# Effects of heavy metals ions on primary photosynthetic processes in Antarctic filamentous alga *Zygnema* sp.

*Short Communication*

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## Abstract

Algae show different extent of resistance to heavy metals. In this study, the resistance of green filamentous alga *Zygnema* sp. (strain EEL201, originally collected at James Ross Island, Antarctica) was tested by chlorophyll fluorescence parameters. The alga was taken from the stock culture cultivated on agar, innoculated to liquid medium and allowed to develop biomass large enough for exposition experiments. Heavy metal treatments consisted of addition of Cd and Zn so that effective concentration was 66 mM. Response of primary photosynthetic processes to Cd, Zn addition was assessed by the exposition time-dependent decrease in potential quantum yield ($F_V/F_M$) photosystem II (PS II) and relative fluorescence decline ratio (RFd). Cd- and Zn-treated *Zygnema* sp. exhibited similar extent of decline of the two parameters associated with inhibition of primary processes of photosynthesis. Negative changes to PS II, however, did not bring full inhibition of PS II functioning since $F_V/F_M$ was about 0.450 after 120 min. exposition. The results might indicate a potential of Antarctic *Zygnema* in the studies focused on resistance to heavy metals and phytoremediation technologies.

***Key words:*** cadmium, zinc, James Ross Island, photosystem II, chlorophyll fluorescence

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## Introduction

In last decades, a great attention has been devoted to the studies focused on eco-physiological characteristics of *Zygnema* sp. from polar regions. They comprised the resistance of the species to desiccation (Holzinger et al. 2014, 2015, Herburger et al. 2015), osmotic stress (Vilumbrales et al. 2013), and high light doses (Thangaraj 2015). Resistance of *Zygnema* sp. to heavy metal ions has not been studied because of their generally low content in polar ecosystems. Recently, however, green algae are considered prospective organisms for phytoremediation of polluted soil and water (Kumar et al. 2015). In this respect, the potential of green algae to accumulate and cope with high doses of heavy metal has got an increasing importance. Filamentous algae are considered effective accumulators of heavy metals. Therefore, physiological studies of the algae under medium to heavy stress caused by heavy metal ions might be useful in the evaluation of phytoremediation capacity of a great variety of species. Therefore, the objective of this study was to evaluate the response of Antarctic strain of *Zygnema* sp. to Cd and Zn added to cultivation medium. Main emphasis was given to the negative changes induced by the heavy metal ions in photosynthetic apparatus, photosystem II in particular. Since the changes could be monitored by chlorophyll fluorescence parameters sensitively (see *e.g.* Komárek et al. 2010), the measurements of potential quantum yield ($F_V/F_M$), and relative fluorescence decline ratio (RFd) were used to evaluate the Cd and Zn effects on primary photosynthetic processes in *Zygnema* sp.

Green algae exhibits a great variety of resistance to heavy metals, Cd and Zn in particular. The ability of green algae to cope with heavy metal stress has been studied in lichen-forming unicellular alga *Trebouxia*. These studies addressed different aspects of tolerance-resistance of several species of the genus involving *e.g.* Cd-induced heat shock protein synthesis (Bačkor et al. 2006), altered chlorophyll content and chloroplast structure (Sanitá di Toppi et al. 2005), reduced growth and chlorophyll synthesis (Piovár et al. 2011). In general, Cd affects negativelly biochemical characteristics of chlorophyll molecules in many algae ranging from micro- to macroalgae (dos Santos et al. 2012).

## Material and Methods

*Zygnema* sp. (strain EEL201) was taken from stock culture of the Laboratory of photosynthetic processes, Masaryk University, Brno. The strain was collected at James Ross Island, Antarctica from the Long-term research plot in 2015. The collection site was a shallow stream passing through the vegetation oasis formed by numerous moss and lichen species (for details see *e.g.* Barták et al. 2015). The site was located close to a seashore at the altitude of 5-8 m a.s.l. Microclimate of the site of the collection is described by Láska et al. (2011). Generally, the northern part of James Ross Island is deglaciated and rich in terrestrial freshwater lakes (Nedbalová et al. 2013), small-area ponds (Váczi et Barták 2011) and streams. All these ecosystems are inhabited by algae and cyanobacteria (*see* Skácelová et al. 2015 for preliminary review).

