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Full Length Research Paper

Use of phosphorus to alleviate stress induced by cadmium and zinc in two submerged macrophytes

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The potential mechanism by which P antagonizes the toxicity of Zn and Cd was investigated in *Elodea nuttallii* and *Hydrilla verticillata* to understand the interactions between P, Cd and Zn pollutants. The two submerged macrophytes were treated with a combination of Cd (0.25 mg L^{-1}) and Zn (1.0 mg L^{-1}) and various concentrations of P (0, 0.05 and 5 mg L^{-1}) for different exposure times (0.5, 1, 2, 4 and 7 days). The toxic effects and oxidative stress caused by the Cd and Zn resulted in a reduction of the total chlorophyll (chlorophyll a and b) and an increase in the content of glutathione (GSH). The activity of catalase (CAT) and the contents of malondialdehyde (MDA) both increased in the 1st day, and then reduced during the following 6 days. However, the activity of guaiacol peroxidase (POD) and the contents of soluble protein both decreased in the first day then increased from the 2nd to 7th days. Each index in the plants treated with Cd and Zn containing P showed similar changing trends to those treated with only Cd and Zn during the 7 days. However, different indices had different response times. At the best response time in each index, the result showed that using P can protect plants from the toxicity and oxidative stress caused by Cd and Zn, which suggested that P can produce an antagonistic response with Cd and Zn to mitigate the oxidative stress to plants. Also, these results suggested that Cd and Zn exerted their toxic effects on the growth of *E. nuttallii* and *H. verticillata,* at least in part, by the induction of oxidative stress and inhibition of photosynthesis. Through comparing the response of the two plants to oxidative stress caused by Cd and Zn, it was found that *E. nuttallii* was more sensitive than *H. verticillata. E. nuttallii* can be regarded as an indicative plant for Cd and Zn polluted waters.

Key words: Cadmium, Zinc, Phosphorus, oxidative stress, antagonize.

INTRODUCTION

Submerged plants, as the main primary producers of water ecosystems, form the basis of a complex food chain. They also indirectly support the food chain by increasing eco-space, inhibiting the biological and non-biological suspended solids, providing dissolved oxygen and improving underwater light conditions. Submerged plants are the basis by which biological diversity is maintained (Elln et al., 2002). However, due to the abundance of heavy metals and P nutrition element in the aquatic environment resulting from agricultural run-off, industrial discharge and biological waste decomposition, the disappearance and recession phenomena of submerged plants are widespread in the world (Melzer, 1999).

Cadmium (Cd) and Zinc (Zn) are commonly found in industrial effluent. They are also harmful to plants at relatively low concentrations (Chakravarty and Srivastava, 1992). The combination of Cd and Zn can reduce the rates of respiration, induce lipid peroxidation and the activity of scavenging enzymes and produce excess reactive oxygen species (ROS) which cause oxidative damage to the proteins and lipids of plants.

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(Parameswaran and Majeti, 2003; Bipasha and Sheela, 1997; Gussarson et al., 1995; Xu et al., 2003)

Phosphorus (P) is the main factor causing eutrophication (Xie, 1989; Peng et al., 1986). Several reports have shown that a high concentration of P causes toxicity to plants by inhibiting their growth, accelerating leaf senescence (Marschner, 1995), impairing leaf-water relations (Bhatti and Loneragen, 1970) and inducing micronutrient deficiency (Marschner, 1995). P at high concentration can also cause damage to the protein, photosynthesis and photorespiration of aquatic plants (Ma et al., 2008; Chen et al., 2002). On the other hand, P is a required element for the growth of plants, taking part in the photo-phosphorylation and carbon assimilation in the photosynthesis (Zhou et al., 1993). Soluble phosphate sources can provide an abundance of soluble P and increase the efficiency of metal-phosphate mineral formation (Berti and Cunningham, 1997; Hettiarachchi et al., 1997; Cooper et al., 1998; Ma et al., 1993).

Although many publications have reported that the combination of Cd and Zn or only P can cause toxicity to plants, there have been no reports investigating interactions of physiological and biochemical mechanisms between Cd + Zn and P. Several studies have shown that P can decrease the toxicity caused by the combination of Cd and Zn (Hettiarachchi and Pierzynski, 1999; Vangronsveld and Cunningham, 1998). They only focused on the use of P to reduce the mobility, solubility, or extractability of Cd and Zn in the soils. However, the physiological and biochemical mechanisms of using P to alleviate the toxicity and oxidative stress resulting from Cd and Zn in aquatic plants have not been reported to our knowledge.

