DOI: 10.32615/ps.2020.035 PHOTOSYNTHETICA 58 (3): 853-861, 2020

Low pH in simulated acid rain promotes the toxicity of copper on the physiological performance in *Sargassum horneri*

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Abstract

In order to investigate the interactive effects of simulated acid rain and copper (Cu) on macroalgae, *Sargassum horneri* was cultured under pH of 8.2, 5.6, 4.5 and Cu concentrations of 0, 25, 100 μg L^{-1}. Under the medium concentration of Cu, moderate pH mitigated the toxicity of Cu on *S. horneri*, while the low pH and high Cu concentration reduced the contents of chlorophyll (Chl) *a* and carotenoids. Furthermore, pH had no interaction with Cu on maximal Chl fluorescence or electron transport efficiency, while effective PSII quantum yield, maximum electron transport rate, and saturating irradiance were obviously affected by pH and Cu, and the interactive effects were significant. Additionally, with the increasing Cu concentration and decreasing pH, nonphotochemical quenching and regulated energy dissipation dropped sharply, while nonregulated energy dissipation increased. Therefore, the high concentration of Cu and low pH had synergistic effects on the photosynthetic performance and growth of *S. horneri.*

Additional key words: environmental problem; metal pollution; photoprotective ability; photosynthetic apparatus; *Sargassum* beds.

Introduction

Acid rain and heavy metal pollution have become serious environmental problems around the world in recent decades. Acid rain is caused mainly by sulfur dioxide (SO_2) and nitrogen oxides (NO_x) emission, and the pH of acid rain is usually lower than 5.6 (Li *et al*. 2017, Wang *et al*. 2018). China has become one of the severely acid-rain polluted countries, especially in the southern and southwestern parts (Ma *et al*. 2019). In Zhejiang Province, acid rain is a serious problem in Ningbo, Jinhua, and Wenzhou, and pH of the acid rain was as low as 3.24 (Gao *et al*. 2016, Li *et al*. 2017). It has already been proven that acid rain is harmful to plants (Li *et al*. 2017). The gene expression could be damaged by acid rain directly or indirectly (Du *et al*. 2017). In addition, the growth, photosynthetic abilities, seedling emergence, and biodiversity declined with the frequency of strong acid rain (Shukla *et al*. 2013, Gao *et al*. 2016).

In addition, heavy metal pollution is mainly caused by anthropogenic activities, including burning garbage, industrial waste, overuse of chemical fertilizer and pesticide; it threatens the environmental ecosystem (Flemming and Trevors 1989, Giordani *et al*. 2005). Furthermore, heavy metal pollution is one of the common contaminants in an estuarine or coastal zone (Milan *et al*. 2016, Gao *et al*. 2017). The concentration of Cu^{2+} in offshore marine environments in China is reportedly from 0.1 to 43.2 μg L^{-1} (Jin *et al*. 2015). Cu is one of essential micronutrients for growth and development of plant (Zou *et al*. 2015), which is necessary for photosynthesis and respiration and it is required for electron transport proteins (Raven *et al*. 1999). However, when Cu concentration is excessive, it inhibits the growth, photosynthetic activity, photosynthetic pigment synthesis, and enzyme activities of algae (Brown and Newman 2003, Lombardi and Maldonado 2011, Rocha *et al*. 2016). Therefore, acid rain and copper pollution are harmful to the growth and development of plants. However, previous studies focused on the higher plants (Gong *et al*. 2019, Liu *et al*. 2019, Ma *et al*. 2019). Less effort has been devoted to the interactive effects of acid rain and copper pollution on marine organisms, especially on macroalgae.

Sargassum horneri (Turner) C. Agardh widely distri-

Received 12 January 2020, *accepted* 14 April 2020.

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Abbreviations: Car – carotenoids; Chl – chlorophyll; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v – variable fluorescence; F_v/F_m – maximum PSII quantum yield; I_k – saturated irradiance; NPQ – nonphotochemical quenching; rETR – relative electron transfer rate; $rETR_{max}$ – maximum electron transport rate; RGR – relative growth rate; $Y_{(II)}$ – actual photochemical efficiency of PSII; $Y_{(NO)}$ – quantum yield of nonregulated energy dissipation; $Y_{(NPQ)}$ – quantum yield of regulated energy dissipation; α – electron transport efficiency.

