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Effects of Copper and Cadmium on Growth, Photosynthesis, and Pigment Content in *Gracilaria lemaneiformis*

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In coastal areas, macroalgae are important primary producers and an integral part of the ecosystem. Coastal waters are most affected by anthropogenic contaminants, and as seaweeds border a high proportion of the world's coastline their responses to contaminants are of obvious importance. Many field studies have suggested that metal concentration in seaweeds respond rather faithfully to gradients of metal concentration in their environments (Haritonidis and Malea 1995; Say et al. 1990), therefore some marine macroalgae have been used as a biomonitors of metal contamination in many coastal water (Villares et al. 2001; Haritonidis and Malea 1999; Ho 1990). Furthermore, they can also be used as a biosorbent material for removing metals from solution and their potential application have found in technological processes, in particular, for industrial and mining waste treatments (Volesky and Holan 1995). Heavy metal pollutants in higher plants and microalgae have been shown to depress photosynthesis (Ralph and Burchett 1998), disrupt electron transport in photosystem II (Shioi et al. 1978; Yruela et al, 2000), reduce pigments concentration (Saygideger, 2000), affect the permeability of the plasma membrane (De Filippis 1979). There is relatively little information on the physiological response of seaweed species to elevated heavy metal (Gledhill et al. 1997; Brown and Newman 2003). Up on exposure to elevated copper concentrations, growth of *Laminaria hyperborea* and *Fucus vesiculosus* has been assessed in the laboratory and appeared to differ in their tolerances (Munda and Hudnik 1986; Hopkin and Kain 1978; Gledhill et al. 1997).

Gracilaria lemaneiformis, an important macroalgae used for sea-vegetable foods and forage, has been used as the biofilters of nutrients because of its high efficiencies in removing inorganic nitrogenous compounds (Fei 1999). It is able to absorb and metabolize different forms of inorganic nutrient, mainly N and P. The macronutrients input into the coastal environments is often accompanied by heavy metal pollutants. However, physiological change of *G. lemaneiformis* in response to the increasing heavy metal remains essentially unknown.

In the present study, *G. lemaneiformis* was cultured in different heavy metal (Cu and Cd) concentration to be assessed the effects of heavy metal on its growth, photosynthesis and pigment content.

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MATERIALS AND METHODS

The red alga *Gracilaria lemaneiformis* (Rhodophyte) was collected from Nanao Island, Shantou, China (23°20' N, 116°55' E). Upon arrival in the laboratory, the seaweed was thoroughly cleaned and then maintained in the aerated seawater (31 ‰ in salinity) enriched with f/2 medium (Mclachlan, 1973) without trace metals and EDTA in the aquarium at 50 μmol m^{-2} s^{-1}(LD cycle 12:12h) and 20 °C. After acclimation for 4 days, healthy samples (0.4-0.8g fresh weight) were cultured in 500ml medium as described above. Heavy metal were added and the nominal concentrations were 0, 2, 5, 10μM for Cu, and 0, 50, 100, 200μM for Cd. All experiments were carried out with three replications. After 96hr exposure to copper and cadmium, the seaweed materials were harvested. Chlorophyll a (Chl a), carotenoids (Car), phycoerythrin (R-PE) and phycocyanin (R-PC) were determined according to Evans (1988). Photosynthetic oxygen evolution was measured by using an oxygen monitor (YSI 5300, U.S.A) with a Clark-type oxygen electrode. The temperature was maintained at 20 °C and illumination was provided by a halogen lamp at PAR of 400 μmol$m^{-2}s^{-1}$. Biomass (fresh weight) was determined by weighing the alga after blotting by absorbent paper. The relative growth rate (R) was calculated by using the following formula:

$R = (\ln FW_t - \ln FW_o)/t,$

where FW_t is the fresh weight of samples at t time, FW_o is the original fresh weight of samples. The results were analyzed according to one-way ANOVA followed by Least Significance Difference test. The confidence level was set at 5%.

