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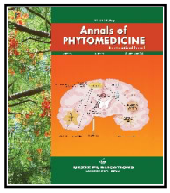

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Phytoremediation approach for uptake and accumulation of Hg in *Isachne globosa* (Thunb.) O. Kuntz to mitigate contamination around an industrial area

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Abstract

Heavy metal pollution of the environment is a global concern. The study aimed to investigate the phytoremediation potential of the native plant *Isachne globosa* (Thunb.) O. Kuntz for removing and bioaccumulating mercury (Hg) from contaminated environments. The conventional methods used for heavy metal removal are often expensive and harmful to the environment and living organisms. On the other hand, phytoremediation is an eco-friendly and cost-effective technology that utilizes plants to remediate metal-contaminated soil or water. In the laboratory, seedlings of *I. globosa* with uniform height and weight were cultivated separately in water spiked with different mercury concentrations. A control group of plants was grown in double distilled water without adding mercury. After a specified period, the entire plants were harvested, and the metal content was analyzed. The obtained data were subjected to a Two-way analysis of variance with a significance level of a 0.05. The results showed that *I. globosa* plants could accumulate mercury, with the highest concentration observed on the 14th day at 25 ppm, reaching a level of 45.860 ppm. These findings suggest that *I. globosa* has significant phytoremediation potential for mercury-contaminated environments. By utilizing native plants like *I. globosa* in phytoremediation efforts, it is possible to mitigate the impact of heavy metal pollution in a sustainable and environmental-friendly manner.

1. Introduction

The increase in population and accelerated industrialization cause serious environmental problems, including producing and releasing toxic waste materials into the environment (Ahmad *et al*., 2021). Consequently, concerns about ecological risks caused by heavy metals have led to intensive research of new economic plant-based remediation technologies. Heavy metals are naturally occurring compounds, but human activities amplify their presence in the environment (Robert *et al*., 2000). Soil pollution caused by heavy metals poses a distinct challenge compared to other biosphere compartments like air and water, as these contaminants tend to persist in the soil for extended periods. Hence, removing and recovering heavy metals from contaminated environments is crucial for environmental protection and mitigating their toxic effects (Angelovicova *et al*., 2014).

Metals are introduced into the environment through natural processes like weathering soils and rocks, volcanic eruptions, and human activities involving mining, processing, or using substances containing metals. Heavy metals can induce oxidative stress in plants, disrupting cell membrane integrity, interfering with nutrient uptake, inhibiting photosynthesis, and decreasing plant chlorophyll (Wu *et al*., 2016). Traditional methods used for reclamation of contaminated environment, such as chemical, physical, and microbial methods, often have high installation and operational costs. However, using metal-accumulating plants in phytoremediation or phytomining processes has gained attention. Phytoremediation is a plant-based technology used for the remediation of metal-contaminated environments. It is a low-cost approach for practical, feasible, and acceptable remediation of metal-contaminated sites. This relies on the ability of certain plants to bioaccumulate the elements (Sharma *et al*., 2016). This technology has been increasingly investigated and employed at sites contaminated with heavy metals like cadmium, mercury, lead, chromium, and many others.

Mercury is one of the most hazardous metals in the environment; once released into the atmosphere, it will remain for 6-24 months and transport thousands of kilometres before depositing in the earth's crust (Wang *et al*., 2012). Mining tradition inputs direct mercury deposition into the environment (Petelka *et al*., 2019). The present study aims to evaluate the ability of *I. globosa* to accumulate mercury from the soil/water by absorbing through roots and translocating to above-ground parts and the stress responses of *I. globosa* to mercury exposure. *I. globosa* is a perennial, fast-growing grass belonging to Poaceae family.

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2. Materials and Methods

2.1 Collection of samples

Soil and plant samples were collected from Permude, Surathkal, around an industrial area of Mangalore, Karnataka (latitude $13^{0}00$ 21.2 N and longitude $74^{0}52'44.4$'E), and screened for the presence of mercury. The plant was authenticated by Dr. K. Gopalakrishna Bhat, Professor of Botany, Udupi. The specimen number of the plant is 18PH106R/01, the herbarium was deposited in the Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences.

