Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2015, 43(1):119-123. DOI:10.15835/nbha4319790

Biological Adsorption and Accumulation Analysis of *Hizikia fusiforme* Response to Copper Stress Conditions

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Abstract

Coastal water pollution is an important environmental problem now days. *Hizikia fusiforme* is cultivated in coastal water, being considered as a healthy food. However, little information exists concerning on this species responses to copper stress conditions. Experiments were conducted to distinguish biological adsorption and biological accumulation of *H. fusiforme* in regard to copper stress; it was determined the long-term stress with lower concentrations of copper (0.25 mg/L and 0.50 mg/L) and short-term stress with higher concentrations of copper (1.5 mg/L and 3.0 mg/L) on *H. fusiforme*. Results suggested that *H. fusiforme* has different response to various copper stresses; lower concentration stress could significantly enhance the growth of *H. fusiforme*, while *H. fusiforme* growth was inhibited and mitigated injured by 0.25-0.50 mg/L copper stress. Under the highest stress, *H. fusiforme* was extremely harmed, the biomass loss was significant and dry weight/fresh weight was also significantly decreased. Results suggested that lower and higher concentrations of copper stress have different impacts on *H. fusiforme*; the biological adsorption amount is lower than that of biological accumulation amount under low copper stress conditions, but the biological adsorption amount is much higher under high concentration copper stress. A better understanding of *H. fusiforme* responses to heavy metal stress should bring more data about its physiological adaptation mechanism under such conditions.

Keywords: algae, biological adsorption, biological accumulation, copper stress, pollution

Introduction

Large algae are mainly distributed in coastal intertidal zones, which are also the primary producers and assure energy transformation of marine ecological system. At the same time, large algae are very important to humans as biological resources. Under the background of global environmental changes, marine organisms and ecological systems are always affected by the interactions of many environment factors (Brierley and Kingsford, 2009). Lately, with the rapid economy development of coastal areas in China, the pollution conditions of inshore were more and more serious, and had become global environmental problems. China is the largest algae cultivation country in the world; some species such as *Laminaria japonica* and *Porphyra* are very famous, but coastal water pollution, especially heavy metal pollution, has damaging effects on large algae cultivation (Tan *et al*., 2002; Wang *et al*., 2009). However, little information exists concerning the physiological responses of large algae such as *Hizikia fusiforme* to heavy metals stress.

Hizikia fusiforme as a unique species in North-East Asia, recon as a sea vegetable. It is rich in trace elements, dietary fiber, polysaccharide sulfate and other bioactive substances (Pugh, 2011; Schepetkin *et al*., 2006). In recent years, with the evaluation and development of *H. fusiforme* food and medicines, the typical heavy metal contents determination and analysis in *H. fusiforme* was gradually attracted people's attention. For the typical heavy metals such as arsenic, lead, cadmium, mercury, zinc and copper, the United Nations, the European Union, Japan and other countries have clear food testing method and evaluation standard (copper ≤ 50 mg/kg) (Besada *et al*., 2009; Dawczynski *et al*., 2007; Rodenas *et al*., 2009), but very few reports of such research appeared in China.

Heavy metals such as Cu^{2+} and other elements exist in natural seawater at lower concentrations. However, the concentration of Cu^{2+} could be increased by industrial activities and the sediments in coastal waters (Connan *et al*., 2011). China offshore pollution is mainly caused by rivers and the pollution can directly increase the soluble heavy metal and trace elements content. At the same time, it can also decrease the pH of the seawater. Some reports showed that the pH of the seawater dropped by 0.1 units since 1975, and the pH of the seawater will continue to drop of 0.2-0.4 units by the end of the century

Received: 09 Jan 2015. Received in revised form: 09 Apr 2015. Accepted: 17 May 2015. Published online: 08 June 2015.

Table 1. The environment factors of copper adsorption

Factors	Light intensity (μmol/m²/s)	Photoperiod (Day/night)	Temperature (°C)	Salinity (‰)	Cu^{2+} concentration in nature seawater (mg/L)	CO_2 (ppm)	pH
Value	151～155	12:12 (6:00～18:00)	23	35	＜0.009	450	7.8

(Doney *et al*., 2009). This result will also induce potential adverse impact on the solubility and uptake of heavy metals, and the growth and metabolic processes of the algae. In addition, Cu^{2+} concentration in coastal water is always fluctuating, and this also affects algae metabolism.