RESULTS AND DISCUSSION

G. lemaneiformis showed significantly limited growth when exposed to Cd concentrations from 100 to 200μM for 96hr ($P<0.05$). The relative growth rate in 50, 100 and 200μM Cd-treatments decreased by 7.0%, 46.3% and 89.4% compared to the control treatment (Fig. 1). Fig. 2 showed that Cu exposure also induced marked growth depression; the relative growth rate after 96hr Cu exposure from 2 to 10μM declined by 35.1%, 71.9% and 71.2% in contrast to the control treatment ($P<0.05$). The reduction in growth could be a consequence of heavy metal interference with a number of metabolic processes associated with normal development (Van Assche and Clijsters 1990; Alia and Saradhi 1991). The present study demonstrated that Cu appeared to be more toxic to *G. lemaneiformis* than Cd, as earlier reported by Lyngby and Brix (1984) and Malea (1994). Copper is an essential element for metabolic processes and actively taken up by the plants, however, when exposed to increasing concentration, uptake may exceed metabolic requirements and resulted in a toxic impact. On the other hand, Cd is a non-essential element for growth, the plant may actively exclude or sequester such metal to minimize the metal toxic impact. Similarly, It had been reported that *Halophila stipulacea* had a lower uptake concentration of metals which do not participated in physiological process (Malea 1994). In addition, the

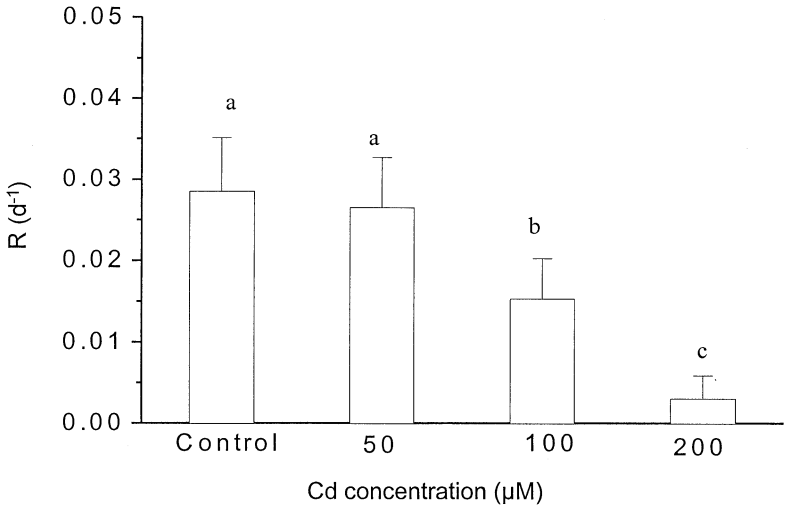

Figure 1. Effects of Cd concentration on growth of *G. lemaneiformis*. Values are means ± SD (n=3). Those with different superscript are significantly different ($P<0.05$).

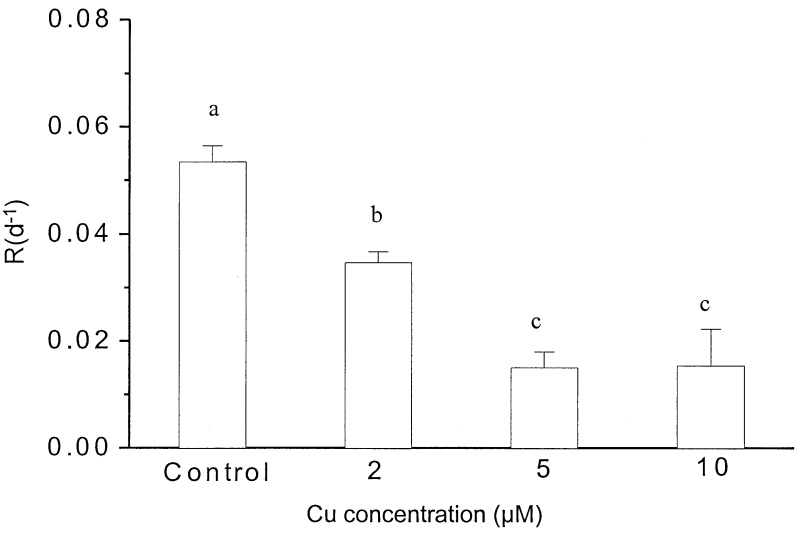

Figure 2. Effects of Cu concentration on growth of *G. lemaneiformis*. Values are means ± SD (n=3). Those with different superscript are significantly different ($P<0.05$).

