EFFECTS OF SINGLE AND COMBINED STRESS OF CU OR ZN ON THE PHOTOSYNTHETIC FLUORESCENCE CHARACTERISTICS OF VALLISNERIA NATANS

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Abstract. For investigating the response of chlorophyll fluorescence characteristics under single and combined of Cu or Zn stress, Vallisneria natans was selected as the experimental subject. V. natans was cultured 21 days under six concentration levels of Cu or Zn. The results showed that $F_{v}/F_{m}$, $F_{v}/F_{o}$, $Y$ (Ⅱ) and $q_{P}$ increased, and other indicators were almost unchanged in low-concentration treatment (Cu≤0.4mg/L, Zn≤4.0mg/L, Cu+Zn≤0.2+2.0 mg/L). V. natans could still maintain normal growth within such a range of heavy metal concentration, and it could maintain relatively a high photosynthetic activity. When heavy metal concentration was higher than the above concentration, the photosynthetic activity was inhibited. $F_{v}/F_{m}$, $F_{v}/F_{o}$, $Y$ (Ⅱ) and $q_{P}$ decreased gradually with the increasing stress concentration and stress time, while $Y$(NO), $Y$ (NPQ), and $q_{N}$ showed different degrees of increase. The combined stress of Cu and Zn had a synergistic effect on the photosynthetic activity. V. natans can be used for ecological restoration of polluted water with low concentration of Cu and Zn.

Keywords: Vallisneria natans, heavy metal stress, chlorophyll fluorescence parameter, synergistic effect

Introduction

Heavy metals such as Cu, Cd, Pb and Cr are very common environmental pollutants, which can negatively and profoundly impact on the species diversity in aquatic ecosystems (Xing et al., 2013; Ji et al., 2018). Certain physiological variables of plants can be positively stimulated by the treatment of heavy metals with low concentration and with a relatively short period of time. However, long-term and high-concentration single heavy metal stress will show strong toxicity to aquatic plants (Gao et al., 2019a). Both Cu and Zn are essential trace elements for plant growth (Nagajyoti et al., 2010). Cu with optimum level can positively influence the production of chlorophyll and development of reproductive organs, while excessive copper will stifle the growth of plant roots, thus reducing the extraction ability of plant roots for other nutrients (Xue et al., 2010). Zn plays an important role in regulating osmotic pressure, maintaining metabolism and promoting substances synthesis in plants. Like Cu, when its concentration reaches a certain threshold, Zn can inhibit plant growth or even cause plant death (Ji et al., 2017).

Vallisneria natans is a very common submerged plant in rivers and lakes in China, and it is considered to be one of the effective species for solving heavy metal pollution in water bodies (Gao et al., 2019b). China has a large number of rivers and lakes in which the pollution of heavy metals is quite complicated. The poisoning effect of submerged plants often comes from the combined stress of multiple heavy metals (Xing et al., 2017). Currently, a large number of studies on submerged plants mainly focus on the enrichment of heavy metals (Upadhyay et al., 2014; Ahmad et al., 2016; Borisova et al., 2017) and...
physiological indicators (Wang et al., 2012). The researches on the fluorescence characteristics of plant chlorophyll are mostly limited to single heavy metal stress in a short period of time, and there are few literature-related to combined stress. In this study, the effects of single and combined heavy metal (using Cu and Zn as pollutants) on chlorophyll fluorescence parameters of *V. natans* were investigated to illuminate the response mechanism of the photosynthetic system, thus providing some guidance for ecological restoration of heavy metal polluted water bodies.

