doi: 10.4149/gpb_2013074

Effect of Pb^{2+} ions on photosynthetic apparatus

Frantisek Sersen[1], Katarina Kralova[1], Matus Pesko[2] and Marek Cigan[1]

[1] *Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*
[2] *Department of Environmental Ecology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*

Abstract. Using model lead compounds $Pb(NO_3)_2$ and $Pb(CH_3CHOO)_2$ the mechanism and the site of action of Pb^{2+} ions in the photosynthetic apparatus of spinach chloroplasts were studied. Both compounds inhibited photosynthetic electron transport (PET) through photosystem 1 (PS1) and photosystem 2 (PS2), while $Pb(NO_3)_2$ was found to be more effective PET inhibitor. Using EPR spectroscopy the following sites of Pb^{2+} action in the photosynthetic apparatus were determined: the water-splitting complex and the $Z^{\bullet}/D^{\bullet}$ intermediates on the donor side of PS2 and probably also the ferredoxin on the acceptor side of PS1, because cyclic electron flow in chloroplasts was impaired by treatment with Pb^{2+} ions. Study of chlorophyll fluorescence in suspension of spinach chloroplasts in the presence of Pb^{2+} ions confirmed their site of action in PS2. Using fluorescence spectroscopy, also formation of complexes between Pb^{2+} and amino acid residues in photosynthetic proteins was confirmed and constants of complex formation among Pb^{2+} and aromatic amino acids were calculated for both studied lead compounds.

Key words: EPR — Fluorescence spectroscopy — Lead(II) acetate — Lead(II) nitrate — Photosynthetic electron transport

Abbreviations: AAA, aromatic amino acids; Chl*a*, chlorophyll *a*; DCPIP, 2,6-dichlorophenol-indophenol; DPC, 2,5-diphenylcarbazide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; LHCII, light harvesting complex of PS2; Q_B, the second plastoquinone electron acceptor; PET, photosynthetic electron transport; PS, photosystem.

Introduction

Lead is a major pollutant in both terrestrial and aquatic ecosystems. Phytotoxicity of this metal is connected with its adverse effects on physiological and biochemical processes in plants including photosynthesis, enzyme activities, nutrient uptake, water status, stomata closure and induction of reactive oxygen species (Sharma and Dubey 2005). Pb ions strongly inhibit the key enzyme of chlorophyll biosynthesis δ-amino laevulinate dehydrogenase (Prasad and Prasad 1987) and enhance chlorophyll degradation due to increased chlorophyllase activity (Drazkiewicz 1994). The inhibition of metalloenzymes is connected with the displacement of the essential metals by Pb.

Correspondence to: Frantisek Sersen, Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská dolina CH2, 845 15 Bratislava, Slovak Republic
E-mail: sersen@fns.uniba.sk

Among the articles dealing with lead toxicity to plants only a small number is focused on its effects on the photosynthetic electron transport (PET). Artificial electron donors of photosystem 2 (PS2) – 2,5-diphenylcarbazide (DPC), NH_2OH, $MnCl_2$ and benzidine – substantially reversed the Pb-induced inhibition of 2,6-dichlorophenol-indophenol (DCPIP) photoreduction as well as the reduction of chlorophyll *a* (Chl*a*) fluorescence in *Nostoc muscorum*. Moreover, inhibition of electron flow also on the acceptor side of the PS2 reaction centre was confirmed at high Pb concentrations what was reflected in an increase in Chl*a* fluorescence (Prasad et al. 1991). Qufei and Fashui (2009) studied the effects of Pb^{2+} ions on the secondary structure and function of PS2 of *Spirodela polyrrhiza* and found that Pb^{2+} ions accumulated in PS2 and damaged its secondary structure, decreased the absorbance of visible light, inhibited energy transfer among amino acids within the PS2 protein-pigment complex, and reduced energy transport from tyrosine residue to Chl*a*. Rashid and Popovic (1990) observed that the degree of PS2

inhibition by Pb^{2+} was higher in the protein depleted (due to the loss of endogenous Ca^{2+}) than in intact PS2, however, the interaction of Pb^{2+} with $CaCl_2$ in protein-depleted PS2 was competitive indicating that Pb^{2+} competes for binding to the Ca^{2+} and Cl^- active sites in the water-splitting complex. These researchers concluded that Pb^{2+}-induced inhibition of PS2 activity was mediated *via* the water-splitting system because Pb^{2+} inhibition of PS2 activity cannot be reversed by $CaCl_2$ but it can be reversed by DPC.

