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Assessment of Phytoremediation Potential of Sadabahar Assisted by Humic Acid against Lead (Pb) Contamination

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ABSTRACT

This study was conducted to examine the phytoremediation potential of Sada Bahar *(Catharanthus roseus)* grown in lead contaminated soil augmented by humic acid. A completely randomized bock designed pot experiment was conducted in which three concentrations of lead comprising (0.1mM, 0.3mM, and 0.5 mM) while humic acid was applied as 2% and 4% (V/W) to explore their effect on growth, physiological, biochemical, enzymatic of plant as well as the accumulation of lead in different tissues of plant. Different eleven combinations of treatments including one as control were designed and each treatment has three replicates. The results showed there was significant negative effect of lead on morphological parameters of plants without humic acid, such as plant height reduced to 16%, 18% and 37% respectively at varying levels of lead while leaf surface area reduced 6% to 9% and biomass 23% to 45% at ($P<0.05$) with highest reduction rate (0.5 mM). Biochemical parameters, including chlorophyll content and photosynthetic rate were also reduced to great extent while catalase, superoxide dismutase, proline content were increased under lead toxicity. However, the application of humic acid mainly at its maximum concentration of 4% alleviated the adverse effects by improving growth performance, and restoring physiological attributes approximately 40-60%. Lead accumulation was noted highest in roots as compared to shoot and leaves by following the pattern Root>shoot>leaves. The bioconcentration factor (BCF) and translocation factor (TF) values confirmed the capacity of *Sada Bahar* to uptake and maintain lead, signifying its potential for phytoremediation. Overall, the findings highlighted that humic acid effectively mitigated lead induced stress and enhanced the phytoremediation efficiency of *Catharanthus roseus* against lead contamination.

INTRODUCTION

Lead (Pb) is the toxic heavy metal, which has high atomic weight and densities five times greater than water (Tchounwou et al., 2012). Toxic metalloids in surroundings are a grave issue that are driven on by both manmade and natural events. Mineral weathering, volcanic eruption and erosion are the few natural events while manmade activities like mining, urbanization, waste deposition and farming practices are great source of heavy metals (Asgari Lajayer et al. 2018). Among all heavy metals, lead has been found a very toxic environmental contaminant which poses threat to whole ecosystem (Pourret and Bollinger, 2018). Due to their high toxicity and bioavailability, they negatively affect soil, air, water and in addition plants also show visible symptoms when exposed to lead, it also accumulates in sediments, enters the food web immediately and affects aquatic organisms (Wang et al., 2020a). Lead pollutants play a significant role in disturbing the climate and bring changes, ultimately leading to higher rates of sickness like mental, breathing, urinary tract infection and heart diseases in humans which increase mortality (Kushwaha et al., 2018). Plants have capacity to absorb the heavy metals and other contaminants thus clean up the soil through the process of accumulation and extraction (Baker et al., 2018). Most of the plant species can absorb metals more quickly and move them to their roots and aerial parts thus allocate the contaminates through hyperaccumulation process (Khan et al., 2016). It has been found by (Das et al., 2020) that Sadabahar (*Catharanthus roseus*) releases different kinds of compounds including monoterpenoid indole alkaloids in its roots, shoots and leaves which make it as drought tolerant and can thrive in hot, dry climatic conditions making it a viable option for phytoremediation Many authors have reported the wide range of strategies to enhance phytoremediation, among them, humic acid a dark, black-colored material formed by microbial breakdown has been found a good option for use to boost the phytoremediation as being ecofriendly (Wang et al., 2010). Since humic substances are organic, non-toxic, and safe for the environment, their capacity to release metals offers a fresh and suitable way to use them in improved phytoextraction methods (Jensen et al., 2011).

The present study aims to determine the potential of Sadabahar under the effect of humic acid grown in lead contaminated soil although a lot of work have been published so far in this field but still limited literature is available on the potential of humic acid in enhancing the phytoremediation potential of plant like Sadabahar.

