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# Study on the tolerance to heavy metal Pb and Pb accumulation of *Sedum aizoon* L.

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**Abstract.** In this study, using *Sedum aizoon* L. as experimental materials. Through hydroponic experiment, the effects of different concentration of  $Pb^{2+}$  solution (0, 20, 60, 100, 140 $mg \cdot L^{-1}$ ) on growth and Lead absorption of *Sedum aizoon* L. was studied. The change law of SOD, POD, and CAT activity influence on chlorophyll. By using atomic absorption spectrometer, the accumulation of Pb was determined. Results showed that with the increase of  $Pb^{2+}$  concentration, the growth of root was inhibited, but plant height was firstly increase and then decrease, the greatest change was 20  $mg \cdot L^{-1}$ . Compared with the control, chlorophyll content increased with concentration of  $Pb^{2+}$  first drop after increased. The activity of SOD, POD and CAT enzyme was increased first and then decreased. The Pb content of root increased with the increase of  $Pb^{2+}$  concentration, but Pb content of stem and leaf increased after the first drop, and the content was the largest when the Pb concentration was 100  $mg \cdot L^{-1}$ . The accumulation of Pb in *Sedum aizoon* L. in vivo as root >> stem > leaf. *Sedum aizoon* L. for Pb tolerated, with concentration of  $Pb^{2+}$  on the transfer coefficient of  $Pb^{2+}$  size order for 60 > 100 > 20 > 140 > 0 ( $mg \cdot L^{-1}$ ).

## 1. Introduction

With the development of economy and society, rapid urbanization and industrialization have caused serious environmental problems, and heavy metal pollution in cities and their surrounding soils has become increasingly serious<sup>[1-2]</sup>.  $Pb^{2+}$  has little mobility in the soil and is potentially harmful when it enters the body through the food chain.  $Pb^{2+}$  accumulates in plants and combines with the sulfhydryl groups of proteins and enzyme active centers, resulting in an imbalance of SOD, POD, CAT and other enzyme activities, causing disturbances in physiological and biochemical processes, ultimately harming plants<sup>[3-5]</sup>.

*Sedum aizoon* L. is a perennial herb of Sedum. It is cold-tolerant and drought-tolerant, easy to plant and manage, and has an important performance of environmental adaptability. It can be studied as a potential heavy metal repairing plant. In order to study the tolerance and accumulation potential of *Sedum chinensis* to Pb under different  $Pb^{2+}$  concentrations, the purpose of this study was to investigate the accumulation potential of *Sedum chinensis* in Pb. To explore the great potential of *Sedum aizoon* L. in environmental protection; at the same time, provide certain basis and reference for the safe production of Sedum.

## 2. Material and method

### 2.1. Material



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*Sedum aizoon* L. was formed by cutting and breeding of the experimental farm of Sichuan Agricultural University.

## 2.2. Method

**2.2.1. Material culture and treatment.** *Sedum aizoon* L. was rinsed with distilled water, and the robust branches were selected and cut into 20 cm cuttings, pre-incubated with 1/4 Hoagland-type complete nutrient solution for 20 days. After the shoots grow stronger than the roots, the cuttings with good growth and the same root length are randomly selected. In this experiment, a total of five  $Pb^{2+}$  concentrations were treated. The  $Pb(NO_3)_2$  was dissolved in distilled water, and then mixed with nutrient solution to prepare culture solution with 5 concentration gradients of 0, 20, 60, 100, 140  $mg \cdot L^{-1}$  to cultivate *Sedum aizoon* L., and adjust the pH to about 5.8. The mixture was cultured in a black plastic crucible of 10 cm  $\times$  10 cm, and each treatment was set to 3 replicates, 3 pots each for each treatment, 3 plants per pot, and 100 ml of the culture solution per pot. During the processing of the experimental materials, the culture solution was changed every 2 days, and the pH value of the culture solution was tested every day to ensure that the pH of the culture solution was about 5.8. During the experimental treatment, the growth state of the plants was observed every 5 days, and samples were taken after continuous treatment for 20 days. The sample was washed repeatedly with tap water for 20 min, then rinsed with deionized water for 3 times to absorb the moisture on the surface of the plant and measure the relevant indicators.

