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Mitigating pb toxicity in *Sesbania sesban* L. through activated charcoal supplementation: a hydroponic study on enhanced phytoremediation

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Abstract

Background Soil contamination by heavy metals is a critical environmental challenge, with Pb being of particular concern due to its propensity to be readily absorbed and accumulated by plants, despite its lack of essential biological functions or beneficial roles in cellular metabolism. Within the scope of phytoremediation, the use of plants for the decontamination of various environmental matrices, the present study investigated the potential of activated charcoal (AC) to enhance the tolerance and mitigation capacity of *S. sesban* seedlings when exposed to Pb. The experiment was conducted as a factorial arrangement in a completely randomized design in hydroponic conditions. The *S. sesban* seedlings were subjected to a gradient of Pb concentrations (0, 0.02, 0.2, 2, and 10 mg/L) within the nutrient solution, alongside two distinct AC treatments (0 and 1% inclusion in the culture media). The study reached its conclusion after 60 days.

Results The seedlings exposed to Pb without AC supplementation indicated an escalation in peroxidase (POX) activity, reactive oxygen species (ROS), and malondialdehyde (MDA) levels, signaling an increase in oxidative stress. Conversely, the incorporation of AC into the treatment regime markedly bolstered the antioxidative defense system, as evidenced by the significant elevation in antioxidant capacity and a concomitant reduction in the biomarkers of oxidative stress (POX, ROS, and MDA).

Conclusions With AC application, a notable improvement was observed in the chlorophyll *a*, total chlorophyll, and plant fresh and dry biomass. These findings illuminate the role of activated charcoal as a viable adjunct in phytoremediation strategies aimed at ameliorating heavy metal stress in plants.

Keywords Heavy metal, Oxidative stress, Soil contamination

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Background

The anthropogenic accumulation of heavy metals within the Earth's crust, attributable to activities, such as the utilization of chemical fertilizers, pesticides, Pb-containing batteries, the combustion of fossil fuels including coal and gasoline, and the discharge of municipal wastewater, has precipitated significant environmental contamination [1]. Pb contamination in soil persists for 150 to 5000 years and is difficult to remediate, leading to long-term accumulation in soil and organisms [2]. The absorption of Pb by plants is associated with negative effects on nutrient uptake, photosynthesis, antioxidant enzymes, metabolic performance, and growth. Excessive lead accumulation can reduce root growth by 42% [3]. Pb toxicity also causes oxidative stress in the roots and shoots of plants by increasing malondialdehyde (MDA), H_2O_2 and electrolyte leakage (EL), which also increases the composition of various enzymatic and non-enzymatic antioxidants [4]. Although various heavy metal decontamination techniques exist, ranging from heat treatment and electro-remediation to soil substitution, precipitation, and chemical leaching, their application is often prohibitively expensive and impractical for agricultural settings. Phytoremediation, leveraging hyper-accumulator plant species capable of thriving in environments with elevated toxic heavy metal concentrations, presents a viable alternative [5; 6]. It is clear that a suitable and cost-effective way to remove lead from the environment is needed. Within this context, the genus Sesbania, part of the Fabaceae (Leguminosae) family, has been identified, with *S. sesban* specifically highlighted for its phytoextraction potential [7; 8]. Recent scholarly endeavors have also concentrated on the application of activated charcoal as a sorbent for heavy metal removal from the upper soil layers. Owing to its micro or macro porous structure, activated charcoal offers an expansive adsorptive surface area [9]. In scenarios of elevated root-zone heavy metal concentrations, the subsequent plant uptake and translocation to shoots can severely disrupt morphological, physiological, and biochemical functions. Activated charcoal, characterized by its porous architecture and significant surface-to-volume ratio, emerges as an efficacious medium for ion separation from the plant root environment [10]. The sorptive efficacy of activated charcoal, fundamentally linked to its surface properties such as area, pore size, and volume is contingent upon its production parameters [11; 12; 13; 14].

The significance of activated charcoal as a versatile medium for the adsorptive removal of heavy metals, such as copper, lead, and nickel from aqueous solutions cannot be overstated. In recent academic discourse, there has been a discernible pivot towards refining the surface characteristics of activated charcoal to enhance its capacity for heavy metal sequestration from contaminated soils [9]. Following the addition of biochar to the soil, the introduced high porosity, stable surface charges, and improved exchange sites play a key role in decreasing the heavy metal uptake in plants. Organic activated carbon also has the potential to ameliorate the toxic effects of heavy metals in plants and soil. High surface area, pore spaces and adsorption rates of activated carbon decrease the mobilization of Pb and its uptake into plants [15]. This multifunctionality positions activated carbon distinctly apart from biochar, which is more commonly associated with augmenting microbial activity and plant growth through mechanisms such as nutrient release, pH modification, and the emission of volatile organic compounds [16]. Furthermore, unlike substrates such as sugar or sawdust that provide resources for heterotrophic organisms, activated carbon is not expected to influence the dynamics of non-native plant populations by altering nutrient cycling rates within ecosystems [17]. Despite the acknowledged utility of activated carbon in environmental remediation, studies exploring its efficacy in mitigating heavy metal toxicity in agricultural soils, particularly in relation to plant physiology and biochemistry, remain limited. This gap in the literature indicates the necessity for comprehensive research to evaluate the potential of activated charcoal in environmental management practices, especially considering the increasing global burden of soil contamination by heavy metals due to industrial and agricultural activities.

Therefore, the objectives of this investigation are: (1) to assess the morphological and biochemical responses of *S. sesban*, a plant known for its phytoextraction capability, to different Pb concentrations; (2) to ascertain the tolerance thresholds of this species to elevated Pb levels, thereby evaluating its suitability for phytoremediation purposes; (3) to examine the positive role of activated charcoal in enhancing the resilience of *S. sesban* seedlings to Pb stress, potentially offering a dual approach to the amelioration of Pb-contaminated environments.

