Effects of Cu^{2+} on the photosynthesis of *Hydrocharis dubia* (Bl.)

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Abstract- **The shortage of water is extremely severe in China and the situation is growing worse because of the water pollution by heavy metal ions which is difficult to be removed with conventional methods. The precipitation of heavy metal ions makes the elimination even more difficult. *Hydrocharis dubi*a (Bl.) grown in 1/2 Hoagland growth medium with different Cu^{2+} concentrations is studied to explore its utility in eliminating Cu^{2+} by investigating the effects of Cu^{2+} on the biomass gain and photosynthesis of *Hydrocharis dubi*a (Bl.). The amounts of Chlorophyll a and b within the leaves, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate are also investigated. The biomass gain decreases with increasing Cu^{2+} concentration and negative gain occurs in *Hydrocharis dubi*a (Bl.) grown in the medium with *p*(Cu^{2+})=5.0mg Ł$^{-1}$. The amounts of Chlorophyll a and b undergo decreasing with increasing Cu^{2+} concentration as well, indicating the synthesis of Chlorophyll is impaired by Cu^{2+} due to the effects of Cu^{2+} on the growth of *Hydrocharis dubi*a (Bl.) which results in the decreasing of the net photosynthetic rate and stomatal conductance.**

***Key words*: Chlorophyll, biomass, photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration**

I. INTRODUCTION

The shortage of water is extremely severe in China considering that almost half of the 600 cities are undergoing water shortage and pollution. It is indicated the surface water overall is medium polluted in *Communique of the Environmental Status of China of 2010*. Among the state-controlled monitoring sections of surface water in 2010, the ratios of I~III and V level of surface water are 51.9% and 20.8%, respectively. Furthermore, heavy metal ions such as lead, mercury and copper ions exceed the standard limits in about 40 surface water sections in rivers of southwest China, the Hai River, the Yangtze River and the Yellow River etc. The utilities of water will be diminished and the ecosystem around the water will be impaired due to water pollution, which enforces human beings to develop more effective and economic methods to purify contaminated water. Despite that plenty of methods have been intensively explored like conventional activated sludge method, the purification technique with aquatic plants has attracted more and more interests due to the lower cost and simple management. The environmental ecosystem will recover or be improved along with recycled water resources [1].

Different from organic chemicals which can be naturally degraded or biodegraded, heavy metal ions in contaminated water will dissolve in water or precipitate into the bottom of water. The adsorption or absorption effects of aquatic plants such as *Typha orientalis* [2], *Hydrocharis dubi*a (Bl.) [3-5], *Eichhornia crassipes* [6], *Myriophyllum aquaticum* [7] are exploited recent years to eliminate heavy metal ions from water. Polluted water, however, with heavy metal ions beyond certain concentrations will be poisonous to plants and the physiological characteristics and photosynthesis of plants will be affected by heavy metal ions. In contrast to intensive investigations in literature about the dependence of physiological characteristics of aquatic plants on heavy metal ions, it is rarely reported about the effects of heavy metal ions on the photosynthesis of plants. Thus, the photosynthesis of *Hydrocharis dubi*a (Bl.) which grows widely in water is explored utilizing 1/2 Hoagland growth medium with different Cu^{2+} concentrations. Measurements of the amounts of Chlorophyll in *Hydrocharis dubi*a (Bl.) leaves, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate at different days reveal the mechanism of the effects of Cu^{2+}on *Hydrocharis dubi*a (Bl.) photosynthesis, which sheds light on the application of *Hydrocharis dubi*a (Bl.) in purification of polluted water.

Ⅱ.Materials and methods

The experimental materials, *Hydrocharis dubi*a (Bl.) are from the pond inside Soochow University, Suzhou, China. After washing, the materials are grown in 1/2 Hoagland growth medium in illumination incubator. In early December, five selected well-grown *Hydrocharis dubi*a (BI.) are cultured in 1/2 Hoagland growth medium with Cu^{2+} concentration *p*(Cu^{2+})=0 mg Ł$^{-1}$(CK), 1.0 mg Ł$^{-1}$(A), 2.5 mg Ł$^{-1}$(B), 5.0 mg Ł$^{-1}$(C), in which Cu^{2+} is prepared from analytically pure $CuSO_4 \cdot 5H_2O$. All measurements are conducted every two days and every single group is repeated three times.

The measurements of Chlorophyll is according to the method of Tanushree Bhattacharya · S. Chakraborty · D. K. Banerjee, 2010[8]. The net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration of the second leaf from roots are measured utilizing LI-6400 portable photosynthesis system by LI-COR, USA under red-blue light source. The flow rate to the sample cell is set as 500 umol s^{-1} and the intensity of illumination is 1500 umol·$m^{-2}s^{-1}$. All measurements are conducted between 8:30am~11:30am under temperature 25~27°C.

