Pol. J. Environ. Stud. Vol. 29, No. 2 (2020), 1129-1135
DOI: *10.15244/pjoes/105977* ONLINE PUBLICATION DATE: 2019-10-04

Original Research

Effects of Cu^{2+} and Hg^{2+} on Growth and Photosynthesis of Two *Scenedesmus* Species

Li-Li Dong[1], Guo-Qing Zhang[1], Wei Li[1], Tao Ding[1], Heng-Xing Wang[1], Gen Zhang[2]*

[1]College of Life and Environment Sciences, Huangshan University, Huangshan City, Anhui Province, P. R. China
[2]Shenzhen GenProMetab Biotechnology Company Limited, Shenzhen City, Guangdong Province, P. R. China

Received: 28 December 2018
Accepted: 26 March 2019

Abstract

Cu^{2+} and Hg^{2+} are two typical contaminants. Previous studies on toxicity of Cu^{2+} and Hg^{2+} to green algae mainly employed concentrations higher than environmental levels. Since the results varied among different strains of the same species, toxicity assessment using local green alga strains might be more accurate for revealing risks of Hg^{2+} and Cu^{2+} to local environments. In the present study, S*cenedesmus quadricauda* and *Scenedesmus acutus* were isolated from the Xin'an River in Huangshan City, China. Both were treated with 0.01-0.15 mg/L Hg^{2+} or 0.5-10 mg/L Cu^{2+}. The results showed that Hg^{2+} and Cu^{2+} were highly toxic. Treatment with 0.1 mg/L Hg^{2+} completely inhibited growth of *S. acutus* and 0.15 mg/L Hg^{2+} inhibited growth of *S. acutus*, but no significant changes were observed in contents of photosynthetic pigments and chlorophyll fluorescence parameters, suggesting that toxicity of Hg^{2+} might not be due to inhibition on photosynthesis. Treatments with 0.5 mg/L Cu^{2+} depressed cell growth, and higher levels of Cu^{2+} decreased contents of photosynthetic pigments (chl-a, car or chl-b) in *S. quadricauda* and *S. acutus*. Moreover, *S. quadricauda* might be more sensitive to heavy metal treatments than *S. acutus*. These results should be useful for evaluating environmental risks of Hg^{2+} and Cu^{2+} to Huangshan City.

Keywords: alga density, chlorophyll, fluorescence, *Scenedesmus quadricauda*, *Scenedesmus acutus*

Introduction

Hg^{2+} and Cu^{2+} are two typical contaminants. Environmental pollution of Hg^{2+} and Cu^{2+} have been observed globally. Hg^{2+} is high toxicity to human beings and environments due to its bioaccumulation and biomagnification within trophic levels [1]. Hg^{2+} could be transformed to methylmercury, which is even more toxic [2]. Hg^{2+} and derivates can cause severe disorders to organisms, including neurological, immunological, cardiac, motor, reproductive and genetic toxicity, and also is associated with Alzeihemer's, Parkinson's, autism, lupus, and amyotrophic lateral sclerosis [3,4]. Recent molecular studies have revealed that Hg^{2+} treatment decreased many critical processes, including ferric iron binding, antioxidant activity, cellular homeostasis, and glutathione metabolism in copepod

*e-mail: zhanggen1988@163.com

[5]. Metabolomics analyses revealed that Hg^{2+} negatively affected ion-osmoregulatory, damaged cell membrane and induced hypoxic stress in fish [1].

Cu^{2+} is an essential micronutrient playing an important role in many metabolic processes, as a cofactor for enzymes. Excess Cu^{2+} initiates oxidative damage and interferes with important cellular components in organisms, which further leads to abnormal Cu^{2+} metabolism and neurodegenerative changes [6].

Algae are important components in aquatic ecosystems. Influences and bioaccumulation of heavy metals in alga cells will affect high trophic levels and the whole ecosystem [7]. Toxicity assessments of Hg^{2+} and Cu^{2+} to green algae demonstrated that Hg^{2+} is the most toxic heavy metal, followed by Cu^{2+} [8]. Both of them inhibited growth, cell permeability, photosynthesis and/or nitrogen fixation in algae [9-14], and altered alga communities [15]. These effects were influenced by environmental conditions and water chemistry, i.e., temperature [16], water hardnees and alkalinity [17]. However, most studies tested the toxicity of Hg^{2+} and Cu^{2+} at concentrations ranging from 0.5 mg/L to 100 mg/L. Obviously, these concentrations were much higher than those in real environments, which could not comprehensively reveal environmental risks of Hg^{2+} and Cu^{2+} pollution in nature.

