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Academic press

2019-07-15

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Liu, M, Korpelainen, H, Dong, L & Yi, L 2019, 'Physiological responses of *Elaeocarpus glabripetalus* seedlings exposed to simulated acid rain and cadmium', *Ecotoxicology and Environmental Safety*, vol. 175, pp. 118-127. <https://doi.org/10.1016/j.ecoenv.2019.03.026>

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<http://hdl.handle.net/10138/328166>

10.1016/j.ecoenv.2019.03.026

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1 **Physiological responses of *Elaeocarpus glabripetalus***  
2 **seedlings exposed to simulated acid rain and cadmium**

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9

10 **Abstract**

11 Combined effects of cadmium (Cd) and acid rain on physiological and biochemical  
12 characteristics in *Eleocarpus glabripetalus* seedlings were investigated under  
13 controlled conditions. The single Cd treatment and the combined Cd and acid rain  
14 treatment increased growth at low Cd concentrations, while decreased growth and  
15 photosynthesis at high Cd concentrations. A low Cd concentration ( $50\text{mg}\cdot\text{kg}^{-1}$ )  
16 combined with different acid rain treatments increased the seedling biomass, while  
17 there was no significant difference in chlorophyll fluorescence and antioxidative  
18 enzyme activities compared with the control plants. A high Cd concentration ( $100$   
19  $\text{mg}\cdot\text{kg}^{-1}$ ) under different acid rain treatments significantly decreased the biomass, the  
20 Fe and Mg content, chlorophyll fluorescence, photosynthetic parameters (the  
21 maximum net photosynthetic rate,  $A_{max}$ , and the light saturation point,  $L_{SP}$ ). Relative  
22 electric conductivity and peroxidase (POD) activity were significantly higher while  
23 the reduced glutathione (GSH) content and catalase (CAT) activity were significantly  
24 lower at high Cd concentration under acid rain when compared to the control plants.  
25 The results indicated that the combination of a high concentration of Cd and acid rain  
26 aggravated the toxic effect of Cd or acid rain alone on the growth and physiological  
27 parameters of *E. glabripetalus* due to serious damage to the chloroplast structure.  
28 However, *E. glabripetalus* showed tolerance to a low concentration of Cd and low pH  
29 induced by acid rain.

30 **Additional Key words:** Interactive effects; Cadmium; Acid rain; Physiology;

31 *Elaeocarpus glabripetalus*.

32 **Abbreviation**

33 *AQY* – apparent quantum; *A<sub>max</sub>* – maximum net photosynthetic rate; CAT – catalase;  
34 Chl – chlorophyll; GSH – glutathione; *F<sub>m</sub>* – maximal fluorescence in dark-adapted  
35 state; *F<sub>m</sub>'* – maximal fluorescence in the light-adapted state; *F<sub>s</sub>* – steady state value of  
36 fluorescence; *F<sub>v</sub>* – variable fluorescence; *F<sub>v</sub>/F<sub>m</sub>* – maximum quantum yield; *F<sub>v</sub>/F<sub>0</sub>* –  
37 potential activity of PSII; *F<sub>0</sub>'* – basal fluorescence after farred illumination; *L<sub>CP</sub>* – light  
38 compensation point; *L<sub>SP</sub>* – light saturation point; *NPQ* – non-photochemical  
39 quenching of fluorescence; PAR – photosynthetically active radiation; *P<sub>n</sub>* – net  
40 photosynthetic rate; POD – peroxidase; PSII – photosystem II; PPFD – photosynthetic  
41 photon flux density; *qP* – photochemical quenching; ROS – reactive oxygen species

42 ***1 Introduction***

43 Cadmium (Cd) is one of the most toxic elements that can be found as a contaminant in  
44 the soil. According to Zhou et al. (2004) and Jin et al. (2010), the concentration of Cd  
45 in some severe polluted soils was higher than 100 mg·kg<sup>-1</sup>. Cadmium contents in soil  
46 have dramatically increased during recent years and its toxicity limits plant growth  
47 and development. Cadmium can be easily taken up by roots and it tends to accumulate  
48 within plant organs (Gill et al., 2012). Cd inhibits plant growth and biomass  
49 production, affects mineral uptake, photosynthesis and the protective system, and  
50 negatively interferes with essential physiological processes (Januškaitienė, 2012,  
51 Parmar et al., 2013, Li et al., 2015, Liu et al., 2017,).

52 In the nature, plants are rarely exposed to a single hazardous stress. Different  
53 contaminants may show additive, antagonistic or synergistic interactions. Acid rain is  
54 one of the well-known environmental problems, which cause negative impacts on  
55 plant growth and health. It has been found that acid rain destroys leaves, causes  
56 deleterious effects on photosynthesis and inhibits growth (Sun et al., 2016, Debnath et  
57 al., 2018). Most agroforestry areas with acid rain pollution have also other  
58 contaminants, such as Cd, leading to combined pollutions effects, which are major  
59 problems for sustainable forestry and environmental safety.

60 There are many previous investigations on the effects of acid rain or Cd alone on  
61 plants (Chmielowska-Bąk et al., 2014, Li et al., 2015, Zheng et al., 2017, Ren et al.,  
62 2018), while only a few studies have reported results on their combined adverse  
63 effects on plant growth ( Liao et al., 2003, Liu et al., 2011, Sun et al., 2012). Such

64 combined pollution could have harmful effects on plant growth through the inhibition  
65 of photosynthesis, because the photosynthetic apparatus appears especially sensitive  
66 to Cd (Gill et al., 2012). Previous studies have found that Cd combined with acid rain  
67 may damage the chloroplast structure, thus reducing photosynthesis (Sun et al., 2012),  
68 and decrease the activities of antioxidative enzymes (Liu et al., 2011). The combined  
69 effect of acid rain and heavy metal exposure would inhibit plant growth through  
70 reducing biomass and mineral uptake (Zhang et al., 2016). Many of the previous  
71 studies on the combined effect of heavy metals and acid rain have focused on crops  
72 and only few on woody plants. Compared with herbaceous plants, woody plants  
73 usually grow slowly with a deep root system and long generation time, and they are  
74 more easily affected adversely by pollution.

75 *Eleocarpus glabripetalus* is a heliophilous and dominant species in forest ecosystems  
76 of China. It is widely distributed in regions affected by severe acid rain but also by Cd  
77 pollution. Our previous studies showed that *E. glabripetalus* seedlings have tolerance  
78 to acid rain (Liu et al., 2015). However, we don't know how it would grow under the  
79 combined pollution of acid rain and Cd. Therefore, the objectives of our present study  
80 are: (1) to investigate the responses of chlorophyll fluorescence and photosynthetic  
81 parameters to the combined pollution of Cd and acid rain; (2) to reveal the activities  
82 of antioxidative enzymes and the content of reduced glutathione (GSH) under the  
83 combined pollution of Cd and acid rain; (3) to show the combined effect on biomass  
84 allocation, and on the content of Cd, Mg and Fe in plants. The results obtained from  
85 this study will enhance understanding of the mechanisms of tolerance to the combined

86 pollution of Cd and acid rain in *E. glabripetalus*.

87

## 88 **2 Materials and methods**

### 89 *2.1. Preparation of simulated acid rain and Cd solution*

90 According to the average pH value of acid rain in southern China (Hangzhou  
91 Municipal Environmental Protection, 2013), two pH values (pH 3.0 and 5.6) were  
92 selected for the experiment. Correspondingly, according to the average concentration  
93 of Cd in polluted soil ( Zhou et al., 2004, Jin et al., 2010), the final concentrations of  
94 50 and 100 mg·kg<sup>-1</sup> were selected. Two solutions were prepared: (1) According to the  
95 general anion composition of rainfall in southern China, the SO<sub>4</sub><sup>2-</sup>/ NO<sub>3</sub><sup>-</sup> ratio is 2,87  
96 (Xu et al., 2011). Considering that the proportion of NO<sub>3</sub><sup>-</sup> has been rising during  
97 recent years, a simulated acid rain solution stock of pH 3.0 was prepared with a  
98 H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> ratio of 1.0:1.0 (v/v), and then used as such or diluted to pH 5.6 to  
99 be utilized as the spraying solution to simulate acid rain. (2) The Cd solutions (0.15  
100 mM and 0.30 mM, calculated for 10 kg soil in the pot and 100-day stress according to  
101 50 and 100 mg·kg<sup>-1</sup>) were prepared by dissolving appropriate quantities of  
102 CdCl<sub>2</sub>·5H<sub>2</sub>O (Chemical Co. Ltd., China) in distilled water (pH 7.0).