Data Processing

All analysis is based on average values of three measurements to reduce experimental error after the processing of original data with Excel. Statistical significance analysis of all the photosynthetic indexes with Cu^{2+} concentration and growth days is conducted with SPSS 11.5, where $P \geq 0.05$ and $P<0.05$ indicate significant or insignificant difference respectively.

Ⅲ. RESULTS AND DISCUSSION

A. Biomass

The biomass of plants will increase in condition of normal growth, whereas the biomass gain will decrease or even become negative if the growth of plants is impaired. In this paper, the biomass of *Hydrocharis dubi*a (Bl.) decreases with increasing Cu^{2+} concentration at the same growth day compared with the control group and significant differences exist as shown in Fig. 1. It indicates the poisonous effect of Cu^{2+} on the growth of *Hydrocharis dubi*a (Bl.), which is also reported in the literature [9].In the control group the biomass grows with increasing days. The results are extremely different at $p(Cu^{2+})$=1.0mg·L^{-1} and $p(Cu^{2+})$=2.5mg·L^{-1}, for which the biomass gain undergoes reducing at first but increases later with more growth days. It can be inferred that *Hydrocharis dubi*a (Bl.) becomes adaptable to these two Cu^{2+} concentrations after few days but its growth is still affected compared with CK. The biomass of *Hydrocharis dubi*a (Bl.) grown in $p(Cu^{2+})$=5.0mg·L^{-1} , however, behaves decreasing with all the observed days in Fig. 2, suggesting that *Hydrocharis dubi*a (Bl.) cannot adapt to such high Cu^{2+} concentration and its grown is threatened due to the poisonous effect of Cu^{2+} which will lead to death eventually.

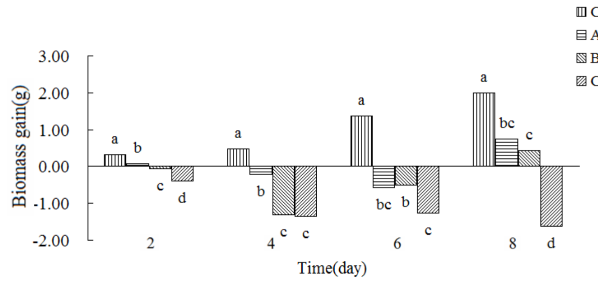

Fig.1.Effects of $p(Cu^{2+})$ on biomass gain under the same growth days.

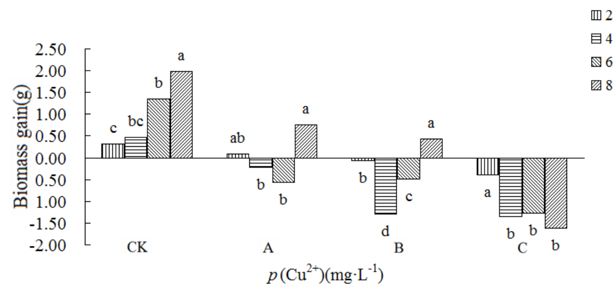

Fig.2.The biomass gain for the same $p(Cu^{2+})$ with increasing growth days.

B. Chlorophyll

Chlorophyll a and b are the two main structures of Chlorophyll which is vital to the photosynthesis of plants. It is revealed in this report that more Cu^{2+} will decrease the amount of Chlorophyll a with the same growth days as indicated in Fig. 3. Except for the case of $p(Cu^{2+})$=1.0mg·L^{-1} at Day 6, the amounts of Chlorophyll a are significantly different with CK in all the other groups and Chlorophyll b almost follows the same trend in Fig. 4 apart from the case of $p(Cu^{2+})$=1.0mg·L^{-1} at Day 2 and 6. Cu^{2+} becomes poisonous to *Hydrocharis dubi*a (Bl.) after absorption from the roots and its growth will be suppressed which is proved by the previous analysis on biomass gain. This effect will deteriorate with increasing Cu^{2+} concentration and the synthesis of Chlorophyll a and b will be impaired. As a result, the photosynthesis of *Hydrocharis dubi*a (Bl.) becomes less active as shown in the following analysis.

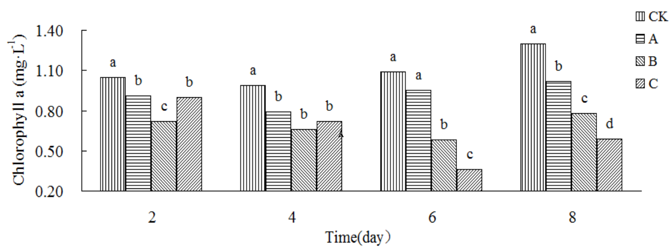

Fig.3.Effects of $p(Cu^{2+})$ on Chlorophyll a under the same growth days.

