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Heavy metal phytoextraction by *Sedum alfredii* is affected by continual clipping and phosphorus fertilization amendment

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Abstract

Improving the efficacy of phytoextraction is critical for its successful application in metal contaminated soils. Mineral nutrition affects plant growth and metal absorption and subsequently the accumulation of heavy metal through hyper-accumulator plants. This study assessed the effects of di-hydrogen phosphates (KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$) application at three levels (22, 88 and 352 mg P/kg soil) on *Sedum alfredii* growth and metal uptake by three consecutive harvests on aged and Zn/Cd combined contaminated paddy soil. The addition of phosphates (P) significantly increased the amount of Zn taken up by *S. alfredii* due to increased shoot Zn concentration and dry matter yield (DMY) ($P < 0.05$). The highest phytoextraction of Zn and Cd was observed in KH_2PO_4 and $NH_4H_2PO_4$ treatment at 352 mg P/kg soil. The amount of Zn removed by phytoextraction increased in the order of 1st clipping < 2nd clipping < 3rd clipping, and for Cd extraction the order was 2nd clipping < 1st clipping < 3rd clipping. These results indicate that the application of P fertilizers coupled with multiple cuttings can enhance the removal of Zn and Cd from contaminated soils by *S. alfredii*, thus shortening the time needed for accomplishing remediation goals.

Key words: soluble phosphates; clipping; phytoremediation; heavy metal; *Sedum alfredii*

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Introduction

Intensive human activities such as modern agriculture, traffic, mining, and industrial activities resulted in accumulation of heavy metals in soils in many areas of the world. Especially, excessive application of low-quality fertilizers, pesticides, sewage sludge and sewage irrigation increased the concentrations of Zn and Cd in many agricultural soils of China (Tang et al., 2010; Wei and Yang, 2010). The concern over the accumulation of heavy metals in agricultural soils has been increasing because of food safety, potential health risks, and its detrimental effects on soil ecosystems (Zheng et al., 2007).

Metal phytoextraction is often considered as a gentle, eco friendly cleanup method. Phytoremediation represents one of the most promising economic opportunities for remediation, based on plant ability to work as bio-pumps, extracting and concentrating particular solutes with simultaneous uptake of large amounts of water (Claus et al., 2007). The amount of metal extraction by plants is limited by two bottleneck processes: (1) cultivation of appropriate plant/crop species on the contaminated sites that can accumulate high metal concentration in plant biomass; and (2) removal of as much as possible harvestable metal-enriched biomass from the sites. All these factors contribute to the total amount of metals extracted from the soil (Bareen and Tahira, 2010). But the slow growing and small biomass of most heavy metal accumulator species limit the use of hyper-accumulator plants for a large scale remediation. The required time for soil cleaning-up is usually 10–15 years or longer.

Phytoextraction is essentially an agronomic approach and its success depends ultimately on agronomic practices such as plant selection, fertilization and irrigation. Therefore, extensive research has been conducted on process optimization by means of chemically improved plant availability and uptake of heavy metals (Meers et al., 2008). A wide range of potential amendments has been proposed in the literature, with considerable attention being paid to aminopolycarboxylic acids such as ethylene diamine tetraacetie acid (EDTA), ethylenediamine disuccinic acid (EDDS), ethyleneglycol bis(2-aminoethyl ether)tetraacetic acid (EGTA), and diethylenetriamine pentoacetic acid (DTPA) (Bareen and Tahira, 2010; Liu et al., 2008; Luo et al., 2006). However, these compounds have received

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increasing criticism due to limiting of plant growth, their environmental persistence and associated risks for leaching (Di Palma and Ferrantelli, 2005; Wu et al., 2004). It is necessary to find alternatives which are environmental friendly and effective.

In addition to adequate plant species of hyperaccumulators, agronomic practices such as intercropping, and fertilization can have a positive effect on heavy metal extraction. Since phytoremediation strategies are often implemented in nutrient-poor soils, in which available nutrients (nitrogen, potassium and phosphorus) are generally limiting, several investigations have been performed based on the hypothesis that phytoextraction of heavy metals can be enhanced by proper fertilization (Barrutia et al., 2009; Grant and Bailey, 1997; Lin et al., 2009; Lu et al., 1998; Ni et al., 2004; Sarwar et al., 2010; Zhao et al., 1998; Zwonitzer et al., 2003). In addition to stimulating plant growth, fertilization can enhance microbial growth such as rhizosphere plant growth-promoting bacteria (Barea et al., 2005; Glick, 2010). These will enhance the availability of heavy metals to plants.

Mowing, clipping and/or harvesting period may also affect plant productivity by the direct effect of defoliation on below-ground biomass (Vinther, 2006). *Sedum alfredii* has been identified as a zinc (Zn)/cadmium (Cd) hyperaccumulator plant native to China, it has exceptional abilities to tolerate and accumulate high concentrations of Zn/Cd, and the characteristics of large biomass, rapid growth, asexual propagation, and it is a perennial plant (Yang et al., 2002, 2006).

The main objective of this study was to find cheaper, environmentally benign chemical compounds to induce metal bioavailability and optimize the time of harvest. We reported here on a 12-month (three consecutive clippings, one growth cycle of *S. alfredii*) study aimed at assessing the influence of two agronomic practices: phosphorus fertilization and clipping on Zn and Cd accumulation in a long naturally aged heavy metal contaminated soil. Different combinations of phosphorous fertilization, clipping aboveground plant biomass, heavy metal concentrations and sustained rate of metal extraction were evaluated.

1 Materials and methods

1.1 Soil collection and characterization

The aged heavy metal contaminated soil used for this study was collected from the surface layer (0–20 cm) in Fuyang County of Hangzhou in Zhejiang Province, China (Brus et al., 2009; Yin et al., 2009). Soil samples were air-dried, then removed stones and coarse plant roots or residues, and ground to pass through a 2-mm nylon sieve. The sample was thoroughly mixed prior to analysis. Selected physical and chemical characteristics of the soil were determined according to Datta et al. (2010) and shown in Table 1. Soil pH was determined by means of the glass-electrode method with a ratio of soil to water of 1:2.5 (*W*/*V*). For particle-size distribution the hydrometer technique was used. Soil organic matter was determined by wet oxidation method. Soil was extracted with 0.5 mol/L $NaHCO_3$, and P in the extracts was determined following the ascorbic Acid-Blue color method. For characterizing potassium (K) status, soil was extracted with 1 mol/L NH_4OAc (pH 7.0) and K concentration in extracts was estimated using a flame photometer. Moist soil was extracted with 2 mol/L KCl solution and available nitrogen (N) in the extracts was determined by steam-distillation method. Total heavy metal was digested with concentrated HNO_3-$HClO_4$-HF in closed Teflon vessels, the available heavy metal was extracted using extractable Mehlich-3, and ion concentration was determined by ICP-OES (Thermo Scientific ICAP 6000 series, Thermo Fisher Scientific Inc., USA).

