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Growth and Zinc Accumulation of *Sedum alfredii* Hance—a Zn Hyperaccumulator as Affected by Phosphorus Application

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Plant species, which are endemic to metalliferous soils, are tolerant of metal toxicity. Two basic strategies of metal tolerance exist: (1) exclusion, whereby plants avoid excessive uptake and transport of metals, and (2) accumulation and sequestration, whereby plants take up large amounts of metal and transfer the metal to the shoots where it is accumulated (Baker, 1981). Plants which possess the second strategy and are able to accumulate greater than 100 times the metal concentrations of normal species have been termed hyperaccumulators (Brooks, et al., 1977; Baker and Brooks, 1989). Approximately 400 hyperaccumulating species of plants have been reported, of which about 18 are Zn hyperaccumulators, defined as containing more than 10 g Zn/kg in shoot dry matter (DM) (Baker et al., 2000; Reeves and Baker, 2000).

Plants that hyperaccumulate metals have attracted attention for many years as geobotanical indicators of mineral deposits. More recently, their ability to extract metals from soil and concentrate them in shoots, has given rise to practical applications such as phytomining and phytoremediation (Cunningham and Ow, 1996; Raskin et al., 1997; Salt et al., 1998; Brooks et al., 1998; Robinson et al., 1997). Hyperaccumulator plants are potential tools to remove excess metals in contaminated soils. Appropriate candidate plants for the remediation of metal contaminated soils by phytoextraction must possess the desirable and exploitable growth characteristics and adaptability to agronomic practices.

Natural surface exposures of Pb/Zn minerals are generally limited in area. But where mining, ore transport, and smelting have occurred, more extensive development of mineral-rich soils has taken place, and the distribution of hyperaccumulators of Zn and (or) Pb may reflect these activities (Reeves and Baker, 2000). Based on a vegetation survey, a new Zn-accumulating ecotype of *Sedum alfredii* Hance, growing naturally in an old Zn/Pb mining region in Quzhou City (29°17′ N, 118°56′ E), Zhejiang province, P. R. China, has been identified (Yang, *et al.*, 2002). Under natural conditions, shoot Zn concentrations in *Sedum*

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alfredii Hance plants grew on old mining soils with total Zn concentrations of 2269-3858 mg/kg soil and available Zn of 105.5-325.4 mg/kg soil ranged 4134-5000 mg/kg.

Phosphorus-zinc interactions in normal plants are well established (Mengel and Kirkby, 1987; Srivastava and Gupta, 1996). In most cases, excessive P can induce zinc deficiency (Cakmark and Marschner, 1986; Paker et al., 1992; Webb and Loneragan, 1988). In Zn-hyperaccumulator, Phosphorus-zinc interactions are still unclear. Zhao *et al.* (1998) reported that P supply had no effects on zinc accumulation in the hyperaccumulator *Thlaspi caerulescens*.

The objective of the reported work was to study the impact of phosphorus application on the growth of *Sedum alfredii* Hance using a pot experiment under greenhouse condition, and to identify Zn accumulation response of *Sedum alfredii* Hance to phosphorus fertilization.

MATERIALS AND METHODS

Stems of *Sedum alfredii* Hance were collected from their naturally growing areas, and transplanted into a basic nutrient solution, then cultured for 30 days for preparing seedlings. The basic nutrient solution contained the following nutrient concentrations (μmol/L): 2000 $Ca(NO_3)_2{\cdot}4H_2O$, 1000 KH_2PO_4, 500 $MgSO_4{\cdot}7H_2O$, 100 KCl, 700 K_2SO_4, 10.0 H_3BO_3, 0.50 $MnSO_4{\cdot}H_2O$, 0.50 $ZnSO_4{\cdot}7H_2O$, 0.20 $CuSO_4{\cdot}5H_2O$, 0.01 $(NH_4)_6Mo_7O_{24}$, 100 Fe-EDTA.