### 103 *2.1 Plant material and stress treatments*

104 The soil, a mixture of reddish brown forest soil, was sampled from the depth of 0-20  
105 cm. Then, the soil sample was air-dried and ground to pass through a 2 mm sieve, and  
106 10 kg was divided into each pot. The initial soil pH was 6.88 and available Cd was  
107 0.082 mg·kg<sup>-1</sup>. Healthy one-year old seedlings of *E. glabripetalus* with height of 40-

108 60 cm and a basal diameter of approximately 0.8-1.0 cm were planted into pots with  
109 an inner diameter of 30 cm and a depth of 40 cm, one seedling per pot. A PVC plate  
110 was placed under each pot. The seedlings were kept under a canopy in the Botanical  
111 Garden of the Zhejiang Agriculture and Forestry University (119°44' E, 30°16' N),  
112 East China. After growing for two months, seedlings were subjected to 9 treatments  
113 of Cd and simulated acid rain, (1) The control group (CK): *E. glabripetalus* seedlings  
114 were watered daily by distilled water (pH 7.0) without Cd. (2) Single treatment with  
115 Cd: soil was sprayed daily with Cd solutions (0.15 mM or 0.30 mM). (3) Single  
116 treatment with simulated acid rain: *E. glabripetalus* seedlings were sprayed daily with  
117 acid rain solutions (pH 3.0 or 5.6) on leaves. (4) Combined treatment with Cd and  
118 simulated acid rain: *E. glabripetalus* seedlings were sprayed with acid rain solutions  
119 (pH 3.0 or 5.6) on leaves and Cd solutions (0.15 mM or 0.30 mM) into soil every day.  
120 For these 9 treatments, the amount of simulated acid rain or distilled water or Cd  
121 solutions was 300 mL per pot every day, as calculated according to precipitation and  
122 evaporation in southeastern China. All treatments were replicated in 12 pots. During  
123 the 100-day experiment, the places of pots were often randomly switched to avoid  
124 differences in microclimate.

125

## 126 2.2 Measurements of chlorophyll fluorescence and photosynthetic parameters

127 After the 100-day experiment, chlorophyll fluorescence was measured from 09:00-  
128 11:30 a.m. using a portable chlorophyll fluorometer (PAM-2000 WALZ, Germany).

129 Three seedlings were selected from each treatment, with measurements performed for

130 a leaf from the upper, middle and lower parts of the plant. The sampled leaves were  
 131 dark-adapted for 20 minutes using a dark leaf clip before each chlorophyll  
 132 fluorescence measurement. The initial fluorescence ( $F_0$ ), which was measured using  
 133 an analytical light of  $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD, was recorded after dark adaptation and,  
 134 subsequently, a saturating pulse ( $6,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD, 2s) was given to measure  
 135 the maximal fluorescence ( $F_m$ ). The steady state value of fluorescence ( $F_s$ ), the  
 136 maximal fluorescence in the light-adapted state ( $F_m'$ ) and the basal fluorescence after  
 137 farred illumination ( $F_0'$ ) were determined by a procedure described by Guo (2005),  
 138 with six measurements conducted per replication. The maximum quantum yield ( $F_v/F_m$ )  
 139 was determined by  $F_v/F_m = (F_m - F_0) / F_m$ .  $F_v$  is variable fluorescence calculated  
 140 as  $F_v = F_m' - F_0'$ . Non-photochemical quenching of fluorescence ( $NPQ$ ) was calculated  
 141 as  $NPQ = (F_m - F_m') / F_m'$ . Potential activity of photosystem II (PSII) ( $F_v/F_0$ ) was  
 142 calculated by  $F_v/F_0 = (F_m - F_0) / F_0$ . Photochemical quenching ( $qP$ ) was calculated by  
 143  $qP = (F_m' - F_s) / (F_m' - F_0')$ .  
 144 Photosynthetic parameters were measured for the same leaves at the same time as for  
 145 chlorophyll fluorescence, using a portable photosynthesis system, Li-6400 (Li-Cor  
 146 Inc. Lincoln, Nebraska, USA). Leaf temperature was  $25^\circ\text{C}$  with a relative humidity of  
 147 80% inside the leaf chamber during measuring. Photosynthetic photon flux density  
 148 (PPFD) was controlled at  $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Ambient  $\text{CO}_2$  concentration was  $400 \pm 5$   
 149  $\mu\text{mol}\cdot\text{mol}^{-1}$ . Responses to photosynthetically active radiation (PAR) were measured at  
 150 0, 10, 20, 50, 80, 100, 200, 300, 500 and  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Net photosynthetic rate  
 151 ( $P_n$ ) - PAR response curves were generated by plotting  $P_n$  as a function of PAR. These

152 curves were modeled by fitting data to a nonrectangular hyperbola (Lambers et al.,  
153 1998) by means of the nonlinear least squares curve-fitting procedure of *Photosyn*  
154 *Assistant software* (Dundee Scientific, Scotland, UK). This model is expressed as  
155 follows:  
156 
$$P_n = [AQY \times PAR + A_{max} - \sqrt{(AQY \times PAR + A_{max})^2 - 4 \times AQY \times A_{max} \times PAR \times K} / 2 \times K] - R_d$$
  
157 where *AQY* is apparent quantum yield [ $\mu\text{mol}(\text{CO}_2) \mu\text{mol}^{-1}(\text{photon})$ ], *PAR* is the  
158 photosynthetic active radiation, *A<sub>max</sub>* is the maximum net photosynthetic rate, *K* is the  
159 curvature factor, and *R<sub>d</sub>* is leaf-level “dark” respiration [ $\mu\text{mol}(\text{CO}_2) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ]. Light  
160 saturation point (*L<sub>SP</sub>*) and light compensation point (*L<sub>CP</sub>*) were estimated.

161

### 162 2.3 Measurements of antioxidant system

163 1 g leaves from control and treated plants were ground in liquid nitrogen, then  
164 extracted with 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM  
165 EDTA, 1% (w/v) PVP, 0.1 mM PMSF, and 0.2% (v/v) Triton X100 for the  
166 measurements of peroxidase (POD) and catalase (CAT) activities and the content of  
167 reduced glutathione (GSH). The homogenate was centrifuged at 14 000 ×g at 4°C for  
168 15 min. The supernatants were used for the enzyme assays. The means ± SE were  
169 calculated from the data of at least 3 independent measurements.

170 POD activity was determined by monitoring the increase in absorbance at 470 nm  
171 during the oxidation of guaiacol (Upadhyaya et al., 1985). The amount of enzyme  
172 producing 1  $\mu\text{mol}/\text{min}$  of oxidized guaiacol was defined as 1 U. CAT activity was  
173 determined by UV spectrophotometry by a direct measurement of  $\text{H}_2\text{O}_2$

174 decomposition at 240 nm, following the procedure of Aebi (1984); 1 U equals the  
175 amount of H<sub>2</sub>O<sub>2</sub> (in μmol) decomposed in 1 min.

176 Reduced (GSH) contents were measured according to Griffith (Griffith, 1980). The  
177 contents were determined in a reaction mixture containing leaf extract, 100 mM  
178 sodium phosphate buffer (pH 7.7) and 100 mM sodium phosphate buffer (pH 6.8)  
179 containing 4 mM 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB). The reaction mixture  
180 was maintained at 25°C for 10 min and the absorbance was read at 412 nm. A  
181 standard graph was drawn using different concentrations of a GSH solution. With the  
182 help of the standard graph, reduced GSH contents in the leaves were calculated.

183 Relative electric conductivity was determined using the conductivity instrument. First,  
184 1 g fresh leaves were cut and completely immersed in 20 ml deionized water, and then  
185 degassed for 15min. The initial conductivity (E<sub>1</sub>) was measured with a conductivity  
186 instrument (LC116, Mettler Toledo Instruments Co., Ltd, Shanghai, China) after 30  
187 mins. The solution was then incubated at 100 ° C for 15 min and cooled to room  
188 temperature, and the final conductivity (E<sub>2</sub>) was determined. The conductivity of  
189 deionized water was also determined as blank conductivity (E<sub>0</sub>). The relative  
190 conductivity was calculated according to the following formula: relative electric  
191 conductivity (%) = (E<sub>1</sub> - E<sub>0</sub>) / (E<sub>2</sub> - E<sub>0</sub>) × 100%.

#### 192 *2.4 Measurements of biomass, and element and Cd contents of plants*

193 Three replicated plants from each treatment were harvested at 100 days. Plant parts,  
194 such as stems, leaves and roots (carefully washed and blotted), were separated and  
195 dried in an oven at 80 °C for 48h to constant weight and weighed. Dry mass

196 accumulation was then calculated. The contents of elements (Mg, Fe and Cd) in the  
197 leaves, stems and roots of *E. glabripetalus* were measured by ICP-AES (*PerkinElmer*  
198 *Inc.*, Shelton, CT, USA) with three replicates per treatment.

