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RESEARCH ARTICLE

Mitigating Heavy Metal Uptake in Wastewater-Irrigated Radish (*Raphanus sativus* L.) through Biochar and L-Methionine Application

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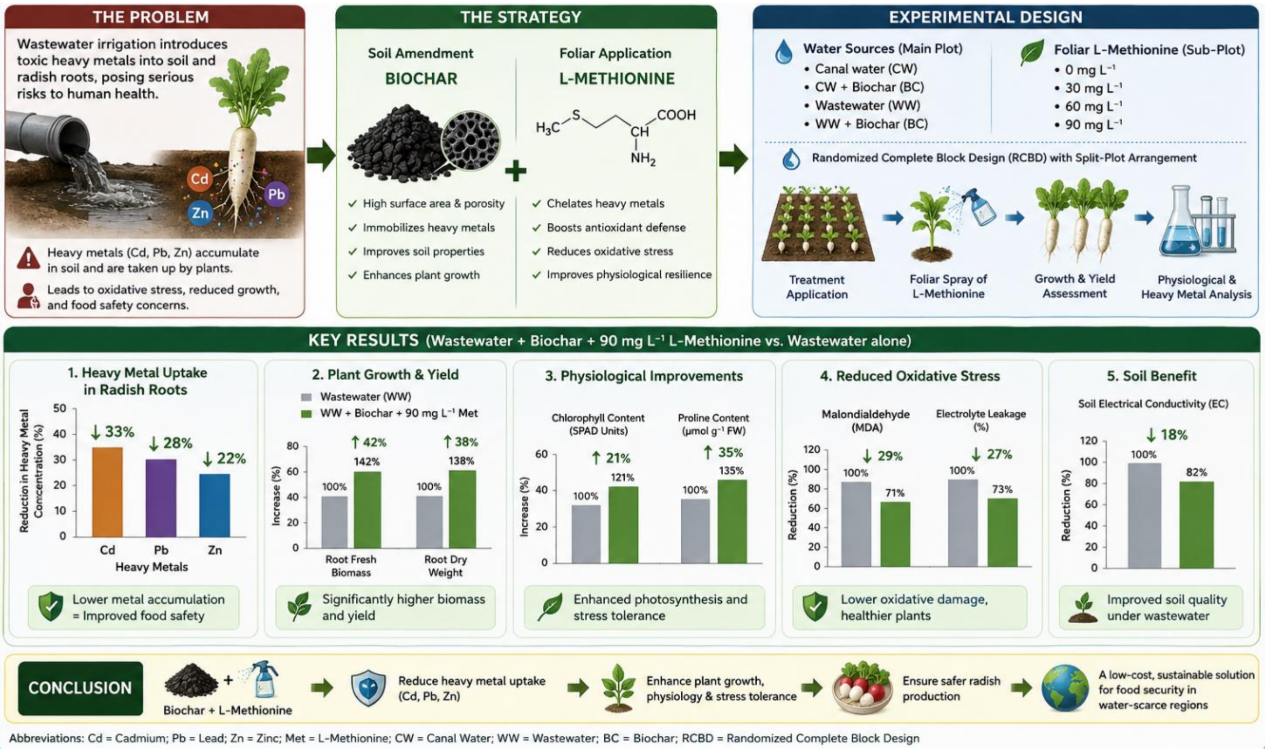
GRAPHICAL ABSTRACT



ABSTRACT

White radish (*Raphanus sativus* L.), a popular and nutritious root crop, is often grown using wastewater irrigation in water-scarce regions, leading to heavy metal accumulation and human health risks. This study evaluated a pragmatic solution using soil-applied biochar and foliar-applied L-methionine to mitigate these hazards. The research was conducted at the Agronomy Farm, University of Agriculture, Faisalabad, using a Randomized Complete Block Design (RCBD) with a split-plot arrangement. Factors included water sources (canal water, canal water + biochar, wastewater, wastewater + biochar) and foliar L-methionine concentrations (0, 30, 60, and 90 mg L^{-1}). The results demonstrated that the combined application of biochar and 90 mg L^{-1} L-methionine significantly enhanced growth and safety profiles. Specifically, the application of biochar and methionine led to a substantial reduction in heavy metal uptake, with Cadmium (Cd) reduced by 33%, Lead (Pb) by 28%, and Zinc (Zn) by 22% in radish roots compared to wastewater irrigation alone. Furthermore, plant productivity improved significantly, with root fresh biomass increasing by 42% and root dry weight by 38% under the wastewater + biochar treatment supplemented with 90 mg L^{-1} methionine. These interventions also improved physiological resilience, as evidenced by higher chlorophyll content and increased stress-tolerance markers like proline. Ultimately, this study implies that integrating biochar with L-methionine serves as a highly effective, low-cost strategy to secure food safety and enhance crop yields in regions forced to rely on contaminated wastewater for agriculture.

Key words: Heavy metal uptake, Electrolyte leakage, Oxidative stress biomarkers, Soil electrical conductivity (EC), Malondialdehyde (MDA) and Proline accumulation

INTRODUCTION

One of the biggest problems of the twenty-first century would be water scarcity. Over two thirds population of the world is predicted to live in places with "stress" levels of water scarcity by 2050 (Hayat et al., 2025a; Falco et al., 2019). It is expected that the effects of climate change would worsen this scarcity, further intensifying the challenge. Population increases and human activities like mining, industry, exploration and urbanization are major contributors to environmental pollution, which deteriorates and contaminates life's fundamental elements in an alarming and punitive manner (Hayat et al., 2025b; Hemat et al., 2025; Jie et al., 2023). The backbone of the human food supply is agriculture, which strives to provide safe goods with the least negative effects on the environment. In addition, the growth of food crops could be threatened by a shortage of soil and water, which are the two natural resources that play key role in agriculture (Akram et al., 2025; Perveen et al., 2010). Approximately 10% of the world's irrigated agricultural land, equating to 20 million hectares of cropland, is irrigated using wastewater (Obijianya et al., 2025; Ungureanu et al., 2020). Pakistan's canal water supply efficacy is decreasing, increasing the country's reliance on groundwater and wastewater. Due to lower fertilizer costs and increased agricultural yield, farmers utilizing wastewater can potentially make a third more money from cultivating the same crops than those using fresh water (Ali et al., 2024; Nasreen & Ashraf, 2020). This wastewater is a rich supply of organic matter that lowers the need for freshwater to irrigate crops. It comes from municipal, industrial and agricultural resources (Hemat al., 2025; Michael-Kordatou et al., 2015). Toxic heavy metals are mostly produced by thermal power plants, industrial processes, insecticides, and municipal solid waste. Heavy metals are poisonous, non-essential, and non-biodegradable compounds with a density more than 5g cm^{-3} or five times that of water (Ling & Na, 2006; Mahmud et al., 2025). They are extremely hazardous to human health even at low concentrations and they cause oxidative stress once they enter the food chain from either natural or man-made sources (Paithankar et al., 2021; Waqas et al., 2024). The soil's ability to absorb ongoing wastewater irrigation reduces the soil's potential to absorb heavy metals, which raises the risk that the metals will leak into the groundwater or be absorbed by plants (Angon et al., 2024; Xu et al., 2024). Biochar is a term used to describe a carbon-rich substance. Usually, biomass such as wood, manure, or leaves, is thermally treated in an oxygen-free atmosphere to produce biochar. The unique physio-chemical properties of biochar have drawn interest because of its wide range of uses in environmental remediation, agriculture, energy production, and climate change mitigation (Premarathna et al., 2019). Pyrolysis and hydrothermal carbonization are widely used techniques to produce biochar from carbon-rich materials. Biochar has demonstrated potential as a useful amendment because it has a number of advantages, including increased soil pH, organic carbon content, increased soil water-holding capacity, decreased contaminant levels, increased crop yields, and a barrier to the uptake and buildup of pollutants (Cheng et al., 2020). Recent studies show that biochar has potential for use in a variety of wastewater treatment applications, including adsorption, redox, catalysis, and biocidal uses. The primary advantages of materials based on biochar are their high porosity, huge surface area, improved ability for ion exchange, and plenty of functional groups. There have been attempts to use various forms of biochar material, such as wheat straw biochar, to remove contaminants from aqueous solutions (Cui et al., 2021). It has a great potential for immobilization and passivation, adsorption and removal of heavy metal contaminants, and enhancement of environmental quality. Biochar's surface is highly charged with negative ions, which enhances its ability to exchange cations. Because heavy metal ions are often positively charged, biochar is more electrostatically attracted to them and can exhibit high adsorption properties (Sizmur et al., 2017). Methionine is an essential amino acid that belongs to the aspartate family and is crucial for maintaining the crop health (Devi et al., 2023; Trovato et al., 2021). Methionine is a plant growth regulator that is also linked to the general growth of plants and is necessary for many elements of photosynthesis (Khan et al., 2019; Zaid et al., 2020). A broad class of signaling molecules known as phytohormones was thought to be essential in the

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positive control of physiological and molecular reactions to heavy metal (HM) stressors (Li et al., 2018). Cadmium (Cd) is among the most hazardous heavy metals (HMs) for various organisms. Because cadmium flows quickly through the soil, plants can readily absorb it and transfer it to other organs (Rahim et al., 2016). When lead (Pb) reacts with the sulphhydryl group (thiol, R-SH), reactive oxygen species (ROS) are produced that lead to oxidative bursts, which are known to limit enzyme activity (Zulfiqar et al., 2019). Arsenic (As), another unnecessary metal, similarly poisons plants at high quantities by limiting biomass production, preventing root length and proliferation, and interfering with a number of metabolic processes that have a significant negative impact on plant growth and reproductive ability (Sharma et al., 2021). The complicated assembly of metallothionein, phytochelatin, and a metal-binding peptide produces nontoxic metabolites that shield cells and organisms (Muller et al., 2015). According to reports, methionine effectively controls how plants grow and develop when they are exposed to environmental cues like drought stress (You et al., 2019).

The main objective of this study was to execute the impact of wastewater irrigation on the growth and yield of radish (*Raphanus sativus* L.). Specifically, the study aimed to evaluate the effects of biochar application and foliar application of L-Methionine under different irrigation treatments. The investigation was conducted at the University of Agriculture Faisalabad's Agronomy Research Farm during winter 2024. Radish growth, biochemical and heavy metals analysis were done and evaluated during the study.

MATERIALS AND METHODS

Experimental site and design

The research experiment was performed at the Agronomy Research Farm, University of Agricultural Faisalabad, Pakistan. The experiment was set up using a Randomized Complete Block Design (RCBD) under a split-plot arrangement with three replications. On November 5, 2023, radish was sowed at a seed rate of 2-3 kg ha^{-1}. Beds measuring 75 cm from bed to bed and 8 cm from plant to plant were used for seeding. Following the growth of radish seedlings, biochar was added to the appropriate treatments. Foliar application of methionine was done after 2 weeks of seed germination. The purpose of the research was to determine how soil applied biochar and foliar methionine responded to wastewater irrigation in terms of radish growth. Prior to the experiment, a physiochemical analysis of soil was conducted on the chosen location. Using an auger, a soil sample was taken from the experimental site at a depth of 30 cm, and a composite sample was created. Testing was done on the chemical and physical characteristics of soil at the Ayub Agriculture Research Institute (AARI), located in Faisalabad.

Table 1: Soil analysis report before sowing crop.

Determination	Value	Units
Ph	8.2	
EC	1.37	mScm^{-1}
Organic matter	1.26	%
Available Phosphorus	9.22	mg kg^{-1}
Available Potassium	224	mg kg^{-1}
Soil Saturation	34	%

The experiment was comprised of 2 factors. Factor A included irrigation source and biochar while factor B included foliar application of methionine. Plant samples were collected at fully grown stage for morphological data.

Electrical conductivity (EC mS cm^{-1})

A soil sample was obtained from the experimental area. The sample was air dried and passed through a 2 mm sieve to remove large particles. Took 200 g of sieved soil, added 250 mL distilled water and mixed it with spatula until the soil reached the point of saturation. The sample was covered with plastic wrap to prevent evaporation. After this, the soil sample was left for 1-2 hours to make it fully saturated. The extract's electrical conductivity (EC) was then assessed using a conductivity meter (Rhoades *et al*., 1996).

pH

To test the pH of the soil, 50 g of air-dried soil and 100 mL of distilled water were added in a glass beaker. After mixing the materials, they were placed aside for an hour. The pH of the soil was then tested using a calibrated pH meter (Thomas *et al*., 1996).

Available phosphorus (mg kg^{-1})

In a 250 mL Erlenmeyer flask, 5 g of soil and 100 mL of a 0.5 M $NaHCO_3$ solution were combined to estimate the amount of accessible phosphorus. A 50 mL volumetric flask was filled with 10 mL of the filtrate and 1 mL of 5 N H_2SO_4 after the flasks had been shaken for around 30 minutes. After adding distilled water to get the total amount to 40 mL, 7 mL of reagent B (ascorbic acid) was added until the mixture turned blue. After ten minutes, a spectrophotometer operating at 882 nm wavelength was used to measure the transmittance (Kuo *et al*., 1996).