The stock culture was cultivated on agar. Several weeks before experiments, *Zygnema* sp. was innoculated to a liquid medium to develop substantial biomass. Cultivation was carried out in a liquid 3N BBM solution under constant temperature of 10°C and the irradiance of

40 μmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation (PAR) with periodic dark phase (16/8 hours). For chlorophyll fluorescence measurements, 1 ml of *Zygnema* sp. was pipetted to the holes of microbiological plates (control). Heavy metal treatment was done by the addition of $CdSO_4$ and $ZnSO_4$ so that the effective concentration was 66 mM in the holes (Cd treatment, Zn treatment).

Chlorophyll fluorescence measurements were done before the addition of heavy metals, immediatelly after (10 min.), and then 2 measurements in intervals of 30 min. and after that 4 in intervals of 1 h. The effects of heavy metal ions on primary photochemical processes were assessed by a slow Kautsky kinetics supplemented with saturation pulses applied in dark- and light-adapted state of *Zygnema* sp. Measurements were taken at 5°C. The microbiological plates with *Zygnema* sp. were predarkened for 5 min. in the measuring compartment of a HFC-010 fluorometer (Photon Systems Instruments, Czech Republic). Then, a saturation pulse was applied to induce maximum chlorophyll fluorescence ($F_M$). Together with measurements of background chlorophyll fluorescence ($F_0$), this allowed to evaluate the capacity of photochemical photosynthetic processes ($F_V/F_M$) in photosystem II. Then, after intervening period of dark (10 s), actinic light was switched on and chlorophyll fluorescence was measured until it reached a constant value ($F_S$) in steady state. From the curve (Kautsky kinetics), maximum value at the P peak was evaluated ($F_P$) so that the relative fluorescence decline ratio (RFd) could be calculated ($RFd = (F_P-F_S)/F_S$).

## Results and Discussion

The effect of Cd and Zn on primary photochemical processes of photosynthesis is demonstrated as a decrease in $F_V/F_M$ values in Fig. 1. The negative effect of Cd, Zn was apparent immediatelly after the addition (10 min.) and was more pronounced with time of exposition (Cd) or showed an irregular pattern (Zn). However, the negative effect of Zn was seen at any time of exposition (Fig. 1). In untreated control, $F_V/F_M$ declined with time, however the range of the decline was rather small (from 0.650 to 0.430, *see* Fig. 1) indicating that potential of photosynthetic processes of *Zygnema* sp. remained quite high throughout the experiment. In Cd-treated *Zygnema* sp., a decrease in $F_V/F_M$ values was apparent with the time of exposition. The decrease is attributed to a partial inhibition of PS II caused either by binding of cadmium to a calcium binding site in PS II (Faller et al. 2005), donor site inhibition of PS II (Voigt et Nagel 2002), or PS II reaction centre inhibition (Küpper et al. 2002).

The extent of $F_V/F_M$ decline found in this *Zygnema* study, however, was smaller than in the data of Bačkor et al. (2007) reported for 24 h Cd-treated *Trebouxia erici* (75 and 59% of control in 5 and 10 mM Cd, respectively). The reason for a smaller decrease in $F_V/F_M$ in *Zygnema* could be the likely smaller amount of Cd ions in cytosol. Thanks to cell wall thickness and paralel arrangement of filaments, the uptake of Cd ions would be lower or prolonged in *Zygnema* compared to *Trebouxia*.

In this study, apparent photosynthesis, however, was low even at the begining of the experiment since the effective quantum yield of PS II had the values below 0.15 ($\Phi_{PSII}$ data not shown here). Low $\Phi_{PSII}$ PS II values were caused by a small difference between $F_M'$ and $F_S$ in all Kautsky kinetics irrespectively of the time of the measurements.

