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Original Research

Effects of Cd^{2+} and Pb^{2+} on Growth and Photosynthesis of Two Freshwater Algae Species

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Abstract

Microalgae are biological indicators of heavy metal pollution. Cadmium (Cd) and lead (Pb) are extremely toxic metals to aquatic organisms. In the present study, single and combined toxicity of Cd^{2+} and Pb^{2+} to *Scenedesmus acutus* and *Schroederia* sp. collected from the famous Xin'an River (Huangshan City) were evaluated. Treatments with 0.5-2.0 mg/L Cd^{2+} significantly reduced *S. acutus* population growth, and treatment with 2.0 mg/L Cd^{2+} significantly decreased *Schroederia* sp. population growth rate, suggesting a higher tolerance of *Schroederia* sp. than *S. acutus* to Cd^{2+} pollution. In addition, Cd^{2+} treatments significantly decreased chlorophyll *a*, carotene contents, and photosynthetic fluorescent parameters $rETR_{max}$ and I_k, demonstrating that the harms on photosynthesis might be the underlying mechanism of Cd^{2+} toxicity to algae. Treatments with 5.0-15.0 mg/L Pb^{2+} did not significantly affected population growth and photosynthetic pigment content. However, combined exposure to Cd^{2+} and Pb^{2+} revealed antagonistic effects on both species. Overall, these results provide basic information to the ecological risk assessments of heavy metal pollution in the Xin'an River Basin.

Keywords: heavy metal toxicity, microalgae, growth, chlorophyll, photosynthesis

Introduction

Heavy metals are typical pollutants and their concentrations are aquatic and soil environments are increasing over the past decades, which are mainly released by human activities such as domestic waste-water, urban and agricultural run-off, and industrial sewage [1]. Due to their persistent, toxic and bioaccumulation characteristics, heavy metals seriously threat human health and environments [2]. Cadmium (Cd) and lead (Pb) are toxic metals and are considered non-essential for most living organisms [3, 4]. Cd^{2+} is a carcinogenic, mutagenic and endocrine disrupting factor [5]. It is a risk factor of lung damages and bone weakness, as well as affects the metabolism and regulation of calcium in organisms [6]. Pb^{2+} is very dangerous to living things and harms the human body system [7]. Although individual heavy metal pollution

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has been investigated, most of heavy metal pollutions are concomitant or joint in the aquatic environments under natural conditions [8]. The free ions of Cd^{2+} and Pb^{2+} often present in freshwater ecosystems simultaneously. Thus, it is necessary to investigate their combined effects to organisms.

Microalgae are not only the most important primary producers, but also the basis of the food webs in most aquatic ecosystems [9]. Due to their high population growth rate, short life cycle and strong heavy metal absorption capacity, microalgae are considered as a promising tool for heavy metal detection and bioremediation [10, 11]. Photosynthesis is the process that light energy is absorbed by light-harvesting complexes and transferred as excitation energy from water to nicotinamide adenine dinucleotide phosphate (NADPH) [12]. Chlorophyll fluorescent parameters are sensitive to changes of environmental factors and provide valuable information about the disruptive mechanisms of pollutions underlying alteration of photosynthesis process [13]. Therefore, in addition to population growth rate, chlorophyll fluorescent parameters can be used as bioindicators to monitor the effects of metal pollution on microalgae.

Huangshan city in China is a famous scenery (the Yellow Mountain) throughout the world. Guan et al. [14] reported that the urban and suburb areas of Huangshan City suffered high risks of Cd and Pb pollutions. Since different alga strains of the same species may vary in sensitivity to heavy metal pollution, it is also essential to evaluate the toxicity of pollutants using the local alga strains. Previously, our group has explored the effects of Cu^{2+} and Hg^{2+} on growth and photosynthesis of eight freshwater algae species collected from the Xin'an River, Huangshan city [15]. However, combined toxicity of Cd^{2+} and Pb^{2+} to the local alga strains have not been reported yet, which are important to evaluate the environmental risks of heavy metal pollutions to the Huangshan scenery. In the present study, we isolated two algae species *Scenedesmus acutus* and *Schroederia* sp. from the Xin'an River, Huangshan City. Next, we investigated the effects of Cd^{2+}, Pb^{2+} and their combined exposure on the growth the photosynthesis parameters of these two algae species. These results aimed to understand the combined toxicity of Cd^{2+} and Pb^{2+} to the local green algae in the Huangshan area, which are important for the ecological risk of assessment of heavy metal pollution in Xin'an River Basin.

