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ORIGINAL PAPER

Zinc conferred cadmium tolerance in *Lemna minor* L. via modulating polyamines and proline metabolism

Xuqiang Qiao · Penghe Wang · Guoxin Shi · Haiyan Yang

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Abstract To investigate the alleviating effects of zinc (Zn) against gradually increasing cadmium (Cd) stress in aquatic environment, dry weight, polyamines and proline contents as well as metabolic enzymes were studied in *Lemna minor* L. after 4 days exposure. Dry weight was significantly decreased as the concentration of Cd increased. Cd stress also increased the putrescine (Put) content, while decreasing spermidine (Spd) content, whereas no significant change was observed in spermine (Spm) content. Hence, the ratio of (Spd + Spm)/Put rapidly reduced. In addition, the activities of arginine decarboxylase (ADC), ornithine decarboxylase and polyamine oxidase (PAO) enhanced accordingly. Cd treatment also induced a continuous accumulation of proline. Meanwhile, pyroline-5-carboxylate synthetase (P5CS) activity increased initially only to decline later and ornithine δ-aminotransferase (OAT) activity was only significantly stimulated at 4 μM Cd, while the proline dehydrogenase (PDH) activity declined. However, Zn supplementation lowered accumulation of Put and proline contents and raised the Spd content, via decreasing the activities the ADC and PAO and keeping the activities of P5CS, OAT and PDH at the control levels, but failed to generate a statistically significant difference in content of dry weight. These results suggested that Zn application can maintain polyamines and proline homeostasis, thus conferring the tolerance of *L. minor* to Cd.

Keywords Zn · Cd · Polyamines · Proline · *Lemna minor* L.

Abbreviations

ADC	Arginine decarboxylase
Agm	Agmatine
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra acetic acid
FW	Fresh weight
OAT	Ornithine δ-aminotransferase
ODC	Ornithine decarboxylase
P5CS	Pyroline-5-carboxylate synthetase
PAO	Polyamine oxidase
PAs	Polyamines
PDH	Proline dehydrogenase
PIS-bound	Perchloric acid insoluble bound
PLP	Pyridoxal phosphate
PMSF	Phenylmethylsulfonylfluoride
PS-conjugated	Perchloric acid soluble conjugated
Put	Putrescine
PVP	Polyvinylpyrrolidone
Spd	Spermidine
Spm	Spermine

Xuqiang Qiao and Penghe Wang are co-first authors.

X. Qiao
College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, Jiangsu, People's Republic of China

P. Wang
Institute of Wetland Ecology, School of Life Science, Nanjing University, Nanjing 210093, Jiangsu, People's Republic of China

G. Shi (✉)
College of Life Science, Nanjing Normal University, Nanjing 210046, Jiangsu, People's Republic of China
e-mail: xuqiang514@126.com

H. Yang
Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences, Nanjing 210046, Jiangsu, People's Republic of China

Introduction

Cadmium (Cd) is a kind of toxic heavy metal to most plants which generates from industrial and agricultural activities and ubiquitously exists in water (Sun et al. 2009). It is readily taken up by aquatic plants and is toxic at cellular, physiological, biochemical, and molecular levels, including growth retardation, inhibition of photosynthesis, induction and inhibition of enzymes, disruptions in water relations, and ultrastructural changes (Ding et al. 2007; Piotrowska et al. 2010; Prasad 1995; Singh et al. 2006; Tripathi et al. 1996; Yang et al. 2010). The trace metal zinc (Zn) is essential to all organisms functioning as a co-factor in a variety of biomacromolecules, including metabolic enzymes, transcription factors and cellular signaling proteins (Mendoza-Cozatl et al. 2005; Morrissey and Guerinot 2009; Palmer and Guerinot 2009). Due to chemical similarity between Cd and Zn, they both can be taken by plants as divalent cations and therefore moderate Zn supply can alleviate the effect caused by Cd in plants. Early studies have demonstrated that supplemented with Zn could suppress Cd accumulation, increase plant biomass, affect energy transfer in photosystem II and photochemical quenching, lower level of reactive oxidative species (ROS), protein and DNA damages (Aravind et al. 2009; Balen et al. 2011; Bunluesin et al. 2007; Malec et al. 2008). However, less is known about polyamines and proline metabolism. In Arabidopsis, differential expression of ADC has been detected under stress conditions like drought, high salinity, mechanical injury, potassium deficiency (Alcázar et al. 2006; Hummel et al. 2004; Peréz-Amador et al. 2002; Urano et al. 2003). In addition, Yang et al. (2011) and Chen et al. (2001) have demonstrated that increased level of proline in response to lead and copper treatment is due to the elevations of OAT or GK activities in wheat and rice seedlings.

