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Lichen Transplantation for Assessing Intra-Urban Environmental Stress: A Case Study in Jelgava, Latvia

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Abstract

Lichen transplantation can reveal spatial variation in biological responses across urban environments, but visible vitality cannot identify specific pollutants. We transplanted the epiphytic lichen *Hypogymnia physodes* from a presumed lower-impact forest area to 20 sites in Jelgava, Latvia and assessed its visible condition monthly for 243 days. The primary endpoint was first-observed necrosis; discoloration was retained as a descriptive secondary state. Thirteen primary transplants developed necrosis, first detected between days 62 and 215. One transplant was right-censored at day 123 after pruning, and six had no observed necrosis by day 243. Kaplan–Meier analysis gave a median time to first-observed necrosis of 184 days (20 primary transplants; 13 events; and 7 censored). A descriptive Urban Stress Index summarized event timing for 19 sites with complete follow-up. Correlations between this index and land-use proportions were statistically non-significant. Exploratory correlations with co-located winter snow Pb and Zn concentrations were negative, contrary to a simple metal-toxicity interpretation. The observed necrosis may reflect altered environmental conditions after transplantation rather than metal accumulation; absent concurrent air and thallus chemistry, microcli-mate measurements, and site replication, pollutant-source attribution is precluded. The approach is therefore best used for exploratory spatial screening and hypothesis generation.

Keywords: *Hypogymnia physodes*; epiphytic lichens; time-to-event analysis; visual vitality; right-censoring; urban microenvironment; exploratory biomonitoring

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1. Introduction

Lichens obtain water and dissolved substances across the thallus surface and respond to both atmospheric deposition and environmental conditions. Their elemental composition, diversity, and vitality have therefore been used to complement instrumental monitoring at spatial scales that fixed stations may not resolve [1,2]. Evidence also links epiphytic lichen diversity with fine particulate matter ($PM_{2.5}$), supporting the inclusion of particulate pollution in urban biomonitoring frameworks [3].

Transplantation standardizes the source material and exposure start by relocating lichens from a lower-impact area to selected monitoring sites. Transplant studies commonly combine chemical accumulation, chlorophyll fluorescence, pigment integrity, or visible condition endpoints [4–7]. Visible discoloration and necrosis are inexpensive to record, but they are semi-quantitative and less specific than chemical or ecophysiological measurements; they should therefore be interpreted as general stress responses rather than diagnostic evidence for a pollutant.

Urban lichen responses may reflect interacting drivers. Here, urban structural composition means the percentage of land around each site assigned to residential, industrial, mixed residential–commercial, public/commercial, and green or surface-water classes. These classes can proxy build density, openness, and nearby activity, but they do not measure emissions. Landscape structure and habitat fragmentation have also been associated with lichen diversity in peri-urban environments [8]. Temperature, humidity, hydration, solar exposure, wind, tree-level microhabitat, and colonization history can be equally important [9,10]. Any association with land use must therefore be treated as exploratory unless concurrent microclimatic and chemical data are available.

The foliose species *Hypogymnia physodes* was chosen because it is widespread in northern Europe and has frequently been used to monitor trace elements and mixed urban deposition [11,12]. In the present experiment, the thalli remained on their original twigs, which were attached to receiving trees. The receiving-tree species was therefore not a new growth substrate; it was considered only as a possible proxy for local placement and microenvironment.

Previous co-located work in Jelgava quantified heavy metals in short-duration winter snow samples and described land-use composition [12–14]. The transplant photographs and site metadata are publicly archived [14]. These external measurements provide environmental context, but snow collected during a seven-day winter interval cannot represent invariant deposition across the full July–March exposure, and no elemental concentrations were measured in the transplanted thalli.

This exploratory study asked whether time to first-observed necrosis varied among 20 Jelgava sites and whether a descriptive timing index showed monotonic associations with land-use composition or co-located winter snow–metal indicators. We hypothesized spatial heterogeneity in visible vitality, while recognizing that the design cannot distinguish pollution effects from microclimatic, placement, or transplant-related stress and cannot identify specific emission sources.

