

Original Research

Studies on Toxicity and Bioaccumulation of Cu²⁺ in Alga *Scenedesmus obliquus* and Its Effect on Life Table Demography of the Rotifer *Brachionus calyciflorus*

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Abstract

The algae- rotifer food chain plays a pivotal role in freshwater dynamics, as well as assessing toxicity in aquatic environments. We investigated the changes in algal cell density, photosynthetic pigments, superoxide dismutase (SOD) activity, and Cu²⁺ bioaccumulation after exposing *Scenedesmus obliquus* to 3.75, 7.5, 15, 30, and 60 µg/L Cu²⁺ for 72 h. We also studied the effects of Cu²⁺-exposed algae on the life table demographic parameters of *Brachionus calyciflorus* after 48 and 96 h of feeding. The results found that, when compared with the control, 3.75 µg/L Cu²⁺ significantly increased algal cell density after 48 h, while 60 µg/L Cu²⁺ significantly reduced algal cell density after 24 h. Increases in exposure time resulted in the chlorophyll-a, chlorophyll-b, and carotenoids showing an initial decrease and then increasing trend when compared to the control. Low concentrations of Cu²⁺ tended to induce increased SOD activity in algal cells, while high concentrations inhibited SOD activity. With increasing Cu²⁺ concentration and time, the Cu²⁺ bioaccumulation in algal cells increased proportionally. The highest bioaccumulation value was 1205 µg/g in 60 µg/L Cu²⁺ treatment after 72 h of exposure, 376.28% higher than pre-exposure level. Algae in the 3.75 µg/L Cu²⁺ treatment group significantly inhibited the population growth of *B. calyciflorus*, algae in the 60 µg/ Cu²⁺ group had the strongest inhibitory effect on the population growth of rotifers, and algae in the 30 µg/L Cu²⁺ group significantly increased the offspring mictic rate. The rotifer population produced adaptive responses to Cu²⁺-exposed algae stress after 96 h of feeding, unlike after 48 h.

Keywords: Cu²⁺, *Scenedesmus obliquus*, bioaccumulation, *Brachionus calyciflorus*, life table demography

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Introduction

Algae are the primary producers in an aquatic ecosystem and the material basis on which other organisms depend. They play a critical role in the aquatic food chain. Among aquatic organisms algae are the first hand indicators when pollutants enter a water body [1]. Contaminated algae inevitably affect zooplankton and fish through bioaccumulation via the food chain, and then eventually affect human health [2]. Heavy metals have many documented impacts on algae, including stunting algal growth and metabolism, inhibiting photosynthesis, reducing photosynthetic pigments, causing cell aberrations, and changing the community structure in natural water bodies [3-5]. *Scenedemus obliquus* is a common planktonic alga, which is sensitive to pollutants, easily obtainable, and reproduces quickly. Scientists use *S. obliquus* to investigate the effects of chemicals on the algae population level over a short time period [6-7].

As basal consumers, rotifers convert a significant fraction of their food into biomass and also play a significant role in maintaining the stability of the community structure and function of water ecosystems [8]. As such, rotifers too are affected by heavy metal pollutants. Due to their rapid reproduction, short generation time, ease of maintenance, and sensitivity to toxicants, the monogonont rotifer *Brachionus calyciflorus* have also been included as a standard freshwater bioassay species and widely used in environmental toxicity monitoring of pollutants [9]. The effects of common heavy metals on the life table demography of rotifer have been studied using *B. calyciflorus*, and excellent indicators such as net reproduction rate, generation time, and population growth rate were obtained for evaluating the chronic toxicity of heavy metals on the rotifers [10-11].

Cu²⁺ is a trace element necessary for physiological activities, but has adverse effects on organisms such as algae and rotifers when present in excess. Previous studies focused on the direct exposure of rotifers to test solutions containing Cu²⁺. Zhao et al. [12] showed that the 24, 48, and 96 h-LC50 of Cu²⁺ exposure to *B. calyciflorus* was 60.06, 23.72, and 15.07 µg/L, respectively. *B. koreanus* exposure to Cu²⁺ significantly up-regulated the expressions of heat shock protein family genes [13]. Salinity, pH, and organic matter content can significantly affect the acute and chronic toxic effects of Cu²⁺ on *B. plicatilis* [14]. However, the population dynamics of rotifers after ingesting Cu²⁺-exposed algae have not been well documented.

Our study aims to replicate a typical algae-rotifer food chain in an aquatic ecosystem with the green alga *S. obliquus* and monogonont rotifer *B. calyciflorus* as the test species. These species were included as standard freshwater bioassay species, designated by the American Society for Testing and Materials (ASTM). First, we analyzed the changes in growth

and physiological indexes of Cu²⁺-exposed algae, and the bioaccumulation of Cu²⁺ in the algae to determine the risk to *S. obliquus*. Furthermore, the effects of Cu²⁺-exposed algae on the life table demography of *B. calyciflorus* were evaluated to provide a better understanding of the toxicity and eco-toxicological evaluations of Cu²⁺ on aquatic ecosystems.

Materials and Methods

Test Organisms

The single-cell green alga *S. obliquus* was obtained from Wuhan Institute of Hydrobiology's algae seed bank (Chinese Academy of Sciences). It was grown in a semi-continuous culture using HB-4 medium and renewed daily at 20%. The culture conditions were as follows: the culture vessel was a 500 mL Erlenmeyer flask, the culture volume was 400 mL, temperature was 26±1°C, the light cycle was L:D = 14:10, and the light intensity was approximately 3000 lux. Algae at the exponential growth stage were centrifuged and re-suspended in the hard synthetic freshwater (96 mg NaHCO₃, 60 mg CaSO₄, 60 mg MgSO₄, and 4 mg KCl in 1 L deionized water). Cell densities of *S. obliquus* suspensions were counted directly using a hemocytometer, and adjusted to the desired densities using the hard synthetic freshwater. The components of the HB-4 medium and the hard synthetic freshwater are described in detail elsewhere [15].

