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**Optimizing Spinach (*Spinacia oleracea* L.) Growth in Lead-Contaminated Environments
with EDTA and GA3 Applications**

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Abstract

Lead (Pb) contamination in soil affects plant nitrogen assimilation and reduces seed germination and plant water relations. Pb exposure also reduces carotenoid, chlorophyll, carbon dioxide, assimilation, and photosynthetic rates in plants. The use of Ethylenediaminetetraacetic acid (EDTA), and Gibberellic acid GA3 is an effective amendment to mitigate this problem. EDTA improves plant growth by increasing the bioavailability of essential ions in the soil, allowing plants to better absorb nutrients. Its efficient, environmentally friendly, and biodegradable nature makes it ideal for enhancing metal uptake and promoting healthy plant development in metal-polluted soils. GA3 improves plant growth by different mechanisms such as stem elongation, flowering and seed germination. The combined application of the GA3 and EDTA as an amendment on spinach still needs scientific justification. This study investigates the combined impact of GA3 and EDTA as an amendment on spinach growth. The 10ppmGA3, 0.5%EDTA, 10ppmGA3 + 0.5%EDTA, 1%EDTA, and 10ppmGA3 + 1%EDTA treatments were administered in four replicates using a completely randomized design (CRD), both with and without Pb stress. Results demonstrate that 10ppmGA3 + 1%EDTA exhibits a rise in spinach shoot fresh weight (97.73%), root fresh weight (89.98%), shoot dry weight (96.72%), and root dry weight (100.00%) over the control in 500Pb stress. The study found that 10ppmGA3 + 1%EDTA significantly increased spinach chlorophyll a (64.11%), chlorophyll b (98.34%), and

total chlorophyll (74.37%) in 500ppmPb stress than the control. In conclusion, 10ppmGA3 + 1%EDTA is the suggested amendment for mitigating Pb stress in spinach.

Keywords: Ethylenediaminetetraacetic acid (EDTA), Gibberellic acid GA3, Lead stress, Antioxidants, Pigments.

Introduction:

Heavy metals lead (Pb) is found in contaminated soil (Netherway et al., 2020). Lead accumulates in soil, air, and water because of anthropogenic activities including fertilizer usage and vehicle emissions (Meena et al., 2020). Plant roots absorb Pb from the soil and store it in an insoluble form (Guo et al., 2022). Higher Pb contamination in soil affects plant nitrogen assimilation and reduces seed germination and plant water relations (Goyal et al., 2020). Pb exposure also reduces carotenoid, chlorophyll, carbon dioxide, assimilation, and photosynthetic rates in plants (Shahzad et al., 2023).

Ethylenediaminetetraacetic acid (EDTA), a strong chelating agent from an amine and four carboxylate groups, significantly improves soil quality and agricultural productivity (Shukla et al., 2022). EDTA improves phytoremediation and soil health by increasing heavy metal solubility, mobility, and bioavailability in contaminated soils (Hamidpour et al., 2020; Li et al., 2020). EDTA chelates protect agricultural plants from lead, cadmium, and nickel. It increases plant growth, photosynthetic activity, and yields in poor nutrient soils or within hydroponic systems by enhancing the accessibility of vital nutrients (Hamidpour et al., 2020; Neocleous et al., 2020). However, EDTA application requires careful management to minimize environmental impact, including heavy metal mobilization risk, soil composition, and pH, ensuring its benefits while mitigating potential environmental concerns (Wang et al., 2021).

Gibberellic acid (GA3), a key plant hormone from the gibberellin family, significantly enhances plant growth and development (SMH et al., 2023). It facilitates seed germination by breaking dormancy and promoting enzyme synthesis, improving embryo energy supply (Ge et al., 2023; Nautiyal et al., 2023). GA3 encourages stem elongation, improving dwarf plants and harvest efficiency. It regulates flowering and fruit growth, extending fruit lifespan and enhancing plant tolerance to abiotic stresses (SMH et al., 2023). However, GA3 application must be managed

carefully to avoid excessive stem growth and potential lodging, stimulating plant development in horticulture and agriculture for improved crop yield and quality (Hedden & Hoad, 2021).

Spinach (*Spinacia oleracea* L.) is a nutrient-rich, dietary vegetable. The leafy green vegetable globally consumes 25 thousand tons annually. It is essential for micronutrients (K, Fe, Mg, Mn, & folic acid) and vitamins (K, C, B2, and A) (Sheikh et al., 2025). The antioxidant activity of spinach is due to flavonoids, a crucial component of water-soluble polyphenols (Murcia et al., 2020). Spinach is rich in omega-3 fatty acids, vitamins E and B6, and dietary fiber which regulates and maintains human tissue (Stephen et al., 2023).

This study examines how EDTA and GA3 improve phytoremediation efficiency and lead-induced stress in spinach grown in contaminated soils. It is hypothesized that both EDTA and GA3 should improve spinach growth under lead stress. This research examines the individual and combined effects of EDTA and GA3 on the growth of spinach plants in Pb stress. This study contributes to the larger objective of crop and ecosystem preservation by addressing this knowledge gap and proposing an environmentally friendly approach to reducing the negative effects of Pb on spinach cultivation.

Material and methods:

Experimental site:

The Department of Botany at Islamia University Bahawalpur, Rahim Yar Khan Campus, performed a pot experiment in its research area (Botanical Garden) to investigate the effects of EDTA-chelated biochar and GA3 on spinach growth. A composite soil test including six samples for the preliminary soil assessment. Table 1 presents the properties of the soil before the experiment.

Table 1. Soil and irrigation water parameters (physio-chemical) before experimentation

Soil	Values	References
pH	8.41	(Page et al., 1983)
EC _e (dS/m)	3.35	(Rhoades, 1996)
Soil organic matter (%)	0.38	(Nelson & Sommers, 1982)
Extractable k (potassium) (µg/g)	111	(Pratt, 2016)
Extractable Na (sodium) (µg/g)	146	(Donald & Hanson, 1998)
Total N (nitrogen) (%)	0.0028	(Bremner, 1996)
Available P (phosphorus) (µg/g)	5.81	(Kuo, 2018)
Texture	Clay Loam	(Gee & Bauder, 2018)

Irrigation	Values	References
pH	7.04	
EC (electrical conductivity) ($\mu\text{S}/\text{cm}$)	464	
<i>Chloride (meq. /L)</i>	<i>0.01</i>	
Bicarbonates (meq. /L)	5.26	(Estefan et al., 2013)
Sodium (mg/L)	104	
Ca+Mg (meq. /L)	3.15	
Carbonates (meq. /L)	0.05	

Treatment plan & experimental design:

Gibberellic acid and EDTA treatments were used with concentrations of 10ppmGA3 + 0.5%EDTA and 10ppmGA3 + 1%EDTA under the lead stress with 500ppm. So, these two levels of remediating treatments were designed to overcome the lead stress from spinach crops. Table 2 represents the detailed treatment plan as follows:

Table 2: Detailed treatment plan to overcome Lead stress in plants.