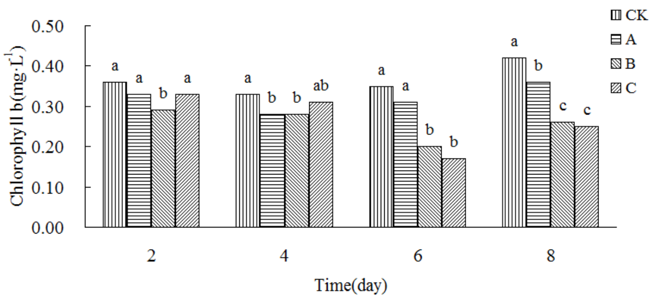

Fig.4. Effects of $p(Cu^{2+})$ on Chlorophyll b under the same growth days.

C. Photosynthesis

Photosynthesis is the basis of all lives and plants will only grow normally via sufficient photosynthesis. Therefore, the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate are measured in this experiment to characterize the effects of Cu^{2+} on the photosynthesis of *Hydrocharis dubi*a (Bl.). The net photosynthetic rate will decrease with increasing Cu^{2+} concentration at the same growth day as shown in Fig. 5. Significant differences exist in all groups compared with CK except for $p(Cu^{2+})$=1.0mg $\cdot L^{-1}$ at Day 2 after statistical analysis. The net photosynthetic rate even becomes negative at Day 8 for $p(Cu^{2+})$=5.0mg $\cdot L^{-1}$, indicating the respiration of *Hydrocharis dubi*a (Bl.) overwhelms the photosynthesis in this case and it will die duo to over consumption. In condition of $p(Cu^{2+})$=1.0mg $\cdot L^{-1}$ and $p(Cu^{2+})$=2.5mg $\cdot L^{-1}$, the gained energy from photosynthesis can still satisfy the loss although the net photosynthetic rate is clearly influenced by Cu^{2+} compared with CK and *Hydrocharis dubi*a (Bl.) can grow slowly in these groups.

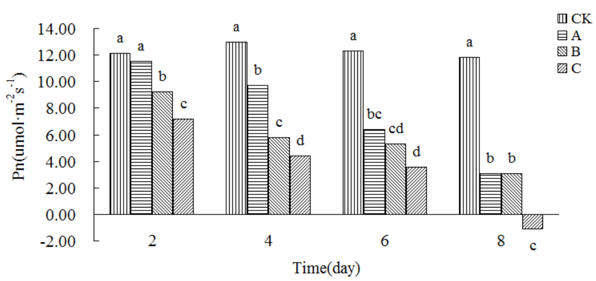

Fig.5. Effects of $p(Cu^{2+})$ on the net photosynthetic rate under the same growth days. Pn: Photosynthetic rate.

Furthermore, the trend of stomatal conductance is almost the same with the net photosynthetic rate from Day 4 to Day 8 as shown in Fig. 6. The stomatal conductance decreases significantly in all three Cu^{2+} concentrations at the same growth day except for Day 2. These values demonstrate the situation of stomas in *Hydrocharis dubi*a (Bl.) which can influence CO_2 concentration critically and thus the photosynthesis of plants.

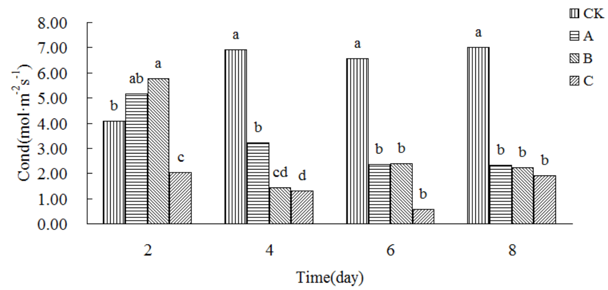

Fig.6. Effects of $p(Cu^{2+})$ on the stomatal conductance under the same growth days. Cond: stomatal conductance

The intercellular CO_2 concentration, however, varies differently with the net photosynthetic rate in this experiment as shown in Fig. 7. Thus intercellular CO_2 concentration is not the key factor of the photosynthesis of *Hydrocharis dubi*a (Bl.) in the experimental conditions owning to the fact that the intercellular CO_2 concentration is extremely sufficient for the photosynthesis. *Hydrocharis dubi*a (Bl.) will not face the shortage of CO_2 during photosynthetic process.