**2.2.2. Measuring indicators and methods.** During the treatment of the experimental materials, the growth of the plants under each treatment was observed every 5 days, and the root length of the plants (measured the longest root length of 2 cm or more) and the plant height were measured, and the root-shoot ratio (R/T) of the plants was measured after the treatment. Determination of antioxidant enzyme activity: Take fresh sample leaves 1.0 g, add 50 mmol PBS (pH 7.8, containing 0.2 mmol EDTA), 2% PVP, quartz sand grinding, centrifuge at 12000 rpm for 20 min, and take the supernatant for the enzyme activity determination. Superoxide dismutase (SOD), catalase (CAT): determined by Xun and Liu<sup>[6]</sup>. Peroxidase (POD): determined according to Cakmark<sup>[7]</sup>. Determination of lead content: 15 min at 110 °C, dried at 75 °C to weight, weighed, pulverized, passed through a 100 mesh sieve. Weigh 0.500 g of plant sample, add nitric acid-perchloric acid (4:1, V:V) for 12 h, digest until the solution is transparent, filter, dilute to 50ml, and measure lead content with iCAP 6300 ICP mass spectrometer<sup>[8]</sup>.

**2.2.3. Data analysis.** Data processing and analysis of variance were performed using Excel2007 and DPS7.05 analysis software. Differential significance analysis was performed according to the least significant difference method (LSD method). Transport coefficient = lead content in the aerial part of the plant / lead content in the root, Endurance index = root elongation of the plant in heavy metal treatment / root elongation of the control  $\times$  100.

## 3. Result and analysis

### 3.1. Effects of Different $Pb^{2+}$ Concentrations on the Growth and Changes of *Sedum aizoon* L.

It can be seen from Table 1 that *Sedum aizoon* L. grows well under the treatment of Pb solution of 0-140  $mg \cdot L^{-1}$ . With the increase of  $Pb^{2+}$  concentration and treatment time, the leaves of the plant are small, thin and gradually yellow, of which  $Pb^{2+}$  When the concentration was 100  $mg \cdot L^{-1}$ , the leaves of the plants were yellower than those of other leaves. With the increase of  $Pb^{2+}$  concentration, the morphology of *Sedum aizoon* L. roots changed significantly compared with the control group, which showed that the root color became darker (light yellow, light brown), The main root becomes thicker and the number increases, the number of lateral roots increases, and the root length shortens. With the increase of  $Pb^{2+}$  concentration and treatment time, root growth showed a downward trend; The variation of plant height with the increase of treatment time showed a decreasing trend. With the

increase of  $Pb^{2+}$  concentration, the growth and variation of plant height increased first and then decreased. Under the treatment of  $20\text{ mg}\cdot\text{L}^{-1}$   $Pb^{2+}$  concentration, the strain the highest growth change was observed, and the growth variation of plant height at the concentration of  $60\text{-}140\text{ mg}\cdot\text{L}^{-1}$  was lower than that of the control group. In the same period of time, the root length and plant height growth variation of each treatment group were significantly different from those of the control group. At the 20th day of treatment, the root length growth change of  $60\text{-}140\text{ mg}\cdot\text{L}^{-1}$   $Pb$  solution was significantly different from that of the previous treatment. On the 10th day, the variation of plant height growth under  $100\text{ mg}\cdot\text{L}^{-1}$   $Pb$  solution was significantly different from that in other time periods. On the 15th day, the plant height growth variation under  $140\text{ mg}\cdot\text{L}^{-1}$   $Pb$  solution There is a significant difference between the amount of change processed with other time periods. It can be seen from Table 1 that with the increase of  $Pb^{2+}$  concentration, the tolerance index of *Sedum aizoon* L. gradually decreased, and the decrease range was the same. When the concentration was  $100\text{ mg}\cdot\text{L}^{-1}$ , the decrease was the largest.

It can be seen from Table 2 that under different  $Pb^{2+}$  concentration treatments, compared with the control group, the dry weight of the seedlings decreased first and then increased. When the concentration was  $20\text{ mg}\cdot\text{L}^{-1}$ , the dry weight was the highest; when  $100\text{ mg}\cdot\text{L}^{-1}$  the dry weight is the smallest. With the increase of  $Pb^{2+}$  concentration, the root dry weight decreased compared with the control group, and the dry weight was the highest when the concentration was  $20\text{ mg}\cdot\text{L}^{-1}$ . After analysis of variance, the difference between root dry weight and dry weight of each treatment group was extremely significant. With the increase of  $Pb^{2+}$  concentration, the root-shoot ratio showed a trend of increasing first and then decreasing. The root-shoot ratio of each treatment group was significantly different from that of the control group. The root-shoot ratio and other treatments at the concentration of  $60\text{ mg}\cdot\text{L}^{-1}$  the difference in root-shoot ratio between the groups was extremely significant; the difference between root-shoot ratio and  $100, 140\text{ mg}\cdot\text{L}^{-1}$  at  $20\text{ mg}\cdot\text{L}^{-1}$  was not significant.