B. calyciflorus individuals were initially isolated from Lake Jinghu (31°33'N, 118°37'E), located in the center of Wuhu city, China. Individuals parthenogenetically reproduced from one strain were continuously cultured in the hard synthetic freshwater for more than one year. Stock rotifer cultures were kept at 25±1°C under natural light and fed with the single-cell green alga *S. obliquus*. The diet was controlled at a density of approximately 1.0 × 10⁶ cells/mL.

Chemicals

The Cu²⁺ concentration was calculated based on the Cu²⁺ content in CuSO₄·5H₂O (Sinopharm Group, analytical grade), and the stock solutions of 1000 µg/L were prepared by dissolving the appropriate amount of CuSO₄·5H₂O into deionized water. The nominal test concentrations, including 0 (the blank control), 3.75, 7.5, 15, 30, and 60 µg/L, were obtained by adding appropriate aliquots of the stock solution to the HB-4 media.

Toxicity Test on *S. obliquus* Exposed to Cu²⁺

S. obliquus were inoculated into HB-4 media solution containing the aforementioned six Cu²⁺ concentrations to ensure that the initial algae density

was 1.0×10^6 cells/mL. The culture conditions were the same as in the pre-culture. Each treatment was conducted in three replicates to confirm reproducibility.

Changes in Algal Cell Densities Induced by Cu²⁺

Algal cell density was counted every 24 h using the microscopic field counting method. After thoroughly shaking, the sample of 0.1 mL algae solution was removed and covered evenly in a hemocytometer, where seven fields of view were photographed and counted under the microscope. To reduce counting errors, each algae culture bottle was sampled twice, then averaged to report the algal cell density of the bottle. All three bottles were counted for each treatment as replicates.

Changes in Photosynthetic Pigments Induced by Cu²⁺

The algae photosynthetic pigments were measured in each treatment every 24 h. Whatman GF/C (1.2 μ m pore size) was used to suction filter 200 mL of algae solution, which was homogenized with 80% acetone. The homogenate was extracted with acetone in the dark at 4 °C for 24 h. After centrifugation, a 15 mL sample of the supernatant was collected, and the absorbance values at 663, 645, and 470 nm were measured with a spectrophotometer. The contents (μ g/mL) of chlorophyll-a (C_a), chlorophyll-b (C_b), and carotenoid (C_k) were calculated according to the following equations [16]:

$$\begin{aligned} C_a &= 12.21\text{OD}_{663} - 2.81\text{OD}_{645}, \\ C_b &= 20.13\text{OD}_{645} - 5.03\text{OD}_{663}, \\ C_k &= (1000\text{OD}_{470} - 3.27C_a - 104C_b) / 229, \end{aligned}$$

...where OD663, OD645, and OD470 represent the optical density of the solution at 663, 645, and 470 nm using a spectrophotometer, respectively.

Changes in Superoxide Dismutase Activity Induced by Cu²⁺

The algal cells' superoxide dismutase (SOD) activity was measured in each treatment every 24 h. The sample of 200 mL of algae solution was centrifuged at 5000 rpm for 7 min, and the algae cells were collected after removing the supernatant. The collected cells were added to 3 mL of pre-chilled 0.05 mol/L phosphate-buffered saline (PBS, pH = 7.8), then crushed with 120 w ultrasonic wave for 5 s intervals under ice bath conditions for 60 cycles. The crushed solution was centrifuged at 8500 rpm for 15 min at 4°C, and the supernatant was the SOD test solution. SOD activity was measured using the photo-reduction reaction method of nitro blue tetrazolium chloride (NBT) described by Li et al. [16] with slight modifications.

Changes of Cu²⁺ Bioaccumulation in Algal Cells

Cu²⁺ accumulation in algal cells in each treatment group was measured every 24 h. In each treatment, one-third of the concentrated algae solution was dried and weighed, 3 mL of nitric acid was added, then was left for 24 h. Next, 0.3 mL of perchloric acid was added before being heated on an electric heating plate at 90°C until the solution was colorless and transparent. The excess nitric acid and perchloric acid evaporated, and the volume of the solution was adjusted to 5 mL with 5% nitric acid. The Cu²⁺ content in the solution was determined using a flame atomic absorption spectrophotometer.

Life Table Experiments on B. calyciflorus Fed with Cu²⁺-exposed Algae

The pre-cultured rotifers were fed with the *S. obliquus* collected from the six Cu²⁺ concentration treatments at a density of 1.0×10^6 cells/mL. The culture conditions were the same as those in the pre-culture. After 48 and 96 h of feeding, the ten neonates (less than 2 h) were randomly selected from each treatment group. The samples were placed in 6 mL glass cups containing 5 mL of the test solution with 1.0×10^6 cells/mL of Cu²⁺-exposed algae. The number of neonates produced via parthenogenesis and the number of original test individuals still alive were recorded every 8 h. Then the neonates were transferred to other glasses and further cultured under the same conditions until the females were identified by carrying the first egg. Every 24 h, the original surviving rotifers were transferred into freshly prepared test solution containing the hard synthetic freshwater and Cu²⁺-exposed algae. The life-table experiments were repeated three times as replicates for each treatment and conducted in darkness at $25 \pm 1^\circ\text{C}$ until each individual of every cohort died.

