

Original Research

# Early Toxic Effects of Cd<sup>2+</sup> on Photosynthetic Activity of Six Freshwater Algae Species

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## Abstract

Cd<sup>2+</sup> is a serious environmental pollutant. Previous studies have investigated the effects of Cd<sup>2+</sup> on animals, plants and algae. However, the tested concentrations were generally higher than the actual environment level. The present study determined the effects of low concentrations of Cd<sup>2+</sup> (0.05 to 0.20 mg/L) on the chlorophyll fluorescent parameters of six local algae species in order to investigate the early toxicity of Cd<sup>2+</sup>. The results showed that treatments with up to 0.20 mg/L Cd<sup>2+</sup> did not significantly affect population growth rate of *S. quadricauda*, *C. pyrenoidosa*, *S. obliquus*, *N. palea*, *S. minutum* and *S. acutus*. Treatments with 0.05-0.20 mg/L Cd<sup>2+</sup> significantly increased F<sub>v</sub>/F<sub>m</sub> in *N. palea* and *S. minutum* and decreased F<sub>v</sub>/F<sub>m</sub> in *S. obliquus* and *S. acutus*, but did not significantly affect F<sub>v</sub>/F<sub>m</sub> in *C. pyrenoidosa*. When exposed to Cd<sup>2+</sup>, rETR<sub>max</sub> was reduced in *S. obliquus*, *C. pyrenoidosa*, *S. acutus* and *S. quadricauda*, but was elevated in *N. palea* and *S. minutum*. Overall, trace Cd<sup>2+</sup> still revealed early toxicity to green algae. *N. palea*, *S. minutum* and *C. pyrenoidosa* were more tolerant to Cd<sup>2+</sup> than *S. obliquus*, *S. acutus* and *S. quadricauda*.

**Keywords:** alga density, chlorophyll, fluorescence, pollution

## Introduction

Due to the industrial disposal of electronic components, environmental pollution of Cd<sup>2+</sup> is a serious global problem [1]. Cd<sup>2+</sup> can be accumulated in organisms and then biomagnified through food chains [2]. Investigations have revealed notable daily intakes and health risks of Cd<sup>2+</sup> exposure through drinking water and vegetable consumption in humans [3], which may correlate with cancer mortality rates [4]. Since Cd<sup>2+</sup> is similar to Ca<sup>2+</sup>, Cd<sup>2+</sup> may interfere with different kinds

of Ca<sup>2+</sup>-dependent metabolic or developmental processes [5, 6] and induce widespread misfolding and aggregation of nascent proteins [7], thus highly intoxicating all kinds of lives [1].

Toxicity of Cd<sup>2+</sup> to animals and plants has been well studied. In animals, Cd<sup>2+</sup> was greatly accumulated in kidneys and livers/hepatopancreas, which then caused pathological disturbances, triggered lipid peroxidation and oxidative stress [1, 8], and suppressed immunity functions [9]. Besides, Cd<sup>2+</sup> displayed genotoxicity to animals, including the calanoid copepod *Acartia tonsa*, the decapod shrimp *Palaemon varians* and the pleuronectiform fish *Solea senegalensis* [10]. In response to Cd<sup>2+</sup> exposure, animals may increase the expression level of transferrin, which can interact with

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and detoxicate Cd<sup>2+</sup> to livers [11]. In plants, exposure to Cd<sup>2+</sup> inhibited cell division [12], seed germination and seedling growth [13], declined photosynthesis activity, chlorophyll content and the Calvin cycle [6, 14, 15], damaged chloroplast [16, 17] and induced genetic toxicity [18].

Algae are primary producers in aquatic ecosystems. Damage to algae cells and bioaccumulation of heavy metals in algae will negatively influence higher trophic levels and finally endanger human beings [19]. Although algae had bioremediation potential in Cd<sup>2+</sup>-polluted areas [20], Cd<sup>2+</sup> revealed obvious toxicity to algae in most cases. As previously reported, 7 mg/L Cd<sup>2+</sup> significantly reduced contents of photosynthetic pigments and induced oxidative stress in *Chlorella vulgaris* [21]. Transcriptome and metabolome studies revealed that exposure to 12.90 mg/L Cd<sup>2+</sup> induced oxidative stress in the freshwater alga *Chlamydomonas reinhardtii* [22]. Similar physiological results were revealed in *Desmodesmus armatus* when treated with 10.45 mg/L Cd<sup>2+</sup> [23]. At higher concentrations of Cd<sup>2+</sup>, more severe effects were observed. For example, treatment with 67.45 mg/L Cd<sup>2+</sup> induced stronger cellular toxic impacts on *Chlamydomonas reinhardtii*, such as inhibition of cellular division and photosynthesis, increase of cell size and cellular granularity [24]. These studies have investigated the effects of Cd<sup>2+</sup> at levels above 5 mg/L. However, in most Cd<sup>2+</sup>-polluted waterbodies, environmental concentrations of Cd<sup>2+</sup> were generally below 0.50 mg/L [25]. These investigations of high concentrations of Cd<sup>2+</sup> could not comprehensively reveal environmental risks of Cd<sup>2+</sup> pollution in nature.

In the present study, the effects of low concentrations of Cd<sup>2+</sup> (0.05 to 0.20 mg/L) on chlorophyll fluorescent parameters in *Scenedesmus quadricauda*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Nitzschia palea*, *Selenastrum minutum* and *Scenedesmus acutus* were investigated, which might reveal the early toxicity of Cd<sup>2+</sup> to green algae. Besides, changes of growth indices were also monitored to compare their sensitivity to low concentration of Cd<sup>2+</sup> with chlorophyll fluorescent parameters. These results would be useful to evaluate risks of trace Cd<sup>2+</sup> to aquatic environments.

