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Determination of the effect of lead toxicity on the morphological and biochemical changes of *Arachis hypogaea L.*

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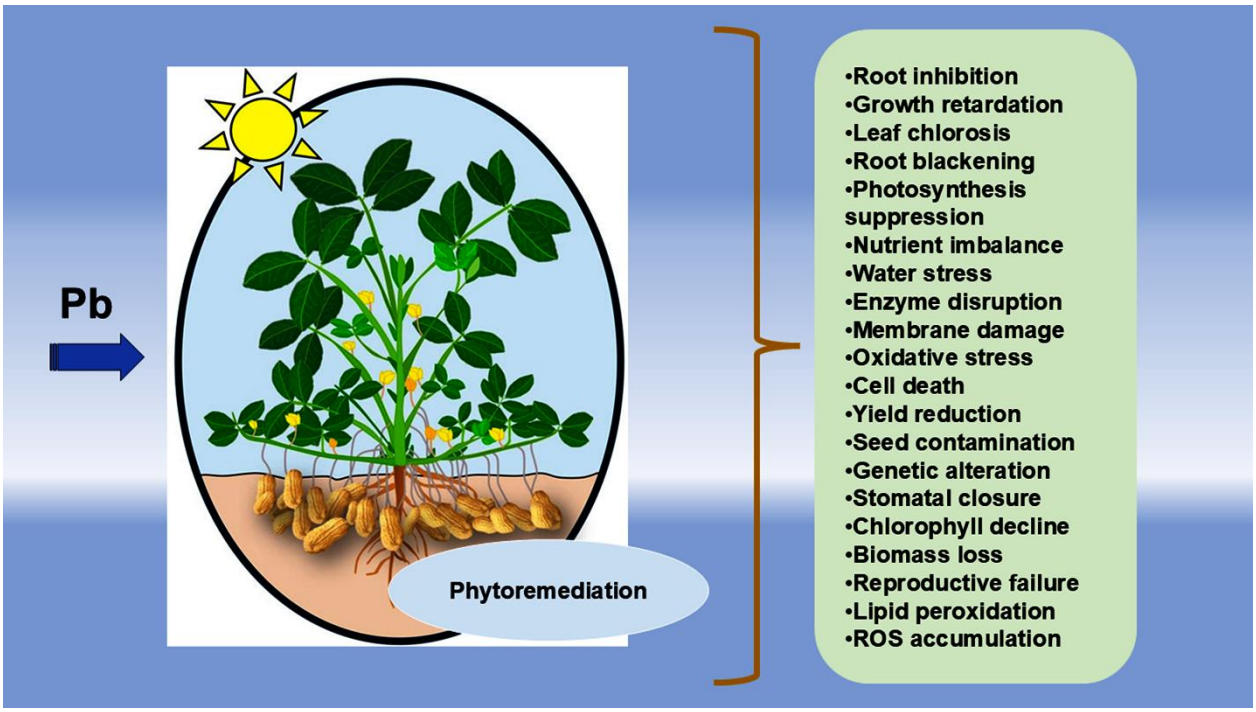
***Abstract*:** This study investigates the impact of lead (Pb) toxicity on the morphological and biochemical characteristics of Arachis hypogaea L. (Peanut). Peanut seedlings were exposed to varying concentrations of 0, 100, 200, 400, and 800 ppm of lead nitrate solution ($Pb(NO_3)_2$). Under controlled, soilless conditions. The results demonstrated a dose-dependent accumulation of Pb in plant tissues, following the order: root > stem > leaf. Elevated Pb levels led to significant reductions in root and stem lengths, as well as decreased fresh weights of roots, stems, and leaves. Biochemically, there was a notable decline in phenolic compounds and photosynthetic pigments, alongside increased oxidative stress. Protein content diminished across all plant parts, while proline levels decreased in roots and stems but increased in leaves at higher Pb concentrations, suggesting a potential protective response. These findings highlight the detrimental effects of Pb toxicity on peanut plant growth and physiology, emphasizing the need for strategies to mitigate heavy metal contamination in agricultural environments.