MATERIAL AND METHODS

A completely Randomized Block Designed (CRBD) pots experiment was conducted in the Department of Environmental Sciences at Bahauddin Zakariya University, Multan under optimized environmental conditions. Soil collected from an agriculture field being in agriculture practice since last 10 years, was air dried, crushed and sieve to 2mm diameter and each pots consisting 2 kg of was filled with water to maintain the moisture contents uniform. Seedlings of Sada bahar (*Catharanthus roseus)* were taken from the nursery of arboriculture wing, Bahauddin Zakariya University, Multan and transplanted to the experimental pots. Each pot was consisting of five healthy and equal sized plants. Eleven treatments combinations were set up comprising 2 and 4% of humic acid along with 0.1mM, 0.3mM and 0.5mM of lead while one was kept as control without both factors. The treatment combinations were T0 (LA0×HA0), T1 (LA1×HA0), T2 (LA2×HA0), T3 (LA3×HA0), T4 (LA0×HA1), T5 (LA0×HA2), T6 (LA1×HA1), T7 (LA1×HA2), T8 (LA2×HA1), T9 (LA2×HA2), T10 (LA3×HA1), and T11 (LA3×HA2) with three replicates of each. Plants were harvested for the assessment of fresh weight of root, shoot and leaved by digital weighing balance while dry weight was measured after keeping the plant samples in oven for 24 hours at 70°C. While photosynthetic rate and chlorophyll contents were measured by following the method of Arnon (1967). Similarly, antioxidant enzymes were calculated by using the method of Beer and Seizer's (1952). Lead in the root, shoot and leaves was measured by drying and crushing the plant sample and digestion. Finally, the bioaccumulation factor (BAF) and translocation factor (TF) were calculated to evaluate the plant ability to uptake and translocate lead within plant tissues by using the formulas:

1. Bioconcentration factor = lead concentration in plant / lead concentration in soil
2. Translocation factor = lead concentration in shoot / lead concentration in root

Data was analyzed by using the statistical software (Statistix8.1) for conducting the Two Way Anova while HSD Tukey's was also applied to observe the significant different between the treatments at $P<0.05$ shown with different compact letter display (CLD). The data was arranged, Tabulated and graphically visualized in the MS Excel 2016.

RESULTS AND DISCUSSION

Effect of Lead and Humic Acid on Growth

As shown in Fig.1& 2, the growth of *Catharanthus roseus* (Sada Bahar) was strongly affected by different levels of lead (Pb) and humic acid (HA). When plants were treated with lead alone, their growth showed a clear decline depending on the concentration used. However, the addition of humic acid helped the plants recover and grow better, even under lead stress.
Plants exposed to 0.1 mM lead showed about a 16% reduction in height compared with the control. This reduction became clearer at higher concentrations, reaching 18% at 0.3 mM and 37% at 0.5 mM Pb. When humic acid was added along with lead, the harmful effect was greatly reduced. With 2% humic acid, plant height improved by around 12% compared to plants treated with lead alone and at 4% humic acid, the height increased by nearly 18% compared to the control. This clearly showed that humic acid promoted plant growth even under heavy metal stress. Lead toxicity also reduced the number of leaves. A 36% decline was recorded at 0.1 mM Pb without humic acid. On the other hand, when humic acid was applied alone, the plants produced a much higher number of leaves about 89% more with 2% HA and up to 133% more with 4% HA compared to the control. When humic acid was applied along with lead, leaf number improved by 40–60% compared to the lead only treatments, showing the protective role of humic acid. A gradual reduction in leaf area was observed with increasing lead concentrations 6%, 8.7% and 9.5% for 0.1, 0.3 and 0.5 mM Pb respectively. However, the application of humic acid improved the leaf area showing an increase of 4.9% with 2% HA and 7.9% with 4% HA compared to the control, indicating a recovery in leaf growth. Lead stress reduced root fresh weight by 7% to 23% compared to the control, while root dry weight dropped by 16%. When humic acid was applied, both parameters improved noticeably, root fresh weight increased by 10% with 2% HA and 15% with 4% HA, while root dry weight improved by 26% when humic acid was applied alone. A similar trend was observed for shoots. Shoot fresh weight declined by 20%, 31%, and 43% at 0.1, 0.3, and 0.5 mM Pb, respectively, but the addition of humic acid helped counteract this loss. The use of 2% HA increased shoot fresh weight by 36%, while 4% HA showed an even greater recovery of about 50% compared to the control. Shoot dry weight also showed improvement, humic acid at 2% reduced the loss to only 12%, and plants with 4% HA performed best. These findings highlighted that applying subsequent concentrations of humic acid without lead gave the most favorable results for growth parameters of plant.

