Physiological and Biochemical Responses of Plants to Rooftop and Ground-level Conditions in Urban Green Spaces

Mehak Shehzad and Adnan Younis

Institute of Horticultural Sciences, University of Agriculture, Faisalabad 38040, Pakistan

Kashf Mehmood

Department of Biological Sciences, Superior University, Lahore 54000, Pakistan

Alanoud T. Alfagham and Saud Alamri

Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia

Afroz Rais

Department of Botany, Sardar Bahadur Khan Women University, Quetta 87300, Pakistan

Shahbaz Khan

Colorado Water Center, Colorado State University, Fort Collins, CO 80523, USA; and Central Great Plains Resources Management Research, USDA-Agricultural Research Service (ARS), Akron, CO 80720, USA

Keywords. climate smart horticulture, plant ecology, stress biology, sustainable green roof system, urban environment

Abstract. An understanding of plant adaptation to rooftop environments is essential for improvising urban greening strategies; however, limited research has systematically examined the physiological and biochemical responses of plants at urban rooftops. This study addressed this gap by evaluating physiological and biochemical alterations of six herbaceous perennial species, Rosa hybrids, Murraya paniculata, Cestrum nocturnum, Pittosporum tenuifolium, Duranta, and Hibiscus rosa sinensis, cultivated in an intensive rooftop garden environment vs. optimal ground conditions. These species, acclimatized to the local climate of Pakistan, were assessed to determine the transpiration rate, photosynthetic rate, chlorophyll contents, and respiration rate using on-site measurements and standardized leaf tissue analyses. The results demonstrated significant differences in physiological and biochemical parameters between rooftop and ground-level plants. Seasonal variations and elevation profoundly influenced plant physiology and significant biochemical alterations observed in response to rooftop conditions. Photosynthetic and transpiration rates were reduced by up to 30% in winter under low light conditions, while summer conditions led to a 25% increase in transpiration rates for rooftop plants. Ground-level plants exhibited the highest respiration rates during the summer season. A leaf analysis revealed that rooftop-grown plants exhibited higher pH levels and 15% increased chlorophyll and ascorbic acid contents but 20% lower water use efficiency compared with their ground-level counterparts. Despite this, rooftop plants showed a 10% higher air pollution tolerance index. Pearson's correlation analysis confirmed a strong positive relationship between most biochemical parameters and physiological activities with increased elevation, except for relative water content, which exhibited a negative correlation under rooftop conditions. The results indicated that those plants on rooftops exhibited reduced water use efficiency compared with that of ground-level plants undergoing distinct physiological and biochemical adaptations and showed more resilience to the pollution index in response to urban rooftop conditions. These findings provide new insights into plant adaptability in urban environments and can transform future strategies for sustainable urban greening and species selection in roof garden systems.

Pakistan's climate presents significant challenges for urban gardening, with temperatures ranging from 35 to 40 °C in summer to 5 to 15 °C in winter and annual precipitation consistently below 400 mm but subject to fluctuations (Jabbar and Yusoff 2022). In cities

like Lahore that are characterized by hot, dry summers and cold, foggy winters, selecting suitable plant species for rooftop gardens is particularly challenging. Even slight changes in elevation can lead to significant alterations in environmental conditions, including variations in shading, humidity, and prolonged sunlight exposure, which impact plant growth and physiological responses (Mansoor et al. 2019).

Plants exhibit various adaptations to cope with environmental stresses through physiological and morphological modifications (de la Paz Pollicelli et al. 2018; Hafeez et al. 2024). These adaptations are crucial for survival, especially under the harsher conditions of rooftops compared with ground-level environments (Ashfaq et al. 2019; Rayner et al. 2016). Recent studies have highlighted microclimatic variations on rooftop gardens such as increased sun exposure and limited water availability, which may not be as pronounced at ground level (Mansour 2014). However, existing research lacks specific insights into how these factors uniquely affect rooftop environments.

Urban greening strategies in semi-arid regions face unique challenges because of extreme temperatures, limited rainfall, and high evapotranspiration rates. While global studies highlight that the benefits of rooftop gardens are usually related to developed countries with less fluctuating climates, semi-arid climatic regions in Pakistan require specific attention because of harsher microclimatic factors, including prolonged dry periods and intense sunlight in summer, chilling winters, and sporadic rainfall during monsoon season. Rooftop plants here must withstand these stresses to offer benefits such as reducing the urban heat island effect and conserving energy. There is a notable research gap in the available literature that would provide a comprehensive understanding of how microclimatic conditions on rooftops impact plant physiological and biochemical responses. Various previous studies have documented the phenomenon of plant adaptations to stress (Mansour 2014; Srivastava et al. 2018), but there are insufficient data on how these adaptations correlate to rooftop conditions. Recent findings by Petra et al. (2020) and Hussien et al. (2023) indicated significant changes in leaf structure and function in response to rooftop temperatures, but comprehensive evaluations of plant performance in these specific environments remain scarce.

The complexity of plant responses to rooftop environments necessitates targeted experimentation to identify species suited for these conditions. Plants that thrive in ground conditions may not perform well on rooftops because of various abiotic factors (Rayner et al. 2016). Research by Cáceres et al. (2024) indicated that succulents and certain herbaceous plants are particularly well-suited for rooftop gardening because of their moisture conservation and temperature regulation properties. This aligns with earlier findings by Morales-Tapia et al. (2019) and Tran et al. (2019), which highlighted the effectiveness of these plants in moderating rooftop microclimates.

Photosynthesis and transpiration, regulated by stomatal activity, are crucial processes influenced by light intensity, CO_2 concentration, and water availability (Buttery and Buzzell 1977; Greer and Halligan 2001).

However, recent research has underscored the impact of microclimatic conditions on these processes, with studies by Kim et al. (2024) and Lee et al. (2024) stating how variations in rooftop environments affect photosynthetic efficiency and transpiration rates. These insights highlight the importance of understanding microclimatic influences to optimize plant selection for rooftop gardening.