Polyamines are polybasic aliphatic amines that play a major role in various physiological and developmental processes in plants (Martin-Tanguy 2001). They may work as an antioxidant, a free radical scavenger and a membrane stabilizer (Larher et al. 2003; Velikova et al. 2000) and contribute in enhancing tolerability (Alcázar et al. 2006; Groppa and Benavides 2008). Moreover, it was found that aquatic plants may accumulate PAs as cellular reducing agents in response to the increase in ROS production triggered by Cd (Verbruggen et al. 2009). Proline has also been widely reported presenting a remarkable accumulation in response to heavy metal exposure among plants (Chen et al. 2001; Radic et al. 2010; Zengin and Munzuroglu 2005). Simultaneously, proline accumulation was demonstrated that it is positively correlated with Put, Spd and titers of total polyamines in Cd-treated pear shoots (Wen et al. 2011).

Lemna minor L., a member of the duckweed family, lives in many types of fresh water ecosystems (Bog et al. 2010). In particular, it has been commonly used as a test organism in ecotoxicological and environmental studies (Horvat et al. 2007; Khellaf and Zerdaoui 2010), owing to the physiological properties (small size, high multiplication rates and vegetative propagation). Therefore, we chose it as the experimental material and polyamines (Put, Spd, Spm and (Spd + Spm)/Put) and proline contents as well as critical metabolic enzymes (ADC, ODC, PAO, P5CS, OAT and PDH) were investigated in detail, aiming to further explore mitigative strategies of Zn on Cd-induced stress responses.

Materials and methods

Plant material and treatments

Lemna minor were collected from unpolluted freshwater bodies in Nanjing, China, washed with distilled water, and acclimated in 1/10 Hoagland solution. They were cultured in a totally enclosed incubator (Forma 3744, UK) at a day/night temperature of 24 ± 2 °C for more than 2 weeks. The illumination procedure consisted of a 16/8 light/dark cycle and aphoton flux density of 50 μmol m^{-2} s^{-1} (Horvat et al. 2007). Plant materials were treated as follows: (1) control: 1/10 Hoagland solution; (2) Cd treatment: 1/10 Hoagland solution containing 1, 2, 3, 4 μM Cd; (3) Zn treatment: 1/10 Hoagland solution containing 20 μM Zn; (4) Cd + Zn treatment: 1/10 Hoagland solution containing 1 μM Cd + 20 μM Zn, 2 μM Cd + 20 μM Zn, 3 μM Cd + 20 μM Zn, 4 μM Cd + 20 μM Zn. After 4 days treatment, the whole plants were sampled. All experiments were performed in triplicate.

Determination of dry weight

The plant samples were thoroughly washed with tap water, rinsed with distilled water and oven-dried to constant weight at 85 °C as described by Chatterjee et al. (1998). The dry weight was expressed as mg g^{-1} fresh weight.

Determination of polyamine

1.5 g fresh weight (FW) of *L. minor* was homogenized in 4 mL of 6 % (v/v) cold perchloric acid (PCA), kept on ice for 1 h, and then centrifuged (4 °C) at 21,000×*g* for 30 min. The pellet was extracted twice with 2 mL 5 % (v/v) PCA and centrifuged again. The three supernatants were pooled and used to determine the contents of free and PS-conjugated polyamines, whereas the pellet was used to determine the contents of PIS-bound polyamines. The

pellet was re-suspended in 5 % (v/v) PCA and hydrolyzed for 24 h at 110 °C in flame-sealed glass ampoules after being mixed with 12 N HCl (1:1, v/v). The hydrolyzates were filtered, dried at 70 °C, and then re-suspended in 1 mL of 5 % (v/v) PCA for analysis of PIS-bound polyamines. For PS-conjugated polyamines, 2 mL of the supernatant were mixed with 2 mL of 12 N HCl and hydrolyzed for 24 h at 110 °C in flame-sealed glass ampoules. The supernatant, hydrolyzed supernatant and the pellet were then benzoylated (Aziz and Larher 1995).

The benzoyl derivatives were separated and analyzed using a HPLC system (Agilent 1100, USA) equipped with an UV detector under the following conditions: 200 mm × 4.6 mm C_{18} reverse-phase column (Kromasil, Sweden); particle size, 5 μm; column temperature, 30 °C; mobile phase, 64 % (v/v) methanol; a flow rate of 0.8 ml min^{-1}, a detected wavelength of 254 nm. The internal standard was 1,6-hexanediamine.