### 199 2.5 Data analysis

200 The significance of differences between the treatments was analyzed by one-way  
201 analysis of variance (ANOVA) using SPSS17.0. The interactions between Cd and acid  
202 rain were analyzed by the two-way analysis of ANOVA. Prior to analysis, the data  
203 were checked for normality and homogeneity of variances. Differences between  
204 treatments were detected and mean values were compared by the Tukey's test ( $p =$   
205 0.05). All data were expressed as means  $\pm$  SE ( $n \geq 3$ ).

## 206 3 Results

### 207 3.1 Effects of Cd and acid rain on chlorophyll fluorescence in *E. glabripetalus* 208 seedlings

209 Fig.1 shows the changes in chlorophyll fluorescence parameters when *E.*  
210 *glabripetalus* seedlings were treated with Cd and acid rain. When treated with acid  
211 rain only, the  $F_v/F_m$ ,  $NPQ$ ,  $qP$  and  $F_v/F_0$  values of *E. glabripetalus* seedlings  
212 increased compared with control plants.  $F_v/F_m$  decreased by 1.20% at pH 5.6 and  
213 increased by 0.41% at pH 3.0.  $NPQ$ ,  $qP$  and  $F_v/F_0$  increased by 8.99%, 2.66% and  
214 0.46%, respectively, at pH 5.6, and increased by 10.99%, 1.75% and 0.37%,  
215 respectively at pH 3.0. When *E. glabripetalus* seedlings were treated with Cd alone,  
216  $F_v/F_m$  decreased by 1.22% and 2.98% at 50 and 100 mg·kg<sup>-1</sup> Cd, respectively, in  
217 comparison with the control plants.  $NPQ$ ,  $qP$  and  $F_v/F_0$  increased by 16.63%, 0.63 %

218 and 1.06%, respectively, at 50 mg·kg<sup>-1</sup> Cd, while they decreased by 8.36%, 2.11% and  
219 9.88%, respectively, at 100 mg·kg<sup>-1</sup> Cd.

220 When *E. glabripetalus* seedlings were treated with acid rain and 50 mg·kg<sup>-1</sup> Cd,  $F_v/F_m$   
221  $F_m$  increased by 0.64% at pH 5.6 and 0.08% at pH 3.0 compared with the control  
222 plants, by 1.86% and 1.88% compared with the single treatment with pH 5.6 acid rain  
223 and the single treatment of 50 mg·kg<sup>-1</sup> Cd, respectively (Fig.1). However, when *E.*  
224 *glabripetalus* seedlings were treated with acid rain and 100 mg·kg<sup>-1</sup> Cd,  $F_v/F_m$   
225 decreased compared with control or acid rain-treated plants.  $NPQ$ ,  $qP$  and  $F_v/F_0$   
226 decreased by 7.05%, 2.51% and 5.80%, compared with control plants. Decreases in  
227  $NPQ$ ,  $qP$  and  $F_v/F_0$  were found in some combined treatments with Cd and acid rain.  
228 For example, when the seedlings were treated with acid rain at pH 3.0 and 100 mg·kg<sup>-1</sup>  
229 Cd,  $NPQ$  decreased by 22.40%, 30.08% and 15.32%, respectively, compared with  
230 control plants and those treated with 100 mg·kg<sup>-1</sup> Cd alone and acid rain at pH 3.0  
231 alone, respectively. The two-way ANOVA analysis indicated that there was no  
232 interaction between Cd and acid rain on chlorophyll fluorescence parameters ( $F_v/F_m$ ,  
233  $NPQ$ ,  $qP$  and  $F_v/F_0$ ). The values of  $NPQ$ ,  $qP$  and  $F_v/F_0$  of *E. glabripetalus* seedlings  
234 kept under the combined treatment with a high concentration of Cd (100 mg·kg<sup>-1</sup>) and  
235 low pH acid rain (pH 3.0) were lower than those in seedlings exposed to a single  
236 treatment with Cd or acid rain, indicating that 100 mg·kg<sup>-1</sup> Cd and pH 3.0 acid rain  
237 have a synergistic effect on most chlorophyll fluorescence parameters.

238 *3.2 Effects of Cd and acid rain on photosynthetic parameters in E. glabripetalus*  
239 *seedlings*

240 When the seedlings were treated with acid rain only,  $A_{max}$  increased at pH 3.0 and pH  
241 5.6 compared with control seedlings ( $p > 0.05$ ) (Table 1).  $A_{max}$  increased by 30.36%  
242 at pH 5.6 and 11.04% at pH 3.0. When the seedlings were treated with Cd alone,  $A_{max}$   
243 increased by 52.65% at 50 mg·kg<sup>-1</sup> Cd, while it decreased slightly at 100 mg·kg<sup>-1</sup> Cd  
244 compared with control seedlings. However, when *E. glabripetalus* seedlings were  
245 treated with acid rain and 50 mg·kg<sup>-1</sup> Cd,  $A_{max}$  increased by 1.49% at pH 5.6 and  
246 15.07% at pH 3.0 ( $p > 0.05$ ), while there was a decrease by 57.96% at pH 5.6 and  
247 39.07% at pH 3.0 under 100 mg·kg<sup>-1</sup> Cd treatment ( $p > 0.05$ ).  $AQY$  increased when the  
248 seedlings were treated with acid rain or Cd treatment only. The combined treatment  
249 with pH 3.0 acid rain increased  $AQY$  by 80% at 50 mg·kg<sup>-1</sup> Cd and by 56% at 100  
250 mg·kg<sup>-1</sup> Cd ( $p < 0.05$ ). Compared with control plants,  $L_{CP}$  decreased in seedlings  
251 treated with acid rain or Cd only ( $p < 0.05$ ). When *E. glabripetalus* seedlings were  
252 treated with pH 3.0 acid rain and Cd,  $L_{CP}$  decreased by 57.89% at 50 mg·kg<sup>-1</sup> Cd and  
253 by 21.05% at 100 mg·kg<sup>-1</sup> Cd.  $L_{SP}$  increased at 50 mg·kg<sup>-1</sup> Cd and decreased at 100  
254 mg·kg<sup>-1</sup> Cd ( $p < 0.05$ ), and acid rain treatment alone decreased  $L_{SP}$  ( $p < 0.05$ ). Under a  
255 combined treatment with acid rain and 100 mg·kg<sup>-1</sup> Cd,  $L_{SP}$  decreased by 65.18% at  
256 pH 5.6 and by 54.31% at pH 3.0 ( $p < 0.05$ ) (Table 1). The two-way ANOVA analysis  
257 indicated that there was no interaction effect between Cd and acid rain on  
258 photosynthetic parameters.

### 259 3.3 Effects of Cd and acid rain on POD and CAT activities, relative electric 260 conductivity and GSH content in *E. glabripetalus* seedlings

261 When *E. glabripetalus* seedlings were treated with acid rain alone, POD and CAT

262 activities increased along with decreasing pH values but no significant difference  
263 were detected compared with the control plants ( $p > 0.05$ ) (Fig.2). The POD activity  
264 increased with an increasing Cd concentration. At  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd, the POD activity  
265 was significantly higher than that of the control plants ( $p < 0.05$ ), with an increase of  
266 63.80%. When the seedlings were treated by both acid rain and Cd, the POD activity  
267 was significantly higher than that of control plants ( $p < 0.05$ ), increases equaling  
268 176.45% at pH 5.6 and 226.05% at pH 3.0 under  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd. The CAT activity  
269 increased at  $50 \text{ mg}\cdot\text{kg}^{-1}$  Cd ( $p > 0.05$ ) while decreased at  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd ( $p < 0.05$ ).  
270 The CAT activity was higher than that of control plants under a combination of pH 3.0  
271 acid rain and  $50 \text{ mg}\cdot\text{kg}^{-1}$  Cd, while the CAT activity was lower under a combination of  
272  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd and pH 3.0 / 5.6 acid rain than that of control plants ( $p < 0.05$ ).  
273 When *E. glabripetalus* seedlings were treated with single acid rain, relative electric  
274 conductivity decreased but showed no significant difference compared with the  
275 control plants ( $p > 0.05$ ), (Fig.2). When treated with Cd alone, relative electric  
276 conductivity increased significantly at  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd ( $P < 0.05$ ). Under the  
277 combined treatment with acid rain and  $100 \text{ mg}\cdot\text{kg}^{-1}$  Cd, relative electric conductivity  
278 increased by 73.93% at pH 5.6 and 165.64% at pH 3.0 ( $p < 0.05$ ).  
279 Compared with the control seedlings, the GSH content decreased under acid rain  
280 (Fig.2). At  $50 \text{ mg}\cdot\text{kg}^{-1}$  Cd ( $p < 0.05$ ), the GSH content increased but decreased at  $100$   
281  $\text{mg}\cdot\text{kg}^{-1}$  Cd ( $p < 0.05$ ). Under the combined treatment with acid rain and  $100 \text{ mg}\cdot\text{kg}^{-1}$   
282 Cd, the GSH content decreased by 21.30% at pH 5.6 and 25.16% at pH 3.0 ( $p < 0.05$ ).  
283 The ANOVA analysis showed that there was a significant interaction effect between

284 Cd and acid rain on POD and CAT activities, and on the GSH content. Under the  
285 combined treatment with a high concentration of Cd and low pH acid rain, the POD  
286 and CAT activities of *E. glabripetalus* seedlings were lower than those under Cd or  
287 acid rain alone, thus indicating that 100 mg·kg<sup>-1</sup> Cd and pH 3.0 acid rain have a  
288 synergistic effect on antioxidant enzymes.

