EFFECT OF Cu^{2+} ON THE RED MICROALGA *RHODELLA RETICULATA*

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Summary. The effect of different Cu^{2+} concentrations on the red microalga *Rhodela reticulata* was studied. *Rhodella* cells cultivated in Cu^{+2} containing media exhibited ability to uptake and accumulate the heavy metal from the suspension. The analyses performed by ICP-AES demonstrated that Cu^{+2} biosorption increased with the increase of the heavy metal concentration in cultivation media. Cu^{+2} had inhibitory effect on algal cells in the order: 0.25> 0.025> 0.0025> 0.00025 mg/ 100ml. Cu^{+2} was toxic for *Rhodella* cells at concentrations up to 0.0025mg/ 100ml as judged by the decrease in growth rate expressed as changes in dry weight and polysaccharide production. Synthesis of the photosynthetic pigments was found to decrease or even was inhibited by the higher amounts of Cu^{+2}. Phycobiliproteins (C phycocyanin and B phycoerytrin) were affected to a lesser extent compared to chlorophyll ”a” and carotenoid contents. Lower Cu^{+2} concentrations influenced positively the growth and photosynthesis of algal cells, which was confirmed by the increase in red and green pigments. It is suggested that polysaccharides produced by algal cells as well as the metabolic activity of bacteria contaminating the non-sterile algal culture resulted in Cu^{+2} immobilization and detoxification.

***Keywords*:** copper influence, *Rhodella reticulata,* biosorption, polysaccharides.

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INTRODUCTION

Copper (Cu^{2+}) is an essential biological element which interferes with numerous physiological processes as a component of different enzymes, taking part in electron flow and redox reactions in plant cell ingredients: cell wall, cytoplasm, mitochondria, chloroplasts (Lolkemia and Vooijs, 1986). Latest investigations demonstrate that cyanobacteria (*Synechocystis*) have metal requirements resulting in Cu^{2+} trafficking to the thylakoids (Cavet et al., 2003). However, it was found, that excessive Cu^{2+} concentration has harmful effects on growth and photosynthesis, destroying the photosynthetic apparatus and plasma membrane permeability of plants (Ouzounidou, 1993; 1994). It was reported that higher copper concentrations are able to inhibit some sulfhydryl – dependent enzymes (Silverberg et al., 1976).

Copper is among the persistent and ubiquitous environmental pollutants, introduced into the environments through anthropogenic and industrial activities. Cu^{2+} pollution of marine aquatic environments occurred as a result of copper use in antifouling paint for ships hulls as an algicide and wood preservatives (Clark, 1992). Algae in aquatic ecosystems are continuously exposed to toxic metals influence. The toxicity of Cu^{2+} to different algal types varies (Fitzgerald and Faust, 1963). Some algal species demonstrated tolerance to different heavy metals possessing mechanisms to prevent their toxic effects on metabolism or damage of intracellular structures. Green algae exposed experimentally to Cu^{+2} bind the heavy metal by protein ligands and sequestered it in the form of intranuclear inclusions (Silverberg et al., 1976), while blue green algae sequester copper ions via synthesis of phytochelation complexes (Gekeler, 1988).

Cell wall constituents have been implicated as being responsible for metal binding in interaction of heavy metals with the algal cells in aqueous solutions where both electrostatic attraction and the formation of complexes play a role (Shiewer and Volesky, 2000).

The dominant role of some algal polysaccharides (carrageenan) in accumulation of metals was proved in microalgae and is considered to be correlated with the ability of the polysaccharides to form gels (Pengfu et al., 2001); the ion exchange properties of the sulfated polysaccharides (Schiewer and Volesky 1995); the degree of sulfatation of the carageenan molecule (Veroy et al., 1980), and the presence of alginate uronic, glucuronic and galacturonic acids (Strong et al., 1982; Bender et al., 1994; Davis et al., 2003).

Considerable information on specific interactions between different green, blue-green algae, and copper exists but only limited data are available for the heavy metal effect on red algae (Pasher et al., 2004).

The aim of this study was to assess the effects of Cu^{2+} toxicity on growth, photosynthesis, polysaccharide production and viability of the red microalgae *Rhodella reticulata*, representatives of Rhodophyta.

