Physiological and Biochemical Responses of *Hydrilla verticillata* under Cu and Zn Stress

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Keywords: The Heavy Metal Stress, *Hydrilla verticillata*, Superoxide Dismutase, Peroxidase, Malondialdehyde.

Abstract: In order to understand the physiological and biochemical responses of *Hydrilla verticillata* under heavy metal stress, single and combined Cu or Zn were used for stress cultivation. The results showed that the activities of SOD, POD and MDA in the first treatment group (Cu<0.2 mg/L, Zn<1.6 mg/L, Cu+Zn<0.1 mg/L + 0.8mg/L) were slightly lower than those in a blank control group. SOD, POD and MDA increased significantly with the increase of heavy metal concentration within 7 days. From the 7th day to the 21st day of culture, they increased first, then decreased. The change range of enzyme activity was the largest under combined stress. The inhibitory effects of heavy metals on *H. Verticillata* were: combined stress > Cu stress > Zn stress.

1 INTRODUCTION

Cu, Zn, Pb and Cd are common heavy metal pollutants in water (Xing, 2013). Heavy metals play a key role in the balance of active oxygen metabolism system in plants (Ji, 2017; Gao, 2019). The types and sources of heavy metals are different, which lead to the complexity of water pollution (Shahid,2017). Therefore, heavy metal polluted water also generally appears in the form of combined stress. Cu and Zn are trace elements for plant growth, which can promote its development and growth at low concentration. However, high concentrations of Cu and Zn will affect the absorption of other nutrients by plants, break the balance of cell metabolism, and seriously curb their physiological growth (Jian, 2016).

Hydrilla verticillata is the dominant species in most lakes in China. Because of its rapid growth, wide distribution and good effect on the enrichment of heavy metals, it has gradually become a pioneer species to solve the problem of heavy metal pollution (Wang, 2020). In this study, the physiological and biochemical characteristics of *H.verticillata* under Cu and Zn stress were discussed in order to provide some basis for the ecological restoration of heavy metal polluted water.

2 EXPERIMENT MATERIAL AND METHOD

2.1 Material

H.verticillata and sediment were collected in Poyang Lake. After removing sundries, the sediment with a thickness of 8 cm was evenly laid in plastic square boxes (34.0 cm long, 22.5 cm wide and 10 cm high). The apical tips of *H.verticillata* with a height of 20 cm were planted in the plastic boxes after washing off the surface attachments, with 6 tips in each box. Then, the plastic boxes were placed into the glass jar (40 cm long, 40 cm wide and 50 cm high). Air-dried tap water was slowly added into the jars to the height of 48 cm for preculture. After the plant growth was stable, it was used in the later experiment.

2.2 Experimental Design

The toxicity of Cu and Zn to *H.verticillata* was about 8:1 from pre-experiment. Naturally, the concentrations of Cu and Zn should be configured according to 1: 8 in water body.

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Gao, G., Yang, W., Fang, B. and Chen, Q.
Physiological and Biochemical Responses of Hydrilla verticillata under Cu and Zn Stress.
DOI: 10.5220/0011189000003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 111-115
ISBN: 978-989-758-595-1

Different concentrations of $CuSO_4$ and $ZnSO_4$ combined stress treatment, and 5 treatment groups (T1, T2, T3, T4 and T5) were set respectively (Table 1). At the same time, a blank control group (CK) was set, and 3 replicates were set in each treatment group. The indexes of stress culture were measured every 7 days, and the experimental period was 21 days.

Table 1: Concentration setting (mg/L).

	Cu	Zn	Cu+Zn
CK	0	0	0+0
T1	0.2	1.6	0.1+0.8
T2	0.4	3.2	0.2+1.6
T3	0.6	4.8	0.3+2.4
T4	0.8	6.4	0.4+3.2
T5	1.0	8.0	0.5+4.0

2.3 Method

Malondialdehyde (MDA) was determined by thiobarbituric acid colorimetry. Superoxide dismutase (SOD) was determined by nitrogen blue tetrazole colorimetry. Peroxidase (POD) was determined by guaiacol.

2.4 Data Analysis

The experimental results were expressed in the terms of the mean and standard deviation more than three parallel data. Excel 2017 was used for the processing and mapping of the original test data. SPSS 19.0 software was used for one-way analysis of variance, and SNK method was used for multiple comparative analysis. $P < 0.05$ indicated significant difference.