GA3	02 levels i.e., 0ppm, 10ppm
EDTA chelated biochar=EDTABC	03 levels i.e., 0%, 0.5%, 1%
Lead (Pb)	Control, 500ppm
T1	0ppmGA3+0%EDTA+ Control Pb
T2	10ppmGA3+0%EDTA+ Control Pb
T3	0ppmGA3+0.5%EDTA+ Control Pb
T4	10ppmGA3+0.5%EDTA+ Control Pb
T5	0ppmGA3+1%EDTA+ Control Pb
T6	10ppmGA3+1%EDTA+ Control Pb
T7	0ppmGA3+0%EDTA+ 500ppm Pb
T8	10ppmGA3+0%EDTA+ 500ppm Pb
T9	0ppmGA3+0.5%EDTA+ 500ppm Pb
T10	10ppmGA3+0.5%EDTA+ 500ppm Pb
T11	0ppmGA3+1%EDTA+ 500ppm Pb
T12	10ppmGA3+1%EDTA+ 500ppm Pb

Fertilizer:

Spinach crops obtained 130, 90, and 110 kg/hectare of K_2O , P_2O_5 , and N in SOP, DAP, and urea. Three N splits and phosphate and potash were applied at sowing. Pb stress was artificially applied after two weeks of germination. Lead nitrate was added to the soil at 500 ppm Pb/kg.

Seed collection, sterilization, and sowing

The spinach seeds used in this investigation were procured from a verified seed vendor. Healthy seeds were chosen, whereas broken or weak seeds were discarded. Prior to planting, seeds were sterilized using 5% sodium hypochlorite and 95% ethanol, followed by three rinses in sterilized deionized water to remove the sterilizing agents. Twenty seeds were planted in five-kilogram soil pots. Following germination thinning, we retained 10 seedlings in each container.

Harvesting & data collection:

Plants were harvested after 50 days of sowing, and the fresh weight of the shoot & root was recorded by using an analytical grade scale. Shoot and root fresh or dry weight was measured after oven drying at 65 °C for 72 hours.

Chlorophyll contents and accessory pigments:

The analysis of fresh spinach leaves' chlorophyll a, b, and total chlorophyll concentration was conducted using Arnon's method (Arnon, 1949) subjected to extraction using a solution of 80% acetone. In order to determine the final chlorophyll concentration, absorbance measurements were made at several wavelengths (663, 645, 480, 537, and 503 nm). (Arnon, 1949), carotenoids (Kirk & Allen, 1965), anthocyanin (Sims & Gamon, 2002) and lycopene (Rao et al., 1998)

Electrolyte leakage (EL)

Electrolyte leakage was measure by (Lutts et al., 1996) by using following equation.

$$\text{Electrolyte Leakage (\%)} = \left(\frac{\text{EC1}}{\text{EC2}} \right) \times 100$$

Antioxidants

The stress markers antioxidants SOD, POD, CAT, APX and MDA activities were measured by following methodologies, MDA by (Dhindsa et al., 1982), POD by (Hori et al., 1997), CAT by (Aebi, 1984), APX by (Nakano & Asada, 1981) and MDA by (Hernández & Almansa, 2002)

Statistical analysis:

To analyze the data, standard statistical methods were used, such as a two-way ANOVA to determine the significance of the treatments and the Tukey test to compare two sets of data ($p <$

116 0.05). Cluster analysis with OriginPro includes convex hull, hierarchical, and Pearson correlation
117 plots (OriginPro, 2021).

118 **Results:**

119 **Growth attributes**

120 Under 0ppmPb, using 10ppmGA3, 0.5%EDTA, 10ppmGA3 + 0.5%EDTA, 1%EDTA, and
121 10ppmGA3 + 1%EDTA treatments showed an increase in the shoot fresh weight (60.87%,
122 33.33%, 68.12%, 53.62%, and 72.46%), root fresh weight (40.58%, 11.59%, 68.12%, 20.29%,
123 and 88.41%), shoot dry weight (33.33%, 6.67%, 53.33%, 20.00%, and 73.33%), and root dry
124 weight (46.43%, 10.71%, 75.00%, 35.71%, and 96.43%) above the control. Compared to the
125 control adding 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments
126 under 500ppmPb stress, caused an increase in the shoot fresh weight (70.45%, 22.73%, 84.09%,
127 34.09%, and 97.73%), root fresh weight (55.44%, 12.26%, 81.35%, 38.17%, and 89.98%), shoot
128 dry weight (47.54%, 47.54%, 88.52%, 47.54%, and 96.72%), and root dry weight (70.91%,
129 10.91%, 78.18%, 27.27%, and 100.00%) (Figure 1A, B, C, and D).