Results and discussion

Plant biomass production

The visual comparison illustrated in Fig. 1 provides a compelling representation of the effects of varying concentrations of Pb on the growth of *S. sesban* seedlings after 60 days, with and without the addition of AC. This figure indicates a clear contrast in the morphological development of the seedlings. On the left, the control and Pb-treated seedlings without AC display a gradient of stress responses, with the least stressed appearing robust and the most stressed exhibiting significant reduction in both root and shoot biomass as Pb concentration increases. In contrast, on the right, the seedlings treated with AC, even at high levels of Pb, maintain a healthier

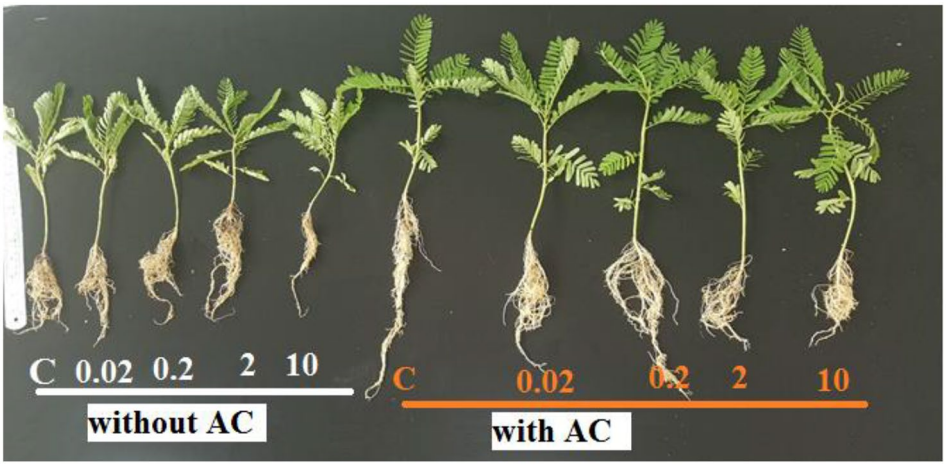

Fig. 1 Comparative root and shoot of control (left) and another treated of *S. sesban* 60 days after initial exposure to levels of Pb and activated charcoal respectively

Table 1 Mean comparison of the effect of concentrations of pb without/with 1% activated charcoal treatments on growth parameters including, shoot fresh weight (FW), root FW, shoot dry weight (DW) and root DW of *S. sesban* after 60 days. Treatment means with same letters do not differ significantly according to LSD test ($p<0.05$). Values are the mean of three replications ± StD of 3 replications

Activated charcoal (AC)	Pb (mg/L)	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)	Total Plant biomass (g)
Control	Control	11.58 ± 0.92ab	8.07 ± 0.46bc	1.50 ± 0.15d	0.78 ± 0.05d	19.65 ± 0.66b
	0.02	9.41 ± 0.72 cd	7.79 ± 0.66bc	1.53 ± 0.15d	0.70 ± 0.04de	17.2 ± 1.54bc
	0.2	8.53 ± 1.17d	6.52 ± 0.45 cd	1.66 ± 0.08d	0.68 ± 0.08de	15.05 ± 1.57 cd
	2	6.11 ± 0.77e	6.16 ± 0.28d	1.50 ± 0.13d	0.82 ± 0.06d	12.27 ± 0.99de
	10	5.35 ± 0.84e	4.07 ± 0.60e	1.36 ± 0.13d	0.58 ± 0.05e	9.42 ± 1.02e
1%	Control	11.04 ± 0.95bc	8.74 ± 0.45b	2.30 ± 0.24c	1.24 ± 0.12c	19.78 ± 1.1b
	0.02	11.03 ± 1.11bc	8.90 ± 0.60b	2.28 ± 0.08c	1.27 ± 0.03c	19.93 ± 0.63b
	0.2	13.09 ± 1.09a	12.63 ± 1.43a	3.15 ± 0.36a	1.77 ± 0.08a	25.72 ± 1.86a
	2	12.59 ± 1.06ab	11.32 ± 1.95a	3.11 ± 0.17ab	1.56 ± 0.07b	23.91 ± 0.97a
	10	12.49 ± 0.71ab	9.05 ± 0.64b	2.80 ± 0.19b	1.22 ± 0.13c	21.54 ± 1.31a

appearance, suggesting that AC has a protective effect against the phytotoxicity induced by Pb.

In the current investigation, the impact of varying concentrations of Pb on the growth parameters of *S. sesban* was quantified, providing an understanding of the interaction between heavy metal stress and plant development. The results delineated in Table 1 reveal a significant decline in root fresh weight (FW) and total plant biomass at Pb concentrations exceeding 0.02 mg/L in the absence of AC, with reductions of 19.2, 23.6, and 49.6% in root fresh weight and with reductions of 22, 38 and 50% in total plant biomass at 0.2, 2, and 10 mg/L of Pb, respectively. These findings are in agreement with the documented inhibitory effects of Pb on seed germination, seedling growth, and biomass accumulation as reported by McComb et al. [8] and Mishra et al. [18], further corroborating the notion that Pb toxicity adversely affects plant vigor. Conversely, the application of AC appears to mitigate these negative outcomes, as evidenced by the increased total plant biomass at Pb concentrations, underscoring AC's potential role in ameliorating Pb-induced phytotoxic effects. This phenomenon is consistent with previous observations of increased plant biomass and growth tolerance indices in the presence of soil amendments capable of sequestering heavy metals [19; 20]. The resultant alterations in root architecture and growth patterns are indicative of a stress response, wherein callose synthesis and vacuolar sequestration serve as immediate defense mechanisms. The mechanism of AC's influence on plant growth is multifaceted. While AC is known to bind organic molecules, thereby potentially reducing the bioavailability of toxicants like Pb, it also indiscriminately binds to signaling molecules, which may disrupt plant-microbe interactions essential for plant health [21; 22; 23].