Available potassium (mg kg^{-1})

50 milliliter centrifuge tube was filled with 5 grams of dirt and 33 milliliters of ammonium acetate solution. The liquid supernatant was extracted by centrifuging the tube after it had been shaken for five minutes. After gathering the extract in a 100 mL volumetric flask, it was diluted with 100 mL of ammonium acetate solution. A flame photometer was used to measure the potassium levels.

Organic matter (%)

Once the soil moisture content was ascertained, 25 g of dry soil was added to a 150 mL flask. Ten milliliters of 1 N $K_2Cr_2O_7$ and twenty milliliters of H_2SO_4 were added to the flask to prepare the soil suspension. After five minutes of heating at 135°C, the mixture was allowed to cool for thirty minutes. After that, the sample was titrated against $FeSO_4$ solution using 200 mL of water and three drops of ferroin as an indicator (Helmke and Sparks, 1996).

Wastewater Physicochemical and Heavy Metal Analysis

The wastewater used for irrigation was sourced from the local municipal drainage system of Faisalabad. Prior to the commencement of the trial, water samples were analyzed at the Ayub Agriculture Research Institute (AARI). The analysis revealed high levels of total dissolved solids (TDS) and heavy metal concentrations exceeding the permissible limits for standard irrigation water. Specifically, the wastewater contained significant concentrations of Zinc (Zn), Cadmium (Cd), and Lead (Pb), which were the primary metals monitored throughout the study.

Biochar Characterization and Application Rate

The biochar used in this study was produced from wheat straw through slow pyrolysis at a temperature of approximately 450-500°C in an oxygen-limited environment. The resulting biochar featured a high surface area and porous structure, optimized for the immobilization of heavy metals. It was characterized by a high pH (alkaline) and a high cation exchange capacity (CEC), facilitating the adsorption of positively charged heavy metal ions. Biochar was applied to the soil at a standardized rate of 10 tons per hectare (10 t ha^{-1}). It was thoroughly incorporated into the top 15 cm of the soil in the designated “wastewater + biochar” and “canal water + biochar” plots prior to sowing.

Climatic Conditions

The field experiment was conducted during the winter season (November 2023 to February 2024) at the Agronomy Research Farm, University of Agriculture, Faisalabad, Pakistan (31.4504° N, 73.1350° E). The region experiences a semi-arid climate. During the growth period, the average temperature ranged from 8°C (minimum) to 24°C (maximum), with low relative humidity and minimal rainfall, typical for the winter cropping cycle in the Punjab plains.

Foliar Spray Methodology

Foliar application of L-methionine was performed at four distinct concentrations: 0 (control), 30, 60 and 90 mg L^{-1}. The first foliar spray was applied two weeks after seed germination (at the 3-4 leaf stage), with a second application following 15 days later to ensure maximum absorption during the critical root development phase. Sprays were conducted during the early morning hours to avoid high evaporation rates and ensure stomatal openness. A manual knapsack sprayer was used to provide a uniform fine mist until the leaves were completely wet (run-off point). A small amount of surfactant (Tween-20) was added to the solution to enhance leaf surface adherence.

Growth Parameters

The following growth parameters were recorded;

Shoot length (cm)

Using a measuring tape, the shoot length was determined from the stem's base to the tip of the longest leaf.

Root length (cm)

The root length was recorded from root tip to base of the stem by using a measuring tape.

Fresh weight of roots (g)

The samples were uprooted and excess soil was removed. The roots were weighed using a precise electric weighing balance.

Fresh weight of shoots (g)

The shoot was carefully cut from the plant, patted dry to remove any excess moisture, and then weighed using an electric balance to determine the fresh weight.

Dry weight of roots (g)

Plant roots were dried in an oven at 50°C for 72 hours and roots dry weight was measured.

Dry weight of shoots (g)

Plant shoots were dried in an oven at 50°C for 72 hours and their dry weight was measured.

Physiological Parameters

Chlorophyll a, b, total chlorophyll contents and carotenoids (mg g^1)

The total chlorophyll and chlorophyll a and b content was measured by Davis in 1979, method whereas carotenoid content was examined by Arnon method (Ahmad et al., 2025; Shah et al., 2021). Fresh plant leaf material (0.1 g) was cut, and an acetone solution (80%) mixed with ethanol (1:1) was utilized. The suspension was filtered to remove turbidity after grinding samples in acetone. Using a spectrophotometer, the absorbance of the samples was determined at three distinct wavelengths: 645 nm, 663 nm, and 480 nm. The contents of carotenoid pigments, total chlorophyll, and chlorophyll a and b were calculated using the following formulas (Datt, 1998; Longjam et al., 2018).

$$\mathbf{Chlorophyll\ a\ (mg\ g-1\ FW) = (12.7 \times OD063 - 2.69 \times OD645) \times V/1000 \times W}$$

$$\mathbf{Chlorophyll\ b\ (mg\ g-1\ FW) = (22.9 \times OD645 - 4.68 \times OD663) \times V/1000 \times W}$$

$$\textbf{Total chlorophyll}\ (\mathbf{mg\ g-1\ FW}) = (\mathbf{20.2} \times \mathbf{OD645} + \mathbf{8.02} \times \mathbf{OD663}) \times \mathbf{V/1000} \times \mathbf{W}$$

1. OD=Optical density
2. V= Volume of acetone used in extract (mL)
3. W=Weight of fresh leaf tissue (mg)

$$\boldsymbol{Carotenoids\ (mg\ g-1\ FW) = (OD480 + 0.114 \times OD663 - 0.638 \times OD645)/2500}$$

Electrolyte leakage (%)

Electrolyte leakage is fundamentally associated with K^+ efflux from plant cells, which is often employed as a biomarker of stress damage in plant tissues and to assess stress tolerance. The proportion of ion leakage of leaves because of stress injury was evaluated using a conductivity meter (Professional Bench Type conductivity (EC), TDS-TEMP Bench Meter, USA), (Lutts et al., 1996). Two to three weeks after stress imposition, leaves from three replications per treatment were gathered and chopped into 1 cm segments. Each sample was washed with deionized water to get rid of any electrolytes that had stuck to the surface. After that, leaf segments were incubated on a revolving shaker at 25 °C (100 rpm) in stoppered vials with 10 mL of deionized water. After three hours, the bathing solution's electrical conductivity was assessed. Conductivity values were obtained during equilibration at 25°C after the samples were autoclaved for 20 minutes at 120°C. The following formula was then used to calculate the EC%.

$$\boldsymbol{EC\% = (L1/L2) \times 100.}$$

Biochemical Parameters

Determination of MDA (µ mol L^{-1})

Malondialdehyde concentration (MDA) was measured using the Thio barbituric acid (TBA) test, which was based on spectrophotometric quantification of the pink complex produced when MDA reacted with two molecules of TBA (De Leon & Borges, 2020; Papastergiadis et al., 2012; Schmedes & Hølmer, 1989). In this process, 0.1 g of leaf tissue were ground in 0.5 ml of 0.1% (w/v) TCA. After homogenized samples were centrifuged for ten minutes at 15,000 x g, the supernatant was moved to a new tube. After collecting the supernatant, 1.5 mL of 20% TCA (containing 0.5% TBA) and 0.5 mL of the supernatant were mixed together. After 15 minutes of incubation at 95°C in a water bath, the mixture was quickly cooled on ice. After generating a red compound in an acidic buffer, the density of the resultant red compound was measured at 532 nm using a spectrophotometer to estimate the levels of MDA.

Proline measurement (µg g^{1})

The reaction of proline with Ninhydrin was used to determine its colorimetric value (Lee et al., 2018; Rosen, 1957). Centrifugation at 12,000 g for 10 minutes was used to remove the residue from the frozen plant material after it had been homogenized in 3% aqueous sulpho salicylic acid (0.01 g 0.5 mL). After carefully stirring and blending 1.25 g of Ninhydrin with 20 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid, the mixture was combined with acetic acid. A test tube containing 1 milliliter of homogenized tissue, 2 milliliters of acid ninhydrin solution, and 2 milliliters of glacial acetic acid was heated to 100 degrees Celsius for an hour. An ice bath was used to halt the response. Until the two phases separated, the extract reaction mixture was vigorously mixed with 2 mL toluene and allowed to sit at room temperature for half an hour. To measure the optical density of the chromophore-containing toluene (1 mL, upper phase), toluene was used as a blank and the mixture was warmed to room temperature. The proline concentration was determined from the standard curve using D-proline.

Determination of Mineral analysis

Using the guidelines provided by AOAC (1990), an Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to assess the amounts of heavy metals in the produced samples. Among the elements selected were zinc (Zn), cadmium (Cd), Copper (Cu), iron (Fe), lead (Pb), Chromium (Cr) and Nickel.

Table 2: summarizes the instrumental working conditions for the following elements:

Elements	Detection limit
Lead	8 (Flame AA)
Cadmium	12 (Flame AA)
Copper	15 (Flame AA)
Iron	5 (Flame AA)
Zinc	1.5 (Flame AA)
Nickel	11 (Flame AA)

Statistical analysis

Experimental data were collected regarding growth, yield, physiological and heavy metals in radish. Data were analyzed by using Fischer's analysis of variance and treatment means were compared using Tukey's Honestly Significant Difference test at a 5% level of confidence by using software statistix 8.1 (Afzal et al., 2013; Ibrahim & Abdullahi, 2023; Njeri Mugwe & Runo, 2026).

RESULTS

Growth Parameters

Root Fresh Weight (g)

The results showed that different irrigation sources and foliar application significantly affect the root fresh weight of radish while the interactive effect was found insignificant (Table 3 & Figure 1). Statistically, the highest root fresh weight was measured where wastewater +biochar was applied whereas the least value was observed under canal water. On the other hand, foliar application of methionine with 90 mg/L significantly increased the root fresh weight followed by 60 mg/L and the lowest root fresh weight was found under control treatment. L-methionine also acts as a growth regulator of cytokinin, brassinosteroids, and auxin, increasing the initiation of roots; helps with the absorption of more nutrients by the plant (El-Awadi et al., 2011). Throughout all irrigation treatments, there was a discernible trend in the mean root fresh weight data that increased with larger concentrations of L-methionine. The WB treatment with 90 mg L^{-1} of L-methionine had the highest mean root fresh weight (618 g), while the CW treatment under control conditions had the lowest mean root fresh weight (330 g). When applied with wastewater or canal water, biochar consistently produced higher root fresh weights than treatments without biochar. The wastewater treatments (WW and WB) demonstrated greater root fresh weights in comparison to the canal water treatments (CW and CB), underscoring the beneficial impacts of wastewater and biochar on the growth of radish.

Table 3: Effect of biochar and methionine on root fresh weight of wastewater irrigated radish

SOV	DF	SS	MS	F	P
Rep	2	6	3		
Treatment	3	357784	119261	638.33	0.0000
*Error Rep*Treatment*	6	1121	187		
Foliar	3	38303	12768	23.42	0.0000
*Treatment*Foliar*	9	4542	505	0.93	0.5209
*Error Rep*Treatment *Foliar*	24	13087	545		
Total	47	414844			

Significant at P≤0.01, Significant at P≤0.05, NS-Non-Significant at P≥0.05

Shoot Fresh weight (g)

The results showed that different irrigation sources and foliar application significantly affect the shoot fresh weight while the interactive effect was found insignificant (Table 4, Figure 1). Statistically, the highest shoot fresh weight was measured where wastewater +biochar was applied whereas the least value was observed under canal water. On the other hand, foliar application of methionine with 90 mg/L significantly increased the shoot fresh weight followed by 60 mg/L and the lowest shoot length was found under control treatment. The treatments had a considerable impact on shoot fresh weight, according to the concise interpretation results. Different combinations of irrigation and biochar greatly affected the shoot fresh weight of radish. Positive effects of biochar observed for beans include increases in the biomass of roots and shoots (Güereña et al., 2015; Sheffield et al., 2024). The main effect of treatment was highly significant (F=45.21, P=0.0002), (Table 25). Similarly, the main effect of applying L-methionine was very significant (F=19.31, P<0.0001), indicating that different L-methionine concentrations topically had a notable effect on shoot fresh weight. The effect of irrigation treatments and biochar on shoot fresh weight was not significantly affected by the varying quantities of L-methionine, as indicated by the non-significant interaction between treatment and foliar application (F=1.89, P=0.1022).

Under control conditions, the CW treatment had the lowest shoot fresh weight (67 g), while the WB treatment, which contained 90 mg L^{-1} of L-methionine, had the highest shoot fresh weight (136.67 g). Compared to control treatments, the application of biochar and the utilization of wastewater, both separately and together, enhanced the shoot's fresh weight.