## Materials and Methods

*S. quadricauda*, *C. pyrenoidosa*, *S. obliquus*, *N. palea*, *S. minutum* and *S. acutus* were isolated from the Xin'an River in Huangshan City, P. R. China. The composition of water quality in the sampling area varied among seasons. The NH<sub>3</sub>-N concentrations were 0.30±0.03, 0.16±0.02, 0.14±0.08 and 0.60±0.10 mg/L, the total phosphorus (TP) content was 0.064±0.003, 0.033±0.001, 0.029±0.009 and 0.068±0.002 mg/L, and the dissolved oxygen (DO) content was 5.83, 8.12, 8.81 and 8.70 mg/L during spring, summer, autumn and winter, respectively. To the best of our knowledge, no

obvious pollution took place in this area. The ordinary heavy metal pollutants (such as Cu, Cr, Cd, Pb and Zn) were not detectable in this area.

Stock alga culture solution was maintained in 500 ml flasks in a light incubator with light cycle of 12:12 h and light intensity of approximately 6,000 lux. The culture temperature was 25±1°C, and BG-11 was used as the culture medium [27]. To suspend alga cells, flasks were shaken manually three times per day.

Four concentrations of Cd<sup>2+</sup>, including 0.05, 0.10, 0.15 and 0.20 mg/L, were prepared by dissolving CdCl<sub>2</sub> (analytic grade) in BG-11 medium. BG-11 medium without the addition of Cd<sup>2+</sup> was used as the control. Algae at the exponential growth stage were used for Cd<sup>2+</sup> treatments. Alga density was determined using a hemocytometer. The initial alga density was adjusted to 1×10<sup>5</sup> cells/mL and then cultured as described above. Alga density was monitored every 24 hours for overall 10 days (8 days for *C. pyrenoidosa*) in order to calculate population growth rate. Each assay was repeated three times independently.

On the fifth day, 100 ml of alga solution was collected from each treatment. After being placed in the dark for 15 min, chlorophyll fluorescence parameters – including maximal photochemical efficiency of PS II (F<sub>v</sub>/F<sub>m</sub>), actual photochemical efficiency of PS II (Yield), maximal relative electron transport rate (rETR<sub>max</sub>), electron transport efficiency (α) and half-saturation light intensity (I<sub>k</sub>) – were determined using a phytoplankton fluorescence instrument (phyto-PAM, Walz-Germany).

Statistical analyses were performed using SPSS 19.0. After homogeneity of variance tests, effects of Cd<sup>2+</sup> on each parameter were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD).

## Results

### Effects of Cd<sup>2+</sup> on Algae Growth

Along with increasing culture time, cell density of all alga species (except *C. pyrenoidosa*) increased in all treatments and the control. Cell density of *C. pyrenoidosa* declined in most treatments and the control at day 8, suggesting that alga populations might be aged (Fig. S1).

There was no significant difference in growth curve of *C. pyrenoidosa*, *N. palea* and *S. acutus* among Cd<sup>2+</sup> treatments and the control. Growth curve of *S. quadricauda*, *S. minutum* and *S. obliquus* seemed a little higher in the control in comparison to Cd<sup>2+</sup> treatments (Fig. S1).

One-way analysis of variance revealed that Cd<sup>2+</sup> did not significantly affect population growth rate of *S. quadricauda*, *C. pyrenoidosa*, *S. obliquus*, *N. palea*, *S. minutum* and *S. acutus* (Fig. S2).