Keywords: Heavy metals, lead, total protein content, chlorophyll, carotenoids



Introduction

Heavy metal contamination has emerged as a critical environmental concern due to its widespread occurrence and long-term persistence in ecosystems. Heavy metals accumulate in diverse environmental mediums, causing bioaccumulation in food chains [1-4]. Long-term discharge of industrial effluents has led to the accumulation of heavy metals, particularly lead (Pb), in soils, ultimately entering the human body through the food chain and causing numerous health issues. Lead is considered the top priority pollutant by the Central Pollution Control Board due to its severe toxic effects, including developmental delays in infants, mental and neurological disorders, kidney damage, gastric issues, hormonal imbalances, and cardiovascular diseases. Major sources of lead contamination include acid batteries, paints, coal industries, automobiles, plastics, and agricultural activities. The lack of effective wastewater treatment and safe disposal methods further aggravates lead pollution [5,6]. These contaminations present significant threats to biodiversity and ecosystem functionality. Among these, lead (Pb) is a particularly harmful pollutant, primarily introduced into the environment through industrial discharge, mining, and the extensive use of lead-containing products [7]. Once released into the soil, lead can be readily absorbed by plants, disrupting essential physiological and biochemical processes and ultimately affecting plant growth and productivity [8-10]. *Arachis hypogaea L.* (commonly known as peanut) is an economically significant leguminous crop cultivated extensively in tropical and subtropical regions for its nutritional and commercial value. However, like many other crop species, it is susceptible to the detrimental effects of heavy metals, including lead [11]. Exposure to elevated levels of lead has been associated with alterations in plant morphology, including reduced root and shoot growth, chlorosis, and inhibited biomass accumulation [12,13]. Moreover, lead stress can include a cascade of biochemical changes, such as accumulating reactive oxygen species (ROS), altered enzymatic antioxidant activity, and disturbances in essential nutrient uptake and metabolism. Understanding the extent of morphological and biochemical alterations caused by lead toxicity in *Arachis hypogaea* is essential for assessing the plant's tolerance mechanisms and for developing effective strategies for phytoremediation or mitigation of heavy metal stress in agricultural systems [14,15]. This study aims to systematically investigate the effect of varying concentrations of lead on the morphological traits and biochemical parameters of *Arachis hypogaea L*, thereby providing valuable insights into the plant's response to heavy metal-induced stress.

Materials and Methods

Plant growth conditions and lead stress treatments

Groundnut seeds were obtained from the S. V. Agricultural College, Tirupati, India. They were planted in clay pots with air-dried red soil and farmyard manure in a 3:1 proportion. The pots were kept in a separate area with a natural photoperiod of 12-14 hours and a temperature of 28 ± 4 °C. They were watered once daily. Following germination, seedlings were trimmed to three per container and kept for 14 days. Fourteen-day-old seedlings were exposed to Pb stress by adding 0, 100, 200, 400, and 800 ppm of lead nitrate solution ($Pb(NO_3)_2$). Both control and treatment pots received daily irrigation with tap water. Water was added carefully, a little less than the field capacity.

Determination of plant growth parameters

The plants were gently removed from their containers and properly cleansed with running tap water. Plant growth was assessed by measuring root and shoot length. Dry weight was measured after drying shoots and roots at 82℃ to a constant weight.

Analysis of Pb and nutrient elements

Plants were uprooted and split into roots and leaves, which were properly washed with tap water and then treated with a 0.2% detergent solution (Tween-20) to remove the waxy/oily later on the sample surface. The samples were washed with 0.1 N HCl, thoroughly washed with water, and then rinsed with double-distilled water. The samples were dried at 80℃ in a hot air oven for 24 hours and pulverised with a mortar and pestle. A 0.5 g oven-dried powder sample of roots and leaves was placed in a 50 ml boiling test tube with 5 ml of 60% concentrated nitric acid. The mixture was then kept at room temperature overnight. Diluted samples were analysed for elements using an ICP-MS (Inductively Coupled Plasma Mass Spectrometry). Replications were kept, and the average was utilised for computations. Elemental concentrations were measured in mg or µg dry weight.

