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Biochar Application Mitigates Heavy Metal Contamination and Enhances Growth and Production of Tomato Under Diverse Wastewater Irrigation Scenarios

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Abstract

Water scarcity is a pressing and emerging geopolitical and societal challenge that has intensified reliance on wastewater (WW) for irrigation, but its contamination with pathogens and noxious compounds such as heavy metals (HMs) poses serious risks to food safety and public health. This study highlights the need to evaluate the health risks associated with the potential consumption of tomatoes irrigated with WW and the role of biochar (BC) in mitigating these risks. Two-year trials were conducted to evaluate tomatoes grown in BC-amended soil under WW irrigation at various locations of Faisalabad, Punjab, Pakistan. Data revealed that escalated use of wastewater caused adverse effects on plant growth (15 to 18.9%) and fruit quality parameters. Moreover, heavy metal concentration in plant tissues was markedly higher in the 2nd year of the trial, likely due to cumulative heavy metal enrichment in wastewater-irrigated soils, and the trend of improvement was Uchkera site, followed by Satyana and UAF Farms. However, BC-treated soil significantly enhanced chlorophyll contents, secondary metabolites production, osmoregulation, antioxidant activities and curtailed reactive oxygen species accumulation, thereby restoring plant growth, biomass production and fruit quality. The present study offers

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compelling evidence for the integration of BC-soil amendment into wastewater-based irrigation systems to promote sustainable food security and safety

Keywords: Heavy metal; biochar, tomato, secondary metabolites, antioxidants, ROS, growth, chlorophyll contents, yield quality.

Graphical abstract

1 Introduction

Bulging human populations and increasing pollution density in urban and peri-urban megacities and agglomerations exert pressure on regional water resources (Soliman, 2021). Climate change also exacerbates the local water demands (Rosa, 2022). Due to climate change, about 2/3rd of the population will face water scarcity, and global food production must be increased up to 50% to feed 9 billion by 2025 (Oliver et al., 2025). Water scarcity tends to be much more accentuated during the current century, thus affecting more than 80% of cultivated land across the globe (UNDRR, 2022). Wastewater (WW) can partly compensate for this shortage by fulfilling water supply demands (Silva, 2023). In developing and underdeveloped countries, many small and large-scale farmers have been compelled to extensively use WW for irrigation due to water scarcity, lack of farmstead amenities and poor infrastructure of water supplies (Balasubramanya et al., 2022). The persistent use of WW for irrigation purposes exacerbates the risk of heavy metal (HMs) pollution in agricultural ecosystems that accelerates their assimilation into edible crops, thereby increasing dietary exposure and causing potential toxicological consequences for human animal and plant health (Haseeb et al., 2023; Hussain et al., 2025; Raza et al., 2021). This approach also deteriorates soil physiochemical properties by altering soil pH, EC, soil microbial activities, soluble salts, nutrient balance, and redox potential (Hussain et al., 2025; Khaskhoussy et al., 2022; Zidan et al., 2024). So, the presence of harmful inorganic, organic chemicals, and noxious HMs (Pb, Cd, Cr, As, Hg, etc.), pharmaceuticals, macro and microplastics and pathogens in WW are linked to biochemical impacts, reduced agriculture productivity and diminished soil fertility (Wang et al., 2021; Ziylan-Yavas et al., 2022). Recent studies have further elaborated that heavy metals induce oxidative stress, disrupt cellular homeostasis, and interfere with enzymatic and physiological processes in plants, thereby severely impairing growth and productivity (Nazir et al., 2022; Zahra et al., 2021). Moreover, phytoremediation-based conceptual frameworks suggest that plants activate detoxification pathways, antioxidant

defense systems, and metal sequestration mechanisms to cope with HM toxicity in agricultural systems (Jalal et al., 2024; Hussan et al., 2025).

Biochar (BC) is an incipient carbon material comprised of low-cost biomass remnants, which has recently acquired significant attention in scientific communities due to its promising potential in remediation of a variety of environmental constraints, such as soil remediation, WW treatments and carbon sequestration (Kulkarni et al., 2023; Ullah et al., 2024). It is formed by the thermal carking of carbon-rich biomass at high temperatures and has a large surface area, more functional groups and high cation exchange capacity that participate plausible role in increasing soil fertility, soil organic matter, microbial community, enzymatic activities, soil porosity, soil water retention and reduces soil bulk density (Li et al., 2022; Ullah et al., 2024). Moreover, the BC application reduces the bioavailability of HMs, and their functional groups on the surface area determine the adsorption and sequestration of HMs (Penido et al., 2019; Wang et al., 2022). However, the potential; value of BC in polluted land is not well-tested on a large scale and mixed results are reported in different field trials due wide range of variations in biochar characterization and field conditions (Li et al., 2025; O'Connor et al., 2018). In addition, biochar has been reported to immobilize heavy metals through adsorption, ion exchange, and complexation mechanisms, thereby reducing their uptake by plants and alleviating metal-induced stress. It also enhances antioxidant enzyme activities and improves plant resilience under contaminated conditions, making it a promising amendment for sustainable agriculture.

Vegetables are regarded as an essential component of the human diet due to their opulent source of fibres, minerals, essential nutrients and antioxidative potential; and their potential use to control and prevent disease risks (Gupta et al., 2021). However, the regular consumption of vegetables grown on contaminated soil, irrigated with WW causes severe impacts on consumers' health (Hussain et al., 2025). Various scientific studies have recognized that vegetables grown in contaminated soil all over the world contain substantial amounts of HMs contaminants that pose severe health ailments (Hussain et al., 2025; Khaskhoussy et al., 2022; Negassa et al., 2025). Going beyond the debate of tomato (*Solanum lycopersicum* L.) as vegetables or fruits, it is regarded as one of the widely cultivated crops globally due to its enriched composition and prolific supply of essential nutrients, dietary fibres, vitamins, minerals, organic acids, antioxidants, phenolic compounds, phytochemicals and essential nutrients, rendering them

pivotal for both human nutrition and agriculture perspective (Lu et al., 2019), however, tomatoes are also on the risk of HMs contamination that compromises its overall growth parameters and yield indices (Alam et al., 2024; Bahiru, 2021; Negassa et al., 2025). Therefore, wastewater irrigation combined with heavy metal contamination poses significant risks to tomato production, affecting both crop quality and food safety. So, this trail explored the effect of BC soil amendment on growth, physiochemical attributes and HMs uptake of WW-treated plants under three different locations in Faisalabad, Pakistan, where HMs contamination of soil and water is a persistent and downplayed issue. It is hypothesized that application of biochar as a soil amendment will improve plant growth, enhance soil physiochemical properties, and reduce heavy metal uptake in wastewater-irrigated soils across the three study locations in Faisalabad, Pakistan

2 Material and Methods

This experiment was carried out at three different locations in Faisalabad, Punjab, Pakistan Uchkera Farm (UF; Lat 31.452°, Long 73.009°), Satyana Farm (SF; Lat 31.3644°, Long 73.1443°) and University of Agriculture, Faisalabad (UAF; Lat 31.4416°, Long 73.0766°) (Fig S1), to investigate the effect of BC to ameliorate the noxious effect of HMs toxicity caused by WW irrigation. The weather data of Faisalabad are shown in Fig. S2. The irrigation treatments at the site of field trails were canal water (CC), WW and WW with BC (WW+BC). The sites have subtropical and semi-arid climate conditions. Fields were ploughed, levelled, and fertilized with NPK (100:50:50). Five tons per hectare BC prepared from wood material at 300°C pyrolysis temperature was incorporated into one plot of each site. Tomato seeds (Bull eye) were sown manually in rows on 10 December during both years (2023-24), with row spacing 75 cm, and plant spacing 35 cm at 1.5-2 cm depth within a net plot size of 1.8 m x 4m. Tomatoes were harvested at BBCH stage 69 (after the end of flowering before fruit development). Plants were gently uprooted, cleaned with tap water and analyzed for morphological and physiochemical attributes.

2.1 Growth and yield attributes

Shoot (SL) and root lengths (RL) were recorded by measuring the from the base of the stem to the tip of the longest leaf, and from the base of the stem to the tip of the longest root, respectively. Shoot fresh weight (SFW), and root dry weight (RDW) were recorded by using a

digital balance, fresh weights were taken immediately after harvest, while dry weights were taken after oven drying the sample at 65°C until constant weight was achieved.

