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Research Article

Physiological Responses and Tolerance of Halophyte *Sesuvium portulacastrum* L. to Cesium

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Cesium (Cs) is a soil contaminant and toxic to the ecosystem, especially the plant species. In this study, we have assessed the potential of a halophyte *Sesuvium portulacastrum* for its Cs tolerance and accumulation. Thirty days old *S. portulacastrum* plants were subjected to different concentrations of Cs (0, 5, 10, 25, 50, and 150 mg·L^{-1} Cs) using cesium chloride. The biomass and photosynthetic pigments were not affected up to 25 mg·L^{-1} Cs treatment while a significant decline in pigment levels was observed at higher concentrations. The Cs treatments increased protein content at low concentrations while higher concentrations were inhibitory. Under Cs exposure, significant induction of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) was observed. The antioxidant enzyme activities were upregulated up to 50 mg·L^{-1} Cs but decreased significantly at 150 mg·L^{-1}. The accumulation of Cs was dose and tissue-dependent as evidenced by a higher accumulation of Cs in leaves (536.10 μg·g^{-1}) as compared to stem (413.74 μg·g^{-1}) and roots (284.69 μg·g^{-1}). The results suggest that *S. portulacastrum* is a hyper-accumulator of Cs and could be useful for the phytoremediation of Cs-contaminated soils.

1. Introduction

The excess level of cesium (Cs) in soil imposes a negative impact on plant growth and development [1]. Cs restricts the uptake of potassium (K) due to its chemical resemblance with potassium and creates severe potassium deficiency followed by subsequent chlorosis in plants [2, 3]. Most of the Cs in the natural atmosphere are nonradioactive Cs-133 and are present in the soil at about 25 μg·g^{-1} soil [4]. The group I alkali metal Cs is mostly nonradioactive Cs-133 but some occur as radioactive isotopes (Cs-137, Cs-134) which enter into the ecosystem [5]. It is not only incorporated into the food chain but also emits β and γ radiations which have long half-lives [6]. Different physical and chemical methods are used for the removal of radionuclides and toxic metals. However, their widespread and large-scale applications have limitations due to high costs and some side effects [7]. Some alkaliphilic bacteria such as *Microbacterium* sp TS-1 can grow in presence of 1.2 M cesium chloride [8]. The studies on ecto- and endo-mycorrhizal fungi revealed that they reduce Cs toxicity by limiting Cs availability to their host plant through immobilization between 10 and 100% of the total Cs activity [9]. Some plant species are endowed with the ability to extract or absorb radionuclides and other heavy metals from the soil. This ability can be utilized for environmental clean-up. Under increasing Cs treatment (0.4 mM, to 3 mM), *Arabidopsis thaliana, Calla*

palustris, Pennisetum purpureum, and Ocimum basilicum showed increased uptake of Cs [10–13], respectively. Similarly, *Sorghum bicolor* accumulated 5270 mg·kg^{-1} in roots and 4513 mg·kg^{-1} in hydroponically grown aerial parts without significant change in plant height and dry weight [14].

Being native flora of saline soils, halophytic plants are better suited to tolerate high salt due to efficient ROS scavenging, compartmentation, and excretion mechanisms to maintain water balance [15, 16]. This mechanism of tolerance in halophytes has also been shown towards metal stress and different inorganic pollutants [17–19]. *S. portulacastrum* is a facultative halophyte that grows luxuriantly on the tropical and subtropical shores of five continents [20]. It is highly tolerant to different abiotic stresses such as salt, drought, heavy metals, and textile dyes [20]. In the last decade, apart from its salt tolerance mechanism, this plant has been extensively studied for its applicability in phytoremediation of heavy metals such as cadmium, arsenic, lead, nickel, copper [21–23], textile dyes [24], and desalination [25]. *Sesuvium* plants rapidly uptake the toxic compounds and translocate them to aerial parts such as leaves. In leaves, these compounds are sequestered into vacuoles without any toxicity [26]. However, the effect of stable Cs on *S. portulacastrum* has not been studied warranting such studies will help to assess toxicity and/or accumulation.

The evaluation of *Sesuvium* for cultivation on Cs-contaminated soil requires a study about Cs toxicity, tolerance, and accumulation in different organs. Therefore, the responses of *Sesuvium* to Cs were studied in hydroponics to provide a homogenous substrate and avoid interference of soil particles. The effect of increasing the concentration of Cs was analyzed by monitoring plant growth in terms of root length, shoot length and fresh weight, and physiological and biochemical status in terms of percent tissue water content, chlorophyll content, phosphate soluble proteins, and antioxidant enzymes. The hypothesis of this study is that *Sesuvium* is a hyper-accumulator of Cs and the objectives are to study the Cs accumulation potential of *Sesuvium* and analyze the effect of Cs on the growth and biochemical responses of *Sesuvium*.