Analysis of biochemical parameters

The biochemical parameters were estimated according to standard methods. Chlorophyll content was described by Arnon (1949) [16]. Carotenoids were estimated following the procedure by Jensen and Jensen (1971) [17]. Total carbohydrates were measured according to Hegde et al. (1962) [18], while total protein content was assessed using the method by Lowry et al. (1951) [19]. Starch content was estimated following Mc Cready et al. (1950) [20], and free amino acids were analyzed using the procedure by Moore and Stein (1948) [21]. Proline content was determined as per Bates et al. (1973) [22]. Nitrate reductase activity was measured using the method by Lin and Kao (1980) [23], and nitrite reductase activity followed the method described by Losada and Panique (1971) [24]. Finally, leaf soluble proteins were quantified according to Laemmli (1970) [25].

Results and Discussion

The present study investigated the biochemical responses of Arachis hypogaea L. under varying concentrations of treatment (0,25, 50, 75, and 100 µg/ml) across three time intervals: the 10^{th}, 20^{th}, and 30^{th} day. Key physiological and biochemical parameters, including chlorophyll content, carotenoids, carbohydrates, proteins, amino acids, starch, proline, and nitrogen assimilation enzymes, were analyzed to assess stress-induced changes. Chlorophyll ‘a’ and ‘b’ contents decreased progressively with increasing treatment concentrations and time. In control samples, both chlorophyll ‘a’ and ‘b’ showed relatively stable values, whereas treatments at 75 and 100 µg/ml exhibited significant reductions, particularly on the 30^{th} day. Total chlorophyll content followed a similar trend, indicating impaired photosynthetic capacity likely due to pigment degradation under stress. Carotenoid levels also declined in a concentration and time-dependent manner. While control plants maintained relatively stable carotenoid content, treated plants, especially at 75 and 100 µg/mL, showed marked reductions by the 30^{th} day. This reduction may be attributed to oxidative damage or disrupted biosynthesis pathways, affecting the photoprotective mechanisms in stressed plants. Total

carbohydrate content showed a noticeable decrease with increasing stress, suggesting that the treatments affected carbon assimilation and storage. Control plants maintained higher carbohydrate levels across all time points, while 100 µg/mL treatment led to the lowest values, indicating impaired photosynthesis or enhanced consumption of carbohydrates under stress conditions. Similarly, protein content was significantly reduced under higher treatment concentrations. Control plants displayed a slight increase on the 20th day, followed by stabilization, whereas treated plants, especially at 100 µg/mL, exhibited a marked decline throughout the study. This suggests that protein synthesis may be hindered, or degradation accelerated, under stress conditions. Free amino acid content also decreased with increasing concentrations. Control samples remained relatively stable, while plants exposed to higher treatments showed a sharp decline by day 30. This could be due to the diversion of amino acids towards stress-related metabolic pathways or inhibited nitrogen assimilation. The total starch content was significantly lower in treated plants, indicating that stress may impair carbohydrate storage mechanisms. The decline was most prominent at 100 µg/mL, with a steady decrease over time. This could result from the combined effects of reduced photosynthesis and increased metabolic demand during stress. Proline content conversely, proline content increased with both treatment concentration and time. Control plants showed a moderate rise in proline levels, while higher concentrations (75 to 100 µg/mL) led to substantial increases, peaking on the 30th day. This accumulation suggests that proline acts as an osmoprotectant and antioxidant, playing a crucial role in stress adaptation. Nitrate reductase and nitrite reductase activities were both inhibited by increasing treatment concentrations. In control plants, both enzymes exhibited peak activity on the 20th day. However, activity significantly declined in treated samples, particularly at 75 and 100 µg/mL. This indicates a disruption in nitrogen metabolism, likely caused by oxidative stress or enzyme inhibition.

Table 1: Effect of Lead nitrate on chlorophyll ‘a’, chlorophyll ‘b’ & total chlorophyll content of *Arachis hypogeae* L. (mg g^{-1} fwt).