2. Materials and Methods

The transplant experiment was conducted from 1 July 2016 to 1 March 2017. Land-use variables and winter snow–metal measurements were incorporated from earlier work at corresponding locations as contextual exploratory predictors [12–14].

2.1. Study Area

The study was conducted in Jelgava, central Latvia, approximately 40 km southwest of Riga. The low-relief city lies along the Lielupe River and its tributaries and has a humid continental climate with cold winters and mild to warm summers. River corridors, vegetation, and heterogeneous built form may create local differences in moisture, solar exposure, and air movement; these variables were not measured during the transplant experiment.

Jelgava contains residential, commercial, industrial, transport, green-space, and river-adjacent environments. These descriptors were used to characterize urban setting rather than to quantify emission intensity. No contemporaneous traffic counts, industrial emissions inventory, or ambient pollutant concentrations were available for the monitoring sites.

2.2. Selection of Monitoring Sites

Twenty monitoring sites were selected to span contrasting land-use and physical settings (Figure 1). Road-adjacent, residential, industrial-location, public/commercial, green-space, and river-adjacent labels describe site context only. The terms do not constitute measured traffic or emissions categories, and no site was designated as a pollution-source class.

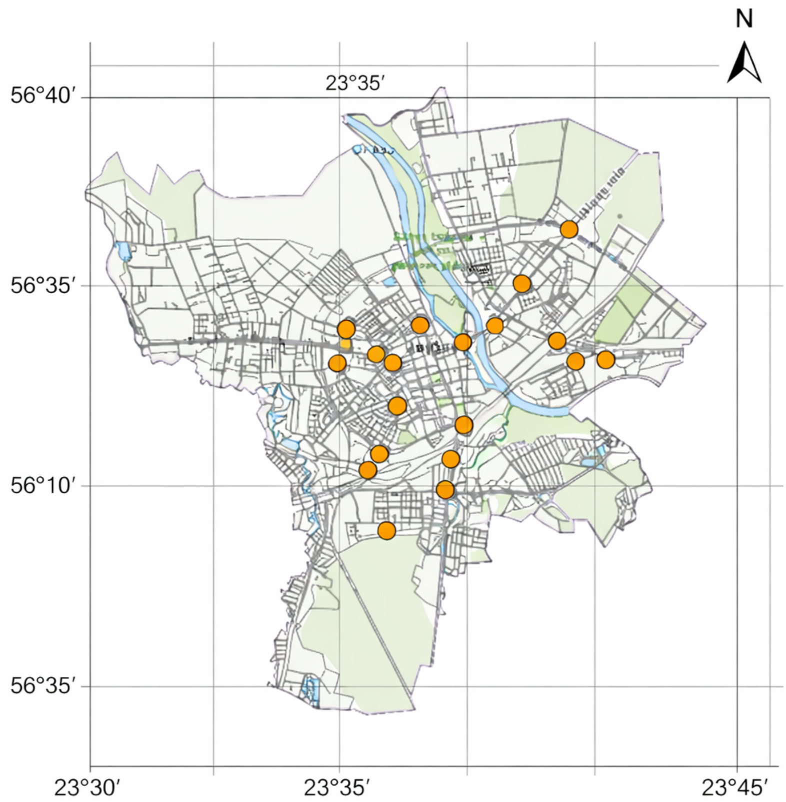

Figure 1. The location of lichen transplants in Jelgava City.

At each site, a receiving-tree branch provided the field support for the lichen-bearing source twig. Attachment height ranged from 1.2–2.2 m above ground. The lichen-bearing source twig remained intact; receiving-tree identity was recorded as a possible microenvironmental descriptor rather than an original-substrate treatment.

The sample size (n = 20 primary transplants) was determined by field logistics rather than an a priori power calculation. One primary transplant was installed per site; the second twig at Site 13 and later replacements at Sites 16 and 17 were used only as qualitative checks and were not independent observations in the analyses.

2.3. Lichen Species and Collection

The epiphytic foliose lichen *Hypogymnia physodes* was selected because of its regional availability and established use in biomonitoring [11,12]. Material was collected from a forested area outside Jelgava. This source area was presumed to be lower impact on the basis of setting and healthy appearance, but concurrent chemical baseline data were not collected; it is therefore described as a source area, not a verified control.

