Short thesis for the degree of Doctor of Philosophy (PhD)

Assessment of Anthropogenic Air Pollution Exposure in Urban Trees

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1. INTRODUCTION

Urbanisation intensity and a substantial increase in human activities are deteriorating urban air quality throughout Central and Eastern Europe. Traffic, household heating, and various industrial processes contribute gaseous air pollutants such as SO_2, NO_2, O_3, and CO, as well as fine particulate matter (PM_{10} and $PM_{2.5}$), affecting urban vegetation through chronic biochemical stress (Evangelopoulos et al., 2020; Tsai et al., 2015; Zhang et al., 2022). Within individual city blocks, pollutant concentrations often vary, reflecting differing emission sources, urban structure, and meteorology.

Plants function as passive biosensors to detect airborne contaminants; they can absorb gases through stomata and the cuticle and accumulate particulates on leaf surfaces (Mulgrew & Williams, 2000; Volkov & Ranatunga, 2006). The dominant gaseous air pollutants, namely SO_2, NO_2 and O_3, disrupt chloroplast membranes, inhibit chlorophyll synthesis and increase ROS production, resulting in oxidative damage to pigment-protein complexes (Jozefíková et al., 2020). Airborne dust accumulates on leaves, potentially blocking stomata, reducing light absorption, and compromising gas exchange, thereby exacerbating the internal physiological stress caused by gaseous pollutants (Adhikary & Sahu, 2023). Changes in chlorophyll (a, b) levels and increased amounts of its degradation products (pheophytins) are an established indicator of pollution intensity and type.

Several indices based on plant bioindicators, notably the Air Pollution Tolerance Index (APTI; Singh et al., 1991), are frequently used to assess urban air quality. However, APTI takes into account only four-leaf parameters: ascorbic acid, total chlorophyll, relative water content and leaf extract pH. Factors such as season, baseline values per species, and non-pollution factors, like drought and extreme temperatures, affect it and limit its applicability across cities (Molnár et al., 2020a; Tripathi & Nema, 2023). Dust accretion has been omitted from APTI, although it influences stomatal function, stability of pigments and temperature of leaves (Gedan, 2017; Guéguen et al., 2019).

This thesis combines atmospheric and plant physiological monitoring of urban air quality in Hungary and introduces a novel bioindicator, the Pigment Integrity-to-Dust Ratio (PIDR). PIDR integrates the ratio of intact chlorophyll to its degradation product pheophytin with the gravimetric leaf dust load, forming a spatially adaptable, mechanistically grounded indicator of urban air pollution stress. This thesis focuses on bioindicator responses in *Ginkgo biloba* and *Hedera helix*, chosen for their known susceptibility, widespread distribution across cities and complementary life forms (deciduous versus evergreen). The work covers Hungarian cities (Debrecen) and one (Budapest), with multi-year monitoring of atmospheric parameters and seasonal measurements of biochemical parameters along pollution gradients.

2. AIMS AND HYPOTHESES

This thesis integrates atmospheric monitoring with plant physiological biomonitoring across four complementary research objectives, each structured around explicitly formulated hypotheses.

Study 1. Effects of COVID-19-induced mobility restriction on air quality in Debrecen (2018–2022)

This study aimed to quantify the effect of reduced anthropogenic mobility during the COVID-19 pandemic on PM_{10} concentrations in Debrecen, Hungary, distinguishing pre-pandemic, pandemic, and post-pandemic periods, and to assess the modulating role of meteorological variables.

H1.1. PM_{10} concentrations were significantly lower during the pandemic and post-pandemic periods relative to the pre-pandemic baseline.

H1.2. PM_{10} concentrations were significantly lower during lockdown months compared to pre- and post-lockdown months.

H1.3. Meteorological variables (wind speed, wind direction) significantly modulate PM_{10} concentration dynamics.

Study 2. Seasonal dynamics of photosynthetic pigments in *G. biloba* as urban biomonitoring indicators in Debrecen

This study aimed to characterise the seasonal variation in photosynthetic pigment concentrations of newly planted *G. biloba* trees along an urban traffic gradient in Debrecen (July–October 2023), and to evaluate their suitability for biomonitoring applications.

H2.1. Chlorophyll concentrations peak in mid-summer (July–August) and decline progressively towards autumn (September–October).