Based on the collected data, age-specific survivorship (l_x) and age-specific fecundity (m_x) were constructed for each replicate using conventional life-table techniques [17]. X was defined as the age interval to calculate the population demographic parameters. Net reproductive rate (R_0), generation time (T), life expectancy at hatching (e_0), and intrinsic rate of population increase (r_m) were calculated according to Sha et al. [18]. The offspring mictic rate (MR) was calculated for each treatment as the proportion of mictic females to all F_1 offspring.

Data Statistical Analysis

SPSS Statistics 16.0 (SPSS, Inc.) and Excel (version 2019) were used to analyze the data. Normality of distribution and homogeneity of variance were first evaluated by Shapiro-Wilk test and Levene *statistic* with non-significant differences ($P > 0.05$), respectively. Differences between the treatments and the

Table 1. Effects of different concentrations of Cu²⁺ and exposure time on the natural logarithm of *Scenedesmus obliquus* densities.

Cu ²⁺ concentration (μg/L)	24 h	48 h	72 h
0	14.6±0.039 ^b	14.7±0.026 ^a	15.1±0.002 ^{bc}
3.75	14.6±0.092 ^b	15.0±0.008 ^b	15.1±0.101 ^c
7.5	14.6±0.063 ^b	14.8±0.093 ^a	15.1±0.015 ^{abc}
15	14.6±0.086 ^b	14.8±0.074 ^a	15.0±0.047 ^{abc}
30	14.6±0.050 ^b	14.8±0.052 ^a	14.9±0.099 ^{ab}
60	14.3±0.087 ^a	14.7±0.029 ^a	14.9±0.061 ^a

Multiple comparison of SNK-q: The same letter indicates that there are no significant differences between two groups in the same column.

controls were analyzed using a one-way analysis of variance (ANOVA), and the interactions of Cu²⁺ concentration × exposure time interactions were investigated by two-way ANOVA, with the significance set to $P < 0.05$. For each parameter, differences between means were evaluated for significance using Duncan's multiple range test. A paired-sample T-test analyzed the feeding time data (48 and 96 h).

Results and Discussion

Effects of Cu²⁺ on the Growth of *S. obliquus*

Previous research has shown that Cu²⁺ had the most apparent inhibitory effect on the growth of *S. obliquus*, when compared with common essential metal ions such as Zn²⁺ and Mn²⁺ [19]. The 96 h median effect concentration (EC50) of Cu²⁺ on the growth of *S. obliquus* was 51 μg/L, and the inhibitory effect of Cu²⁺ on the growth of *S. obliquus* was more significant than that of *Chlorella pyrenoidosa* and *Closterium lunula* [20]. In our study, when compared to the control, 3.75 μg/L Cu²⁺ significantly promoted the growth of *S. obliquus* after 48 h of exposure ($P < 0.05$). 60 μg/L Cu²⁺, however, significantly inhibited the growth of *S. obliquus* after 24 h of exposure ($P < 0.05$) (Table 1). There are many sulfur-containing groups on the cell

wall of *S. obliquus*, and Cu²⁺ has a powerful affinity for sulfur-containing groups, which may explain why *S. obliquus* growth is highly sensitive to Cu²⁺ [21].

Generally, there are two aspects attributing to the effect that Cu²⁺ has on the biochemical processes of algae. Cu²⁺ is an essential element of life that directly participates in the biochemical processes (e.g., respiration and photosynthesis) in algae cells. Nevertheless, Cu²⁺ can affect certain enzyme activities in algae cells, thus affecting algal nutrient absorption [22]. Previous studies have found that 1 and 10 μg/L Cu²⁺ significantly promoted the growth of *Microcystis aeruginosa*, while 0.01 and 100 μg/L Cu²⁺ severely inhibited growth and the synthesis of algal toxin [23]. We determined that 3.75 μg/L Cu²⁺ significantly promoted the growth of *S. obliquus* after 48 h ($P < 0.05$) and 60 μg/L Cu²⁺ significantly inhibited its growth after 24 and 72 h ($P < 0.05$) (Table 1). These results show a dose-response characteristic of low concentration growth promotion and high concentration inhibition of algae growth. This suggests that a small amount of Cu²⁺ is necessary for the algae metabolism process as chlorophyll cannot form in the absence of Cu²⁺. An appropriate amount of Cu²⁺ can accelerate the formation of chlorophyll, thereby accelerating the intensity of photosynthesis and the accumulation of carbohydrates. A large amount of Cu²⁺, however, changes the *S. obliquus* protoplasmic membrane permeability. This

Table 2. Regression curves of the natural logarithm of *Scenedesmus obliquus* densities along with time at different concentrations of Cu²⁺.

Cu ²⁺ concentrations (μg/L)	Regression functions	Significance test	
0	Y = -0.078X ² + 0.604X + 13.960	R ² = 0.942	P = 0.000
3.75	Y = -0.120X ² + 0.763X + 13.921	R ² = 0.957	P = 0.000
7.5	Y = -0.106X ² + 0.685X + 13.947	R ² = 0.941	P = 0.000
15	Y = 0.123X ² + 0.711X + 13.949	R ² = 0.925	P = 0.000
30	Y = -0.126X ² + 0.097X + 13.937	R ² = 0.936	P = 0.000
60	Y = -0.049X ² + 0.488X + 13.894	R ² = 0.924	P = 0.000

Y is the natural logarithm of *Scenedesmus obliquus* density (ln N), X is the culture time (h).

Table 3. Effects of exposure time, Cu²⁺ concentration and their interaction on the natural logarithm of *Scenedesmus obliquus* densities.

