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COMPARISON OF PHYTOTOXICITY OF LEAD AND TIN ORGANIC COMPOUNDS BY MEANS OF LUMINESCENCE METHODS*

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Abstract. The effect of organic-metallic compounds including lead, $(C_6H_5)_3PbCl$, and tin, $(C_6H_5)_3SnCl$, on cucumber leaves and green algae *Scenedesmus quadricauda* were studied. Both compounds, in concentration of 2 μmol dm^{-3}, caused increased emission of ultra weak chemiluminescence (UCL) of cucumber leaf discs after 20 hours of treatment, and activity of tin triphenyl chloride was stronger than that of lead triphenyl chloride. Chilling at high light of the studied discs treated by both compounds increased UCL intensity more than the control ones. Suspension of green algae *Scenedesmus quadricauda* treated for 1 h, 2 h and 4 h by $(C_6H_5)_3SnCl$ in concentration 0.5 μmol dm^{-3} decreased the value of the L_D parameter indicating inhibition of photosynthetic electron transport. However, after 20 h of treatment, adaptation of *Scenedesmus quadricauda* to the applied tin was observed. Otherwise, $(C_6H_5)_3PbCl$ caused a stronger decrease of L_D depending on duration of treatment.

Keywords: *Cucumis sativus* L., delayed luminescence, *Scenedesmus quadricauda* (Turp.), ultra-weak chemiluminescence, organotin and organolead compounds

INTRODUCTION

There is an increasing body of literature on plant responses to heavy metals, due to the intensified awareness of and concern over contamination or pollution of the environment (Dżugan and Pasternakiewicz 2005). Direct or indirect toxicities to plants have been reported. Both tin and lead are generally unreactive metals and they are quite easily oxidised, tin usually to tin(IV) and lead to lead(II). Results of controlled studies of acquisition of lead and tin by plants, mainly in

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inorganic forms, have been reviewed (Krupa and Baszyński 1995, Memon 2001, Prasad 2004). These metals are relatively low toxic for plants in this from. Roots seem to be particularly affected, perhaps because they reportedly attain considerably higher concentrations than those in shoots (Boucher and Carpentier 1999, Mohammed and Markert 2005). Lead mainly affects, only in high concentration, mitochondrial respiration and photosynthesis by disturbing electron transfer reactions (Woolery and Lewin 1976, Olivares 2003, Xiong *et al.* 2006). Length of corn root was reduced by 48% at 500 ppm Pb, and wheat root weight reduced by 22% at 1000 ppm. Burzyński and Kłobus (2004) showed very slight effect of Pb in concentration of 1 mM on assimilation of CO_2, stomata conductivity and internal CO_2 concentration in cucumber plants. Furmanek and Andrzejewska-Ponomarev (2006) showed increased content of malone dialdehyde in tomato plants treated by lead nitrate, which indicated lipid peroxidation in biomembranes. Tin is absorbed by plant root hairs, but generally is not redistributed elsewhere in the plant. Inorganic tin is generally unavailable to plants and algae, but at high concentrations some toxic effects were observed (Romney *et al.* 1975, Fargasova 1994).

Compounds containing a metal and an organic radical interact with living organisms and exhibit toxic action. Such organic tin and lead compounds are, in general, considerably more toxic than inorganic compounds of the metals (Przestalski *et al.* 2000). They markedly contaminate the environment and come from many sources; in particular from the paint industry and plant protection chemicals. The literature on the effect of organotins and organoleads on membranes, especially model lipid membranes, is poor. Amphiphilic character of organometallic compounds enables them to intercalate and penetrate cell membranes, potentially affecting various vital cell functions (Gabrielska *et al.* 2004). Compound adsorption onto the membranes depends on the compound properties, as well as on the membrane composition and state. When adsorbing onto the lipid surface, phenyltins localize at areas where lipid bilayer organization is compatible with the compound spatial requirements (Langner *et al.* 2000, Hładyszowski *et al.* 2002). Oxidation of lipids can by detected by the chemiluminescence method. Application of chemiluminescence to the study of lipid peroxidation reactions is based on the occurrence of short-lived free radicals and excited states derived from side reactions of the lipid peroxidation process (Boveris *et al.* 1981). Thus, the light emission yield is extremely low. Chemiluminescence is induced or enhanced by conditions that normally increase lipid peroxidation or that create a peroxidative stress, i.e. toxic effect of hyperbaric oxygen or infusion to the intact organ with organic hydroperoxides. The higher quantum yield of light-induced emission allows a better study of the photoemissive species occurring in the chemiluminescence system. The purpose of this work was detection of toxic symptoms in cucumber plants and green algae subjected to organotin and organolead compounds, i.e. $(C_6H_5)_3PbCl$ and $(C_6H_5)_3SnCl$ in a low concentration using very sensitive luminescence methods: ultraweak chemiluminescence and delayed luminescence.