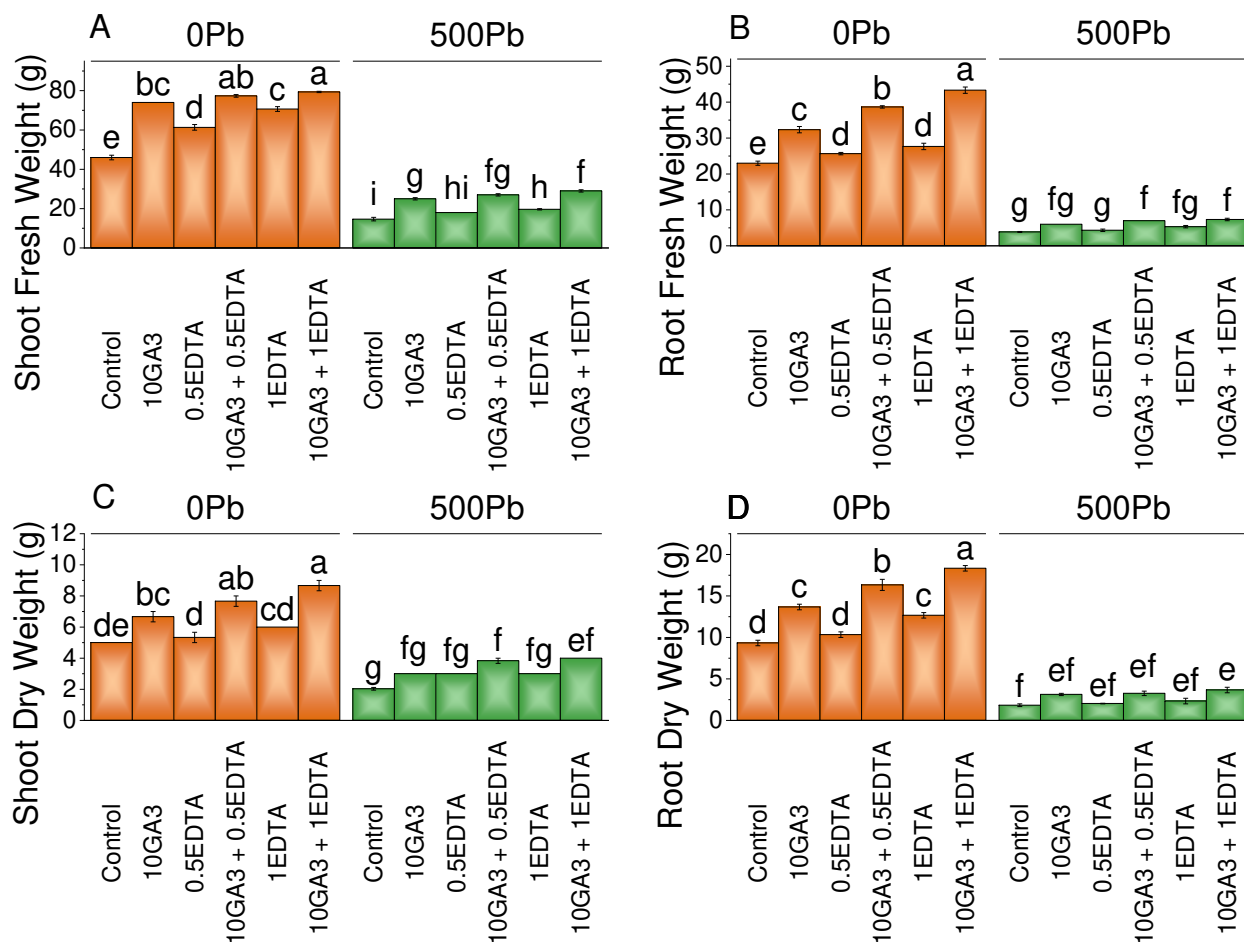


Figure 1. Impact of gibberellic acid (GA3) and EDTA on the fresh weight of shoots (A), roots (B), shoots (C), and roots (D) of spinach plants cultivated in environments with 0Pb and 500Pb stress, respectively. The standard error of four replicates is shown by the bars. Different letters on the bars showed significant differences when the Tukey test was performed at $p \leq 0.05$.

Chlorophyll content and carotenoids

Adding 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments yielded a significant improvement in chlorophyll a (33.08%, 28.07%, 35.85%, 29.38%, and 40.34%), chlorophyll b (37.13%, 8.93%, 38.55%, 23.30%, and 60.30%), total chlorophyll (34.66%, 20.60%, 36.91%, 27.01%, and 48.13%), and carotenoids (52.59%, 7.60%, 74.53%, 34.56%, and 81.87%) over the control under 0Pb stress. Under 500Pb stress, the increase in chlorophyll a (59.72%, 39.62%, 63.51%, 42.13%, and 64.11%), chlorophyll b (37.61%, 25.60%,

79.54%, 26.51%, and 98.34%), total chlorophyll (53.09%, 35.42%, 68.32%, 37.45%, and 74.37%), and carotenoids (55.59%, 44.83%, 64.04%, 54.19%, and 92.97%) were recorded by applying 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments compared to the control (Figure 2A, B, C, and D).

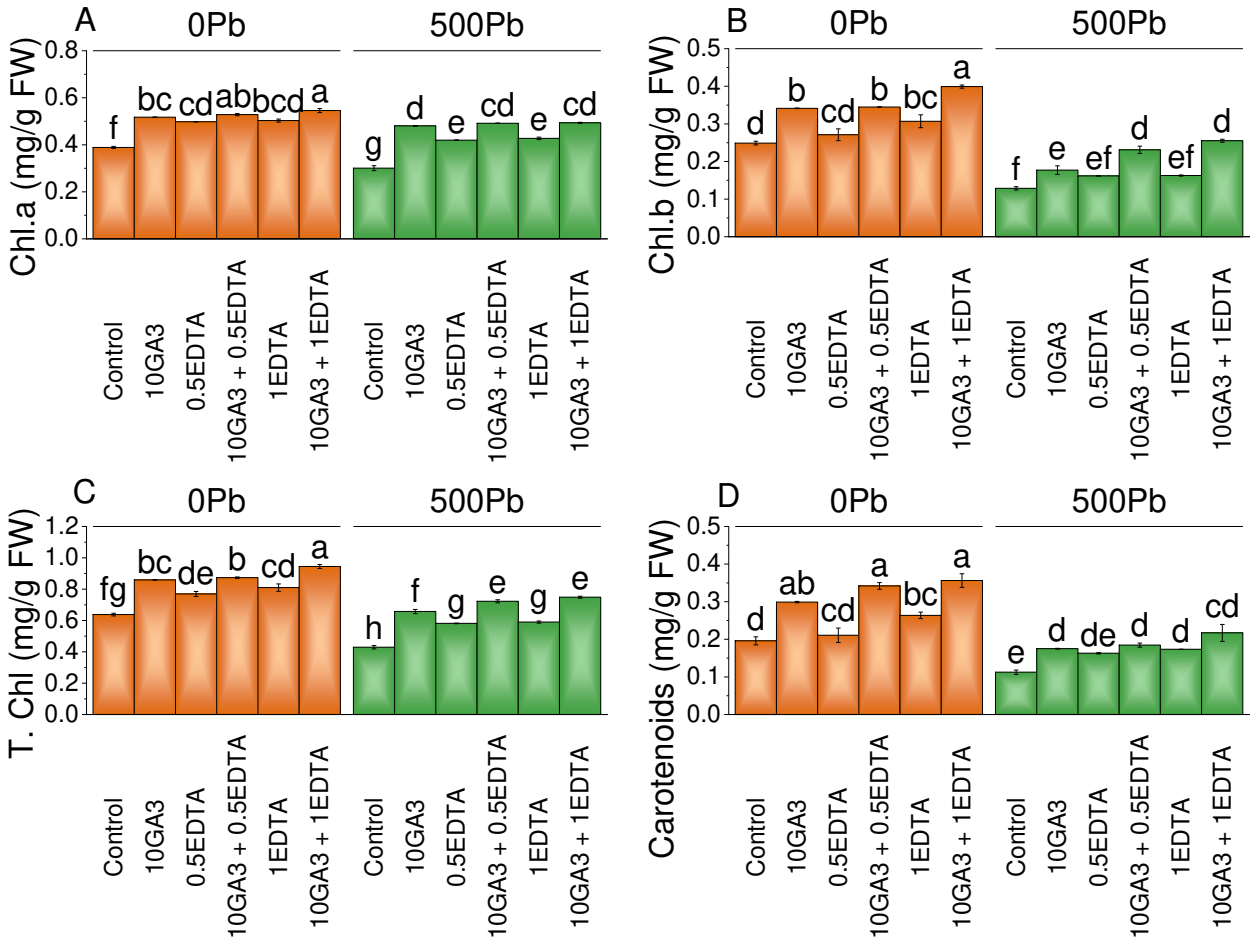


Figure 2. Results showing the impact of gibberellic acid (GA3) and EDTA on several plant pigments in spinach cultivated in 0Pb and 500Pb stress, including chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D). The bars show the averages ($\pm$ SE) of four separate experiments. The Tukey test showed that there were significant changes in the various letters on the bars at $p < 0.05$.

Anthocyanin, Lycopene, and Electrolyte leakage

Under no stress, applying 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments increased anthocyanin (52.30%, 34.87%, 85.98%, 43.69%, and 94.07%), and caused a

decrease in lycopene (26.93%, 13.94%, 31.67%, 23.42%, and 66.46%), and electrolyte leakage (71.55%, 45.65%, 82.14%, 55.41%, and 96.74%) than the control. Under 500Pb stress, the increase in anthocyanin (46.57%, 11.97%, 49.40%, 24.05%, and 74.32%), and decrease in lycopene (23.59%, 8.62%, 28.26%, 19.57%, and 42.98%), and electrolyte leakage (20.34%, 6.96%, 32.08%, 16.19%, and 37.41%) were recorded over the control with 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments (Figure 3A, B, and C).