2.2 Biochemical analysis

Photosynthetic pigment contents were determined by using the method of Arnon (1949) and carotenoid content was measured by following the procedure of Mercadante and Rodríguez-Amaya (1991). For this purpose, a fresh leaf sample (0.1 g) was ground in 2 mL of 80% acetone, and then the volume was maintained up to 10 mL by using 80% acetone, and the absorbance was noted at 663, 645 and 480 nm. Anthocyanin (Antho) contents for both shoot and root samples were determined by opting the protocol of Starck and Wray (1989). For this purpose, fresh leaves and root samples (0.1 g) were boiled for 1 hour at 50 ºC in acidified methanol and optical density was noted at 535 nm. The phenolic (Phe) contents were recorded by adopting the technique outlined by Julkunen-Tiitto (1985). A 0.1 (g) of fresh leaves and root samples were ground in acetone (80%). After that 1 mL (H_2O_2), 2,5 mL (Na_2CO_3) and 0.5 mL (Folin phenol reagent) were added to 100 µL of extract, and absorbance was observed at 750 nm. Moreover, proline (Pro) contents were recorded by using the approach described by Bates et al. (1973). A 0.1 g of fresh leaves and fresh roots samples were ground in 2 mL sulpho-salicylic acid, and after that 1 mL of ninhydrin and I mL of glacial acetic acid were poured into a test tube. Then 2 mL of toluene was added to each sample mixture after a 1-hour water bath (100 ºC), and absorbance was observed at 520 nm. Malondialdehyde (MDA) content for both shoot and root parts was observed according to the methodology of Heath and Packer (1968). A fresh sample of each plant part (0.1 g) was ground in trichloroacetic acid (1%). After that 20% trichloroacetic acid, 0.5% thiobarbituric acid were mixed in 1 mL of homogenized sample. The samples were boiled in a water bath of 50 minutes at 95 ºC, and absorbance was recorded at 532 nm and 600 nm. Hydrogen peroxide (H_2O_2) estimation was done by using the procedure of Velikova et al. (2000). For this purpose, fresh leaves and root samples were homogenized in 0.1% trichloroacetic acid, and then 0.5 mL from this sample mixture was vortexed with phosphate buffer (pH 7.0) and potassium iodide solution. The absorbance was noted at 390 nm.

2.3 Heavy metal analysis

The HMs contents Hg, Cd, Ni, Pb, Co, Cu and Zn of tomato leaves were determined by using atomic absorption spectroscopy. Dried plant samples (0.5 g) were digested in HNO_3, and heated

until a clear color was observed. Then the digested samples were filtered, and the volume was maintained up to 50 mL.

2.4 Statistical analysis

The trails were set up using a Randomized Complete Block Design with three replicates. A two-way Analysis of Variance was done by using Statistix 8.1, and a comparison of means was done by opting Tukey HSD test at a P value (0.05). Heatmap and PCA analysis were performed by using origin and R-studio software.

3 Results

3.1 Soil physicochemical attributes and water quality

Soil physicochemical attributes indicated significantly higher heavy metal (HMs) contamination in wastewater (WW) sources compared to the control (CW). Maximum concentrations of Co, Cr, Pb, and Zn were observed in UF and SF WW, suggesting inputs from urban and industrial discharges. Although Cr and Hg remained relatively consistent across locations, their concentrations in WW exceeded safe limits (Fig. S3). Water quality parameters varied among sites (UAF farms, Paharang, Madhuana, and Canal water). Turbidity, total solids, total dissolved solids, and electrical conductivity were highest in Madhuana drain water and lowest in Canal water. Chemical oxygen demand and biological oxygen demand were highest at the UAF site and lowest at Paharang. Iron content was highest in Madhuana, whereas bicarbonates, sulphates, phosphate, and chloride were highest at the UAF site. The pH ranged from 6.8 (Canal water) to 7.9 (UAF and Paharang) (Table S1).

3.2 FTIR and biochar characterization

Fourier transform infrared spectroscopy of wood biochar (BC) confirmed the presence of key functional groups (Fig. S4). The material exhibited pore sizes ranging from 2 to 50 nm, dominated by micro- and mesopores. Carbonyl (C=O) and aromatic (C=C) groups were identified at 1700 cm^{-1} and 1600 cm^{-1}, respectively. Broad peaks at 2000 cm^{-1} and 3400 cm^{-1} corresponded to C–H stretching and hydroxyl (–OH) groups. Peaks at 1300–1000 cm^{-1} and 800 cm^{-1} indicated C–O stretching and C–H bending, confirming the presence of oxygen-containing aromatic groups that facilitate HM adsorption.

3.3 Growth and yield parameters

Tomato growth and yield parameters, including shoot length, fruit diameter, fruit length, and yield per plant, differed significantly among locations and treatments during 2023 and 2024, while their interaction was non-significant (Table 1). Wastewater application reduced shoot length at UAF (18.6 and 18.9%), Uchkera (15.3 and 17.8%), and Satyana (16.5 and 17.5%) compared to CW. Biochar application mitigated these reductions across all locations. Fruit diameter and length were also reduced under WW, with the greatest decline at Uchkera, followed by Satyana and UAF; however, WW+BC improved these traits relative to WW alone. Yield per plant decreased under WW at UAF (15.3 and 16.5%), Uchkera (16.2 and 18.9%), and Satyana (11.4 and 14.6%), whereas BC application alleviated these effects. Overall, yield per plant was highest at UAF, followed by Satyana and Uchkera (Table 1).

3.4 Chlorophyll contents

Chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll) was significantly influenced by locations and treatments during both years, with non-significant interactions except for chlorophyll b (Table 2). Wastewater application reduced chlorophyll contents, whereas WW+BC enhanced them. Chlorophyll a increased by 20.6–26.4% in 2023 and 17.9–32.0% in 2024 under WW+BC compared to WW. Chlorophyll b increased by 29.4–39.1% in 2023 and 20.3–40.9% in 2024. Total chlorophyll decreased under WW at UAF (31.3 and 34.4%), Uchkera (46.0 and 49.3%), and Satyana (38.9 and 37.6%), while BC application mitigated these reductions.

3.5 Proline, anthocyanin, and phenolic contents

Phenolic, proline, and anthocyanin contents in roots and shoots showed significant variation across locations and treatments during both years, with limited interaction effects (Fig. 1-3). Wastewater application increased these parameters, while WW+BC further enhanced them compared to WW alone. Root and shoot phenolic contents generally increased under WW+BC across locations, with minor exceptions at Satyana (Fig 1). Root anthocyanin increased by 23.7–57.6% in 2023 and 11.1–44.4% in 2024, and shoot anthocyanin increased by 9.09–43.9% in 2023 and 6.5–78.4% in 2024 under WW+BC relative to WW (Fig. 2). Root and shoot proline

contents generally increased under WW+BC across locations, but higher was noted in Uchkera followed by Satyana and UAF (Fig 3).

3.6 Oxidative damages

Oxidative stress indicators (H_2O_2 and MDA) in roots and shoots were significantly affected by treatments and locations during both years, while their interaction was non-significant. Wastewater application increased H_2O_2 and MDA levels, whereas BC application reduced these effects. Root H_2O_2 increased under WW at UAF (31.4 and 16.9%), Uchkera (20.3 and 17.1%), and Satyana (25.4 and 6.9%) compared to CW. However, WW+BC reduced these levels. Root MDA decreased by 9.1–15.5% in 2023 and 6.5–15.4% in 2024 under WW+BC compared to WW. The highest oxidative stress was observed at Uchkera, followed by Satyana and UAF (Fig. 4–5).

3.7 Heavy metal contents

Heavy metal accumulation (Cd, Cu, Cr, Ni, Pb, Hg, Co, and Zn) in tomato varied significantly with treatments and locations during 2023 and 2024, while interaction effects were mostly non-significant except for Ni and Co. Wastewater application increased HM concentrations compared to CW, whereas WW+BC reduced their accumulation. The highest HM concentrations were recorded at Uchkera, followed by Satyana and UAF, and were generally higher in 2024 than in 2023 (Fig. 6–8).