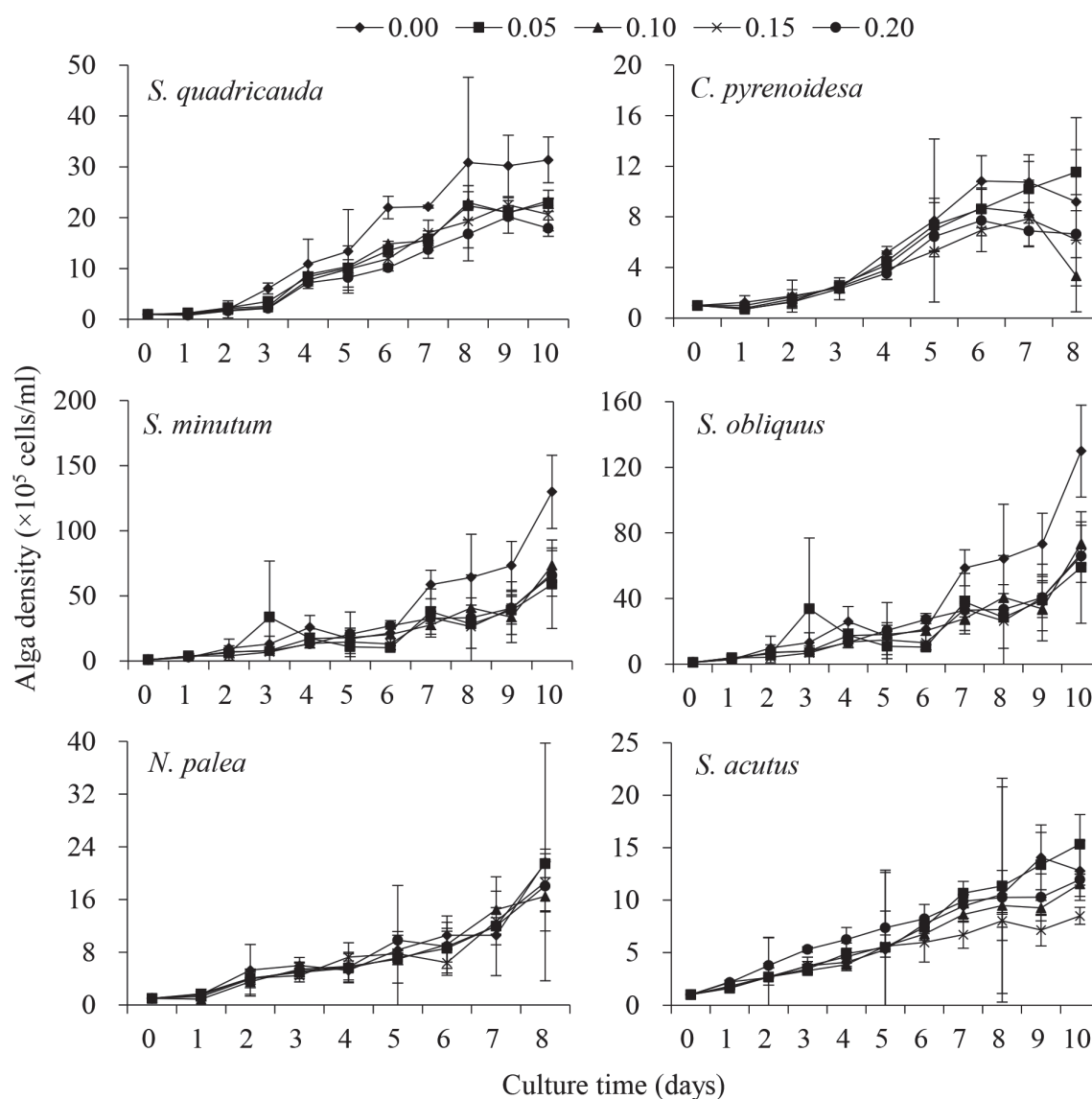


Fig. S1. Growth curves of *S. quadricauda*, *C. pyrenoidesa*, *S. obliquus*, *N. palea*, *S. minutum* and *S. acutus* in treatments with 0.05, 0.10, 0.15 and 0.20 mg/L Cd<sup>2+</sup>. Data represent mean±SD (n = 3).

#### Effects of Cd<sup>2+</sup> on Chlorophyll Fluorescent Parameters

Compared with the control, treatments with 0.05-0.20 mg/L Cd<sup>2+</sup> significantly increased  $F_v/F_m$  of *N. palea* and *S. minutum*, but decreased  $F_v/F_m$  of *S. obliquus* and *S. acutus*. No significant difference in  $F_v/F_m$  of *C. pyrenoidesa* was detected among Cd<sup>2+</sup> treatments and the control. Treatments with 0.15-0.20 mg/L Cd<sup>2+</sup> significantly reduced  $F_v/F_m$  of *S. quadricauda* (Fig. 1). For all alga species, the changes of yield among Cd<sup>2+</sup> treatments and the control were exactly identical to those of  $F_v/F_m$  (Fig. 2, Table S1).

In comparison to the control, treatments with 0.05-0.2 mg/L Cd<sup>2+</sup> significantly reduced  $rETR_{max}$  of *S. obliquus* and *C. pyrenoidesa*, but elevated those in *N. palea* and *S. minutum*. Treatments with

0.10-0.20 mg/L Cd<sup>2+</sup> significantly reduced  $rETR_{max}$  of *S. acutus*. Similar results were revealed in *S. quadricauda* exposed to 0.15-0.20 mg/L Cd<sup>2+</sup> (Fig. 3, Table S1).

All Cd<sup>2+</sup> treatments significantly decreased  $\alpha$  in *S. obliquus* and *S. acutus*, but did not affect  $\alpha$  in *N. palea* and *C. pyrenoidesa*. Treatments with 0.15-0.20 mg/L Cd<sup>2+</sup> significantly reduced  $\alpha$  of *S. quadricauda* and treatments with 0.10-0.20 mg/L Cd<sup>2+</sup> significantly increased  $\alpha$  of *S. minutum* (Fig. 4, Table S1).

In response to Cd<sup>2+</sup> treatments,  $I_k$  significantly decreased in *S. obliquus*, increased in *S. minutum*, but did not change in *N. palea*. When exposed to 0.10-0.20 mg/L Cd<sup>2+</sup>,  $I_k$  was significantly reduced in *S. acutus*, *C. pyrenoidesa* and *S. quadricauda* (Fig. 5, Table S1).

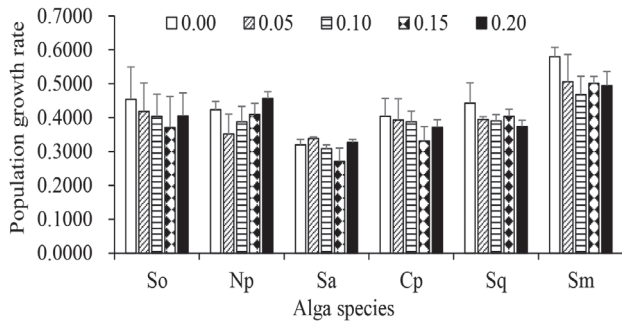


Fig. S2. Effects of Cd<sup>2+</sup> (mg/L) on population growth rate of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. No significant differences were detected among Cd<sup>2+</sup> treatments and control within the same species.

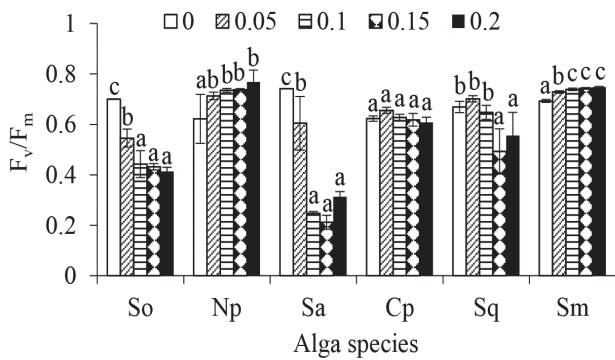


Fig. 1. Effects of Cd<sup>2+</sup> (mg/L) on maximal photochemical efficiency of PS II (F<sub>v</sub>/F<sub>m</sub>) of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. Different letters above bars represent significant differences among treatments with Cd<sup>2+</sup> and the control within the same species.

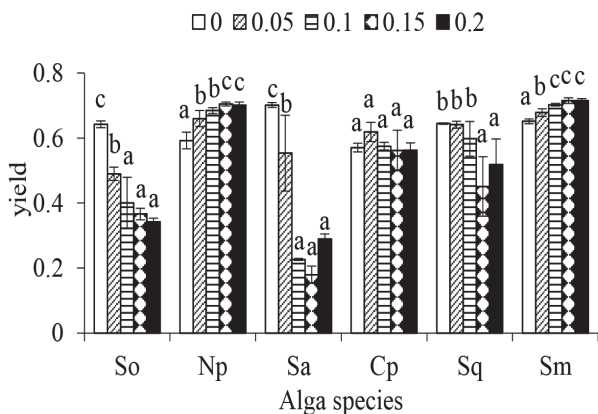


Fig. 2. Effects of Cd<sup>2+</sup> (mg/L) on actual photochemical efficiency of PS II (yield) of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. Different letters above bars represent significant differences among treatments with Cd<sup>2+</sup> and the control within the same species.

