Corn-derived biochar mitigates oxidative stress and increases the content of essential elements in lettuce leaves grown in phthalate-polluted soil

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Abstract

Phthalic acid esters (PAEs) are recognized markers of microplastic pollution of the environment. The study assessed the effects of different biochars (BC) derived from sewage sludge (SS), corn residues (CR), sunflower (SF), and residues from biogas production (BG) on lettuce grown in PAEs-polluted soil. The BC varied in composition, porosity, and carbon structure, with CR-BC exhibiting the highest surface area and optimal aliphatic carbon content, making it the most effective for soil application. SS had the highest heavy metal and PAHs content, though within safe limits. Elevated phosphate levels in lettuce leaves, influenced by high PAHs, ash, and metal content in BC, were associated with increased CAT activity, indicating oxidative stress. A strong positive correlation was found between Cd and phosphate content, especially in SS-treated plants, and between phosphate and B. CR-BC limited heavy metal uptake while promoting beneficial nutrient interactions (such as between Ca and Mg). PAEs accumulation in lettuce was strongly negatively correlated with phosphate and B levels, suggesting these elements reduce pollutant uptake. Among treatments, CR-BC significantly reduced PAEs accumulation in lettuce leaves, which is critical for food safety. CR-BC also enhanced lettuce biomass, chlorophyll content, and nutrient uptake, and it decreased oxidative stress (lower levels of MDA and enhanced antioxidant enzyme activity of SOD and CAT). Conversely, BG-BC negatively affected plant growth, likely due to its high pH. Overall, the findings highlight the importance of BC feedstock properties, with corn-derived BC offering the most beneficial effects on plant health and pollutant mitigation in polluted soils.

Keywords: PAEs; vegetables; chlorophyll; enzymatic activity;

1. Introduction

Phthalic acid esters (PAEs) are a group of plasticizers widely used in the industry (Chen et al., 2024; Wang et al., 2023) and are recognized as the indicators of environmental pollution by plastic. PAEs are typically classified into low molecular weight (LMW) compounds such as dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), and butyl benzyl phthalate (BBP), high molecular weight (HMW) PAEs, including di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP) (Guo et al., 2024). PAEs are currently polluting all components of the environment: water, soil, and air (Tuan Tran et al., 2022; Xiong and Pei, 2021). Therefore, monitoring the fate of the six PAEs mentioned above is strongly recommended. PAEs can be absorbed by plants both from the air through leaves and from the soil through roots (Zhao et al., 2022), leading to physiological, morphological, cellular, and metabolic changes in plants. Additionally, PAEs in plants can generate reactive oxygen species (ROS) and other intra- or extracellular free radicals. The main ROS produced - superoxide radicals (O_2^{*-}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{*}OH$), and singlet oxygen ($^{1}O_2$) (Gupta et al., 2018) - cause oxidative stress and disrupt plant metabolic activity. This includes reduced chlorophyll content, inhibited growth and development, damage to cell membranes (Sharma and Kaur, 2020), and even a decrease in vitamin content in plants (Wang et al., 2015).

Biochar (BC), a material that has gained popularity in recent years, is produced by heating biomass (including waste materials) in an oxygen-free atmosphere (Xie et al., 2022). Biochar has many applications, including carbon sequestration, use as an alternative fuel, support for immobilization processes, and as a sorbent. It is also widely used as a soil amendment to improve soil quality (Xia et al., 2024). The addition of biochar to soil increases soil pH (Joseph et al., 2021), enhances the retention of nutrients such as K, Mg, P, N, Fe, and Cu (Wang et al., 2020), and improves soil moisture by reducing water evaporation (Gao et al., 2021). At the same time, biochar immobilizes pollutants that could harm plants (Y. He et al., 2023). However, BC may contain substances that, once released, can be harmful to the environment. These substances include polycyclic aromatic hydrocarbons (PAHs), which are formed during the pyrolysis process (Reizer et al., 2022), and heavy metals (HM), whose presence in BC results from their occurrence in the feedstock (Hilber et al., 2017). Due to their toxic effects on plants, animals, and humans (Molina and Segura, 2021), the International Biochar Initiative (IBI) has established maximum permissible concentrations of both PAHs (ranging from 20 to 300 mg/kg) and HM in BC (IBI International Biochar Initiative, 2015).

According to our previous studies, BC can be successfully applied for the immobilization of PAEs in the soil, reducing their bioavailability towards vegetables, such as lettuce and radish (Sokołowski et al., 2024a, 2024b). The present study aimed to evaluate the response of vegetables, with lettuce as the most consumed plant representative, to the BC amendment of PAE-polluted soil. This research analyzed the impact of six priority PAEs and the addition of various biochars to the soil on the content of various pigments, like chlorophylls and carotenoids, and the activity of various antioxidative enzymes in lettuce leaves. The content of micro- and macroelements in the leaves and roots of lettuce growing in PAE-polluted soil and enriched with various types of biochar was also examined. Biochars were designed to adsorb phthalates and reduce their harmful effects on the plant. Additionally, microscopic photos were taken to observe the number of chloroplasts in the leaf cells. The obtained results will be helpful in the understanding of the effect of both PAEs and BC amendment of PAE-polluted soil on plant growth.

2. Materials and methods

2.1. Biochar preparation and analysis

Four different types of biochar were used in the studies. All materials were pyrolyzed for 3 hours in a slow pyrolysis process (heating rate 3 ºC min^{-1}) at 600 °C in a nitrogen atmosphere (630 cm^3 min^{-1}). As feedstock, four different waste materials: corn (*Zea* mays) stalks (material labeled CR), sunflower (*Helianthus L.*) stalks (SF), sewage sludge from a municipal wastewater treatment plant (SS), and residues from biogas production (BG) were used. The pH was determined by a digital pH meter HQ430d Benchtop Single Input (HACH, USA) by measuring the pH of the solution obtained by mixing biochar with distilled ultrapure water in a ratio of 1:10 (wt/wt), shaken for 24 h, and centrifuged. The ash content (mineral fraction) was calculated from a difference in biochar mass before and after heating 1 g of BC at 760 °C in the furnace (MagmaTherm) for 6 h (according to ASTM D3174-12 procedure). The carbon, hydrogen, and nitrogen content was determined in a CHN/CHNS EuroEA3000 Elemental Analyser (EuroVector). The oxygen content was calculated as the difference in ash and C, H, and N content. For the determination of total organic carbon (TOC), Shimadzu SSM-5000A was used. ASAP 2420 Analyzer (Micromeritics, USA) was applied to determine the BC surface area and porosity. The functional groups on the BC surface were analyzed using X-ray photoelectron spectroscopy (XPS) (analytical system UHV Prevac, Poland). Microwave mineralization in Start D Microwave Digestion System Milestone and analysis by Thermo Scientific iCAP™ 7000 ICP-OES was used for the determination of metal concentration in biochars. The content of two fractions of PAHs in BC: total and bioavailable, was examined. The total concentration of PAHs (c_{total}) was determined using accelerated solvent extraction (DIONEX ASE 350, ThermoScientific). The bioavailable PAHs (c_{free}) were analyzed using polyoxymethylene (POM) passive samplers according to the procedure presented by Oleszczuk et al. (Oleszczuk et al., 2016). In both cases, gas chromatography with mass spectroscopy (ThermoScientific ISQ with TRACE 1300) was used to analyze the PAHs concentration in BC.

2.2. Lettuce cultivation

For the laboratory experiment, agricultural soil was used, whose properties were presented in the previous paper (Sokołowski et al., 2024a). The following variations were tested:

1) Control, e.g., soil without PAEs, and the results were labeled as “soil”,
2) PAEs polluted soil labeled as “PAEs”,
3) PAEs polluted soil amended with BG labeled as “BG-BC”,
4) PAEs polluted soil amended with SS labeled as “SS-BC”,
5) PAEs polluted soil amended with SF labeled as “SF-BC”,
6) PAEs polluted soil amended with CR labeled as “CR-BC”.

340 g of air-dried, homogenized, and sieved soil were put into glass containers (500 mL, 12.5 cm diameter, 6.3 cm height), mixed with 3.4 g of biochar and spiked with 1 mL of PAEs mixture containing 1 $\mu L\ mL^{-1}$ of each tested compound (DMP, DEP, DBP, BBP, DEHP, DNOP). Samples were watered with tap water to reach 65% of the soil water holding capacity. In the experiment, five sprouted seeds of lettuce (*Lactuca sativa L.*) (sprout length 3 mm) were placed into the soil. Soil moisture was monitored by weighing the samples three times a week. Plants were cultivated for six weeks in the growth chamber Conviron GEN100 in constant conditions: photoperiod 12 h light/12 h darkness, temperature 22 °C/18 °C day/night, and constant humidity 65%. After the experiment, lettuce samples were cut to separate the roots and leaves.

2.3. PAEs content determination

The content of six tested PAEs was determined in freeze-dried lettuce roots and leaves via the GC-MS/MS procedure described in (Sokołowski et al., 2024b).

2.4. Chlorophyll and carotenoid content determination

The chlorophyll content in lettuce leaves was determined using the method described by Li et al. (Li et al., 2020). A 0.5 g sample of randomly selected leaves from the center of the plant was homogenized with 5 mL of cold acetone. The resulting mixture was then filtered and centrifuged. The absorbance of the supernatant was measured at 645 nm and 663 nm using an Agilent Technologies Cary 300 UV-VIS spectrophotometer. The chlorophyll content was calculated using the following formulas:

$Chl\ a = 12.7A_{663} - 2.7A_{645}$,

$Chl\ b = 22.9A_{645} - 4.68A_{663}$,

$Total\ Chl = 20.2A_{645} + 8.02A_{663}$,

where A is the absorbance at the respective wavelength (nm).

