# The effect of copper on the daily growth rate and photosynthetic efficiency of the brown macroalga *Padina boergesenii*

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## ABSTRACT

The effect of copper (Cu) on the daily growth rate (DGR) and photosynthetic efficiency (PE) of the brown macroalga *Padina boergesenii* was studied under controlled laboratory conditions. The DGR was measured as an increase in fresh weight while the PE was measured as the ratio of variable to maximal chlorophyll fluorescence ($F_v/F_m$). *Padina boegesenii* were exposed to 25, 50, 100 and 500µg Cu/l for 21 days. The DGR and PE of *P. boergesenii* decreased significantly with an increase in copper concentration in the growth media. This study shows that copper is acutely toxic to *P. boergesenii* at concentrations higher than 500µg Cu/l. Low concentration of copper is toxic to *P. boergesenii* after a prolonged exposure of 21 days. Similarly, exposure time has a significant negative effect on both DGR and PE.

## INTRODUCTION

The effect of heavy metals on aquatic organisms is currently attracting wide attention, particularly in studies related to industrial and anthropogenic pollution. The enrichment of coastal waters with trace metals through sewage and other anthropogenic sources has become a severe problem. This situation has resulted in numerous studies of the effects of heavy metals on benthic marine algae, especially in coastal areas (e.g. Strömgren, 1979a,b, 1980; Markham et al., 1980; Filho et al*.,* 1996). Accumulation of heavy metals in marine environments has been extensively studied using marine macroalgae due to their ability to concentrate and tolerate high metal levels. Marine macroalgae have been shown to be good bioindicators of heavy metal contamination in seawater (Bryan, 1983; Soderlund et al*.,* 1988). There has recently been an increase in the use of marine algae as test organisms in laboratory studies of marine pollution (Fletcher, 1991). Most of these experimental studies under controlled conditions have been conducted on species from temperate regions (Markham et al., 1980; Anderson and Hunt, 1988; Pellegrini et al*.,* 1993) and data on tropical species is limited.

The use of copper in antifouling paints, in the treatment of diseases of fishes, in agricultural chemicals, and as algalcides has increased the need to study the effects of this metal on aquatic organisms (Clark, 1986). The most extensive research on the effects of copper has been directed to fishes, because of their economic value, and few studies exist on the effect of copper on macroalgae.

## MATERIALS AND METHODS

### Study site

The brown macroalgae *Padina boergesenii* used in this experiment were collected at Bawe Island, 6km west of Zanzibar town. Water currents from the coastal area of Zanzibar town flows in a northerly direction and this may therefore prevent pollutants from reaching Bawe Island. Experimental work was conducted at the Institute of Marine Sciences, Zanzibar.

### Experimental setup

The experiments were conducted indoors. All glassware used during the experiment was washed and soaked overnight in 1N $HNO_{3,}$ then soaked overnight in de-ionised water. Seaweeds were cultivated in natural filtered seawater that was changed twice a week. Algae with known weight (about 4g/l) were cultivated in 2-litre glass beakers. Fluorescent tubes were used to provide extra light. Algae were acclimatised for 24 hours before heavy metal exposure. Each flask was aerated with ambient air to provide water movement. The culturing vessels were covered with a cellulose membrane in order to avoid extended evaporation and entrance of foreign particles. Temperature, pH and salinity was measured using a thermometer; a pH meter and refractometer respectively.

Toxicity of copper was tested using six replicates, with each set-up having a corresponding control. Each set-up had a control with no copper, 25, 50, 100 and 500 µg Cu/l. During selection of suitable exposure concentrations before the start of the experiments algae were subjected to higher concentrations of copper (1000µg Cu/l), but all the algal thalli died after a few days of exposure. During days 4, 7, 14 and 21 the photosynthetic efficiencies and daily growth rates of experimental algae were measured.

### Determination of daily growth rate (DGR) of *Padina boergesenii*

The algae were carefully removed from the flasks using a piece of soft plastic-lined forceps and the excess water was blotted on filter paper. The macroalgal DGRs were expressed as a percentage increase in fresh weight and were calculated according to the equation $DGR = 100[(W_t/W_o)^{1/t}–1]$ (Lignell et al*.,* 1987; Huglund et al.,1996) where $W_o$ is the initial biomass and $W_t$ is the biomass at day t. The DGR was expressed as the percentage of the growth rate recorded from control algae.