Petra et al. (2020) conducted a comparative anatomical study of Vinca plants grown on rooftops vs. ground level and revealed distinct physiological and morphological differences. Despite these differences, rooftop-grown Vinca showed high resilience, suggesting that some, but not all, plants within specific species can adapt to rooftop conditions. This is supported by recent research that highlighted that woody species generally exhibit greater adaptability to urban environments, but that only a few can thrive under the specific challenges of rooftop cultivation (de Oliveira Santos et al. 2024; Kim et al. 2022). Plants growing on rooftops may not perform as efficiently as those on the ground and undergo various physiological and biochemical changes to adapt to the differing environmental conditions. This article aimed to fill the gap in knowledge by evaluating plant performance in rooftop environments and identifying species well-suited for intensive rooftop gardening in semi-arid regions. By investigating physiological responses and analyzing biochemical differences, this study provides practical insights for optimizing rooftop gardening practices and enhancing sustainable urban horticulture. The findings will improve the selection of plant species that can effectively adapt to and thrive in the challenging conditions of rooftop gardens.

Materials and Methods

For this study, six plant species were carefully selected from an intensive rooftop garden and compared with similar species grown in a conventional garden setting. Species included Rosa hybrids, Hibiscus rosa sinensis, Duranta erecta, Cestrum nocturnum, Murraya paniculata, and Pittosporum tenufolium. All these species were selected because of their high adaptability in local climate, hardiness, and wide use in typical garden landscapes. The plant names, geographical distribution, and botanical descriptions are listed in Table 1. The influence of shade was a pivotal consideration at every stage of observation and was considered for all physiological analyses. To ensure the precision of plant responses in both locations, similar shading conditions were provided to the plants on the ground. Additionally, several factors were assumed to be optimal and consistent for both locations, including the plant growth media, which was considered ideal with respect to media pH and composition, and all cultural and maintenance practices, such as watering, fertilization, and pesticide management, were maintained at optimal levels.

Site description. A 150-m² rooftop garden in a residential colony in Lahore, Pakistan, was selected. The roof was constructed in 2016, and it had sustained a variety of plant species for more than 3 years. The house was west-oriented, receiving 6 to 8 h of direct sunlight in the summer from two sides, with one side fully exposed and the other partially shaded. The roof had a slight gable with 5- to 7-cm slope at the northern corner. More than 75% of the area was covered with vegetation, including perennial shrubs, ornamental plants, and groundcovers. The roof was protected by multiple preparatory layers, including an inner waterproof layer (thickness, 2-3 mm), drainage layer (depth, 1-1.5 inches), and root barrier (thickness, 1.5 mm). The biochemical characteristics of the growth medium included a pH of 6.8, electrical conductivity of 310.5 mS/m, porosity of 45.2%, and bulk density of 1.89 g/ m³. The growth medium was composed of a mixture of sand, leaf compost, and garden soil in a 30:30:40 v/w proportion.

Microclimatic data. To provide a comprehensive understanding of plant response, microclimatic factors were critically considered across both rooftop and ground environments during this study. Data on temperature, humidity, wind speed, and solar radiation were recorded using environmental sensors placed at both locations. In summer, rooftop temperatures averaged $38 \,^{\circ}\text{C} \pm 2$, with lower humidity (45%) and higher wind speeds (12 km/h) compared with the ground, where temperatures were $35 \,^{\circ}\text{C} \pm 1.5$ on average, humidity was 50% to 55%, and wind speeds were 7 km/h. In winter, rooftop temperatures dropped to 15°C, which was notably lower than the ground temperature (18 °C), relative humidity (55% vs. 60%), and moderate wind speeds (8-10 km/h vs. 6 km/h). Rooftop plants also received more hours of direct sunlight on average (±2-2.5 h) in both seasons. These measurements were taken over the same time periods as the physiological and biochemical observations to ensure consistency.

Physiological assessment. The transpiration rate, respiration rate, stomatal conductance, and photosynthetic rate were measured by placing an intact leaf of the plant in the chamber of an infrared gas analyzer as described by Field and Mooney (1990). Water use efficiency (WUE) was calculated by taking the photosynthetic rate-to-transpiration rate ratio.

Anticipated performance index. Evaluation of plants, based on their anticipated performance index, involved a combined assessment of its morphological and socio-economic characteristics with resultant values of pollution tolerance index. The anticipated performance index (API) evaluation scale used for this study was described by Correa-Ochoa et al. (2022) and involved a grading system (+ or -) fixed for each specific plant characteristic (Table 2).

Air pollution tolerance index. Globally, the air pollution tolerance index (APTI) is used as standardized criteria for evaluating the tolerance of plants to dust and particulate matter. It includes biochemical evaluations of plant leaves, including the pH of leaf extract, chlorophyll contents, ascorbic acid contents, and relative water contents (Table 3).

The APTI of plants was determined by quantification of the following biochemical parameters of plants using a previously described protocol (Correa-Ochoa et al. 2022):

$$APTI = \left(\frac{A(T+P) + R}{10}\right) \qquad [1]$$

where A represents the ascorbic contents $(mg \cdot g^{-1})$, T represents the total chlorophyll contents $(mg \cdot g^{-1})$, P represents the pH of the leaf extract, and R represents the relative water contents of leaves (%).

Sample collection. To estimate the air tolerance index of rooftop plants, leaf samples were collected during two growing seasons. The first batch was collected in summer during May to July, and the second batch was collected in early fall (October-December). Leaves were selected as the representative plant part for the determination of APTI because they have high surface areas and are easy to manage in the experiment. Leaves were randomly selected from each plant species before rain (pre-monsoon) or after a week of rain. Samples were taken in a paper bag and brought to the laboratory. Leaves were washed thoroughly with tap water and then with distilled water to remove dirt and adhered particulate matter. Cleaned samples were used for further experimentation.

pH. Fresh leaf samples were ground into a paste form with distilled water and homogenized in an electric stirrer. The homogenized mixture was sieved, and pH was measured with a digital pH meter (Systemics System 361).

Relative water content. The relative water content (RWC) in plants refers to the water content in a particular part of a plant relative to its biomass accumulation (i.e., dry matter content). The RWC of samples was measured according to the protocol defined by Henson et al. (1989) as follows:

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$
 [2]

where FW represents fresh weight (g), TW represents turgid weight (g), and DW represents dry weight (g).

The fresh weight (FW) of washed samples was obtained. To estimate turgid weight (TW), samples were soaked in water for 24 h, and then TW was recorded. After that, samples were dried in a dehydrator for 2 to 3 d to obtain the dry weight (DW).

Total chlorophyll content. The total chlorophyll content was estimated using fresh leaves and an infrared gas analyzer. An infrared light analyzer measured absorption spectrometry at two wavelengths (A663 and A645) depending

Received for publication 23 Sept 2024. Accepted for publication 15 Nov 2024.