Arachis hypogeae L.	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Chlorophyll ‘a’	Control	1.210± 0.121	1.281±0.110	1.192±0.301
	25 µM/L	1.195 ± 0.012	1.169 ± 0.151	1.121 ± 0.522
	50 µM/L	1.112 ± 0.264	1.158 ± 0.082	1.128 ± 0.230
	75 µM/L	1.136 ± 0.039	0.910 ± 0.085	0.848 ± 0.268
	100 µM/L	0.914 ± 0.138	0.729 ± 0.023	0.708 ± 0.085
Chlorophyll ‘b’	Control	1.191 ± 0.058	1.180 ± 0.021	1.187 ± 0.025
	25 µM/L	1.188 ± 0.043	1.153 ± 0.045	1.199 ± 0.049
	50 µM/L	1.080 ± 0.081	1.069 ± 0.103	1.017 ± 0.058
	75 µM/L	1.039 ± 0.022	0.911 ± 0.118	0.721 ± 0.081
	100 µM/L	0.784 ± 0.054	0.664 ± 0.022	0.581 ± 0.088
Total chlorophyll	Control	2.415 ± 0.177	2.683 ± 0.141	2.111 ± 0.101
	25 µM/L	2.157 ± 0.127	2.201 ± 0.114	2.199 ± 0.192
	50 µM/L	1.757 ± 0.111	1.622 ± 0.119	1.518 ± 0.121
	75 µM/L	1. 671 ± 0.142	1.444 ± 0.123	1.213 ± 0.912
	100 µM/L	0.955 ± 0.154	0.819 ± 0.181	0.902 ± 0.110

The results demonstrated a concentration and time-dependent decline in chlorophyll content in Arachis hypogaea L. under increasing levels of the applied treatment. In control plants, chlorophyll a remained relatively stable, peaking slightly on the 20th day (1.283 ± 0.110 µg/mL) before slightly decreasing by day 30 (1.190 ± 0.302 µg/mL). However, a notable reduction was observed in plants treated with 75 and 100 µm/L, where chlorophyll a dropped to 0.845 ± 0.265 and 0.706 ± 0.083 µg/mL, respectively, by the 30th day. Chlorophyll b followed a similar trend, remaining stable in the control group (1.16-1.17 µg/mL), while declining progressively in higher concentrations, with the 100 µg/mL treatment reducing levels to 0.580 ± 0.087 µg/mL by day 30. Total chlorophyll content also showed a marked decrease with increasing concentrations and time, with the most significant reductions at 75 and 100 µg/mL, where values dropped to 1.212 ± 0.911 and 0.901 ± 0.109 µg/mL, correspondingly, by the end of the experimental period. These results suggest that elevated treatment concentrations negatively impact chlorophyll synthesis or stability, likely due to oxidative stress, disruption of chloroplast structure, or inhibition of key biosynthetic enzymes. Overall, the observed reductions in chlorophyll pigments point to impaired photosynthetic efficiency in response to increased stress levels.

Table 2: Effect of Lead nitrate on total carotenoids content of *Arachis hypogeae* L. (mg g^{-1} fwt).

Arachis hypogeae L	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Total carotenoids	Control	1.751 ± 0.271	1.852 ± 0.221	1.755 ± 0.272
	25 µM/L	1.685 ± 0.110	1.620 ± 0.111	1.588 ± 0.116
	50 µM/L	1.322 ± 0.120	1.282 ± 0.214	0.955 ± 0.123
	75 µM/L	0.781 ± 0.126	0.744 ± 0.113	0.623 ± 0.125
	100 µM/L	0.530 ± 0.024	0.454 ± 0.120	0.322 ± 0.118

The total carotenoid content in Arachis hypogaea L. showed a clear decline with increasing concentrations of the treatment and for 30 days. In control plants, carotenoid levels remained relatively stable, ranging from 1.749 ± 0.269 µg/mL on the 10th day to 1.753 ± 0.270 µg/mL on the 30th day, indicating no significant fluctuation in pigment content under normal conditions. However, a progressive and concentration-dependent reduction was observed in treated plants. At 25 µM/L, carotenoid levels decreased moderately to 1.586 ± 0.114 µg/mL by day 30, while at 50 µM/L, a sharper decline was noted, reaching 0.954 ± 0.121 µg/mL. The most substantial reductions were recorded at 75 and 100 µM/L, with carotenoid content dropping to 0.623 ± 0.123 µg/mL amd 0.320 ± 0.115 µg/mL, respectively, by the end of the experiment. This decreasing trend suggests that higher concentrations of the treatment may inhibit carotenoid biosynthesis or promote degradation, possibly due to oxidative stress or damage to plastids where carotenoids are synthesized and stored. As carotenoids play a vital role in photoprotection and antioxidant defense, their reduction implies that the plant's ability to mitigate photooxidative damage may be compromised under elevated stress levels.

Table 3: Effect of Lead nitrate on total carbohydrate content of *Arachis hypogeae* L. (mg g^{-1} fwt).