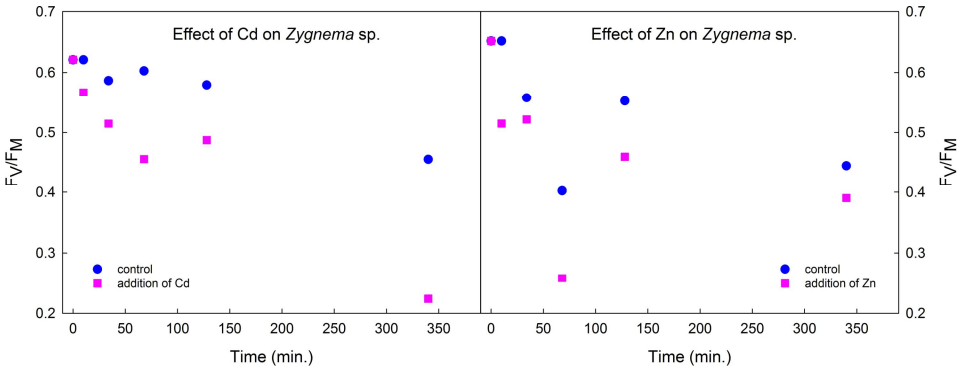


**Fig. 1.** Effect of cadmium (Cd, left panel) and zinc (Zn, right panel) addition on $F_V/F_M$ (potential photosynthetic quantum yield of photosystem II) in *Zygnema* sp.

Relative fluorescence decline ratio (RFd) remained more or less constant in control having the values ranging between 1.2 and 1.4 (Fig. 2). Effects of Cd and Zn was seen immediately after the addition and was demonstrated as a decrease in RFd values. The decline reached minimum values of 0.8 in Cd- and 0.6 in Zn-treated *Zygnema* sp. Then, with the exposition time higher than 120 min., RFd rather increased showing, however lower values then untreated control.

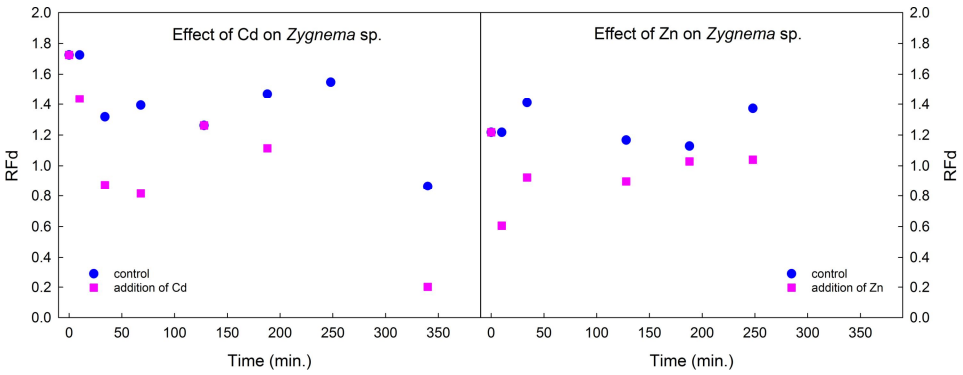


**Fig. 2.** Effect of cadmium (Cd, left panel) and zinc (Zn, right panel) addition on RFd (relative fluorescence decline ratio) in *Zygnema* sp.

Both $F_V/F_M$ and RFd data showed that Cd and Zn treatments (66 mM) did not lead to full inhibition of primary photosynthetic processes. Cd- and Zn-treated *Zygnema* sp. exhibited a decrease in $F_V/F_M$ and RFd values, however, PS II centers were still exhibiting photosynthetic activity even after 5 h 40 min. exposition to 66 mM Cd and Zn. This supports a hypothesis of a relatively high resistance of *Zygnema* sp. to heavy metals ions. Similarly, algae of genus *Trebouxia* exhibit relatively high resistance to heavy metals (Bačkor et al. 2007) and osmotic stress (Váczi et Barták 2006). Algae do possess several physiological mechanisms to limit toxic effects

of heavy metal ions. Among them, production of phytochelatins plays major role as shown for Zn-treated *Stigeoclonium tenue* by Pawlik-Skowronska (2003 a, b). Increased synthesis of glutathione and the enzymes related to glutathione synthesis pathway is also reported in Cd-treated algae (Domínguez et al. 2003).

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