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The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione

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ABSTRACT

Two ecotypes of *S. alfredii* [Pb accumulating (AE) and Pb non-accumulating (NAE)] differing in their ability in accumulating Pb were exposed to different Pb levels to evaluate the effects on plant length, photosynthetic pigments, antioxidant enzymes (SOD and APX), cysteine, non-protein thiols (NP-SH), phytochelatins (PCs) and glutathione (GSH) vis-à-vis Pb accumulation. Both ecotypes showed significant Pb accumulation in roots, however only the AE showed significant Pb accumulation in shoots. We found that both AE and NAE of *S. alfredii*-induced biosynthesis of GSH rather than phytochelatins in their tissue upon addition of even high Pb levels (200 μM). Root and shoot length were mostly affected in both ecotypes after addition of higher Pb concentrations and on longer durations, however photosynthetic pigments did not alter upon addition of any Pb treatment. Both superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities of AE were higher than NAE. The levels of cysteine and NP-SH were also higher in AE than in NAE. Hence, the characteristic Pb accumulation of ecotypes differed presumably in relation to their capacity for detoxification of Pb. These results suggest that enzymatic and non-enzymatic antioxidants play a key role in the detoxification of Pb-induced toxic effects in *Sedum alfredii*. This plant can be used as an indicator species for Pb contamination.

1. Introduction

Lead (Pb) is a hazardous heavy metal pollutant that originates from various sources, which include paints, gasoline additives, Pb smelting and refining, pesticide production, etc. [1,2]. Many studies have been conducted on the use of plants for the removal of Pb from the environment in an ecological and cost-effective way. This includes phytoextraction, which is a type of phytoremediation that involves the removal of heavy metals from contaminated soils/ground water with plants that accumulate large amounts of heavy metals in their shoot part [3,4]. Heavy metals can cause serious damages-even at very low doses by replacing essential elements involved in biological reactions [5,6]. Despite the significant problems caused by Pb for agriculture, Pb tolerance mechanisms of plants are not well understood to date.

Metal detoxification and tolerance in plants can be achieved by numerous mechanisms, such as chelation by metal-binding compounds, metal deposition in vacuoles, alterations of membrane structures, and synthesis of stress metabolites [7]. Phytochelatins (PCs), a class of small thiol (SH)-rich peptides, bind heavy metals and metalloid through thiolate coordination [8,9]. Due to their ability to bind metals, PCs are generally considered to be an important cellular chelating agent. However, there is now considerable debate on the role of PCs in metal detoxification and tolerance of higher plants [10,11]; since recent studies have demonstrated that metal hyperaccumulation phenotype of the plants does not correlate with PCs [12].

Sedum alfredii H., grown in old Pb/Zn mined area of southeast China, has been identified as a Zn/Cd hyperaccumulator [13], and is also recognized as a Pb-accumulating species. It could accumulate up to 514 mg kg^{-1} and 13,922 mg kg^{-1} DW of Pb in shoot and root, respectively under hydroponic conditions [14]. Sun et al. [12,15,16] reported that they could not find PCs in the leaf, stem and root tissues of the mine population of *S. alfredii* in the presence of Cd, Zn or Pb at certain concentrations. However, Zhang et al. [17] confirmed PC formation could be induced only in the stem and root when exposed to 700 μM Pb. The main objective of our present study was to investigate, whether PCs in different plant tissues can be induced in Pb accumulating ecotype (AE) of *S. alfredii*, and also whether they can play a role in Pb detoxification and tolerance or not.

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In order to validate previous outcome in more detail, we used different Pb concentrations and time durations to re-examine PC induction in *S. alfredii*. In our present study, we investigated cysteine, non-protein thiols (NP-SH), glutathione (GSH), and PC synthesis in AE and non-lead accumulating ecotype (NAE) of *S. alfredii*.

2. Materials and methods

2.1. Plant material, growth condition and treatment

The Pb accumulating ecotype (AE) of *S. alfredii* was collected from an old Pb/Zn mined area and the non-accumulating ecotype (NAE) was obtained from a tea garden of Hangzhou in Zhejiang province of China. Healthy and equal-sized shoots of *Sedum alfredii* H. were chosen and grown in 2.5 L pots for 2 weeks in distilled water for the initiation of new roots. Plants were grown in a glasshouse with natural light, day/night temperature of 30/25 °C and day/night humidity of 70/90%. The pH of nutrient medium was adjusted to 5.5 using 0.1 M NaOH or 0.1 M HCl and was continuously aerated with an aquarium pump and renewed after every fourth day during the experiment. The composition of the nutrient medium used for Pb treatment was as follows: 2000 μM KNO_3, 50 μM KCl, 500 μM $Ca(NO_3)_2{\cdot}4H_2O$, 200 μM $MgSO_4{\cdot}7H_2O$, 100 μM NH_4NO_3, 25 μM KH_2PO_4, 12 μM H_3BO_3, 2.0 μM $MnSO_4{\cdot}H_2O$, 0.5 μM $ZnSO_4{\cdot}7H_2O$, 0.2 μM $CuSO_4{\cdot}5H_2O$, 0.1 μM Na_2MoO_4, 0.1 μM $NiSO_4$, 20 μM Fe-EDTA.