MATERIAL AND METHODS

Plants of cucumber (*Cucumis sativus* L. cv. Dar) were grown in controlled conditions of light (PPFD 200 μmol $m^{-2} s^{-1}$, photoperiod 12 h) and temperature (22°C/18°C, day/night). 18 discs (Ø 30 mm) were cut from the second leaves of cucumber plants and divided in three series with 6 in each series. The first were put into Petri dishes with distilled water (control), and the second and third − into solution of 2 μmol dm^{-3} $(C_6H_5)_3PbCl$ (lead triphenyl chloride) or $(C_6H_5)_3SnCl$ (tin triphenyl chloride), respectively. After 20 h of treatment in darkness at temperature of 21°C the intensity of ultraweak chemiluminescence (UCL) per 1 g of dry mass of leaf disc was measured (experiment 1) using a special high sensitive single photon counter (Fig. 1) described by Murkowski (2002). Next, the dishes with the three series of discs were subjected to intense light in the range of photosynthetically active radiation (PPFD 1200 μmol m^{-2} s^{-1}) at temperature of 3°C for 2 h. After this stress they were incubated in darkness for 2 h at temperature of 20°C and UCL was measured (experiment 2). After the measurements the discs were put into dishes with water and placed at 20°C in darkness (recovery after chill and high light stress), then UCL was measured after 20 h (experiment 3).

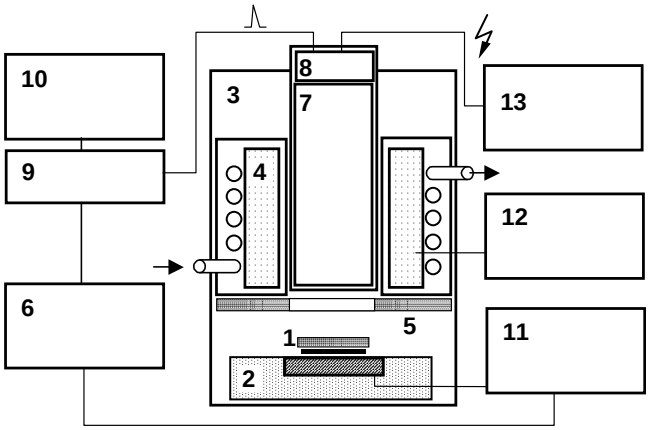

Fig. 1. Ultraweak biochemiluminescence measurement set
1 – leaf disc covered by an organic glass plate; 2 – drawer with thermostatic table for investigated biological samples (range from 30ºC to 80ºC, precision ± 0,5ºC); 3 – lightproof measuring camera; 4 – thermoelectric battery (Peltier elements) with a water cooler; 5 – thermo-insulating plate with organic glass window; 6 – programmer of measurement cycle; 7 – photomultiplier EMI 9558B (photocathode S20); 8 – pulse preamplifier; 9 – counter; 10 – printer; 11 – power supply of the thermoelectric battery; 12 – power supply of the thermoelectric cooler; 13 – high voltage power supply.