Materials and Methods

Sample Collection

S. acutus and *Schroederia* sp. were isolated from Xin'an River, Huangshan City, P. R. China and then cultured in 500 mL glass flasks containing 300 mL of BG-11 medium [16] at 25±1ºC. The composition of BG-11 medium consists of: (g/L) $NaNO_3$ (1.5), K_2HPO_4 (0.04), $MgSO_47H_2O$ (0.075), $CaCl_2H_2O$ (0.036), Na_2CO_3 (0.02), Citric acid (0.006), EDTA (0.001), and 1 mL of trace elements solution having the following composition (g/L): H_3BO_3 (2.86), $MnCl_24H_2O$ (1.81), $ZnSO_47H_2O$ (0.222), $NaMoO_42H_2O$ (0.39), $CuSO_45H_2O$ (0.079), $Co(NO_3)_26H_2O$ (0.0494). The photoperiod was 12 h: 12 h (light: dark) with light intensity of approximately 120 μmol photons $m^{-2}s^{-1}$. During the culture period, algae solutions were manually shaken for three times per day.

Exposure to Heavy Metals

$CdCl_2$ and $PbCl_2$ (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) were used for heavy metal exposure and the stock solutions were 1 g/L in distilled water. Single exposure to Cd^{2+} (0.50, 1.00 and 2.00 mg/L), Pb^{2+} (5.00, 10.00 and 15.00 mg/L) and combined exposure to Cd^{2+} + Pb^{2+} (0.25 mg/L Cd^{2+} + 2.50 mg/L Pb^{2+}, 0.50 mg/L Cd^{2+} + 5.00 mg/L Pb^{2+} and 1.00 mg/L Cd^{2+} + 7.50 mg/L Pb^{2+}) were conducted. Meanwhile, the control experiment was included, in which no Pb^{2+} and Cd^{2+} were added. Algae at the exponential growth phase were used for heavy metal exposure. Cell density was determined using a hemocytometer and then the initial alga density was adjusted to 1×10^5 cells/mL. The culture system was 500 mL glass flasks containing 300 mL of media. Each assay was repeated three times independently. Cell density was monitored every 24 hours for 4 days and population growth rate was calculated using the equation described by Patiño et al. [17].

Measurements of Chlorophyll Contents and Photosynthesis Fluorescence Parameters

For culture for four days, 150 mL of alga solution was filtered onto Whatman GF/F 0.22 μm pore-sized membranes using a vacuum filtration system. The harvested algae were homogenized in 5 mL of 95% methanol solution and then placed in dark at 4ºC for 12 hours to extract chlorophylls. After centrifugation at 3,000 rpm for 10 min, absorbance of supernatants at 480 nm, 510 nm, 635 nm, 652 nm, 665 nm, 668 nm and 750 nm was determined using a spectrophotometer (TU-1901, Beijing, China). Contents of chlorophyll *a* (chl-*a*), chlorophyll *c* (chl-*c*) and carotenoids(car) were calculated according to Parsons and Strickland [18].

After exposure for four days, 15 mL of alga solution was collected for measurements of photosynthesis fluorescence parameters. Before each measurement, the alga cells were adapted to darkness for 10 min to complete re-oxidize PSII electron acceptor molecules. Afterwards, Chl-*a* fluorescence was measured using a Phyto-PAM pulse amplitude modulated fluorometer (Walz, Germany). Maximal photochemical efficiency of PSII (F_v/F_m), actual photochemical efficiency of PSII (Yield), maximal relative electron transport rate

($rETR_{max}$), electron transport efficiency (α) and half-saturation light intensity (I_k) were calculated according to Dao and Beardall [19].

Data Analysis

All data were analyzed using SPSS 23.0. Effects of Cd^{2+} and Pb^{2+} on the tested parameters were evaluated using the one-way analysis of variance (ANOVA), followed by LSD multiple comparison. Two-way ANOVA was conducted in order to detect the interactive effects of Cd^{2+} and Pb^{2+} on the parameters. For all statistical tests, significant threshold of P values was 0.05.

Results

Effects of Cd^{2+} and Pb^{2+} on Algae Growth

Apart from treatments with 1.00 and 2.00 mg/L Cd^{2+}, alga densities in other treatments increased during the four days (Fig. S1). In single exposure to Cd^{2+}, population growth rates of *S. acutus* significantly decreased in all treatments (0.50-2.00 mg/L) compared to control (Fig. 1). Population growth rate of *Schroederia* sp. significantly increased at 0.5 mg/L Cd^{2+} and then decreased significantly at 2.00 mg/L Cd^{2+}. In single exposure to Pb^{2+}, all treatments (5.0-15.0 mg/L) did not significantly impact population growth rate in both two microalgae species. Combined Cd^{2+} and Pb^{2+} exposure (from 0.25 mg/L Cd^{2+} + 2.5 mg/L Pb^{2+} to 1.0 mg/L Cd^{2+} + 7.5 mg/L Pb^{2+}) showed no significant effects on population growth rate of *S. acutus*. However, treatments with 0.25 mg/L Cd^{2+} +2.5 mg/L Pb^{2+} and 1.0 mg/L Cd^{2+} + 7.5 mg/L Pb^{2+} significantly decreased population growth rate of *Schroederia* sp.