Photosynthetic pigments

The photosynthetic pigments serve as critical indicators of plant health and are directly implicated in the plant's response to environmental stressors, such as heavy metal

toxicity. In this study, a discernible decrease in chlorophyll *a* content was observed at the highest concentration of Pb (10 mg/L), showing a substantial reduction by 46% when compared to the control group (Table 2). This deleterious effect of Pb on chlorophyll *a* content is consistent with the findings of Malar et al. [19], who reported a gradual decline in chlorophyll content with increasing Pb concentrations. The decrement in chlorophyll levels under heavy metal stress is often associated with the peroxidation of chloroplast membranes because of elevated ROS levels, which can disrupt the chlorophyll biosynthesis pathway by impairing the uptake of essential elements like magnesium and iron, integral to the chlorophyll molecule [21; 24]. Conversely, the application of AC was found to significantly increase chlorophyll *a* content across all tested Pb concentrations compared to Pb treatment only, suggesting a protective and restorative effect of AC on the photosynthetic apparatus. The mechanism behind this increase may be attributed to the adsorptive properties of AC, which potentially reduces the bioavailability of Pb, thus alleviating its inhibitory effects on the photosynthetic machinery. The AC appears to foster a more favorable environment for the maintenance or even enhancement of chlorophyll *a* synthesis, as evidenced by the increase in chlorophyll *a* content in the presence of AC compared to Pb treatment only. Chlorophyll *b* content exhibited a decrease at concentrations of 0.2, 2, and 10 mg/L Pb, yet the presence of AC resulted in a stabilization of chlorophyll *b* levels, indicating the potential of AC in mitigating the stress-induced alterations in chlorophyll *b* synthesis. The total chlorophyll content followed a similar trend to that of chlorophyll *a*, with the most significant reduction observed at the highest Pb concentration. The addition of AC, however, led to an overall increase in total chlorophyll content compared to lead treatment only, reinforcing the assumption that AC might be exerting a protective effect by modulating the availability of Pb or by influencing the expression of genes responsible for chlorophyll synthesis. The ratio of chlorophyll *b* to chlorophyll *a* decreased under medium Pb treatments (0.2 and 2 mg/L), which might indicate a selective vulnerability of chlorophyll *b* or a differential regulation of the biosynthesis of these pigments under metal stress. However, the presence of AC with Pb treatments appeared to attenuate the reduction in this ratio, suggesting a complex interplay between AC and the biosynthesis or degradation of chlorophyll molecules under heavy metal stress. In the absence of AC, an increase in protein content was observed at all Pb concentrations, except the lowest (0.02 mg/L). According to Gopal and Rizvi [25] as well as Malar et al. [19], heavy metal stress can impair physiological processes including photosynthesis, respiration and membrane integrity. Plants respond to heavy metal stress by activating various resistance mechanisms, including the enhanced production of both nonenzymatic and enzymatic antioxidants [26]. This increase in protein content could be attributed to the synthesis of antioxidant enzymes and other stress proteins in response to Pb exposure. This protein accumulation likely reflects the plant's attempt to mitigate the toxic effects of Pb on its metabolism. Also, the presence of AC with Pb treatments led to an increase in protein content, indicating a possible ameliorative effect of AC on the protein synthesis machinery or a reduction in protein degradation rates, which could be a response to a lessened oxidative stress environment.

Oxidant and antioxidant system parameters

In this study, we evaluated various biochemical, photosynthetic and growth parameters under activated charcoal (AC)-treatment in Pb-contaminated substrates in *S. sesban.* We presented the oxidant and antioxidant system parameters of *S. sesban* in Figs. 2, 3 and 4. The manifestation of oxidative stress under conditions of heavy metal stress, as evidenced by the imbalanced generation and scavenging of reactive oxygen species (ROS)

Table 2 Comparison of different concentrations of pb without/with 1% activated charcoal treatments on photosynthetic pigments including, Chlorophyll (Chl.) *A*, *b*, total and Chl. *b/a* ratio and soluble protein of *S. sesban* after 60 days. Treatment means with same letters do not differ significantly according to LSD test ($p<0.05$). Values are the mean of three replications ± StD of 3 replications

Activated charcoal	Pb (mg/L)	Chl. a (mg/g FW)	Chl. b (mg/g FW)	Chl. T (mg/g FW)	Ratio Chl. b/a	Soluble protein (mg/g FW)
Control	Control	1.74 ± 0.12b	0.87 ± 0.06ab	2.61 ± 0.18b	0.50 ± 0.00a	1.15 ± 0.039f
	0.02	1.67 ± 0.24b	0.79 ± 0.05b	2.46 ± 0.28b	0.48 ± 0.04a	1.14 ± 0.030f
	0.2	1.68 ± 0.25b	0.56 ± 0.05c	2.24 ± 0.30b	0.34 ± 0.02b	1.23 ± 0.040e
	2	1.71 ± 0.02b	0.58 ± 0.06c	2.29 ± 0.07b	0.34 ± 0.03b	1.28 ± 0.016de
	10	0.94 ± 0.08c	0.48 ± 0.08c	1.42 ± 0.15c	0.51 ± 0.05a	1.31 ± 0.055bcd
1%	Control	2.66 ± 0.18a	0.93 ± 0.03a	3.59 ± 0.20a	0.35 ± 0.01b	1.33 ± 0.030bcd
	0.02	2.75 ± 0.28a	0.86 ± 0.05ab	3.61 ± 0.31a	0.31 ± 0.02b	1.37 ± 0.063abc
	0.2	2.97 ± 0.17a	0.88 ± 0.13ab	3.85 ± 0.28a	0.29 ± 0.04b	1.30 ± 0.030 cd
	2	2.74 ± 0.29a	0.82 ± 0.04ab	3.56 ± 0.32a	0.30 ± 0.02b	1.40 ± 0.018a
	10	2.74 ± 0.20a	0.83 ± 0.03ab	3.56 ± 0.23a	0.30 ± 0.01b	1.38 ± 0.043ab