Treatment of PS2 membranes isolated from spinach with Pb^{2+} resulted in dissociation of three extrinsic polypeptides (17 kDa, 23 kDa and 33 kDa) which protect the binding sites of native inorganic cofactors (Cl^-, Ca^{2+} and Mn^{2+}) in the oxygen evolving complex. However, dissociating action of Pb^{2+} could be strongly prevented by Ca^{2+} *(*Rashid et al. 1994). According to Ahmed and Tajmir-Riahi (1993) conformational changes in light-harvesting chlorophyll (LHCII) subunits induced by Pb treatment may lead to incomplete assembly followed by degradation. High affinity of Pb^{2+} to the *N*- and *S*-donor ligands occurring in proteins results in damage of the photosynthetic apparatus. Miles at al. (1972) found that some lead salts inhibited PET in spinach chloroplasts and fluorescence induction curves indicated as the primary site of inhibition the donor side of PS2.

Study of Pb^{2+} effects on energy distribution and photochemical activity of spinach chloroplasts showed a decrease of the absorption band intensity of chloroplasts in red and blue region, decline of fluorescence quantum yield nearby 680 nm as well as the decline of excitation band nearby 440 nm. Pb^{2+} ions decreased absorption of light in spinach chloroplasts, inhibited excitation energy to be absorbed by LHCII and transferred to PS2. They reduced the conversion of light energy to electron energy, and decelerated electron transport, water photolysis and oxygen evolution. On the other hand, photoreduction activities of PS1 were little changed (Wu et al. 2008).

The goal of this work was to find the mechanism and the site of action of Pb^{2+} ions in photosynthetic apparatus using model lead compounds $Pb(NO_3)_2$ – lead(II) nitrate, and $Pb(CH_3CHOO)_2$ – lead(II) acetate.

Material and Methods

All chemicals used in this study were of analytically grade. 2,6-Dichlorophenol-indophenol (DCPIP), 1,5-diphenylcarbazide (DPC), TRIS, $MgCl_2$, saccharose, 1,1`-dimethyl-4,4`-bipyridinium dichloride hydrate (methyl viologen) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) were purchased from Centralchem (Slovakia). $Pb(NO_3)_2$ and $Pb(CH_3COO)_2 \times 3H_2O$ supplied from Lachema (Czech Republic) were recrystallized before experiments.

Chloroplasts were prepared from fresh market spinach by the method published by Šeršeň at al. (1990) using TRIS buffer (20 mmol/l TRIS, 0.4 mmol/l saccharose, 20 mmol/l $MgCl_2$, pH = 7.2). The chlorophyll content was determined according to Lichtenthaler (1987).

The PET through PS2, from H_2O to plastoquinone Q_B, was monitored spectrophotometerically (Genesis 6, Thermo Scientific, USA) as a photoreduction of DCPIP according to Kráľová et al. (1992). DPC, an artificial donor of PS2, was used for the detection of PET through PS2 (from intermediate Z to Q_B) according to Xiao et al. (1997) and Šeršeň at al. (2000). The rate of PET through PS1 was detected spectrophotometrically by the photooxidation of $DCPIPH_2$ according to Šeršeň at al. (2000) using methyl viologen as a final electron acceptor of PS1. Chlorophyll concentration in chloroplast suspension was 30 mg/l.

Chlorophyll fluorescence of spinach chloroplasts at room temperature was recorded by spectrofluorimeter FSP 920 (Edinburgh Instruments, UK) using excitation wavelength λ_{ex} = 436 nm. Fluorescence of aromatic amino acids was monitored by spectrophotometer F-2000 (Hitachi, Tokyo, Japan) using excitation wavelength λ_{ex} = 275 nm according to our previous work (Šeršeň and Kráľová 2001). Both fluorescence experiments were performed in 1 cm fluorescence cell in the right-angle arrangement. The chlorophyll concentration in chloroplast suspension was 10 mg/l. In low temperature experiments a chloroplast suspension applied on filter paper was inserted into a Dewar finger with liquid nitrogen and spectra were scanned using a device F-4500 (Hitachi, Tokyo, Japan).

EPR measurements were performed by an instrument ERS 230 (ZWG AdW, Berlin, Germany) operating in the X-band according to Šeršeň and Kráľová (2001). The chlorophyll concentration in chloroplast suspension was 4.0 g/l.

