

Faculté des bioingénieurs

Coupling biomonitoring methods to assess trace element deposition

A new interpretative scale of lichen sensitivity to trace element pollution

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Table of contents

Acknowledgements	1
Table of contents.....	2
List of figures.....	4
List of tables	7
1. Introduction	9
2. State of the art	10
2.1. Atmospheric pollution.....	10
2.1.1. Air pollution in context: past lessons, present challenges	10
2.1.2. Cycle of air pollutants in the atmosphere.....	10
2.1.3. Transport of air pollutants in the atmosphere	11
2.1.4. Deposition of air pollutants in ecosystems.....	12
2.1.5. Impacts of air pollutants.....	13
2.2. Trace element pollution	15
2.2.1. Characterisation of trace elements	15
2.2.2. Sources and evolution of atmospheric trace element emissions	15
2.2.3. Variable transport and deposition.....	18
2.2.4. Roles and risks associated with trace elements	18
2.2.5. Atmospheric monitoring of trace elements in Wallonia.....	20
2.3. Biomonitoring using lichens	22
2.3.1. Lichens: a symbiotic organism.....	22
2.3.2. Lichen ecology	24
2.3.3. Bioindication.....	25
2.3.4. Bioaccumulation	26
2.3.5. Coupling biomonitoring methods.....	29
3. Objectives of the study	31
4. Materials and methods	32
4.1. Study area.....	32
4.1.1. Geographical, climatic and historical context.....	32
4.1.2. Sampling sites	32
4.2. Bioindication methods	34
4.2.1. Choice of sites and trees	34
4.2.2. Lichen identification and sampling	35
4.3. Bioaccumulation methods	36

4.3.1. Sampling	36
4.3.2. Samples preparation and acid digestion.....	36
4.3.3. Chemical analysis.....	37
4.3.4. Quality control.....	37
4.4. Data treatment and statistical analysis.....	39
4.4.1. Bioindication indices.....	39
4.4.2. Bioaccumulation metrics	40
4.4.3. Statistical analysis.....	41
4.5. Complementary data	42
5. Results and discussions.....	44
5.1. Bioindication method.....	44
5.1.1. Lichen diversity and abundance differences across contrasted cities	44
5.1.2. Species occurrences and distribution across sites.....	47
5.1.3. Community contrasts per sites: NMDS based approach	49
5.2. Bioaccumulation method	52
5.2.1. Trace element concentrations.....	52
5.2.2. Geochemical Indices for evaluating trace element contamination	53
5.2.3. Exploring relationships between trace elements	58
5.3. Coupling biomonitoring methods	61
5.3.1. Exploring correlations between lichen diversity and pollutant variables	61
5.3.2. Linking species diversity to pollution sensitivity	64
5.3.3. Assessment of the sensitivity scale to trace-element pollution	68
6. Conclusions and perspectives.....	72
6.1. Conclusions of the study	72
6.2. Perspectives of the study	73
7. References.....	74
8. Appendices.....	100

List of figures

Figure 1: Simplified illustration providing an overview of emissions, transport and deposition processes of atmospheric pollutants.....	11
Figure 2: Atmospheric lifetimes (vertical axis) and spatial scales of transport (horizontal axis) for common air pollutants, adapted from Pryor et al. (2015).....	12
Figure 3: Illustration of dry and wet deposition processes, including turbulent diffusion, sedimentation and scavenging mechanisms (Leelőssy et al., 2014).	13
Figure 4: Sectoral contributions to atmospheric trace element emissions in the EU28 (2019), adapted from Bai et al. (2023).	16
Figure 5: Trends in atmospheric trace element emissions in the EU28 (1990-2023), based on NEC Directive inventory data (European Environment Agency, 2025)	17
Figure 6: Simplified diagram of biomagnification of trace elements through ecosystems and living organisms.	19
Figure 7: Spatial distribution of the 16 monitoring stations in the "éléments métalliques" (EM) network coordinated by the Walloon Air and Climate Agency (AWAC, 2023), highlighting the concentration of the stations along the Sambre-Meuse industrial axis.	21
Figure 8: Illustration of the nutritional exchanges between the photobiont and the mycobiont in a lichen symbiosis. The black arrows represent metabolic processes and nutrient exchanges carried out by both photobionts (cyanobacteria and algae); the blue dashed arrows indicate processes specifically carried out by the cyanobacteria. Adapted from (Van Haluwyn et al., 2022).	22
Figure 9: Illustration of the typical structure of the lichen thallus in cross-section (https://biogeoscience.eu).	23
Figure 10: Examples of different morphological thalli: (1) crustose; (2) foliose; (3) fruticose; (4) complex.	23
Figure 11: Illustration and images of lichen reproductive structures: sexual reproduction via apothecium (left); vegetative reproduction via soredia (middle); vegetative reproduction via isidia (right).	24
Figure 12: Illustration of the field measurement protocol for calculating the frequency based index using a 5 squares grid (10×10 cm squares) placed at 1 m height on four cardinal trunk faces, following the European prEN16413 protocol; (b) field application using a rope based grid (Truong, 2013).	25
Figure 13: Possible locations and uptake mechanisms of trace elements in the lichen thallus: (1) particulate entrapment on the surface and in intercellular spaces of the fungal biont; (2) intercellular uptake of trace elements; (3) extracellular adsorption to functional groups of the fungal cell walls; (4) extracellular complexation to organic acids such as parietinic acid (Honegger, 1991).	27
Figure 14: Ternary plot illustrating the landscape structure (percentage of artificialized, agricultural and forested areas) for every municipality, highlighting the contrasting gradients of land use.....	33