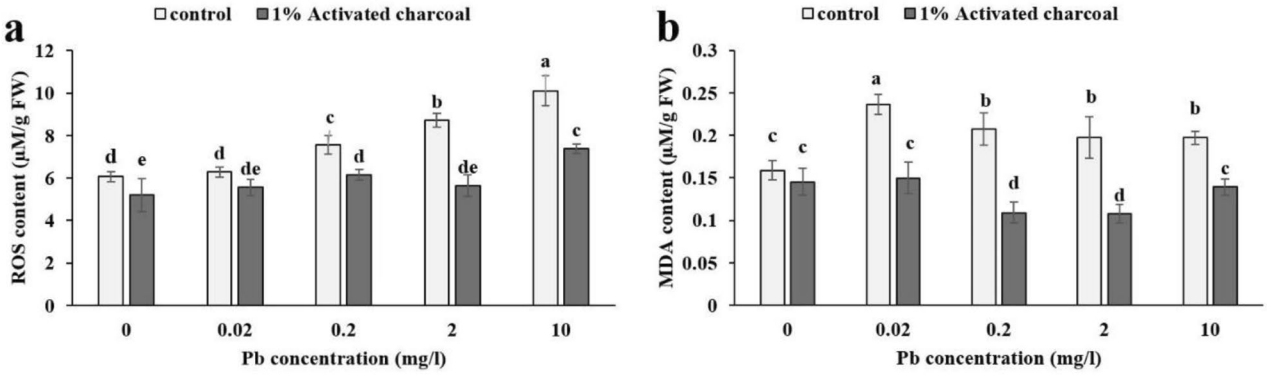

Fig. 2 Mean comparison of the effect of concentrations of Pb, without/with 1% activated charcoal treatments on ROS content (**a**) and MDA content (**b**) of *S. sesban* after 60 days. Treatment means with same letters do not differ significantly according to LSD test ($p<0.05$). Values are the mean of three replications ± StD of 3 replications

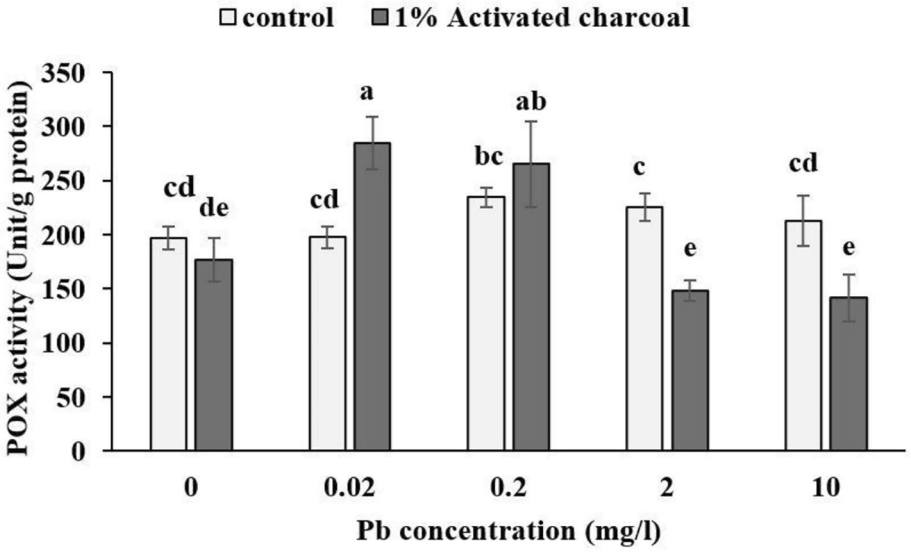

Fig. 3 Mean comparison of the effect of concentrations of Pb without/with 1% activated charcoal treatments on POX enzyme activity of *S. sesban* after 60 days. Treatment means with same letters do not differ significantly according to LSD test ($p<0.05$). Values are the mean of three replications ± StD of 3 replications

[27] that was evaluated in the leaves of *S. sesban* grown under the application of AC as well as Pb-contaminated, are presented in Fig. 2a. In the absence of AC, the results revealed a significant elevation in ROS levels by 25, 30, and 31% in *S. sesban* seedlings exposed to 0.2, 2, and 10 mg/L concentrations of Pb, when compared to the control group, respectively (Fig. 2a). Similarly, malondialdehyde (MDA) content, a marker of lipid peroxidation and thus cell membrane integrity damage, increased significantly by 44, 31, 25, and 25% across the 0.02, 0.2, 2, and 10 mg/L Pb concentrations, respectively (Fig. 2b). Conversely, the application of activated charcoal (AC) significantly reduced the levels of ROS and MDA compared to the control. For example under 0, 0.02, 0.2, 2, and 10 mg/L of Pb conditions, the application of AC decreased ROS levels by 14, 7, 22, 29, and 7% respectively, compared to the control. Additionally, it decreased MDA levels by 13, 35, 48, 45, and 30% in comparison to the control, respectively (Fig. 2b).

