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Integrated Fertilization Regimes Boost Heavy Metals Accumulation and Biomass of *Sedum alfredii* Hance

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ABSTRACT

The hyperaccumulator *Sedum alfredii* Hance (*S. alfredii*) may be employed for zinc (Zn) and cadmium (Cd)-polluted soil remediation. However, the low phytoremediation efficiency, related to the low biomass production, limits its use with that purpose. In this experiment, nitrogen (N), phosphorus (P), and potassium (K) fertilizers, and organic manure were applied to investigate the phytoremediation ability of *S. alfredii*. Hydroponic and pot experiments were conducted using Zn-Cd polluted soil. The hydroponic experiment indicated that appropriate fertilizer application could increase ($p < 0.05$) the amount of accumulated Zn and Cd in *S. alfredii*. When N supply ranged from 0.5 to 2.5 mmol L⁻¹, it could improve growth and accumulation of Zn and Cd in whole plants of *S. alfredii*. The 1 mmol L⁻¹ N was an optimal N dosage for shoot biomass production and Cd accumulation in shoots, while the 2.5 mmol L⁻¹ was an optimal N dosage for Zn accumulation in shoots. Both low (<0.05 mmol L⁻¹) and high (>0.8 mmol L⁻¹) P supply decreased growth, and Zn/Cd accumulation in whole plants of the studied species. The 0.1 mmol L⁻¹ P was an optimal dosage for *S. alfredii* biomass production and Zn/Cd accumulation in shoots. The supply levels within the range from 0.3 to 1 mmol L⁻¹ K could significantly improve the biomass production of *S. alfredii* and its capability to accumulate Zn and Cd in the biomass. The 0.5 mmol L⁻¹ K was an optimal dosage for the whole biomass production and Zn accumulation in shoots, while the 1 mmol L⁻¹ was an optimal K dosage for Zn accumulation in shoots, which was 17.2% higher than the control. Moreover, the soil pot experiment showed that the combination of organic (fermented manure) and inorganic fertilizers made significant effects on the Zn and Cd-polluted soil remediation by *S. alfredii*. These effects varied, however, with the application of different proportions of N, P, K and organic matter. The Zn accumulation by *S. alfredii* reached the highest efficiency ability under the highest fertilizer mixing rate (N: 50 mg kg⁻¹, P: 40 mg kg⁻¹, K: 100 mg kg⁻¹, organic matter: 1%). Even more, *S. alfredii* showed the strongest ability to accumulate Cd with a lower fertilizer mixing rate (N: 25mg kg⁻¹, P: 20mg kg⁻¹, K: 50 mg kg⁻¹, organic matter: 0.5%).

KEYWORDS

Sedum alfredii Hance; zinc; cadmium; phytoremediation; fertilizer application



1 Introduction

Potentially hazardous metals (PHMs) contamination in soils continue to attract researchers concern, as they impose detrimental effects on environment, and further threaten sustainable use of agro-environmental resources, which affects human being's health [1]. The PHMs in the soils are hard to remove because of its non-biodegradation [2]. Traditional physico-chemical methods for PHMs-contaminated soils' remediation include the replacement of the polluted soils, combination with clean soils and washing with soil chelating agents [3]. However, such measures could destroy soil structure, reduce soil stability and lead to new PHMs into the soils. Hyperaccumulators can effectively absorb soil heavy metals and accumulate them in roots and shoots. Therefore, phytoremediation regarded as an economical and environment friendly option is being constantly applied to reduce PHMs in agricultural soils [4–7].

Sedum alfredii Hance characterizing its high Zn/Cd uptake ability, is a perennial hyperaccumulator [8]. This species can use secreted rhizosphere protons and special organic matter to acidify rhizosphere environments, chelate and dissolve heavy metals in soil, and increase accumulation [9]. Previous research has proved that *S. alfredii* possesses many advantages such as perennial growth and a strong enrichment ability for soil PHMs remediation [10]. However, the low biomass production and low phytoremediation efficiency limit use of most hyperaccumulators [11,12]. Up to date, a study reported that the application of 1 mmol L⁻¹ N in the form of Ca(NO₃)₂ significantly increased the shoot of dry weight yield of *Sedum plumbizincicola* seedlings grown in Cd contaminated soils [13]. Similarly, Zn accumulation significantly raised under the 2.5 mmol L⁻¹ N concentration in *Pentas lanceolata* [14].

As one of the most traditional agronomic management measures, fertilization is considered vital to improve soil nutrient status and promote crops growth, development and yield [15]. Soil nutrients can affect their bioavailability by influencing the translocation, adsorption and the forms of PHMs in soils, which will further affect the absorption and accumulation of PHMs by plants [16]. Organic fertilizers (e.g., composted pig manure) can increase the content of soil nutrients, promote plant growth and also play a vital role in the PHMs bioavailability in soils [17]. However, previous researches related to nutrient regulation by hyperaccumulators mainly focus on the selection of conventional fertilizers and individual element application rates [18,19]. Liu et al. showed that the application of chicken manure significantly increased Cd uptake in the shoots of *Carpobrotus rossii* compared to the control [20]. A similar study showed that the application of pig manure compost (15 g kg⁻¹) significantly increased shoot Cd accumulation in *Sedum alfredii* [21]. Few studies were carried out to study the PHMs uptake efficiency ability under the combination of different levels of organic manure and chemical fertilizers.

