The effects of lead on photosynthetic performance of waxberry seedlings (*Myrica rubra*)

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Abstract

The photosynthesis was investigated 30 d after Pb treatment in *Myrica rubra* seedlings. The Pb treatment resulted in significantly increased Pb concentrations in shoots. Low Pb concentration exposure (≤2 mM) reduced the net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) without affecting the intercellular CO_2 concentration (C_i), chlorophyll (Chl) content, and Chl fluorescence parameters. At 10 d after severe Pb treatment (≥4 mM), P_N was inhibited and accompanied by Chl damage, while at 30 d, the inhibition of P_N was followed by an increase of C_i and a decrease of g_s, E, Chl content, and Chl fluorescence parameters. *M. rubra* showed a promising prospect for use in the soil phytoremediation, when Pb concentration is low, but the remediation efficiency of *M. rubra* is limited if Pb exceeds 2 mM.

Additional key words: chlorophyll fluorescence; lead stress; photosynthesis.

Introduction

Lead (Pb) is one of the most common heavy metal pollutants in the environment according to the United States Environmental Protection Agency (Watanabe, 1997). The Ministry of Land and Resources of China (MLRC) and the Ministry of Environmental Protection of China (MEPC) conducted a national soil pollution survey from 2005 to 2013. The survey results show that nearly 1.5% of all survey points across mainland China were polluted by Pb, and 1.1, 0.2, 0.1, and 0.1% of these survey points were suffering minor, low, moderate, and serious Pb pollution, respectively (MLRC and MEPC 2014). Pb released from industrial units, metallurgical operations, and mining activities affects plant growth, directly or indirectly. Pb pollution influences morphological, physiological, and biochemical processes. It results in a decreased seed germination and plant growth as well as an increase in lipid peroxidation, oxidative stress, and DNA damage (Sharma and Dubey 2005, Sengar *et al.* 2008, Gupta *et al*. 2009, Yadav 2010, Shahid *et al.* 2011). The Pb-induced damages to photosynthesis may be caused by a reduction in photosynthetic pigments (Drazkiewicz 1994, Sharma and Dubey 2005, Hussain *et al.* 2006) and changes in the fine structure of chloroplasts (Fargašová 2001). This may also inhibit the photosynthetic electron transport system (Rashid *et al.* 1994), cause changes in ratios of unsaturated lipids, saturated lipids, and protein of chloroplast membranes (Stefanov *et al.* 1995, Skórzyńska-Polit and Baszyński 1997), and inhibit Calvin cycle enzymes (Parys *et al.* 1998).

In general, 5–7 years old trees can grow to heights of 5–15 m, with an annual biomass production of 150 t ha^{-1} (Caparròs *et al*. 2008). Recently, because of the large amount of biomass production, outstanding growth rate, and their high accumulation and tolerance to heavy metals, *Paulownia tomentosa*, *Myrica rubra,* and other tree species have been considered as effective plants for their

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Abbreviations: AOS – activated oxygen species, C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; F_0 – minimal fluorescence of the dark-adapted state; Φ_{PSII} – effective quantum yield of PSII photochemistry; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; WUE – water-use efficiency.

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use in phytoremediation (He *et al.* 2004, Doumett *et al.* 2008, Witters *et al.* 2009, Tzvetkova *et al.* 2015). The massive production of biomass in areas with a high Pb concentration allows *M. rubra* to remove significant amounts of Pb from polluted soils. Previously, we showed that *M. rubra* seedlings could grow normally in Hoagland solution with 1 mM Pb and accumulated 1,108 mg(Pb) kg^{-1} in leaf tissue (He *et al.* 2004). Subsequently, studies have shown that 2 mM Pb could induce antioxidant defense mechanisms that could prevent oxidative damage and increase the soluble sugar content allowing *M. rubra* seedlings to minimize dehydration damage (He *et al.* 2009). Although some information on strong Pb tolerance of *M. rubra* seedling has been provided, the relationship between Pb and the functioning of the photosynthetic apparatus needed further detailed analyses.

The present study investigated the effects of elevated Pb stress on Pb accumulation and photosynthesis of *M. rubra* seedlings. Our specific objective was to determine and analyze the changes in Chl content, gas exchange, and Chl fluorescence parameters in order to evaluate the tolerance of *M. rubra* seedlings to serious Pb stress over a long period.