The content of carotenoids in leaf samples was determined based on the Lichtenthaler method (Lichtenthaler, 1987), using the equation:

$Carotenoids = (1000A_{470} - 1.9Chl\ a - 63.14Chl\ b)/214$,

Where A_{470} is the absorbance at 470 nm, Chl a, and Chl b are the content of chlorophyll a and chlorophyll b, respectively. The results obtained from these calculations were then converted into the content in 1 g of fresh leaf mass.

2.5. Metal ions determination in lettuce samples

The concentration of micro- and macronutrients and heavy metals in the soil and the roots and leaves of lettuce samples was determined by ICP-MS (Agilent 8900 ICP-MS Triple Quad). Solutions for the analysis were obtained by microwave mineralization in the HNO_3 (PreeKem Topex+) of 0.1 g of freeze-dried roots and leaves.

2.6. Microscopic studies

Chloroplast observation was performed using a Motic BA310E optical microscope equipped with a Moticam 3.0MP camera. A fragment of leaf tissue was examined at 1000x magnification using an oil immersion objective. Images were captured using Motic Images Plus 2.0 software.

2.7. Antioxidative enzymes activity

The activities of enzymes were determined using: SOD Assay Kit® (Cat. No. 19160-1KT-F, Merck, Germany), Catalase Assay Kit® (CAT100-1KT, Merck, Germany), Phosphate Assay Kit® (MAK308-1KT, Merck, Germany), Antioxidant Assay Kit® (MAK334-1KT, Merck, Germany) and Lipid Peroxidation (MDA) Assay Kit® (MAK568-1KT, Merck, Germany), according to the protocols provided by manufacturer.

2.8. Quality assurance/quality control

To ensure the accuracy and reliability of phthalates quantification in soil and plant tissues, all analytical procedures were performed under strict QA/QC protocols. Certified reference materials (Merck, Poland) and matrix-matched standards were used. The QuEChERS method validation procedure ensured good linearity (>0.997), recovery (97.2–99.1 %), and satisfactory inter- and intraday precision (~4%). Blanks and triplicates were included in each batch to monitor contamination and precision. For pigment analysis and enzymatic activity assays, triplicate measurements were performed, and standard curves ensured linearity (>0.995). Microscopic analyses were conducted using standardized staining protocols and imaging settings, with independent reviewers confirming qualitative observations. All equipment was routinely calibrated, and data were subjected to statistical validation to confirm reproducibility and significance.

2.9. Statistical analysis

All tests were carried out in three replicates. The presented results are the mean value of three measurements ± standard deviation (SD). Means followed by the same letter within the series indicate no significant differences ($p < 0.05$) (Tukey's test). Two-tailed Pearson correlation analysis was performed to test the variables. The significance of the correlation factors was evaluated at a significance level of 0.01 and 0.05.

3. Results and discussion

3.1. Biochar properties

The properties of the obtained BC varied significantly depending on the feedstock. All BCs were characterized by an alkaline pH, and carbon was the predominant element in all four tested materials (Table 1), with the highest content observed in sunflower-derived BC (SF, 86.7%) and the lowest in biogas residue-derived BC (BG, 25.9%). SF and CR had the highest TOC, indicating their potential use as carbon sources for soil microorganisms. Ash content also varied substantially, ranging from 6.7% in SF to 62.8% in sewage sludge-derived BC. SS also had the highest oxygen content, suggesting a greater presence of mineral fractions, such as metal oxides. Inorganic components in BC play a vital role in plant development by serving as sources of essential minerals (Bilias et al., 2024).

The BCs also differed in porosity. CR had the highest specific surface area (90.1 m^2/g), while BG had the lowest (below 1 m^2/g). A lower S_{BET} limits the capacity of BC to adsorb both toxins and nutrients in soil. BG also contained the highest proportion of bioavailable PAHs, while SS had the highest total PAHs content (766.5 μg/kg), though still below the International Biochar Initiative (IBI) recommended limit (6 mg/kg for Σ16 EPA-PAHs) (Adánez-Rubio et al., 2021). CR showed the lowest total PAHs content (75.76 μg/kg), approximately ten times lower than SS. Notably, high total PAH content does not necessarily correlate with a high bioavailable fraction, highlighting variability in PAHs bioavailability across BC types.

Analysis of surface functional groups (Figure 1) revealed that the percentage of C=C sp^2 carbon followed the order: BG > SF> SS> CR, whereas for C-C sp^3 carbon, CR>SS> BG> SF. These results indicate that a predominance of aliphatic carbon is advantageous for soil application, as aliphatic C is more readily mineralized by soil microorganisms. Among oxygen-containing functional groups, hydroxyl groups (-OH) are key to BC hydrophilicity (Fan et al., 2022), and their highest abundance was found in SS and CR. Overall, CR was characterized by the lowest percentage of C=C sp^2 and the highest C-C sp^3 carbon, along with the presence of carbonates. Carbonyl groups were dominant in BG and SF, while carboxyl groups prevailed in SS. Hydroxyl groups were attached to the aromatic moieties in CR and aliphatic chains in both CR and SF. Another important safety parameter is metal content (Table S1). As expected, SS had the highest content of metals, including essential micronutrients like Fe, Cu, Mn, Ni, and Zn, at levels several orders of magnitude higher than in other BCs. While metal concentrations in all BCs remained below the IBI recommendations (IBI International Biochar Initiative, 2015), the high Zn content in SS warrants attention. Zinc is crucial for photosynthesis, and its supplementation is often recommended (Stanton et al., 2022). CR had the lowest overall metal content, although its Fe level was higher than in the other plant-derived BC.

Soil enriched with CR was characterized by the highest content of macroelements (Mg, K, and Ca), microelements (Fe and Mn), and HM (Co, Cr, Cd, and Pb). In contrast, the SS-BC amendment was associated with the lowest overall levels of macro- and microelements (Mg, Fe, and Mn) in the soil. Additionally, SS contributed the highest concentrations of P, Cu, and Zn to the soil (Table S2).

Table 1. Physicochemical properties of the obtained biochars.

Material	pH	Ash (%)	C (%)	H (%)	N (%)	O (%)	TOC (mg/g)	S_{BET} (m^2/g)	C_{free} (ng/L)	C_{total} (μg/kg)
BG	10.7c	25.9c	62.9b	1.20b	2.57c	7.43c	631b	0.94a	0.86d	345.9c
SS	8.06a	62.8d	23.5a	0.57a	3.66d	9.50d	327a	1.14b	0.46c	766.5d
SF	9.26b	6.70a	86.7d	1.67c	0.96a	3.96a	878c	26.1c	0.12a	270.4b
CR	9.60b	14.1b	76.7c	2.71d	1.30b	5.18b	818c	90.1d	0.16b	75.76a

TOC – total organic carbon

S_{BET} – specific surface area

C_{free} – a bioavailable fraction of PAHs

C_{total} – total content of PAHs

The same letters (a–d) in columns are assigned to the same homogeneous groups, n = 3

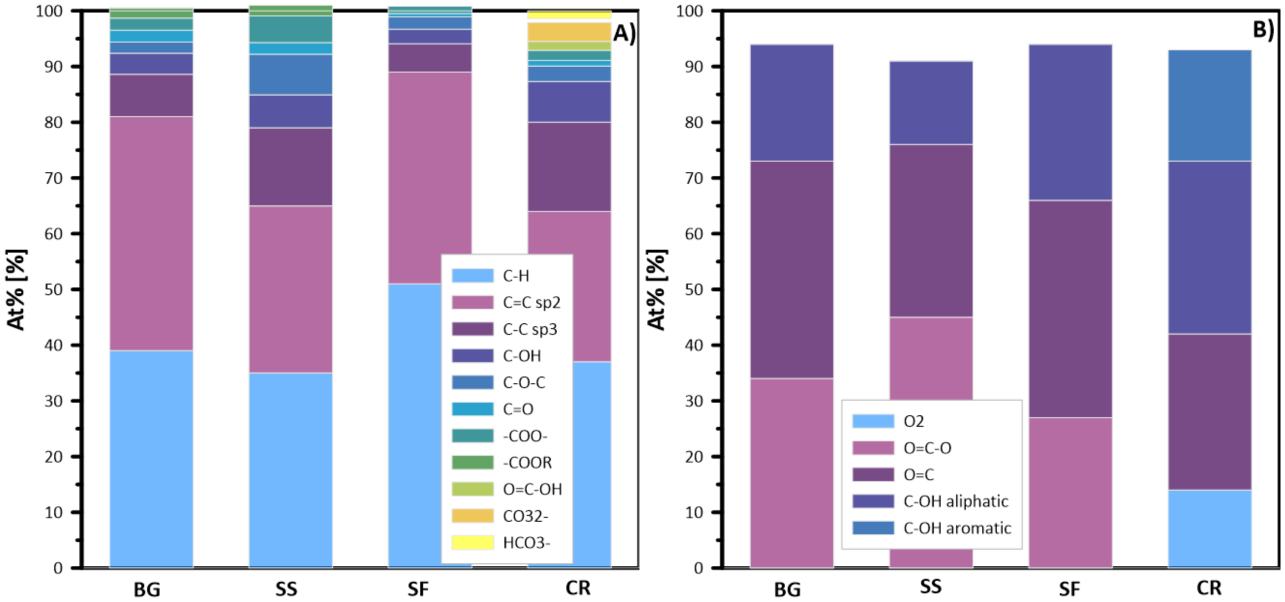


Figure 1. Results of A) C1s and B) O1s XPS analysis of the obtained biochars.