In this study, hydroponic and pot experiments were carried out containing different application rates of N, P and K fertilizers with organic manure. The aims of this research were (1) to reveal the biomass production of *S. alfredii*, and its absorption and accumulation of Zn and Cd, (2) to explore an optimum fertilization practice for improving the absorption efficiency of Zn and Cd, and (3) to improve the effective use of *S. alfredii* on heavy metal-contaminated soils. Specifically, the following hypotheses were tested: (1) Increasing the supply of N, P and K improve the biomass production of *S. alfredii* and its capability to accumulate Zn and Cd in its tissues; (2) The combination of organic (fermented manure) and inorganic fertilizers make significant effect on Zn and Cd-polluted soil remediation by *S. alfredii*.

2 Materials and Methods

2.1 Plant Materials

The seedlings of *S. alfredii* were collected from the trial field of the Institute of Soil Science, Chinese Academy of Sciences in Fuyang (30°05'N, 119°95'E), Zhejiang Province. They were kept under greenhouse conditions before the experiment. After pre-culture, homogeneous *S. alfredii* individuals with a length of ~5 cm, and uniform size, leaves, and buds were chosen as testing materials for this study.

2.2 Soil Samples

In this study, contaminated soil was collected from polluted agriculture lands close to the mining factory in Fuyang County, Zhejiang Province, Southeastern China. Soil samples were collected from the topsoil (0–20 cm) within an area was 20 m × 20 m. The double diagonal method was used for soil sampling at 5 points. Then soil samples were stored in sealed plastic bags [22]. The farmland was infertile due to the contamination from the nearby smelting plant. All soil samples were ground and sieved through a 5-mm mesh. Physico-chemical characteristics of the tested soils are shown in [Tab. 1](#).

Table 1: Available nutrients, and total Zn and Cd values on the collected soils. Each value is the mean of n = 5

Variable								
pH	Organic matter (g kg ⁻¹)	Alkaline N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Total Zn (mg kg ⁻¹)	Available Zn (mg kg ⁻¹)	Total Cd (mg kg ⁻¹)	Available Cd (mg kg ⁻¹)
7.6	47.4	52.4	8.9	5.9	1637.8	28.5	6	0.6

2.3 Study Methods

2.3.1 Hydroponic Experiment

The components of the complete nitrogenous nutrient solution used in this study were the same as those described in Deng et al. study [23]. The treatments consisted of five concentrations of N: 0, 0.5, 1, 2.5, 5, 10 mol L⁻¹. The nitrogenous element was added in the form of NH₄NO₃. Each treatment had 4 replicates.

The components of the complete phosphorus nutrient solution followed those as described by Chen and Lin's work [24]. The treatments included eight concentrations of P: 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mol L⁻¹. P was added in the form of NaH₂PO₄. Each treatment had 4 replicates.

The components of the complete potassic nutrient solution were as in the Kashem and Kawai's study [25]. The treatments consisted of five concentrations of K: 0, 0.3, 0.5, 1, and 2 mol L⁻¹. Potassium was added in the form of KCl. Each treatment had 4 replicates.

The hydroponic experiment was performed in a greenhouse. After 7 days of pre-culture in a nutrient solution, plants were treated with different N, P and K application levels. Part of the nutrient solution was collected daily to measure the pH values, which were adjusted to pH 5.8 with 0.1 mol L⁻¹ NaOH/0.1 mol L⁻¹ HCl. It was continuously airtreated during any 24 h period. Each solution was refreshed every 5 days. The plant growth conditions and disease symptoms were recorded over the whole growing period. The study lasted 40 days. Both before and after the experiments, whole plant samples were obtained. They were repeatedly washed using deionized water. Thereafter, samples were divided into aboveground parts and roots, and rinsed using tap water repeatedly. Roots were treated with Na-EDTA (20 mmol L⁻¹) for 15 min to remove Zn²⁺ and Cd²⁺ from the root surface. The dry weight of plant parts was measured. They were ground to pass through a 0.25 mm sieve.

2.3.2 Soil Experiment

Different treatments regarding N, P, K and organic matter levels are described in [Tab. 2](#). Each treatment had 4 replicates. Nitrogen was added as NH₄NO₃, while P and K were applied in the form of K₂HPO₄. The nutrient concentration of the organic fertilizer (i.e., fermented pig manure) was 1.36, 2.31 and 0.84 g kg⁻¹ for N, P, and K, respectively. The concentrations of total Zn and Cd in soils were 1500 mg kg⁻¹ and 19 mg kg⁻¹, respectively. The base fertilizer was applied mixed in plastic pots. Each pot had 3 uniform-size plants. After 5 months, the shoot and root plant parts were collected. The weight of plant samples was measured, and then they were treated as mentioned above.

2.4 Sample Determination Methods

Plant samples were treated with a mixture of HNO₃ and HClO₄. The total Zn and Cd concentrations of plants were determined by an ICP-OES.

Table 2: Treatments of the soil pot experiment

Treatment	1	2	3	4	5	6	7	8	9
N (mg kg ⁻¹)	0	50	100	0	50	100	0	25	50
P (mg kg ⁻¹)	0	0	0	40	40	40	0	20	40
K (mg kg ⁻¹)	0	0	0	100	100	100	0	50	100
Organic matter (%)	0	0	0	0	0	0	1	0.5	1

2.5 Statistical Analysis

One-way ANOVAs were applied to determine the effects of various nutrient concentrations on dry weight yield, and Zn and Cd concentrations and contents of *S. alfredii*. The Duncan's multiple comparison test was used when F tests were significant at $p < 0.05$ [26]. Data were analysed using SPSS 20.0 software.