In this paper, we selected two submerged plants *Elodea nuttallii* and *Hydrilla verticillata* as our experimental samples. They were treated with certain concentrations of Cd, Zn and P and with different exposure times. Our objective is to investigate the interactions between Cd + Zn and P to find the physiological and biochemical mechanisms that allow P to alleviate oxidative stress resulting from Cd and Zn in these aquatic plants. Furthermore, the recession mechanism in submerged plants caused by Cd, Zn and P was studied in order to provide a theoretical basis for resuming submerged plants. By comparing the responses of *E. nuttallii* and *H. verticillata* to combined pollution by Cd and Zn, an indicative plant can be selected which is more tolerant yet sensitive to polluted waters. In the present experiment, the photosynthetic system, lipid peroxidation, antioxidant, soluble protein and antioxidative enzymes were analyzed to demonstrate that P can alleviate the toxicity and oxidative stress resulting from Cd and Zn.

MATERIALS AND METHODS

Plant culture and treatment

E. nuttallii and *H. verticillata* seedlings at similar stages of growth were procured from Taihu in Suzhou, Jiangsu Province, China. Before the experiments, plants were cultured for at least two weeks in a primary culture medium for acclimatization in tanks 1 m long, 2 m wide and 1 m deep. The primary culture medium contained 1% Hoagland's trace elements (Hoagland and Arnon, 1950) and the macro-nutrition elements adapted from Hoagland's solution, including 0.05 mM $Ca(NO_3)_2$, 0.007 mM KCl, 0.2 mM $MgSO_4$ and 0.7 mM K_2SO_4.

After two weeks' acclimatization and when seedlings had grown to a length of about 15 cm, they were selected for uniformity and transplanted to a modified 1% Hoagland nutrient solution in red plastic kegs with a photoperiod of 8 h/16 h (day/night) at a temperature of 25 ± 2 ℃. The red kegs covered by black plastic bags to inhibit algae growth had a capacity of 4 L. Cd as $CdCl_2 \cdot 2.5H_2O$, Zn as $ZnSO_4 \cdot 7H_2O$ and P as KH_2PO_4 were added to the nutrient solution in three treatment schemes:

1. τCd + Zn (0.25 mgL^{-1} Cd+1 mgL^{-1} Zn) as control;
2. υCd + Zn + LP (0.25 mgL^{-1} Cd+1 mgL^{-1} Zn+0.05 mgL^{-1}P);
3. ϖCd + Zn + HP (0.25 mgL^{-1} Cd +1 mgL^{-1} Zn + 5 mgL^{-1}P).

The selected concentrations of Cd and Zn were related to Surface water quality standards in China and P was chosen from the level of eutrophication of lakes in China (Jin, 1990). Plants cultured with the three treatments were harvested at 0 (control), 0.5, 1, 2, 4 and 7 days, respectively. Throughout the acclimatization and experimental periods, the solution was replaced every 2 days and the pH of the solution was adjusted to 5.5 (± 0.1) at the same time using 1 M HCl or 1 M NaOH. Every treatment was replicated three times. During the experimental periods, plants were rinsed with distilled water, blotted, and frozen immediately in liquid nitrogen for storage at –80℃. The two kinds of plant had the same treatment, and were carried out under the same conditions for uniformity.

Determination of photosynthetic pigments

A 0.2 g sample of plant material was ground in liquid nitrogen and then extracted with 10 ml of 95% ethanol for 24 h in darkness, at 4℃. After centrifugation at 10,000 *g* for 10 min, the absorbance of the supernatant at 649, 665 and 470 nm was measured. The chlorophyll (*a*/*b*) concentrations were calculated as described by Van Dijk and Roelofs (1988).

Enzyme preparation

A 0.5 g sample of plant material was ground in liquid nitrogen and homogenized with 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 20 min at 4℃, and the supernatant was used for the enzyme assays.

The protein content was quantified according to the method of Bradford (1976), using a standard curve generated with bovine serum albumin.

