) causing reduction in reproduction (O'kelly, 1974). The members of Chlorophyceae are sensitive to Cu toxicity, at higher concentrations (Sunda and Huntsman, 1995). The leachate from AF coating is considered as one of the major sources of increased Cu levels in the marine environment (Hall *et al*., 1988; Claisse and Alzieu, 1993; Valkirs *et al*., 2003). At community level, chronic Cu pollution alters the dominance and influences the biodiversity of algae (Thomas and Seibert, 1977).

Since Cu based AF coatings have been largely used as booster biocide, in this study effects of Cu (I) oxide on growth and biochemical characteristics of *D. tertiolecta* and *T. suecica* were investigated.

Materials and Methods

Test species, *Dunaliella tertiolecta* Butcher (C-010) and *Tetraselmis suecica* (Kylin) Butcher (P-004) were procured from the Korea Marine Microalgae Culture Center (KMCC). Copper (I) oxide (99%) was purchased from Shin Yo Pure Chemicals, Japan. Guillard's f/2 medium (Guillard and Ryther, 1962) was prepared with 25 ‰ of filtered seawater (0.45 µm) and used in the bioassay experiments. Experiments were conducted in 250 ml conical flasks with 150 ml of f/2 medium (pH 7.5). To prepare stock solution, 100 mg of Cu (I) oxide was mixed with f/2 medium. Firstly, range finding tests were conducted with 0.01, 0.1, 1.0, 10.0, 100.0 mg l^{-1} of Cu (I) oxide concentrations. From this seven experimental concentrations were fixed (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 mg l^{-1}. The Cu levels in prepared Cu (I) oxide constituted f/2 medium ranged from 0.055-1.776 mg l^{-1}. Considering the environmental relevancy and preliminary range finding test results, the above concentrations were selected. Culture flasks were kept in culture chamber under fluorescent light (55 µE m^{-2} s^{-1}) with 12:12 LD cycle at 20±1 ºC.

In preliminary experiments, algal bioassay was conducted for 10 days and every alternative day cell number was measured under microscope (Olympus CK-II). Simultaneously optical density of the culture was also read on UV/VIS spectrophotometer (Ultrospec 3000, Pharmacia) at 620 nm. Between cell number and optical density of the culture a linear regression was observed with r= 0.99, hence in all further experiments optical density was estimated to determine the cell numbers. Chlorophyll (*a* and *b*) contents were estimated (4-10 d) with five ml of culture aliquots (Strickland and Parsons, 1972). Similarly, protein and carbohydrate contents were estimated using standard methods (Lowry *et al*., 1951; Dubois *et al*., 1956).

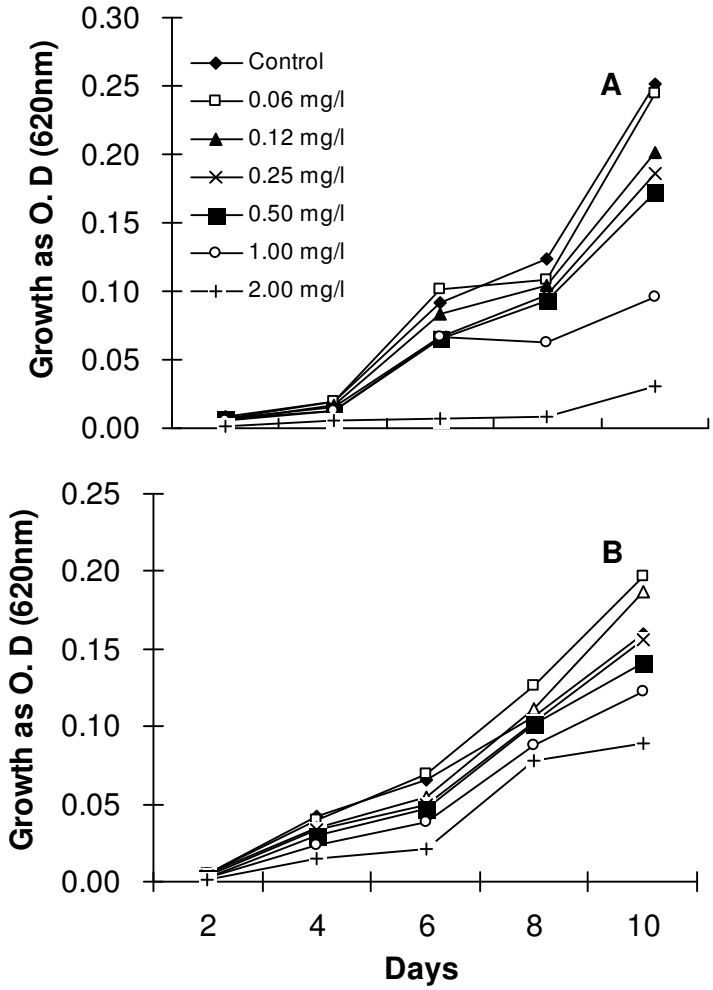

Fig. 1: Effect of copper (I) oxide on growth of *T. suecica* (A) and *D. tertiolecta* (B).