Published online 13 Dec 2024.

Authors are thankful to the Researchers Supporting Project number (RSP2025R194), King Saud University, Riyadh, Saudi Arabia.

S.K. is the corresponding author. E-mail: shahbaz. khan@colostate.edu.

This is an open access article distributed under the CC BY-NC license (https://creativecommons. org/licenses/by-nc/4.0/).

Table 1. Botanical description and geographical distribution of selected plant species.

No.	Botanical name	Common name	Family	Origin	Status in Pakistan
1	Rosa hybrids	Rose/ghulab	Rosaecae	Central Asia	Indigenous, hardy perennials
2	Cestrum nocturnum	Raat ki rani	Solanaceae	West Indies	Naturalized, hardy perennial invasive in some areas
3	Murraya paniculata	Marwa	Rutaceae	South Asia	Indigenous, hardy perennials, invasive
4	Hibiscus rosa sinensis	Hibiscus	Malvaceae	West Africa	Naturalized
5	Duranta spp.	Duranta	Verbenaceae	America	Exotic, frost-sensitive, summer hardy
6	Pittosporum tenufolium	Silvery	Rhamnaceae	Australia	Exotic, hardy

on the type of chlorophyll (either a or b). A protocol for quantification of the total chlorophyll content described by Arnon (1949) involved the following formulas:

Chlorophyll
$$a = (12.7 \times A663)$$

 $- (2.29 \times A645)$ [3]
Chlorophyll $b = (22.9 \times A645)$
 $- (4.68 \times A663)$ [4]

Total Chlorophyll Contents
$$\left(\frac{mg}{mg}\right)$$

$$(g) = (20.2 \times A645) + (8.02 \times A663)$$
[5]

Ascorbic acid content. For quantification of vitamin C/ascorbic acid, fresh samples were dried in a dehydrator for 72 h. Dried samples were ground and sieved through a 150- μ m sieve. Quantification was performed using a volumetric method described by Sadasivam (1996). Then, 100 g of fine powder was placed in a 100-mL conical flask and dissolved in 4% oxalic acid, and the volume 100 mL was made in the conical flask. Aliquots were agitated in an electronic stirrer to make a homogenized mixture and titrated against bromide dye. The amount of dye used was equivalent to the amount of ascorbic acid present.

Statistical analysis. A statistical analysis was performed using an analysis of variance (ANOVA) technique in Microsoft Excel 2007 (Redmond, WA, USA). Furthermore, Pearson's correlation analysis was used to identify the extent of correlation among the physiological and biochemical parameters of plants in response to elevation. If the correlation coefficient r^2 was less than 0 (i.e., $r^2 <$ 0), then it indicated a negative relation stated as increase in one parameter caused a decrease in other parameter and vice versa. If $r^2 \ge 0$, then it indicated positive dependency of both parameters on each other.

Table 2. Anticipated	performance	index	scoring
criteria.			

Grade	Score	Category
0	Up to 30	Not recommended
1	<u>3</u> 1–40	Very poor
2	41-50	Poor
3	51-60	Moderate
4	61-70	Good
5	71-80	Very good
6	81-90	Excellent
7	91-100	Best

Results

Physiological assessment

For each species, three representative plants were selected and kept under observation in winter and summer, respectively. A physiological assessment of each plant with respect to its location is mentioned separately (Table 4).

Roses. Roses are one of the most popular outdoor plants in Pakistan. They are day-neutral plants and generally thrive well in sunny locations with good drainage. The results showed a notable difference in photosynthetic rates at both locations. The photosynthetic rate was 12.69 μ mol CO₂ m⁻²·s⁻¹ on the rooftop and 8.99 μ mol CO₂ m⁻²·s⁻¹ in a standard garden in summer. In winter, the photosynthetic rate was slower but consistent ($\pm 8 \ \mu mol CO_2 \ m^{-2} \cdot s^{-1}$). In summer, the rooftop transpiration rate was 8.61 mmol H₂O m⁻²·s⁻¹, with a respiration rate of 4.35 CO₂ m⁻²·s⁻¹ and stomatal conductance of 0.99 mmol $H_2O \text{ m}^{-2} \cdot \text{s}^{-1}$. The winter trends of roses and other species are shown in Table 4. An ANOVA revealed statistically significant differences among all physiological parameters of rose species growing in different locations. The recorded photosynthetic rate, transpiration rate, and stomatal conductance were higher on the rooftop compared with those under ground conditions in both seasons. Rooftop plant WUE was less in summer, thus revealing that rose species required more frequent watering during summer.

Hibiscus belongs to a group of longduration perennial flowering plants that grow well in almost all parts of Pakistan. The results showed that the transpiration rate, respiration rate and stomatal conductance were comparatively high on the rooftop in summer and in winter. All winter physiological parameters were statistically the same in both locations. A statistical analysis revealed no significant difference in the physiological activity of *Hibiscus* plants in both locations except for respiration rate, which was comparatively higher in summer on the rooftop and statistically different from that of ground-grown plants.

Duranta erecta. In the warm, arid climate of Pakistan, this non-native plant is commonly used for corners and borders. A statistical analysis revealed that the photosynthetic rate, transpiration rate, WUE, and stomatal conductance of *Duranta erecta* significantly differed in summer; however, in winter, the transpiration rate and respiration rate were statistically similar in both locations.

Silvery is a very versatile shrub used as a hedge for contrasting color themes. Its creamy and velvety appearance and lavender-colored flower give an overall soft and appealing appearance of landscapes. A statistical analysis revealed that the photosynthetic rate, respiration rate, and stomatal conductance of *Silvery* did not show significant differences in both seasons, whereas the transpiration rate was statistically significant in summer and WUE was

Table 3. Standard for gradation of plant species based on the air pollution tolerance index (APTI), morphology, and socio-economic value.