3.8 Heatmap, Mantel, and chord correlation

Correlation analysis indicated that CW treatments were negatively associated with SMDA, RMDA, Co, Hg, Cr, Zn, SHO, RHO, Ni, proline, phenolics, anthocyanins, Pb, and Cd, while showing positive relationships with other parameters during both years. In contrast, WW and WW+BC treatments exhibited opposite trends (Fig. 9). Network analyses showed more dispersed and weakly connected clusters in 2023, whereas 2024 exhibited a dense and highly interconnected network with fewer isolated clusters (Fig. 10).

4 Discussion

To address this pressing issue, an experiment was undertaken to elucidate the fate and transport of WW-borne HMs applied through irrigation to the tomato crop grown on three different locations of Faisalabad, having either received no biochar or wood biochar amendment. Data regarding soil and water samples showed higher accumulation of HMs (Fig. 7-9) as compared to CWT.

Data regarding growth and yield attributes revealed significant heterogeneity in growth and yield indicators across WW sources. The maximum reduction in all these attributes was observed when the WW of the Uchkera site was supplied for irrigation. This decline might be attributed to HMs-induced toxicity, which potentially impairs root architecture (reduced root counts, length, and curtailed biomass), disrupts physiological homeostasis, restricts nutrient disruption, ROS production, and photosynthesis impairment, ultimately compromising plant growth and productivity (Manzoor et al., 2023; Umar et al., 2025) (Table 1). Conversely, BC amendment WW significantly improved plant growth and yield traits by restricting the uptake, assimilation, and bioavailability of HMs irrespective of location differences. This decline is due to the putative role of BC in HMs' electrostatic interaction, complexation, pore occlusion, surface adsorption, ion exchange, and precipitation, hydrogen bonding, which collectively restrict HMs' translation and improve plant growth and yield attributes (Pallewatta et al., 2025). Moreover, the other reason for improved growth and yield attributes due to BC application might be linked to its plausible role in stabilizing soil structure, improving water retention, maintaining aeration, regulating nutrient availability, and enhancing microbial activity (Ullah et al., 2024). So, the lower bioavailability of HMs, higher biomass production, and improved soil physicochemical attributes with application of BC+WW resulted in improved photosynthetic pigments, as shown in Table 2. However, WW application deteriorated photosynthetic efficiency and reduced photosynthetic pigments accretion due to the presence of HMs translocation. The reason for this reduction might be associated with HMs induced chlorosis, necrosis, biomass reduction and reactive oxygen species (ROS) production (Ali et al., 2024; Wang et al., 2024). So, the elevated level of ROS in WW-irrigated plants triggered robust activation of antioxidant defense pathways, maintained osmoregulatory balance, regulated redox homeostasis, stabilized the osmoregulation, and enhanced secondary metabolite production (Fig 2-5). The current study corroborated the findings of Sharma et al. (2024), who also noted higher osmolyte, secondary metabolite, and

antioxidant turnover with overproduction of ROS under HMs stress. So, the higher anthocyanin, proline, phenolics, ROS, and antioxidants were affirmed in tomato grown in Uchkera side, followed by Satyana and UAF farms. However, BC-soil amendment quickly doused the ROS by restricting the HMs bioavailability and improved secondary metabolites and antioxidant production. The present study aligns with the findings of Różyło et al. (2017) and Regmi et al. (2023), who reported a significant increase in phenolic compounds, flavonoids, and phytochemical profiles in wheat and Viola cornuta (viola) plants, respectively, following BC application. The enhancement in secondary metabolite production might be attributed to BC-induced improvement in soil water retention capacity, enhanced soil aeration, superior physicochemical properties and higher microbial activities; all these factors facilitated higher nutrient uptake, translocation and assimilation in different plant tissues. These nutrients are cofactors of different enzymes and a structural part of various biomolecules, thereby supporting key developmental and physiological processes that underpin the biosynthesis of secondary metabolites. So, the higher HMs assimilation capacity was also observed in WW-treated plants (Fig. 5-8). However, WW treatment, especially Uchkera, followed by Satyana and UAF farms, significantly enhanced HMs uptake, which results in poor root architecture, disrupts physiological homeostasis, and impairs photosynthetic processes, which provide energy for secondary metabolite production (Manzoor et al., 2023). The relation of HMs accumulation in WW-irrigated areas and the effect of CWT and BC on physiological, biochemical processes are represented in Fig. 9-10. Results revealed that WW can be used as a viable irrigation source under water-limited conditions, however, its application induces sublethal toxicity, potentially disturbing soil health and plant physicochemical processes. However, BC soil amendment can efficiently manage HMs toxicity by attributing electrostatic interaction, complexation, pore occlusion, surface adsorption, ion exchange, precipitation, hydrogen bonding, and chelating functional groups.

5 Conclusions

The current study underscores the dual reality of wastewater irrigation in water-scarce agroecosystems, which can compensate for freshwater demand but potentially induces a sublethal level of heavy metal stress that compromises plant health and fruit quality indices. The toxic effect of Uchkera side wastewater was more pronounced, followed by Stayana and UAF

farms. Overall, the effect of canal water was more beneficial than compared of wastewater due to a lower profile of heavy metals. However, wood-derived biochar soil incorporation reduced heavy metal bioavailability through electrostatic interaction, complexation, pore occlusion, surface adsorption, ion exchange, precipitation, hydrogen bonding, and chelating functional groups. Biochar soil amendment enhances plants' chlorophyll contents, phenolics, antioxidants, flavonoids, reduced reactive oxygen species production and heavy metals uptake, thereby restoring plant growth and productivity. These insights not only reinforce the potential of BC as a cost-effective soil fertilizer but also call for its integration into sustainable water reuse practices. The sequential and gradual contamination of the food chain with wastewater irrigation can also cause severe consequences on the circular economy and compromise human health, so preventive measures can be taken to avoid heavy metal contamination. Future studies should be conducted to explore the biochar's impact on multi-cropping systems in varied climatic zones.

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Author contributions

FR and NZ were responsible for supervising this research jointly. CQ, RS and NZ provided constructive guidelines on the development of research study. FR and MS conducted the study and GM and MS helped in laboratory work. PM, EMHW, IY and JKS helped me in data analysis, reviewed and editing the manuscript. All authors contributed to the improvement of this manuscript through proofreading.

Availability of data and material

Data are available on request to the corresponding author.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

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462 **Table 1:** Effect of biochar on growth and yield attributes of tomato under different wastewater
463 scenarios during both years.

Treatments	Shoot length (cm)		Fruit diameter (cm)		Fruit length (cm)		Fruit weight (kg plant^{-1})	
	2023	2024	2023	2024	2023	2024	2023	2024
UAF+CWT	72±2.5a	69±1.9a	4.07±0.11a	3.97±0.05a	5.12±0.06a	4.93±0.11a	1.33±0.07a	1.27±0.03a
UAF+WWT	58±1.8de	56±1.4de	3.59±0.01de	3.47±0.06de	4.56±0.06c-e	4.43±0.05c-e	1.13±0.05b-d	1.06±0.06bc
UAF+WWT+BC	65±2.0bc	63±2.2bc	3.79±0.08b-d	3.65±0.08b-d	4.71±0.11b-d	4.60±0.04b-d	1.22±0.02a-c	1.14±0.03b
Uchkera+CWT	65±2.6bc	64±2.3a-c	3.82±0.07bc	3.73±0.06bc	4.74±0.10bc	4.62±0.08bc	1.21±0.04a-c	1.13±0.04b
Uchkera+WWT	55±2.6e	52±1.5e	3.52±0.10e	3.39±0.10e	4.43±0.11e	4.27±0.06e	1.02±0.03d	0.92±0.03d
Uchkera+WW+BC	61±1.2c-e	59±1.7cd	3.76±0.06cd	3.63±0.05cd	4.64±0.05c-e	4.49±0.09cd	1.12±0.04b-d	1.06±0.05bc
Satyana+CWT	68±2.1ab	67±3.0ab	4.00±0.09ab	3.85±0.10ab	4.90±0.10b	4.77±0.09ab	1.25±0.05ab	1.16±0.06ab
Satyana+WWT	57±2.0de	55±2.6de	3.61±0.05c-e	3.46±0.08de	4.52±0.06de	4.39±0.06de	1.10±0.41cd	0.99±0.03cd
Satyana+WWT+BC	62±1.7b-d	60±2.1b-d	3.73±0.04c-e	3.61±0.30cd	4.67±0.06cd	4.54±0.05cd	1.14±0.46b-d	1.08±0.02bc
Significance								
Location	**	**	**	**	**	**	**	**
Treatments	**	**	**	**	**	**	**	**
Location x Treatments	ns	ns	ns	ns	ns	ns	ns	ns

464

465 **Table 2:** Effect of biochar on chlorophyll contents of tomato under different wastewater
466 scenarios during both years.