Results and Discussion

In all the Cu (I) oxide concentrations tested growth characteristics of *T. suecica* and *D. tertiolecta* were not completely inhibited (Fig. 1). In low concentrations of Cu (I) oxide, 3-18% increase in growth rate was observed. A maximum growth decrease (88%) was observed after 10 days exposure to 2 mg l^{-1}, whereas at 1 mg l^{-1} level it was 50%. After 48 hr exposure, 30% of growth of *T. suecica* grown in 0.12 mg l^{-1} was reduced. Experimental period from 6-10 days showed high growth rate in low Cu (I) oxide concentrations when compared to respective controls with 23% increase (Fig. 1). In *D. tertiolecta* cultures exposed to 2 mg l^{-1} of Cu (I) oxide for 10 days, a maximum of 44% growth decreased.

In this study, we compared growth rate of *D. tertiolecta* and *T. suecica*, in which *T. suecica* was found to be sensitive to Cu (I) oxide. The 96 hr EC_{50} concentrations estimated for *T. suecica* and *D. tertiolecta* were 1.30 and 1.34 mg l^{-1} respectively. In both *T. suecica* and *D. tertiolecta* cultures, the relationship with optical density and cell number is shown in Fig. 2. The cell numbers were proportionate to optical density, so optical density was measured to estimate the growth rate as reported by Hahm *et al*. (2002) for a marine microalga *Skeletonema costatum*.

T. suecica cultures showed 3-26% increase in chlorophyll *a* at low concentrations. In all Cu (I) oxide levels, chlorophyll *b* concentrations in *T. suecica* cultures were decreased (18-35%) during 2-10 days exposure time. Chlorophyll *a* content in *D. tertiolecta* exposed to a low concentration (0.0625 mg l^{-1}) of Cu (I) oxide was increased up to 6 days. But it decreased to 50% at 2 mg l^{-1} after 10 days exposure. In *D. tertiolecta* cultures exposed to Cu (I) oxide for 2-10 days, 3-12 % of chlorophyll *b* contents were decreased. After 10 days exposure to low concentrations of Cu (I) oxide chlorophyll *b* decreased to a minimum of 3% (Fig. 4). Copper (I) oxide had more or less similar effect on chlorophyll *a* and *b* contents of *D. tertiolecta* whereas impact was comparatively more on *T. suecica* (Fig. 3 and 4).

In all the Cu (I) oxide concentrations tested carbohydrate contents of *T. suecica* cultures were decreased when compared to control (Fig. 5). At 2 mg l^{-1}, a maximum of

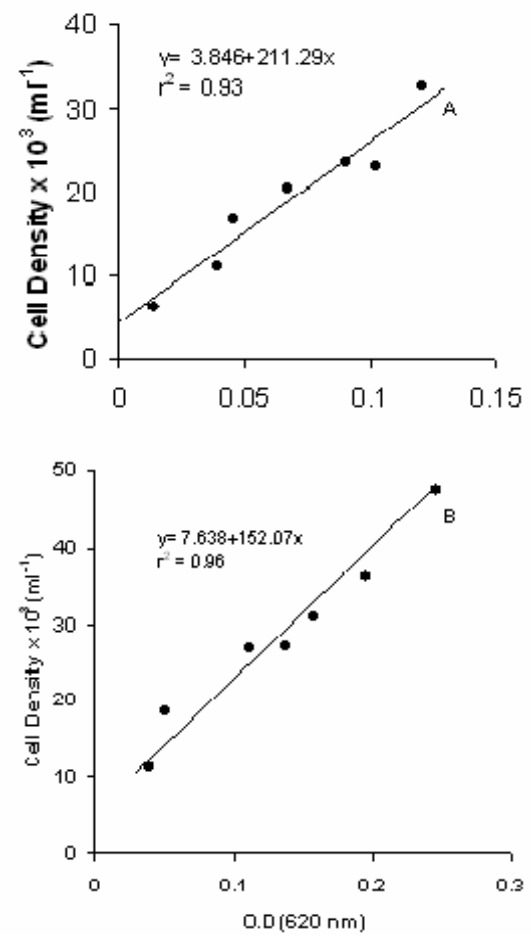

Fig. 2: Relationship among the cell densities and optical densities of *T. suecica* (A) and *D. tertiolecta* (B).

