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Effect of $(C_6H_5)_3PbCl$ and $(C_6H_5)_3SnCl$ on Delayed Luminescence Intensity, Evolving Oxygen and Electron Transport Rate in Photosystem II of *Chlorella vulgaris*

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Abstract The effect of the organometallic compounds containing lead, $(C_6H_5)_3PbCl$, and tin, $(C_6H_5)_3SnCl$, on *Chlorella* green algae photosystem II was studied. Suspension of the algae treated with $(C_6H_5)_3SnCl$ at concentrations of 1.0 and 4.0 μmol dm^{-3} for 22 h revealed a decrease in most physiological parameters studied, particularly in decasecond component of delayed chlorophyll luminescence, photosynthetic electron transport rate and diluted oxygen concentration, which implies an inhibition of photosynthetic electron transport as well as oxygen evolving system. On the other hand, $(C_6H_5)_3PbCl$ caused stronger inhibition than $(C_6H_5)_3SnCl$, particularly in the higher concentration.

Keywords Chlorophyll fluorescence · Delayed luminescence · Lead · Organolead · Organotin · Tin

Heavy metals, especially copper, nickel, lead and zinc, negatively affect aquatic environments (Bilgrami and Kumar 1997; Boucher and Carpentier 1999; Danilov and Ekelund 2001). They disturb ion balance and cell transport processes, cause degradation of photosynthetic pigments, give rise to oxidation stress, and, particularly, disrupt photosynthesis and respiration of eukaryotic algae (Poskuta et al. 1996; Memon et al. 2001). The impact varies depending on the nature of the affected organisms. Since heavy metals accumulate in sediments, extensive knowledge on their effects on the aquatic biota is needed. In this context, the use of model organisms (often unicellular), which allows for rapid assessment of pollutants in freshwater, can be of advantage. We aim to determine whether photosynthesis can be used as set of sensitive physiological parameters in the toxicological studies on *Chlorella*, a green unicellular alga, found in both fresh and marine waters. Its physiology, biochemistry and photosynthetic apparatus are similar to those in higher plants but its growth is very quick. For these reasons *Chlorella* is often studied in various investigations on metabolism and stress (Lewis and McCourt 2004; El Khachia et al. 2008).

Compounds containing a metal and an organic radical interact with living organisms and exhibit toxic action. Such organic compounds of tin and lead are, in general, considerably more toxic than inorganic compounds of the metals (Przestalski et al. 2000). Organic tin and lead compounds exhibit reactivity in the lipid phase of membranes, so they may be toxic for plants (Fargasova and Kizlink 1996; Olivares 2003). These compounds influence chloroplast membranes inhibiting reactions of photosynthesis. In our previous paper, phenylolead applied at the concentration of 0.5 μmol dm^{-3} inhibited photosynthetic electron transport of *Scenedesmus quadricauda*, particularly after 20-h long treatment, while in the case of phenylotin such effect was not observed (Murkowski and Skórska 2008). The light phase of photosynthesis is very susceptible to environmental stresses, which can be observed using chlorophyll delayed luminescence and chlorophyll fluorescence (Devlin et al. 1983; Schreiber et al. 1994; Murkowski 2002; Skórska and Swarcewicz 2006; Berden-Zrimec et al. 2007). The aim of this work was to trace inhibition symptoms in the *Chlorella* green algae photosystem II subjected to organic-tin and organic-lead compounds, i.e. $(C_6H_5)_3PbCl$ and $(C_6H_5)_3SnCl$

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applied in low concentrations, using very sensitive luminescence methods.