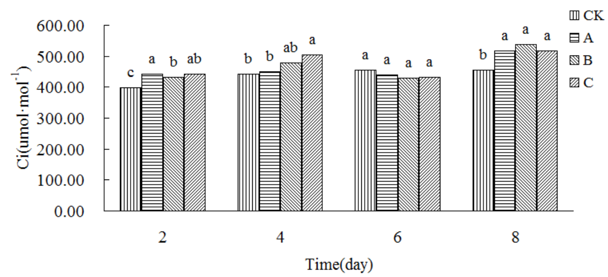

Fig.7. Effects of $p(Cu^{2+})$ on the intercellular CO_2 concentration under the same growth days. Ci: intercellular CO_2 concentration

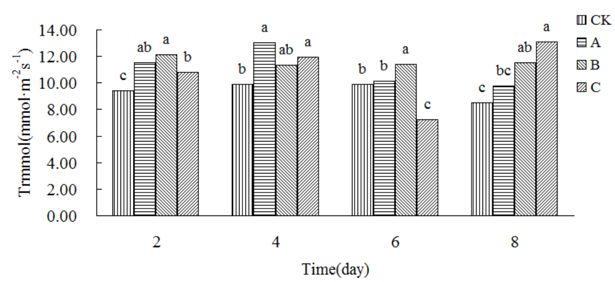

Fig.8. Effects of $p(Cu^{2+})$ on the transpiration rate under the same growth days. Trmmol: Transpiration rate

The transportation of water is affected by the stomatal conductance as well, which is demonstrated in the transpiration rate. The measured transpiration rates are significantly different with CK for *Hydrocharis dubi*a (Bl.) grown in the medium with Cu^{2+}. The transpiration rates increases with increasing Cu^{2+}

concentration except for $p(Cu^{2+})$=5.0mg L^{-1} at Day 6, of which the value is clearly lower than the other three groups. Overall, the transpiration rate is dependent on Cu^{2+} concentration in growth medium.

Ⅳ. CONCLUSION

The growth of *Hydrocharis dubi*a (Bl.) is affected poisonously by Cu^{2+} absorbed from the roots and the biomass gain will decrease as a result. More Cu^{2+} will also reduce the amounts of Chlorophyll a and b, thus the net photosynthetic rate undergoes decreasing. The effects of Cu^{2+} on the stomatal conductance are investigated as well in Figs. 5 and 6, which will bring on the decrease of net photosynthetic rate in this report. The intercellular CO_2 concentration is not the critical factor to the photosynthesis of *Hydrocharis dubi*a (Bl.) in the conditions we exploited due to the sufficiency of CO_2.

It is demonstrated in this paper that *Hydrocharis dubi*a (Bl.) can be adaptable to low Cu^{2+} concentration after some growth time and grow eventually, but it is not applicable to high Cu^{2+} concentration which will conduct to the death of *Hydrocharis dubi*a (Bl.). Hence, *Hydrocharis dubi*a (Bl.) is not suitable for be cultivated in water with very high Cu^{2+} concentration and its application for purification of polluted water may be only limited to situation with low Cu^{2+} concentration.

REFERENCES

[1] Shutes R.B.E, " Artificial wetlands and water quality improvement," Environment International. **26 :** 441-447, 2001.

[2] Yongjie HUANG, Dengyi LIU, Youbao WANG, Xingming WANG, Jing LI, "Heavy metals accumulation by hydrophytes," Chinese Journal of Ecology. **25 (5) :** 541-545, 2006.

[3] Haiqing HAO, Guoxin SHI, "Toxic effect of Zinc on *Hydrocharis dubia*," Chinese Journal of Applied & Environmental Biology. **7 (6)** :526-531,2001.

[4] Chunxia DING, Guoxin SHI, Qinsong XU, Haiyan YANG, Ye XU, Yingxiao XU, "Physiological and structural responses of *Hydrocharis dubia* leaves to different concentrations of Pb^{2+} stress," Guihaia. **29(6)** : 768-773, 2009.

[5] Dingtian YANG, Guoxin SHI, Wenpeng YOU, Fuchen CHANG, Kaibing XIE, "The Study of Cr^{6+} Pollution Affecting Chlorophyll and Activity of Enzymes in *Hydrocharis dubia* (B1.) Backer," Journal of Nanjing Normal University (Natural Science). **22 (2)** : 92-96, 1999.

[6] Caiyun TAN, Yuman LIN, Zuliang CHEN, "The Use of *Eichhornia crassipes* for the Removal of Heavy Metals from Aqueous Solutions,"Journal of Subtropical Resources and Environment. **06 :** 47-52, 2009.

[7] Hussner A , "Growth and photosynthesis of four invasive aquatic plant species in Europe," European Weed Research Society Weed Research **49 :** 506-515, 2009.

[8] Tanushree Bhattacharya, Chakraborty S, Banerjee D.K, **"**Heavy metal uptake and its effect on macronutrients, chlorophyll, protein, and peroxidase activity of Paspalum distichum grown on sludge-dosed soils," Environ Monit Assess. **169:**15-26, 2010.

[9]Hongjing LI, Suxia XIE, Tianyu LI, "The Toxic Effect of Hg^{2+} on *Hydrocharis dubia* BL. Backer," Journal of Nanjing Normal University (Natural Science). **23(3)**:87-90, 2003.