Arachis hypogeae L	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Total carbohydrates	Control	20.231 ± 0.185	19.361 ± 0.439	18.745 ± 0.261
	25 µM/L	19.134 ± 0.152	18.184 ± 0.180	13.187 ± 0.135
	50 µM/L	18.019 ± 0.130	17.702 ± 0.150	14.904 ± 0.131
	75 µM/L	17.999 ± 0.236	16.613 ± 0.338	13.819 ± 0.179
	100 µM/L	16.829 ± 0.030	15.719 ± 0.132	12.219 ± 0.193

The total carbohydrate content in Arachis hypogaea L. exhibited a gradual decline with increasing treatment concentrations and over time, indicating a stress-induced disruption of carbohydrate metabolism. Control plants showed a modest, natural reduction in carbohydrate levels from 19.230 ± 0.184 mg/g on day 10 to 17.744 ± 0.259 mg/g by day 30. In contrast, treated plants experience more significant decreases, particularly at higher concentrations. At 25 µM/L, carbohydrate levels declined steadily to 12.186 ± 0.134 mg/g by day 30, while at 50 µM/L, a similar downward trend was observed, ending at 13.902 ± 0.130 mg/g. More pronounced reductions were recorded at 75 and 100 µM/L, with carbohydrate content dropping to 12.816 ± 0.176 and 11.216 ± 0.192 mg/g, respectively, on the 30th day. These results suggest that elevated treatment levels may interfere with photosynthesis and carbohydrate synthesis or promote carbohydrate degradation, potentially due to oxidative stress or metabolic reprogramming under stress conditions. The reduction in carbohydrate content could also indicate a shift in energy allocation towards defense mechanisms rather than growth and storage, highlighting the plant's physiological response to prolonged exposure to the stressor.

Table 4: Effect of Lead nitrate on total protein content of *Arachis hypogeae* L (mg g^{-1} fwt).

Arachis hypogeae L	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Total protein content	Control	14.829 ± 0.221	16.872 ± 0.228	15.097 ± 0.335
	25 µM/L	12.828 ± 0.413	11.366 ± ` 0.132	10.228 ± 0.091
	50 µM/L	11.180 ± 0.092	9.177 ± 0.117	8.183 ± 0. 196
	75 µM/L	10.811 ± 0.116	9.641 ± 0.038	8.165 ± 0.121
	100 µM/L	8.911 ± 0.111	8.166 ± 0.181	6.193 ± 0.164

The total protein content in Arachis hypogaea L. declined progressively with increasing concentrations of the treatment and throughout the experiment, suggesting a disruption in protein synthesis or enhanced degradation under stress. In control plants, protein levels increased slightly from 13.828 ± 0.220 mg/g on the 10th day to a peak of 15.870 ± 0.225 mg/g on the 20th day, before dropping to 14.095 ± 0.334 mg/g by day 30, possibly reflecting a natural metabolic adjustment over time. However, in treated plants, protein content showed a steady and concentration-dependent reduction. At 25 µM/L and 75 µM/L, where final values were 7.182 ± 0.195 mg/g and 7.164 ± 0.120 mg/g, respectively. The most significant decline occurred at 100 µM/L, where protein content fell from 7.910 ± 0.110 mg/g on day 10 to just 6.190 ± 0.160 mg/g by the end of the experiment. These results indicate that higher concentrations of the treatment may impair protein biosynthesis machinery or increase proteolytic activity, potentially due to oxidative stress, cellular damage, or a shift in metabolic priorities under adverse conditions. The observed protein depletion aligns with typical stress responses in plants, reflecting reduced growth and diminished metabolic function.

Table 5: Effect of Lead nitrate on free amino acids content of Arachis hypogeae L (mg g-1 fwt).