Acknowledgements: This work was supported by Technology Innovation Development Program of Yantai (2020MSGY058), the National Key R&D Program of China (2017YFC0506200), Shandong Province Science Foundation for Youths (ZR201807120023), Key Research and Development Program of Yantai (2018ZHGY082).

butes on rocks from lower intertidal to subtidal zones along the coastline of the northern Pacific (Komatsu *et al*. 2014, Xu *et al*. 2018) (Fig. 1). This brown seaweed is an important contributor to the primary production in coastal zones for forming up *Sargassum* beds, an important marine ecosystem for providing spawning, nursing, and feeding for other marine organisms (Miki *et al*. 2016, Xu *et al*. 2017). The fast-growing *S. horneri* is usually chosen to construct *Sargassum* beds in Japan and Korea (Uji *et al*. 2016). Unfortunately, in recent years, *Sargassum* beds have degenerated rapidly in the world (Miki *et al*. 2016). The Nanji Island, Zhejiang Province, China (27°27'7N, 121°05'5E) was once a place of rich *S. horneri*; however, *Sargassum* beds have declined in vast areas in recent decades, which was caused mainly by environmental changes in the factors including the elevated sea water temperature, and land reclamation (Komatsu *et al*. 2014). In addition, acid rain might also pose a threat on *Sargassum* beds as it frequently occurred in Zhejiang Province, China. Besides, the physiology of *S. horneri* could be harmed when exposed to high Cu concentration. Therefore, in this study, the impacts of acid rain and Cu pollution on the physiological characteristics of *S. horneri* were analyzed to find the reasons of the degradation and to provide supports for saving and remedying the *Sargassum* beds.

Materials and methods

Plants and culture conditions: *S. horneri* was sampled in March 2019 from Gouqi Island, Zhejiang Province (30°42'N, 122°48'E) (Fig. 1). The thalli were cleaned on site directly, and delivered to laboratory in cooler box by airplane within 48 h. After arrival at laboratory, the thalli were rinsed gently with filtered seawater at least three times to remove epiphytes, sediments, and other grazers. Afterwards, they were cultured in enriched natural seawater (the concentration of $NaNO_3$ and $NaPO_4 \cdot 12\ H_2O$ was 100 μM and 10 μM, respectively). The precultural conditions set temperature of 16 ± 1°C, light intensity of 100 μmol(photon) $m^{-2}\ s^{-1}$, and salinity of 30‰, with a light/dark period of 12/12 h, and constant aeration with ambient air. After being incubated for 7 d, the lateral branch of *S. horneri* was cut off for the experiment.

Experimental design: Approximately 0.5 g of thalli fresh mass (FM) were placed in 1-L flasks and cultured at crossed three concentrations of Cu [0, 25, and 100 μg(Cu) L^{-1}] and three pH levels (4.5, 5.6, and 8.2) for 7 d.

Cu concentration [μg L^{-1}]	pH level
0	4.5
	5.6
	8.2
25	4.5
	5.6
	8.2
100	4.5
	5.6
	8.2

The medium was made from natural seawater with addition of 100 μM $NaNO_3$ and 10 μM $NaPO_4 \cdot 12\ H_2O$, and the concentration of Cu in natural seawater was 1.28 μg L^{-1}, the Cu concentration in treatments was measured by an atomic absorption spectrophotometer (*AA240*, *Varian*, USA). The pH levels were made by the mixed acid solution [mix of H_2SO_4 (1 M) and HNO_3 (1 M) in a 2:1 ratio (Li *et al*. 2017)]. The cultural conditions were set at temperature of 20°C, light intensity of 100 μmol(photon) $m^{-2}\ s^{-1}$, and salinity of 30‰, with the light/dark period of 12/12 h and continuous aeration with ambient air. After 7 d, the growth and physiological responses of *S. horneri* were determined.

Relative growth rate (RGR): The RGR was calculated as follows: RGR = 100 × $\ln(W_t/W_0)/t$, where W_0 is the initial FM and W_t is the final FM after t days. Before weighting the algae, samples were softly blotted using filter paper to remove excess water.

Chlorophyll (Chl) *a* and carotenoid (Car) content: Approximately 0.01 g of FM of *S. horneri* were ground in methanol (3 mL) with quartz sand, then the volume was fixed to 10 mL with methanol and placed in darkness for 24 h at 4°C. Afterwards, the samples were centrifuged for 10 min at 5,000 rpm at 4°C, then the absorbance values of the sample were obtained by a scanning spectrophotometer (*UV6100A*, Y*uanxi Instrument Co*., *Ltd*., China), and the contents of pigments were determined according to Porra (2002) and Parsons and Strickland (1963): Chl *a* [μg mL^{-1}] = $16.29 \times (A_{665} - A_{750}) - 8.54 \times (A_{652} - A_{750})$, Car [μg mL^{-1}] = $7.6 \times [(A_{480} - A_{750}) - 1.49 \times (A_{510} - A_{750})]$.