Materials and methods

Plant material, growth conditions and harvest: Self-rooted, one-year-old and uniform seedlings of *M. rubra* were collected from Dongkui bayberry farm in Nanning, Guangxi Province, China. Old leaves were removed to minimize transpiration and the seedlings were transferred into a greenhouse with a relative humidity of no more than 60%, 30/25°C day/night temperature, and PPFD of 150 μmol m^{-2} s^{-1}. Each plant was cultivated in a plastic pot with 5 L of half-strength Hoagland nutrition solution (pH 5.4). The nutrition solution was aerated 20 min per hour, adjusted to pH 5.4 with 0.1 mM HCl per day and renewed once per week. After one month, 40 seedlings were divided into four groups of ten and cultivated with nutrition solution supplemented with 0, 2, 4, and 6 mM $Pb(NO_3)_2$ (*Sigma-Aldrich Biotechnology*, St. Louis, MO, USA) for 30 d, respectively. Tissue samples of stems and leaves were collected separately at 10 and 30 d after the beginning of Pb treatments. The leaves were divided into two parts, which were used for the analysis of chlorophyll (Chl) and Pb content, respectively.

Pb concentration: The stem and leaf samples were washed with de-ionized water. Subsequently, these tissue samples were dried in oven for 1 h at 105°C to inactivate enzymes and to denature proteins rapidly. Then samples were incubated at 70°C for 3 d. Dried tissues were weighed and digested with nitric acid. The Pb concentration of each sample was determined by flame atomic absorbance spectrometry (*ZEEnit-700p,* Jena, Germany) and expressed in mg(Pb) kg^{-1}(dry mass, DM).

Chl content: Leaf samples were cut into small pieces, less than 2 × 5 mm. Then, 0.1 g of fresh mass (FM) of each sample was precisely weighed and placed in a flask with 10 mL of mixed solution [acetone:ethanol:water with a ratio of 4.5:4.5:1 (v/v/v), respectively]. All flasks were placed in darkness for 24 h, and shaken once every 4 h during this period. Absorbance of the supernatant was recorded at 645 and 663 nm using a spectrophotometer (*UV–1800*, *Shimadzu Corp.*, Kyoto, Japan). Chl content in leaf samples was calculated according to Arnon (1949). The Chl *a*/*b* ratio was defined as the ratio of the mean Chl *a* and mean Chl *b* content.

Gas-exchange parameters were measured at 10 and 30 d of the Pb treatment. The light-saturated net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) of young, fully expanded leaves were measured using a portable photosynthesis system (*LI–6400*, *Li–Cor*, *Inc.*, Lincoln, NE, USA). Water-use efficiency (WUE) was defined as the ratio of P_N/E. Artificial illumination was supplied to each leaf from a red/blue LED light source, and 340 cm^3 m^{-3} of CO_2 was supplied by a *6400* CO_2 mixer (*Li–Cor*, *Inc.*, Lincoln, NE, USA). Measurements were made between 11:00–13:00 h of the local time.

Chl fluorescence parameters were measured at a fixed time (11:00 h) by a portable fluorimeter (*PAM–2000, Walz*, Effeltrich, Germany). To obtain the maximum potential fluorescence of PSII, the leaves were adapted to darkness for 15 min before the measurements. The minimum fluorescence level in the dark-adapted state (F_0) was measured based on the intensity of the modulated light, which was sufficiently low and caused no significant variable fluorescence. When the actinic light was removed, the minimal fluorescence level in the light-adapted state (F_0') was measured by illuminating the leaves with 3 s of far red light. The maximal fluorescence levels in the dark-adapted (F_m) and light-adapted (F_m') states were determined before and after the actinic illumination by applying 1-s saturating 'white light' to close all of the reaction centers. The following values were determined: the maximum quantum yield of PSII photochemistry: $F_v/F_m = (F_m - F_0)/F_m$, the effective quantum yield of PSII photochemistry: $\Phi_{PSII} = (F_m' - F_s)/F_m'$, photochemical quenching coefficient: $q_P = (F_m' - F_s)/(F_m' - F_0')$, and nonphotochemical quenching coefficient: $q_N = (F_m - F_m')/(F_m - F_0')$.