Sources of variances	SS	DF	MS	F value	P value
Exposure time (A)	12.339	3	4.113	384.374	0.000
Cu ²⁺ concentration (B)	0.26	5	0.052	4.853	0.001
Interaction of A×B	0.291	15	0.019	1.814	0.060
Error	0.514	48	0.011		

has noticeable toxic effects on the algae, such as poor growth, photosynthesis inhibition, and even death of the algae [4].

There is a good regression relationship between the natural logarithmic value of algal density and exposure time in different treatments, indicating that low concentrations of Cu²⁺ may have toxic effects on algae, increasing with exposure time (Table 2). A two-way ANOVA determined that both exposure time and Cu²⁺ concentration had significant effects on the logarithmic value of algal density ($P < 0.01$), but the interaction between exposure time and Cu²⁺ concentration did not (Table 3). Previous research found that the inhibitory effect of Pb²⁺ on the growth of *S. obliquus* mainly occurs late in the culture period, while Cr³⁺ and Cr⁶⁺ show toxicity throughout the culture period [24]. These findings further show that Cu²⁺ at a certain concentration can promote the growth of algae, but over time Cu²⁺ bioaccumulation increases causing a toxic effect on algae. This complex relationship is evident through the complex results.

Effect of Cu²⁺ on Photosynthetic Pigments and SOD Activity of *S. obliquus*

A previous study found that the chlorophyll-a, chlorophyll-b, and carotenoids in *Dunaliella salina* cells decreased significantly with the increase of Cu²⁺ concentration [25]. However, we found that the effects of Cu²⁺ on photosynthetic pigments of *S. obliquus* differed (Fig. 1). After 24 h, the chlorophyll a, chlorophyll b, and carotenoids in each treatment were all lower than in the control. After 48 h, the chlorophyll-a in the five treatments were all higher than that of the control, especially in the 3.75 µg/L. After 48 h, the chlorophyll-b in 3.75, 7.5, and 60 µg/L were lower than that of control, but higher in 15 and 30 µg/L. After 48 h, the carotenoids in all five treatments were approximately the same as the control. After 72 h, the highest values of chlorophyll-a, chlorophyll-b, and carotenoids were 2.95, 1.20, and 1.68 µg/mL in the 7.5, 30, and 30 µg/L treatments, respectively (Fig. 1). Photosynthetic pigment plays a vital role in the growth of algae and reflects the strength of algae photosynthesis. The reduction of photosynthetic pigments is one of the crucial characteristics of algae suffering from metal exposure [26]. Here, longer exposure time resulted in photosynthetic pigments in

each treatment group first decreasing then increasing, when compared to the control. This was especially seen in the change of chlorophyll-a in the low concentration group, suggesting that recovery of algae photosynthesis and adaptation of algae growth to toxicity of Cu²⁺ occurred, which agreed with the results obtained by Kondzior and Butarewicz [27].

Yang et al. [24] found that Pb²⁺, Cr³⁺, and Cr⁶⁺ increased the O₂⁻ content in *S. obliquus* cells and

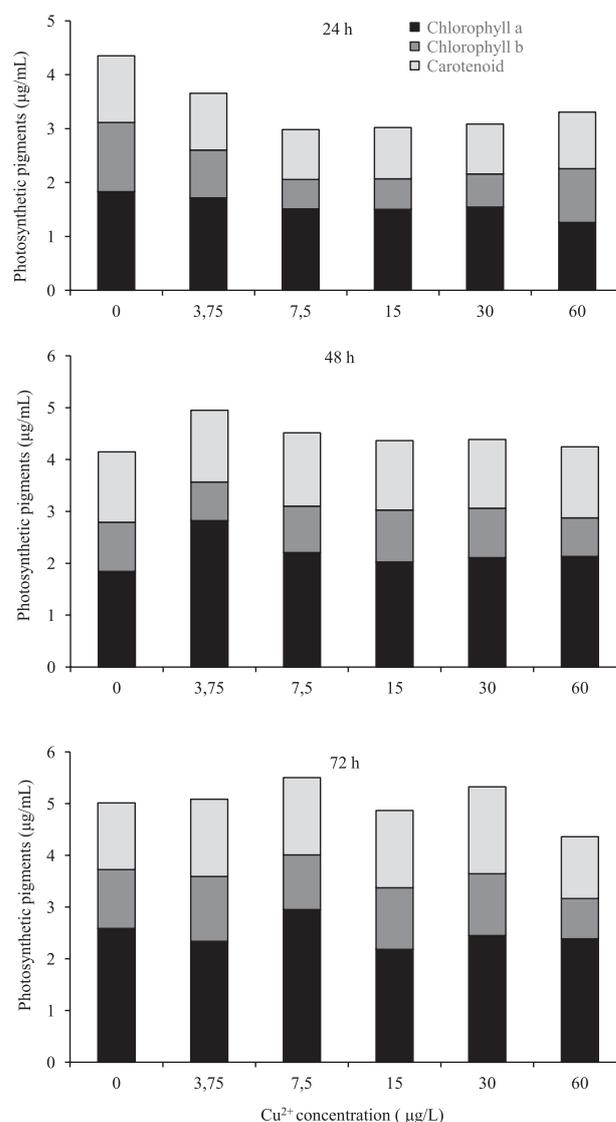


Fig. 1 Effects of Cu²⁺ concentrations and exposure time on the photosynthetic pigments in *Scenedesmus obliquus*.