H2.2. Pheophytin concentrations increase concomitantly with seasonal chlorophyll decline, reflecting progressive chlorophyll degradation.

H2.3. Initial exposure to urban air pollutants induces a short-term reduction in chlorophyll and carotenoid concentrations, with prolonged exposure resulting in cumulative pigment loss.

Study 3. Development and validation of the Pigment Integrity-to-Dust Ratio (PIDR) in Budapest

This study aimed to develop and pilot-test PIDR as a novel composite bioindicator integrating chlorophyll degradation status and leaf dust load in *G. biloba* leaves collected across a traffic intensity gradient in Budapest (2023–2024).

H3.1. PIDR shows greater spatial contrast in the pollution-stress signal than APTI alone.

H3.2. PIDR captures temporal changes in leaf physiological condition between sampling periods.

H3.3. PIDR reflects pollutant-specific stress patterns corresponding to measured air pollutant concentrations.

Study 4. Seasonal and spatial air pollution tolerance of *Hedera helix* in Debrecen

This study aimed to assess seasonal and spatial variation in dust deposition and leaf physiological parameters of *H. helix* at 36 urban bus and tram stops across Debrecen over a 12-month sampling period (July 2023 – June 2024).

H4.1. Leaf dust deposition is significantly higher at high-traffic sites compared to the low-exposure reference site.

H4.2. Pigment concentrations and APTI values reflect spatial gradients in urban air pollution intensity.

H4.3. Leaf physiological parameters correlate with distance from the city centre.

H4.4. Spatial differences in physiological parameters reflect the intensity gradient of urban air pollution.

3. MATERIALS AND METHODS

3.1. Study areas and species

Fieldwork was conducted in two Hungarian cities: Debrecen (47°32'N, 21°37'E; the second-largest city in Hungary, population ~200,000; humid continental climate, Köppen Dfb) and Budapest. In Debrecen, *G. biloba* leaves were collected monthly from 28 trees along a 1,200-m western bypass transect (July–October 2023), and *H. helix* leaves were collected monthly from ivy-covered steel mesh structures at 36 bus and tram stops across the city (July 2023–June 2024). In Budapest, *G. biloba* samples were collected from three sites representing a traffic intensity gradient: a high-traffic site (Rákóczi Avenue), a moderate-traffic site (Dembinszky Road), and a low-traffic reference site (Budatétény Rose Garden) in July and September 2023 and September 2024. Atmospheric PM_{10} concentration data for Debrecen (2018–2022) were obtained from three monitoring stations operated by the Hungarian Meteorological Service (Kalotaszeg tér, Hajnal Street, Debrecen Klinika Campus).

Fig. 1. Sampling layout in Debrecen city. A total of 28 trees were selected and sampled along the marked line. The red star marks the null point from which the distances of each sampling point were measured. The observation stations: Station 1, Kalotaszeg tér; Station 2, Hajnal Street; and Station 3, Debrecen Klinika Campus for monitoring air pollutants are indicated in the insert.

Fig. 2. Location of sampling sites and air pollution monitoring stations: Station 1 (S_1), Kalotaszeg tér; Station 2 (S_2), Hajnal Street; and Station 3 (S_3), Debrecen Klinika Campus. Two weather stations, M_1: Macs Met (Agrometeorological Observatory of Debrecen) and M_2: International Airport of Debrecen (HMS). The map was created using ArcMap version 10.4.