Figure 15: Spatial distribution of the 41 sampling sites in Wallonia categorized by land use type: forested site (green), agricultural sites (yellow), transitional sites (orange), and urban sites (red). The number of sites (n) at each location is indicated.	34
Figure 16: Schematic representation of the lichen bioindication sampling method. A 10 × 50 cm grid, composed of five 10 × 10 cm squares, is placed at 1 m above ground level on each cardinal side of the tree trunk. Observations are conducted within each square.....	35
Figure 17: Frequency across sites (n = 41 sites) is plotted against the percentage of grid squares occupied by each species within a site (n = 80 grid squares per site). Species present in at least 20% of the sites are shown in blue, while those present in fewer than 20% are shown in grey. Squares and triangles represent species within the two suballiances of <i>Xanthorion parietinae</i> : the <i>Physcienion adscendentis</i> (squares) and <i>Pleurostiction acetabuli</i> (triangles).	47
Figure 18: Non-metric multidimensional scaling ordination based on lichen species composition across 41 sites: (a) Red coloured sites are in urban areas, orange coloured sites are transitional urban-rural areas, yellow coloured sites are agricultural and the green coloured site is forested. The sites are labelled with three letter codes; (b) Each point represents a species coded by a unique three letter abbreviation (see Appendix C.1).	50
Figure 19: Distribution of trace element concentrations [$\mu\text{g}\cdot\text{g}^{-1}$] on a logarithmic scale. The naturalness and alteration thresholds proposed by Bargagli & Nimis (2002) are indicated in green and red, respectively.	53
Figure 20: Distribution of enrichment factors on a logarithmic scale. Thresholds of enrichment, ranging from lowest to highest, are indicated by the green, yellow and red dashed lines, corresponding to enrichment factor values of 0.5, 2 and 10, respectively.....	54
Figure 21: Pollution load index (PLI) values for the different studied cities. Pollution classes, as defined in Table 23 (see Appendix E.6), are shown in colour: green for unpolluted sites (PLI < 1) and yellow for low to moderate pollution (PLI > 1).....	57
Figure 22: Principal component analysis biplot of the considered trace elements, where each arrow corresponds to a specific element.	58
Figure 23: Scores of the studied sites projected onto the principal component analysis biplot of the trace elements. Red coloured sites are in urban areas, orange coloured sites are transitional urban-rural areas, yellow coloured sites are agricultural and the green coloured site is forested. The sites are labelled with three-letter codes.	59
Figure 24: Principal component analysis biplot of synthetic pollution indices (ΣEF and PLI), atmospheric pollutants (NO_2 , PM_{10} and O_3), diversity metrics (LDV and Shannon's index) and land-use variables (LCA and % artificialised in a 50 m radius).....	62
Figure 25: Scores of the studied sites projected onto the principal component analysis biplot of all variables. Red coloured sites are in urban areas, orange coloured sites are transitional urban-rural areas, yellow coloured sites are agricultural and the green coloured site is forested. The sites are labelled with three-letter codes.	63
Figure 26: Canonical correspondence analysis (CCA) of lichen species composition in relation to environmental variables. The left panel shows the ordination of environmental variables, including pollution indicators (PLI, ΣEF), atmospheric pollutant concentrations (PM_{10} , NO_2 , O_3), ecological variables (LDV, Shannon diversity) and land-use proxies (%A50m, LCA). The	

right panel displays species scores in the CCA space, highlighting the associations between specific lichen taxa and environmental gradients.	64
Figure 27: Canonical correspondence analysis biplot of species scores on CCA1 (x-axis) and CCA2 (y-axis). The green to red background reflects the transformed coordinates of the species onto the y' axis (green = sensitive; red = tolerant). The black arrow indicates the sensitivity vector (y') with opposed direction and magnitude to the ΣEF vector and the dashed line perpendicular to the sensitivity vector (y') separates the trace element high influence (red) from low influence (green) areas.	66
Figure 28: Scatterplot of lichen species sensitivity scores extracted from the NO_2 (x-axis) and ΣEF (y-axis) gradients, with points coloured according to literature-derived NO_2 sensitivity classes (Gombert et al., 2006). The trendline is shown with the corresponding equation and R^2 value.	70
Figure 29: Distribution of LCA values for the 41 sites.	101
Figure 30: Visualisation of two different values of LCA : CHA11 with a green pocket in an urban environment reflected by a low LCA (A) and MAR11 with an artificial area surrounded by less artificialized area (B).	102
Figure 31: Comparison of the effect of normalisation (UCC versus estimation of geochemical background) for the studied geochemical indices (PLI and ΣEF)	103
Figure 32: Estimation of the geochemical background values using the mirror method: example of Pb	104
Figure 33: Spatial distribution of sampling sites and ΣEF values for each site	119
Figure 34: Spatial distribution of sampling sites and PLI values for each site	119
Figure 35: Spatial distribution of sampling sites and NO_2 values for each site	120
Figure 36: Spatial distribution of sampling sites and PM_{10} values for each site	120
Figure 37: Spatial distribution of sampling sites and LDV values for each site	121
Figure 38: Spatial distribution of sampling sites and Shannon's index values for each site .	121