After 14 d of pre-culture, the young plants (fourteen) of both populations were exposed to different concentrations of Pb (0, 5, 25, 100, 200 μM, as $Pb(NO_3)_2$) and maintained in 100% Hoagland's solution [18] in 2.5 L black plastic pots under above mentioned laboratory conditions for a period of 1, 3 and 5 d. KH_2PO_4 concentration was adjusted to 0.025 mM in order to prevent precipitation of Pb [14]. The experiment was randomly arranged with each treatment in triplicate. Pots without Pb served as control. After harvesting, plants were washed with double distilled water, blotted dry and used for the study of various parameters. All chemicals used were of analytical grade purchased from Sigma Chemical Company (USA) and Chemical Factory, Shanghai (China).

2.2. Plant growth analysis

Plants were harvested after 1, 3 and 5 d of treatment, and morphological parameters (root and shoot length) were measured by Vernier calipers; both expressed in cm plant^{-1}. After that, plants were washed thrice with distilled water and finally with de-ionized water. Roots and primary leaves were collected for enzyme analysis and 1 g of fresh samples were frozen in liquid nitrogen and stored at −80 °C.

2.3. Metal estimation

At the time of harvest, roots of intact plants were washed with distilled water for metal analysis, and immersed in 20 mM Na-EDTA for 15–20 min to remove adsorbed Pb adhering to the root surface. Then, plants were washed thrice with distilled water and finally with de-ionized water, and oven-dried at 70 °C for approximately 72 h. Pb concentration was estimated in both roots and shoots of both varieties (AE/NAE). 0.1 g of plant sample was digested with 5 ml HNO_3 and 1 ml $HClO_4$ in closed teflon vessels until a clear digest was obtained. The digested material was washed into 50 ml flask and made up to 50 ml volume using de-ionized water. Metal concentrations in plant samples were determined using Integrated Couple Plasma Mass Spectrophotometer (Agilent 7500a). The tissue Pb concentration was expressed as mg kg^{-1} DW.

2.4. Determination of total chlorophyll and carotenoid concentration

Plant material (100 mg) was ground in chilled 80% acetone in dark conditions. After centrifugation at 10,000 × *g* for 10 min at 4 °C, absorbance of the supernatant was taken at 663, 645, 510 and 480 nm. The concentration of chlorophylls was estimated by the method of [19] and that of carotenoid concentration by using the formula given by [20].

2.5. Activities of antioxidant enzymes

For enzyme assays, 0.3 g leaves were ground with 3 ml ice-cold 25 mM Hepes buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM AsA and 2% PVP. The homogenate was centrifuged at 4 °C for 20 min at 12,000 × *g* and the resulting supernatants were used for determination of enzyme activity. All spectrophotometer analyses were conducted on a SHIMADZU UV-2410 PC spectrophotometer.

2.5.1. Assay of SOD activity (EC 1.15.1.1)

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) [21]. 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 130 mM methionine, 750 μM NBT, 20 μM riboflavin, 0.1 mM EDTA and 0.1 ml of plant extract. Reaction was started by adding 20 μM riboflavin and placing the tubes under 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal colour, served as control. Reaction was stopped by switching off the light, and the tubes were covered with a black cloth. A non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm, and one unit of SOD was defined as being present in the volume of extract that caused inhibition of the photo-reduction of NBT by 50%.

2.5.2. Assay of APX activity (EC 1.11.1.11)

Ascorbate peroxidase (APX) activity was measured according to [22]. The assay depends on the decrease in absorbance at 290 nm as ascorbate was oxidized. 2 ml reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM hydrogen peroxide, 0.1 mM EDTA and 0.1 ml supernatant. The reaction was started by adding hydrogen peroxide (H_2O_2).

2.6. Total cysteine concentration

Plant material (800 mg) was homogenized in 5% chilled perchloric acid and centrifuged at 10,000 × *g* for 10 min at 4 °C. Cysteine concentration was measured in supernatant using acid ninhydrin reagent at 560 nm according to the method of Gaitonde [23].

2.7. Non-protein thiols (NP-SH) concentration

NP-SH concentration was measured following the method of Ellman [24]. Plant material (100 mg) was homogenized in 5% sulfosalicylic acid. After centrifugation at 10,000 × *g* for 15 min at 4 °C, NP-SH concentration was measured in the supernatant by reaction with Ellman reagent and absorbance was recorded at 412 nm.

2.8. HPLC analysis of phytochelatins (PCs) and glutathione (GSH)

Plant tissues were extracted with an equal volume (1 ml g^{-1} FW) of 10% (w/v) of 5-sulfosalicylic acid (SSA) at 0 °C, as described previously [25]. The extracts were centrifuged at 10,000 × *g* for 1 min and the supernatants were kept at 0 °C for 30 min just before HPLC analysis. The separation of PCs was carried out by the post-column method of Mendum et al. [26], with some modifications

Table 1
Effect of different lead concentrations on plant length (cm) of accumulator (AE) and non-accumulator (NAE) ecotype of *S. alfredii* H.