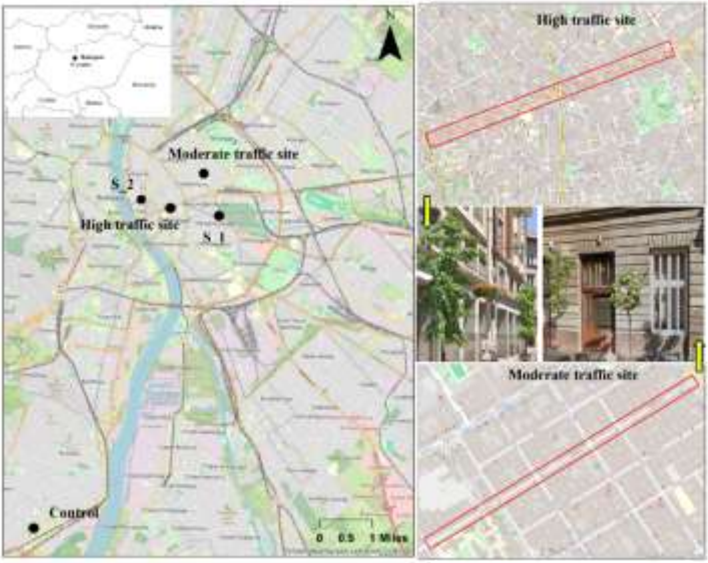

Fig 3. Map of the Budapest study area showing sampling and monitoring locations. The map was created using ArcMap version 10.4. Leaf samples of Ginkgo biloba were collected from three different sites: the control at Budatétényi Rose Garden (C), a site with moderate traffic in Dembinszky Street, and a site with high traffic in Rákóczi Avenue. Air pollutant data (CO, NO_2, O_3, PM_{10}, $PM_{2.5}$, SO_2) were obtained from nearby National Air Quality Monitoring Network stations: Budatétény Rose Garden (C), Station 1 (S1) in Teleki Square, and Station 2 (S2) in Erzsébet Square.

3.2. Dust deposition analysis

Leaf area was determined by scanning individual leaves and analysing scanned images in ImageJ (v. 1.52a). Each leaf was washed with 250 mL of deionised water for 10 minutes on an orbital shaker, followed by a 50 mL rinse; the pooled 300 mL suspension was sieved through a 150 µm mesh to remove coarse debris, then vacuum-filtered through pre-weighed glass microfibre filters (5–8 µm pore size; Munktell 392). Filters were dried to constant weight (±0.1 mg), and dust accumulation was expressed as µg cm^{-2} leaf area (Weerakkody et al., 2017; Szabó et al., 2023).

3.3. Photosynthetic pigment analysis

Fresh leaf tissue (20 mg FW) was homogenised in 5 mL of 96% ethanol at 2,500 RPM for 4 minutes, centrifuged, and the supernatant stored at 4°C in darkness. Absorbances were measured at 470, 649, 665, and 750 nm using a UV/VIS spectrophotometer (BOECO S220) in 1-cm quartz cuvettes following Lichtenthaler et al. (1981). Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid concentrations were calculated according to established equations and expressed as mg g^{-1} fresh weight. Pheophytin concentrations were determined spectrophotometrically before and after acidification with 10 M HCl (~50 µL per extract), which demetallates intact chlorophyll to pheophytin, enabling measurement of pre-existing pheophytin (Vernon, 1960). All extractions were performed under subdued light to prevent photodegradation.

3.4. Air Pollution Tolerance Index (APTI)

APTI was calculated for *H. helix* and *G. biloba* following Singh et al. (1991), integrating four leaf parameters: ascorbic acid concentration (AAC), total chlorophyll (TChl), relative water content (RWC), and leaf extract pH. Classification of species as sensitive (APTI < 16) or tolerant (APTI > 16) was based on established thresholds (Molnár et al., 2020; Simon et al., 2021).

3.5. Pigment Integrity-to-Dust Ratio (PIDR)

PIDR is a novel composite bioindicator developed in this thesis that simultaneously quantifies internal biochemical damage (chlorophyll-to-pheophytin ratio) and external particulate exposure (gravimetric leaf dust load). It is calculated as:

$$\text{PIDR Ginkgo} = \frac{\left(\frac{\text{Chl-a}}{\text{Pheo-a}} + \frac{\text{Chl-b}}{\text{Pheo-b}}\right)}{2 \times \text{Dust load } (\mu\text{g/cm}^{-2})}$$

Raw PIDR values were normalised as fold-changes relative to the control-site median for each sampling date, then classified into stress categories using inverse hyperbolic sine (asinh)-transformed thresholds (0%, 50%, and 80% pigment loss relative to control). Zone-level deviations were summarised using median ± Median Deviation from Control (MDC) statistics to minimise the influence of outliers. Accordingly, stress categories were defined as: Minimal to No Stress: $\Delta i \geq$

T1.0, Low stress: T0.50 < Δi < T1.0, Moderate Stress: T0.20 < Δi ≤ T0.50, Severe Stress: Δi ≤ T0.20

3.6. Statistical analysis

Statistical analyses were conducted in IBM SPSS Statistics (version 21) and R v. 4.1.2. Temporal comparisons of PM_{10} across pre-pandemic, pandemic, and post-pandemic periods used one-way ANOVA with Tukey HSD post-hoc tests; wind-rose analyses were performed using WRPLOT View. Seasonal pigment dynamics were assessed by one-way ANOVA (Debrecen *G. biloba*) and two-way ANOVA (*H. helix*, factors: site × season). Spatial autocorrelation between biochemical parameters and distance from the city centre was tested by Pearson and Spearman rank correlations. Multivariate patterns were examined by Principal Component Analysis (PCA). PIDR comparisons across sites were based on nonparametric statistics (median, MDC) after an asinh transformation.