For the pot experiment, the topsoil (0~20 cm) of the old-mining soil, in which the Zn-accumulating ecotype of *Sedum alfredii* Hance naturally grew in Quzhou City (29°17′ N, 118°56′ E), Zhejiang province, P. R. China, was collected, and air-dried, dissagregated and passed though a 0.25mm nylon screen. The important properties of the test soil were as follows: pH(H_2O) 5.24, total organic carbon 16800 mg/kg soil, total nitrogen 1440 mg/kg soil, total phosphorus 2570 mg/kg soil, hydrolizable nitrogen 149.9 mg/kg soil, available phosphorus 6.2 mg/kg soil, total zinc 2326 mg/kg soil, and extractable zinc 31.1 mg/kg soil.

The pot experiment grown with *Sedum alfredii* Hance plants included five treatments 0 (CK), 31, 62, 124, 248 mg P/kg soil (added as NaH_2PO_4). The supplied doses of N (in urea) and K (in KCl) were equal for all treatments in the pot experiment: 56 mg N/kg soil, and 39 mg K/kg soil applied as basal fertilization. One thousand grams of the prepared soil were placed in each plastic pot. The treatments were quadruplicated and randomly arranged under greenhouse conditions. Three seedlings of similar length were selected and transplanted into each plastic pot. During the experimental period, deionized water was added to compensate for evapotranspiration losses when it was needed. Plant shoots were harvested after 10 weeks growth and washed with deionized water.

Plant tissues were oven-dried at 70°C to constant weight, recorded the weight by each pot, and ground in a stainless steel mill. Dry plant material was placed in porcelain crucibles and mineralized by oven-drying digestion methods. Digested samples were dissolved in 6.0 mol/L HCl, filtered and brought to 25 ml. For the determination of total soil Zn concentrations, the homogenized sample (0.5 g) was digested with aqua regia and diluted to 100 ml with 2% HNO_3. The extractable Zn in soils was extracted by 0.005 mol/L DTPA. Zinc in digesting solution of plant materials and soils, and in extracting solution of soils were measured with a flaming atomic absorption spectrophotometer (Shimazu AA-6800). Soil pH and soil N, P, and total organic carbon concentrations were measured with conventional methods (Committee of Agrochemistry, 1983).

The significance of differences between the means of the treatments was evaluated by one-way analysis of variance followed by Duncan's multiple range test at 5% significance.

RESULTS AND DISCUSSION

Shoot dry weights of *Sedum alfredii* Hance were significantly ($p<0.05$) affected by phosphorus application, with the greatest growth occurring at 31 P mg/kg soil supplied (Figure 1). Shoot biomass was significantly greater for phosphorus application (except the 248 P mg/kg treatment) than for no phosphorus application. Shoot biomass of the 31 P mg/kg treatment was significantly greater than those of the 62, 124, 248 P mg/kg treatments. These results indicated that phosphorus application could significantly enhance the growth of *Sedum alfredii* Hance at appropriate doses of P supply, and excessive P application could significantly inhibit growth. Therefore, P application rates must be adjusted according to the available P levels in the contaminated soil to avoid the detrimental effects of excessive P application.

Zinc concentration in shoots of *Sedum alfredii* Hance were also significantly ($p<0.05$) affected by P application, with the highest concentration occurring at 31 mg/kg supplied (Table 1). Zn concentration in shoots of *Sedum alfredii* with 31 mg/kg treatment was significantly higher than that of the no phosphorus application. Zn concentration in shoots of *Sedum alfredii* with 248 mg/kg treatment was significantly lower than those of the 31 or 62 mg/kg treatments. These results indicated that P application could significantly increase Zn