Table 1 Physico-chemical properties of tested soil

Parameter	Value
pH (soil:water, 1:2.5, *W*/*V*)	7.8 ± 0.2
Organic matter (g/kg)	46.7 ± 3.1
Available nitrogen (mg/kg)	68.1 ± 4.9
Available phosphorus (mg/kg)	26.7 ± 2.4
Available potassium (mg/kg)	72.3 ± 2.4
Sand (2–0.05 mm) (%)	55.63 ± 0.69
Silt (0.05–0.002 mm) (%)	38.16 ± 0.47
Clay (< 0.002 mm) (%)	6.32 ± 0.12
Texture	Sandy loam
Total metals (mg/kg)	
Zn	1678.4 ± 34.5
Cd	6.45 ± 0.43
Available metals (mg/kg)	
Zn	436.2 ± 7.9
Cd	2.14 ± 0.24

1.2 Experimental design

A randomized pot experiment was conducted in glasshouse. Polyethylene pots were filled with 1 kg air-dry soil. A filter paper was placed at the bottom of each pot to cover the holes and prevent the loss of soil. Four types of phosphate (P) fertilizers were tested, including KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$, each at the rates of 0, 22, 88 and 352 mg P/kg soil. The P fertilizer was dissolved in distilled water and then mixed with soil. In addition, 200 mg N (as urea) and 200 mg K (as K_2SO_4)/kg soil (based on air-dry weight) were applied as base fertilizers. The control was also applied with N and K but zero P fertilizer. Each treatment was replicated three times. The moisture of fertilized soils in the pot was then adjusted with distilled water to 50% of water holding capacity (WHC) and maintained at this moisture level for three months of soil aging.

1.3 Seedling preparation and pot experiments

S. alfredii plants were collected from Pb/Zn mining area in Zhejiang Province of China. Healthy and equally-sized shoots of *S. alfredii* were chosen and grown in plastic culture pots for 3 weeks in modified Hoagland nutrient medium for the initiation of new roots. After pre-culture, five uniform and healthy seedlings with fine root of *S. alfredii* were transplanted in each pot on June 22, 2009. Pots were watered daily up to 50%–65% of the maximal WHC. Pot experiments were performed in a glasshouse with natural light, relative humidity varied between 50%

and 70%.

1.4 Plant harvest

At harvest, shoots were cut at their base, using scissors, reserving stem stubble 2–3 cm height, keeping the new roots grew out of small buds, in order for the plant to continue to grow. The shoots of the first, second and third crops were harvested in October 2009, February 2010 and June 2010, respectively. The growth period between each harvest was four months. The harvested shoot was thoroughly washed with running tap water and rinsed with ultra pure water three times to remove any soil particles attached to plant surfaces, oven-dried in paper bags at 75°C for 72 hr. The dried tissues were then weighed immediately upon removal from a desiccator, and ground into powder less than 0.25 mm with an agate ball mill (Retsch RS100, Germany) for the analysis of heavy metals and other elements.

1.5 Plant and soil analysis

About 0.2 g of sub-samples was digested with 5 mL concentrated HNO_3 (Sinopharm Chemical Reagent Company, Shanghai, China) and 1 mL $HClO_4$ (Zhenxing Chemical Reagent Company, Shanghai, China) in closed Teflon vessels until clear. The digested material was washed into 100 mL flask which was made up to volume (100 mL) using de-ionized water. Metal concentrations in the digested samples were determined using ICP-OES (Thermo Scientific ICAP 6000 series, Thermo Fisher Scientific Inc., USA). Working standard solutions for analysis of the elements were prepared with serial dilutions of stock standard solutions Cd (GBW(E)080628), Zn (GBW(E)0806286), P (GBW(E)081229). For quality control, certified reference peach leaf (GBW08501) was also analysed in triplicate using the same method in each batch of analyses. The analyses results were only accepted when the measured standard concentrations were within 95% to 105% of the certified value.

1.6 Statistical analysis

All data statistical analysis was carried out using the SPSS statistical package (version 13.0). All values reported are means of three independent replications. Data were tested at a significant level of $P < 0.05$ by two-way ANOVA. Graphical work was performed using Origin v.7.5.

2 Results

2.1 Plant growth

The dry matter yield (DMY) of the first harvest increased with P application rate for all the P fertilizers except for KH_2PO_4 at 22 mg P/kg soil (Fig. 1A). When KH_2PO_4, $Ca(H_2PO_4)_2$ or NaH_2PO_4 was applied at 352 mg P/kg soil or $NH_4H_2PO_4$ at 88 mg P/kg soil, the DMY increased by 46%, 62%, 54% and 78% respectively, as compared to the control. The DMY of $NH_4H_2PO_4$ treatment was significantly higher than any of the other three P sources at the application rates of 22 and 88 mg P/kg soil ($P <$ 0.05).