Thalli were collected with 15–20 cm sections of their original twigs. Only thalli without visible baseline discoloration, necrosis, tissue loss, or detachment were selected. Retaining the original twig reduced handling and avoided treating the receiving-tree bark as a new substrate.

2.4. Transplantation Procedure

Transplantation began on 1 July 2016. Source twigs bearing visually intact *H. physodes* were installed at 20 urban sites and followed for 243 days.

Each lichen-bearing source twig constituted one exposure unit for visual assessment; no analytical sample mass was required because neither elemental nor other chemical analysis of the thalli was performed. One primary exposure unit was installed at each site. A second parallel twig at Site 13 was photographed as a qualitative consistency check. Because this twig was not part of a replicated site-level design, it was excluded from all numerical analyses.

All source twigs were attached to receiving-tree branches at 1.2–2.2 m above ground and oriented in a south-westerly (SW) direction. The same field support and attachment method were used throughout, but attachment height was not constant. Distances to potential micro-contamination sources were not systematically recorded. Standardization reduced, but could not eliminate, variation in shade, solar exposure, wind, moisture, and local exposure geometry among sites.

Thin metal wire was used at every site to secure each source twig to the receiving-tree branch without direct contact with the thallus (Figure 2). At Site 6, pruning removed the transplant after 123 days; this observation was right-censored at the last documented inspection and excluded from the complete-follow-up index analysis.

(**a**) (**b**)

Figure 2. Examples of source twigs bearing *Hypogymnia physodes* after attachment at (**a**) Jelgava railway station and (**b**) Lielā iela/Dobeles iela.

Georeferenced photographs, site metadata, and land-use variables are available in the public Mendeley Data repository [14].

2.5. Vitality Assessment and Monitoring Schedule

Transplants were inspected approximately monthly. Each observation was compared with the baseline photograph and assigned to an operational visual state (Table 1). The original field protocol did not use a calibrated color card, a quantitative damaged-area threshold, observer blinding, or inter-observer reliability testing. Accordingly, the observations are treated as semi-quantitative. The recorded inspection date is the first detection date; the true onset lies between that inspection and the preceding visit.

The primary endpoint was first-observed necrosis. Discoloration without necrosis was retained as a secondary descriptive state and was not counted as a primary event. Biological tissue loss or detachment recorded as deterioration would count as an event; loss attributable to pruning or other external mechanical disturbance was censored. Transplants without necrosis by day 243 were right-censored at day 243.

Table 1. Operational criteria used for the semi-quantitative visual assessment.

State	Operational Visual Criterion	Analytical Treatment
No visible damage	Comparable with baseline; no persistent discoloration, blackening, tissue loss, or detachment.	At risk for necrosis
Discoloration	Persistent departure from the baseline grey-green color without visible tissue death or loss.	Secondary descriptive state; not an event
Necrosis	Persistent dark-brown or blackened damaged area and/or local tissue death visible in the monthly photograph.	Primary event at first detection
Detachment/loss	Partial or complete thallus loss. Biological deterioration was an event; documented pruning or mechanical loss was censoring.	Event or censoring, according to cause

Note: Photographs supported within-site comparison with the baseline but were not color-calibrated; no blinded or duplicate scoring was performed.

For terminology used throughout the text and tables, the operational categories "discoloration" and "no visible damage" correspond, respectively, to the legacy Figure 3 labels "color change" and "good vitality"; the latter figure label denotes the absence of visible damage and does not imply independently verified physiological vitality.

