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Long term effects of copper upon physiological processes and growth of Chlorella saccharophila (Krüger) Migula and Cyanidium caldarium Geitler

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ABSTRACT. — It has been studied long term effects of copper upon physiological processes and growth of *Chlorella saccharophila* (Krüger) Migula and *Cyanidium caldarium* Geitler, two algae which have the optimum of pH at 6,3 and 1,5 respectively. The algae differed markedly in their resistance to the copper. Infact, growth of *Chlorella saccharophila* showed a lag time in presence of sublethal copper concentrations (0,25-1 mg/l). By contrast, *Cyanidium caldarium* was about 4000 fold more tolerant to the metal. In both algae photosynthesis was more sensitive to applied copper than was respiration.

INTRODUCTION

Copper is essential to all organisms as a constituent of many metallo-enzymes, mostly those concerned with oxidation, but it is a poison when present in excess (Bowen, 1966). Since toxic effects may be seen at concentrations below 1 ppm in the nutrient solution, copper salts are widely used in the control of microorganisms. The mode of action of copper is not strictly specific and is still the object of discussion (Gadd and Griffiths, 1978; Samuellsson and Oquist, 1980). Inhibition of many enzymes and physiological processes such as photosynthesis and respiration have been recognized mostly during experiments of short duration (McBrien and Hassal, 1967; Overnell, 1975). The present paper deals with long term effects of copper upon photosynthesis, respiration and growth of *Chlorella saccharophila* (Krüger) Migula and *Cyanidium caldarium* Geitler, two algae which have optimum growth rates at different pH's: 6,3 and 1,5 respectively. Moreover, *Cyanidium caldarium* Geitler lives in acidic springs which often carry high levels of heavy metals (White *et al.*, 1963).