### 289 *3.4 Effects of Cd and acid rain on biomass in E. glabripetalus seedlings*

290 Table 2 shows the biomass accumulation of *E. glabripetalus* exposed to simulated  
291 acid rain and Cd. A acid rain treatment alone increased the total biomass by 15.92% at  
292 pH 5.6 and 20.66% at pH3.0 compared with the control plants. The biomass  
293 allocation of plants kept in control conditions was 2.99% in leaves, 46.70% in stem  
294 and 25.31% in roots. The root biomass increased by 34.98% at pH 5.6, while there  
295 was more biomass in leaves at pH 3.0 (37.30%). When the seedlings were treated by  
296 Cd alone, the stem biomass reduced by 38.03% and 41.47% at 50 and 100 mg·kg<sup>-1</sup>  
297 Cd, respectively, in comparison with control plants. Also, 50 mg·kg<sup>-1</sup> Cd increased the  
298 total biomass by 20.37% while 100 mg·kg<sup>-1</sup> Cd decreased the total biomass by  
299 11.00%. The combined acid rain and Cd stress decreased biomass allocation into the  
300 stem. For example, the proportion of biomass in stem was 37.70% and 37.93% at pH  
301 3.0 and 5.6 under 100 mg·kg<sup>-1</sup> Cd treatment, respectively. The total biomass increased  
302 under the combined 50 mg·kg<sup>-1</sup> Cd and acid rain treatment ( $p > 0.05$ ), while it  
303 decreased under 100 mg·kg<sup>-1</sup> Cd and acid rain ( $p < 0.05$ ).

### 304 *3.5 Effects of Cd and acid rain on Mg, Fe and Cd contents in E. glabripetalus*

305 *seedlings*

306 Acid rain treatment alone decreased significantly the Mg content of stems and leaves  
307 compared with the control plants, while the slight decrease in roots was not significant  
308 (Table 3). When seedlings were treated by Cd alone, the Mg content of roots and  
309 stems decreased but not significantly ( $p > 0.05$ ). However, 50 mg·kg<sup>-1</sup> Cd increased  
310 the Mg content of leaves by 6.82%, while 100 mg·kg<sup>-1</sup> Cd decreased it by 30.68% ( $p <$   
311 0.05) in comparison with the control plants. When the seedlings were treated with the  
312 combined stress of pH 5.6 acid rain and Cd, the Mg content of roots decreased  
313 significantly ( $p < 0.05$ ), by 16.20% and 12.55% at 50 and 100 mg·kg<sup>-1</sup> Cd,  
314 respectively. Similarly, the Mg content of leaves decreased significantly compared  
315 with control plants ( $p < 0.05$ ) under the combined stress of acid rain and Cd. The total  
316 Mg content of plants decreased under acid rain or Cd stress alone and under the  
317 combined stress.

318 The acid rain treatment increased the Fe content in roots compared with the control  
319 plants ( $p < 0.05$ ) (Table 4). When the seedlings were treated by Cd alone, the Fe  
320 content of roots decreased significantly at 100 mg·kg<sup>-1</sup> Cd ( $p < 0.05$ ). However, 50  
321 mg·kg<sup>-1</sup> Cd increased the Fe content of stems ( $p < 0.05$ ). When the seedlings were  
322 exposed to pH 5.6 acid rain and 100 mg·kg<sup>-1</sup> Cd, the Fe content of roots decreased  
323 significantly compared with control plants ( $p < 0.05$ ). Mostly, there were no  
324 significant differences in the Fe contents of stems and leaves between the treatments  
325 and controls. The total Fe content of plants increased under acid rain stress alone  
326 (pH3.0,  $p < 0.05$ ) and under the combination of 50mg·kg<sup>-1</sup> Cd and acid rain, while it  
327 decreased under the combined stress of 100mg·kg<sup>-1</sup> Cd and acid rain ( $p < 0.05$ ).

328 The Cd content of plants was higher at 100 mg·kg<sup>-1</sup> Cd compared with 50 mg·kg<sup>-1</sup> Cd  
329 (Table 5). Most Cd distributed into roots under exposure to Cd alone, with the  
330 proportions equaling 91.27% and 91.45% at 50 and 100 mg·kg<sup>-1</sup> Cd, respectively.  
331 Acid rain was found to decrease the total Cd content of plants compared with the  
332 single Cd treatment. The total Cd content of plants decreased by 24.82% under the  
333 combined treatment with 50 mg·kg<sup>-1</sup> Cd and pH 3.0. However, in most cases, there  
334 were no significant differences between the effects of the single Cd treatment and the  
335 combined treatment on roots, stems or leaves.

336

#### 337 **4 Discussion**

338 It has been reported that single treatments with Cd or acid rain decrease  
339 photosynthesis (Parmar et al., 2013, Li et al., 2015, Sun et al., 2016). Chlorophyll  
340 fluorescence and photosynthetic parameters are easily affected by environmental  
341 stress and they are commonly used as sensitive indicators of a plant's photosynthetic  
342 performance. In our study on *E. glabripetalus* seedlings, the treatment by acid rain  
343 only increased  $F_v/F_m$ ,  $NPQ$ ,  $qP$ ,  $F_v/F_0$ ,  $A_{max}$  and  $AQY$ , which showed that the  
344 absorption of light energy, electron transport, conversion of light energy and net  
345 photosynthesis rate increased (Fig. 1 and Table 1). The results were in agreement with  
346 previous reports on the effects of acid rain on the photosynthesis in *E. glabripetalus*  
347 (Liu et al., 2015). Both Cd treatments decreased  $F_v/F_m$  and the 100 mg·kg<sup>-1</sup> Cd  
348 treatment decreased  $NPQ$ ,  $qP$ ,  $F_v/F_0$ ,  $A_{max}$  and  $AQY$ . These results showed that high  
349 Cd concentrations decrease the light reaction of photosynthesis, conversion of light  
350 energy and net photosynthesis rate, resulting in the inhibition of photosynthesis in *E.*

351 *glabripetalus* seedlings. The effects of Cd on *E. glabripetalus* seedling are similar to  
352 most reports on other plant species (Santos et al., 2012, Xue et al., 2013). An acid rain  
353 treatment alone or a high Cd concentration decreased  $L_{CP}$  and  $L_{SP}$  of *E. glabripetalus*  
354 seedlings, which indicated enhanced and reduced light use efficacy at low and high  
355 light levels, respectively. Interestingly, the combined treatment of Cd and acid rain did  
356 not alleviate the effect induced by the Cd treatment alone; instead, the combination  
357 decreased the chlorophyll fluorescence parameters induced by the Cd treatment. Thus,  
358 the combined stress led to a more serious decrease in the photosynthesis of *E.*  
359 *glabripetalus* seedlings. In addition, the degree of change in chlorophyll fluorescence  
360 and photosynthetic parameters in seedlings treated with Cd and acid rain depended on  
361 the concentration of Cd and the pH value used to simulate acid rain. In most cases, the  
362 combined treatment with Cd and acid rain decreased  $NPQ$ ,  $qP$  and  $F_v/F_0$ , and there  
363 was a greater inhibition in the absorption of light energy, electron transport and light  
364 reaction than under the Cd treatment alone (Fig.1).

365 Using two-way ANOVA analyses, we found that there are no significant interactive  
366 effects between Cd and acid rain on chlorophyll fluorescence and photosynthetic  
367 parameters mentioned above. However, the values of  $NPQ$ ,  $qP$ ,  $F_v/F_0$ ,  $A_{max}$  and  $L_{SP}$   
368 in *E. glabripetalus* seedlings exposed to the combined treatment with a high  
369 concentration of Cd ( $100 \text{ mg}\cdot\text{kg}^{-1}$ ) and low pH acid rain (pH 3.0) decreased more than  
370 those in seedlings exposed to other treatments, indicating that a high concentration Cd  
371 and low pH acid rain show a synergistic effect on most chlorophyll fluorescence  
372 parameters of *E. glabripetalus*. Our result is different from a previous report that

373 showed that there is an interactive effect of Cd and acid rain on chlorophyll  
374 fluorescence parameters in soybean seedlings (Sun et al., 2012). It has been  
375 previously shown that a high amount of Cd in soil can inhibit the chlorophyll  
376 biosynthesis, lead to the reduction in chlorophyll contents and photoactivation of PSII  
377 (Sun et al., 2012, Dias et al., 2013, Li et al., 2015). We conclude that in *E.*  
378 *glabripetalus* seedlings, the combination of low pH acid rain and a high Cd  
379 concentration would damage the chloroplast structure and lead to a greater decrease in  
380 the chlorophyll fluorescence parameters, net photosynthesis rate and light use  
381 efficacy.