Toxicity of pollutants to green algae varied among species [14] and strains [17, 18]. Investigations on more species and strains from local water bodies might more accurately evaluate pollution on local environments [17]. *S. quadricauda* and *S. acutus* are two dominant alga species in aquatic environments. In the present study, *S. quadricauda* and *S. acutus* isolated from the Xin'an River (Huangshan, China) were employed as model organisms. *S. quadricauda* and *S. acutus* were treated with trace amounts of Hg^{2+} and Cu^{2+}. Thereafter, changes of growth indices, chlorophyll contents and photosynthetic parameters were compared. These results would be useful for evaluating risks of trace Hg^{2+} and Cu^{2+} to local environments.

Materials and Methods

S. quadricauda and *S. acutus* were isolated from the Xin'an River in Huangshan City, P. R. China, and then cultured in 500 ml flasks containing 300 ml of BG-11 medium [19] at 25±1ºC. The photoperiod was 12 h: 12 h (light: dark) with light intensity of 6,000 lux. Algae were manually shaken three times per day.

For treatments with heavy metals, algae at the exponential growth stage were harvested and pooled. Alga density was determined using a hemocytometer and then the initial alga density was adjusted to 1×10^5 cells/mL. Next, $HgCl_2$ and $CuCl_2$ (analytic grade) were used as the metal sources. Four concentrations of Hg^{2+} (0.01, 0.05, 0.1, 0.15 mg/L) and Cu^{2+} (0.5, 1, 5, 10 mg/L) were prepared. BG-11 media without additional Cu^{2+} or Hg^{2+} were included as the control. Each assay was repeated three times independently. Alga density was monitored every 24 hours for 10 days to calculate population growth rate.

Contents of photosynthetic pigments were determined on day 10. Briefly, 150 ml of algae solution were sampled from each treatment and then vacuum filtrated on 0.22-μm filter membrane (Whatman GF/F). Algae were grounded in 10 ml of 95% ethanol and then extracted at 4ºC in dark for 12 hours. After centrifuging at 5,000 rpm for 10 min, absorbance of supernatants at 665 nm, 649 nm and 647 nm was determined using a spectrophotometer. Contents of chlorophyll a (chl-a), chlorophyll b (chl-b) and carotenoids (car) were calculated according to Yang et al. [20].

On day 5, chlorophyll fluorescence parameters were determined. Briefly, 100 ml of algae solution was collected and then placed in the dark for 15 min. Afterward, chlorophyll fluorescence parameters, including maximal photochemical efficiency of PSII

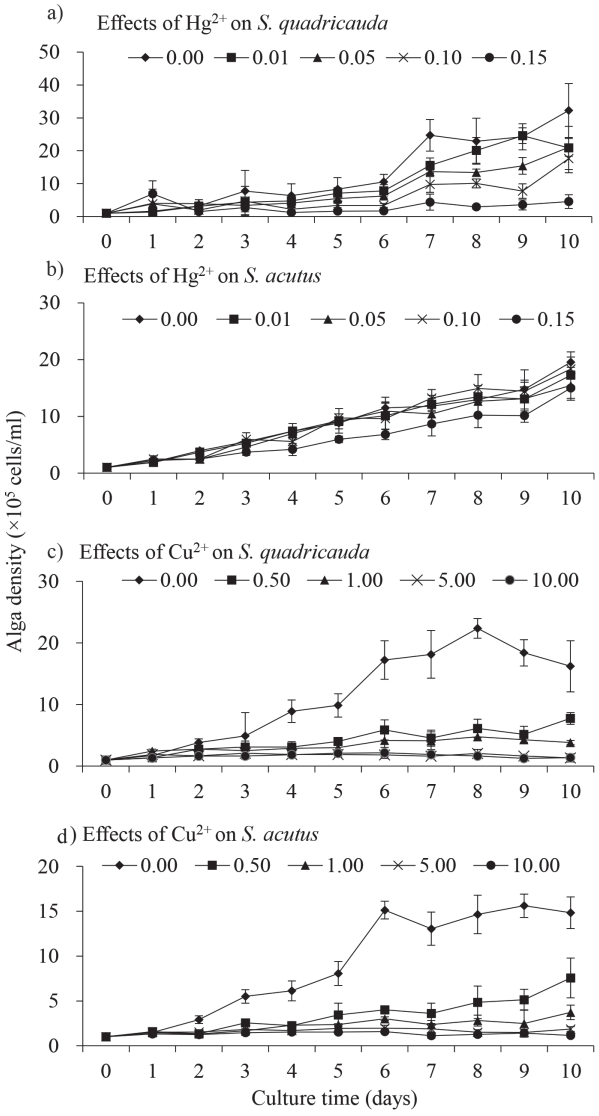

Fig. S1. Growth curves in treatments with Hg^{2+} (mg/L) and Cu^{2+} (mg/L). Data represent mean±SD.