Effects of Cd^{2+} and Pb^{2+} on Chlorophyll Contents

Compared with the control, Chl-*a* and Chl-*c* contents of *S. acutus* significantly decreased in treatments with 1.0 and 2.0 mg/L Cd^{2+}. For *Schroederia* sp., Cd^{2+} showed negative effects on Chl-*a* and Chl-*c* contents at all tested concentrations. Car contents significantly declined in treatment with 2.0 mg/L Cd^{2+} for *S. acutus*, but in treatments with 0.50 and 1.00 mg/L Cd^{2+} for *Schroederia* sp. In single Pb^{2+} experiment, there were no significant effect on Chl-*a*, Chl-*c* and Car contents at concentrations from 5.0 to 15.0 mg/L (Fig. 2).

Joint exposure to Cd^{2+} + Pb^{2+} significantly reduced Chl-*a*, Chl-*c*, and Car contents in *S. acutus* at all tested concentrations. For *Schroederia* sp., significant increases of Chl-*a* and Chl-*c* contents were observed in treatment with 0.25 mg/L Cd^{2+} + 2.50 mg/L, but no significant differences were detected in other concentrations.

Effects of Cd^{2+} and Pb^{2+} on Photosynthesis Fluorescence Parameters

In Cd^{2+} treatments, for *S. acutus*, $rETR_{max}$ and I_k significantly decreased at all Cd^{2+} concentrations, both F_v/F_m and Yield decreased significantly at 0.50 and 2.00 mg/L Cd^{2+}, and α level only significantly reduced at 0.50 mg/L Cd^{2+} (Fig. 3). For *Schroederia* sp., $rETR_{max}$, F_v/F_m and I_k were significantly lower at all test concentrations than in the control. However, Cd^{2+} at all the tested concentrations did not significantly affect α. Yield significantly decreased only at 1.00 mg/L Cd^{2+}.

In Pb^{2+} treatments, for *S. acutus*, Pb^{2+} did not significantly affect $rETR_{max}$, α, I_k and Yield in all treatment, but all F_v/F_m significantly decreased compared

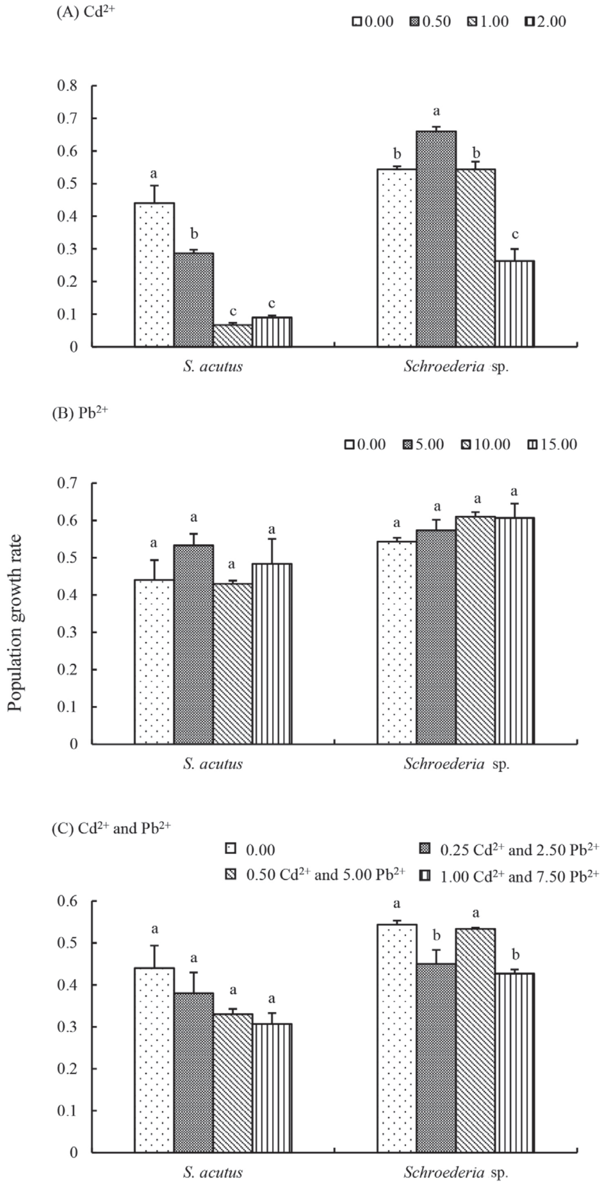

Fig. 1. Effects of Cd^{2+} (mg/L) and Pb^{2+} (mg/L) on population growth rate of *Scenedesmus acutus* and *Schroederia* sp. (mean±SD). Different letters above bars represent significantly different.