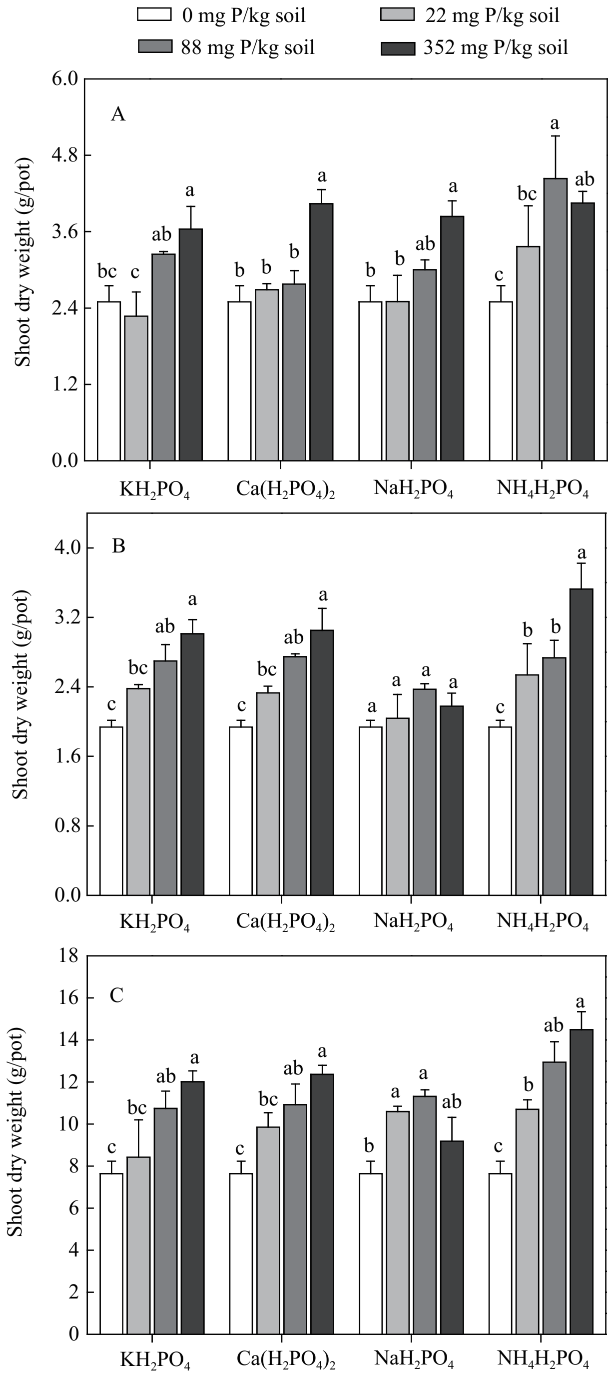

Fig. 1 Effect of different phosphorus fertilizer types and dosages on shoots dry weight of *Sedum alfredii*. (A) first harvest; (B) second harvest; (C) third harvest. Data represents means ± SD (n = 3). Different letters on each bar indicate significant difference among different levels of the same phosphate types ($P < 0.05$).

At the second harvest, the DMY significantly increased by the application of P fertilizers at the levels of 22, 88 and 352 mg P/kg soil, but there was no distinct increase for NaH_2PO_4 ($P < 0.05$). The increment was 55%, 57%, 22% and 82%, respectively, at each higher fertilizer level (Fig. 1B). The lowest biomass yield occurred in the treatments of NaH_2PO_4 for all the P levels, and the difference between NaH_2PO_4 and any other P source was significant at 352 mg P/kg soil ($P < 0.05$). At the third clipping, the plants were lusher and had more tender branches as compared with the first two harvests (Fig. 2). DMY of *S. alfredii* increased from previous cuttings for all the treatments, with the highest DMY increase averaged by 1.6, 1.6, 1.5 and 1.9 folds, respectively for the four P fertilizers, as compared to the control (Fig. 1C). The shoot DMY of *S. alfredii* plants in the treatments of KH_2PO_4, $Ca(H_2PO_4)_2$ and $NH_4H_2PO_4$ was significantly higher than that of NaH_2PO_4 at 352 mg P/kg soil. After three clippings, plants continued to grow better with P fertilizers, particularly $NH_4H_2PO_4$.

2.2 Heavy metal concentration in shoots

2.2.1 Zinc

Phosphorus fertilization resulted in higher uptake of Zn by *S. alfredii* as compared to the control. The increase in shoot Zn concentration reached a significant level when

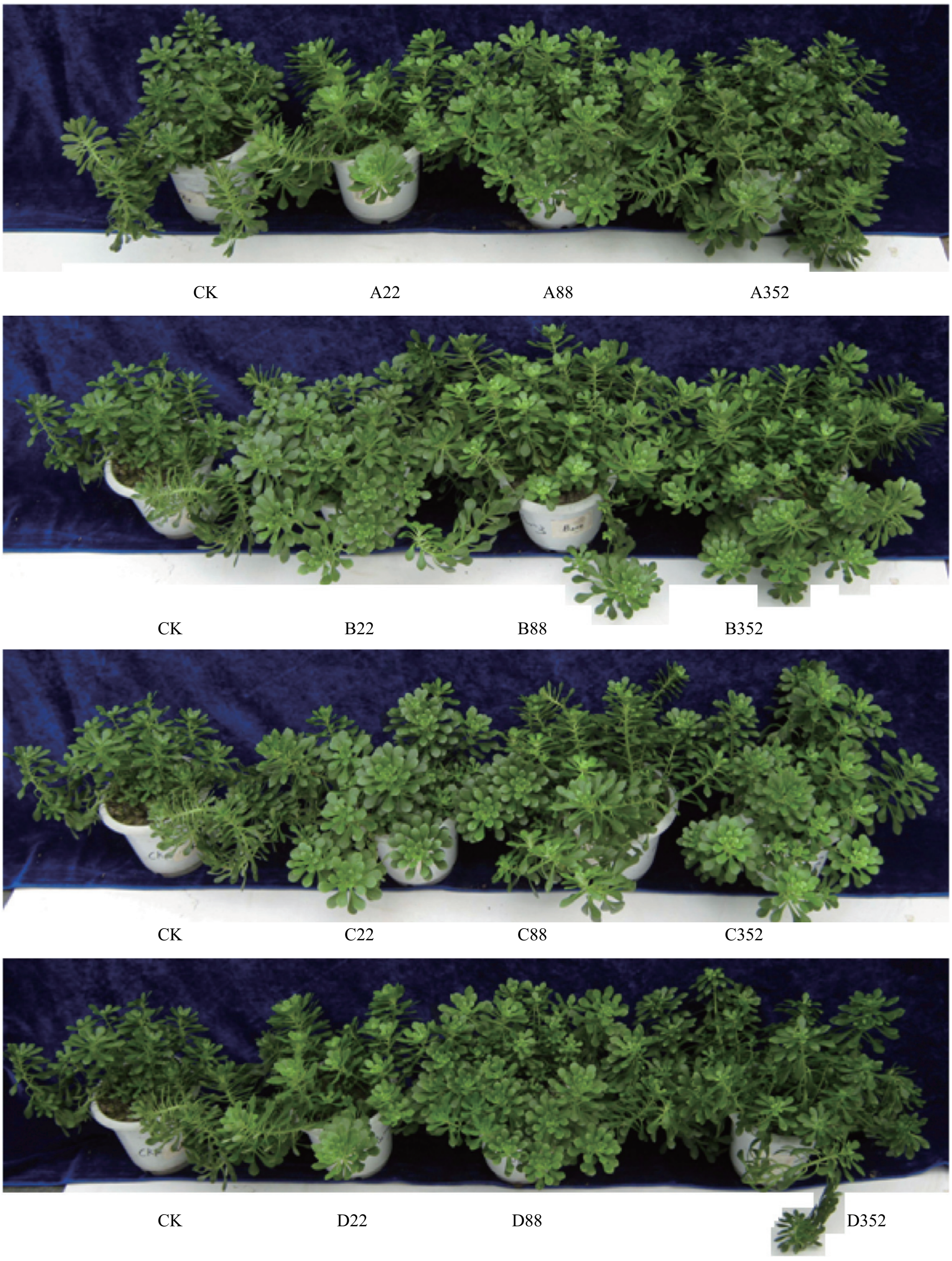

Fig. 2 Photographs of *Sedum alfredii* at the third clipping after 120 days of growth. CK: control; A22, A88 and A352: KH_2PO_4 at 22, 88 and 352 mg P/kg soil, respectively; B22, B88 and B352: $Ca(H_2PO4)_2$ at 22, 88 and 352 mg P/kg soil, respectively; C22, C88 and C352: NaH_2PO_4 at 22, 88 and 352 mg P/kg soil, respectively; D22, D88 and D352: $NH_4H_2PO_4$ at 22, 88 and 352 mg P/kg soil, respectively.