2. Materials and Methods

2.1. Plant Material and Growth Conditions. In the present study, a facultative halophyte *S. portulacastrum* L. was assessed for accumulation and tolerance to stable Cs (^{133}Cs). The shoots (4–5 cm long) of naturally grown *S. portulacastrum* were collected from the coastal areas of Mumbai, India (19°03′51.6″N 72°58′44.2″E). The plants were surface sterilized and hydroponically maintained as per Nikalje et al. [19]. The experiments were carried out in a plant growth chamber (Sanyo, Japan) with 14 h: 10 hr light/dark cycle, 25/22°C day/night temperature, light intensity 150 μE·m^{-2}·S^{-1}, and relative humidity 65–75%. After four weeks of growth, seedlings were subjected to different Cs treatments.

2.2. Cesium Treatment. The rooted shoots were transferred (twelve plants per treatment) into 500 ml half-strength Hoagland's nutrient solution added with Cs at various concentrations: 0, 5, 10, 25, 50, and 150 mg·L^{-1} using cesium chloride. The plants were harvested after 28 days. The plant parts viz. root, stem, and leaves were separated and used for further analysis.

2.3. Growth and Biochemical Attributes. Plant growth was measured in terms of root length, shoot length, and root and shoot percent tissue water content (%TWC). The fresh weight (FW) was immediately taken; leaves, stem, and roots were oven dried separately at 60°C till constant dry weight (DW).

Percent tissue water (%TWC) content was calculated using the following formula [26]:

$$\%\text{TWC} = \left[\frac{(\text{FW} - \text{DW})}{\text{FW}}\right] \times 100. \quad (1)$$

For the estimation of Chl *a*, Chl *b,* and total chlorophyll, the leaves (300 mg) were crushed in 5 ml chilled 80% acetone in precooled mortar and pestle in the dark. After centrifugation, the absorbance of the supernatant was taken at 645 and 663 nm [27].

The extraction and estimation of soluble protein content were performed as per Lowry's method [28] where Bovin serum albumin served as standard. The antioxidant enzymes activities such as superoxide dismutase [29], catalase [30], ascorbate peroxidase [31], and glutathione reductase [32] were performed with some modifications given by Nikalje et al. [19].

2.4. Cesium Quantification. After oven drying at 60°C, the plant samples (roots, stem and leaves separately) were finely ground, weighed (quantity in *g*), and digested using 10 ml of the di-acid mixture (HNO_3: $HClO_4$) (5: 1) on a hot plate. The Cs content in the digested extract was determined by atomic absorption spectrophotometer (GBC906AA, Australia) using Cs hollow cathode lamp at 852 nm wavelength.

2.5. Experimental Design and Statistical Analyses. All the experiments were performed in completely randomized design in triplicates. A total of 12 plants were subjected to each treatment and three biological and technical replicates were used for each studied parameter. The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 20.0. The treatment means were compared by using Duncan's multiple range test (DMRT) at $p \leq 0.05$ and data were expressed as mean ± SE.

3. Results

3.1. Plant Growth and dry Biomass. S. portulacastrum plants were challenged with different levels of Cs viz, 0, 5, 10, 25, 50, and 150 mg·L^{-1}. The results showed that up to 25 mg·L^{-1} Cs, plants did not exhibit significant growth retardation but slight growth enhancement was observed at 5 and 10 mg·L^{-1}

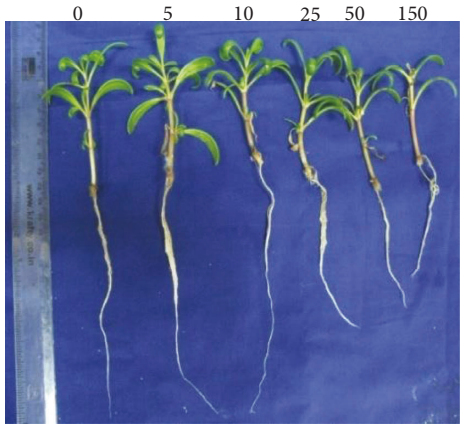

Figure 1: Effect of cesium on the growth of *S. portulacastrum*. Plants were subjected to different concentrations of Cs (0, 5, 10, 25, 50, and 150 mg·L^{-1}) and harvested after 28 days of treatment for growth analysis.

