Scientific Reports

https://doi.org/10.1038/s41598-026-51657-x

Article in Press

Microbe-biochar interaction in improving plant growth and water reuse in a green wall system

Received: 27 December 2025

Accepted: 29 April 2026

Published online: 23 June 2026

Cite this article as: Zarei A., Karimian Z., Meghdari Z. *et al.* Microbe-biochar interaction in improving plant growth and water reuse in a green wall system. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-51657-x

Atefeh Zarei, Zahra Karimian, Zahra Meghdari & Ava Heidari

Microbe-Biochar Interaction in improving Plant Growth and Water Reuse in a Green Wall System

Atefeh Zarei[1], Zahra Karimian[*2], Zahra Meghdari[3], Ava Heidari [1]

1. Department of Environmental Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Horticultural science and Landscape Architecture, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran (*Corresponding author: zkarimian@um.ac.ir)
3. Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

Abstract

Given the increasing scarcity of potable water and the rising generation of domestic and industrial wastewater, the reuse of greywater to meet part of plant water requirements, along with its environmentally friendly approach, has gained increasing attention. The present study was conducted in 2023 to investigate the effects of microorganisms and biochar on the establishment and growth of *Crassula capitella* Thunb., as well as on greywater treatment. Cyanobacteria (C0, C0.4, and C0.8 g), mycorrhiza (M0, M5, and M10 g), and biochar (B0, B5, and B10 g) were evaluated under two irrigation regimes including municipal water (MW) and greywater (GW) within a green wall system in Mashhad, Iran. The results indicated that the combined treatments and irrigation water type had statistically significant effects on most of the measured traits. The C0.4–M10–B0 treatment increased chlorophyll content by more than 97% (from 4.93 to 9.74 µg g^{-1} FW), while the C0–M10–B5 treatment increased RFW and RDW by 189% and 298%, respectively (from 2.530 to 7.309 g and from 0.353 to 1.407 g), compared to their respective minimum values. The initial wastewater COD (376 mg/L) was 118% greater than the COD measured after treatment with C0.4–M10–B0 (173 mg/L). Overall, the C0–M10–B5 and C0.4–M10–B0 treatments showed superior performance in improving plant establishment and growth of *C. capitella* in the green wall system, while the C0.4–M10–B0 and C0.4–M5–B5 treatments were more effective in greywater treatment. Based on these findings, the

integrated use of cyanobacteria, mycorrhizae, and biochar may contribute to the establishment of Crassula in green wall systems and to urban water resource management under the conditions tested in Mashhad, Iran. However, further studies are needed to assess the applicability of these results to other cities or environmental conditions.

Keywords: Cyanobacteria, Mycorrhiza, Pollution tolerance, Gray water

Introduction

Rapid urbanization over recent decades, combined with changing patterns of water consumption and increasing volumes of domestic wastewater, has posed significant challenges to sustainable urban water management. These developments have contributed to declining water quality, greater pressure on wastewater collection and treatment infrastructure, and increased pollutant loads entering the environment (Spencer, 2021; Kummu et al., 2019). In many regions, limited renewable water resources and decreasing precipitation have made the reuse of urban wastewater not merely an option but a necessity. According to reports by the United Nations Environment Programme (UNEP) and UN-Habitat, approximately 90% of wastewater generated in developing countries in 2010 was discharged into nearby water bodies without adequate treatment, largely due to insufficient treatment facilities (UNEP, 2010). More recent assessments, however, indicate substantial progress, with an estimated 56% of domestic wastewater being safely treated worldwide by 2020 (WHO, 2021).

Among the various wastewater streams, domestic wastewater and particularly greywater accounts for a considerable proportion. Compared with blackwater, greywater contains a relatively low pathogenic load, while its composition of organic matter, surfactants, oils, and nitrogen and phosphorus compounds makes it well suited for biological treatment and reuse (Rubel et al., 2025; Gilboa & Friedler, 2008). Greywater originates from activities such as handwashing, bathing, laundry, and kitchen use, and

represents a significant share of household wastewater generation (Boano et al., 2020). Its reuse can enhance the flexibility and resilience of local water systems while reducing demand on freshwater supplies and lowering wastewater conveyance and treatment costs (Van de Walle et al., 2023). In recent years, treated greywater has therefore attracted growing interest for applications such as urban agriculture (Eregno et al., 2017) and the irrigation of urban green spaces (Neale et al., 2020).

In line with these emerging needs, decentralized nature-based solutions that integrate vegetation into built environments have gained attention as practical systems for onsite greywater treatment, providing a logical transition from broader water challenges to building-scale applications. An innovative approach to wastewater reuse that simultaneously enhances urban environmental quality is the integration of green walls and plant-based treatment systems. Green walls, as vertical vegetation systems, not only improve the aesthetic and microclimatic conditions of urban areas but also contribute to wastewater treatment, pollutant filtration, mitigation of the urban heat island effect, and improvement of air quality (Manso et al., 2021). Numerous studies have demonstrated that plant species used in green wall systems can absorb and immobilize a portion of the chemical contaminants present in greywater, thereby improving the quality of reclaimed water (Costamagna et al., 2025; Abd-ur-Rehman et al., 2022; Bakheet et al., 2020).

Research further suggests that green walls function as a form of constructed wetland and represent an environmentally friendly technology capable of effectively treating greywater, with demonstrated potential for treating wastewater with relatively high organic loads (Addo-Bankas et al., 2021). In such systems, the selection of suitable plant species, along with optimized substrate composition and operational conditions, plays a critical role in achieving high treatment efficiency (Prodanovic et al., 2019). Additionally, outdoor green wall systems have been shown to maintain high pollutant

removal efficiency even under winter conditions when treating synthetic greywater (Boano et al., 2020).

Beyond the role of plants, microorganisms are essential contributors to biological treatment processes and can substantially enhance the performance of plant-based systems. Cyanobacteria, for instance, are capable of fixing atmospheric nitrogen and producing extracellular polysaccharides (Alvarez et al., 2023), which improve photosynthetic efficiency (Grzesik et al., 2017), enhance soil structure, and increase the adsorption capacity for various organic and inorganic pollutants. Through these mechanisms, cyanobacteria can improve substrate quality and overall system performance (Muñoz-Rojas et al., 2018), while also reducing biochemical oxygen demand (BOD), chemical oxygen demand (COD), and other key water quality parameters (Choque-Quispe et al., 2025).

Mycorrhizal fungi, as one of the most important plant-associated symbiotic microorganisms, also play a fundamental role in wastewater treatment systems. By expanding the effective root absorption area, enhancing nutrient transfer, improving plant tolerance to salinity and pollution stress, and immobilizing contaminants in the rhizosphere, mycorrhiza significantly contribute to treatment efficiency (Hawkins et al., 2023; Giovannini et al., 2020; Deja-Sikora et al., 2020). Improved uptake of nitrogen, phosphorus, and micronutrients further promotes plant growth in low-quality or contaminated substrates (Tian et al., 2024; Dou et al., 2019). Evidence suggests that the combined presence of plants, cyanobacteria, and mycorrhiza can reduce chemical pollutant concentrations, enhance plant performance, and improve the stability of treatment systems (Khoshyomn et al., 2024).

Biochar represents another complementary component with the potential to enhance plant-based wastewater treatment systems. Owing to its porous structure, high specific surface area, and abundance of functional groups,

biochar is capable of adsorbing and stabilizing a wide range of pollutants, including heavy metals, organic compounds, and nitrogen and phosphorus containing substances (Shah et al., 2024; Sutar et al., 2022; Munawar, 2021). In addition, biochar provides a favorable habitat for microbial communities, thereby increasing the biological efficiency of treatment processes (He et al., 2020). Its incorporation into soil or growth substrates has also been shown to improve plant growth, enhance physiological performance (Li et al., 2022), increase water-holding capacity, and reduce heavy metal uptake from reclaimed wastewater (Kamran et al., 2020).

In studies examining plant responses to alternative irrigation sources such as greywater, physiological and biochemical indicators are commonly evaluated to better understand plant tolerance mechanisms. Traits such as RWC, plant weight, and photosynthetic pigments are widely used to assess plant water status (Noreen et al., 2025), growth performance (Anangadan et al., 2025), and photosynthetic efficiency (Anangadan et al., 2024) under stress conditions. Biochemical markers, including ascorbic acid and enzyme activity, are frequently measured as indicators of antioxidant defense responses to oxidative stress (Jozay et al., 2024; Ma et al., 2022). In addition, determining leaf nutrient concentrations helps evaluate plant nutritional status and nutrient uptake efficiency, particularly in studies involving soil amendments and beneficial microorganisms that influence nutrient availability (Tian et al., 2024). Integrative indices such as APTI are also used to assess overall plant tolerance to environmental stress (Karimian et al., 2023). Meanwhile, changes in greywater quality parameters (e.g., EC, BOD, COD, and pH) are commonly analyzed to determine the potential role of plant-based systems in wastewater improvement (Aylan et al., 2052; Ouhsassi et al., 2020). Therefore, these indicators were evaluated in the present study to assess both plant tolerance and the contribution of treated plants to greywater purification under the combined application of biochar, cyanobacteria, and mycorrhiza.

Increasing water scarcity and the growing production of urban wastewater highlight the urgent need for sustainable and cost-effective solutions for water treatment and reuse. Among emerging approaches, engineered systems such as green walls offer a promising strategy for integrating nature-based solutions in urban environments. This study investigates the interactions among *Crassula capitella*, microorganisms (cyanobacteria and mycorrhiza), and biochar within a green wall irrigated with greywater. *Crassula capitella* was selected due to its succulent nature and Crassulacean Acid Metabolism (CAM), which enhances water-use efficiency and tolerance to water stress by enabling nocturnal stomatal opening. These traits make it well-suited for green wall systems irrigated with greywater, where water availability and quality may vary (Lüttge, 2004).

Previous studies have documented the individual contributions of biochar, cyanobacteria, and mycorrhizal fungi in improving soil quality, promoting plant growth, and enhancing water treatment. However, knowledge about their synergistic interactions, particularly in confined and engineered settings like green walls, remains limited. Biochar provides a favorable habitat for microbial colonization, potentially enhancing microbial activity, while the co-existence of cyanobacteria and mycorrhiza may improve nutrient cycling in complementary ways. Here, we systematically examine the combined effects of biochar, cyanobacteria, and mycorrhizal fungi in a greywater-irrigated green wall, addressing a critical gap in our understanding of integrated nature-based solutions for urban water management.

Materials and Methods

Study area

This study was conducted in Mashhad, Razavi Khorasan Province, Iran, located at 36°59′ N latitude and 59°36′ E longitude in northeastern Iran. The city experiences maximum summer temperatures of up to 43 °C and minimum winter temperatures as low as −23 °C. The mean annual precipitation is

approximately 253 mm, and the elevation is 995 m above sea level (Salahi et al., 2016). Mashhad is the second-largest city in Iran, with an estimated population of about 3.5 million. As the country's most important religious and pilgrimage destination, it is estimated to receive approximately 27 million religious tourists annually.

The experiment was conducted over a five-month period, from early June to mid-November 2023, on the eastern side of the Faculty of Natural Resources and Environment at Ferdowsi University of Mashhad (Figure 1).