Results

Pb accumulation in leaves and stems of *M. rubra* seedlings increased significantly with increasing external Pb concentrations at 30 d after the beginning of Pb exposure. After the treatment by 2 mM Pb, only 56.09 and 86.42 mg(Pb) kg^{-1} accumulated in leaves and stems, respectively. However, the corresponding values at 4 mM Pb were substantially higher by up to 9.9 and 34.5 times of the above amounts. After the treatment by 6 mM Pb, the concentration of Pb reached 1,211 and 3,474 mg kg^{-1} in leaf and stem tissues, respectively (Table 1). The ratios of Pb in leaf to stem tissues affected by 2, 4, and 6 mM Pb were 0.64, 0.18, and 0.34, respectively.

Chl concentration: Chlorosis is a general symptom of heavy metal damage in plants. At 10 d after the treatment, the contents of total Chl and Chl *a* were significantly lower than that in controls, but only in the seedlings exposed to 6 mM Pb (Table 2), while Chl *b* content was stable under all of the Pb concentrations. It should be noted that a significant reduction of the Chl *a*/*b* ratio was observed only in the seedlings after 10 d of their exposure to 6 mM Pb. After 30 d from the exposure, the content of total Chl, Chl *a*, and Chl *b* did not change at 2 mM Pb, and decreased significantly at 4–6 mM Pb (Table 2). For Pb exposure over 2 mM, the Chl content was reduced in a concentration- and time-dependent manner. Compared with controls, the Chl *a* and Chl *b* contents were reduced by 24.9 and 12.3%, respectively, at 30 d after the treatment with 6 mM Pb (Table 2).

Gas exchange: At 10 d after the treatment, P_N decreased with the increased Pb concentration except for 2 mM Pb exposure. However, g_s, E, C_i, and WUE did not change compared with controls even at 6 mM Pb (Table 3). At 30 d after the treatment, P_N, g_s, E, and WUE were reduced with the increasing Pb stress, but C_i showed a significantly increasing trend. P_N, E, g_s, and WUE under 6 mM Pb decreased by 69.3, 57.5, 56.5, and 30.9% compared with controls, respectively, at 30 d after the treatment, while C_i increased by 22.4% under the same conditions (Table 3).

Chl fluorescence: During the first 10 d of the treatment, F_0, F_v/F_m, Φ_{PSII}, q_P, and q_N remained stable, which indicated the highest Pb supply had no effect on Chl fluorescence of the seedlings for a relative short time period. At 30 d of the treatment, F_0 showed an increasing trend with 4 and 6 mM Pb, which was likely due to inactivation of reaction centers (Fig. 1*A*). The F_v/F_m value is an indicator of the maximum primary photosynthetic quantum efficiency of PSII, while Φ_{PSII} measures the effective yield of light quantum absorbed by PSII that is used in photochemistry. At 30 d after the treatment, F_v/F_m reached 0.79–0.83 at each Pb treatment, similar to those of the control, while Φ_{PSII} decreased significantly with a further increase of the Pb concentration, which was accompanied by the decline of q_P and q_N. Under 6 mM Pb for 30 d, Φ_{PSII}, q_P, and q_N decreased by 48.6, 42.8, and 25.9% compared with the controls, respectively (Fig. 1).

Table 1. Pb contents in *Myrica rubra* seedlings treated with 0, 2, 4, or 6 mM Pb for 30 d. Results are means ± SD (*n* = 3). Means with *different letters* within the same column are significantly different from one another (*p*<0.05). DM – dry mass.

Treatment time [d]	Pb concentration [mM]	Pb content [mg kg^{-1}(DM)] Stem	Leaf
30	0	55.34 ± 7.72^c	0^c
	2	86.42 ± 4.34^c	56.09 ± 6.44^c
	4	$2{,}985 \pm 66^b$	552 ± 34.18^b
	6	$3{,}473 \pm 165^a$	$1{,}210 \pm 55^a$

Table 2. Chlorophyll (Chl) content and Chl *a/b* ratio of *Myrica rubra* seedlings treated with 0, 2, 4, or 6 mM Pb for 10 and 30 d. The Chl *a*/*b* ratio was defined as the ratio of mean Chl *a* and mean Chl *b* content. Results are means ± SD (*n* = 3). Means with *different letters* within the same column are significantly different from one another (*p*<0.05). FM – fresh mass.