The adsorption of algae upon heavy metals can be divided into two categories: inactive algae adsorption and activated algae adsorption. Inactive algae adsorption rate and amount are affected by environmental temperature, pH, particle size and ion competition (Stirk *et al*., 2000). The adsorption processes of activity algae on heavy metals can divide into two steps: first step is called biological adsorption, and this step is very quickly and reversibly, the feature is that heavy metal ions adsorption is on the cell surface. The second step is very slow and is called biological accumulation. It is mainly similar with the heavy metal ions absorption into the cells.

At present, the researches on *Hizikia fusiforme* responding to heavy metal stress were always focus on photosynthesis, growth and development, enzyme activity analysis and evaluation, and no information exists concerning the adsorption and accumulation mechanism of this species to heavy metal (Alahverdi *et al*., 2012; Zhu *et al*., 2011).

The aim of this paper was to distinguish biological adsorption and biological accumulation of *Hizikia fusiforme* to copper stress and to determine the biomass and Cu^{2+} concentration changes to long-term of lower concentration copper stress and short-term of higher concentration copper stress.

Materials and methods

Plant material and copper stress treatments

Hizikia fusiforme adopted in this experiment was cultivated by Lufeng breeding institute in Zhejiang Province of China. Uniform samples (morphology and plant height) were screened, impurity were removed, and then clamped the seedlings by 15 cm of 100 polyethylene ropes; each rope contained 7 seedlings before treatments. When *H. fusiforme* seedlings were cultured for 24 h in natural seawater, each group was used for copper stress: (1) lower concentration stress conditions (0.25 mg/L and 0.5 mg/L) for 7 d, and biomass of the samples was measured in 1 d, 3 d, 5 d and 7 d during stress periods; (2) higher concentration stress conditions (1.5 mg/L and 3.0 mg/L) for 24 h, and then biomass of the samples was measured. Four replications were used in each stress condition. Seawater was replaced every 24 h, and the environment factors during experiment were presented in Table 1.

Measurement of H. fusiforme biomass changes to copper stress

The samples were washed with tap water followed by distilled water and then put into the gauze bag (15 × 20 cm) which was soaked in distilled water, then centrifuged at 1,400 r for 2 min. Fresh weight (G initial total mass) was measured after removing excess water from the algae surface (Hai-Zao *et al*., 2009; Zhu *et al*., 2011).

Y net increase = G initial total mass after stress treatment - G initial total mass

Y net rate of increase = (G initial total mass after stress treatment - G initial total mass - G rope) / (G initial total mass after stress treatment - G rope) × 100%.

Measurement of H. fusiforme biological accumulation after copper stress

The samples were washed with tap water followed by distilled water and then put into the gauze bag which was soaked in distilled water (15 × 20 cm), then centrifuged at 1,400 r for 2 min. Rope was removed, then the fresh seedling weight and rope weight were measured. Control groups were divided into two groups and one was over-dried at 105 °C for 20 min, then dried at 80 °C for 48 h to a constant weight (C). The other group was leached by 10 mM HCl (remove alginic acid) for 60 min, washed with tap water twice, and then leached by 10 mM EDTA-2Na (remove Cu^{2+}) for 30 min, and was over-dried at 105 °C for 20 min, and then dried at 80 °C for 48 h to a constant weight (T). Control group was called the first group in this experiment, and the others were called from the second group to fifth group according to the Cu^{2+} concentration (from low to high).

Y_C% = (G dry weight / G fresh weight) × 100%.

Y_T% = (G dry weight after treatment / G fresh weight before treatment) × 100%.

Cu^{2+} concentration after stress treatments was measured according to the standard method (GB/T 5009.13-2003).

Data analysis

The data were analyzed by one-way ANOVAS using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). The means and standard errors (SE) were reported.