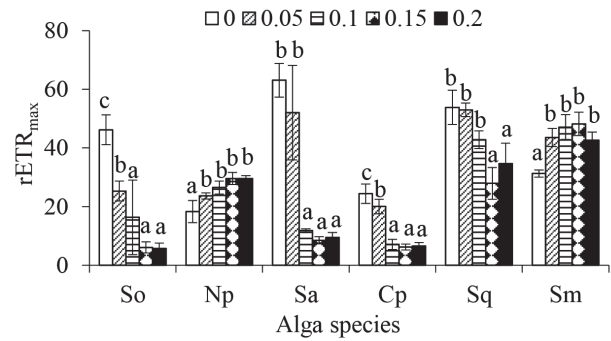


Fig. 3. Effects of Cd<sup>2+</sup> (mg/L) on maximal relative electron transport rate (rETR<sub>max</sub>) of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. Different letters above bars represent significant differences among treatments with Cd<sup>2+</sup> and the control within the same species.

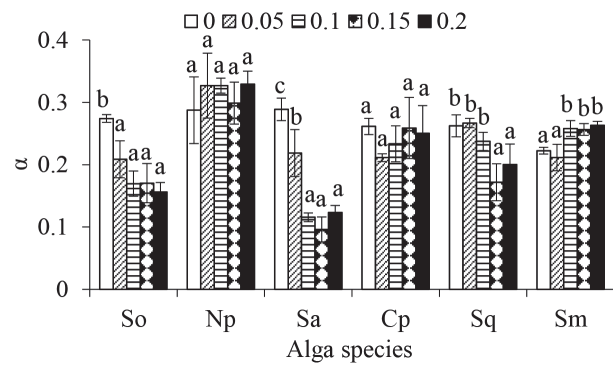


Fig. 4. Effects of Cd<sup>2+</sup> (mg/L) on electron transport efficiency (α) of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. Different letters above bars represent significant differences among treatments with Cd<sup>2+</sup> and the control within the same species.

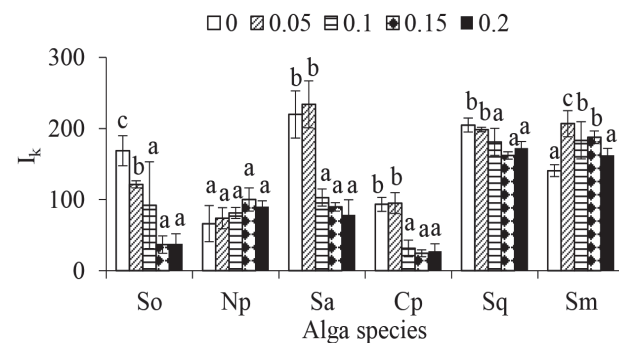


Fig. 5. Effects of Cd<sup>2+</sup> (mg/L) on half-saturation light intensity (I<sub>k</sub>) of green algae (mean±SD, n = 3). Sq: *S. quadricauda*. Cp: *C. pyrenoidosa*. So: *S. obliquus*. Np: *N. palea*. Sm: *S. minutum*. Sa: *S. acutus*. Different letters above bars represent significant differences among treatments with Cd<sup>2+</sup> and the control within the same species.

Table S1. Effects of Cd<sup>2+</sup> on chlorophyll fluorescent parameters in *S. obliquus*, *N. palea*, *S. acutus*, *C. pyrenoidosa*, *S. quadricauda* and *S. minutum* (mean  $\pm$  standard deviation). Different letters represent significant difference among treatments with Cd<sup>2+</sup> and the control within the same species.