Treatments	Chlorophyll a (mg g^{-1} FW)		Chlorophyll b (mg g^{-1} FW)		Total chlorophyll (mg g^{-1} FW)	
	2023	2024	2023	2024	2023	2024
UAF+CWT	1.9±0.11ab	2.0±0.20a	1.1±0.07a	1.0±0.04a	3.1±0.04a	3.0±0.20a
UAF+WWT	1.3±0.25c-e	1.3±0.10cd	0.8±0.05cd	0.7±0.05cd	2.1±0.20c	2.0±0.78cd
UAF+WWT+BC	1.6±0.26bc	1.5±0.20bc	1.1±0.15ab	0.9±0.05ab	2.6±0.15b	2.4±0.15bc
Uchkera+CWT	1.5±0.15c	1.6±0.15a-c	1.2±0.05a	0.8±0.04ab	2.7±0.20ab	2.5±0.19b
Uchkera+WWT	0.9±0.25f	0.8±0.15e	0.6±0.07cd	0.4±0.06e	1.5±0.18e	1.3±0.21e
Uchkera+WW+BC	1.1±0.11ef	1.1±0.10de	0.8±0.10cd	0.6±0.02cd	1.9±0.20cd	1.7±0.09de
Satyana+CWT	2.0±0.15a	1.8±0.10ab	0.8±0.08bc	0.8±0.06ab	2.8±0.10ab	2.6±0.04ab
Satyana+WWT	1.1±0.25d-f	1.0±0.23de	0.6±0.05cd	0.6±0.06d	1.7±0.26de	1.6±0.25de

Satyana+WWT+BC	1.4±0.28cd	1.2±0.20c-e	0.7±0.05cd	0.7±0.06cd	2.2±0.32c	1.9±0.18d
Significance						
Location	**	**	**	**	**	**
Treatments	**	**	**	**	**	**
Location x Treatments	ns	ns	*	*	ns	ns

467

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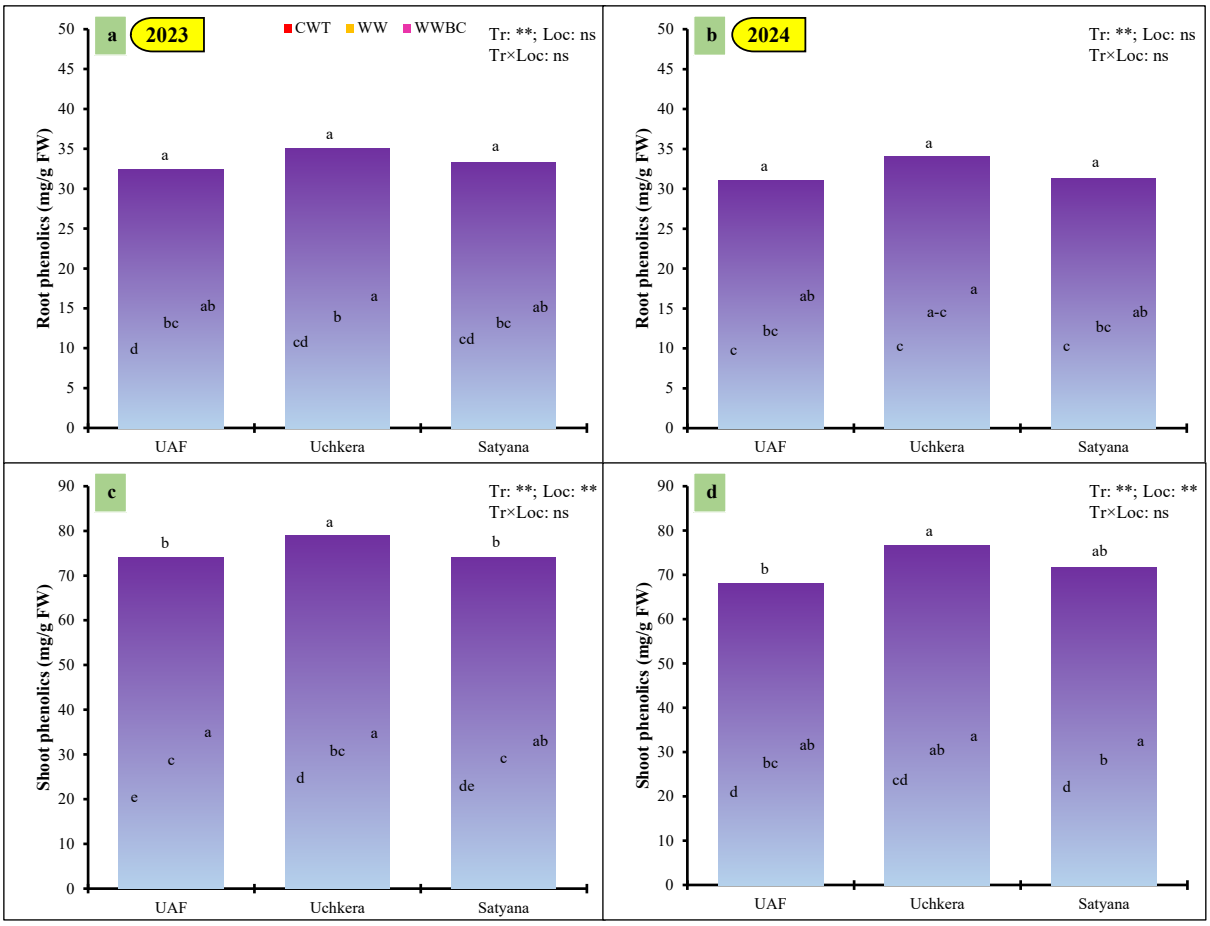


469

470 **Fig:1:** Effect of biochar on root phenolics and shoot phenolics contents of tomato under different

471 wastewater scenarios during both years.