***In vivo* Chl fluorescence**: Chl fluorescence parameter of PSII of *S. horneri* was measured with a pulse amplitude modulated fluorometer (*Diving-PAM*, *Walz*, Germany). The actinic light was set as 104 μmol(photon) $m^{-2}\ s^{-1}$, which was approximately consistent with the culture light intensity, and the saturating pulse was 5,640 μmol(photon) $m^{-2}\ s^{-1}$. After 15-min dark adaption, the minimal fluorescence level (F_0) was measured with measuring light at the low frequency, the maximal fluorescence (F_m) was obtained

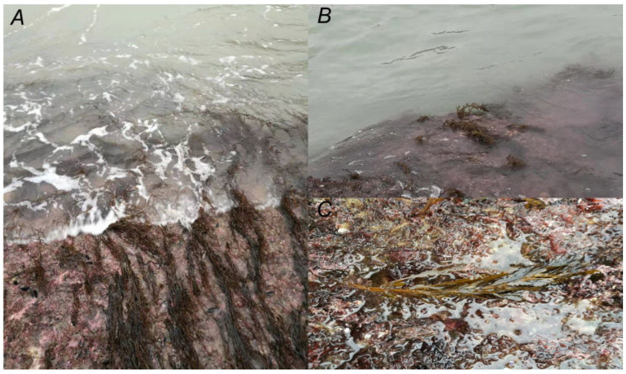

Fig. 1. *Sargassum horneri* in the intertidal zone of Gouqi Island, Zhejiang Province during low tides.

by applying a saturating pulse, the maximal fluorescence level (F_m') was determined by applying a saturating pulse of actinic light [104 μmol(photon) m^{-2} s^{-1}], and the Chl fluorescence yield (F_s) was obtained during the actinic irradiance. The following parameters were calculated based on the studies of Belshe *et al*. (2007) and Wang *et al*. (2009): (*1*) maximum PSII quantum yield, $F_v/F_m = (F_m - F_0)/F_m$; (*2*) effective quantum yield of PSII, $Y_{(II)} = F_v'/F_m' = (F_m' - F_s)/F_m'$; (*3*) nonphotochemical quenching, $NPQ = (F_m/F_m') - 1$; (*4*) quantum yield of regulated energy dissipation, $Y_{(NPQ)} = F_s/F_m' - F_s/F_m$; (*5*) quantum yield of nonregulated energy dissipation, $Y_{(NO)} = F_s/F_m$.

The rapid light curves (RLCs) can be obtained by a series of 20-s light exposures with increasing irradiance [0, 38, 104, 186, 322, 463, 621, 893; and 1,189 μmol(photon) m^{-2} s^{-1}]. Relative electron transfer rate, $rETR = F_v'/F_m' \times AF \times PAR \times 0.5$, where AF is the light absorption capacity, and PAR is the actinic photosynthetically active radiation. The parameters of the RLCs were calculated following the rETR curves following the models (Eilers and Peeters 1988):

$$rETR = I/(aI^2 + bI + c),$$

$$I_k = (c/a)^{1/2},\ \alpha = 1/c,$$

$$rETR_{max} = 1/[b + 2(ac)^{1/2}]$$

where $rETR_{max}$ is the maximum electron transport rate, α is the electron transport efficiency, *I* is the incident irradiance, I_k is the saturated irradiance, and *a*, *b*, and *c* are the adjustment parameters.

Statistical analyses: Significance among treatments was tested using one-way or two-way analysis of variance (*ANOVA*, *Tukey*'s post-hoc test) test for differences using the *SPSS 19.0*. The significant level was set at 0.05. All data were expressed as the mean ± standard deviation (SD, $n \geq 3$).

Results

Growth: After being cultured under different pH levels and Cu concentrations for 7 d, the growth of *S. horneri* was determined. Results showed that pH and Cu exerted a main effect on the RGR, and both had a significant interactive effect as revealed in the two-way *ANOVA* (Table 1). In addition, the medium Cu concentration (25 μg L^{-1}) did not affect the RGR significantly, while high Cu concentration (100 μg L^{-1}) significantly reduced the RGR of *S. horneri* as shown in the post hoc *Tukey*'s comparison (Fig. 2). Moreover, the RGR decreased with the decrease of pH at all conditions, except at pH 5.6 and the low Cu (0 μg L^{-1}) in which RGR was 8.3%

Table 1. Results of two-way analysis of variance for the effects of pH and Cu on the *Sargassum horneri*. pH × Cu – the interactive effect between these two factors; df – degrees of freedom; *F* – the value of the *F* statistic.

Source	df	*F*	*P* value	Source	df	*F*	*P* value
RGR				Chl *a*			
pH	2	159.306	< 0.01	pH	2	0.667	> 0.05
Cu	2	85.319	< 0.01	Cu	2	16.000	< 0.01
pH × Cu	4	17.401	< 0.01	pH × Cu	4	4.434	< 0.05
Car				F_v/F_m			
pH	2	12.488	< 0.01	pH	2	39.471	< 0.01
Cu	2	37.727	< 0.01	Cu	2	2.983	> 0.05
pH × Cu	4	8.396	< 0.01	pH × Cu	4	0.870	> 0.05
$Y_{(II)}$				NPQ			
pH	2	22.317	< 0.01	pH	2	103.892	< 0.01
Cu	2	18.875	< 0.01	Cu	2	22.523	< 0.01
pH × Cu	4	3.489	< 0.05	pH × Cu	4	21.721	< 0.01
$Y_{(NO)}$				$Y_{(NPQ)}$			
pH	2	44.477	< 0.01	pH	2	26.386	< 0.01
Cu	2	0.511	> 0.05	Cu	2	2.367	> 0.05
pH × Cu	4	1.980	> 0.05	pH × Cu	4	3.085	< 0.05
α				$rETR_{max}$			
pH	2	49.910	< 0.01	pH	2	31.790	< 0.01
Cu	2	0.562	> 0.05	Cu	2	36.823	< 0.01
pH × Cu	4	0.795	> 0.05	pH × Cu	4	4.220	< 0.05
I_k							
pH	2	11.878	< 0.01				
Cu	2	18.450	< 0.01				
pH × Cu	4	4.494	< 0.01				

