

# Assessment of Aesthetic Quality of Outdoor Green Walls: Investigating the Impact of Plant Growth-Promoting Bacteria and Recycled Water Irrigation

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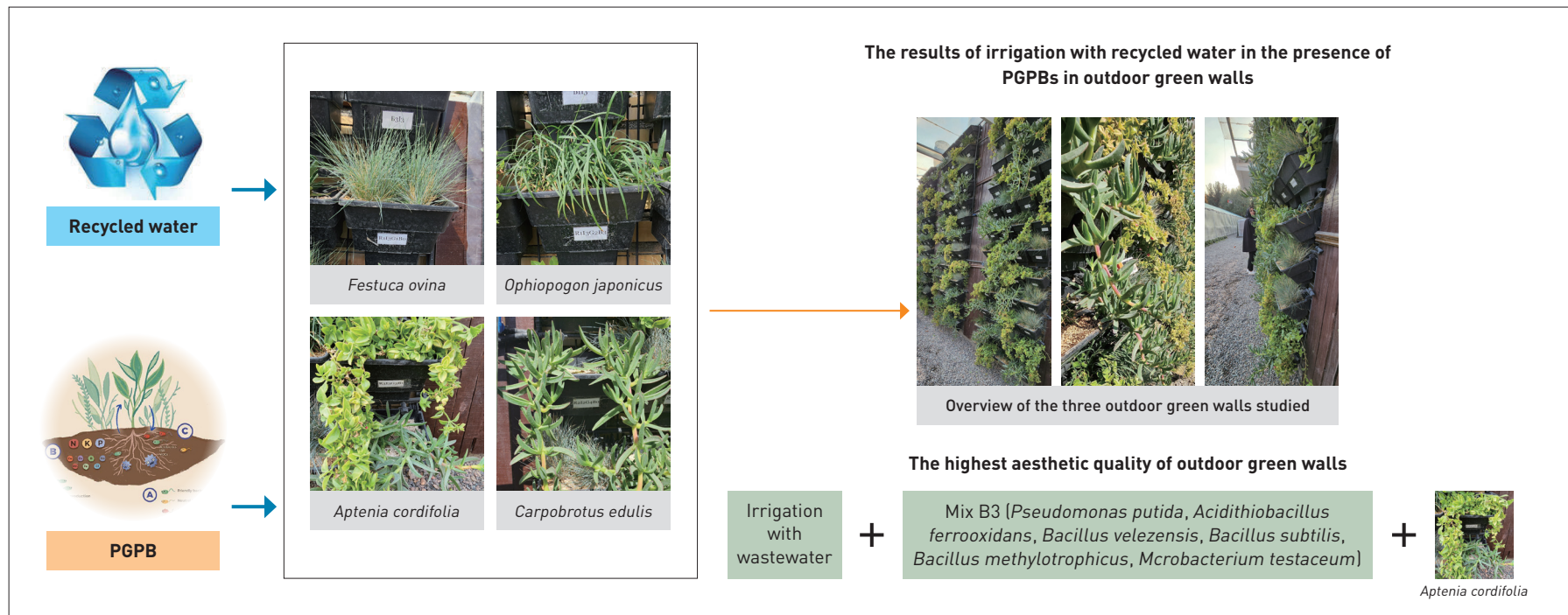
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## GRAPHICAL ABSTRACT



## ABSTRACT

Outdoor green walls are gaining popularity for enhancing building aesthetics and promoting healthy and sustainable urban environment. However, concerns about their high water consumption in the context of ongoing water crises highlight the need for alternative water sources, such as recycled water. This research investigated the effects of recycled water irrigation and plant growth-promoting bacteria (PGPB) on four plant species

for green walls in Mashhad, Iran: *Festuca ovina*, *Ophiopogon japonicus*, *Aptenia cordifolia*, and *Carpobrotus edulis*. Over nine months (March to December 2022), researchers conducted experiments using three water types (graywater, wastewater, and urban water) as the main factor and four bacterial treatments (Mix B1, Mix B2, Mix B3, and a control) as the sub-factor, with three replications of split-plot layout based on a randomized complete

block design. Results showed significant differences in aesthetic qualities, with optimal visual quality achieved by applying Mix B2 or B3 with wastewater for *Aptenia cordifolia*, *Carpobrotus edulis*, and *Festuca ovina*; and *Ophiopogon japonicus* performed best with Mix B1 and graywater. The research highlights the potential of using *Aptenia cordifolia* with Mix B3 and wastewater irrigation for outdoor green walls, especially in arid and semi-arid climates.

## KEYWORDS

Graywater; Plant Growth-Promoting Bacteria; Green Wall; Sustainable Green Space; Wastewater Consumption

## HIGHLIGHTS

- Finds that plant growth-promoting bacteria and recycled water can enhance the aesthetic quality of green walls
- Finds that plant growth-promoting bacteria has positive effects on plant growth, flowering, and overall coverage
- Recommends plant, bacteria strains, and irrigation combination for green walls under arid and semi-arid climates

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## 1 Introduction

Combining the aesthetic appeal of landscaping with the environmental benefits of vertical gardens help improve urban environments. When properly designed and maintained, these vertical landscapes can transform the visual appearance of buildings, improve urban aesthetics, enhance biodiversity, and contribute to oxygen production and fresh food growth in the surrounding environment<sup>[1]~[5]</sup>. Existing research has shown that plant coverage on vertical surfaces effectively absorbs particulates and carbon dioxide<sup>[6]</sup>, which can significantly improve overall air quality in urban areas<sup>[7]</sup>.

Vertical gardens allow humans to recreate a life system very similar to natural environments, as adding nature back to places where it has been removed<sup>[8]</sup>. As a form of green walls, vertical gardens are categorized into green facades and living walls<sup>[9]</sup>.

Green facades use annual climbing plants that creep up or hang down the walls. Living walls utilize vertical structures to support plant growth and display diverse aesthetics on the wall by selecting different plants, varied design patterns, colors, textures, shapes, and leaf densities. The idea of living wall is easily compatible with the concept of hydroponic systems by providing continuous irrigation and adequate nutrition.

Plants in green walls have architectural, aesthetic, engineering, and climate control functions<sup>[10]</sup>. Examining plant growth, color, flowering, leaf structure, and root growth is very important for the stability of the wall<sup>[11]</sup>. However, vertical growth systems often face several challenges, including stability, high initial capital, the need for automated irrigation equipment, intensive maintenance, and limited vegetation types because of uneven lighting<sup>[12][13]</sup>. Particularly, plant establishment, survival, and persistence in green walls pose difficulties, especially in environments with climate and growth constraints and stressful conditions such as high temperatures, moisture deficiency, and nutrient shortages. Studies indicate that the application of plant growth-promoting bacteria (PGPB) can significantly enhance plant survival and increase growth and overall performance in green walls<sup>[14][15]</sup>. Luana Alves de Andrade et al. demonstrated that specific bacterial strains such as *Pseudomonas* spp. and *Bacillus* sp. have the ability to promote root growth and nutrient absorption, and improve plant performance<sup>[16]</sup>. Properly integrating these bacteria into the design and maintenance of green walls can improve their flexibility, durability, and ecological performance.

Considering stressful conditions, the type of irrigation water also plays a vital role in the success of green walls<sup>[17]</sup>. In addition, the limitation of fresh water for irrigation, particularly in countries located in arid regions, is a global challenge. To address this situation, the reuse of treated wastewater can be beneficial for the environment and have favorable psychological effects on society, contributing to sustainable urban development<sup>[17][18]</sup>. Here, wastewater refers to the water resulting from human activities, e.g., domestic sewage, industrial sewage, and potable water discharged from sanitation systems<sup>[19]</sup>. In water-scarce regions, these resources can be used to supplement or replace fresh water for applications that do not require drinking water quality, particularly agricultural irrigation<sup>[20]</sup>. This approach offers an important opportunity to bridge the gap between water demand and supply, with the potential to meet over 13% of total water needs if properly treated and recycled<sup>[21]</sup>.