List of tables

Table 1: Scale for interpreting trace-metal concentrations (expressed in $\mu\text{g}\cdot\text{g}^{-1}$ of dry weight) in lichens, reflecting environmental alterations and deviations from natural background levels (Nimis et al., 1993).....	28
Table 2: Noise [%] of analytical blanks (n=3)	37
Table 3: Mean and standard deviation recovery rates values [%] of the 14 studied elements in the IAEA336 samples (n=3)	38
Table 4: Mean and standard deviation of calculated lichen diversity indices, with corresponding municipal surface area and population density. Data from the municipalities were recovered from <i>Institut wallon de l'évaluation, de la prospective et de la statistique</i> (IWEPS, 2025).....	44
Table 5: Comparison of biodiversity indices (specific richness and lichen diversity value) across different regions, with values reported in other studies.	46
Table 6: Comparison of the mean and standard deviation of enrichment factor values for the studied trace elements for: (a) area near a nickel smelter in Slovakia (Kováčik et al., 2023); (b) a mountain region in China (Liu et al., 2016) and; (c) the Wallon region (Matthée, 2024).	55
Table 7: Mean and standard deviation of pollution load index values for cities exceeding the threshold. A ranking of the trace elements with the highest contamination factors is shown for each city, along with the main origin of these elements.	57
Table 8: Permutation based ANOVA p-values for each explanatory variable in both global (all predictors included) and partial (conditioning on all other predictors) canonical correspondence analysis models. P-values < 0.05 are shown in bold.	65
Table 9: List of species that are tolerant (A) with $y' < -0.4$, intermediate (B) with $-0.4 < y' < 0.4$ or sensitive (C) with $y' > 0.4$ to trace element pollution.	67
Table 10: Correlation matrix between the studied geochemical indices (PLI_{UCC} , $\text{PLI}_{\text{estimated background}}$ and $\Sigma\text{EF}_{\text{UCC}}$)	103
Table 11: Coordinates of the 41 sampling sites in the study area	105
Table 12: Three letter code abbreviation of the species in our dataset and authorities associated	106
Table 13: Sensitivity scale proposed of species to trace elements proposed by Agnan (2017)	109
Table 14: Sensitivity scale of the species to NO_2 derived from the canonical correspondence analysis ordination.....	111
Table 15: Alpha diversity metrics (species richness, lichen diversity value, Shannon's index) for the 41 sites in the study area.....	112
Table 16: Spearman correlation between the trace elements of interest. Values of $\rho > 0.7$ are in bold.	113
Table 17: Enrichment factors of the trace elements of interest for the 41 sites (first part).	113
Table 18: Enrichment factors of the trace elements of interest for the 41 sites (second part)	115

Table 19: Pearson correlation (r) between the enrichment factor of the trace elements and ΣEF	116
Table 20: Comparison mean and standard deviation values of concentrations [$\mu g \cdot g^{-1}$] in trace elements for a polluted area (Çobanoğlu & Kaan, 2024), an unpolluted area (Bergamaschi et al., 2004) and a heterogeneous area (Agnan, 2017).	116
Table 21: Thresholds of naturalness and alteration proposed by Bargagli & Nimis (2002) in [$\mu g \cdot g^{-1}$]	117
Table 22: Ranges of contamination factors (CF) and associated levels of contamination....	117
Table 23: Ranges of pollution load index (PLI) and associated interpretation of pollution....	118
Table 24: Spearman correlation between the geochemical indices (PLI and ΣEF), diversity metrics (LDV and Shannon's index), pollutants (NO_2 , O_3 , PM_{10}) and land use variables (LCA and %A50m)	118
Table 25: Overview of land cover and demographics in cities within the study area.....	122

1. Introduction

Air pollution remains one of the most widespread environmental challenges in Europe, with serious consequences for ecosystems, human health and atmospheric processes (e.g., radiative forcing, smog formation, ozone depletion). Among the various pollutants, trace elements such as cadmium, lead, zinc and chromium are particularly concerning. Although these elements often receive less specific attention in routine air quality monitoring, they pose significant ecological threats. This is explained by their ability to persist in the environment, their inherent toxicity and their tendency to accumulate in living organisms over time. In Wallonia, air quality monitoring mainly relies on fixed stations that measure particulate matter, but not the specific concentration of trace elements, even though such elements can be transported by particulates and pose distinct environmental and health risks. While these stations provide continuous data, their limited number means they offer only a sparse and incomplete picture of atmospheric deposition, especially in areas with mixed land uses and diverse sources of emissions.

In this context, using lichens for biomonitoring offers a valuable and complementary approach. Biomonitoring refers to the use of living organisms to assess the presence and impact of pollutants in the environment over time. Lichens are highly responsive to changes in air quality. They reflect environmental conditions through shifts in their species composition (bioindication) and through pollutant absorption into their tissues (bioaccumulation). Because of this dual capacity, lichens are considered effective indicators of air pollution. However, even though both approaches are well-established, few studies have used them together to explore their combined potential.

This thesis explores the potential of combining both methods of biomonitoring (bioindication and bioaccumulation) to enhance the assessment of atmospheric trace element pollution. The main goal is to assess how sensitive different lichen species are to atmospheric trace elements. To address this, the study follows three connected lines of inquiry. First, the analysis focuses on the diversity and structure of lichen communities across sites that vary in land use and urbanisation. Second, trace element concentrations are measured in the common epiphytic lichen *Xanthoria parietina* and geochemical indices are applied to evaluate pollution levels. Third, ecological (qualitative) and chemical (quantitative) data are combined using multivariate analyses to assign sensitivity scores to species and investigate how pollutant loads influence community composition.

Through this combined approach, this thesis aims to contribute to a clearer understanding of how lichens respond to and record atmospheric trace element pollution. It addresses a key gap in current research: the lack of species-level sensitivity classifications to atmospheric trace element pollution.