Arachis hypogeae L.	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Free aminoacids content	Control	5.330 ± 0.122	5.910 ± 0.220	5.810 ± 0.122
	25 µM/L	5.121 ± 0.251	4.550 ± 0.111	4.212 ± 0.214
	50 µM/L	4.931 ± 0.121	4.511 ± 0.181	4.219 ± 0.912
	75 µM/L	4.731 ± 0.113	3.819 ± 0.133	3.172 ± 0.119
	100 µM/L	3.200 ± 0.122	3.183 ± 0.161	3.116 ± 0.192

The free amino acid content in Arachis hypogaea L. exhibited a declining trend with increasing treatment concentrations, indicating that elevated stress levels may disrupt nitrogen metabolism or protein turnover. In control plants, amino acid content showed a slight increase from 4.330 ± 0.121 mg/g on the 10th day to 4.908 ± 0.219 mg/g on the 20th day, followed by a minor reduction to 4.809 ± 0.120 mg/g by day 30, likely reflecting normal metabolic fluctuations. In contrast, plants exposed to the treatment displayed a concentration and time-dependent reduction. At 25 µM/L, free amino acids decreased gradually to 3.211 ± 0.212 mg/g by day 30, while at 50 µM/L, levels dropped to 3.218 ± 0.910 mg/g. More pronounced reductions were observed at 75 and 100 µM/L, with final values of 2.172 ± 0.190 mg/g, respectively. These results suggest that higher treatment concentrations may suppress amino acid biosynthesis or accelerate their utilization in stress-response pathways such as proline synthesis or the production of secondary metabolites. The significant reduction at higher doses also implies a possible disruption in protein degradation or transamination processes, ultimately affecting plant growth and stress resilience.

Table 6: Effect of Lead nitrate on total starch content of Arachis hypogeae L. (mg g-1 fwt).

Arachis hypogeae L.	Concentration µM/L	10th DAY	20th DAY	30th DAY
Total starch content	Control	7.821 ± 0.131	8.770 ± 0.035	6.990 ± 0.159
	25 µM/L	6.899 ± 0.011	6.211 ± 0.134	5.217 ± 0.102
	50 µM/L	6.135 ± 0.131	5.816 ± 0.119	4.819 ± 0.092
	75 µM/L	5.306 ± 0.134	4.890 ± 0.053	3.902 ± 0.089
	100 µM/L	4.332 ± 0.065	4.130 ± 0.008	3.184 ± 0.020

The total starch content in Arachis hypogaea L. decreased progressively with increasing treatment concentration and over time, indicating a stress-induced disruption of carbohydrate storage metabolism. In control plants, starch levels peaked on the 20th day at 7.771 ± 0.033 mg/g before decreasing to 5.990 ± 0.157 mg/g by the 30th day, likely reflecting normal starch mobilization during plant growth. However, treated plants exhibited a significant concentration-dependent reduction in starch accumulation. At 25 µM/L, starch content fell steadily to 4.216 ± 0.100 mg/g by day 30, while more pronounced declines were seen at 50 µM/L 3.819 ± 0.091 mg/g and 75 µM/L 2.901 ± 0.087 mg/g. The most substantial reduction was observed at 100 µM/L, where starch content dropped from 3.331 ± 0.064 mg/g on day 10 to just 2.183 ± 0.018 mg/g by the end of the experiment. This decreasing trend suggests that elevated stress levels may impair photosynthate production or hinder starch biosynthesis, possibly due to reduced photosynthetic activity or enzymatic inhibition. Additionally, stress conditions may promote starch degradation as an energy source to support stress-responsive metabolic processes, further contributing to the decline in starch reserves.

Table 7: Effect of Lead nitrate on total proline content of *Arachis hypogeae* L. (mg g^{-1} fwt).

Arachis hypogeae L.	Concentration µM/L	10th DAY	20th DAY	30th DAY
Total proline	Control	0.973 ± 0.089	1.122 ± 0.159	2.253 ± 0.150
	25 µM/L	1.277 ± 0.022	1.300 ± 0.257	2.722 ± 0.172
	50 µM/L	1.384 ± 0.123	1.655 ± 0.123	2.800 ± 0.718
	75 µM/L	1.588 ± 0.212	1.712 ± 0.666	3.216 ± 0.221
	100 µM/L	1.722 ± 0.136	2.477 ± 0.231	3.418 ± 0.132