	Parameter	Assessment values	Gradation
Tolerance	APTI	9–12	+
		12.5–15	++
		15.5–18	+ + +
		18.5–21	++++
		21.5-24	+++++
Growth pattern	Plant type	Evergreen	+
*	• •	Deciduous	_
	Canopy	Thin/rough/sparse	_
		Partially thick/less disperse	+
		Thick and disperse	++
	Height	Small	_
	-	Medium	+
		Big	++
Leaf morphology	Leaf size	Small	_
		Medium	+
		Large	++
Frowth pattern	Texture	Smooth	_
Growth pattern		Rough/brittle	+
Leaf morphology	Hardiness	Hardy	+
		Delineate	_
	Economic value	Fewer than three uses	_
Leaf morphology		Three or four uses	+
		Five or more	++

	of herbaceous			

		Sur	nmer	Winter		
Plant	Parameter	Roof garden	Standard garden	Roof garden	Standard garden	
Rosa hybrids	Photosynthesis rate	12.69 ± 0.52 a	9.89 ± 0.33 b	8.99 ± 0.15 a	8.69 ± 0.30 a	
2	Transpiration rate	$8.61 \pm 0.3 a$	$4.12 \pm 0.11 \text{ b}$	1.35 ± 0.25 b	1.98 ± 0.20 a	
	Respiration rate	$4.35 \pm 0.1 \text{ b}$	5.64 ± 0.1 a	3.31 ± 0.11 a	$1.28 \pm 0.16 \text{ b}$	
	Stomatal conductance	0.99 ± 0.32 a	$0.81 \pm 0.61 \text{ b}$	0.36 ± 0.22 b	0.98 ± 0.21 a	
	Water use efficiency	$1.47 \pm 0.1 \text{ b}$	2.4 ± 0.03 a	6.59 ± 1.08 a	$4.38 \pm 0.5 \text{ b}$	
Cestrum nocturnum	Photosynthesis rate	8.59 ± 1.69 a	7.77 ± 0.65 a	3.88 ± 0.08 a	$8.73 \pm 1.54 \text{ a}$	
	Transpiration rate	2.34 ± 1.25 a	1.06 ± 0.88 a	2.24 ± 0.09 a	$3.08 \pm 1.3 \text{ a}$	
	Respiration rate	4.68 ± 1.25 a	$2.6 \pm 0.65 \text{ b}$	4.51 ± 0.05 a	4.11 ± 1.41 a	
	Stomatal conductance	0.10 ± 0.24 a	$0.77 \pm 0.5 \ a$	0.44 ± 0.13 a	$0.35 \pm 0.08 \ a$	
	Water use efficiency	$3.63 \pm 1.8 \text{ b}$	$7.33 \pm 3.7 \text{ a}$	1.73 ± 0.002 a	2.83 ± 1.01 a	
Murraya paniculta	Photosynthesis rate	$8.63 \pm 0.3 a$	$5.89 \pm 2.5 \text{ b}$	4.18 ± 0.03 a	$4.69 \pm 0.9 a$	
~ 1	Transpiration rate	2.61 ± 0.39 a	1.12 ± 0.6 a	2.35 ± 0.5 a	1.98 ± 0.66 a	
	Respiration rate	4.35 ± 0.4 a	5.64 ± 0.05 a	$3.31 \pm 0.6 a$	$1.28 \pm 0.66 \text{ b}$	
Tibicaus rosa sinansis	Stomatal conductance	0.99 ± 0.9 a	$0.81 \pm 2.1 \text{ a}$	$0.36 \pm 0.3 \text{ b}$	0.78 ± 0.28 a	
	Water use efficiency	$3.3 \pm 0.4 \text{ b}$	5.26 ± 1.1 a	$1.77 \pm 0.71 \text{ b}$	$2.3 \pm 1.8 \text{ a}$	
Hibiscus rosa sinensis	Photosynthesis rate	32.64 ± 5.9 a	27.16 ± 7.1 a	10.16 ± 4.74 a	12.06 ± 1.2 a	
	Transpiration rate	$8.16 \pm 0.5 a$	$6.98 \pm 1.8 \text{ a}$	1.69 ± 0.9 a	$2.14 \pm 1.1 a$	
	Respiration rate	4.33 ± 1.2 a	$1.03 \pm 0.88 \ b$	1.836 ± 1.0 a	1.98 ± 0.59 a	
	Stomatal conductance	1.65 ± 0.4 a	2.19 ± 0.7 a	$0.98 \pm 0.1 \ a$	0.97 ± 0.12 a	
	Water use efficiency	$4 \pm 0.6 a$	3.89 ± 0.88 a	6.01 ± 4.2 a	$5.63 \pm 3.1 \text{ a}$	
Golden duranta	Photosynthesis rate	11.14 ± 1.2 a	$7.82 \pm 0.6 \text{ b}$	$9.12 \pm 0.2 \text{ a}$	$7.88 \pm 0.67 \text{ b}$	
	Transpiration rate	$4.13 \pm 0.5 a$	$1.19 \pm 0.69 \text{ b}$	2.03 ± 0.58 a	2.11 ± 0.7 a	
	Respiration rate	0.51 ± 0.26 a	0.45 ± 0.39 a	0.24 ± 0.08 a	0.26 ± 0.06 a	
	Stomatal conductance	8.92 ± 0.2 a	8.31 ± 0.64 a	7.65 ± 0.4 a	$4.12 \pm 0.4 \text{ b}$	
	Water use efficiency	$2.69 \pm 0.9 \text{ b}$	6.57 ± 1.1 a	4.49 ± 0.9 a	$3.73 \pm 1.05 \text{ b}$	
Pittosporum tenufolium	Photosynthesis rate	19.36 ± 1.12 a	18.01 ± 1.65 a	16.13 ± 0.98 a	$14.42 \pm 0.56 \text{ a}$	
* v	Transpiration rate	3.66 ± 0.7 a	$1.21 \pm 0.11 \text{ b}$	$0.89 \pm 0.5 \ a$	0.39 ± 0.31 a	
	Respiration rate	5.31 ± 1.09 a	6.12 ± 0.17 a	6.14 ± 0.78 a	5.21 ± 0.54 a	
	Stomatal conductance	9.065 ± 1.03 a	8.82 ± 0.12 a	$5.45 \pm 0.8 \ a$	4.46 ± 0.51 a	
	Water use efficiency	$5.29 \pm 1.08 \text{ b}$	14.88 ± 0.01 a	$16.13 \pm 2.9 \text{ a}$	$14.42 \pm 0.88 \ b$	

Parameters with the same letters indicate a nonsignificant difference in similar growing seasons when $P \ge 0.05$. Parameters with different letters indicate a significant difference in the same growing season when $P \le 0.05$.

the only parameter that showed a significant difference among plants in both seasons.

Murraya paniculata L. Rutaceae is an ornamental evergreen shrub typically known for its sweet scent. The photosynthetic rate and transpiration rate in summer and respiration rate and stomatal conductance in winter were statistically significant, while WUE varied significantly in both seasons.