### Determination of photosynthetic efficiency (PE)

The ratio of variable chlorophyll fluorescence ($F_v$) to maximal chlorophyll fluorescence ($F_m$) is proportional to the quantum yield of photochemistry and gives an indication of the efficiency of the photosynthetic apparatus of macroalgae (Mtolera, 1996). The higher the value of $F_v$ / $F_m$ the higher the photosynthetic efficiency. A reduction in $F_v$ / $F_m$ indicates a decreased efficiency of the Photo system II (PS II). Such a situation may be induced by several factors including herbicides and pollutants, such as heavy metals like copper.

In this study PE was measured on algae exposed to heavy metals using the Hanstech's Plant Efficiency Analyser (PEA) (Great Britain) after 4, 7, 14 and 21 days. A piece of the alga thallus was attached on the leaf holder of the Plant Efficiency Analyser equipment and subjected to dark conditions for about 10 minutes. Thereafter its fluorescence characteristics were measured. This was repeated six times for each treatment concentration.

### Statistical tests

A factorial design (two way ANOVA with replication) according to Zar (1984) was used to test whether there were any significant effects of copper concentration and days of exposure on the DGR and PE of *P. boergesenii.* It was also used to test if concentration of copper had an interaction with days of exposure in affecting the DGR and PE of *P. boergesenii*.

## RESULTS

Salinity, pH and temperature ranged from 35–37‰ , 7.97–8.34 and 25–27°C respectively.

### Daily growth rate (DGR) of *Padina boergesenii* exposed to copper

Daily growth rates as percentage per day results are presented in Figure 1. Generally the DGRs decreased significantly ($F_{(4, 40)}$=28.73; p<0.000) with an increase in copper concentration in the growth media. As number of days of exposure increased the inhibition in DGR also increased significantly ($F_{(3,120)}$=60.83; p<0.000). There was also a significant interaction ($F_{(12, 120)}$=3.82, p<0.001) between copper concentration and algal exposure time in decreasing the DGR of *P. boergesenii*.

Table 1 shows the statistical results of comparisons of mean daily growth rates (DGRs) of different concentrations. The highest mean DGR occurred during day 4 for algal thalli that were exposed to 25µg Cu/l, which corresponds to a growth rate of 119.3±19.2% of the control. The lowest daily growth rate occurred after 21 days of exposure to 500µg/l of copper corresponding to 23.4±0.7% of control. For algal thalli exposed to 500µg/l of copper the DGR was highly inhibited.

Other changes were observed at 100 and 500µg Cu/l, where the culturing media turned light yellow from colourless. Thalli treated with 500µg Cu/l started to shed their fine hairy structures situated at their undersides immediately after four days of exposure.

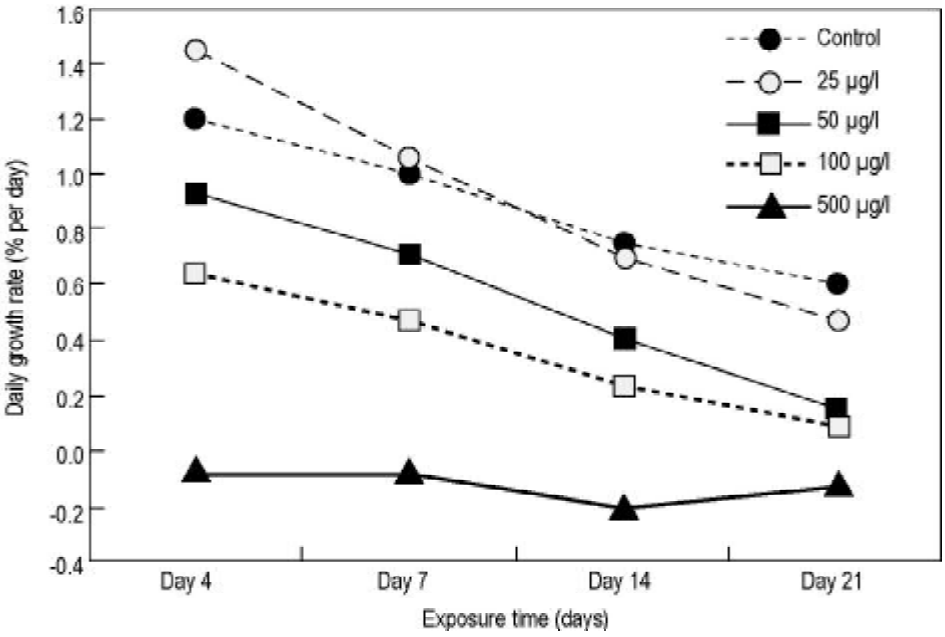



Figure 1. Daily growth rate of *Padina boergesenii* exposed to different concentrations of copper for 21 days