Effect of Lead and Humic Acid on Biochemical and Enzymatic Parameters of Plant

Lead exposure had a clear negative effect on the chlorophyll content and photosynthetic performance of *Catharanthus roseus*, while the application of humic acid (HA) helped to lessen these toxic effects and improved the physiological condition of the plants.

As presented in Fig.3, lead stress caused a significant concentration dependent decline in chlorophyll content. Chlorophyll levels decreased by 7%, 16% and 28% at 0.1 mM, 0.3 mM and 0.5 mM Pb, respectively ($p < 0.05$) as compared to control. But when humic acid applied alone enhanced chlorophyll synthesis, showing increase of 4.4% with 2% HA and 8% with 4% HA, indicating its positive influence on pigment formation even in the absence of stress. When humic acid was combined with lead, the reduction in chlorophyll was notably alleviated. At 0.1 mM Pb with 4% HA, chlorophyll content was nearly restored to control level, showing only a minor reduction of 0.8%. At the highest Pb concentration (0.5 mM), chlorophyll loss dropped from 28% (Pb alone) to only 12% with 4% HA. These findings indicate that humic acid, especially at 4%, effectively lessened lead induced chlorophyll degradation and maintained pigment stability under stress conditions. A similar pattern was observed for photosynthetic rate. Lead exposure reduced photosynthesis by 10%, 24%, and 42% at 0.1, 0.3, and 0.5 mM Pb, respectively, compared with the control. Conversely, humic acid alone promoted photosynthetic activity, showing an increase of 7.2% at 2% HA and 12% at 4% HA compared to the untreated control, reflecting its stimulatory effect on plant metabolism. When applied in combination with lead, humic acid clearly alleviated the stress-induced decline. At 0.5 mM Pb, the addition of 2% HA improved photosynthesis by 11% compared to Pb alone, while 4% HA improved it by about 5%. Overall, humic acid, particularly at 4%, consistently reduced the inhibitory impact of lead and maintained higher photosynthetic efficiency in stressed plants. Lead exposure activated a strong oxidative stress response in *Catharanthus roseus*, reflected by a noticeable increase in antioxidant enzyme activities. SOD activity rose by 21% under 0.1 mM Pb treatment compared to the control and similar trends were observed for catalase (CAT) activity, confirming the plant's defensive response to reactive oxygen species generated by lead toxicity. However, the addition of humic acid significantly moderated these responses. At 2% HA, SOD and CAT activities decreased moderately compared to Pb only treatments, while 4% HA led to a more distinct reduction, bringing enzyme activity closer to control levels. A similar trend was found in proline accumulation. Lead stress alone markedly increased proline levels reflecting enhanced osmotic and oxidative stress in the plant. With the addition of 2% humic acid, proline accumulation decreased moderately while 4% HA resulted in a stronger reduction, suggesting that the plants were under less stress. The application of humic acid, especially at 4%, significantly alleviated these effects enhancing chlorophyll and photosynthetic rate, while lowering antioxidant enzyme and proline accumulation. These results highlight the crucial role of humic acid in improving physiological resilience and mitigating lead induced oxidative stress in *Catharanthus roseus*