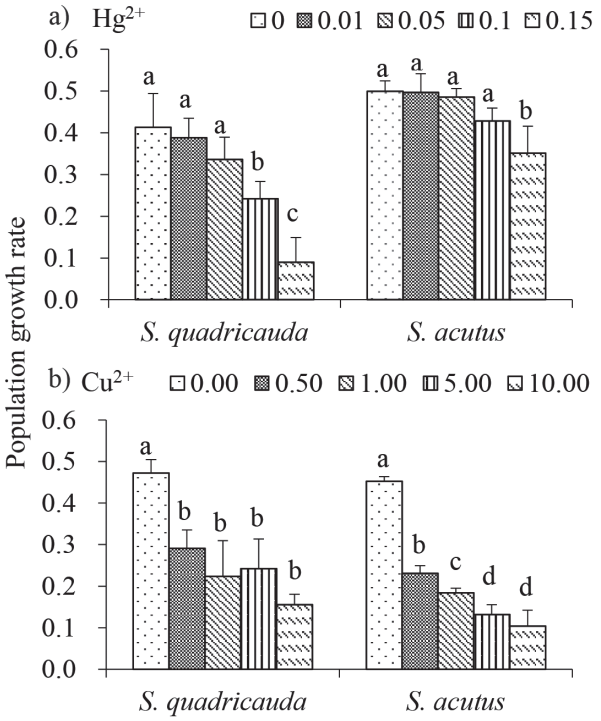

Fig. 2. Effects of Hg^{2+} (mg/L) and Cu^{2+} (mg/L) on population growth rate of *S. quadricauda* and *S. acutus* (mean±SD). Different letters above bars represent significant differences.

(F_v/F_m), actual photochemical efficiency of PSII (yield), maximal relative electron transport rate ($rETR_{max}$), initial slope rate (α) and half-saturation light intensity (I_k) were determined using a phytoplankton fluorescence instrument (phyto-PAM, Walz-Germany).

After testing homogeneity of variance, the effects of Cu^{2+} or Hg^{2+} on each parameter were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) using SPSS 19.0.

Results

Effects of Cu^{2+} and Hg^{2+} on Algae Growth

Along with time, alga density increased gradually (Fig. S1), but population growth rate decreased with the elevation of Hg^{2+} and Cu^{2+} concentrations. Compared with the control, treatments with 0.1-0.15 mg/L Hg^{2+} and 0.5-10 mg/L Cu^{2+} significantly decreased population growth rate of *S. quadricauda*. Treatments with 0.15 mg/L $Hg^{2+}$and 0.5-10 mg/L Cu^{2+} significantly reduced population growth rate of *S. acutus* (Fig. 2).

Effects of Cu^{2+} and Hg^{2+} on Contents of Photosynthetic Pigments

At all tested concentrations, Hg^{2+} did not significantly affect contents of chl-a, car and chl-b in either *S. quadricauda* or *S. acutus* (P>0.05)

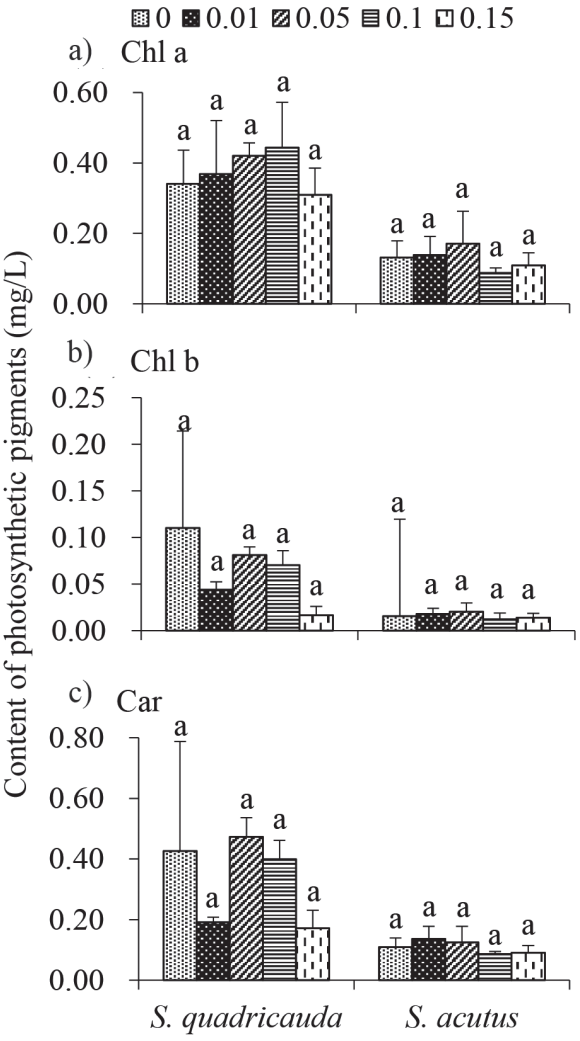

Fig. 3. Effects of Hg^{2+} (mg/L) on contents of photosynthetic pigments in *S. quadricauda* and *S. acutus* (mean±SD). Different letters above bars represent significant differences.