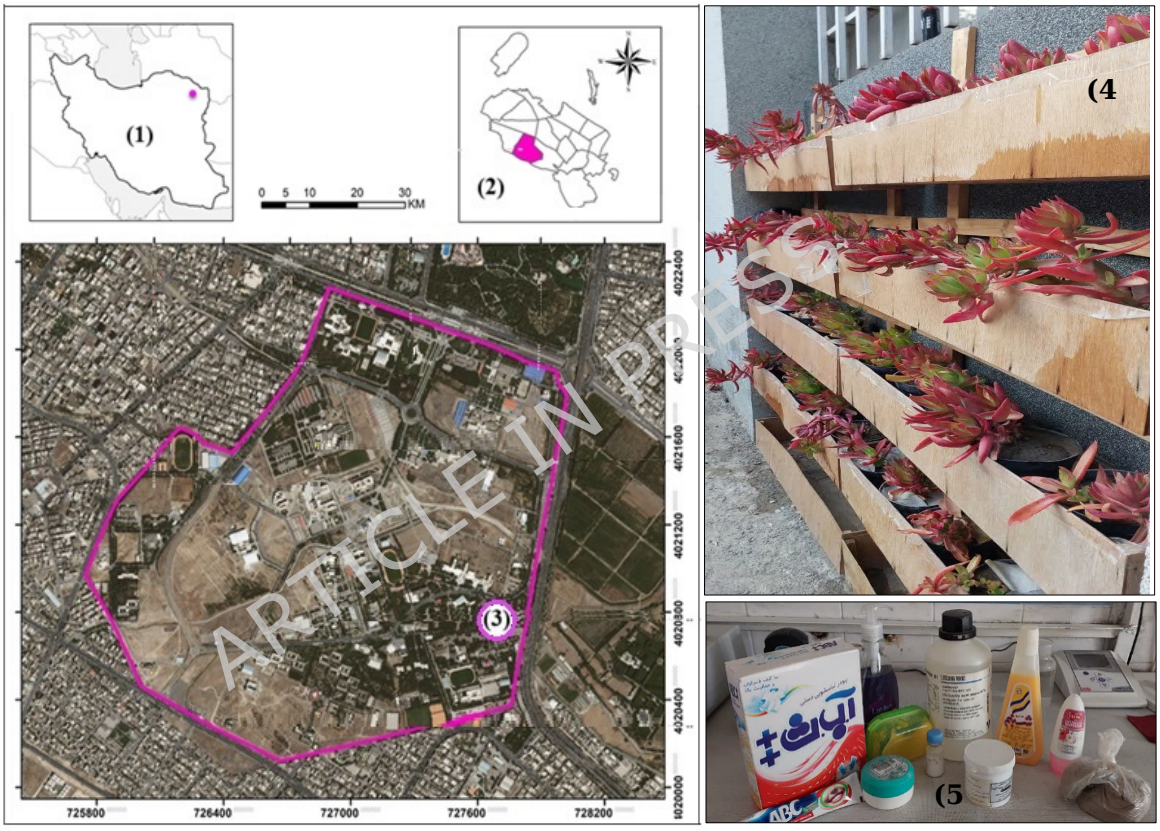


Figure. 1. Location of study sites (3) in Mashhad city/Razavi Khorasan Province (2), Iran (1) (Source: Google map, 2025). Green wall (4) and the materials of green wastewater (5), (Source: Authors, 2025)

Preparation of cyanobacteria, mycorrhizae, and biochar

For cyanobacterial propagation, a culture medium containing BG11 powder was used. The cultures were transferred to a growth chamber under

controlled conditions with a 16 h light/8 h dark photoperiod, light intensity of 1600 lux (fluorescent lamps), and a temperature of 30 ± 2 °C (Zangeneh et al., 2022). A light microscope (Echo UM-210 BD) was used to determine the morphological characteristics of the cyanobacteria (Desikachary, 1959).

Cyanobacterial inoculation into the soil was carried out following the method reported by Zangeneh et al. (2021). Initially, fresh and dry weights were measured, with dry weight determined after oven-drying for 24 h at 105 °C. Subsequently, the required amount of cyanobacterial biomass was mixed with 50 cc of distilled water and placed on a magnetic stirrer at 0 °C for 24 h. Finally, the resulting suspension was applied to the soil surface in three stages using a sterile syringe.

Cyanobacteria were applied after successful plant establishment, approximately one month after planting, at rates of 0, 0.4, and 0.8 g per pot, distributed in three stages over the soil surface. Based on light microscopic observations, the cyanobacterium used in this study was identified as Oscillatoria sp., characterized by filamentous, straight, unbranched trichomes, lacking heterocysts, with slightly curved apical cells and occasionally surrounded by a thin mucilaginous sheath (Komárek and Anagnostidis, 2005; Whitton, 2012).

The mycorrhizal fungus used in this study was purchased from Zist Fanavaran Touran Company (Shahroud, Semnan Province, Iran). Mycorrhizawere applied at rates of 0, 5, and 10 g per pot and incorporated into the soil in layers at the center of the pots to facilitate symbiosis with plant roots (Markram-Kashtiban et al., 2019).

Biochar was obtained from Tarbiat Modares University, Mazandaran Branch, Noor County, Iran. The biochar was produced via pyrolysis of wood residues from three plant species including *Parrotia persica*, *Populus* spp., and *Eucalyptus* spp. at 600 °C with a heating rate of 10 min^{-1} under an argon atmosphere for 1 h. The physicochemical properties of the biochar used in this study are presented in Table 1. Biochar treatments were applied at rates

of 0, 5, and 10 g per pot were incorporated into the potting soil and thoroughly mixed prior to planting.

Preparation of greywater

The materials used for the preparation of synthetic greywater were adopted from the study by Diaper et al. (2008) and are listed in Table 3. Secondary treated wastewater was obtained from the Mashhad Industrial Town (Kalat) wastewater treatment plant. To prepare the irrigation treatments, 2L of the treated effluent was thoroughly mixed in a container and then diluted with 100 L of municipal water. The chemical oxygen demand (COD) of the final greywater was 376 mg/L, determined using the method described by Phattore et al. (2019). The pH was measured using a Testo 230 pH meter as 8.98, and the electrical conductivity (EC) was measured using an EC meter as 1593 µS/cm.

Parameters	Biochar	Soil
pH	7.86	8.83
EC (µS/cm)	316.3	343
Carbon (%)	73.41	-
Hydrogen (%)	2.71	-
Nitrogen (%)	0.37	-
Sulfur (%)	0.00	-
Oxygen (%)	23.51	-
Clay (%)	-	40
Silt (%)	-	40
Sand (%)	-	20
WHC (%)	84	

Table 1. Analysis of important biochar and soil compounds in

Table 2. Abbreviations of treatments, and their rate in

	Irrigation and Combined treatments	
	Municipal water (MW)	Gray water (GW)
1	C0.4–M5–B5	C0.4–M5–B5
2	C0.4–M10–B0	C0.4–M10–B0
3	C0.0–M5–B0	C0.0–M5–B0
4	C0.4–M10–B10	C0.4–M10–B10
5	C0.8–M0–B5	C0.8–M0–B5
6	C0.0–M10–B5	C0.0–M10–B5
7	C0.8–M5–B10	C0.8–M5–B10
8	C0.8–M10–B5	C0.8–M10–B5
9	C0.4–M0–B0	C0.4–M0–B0
10	C0.0–M5–B10	C0.0–M5–B10
11	C0.0–M0–B5	C0.0–M0–B5
12	C0.4–M0–B10	C0.4–M0–B10

C: Cyanobacteria, M: Mycorrhiza and B: Bochar treatments

Table 3. Recipe of synthetic gray water (Source: Diaper et al., 2008).

Ingredient	Amount in 100L (g) unless otherwise stated	Product used
Sunscreen OR	1.5	UV TripleGuard
Moisturiser	1.0	Dove
Toothpaste	3.25	Colgate Maximum Cavity Protection (regular)
Deodorant	1.0	Mum
Na_2SO_4	3.5	Analytical grade
$NaHCO_3$	2.5	Analytical grade
Na_2PO_4	3.9	Analytical grade
Clay (Unimin)[1]	5.0	Industrial grade
Vegetable Oil	0.7	Coles Own brand
Shampoo/hand wash	72	Palmolive
Laundry	15	Omo High Performance/ Omomatic concentrate
Boric acid	0.14	Analytical grade
Lactic acid	2.8	Analytical grade

Secondary effluent	2 L	Eastern Treatment Plant at Carrum (from secondary clarifier)

Green wall setup, plant material, and growth substrate

The green wall was constructed from wood with dimensions of 80 cm in length, 15 cm in width, and 15 cm in height, arranged in five vertical tiers, and installed at the study site. The soil was obtained from the research greenhouses of the Faculty of Agriculture, FUM, Mashhad. Pots and soil with a clay loam texture were sterilized using an autoclave at 121 °C for 60 min, and each pot was filled with 1 kg of soil (Table 1). Additionally, no chemical fertilizers or pesticides were applied during the experiment to avoid interference with the microbial treatments. The planted plants were also irrigated with NPK fertilizer (20-20-20, g/l) every two weeks. After soil inoculation with mycorrhiza (M) and the addition of biochar (B) and cyanobacteria (C), cuttings of *Crassula capitella* containing young and healthy roots were obtained from the greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The plant species (*Crassula capitella* Thunb, family: Crassulaceae) were identified and authenticated by at Ferdowsi University of Mashhad Herbarium (FUMH, Taxonomist: Mr. Mohammad Reza Joharchi), Mashhad, Iran. A voucher specimen (Code: E-1718 FUMH) has been deposited in the herbarium.

The pots were labeled according to the experimental treatments and randomly arranged on the green wall. According to the experimental design, half of the pots on one side of the green wall were irrigated with municipal water (MW), while the pots on the opposite side were irrigated with greywater (GW) (Figure 1).

Measurement of leaf relative water content (RWC)

Leaf relative water content (RWC) was determined according to the method described by Ritchie et al. (1990). Fresh weight (FW) of the leaves was

measured immediately after sampling. To determine turgid weight (TW), each leaf was immersed in 50 mL of distilled water in a sealed container and kept at 4 °C for 24 h. The leaves were then oven-dried at 70 °C for 48 h to determine dry weight (DW). Finally, leaf relative water content was calculated using the following equation:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Measurement of Fresh and Dry Weight of Shoot and Root

To determine the fresh weight of the shoot, the plant was cut at the soil surface and weighed using a balance with 0.001 g precision. Roots were gently removed from the soil, washed with water to remove adhering particles, blotted with paper towels to remove excess water, and then weighed using the same balance. Both shoots and roots were oven-dried at 70 °C for 48 hours, and the dry weight was recorded afterward (Bates et al., 2007).

Electrolyte Leakage (EC) and pH of Plant and Soil

To measure tissue electrolyte leakage, approximately 1 g of young leaf tissue was added to 20 mL of distilled water and kept at 24 °C for 24 hours. The electrical conductivity of the solution was recorded as initial leakage (EC1) using an EC meter. Samples were then autoclaved at 120 °C for 15 minutes, allowed to cool, and the final leakage (EC2) was measured. Electrolyte leakage ($\mu S \cdot cm^{-1}$) was calculated using the following formula (Huang & Yu, 2008):

$$EC = (EC_1 / EC_2) \times 100$$

For plant pH measurement, 4 g of leaf tissue was ground with 40 mL of distilled water in a porcelain mortar, centrifuged at 3000 rpm for 5 minutes, and the pH of the supernatant was measured using a pH meter (Chen et al., 2022). Soil pH was determined by preparing a 1:25 soil-to-distilled water suspension, shaking it at room temperature for 30 minutes, and then measuring the pH using a pH meter (Milanpoor et al., 2020).