Cestrum nocturnum did not show any statistical difference in physiological activity in winter; however, in summer, only the transpiration rate and WUE showed notable differences. The transpiration rate was high on the rooftop but WUE was low, similar to other rooftop-grown plants.

APTI and API of plant species on the rooftop

The APTI for each plant species was determined based on the biochemical analysis of four key factors that contribute to plant capacity to endure pollution. Among these factors, pH is a crucial indicator of plant resistance to air pollutants, with higher pH levels generally signifying enhanced tolerance and better adaptation to pollutant exposure (Lu et al. 2018). The pH values of the plant extracts ranged from 4.5 to 8.1 (Table 5).

The results demonstrated that plants grown on the rooftop exhibited higher APTI values compared with those of plants grown at ground level, indicating an improved ability to tolerate pollution under elevated conditions. Specifically, *Hibiscus* showed the highest APTI on the rooftop, with a ground-level APTI of 10.8 ± 1.25 and rooftop APTI of 9.52 ± 1.63 . *Murraya* followed, with APTI values of 10.3 ± 2.01 at ground level and 9.15 ± 1.1 on the rooftop. *Silvery* plants had APTI values of 10.1 ± 1.98 at ground level and 9.7 ± 1.54 on the rooftop, while *Roses* and *Duranta* had comparatively lower APTI values, with *Roses* showing 9.2 ± 2.25 at ground level and 8.9 ± 0.89 on the rooftop and *Duranta* showing 9.1 ± 1.36 at ground level and 7.2 ± 1.87 on the rooftop.

The APTI of rooftop plants demonstrated a strong positive correlation with RWC and total chlorophyll content in leaf samples. In contrast, APTI was negatively related to plant pH, which contradicted the findings of a previous study (Lu et al. 2018). These results confirm the research findings of Alotaibi et al. (2020), who observed a similar correlation among green belt plants in Riyadh, UAE, and confirmed a robust positive relationship between APTI and ascorbic acid as well as relative water content through a regression analysis.

The pH of the plants did not show a significant impact on overall physiological activity, except in relation to ascorbic acid and the transpiration rate. This finding underscores the complexity of plant responses to rooftop environments and highlights the importance of specific biochemical attributes, such as chlorophyll content and relative water content, to determining pollution tolerance. The results demonstrated that while pH has limited influence on plant physiology, other factors such as ascorbic acid and chlorophyll contents play substantial roles in enhancing pollution tolerance and overall plant health.

Malav et al. (2022) assessed APTI and API of different crop plants and reported that APTI of plants was significantly correlated with ascorbic acid, chlorophyll, and relative water contents. However, herbaceous crops, trees, and shrubs have different mechanisms and performance indexes compared with those of ornamental shrubs and garden plants.

The biochemical analysis results advocate that while rooftop environments generally enhance the pollution tolerance of the studied species, the extent of this enhancement varies among different plants. *Hibiscus, Murraya*, and *Silvery* exhibited notable improvements in their pollution tolerance when grown on a rooftop, whereas *Roses* and *Duranta* showed less pronounced effects. This variability underscores the importance of selecting suitable plant species for rooftop gardens to optimize their potential for mitigating pollution.

Pearson's correlation analysis

Pearson's correlation analysis was performed to evaluate the relationships between physiological and biochemical attributes of rooftop-grown plants (Table 6). Pearson's correlation coefficient (r^2) explains the extent of the relationship that exists between two distinctive parameters; $r^2 < 0$ indicates a negative relation stated as increase in one parameter caused a decrease in other parameter and vice versa. If $r^2 \ge 0$, then a positive

Table 5. Air pollution tolerance index assessment of herbaceous perennials under rooftop and ground conditions.

Plant	Biochemical parameter	Roof garden	Standard garden
Rosa hybrids	Tch (mg g^{-1})	1.31 ± 0.98 a	$1.25 \pm 0.25 \text{ b}$
-	AA $(mg \cdot g^{-1})$	3.01 ± 2.54 a	$2.43 \pm 1.95 \text{ a}$
	pH	7.7 ± 1.14 a	$8.35 \pm 0.95 a$
	RWC (%)	$57.18 \pm 3.65 \text{ b}$	65.6 ± 1.25 a
	APTI	9.2 ± 2.25 a	$8.9 \pm 0.89 \ a$
Cestrum nocturnum	Tch $(mg \cdot g^{-1})$	1.86 ± 1.12 a	$0.99 \pm 0.47 \ b$
	AA $(mg \cdot g^{-1})$	3.26 ± 2.14 a	$0.9 \pm 1.32 \text{ b}$
	pH	6.8 ± 0.88 a	$6.3 \pm 0.76 \text{ a}$
	RWC (%)	$63.18 \pm 4.36 \text{ b}$	$71.6 \pm 1.1 \text{ a}$
	APTI	9 ± 2.69 a	$7.9 \pm 1.32 \text{ b}$
Murraya paniculata	Tch $(mg \cdot g^{-1})$	2.16 ± 2.09 a	2.01 ± 0.25 a
	AA $(mg \cdot g^{-1})$	4.2 ± 2.1 a	$2.3 \pm 1.1 \text{ b}$
	pH	$6.7 \pm 0.5 a$	6.2 ± 0.54 a
	RWC (%)	$66.18 \pm 3.1 \text{ b}$	74.6 ± 0.88 a
	APTI	10.3 ± 2.01 a	$9.15 \pm 1.1 \text{ b}$
Hibiscus rosa sinensis	Tch $(mg \cdot g^{-1})$	2.86 ± 1.2 a	$1.91 \pm 0.78 \text{ b}$
	AA $(mg \cdot g^{-1})$	3.74 ± 1.25 a	$2.4 \pm 1.63 \text{ b}$
	pH	6.3 ± 0.89 a	$5.8 \pm 1.07 \text{ a}$
	RWC (%)	$64.18 \pm 2.65 \text{ b}$	72.6 ± 1.41 a
	APTI	10.8 ± 1.25 a	9.52 ± 1.63 a
Golden duranta	Tch (mg \cdot g ⁻¹)	$1.7 \pm 0.6 a$	$1.3 \pm 1.02 \text{ a}$
	AA $(mg \cdot g^{-1})$	$4.2 \pm 1.1 \text{ a}$	$3.2 \pm 1.87 \text{ b}$
	pH	6.5 ± 0.65 a	6.05 ± 1.31 a
	RWC (%)	65.21 ± 3.1 a	$55.82 \pm 1.65 \text{ b}$
	APTI	9.1 ± 1.36 a	$7.2 \pm 1.87 \text{ b}$
Pittosporum tenufolium	Tch $(mg \cdot g^{-1})$	$0.54 \pm 0.5 \ a$	$0.89 \pm 0.69 \ a$
-	AA (mg·g ^{-1})	$3.16 \pm 1.03 \text{ a}$	$2.47 \pm 1.54 \text{ b}$
	pH	$5.8 \pm 1.2 \text{ b}$	6.7 ± 0.98 a
	RWC (%)	78.8 ± 3.21 a	80.36 ± 1.32 a
	APTI	10.1 ± 1.98 a	9.7 ± 1.54 a