Analysis of ADC, ODC and PAO activities

The ADC and ODC activities were determined according to Zhao et al. (2003) with some modifications. 1.5 g fresh plant material was homogenized in 50 mM phosphate buffer (pH 6.3) containing 0.1 mM phenylmethylsulfonylfluoride (PMSF), 40 μM pyridoxal phosphate (PLP), 5 mM dithiothreitol (DTT), 5 mM ethylene diamine tetra acetic acid (EDTA), 20 mM ascorbic acid (Vc) and 40 μM polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 12,000×*g* for 40 min and the supernatants were used for the enzyme assay. For ADC and ODC measurement, Reaction mixture (1.5 mL) consisted of 1 mL of the assay buffer with 100 mM Tris–HCl (pH 8.5), 5 mM EDTA, 40 μM pyridoxal phosphate and 5 mM DTT, 0.3 mL of either the ADC or ODC enzyme extract and 0.2 mL of 25 mM L-arginine (L-ornithine). The reaction mixture was incubated at 37 °C for 60 min, and centrifuged (4 °C) at 3,000×*g* for 10 min after which 0.5 mL of the supernatant was mixed with 1 mL of 2 mM NaOH, and then 10 μL benzoyl chloride was added to the mixture and stirred continuously for 20 s. After the reaction proceeded at 25 °C for 60 min, 2 mL of saturated NaCl and 2 mL of ether were added to the reaction mixture and stirred thoroughly, then centrifuged (4 °C) at 1,500×*g* for 5 min, 1 mL of ether phase was collected and evaporated at 50 °C. The remainder was dissolved in 0.5 mL of methanol (HPLC grade), and its absorption value at 254 nm was measured by a spectrophotometer (Thermo GENESYS 10, USA) for ADC (the solution was diluted into 20 mL fords before measuring) and an HPLC system (Agilent 1100, USA) for ODC respectively. A standard curve with Agmatine (Agm) or Put was used to calculate the activity of ADC (ODC). ADC and ODC activities were expressed as μmol Agm g^{-1} FW h^{-1} (U) and μmol Put g^{-1} FW h^{-1} (U) respectively.

PAO activity was determined by the improved method of Smith (1972) described by Wang et al. (2004) with some modifications. 0.5 g fresh plant material was ground on ice, in 1.6 mL phosphate buffer (0.1 mM, pH 6.5); then separated centrifugally at 10,000×*g* for 20 min at 4 °C. The supernatants were used to assay enzyme activity. For PAO measurement, 3 mL of reaction mixture consisted of 2.5 mL phosphate buffer (pH 6.5), 0.2 mL chromogenic reagent [25 mL *N*,*N*-dimethylaniline and 10 mg 4-aminoantiprine in 100 mL phosphate buffer (0.1 mM, pH 6.5)], 0.1 mL horseradish peroxidase (250 U mL^{-1}) and 0.2 mL enzyme extract. Then 0.1 mL PAs (10 mM Spd + 10 mM Spm) was added. The reaction was conducted at 25 °C for 30 min, and measured spectrophoto-metrically at 550 nm where 0.001 ΔA_{550} g^{-1} FW min^{-1} was equal to one enzyme activity unit (U).

Determination of proline

Proline content was estimated using ninhydrine acid reagent according to Bates et al. (1973). Plant samples (0.5 g) were homogenized with 5 mL sulfosalicylic acid (3 %, w/v) in a cold mortar and pestle. The homogenate was centrifuged (4 °C) at 10,000×*g* for 15 min, and 2 mL of the supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrine. After agitation, the reaction mixture was incubated at 100 °C for 30 min. After cooling, 4 ml of toluene was added to each tube and vortexed for 30 s. The chromophore containing toluene was separated and absorbance was taken at 520 nm in spectrophotometer against toluene blank. The concentration of proline was quantified using the standard curve of L-proline and expressed as μg g^{-1} FW.

Analysis of P5CS, OAT and PDH activities

P5CS activity was assayed by the method of Smith et al. (1984) with some modifications. 2.0 g fresh plant material was grounded in 2 mL TD buffer containing 50 mM Tris–HCl buffer (pH 7.0), 1 mM dithiothreitol and 10 % glycerol. After centrifugation (4 °C) at 14,000×*g* for 20 min, the supernatant was collected and precipitated by adding solid ammonium sulfate (40 % saturation). Then, the soluble fraction obtained by centrifugation (14,000×*g* for 20 min at 4 °C) was saturated with dry ammonium sulfate to a concentration of 80 %. After centrifugation at 14,000×*g* for 15 min at 4 °C, the pellet was collected and completely dissolved with 1 mL TD buffer. The crude enzyme solution was obtained after a 24 h dialysis against TD buffer at 4 °C. A total volume of 1 mL assay mixture containing 50 mM glutamate, 10 mM ATP, 20 mM $MgCl_2$, 100 mM oxammonium hydrochloride, 50 mM Tris–HCl buffer (pH 7.0)

and an appropriate amount of enzyme was incubated at 37 °C for 30 min and then the reaction was stopped by adding 1 mL stop solution (5.5 % $FeCl_3$, 2.0 % $HClO_4$, 2 M HCl). The precipitate was removed by centrifugation, and the absorbance of the supernatant at 535 nm was recorded against a blank identical to the one mentioned above but lacking ATP. 0.01 ΔA_{535} h^{-1} at 535 nm was defined as one unit (U) of P5CS activity.