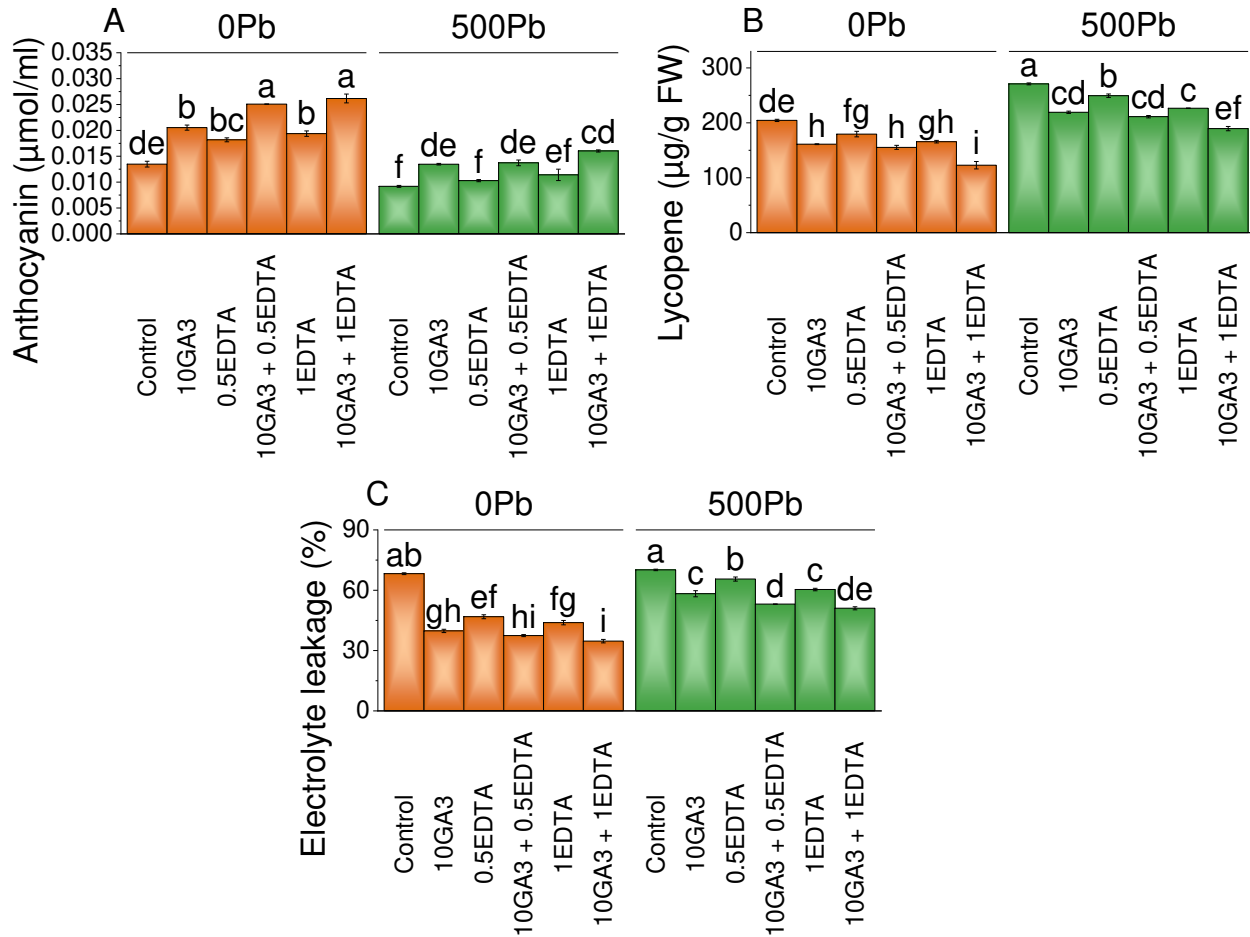


Figure 3. Effects of gibberellic acid (GA3) and EDTA on anthocyanin (A), lycopene (B), and electrolyte leakage (C) in spinach plants grown in environments with 0Pb and 500Pb stress. The standard error of four replicates is shown by the bars. A variety of letters on the bars showed significant differences when the Tukey test was performed at $p \leq 0.05$.

POD, SOD, and CAT

Under 0Pb, applying 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments showed decrease in the POD (44.44%, 14.35%, 68.03%, 30.69%, and 96.03%), SOD (39.18%, 16.67%, 55.55%, 25.93%, and 80.30%), and CAT (38.10%, 26.09%, 45.00%, 31.82%, and 52.63%) over the control. Compared to the control adding 10GA3, 0.5EDTA, 10GA3 + 0.5EDTA, 1EDTA, and 10GA3 + 1EDTA treatments under 500Pb stress, caused decrease in the POD (40.21%, 16.24%, 54.55%, 24.77%, and 67.90%), SOD (19.75%, 10.23%, 27.63%, 14.12%, and 32.88%), and CAT (15.38%, 7.14%, 20.00%, 11.11%, and 25.00%) (Figure 4A, B, and C).