3.2. Properties of lettuce cultivated in the PAE-polluted soil

3.2.1. Content of PAEs in the lettuce

In the control sample (without any PAEs), the content of six PAEs (Σ6 PAEs), both in the roots and leaves of lettuce, was below the limit of detection (LOD) (Table 2). The addition of BG and SF to the soil did not affect the concentration of six PAEs in lettuce leaves. SS slightly increased their content, with the highest concentration reaching 52.8 µg/g dry mass. The most efficient BC in reducing PAEs in lettuce leaves was CR. However, the impact of tested BC on PAEs content in lettuce roots differed. SF was the only BC that lowered the content of six PAEs in lettuce roots. In contrast, BG, SS, and CR increased PAEs levels in the roots. The obtained levels of six PAEs were below the range observed for leafy (~75 µg/g) and root vegetables (~170 µg/g) collected from plastic film greenhouses and open fields in China (Feng et al., 2025). From a practical perspective, the key concern is the PAEs content in the lettuce leaves rather than in the roots. Therefore, CR is recommended as an effective BC for immobilizing PAEs in soil and reducing their bioavailability to edible plant parts.

To study the transport and translocation of PAEs and metals in the lettuce leaves in comparison to lettuce roots, the Translocation Factor, T_F, was used:

$$T_F = \frac{c_L}{c_R}$$

Where c_L is the concentration of PAEs and metals in lettuce leaves, c_R is the concentration in the lettuce roots (Boros-Lajszner et al., 2020). T_F values indicate that under PAEs pollution, the transport of PAEs to lettuce leaves was facilitated. In general, BC amendments slightly reduced this translocation. However, a slight increase in PAEs concentration in the leaves was observed with the use of SF. CR proved to be the most effective BC in immobilizing PAEs, as it resulted in lower concentration in both roots and leaves, along with reduced translocation from roots to leaves.

Table 2. Content of six PAEs in lettuce roots and leaves.

Sample	Σ6 PAEs in lettuce roots	Σ6 PAEs in lettuce leaves	T_F
	(μg/g dry mass)		(-)
Soil	<LOD	<LOD	-
PAEs	13.4 ± 0.411b	49.5 ± 0.850b	3.72b
BG-BC	14.5 ± 0.536c	50.4 ± 0.283b	3.48b
SS-BC	15.6 ± 0.485d	52.8 ± 1.64c	3.38b
SF-BC	11.8 ± 0.264a	49.9 ± 0.776b	4.23b
CR-BC	17.5 ± 0.612e	28.4 ± 0.660a	1.62a

LOD – limit of detection

The same letters (a-e) in columns are assigned to the same homogeneous groups, n = 3

3.2.2. Fresh and dry mass of lettuce

Soil contamination with PAEs did not significantly affect the mass of lettuce compared to plants grown in non-polluted soil. Only a slight decrease in leaf fresh mass and a slight increase in both fresh and dry root mass, as well as leaf dry mass, were observed in the presence of PAEs (Table S3). The BC amendment did not influence the fresh mass of the lettuce leaves. Contrary to findings in the literature, where BC addition to the soil increases both fresh and dry mass (Massaccesi et al., 2024), our study involved lettuce grown in PAE-polluted soil.

Among the tested biochars, CR-BC was the most effective - plants grown with its addition showed the highest fresh leaf mass and the greatest fresh and dry root mass. Even under PAEs contamination, lettuce growth was not significantly hindered, and BC, as a nutrient source, contributed to increased biomass. In contrast, BG-BC caused a notable reduction in lettuce mass, likely due to its high pH (10.7), which may disrupt soil biological processes. For instance, enzymatic activities such as those of glycosidases (optimal pH 4-6), and proteolytic or oxidative enzymes (optimal pH 7-9), as well as soil microbial activity (optimal pH 5.5-8.8), could be negatively impacted (Neina, 2019). Overall, these results suggest that BC application to PAE-polluted soil can enhance lettuce growth through nutritional and detoxifying effects, though the outcome strongly depends on the properties and feedstock of the biochar used.

To establish the effect of PAEs on the lettuce in BC-amended soil, the Tolerance Index (T_i) based on plant yield was used:

$$T_i = \frac{m_{R/L}}{m_{CR/CL}}$$

Where $m_{R/L}$ is the dry mass of the lettuce roots or leaves (g), and $m_{CR/CL}$ is the dry mass of the lettuce roots or leaves in the control. T_i describes the ability of plants to counter the adverse effects of soil pollution (Boros-Lajszner et al., 2020).

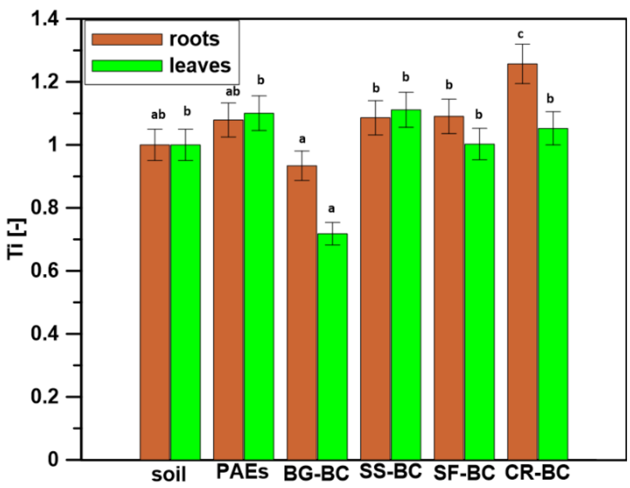


Figure 2. Tolerance index (T_i) for lettuce grown in PAEs-polluted soil amended with BC.

The T_i values did not change following PAEs pollution, indicating that at the tested PAEs concentration, lettuce was able to grow (Figure 2). However, two amendments had a significant impact on lettuce mass: BG-BC harmed leaves, while CR-BC had a positive effect on roots. These results highlight that the type of biochar is strongly linked to the observed changes.

3.2.3. Content of pigments

Chlorophyll a (Chl a) and chlorophyll b (Chl b) coexist in plant tissues at a ratio of 3:1, although their content may vary depending on environmental conditions. Low chlorophyll content can serve as an indicator of environmental pollution and stress (Ghosh et al., 2018). The presence of PAEs in the soil can alter the content of photosynthesis pigments like Chl a, Chl b, and carotenoids in plants. Carotenoids, another group of plant pigments, are found in both photosynthetic tissues like leaves and in non-photosynthetic organs such as roots and flowers (Dhami and Cazzonelli, 2020). These pigments protect cells from damage caused by visible radiation and assist in transferring energy to chlorophyll during photosynthesis (Sun et al., 2022). Four major carotenoids - lutein (40-57%), β-carotene (25-40%), violaxanthin (9-20%), and neoxanthin (5-15%) are typically found in chloroplasts, forming chlorophyll-carotenoid-protein complexes (Gross, 1991). In lettuce, the total carotenoid content has been estimated at 68 μg per gram of fresh weight, with molar ratios of light-harvesting pigments as follows: Chl a (100), Chl b (67.0), total carotenoids (49.7), lutein (26.7), β-carotene (1.70), neoxanthin (9.50), and violaxanthin (9.80) (Gross, 1991). Our results show that both PAEs and BC types significantly affect pigment content in lettuce leaves (Table 3). The highest pigment concentrations were found in plants grown in PAE-contaminated soil without any biochar addition. These findings contrast with those of Ma et al. (Ma et al., 2018), who observed reduced Chl a and carotenoid content in lettuce under various PAEs, although similar to our results, they noted no effect of DEHP on Chl b content.

Only CR had a positive effect on Chl a levels, while both SF and CR increased Chl b content. The lowest Chl a and Chl b levels were found in leaves from plants grown in soil amended with sewage sludge, which contained high levels of phosphorus and PO_4^{3-}. Excessive phosphate may lead to chlorosis in plants (Fan et al., 2021). Moreover, reduced chlorophyll content can occur when metal ions such as Cu^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+} replace Mg^{2+} in the chlorophyll molecule (Myśliwa-Kurdziel and Strzałka, 2002). All tested biochars reduced carotenoid content in comparison to the control sample, contrary to results by Jabborova et al. (Jabborova et al., 2021), who reported increased carotenoid levels in lettuce leaves under the BC amendment; however, their study did not assess BC effects under PAE pollution.

Under normal growing conditions, the chlorophyll/carotenoid ratio remains stable. However, under high temperatures, nutrient stress, or during leaf senescence, chlorophyll content declines more rapidly than carotenoids, altering the ratio (C. He et al., 2023). Thus, chlorophyll and carotenoid levels can be used to assess plant physiological responses to environmental stress. The highest chlorophyll/carotenoid ratio was found in leaves exposed only to PAEs, suggesting delayed aging in these plants. The lowest ratio was observed in the control sample. The addition of all BC types to PAE-polluted soil reduced the chlorophyll/carotenoid ratio, yet still mitigated plant aging compared to the untreated control.

Table 3. Content of photosynthesis pigments in lettuce leaves.