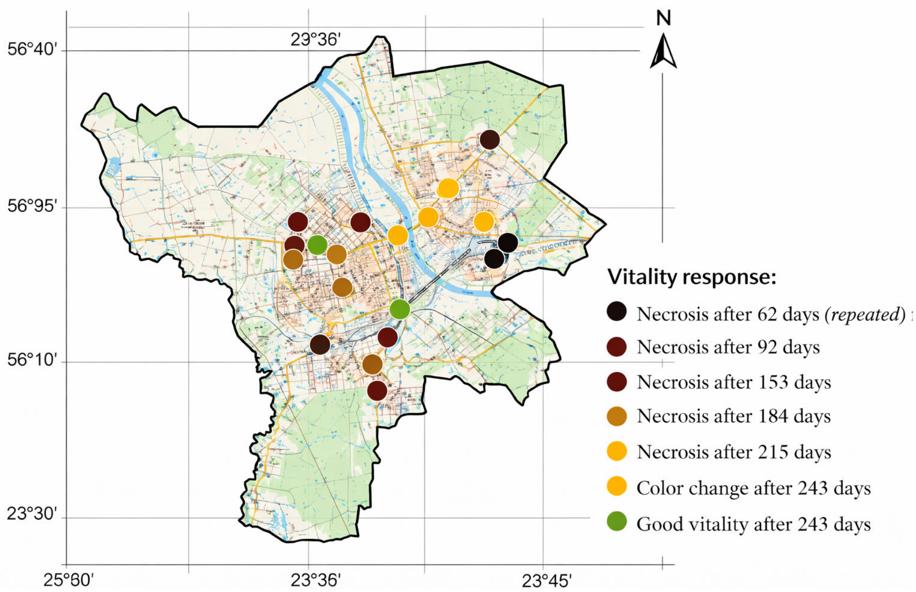

Figure 3. Spatial distribution of the recorded primary-transplant vitality outcomes in Jelgava. For consistency with the operational categories defined in Table 1, the legacy figure label "color change after 243 days" corresponds to "discoloration; no necrosis at day 243", and "good vitality after 243 days" corresponds to "no visible damage at day 243". These figure labels describe visual condition only and should not be interpreted as quantitative measures of physiological vitality. The repeat notation at Sites 16 and 17 is qualitative only.

2.6. Repeated Transplants and Quality Control

After early necrosis at Sites 16 and 17, replacement transplants were installed and monitored with the same visual criteria. These replacements, and the second twig at Site 13, were not part of the one-primary-transplant-per-site design. They are reported only as qualitative observations and were excluded from Kaplan–Meier estimation, descriptive index calculations, correlations, and sensitivity tests.

2.7. Data Analysis

Analyses were performed in Python 3.13.9 using pandas 2.3.3, NumPy 2.3.5, SciPy 1.16.3, lifelines 0.30.1, matplotlib 3.10.6, and statsmodels. The archived analysis notebook documents the Python environment and the versions of the listed packages except statsmodels, for which the exact version was not recorded. Two-sided *p*-values are reported as exploratory, without multiplicity adjustment, and no *p*-value is interpreted as evidence of causality.

2.7.1. Endpoint and Censoring

The time-to-event outcome was the number of days from transplantation to first-observed necrosis in *H. physodes.* The 13 primary transplants with necrosis were events. Site 6 was censored at day 123 after pruning; the six primary transplants with discoloration only or no visible damage were censored at day 243. Monthly observations produce interval uncertainty around the true onset, although the recorded first-detection day was used for analysis.

2.7.2. Descriptive Statistics

Event counts, censoring counts, and first-detection times were summarized for the 20 primary transplants. The descriptive Urban Stress Index (USI) was summarized only for 19 sites with complete 243-day follow-up; Site 6 was excluded because its outcome after day 123 was unknown. Repeated transplants were excluded.

2.7.3. Kaplan–Meier Analysis

The Kaplan–Meier product-limit estimator was used to estimate the probability of remaining free of observed necrosis [15]. The analysis included all 20 primary transplants and accommodated right-censoring. Because prespecified groups were small and no group comparison was central to the revised aims, no log-rank test was retained.

2.7.4. Descriptive Urban Stress Index

For a compact descriptive ranking among sites with complete follow-up, USI was calculated as:

$$USI_i = \frac{243 - T_i}{243}$$

where T_i is the recorded day of first-observed necrosis at site i.

For sites without observed necrosis, T_i was set to 243, giving USI = 0.

USI ranges from 0 (no observed necrosis by day 243) to values approaching 1 (earlier detection). It is not a validated physiological scale and discards information about discoloration, observation intervals, and uncertainty; it was used only for descriptive and exploratory analyses.