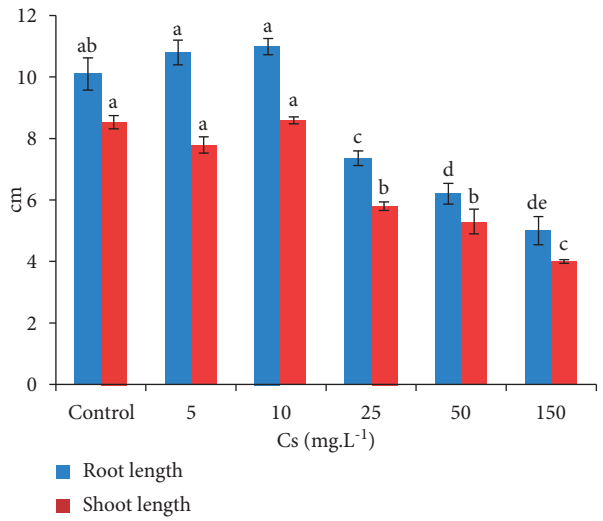

Figure 2: Effect of cesium on root and shoot length of *S. portulacastrum*. All the values are mean of twelve readings ± S.E. One-way ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values in root or shoot (DMRT $p \leq 0.05$).

Cs. When Cs was applied beyond 50 mg·L^{-1}, chlorosis was observed, and growth was significantly inhibited (Figure 1). At 5 and 10 mg·L^{-1} Cs, it was seen that the root length was higher as compared to control and other treatments while there were no significant changes in shoot length (Figure 2). On application of 25, 50, and 150 mg·L^{-1} Cs, the length of both root and shoot was decreased gradually. At 150 mg·L^{-1} Cs treatment the root and shoot length decreased significantly by 1.83-fold and 1.8-fold, respectively, at $p \leq 0.05$ (Figure 2).

3.2. Percent Tissue Water Content (%TWC). The percent tissue water content of both the root and shoot showed a similar trend (Figure 3). The %TWC increased gradually up to 25 mg·L^{-1} Cs while it decreased significantly at 150 mg·L^{-1}

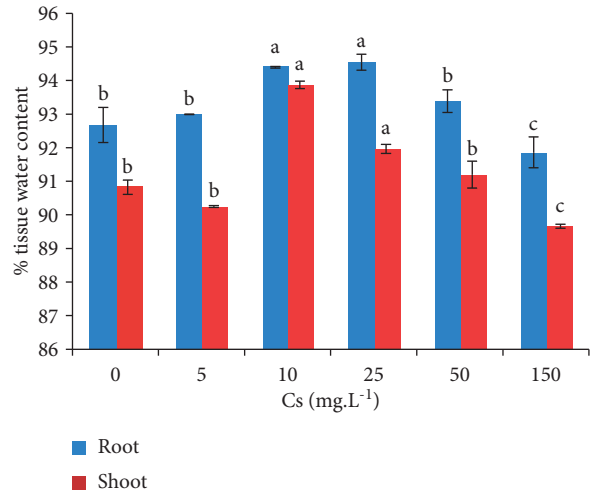

Figure 3: Effect of cesium on % tissue water content of *S. portulacastrum*. All the values are mean of twelve readings ± S.E. One-way ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values in root or shoot (DMRT $p \leq 0.05$).

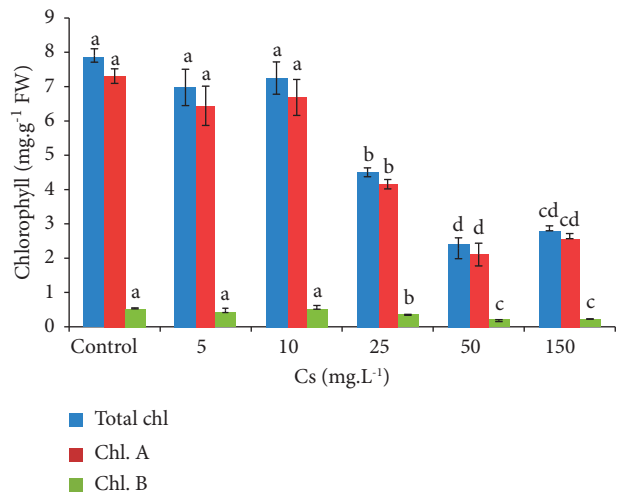

Figure 4: Effect of cesium on chlorophyll content of *S. portulacastrum*. All the values are mean of twelve readings ± S.E. One-way ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values in total chlorophyll, chlorophyll-a, or chlorophyll-b (DMRT $p \leq 0.05$).