Treatment time [d]	Pb treatment [mM]	Chl content [mg kg^{-1}(FM)] Total Chl	Chl *a*	Chl *b*	Chl *a/b*
10	0	5.80 ± 0.53^a	4.10 ± 0.11^{ab}	1.71 ± 0.13^a	2.40
	2	6.18 ± 0.32^a	4.32 ± 0.07^a	1.92 ± 0.11^a	2.25
	4	5.52 ± 0.40^{ab}	3.91 ± 0.28^{ab}	1.66 ± 0.14^a	2.35
	6	4.91 ± 0.04^b	3.50 ± 0.03^b	1.96 ± 0.35^a	1.79
30	0	5.71 ± 0.29^a	4.18 ± 0.16^a	1.54 ± 0.08^{ab}	2.72
	2	5.71 ± 0.19^a	4.08 ± 0.12^a	1.58 ± 0.06^a	2.58
	4	4.53 ± 0.17^b	3.28 ± 0.11^b	1.34 ± 0.05^b	2.46
	6	4.41 ± 0.13^b	3.14 ± 0.08^b	1.35 ± 0.01^b	2.32

Discussion

Photosynthesis serves as an important determinant of the yield, but is very vulnerable to heavy metal stress. Pb^{2+} inhibits the entire process of photosynthesis in plants, including photochemical (Fargašová 2001, Wu *et al.* 2008a) and carbon-based reactions (Wu *et al.* 2008b). Changes of photosynthetic indices can be used to determine a tolerance of a plant species to Pb. The present study dealt with the response of photosynthesis in *M. rubra* seedlings to severe Pb stress. Our results may provide important information related to a remediation of Pb polluted soils.

Zu *et al.* (2004) reported that the normal content of Pb in shoots of plants is 5 mg kg^{-1}. A plant that can accumulates Pb concentrations >1,000 mg kg^{-1}, when grown on metal rich soils, is called a hyperaccumulator of Pb (Baker *et al.* 1989, Ali *et al.* 2013). Aside from their strong ability to adsorb Pb, hyperaccumulators should have positive mechanism(s) for translocating larger than average amounts of metals up to the leaves rather than moving metals into the stems or roots (Nicoletta and Navari-Izzo 2011). A large body of evidence indicates that two factors can create a potential for a hyperaccmulator to accumulate greater amounts of metals than average in shoots than roots. These are a positive upward transport of heavy metals in plants and a reliance on enhanced xylem loading and chelation of metals along with other small molecule organic compounds (Axelsen and Palmgren 1998, Mils *et al.* 2003, Callahan *et al.* 2006). At 6 mM Pb treatment, the accumulation of Pb in leaf and stem tissues reached 1,211 and 3,474 mg kg^{-1}, respectively (Table 1). This observation suggests that seedlings of *M. rubra* have a relatively strong ability to absorb and accumulate Pb. However, the seedlings of *M. rubra* stored more Pb in stems than in leaves. The ratios of leaf and stem Pb were less than 1 which suggested that the upward transportation mechanism from stem to leaf of *M. rubra* depended passively on the concentration of Pb supplied, and was not closely related to the positive upward transportation of heavy metal as expected for the potential hyperaccumulator.

The total Chl, Chl *a*, and Chl *b* content of *M. rubra* remained stable during 30 d of 2 mM Pb treatment. However, with 4 mM Pb, the total Chl, Chl *a*, and Chl *b* content declined in the seedlings after 30 d. Under the treatment with 6 mM Pb, the Chl content decreased in seedlings at day 10, but the Chl *b* content did not change. Therefore, the effects of Pb on the Chl concentration largely depended on the Pb concentrations and the time of exposure to Pb stress. In the present study, our data suggest that the major reason for the decrease of total Chl and Chl *a*/*b* was the decline of Chl *a*. According to Prasad *et al.* (1998), Pb induced a greater decline in total Chl and Chl *a* content than that in the Chl *b* content and the Chl *a*/*b* ratio. Gajić *et al.* (2009) assessed the tolerance of *Ligustrum ovalifolium* Hassk. to Pb and found that the Chl *a* was more sensitive to Pb than Chl *b.* Li *et al.* (2012) observed that increased Pb supply resulted in the decrease in Chl *a* and carotenoids in the seedlings of rice, but no decrease in Chl *b* was observed. Our results agree with the above findings. It is generally accepted that this heavy metal reduces the Chl content by inhibiting the activity of enzymes that participate in Chl synthesis (Moustakas 1994). The replacement of the central Mg in Chl molecule with Pb is another explanation for the decline in Chl content (Prasad, 1998). Wu *et al*. (2008a) also speculated that Pb might prevent photosynthetic light harvesting in the affected Chl molecules by the substitution of Mg with Ca. Shakoor *et al*. (2014) proposed that the decline in Chl content under Pb stress was caused by an oxidative response, because citric acid, an antioxidant, induced diminution of damage in *Brassica napus* under Pb stress. Our previous research demonstrated that the 2 mM Pb^{2+} treatment had no apparent effect on the content of total soluble proteins, but increased the activities of superoxide dismutase and peroxidase as well as the content of soluble sugars (He *et al.* 2009). Thus, it was suggested that the antioxidant defense mechanism in the seedlings of *M. rubra* may be involved in the protection of Chl against Pb stress.