P application rates were 88 mg/kg or higher (except for $NH_4H_2PO_4$). On average, shoot Zn concentrations increased by 85%, 50%, 46% and 70%, respectively, for the application of KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$. The highest Zn concentrations in shoots were observed with KH_2PO_4 and $NH_4H_2PO_4$ treatment at 352 mg P/kg soil, which were significantly higher than those with any other P sources ($P < 0.05$) (Fig. 3A).

After the second harvest, Zn continued to accumulate in shoot, but the increase was not significant at the application rates of 22 and 88 mg P/kg soil (except for $NH_4H_2PO_4$). However, at 352 mg P/kg soil Zn accumulation in shoot was significantly enhanced regardless of P sources ($P < 0.05$). The increases averaged 80%, 107%, 84% and 113%, respectively for the application of KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$. The highest Zn concentrations in shoots were found with the treatments of $Ca(H_2PO_4)_2$ and $NH_4H_2PO_4$ at 352 mg P/kg soil, which were significantly higher than any other P source ($P < 0.05$). Shoot Zn concentration at second cropping was significantly higher than the first crop, and the increase averaged 2.2, 3.1, 2.9, and 2.9 folds at 352 mg P/kg soil for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$, respectively (Fig. 3B).

At the third clipping, the increase in shoot Zn concentration at 22 and 88 mg P/kg soil was not significant, some even lower than the control (Fig. 3C). However, when P application rate reached 352 mg/kg soil, shoot Zn concentration increased by 143%, 95%, 69% and 91%, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$. The highest shoot Zn concentration occurred in the treatments of KH_2PO_4, $Ca(H_2PO_4)_2$ and $NH_4H_2PO_4$ at 352 mg P/kg soil. Shoot Zn concentrations of the third crop were similar to those of the first crop at low P application levels (up to 88 mg/kg soil), but the average values were increased 1.9, 1.9, 1.7 and 1.6 folds, respectively at the application rate of 352 mg P/kg soil for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ (Fig. 3), although they were lower than those of the second clipping, by 14% to 53%.

2.2.2 Cadmium

Cadmium concentrations in shoot generally decreased with increasing P application rate (Fig. 4). Shoot Cd concentration of control was 47 mg/kg. At the first clipping, Cd concentration in the shoot of *S. alfredii* declined by the application of water soluble P (except KH_2PO_4 and $Ca(H_2PO_4)_2$ which resulted in a slight increase), and a marked decrease occurred at 352 mg P/kg soil. The lowest Cd level (only 30 mg/kg) was found in the treatments of $Ca(H_2PO_4)_2$ at 352 mg P/kg soil.

At the second clipping, Cd concentration was significantly lower at 22 and 88 mg P/kg soil, respectively as compared to control, although the amount had slightly increased at 352 mg P/kg soil of $Ca(H_2PO_4)_2$ and $NH_4H_2PO_4$ ($P < 0.05$). Shoot Cd concentration was not significantly different between the second and the first clippings for the control, but declined from 3% for $Ca(H_2PO_4)_2$ at 88 mg P/kg soil to 27% for KH_2PO_4 at

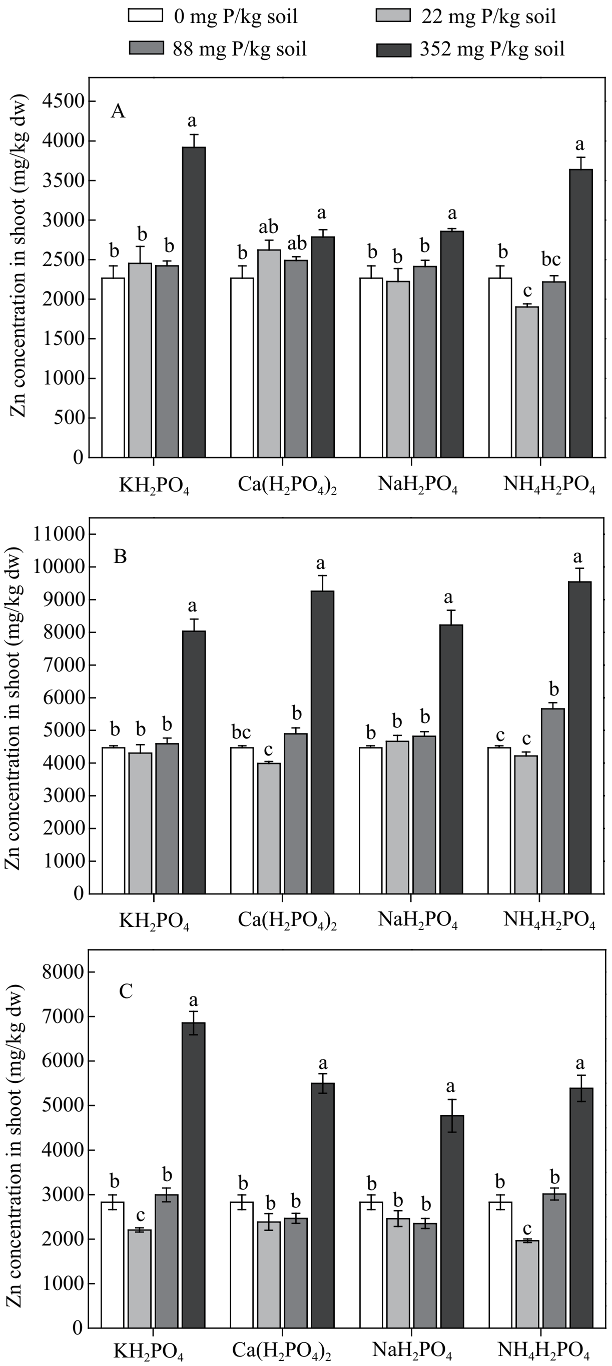

Fig. 3 Effect of different phosphorus fertilizer types and dosage on Zn concentration in shoots of *Sedum alfredii*. (A) first harvest, (B) second harvest, (C) third harvest. Data represents means ± SD (n=3). Different letters on each bar indicate significant difference among different levels of the same phosphate types ($P < 0.05$).

22 mg P/kg soil. On the other hand, Cd concentration increased by 9% for KH_2PO_4 and by 65% for NaH_2PO_4 at 352 mg P/kg soil.