higher than that of pH 8.2 group. The RGR at pH 4.5 was significantly lower than those at pH 5.6 and 8.2. However, no significant difference was found between pH 5.6 and 8.2.

Chl *a* and Car content: As shown in Fig. 3, the decrease in pH had no negative effects on the contents of Chl *a*, while higher Cu concentrations (25 and 100 µg L^{-1}) reduced significantly the contents of Chl *a*, except at pH 5.6, at which Cu had no significant effects on the contents of Chl *a*. Furthermore, pH and Cu showed an interactive effect on the contents of Chl *a* as indicated in the two-way *ANOVA* (Table 1). The low pH worsened the negative effects of Cu on the contents of Chl *a*. The lower pH and higher Cu concentrations were negatively related to the Car contents. pH and Cu were observed as the major influencing factors on the contents of Car as disclosed in two-way *ANOVA* (Table 1); both showed the significant interactive effect, and low pH aggravated the decrease of Car caused by excessive Cu.

Chl fluorescence parameters: The F_v/F_m and NPQ of *S. horneri* under different conditions were measured (Fig. 4). As shown in the two-way *ANOVA*, pH exerted a significant effect on F_v/F_m, but Cu concentration had no significant effect. Furthermore, pH had no interactive effect with Cu concentration (Table 1). When the pH was lowered to 4.5, F_v/F_m declined markedly, while higher pH (5.6 and 8.2) had no significant effects on F_v/F_m.

In the meantime, pH showed significant interactive effects with Cu concentration, and both pH and Cu concentration played main effects on NPQ (Table 1). At the seawater pH 8.2, relatively higher Cu concentration (25 µg L^{-1}) induced higher NPQ. With decrease in pH and increase in Cu concentration, the NPQ was reduced rapidly, indicating that pH and Cu concentration inhibited the photoprotective ability of *S. horneri*.

As shown in Fig. 5, $Y_{(II)}$, $Y_{(NPQ)}$, and $Y_{(NO)}$ were significantly affected by pH (Table 1), of which $Y_{(II)}$ was also affected by Cu concentration, and had obvious interactive effect with pH. pH promoted the inhibition of Cu concentration on $Y_{(II)}$. However, Cu had no significant effects on $Y_{(NPQ)}$ and $Y_{(NO)}$, and had no interaction with pH. As for $Y_{(NPQ)}$, when Cu concentration increased to 25 µg L^{-1} at normal pH (8.2), $Y_{(NPQ)}$ was higher than that at other treatment conditions, higher Cu concentration and lower pH significantly reduced the $Y_{(NPQ)}$ value. In

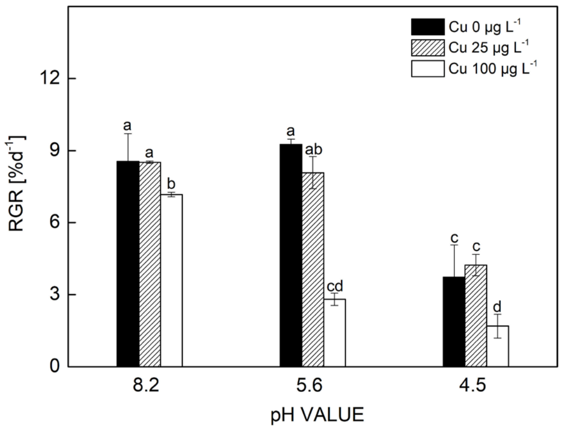

Fig. 2. The relative growth rate (RGR) of *Sargassum horneri* grown under different treatments for 7 d. *Different letters* indicate significant differences (P<0.05).