**Materials and Methods**

**Experimental materials**

*V. natans* and bottom mud required for the experiment were collected from Poyang Lake. After removing debris from the bottom mud, it was evenly spread in a plastic square box (length 34.0 cm, width 22.5 cm, height 10 cm), and the thickness of bottom mud is 8 cm. Individuals of *V. natans* with relatively intact leaves and robust growth were selected. The leaf length and the root length was trimmed to 15 cm and 3 cm, respectively. After the attachments on the leaf surface were washed away, these plants were evenly planted in plastic boxes (12 plants per box), and then these plastic boxes were placed in glass tanks (length 40 cm, width 40 cm, height 50 cm). Tap water was added into the tanks to a height of 40 cm for pre-cultivation of submerged plants, and these plants was used for experiments after the plants' growth status was stable (*Figure 1*).

![Figure 1. Diagram of the experimental culture and equipment](image-url)

**Experimental design**

Pre-experiment: Different concentrations of CuSO₄ and ZnSO₄ solution were added into the glass tanks for treatment. The concentrations of Cu and Zn were divided into different levels (5.0 mg/L, 10.0 mg/L, 15.0 mg/L and 20.0 mg/L for Cu; 5.0 mg/L, 10.0 mg/L, 20.0 mg/L, 40.0 mg/L, 50.0 mg/L, 75.0 mg/L and 100.0 mg/L for Zn). Root and leaf damage of the experimental subjects were recorded every 24 h. The results were shown in *Table 1*.

When 5.0 mg/L and 10.0 mg/L concentrations of Cu were applied, the total damage of *V. natans* was 11 and 14, respectively. In contrast, when the same total damage occurred, the applied concentrations of Zn were 50.0 mg/L and 100.0 mg/L, respectively. It demonstrated that the toxicity of Cu for *V. natans* was around ten times higher than that
of Zn. Naturally, the concentrations of Cu and Zn should be configured according to 1:10 in water body.

Table 1. Influence of Cu or Zn stress on the growth of V. natans

| Concentration of heavy metals (mg/L) | 0 h Root damage | 24 h Root damage | 48 h Root damage | 72 h Root damage | Total damage |
|-------------------------------------|----------------|----------------|----------------|----------------|--------------|
| Cu                                  |                |                |                |                |              |
| 5                                   | 1              | 1              | 1              | 2              | 3            |
| 10                                  | 1              | 1              | 1              | 2              | 3            |
| 15                                  | 1              | 1              | 2              | 3              | 3            |
| 20                                  | 1              | 1              | 2              | 4              | 4            |
| Zn                                  |                |                |                |                |              |
| 5                                   | 1              | 1              | 1              | 1              | 1            |
| 10                                  | 1              | 1              | 1              | 1              | 1            |
| 20                                  | 1              | 1              | 1              | 1              | 2            |
| 40                                  | 1              | 1              | 1              | 2              | 9            |
| 50                                  | 1              | 1              | 1              | 2              | 10           |
| 75                                  | 1              | 1              | 2              | 1              | 13           |
| 100                                 | 1              | 1              | 2              | 3              | 14           |

Root damage: 1 represents normal growth (the roots are long and robust); 2 represents mild damage (part of the root tip is deformed, and the color of root hair turns darker); 3 represents moderate damage (the color of taproot turns black). 4 represents severe damage (the root system is completely black, and lots of roots fall off).

Leaf damage: 1 represents normal growth (the leaves are smooth and flat, and the color is emerald green). 2 represents mild damage (the green color of the leaves fades, and the chlorosis phenomenon occurs). 3 represents moderate damage (shrinkage and fold occurs in certain parts of the leaves). 4 represents severe damage (the leaves are completely chlorotic and withered).

Formal experiment: CuSO₄ and ZnSO₄ solutions were added into the glass tank for single or combined stress treatments. Five treatment groups were set up according to different concentrations, and a control group (CK) was set at the same time (Table 2). There were three replicates for each treatment group. Photosynthetic fluorescence was measured every 7 days after stress cultivation, and the experimental period was 21 days in total. The temperature of water is between 22 and 29 °C and PH was 6.8. The experiment was completed from May to June in 2021.