Ecotypes	Organ	Days	CK	Pb 5 μM	Pb 25 μM	Pb 100 μM	Pb 200 μM
AE	Root	1	21 ± 0.02	20 ± 0.03	19 ± 0.02	18 ± 0.02	16 ± 0.02
		3	21 ± 0.02	20 ± 0.02	17 ± 0.02	14 ± 0.01	12 ± 0.01
		5	22 ± 0.03	21 ± 0.01	16 ± 0.02	14 ± 0.02	10 ± 0.01
	Shoot	1	16 ± 0.02	15 ± 0.02	14 ± 0.01	13 ± 0.02	12 ± 0.01
		3	17 ± 0.02	16 ± 0.02	13 ± 0.01	12 ± 0.01	11 ± 0.01
		5	19 ± 0.03	18 ± 0.03	12 ± 0.01	11 ± 0.01	10 ± 0.01
NAE	Root	1	17 ± 0.03	16 ± 0.02	15 ± 0.03	13 ± 0.01	12 ± 0.01
		3	18 ± 0.02	17 ± 0.02	14 ± 0.01	12 ± 0.01	11 ± 0.01
		5	21 ± 0.03	18 ± 0.02	13 ± 0.01	12 ± 0.01	10 ± 0.01
	Shoot	1	15 ± 0.01	14 ± 0.02	13 ± 0.01	12 ± 0.01	11 ± 0.01
		3	16 ± 0.02	15 ± 0.02	12 ± 0.01	10 ± 0.01	9 ± 0.01
		5	18 ± 0.02	16 ± 0.02	11 ± 0.01	10 ± 0.01	8 ± 0.01

Values are mean of three individual replicates ± S.D. CK: control.

[27]. In brief, 20 μl samples were injected into a reverse-phase column (Hibar Lichrosorb RP-18, Cica-Merck, Darmstadt, Germany) and connected to an HPLC pump (L-7110, Hitachi, Japan), and the column was eluted with a linear gradient of acetonitrile in 0.1% (w/v) trifluoroacetic acid at a flow rate of 0.5 ml min^{-1}. The gradient program for acetonitrile was 0% for 4 min, 0–10% for 4 min, and then 10–20% (v/v) for 40 min. The column eluent was derivatized with 75 mM 5,5′-dithiobis (2-nitrobenzoic acid) in 50 mM potassium phosphate (pH 7.6) at a flow rate of 1 ml min^{-1} and monitored at 412 nm, using a UV–visible detector (L-7420, Hitachi). The retention times of PC peptides were identified with corresponding authentic (γEC)*n*G peptides (*n* = 2–5). Concentrations of PCs were expressed as μM of sulfhydryl equivalent per kilogram of fresh weight of plant tissue, using GSH as a standard.

2.9. Statistical analysis

Data were tested by analysis of variance (ANOVA) using SAS software (SAS Institute, Cary, NC). All data presented are the mean values. The measurement was done with three replicates. Statistical assays were carried out by ANOVA and means were compared by the least significant difference (LSD). Comparisons with *p* values < 0.05 were considered significantly different. Graphical work was carried out using Origin v.7.0.

3. Results and discussion

3.1. Effect of Pb on plant growth

In general, Pb treatment reduced the plant length in both ecotypes of *S. alfredii* (Table 1) as compared to control. In case of lower Pb concentration (5 μM), both ecotypes grew well at all time durations. However, with increase in concentration, symptoms of Pb toxicity in both ecotypes of *S. alfredii* were recorded, i.e. significant decrease in root and shoot length after 3 and 5 d. A concentration and time dependent decline in plant growth upon Pb exposure has also been reported for wheat [28], *Zea mays* [29] and radish [30]. Growth retardation may be attributed to disturbance to nutrient metabolism [30] and disturbed photosynthesis [31].

3.2. Absorption of Pb

S. alfredii seedlings raised under increasing concentrations of Pb showed concentration and time dependent accumulation of Pb concentration (Table 2). The Pb levels in both roots and shoots of AE showed positive and linear relationships with Pb treatments, and the maximum Pb concentration after 5 d was 53,775 mg kg^{-1} DW in roots and 2506 mg kg^{-1} DW in shoots at 200 μM Pb. The Pb concentration in roots and shoots of NAE also increased with the increase in Pb treatment, and the maximum Pb concentration in root and shoots after 5 d were 66,847 and 66 mg kg^{-1} DW, respectively, at 200 μM. Hence, compared to AE plants, most of the Pb accumulated in root tissue of the NAE, indicating that the translocation of Pb from root to shoot was lower in NAE ecotype. Such a concentration and time dependent increase in Pb accumulation has been previously reported in *Ceratophyllum demersum* [32] and *Zea mays* [29]. Such a high increase in Pb levels suggests that plants possess significant potential to tolerate Pb and that plants presumably rely on more than a single mechanism to achieve this. Therefore, we monitored some important enzymes and metabolites involved in antioxidant defense to test the point.