4. Effect of biochar and methionine on shoot fresh weight of wastewater irrigated radish.

SOV	DF	SS	MS	F	P
Replication	2	109.1	54.56		
Treatment	3	8353.4	2784.47	45.21	0.0002
Error Rep*treatment	6	369.5	61.59		
Foliar	3	4217.9	1405.97	19.31	0.0000
Treatment*Foliar	9	1241.0	137.89	1.89	0.1022
Error Rep*Treatment*Foliar	24	1747.3	72.81		
Total	47	16038.3			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

Root Dry Weight (g)

The results showed that different irrigation sources and foliar application significantly affect the root dry weight while the interaction was found insignificant (Table 5 & Figure 1). Statistically, the highest shoot length was measured where wastewater +biochar was applied whereas the least value was observed under canal water. On the other hand, foliar application of methionine with 90 mg/L significantly increased the root dry weight followed by 60 mg/L and the lowest shoot length was found under control treatment. The findings of the concise interpretation are demonstrating that the primary effects of the treatment (biochar application and irrigation) as well as the foliar L-methionine were very significant for root dry weight. Root dry weight was considerably impacted by various combinations of irrigation and biochar, as indicated by the treatment effect, which had an F-value of 638.33 and a *p*-value of less than 0.0001, (Table 25). Root dry weight was shown to be considerably influenced by various quantities of L-methionine, as demonstrated by the significant foliar application effect, which had an F-value of 23.42 and a P-value of less than 0.0001. Biochar and methionine affected the radish plants' growth, yield, and uptake of heavy metals while they were irrigated with wastewater. When radish plants were treated with biochar and methionine, their dry weight increased relative to the plants that did not get these treatments (Nazli et al., 2020).

5. Effect of biochar and methionine on root dry weight (g) of wastewater irrigated radish.

SOV	DF	SS	MS	F	P
Rep	2	0.3928	0.1964		
Treatment	3	30.0722	10.0241	45.21	0.0000
Error Rep*Treatment	6	1.3303	0.2217		
Foliar	3	15.1844	5.0615	19.31	0.0000
Treatment*Foliar	9	4.4677	0.4964	1.89	0.1022
Error Rep*Treatment *Foliar	24	6.2904	0.2621		
Total	47	57.7379			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

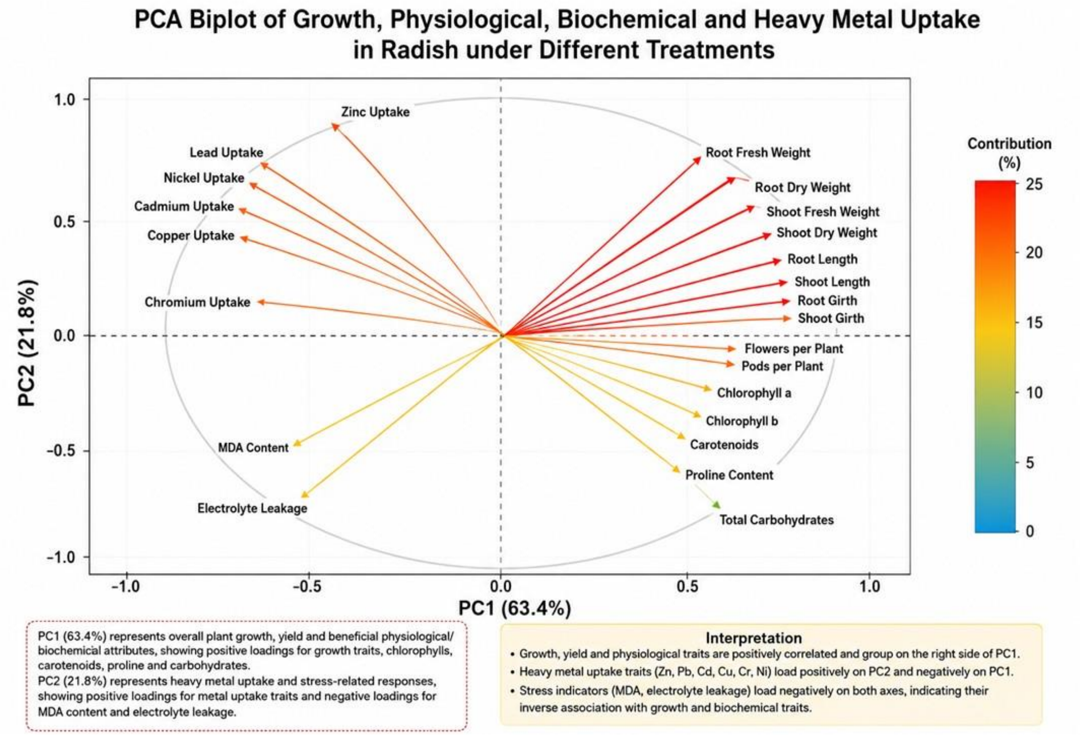
Shoot Dry Weight (g)

The results showed that different irrigation sources and foliar application significantly affect the shoot dry weight while the combine effect was found insignificant (Table 6 & Figure 2). Statistically, the highest shoot length was measured where wastewater +biochar was applied whereas the least value was observed under canal water. On the other hand, foliar application of methionine with 90 mg/L significantly increased the shoot length followed by 60 mg/L and the lowest shoot length was found under control treatment, (Figure 1). The results showed that the foliar application of L-methionine, irrigation, and biochar treatments had a substantial effect on the shoot dry weight of radish. A strong effect on shoot dry weight was seen with varied combinations of irrigation and biochar, as indicated by the highly significant treatment effect (F=45.21, P<0.0001). The favorable impacts of both biochar and wastewater were highlighted by the order of increase in mean shoot dry weight: canal water<canal water + Biochar<wastewater<wastewater + Biochar. Higher concentrations of L-methionine resulted in larger shoot dry weight. Similarly, the foliar spray of L-methionine was likewise very significant (F=19.31, P<0.0001), (Table 25). The comparison to fresh water-irrigated control plants, the shoot's dry weight increased considerably (Sadak et al., 2015).

6. Effect of biochar and methionine on shoot dry weight of wastewater irrigated radish.

SOV	DF	SS	MS	F	P
Rep	2	0.3928	0.1964		
Treatment	3	30.0722	10.0241	45.21	0.0000
Error Rep*Treatment	6	1.3303	0.2217		
Foliar	3	15.1844	5.0615	19.31	0.0000
Treatment*Foliar	9	4.4677	0.4964	1.89	0.1022
Error Rep*Treatment *Foliar	24	6.2904	0.2621		
Total	47	57.7379			

Significant at P≤0.01 Significant at P≤0.05 & NS-Non-Significant at P≥0.05

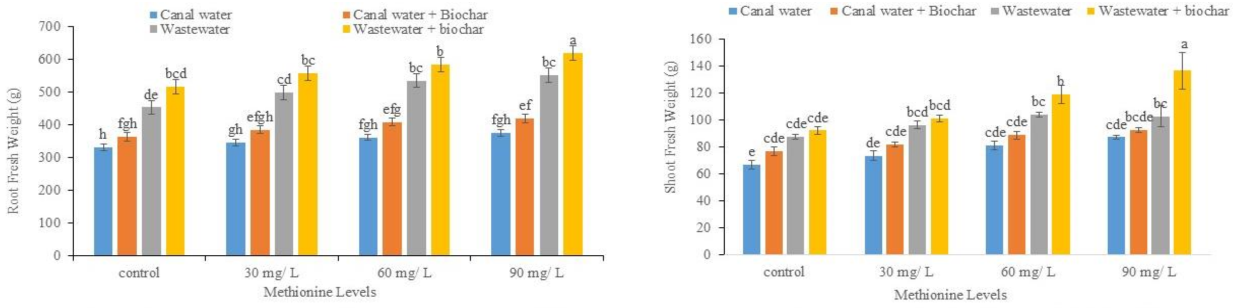


PC1 (63.4%) represents overall plant growth, yield and beneficial physiological/ biochemical attributes, showing positive loadings for growth traits, chlorophylls, carotenoids, proline and carbohydrates.
PC2 (21.8%) represents heavy metal uptake and stress-related responses, showing positive loadings for metal uptake traits and negative loadings for MDA content and electrolyte leakage.

Interpretation

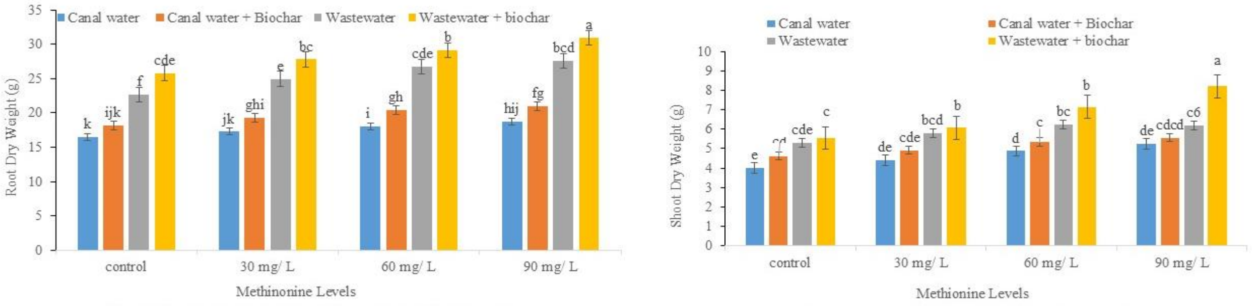
- Growth, yield and physiological traits are positively correlated and group on the right side of PC1.
- Heavy metal uptake traits (Zn, Pb, Cd, Cu, Cr, Ni) load positively on PC2 and negatively on PC1.
- Stress indicators (MDA, electrolyte leakage) load negatively on both axes, indicating their inverse association with growth and biochemical traits.

Fig. 1: Principal Component Analysis (PCA) biplot illustrating the multivariate relationships among growth, physiological, biochemical, oxidative stress, and heavy metal uptake traits in radish under different treatment conditions. PC1 (63.4%) primarily explained variation associated with enhanced plant growth and beneficial physiological and biochemical attributes, including root and shoot biomass, chlorophyll content, carbohydrates, and carotenoids, while negatively correlating with stress-induced parameters such as electrolyte leakage and malondialdehyde (MDA) content. PC2 (21.8%) was mainly associated with heavy metal accumulation traits (Zn, Pb, Ni, Cd, Cu, and Cr uptake) and stress-response indicators. The directional clustering and vector lengths indicate the magnitude and correlation of each variable, highlighting the contrasting influence of stress-related traits versus growth-promoting characteristics in radish plants under varying treatments.



Comparison of treatment means for radish root fresh weight.

Comparison of means for shoot fresh weight of radish.



Comparison of means for root dry weight of radish.

Comparison of means for shoot dry weight.

Fig. 2: Conceptual framework and physiological mechanisms of heavy metal mitigation in radish (Raphanus sativus L.) through soil-applied biochar and foliar-applied L-methionine.

Root Length (cm)

The majority of radish plant root system is found in the first 12 inches of soil. However, it can reach a depth of up to 2 feet. Treatment effect was very significant (F=15.63, P=0.0031) (Table 25), suggesting that the root length was significantly impacted by various irrigation and biochar combinations, (Table 6 & Figure 3). The results demonstrate the beneficial impacts of both biochar and wastewater, as the mean root length grew in the following order: canal water<Canal water + Biochar<Wastewater<Wastewater + Biochar. Likewise, there was a substantial increase in root length with increasing quantities of L-methionine applied topically (F=5.54, P=0.0049). The results indicate that the combined effect of these factors on root length was not different from their separate effects. The interaction between the treatments was not significant (F=1.01, P=0.4601). Additionally, wastewater treatments had longer root lengths than canal water treatments, highlighting the advantages of both biochar and wastewater for the growth of radish. When comparing the control to the highest L-methionine content, the root length grew by 33% in canal water, 8.3% in canal water + Biochar, 36.4% in wastewater, and 13.3% in wastewater + Biochar. The application of biochar has significantly increased the root length by improving soil structure (An et al., 2022; Ruan et al., 2024; Xiang et al., 2017).

Shoot Length (cm)

The results showed that different irrigation sources and foliar application significantly affect the shoot length of radish while the interaction of foliar and treatment was found insignificant (Table 7 & Figure 3). Statistically, the highest shoot length was measured where wastewater + biochar was applied whereas the least value was observed under canal water. On the other hand, foliar application of methionine with 90 mg/L significantly increased the shoot length followed by 60 mg/L and the lowest shoot length was found under control treatment. The wastewater + biochar treatment with 90 mg/L L-methionine had the highest mean shoot lengths (mean=35), followed by the canal water with biochar treatment (mean=25). The findings of the concise interpretation showed that there were significant effects on radish shoot length from both foliar L-methionine sprays (*f=46.51, p<0.0001*) and irrigation treatments (*f=115.38, p<0.0001*), (Table 25). The results indicate that there was no significant deviation in the combined effect of the treatments and foliar spray from their separate effects (*f=1.78, p=0.1240*). In comparison to the control, the Canal water + Biochar treatment increased by 56.25% when 30 mg/L methionine was added, while the Wastewater and Wastewater + Biochar treatments increased by 68.75% and 118.75%, respectively. The application of biochar and various irrigation techniques had a considerable impact on shoot length. Biochar has positive effect on plant shoot length (Agarwal et al., 2022).