Table 3. The change of light-saturated net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), internal CO_2 concentration (C_i), and water-use efficiency (WUE) of *Myrica. rubra* seedlings treated with 0, 2, 4, or 6 mM Pb after 10 and 30 d. Water-use efficiency (WUE) was defined as the ratio of P_N/E. Results are means ± SD (n = 3). Means with *different letters* within the same column are significantly different from one another (p<0.05).

Treatment time [d]	Pb treatment [mM]	P_N [µmol(CO_2) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	g_s [mol (H_2O) m^{-2} s^{-1}]	C_i [µmol(CO_2) mol^{-1}]	WUE [mol(CO_2) mol(H_2O)$^{-1}$]
10	0	3.27 ± 0.22^a	0.875 ± 0.068^a	45.68 ± 2.83^a	281.95 ± 19.50^a	3.74 ± 0.41^a
	2	3.00 ± 0.42^{ab}	0.885 ± 0.133^a	46.00 ± 7.00^a	284.16 ± 29.37^a	3.39 ± 0.33^a
	4	2.58 ± 0.21^b	0.806 ± 0.129^a	41.17 ± 3.17^a	298.53 ± 5.94^a	3.20 ± 0.15^a
	6	2.55 ± 0.31^b	0.736 ± 0.064^a	40.16 ± 3.14^a	309.26 ± 10.71^a	3.46 ± 0.39^a
30	0	3.22 ± 0.26^a	0.400 ± 0.058^a	43.33 ± 6.83^a	274.10 ± 8.33^b	8.05 ± 0.39^a
	2	1.68 ± 0.43^b	0.236 ± 0.017^b	25.17 ± 0.65^b	303.40 ± 10.01^{ab}	7.13 ± 0.12^a
	4	1.37 ± 0.31^b	0.251 ± 0.030^b	27.50 ± 3.50^b	331.70 ± 20.59^a	5.46 ± 0.47^b
	6	0.95 ± 0.24^b	0.170 ± 0.032^b	18.84 ± 2.83^b	335.60 ± 15.64^a	5.56 ± 0.36^b