Graywater, a sort of recycled water, accounts for approximately 54% to 86% of the wastewater generated by a

household<sup>[22]</sup>. Its composition varies depending on the source, including those from kitchen, bathroom, handwash basin, and laundry. Using recycled water for irrigation not only helps conserve water resources<sup>[23]</sup>, but also enhances plant health by substituting chemical fertilizers<sup>[24][25]</sup>. Meanwhile, applying metal-resistant PGPB has been proven to be an efficient way to improve biomass accumulation and plant tolerance to heavy metals in recycled water<sup>[26]</sup>. However, despite all these advantages, improper management may also pose public health risks from pathogens, heavy metal accumulation, and soil salinization, thus causing public resistance. These issues require careful planning that considers agricultural and horticultural factors (e.g., PGPB), design factors, and plant factors in soil filtration<sup>[27]</sup> for pollutants removal.

Although much research has focused on the environmental and functional benefits of green walls, few studies have investigated how factors such as bacterial treatments and recycled water irrigation impact plant performance and aesthetic quality, especially their combined effects in green walls, which remain largely unexplored. To address this research gap, this study specifically investigates the impact of PGPB and recycled water irrigation on plant aesthetics, including leaf health, color, density, and vibrancy of vegetation, aiming to provide new insights into how to apply sustainable practices of green walls.

## 2 Materials and Methods

### 2.1 Experiment Site

The research was carried out in Mashhad, the second-largest and most populous city situated in northeastern Iran. Mashhad experiences a semi-arid climate characterized by cold winters and hot, dry summers. The average annual precipitation hovers around 255 mm. The annual temperatures range from 4°C to 22°C, with a reported relative humidity of approximately 40%<sup>[28]</sup>. Specifically,

the experiment site was on a 15-meter wall in the outdoor area of the Pirsha Greenhouse located north of Mashhad.

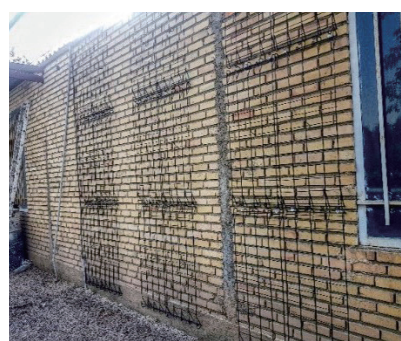
### 2.2 Green Wall System Design

In this experiment, vertical planting panels constructed with steel wire mesh were installed on a wall facing east-west without any shading. The vertical planting system utilized in this project is the Elmich system, an original Malaysian design<sup>[29]</sup>. The wall design adopted polypropylene plastic pots, measuring 19 cm in width, 19 cm in length, and 21 cm in height. Taking into account the types of ornamental plants and the specified treatments of PGPB and irrigation, this experiment set 3 replications (R1, R2, and R3). Each replication includes 3 walls, each of which comprises 2 vertical panels. Within each panel, 2 columns and 4 rows of experiment units were arranged. Each experiment unit consisted of 1 pot with 2 plants of the same species. This experiment, in total, encompassed 144 experimental units (Fig. 1).

This research was conducted as 4 separate experiments for selected ornamental species, in the form of split plots based on a completely randomized block design in 2022 (Fig. 2). The culture medium (substrate) used in all experimental units was the same, consisting of 25% cocopeat, 5% vermicompost, 55% perlite, 10% vermiculite, and 5% zeolite. The percentage of organic matter in the culture medium was 20%. This amount aligns with the recommendation by *Green Roof Guidelines* from FLL<sup>①[30]</sup>.

① The *Green Roof Guidelines* was published by Landscape Development and Landscaping Research Society e. V. (FLL), Germany, providing internationally recognized guidance for green roof planning, construction, and maintenance.

1. The experimental design and layout. The photo on the left depicts experimental units with a drainage layer made of Leica material at the bottom. The photo on the right shows the 3 walls, as of 6 vertical panels.
2. Views of the experiment's green walls (photographed in spring, summer, and autumn, 2022, from left to right).



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### 2.2.1 Irrigation Water Treatments

Considering the quality of irrigation water the main factor, three levels of recycled water treatments were applied: graywater (I1) collected from rainwater and a special dual sink for washing fruits and vegetables; wastewater (I2) sourced from the sewage effluent from domestic sewage, industrial wastewater, and water discharged from sanitation systems that originated and discharged into the Kashfroud river in Mashhad; and urban water (I3, control). The waters utilized were gathered in three tanks and administered through drip irrigation system. The irrigation rate was set at 70% of the field capacity of soil, adjusted according to the discharge rate of the drippers.

### 2.2.2 Plant Growth-Promoting Bacterial Treatments

This research included 4 biological strains of bacteria: Mix B1 (*Pseudomonas fluorescens*, *Azospirillum lipoferum*, *Thiobacillus thioparus*, and *Azotobacter chroococcum*), Mix B2 (*Paenibacillus polymyxa*, *Pseudomonas fildensis*, *Bacillus subtilis*, *Achromobacter xylosoxidans*, and *Bacillus licheniform*), Mix B3 (*Pseudomonas putida*, *Acidithiobacillus ferrooxidans*, *Bacillus velezensis*, *Bacillus subtilis*, *Bacillus methylotrophicus*, *Microbacterium testaceum*), and non-inoculated controls (B0). The bacteria were strategically inoculated around the plant root perimeter within the substrate. Each plant received 20 ml of the biofertilizer. The bacteria utilized in this experiment were procured from the Soil Biology and Biotechnology Laboratory at the Golestan Agricultural and

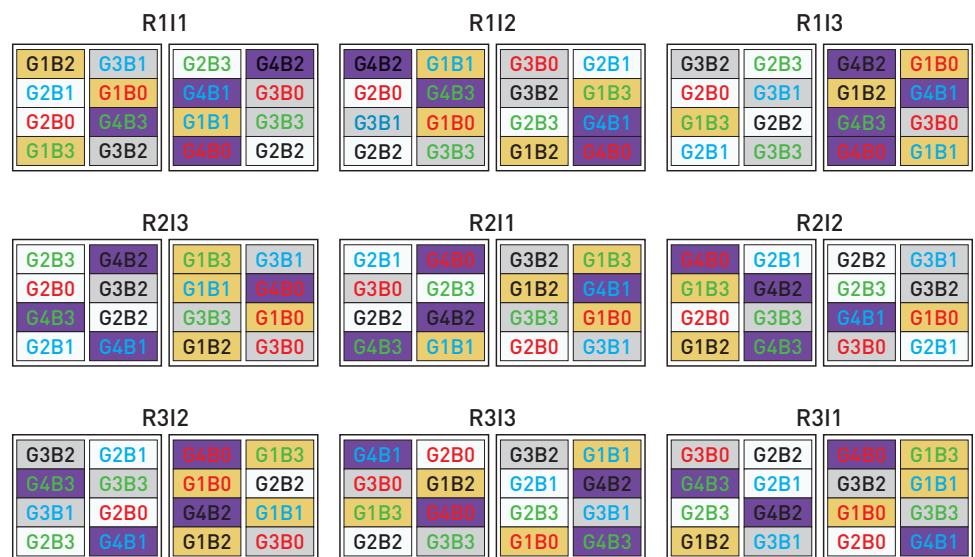
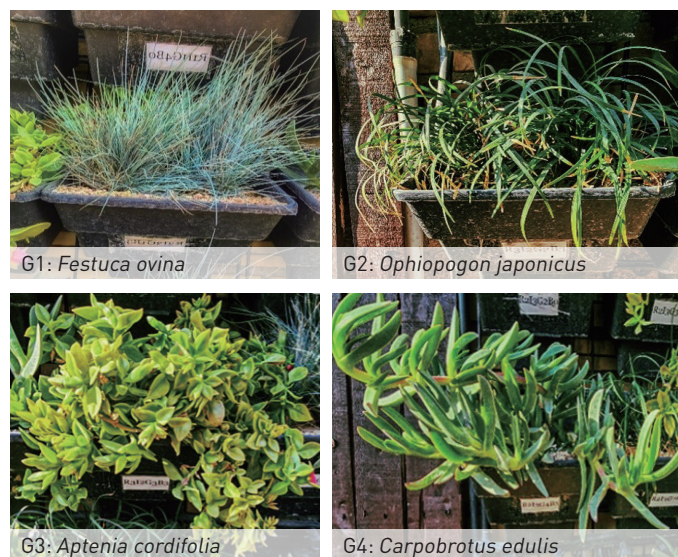
Natural Resources Research Center LBSG, specifically identified by isolate number 041011 in the laboratory bank. The bacteria were extracted from the rhizosphere of agronomy plants like soybean and wheat<sup>[31]</sup>. Except for the non-inoculated controls, the substrates around each plant specimen were inoculated with 20 ml of bacterial suspension in the irrigation water to obtain a bacterial concentration of 108 CFU/ml. The biofertilizer was injected evenly onto the substrate surface around each plant's root zone using a syringe, two weeks after planting. The plants were cultivated in March 2022.