2.7.5. Exploratory Environmental Associations

Spearman rank correlations related USI to land-use percentages and site-level winter snow concentrations reported for corresponding locations [12–14]. Snow samples represented a short winter interval and were not assumed to describe deposition throughout the 243-day exposure. Only nominal two-sided *p*-values are shown; with $n \leq 19$, correlated predictors, and no multiplicity adjustment, effect directions and magnitudes are hypothesis-generating. No multivariable model was retained because the sample size and one-transplant-per-site design did not support stable estimation.

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3. Results

Among the 20 primary transplants, 13 developed visible necrosis and 7 observations were right-censored. First-observed necrosis occurred between days 62 and 215. Site 6 was censored at day 123 after pruning; four sites showed discoloration without necrosis at day 243, and two retained no visible damage through day 243.

3.1. Overall Vitality Outcomes

Five primary transplants had first-observed necrosis by day 92, five between days 153 and 184, and three at day 215. Four sites showed discoloration without necrosis, two showed no visible damage, and one was removed by pruning (Table 2).

Table 2. Visual outcomes of transplanted *Hypogymnia physodes* at the Jelgava monitoring sites.

No.	Monitoring Site	Outcome	Event/Censoring Day
16	Prohorova iela/Neretas iela	Necrosis	62
16 *	Prohorova iela/Neretas iela	Necrosis (repeat; qualitative)	31
17	Langervaldes Park	Necrosis	62
17 *	Langervaldes Park	Necrosis (repeat; qualitative)	90
1	Viskaļu iela/Lāču iela	Necrosis	92
3	Lietuvas šoseja/Savienības iela	Necrosis	92
13	Auseklа iela/Blaumaņa iela	Necrosis	92
2	Lietuvas šoseja/Platones iela	Necrosis	153
5	Tērvetes ielas railway crossing	Necrosis	153
12	Satiksmes iela/Ganību iela	Necrosis	153
19	Rīgas iela, RAF residential area	Necrosis	153
11	Dobeles šoseja/Satiksmes iela	Necrosis	184
7	Tērvetes iela/Pavasara iela	Necrosis	215
8	Lielā iela/Ozola skvērs	Necrosis	215
10	Aspazijas iela/Asteru iela	Necrosis	215
14	Lielā iela/Driksas tilts	Discoloration; no necrosis	243
15	Rīgas iela/Brīvības bulvāris	Discoloration; no necrosis	243
18	Aviācijas iela	Discoloration; no necrosis	243
20	Rīgas iela/TC Valdeka	Discoloration; no necrosis	243
4	Jelgava railway station	No visible damage	243
9	Lielā iela/Dobeles iela	No visible damage	243
6	Rūpniecības iela/Tērvetes iela	Removed by pruning; censored	123

* Replacement transplant, reported qualitatively only. Site 6 was right-censored at day 123; discoloration-only and primary transplants with no visible damage were right-censored at day 243 for the necrosis endpoint.

The primary transplants at Sites 16 and 17 developed necrosis by day 62. Their replacement transplants also developed necrosis (days 31 and 90, respectively), but these non-random repeats were used only as qualitative observations and did not change any estimates.

First-observed necrosis occurred at days 92, 153, 184, or 215 among the remaining event sites (Table 2). The monthly schedule means these values are detection times rather than exact biological onset times.

At day 243, Sites 14, 15, 18, and 20 showed discoloration without necrosis, whereas Sites 4 and 9 showed no visible damage. These six observations were censored at day 243 for the primary endpoint.

3.2. Time-to-Event Survival Analysis

The Kaplan–Meier estimate declined at the five recorded event times (62, 92, 153, 184, and 215 days; Figure 4). The analysis included 20 primary transplants, 13 events, and 7 censored observations.