7. Effect of biochar and methionine on root length of wastewater irrigated radish.

SOV	DF	SS	MS	F	P
Rep	2	4.167	2.0833		
Treatment	3	147.167	49.0556	15.63	0.0031
Error Rep*Treatment	6	18.833	3.1389		
Foliar	3	43.667	14.5556	5.54	0.0049
Treatment*Foliar	9	23.833	2.6481	1.01	0.4601
Error Rep*Treatment *Foliar	24	63.000	2.6250		
Total	47	300.667			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

Table 8: Effect of biochar and methionine on shoot length (cm) of wastewater irrigated radish.

SOV	DF	SS	MS	F	P
Rep	2	10.04	5.021		
Treatment	3	2064.90	688.299	115.38	0.0000
Error Rep*Treatment	6	35.79	5.965		
Foliar	3	679.23	226.410	46.51	0.0000
Treatment*Foliar	9	78.19	8.688	1.78	0.1240
Error Rep*Treatment *Foliar	24	116.83	4.868		
Total	47	2984.98			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

Root Girth (cm)

Measurements of root girth revealed clear differences between treatments; the Wastewater + Biochar treatment with 90 mg/L L-methionine had the highest mean (mean=12), closely followed by Canal water with Biochar (mean=10). Concise interpretation statistical analysis showed that both foliar L-methionine sprays (*f=8.54, p=0.0005*) and irrigation treatments (*f=39.87, p=0.0002*) had a significant impact on the girth of radish roots, (Table 8 & Figure 3). These findings suggest that foliar spraying with L-methionine and applying biochar, especially when combined with wastewater, both considerably improve root girth. Although foliar application and treatments had an interaction effect, it was not statistically significant (*f=1.34, p=0.2711*), indicating that the overall effect did not differ significantly from the effects of each treatment alone. Biochar application has positive impact on root girth by increasing porosity of soil (Upadhyay et al., 2024).

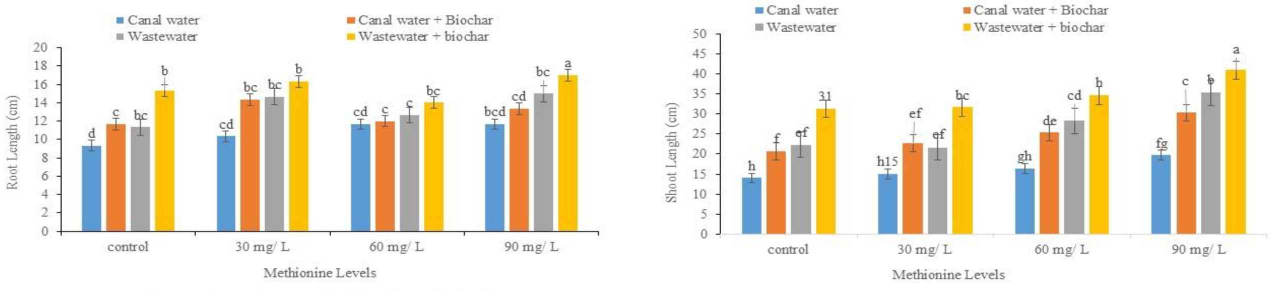
Shoot Girth (cm)

The findings of the analysis of variance (ANOVA) showed that the radish shoot girth was significantly impacted by both foliar L-methionine sprays (*f=6.14, p=0.003*) and irrigation treatments (*f=19.77, p=0.0016*), (Table 9 & Figure 2 and 3). These results imply that foliar L-methionine spraying and the application of biochar, particularly when combined with wastewater, greatly improve shoot girth. The results indicate that there was no significant difference in the combined influence of the treatments and foliar application (*f=0.52, p=0.8484*) when compared to their individual effects. These treatments consistently showed significant improvements in shoot girth at higher L-methionine levels (60 mg/L and 90 mg/L), with the Wastewater + Biochar treatment showing a 28.57% increase and the Wastewater treatment showing a 14.29% increase compared to the control. It was observed that the higher level of sewage water significantly affected the vegetative yield of radish crop and provides maximum return at 100% sewage water. Plant height, root length, girth and yield of radish crop significantly increased with the increase of sewage content in the blended water. The wastewater water also increased the marketable yield of the radish crop (Almaramah et al., 2024; Isaac et al., 2009).

Table 9: Effect of biochar and methionine on Root Girth (cm) of wastewater irrigated radish.

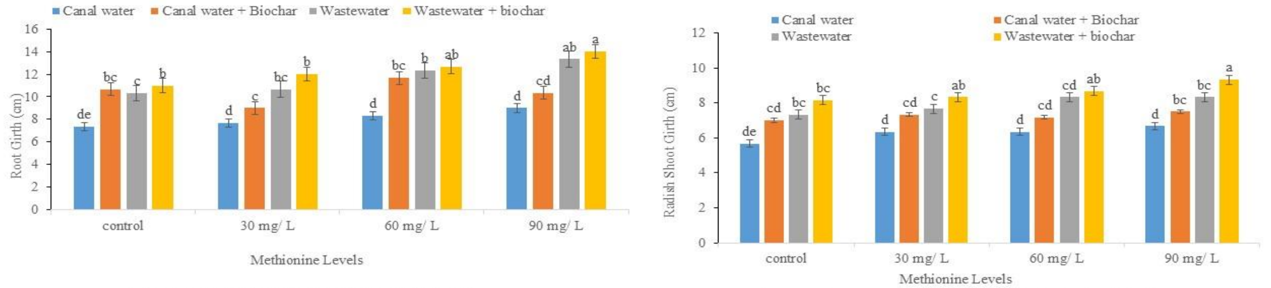
SOV	DF	SS	MS	F	P
Rep	2	6.167	3.0833		
Treatment	3	129.562	43.1875	39.87	0.0002
Error Rep*Treatment	6	6.500	1.0833		
Foliar	3	32.729	10.9097	8.54	0.0005
Treatment*Foliar	9	15.354	1.7060	1.34	0.2711
Error Rep*Treatment *Foliar	24	30.667	1.2778		
Total	47	220.979			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.



Comparison of means for Root Length (cm).

Comparison of means for Radish Shoot Length.



Comparison of means for Root Girth (cm).

Comparison of means of Shoot Girth (cm) of Radish.

Fig. 3: Graphical representation of the synergistic effects of soil-applied biochar and foliar-applied L-methionine on the growth and heavy metal mitigation of radish (Raphanus sativus L.) under wastewater irrigation.

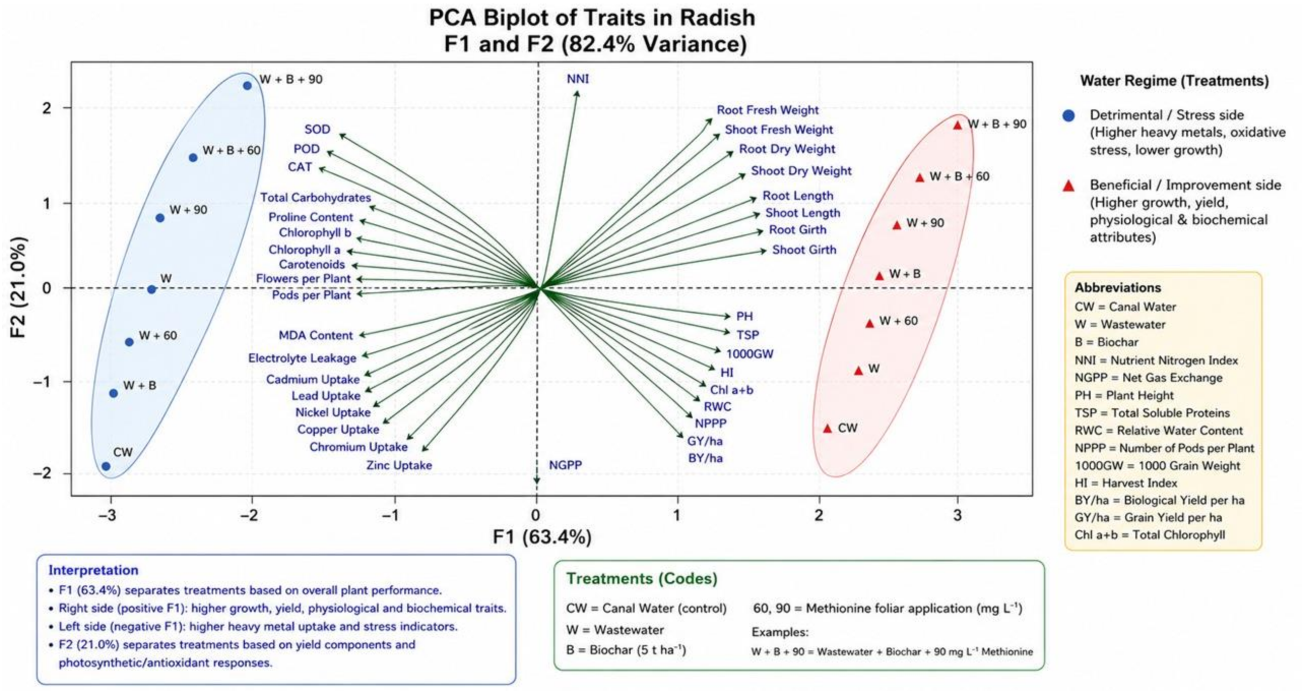


Fig. 4: Principal Component Analysis (PCA) biplot illustrating the relationship between growth, physiological, and biochemical traits in radish across different water regimes and soil amendments. The first two principal components (F1 and F2) account for 82.4% of the total observed variance.

Table 10: Effect of biochar and methionine on shoot girth of wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	2.1979	1.099		
Treatment	3	36.7656	12.2552	19.77	0.0016
Error Rep*treatment	6	3.7188	0.6198		
Foliar	3	5.3073	1.7691	6.14	0.003
treatment*foliar	9	1.3385	0.1487	0.52	0.8484
Error Rep*treatment*foliar	24	6.9167	0.2882		
Total	47	56.2448			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

Number of flowers per plant

The wastewater + biochar treatment with 90 mg/L, L-methionine had the highest mean flower counts per plant (mean=18), followed by the wastewater treatment (mean=15). The mean flower counts per plant varied throughout treatments. With a mean of 13, canal water and canal water + Biochar treatments showed decreased mean flower counts. Concise interpretation statistical analysis showed, (Table 10, (Figure 4), that the quantity of flowers per plant was significantly impacted by both foliar L-methionine sprays (*f=29.42, p<0.0001*) and irrigation treatments (*f=29.79, p=0.0005*). These findings show that applying biochar, particularly when combined with wastewater, and foliar spraying radish plants with L-methionine greatly increase their capacity to flowering. Saxena et al., (2013) found that % germination, root length, shoot length, number of flowers per plant, number of pods per plant, tolerance to high temperatures and harsh light conditions, and number of seeds were all shown to have grown greatly with the use of biochar. Beans have also been shown to exhibit increases in the root: shoot ratio in response to the use of biochar (Farhangi-Abriz & Torabian, 2018; Rezaie et al., 2019; Sheffield et al., 2024). Furthermore, foliar application and treatments had a significant interaction effect (*f=2.79, p=0.0214*), indicating that the combined influence of these factors on flower output is different from the sum of their individual effects (Figure 4).

Table 11: Effect of biochar and methionine on flowers per plant of wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	43.875	21.9375		
Treatment	3	262.562	87.5208	29.79	0.0005
Error Rep*treatment	6	17.625	2.9375		
Foliar	3	117.063	39.0208	29.42	0
treatment*foliar	9	33.354	3.706	2.79	0.0214
Error Rep*treatment*foliar	24	31.833	1.3264		
Total	47	506.312			

Significant at P≤0.01, Significant at P≤0.05, Non-Significant at P≥0.05

Numbers of pods per plant

The major effects of foliar application (*f=170.89, p=0*) and treatment (*f=71.43, p=0*) are both extremely significant, according to the results, indicating that both factors have a considerable effect on the number of pods per plant. The interaction between foliar application and treatment, however, is not significant (*f=0.32, p=0.9597*), suggesting that the sum of their separate impacts does not significantly affect the number of pods, (Table 11 & Figure 3 and 4). 19.313 is the grand mean of the number of pods per plant.

Moderate variability in the results is shown by the coefficients of variation (CV), which are 6.16% for the treatment impact and 3.95% for the combined treatment and foliar application effects. Germination %, root length, shoot length, number of flowers per plant, number of pods per plant, tolerance to high temperatures and harsh light conditions, and number of seeds were all shown to have grown greatly with the use of biochar (Rani & Kayang, 2023; Saxena et al., 2013; Yadav et al., 2026).