3 Results

3.1 Biomass Production, and Zinc and Cadmium Absorption by *S. alfredii* at Different N Doses

The results showed that the dry weights of the aboveground parts (shoots) varied from 0.020 g to 0.301 g, and the dry weights of the roots were in the range of 0.005 g to 0.0301 g (Fig. 1). The highest shoot dry weight was observed under 1 mmol L⁻¹ N, while treatment of 2.5 mmol L⁻¹ N showed the highest root biomass. In addition, total biomass (shoot+root) of *S. alfredii* plants was significantly reduced by N application of 10 mmol L⁻¹.

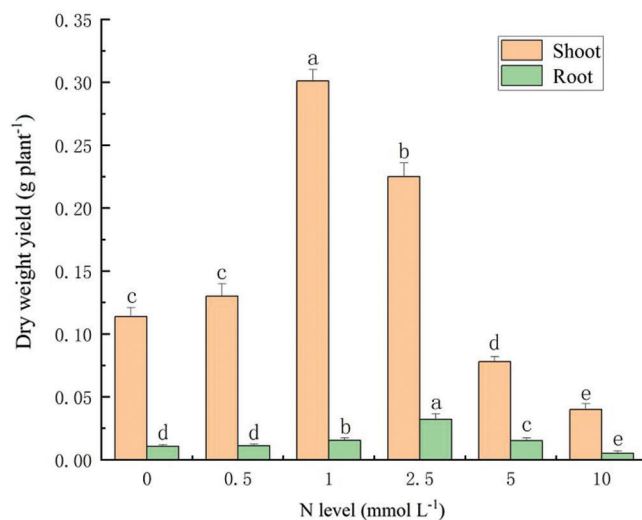


Figure 1: Biomass production of *S. alfredii* at different N application rates. Data are average + standard deviation of n = 4. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

At 2.5 and 5 mmol L⁻¹ N, Cd concentrations in shoots of *S. alfredii* reached the highest value of 2680 mg kg⁻¹, while Zn concentrations in the root had the highest value of 14876 mg kg⁻¹ at 10 mmol L⁻¹ N (Figs. 2A and 2B). With increasing N supply, Zn accumulation in the different plant parts of *S. alfredii* and Cd accumulation in the shoot increased first, and then started to decrease (Figs. 2C and 2D). The highest accumulation of Zn (2.1735 mg per plant) was in the shoot under 2.5 mmol L⁻¹ N. Meanwhile, the accumulation of Cd in the shoot of *S. alfredii* reached the peak of 0.5513 mg per plant under the N level of 1 mmol L⁻¹.

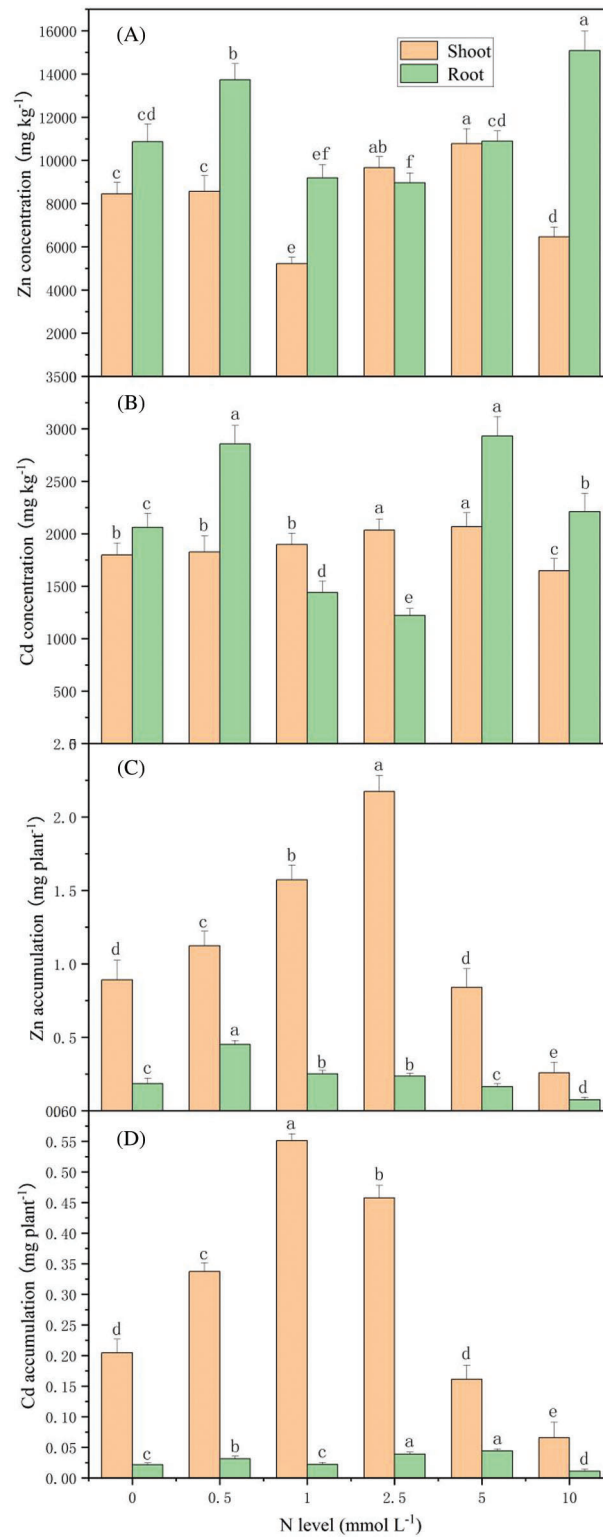


Figure 2: Zn and Cd concentration and accumulation by *S. alfredii* at different N applications. Data are average + standard deviation of $n = 4$. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

3.2 Biomass Production, and Zinc and Cadmium Absorption by *S. alfredii* at Different P Doses

With the increasing P concentration in solution, the dry weight of *S. alfredii* increased first then decreased (Fig. 3). The biomass production of both the shoot and root reached a peak at 0.1 mmol L⁻¹ P, which were 0.559 g per plant and 0.129 g per plant, respectively. Compared with the control (shoot biomass was 0.4366 g plant⁻¹, root biomass was 0.0856 g plant⁻¹), the P concentration from 0.05 mmol L⁻¹ to 1.6 mmol L⁻¹, kept *S. alfredii* growing normally without toxic effects.

