

# Effects of Cadmium Stress on Photosynthetic Physiological Characteristics of Two *Rorippa* Species

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## Authors' contributions

This work was carried out in collaboration among all authors. Author XYF designed the study. Author YYN performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WLY and CH managed the analyses of the study. Author CH managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

The photosynthetic physiological characteristics of *Rorippa amphibia* and *Rorippa sylvestris* were studied by pot experiment under Cd stress, and 7 Cd treatments were 0, 1, 5, 10, 50, 100, 200 ( $\text{mg}\cdot\text{kg}^{-1}$ ) respectively. The results showed that, (1) The Chla, chl<sub>b</sub>, and Ch(la+b) changed little when the Cd concentration in the soil was less than  $100 \text{ mg}\cdot\text{kg}^{-1}$ . There was no significant difference among the treatments. When Cd concentration was more than or equal to  $100 \text{ mg}\cdot\text{kg}^{-1}$ , all indexes decreased significantly, Chla/b increased gradually with the increase of Cd stressful concentration; (2) Net photosynthetic rate ( $P_n$ ), stomatal conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) first increased and then decreased with the increase of Cd stressful concentration, while intercellular carbon dioxide concentration ( $C_i$ ) increased significantly; (3) The photochemical quenching coefficient (qP) and electron transfer rate (ETR) decreased gradually with the increase of Cd stressful concentration, while the non-photochemical quenching coefficient (NPQ) increased with the

increase of Cd stress concentration. It indicated that the photosynthetic mechanism of leaves was damaged; the maximum photochemical quantum yield (Fv/Fm) and potential activity (Fv/Fo) of PSII decreased gradually with the increase of Cd stress concentration. When Cd concentration was more than 50 mg·kg<sup>-1</sup>, the decrease was very obvious, showing photoinhibition. The concentration of Cd below 50 mg·kg<sup>-1</sup> had little effect on photosynthesis. These results provide a theoretical application by using these two species of *Rorippa* to ecological restore the Cd contaminated farmland and abandoned mines.

**Keywords:** Cadmium stress; *Rorippa amphibia*; *Rorippa sylvestris*; photosynthetic; chlorophyll fluorescence.

## 1. INTRODUCTION

With the rapid development of industry, agriculture and economy in China, the problem of environmental pollution is becoming more and more serious. Heavy metal pollution is an important part of environmental pollution. Cadmium (Cd) pollution is one of the "five toxic" elements with the largest pollution area, the widest range and the most serious harm. It mainly comes from the application of chemical fertilizers and pesticides, the irrigation of industrial wastewater and sewage, mining, atmospheric sedimentation, etc [1]. The results show that photosynthesis of plants is sensitive to Cd [2]. For non Cd tolerant plants, Cd can hinder the biosynthesis of chlorophyll [3], destroy the structure and function of photosynthetic organs, and significantly reduce the photosynthesis rate of plants; It also interferes with electron transfer and ATP synthesis, and inhibits the activities of key enzymes in the dark reaction stage [4,5]; In addition, the stomatal behavior of plants can be controlled by changing the water potential and turgor pressure of mesophyll cells [6], thus reducing the gas exchange rate and some gas exchange parameters. However, for Cd tolerant plants, photosynthesis was not significantly inhibited under Cd stress, For example *Viola baoshanensis*, *Solanum nigrum* and other plants [7,8]; Therefore, the effect of Cd on Photosynthesis of plants is closely related to the tolerance of plants to Cd. In addition, the photosynthetic responses of different Cd tolerant plants to Cd stress were also significantly different, For example, chlorophyll content and net photosynthetic rate of *Solanum nigrum* and *brassi cajuncea* had no significant difference under low Cd concentration stress and normal growth environment, but some gas exchange parameters (transpiration rate and stomatal conductance) showed opposite trend [7,9]; For example, the chlorophyll content of *Rorippa globosa* first increased and then decreased with the aggravation of Cd stress [10], The chlorophyll

content of *Solanum nigrum* decreased with the increase of Cd stress [7]. In conclusion, the response of photosynthetic characteristics of different plants to Cd stress was very different.