Results

The biomass changes of H. fusiforme under copper stress conditions

The biomass growth rate of the control group (Cu^{2+} ＜ 0.009 mg/L) sample increased with increasing culturing time during 7 d, and the biomass growth rate reached 9.49% at 7 d. A similar trend of biomass growth rate to that of the control group was observed also under 0.25 mg/L stress group, but the value at 1 d was less than that of the control group, and the values were much higher than for control group at the other 3 culturing days, the biomass growth rate reached 11.73% at 7 d. The biomass growth rate of the 0.5 mg/L stress group presented a negative growth trend, the value was sharply decreased at 5 d and the biomass was lost at the extreme at 7 d, when it reached -40.83%. The biomass growth rate of the 1.5 mg/L stress group and 3 mg/L stress group were both decreased since 1 d of stress treatment, more under the 3 mg/L stress group, the value of that being 17.56%. The biomass growth rate decreased with the increasing copper concentration at 1 d in all five groups (Fig. 1).

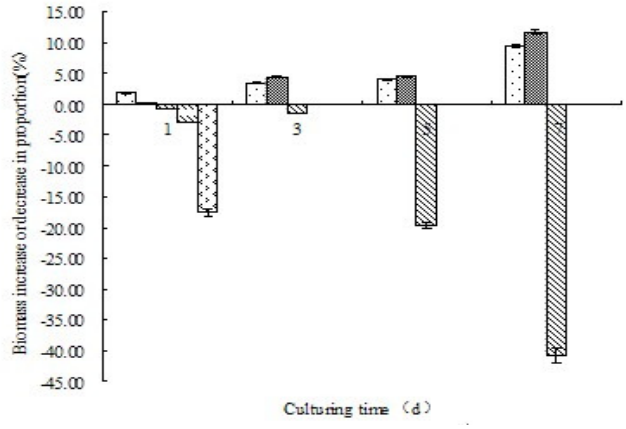

Fig. 1. The biomass changes of *Hizikia fusiforme* under copper stress conditions

Note: control group (Cu^{2+} < 0.009 mg/L); ( ) 0.25 mg/L copper stress group; ( ) 0.5 mg/L copper stress group; ( ) 1.5 mg/L copper stress group; ( ) 3 mg/L copper stress group; Bars represent mean ± s.e

DW/FW of each stress group before treatment (C) and after treatment (T)

The C and T values for the control group were the highest at 1 d: 13.41% and 7.26%, respectively. The minimum T value appeared at 3 d. However, C value appeared at 5 d. The values reached 10.07% and 7.18% at the end of the culturing days. The changing trends of C and T values were different and the changes showed that the biomass loss was significant after treatments with HCl and EDTA-Na (Fig. 2A).

The C value and T value of the 0.25 mg/L copper stress group were the highest at 1 d: 12.71% and 6.97%, respectively. The minimum T value appeared at 3 d (5.51%), and then increased slowly at 5 d and 7 d. The minimum C value appeared at 5 d (10.44%) and then increased. The values reached 10.64% and 6.94% at the end of the culturing period. A similar trend was found with the control group during the culturing days, and 0.25 mg/L of Cu^{2+} had a significant effect on C value (Fig. 2B).

The change trend of C value and T value in the 0.5 mg/L stress group was similar with that of the 0.25 mg/L stress group. The T value decreased from 6.89% to 5.76% at 3 d, and the C value decreased from 12.21% (max) to 11.21% (min) at 5 d. The values reached 11.72% and 6.86% at the end of the culturing period, respectively. The changes showed that the biomass loss was significant after being treated with HCl and EDTA-Na (Fig. 2C).

The C values were 10.96% and 10.33%, while the T values were 6.50% and 6.04% in the 1.5 mg/L stress group and 3 mg/L stress group after 24 h copper stress. The C values decreased with increasing copper concentration at 1 d stress in all five groups (Fig. 2D).

The Cu^{2+} content before treatment (C) and after chemical treatment (T)

Copper adsorption content of C and T values of the control group decreased from 1 d to 5 d, and then increased from 5 d to 7 d. All of the T values were higher than C values during the culturing days. The max C and T values were both reached at 1 d, which were 10.4 mg/kg and 11.6 mg/kg. The min C and T values were reached at 5 d, and were 4.8 mg/kg and 7.6 mg/kg.

