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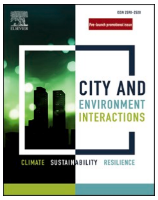

Research Articles

Assessment of air pollution tolerance of *Hedera helix* in urban areas

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ABSTRACT

Rapid urbanisation and industrial development have increased air pollution in cities, reduced green spaces, and affected human health and ecosystem functions. Vegetation, such as trees and climbing plants, mitigate pollution by capturing airborne particulate matter. The main objective of this study was to examine spatial and seasonal patterns of urban air pollution in Debrecen, Hungary, based on leaf surface dust deposition on common ivy (*Hedera helix* L.) as an indicator and to assess its physiological responses of at urban sites. *Hedera helix* leaves were collected from bus stops across the city. Seasonal variations in the pigment, ascorbic acid, and water contents, pH, dust deposition, and Air Pollution Tolerance Index (APTI) were analysed. Dust showed the largest relative increase (458–3042%) at urban sampling sites, whereas the carotenoid (54.8–132.7%) and ascorbic acid contents (113–133%) increased moderately. Chlorophyll varied between − 62.9% and + 64.8% compared to the control. The dust and pigment contents in the leaves decreased with increasing distance from the city centre, while the ascorbic acid content increased. APTI values classified *H. helix* as an air pollution-sensitive species, with variations across sites and seasons. Variance analysis and principal component analysis highlighted the seasonal dynamics of leaf physiological traits. These findings demonstrate that *H. helix* effectively reflects urban air pollution and environmental stress, supporting its use as a bioindicator in urban ecosystems.

1. Introduction

Rapid urbanisation and industrial activities have intensified anthropogenic air pollution in urban environments, leading to higher to environmental quality and human health risks. Urbanisation refers to the movement of people from rural areas to cities, a trend significantly influencing global development. This change often decreases green spaces and increases air pollution, making it important to understand the effects of pollutants on health and the environment[1]. As cities are expected to keep growing, urban planning and development should be addressed through a comprehensive, large-scale approach[2].

To better understand the impact of air pollution, the main types of common air pollutants and their associated health and environmental effects need to be reviewed. Particulate matter (PM) is a mixture of solid and liquid particles in the air, including coarse (PM_{10}) and fine ($PM_{2.5}$) fractions. $PM_{2.5}$ remains airborne for long periods and enters the lungs and bloodstream, contributing to inflammation and organ damage. It is one of the leading causes of premature death and has been linked to over four million deaths worldwide annually. Urbanisation often leads to a higher population density, which is linked to increased airborne PM levels [3]. Green spaces and plants reduce PM by capturing particles on their surfaces, either temporarily or permanently and promoting particle dispersion through airflow dynamics in vegetated areas[4]. However, urban air pollution affects human health and induces physiological stress in plants, limiting the effectiveness of green spaces in mitigating PM.

Many studies have investigated how green spaces, for example, trees, support public health, emphasising the important roles that urban greenery plays in lowering health risks and improving the well-being of city populations[5,6]. Among the different types of urban greenery, green walls offer unique benefits. They cover large wall surfaces without blocking airflow and can be placed near pollution hotspots, such as busy roads with heavy pedestrian activity; thus, it is a practical method for increasing plant coverage in polluted cities without taking up valuable

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Fig. 1. An ivy-covered bus stop in Debrecen city (Hungary) (Future of Debrecen, n. d).

space [7]. Since these walls absorb and interact with air pollutants, they clean the air naturally. For this reason, green walls are often considered an important component of nature-based urban solutions, but their effectiveness in PM mitigation under real urban conditions is not nearly as extensively researched as that of traditional green spaces[8].

Climbing plants, such as ivy, anchor their roots into supporting structures or walls, creating patchy green coverings. These plants are typically very resilient and withstand atmospheric conditions, including high temperatures, strong winds and heavy rainfall common in harsh climates[9]. The evergreen nature of common ivy (*Hedera helix* L.) means that it is exposed to urban air pollutants year-round. Its wall-growing habit keeps leaves in proximity to traffic emissions, and its dense leaf surface may enhance dust deposition [10]. It typically grows on urban walls worldwide, making its potential bio-protective role highly relevant. *Hedera* spp. have already been studied in relation to air pollution. In one study, researchers analysed *H. helix* leaves collected from roadside walls to determine whether they could absorb dust and pollutants that contribute to the deterioration of stone surfaces and pose health risks in urban environments and found that *H. helix* captured airborne particles, particularly in areas with heavy traffic [11]. Another study evaluated the composition of atmospheric dust that had accumulated over a specific period on the leaves of *H. helix* and *Senecio cineraria* and their potential use as bio-monitors [12]. Bioindicator studies using trees have already been conducted in Debrecen, Hungary [13,14]. In addition, research on different *Rosa* cultivars has been conducted to assess air pollution tolerance in this city [15].

The Air Pollution Tolerance Index (APTI) is a measure used to assess how well plant species withstand air pollutants. It combines four leaf-related parameters – ascorbic acid, chlorophyll, relative water content (RWC) and pH – into a single value. These leaf physiological parameters were selected because they provide complementary information on the air pollution stress responses of plants. Ascorbic acid and chlorophyll are related to oxidative stress protection and photosynthetic performance, and the RWC and pH reflect leaf water balance and metabolic stability under environmental stress conditions. Higher APTI values indicate more tolerant plants, and lower values indicate more sensitive ones, enabling species classification from sensitive to tolerant [16]. Based on previous studies, *H. helix* has been classified into different categories according to its APTI values, but the study conditions also varied [16,17].

Although the dust deposition and pollution tolerance of *Hedera* spp. have been investigated, limited information is available on its seasonal physiological responses under real urban exposure conditions, especially in Central European urban environments, such as Debrecen. Moreover, the integrated evaluation of leaf physiological parameters and dust deposition in the context of green wall applications remains unexplored. Therefore, this study addressed this gap by providing an integrated, seasonally resolved, in situ assessment of physiological responses and surface dust deposition of *H. helix* under real urban exposure conditions, offering a novel perspective on plant–pollution interactions in urban environments. Accordingly, this study aimed to determine the urban air pollution patterns through leaf surface dust deposition on *H. helix* and to evaluate its physiological responses. We hypothesised that dust deposition on leaf surfaces would be higher in traffic-influenced urban areas (e.g. city centre, major roads, and intersections) compared to less polluted control sites and less polluted urban sites (e.g. residential areas and side streets). In addition, the pigment content and APTI values were expected to reflect spatial variation in air pollution levels, and measurable leaf parameters were presumed to correlate with the distance from the city centre. These relationships were expected to reflect spatial differences in the urban air pollution intensity.

2. Material and methods

2.1. Study site

Debrecen (47.5°N, 21.5°E) is located in eastern Hungary, on the Great Hungarian Plain, at approximately 120 m above sea level. These geographical conditions contribute to the formation of the urban heat island effect. With a population of around 200,000, Debrecen is the second-largest city in the country and functions as a key cultural, educational, and economic centre in northeastern Hungary[18]. The city's topography is uniform with no significant elevation differences. The region is characterised by four distinct seasons, typical of Central European climatic conditions. The average annual temperature is 10°C with a typical yearly temperature variation of 22.9°C. Annual precipitation reaches 549 mm, and the city receives about 2000 h of sunshine per year[19,20]. According to the Köppen–Geiger classification, Debrecen has a humid continental climate (Dfb, bordering on Dfa) [21]. Most parts of the city are situated on sandy soil of the Nyírség region, but the southwestern and western edges extend into the loess-covered Hajdúhát area. The dominant wind direction, from the north–northwest, often transports dust from nearby agricultural zones[22]. In addition, long-range transport of Saharan dust occasionally affects the city's air quality[23]. During the hot season, the 24-h limit values for PM are frequently exceeded.