*Rorippa sylvestris* and *Rorippa amphibia* are two perennial herbs *Brassicaceae Rorippa*. *R. sylvestris* is native to Europe, ZHANG SHU MEI was first found in the lawn of Dalian green belt in 2006. *R. sylvestris* is native to Europe and southwest Asia, and has been recorded in Yining, Xinjiang in China, Shenyang area was found in the campus lawn of Shenyang Agricultural University for the first time in 2015 [11]. *R. sylvestris* and *R. amphibia* both take the lawn as the distribution center and form the dominant community. Because the lateral roots are very developed and the root tiller reproduction ability is very strong, they have strong invasion, and are recognized as alien invasive plants and regional invasive plants respectively [12]. Using the characteristics of strong vitality of invasive plants, planting in heavy metal contaminated soil for remediation will turn disaster into blessing. Perennial herbaceous plants have the advantages of one-time planting and multiple harvest, low cost of soil remediation and quick effect. The cultivation experiment and field investigation showed that two specifications of *Rorippa* is a raceme with a large number of small flowers and bright yellow. It has high ornamental value, and has the characteristics of fast growth, resistance to pruning, trampling, ecological breadth and strong stress resistance. It is also a very good garden and woodland cover plant. Therefore, The photosynthetic physiological characteristics of *R. amphibia* and *R. sylvestris* were studied under Cd stress, and the effects of Cd Stress on photosynthetic pigment content, gas exchange parameters and chlorophyll fluorescence parameters were analyzed, in order to evaluate the tolerance of two specifications of *Rorippa* to Cd and provide good plant materials for ecological restoration of Cd contaminated farmland soil and abandoned mines.

## 2. MATERIALS AND METHODS

### 2.1 Test Soil

The experiment was carried out in the plastic greenhouse of the scientific research base of Shenyang Agricultural University (41°51'N, 123°34'E). The climate was continental monsoon, the annual average temperature was 8.1 °C, and the precipitation was 721.9 mm. The physical and chemical properties of cultivated soil are shown in Table 1.

### 2.2 Materials and Treatment

In May 2019, after air drying, grinding and sieving (4 mm), 7 kg soil was weighed and put into plastic flowerpots with diameter of 13cm x depth of 10cm. Seven experimental gradients were set up: 0 (CK, 1, 5, 10, 50,100,200) (mg·kg<sup>-1</sup>), Each experimental gradient was repeated 10 times. Cadmium (CdCl<sub>2</sub>·2.5H<sub>2</sub>O, analytically pure) was added to the soil in the form of aqueous solution, and then stored for 1 month. At the end of May, the root segments of *R. sylvestris* and *R. amphibia* were excavated in the campus, with a length of 3cm and a diameter of 3 mm, and planted in the hole tray. The soil was 1/2 river sand +1/2 peat. After 10 days of slow growth, the seedlings with the same growth were selected and planted in the flowerpot. Three plants were planted in each pot. The day/night temperature was 25°C/18°C, the light cycle was 12 h, the light/12 h dark, and the soil moisture content was maintained at about 85%. After treatment for 70 days, the indexes were determined.

### 2.3 Determination Index

The specific determination index was as follows.

The chlorophyll content was determined by acetone ethanol mixed solution extraction method [13];

Fluorescence parameters were measured by chlorophyll fluorescence meter (Pam-2500). It was carried out from 8:00 am to 11:00 am. The parameters included initial fluorescence (F<sub>0</sub>), maximum fluorescence (F<sub>m</sub>), electron transfer rate (ETR), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) [14]; According to the measured data, the maximum photochemical quantum yield (F<sub>v</sub> / F<sub>m</sub>) and the potential activity

(F<sub>v</sub> / F<sub>0</sub>) of PSII can be calculated [15].