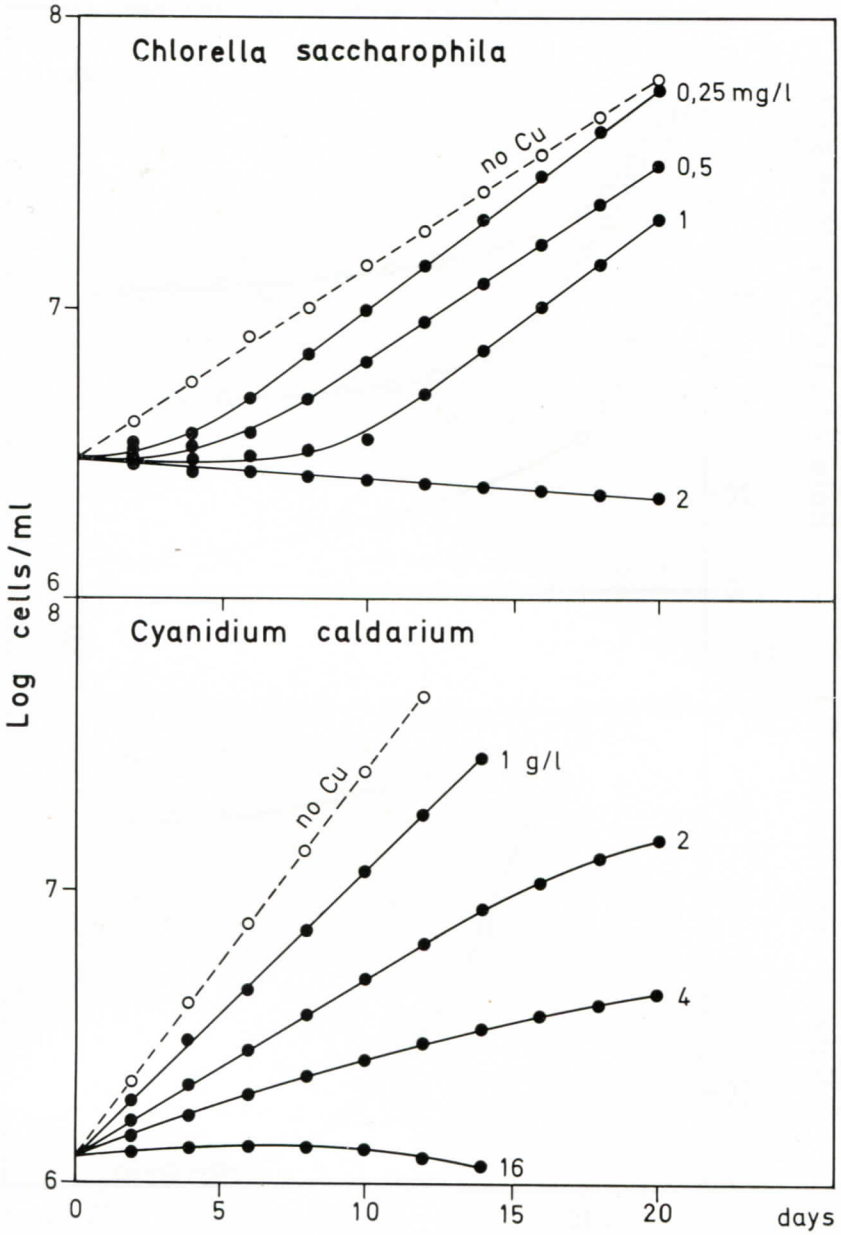

Fig. 1 — Growth rates of *Chlorella saccharophila* and *Cyanidium caldarium* as a function of time at various concentrations of copper. The figures on the curves denote copper concentration.

— Velocità di crescita di *Chlorella saccharophila* e *Cyanidium caldarium* in funzione del tempo alle concentrazioni di rame riportate in figura.