472

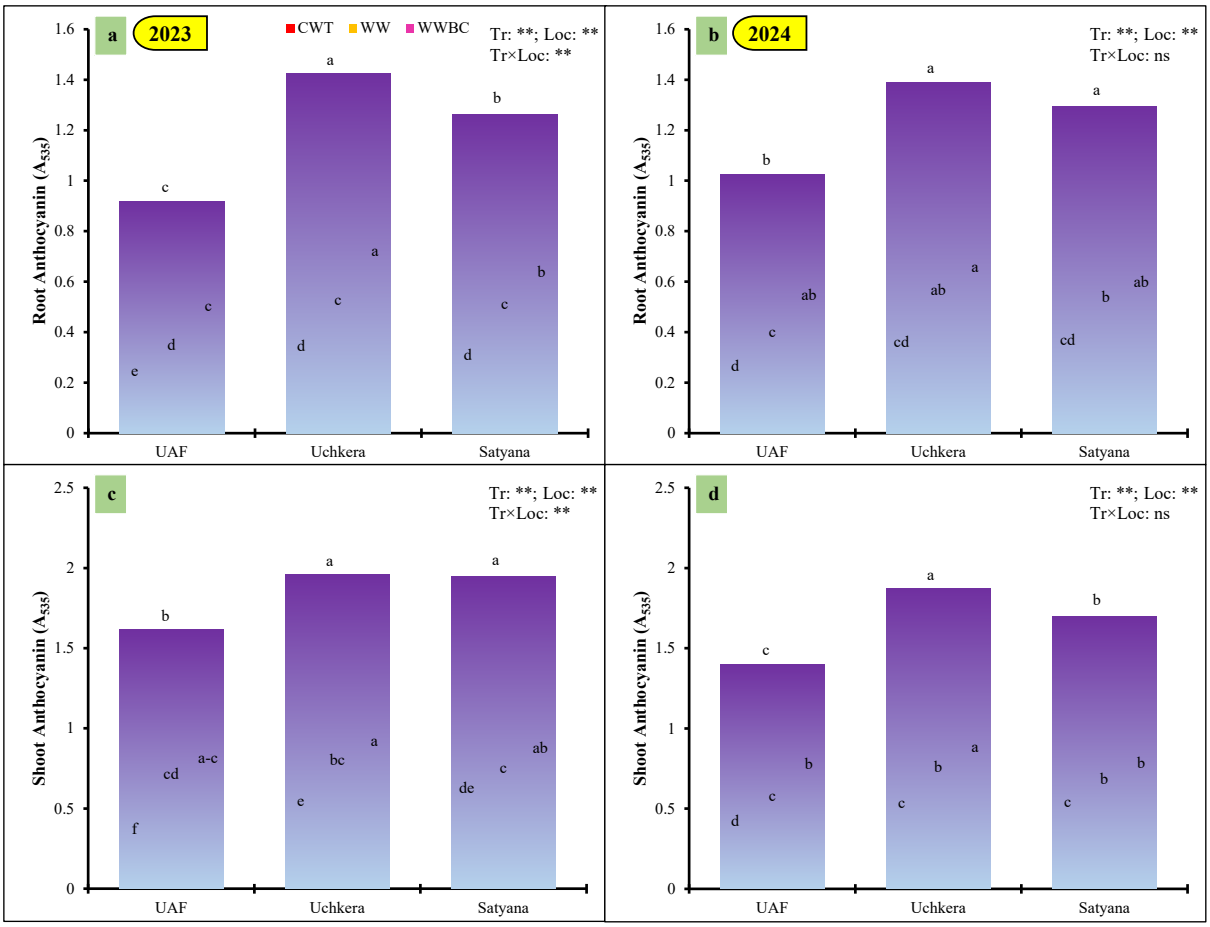


Fig:2: Effect of biochar on root anthocyanin and shoot anthocyanin contents of tomato under different wastewater scenarios during both years.

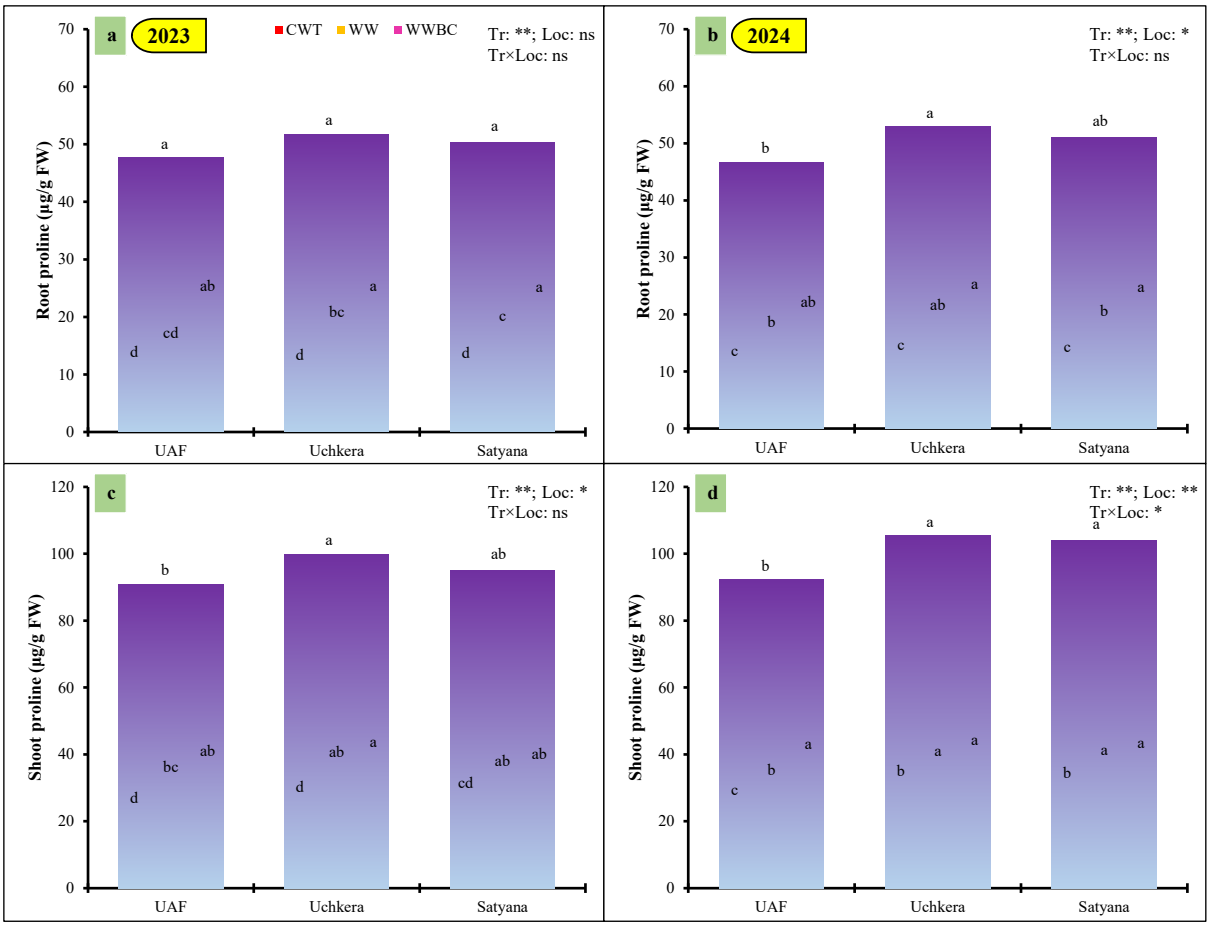


Fig:3: Effect of biochar on root proline and shoot proline contents of tomato under different wastewater scenarios during both years.

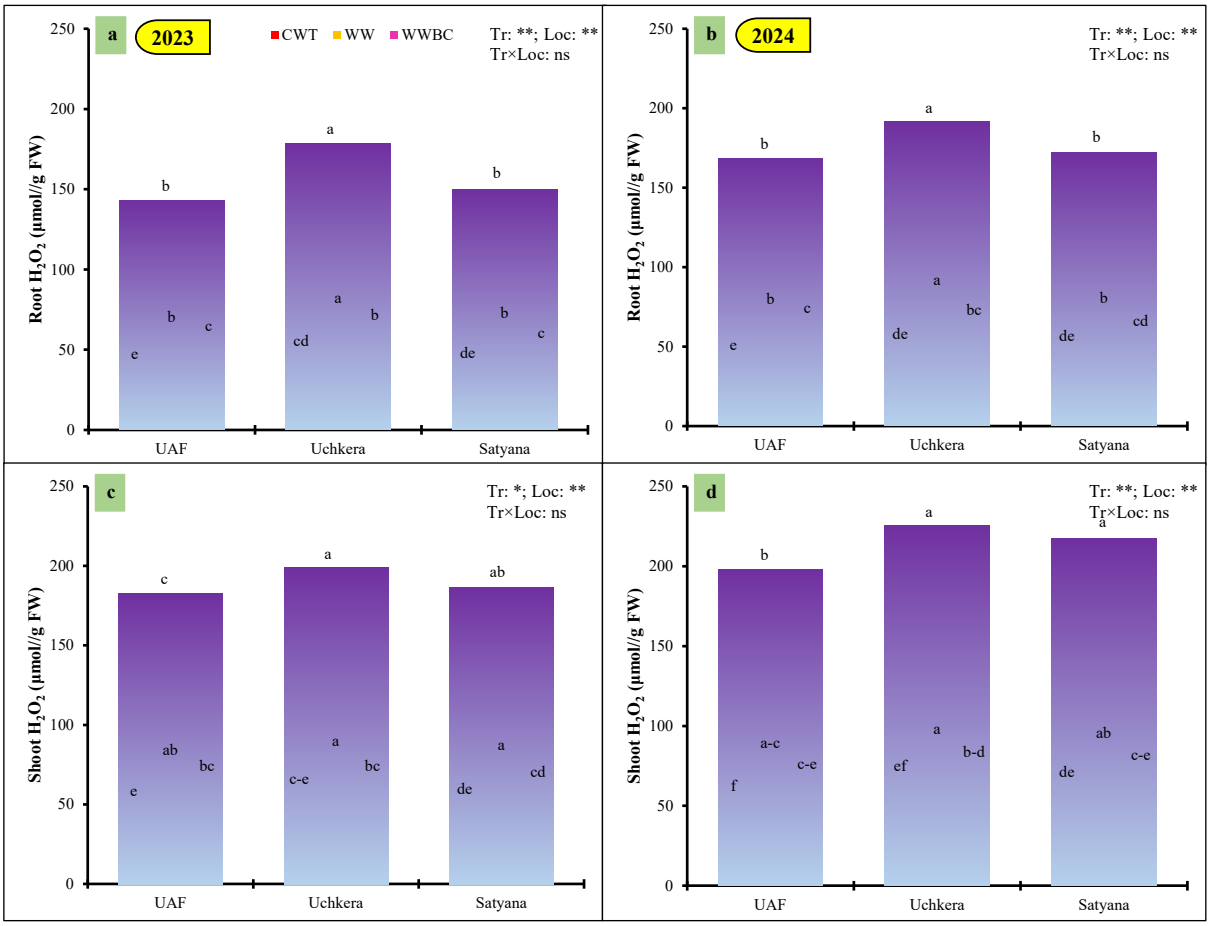


Fig:4: Effect of biochar on root H_2O_2 and shoot H_2O_2 contents of tomato under different wastewater scenarios during both years.

