Toxicity of Cu, Pb, and Zn on Seed Germination and Young Seedlings of Wheat (Triticum aestivum L.)

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Abstract. Seeds and young seedlings of wheat (*Triticum aestivum L.*) under exposure to Copper (Cu), lead (Pb) and Zinc (Zn) were studied by employing a hydroponic experiment. Addition of Cu or Pb or Zn to the solution inhibited seed germination, plumule and radicle elongation. The toxic of metals to seed germination parameters can be arranged in the rank order of inhibition as follows: Cu > Pb >> Zn. This study showed the contents of chlorophyll and soluble protein in young seedlings of wheat were decreased after 4 d of all heavy metal treatments. Among the tested metals, the toxic of metal to young seedlings was found similar to seeds. All heavy metal concentrations in seedlings increase with their increase in the medium and the duration of treatments. On the other hand, the results demonstrated that the excess accumulation of Cu in leaf and roots could reduce accumulations of zinc (Zn); the excess accumulation of Pb decreased the levels of Cu or Zn in leaf and roots; the excess accumulation of Zn had no influence on Cu accumulations in leaf and roots.

Keywords: Heavy metals, Seed, Germination parameters, Seedling

1 Introduction

Toxic heavy metals have no function to organism and can be highly toxic when their concentrations are exceeded threshold value. Other heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species [4, 5]. Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals [6]. Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants [7, 8]. Most of these studies have been conducted using seedlings or adult plants. In a few studies, the seeds have been exposed to the metals [5]. All data shows heavy metals delay the normal growth periods of plants.

Seed germination is a process of crucial importance and as such it must be tightly regulated. In the plant's life cycle, seed is a stage that is well protected against various stresses. However, soon after imbibitions and subsequent vegetative developmental processes, they become stress sensitive in general. Therefore, seeds are equipped with sensing mechanisms that allow it to obtain the information required to assure that germination will only occur when environmental factors are favorable to complete developmental processed. The effects of metals in seed germination had been investigated. Li et al. [9] reported toxic heavy metals could significantly inhibit *Arabidopsis thaliana* seed germination, plumule and radicle growth.

The accumulation of metals, the content of total soluble protein, and chlorophyll in wheat seedlings were further examined in order to disclose the toxicity mechanisms of metals in plants. Thereafter, it could be helpful to improve the plant growth security in polluted soil.

2 Materials and methods

2.1 Plant materials and heavy metal treatments

The heavy metals used in this study (Cu, Pb and Zn) were in the form of nitrates. Seven concentrations (between 0 and 250 mM) of each metal were used in this study. The selected concentrations of Cu, Pb and Zn were 0.5 1, 10, 50, 100 and 250 mM, respectively. For each treatment, the pH was adjusted to 6.5. Wheat seeds were purchased from a local seed market. The seeds were sterilized in 10% Na-hypochlorite solution for 20 min to prevent fungal growth, washed with distilled water for several changes.

A second hydroid culture experiment was performed to determine whether the supply of Cu, Pb and Zn would inhibit seedling growth (e.g. Chlorophyll and soluble protein content) or whether descend mineral nutrient metal uptake. Ten-day-old wheat seedlings were exposed to Cu, Pb and Zn (1 and 50 mM, respectively) and studied during 4 d.

2.2 Determination of germination parameters

Seed germination and shoot/root elongation test on filter paper was carried out in glass Petri dishes (90 × 15 mm) with three layers of filter paper on the bottom. Each dish contained 9 mL of metal solution or 9 mL of distilled water (control), and 100 seeds, covered by lid. Petri dishes containing seeds were incubated for 8 d in a dark chamber at 28 ºC ± 1 ºC. Number of geminated seeds was counted 3 d after the treatments. Radicle and plumule lengths were recorded at 4 d, 6d and 8d. Each treatment was in triplicate.

2.3 Determination of metal contents

The uniform wheat seedlings at 4 d after the treatments were immerged in ice Tris-Mes solution for 15 min, rinsed with demonized water thoroughly, and then plant material was dried at 70 °C for 2 d, weighed and then milled. Samples were digested in a 3 : 1 mixture of HNO_3 : $HClO_4$. The digests were used to determine cations, by ICP-OES. All measurements were repeated in triplicate.