To prevent the leaching of the inoculated bacteria from the planting beds, the experimental units were irrigated up to 80% of the field capacity every 7 days in spring, every 3 days in summer, and every 10 days in winter (maintaining 300 ml for each plant). To ensure maintaining the bacteria within the soil system, irrigation was controlled to prevent drainage flow.

### 2.2.3 Selected Ornamental Hyperaccumulator Plants

The thoughtful selection of plant diversity, as evidenced in the study of Nektarios et al.<sup>[32]</sup>, facilitates not only survival and growth but also flowering performance. There are documented cases where crassulacean acid metabolism (CAM) plants such as succulents, exhibit resilience in water-limited conditions. In this study, two plants from the CAM species and two grass species were specifically chosen to achieve a judicious plant diversity, catering to both aesthetic considerations and overall ecological performance (Figs. 3, 4). Additionally, for the first time, an attempt was made to

3. The appearance conditions of the four experimental plant species studied in the fall under B3 and I2 treatments.
4. Schematic diagram of the experiment.



challenge the cold-season grasses and their sustainability during the warm season, alongside CAM plants. The plants selected in this research are particularly effective for phytoremediation of pollution, and may have a stabilizing phytoremediation effect for areas contaminated by metals.

1) *Festuca ovina* (G1): It is a native evergreen grass consisting of a loosely tufted, soft yet firm clump of needle-like leaves 10 ~ 25 cm long, forming a singular mass<sup>[33]</sup>.

2) *Ophiopogon japonicus* (G2): It is an evergreen perennial plant, native to China, Vietnam, India, and Japan, and hardy and semi-hardy to cold, tolerating temperatures down to  $-5^{\circ}\text{C}$ <sup>[34]</sup>.

3) *Aptenia cordifolia* (G3): It is a succulent, perennial plant abundantly used in green spaces in southern Iran. This plant is resistant to heat and drought, with light pink, white, and red flowers that bloom throughout the year. Full sun exposure is needed<sup>[35]</sup>.

4) *Carpobrotus edulis* (G4): It is a succulent, creeping perennial plant, native to South Africa, and thrives in full sun exposure and extremely hot, dry conditions. It adapts to various soils but prefers well-drained conditions<sup>[36]</sup>.

## 2.3 Measurements

The aesthetic quality of the plants was evaluated once per month by four trained evaluators (two female and two male, who were familiar with horticulture and landscape) with the evaluation criteria proposed by Monterusso et al.<sup>[37]</sup>. Accordingly, the aesthetic quality of plants in each experimental unit was scored from 1 to 5, where 1 represents severely stressed and completely dry; 2 represents stressed, less than 50% of leaves contain green pigment; 3 represents mildly stressed, 50% of leaves have green pigment; 4 represents low stress, more than 50% of leaves appear healthy; and 5 represents no stress, all leaves appear healthy. At the end of the experiment, plants that were completely dry were reported as dead (Table 1).

It is worth mentioning that each month the aesthetic quality of both grasses were assessed by the same trained evaluators according to the National Turfgrass Evaluation Program (NTEP) guidelines<sup>[38]</sup>. It applied the visual grading system with scores ranging from 1 to 9. The aesthetic quality of the grass was evaluated with a personal estimation of visual attributes

**Table 1: Evaluation criteria for aesthetic quality of green wall plants**

Indicator	Score				
	1	2	3	4	5
Plant status	80% or more of the leaves fallen	Up to about 66% of the leaves fallen	Up to about 50% of the leaves fallen	80% or more of the plant is erect, monoecious, and with little dormancy	95% or more of the plant is static, uniform, attractive, very ornamental, and without dormancy
Leaf rolling	100% rolling	Up to about 75% rolling	Up to about 50% rolling	Up to about 25% rolling	No rolling
Landscape performance	<ul style="list-style-type: none"> <li>· No or very little ornamental value of the landscape appearance</li> <li>· Poor growth and shoot color, high rate of dormancy or disease/ insect damage</li> </ul>	<ul style="list-style-type: none"> <li>· Much less than the average landscape value and appearance</li> <li>· Very Poor vigor with major problems in plant growth or disease/insect damage appeared</li> </ul>	<ul style="list-style-type: none"> <li>· Below the average landscape value and appearance</li> <li>· Poor vigor with major problems in plant growth or disease/ insect damage appeared</li> </ul>	<ul style="list-style-type: none"> <li>· The appearance and value of the landscape is above the average level</li> <li>· Few problems in the appearance of plant growth and disease/ insect damage</li> </ul>	<ul style="list-style-type: none"> <li>· Outstanding ornamental value of the landscape appearance</li> <li>· Good vigor, shoot color and flowering, with very little or no disease/insect damage</li> </ul>
Flowering	No flowering	Up to about 25% flowering	Up to about 50% flowering	Up to about 75% flowering	Up to about 95% flowering

### NOTES

1. This research set standardized guidelines or pre-established benchmarks for plant health, growth, and aesthetics for evaluators to determine the "average" status and minimize personal bias.
2. Contents were sourced from Ref. [37].

**Table 2: Aesthetic quality indicators of grasses on the green walls**

Indicator	Definition	Measurement	Influence on quality/performance
<b>Establishment</b>	Proximity of tillers to the mother plant, promoting root and seedling growth	Closer tillers proximity means faster root formation	Better establishment leads to uniform, homogeneous grass cover
<b>Density</b>	Number of grass tillers per unit area, influenced by genetics and environment	High plant density correlates with closely spaced individuals in the growing area, referring to the distribution of <i>Cynodon dactylon</i> and <i>Agrostis stolonifera</i>	Higher density leads to more robust, resilience
<b>Color</b>	Grass surface's light reflection, indicating plant health and vigor	Green indicates healthy status, yellow presents nutrient deficiency or stress	Significantly impact the visual quality and reflect plant health
<b>Texture</b>	Leaf width categorized as fine-leaf (fine texture) or broad-leaf (coarse texture), affecting softness/roughness	Fine-leaf texture often correlates with higher density	Soft, fine texture leads to better grass quality
<b>Softness</b>	Touch to find out whether the plant is soft or not	Soft-leaf texture often correlates with higher density	Softness leads to better grass quality
<b>Thatch</b>	Layer of grass clippings between the soil surface and the green vegetation of the grass	Rated 1 ~ 9 (1 represents high thatch/low quality while 9 means low thatch/high quality)	High thatch negatively influences grass health

**NOTE**

Contents were sourced from Ref. [38].

including establishment, density, color, texture, softness, and thatch (Table2).

Except for the aforementioned indicators, additional measurements were necessary to quantify plant distribution and spatial growth patterns, providing a comprehensive assessment of plant performance in the green wall system. Shape, representing the overall quality of the plant, was graded by the evaluators based on factors such as visual appearance, uniformity, and plant vigor. Plant coverage, meaning the horizontal and vertical coverage of plants for each experimental unit, was measured using 20 cm × 20 cm quadrats. Each quadrat was divided into 100 compartments (2 cm × 2 cm). Survival percentage represents the percentage of plant survival days to the total days of the experiment<sup>[39]</sup>, which was measured at the end of the experiment. Weeds evaluated in the experimental units were monitored monthly with manual counting<sup>[40]</sup>. The substrate settlement, the reduction in substrate volume over time, was measured using a straight line from the surface of the substrate to the edge of the pot.

For statistical analysis, JMP 8 software was employed. Data analysis was conducted using analysis of variance (ANOVA) and mean comparison with Tukey's test at a significance level of at least 5%.