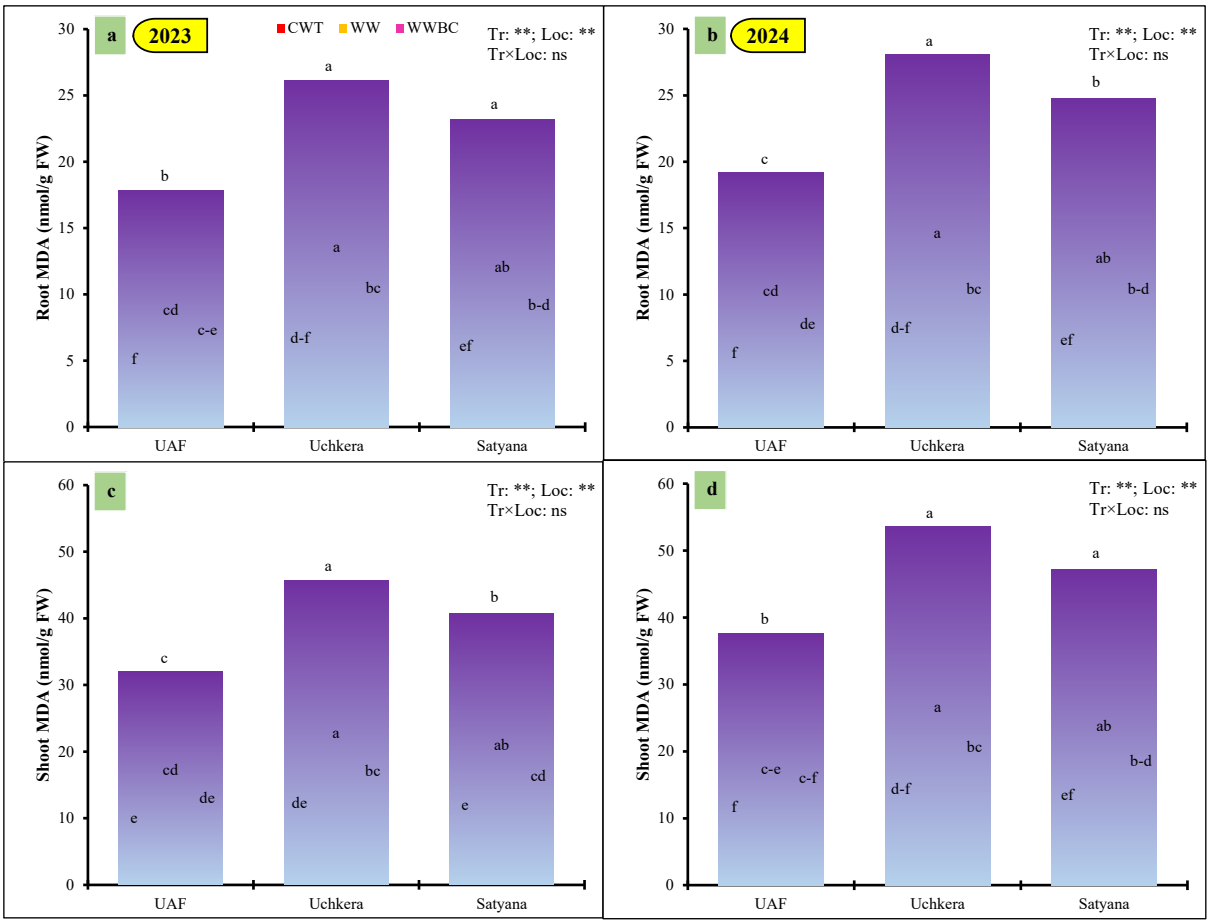


Fig:5: Effect of biochar on root MDA and shoot MDA contents of tomato under different wastewater scenarios during both years.