2.4 Determination of Chlorophyll and protein

Total chlorophylls were extracted with 80% acetone and estimated according to Arnon [10]. Total soluble protein content was determined by the method of Bradford [11] using BSA as a standard.

3 Results and Discussion

3.1 Effects of heavy metals on wheat seed germination

Table 1. Seed germination rate (%) compared to the control at different heavy metal concentration.

Metal dose (mM)	Cu		Pb		Zn	
	Germination rate (%)	Inhibitory rate (%)	Germination rate (%)	Inhibitory rate (%)	Germination rate (%)	Inhibitory rate (%)
0	100.0 ± 5.6	0	100.0 ± 5.6	0	100.0 ± 5.6	0
0.5	95.1 ± 2.6	4.9	94.7 ± 1.5	5.3	99.3 ± 0.6	0.6
1.0	93.7 ± 1.2	6.3	90.3 ± 2.4	9.7	99.3 ± 0.6	0.7
10.0	73.3 ± 7.6	26.7	86.1 ± 1.2	13.9	98.1 ± 1.7	1.9
50.0	35.2 ± 5.1	64.8	46.3 ± 1.0	53.7	91.3 ± 1.5	8.7
100.0	n.d.	100	18.7 ± 6	81.3	82.3 ± 2.5	17.7
250.0	n.d.	100	n.d.	100.	52.1 ± 1.9	47.9

The data of seed germination rate are means s.e. from three experiments.

The germination percentages of wheat seeds exposed to different concentrations of metals were given in Table 1. The seed germination rate over control decreased significantly with increasing metal concentration. The toxic of metals to seed germination parameters can be arranged in the rank order of inhibition as follows: Cu > Pb >> Zn. Germination inhibitory rate of Cu increased by 4.9%, 6.3%, 26.7%, 64.8%, 100% and 100% at 0.5, 1, 10, 50, 100 and 250 mM, respectively; the increase was 0.6%, 0.7%, 1.9%, 8.7%， 17.7% and 47.9% at 0.5, 1, 10, 50, 100 and 250 mM, respectively, for Zn; and the increase 5.3%, 9.7%, 13.9%, 53.7, 81.3% and 100% at 0.5, 1, 10, 50, 100 and 250 mM, respectively, for Pb. The seed germination rate was found no affected by Cu and Pb at lower concentrations of 0.5 and 1 mM, while significantly affected by Cu and Pb at higher concentration of metal ranging from 10 to 250 mM. Wheat seed was able to germinate at all Zn concentration evaluated, even at a concentration 250 mM in this study. Jadia and Fulekar [12] obtained similar results in a study using Cd, Cu, Ni, Pb and Zn on sunflower seeds. Salvatore et al. [3] also reported low metal concentrations were observed to have no effect on seed germination while the inhibiting effect of heavy metals on seed germination was tested at high concentrations, such as to reduce the germination percentage.

3. 2 Effects of heavy metals on seedling growth

In the present experiment, the toxicological effects of metals (Cu, Pb, Zn) on the wheat seedling root and shoot growth were observed. The inhibitory effects of metals on the radicle and plumule lengths of young seedlings were evaluated (Table 2 and 3). Based on these values, metals can be arranged in a rank order of inhibition as follows: Cu > Pb >> Zn. The metal toxic rank of plumule elongation is similar with the result of radicle elongation; the only difference is the three metal concentrations which caused significant inhibition for plumule growth was much higher than that for radicle growth. That can be explained that metal accumulation in roots of wheat seedling was more than those of in leaves under the same metal treatment.

Table 2. Effect of metals on radicle elongation of wheat on filter Paper.