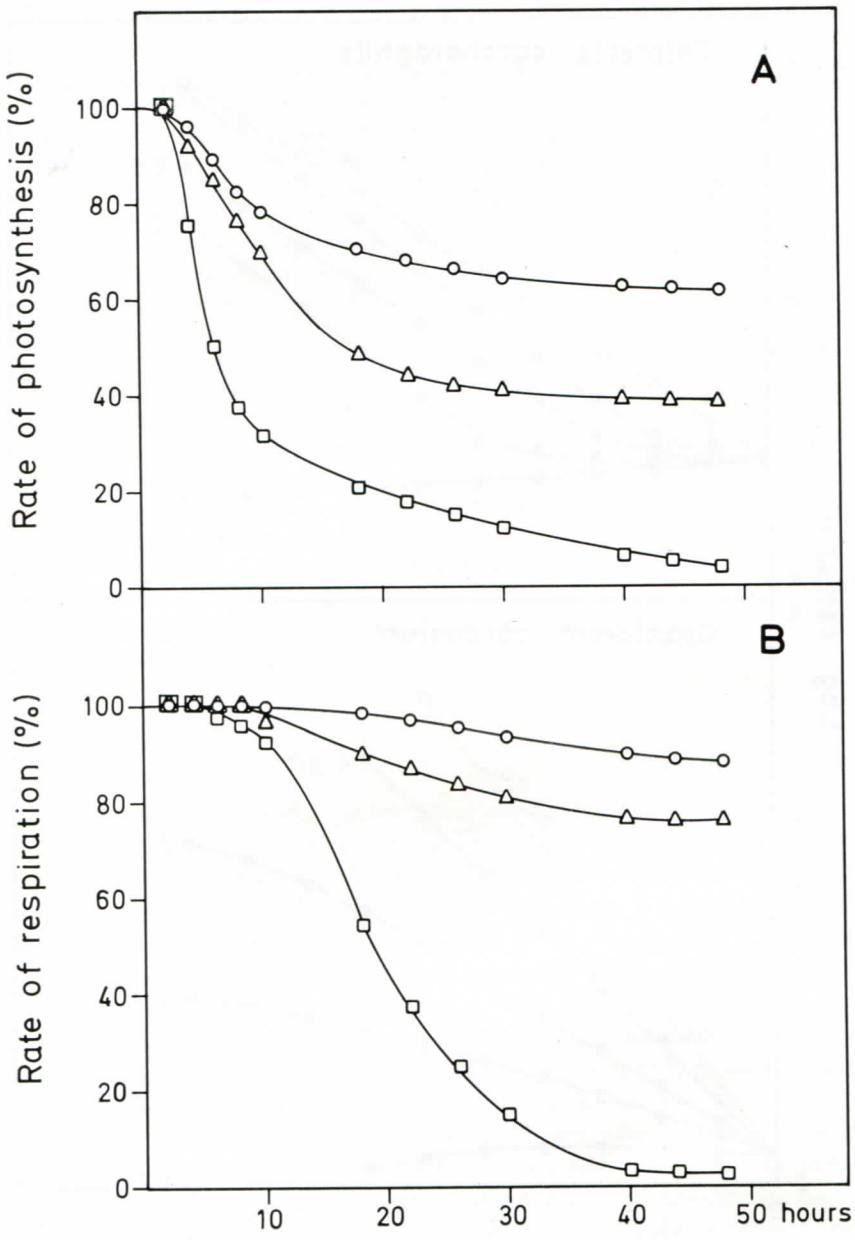

Fig. 2 — *Chlorella saccharophila.* Rates of photosynthesis (A) and respiration (B) in the medium as function of time at various level of added copper salt: ○—○ 0,5 mg/l; △—△ 1 mg/l; □—□ 2 mg/l. Ordinates: rates in per cent of that in Cu-free medium.

— *Chlorella saccharophila.* Velocità di fotosintesi (A) e respirazione (B) nel terreno di coltura in funzione del tempo a varie concentrazioni di rame: ○—○ 0,5 mg/l; △—△ 1 mg/l; □—□ 2 mg/l. Ordinate: velocità espresse in per cento di quelle ottenute nel terreno senza rame.