F<sub>v</sub>/F<sub>m</sub> (Maximum photochemical quantum yield of PSII) =  $\frac{F_m - F_0}{F_m}$

F<sub>v</sub>/F<sub>0</sub> (Potential activity of PSII) =  $\frac{F_m - F_0}{F_0}$

Choose a sunny, windless day, Photosynthetic gas exchange parameters were measured by li-6800 photosynthetic apparatus. The leaves were all mature functional leaves in the same direction. Three plants were tested for each treatment, and three leaves were determined for each plant, Net photosynthetic rate ( $P_n$ /μmol·m<sup>-2</sup>·s<sup>-1</sup>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>/10-6), stomatal conductance ( $G_s$  / mol·m<sup>-2</sup>·s<sup>-1</sup>) and transpiration rate ( $T_r$  / mmol·m<sup>-2</sup>·s<sup>-1</sup>) were measured three times.

### 2.4 Data Processing

SPSS 23.0 and Excel 2017 were used to process the data, One way ANOVA and Duncan multiple comparison were used to analyze the significance.

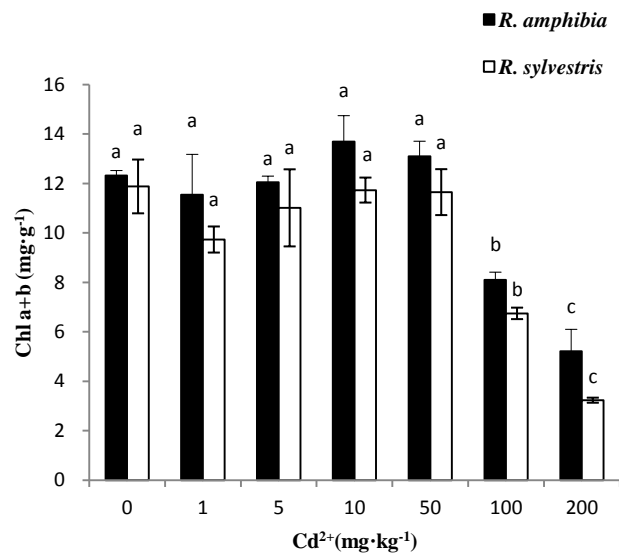
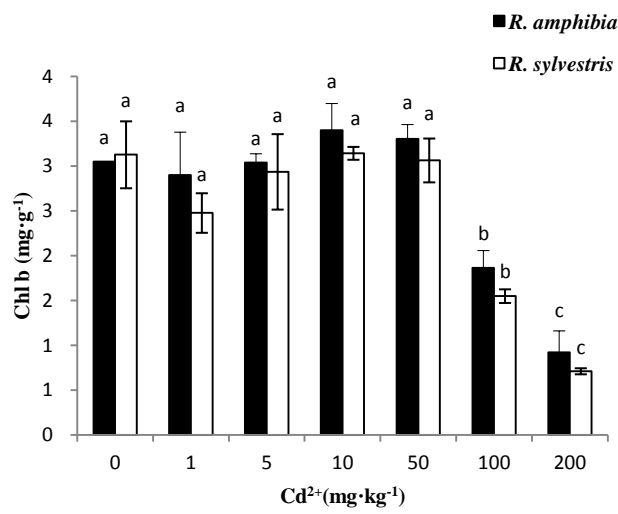
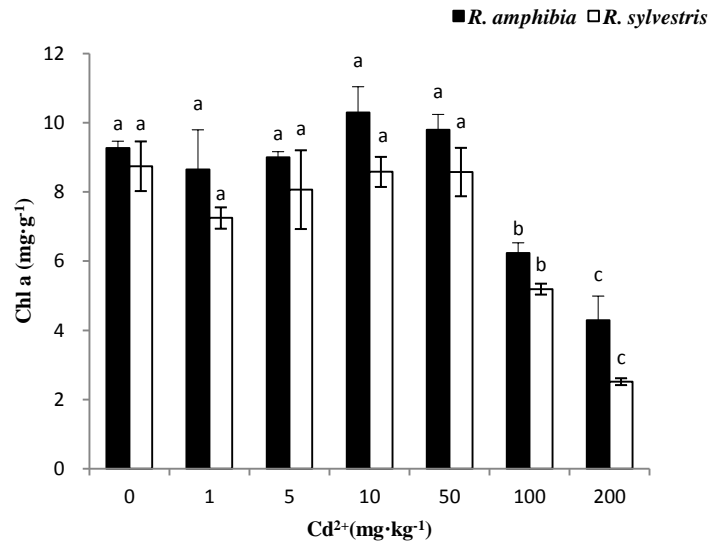
## 3. RESULTS AND DISCUSSION