Table 12: Effect of biochar and methionine on numbers of pods per plant of wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	9.5	4.75		
Treatment	3	303.562	101.187	71.43	0
Error Rep*treatment	6	8.5	1.417		
Foliar	3	299.063	99.688	170.89	0
treatment*foliar	9	1.688	0.188	0.32	0.9597
Error Rep*treatment*foliar	24	14	0.583		
Total	47	636.312			

Grand Mean 19.313, CV (Rep*treatment) 6.16, CV(Rep*treatment*foliar) 3.95

Chlorophyll a content (mg g^{-1})

Chlorophyll a content is considerably impacted by different combinations of irrigation and biochar, as indicated by the results, which show that the primary effect of the treatment (Factor A) is statistically significant (*p=0.0083*) with an F-value of 10.53, (Table 12, Figure 3 and 5). F-value of 39.28 indicates that the foliar application of L-methionine (Factor B) also has a highly significant effect (*p<0.0001*), suggesting that different quantities of L-methionine also have a considerable impact on the amount of chlorophyll a. Increased L-methionine levels influence phytohormones, which ultimately increases the chlorophyll content and chloroplast development (Cortleven & Schmülling, 2015; Montanaro et al., 2022; Skowron et al., 2025). The relationship between foliar L-methionine application and irrigation treatments (treatment*foliar) is, however, not significant (*p=0.9933*), with an F-value of 0.19. This suggests that the sum of these impacts, apart from their separate contributions, does not considerably affect the amount of chlorophyll a, (Figure 4).

Chlorophyll b (mg g^{-1})

The combination of irrigation and biochar application considerably impacts the chlorophyll b content in radish. The main effect of treatment (Factor A) has an F-value of 69.93 and is highly significant (*p<0.0001*). Likewise, the application of L-methionine topically (Factor B) has an even more notable effect (*p<0.0001*), with an F-value of 225.91, indicating that variations in L-methionine concentration have a substantial impact on the amount of chlorophyll b, (Table 13 & Figure 4). The application of L-methionine promoted phytohormones which can increase photosynthetic activity leading to improved yield (Rouphael et al., 2017). Moreover, (Table 25), the treatment foliar interaction (*p=0.0037*) and F-value of 3.9 indicate the significance of this relationship between the treatment and foliar application. This suggests that, in addition to their respective contributions, the combined impacts of irrigation treatments and the foliar application of L-methionine considerably affect chlorophyll b content (Figure 4).

Carotenoids content (mg g^{-1})

Different combinations of irrigation and biochar considerably affect the carotenoid concentration in radish, as shown by the statistically significant main effect of treatment with an F-value of 12.95 (*p=0.005*). The application of biochar improved total chlorophyll, carotenoid and protein content of plant under different irrigation systems (Younis et al., 2015). An F-value of 5.43 indicates that the foliar treatment of L-methionine also has a significant effect (*p=0.0054*), indicating a significant influence of various quantities of L-methionine on carotenoid content. However, the correlation between foliar L-methionine application and irrigation treatments (treatment*foliar) is not statistically significant (*F-value=1, p=0.4662*), (Table 14 & Figure 5). This shows that the combined effects of these factors do not significantly change the carotenoid content above and beyond what they each contribute on their own (Kothari & Schweiger, 2022; Molner et al., 2024).

Table 12. Effect of biochar and methionine on chlorophyll a content of wastewater irrigated radish.

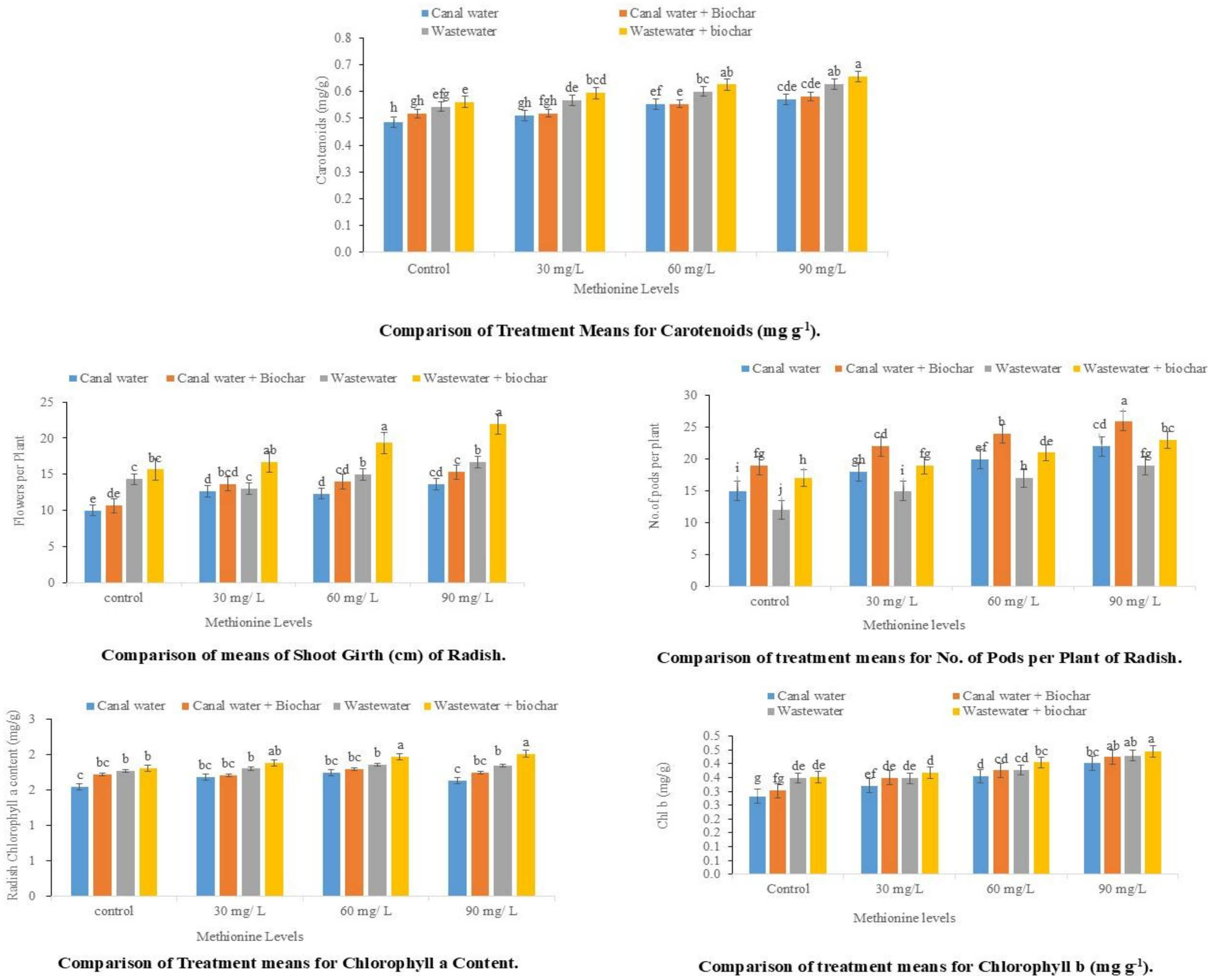
Source	DF	SS	MS	F	P
Rep	2	3.15E-06	1.58E-06		
Treatment	3	4.06E-05	1.35E-05	16.47	0.0027
Error Rep*treatment	6	4.93E-06	8.22E-07		
Foliar	3	9.35E-06	3.12E-06	2.47	0.0864
treatment*foliar	9	1.19E-05	1.32E-06	1.05	0.433
Error Rep*treatment*foliar	24	3.03E-05	1.26E-06		
Total	47	1.00E-04			

Significant at P≤0.01, Significant at P≤0.05 & NS-Non-Significant at P≥0.05

Heavy Metal Analysis

Zinc Uptake (ppm)

The concise interpretation results indicated that the treatment factor had a highly significant effect on zinc content with an *F-value* of 327.57 and a *p-value* of 0, suggesting that different irrigation and biochar treatments substantially influenced zinc accumulation in radish. Similarly, the foliar application of L-Methionine significantly affected zinc content, with an *F-value* of 10.72 and a *p-value* of 0.0001, highlighting the importance of L-Methionine concentration in zinc uptake. However, the interaction between these factors (treatment*foliar) was not significant, with an F-value of 2.05 and a *p-value* of 0.0775, (Table 25, Figure 6), indicating that the combined effect of these treatments on zinc content is not significantly different from their individual effects, (Table 15). The mean square errors for the replications and treatments and their interaction were 6.53 and 8.29, respectively. The grand mean zinc content across all treatments was 29.938 ppm, with coefficients of variation (CV) for the errors associated with replications and treatments and their interaction being 8.54% and 9.62%, respectively. These findings suggest that irrigation, biochar application, and foliar L-Methionine application significantly impact zinc content in radish, but their combined effects do not differ significantly from their individual impacts. Biochar has a high surface area and porosity, which can adsorb zinc ions and make them more available to plant roots. Additionally, biochar can increase the cation exchange capacity of the soil, facilitating the uptake of zinc by plants without causing toxicity (Puga et al., 2015).



Comparison of Treatment Means for Carotenoids (mg g^{-1}).

Comparison of means of Shoot Girth (cm) of Radish.

Comparison of treatment means for No. of Pods per Plant of Radish.

Comparison of Treatment means for Chlorophyll a Content.

Comparison of treatment means for Chlorophyll b (mg g^{-1}).

Fig. 5: Growth and photosynthetic pigment response of radish to biochar and methionine treatments. Comparison of treatment means for shoot girth, number of pods and pigment concentrations (Chl a, Chl b and Carotenoids) across four methionine concentrations and two irrigation sources with/without biochar.

Table 13: Effect of biochar and methionine application on chlorophyll b content of wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	5.91E-08	2.95E-08		
Treatment	3	6.06E-06	2.02E-06	69.93	0
Error Rep*treatment	6	1.73E-07	2.89E-08		
Foliar	3	2.68E-05	8.93E-06	225.91	0
treatment*foliar	9	1.39E-06	1.54E-07	3.9	0.0037
Error Rep*treatment*foliar	24	9.49E-07	3.95E-08		
Total	47	3.54E-05			

Significant at P≤0.01, Significant at P≤0.05 & NS-Non-Significant at P≥0.05

Table 14: Effect of biochar and methionine application on carotenoids content of wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	2.88E-10	1.44E-10		
Treatment	3	3.92E-09	1.31E-09	12.95	0.005
*Error Rep*treatment*	6	6.05E-10	1.01E-10		
Foliar	3	9.76E-10	3.26E-10	5.43	0.0054
*treatment*foliar*	9	5.40E-10	6.00E-11	1	0.4662
*Error Rep*treatment*foliar*	24	1.44E-09	6.00E-11		
Total	47	7.77E-09			

Chromium content (ppm) in Radish

The concise interpretation results indicated that the treatment factor had a highly significant effect on chromium content, with an *F-value* of 27.96 and a *p-value* of 0.0006, demonstrating that different irrigation and biochar treatments substantially influenced chromium accumulation in radish. In contrast, the foliar application of L-Methionine did not significantly affect chromium content, as indicated by an *F-value* of 1.2 and a p-value of 0.3301. The interaction between these factors was also not significant, with an F-value of 0.25 and a p-value of 0.9819, indicating that the combined effect of these treatments on chromium content is not significantly different from their individual effects, (Table 16). The mean square errors for replications and treatments and their interaction were 0.01143 and 0.00864, respectively. These findings suggest that while irrigation and biochar application significantly impact chromium content in radish, the foliar application of L-Methionine does not, and their combined effects are not significantly different from their individual impacts. Biochar can alter soil chemistry, affecting the speciation of chromium in the soil solution. Changes in chromium speciation can impact its availability to plants (Al-Wabel et al., 2024; Rafique et al., 2022).

Table 15: Effect of biochar and methionine on zinc uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	7.12	3.56		
Treatment	3	6421.73	2140.58	327.57	0
Error rep*treatment	6	39.21	6.53		
Foliar	3	266.73	88.91	10.72	0.0001
treatment*foliar	9	153.02	17	2.05	0.0775
Error rep*treatment*foliar	24	199	8.29		
Total	47	7086.81			

Grand Mean 29.938, CV (rep*treatment) 8.54, CV (rep*treatment*foliar) 9.62

Table 16: Effect of biochar and methionine on chromium uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.01546	0.00773		
Treatment	3	0.95834	0.31945	27.96	0.0006
Error Rep*treatment	6	0.06855	0.01143		
Foliar	3	0.03117	0.01039	1.2	0.3301
treatment*foliar	9	0.01952	0.00217	0.25	0.9819
Error Rep*treatment*foliar	24	0.20738	0.00864		
Total	47	1.30043			

Copper uptake (ppm) in Radish

The concise interpretation showed that the treatment factor had a highly significant effect on copper content, with an F-value of 24.92 and a p-value of 0.0009, indicating that different irrigation and biochar treatments substantially influenced copper accumulation in radish. The foliar application of L-Methionine also significantly affected copper content, with an F-value of 10.77 and a p-value of 0.0001. However, the interaction between these factors was not significant, with an F-value of 0.22 and a p-value of 0.989, indicating that the combined effect of these treatments on copper content is not significantly different from their individual effects, (Table 17). The mean square errors for replications and treatments and their interaction were 3.3542 and 1.4931, respectively. The grand mean copper content across all treatments was 14.625 ppm, with coefficients of variation (CV) for the errors associated with replications and treatments and their interaction being 12.52% and 8.35%, respectively. These findings suggest that both irrigation and biochar application and foliar L-Methionine application significantly impact copper content in radish, but their combined effects are not significantly different from their individual impacts. Soil amendment with biochar demonstrated positive results for Cu stabilization in aged Cu-contaminated soil, thereby reducing its accumulation and translocation in plants and mitigating livestock feed security risks (Korai et al., 2025; Nie et al., 2024; Rehman et al., 2019).