(Fig. 3). In comparison, all these parameters were significantly affected by exposure to Cu^{2+}. Treatments with 0.5-10 mg/L Cu^{2+} significantly decreased contents of chl-a, chl-b and car in *S. quadricauda*, except car in treatment with 0.5 mg/L, which was not significantly different from the control. Treatment with 0.5 mg/L Cu^{2+} significantly increased contents of all pigments in *S. acutus*, but treatments with 5 and 10 mg/L Cu^{2+} significantly suppressed contents of chl-a and car (Fig. 4).

Effects of Cu^{2+} and Hg^{2+} on Parameters of Chlorophyll Fluorescence in Algae

In response to treatments with 0.01-0.15 mg/L Hg^{2+}, no significant differences were detected in all tested chlorophyll fluorescence parameters in both *S. quadricauda* and *S. acutu* (Fig. 5).

In *S. quadricauda*, treatment with 0.5 mg/L Cu^{2+} significantly induced F_v/F_m, yield, $rETR_{max}$ and I_k,

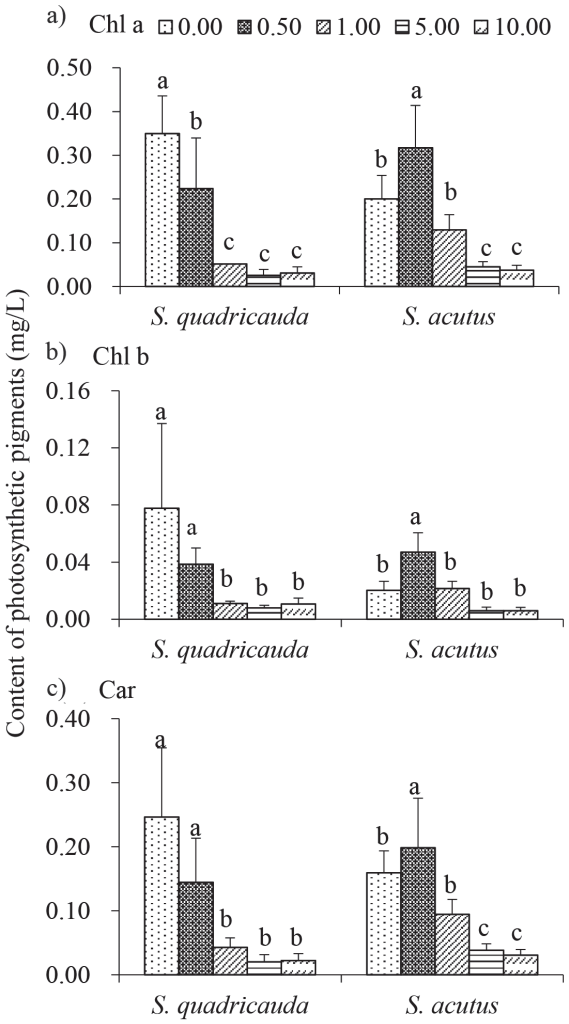

Fig. 4. Effects of Cu^{2+} (mg/L) on contents of photosynthetic pigments in *S. quadricauda* and *S. acutus* (mean±SD). Different letters above bars represent significant differences.