Table 2. Concentration settings of Cu and Zn in water

| Heavy metal | Treatment group concentration (mg/L) |
|------------|-------------------------------------|
|            | CK  | T1    | T2    | T3    | T4    | T5    |
| Cu         | 0   | 0.2   | 0.4   | 0.6   | 0.8   | 1.0   |
| Zn         | 0   | 2.0   | 4.0   | 6.0   | 8.0   | 10.0  |
| Cu+Zn      | 0+0 | 0.1+1.0 | 0.2+2.0 | 0.3+3.0 | 0.4+4.0 | 0.5+5.0 |

Measuring method

The leaf fluorescence parameters were measured by the underwater modulation fluorescence instrument (Diving-PAM). Three plants were randomly selected from each treatment group, and the leaves were clamped with dark leaf clamps. Wincontrol software was used for data acquisition after dark adaptation for 20 min. The specific measurement method was included in the literature (Gao et al., 2019a).
Data analysis

The experimental result is expressed as the form of mean ± standard error. Excel 2017 is used in the processing and drawing of experimental data. SPSS 19.0 software is used to conduct one-way analysis of variance. The SNK method is used for multiple comparison analysis. Two-way analysis of variance was used to judge whether the interactions of factors were significant.

Result and Analysis

Effects of Cu or Zn on plant growth

There was no significant difference in plant height after single metal stress (Cu≤0.4 mg/L or Zn≤4 mg/L) compared with CK. The combined stress of Cu and Zn (0.1+1.0 mg/L) was beneficial to plant growth, and had antagonistic effect on plant growth. When the concentration exceeded T2 group, the plant growth was significantly inhibited and showed a significant downward trend (P < 0.05); Under combined stress, the decline was the most.

Effects of Cu or Zn on Fv/Fm and Fv/Fo

After the stress treatment, Fv/Fm and Fv/Fo of V. natans were measured, and the change trend of Fv/Fm and Fv/Fo was analyzed. The results were shown in Fig. 2.

Figure 2. Effects of different heavy metal concentrations on Fv/Fm and Fv/Fo. Note: Different lowercase letters in each line indicate significant differences at P < 0.05 among different treatment groups.
After 7 days’ stress, \( F_{v}/F_{m} \) and \( F_{v}/F_{o} \) showed an upward trend when heavy metal concentration was lower than that of T2, then these two variables decreased gradually. The changes of \( F_{v}/F_{m} \) and \( F_{v}/F_{o} \) under low concentration treatment were insignificant, and the minimum values were all observed in T5 group. Compared with CK, for T5 group, \( F_{v}/F_{m} \) decreased by 25.10%, 24.98% and 32.57%, and \( F_{v}/F_{o} \) decreased by 63.82%, 63.66% and 71.77%. For subjects with 14 days’ and 21 days’ cultivation, only \( F_{v}/F_{m} \) and \( F_{v}/F_{o} \) in T1 group were slightly higher than that of CK, and the values of other groups decreased significantly according to the increase of heavy metal concentrations. For subjects of T5 group with 14 days’ cultivation, \( F_{v}/F_{m} \) were 56.34%, 60.74% and 31.44% of CK, and \( F_{v}/F_{o} \) were 18.28%, 17.18% and 8.25% of CK. For subjects with 21 days’ cultivation, \( F_{v}/F_{m} \) and \( F_{v}/F_{o} \) in different groups changed more widely, and these two parameters stressed by combined stress in T5 group couldn’t be determined. Overall, heavy metals had significant effects on \( F_{v}/F_{m} \) and \( F_{v}/F_{o} \) (\( P<0.05 \)), and the amplitude of variation of \( F_{v}/F_{o} \) was higher than that of \( F_{v}/F_{m} \). The impact caused by single stress of Cu or Zn was lower than that of combined stress.