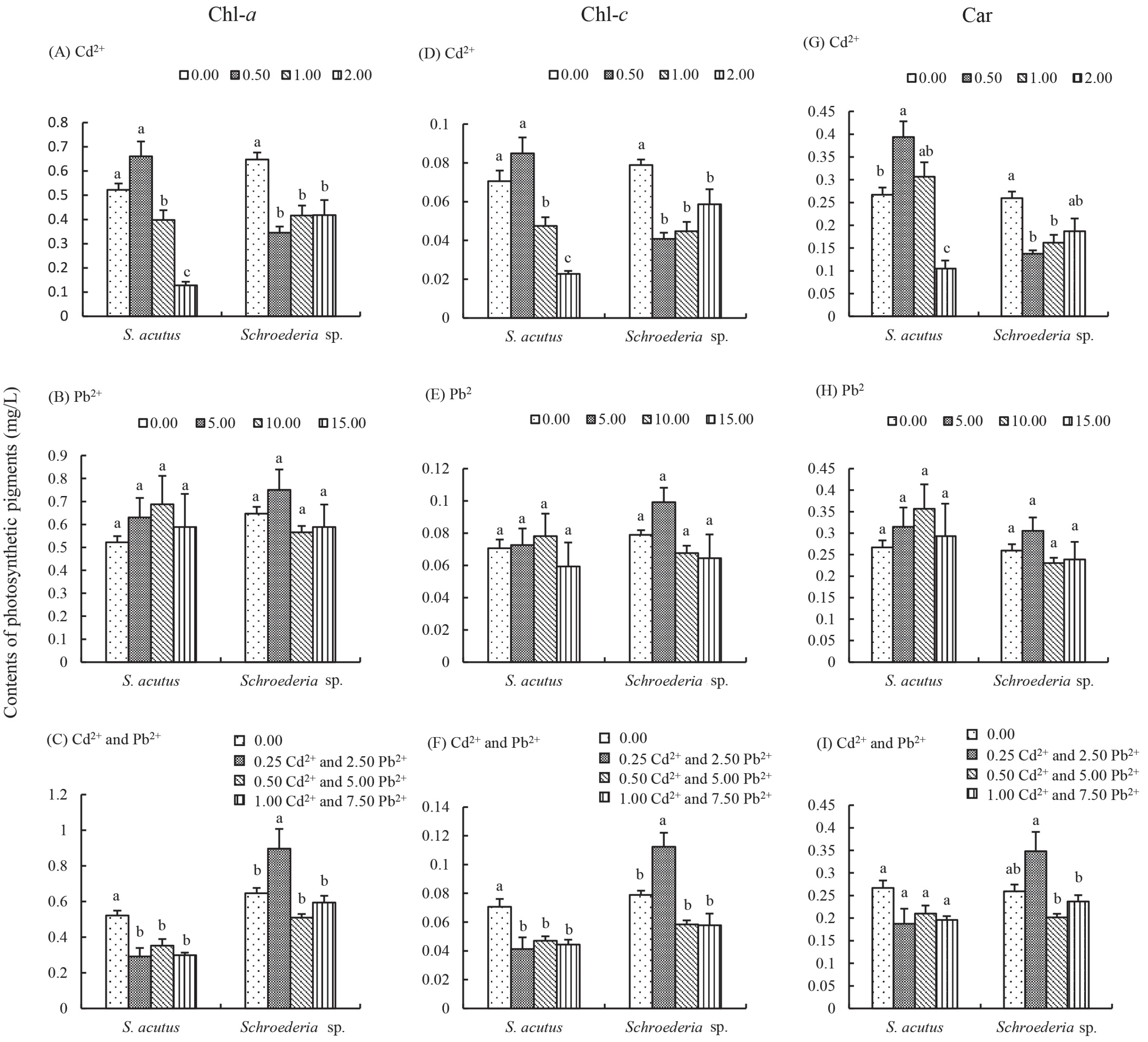

Fig. 2. Effects of Cd^{2+} (mg/L) and Pb^{2+} (mg/L) on chlorophyll contents of *Scenedesmus acutus* and *Schroederia* sp. (mean±SD). Different letters above bars represent significantly different.

with the control. For *Schroederia* sp., there were also no significant changes in $rETR_{max}$, α, I_k and yield values at all tested concentration. However, significant increases of F_v/F_m levels occurred in 10.00 and 15.00 mg/L Pb^{2+} exposure.

In combined exposure, for *S. acutus*, significant decreases of α, F_v/F_m and Yield parameters of were found in treatment with 0.25 mg/L Cd^{2+} + 2.50 mg/L Pb^{2+} and 0.50 mg/L Cd^{2+} + 5.00 mg/L Pb^{2+}. No significant difference in I_k was observed between all treatments and the control. For *Schroederia* sp., α values increased in all combined treatments. Treatments with 0.50 mg/L Cd^{2+} + 5.00 mg/L Pb^{2+} and 1.00 mg/L Cd^{2+} + 7.50 mg/L Pb^{2+} significantly increased Yield. However, there was no difference between all joint exposure and the control in $rETR_{max}$, F_v/F_m and I_k.