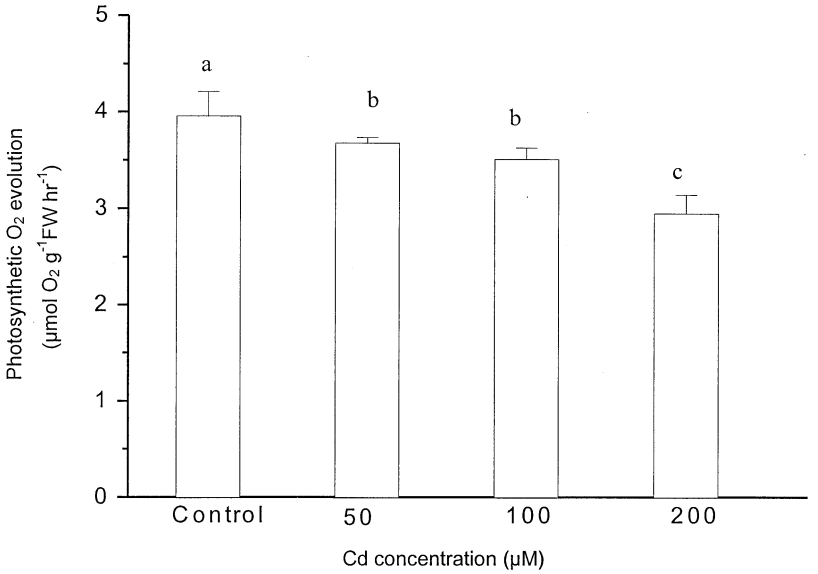

Figure 3. Effects of Cd concentration on photosynthetic O_2 evolution in *G. lemaneiformis*. Values are means±SD (n=3). Those with different superscript are significantly different ($P<0.05$).

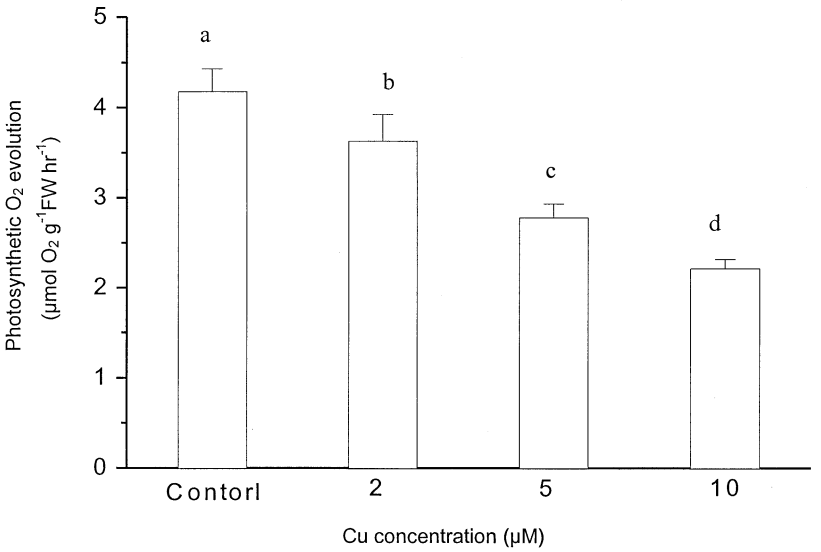

Figure 4. Effects of Cu concentration on photosynthetic O_2 evolution in *G. lemaneiformis*. Values are means±SD (n=3). Those with different superscript are significantly different ($P<0.05$).

Table 1. Pigment content (mg/g FW) in *G. lemaneiformis* when exposed to elevated Cd concentrations for 96hr. Data are means ± SD (n=3).

	Chl a	Car	R-PC	R-PE
Control	0.15 ± 0.02^{a}	0.04 ± 0.01^{a}	0.15 ± 0.03^{a}	1.04 ± 0.02^{a}
50μM	0.13 ± 0.02^{a}	0.03 ± 0.01^{ab}	0.08 ± 0.02^{b}	1.12 ± 0.19^{a}
100μM	0.13 ± 0.01^{a}	0.04 ± 0.00^{a}	0.02 ± 0.01^{c}	0.68 ± 0.22^{b}
200μM	0.09 ± 0.01^{b}	0.02 ± 0.00^{b}	0.02 ± 0.01^{c}	0.49 ± 0.08^{b}

Those with different superscript are significantly different ($P<0.05$).

Table 2. Pigment content (mg/g FW) in *G. lemaneiformis* when exposed to elevated Cu concentrations for 96hr. Data are means ± SD (n=3).