3 RESULTS AND ANALYSIS

3.1 Effects of Cu and Zn Stress on SOD

Single and combined stress of Cu and Zn had significant effects on SOD of *H.verticillata* ($P < 0.05$). From T1 to T5, the SOD activity showed an upward trend and reached the maximum at T5 (Figure 1).

There was no significant change in T1 and T2 compared with CK, and the activity of SOD at T1 was lower than that at CK. When the stress duration was 14 days, the maximum of SOD under Cu single stress and combined stress were 45.91 U/mg and 45.80 U/mg respectively, and appeared at T4. The maximum of SOD under Zn single stress was 45.55 U/mg and appeared at T5.

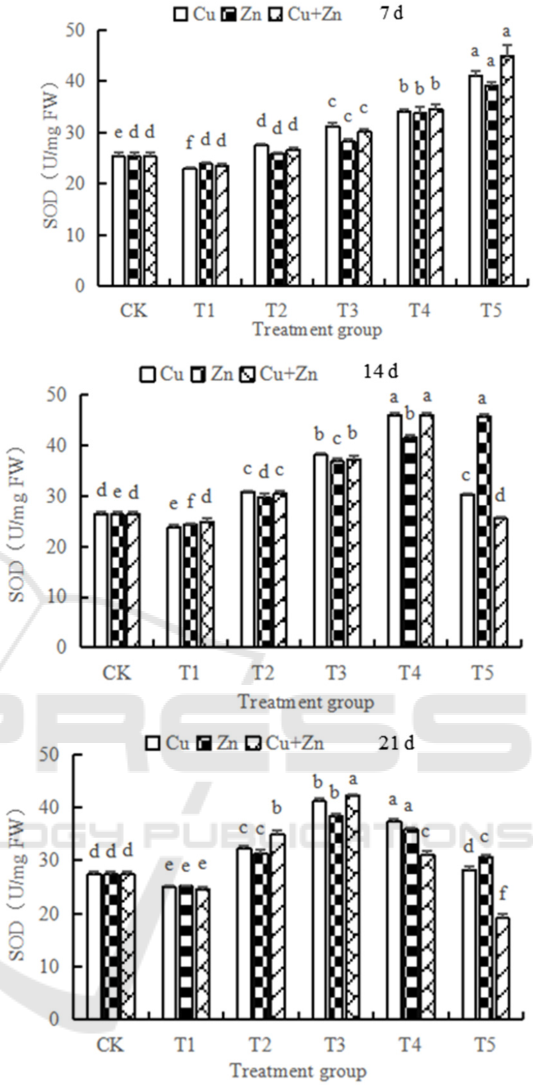


Figure 1: Effects of different heavy metals stress on SOD.

Under combined stress, the activity of SOD at T5 decreased below than that at CK. After 21 days' stress culture, the maximum of SOD all appeared at T3 under three stress modes, which increased 50.47%, 40.38% and 54.35% respectively compared with CK, and then decreased gradually. On the whole, the inhibition of Cu and Zn Stress on SOD was as follows: combined stress > Cu Stress > Zn stress.

3.2 Effects of Cu and Zn Stress on POD

After 7 days' stress, the activity of POD gradually increased with the increase of heavy metal concentration (Figure 2).

The change of POD at T1 was not significant compared with CK, which indicated that this concentration range had a weaker effect on *H.verticillata*. The values of POD at T5 reached the maximum, which were 30.11 U/mg, 30.82 U/mg and 35.05 U/mg respectively. After 14 days' stress culture, the values of POD increased from T1 to T4, then decreased at T5. The maximum values of POD increased 91.12%, 84.05% and 117.28% respectively compared with CK. When the culture time reached 21 days, they reached the maximum at T3 under three treatments, which were 31.87 U/mg, 29.66 U/mg and 33.64 U/mg respectively. The values at T1 were smaller than those at CK under three stress modes. On the whole, there were significant differences in POD activity under heavy metal stress.