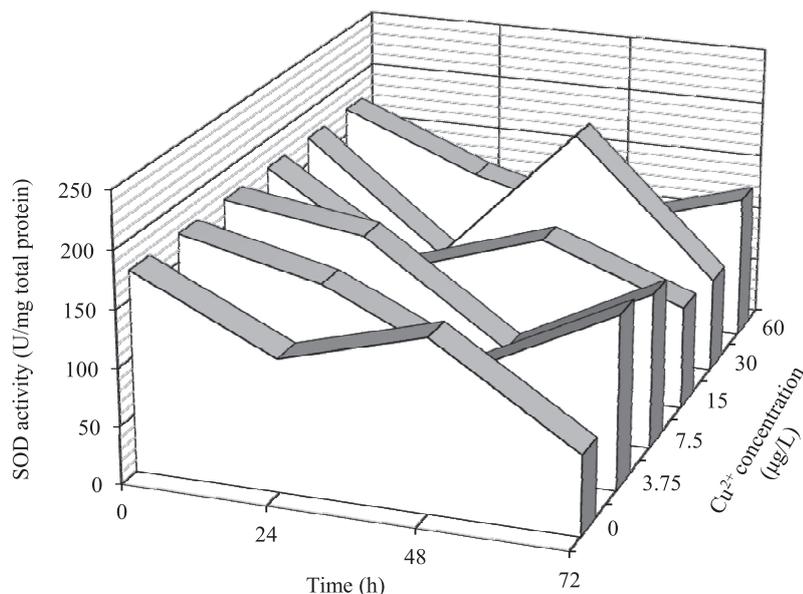


Fig. 2 Effects of Cu^{2+} concentration and exposure time on the SOD activity in *Scenedesmus obliquus*.

enhanced the lipid peroxidation of algal cell membranes, adversely affecting the algal cells. SOD can eliminate O_2^- through disproportionation and is important for biological protection. Its activity could be significantly induced by most toxicants [28]. We found that under longer exposure times, SOD activities in the control, 15, and 30 $\mu\text{g/L}$ treatments increased then decreased, similar to the reaction of photosynthetic pigments. The reverse occurred in 3.75, 7.5, and 60 $\mu\text{g/L}$. SOD activity generally increased at low concentrations of Cu^{2+} and was inhibited at high concentrations, indicating that SOD can play a significant role in low-level oxidative stress rather than high-level [29].

Bioaccumulation of Cu^{2+} in *S. obliquus*

Several studies have revealed that a variety of algae (e.g., *S. quadricauda*, *Chlorella*, and *M. aeruginosa*) can bioaccumulate metals at high amounts [30-31]. In our study, the Cu^{2+} in the algae before exposure was 253 $\mu\text{g/g}$. The Cu^{2+} bioaccumulation in the control decreased after exposure, and the lowest value was 115 $\mu\text{g/g}$ after 48 h, 54.54% lower than pre-exposure level. In the 3.75-60 $\mu\text{g/L}$, however, greater Cu^{2+} bioaccumulation occurred in the algal cells. The bioaccumulation increased in approximate proportion with the increase of Cu^{2+} concentration and exposure time. The highest value was 1205 $\mu\text{g/g}$ in 60 $\mu\text{g/L}$ after 72 h, 376.28% higher than pre-exposure level (Fig. 3). The results exhibited that Cu^{2+} , even at a lower level, is bioaccumulated by *S. obliquus* and can reach a higher level. This phenomenon may have toxic effects on zooplankton, such as rotifers. Therefore, the toxic effects of heavy metals at low concentrations cannot be ignored, especially in algae-rich waters.

Effects of Cu^{2+} -exposed Algae on Life Table Demography of Rotifer

The life table parameters of *B. calyciflorus* were significantly affected by single and mixed metals [32]. Zhao et al. [12] found that the survival, reproductive, net reproduction, and population growth rates of *B. calyciflorus* significantly decreased after exposure to 60 $\mu\text{g/L}$ Cu^{2+} , while the mictic rate significantly increased. Scientists believe that the population growth of *B. calyciflorus* is significantly affected by food quality, such as the type and density of algae [33].

Our results show that algae collected in different Cu^{2+} concentrations significantly affected the life table parameters of *B. calyciflorus* ($P < 0.05$). After 48 h of feeding, algae in 3.75, 7.5, 15, and 60 $\mu\text{g/L}$ significantly reduced rotifer life expectancy, net reproduction rate, population growth rate, and average life span ($P < 0.05$). Algae in 7.5 $\mu\text{g/L}$ experienced significantly reduced generation time ($P < 0.05$) and those in 30 $\mu\text{g/L}$ experienced a significantly increased offspring mictic rate ($P < 0.05$) (Table 4). After 96 h of feeding, algae in 3.75 $\mu\text{g/L}$ had significantly reduced net reproduction and population growth rates ($P < 0.05$) in the rotifers. Algae in 7.5 $\mu\text{g/L}$ showed significantly reduced life expectancy, net reproduction rate, generation time, and population growth rate ($P < 0.05$). Algae in 15 $\mu\text{g/L}$ had a significantly reduced population growth rate and increased offspring mictic rate ($P < 0.05$). Algae in 30 $\mu\text{g/L}$ showed a significantly increased offspring mictic rate ($P < 0.05$). Finally, algae in 60 $\mu\text{g/L}$ exhibited significantly reduced life expectancy, net reproduction rate, and population growth rate ($P < 0.05$) (Table 4).