Cs. At 50 mg·L^{-1}, the %TWC was not significantly different from that of the control.

3.3. Chlorophyll Pigments. The chlorophyll pigments were highly susceptible to high Cs treatment (Figure 4). The total chlorophyll, chlorophyll-a, and chlorophyll-b contents were maintained up to 10 mg·L^{-1} Cs concentration. However, Cs treatment of 25 mg·L^{-1} and above caused a gradual decrease in the content of chlorophyll pigments. As compared to the control, the total chlorophyll, chlorophyll-a, and Chlorophyll-b content was decreased at 150 mg·L^{-1} Cs by 2.08, 2.76, and 2.7-fold, respectively, at $p \leq 0.05$ (Figure 3).

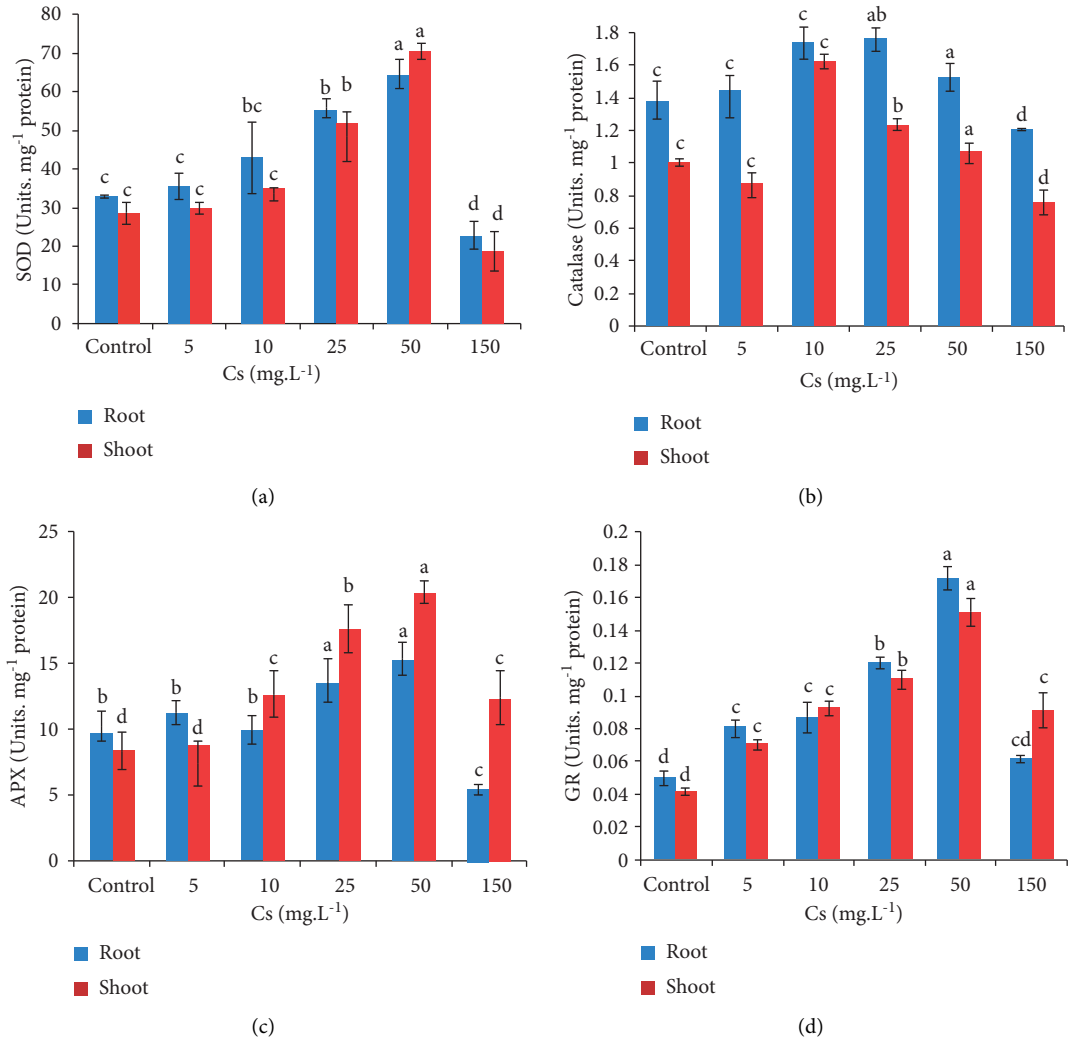

Figure 5: Effect of cesium on antioxidant enzymes activity of *S. portulacastrum*: (a) superoxide dismutase (SOD), (b) catalase (CAT), (c) ascorbate peroxidase (APX), and (d) glutathione reductase (GR). All the values are mean of twelve readings ± S.E. One-way ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values of antioxidant enzymes (DMRT $p \leq 0.05$).

3.4. Antioxidant Enzyme Activities. Both root and shoot tissues showed similar antioxidant enzyme activities viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) under Cs exposure (Figures 5(a)–5(d)). The antioxidant enzyme activities were significantly increased up to 50 mg·L^{-1} Cs, treatment while, higher concentration (150 mg·L^{-1}) induced a significant decrease in enzyme activities. The SOD activity was increased by 2-fold in the root and 2.5-fold in the shoot at 50 mg·L^{-1} Cs while it was decreased by 1.4–1.5-fold in the root and shoot at 150 mg L^{-1} Cs (Figure 5(a)). The CAT activity was increased by 2.1-fold in the root