2.2 Experimental design

The experiment was carried out in a hydroponic system with different mercury concentrations. Mercury was taken as mercuric chloride at 5, 10, 25, 50, and 100 ppm concentration ranges in different polythene tubs. Double distilled water was kept as a control sample. The seedlings of *I. globosa* plants were collected from the non-contaminated region, having similar biomass (height 15-30 cm, weight 1-3 g), were washed and transferred to the mercury spiked water (n = 30). Sampling was done on the 0^{th}, 1^{st}, 7^{th}, 14^{th}, 21^{st}, and 30^{th} days to check the accumulation of spiked metal.

2.3 Analysis of heavy metal

Plant and soil samples collected from the study region were brought to the laboratory, dried, and subjected to acid digestion with three different acids with a 2:2:5 ratio of Conc. HNO_3, $HClO_4$ and H_2SO_4. Samples were digested at 95 ± 5°C in a hot plate for 5-6 h (Tomiyasu *et al*., 2005). When samples became colorless, it was cooled, and the volume was made up to 25 ml with double distilled water, filtered through Whatman filter paper, and stored for further analysis with AAS.

2.4 Mercury analysis

The samples were analyzed at NITK Surathkal, with atomic absorption spectrophotometer (AAS), and Poornayu Research Labs, Bangalore, with AOAC/ICPOES system.

2.5 Abiotic stress indicators

2.5.1 Chlorophyll content measurement

The chlorophyll content was measured by the method (Arnon, 1949; Rajalakshmi *et al*., 2015). About 0.2 g of the leaf of *I. globosa* was taken from the plant and washed with tap water and double distilled water. The tissue samples were cut into small pieces and homogenized with 80% prechilled acetone. The extract was centrifuged for 5 min at 6000 rpm; the step was repeated until the green color disappeared. OD was read at 663 and 645 nm, using 80% acetone as blank in the UV spectrophotometer. The total chlorophyll concentration, chlorophyll a, and chlorophyll b were calculated.

2.5.2 Proline content measurement

Proline content was measured by using Bates *et al*. 1973 method. 0.5 g of the plant tissue was homogenized with 3% sulfosalicylic acid and centrifuged at 6000 rpm for 5 min. A mixture of sulfosalicylic acid, glacial acetic acid, and acid ninhydrin was prepared, and an equal homogenate volume was added. Incubate at 100^{0}C for 60 min in a water bath. The reaction was arrested by keeping it in an ice bath. 4 ml of toluene was added and stirred vigorously. The absorbance of the chromophore region was measured at 520 nm, keeping the reaction mixture blank.

2.5.3 Sample preparation

Fresh leaf samples (0.2 g) were homogenized in 5 ml pre-chilled 0.4 M PBS (pH 7.8) and centrifuged at 10000 4°C for 20 min. Supernatant enzyme solutions were separated.

2.5.3.1 Estimation of superoxide dismutase (SOD)

SOD activity of the plant sample was based on the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) by Beauchamp and Fridovich (1971) standard procedure. The reaction mixture contained 10 mM potassium phosphate buffer, NBT, methionine, riboflavin, and enzyme extract (procedure mentioned above). The tubes were kept under white fluorescent light for 10 min. The absorbance was read at 560 nm, and the enzyme activity was expressed in units per mg of protein.

2.5.3.2 Estimation of catalase

Based on the procedure of Loggini *et al*. (1999), the reaction mixture contained 10 mM PBS (pH 7.0), 0.3% H_2O_2, and 0.1 ml of enzyme solution was added (procedure mentioned above). The decrease in absorbance was measured at 240 nm. Catalase activity was expressed as changes in absorbance per mg protein per minute.

2.5.3.3 Estimation of glutathione

To start the reaction, 1 ml of the extract (procedure mentioned above), DTNB was added to initiate the reaction. Phosphate buffer was added, and the tubes were shaken well; absorbance was read at 412 nm.