Metal	Dose (mM)	4 d Radicle length (cm)	4 d Inhibitory rate (%)	6 d Radicle length (cm)	6 d Inhibitory rate (%)	8 d Radicle length (cm)	8 d Inhibitory rate (%)
Cu	0	3.7±0.9	0.0	7.6 ± 0.5	0.0	9.7 ± 0.3	0.0
	0.5	1.7 ± 0.5	54.1	1.8 ± 0.2	76.3	2.2 ± 0.1	77.3
	1.0	0.8 ± 0.1	78.4	1.2 ± 0.2	84.2	1.4 ± 0.3	85.6
	10.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	50.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	100.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
Pb	0	3.7	0.0	7.6 ± 0.5	0.0	9.7 ± 0.3	0.0
	0.5	3.4 ± 0.2	8.1	6.4 ± 0.2	15.8	7.9 ± 0.5	18.6
	1.0	2.5 ± 0.1	32.4	5.9 ± 0.7	22.4	6.0 ± 0.3	38.1
	10.0	0.2 ± 0.0	94.6	0.1 ± 0.0	98.7	0.2 ± 0.1	97.9
	50.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	100.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
Zn	0	3.7 ± 0.2	0.0	7.6 ± 0.5	0.0	9.7 ± 0.3	0.0
	0.5	4.5 ± 0.1	-21.6	5.0 ± 0.2	34.2	6.2 ± 0.4	36.1
	1.0	3.6 ± 0.1	2.7	4.8 ± 0.4	36.8	6.1 ± 0.7	37.1
	10.0	0.6 ± 0.0	83.8	0.7 ± 0.5	90.8	0.8 ± 0.2	91.8
	50.0	0.3 ± 0.0	91.9	0.3 ± 0.1	96.1	0.3 ± 0.1	96.9
	100.0	0.2 ± 0.0	94.6	0.2 ± 0.0	97.4	0.2 ± 0.0	97.9
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0

The data of radicle elongation are means ± s. e. from three experiments.

Fargašová [4] reported mustard seedlings (*Sinapis alba L.*) exhibited some symptoms of metal toxicity (e.g. reduced growth, chlorosis). The toxicity effect of the trace elements on the mustard seedling growth was, in descending order of damage, Cu > Se > Cd > Zn >> Pb. Cu is introduced as very toxic metals to many plants [13] and this statement was fully confirmed during our experiments with wheat seedlings. A good agreement for the Cu inhibitory effect was found for the root and shoot elongation of wheat seedlings as compared with those reported by Fargašová [4] for mustard seedlings. However, the difference was in the position of Zn, which was for mustard introduced as more toxic than Pb. Zinc indicated the lowest inhibitory effect on the root and shoot elongation of wheat in our tests corresponds with the values introduced by Mahmood et al. [14]. Even, Zn at 0.5 mM concentration promoted the root growth over the control root size by approximately 22.9% at the 4 dth of treatment. The similar effect also was reported by Jadia and Fulekar [12].

Table 3. Effect of metals on plumule elongation of wheat on filter Paper.

Metal	Dose (mM)	4 d Plumule length (cm)	4 d Inhibitory rate (%)	6 d Plumule length (cm)	6 d Inhibitory rate (%)	8 d Plumule length (cm)	8 d Inhibitory rate (%)
Cu	0	3.2±0.5	0.0	6.5±1.3	0.0	10.1±1.8	0.0
	0.5	2.9±0.5	9.4	3.2±1.0	50.8	6.8±0.8	32.7
	1.0	1.3±0.2	59.4	3.2±0.8	50.8	5.7±0.7	43.6
	10.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	50.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	100.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
Pb	0	3.2±0.5	0.0	6.5±1.3	0.0	10.1±1.8	0.0
	0.5	2.9±0.4	9.4	4.5±0.7	30.8	8.6±1.3	14.9
	1.0	2.7±0.5	15.6	4.2±0.8	35.4	8.2±1.8	18.8
	10.0	0.6±0.1	81.3	2.3±0.7	64.6	4.0±1.0	60.4
	50.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	100.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0
Zn	0	3.2±0.5	0.0	6.5±1.3	0.0	10.1±1.8	0.0
	0.5	2.9±0.5	9.4	3.5±1.7	46.2	7.6±1.2	24.8
	1.0	2.1±0.3	34.4	2.7±0.8	58.5	6.7±1.0	33.7
	10.0	1.4±0.3	56.3	2.1±0.4	67.7	2.9±1.0	71.3
	50.0	0.7±0.1	78.1	1.1±0.2	83.1	1.7±0.5	83.2
	100.0	0.2±0.1	93.8	0.3±0.1	95.4	0.4±0.1	96.0
	250.0	n.d.	100.0	n.d.	100.0	n.d.	100.0

The data of plumule elongation are means ± s. e. from three experiments.