and 1.8-fold in the shoot at 50 mg·L^{-1} Cs while 150 mg·L^{-1} Cs induced a decrease in activity by 1.7-fold in the root and 1.2-fold in the shoot (Figure 5(b)). A similar trend was observed in the case of APX activity which recorded an increase of 1.5-fold in root and 2.4-fold in the shoot at 50 mg·L^{1} Cs and a decrease of 1.9-fold in root and 1.5-fold in the shoot at 150 mg·L^{-1} Cs (Figure 5(c)). Compared to other enzymes, the GR activity showed higher activity (3.4-fold in the root and 5-fold in the shoot at 50 mg·L^{-1} Cs) whereas 150 mg·L^{-1} Cs induced a decrease of 0.8-fold in the root. Although GR activity was decreased at 150 mg·L^{-1} Cs in the shoot, it was still 3-fold higher than control plants (Figure 5(d)).

3.5. Cesium Accumulation in Different Plant Parts. The accumulation of Cs in different parts of the plant (root, stem, and leaves) is depicted in Figure 6; The Cs accumulation was higher in aerial parts (leaves and stem) as compared to underground parts (root). In leaves, the Cs level was highest (536.10 μg·g^{-1}) followed by the stem (413.74 μg·g^{-1}) and roots (284.69 μg·g^{-1}) suggesting the dose and tissue-specific nature of Cs accumulation in *Sesuvium*.

4. Discussion

The presence of metal toxicants including radiocesium in soil, water, and air can cause serious consequences to human health and the environment [33]. A recent survey on heavy

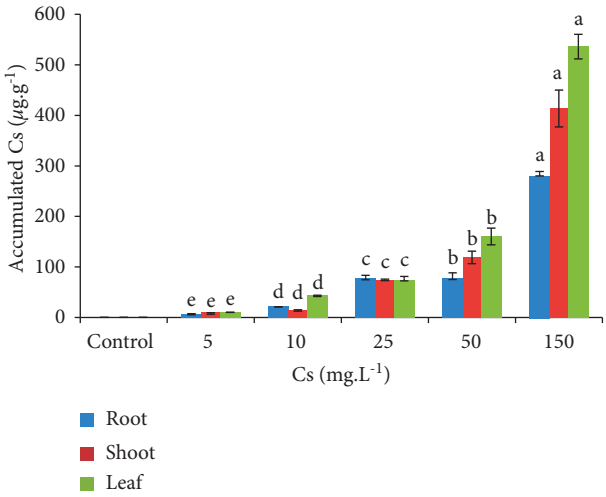

FIGURE 6: Cesium accumulation in root, stem, and leaves of *S. portulacastrum*. All the values are mean of twelve readings ± S.E. One-way ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values in root, stem, or leaves (DMRT $p \leq 0.05$).