MATERIALS AND METHODS

The non – axenic culture of the red alga *Rhodella reticulata* (Rhodophyta, strain UTEX LB2320 from the algal collection of the Department of Botany, University of Austin, Texas, USA) was used in the experiments. *Rh. reticulata* was intensively cultivated for 96 h in Brody Emerson modified medium at light intensity of 260 $\mu Em^{-2}s^{-1}$ and temperature 27 ºC. Suspension was aerated with 1% CO_2 as described by Georgiev et al. (1987). Influence of increasing Cu^{+2} concentrations ($CuSO_4$ 10^{-6}, $CuSO_4 10^{-5}$; $CuSO_4 10^{-4}$, $CuSO_4 10^{-3}$ respectively Cu^{2+}: 0.000025; 0.00025; 0.0025; 0.025 mg L^{-1}) on microalgal growth was monitored for 96 hours and expressed as changes in dry weight, polysaccharide production, and pigment contents. Pigments (chlorophyll"a" and carotenoids) were extracted with methanol and their absorbance spectra were determined according to the McKinney method. Phycobiliproteins (phycoerythrin, alo and c- phycocyanin) were determined spectrophotometrically (Spectrophotometer Carl Zeiss Iena, Germany) according to Pekarkova et al. (1988). Accumulated extracellular polysaccharides were assayed according to the method of Simon et al. (1993); viscosity was measured using a Viscosimeter B3 (Veb kombinat medizin und labortechnik, Leipzig, MLW Prufgerarate – werk Medingen sitz freital, Germany).

Measurements of Cu^{+2} concentrations were performed using Spektroflame D (Spektro Analytical Instruments, Kleve, Germany) ICP-AES (atomic emission spectrometer with inductively coupled plasma). The instrument operating parameters were: Plasma power 1000 W; Coolant gas flow 14 L/min; Auxillary gas flow 1 L/min; Nebuliser gas Flow 0.8 L/min; Nebuliser Cross-flow; Sample uptake 2 ml/min. Rinsing with nitric acid for 30 s was performed between sample measurements. Calibration of the spectrometer was done using freshly prepared solutions of Cu^{+2} (stock solution 1000 mg/L, Merck) in the concentration range 0.01 - 10 mg/L.

Supernatant was measured directly – firstly algal cells were digested with 2 ml concentrated nitric acid and then diluted to 25 mL with bidestilled water.

The viability of *Rhodella* cells exposed to Cu^{2+} was determined after seeding of 200µl treated algal material on agar plate and subsequent cultivation.

All experiments were repeated at least three times and data presented are means of three separate experiments.

RESULTS

Cu^{+2} has specific influence on *Rhodella reticulata* culture grown in laboratory conditions of intensive cultivation. Toxic effect of the metal proved to be higher at increasing metal concentrations in the medium (from 0.000025 to 0.025 mg L^{-1}) demonstrating proportional inhibition of the algal growth expressed as dry weight

and polysaccharide production. (Fig.1). The toxic Cu^{+2} concentration (0.025 mg L^{-1}) decreased the algal productivity by 88% while the lowest concentration applied (0.000025 mg L^{-1}) slightly influenced this parameter (33%).

Toxic effect of Cu^{+2} on photosynthetic structures of *Rhodella* cells depended on the heavy metal concentrations. It was found that 0.0025 mg L^{-1} Cu^{2+}decreased dry weight, chlorophyll "a"content and carotenoids. In addition, it inhibited phycobiliprotein synthesis. Algal cells treated with Cu^{+2} bellow this concentration, had higher content of pigments (chlorophyll"a", carotenoids, phycoerytrin allophycocyanin, C-phycocyanin) compared with the control (Fig.2).

Measurements of Cu^{+2} quantities in the algal suspension using a ICP-AES spectrometer demonstrated the potential of *Rhodella* cells to uptake and accumulate Cu^{2+}. The amount of the heavy metal absorbed by the algal cells was: $>2.3.10^{-7}$g> 0.0024mg> 0.059mg> 1.06 mg. The enhancement of the effect registered was related to the increase of Cu^{+2} concentrations added (0.000025; 0.00025; 0.0025; 0.025 mg L^{-1}) (Table 1).