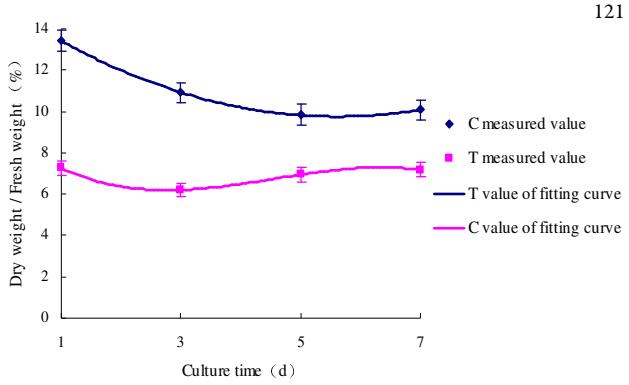

Fig. 2A. DW/FW of the control group before treatment (C) and after treatment (T)

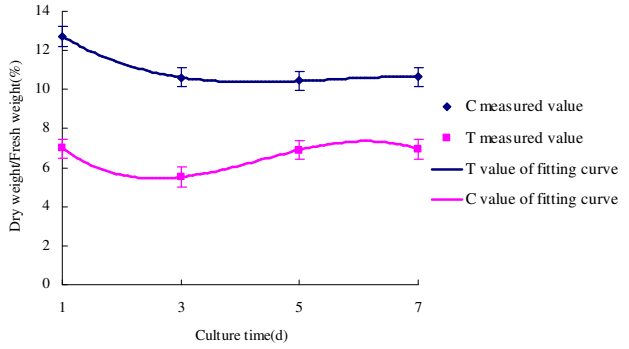

Fig. 2B. DW/FW of the 0.25 mg/L copper stress group before treatment (C) and after treatment (T)

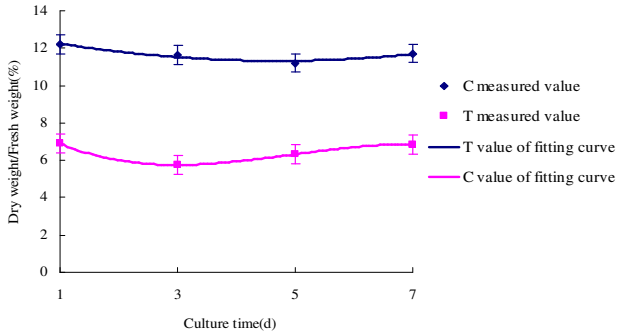

Fig. 2C. DW/FW of the 0.5 mg/L copper stress group before treatment (C) and after treatment (T)

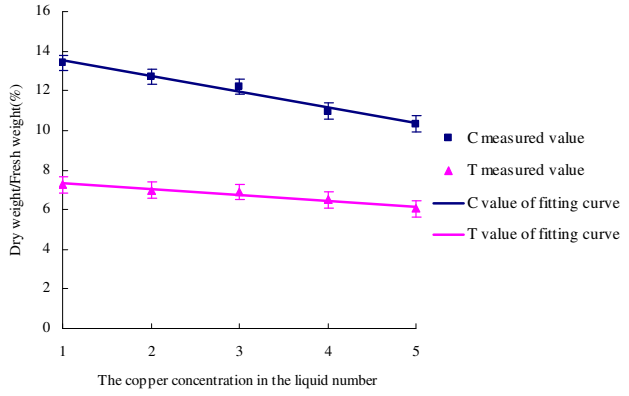

Fig. 2D. DW/FW of each group before treatment (C) and after treatment (T) on the first day of cultivation time

The results showed that the biological adsorption and accumulation amount of the Cu^{2+} significantly decreased with the increasing culturing time (Fig. 3A).