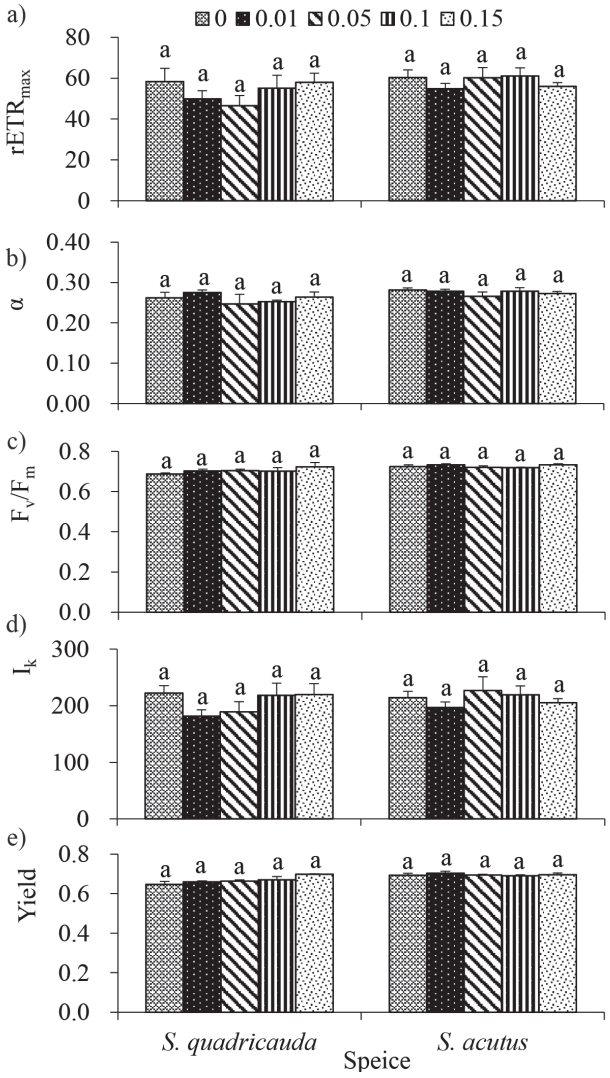

Fig. 5. Effects of Hg^{2+} (mg/L) on chlorophyll fluorescence parameters in *S. quadricauda* and *S. acutus* (mean±SD). Different letters above bars represent significant differences.

which were then gradually declined with elevated concentration (Fig. 6). α was not affected in treatments with 0.5 and 1 mg/L Cu^{2+}, but also declined at 1-10 mg/L Cu^{2+}. In contrast, effects of Cu^{2+} on chlorophyll fluorescence parameters of *S. acutu* were more complicated. Treatments with 0.5 and 1.0 mg/L Cu^{2+} significantly elevated α, F_v/F_m and yield. All these indices were significantly lower in treatment with 5 and 10 mg/L than those in the control. Treatment with 0.5 mg/L Cu^{2+} increased $rETR_{max}$ but did not affect I_k. Both indices were significantly reduced at 1-10 mg/L Cu^{2+} (Fig. 6).

Discussion

Hg^{2+} is highly toxic to green algae. Treatment with 0.1 mg/L Hg^{2+} completely inhibited growth and significantly decreased photosynthesis in *S. acutus* [21]. Similarly, in the present study, treatment with 0.1 mg/L Hg^{2+} significantly inhibited growth of *S. quadricauda* and 0.15 mg/L Hg^{2+} significantly inhibited growth of *S. acutus*, displaying toxicity of Hg^{2+} to environments.

To explore mechanisms underlying inhibition of Hg^{2+} to alga density, contents of photosynthetic pigments and changes of chlorophyll fluorescence parameters were monitored. No significant changes in content of photosynthetic pigments were detected in Hg^{2+} treatments, which seemed conflicted with the suppressed alga density. It has been revealed that treatment with Hg^{2+} increased cell size of live *T. weissflogii* cells [22]. Enlarged alga cells could synthesize more photosynthetic pigments, which might supplement loss of pigment content caused by reduced cell density.

As previously reported, Hg^{2+} decreased PSII quantum yield in *M. pteropus* [23], inhibited the transfer of excitation energy within phycobilisomes in S*pirulina platensis* [24] and decreased the capacity of photosystem to dissipate the excitation light energy via the photochemical pathway in *M. aeruginosa* [25].

