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RESPONSES OF PHOTOSYSTEM I AND II ACTIVITIES OF *MICROSORUM PTEROPUS* BLUME TO Pb^{2+} TOXICITY

DENG CHUN-NUAN* AND FAN TAO

Key Lab of Plateau Lake Ecology & Global Change, College of Tourism and Geographic Science, Yunnan Normal University, Kunming 650500, China

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Abstract

Effects of Pb^{2+} on photosystem activities, cyclic electron flow (CEF) and proton motive force of aquatic fern *Microsorum pteropus* Blume were evaluated. Roots and shoots of *M. pteropus* accumulated high concentration of Pb^{2+} after exposure to 5 mg/l Pb^{2+} for 7 days. Quantum yield of photosystem I (PSI) and II (PSII) decreased with increasing Pb^{2+} concentration.

Lead (Pb^{2+}) is one of the most common toxic heavy metals in water environments. Pb^{2+} may reach around 1000-folds above its natural levels in contaminated water (Belatik *et al*. 2013). High concentration of Pb^{2+} disturbs many physiological processes in plants (Leal-Alvarado *et al*. 2016). Pb^{2+} affects photosynthetic pigments, photosynthetic apparatus, enzymic activity and causes retardation of plant growth (Islam *et al*. 2008). Pb^{2+} was found to reduce oxygen evolution (Wu *et al.* 2008). Pb^{2+} interacts with the water oxidation complex and thus perturbs charge recombination and electron transport (Belatik *et al.* 2013). Some studies revealed that some heavy metals damage PSI reaction centers (Kojima *et al.* 1987) and decrease the PSI activity (Qian *et al.* 2009). It was also reported that Pb had limited toxicity to plants and had no significant effect on quantum yield or PSII photochemical efficiency (Dao and Beardall, 2016).

It is generally considered that photosystem I (PSI) is less sensitive than PSII under various environmental stresses, however, the influence of Pb^{2+} on the PSI and PSII of plant is still largely unknown. Effects of Pb^{2+} on the activities in PSI and PSII, membrane potential ($\Delta\psi$) and proton gradient (ΔpH) of *Microsorum pteropus* were examined. The Dual-PAM-100 system was used to probe the effects of Pb^{2+} on PSI and PSII functions and membrane potential ($\Delta\psi$) and proton gradient (ΔpH) monitored by P515 signal detector.

The aquatic fern *M. pteropus* plants were grown in Pb-free water at 25°C under 100 µmol photons/m^2/s illumination with a 12 : 12 hrs light: dark cycle. Lead nitrate ($Pb(NO_3)_2$) of analytical grade was dissolved in distilled water and diluted to a series of desired Pb^{2+} concentrations (0 - 5 mg/l). Tested plants were grown in solution containing various concentrations of Pb^{2+}. The treatment without Pb^{2+} was used as the control. PSI and PSII activities of the plants were measured after 1, 3, 5 and 7 days of Pb^{2+} stress experiments. The membrane potential and the proton gradient, lead content in plant tissue were measured at the end of experiment. The Pb^{2+} content in plants was determined by inductively coupled plasma mass spectrometry (ICP-MS) after 7 days of exposure to Pb^{2+}.

PSI and PSII activities of *M. pteropus* leaves were measured simultaneously using a Dual-PAM-100 system (Heinz Walz GmbH, Effeltrich, Germany). Cyclic electron flow (CEF) and yield of cyclic electron flow [Y(CEF)] were calculated from the data of slow induction curve. The quantum yield of cyclic electron flow [Y(CEF)] was the difference between Y(I) and Y(II). Transthylakoid proton gradient (ΔpH) and membrane potential ($\Delta\psi$) can be measured automatically

∗ Author for correspondence: <dengchunnuan@126.com>.

by extended P515/535 emitter-detector modules of the Dual-PAM-100 system (Schreiber and Klughammer 2008).

After 7 days of Pb^{2+} treatment, the concentration of Pb^{2+} accumulated in plant tissues increased with increasing Pb^{2+} concentration in water (Table 1). Pb^{2+} content in shoot and root of *M. pteropus* grown in 5 mg/l Pb^{2+} solution was 2358 and 3396 μg/g dry tissue, respectively, indicating the hyperaccumulation of Pb of *M. pteropus*.

Table 1. Pb^{2+} accumulation in shoot and root of *M. pteropus* after exposure to different content of Pb^{2+}.

Pb^{2+} (mg/l)	Shoot (μg/g dry tissue)	root(μg/g dry tissue)
control	0	0
0.05	73 ± 8	81 ± 12
0.1	52 ± 18	200 ± 43
0.5	310 ± 56	829 ± 125
1	649 ± 92	1055 ± 187
5	2358 ± 645	3396 ± 623

Energy conversion in PSI and PSII was significantly influenced by Pb^{2+} (Table 2). Y(I) and Y(II) decreased gradually with exposure time. Y(I) (quantum yield of PSI) decreased with increasing Pb^{2+} concentration, accompanied with the increase of Y(ND) (PSI donor side limitation). Y(II) (quantum yield of PSII) significantly decreased and Y(NO) (quantum yield of non-light-induced non-photochemical fluorescence quenching) increased as Pb^{2+} concentration increased. Interestingly, Y(I) was adversely affected much more than Y(II) by Pb^{2+}. For example, Y(I) decreased by 35%, while Y(II) decreased by 19% for 5 mg/l Pb^{2+} treatment in comparison with the control. The yield of cyclic electron flow [Y(CEF)] was also inhibited by various concentrations of Pb^{2+}.