OAT activity was assayed with ninhydrin according to Kim et al. (1994). 1.0 g fresh plant material was immediately homogenized in 100 mM potassium phosphate buffer (pH 7.9) containing 1 mM EDTA, 15 % glycerol and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 14,000×*g* for 15 min at 4 °C and the supernatant was collected for OAT activity measurement. 1 mL of the reaction mixture consisted of 50 mM Tris–HCl (pH 8.0), 50 mM L-ornithine, 5 mM β-ketoglutarate, 0.05 mM pyridoxal phosphate and the appropriate amount of crude enzyme extract was incubated at 37 °C for 20 min. After the addition of 0.3 mL of 3 M perchloric acid and 0.2 mL of 2 % ninhydrin, the reaction was stopped by boiling for 5 min. The precipitate was collected by centrifugation (14,000×*g*, 30 min, 4 °C) and completely dissolved with 1.5 mL of ethanol, and the absorbance of 0.01 at 510 nm was defined as one unit (U) of OAT activity.

PDH activity was measured as described by Rena and Splittstoesser (1975) with a slight modification. 0.5 g fresh plant material was homogenized in the ice-cold extraction buffer (100 mM sodium phosphate, 1 mM cysteine, 0.1 mM EDTA, pH 8.0). After centrifugation at 14,000×*g* for 10 min at 4 °C, the supernatant was used as crude enzyme preparation for measurement of PDH activity. The crude extraction was incubated in the reaction buffer [100 mM Na_2CO_3–$NaHCO_3$, 10 mM nicotinamide adenine dinucleotide (NAD), 20 mM L-proline, pH 10.3] at 32 °C for 5 min, and then PDH dependent NAD reduction was monitored at 340 nm for 4 min. 0.001 ΔA_{340} min^{-1} was defined as one unit (U).

Statistical analysis

All values are expressed as mean ± standard deviation from three individual experiments. The data were subjected to an analysis of variance in SPSS Statistics 17.0. The correlation coefficients were expressed using r values.

Results

Effects of Zn on contents of dry weight under Cd stress

As shown in Fig. 1, The dry weight of *L. minor* decreased markedly in response to single Cd concentration compared with the control ($r = 0.9889$, $P < 0.01$). In contrast, the application of Zn weakened the decline to a certain extent, but no significant change was observed.

Effects of Zn on levels of polyamines under Cd stress

In comparison to the control plants, single Cd stress induced a massive accumulation of Put (Fig. 2a, b) ($r_{put} = 0.8857$, $P < 0.05$; $r_{spd} = -0.9884$, $P < 0.01$). When plants were treated with 4 μM Cd, the content of Put reached the peak at 849.74 nmol g^{-1} FW, and it was 57.06 % higher than control plants. The content of Spd reduced dramatically under single Cd stress ($r_{spd} = -0.9746$, $P < 0.01$), and it was only 293.80 nmol g^{-1} FW, 79.34 % of the control, when the fronds were grown in 4 μM Cd. However, as the concentration of Cd continued to rise, no outstanding changes were observed in Spm content (Fig. 2c). Therefore, due to the combined action of Put, Spd and Spm under induced Cd stress, the ratio of total (Spd + Spm)/Put decreased fiercely with the increase of the Cd concentrations (Fig. 2d). Zn addition significantly decreased the content of Put and increased the content of Spd (Fig. 1a), except for the 1 μM Cd treatment, but failed to generate a statistically significant difference in the content of Spm. So, application of Zn restored the Cd-induced decline in the (Spd + Spm)/Put ratio (Fig. 2).