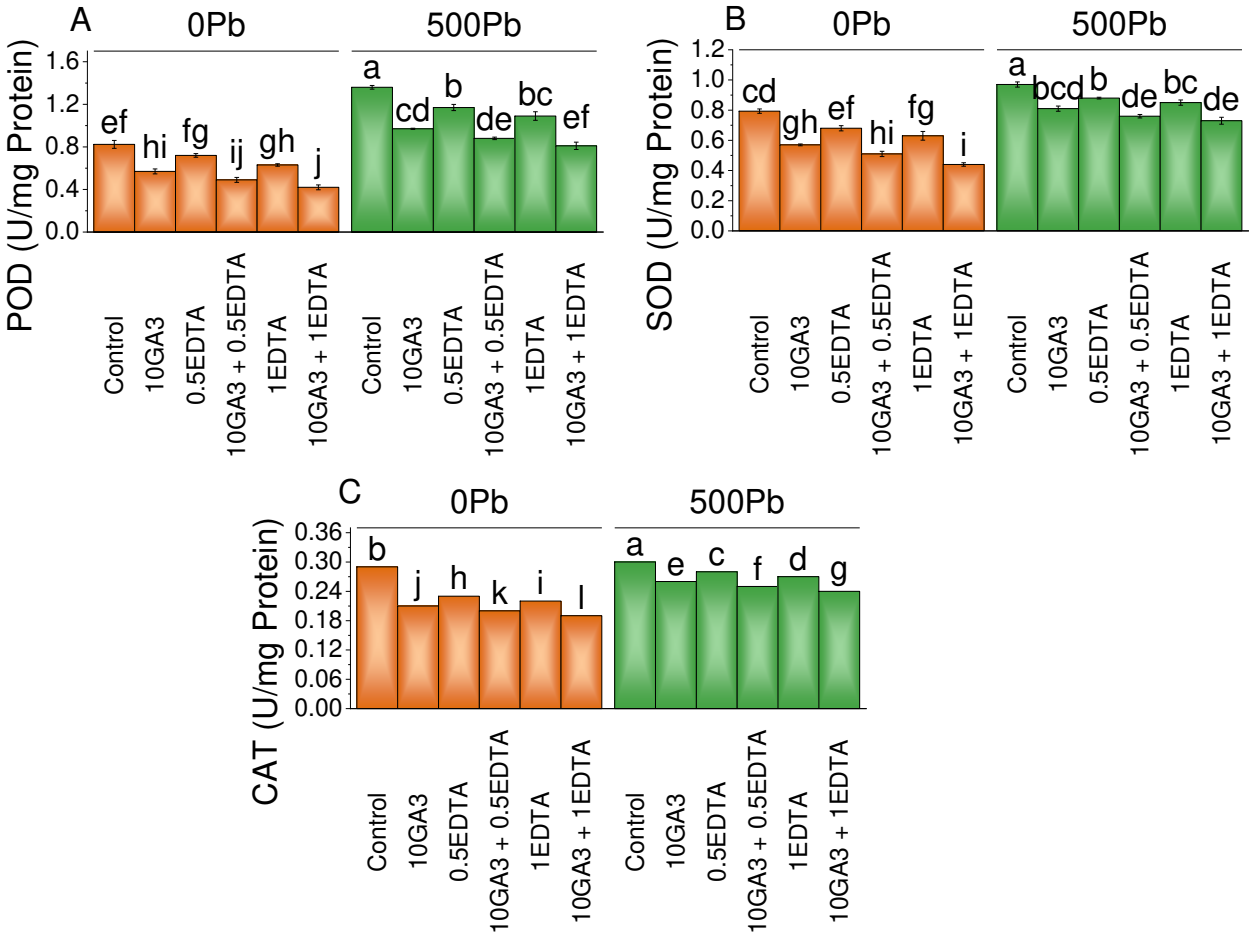


Figure 4. Gibberellic acid (GA3) and EDTA's impact on peroxidase (POD), superoxide dismutase (SOD) (B), and catalase (CAT) (C) in spinach plants exposed to 0Pb and 500Pb stresses, respectively. The bars show the averages ($\pm$ SE) of four separate experiments. The Tukey test showed that there were significant variations in the various letters on the bars at $p < 0.05$.

APX, MDA, and H₂O₂

Under 0Pb, the decrease in APX (49.76%, 95.39%, 23.13%, and 83.71%), MDA (38.99%, 3.27%, 79.68%, 12.76%, and 71.32%), and H₂O₂ (51.32%, 33.72%, 61.97%, 41.98%, and 74.24%) were measured over the control with GA, GABA, and GA+GABA treatments. Under Pb toxicity, adding GA, GABA, and GA+GABA treatments showed a decrease in APX (47.45%, 20.81%, 68.32%, 31.34%, and 89.17%), MDA (47.15%, 19.08%, 66.06%, 31.16%, and 60.18%), and H₂O₂ (16.67%, 6.25%, 21.43%, 10.19%, and 29.35%) over the control (Figure 5A, B, and C).

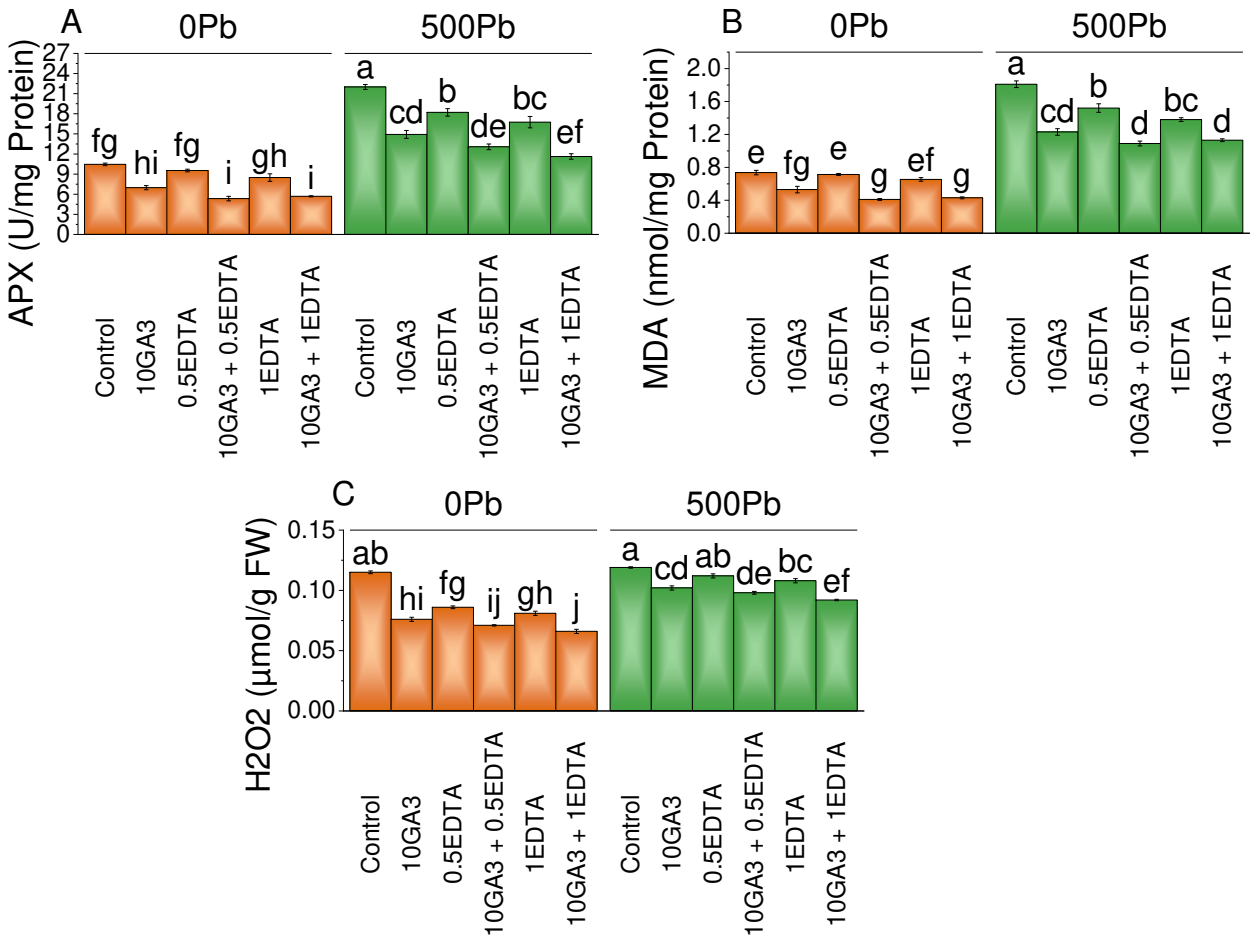


Figure 5. Gibberellic acid (GA₃) and EDTA affected ascorbate peroxidase (APX) A, malondialdehyde (MDA) B, and hydrogen peroxide (H₂O₂) C levels in spinach plants cultivated in environments with 0Pb and 500Pb stress, respectively. The means ± SE of four replicates are