Table 2
Lead concentrations (mg kg^{-1} DW) in root and shoots under control (CK) and different lead treated two ecotypes of *S. alfredii* H. after 1, 3 and 5 d of treatment.

Ecotypes	Organ	Days	CK	Pb 5 μM	Pb 25 μM	Pb 100 μM	Pb 200 μM
AE	Root	1	ND	3691d ± 44.2	12146c ± 689.0	18379b ± 556.0	25813a ± 342.0
		3	ND	4580d ± 41.0	12860c ± 290.5	37005b ± 150.5	40985a ± 669.5
		5	ND	5094d ± 71.0	15306c ± 434.0	38209b ± 1181.0	53775a ± 1025.5
	Shoot	1	ND	34d ± 1.9	204b ± 0.7	219b ± 35.9	610a ± 14.3
		3	ND	35c ± 0.5	322c ± 7.7	398b ± 2.3	1894a ± 16.0
		5	ND	163d ± 2.5	416c ± 6.2	627b ± 9.1	2506a ± 47.5
NAE	Root	1	ND	1302d ± 43.5	13077b ± 235.5	13718b ± 1754.1	23292a ± 874.7
		3	ND	2894c ± 104.4	14467c ± 343.0	27019b ± 561.0	29039a ± 803.7
		5	ND	3928d ± 15.1	20611c ± 565.4	63156b ± 677.4	66847a ± 625.8
	Shoot	1	ND	5c ± 0.2	8b ± 0.2	10b ± 0.7	17b ± 1.5
		3	ND	8cd ± 0.10	9c ± 0.9	34a ± 1.5	27b ± 1.8
		5	ND	12d ± 0.7	17c ± 0.6	42a ± 1.3	66a ± 0.8

AE: accumulating ecotype; NAE: non-accumulating ecotype; ND: not detected. Values are mean of three individual replicates ± SD in a row followed by a different letter for each plant species are significantly different ($p < 0.05$) according to LSD test.