These findings indicate the role of AC in enhancing the oxidative stress defense mechanism of *S. sesban* seedlings, likely through the adsorption of Pb and reduction of its bioavailability to the plant tissues. The observed increase in ROS and MDA in response to Pb exposure corroborates the established understanding that heavy metals disrupt cellular homeostasis by promoting excessive ROS production, leading to oxidative stress [28; 29]. The elevation in oxidative stress markers in the absence of AC indicates the toxicity impact of Pb on plant physiological processes, in agreement with findings from Iqbal

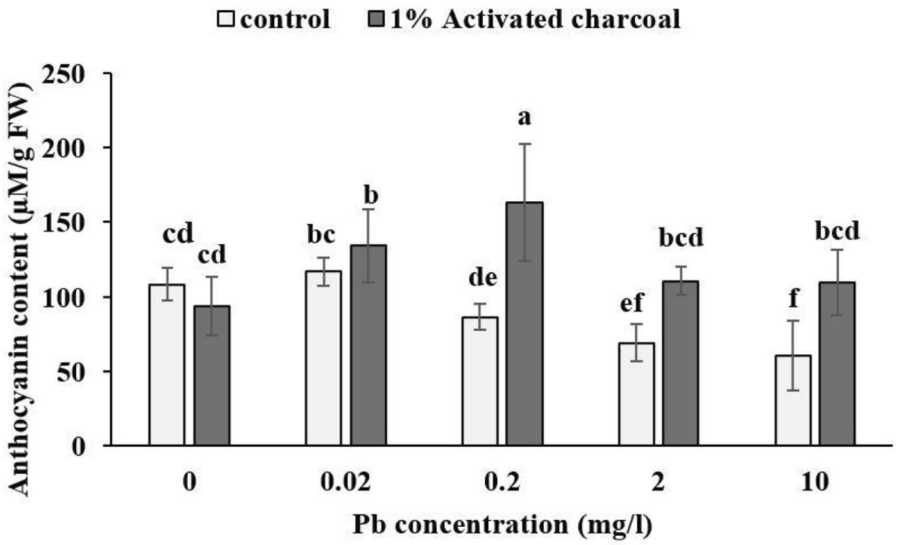

Fig. 4 The mean comparison of the effects of concentrations of Pb without/with 1% activated charcoal treatments on anthocyanin content of *S. sesban* after 60 days. Treatment means with same letters do not differ significantly according to LSD test ($p^{*}0.05$). Values are the mean of three replications ± StD of 3 replications

et al. [30], who reported a positive relationship between lead treatment and ROS amount in spinach. Moreover, Methela et al. [24] highlighted the detrimental impacts of Pb on plant growth and development, notably through impairment of cell membrane integrity, disruption of photosynthetic systems, and the induction of excessive ROS production. The effect of AC on ROS and MDA levels suggests its potential role in sequestering Pb, thereby limiting its phytoavailability and the consequent oxidative stress. This assumption is supported by the properties of AC, which reduce the concentration of bioavailable heavy metals within the rhizosphere. This mechanism likely contributed to the observed reduction in oxidative stress markers. The efficacy of AC in this context aligns with its known capacity for adsorbing pollutants, thereby providing a physical barrier that impedes the entry of toxic metals into the plant system and facilitates the maintenance of redox homeostasis. In contaminated soils, AC can be used as an organic amendment to immobilize heavy metals, reduce their bioavailability, and improve soil quality [31; 32].

In the context of phytoremediation, plants have evolved an array of defense mechanisms to contend with the deleterious effects of heavy metal toxicity. These mechanisms encompass both enzymatic and non-enzymatic antioxidants. These antioxidants collectively mitigate oxidative damage by scavenging reactive oxygen species (ROS), thus conferring protection against the oxidative stress imposed by heavy metal exposure [26; 28; 29]. The Fig. 3 reveals the influence of Pb concentrations, both without and with 1% activated charcoal (AC) treatment, on POX enzyme activity after 60 days. Notably, in plants treated with 0.02 and 0.2 mg/L Pb with AC, a substantial increase in POX activity was observed, by 44 and 35% in comparison to the untreated control, respectively.

This suggests that the presence of AC may amplify the plant's enzymatic response, potentially by modulating the bioavailability of Pb, thereby reducing its cytotoxic effects and enhancing the plant's inherent detoxification pathways. The augmentation of POX activity in the presence of AC can be interpreted as a stress response, facilitating the scavenging of H_2O_2, thus preventing the propagation of free radicals and protecting cellular components from oxidative damage. This enhanced enzymatic activity in the presence of AC might also be attributable to a direct effect of AC on the enzyme's stability or on the expression of genes encoding antioxidant enzymes. Our findings from this investigation align with previous research, which has demonstrated that the application of AC in contaminated media can reduce Pb absorption and induce plant antioxidant systems [26]. Furthermore, these results corroborate the hypothesis that AC can act as a modulator of metal stress, enhancing the plant's resilience to heavy metal exposure. While, the significant decrease in POX activity in AC-amended treatments under 2 and 10 mg/L Pb (high levels of Pb+AC), as revealed by the statistical analysis (Fig. 3), indicates the potential of AC as a valuable amendment in the phytoremediation of Pb-contaminated environments. The content of ROS and MDA in these treatments compared to Pb treatments alone was the only result of the induction of the antioxidant system. Therefore, the reduction of POX may be due to the increase of other mechanisms of resistance in plants to heavy metals. The different

responses of different Pb levels in the presence of AC can be related to the difference in Pb uptake mechanisms in *S. sesban*.