Determination of Photosynthetic Pigment Content

The total chlorophyll content of the samples was determined using the Lichtenthaler method (1978). In this method, 1 g of fresh leaf tissue was homogenized with 44 mL of 80% acetone. The homogenate was centrifuged at 3000 rpm for 5 minutes. The absorbance of the supernatant was measured at wavelengths of 470, 645, and 663 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and carotenoid contents were calculated using the following formulas:

ChloropHyll a[mg/] = (19.3 × A663 - 0.86 × A645) V/100 W
ChloropHyll b[mg] = (19.3 ×A645 - 3.6 × A663) V/100 W
Carotenoids[mg] = 100(A470) - 3.27 (mg Chl. a) – 104 (mg Chl. b)/ 227

Measurement of Hydrogen Peroxide (H_2O_2)

To determine leaf hydrogen peroxide content, 0.07 g of leaf tissue was homogenized in an ice bath with 5 mL of 1% trichloroacetic acid (TCA). The resulting suspension was centrifuged at 12,000 rpm for 15 minutes at 4 °C. Then, 0.5 mL of the supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer and 1 mL of 1 M potassium iodide (KI). The absorbance of the reaction mixture was measured at 390 nm using a spectrophotometer. The H_2O_2 content in the sample was determined by comparison with a standard curve prepared with known concentrations of H_2O_2 (Patterson et al., 1984).

Measurement of Polyphenol Oxidase (PPO) Enzyme Activity

For enzyme extraction, 0.1 g of leaf tissue was selected and powdered using liquid nitrogen. The powdered tissue was mixed with a suitable buffer solution and centrifuged for 15 minutes to separate the enzyme extract from the solid material. From the resulting extract, 300 µL was collected and stored in a microtube at –20 °C. These extracts were stable and usable for up to 45 days. To assess polyphenol oxidase (PPO) activity, a reaction mixture containing phosphate buffer, pyrogallol, and the enzyme extract was prepared according to the method of J. Raymond (1993). The absorbance

change due to the formation of purpurogallin was measured every 5 seconds at 420 nm for one minute using a spectrophotometer. PPO activity was expressed as enzyme units (U mg^{-1} protein).

Determination of Ascorbic Acid Content in Plants

To measure the ascorbic acid content, 1 g of leaf tissue from each plant was homogenized with 20 mL of an extracting solution containing 5% metaphosphoric acid and centrifuged at 8000 rpm for 20 minutes at 4 °C. Then, 1 mL of the clear supernatant was mixed with 0.5 mL of DCIP to oxidize ascorbic acid to dehydroascorbic acid. To measure the oxidized ascorbic acid, 0.5 mL of distilled water was added to 1 mL of the centrifuged solution. For the blank solution, 1 mL of 5% metaphosphoric acid was combined with 0.5 mL of DCIP. Subsequently, 1 mL of 1% thiourea was added to the samples in three final steps. Then, 1 mL of 20% sulfuric acid was added to the blank solution and 1 mL of 10 mM DNPH solution was added to the remaining samples. The samples were incubated in a 50 °C water bath for 1 hour and then placed in an ice bath for 20 minutes. Next, 2.5 mL of 85% sulfuric acid was added, and the test tubes were again kept in an ice bath for 30 minutes.

The absorbance of each sample was measured at 520 nm using a UV–VIS spectrophotometer, compared to the blank. Finally, ascorbic acid content was calculated based on the standard curve prepared from the measured absorbance values (Hewitt, 1961).

Measurement of Sodium, Potassium, Phosphorus, Nitrogen, and Protein in Plants

To determine sodium (Na) and potassium (K) contents and prepare extracts for ICP analysis, 0.3 g of ground plant sample was weighed and placed in a crucible for 4 hours in a muffle furnace at 450 °C. After cooling, the resulting

ash was mixed with 10 mL of 0.1 N hydrochloric acid and heated on a hot plate at 80 °C for 10 minutes. Sodium and potassium concentrations in the plant extract were measured using a flame photometer, and the percentage of Na and K in the plant sample was calculated (Hovre, 1961).

For phosphorus (P) determination, the same procedure was followed for ashing and extracting the sample. Then, 0.5 mL of the extract was mixed with 4 mL of solution B (a combination of specific chemicals), and the absorbance was measured at 660 nm using a spectrophotometer. The phosphorus content was calculated based on the measured absorbance (Murphy & Riley, 1962).

Total nitrogen (N) and protein content were determined using the macro-Kjeldahl method. Briefly, 0.3 g of ground plant sample was weighed and mixed with 1.1 g of catalyst (a mixture of potassium sulfate, copper sulfate, and selenium powder) and 5 mL of concentrated sulfuric acid. The mixture was digested at 500 °C for 1 hour. After cooling, 20 mL of 10 N sodium hydroxide and boric acid solution were added. Nitrogen was distilled and titrated with 0.005 N sulfuric acid. Nitrogen and protein contents were calculated using the following formulas (Bradstreet, 1954):

ppm= V×0.005×50×14×1000(0.3×20), V=%N= ppm / 10000

Where V is the volume of acid (mL) used for titration Protein%= %N×6.25

Calculation of Anticipated Performance Index (APTI)

The Anticipated Performance Index (APTI) is an empirical index used to evaluate plant tolerance to air pollutants. Based on this index, plants are categorized into sensitive, moderate, and tolerant groups in response to air pollution. Higher APTI values indicate greater tolerance of plants to air pollutants (Karimian et al., 2023). To calculate APTI, the values of specific traits, including leaf chlorophyll content, leaf pH, relative water content (RWC), and ascorbic acid content, are required. The measurement methods for these traits have been described in the previous sections. Finally, the APTI value, which reflects the plant's tolerance to air pollution, was calculated using the following formula (Singh, 1983):

APTI = Acid ascorbic (Chlorophyll + pH) + RWC /10

Experimental Design and Data Analysis

In this study, four factors including mycorrhiza, cyanobacteria, biochar, and irrigation type were considered. A full factorial design based on three levels for each factor (3×3×3) would result in 27 treatment combinations. However, to reduce the number of experimental runs while maintaining adequate representation of the experimental domain and factor interactions, a Response Surface Methodology (RSM)-based optimal design was generated using Design-Expert software (Version 13.0.5.0) (Montgomery et al., 2016). The software-generated design matrix selected 13 treatment combinations as representative and well-distributed points within the factor space. This approach allowed efficient exploration of the main effects and interactions among factors while minimizing the total number of experimental runs. It should be noted that the application of RSM in this study was primarily aimed at optimizing the selection of treatment combinations rather than performing full response surface modeling.

Due to the nature of the optimal design, the experimental matrix does not strictly follow a full factorial structure and does not necessarily include a single complete control treatment (C0, M0, B0). Instead, treatments with zero or low levels of individual factors were included, providing a relative baseline for comparison and enabling the evaluation of interaction effects within the experimental domain. The selected treatments (Table 2) were applied under two irrigation regimes, municipal water (MW) and greywater (GW), each with three replicates, resulting in a total of 78 experimental units. This level of replication provided sufficient statistical comparison while considering greenhouse space and resource limitations. However, we acknowledge that a higher number of replicates could further reduce variability and strengthen the robustness of the results, and this limitation has been noted in the revised manuscript.

At the end of the experiment, the collected data were subjected to analysis of variance (ANOVA) to evaluate the effects of treatments. In addition, relationships among measured variables were examined using correlation analysis in Minitab 22 software, and only correlation coefficients greater than 0.6 were considered significant. Furthermore, Principal Component Analysis (PCA) was performed to investigate the multivariate relationships among variables and to better understand the combined effects of treatments on plant physiological traits and water quality parameters (The results of the PCA analysis are available in the supplementary file).

Results

The results of this study showed that the combined treatments had significant effects on the COD, pH, and EC of the outlet wastewater at the 1% probability level. In addition, the effects of these treatments were statistically significant ($p \leq 0.05$) for all measured traits except RWC, SFW, RDW, and soil EC. The irrigation type also showed significant effects on most traits at least at the 5% level, except for SFW, RDW, total chlorophyll, and plant EC. The interaction effects between combined treatments and irrigation type were significant at the 1% level for RFW and RDW, soil EC and pH, PPO activity, H_2O_2 content, and ascorbic acid concentration (Table 4).

Effects on Graywater Quality Parameters (COD, pH, and EC)

The mean comparison among the combined treatments showed that the highest COD value (475.67 mg L^{-1}) in the C0.4–M0–B10 treatment significantly differed from the lowest value (172.67 mg L^{-1}) observed in the C0.4–M10–B0 treatment. In contrast, most of the other treatments showed no significant differences from one another. The highest COD concentration was recorded under C0.4–M0–B10 (475.67 mg O_2 L^{-1}), which was about 175% higher than the minimum observed value. Conversely, C0.4–M10–B0 produced the lowest COD (172.67 mg O_2 L^{-1}), about 64% lower than the maximum (Table 5).

Table 4. ANOVA of plant and soil traits of *Crassula capitella* under treatments

* and ** indicate significance at the 5% and 1% probability levels, respectively, and ns indicates non-significance.

The highest pH value of the outlet wastewater in *C. capitella* was observed in the C0.4–M10–B0 treatment (9.45), whereas the lowest pH value (8.45) occurred in the C0.4–M10–B10 treatment. Overall, most pH values ranged between 8.45 and 8.92. Based on the minimum and maximum recorded values, the outlet wastewater pH exhibited a variation of approximately 11.9% across treatments. Additionally, the treatment showing the minimum pH did not differ significantly from the pH of the influent wastewater (Table 5).

The mean comparison of EC values among treatments indicated that the highest EC (11286.7 µS cm^{-1}) was recorded in the C0.4–M5–B5 treatment, whereas the lowest EC (4180.3 µS cm^{-1}) was observed in the C0–M10–B5 treatment. Most EC values ranged between 4704.3 and 8760 µS cm^{-1}. Based on the minimum and maximum observed values, EC showed an overall variation of approximately 170% among the treatments. The treatment with

Sources	DF	EC-Plant (µS/cm)	EC-Soil (µS/cm)	pH-Plant	pH-Soil	PPO (U/mg)	APTI
Combined treatments	12	189.87**	189828 ns	0.19816**	0.24951**	2620.0**	60.3*
Water type	1	121.77ns	46055628**	4.87000**	1.31482**	4314.3*	313.27*
Sample×Water type	12	70.820ns	736703**	0.11892ns	0.21106**	2098.0*	43.5ns
Error	52	47.730	153913	0.06804	0.07579	974.30	30.81
Total	77						

Sources	DF	RWC (%)	SFW (g)	RFW (g)	RDW (g)	Chl. (µg/g fw)	H_2O_2 (µmol/g fw)	Ascorbic acid (mg/g fw)
Combined treatments	12	77.89 ns	1340.7 ns	4.099*	0.15299 ns	12.103**	0.01037*	48.500**
Water type	1	995.97 **	280.0 ns	12.146 **	0.15101 ns	8.225 ns	0.02565*	196.69**
Sample×Water type	12	28.42 ns	2301.1 ns	6.355* *	0.28851 **	9.429 ns	0.01146**	54.730**
Error	52	52.22	1303.7	1.961	0.09609	5.1480	0.00463	20.050
Total	77	-	-	-	-	-	-	-

the minimum EC did not show a noticeable difference compared with the EC of the influent wastewater (1593 µS cm^{-1}) (Table 5).

Effects on Plant Growth and Biomass ***(Shoot and root fresh and dry weight)***

Although no statistically significant differences were detected among treatments, the C0.4–M10–B0 treatment under graywater irrigation exhibited the highest shoot fresh weight (137.35 g), whereas the lowest value (48.68 g) was recorded in the C0.8–M5–B10 treatment under municipal water. Based on the minimum and maximum observed values, SFW showed an overall variation of approximately 182% across treatments.

The highest root fresh weight (RFW) value (7.31 g) was observed in the C0–M10–B5 treatment irrigated with municipal water, whereas the lowest RFW (2.53 g) occurred in the C0.8–M5–B10 treatment under the same irrigation source. Based on the minimum and maximum recorded values, RFW showed an overall variation of approximately 189% across treatments. Similarly, root dry weight (RDW) ranged from 0.353 g in the C0.8–M5–B10 treatment to 1.407 g in the C0–M10–B5 treatment, corresponding to a variation of about 299% between the minimum and maximum values. On average, plants irrigated with municipal water produced slightly higher RFW (15%) and RDW (10%) than those irrigated with graywater (Table 6).