All above mentioned experiments (except low temperature fluorescence measurements) were carried out at 25°C.

Results and Discussion

Both lead compounds inhibited DCPIP photoreduction. Their effectiveness expressed as IC_{50} value, i.e. concentration causing 50% decrease of DCPIP photoreduction related to control chloroplasts, was 337 μmol/l for lead(II) nitrate and 643 μmol/l for lead(II) acetate. It means that PET through PS2 was restricted and application of an ionic compound $Pb(NO_3)_2$ resulted in significantly higher PET inhibition than application of a chelate $Pb(CH_3CHOO)_2$. Similar inhibition of DCPIP photoreduction caused by Pb^{2+} was also observed by Miles at al. (1972), Rashid and Popovic (1990), Prasad et al. (1991) and Romanowska et al. (2012). The higher inhibitory effect of $Pb(NO_3)_2$ can be connected with its easier dissociation in water sur-

roundings in comparison with $Pb(CH_3COO)_2$ what is reflected in formation constants ($k_1 = [ML]/[M]\cdot[L]$ and $k_2 = [ML_2]/[M]\cdot[L]^2$) of these compounds, where [M],[L], [ML], and $[ML_2]$ are concentrations of Pb^{2+}, ligand and complexes of metal with one and two ligands, respectively. For $Pb(NO_3)_2$, $k_1 = 1.78 – 2.24$ and $k_2 = 2.51– 3.16$ (Smith and Martel 1976). For $Pb(CH_3COO)_2$, $k_1 = 141$ and $k_2 = 3162$ (Martel and Smith 1976).

In order to determine if PS2 is damaged on the donor or on the acceptor side of PS2, an experiment with DPC was carried out. It is known that artificial electron donor DPC can supply electrons in the site of intermediate Z of the PET chain. After adding DPC to chloroplasts in which DCPIP reduction was inhibited up to 90% by studied lead compounds, the photoreduction of DCPIP was completely restored. This indicates that the section from the intermediate Z to the secondary quinone acceptor Q_B in the PET chain was not damaged. Consequently, it can be concluded that the donor side of PS2 is damaged in the section between the water-splitting complex and the intermediate Z.

For determination of the site of Pb^{2+} action also EPR technique was used. In Fig. 1b and 1c EPR spectra of chloroplasts treated with studied lead compounds are presented. The comparison of these spectra with EPR spectrum of control chloroplasts (Fig. 1a) showed that Pb^{2+} decreased both EPR signals II, i.e. signal II_{slow} and signal $II_{very\ fast}$. Signal II_{slow} (g = 2.0046, $\Delta B_{PP} \sim 2$ mT) is good observable in EPR spectrum of control chloroplasts recorded in the dark (Fig. 1a, full line) and it belongs to intermediate $D^{\bullet}$, which is a tyrosine residue in the 161st position of D_2 protein on the donor side of PS2 (Debus 1988a). The decrease of signal II_{slow} by studied Pb^{2+} compounds can be observed in Figs. 1b,c – full lines). As mentioned above, Pb^{2+} also decreased the signal $II_{very\ fast}$ (Fig. 1b,c – dotted lines) which belongs to the intermediate $Z^{\bullet}$, which is a tyrosine residue in the 161st position of D_1 protein on the donor side of PS2 (Debus 1988b). Signal $II_{very\ fast}$ (g = 2.0046, $\Delta B_{PP} \sim 2$ mT) is good observable in EPR spectra of control chloroplasts (Fig. 1a) as a difference between spectrum recorded in the dark (Fig. 1a – full line) and spectrum recorded in the light (Fig. 1a – dotted line).

The above-mentioned tyrosines $Z^{\bullet}$ and $D^{\bullet}$ mediate the electron transfer from water-splitting complex to the core of PS2 (P680). The decrease of intensities of both EPR signals belonging to redox-active tyrosines $Z^{\bullet}$ and $D^{\bullet}$ suggests that these intermediates or their near surroundings were damaged by the studied lead compounds and consequently, PET was inhibited. This assumption was also confirmed by the fact, that DPC restored DCPIP photoreduction in chloroplasts treated with the studied lead compounds.