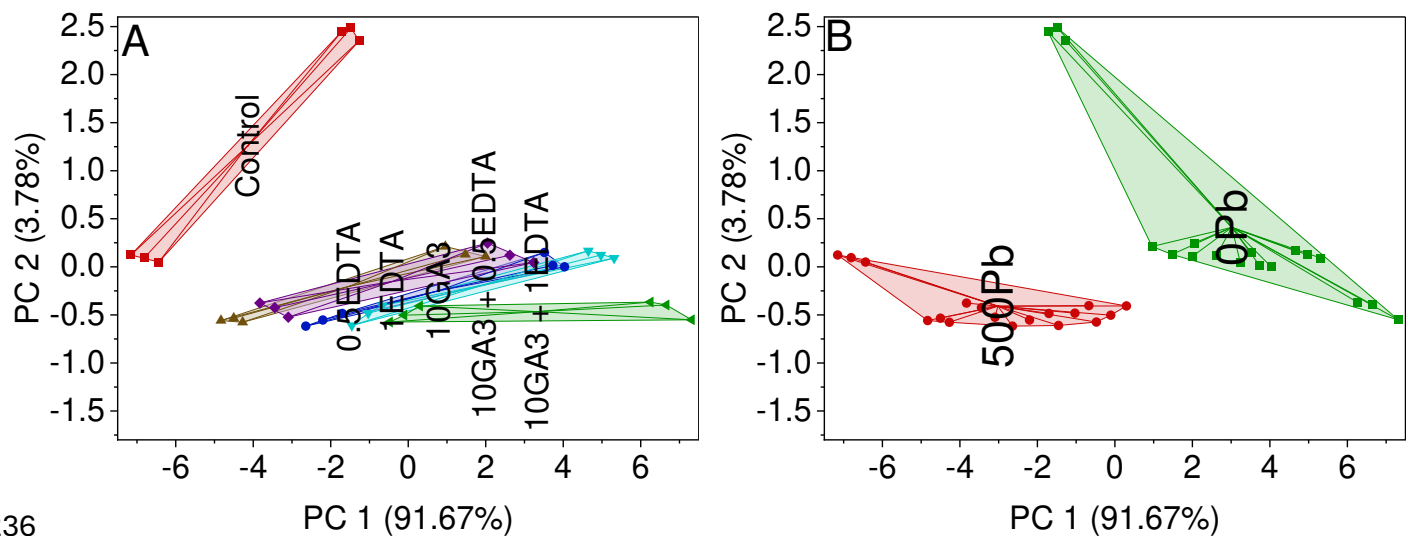
shown by the bars. There were significant changes in the various letters on the bars when the Tukey test was performed at $p \leq 0.05$.

Convex Hull and Hierarchical Cluster Analysis

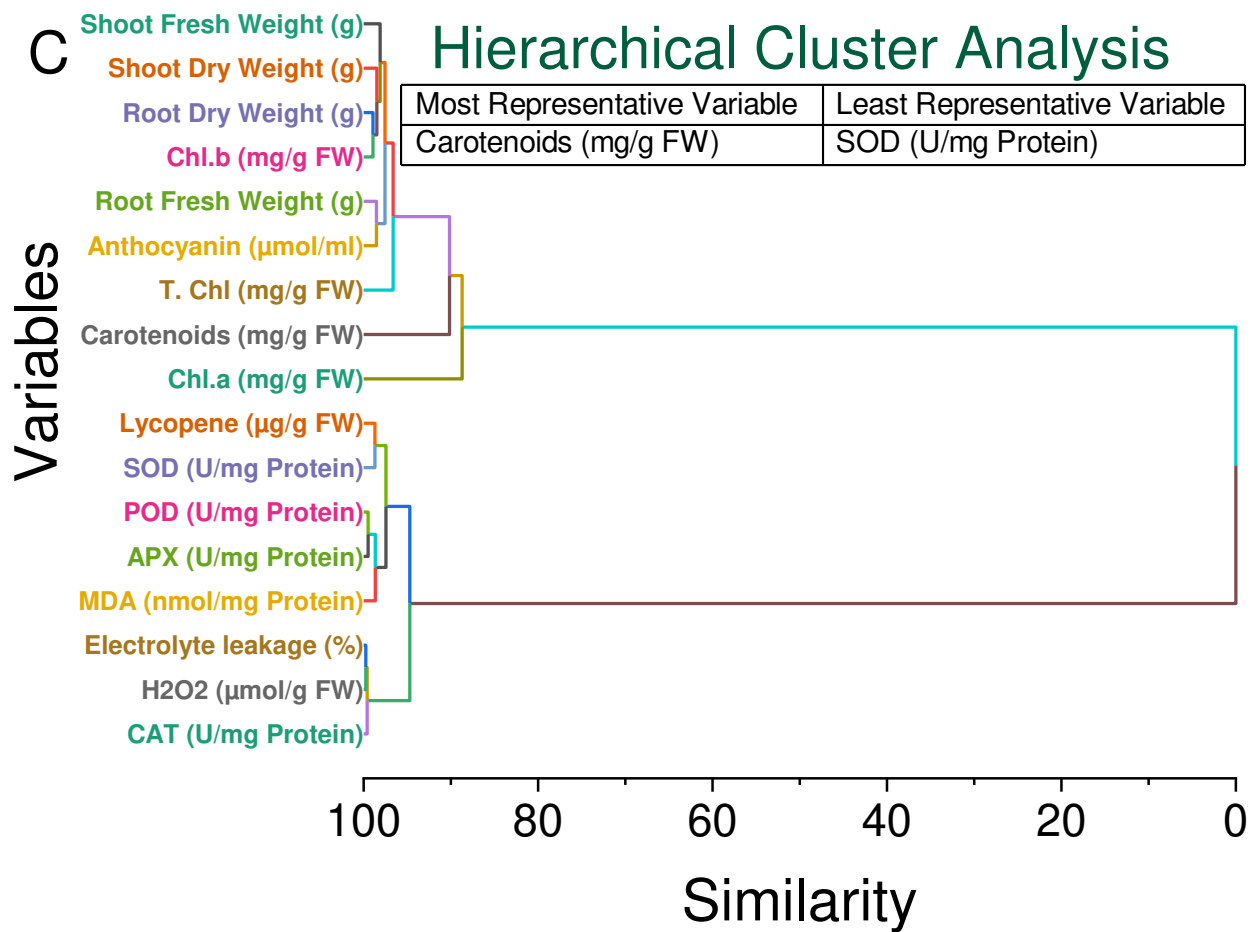
Major values such as (-1.26227, 2.35225) and (-7.15773, 0.12262) indicate the extremes of the scores for the Control group, which show a large distribution over both main components. There is a lot of variation in the control condition, and this variety is captured by the convex hull. Although they extend significantly along both the positive and negative axes of PC2, the scores of the 10GA3 group are more concentrated around the origin. A more concentrated distribution is shown by the convex hull, which includes important locations such as (4.03678, 0.00057) and (-2.64209, -0.617), in comparison to the control. Scores in the 0.5EDTA condition deviate significantly along both major components and are dispersed about the origin. The convex hull is defined by points like (1.99878, 0.10754) and (-4.83195, -0.55895), which show a modest dispersion of scores. Distribution of scores around the origin is seen in the 10GA3 + 0.5EDTA group, with substantial extension along PC1 and to a lesser amount along PC2. Similar to the 0.5EDTA group, but with a different form, points such as (5.31617, 0.09074) and (-1.45402, -0.61018) define the convex border for this condition. There is a substantial range of scores for the 1EDTA group from the origin; the convex hull is established at major places like (3.23906, 0.04517) and (-3.82838, -0.37742). A similar distribution to the 0.5EDTA condition is shown in this group, suggesting that their variability is also comparable. At last, the 10GA3 + 1EDTA condition displays scores spread that is comparable to previous mixed situations; the convex border is formed by points such as (7.31772, -0.55097) and (-0.4757, -0.57542). According to Figure 6A, this points to a score distribution that incorporates features of both 10GA3 and 1EDTA. Coordinates for the 0Pb group range from (-1.72308, 2.44509) to (7.31772, -0.55097) in PC 2, and the points tend to cluster in the positive area of PC 1. In contrast, the 500Pb group is found in PC 2's variable regions and the negative area of PC 1. Within this category, you may find points between (-0.40758, 0.29941) and (-7.15773, 0.12262). When building the convex hull, the outlying points of every set are taken into account. These coordinates are part of the 0Pb set: (7.31772, -0.55097), (4.03678, 5.68755E-4), and (-1.72308, 2.44509). Points (-7.15773, 0.12262), (-6.43606, 0.0496), and (-0.66932, -0.40431) are the extremes for the 500Pb group (Figure 6B). With a similarity score of 0.24634, the variables electrolyte leakage and H2O2 are strongly related to one another. A similarity score of 0.4092 indicates that these variables are further clustered with a