Cd <sup>2+</sup> (mg/L)	Fv/Fm	Yield	rETRmax	$\alpha$	Ik
<i>S. obliquus</i>					
0.00	0.70 $\pm$ 0.00 <sup>c</sup>	0.64 $\pm$ 0.01 <sup>c</sup>	46.19 $\pm$ 5.07 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>b</sup>	168.73 $\pm$ 21.21 <sup>c</sup>
0.05	0.55 $\pm$ 0.04 <sup>b</sup>	0.49 $\pm$ 0.02 <sup>b</sup>	25.30 $\pm$ 3.41 <sup>b</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	121.41 $\pm$ 4.88 <sup>b</sup>
0.10	0.44 $\pm$ 0.05 <sup>a</sup>	0.40 $\pm$ 0.08 <sup>a</sup>	16.37 $\pm$ 12.71 <sup>a</sup>	0.17 $\pm$ 0.02 <sup>a</sup>	92.05 $\pm$ 61.02 <sup>a</sup>
0.15	0.43 $\pm$ 0.01 <sup>a</sup>	0.37 $\pm$ 0.02 <sup>a</sup>	6.10 $\pm$ 1.88 <sup>a</sup>	0.17 $\pm$ 0.03 <sup>a</sup>	36.75 $\pm$ 12.27 <sup>a</sup>
0.20	0.41 $\pm$ 0.02 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>a</sup>	5.78 $\pm$ 1.78 <sup>a</sup>	0.16 $\pm$ 0.02 <sup>a</sup>	37.81 $\pm$ 14.27 <sup>a</sup>
<i>N. palea</i>					
0.00	0.62 $\pm$ 0.10 <sup>a</sup>	0.59 $\pm$ 0.03 <sup>a</sup>	18.34 $\pm$ 3.79 <sup>a</sup>	0.29 $\pm$ 0.05 <sup>a</sup>	66.16 $\pm$ 25.48 <sup>a</sup>
0.05	0.71 $\pm$ 0.01 <sup>b</sup>	0.66 $\pm$ 0.02 <sup>b</sup>	23.65 $\pm$ 1.06 <sup>b</sup>	0.33 $\pm$ 0.05 <sup>a</sup>	73.90 $\pm$ 14.80 <sup>a</sup>
0.10	0.74 $\pm$ 0.01 <sup>b</sup>	0.68 $\pm$ 0.01 <sup>c</sup>	26.52 $\pm$ 2.22 <sup>b</sup>	0.33 $\pm$ 0.01 <sup>a</sup>	81.26 $\pm$ 7.73 <sup>a</sup>
0.15	0.74 $\pm$ 0.00 <sup>b</sup>	0.70 $\pm$ 0.01 <sup>c</sup>	29.59 $\pm$ 2.02 <sup>b</sup>	0.30 $\pm$ 0.03 <sup>a</sup>	100.15 $\pm$ 16.25 <sup>a</sup>
0.20	0.77 $\pm$ 0.05 <sup>b</sup>	0.70 $\pm$ 0.01 <sup>c</sup>	29.60 $\pm$ 0.98 <sup>b</sup>	0.33 $\pm$ 0.02 <sup>a</sup>	90.21 $\pm$ 8.08 <sup>a</sup>
<i>S. acutus</i>					
0.00	0.74 $\pm$ 0.00 <sup>c</sup>	0.70 $\pm$ 0.01 <sup>c</sup>	63.09 $\pm$ 5.73 <sup>b</sup>	0.29 $\pm$ 0.02 <sup>c</sup>	219.82 $\pm$ 33.22 <sup>b</sup>
0.05	0.60 $\pm$ 0.11 <sup>b</sup>	0.55 $\pm$ 0.12 <sup>b</sup>	52.01 $\pm$ 16.09 <sup>b</sup>	0.22 $\pm$ 0.04 <sup>b</sup>	234.02 $\pm$ 32.90 <sup>b</sup>
0.10	0.25 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.00 <sup>a</sup>	11.84 $\pm$ 0.63 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	102.89 $\pm$ 11.89 <sup>a</sup>
0.15	0.21 $\pm$ 0.03 <sup>a</sup>	0.18 $\pm$ 0.03 <sup>a</sup>	8.54 $\pm$ 1.21 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>a</sup>	89.64 $\pm$ 6.00 <sup>a</sup>
0.20	0.31 $\pm$ 0.02 <sup>a</sup>	0.29 $\pm$ 0.02 <sup>a</sup>	9.53 $\pm$ 1.70 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	78.53 $\pm$ 21.33 <sup>a</sup>
<i>C. pyrenoidosa</i>					
0.00	0.62 $\pm$ 0.01 <sup>a</sup>	0.57 $\pm$ 0.01 <sup>a</sup>	24.43 $\pm$ 3.31 <sup>c</sup>	0.26 $\pm$ 0.01 <sup>a</sup>	93.35 $\pm$ 9.85 <sup>b</sup>
0.05	0.66 $\pm$ 0.01 <sup>a</sup>	0.62 $\pm$ 0.03 <sup>a</sup>	20.05 $\pm$ 2.47 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>a</sup>	95.12 $\pm$ 14.67 <sup>b</sup>
0.10	0.63 $\pm$ 0.01 <sup>a</sup>	0.58 $\pm$ 0.01 <sup>a</sup>	7.12 $\pm$ 1.75 <sup>a</sup>	0.23 $\pm$ 0.03 <sup>a</sup>	31.37 $\pm$ 11.63 <sup>a</sup>
0.15	0.62 $\pm$ 0.03 <sup>a</sup>	0.56 $\pm$ 0.06 <sup>a</sup>	6.25 $\pm$ 1.00 <sup>a</sup>	0.26 $\pm$ 0.05 <sup>a</sup>	24.61 $\pm$ 4.77 <sup>a</sup>
0.20	0.61 $\pm$ 0.02 <sup>a</sup>	0.56 $\pm$ 0.02 <sup>a</sup>	6.55 $\pm$ 1.24 <sup>a</sup>	0.25 $\pm$ 0.04 <sup>a</sup>	27.34 $\pm$ 10.39 <sup>a</sup>
<i>S. quadricauda</i>					
0.00	0.67 $\pm$ 0.02 <sup>b</sup>	0.64 $\pm$ 0.00 <sup>b</sup>	53.83 $\pm$ 5.83 <sup>b</sup>	0.26 $\pm$ 0.02 <sup>b</sup>	204.81 $\pm$ 9.75 <sup>b</sup>
0.05	0.70 $\pm$ 0.01 <sup>b</sup>	0.64 $\pm$ 0.01 <sup>b</sup>	52.99 $\pm$ 2.29 <sup>b</sup>	0.27 $\pm$ 0.01 <sup>b</sup>	198.56 $\pm$ 3.06 <sup>b</sup>
0.10	0.65 $\pm$ 0.03 <sup>b</sup>	0.60 $\pm$ 0.05 <sup>b</sup>	42.82 $\pm$ 3.03 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>b</sup>	181.01 $\pm$ 19.15 <sup>a</sup>
0.15	0.49 $\pm$ 0.09 <sup>a</sup>	0.45 $\pm$ 0.09 <sup>a</sup>	27.93 $\pm$ 5.42 <sup>a</sup>	0.17 $\pm$ 0.03 <sup>a</sup>	162.01 $\pm$ 5.17 <sup>a</sup>
0.20	0.56 $\pm$ 0.09 <sup>a</sup>	0.52 $\pm$ 0.08 <sup>a</sup>	34.71 $\pm$ 6.96 <sup>a</sup>	0.20 $\pm$ 0.03 <sup>a</sup>	172.60 $\pm$ 9.04 <sup>a</sup>
<i>S. minutum</i>					
0.00	0.69 $\pm$ 0.01 <sup>a</sup>	0.65 $\pm$ 0.01 <sup>a</sup>	31.28 $\pm$ 1.25 <sup>a</sup>	0.22 $\pm$ 0.01 <sup>a</sup>	140.72 $\pm$ 8.20 <sup>a</sup>
0.05	0.73 $\pm$ 0.01 <sup>b</sup>	0.68 $\pm$ 0.01 <sup>b</sup>	43.55 $\pm$ 3.09 <sup>b</sup>	0.21 $\pm$ 0.02 <sup>a</sup>	206.74 $\pm$ 18.33 <sup>c</sup>
0.10	0.74 $\pm$ 0.00 <sup>c</sup>	0.70 $\pm$ 0.00 <sup>c</sup>	47.10 $\pm$ 4.25 <sup>b</sup>	0.26 $\pm$ 0.01 <sup>b</sup>	183.32 $\pm$ 26.07 <sup>b</sup>
0.15	0.74 $\pm$ 0.00 <sup>c</sup>	0.72 $\pm$ 0.01 <sup>c</sup>	48.20 $\pm$ 3.98 <sup>b</sup>	0.26 $\pm$ 0.01 <sup>b</sup>	187.73 $\pm$ 8.84 <sup>b</sup>
0.20	0.75 $\pm$ 0.00 <sup>c</sup>	0.72 $\pm$ 0.01 <sup>c</sup>	42.66 $\pm$ 2.77 <sup>b</sup>	0.26 $\pm$ 0.01 <sup>b</sup>	162.24 $\pm$ 9.92 <sup>a</sup>

