

Preliminary screening of growth performance, biochemical profiling and accumulation capacity of *Senna tora* and *S. uniflora* to lead stress

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DOI: 10.18811/ijpen.v11i04.13

ABSTRACT

Lead is a non-essential element that has a detrimental effect on plant growth and metabolism when accumulated in higher quantities. In the present study, we investigate effect of lead on *Senna tora* (L.) Roxb. and *Senna uniflora* (Mill.) H.S. Irwin and Barneby, those grow dominantly on banks water bodies in the Kolhapur district, Maharashtra, India. We assessed the relative effects of varying lead concentrations on the growth of seedlings, biochemicals and accumulation capacity of both species. In both species, the seedling growth was reduced in response to lead treatment at concentrations 5, 10, 20 and 40 mg kg⁻¹ of soil and the germination of seed, root and shoot growth, fresh and dry biomass were highly diminished at 40 mg of lead kg⁻¹ of soil. The growth parameters decreased with the increased accumulation of lead (i.e., 0.073, 0.110, 0.149 and 0.170 mg g⁻¹ and 0.122, 0.169, 0.212 and 0.429 mg g⁻¹ at 5, 10, 20 and 40 mg kg⁻¹ of soil) in *S. tora* and *S. uniflora*, respectively. Decline in the content of chlorophyll, reducing sugar, total sugar and protein was a common feature while, proline content increased at 5, 10, 20 and 40 mg of lead kg⁻¹ of soil for *S. tora* and *S. uniflora*, respectively. In both plant species, an increase in lead concentration resulted in a decrease in the bioconcentration factor and tolerance index. The growth performance and biochemicals were impaired due to different concentrations of lead.

Keywords: Accumulation capacity, biochemicals, growth performance, lead, *Senna tora*, *Senna uniflora*.

Highlights

- Both species (*Senna tora* and *Senna uniflora*) investigated in the present study are growing dominantly along the sides of water bodies in Kolhapur district.
- Lead is a toxic heavy metal that alters the development of plants.
- An increase in lead concentrations is directly related to a decrease in the growth of germinating seeds, their length and biomass of seedlings.
- Similarly, there is a decrease in concentrations of total chlorophyll, reducing sugars, total sugars and protein levels, while proline levels increase.
- The accumulation capacity of lead in *Senna tora* and *Senna uniflora* seedlings increased with the increase in concentration of lead.

International Journal of Plant and Environment (2025);

ISSN: 2454-1117 (Print), 2455-202X (Online)

INTRODUCTION

Lead is considered as a major and effective pollutant of the environment. Besides mining and smelting, lead is also added to the environment by use of lead-containing paints, gasoline, explosives, disposal of sewage sludges, electronics, plumbing and cookware (Chaney and Ryan, 1994; Mishra *et al.*, 2025). Despite several efforts and control measures to restrain the input of lead in the environment, it has become an acute environmental problem and a threat to human life in many countries. As lead is a major environmental contaminant arising from industrial activities, its accumulation in soil is expected to emerge as a critical environmental concern in future (Yang *et al.*, 2000). As per MAFF (1993) report, the permissible limit for lead in the United Kingdom for food is 1 mg kg⁻¹. Lead is naturally found in soil at very low concentrations and it may extend from 20-50 mg kg⁻¹ in uncontaminated soils (Nriagu, 1978). In the United Kingdom, soils with 400-800 mg kg⁻¹ of lead content are considered highly polluted. Therefore, crops grown on such soils will contain a higher lead concentration than the legal limit if the pH is below 6.0 (MAFF, 1993). After arsenic, lead has attained second rank due to its intense risk of instigating toxicity (Gaya and Ikechukwu, 2016).

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How to cite this article: Matade, N.S., Pawar, K.B. (2025). Preliminary screening of growth performance, biochemical profiling and accumulation capacity of *Senna tora* and *S. uniflora* to lead stress. *International Journal of Plant and Environment*. 11(4), 765-770.

Submitted: 10/12/2024 **Accepted:** 30/09/2025 **Published:** 31/12/2025

Lead contamination poses a major environmental concern, adversely impacting the growth and overall development of plants (Gupta *et al.*, 2024). Burton *et al.*, (1984) have stated that the toxicity of lead on plants rapidly inhibits the root growth, and shows stunted growth, and chlorosis of plants. Physiological processes of plants that are affected by lead when it enters the cell, even in small amounts. Inhibited enzyme activities, imbalance of water, altered state of hormones, impaired membrane permeability, and disrupted mineral nutrition are the disorders caused by the toxicity of lead, which disturbs the

physiological status of plants and leads to death due to a high concentration of lead (Seregin and Ivanov, 2001; Ernst, 1998).