Thus, PET interruption results in a disability to supply electrons to PS1 and consequently a great increase of the signal I is observable in EPR spectra of chloroplasts treated

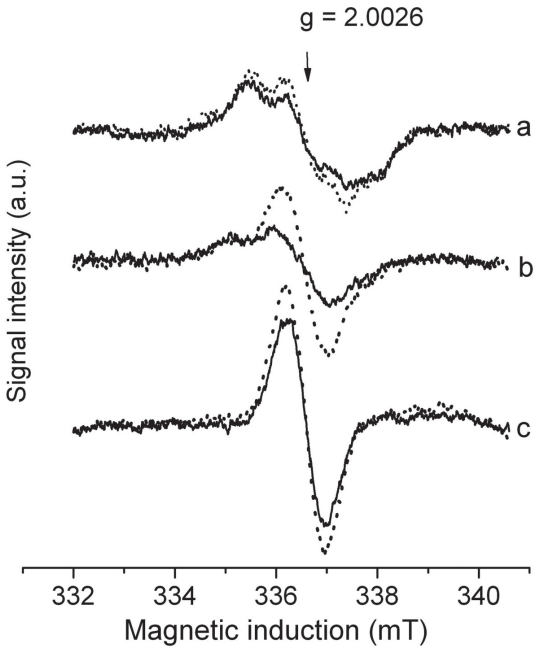

Figure 1. EPR spectra of untreated spinach chloroplasts (a) and chloroplasts treated with 0.05 mol/l lead(II) acetate (b) or lead(II) nitrate (c) recorded in the dark (full lines) and in the light (dotted lines).

with Pb^{2+} (Fig. 1b,c – dotted lines). The EPR signal I (g = 2.0026, $\Delta B_{PP} = 0.8$ mT) belongs to oxidized core of PS1 ($P700^+$, i.e. chlorophyll *a* dimer) (Hoff 1979). In control chloroplasts, if PET between PS2 and PS1 is not interrupted, signal I is almost invisible (its intensity is very low). In EPR spectra of chloroplasts treated by the studied lead compounds increased intensities of signal I can be observed also in the dark (Fig. 1b,c – full lines) indicating that PS1 could be impaired. A damage of PS1 was also confirmed by an experiment using photooxidation of $DCPIPH_2$ (reduced 2,6-dichlorophenol indophenol serves as artificial electron donor for PS1). In control chloroplasts treated by DCMU, a herbicide which interrupts PET from PS2 to PS1 but does not damage PS1, application of $DCPIPH_2$ restored PET through PS1. On the contrary, in chloroplasts treated with studied lead compounds PET through PS1 did not occur. Consequently, appearance of EPR signal I already in the dark suggested that Pb^{2+} impaired the non-cyclic and cyclic electron flow through PS1.

Moreover, using EPR spectroscopy it was manifested that both studied lead compounds released Mn^{2+} ions from the manganese cluster of water-splitting complex into interior of thylakoid membranes. In Fig. 2 are recorded EPR spectra of free Mn^{2+} ions in spinach chloroplasts treated with studied lead compounds, which consist from six lines of the fine structure. EPR spectra of Mn^{2+} ions cannot be observable in

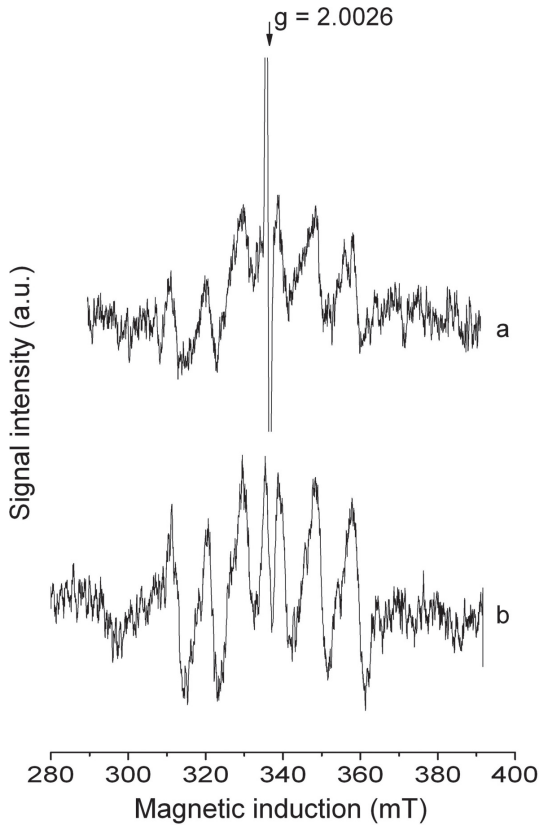

Figure 2. EPR spectrum of spinach chloroplasts treated with 0.05 mol/l of $Pb(NO_3)_2$ (a) or $Pb(CH_3COO)_2$ (b).

intact chloroplasts due to their strong spin-spin interaction in the manganese cluster. The narrow singlet line at g = 2.0026 (between manganese lines 3 and 4) belongs to $P680^+$.