Interactive Effects of Cd^{2+} and Pb^{2+}

Two-way ANOVA was performed to test the interactive effects of Cd^{2+} and Pb^{2+} on all tested parameters (Tables 1 and 2). For *S. acutus*, Cd^{2+} and Pb^{2+} showed significant interactive effects on Chl-*a*, Car contents and all photosynthetic fluorescence parameters, but not on population growth rate and Chl-*b* content (Table 1). For *Schroederia* sp., interaction between Cd^{2+} and Pb^{2+} significantly affected population growth rate, $rETR_{max}$, F_v/F_m and I_k, but not influence Chl-*a*, Chl-*c*, Car contents, α and Yield (Table 2).

Discussion

Single exposure to 5.0-15.0 mg/L Pb^{2+} did not significantly stimulate the growth of *S. acutus* and *Schroederia* sp. in this study, consistent with Stewart [20], in which no visual effects of up to 10 mg/L Pb^{2+} on vegetative morphology or development of reproductive structures in *Platythamnion peetinatum*, *Platysiphonia decumbens*, and *Pleonosporium squarrulosum*. However, treatments with 0.5-2.0 mg/L Cd^{2+} significantly depressed *S. acutus* growth and treatment with 2.0 mg/L Cd^{2+} significantly reduced *Schroederia* sp. growth in the present study. These results indicated that Cd^{2+} is much toxic than Pb^{2+} to green algae. Similarly, Alho et al. [21] revealed that Cd^{2+} is greatly more toxic to the alga *Raphidocelis subcapitata* than Pb^{2+}. Li et al.

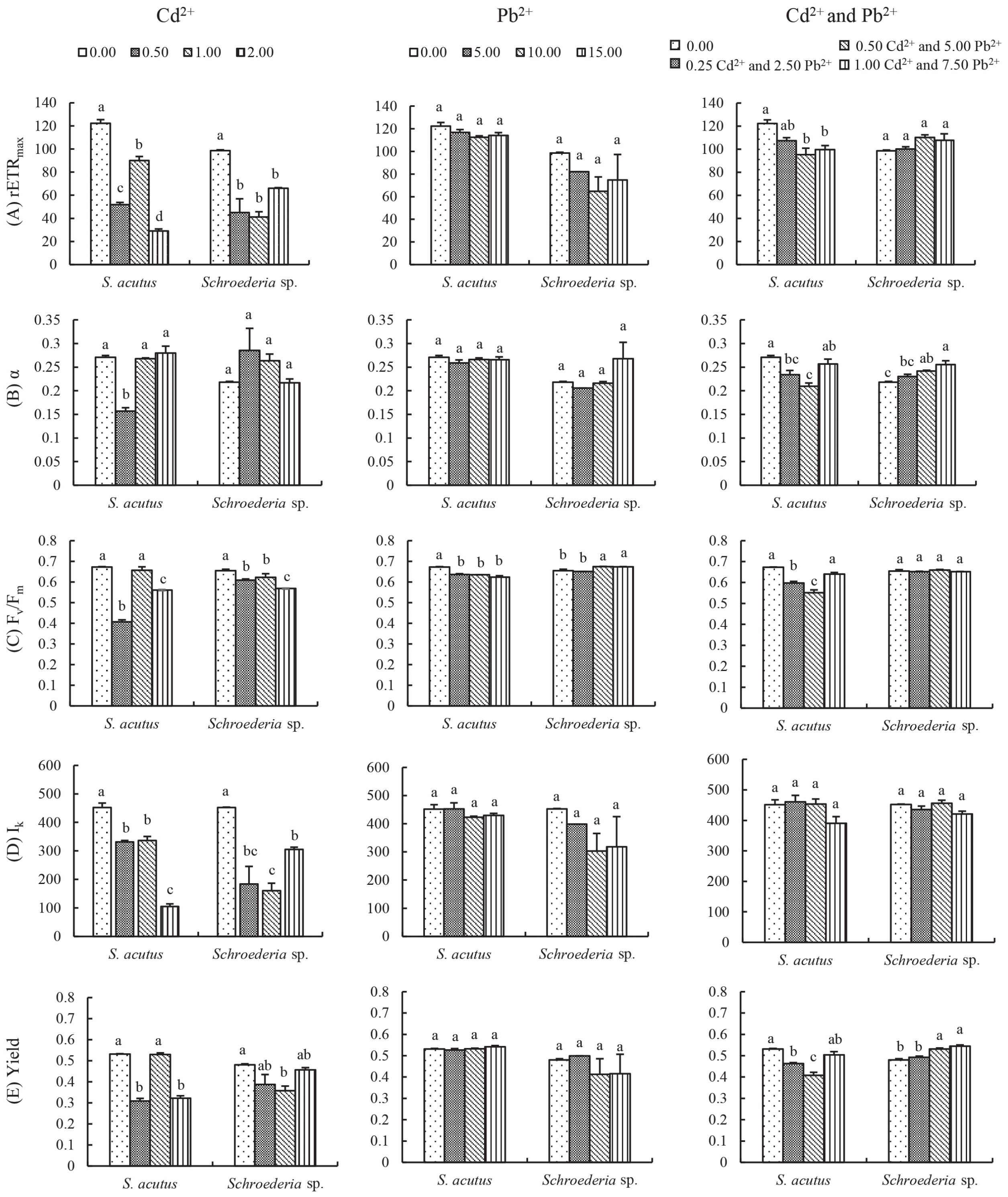

Fig. 3. Effects of Cd^{2+} (mg/L) and Pb^{2+} (mg/L) on photosynthetic fluorescent parameters of *Scenedesmus acutus* and *Schroederia* sp. (mean±SD). Different letters above bars represent significantly different.