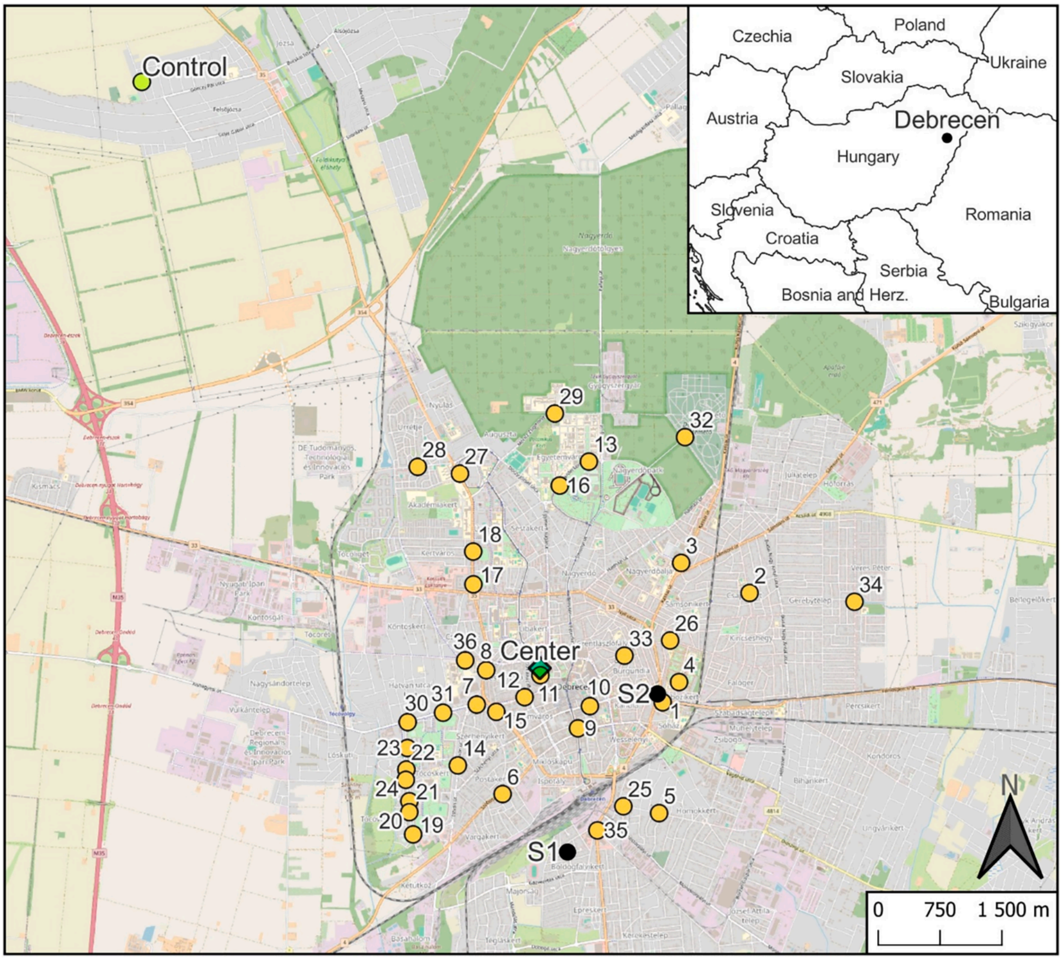

Fig. 2. Location of sampling sites and air pollution monitoring stations (S1 – Kalotaszeg tér, S2 – Hajnal utca) in Debrecen (Hungary).

2.2. Sampling

In 2020, as part of the “Future of Debrecen – Green Bus Stop” project, 50 bus stops across the city of Debrecen, Hungary, were planted with *H. helix* by the Green Working Group to match the new eco-friendly buses. All individual plants were grown locally by DEKERT Nonprofit Ltd. (Debrecen, Hungary), and the plants helped reduce traffic-related pollutant levels and limit pollutant transport by foliar interception

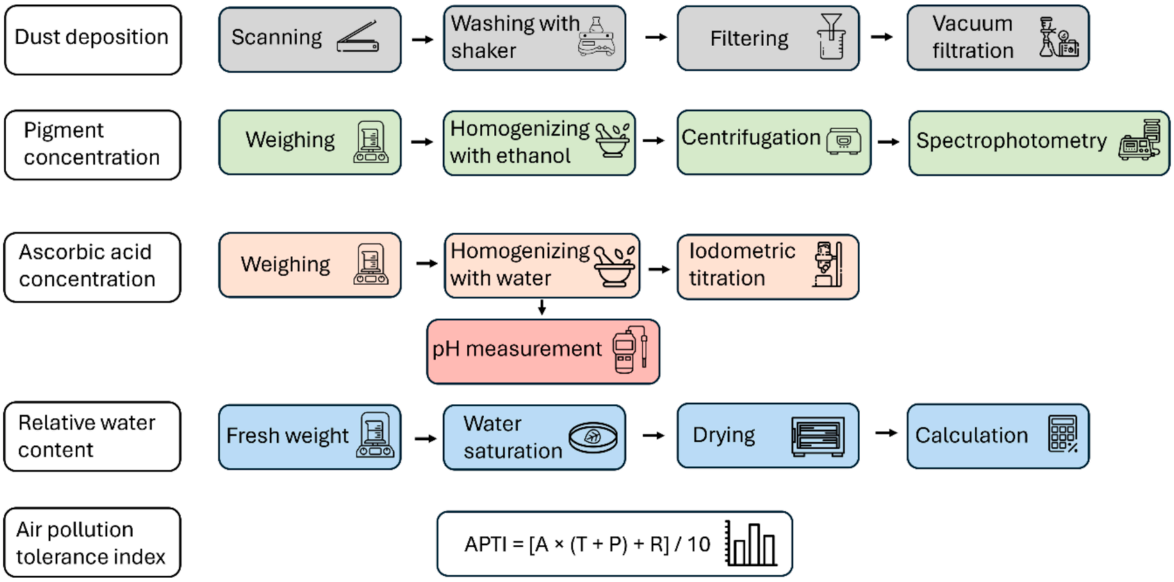

Fig. 3. Workflow diagram of laboratory methods used for the analysis of leaf-based air pollution indicators. Workflow diagram was made by The Noun Project (https://thenounproject.com).

(Fig. 1) [24]. This initiative offered a valuable opportunity to implement comprehensive and systematically organised biomonitoring. All plants were installed as part of the same urban greening program and supplied by the same provider, ensuring a similar plant age and initial growing conditions at each site. The steel mesh support structures were uniform in design and maintained under the same municipal management regime. At each stop, the ivy walls were located on the side opposite the road, ensuring comparable exposure conditions.

We collected samples from ivy-covered steel mesh structures at 36 bus and tram stops in Debrecen, representing common urban locations with varying traffic exposure levels (Fig. 2, Supplementary Information Table 1). Although 50 bus stops were originally planted within the framework of the project, at the time of sampling, only 36 sites had sufficiently developed with intact ivy cover suitable for standardised leaf sampling. Sites with incomplete plant cover or maintenance-related disturbances were excluded, and all bus and tram stops with established and comparable ivy cover were included in the study. The control site was located in the suburban district of Debrecen-Józsa, representing a residential environment with a lower traffic intensity and reduced direct vehicular emission exposure compared to inner-city sampling sites. Climatic conditions and horticultural maintenance practices were comparable to those of the other sampling locations. Although the site cannot be considered pollution-free, it served as a relatively low-exposure reference in the same urban–regional context. The sampling period lasted one year, from July 2023 to June 2024. During the first week of each month, samples were collected between 8 and 10 a.m. At each site and sampling date, 15 fully developed, visually healthy leaves of a similar age and size were collected randomly from different parts of the ivy wall to ensure spatial representativeness. These 15 leaves constituted 1 composite biological sample per site per month. Given the limited biomass available at each site and the need to perform multiple physiological and biochemical analyses from the same sampling event, 15 leaves were considered an optimal compromise between analytical requirements and minimising vegetation disturbance. The collected samples were placed in labelled plastic bags and transported to the laboratory for further analyses.

2.3. Laboratory work

We studied several parameters related to leaf physiology and air pollution exposure. Specifically, the amount of settled dust on the leaf surface, pigment (chlorophyll and carotenoid) contents, ascorbic acid content (AAC), pH and RWC were measured [25]. Based on these variables, the APTI was also calculated[26](Fig. 3).