Similar results were obtained when the plants of *S. alfredii* were periodically clipped (Fig. 4C). P fertilization resulted in significantly lower Cd concentration in shoots for all the P sources and rates (except for 352 mg P/kg soil as KH_2PO_4) compared to control ($P < 0.05$). Shoot Cd level of the third harvest were 31 mg/kg in control and 17 mg/kg at 22 mg P/kg soil in the treatment with $NH_4H_2PO_4$, the lowest of the three clippings. Shoot Cd concentration declined by 14% to 59% in the second harvest and by 21% to 58% in the third harvest.

2.3 Phosphorus concentration in shoots

Phosphorus concentrations in the shoots of *S. alfredii* increased significantly ($P < 0.05$) with P supply (Fig. 5), and peaked at 352 mg P/kg soil except for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 with the first clipping. Shoot P concentration increased in the first clipping by 1.3, 1.2, 1.1 and 1.3 folds, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ at 352 mg P/kg soil, as compared with control. The corresponding shoot P increases were 1.5, 1.5, 1.7 and 1.7 folds in the second clipping. The difference in shoot P concentration between control and the highest P rate was the greatest in the third clipping, an increase by 192%, 89%, 113% and 136% for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$, respectively.

There were variations of shoot P concentration among the three harvests. The highest P concentration was observed in the second clipping samples. For example, shoot P concentration in control was 2548 mg/kg, which was 1.5 times of the first clipping. After the third harvest, shoot P concentration decreased by 27% in control and 5%–25% in the 22 mg/kg P treatment, but increased at higher P levels than the first harvest. Shoot P concentration of the third harvest sample was lower than that in the second harvest samples, only 0.5, 0.4–0.7, 0.5–0.7 and 0.6–0.9 folds, respectively at 0, 22, 88 and 352 mg P/kg soil treatments.

2.4 Heavy metal extraction by shoots

2.4.1 Zinc

Table 2 shows Zn accumulation by aerial parts of *S. alfredii* after one year. After the first clipping, Zn accumulation in the shoots of control was 5.6 mg/pot. Shoot Zn extraction increased significantly with P application rate for all the P sources ($P < 0.05$). The highest Zn extraction after one planting season reached up to 14.8 mg/pot with $NH_4H_2PO_4$ at 352 mg P/kg soil. Shoot Zn extraction averagely increased by 2.5, 2.0, 2.0 and 2.6 folds, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ at 352 mg P/kg soil, as compared to control.

At the second harvest, the increase in shoot Zn accumulation was more significant due to higher elemental concentration. Zn accumulation in shoot increased with increasing P application rate and reached 2.8, 3.5, 2.1 and 4.2 folds, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ at 352 mg P/kg soil as compared to control. The maximum Zn extraction was 36 mg/pot at 352 mg P/kg soil as $NH_4H_2PO_4$. The increase of Zn accumulation at the second clipping was 1.3–2.0, 1.6–2.0, and 1.6–2.6 folds, respectively at 22, 88 and 352 mg P/kg soil, as compared to the first clipping.

Uptake of Zn by plant increased significantly in the third clipping mainly because of higher biomass yield. Zn accumulation in control plants were 21.8 mg/pot, which was increased by 3.9 and 2.5 folds after the first and second clippings. At 352 mg P/kg soil, Zn extraction was increased by 4.0, 3.1, 2.2 and 3.8 folds in KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ treatments, respectively. There were significant increases in Zn accumulation in the third harvest. The average Zn extraction in control was augmented by 289% at the first harvest and 152% at the second harvest. The Zn accumulation at the third clipping was augmented by 228%–348%, 270%–310%, and 325%–519%, respectively at 22, 88 and 352 mg P/kg soil as compared to the first clipping. The increase at the third clipping was 1.8–2.5, 2.0–2.6 and 2.3–3.6 folds, respectively for 22, 88 and 352 mg P/kg soil over the second clipping.

2.4.2 Cadmium

Cadmium extraction by shoots in control was 119 μg/pot after the first clipping (Table 3). The amount of shoot Cd slightly increased with P application rate, and reached on average 1.2, 1.0, 1.1 and 1.4 folds, respectively, for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ at 352 mg P/kg soil. The highest Cd extraction after one planting season reached up to 162.6 μg/pot for the treatment of $NH_4H_2PO_4$ at 352 mg P/kg soil.

After the second harvest, Cd accumulation was slightly

Table 2 Zinc extraction from soil by shoots of *Sedum alfredii* after one year growth*

P source	Harvest	Zinc extraction (mg/pot)			
		0 mg P/kg soil	22 mg P/kg soil	88 mg P/kg soil	352 mg P/kg soil
KH_2PO_4	1st	5.62 ± 0.30 c	5.32 ± 0.07 c	7.95 ± 0.23 b	14.17 ± 0.24 a
	2nd	8.63 ± 0.25 b	10.47 ± 1.01 b	12.51 ± 1.71 b	24.56 ± 3.28 a
	3rd	21.84 ± 2.99 bc	18.61 ± 4.01 c	32.44 ± 4.02 b	87.32 ± 5.45 a
	Total	36.09	34.40	52.90	126.06
$Ca(H_2PO_4)_2$	1st	5.62 ± 0.30 c	7.12 ± 0.45 b	6.90 ± 0.31 b	11.33 ± 0.51 a
	2nd	8.63 ± 0.25 b	9.35 ± 0.04 b	13.52 ± 0.98 b	29.81 ± 3.86 a
	3rd	21.84 ± 2.99 bc	23.36 ± 0.69 b	26.93 ± 2.78 b	68.14 ± 5.19 a
	Total	36.09	39.83	45.35	109.28
NaH_2PO_4	1st	5.62 ± 0.30 c	5.80 ± 0.44 c	7.22 ± 0.10 b	11.06 ± 0.42 a
	2nd	8.63 ± 0.25 b	10.36 ± 1.10 b	11.48 ± 0.43 b	17.92 ± 1.62 a
	3rd	21.84 ± 2.99 bc	26.05 ± 1.37 b	26.62 ± 2.02 b	46.83 ± 5.53 a
	Total	36.09	42.21	45.32	75.81
$NH_4H_2PO_4$	1st	5.62 ± 0.30 c	6.35 ± 0.47 c	9.71 ± 0.44 b	14.80 ± 0.83 a
	2nd	8.63 ± 0.25 c	10.70 ± 1.69 bc	15.42 ± 0.69 b	36.02 ± 4.09 a
	3rd	21.84 ± 2.99 c	21.07 ± 1.11 c	39.14 ± 3.97 b	83.04 ± 5.73 a
	Total	36.09	38.12	64.27	133.86

* Data are represented as mean ± SD ($n = 3$), different letters on each row mean significant differences, 2-way ANOVA ($P < 0.05$).