### 3 Results

#### 3.1 Mean Comparisons of Irrigation Water Types and Bacteria Strains on Plants

The ANOVA results (Table 3) for *Festuca ovina* revealed that all traits, except those related to texture, and the number of weeds, exhibited statistically significant differences. Regarding substrate settlement, only the simple effect of irrigation water showed a significant difference. In traits of softness, color, overall quality, and thatch, in addition to the simple effects of bacteria, the interaction effects of irrigation water and bacteria treatments were statistically significant.

According to ANOVA results (Table 4), the qualitative data of *Ophiopogon japonicus* suggested that the simple effects of irrigation water and bacteria were significantly different in the overall quality. Meanwhile, for establishment, density, and color, only the effects of various bacterial strains were found to be significant. In terms of plant coverage, softness, and substrate settlement, solely the simple effects of irrigation water were statistically significant. However, when considering softness, thatch, and number of weeds, not only were the simple effects of irrigation water and bacteria significant, but their interaction effects also

**Table 3: The ANOVA mean square results of different treatments on the aesthetic quality indicators of *Festuca ovina***

Sources of variation	df	Establishment	Density	Plant coverage	Softness	Texture	Color	Overall quality	Thatch	Number of weeds	Substrate settlement (cm)
Block	2	0.23	0.50	10,412.40	0.29	0.01	0.04	0.007	0.02	1.36	0.11
Irrigation	2	2.46ns	2.00ns	14,069.82ns	1.64ns	1.42ns	0.71ns	1.83ns	1.36ns	33.69ns	0.86**
Main factor error	4	0.37	0.98	12,325.10	0.60	1.26	0.44	0.51	0.52	9.11	0.04
Bacteria	3	1.52ns	0.39ns	12,977.23ns	1.07**	0.67ns	2.98**	2.39**	2.81**	4.62ns	0.21ns
Irrigation × bacteria	6	2.09**	1.28*	23,507.40*	0.64*	0.27ns	0.69*	2.81**	1.17*	6.62ns	0.85ns
Sub factor error	18	0.48	0.36	6,190.72	0.17	0.34	0.23	0.25	0.36	12.12	0.33

**NOTE**

\* indicates significance at the level of 5%; \*\* indicates significance at the level of 1%; ns means non-significant.

**Table 4: The ANOVA mean square results of different treatments on the aesthetic quality indicators of *Ophiopogon japonicus***

Sources of variation	df	Establishment	Density	Plant coverage	Softness	Texture	Color	Overall quality	Thatch	Number of weeds	Substrate settlement (cm)
Block	2	1.29	0.39	12,349.82	0.45	0.17	0.86	0.46	0.64	1.86	0.02
Irrigation	2	3.39ns	5.07ns	89,845.80*	7.25*	5.43*	2.33ns	4.88*	14.07*	20.02*	0.87**
Main factor error	4	2.46	1.53	10,667.99	0.73	0.53	1.26	0.64	1.06	1.77	0.02
Bacteria	3	4.45*	3.72*	24,539.28ns	3.33*	2.42ns	2.51*	3.81*	4.95**	1.66**	1.22ns
Irrigation × bacteria	6	1.03ns	0.85ns	6,959.26ns	1.26*	0.30ns	0.65ns	0.70ns	2.88**	4.36**	0.78ns
Sub factor error	18	1.27	0.98	8,533.91	0.68	0.82	0.59	0.90	0.71	0.32	0.50

**NOTE**

\* indicates significance at the level of 5%; \*\* indicates significance at the level of 1%; ns means non-significant.

proved to be statistically significant. It is noteworthy that for survival percentage, the simple effects of irrigation water and bacteria did not exhibit statistically significant differences, as all tested plants demonstrated a 100% survival rate.

The ANOVA results (Table 5) for *Aptenia cordifolia* showed that the simple effects of irrigation water have a significant difference in leaf rolling. The simple effects of irrigation water and bacteria also demonstrated significant differences in plant coverage and substrate settlement. Additionally, for the indicators of shape, flowering, and landscape performance, both the simple effects of

bacteria and the interaction effects of irrigation water and bacteria were statistically significant.

For ANOVA results of *Carpobrotus edulis* (Table 6), significant differences were evident in the simple effects of irrigation water and bacteria in substrate settlement. Moreover, for leaf rolling, not only were the simple effects of bacteria significant, but their interaction effects were also proved to be statistically significant. Furthermore, for traits related to shape and landscape performance, only the simple effects of bacteria demonstrated a significant difference. However, in terms of plant coverage, both the simple effects of

**Table 5: The ANOVA mean square results of different treatments on the aesthetic quality indicators of *Aptenia cordifolia***

Sources of variation	df	Shape	Flowering	Leaf rolling	Landscape performance	Plant coverage	Substrate settlement (cm)
Block	2	0.09	0.19	0.02	0.23	35,364.00	0.01
Irrigation	2	1.27ns	1.27ns	2.63*	1.30ns	132,132.00*	2.98*
Main factor error	4	0.30	0.25	0.21	0.23	8,288.00	0.18
Bacteria	3	0.57**	1.49**	0.52ns	0.58**	115,938.00*	6.49**
Irrigation × bacteria	6	0.31**	0.59**	0.29ns	0.45**	51,640.72ns	0.17ns
Sub factor error	18	0.07	0.08	0.35	0.08	31,950.40	0.13

**NOTE**

\* indicates significance at the level of 5%; \*\* indicates significance at the level of 1%; ns means non-significant.

**Table 6: The ANOVA mean square results of different treatments on the aesthetic quality of *Carpobrotus edulis***

Sources of variation	df	Shape	Leaf rolling	Landscape performance	Plant coverage	Substrate settlement (cm)
Block	2	0.12	0.04	0.15	15,557.31	0.11
Irrigation	2	0.53ns	0.30ns	0.07ns	16,212.00ns	2.54*
Main factor error	4	0.05	0.03	0.03	17,161.30	0.23
Bacteria	3	1.09**	0.30**	0.45*	35,315.46**	2.97**
Irrigation × bacteria	6	0.31ns	0.15*	0.13ns	21,866.30*	0.24ns
Sub factor error	18	0.15	0.04	0.10	6770.75	0.19

**NOTE**

\* indicates significance at the level of 5%; \*\* indicates significance at the level of 1%; ns means non-significant.