As compared to monocot roots, lead accumulates in greater amounts in dicots (Huang and Cunningham, 1996). *Senna tora* and *S. uniflora* are the prominent species growing along the sides of water bodies in the Kolhapur district. The screening of these two test species with respect to accumulation capacity may help to check their phytoremediation potential. Hence, an attempt has been made to check the detrimental impacts of lead on the growth efficiency, biochemical analysis and accumulation capacity of *Senna tora* and *Senna uniflora*.

MATERIAL AND METHODS

Collection of plant material and preparation of a stock solution of lead

Soil samples and dried pods of *S. tora* and *S. uniflora* were collected from their natural population in December 2023 from different sites of Rajaram Lake, Kolhapur (16°40'56"N, 74°15'49" E) (Fig.1). For treatment purpose, 1.614 grams of Lead (II) nitrate $Pb(NO_3)_2$ (Sisco Pvt. Ltd.) was dissolved in 1000 ml of deionised water for the preparation of a stock solution. Solutions of different concentrations viz. 25ppm, 50ppm, 100ppm and 200ppm of Lead nitrate were prepared by diluting 2.5ml, 5ml, 10ml and 20ml of the stock solution to 100ml with deionised water (Anand, 2018).

Soil Bioassay

For the soil bioassay, 1kg of dry soil was placed in plastic trays with dimensions (22cm x 17cm x 4.2 cm), and the soil was then moistened with water. The seeds were surface sterilised by treating with 0.1% $HgCl_2$ for few seconds. Thirty seeds of test species were sown in each tray and supplied with 30 ml of lead solutions for treatment and 30 ml of deionised water for control on alternate days (for five days). The lead content in the soil is 0.970 mg g^{-1} . A total of 150ml of 25, 50, 100 and 200ppm solution of Pb was applied, and the concentration of Pb became 5, 10, 20 and 40 mg kg^{-1} of soil. The seeds were incubated at a temperature (26°C) under natural light and dark conditions. Ten-day old seedlings were uprooted carefully, and the number of seeds germinated, root length, shoot length, fresh weight and dry weight were recorded. For the bioassay each treatment and control was arranged in triplicate.

Biochemical Analysis

Total chlorophyll

Five hundred milligrams of leaf material was extracted using 80% acetone and then centrifuged at 3000 rpm, and the pellet was re-extracted with 80% acetone. Absorbance of extract was recorded at 645 and 663nm and the outcomes were given in mg g^{-1} fresh weight (Arnon, 1949).

Reducing sugar and total sugar

For estimation of sugars, 500 mg of powder of dry material was extracted with 80% ethanol and filtered. Filtrate was condensed, decolourised, and diluted with 40 ml of distilled water and it was used for the estimation of reducing sugar. 20ml of filtrate and 4ml of concentrated HCl was autoclaved and neutralised with sodium carbonate and filtered. Filtrate (0.4ml) along with standard glucose solutions was served with the reagent Somogyi's alkaline copper tartarate and Arsenomolybdate. Absorbance of the reaction mixture was recorded at 560nm and mg g^{-1} dry matter was the unit of measurement (Nelson, 1944).

Protein

Soluble protein content of seedlings was estimated by the Lowry *et al.*, (1951) method. Five hundred mg of dry powder of plant material was extracted with 0.1M Phosphate buffer (pH 5.8), and the filtrate was centrifuged. Extract was treated with Reagent C and Folin and Ciocalteu phenol reagent. After 30 minutes of incubation, absorbance was determined at 660nm, and the values were presented as mg g^{-1} dry weight.

Proline

Dry plant material powder (500 mg) was homogenised with 10ml 3% sulfosalicylic acid and filtered. Filtrate (0.5 ml) was added with acid ninhydrin reagent (2ml) and glacial acetic acid (2ml) in a test tube for one hour at 100°C in a boiling water bath. The process was ended by dipping the test tube in an ice bath, subsequently four millilitres of toluene was added and mixed vigorously. The proline absorbance was evaluated at 520nm and was expressed in mg g^{-1} dry weight (Bates *et al.*, 1973).

Accumulation of lead

Oven-dry powder (100 mg) of plant material was digested using 69-70% nitric acid and 60% perchloric acid. Lead content was measured by using an Atomic Absorption Spectrophotometer



Fig. 1: A) Population of *S. tora*, B) Population of *S. uniflora* and C) Site of Rajaram Lake (Kolhapur)

(Systronics [India] Ltd AAS- 263), and mg g^{-1} dry weight was the unit of measurement (Toth *et al.*, 1948).

