EFFECTS OF LEAD STRESS ON THE CHLOROPHYLL CONTENT AND PHOTOSYNTHETIC FLUORESCENCE CHARACTERISTICS OF *VALLISNERIA NATANS*

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(Received 9th Dec 2018; accepted 4th Mar 2019)

Abstract. In order to reveal the effects of lead stress on the chlorophyll content and fluorescence characteristics of *Vallisneria natans*, six Pb concentration levels were applied to a culture in clean river sand and water. The results showed that Pb could promote the synthesis of chlorophyll at a low concentration ($Pb \leq 10$ $mg \cdot L^{-1}$) in one day. *V. natans* had certain tolerance to the stress of low Pb concentration. With the prolongation of stress time and increasing Pb concentration, the leaves of *V. natans* gradually lost their green color. Chlorophyll a decreased more than chlorophyll b. It had the least effect on carotenoids. Fv/Fm, Fv/Fo, qP, Y(II) and ETR decreased significantly ($Pb > 10 mg \cdot L^{-1}$) in seven days. However, *qN*, *Y(NO)* and *Y(NPQ)* showed an upward trend. The efficiency of the leaves using light energy decreased noticeably, and the electron transport of PSII was blocked severely. It can be speculated that *V. natans* may be used in the phytoremediation of waters contaminated by a low concentration of lead.

Keywords: *Vallisneria natans, lead stress, photosynthetic pigment, fluorescence characteristics, rapid light curve*

Introduction

In recent years, heavy metal pollution has become prominent with mining, smelting, electroplating, and different types of wastewater, with solid wastes being discharged into water bodies (Ji et al., 2018). Due to the fact that these cannot degrade or be decomposed, heavy metals are accumulated in living organisms, impair the health of animals and humans with biological amplification through the food chain (Xu et al., 2003). As one of the “five poisonous” heavy metal elements, lead (Pb) is a non-essential element utilized during plant growth and metabolism, and otherwise has toxic effects (Sharma and Dubey, 2005; Bisht et al., 2013; Ansari et al., 2017; Shahid et al., 2016), inhibiting the progress of photosynthesis and reducing the activity of chlorophyllase.

Submerged macrophyte, as important primary producers in aquatic ecosystems, not only provide food, habitat and breeding sites for aquatic animals, but also can get rid of N, P and other nutrients (Song et al., 2011). It also has ability to adsorb and accumulate heavy metals (Pan et al., 2011; Chen et al., 2017), used to remove Pb from water (Li et al., 2011). The nutrient absorption, secondary metabolism and antioxidant response of *Vallisneria natans* (*V. natans*) under Pb stress were studied (Wang et al., 2011, 2012) Under Pb stress, malondialdehyde content increased and total chlorophyll and carotenoids decreased. *V. natans* had a certain resistance to Pb stress, and the key enzymes of nitrogen and phosphorus metabolism were more sensitive to the response of Pb stress (Yu et al., 2016). At present, the research focus on mainly the enrichment of heavy metals (Liang et al., 2016; Xue et al., 2010), physiological and biochemical effects (Yu et al., 2016; Xu et al., 2006; Min et al., 2012), ultrastructure (Xu et al., 2004; Shi et al., 2000), etc. While the mechanisms of submerged macrophyte enduring heavy metals from photosynthetic and chlorophyll fluorescence are rarely reported.

V. natans is a perennial herbaceous plant perennial in China with high economic value (Wang et al., 2006), strong regeneration ability, used in water ecological restoration project widely (Gu et al., 2017; Wang et al., 2009). In 1980, professor Schreiber invented the pulse amplitude modulated. Due to the rapid, simple, sensitive, reliable and non-interference characteristics of the modulated fluorescence technology, it can reflect the “intrinsic” characteristics of the photosynthetic system (Hu et al., 2017), regarded as the effective probe in studying the relationship between plant photosynthesis and the degree of environmental stress (Janssen et al., 1992). The aims of this study were to investigate the intrinsic mechanism of photosynthetic system response under Pb stress, and provide some basic data and theoretical basis for the ecological restoration of Pb contaminated waters.