Lead Accumulation in Plant Tissues

Lead accumulation in *Catharanthus roseus* differed markedly across plant parts presenting that the roots acted as the main site for lead storage. A concentration dependent increase in Pb uptake was observed and the addition of humic acid (HA) further enhanced the plant ability to preserve lead especially in the roots. In plants treated with 0.1 mM lead alone, root Pb accumulation increased by about 12% compared with the control. At 0.3 mM lead, the accumulation rose to 24% and at 0.5 mM lead, it reached 35% higher than the control. When humic acid was added, this effect became more pronounced 2% humic acid with 0.5 mM lead increased root lead concentration by approximately 25% compared to lead alone while 4% humic acid raised it by nearly 40% relative to the control. This shows that humic acid not only improved lead uptake but also enhanced its immobilization within the root tissues. In shoots, a similar increasing pattern was observed, lead alone at 0.1, 0.3 and 0.5 mM caused 10%, 18% and 27% increases in shoot lead levels over the control, respectively. When humic acid was added, the results improved notably 2% humic acid increased shoot lead content by 18% while 4% humic acid raised it by 35% compared to lead only treatments. The lowest accumulation of lead occurred in the leaves. Across treatments, lead levels in leaves increased from 1% to 20% compared to the control. At 0.1 mM lead alone lead accumulation was minimal (around 5% increase), while at 0.5 mM lead, it rose to 15%. However, the addition of 2% humic acid raised leaf lead content by 12%, and 4% humic acid further increased it by 20% compared with the control. This indicates that humic acid, especially at 4% enhanced the mobility of lead toward the aerial parts but at a much lower rate than in roots and shoots (Fig.4).

Lead Bioaccumulation (BAF) and Translocation (TF) Factors

The bioaccumulation of lead in *Catharanthus roseus* varied noticeably across treatments, value greater than 1 indicates efficient metal uptake. The results showed that plants exposed to 0.1 mM lead combined with 4% humic acid achieved the highest BAF value, highlighting a strong ability of the plant to absorb and accumulate lead. This treatment recorded an 83% higher BAF compared to the corresponding lead only treatment. When 2% humic acid was applied with 0.1 mM lead, BAF increased by around 46% compared to lead alone. At 0.3 mM lead, plants treated with 2% humic acid exhibited a 24% increase in BAF compared to the lead-only treatment, while those treated with 4% humic acid showed an even higher 31% increase, indicating that humic acid effectively enhanced metal accumulation across all lead levels. Similarly, at 0.5 mM lead, the application of 2% humic acid increased BAF by 19% and 4% humic acid maintained BAF values greater than 1, confirming the plants continued ability to accumulate lead even under high stress. Therefore, humic acid significantly enhanced the bioaccumulation capacity of *Catharanthus roseus*, especially at 4% making the plant more efficient in extracting lead from the soil and storing it in its tissues. Similarly, translocation factor reflects the plants' ability to transfer absorbed lead from roots to its aerial parts (shoots and leaves). TF values greater than 1 suggests efficient movement of metals within the plant. In this study, *Catharanthus roseus* showed improved translocation ability when treated with humic acid. At 0.1mM lead the addition of 2% humic acid increased TF by 12% while 4% humic acid further enhanced it by 26% compared to lead only plants the highest TF recorded among all treatments. When plants were exposed to 0.3mM lead, the combination with 4% humic acid resulted in a 13% higher TF compared to lead alone, indicating better internal movement of lead toward shoots and leaves. At the highest lead level (0.5 mM lead), the addition of 2% humic acid raised TF by 13% while 4% humic acid caused a modest 4% improvement compared to lead only plants. Although the increase at this level was smaller the TF values remained above 1, indicating that humic acid continued to facilitate lead movement even under severe stress.

Figure 1

Effect of lead and humic acid on plant height, number of leaves, and leaf surface area of Sadabahar (Catharanthus roseus) under different treatments conducted by Two Way ANOVA, different letters show significant differences between the treatments analyzed through HSD Tukey's test at P>0.05.