Leaf samples were scanned (HP Laser MFP 432fdn; HP Inc., Palo Alto, CA, USA) in black and white at 300 dpi, and the surface area of each leaf was calculated using a computer-based algorithm. Dust was quantified on the adaxial (upper) surface, as the abaxial (lower) surface typically retains less dust due to its fibrous texture. Each set of leaf samples was placed in a 500-mL plastic container with 250 mL of deionised water. Using an analogue shaker (GFL 3015; GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany), the samples were mixed in a circular motion for 10 min, and the resulting suspension was filtered through a 100-μm mesh. This procedure was repeated with an additional 50 mL of deionised water, producing 300 mL of a suspension. The combined solution was filtered (Munktell 392; Ahlstrom-Munksjö, Espoo, Finland) with a vacuum using a BOECO R-300 (BOECO, Hamburg, Germany) pump. Dust accumulation was expressed as particulate mass normalised to the leaf surface area (mg cm^{-2}) and determined using a composite sample of 15 leaves.

The chlorophyll (Chl) content was determined using a photometric method based on a colour reaction. Approximately 0.02 g of leaf tissue was weighed on a precision analytical balance, and samples were mixed with several drops of 96% ethanol in a porcelain mortar. To aid mechanical grinding and preserve pigment stability, quartz sand and magnesium oxide were added to samples. After homogenisation, the samples were rinsed quantitatively with 5 mL of 96% ethanol and immediately transferred into centrifuge tubes. Samples were centrifuged at 1500 rpm for 3 min using an IEC Centra MP4 centrifuge. Finally, light absorbance was measured (optical density) at wavelengths of 470, 649, 665, and 750 nm using a BOECO S-220 spectrophotometer (BOECO, Hamburg, Germany). The absorbance values represent the light intensity reduction and directly relate to the pigment content [27]. The chlorophyll and carotenoid (Car) contents were measured in three technical replicates using extract aliquots derived from the same composite biological sample collected at each site and sampling event. The pigment content was calculated on a fresh mass basis. The chlorophyll values were derived using the formula described by Lichtenthaler [28]:

$\mathrm{TChl}\ (\mathrm{mg\ g^{-1}}) = (5.24 \times E_{665} + 22.24 \times E_{649}) \times (V / m) \times 1000$,

where.

V. Is the volume of the extract, i.e., 5 mL

m is the mass of the weighed sample (g);

E_{649} represents the difference in absorbance measured at 649 and 750 nm;

E_{665} represents the difference in absorbance measured at 665 and 750 nm.

Carotenoid values were derived using the formula described by Lichtenthaler [28]:

$\mathrm{Car}\ (\mathrm{mg\ g^{-1}}) = (1000E_{470} - 2{,}13 \times \mathrm{Chl_a} \times 97.64 \times \mathrm{Chl_b}) / 209 \times (V / m)$,

where.

V. Is the volume of the extract, i.e., 5 mL

m is the mass of the weighed sample (g);

E_{470} represents the difference in absorbance measured at 470 and 750 nm.

The AAC was determined by iodometric titration[29]. Approximately 2 g of fresh leaf tissue was weighed using an analytical balance, finely chopped and homogenised in 50 mL of deionised water. The mixture was filtered, and the filtrate was diluted to a final volume of 100 mL in a volumetric flask. The pH of the extract was measured using a digital pH meter (Hach HQ40d; Hach Company, Loveland, CO, USA) in three replicates.

For the titration, 20-mL aliquots of the extract were transferred into separate containers and mixed with 1 mL of 0.5% starch solution as an indicator. An automatic burette was used to gradually add 0.0025 M iodine solution to each sample, and the process was performed in triplicate. The reaction between iodine and ascorbic acid was visually monitored. When all of the ascorbic acid had been oxidised, excess iodine was bound in a blue complex with starch helices. The endpoint of the titration was identified when the blue coloration remained stable for at least 20 s. AAC was determined from a single composite leaf extract prepared per site and sampling event. Iodometric titration was performed for each extract in three technical replicates to improve measurement precision.

The AAC was calculated using the formula below:

$\mathrm{AAC}\ (\mathrm{mg\ g^{-1}}) = c \times V \times M \times 5 / m$,

where.

c is the molarity of the iodine solution (mol L^{-1});

V. Is the volume of iodine consumed during titration (mL)

M is the molar mass of ascorbic acid (176 g mol^{-1});

and m is the mass of the sample analysed (g).

The RWC of leaf samples was determined by measuring the fresh weight (FW), turgid weight (TW), and dry weight (DW). The FW of the leaves was recorded using an analytical balance. The samples were then submerged in deionised water for 24 h to achieve full turgidity. After this saturation period, the turgid weight was measured. The leaves were

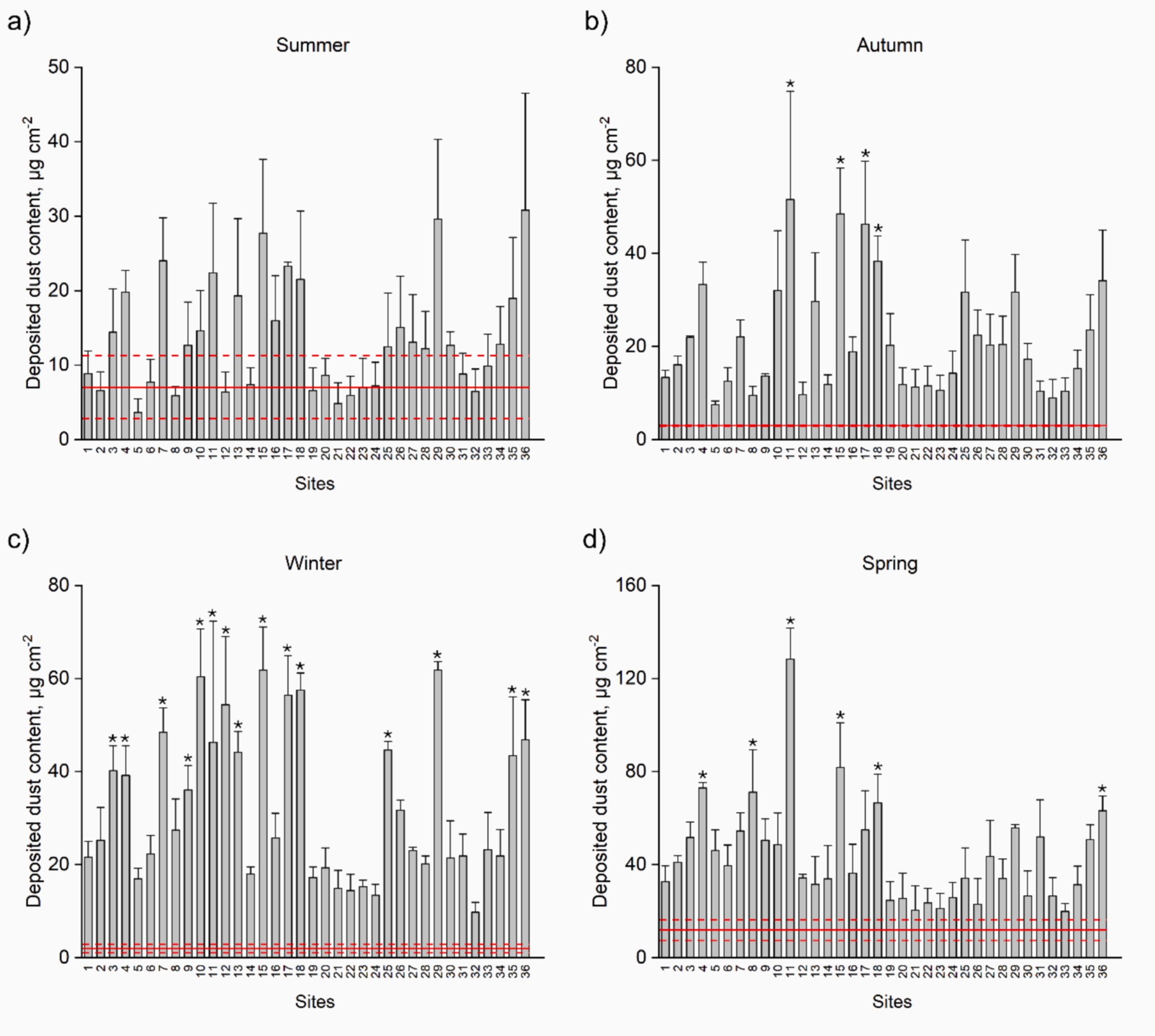

Fig. 4. Deposited dust content on leaf surfaces in summer (a), autumn (b), winter (c) and spring (d). Red lines indicate the mean ± standard error of the control, and significant differences ($p < 0.05$) from the control sample are marked with an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oven-dried at 70°C until they reached a constant mass, and the DW was recorded[29]. Three independent leaves were randomly selected from the composite biological sample to determine the RWC, which was calculated using the following formula:

RWC (%) = (FW − DW) / (TW − DW) × 100.