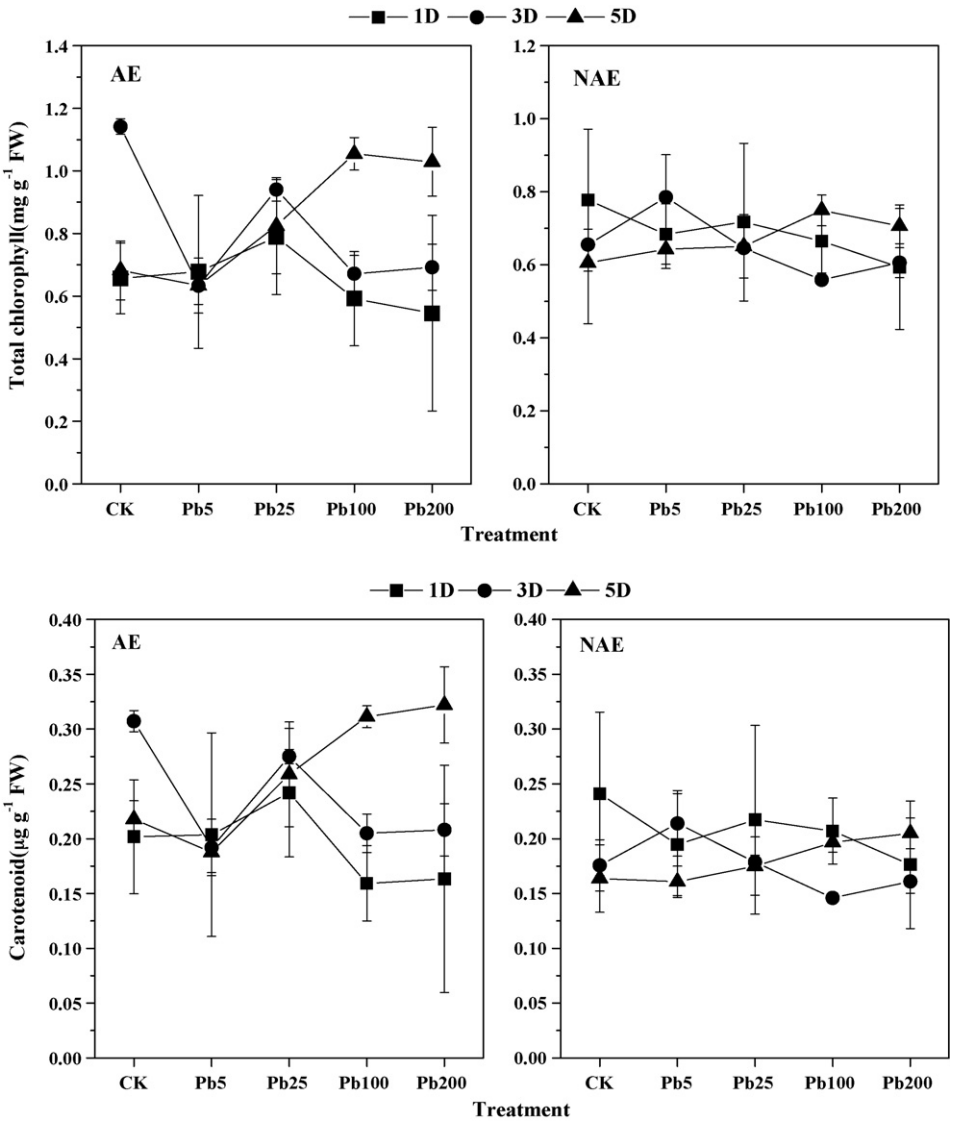

Fig. 1. Changes in total chlorophyll and carotenoids concentration in two ecotypes of *S. alfredii* H. in respond to different Pb treatment after 1, 3 and 5 d. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control. Data are means ± SD of three individual replicates.

3.3. Effect of Pb on total chlorophyll and carotenoids concentration

Photosynthetic pigments (total chlorophyll and carotenoids) exhibited a similar response upon Pb exposure (Fig. 1). No significant decrease on the level of total chlorophyll and carotenoids of two ecotypes was observed at any of the Pb treatments after 1 d. The total chlorophyll and carotenoid concentration of two ecotypes declined with increasing of Pb levels after 3 d, except in NAE at 5 μM. However, both total chlorophyll and carotenoid showed rise with increase in Pb levels after 5 d in AE. The maximum total chlorophyll and carotenoid of two ecotypes was observed at 100 μM after 5 d, which was about 54% and 48% higher, respectively in AE. The result of no significant effect to photosynthetic pigments is in contrast to the previous works [32]. The two varieties also did not differ significantly in effect on to photosynthetic pigments. In NAE this seems to be due to low accumulation of Pb in shoots and thus, root confinement of Pb in NAE may be an adaptive strategy. In AE, which accumulated significant amount of Pb in the shoots, no significant effects points to its higher tolerance potential. *Sedum alfredii* is a well known Cd/Zn hyperaccumulator [15], hence it might be having tolerance to other metals as well.

3.4. Effect of Pb on Superoxide dismutase and ascorbate peroxidase activity

Antioxidant enzymes examined in our study viz., SOD and APX showed varying responses with induction at various concentrations and time of treatment. SOD activity of AE was higher than NAE in the absence of treatment. SOD activity in roots of both ecotype declined as the Pb concentration increased after 1 d, whereas the change was not significant after 3 and 5 d (Fig. 2). In shoots also, the SOD activity declined after 1 d in both ecotypes except at 100 μM in AE. After 3 and 5 d, AE did not show a significant change in SOD activity, whereas NAE showed a decline after 3 d and no change after 5 d. APX activity in roots showed significant increase after 3 and 5 d at 25 μM Pb in AE, whereas in NAE it did not show any significant changes (Fig. 3). In shoots, APX activity showed variations but not significantly in AE, whereas in NAE, the activity showed a declining trend. A variety of proteins function as scavengers of superoxide and hydrogen peroxide. These include among others, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) [33]. An enhancement noticed in the activities of these enzymes under Pb stress would have lead to an enhanced capacity to resist Pb-induced oxidative burst in the plants [34]. Although Pb is not