Bioconcentration factor (BCF)

Bioconcentration factor was determined by the formula given by Siyar *et al.*, (2022).

$\text{BCF} = \text{Heavy metal concentration in plant (mg/kg)} / \text{Heavy metal concentration in soil (mg/kg)}$.

Tolerance Index (TI)

Tolerance index is measured using the formula given by Bhat *et al.*, (2022).

$\text{TI (\%)} = \text{Fresh weight of plants cultivated in metal solution} / \text{Fresh weight of plants cultivated in control solution} \times 100$.

STATISTICAL ANALYSIS

All values are the means of triplicates with standard error. The statistical analysis like mean value, standard deviation and standard error were derived using Microsoft Excel Software (MS Office version 2010).

RESULTS AND DISCUSSION

Effect of lead concentration on seed germination and growth parameters

Due to the treatment with lead, the number of germinated seeds of *S. tora* decreased with an increase in lead concentration. The highest reduction in seed germination was observed at 40 mg kg^{-1} of soil while it was lowest at 5 mg kg^{-1} of soil. Same pattern of reduction was also noticed in *S. uniflora* including more reduction at 40 mg of lead (Fig.2). The reduction in length of root and shoot is observed as a consequence of different concentrations of lead with maximum decrease in the same was due to 40 mg of lead (Fig.3). Fresh weight and dry weight decreased due to treatment of lead at $5, 10, 20$ and 40 mg kg^{-1} soil in seedlings of both *S. tora* and *S. uniflora* respectively (Fig.4). Effect of lead on growth performance was found to be more prominent in case of *S. uniflora* due to more accumulation of lead as compared to *S. tora*. Lead is readily absorbed and stored in different plant parts, but it is not an essential element and is not involved in cell metabolism. It adversely affects plants by causing toxic symptoms such as inhibition of growth, detrimental impacts on photosynthesis, roots darkening, disturbance in nutrition with minerals and the balance of water and alterations in hormones and structure and permeability of membrane (Nas and Ali, 2018). Recurrently the adverse effect of lead is assessed by morphological parameters like growth and biomass of seedlings. We have tried to evaluate the growth performance based on the accumulation of lead. Findings of the present work are in accordance with the reports in which growth performance and accumulation capacity are appraised parameters under the impact of varying concentrations of lead. Kumar and Jayaraman (2014) have analysed effect of varying levels of lead nitrate ($25, 50, 75, 100$ and 150 mg kg^{-1} soil) on Black gram [*Vigna mungo* (L.) Hepper] by using soil bioassay and showed the reduction in viability percentage, length of root, length of shoot, fresh and dry biomass of 15 days old seedlings of Black gram as per the increase in the concentrations of lead. Singh *et al.*, (2012) have

described the reduction of total chlorophyll content (0.418, 0.380, 0.351) due to treatment of $10, 50$ and $100 \mu\text{M}$ lead as compared to control (0.485) in seedlings of *Vigna mungo* L. by using pot culture experiment.

Effect of lead concentration on biochemical profiling

The total chlorophyll content of seedlings of *S. tora* and *S. uniflora* was reduced due to different concentration of lead with more

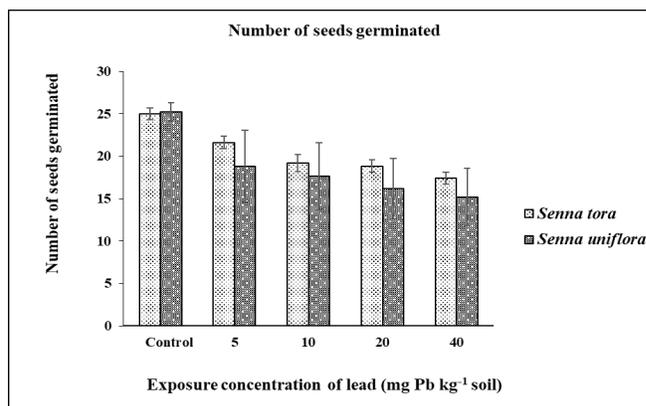


Fig. 2: Impact of varying amounts of lead on the number of seeds germinated of *S. tora* and *S. uniflora*