Table 1. Two-way ANOVA analysis of Cd^{2+} and Pb^{2+} effects on population growth, chlorophyll contents and photosynthetic fluorescent parameters in *Scenedesmus acutus*.

Parameters	Type III SS	d.f.	MS	F value	P value
Population growth rate					
Cd^{2+}	0.34	3	0.113	20.39	<0.001
Pb^{2+}	0.107	4	0.027	4.831	0.007
$Cd^{2+}\times Pb^{2+}$	0.002	1	0.002	0.337	0.568
Chlorophyll a					
Cd^{2+}	0.447	3	0.149	6.208	0.004
Pb^{2+}	0.071	4	0.018	0.74	0.576
$Cd^{2+}\times Pb^{2+}$	0.13	1	0.13	5.428	0.03
Chlorophyll c					
Cd^{2+}	0.006	3	0.002	6.535	0.003
Pb^{2+}	0.002	4	0.000	1.165	0.356
$Cd^{2+}\times Pb^{2+}$	0.001	1	0.001	3.623	0.071
Carotenoids					
Cd^{2+}	0.108	3	0.036	5.255	0.008
Pb^{2+}	0.042	4	0.01	1.528	0.232
$Cd^{2+}\times Pb^{2+}$	0.04	1	0.04	5.876	0.025
Maximal relative electron transport rate ($rETR_{max}$)					
Cd^{2+}	14181.167	3	4727.056	114.834	<0.001
Pb^{2+}	1330.408	4	332.602	8.080	<0.001
$Cd^{2+}\times Pb^{2+}$	1794.141	1	1794.141	43.585	<0.001
Initial slope rate (α)					
Cd^{2+}	0.031	3	0.01	38.411	<0.001
Pb^{2+}	0.001	4	0	1.330	0.293
$Cd^{2+}\times Pb^{2+}$	0.003	1	0.003	11.726	0.003
Maximal photochemical efficiency (F_v/F_m)					
Cd^{2+}	0.121	3	0.04	161.556	<0.001
Pb^{2+}	0.011	4	0.003	11.271	<0.001
$Cd^{2+}\times Pb^{2+}$	0.025	1	0.025	98.612	<0.001
Half-saturation light intensity (I_k)					
Cd^{2+}	178492.570	3	59497.523	58.091	<0.001
Pb^{2+}	17762.461	4	4440.615	4.336	0.011
$Cd^{2+}\times Pb^{2+}$	11076.998	1	11076.998	10.815	0.004
Actual photochemical efficiency (Yield)					
Cd^{2+}	0.153	3	0.051	129.726	<0.001
Pb^{2+}	0.008	4	0.002	5.185	0.005
$Cd^{2+}\times Pb^{2+}$	0.008	1	0.008	20.937	<0.001

d.f.: degrees of freedom; SS: sum of square; MS: mean square.

Table 2. Two-way ANOVA analysis of Cd^{2+} and Pb^{2+} effects on growth, chlorophyll contents and photosynthetic fluorescent parameters in *Schroederia* sp.

Parameters	SS	d.f.	MS	F value	P value
Population growth rate					
Cd^{2+}	0.24	3	0.08	30.85	<0.001
Pb^{2+}	0.035	4	0.009	3.368	0.029
Cd^{2+}×Pb^{2+}	0.018	1	0.018	7.098	0.015
Chlorophyll a					
Cd^{2+}	0.239	3	0.08	4.488	0.015
Pb^{2+}	0.147	4	0.037	2.069	0.123
Cd^{2+}×Pb^{2+}	0.003	1	0.003	0.158	0.695
Chlorophyll c					
Cd^{2+}	0.005	3	0.002	6.516	0.003
Pb^{2+}	0.003	4	0.001	2.788	0.054
Cd^{2+}×Pb^{2+}	5.370E-6	1	5.370E-6	0.02	0.888
Carotenoids					
Cd^{2+}	0.041	3	0.014	4.831	0.011
Pb^{2+}	0.024	4	0.006	2.158	0.111
Cd^{2+}×Pb^{2+}	0.000	1	0	0.09	0.768
Maximal relative electron transport rate ($rETR_{max}$)					
Cd^{2+}	2418.812	3	806.271	2.048	0.139
Pb^{2+}	9850.299	4	2462.575	6.255	0.002
Cd^{2+}×Pb^{2+}	5004.125	1	5004.125	12.712	0.002
Initial slope rate (α)					
Cd^{2+}	0.012	3	0.004	2.308	0.107
Pb^{2+}	0.009	4	0.002	1.370	0.28
Cd^{2+}×Pb^{2+}	0.001	1	0.001	0.445	0.512
Maximal photochemical efficiency (F_v/F_m)					
Cd^{2+}	0.017	3	0.006	88.488	<0.001
Pb^{2+}	0.004	4	0.001	16.615	<0.001
Cd^{2+}×Pb^{2+}	0.002	1	0.002	34.767	<0.001
Half-saturation light intensity (I_k)					
Cd^{2+}	87048.937	3	29016.312	3.190	0.046
Pb^{2+}	178192.759	4	44548.190	4.898	0.006
Cd^{2+}×Pb^{2+}	79648.679	1	79648.679	8.757	0.008
Actual photochemical efficiency (Yield)					
Cd^{2+}	0.018	3	0.006	.817	0.5
Pb^{2+}	0.089	4	0.022	2.993	0.044
Cd^{2+}×Pb^{2+}	0.012	1	0.012	1.563	0.226