Materials and methods

Materials cultivation and treatment

Whole plants of *V. natans* were collecting from Nanjishan nature reserve of Poyang Lake, China, on June 5th in 2017. Plants (20 cm length) were planted in plastic buckets, adding 1/10 Hoagland nutrient solution, and acclimated for two weeks. Then, *V. natans*, healthy and consistent growth, were transplanted into eighteen transparent glass jars (50 cm × 40 cm × 40 cm) with 10 cm thick river sand paved at the bottom (*Fig. 1*). 50 L distilled water and Pb solution with different concentration levels were injected into the jars. Pb was added in the form of $Pb(NO_3)_2$ with a concentration level of 0, 1, 10, 20, 50 and 80 $mg \cdot L^{-1}$, 0 $mg \cdot L^{-1}$ was the control group (CK), three repetitions for each level.

***Figure 1**. Diagram of experimental device*

Determination of chlorophyll content

The determination of photosynthetic pigment was used by 95% ethanol extraction (Li, 2000). The value of absorbance was measured at 665, 649 and 470 nm, respectively. The concentrations of chlorophyll a (Ca), chlorophyll b (Cb), carotenoid (Cc) and total chlorophyll (Ct) in the extract were calculated respectively by *Equations 1-4.* The content of Ca, Cb, Cc and Ct ($mg \cdot g^{-1}$) was calculated by *Equation 5.*

DOI: http://dx.doi.org/10.15666/aeer/1702_41714181
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$$C_a(mg \bullet L^{-1}) = 13.95A_{665} - 6.88A_{649} \quad \text{(Eq.1)}$$

$$C_b(mg \bullet L^{-1}) = 24.96A_{649} - 7.32A_{665} \quad \text{(Eq.2)}$$

$$C_c(mg \bullet L^{-1}) = \frac{1000A_{470} - 2.05C_a - 114.8C_b}{245} \quad \text{(Eq.3)}$$

$$C_t(mg \bullet L^{-1}) = C_a + C_b \quad \text{(Eq.4)}$$

$$Chlorophyl\ content(mg \bullet g^{-1}FW) = \frac{C_t(mg \bullet L^{-1}) \times total\ amount\ of\ extract(ml)}{sample\ fresh\ weight(g)} \quad \text{(Eq.5)}$$

Determination of fluorescence parameters

The chlorophyll fluorescence characteristics of *V. natans* leaves were determined by the underwater modulation fluorescence instrument (DIVING-PAM), produced by WALZ company in German. Before the measurement, the dark clips were clamped on the leaves of *V. natans* in situ. After dark adaptation for 20 min, the clips and detection light were opened. First, the induction curve was determined, the minimum fluorescence (Fo) and maximum fluorescence (Fm) were obtained. The biggest actinic light efficiency of PSII (Fv/Fm), effective quantum yield (Y(II)), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (qN) and quantum yield of regulatory energy dissipation (Y(NPQ)) and quantum yield of non-regulatory energy dissipation (Y(NO)) were calculated automatically by the selected system mode. Fv/Fo = (Fm - Fo) / Fo. Then, the rapid light curve was measured. The gradients of photosynthetic active radiation (PAR) were 0, 100, 200, 300, 400, 600, 800, 1000 and 1200 μmol·m^{-2}·s^{-1} respectively.

Statistical analysis

Excel 2017 was used to process the experimental data and draw graphics. SPSS19.0 was used for one-way analysis of variance. The Duncan method was used for multiple comparisons. $P < 0.05$ means significant difference.

Results

Effects on photosynthetic pigment under Pb stress

As shown in *Figure 2A,* Ct, Ca and Cb after one day under Pb stress of 1 mg·L^{-1} were increased significantly compared with the control group (CK). The increase of Ct, Ca and Cb under Pb stress of 10 mg·L^{-1} was not significant compared with the CK ($P > 0.05$). Ct, Ca, Cb, Cc and Ca/Cb were reduced significantly when Pb was over 20 mg·L^{-1}.