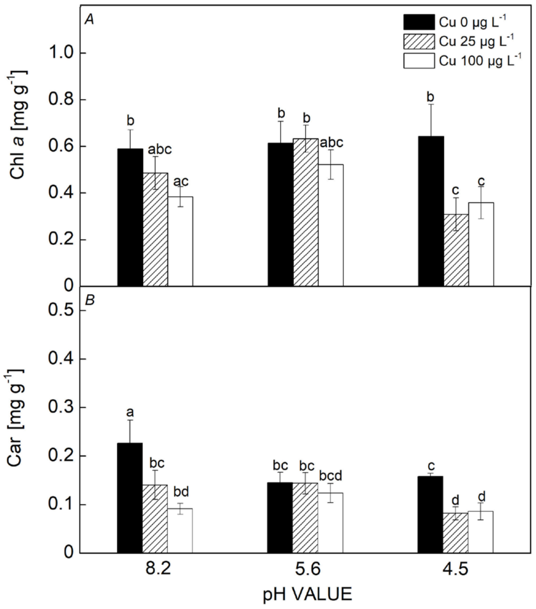

Fig. 3. The chlorophyll (Chl) *a* (*A*) and carotenoid (Car) (*B*) contents of *Sargassum horneri* grown under different treatments for 7 d. *Different letters* indicate significant differences (P<0.05).

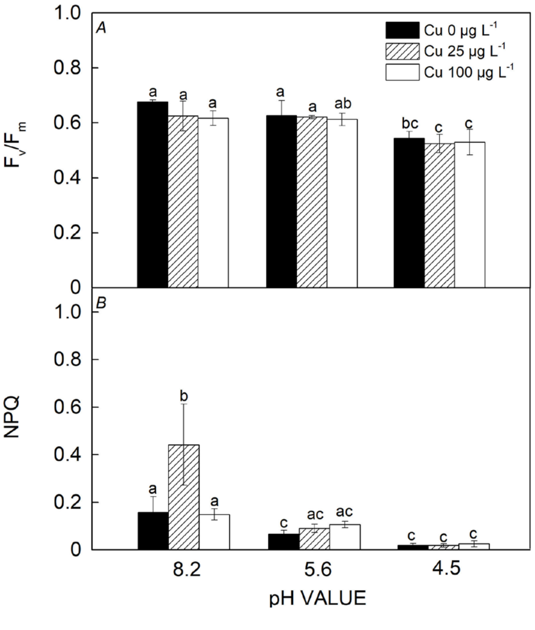

Fig. 4. The maximum PSII quantum yield (F_v/F_m) (*A*) and nonphotochemical quenching (NPQ) (*B*) of *Sargassum horneri* grown under different treatments for 7 d. *Different letters* indicate significant differences (P<0.05).

regards to $Y_{(NO)}$, lower pH induced higher $Y_{(NO)}$, while Cu concentration showed no such a significant effect (Fig. 5).

Effects of light intensity on NPQ of *S. horneri* were changed by pH and Cu concentration (Fig. 6). Under normal pH (8.2) condition, Cu concentration at 25 µg L^{-1} induced significantly higher NPQ, while that at 100 µg L^{-1} inhibited NPQ, indicating that higher Cu concentration was harmful to NPQ (Fig. 6*A*). Additionally, NPQ was sensitive to pH. When the pH was lower than 8.2, there was no obvious difference between all the treatments, indicating that pH (5.6 and 4.5) could damage photoprotective ability of *S. horneri*.

Photosynthetic parameters calculated from the rapid light curve (RLC) of *S. horneri* showed that lower pH (4.5) significantly inhibited the quantum efficiency of photosynthesis (α), while higher pH (≥ 5.6) did not (Table 2). As indicated in the two-way *ANOVA*, pH affected α obviously, but showed no significant interaction with Cu concentration (Table 1). Under a given Cu concentration, lower pH (5.6 and 4.5) could increase the maximum electron transport rate ($rETR_{max}$). Higher pH at 8.2 decreased the $rETR_{max}$ with the increase of Cu concentration, significantly. Besides, when the pH was 5.6, only the high Cu concentration (100 µg L^{-1}) reduced the $rETR_{max}$ significantly, and at low pH (4.5), Cu concentration had no significant effects on $rETR_{max}$ (Fig. 7, Table 2). Furthermore, pH and Cu had clearly an interactive effect on saturating irradiance (I_k) and both exerted main effects (Table 1). Except at pH 8.2, I_k decreased with the increase of Cu concentration. For example, I_k of the medium Cu concentration (25 µg L^{-1}) was 34.5% higher than that of the control. Additionally, under a given Cu concentration condition, low pH (5.6 and 4.5) boosted the I_k value.