**Effects of Cu or Zn stress on \( Y(II) \), \( Y(NPQ) \) and \( Y(NO) \)**

As shown in Fig. 3, \( Y(II) \) increased gradually when applied concentration was lower than that of T2. When the concentration was higher than that of T2, \( Y(II) \) decreased significantly. Compared with the single stress of Cu or Zn, the variation amplitude of \( Y(II) \) resulted from combined stress was relatively greater. In addition, \( Y(II) \) in T2 and T5 groups were 1.12 and 0.64 times of those in CK, respectively. It meant that the transfer of photosynthetic electrons of \( V. \) natans could be improved by the treatment of heavy metals with low concentration (\( \leq T2 \)), and the treatment could also enhance the effective quantum yield to a certain extent. However, the high concentration would damage the reaction of photosynthetic system inside \( V. \) natans, thus influencing the photosynthesis. Among the three types of heavy metal stress, the combined stress had the greatest impact on \( Y(II) \).

For subjects with 7 days’ cultivation, when applied concentration was higher than that of T2, \( Y(II) \) increased with the increase of the heavy metal concentration. The minimum values of \( Y(II) \) were all observed in the T5 group for all three types of stress, and these values were 72.60%, 72.87% and 63.51% of CK. \( Y(NO) \) and \( Y(NPQ) \) change insignificantly in T2 group (\( P>0.05 \)). However, in T3, T4, and T5 groups, two values mentioned above showed a different degree of upward trend. In T5 group, \( Y(NO) \) and \( Y(NPQ) \) under combined stress were 1.64 and 3.40 times that of CK. For subjects within 21 days’ cultivation, \( Y(II) \) decreased significantly, and \( Y(NO) \) increased dramatically with the increase of heavy metal concentrations. The impact caused by combined stress was significantly higher than that of single stress of Cu or Zn. The increase of \( Y(NPQ) \) was much greater than that of \( Y(NO) \). After 21 days’ cultivation, \( Y(II) \), \( Y(NO) \) and \( Y(NPQ) \) treated by combined stress couldn’t be determined in T5 group.

**Effects of Cu or Zn stress on \( qP \) and \( qN \)**

As shown in Fig. 4, the values of \( qP \) in T1 group were higher than that of CK. The longer stress time of heavy metals would cause a greater decrease of \( qP \). For subjects with 21 days’ cultivation, \( qP \) couldn’t be determined in T5 group under combined stress. For subjects with 14 days’ cultivation, \( qN \) slightly increased with the increase of heavy metal concentrations until the applied concentration was higher than that of T2. For subjects with 21 days’ cultivation, \( qN \) showed an upward trend under the single stress, while the
same parameter under combined stress increased first, reaching the maximum value (0.617) in T3 group, and then decreased. The value of qN in T5 group couldn’t be determined. The variation amplitude of qN resulted from combined stress was relatively greater.

**Figure 3.** Effects of different heavy metal concentrations on Y(II), Y(NO) and Y(NPQ). Note: Different lowercase letters in each line indicate significant differences at P < 0.05 among different treatment groups.
Figure 4. Effects of different heavy metal concentrations on qP and qN. Note: Different lowercase letters in each line indicate significant differences at $P<0.05$ among different treatment groups.