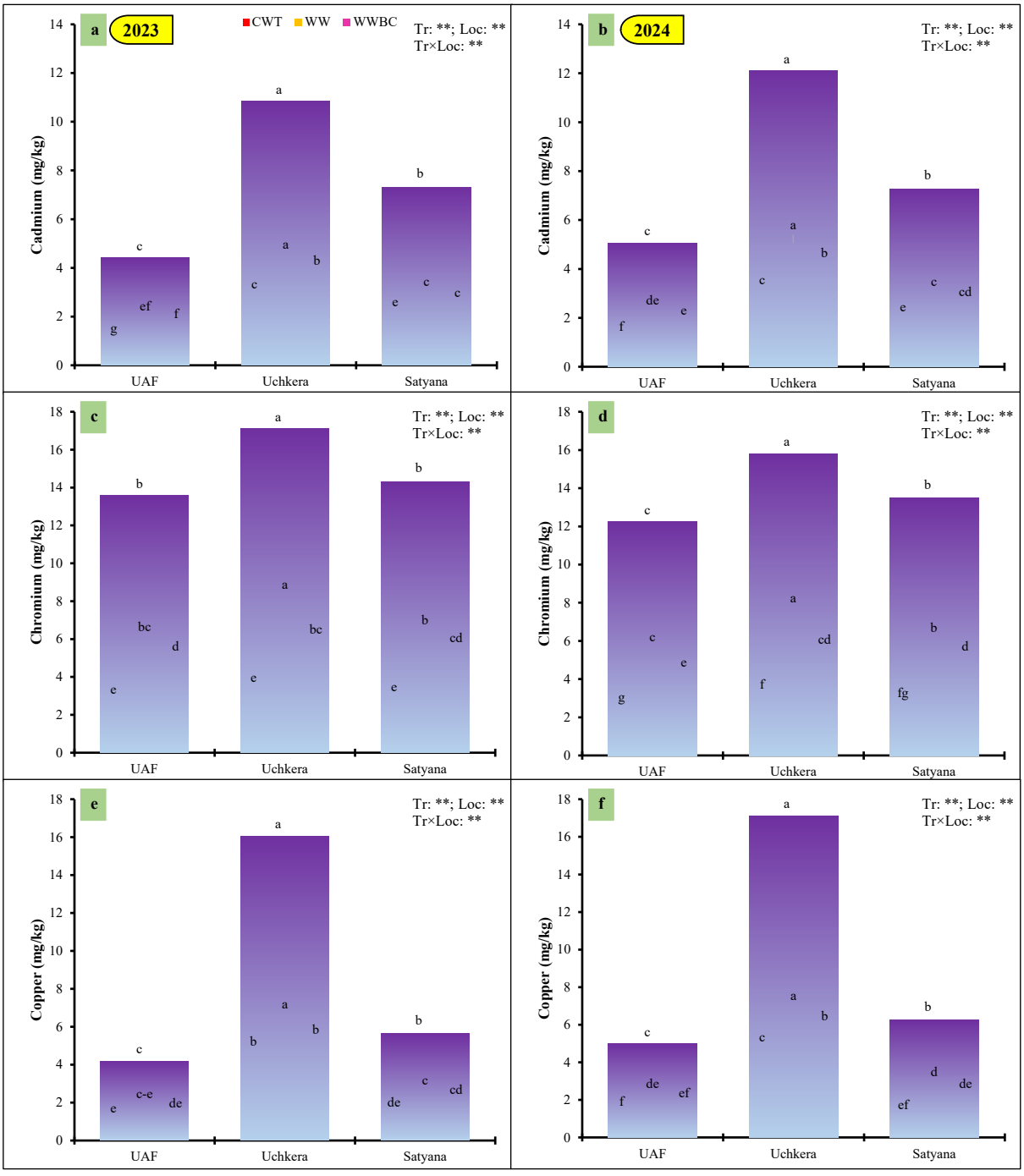
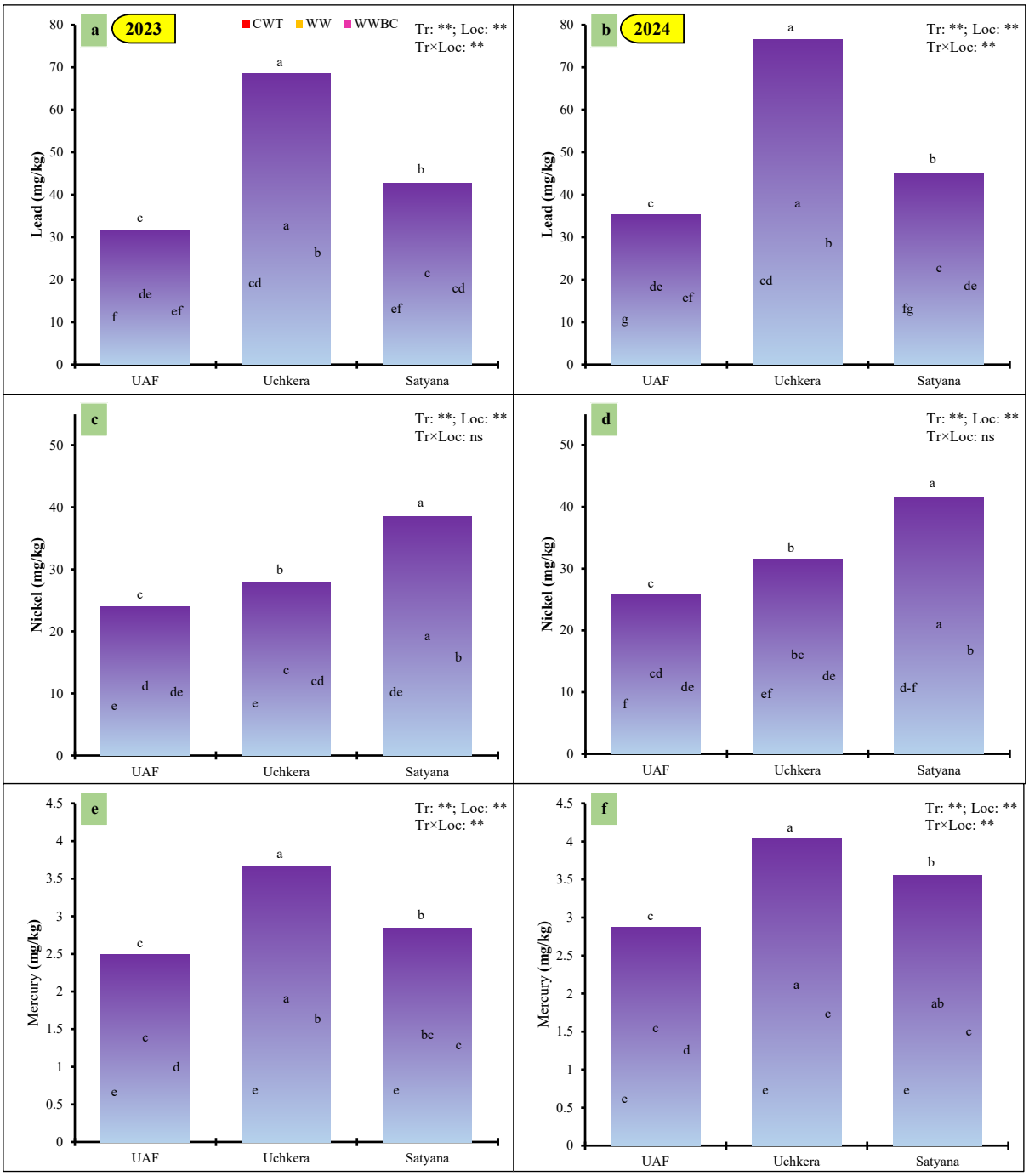


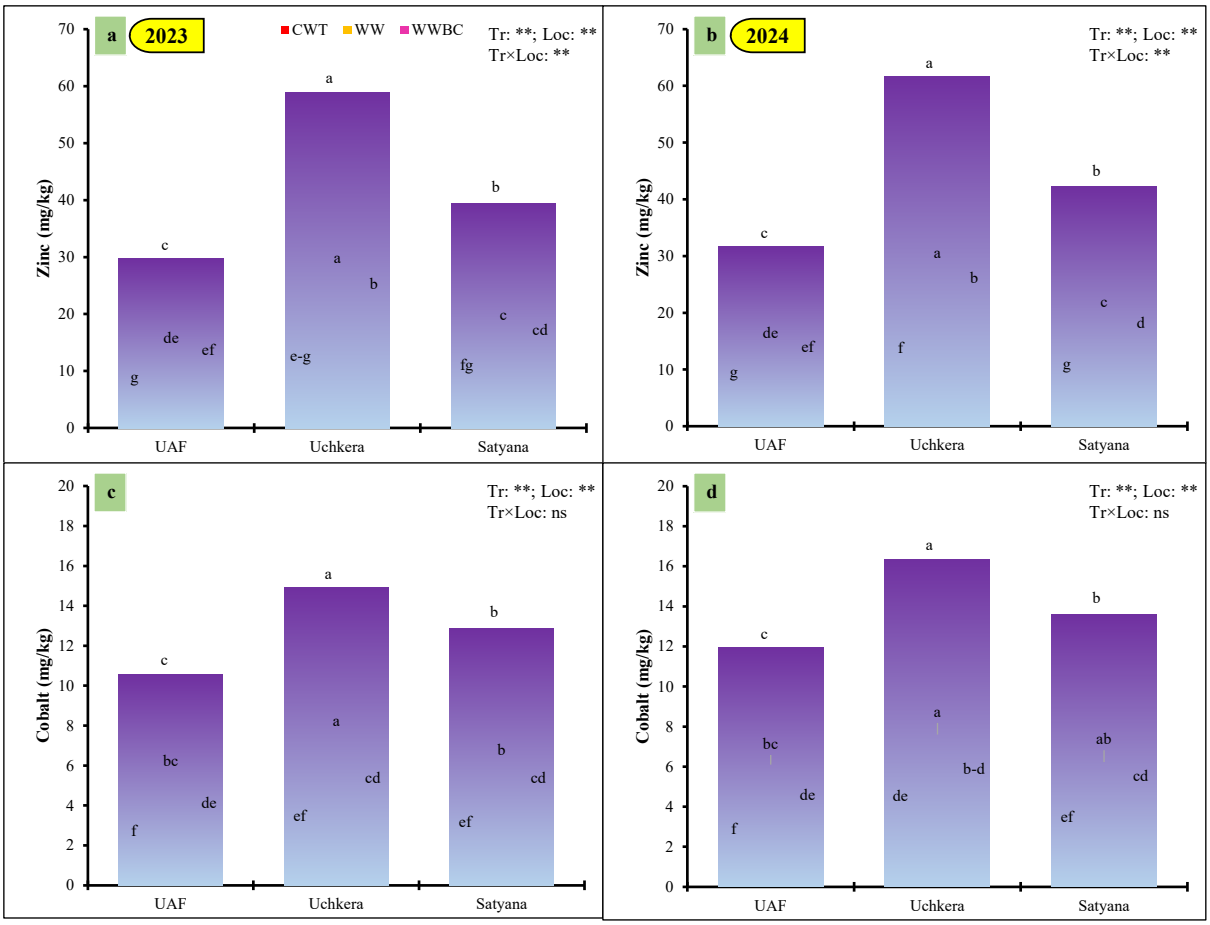
Fig:6: Effect of biochar on calcium, chromium, and copper contents of tomato under different wastewater scenarios during both years.



495

496 **Fig: 7:** Effect of biochar on lead, nickel, and mercury contents of tomato under different

497 wastewater scenarios during both years.