**Table 1. Results of (SNK/Tukey) multiple comparison test for similarity of means for PE and DGR of *Padina boergesenii* between different concentrations of copper**

| | | Control | 25 | 50 | 100 | 500 |
|---|---|---|---|---|---|---|
| Concentration (µg Cu/l) | Mean PE | 0.750 | 0.713 | 0.670 | 0.603 | 0.429 |
| | Mean DGR | 0.892 | 0.922 | 0.553 | 0.361 | -0.127 |
| Control | PE | | *** | *** | *** | *** |
| | DGR | | ** | *** | *** | *** |
| 25 | PE | | | n.s. | *** | *** |
| | DGR | | | *** | *** | *** |
| 50 | PE | | | | *** | *** |
| | DGR | | | | ** | ** |
| 100 | PE | | | | | n.s. |
| | DGR | | | | | *** |
| 500 | PE | | | | | |
| | DGR | | | | | |

n.s. =non significant; *=significant p<0.01; **highly significant at p<0.001; ***very highly significant at p<0.0001.

In addition, the thin outer layer of the thallus started to wear off at the start of the third week of exposure.

**Photosynthetic efficiency (PE) of copper-exposed *Padina boergesenii***

Results of the effect of copper on the PE of *P. boergesenii* are presented as the mean values of $F_v/F_m$ in Figure 2. It was observed that both concentration and exposure time had a significant ($F_{(4, 40)}$=28.73; p=<0.000 and $F_{(3, 120)}$=62.85; p<0.000 respectively) effect in inhibiting the photosynthetic efficiency. There was also a significant interaction ($F_{(12, 120)}$ =6.01; p<0.000) between copper concentration and exposure time in inhibiting the photosynthetic efficiency of *P. boergesenii*. The mean PE for the controls was markedly stable throughout the experiment. In the presence of 25µg/l copper during day four the mean PE was the highest, equivalent to 105.0±1.5% of the control.

Table 1 also shows the results of statistical comparison test of mean PE between different treatments (concentrations) of copper. The lowest photosynthetic efficiencies were recorded in plants that were exposed to 500µg/l of copper (Figure 2) equivalent to 46.5±2.1% of the control. Nevertheless the mean photosynthetic efficiencies of every treatment (exposure concentrations) were significantly different from each other, (p<0.05).