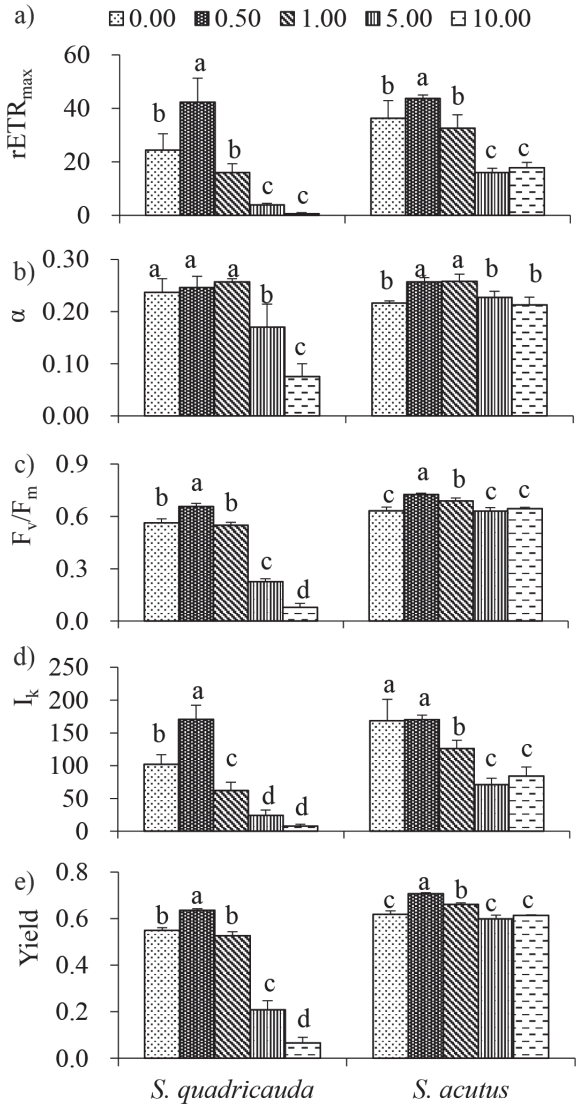

Fig. 6. Effects of Cu^{2+} (mg/L) on chlorophyll fluorescence parameters in *S. quadricauda* and *S. acutus* (mean±SD). Different letters above bars represent significant differences.

However, treatments with up to 370 nM Hg^{2+} almost did not affect chlorophyll fluorescence parameters in *C. vulgaris* and *P. biwae* [25]. In the present study, chlorophyll fluorescence parameters were not significantly affected by Hg^{2+} treatments, suggesting that the photosynthic system was quite tolerant to Hg^{2+} in these two species.

Together with the unchanged contents of photosynthetic pigments, the present study demonstrated that inhibition of photosynthesis might not be the primary reason underlying toxicity of Hg^{2+} to growth of *S. acutus* and *S. quadricauda*. Several possibilities might explain the depressed alga density in response to Hg^{2+}, including inhibiting active transport of nutrients, nitrogen starvation [21], generation of reactive oxygen species (ROS) and oxidative damage [26]. Howover, more investigations are required to clarify this issue.

Cu^{2+} is an essential element to the photosynthetic process. In the present, contents of chl-a, chl-b and car, rETRmax, α, Fv/Fm and yield increased in *S. acutus* treated with 1 mg/L Cu^{2+}, suggesting a promotive effect of a low level of Cu^{2+} on photosynthesis. Similar effects were also reported in plant species, such as cereal crops [27,28]. Increased contents of pigments might be attributed to the promotion of Cu^{2+} on terpenoid biosynthesis [29].

Similar to previous reports [8,30], treatments with Cu^{2+} depressed cell growth and decreased contents of photosynthetic pigments (chl-a, car or chl-b) in *S. quadricauda* and *S. acutus*, demonstrating that high levels of Cu^{2+} triggered severe environmental concerns. A high level of Cu^{2+} blocked the electron transport and subsequently inhibited PSII activity [31], which further decreased F_v/F_m and adversely affected photosynthesis of algae [31-35]. In the present study, treatments with high levels of Cu^{2+} decreased contents of photosynthetic pigments, $rETR_{max}$ and I_k in both *S. quadricauda* and *S. acutus*, suggesting that the photosynthetic process was inhibited. Inhibition of Cu^{2+} to pigment accumulation and retarded chlorophyll integration into the photosystems [36] through competing with Mg^{2+} [37]might explain these phenomena. Overall, these results together suggested that Cu^{2+} affected alga growth probably through a mediating photosynthesis process.

Sensitivity to heavy metals differed among alga species. In the present study, contents of photosynthetic pigments decreased or did not change in *S. qudriauda*, but increased in *S. acutus*, in response to treatment with 0.5 mg/L Cu^{2+}. When exposed to 0.1 mg/L Hg^{2+}, population growth rate decreased in *S. quadricauda*, but did not change in *S. acutus*. These results suggested that *S. quadricauda* might be more sensitive to heavy metal pollution than *S. acutus*.

Conclusions

Both Cu^{2+} and Hg^{2+} significantly inhibited growth of *S. quadricauda* and *S. acutus*. Treatments with Hg^{2+} did not affect but treatments with Cu^{2+} significantly reduced contents of photosynthetic pigments and chlorophyll fluorescence parameters. Inhibition of photosynthesis might be a major reason underlying toxicity of Cu^{2+} to growth of *S. acutus* and *S. quadricauda*.

Acknowledgements

This work was supported by the General Project of Natural Science Research in Universities of Anhui Province (KJHS2019B02), the National Natural Science Foundation of China (No. 31600317), the Anhui Quality Engineering Project (2015SJJD022), the College Students' Innovative Project of Anhui Province (201810375112, 201810375117).