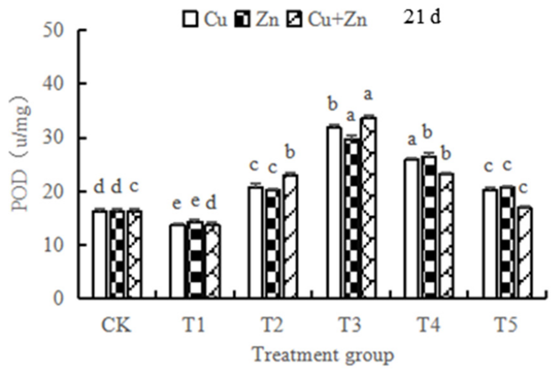
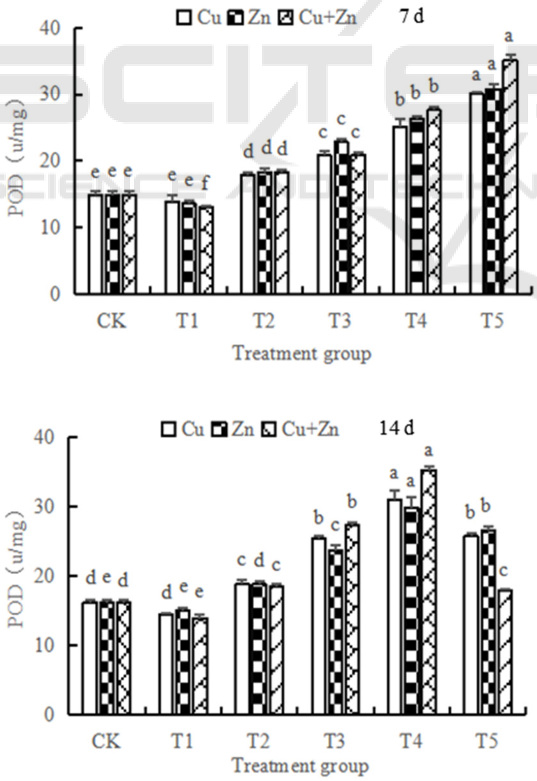
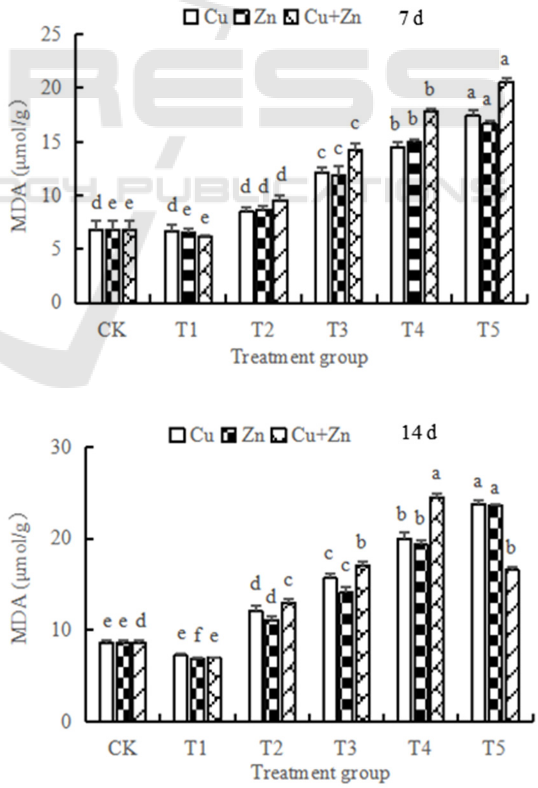


Figure 2: Effects of different heavy metals stress on POD.

3.3 Effects of Cu and Zn stress on MDA

Different concentrations of heavy metals had significant effects on MDA ($P < 0.05$). After 7 days' stress, the activity of MDA increased significantly with the increase of heavy metal concentration (Figure 3).





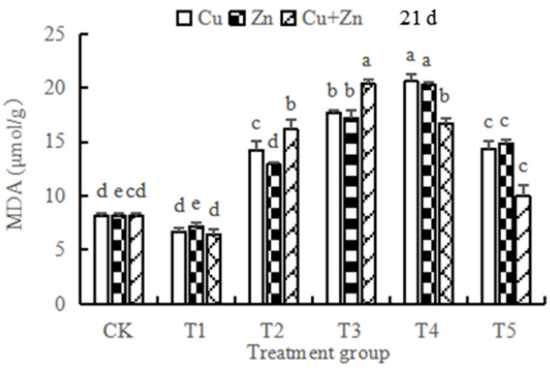


Figure 3: Effects of different heavy metals stress on MDA.