2. State of the art

2.1. Atmospheric pollution

2.1.1. Air pollution in context: past lessons, present challenges

Air pollution is not a new phenomenon, during the Industrial Revolution, when steam engines and coal combustion became widespread, many cities experienced repeated episodes of severe smog. One of the most infamous cases occurred in London in December 1952, when thick industrial smoke caused approximately 4,000 premature deaths (Jacobson, 2012). This event starkly highlighted the deadly consequences of unregulated emissions.

Despite major advances in environmental laws, air pollution remains a serious global health problem. The World Health Organization (WHO) estimates that 6.7 million people worldwide die prematurely each year from diseases linked to air pollution exposure (WHO, 2025). No region is completely safe: remote areas can still suffer from long-range pollutant transport and urban centres continue to struggle with high local emissions.

In rapidly industrializing countries where fossil fuels dominate the energy mix, air quality often falls below international health standards. India provides a clear example: although national limits may label certain pollution levels as “safe”, fine particulate matter (PM) concentrations frequently exceed WHO guidelines. Studies suggest that air pollution accounts for 7.2% of all annual deaths in India, including about 33,000 premature fatalities each year in just ten major cities (Kurtkoti, 2024).

These examples expose a paradox: even with air quality regulations in place, current thresholds may not fully protect public health. Therefore, understanding the sources, composition and atmospheric behaviour of pollutants is essential for developing more effective strategies to improve air quality and reduce health risks worldwide.

2.1.2. Cycle of air pollutants in the atmosphere

Air pollutants originate from both anthropogenic and natural sources, including road traffic, industrial processes, agriculture, volcanic activity and forest fires. Once emitted into the atmosphere, these substances exist in three physical states (gas, liquid or solid) each determining their transport, transformation and eventual deposition. Gaseous pollutants, such as sulphur dioxide (SO₂), nitrogen oxides (NO_x) or volatile organic compounds (VOCs), can remain airborne for extended periods, during which they may undergo photochemical transformations depending on atmospheric conditions. Liquid phase pollutants, present in droplets, clouds or fog, are often transformed chemically before being removed by precipitation. Solid pollutants, mainly PM, act as vectors for metals and mineral dust, with transport distance varying by size: coarse particles settle near the source, while finer particles (PM_{2.5}) can travel hundreds of kilometres. Figure 1 illustrates the simplified cycle of air pollution from emission sources to effects upon deposition.

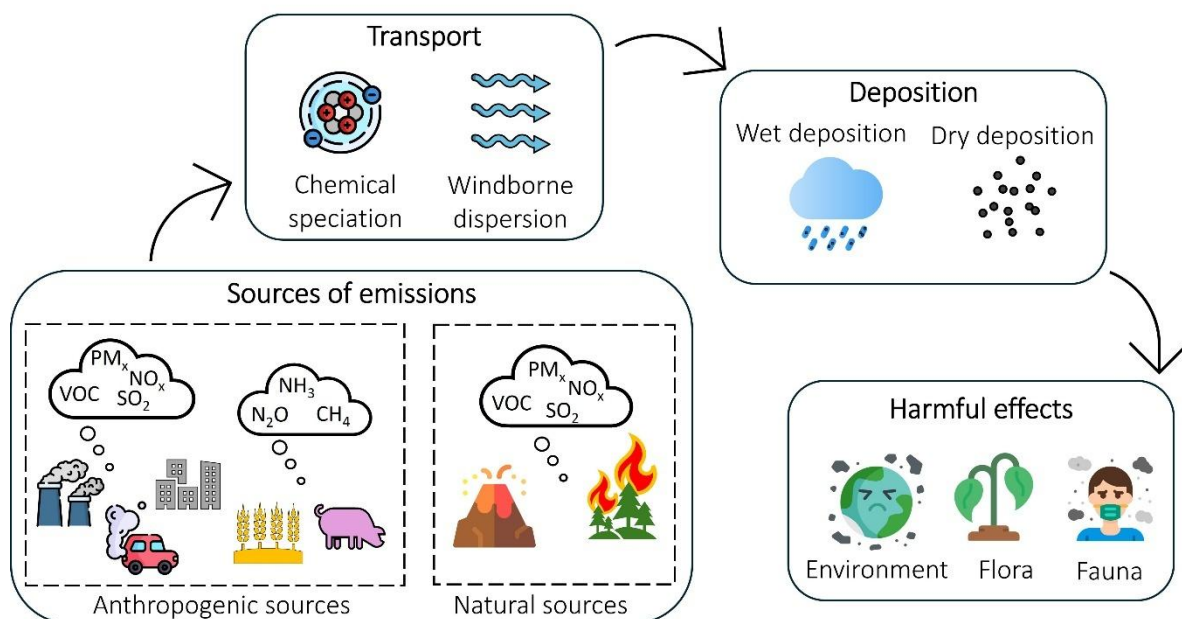


Figure 1: Simplified illustration providing an overview of emissions, transport and deposition processes of atmospheric pollutants.

A further distinction is made between primary pollutants, emitted directly from identifiable sources and secondary pollutants, which form through atmospheric reactions. While primary pollutants such as, methane (CH_4), ammonia (NH_3) or nitrous oxide (N_2O), can be directly attributed to emission sources and are easier to regulate, secondary pollutants (e.g., tropospheric ozone) are influenced by meteorological variables such as sunlight, humidity and temperature, making them harder to predict and control (Godish, 1997; Sitaras & Siskos, 2008). Understanding both their physical state and chemical reactivity is essential to assess their environmental fate and potential health impacts.