Table 3 Cadmium extraction from soil by shoots of *Sedum alfredii* after one year growth*

P source	Harvest	Cadmium extraction (μg/pot)			
		0 mg P/kg soil	22 mg P/kg soil	88 mg P/kg soil	352 mg P/kg soil
KH_2PO_4	1st	119.01 ± 14.43 ab	114.32 ± 2.85 b	128.84 ± 4.03 ab	143.32 ± 6.73 a
	2nd	88.73 ± 1.70 b	92.10 ± 2.58 b	94.83 ± 9.75 b	130.90 ± 16.73 a
	3rd	240.34 ± 30.95 b	181.63 ± 40.05 b	241.34 ± 29.53 b	408.04 ± 30.34 a
	Total	448.08	388.05	465.01	682.26
$Ca(H_2PO_4)_2$	1st	119.01 ± 14.43 a	131.44 ± 9.66 a	114.54 ± 9.05 a	123.41 ± 5.10 a
	2nd	88.73 ± 1.70 b	85.62 ± 3.61 b	109.41 ± 7.10 b	149.33 ± 20.95 a
	3rd	240.34 ± 30.95 ab	203.11 ± 8.98 b	195.72 ± 21.11 b	314.02 ± 20.67 a
	Total	448.08	420.17	419.67	586.76
NaH_2PO_4	1st	119.01 ± 14.43 ab	99.90 ± 5.19 b	128.91 ± 2.13 a	132.41 ± 4.74 a
	2nd	88.73 ± 1.70 b	85.42 ± 8.22 b	96.13 ± 11.04 b	124.12 ± 5.74 a
	3rd	240.34 ± 30.95 a	243.05 ± 14.85 a	215.02 ± 16.94 a	242.24 ± 29.52 a
	Total	448.08	428.37	440.06	498.77
$NH_4H_2PO_4$	1st	119.01 ± 14.43 c	126.92 ± 4.90 bc	147.82 ± 6.71 ab	162.60 ± 15.50 a
	2nd	88.73 ± 1.70 b	104.71 ± 16.77 b	111.42 ± 6.67 b	175.02 ± 7.17 a
	3rd	240.34 ± 30.95 bc	186.10 ± 11.15 c	265.91 ± 23.57 ab	320.04 ± 16.21 a
	Total	448.08	417.73	525.15	657.66

* Data are represented as mean ± SD (n = 3), different letters on each row means significant differences, 2-way ANOVA ($P < 0.05$).

enhanced at lower P levels, but there was no significant difference between control and 22/88 mg P/kg soil. Application of 352 mg P/kg soil caused a significant increase in Cd extraction over control ($P<0.05$). Shoot Cd extraction increased on average by 1.5, 1.7, 1.4 and 2.0 folds, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ at 352 mg P/kg soil as compared to control. However, Cd extraction was reduced by 15%–35% and 5%–26% in the second clipping for the treatments of 22 and 88 mg P/kg soil respectively as compared to the first clipping.

Cd extraction in control treatment after third harvest was 240.3 μg/pot, which were 2.0 and 2.7 folds more than the first and second clipping. There were no significant changes in Cd accumulation at 22 and 88 mg P/kg soil compared to control for each P level ($P < 0.05$). However, application of 352 mg P/kg soil significantly increased Cd extraction ($P < 0.05$), by 1.7, 1.3, 1.0 and 1.3 folds, respectively for KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$. Cd accumulation was significantly increased at the third harvest, by 47%–143%, 67%–87%, and 83%–185%, respectively at 22, 88 and 352 mg P/kg respectively soil from the first clipping, and by 1.8–2.9, 1.8–2.6 and 1.8–3.1 folds, respectively at 22, 88 and 352 mg P/kg soil from the second clipping.

3 Discussion

3.1 Clipping changes in plant growth and metal extraction

The seasonal variation of shoot biomass and metal concentrations in the plants of *Sedum alfredii* suggest that either the availability of metals in the soil has a seasonal pattern or growth dilution/shunting may occur in plant tissues. Thereby, differences in plant behaviors were noticed especially in the different clippings in this study. Study on *Thlaspi caerulescens* has shown that shoot biomass increases with successive cropping, particularly at third one, whereas the biomass of maize at second and third harvest was significantly lower than first cropping (Lombi et al., 2001). The present study showed that shoot dry weight and heavy metal concentration were sharply changed. The highest biomass occurred at the third clipping, while the lowest biomass was at the second clipping. These were related with different growth seasons of plant. The first growing period was June to October, and the ambient temperature was high and sunlight was strong, which was not suitable for *S. alfredii* growth. From November to following February was winter in China, ambient temperature was the lowest and *S. alfredii* was in dormant period, so the dry weight was the least at the second harvest. The most appropriate growth period was spring to mid-summer. The rootstock of plant was stolon and many small buds grew after winter cold, and covered nearly 60% of the land surface. After two clippings, the root system of *S. alfredii* was very strong, able to absorb more nutrients and promoting shoot growth. Therefore the shoot biomass at the third harvest was 3–5 folds higher than the first two harvests.

Phytoextraction is a remediation technique that refers to the durability notion of shoots and used plants have to keep a similar behavior toward metals in due course of time. Few studies have been conducted on heavy metal accumulation by hyper-accumulators with consecutive harvests. Hyper-accumulator species have been reported to extract a similar amount of heavy metals in consecutive crops over different seasons and years (Bidar et al., 2009; Jiang et al., 2010; Lombi et al., 2001). Lombi et al. (2001) found that Zn or Cd concentrations in the shoots of *T. caerulescens* do not decrease with cropping. Light fluctuations of Zn and Cd concentrations in washed shoots of *Trifolium repens* have been observed over the seasons and over the years. But great changes of Zn and Cd concentrations in washed shoots of *Lolium perenne* have been observed over time (Bidar et al., 2009). In the present study the highest Zn concentration was measured at the second harvest and lowest was at the first harvest. The difference was 1.7–3.1 folds. Zinc concentration in control plants was 1965 mg/kg at the first harvest, but increased 2.3 folds in the second