## Discussion

Toxicity of pollutants to green algae varied among species [28] and strains [29]. In the present study, based on locally collected alga strains, the as-obtained toxic assessment results should be more accurate to evaluate environmental risk of Cd<sup>2+</sup> to the local environments [29]. Branco et al. [30] revealed that IC<sub>50</sub> of Cd<sup>2+</sup> to *N. palea* was 0.0276 mg/L. Zhou, and Xiang [31] suggested that 0.001 mg/L significantly reduced growth of *S. minutum*. However, treatment with up to 0.20 mg/L Cd<sup>2+</sup> did not affect the population growth rate of *N. palea* and *S. minutum* in the present study, suggesting that these two species from the Xin'an river might be more tolerant to Cd<sup>2+</sup> than those used in Branco et al. [30] and Zhou and Xiang [31].

As previously reported, treatments with 6.74 mg/L, 0.72 mg/L, 1 mg/L and 0.51 mg/L Cd<sup>2+</sup> suppressed growth of *S. quadricauda* [32], *C. pyrenoidosa* [33], *S. obliquus* [34] and *S. acutus* [35], respectively. In the present study, treatments with 0.20 mg/L Cd<sup>2+</sup> did not affect the population growth rate of these species, which might be due to the low level of Cd<sup>2+</sup>. However, chlorophyll fluorescent parameters were more or less affected by treatments with 0.05-0.20 mg/L Cd<sup>2+</sup>, suggesting that chlorophyll fluorescent parameters were more sensitive to Cd<sup>2+</sup> pollution compared with growth parameters. These results were consistent with the previous viewpoint that photosystem II is one of the most sensitive indices to environmental stress [26]. Thus, we proposed that chlorophyll fluorescent parameters might be used as biomarkers to monitor early toxicity of Cd<sup>2+</sup> in green algae.

Cd<sup>2+</sup> has been reported to inhibit chlorophyll fluorescent parameters of *Solanum melongena* [36], *Robinia pseudoacacia* [37] and *Arabidopsis thaliana* [38]. In response to Cd<sup>2+</sup> treatments, F<sub>v</sub>/F<sub>m</sub>, yield,  $\alpha$  and I<sub>k</sub> all significantly decreased in *S. obliquus*, *S. acutus* and *S. quadricauda*, suggesting that the whole photosynthetic system was damaged by Cd<sup>2+</sup> in these species. However, all these indices were not significantly reduced by Cd<sup>2+</sup> in *N. palea* and *S. minutum*, indicating that those two species might be more tolerant to Cd<sup>2+</sup> pollution than *S. obliquus*, *S. acutus* and *S. quadricauda*.

Moreover, in *N. palea* and *S. minutum*, treatment with 0.05 mg/L Cd<sup>2+</sup> even increased F<sub>v</sub>/F<sub>m</sub>, yield and rETR<sub>max</sub>, compared with the control (Table S1). Two possibilities might explain these results. First, as reported in *Juncus acutus*, treatment with Cd<sup>2+</sup> inhibited the electron transport rate and led to the accumulation of extra energy in chlorophylls. The accumulated energy was then dissipated in the forms of heat and/or fluorescence, displaying enhanced fluorescent intensity, which might affect the determination of chlorophyll fluorescent parameters [39]. Second, treatments with low concentrations of Cd<sup>2+</sup> have been reported to increase chlorophyll content in *Lolium multiflorum* [40] and *Lactuca sativa* [41]. Accumulation of chlorophyll should

also increase the chlorophyll fluorescent parameters. However, more investigations are still required to validate these hypotheses in *N. palea* and *S. minutum*.

In response to Cd<sup>2+</sup> treatments, I<sub>k</sub> and rETR<sub>max</sub> decreased significantly in *C. pyrenoidosa*, but  $\alpha$ , F<sub>v</sub>/F<sub>m</sub> and yield were not affected. rETR<sub>max</sub> represents the maximal relative electron transport rate [42]. A decrease of rETR<sub>max</sub> suggested that electron transport to the PS II reaction center was blocked. The subsequent accumulation of electrons should be harmful to alga cells and trigger photoinhibition, reducing I<sub>k</sub> simultaneously. Both F<sub>v</sub>/F<sub>m</sub> and yield represent photosynthetic ability. F<sub>v</sub>/F<sub>m</sub> and yield did not change under Cd<sup>2+</sup> stress in *C. pyrenoidosa*, suggesting that photoinhibition might not occur. Electron transport efficacy  $\alpha$  remained stable to Cd<sup>2+</sup> exposure, which might make up for the reduction of rETR<sub>max</sub>.

The Chinese Environmental Quality Standard for Surface Water (GB3838-2002) stipulates that the maximum limit concentration of Cd is 0.001 mg/L, 0.005 mg/L and 0.01 mg/L in Class I, Class II-IV and Class V surface water, respectively [43]. The criterion of maximum concentration (CMC) and continuous concentration (CCC) of Cd in the freshwater ecosystem was 0.4  $\mu$ g/L and 0.2  $\mu$ g/L in the United States of America, respectively [44]. The criterion of Cd in fresh ecosystems in Canada [45], Malaysia (Class II) [46] and Europe [47] were 0.008  $\mu$ g/L, 10  $\mu$ g/L and <0.07  $\mu$ g/L, respectively. In the present study, treatment with 0.05 mg/L Cd significantly inhibited one or more chlorophyll fluorescent parameters in *S. obliquus*, *S. acutus* and *C. pyrenoidosa*. Considering that the safe concentration of pollutants should be 10 times lower than the no-observed-effect concentration (NOEC) [45], the criterion of Cd in surface water should be lower than 0.005 mg/L. Thus, the concentration of Cd in the Chinese Environmental Quality Standard for Surface Water should be re-evaluated.