2.1.3. Transport of air pollutants in the atmosphere

Once emitted into the atmosphere, air pollutants are transported through a combination of physical and meteorological processes. Diffusion drives the movement of molecules from high to low concentration areas, influenced by temperature and pressure gradients (Vallero, 2024). Advection, caused by wind patterns, moves pollutants horizontally and vertically, dispersing or concentrating emissions depending on direction and speed. Turbulence in the atmospheric boundary layer, often driven by convection and wind shear, further redistributes pollutants. Local weather phenomena can modify these dynamics significantly: temperature inversions¹ trap pollutants near the surface in urban valleys (Rendón

¹ A temperature inversion occurs when a layer of warmer air sits above cooler air near the ground, preventing vertical mixing and causing pollutants to stay trapped near the surface (Britannica, 2025).

et al., 2014), while urban heat islands² generate atmospheric circulations that concentrate pollutants in dense city centres.

The spatial scale of pollutant transport depends heavily on these mechanisms, but also on pollutant properties. While coarse particulate matter settles near the source, fine aerosols and long-lived gases may travel across regions, continents, or even hemispheres. One striking example is the transatlantic transport of Saharan mineral dust over the North Atlantic Ocean. This process plays an essential role in marine biogeochemistry, fertilizing phytoplankton and influencing primary productivity (Roiger et al., 2012).

This variability in transport capacity is largely governed by a pollutant's atmospheric lifetime, the average time it remains airborne before being removed through deposition or chemical reaction (Hobbs, 2000; Jacob, 2025). Lifetimes vary from seconds, for highly reactive species, to years, for inert gases such as chlorofluorocarbons (CFCs) and strongly determine how far pollutants can travel. As shown in Figure 2, short-lived species remain near their sources, while more persistent ones can undergo long-range transport. However, atmospheric lifetimes are often only approximations: especially for secondary pollutants, whose formation and removal depend on complex atmospheric chemistry (Pryor et al., 2015). This uncertainty complicates emission source attribution and dispersion modelling.

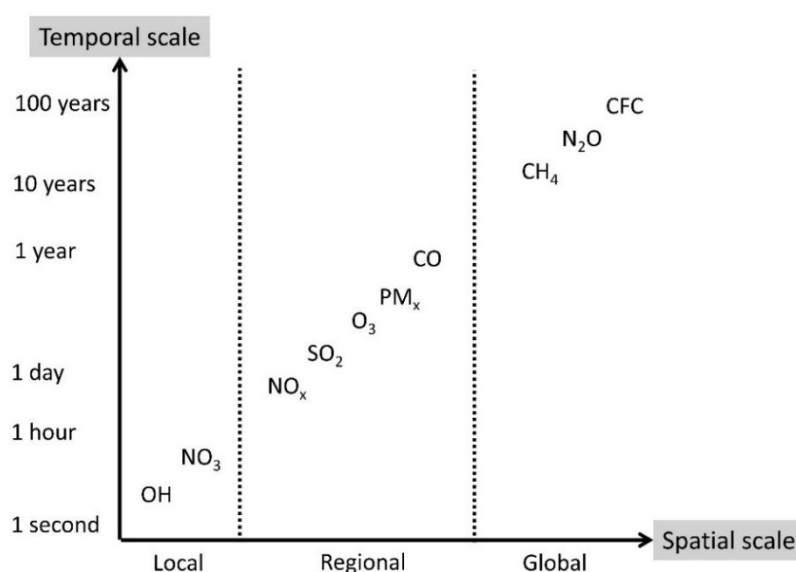


Figure 2: Atmospheric lifetimes (vertical axis) and spatial scales of transport (horizontal axis) for common air pollutants, adapted from Pryor et al. (2015).

2.1.4. Deposition of air pollutants in ecosystems

Once transported through the atmosphere, air pollutants are eventually removed via deposition processes, marking the end of their atmospheric lifetime. Deposition plays a key role in shaping air quality and affecting ecosystems and human health. It occurs primarily

² An urban heat island, is a metropolitan area that's a lot warmer than the rural areas surrounding it and thus possibly concentrating pollutants near the ground (National Geographic, 2025).

through two pathways (dry and wet deposition) each influenced by meteorological conditions and the physical and chemical properties of the pollutants (Figure 3).