The damage of photosynthetic apparatus was also confirmed by fluorescence experiments. In Fig. 3 fluorescence emission spectra of chloroplasts treated by Pb^{2+} compounds are presented. From these fluorescence emission spectra recorded at 25°C (Fig. 3A) and at temperature of liquid nitrogen (Fig. 3B) it is evident that both studied lead compounds caused a decrease of chlorophyll fluorescence. It is known that emission band at 684 nm belongs to Chl*a* occurring in pigment protein complexes in PS2 (Govindjee 1995). On the other hand, the shoulder at 740 nm is caused by fluorescence emission of Chl*a* in pigment-protein complexes in PS1 (Govindjee 1995). From Fig. 3A it seems that studied lead compounds quench Chl*a* fluorescence in both photosystems, but especially in PS2. To confirm this assumption, fluorescence measurements were carried out at liquid nitrogen temperature. Because the method used for low-temperature fluorescence measurements (deposition of chloroplasts on filter paper, which was then immersed in a Dewar finger with liquid nitrogen) did not provide reproducible results concerning the absolute values of fluorescence intensity, we used the ratio of signal intensity from Chl*a* in PS1 to that in PS2. For control sample this ratio is about 1.1. On the other hand, in chloroplasts treated with Pb^{2+} it significantly increased to 1.3–1.5, however only at higher lead concentrations (6.8 mmol/l of Pb^{2+}). Fig. 3B shows typical fluorescence spectra obtained at liquid nitrogen temperature (the spectra were normalized to the signal originating from PS1 chlorophylls). From these measurements we conclude that the Pb^{2+} interacts mainly with pigment-protein complexes in PS2. This result is in accordance with the finding of Miles et al. (1972)

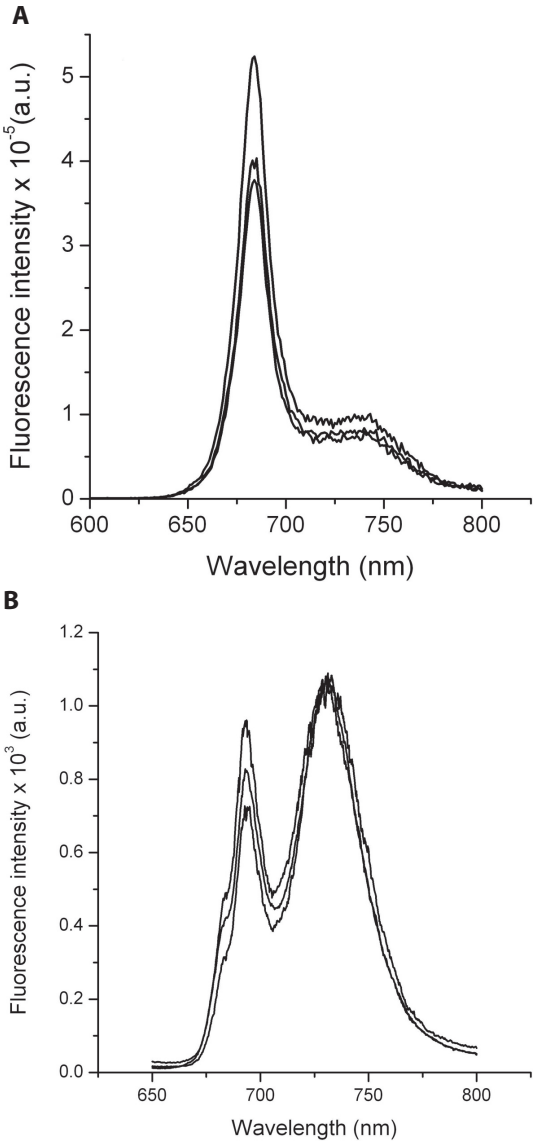

Figure 3. Fluorescence emission spectra of Chl*a* of untreated chloroplasts (upper line) and chloroplasts treated by $Pb(CH_3COO)_2$ (middle line) and $Pb(NO_3)_2$ (bottom line). **A.** Spectra recorded at 25°C (lead concentration: 0.034 mol/l). **B.** Spectra recorded at temperature of liquid nitrogen (lead concentration: 0.068 mol/l).

that Pb^{2+} interacts with PS2 components occurring between P680 and the water oxidizing complex.