To calculate the APTI, we applied the formula developed by Singh and Rao [26], using their original notations:

APTI = [A × (T + P) + R] / 10,

where.

A is the ascorbic acid content (mg g^{-1});

T is the total chlorophyll content (mg g^{-1});

P is the pH;

R is the RWC.

Three air pollution monitoring stations are located in Debrecen, and they were used to measure the CO, NO, NO_2, NO_x, PM_{10}, $PM_{2.5}$ and SO_2 pollutant levels in the city. The monitoring stations are situated in the northern (Klinika), central (Hajnal utca) and southern (Kalotaszeg tér) parts of the city. In our study, only the Kalotaszeg tér (Station 1) and Hajnal utca (Station 2) stations were included (Fig. 2) because the Klinika station had no data for 2023. The city was divided into two parts, and the sampling sites were compared to the monitoring station closest to them. Distance identification and all maps were produced using QGIS version 3.44.0 software[30]. Meteorological data were obtained from the Debrecen Airport monitoring station[31].

2.4. Statistical analysis

Monthly data were aggregated into seasonal groups to reduce short-term temporal variability and emphasise broader seasonal environmental patterns. Statistical analyses were performed using IBM SPSS Statistics (version 21) and Canoco for Windows 4.5[32] for the Levene test, analysis of variance (ANOVA), Dunnett's test, and correlation and regression analyses. Principal component analysis (PCA) was performed in R software (version 4.1.2) by the R Foundation for Statistical Computing (2021) [33]. The normality of the data distribution was assessed using the Shapiro–Wilk test. The homogeneity of variances was tested with Levene's test. The dust content on the leaf surface, pigment content, AAC, RWC, pH and APTI of the studied areas were compared by a two-way ANOVA, in which one factor was the studied sites and the other factor was the studied seasons. Differences from the control were tested with a pairwise Dunnett's test. Pearson correlation and regression analyses were performed between the measured physiological parameters (dust deposition on leaf surfaces, pigment content, AAC, RWC, pH and APTI) and the distance from the city centre, as well as with the air

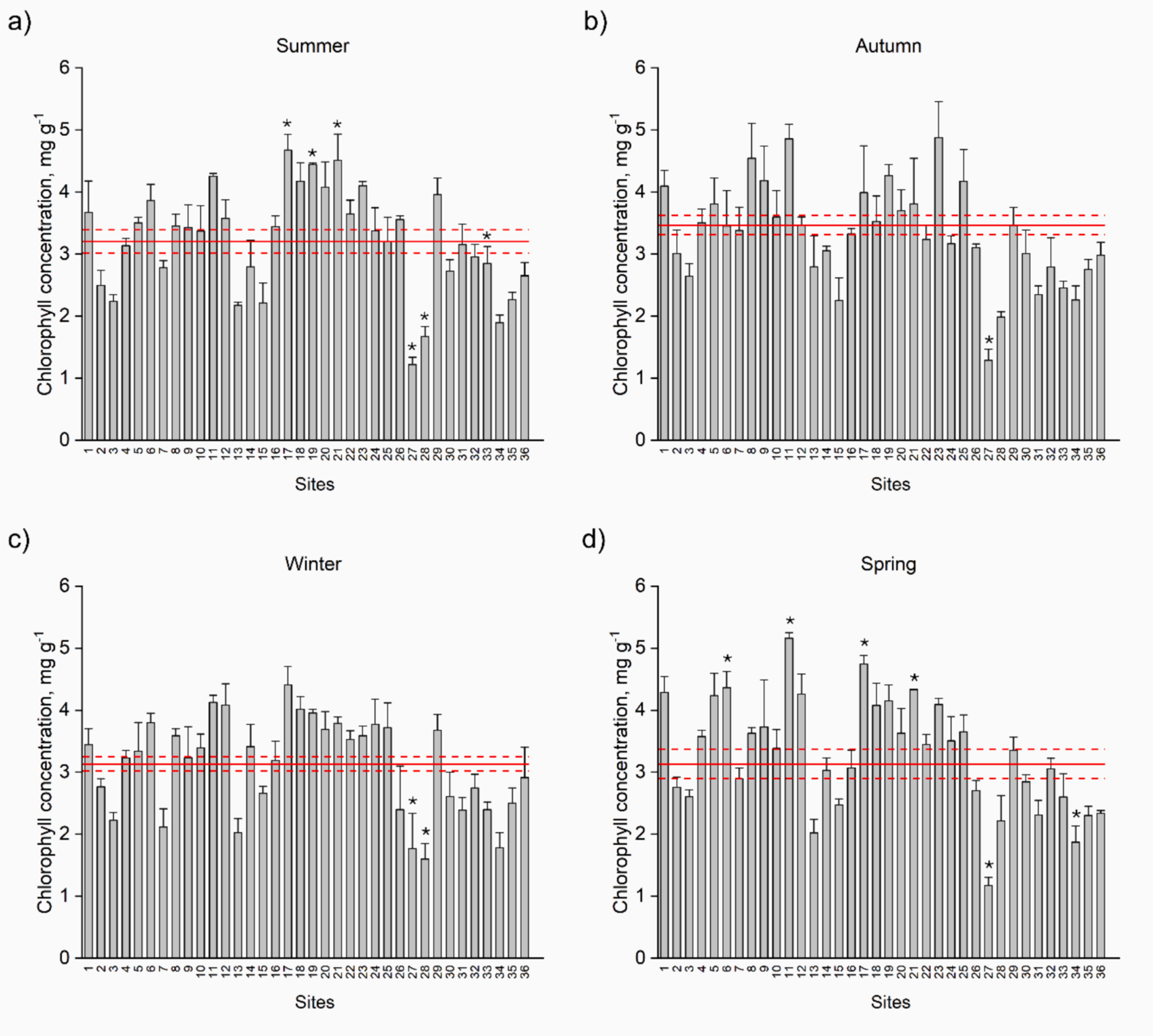

Fig. 5. Chlorophyll content of leaf samples in summer (a), autumn (b), winter (c) and spring (d). Red lines indicate the mean ± standard error of the control, and significant differences ($p < 0.05$) from the control sample are marked with an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pollution data from the two monitoring stations in Debrecen, including precipitation, temperature and wind parameters. Correlation and regression analyses were performed with leaf dust deposition and leaf size. PCA was performed to examine seasonal variations and relationships among dust deposition, pigment content, RWC, pH and AAC.

3. Results

3.1. Differences in parameters among sites and seasons

Significant differences were found in the dust content on leaf surface, chlorophyll and carotenoid contents, and AAC among sites and seasons (Supplementary Information Table 2). Significant differences in the dust content were observed compared to the control. The differences were positive across seasons, with the most pronounced effects observed in winter and moderate differences in autumn and spring (Fig. 4).

Significant differences in the chlorophyll content were observed compared to the control, but these differences were found only at a few sites in summer, autumn and spring. The chlorophyll content fluctuated around the control level across seasons (Fig. 5).

Significant differences in the carotenoid content were observed compared to the control in autumn, winter and spring seasons. The carotenoid content fluctuated around the control level across seasons but not significantly ($p > 0.05$) (Fig. 6).

A significant difference in AAC was observed compared to the control but only in spring. AAC fluctuated around the control across the seasons but not significantly ($p < 0.05$), except in autumn, when it was higher than the control at all sites (Fig. 7).

