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Interactive effects of temperature and copper on photosystem II photochemistry in *Chlorella vulgaris*

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ABSTRACT

In natural aquatic ecosystems, temperature conditions may undergo changes depending on the depth of the water column or micro-environmental conditions. In this study, copper effect on the photosynthetic performance of *Chlorella vulgaris* was investigated at different temperatures by using chlorophyll *a* fluorescence transients and fluorescence imaging parameters. Copper as a pollutant is known to be an inhibitor of photosystem II (PSII) photochemistry; therefore it was important to know how the change of temperature may alter this effect. PSII photochemistry was investigated when *C. vulgaris*, affected by different copper concentrations, was exposed to 24, 28 and 31 °C. Increase of temperature induced higher alterating effects to PSII quantum yield, primary photosynthetic electron transport from water splitting system and consequently higher decrease of total photosynthetic performance if compared to copper effect alone. Additional temperature effect to copper inhibition increased energy dissipation via non-photochemical pathway. In this study we indicated that, when *C. vulgaris* changes temperature conditions, inhibitory effect of copper also undergoes changes. For natural aquatic system we may suppose, when algae are distributed at different depths of water column, that toxicity effect will be dependent to the temperature conditions of the site.

1. Introduction

Copper (Cu) is, at low concentration, an essential component of various plants' enzymes, particularly those involved in the photosynthetic and respiratory electron transport processes [1–3]. Although Cu is essential for plant cellular metabolism, it may also induce toxic effects when present at higher concentrations [4–6]. In aquatic ecosystems Cu represents a widespread contaminant released mostly by anthropogenic activities [7]. Cu is known to inhibit water splitting system [8] and to reduce chlorophyll synthesis [9]. For such inhibition, Cu is known to interact with the Q_B and Pheo electron transport sites, preventing the conversion of absorbed light energy by chlorophyll (Chl) antenna complex into photosystem II (PSII) electron transport [10–12]. PSII activity appeared to be the most sensitive site of Cu inhibitory effect [5]. Some ecological factors are known to alter Cu toxicity. For example, high light intensity may increase Cu toxicity by its inhibitory effect on photosynthesis [13,14]. The pH of the extracellular environment can also affect Cu toxicity by influencing both Cu chemistry and organism physiology [15].

In aquatic ecosystems, temperature undergoes rapid variations which may change the conditions for optimal cellular metabolism of algae. In large aquatic ecosystems, the stratified water column may show important differences of temperature even on a short distance [16]. Temperature has an effect on both biochemical and hydrodynamic processes of pond systems. Solar radiation during the daylight hours heats up the top water layer, causing thermal stratification with a consequence of the warmer and lighter water overlaying the cooler and denser deeper water [17]. Thermal stratification also localizes algae into bands of 10–15 cm width that move up and down through the water column in response to changes in light intensity. Temperature has been shown to enhance the toxic effects of some pollutants on algae [18,19]. For metals, the elevated toxicity found at higher temperatures may only be partially explained by the higher uptake rate of metals [20]. However, there is no information on temperature interaction with Cu inhibitory effect on algae photosynthetic activity.

For Cu concentrations having inhibitory effects on *Chlorella vulgaris*, *Chlamydomonas reinhardtii* and *Selenastrum capricornutum*, Chl *a* fluorescence induction kinetic was found to be altered, indicating an alteration of PSII photochemistry [21]. Photosynthetic fluorescence indicators based on the Chl *a* fluorescence kinetic provide useful information on the structural and functional alterations of the photosynthetic apparatus when algae are exposed to different exogenous effectors [21–23].

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In this study we attempted to show how an alteration of temperature may change Cu toxic effect on energy balance in primary photosynthetic processes in *Chlorella vulgaris* (*C. vulgaris*), concerning conversion of light energy in photosynthetic electron transport and dissipation of energy via non-photochemical process. Such alteration of Cu toxicity by temperature effect will help to understand the possible micro-environmental differences forming heterogenic distribution of toxicity in large water reservoir having the same concentration of pollutants.

2. Material and methods

2.1. Algal culture

The microalga *C. vulgaris* was grown in sterile BG11 liquid medium [24]. The cells were grown under continuous and constant light intensity (100 μmol m^{-2} s^{-1}, SYLVANIA GRO-LUX Wide Spectrum light F40/GRQ/AQ/WS) at 24 °C. The stock culture was aerated with bubbling air. Aliquots of algal samples were used when cellular cultures were in their exponential growth phase.