90% reduction was observed. From 2 to 10 days exposure time, reduction in carbohydrate contents of *D. tertiolecta* cultures was found to be less than 50% at 2 mg l^{-1} (Fig. 5). Results showed increase in carbohydrate contents during increased experimental time.

Protein content in *T. suecica* grown in low concentrations of Cu (I) oxide, a maximum of 33% was decreased. Above 0.250 mg l^{-1} levels comparatively very less protein contents were observed in *D. tertiolecta* cultures. When compared to carbohydrate, protein content was sensitive to Cu (I) oxide toxicity (Fig. 6).

It has been reported that the cell division and other biochemical composition of *S. capricornutum* were decreased due to the toxicity of ionic copper (Mi-Kyung and Smith, 2001). Copper induced reduction in growth rate was also reported in *Asterionella glacialis* and *Chlorella pyrenoidosa* (Pistocchi *et al*., 1997). Cu II ions initially affect the osmotic permeability of the outer cell membranes (Ragan *et al*., 1979; DeFilippis, 1979). Cu ion transported into cytoplasm where it affects the photosynthetic sites and uncouples the electron transport to NADP in photosystem II (Cedeno-Maldonado *et al*., 1972). As reported in above studies, in the present study also the highest Cu (I) oxide test concentration administered to *T. suecica* and *D. tertiolecta* was not lethal but altered the growth and biochemical compositions.

In *T. suecica*, both growth and chlorophyll pigment contents decreased in all Cu (I) oxide concentrations. Azeez and Banerjee (1986) also reported decreased chlorophyll contents in two Cyanophytes, *Spirulina platensis* and *Anacystis nidulens* due to Cu toxicity. As the ionic Cu increases, Cu is bound to chloroplast membranes and other cell proteins causing reduction in chlorophyll pigments (Cedeno-Maldonado and Swader, 1974; Khobot'yev *et al.,* 1976; Rai *et al*., 1990; Rijstenbil *et al*., 1994). Higher concentrations of Cu, produce irreversible damage to chloroplast lamellae (Overnell, 1975), preventing photosynthesis and eventually causing the death of cell.

In the present study, during increasing exposure time, Cu effect gradually reduced. In 4 days exposed *T. suecica* cultures very less chlorophyll content was estimated. But in 10 days old cultures exposed to 2 mg l^{-1} of Cu (I) oxide, 22% chlorophyll *a* content was observed. This may be due to the accumulation of Cu onto algal cells in higher concentrations which greatly reduced the available Cu concentration and its toxicity to surviving cells in the final stages of experiment.

Copper affects the distribution of biochemicals such as proteins, lipids and free fatty acids in algae (Lage *et al*., 1994, 1996). At low concentrations of Cu (I) oxide protein content decreased to a maximum of 33%. When compared to carbohydrates, protein content was much decreased due to Cu (I) oxide toxicity. Among the two species tested, protein content in *D. tertiolecta* had less impact by Cu toxicity.

Overnell (1975) reported decrease in growth of *D. tertiolecta* and *D. primulecta* exposed to Cu $^{2+}$ ions. But in our studies Cu (I) oxide was used so LC_{50} values estimated were in mg l^{-1}levels. In low concentrations of Cu (I) oxide no toxic effect was observed on growth but the biochemical composition was found to be altered. As reported by Lupi *et al*. (1998), both carbohydrate and protein contents declined in cultures exposed to higher concentrations of Cu.

The low Cu (I) oxide concentrations used (0.055-0.111 mg l^{-1} of Cu) in this study were environmentally relevant as reported in earlier studies (Danielsson, 1980; Hall *et al*., 1988; Claisse and Alzieu, 1993; Valkirs *et al*., 2003). Especially, in enclosed marinas and harbors where tidal influence is less the Cu levels are expected to be very high.

In antifouling marine paints typically up to 40% (wt.) Cu (largely in the form of Cu (I) oxide) is being used. Excessive Cu concentrations observed along the coastal and harbor areas of different parts of the world are mainly due to Cu based AF paints (Claisse and Alzieu, 1993; Valkirs *et al*., 2003). This may lead to elimination of susceptible microalgal species causing phenomenal changes like incidence of monospecific fouling communities.