3.2. Spatiality in the physiological parameters

A significant correlation was observed between the measured parameters (dust, chlorophyll and carotenoid contents and AAC) and distance from the city centre (Supplementary Information Table 3). Regression analyses were performed following correlation analysis to examine the spatial relationships between measured parameters and the distance from the city centre. Significant spatial relationships were observed between distance and leaf surface dust accumulation as well as

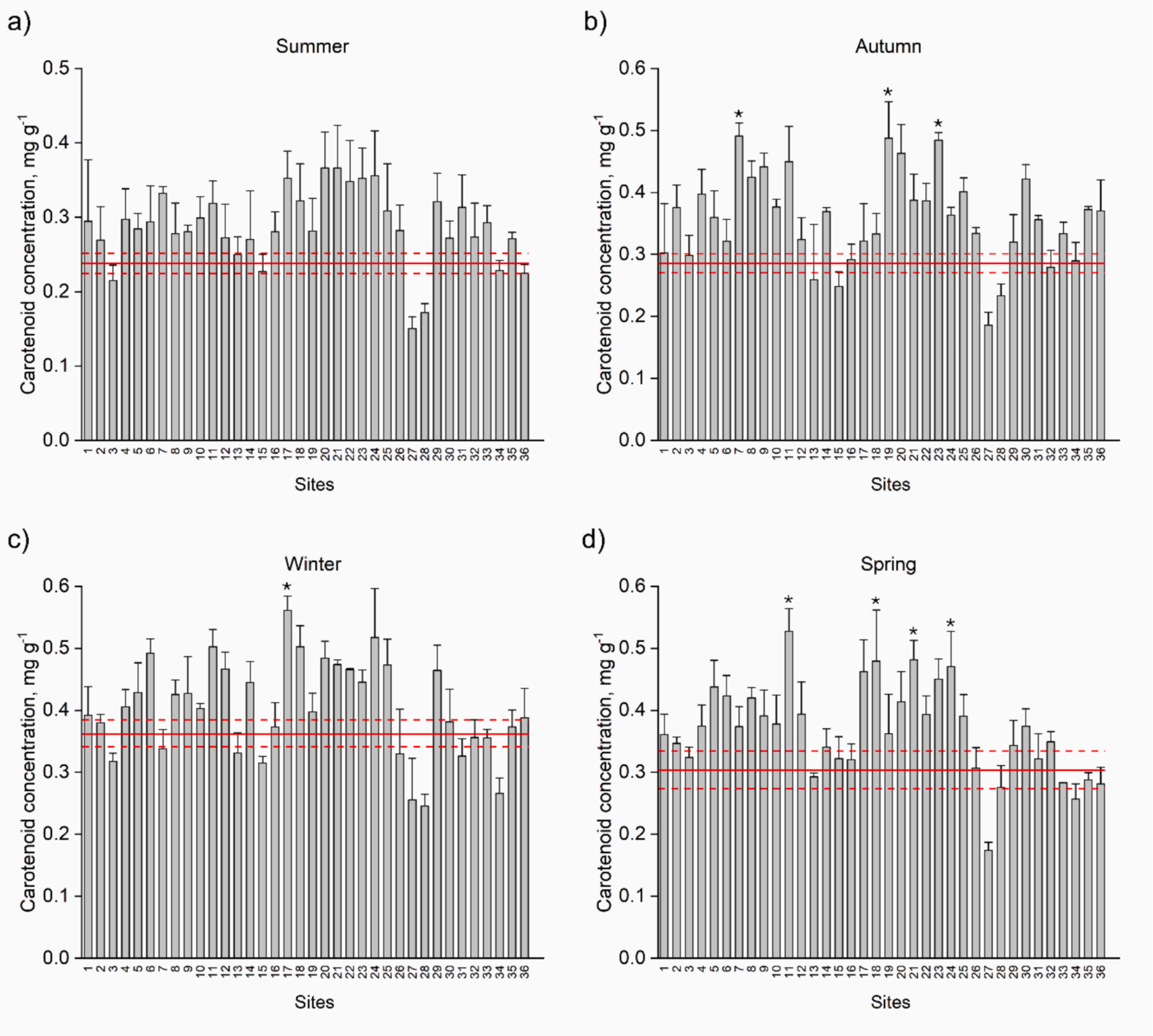

Fig. 6. Carotenoid content of leaf samples in summer (a), autumn (b), winter (c) and spring (d). Red lines indicate the mean ± standard error of the control, and significant differences ($p < 0.05$) from the control sample are marked with an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

physiological parameters. Dust accumulation on the leaf surface decreased with increasing distance with significant correlations detected in winter and spring ($p < 0.01$), whereas correlations were weak and not statistically significant in summer or autumn (Fig. 8). Similarly, the chlorophyll and carotenoid contents showed significant negative correlations with distance. For the chlorophyll content, significant relationships were observed in autumn, winter and spring ($p < 0.05$), and carotenoid content was significantly associated with distance in autumn and spring ($p < 0.05$). In contrast, AAC exhibited a significant positive correlation with distance from the city centre, increasing with distance, particularly in winter ($p \leq 0.01$) (Table 1.).

A significant negative correlation ($p < 0.001$) was observed between the leaf surface area and deposited dust in each season (Fig. 9). Linear regression analysis explained a small proportion of the variance in each case, indicating a weak predictive relationship.

3.3. Principal component analysis

PCA revealed seasonal differences in the relationships among leaf surface dust deposition, pigment content, RWC, pH and AAC (Fig. 10; Supplementary Information Tables 4–7). In summer, the first two principal components explained 68.3% of the total variance. PC1 separated pigment-related variables (chlorophyll and carotenoid contents) and pH from dust, RWC and AAC. PC2 was primarily defined by an inverse relationship between dust and RWC with dust loading negatively and RWC positively.

In autumn, the first two components explained 55.5% of the total variance. PC1 separated pigment variables from dust and AAC, and PC2 was mainly characterised by negative loadings of dust and AAC and a positive loading of pH. RWC made a moderate contribution to PC2 and was positioned separately from dust.

In winter, the explained variance of the first two components was highest (71.9%). PC1 was strongly associated with the chlorophyll and carotenoid contents (positive loadings), whereas AAC loaded negatively. PC2 was defined by the close alignment of dust and RWC (positive loadings), while pH loaded in the opposite direction.

In spring, the first two components explained 57.9% of the total variance. PC1 was primarily driven by the chlorophyll and carotenoid contents, with AAC loading negatively. PC2 was mainly influenced by dust (negative loading), while pH showed a positive loading. AAC also contributed negatively. Across seasons, the chlorophyll and carotenoid contents consistently clustered together, whereas the relative positioning of dust, RWC and AAC varied seasonally.