As shown in *Figure 2B,* Ct, Ca and Cb were lower slightly after seven days under 1 mg·L^{-1} Pb. Above 10 mg·L^{-1} Pb stress, Ct, Ca, Cb and Cc decreased significantly ($P < 0.05$). It was respectively 45.89%, 39.20%, 58.01% of the CK under Pb stress of 80 mg·L^{-1}. Ca/Cb appeared in overall downward trend. Ca was more sensitive than Cb under Pb stress, and decreased sharply. The damage of Pb stress on chlorophyll was

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larger than carotenoids. The leaves of *V. natans* lost green color gradually with the increase of Pb concentration.

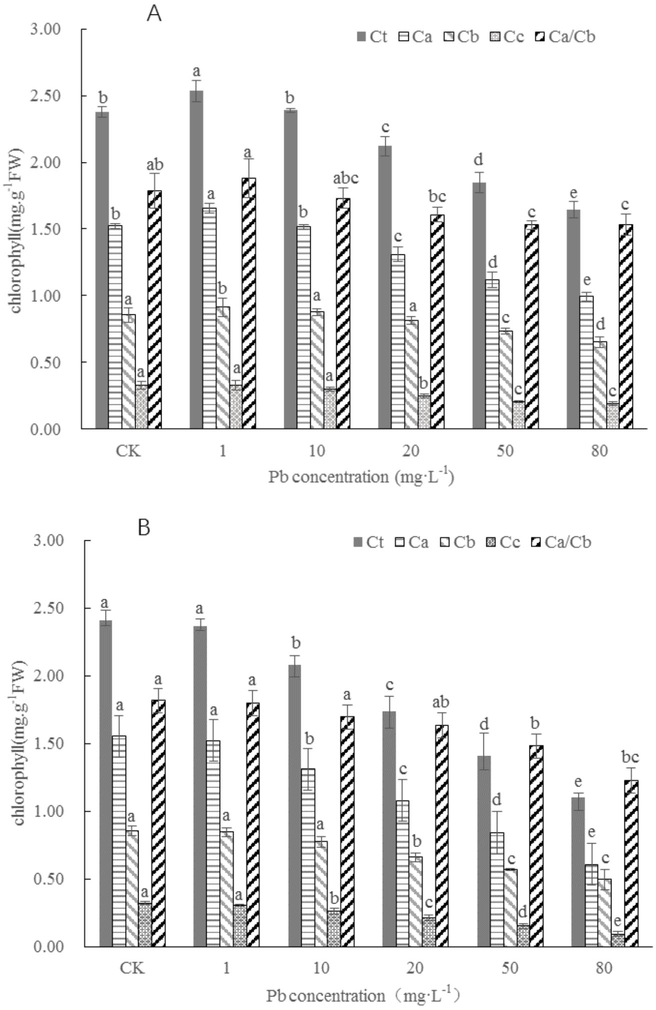

***Figure 2**. Varieties of Ct, Ca, Cb, Cc and Ca/Cb on V. natans under Pb stress. (Different lowercase letters represent the significant differences (P < 0.05) in different treatment groups, the same below. A represents one day stress, B represents seven days stress)*

Effect on Fo, Fm, Fv/Fm and Fv/Fo under Pb stress

As shown in *Figure 3*, Fo decreased with the increase of Pb compared with the CK after one day, but the difference was not significant. Fm was less than the CK. Fm had little difference at Pb stress of 20-80 mg·L^{-1}. Fv/Fm was lower than that of the CK except at Pb stress of 1 mg·L^{-1}. Fv/Fm had significant difference ($P < 0.05$) under Pb stress of 10-80 mg·L^{-1}. The change trend of Fv/Fo was similar with Fv/Fm.

After seven days, the increase of Fo was significant under the high Pb stress. Under Pb stress of 80 mg·L^{-1}, the increase of Fo was 28.32% compared with the CK. Fm was smaller than the CK, also smaller than that of the same Pb stress after one day. Fm was 39.58%, 37.23%, 31.47% of the CK at Pb stress of 20-80 mg·L^{-1}. Fv/Fm was lower than the CK. Conversion efficiency of PS II primary light energy decreased with the increase of Pb. Fv/Fo was also lower significantly than the CK ($P < 0.05$).