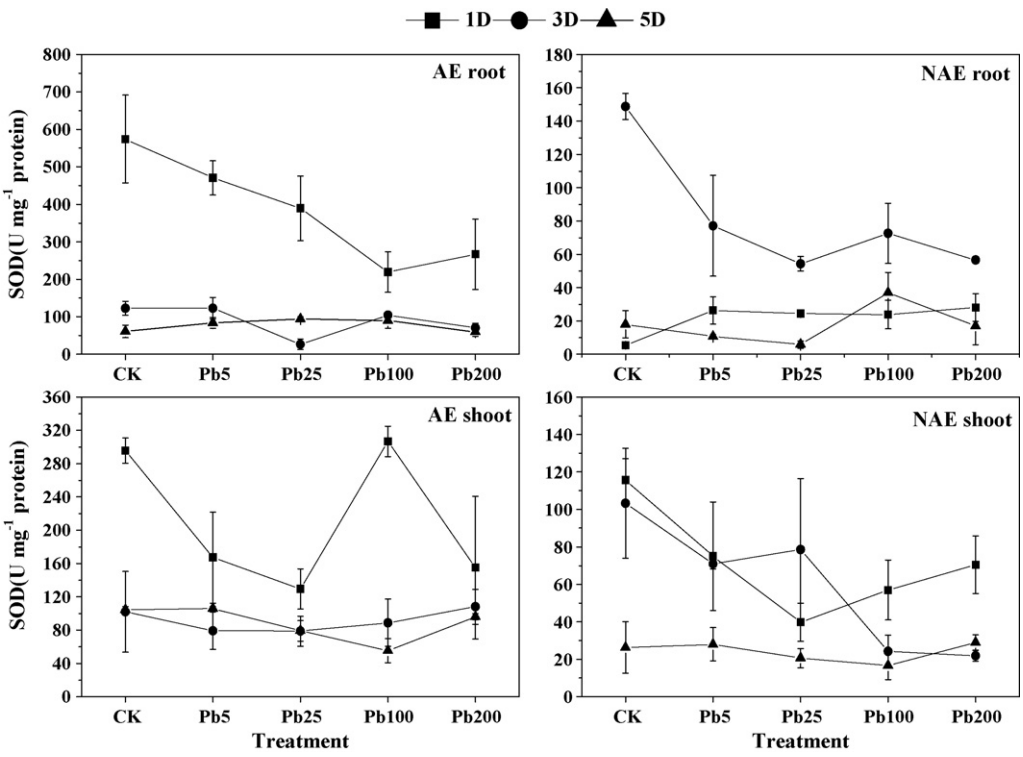

Fig. 2. Effect of different Pb concentration on superoxide dismutase (SOD) activity in root and shoots after 1, 3 and 5 d of treatment in two ecotypes of *S. alfredii* H. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control. Data are means ± SD of three individual replicates.

a redox-active metal, its role in stimulation of oxidative stress has been suggested [32]. Increase in SOD and APX activities could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via an increase in levels of superoxide radicals [35,36]. The effect of Pb stress on SOD and PAX expression is likely to be governed by the tissue and sub cellular sites at which oxidative stress is governed as supported by the higher activity of SOD in roots than in shoots of Pb stressed plants.

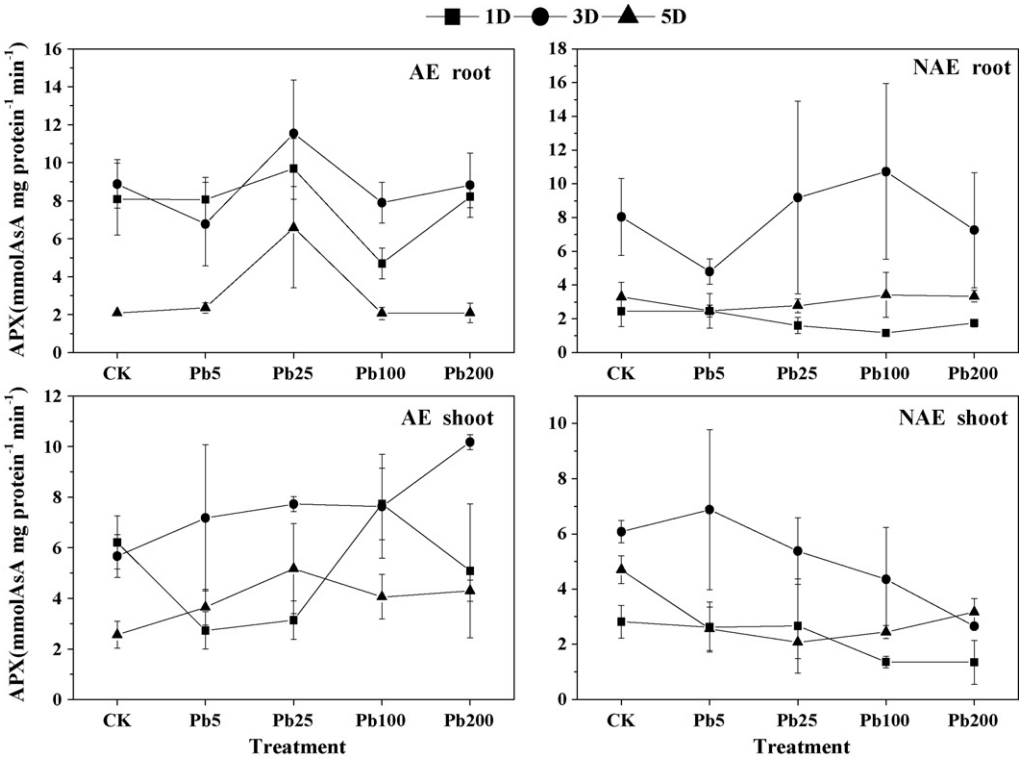

Fig. 3. Effect of different Pb concentration on ascorbate peroxidase (APX) in root and shoots after 1, 3 and 5 d of treatment in two ecotypes of *S. alfredii* H. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control. Data are means ± SD of three individual replicates.