3.3 Cu, Pb and Zn in wheat plants

In general, all heavy metal concentrations in seedlings increase with their increase in the medium and the duration of treatments (Table 4). The heavy metals, Cu, Pb and Zn were taken up by shoot and root both; and all metal concentrations were found to be higher in roots. The heavy metals were untaken by the wheat plants in the following order: Zn > Cu > Pb. Jadia and Fulekar [12] demonstrated the heavy metals were accumulated by the sunflower plants in the following order: Zn > Cu > Cd > Ni > Pb and this rank order corresponds with the results obtained for wheat in here presented study. On the other hand, the results demonstrated that the excess accumulation of Cu in leaf and roots could reduce the level of zinc (Zn); the excess accumulation of Pb decreased the level of Cu or Zn in leaf and roots; the excess accumulation of Zn had no influence on Cu accumulations in leaf and roots.

Normal and phytotoxic concentrations of Pb, Zn and Cu were reported by Levy et al. [15], which were 0.5 - 10 and 30 - 300mg kg^{-1} for Pb, 3 - 30 and 20 - 100mg kg^{-1} for Cu, and 10 - 150 and > 100 mg kg^{-1} for Zn. In the present study, Cu and Pb were observed that they could interfere with the absorption process of Zn or Cu as nutrient element when heavy metal concentration in wheat seedling is higher than the normal or phytotoxic levels.

Heavy metals can bind strongly to oxygen, nitrogen and sulphur atoms. Because of these feature, heavy metals can inactivate enzymes by binding to cysteine residues and block the essential biological function of enzyme. Heavy metal can also displace the essential metal ions in bimolecular. Many enzymes contain metals in positions important for their activity. Therefore, the displacement of one metal by another will normally also lead to inhibition or loss of enzyme activities [16]. Goyer [17] introduced lead may interact metabolically with nutritionally essential metals and replace zinc on heme enzymes.

The displacement mechanism of toxicity is best explained by classification of the metal ions according to their binding preference, i.e. oxygen-seekong class A metals, nitrogen- or sulfur- seeking class B metals, and the bordenline metals show more or less the same preference of bonding to O-, S-or N-containing ligands [18]. The class B (Cu and Pb) metals readily displace either borderline (Zn) or class A (Mg or Ca) metals from essential binding sites through much more tenacious bonding mechanisms. Borderline metals become toxicants when they displace Class A metals from essential binding sites in biomolecular, bonding to these sites more strongly. Besides, they are the most effective binders to SH group and nitrogen containing groups at the catalytically active centers in the enzymes.

Table 4. Effect of metals on the accumulation of metals in leaves and in roots.