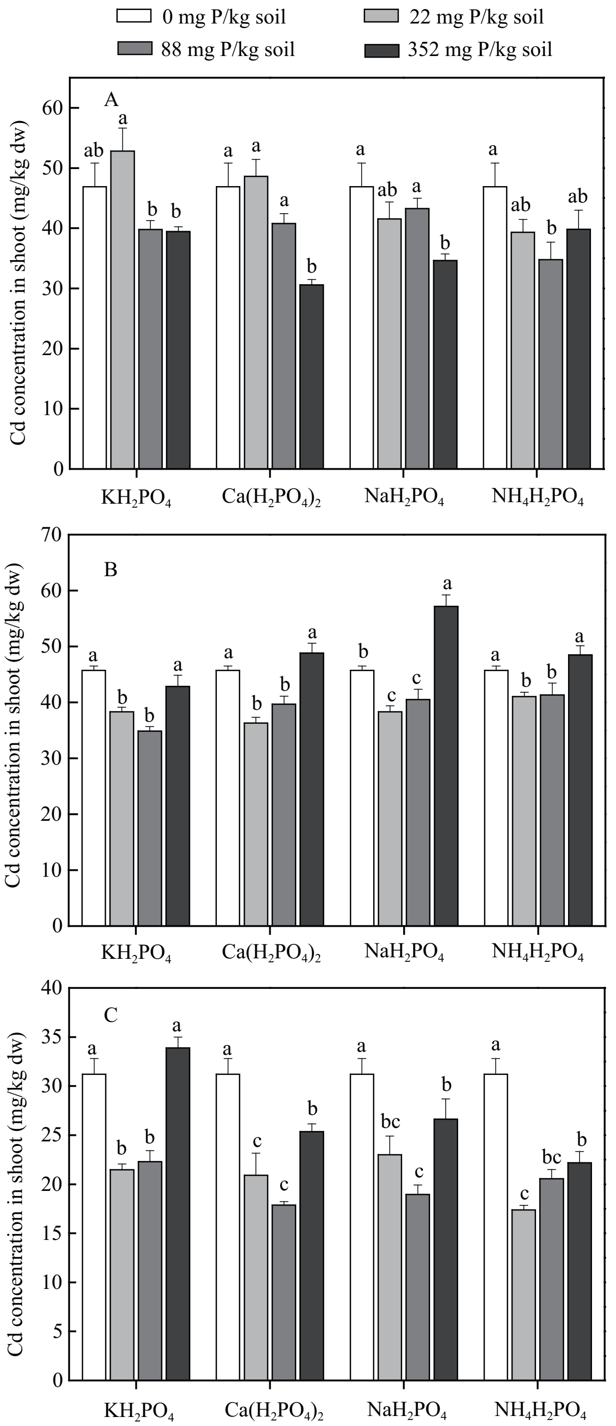

Fig. 4 Effect of different phosphorus fertilizer types and dosages on Cd concentration in shoots of *Sedum alfredii*. (A) first harvest, (B) second harvest, (C) third harvest. Data are represented as mean ± SD (n=3). Different letters on each bar indicate significant difference among different levels of the same phosphate types ($P < 0.05$).

harvest. We speculate that the root growth increased after the first harvest thus increased contact area of soil, so that roots could take up more available Zn in soil. At the same time, low-molecular-mass organic acids produced at soil-root interface (rhizosphere) may also play an important role in the availability of Zn to plants (Barea et al., 2005). Zn concentration at the third clipping was lower than the second clipping. This may be generally referred to as a “dilution effect”, resulting from metal slower uptake than increased dry weight of the leaves (Bidar et al., 2009; Brekken and Steinnes, 2004; Duman et al., 2007). Another possible explanation could be via three time’s consecutive harvests, together with decreasing available Zn concentration for long aged soil, leading to lower Zn content in shoots.

In contrary, Cd concentration in shoots of *S. alfredii* was decreased with increasing clippings (except for the second clipping at 352 mg P/kg soil). Shoot Cd concentrations significantly decreased with consecutive clippings at different P sources and levels, perhaps due to a decrease in available Cd concentrations of soil as phytoremediation

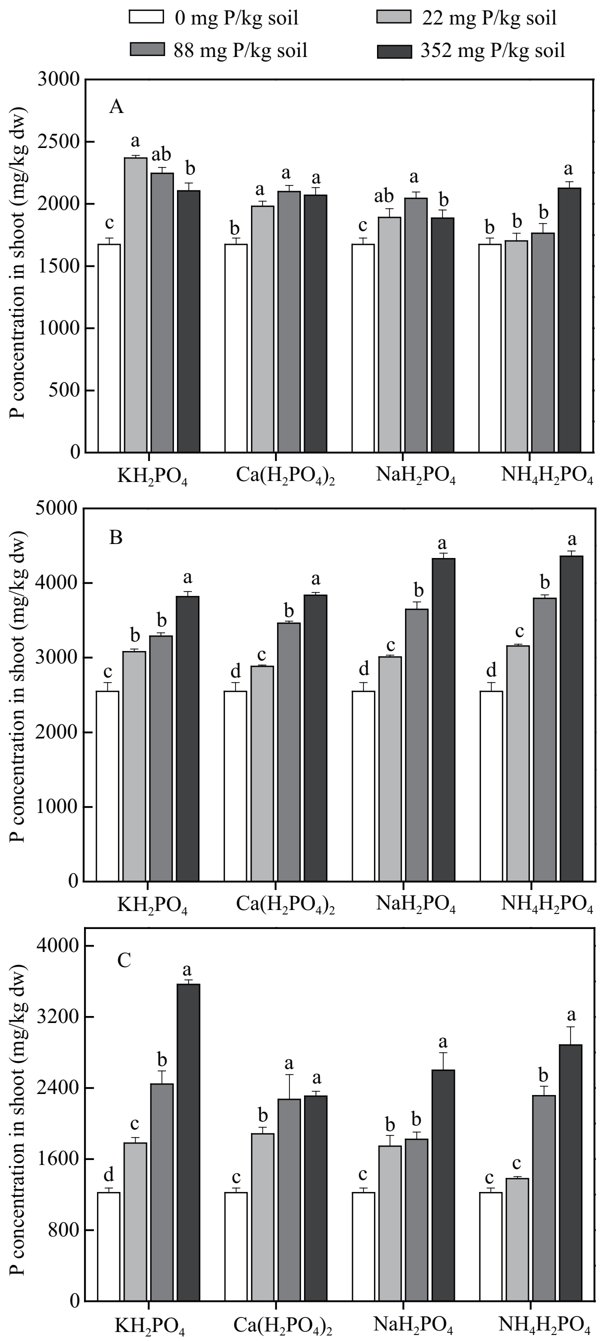

Fig. 5 Effect of different phosphorus fertilizer types and dosages on P concentration in shoots of *Sedum alfredii*. (A) first harvest, (B) second harvest, (C) third harvest. Data are represented as means ± SD (n=3). Different letters on each bar indicate significant difference among different levels of the same phosphate types ($P < 0.05$).

proceeded. Similar results have also been reported by Joner and Leyval (2001) in maize and Jiang et al. (2010) in *Sedum plumbizincicola*. It is interesting to mention that, phytoextraction efficiency for Zn and Cd did not decrease with consecutive clipping. At the third cropping, which produced the highest biomass, extraction of Zn and Cd in the shoots was also the highest. This suggests that optimization of the growing conditions including appropriate nutrient levels plus phosphorus fertilization and multiple harvests might result in an increased yield without decreasing metal concentration.