Parameters with the same letters indicate a nonsignificant difference in similar growing seasons when $P \ge 0.05$. Parameters with different letters indicate a significant difference in the same growing season when $P \le 0.05$.

AA = ascorbic acid; APTI = air pollution tolerance index; RWC = relative water content; Tch = total chlorophyll content.

correlation is indicated; however, any r^2 value > 0.5 indicates a strong positive correlation between both parameters. The analysis revealed that ascorbic acid, total chlorophyll content, and RWC were positively correlated with APTI. Specifically, higher ascorbic acid, total chlorophyll content, and RWC were positively associated with APTI. This suggests that roof-top conditions have a pronounced effect on the biochemical functioning of plants by retaining higher ascorbic acid levels and total chlorophyll contents to overcome stress conditions, indicating their crucial role in pollution resistance.

Conversely, WUE exhibited a negative correlation with the transpiration rate. This finding implies that higher transpiration rates may lead to decreased WUE. However, WUE showed a positive relationship with stomatal conductance, total chlorophyll content, and RWC, indicating that an effective stomatal function and elevated chlorophyll content could contribute to improved WUE under rooftop conditions.

Discussion

This study aimed to assess the physiological and biochemical responses of various plant species under rooftop and ground conditions. The methodology involved selecting representative plants from each species and observing their physiological parameters across winter and summer seasons. Parameters such as photosynthetic rate, transpiration rate, stomatal conductance, and WUE were measured. Additionally, APTI was computed based on biochemical factors, including pH, chlorophyll content, and ascorbic acid level.

Figure 1 shows a comparison of computed biochemical values of each plant at both locations. Among all plants studied, higher chlorophyll and ascorbic acid contents were observed in rooftop plants compared with those of plants grown on the ground. A similar positive correlation was computed through Pearson's correlation analysis. According to Verma et al. (2011), the chlorophyll content serves as a primary indicator of plant stress because it is one of the first components affected by adverse conditions; this was later supported by Morales-Tapia et al. (2019). Similarly, Swain et al. (2016) found that a higher chlorophyll content is associated with increased tolerance to air pollution. Our findings align with those of these studies, indicating that rooftop conditions enhance chlorophyll density and ascorbic acid levels, contributing to an improved APTI.

However, these results contrast with those of Gupta and Khadka (2016), who reported that high pollution stress leads to reduced chlorophyll contents because of oxidation and degradation of chlorophyll molecules. This discrepancy may be attributed to varying microclimatic environments, which may elevate certain stress factors compared with groundlevel conditions. Similarly, longer periods of sunlight exposure on rooftops could potentially enhance photosynthetic efficiency despite higher stress levels.

Rooftop plants demonstrated higher transpiration rates but lower WUE compared with those of ground-grown plants (Fig. 1). This suggests that while rooftop plants transpire more, they have lower WUE, in agreement with the findings of Rabbani and Kazemi (2022). A statistical analysis revealed a significant difference in WUE between rooftop and ground plants (P < 0.05). This finding corroborates the observation that herbaceous plants under rooftop conditions are more prone to water stress and require more frequent irrigation, particularly under prolonged daylight conditions.

The APTI of rooftop plants was consistently higher than that of ground plants, indicating better air pollution tolerance. The performance index (API) was also assessed, showing that *Hibiscus* exhibited the highest API with values

Table 6. Correlation analysis of physiological and biochemical attributes of plants growing in the rooftop environment.

	pН	RWC	AA	Tch	APTI	PR	TR	RR	SC	WUE
pН	1									
RWC	-0.87	1								
ASC	0.294	-0.350	1							
Tch	-0.23	-0.10	0.585	1						
APTI	-0.60	0.603	0.50	0.56	1					
PR	-0.44	0.196	0.020	0.75	0.423	1				
TR	0.39	-0.475	0.146	0.41	-0.14	0.602	1			
RR	-0.04	0.229	0.182	0.179	0.410	0.224	0.038	1		
SC	-0.615	0.68	-0.605	-0.360	0.088	0.071	-0.206	-0.455	1	
WUE	-0.915	0.877	-0.2669	0.216	0.633	0.44	-0.491	0.418	0.364	1

AA = ascorbic acid; APTI = air pollution tolerance index; PR = photosynthetic rate; RR = respiration rate; RWC = relative water content; SC = stomatal conductance; Tch = total chlorophyll content; TR = transpiration rate; WUE = water use efficiency.

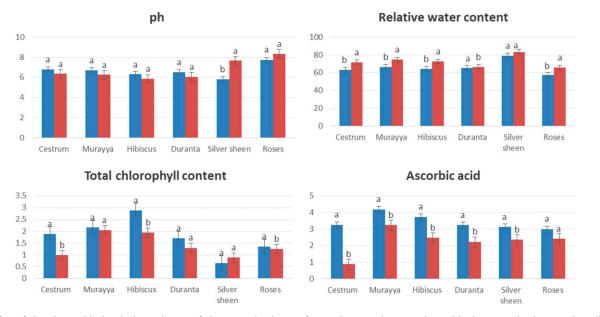


Fig. 1. Effect of elevation on biochemical constituents of plants growing in a rooftop environment in comparison with plants growing in ground conditions.

exceeding 10, whereas other species such as *Rosa* hybrids and *Murraya paniculata* had lower values but were still categorized as "good" performers (Table 7). Statistical comparisons revealed significant differences in APTI among the species (ANOVA, P < 0.05), with *Hibiscus* and *Murraya paniculata* showing the most robust performance under rooftop conditions.