Sample	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll/carotenoids
		(µg/g fresh mass)		(-)
Soil	369.2 ± 6.5bc	96.04 ± 0.46b	138.9 ± 2.38e	3.349a
PAEs	483.8 ± 8.1d	139.0 ± 3.30d	146.1 ± 1.29f	4.264c
BG-BC	295.6 ± 7.0a	93.19 ± 1.32a	103.4 ± 0.75b	3.762b
SS-BC	289.5 ± 4.5a	91.11 ± 1.39a	100.6 ± 0.86a	3.782b
SF-BC	357.1 ± 6.6b	98.67 ± 2.22b	116.6 ± 2.86c	3.908bc
CR-BC	380.6 ± 5.7c	110.7 ± 1.82c	124.1 ± 3.39d	3.959bc

The same letters (a–f) in columns are assigned to the same homogeneous groups, n = 3

3.2.4. Metal content and its translocation within lettuce

The mineral composition analysis of lettuce roots and leaves revealed some differences between samples (Table S4). Both soil contamination with PAEs and the addition of BC affected the mineral content of the harvested plants. Some of the changes were beneficial, such as a reduction in the concentrations of HM, like Cd, Pb, Hg, and Cr, and an increase in essential elements such as K, P, Ca, Mg, and Fe. Enriching the soil with various types of BC increased the content of K and B (all BC types), P (BG-BC), Cu and Zn (SS-BC, SF-BC, and CR-BC), Mn (BG-BC, SF-BC, and CR-BC), and Ca (CR-BC) in lettuce. In a study conducted by Bilias et al. (Bilias et al., 2024), sewage sludge-derived BC significantly increased the P concentration in lettuce, decreased Fe and Cu levels, and did not affect Ca, Mg, Zn, and Mn concentrations.

The addition of BC to the soil also reduced the content of HM: Pb (reduced by BG-BC, SF-BC, and CR-BC), Cd (reduced by all BC types), Hg (reduced in roots by SS-BC and CR-BC, and in leaves by SS-BC and SF-BC), and Cr (reduced by BG-BC and CR-BC). Similar results were reported by Ibrahim et al. (Ibrahim et al., 2019), where peanut shell- and corn-cob-derived BC reduced the accumulation of Cr, Cd, Pb, Ni, and As in lettuce. The highest levels of Mg, Fe, and Ca were observed in lettuce leaves from plants grown in PAE-polluted soil without any BC addition. Similar high levels of Fe (194.76 mg/kg) and other metals (following the ratio Zn > Cu > Ni> Pb > Cr > Cd) were noted in lettuce grown in PAEs-polluted soil (Atamaleki et al., 2021). Mg and Fe are essential for chlorophyll synthesis, and their elevated levels correlate with the highest concentrations of both Chl a and Chl b in these plants.

Considering T_F values (Table 4), the BG-BC amendment enhanced the translocation of macroelements (Mg and P), microelements (Fe, Cu, Mn), and heavy metal (Co) to the lettuce leaves. In contrast, CR-BC reduced the translocation of Cd and Pb from roots to leaves. The greatest reduction in T_F was observed for B (from 14 to 2-3), an essential element involved in cell wall biosynthesis and structural integrity (Petridis et al., 2013).

Table 4. T_F of selected micro- and macroelements in lettuce. Numbers followed by different letters are statistically different $p \leq 0.05$ (Tukey's test).

	macroelements				microelements					heavy metals				
	Mg	P	K	Ca	Fe	Cu	B	Zn	Mn	Co	Cr	Cd	Pb	Hg
Soil	0.341a	1.104c	1.873c	0.755b	0.311b	0.747ab	14.234b	0.631a	0.694a	0.944b	0.343b	2.439a	4.644c	1.568a
PAEs	0.427b	0.974b	1.715bc	0.915c	0.350b	1.087b	2.010a	0.854ab	0.805a	0.136a	0.260ab	0.846bc	0.945a	2.587b
BG-BC	0.621c	1.273d	1.891c	0.935c	0.452c	1.889c	2.547a	1.022b	1.160c	2.176c	0.072a	1.132b	1.283ab	3.194b
SS-BC	0.366a	0.779a	1.205a	0.802b	0.294b	0.461a	1.248a	1.652c	0.755a	1.083b	0.427b	0.613bc	1.382ab	1.346a
SF-BC	0.375a	0.827a	1.154a	0.764b	0.246ab	0.638ab	1.767a	0.676a	0.715a	0.632ab	0.886c	0.492c	2.033b	1.679a
CR-BC	0.355a	0.961b	1.554b	0.435a	0.199a	0.622ab	2.753a	0.456a	0.983b	1.060b	0.219b	0.818bc	1.457ab	2.963b

3.2.5. Microscopic studies

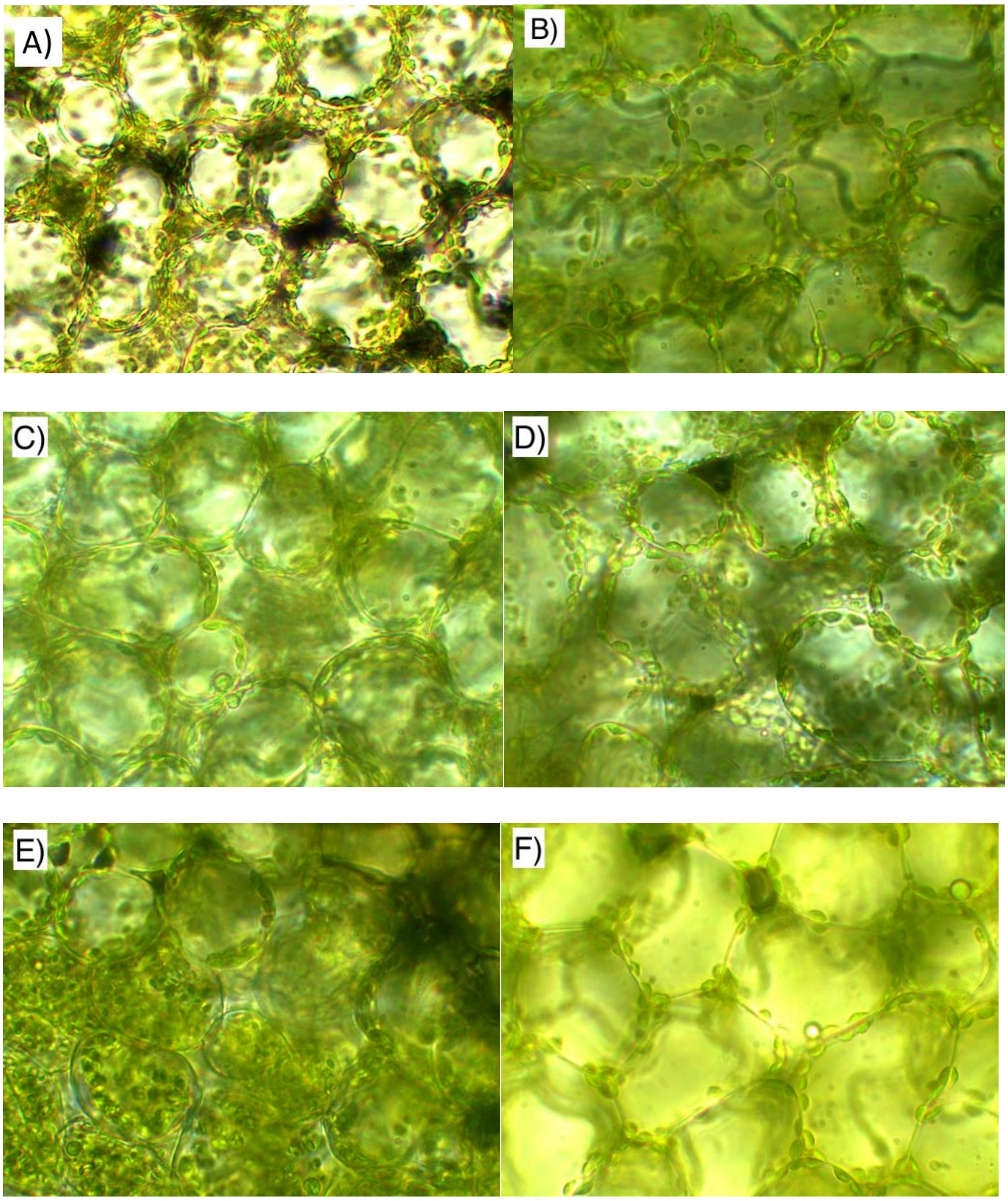
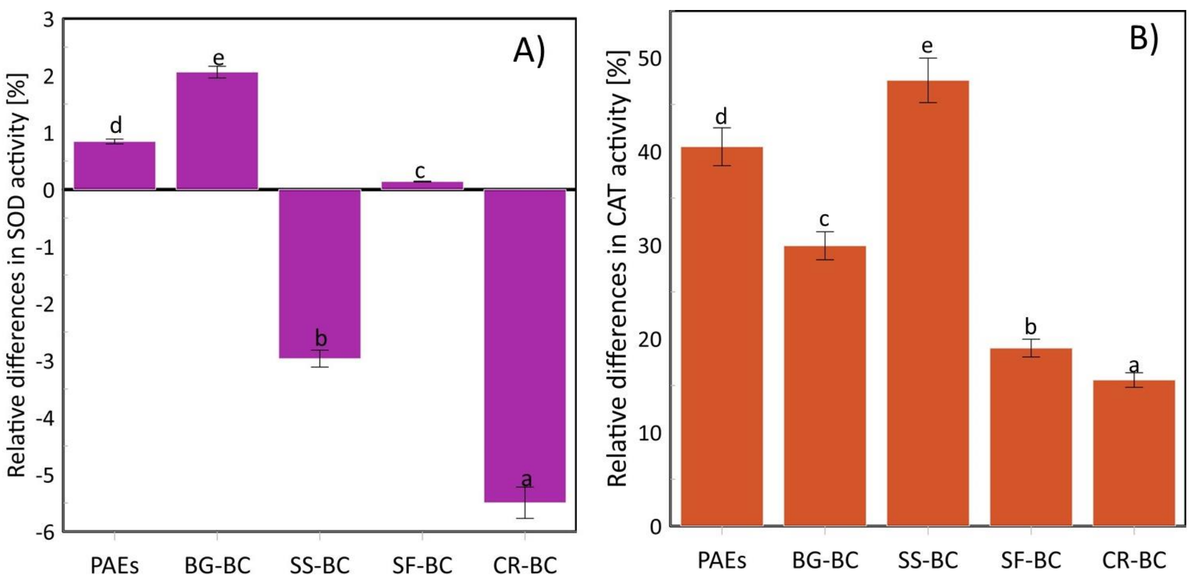


Figure 3. The microscopic photos of lettuce leaf cells: A) Soil, B) PAEs, C) BG-BC, D) SS-BC, E) SF-BC, F) CR-BC.