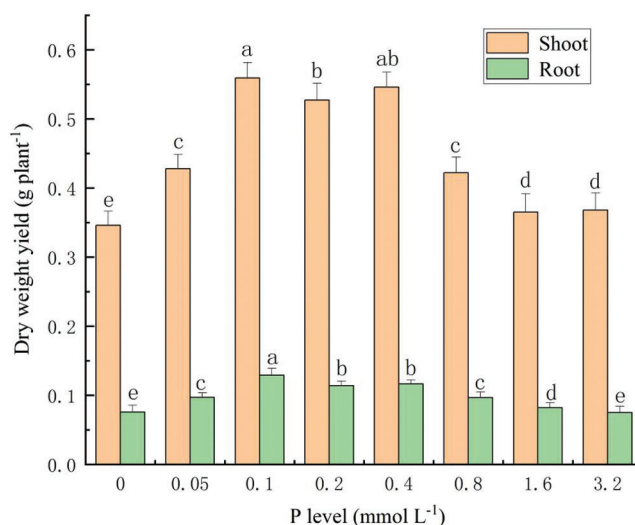


Figure 3: Biomass production of *S. alfredii* at different P application rates. Data are average + standard deviation of n = 4. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

The increasing amount of P application improved the studied heavy metal contents in the plant's roots (Fig. 4). Furthermore, when the P supply level was 0.4 mmol L⁻¹, the concentrations of Zn and Cd in the roots peaked at 15035 and 5334 mg kg⁻¹, respectively. Moreover, with the increasing concentrations of P, the uptake of heavy metals in the shoots and roots of *S. alfredii* presented a trend of increasing firstly and then decreasing. The accumulation of Zn and Cd on the plant's shoots reached the maximum values of 9.746 and 4.192 mg per plant, respectively, when the P concentration was 0.1 mmol L⁻¹. The highest amounts of Zn and Cd in the roots were 1.579 and 0.513 mg per plant, respectively, when the P concentration was 0.4 mmol L⁻¹.

3.3 Biomass Production, and Zinc and Cadmium Absorption by *S. alfredii* at Different K Doses

The different K supplies significantly influenced biomass production of the different parts of *S. alfredii* (Fig. 5). When the K concentration was 0.5 mmol L⁻¹, shoot and root biomasses reached a peak of 0.576 g per plant and 0.0918 g per plant, respectively. However, under the K treatment of 2 mmol L⁻¹, the shoot biomass was reduced to 0.405 g per plant.

In the K treatment of 1 mmol L⁻¹, Zn and Cd shoot concentrations reached the highest values of 17015 mg kg⁻¹ and 7710 mg kg⁻¹, respectively. With the K application of 0.3 mmol L⁻¹, the highest Zn and Cd root concentrations were 7725 mg kg⁻¹ and 2658 mg kg⁻¹, respectively (Figs. 6A and 6B). However, root Cd concentrations were similar at 0.3 and 0.5 mmol L⁻¹. With the increasing K supply, Zn and Cd accumulations on shoots and roots showed the same trend, which initially increased but then decreased (Figs. 6C, 6D). The Zn accumulation of the shoot reached a peak value of 9.239 mg per plant, at a K concentration of 0.5 mmol L⁻¹. However, the shoot Cd accumulation reached the maximum of 4.071 mg per plant under the treatment of 1 mmol L⁻¹ K. Zn and Cd accumulations on roots reached their greatest values at 0.5 mmol L⁻¹ K.