Effects of Zn on the activities of ADC, ODC and PAO under Cd stress

It can be seen from Fig. 2a that ADC activity increased markedly under Cd treatment ($r = 0.8818$, $P < 0.05$)

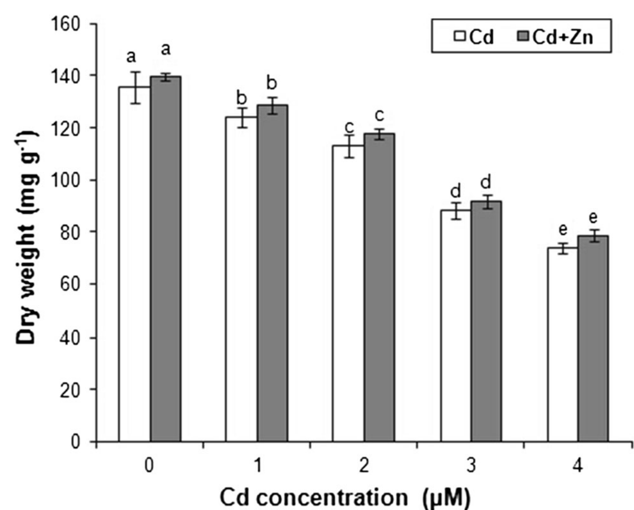

Fig. 1 Effects of Zn on contents of dry weight under Cd stress in *L. minor*. Data were expressed as mean ± SD of triplicates. Value designated over the *bars* in *different letter* are significant different at $P < 0.05$

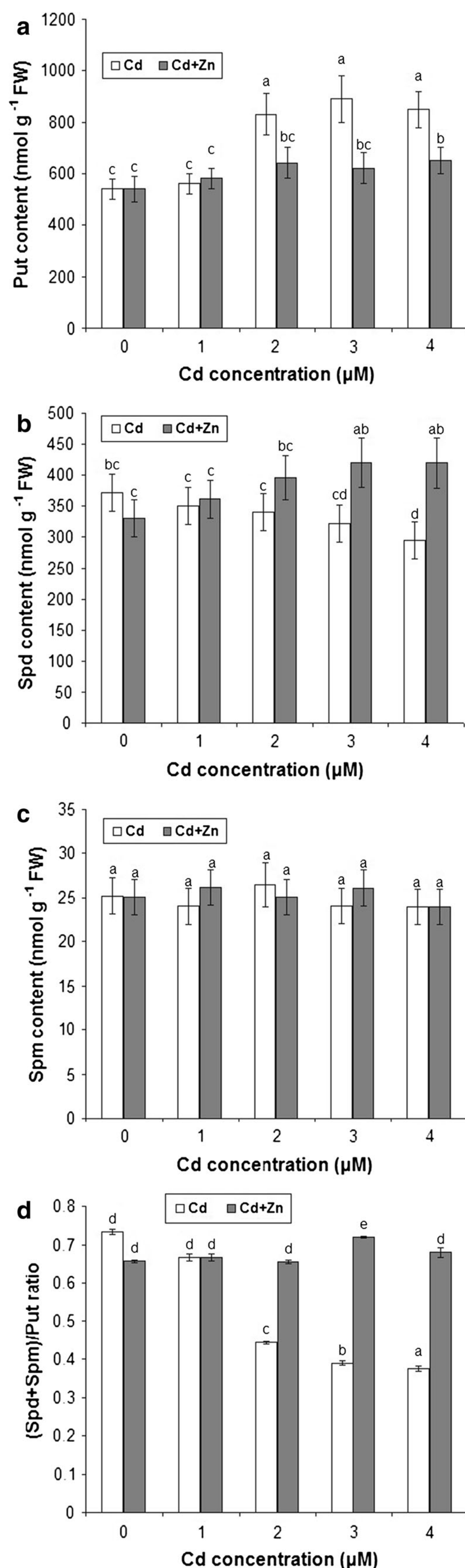

Fig. 2 Effects of Zn on levels of polyamines under Cd stress in *L. minor*. **a** Put content. **b** Spd content. **c** Spm content. **d** (Spd +Spm)/Put ratio. Data were expressed as mean ± SD of triplicates. Value designated over the *bars* in *different letter* are significant different at $P < 0.05$

(Fig. 3a). When plants were treated with 3 μM Cd, ADC activity reached the peak at 22.22 U g^{-1} FW, and it was 1.61-fold higher than control plants. ODC and PAO activity changed in a similar pattern as ADC activity under single Cd stress (Fig. 3b, c). The maximum inductions were being 270.55 and 150.01 %, respectively, following the application of 4 and 3 μM Cd. However, Zn application distinctly weakened the enhancement of ADC and PAO activities, while there was no statistically significant difference in ODC activity (Fig. 3).

Effects of Zn on level of proline under Cd stress

As shown in Fig. 4, the proline content of *L. minor* increased conspicuously with the increase of the Cd concentrations, reaching its peak value at 3 μM Cd. However, it decreased when fronds were treated with 4 μM Cd. Compared to single Hg-treated fronds, the application of Zn dramatically reduced the proline content, but there is no remarkable difference among Zn added groups (Fig. 4).