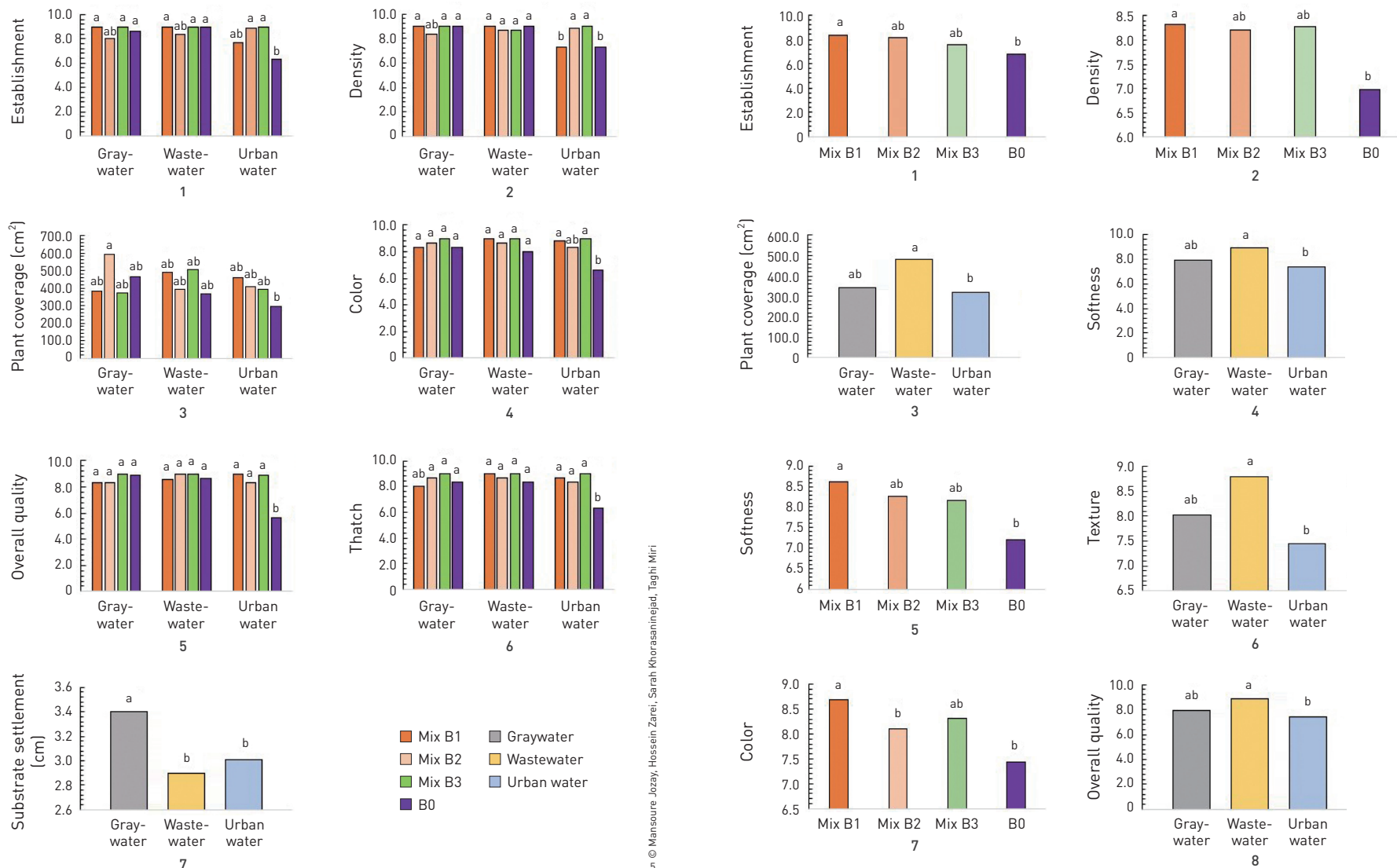
bacteria and the interaction effects of irrigation water and bacteria showed a statistically significant difference.

### 3.2 Qualitative Evaluation

#### 3.2.1 The Effect of Irrigation Water Types and Bacterial Strains on *Festuca ovina*

In *Festuca ovina*, irrigation with wastewater and graywater led to higher establishment and density compared with urban water (Fig. 5-1). Additionally, the control treatment (without bacterial application) performed better in increasing density and

establishment for urban water with the presence of Mix B2 and Mix B3 (Figs. 5-1, 5-2). Figures 5-3 ~ 5-6 show that the plant coverage, color, overall quality, and thatch are associated with the treatment of PGPB; in addition, irrigation with all three different water types inoculated with bacterial strains did not result in a significant difference. It is noteworthy that the lowest quality in terms of thatch amount was observed in urban water without bacterial presence. Regarding substrate settlement, graywater irrigation resulted in the highest amount of substrate reduction compared to the other two sources (Fig. 5-7). It seems that irrigation with wastewater in



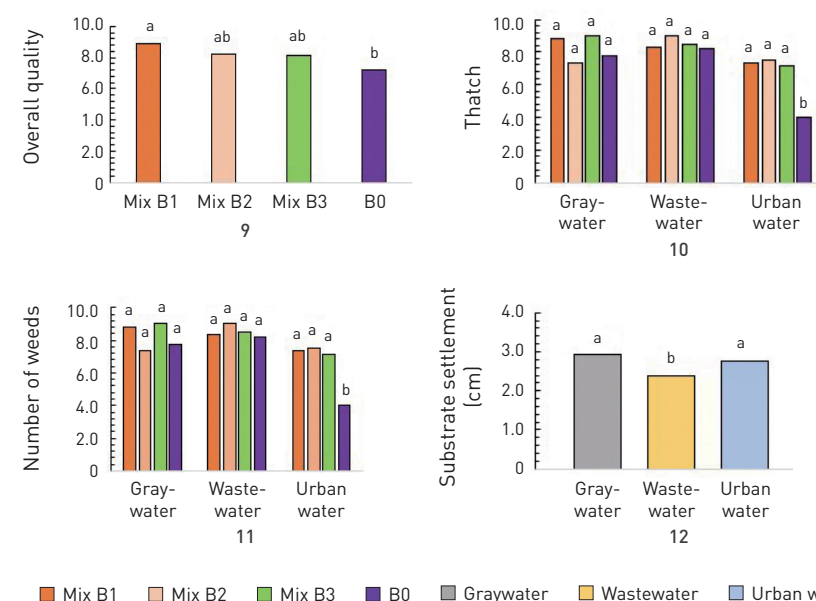
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5. Aesthetic quality evaluation of *Festuca ovina* (indicators with entirely different letters are significantly different, whereas those sharing at least one letter are not).

*Festuca ovina* performed similarly to urban water, which did not have significant effect on substrate settlement.

### 3.2.2 The Effect of Irrigation Water Types and Different Strains of Bacteria on *Ophiopogon japonicus*

Results from the analysis of the mean square effects of bacterial strains on *Ophiopogon japonicus* showed that Mix B1 has been effective in increasing establishment and density (Figs. 6-1, 6-2). On average, the Mix B1 increased 57.1% of the establishment and 37.1% of the density, compared with B0, suggesting that the presence of PGPB benefits density and establishment of *Ophiopogon japonicus*. Regarding plant coverage (Fig. 6-3), softness (Fig. 6-4), texture (Fig. 6-6), and overall quality (Figs. 6-8, 6-9), irrigation with wastewater and graywater has created better performance



6. Aesthetic quality evaluation of *Ophiopogon japonicus* (indicators with entirely different letters are significantly different, whereas those sharing at least one letter are not).

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compared with urban water. Inoculation of the substrate with Mix B1 increased softness, color, and overall quality compared with B0 (Figs. 6-5, 6-7, and 6-9). It is worth noting that the control treatment (urban water and B0) could not effectively enhance the qualitative traits of the plant (Figs. 6-3 ~ 6-9).

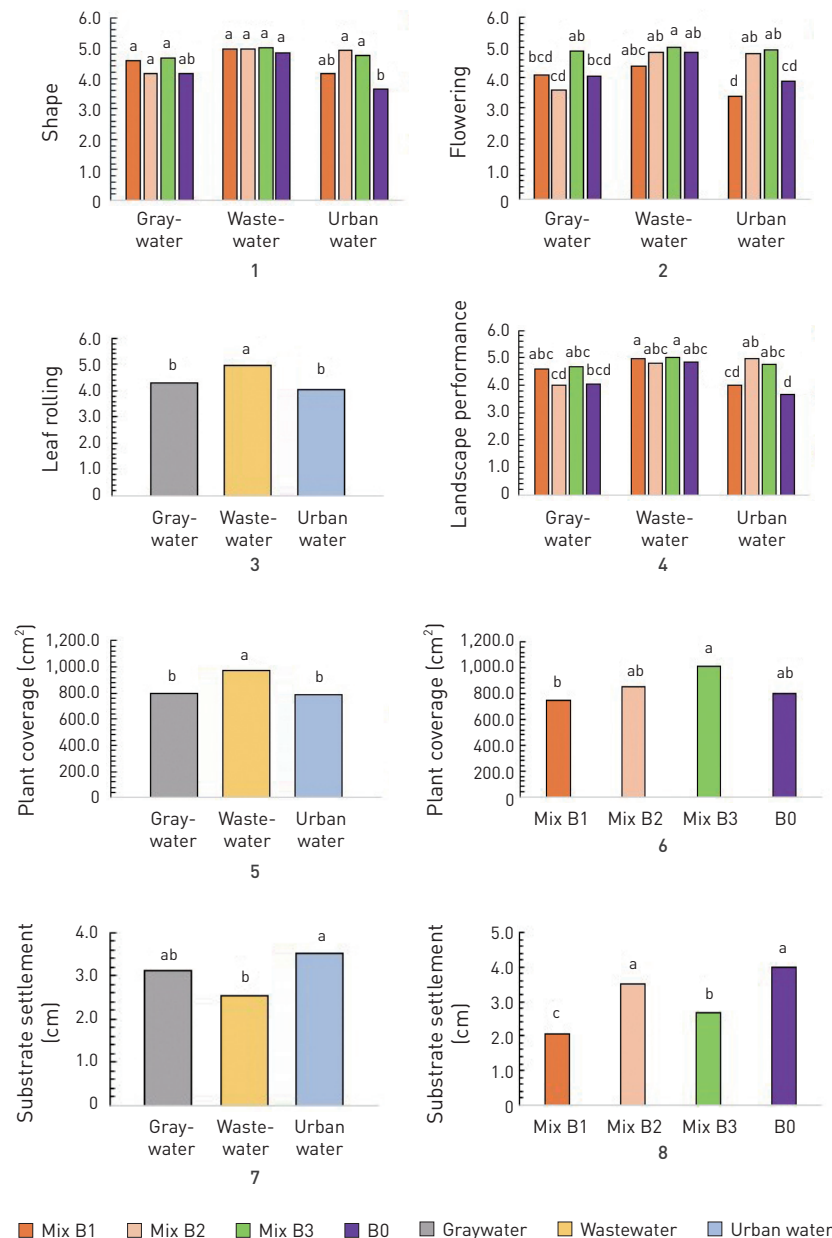
Figure 6-10 illustrates that the highest amount of thatch is related to B0 and urban water irrigation. However, statistically, the inoculated substrate with all bacterial strains with wastewater and graywater performed similarly, producing less thatch compared with B0. Additionally, the smallest number of weeds was observed in B0 with urban water irrigation (Fig. 6-11). Irrigation with wastewater created the least substrate settlement, while graywater performed similarly to urban water without creating a statistically significant difference (Fig. 6-12).