Above results showed that PSI of *M. pteropus* was more sensitive than PSII to the toxicity of Pb^{2+} and was a major target for Pb^{2+} stress. This is in contrast to previous studies which reported that PSI is less affected than PSII under environmental stresses such as heavy metals (Wu *et al.* 2008). The donor side limitation [Y(ND)] increased by Pb^{2+} toxicity (Table 2). Similarly, Wodala *et al*. (2012) reported similar result. The target sites of heavy metals in PSI seems to be dependent on the metal species. For example, Pb^{2+} decreased the active P700 content by 28% (Wong and Govindjee 1976) but Cu^{2+} did not affect the function of P_{700} in isolated thylakoids (Šeršeň *et al.* 1997). Murthy and Mohanty (1993) reported that 6 μM mercury, an inhibitor of plastocyanin, reduced 50% of the activity of the whole electron transport chain, suggesting the presence of an inhibition site between PSII and PSI. The inhibition site of Pb^{2+} may also be associated with the inactiviation of plastocyanin (Belatik *et al.* 2013). The significant inhibition of Pb^{2+} of PSI can be also attributed to its strong binding to PSI.

This study also showed that the Pb^{2+} exposure caused inhibition of the cyclic electron flow (CEF). Cyclic electron flow is involved in ΔpH generation, and it is important for induction of non-photochemical quenching (Joliot and Johnson 2011). Belatik *et al.* (2013) found that Pb^{2+} had strong binding ability to PSI complex and might bind the plastocyanin. Plastocyanin is an extrinsic polypeptide containing Cu ion (Katoh and Takamiya 1964). Pb^{2+} cations could mimic the effect of Ca^{2+} and Zn^{2+} at specific molecular targets (Morales *et al.* 2011) and could disturb the Cu binding site of plastocyanin (Belatik *et al.* 2013). This could explain the vulnerability of PSI to Pb^{2+} and the significant decrease of CEF under Pb^{2+} stress.

Δψ showed an increasing trend with increasing Pb^{2+} concentration (Table 3). Δψ increased by 12% from 18.76 $\Delta I/I \times 10^{-3}$ for the control to 23.02 $\Delta I/I \times 10^{-3}$ at 5 mg/l Pb^{2+}. ΔpH decreased slightly but not significantly at 0.05 mg/l Pb^{2+} and then changed little as Pb^{2+} concentration increased further.

Table 2. Quantum yield of PSI, PSII and cyclic electron flow of M. *pteropus* under various concentrations of Pb^{2+} after 7 days exposure.

Pb^{2+} (mg/l)	Y(I)	Y(ND)	Y(NA)	Y(II)	Y(NO)	Y(NPQ)	Y(CEF)
0	0.365 ± 0.042	0.480 ± 0.045	0.155 ± 0.019	0.306 ± 0.008	0.326 ± 0.022	0.368 ± 0.015	0.059 ± 0.034
0.05	0.295 ± 0.011	0.518 ± 0.027	0.187 ± 0.038	0.262 ± 0.011*	0.377 ± 0.005	0.360 ± 0.012	0.033 ± 0.009
0.1	0.302 ± 0.013	0.541 ± 0.022	0.157 ± 0.010	0.255 ± 0.002*	0.333 ± 0.010	0.413 ± 0.012	0.047 ± 0.011
0.5	0.277 ± 0.034*	0.502 ± 0.018	0.221 ± 0.042	0.240 ± 0.015*	0.380 ± 0.021	0.380 ± 0.007	0.037 ± 0.026
1	0.279 ± 0.039*	0.515 ± 0.009	0.205 ± 0.036	0.246 ± 0.028*	0.366 ± 0.041	0.388 ± 0.013	0.033 ± 0.013
5	0.271 ± 0.004*	0.549 ± 0.014*	0.180 ± 0.010	0.268 ± 0.007*	0.395 ± 0.014*	0.337 ± 0.016	0.004 ± 0.009*

Y(I), quantum yield of PSI; Y(ND), donor side limitation of PSI; Y(NA), acceptor side limitation of PSI; Y(II), quantum yield of PSII; Y(NO), non-regulated energy dissipation; Y(NPQ), regulated energy dissipation; Y(CEF), yield of cyclic electron flow. *Represents $p < 0.05$.

Table 3. Effect of Pb^{2+} on membrane potential (Δψ) and proton gradient (ΔpH) components of the overall proton motive force (*pmf*).

Pb^{2+}(mg/l)	Δψ (Δ*I/I*)	ΔpH(Δ*I/I*)
0	18.76 ± 1.76	5.84 ± 0.86
0.05	18.10 ± 3.86	2.77 ± 0.42
0.5	19.94 ± 1.18	3.76 ± 0.16
5	23.02 ± 5.48	2.99 ± 0.08

ΔpH component is the key regulatory signal for initiation of nonphotochemical quenching of excitation energy (Kanazawa and Kramer 2002). Pb^{2+} at 0.05 - 5 mg/l does not exert significant adverse effects on H^+ efflux from the lumen to the stroma via thylakoid ATP-ase, which agrees with the little change of Y(NPQ) (Table 2). Some other environmental stresses can decrease the membrane potential and proton gradient (Antal *et al.* 2011), which lowers the photosynthetic rate (Tang *et al.* 2001). In addition, the slight decrease of ΔpH might be due to the inactivation of CEF. Because Δψ and ΔpH are the two main components of proton motive force, the increase of Δψ paralleled with slight decrease of ΔpH implies that Δψ was enhanced to counteract the adverse effects produced by decreases of ΔpH and CEF so as to meet the energy demand required for ATP synthesis.

Briefly, *Microsorum pteropus* can accumulate high content of Pb in its root and shoot. Y(I) declined faster than Y(II) under Pb^{2+} stress. Y(CEF) decreased along with the increase of treatment time. PSI activity was more affected than PSII due to the inactivation of CEF around PSI under Pb^{2+} stress.

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