	Chl a	Car	R-PC	R-PE
Control	0.26 ± 0.02^{a}	0.06 ± 0.01^{a}	0.15 ± 0.01^{a}	1.30 ± 0.11^{a}
2μM	0.26 ± 0.05^{a}	0.07 ± 0.01^{a}	0.12 ± 0.03^{ab}	1.24 ± 0.25^{a}
5μM	0.27 ± 0.03^{a}	0.06 ± 0.01^{a}	0.11 ± 0.03^{ab}	1.31 ± 0.11^{a}
10μM	0.24 ± 0.02^{a}	0.06 ± 0.01^{a}	0.08 ± 0.02^{b}	0.84 ± 0.09^{b}

Those with different superscript are significantly different ($P<0.05$).

lower solubilities of Cd may be lead to greater chelation and adsorption on particles in the culture medium. This could also help to explain the greater toxicity in Cu treatment than in Cd treatment.

Cd treatment from 50 to 200 μM had a significant effect on photosynthesis of *G. lemaneiformis* samples relative to the control ($P<0.05$), and exposure to Cd concentrations from 50 to 200μM resulted in the reduction in photosynthetic O_2 evolution by 7.0%, 11.1% and 25.2%, respectively, with respect to the control response(Fig.3). The photosynthetic O_2 evolution with the elevated Cu concentration from 2 to 10 μM had an apparent decline ($P<0.05$), reaching up to 86.9%, 66.5% and 52.9% of that of the control treatment (Fig. 4). It was generally accepted that high heavy metal concentration led to photosynthetic depression (Greger and Ögren 1991; Yruela et al. 2000). Cu had a regulatory role in photosynthetic electron transport as part of polypeptides involved in electron transport, or as a stabilizer of lipid environment close to electron carriers of PSII complex (Maksymiec 1997; Barón et al. 1995). Pätsikka et al (2001) determined that the excess Cu damaged the proteins of the oxygen-evolving complex at the donor side of PSII, causing rapid loss of PSII activity in the light. Cd damaged the photosynthetic apparatus, in particular the light harvesting complex II (Krupa 1988), the photosystem II and I (Siedlecka and Baszynsky 1993; Siedlecka and Krupa 1996).

Effects of Cd and Cu on pigment content of *G. lemaneiformis* after 96hr exposure

are shown in Tables.1 and 2, and a significant decline in Chl a and Car content was observed when *G. lemaneiformis* was exposed to 200μM Cd concentration ($P<0.05$). In constrast, Chl a and Car content remained relatively stable with the increasing Cu concentration (2-10μM).

Cd and Cu treatment induced a marked decrease in R-PC content. R-PC content decreased by 51.0%, 89.9% and 90% in exposure from 50 to 200μM Cd, 23.7%, 29.0% and 50.5% in exposure from 2 to 10μM Cu, respectively, in comparison with the control treatment (Tables.1 and 2). *G. lemaneiformis* when exposed to 100 and 200μM Cd concentration maintained a lower R-PE content. 2 and 5μM Cu had a insignificant affect on R-PE content in *G. lemaneiformis*, whereas 10μM Cu concentration led to a sharply decline in R-PE content ($P<0.05$). Ouzounidou (1993) reported that the chlorophyll content decreased at elevated Cd levels. The reduced chlorophyll content in Cd treated samples had been found to be associated with Mg and Fe deficiency in the process of chlorophyll biosynthesis (Greger and Ogren 1991). Cd had also been shown to inhibit the chlorophyll synthesis by affecting the activity of photochlorophyllide reductase (Stobart et al. 1985). Chl a maintained stable in exposure from 2 to 10μM Cu concentration, which may be due to the formation of Cu-substituted chlorophyll (Prasad et al. 2001; Küpper et al. 1996). In this study Cu and Cd treatments had a more apparent effects on R-PC and R-PE than Chl a and Car, which indicated that R-PC and R-PE were more sensitive to Cu or Cd treatment. R-PC and R-PE located in phycobilisomes were the main accessory pigments in the red algae. They transferred light to reaction center of photosystem I and II by overlapping their respective absorption and fluorescence spectra between bilipigments and chlorophyll a at the reaction center (Rodrigo and Robaina 1997). A decrease of R-PC and R-PE content indicated that the phycobilisomes could have been damaged in high Cu and Cd concentration, which resulted in a decrease of light energy absorbed by phycobilisomes. This could also explain the photosynthetic inhibition in high Cu and Cd concentrations.

Our results provided additional evidence that Cu was more toxic to *G. lemaneiformis* than Cd, and R-PE and R-PC were more sensitive to Cu and Cd than Chl a and Car. The phycobilisome damage under high Cu and Cd concentration resulted in a decline of light energy absorbed to inhibit photosynthesis. Further works would be needed to determine how Cu and Cd affected the phycobilisomes.

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