2.5.3.4 Estimation of malondialdehyde (MDA)

MDA was measured to determine the extent of membrane damage, which is the final by-product of lipid peroxidation. Fresh leaf samples of desired weight were homogenized in 5% trichloroacetic acid (TCA) and centrifuged at 12000 rpm for 15 min. The supernatant contained enzyme solution. 0.5 ml supernatant was added to 2 ml of TCA-TBA (Thiobarbituric acid) mixture, placed in a boiling water bath for 15 min, and immediately transferred to an ice bath to stop the reaction. The amount of malondialdehyde was determined by measuring the absorbance of the supernatant at 535 nm.

2.6 SEM-EDX analysis

The sample analysis was done at Mangalore University, DST-PURSE laboratory. The procedure for analysing SEM/EDX is as follows cutting and mounting the dried samples, transferring them to the SEM chamber for imaging, obtaining EDX spectra to identify elements, and generating elemental maps for spatial distribution analysis.

2.7 Statistical analysis

Tukey's analysis evaluated differences in the measured parameters of the samples. The analysis results were evaluated at a $p<0.05$ significance level. Graphpad prism was used for this analysis.

3. Results

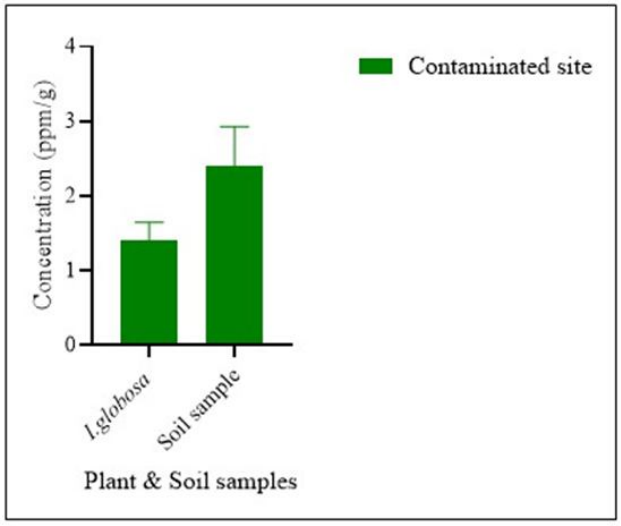

Figure 1: Hg samples collected from the study area.

In Figure 1, it was shown that the plant and soil samples collected from the industrial belt region showed maximum mercury in soil and comparatively less in plant samples. This indicates that the study region chosen was contaminated and needs to be remediated.

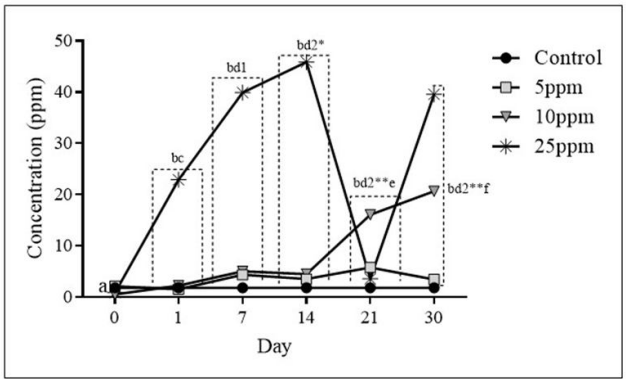

Figure 2: Mercury accumulation in *I. globosa* for 30 days. There is a significant difference between the control and 25 ppm samples on the 14th day. The data represented in Mean ± SD, Subscripts a, b, c, d, e, f, Numbers 1,2, and *, ** indicates a significant difference at $p<0.05$.

The plant sample was spiked with mercury-treated water with different concentrations. In this Figure 2, we can analyze that *I. globosa* can accumulate mercury from the spiked water. The study was limited to 30 days. In contrast, the plants could take up maximum metal (45.560 ppm) on the 14th day at 25 ppm concentration.