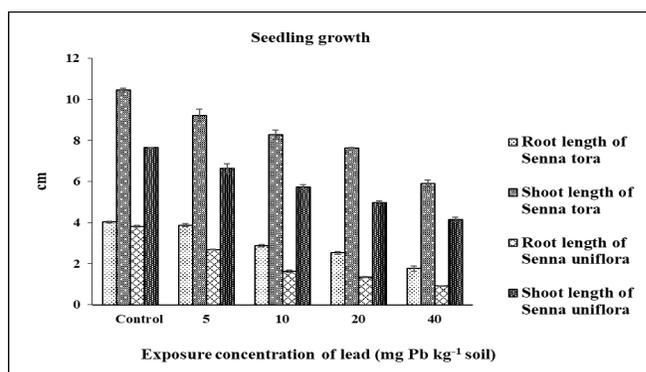


Fig. 3: Impact of varying amounts of lead on the seedling growth of *S. tora* and *S. uniflora*

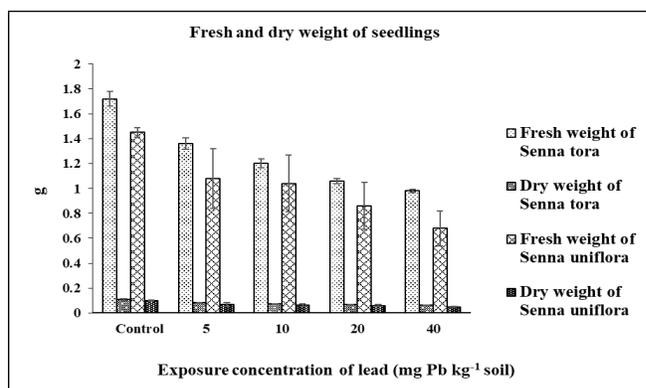


Fig. 4: Impact of varying amounts of lead on the fresh and dry weights of *S. tora* and *S. uniflora* seedlings

decrease in the content of chlorophyll at 40 mg kg⁻¹ soil in *S. tora* and in *S. uniflora* as compared to control samples (Fig.5). In *S. tora* lead at 40 mg kg⁻¹ soil caused more decrease in the contents of reducing and total sugars and protein as compared to other concentrations of lead (Fig. 6). In *S. uniflora* 40 mg of lead kg⁻¹ of soil caused maximum reduction in the contents of reducing sugar, total sugar and protein content which is succeeded by 20, 10 and 5 mg of lead kg⁻¹ of soil (Fig.7). The proline content of seedlings of *S. tora* and *S. uniflora* was increased in response to different concentrations of lead with maximum increase at 40 mg of lead (Fig.8). The overall increased accumulation of lead in seedlings of *S. tora* and *S. uniflora* was observed in different concentrations of lead like 5, 10, 20 and 40 mg (Fig.9). The synthesis of chlorophyll is hampered by the reduction in the adoption of crucial elements like magnesium and iron by the plants (Burzynski, 1987). The plants supplied with lead showed chlorophyll breakdown, which is boosted due to enhanced activity of the enzyme chlorophyllase and chlorophyll- b is affected adversely than chlorophyll a (Drazkiewicz, 1994). As per the report of Alia and Saradhi (1991), the uptake of proline is considered as an indicator of environmental as well as heavy metal stress and has protective roles. Zengin and Munzuroglu (2005) have observed reduced chlorophyll content and substantially increased proline content between 4.6% and 21.2% in the leaves of seven-day old bean seedlings (*Phaseolus vulgaris* L.) after the treatment of 1.5, 2.0 and 2.5 mM of lead. Hatamian *et al.*, (2020) showed that proline content was increased highly in leaves of *Celtis australis* seedlings, which accumulates the highest proline in leaves when treated with 30 mg L⁻¹ lead. Choudhury *et al.*, (2016) have noted 28.9 mg/kg and 104.25 mg/kg accumulation of lead in Indian mustard and Marigold, respectively, when grown in soil collected from contaminated Buriganga riverbed sediments. Herlina (2018) carried out a pot experiment with *Cordyline fruticos* L. under various concentrations of lead 250, 500 and 750 mg lead kg⁻¹ soil and showed that the high concentrations of Pb lead to a reduction of growth parameters and increased accumulation of lead in roots and leaves. Akpan *et al.*, (2022) have stated that *Abelmoschus esculentus* accumulated lead as 2.790, 4.742, 7.440, 12.700 and 13.690 mg/kg in stem, 9.610, 13.847, 18.561, 22.351 and 29.630 mg/kg in roots and 18.662, 23.464, 24.051, 29.698 and 37.191mg/kg in leaves at 10, 20, 30, 40 and 50ppm lead respectively after 7 weeks of treatment with the use of pots filled with 5 kg soil.