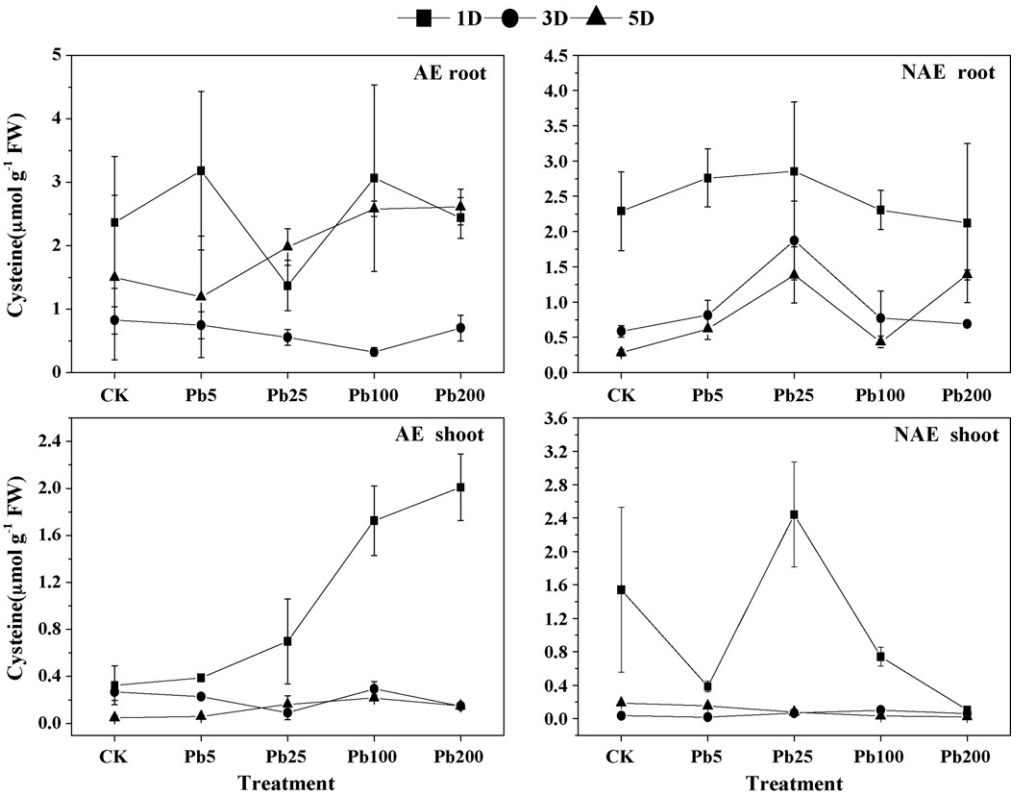

Fig. 4. Effect of different Pb concentration on total cysteine content in root and shoots after 1, 3 and 5 d of treatment in two ecotypes of *S. alfredii* H. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control. Data are means ± SD of three individual replicates.

3.5. Effect of Pb on cysteine and non-protein thiol concentration

Cysteine and non-protein thiols (NP-SH) constitute non-enzymatic antioxidants of the plants. In our present investigation, cysteine content decreased with increase in duration, with the maximum cysteine levels being noticed after 1 d in both ecotypes, except in AE root at 25 μM after 3 d. As a whole, cysteine content in roots of both ecotypes was higher than shoots. The cysteine level in the shoots of AE increased by 20–519% in comparison to control after 1 d (Fig. 4). However, at 3 and 5 d, cysteine levels did not change significantly in both ecotypes in shoots. Thiol compounds such as cysteine (Cys), GSH and PCs play an important role in heavy metal detoxification in plants. These thiols are biological active compounds whose function is to prevent oxidative stress in plant cells. Increase in cysteine content in response to Pb toxicity has been observed earlier in *H. verticillata* and *V. spiralis* [8,37]. This increase in the level of thiols may be due to stimulation of enzymes of sulfate reduction pathway such as APS reductase and serine acetyltransferase [38]. Augmented sulfur uptake and transport may also account for induced cysteine levels. It is well established that entry of heavy metals such as Cd into plant stimulates sulfate absorption [39]. Decrease observed at longer duration is possibly due to its higher rate of consumption for GSH synthesis. Decrease in cysteine content in response to Pb toxicity has been observed earlier [32,37].

NP-SH includes cysteine and glutathione (GSH) in combination and its increase indicates-induced synthesis of all or some of these constituents. NP-SH content in Pb exposed plants showed significant induction in roots and shoots of both ecotypes (Fig. 5). The level in roots of AE increased with increasing Pb concentration after 1 or 3 d of treatment. The maximum level was 0.59 and 0.95 μmol g^{-1} FW (39 and 153% higher than control) at 200 μM after 1 and 3 d. The increment was significant as compared to control ($p < 0.05$). The maximum level in roots of NAE was 0.69, 0.62 and 0.52 μmol g^{-1} FW at 100 μM after 1, 3 and 5 d. However, the NP-SH levels in shoots of two ecotypes had no significant difference with increase in Pb concentration. The highest levels of NP-SH were recorded in leaves of both ecotypes after 1 d. The increase in the levels of NP-SH seems to be directly correlated to the increase in cysteine levels and presumably sulfate uptake and assimilation. The enhancement in NP-SH concentration possibly also reflects a defense reaction to enhanced production of ROS [40].

3.6. Effect of Pb on phytochelatins and glutathione induction

In our Pb treated samples, we did not find any phytochelatin initiation at any Pb concentration after 5 d of treatment in both ecotype of *S. alfredii*, however we found some GSH in the Pb treated samples in both ecotypes (Table 3). After exposure to increasing Pb concentration, GSH levels increased in both ecotypes. Maximum GSH levels was recorded in the roots of AE of *S. alfredii*, i.e. 1.561 nmol kg^{-1} FW, as compared to control. In case of shoots, GSH concentration was much higher upon addition of Pb concentration in NAE as compared to AE.