Materials and Methods

A suspension of the *Chlorella* green algae grown at the temperature of 22°C in the light (PPFD[1] 80 μmol m^{-2} s^{-1}), photoperiod 12 h/12 h (day/night), aired continuously, and in the phase of logarithmic growth was used for the measurements. The single sample contained 100 cm^3 of suspension in a glass, and one series comprised six samples. All the samples were divided into five groups, one of them being the control, diluted with distilled water (1:1). Two groups were treated with $(C_6H_5)_3PbCl$ at the final concentrations 1.0 and 4.0 μmol dm^{-3}, respectively. In the other two groups the final concentration of $(C_6H_5)_3SnCl$ was 1.0 and 4.0 μmol dm^{-3}, respectively. All measurements were done after 22-h long treatment in the dark. Both compounds, >98% purity, had been supplied by Merck. A decay kinetics of chlorophyll delayed luminescence (DL) in the range from 1 to 21 s was measured using the special luminometer (Murkowski 2002; Murkowski and Skórska 2008). Before measurements, the samples were incubated for 15 min under weak light of a tungsten lamp, PPFD 1 μmol m^{-2} s^{-1}. Intensity of oxygen evolving in each sample was measured by means of the LDO HQD40 portable luminescence oxygen meter (Hach Lange, Dublin) at PPFD 1200 μmol m^{-2} s^{-1}. Thereafter, all the samples were infiltrated through the Whatman filter GF/A, and chlorophyll fluorescence was measured (ETR) using a pulse-amplitude modulated fluorescence based method, where the variable fluorescence at 665 nm is monitored (Schreiber et al. 1994). Measurements were performed using a PAM-200 fluorometer (Walz, Effeltrich, Germany) for detecting signals directly above the biofilter. After 10 min of dark adaptation, the sample was illuminated for 4 min with actinic light at PPFD 120 μmol m^{-2} s^{-1}, the quantum yield was detected by applying a single saturation pulse of PPFD 3,000 μmol m^{-2} s^{-1}. The measurements were done in six biological replications. Means for each series were calculated and statistically elaborated using one-way ANOVA. Post-hoc analysis allowed separation of homogenous groups by means of Newman–Keuls ($p < 0.05$), which are marked by the same letters on the diagrams.

Results and Discussion

In our experiments on a suspension of *Chlorella* green algae treated with $(C_6H_5)_3PbCl$ for 22 h at the concentration of 1.0 μmol dm^{-3}, a decrease of the delayed luminescence compared to the control was observed, while $(C_6H_5)_3SnCl$ did not affect DL intensity (Fig. 1a). Both compounds at 4.0 μmol dm^{-3} reduced the DL intensity more deeply, particularly the one containing lead (Fig. 1b). Values of the decasecond component of DL, marked as N6 (Fig. 2a), decreased to 89% and 50% of the control in the green algae treated with triphenylotin in 1.0 and 4.0 μmol dm^{-3}, and to 77% and 31%—in those treated with triphenylolead, respectively. The kinetics of delayed luminescence decay in the range of 0.5–17 s is a very sensitive indicator of herbicides, inhibitors of photosynthesis action on electron transport in the photosystem II (Murkowski 2002).

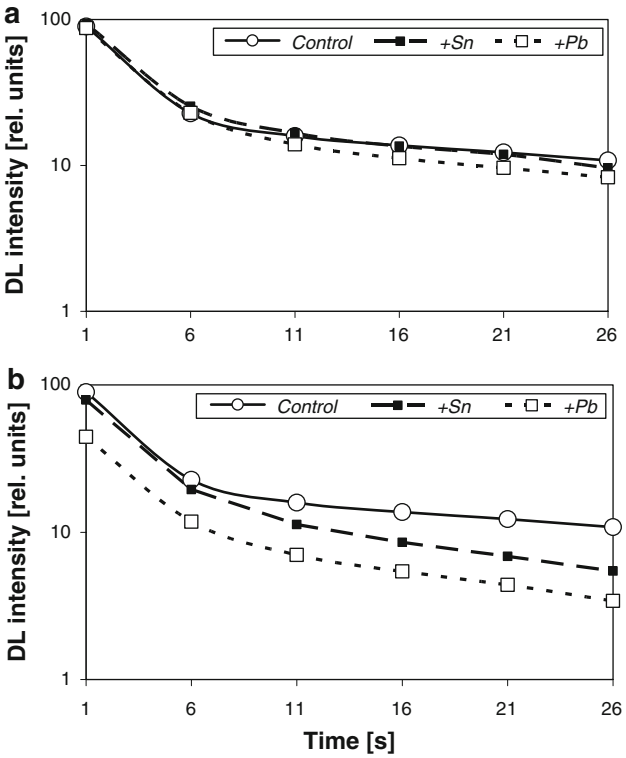

Fig. 1 Intensity of chlorophyll delayed luminescence in cells of *Chlorella* green algae after 22 h incubation with triphenylotin or triphenylolead at 1 μmol dm^{-3} (**a**) or 4 μmol dm^{-3} (**b**)

The results of DOC measurements indicate a strong inhibition of the oxygen evolving system caused by the studied compounds at the higher concentrations. In addition, triphenylolead at 1.0 μmol dm^{-3} caused a deeper than triphenylotin reduction of this parameter (Fig. 2b). The electron transport rate (ETR) measured by chlorophyll fluorescence, was reduced by 40% or 57% in the green algae treated with the triphenylolead, and by 20%—in the triphenylotin (Fig. 2c). ETR parameter as photosynthetic electron transport rate, is related to the physiological state of plants and phytoplankton (Schreiber et al. 1994; Berden-Zrimec et al. 2007). Effects of heavy metals on the most

[1] PPFD—photosynthetic photon flux density.