Effect of lead on bioconcentration factor and tolerance index

The bioconcentration factor (BCF) of *S. tora* and *S. uniflora* was observed to be the highest at 5 mg lead. An increase in the concentration of metal solutions resulted in a decrease in the tolerance index of both species investigated. The TI of *S. tora* is highest at 5 mg kg⁻¹ of soil and lowest at 40 mg kg⁻¹ of soil. At 5, 10, 20 and 40 mg kg⁻¹ of soil the tolerance index declined in *S. uniflora*. The maximum BCF and TI values were observed in *S. uniflora* as compared to *S. tora* in response to the toxicity of lead (Table.1). The movement of heavy metals from soil to plants is referred to as the bio-concentration factor. The BCF greater than 1 indicates higher heavy metal deposition in plants than in

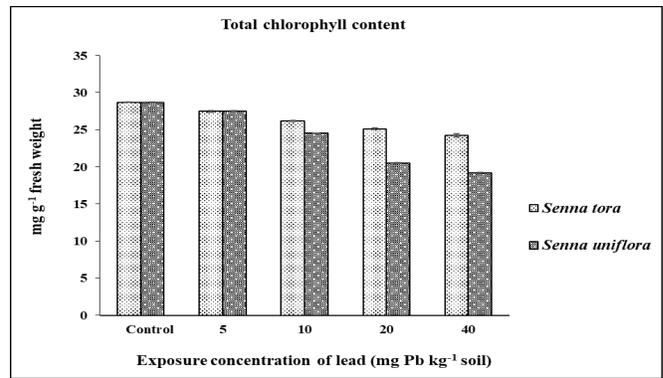


Fig. 5: Impact of varying amounts of lead on total chlorophyll contents of *S. tora* and *S. uniflora* seedlings

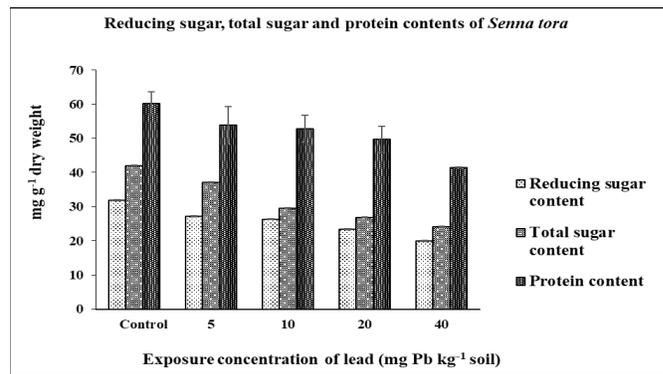


Fig. 6: Impact of varying amounts of lead on reducing sugar, total sugar and protein contents of *S. tora* seedlings

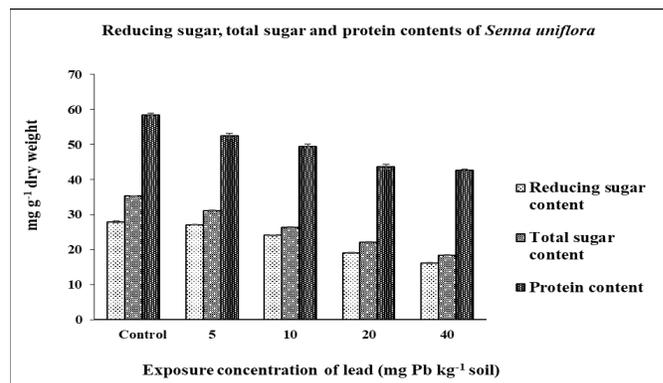


Fig. 7: Impact of varying amounts of lead on reducing sugar, total sugar and protein contents of *S. uniflora* seedlings

soil whereas when the BCF is less than 1, there are more heavy metals in the soil than in plants (Lugwisha, 2016). As per our work findings the BCF is higher than 1 for both test species; it can be said that heavy metals accumulation is more in plants than in the soil. According to Luthansa *et al.*, (2021), BCF is separated into three groups: the indicators (BCF equal to 1), the accumulator (BCF greater than 1) and the excluder (BCF less than 1). Hence, *S. tora* and *S. uniflora* can accumulate Pb as their BCF >1. The TI higher than 0.5 indicates that the plant has good tolerance and grows well (Wu *et al.*, 2017).