Table 1. Effects of Different  $Pb^{2+}$  Concentrations on Root Length, Plant Height Growth and Endurance Index of *Sedum aizoon* L.

$Pb^{2+}$ ( $\text{mg}\cdot\text{L}^{-1}$ )	Root elongation variation (cm)				Plant height growth variation (cm)				Endurance index
	5d	10d	15d	20d	5d	10d	15d	20d	
0	2.17 aA	1.63 aA	1.19 aA	0.91 aA	1.44 bB	0.93 bB	0.82 bB	0.81 bB	100
20	1.93 bB	1.59 bB	0.83 bB	0.75 bB	1.93 aA	1.61 aA	1.31 aA	1.14 aA	86.44
60	1.78 bB	1.50 bB	0.71 bB	0.51 cC	1.11 cC	0.74 cC	0.73 cC	0.54 cC	76.27
100	1.51 cC	0.97 cC	0.53 cC	0.39 dC	0.84 dD	0.72 cC	0.54 dD	0.33 dD	57.62
140	1.52 dC	0.72 dD	0.28 dD	0.28 eD	0.81 dD	0.53 dD	0.34 eE	0.31 dD	47.46

The difference between the English capital letters and the letters in the same column indicates that the difference between the indicators in the different treatments is extremely significant ( $P<0.01$ ), and the lowercase letters indicate that there is a significant difference between the indicators in different treatments ( $P<0.05$ ). The same below.

Table 2. Effects of Different  $Pb^{2+}$  Concentrations on the Dry Weight and Root-shoot Ratio of *Sedum aizoon* L.

$Pb^{2+}$ ( $\text{mg}\cdot\text{L}^{-1}$ )	Seedling weight (g)	root weight (g)	root shoot ratio
0	3.202 bB	1.036 cC	0.48 dD
20	4.044 aA	1.771 aA	0.56 bcBC
60	2.219 cC	1.217 bB	0.64 aA
100	1.766 eE	0.889 dD	0.58 bB
140	1.907 dD	0.727 eE	0.53 cC

### 3.2. Effects of Different $Pb^{2+}$ Concentrations on the Activity of *Sedum aizoon* L. SOD, POD and CAT

It can be seen from Table 3 that with the increase of  $Pb^{2+}$  concentration, the activity of SOD, POD and CAT decreased first and then increased. When the concentration was  $100 \text{ mg}\cdot\text{L}^{-1}$ , the enzyme activity decreased to the lowest point. Compared with the group, it was reduced by 10.37%, 21.65% and 46.63%, respectively. However, the CAT activity was lower than the control level; When the concentration was  $140 \text{ mg}\cdot\text{L}^{-1}$ , the increase of POD activity was higher than that of the control group, which was 69.74% higher than that of the control group. After variance analysis, the difference of SOD activity between treatment groups was extremely significant; the POD and CAT activities of each treatment group were significantly different from those of the control group; the POD activity under the treatment of  $20 \text{ mg}\cdot\text{L}^{-1}$  concentration and  $60 \text{ mg}\cdot\text{L}^{-1}$  concentration treatment The difference in POD activity was not significant, and the difference between CAT activity at  $60 \text{ mg}\cdot\text{L}^{-1}$  concentration and CAT activity at  $100 \text{ mg}\cdot\text{L}^{-1}$  concentration was not significant.

Table 3. Effects of different  $Pb^{2+}$  concentrations on *Sedum aizoon* L. SOD, POD and CAT.

$Pb^{2+}$ concentration ( $\text{mg}\cdot\text{L}^{-1}$ )	SOD activity ( $\text{U}\cdot\text{g}^{-1}\text{FW}$ )	POD activity ( $\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}\text{FW}$ )	CAT activity ( $\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}\text{FW}$ )
0	243.120 aA	0.813 bB	1.617 aA
20	237.097 bB	0.747 cC	1.243 bB
60	225.773 cC	0.710 cC	0.927 dD
100	217.930 eE	0.637 dD	0.863 dD
140	222.330 dD	1.380 aA	1.067 cC