Vitality test of *Rhodella* cells manifested algal colonies growing on an agar medium after sowing material treated with different Cu^{+2} concentrations. The persistor cells produced colonies 14 days after inoculation. Their recultivation in a liquid medium resulted in growth of colonies regardless the heavy metal pre-treatment. Our results confirmed the suggestion that this alga possesses mechanisms for detoxi-

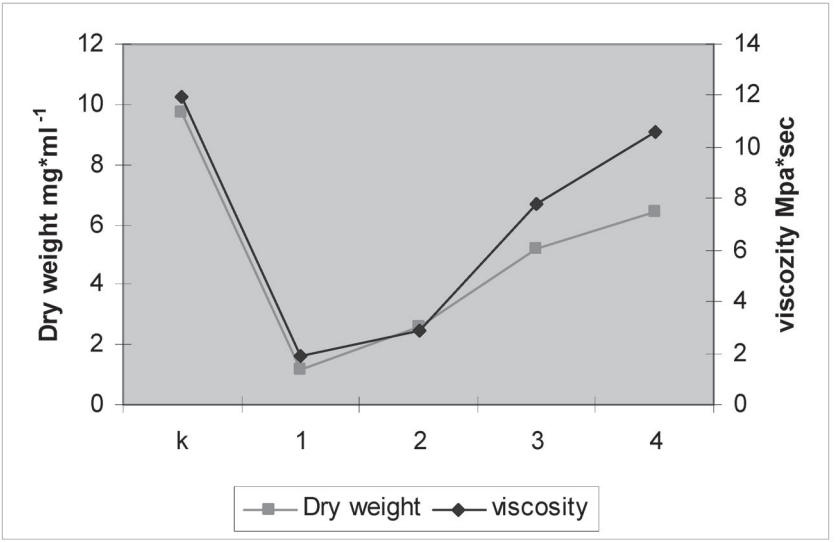
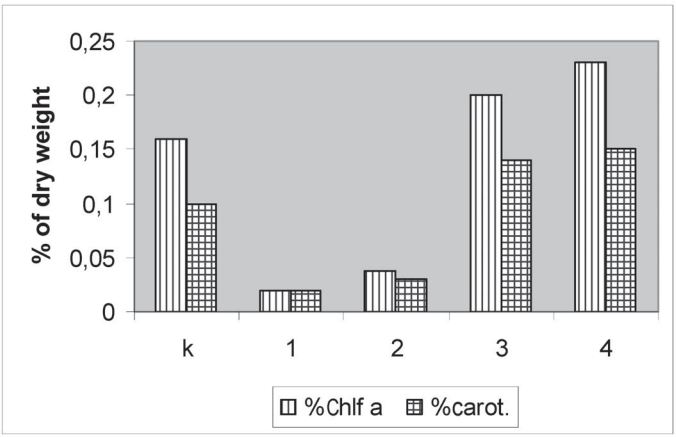


Figure1. Effect of copper on dry weight and viscosity of *Rhodella reticulata*
Legend: k – control *Rh. reticulat*; 1 – *Rh. reticulata* + $CuSO_4 \times 10^{-3}$M; 2 – *Rh. reticulata* +$CuSO_4 \times 10^{-4}$M; 3 – *Rh. reticulata* +$CuSO_4 \times 10^{-5}$M; 4 – *Rh. reticulata* +$CuSO_4 \times 10^{-6}$M.

A



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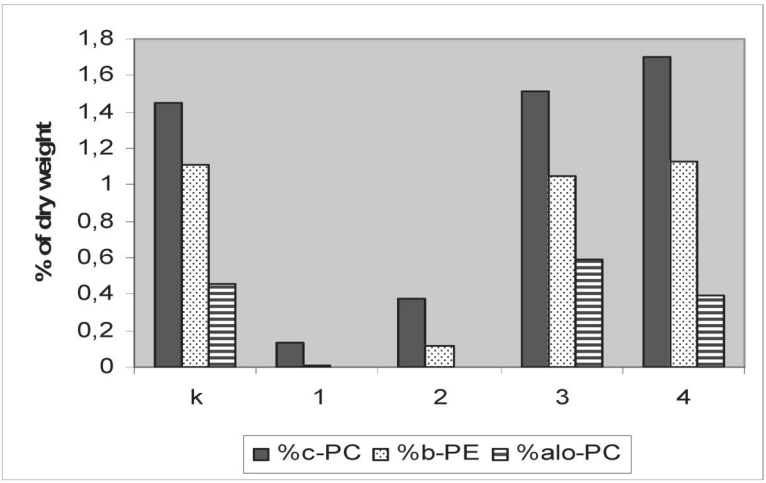