Suspension of green algae *Scenedesmus quadricauda* (Turp.) grown on L_5m nutrient at temperature of 18°C in light (PPFD 80 μmol m^{-2} s^{-1}), photoperiod 12 h/12 h

(day/night) continuously air inflating and in the phase of logarithmic growth was used for the measurements (Prokowski 2002). Concentration of chlorophyll in the suspension was 28 mg m^{-3}. The single sample was 9 cm^3 of suspension in a glass dish, and a measurement series was eight samples. For each sample of one series (control) – 1 cm^3 of water was added to suspension, for the second series – 1 cm^3 of 5 μmol dm^{-3} $(C_6H_5)_3PbCl$, and for the third one – 1 cm^3 of 5 μmol dm^{-3} $(C_6H_5)_3SnCl$. Final concentration of these compounds was 0.5 μmol dm^{-3}. Before measurements green algae were incubated at temperature 20°C in weak light of a tungsten lamp, PPFD 1 μmol m^{-2} s^{-1}. Measurements of decay kinetics of chlorophyll delayed luminescence were done after 1 h, 2 h, 4 h and 20 h of treatment, by means of a special luminometer (Fig. 2) described in detail by Murkowski and Prokowski (1987, 1994, 2003). The rate of chlorophyll luminescence decay, proportional to photosynthetic electron transport rate (L_D parameter), was calculated according to Murkowski (2002).

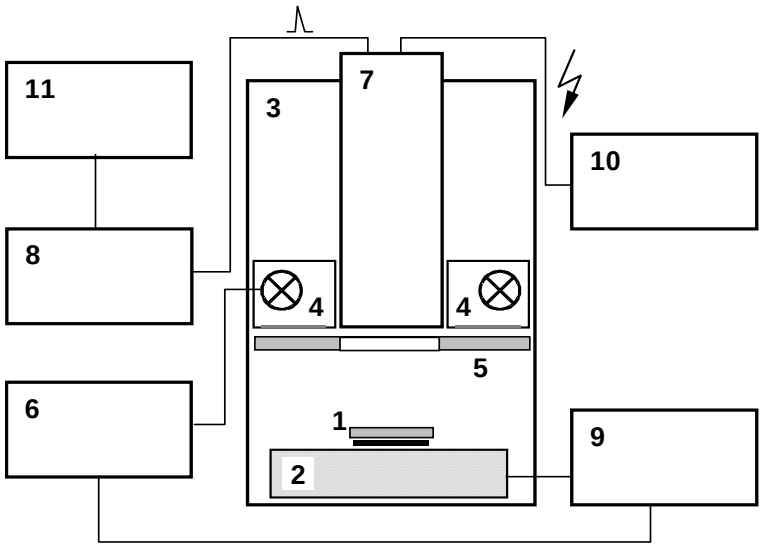

Fig. 2. Special luminometer for recording chlorophyll delayed luminescence decay
1 – investigated leaf disc covered by a glass plate; 2 – drawer with table for investigated sample; 3 – light-proof measuring camera; 4 – illuminator; 5 – mechanical diaphragm; 6 – power supply of the illuminator; 7 – photomultiplier Zeiss S52FC20 (photocathode S20) with preamplifier; 8 – counter; 9 – power supply of thermoelectric battery; 10 – high voltage power supply; 11 – printer

All measurements were done in 6-8 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

The discs cut from leaves of cucumber plants, passive infiltrated $(C_6H_5)_3PbCl$ solution, had increased UCL by 85% and the discs in $(C_6H_5)_3SnCl$ solution – by 160% in comparison with the control (Fig. 3A). Chilling at high light of leaf discs caused high increase of UCL indicating further oxidation of biomembrane lipids. It was especially higher in the discs treated with tin and lead organic compounds - by 134% and 124%, respectively, in comparison with the control (Fig. 3B). After recovery, UCL of the control discs drastically decreased below the initial level, while of those treated with tin compounds further increased and was higher almost 19-fold than the control. UCL intensity of the discs treated with lead compounds increased slightly after recovery and was 14-fold higher than the control (Fig. 3C). Such changes indicate a strong permanent lipid oxidation process.