According to the correlation analysis (Table 8), RDW showed a strong positive correlation with RFW (r=0.801) and shoot fresh weight (SFW; r=0.679). In addition, RFW was positively correlated with SFW (r=0.657). All correlations were statistically significant at the 1% level. These results indicate that increases in RDW were associated with higher RFW and SFW, and increasing RFW also resulted in improved SFW.

Table 5. Mean comparison between measured traits in gray water effluent under combined treatments

Combined treatments	COD (mg O_2/l)	EC (µS/cm)	pH
C0.4–M5–B5	195.000^{b}	11286.7^{a}	8.49667^{b}
C0.4–M10–B0	172.667^{b}	7153.3^{ab}	9.45433^{a}
C0.0–M5–B0	289.000^{ab}	4704.3^{b}	8.52667^{b}
C0.4–M10–B10	278.100^{b}	8760.0^{ab}	8.45000^{b}
C0.8–M0–B5	334.767^{ab}	6893.3^{ab}	8.70333^{ab}
C0–M10–B5	254.000^{b}	4180.3^{b}	8.92667^{ab}
C0.8–M5–B10	319.000^{ab}	7160.0^{ab}	8.54333^{b}
C0.8–M10–B5	332.333^{ab}	6581.3^{ab}	8.77000^{ab}
C0.4–M0–B0	222.333^{b}	7663.3^{ab}	8.55333^{b}
C0–M5–B10	245.667^{b}	5470.0^{b}	8.78667^{ab}
C0–M0–B5	195.333^{b}	5740.0^{b}	8.77000^{ab}
C0.8–M5–B0	304.667^{ab}	6826.7^{ab}	8.47000^{b}
C0.4–M0–B10	475.667^{a}	8373.3^{ab}	8.45000^{b}

Effects on Plant Water Status and Photosynthetic Pigments (RWC and Chlorophyll Content)

Mean comparison among the combined treatments showed that the highest relative water content (RWC) was obtained in the C0.4–M0–B10 treatment under municipal water irrigation (90.46%), whereas the lowest RWC (70.28%) was recorded in the C0.8–M10–B5 treatment irrigated with graywater. Based on the minimum and maximum recorded values, RWC varied by approximately 28.7% across treatments. On average, irrigation with municipal water resulted in higher RWC compared with graywater irrigation (Fig. 2, E).

The highest chlorophyll content was recorded in the C0.4–M10–B0 treatment irrigated with graywater (11.366 µg g^{-1} FW), whereas the lowest value (3.508 µg g^{-1} FW) was observed in the C0.8–M5–B10 treatment irrigated with municipal water. Based on the minimum and maximum recorded values, chlorophyll content varied by approximately 224% across treatments. Overall, plants irrigated with graywater exhibited slightly higher chlorophyll levels compared with those irrigated with municipal water (Table 7).

Table 6. Means comparison between measured traits in *Crassula capitella* for the interaction of Combined treatment×Water type (municipal water (MW) and graywater (GW)).

Combined treatments	Water type	RFW (g)	RDW (g)	EC Soil (µS/cm)	pH Soil
C0.4–M5–B5	MW	5.789^{abc}	0.949^{ab}	718.77^{abc}	8.37^{abcd}
	GW	4.802^{abc}	0.861^{ab}	812.67^{abc}	8.56^{abcd}
C0.4–M10–B0	MW	5.918^{abc}	0.770^{ab}	1536.00^{abc}	8.23^{abcd}
	GW	6.817^{abc}	1.102^{ab}	437.17^{bc}	9.06^{a}
C0.0–M5–B0	MW	7.263^{a}	1.204^{ab}	1545.67^{abc}	7.780^{d}
	GW	4.963^{abc}	0.781^{ab}	535.23^{bc}	8.73^{ab}
C0.4–M10–B10	MW	5.702^{abc}	0.855^{ab}	812.00^{abc}	8.35^{abcd}
	GW	4.363^{abc}	0.692^{ab}	1006.00^{abc}	8.33^{abcd}
C0.8–M0–B5	MW	5.951^{abc}	1.053^{ab}	1342.00^{abc}	8.29^{abcd}
	GW	5.598^{abc}	0.994^{ab}	324.07^{c}	8.57^{abcd}
C0–M10–B5	MW	7.309^{a}	1.407^{a}	1405.33^{abc}	7.75^{d}
	GW	4.438^{abc}	0.755^{ab}	1095.00^{abc}	8.16^{bcd}
C0.8–M5–B10	MW	2.530^{c}	0.353^{b}	1410.33^{abc}	8.21^{abcd}
	GW	7.283^{a}	1.296^{ab}	844.33^{abc}	8.70^{abc}
C0.8–M10–B5	MW	6.931^{abc}	1.370^{a}	861.57^{abc}	8.39^{abcd}
	GW	4.127^{abc}	0.781^{ab}	782.33^{abc}	8.07^{bcd}
C0.4–M0–B0	MW	5.609^{abc}	0.813^{ab}	1634.00^{ab}	8.06^{bcd}
	GW	2.643^{bc}	0.479^{ab}	1002.00^{abc}	7.85^{cd}
C0–M5–B10	MW	4.782^{abc}	0.713^{ab}	1039.67^{abc}	8.27^{abcd}
	GW	4.348^{abc}	0.551^{ab}	636.67^{abc}	8.51^{abcd}
C0–M0–B5	MW	7.089^{a}	0.730^{ab}	1242.00^{abc}	8.35^{abcd}
	GW	5.954^{abc}	1.114^{ab}	887.67^{abc}	8.47^{abcd}
C0.8–M5–B0	MW	6.745^{abc}	1.324^{ab}	1857.33^{a}	8.10^{bcd}
	GW	5.780^{abc}	0.960^{ab}	576.33^{bc}	8.59^{abcd}
C0.4–M0–B10	MW	6.833^{abc}	0.940^{ab}	925.00^{abc}	8.12^{bcd}
	GW	7.072^{ab}	0.971^{ab}	1072.33^{abc}	8.06^{bcd}

***Effects on Oxidative Stress and Membrane Stability* (H₂O₂ Content and EC)**

Based on the mean comparison test, the highest hydrogen peroxide (H_2O_2) content was observed in the C0.4–M10–B0 treatment (0.387 µmol g^{-1} FW), whereas the lowest value (0.0913 µmol g^{-1} FW) was recorded in the C0.4–M0–B0 treatment. Both treatments were irrigated with municipal water. Based on the minimum and maximum recorded values, H_2O_2 content varied by approximately 324% across treatments. Overall, plants irrigated with municipal water exhibited higher H_2O_2 concentrations than those irrigated with graywater (Fig. 2, D).

Combined treatments	EC-plant	pH-plant	Chlorophyl
C0.4–M5–B5	82.79^{bc}	5.32^{b}	4.19^{b}
C0.4–M10–B0	92.06^{abc}	5.44^{b}	9.74^{a}
C0.0–M5–B0	89.96^{abc}	5.62^{ab}	7.56^{ab}
C0.4–M10–B10	95.13^{ab}	5.55^{ab}	5.96^{ab}
C0.8–M0–B5	92.64^{abc}	5.81^{ab}	5.35^{ab}
C0–M10–B5	96.30^{ab}	5.99^{a}	5.27^{ab}
C0.8–M5–B10	83.30^{bc}	5.58^{ab}	5.85^{ab}
C0.8–M10–B5	80.70^{c}	5.44^{b}	7.00^{ab}
C0.4–M0–B0	95.79^{ab}	5.51^{ab}	5.49^{ab}
C0–M5–B10	90.13^{abc}	5.72^{ab}	6.60^{ab}
C0–M0–B5	95.13^{ab}	5.75^{ab}	4.93^{b}
C0.8–M5–B0	94.97^{ab}	5.48^{ab}	6.90^{ab}
C0.4–M0–B10	98.42^{a}	5.66^{ab}	5.37^{ab}

The highest plant EC value (105.25 µS/cm) was recorded in the C0.4–M0–B10 treatment, whereas the lowest value (77.91 µS/cm) occurred in the C0.4–M5–B5 treatment; both were irrigated with graywater. Across all treatments, plant EC values ranged from 77.91 to 105.25 µS/cm, showing no significant differences among most treatments. Based on the minimum and maximum values, plant EC varied by approximately 35.1% across treatments. The overall effect of graywater irrigation on plant EC, compared with municipal water, was negligible (Table 7).

Table 7. Means comparison of some traits in *Crassula capitella* under main effect of combined treatments

Mean comparison among the combined treatments showed that the highest soil EC was recorded in the C0.8–M5–B0 treatment (1857.33 µS/cm), whereas the lowest value (324.07 µS/cm) occurred in the C0.8–M0–B5 treatment; both received graywater irrigation. Across the remaining treatments, soil EC values generally ranged from 437.17 to 1634 µS/cm, without distinct significant differences among most of them. Based on the minimum and maximum values, soil EC varied by approximately 473% across treatments. Overall, graywater irrigation tended to increase soil EC compared with municipal water irrigation (Table 6).

Table 8. Correlation coefficients (r>0.30) among the measured traits of *Crassula capitella* under treated irrigated by municipal water and gray water (n=78, P<0.000).

Sample 1	Sample 2	Correlation	P-Value
APTI	Ascorbic acid	0.88	0.000
RDW	RFW	0.801	0.000
RDW	SFW	0.679	0.000
RFW	SFW	0.657	0.000
Ascorbic acid	H_2O_2	0.423	0.000
RWC	pH Plant	0.41	0.000
APTI	H_2O_2	0.397	0.000
Chlorophyll	pH Soil	0.354	0.002
EC Soil	pH Plant	0.343	0.002
Ascorbic acid	EC Soil	0.337	0.003
PPO	pH Plant	0.335	0.003
Ascorbic acid	SFW	-0.301	0.008
RWC	pH Soil	-0.314	0.005
Ascorbic acid	RDW	-0.318	0.005
EC Soil	pH Soil	-0.655	0.000

Effects on Antioxidant Metabolism (Ascorbic Acid Content and PPO Activity)

The combined treatments showed significant differences in ascorbic acid content. The highest ascorbic acid concentration (22.64 mg g^{-1} FW) was observed in the C0.8–M5–B10 treatment irrigated with municipal water, whereas the lowest value (1.87 mg g^{-1} FW) occurred in the C0–M10–B5 treatment irrigated with graywater. Based on the minimum and maximum recorded values, ascorbic acid content varied by approximately 1111% across treatments. In general, plants irrigated with municipal water exhibited higher ascorbic acid levels than those irrigated with graywater (Fig. 2, A).