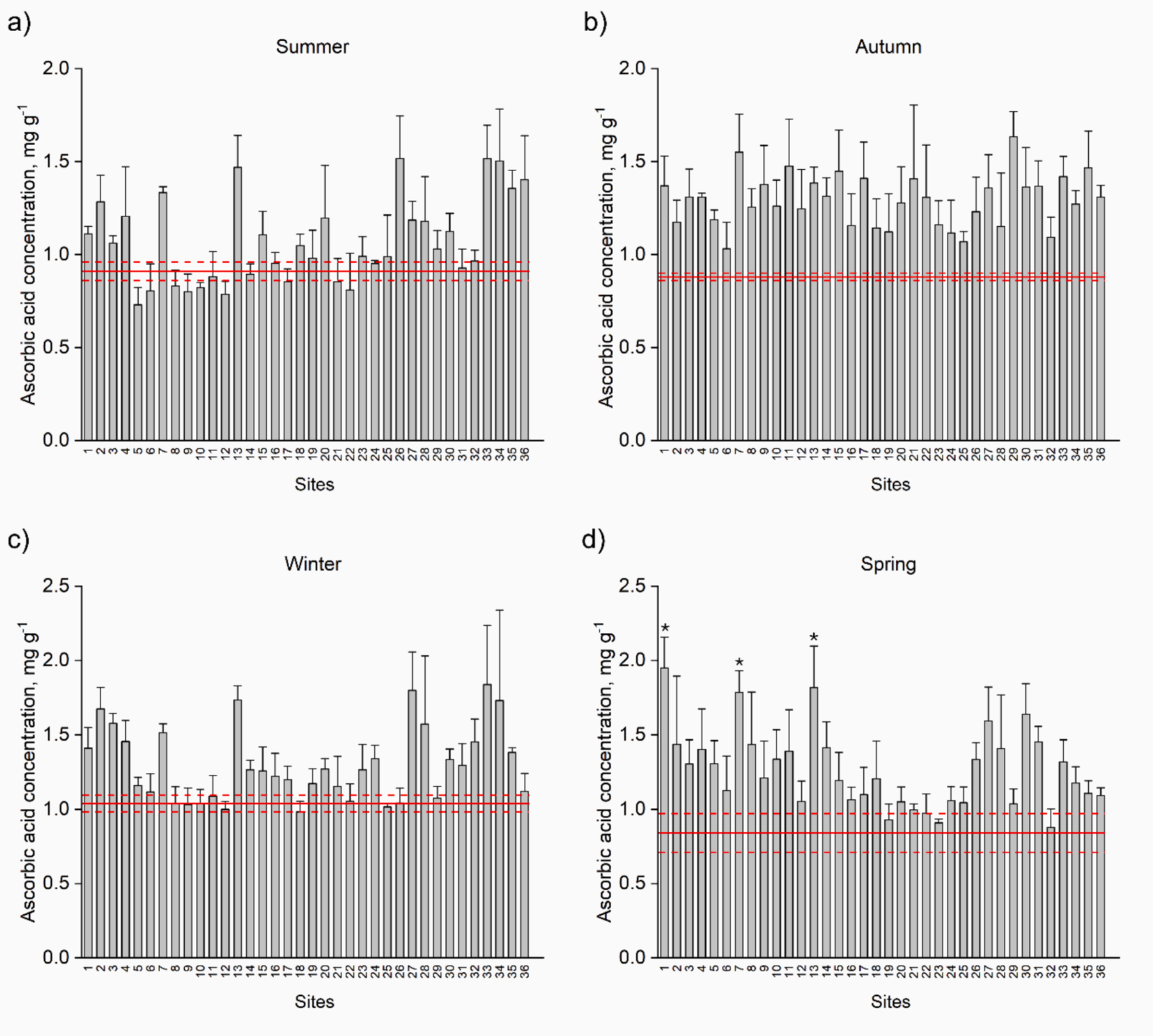

Fig. 7. Ascorbic acid content of leaf samples in summer (a), autumn (b), winter (c) and spring (d). Red lines indicate the mean ± standard error of the control, and significant differences ($p < 0.05$) from the control sample are marked with an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. *Air pollution tolerance Index*

APTI differed significantly from the control in certain urban sites with the highest number of affected bus stops occurring in winter (Supplementary Information Table 2). APTI ranged between 6.6 and 12.0 in all seasons, placing plants in the sensitive category. The highest APTI values were recorded in winter (Fig. 11). There were significant differences in APTI among seasons. Post hoc Bonferroni analysis revealed that summer did not differ significantly from autumn, and winter did not differ significantly from spring. APTI increased from summer to winter, followed by a decrease in spring.

3.5. *Correlation with air pollutants and meteorological data*

The carotenoid contents at several locations showed a positive correlation with CO levels. In contrast, we found a negative correlation between temperature and the carotenoid content. There was a positive relationship between the carotenoid content and wind speed. The correlations between carotenoids and other air pollutants, such as NO, NO_2, NO_x, PM_{10}, $PM_{2.5}$ and SO_2, were weaker or mixed. Although the other parameters did not correlate as strongly with the values measured at the stations as the chlorophyll content did, certain patterns were observed: in studied area 31, dust, chlorophyll content and AAC correlated with CO, showing negative correlations (Supplementary Information Tables 8–10).

4. Discussion

Plant physiological responses in the urban environment are primarily structured by spatial pollution gradients and seasonal environmental variability. Although both spatial location and seasonal variation influenced plant physiological traits, seasonal environmental variability generally had a stronger effect on biochemical parameters, indicating that temporal climatic factors play a primary role in modulating plant responses.

4.1. *Spatial variation in plant physiological responses*

Significant differences were found between the control and urban sites in dust deposition, chlorophyll content and carotenoid content. The dust content was significantly higher at urban sites, confirming stronger exposure to traffic- and industry-related PM. Regression analysis supported this pattern, showing decreasing dust deposition with increasing distance from the city centre in winter and spring. These findings align

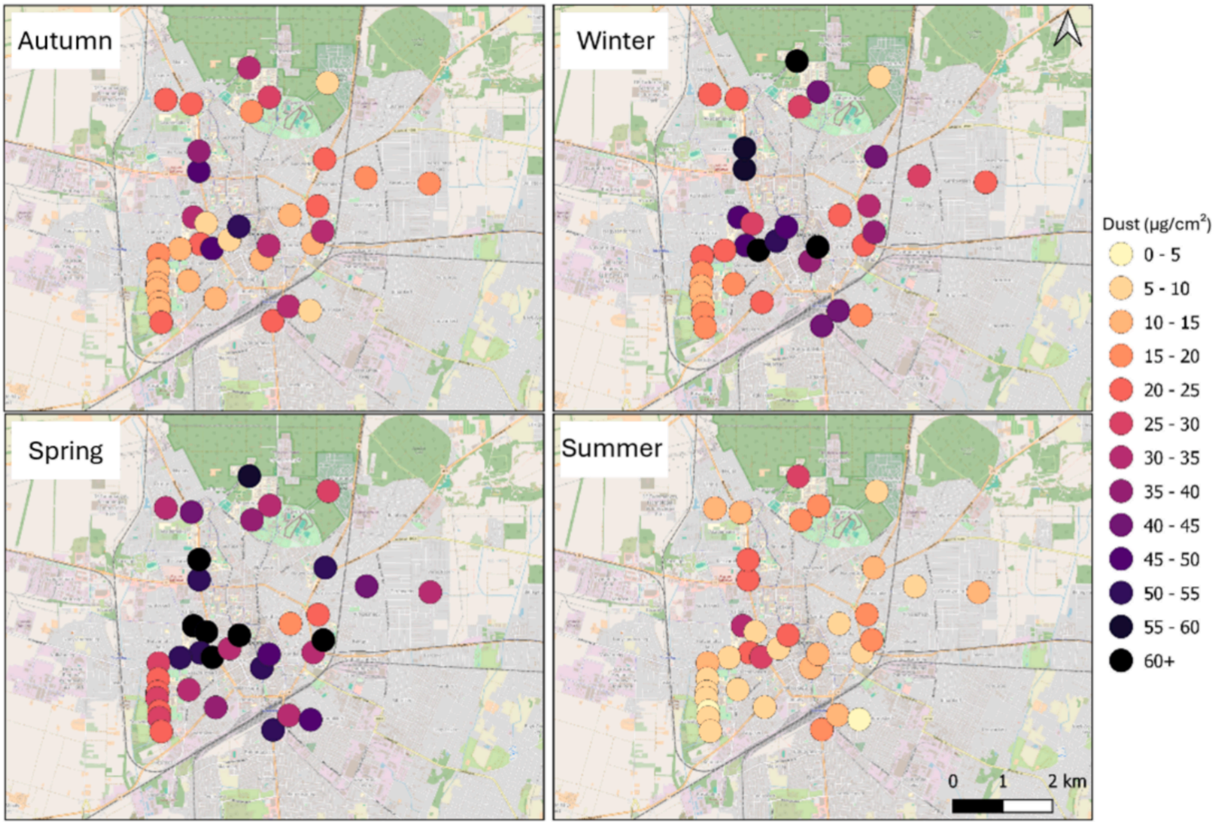

Fig. 8. Spatial distribution of particulate matter in the city.

Table 1
Regression parameters between distance from the city centre and measured physiological and environmental variables across seasons. Notations: Chl = chlorophyll content, Car = carotenoid content, AAC = ascorbic acid content. An asterisk indicates statistical significance ($p < 0.05$).

	Summer		Autumn		Winter		Spring	
	p	R^2	p	R^2	p	R^2	p	R^2
Dust	0.215	0.031	0.069	0.064	0.007*	0.192	0.001*	0.261
Chl	0.116	0.071	0.030*	0.131	0.047*	0.111	0.032*	0.128
Car	0.249	0.039	0.048*	0.110	0.069	0.094	0.038*	0.121
AAC	0.120	0.070	0.131	0.066	0.007*	0.195	0.257	0.038

with previous observations of elevated urban particulate exposure in Debrecen and Vienna[13,27,34].