The interaction of Pb^{2+} with photosynthetic proteins was observed by fluorescence quenching of aromatic amino acids (AAA) occurring in these proteins. In Fig. 4A fluorescence emission spectra of AAA treated with Pb(CH3CHOO)2 are shown. The intensity of fluorescence emission band at λ = 334 nm decreased with increasing Pb^{2+} concentration. This fluorescence quenching is due to the formation of Pb^{2+} complexes with AAA. Fig. 4B presents dependences of the decrease of AAA fluorescence intensity (expressed as % of control) on the concentrations of $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$. The differences between fluorescence quenching caused by both investigated Pb^{2+} compounds were within experimental error. From the presented dependence following apparent stability constants of Pb^{2+} complexes with AAA were calculated according to Šeršeň and Kráľová (2001): $k_1 = 6942 \pm 1843$ ($r^2 = 0.9617$) for $Pb(NO_3)_2$ and $k_1 = 6067 \pm 1666$ ($r^2 = 0.9591$) for $Pb(CH_3COO)_2$. These calculations were carried out with respect to above presented formation constants k_2 of studied lead compounds, i.e. 3.16 for $Pb(NO_3)_2$ and 3162 for $Pb(CH_3COO)_2$, respectively. The differences between apparent stability constants evaluated for both studied Pb^{2+} compounds did not differ significantly at $p = 0.05$. Stability constant for the complex Pb^{2+}-tryptophan published by Lone (2010) determined by pH-metric titrations is k = 7585. Lower values of k determined in our experiment could be connected with the fact that in our experiments trypthophan residues as well residues of further aromatic amino acids (tyrosine and phenylalanine) are constituents of photosynthetic proteins. The apparent stability constants of Hg^{2+} and Cd^{2+} with aromatic amino acids determined previously using fluorescence method were 10200 and 3700, respectively (Šeršeň and Kráľová 2001). The stability of metal ion-cellular target complexes increases with the magnitude of the covalent and ionic index. The metals ions Hg^{2+}, Pb^{2+} and Cd^{2+} have a similar ionic index with a descending order of covalent index: $Hg^{2+} > Pb^{2+} >$ and Cd^{2+} (Nieboer et al. 1999). This order is similar to that of toxicity estimated by IC_{50} values (28.337 and 1000 μmol/l for Hg and Cd, respectively) as well as stability constants of metal-AAA complexes in spinach chloroplasts determined by fluorescence spectroscopy (Šeršeň and Kráľová 2001).

Conclusion

It was found that lead(II) nitrate and lead(II) acetate inhibited PET through PS2 and PS1. Using EPR spectroscopy the sites of action of Pb^{2+} were found to be the water-splitting complex and the Z/D intermediates on the donor side of PS2. This fact was first time directly confirmed in this study by registration of Mn^{2+} ions, which were released from manganese cluster. Beside this, the acceptor side of PS1 is impaired (ferredoxin and cyclic electron flow). The mechanism of inhibitory action of $Pb(NO_3)_2$ and $Pb(CH_3CHOO)_2$ consists in the formation of complexes between Pb^{2+} and amino acid residues in photosynthetic proteins as was confirmed by fluorescence spectroscopy. Our results are in a good accordance with former findings of Miles et al. (1972), Rashid and Popovic (1990), Prasad et al. (1991) and Romanowska et al. (2012).