Based on the mean comparison test, the highest polyphenol oxidase (PPO) activity (180.53 U mg^{-1} protein) was recorded in the C0–M10–B5 treatment irrigated with municipal water, whereas the lowest value (33.87 U mg^{-1} protein) occurred in the C0–M0–B5 treatment irrigated with graywater. Based on the minimum and maximum recorded values, PPO activity varied by

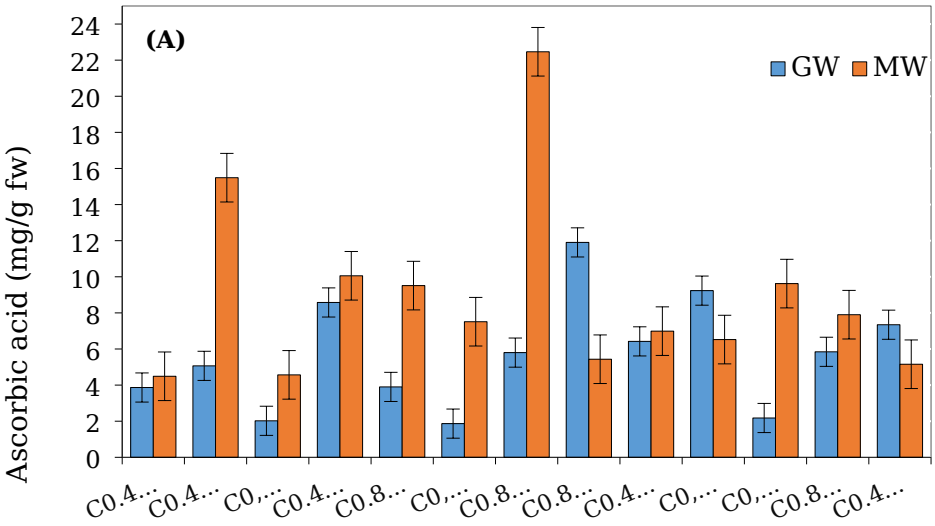
approximately 433% across treatments. In general, plants irrigated with municipal water exhibited higher PPO activity than those irrigated with graywater (Fig. 2, B).

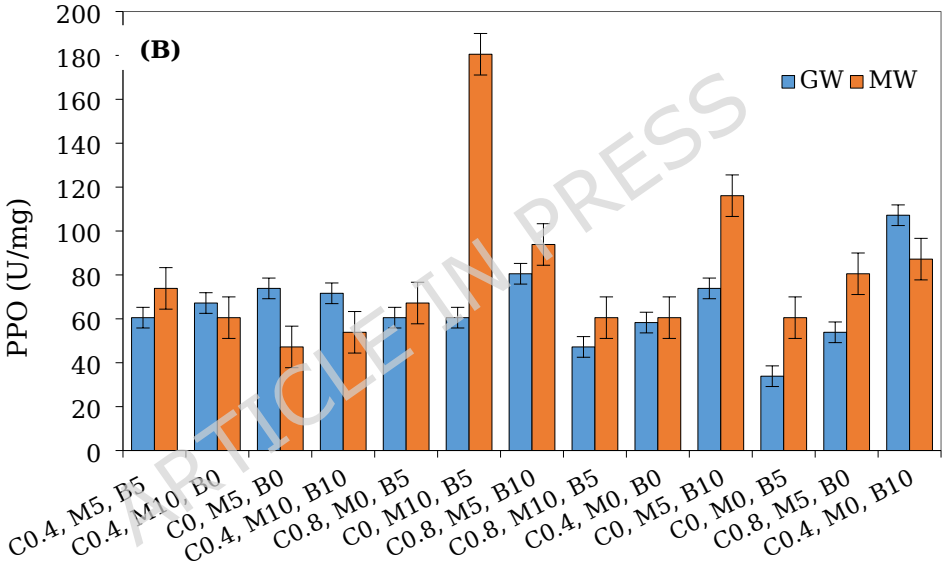
Air Pollution Tolerance Index (APTI)

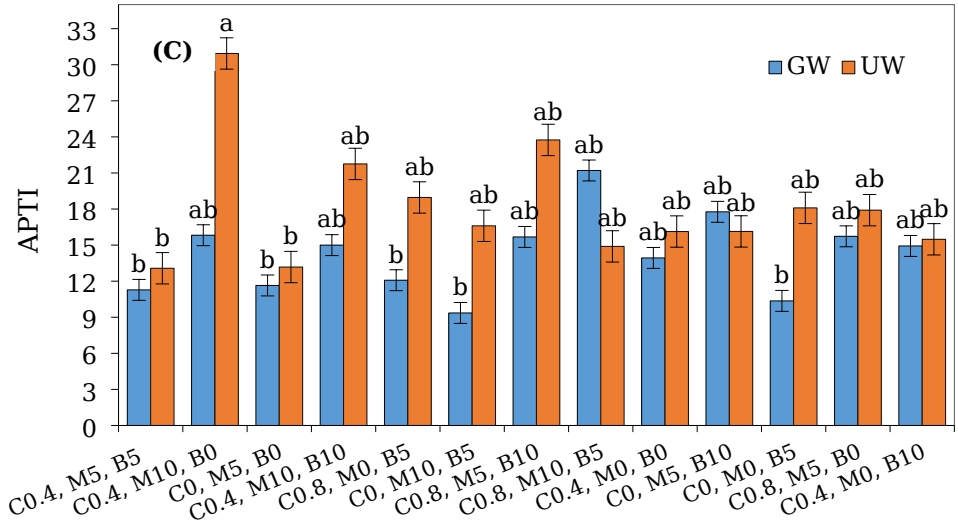
The highest Air Pollution Tolerance Index (APTI) value (30.94) was observed in the C0.4–M10–B0 treatment irrigated with municipal water, whereas the lowest value (9.36) occurred in the C0–M10–B5 treatment irrigated with graywater. Based on the minimum and maximum values, APTI varied by approximately 230% across treatments. On average, plants irrigated with graywater exhibited lower APTI values than those irrigated with municipal water. According to the correlation analysis, APTI showed a strong positive correlation with ascorbic acid content (r=0.88), indicating that increases in ascorbic acid were associated with higher APTI values (Fig. 2, C).

Effects on Plant Nutrient Uptake (N, P and K Content)

As shown in Figure 3, which illustrates the effects of combined treatments on nutrient concentrations in plant tissues, the highest nitrogen content (26,519 ppm) was observed in the C0.8–M0–B5 treatment irrigated with gray water. In contrast, the lowest nitrogen content (6,183 ppm) was recorded in the C0–M0–B5 treatment irrigated with municipal water. The highest phosphorus concentration (0.47 ppm) was found in the C0–M10–B5 treatment irrigated with gray water, while the lowest value (0.12 ppm) occurred in the C0.8–M10–B5 treatment irrigated with municipal water. The maximum (70,622 ppm) and minimum (20,382.2 ppm) potassium concentrations were observed in the C0–M5–B10 and C0.8–M0–B5 treatments irrigated with municipal water, respectively (Fig. 3).







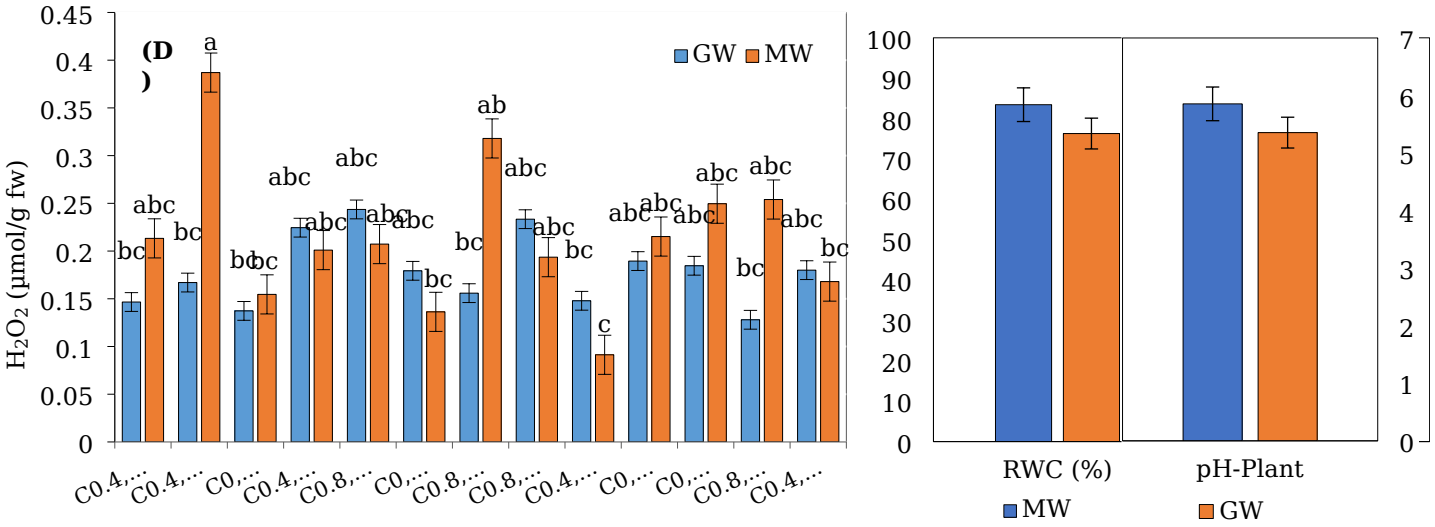
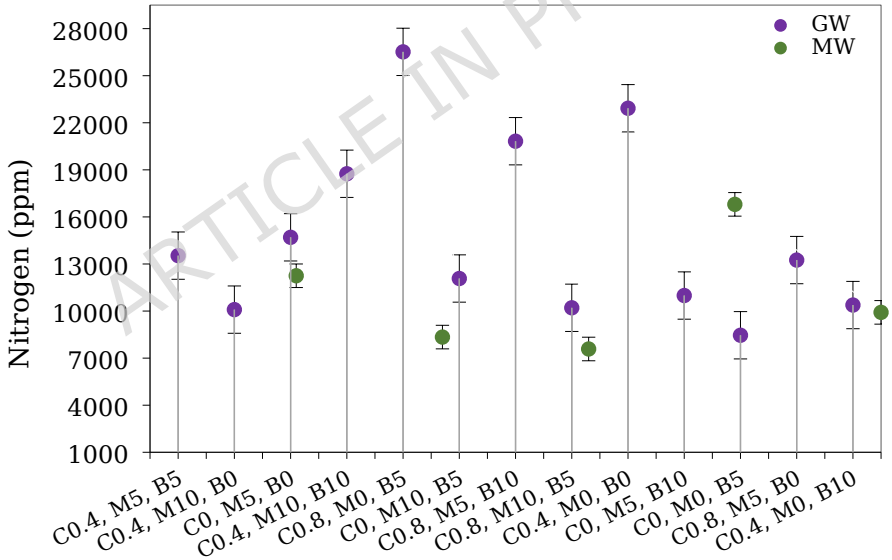


Figure 2. Ascorbic acid (A), APTI (B), PPO (C), and H_2O_2 (D) under the combined treatment×water type interaction, and RWC and plant pH (E) under the main effect of water type in *Crassula capitella*