d.f.: degrees of freedom; SS: sum of square; MS: mean square.

[22] also revealed that *Chlamydomonas reinhardtii* was more tolerant to Pb^{2+} (EC_{50}: 29.48±8.83 mg/L) than to Cd^{2+} (EC_{50}: 12.48±1.30 mg/L) after 96 h of exposure. *S. acutus* and *Schroederia* sp. used in our experiment belong to the Chlorophyceae which has been considered tolerant to metals generally [23]. In particular, *S. acutus* has an higher ability to withstand metal concentrations than other algae species (Stokes et al. 1973) and phycoremediation potential of Pb^{2+} pollution [24]. To the best of our knowledge, there is no report investigating the toxicity of heavy metals to *Schroederia*. The present results indicated that *Schroederia* sp. is more tolerant to Cd^{2+} pollution than *S. acutus*, displaying a possibility to remediate Cd^{2+} pollution. However, more investigations are required to test its capacity of Cd^{2+} accumulation.

The changes of the photosynthetic pigments and biochemical contents can be used to monitor toxicity of heavy metals [25]. Both Cd^{2+} and Pb^{2+} caused the decrease of chlorophyll contents in marine algae [26]. Ismaiel and Said [27] observed that Chl-*a*, Chl-*b* and Car contents of *Pseudochlorella pringsheimii* were highly repressed in response to Cd^{2+} exposure (12-300 μM), whereas Pb^{2+} highly stimulated their contents at low concentrations (5-100 μM), but inhibited their contents at high concentrations (300-500 μM). Partially similarly, in the present study, Cd^{2+} also revealed inhibitory effects on Chl-*a*, Chl-*c* and Car contents in both algae species, demonstrating the high toxic of Pb^{2+} to the synthesis of photosynthetic pigments. Differently, Pb^{2+} treatments did not significantly influence contents of photosynthetic pigments in the present study, suggesting different mechanisms in Pb^{2+} metabolism between the two algae species used in this study and *P. pringsheimii*.

The photosynthetic fluorescence parameters are most useful indices to assess the photosynthetic status of algae under stress conditions [28]. The inhibition of chlorophyll synthesis may alter the light harvesting complexes responsible for light energy transfer to PSII reaction center [29], which could be reflected by determining the photosynthetic fluorescence parameters. In general, F_v/F_m and Yield values represent the fluorescent yield and the $rETR_{max}$ value indicates the photosynthetic electron transport. In the present study, treatment with 1.00 mg/L Cd^{2+} did not change F_v/F_m and Yield in *S. acutus*, but significantly decreased $rETR_{max}$ and I_k, suggesting an impairment of PSII system. In *Schroederia* sp., α value was not affected but $rETR_{max}$, F_v/F_m and I_k decreased significantly in all Cd^{2+} treatments, suggesting that Cd^{2+} damaged both PSII reaction center and the electron transport chain in *Schroederia* sp. However, in Pb^{2+} treatments, $rETR_{max}$, α, I_k and Yield values did not alter significantly in both two algae species, further indicating that Pb^{2+} did not harm the photosynthesis system in freshwater green algae. Differently, 10 mg/L Pb^{2+} completely suppressed the photosynthesis in the marine diatom *Phaeodactylum tricornutum* [30].

There are roughly four types of interactions between heavy metals: antagonism, synergy, addition and sensitization [31]. Devi Prasad and Devi Prasa [32] reported that combination of Cd^{2+} and Pb^{2+} revealed antagonism on the growth of *Ankistrodesmus falcatus* when compared to single exposure. In the present study, single exposure to 1.00 mg/L Cd^{2+} significantly decreased, but combined exposure to 1.00 mg/L Cd^{2+} + 7.50 mg/L Pb^{2+} did not affect *S. acutus* growth rate, suggesting that Pb^{2+} alleviated the toxicity of Cd^{2+} to *S. acutus*. Similar antagonism was also observed on $rETR_{max}$, Yield and I_k in combined exposure to *S. acutus* and *Schroederia* sp. Broadly reported, Cd^{2+} and Pb^{2+} showed antagonistic effects on Chinese watermelon [33], soybean [34] and *Microcystis aeruginosa* [35]. The underlying mechanism might be associated with the competition between Cd^{2+} and Pb^{2+}. Kola and Wikinson [36] demonstrated that Pb^{2+} inhibited partially Cd^{2+} uptake by *Chlamydomonas reinhardtii*.