382 In general, antioxidant metabolism is regarded as an important mechanism that helps  
383 to reduce the excessive accumulation of reactive oxygen species (ROS) induced by  
384 environment stress (Chaoui et al., 1997, Zhang et al., 2015). Acid rain or Cd stress  
385 can induce membrane damage, increase membrane permeability and the accumulation  
386 of free radicals in plants (Chaoui et al., 1997, Mobin and Khan, 2007, Debnath et al.,  
387 2018). However, active oxygen-scavenging systems can protect membranes from the  
388 deleterious effects (Jaleel et al., 2009). Previous studies have indicated that activities  
389 of antioxidant enzymes and relative electric conductivity are correlated with plant  
390 tolerance to abiotic stresses. Cd-induced damage has been shown to be negatively  
391 correlated with the capacity to increase POD and CAT activities and relative electric  
392 conductivity in many plants (Liu et al., 2017). According to our research, single acid  
393 rain increased the POD and CAT activities in leaves. Generally, Cd toxicity can  
394 increase (Srivastava et al., 2014) or decrease (Hassan et al., 2005) POD or CAT

395 activities depending on the stress intensity and duration or on the plant's  
396 developmental stage (Liu et al., 2017). However, a low Cd concentration (50 mg·kg<sup>-1</sup>)  
397 increased CAT activity, while a high Cd concentration (100 mg·kg<sup>-1</sup>) decreased the  
398 CAT activity and relative electrolyte leakage in leaves. This is in agreement with a  
399 report on soybean published by Liu (2011). Similarly to single Cd or acid rain  
400 treatments, the combined treatment with acid rain and Cd increased the POD activity  
401 significantly ( $p < 0.05$ ). The combination of a high Cd concentration (100 mg·kg<sup>-1</sup>)  
402 and acid rain (pH 3.0 and 5.6) inhibited the CAT activity ( $p < 0.05$ ), while the POD  
403 activity and relative electrolyte leakage increased (Fig.2). Thus, a moderate Cd  
404 concentration and acid rain induced lipid peroxidation and increased POD and CAT  
405 activities, while a high Cd concentration (100 mg·kg<sup>-1</sup>) inhibited the CAT activity and  
406 increased relative electrolyte leakage. The same effects have been observed in wheat  
407 treated with La<sup>3+</sup> (d'Aquino et al., 2009) and soybean treated with La<sup>3+</sup> and acid rain  
408 (Zhang et al., 2015). The increased POD and the decreased CAT could not alleviate  
409 the peroxidation damage to the cell membrane. The deleterious effects on the cell  
410 membrane aggravated the electrolyte leakage from the cytoplasm (the membrane  
411 permeability), as indicated by the increased relative electrolyte leakage. In addition,  
412 we analyzed the interaction between Cd and acid rain on the antioxidant enzyme  
413 system of *E. glabripetalus* seedlings and found that there was a synergistic effect of  
414 Cd and acid rain on the antioxidant enzyme system of *E. glabripetalus* seedlings.  
415  
416 GSH is a major low molecular-weight free thiol tripeptide in plant and animal cells. It

417 is involved in the cellular defense against the toxic action of oxyradicals, salinity,  
418 acidity and heavy metal (David et al., 2005). GSH serves as an important defense line  
419 against ROS to protect cells from oxidative stress damage and to modify metal  
420 toxicity . It has been reported that the GSH content of a cell could either decrease or  
421 increase under metal exposure (Maity et al., 2018, Kim et al., 2018). When *E.*  
422 *glabripetalus* seedlings were treated with either acid rain or a high concentration of  
423 Cd (100 mg·kg<sup>-1</sup>), the GSH content decreased. Also the combination of acid rain and  
424 100mg·kg<sup>-1</sup> Cd decreased the GSH content ( $p < 0.05$ ). This result indicated that GSH  
425 is involved in the cellular defense against acid rain and Cd stress and alleviates  
426 oxidative stress. It may be an antioxidant intervention strategy to prevent oxidative  
427 stress under Cd and acid rain stress.

428 The total biomass increased when *E. glabripetalus* seedlings were treated by acid rain,  
429 as also reported in our previous study (Liu et al., 2015). The reason may be that  
430 nitrogen (N) is added into the soil in the acid rain, which then accelerates the biomass  
431 accumulation. We found that a high pH value enhances biomass accumulation in  
432 leaves, while a low pH value facilitates root growth. However, a low Cd concentration  
433 (50 mg·kg<sup>-1</sup> Cd) (with or without acid rain) increases the total biomass, while a high  
434 Cd concentration (100 mg·kg<sup>-1</sup>) (with or without acid rain) decreases the total  
435 biomass. It has been found also in soybean that a low Cd concentration shows a  
436 positive effect on biomass accumulation (Liu et al., 2011). Yet, despite the different  
437 effects of Cd and acid rain the, two-way ANOVA analysis indicated that there was no  
438 significant interaction between Cd and acid rain on the total biomass of *E.*

439 *glabripetalus* seedlings.

440 Cd, which is a common heavy metal pollutant in soil, could accumulate within plant  
441 organs and negatively affect essential physiological processes, thus limiting plants'  
442 productivity (Gill et al., 2012). The exposure of *E. glabripetalus* to Cd stress leads to  
443 an accumulation of Cd mainly in roots (> 90%). These results are consistent with  
444 other reports showing that Cd is mainly retained in roots (Gill et al., 2012, Dias et al.,  
445 2013). Partitioning of heavy metals in different plant organs is a strategy to avoid  
446 toxicity for a plant (López-Climent et al., 2011, Dias et al., 2013).

447 Acid rain was found to decrease the total Cd content of plants compared with the  
448 single treatment of Cd. The total Cd content in plants decreased evenly by 24.8% at  
449 50 mg·kg<sup>-1</sup> Cd and pH 3.0 acid rain. There is a competition between H<sup>+</sup> and Cd<sup>2+</sup>  
450 uptake (Li et al., 2009). At low pH, Cd is likely to form insoluble precipitates with thiol  
451 base synthesized by sulphur ions under acid rain, which may inhibit the absorption of  
452 Cd and affect the Cd transportation in plants. However, in most cases, there were no  
453 significant differences between the effects of single Cd treatments and combined  
454 treatments on roots, stems or leaves. We suggest that an acid rain treatment inhibits *E.*  
455 *glabripetalus* seedlings from absorbing more Cd, leading to a higher accumulation of  
456 Cd in roots when compared to a single Cd treatment.

457 It is important to notice that Cd influences the uptake of other mineral nutrients in  
458 plant (Dias et al., 2013). In this study, we detected a significant reduction of Fe and  
459 Mg only at a high Cd concentration, which suggests that Cd interferes with the  
460 translocation of micronutrients to leaves and roots. Fe plays an important role in

461 electron transfer, because it is a cofactor in the photosynthetic processes, such as PSII  
462 and PSI. Although acid rain increases the total amount of Fe in plants, Cd-induced Fe  
463 deficiency affects the photosynthetic electron transport. In agreement with this, the  
464 reduction in  $F_v/F_m$  may be associated to the decreased amount of Fe. These results  
465 are similar to those reported in other plant species (López-Millán et al., 2009, Dias et  
466 al., 2013). Fe and Cd have similar binding sites at the cell; therefore, a high  
467 concentration of Cd leads to a decrease in Fe absorption by the cell. Mg is an essential  
468 component of the chlorophyll (Chl) molecule with a very important role in  
469 photosynthesis, such as Chl biosynthesis and photochemical reactions (Peng et al.,  
470 2015). In addition, Mg acts as a cofactor for many enzymes and functions in many  
471 other physiological processes, such as the formation of reactive oxygen species (ROS)  
472 and related photooxidative damage (Verbruggen and Hermans, 2013, Peng et al.,  
473 2015). The total Mg content in plants decreased under acid rain, Cd stress and the  
474 combined acid rain and Cd stress. The decrease in Mg might cause an increase in the  
475 production of ROS and antioxidant enzymes and, furthermore, impaired  
476 photosynthesis (Peng et al., 2015). Our results clearly showed that under a low Cd  
477 concentration and acid rain, decreases in Mg in the roots and leaves of *E.*  
478 *glabripetalus* caused decreases in  $A_{max}$  and  $L_{CP}$ , and increases in the POD and CAT  
479 activities, GSH content and relative electric conductivity.