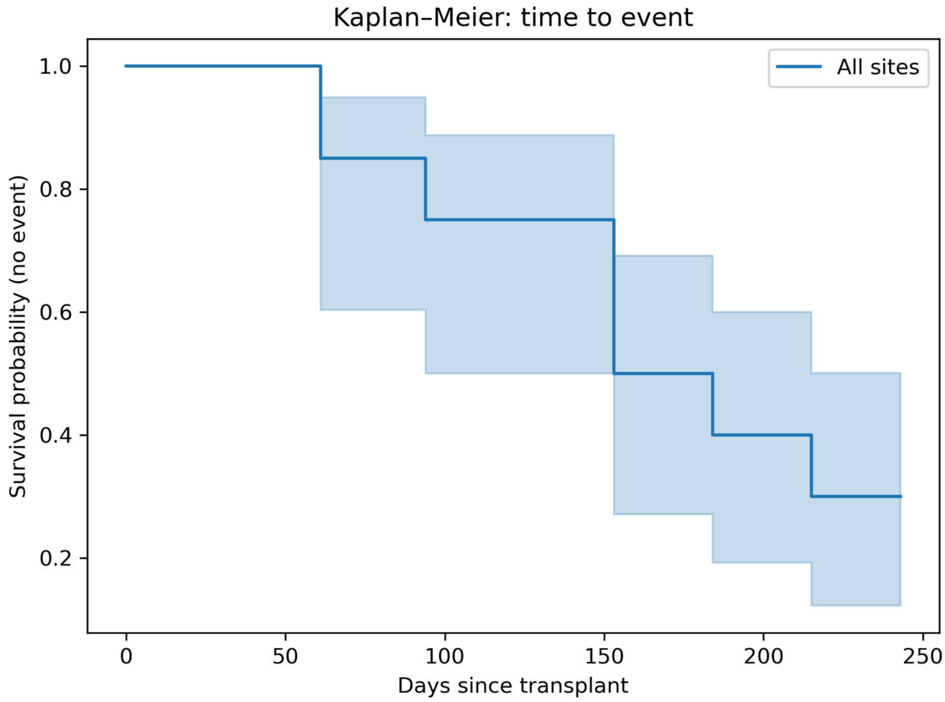

Figure 4. Kaplan–Meier estimate of time to first-observed necrosis for 20 primary lichen transplants. Tick marks denote censoring; the shaded band is the approximate 95% confidence interval.

The estimated median time to first-observed necrosis was 184 days. The estimated probability of remaining necrosis-free at day 243 was 0.321. Confidence intervals were wide, reflecting the small sample and censoring.

3.3. Urban Stress Index

For the 19 sites with complete 243-day follow-up, USI ranged from 0 to 0.745 (mean 0.285, SD 0.276; median 0.243; and interquartile range 0–0.496). The index summarizes only necrosis timing and was not interpreted as a calibrated measure of pollution or physiological injury.

3.4. Associations with Urban Structure

None of the reported land-use correlations with USI was statistically significant: public/commercial development ($\rho = -0.26$, $p = 0.265$), green/water cover ($\rho = -0.11$, $p = 0.634$), low-density residential cover ($\rho = 0.19$, $p = 0.416$), mixed residential–commercial cover ($\rho = -0.03$, $p = 0.891$), and industrial cover ($\rho = 0.02$, $p = 0.925$).

These estimates do not support a monotonic association between USI and any single land-use proportion in this sample. The directions are reported for completeness, not as evidence of protective or harmful effects.

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Alternative explanations include local shade, moisture, solar exposure, wind, placement on the receiving tree, unmeasured emissions, and random variation from having one primary transplant per site.

3.5. Associations with Heavy Metals

Exploratory correlations with February 2017 snow indicators were negative for Pb ($\rho = -0.57$, nominal $p = 0.008$) and Zn ($\rho = -0.49$, nominal $p = 0.028$), while Cr was also negative but did not meet the 0.05 threshold ($\rho = -0.44$, $p = 0.051$). These nominal p-values were not adjusted for multiple testing.

The directions are counter to a simple hypothesis in which greater metal deposition produces earlier necrosis. They may reflect temporal mismatch, spatial context, correlated predictors, exposure geometry, or chance. They do not show a protective effect of metals and do not identify a cause of lichen damage.