4. RESULTS AND DISCUSSION

4.1. Effect of COVID-19 lockdown on PM_{10} concentrations in Debrecen

Analysis of PM_{10} data from three monitoring stations over 2018–2022 revealed that pandemic-period PM_{10} concentrations were significantly lower than pre-pandemic levels at two of the three stations ($p < 0.05$). This reduction was most pronounced during the strict lockdown months (March–April 2020), when vehicular traffic was substantially curtailed. Notably, Station 1 (Kalotaszeg tér) recorded elevated PM_{10} in spring 2021 during the second lockdown (March–May 2021: 57–66 $\mu g\ m^{-3}$), attributable to low wind speeds and a stable boundary layer that caused localised pollutant accumulation near a major traffic junction, illustrating that meteorological conditions can override emission reductions at specific urban sites. The post-pandemic period showed an intermediate pattern, with PM_{10} partially recovering towards pre-pandemic levels as mobility normalised. These findings are consistent with observations from other CEE cities, where reductions in NO_2 and PM during COVID-19 lockdowns confirmed the dominant role of traffic emissions in urban particulate loading (Salma et al., 2020; Shi et al., 2021).

Wind direction and speed were significant predictors of PM_{10} concentrations ($p < 0.05$). North-northwest winds, which are the prevailing direction in Debrecen, were associated with elevated PM_{10} at downwind stations, while high wind speeds ($> 3\ m\ s^{-1}$) consistently reduced PM_{10} levels by promoting atmospheric dispersion. Transboundary PM transport, including episodic Saharan dust intrusions in spring, contributed to temporary PM_{10} peaks that were not attributable to local emission sources, complicating the interpretation of lockdown effects during spring 2020.

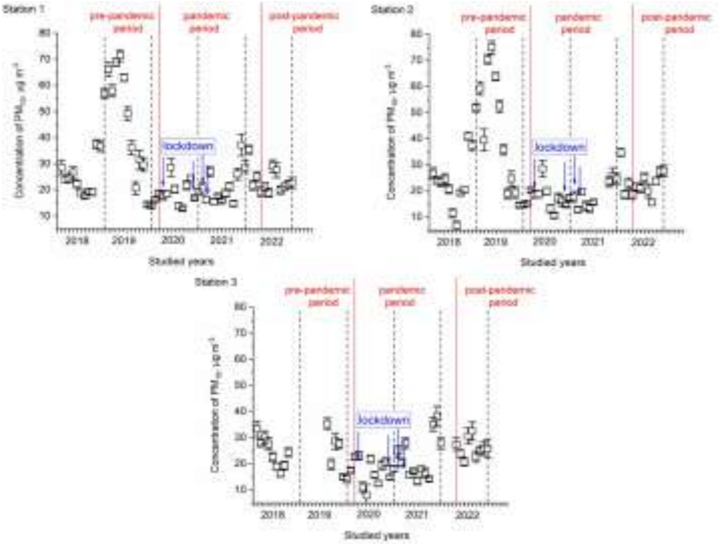

Fig. 4. Daily concentration of PM_{10} (mean ± SE) during the three studied periods (pre-pandemic, pandemic, and post-pandemic) in each month. Pre-pandemic period: before March 2020, pandemic period: March 2020–February 2022, post-pandemic period: after March 2022. Notations: blue arrows indicate the time of lockdowns.

4.2. Seasonal pigment dynamics in *G. biloba* in Debrecen

During our experiments, the highest levels of both chlorophylls and total chlorophyll were detected in July, decreasing progressively throughout August and September until October. Our findings align with leaf development and ageing. Following the opening of leaves in May, cell production and leaf size increase, and chloroplast disassembly begins via the dephytonase pathway, which converts chlorophylls into pheophytin, a derivative of chlorophyll. Pheophytin concentrations increase from July to October. Pheophytin b accumulates fastest, suggesting that in newly transplanted urban *G. biloba*, the decline in total chlorophyll during autumn occurs in a standard, predictable way.