480

## 481 **5 Conclusion**

482 (1) Combined treatment of a high concentration of Cd ( $100 \text{ mg}\cdot\text{kg}^{-1}$ ) and low pH acid  
483 rain (pH 3.0) decreased  $NPQ$ ,  $qP$ ,  $F_v/F_0$ ,  $A_{max}$  and  $L_{SP}$  in *E. glabripetalus* seedlings,  
484 indicating that Cd and acid rain have a synergistic effect on most chlorophyll  
485 fluorescence and photosynthesis parameters. (2) Combined treatment of a moderate  
486 concentration of Cd ( $50 \text{ mg}\cdot\text{kg}^{-1}$ ) and acid rain increased POD and CAT activities,  
487 GSH content and relative electric conductivity, while the combined stress with a high  
488 concentration of Cd and low pH acid rain decreased the GSH content and CAT  
489 activity. (3) Combined treatment of high concentration of Cd ( $100 \text{ mg}\cdot\text{kg}^{-1}$ ) and acid  
490 rain decreased the total biomass and changed biomass allocation into different plant  
491 organs. (4) Cd mainly accumulated in roots, and acid rain decreased the total Cd  
492 content. (5) Combined treatment of a high concentration of Cd ( $100 \text{ mg}\cdot\text{kg}^{-1}$ ) and acid  
493 rain decreased Fe and Mg contents, further resulting in the inhibition of  
494 photosynthesis, increases or decreases in the antioxidant enzyme activities, and  
495 reduction in biomass.

496 According to our results, a high concentration of Cd ( $100 \text{ mg}\cdot\text{kg}^{-1}$ ) and low pH acid  
497 rain (pH 3.0) have toxic effects on the physiological processes of plants. Our study  
498 provides novel insights into the combined effects of Cd and acid rain on woody plants  
499 and might also serve as a guide to evaluate forest restoration and biological safety in  
500 areas with Cd pollution in acid rain.

## 501 **Acknowledgements**

502 The study was supported by the Natural Science Foundation of Zhejiang Province  
503 (2015C33020), the National Key Research and Development Program of China

504 (2016YFD0600201) and the National Natural Science Fund (31570611, 31100325).

## 505 **References**

- 506 **Aebi H. 1984.** Catalase in vitro. *Methods in Enzymology*: Academic Press, New York.
- 507 **Chaoui A, Mazhoudi S, Ghorbal MH, Ferjani EE. 1997.** Cadmium and zinc induction of lipid  
508 peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.).  
509 *Plant Science*, **127**: 139e147.
- 510 **Chmielowska-Bąk J, Lefèvre I, Lutts S, Kulik A, Deckert J. 2014.** Effect of cobalt chloride on soybean  
511 seedlings subjected to cadmium stress. *Acta Societatis Botanicorum Poloniae*, **83**: 201-207.
- 512 **d'Aquino L, de Pinto MC, Nardi L, Morgana M, Tommasi F. 2009.** Effect of some light rare earth  
513 elements on seed germination, seedling growth and antioxidant metabolism in *Triticum*  
514 *durum*. *Chemosphere*, **75**: 900-905.
- 515 **David MC, Herminia LT, Andrea HN, Rafael MS. 2005.** Sulfur assimilation and glutathione metabolism  
516 under cadmium stress in yeast, protists and plants. *FEMS Microbiology Reviews*, **29**: 653-671.
- 517 **Debnath B, Irshad M, Mitra S, Li M, Rizwan HM, Liu S, Pan T, Qiu D. 2018.** Acid rain deposition  
518 modulates photosynthesis, enzymatic and non-enzymatic antioxidant activities in tomato.  
519 *International Journal of Environmental Research*, **12**: 203-214.
- 520 **Dias M, Monteiro C, Moutinho Pereira J, Correia C, Gonçalves B, Santos C. 2013.** Cadmium toxicity  
521 affects photosynthesis and plant growth at different levels. *Acta Physiologiae Plantarum*, **35**:  
522 1281-1289.
- 523 **Gill SS, Khan N, Tuteja N. 2012.** Cadmium at high dose perturbs growth, photosynthesis and nitrogen  
524 metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in  
525 garden cress (*Lepidium sativum* L.). *Plant Science*, **182**: 112–120.
- 526 **Griffith OW. 1980.** Determination of glutathione and glutathione disulfide using glutathione reductase  
527 and 2-vinylpyridine. *Analytical Biochemistry*, **106**: 207-212.
- 528 **Guo DP, Guo YP, Zhao JP, Liu H, Peng Y, Wang QM, Chen JS, Rao GZ. 2005.** Photosynthetic rate and  
529 chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsatsai*) after turnip  
530 mosaic virus infection. *Plant Science*, **168**: 57-63.
- 531 **Hangzhou Municipal Environmental Protection B. 2013.** Report on the State of the Environment in  
532 China. Hangzhou, China (In Chinese). 2012.
- 533 **Hassan MJ, Shao G, Zhang G. 2005.** Influence of cadmium toxicity on growth and antioxidant enzyme  
534 activity in rice cultivars with different grain cadmium accumulation. *Journal of Plant Nutrition*,  
535 **28**: 1259-1270.
- 536 **Jaleel CA, Manivannan P, Wahid A, Farooq M, Somasundaram R, Panneerselvam R. 2009.** Drought  
537 stress in plants: a review on morphological characteristics and pigments composition.  
538 *International Journal of Agriculture & Biology*, **11**: 100-105.
- 539 **Januškaitienė I. 2012.** The effect of cadmium on several photosynthetic parameters of pea (*Pisum*  
540 *sativum* L.) at two growth stages. *Žemdirbystė-Agriculture*, **99**: 71-76.
- 541 **Jin CX, Zhou QX, Zhou QX, Fan J. 2010.** Effects of chlorimuron-ethyl and cadmium on biomass growth  
542 and cadmium accumulation of wheat in the Phaozem area, Northeast China. *Bulletin of*  
543 *Environmental Contamination and Toxicology*, **84**: 395-400.
- 544 **Kim H, Kim JS, Kim PJ, Won EJ, Lee YM. 2018.** Response of antioxidant enzymes to Cd and Pb  
545 exposure in water flea *Daphnia magna*: differential metal and age — specific patterns.

546 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **209**: 28-36.

547 **Lambers H, Chapin FS, Pons TL. 1998.** *Plant physiological ecology*: Springer, New York.

548 **Li LZ, Zhou DM, Wang P, Jin SY, Peijnenburg WJ, Reinecke AJ, van Gestel CA. 2009.** Effect of cation

549 competition on cadmium uptake from solution by the earthworm *Eisenia fetida*.

550 *Environmental toxicology and chemistry*, **28**: 1732-8.

551 **Li SL, Yang WB, Yang TT, Chen Y, Ni WZ. 2015.** Effects of cadmium stress on leaf chlorophyll

552 fluorescence and photosynthesis of *elsholtzia argyi*—a cadmium accumulating plant.

553 *International Journal of Phytoremediation*, **17**: 85-92.

554 **Liao BH, Liu HY, Lu SQ, Wang KF, Probst A, Probst JL. 2003.** Combined toxic effects of cadmium and

555 acid rain on *Vicia faba* L. *Bulletin of Environmental Contamination and Toxicology*, **71**: 998-

556 1004.

557 **Liu HJ, Zhang CX, Wang JM, Zhou CJ, Feng H, Mahajan MD, Han XR. 2017.** Influence and interaction

558 of iron and cadmium on photosynthesis and antioxidative enzymes in two rice cultivars.

559 *Chemosphere*, **171**: 240-247.

560 **Liu MH, Yi LT, Yu SQ, Yu F, Yin XM. 2015.** Chlorophyll fluorescence characteristics and the growth

561 response of *Elaeocarpus glabripetalus* to simulated acid rain. *Photosynthetica*, **53**: 23-28.

562 **Liu TT, Wu P, Wang LH, Zhou Q. 2011.** Response of soybean seed germination to cadmium and acid

563 rain. *Biological Trace Element Research*, **144**: 1186-96.

564 **López-Climent MF, Arbona V, Pérez-Clemente RM, Gómez-Cadenas A. 2011.** Effects of cadmium on

565 gas exchange and phytohormone contents in citrus. *Biologia Plantarum*, **55**: 187-190.

566 **López-Millán AF, Sagardoy R, Solanas M, Abadía A, Abadía J. 2009.** Cadmium toxicity in tomato

567 (*Lycopersicon esculentum*) plants grown in hydroponics. *Environmental and Experimental*

568 *Botany*, **65**.