### 3.3. Effects of Different $Pb^{2+}$ Concentrations on Absorption and Accumulation of Pb in *Sedum aizoon* L.

It can be seen from Table 4 that with the increase of  $Pb^{2+}$  concentration, the Pb content accumulated in roots also increased; The Pb content in stems and leaves showed a trend of increasing first and then decreasing with the increase of  $Pb^{2+}$  concentration. The Pb content was the highest when the concentration was  $100 \text{ mg}\cdot\text{L}^{-1}$ . The accumulation of Pb in *Sedum aizoon* L. was root  $\gg$  stem > leaf. The difference in Pb content in roots, stems and leaves between treatments was extremely significant. The transport coefficient of  $Pb^{2+}$  increased first and then decreased with the increase of  $Pb^{2+}$  concentration. The transport coefficient of  $Pb^{2+}$  in *Sedum aizoon* L. was  $60 > 100 > 20 > 140 > 0$  ( $\text{mg}\cdot\text{L}^{-1}$ ).

Table 4. Effects of Different  $Pb^{2+}$  Concentrations on Absorption and Accumulation of Pb in *Sedum aizoon* L.

$Pb^{2+}$ concentration ( $\text{mg}\cdot\text{L}^{-1}$ )	Lead content ( $\text{mg}\cdot\text{kg}^{-1}$ )			Transport coefficient
	root	stem	leaf	
0	0.420 eE	0.023 eE	0.002 eE	0.055
20	56.447 dD	5.470 dD	4.240 dD	0.170
60	126.593 cC	12.430 cC	10.733 cC	0.188
100	166.457 bB	15.597 aA	13.483 aA	0.174
140	213.300 aA	13.773 bB	11.337 bB	0.119

## 4. Discussion and conclusion

In this experiment, with the increase of  $Pb^{2+}$  concentration, the leaves of the plants were small and thin, and gradually became green and yellow. Compared with the control, the root morphology changed significantly, mainly due to the darkening of the roots (light yellow, light brown), and the main roots increased. The number is coarse and the number is increased, the number of lateral roots is increased,

and the root length is shortened. It can be seen that the growth of root length of *Sedum aizoon* L. was inhibited as the concentration of  $Pb^{2+}$  increases.

Antioxidant protective enzyme systems such as SOD, POD and CAT in plants can eliminate active oxygen free radicals generated under stress and avoid oxidative damage [9-10]. The results of this experiment showed that the activities of SOD, POD and CAT decreased first and then increased with the increase of  $Pb^{2+}$  concentration. When the concentration of  $Pb^{2+}$  was  $100\text{ mg}\cdot\text{L}^{-1}$ , the enzyme activity decreased to the lowest point. CAT activity was lower than the control level, indicating that CAT is more sensitive to  $Pb^{2+}$ . This change in enzyme activity may be related to the heavy metal ions of the plant involved in the redox cycle, which causes changes in reactive oxygen species. In a certain concentration range, active oxygen can act as a signalling molecule to promote enzyme activity.

In this experiment, a sticky brown precipitate on the bottom of the container was observed and increased with increasing  $Pb^{2+}$  concentration. The root secretions chelate, adsorb and encapsulate  $Pb^{2+}$ . It is precipitated outside the root, which effectively reduces the toxic effect of  $Pb^{2+}$  on plants. The experimental results show that the accumulation of Pb by *Sedum aizoon* L. increases with the increase of Pb concentration. The Pb content accumulated in the roots of this herb is much higher than that of the aerial parts (stems and leaves). It can be seen that the transport of  $Pb^{2+}$  in plants is hindered, and the damage to the aerial parts is less, and the plant tolerance is improved to some extent. In this study, the transport coefficient of  $Pb^{2+}$  increased first and then decreased with the increase of  $Pb^{2+}$  concentration, and the transport coefficient was the highest when  $Pb^{2+}$  concentration was  $60\text{ mg}\cdot\text{L}^{-1}$ . It can be speculated that when  $Pb^{2+} > 60\text{ mg}\cdot\text{L}^{-1}$ , the ability of *Sedum aizoon* L. to transfer heavy metals from the underground part to the aboveground part will decrease with the increase of  $Pb^{2+}$  concentration.

The root elongation method was used to determine the tolerance of *Sedum aizoon* L. to Pb. The greater the tolerance index, the stronger the plant's ability to withstand heavy metals. The experimental results showed that the tolerance index gradually decreased with the increase of  $Pb^{2+}$  concentration. In this experiment, the accumulation of Pb in the aerial part of *Sedum aizoon* L. did not reach the standard of hyperaccumulators. It can be seen that *Sedum aizoon* L. is not a Pb hyperaccumulator, but its accumulation is more than 10 times that of ordinary plants, and it has Pb super tolerance.

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