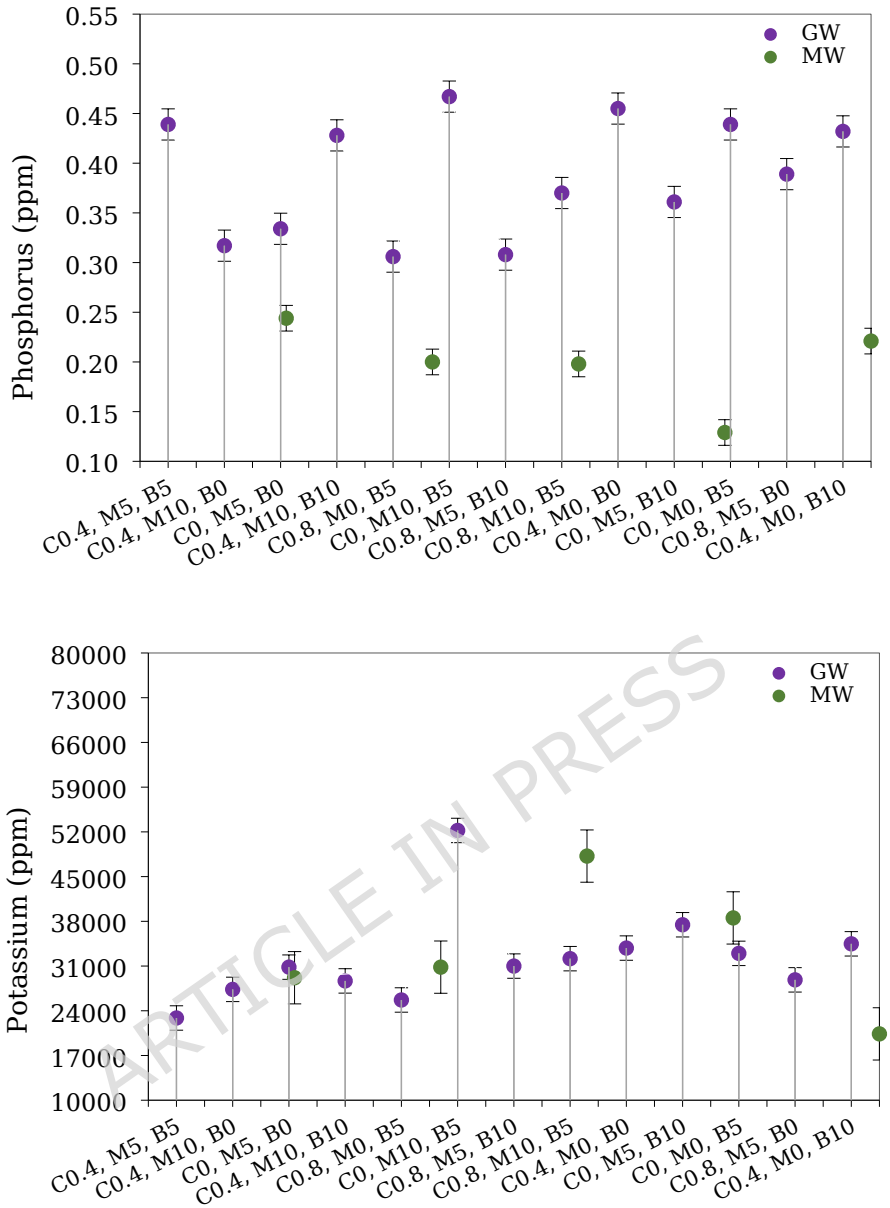


Figure 3. Concentration of Nitrogen, Phosphorous and Potassium (Combined treatment in *Crassula capitella* under UW and GW

Discussion

The findings of this study clearly demonstrate that the combined application of cyanobacteria, mycorrhiza, and biochar exerts significant influence on both the growth performance of *Crassula capitella* in a gray water–irrigated green wall system and the quality of the treated wastewater. The results collectively suggest that plant physiological responses, substrate

modifications induced by microbial and organic amendments, and the chemical characteristics of gray water interact to shape plant performance and treatment efficiency.

The high water-holding capacity of the biochar used in this study (84%) reflects its inherently porous structure, which likely contributed to improved moisture retention in the growth medium and enhanced water availability to plants. This is consistent with previous reports highlighting the role of porous biochar in improving substrate physical properties (Gul-Lalay et al., 2024; Moradi et al., 2024).

Graywater Quality Parameters

One of the most notable outcomes was the substantial variation in COD removal efficiency among treatments. The C0.4–M10–B0 treatment yielded the lowest COD concentration (172.67 mg L^{-1}), corresponding to a purification efficiency more than 2.75 fold higher than the treatment with the highest COD value. This enhanced performance aligns with literature demonstrating that mycorrhiza and cyanobacteria have strong biodegradation capacities, contributing to COD reductions of up to 74.4% (Du et al., 2019) and 77% (Ouhsassi et al., 2020), respectively.

The pH of the treated gray water remained alkaline (8.45–9.45), with the highest value occurring in C0.4–M10–B0. Because the biochar applied in this experiment was derived from woody biomass (*Parrotia persica*, poplar, eucalyptus), its buffering capacity and tendency to shift soil and effluent pH toward neutral to alkaline levels (Lievens et al., 2018) likely contributed to these results. The observed pH shift is also consistent with the alkalinizing effects reported for cyanobacteria (Mahran and Razek, 2024), biochar (Aylan et al., 2025), and mycorrhiza (Wang et al., 2024).

Substantial differences in outlet EC up to 2.7 fold indicated marked treatment-dependent variation in salinity. Although all EC values remained within the saline range, these differences likely reflect ion release from

biochar, mineralization by cyanobacteria, and rhizosphere-driven nutrient mobilization under mycorrhizal colonization. Similar impacts of gray water irrigation on soil EC and nutrient dynamics have been reported by Sulayman et al. (2025).

Plant Water Status and Growth Performance

The highest RWC (90.46%) was obtained under C0.4–M0–B10 (municipal water), approximately 1.3 times higher than the lowest RWC recorded under gray water irrigation. These differences suggest that gray water–induced osmotic stress reduced leaf RWC, consistent with prior studies (Lubbe et al., 2016). The enhanced RWC in treatments with higher biochar content supports previous findings that biochar improves water-holding capacity and reduces plant water stress (Noreen et al., 2025).

In terms of biomass production, SFW was highest in C0.4–M10–B0, 2.8 times greater than the lowest value. The growth-promoting effects of cyanobacteria on shoot biomass (Bello et al., 2021) and the positive influence of gray water irrigation reported in other species (Mekki et al., 2015; Anangadan et al., 2025) help explain this response. Conversely, excessive cyanobacterial concentrations can suppress growth (Massa et al., 2024), which may clarify variation among treatments.

Root biomass (RFW and RDW) reached its maximum in the C0–M10–B5 treatment under municipal water irrigation—about 3–4 times higher than the minimum. This outcome is consistent with the known capacity of mycorrhiza (Hawkins et al., 2023) and biochar (Pu et al., 2022; Wu et al., 2021) to enhance root architecture, substrate porosity, and nutrient uptake (Tian et al., 2024; Kamran et al., 2020).

Photosynthetic Pigments, Oxidative Stress and Membrane Stability

Chlorophyll content peaked in C0.4–M10–B0 (a 3.2-fold increase over the lowest value). The stimulation of chlorophyll by mycorrhiza under both optimal and stressful conditions (Farghaly et al., 2022), along with reduced

heavy metal uptake mediated by mycorrhiza (Gupta et al., 2021), likely contributed to these results. Cyanobacterial treatments are also known to increase chlorophyll and delay senescence (Santini et al., 2021). The mixed effects of gray water on chlorophyll reported in earlier studies (Anangadan et al., 2024; MKafay et al., 2022) are consistent with the variable responses observed here.

H_2O_2 levels showed a clear trend: the lowest value (in C0.4–M0–B0) was more than four times lower than the maximum. Cyanobacterial extracts are known to reduce oxidative stress by limiting H_2O_2 accumulation (Abdelaziz et al., 2022), although in this experiment, neither biochar nor mycorrhiza effectively decreased H_2O_2.

Plant EC and soil EC varied substantially among treatments, with soil EC showing a 5.7-fold range. Biochar's ability to enhance membrane stability (Gao et al., 2024) and reduce soil EC over time (Sun et al., 2022), along with cyanobacterial and mycorrhizal effects on membrane integrity (Alharbi et al., 2023; Wu et al., 2024), helps explain these patterns. Variations in plant and soil pH also corresponded with known pH-modulating effects of mycorrhiza (Feng et al., 2023; Farhaoui et al., 2025) and gray water irrigation (Anangadan et al., 2024), as well as the alkalinity of biochar (Ahmed et al., 2024).

Antioxidant Metabolism

Ascorbic acid content exhibited more than a twelvefold difference among treatments, with the highest levels recorded in C0.8–M15–B10. The increase aligns with the well-established role of mycorrhiza in improving plant antioxidant capacity under stress (Ma et al., 2022), the stimulatory effects of acidified biochar on nutrient uptake and ascorbic acid accumulation (Shahzad et al., 2023), and the positive influence of cyanobacteria on antioxidant levels (Gashash et al., 2022). Improved nutrient accessibility is

known to enhance antioxidant responses (Mihoub et al., 2017), which likely contributed to these findings.

APTI values exhibited strong treatment dependence, with C0.4–M10–B0 showing the highest value (30.94), more than three times the minimum. Higher APTI is closely linked to elevated ascorbic acid content and reduced oxidative stress (Karimian et al., 2023), suggesting that treatments enhancing physiological resilience also improved air pollution tolerance.

Similarly, PPO activity varied by more than fivefold, peaking in C0–M10–B5. Increases in PPO activity mediated by biochar and mycorrhiza have been widely reported as part of plant defense responses (Bhatt et al., 2024), and gray water irrigation itself can stimulate PPO (Jozay et al., 2024), consistent with the patterns observed here.

Plant Nutrient Uptake (N, P and K Content)

Under gray water irrigation, nitrogen content peaked in C0.8–M0–B5, while phosphorus was highest in C0–M10–B5 and potassium in C0–M5–B10 (municipal water), each showing 214%, 53% and 245% than minimum value, respectively. These results reflect the functional roles of the applied amendments: cyanobacteria fix nitrogen and solubilize phosphorus (Kollmen and Strieth, 2022), thereby enhancing plant nutrient status (Jing et al., 2025); biochar improves nutrient retention and availability, including increases in K uptake (Gao et al., 2024; Zhu et al., 2025); and mycorrhiza facilitate organic nitrogen decomposition (Pellitier et al., 2021), P uptake (Wu et al., 2024), and K transport (Alizadeh et al., 2025). Although gray water provides nutrients such as N and P and may reduce fertilizer demand, excessive concentrations can lead to leaching and eutrophication (Jaramillo et al., 2017). In green wall systems, gray water irrigation has been associated with increased soil mineral content and biomass (Anangadan et al., 2024), consistent with the responses observed here.

Taken together, the integrated responses observed across physiological, biochemical, and substrate-related traits point toward a shared mechanistic pathway through which cyanobacteria, mycorrhiza, and biochar collectively enhance pollutant tolerance and graywater purification performance. Specifically, the simultaneous increase in antioxidant metabolites (ascorbic acid), enzymatic defenses (PPO), and APTI together with reduced oxidative pressure (lower H_2O_2) and improved water status (higher RWC) indicates that these amendments stabilized cellular redox balance and membrane integrity under graywater-induced osmotic and chemical stress. Such stabilization is well known to increase plant tolerance to complex pollutants and to maintain photosynthetic and metabolic activity under stressful wastewater conditions (Ahsan et al., 2025). Furthermore, the porosity, adsorption capacity, and ion-buffering properties of biochar, combined with nutrient mobilization by mycorrhiza and nitrogen/phosphorus provision by cyanobacteria, likely optimized rhizosphere conditions for microbial degradation processes, thereby contributing directly to enhanced COD removal. These converging physiological and substrate-mediated processes provide a coherent mechanistic explanation for why treatments that supported higher antioxidant activity, better membrane stability, and improved water balance also corresponded to the highest graywater purification efficiency.

Conclusion

The results of this study indicate that, among the tested treatments, C0.4–M10–B0 and C0.8–M0–B5 were associated with relatively improved establishment and growth of *Crassula capitella* irrigated with greywater in a green wall system. This response was reflected in higher values of growth and stress-related parameters, including fresh and dry biomass, relative water content, PPO activity, APTI, chlorophyll content, and H_2O_2 concentration. With respect to greywater treatment, C0.4–M10–B0 achieved the lowest COD and C0.4–M5–B5 also showed low COD, indicating effective organic matter removal, although it resulted in higher EC values.

In the context of increasing water scarcity in semi-arid and densely populated cities such as Mashhad, Iran, these findings suggest that greywater reuse, combined with selected microorganisms and biochar, can serve as a supplementary water source for irrigating green wall systems. The integrated application of phytoremediation and bioremediation may help reduce environmental risks associated with untreated wastewater while supporting building-scale green infrastructure.