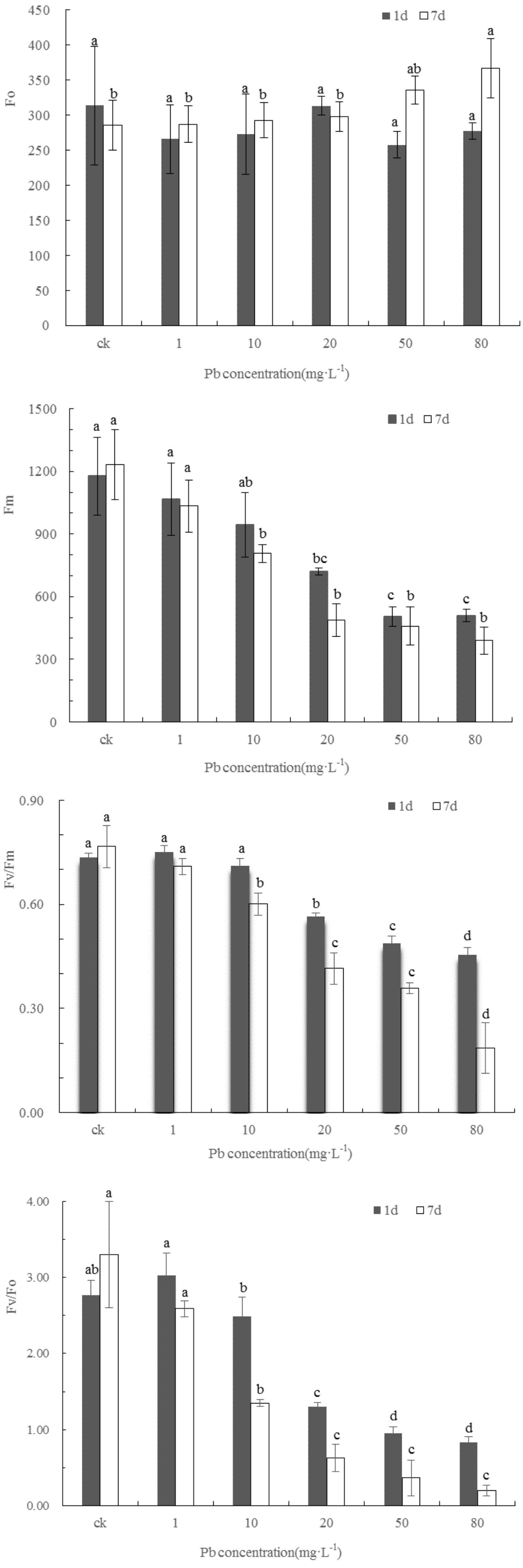

Figure 3. *Effects on Fo, Fm, Fv/Fm and Fv/Fo of V. natans*

Effects on qP, qN on leaves of V. natans under Pb stress

The value of qP was in a downward trend with the increase of stress intensity (*Fig. 4*). It had no significant difference under Pb stress of 0-10 mg·L^{-1} ($P > 0.05$) after one day. It decreased to 82.67% of the CK under Pb stress of 80 mg·L^{-1} after one day, and 37.36% after seven days. The trend of qN was opposite to qP. qN increased slowly with the increase of stress concentration. It peaked under Pb stress of 80 mg·L^{-1}, 141.3% higher than the CK.