### 3.2.3 The Effect of Irrigation Water Types and Bacterial Strains on *Aptenia cordifolia*

The results suggested that bacterial application has induced a statistically significant difference in the shape of *Aptenia cordifolia* and its landscape performance compared with B0 (Figs. 7-1, 7-4), resulted in a more aesthetically pleasing and appealing shape. Statistically, treatments involving Mix B3 and wastewater were found to be more effective in flowering (Fig. 7-2). The use of Mix B1 and urban water resulted in the smallest number of flowers and a lower statistical rank than B0 in terms of flower formation. Regarding leaf rolling, wastewater led to more twisted yet healthy leaves (Fig. 7-3). As depicted in Figs. 7-5 ~ 7-8, the treatment involving Mix B3 and wastewater produced a larger coverage area and minimized substrate settlement.

### 3.2.4 The Effect of Irrigation Water Types and Bacterial Strains on *Carpobrotus edulis*

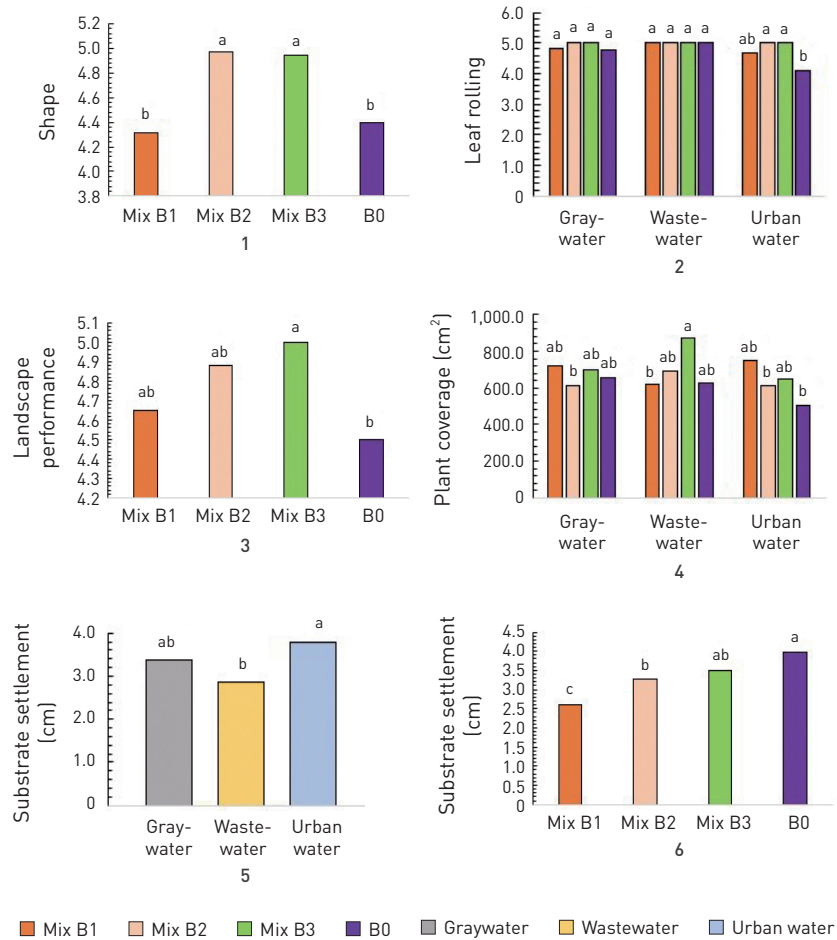
Figure 8-1 shows that for *Carpobrotus edulis*, inoculation of the substrate with Mix B2 and B3 led to the creation of a more suitable plant shape, while the Mix B1 was not very successful in shaping, with a similar effect to B0. Plants irrigated with urban water and B0 treatment showed more leaf curling (Fig. 8-2). As showed in Fig. 8-3, the application of PGPB created more visual appeal and a more attractive landscape view than B0. It appears that the inoculation of bacterial strains into the substrate, coupled with all three water types, proved successful in augmenting the plant coverage area in *Carpobrotus edulis* compared with B0 (Fig. 8-4). Notably, irrigation with wastewater, and application of Mix B1, yielded the least soil settlement, while B0 and urban water exhibited the highest substrate settlement (Figs. 8-5, 8-6).



7. Aesthetic quality evaluation of *Aptenia cordifolia* (indicators with entirely different letters are significantly different, whereas those sharing at least one letter are not).

## 4 Discussion

In this study, a combination of bacterial strains and the utilization of wastewater were employed to fulfill the nutritional requirements of plants, offering a viable alternative to chemical fertilizers. The flowering of various plant species on green wall was introduced as a decorative element. The inclusion of Mix B3 in the substrate and irrigation with wastewater resulted in increased flowering in *Ophiopogon japonicus* and *Aptenia cordifolia*, while *Festuca ovina* exhibited enhanced flowering in Mix B3 substrate



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8. Aesthetic quality evaluation of *Carpobrotus edulis* (indicators with entirely different letters are significantly different, whereas those sharing at least one letter are not).

coupled with graywater irrigation. The advantages of using ornamental plants with aesthetic features are evident, as their leaves, flowers, flower patterns, fruits, and fragrance visually please people, adding scenic value to it<sup>[41]</sup>. Utilizing ornamental species in wastewater treatment systems provides an efficient means of removing pollutants<sup>[42]</sup>. Furthermore, this can significantly enhance the visual quality of green wall landscape, which is often undervalued but with positive psychological and social implications in daily life<sup>[43]</sup>. In this study, the plants with the lowest visual appeal determined by statistical ranking were cultivated in a substrate without bacteria and irrigated with urban water. Remarkably, the introduction of bacterial strains resulted in a significant enhancement in the coloration of the plants. The most aesthetically pleasing experimental units, featuring succulent plants, were cultivated in a medium enriched with Mix B2 and Mix B3. Conversely, for slender grassy plants during colder seasons, the highest visual quality was observed in the medium

containing bacteria B1 and graywater irrigation. It appears that the use of bacterial strains and recycled waters significantly enhances the aesthetic value of plants. In this experiment, the presence of bacteria in the growing medium seems to have resulted in increased nutrient availability for the plants, thereby substantially improving growth characteristics.

Moreover, it was able to enhance visual and qualitative characteristics such as leaf color, flowering, scenic quality, and coverage. The use of PGPB in cultivating plants on outdoor green walls increases their growth and health. Martinez-Garcia et al. demonstrated the positive impact of PGPB on nutrient absorption and overall plant performance<sup>[44]</sup>. These beneficial bacteria establish symbiotic relationships with plants, promoting root growth, nutrient absorption, and resistance to diseases.