Table 3
Effect of different Pb concentrations on GSH induction (nmol kg^{-1} FW) in root and shoots of two ecotypes of *S. alfredii* H. after 5 d of Pb treatment.

Lead Conc.	AE		NAE	
	Root	Shoot	Root	Shoot
CK	0.050 ± 0.01	0.031 ± 0.01	0.098 ± 0.03	0.022 ± 0.01
5 μM	0.504 ± 0.05	0.047 ± 0.04	0.263 ± 0.04	0.058 ± 0.02
25 μM	0.739 ± 0.05	0.049 ± 0.04	0.620 ± 0.06	0.083 ± 0.02
100 μM	1.204 ± 0.13	0.050 ± 0.05	0.824 ± 0.07	0.086 ± 0.03
200 μM	1.561 ± 0.20	0.156 ± 0.08	1.035 ± 0.10	0.196 ± 0.09

Values are mean of three individual replicates ± SD. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control.

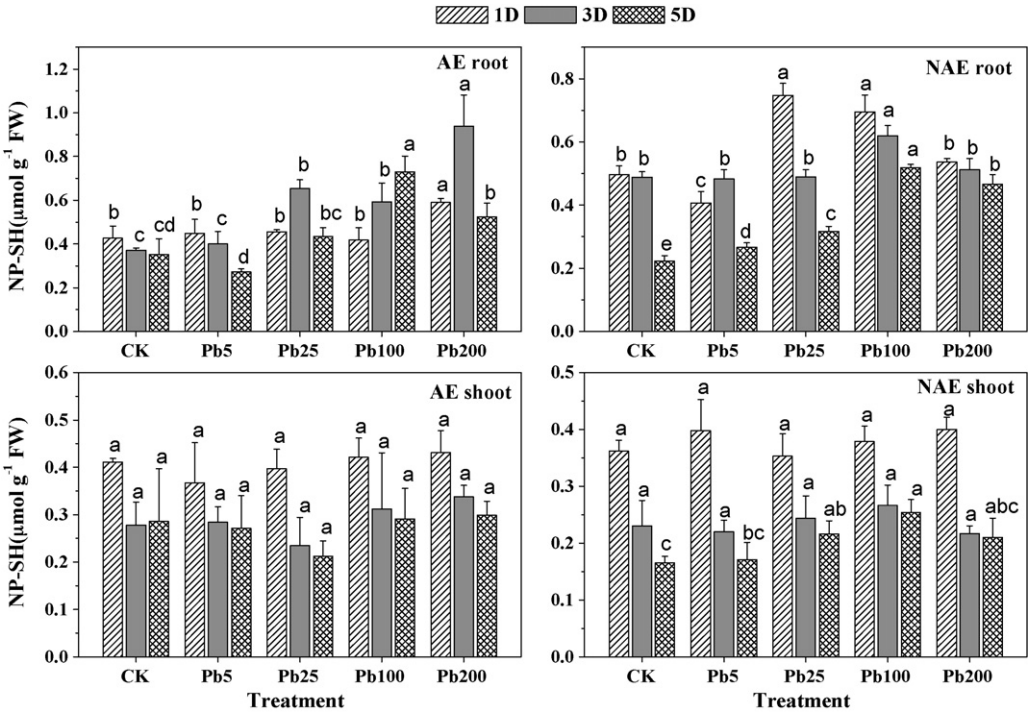

Fig. 5. Effect of different Pb concentration on non-protein thiol (NP-SH) content in root and shoots after 1, 3 and 5 d of treatment in two ecotypes of *S. alfredii* H. AE: accumulating ecotype; NAE: non-accumulating ecotype; CK: control. Data are means ± SD of three individual replicates. Mean values followed by different letters (a, b, c, d and e) are significant difference at ($p < 0.05$).

From our result it is again clear that *S. alfredii* (both AE and NAE) did not synthesize PCs. However, huge amount of Pb was accumulated in the root and shoots. Furthermore, after Pb exposure, no PCs were detected in any tissues of both ecotype plants. Similar type of results were reported previously [41,42] that PC accumulation was higher in non-metallicolocus plants than in hypertolerant plants of *T. caerulescens*, suggesting that PC-based sequestration is not essential for constitutive tolerance for any metals. Since, Pb did not induce any PC in both ecotype of *S. alfredii*, these ecotypes developed extensively the capacity to synthesize GSH, which is not only a major antioxidant but can also chelate and detoxify metals [32]. The increase in GSH concentration has been reported [12] in *S. alfredii* and in *Arabidopsis* trichome cells [43]. Thus our observation suggests that in the absence of PCs, GSH may play an important role of detoxification mechanism in both ecotypes of *S. alfredii* under Pb stress.

4. Conclusion

It is concluded that *Sedum alfredii* can accumulate high amount of available Pb from the hydroponic medium. Pb accumulation resulted in oxidative stress that was, however, efficiency controlled due to significant increase in antioxidants. So, both varieties (AE/NAE) can be grown in the Pb polluted area as an indicator.

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