Microscopic images of lettuce leaf cells (Figure 3) reveal differences between the various soil amendments. In all samples, chloroplasts were located near the cell walls; however, the number of chloroplasts varied between treatments. PAEs can alter the internal structure of chloroplasts (Zhuang et al., 2024). Leaf samples from plants grown in control soil had the highest number of chloroplasts (Figure 3A). Plants grown in PAEs-contaminated soil amended with BG and SS (Figures 3B, 3C, and 3D, respectively) showed a slightly lower number of chloroplasts. The lowest number of chloroplasts was observed in the sample from PAE-polluted soil amended with CR (Figure 3F).

3.2.6. Enzymatic activity of lettuce

Plant cells contain various enzymatic and non-enzymatic antioxidants that protect them from the harmful effects of ROS. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), peroxiredoxins (Prx), and thioredoxins (Trx), along with non-enzymatic antioxidants such as vitamins (e.g., vitamin C, ascorbic acid, vitamin E, tocopherols, provitamin A, carotenes) and glutathione (GSH), work together to defend plants against oxidative stress (Gupta et al., 2018). SOD, the first line of defense against superoxide radicals, catalyzes their dismutation into O_2 and H_2O_2, whereas CAT catalyzes the decomposition of hydrogen peroxide into H_2O and O_2 (Elavarthi and Martin, 2010).



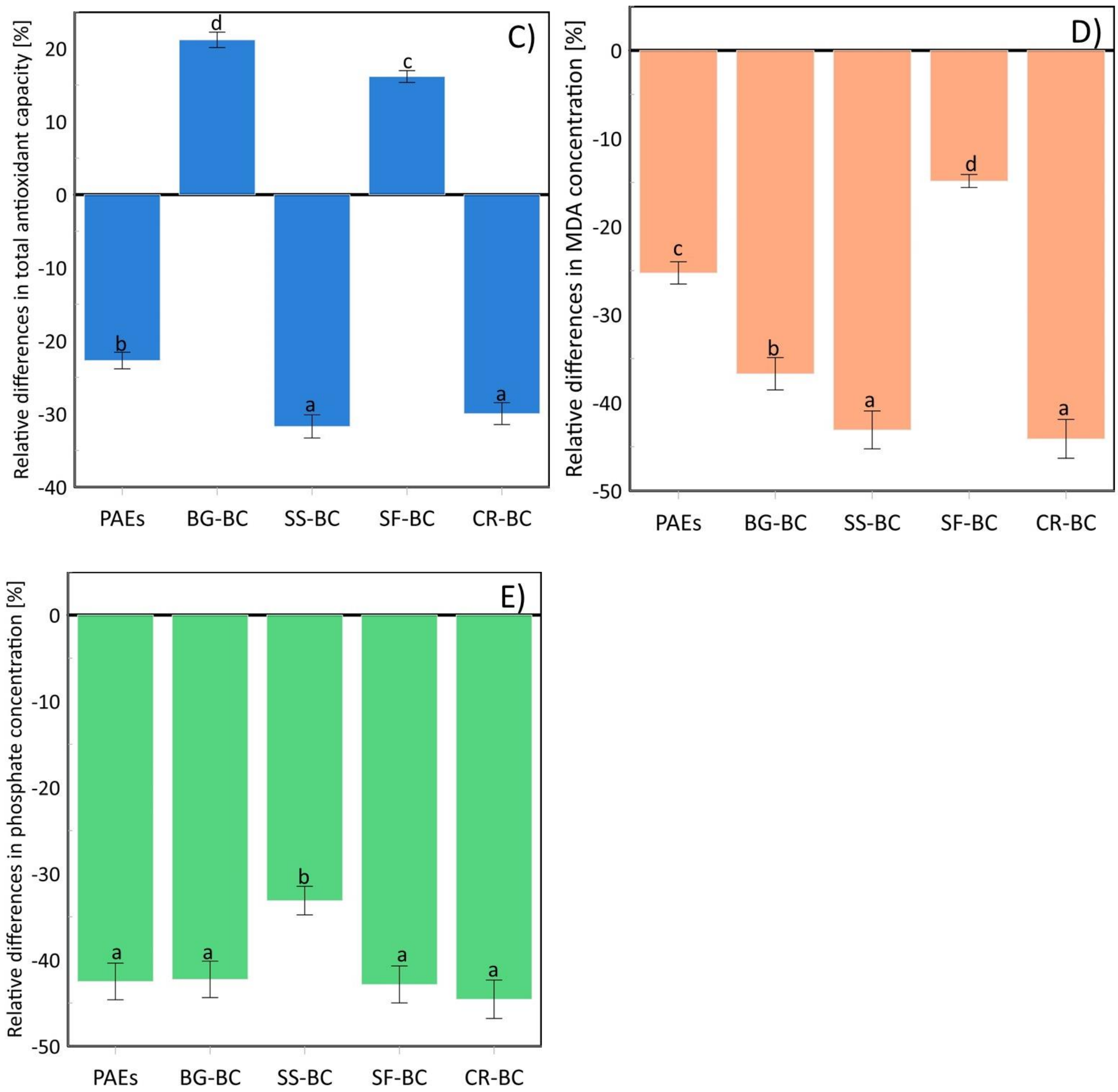


Figure 4. The relative enzymatic activity in the lettuce leaves in comparison to the control A) SOD, B) CAT, C) TAC, D) MDA, and E) Phosphates. Numbers followed by different letters are statistically different, $p \leq 0.05$ (Tukey's test).

PAE pollution has induced oxidative stress in lettuce leaves, as evidenced by higher SOD activity compared to the blank sample (Figure 4A). The lowest SOD activity (5.5% lower than in the control soil) was observed in plants grown in the CR-BC, indicating that biochar derived from corn helped alleviate oxidative stress in the tested plants. A similar, though less effective, result was noted for SS-BC. These findings are consistent with previous reports showing that biochar amendments reduce oxidative stress in lettuce exposed to salt stress (Sahin et al., 2025). The highest SOD activity - only slightly higher than in the blank sample (by 2.1%) - was recorded in lettuce leaves from the BG and SF treatments. Once again, BG-BC appeared to have a negative impact on lettuce properties.

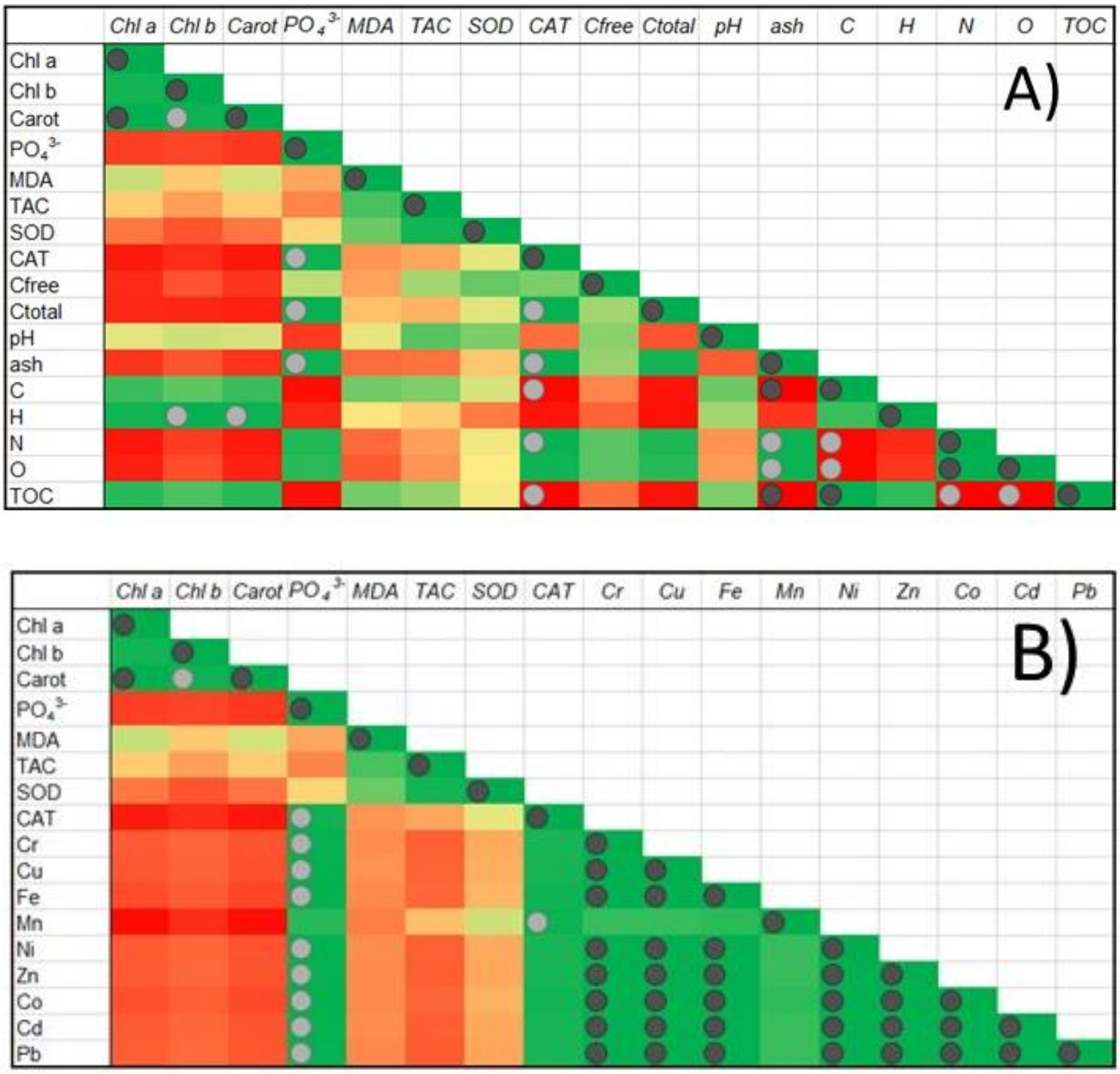
Soil contamination with PAEs resulted in increased CAT activity in all samples (Figure 4B), confirming the oxidative stress in lettuce. PAE pollution led to a 40% increase in CAT activity. Additionally, the addition of SS further increased CAT activity by up to 47%, likely due to the high content of HM in this material, which is known to induce oxidative stress (Bhavya et al., 2022). The beneficial effect of BC amendments in reducing oxidative stress, evidenced by decreased CAT activity, has been highlighted in the literature (Helaoui et al., 2023; Sahin et al., 2025). In this study, the addition of all other types of BC reduced CAT activity in the tested plants compared to the sample exposed to PAEs alone. This suggests that plants grown in soil amended with these biochars experienced less oxidative stress. The lowest CAT activity was observed in leaves from the CR treatment, being only 15% higher than in the blank sample but 22% lower than in the PAE-contaminated soil.