498

499 **Fig:8:** Effect of biochar on zinc and cobalt contents of tomato under different wastewater

500 scenarios during both years.

501

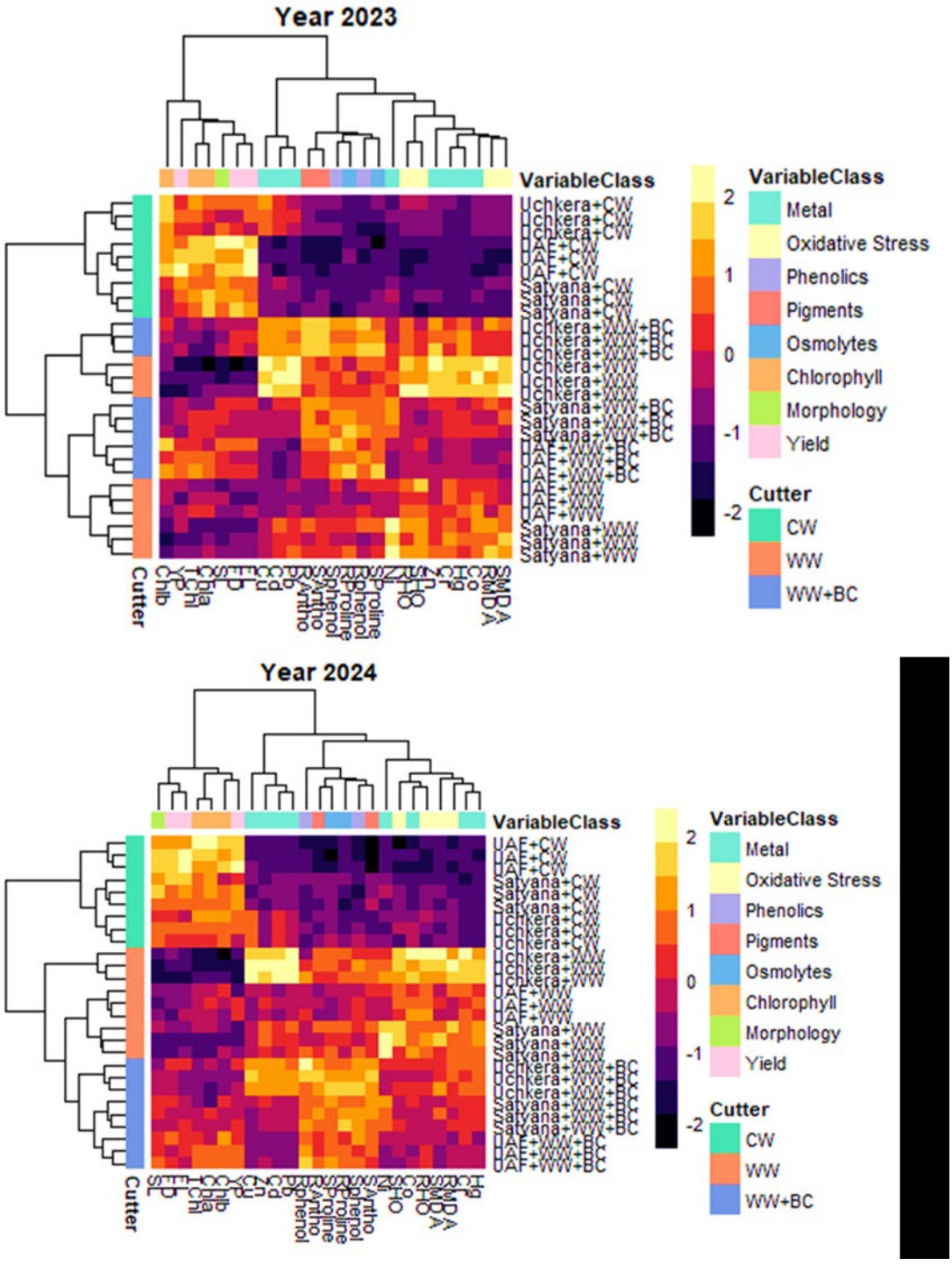


Fig: 9: Heatmap correlation matrix on the effect of biochar on growth, yield, biochemical and heavy metal contents of tomato under different wastewater scenarios during both years.

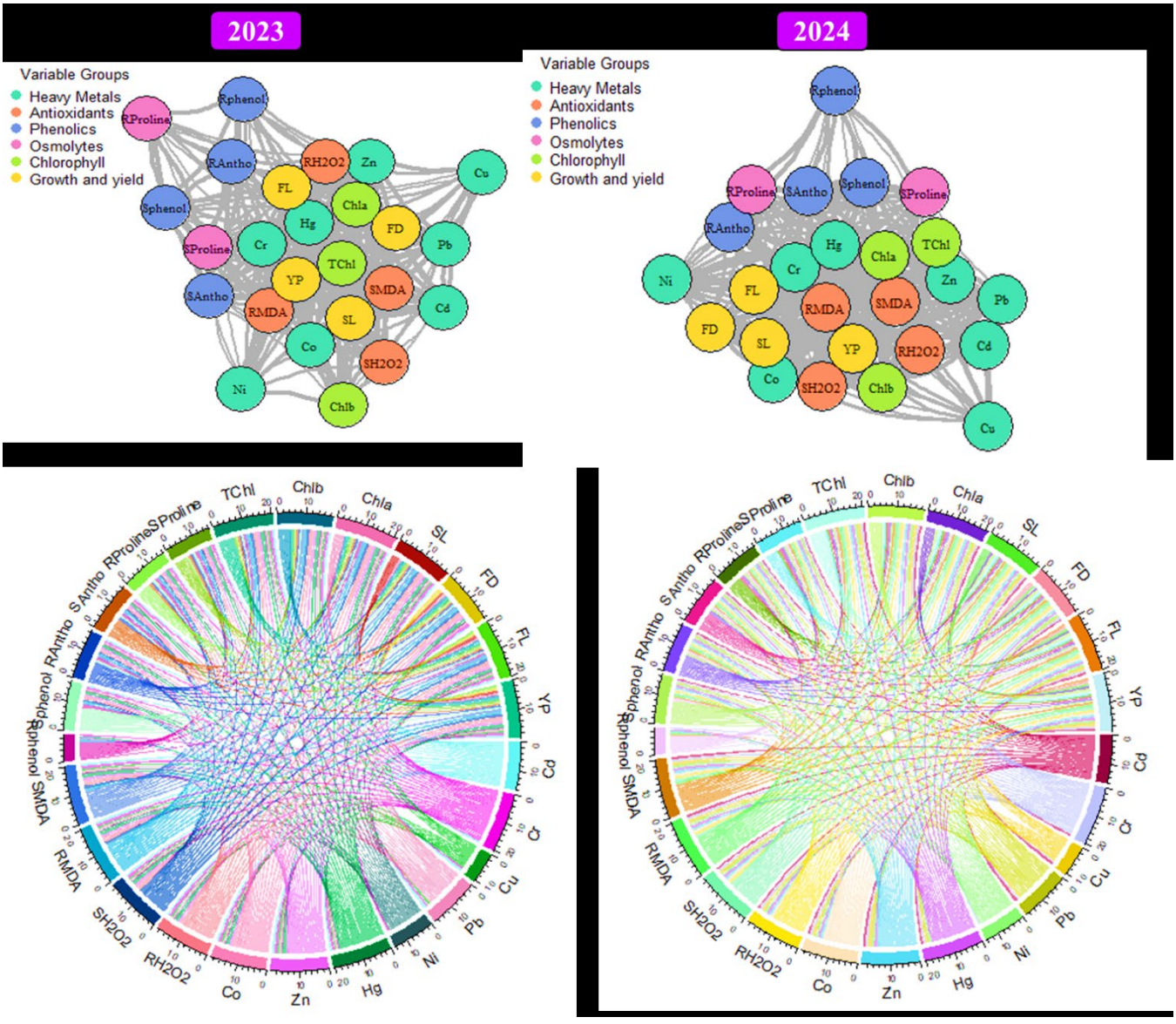


Fig: 10: Merchant and chord diagrams on the effect of biochar on growth, yield, biochemical and heavy metal contents of tomato under different wastewater scenarios during both years.

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Biochar application mitigates heavy metal contamination and enhances growth and production of tomato under diverse wastewater irrigation scenarios

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