Assay of CAT and POD activity

CAT activity was determined by measuring the change of absorbance at 240 nm that accompanied the consumption of H_2O_2 (Aebi, 1974). The 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and 100–150 µl of enzyme extract. The decrease of absorbance at 240 nm was measured. The activity was calculated using the extinction coefficient ε = 0.0394 $cm^2 \mu mol^{-1}$. One unit of CAT activity was defined as the amount required to decompose 1 nmolH_2O_2 min^{-1} mg^{-1}fresh weight.

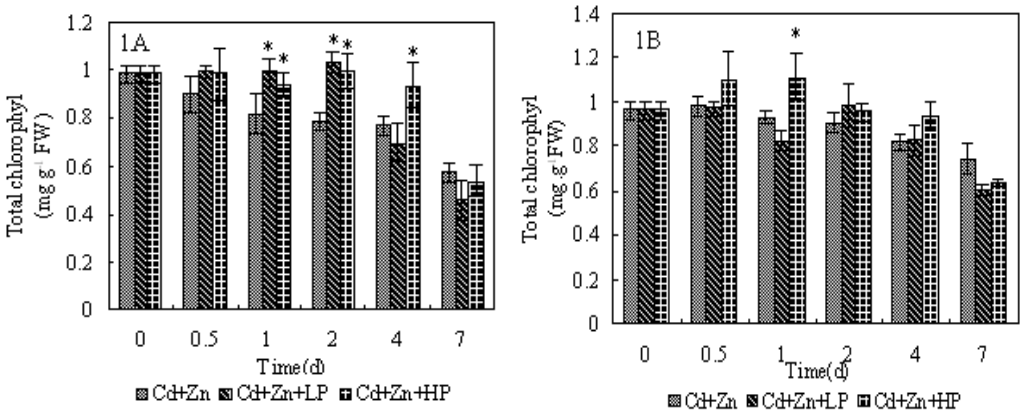

Figure 1. Effects of Cd, Zn, P on the total chlorophyll content. The values are mean ± S.E. (n = 6). Asterisks (*) indicate that the mean values are significantly different between the treated samples and controls ($P < 0.05$).

POD activity was determined as oxidation of guaiacol by H_2O_2 (Upadhyaya et al., 1985). The reaction mixture was 2.5 ml of 50 mM potassium phosphate buffer (pH 6.1), 0.1 ml of 3% H_2O_2, 0.2 ml of 1% guaiacol and 10–20 µl of enzyme extract. The increase in absorbance at 420 nm was measured. The activity was calculated using the extinction coefficient ε = 26.6 mM^{-1} cm^{-1}. One unit of POD activity was defined as the amount required to decompose 1 µmol guaiacol min^{-1} mg^{-1} fresh weight.

Metabolite determination

The level of lipid peroxidation in plant leaves was determined by estimation of the malondialdehyde (MDA) content based on the method of Heath and Packer (1968). Briefly, 0.5 g of plant material was ground with 5 ml of 1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 *g* for 10 min, then 0.5 ml of the supernatant was mixed with 2 ml of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 96℃ for 30 min, stopped at 0℃, then centrifuged at 10,000 g for 10 min. The amount of MDA equivalent of thiobarbituric acid-reactive substance was calculated from the difference in absorbance at 532 and 600 nm using the extinction coefficient ε = 155 mM^{-1} cm^{-1}.

Plants material (0.5 g) was ground into powder in liquid nitrogen for the GSH assays. A sample of the powder was mixed well with 10 ml of 0.0005 M ethylene diamine tetra acetic acid (EDTA-Na_2) containing 3% TCA. The homogenate was centrifuged at 10,000 g for 20 min at 4℃ and the supernatant pH adjusted to 6.0-8.0 with 1.0 M NaOH. The glutathione pool was assayed as described by Guri (1983). For GSH assay, the 3 ml of reaction mixture contains 1 ml distilled water, 1 ml supernatant, 1 ml 0.2 M phosphate buffer (pH 7.0); for comparison with the control, 0.1 ml 5, 5-dithiobis (2-nitrobenzoic acid) was added to the reaction mixture and the absorbance was measured spectrophotometrically after 30 min at 412 nm. The amount of GSH was measured by a standard curve prepared in the range 0-8.0 µM using GSH.

Statistics

Each result shown in the figures was the mean of at least six replicated treatments. Standard deviation and Student's *t*-test calculated by SPSS 13.0 were used to examine the differences between each treatment and the level of statistical significance was set at P≤0.05.