Concentrations of the remaining carotenoids remain near the levels observed during July until the month of August, and the total amount of chlorophyll decreases to negligible levels in September-October in tandem with the pigments described earlier. This might suggest a maintained capacity to maintain carotenoid levels, protecting the leaf from photoinhibition throughout the summer peak of photosynthetic rates, before they decline along with chlorophylls. The APTI index follows the trends observed for chlorophylls, decreasing in the summer-to-autumn timeframe. We found lower values at locations near street intersections with heavy traffic. This first provides spatial evidence that the trees are suffering from air pollution, at least within the chosen transect line. Spatial regression analysis confirmed a significant negative correlation between APTI and proximity to the major road intersections ($p < 0.05$) during September and October, indicating that pollution-mediated stress was superimposed on the background autumnal senescence signal.

Table 1. Values of the APTI parameters and significance differences among months of 2023 by using ANOVA (mean of:15 - 20 leaf samples per month per tree of 28 trees)

Parameters	Months	Mean ± SE	F	*p*
pH	July	3.52 ± 0.16	92.121	<0.001
	August	3.93 ± 0.32		
	September	4.46 ± 0.34		
	October	4.91 ± 0.46		
Total chlorophyll, mg g^{-1}	July	0.94 ± 0.47	2.479	0.065
	August	0.78 ± 0.47		
	September	0.81 ± 0.33		
	October	0.64 ± 0.31		
Ascorbic acid, mg g^{-1}	July	5.08 ± 1.84	11.918	<0.001
	August	5.94 ± 2.07		
	September	7.44 ± 2.57		
	October	8.34 ± 2.42		
Relative water content,%	July	86.43 ± 3.45	0.119	0.949
	August	86.97 ± 7.10		
	September	86.89 ± 4.31		
	October	87.21 ± 4.6 1		

4.3. PIDR as a novel bioindicator of urban pollution stress in Budapest

PIDR values at the high-traffic Rákóczi Avenue site were significantly lower (fold change < 1.0) relative to the Budatétény Rose Garden control during both sampling years, indicating elevated chlorophyll degradation coupled with higher dust accumulation at the traffic-exposed site. The moderate-traffic Dembinszky Road site showed intermediate PIDR values. Stress classification based on asinh-transformed thresholds assigned high-traffic trees predominantly to the 'low stress' and 'moderate stress' categories in September 2023 and September 2024, respectively, while control-site trees were consistently classified as 'minimal to no stress'.

PIDR demonstrated greater spatial contrast between sites than APTI alone, as the combined chlorophyll–dust signal amplified site differentiation relative to APTI's four-parameter composite. Temporal changes in PIDR between July 2023 and September 2023 at the Rákóczi site reflected the seasonal accumulation of traffic-derived dust, while the inter-annual comparison (September 2023 vs. September 2024) indicated a decline in pigment integrity at the dense-traffic site despite year-to-year variation in PM_{10} levels. Correlation of PIDR with monitored air pollutant concentrations (CO, NO_2, O_3, PM_{10}, $PM_{2.5}$, SO_2) showed the strongest associations with CO at the high-traffic site ($\beta = -0.80$), O_3 at the moderate-traffic site, and $PM_{2.5}$ at the control site, confirming that PIDR reflects site-specific pollutant stress patterns. Two methodological artefacts were identified in the September 2023 data: at the high-traffic Rákóczi site, post-rainfall dust wash off artificially reduced the dust-load denominator, inflating PIDR independently of biological stress; at the moderate-traffic Dembinszky site, near-zero Pheo-*a*-a values (0.019 mg g^{-1}, below the detection limit) inflated the Chl-*a*/Pheo-*a* ratio independently of true physiological condition. Both September 2023 values are therefore excluded from quantitative temporal comparisons.