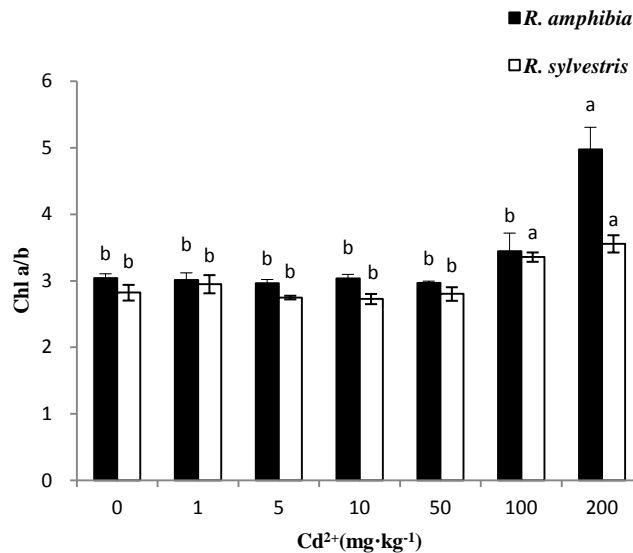
### 3.1 Effects of Cadmium Stress on Chlorophyll Content of 2 Specifications of *Rorippa*

As shown in Fig. 1, Chla, Chlb and Chl (a+b) of *R. sylvestris* and *R. amphibia* decreased with the increase of Cd stress. Chla, Chlb and Chl (a+b) decreased significantly at 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>, respectively ( $P < 0.05$ ). Compared with CK, *R. amphibia* decreased by 33%, 39% and 34% at 100 mg·kg<sup>-1</sup>, respectively, At 200 mg·kg<sup>-1</sup>, the decrease rates were 54%, 70% and 58%, respectively, At 100 mg·kg<sup>-1</sup>, the decrease rates of *R. sylvestris* were 41%, 50% and 43%, respectively, and 71%, 77% and 73% at 200 mg·kg<sup>-1</sup>. The results showed that Cd had no significant effect on Chla, Chlb and Chl (a+b) contents under the stress of 1 mg·kg<sup>-1</sup> - 50 mg·kg<sup>-1</sup>, while the contents of chlorophyll were significantly affected by Cd at high concentrations (100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>). It can also be seen from Fig. 1 that Chl (a/b) increased with the increase of Cd stress, indicating that the inhibition of Cd on Chla was weaker than that of Chlb.

**Table 1. Basic physical and chemical properties of the soil**

<b>pH</b>	<b>Total nitrogen (g·kg<sup>-1</sup>)</b>	<b>Total phosphorus P (g·kg<sup>-1</sup>)</b>	<b>Total potassium (g·kg<sup>-1</sup>)</b>	<b>Organic matter (g·kg<sup>-1</sup>)</b>	<b>Available nitrogen (mg·kg<sup>-1</sup>)</b>	<b>Available phosphorus (mg·kg<sup>-1</sup>)</b>	<b>Available potassium (mg·kg<sup>-1</sup>)</b>	<b>Cd concentration (mg·kg<sup>-1</sup>)</b>
7.14	0.69	0.25	9.97	1.83	8.97	58.32	54.56	0.12





**Fig. 1. Chlorophyll content of two species of *Rorippa* under Cd stress**

Note: Lowercase letters in the Fig. stand for the significant difference at 0.05 level