bigger group that includes CAT. The fact that POD and APX have a clustering value of 0.51683 indicates that they are closely related. There is a strong relationship between root dry weight and chlorophyll b, as seen by their high similarity value of 1.06683. The clustering of lycopene and SOD, which has a similar score of 1.28694, further demonstrates their significant association. With a similar score of 1.34042, MDA forms a substantial cluster with its linked variables. There is a strong clustering relationship between anthocyanin and root fresh weight (1.46529). The linked variables and shoot dry weight have a value of 1.51378, suggesting a strong relationship between them. Launch new weight clusters with 1.89173 as their shared variable value. Total chlorophyll (T. Chl) and carotenoids both exhibit substantial grouping; the former has a similarity value of 3.37689 and the latter is 9.83623. With a similarity score of 11.29028, chlorophyll an is clustered, suggesting a strong but separate link. Chlorophyll and total chlorophyll exhibit the strongest grouping, with extremely high similarity values of 88.70972 and 94.7133, respectively, as seen in Figure 6C.

236



237



238 **Figure 6.** Cluster plot convex hull for treatments (A), lead (Pb) levels (B), and hierarchical
 239 cluster plot (C) for studied attributes.

240 Pearson Correlation Analysis

A substantial positive connection was observed between root fresh weight (0.96124), shoot dry weight (0.96138), and root dry weight (0.97103) with respect to shoot fresh weight. Although the correlation between chlorophyll a (0.68915) and shoot fresh weight was less than that between chlorophyll b (0.9591), both chlorophylls showed a positive correlation with shoot fresh weight. Shoot fresh weight was also positively correlated with total chlorophyll (0.8865) and carotenoids (0.76652). There was a very significant positive relationship between anthocyanin and shoot fresh weight (0.92847). Lycopene showed an interesting inverse association with shoot fresh weight, with a substantial negative correlation of -0.94188. All three antioxidant enzymes—SOD (-0.93234), CAT (-0.83393), and electrolyte leakage (-0.82809)—exhibited a negative connection. Similarly, there was a high negative connection between shoot fresh weight (-0.96267) and MDA (-0.85523), both of which are indicators of oxidative stress. Several variables, including root fresh weight, shoot dry weight, chlorophyll b, and total chlorophyll, were positively linked with root fresh weight. A tight relationship was also shown by the substantial positive correlation it had with anthocyanin (0.97199). Root fresh weight (-0.96714), electrolyte leakage (-0.91335), POD (-0.95787), SOD (-0.98162), and CAT (-0.91837) all demonstrated a strong negative correlation with lycopene. The following variables showed strong correlations with shoot dry weight: anthocyanin (0.94663), total chlorophyll (0.94913), root dry weight (0.97756), and chlorophyll b (0.96456). A strong negative correlation (-0.97978) was observed for lycopene once again, as were electrolyte leakage (-0.89534), POD (-0.98205), and SOD (-0.96108). There was a considerable positive connection between root dry weight and anthocyanin (0.94127), total chlorophyll (0.94075), and chlorophyll b (0.9796). On the other hand, there were substantial negative associations for lycopene (-0.97335), electrolyte leakage (-0.87166), POD (-0.97468), and SOD (-0.95966). Chlorophyll an exhibited weaker correlation with the other variables, demonstrating stronger associations with total chlorophyll (0.93228) and chlorophyll b (0.78363), although it did positively correlate with shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. Anthocyanin ($r=0.95229$) and total chlorophyll ($r=0.95504$) were strongly positively correlated with chlorophyll b. There was an inverse relationship between chlorophyll b and lycopene, electrolyte leakage, and antioxidant enzymes. A robust positive connection was observed between total chlorophyll and carotenoids ($r=0.80312$) and anthocyanin ($r=0.93827$). Total chlorophyll was negatively correlated with lycopene, electrolyte leakage, POD, SOD,

CAT, APX, and MDA. Although carotenoids' associations with other factors were weaker, they did have a moderate positive connection with anthocyanin (0.85475). Apart from lycopene, electrolyte leakage, and antioxidant enzymes, anthocyanin showed strong positive correlations with most of the variables. Most variables showed strong negative correlations with lycopene, suggesting a clear inverse relationship with the parameters that were studied. The strong negative correlations observed between electrolyte leakage, POD, SOD, CAT, APX, MDA, and H₂O₂ and most of the variables indicate that elevated levels of these stress markers and antioxidant enzymes are linked to decreased values of other biochemical and physiological characteristics (Figure 7).