The combined antioxidative activity of these compounds is referred to as total antioxidant capacity (TAC) (Bartosz, 2003), which serves as an indicator of a plant’s response to environmental stress. A lower TAC value (e.g., reduced levels of antioxidative enzymes) suggests that the plant is less resistant to oxidative stress and was grown under less stressful conditions. TAC is determined by the concentration of all antioxidant compounds, with SOD and CAT activities being the primary contributors. The results for TAC (Figure 4C) showed a strong correlation with SOD activity in the tested plants. The highest TAC value was observed in plants grown in PAE-polluted soil with BG amendment (2687.3 μmol). In contrast, a 56–57% reduction in TAC was noted for SS-BC and CR-BC treatments, confirming that the addition of sewage sludge- or corn-derived biochar mitigates oxidative stress in lettuce grown not only in HM-polluted soil, as reported in the literature (Ibrahim et al., 2019), but also in PAE-polluted soil.

The determination of MDA concentration in lettuce leaves was another method used to assess oxidative stress in lettuce grown in PAE-polluted soil amended with BC. MDA, a marker of lipid peroxidation and loss of membrane integrity, is formed both non-enzymatically (induced by ROS) and enzymatically (through hydroperoxide activity followed by lipoxygenase action) (Morales and Munné-Bosch, 2019). Higher MDA concentrations in plants indicate greater oxidative stress. In our study, the highest MDA concentration was observed in plants from the control sample (Figure 4D), while BC addition reduced MDA levels, indicating decreased lipid peroxidation. The lowest MDA concentration was recorded in plants treated with CR-BC (2.53 μmol/L). The reduction in MDA content due to BC addition was consistent with findings by Younis et al. (Younis et al., 2015), who reported that BC application reduced MDA levels in spinach grown in Ni-contaminated soil. However, sunflower-derived BC was the exception, increasing MDA concentration by 14%.

Determining the free phosphate concentration liberated from peptides, proteins, or phospholipids may give some information about plant development. P is an essential constituent of many compounds in plants like phospholipids, nucleic acids, and ATP, and also takes part in many metabolic processes (Smith et al., 2003). The highest phosphate concentration was determined in leaves from plants from the blank sample (Figure 4E), and the lowest, again, in plants from the sample with PAEs and CR-BC amendment (a 55% reduction). The BC amendment lowered the phosphate content in all samples. The highest phosphate concentration was determined in the lettuce that grew on the soil with SS-BC addition. It may be the result of a high concentration of P in BC obtained from sewage sludge (2.3 %At). It is also confirmed by Woldetsadik et al., (Woldetsadik et al., 2017) that BC rich in P is a significant source of P and has a positive effect on the P concentration in lettuce tissues.

3.2.7. Interaction of PAEs, BC, soil, and lettuce properties



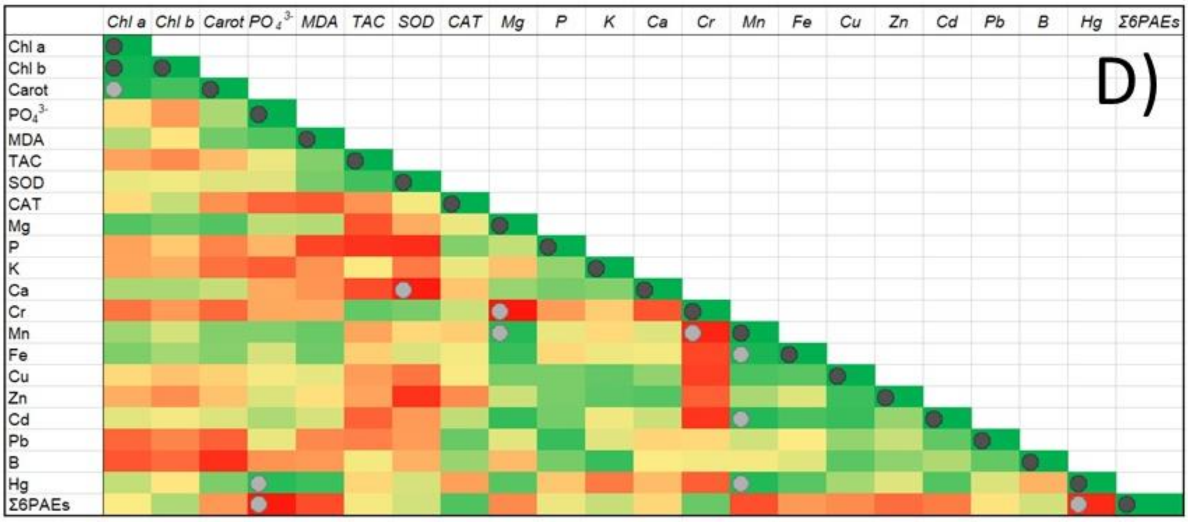


Figure 5. Heatmap visualization for Pearson correlations: A) between lettuce response and BC properties, B) between lettuce response and metals content in BC, C) between lettuce response and metals and PAEs content in lettuce leaves, D) between lettuce response and metals and PAEs content in lettuce roots. • $p < 0.05$, • $p < 0.01$, the intensity of colors: green (positive correlation) and red (negative correlation) shows the differences.

The properties of BC added to the soil contaminated with PAEs affected the plants' properties. Aromatic moieties in BC promoted the photosynthetic activity of Chl b and carotenoids ($p < 0.05$, Figure 5A). Higher levels of total PAHs and ash content in BC increased the concentration of PO_4^{3-} in lettuce leaves. An increase in metal content in BC (including Cr, Cu, Fe, Ni, Zn, Co, Cd, and Pb) was also associated with elevated phosphate levels in lettuce leaves (Figure 5B). The PO_4^{3-} concentration in lettuce leaves was correlated with CAT activity ($p < 0.05$) (Figure 5B). The increased phosphate concentration may reflect a plant response to oxidative stress, such as the simultaneous rise in HM content in BC and CAT activity in lettuce. ROS can damage phospholipid membranes, leading to the release of PO_4^{3-} (Pamplona, 2008). Additionally, a higher Mn content in BC enhanced CAT activity in lettuce leaves (Figure 5B). Excess of Mn in soil can induce oxidative stress in plants, triggering an increase in antioxidant enzyme activities, including CAT (Srivastava and Dubey, 2011).

There are also some correlations between mineral composition and the enzymatic activity of lettuce. A very strong positive correlation ($p < 0.01$) was observed between Cd and phosphate content in lettuce leaves (Figure 5C), along with significant positive correlations ($p < 0.05$) between phosphate and B concentrations. The promoting effect of combined BC and phosphorus fertilizer on the Cd uptake was confirmed by Li et al. (Li et al., 2022). In our study, the highest Cd concentration was found in plants from the sample amended with SS addition, which also had the highest P content among the tested materials. Significant positive correlations were also observed between the content of various metals in lettuce leaves, for example between Ca and Mg (Figure 5C). Such correlations were also reported by Fan et al., (Fan et al., 2021). Higher SOD activity was associated with higher Fe concentration in leaves (Figure 5C). In lettuce roots, the concentration of certain metals was correlated with others. For example, higher Mg content was associated with reduced Cr levels, whereas higher Cr content led to reduced Mn content (Figure 5D). Additionally, Mn increased the concentrations of Mg, Fe, Cd, and Hg in roots. The concurrent increase in Mn, Mg, and Fe supports the concept of cross-talk between macro- and microelements in plants, as noted by Fan et al. (Fan et al., 2021).