These results confirmed the oxidation effect of lead applied in the form of inorganic compounds on plants, described by other authors. Increased lipid peroxidation has been found in roots of the plants *Tithonia diversifolia* growing on roadsides with heavy urban traffic, as well as levels of Pb in leaves and roots considered as typical of contaminated plants, but without visible symptoms of damage to the leaves (Olivares 2003). In the studies of Zacchini (2003) callus cultures of maize cv. Samodek were exposed for 22 months to lead (500 μmol dm^{-3} lead chloride) and oxidative damage and antioxidative response were evaluated. Inductively coupled plasma emission spectroscopy analysis showed that lead entering the cells was accumulated, but its internal concentration was maintained 10-fold less than the external one. Increase of lipid peroxide content indicated that cells underwent a stress condition due to an oxidative attack, counteracted by an increase of antioxidative defence enzyme activity - ascorbate peroxidase and glutathione reductase. Furmanek and Andrzejewska-Ponomarev (2006) found increased amount of secondary products of lipid peroxidation in the roots of tomato plants treated by $Pb(NO_3)_2$ in concentration of 1 mmol dm^{-3}.

In our experiments on suspension of green algae *Scenedesmus quadricauda* treated for 1 h, 2 h and 4 h by $(C_6H_5)_3SnCl$ at concentration of 0.5 μmol dm^{-3} decrease of the measured L_D parameter by 17%, 13% and 11%, respectively, compared to the control (Fig. 4) was observed. However, after 20 h of the treatment the measured value was almost the same as the control. It indicated adaptation of *Scenedesmus quadricauda* to the applied tin compound. Otherwise, in the case of $(C_6H_5)_3PbCl$ in the applied concentration, decrease of L_D was dependent on time of treatment. Reduction of L_D value was equal to ca. 19%, 31%, 43% and 66%, respectively, after 1 h, 2 h, 4 h and 20 h of organolead treatment.

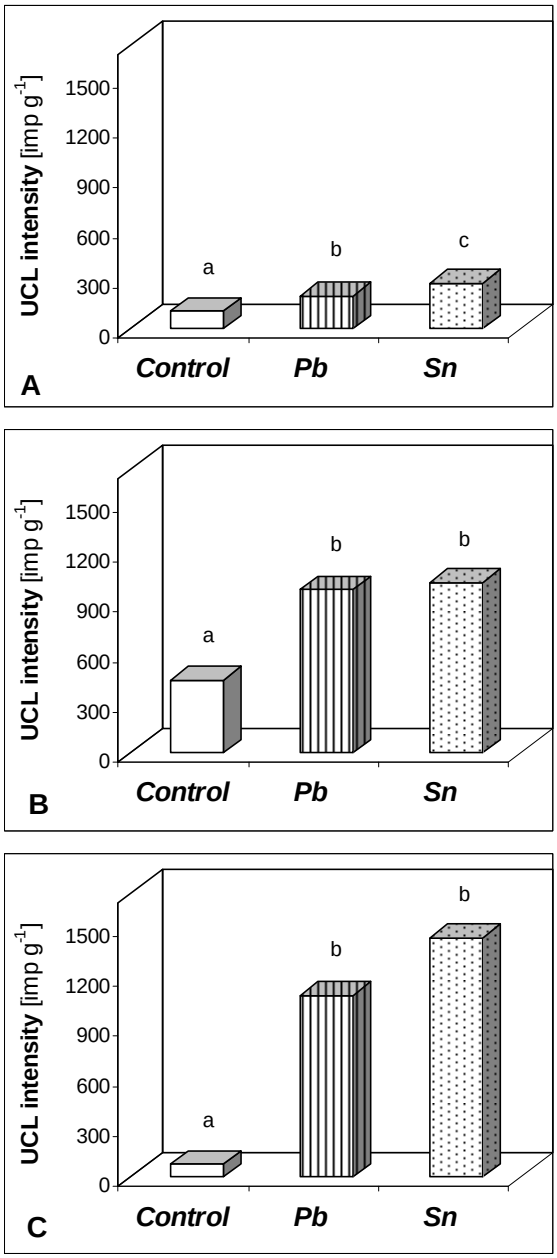

Fig. 3. Values of UCL intensity per 1 g of dry mass of cucumber leaf disc after 20 h treatment by 2 μmol dm^{-3} of $(C_6H_5)_3PbCl$ or $(C_6H_5)_3SnCl$ in darkness at temperature of 21°C (A, experiment 1), after 2 h at 3°C at PPFD 1200 μmol m^{-2} s^{-1} (B, experiment 2), and after 20 h of recovery (C, experiment 3)