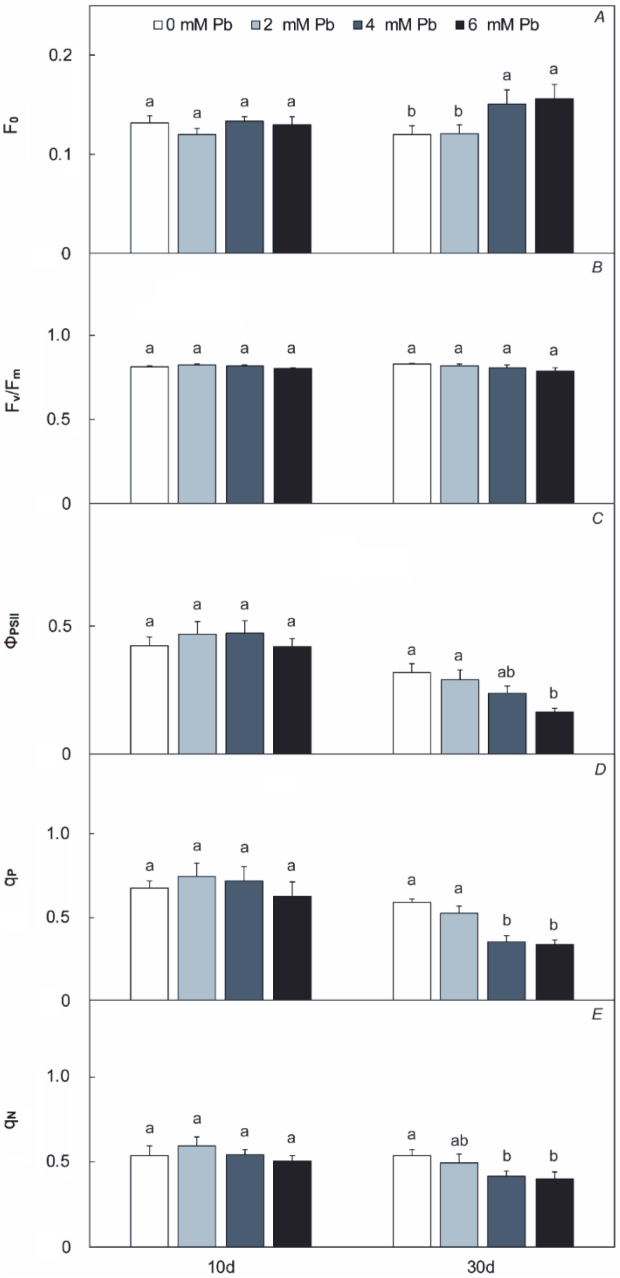

Fig. 1. The change of minimal fluorescence (F_0), maximal quantum yield of PSII photochemistry (F_v/F_m), effective photochemical efficiency of PSII (Φ_{PSII}), photochemical quenching coefficient (q_P), and nonphotochemical quenching coefficient (q_N) of *Myrica rubra* seedlings treated with 0, 2, 4, or 6 mM Pb for 10 and 30 d. Bars are the means ± SD (n = 3). Bars with *different letters* are significantly different from one another (p<0.05).

P_N is a combined reflection of the comprehensive process of photosynthesis, including light trapping, electron transfer, photosynthetic phosphorylation, and CO_2 assimilation. Although the photosynthetic parameters (g_s, E, C_i, and WUE) and Chl fluorescence parameters (F_0, F_v/F_m, Φ_{PSII}, q_P, and q_N) in the seedlings after 10 d of 4 mM and 6 mM Pb treatments showed no significant differences when compared with the control, P_N declined significantly under the same Pb stress. P_N was more sensitive to Pb than the other photosynthetic parameters (*i.e.*, g_s, E, C_i, and WUE). The reductions of Chl *a* content at 10 d after the treatment suggest that the decline of P_N in a relatively short time of treatment might be caused by damage of Chl. Chl is more sensitive to Pb than stomatal regulation and photochemical reactions. At 30 d after the treatment, the inhibition of P_N induced by Pb was in accordance with the decreasing trends of g_s and E, which indicated that stomatal closure was another factor causing the decrease in P_N for a relatively longer time period apart from Chl damage. Ahmad *et al*. (2008) treated the seedlings of mung bean (*Vigna radiata*) by 50 mg(Pb) L^{-1} for 10 d and concluded that stomatal limitation was the main factor causing photosynthetic inhibition by Pb. *Li et al.* (2012) also obtained similar results in rice seedlings treated by 50–200 µM Pb for 14 d. In contrast, the reduced g_s did not lead to a decrease in C_i. Intercellular CO_2 concentration represents a balance of CO_2 between CO_2 entering through the stoma and CO_2 consumed by assimilation. The increased in C_i and the decrease in g_s in the seedlings at 30 d after the treatment with 4–6 mM Pb suggests that carbon assimilation was inhibited; this means the drastic P_N reduction over longer time periods (Farquhar and Sharkey. 1982, Koyro *et al.* 2013). Rubisco is the most important enzyme related to carbon assimilation in photosynthesis. Van Assche and Clijsters (1990) reported that Pb inhibited the activity of Rubisco. Wu *et al.* (2008b) found that Ca^{2+}-ATPase, Rubisco activase, and the carboxylation activity of Rubisco were inhibited in Pb-treated spinach; this suggested that Pb impaired CO_2 assimilation by inhibiting the transformation from active chemical energy to stable forms of energy.