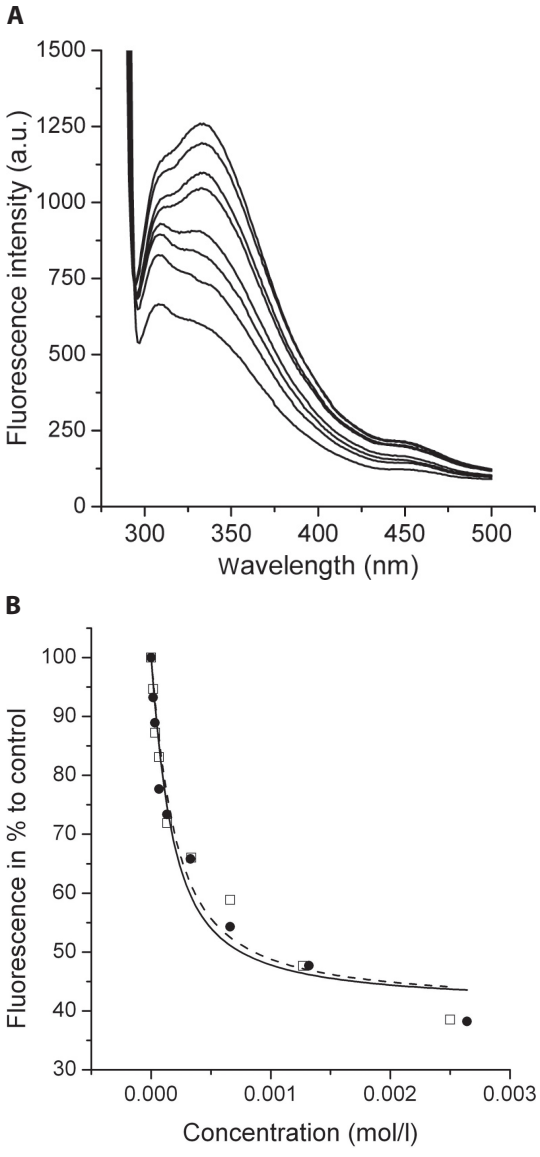

Figure 4. A. Fluorescence emission spectra of aromatic amino acids in untreated spinach chloroplasts and in presence of $Pb(CH_3COO)_2$: 0, 16.75, 33.5, 67,134, 335, 670, 1340 and 2680 μmol/l (curves from top to bottom). **B.** Dependence of the fluorescence intensity of aromatic amino acids on the concentration of $Pb(NO_3)_2$ (squares and full line) and $Pb(CH_3COO)_2$ (circles and dashed line).

Acknowledgement. Authors thank Dr. Andrej Pavlovič from Department of Biophysics, Faculty of Science, Palacky University in Olomouc, for liquid nitrogen fluorescence measurements.

No conflicts of interest, financial or otherwise, are declared by the author(s).

References

Ahmed A., Tajmir-Riahi H. A. (1993): Interaction of toxic metal ions Cd^{2+}, Hg^{2+}, and Pb^{2+} with light harvesting proteins of chloroplast thylakoid membranes: An FTIR spectroscopic study. J. Inorg. Biochem. **50,** 235–243 http://dx.doi.org/10.1016/0162-0134(93)80050-J

Debus R. J., Barry D. A., Babcock G. T., McIntosh L. (1988a): Site-directed mutagenesis identifies a tyrosine radical involved in the photosynthetic oxygen-evolving system. Proc. Natl. Acad. Sci. USA **85,** 427–430 http://dx.doi.org/10.1073/pnas.85.2.427

Debus R. J., Barry D. A., Sithole I., Babcock G. T., McIntosh L. (1988b): Directed mutagenesis indicates that donor to $P680^{+}$ in photosystem II is tyrosine-161 of the D1 polypeptide. Biochemistry **27,** 9071–9074 http://dx.doi.org/10.1021/bi00426a001

Drazkiewicz M. (1994): Chlorophyll-occurrence, functions, mechanism of action, effects of internal and external factors. Photosynthetica **30,** 321–331

Govindjee (1995): Sixty-three years since Kautsky: Chlorophyll *a* fluorescence. Aust. J. Plant. Physiol. **22,** 131–160 http://dx.doi.org/10.1071/PP9950131

Hoff A. J. (1979): Application of ESR in photosynthesis. Phys. Rep. **54,** 75–200; DOI: 10.1016/0370-1573(79) 90016-4

Kráľová K., Šeršeň F., Sidóová E. (1992): Photosynthesis inhibition produced by 2-alkylthio-6-R-benzothiazoles. Chem. Pap. **46,** 348–350

Lichtenthaler H. K. (1987): Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. **148,** 350–382 http://dx.doi.org/10.1016/0076-6879(87)48036-1

Lone M. A. (2010): Studies on complexes of TI(I), Pb(II) and Bi(III) with some potential bioligands. Ph.D. thesis. The University of Kashmir, Srinagar