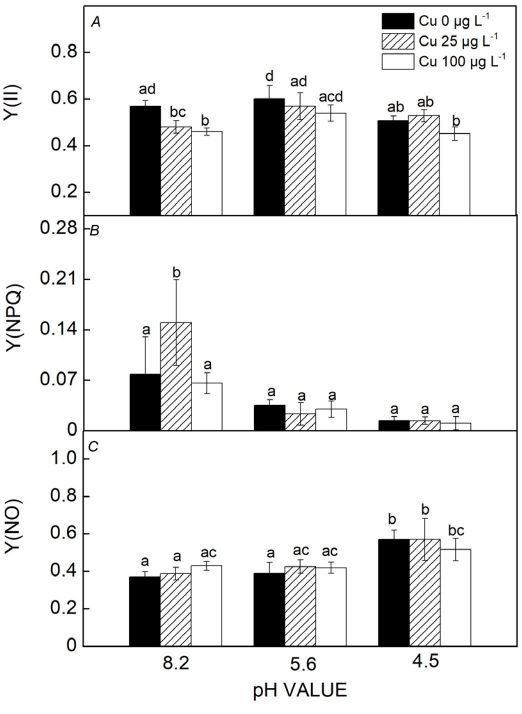

Fig. 5. The actual photochemical efficiency of PSII ($Y_{(II)}$) (*A*), quantum yield of regulated energy dissipation ($Y_{(NPQ)}$) (*B*), and quantum yield of nonregulated energy dissipation ($Y_{(NO)}$) (*C*) of *Sargassum horneri* grown under different treatments for 7 d. *Different letters* indicate significant differences (*P*<0.05).

Discussion

Growth: At present, acid rain and copper pollution are widely receiving attentions for their harmful effects to marine ecosystem (Gao *et al*. 2016, Li *et al*. 2017, Couet *et al*. 2018). *S. horneri* distributes mainly on rocky shores in the intertidal zone (Fig. 1), experiencing daily in and out of seawater. Thus, the chance of meeting acid rain and Cu pollution is markedly increasing. In this study, we found that the RGR of *S. horneri* was not reduced significantly by medium Cu concentration (25 µg L^{-1}) and pH (5.6), while the higher Cu concentration (100 µg L^{-1}) or lower pH (4.5) obviously inhibited the growth unlike low Cu concentration (0 µg L^{-1}) and higher pH (8.2) did (Fig. 2). The growth performance of algae is an important indicator to the physiological characteristics, and can reflect integrative physiology of the plant. Reportedly, no significant difference was shown in growth rate of *Ulva prolifera* between pH 8.2 and pH 4.4 (Li *et al*. 2017). A short-term acid rain could inhibit drastically the photosynthetic properties of *Ulva conglobata* and even damage the thalli irreversibly under low pH condition (pH 3), while the *Corallina* sp. could survive and regulate the pH of ambient seawater (Gao *et al*. 2016). In this study, we showed that *S. horneri* was more sensitive to acid rain compared to *Corallina* sp. and *U. prolifera*. Additionally, Cu is an essential micronutrient for the growth and development of

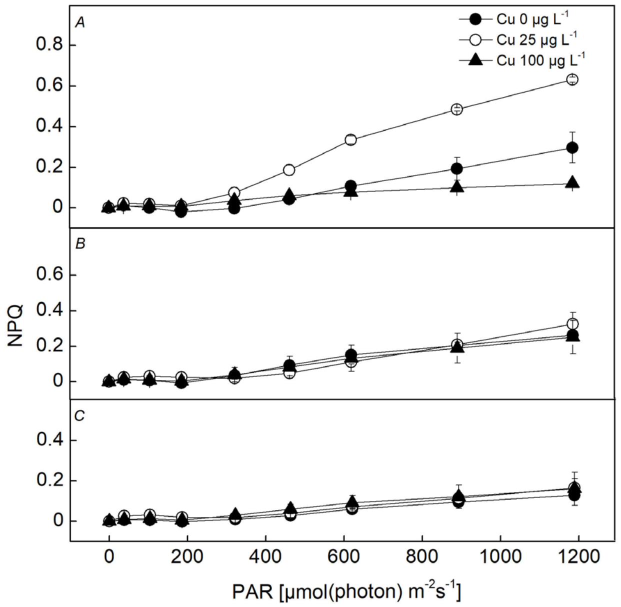

Fig. 6. Effects on nonphotochemical quenching (NPQ) as a function of light intensity of *Sargassum horneri* grown under different treatments for 7 d. (*A*) pH 8.2; (*B*) pH 5.6; (*C*) pH 4.5.

Table 2. Photosynthetic parameters calculated from the rapid light curve (RLC) of *Sargassum horneri* grown under different treatments for 7 d. α – quantum efficiency of photosynthesis; rETR$_{max}$ – the maximum electron transport rate; I_k – saturated irradiance. *Different letters* indicate significant differences between different treatments.

pH level	Cu concentration [µg L^{-1}]	α	rETR$_{max}$	I_k
8.2	0	0.369 ± 0.013^{a}	44.21 ± 1.26^{ad}	119.51 ± 6.08^{ac}
	25	0.344 ± 0.021^{a}	36.52 ± 2.22^{bd}	160.73 ± 33.55^{b}
	100	0.349 ± 0.019^{a}	30.34 ± 0.59^{b}	84.78 ± 5.16^{c}
5.6	0	0.347 ± 0.033^{a}	58.87 ± 5.15^{c}	166.78 ± 34.86^{b}
	25	0.344 ± 0.024^{a}	51.98 ± 6.31^{c}	165.07 ± 10.45^{b}
	100	0.344 ± 0.034^{a}	38.46 ± 2.85^{ad}	116.43 ± 9.11^{a}
4.5	0	0.267 ± 0.024^{b}	45.19 ± 4.24^{ad}	168.55 ± 3.95^{b}
	25	0.281 ± 0.024^{b}	44.61 ± 5.86^{a}	153.76 ± 17.62^{ab}
	100	0.264 ± 0.032^{b}	38.82 ± 3.55^{ad}	148.03 ± 13.89^{ab}