3.2 Phosphorus fertilizer on metal accumulation

Phosphorus-zinc interactions in normal plants are well established, especially in cereal crops and vegetables (Lu et al., 1998). In most cases, excessive P can induce zinc deficiency. The results from this study indicate that Zn concentration was considerably increased with P levels, especially at 352 mg P/kg soil (Fig. 3). These results did not agree with Ni et al. (2004) who have found that *S. alfredii* shoot biomass and Zn concentration at 31 mg P/kg treatment is significantly greater than others. This difference might be due to difference of soil characteristics and phosphate forms. Zhao et al. (1998) reported that foliar P spray has little effect on the concentrations of total and soluble Zn in the shoots of hyper-accumulator *Thlaspi caerulescens*. In contrast, the total Zn concentration in the roots is increased significantly by the foliar P spray, particularly in the highest Zn treatment. They have inferred that a close positive correlation does exist between insoluble P and insoluble Zn in roots, the slope of the linear regression is 0.3, a value close to P:Zn ratio of 0.31 in $Zn_3(PO_4)_2$. Zn precipitated with inorganic phosphate in root can suppress the transport of P from roots to shoots (Zhao et al., 1998). But in our results, P was continuously transported from roots to the shoots up to 22–352 mg P/kg soil; the detailed reasons for the phenomenon will be further studied. These results indicated that "phosphorus induced Zn requirement" was evident in *S. alfredii*. These results confirmed previous studies on Indian mustard in which shoot Zn concentration does increase after P supply in solution increases (Hamlin et al., 2003). Zn concentrations in all wheat cultivars increase when the P level in the soil is increased from 0 to 25 μg P/g soil (Imtiaz et al., 2006). The level of P does affect the metabolism of nucleic acid, protein, sugar, organic acids and other organic molecules in plants, and these metabolites are often major zinc binding sites. Therefore, the increase of phosphorus will affect the binding form of zinc in plants and transport/physiological functions. Zhao et al. (1998) found that precipitation of Zn with inorganic phosphate or phytate is unlikely to play a significant role in Zn tolerance in shoots of *T. caerulescens*. A significant exponential relationship between Cd/Zn uptake rate and cellular P concentration in *Microcystis aeruginosa* has been observed (Zeng and Wang, 2009). In our study there was a significant positive relationship between total P and Zn concentration in shoots of *S. alfredii* with all species and levels of phosphate. The simulation equation was $Y = 2.204X - 1615.80$ ($R^2 = 0.79$, $P < 0.0001$) (Fig. 6). *S. alfredii* is a natural inhabitant of old Pb/Zn mined sites (the corresponding soils contained the total Zn of 3858 mg/kg, DTPA-extractable Zn were about 325.4 mg/kg). It has an exceptional ability to tolerate and accumulate high concentration of Zn (shoots contained about 5000 mg/kg Zn) (Yang et al., 2002). The tested soil total and Mehlich-3 extractable Zn concentrations were 1678 and 436 mg/kg, respectively, which would not inhibit plant growth, therefore in this study Zn accumulation is also very high. The application of P at appropriate rates may be useful an approach to enhance the growth and Zn accumulation in *S. alfredii* for phytoremediation of the Zn contaminated soils.

The variation of Cd in shoots was different from that of zinc concentration under tested P fertilizer treatments. The general trend of Cd concentration decreased with increasing P level. Similar results have been obtained by Dheri et al. (2007), who indicated that the application of P decreases Cd concentration in shoot of *Spinacia oleracea*, and the decrease is higher in sandy soil than silty-loam soil. Basta et al. (2001) have reported that plant-tissue concentration of Cd is consistently reduced in the presence of soluble P, possibly through the formation of mixed metal phosphates, which can have restricted Cd uptake by plants. The results indicated that *in situ* immobilization of Cd occurred in the phosphate amended soils, probably due to the phosphate-induced Cd^{2+} adsorption and precipitation of Cd as $Cd(OH)_2$ and $Cd_3(PO_4)_2$ with the addition of KH_2PO_4 (Bolan et al., 2003). Another explanation could be due to the fact that Cd and Zn are in the same group in the periodic table of the elements; they may compete with each other for exchange sites on root surface and for the transporter inside the plant. The tested soil had high Zn concentration and higher Zn^{2+} restricted root Cd^{2+} absorption. Most soluble phosphate significantly increased the phytoextraction capacities of *S. alfredii* to Cd due to the increase in shoot dry weight. However, Yu and Zhou (2009) have found that Cd accumulation in the leaves and shoots of *Mirabilis jalapa* significantly decreases with increasing P concentrations from 20 to 500 mg/kg, P and Cd may deposit in plants to tolerate Cd toxicity for

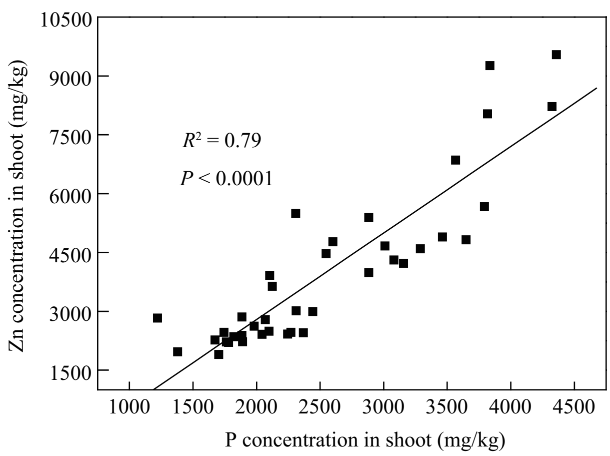

Fig. 6 A correlation analysis of phosphorus concentration and zinc concentration in shoot of *Sedum alfredii* in a zinc-contaminated soil for three clippings. The data was four species of phosphate at each mowing ($n = 39$).

reducing the degree of structural damage in plants.

4 Conclusions

The results from this study showed that hyperaccumulator plant *S. alfredii* is effective in phytoextraction of Zn and Cd from moderately contaminated soils if applied with soluble P fertilizers. Higher application rates (up to 352 mg P/kg soil) of P in the forms of KH_2PO_4, $Ca(H_2PO_4)_2$, NaH_2PO_4 and $NH_4H_2PO_4$ did not show negative effect on plant growth. The application of P fertilizers increased shoot biomass and Zn concentration but decreased plant Cd concentration. Zn phytoextraction by *S. alfredii* was significantly enhanced by P fertilization. The extent of biomass increase was higher than the reduction in plant Cd concentrations, thus increasing extraction capacity of Cd by *S. alfredii* with P fertilization. Moreover, there was a significant positive correlation between shoot P and Zn concentration. As for different harvests, the largest biomass occurred at the third clipping, the highest Zn concentration was observed at the second clipping, and the highest Cd concentration was measured at the first clipping. The metal extraction appeared to increase with clipping time. Among the different P sources, KH_2PO_4 and $NH_4H_2PO_4$ were superior to the others. Consecutive harvesting apparently enhanced the extraction Zn and Cd from contaminated soil by *S. alfredii*.

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