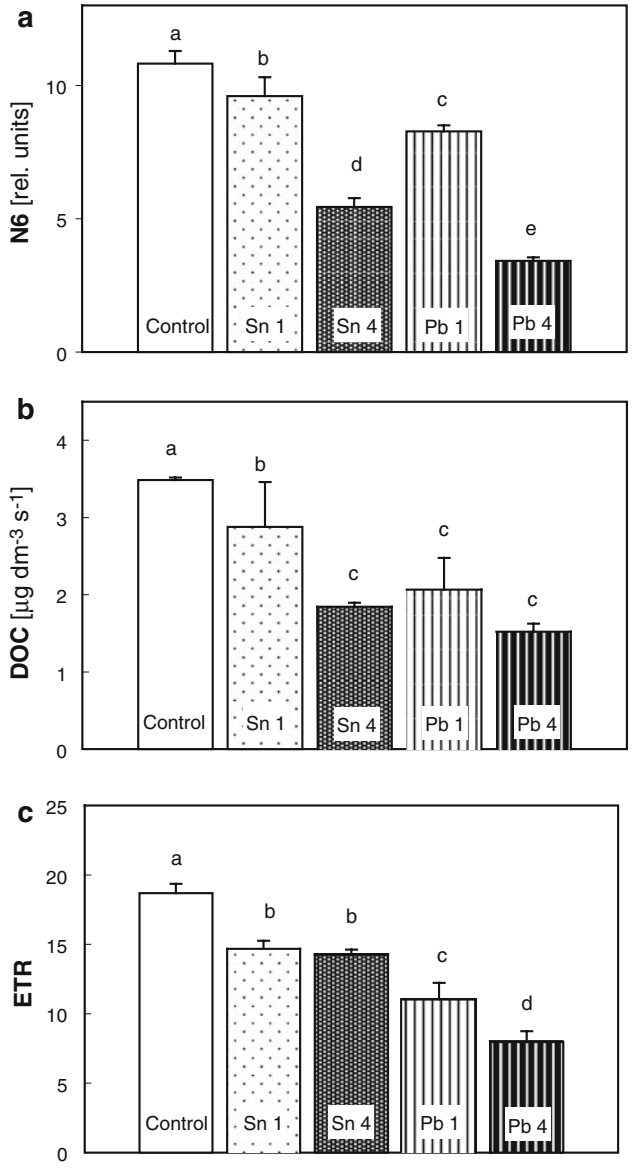

Fig. 2 Intensity of the decasecond component (N6) of chlorophyll delayed luminescence (**a**), intensity of oxygen evolving (**b**) and values of the photosynthetic electron transport rate (**c**) of *Chlorella* incubated for 22 h with triphenylotin or triphenylolead at 1.0 µmol dm^{-3} (Sn 1, Pb 1) or 4.0 µmol dm^{-3} (Sn 4, Pb 4). *Vertical segments* present the values of standard deviations. Means marked by the *same letters* do not differ ($p < 0.05$)

susceptible part of the photosynthetic electron transport chain between PQ_A do PQ_B were reported (Rutherford and Inoue 1984; Krupa and Baszyński 1995). The applied in our experiments lead- and tin-containing organic compounds showed a similar effect as such herbicides as triazines, urea, diazines (Devlin et al. 1983; Murkowski 2002; Skórska and Swarcewicz 2006). Such herbicide attaches its particle into the molecule of D1 (32 kDa) protein inhibiting electron transport between acceptors PQ_A and PQ_B in the photosystem II, which causes a decrease of non-cyclic photophosphorylation rate and CO_2 assimilation and changes the intensity of chlorophyll fluorescence (Rutherford and Inoue 1984).