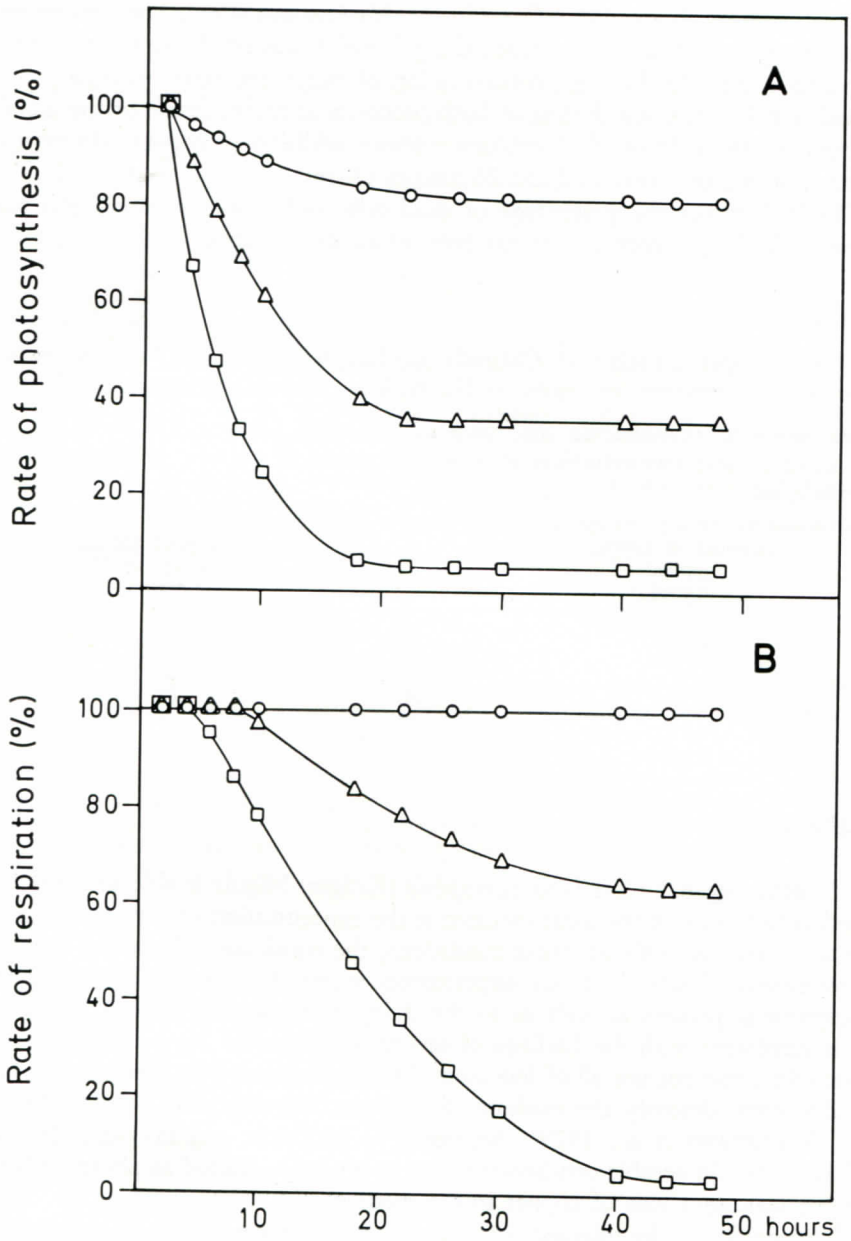

Fig. 3 — *Cyanidium caldarium*. Rates of photosynthesis (A) and respiration (B) in the medium as function of time at various level of added copper salt: ○—○ 1 g/l; △—△ 4 g/l; □—□ 16 g/l. Ordinates in per cent of that in Cu-free medium.

— *Cyanidium caldarium*. Velocità di fotosintesi (A) e respirazione (B) nel terreno di coltura in funzione del tempo a varie concentrazioni di rame: ○—○ 1 g/l; △—△ 4 g/l; □—□ 16 g/l. Ordinate: velocità espresse in per cento di quelle ottenute nel terreno senza rame.

The photosynthesis rate of *Cyanidium caldarium* was affected only when concentration of copper in the medium reached 1 g/l, which was similar to the concentration of hydrogen ions. At the same concentration of metal, the respiration rate was not affected. Finally, constant decline of both processes at higher levels of the metal was observed. In the culture of *Cyanidium* without addition of copper, photosynthesis and respiration rates were 172 and 26 nmoles O_2/min/10^8 cells respectively.

Table 1 shows the percentage of dead cells and copper sorption of *Chlorella saccharophila* in presence of various concentrations of copper in the medium.

TABLE 1

Dead cells copper sorption of Chlorella saccharophila after 48 hours of exposition to various concentrations of copper in the medium. The system contained 10^7 cells/ml.

Cellule morte e assorbimento del rame di Chlorella saccharophila dopo 48 ore di esposizione a varie concentrazioni di rame nel mezzo di coltura. Il sistema conteneva 10^7 cellule/ml.

Amount of copper applied µg/ml	% dead cells in the system	% copper applied taken up by the algae
0,5	15	70
1	20	42
2	40	25
10	85	4

DISCUSSION

As other algae, *Chlorella saccharophila* (Krüger) Migula is able to re-establish a normal growth rate in the same medium if the concentration of copper added to the medium is not too high. In these conditions, the continuous decline of the rate of photosynthesis observed in our experiments seems due to the inhibition of the photosynthetic process as well as to the dying of some cells (Fig. 1 and Tab. 1). This is consistent with the findings of several authors who have demonstrated that copper kills some but not all of inoculum. Dead cells, absorbing copper more rapidly then live ones, detoxify the medium (STEEMANN NIELSEN and WIUM ANDERSEN, 1970; ALBERTANO *et al.*, 1979). According to MCBRIEN and HASSAL (1967), the respiration rate in aerobic conditions is not as severely affected as photosynthesis by the same concentrations of copper in the medium.

Noteworthy is the tolerance of *Cyanidium caldarium* Geitler to very high concentrations of copper in the medium, in spite of the fact that low pH increases the availability of metal ions (GADD and GRIFFITHS, 1978).

As matter of fact, pH can have a considerable effect on both availability and toxicity of heavy metals. We are further investigating whether tolerance to heavy metals is a general feature of acid-tolerant species.

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RIASSUNTO

Effetti a lungo termine del rame sui processi fisiologici e sull'accrescimento di Chlorella saccharophila (Krüger) Migula e Cyanidium caldarium Geitler. - Sono stati studiati gli effetti a lungo termine del rame sui processi fisiologici e sull'accrescimento di *Chlorella saccharophila* (Krüger) Migula e *Cyanidium caldarium* Geitler, due alghe il cui optimum di crescita è a pH 6,3 e 1,5 rispettivamente. Le alghe differiscono notevolmente nella loro resistenza al rame. Infatti, concentrazioni subletali (0,25-1 mg/l) di rame determinano una lag-fase nella crescita di *Chlorella saccharophila.* Sono necessarie, invece, concentrazioni 4000 volte maggiori del metallo per diminuire la crescita di *Cyanidium caldarium.* In entrambe le alghe la fotosintesi si è rivelata più sensibile al rame della respirazione.