One of the most important physiological traits of bacteria that facilitate plant growth is the presence of the enzyme 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase<sup>[45]</sup>. This enzyme supports plant growth under stress conditions. Research has shown that PGPB possessing ACC deaminase can break down ACC, the precursor of plant ethylene, into ammonia and  $\alpha$ -ketobutyrate, thereby reducing the level of ethylene production in stressed plants. In other words, these PGPB act as a sink for ACC<sup>[46]</sup>. It appears that the bacterial strains in this study, in addition to being selected for their ability to absorb metals and pollutants in plants, also contributed to plant growth and stability under the stressful conditions of vertical green systems and visual appeal enhancement by generating more biomass.

Regarding the selection of plant cover, attention to factors such as root depth and plant productivity is crucial<sup>[47]</sup>; and it is a primary role of plant cover to create a habitat for microorganisms<sup>[48]</sup>. Plant cover transports a limited amount of oxygen to the root zone, allowing aerobic bacteria to establish in the root area and decompose organic matters<sup>[49]</sup>. Nutrients and organic matters are absorbed and decomposed by densely populated microbial communities<sup>[50]</sup>. The presence of PGPB enhances the resilience and adaptability of plants, enabling them to thrive even in challenging urban environments.

## 5 Conclusions

The research on the aesthetic quality of outdoor green walls explored the impact of PGPB and recycled water types. Key findings include the enhancement of plant health and appearance through PGPB application and the effectiveness of certain recycled water types in supporting plant growth without compromising

aesthetics. The results of this study demonstrate that substrates without bacteria alone cannot establish optimal plant growth. However, in combination with bacteria and the use of wastewater and graywater, these substrates can enhance the survival and growth of plants on green walls. Substrates containing mixed strains of bacteria significantly outperformed the control substrate, with the highest coverage area (the most critical factor for green walls) obtained from the bacterial-inoculated substrates with Mix B2 and Mix B3. Among the studied water types, wastewater showed the best performance. A nutrient-rich substrate can be employed to create high-quality green walls with aesthetic traits such as good coverage, establishment, and optimal growth. The outcomes of this research underscore the potential of recycled waters as a sustainable and enduring option, contributing to the conservation of water resources and diminishing reliance on fresh water sources. Effective treatment and management of recycled water emerge as crucial factors in maintaining plant health and fostering the long-term sustainability of outdoor green walls. The findings of this study suggest that planting *Aptenia cordifolia* instead of less resilient plants, with the of substrate inoculated with Mix B3 and wastewater irrigation, can achieve better aesthetic appeal in outdoor green walls, particularly in dry and semi-arid climates. Outdoor green walls, with their aesthetic appeal, offer a promising approach to purifying graywater and wastewater in urban landscapes. However, the widespread acceptance of green walls for recycled water treatment hinges on their efficacy in removing emerging pollutants from recycled water sources. This study highlights the innovative combination of PGPB and recycled waters, offering a sustainable, eco-friendly approach to creating urban green walls. Overall, the research contributes to urban gardening by demonstrating how biological treatments and water recycling can improve both the sustainability and visual quality of green infrastructure.

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**Competing interests** | The authors declare that they have no competing interests.

## REFERENCES

- [1] Kazemi, F., & Jozay, M. (2024). Developing a sustainable nature-based agricultural vertical system in cadmium polluted urban environments. *Ecological Engineering*, (208), 107385.
- [2] Naserian, M. M., Khodabakhshian, R., Kazemi, F., & Jozay, M. (2024). Solar thermo-visual gain optimization of a building using a novel proposed nature-based green system. *Journal of Thermal Analysis and Calorimetry*, (149), 1109–1123.
- [3] 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.
- [4] Ogut, O., Tzortzi, N. J., & Bertolin, C. (2022). Vertical green structures to establish sustainable built environment: A systematic market review. *Sustainability*, 14(19), 12349.
- [5] Yeom, S., Kim, H., Hong, T., Ji, C., & Lee, D.-E. (2022). Emotional impact, task performance and task load of green walls exposure in a virtual environment. *Indoor Air*, 32(1), e12936.
- [6] Kazemi, F., Rabbani, M., & Jozay, M. (2020). Investigating plant and air-quality performances of an internal green wall system under hydroponic conditions. *Journal of Environmental Management*, (275), 111230.
- [7] Irie, T. (2022). The cooling effect of green infrastructure in mitigating nocturnal urban heat islands: A case study of Yoyogi Park and Meiji Jingu Shrine in Tokyo. *Landscape Research*, 47(5), 559–583.
- [8] Blanc, P. (2008). *The Vertical Garden: From Nature to the City* (G. Bruhn, Trans.). W. W. Norton & Company.
- [9] Manso, M., & Castro-Gomes, J. (2015). Green wall systems: A review of their characteristics. *Renewable and Sustainable Energy Reviews*, (41), 863–871.
- [10] Hasim S, I. (2009). *Indonesian Ornamental Plants* [Tanaman Hias Indonesia]. Penebar Swadaya. Jakarta.
- [11] Bustami, R. A., Belusko, M., Ward, J., & Beecham, S. (2018). Vertical greenery systems: A systematic review of research trends. *Building and Environment*, (146), 226–237.
- [12] Widyawati, N. (2018). *Verticulture: The Art and Innovation of Vertical Gardening* [Vertikultur: Seni dan Inovasi Berkebun Vertikal]. Penerbit ANDI.
- [13] Jozay, M., Kazemi, F., & Fotovat, A. (2023). Performance of *Frankenia thymifolia* as a ground cover plant species and its effect on physicochemical characteristics of recycled substrates in four different seasons in external green wall systems. *Journal of Plant Environmental Physiology*, 18(69), 1–25.
- [14] Jozay, M., Zarei, H., Khorasaninejad, S., & Miri, T. (2024). The impact of plant growth-promoting bacteria on the functional and thermodynamic traits of *Aptenia cordifolia* and *Carpobrotus edulis* under recycled water irrigation in external green wall conditions. *Agricultural Water Management*, (306), 109198.
- [15] Liu, J., Fimognari, L., de Almeida, J., Jensen, C. N. G., Compant, S., Oliveira, T., Baelum, J., Pastar, M., Sessitsch, A., Moelbak, L., & Liu, F. (2023). Effect of *Bacillus paralicheniformis* on soybean (*Glycine max*) roots colonization, nutrient uptake and water use efficiency under drought stress. *Journal of Agronomy and Crop Science*, 209(4), 547–565.
- [16] de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., & Rigobelo, E. C. (2023). Plant growth-promoting rhizobacteria for sustainable agricultural production. *Microorganisms*, 11(4), 1088.
- [17] Jozay, M. (2024). *Combining green space and urban gardening in external green wall under the influence of growth-promoting bacteria and types of recycled water* [Doctoral