Correlation analysis of various indexes

According to Pearson correlation analysis (Table 3), for subjects within 14 days’ cultivation, the correlation between $Fv/Fm$, $Fv/Fo$ and heavy metal concentrations was insignificant. For subjects with 14 to 21 days’ cultivation, a significant negative correlation was observed for the single stress of Cu or Zn. Under combined stress of Cu and Zn, there was also a very significant negative correlation between $Fv/Fm$, $Fv/Fo$ and heavy metal concentrations at the level of $P<0.01$. A significant negative correlation between $Fv/Fo$ and heavy metal concentrations could be observed from the data of all three types of treatment. For subjects with 7 days’ cultivation, the correlation between $Y(II)$ and heavy metal concentrations was insignificant. For subjects with 14 days’ cultivation, the negative correlation was significant for single stress and was extremely significant for combined stress. For subjects with 21 days’ cultivation, the negative correlation was extremely significant for both single stress of Cu and combined stress, and was significant for the single stress of Zn. $Y(NO)$ in the whole cultivation stage was significantly and positively correlated with heavy metal concentrations at the $P<0.05$ level. For subjects within 14 days’ cultivation, a significant positive correlation was observed for the single stress of Cu or Zn. The positive correlation observed was extremely significant within 14-21 days. The correlation was insignificant for combined
stress. For subjects within 7 days’ cultivation, there was no significant correlation between \(q_P\) and heavy metal concentrations, while after 7 days’ cultivation, a significant negative correlation was observed. \(q_N\) was significantly and positively correlated with heavy metal concentrations under the single stress, while the correlation was not significant under combined stress.

| Table 3. Correlation of various indicators under Cu or Zn stress |
|---------------------------------------------------------------|
| Indicator | \(F_v/F_m\) | \(F_v/F_o\) | \(Y(\text{II})\) | \(Y(\text{NO})\) | \(Y(\text{NPQ})\) | \(q_P\) | \(q_N\) |
|-----------|-------------|-------------|----------------|----------------|----------------|--------|--------|
| 7d        |             |             |                 |                 |                 |        |        |
| Cu        | -0.872      | -0.789      | -0.858          | 0.694*          | 0.856*         | -0.827 | 0.871* |
| Zn        | -0.870      | -0.807      | -0.800          | 0.706*          | 0.861*         | -0.800 | 0.820* |
| Cu+Zn     | -0.869      | -0.724      | -0.842          | 0.858*          | 0.876          | -0.817 | 0.825* |
| 14d       |             |             |                 |                 |                 |        |        |
| Cu        | -0.937      | -0.899      | -0.940*         | 0.975*          | 0.984*         | -0.932 | 0.987* |
| Zn        | -0.964      | -0.918      | -0.951*         | 0.937*          | 0.930*         | -0.933 | 0.989* |
| Cu+Zn     | -0.955      | -0.941      | -0.963**        | 0.668*          | 0.580          | -0.947 | 0.473  |
| 21d       |             |             |                 |                 |                 |        |        |
| Cu        | -0.959*     | -0.927**    | -0.924**        | 0.864*          | 0.929**        | -0.867 | 0.950* |
| Zn        | -0.937*     | -0.921**    | -0.943*         | 0.939*          | 0.921**        | -0.869 | 0.922* |
| Cu+Zn     | -0.950**    | -0.893**    | -0.919**        | 0.622*          | 0.666          | -0.827 | 0.437  |

* indicates significant correlation at \(P<0.05\). ** indicates extremely significant correlation at \(P<0.01\)

According to the two-way ANOVA (Table 4), Cu had an extremely significant effect on \(F_v/F_o\) and \(Y(\text{II})\) \((P < 0.01)\). It had significant effect on \(F_v/F_m\), \(Y(\text{II})\), \(Y(\text{NO})\), \(Y(\text{NPQ})\), \(q_P\) and \(q_N\) \((P < 0.05)\). Zn had an extremely significant effect on \(F_v/F_m\) and \(Y(\text{II})\) \((P < 0.01)\), and had a significant effect on \(F_v/F_o\), \(Y(\text{NPQ})\) and \(q_P\) \((P < 0.05)\). From the mean square value, the difference between groups caused by the change of Cu was greater than that caused by Zn. The interaction effect of the two factors only had an extremely significant effect on \(F_v/F_m\), \(F_v/F_o\) and \(Y(\text{II})\) \((P < 0.01)\). It had a significant effect on \(Y(\text{II})\) and \(q_P\) \((P < 0.05)\). The difference between groups caused by combined stress was the largest.