Effects of Zn on the activities of P5CS, OAT and PDH under Cd stress

P5CS activity increased initially, reaching peak values at 2 μM Cd. However, as the concentration of Cd continued to rise, it declined afterwards (Fig. 5a). However, OAT activity was only significantly stimulated at 4 μM Cd, which was 1.18-fold higher than that of control plants (Fig. 5b). In contrast, PDH activity was inhibited with increasing Cd concentration (Fig. 5c). Application of Zn displayed a significant decrease in P5CS activity, except for the 4 μM Cd treatment. When the fronds were treated with 4 μM Cd, P5CS activity was slightly increased whereas OAT activity was decreased progressively combined with Zn addition. Additionally, Zn supplementation failed to generate a statistically significant difference in PDH activity compared to the Cd treatments only (Fig. 5).

Discussion

Reduction in biomass production is general response of higher plants to heavy metal toxicity (Ouariti et al. 1997). In our experiments, dry weight of *L. minor* was

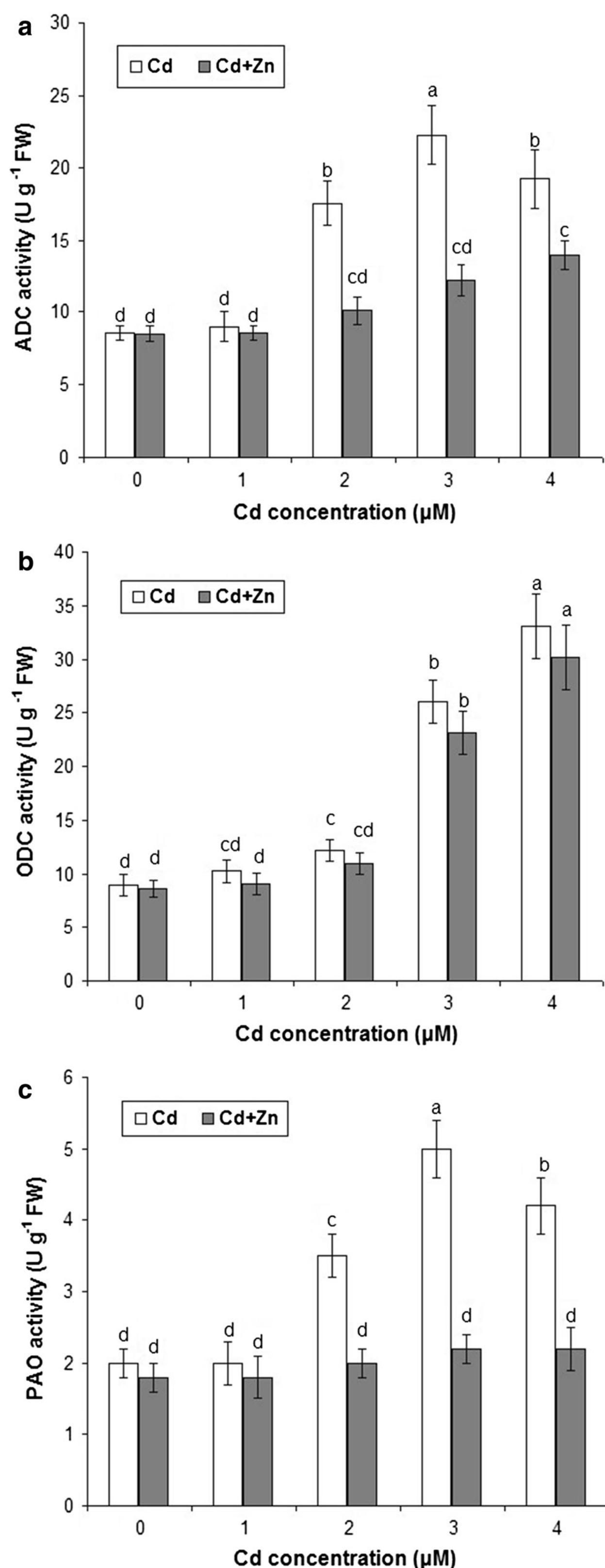

Fig. 3 Effects of Zn on the activities of ADC (**a**), ODC (**b**) and PAO (**c**) under Cd stress in *L. minor*. Data were expressed as mean ± SD of triplicates. Value designated over the *bars* in *different letter* are significant different at $P < 0.05$