Table 17: Effect of biochar and methionine on copper uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	11.375	5.6875		
Treatment	3	250.75	83.5833	24.92	0.0009
Error Rep*treatment	6	20.125	3.3542		
Foliar	3	48.25	16.0833	10.77	0.0001
treatment*foliar	9	2.917	0.3241	0.22	0.989
Error Rep*treatment*foliar	24	35.833	1.4931		
Total	47	369.25			

Grand Mean 14.625, CV (Rep*treatment) 12.52, CV (Rep*treatment*foliar) 8.35

Cadmium content (ppm) in Radish

The concise interpretation indicated that the treatment factor had a highly significant effect on cadmium content, with an F-value of 57.68 and a p-value of 0.0001, suggesting that different irrigation and biochar treatments substantially influenced cadmium accumulation in radish. The foliar application of L-Methionine also significantly affected cadmium content, with an F-value of 29.06 and a p-value of 0, demonstrating its strong impact on cadmium uptake, (Table 18 Figure 6). However, the interaction between these factors was not significant, with an F-value of 1.55 and a p-value of 0.1878, indicating that the combined effect of these treatments on cadmium content is not significantly different from their individual effects. The mean square errors for replications and treatments and their interaction were 0.00005 and 0.00003, respectively. The grand mean cadmium content across all treatments was 0.0219 ppm, with coefficients of variation (CV) for the errors associated with replications and treatments and their interaction being 31.27% and 26.01%, respectively. These findings suggest that both irrigation and biochar application and foliar L-Methionine application significantly impact cadmium content in radish, but their combined effects are not significantly different from their individual impacts (Mondal et al., 2025; Nessem et al., 2023; Zhang et al., 2025). The application of wheat straw biochar in rice plant had greatly decreased the uptake of cadmium from root to shoot (Chen et al., 2016).

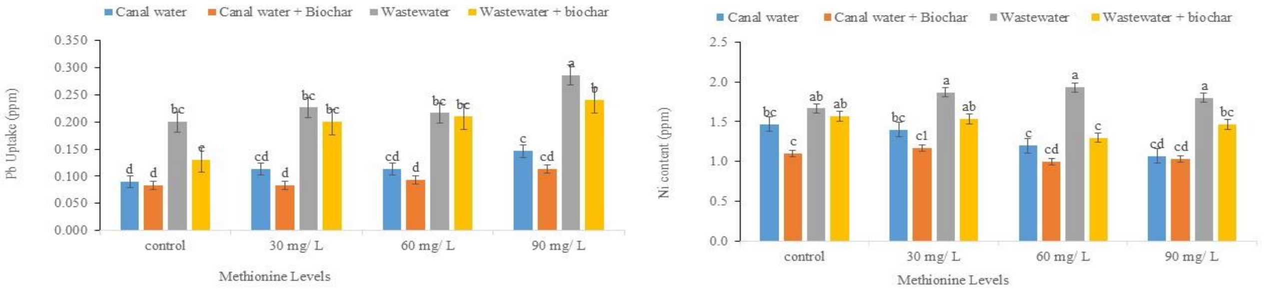
Table 18: Effect of biochar and methionine on Cadmium uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.00012	0.00006		
Treatment	3	0.00808	0.00269	57.68	0.0001
Error Rep*treatment	6	0.00028	0.00005		
Foliar	3	0.00282	0.00094	29.06	0
treatment*foliar	9	0.00045	0.00005	1.55	0.1878
Error Rep*treatment*foliar	24	0.00078	0.00003		
Total	47	0.01253			

Grand Mean 0.0219, CV (Rep*treatment) 31.27, CV (Rep*treatment*foliar) 26.01

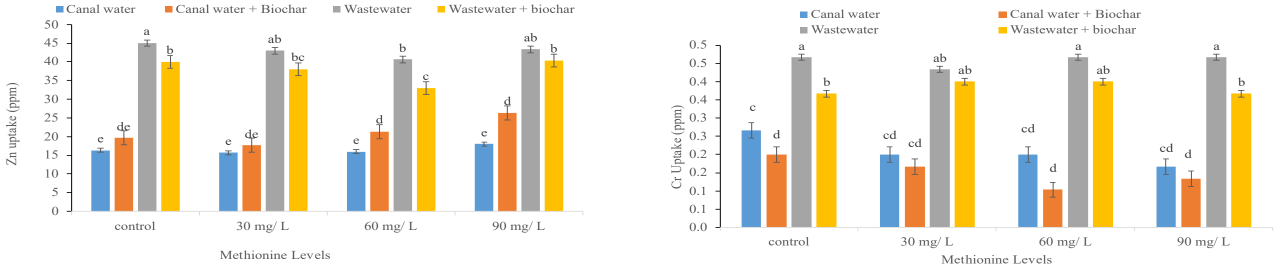
Lead content (ppm) in Radish

The concise interpretation revealed that the treatment factor had a significant effect on lead content, with an F-value of 20.29 and a p-value of 0.0015, indicating that different irrigation and biochar treatments significantly influenced lead accumulation in radish. Similarly, the foliar application of L-Methionine also had a significant impact on lead content, with an F-value of 6.32 and a p-value of 0.0026, demonstrating its effect on lead uptake, (Table 19 Figure 6). However, the interaction between these factors (treatment*foliar) was not significant, with an F-value of 0.6 and a p-value of 0.7834, suggesting that the combined effect of these treatments on lead content is not significantly different from their individual effects. The mean square errors for replications and treatments and their interaction were 0.00254 and 0.0016, respectively. The grand mean lead content across all treatments was 0.1592 ppm, with coefficients of variation (CV) for the errors associated with replications and treatments and their interaction being 31.65% and 25.14%, respectively. These findings indicate that both irrigation and biochar application and foliar L-Methionine application significantly influence lead content in radish, with their combined effects not differing significantly from their individual impacts. The application of biochar decreased the extractable amounts of Pb by raising pH levels, which cause metal precipitation and therefore decrease heavy metal solubility (Puga et al., 2015).



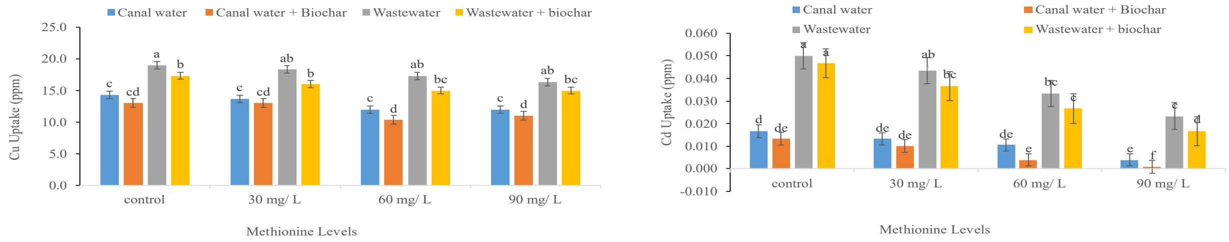
Comparison of Treatment Means for Lead uptake in Radish.

Comparison of Treatment Means for Ni uptake of Radish.



Comparison of Treatment Means for Zinc uptake in Radish.

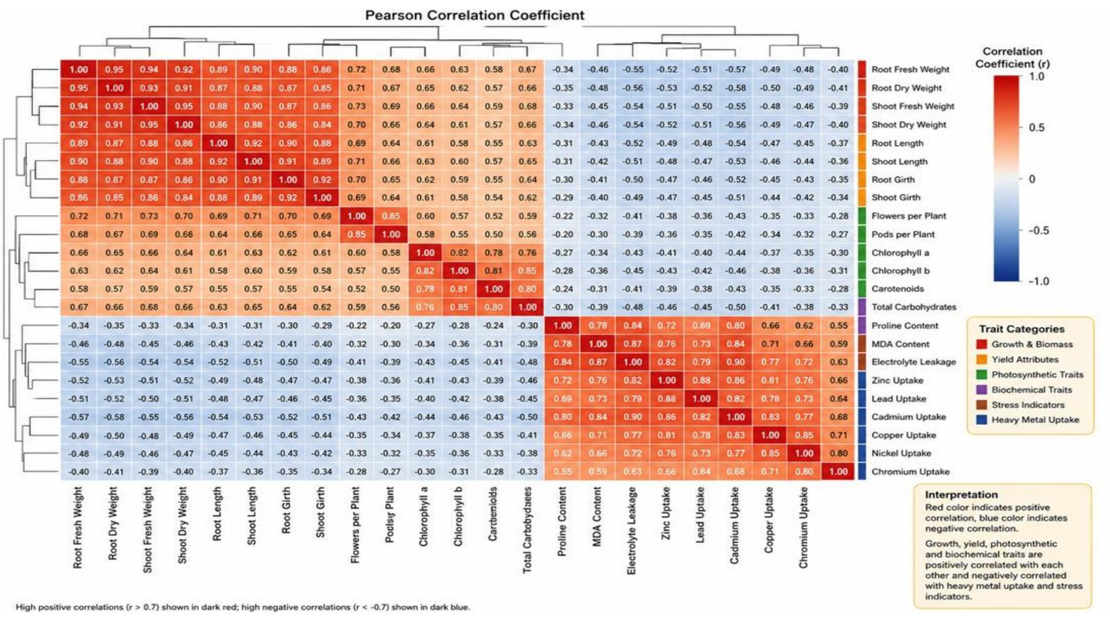
Comparison of Treatment Means for Chromium uptake in Radish



Comparison of Treatment Means for Copper uptake in Radish

Comparison of Treatment Means of Cd uptake in Radish.

Fig. 6: Impact of biochar application and varying levels of methionine (control, 30, 60, and 90 mg/L) on the heavy metal uptake (ppm) of radish irrigated with canal water and wastewater. Panels display the comparative accumulation of Zinc (Zn), Chromium (Cr), Copper (Cu), Cadmium (Cd), Lead (Pb) and Nickel (Ni) in plant tissues. Different letters indicate significant differences between treatments ($P < 0.05$), with data generally illustrating that biochar and higher methionine levels contribute to a reduction in toxic metal accumulation compared to untreated wastewater irrigation. Grand Mean 0.1592, CV (Rep*treatment) 31.65, CV (Rep*treatment*foliar) 25.14.