RESULTS AND DISCUSSION

Effects of phosphorus on the photosynthesis of Cd and Zn polluted plants

Chlorophyll a and b are major participants in the photosynthesis. The effect of the P concentration on the total content of chlorophyll (chlorophyll a and b) in the leaves of *E. nuttallii* and *H. verticillata* plants was studied (Figure 1A and 1B). As shown in Figure 1A, the content of total chlorophyll in the Cd + Zn (control) treatment decreased significantly during the treatment phase in leaves of *E. nuttallii*; the total chlorophyll decreased by about 42% at the 7th day compared with that in the plants without Cd, Zn and P. As shown in Figure 1B, the content of total chlorophyll in the leaves of *H. verticillata* in the Cd + Zn (control) also had a decreasing trend. The results were similar to those reported previously. Padmaja et al. (1990) had speculated that adding Cd and Zn reduced δ-aminolevulinic acid dehydratase (ALAD) activity; Kupper et al. (1998) had reported that Mg^{2+} which is the central ion in the chlorophyll is replaced by the Cd and Zn; Baryla et al. (2001) and Benavides et al. (2005) had reported that the reason for the loss of chlorophyll was the distortion of the chlorophyll ultrastructure, inhibition of the synthesis of photosynthetic pigments and enzymes of the Calvin cycle, or reduction in the chloroplast density and size which led to the damage of the photosynthetic cycle; Xun (2004) had reported that the Cd and Zn weakened the capacity of resisting ROS, resulting in lipid peroxidation or deesterification, and then led to damages to structure of the chloroplast membrane. Jiang et al. (2007) had reported that the combined pollution by Cd and Zn caused the structure of the chloroplast to be damaged.

However, the content of total chlorophyll in the leaves of *E. nuttallii* for the Cd + Zn + P treatments (P at different concentrations) increased first and then decreased. By

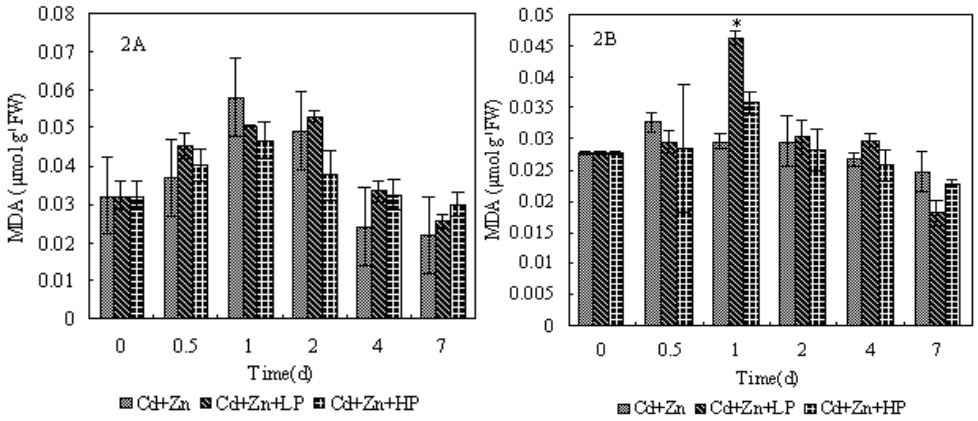

Figure 2. Effects of Cd, Zn, P on the MDA content. Values are mean ± S.E. (n = 6). Asterisks (*) indicate that the mean values are significantly different between treatments and controls ($P < 0.05$).

the end of the 7th day, it had decreased significantly by 55 and 45%, respectively compared with that in the plants without Cd, Zn and P. At the end of the 2nd day, the contents of total chlorophyll for the Cd+Zn+LP and Cd+Zn+HP treatments were 1.03 and 1.00 mg g^{-1} FW, respectively, which were about 0.32-fold and 0.28-fold higher than the control. At the end of the 4th day, the content of total chlorophyll in the Cd + Zn + HP treatment was 0.94 mg g^{-1} FW, which was 0.24-fold higher than the control. The content of total chlorophyll in the leaves of *H. verticillata* (Figure 1B) after treatment with Cd + Zn + HP increased within the 1st day and then decreased during the following 6 days. At the end of the 1st day, the content of total chlorophyll for the Cd + Zn + HP treatment was significantly higher (20%) than the control. We speculated that the external P may promote plant growth and increase the yields so that the concentration of Cd and Zn in the plants was perhaps diluted with respect to the control plants, and the toxicity was reduced; this may be called the “dilution effect” (Olsen, 1972). The external P can form phosphate deposits with Cd and Zn, and then decrease the restraint caused by Cd and Zn to protochlorophyll-isocyanate-reductase and aminohexan-γ-valerianic acid, both enzymes being active in the biosynthesis of chlorophyll (Stobart and Griffiths, 1985). Alternatively, the external P can protect the central Mg^{2+} ion in the chlorophyll from replacement by Cd^{2+} or Zn^{2+}.