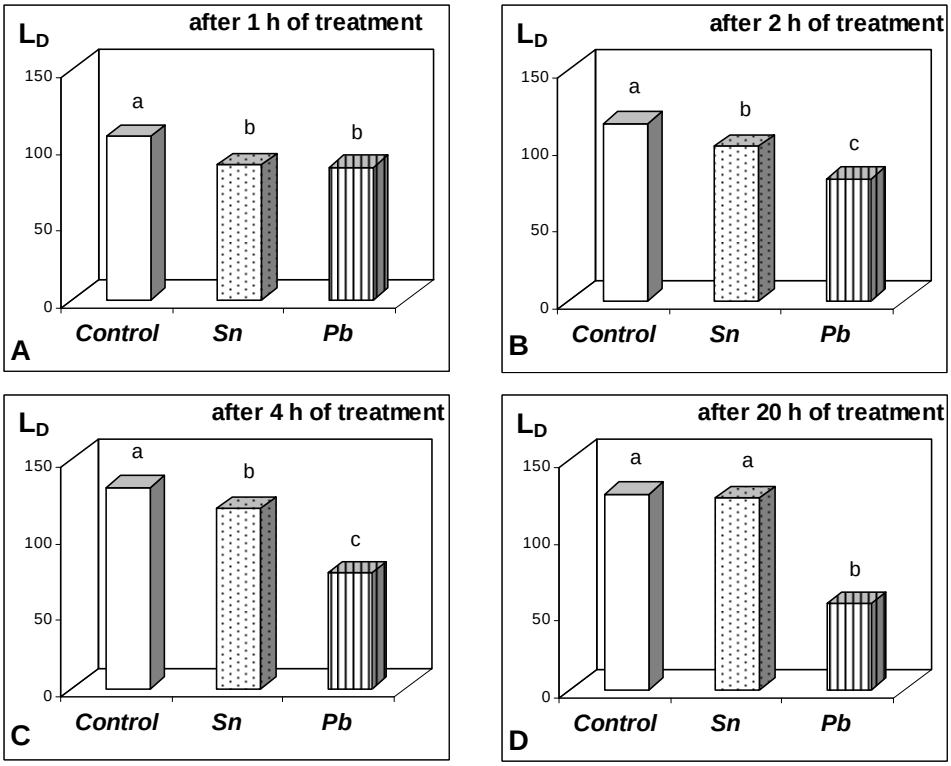

Fig. 4. Values of L_D, rate of delayed luminescence decay of *Scenedesmus quadricauda*, measured after 1 h (A), 2 h (B), 4 h (C) and 20 h (D) of treatment by 0.5 μmol dm^{-3} of $(C_6H_5)_3PbCl$ or $(C_6H_5)_3SnCl$

Lowering of L_D value indicates inhibition of photosynthetic electron transport. 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 Photosystem II (Murkowski and Skórska 1997, Murkowski 2002). The applied lead and tin organic compounds showed similar effect as this type of herbicides, i.e. triazines, urea, diazines. Such a herbicide attaches its particle into molecule D1 (32 kDa) protein inhibiting electron transport between acceptors PQ_A and PQ_B in photosystem II, which causes a decrease of non-cyclic photophosphorylation rate and CO_2 assimilation and changes the intensity of chlorophyll fluorescence.

Some authors studied effects of lead and tin in inorganic forms, at higher concentration than in our experiment, on green algae. Poskuta et al. (1996) showed photosynthesis inhibition of *Chlorella pyrenoidosa* after 24, 48 and 72 h treatment by Pb in concentrations of 0.5, 1.0 and 2.0 mmol dm^{-3}. The magnitudes of inhibition increased with increasing Pb concentration and time of exposure. A similar pattern of inhibition was observed for biosynthesis of chlorophyll, but the magnitudes of inhibition