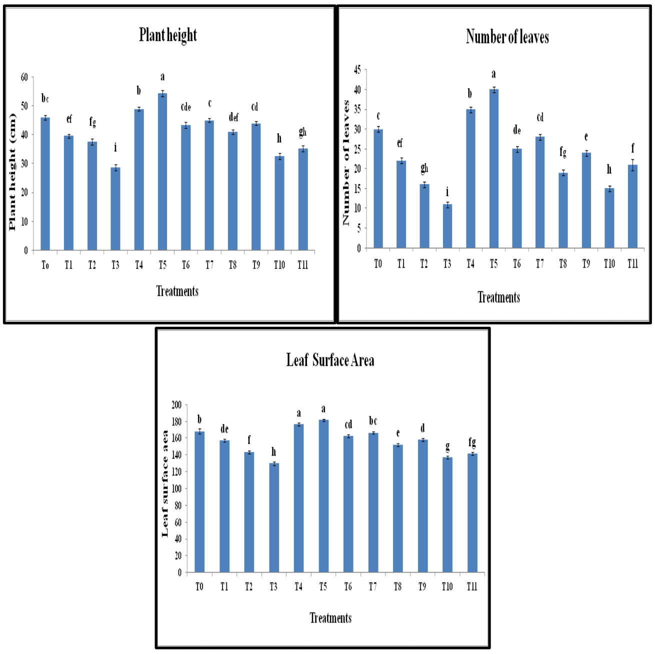


Figure 2

Effect of lead and humic acid on root, shoot fresh and dry weights of Sadabahar (Catharanthus roseus) under different treatments conducted by Two Way ANOVA, the significant difference between the treatments has been shown with different letters conducted through HSD Tukey's test at P>0.05.

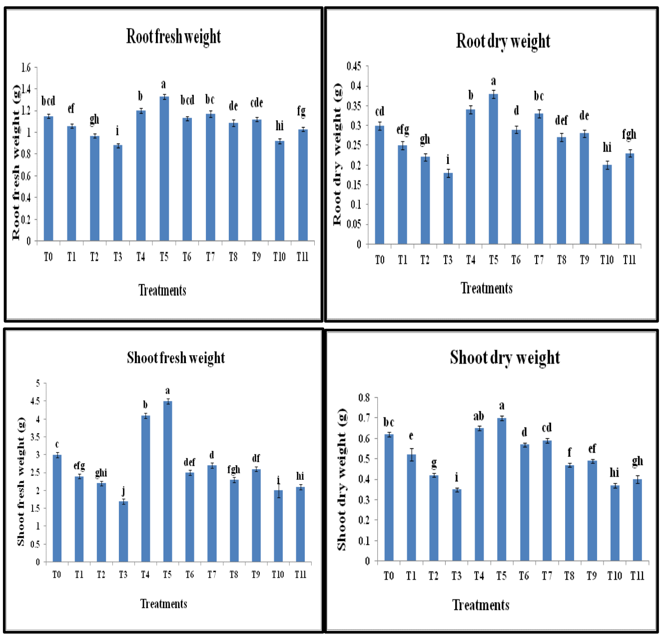


Figure 3

Effect of lead and humic acid on chlorophyll content, photosynthetic rate, superoxide dismutase, catalase, proline content of Sadabahar (Catharanthus roseus) under different treatments conducted by Two Way ANOVA, the significant difference between the treatments has been shown with different letters conducted through HSD Tukey's test at P>0.05.