Anthocyanins, a class of flavonoid compounds, play a pivotal role in plant responses to environmental stressors, including heavy metal toxicity. These secondary metabolites are well documented for their antioxidative capabilities, providing a cellular defense mechanism against oxidative stress. The graphical data presented in Fig. 4 indicate a differential impact of Pb concentrations on anthocyanin content in *S. sesban* after 60 days, both in the absence and presence of 1% activated charcoal (AC). Without AC, a decrement in anthocyanin content by 36 and 44% was observed at Pb concentrations of 2 and 10 mg/L, respectively. On the other hand, in the presence of AC, there was an increment by 24 and 51% at these respective concentrations compared to the control. This dichotomy in plant response elucidates the potential modulatory effect of AC on anthocyanin biosynthesis under heavy metal stress. Anthocyanins are a class of antioxidant stress compounds whose content was affected by Pb and AC treatments. Notably, AC treatment led to a greater increase in anthocyanin content in *S. sesban* plants, resulting more pronounced at 0.02 and 0.2 mg/L Pb concentrations, accompanied by an elevation in POX activity. Therefore, the high anthocyanin content as well as the enhancement in POX activity observed in plants exposed to both Pb and AC, may play a protective role against Pb-induced cellular damage, potentially due to the enhancing effects of AC. The enhancement of anthocyanin content in the presence of AC may be attributed to the adsorbent's capacity to influence the expression of genes involved in the anthocyanin biosynthetic pathway, or it could be the result of indirect changes in the modified physicochemical and biological properties of the soil imparted by AC, which can alter Pb to plant nutrient uptake and stress signaling pathways. Studies such as those by Cao et al. [33], Mariana et al. [34], and Rahi et al. [31] corroborate the role of AC in augmenting plant antioxidant defense systems, suggesting that the presence of AC could either directly enhance gene expression related to antioxidant pathways or modulate the activity of oxidative processes, thus influencing the synthesis of anthocyanins. The observed variations in anthocyanin levels under different Pb concentrations, with and without the addition of AC, may also reflect the differential absorption mechanisms that plants employ under varying degrees of heavy metal stress. AC's ability to modify soil properties might result in a more conducive environment for root growth and function, thereby enhancing the plant's resilience to Pb stress. Furthermore, the physicochemical changes induced by AC in the soil matrix could lead to reduced availability of lead for plant uptake, thereby lessening the intensity of stress experienced by the plant and allowing for a more robust synthesis of protective anthocyanins. The implications of our findings are twofold. Firstly, they provide empirical evidence supporting the beneficial application of AC in contaminated soils to bolster plant defense mechanisms against heavy metal toxicity. Secondly, they contribute to the broader understanding of the interaction between soil amendments and plant physiological processes, specifically regarding the synthesis of critical antioxidative compounds such as anthocyanins. The positive influence of AC on substrates efficacy, as suggested by Rahi et al. [31], indicates the potential for AC to serve as a soil conditioner, enhancing the overall health and stress tolerance of plants in contaminated environments.

The correlation matrix detailed in Table 3 indicates a comprehensive view of the interrelationships among various physiological and growth parameters of *S. sesban* seedlings, cultivated in hydroponic media subjected to different concentrations of Pb with and without the application of AC. The statistical significance of these correlations shaded light on the multifaceted interactions between plant stress responses and growth under heavy metal stress, moderated by the application of AC. Analyzing the data, a robust inverse relationship was evident between ROS and the chlorophyll content (both Chl. *a* and total chlorophyll), reinforcing the hypothesis that elevated ROS levels, indicative of oxidative stress, are detrimental to chlorophyll synthesis and thus photosynthetic capacity. This observation is in line with previous findings which indicated that oxidative stress precipitated by heavy metal exposure compromises chloroplast integrity, leading to reduced chlorophyll levels and impaired growth [19]. MDA levels, serving as a proxy for lipid peroxidation, also inversely correlated with chlorophyll levels, further substantiating the negative impact of oxidative stress on chlorophyll content. Conversely, anthocyanin levels exhibited a negative correlation with ROS and MDA, suggesting a protective, antioxidative role for anthocyanins in mitigating oxidative stress, a relationship that has been observed in other studies as well [35]. Interestingly, the correlation between chlorophyll *b* (Chl. *b*) and ROS was notably stronger than that of chlorophyll *a* (Chl. *a*), suggesting a greater susceptibility of Chl. *b* to oxidative damage. The imbalance in the Chl. *b*/*a* ratio under stress may reflect an adaptive response to maintain photosynthetic efficiency under suboptimal conditions [36]. From a growth perspective, the strong negative correlations of both ROS and MDA with shoot and root biomass parameters (FW and DW) corroborate the adverse effects of Pb-induced oxidative stress on plant development. However, the presence of AC appears to confer a protective effect, as indicated by the positive correlations between chlorophyll content and biomass in AC-amended treatments (Table 3), aligning with studies

Table 3 Correlation matrices showing relationships between measured parameters of *S. sesban* seedlings grown in hydroponic media after 60 days in response to different concentrations of pb without/with 1% activated charcoal treatments. ROS: reactive oxygen species; MDA: malondialdehyde; POX: peroxidases; Antho: anthocyanin; Chl. *A*: chlorophyll *a*; Chl. *B*: chlorophyll *b*; total chlorophyll: Chl. T; fresh weight: FW; dry weight: DW; carotenoids: Car; shoot fresh weight (FW); root FW, shoot dry weight (DW) and root DW.