Phosphate concentrations in lettuce leaves also increased with higher Hg levels in the roots. Conversely, high Ca concentrations in roots were associated with decreased SOD activity in the leaves. Both Chl b and carotenoid contents increased with rising levels of Chl a. The correlation between Chl a and Chl b was stronger ($p < 0.01$) than that between Chl a and carotenoids ($p < 0.05$) (Figures 5C and 5D).

The content of the six tested PAEs in lettuce leaves and roots was correlated with the mineral composition of plants (Figure 5C and Figure 5D). The bioavailability of PAEs in lettuce leaves showed a very strong negative correlation ($p < 0.01$) with phosphate concentration (Figure 5C). The uptake and translocation of Cd from roots to leaves were inhibited by the presence of PAEs. A significant negative correlation ($p < 0.05$) was also observed between B concentration and PAEs concentration in lettuce leaves. In lettuce roots, PAEs concentration was negatively correlated with phosphate concentration and Hg content (Figure 5D).

Conclusions

Lettuce cultivated in PAEs polluted soil exhibited oxidative stress as evidenced by increased SOD and CAT activities. The addition of BC to the soil alleviated oxidative stress in plants. The presence of PAEs in the soil also affected the other physiological parameters of lettuce. Although the highest contents of Chl a and Chl b were observed in the lettuce leaves grown in the soil contaminated with PAEs, these plants had the lowest mass. The beneficial effects of enriching the soil with BC were also evident in the mineral composition of the lettuce. All tested BC types increased the concentrations of certain macro- and microelements while reducing heavy metal content. The most beneficial effects were observed with biochar obtained from corn. Plants grown in the CR-BC-amended soil had the highest biomass, were not exposed to oxidative stress, and accumulated fewer PAEs compared to plants grown in the soil contaminated with PAEs and without any BC addition.

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Author Contribution

A.S. Investigation, Visualization, Writing original draft; P.P. Investigation; M.D. Investigation; J.S. Investigation; P.O. Supervision; Y.G. Supervision; B.C. Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Visualization; Writing - original draft; and Writing - review & editing.

Declaration of Competing Interest

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.

Supporting Information Description

In the Supporting Information, three tables (Table S1-S4) are attached.

References

Adánez-Rubio, I., Fonts, I., De Blas, P., Viteri, F., Gea, G., Alzueta, M.U., 2021. Exploratory study of polycyclic aromatic hydrocarbons occurrence and distribution in manure pyrolysis products. Journal of Analytical and Applied Pyrolysis 155, 105078. https://doi.org/10.1016/j.jaap.2021.105078

Atamaleki, A., Yazdanbakhsh, A., Fallah, S., Hesami, M., Neshat, A., Fakhri, Y., 2021. Accumulation of potentially harmful elements (PHEs) in lettuce (Lactuca sativa L.) and coriander (Coriandrum sativum L.) irrigated with wastewater: a systematic review and meta-analysis and probabilistic health risk assessment. Environ Sci Pollut Res 28, 13072–13082. https://doi.org/10.1007/s11356-020-12105-z

Bartosz, G., 2003. Total antioxidant capacity, in: Spiegel, H.E., Nowacki, G., Hsiao, K.-J. (Eds.), Advances in Clinical Chemistry. Elsevier, pp. 219–292. https://doi.org/10.1016/S0065-2423(03)37010-6

Bhavya, G., Hiremath, K.Y., Jogaiah, S., Geetha, N., 2022. Heavy metal-induced oxidative stress and alteration in secretory proteins in yeast isolates. Arch Microbiol 204, 172. https://doi.org/10.1007/s00203-022-02756-6

Bilias, F., Tsolis, V., Zafeiriou, I., Koukounaras, A., Kalderis, D., Chlouveraki, E., Gasparatos, D., 2024. Effects of Sewage Sludge Biochar and a Seaweed Extract-Based Biostimulant on Soil Properties, Nutritional Status and Antioxidant Capacity of Lettuce Plants in a Saline Soil with the Risk of Alkalinization. J Soil Sci Plant Nutr 24, 7271–7287. https://doi.org/10.1007/s42729-024-02039-7

Boros-Lajszner, E., Wyszkowska, J., Kucharski, J., 2020. Application of white mustard and oats in the phytostabilisation of soil contaminated with cadmium with the addition of cellulose and urea. J Soils Sediments 20, 931–942. https://doi.org/10.1007/s11368-019-02473-6

Chen, L., Yu, L., Han, B., Li, Y., Zhang, J., Tao, S., Liu, W., 2024. Pollution characteristics and affecting factors of phthalate esters in agricultural soils in mainland China. Journal of Hazardous Materials 466, 133625. https://doi.org/10.1016/j.jhazmat.2024.133625

Dhami, N., Cazzonelli, C.I., 2020. Environmental impacts on carotenoid metabolism in leaves. Plant Growth Regul 92, 455–477. https://doi.org/10.1007/s10725-020-00661-w

Elavarthi, S., Martin, B., 2010. Spectrophotometric Assays for Antioxidant Enzymes in Plants, in: Sunkar, R. (Ed.), Plant Stress Tolerance: Methods and Protocols. Humana Press, Totowa, NJ, pp. 273–280. https://doi.org/10.1007/978-1-60761-702-0_16

Fan, M., Li, C., Shao, Y., Zhang, S., Gholizadeh, M., Hu, X., 2022. Pyrolysis of cellulose: Correlation of hydrophilicity with evolution of functionality of biochar. Science of The Total Environment 825, 153959. https://doi.org/10.1016/j.scitotenv.2022.153959

Fan, X., Zhou, X., Chen, H., Tang, M., Xie, X., 2021. Cross-Talks Between Macro- and Micronutrient Uptake and Signaling in Plants. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.663477

Feng, N.-X., Pan, B., Huang, H.-J., Huang, Y.-T., Lyu, H., Xiang, L., Zhao, H.-M., Liu, B.-L., Li, Y.-W., Cai, Q.-Y., Li, D.-W., Mo, C.-H., 2025. Uptake, translocation, and biotransformation of phthalate acid esters in crop plants: A comprehensive review. Journal of Hazardous Materials 489, 137580. https://doi.org/10.1016/j.jhazmat.2025.137580

Gao, Y., Shao, G., Yang, Z., Zhang, K., Lu, J., Wang, Z., Wu, S., Xu, D., 2021. Influences of soil and biochar properties and amount of biochar and fertilizer on the performance of biochar in improving plant photosynthetic rate: A meta-analysis. European Journal of Agronomy 130, 126345. https://doi.org/10.1016/j.eja.2021.126345

Ghosh, P., Das, P., Mukherjee, R., Banik, S., Karmakar, S., Chatterjee, S., 2018. EXTRACTION AND QUANTIFICATION OF PIGMENTS FROM INDIAN TRADITIONAL MEDICINAL PLANTS: A COMPARATIVE STUDY BETWEEN TREE, SHRUB AND HERB.

Gross, J., 1991. Carotenoids, in: Pigments in Vegetables. Springer US, Boston, MA, pp. 75–278. https://doi.org/10.1007/978-1-4615-2033-7_3

Guo, W., Zhang, Z., Zhu, R., Li, Z., Liu, C., Xiao, Hongwei, Xiao, Huayun, 2024. Pollution characteristics, sources, and health risks of phthalate esters in ambient air: A daily continuous monitoring study in the central Chinese city of Nanchang. Chemosphere 353, 141564. https://doi.org/10.1016/j.chemosphere.2024.141564

Gupta, D.K., Palma, J.M., Corpas, F.J. (Eds.), 2018. Antioxidants and Antioxidant Enzymes in Higher Plants. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-75088-0

He, C., Sun, J., Chen, Y., Wang, L., Shi, S., Qiu, F., Wang, S., Tagesson, T., 2023. A new vegetation index combination for leaf carotenoid-to-chlorophyll ratio: minimizing the effect of their correlation. International Journal of Digital Earth 16, 272–288. https://doi.org/10.1080/17538947.2023.2168772

He, Y., Zhao, X., Zhu, S., Yuan, L., Li, X., Feng, Z., Yang, X., Luo, L., Xiao, Y., Liu, Y., Wang, L., Deng, O., 2023. Conversion of swine manure into biochar for soil amendment: Efficacy and underlying mechanism of dissipating antibiotic resistance genes. Science of The Total Environment 871, 162046. https://doi.org/10.1016/j.scitotenv.2023.162046

Helaoui, S., Boughattas, I., Mkhinini, M., Ghazouani, H., Jabnouni, H., Kribi-Boukhris, S.E., Marai, B., Slimani, D., Arfaoui, Z., Banni, M., 2023. Biochar application mitigates salt stress on maize plant: Study of the agronomic parameters, photosynthetic activities and biochemical attributes. Plant Stress 9, 100182. https://doi.org/10.1016/j.stress.2023.100182

Hilber, I., Bastos, A.C., Loureiro, S., Soja, G., Marsz, A., Cornelissen, G., Bucheli, T.D., 2017. The different faces of Biochar: contamination risk versus remediation tool. Journal of Environmental Engineering and Landscape Management 25, 86–104. https://doi.org/10.3846/16486897.2016.1254089

IBI International Biochar Initiative, 2015. Standardized product definition and product testing guidelines for biochar that is used in soil.