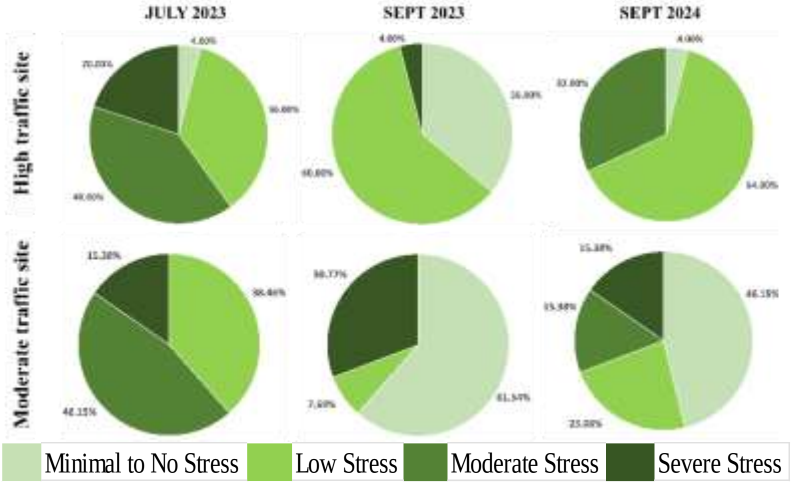

Fig. 5. Stress classifications of *G. biloba* leaves in moderate and high traffic sites and sampling dates July 2023, September 2023, and September 2024. Stress levels were classified as fixed fractions of the asinh-transformed control median: severe (≤0.20), moderate (0.20-0.50), low (0.50-1.0), and minimal-to-no stress (≥1.0×).

4.4. Seasonal and spatial air pollution tolerance of *H. helix* in Debrecen

Two-way ANOVA revealed significant effects of both site ($p < 0.001$) and season ($p < 0.01$) on all measured physiological parameters, with significant site × season interactions for dust, chlorophyll, and carotenoids, indicating that pollution-related physiological responses of *H. helix* are season-dependent. Dust deposition was significantly higher ($p < 0.001$) at inner-city sites with heavy traffic compared to the Debrecen-Jozsa reference site. Dust accumulation peaked in autumn–winter, coinciding with the residential heating season and reduced precipitation-driven leaf-washing events.

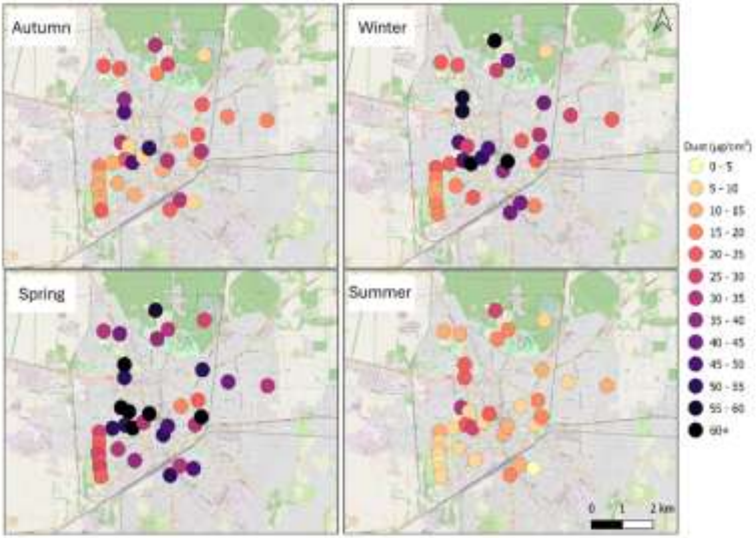

Fig. 6. Spatial distribution of particulate matter in the city

Chlorophyll and carotenoid concentrations were significantly lower at high-traffic sites relative to the reference site in winter and spring ($p < 0.05$). APTI values for *H. helix* were consistently below 16 across all sites and seasons, indicating the species is sensitive to air pollution-a finding consistent with its utility as an early-warning bioindicator. PCA separated sites along PC1, which was dominated by dust load, chlorophyll, and carotenoid loadings, accounting for 46–56% of variance across seasons. Pearson correlations between leaf physiological parameters and distance from the city centre were significant for dust ($r = -0.43$ to -0.61, $p < 0.05$) and chlorophyll ($r = 0.31$ to 0.47, $p < 0.05$) in winter and spring seasons, but non-significant in summer, reflecting the seasonal dominance of heating-related emissions in winter.