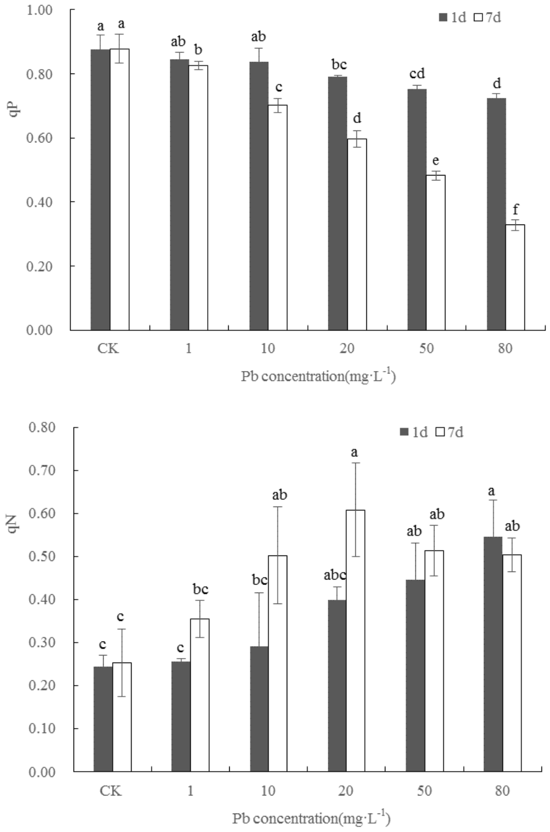

Figure 4. *Effects on qP and qN on V. natans under Pb stress*

Effect on Y(II), Y(NO), Y(NPQ) under Pb stress

Y(NO) increased slowly with the increase of stress concentration. The increase of Y(NO) was significant over 20 mg·L^{-1} Pb after one day. Under the same stress concentration, Y(NO) after seven days was higher than that after one day, which was 1.01, 1.01, 1.05, 1.08, 1.14 and 1.3 times respectively. Y(NPQ) showed an overall upward trend. Under Pb stress of 80 mg·L^{-1}, it peaked 4.05 times of the CK, then decreased significantly ($P < 0.05$) (*Fig. 5*).

Response of rapid light curves under Pb stress

The relative electron transfer rate (ETR) increased rapidly, then decreased and flattened. It behaved as light suppression. ETR increased compared with the CK under Pb stress of 1 mg·L^{-1} and 10 mg·L^{-1} after one day (*Fig. 6A*). It indicated low Pb could stimulate transmission of the electron. *V. natans* had certain resistance to low concentration. ETR was smaller than the CK under Pb stress of 20-80 $mg \cdot L^{-1}$. The maximum of ETR appeared when PAR was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. ETR was 91.42%, 72.78%, 50.3% of the CK.

ETR in each treatment group was lower than the CK after seven days (*Fig. 6B*). ETR decreased significantly ($P < 0.05$) with the prolongation of stress duration. The change trend was similar to that of qP. The proportion of real electron transfer in light reaction center and absorbing light used in photochemical process reduced. Electron transfer in the leaves was significantly inhibited so photosynthetic efficiency weakened (Gan et al., 2017).