2.2. Algal exposure to $CuSO_4$

Aliquots of 50 mL of the algal culture (1×10^6 cells/mL) in growing media were exposed to 0, 1, 5, 10 and 20 μmol/L of $CuSO_4$ and incubated in the condition as described above for 24 h. The free ionic Cu^{2+} is known to be the main toxic Cu species in *C. vulgaris* [25] and toxicity of metals in algae is directly dependent on the concentration of free metal ion rather than the total metal concentration [26]. Therefore, the free ionic Cu^{2+} concentration was determined for temperatures of 24–31 °C using the chemical equilibrium calculation software Visual MINTEQ 2.61. The concentrations of Cu^{2+} at each tested temperatures are shown in Table 1.

2.3. Determination of total chlorophyll content

Chlorophyll content was extracted from 1 mL of culture using 1 mL 100% methanol. The extracts was heated at 65 °C for 10 min and separated by centrifugation. Quantitative determination of chlorophyll content was performed according to Lichtenthaler [27] using a UV/VIS spectrometer (Lambda 40, Perkin Elmer).

2.4. Chlorophyll (Chl) a fluorescence transient

All Chl *a* fluorescence measurements were conducted at room temperature with a portable fluorimeter (Plant Efficiency Analyzer, built by Hansatech Instruments Ltd. King's Lynn Norfolk, UK). Samples of algal cells equivalent to 10 μg of total Chl were uniformly placed on a 13-mm glass fiber filter (Millipore #AP20 013 00) by using low pressure filtration to avoid physiological stress effect and to obtain reproducible results. Five measurements were made. The samples were dark adapted for at least 15 min before the measurements were started, to allow the PSII reaction centers to open (re-oxidize) and the electron transport chain to be fully oxidized. The measurement consisted of a single strong 6 s light pulse (3500 μmol photons m^{-2} s^{-1}, an excitation intensity sufficient to ensure closure of all PSII reaction centers) provided by an array of six light-emitting diodes (peak 650 nm) in the PEA instrument focused on the sample surface (4 mm^2). The Chl *a* fluorescence emission induced by the strong light pulses was measured and digitized between 10 μs and 6 s by the instrument.

2.5. Heat treatment

Samples of algal cells equivalent to 10 μg of total Chl were heated at 28 and 31 °C for 60 min (photon flux density was 100 μmol m^{-2} s^{-1}) and then uniformly placed on glass fiber filter Millipore #AP20 013 00) by using low pressure filtration to avoid physiological stress and to obtain reproducible results. Five measurements were made. The samples were dark adapted for 5 min before the measurements were started. Chl *a* fluorescence transients induced by 6 s light pulses were measured.

2.6. Intracellular [Cu] determination

After 24 h of Cu treatments, algal cells were recuperated and filtered on a 0.45 μm filter previously dried and weighted. Filters were dried at 95 °C for 24 h, weighted to calculate algal dry weight and then placed in an acid-washed glass tube in which 4 mL HNO_3 and 500 μL H_2O_2 was added. The samples were allowed to digest 48 h at room temperature before being diluted in nanopure water for ICP-AES quantification of Cu. Cu concentrations were normalized to the dry weight.

2.7. Chlorophyll a fluorescence parameters

Several parameters can be derived from the polyphasic Chl *a* fluorescence rise OJIP that provide information about photosynthetic fluxes. A model has been proposed for the analysis of the polyphasic rise OJIP that has been shown to be a valuable tool in plant and algae vitality monitoring [28]. From the fluorescence transient measured during the first second of illumination several phenomenological and biophysical expressions leading to a description of a photosynthetic sample in a given physiological state were calculated. One of the parameters calculated is the maximum yield of primary photochemistry of PSII (F_V/F_M). It corresponds to the efficiency by which an absorbed photon will be trapped by PSII reaction centers. It has frequently been used as a measure of the maximum efficiency of PSII photochemistry [29]. The absorption of photons (ABS) per active reaction center (RC) showing the antenna size was estimated by the ratio ABS/RC = $((F_K - F_o)/0.250)\cdot(1/(F_J - F_o))\cdot(F_M/(F_M - F_o))$. The performance index (PI) was one of the Chl fluorescence parameters that provide useful and quantitative information about the state of the photosynthetic organisms and their vitality.

Table 1
The free ionic Cu^{2+} concentration determined for temperatures of 24–31 °C using the chemical equilibrium calculation software Visual MINTEQ 2.61.

	Temperature					
	24 °C		28 °C		31 °C	
Copper treatment	[Cu^{2+}] (nM)	Log Cu^{2+} activity	[Cu^{2+}] (nM)	Log Cu^{2+} activity	[Cu^{2+}] (nM)	Log Cu^{2+} activity
Control	0.03	−10.77	0.03	−10.75	0.03	−10.73
1 μM	0.25	−9.84	0.27	−9.81	0.29	−9.79
5 μM	13.03	−8.13	12.5	−8.15	12.07	−8.16
10 μM	38.1	−7.66	36.81	−7.68	35.73	−7.69
20 μM	90.77	−7.28	89.76	−7.29	88.81	−7.3