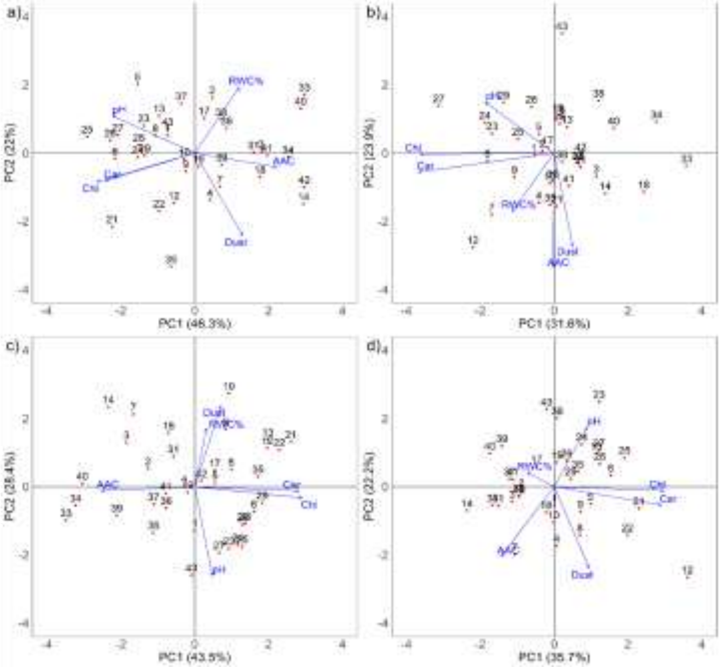

Fig. 7. PCA biplot of sample sites in a) Summer, b) Autumn, c) Winter, and d) Spring

5. CONCLUSION

In this thesis, urban air quality biomonitoring is explored through a novel combination of atmospheric pollution dynamics and plant physiological responses under Central European urban conditions. The major conclusions are as follows: Vehicle traffic volume reductions induced by the COVID-19 pandemic led to a remarkable reduction in PM_{10} in Debrecen. This demonstrates traffic's major role as the most significant source of locally anthropogenic particles. Meteorological factors influence daily PM_{10} levels to a far greater extent (e.g., wind speed and direction) than pollution emission values, whereas natural particulate pollution sources, such as Saharan dust episodes, represent a significant natural error component in Central European air quality studies.

G. biloba exhibits a robust and reproducible seasonal chlorophyll degradation sequence (chlorophyll → pheophytin) during the July–October vegetation period in an urban setting. Newly established trees (< 2 years post-planting) displayed measurable spatial gradients in pigment concentrations corresponding to traffic intensity, demonstrating that even recently planted urban trees can serve as valid biomonitoring tools and that pheophytin accumulation provides a reliable senescence-independent marker of chlorophyll degradation stress.

PIDR, developed and validated as part of this doctoral work, provides a more sensitive and mechanistically interpretable bioindicator of urban pollution stress than APTI alone. By explicitly linking the chlorophyll degradation ratio to the gravimetric dust load, PIDR differentiates fine-scale spatial pollution gradients with greater contrast than APTI, reflects temporal changes in physiological condition between sampling periods, and corresponds to monitored pollutant concentrations. The index is dimensionless, readily accessible using low-cost gravimetric and spectrophotometric methods, and proposed as a complementary tool - not a replacement for-APTI for urban tree biomonitoring.

H. helix, deployed on standardised green wall structures across Debrecen's public transport network, demonstrated consistent seasonal and spatial variation in dust deposition and photosynthetic pigment concentrations. Its APTI-classified sensitivity, broad urban distribution, evergreen habit, and quantifiable particulate accumulation collectively validate its application as a passive, city-scale air quality biomonitor capable of year-round surveillance, including winter pollution episodes undetectable by deciduous species.

Collectively, these findings demonstrate that tree-based biochemical biomonitoring-when supported by methodologically rigorous index development, seasonal stratification, and atmospheric monitoring validation-can provide quantitative, spatially portable, and ecologically interpretable records of urban pollution exposure. This work contributes methodological advances applicable to urban greening policy, green infrastructure planning, and environmental health surveillance in Central and Eastern European cities.

6. NEW SCIENTIFIC RESULTS

•PM_{10} levels indicated heterogeneous patterns characterised by variations including decreases, slight increases, or stability, contingent upon the specific sampling sites under consideration.

•The pigment composition of *G. biloba* exhibits a marked response to traffic-related air pollutant gradients. Our findings suggest that *G. biloba* can serve as a reliable and accurate bioindicator of long-term air quality in urban environments.

•This study tested the Pigment Integrity-to-Dust Ratio (PIDR) as a novel bioindicator that incorporates two key elements of plant stress: (i) the ratio of chlorophyll to its degradation products (pheophytins) and (ii) the quantity of dust deposited on the leaf surface.