## Conclusions

Treatments with up to 0.20 mg/L Cd<sup>2+</sup> did not significantly affect population growth of *S. quadricauda*, *C. pyrenoidosa*, *S. obliquus*, *N. palea*, *S. minutum* and *S. acutus*. However, exposure to 0.05-0.20 mg/L Cd<sup>2+</sup> significantly reduced chlorophyll fluorescent parameters of *S. obliquus*, *S. acutus* and *S. quadricauda*, but not of *N. palea* and *S. minutum*. *N. palea*, *S. minutum* and *C. pyrenoidosa* were more tolerant to Cd<sup>2+</sup> than *S. obliquus*, *S. acutus* and *S. quadricauda*.

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### Conflict of Interest

There is no conflict of interest in this research.

### References

- MOISEENKO T.I., GASHKINA N.A. Biogeochemistry of cadmium: anthropogenic dispersion, bioaccumulation, and ecotoxicity. *Geochem. Int.* **56** (8), 798, **2018**.
- ZHU D., WU L., CHRISTIE P., KE X., LUO Y., LI Z. Ecotoxicity of cadmium in a soil collembolan-predatory mite food chain: can we use the <sup>15</sup>N labeled litter addition method to assess soil functional change. *Environ. Pollut.* **219**, 37, **2016**.
- HUANG Y., HE C., SHEN C., GUO J., YANG Z. Toxicity of cadmium and its health risks from leafy vegetable consumption. *Food Funct.* **8** (4), 1373, **2017**.
- SATARUG S., GARRETT S.H., SENS M.A., SENS D.A. Cadmium, environmental exposure, and health outcomes. *Cien. Saude. Colet.* **16** (5), 2587, **2010**.
- ANDOSCH A., AFFENZELLER M.J., LÜTZ C., LÜTZ-MEINDL U. A freshwater green alga under cadmium stress: ameliorating calcium effects on ultrastructure and photosynthesis in the unicellular model *Micrasterias*. *J. Plant Physiol.* **169** (15), 1489, **2012**.
- LAVOIE M., CAMPBELL P.G., FORTIN C. Predicting cadmium accumulation and toxicity in a green alga in the presence of varying essential element concentrations using a biotic ligand model. *Environ. Sci. Technol.* **48** (2), 1222, **2014**.
- TAMÁS M.J., FAUVET B., CHRISTEN P., GOLOUBINOFF P. Misfolding and aggregation of nascent proteins: a novel mode of toxic cadmium action in vivo. *Curr. Genet.* **64** (1), 177, **2018**.
- MOSBAH A., GUERBEJ H., BOUSSETTA H., BOURAOUI Z., BANNI M. Protective effects of dietary garlic powder against cadmium-induced toxicity in sea bass liver: a chemical, biochemical, and transcriptomic approach. *Biol. Trace Elem. Res.* **183** (2), 370, **2018**.
- SUN M., TING LI Y., LIU Y., CHIN LEE S., WANG L. Transcriptome assembly and expression profiling of molecular responses to cadmium toxicity in hepatopancreas of the freshwater crab *Sinopotamon henanense*. *Sci. Rep.* **6**, 19405, **2016**.
- PAVLAKI M.D., ARAÚJO M.J., CARDOSO D.N., SILVA A.R.R., CRUZ A., MENDO S., SOARES A.M.V.M., CALADO R., LOUREIRO S. Ecotoxicity and genotoxicity of cadmium in different marine trophic levels. *Environ. Pollut.* **215**, 203, **2016**.
- WANG J., WANG J., SONG W., YANG X., ZONG W., LIU R. Molecular mechanism investigation of the neutralization of cadmium toxicity by transferrin. *Phys. Chem. Chem. Phys.* **18** (5), 3536, **2016**.
- KÜPPER H., LOMBI E., ZHAO F.J., MCGRATH S.P. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta.* **212** (1), 75, **2000**.
- WU S., SHEN C., YANG Z., LIN B., YUAN J. Tolerance of *Ricinus communis* L. to Cd and screening of high Cd accumulation varieties for remediation of Cd contaminated soils. *Int. J. Phytoremediation.* **18** (11), 1148, **2016**.
- VAN ASSCHE F., CLIJSTERS H. Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **13** (3), 195, **1990**.
- CHANG Y.S., CHANG Y.J., LIN C.T., LEE M.C., WU C.W., LAI Y.H. Nitrogen fertilization promotes the phytoremediation of cadmium in *Pentas lanceolata*. *Int. Biodeter. Biodegr.* **85**, 709, **2013**.
- KÜPPER H., PARAMESWARAN A., LEITENMAIER B., TRTÍLEK M., SETLÍK I. 2007. Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* **175** (4), 655, **2007**.
- HE S., YANG X., HE Z., BALIGAR V.C. Morphological and physiological responses of plants to cadmium toxicity: a review. *Pedosphere*, **27** (3), 421, **2017**.
- KRANNER I., COLVILLE L. Metals and seeds: Biochemical and molecular implications and their significance for seed germination. *Environ. Exp. Bot.* **72** (1), 93, **2011**.
- BISHOP W.M., WILLIS B.E., RICHARDSON R.J., COPE W.G. The presence of algae mitigates the toxicity of copper-based algacides to a nontarget organism. *Environ. Toxicol. Chem.* **37** (8), 2132, **2018**.
- ZHANG H.M., GENG G., WANG J.J., XIN Y., ZHANG Q., CAO D.J., MA Y.H. The remediation potential and kinetics of cadmium in the green alga *Cladophora rupestris*. *Environ. Sci. Pollut. Res. Int.* **26** (1), 775, **2019**.
- CHENG J., QIU H., CHANG Z., JIANG Z., YIN W. The effect of cadmium on the growth and antioxidant response for freshwater algae *Chlorella vulgaris*. SpringerPlus, **5** (1), 1290, **2016**.
- JAMERS A., BLUST R., DE COEN W., GRIFFIN J.L., JONES O.A.H. An omics based assessment of cadmium toxicity in the green alga *Chlamydomonas reinhardtii*. *Aquat. Toxicol.* **126**, 355, **2013**.