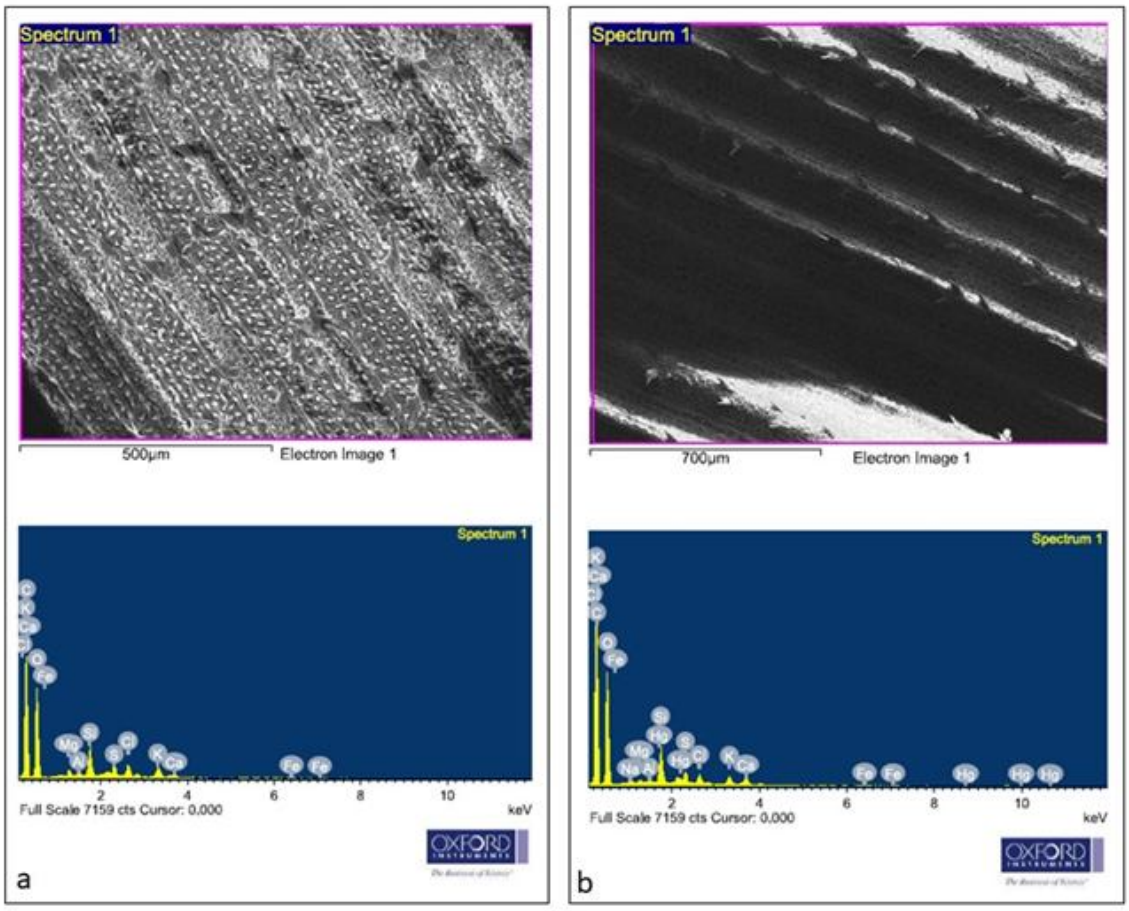

Figure 3: SEM/EDX images of *I. globosa* (a) Control, (b) Hg treated.

SEM analysis was performed to evaluate morphological changes after exposure to Hg metal. The results revealed that heavy metal exposure leads to changes in surface morphology due to metal adsorption. Further, EDX spectra showed the presence of Hg peaks on the surface of treated plants.