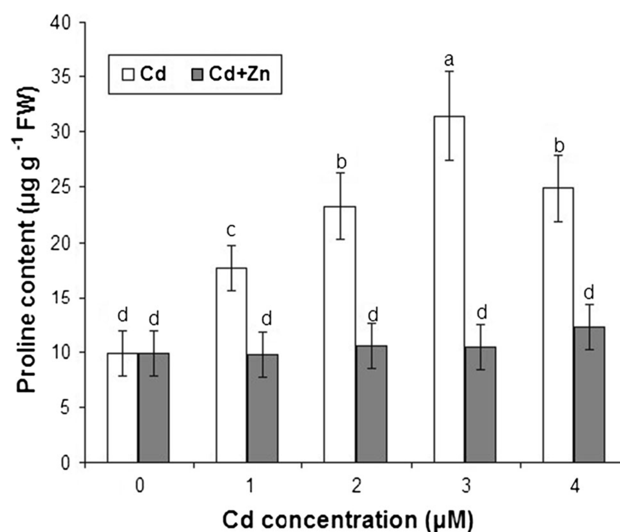

Fig. 4 Effects of Zn on levels of proline under Cd stress in *L. minor*. Data were expressed as mean ± SD of triplicates. Value designated over the *bars* in *different letter* are significant different at $P < 0.05$

significantly inhibited. Similar results have been shown by plant dry weight of rice, ryegrass and *Vetiveria zizanioides* (Hassan et al. 2005; Xu et al. 2006, 2009). However, a decline in dry weight was weakened by Zn addition in the present study (Fig. 1), suggesting that Zn addition contributes to an increased tolerance capacity to Cd poisoning.

The homeostasis of inner polyamines metabolism in plants is essential for cell division, growth and death (Alcázar et al. 2010). In plants, polyamines levels depend not only on their synthesis but also on their degradation. The initial step in polyamines biosynthesis is the decarboxylation of arginine or ornithine to produce Put by ADC or ODC activities. Then, Spd and Spm synthesize on base of Put (Martin-Tanguy 2001). In addition, PAO activity which oxidizes Spd and Spm at their secondary amino groups participates in the polyamines degradation (Alcázar et al. 2010). Early studies had pointed out polyamines homeostasis was disturbed by Cd stress in *H. dubia and P. crispus* (Yang et al. 2010, 2013), and the results of our study are in accordance with the idea, which revealed that Cd stress resulted in a sharp increase in Put content and a significant decrease in Spd content and (Spd + Spm)/Put ratio (Fig. 2). However, Spm content remained constant with the increasing concentration of Cd. This could be a result of increases in ADC, ODC and PAO activities (Fig. 3), which accelerated the synthesis of Put and degradation of Spd, as suggested by a number of reports (Groppa et al. 2007; Moschou et al. 2008; Yang et al. 2010). Nevertheless, Zn addition effectively maintained the polyamines metabolism balance. It decreased Put content and increased Spd content, via reducing ADC and PAO activities. Unfortunately, no changes in Spm content and ODC activity were shown.