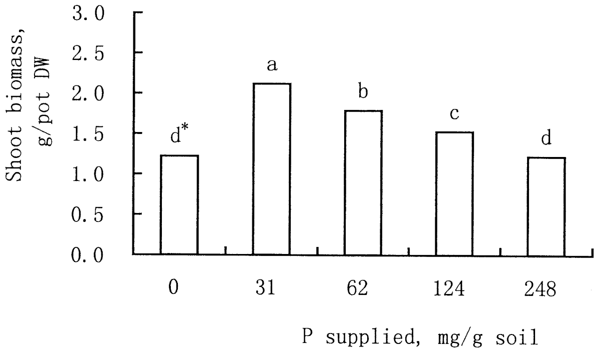

Figure 1. Shoot biomass of *Sedum Alfredii* as affected by P application. ("*": means marked with different letters are significantly different at 0.05 level by Duncan's test)

Table 1. Effects of P application on Zn concentration and accumulation in shoots of *Sedum alfredii* Hance.

P supplied (mg/kg soil)	Zn concentration (mg/g DW)	Zn accumulation (mg/pot)	Bioaccumulation factor (BF*)
0	11.34 bc**	14.07 c	4.88
31	14.90 a	31.70 a	6.41
62	13.21 ab	23.59 b	5.68
124	12.04 bc	18.42 bc	5.18
248	10.99 c	13.29 c	4.72

"BF*": ratio of Zn concentration in shoots to total Zn concentration in the initial soil (2.326 mg/g soil); "**": means followed by different letters are significantly different at 0.05 level by Duncan's test.

concentrations in shoots of *Sedum alfredii* at appropriate doses of P supply, and excessive P application could significantly reduce Zn concentrations.

The Zn bioaccumulation factor (BF) was calculated as the ratio of Zn concentration in shoots to the total Zn concentration in the initial soil (Table 1). The bioaccumulation factor was larger for P application (except for the 248 mg/kg treatment) than for no P application. The BF values (4.72-6.41) were larger than those (1.25-1.94) grown under natural conditions as reported by Yang *et al* (2002). This data suggests that P application may enhance the phytoextraction of Zn in contaminated soils by *Sedum alfredii* Hance.

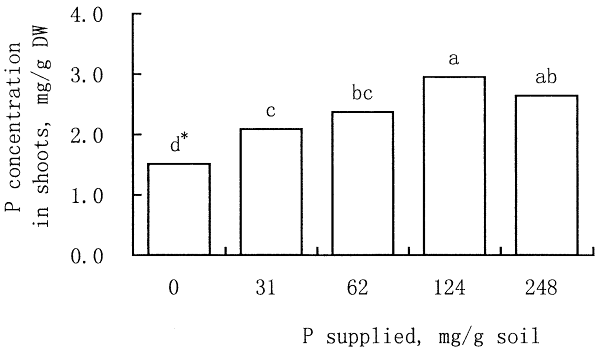

Figure 2. P concentration in shoots of *Sedum Alfredii* Hance as a result of P application. ("*": means marked with different letters are significantly different at 0.05 level by Duncan's test)

Zinc accumulation in shoots was calculated by Zn concentration in shoots × dry weight, which is an important parameter for evaluating the potential effectiveness of hyperaccumulating plant species in phytoremediation. Zn accumulation in shoots was significantly different among the treatments as a result of P application, with the largest Zn accumulation occurring at 31 mg/kg (Table 1). Zn accumulation in shoots of *Sedum alfredii* Hance with 31 or 62 P mg/kg treatment was significantly greater than that of no P application. This result implied that P application could enhance the phytoremediation of Zn contaminated soils.

Phosphorus concentrations in shoots of Sedum alfredii Hance increased significantly ($p<0.05$) with P supply (Figure 2), and peaked at the 124 mg/kg dose. As dry weight, Zn concentration and Zn accumulation were peaked at 31 mg/kg, P phosphorus concentration in shoots was 2.09 mg/g DW which can be regarded as the appropriate level for growth and Zn accumulation.

Phosphorus application can enhance the growth of *Sedum alfredii* Hance – a Zn hyperaccumulator, and increase Zn concentrations and Zn accumulation in shoots. In this experiment, the appropriate phosphorus concentration in shoots of *Sedum alfredii* Hance is about 2.0 mg/g DW, which can be used as an index for P application in practice.

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