Conflict of Interest

The authors declare no conflict of interest.

References

1. CAPPELLO T., PEREIRA P., MAISANO M., MAUCERI A., PACHECO M., FASULO S. Advances in understanding the mechanisms of mercury toxicity inwild golden grey mullet (*Liza aurata*) by ^{1}HNMR-based metabolomics. Environ. Pollut. **219**, 139, **2016**.
2. BRANCO V., CAITO S., FARINA M., TEIXEIRA DA ROCHA J., ASCHNER M., CARVALHO C. Biomarkers of mercury toxicity: past, present, and future trends. J.Toxicol. Environ. Health Part B Crit. Rev. **20** (3), 119, **2017**.
3. BERNHOFT R.A. Mercury toxicity and treatment: a review of the literature. J. Environ. Public Health, **2012**, 460508, **2012**.
4. KALITA J., KUMAR V., MISRA U.K., BORA H.K. Memory and learning dysfunction following copper toxicity: biochemical and immunohistochemical basis. Mol. Neurobiol. **55** (5), 3800, **2018**.
5. WANG M., LEE J.S., LI Y. Global proteome profiling of a marine copepod and the mitigating effect of ocean acidification on mercury toxicity after multigenerational exposure. Environ. Sci. Technol. **51** (10), 5820, **2017**.
6. PAL A. Copper toxicity induced hepatocerebral and neurodegenerative diseases: an urgent need for prognostic biomarkers. Neurotoxicology, **40** (1), 97, **2014**.
7. BISHOP W.M., WILLIS B.E., RICHARDSON R.J., COPE W.G. The presence of algae mitigates the toxicity of copper-based algaecides to a non-target organism. Environ. Toxicol. Chem. **37** (8), 2132, **2018**.
8. KUMAR K.S., DAHMS H.U., LEE J.S., KIM H.C., LEE W.C., SHIN K.H. Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. Ecotoxicol. Environ. Saf. **104** (2), 51, **2014**.
9. MIAO L., YAN W., ZHONG L., XU W. Effect of heavy metals (Cu, Pb, and As) on the ultrastructure of *Sargassum pallidum* in Daya Bay, China. Environ. Monit. Assess. **186**, 87, **2014**.
10. ZHANG W., TAN N.G., LI S.F. NMR-based metabolomics and LC-MS/MS quantification reveal metal-specific tolerance and redox homeostasis in *Chlorella vulgaris*. Mol. Biosyst. **10**, 149, **2014**.
11. HOOK S.E., OSBORN H.L., GISSI F.,MONCUQUET P., TWINE N.A., WILKINS M.R., ADAMS M.S. RNA-Seq analysis of the toxicant-induced transcriptome of the marine diatom, *Ceratoneis closterium*. Mar. Genomics **16** (1), 45, **2014**.
12. KIANI S., FARHADIAN O., SOOFIANI N.M. Effect of heavy metals (Cadmium, Copper, Lead and Nickel) on Chlorophyll a and biomass of green algae *Scenedesmus quadricauda*. J. Fisheries Sci. Technol., **3** (3), **2014**.
13. JIANG Y., ZHU Y., HU Z., LEI A., WANG J. Towards elucidation of the toxic mechanism of copper on the model green alga *Chlamydomonas reinhardtii*. Ecotoxicology, **25** (7), 1417, **2016**.
14. WU H., WEI G., TAN X., LI L., LI M. Species-dependent variation in sensitivity of *Microcystis* species to copper sulfate: implication in algal toxicity of copper and controls of blooms. Sci. Rep. **7**, 40393. **2017**.
15. MORIN S., LAMBERT A.S., RODRIGUEZ E.P., DABRIN A., COQUERY M., PESCE S. Changes in copper toxicity towards diatom communities with experimental warming. J. Hazard. Mater. **334**, 223, **2017**.
16. PEREIRA C.M.S., DERUYTTER D., BLUST R., DE SCHAMPHELAERE K.A.C. Effect of temperature on chronic toxicity of copper, zinc, and nickel to, *Daphnia magna*. Environ. Toxicol. Chem. **36** (7), 1909. **2017**.
17. FAWAZ E.G., SALAM D.A. KAMAREDDINE L. Evaluation of copper toxicity using site specific algae and water chemistry: field validation of laboratory bioassays. Ecotoxicol. Environ. Saf. **155**, 59, **2018**.
18. TWISS M.R., NALEWAJKO C. Influence of phosphorus nutrition on copper toxicity to three strains of *Scenedesmus acutus* (chlorophyceae). J. Phycol. **28** (3), 291, **2010**.
19. CHEN F., LIU Z., LI D., CHENG P., CHEN S. Using ammonia for algae harvesting and as nutrient in subsequent cultures. Bioresour. Technol. **121**, 298, **2012**.
20. YANG X., LI X., ZHOU Y., ZHENG W., YU C., ZHENG T. Novel insights into the algicidal bacterium dh77-1 killing the toxic dinoflagellate *Alexandrium tamarense*. Sci. Total Environ. **482-483** (1), 116, **2014**.
21. HUISMAN J., HOOPEN H.J.G.T., FUCHS A. The effect of temperature upon the toxicity of mercuric chloride to *Scenedesmus acutus*. Environ. Pollut. Ecol. Biol. **22** (2), 133, **1980**.
22. WU Y., ZENG Y., QU J.Y., WANG W.X. Mercury effects on *Thalassiosira weissflogii*: applications of two-photon excitation chlorophyll fluorescence lifetime imaging and flow cytometry. Aquat. Toxicol. **110-111**, 133, **2012**.
23. DENG C., ZHANG D., PAN X., CHANG F., WANG S. Toxic effects of mercury on PSI and PSII activities, membrane potential and transthylakoid proton gradient in *Microsorium pteropus*. J. Photochem. Photobiol. B. **127**, 1, **2013**.
24. MURTHY S.D.S., SABAT S.C., MOHANTY P. Mercury induced inhibition of photosystem II activity and changes in the emission of fluorescence from phycobilisomes in intact cells of the cyanobacterium, *Spirulina platensis*. Plant Cell Physiol. **30**, 1153, **1989**.
25. JUNEAU P., DEWEZ D., MATSUI S., KIM S.G., POPOVIC R. Evaluation of different algal species sensitivity to mercury and metolachlor by PAM-fluorometry. Chemosphere, **45** (4-5), 589, **2001**.
26. ELBAZ A., WEI Y.Y., MENG Q., ZHENG Q., YANG Z.M. Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*. Ecotoxicology, **19** (7), 1285, **2010**.
27. BOGOEVA I. Pigment content in cereal crops grown on copper polluted soil. Bul. J. Agr. Sci. **4**, 767, **1998**.
28. BOGOEVA I., BONCHEVA E. Pigment content in cereal crops grown in condition of copper pollution and *Fusarium culmorum* infection. Agr. Sci. **2007**.
29. DROPPA M., TERRY N., HORVATH G. Variation in photosynthetic pigments and plastoquinone contents in sugar beet chloroplasts with changes in leaf copper content. Plant Physiol. **74**, 717, **1984**.
30. KOVÁČIK J., KLEJDUS B., HEDBAVNY J., BAČKOR M. Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). Plant Sci. **178** (3), 307, **2010**.
31. QIAN H., YU S., SUN Z., XIE X., LIU W., FU Z. Effects of copper sulfate, hydrogen peroxide and n-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and microcystin disposition