Comparing the Figures 1A and 1B, it can be seen that the changes of total chlorophyll content in the leaves of *H. verticillata* was less significant than that of *E. nuttallii*. This showed that *E. nuttallii* was more sensitive than *H. verticillata.*

Effects of phosphorus on the lipid peroxidation of Cd and Zn polluted plants

Malondialdehyde (MDA) is a secondary end product of polyunsaturated fatty acid oxidation and has been used to detect the degree of lipid peroxidation. The effects of phosphorus on the MDA content of leaves of *E. nuttallii* and *H. verticillata* plants are shown in Figures 2A and 2B. In the 1st day, the MDA contents after the three treatments were 0.058, 0.050 and 0.047 μmol g^{-1}FW, respectively, which showed that the MDA in the control was higher than those for the other two treatments. The high MDA content in the control indicated the enhancement of lipid peroxidation in metal-exposed plants, causing damage to tissue development and function. The lipid peroxidation due to increased production of ROS (Tappel, 1973; Kappus, 1985) or redox active metal ions (Weckx and Clijsters, 1996), or initiated by the iron-containing enzyme lipoxygenase (Thompson et al., 1987) led to membrane destabilization. The addition of P decreased the MDA content indicating that P can protect the lipid from being damaged by the combination of Cd and Zn.

The MDA content in the *H. verticillata* plants is shown in Figure 2B. At 0.5 days and the 7th day, the MDA contents in the treatments containing P were lower than those in the controls, which indicated that the addition of P alleviated the lipid peroxidation. We speculate that the P can alleviate the lipid peroxidation by participating in the formation of some enzymes. There has been no report on the mechanism by which P reduces the lipid peroxidation. We speculate that P can take part in the formations of some enzymes which can protect the membrane or that P can form phosphate precipitation with Cd and Zn to prevent them from entering the plant cell, or that P can promote plant growth and development and thus dilute the concentration of Cd and Zn, thus reducing their toxicity, or maybe the P changed the DNA in plants. The mechanism needs further study.

With the prolonged exposure time, increased MDA for the three treatments suggests that the Cd and Zn level stimulate lipid peroxidation, causing damage to tissue development and function, and decreased MDA indicates

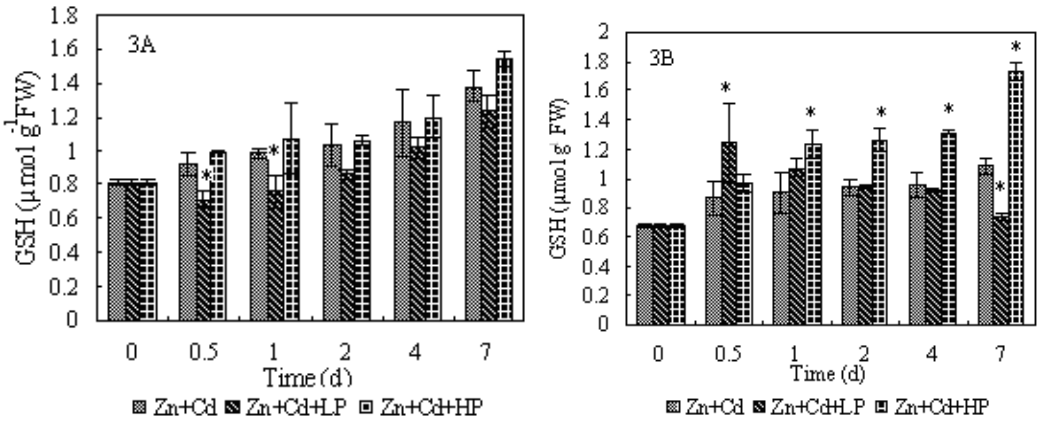

Figure 3. Effects of Cd, Zn, P on the contents of GSH. Values are mean ± S.E. (n = 6). Asterisks (*) indicate that mean values are significantly different between treatments and controls ($P < 0.05$).