Ibrahim, M., Li, G., Chan, F.K.S., Kay, P., Liu, X.-X., Firbank, L., Xu, Y.-Y., 2019. Biochars effects potentially toxic elements and antioxidant enzymes in *Lactuca sativa* L. grown in multi-metals contaminated soil. Environmental Technology & Innovation 15, 100427. https://doi.org/10.1016/j.eti.2019.100427

Jabborova, D., Kadirova, D., Narimanov, A., Wirth, S., 2021. Beneficial effects of biochar application on lettuce (Lactuca sativa L.) growth, root morphological traits and physiological properties. https://doi.org/10.21276/ap.2021.10.2.13

Joseph, S., Cowie, A.L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M.L., Graber, E.R., Ippolito, J.A., Kuzyakov, Y., Luo, Y., Ok, Y.S., Palansooriya, K.N., Shepherd, J., Stephens, S., Weng, Z. (Han), Lehmann, J., 2021. How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. GCB Bioenergy 13, 1731–1764. https://doi.org/10.1111/gcbb.12885

Li, J., Zhang, S., Ding, X., 2022. Biochar combined with phosphate fertilizer application reduces soil cadmium availability and cadmium uptake of maize in Cd-contaminated soils. Environ Sci Pollut Res 29, 25925–25938. https://doi.org/10.1007/s11356-021-17833-4

Li, J., Zhou, X., Zhou, J., Shang, R., Wang, Y., Jing, P., 2020. Comparative Study on Several Determination Methods of Chlorophyll Content in Plants. IOP Conf. Ser.: Mater. Sci. Eng. 730, 012066. https://doi.org/10.1088/1757-899X/730/1/012066

Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, in: Methods in Enzymology, Plant Cell Membranes. Academic Press, pp. 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Ma, T., Zhou, W., Chen, L., Wu, L., Christie, P., Liu, W., 2018. Toxicity of phthalate esters to lettuce (Lactuca sativa) and the soil microbial community under different soil conditions. PLOS ONE 13, e0208111. https://doi.org/10.1371/journal.pone.0208111

Massaccesi, L., Nogués, I., Mazzurco Miritana, V., Passatore, L., Zacchini, M., Pietrini, F., Carloni, S., Marabottini, R., Moscatelli, M.C., Marinari, S., 2024. Short-term effects of biochar and compost on soil microbial community, C and N cycling, and lettuce (*Lactuca sativa* L.) yield in a Mediterranean environment. Applied Soil Ecology 199, 105411. https://doi.org/10.1016/j.apsoil.2024.105411

Molina, L., Segura, A., 2021. Biochemical and Metabolic Plant Responses toward Polycyclic Aromatic Hydrocarbons and Heavy Metals Present in Atmospheric Pollution. Plants 10, 2305. https://doi.org/10.3390/plants10112305

Morales, M., Munné-Bosch, S., 2019. Malondialdehyde: Facts and Artifacts. Plant Physiology 180, 1246–1250. https://doi.org/10.1104/pp.19.00405

Myśliwa-Kurdziel, B., Strzałka, K., 2002. Influence of Metals on Biosynthesis of Photosynthetic Pigments, in: Prasad, M.N.V., Strzałka, Kazimierz (Eds.), Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Springer Netherlands, Dordrecht, pp. 201–227. https://doi.org/10.1007/978-94-017-2660-3_8

Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Applied and Environmental Soil Science 2019, 5794869. https://doi.org/10.1155/2019/5794869

Oleszczuk, P., Kuśmierz, M., Godlewska, P., Kraska, P., Pałys, E., 2016. The concentration and changes in freely dissolved polycyclic aromatic hydrocarbons in biochar-amended soil. Environmental Pollution 214, 748–755. https://doi.org/10.1016/j.envpol.2016.04.064

Pamplona, R., 2008. Membrane phospholipids, lipoxidative damage and molecular integrity: A causal role in aging and longevity. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1777, 1249–1262. https://doi.org/10.1016/j.bbabio.2008.07.003

Petridis, A., Gasparatos, D., Haidouti, C., Paschalidis, C., Zamanidis, P., 2013. Effects of Nitrogen and Boron Fertilization on Lettuce Mineral Nutrition in a Calcareous Soil. Communications in Soil Science and Plant Analysis 44, 733–740. https://doi.org/10.1080/00103624.2013.748125

Reizer, E., Viskolcz, B., Fiser, B., 2022. Formation and growth mechanisms of polycyclic aromatic hydrocarbons: A mini-review. Chemosphere 291, 132793. https://doi.org/10.1016/j.chemosphere.2021.132793

Sahin, O., Gunes, A., Deniz Yagcıoglu, K., Kadioglu, Y.K., 2025. Exploring the impact of rice husk biochar on oxidative mechanisms and salt stress tolerance in lettuce plants. Journal of Plant Nutrition 48, 607–616. https://doi.org/10.1080/01904167.2024.2408419

Sharma, R., Kaur, R., 2020. Physiological and metabolic alterations induced by phthalates in plants: possible mechanisms of their uptake and degradation. Environmental Sustainability 3, 391–404. https://doi.org/10.1007/s42398-020-00141-x

Smith, F.W., Mudge, S.R., Rae, A.L., Glassop, D., 2003. Phosphate transport in plants. Plant and Soil 248, 71–83. https://doi.org/10.1023/A:1022376332180

Sokołowski, A., Dybowski, M.P., Oleszczuk, P., Gao, Y., Czech, B., 2024a. Biochar mitigates the postponed bioavailability and toxicity of phthalic acid esters in the soil. Science of The Total Environment 945, 173933. https://doi.org/10.1016/j.scitotenv.2024.173933

Sokołowski, A., Dybowski, M.P., Oleszczuk, P., Gao, Y., Czech, B., 2024b. Fast and reliable determination of phthalic acid esters in soil and lettuce samples based on QuEChERS GC–MS/MS. Food Chemistry 440, 138222. https://doi.org/10.1016/j.foodchem.2023.138222

Srivastava, S., Dubey, R.S., 2011. Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. Plant Growth Regul 64, 1–16. https://doi.org/10.1007/s10725-010-9526-1

Stanton, C., Sanders, D., Krämer, U., Podar, D., 2022. Zinc in plants: Integrating homeostasis and biofortification. Molecular Plant 15, 65–85. https://doi.org/10.1016/j.molp.2021.12.008

Sun, T., Rao, S., Zhou, X., Li, L., 2022. Plant carotenoids: recent advances and future perspectives. Mol Horticulture 2, 3. https://doi.org/10.1186/s43897-022-00023-2

Tuan Tran, H., Lin, C., Bui, X.-T., Ky Nguyen, M., Dan Thanh Cao, N., Mukhtar, H., Giang Hoang, H., Varjani, S., Hao Ngo, H., Nghiem, L.D., 2022. Phthalates in the environment: characteristics, fate and transport, and advanced wastewater treatment technologies. Bioresource Technology 344, 126249. https://doi.org/10.1016/j.biortech.2021.126249

Wang, D., Jiang, P., Zhang, H., Yuan, W., 2020. Biochar production and applications in agro and forestry systems: A review. Science of The Total Environment 723, 137775. https://doi.org/10.1016/j.scitotenv.2020.137775

Wang, Huan, Li, C., Yan, G., Zhang, Y., Wang, Haiyan, Dong, W., Chu, Z., Chang, Y., Ling, Y., 2023. Seasonal distribution characteristics and ecological risk assessment of phthalate esters in surface sediment of Songhua River basin. Environmental Pollution 337, 122567. https://doi.org/10.1016/j.envpol.2023.122567

Wang, J., Chen, G., Christie, P., Zhang, M., Luo, Y., Teng, Y., 2015. Occurrence and risk assessment of phthalate esters (PAEs) in vegetables and soils of suburban plastic film greenhouses. Science of The Total Environment 523, 129–137. https://doi.org/10.1016/j.scitotenv.2015.02.101

Woldetsadik, D., Drechsel, P., Marschner, B., Itanna, F., Gebrekidan, H., 2017. Effect of biochar derived from faecal matter on yield and nutrient content of lettuce (Lactuca sativa) in two contrasting soils. Environ Syst Res 6, 2. https://doi.org/10.1186/s40068-017-0082-9

Xia, F., Zhang, Z., Zhang, Q., Huang, H., Zhao, X., 2024. Life cycle assessment of greenhouse gas emissions for various feedstocks-based biochars as soil amendment. Science of The Total Environment 911, 168734. https://doi.org/10.1016/j.scitotenv.2023.168734

Xie, Y., Wang, L., Li, H., Westholm, L.J., Carvalho, L., Thorin, E., Yu, Z., Yu, X., Skreiberg, Ø., 2022. A critical review on production, modification and utilization of biochar. Journal of Analytical and Applied Pyrolysis 161, 105405. https://doi.org/10.1016/j.jaap.2021.105405

Xiong, Y.-H., Pei, D.-S., 2021. A review on efficient removal of phthalic acid esters via biochars and transition metals-activated persulfate systems. Chemosphere 277, 130256. https://doi.org/10.1016/j.chemosphere.2021.130256

Younis, U., Athar, M., Malik, S., Shah, M., Mahmood, S., 2015. Biochar impact on physiological and biochemical attributes of Spinach (Spinacia oleracea L.) in nickel contaminated soil. Global Journal of Environmental Science and Management 1, 245–254. https://doi.org/10.7508/gjesm.2015.03.007

Zhao, F., Ma, Z., Ping, H., He, Z., Li, B., Gao, Y., Li, C., 2022. Tissue distribution of phthalates in celery under different cultivation patterns and associated dietary exposure. Environmental Pollution 292, 118391. https://doi.org/10.1016/j.envpol.2021.118391

Zhuang, H., Li, Z., Wang, M., Liu, B., Chu, Y., Lin, Z., 2024. Effects of microplastics and combined pollution of polystyrene and di-n-octyl phthalate on photosynthesis of cucumber (Cucumis sativus L.). Science of The Total Environment 947, 174426. https://doi.org/10.1016/j.scitotenv.2024.174426