Some authors have studied the effects of lead and tin in inorganic forms on green algae, but they applied them at higher concentrations than those in our experiment. Poskuta et al. (1996) reported a photosynthesis inhibition of *Chlorella pyrenoidosa* after 24-, 48- and 72-h treatment by Pb at 0.5, 1.0 and 2.0 mmol dm^{-3}, i.e. at least 500 times more than us. The magnitudes of inhibition increased with increasing lead concentrations and the time of exposure. A similar pattern of inhibition was observed for chlorophyll biosynthesis, but the magnitudes of inhibition were lower. Mohammed and Markert (2005) presented toxic effects of $Pb(NO_3)_2H_2O$ on the biomass of the green alga *S. quadricauda*. The growth was gradually decreased with Pb at 15, 20 and 25 mg dm^{-3}, while at 30 mg dm^{-3} the effect was more pronounced. There were differences in toxic effects of the lead depending on the concentration and the time of treatment. Bilgrami and Kumar (1997) reported that the lead at 100 mg dm^{-3} did not affected growth of *Chlorella v.* and *S. quadricauda*, however, at 10,000 mg dm^{-3} the growth of phytoplankton was inhibited. *S. quadricauda* expressed higher tolerance to this metal than *Chlorella*. Lamaia et al. (2005) studied the toxicity and accumulation of lead in a common filamentous green alga, *Cladophora fracta*. They were cultured in a medium, which was supplemented with 5, 10, 20, 40 or 80 mg dm^{-3} of $Pb(NO_3)_2$ and were separately harvested after 2, 4, 6 and 8 days. The toxicity symptoms of Pb in *C. fracta* showed damage, reduced number of chloroplasts, disintegrated cell wall and death. The accumulation study showed that there were significant increases of metal levels inside algae tissue when the exposure time and concentration were elevated, and the lowest total chlorophyll content was found in the algae exposed to 80 mg dm^{-3} of Pb.

In a study on inorganic tin compounds, Fargasova (1994) showed toxic and inhibitory effects of Sn(II) ($SnCl_2{\cdot}H_2O$) and Sn(IV) (Na_2SnO_3) on the alga *S. quadricauda*. Inhibition of growth rates was observed and the Sn^{4+} ion also had lower inhibitory effects than the Sn^{2+} ion. The influence of inorganic tin compounds on the unicellular *cyanobacterium Synechocystis aquatilis* was studied by Pawlik-Skowrońska et al. (1997). Both Sn(II) and Sn(lV), used as chlorides (10 mg dm^{-3}), inhibited the growth and chlorophyll a content of the cyanobacterium cultures, but only under alkaline conditions. Generally, the observed tin toxicity increased with the increase of metal concentration, time of exposure and pH value of the medium (in the range 7–9.8). Sn(II) seemed to be more toxic than Sn(IV). At the lowest concentration, 1 mg dm^{-3}, Sn(II) caused a 36% and 40% decrease in growth and chlorophyll content, respectively, after 96 h exposure at pH 9.8, while Sn(IV) caused even a

slight increase of both physiological parameters (hormetic effect). Similar increases in growth and chlorophyll content were also observed at the high concentration (10 mg dm^{-3}) of Sn (II) and Sn(IV). Bilgrami and Kumar (1997) studied the impact of Cu, Pb and Zn on the growth of *Closterium acerosum*, *Pediastrum simplex*, *Chlorella vulgaris* and *S. quadricauda*. At the concentration of 100 mg dm^{-3} these metals were not toxic, however, at 10,000 mg dm^{-3} the growth of phytoplankton was inhibited. Cu was the most toxic element followed by Pb and Zn. Moreover, *S. quadricauda* expressed the highest tolerance to these metals, while the most susceptible was *C. acerosum*.

Simultaneous fluorescence and photoacoustic measurements have been used to study the effects of metal ions (copper, lead, and mercury) during dark incubation of thylakoid membranes (Boucher and Carpentier 1999). The values of the chlorophyll fluorescence parameters strongly decreased in the presence of the metal ions coinciding with an increase in the non-photochemical deexcitation rate constant $k(N)$. It was observed that photosynthetic energy storage measured by photoacoustic spectroscopy also decreased but a large portion of energy storage remained unaffected even at the highest metal ion concentrations used. A maximal inhibition of photosynthetic energy storage of 80% and 50% was obtained with Hg^{2+}- and Cu^{2+}-treated thylakoids, respectively, while energy storage was insensitive to Pb^{2+}. The results are consistent with the known predominant inhibition of the donor side of photosystem II by the metal ions. The insensitive portion of energy storage is attributed to the possible recurrence of cyclic electron transport around photosystem II that would depend on the extent of inhibition produced on the acceptor side by the metal ion used (Boucher and Carpentier 1999).

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