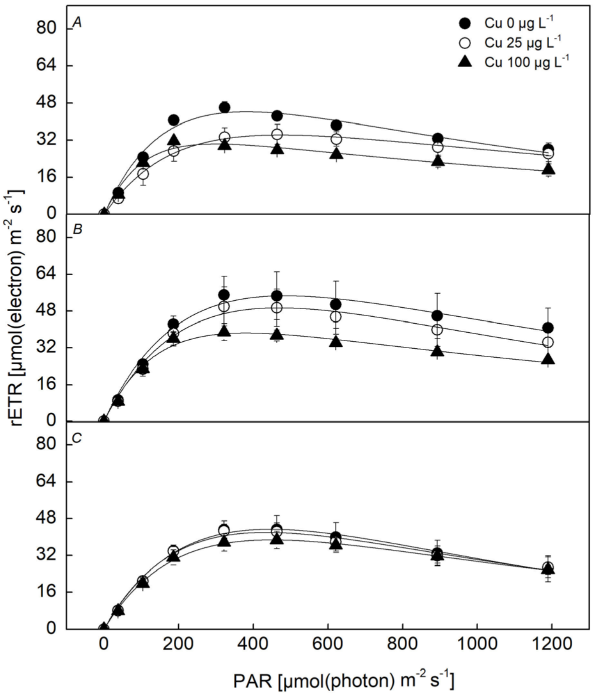

Fig. 7. Rapid light curve (RLC) of *Sargassum horneri* grown under different treatments for 7 d. (*A*) pH 8.2; (*B*) pH 5.6; (*C*) pH 4.5. rETR – relative electron transfer rate; PAR – photosynthetically active radiation.

macroalgae (Raven *et al*. 1999). Unfortunately, excessive Cu would be toxic to the plant (Han *et al*. 2008), such as *U. prolifera*, for which 0.5 µM Cu inhibited significantly the growth rate (Gao *et al*. 2017), while the growth of brown macroalga *Hizika fusiformis* was not reduced significantly at Cu concentration of < 0.05 mg L^{-1} (Zhu *et al*. 2011). Compared to *U. prolifera* and *H. fusiformis*, *S. horneri* is easily affected by Cu concentration as shown in this study. Nevertheless, *Sargassum* species have a highly efficient capacity of heavy metal bioaccumulation (Jothinayagi and Anbazhagan 2009). Macroalgae in the intertidal zone can regulate their physiological properties to mitigate the damage from acid rain and Cu stress, but this adaptive capacity is species dependent, thus more works are needed in the future. As indicated in our two-way *ANOVA*, pH had the significant interaction with Cu concentration on the growth of *S. horneri*, and frequent occurrence of acid rain and Cu pollution may be one of the causes of *Sargassum* bed recession.

Photosynthetic performance: Photosynthesis is easily affected by environmental stress. In this study, we showed that pH exerted a main effect, but Cu concentration had no significant effect on F_v/F_m or α, and their interaction was not obvious (Table 1). The phenomena indicated that the maximum photochemical quantum yield of PSII and quantum efficiency of photosynthesis were more easily affected by pH than by Cu concentration. The low pH induced by acid rain could disturb the acidity of the cell surface and influence the membrane electrochemical potential and enzyme activities, while algae need to allocate more energy to transport ions against the acid-base perturbation, leading to decreased photosynthetic performance and growth (Milligan *et al*. 2009, Flynn *et al*. 2012, Liu *et al*. 2014, Gao *et al*. 2019). It has been reported that acid rain had an adverse effect on photosynthesis of *U. prolifera* (Li *et al*. 2017), and the biodiversity of macroalgae and community structure could be altered by acid rain (Gao *et al*. 2016). Furthermore, $Y_{(II)}$, rETR$_{max}$, and I_k were obviously affected by pH and Cu, and the interactive effects were significant (Table 1). In addition, as shown in Fig. 3 and Table 1, the pH significantly affected the Chl *a* content of *S. horneri*, and had an interactive effect with Cu. The reasons might be that low pH could increase the concentration of free Cu^{2+} in the natural seawater and the excessive Cu ions could induce the loss of K^+ and inhibit the uptake of essential nutrient elements by increasing the permeability of the plasma membrane (Lidon and Henriques 1993, Millero 2009). At pH 5.7, the free hydrated Cu^{2+} was about 98% of total Cu with only 1.6% from $CuOH^+$ (Gao *et al*. 2014). The toxicity of metal pollution is mainly caused by the free hydrated metal ion, rather than total metal concentration (Campbell 1995). Roy-Arcand *et al*. (1989) pointed out that acid rain and metal treatment could cause the main changes in ultrastructure of algal cells, especially the chloroplasts and mitochondria. Tarhanen (1998) also found that the thylakoid and mitochondrial cristae located in chloroplasts and mitochondria, respectively, were swollen that was caused by acid rain and a mixture of copper and nickel. Besides, the Chl *a* content of *U. prolifera* was significantly reduced by low pH (Li *et al*. 2017). However, Costa *et al*. (2016) found that Cu had positive influence on photosynthetic pigments of *Sargassum cymosum*, which is contrary to the results of the present study. The photosynthetic pigment contents could be related to the metabolic biosynthesis of antioxidant compounds, the decrease of photosynthetic pigments contents caused by Cu were the consequence of reduced biosynthesis of enzymes and chloroplasts (Santos *et al*. 2012). In the present study, we found that medium pH at 5.6 could mitigate the harm of excessive Cu (Fig. 3), implying that on the cell surface the competition between H^+ and Cu^{2+} exceeded the increased availability of Cu^{2+} induced by reduced pH in *S. horneri* (Franklin *et al*. 2000, Gao *et al*. 2017). The degradation of Chl *a* caused by lower pH and higher contents of Cu may be one of the causes of inhibition of photosynthesis.