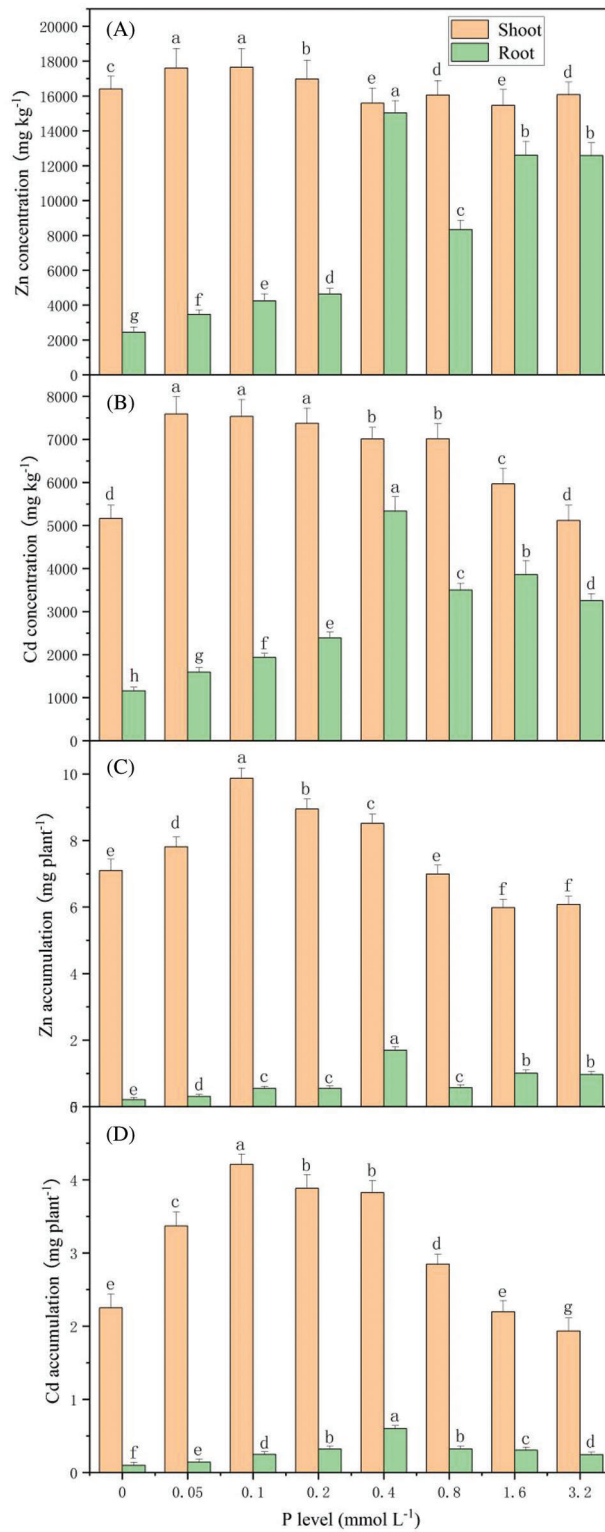


Figure 4: Concentration and accumulation of Zn and Cd on *S. alfredii* with different P applications. Data are average + standard deviation of $n = 4$. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

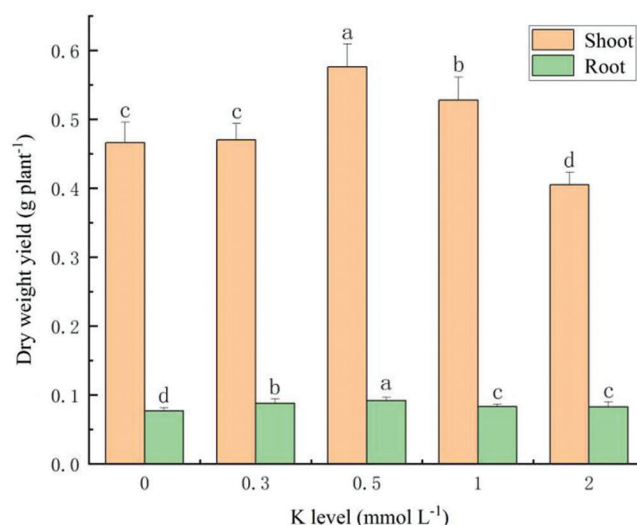


Figure 5: Biomass production of *S. alfredii* at different K application rates. Data are average + standard deviation of $n = 4$. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

3.4 Effects of Different Fertilization Treatments on Growth of and Zn and Cd Removal by *S. alfredii* Grown on Soil Pots

When only N was applied as a fertilizer, the dry matter yield of *S. alfredii* varied significantly and gradually decreased with increasing levels of that fertilizer (Fig. 7, see Tab. 2). Compared with the control treatment (2.44 g pot⁻¹), growth of *S. alfredii* was significantly reduced under the high N level (100 mg kg⁻¹) to 1.23 g pot⁻¹. In the treatments where phosphate and potassium fertilizers were combined with different concentrations of nitrogen fertilizers (i.e., treatments 4–6: Tab. 2), the additional application of P and K significantly improved the biomass yield of *S. alfredii*. Dry matter yield of *S. alfredii* in treatment 5 (50 mg kg⁻¹ N combined with P and K fertilizers) reached a value of 2.86 g pot⁻¹, which did not differ with that in treatment 4. At the same levels of P and K concentrations, however, biomass yield of *S. alfredii* was once again reduced at the highest N concentration (i.e., 100 mg kg⁻¹) in treatment 6.

The organic fertilizer was applied as composted pig manure with different concentrations of the inorganic fertilizers (treatments 7–9: Tab. 2). The dry matter of *S. alfredii* reached a maximum of 4.36 g pot⁻¹ in treatment 9 [mixed application of fertilizers at the highest concentrations (i.e., N: 50 mg kg⁻¹, P: 40 mg kg⁻¹, K: 100 mg kg⁻¹, organic matter: 10 g kg⁻¹)], indicating that the organic fertilizer application significantly promoted growth of *S. alfredii*.

Compared with the control treatment (treatment 1), the Zn concentration of different fertilization (treatments 2–9) decreased in *S. alfredii* (Tab. 3). With a high amount of N (treatment 3) application, the Cd concentration of *S. alfredii* reached a maximum of 193 mg kg⁻¹; in mixed applications of fertilizers at the highest concentrations (treatment 9), the Cd concentration was only of 140 mg kg⁻¹. When only N fertilizer was applied (treatments 1–3), increasing N concentrations reduced the Zn concentration and accumulation of Zn and Cd in *S. alfredii*. In the treatments of mixed application of inorganic fertilizers (treatments 4–6), K and P fertilizer application (under the same N application) increased significantly the accumulation of heavy metals in plants. Organic fertilizer application (treatments 7–9) increased the accumulation of Zn and Cd in plants. In treatment 9 [mixed application of fertilizers at the highest concentrations (N: 50 mg kg⁻¹, P: 40 mg kg⁻¹, K: 100 mg kg⁻¹, organic matter: 10 g kg⁻¹)], Zn accumulation reached the highest value of 50.67 mg pot⁻¹. Meanwhile, Cd accumulation reached a maximum of 0.71 mg pot⁻¹ in treatment 8 [mixed application of fertilizers (N: 25 mg kg⁻¹, P: 20 mg kg⁻¹, K: 50 mg kg⁻¹, organic matter: 5 g kg⁻¹)]. However, this value did not differ from that when only the organic fertilizer was applied (i.e., treatment 7).