•Our results demonstrated that PIDR is a reliable indicator of the response to physiological stress, based on pigment concentration changes and deposited dust concentration.

•We found that PIDR provides a more direct interpretation than APTI in this dataset, appearing as an advance to the current bioindicators, as it reflects complex stresses of urban environments.

•APTI values classified *H. helix* as an air pollution-sensitive species, with variations across sites and seasons.

•The concentrations of dust and pigments in the leaves decreased with increasing distance from the city centre, while the ascorbic acid content increased.

•These findings demonstrate that *H. helix* effectively reflects urban air pollution and environmental stress, supporting its use as a bioindicator in urban ecosystems. Its evergreen habit, particulate matter retention capacity, and sensitivity to environmental change make it a promising model for assessing spatial and seasonal variability in urban air quality.

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Subject: PhD Publication List

Candidate: Semonti Mukherjee
Doctoral School: Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. Sipos, B., Molnár, V. É., **Mukherjee, S.**, Bibi, D., Bárány, F. Z., Oláh, V., Tóthmérész, B., Magura, T., Simon, E.: Assessment of air pollution tolerance of Hedera helix in urban areas.
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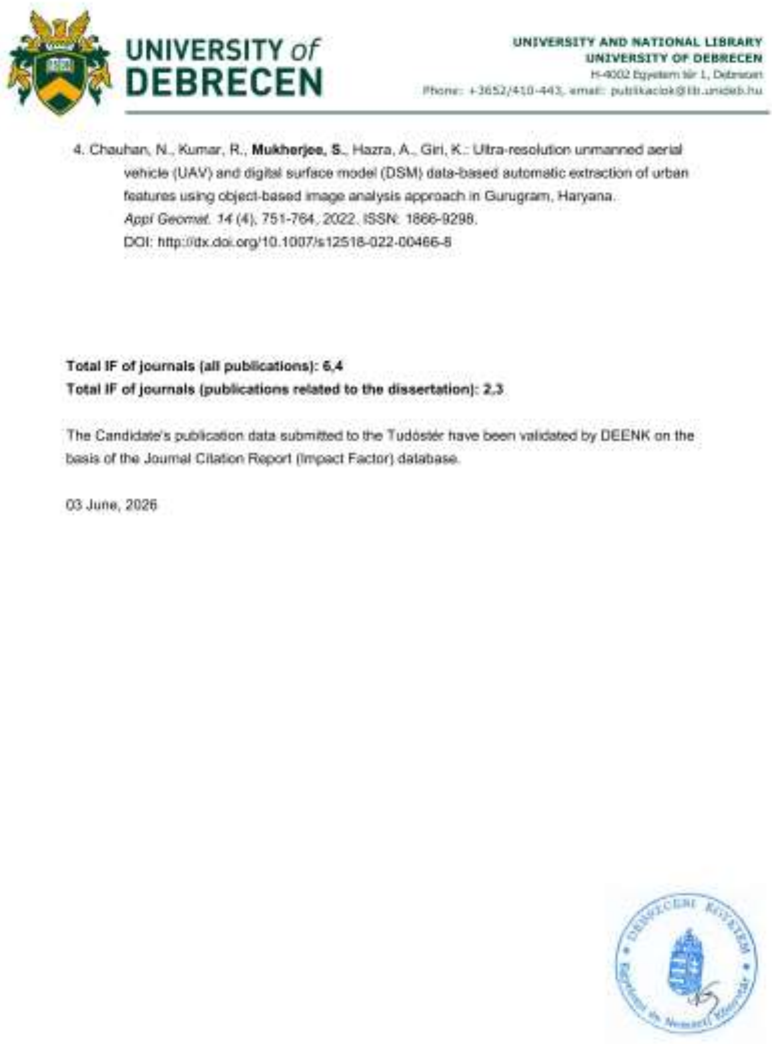

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4. Chauhan, N., Kumar, R., **Mukherjee, S.**, Hazra, A., Giri, K.: Ultra-resolution unmanned aerial vehicle (UAV) and digital surface model (DSM) data-based automatic extraction of urban features using object-based image analysis approach in Gurugram, Haryana.
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Total IF of journals (all publications): 6,4
Total IF of journals (publications related to the dissertation): 2,3

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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