Overall, this study provides evidence that the combined use of cyanobacteria, mycorrhizae, and biochar – particularly the treatments C0.4–M10–B0 and C0–M10–B5 for plant growth, and C0.4–M10–B0 and C0.4–M5–B5 for greywater treatment – has potential to support greywater-irrigated green wall systems. However, as the findings are based on a specific experimental scale and local conditions, further long-term studies under diverse environmental and operational settings are

Author Contribution Declaration

Atefeh Zarei: Field and Experimental works, Software, Investigation, Data curation, Writing–review & editing. Zahra Karimian**:** Writing–original draft, Investigation, Project administration, Software, Data curation and Writing–review & editing. Zahra Meghdari: Experimental works. Ava Heidari: Conceptualization and study design

Funding Declaration

This study was supported by a grant from Ferdowsi University of Mashhad-Iran [N./ 55591].

Data Availability Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Abd-ur-Rehman, H. M., Deletic, A., Zhang, K., & Prodanovic, V. (2022). The comparative performance of lightweight green wall media for the removal of xenobiotic organic compounds from domestic greywater. *Water Research*, 221, 118774. https://doi.org/10.1016/j.watres.2022.118774

Addo-Bankas, O., Zhao, Y., Vymazal, J., Yuan, Y., Fu, J., & Wei, T. (2021). Green walls: A form of constructed wetland in green buildings. *Ecological Engineering*, 169, 106321.https://doi.org/10.1016/j.ecoleng.2021.106321

Ahmad, S., Zhai, X., Wang, M., Shi, Y., Chen, Y., Liang, Q., He, B., & Wen, R. (2024). Biochar amendments improve soil functionalities, microbial community and reduce Pokkah boeng disease of sugarcane. *Chemical and Biological Technologies in Agriculture*, 11, 28. https://doi.org/10.1186/s40538-024-00546-4

Ahsan, M., Younis, A., Saeed, M. F., Jamal, A., Shaheen, M. R., Tariq, U., et al. (2026). *Journal of Horticultural Science and Biotechnology,* 101(2), 321–340. https://doi.org/10.1080/14620316.2025.2538508

Alharbi, K., Hafez, E. M., El-Dein Omara, A., & Nehela, Y. (2023). Composted bagasse and/or cyanobacteria-based biostimulants maintain barley growth and productivity under salinity stress. *Plants*, 12, 1827. https://doi.org/10.3390/plants12091827

Alizadeh, Z., Heidari, P., & Asghari, H. R. (2025). Exploring the influence of symbiosis between arbuscular mycorrhizal fungi and beans on potassium uptake and the activity of AKT and HKT genes. *Scientific Reports*, 15, 19169. https://doi.org/10.1038/s41598-025-04385-7

Álvarez, C., Jiménez-Ríos, L., Iniesta-Pallarés, M., Jurado-Flores, A., Molina-Heredia, F. P., Ng, C. K. Y., & Mariscal, V. (2023). Symbiosis between cyanobacteria and plants: From molecular studies to agronomic applications. *Journal of Experimental Botany*, 74, 6145–6157. https://doi.org/10.1093/jxb/erad278

Anangadan, S. M., Pradhan, S., Saththasivam, J., McKay, G., & Mackey, H. R. (2024). Evaluation of greywater as a sustainable source of irrigation for ornamental crops in green walls—A study of plant and soil using *Ruellia tuberosa*. *Sustainability*, 16, 1183. https://doi.org/10.3390/su16031183

Bakheet, B., Prodanovic, V., Deletic, A., & McCarthy, D. (2020). Effective treatment of greywater via green wall biofiltration and electrochemical disinfection. *Water Research*, 185, 116228. https://doi.org/10.1016/j.watres.2020.116228

Bello, A. S., Ben-Hamadou, R., Hamdi, H., Saadaoui, I., & Ahmed, T. (2021). Application of cyanobacteria (*Roholtiella* sp.) liquid extract for the alleviation of salt stress in bell pepper (*Capsicum annuum* L.) plants grown in a soilless system. *Plants*, 11, 104. https://doi.org/10.3390/plants11010104

Bhatt, B., Gupta, S. K., Mukherjee, S., & Kumar, R. (2024). A comprehensive review on biochar against plant pathogens: Current state-of-the-art and future research perspectives. *Heliyon*, 10. 10.1016/j.heliyon.2024.e37204

Boano, F., Caruso, A., Costamagna, E., Fiore, S., Demichelis, F., Galvão, A., Pisoeiro, J., Rizzo, A., & Masi, F. (2020). Assessment of the treatment performance of an open-air green wall fed with graywater under winter conditions. *ACS ES&T Water*, 1, 595–602. https://doi.org/10.1021/acsestwater.0c00117

Bradstreet, R. B. (1954). Kjeldahl method for organic nitrogen. *Analytical Chemistry*, *26*(1), 185-187. https://doi.org/10.1021/ac60085a028

Choque-Quispe, Y., Solano-Reynoso, A. M., Choque-Quispe, D., Ligarda-Samanez, C. A., Ramos-Pacheco, B. S., Flores-Ccorisapra, Y., & Carhuarupay-Molleda, Y. F.

(2025). Optimization of the flocculating capacity of a new micro-atomization extract from a *Nostoc* cyanobacterium species from the High Andes. *Chemical Engineering Journal Advances*, 100777. https://doi.org/10.1016/j.ceja.2025.100777

Costamagna, E., Rizzo, A., Fiore, S., & Boano, F. (2025). Resilience to flow variability of an open-air green wall for greywater treatment. *Journal of Environmental Management*, 380, 125114. https://doi.org/10.1016/j.jenvman.2025.125114

Deja-Sikora, E., Kowalczyk, A., Trejgell, A., Szmidt-Jaworska, A., Baum, C., Mercy, L., & Hrynkiewicz, K. (2020). Arbuscular mycorrhiza changes the impact of potato virus Y on growth and stress tolerance of *Solanum tuberosum* L. in vitro. *Frontiers in Microbiology*, 10, 2971. https://doi.org/10.3389/fmicb.2019.02971

Diaper, C., Toifl, M., & Storey, M. (2008). Greywater technology testing protocol. In CSIRO: water for a healthy country national research flagship. Canberra, Australia: CSIRO Australia.

Dou, W. Q., He, H., Song, W. P., Wang, S. G., & Dai, D. W. (2019). Treatment of Simulated Saline Wastewater from the Coal Chemical Industry Using Ecological Floating Beds Enhanced with Arbuscular Mycorrhiza. *Huan Jing ke Xue= Huanjing Kexue*, *40*(2), 761-767. https://doi.org/10.13227/j.hjkx.201811028

Dou, W.-Q., He, H., Song, W.-P., Wang, S.-G., & Dai, D.-W. (2019). Treatment of simulated saline wastewater from the coal chemical industry using ecological floating beds enhanced with arbuscular mycorrhiza. *Huanjing Kexue*, 40, 761–767. https://doi.org/10.13227/j.hjkx.201811028

Elakbawy, W. M., Shanab, S. M., & Shalaby, E. A. (2022). Enhancement of plant growth regulators production from microalgae cultivated in treated sewage wastewater. *BMC Plant Biology*, 22, 377. https://doi.org/10.1186/s12870-022-03764-w

Eregno, F. E., Moges, M. E., & Heistad, A. (2017). Treated greywater reuse for hydroponic lettuce production in a green wall system: Quantitative health risk assessment. *Water*, 9, 454. https://doi.org/10.3390/w9070454

European Union. (2020). Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse. *Official Journal of the European Union*, L177, 32–55.

Farghaly, F. A., Nafady, N. A., & Abdel-Wahab, D. A. (2022). The efficiency of arbuscular mycorrhiza in increasing tolerance of *Triticum aestivum* L. to alkaline stress. *BMC Plant Biology*, 22, 490. https://doi.org/10.1186/s12870-022-03790-8

Farhaoui, A., Taoussi, M., Laasli, S.-E., Legrifi, I., El Mazouni, N., Meddich, A., Hijri, M., & Lahlali, R. (2025). Arbuscular mycorrhizal fungi and their role in plant disease control: A state-of-the-art. *The Microbe*, 100438. https://doi.org/10.1016/j.microb.2025.100438

Feng, Z., Liu, X., Qin, Y., Feng, G., Zhou, Y., Zhu, H., & Yao, Q. (2023). Cooperation of arbuscular mycorrhizal fungi and bacteria to facilitate the host plant growth dependent on soil pH. *Frontiers in Microbiology*, 14, 1116943. https://doi.org/10.3389/fmicb.2023.1116943

Gao, Z.-W., Ding, J., Ali, B., Nawaz, M., Hassan, M. U., Ali, A., Rasheed, A., Khan, M. N., Ozdemir, F. A., & Iqbal, R. (2024). Putting biochar in action: A black gold for efficient mitigation of salinity stress in plants—Review and future directions. *ACS Omega*, 9, 31237–31253. https://doi.org/10.1021/acsomega.3c07921

Gilboa, Y., & Friedler, E. (2008). UV disinfection of RBC-treated light greywater effluent: Kinetics, survival and regrowth of selected microorganisms. *Water Research*, 42(4–5), 1043–1050. https://doi.org/10.1016/j.watres.2007.09.027

Grzesik, M., Romanowska-Duda, Z., & Kalaji, H. M. (2017). Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants under limited synthetic fertilizers application. *Photosynthetica*, 55, 510–521. https://doi.org/10.1007/s11099-017-0716-1

Gul-Lalay, N., Ullah, S., Shah, S., Jamal, A., Saeed, M. F., Mihoub, A., ... & Radicetti, E. (2024). Combined effect of biochar and plant growth-promoting rhizbacteria on physiological responses of canola (*Brassica napus* L.) subjected to drought stress. Journal of Plant Growth Regulation, 43(6), 1814-1832. https://doi.org/10.1007/s00344-023-11219-1

Gupta, S., Thokchom, S. D., & Kapoor, R. (2021). Arbuscular mycorrhiza improves photosynthesis and restores alteration in sugar metabolism in *Triticum aestivum* L. grown in arsenic contaminated soil. *Frontiers in Plant Science*, 12, 640379. https://doi.org/10.3389/fpls.2021.640379

Havre, G. N. (1961). The flame photometric determination of sodium, potassium and calcium in plant extracts with special reference to interference effects. *Analytica Chimica Acta*, *25*(6), 557-566. https://doi.org/10.1016/0003-2670(61)80134-7

Hawkins, H.-J., Cargill, R. I. M., Van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., Soudzilovskaia, N. A., & Kiers, E. T. (2023). Mycorrhizal mycelium as a global carbon pool. *Current Biology*, 33, R560–R573. 10.1016/j.cub.2023.02.027

He, P., Zhang, H., Duan, H., Shao, L., & Lü, F. (2020). Continuity of biochar-associated biofilm in anaerobic digestion. *Chemical Engineering Journal*, 390, 124605. https://doi.org/10.1016/j.cej.2020.124605

Hewitt, E. J., & Dickes, G. J. (1961). Spectrophotometric measurements on ascorbic acid and their use for the estimation of ascorbic acid and dehydroascorbic acid in plant tissues. *Biochemical Journal*, *78*(2), 384. : https://doi.org/10.1042/bj0780384

Jaramillo, M. F., & Restrepo, I. (2017). Wastewater reuse in agriculture: A review about its limitations and benefits. *Sustainability*, 9, 1734. https://doi.org/10.3390/su9101734

Jing, H., Zhou, N., Tantai, Y., & Zhao, Y. (2025). Artificial cyanobacteria crusts can improve soil fertility and plant growth in a semi-arid area, northern China. *Journal of Arid Land*, 17, 808–822. https://doi.org/10.1007/s40333-025-0019-5

Jozay, M., Zarei, H., Khorasaninejad, S., & Miri, T. (2024). Exploring the impact of plant growth-promoting bacteria in alleviating stress on *Aptenia cordifolia* subjected to irrigation with recycled water in multifunctional external green walls. *BMC Plant Biology*, 24, 802. https://doi.org/10.1186/s12870-024-05511-9

Karimian, Z., Hozhabralsadat, M. S., Heidari, A. (2023). Evaluating the ability of green roof plants in capturing air pollutants using biogas-digestate: Exploring physiological, biochemical, and anatomical characteristics. *Environmental Pollution*, 333, 122071. https://doi.org/10.1016/j.envpol.2023.122071

Khoshyomn, S., Heidari, A., Farzam, M., Shariatmadari, Z., & Karimian, Z. (2024). Integrated approaches for heavy metal–contaminated soil remediation: Harnessing the potential of *Paulownia elongata* SY Hu, *Oscillatoria* sp.,

arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*), and iron nanoparticles. *Environmental Science and Pollution Research*, 31, 19595–19614. https://doi.org/10.1007/s11356-024-32380-4

Komárek, J. and Anagnostidis, K. (2005). Cyanoprokaryota.-2. Teil/ 2nd part: Oscillatoriales. In: Büdel, B., Gartner, G., Krienitz, L. and Schagerl, M., Eds., Süßwasserflora von Mitteleuropa 19/2, Elsevier, Heidelberg, 759.