Furthermore, there were significant differences in rotifer life table parameters between 48 and 96 h of feeding for algae collected with the same Cu^{2+}

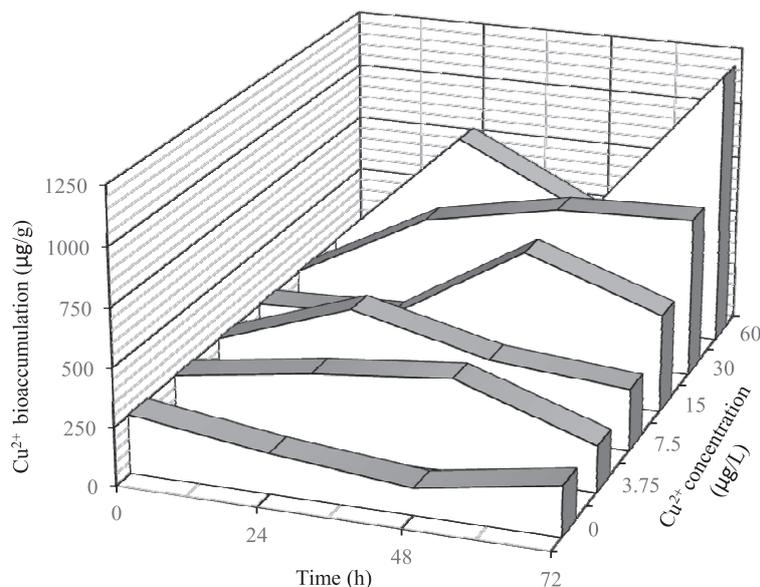


Fig. 3 Effects of Cu²⁺ concentration and exposure time on the Cu²⁺ bioaccumulation in *Scenedesmus obliquus*.

concentration. The life expectancy, net reproduction rate, and average lifespan after 96 h were significantly higher than those of 48 h in 7.5 µg/L ($P < 0.05$). The generation time of 96 h was significantly higher than that of 48 h in 3.75, 7.5, and 15 µg/L ($P < 0.05$). Additionally, the offspring mictic rate of 96 h was significantly higher than that of 48 h in 3.75 and 30 µg/L ($P < 0.05$) (Table 4). These results indicate that the feeding time has a significant effect on the rotifer life table parameters. With a longer feeding time, the life table parameters increased in most treatments,

suggesting that the inhibitory effect of Cu²⁺-rich algae on rotifer population growth decreased after 96 h. This may be due to the fact that during the longer feeding times, rotifers gradually adapted to Cu²⁺-exposed algae. Their defense mechanism may have been activated to protect the organism. Particularly, the increased offspring mictic rate reveals that the rotifer had transformed from asexual reproduction to sexual reproduction and prepared to produce dormant eggs to survive the unfavorable environment; this is a general rotifer response to many of environmental stresses [34].

Table 4. Effects of different Cu²⁺ concentration and feeding time on life table parameters of *Brachionus calyciflorus*.

Cu ²⁺ (µg/L)	e_0 (h)	R_0 (ind.)	T (h)	r_m (d ⁻¹)	Life (h)	MR (%)
Fed for 48 h						
0	116.0±9.4 ^c	6.8±1.0 ^a	81.8±4.7 ^a	0.64±0.03 ^a	104.0±9.4 ^a	0.01±0.01 ^a
3.75	86.4±5.4 ^b	2.7±0.2 ^b	63.3±0.6 ^b	0.39±0.03 ^b	74.4±5.4 ^b	0.00±0.00 ^a
7.5	77.6±2.4 ^{ab}	2.6±0.3 ^b	57.8±2.4 ^b	0.41±0.03 ^b	65.6±2.4 ^b	0.02±0.01 ^{ab}
15	90.4±2.1 ^b	3.3±0.4 ^b	67.0±4.1 ^{ab}	0.46±0.05 ^b	78.4±2.1 ^b	0.02±0.01 ^{ab}
30	112.8±9.8 ^c	5.5±0.9 ^a	81.4±6.7 ^a	0.55±0.02 ^{ab}	100.8±9.8 ^a	0.08±0.04 ^b
60	66.4±5.1 ^a	1.9±0.8 ^b	67.0±1.1 ^{ab}	0.15±0.12 ^c	54.4±5.1 ^c	0.03±0.03 ^{ab}
Fed for 96 h						
0	116.8±9.4 ^a	6.8±1.0 ^a	81.8±4.8 ^a	0.64±0.03 ^a	104.0±9.4 ^{abc}	0.01±0.01 ^a
3.75	97.8±11.7 ^a	2.9±0.3 ^b	82.3±5.4 ^a	0.31±0.01 ^b	85.8±11.7 ^a	0.04±0.01 ^a
7.5	126.0±0.0 ^b	4.3±0.0 ^b	103.2±0.0 ^b	0.35±0.00 ^b	114.0±0.0 ^b	0.00±0.00 ^a
15	124.0±9.6 ^{ab}	4.5±0.9 ^{ab}	97.9±4.3 ^{ab}	0.38±0.05 ^b	112.0±9.6 ^{ab}	0.10±0.03 ^b
30	119.6±1.4 ^{ab}	5.9±0.9 ^a	84.6±2.8 ^{ab}	0.53±0.03 ^a	107.6±1.4 ^{ab}	0.46±0.02 ^c
60	90.0±11.5 ^c	2.8±0.9 ^b	82.0±13.7 ^a	0.27±0.07 ^b	78.0±11.5 ^c	0.02±0.25 ^a

The different letters signify significant difference between two concentrations of pollutant with the same feeding time (SNK).