- POKORA W., BAŚCIK-REMISIEWICZ A., TUKAJ S., KALINOWSKA R., PAWLIK-SKOWROŃSKA B., DZIADZIUSZKO M., TUKAJ Z. Adaptation strategies of two closely related *Desmodesmus armatus* (green alga) strains contained different amounts of cadmium: a study with light-induced synchronized cultures of algae. *J. Plant Physiol.* **171** (2), 69, **2014**.
- SAMADANI M., PERREAULT F., OUKARROUM A., DEWEZ D. Effect of cadmium accumulation on green algae *Chlamydomonas reinhardtii* and acid-tolerant *Chlamydomonas* cpcc 121. *Chemosphere*, **191**, 174, **2018**.
- VYMAZAL J. Toxicity and accumulation of cadmium with respect to algae and cyanobacteria: a review. *Environ. Toxicol. Water Qual.* **2** (4), 387, **1987**.
- PLEKHANOV S.E., CHEMERIS Y.K. Early toxic effect of zinc, cobalt, and cadmium on photosynthetic activity of green alga *Chlorella pyrenoidosa* chick s-39. *Izv. Akad. Nauk Ser. Biol.* **30** (5), 610, **2003**.
- 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**.
- 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**.
- 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**.
30. BRANCO D., LIMA A., ALMEIDA S.F., FIGUEIRA E. Sensitivity of biochemical markers to evaluate cadmium stress in the freshwater diatom *Nitzschia palea* (Kützing) W. Smith. *Aquatic Toxicology*, **99** (2), 109, **2010**.
  31. ZHOU H., XIANG S. The effect of copper, zinc, lead, cadmium on the growth and the ultrastructure of *Selenastrum minutum*. *Journal of Zhejiang University*, **25** (2), 85, **1998**.
  32. BIŠOVÁ K., HENDRYCHOVÁ J., CEPÁK V., ZACHLEDER V. Cell growth and division processes are differentially sensitive to cadmium in *Scenedesmus quadricauda*. *Folia Microbiol.* **48** (6), 805, **2003**.
  33. NUGROHO A.P., HANDAYANI N.S.N., PRAMUDITA I.G.A. Combined effects of copper and cadmium on *Chlorella pyrenoidosa* H. Chick: subcellular accumulation, distribution, and growth inhibition. *Toxicol. Environ. Chem.* **99** (9-10), 1368, **2017**.
  34. CAIN J.R., PASCHAL D.C., HAYDEN C.M. Toxicity and bioaccumulation of cadmium in the colonial green alga *Scenedesmus obliquus*. *Arch. Environ. Contam. Toxicol.* **9** (1), 9, **1980**.
  35. TORRICELLI E., GORBI G., PAWLIK-SKOWRONSKA B., DI TOPPI L.S., CORRADI M.G. Cadmium tolerance, cysteine and thiol peptide levels in wild type and chromium-tolerant strains of *Scenedesmus acutus* (chlorophyceae). *Aquat. Toxicol.* **68** (4), 315, **2004**.
  36. SINGH S., PRASAD S.M. Growth, photosynthesis and oxidative responses of *Solanum melongena* L. seedlings to cadmium stress: mechanism of toxicity amelioration by kinetin. *Sci. Hortic.-Amsterdam.* **176**, 1, **2004**.
  37. DEZHBAN A., SHIRVANY A., ATTAROD P., DELSHAD M., KHOSHNEVIS M. Cadmium and lead effects on chlorophyll fluorescence, chlorophyll pigments and proline of *Robinia pseudoacacia*. *J. For. Res.* **26** (2), 323, **2015**.
  38. MARTÍNEZ-PEÑALVER A., GRAÑA E., REIGOSA M.J., SÁNCHEZ-MOREIRAS A.M. The early response of *Arabidopsis thaliana* to cadmium- and copper-induced stress. *Environ. Exp. Bot.* **78**, 1, **2012**.
  39. SANTOS D., DUARTE B., CAÇADOR I. Biochemical and photochemical feedbacks of acute Cd toxicity in *Juncus acutus* seedlings: The role of non-functional Cd-chlorophylls. *Estuar. Coast. Shelf Sci.* **167**, 228, **2015**.
  40. SUN Y.Y., PING G., SHAN H., SHI J.M. Effects of Cd stress on Cd accumulation, physiological response and ultrastructure of *Lolium multiflorum*. *Pratacult. Sci.* **33** (8), 1589, **2016**.
  41. AZZI V., KANSO A., KAZPARD V., KOBEISSI A., LARTIGES B., SAMRANI A.E. *Lactuca sativa* growth in compacted and non-compacted semi-arid alkaline soil under phosphate fertilizer treatment and cadmium contamination. *Soil Till. Res.* **165**, 1, **2017**.
  42. ROLEDA M.Y. Photosynthetic response of Arctic kelp zoospores exposed to radiation and thermal stress. *Photochem. Photobiol. Sci.* **8** (9), 1302, **2009**.
  43. NATIONAL ENVIRONMENTAL PROTECTION BUREAU OF CHINA. National Standards of the People's Republic of China: Environmental Quality Standards for Surface Water. GB3838-2002. **2002**.
  44. UNITED STATE ENVIRONMENTAL PROTECTION AGENCY. National Recommended Water Quality Criteria. Office of Science and Technology, Washington, DC. **2005**.
  45. CANADIAN ENVIRONMENTAL QUALITY GUIDELINES. Canadian Council of Ministers of the Environment, Winnipeg, 61, **1999**.
  46. DEPARTMENT OF ENVIRONMENT. Malaysia Environment Quality Report 2009. Department of Environment, Ministry of Natural Resources and Environment, Malaysia, ISSN 0127-6433, 90, **2009**.
  47. UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE. Standard statistical classification of surface freshwater quality for the maintenance of aquatic life. In: Reading in International Environment Statistics. United Nations Economic Commission for Europe, United Nations, New York and Geneva. **1994**.