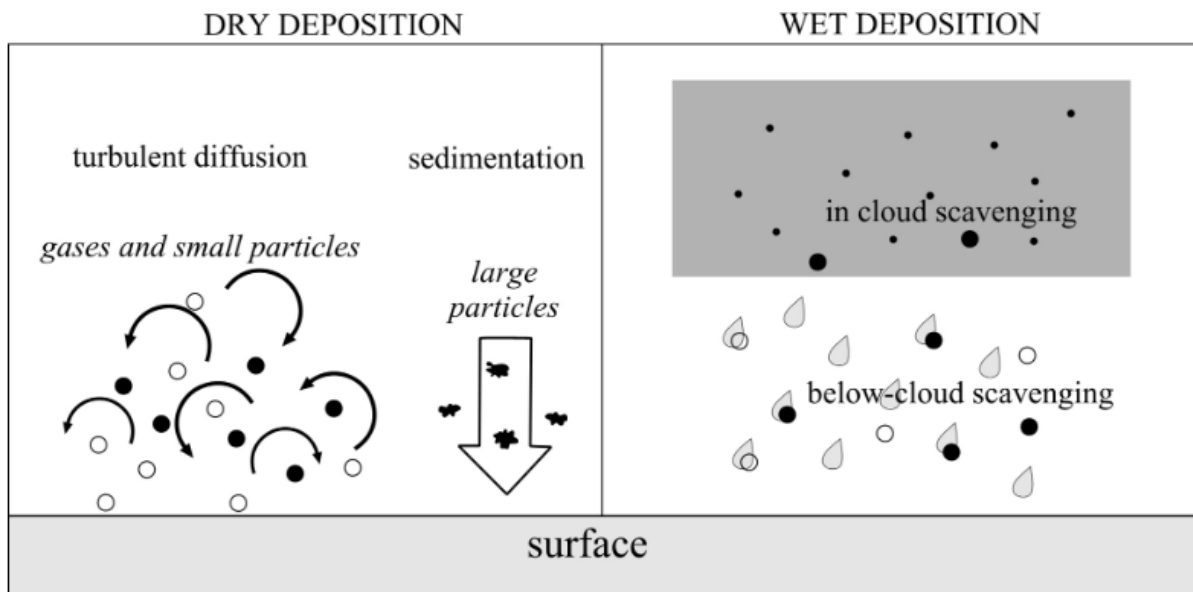


Figure 3: Illustration of dry and wet deposition processes, including turbulent diffusion, sedimentation and scavenging mechanisms (Leelőssy et al., 2014).

Dry deposition occurs without precipitation and is strongest near emission sources. Coarse dust particles rich in aluminosilicate and iron-oxide minerals, larger than $2.5\ \mu\text{m}$, settle rapidly by gravity and turbulence, despite aluminium and iron being relatively light elements, because they occur in high density mineral phases (Nriagu, 1989). Fine particles, smaller than $1\ \mu\text{m}$ or gases deposit by interception and adsorption onto rough surfaces (vegetation, buildings) rather than by settling (Abbatt, 2003; Amicarelli et al., 2021; Lindén et al., 2023). In dry conditions, any deposited material can be resuspended by wind or human disturbance, obscuring true deposition rates (Jickells et al., 2016).

Wet deposition, on the other hand, involves the scavenging of pollutants by precipitation. Particles and gases are either incorporated into cloud droplets (in cloud scavenging) or captured as raindrops or snowflakes fall (below cloud scavenging). This mechanism is particularly relevant for fine aerosols that may be transported over long distances before removal (J. Nriagu et al., 1989). As a result, wet deposition often dominates in remote or high altitude ecosystems, where it contributes to the accumulation of pollutants far from their sources, affecting soil and water chemistry.

2.1.5. Impacts of air pollutants

Air pollutants exert pressure on ecosystems at global, regional and local scales, contributing to widespread environmental degradation.

Global impacts

Global impacts such as enhanced radiative forcing, ozone depletion and delayed stratospheric recovery are primarily driven by long-lived atmospheric pollutants. Short-term warming is amplified by increased stratospheric water vapour and additional tropospheric ozone, largely linked to CH₄ emissions (Mar et al., 2022). Ozone layer recovery is delayed by the presence of N₂O, which also acts as a potent greenhouse gas (UN environment program, 2024). High global warming potential and catalytic ozone destruction are associated with CFCs, which release chlorine radicals upon photolysis (US EPA, 2015).

Regional impacts

At regional levels, atmospheric pollutants such as SO₂, NO_x and NH₃ drive acidification and eutrophication. These processes alter soil chemistry and disrupt microbial and plant communities (Visgilio & Whitelaw, 2007). Nitrogen enrichment in particular, reduces biodiversity in terrestrial ecosystems and promotes hypoxic zones in aquatic systems due to algal overgrowth (Jaworski et al., 1997). The concept of critical loads was introduced to define the threshold of pollutant deposition beyond which ecosystems suffer long term damage (Fuhrer et al., 1997; Nilsson, 1988). While widely used for acidifying and eutrophying pollutants, this framework remains poorly adapted to particulate bound elements, due to the complexity of their atmospheric behaviour and chemical speciation (oxidation state, solubility and molecular form).

Local impacts

At the local level, pollution effects can be more subtle and less predictable. Ground-level ozone, a secondary pollutant formed through photochemical reactions involving NO_x and VOCs, causes respiratory illnesses in humans and oxidative damage in vegetation (Jacobson, 2012). Its formation is influenced by temperature, solar radiation and urban emissions, making it particularly problematic in densely populated areas.

PM, a key component of aerosols, is not a single pollutant but a complex mixture of particles that differ in size, shape and chemical composition. Typically, PM is classified into PM₁₀ (aerodynamic diameter ≤ 10 µm) and PM_{2.5} (aerodynamic diameter ≤ 2.5 µm), with the finer particles remaining airborne longer and posing greater health risks (Belgian Interregional Environment Agency, 2025). Chemically, PM includes both organic compounds, such as polycyclic aromatic hydrocarbons and n-alkanes (Lyu et al., 2019) and inorganic substances, notably metals (Inerb et al., 2022; Popouen et al., 2022).

Although their toxicity is known, metals in PM are rarely monitored individually. Most air quality networks report only total PM mass, ignoring its chemical composition. This limits our understanding of which components drive toxicity and hampers the development of targeted regulations. Public health assessments, including those by the WHO, continue to evaluate PM as a bulk pollutant, without accounting for its varying biological effects (WHO, 2007) (WHO, 2007). Addressing this gap requires more refined analytical techniques and a deeper understanding of metallic elements within the PM matrix to inform effective environmental and public health policy.

2.2. Trace element pollution

2.2.1. Characterisation of trace elements

For many years, the term “heavy metals” has been used to describe metallic elements, good thermal and electrical conductors capable of forming cations, with densities exceeding $5 \text{ g}\cdot\text{cm}^{-3}$ (Duffus, 2002). However, this definition fails to encompass certain lower density metals and excludes metalloid elements that are also of interest. By contrast, the term “trace element” is often defined as any element making up less than 0.1% of the Earth's crust by mass, but this strict definition excludes some important elements having higher mass. These relatively abundant elements can still act as micropollutants in the environment making this definition too simple for environmental studies.