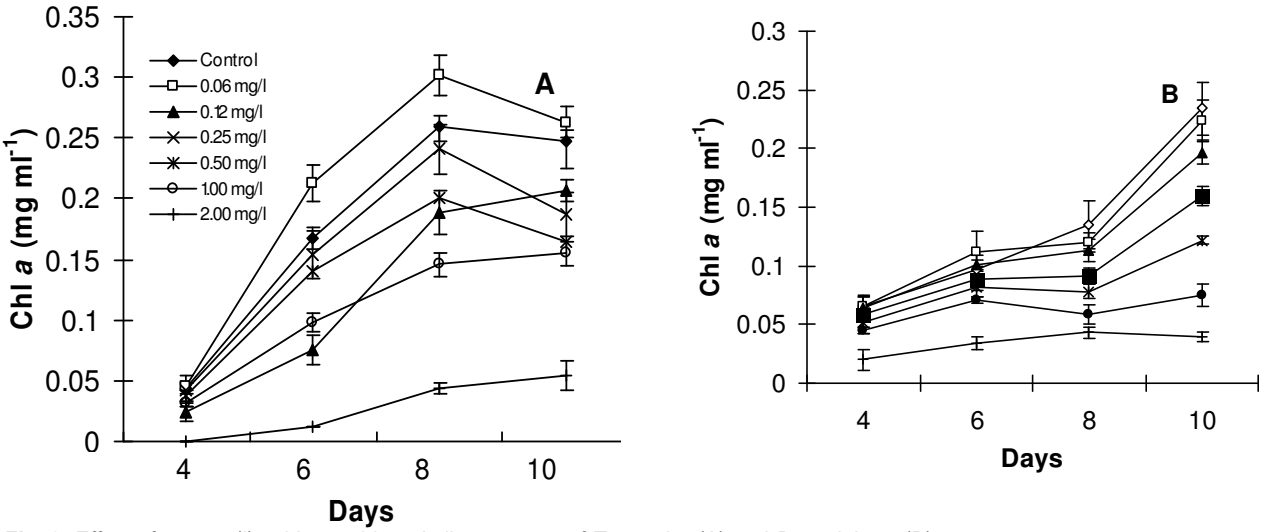

Fig. 3: Effect of copper (I) oxide on chlorophyll a contents of *T. suecica* (A) and *D. tertiolecta* (B).

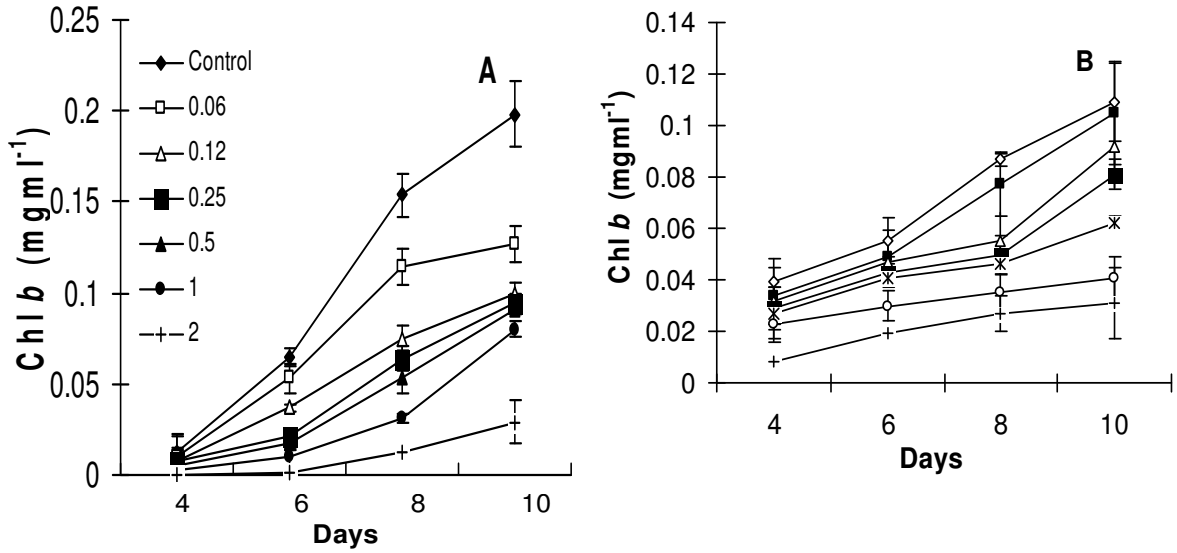

Fig. 4: Effect of copper (I) oxide on chlorophyll b contents of *T. suecica* (A) and *D. tertiolecta* (B).