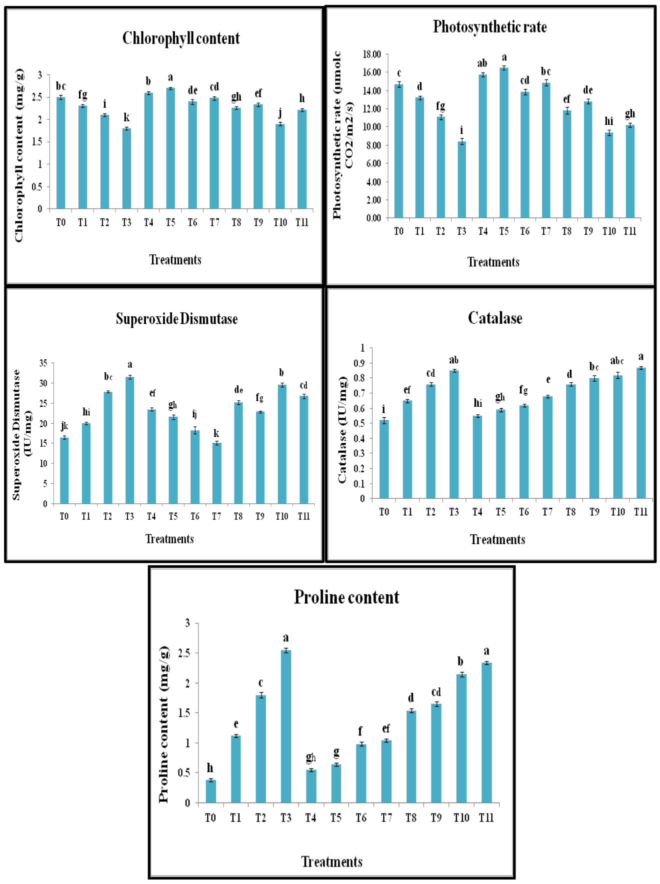


Figure 4

Effect of lead and humic acid on lead in root, shoot, leaves of plants and in soil, conducted by Two Way ANOVA, the significant difference between the treatments has been shown with different letters conducted through HSD Tukey's test at P>0.05.

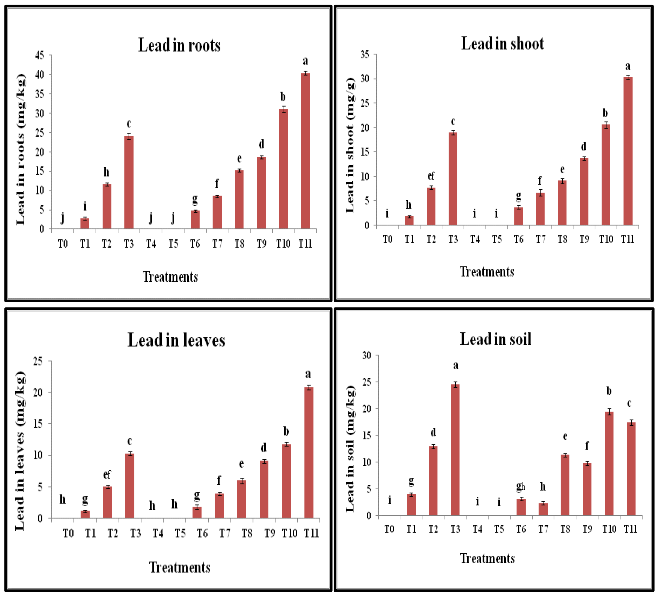
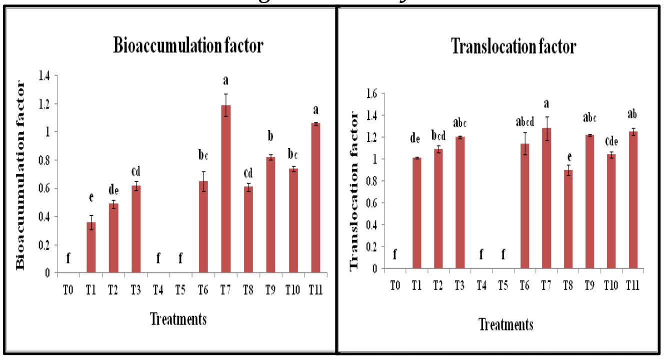


Figure 5

Translocation factor & bioconcentration factor showing the transportation of lead within parts of Sadabahar (Catharanthus roseus) under different treatments conducted by Two Way ANOVA, the significant difference between the treatments has been shown with different letters conducted through HSD Tukey's test at P>0.05.