Table 1: Bio-concentration factor and Tolerance index in *S. tora* and *S. uniflora* seedlings for different concentrations of lead

Treatments mg lead kg ⁻¹ of soil	Bio-concentration factor		Tolerance Index	
	<i>S. tora</i>	<i>S. uniflora</i>	<i>S. tora</i>	<i>S. uniflora</i>
5	14.67 ± 0.657	24.4 ± 0.346	79.8 ± 4.139	89.78 ± 1.986
10	11.03 ± 0.498	16.9 ± 0.173	70.32 ± 1.598	86.48 ± 1.333
20	7.45 ± 0.076	10.62 ± 0.176	62.45 ± 2.945	71.32 ± 4.502
40	4.25 ± 0.043	10.72 ± 0.023	57.24 ± 1.957	56.21 ± 1.443

All values are means of triplicate ± Standard error*

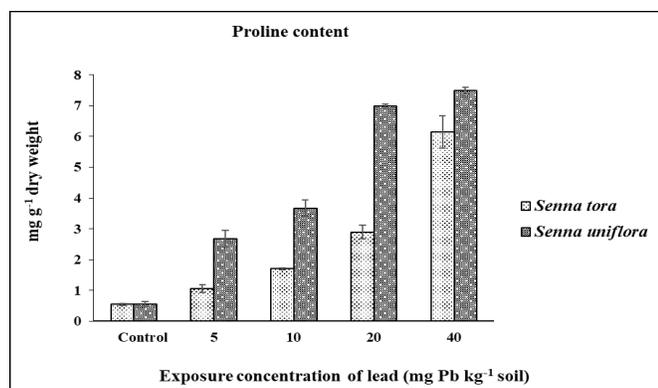


Fig. 8: Impact of varying amounts of lead on the proline content of *S. tora* and *S. uniflora* seedlings

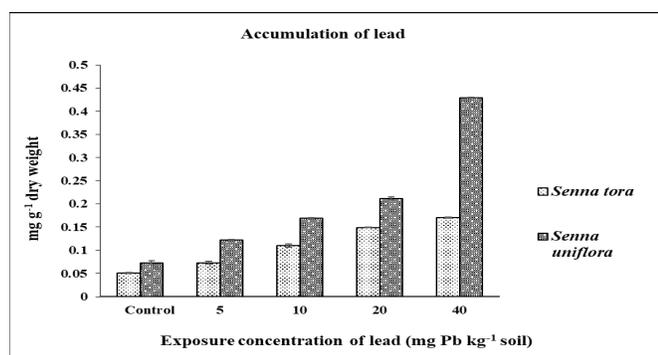


Fig. 9: Accumulation of lead in *S. tora* and *S. uniflora* seedlings

CONCLUSION

Based on current findings, it can be concluded that, though test species *S. tora* and *S. uniflora* are growing dominantly on lake sides, the treatment with different levels of lead has caused a detrimental impact on the growth performance of both species. As the amount of lead in the soil increased, both plants showed a clear reduction in seed germination, root length, shoot length, fresh weight and dry weight. Between two species, *S. uniflora* was more sensitive, showing growth reduction and higher lead accumulation than *S. tora*. The increase in accumulation of lead showed a negative impact on biochemical parameters, which caused a reduction in total chlorophyll, reducing sugars, total sugars and protein contents, whereas a notable increase in the proline content was observed in both species. Both plants

absorbed lead from the soil, with the highest uptake at 40 mg kg⁻¹. The highest bio-concentration factor and tolerance index were observed at 5 mg kg⁻¹. *S. uniflora* absorbed more lead than *S. tora*, but its growth was affected more severely. The highly toxic effect in *S. tora* and *S. uniflora* was observed at 40 mg kg⁻¹ of soil. The study demonstrates that among the two species, lead accumulation capacity, bioconcentration factor and tolerance index were found to be higher in *S. uniflora* than *S. tora*. *S. uniflora* acts as an efficient accumulator of lead, while *S. tora* shows better tolerance. The test species could be valuable for eco-friendly management of polluted soils and may be deployed in phytoremediation techniques for lead.

ACKNOWLEDGEMENT

The authors are grateful to the Head of the Department of Botany, Shivaji University, Kolhapur, India for providing facilities for the research work

AUTHOR'S CONTRIBUTION

Matade N.M.: Collected material, performed the experiment, analysed the data and drafted the manuscript. Pawar K.B.: supervised, edited and revised manuscript.

CONFLICT OF INTEREST

Author states that they have no conflict of interest.

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