569 **Maity S, Banerjee R, Goswami P, Chakrabarti M, Mukherjee A. 2018.** Oxidative stress responses of

570 two different ecophysiological species of earthworms (*Eutyphoeus waltoni* and *Eisenia fetida*)

571 exposed to Cd-contaminated soil. *Chemosphere*, **203**: 307-317.

572 **Mobin M, Khan NA. 2007.** Photosynthetic activity, pigment composition and antioxidative response of

573 two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to

574 cadmium stress. *Journal of Plant Physiology*, **164**.

575 **Parmar P, Kumari N, Sharma V. 2013.** Structural and functional alterations in photosynthetic

576 apparatus of plants under cadmium stress. *Botanical Studies*, **54**: 45.

577 **Peng HY, Qi YP, Lee J, Yang LT, Guo P, Jiang HX, Chen LS. 2015.** Proteomic analysis of *Citrus sinensis*

578 roots and leaves in response to long-term magnesium-deficiency. *BMC Genomics*, **16**: 253.

579 **Ren X, Zhu J, Liu H, Xu X, Liang C. 2018.** Response of antioxidative system in rice (*Oryza sativa*) leaves

580 to simulated acid rain stress. *Ecotoxicology and Environmental Safety*, **148**: 851-856.

581 **Santos RW, Schmidt EC, Martins RDP, Latini A, Maraschin M, Horta PA, Bouzon ZL. 2012.** Effects of

582 cadmium on growth, photosynthetic pigments, photosynthetic performance, biochemical

583 parameters and structure of chloroplasts in the agarophyte *Gracilaria domingensis*

584 (Rhodophyta, Gracilariales). *American Journal of Plant Sciences*, **3**: 1077-1084

585 **Srivastava RK, Pandey P, Rajpoot R, Rani A, Dubey RS. 2014.** Cadmium and lead interactive effects on

586 oxidative stress and antioxidative responses in rice seedlings. *Protoplasma*, **251**: 1047-1065.

587 **Sun JW, Hu HQ, Li YL, Wang LH, Zhou Q, Huang XH. 2016.** Effects and mechanism of acid rain on plant

588 chloroplast ATP synthase. *Environmental Science & Pollution Research*, **23**: 1-11.

589 **Sun ZG, Wang LH, Chen MM, Wang L, Liang CJ, Zhou Q, Huang XH. 2012.** Interactive effects of

590 cadmium and acid rain on photosynthetic light reaction in soybean seedlings. *Ecotoxicology*  
591 *and Environmental Safety*, **79**: 62-68.

592 **Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN. 1985.** Effect of paclobutrazol on the  
593 activities of some enzymes of activated oxygen metabolism and lipid peroxidation in  
594 senescing soybean leaves. *Journal of Plant Physiology*, **121**: 453-461.

595 **Verbruggen N, Hermans C. 2013.** Physiological and molecular responses to magnesium nutritional  
596 imbalance in plants. *Plant and Soil*, **368**: 87-99.

597 **Xu H, Bi XH, Feng YC, Lin FM, Jiao L, Hong SM, Liu WG, Zhang XY. 2011.** Chemical composition of  
598 precipitation and its sources in Hangzhou, China. *Environmental Monitoring and Assessment*,  
599 **183**: 581-592.

600 **Xue XC, Gao HY, Zhang LT. 2013.** Effects of cadmium on growth, photosynthetic rate and chlorophyll  
601 content in leaves of soybean seedlings. *Biologia Plantarum*, **57**: 587-590

602 **Zhang X, Wang L, Zhou A, Zhou Q, Huang X. 2016.** Alterations in cytosol free calcium in horseradish  
603 roots simultaneously exposed to lanthanum(III) and acid rain. *Ecotoxicology and*  
604 *Environmental Safety*, **126**: 62-70.

605 **Zhang XB, Du YP, Wang LH, Zhou Q, Huang XH, Sun ZG. 2015.** Combined effects of lanthanum (iii) and  
606 acid rain on antioxidant enzyme system in soybean roots. *PLOS ONE*, **10**: e0134546.

607 **Zheng S, Pan T, Ma C, Qiu D. 2017.** Differential gene expression of longan under simulated acid rain  
608 stress. *Bulletin of Environmental Contamination and Toxicology*, **98**: 726-731.

609 **Zhou QX, Kong FX, Zhu L. 2004.** *Ecotoxicology (in Chinese)*. Science Press, Beijing.

610

611 Table 1 Effects of Cd and simulated acid rain on photosynthetic parameters, including  
 612 the maximum net photosynthetic rate ( $A_{max}$ ), apparent quantum yield ( $AQY$ ), light  
 613 saturation point ( $L_{SP}$ ) and light compensation point ( $L_{CP}$ ), in *Elaeocarpus*  
 614 *glabripetalus* seedlings.

615

Cd (mg·kg <sup>-1</sup> )	Acid rain pH	$A_{max}$ ( $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$AQY$ ( $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$L_{CP}$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$L_{SP}$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
0	7.0	4.71±0.02ab	0.025±0.001c	30.4±0.2ab	500.8±34.3a
	5.6	6.14±0.04a	0.053±0.001ab	24.0±0.3b	260.8±20.6bc
	3.0	5.23±0.18ab	0.025±0.001c	25.6±0.2ab	364.8±29.7b
50	7.0	7.19±0.04a	0.281±0.001a	14.4±0.9c	526.4±32.9a
	5.6	4.78±0.03ab	0.063±0.001ab	17.6±1.0bc	468.8±27.3ab
	3.0	5.42±0.19ab	0.045±0.001b	12.8±1.0c	569.6±47.3a
100	7.0	4.69±0.03b	0.149±0.001a	16.0±0.6bc	96.0±14.5c
	5.6	1.98±0.01c	0.019±0.002c	38.4±0.9a	174.4±13.3c
	3.0	2.87±0.01c	0.039±0.001bc	24.0±0.5b	228.8±28.6bc

616 Data are expressed as mean values ± SE of three replicates. Significant differences at  $p < 0.05$  are showed with  
 617 different letters in each column.

618 Table 2 Effects of Cd and simulated acid rain on the biomass in *Elaeocarpus*  
 619 *glabripetalus* seedlings

620

Cd (mg·kg <sup>-1</sup> )	Acid rain pH	Leaf biomass (g)	Stem biomass (g)	Root biomass (g)	Total biomass (g)
0	7.0	6.66±0.42b	11.12±0.99a	6.02±0.52b	23.80±1.76b
	5.6	7.64±0.84ab	10.33±0.87ab	9.65±0.82a	27.62±2.39a
	3.0	10.71±0.98a	10.25±0.97ab	7.75±0.85ab	28.71±0.14a
50	7.0	9.02±0.38ab	10.90±0.36a	8.74±0.67ab	28.65±1.10a
	5.6	7.92±0.78ab	9.34±0.56ab	7.34±0.51ab	24.60±0.23ab
	3.0	7.37±0.85ab	10.01±0.95ab	6.98±0.43ab	24.36±2.24ab
100	7.0	6.55±0.89b	8.78±0.42ab	5.84±0.67b	21.17±2.21bc
	5.6	6.61±0.88b	7.64±0.81b	6.37±0.78ab	20.62±2.88bc
	3.0	5.98±0.96b	7.06±0.67b	5.58±0.45b	18.62±2.76c

621 Data are expressed as mean values ± SE of three replicates. Significant differences at  $p < 0.05$  are showed with  
 622 different letters in each column.

623

624 Table 3 Effects of Cd and simulated acid rain on the Mg content in *Elaeocarpus*  
 625 *glabripetalus* seedlings

626

Cd (mg·kg <sup>-1</sup> )	Acid rain pH	Leaf Mg (mg·kg <sup>-1</sup> )	Stem Mg (mg·kg <sup>-1</sup> )	Root Mg (mg·kg <sup>-1</sup> )	Total Mg (mg·kg <sup>-1</sup> )
0	7.0	0.88±0.07a	0.69±0.05ab	8.21±0.12a	9.78±0.23a
	5.6	0.71±0.08c	0.65±0.06b	8.16±0.036ab	9.52±0.32a
	3.0	0.86±0.05b	0.56±0.04c	7.29±0.14ab	8.71±0.24b
50	7.0	0.94±0.01a	0.66±0.04b	7.23±0.41ab	8.83±0.13ab
	5.6	0.61±0.08d	0.61±0.03b	6.88±0.63b	8.10±0.12c
	3.0	0.63±0.05d	0.71±0.02ab	7.48±0.46ab	8.82±0.15ab
100	7.0	0.61±0.08d	0.67±0.07b	7.42±0.53ab	8.70±0.35b
	5.6	0.65±0.03cd	0.75±0.05a	7.18±0.55b	8.58±0.53bc
	3.0	0.64±0.08cd	0.64±0.04b	7.54±0.46ab	8.82±0.14ab

627 Data are expressed as mean values ± SE of three replicates. Significant differences at  $p < 0.05$  are showed with  
 628 different letters in each column.