The C and T values for the first stress group were significantly higher than that of the control group at 1 d: 29.0 mg/kg and 49.6 mg/kg, respectively. Both C and T values significantly increased from 1 d to 3 d, and then decreased from 3 d to 5 d. The maximum values were 79.2 mg/kg and 96.4 mg/kg, reached at the end of the culturing days. The changing trends of C value and T value were similar in this group, however, the T values were higher than C values at the same culturing day (Fig. 3B).

The changing trends of C value and T value were different in the 0.5 mg/L stress group. The C value and T value were 224.0 mg/kg and 73.8 mg/kg at 1 d, and were significantly higher than that of the control group and 0.25 mg/L stress group. The C values showed irregular changes, but the T value increased gradually during the whole culturing time and reached the maximum value of 230.0 mg/kg at 7 d (Fig. 3C).

The C value and T value increased with increasing copper concentration at 1 d in all of the five stress groups. The C values were higher than T values, especially for higher concentration gradients of copper stress (1.5 mg/kg and 3 mg/kg), except for 0.25 mg/L of copper stress group, which had C value lower than T value (Fig. 3D).

Discussions

In natural conditions, the environment of algae growth has higher or lower conditions than the optimal growth factors, such as the periodical changes of light intensity, temperature and other environmental factors. Long and short term stress conditions together with stress intensity may also have different impacts on algae growth. Several reports have indicated that only the changes of long term growth factor could induce photosynthesis, as well as other key metabolic process changes within plants (Hai-Zao, 2009; Ruan, 2001; Zou *et al*., 2005). Results obtained from the current study clearly demonstrated the different responses of *H. fusiforme* to long-term of lower concentration copper stress (0.25 mg/L and 0.50 mg/L) and short-term of higher concentration copper stress (1.5 mg/L and 3.0 mg/L). In addition, artificial culture conditions in the experiment also enhanced the interference factor and provided data about suitable environmental conditions for *H. fusiforme* growth and development.

H. fusiforme lives in alternation of wetting and drying conditions due to the environments of large algae growth. At this time, *H. fusiforme* produced and secreted algin in order to adapt to the changing environment. Under drying conditions, *H. fusiforme* slowed the water loss and kept cell activity, while slowed water absorption under wetting conditions (Zou *et al*., 2005). In addition, algin is an important biological active component of large algae, which account for 48.28-63.73% of the dry weight of *H. fusiforme* (Wang, 2009).

In the current research, biological accumulation of Cu^{2+} must finish the process of biological adsorption by algin in *H. fusiforme*. While for other large algae such as *Sargassum* and *Padina*, the main factor affecting the adsorption of heavy metals is the cell wall polysaccharides, and the formation of functional groups such as S=O and N–H in the cell wall (Andradea *et al*., 2010; Plaza *et al*., 2011; Romera *et al*., 2007; Sheng *et al*., 2004). In this study, the samples treated with 0.01 mol/L HCl and EDTA-Na could effectively retained the copper in tissue cells. This design can distinguish biological adsorption and biological accumulation of *H. fusiforme* to copper stress.

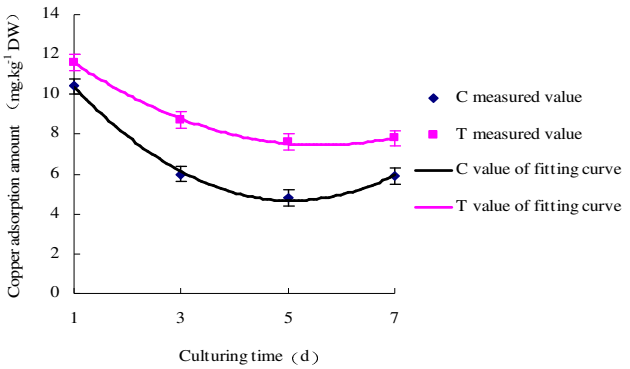

Fig. 3A. The Cu^{2+} content of the control group before treatment (C) and after chemical treatment (T)