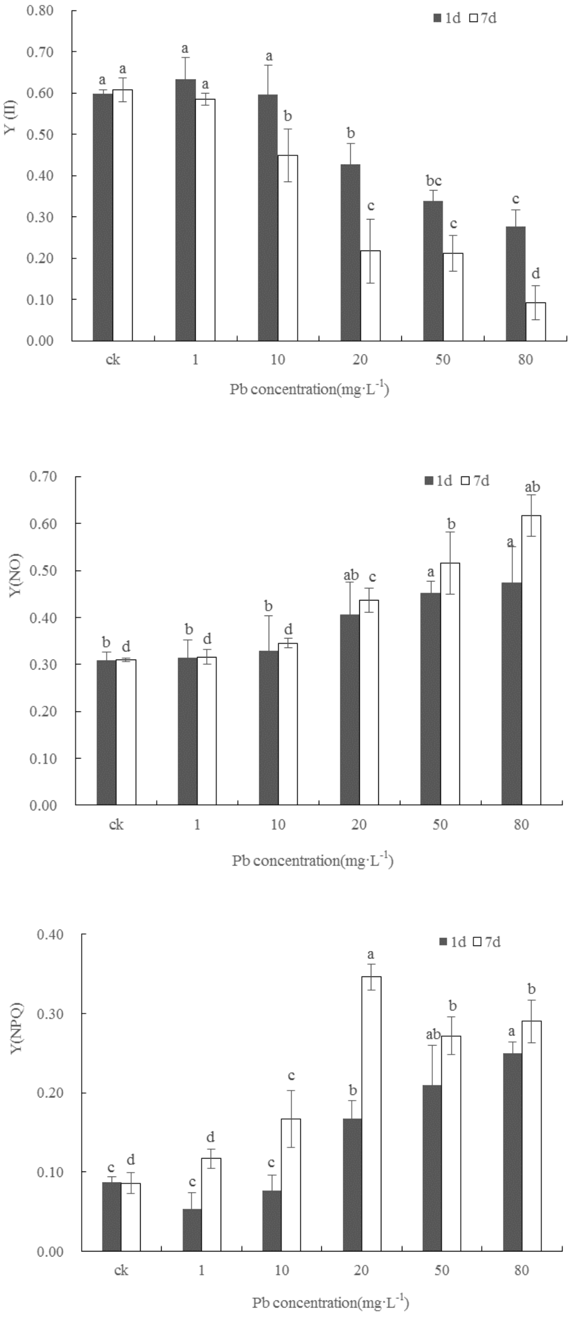

Figure 5. *Effects on Y(II), Y (NO) and Y(NPQ) of V. natans under Pb stress*

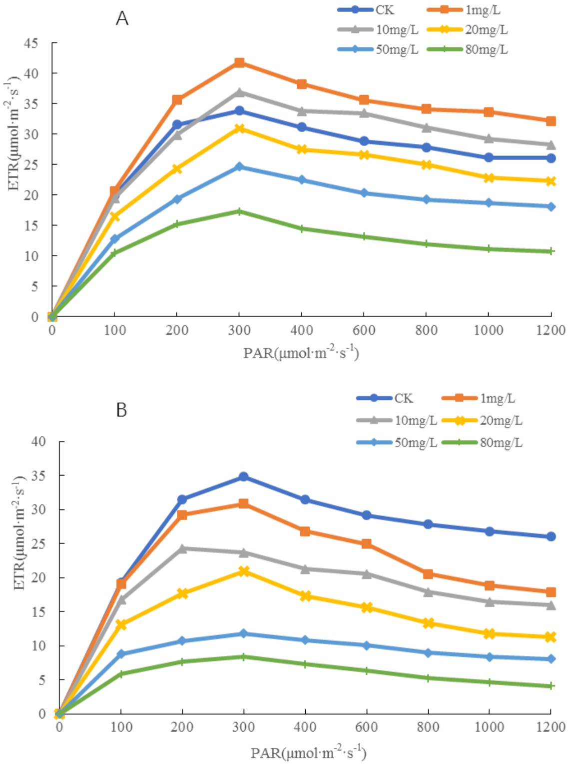

Figure 6. *Effects on ETR of V. natans under Pb stress. (A represents one day stress, B represents seven days stress)*

Discussion

Chlorophyll content is an important index to measure leaf senescence. The degree of reduction can also reflect the situation of Pb poisoning in plants. After one day under 1 mg·L^{-1} Pb, the synthesis of chlorophyll was enhanced because activated microorganisms in the water improved the supply condition of water nutrients (Yu et al., 2016), and Ca/Cb increased which may be a kind of resistance caused by Pb accumulation in *V. natans* to slow down the aging speed of the leaves.

After seven days, Ca decreased faster than Cb. Photosynthetic pigment decreased with the increase of Pb concentration. The main reason may be that Pb binds to the sulfhydryl of related enzyme in the chloroplast (Assche and Clijsters, 1990), destroyed the chloroplast structure. At the same time, with the accumulation of Pb, the combination of Pb and some enzyme (original chlorophyll reductase and porphobilinogen deaminase) that synthesize chlorophyll in cells (Asgharipour., 2011), make the enzyme activity blocked and chlorophyll synthesis inhibited.