were smaller. Mohammed and Markert (2005) presented toxic effects of $Pb(NO_3)_2$ H_2O on the biomass of green alga *Scenedesmus 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 lead metal and its concentration and the time of exposure. Bilgrami and Kumar (1997) studied the impact of Pb on the growth of *Chlorella vulgaris* and *Scenedesmus quadricauda*. At concentration of 0.1 g m^{-3} this metal was not toxic, however, at concentration of 10 g m^{-3} the growth of phytoplankton was inhibited. S. *quadricauda* expressed highest tolerance to the metal. Lamaia *et al.* (2005) studied the toxicity and accumulation of lead (Pb) 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 and reduced number of chloroplasts, disintegrated cell wall and death. There were significant decreases in the relative growth and total chlorophyll content when the exposure time and concentration were increased. The accumulation study showed that there were significant increases of metal levels in algal tissue when the exposure time and concentration were increased. The lowest total chlorophyll contents were found in 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) ($SnCl_2.H_2O$) and Sn(IV) (Na_2SnO_3) on the alga *Scenedesmus quadricauda*. Inhibition of growth rates of *Scenedesmus quadricauda* was observed and the Sn^{4+} ion also had lower inhibitory effects on growth rates than the Sn^{2+} ion. The influence of inorganic tin compounds on the unicellular cyanobacterium *Synechocystis aquatilis* was studied by Pawlik-Skowronska *et al.* (1997). Both Sn(II) and Sn(lV), used as chlorides (at concentrations of 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 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 a 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 a content were also observed at a high Sn (II) and Sn(IV) concentration (10 mg dm^{-3}).

CONCLUSIONS

1. The studied organolead and organotin compounds in concentration of 2 μmol dm^{-3} probably initiated free radical oxidation of biolipids in the leaves of cucumber, observed as increased intensity of ultra-weak chemiluminescence. Solution of $(C_6H_5)_3SnCl$ showed higher prooxidation activity compared to the $(C_6H_5)_3PbCl$ compound.

2. The studied compounds applied in concentration of 0.5 μmol dm^{-3} inhibited photosynthetic electron transport of *Scenedesmus quadricauda*, measured as decrease of L_D parameter of chlorophyll delayed luminescence, which indicated similar activity as herbicides – inhibitors of photosynthesis; $(C_6H_5)_3PbCl$ caused stronger effect than $(C_6H_5)_3SnCl$.

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ABBREVIATIONS: AOS − active oxygen species; DL – delayed luminescence; L_d – rate of delayed luminescence decay; PPFD − photosynthetic photon flux density; PS II − photosystem II; UCL − ultraweak chemiluminescence.

PORÓWNANIE FITOTOKSYCZNOŚCI ORGANICZNYCH ZWIĄZKÓW OŁOWIU I CYNY METODAMI LUMINESCENCYJNYMI

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S t r e s z c z e n i e . W pracy badano wpływ związków organometalicznych, zawierających ołów $(C_6H_5)_3PbCl$ i cynę $(C_6H_5)_3SnCl$ na liście ogórka oraz glony *Scenedesmus quadricauda*. Oba związki o stężeniu 2 μmol dm^{-3} spowodowały zwiększoną emisję ultrasłabej chemiluminescencji (UCL) krążków liści ogórka po 20 godzinach działania, a aktywność chlorku trifenylocyny była większa niż chlorku trifenyloołowiu. Krążki poddane stresowi chłodu przy silnym świetle, traktowane badanymi związkami wykazały zwiększone natężenie UCL w porównaniu z kontrola. Zawiesina glonów *Scenedesmus quadricauda* poddana działaniu $(C_6H_5)_3SnCl$ o stężeniu 0,5 μmol·dm^{-3} w ciągu 1 h, 2 h lub 4 h miała obniżone wartości parametru L_D wskazującego na inhibicję fotosyntetycznego transportu elektronów, jednak po 20 h działania tego związku zaobserwowano adaptację *Scenedesmus quadricauda* do cyny. W przypadku związku ołowiu $(C_6H_5)_3PbCl$ nastąpiło dalsze obniżenie L_D zależne od czasu jego działania.

S ł o w a k l u c z o w e : *Cucumis sativus* L., opóźniona luminescencja, *Scenedesmus quadricauda* (Turp.), ultrasłaba chemiluminescencja, związki organiczne cyny i ołowiu