Li, F., Wichmann, K., & Otterpohl, R. (2009). Review of the technological approaches for grey water treatment and reuses. *Science of the Total Environment*, 407, 3439–3449. https://doi.org/10.1016/j.scitotenv.2009.02.004

Li, X., Yao, T., Huang, X., Li, P., Du, S., Wang, W., ... & Shao, X. (2022). Biochar increases rice yield by improving root morphological and root physiological functions in heavily saline-sodic paddy soil of northeast China. *BioResources*, *17*(1), 1241. https://doi.org/10.15376/biores.17.1.1241-1256

Lubbe, E., Rodda, N., & Naidoo, S. (2016). Effects of greywater irrigation on germination, growth and photosynthetic characteristics in selected African leafy vegetables. *Water SA*, 42(2), 203–212. 10.4314/wsa.v42i2.04

Lüttge, U. (2004). Ecophysiology of crassulacean acid metabolism (CAM). *Annals of botany*, 93(6), 629-652. https://doi.org/10.1093/aob/mch087

Ma, W.-Y., Qin, Q.-Y., Zou, Y.-N., Kuča, K., Giri, B., Wu, Q.-S., Hashem, A., Al-Arjani, A. B. F., Almutairi, K. F., & Abd-Allah, E. F. (2022). Arbuscular mycorrhiza induces low oxidative burst in drought-stressed walnut through activating antioxidant defense systems and heat shock transcription factor expression. *Frontiers in Plant Science*, 13, 1089420. https://doi.org/10.3389/fpls.2022.1089420

Mahran, R. E., Razek, T., & Mohamed, S. M. (2024). Phycoremediation of greywater using *Spirulina platensis* and the potential applications of the produced biomass. *Journal of Environmental Science*, 53, 3277–3303. 10.21608/jes.2024.311199.1838

Manso, M., Teotónio, I., Silva, C. M., & Cruz, C. O. (2021). Green roof and green wall benefits and costs: A review of the quantitative evidence. *Renewable and Sustainable Energy Reviews*, 135, 110111. https://doi.org/10.1016/j.rser.2020.110111

Massa, A., Santos, É., Martins, D., Azevedo, J., Reimão, M., Almeida, A., Azevedo, R., Pinto, E., Vasconcelos, V., & Campos, A. (2024). Toxic and non-toxic cyanobacterial biomass as a resource for sustainable agriculture: A lettuce cultivation experiment. *Environmental Research*, 262, 119942. https://doi.org/10.1016/j.envres.2024.119942

Mekki, A., Fki, F., Kchaou, M., & Sayadi, S. (2015). Short-term effects of gray water on a Mediterranean sandy soil. *CLEAN – Soil, Air, Water*, 43, 754–760. https://doi.org/10.1002/clen.201400049

Mihoub, A., Daddi Bouhoun, M., Naeem, A., & Saker, M. L. (2017). Low-molecular weight organic acids improve plant availability of phosphorus in different textured calcareous soils. Archives of Agronomy and Soil Science, 63(7), 1023-1034. https://doi.org/10.1080/03650340.2016.1249477

Moradi-Choghamarani, F., Moosavi, A. A., & Sepaskhah, A. R. (2024). Sugarcane bagasse-derived biochar potential to improve soil structure and water availability in texturally different soils. *Scientific Reports*, 14, 26541. https://doi.org/10.1038/s41598-024-77700-3

Moradi-Choghamarani, F., Moosavi, A. A., & Sepaskhah, A. R. (2024). Sugarcane bagasse derived biochar potential to improve soil structure and water availability in texturally different soils. *Scientific Reports*, *14*(1), 26541. https://doi.org/10.1038/s41598-024-77700-3

Munawar, M. A., Khoja, A. H., Naqvi, S. R., Mehran, M. T., Hassan, M., Liaquat, R., & Dawood, U. F. (2021). Challenges and opportunities in biomass ash management and its utilization in novel applications. *Renewable and Sustainable Energy Reviews*, 150, 111451. https://doi.org/10.1016/j.rser.2021.111451

Muñoz-Rojas, M., Román, J. R., Roncero-Ramos, B., Erickson, T. E., Merritt, D. J., Aguila-Carricondo, P., & Cantón, Y. (2018). Cyanobacteria inoculation enhances carbon sequestration in soil substrates used in dryland restoration. *Science of the Total Environment*, 636, 1149–1154. https://doi.org/10.1016/j.scitotenv.2018.04.265

Murphy, J. A. M. E. S., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, *27*, 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

Neale, M. R., Sharvelle, S., Arabi, M., Dozier, A., & Goemans, C. (2020). Assessing tradeoffs of strategies for urban water conservation and fit-for-purpose water. *Journal of Hydrology X*, 8, 100059. https://doi.org/10.1016/j.hydroa.2020.100059

Noreen, A., Jahan, S., Kamran, A., Hashem, A., Avila-Quezada, G. D., & Abd-Allah, E. F. (2025). Physiological and yield responses of *Camelina sativa* to modified biochar under drought stress. *Scientific Reports*, 15(1), 38764. https://doi.org/10.1038/s41598-025-22618-7

Ouhsassi, M., Khay, E. O., Bouyahya, A., El Ouahrani, A., Harsal, A. E., & Abrini, J. (2020). Evaluation of self-purifying power of cyanobacteria *Pseudanabaena galeata*: Case of dairy factory effluents. *Applied Water Science*, 10(7), 1–10. https://doi.org/10.1007/s13201-020-01270-8

Patterson, Brian D, Elspeth A MacRae, and Ian B Ferguson. 1984. 'Estimation of hydrogen peroxide in plant extracts using titanium (IV)', *Analytical biochemistry*, 139, 92-487. https://doi.org/10.1016/0003-2697(84)90039-3

Pellitier, P. T., Ibáñez, I., Zak, D. R., Argiroff, W. A., & Acharya, K. (2021). Ectomycorrhizal access to organic nitrogen mediates CO_2 fertilization response in a dominant temperate tree. *Nature Communications*, 12, 5403. https://doi.org/10.1038/s41467-021-25652-x

Prodanovic, V., Wang, A., & Deletic, A. (2019). Assessing water retention and correlation to climate conditions of five plant species in greywater treating green walls. *Water Research*, 167, 115092. https://doi.org/10.1016/j.watres.2019.115092

Pu, C., Yang, G., Li, P., Ge, Y., Garran, T. A., Zhou, X., Shen, Y., Zheng, H., Chen, M., & Huang, L. (2022). Arbuscular mycorrhiza alters the nutritional requirements in *Salvia miltiorrhiza* and low nitrogen enhances the mycorrhizal efficiency. *Scientific Reports*, 12, 19633. https://doi.org/10.1038/s41598-022-17121-2

Santini, G., Biondi, N., Rodolfi, L., & Tredici, M. R. (2021). Plant biostimulants from cyanobacteria: An emerging strategy to improve yields and sustainability in agriculture. *Plants*, 10, 643. https://doi.org/10.3390/plants10040643

Shah, T., Irshad, M. K., Javed, W., Sheraz, M., Muhmood, A., Noman, A., & Shang, J. (2024). Potential of iron oxide-modified biochar in simultaneous mitigation of

lead and nickel toxicity in wheat (*Triticum aestivum* L.). *South African Journal of Botany*, 175, 32–43. https://doi.org/10.1016/j.sajb.2024.10.014

Shahzad, A. S., Younis, U., Naz, N., Danish, S., Syed, A., Elgorban, A. M., Eswaramoorthy, R., Huang, S., & Battaglia, M. L. (2023). Acidified biochar improves lead tolerance and enhances morphological and biochemical attributes of mint in saline soil. *Scientific Reports*, 13, 8720. https://doi.org/10.1038/s41598-023-36018-2

Singh, S. K., & Rao, D. N. (1983, November). Evaluation of plants for their tolerance to air pollution. In *Proceedings of symposium on air pollution control* (Vol. 1, No. 1, pp. 218-224). Indian Association for Air Pollution Control.

Spencer, J. (2021). The sustainable development goals. In *Design for global challenges and goals* (pp. 12-25). Routledge. https://doi.org/10.4324/9781003099680

Sulaiman, M. O., Mackey, H. R., Al-Ghouti, M. A., Saththasivam, J., & Abu-Dieyeh, M. H. (2025). Perspectives of utilizing greywater in agricultural irrigation with a special reference to vegetated wall agrosystems. *Water*, 17, 103. https://doi.org/10.3390/w17010103

Sun, Z., Hu, Y., Shi, L., Li, G., Pang, Z., Liu, S., Chen, Y., & Jia, B. (2022). Effects of biochar on soil chemical properties: A global meta-analysis of agricultural soil. *Plant, Soil and Environment*, 68, 272–289. https://doi.org/10.17221/522/2021-PSE

Sutar, S., Patil, P., & Jadhav, J. (2022). Recent advances in biochar technology for textile dyes wastewater remediation: A review. *Environmental Research*, 209, 112841. https://doi.org/10.1016/j.envres.2022.112841

Tian, K., Lintern, A., Palacios, Y. M., & Winfrey, B. (2024). A mycorrhizal inoculation strategy that improves the morphology of biofilter plants. *Blue-Green Systems*, 6, 247–263. https://doi.org/10.2166/bgs.2024.025

United Nations Environment Programme. (2010). *Sick water? The central role of wastewater management in sustainable development*.

Van de Walle, A., Kim, M., Alam, M. K., Wang, X., Wu, D., Dash, S. R., & Kim, J. (2023). Greywater reuse as a key enabler for improving urban wastewater management. *Environmental Science and Ecotechnology*, 16, 100277. https://doi.org/10.1016/j.ese.2023.100277

Whitton, B. A. (Ed.). (2012). Ecology of cyanobacteria II: their diversity in space and time. Springer Science & Business Media. https://doi.org/10.1007/978-94-007-3855-3

Wu, Y., Chen, C., & Wang, G. (2024). Inoculation with arbuscular mycorrhizal fungi improves plant biomass and nitrogen and phosphorus nutrients: A meta-analysis. *BMC Plant Biology*, 24, 960. https://doi.org/10.1186/s12870-024-05638-9

Zhu, Z., Zhang, Y., Tao, W., Zhang, X., Xu, Z., & Xu, C. (2025). The biological effects of biochar on soil's physical and chemical characteristics: A review. *Sustainability*, 17, 2214. https://doi.org/10.3390/su17052214