Chlorophylls and carotenoids function as integrated components of the photosynthetic apparatus[28], and carotenoids contribute to photoprotection under stress[35,36]. The consistently strong positive correlation between the two pigments ($p < 0.001$) confirmed their coordinated regulation across sites and seasons. However, spatial variation compared to the control did not follow a uniform directional trend. Instead, pigment responses differed among sampling sites, reflecting local heterogeneity in pollution intensity, microclimate and vegetation structure. Similar spatially variable pigment patterns have been reported under heterogeneous urban stress exposure [37].

Although reduced pigment contents under elevated pollution have frequently been described[38,39], and higher pigment contents at reference sites have also been observed[40], other studies have reported pigment content increases under moderate stress conditions[27,41]. Therefore, the heterogeneous spatial distribution of our sampling sites may mask a simple linear relationship. The observed variability suggests the regulated adjustment of the photosynthetic system rather than uniform pigment degradation[42].

The carotenoid content showed a positive association with CO levels. Although the direct regulation of carotenoid metabolism by CO has not been demonstrated, previous research has indicated that CO can influence chlorophyll-related physiological processes[43]. This association may reflect oxidative or pollution-related signalling effects rather than direct metabolic control.

A significant negative correlation between leaf surface area and dust deposition was also detected. Smaller leaves may retain proportionally more particulates due to their aerodynamic characteristics and boundary layer effects, consistent with previous findings on leaf morphology and dust retention[44].

4.2. Seasonal variation in plant physiological responses

Seasonal environmental variability influenced antioxidant regulation and the coordination among physiological traits. The content of ascorbic acid, a key non-enzymatic antioxidant, showed pronounced seasonal differences with higher contents at urban sites in spring and a significant positive correlation with distance during winter. Given its established role in oxidative protection and metabolic regulation [45–48], these fluctuations likely reflect the seasonal modulation of antioxidant capacity under variable urban environmental conditions. Winter-specific spatial trends may also be influenced by seasonal pollution sources and urban microclimatic effects, including residential

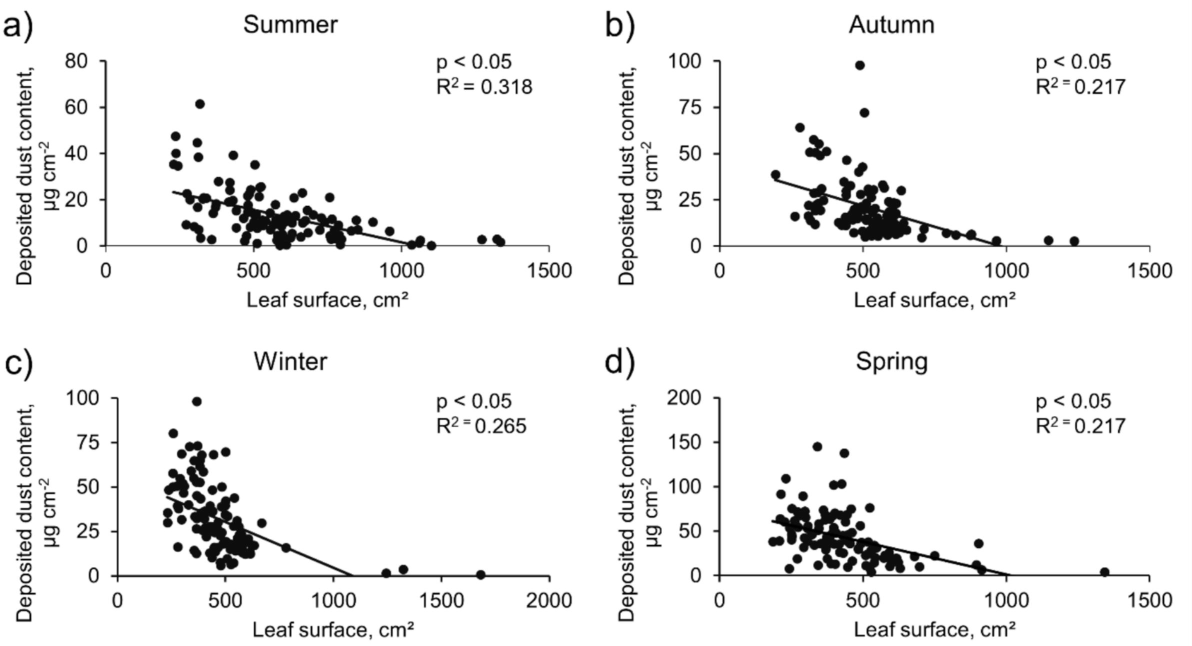

Fig. 9. Scatter plot with regression line between leaf surface and deposited dust content in a) summer, b) autumn, c) winter and d) spring.

heating and the urban heat island phenomenon described for Debrecen [49].

In addition to antioxidant dynamics, seasonal shifts reorganised the relationships among pigment content, RWC, dust deposition and pH. These associations were not constant across summer, autumn, winter and spring, indicating physiological coordination changes throughout the year rather than following a single, stable pattern. In summer, the pigment content and hydration status formed a dominant structural component, and dust deposition showed an opposite relationship to RWC. This suggests that surface particulate load and leaf water balance interact differently under peak vegetative conditions. AAC did not consistently follow pigment dynamics during this period, indicating the partially independent regulation of antioxidant responses.

Autumn was characterised by more complex trait relationships, with dust and AAC contributing more prominently to the overall differentiation among variables. The hydration status appeared less directly related to dust compared to summer, suggesting that transitional environmental conditions influence water regulation through multiple interacting factors. Comparable seasonal adjustments in physiological regulation under changing environmental exposure have been previously described[50,51].

In winter, trait relationships were more clearly structured with dust deposition and RWC aligned and AAC remaining separate from pigment variables. This seasonal reorganisation may reflect temperature-related changes in transpiration and antioxidant regulation[52,53]. In spring, pigments again formed a coherent group, and dust and AAC contributed more strongly to trait differentiation. The shifting position of AAC across seasons indicates dynamic antioxidant adjustment in response to changing environmental conditions.

The carotenoid content showed a negative association with temperature, suggesting enhanced photoprotective investment under cooler conditions, consistent with the results of previous studies[54]; Kreslavski et al., 2023). Wind speed was positively associated with the carotenoid content, reflecting mechanical stimulation or altered pollutant redistribution under stronger airflow. Mechanical stress effects on pigment and secondary metabolite contents have been reported in several plant systems [55–57].

Overall, the seasonal results demonstrate that antioxidant regulation, pigment coordination, hydration status and particulate deposition interact dynamically under fluctuating urban environmental conditions. The data indicate seasonally modulated physiological adjustment, rather than reflecting a uniform stress gradient.

4.3. Air pollution tolerance and bioindicator potential

The APTI values of *H. helix* consistently fell within the sensitive category according to Singh et al. (1983), indicating limited physiological tolerance to urban air pollution. Published data on the APTI of this species are scarce. Chauhan et al. [17] reported a moderately tolerant value (10.92), but their assessment was based on indoor conditions. In a broader survey of climbing species, Pandey et al. [58] demonstrated substantial interspecific variability in tolerance categories. Similarly, urban studies in Budapest showed that ornamental rose cultivars had APTI values within the sensitive range [15], and deciduous tree species, such as *Acer platanoides*, displayed spatial differences in APTI across urban gradients[40].

In the present study, the APTI of *H. helix* was primarily informative rather than indicative of a high tolerance. Although its physiological sensitivity suggests limited suitability as a pollution-tolerant planting species, this characteristic may enhance its value as an urban air quality bioindicator. Previous research has demonstrated its substantial PM retention capacity, including fine (<2.5 µm) and ultrafine (<1 µm) particles at high surface densities [11]. Therefore, despite its sensitive classification, *H. helix* may contribute to urban greening strategies through complementary ecological functions, combining its bioindication potential with effective particle interception.