629 Table 4 Effects of Cd and simulated acid rain on the Fe content in *Elaeocarpus*  
 630 *glabripetalus* seedlings

631

Cd (mg·kg <sup>-1</sup> )	Acid rain pH	Leaf Fe (mg·kg <sup>-1</sup> )	Stem Fe (mg·kg <sup>-1</sup> )	Root Fe (mg·kg <sup>-1</sup> )	Total Fe (mg·kg <sup>-1</sup> )
0	7.0	3.08±0.16b	3.21±0.23bc	6.67±0.62c	12.96±0.56b
	5.6	2.88±0.19b	3.35±0.22bc	9.50±0.31b	15.70±0.67ab
	3.0	3.09±0.21b	3.28±0.06bc	14.50±0.95a	20.87±0.87a
50	7.0	3.33±0.01ab	4.33±0.08a	6.83±0.22c	14.40±0.34ab
	5.6	3.35±0.34ab	3.59±0.15b	7.81±0.35c	14.70±0.44ab
	3.0	3.81±0.38a	4.49±0.02a	6.46±0.21c	14.76±0.49ab
100	7.0	2.79±0.11b	3.09±0.23c	4.82±0.41d	10.70±0.31c
	5.6	2.59±0.26b	3.02±0.11c	3.58±0.32d	9.19±0.24c
	3.0	2.65±0.02b	3.43±0.16bc	5.47±0.03c	12.55±0.35c

632 Data are expressed as mean values ± SE of three replicates. Significant differences at  $p < 0.05$  are showed with  
 633 different letters in each column.

634

635

636 Table 5 Effects of Cd and simulated acid rain on Cd content in *Elaeocarpus*

637 *glabripetalus* seedlings

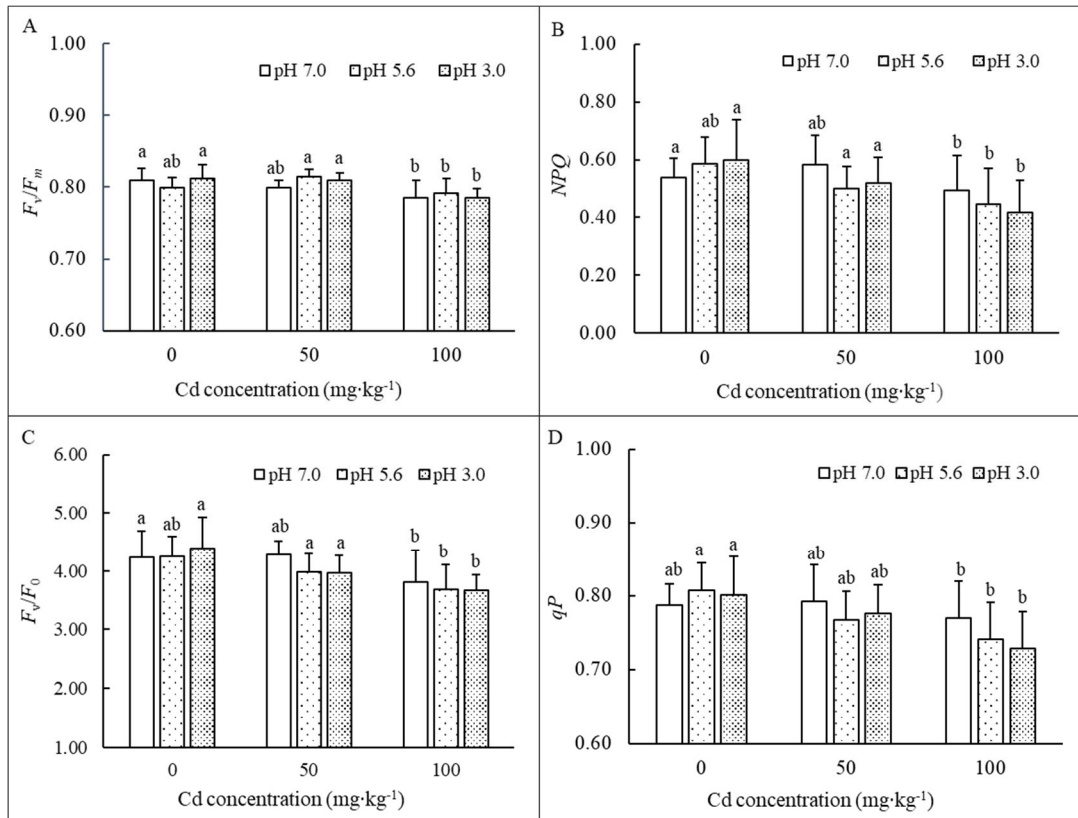
638

Cd (mg·kg <sup>-1</sup> )	Acid rain pH	Leaf Cd (mg·kg <sup>-1</sup> )	Stem Cd (mg·kg <sup>-1</sup> )	Root Cd (mg·kg <sup>-1</sup> )
50	7.0	0.010±0.006ab	0.29±0.04a	3.25±0.24ab
	5.6	0.008±0.0006b	0.23±0.05ab	2.77±0.28ab
	3.0	0.007±0.0002b	0.12±0.01b	2.55±0.16b
100	7.0	0.027±0.005a	0.33±0.03a	3.82±0.32a
	5.6	0.017±0.006ab	0.35±0.05a	3.17±0.42ab
	3.0	0.014±0.003ab	0.32±0.03a	3.49±0.25ab

639 Data are expressed as mean values ± SE of three replicates. Significant differences at  $p < 0.05$  are showed with

640 different letters in each column.

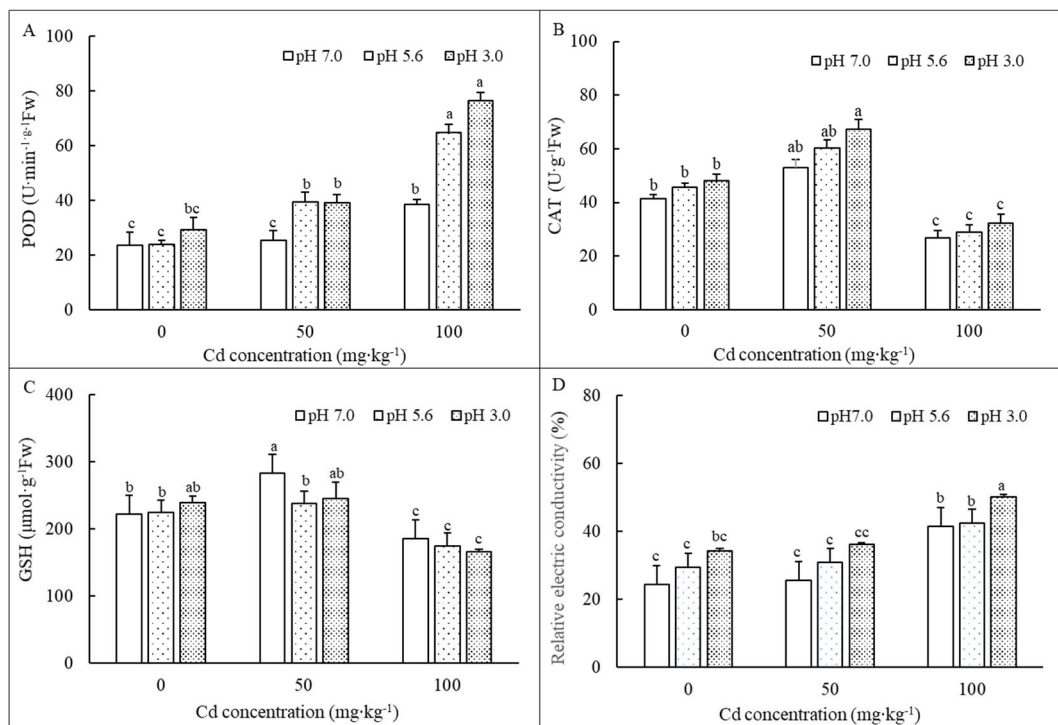
641 Fig.1 Effects of Cd and simulated acid rain on the maximum quantum yield ( $F_v/F_m$ ),  
 642 non-photochemical quenching of fluorescence ( $NPQ$ ), potential activity of PSII ( $F_v/F_0$ )  
 643 and photochemical quenching ( $qP$ ) in *Eleocarpus glabripetalus* seedlings.  
 644 Significant differences at  $P < 0.05$  are shown with different letters.



645

646

647 Fig.2 POD and CAT activities, GSH contents and relative electric conductivity in  
 648 *Elaeocarpus glabripetalus* seedlings under acid rain and Cd treatments. Different  
 649 lowercase letters denote significant differences between treatments at the 5% level.



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