### 3.2 Effects of Cadmium Stress on Photosynthetic Parameters of 2 Specifications of *Rorippa*

It can be seen from Fig. 2 that with the increase of Cd stress concentration, the net photosynthetic rate ( $P_n$ ), transpiration rate ( $T_r$ ) and stomatal conductance ( $G_s$ ) of 2 specifications of *Rorippa* increased first and then decreased. 2 specifications of *Rorippa* was significantly higher than that of CK in 10 mg·kg<sup>-1</sup> and 50 mg·kg<sup>-1</sup> groups, The  $P_n$  of *R. amphibia* was significantly lower than that of CK in other Cd treatments;  $P_n$  of *R. sylvestris* decreased significantly at 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup> ( $P<0.05$ ); The  $T_r$  of 2 specifications of *Rorippa* was significantly higher than that of CK at 10 mg·kg<sup>-1</sup> and 50 mg·kg<sup>-1</sup>, and decreased significantly at 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup> and significantly lower than that of CK ( $P<0.05$ ), Under the low concentration (1 mg·kg<sup>-1</sup>- 5 mg·kg<sup>-1</sup>) of Cd treatment, There was no significant difference between *R. amphibia* and CK ( $P<0.05$ ), *R. sylvestris* was significantly lower than CK ( $P<0.05$ ); The change trend of  $G_s$  of 2 specifications of *Rorippa* is similar to  $P_n$ , With the increase of Cd concentration,  $G_s$  first increased and then decreased, At 10 mg·kg<sup>-1</sup> and 50 mg·kg<sup>-1</sup>, it reached the highest value, and decreased to the minimum value at 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>. The change trend of  $C_i$  is just opposite to  $P_n$ , For each experimental gradient of 2 specifications of *Rorippa*,  $C_i$  increased significantly compared with CK; Under 200

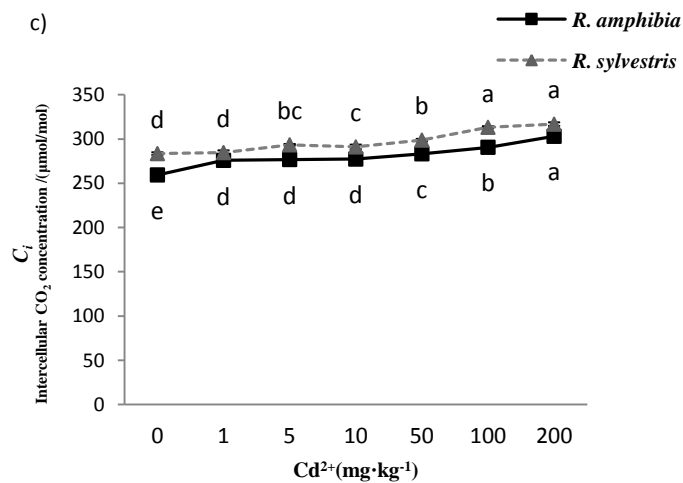
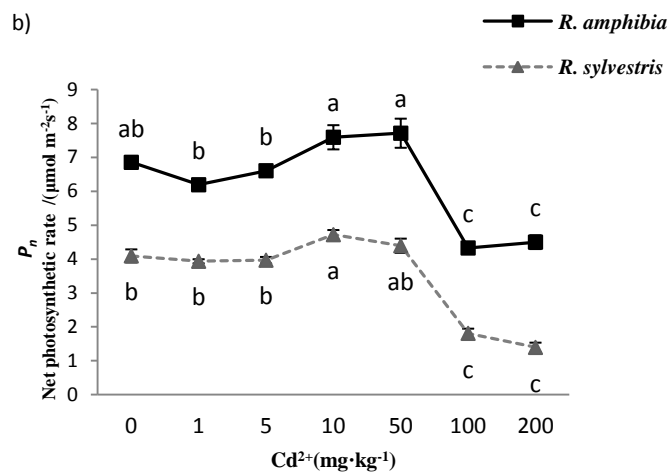
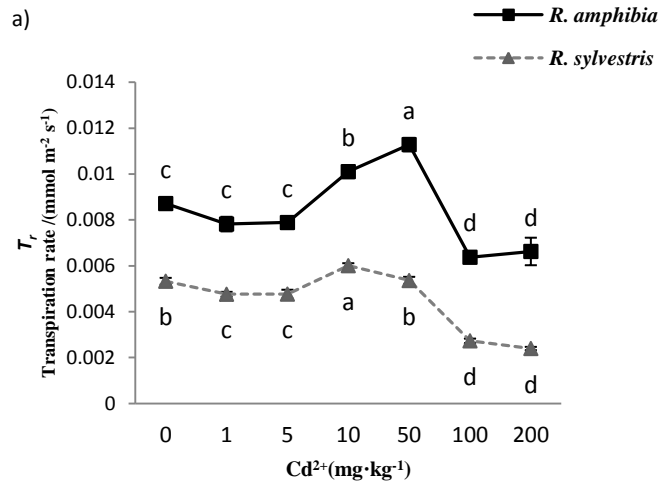
mg·kg<sup>-1</sup> Cd stress, *R. amphibia* reached the maximum and was significantly higher than that of all other treatments ( $P<0.05$ ); Under Cd stress of 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>, *R. sylvestris* was significantly higher than that of other treatments ( $P<0.05$ ). At the same time, as can be seen from Fig. 2, the decrease of  $G_s$ ,  $T_r$  and chlorophyll contents and the increase of  $C_i$  caused by Cd stress were different among different *Rorippa* varieties. The effect of Cd on photosynthesis of *R. amphibia* was less than that of *R. sylvestris*.