3.1 Proline content

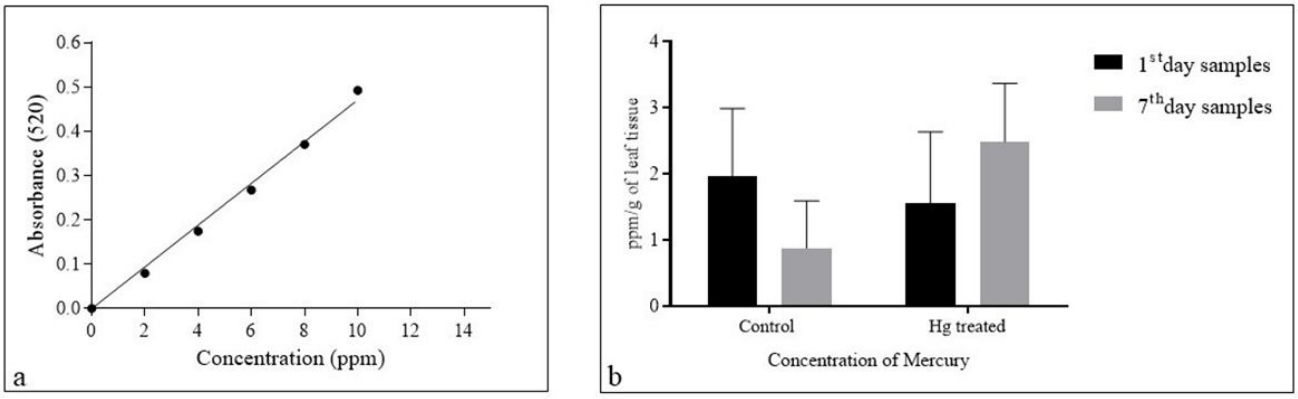

Figure 4: (a) Standard proline, (b) Proline content in Hg treated *I. globosa*.

Proline content is a stress-induced parameter to check the metal stress in mercury-treated plants. Proline content in control plants and mercury-treated plants, there is increased proline content in mercury-treated plants after 7th day of mercury exposure. Heavy metal stress leads to plant proline accumulation (Alia *et al*., 1991; Theriappan *et al*., 2011).

Figure 4 (b) shows the proline content in control and Hg-treated samples. The proline content in the control group was measured as 2.597 µmol/g on the 0th day and slightly decreased to 2.51 µmol/g on the 7th day. This indicates that the proline content remained relatively stable under normal conditions (without Hg treatment). In the Hg-treated group, the proline content was initially measured as 2.59 µmol/g on the 0th day, comparable to the control group. However, on the 7th day, the proline content significantly increased to 3.4 µmol/g. This indicates that the plants responded to the Hg-induced stress by increasing the proline accumulation.

3.2 Chlorophyll content

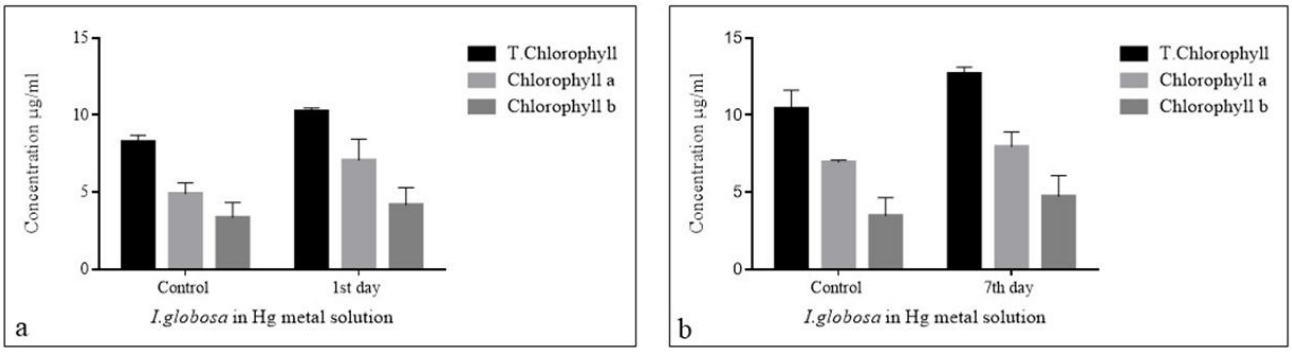

Figure 5: (a) Chlorophyll content in *I. globosa* treated with Hg metal for 24 h, (b) Chlorophyll content in *I. globosa* treated with Hg metal for 7 days. The values are given as Mean ± SD.