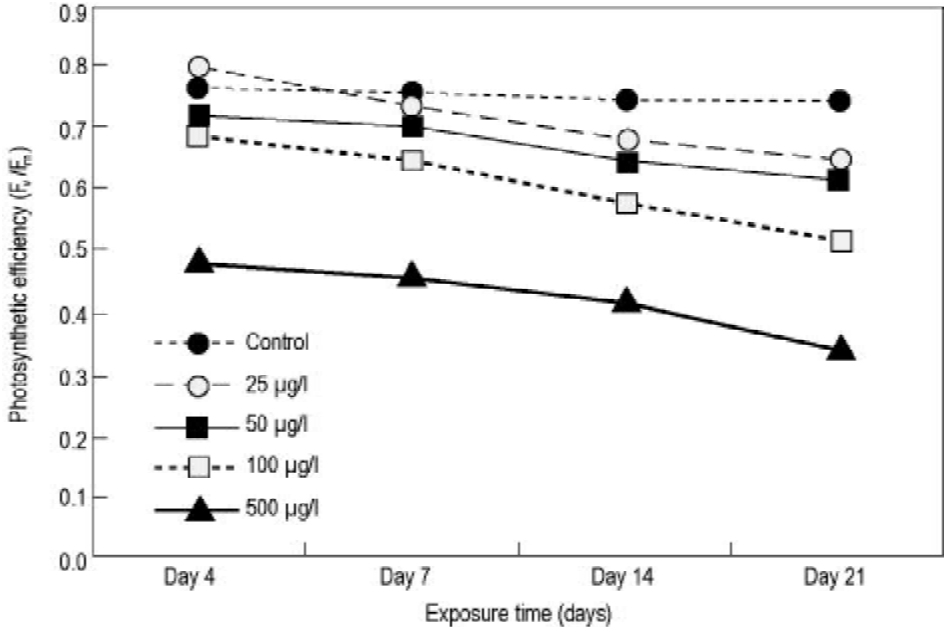



Figure 2. Photosynthetic efficiency of *Padina boergesenii* exposed to different concentrations of copper for 21 days

## DISCUSSION

This study shows that the daily growth rate and photosynthetic efficiency of copper were reduced with an increase in copper concentration in the growth media (Figure 1 and 2). Similarly, the DGR and PE decreased with increase in exposure time (Figure 1

and 2). Probably accumulation of copper, which was taken up by the algal tissue, was responsible for the observed inhibition of both DGR and PE.

Concentration of copper in the growth media was directly proportional to the inhibition of DGR and PE of *P. boergesenii*. The mechanism of copper toxicity in this experiment was not studied, but Stauber and Florence (1987) pointed out that copper may interfere with cell permeability or the binding of essential metals. Following copper transport into the cytosol, copper may react with SH enzyme groups and free thiols (e.g. glutathione), disrupting enzyme active sites and cell division hence reducing growth. Copper may also exert its toxicity in subcellular organelles, interfering with mitochondrial electron transport, respiration, ATP production and photosynthesis in the chloroplasts. Copper ions are also likely to be transported to chloroplasts via cytoplasm where it inhibits photosynthesis by uncoupling electron transport to $NADP^+$. Sorentino (1979) again mentioned that when ionic concentration increases, copper is bound to chloroplast membranes and other cell proteins, causing degradation of chlorophyll and other pigments. At higher concentrations copper produces irreversible damage to chloroplast lamellae preventing photosynthesis and may eventually cause plant death. Copper ions are also known to affect the permeability of the plasmalemma, which results in the loss of potassium ions from the cell (McBrien and Hassall, 1967; O Kelly, 1974).

The highest daily growth rate and photosynthetic efficiency were expected to occur in controls, as no copper was added. However, it was observed that algae that were subjected to low concentrations of copper (25µg Cu/l) exhibited higher daily growth rate and photosynthetic efficiency than those of the control group, especially during the first earlier 4 days of exposure (Figure 1 and 2). Copper acts as a micronutrient when present at low concentrations (Sorentino, 1979) enhancing the growth of the algae. Brand et al. (1986) and Verweij et al. (1992) observed better growth of phytoplankton in the presence of low copper concentrations. It has also been observed by Strömgren (1979a) that the brown alga, *Ascophyllum nodosum* was unaffected by copper at concentrations of 33µg/l or less for 11 days of exposure.

Differing copper concentrations have been shown to affect growth and photosynthesis process. For example, at 28.58µg/l of copper the growth of the cyanophyte *Lyngbya nigra* was inhibited, however photosynthesis and respiration were inhibited severely at 44.8µg/l of copper (Gupta and Arora, 1978). This situation may explain what has been observed in this experiment, whereby growth rate was more inhibited compared to photosynthetic efficiency.

The observed colour change of the growth media at higher concentrations of copper might be due to loss of some pigments by the algae. A similar phenomenon has been reported by Gross et al. (1970) to occur in a fresh water green alga *Chlorella*. He observed the loss of photosynthetic colour pigments by this alga when exposed to excessive concentration of copper. Markham et al. (1980) reported significant loss of pigments from *Asteriolla formosa* when cadmium was added to the media. No other specific study has been done using *Padina boergesenii* to support the observed results.

## CONCLUSION

Copper is acutely toxic to the macroalga *Padina boergesenii* at high concentrations, affecting both algal growth and photosynthesis. When *P. boergesenii* is exposed to low concentrations of copper for a long time it may exhibit toxic effects, hence inhibiting photosynthetic activity and growth. At very low concentration copper acts as a micro-nutrient, favouring some physiological activities of the algae. It is concluded that the degree of toxicity depends on both the concentration and exposure time.

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