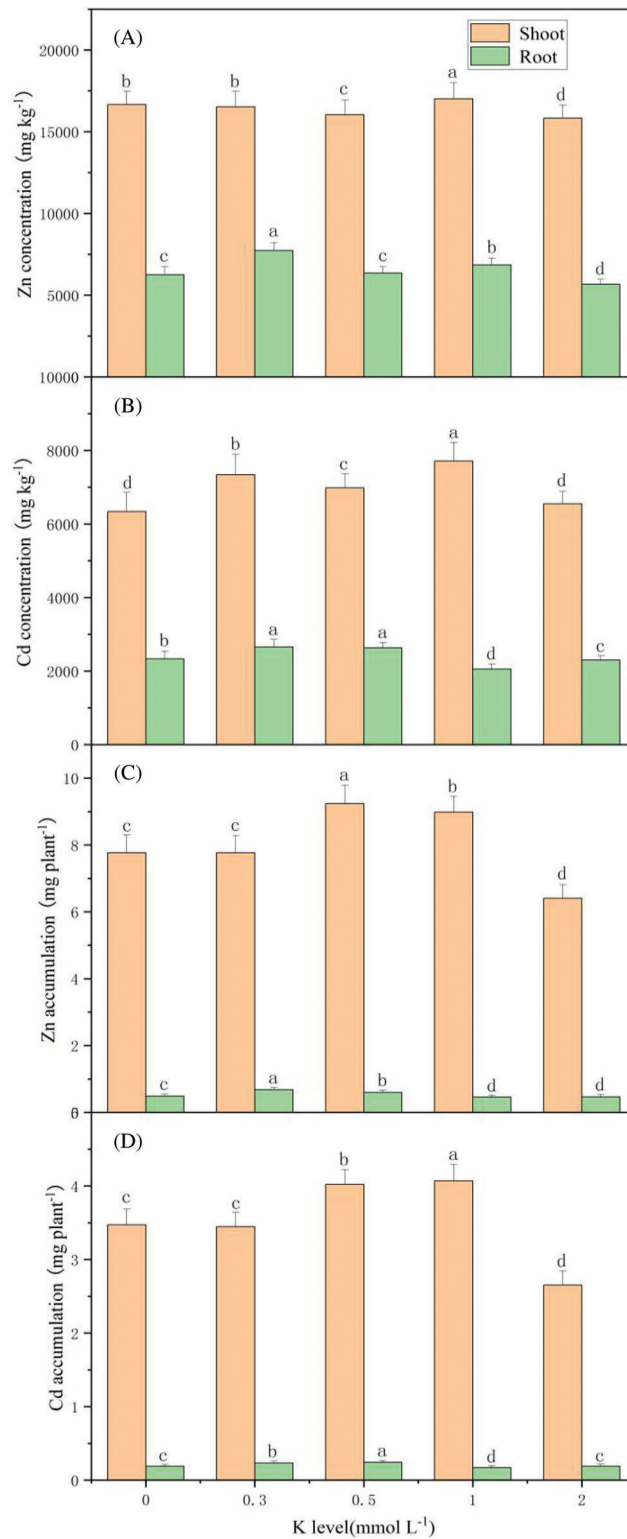


Figure 6: Zn and Cd concentration and accumulation on *S. alfredii* at different K applications. Means are average + standard deviation of $n = 4$. Different letters in the same color-histogram indicate significant differences at $\alpha = 0.05$

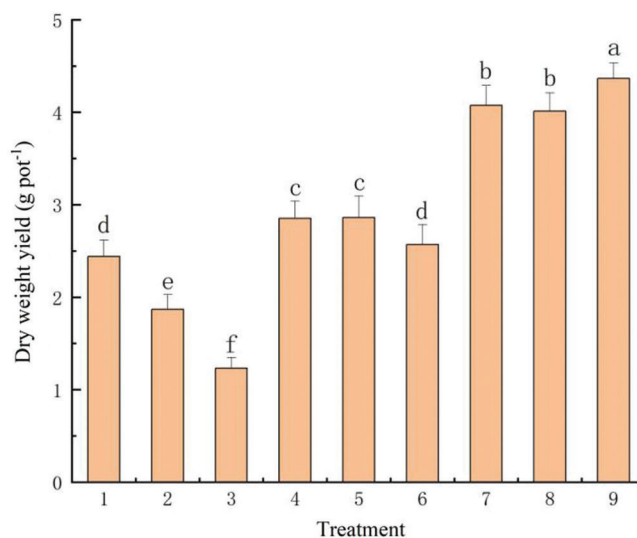


Figure 7: Effect of different fertilizer treatments on biomass of *S. alfredii*. Data are average + standard deviation of $n = 4$. Different letters indicate significant differences at $\alpha = 0.05$

Table 3: Effect of different fertilizer treatments on Zn and Cd concentrations and accumulations by *S. alfredii*