DISCUSSION

The present study revealed that increasing concentrations of lead (Pb) significantly reduced the growth and physiological performance of Sada Bahar (*Catharanthus roseus*). Plant height, shoot length, and biomass were particularly affected under higher levels of lead exposure. However, the application of humic acid effectively alleviated these adverse effects, improving plant height and promoting overall growth under stress conditions. These findings are consistent with previous studies by Qurban et al. (2021) and Rashid et al. (2024), who also reported that heavy metal stress leads to a dose dependent decline in Sada Bahar *(Catharanthus roseus)* growth attributes, including height, leaf development, and biomass accumulation. While earlier studies mainly employed synthetic chelators such as EDTA to enhance metal uptake, the present research utilized humic acid as a natural and environmentally safe amendment. Unlike EDTA, which can increase metal solubility but also cause environmental risks, humic acid improved plant growth and mitigated lead induced toxicity. The treated plants exhibited enhanced root and shoot dry weights even under lead stress, suggesting that humic acid strengthened the plant defense mechanisms and improved tolerance to heavy metal toxicity. Similar protective effects were also observed by Khan et al. (2020) and Nabaei et al. (2019), who reported that treatments such as sodium nitroprusside (SNP) and melatonin improved growth and biomass production in cadmium stressed plants. Lead toxicity was also found to inhibit photosynthetic efficiency in *Catharanthus roseus*, with greater damage observed at higher lead concentrations. This agrees with findings by Subhashini and Swamy (2017), who reported that lead interferes with chlorophyll biosynthesis and stomatal regulation, resulting in reduced photosynthetic activity. Unlike their study, however, the present work demonstrated that the addition of humic acid mitigated these effects and sustained photosynthetic performance, highlighting its potential role as a natural biostimulant under metal stress.

The results further indicated that lead exposure activated the antioxidant defense system of Sada Bahar (*Catharanthus roseus)*, as shown by a marked increase in Catalase (CAT) activity compared to the untreated control. Interestingly, humic acid application alone caused a slight reduction in CAT activity, but when applied with lead, it stabilized the oxidative stress response, suggesting a modulatory effect. These results align with those of Nabaei et al. (2019), who observed that certain biostimulants could enhance the antioxidant capacity of plants under cadmium stress conditions.

Lead accumulation patterns in this study revealed that the roots of Sada Bahar served as the primary site of lead sequestration, with accumulation increasing proportionally with lead concentration. The addition of humic acid significantly enhanced lead uptake and promoted its translocation from roots to aerial tissues. These findings are supported by earlier studies by Pandey et al. (2007) and Barbosa et al. (2019), who reported similar metal retention in roots when Sada Bahar was grown in contaminated soil. However, unlike those studies where the plant functioned mainly as a Phyto stabilizer with limited metal transfer the present results demonstrated that humic acid effectively improved both the bioaccumulation factor (BAF > 1) and translocation factor (TF > 1). This indicates enhanced lead mobility from roots to shoots and leaves, highlighting the plant potential as a phytoextraction rather than merely a stabilizer.

Overall, these findings suggest that Sada Bahar (*Catharanthus roseus*), when supplemented with humic acid particularly at 4% is capable of tolerating and accumulating higher concentrations of lead while maintaining improved growth performance. These findings suggest their strong potential for phytoremediation while enhancing environmental aesthetics.

CONCLUSION

From the findings of this study, it can be concluded that lead stress negatively affects and alter the plant growth, morphological, biochemical and enzymatic activities, while humic acid can play a significant role in alleviating the plant biological functions under stressed conditions as in this study maximum concentrations of lead posed serious reduction in all parameters of plant while it was observed being mitigated at the highest concentration 4% of humic acid showing that it can enhance the potential of plants to withstand with the abiotic stress like lead and other heavy metals.

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