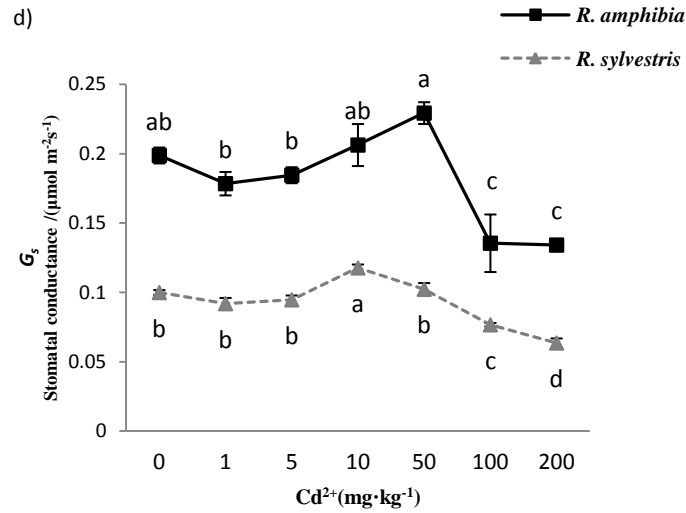
### 3.3 Effects of Cadmium Stress on Chlorophyll Fluorescence Parameters of 2 Species of *Rorippa*

As shown in Table 2, Fv/Fo and Fv / Fm of two species of *Rorippa* did not change significantly in the range of 0 mg·kg<sup>-1</sup> - 50 mg·kg<sup>-1</sup>, In the treatment of 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>, it decreased significantly; ETR and qP decreased with the increase of Cd stress concentration, From 5 mg·kg<sup>-1</sup>, it decreased significantly; NPQ increased gradually with the increase of Cd stress concentration, and reached the maximum value at 100 mg·kg<sup>-1</sup> and 200 mg·kg<sup>-1</sup>, respectively, and was significantly higher than that of other treatment groups ( $P<0.05$ ), From this we can see that, When *R. amphibia* was treated with 0 mg·kg<sup>-1</sup> - 50 mg·kg<sup>-1</sup> and *R. sylvestris* was treated with 0 mg·kg<sup>-1</sup> - 100 mg·kg<sup>-1</sup>, The results showed that the leaves could maintain normal light conversion efficiency

and stable PS potential light activity, The leaves could still maintain normal light conversion efficiency and stable PSII potential light activity, However, PSII photoresponse center was more sensitive to Cd, and its opening degree was

decreased under Cd stress, However, two species of *Rorippa* also try their best to improve the thermal dissipation capacity of the reaction center under PSII, so as to reduce the damage caused by photoinhibition.