Due to the inherent heterogeneity of urban environments, plant physiological responses may be influenced by multiple environmental factors in addition to air pollution, including microclimatic conditions, surface characteristics and local environmental variability. At the same time, the consistent spatial patterns observed across sites differing in traffic intensity and distance from the city centre suggest that air pollution plays an important role in shaping these responses. Therefore, the results should be interpreted as reflecting the combined effects of urban environmental conditions, with air pollution as a key contributing factor.

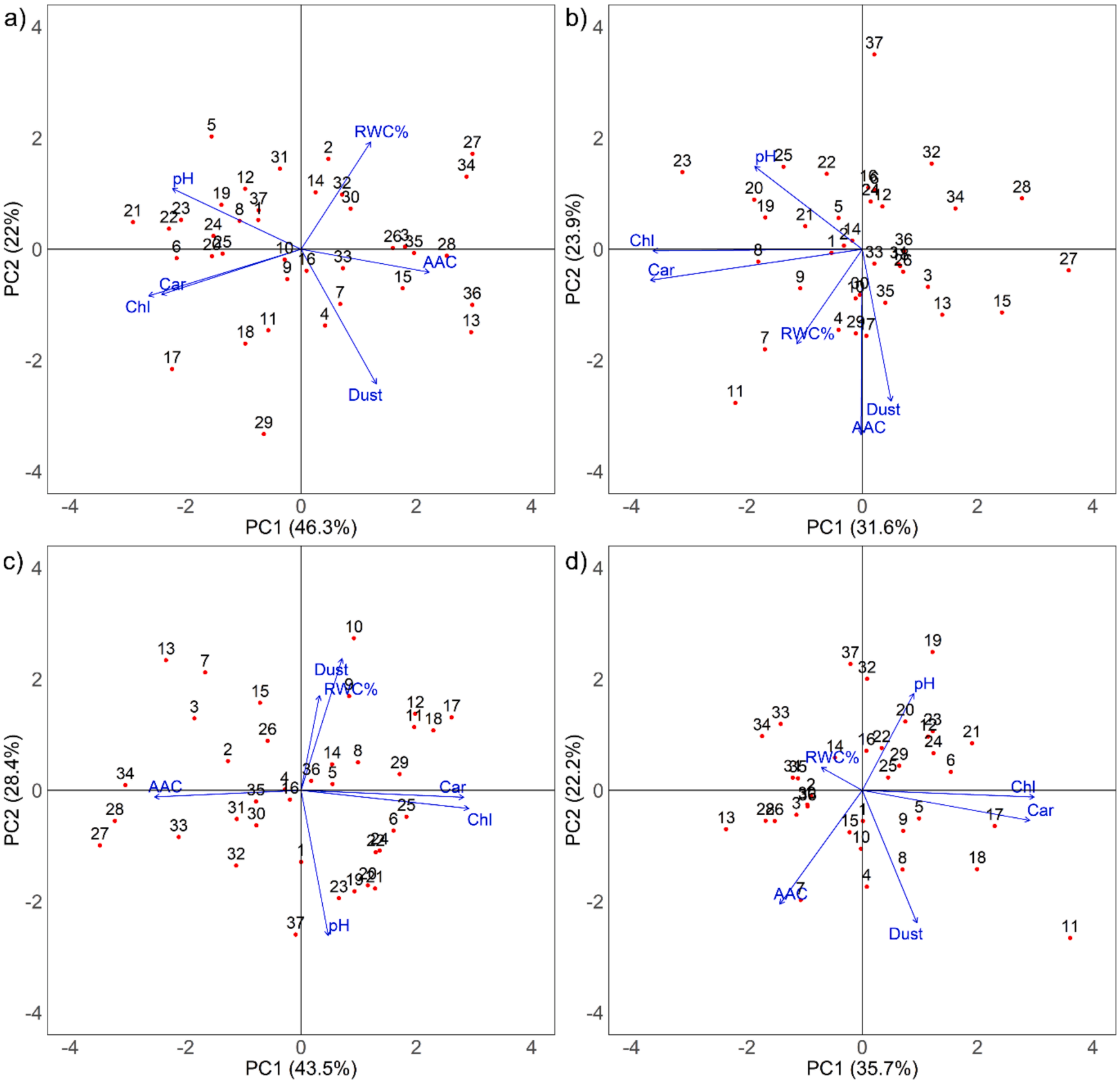

Fig. 10. PCA biplot of sample sites in summer (a), autumn (b), winter (c) and spring (d) based on leaf surface dust deposition, pigment content, RWC, pH and AAC.

5. Conclusion

This study demonstrated that urban environmental conditions, including particulate deposition and meteorological variability, significantly influence leaf biochemical characteristics and reorganise physiological coordination across seasons. Plant responses to urban pollution were not uniform but depended on the combined effects of spatial pollution gradients and seasonal climatic factors. The associations between biochemical parameters and environmental variables, such as carbon monoxide, temperature and wind speed, highlight the complex interactions between atmospheric conditions and plant metabolic regulation. The results suggest that oxidative stress responses and photoprotective pigment dynamics play key roles in seasonal adjustment under urban exposure.

These findings support the relevance of *H. helix* as a bioindicator for assessing spatial and seasonal variability in urban air quality. Although its physiological sensitivity limits its use as a pollution-tolerant planting species, it exhibits measurable physiological responses to environmental stress and persists under urban conditions, supporting its suitability for biomonitoring applications. Given its evergreen habit and widespread occurrence in urban landscapes, *H. helix* represents a promising model for year-round air quality assessment.

From a practical perspective, these characteristics suggest that *H. helix* can be effectively integrated into urban environments as a multifunctional species. As a climbing plant, it is not restricted to container-based planting and can extend over vertical structures, such as walls, fences and metal frameworks (e.g., bus stops, bicycle shelters or shopping cart storage areas), increasing its spatial coverage and functional efficiency. In such applications, it can contribute to urban greening and simultaneously serve as a passive bioindicator, enabling

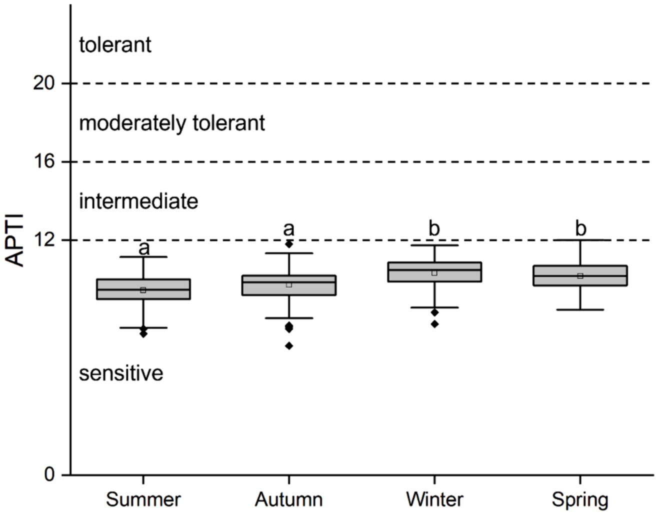

Fig. 11. APTI for *H. helix* leaves across all seasons. Different letters above the boxplots indicate significant differences between groups ($p < 0.05$).

the detection of fine-scale spatial and seasonal variations in air pollution. In this context, these findings have direct implications for urban environmental management, supporting the application of *H. helix* in biomonitoring programs and emphasising that the species used in green infrastructure should be selected based on their functional role, whether for pollution mitigation or environmental monitoring. Broader geographical validation could further validate its biomonitoring potential.

CRediT authorship contribution statement

Bianka Sipos: Writing – original draft, Methodology, Investigation, Data curation. **Vanda Éva Abriha-Molnár:** Methodology, Investigation, Data curation. **Semonti Mukherjee:** Methodology, Investigation, Data curation. **Dina Bibi:** Methodology, Investigation, Data curation. **Fanni Zsófia Bárány:** . **Viktor Oláh:** Writing – review & editing. **Béla Tóthmérész:** Writing – review & editing. **Tibor Magura:** Writing – review & editing. **Edina Simon:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Edina Simon reports was provided by University of Debrecen. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cacint.2026.100371.

Data availability

Data will be made available on request.

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