Chlorophyll content Figures 5 (a, b) in control and mercury-treated plants shows a significant difference between control and treated plant samples. On the 1st day of Hg exposure, the total chlorophyll content in the control group was measured as 8.746 µg/g, which increased to 10.465 µg/g. On the 7th day of Hg exposure, the total chlorophyll content in the control group further increased to 12.828 µg/g. These results suggest that in the control group, the total chlorophyll content increased over time, possibly due to normal growth and development processes.

On the 1st day of Hg exposure, chlorophyll a, and chlorophyll b levels increased in the control group. This indicates active chlorophyll synthesis and pigment accumulation in response to Hg exposure. On the 7th day of Hg exposure, the levels of chlorophyll a decreased slightly compared to the 1st day, while the levels of chlorophyll b remained relatively stable. This suggests a potential alteration in the chlorophyll composition or degradation processes in response to prolonged Hg exposure. The control group showed an overall increase in total chlorophyll content over time, indicating normal plant growth and pigment synthesis. In contrast, the Hg-treated samples showed alterations in chlorophyll levels, which could be attributed to the stress caused by Hg exposure.

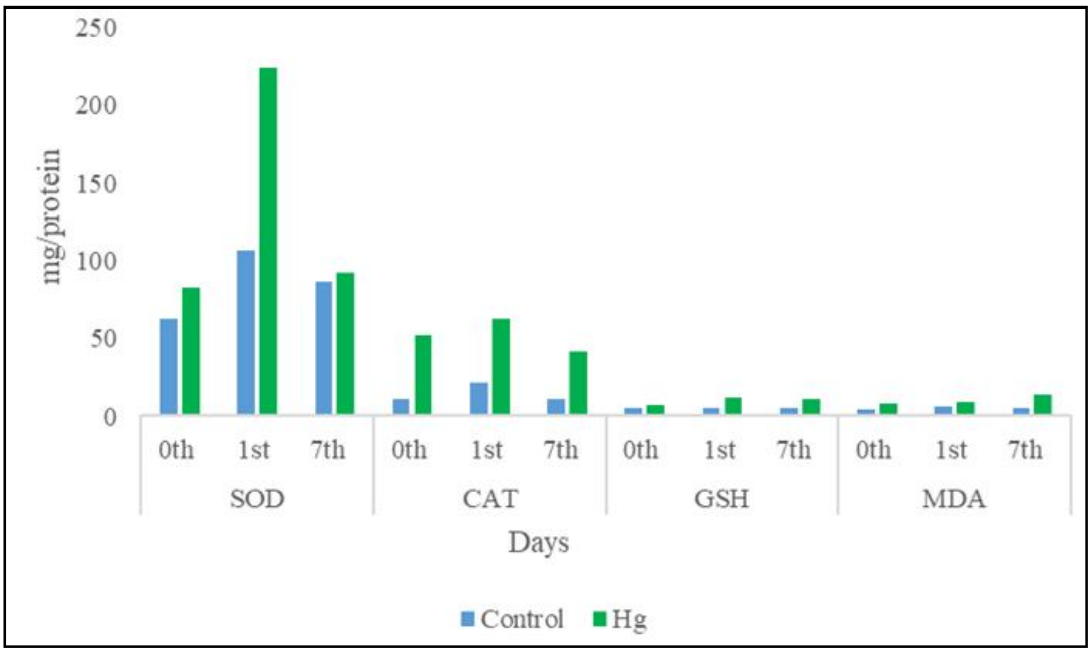

Figure 6: Abiotic stress indicators for Hg treated *I. globose* at different intervals.

The data (Figure 6) shows the levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) in control and Hg-treated samples at different time points (0th, 1st, and 7th day). The activity of SOD on the 1st day, the SOD activity significantly increased compared to the 0th day in both groups. However, on the 7th day, the SOD activity decreased compared to the 1st day. This indicates that the plants responded to the stress caused by Hg exposure by increasing SOD activity, which detoxifies superoxide radicals. The activity of CAT showed a similar trend to SOD. CAT activity increased significantly on the 1st day in Hg-treated samples. However, on the 7th day, CAT activity decreased compared to the 1st day. This suggests that CAT was activated to counteract the oxidative stress induced by Hg exposure. Glutathione plays a crucial role in the detoxification of reactive oxygen species. In the Hg-treated group, GSH levels significantly increased on the 1st day compared to the 0th day. This indicates that the plants responded to Hg-induced oxidative stress by increasing GSH production. Malondialdehyde (MDA) is a marker of lipid peroxidation, which is a result of oxidative damage. In the Hg-treated group, MDA levels increased significantly on the 1st day compared to the 0th day. This suggests that Hg exposure increased the plants' lipid peroxidation and oxidative damage.