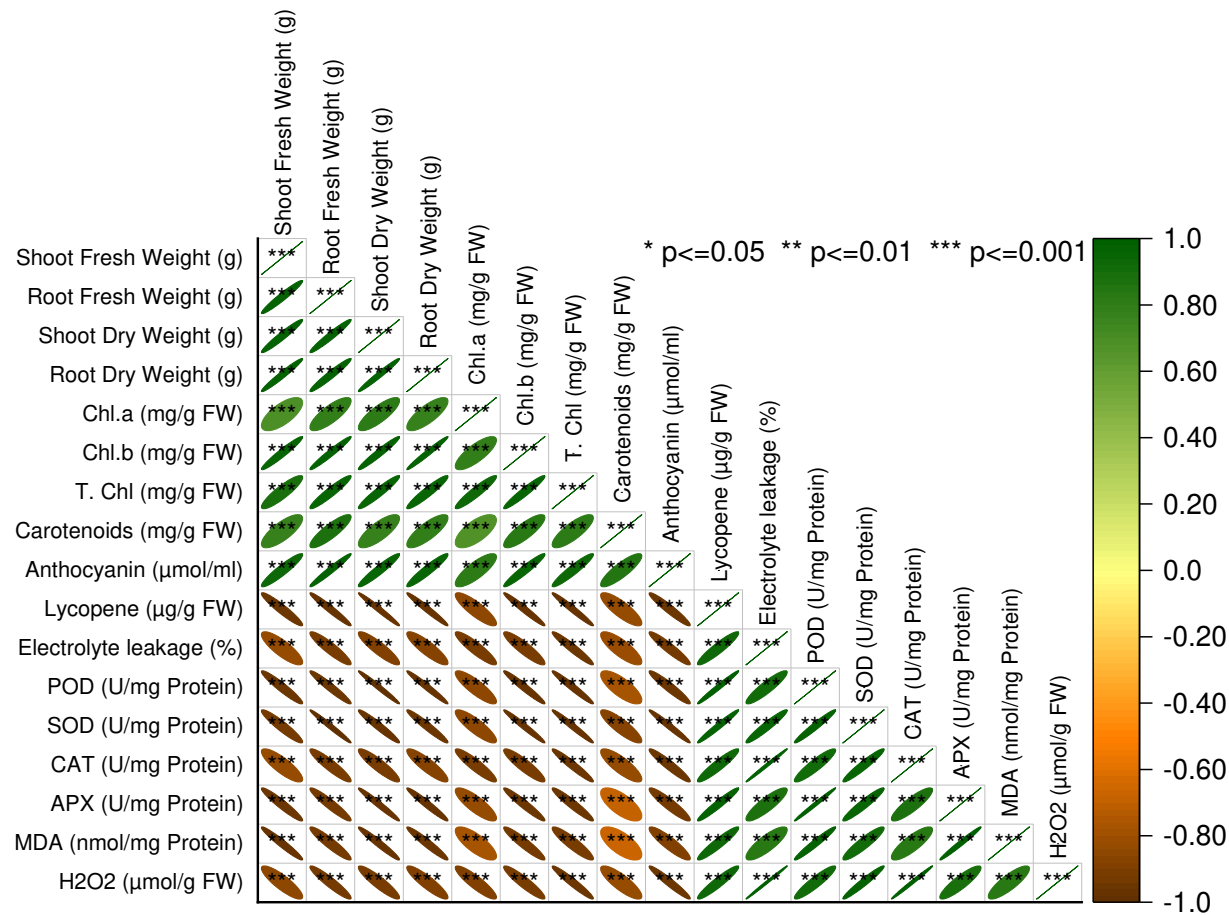


Figure 7. Pearson correlation for studied attributes.

Discussion:

Heavy metal contamination of soil impairs vital physiological functions of plants, resulting in diminished growth, chlorosis, oxidative stress, and sometimes even plant mortality (Magray et al., 2023). The current study focused on examining the effects of GA3 and EDTA treatments on a variety of physiological and biochemical parameters in spinach under no-stress (0Pb) and Pb (500ppmPb) stress. The purpose of this study is to select the best application of GA3 and EDTA both in single and combination on the growth of spinach under 0Pb and 500ppmPb stress. Gibberellic acid (GA3) is a plant growth-promoting hormone that improves cell growth and division, GA3 promotes shoot and root weight by stimulating hydrolytic enzymes to break starch into sugars (Sharma et al., 2024). EDTA improves nutrient accessibility by forming complexes with metal ions and making them more accessible to plants (Kamal et al., 2023). The rise in chlorophyll and carotenoid content under both 0Pb and 500ppmPb stress showed that GA3 and EDTA treatment upgrade photosynthetic productivity. EDTA may also reduce Pb uptake or its translocation to aerial parts under lead (Pb) stress, reducing toxicity and enhancing growth (Ur Rahman et al., 2024). GA3 raises chlorophyll content by increasing the expression of genes involved in the biosynthesis of chlorophyll (Anwar et al., 2023). Carotenoids advance photosynthetic contraption by diminishing reactive oxygen species (ROS) and creating more energy. EDTA may facilitate the absorption of essential micronutrients like magnesium and iron, which are required for the biosynthesis of chlorophyll and carotenes (Saman et al., 2022). Under Pb stress, the combined treatments' capacity to maintain higher levels of carotenoid and chlorophyll suggests that they protect against Pb-induced oxidative damage and preserve photosynthetic activity. Anthocyanins, known for their cell reinforcement properties, expanded altogether with the combination of GA3 and EDTA application. GA3's capacity to improve gene expression in flavonoid biosynthesis pathways prompts an increase in anthocyanins, which scavenge ROS and alleviate oxidative damage under Pb stress (Dong et al., 2023). The decrease in lycopene content might demonstrate anthocyanin synthesis. The application of GA3+EDTA can further improve cell membrane stability and integrity by lessening electrolyte leakage, due to decreased lipid peroxidation and receptive oxygen species (ROS). This decrease in the activities of antioxidant enzymes like catalase, superoxide dismutase, and peroxidase indicates that oxidative stress, which is typically elevated in response to elevated ROS levels, has decreased. The need for high cell reinforcement compound action is reduced by this decrease in enzyme

activity, indicating that treatment effectively reduces ROS production. EDTA lessens Pb bioavailability and ROS synthesis, while GA3 improves plant cell reinforcement frameworks (Castro-Camba et al., 2022). The treatment with GA3 and EDTA lowers the levels of APX, MDA, and H2O2, which also lowers lipid peroxidation and oxidative pressure, indicating a significant reduction in oxidative stress. APX activity decreases because of treatment, indicating lower MDA and H2O2 levels and cellular oxidative damage. This is due to increased anthocyanins and carotenoids, which fortify cells and lessen ROS creation, consequently diminishing lipid peroxidation and advancing the ingestion of water and monodehydroascorbate.

Conclusion

To sum up, spinach plants may have their lead effects mitigated by improving their growth characteristics and stress responses when treated with 10%GA3+1%EDTA. There may be synergies at work here since a 10%GA3+1%EDTA treatment boosts yield, chlorophyll content, carotenoids, and anthocyanin levels. To confirm that 10%GA3+1%EDTA is the most effective amendment for reducing lead stress in spinach, more field studies are needed.

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Author Contributions:

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Uzma Younis: Validation, Data Curation, Writing – Review & Editing, Visualization.

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Consent to Participate

All participants in this study provided informed consent prior to their involvement. The purpose, procedures, potential risks, and benefits of the study were clearly explained, and participation was voluntary. Participants were assured of confidentiality and their right to withdraw at any time without consequences.

Consent for Publication

Not applicable

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available on request (restricted access):

"The datasets used in this study are available from the corresponding author upon reasonable request.

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