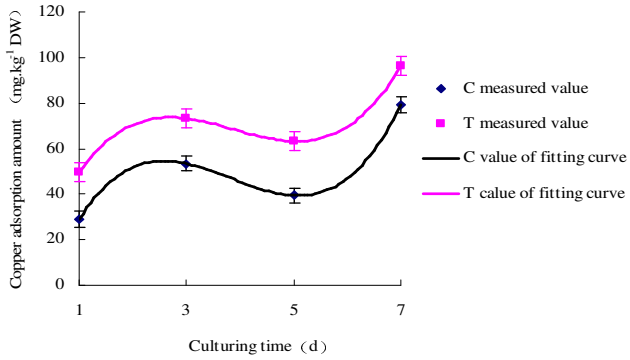

Fig. 3B. The Cu^{2+} content of the 0.25 mg/L copper stress group before treatment (C) and after chemical treatment (T)

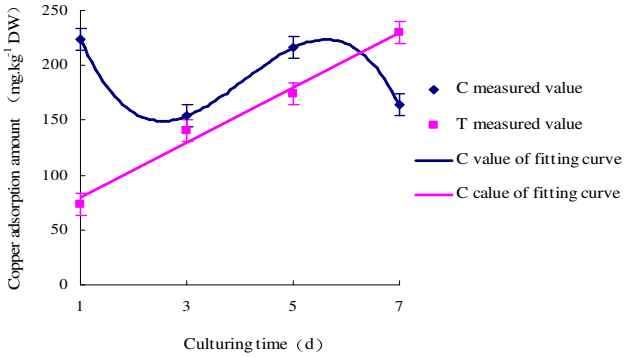

Fig. 3C. The Cu^{2+} content of the 0.5 mg/L copper stress group before treatment (C) and after chemical treatment (T)

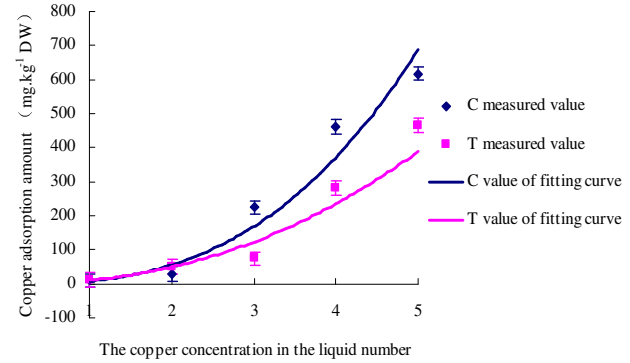

Fig. 3D. The Cu^{2+} content of each group before treatment (C) and after treatment (T) on the first day of cultivation time

Biological adsorption and accumulation of *H. fusiforme* to 0.25 mg/L copper stress was significantly higher than that of the control group, and this was mainly depend on the stress time. The phenomenon reflects that *H. fusiforme* has strong resistance to 0.25 mg/L copper stress, and copper can be used as a necessary trace element for *H. fusiforme* growth under lower stress concentration (Yruela *et al*., 2000). At the same time, the phenomenon also shows the good adsorption capacity on copper ion of algal adhered on the surface of *H. fusiforme* and the rapid transfer ability of mannuronic acid on copper ion. However, under higher copper concentration and short time stress conditions, biological adsorption and accumulation of *H. fusiforme* increased. The values of the adsorption were 211.2 times and 159.6 times of the control group under 1.5 mg/L and 3 mg/L, indicating that physical adsorption of copper may be closely related to the algin outside of the epidermis, which needs further research.

Conclusions

In summary, this study clearly showed that *Hizikia fusiforme* had different responses to various copper stresses, whereas lower concentration could significantly enhance its growth. Under high copper concentration (>0.5 mg/L) stress, *H. fusiforme* was extremely harmed, the biomass loss was significant and dry weight/fresh weight significantly decreased. Results suggested that the stress induced by lower and higher copper concentrations have different impacts on *H. fusiforme* and the biological adsorption is lower than that of biological accumulation under low copper stress conditions, while the biological adsorption is much higher under high concentration copper stress. A better understanding of *H. fusiforme* responses to heavy metal stress should bring more clarification upon its physiological adaptation mechanism and this data might facilitate the effective utilization of this species in seawater.

Acknowledgements

The research was supported by National Natural Science Foundation of China (31070322, 31270541).

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