The F_0 is an emission from excited antenna Chl of PSII, which represents energy liberation in competition with excitation energy transfer to PSII. When PSII maintains in active state, F_0 is minimal. The increase of F_0 in the seedlings under 4–6 mM Pb for 30 d indicated that energy transfer from the LHC to PSII was severely inhibited. Because F_0 originates from Chl *a* antennae in the LHCII (Karukstis 1991), it is supposed that the increase of F_0 might be related to the loss of Chl *a* observed in the seedlings under the same Pb stress (Table 2). The measure of maximum photochemical efficiency (F_v/F_m) can be used to estimate the potential efficiency of the primary photochemical reactions when all of the PSII centers were open. The decline of F_v/F_m reflects the structural damage to PSII and an increase in nonphotochemical energy dissipation as heat. Björkman and Demmig (1987) proposed that if F_v/F_m > 0.8, a plant is healthy and the severe damage of PSII does not occur. In the present study, F_v/F_m remained stable within 0.79–0.83 during the 30 d of Pb treatments. We expected that the Pb of 2–6 mM for 30 d would not cause severe damage to PSII efficiency in leaves of the treated seedlings. Φ_{PSII} measures the actual efficiency of the activity of PSII under stress. At 10 d after the treatment, Φ_{PSII} was virtually unaltered by Pb. At 30 d, a significant reduction of Φ_{PSII} was observed only in the seedlings under 6 mM Pb stress. Furthermore, Φ_{PSII} was more sensitive than F_v/F_m in seedlings with the same Pb treatments. A similar relationship between Φ_{PSII} and F_v/F_m has also been reported for *Vigna umbellata* seedlings under 0.2–0.6 mM Mn (Subrahmanyam and Rathore 2000) and in seedlings of *Amaranthus tricolor* under 0.5–4.0 mmol(Cu) kg^{-1} (Ke *et al.* 2007). Reduction of Φ_{PSII} could be explained as a decreased capacity of the plants to assimilate CO_2 or as a result of oxidative phosphorylation (Subrahmanyam and Rathore 2000). The q_P indicated the use of absorbed light energy through photochemical processes, and could be used to approximate the proportion of PSII reaction centers that are open. The q_N reflected excess nonradiative energy dissipation as heat from the photosynthetic apparatus that could minimize the production of oxidant molecules and protect the plant against photo-oxidative damage. The sum of the two quenching parameters should be 1, *i.e.*, q_P and q_N represent full quenching, and the decline of q_P was accompanied by an increase of q_N induced by light, temperature, heavy metal, or other environmental stress. However, other reports contradict this finding. Meneguelli-Souza *et al.* (2016) observed that q_P and q_N decreased significantly in water hyacinth (*Eichhornia crassipes*) on day 4 after treatment in the presence of 2.0 and 20 mg(As^{5+}) L^{-1}. Tanyolac *et al.* (2007) found that both q_P and q_N showed decreasing trends in maize leaves exposed to 1.5 mM Cu for 12 d. Those authors suggested that the observed decrease in q_N might be associated with the AOS generation caused by Cu stress. Jamil *et al.* (2007) found that q_P and q_N declined in radish as the salt concentration increased, and concluded that stomatal closure by NaCl induced the inhibition of q_N.

In our previous study, only one of three seedlings of *M. rubra* survived for 2 months under 5 mM Pb (He *et al.* 2004). Therefore, the treatment with 2–6 mM Pb would be expected to result in relatively serious damage to the seedlings of *M. rubra.* The Chl content along with photosynthetic and Chl fluorescence parameters of *M. rubra* seedlings exposed to 2–6 mM Pb were investigated in the present study over a period of 30 d to understand the tolerance of photosynthesis in woody plants to serious Pb pollution conditions, such as waste water discharge from mining enterprises. Chl *a* was more strongly affected by Pb than Chl *b* in *M. rubra* seedlings. In the case of photosynthetic parameters, P_N was more sensitive than g_s, E, and C_i to Pb stress. With an increased amount of Pb, P_N was observed to initially decline with 4 mM Pb at 10 d after the treatment. The inhibition of Chl *a* content was observed with 6 mM Pb at 10 d after the treatment, while g_s and E declined with 2–4 mM Pb stress at 30 d after the treatment. Additionally, the declines of Chl fluorescence parameters occurred finally with 4–6 mM Pb 30 d after the treatment.

In conclusion, as *M. rubra* exhibited a strong ability to accumulate Pb and showed tolerance to 2 mM Pb. This species can provide a good plant material for the phytoremediation of soil with a low concentration of Pb. However, if Pb concentration exceeded 2 mM, the Chl content, stomatal conductance, and PSII function were inhibited. It indicates that the use of *M. rubra* is limited for the remediation of soils severely polluted with Pb.

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