Time (d)	Heavy metal accumulation (mg gDW^{-1})													
	Cu treatment (mM)				Pb treatment (mM)						Zn treatment (mM)			
	1		50		1			50			1		50	
	Cu	Zn	Cu	Zn	Pb	Cu	Zn	Pb	Cu	Zn	Cu	Zn	Cu	Zn
Leaves														
0	0.012 ± 0.002	0.089 ± 0.011	0.012 ± 0.001	0.089 ± 0.002	0.004 ± 0.001	0.012 ± 0.011	0.089 ± 0.031	0.004 ± 0.001	0.012 ± 0.004	0.089 ± 0.011	0.012 ± 0.001	0.089 ± 0.021	0.012 ± 0.004	0.089 ± 0.035
1	0.029 ± 0.013	0.105 ± 0.061	1.562 ± 0.101	0.071 ± 0.011	0.014 ± 0.006	0.017 ± 0.018	0.168 ± 0.009	0.021 ± 0.014	0.024 ± 0.009	0.229 ± 0.081	0.079 ± 0.031	0.282 ± 0.065	0.026 ± 0.011	4.693 ± 1.801
2	0.067 ± 0.021	0.108 ± 0.027	5.317 ± 0.206	0.218 ± 0.013	0.027 ± 0.008	0.041 ± 0.016	0.165 ± 0.081	0.034 ± 0.013	0.035 ± 0.011	0.170 ± 0.091	0.072 ±0.027	0.292 ± 0.078	0.025 ± 0.009	5.765 ± 1.105
3	0.114 ± 0.001	0.121 ± 0.001	6.546 ± 0.401	0.179 ± 0.043	0.056 ± 0.003	0.042 ± 0.011	0.206 ± 0.161	0.137 ± 0.051	0.017 ± 0.007	0.099 ± 0.044	0.031 ± 0.021	0.302 ± 0.032	0.032 ± 0.021	8.521 ± 2.563
4	0.373 ± 0.022	0.080 ± 0.021	7.670 ± 0.541	0.135 ± 0.031	0.065 ± 0.005	0.012 ± 0.034	0.088 ± 0.081	0.153 ± 0.042	0.013 ± 0.005	0.093 ± 0.034	0.016 ± 0.009	0.295 ± 0.034	0.031 ± 0.019	9.302 ± 1.536
Roots														
0	0.012 ± 0.007	0.089 ± 0.054	0.012 ± 0.001	0.089 ± 0.033	0.004 ± 0.001	0.012 ± 0.001	0.089 ± 0.041	0.004 ± 0.001	0.012 ± 0.001	0.089 ± 0.012	0.012 ± 0.001	0.089 ± 0.001	0.012 ± 0.014	0.089 ± 0.031
1	0.469 ± 0.032	0.332 ± 0.142	2.828 ± 0.931	0.071 ± 0.054	1.241 ± 0.912	0.038 ± 0.011	0.178 ± 0.013	1.347 ± 0.931	0.038 ± 0.007	0.597 ± 0.074	0.072 ± 0.037	1.129 ± 0.001	0.021 ± 0.011	14.539 ± 3.652
2	1.318 ± 0.873	0.449 ± 0.213	7.601 ± 1.032	0.156 ± 0.076	1.512 ± 0.986	0.027 ± 0.011	0.284 ± 0.073	1.699 ± 0.531	0.078 ± 0.015	0.575 ± 0.067	0.074 ± 0.023	1.567 ± 0.001	0.018 ± 0.005	19.155 ± 4.051
3	1.329 ± 0.972	0.371 ± 0.175	8.188 ± 2.043	0.129 ± 0.056	1.742 ± 0.838	0.045 ± 0.043	0.478 ± 0.053	2.263 ± 0.801	0.024 ± 0.013	0.303 ± 0.015	0.047 ± 0.017	2.100 ± 0.001	0.017 ± 0.001	22.514 ± 5.352
4	1.427 ± 0.874	0.334 ± 0.231	9.115 ± 2.045	0.125 ± 0.063	2.043 ± 0.971	0.036 ± 0.031	0.317 ± 0.034	3.123 ± 1.454	0.022 ± 0.009	0.273 ± 0.017	0.038 ± 0.012	2.663 ± 0.001	0.016 ±0.003	23.933 ± 6.432

The data of metal concentrations are means ± s. e. from three experiments.

3.4 Effects of metals on total soluble protein and chlorophyll (a+b) content of wheat

The alteration of total soluble protein level in leaves of wheat seedlings was presented in Table 5. Total soluble protein levels and the inhibition rate in leaves decreased significantly with increasing concentrations and treatment time of metals in solution. The toxicity effect of metal on total soluble protein was arranged in a rank order of inhibition as follow: Cu> Pb >Zn. There was a negative correlation between metal concentration and total soluble protein content in leaves of wheat seedlings.

The data of the chlorophyll content of the wheat seedlings exposed to differing concentrations of metals were also shown in Table 5. Heavy metals accumulation in plants can result in a decrease of the chlorophyll concentration in the leaves/stems [19]. In the present study, chlorophyll (a+b) content declined progressively with increasing concentrations of heavy metals. Cu accumulation showed a significant inhibitory effect on chlorophyll (a+b). An insignificant decrease in chlorophyll content in wheat seedlings was observed after Zn and Pb accumulation, especially Zn. The statement was supported by Shakya et al. report [20]. Zinc has long been considered to be one of the least toxic metals in the environment [21]. Compared with level of metal application, the toxicity of metals is distinguished from different applied concentrations. The most toxic metal among three metals is Cu at 1 mM dose. Pb is the most toxic metal at 50 mM, although, Pb accumulation in leaves is obviously lower than others metals accumulation after 4 d treatment. High concentrations of Cu are known to activate oxidative damage and alter cell-membrane properties by lipid peroxidation, thereby demonstrating the inhibitory effect on the enzymes involved in chlorophyll production. Lead can be toxic to photosynthetic activity, chlorophyll synthesis and antioxidant enzymes [22].