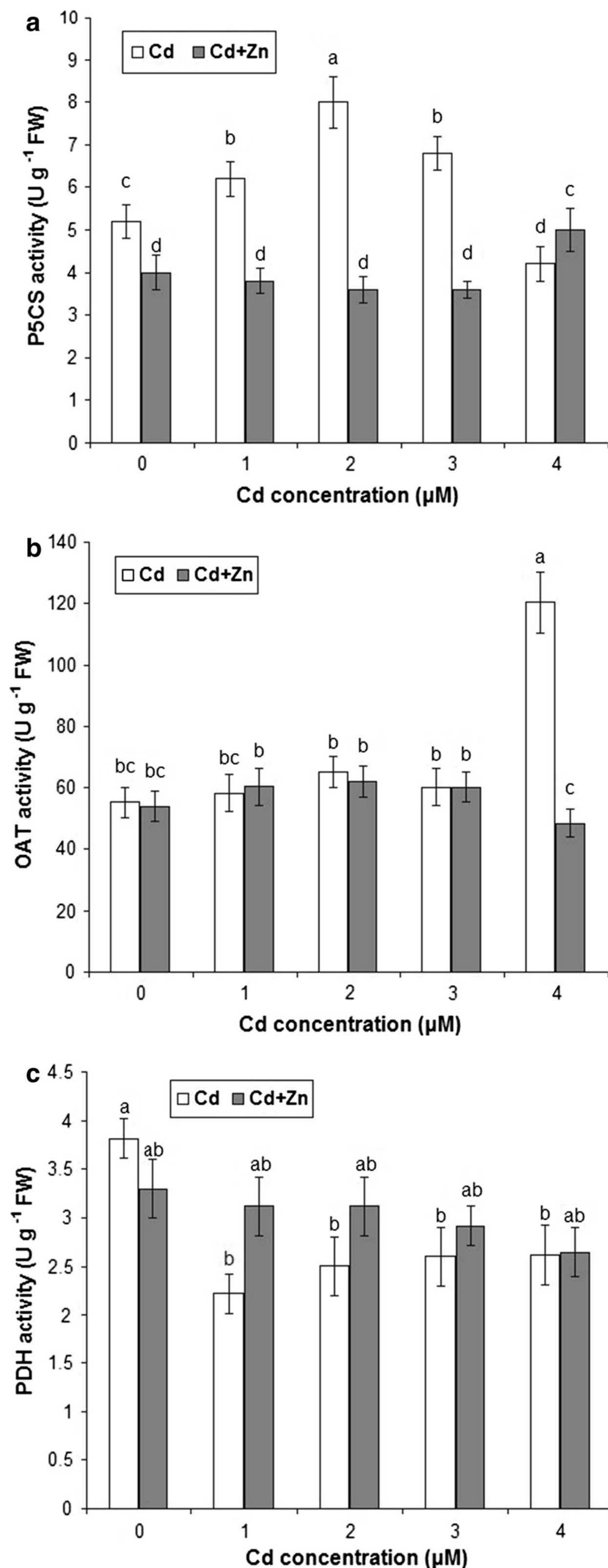

Fig. 5 Effects of Zn on the activities of P5CS (**a**), OAT (**b**) and PDH (**c**) under Cd stress in *L. minor*. Data were expressed as mean ± SD of triplicates. Value designated over the *bars* in *different letter* are significant different at $P < 0.05$

As a result, the (Spd + Spm)/Put ratio was elevated compared to single Cd treatments. Endogenous Put content is generally considered that it may affect the redox state and a mass accumulation of Put is toxic in plants (Górecka et al. 2007; Groppa et al. 2001; Panicot et al. 2002). Spd acts as a protectant for the plasma membrane against stress damage by maintaining membrane integrity, preventing the activation of superoxide-generating NADPH oxidases and/or inhibiting protease and RNase activity (Roussos and Pontikis 2007; Roy et al. 2005). The elevation of the (Spd + Spm)/Put ratio is confirmed as a criterion of tolerance properties in plants regarding salt, osmotic, heat, chilling and heavy metals stresses (Bouchereau et al. 1999). So, it is safe to draw a conclusion that the decreasing of Put and the increasing of Spd and (Spd + Spm)/Put ratio were critical in improving Cd tolerance of *L. minor*, which also would be in agreement with the idea of a protective role for Zn against Cd stress.

Though consensus had not been achieved on the exact roles of proline, it was agreed that the proline accumulation is a common physiological response to various metal ions (Verbruggen and Hermans 2008). A similar result was observed in the present study in relation to proline content under single Cd stress (Fig. 4). The coordinate regulation of proline biosynthesis is achieved via two different pathways from either glutamate or orinithine/ariginine and proline degradation (Szabados and Savouré 2010). The rate-limiting step in glutamate pathway is P5CS enzyme activity, while in the ornithine pathway is OAT activity (Kishor et al. 2005). Meanwhile, PDH activity participates in the proline catabolism (Kishor et al. 2005). The present data in *L. minor* suggest that Cd induced effect on proline accumulation may be explained partially by the glutamate pathway involved in the elevation of P5CS activity (except for 4 μM Cd treatment) and the ornithine pathway involved in the sole stimulation of OAT activity under 4 μM Cd. A decline seen in PDH activity also participated in suppression of proline catabolism (Fig. 5). Nonetheless, Zn supplementation performed a suppressive effect on proline accumulation in *L. minor* exposed to Cd, by modulating P5CS and OAT activities and inhibiting the reduction in PDH activity. Consequently, application of Zn contributed to the proline level intrinsic balance, indicating that it is closely implicated in the protection of *L. minor* from Cd stress. In plants, proline accumulation was also closely linked with polyamines catabolism in plants exposed to abiotic stresses (Aziz et al. 1998; Bouchereau et al. 1999), owing to the reason that proline and Put share a common substrate, glutamate (Seki et al. 2007; Sharma and Dietz 2006; Simon-Sarkadi et al. 2006). In the present study, synchronous changes of proline and Put were observed in single Cd stress and Zn added groups, which were in good

agreement with the viewpoint. Simultaneously, this effect supported this standpoint that proline and polyamines metabolism might exert mutual regulatory mechanism (Aziz et al. 1998).

In conclusion, Cd altered the dry weight, polyamines and proline contents as well as metabolic enzymes, inducing metabolic disturbances in *L. minor*. Application of Zn alleviated Put and proline accumulation, increased the dry weight and Spd contents and the (Spd + Spm)/Put ratio, which indicated that Zn were involved in the adaptation of *L. minor* to Cd-induced stress.

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