that there may be some antioxidative response alleviating or preventing lipid perxoidation (Nimptsch, 2007; Pflugmacher, 2007). Comparing the changes of MDA with exposure time, it was found that MDA in the leaves of *H. verticillata* was less significant than in those of *E. nuttallii*. It showed that *E. nuttallii* was more sensitive than *H. verticillata.*

Effects of phosphorus on the GSH content of Cd and Zn polluted plants

GSH is the most abundant cellular SH in most living organisms and is involved in metal stress in a number of ways. The GSH contents of *E. nuttallii* and *H. verticillata* plants are shown in Figures 3A and 3B, respectively. Figure 3A shows that with prolonged exposure time, the GSH content of *E. nuttallii* increased. It was speculated that GSH might serve as an antioxidant or a metal chelator involved in Cd and Zn detoxification (Perrin and Watt, 1971). No significant differences were detected between Cd + Zn + P and controls during the final 5 days. But within the 1st day, the GSH contents for the Cd + Zn + LP treatments were significantly reduced compared with the controls. It was commonly suggested that GSH served as a precursor in the biosynthesis of PCs (phytochelatins). PC synthesis induced by metals was accompanied by a rapid depletion of total GSH in plant cell suspensions and in intact plants (Jackson et al., 1992; Gupta et al., 1998).

Figure 3B shows that the GSH content of the Cd + Zn (control) treatment increased slowly during the exposure time, but GSH contents of the Cd + Zn + HP treatment increased rapidly in the 1st day, then became constant from 1 to 4 days, with finally a rapid increase from 4 to 7 days. The GSH content in the Cd + Zn + LP treatment reached a peak at the 0.5 days, and then decreased from 0.5 days to 7 days. In the Cd + Zn + HP treatment, no significant difference was detected compared with the control during the first 12 h, but in the 1st, 2nd ,4th and 7th days, the GSH contents increased significantly being 37, 34, 38 and 60% higher than in the controls, respectively. May et al. (1998) had reported that the GSH biosynthesis process required the participation of P. We speculated that using P can stimulate the recycling of GSH by the ASC-GSH cycle.

Effects of phosphorus on the soluble protein contents of Cd and Zn polluted plants

Figure 4A shows that the soluble protein content in the *E. nuttallii* did not increase first and then decrease as we expected. The soluble protein contents for the three treatments all reached minimum values in the 1st day, decreasing by 57, 44 and 29% compared with the treatment without Cd, Zn and P. Hu et al. (2005) speculated that the accumulation of Cd and Zn in the plants led to a breakdown of protein synthesis systems, or the inhibition of protein synthesis or the speeding up of the decomposition of protein. In this case, the soluble protein contents increased from 1 to 7 days, which could be due to the resistance of the plants to stress and to the induction of stress proteins (Toppi and Gabbrielli, 1999). In the first day, the soluble protein contents in the treatment solutions containing P were significantly higher than those in the control. This significant increase also appeared in the 2nd day between the Cd + Zn + HP treatment and controls and in the 7th day between Cd + Zn + LP treatment and controls. Considering that P is an essential nutrient element for the plants' growth and production, and the component of many important compounds such as nucleic acid, protein and phospholipids (Huang, 2004), we proposed that the P has participated in the formation of some protein to alleviate the toxicity of the heavy metal or that the vacuoles have had the special function of compartmentalizing heavy metals (Loneragan et al., 1979; Cotter-Howells and Capron, 1996; Laperche and Traina, 1998), so reducing