4 conclusion

In the present study, addition of Cu or Pb or Zn inhibited seed germination, plumule and radicle elongation. The toxic of metals to seed germination parameters can be arranged in the rank order of inhibition as follows: Cu > Pb >> Zn. All heavy metal concentrations in seedlings increase with their increase in the medium and the duration of treatments. The toxic of metals to young seedlings was found similar to seeds. And heavy metal treatments could reduce the contents of chlorophyll and soluble protein in young seedlings of wheat. On the other hand, the results demonstrated that the excess accumulation of Cu in leaf and roots could reduce accumulations of zinc (Zn); the excess accumulation of Pb decreased the levels of Cu or Zn in leaf and roots; the excess accumulation of Zn had no influence on Cu accumulations in leaf and roots.

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Table 5. Effect of metals on total soluble protein and chlorophyll (a+b) content of the leaves.

Metal	Time (d)	Dose (mM) 1				Dose (mM) 50			
		Chl (a+b) (mg/g FW)	Inhibitory rate (%)	Soluble protein (mg/g FW)	Inhibitor y rate (%)	Chl (a+b) (mg/g FW)	Inhibitory rate (%)	Soluble protein (mg/g FW)	Inhibitory rate (%)
Cu									
	0	4.43 ± 0.03	0.0	1.26 ± 0.01	0.0	4.43 ± 0.03	0.00	1.26 ± 0.01	0.0
	1	4.33 ± 0.11	2.2	0.98 ± 0.02	21.6	3.95 ± 0.31	11.3	0.71 ± 0.07	43.6
	2	4.36 ± 0.09	2.5	0.88 ± 0.02	29.7	5.69 ± 0.21	-28.6	0.61 ± 0.03	51.6
	3	2.98 ± 0.06	33.3	0.82 ± 0.03	35.1	4.38 ± 0.25	1.5	0.39 ± 0.0	69.3
	4	2.94 ± 0.03	34.1	0.50 ± 0.06	60.1	2.96 ± 0.08	33.4	0.22 ± 0.06	82.5
Pb									
	0	4.43 ± 0.03	0.0	1.26 ± 0.01	0.0	1.26 ± 0.01	0.0	1.26 ± 0.01	0.0
	1	4.45 ± 0.32	0.0	0.92 ± 0.03	26.8	1.09 ± 0.09	13.1	1.09 ± 0.08	13.1
	2	4.42 ± 0.21	0.0	0.91 ± 0.06	27.3	1.06 ± 0.06	15.3	1.06 ± 0.05	15.3
	3	3.22 ± 0.15	27.2	0.92 ± 0.04	26.4	0.84 ± 0.05	33.4	0.84 ± 0.04	33.4
	4	3.59 ± 0.12	19.1	0.75 ± 0.05	40.1	0.78±0.03	37.6	0.78 ± 0.03	37.6
Zn									
	0	4.43 ± 0.03	0.00	1.26 ± 0.01	0.0	4.43 ± 0.01	0.00	1.26 ± 0.01	0.0
	1	3.66 ± 0.07	17.2	0.87 ± 0.03	30.6	6.45 ± 0.14	-45.2	0.65 ± 0.04	48.2
	2	3.04 ± 0.05	31.4	0.80 ± 0.07	35.9	5.21 ± 0.26	-18.4	0.76 ± 0.03	39.2
	3	3.22 ± 0.03	27.3	0.97 ± 0.03	22.4	5.11 ± 0.32	-15.4	0.39 ± 0.06	69.0
	4	4.57 ± 0.06	-3.1	0.62 ± 0.05	50.8	4.15 ± 0.17	6.5	0.55 ± 0.05	55.9

The data of chlorophyll and protein contents are means ± s. e. from three experiments.

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