**Fig. 2. Changes of photosynthetic parameters of two species of *Rorippa* under Cd stress.**  
**a) The net photosynthetic rate (Pn); b) the transpiration rate (Tr); c) Inter-cellular CO<sub>2</sub> concentration (C<sub>i</sub>); d) the stomatal conductance (G<sub>s</sub>)**

Note: Lowercase letters in the Fig. stand for the significant difference at 0.05 level

**Table 2. Chlorophyll fluorescence parameters of 2 species of *Rorippa* under Cd stress**

Materials	Cd <sup>2+</sup> (mg·kg <sup>-1</sup> )	F <sub>v</sub> /F <sub>o</sub>	F <sub>v</sub> /F <sub>m</sub>	ETR	qP	NPQ
<i>R. amphibia</i>	0 (CK)	2.875±0.144a	0.742±0.009a	159.678±1.672a	0.821±0.007a	0.331±0.045b
	1	2.700±0.101a	0.730±0.008a	158.971±2.680a	0.817±0.010a	0.392±0.035b
	5	2.686±0.106a	0.729±0.009a	149.975±1.147b	0.763±0.009b	0.411±0.042b
	10	2.700±0.100a	0.730±0.008a	148.617±0.939b	0.756±0.017b	0.405±0.045b
	50	2.584±0.042a	0.721±0.008ab	147.217±2.910b	0.741±0.010b	0.437±0.029b
	100	2.247±0.085b	0.692±0.008bc	145.956±2.467b	0.747±0.014b	0.621±0.039a
<i>R. sylvestris</i>	0 (CK)	3.125±0.070a	0.756±0.003a	157.086±3.124a	0.830±0.004a	0.764±0.016b
	1	3.127±0.072a	0.756±0.004a	160.825±1.467a	0.834±0.009a	0.805±0.018b
	5	2.918±0.072a	0.743±0.004a	149.867±1.617ab	0.774±0.004b	0.771±0.031b
	10	2.941±0.030a	0.749±0.003a	149.833±3.552ab	0.788±0.008b	0.661±0.005b
	50	3.057±0.111a	0.749±0.002a	150.167±1.919ab	0.772±0.006b	0.672±0.060b
	100	2.552±0.089b	0.719±0.003b	142.867±7.781b	0.745±0.038b	0.694±0.028b
	200	1.853±0.141c	0.650±0.011c	108.725±5.963c	0.700±0.017c	1.111±0.088a

Note: Lowercase letters in the table stand for the significant difference at 0.05 level

### 3.4 Discussion

Effects of cadmium stress on chlorophyll content of 2 specifications of *Rorippa*. chloroplast pigment is the basic substance of photosynthesis in plants, and its content changes can reflect the potential of photosynthesis. Some studies have shown that Cd stress can make the leaves yellow [16]. This may be due to the indirect inhibition of chlorophyll biosynthesis by Cd stress, which is seriously inhibited at higher Cd concentrations, Chla and Chlb, as antenna pigments, play a leading role in the absorption and transmission of light energy. In this study, both of them showed a

downward trend, It may be that Cd combines with -SH of many enzymes in chloroplast or substitutes the main components of chloroplast, such as Fe<sup>2+</sup>, Zn<sup>2+</sup> and Mg, which destroys chloroplast structure and accelerates the decomposition of chlorophyll [7,11] When the chlorophyll content is insufficient, the Chl(a/b) will be adjusted to maintain a higher photosynthetic rate, In this study, Chl(a/b) increased gradually, which should be a defense mechanism of two species of *Rorippa* against Cd toxicity [17]. In addition, studies have shown that, Reducing the synthesis of light harvesting chlorophyll protein (Chlb) can reduce the accumulation of reactive oxygen species and enhance their tolerance [18].



Effects of cadmium stress on gas exchange parameters of 2 specifications of *Rorippa*.  $P_n$ ,  $T_r$ ,  $C_i$ ,  $G_s$  can reflect the response of plant photosynthetic physiology to stress, which is the basic index to evaluate the photosynthesis intensity. It is generally believed that there are two main reasons for the change of  $P_n$ : stomatal factor and non stomatal factor [19]. The value of  $C_i$  is used as the basis to judge the stomatal limitation and non stomatal limitation, Only when  $C_i$ ,  $P_n$  and  $G_s$  decrease, it can be considered that the decrease of  $P_n$  is mainly caused by the decrease of stomatal conductance; On the contrary, If the decrease of  $P_n$  is accompanied by the increase of  $C_i$ , it can be considered that the decrease of  $P_n$  is caused by non stomatal limitation [20]. In this study,  $P_n$  and  $G_s$  of two species of *Rorippa* decreased with the increase of Cd stress concentration in soil, and  $C_i$  increased at the same time, This indicated that the decrease of photosynthesis in Cd stressed environment was not only due to the decrease of  $G_s$  and  $CO_2$  supply, but also due to the non stomatal factors hindering the utilization of  $CO_2$ , resulting in the accumulation of intercellular  $CO_2$ . In addition, the factors causing the decrease of  $P_n$  are very complex, such as the inhibition of chlorophyll synthesis, and the decrease of  $P_n$  content may be one of the important reasons for the decrease of  $P_n$ . And the results show that,  $Cd^{2+}$  can affect the electron transfer and  $CO_2$  fixation in photosynthesis, which makes the chlorophyll synthesis unable to be normal, resulting in the inhibition of photosynthesis [21].