Martel A. E., Smith R. M. (1976): Other organic ligands. In: Critical Stability Constants. Vol. 3, p. 6, Plenum Press

Miles C. D., Brandle J. R., Daniel D. J., Chu-Der O., Schnare P. D., Uhlik D. J. (1972): Inhibition of photosystem II in isolated chloroplasts by lead. Plant Physiol. **49,** 820–825 http://dx.doi.org/10.1104/pp.49.5.820

Nieboer E., Fletcher G. G., Thomassen Y. (1999): Relevance of reactivity determinants to exposure assessment and biological monitoring of the elements. J. Environ. Monit. **1,** 1–14 http://dx.doi.org/10.1039/a808849g

Prasad D. D. K., Prasad A. R. K. (1987): Altered δ-aminolaevulinic acid metabolism by lead and mercury in germinating seedlings of Bajra (*Pennisetum typhoideum*). J. Plant Physiol. **127,** 241–249 http://dx.doi.org/10.1016/S0176-1617(87)80143-8

Prasad S. M., Singh J. B., Rai L. C., Kumar H. D. (1991): Metal-induced inhibition of photosynthetic electron transport chain of the cyanobacterium *Nostoc muscorum*. FEMS Microbiol. Lett. **82,** 95–100 http://dx.doi.org/10.1111/j.1574-6968.1991.tb04846.x

Qufei L., Fashui H. (2009): Effects of Pb^{2+} on the structure and function of photosystem II of *Spirodela polyrrhiza*. Biol. Trace Elem. Res. **129,** 251–260 http://dx.doi.org/10.1007/s12011-008-8283-8

Rashid A., Popovic R. (1990): Protective role of $CaCl_2$ against Pb^{2+} inhibition in Photosystem II. FEBS Lett. **271,** 181–184 http://dx.doi.org/10.1016/0014-5793(90)80401-4

Rashid A., Camm E. L., Ekramoddoullah K. M. (1994): Molecular mechanism of action of Pb^{2+} and Zn^{2+} on water oxidizing complex of photosystem II. FEBS Lett. **350,** 296–298 http://dx.doi.org/10.1016/0014-5793(94)00789-6

Romanowska E., Wasilewska W., Fristedt R,. Vener A. V, Zienkiewicz M. (2012): Phosphorylation of PSII proteins in maize thylakoids in the presence of Pb ions. J. Plant Physiol. **169,** 345–352 http://dx.doi.org/10.1016/j.jplph.2011.10.006

Šeršeň F., Balgavý P., Devínsky F. (1990): Electron spin resonance study of chloroplast photosynthetic activity in the presence of amphiphilic amines. Gen. Physiol. Biophys. **9,** 625–633

Šeršeň F., Kráľová K., Macho V. (2000): New findings about the inhibitory action of phenylcarbamates and phenylthiocarbamates on photosynthetic apparatus. Pest. Biochem. Physiol. **68,** 113–118 http://dx.doi.org/10.1006/pest.2000.2499

Šeršeň F., Kráľová K. (2001): New facts about $CdCl_2$ action on photosynthetic apparatus of spinach chloroplasts and its comparison with $HgCl_2$ action. Photosynthetica **39,** 575–580 http://dx.doi.org/10.1023/A:1015612330650

Sharma P., Dubey R. S. (2005): Lead toxicity in plants. Braz. J. Plant Physiol. **17,** 35–52 http://dx.doi.org/10.1590/S1677-04202005000100004

Smith R. M., Martel A. E. (1976): Inorganic complexes. In: Critical Stability Constants. Vol. 4, p. 51, Plenum Press http://dx.doi.org/10.1007/978-1-4757-5506-0

Wu X., Hong F., Liu C., Su M., Zheng L., Gao F., Yang F. (2008): Effects of Pb^{2+} on energy distribution and photochemical activity of spinach chloroplast. Spectrochim. Acta A: Mol. Biomol. Spectrosc. **69,** 738–742 http://dx.doi.org/10.1016/j.saa.2007.05.047

Xiao R., Ghosh S., Tanaka A. R., Greenberg B. M., Dumbroff E. B. (1997): A rapid spectrophotometric method for measuring Photosystem I and Photosystem II activities in a single sample. Plant Physiol. Biochem. **35,** 411–417

Received: July 16, 2013
Final version accepted: October 1, 2013