Treatment	Zn concentration ($\text{mg}\cdot\text{kg}^{-1}$)	Zn accumulation ($\text{mg}\cdot\text{pot}^{-1}$)	Cd concentration ($\text{mg}\cdot\text{kg}^{-1}$)	Cd accumulation ($\text{mg}\cdot\text{pot}^{-1}$)
1	$12510 \pm 619.01\text{a}$	$30.54 \pm 1.62\text{c}$	$186 \pm 9.49\text{b}$	$0.45 \pm 0.02\text{cd}$
2	$12037 \pm 631.97\text{b}$	$22.5 \pm 1.09\text{e}$	$179 \pm 9.1\text{cd}$	$0.33 \pm 0.02\text{e}$
3	$11856 \pm 741.52\text{b}$	$14.62 \pm 0.71\text{f}$	$193 \pm 12.05\text{a}$	$0.24 \pm 0.02\text{f}$
4	$11373 \pm 752.98\text{c}$	$32.44 \pm 2.03\text{c}$	$178 \pm 8.54\text{cd}$	$0.51 \pm 0.03\text{c}$
5	$11313 \pm 619.47\text{c}$	$32.38 \pm 1.78\text{c}$	$187 \pm 9.16\text{b}$	$0.54 \pm 0.03\text{c}$
6	$11326 \pm 713.54\text{c}$	$27.09 \pm 1.53\text{d}$	$168 \pm 8.74\text{d}$	$0.43 \pm 0.02\text{d}$
7	$11536 \pm 699.81\text{bc}$	$46.99 \pm 2.08\text{b}$	$170 \pm 8.67\text{d}$	$0.69 \pm 0.04\text{a}$
8	$11153 \pm 551.39\text{d}$	$44.75 \pm 2.06\text{b}$	$183 \pm 9.96\text{c}$	$0.71 \pm 0.04\text{a}$
9	$12609 \pm 685.93\text{bc}$	$50.67 \pm 2.14\text{a}$	$140 \pm 7.42\text{e}$	$0.61 \pm 0.03\text{b}$

Note: Different letters within each column indicate significant differences at $\alpha=0.05$.

4 Discussion

Optimal applications of N improve the chlorophyll level in plant leaves, strengthen N assimilation and consequently enhance photosynthetic rates. However, excessive amounts of N application can decrease the assimilation rate and further reduce photosynthetic rates [27,28]. In our experiment, N was added in the form of NH_4NO_3 , which included nitrate-N and ammonium-N. Our results agree with previous findings that the demand of N for growth of *S. alfredii* was low [29–31]. The biomass production and Zn and Cd accumulations were not in line with the increasing N doses. We found that the biomass of shoots and roots of *S. alfredii* was obviously enhanced within the suitable N concentration range (0.5 to 2.5 mmol L^{-1}), while the biomass production of whole plants and their plant parts decreased at higher levels of N concentration (5 to 10 mmol L^{-1}). Our finding was in line with that of Liu et al. [20], who reported that the translocation ability of Cd by *Carpobrotus rossii* significantly increased under an optimal

concentration of nitrogenous fertilizer. Similarly, Cd accumulation significantly increased under the 32 mM nitrogen treatment, obtaining the maximum concentration of Cd (622.9 mg kg^{-1}) in the roots of the *Pentstemon lanceolatus* with a total Cd amount of $27.9 \text{ } \mu\text{g}$ [32]. Similarly, this may also be related to the pH value caused by suitable N dosages in the nutrient solution, which further influenced the root function for nutrient uptake [33]. In this study, both low and high N concentrations adversely affected Zn and Cd uptake in *S. alfredii*, which was in agreement with the results of Hao et al. [34]. These authors also showed that both low and high N concentrations could severely restrict Mn uptake in rice (IR68144). Additionally, both low and high N concentrations altered Fe uptake in brown rice (IR64). These results were mainly attributed to the fact that high N concentrations decreased plant growth, and the low N concentrations did not meet the N requirements for plant growth [35].

The level of phosphorus in plants affects the metabolism of organic molecules such as nucleic acids, proteins, sugars and organic acids [36]. Clarkson et al. [37] proposed that the optimal P demand of general plants was at the range of $3\text{--}5 \text{ g kg}^{-1}$, which could promote photosynthesis, respiration and energy storage in plants as to help healthy plant growth. At the same time, Singh et al. [38] reported that when the P concentrations of leaves and shoots are $12\text{--}45 \text{ g kg}^{-1}$ and $9\text{--}30 \text{ g kg}^{-1}$, respectively, plants may be poisoned for the application of excessive phosphorus fertilizer. This is because of it can consume a lot of sugar and energy due to the strong respiration of plants, and further lead to the imbalance between the aboveground part and the root growth ratio [39]. When the root quantity is very large, but the root length is short, it will also have adverse effects on crop growth, yield and quality [39]. However, our experimental results reported that the growth of *S. alfredii* was not highly impacted at high P concentrations. This is mainly due to the fact that there is a regulating mechanism of P concentration in *S. alfredii*, which helps healthy growth of this species under high P concentrations [40]. Millikan et al. [41] reported that Zn demand increased with rising P doses. Similarly, we found that the addition of P ($0\text{--}0.8 \text{ mmol L}^{-1}$) significantly improved the absorption of Zn and Cd, and their translocation and accumulation from the root to shoot of *S. alfredii*. This further suggested that the increase of P dosage could satisfy the high demands of Zn in the shoots of the studied plant species as it was found in other studies [42]. However, our experiment indicated that the ability to accumulate Zn and Cd was weakened in the shoot under a relatively high P concentration ($\geq 1.6 \text{ mmol L}^{-1}$), which differed with the tendency of the plant biomass production of *S. alfredii* at these P concentrations. The availability of Zn and Cd in the plant might have been limited by insoluble phosphate existing in the shoot and leaves [42].