Effects of cadmium stress on chlorophyll fluorescence parameters of 2 species of *Rorippa*. As an indicator of PSII photochemical reaction, chlorophyll fluorescence can effectively reflect the regulation process of plant photosynthetic mechanism. By analyzing the fluorescence parameters, we can understand the physiological changes of plants and the effective information of light energy utilization [22]. Studies have shown that, Cd stress inhibited PSII photosynthetic activity and electron transport [23]. In this study, The changes of  $F_v/F_m$  and  $F_v/F_o$  were not obvious in the treatment of  $1\text{ mg}\cdot\text{kg}^{-1}$  -  $50\text{ mg}\cdot\text{kg}^{-1}$ , which indicated that the damage degree of PSII reaction center was small under the treatment of  $1\text{ mg}\cdot\text{kg}^{-1}$  -  $50\text{ mg}\cdot\text{kg}^{-1}$ , These two species of *Rorippa* can still maintain a certain light energy conversion efficiency and photosynthetic activity, With the further increase of Cd concentration, it reached  $100\text{ mg}\cdot\text{kg}^{-1}$  and  $200\text{ mg}\cdot\text{kg}^{-1}$ ,  $F_v/F_m$  and  $F_v/F_o$  decreased significantly, indicating that the primary photochemical efficiency and energy

transfer efficiency from antenna pigment to PSII reaction were significantly affected by Cd stress, resulting in photo inhibition. ETR can reflect the actual photochemical reaction efficiency of plants on PSII, in this study, The ETR of the two species of *Rorippa* decreased significantly with the increase of Cd stress, this is similar to the research results of Xu Xiaoxun [24] and Su Xiurong [25]. Cd stress led to the destruction of the photosynthetic electron transport chains in the leaves of the two species. The electron cannot be transferred from PSII to PSI effectively, at the same time, PSII reaction center could not accept new photons. Once the photons accumulated too much, the cell assimilation capacity (NADPH and ATP) of leaves could not be formed normally, so ETR decreased, this may be the reason for the decrease of photosynthetic rate of the two species of *Rorippa*, which is consistent with the results of Vassilev and Yordanov [26]. qP (photochemical quenching coefficient) can reflect the proportion of light energy absorbed by antenna pigment in PSII image to participate in photochemical reaction. In this study, qP decreased significantly with the increase of Cd stress concentration, Wu Kun et al. Considered that this was an adaptive phenomenon of plants to prevent excessive reduction of QA, the original electron receptor of PSII ring, when plants were exposed to Cd stress [27].

#### 4. CONCLUSIONS

To sum up, it can be concluded that the photosynthetic physiological characteristics of 2 Species of *Rorippa* had been less affected, when the concentration of Cd was below  $50\text{ mg}\cdot\text{kg}^{-1}$ , with the increase of Cd concentration, the chlorophyll content and  $P_n$  increased gradually. When Cd concentration increased further during  $100\text{ mg}\cdot\text{kg}^{-1}$  and  $200\text{ mg}\cdot\text{kg}^{-1}$ , the PSII reaction center of the two species of *Rorippa* suffered from relative degree of photoinhibition, and the utilization ability of weak light and absorption light was slightly weakened, which eventually led to the decline of photosynthetic capacity of the two species of *Rorippa*. This research indicated that *R. amphibia* and *R. sylvestris* have stronger tolerance to Cd, and can be used for remediation of Cd contaminated soil.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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