The maximum values of MDA were 17.41 μmol/g, 16.71μmol/g, 20.56 μmol/g respectively at T5 under three stress modes. There was no significant change at T1 compared with CK. After 14 days' stress, the values of MDA still maintained an upward trend under single stress of Cu and Zn, and reached the maximum values at T5. However, it reached the maximum of 24.51 μmol/g at T4 combined stress and began to decrease at T5. After 21 days' stress, the maximum values of Cu and Zn single stress were 20.58 μmol/g and 20.25 μmol/g respectively at T4. The maximum value of combined stress was 20.35 μmol/g at T3. In the three modes, the change range of combined stress was greater than that of single stress.

4 DISCUSSION

SOD plays an important role in improving plant resistance to stress. In general, the toxicity of heavy metals leads to excessive O_2^- in plants. In order to eliminate this effect, plants catalyze the conversion of O_2^- to H_2O_2 by increasing SOD activity to protect cells from damage (Parveen, 2017). Therefore, the activity of SOD increased gradually with the increase of heavy metals concentration. In this study, the activity of SOD after 7 days' stress culture accorded with this characteristic. But the activities at T5 on the 14th day, T4 and T5 on the 21st day showed downward trends, which might be because the stress of heavy metals has exceeded the tolerance of *H.verticillata* and inhibited the enzyme activity of the plant itself. Cu and Zn are elements required for plant growth and can participate in their normal physiological activities, and are conducive to the exchange of cellular materials inside and outside the cells. This is also the reason why the values of SOD at T1 in the three treatments are slightly lower than those of CK. In addition, the change range of SOD under combined stress is greater than that under single stress, which indicates that the existence of Zn promotes the absorption of Cu by plant cells and aggravates the toxicity of heavy metals.

POD catalyzes the decomposition of H_2O_2 into H_2O and O_2 (Cao, 2004). The change law of POD is very similar to that of SOD in this study. In the early stage of the experiment, POD increased significantly with the increase of heavy metals, but began to decrease under the influence of high concentration for a long time. On the 21st day, a significant decrease of POD was observed in T4 and T5, which indicated that high concentration of heavy metal stress destroyed the normal function of enzyme, weakened the ability of enzyme system to scavenge reactive oxygen. It is similar to the reason for the decrease of SOD.

The content of MDA represents the degree of cell membrane oxidation (Tang, 2010), which is used to reflect the damage degree of membrane lipid in the process of plant stress or aging. The higher the content of MDA, the more serious the inhibition of plants (Wang, 2004). The content of MDA in *H.verticillata* increased significantly with the concentration of heavy metals and the duration of poisoning, which indicated that the life activities of plants were significantly inhibited by heavy metals. On the 21st day of treatment, the value of MDA reached the maximum under the single stress of Cu or Zn at T4, but the combined stress reduced its activity, which may be due to the increased toxicity caused by the interaction between Cu and Zn, and the toxic degree increased significantly, resulting in the destruction of the enzyme protection system of the plant itself. Therefore, the content of MDA decreased significantly in T5 treatment group. The change range of SOD, POD and MDA under combined stress is the greatest, followed by Cu single stress which may be that Cu is a very active oxidative and reduced transition metal element, and Cu can absorb or release an electron (Cu^{2+} /Cu^{+}) to produce free radicals causing damage (Li, 1993), while the chemical properties of Zn are much weaker than that of Cu.

5 CONCLUSIONS

The activities of SOD, POD and MDA increased significantly with the increase of heavy metal stress in the early stage (within 7 days). From the 7th day to the 21st day, the indexes in high concentration treatment group showed a significant downward trend. The inhibition on *H.verticillata* was the largest under Cu and Zn combined stress, followed by Cu stress. It is the smallest under Zn stress.

ACKNOWLEDGEMENTS

This study was financially supported by the General Project of Jiangxi Science and Technology Department (20212BAB204402), and the 18th "Challenge Cup" Science and Technology Competition of of Nanchang Institute of Technology.

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