The parameters of chlorophyll fluorescence kinetic have been widely recognized as one of the good indicators of plant resistance. The fluorescence parameters of plant growing under normal conditions were stable (Roháček, 2002). Fo was related with the activity of PS II light reaction center. In this research, Fo increased obviously when Pb concentration was over 20 mg·L^{-1}, which may be caused by the damage of the PS II reaction center of *V. natans* leaves (Gan et al., 2017). Fv/Fm indicated the light conversion efficiency of PS II reaction center. Fv/Fo indicated the potential activity of PS II. The change trend of Fv/Fo change trend is similar with and Fv/Fm. Fv/Fo is more sensitive to change of the photosynthetic efficiency. Conversion efficiency of PS II primary light energy decreased with the increase of Pb. The low Pb concentration had a weak effect on the potential maximum photosynthetic capacity of the leaves, and *V. natans* had a definite resistance to adversity. High Pb concentration hampered the light energy conversion efficiency of PS II reaction center, was unfavorable for the leaves to capture light energy into chemical energy.

After seven days, Fv/Fm, qP, Y(II) and ETR was 54.1%, 67.99%, 35.69% and 67.99% of the CK respectively under Pb stress of 20 $mg \cdot L^{-1}$, 24.25%, 37.36%, 15.3% and 24.14% of the CK respectively under Pb stress of 80 $mg \cdot L^{-1}$. This showed that with the prolongation of stress duration, the photosynthetic activity of PS II reaction center in *V. natans* leaves had been irreversibly damaged (Liu et al., 2017). It had basically lost photosynthetic capacity.

The decrease of qP indicated that the electron transportation from the oxidation side of PS II to the reaction center was blocked. The electrons used for photosynthesis decreased and the light energy dissipated in heat or other forms increased, which was consistent with the decrease of Y(II) (Zhang., 2016). qN reflects the plant’s ability to dissipate excess light energy as heat and reflects the light protection ability of plants. The value of qN gradually increased with the increase of Pb level. *V. natans* would start self-protection mechanism, dissipate excess light energy absorbed by the antenna pigment as heat energy to reduce the damage of chloroplasts and other photosynthetic organs when it was threatened by the heavy metal (Qian et al., 2011; Wu et al., 2016; Janssen et al., 1992). With the prolongation of stress duration, the damage of leaves was severe under high Pb stress (>20 $mg \cdot L^{-1}$). Dead leaves appeared. It was beyond the scope of self-protection.

Light quantum of adsorbed by PS II reaction center transferred and dissipated in three ways (Kramer et al., 2004). The sum of all light quantum yield closed to 1, that is, Y(II)+Y(NO) +Y(NPQ)=1. The proportion of Y(II) was the smallest with the increase of Pb concentration. Excessive Pb directly inhibited the transportation of photosynthetic electron, thus reduce the percentage of energy conversion in photochemical way (Qian et al., 2011). Y(NO) is an important indicator of light damage. Its higher level indicates that the photochemical energy conversion and protective regulatory mechanisms (such as heat dissipation) are not sufficient to completely consume light energy absorbed by the plant. With the prolongation of stress time, it increased, indicating that *V. natans* had been damaged. Y(NPQ) is an important indicator of light protection. The higher of Y(NPQ) indicated that the light intensity accepted by the leaves was excess, the plant can adjust (such as the excess light energy dissipated into heat) in order to protect themselves. This research showed that Y(NPQ) after seven days was significantly higher than that after one day. It received excess light. The utilization of light energy by the plant was weakened under Pb stress. The plant consumed excessive heat to protect itself and adapt to the environment by increasing the quantum yield of regulatory energy dissipation (Li et al., 2005).

Conclusions

Vallisneria natans can carry out relatively normal physiological activities ($Pb \leq 10 mg \cdot L^{-1}$). It can be speculated that *V. natans* are used as repairing species in low concentration Pb contaminated waters. This study was conducted in the period of vigorous growth, the period of flowering and decay of *V. natans* should be studied in future.

Acknowledgements. This study was supported by Jiangxi Provincial Education Department (GJJ170978), National Natural Science Foundation of China (51369024, 51769016), Jiangxi Provincial Technology Department (20161BAB216109).

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