metal pollution of global rivers and water lakes revealed that the concentration of heavy metals in these water bodies is exceeding significantly the standard threshold limits as per World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA) [34]. Phytoremediation is one of the plant-based approaches to manage environmental toxicity [35]. The results of our study demonstrated that the impact of Cs depends on the level of accumulation and localization besides the tolerance of the plant species. It was observed that the growth of root and shoot along with their relative water content was not affected at 25 $mg{\cdot}L^{-1}$ of Cs in *Sesuvium*. In *Calendula alata,* Borghei et al. [36] observed maintenance of plant growth, root length, shoot length, and chlorophyll contents at lower concentrations up to 10 $mg{\cdot}L^{-1}$ Cs but beyond this concentration, growth was retarded. Higher Cs concentration (3 M Cs) also showed a reduction in shoot growth in the Cs hyper-accumulator *Pennisetum purpureum* [10]. Excessive Cs has also been found to induce black roots and slow growth in *Brassica juncea* seedlings [37]. Higher concentrations of Cs affected the number of germinated seeds and roots and the shoot length of seedlings [38]. Our results showed that increasing the concentration of Cs caused a reduction in the total chlorophyll content including, chlorophyll-a and chlorophyll-b. The Cs-induced inhibition of chlorophyll content has also been observed in other plant species, such as *Spinacia oleracea* [39] *Nitella pseudoflabellata* [40], *Salix paraplesia* [41], and *Brassica juncea* [37].

The toxic metals induce different morphological, physiological, and biochemical dysfunctions by the generation of reactive oxygen species which impose damaging effects on plants [42]. In Arabidopsis, exogenous application of a small chemical compound namely CsToAcE increased Cs accumulation and tolerance. The CsToAcE1 interacts with a plant protein BETA GLUCOSIDASE 23 (AtβGLU23) and suppresses it. This protein negatively regulates plant response to Cs [43]. Plants cope with the toxic effects of heavy metals by the induction of an efficient antioxidant defense system. Our results indicated that higher antioxidant enzymatic activity (GR activity, 3.4-fold higher in root and 5-fold in the shoot, at 50 $mg{\cdot}L^{-1}$ of Cs) in *S. portulacastrum* suggesting the role of efficient antioxidant enzyme system in case of Cs tolerance. Enhanced induction of antioxidant defense (SOD, POD, CAT, and APX) has also been shown under Cs treatment in *Brassica juncea* [44]. Recently, Adams et al. [1] have observed Cs-induced higher glutathione accumulation without reduction in the Cs accumulation pattern in *Arabidopsis thaliana*. The Cs being the competitor of potassium ion (K^+) competes with potassium for uptake. The unavailability of potassium causes growth retardation in plants [45, 46]. In *Gladiolus grandiflora*, supplementation of potassium showed alleviation of Cd-induced toxicity [47]. Mostofa et al. [48] stated that improvement of plant potassium use efficiency can increase plant performance in Cs-contaminated soil. On the contrary, in the case of halophytes instead of potassium and chloride, sodium is an important macronutrient for their growth [49]. This could be the probable reason for the high tolerance of Cs in *S. portulacastrum*. A significantly higher amount of Cs was accumulated in the aerial parts (536.10 $\mu g{\cdot}g^{-1}$ in leaves and 413.74 $\mu g{\cdot}g^{-1}$ in the stem) as compared to the underground parts (284.69 $\mu g\ g^{-1}$ in roots) suggesting that root-to-shoot translocation of Cs is rapid in *S. portulacastrum*. Such efficient and rapid translocation of toxic compounds from root to shoot is a prerequisite for effective phytoremediation as previously suggested by other researchers [37, 44]. Our results suggest that the facultative nature of *S. portulacastrum* helps it to grow in both saline and nonsaline habitats offering an additional advantage over true halophytes which require a certain amount of salt for growth. Such halophytic species also can be useful as candidates for deciphering the mechanism of salt and metal tolerance, and also for isolating stress-responsive genes which can be applied to enhance the tolerance of sensitive, glycophytic plants [50].

5. Conclusions

This study concludes that *S. portulacastrum* is a hyper-accumulator of toxic Cs metal. The higher accumulation of Cs in aerial plant parts and less alteration in growth revealed the potential of *Sesuvium* as a suitable candidate for the phytoremediation of Cs-contaminated soil. The efficient antioxidant enzyme system and maintenance of growth are the key components of Cs tolerance in *Sesuvium*. It is recommended that *S. portulacastrum* can be cultivated in Cs-contaminated soils and near nuclear power plants for phytoremediation. Further studies are required to understand the precise molecular mechanism of Cs tolerance, the involvement of *Sesuvium*-associated microbes, and validation through field experiments to confirm the phytoremediation ability of *Sesuvium*.

Data Availability

All relevant data are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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