	ROS	MDA	POX	Antho	Chl. a	Chl. b	Chl. T	Chl. b/a	Protein	Shoot FW	Root FW	Shoot DW
MDA	0.487**											
POX	0.052	0.144										
Antho	-0.633**	-0.511**	0.281									
Chl.*a*	-0.674**	-0.749**	-0.074	0.666**								
Chl.*b*	-0.841**	-0.534**	-0.199	0.644**	0.764**							
Chl. T	-0.733**	-0.736**	-0.102	0.687**	0.992**	0.840**						
Chl.*b/a*	0.283	0.555**	-0.089	-0.368*	-0.772**	-0.221	-0.693**					
Protein	-0.073	-0.635**	-0.151	0.114	0.527**	0.098	0.463**	-0.594**				
Shoot FW	-0.788**	-0.713**	-0.224	0.730**	0.790**	0.822**	0.826**	-0.402*	0.244			
Root FW	-0.720**	-0.700**	-0.08	0.774**	0.846**	0.758**	0.861**	-0.534**	0.316	0.844**		
Shoot DW	-0.494**	-0.816**	-0.19	0.607**	0.878**	0.582**	0.854**	-0.707**	0.630**	0.768**	0.863**	
Root DW	-0.590**	-0.862**	0.015	0.683**	0.893**	0.631**	0.875**	-0.694**	0.601**	0.738**	0.883**	0.935**

Notes: **and * Correlation is significant at the 0.01 and 0.05 level

that highlight the ameliorative influence of AC on plant growth in heavy metal-stressed environments [37].

The summary of the effect of Pb without/with 1% activated charcoal treatments on *S. sesban* plants is summarized in Fig. 5. The presence of Pb was found to exacerbate the production of ROS and MDA, indicative of oxidative stress, which in turn compromised the integrity of chloroplast membranes and reduced chlorophyll content (Table 2) and a consequent reduction in plant biomass (Table 1), as highlighted by the negative correlations among oxidative stresses markers and both shoot and root weights (Table 3). Concurrently, our findings revealed that the application of AC markedly reduced the adverse effects of Pb. The treatment with AC (1%) was shown to enhance the chlorophyll *a* and *b* content (Table 2) and plant biomass (Table 1), in compared to not using AC (control). This beneficial effect is attributed to the adsorptive characteristics of AC, which likely limited the bioavailability of Pb and alleviated the associated oxidative stress, thus enabling healthier growth patterns and improved biomass production in the seedlings. The study also brought to the fore the protective role of anthocyanins, whose levels inversely correlated with those of ROS and MDA, underscoring their importance as antioxidants in the plant's defense arsenal against heavy metal toxicity. The ability of AC to increase the plant's resilience is evidenced by the seedlings' improved enzymatic responses and growth metrics, suggesting its utility as a valuable soil amendment.

Conclusion

This research showed that the elevated oxidative stresses were the major cause of phyto-toxicity under Pb treatments in *S. sesban*. Major oxidative stress biomarkers, such as decreased chlorophyll content, ROS generation and high lipid peroxidation (MDA), were highlighted under 0.2 mg/L Pb treatments in *S. sesban* plants. Plants have responded to Pb stress by growth decrease. The shoots and roots of the plant showed symptoms of toxicity as evidenced by a decrease in biomass with increasing Pb concentration. The AC significantly inhibited the stress in plants exposed to high levels of Pb. These results collectively recommended the use of AC in the remediation of soils contaminated with heavy metals, providing dual benefits of enhancing plant growth while safeguarding environmental health. The study thus contributes to the growing body of evidence that supports the application of AC as a soil conditioner, with implications for sustainable agriculture and the preservation of ecological integrity. However, extended dose-dependent investigations are required to define proper AC doses that induce positive effects on plants' growth under different environmental conditions.

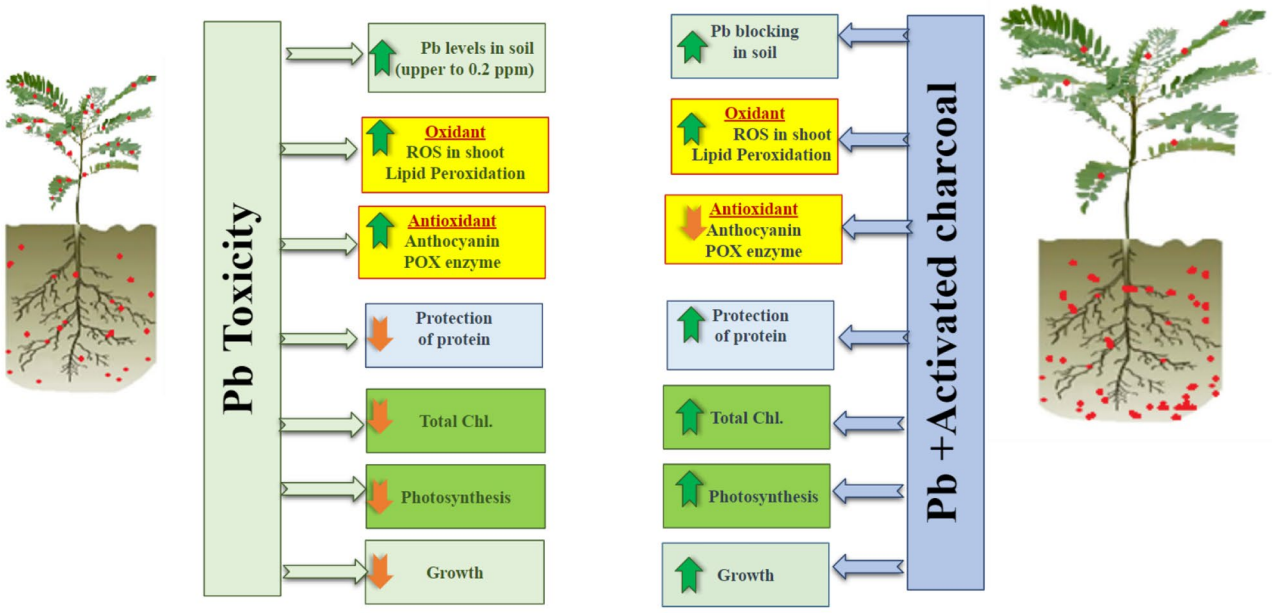

Fig. 5 The summary of the effect of Pb without/with 1% activated charcoal treatments growth and physiology in *S. sesban* after 60 days