In previous studies evaluating the effects of heavy metals on the chronic toxicity of rotifers, they are usually directly exposed to test solutions containing algae and metals. To avoid the concentration change caused by bioaccumulation of metals in algae, the test solution needs to be replaced periodically (usually 12 or 24 h) with fresh solution [35]. The long-term exposure of algae to water that contains metals can cause adsorption and bioaccumulation of said metals. This can lead to rotifers ingesting algae that has bioaccumulated more metal than is in the water, resulting in more serious toxic effects. Our previous research showed that, when exposed to the algae solution containing 10 µg/L Cu²⁺, the life table parameters and the mictic rate of rotifers did not significantly differ from the control [32]. Here, however, the algae in the lowest concentration (3.75 µg/L Cu²⁺) significantly reduced the net reproduction rate and population growth rate of rotifers, indicating that the toxicity of Cu²⁺-riched algae to rotifers exceeds that of Cu²⁺-containing test solutions. Although the concentration of Cu²⁺ in most bodies of water is quite low, usually maintaining ng/L- µg/L grade [36], its toxic effects on rotifers through the food chain is a concern in light of these results. Further research is needed to examine whether similar impacts might occur in rotifers exposed to other metals at lower concentration that are currently thought to be safe.

Conclusions

Traditional eco-toxicological rotifer studies have estimated the toxic effects of metals on rotifers via direct exposure. However, researchers have neglected the ways in which rotifers are impacted by metals accumulated in algae, a common food source. The model food chain selected in this study consisted of *S. obliquus*, the primary producer, and *B. calyciflorus*, the primary consumer. We identified effects of Cu²⁺ on the cell densities and physiological indexes (e.g., photosynthetic pigment and SOD activity growth of *S. obliquus*). We also investigated the bioaccumulation of Cu²⁺ in algal cells and its impacts on life table demography of *B. calyciflorus*. Our results demonstrate that Cu²⁺ exposure had different impacts on the growth of *S. obliquus*, depending on the concentration of Cu²⁺, and Cu²⁺-exposed alga had toxic effects on the population growth of the rotifers. The toxicity of Cu²⁺ bioaccumulation can be expected to provide primary data for the ecological risk assessment of Cu²⁺ in natural water.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. SAIKIA M.M.K., LOHAR M.P. Structural and physico-chemical correlation of algal community of a water body affected by pulp and paper mill effluents. *Global J. Sci. Front. Res.* **12**, 1, **2012**.
2. MALIK N., BISWAS A.K., RAJU C.B. Plankton as an indicator of heavy metal pollution in a freshwater reservoir of Madhya Pradesh, India. *Bull. Environ. Contam. Toxicol.* **90**, 725, **2013**.
3. ALHO L.O.G., GEBARA R.C., PAINA K.A., SARMENTO H., MELÃO M.D.G.G. Responses of *Raphidocelis subcapitata* exposed to Cd and Pb: mechanisms of toxicity assessed by multiple endpoints. *Ecotoxicol Environ Saf.* **169**, 950, **2019**.
4. WANG H., EBENEZER V., KI J.S. Photosynthetic and biochemical responses of the freshwater green algae *Closterium ehrenbergii* Meneghini (Conjugatophyceae) exposed to the metal coppers and its implication for toxicity testing. *J. Microbiol.* **56**, 426, **2018**.
5. WANG S.G., DIAO X.J., HE L.S. Effects of algal bloom formation, outbreak, and extinction on heavy metal fractionation in the surficial sediments of Chaohu Lake. *Environ. Sci. Pollut. Res.* **22**, 14269, **2015**.
6. YAO K., LV X.H., ZHENG G.Q., CHEN Z.H., JIANG Y.L., ZHU X.S., WANG Z.Y., CAI Z.H. Effects of carbon quantum dots on aquatic environments: comparison of toxicity to organisms at different trophic levels. *Environ. Sci. Technol.* **52**, 14445, **2018**.
7. XIONG J.Q., GOVINDWAR S., KURADE M.B., PAENG K.J., ROH H.S., KHAN M.A., JEON B.H. Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, *Scenedesmus obliquus*. *Chemosphere.* **218**, 551, **2019**.
8. DIAS J.D., BONECKER C.C., MIRACLE M.R. The rotifer community and its functional role in lakes of a neotropical floodplain. *Int. Rev. Hydrobiol.* **99**, 72, **2014**.
9. RICO-MARTÍNEZ R., ARZATE-CARDENAS M.A., ALVARADO-FLORES J., PÉREZ LEGASPI I.A., SANTOS-MEDRANO G.E. Rotifers as models for ecotoxicology and genotoxicology. In book: *Invertebrates-Experimental Models in Toxicity Screening*, Chapter:4, Publisher: In Tech-Open Acces Publishers, Editors: Larramendy M. **57**, **2016**.
10. DAHMS H.U., HAGIWARA A., LEE J.S. Ecotoxicology, ecophysiology, and mechanistic studies with rotifers. *Aquat. Toxicol.* **101**, 1, **2011**.
11. SANTOS-MEDRANO G.E., RICO-MARTÍNEZ R. Lethal effects of five metals on the freshwater rotifers *Asplanchna brighwellii* and *Brachionus calyciflorus*. *Hidrobiológica.* **23**, 82, **2013**.