Thus, the designation “trace metal elements” was adopted to reflect both their metallic character (excluding metalloids) and their low crustal abundance (Alloway, 2013). Throughout this manuscript, we will use “trace elements” (TEs) to refer collectively to all studied trace metal elements, emphasizing their status as minor constituents and highlighting their role in atmospheric pollution. The TEs under consideration span several chemical families:

- Transition metals include cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), titanium (Ti), vanadium (V) and zinc (Zn).
- Post-transition metals include aluminium (Al), lead (Pb) and tin (Sn).
- Metalloids are represented by arsenic (As) and antimony (Sb).

Importantly, the toxicity and mobility of TEs depend not only on their concentration but also on their chemical speciation, which refers to the oxidation state, solubility and molecular form in which they occur (Templeton et al., 2000; Tipping et al., 1998). Since these forms determine how readily an element is absorbed by organisms (bioavailability) and how harmful it can be (toxicity), speciation needs to be well understood to better assess TE pollution (Reeder et al., 2006).

2.2.2. Sources and evolution of atmospheric trace element emissions

Natural sources

Atmospheric TEs can originate from several key natural processes, listed below in approximate order of their relative contribution to global emissions (Nriagu, 1989; Rauch & Pacyna, 2009):

- Terrigenous sources: weathering of rocks and wind erosion mobilise lithogenic particles rich in Al, Co, Cr, Fe, Mn, Ni and V.
- Marine sources: sea spray and wave action generate aerosols through bubble bursting at the ocean surface, entraining dissolved and particulate trace elements such as Cu, Fe, Ni, Pb, Sb, V and Zn into the atmosphere (O'Dowd & de Leeuw, 2007).

- Volcanic activity: eruptions release substantial amounts of trace metals, including As, Cd, Cr, Cu, Hg, Ni and Pb. These emissions are highly variable over time.
- Biogenic sources: biomass burning can emit trace quantities of Cu, Pb and Zn, often in episodic but significant pulses (Andreae & Merlet, 2001).

Although crucial to the baseline levels of atmospheric TEs, these natural sources exhibit temporally constant and spatially diffuse fluxes, resulting in lower and more uniform concentrations compared to anthropogenic emissions.

Anthropogenic sources

Human activities now constitute the dominant source of atmospheric TEs, particularly in industrialised regions (Nriagu & Pacyna, 1988; Pacyna et al., 2007). Emissions originate from a range of sectors, with metallurgy, fossil fuel combustion, transport and agriculture standing out as major contributors. These sectors influence TE emissions through processes like metal smelting, coal burning, vehicular traffic (both exhaust and non-exhaust) and the use of agrochemicals. A recent assessment by Bai et al. (2023) quantified the sectoral distribution of eight key anthropogenic trace elements across the 28 member states of the European Union (EU28) in 2019. For each element, the chart (Figure 4) shows the percentage of its total emissions attributable to each sector.

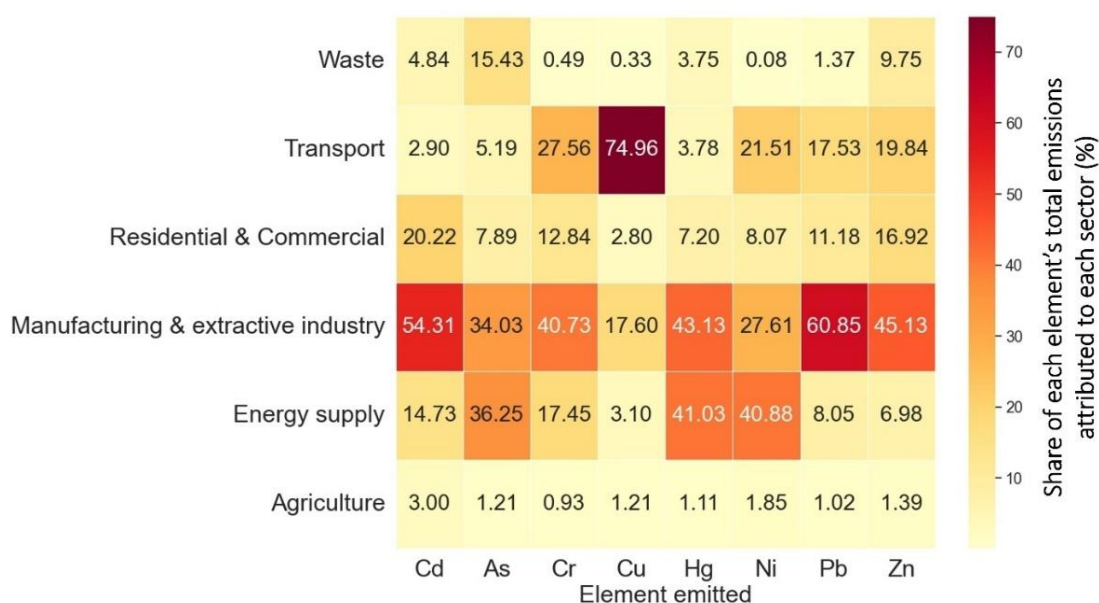


Figure 4: Sectoral contributions to atmospheric trace element emissions in the EU28 (2019), adapted from Bai et al. (2023).