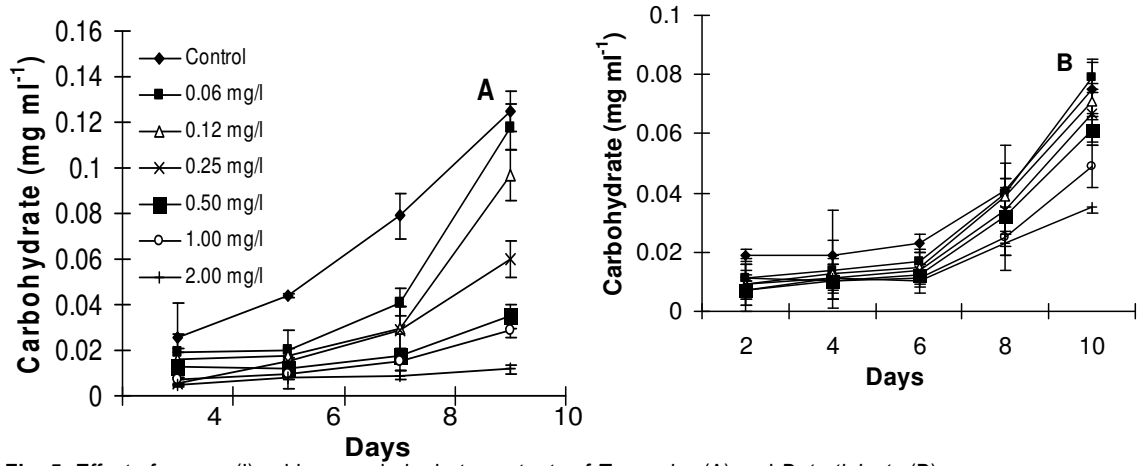

Fig. 5: Effect of copper (I) oxide on carbohydrate contents of *T. suecica* (A) and *D. tertiolecta* (B).

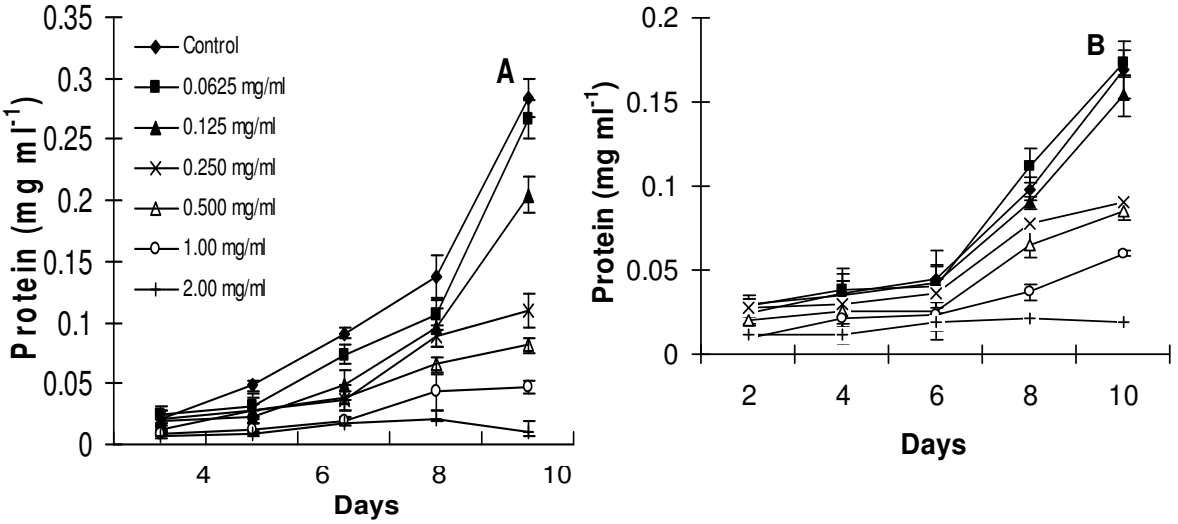

Fig. 6: Effect of copper (I) oxide on protein contents of *T. suecica* (A) and *D. tertiolecta* (B).

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