Root Fresh Weight	1.00	0.95	0.94	0.92	0.89	0.90	0.88	0.86	0.72	0.68	0.66	0.63	0.58	0.67	-0.34	-0.46	-0.55	-0.52	-0.51	-0.57	-0.49	-0.48	-0.40
Root Dry Weight	0.95	1.00	0.93	0.91	0.87	0.88	0.87	0.85	0.71	0.67	0.65	0.62	0.57	0.66	-0.35	-0.48	-0.56	-0.53	-0.52	-0.58	-0.50	-0.49	-0.41
Shoot Fresh Weight	0.94	0.93	1.00	0.95	0.88	0.90	0.87	0.86	0.73	0.69	0.66	0.64	0.59	0.68	-0.33	-0.45	-0.54	-0.51	-0.50	-0.55	-0.48	-0.46	-0.39
Shoot Dry Weight	0.92	0.91	0.95	1.00	0.86	0.88	0.86	0.84	0.70	0.66	0.64	0.61	0.57	0.66	-0.34	-0.46	-0.54	-0.52	-0.51	-0.56	-0.49	-0.47	-0.40
Root Length	0.89	0.87	0.88	0.86	1.00	0.92	0.90	0.88	0.69	0.64	0.61	0.58	0.55	0.63	-0.31	-0.43	-0.52	-0.49	-0.48	-0.54	-0.47	-0.45	-0.37
Shoot Length	0.90	0.88	0.90	0.88	0.92	1.00	0.91	0.89	0.71	0.66	0.63	0.60	0.57	0.65	-0.31	-0.42	-0.51	-0.48	-0.47	-0.53	-0.46	-0.44	-0.36
Root Girth	0.88	0.87	0.87	0.86	0.90	0.91	1.00	0.92	0.70	0.65	0.62	0.59	0.55	0.64	-0.30	-0.41	-0.50	-0.47	-0.46	-0.52	-0.45	-0.43	-0.35
Shoot Girth	0.86	0.85	0.86	0.84	0.88	0.89	0.92	1.00	0.69	0.64	0.61	0.58	0.54	0.62	-0.29	-0.40	-0.49	-0.47	-0.45	-0.51	-0.44	-0.42	-0.34
Flowers per Plant	0.72	0.71	0.73	0.70	0.69	0.71	0.70	0.69	1.00	0.85	0.60	0.57	0.52	0.59	-0.22	-0.32	-0.41	-0.38	-0.36	-0.43	-0.35	-0.33	-0.28
Pods per Plant	0.68	0.67	0.69	0.66	0.64	0.66	0.65	0.64	0.85	1.00	0.58	0.55	0.50	0.56	-0.20	-0.30	-0.39	-0.36	-0.35	-0.42	-0.34	-0.32	-0.27
Chlorophyll a	0.66	0.65	0.66	0.64	0.61	0.63	0.62	0.61	0.60	0.58	1.00	0.82	0.78	0.76	-0.27	-0.34	-0.43	-0.41	-0.40	-0.44	-0.37	-0.35	-0.30
Chlorophyll b	0.63	0.62	0.64	0.61	0.58	0.60	0.59	0.58	0.57	0.55	0.82	1.00	0.81	0.85	-0.28	-0.36	-0.45	-0.43	-0.42	-0.46	-0.38	-0.36	-0.31
Carotenoids	0.58	0.57	0.59	0.57	0.55	0.57	0.55	0.54	0.52	0.50	0.78	0.81	1.00	0.80	-0.24	-0.31	-0.41	-0.39	-0.38	-0.43	-0.35	-0.33	-0.28
Total Carbohydrates	0.67	0.66	0.68	0.66	0.63	0.65	0.64	0.62	0.59	0.56	0.76	0.85	0.80	1.00	-0.30	-0.39	-0.48	-0.46	-0.45	-0.50	-0.41	-0.38	-0.33
Proline Content	-0.34	-0.35	-0.33	-0.34	-0.31	-0.31	-0.30	-0.29	-0.22	-0.20	-0.27	-0.28	-0.24	-0.30	1.00	0.78	0.84	0.72	0.69	0.80	0.66	0.62	0.55
MDA Content	-0.46	-0.48	-0.45	-0.46	-0.43	-0.42	-0.41	-0.40	-0.32	-0.30	-0.34	-0.36	-0.31	-0.39	0.78	1.00	0.87	0.76	0.73	0.84	0.71	0.66	0.59
Electrolyte Leakage	-0.55	-0.56	-0.54	-0.54	-0.52	-0.51	-0.50	-0.49	-0.41	-0.39	-0.43	-0.45	-0.41	-0.48	0.84	0.87	1.00	0.82	0.79	0.90	0.77	0.72	0.63
Zinc Uptake	-0.52	-0.53	-0.51	-0.52	-0.49	-0.48	-0.47	-0.47	-0.38	-0.36	-0.41	-0.43	-0.39	-0.46	0.72	0.76	0.82	1.00	0.88	0.86	0.81	0.76	0.66
Lead Uptake	-0.51	-0.52	-0.50	-0.51	-0.48	-0.47	-0.46	-0.45	-0.36	-0.35	-0.40	-0.42	-0.38	-0.45	0.69	0.73	0.79	0.88	1.00	0.82	0.78	0.73	0.64
Cadmium Uptake	-0.57	-0.58	-0.55	-0.56	-0.54	-0.53	-0.52	-0.51	-0.43	-0.42	-0.44	-0.46	-0.43	-0.50	0.80	0.84	0.90	0.86	0.82	1.00	0.83	0.77	0.68
Copper Uptake	-0.49	-0.50	-0.48	-0.49	-0.47	-0.46	-0.45	-0.44	-0.35	-0.34	-0.37	-0.38	-0.35	-0.41	0.66	0.71	0.77	0.81	0.78	0.83	1.00	0.85	0.71
Nickel Uptake	-0.48	-0.49	-0.46	-0.47	-0.45	-0.44	-0.43	-0.42	-0.33	-0.32	-0.35	-0.36	-0.33	-0.38	0.62	0.66	0.72	0.76	0.73	0.77	0.85	1.00	0.80
Chromium Uptake	-0.40	-0.41	-0.39	-0.40	-0.37	-0.36	-0.35	-0.34	-0.28	-0.27	-0.30	-0.31	-0.28	-0.33	0.55	0.59	0.63	0.66	0.64	0.68	0.71	0.80	1.00

Interpretation

Red color indicates positive correlation, blue color indicates negative correlation.

Growth, yield, photosynthetic and biochemical traits are positively correlated with each other and negatively correlated with heavy metal uptake and stress indicators.

High positive correlations (r > 0.7) shown in dark red; high negative correlations (r < -0.7) shown in dark blue.

Fig. 7: A Pearson correlation coefficient (r) heat map and cluster analysis representing the interrelationships between various morphological, biochemical, and stress-related traits in radish. The color gradient indicates the strength and direction of the correlation, ranging from dark red (strong positive to 1.0) to dark blue (strong negative to -1.0).

Table 19: Effect of biochar and methionine on Lead uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.002	0.001		
Treatment	3	0.15448	0.05149	20.29	0.0015
Error Rep*treatment	6	0.01523	0.00254		
Foliar	3	0.03035	0.01012	6.32	0.0026
treatment*foliar	9	0.00867	0.00096	0.6	0.7834
Error Rep*treatment*foliar	24	0.03843	0.0016		
Total	47	0.24917			

Ni Uptake (ppm) in Radish

The concise interpretation indicated that the treatment factor had a significant effect on nickel content, with an F-value of 22.55 and a p-value of 0.0011, demonstrating that different irrigation and biochar treatments significantly influenced nickel accumulation in radish. However, the foliar application of L-Methionine did not show a significant effect on nickel content, with an F-value of 0.68 and a p-value of 0.5707. The interaction between these factors (treatment*foliar) was also not significant, with an F-value of 0.58 and a p-value of 0.8011, suggesting that the combined effect of these treatments on nickel content is not significantly different from their individual effects. The mean square errors for replications and treatments and their interaction were 0.05243 and 0.08203, respectively, (Table 20 & Figure 6). The grand mean nickel content across all treatments was 1.4042 ppm, with coefficients of variation (CV) for the errors associated with replications and treatments and their interaction being 16.31% and 20.40%, respectively. These findings indicate that while irrigation and biochar application significantly influence nickel content in radish, foliar L-Methionine application and their combined effects do not show significant impacts on nickel uptake. Plant growth can be improved by using biochar. Ni and Zn availability in soils as well as plant uptake of heavy metals can both be decreased by using biochar (Kang et al., 2022).

Table 20: Effect of biochar and methionine on Nickle uptake in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.13115	0.06558		
Treatment	3	3.54617	1.18206	22.55	0.0011
Error Rep*treatment	6	0.31456	0.05243		
Foliar	3	0.1684	0.05613	0.68	0.5707
treatment*foliar	9	0.42687	0.04743	0.58	0.8011
Error Rep*treatment*foliar	23	1.88667	0.08203		
Total	46				

Grand Mean 1.4042, CV (Rep*treatment) 16.31, CV (Rep*treatment*foliar) 20.40

Biochemical Parameters

Proline content (µg g^{-1})

The Analysis of Variance (ANOVA) for proline content in radish reveals significant effects from the treatments and foliar applications. Proline content was significantly impacted by the treatment factor, with a p-value of 0 and an F-value of 652.68. F-value of 1544.68 and p-value of 0 indicated a substantial influence from the foliar application as well. Furthermore, with an F-value of 61.15 and a p-value of 0, the interaction between the therapy and foliar spray was significant, (Table 21 & Figure 4). High experimental precision was shown by the coefficients of variation for the errors related to the treatment and treatment-foliar interactions, which were 4.35% and 1.38%, respectively. Proline content had a grand mean of 10.742. lants treated with biochar had a considerable reduction in proline content (Yildirim et al., 2021).

Table 21: Effect of biochar and methionine on Proline in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.912	0.456		
Treatment	3	426.962	142.321	652.68	0
Error Rep*treatment	6	1.308	0.218		
Foliar	3	101.692	33.897	1544.68	0
treatment*foliar	9	12.077	1.342	61.15	0
Error Rep*treatment*foliar	24	0.527	0.022		
Total	47	543.477			

Grand Mean 10.742, CV (Rep*treatment) 4.35, CV(Rep*treatment*foliar) 1.38

Total carbohydrate content (g)

There are notable effects from both treatments and foliar applications, according to the concise interpretation for the total carbohydrate content in radish. The F-value of 5201 and the p-value of 0 indicate that the treatment factor had a substantial impact on the amount of carbohydrates. Foliar application also shown a substantial impact, as indicated by a p-value of 0 and an F-value of 1491.78, (Table 22 & Figure 5). With an F-value of 2.59 and a p-value of 0.0302, the interaction between treatment and foliar application was significant but less strong. Exact experimental results were indicated by the low coefficients of variation for the errors related to the treatment and treatment-foliar interactions, which were 0.51% and 1.08%, respectively. 5.6458 was the grand mean of the total carbohydrate content. The amount of total carbohydrates in leaves was significantly affected by biochar, and at five and ten percent, respectively, a single application of biochar enhanced the total soluble sugar content (Qian et al., 2019).

MDA Content (μ mol L^{-1})

The concise interpretation showed a significant impact from the foliar sprays and treatments on the malondialdehyde (MDA) level in radish. The experiment, structured with irrigation and biochar application and foliar application of methionine revealed that the treatment factor had a highly significant impact on MDA content, indicated by an F-value of 164.89 and a p-value of 0. MDA content was similarly considerably impacted by foliar treatment, with a p-value of 0 and an F-value of 30.97, (Table 23 & Figure 5). With an F-value of 0.64 and a p-value of 0.7551, the interaction between treatment and foliar spray, however, was not significant. Moderate variability was suggested by the coefficients of variation for the errors related to the treatment and treatment-foliar interactions, which were 7.72% and 12.43%, respectively. 5.0938 was the grand mean of the MDA content. Application of foliar Methionine was successful in reducing the amount of MDA contents in plants (Mehak et al., 2021).

Table 22: Effect of biochar and methionine on Total Carbohydrate content in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	0.2517	0.12583		
Treatment	3	13.0025	4.33417	5201	0
Error Rep*treatment	6	0.005	0.00083		
Foliar	3	16.7825	5.59417	1491.78	0
treatment*foliar	9	0.0875	0.00972	2.59	0.0302
Error Rep*treatment*foliar	24	0.09	0.00375		
Total	47	30.2192			

Grand Mean 5.6458, CV (Rep*treatment) 0.51, CV(Rep*treatment*foliar) 1.08

Table 23: Effect of biochar and methionine on MDA content in wastewater irrigated radish.

Source	DF	SS	MS	F	P
Rep	2	2.281	1.1406		
Treatment	3	76.432	25.4774	164.89	0
Error Rep*treatment	6	0.927	0.1545		
Foliar	3	37.266	12.4219	30.97	0
treatment*foliar	9	2.297	0.2552	0.64	0.7551
Error Rep*treatment*foliar	24	9.625	0.401		
Total	47	128.828			

Grand Mean 5.0938, CV (Rep*treatment) 7.72, CV(Rep*treatment*foliar) 12.43

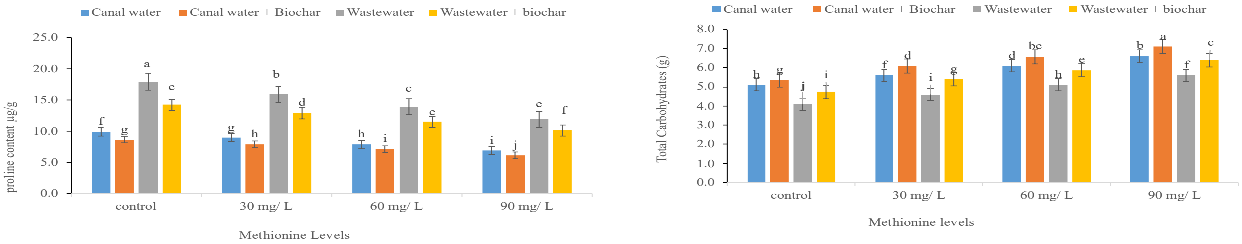
Electrolyte Leakage %

The results concise interpretation demonstrated that the treatments have a highly significant impact on the proportion of electrolyte leakage, with an F-value of 13670.5 and a P-value of 0. Similarly, a significant effect is suggested by the foliar application's F-value of 10142.4 and P-value of 0. With an F-value of 270.93 and a P-value of 0, the interaction between the treatment and foliar application also demonstrates a significant impact, (Table 24 & Figure 5). In comparison to the major sources, the error terms related to replication, treatment, and their interactions exhibit comparatively low MS values, suggesting minimal variance. Overall, the findings imply that the electrolyte leakage % in the radish plants is greatly impacted by the treatments, foliar applications, and their interactions. Plants that are exposed to heavy metals in wastewater have increased oxidative stress and electrolyte leakage, the application of methionine, significantly reduced the amount of these stresses and improved plant growth (Bhat et al., 2025; Tiwari & Sarangi, 2015; Zhou et al., 2020).

Table 24: Effect of biochar and methionine on Electrolyte leakage in wastewater irrigated radish.