Figure 2. Algal pigments (A, B) after exposure of *Rhodella* to different $CuSO_4$ concentrations. Legend: k – control *Rh. reticulata*; 1. *Rh. reticulata*+ $CuSO_4$ x 10^{-3}M; 2. *Rh. reticulata* +$CuSO_4$ x 10^{-4}M; 3. *Rh. reticulata* +$CuSO_4$ x 10^{-5}M; 4. *Rh. reticulata* +$CuSO_4$ x 10^{-6}M.
c-Phycocyanin; **b**-Phycoeritrin; **alo**-Phycocyanin

Table 1. Cu^{2+} concentration in *Rhodella* cells and supernatant measured by ICP AES

Accumulated Cu^{2+} mg/ mg	control	Cu^{2+} (10^{-3}M)	Cu^{2+} (10^{-4}M)	Cu^{2+} (10^{-5}M)	Cu^{2+} (10^{-6}M)
algal cells	<0.0015 mg	1.06 mg	0.059 mg	0.0024 mg	$2.3*10^{-7}$g
supernatant	<0.0006	<0.0008	<0.00078	<0.0006	<0.0006

fying the heavy metal and that the effect of Cu^{2+} on *Rhodella reticulata* is algistatic. The toxic effect of Cu^{2+} on the physiological parameters of *Rhodella reticulata* depended on the initial amounts of Cu^{+2} to which the algal cells were exposed as well as on the presence of contaminating bacteria during the non-sterile algal cultivation.

DISCUSSION

Rhodella reticulata is a representative of Rhodophyta, which during growth in non-axenic culture is able to produce copious amounts of extracellular polysaccharides that cover algal cells and are released into the medium. Extracellular polysaccharides encase the algal cells and could be responsible for their heavy metal stress protection by binding and stopping its diffusion.

Chemical analysis of polysaccharides of some heavy metal resistant algae showed that the presence of galacturonic and glucuronic acids is related to the metal – binding capacity of some blue green algae – *Aphanothece halophytica* and *Oscillatoria* (Pengfu et al., 2001). Similarly strong metal binding capacity was demonstrated for the carboxyl groups of uronic acids (carboxylated polysaccharides) (Kaplan et al. 1987) as well as for other negatively charged functional groups: amino-, thio-, hydroxo- and hydroxo–carboxylic acids (Xue et al., 1988). Regarding these data it can be suggested that the metal binding activity of *Rhodella* could be related to the carboxyl groups of galacturonic acids in the polysaccharide produced by the alga (Dubinsky et al., 1992).

Rhodella reticulata exposed to Cu^{+2} during intensive cultivation uptakes and accumulates the heavy metal. Similarly to other algae (Stokes et al., 1973) this red microalga exhibited tolerance to heavy metal presence. It was demonstrated by the observation that *Rhodella* cells did not exclude Cu^{2+} but rather accumulated it in high quantity, and meanwhile the cell division and culture growth remained unaffected.

Rhodella cells possess a mechanism for heavy metal detoxification. The toxic effect of Cu^{+2} on *Rhodella reticulata* could be defined as algistatic. We suggest that the polysaccharide it produces is able to encase Cu^{+2} and this could be related to algal detoxification properties. Partial inhibition of the electron transport chain in the cells was registered, but this did not damage phycobilisomes which was confirmed by the preserved photosynthetic activity of the treated cells (Fig. 2).

Some authors suggested that Cu^{+2} toxicity towards algae is due to the formation of insoluble forms of the heavy metal in some solutions (Maloney and Palmer, 1956), which can be precipitated by bacteria. Having in mind the presence of bacteria contaminating non-axenic *Rhodella* growth (Toncheva – Panova and Ivanova, 2002) it could be suggested that detoxification of Cu^{+2} was a result of the action of polysaccharides as well as the activity of bacterial microflora in the cultivation media.

Our results are encouraging evidence for the usefulness of red microalgae *Rhodella reticulata* and its product - heteropolysaccharide, for different applications.

Experiments with synthesized nanocomposites, containing red algal heteropolysaccharide as a natural organic polymer are in progress. It has been proved that this material can be applied as a carrier in sol-gel nano matrices for cell immobilization, for biosorption of heavy metals, etc.

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