Photoprotective abilities: The photosynthetic apparatus is the most sensitive to environmental stress (Berry and Björkman 1980), and low pH or higher Cu concentration can cause imbalance between absorption and utilization of light energy, resulting in inhibition of photosynthesis and damage of PSII by producing active oxygen species (AOS) (Wilson *et al*. 2006). In order to avoid the photodamage, excess absorbed light can be dissipated through nonphotochemical quenching (NPQ). Under normal conditions, $Y_{(II)} + Y_{(NPQ)} + Y_{(NO)} = 1$. In the equation, $Y_{(II)}$ is the effective PSII quantum yield, representing that absorbed quanta are converted into chemically fixed energy; $Y_{(NPQ)}$ is the quantum yield for dissipation by downregulation. A high $Y_{(NPQ)}$ value means that the plant can protect itself from photon flux density by regulation; while a high $Y_{(NO)}$ indicates that the plant has already been photodamaged (Wang *et al*. 2009). The present study showed that pH and Cu could damage the photoprotective mechanism system. As shown in Fig. 4, Cu concentrations at 25 μg L^{-1} significantly induced NPQ and $Y_{(NPQ)}$ at pH 8.2, indicating that the medium Cu concentration caused a stress to *S. horneri*, and more importantly, *S. horneri* could protect itself from photodamage by dissipating excessive excitation energy. In order to defense against the stress of heavy metals, macroalgae have evolved a series of protective mechanisms (Mata *et al.* 2009, Gao *et al.* 2017), including cell wall exclusion of metal ions, and chelate with intracellular phytochelatins or polyphosphate (Knauer *et al.* 1997, Franklin *et al.* 2000). However, with the increase of Cu concentration and decrease of pH, NPQ and $Y_{(NPQ)}$ dropped sharply, while $Y_{(NO)}$ increased. The reason might be that the photoprotective mechanism had been damaged by the Cu and pH. Gao *et al*. (2017) also reported that the protective mechanisms had been beaten by excess Cu (2 μM), accompanied by declined growth, rETR, and net photosynthetic rate, which is in accordance with the results of this study (Figs. 2, 4, 7). The negative effect of Cu on the photoprotective mechanism was magnified under low pH condition, as depicted in Fig. 5. The reasons might be that low pH aggravated the acid-base perturbation around the cell surface, and induced more free Cu^{2+} ions in the culture (Franklin *et al*. 2000), resulting in more vulnerable cells to pH and Cu (Gao *et al*. 2017). Our study demonstrated that the extent of effects of pH and Cu on *S. horneri* was dosage dependent: under the medium concentration of Cu (25 μg L^{-1}), moderate decrease of pH (5.6) could mitigate the toxicity of Cu, while lower pH (4.5) aggravated the toxicity of Cu at high Cu concentration (100 μg L^{-1}). Similar finding was reported by Gao *et al*. (2017).

Conclusions: The present study showed that simulated acid rain and Cu had synergistic effects on the photosynthetic performance, photoprotective ability, and growth of *S. horneri*. Under the medium concentration of Cu (25 μg L^{-1}), moderate decrease of pH (5.6) could mitigate the toxicity of Cu, while lower pH (4.5) aggravated the toxicity of Cu at high Cu concentration (100 μg L^{-1}) on *S. horneri*. As the global ocean temperature rising, which has been obviously accepted by most of scientists in the world, and increased human activities along costal zones, *Sargassum* beds have been severely degraded in recent years (Komatsu *et al*. 2014), we should pay more attention seriously on acid rain and metal pollution in the world, and many further works are demanded.

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