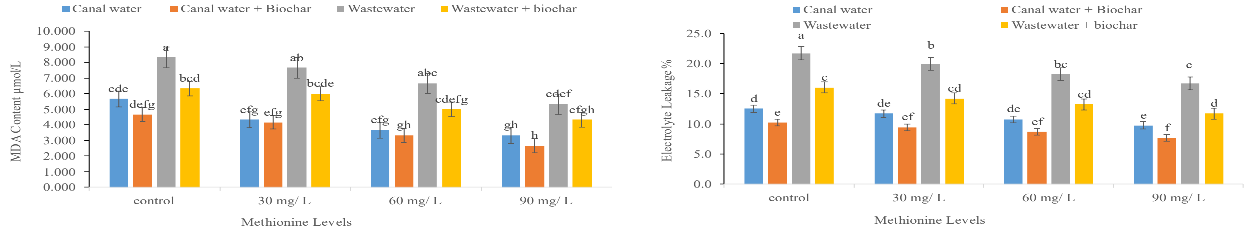
Source	DF	SS	MS	F	P
Rep	2	1.809	0.904		
Treatment	3	692.071	230.69	13670.5	0
*Error Rep*treatment*	6	0.101	0.017		
Foliar	3	88.746	29.582	10142.4	0
*treatment*foliar*	9	7.112	0.79	270.93	0
*Error Rep*treatment*foliar*	24	0.07	0.003		
Total	47	789.908			

Grand Mean 13.294, CV (Rep*treatment) 0.98, CV (Rep*treatment*foliar) 0.4.



Comparison of Treatment Means for Proline in Radish.

Comparison of Treatment Means for Total Carbohydrates in Radish.



Comparison of Treatment Means for MDA content in Radish.

Comparison of Treatment Means for Electrolyte Leakage for Radish

Fig. 5: Influence of biochar and exogenous methionine (0, 30, 60, and 90 mg/L) on biochemical stress markers and nutritional content in radish plants irrigated with canal water or wastewater. The panels represent the comparison of treatment means for Proline content (mu g/g), Total Carbohydrates (g), Malondialdehyde (MDA) content (mu mol/L) and Electrolyte Leakage (%). Results indicate that while wastewater irrigation increases oxidative stress (higher MDA and Electrolyte Leakage), the application of biochar and increasing levels of methionine effectively mitigate these stress responses while enhancing carbohydrate accumulation.

Table 25: ANOVA Summary of Growth, Physiological, Biochemical, and Heavy Metal Parameters in Wastewater-Irrigated Radish.

Parameter	Treatment F-value	Treatment P-value	Foliar F-value	Foliar P-value	Treatment × Foliar F-value	Interaction P-value	Significance Summary
Root Fresh Weight (g)	638.33	0.0000	23.42	0.0000	0.93	0.5209	Treatment and foliar significant; interaction NS
Shoot Fresh Weight (g)	45.21	0.0002	19.31	0.0000	1.89	0.1022	Treatment and foliar significant; interaction NS
Root Dry Weight (g)	45.21	0.0000	19.31	0.0000	1.89	0.1022	Treatment and foliar significant; interaction NS
Shoot Dry Weight (g)	45.21	0.0000	19.31	0.0000	1.89	0.1022	Treatment and foliar significant; interaction NS
Root Length (cm)	15.63	0.0031	5.54	0.0049	1.01	0.4601	Treatment and foliar significant; interaction NS
Shoot Length (cm)	115.38	0.0000	46.51	0.0000	1.78	0.1240	Treatment and foliar significant; interaction NS
Root Girth (cm)	39.87	0.0002	8.54	0.0005	1.34	0.2711	Treatment and foliar significant; interaction NS
Shoot Girth (cm)	19.77	0.0016	6.14	0.0030	0.52	0.8484	Treatment and foliar significant; interaction NS
Number of Flowers per Plant	29.79	0.0005	29.42	0.0000	2.79	0.0214	Treatment, foliar, and interaction significant
Number of Pods per Plant	71.43	0.0000	170.89	0.0000	0.32	0.9597	Treatment and foliar significant; interaction NS
Chlorophyll a (mg g⁻¹)	16.47	0.0027	2.47	0.0864	1.05	0.4330	Only treatment significant
Chlorophyll b (mg g⁻¹)	69.93	0.0000	225.91	0.0000	3.90	0.0037	Treatment, foliar, and interaction significant
Carotenoids Content (mg g⁻¹)	12.95	0.0050	5.43	0.0054	1.00	0.4662	Treatment and foliar significant; interaction NS
Zinc Uptake (ppm)	327.57	0.0000	10.72	0.0001	2.05	0.0775	Treatment and foliar significant; interaction NS
Chromium Uptake (ppm)	27.96	0.0006	1.20	0.3301	0.25	0.9819	Only treatment significant
Copper Uptake (ppm)	24.92	0.0009	10.77	0.0001	0.22	0.9890	Treatment and foliar significant; interaction NS
Cadmium Uptake (ppm)	57.68	0.0001	29.06	0.0000	1.55	0.1878	Treatment and foliar significant; interaction NS
Lead Uptake (ppm)	20.29	0.0015	6.32	0.0026	0.60	0.7834	Treatment and foliar significant; interaction NS
Nickel Uptake (ppm)	22.55	0.0011	0.68	0.5707	0.58	0.8011	Only treatment significant
Proline Content (µg g⁻¹)	652.68	0.0000	1544.68	0.0000	61.15	0.0000	Treatment, foliar, and interaction significant
Total Carbohydrate Content (g)	5201.00	0.0000	1491.78	0.0000	2.59	0.0302	Treatment, foliar, and interaction significant

NS=Non-significant; Significant at P≤0.05; Highly significant at P≤0.01

DISCUSSION

The significant reduction in heavy metal (Cd, Pb, and Zn) uptake, alongside enhanced radish growth observed in this study, can be attributed to a synergistic, dual-layered defense mechanism established by soil-applied biochar and foliar-applied L-methionine, (Figure 7). This integrative strategy operates across both soil and plant physiological domains, providing a comprehensive mitigation pathway against heavy metal stress in wastewater-irrigated systems (Ahmad et al., 2021; Hasanuzzaman et al., 2020). At the soil level, biochar acts as a highly effective immobilization agent through multiple physicochemical mechanisms. Its porous structure, high surface area, and abundance of functional groups make it an efficient adsorbent for heavy metals (Tan et al., 2022; Sohi et al., 2020). The presence of negatively charged functional groups such as carboxyl (-COOH) and hydroxyl (-OH) facilitates electrostatic attraction and complexation with positively charged metal ions like Cd^{2+} and Pb^{2+} (Wang et al., 2021; Yan et al., 2025). This adsorption process significantly reduces the mobility and bioavailability of metals in soil (Beesley et al., 2022; Zhou et al., 2020).

Moreover, biochar application is widely reported to increase soil pH, which plays a crucial role in metal immobilization (Al-Wabel et al., 2024; Li et al., 2022). Elevated pH promotes the precipitation of metals into insoluble forms such as hydroxides and carbonates, thereby limiting their uptake by plants (Chen et al., 2022; Aktar et al., 2023). Additionally, biochar enhances cation exchange capacity (CEC), strengthening its ability to retain heavy metals within the soil matrix (Mousavi & Sedaghat et al., 2025). Biochar also improves soil physical properties, including water retention, aeration, and microbial activity (Lehmann et al., 2021; Joseph et al., 2021). Enhanced microbial populations contribute to heavy metal immobilization through biosorption and transformation processes (Yan et al., 2025). These combined effects create a strong barrier that restricts heavy metal translocation into plant roots. While biochar functions externally, L-methionine provides internal physiological protection against residual metal stress. As a sulfur-containing amino acid, methionine serves as a precursor for glutathione (GSH), a key antioxidant involved in detoxification pathways (Hasanuzzaman et al., 2020; Dorion et al., 2021). Glutathione further contributes to the synthesis of phytochelatins, which chelate heavy metals and sequester them into vacuoles (Clemens & Ma, 2016; Sharma et al., 2021). This process of biochelation is essential for reducing cytosolic toxicity. By binding heavy metals, phytochelatins prevent their interaction with cellular components such as proteins and membranes (Shukla et al., 2024). Consequently, plants maintain metabolic stability under stress conditions.

Methionine also enhances antioxidant defense systems. The observed reduction in malondialdehyde (MDA) levels indicates decreased lipid peroxidation and improved membrane stability (Ali et al., 2022; Ma et al., 2026). Furthermore, methionine contributes to the biosynthesis of ethylene and polyamines, which regulate plant growth and stress responses. The application of 90 mg L^{-1} methionine significantly increased chlorophyll content, (Table 26), reflecting improved photosynthetic efficiency (Raza et al., 2022). This preservation of the photosynthetic apparatus ensures sustained plant growth even under heavy metal stress. The synergy between biochar and methionine lies in their complementary roles: biochar reduces metal entry into the plant system, while methionine detoxifies metals that bypass the soil barrier. This dual mechanism results in enhanced stress tolerance compared to individual treatments (Qadir et al., 2020).

Table 26: Effect of Biochar and L-Methionine on Root Biomass and Length.

Treatment (Factor A)	Foliar Methionine (mg L^{-1})	Root Fresh Weight (g)	Root Dry Weight (g)	Root Length (cm)
Wastewater + Biochar	90	284.5 a	28.6 a	24.2 a
	60	262.1 b	25.4 b	22.8 b
	30	245.8 c	23.1 bc	21.5 c
Wastewater (Only)	0	212.4 e	20.8 d	18.4 e
Canal Water + Biochar	90	278.2 a	27.9 a	23.9 a
Canal Water (Control)	0	235.6 d	22.5 c	20.2 d

(Means with different letters indicate significant differences at $P \leq 0.05$).

The findings of this study align with recent advancements in sustainable remediation strategies. Biochar has been widely recognized as a “green adsorbent” due to its efficiency and environmental compatibility (Al-Wabel et al., 2024; Sohi et al., 2020). Similar reductions in heavy metal uptake have been reported across various crops (Zhou et al., 2020). Zhang et al. (2025) demonstrated significant cadmium reduction in maize using soil amendments, supporting the results observed in this study. Similarly, Le et al. (2026) reported decreased Pb and Zn accumulation in vegetables following biochar application. However, most studies focus solely on soil amendments. The novelty of this research lies in combining biochar with foliar-applied methionine. Integrated approaches have recently gained attention for their superior effectiveness (Kour et al., 2022; Abbas et al., 2024). Wastewater irrigation often introduces nutrient imbalances despite providing essential nutrients (Drechsel et al., 2022). Methionine supplementation helps restore metabolic balance and improves nutrient assimilation (Ali et al., 2019). Thus, this combined treatment not only mitigates toxicity but also enhances plant growth.

Radish is particularly vulnerable to heavy metal accumulation because its edible taproot is in direct contact with contaminated soil, (Table 27). This increases the risk of metals entering the food chain (WHO, 2020; FAO, 2021). Heavy metals such as Cd and Pb can accumulate to toxic levels in root vegetables, posing serious health risks (Khan et al., 2021). Therefore, reducing metal uptake is critical for food safety. The combined biochar-methionine treatment significantly reduced metal accumulation (Pb by 28% and Cd by 33%), ensuring safer consumption levels. Similar findings have been reported in recent studies (Wan et al., 2024; Mohamed et al., 2023). In water-scarce regions like Pakistan, wastewater irrigation is often unavoidable (Qadir et al., 2020). This study provides a practical solution by enabling safe use of wastewater while minimizing health risks.

Table 27: Heavy Metal Concentration in Radish Roots (ppm).

Treatment (Factor A)	Zinc (Zn)	Cadmium (Cd)	Lead (Pb)
Wastewater (Only)	38.42 a	0.033 a	0.221 a
Wastewater + Biochar	29.97 c	0.022 c	0.159 c
Canal Water (Control)	21.15 d	0.012 d	0.084 d

(Tukey grouping shows biochar significantly reduced metal concentrations compared to wastewater alone).

Conclusions

In conclusion, the study conducted at the Agronomy Research Farm, University of Agriculture, Faisalabad, demonstrated that the combined application of biochar and L-methionine significantly enhanced the growth, yield, and stress tolerance of wastewater-irrigated radish (*Raphanus sativus* L.). Using a Randomized Complete Block Design (RCBD) with three replications, treatments involving biochar and higher methionine concentrations (60 and 90 mg L^{-1}) produced the greatest improvements in root length, root fresh and dry weight, shoot fresh and dry weight, chlorophyll (a, b, total) and carotenoids. Proline and MDA levels increased, indicating enhanced stress resilience. Soil electrical conductivity (EC) also improved with biochar application, reflecting better nutrient availability. Importantly, heavy metals (Zn, Cd, Pb, Ni, Cu) remained below plant toxicity thresholds, and their uptake was significantly reduced with biochar and methionine. These findings confirm that integrating biochar with 90 mg L^{-1} L-methionine is an effective strategy for improving productivity and ensuring food safety under wastewater irrigation conditions.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Data Availability

Data will be available from the corresponding author upon request.

Ethics Statement

No animals or human subjects are involved in this experimentation; therefore, no ethical approval is required.

Authors Contribution

Ayesha Yousaf did a significant contribution to this work. Ayesha Yousaf, Fahd Rasul, Muhammad Khızar Hayat, Fazarıa Arshad and Md. Nahid Mahmud participated in the conception, study design, execution and interpretation, writing initial and final draft. All these authors took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published. All of these authors are agreed on the journal to which the article has been submitted and agree to be accountable for all aspects of the work.

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