in *Microcystis aeruginosa*. Aquat. Toxicol. **99** (3), 405, **2010**.
32. MYKHAYLENKO N.F., ZOLOTAREVA E.K. The effect of copper and selenium nanocarboxylates on biomass accumulation and photosynthetic energy transduction efficiency of the green algae *C*hlorella vulgaris. Nanoscale Res. Lett. **12** (1), 147, **2017**.
33. MARANGONI L.F.B., MARQUES J.A., DUARTE G.A.S., PEREIRA C.M., CALDERON E.N., CASTRO C.B.E.,BIANCHINI A. Copper effects on biomarkers associated with photosynthesis, oxidative status and calcification in the Brazilian coral *Mussismilia harttii*, (Scleractinia, Mussidae). Mar. Environ. Res. **130**, 248, **2017**.
34. ROCCHETTA I., KÜPPER H. Chromium- and copper-induced inhibition of photosynthesis in *Euglena gracilis* analysed on the single-cell level by fluorescence kinetic microscopy. New Phytol. **182** (2), 405, **2009**.
35. BAUMANN H.A., MORRISON L., STENGEL D.B. Metal accumulation and toxicity measured by PAM-Chlorophyll fluorescence in seven species of marine macroalgae. Ecotoxicol. Environ. Saf. **72**, 1063, **2009**.
36. CASPIV., DROPPA M., GÁBOR HORVÁTH, MALKIN S., MARDER J. B., RASKIN V.I. The effect of copper on chlorophyll organization during greening of barley leaves. Photosynth. Res. **62** (2), 165, **1999**.
37. PÁDUA M., CAVACO A.M., AUBERT S., BLIGNY R., CASIMIRO A. Effects of copper on the photosynthesis of intact chloroplasts: interaction with manganese. Physiol. Plant. **138** (3), 301, **2010**.