The published February cluster-mean ranges provide concentration context (Table 3) [13]. They are not the site-level distributions used in the correlation analysis. No elemental analysis of transplant thalli was performed, so uptake and internal dose cannot be evaluated.

Table 3. Published February 2017 snow concentration context and exploratory association with USI.

Element	Published Cluster-Mean Range (μg L^{-1})	Spearman ρ	Nominal p
Pb	6.0–31.9	−0.57	0.008
Zn	76.5–680.6	−0.49	0.028
Cr	1.2–14.0	−0.44	0.051

Note: Concentration ranges are published cluster means [13], not site-level distributions. Site-level co-located values were used for exploratory correlations; p-values were not adjusted for multiplicity.

3.6. Attachment-Tree Sensitivity Check

The attachment-tree groups were small and unequal. Because the lichens remained on their original twigs, receiving-tree species was not a substrate treatment and could only proxy local placement or microenvironment.

An exploratory Kruskal–Wallis check did not detect differences among the receiving-tree groups represented by at least three sites ($p > 0.05$). This result is not evidence that microenvironmental effects were absent; the design had limited power and no direct measurements of tree-level conditions.

4. Discussion

4.1. Integrated Interpretation of Findings

The revised analysis shows substantial spatial variation in the timing of visible necrosis: 13 of 20 primary transplants developed the event, and the estimated median first-detection time was 184 days. This is evidence of heterogeneous transplant outcomes, not of a quantified urban pollution gradient.

Several mechanisms could generate the observed pattern. Atmospheric contaminants may contribute, but hydration cycles, humidity, solar exposure, temperature, wind, source-twig condition, receiving-tree placement, and random variation are plausible alternatives [5,6,9]. These factors were not measured and cannot be separated by the present design. Consequently, the observed necrosis could reflect transplant injury or mortality caused by altered environmental conditions after relocation rather than by metal accumulation; the present design cannot distinguish these alternatives.

The USI provides a transparent descriptive ranking of event timing, while Kaplan–Meier analysis appropriately retains censored observations. However, USI is not a validated ecophysiological endpoint and should not be read as a direct measure of exposure or dose.

4.2. Road-Adjacent Spatial Patterns

Some early events occurred at road-adjacent sites, a pattern compatible with literature reporting lichen responses to PM and vehicle-related pollutants [3,9,16–18]. Comparable urban studies have also shown that lichen outcomes can vary with both pollution and local habitat [10,19,20].

The present study did not measure vehicle counts, ambient PM, NO_x, or other traffic tracers. We therefore replaced "high-traffic" with "road-adjacent" and removed source-specific claims. The early events at Sites 16 and 17, including qualitative replacement observations, justify follow-up monitoring but do not identify traffic or an industrial source.

Particulate matter was not included because no PM instrumentation or filters were deployed in the original low-cost visual-monitoring design. This omission is important: lichens can retain airborne particles, and particle loading can be quantified chemically or magnetically [21–23]. Future studies should therefore pair transplants with co-located PM2.5/PM10 monitoring or passive particle characterization.

Visible necrosis is a macroscopic endpoint and was not linked to chlorophyll fluorescence, membrane integrity, oxidative stress markers, or thallus chemistry. Previous transplant work demonstrates the value of such measurements [4–6], and their absence limits mechanistic interpretation here.

Accordingly, similarity with other cities is limited to the general observation that urban lichen responses are spatially heterogeneous and context-dependent [10,19,20]. The Jelgava data do not demonstrate that the same pollutant sources or physiological pathways were operating.

4.3. Seasonal Snow Deposition and Metal Associations

The snow measurements were collected during a short winter interval within the transplant period [13,14]. They are contextual deposition indicators, not time-invariant exposure estimates for July–March. Weather, road maintenance, snow age, and episodic emissions may have altered their spatial pattern.

The negative Pb and Zn correlations contradict a direct monotonic metal-toxicity interpretation. Because the transplants were not analyzed for elemental content, the results cannot establish uptake. Multiple testing, small sample size, shared spatial structure, and temporal mismatch offer plausible explanations for the nominal associations [11,12,24].