4. Discussion

The samples collected from the study area were detected with mercury in soil and plant. The plant chosen for the study was able to uptake and store mercury. The study was conducted for 30 days with different metal concentrations and n_i 30 sample size. The plants could survive up to 25 ppm concentration throughout the study period, whereas plants could not survive with increased concentration. Many studies have proven that mercury can be uptaken by assimilation from topsoil into roots *via* transpiration stream; its availability in soil is low because of less solubility (Baya *et al*., 2010). Many plant species, like *Cyperus rotundus*, *Chromoleana odorata*, and *Lemna minor* are proven suitable for use in phytoremediation studies. These are also used for health benefits (Maroti *et al*., 2022). *Centella asiatica,* mainly known as a medicinal plant, also can remove many metals like Cd, Cr, and Pb (Dolly *et al*., 2022; Mazumdar *et al*., 2021).

The increased proline content in the Hg-treated group suggests its role as an essential osmoprotectant and stress marker. Proline acts as a compatible solute, helping to maintain cellular osmotic balance and protecting against oxidative damage caused by heavy metal toxicity. Its accumulation is often associated with stress tolerance mechanisms in plants. The higher proline content in the Hg-treated group on the 7th day suggests that proline plays a role in mitigating the harmful effects of Hg by acting as an antioxidant and protecting cellular structures from oxidative stress. The increase in proline content could be an adaptive response to counteract the toxic effects of Hg and maintain cellular homeostasis. *P. obtusa,* a medicinal plant, has proven potential for treating oxidative stress (Sapna *et al*., 2022). Increased proline content in the plants indicates the plants ability to respond to metal stress. Proline is an important biochemical marker for stress tolerance in plants, and its accumulation can be used to indicate plant responses to heavy metal toxicity.

Hg exposure also induced oxidative stress in the plants, as evidenced by the increased activities of SOD and CAT and elevated levels of GSH and MDA. Increasing SOD activity is due to oxidative stress and protecting the plants from oxidative damage (Malecka *et al*. 2012; Malar *et al*. 2014; Jyothilekshmi *et al*. 2022). Superoxide radicles are the first radicles produced under stress. Heavy metals induce oxidative stress in plants and direct inactivation of reactive radicles (Dutta *et al.,* 2018). The plants showed a potent antioxidant response by increasing the activities of SOD and CAT (Shahid *et al*., 2017) and elevating GSH levels to counteract the Hg-induced oxidative damage (Yasheshwar *et al*., 2022). However, despite these

defense mechanisms, lipid peroxidation still occurred, as evidenced by the increased MDA levels; a study by Navabore *et al.* (2020) states that an increase in superoxide leads to damage to the membrane and an increase in MDA level. These findings highlight the plants' ability to respond and adapt to heavy metal stress and suggest the potential for oxidative damage under prolonged exposure.

The study was conducted under controlled laboratory conditions; the field studies would preferably assess the practical applicability of the efficacy of the plant for remediating contaminated environments. Further studies should concentrate on the disposal of metal accumulated plants to prevent recontamination.

5. Conclusion

The study investigated the potential of *I. globosa* for the phytoremediation of mercury-contaminated environments. Plants exhibited maximum uptake of metal at 25 ppm concentration; exposure to Hg-induced stress by increasing the proline content and chlorophyll content. It also induced oxidative stress by increasing the activity of SOD and catalase enzymes. By utilizing native plants like *I. globosa*, it is possible to mitigate the impact of heavy metal pollution in a sustainable and environmentally friendly manner.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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