| Table 4. Variance analysis of Cu, Zn and their interaction on physiological indexes |
|-----------------------------------------------|
| Parameters | Cu | \(P\) | Zn | \(P\) | Cu×Zn | \(P\) |
|------------|----|-------|----|-------|-------|-------|
| \(F_v/F_m\) | 45.428 | 0.017* | 36.521 | 0.009** | 68.526 | 0.007** |
| \(F_v/F_o\) | 37.645 | 0.008** | 31.432 | 0.007* | 57.435 | 0.009** |
| \(Y(\text{II})\) | 46.273 | 0.009** | 21.435 | 0.005** | 31.971 | 0.015* |
| \(Y(\text{NO})\) | 1.439 | 0.024* | 10.366 | 0.161 | 21.765 | 0.126 |
| \(Y(\text{NPQ})\) | 7.578 | 0.043* | 8.163 | 0.018* | 4.462 | 0.208 |
| \(q_P\) | 31.301 | 0.032* | 3.750 | 0.042* | 1.756 | 0.027* |
| \(q_N\) | 7.406 | 0.045* | 2.953 | 0.143 | 0.873 | 0.234 |

*Represents a significant correlation at 0.05 level (bilateral). ** Represents a very significant correlation at the level of 0.01 (bilateral)
Discussion

Heavy metals can affect the normal activities of chloroplasts and thylakoids of *V. natans*, interfere with the synthesis of chlorophyll and control the electron transfer of PSII, thus causing changes in the photosynthetic activity and fluorescence parameters (Kalaji et al., 2016).

\( F_{\text{V}}/F_{\text{m}} \) is the maximum light quantum yield, which indicates the relative light energy used in photosynthesis. It can be used to evaluate the adaptability of plants to abiotic stress environment (Li et al., 2016). For subjects within 7 days’ cultivation under the stress of heavy metals, \( F_{\text{V}}/F_{\text{m}} \) and \( F_{\text{V}}/F_{\text{o}} \) showed an upward trend when applied concentration was lower than that of T2. Low concentration of heavy metals could promote the antioxidant enzyme system and increased the activity of antioxidant enzymes in plant body which is beneficial to resist oxidative damage (Li et al., 2016), so a small amount of Cu and Zn could promote the growth of *V. natans*.

However, for subjects within 7-14 days’ cultivation, \( F_{\text{V}}/F_{\text{m}} \) and \( F_{\text{V}}/F_{\text{o}} \) increased only when applied concentration was lower than that of T1. It meant the same treatment concentration would also have a certain degree of inhibitory effect on *V. natans* under long-term stress. When the concentration of heavy metals exceeded the threshold, \( F_{\text{V}}/F_{\text{m}} \) and \( F_{\text{V}}/F_{\text{o}} \) showed a downward trend for all three types of stress. There were two reasons: the increase of the heavy metal concentration lead to the decrease of protein activity on the chloroplast thylakoid membrane of the leaves (Assche and Clijsters, 1990). This hindered electron transfer, thus reducing the number of electrons involved in CO2 fixation in photosynthesis, thus \( F_{\text{V}}/F_{\text{m}} \) decreased (Gao et al., 2019a). The high concentration of heavy metals would interfere with the photosystem reaction center of *V. natans*, and affected the activity of the photosynthetic system by controlling the electrons at the water cracking end, resulting in the decrease of fluorescence level and the decrease of \( F_{\text{V}}/F_{\text{o}} \) (Rai et al., 2016). In this study, \( F_{\text{V}}/F_{\text{o}} \) had a greater range of change, indicating that Cu and Zn had a much greater impact on the water cracking end than \( F_{\text{V}}/F_{\text{m}} \). For the groups treated with the same concentration of heavy metals, the variation ranges of \( F_{\text{V}}/F_{\text{m}} \) and \( F_{\text{V}}/F_{\text{o}} \) under combined stress was larger than that under single stress, and there was significant difference between Cu and Zn groups. This might be related to the types of heavy metals. Cu can affect the chlorophyll content, and Zn can adjust the balance of cell osmotic pressure (Zhang et al., 2016). Low-concentration treatment would not cause too much negative impact on plants, but with the increase of Zn concentration, the osmotic pressure balance was broken in plants, which aggravated the toxicity to chloroplasts and interfered with the photosynthesis (Momchil et al., 2018). High concentration of Zn could also hinder the synthesis of certain proteins, weaken the resistance of the plant itself, and increase the toxicity of other heavy metals (Xu et al., 2006).