- dissertation]. Gorgan University of Agricultural Sciences and Natural Resources.
- [18] UNESCO, & UN-Water. (2020). *United Nations world water development report 2020: Water and climate change*.
- [19] Kaushal, M., Patil, M. D., & Wani, S. P. (2018). Potency of constructed wetlands for deportation of pathogens index from rural, urban and industrial wastewater. *International Journal of Environmental Science and Technology* (15), 637–648.
- [20] Helmecke, M., Fries, E., & Schulte, C. (2020). Regulating water reuse for agricultural irrigation: Risks related to organic micro-contaminants. *Environmental Sciences Europe*, (32), 10.
- [21] Alhamed, H., Biad, M., Saad, S., & Masaki, M. (2018). *Business opportunities report for reuse of wastewater in Morocco*. Netherlands Enterprise Agency.
- [22] Roshan, A., & Kumar, M. (2020). Water end-use estimation can support the urban water crisis management: A critical review. *Journal of Environmental Management*, (268), 110663.
- [23] Chojnacka, K., Witek-krowiak, A., Moustakas, K., Skrzypczak, D., Mikula, K., & Loizidou, M. (2020). A transition from conventional irrigation to fertigation with reclaimed wastewater: Prospects and challenges. *Renew. Renewable and Sustainable Energy Reviews*, (130), 109959.
- [24] Thomaidi, V., Petousi, I., Kotsia, D., Kalogerakis, N., & Fountoulakis, M. S. (2022). Use of green roofs for greywater treatment: Role of substrate, depth, plants, and recirculation. *Science of The Total Environment*, 807(3), 151004.
- [25] Rizzo, L., Gernjak, W., Krzeminski, P., Malato, S., McArdeil, C. S., Perez, J. A. S., Schaar, H., & Fatta-Kassinos, D. (2020). Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. *Science of The Total Environment*, (710), 136312.
- [26] Jozay, M., Zarei, H., Khorasaninejad, S., & Miri, T. (2024). Maximising CO<sub>2</sub> sequestration in the city: The role of green walls in sustainable urban development. *Pollutants*, 4(1), 91–116.
- [27] Li, X., Chang, Z., Lian, X., Meng, G., Ma, J., Guo, R., & Wang, Y. (2022). Phytoremediation of cadmium-contaminated alkaline soil using the ornamental hyperaccumulator *Mirabilis jalapa* L. enhanced by double harvesting: A field study. *Environmental Science and Pollution Research*, (29), 1–8.
- [28] National Centers for Environmental Information. (2019). *Climate information of the city of Mashhad*.
- [29] Elmich Pte Ltd. (2008). *Elmich green wall*.
- [30] FLL. (2018). *Green roof guidelines: Guidelines for the planning, construction and maintenance of green roof*.
- [31] Ju, W., Liu, L., Fang, L., Cui, Y., Duan, C., & Wu, H. (2019). Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. *Ecotoxicology and Environmental Safety*, (167), 218–226.
- [32] Nektarios, P. A., Ntoulas, N., Nydrioti, E., Kokkinou, I., Bali, E. M., & Amountzias, I. (2015). Drought stress response of *Sedum sediforme* grown in extensive green roof systems with different substrate type and depths. *Scientia Horticulturae*, (181), 52–61.
- [33] Yang, X., Gao, Y., Gan, T., Yang, P., Cao, M. & Luo, J., (2022). Elevated atmospheric CO<sub>2</sub> enhances the phytoremediation efficiency of tall fescue (*Festuca arundinacea*) in Cd-polluted soil. *International Journal of Phytoremediation*, 24(12), 1273–1283.
- [34] Janani, K., Sivarajasekar, N., Muthusaravanan, S., Ram, K., Prakashmaran, J., Sivamani, S., Dhakal, N., Shahnaz, T., & Selvaraju, N. (2019). Optimization of EDTA enriched phytoaccumulation of zinc by *Ophiopogon japonicus*: Comparison of Response Surface, Artificial Neural Network and Random Forest models. *Bioresource Technology Reports*, (7), 100265.
- [35] Esfandiari, M., Hakimzadeh Ardakani, M. A., & Sodaiezadeh, H. (2020). Investigation of heavy metal Contamination in the ornamental plants of green spaces in the city of Yazd. *Journal of Ornamental Plants*, 10(4), 213–222.
- [36] Cheng, M., Kopittke, P. M., Wang, A., Sale, P. W. G., & Tang, C. (2018). Cadmium reduces zinc uptake but enhances its translocation in the cadmium-accumulator, *Carpobrotus rossii*, without affecting speciation. *Plant and Soil*, (430), 219–231.
- [37] Monterusso, M. A., Rowe, D. B., & Rugh, C. L. (2005). Establishment and persistence of *Sedum* spp. and native taxa for green roof applications. *HortScience*, 40(2), 391–396.
- [38] Morris, K. N., & Shearman, R. C. (2000). *NTEP turfgrass evaluation guidelines*. National Turfgrass Evaluation Program.
- [39] Razzaghmanesh, M., Beecham, S., & Kazemi, F. (2014). Impact of green roofs on stormwater quality in a South Australian urban environment. *Science of the Total Environment*, (470–471), 651–659.
- [40] Kazemi, F., & Jozay, M. (2020). The effect of organic and inorganic mulches on growth and morphophysiological characteristics of *Gaillardia* sp. *Desert*, 25(2), 155–164.
- [41] Zhuang, J., Qiao, L., Zhang, X., Su, Y., & Xia, Y. (2021). Effects of visual attributes of flower borders in urban vegetation landscapes on aesthetic preference and emotional perception. *International Journal of Environmental Research and Public Health*, 18(17), 9318.
- [42] Brix, H., & Arias, C. A. (2005). The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. *Ecological Engineering*, 25(5), 491–500.
- [43] Silvestri, G., & Aliata, F. (2001). *The Landscape as a Figure of Harmony* [El paisaje como cifra de armonía]. Ediciones Nueva Visión.
- [44] Martinez-Garcia, M., Fernández-Jiménez, N., Santos, J. L., & Pradillo, M. (2020). Duplication and divergence: New insights into AXR1 and AXL functions in DNA repair and meiosis. *Science Reports*, (10), 8860.
- [45] Etesami, H., Alikhani, H., & Hosseini, H. M. (2015). Indole-3-Acetic Acid and 1-Aminocyclopropane-1-Carboxylate Deaminase: Bacterial Traits Required In Rhizosphere, Rhizoplane and/or Endophytic Competence by Beneficial Bacteria. In: D. K. Maheshwari (Ed.), *Bacterial Metabolites in Sustainable Agroecosystem* (pp. 183–258). Springer.
- [46] Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, (169), 30–39.
- [47] Calheiros, C. S., & Stefanakis, A. I. (2021). Green roofs towards circular and resilient cities. *Circular Economy and Sustainability*, (1), 395–411.
- [48] Samal, K., Kar, S., Trivedi, S., & Upadhyay, S. (2021). Assessing the impact of vegetation coverage ratio in a floating water treatment bed of *Pistia stratiotes*. *SN Applied Sciences*, (3), 120.
- [49] Hassan, I., Chowdhury, S. R., Prihartato, P. K., & Razzak, S. A. (2021). Wastewater treatment using constructed wetland: Current trends and future potential. *Processes*, 9(11), 1917.
- [50] Opitz, J., Alte, M., Bauer, M., & Peiffer, S. (2021). The role of macrophytes in constructed surface-flow wetlands for mine water treatment: A review. *Mine Water and the Environment*, (40), 587–605.

# 植物根际促生细菌与再生水灌溉对户外绿墙美学质量影响评估

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## 摘要

户外绿墙因其在提升建筑美学价值及促进健康、可持续的城市环境方面的优势而日益受到关注。然而, 在全球水资源危机持续加剧的背景下, 其高耗水问题引发了对应用替代水源(如再生水)的探讨。本研究探讨了植物生长促进细菌(PGPB)与再生水灌溉——三种灌溉水源(灰水、废水及城市用水)及不同细菌组合(Mix B1、Mix B2、Mix B3及对照组)——对伊朗马什哈德城市绿墙系统中四种植物生长及景观效果的影响, 包括羊茅(*Festuca ovina*)、麦冬(*Ophiopogon japonicus*)、心叶日中花(*Aptenia cordifolia*)和莫邪菊(*Carpobrotus edulis*)。实验于2022年3~12月开展, 以随机分配分区布置三组绿墙。研究表明, 不同灌溉水源与细菌组合对植物的景观效果产生了显著影响。其中, 心叶日中花、莫邪菊和羊茅在废水灌溉条件下, 与Mix B2或Mix B3配合使用时表现出最佳视觉效果; 麦冬在灰水灌溉与Mix B1处理下的景观效果最佳。研究结果突显了心叶日中花在Mix B3与废水灌溉条件下的优异表现, 认为该组合尤其适用于干旱及半干旱气候条件下的户外绿墙建设。

## 关键词

灰水; 植物根际促生细菌; 绿墙; 可持续绿色空间; 再生水利用

## 文章亮点

- 发现植物根际促生细菌和再生水灌溉可显著提升绿墙的美学质量
- 发现植物根际促生细菌对植物的生长、开花和整体覆盖度具有促进作用
- 推荐了适合干旱和半干旱气候下绿墙种植的植物种类、细菌菌株和灌溉组合

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