12. ZHAO H.Y., YANG J.X., LU Z.H., WANG X. The toxic effect of Cu²⁺ on rotifer *Brachionus calyciflorus*. J. Nanjing Norm. Univ. (Nat. Sci. Edit.). **25**, 86, **2002**.
13. JUNG M.Y., LEE Y.M. Expression profiles of heat shock protein gene families in the monogonont rotifer *Brachionus koreanus*-exposed to copper and cadmium. Toxicol. Environ. Health Sci. **4**, 235, **2012**.
14. ARNOLD W.R., DIAMOND R.L., SMITH D.S. The effects of salinity, pH, and dissolved organic matter on acute copper toxicity to the rotifer, *Brachionus plicatilis* ("L" strain). Arch. Environ. Contam. Toxicol. **59**, 225, **2010**.
15. XU X.P., XI Y.L., LI J.Y. Effects of deltamethrin and endosulfan on the population growth of *Brachionus calyciflorus* at different algal (*Scenedesmus obliquus*) densities. Pol. J. Environ. Stud. **28**, 407, **2019**.
16. LI H.S., SUN Q., ZHAO S.J., ZHANG W.H. The experiment principle and technique for plant physiology and biochemistry. Beijing: Higher Education Press. 125, **2000**.
17. HUANG L., XI Y.L., ZHA C.W., ZHAO L.L., WEN X.L. Effects of dieldrin and 17 β -estradiol on life history characteristics of freshwater rotifer *Brachionus calyciflorus* Pallas. J. Freshwater Ecol. **27**, 381, **2012**.
18. SHA J.J., YOU W., CHEN H.M., WANG M., WANG H., LI X.H., QI L., TANG X.X. Using population demographic parameters to assess impacts of two polybrominated diphenyl ethers (BDE-47, BDE-209) on the rotifer *Brachionus plicatilis*. Ecotoxicol. Environ. Saf. **119**, 106, **2015**.
19. WANG L., LIU R., LI W.H., CHEN L., ZHAO H.J., ZHENG Q.S. Effects of stresses of different heavy metals on growth and chlorophyll II fluorescence of *Scenedesmus obliquus*. J. Ecol. Rural Environ. **31**, 743, **2015**.
20. ZHANG W., YAN H., WU Z.L. Toxic effects of copper on inhibition of the growths of unicellular green algae. China Environ. Sci. **21**, 4, **2001**.
21. MANGA V., STOCK N.L., GUÉGUEN C. Molecular characterization of phytoplankton dissolved organic matter (DOM) and sulfur components using high resolution Orbitrap mass spectrometry. Anal. Bioanal. Chem. **408**, 1891, **2016**.
22. ALLEN M.D., KROPAT J., TOTTEY S., DEL CAMPO J.A., MERCHANT S. Manganese deficiency in *Chlamydomonas* results in loss of photosystem II and MnSOD function, sensitivity to peroxides, and secondary phosphorus and iron deficiency. Plant Physiol. **143**, 263, **2007**.
23. LEI Z., CHEN R., WANG S., XUE T., WANG X.C. Effect of copper stress on cell proliferation and production of microcystin *Microcystis aeruginosa*. Acta Sci. Circum. **37**, 1993, **2017**.
24. YANG G.Y., WAN L.L., LEI X.Q., WANG Y.J., LI A.F., ZHANG C.W. Effects of lead and chromium on the growth, photosynthetic performance, and antioxidant activity of *Scenedesmus obliquus*. Acta Sci. Circum. **34**, 1606, **2014**.
25. GUO H.S., LING N., LIU X.R., CAO X.M., LANG L., QI Z., CUI D., LIU B., SONG D.X., JI C.F. Physiological response of *Dunaliella salina* to heavy metal copper stress. J. of Harbin Univ. Commer. (Nat. Sci. Edit.). **35**, 5, **2019**.
26. DUAN C.X., ZHANG B.Y., WU S.C., FENG J.H., WANG G.C. Effect of cadmium on photosynthesis of *Scenedesmus obliquus*. Oceanol. Limnol. Sin. **46**, 351, **2015**.
27. KONDZIOR P., BUTAREWICZ A. Effect of Heavy Metals (Cu and Zn) on the content of photosynthetic pigments in the cells of algae *Chlorella Vulgaris*. J. Ecol. Eng. **19**, 18, **2018**.
28. YANG H., HUANG Z.Y. Activities of antioxidant enzymes and Zn-MT-like proteins induced in *Chlorella vulgaris* exposed to Zn²⁺. Acta Ecol. Sin. **32**, 7117, **2012**.
29. NEL A., XIA T., LUTZ M., LI N. Toxic potential of materials at the nanolevel. Science. **311**, 622, **2006**.
30. KUMAR R.M., FRANKILIN J., RAJ S.P. Accumulation of heavy metals (Cu, Cr, Pb and Cd) in freshwater micro algae (*Chlorella sp.*). J. Environ. Sci. Eng. **55**, 371, **2013**.
31. SINGH N.K., RAGHUBANSHI A.S., UPADHYAY A.K., RAI U.N. Arsenic and other heavy metal accumulation in plants and algae growing naturally in contaminated area of West Bengal, India. Ecotoxicol. Environ. Saf. **130**, 224, **2016**.
32. XU X.P., XI Y.L., HUANG L., XIANG X.L. The life-table demographic response of freshwater rotifer *Brachionus calyciflorus* to multi-metal (Cu, Zn, Cd, Cr, and Mn) mixture interaction. Bull. Environ. Contam. Toxicol. **93**, 165, **2014**.
33. KUEFLER D., AVGAR T., FRYXELL J.M. Rotifer population spread in relation to food, density and predation risk in an experimental system. J. Anim. Ecol. **8**, 323, **2012**.
34. FRANCH-GRAS L., GARCÍA-ROGER E.M., SERRA M., JOSÉ CARMONA M. Adaptation in response to environmental unpredictability. Proc. Biol. Sci. **284**, 0427, **2017**.
35. SARMA S.S.S., MARTÍNEZ-JERÓNIMO F., RAMÍREZ-PÉREZ T., NANDINI S. Effect of cadmium and chromium toxicity on the demography and population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera). J. Environ. Sci. Health. Part A. **41**, 543, **2006**.
36. FANG T., YANG K., LU W.X., CUI K., LI J., LIANG Y.Y., HOU G.J., ZHAO X.X., HUI L. An overview of heavy metal pollution in Chaohu Lake, China: enrichment, distribution, speciation, and associated risk under natural and anthropogenic changes. Environ. Sci. Pollut. Res. **26**, 29585, **2019**.