Our findings align with those of Yadav and Pandey (2020), who observed that APTI correlates strongly with ascorbic acid, chlorophyll, and relative water contents in green belt trees. This supports the notion that these biochemical factors are crucial for assessing plant resilience to pollution. However, the contrasting findings of Rai et al. (2016) and Lu et al. (2018) suggest that while high pH and chlorophyll contents can indicate increased tolerance, they do not universally apply to all stress conditions. This highlights the need for further investigations of the specific stressors that affect rooftop plants. This study had several limitations. The assessments of physiological and biochemical parameters were restricted to a limited number of plant species and environmental conditions. This may have limited the generalization of these findings to other species and urban rooftop environments. Additionally, this study did not account for potential interactions between plant species and urban pollutants, which could further influence plant performance. Seasonal variations beyond winter and summer, such as transitional periods, were not considered.

Conclusion

Our hypothesis posited that plants on rooftops exhibit reduced efficiency compared with that of ground-level plants and undergo distinct physiological and biochemical adaptations in response. This research provides a preliminary understanding of plant responses to rooftop conditions and highlights the importance of selecting suitable species for urban rooftop gardens in semi-arid regions. Despite variations in physiological and biological activities, all selected plant species thrived well and demonstrated high levels of adaptability to rooftop environments, suggesting that with proper maintenance, these species can be suggested as good performers in urban rooftop settings. These findings indicate that species with higher APTI and favorable biochemical profiles, such as Hibiscus sinensis and Murraya paniculata, are well-suited for rooftop gardening. This research investigated these adaptations over two consecutive seasons to gain fundamental insights into the challenges of rooftop environments. Future research should expand the range of plant species and environmental variables to validate the findings across diverse rooftop gardens. Investigating the morphological and anatomical adaptations of plants will provide deeper insights into their resilience

mechanisms. Additionally, studies exploring the interactions between different plant species and urban pollutants could enhance our understanding of plant performance in complex urban environments. Longitudinal studies examining the long-term sustainability and maintenance needs of rooftop gardens would also be beneficial.

References Cited

- Alotaibi MD, Alharbi BH, Al-Shamsi MA, Alshahrani TS, Al-Namazi AA, Alharbi SF, Alotaibi FS, Qian Y. 2020. Assessing the response of five tree species to air pollution in Riyadh City, Saudi Arabia, for potential green belt application. Environ Sci Pollut Res Int. 27(23): 29156–29170. https://doi.org/10.1007/s11356-020-09226-w.
- Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24(1):1–15. https://doi.org/ 10.1104/pp.24.1.1.
- Ashfaq S, Ahmad M, Zafar M, Sultana S, Bahadur S, Ullah F, Zaman W, Ahmed SN, Nazish M. 2019. Foliar micromorphology of Convolvulaceous species with special emphasis on trichome diversity from the arid zone of Pakistan. Flora. 255:110–124. https://doi.org/10.1016/ j.flora.2019.04.007.
- Buttery B, Buzzell R. 1977. The relationship between chlorophyll content and rate of photosynthesis in soybeans. Can J Plant Sci. 57(1): 1–5. https://doi.org/10.4141/cjps77-001.
- Cáceres N, Robbiati FO, Suárez M, Hick EC, Matoff E, Jim CY, Galetto L, Imhof L. 2024.

Table 7. Anticipated performance index (API) of selected plant species.

Plant	Botanical name	APTI	Plant height	Canopy	Plant type	Texture	Leaf size	Economic value	Hardiness	Total (+)	Scoring (%)	API grade ⁱ
Roses	Rosa hybrids	+	+	_	+	+	+	+	+	7	43.75	2
Moon flower	Cestrum nocturnum	+	++	-	+	-	_	_	+	5	31.25	1
Murraya	Murraya paniculata	+	+	+	+	_	_	+	+	6	37.5	1
Hibiscus	Hibiscus rosa sinensis	+	++	++	+	+	+	+	+	10	62.5	4
Golden duranta	Duranta spp.	+	+	+	+	_	_	_	+	5	31.25	1
Silvery	Pittosporum tenufolium	+	+	+	+	_	_	_	+	5	31.25	1

¹ API grades: 0 = not recommended; 1 = very poor; 2 = poor; 3 = moderate; 4 = good; 5 = very good; 6 = excellent; 7 = best.

Growth performance of multi-species plant mixtures on an extensive vegetated roof: A two-year experimental study. Urban Ecosyst. 27(4):1207–1223. https://doi.org/10.1007/s11252-023-01498-7.

- Correa-Ochoa M, Mejia-Sepulveda J, Saldarriaga-Molina J, Castro-Jiménez C, Aguiar-Gil D. 2022. Evaluation of air pollution tolerance index and anticipated performance index of six plant species, in an urban tropical valley: Medellin, Colombia. Environ Sci Pollut Res Int. 29(5):7952–7971. https://doi.org/10.1007/s11356-021-16037-0.
- de la Paz Pollicelli M, Idaszkin YL, Gonzalez-José R, Márquez F. 2018. Leaf shape variation as a potential biomarker of soil pollution. Ecotoxicol Environ Saf. 164:69–74. https://doi.org/ 10.1016/j.ecoenv.2018.08.003.
- de Oliveira Santos TD, Pacheco FAL, Fernandes LFS. 2024. A systematic analysis on the efficiency and sustainability of green facades and roofs. Sci Total Environ. 932:173107. https:// doi.org/10.1016/j.scitotenv.2024.173107.
- Field CB, Mooney HA. 1990. Leaf chamber methods for measuring photosynthesis under field conditions. Remote Sens Rev. 5(1):117–139. https://doi.org/10.1080/02757259009532125.
- Jabbar M, Yusoff MM. 2022. Assessing the spatiotemporal urban green cover changes and their impact on land surface temperature and urban heat island in Lahore (Pakistan). GES. 15(1): 130–140. https://doi.org/10.24057/2071-9388-2021-005.
- Greer DH, Halligan EA. 2001. Photosynthetic and fluorescence light responses for kiwifruit (*Actinidia deliciosa*) leaves at different stages of development on vines grown at two different photon flux densities. Funct Plant Biol. 28(5): 373–382. https://doi.org/10.1071/PP00146.
- Gupta RS, Khadka B. 2016. Evidence for the presence of key chlorophyll-biosynthesis-related proteins in the genus Rubrobacter (*Phylum Actinobacteria*) and its implications for the evolution and origin of photosynthesis. Photosynth Res. 127(2):201–218. https://doi.org/10.1007/ s11120-015-0177-y.
- Hafeez MB, Ghaffar A, Zahra N, Ahmad N, Hussain S, Li J. 2024. Plant growth promoters boost the photosynthesis related mechanisms and secondary metabolism of late-sown wheat under contrasting saline regimes. Plant Stress. 12:100480. https://doi.org/10.1016/j.stress.2024. 100480.
- Henson I, Jensen C, Turner N. 1989. Leaf gas exchange and water relations of lupins and wheat. I. Shoot responses to soil water deficits. Funct Plant Biol. 16(5):401–413. https://doi.org/10.1071/ PP9890401.