Potassium is a necessary component for plant growth. Plants selectively absorb water-soluble potassium ions from the soil through roots. Potassium is concentrated in the most active organs and tissues, such as buds and young leaves [43]. Potassium absorption from soil is mainly used to promote enzyme activation and protein synthesis, thus promoting plant growth and development [44]. In our results, K fertilization (from 0 to 0.5 mmol L^{-1}) enhanced total biomass production of *S. alfredii*. However, *S. alfredii*'s growth was reduced at higher K concentrations ($>1 \text{ mmol L}^{-1}$). Excessive application of potassium fertilizer leads to a decrease in the absorption of magnesium and calcium by plants, causing plant pathological symptoms and affecting plant development [45]. However, at 1 mmol L^{-1} K in our study, Zn and Cd contents of the shoots reached the maximum values. This is in agreement with the results of Wang et al. [46], who found that the shoot growth of *Solanum nigrum* L. was restrained under high K concentrations, while the content of Cd increased significantly. However, what was different with Wang's findings is that in the high K treatment (2 mmol L^{-1}), our results showed that the content of dry matter declined, and Zn and Cd accumulations of the plant were reduced. This was probably because different kinds of hyperaccumulators have different demands for K amounts. A previous study reported that K fertilization induced the increase of Cd content in the soil and therefore enhanced the ability of the plant in absorbing Cd [47]. In addition, some studies reported that the addition of K could increase the content of Cd at the exchangeable state, by competing adsorption sites with Cd in soil particles [48]. Our results showed that

the optimal concentration of K was 0.3-1 mmol L⁻¹ for *S. alfredii*. Proper K application can significantly promote the absorption of Zn and Cd and their transport from the root to the shoot. This might be due because of the K fertilizer could induce Cl⁻ and SO₄²⁻ combination with heavy metal ions in the soil [49].

Previous studies indicated that fertilization could greatly improve soil fertility, thereby increasing crop yields [50,51]. Fertilization was also a key method to improve the accumulation of heavy metals in plants. Similarly, plants respond differently to different doses of Nie et al. [52] reported that appropriate amounts of N and K fertilizers can increase the absorption of the heavy metal Pb in the soil by increasing plant chlorophyll content, strengthening plant photosynthesis, and then increasing plant biomass yield. Sun et al. [53] found that appropriate addition of P can increase the biomass of our studied plant species and the uptake of heavy metals. However, in the present study, with sufficient nutrients in the potting soil, the application of inorganic N, and P and K fertilizers did not significantly promote the growth of *S. alfredii* and its capability of accumulating Zn and Cd. Under high N concentrations, the plant's biomass and heavy metal accumulation decreased significantly. Therefore, in the remediation of soil heavy metal pollution, it is necessary to keep an appropriate level of N in the soil [53]. Zhang et al. [54] identified that the application of organic fertilizer could increase soil nutrients and decrease the content of exchangeable Cd, and then weakened the Cd activity. The treatment of organic fertilizer in this experiment promoted the growth of *S. alfredii*, thus increasing Zn and Cd accumulation. Meanwhile, the heavy metal concentrations in the plant all declined to some extent. However, the variation in the concentrations of Zn and Cd were unnoticeable with the application of inorganic and organic fertilizers. This differs from the results of Guo [55], who found that the organic fertilizer is more capable of strengthening the mobility of heavy metals, thus enhancing their accumulation compared with the inorganic fertilizers in soils. It is likely that decomposition and activation of various organic fertilizers lead to different degrees of transformation of heavy metals to an exchangeable form in soils, thus affecting the availability of soil Zn and Cd [56,57]. Therefore, selecting the proper type of organic fertilizer that can promote the enrichment of heavy metals in *S. alfredii* is particularly critical for remediation of heavy metal contaminated soils.

In the current study, hydroponics and pot experiments were used to reveal the effects of different nutrient supplies on growth of *S. alfredii* and its uptake of Zn and Cd. In the hydroponics experiment, the increasing N, P and K supplies (within a suitable range of concentrations) could accelerate the growth of *S. alfredii*, thus enhancing the absorption of Zn and Cd. However, the result of the pot experiment showed that P and K fertilizers did not significantly promote growth and accumulation of the target heavy metals by *S. alfredii*, while the N fertilizer significantly reduced plant growth and the removal of Zn and Cd. The results of different experiments are diverse, owing to the difference in the available nutrient amounts and Zn/Cd in soil and hydroponics experiments, or the nutrient interactions in soils [58]. Therefore, more research needs to be conducted to remediate Zn/Cd-polluted soils by hyperaccumulators.

5 Conclusions

The hydroponic experiment showed that suitable amounts of N fertilizer (0.5–2.5 mmol L⁻¹) improved Zn and Cd uptake by *S. alfredii*. However, an excessive amount of N application resulted in a decrease in growth of *S. alfredii*. Similarly, appropriate P addition within a range of 0.05-0.8 mmol L⁻¹ increased plant growth and the studied heavy metals uptake of *S. alfredii*. The optimum K supply level was between 0.3 and 1 mmol L⁻¹, which significantly improved the biomass and the ability to enrich heavy metals in the plant.

The soil experiment revealed that the single inorganic nitrogen fertilizer application did not improve the biomass and the ability to enrich Zn and Cd in the studied plant species. The application of inorganic phosphate and potassium fertilizers or the organic fertilizer could promote plant growth and the ability to absorb Zn and Cd. The combination of the organic and inorganic fertilizers made the most significant

effect on Zn and Cd phytoremediation. For Zn, the highest accumulation value was found at a high fertilizer application dose (N: 50 mg kg⁻¹, P: 40 mg kg⁻¹, K: 100 mg kg⁻¹, organic matter: 1%), while the strongest ability to accumulate Cd was found at a relatively low fertilizer application rate (N: 25 mg kg⁻¹, P: 20 mg kg⁻¹, K: 50 mg kg⁻¹, organic matter: 0.5%).

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