Proline content in Arachis hypogaea L. increased significantly with both rising treatment concentrations and overtime, reflecting the plant's adaptive response to stress. In control plants, proline levels rose gradually from 0.971± 0.086 mg/g on the 10th day to 2.251 ± 0.151 mg/g by the 30th day, suggesting a natural role for proline accumulation in development or mild stress adaptation. However, treated plants exhibited a more pronounced and concentration-dependent increase in proline content. At 25 µM/L, proline reached 2.720±0.170 mg/g by day 30, while at 50 µM/L and 75 µM/L, levels climbed further to 2.798±0.716 and 3.215±0.220 mg/g, respectively. The highest accumulation occurred at 100 µM/L, where proline content peaked at 3.415±0.130 mg/g on the 30th day. This marked increase under higher concentrations indicates enhanced proline biosynthesis or reduced catabolism as part of the plant's stress defence strategy. Proline is well known for its role in osmotic adjustment, stabilization of proteins and membranes, and scavenging of reactive oxygen species, making its accumulation a reliable biochemical marker of abiotic stress tolerance.

Table 8: Effect of Lead nitrate on nitrate reductase activity of Arachis hypogeae L. (mg g^{-1} fwt).

Arachis hypogeae L.	**Concentration µM/L**	**10th DAY**	**20th DAY**	**30th DAY**
Nitrate reductase activity	Control	2.544 ± 0.009	3.855 ± 0.171	3.195 ± 0.151
	25 µM/L	2.852 ± 0.011	2.755 ± 0.016	2.599 ± 0.105
	50 µM/L	2.533 ± 0.110	2.394 ± 0.083	2.155 ± 0.014
	75 µM/L	1.744 ± 0.132	1.493 ± 0.355	1.136 ± 0.107
	100 µM/L	1.664 ± 0.110	1.377 ± 0.111	1.199 ± 0.234

Nitrate reductase activity in Arachis hypogaea L. showed significant variation in response to different treatment concentrations and time points, reflecting the impact of stress on nitrogen metabolism. In control plants, the activity peaked on the 20th day at 3.853±0.170 µmol NO2- g-1FW h-1 before slightly declining to 2.194±0.150 µmol NO2- g-1 FW h-1 by day 30, indicating a healthy, functioning nitrogen assimilation process under normal conditions. However, treated plants exhibited a concentration-dependent suppression of nitrate reductase activity. At 25 µM/L, enzyme activity remained relatively stable but lower than the control, declining to 2.595±0.103 µmol NO2- g-1 FW h-1 on the 30th day. A more pronounced reduction was observed at 50 µM/L 2.150±0.012, and the inhibitory effect was even greater at 75 µM/L and 100 µM/L, where enzyme activity dropped to 1.134±0.105 and 1.195±0.231 µmol NO2- g-1 FWh-1, respectively, by the final day. These results suggest that higher concentrations of the stress agent may impair nitrate assimilation by inhibiting the enzyme responsible for converting nitrate to nitrite, a critical step in nitrogen utilization. This suppression could result from oxidative damage to the enzyme, disruption of associated metabolic pathways, or an overall reduction in nitrogen uptake and assimilation under stress conditions.

Table 9: Effect of Lead nitrate on nitrite reductase of *Arachis hypogeae* L. (mg g^{-1} fwt).

Arachis hypogeae L.	Concentration µM/L	10th DAY	20th DAY	30th DAY
Nitrite reductase	Control	3.122± 0.010	3.574 ± 0.259	3.198 ± 0.150
	25 µM/L	3.131 ± 0.122	3.124 ± 0.157	2.847 ± 0.002
	50 µM/L	2.799 ± 0.123	2.522 ± 0.310	2.333 ± 0.118
	75 µM/L	2.222 ± 0.114	2.149 ± 0.236	1.899 ± 0.101
	100 µM/L	1.899 ± 0.210	1.712 ± 0.019	1.459 ± 0.758