Materials and methods

This study was conducted in the research greenhouse at the University of Jiroft in 2020. *S. sesban* seeds were soaked overnight and subsequently sown in seedling trays filled with a peat moss and perlite mixture in a 3:1 ratio. Upon reaching the 5-leaf stage, the seedlings were transplanted into three-liter pots comprised of sand and perlite. Each pot housed four seedlings, which were irrigated with a half-strength Hoagland's nutrient solution for two weeks. This research was conducted as a factorial experiment (2×2) in the form of a completely randomized design with three replicates for biochemical parameters and three pots (three replicates) for growth parameters where each replicate was taken from the average of 3 plants. Factors included 5 levels of lead and 2 levels of activated carbon (AC). *S. sesban* seedlings received nutrient solutions containing different concentrations of lead (Pb), (0, 0.02, 0.2, 2, and 10 mg/L), alongside two levels of activated charcoal in the culture media (0 and 1% w/w), with each treatment replicated three times. The experiment concluded after 60 days. Plant height was measured using a ruler, and the count of buds, flowers, pods, and leaves was recorded for each plant. The fifth leaf from each plant was detached, immediately frozen in liquid nitrogen, and subsequently stored in an ultra-low temp freezer (Jal Tajhiz production) at -80 °C for the assessment of physiological and biochemical characteristics. Following the conclusion of the experiment, plants were carefully removed from the pots and the roots were thoroughly washed with distilled water to eliminate all remaining growth medium.

Measurement of total ROS

Reactive Oxygen Species (ROS) content was measured following the methodology described by Bindschedler et al. [38]. Briefly, 0.01 g of leaf tissue was thoroughly homogenized in 1 mL of ice-cold phosphate buffer (50 mM, pH 7.2). The resulting homogenate was then centrifuged at 10,000 × g for 20 min at a temperature of 4 °C. Subsequently, 100 µL of the supernatant was promptly mixed with 900 µL of xylenol orange reagent. This mixture was incubated at room temperature for a duration ranging from 30 to 60 min. The absorbance of the solution was measured at a wavelength of 560 nm using a spectrophotometer (Rayleigh, UV-1601). For the blank control, 100 µL of distilled water was combined with 900 µL of xylenol orange reagent. Hydrogen peroxide (H_2O_2) at a concentration of 30% served as the standard. The ROS content was quantified and expressed in µmol per gram of fresh weight (µmol g^{-1} FW). The H_2O_2 content was assessed according to the protocol established by Tirani and Haghjou [39], complementing the ROS measurement to provide a comprehensive evaluation of oxidative stress within the plant tissue.

Determination of lipid peroxidation

The assessment of lipid peroxidation in plant leaves was conducted by measuring the malondialdehyde (MDA) content according to Tirani and Haghjou [39]. Initially, 100 mg of leaf tissue was homogenized in 2 mL of 0.1% trichloroacetic acid (TCA). This mixture was then centrifuged at 10,000 × g for 20 min. Subsequently, 2 mL of 20% TCA, which contained 0.5% thiobarbituric acid (TBA), was added to 500 µL of the supernatant derived

from the centrifugation process. The resultant mixture was heated at 95 °C for 15 min and then promptly cooled in an ice bath. The absorbance of the cooled mixture was measured at 532 nm using a spectrophotometer. To correct for nonspecific absorption, the absorbance value at 600 nm was subtracted from the initial measurement. The extent of lipid peroxidation was quantified and expressed as micromoles of MDA formed, utilizing an extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of antioxidants

The evaluation of anthocyanin content was conducted utilizing acidified methanol, as outlined by Wagner [40]. The activity of peroxidase (POX) in *S. sesban* leaf tissues was determined following the methodology described by Tirani et al. [41].

Determination of total protein content

The Bradford [42] assay was employed to determine total protein content, utilizing Coomassie Brilliant Blue G-250 as the protein-binding dye. Bovine serum albumin was used as the standard. All spectrophotometric analyses were conducted using a Rayleigh (UV-1601) spectrophotometer.

Determination of photosynthetic pigments

The estimation of photosynthetic pigments was carried out in accordance with the protocols described by Lichtenthaler and Buschmann [43]. This involved measuring the absorbance of chlorophyll extracts at specific wavelengths (665.2 nm for Chl. *a*, and 652.4 nm for Chl. *b*) to assess the total chlorophyll content as well as the concentrations of chlorophyll *a* and *b* separately.

Statistical analyses

Statistical analyses of the collected data were performed using the Statistical Analysis System (SAS Version 9.4). The data underwent a two-way Analysis of Variance (ANOVA) with three replications to discern significant differences among treatments. Post hoc comparisons were facilitated by Tukey's test, with a significance level set at $p \leq 0.05$, to accurately identify variations in response among the different experimental groups.

Author contributions

M.M.T. and B.P.M. were responsible for conceiving and designing the experiments, laying the groundwork for the study. M.M.T. A.S. and M.A. took on the hands-on task of performing the experiments, ensuring their execution according to the design. The data analysis was carried out by A.S., M. A., and M.M.T., who delved into the results and extracted meaningful insights. The writing and proofreading of the final paper were undertaken by A.S. and M.M.T., ensuring the manuscript was well-articulated and error-free. All authors reviewed the completed manuscript and gave their consent for its publication, signifying their agreement with the content and findings reported in the study. All authors reviewed the manuscript.

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Data availability

Correspondence and requests for materials should be addressed to A.S.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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