We therefore do not interpret the snow correlations as convergent evidence of metal-driven biological stress. They are reported as counterintuitive, exploratory findings that motivate a future design with repeated seasonal deposition measurements and paired thallus chemistry.

The limited snow–metal context should also be interpreted against the long-term decline in atmospheric metal deposition across Europe. Between 1990 and 2010, median concentrations in European moss biomonitors decreased by 77% for Pb and 34% for Zn [25]. Such lower contemporary deposition can reduce the contrast detectable by a visible-condition endpoint in a low-emission urban setting; the method may be more discriminating near active point sources or in regions with higher present-day metal emissions. However, the European trend does not establish a Jelgava-specific temporal trend.

4.4. Role of Green/Blue Infrastructure

The correlation between green/water cover and USI was small and non-significant ($\rho = -0.11$, $p = 0.634$). Although vegetation, openness, and water can modify dispersion and microclimate, the present data do not demonstrate a buffering effect [9,24,26]. Green/blue infrastructure is retained only as a hypothesis for future testing with replicated transplants and direct microclimate measurements.

To improve reliability in future applications, we recommend a minimum field protocol with three safeguards. First, install at least three independent transplants per site together with source-area reference units, and standardize attachment height, aspect, canopy exposure, and distance from roads or point sources. Second, use calibrated photographs or objective vitality measurements (e.g., chlorophyll fluorescence), with blinded duplicate scoring and predefined damage thresholds. Third, record co-located temperature, humidity, light, wind, and PM2.5/PM10, and pair these measurements with elemental analysis of exposed thalli. These measures would reduce confounding by microenvironment and placement while linking visible change to independently measured exposure.

4.5. Methodological Contribution

The main methodological contribution is the use of a time-to-event framework for visual vitality records. Kaplan–Meier estimation handles incomplete follow-up without recoding censored observations as events. USI may assist descriptive ranking, but survival estimates should remain primary because they preserve censoring information.

4.6. Limitations

The study has substantial limitations: one primary exposure unit per site; no independent site-level replication; a presumed rather than chemically verified source area; semi-quantitative visual scoring without color calibration, blinding, a damaged-area threshold, or inter-observer validation; monthly interval uncertainty; and no concurrent traffic counts, particulate-matter measurements, air or thallus chemistry, humidity, temperature, light, or wind measurements. All units were attached to receiving-tree branches using the same method, but heights varied between 1.2 and 2.2 m, cardinal orientation was fixed to SW rather than randomized, and distances to potential micro-contamination sources were not systematically recorded. Receiving-tree species and attachment geometry may confound local responses.

The sample was small for environmental association testing, several correlated predictors were examined without multiplicity correction, and the USI simplifies biological state and censoring. Winter snow measurements cover only a short portion of the exposure period. The second twig at Site 13 and repeats at Sites 16 and 17 were qualitative only. Finally, the exact legacy software environment was not archived. Together, these constraints limit inference to descriptive spatial variation and exploratory hypotheses; they preclude causal or pollutant-source attribution.

5. Conclusions

Monthly observations of 20 primary *Hypogymnia physodes* transplants documented marked within-city variation in its visible condition. Thirteen developed necrosis, seven were censored, and the Kaplan–Meier median time to first-observed necrosis was 184 days.

Because microclimatic and placement conditions were not monitored, the observed necrosis may reflect injury or mortality following environmental change after transplantation rather than metal accumulation.

Land-use correlations were non-significant, and negative associations with winter snow Pb and Zn were counter to a simple toxicity hypothesis. The results therefore do not identify traffic, metals, industry, or green infrastructure as causes of the observed pattern.

Visible-condition transplantation can support low-cost exploratory screening, but mechanistic interpretation requires replicated site-level transplants, standardized place-

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ment, objective vitality scoring, co-located microclimate and PM measurements, and paired chemical analysis of thalli.

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Abbreviations

The following abbreviations are used in this manuscript:

Cr	Chromium
Pb	Lead
PM	Particulate matter
$PM_{2.5}$	Fine particulate matter with an aerodynamic diameter $\leq$ 2.5 μm
SD	Standard deviation
SW	South-west/south-westerly
USI	Urban Stress Index
Zn	Zinc

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