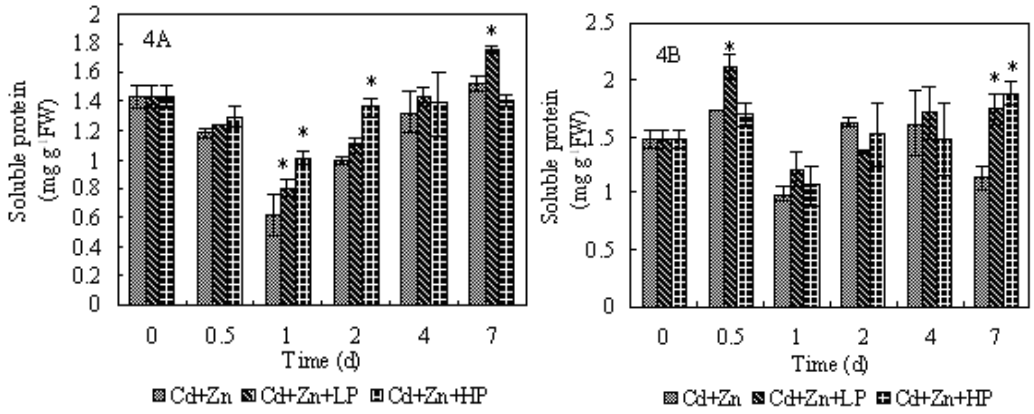

Figure 4. Effects of Cd, Zn and P on the content of soluble protein. Values are mean ± S.E. (n = 6). Asterisks (*) indicate that the mean values are significantly different between treatments and controls (P < 0.05).

its toxicity.

The soluble protein content of the *H. verticillata* plants are shown in the Figure 4B. The trend showed an initial decrease followed by an increase. Compared with the results from *E. nuttallii*, the trend was not obvious. In the 7th day, the content of soluble protein in the samples exposed to the solutions containing P were significant higher (55, 66%) than that for the controls.

Effects of phosphorus on the POD and CAT activity of Cd and Zn polluted plants

The presence of reactive oxygen species (ROS) can be induced by various sources of stress (Mittler et al., 2004), and excess ROS can cause oxidative damage to lipids, protein and DNA. In response to the oxidative stress, the plants form many anti-oxidation defensive systems to signal the toxicity, including enhancement or suppression of the activities of anti-oxidative enzymes. Among the anti-oxidative enzymes, both CAT and POD can decompose H_2O_2 to water and oxygen. CAT is located mainly in the peroxisomes and mitochondria, while POD is located in the cytosol, the cell wall, vacuolar and extracellular spaces (Mishra et al., 2006). In this paper, we investigated these two indicators to estimate the anti-oxidative mechanism of the plants in response to combinations of Cd, Zn and P at different exposure times.

The POD activities of the two kinds of plants are shown in Figures 5A and 5B. The POD activities of *E. nuttallii* decreased over the first 0.5 days and then increased from 12 h to 7 days. Ke (2002) found the same result, and they speculated that the POD was inhibited at the beginning of the trial. In the first 12 h, the POD activities in the Cd + Zn + LP and Cd + Zn + HP treatments were 342.55 and 327.66 U g^{-1} FW, which were 69 and 61% higher, respectively, than that in the controls. We speculate that P promoted the formation of protein, confirming the results found for the soluble proteins. Perhaps P can form phosphate deposits with Cd and Zn to reduce its bio-availability to plants, thus decreasing the toxicity caused by Cd and Zn. During the remaining exposure time, the POD activities in the treatment containing P were not significantly different from those in the controls.

The POD activity of *H. verticillata* plants are shown in Figure 5B, which shows a similar trend to that of *E. nuttallii*. However, the minimum values appeared at the end of the 1st day instead of after 0.5 days. During the first 12 h, there are significant activity increases in the two treatments compared with the control. At the 7th day, the POD activities increased by 63 and 30%, respectively in the Cd + Zn + LP and Cd + Zn + HP treatments compared with the control.

As shown in Figure 6A, the *E. nuttallii* treated with Cd + Zn + HP in the growth medium did not cause significant alterations of the CAT activity during the exposure phase. However, the activity of CAT in the plants treated with Cd + Zn and Cd + Zn + LP increased significantly in the 1st day, and then decreased from 1 to 7 days. In the first day, the CAT activity in the Cd + Zn + HP was significantly reduced compared with that of the control. During the following days, it increased compared with the controls, most significantly in the 2nd and 3rd day.

Similarly, Figure 6B shows that the activities of CAT in the *H. verticillata* at first increased and then decreased. In the Cd + Zn + HP treatment, the CAT activity was significantly increased compared with the control during the first 12 h. In the first day and 7th day, the CAT activities were significantly higher (20 and 74%) than that in controls. But from 2nd to 4th days, no significant difference was observed between treatments and controls.