- Hussien A, Jannat N, Mushtaha E, Al-Shammaa A. 2023. A holistic plan of flat roof to greenroof conversion: Towards a sustainable built environment. Ecol Eng. 190:106925. https:// doi.org/10.1016/j.ecoleng.2023.106925.
- Kim B, Hwang S, Lee Y, Shin S, Kim K. 2022. Comparative analysis of environmental standards to install a rooftop temperature monitoring station. Sci Rep. 12(1):22401. https://doi.org/ 10.1038/s41598-022-27070-5.
- Kim SH, Park CY, Choi JY, Park C. 2024. Exploring maladaptive patterns of small-scale green roofs through evaluation in a capacity of heat mitigation: A case study in Seoul. Build Environ. 266:112052. https://doi.org/10.2139/ssrn. 4769755.
- Lee E, Seo Y, Woo DK. 2024. Enhanced environmental and economic benefits of green roofs in a humid subtropical region under future climate. Ecol Eng. 201:107221. https://doi.org/ 10.1016/j.ecoleng.2024.107221.
- Lu S, Yang X, Li S, Chen B, Jiang Y, Wang D, Xu L. 2018. Effects of plant leaf surface and different pollution levels on PM2. 5 adsorption capacity. Urban Forest Urban Green. 34:64–70. https://doi.org/10.1016/j.ufug.2018.05.006.
- Malav LC, Kumar S, Islam S, Chaudhary P, Khan SA. 2022. Assessing the environmental impact of air pollution on crops by monitoring air pollution tolerance index (APTI) and anticipated performance index (API). Environ Sci Pollut Res Int. 29(33):50427–50442. https://doi.org/ 10.1007/s11356-022-19505-3.
- Mansoor U, Fatima S, Hameed M, Naseer M, Ahmad MSA, Ashraf M, Ahmad F, Waseem M. 2019. Structural modifications for drought tolerance in stem and leaves of *Cenchrus ciliaris* L. ecotypes from the Cholistan Desert. Flora. 261:151485. https://doi.org/10.1016/j.flora.2019. 151485.
- Mansour MMF. 2014. The plasma membrane transport systems and adaptation to salinity. J Plant Physiol. 171(18):1787–1800. https://doi. org/10.1016/j.jplph.2014.08.016.
- Morales-Tapia P, Gambardella M, Gómez M, Montenegro G. 2019. Morpho-anatomical adaptations of Argylia radiata (L.) D. Don to an arid environment. Flora. 258:151440. https://doi. org/10.1016/j.flora.2019.151440.
- Petra SA, Georgescu MI, Manescu CR, Toma F, Badea ML, Dobrescu E, Popa VI. 2020. Leaves anatomical and physiological adaptations of *Vinca major* 'Variegata' and *Hedera helix* L. to specific roof garden conditions. Not Bot Horti Agrobo. 48(1):318–328. https://doi. org/10.15835/nbha48111784.
- Rabbani M, Kazemi F. 2022. Water need and water use efficiency of two plant species in soil-

containing and soilless substrates under green roof conditions. J Environ Manage. 302(Pt A): 113950. https://doi.org/10.1016/j.jenvman. 2021.113950.

- Rai R, Agrawal M, Agrawal S. 2016. Impact of heavy metals on physiological processes of plants: With special reference to photosynthetic system. Plant Resp Xenobiotics. 127–140. https://doi.org/10.1007/978-981-10-2860-1_6.
- Rayner JP, Farrell C, Raynor KJ, Murphy SM, Williams NS. 2016. Plant establishment on a green roof under extreme hot and dry conditions: The importance of leaf succulence in plant selection. Urban Forest Urban Green. 15:6–14. https://doi.org/10.1016/j.ufug.2015. 11.004.
- Sadasivam S. 1996. Biochemical methods. New Age International Publishers, New Delhi, India.
- Srivastava RP, Dixit P, Singh L, Verma PC, Saxena G. 2018. Comparative morphological and anatomical studies of leaves, stem, and roots of *Selinum vaginatum* CB Clarke and *Selinum tenuifolium* Wall. Flora. 248:54–60. https://doi. org/10.1016/j.flora.2018.08.017.
- Swain S, Mallick SN, Prasad P. 2016. Effect of industrial dust deposition on photosynthetic pigment chlorophyll and growth of selected plant species in Kalunga Industrial areas, Sundargarh, Odisha. Intl J Botany Stud. 1:2455–2541X. https://www.researchgate.net/publication/ 330701478_Effect_of_industrial_dust_deposition_ on_photosynthetic_pigment_chlorophyll_and_ growth_of_selected_plant_species_in_Kalunga_ Industrial_areas.
- Tran S, Lundholm JT, Staniec M, Robinson CE, Smart CC, Voogt JA, O'Carroll DM. 2019. Plant survival and growth on extensive green roofs: A distributed experiment in three climate regions. Ecol Eng. 127:494–503. https://doi. org/10.1016/j.ecoleng.2018.09.027.
- Verma P, Chandawat D, Solanki H. 2011. Seasonal variation in physico-chemical and phytoplankton analysis of Kankaria Lake. Life Sci. 19:842–854. https://www.researchgate. net/publication/249648994_SEASONAL_ VARIATION_IN_PHYSICO-CHEMICAL_ AND_PHYTOPLANKTON_ANALYSIS_ OF_KANKARIA_LAKE.
- Yadav R, Pandey P. 2020. Assessment of air pollution tolerance index (APTI) and anticipated performance index (API) of roadside plants for the development of greenbelt in urban area of Bathinda City, Punjab, India. Bull Environ Contam Toxicol. 105(6):906–914. https://doi. org/10.1007/s00128-020-03027-0.