Conclusions

Treatments with 0.5-2.0 mg/L Cd^{2+} significantly inhibited pollution growth of *S. acutus*, but only treatment with 2.0 mg/L Cd^{2+} significantly suppressed *Schroederia* sp. growth due to influences on photosynthetic process. Treatments with 5.0-15.0 mg/L Pb^{2+} did not significantly affect both species. Combined exposure revealed that Pb^{2+} alleviate the toxicity of Cd^{2+} to green algae.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Supplementary Material

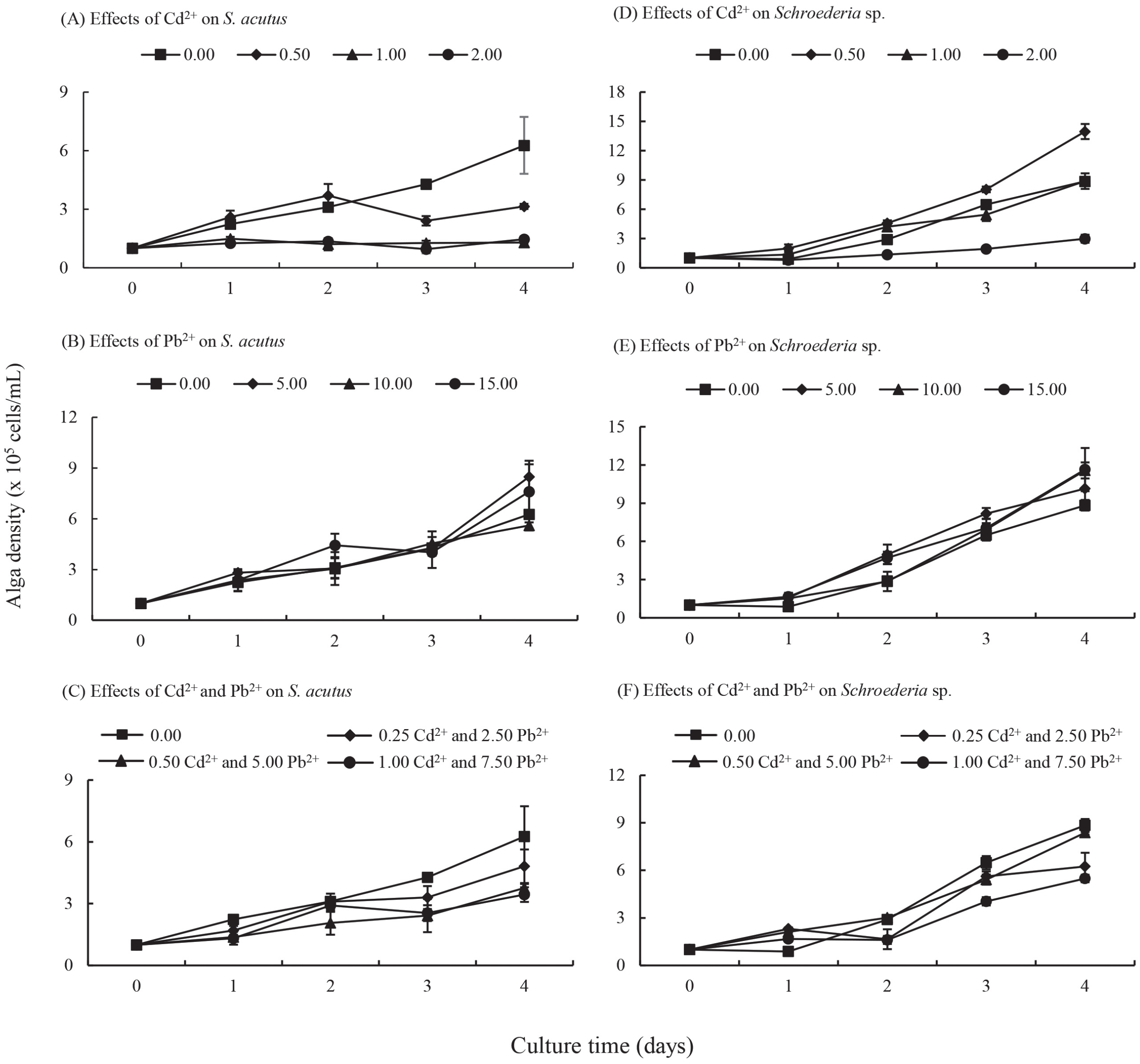

Fig. S1. Growth curves of *Scenedesmus acutus* and *Schroederia* sp. in treatments with Cd^{2+} (mg/L) and Pb^{2+} (mg/L). Data represent mean±SD.