Both POD and CAT are induced proteins, and their high activities showed that the plants can resist, tolerate and clear the oxidative stress caused by Cd and Zn. Our results showed that the addition of P can increase the

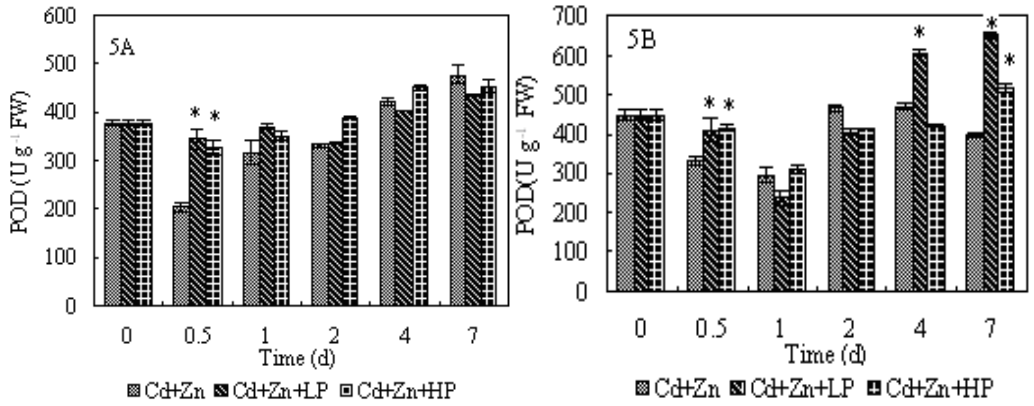

Figure 5. Effects of Cd, Zn, P on the activities of POD. Values are mean ± S.E. (n = 6). Asterisks (*) indicate that the mean values are significantly different between treatments and controls ($P < 0.05$).

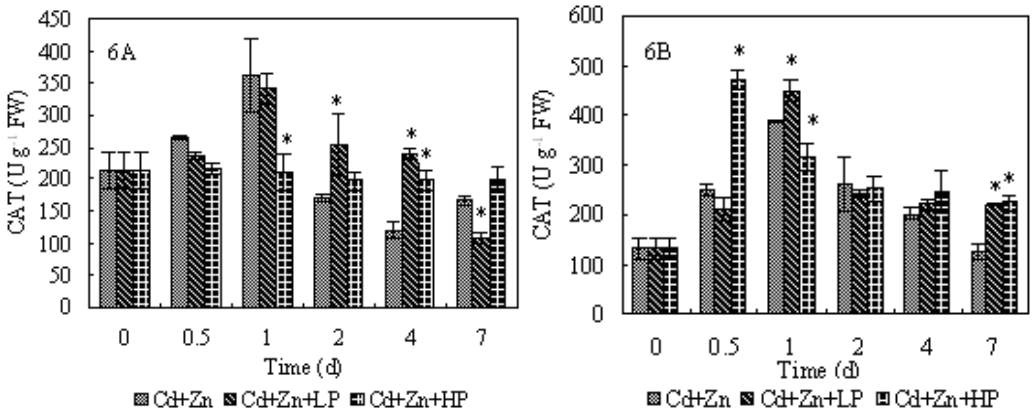

Figure 6. Effects of Cd, Zn, P on the activities of CAT. Values are mean ± S.E. (n = 6). Asterisks (*) indicate that the mean values are significantly different between treatments and controls ($P < 0.05$).

activities of POD and CAT, indicating that P can be used to alleviate the toxicity caused by Cd and Zn. However, the mechanism has not been clear until now.

Conclusion

The co-existence of different elements in the ecosystem can lead to interactions that may be additive, antagonistic or synergistic. Our results suggest that the interaction between P and Cd + Zn is antagonistic. Cadmium and Zinc can lead to a decline of photosynthetic efficiency and an increase of excess ROS causing oxidative stress to plants. The oxidative stress induced by cadmium and zinc can be partially alleviated by the addition of P. These results further explain in freshwater polluted by heavy metal and eutrophication, the main factor which leads to the deterioration of the submerged macrophytes was the presence of heavy metals, not P. Comparing the two submerged macrophytes, it was found that *E. nuttallii* is more sensitive than *H. verticillata*. Accordingly, *E. nuttallii* is proposed as an indicative plant for polluted waters.

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