Light quantum of adsorbed by PS II reaction center transferred and dissipated in effective quantum yield \( Y(\text{II}) \), non-photochemical quenching coefficient \( q_N \), and regulated energy dissipation quantum \( Y(\text{NPQ}) \) (Qian et al., 2011). The values of \( Y(\text{II}) \) always increased first and then decreased, while the values of \( Y(\text{NO}) \) and \( Y(\text{NPQ}) \) hardly changed when applied concentration was lower than that of T2. However, when the concentration was higher than that of T2, \( Y(\text{NO}) \) and \( Y(\text{NPQ}) \) increased significantly for the subjects within 14 days’ cultivation, and the rise of \( Y(\text{NPQ}) \) was higher than \( Y(\text{NO}) \). It indicated that heavy metal stress at this time had already threatened the growth of *V. natans*, and plants consumed too much energy through self-regulation to resist unfavorable conditions (Gao et al., 2019a). For the subject with 14 to 21 days’ cultivation under the combined stress, \( Y(\text{NO}) \) and \( Y(\text{NPQ}) \) had a decline in high concentration.
treatment. The possible reason is that due to the long-term, high-concentration heavy metal stress, the normal physiological activity of *V. natans* was interfered, resulting in the weakening of the photosynthetic ability (Ozffidan et al., 2018). The energy conversion and electron transfer in the photosystem under combined stress were more severely affected. It might be that the presence of Zn promoted the absorption of Cu by plants, which made plants suffer more from the outside environment. Similarly, the variation of increasing trend of *Y(NO)* and *Y(NPQ)* caused by combined stress was greater than by single stress.

The values of *qP* decreased significantly with the increase of the concentration of heavy metals, indicating that the electron flow from the oxidation side of PS II to the reaction center was inhibited in the photosystem, which further caused a decrease in the rate of photosynthesis (Wang et al., 2010; Sun et al., 2020). The variation of *qP* caused by combined stress was greater, which indicated the combined stress has a greater impact on the actinic photoelectron transport of *V. natans*. When applied concentration was lower than that of T2, the values of *qN* hardly changed. The possible reason was that the heavy metal concentration was low, which didn’t affect the normal physiological activities of plants (Mobin and Khan, 2014). However, when applied concentration increased to a higher level (>T2), the values of *qN* increased gradually. It means that the PSII reaction center of leaves absorbs light energy for natural pigments, mostly for heat dissipation (Hou et al., 2018). The increase of *qN* has a positive effect on the protection of plants themselves. For subjects with 21 days’ cultivation, *qP* and *qN* couldn’t be determined in T5 group treated by combined stress. This means the plant photosynthetic function is completely impaired.

**Conclusions**

*V. natans* could still have normal photosynthetic activity in the water environment with low concentration of heavy metals (Cu≤0.4 mg/L, Zn≤4.0 mg/L, Cu+Zn≤0.2+2.0 mg/L). *Fv/Fm*, *Fv/Fo*, *Y(II)* and *qP* increased slightly, so it could promote the photosynthetic cooperation of plants to a certain extent, and the other indicators were almost unchanged. Under the stress of higher concentration of heavy metals, the tolerance of *V. natans* to combined stress was smaller than that of single stress. Therefore, in the three stress treatments, the inhibition of combined stress on the photosynthetic activity was the highest, and had a synergistic effect.

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