Nitrate reductase activity in Arachis hypogaea L. was significantly influenced by increasing concentrations of treatment, showing a consistent decline across time points, especially at higher levels. In control plants, enzyme activity rose from 3.121 ± 0.009 µmol NO_2-g^{-1} FW h^{-1} on the 10th day to a peak of 2.573 ± 0.258 µmol NO^{-2} g^{-1} FW h^{-1} on day 20, followed by a slight decline to 2.197 ± 0148 µmol NO_2^- g^{-1} FW h^{-1} on the 30th day. This pattern indicates normal metabolic regulation under non-stressed conditions. However, treatment exposure caused a concentration and time-dependent decrease in nitrite reductase activity. At 25 µM/L, activity decreased modestly to 2.845 ± 0.001 by day 30, whereas at 50 µM/L and 75 µM/L, the enzyme levels dropped more substantially, reaching 2.330 ± 0.116 and 1.895 ± 0.100 µmol NO_2^- g^{-1} FW h^{-1}, respectively. The most marked decline occurred at 100 µM/L, where activity fell from 1.896 ± 0.208 on day 10 to just 1.455 ± 0.756 µmol NO_2^- g^{-1} FW h-1 by the 30th day. These observations suggest that higher treatment levels may impair nitrite reduction, possibly by disrupting enzyme synthesis, cofactor availability, or electron transport systems. This decline in nitrite reductase activity, along with the suppression of nitrate reductase noted earlier, points to a broader inhibition of the nitrogen assimilation pathway under stress conditions.

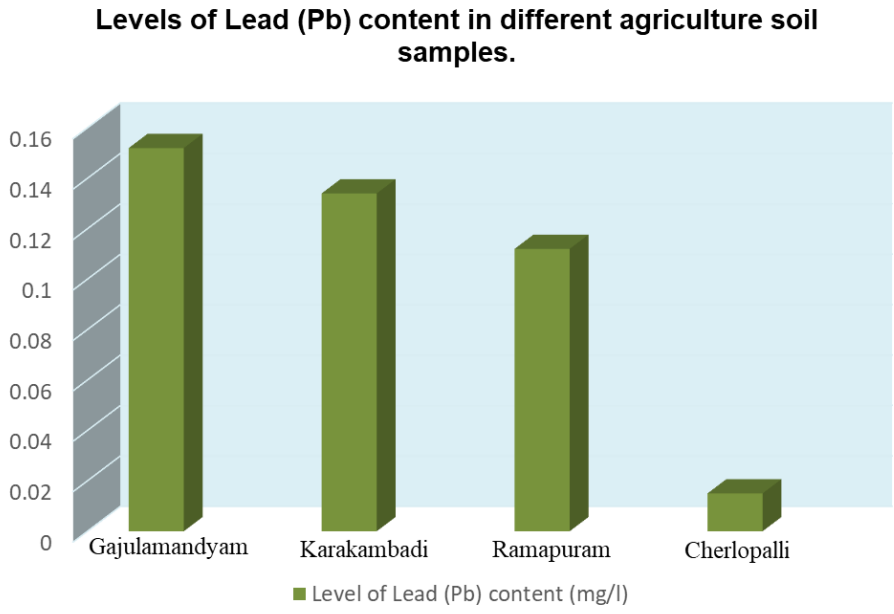


Figure showing levels of lead content in different agricultural soil samples

Conclusion

This study examined the biochemical responses of Arachis hypogaea L. under different lead treatment concentrations. Key parameters such as chlorophyll content, carotenoids, carbohydrates, proteins, amino acids, starch, proline, and nitrogen assimilation enzymes were analyzed to assess stress-induced changes. Results showed that chlorophyll and carotenoid content decreased with increasing treatment concentrations and time, indicating impaired photosynthetic capacity. Total carbohydrate content decreased with increasing stress, suggesting that the treatments affected carbon assimilation and storage. Protein content was significantly reduced under higher treatment concentrations, suggesting that protein synthesis may be hindered or degradation accelerated under stress conditions. Free amino acid content decreased with increasing concentrations, while total starch content was significantly lower in treated plants. Proline content increased with both treatment concentration and time, suggesting that proline acts as an Osmo osmoprotectant and antioxidant. Nitrate reductase and nitrite reductase activities were inhibited by increasing treatment concentrations, suggesting a disruption in nitrogen metabolism. Overall, the results demonstrate that Arachis hypogaea L. undergoes significant physiological and biochemical alterations under increasing stress levels. Reduced photosynthetic pigments, compromised nitrogen metabolism, and altered primary metabolite levels indicate that higher concentrations of the treatment negatively impact plant growth and function. Proline accumulation appears to be a consistent stress response, potentially offering protection against cellular damage. These findings highlight the sensitivity of groundnut plants to chemical stress and underscore the importance of monitoring such parameters for evaluating plant stress tolerance. These findings highlight the sensitivity of groundnut plants to chemical stress and emphasize the importance of monitoring these parameters for evaluating plant stress tolerance.

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