Manufacturing and extractive industries alone account for over 40% of Cd, Cr, Hg, Pb and Zn emissions. The energy sector contributes substantially to Hg and Ni, reflecting their prevalence in fossil fuels (Joensuu, 1971; Maciejczyk et al., 2021). Transportation is a major source of emission from combustion engines but also from non-exhaust emissions: nearly 75% of Cu emissions originate from this sector, mostly from brake and tire wear (Fussell et al., 2022). Residential heating is particularly relevant for Cd and Zn, while waste incineration

remains a significant emitter of As and Zn. The agricultural sector contributes less than 5% for any of the mentioned TEs emissions but may still be locally important (Alengebawy et al., 2021).

Temporal evolution of trace element emissions

Emissions of atmospheric TEs across the EU28 have significantly decreased since 1990, reflecting the combined effects of environmental regulation, technological improvements and structural changes in key industrial sectors (European Environment Agency, 2025; Figure 5Figure 1).

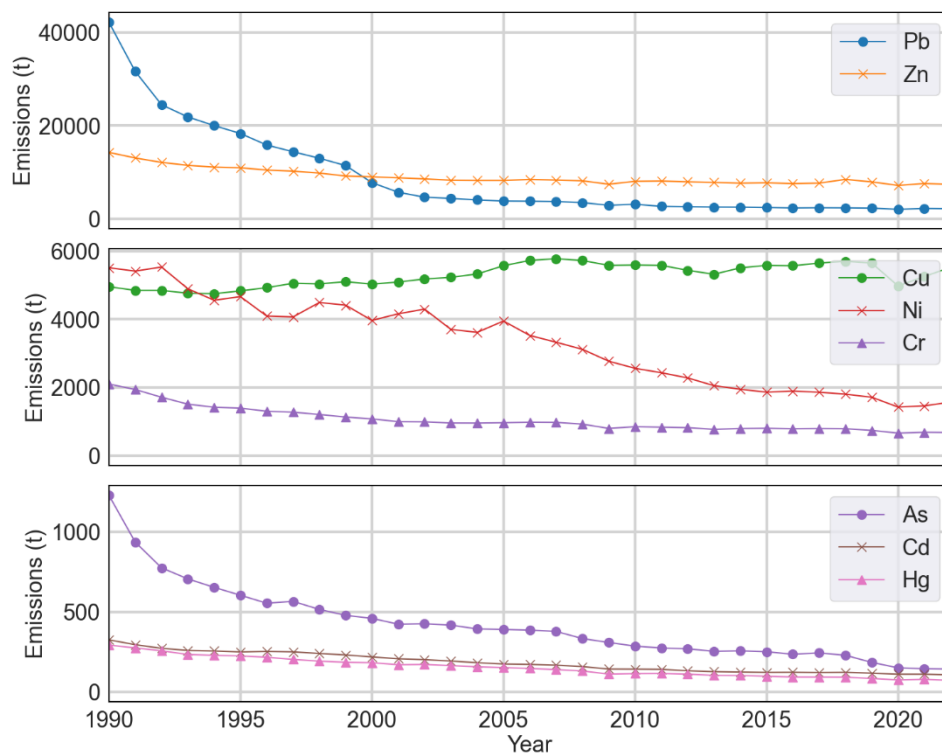


Figure 5: Trends in atmospheric trace element emissions in the EU28 (1990-2023), based on NEC Directive inventory data (European Environment Agency, 2025)

Emissions of Pb showed the most important decline after the EU-wide ban on leaded gasoline, though residual emissions persist from metallurgy and waste incineration (Li et al., 2017; Zhang et al., 2024). Emissions of Ni and Cr have also decreased, mainly due to the phase-out of heavy fuel oils and improved filtration technologies in the metal sector (Bai et al., 2023). The most toxic and tightly regulated elements (As, Cd and Hg) have declined steadily due to stricter regulations but also from reduced coal use and cleaner waste/metal processing (Nalbandian, 2012; Wang et al., 2021).

In contrast, emissions of Zn and Cu, mostly linked to industrial activities and transport-related abrasion, have declined as much as the other elements. They remain relatively high due to their widespread and ongoing use in infrastructure and manufacturing (European Environment Agency, 2020; Wallonair, 2023).

Although anthropogenic emissions of TEs have declined, current atmospheric concentrations remain at levels that pose significant environmental and health hazards (World Health Organisation, 2021). Their persistence in the atmosphere points to spatially uneven pathways of dispersion and fallout.

2.2.3. Variable transport and deposition

The environmental impact of TEs often extends far beyond their emission source due to differences in atmospheric transport and deposition, which depend on the chemical speciation (Templeton et al., 2000). TEs like Pb and Cd, mostly bound to coarse particles, deposit quickly near sources and cause local contamination (Pacyna & Pacyna, 2001). Others, such as Hg or Zn, are associated with fine aerosols (0.01-20 µm) that can travel longer distances, especially when incorporated into cloud droplets via nucleation processes (Desboeufs, 2001). Their residence time ranges from days to months in the troposphere and up to a year in the lower stratosphere (Steinnes & Friedland, 2006).

Solubility influences the atmospheric mobility of TEs by facilitating their dissolution into cloud droplets and transport via wet deposition. More soluble elements, like Cu and Zn, are easily mobilised, while insoluble ones, such as Fe and Al, deposit closer to their source (Jambon & Thomas, 2009).

A key example is Hg: emitted mainly as Hg⁰, it persists in the atmosphere for months before oxidising into Hg²⁺ and depositing far from its source, including in polar latitudes or high-altitude environments (Driscoll et al., 2013).

While declining emissions are encouraging, they are not sufficient: TEs remain highly mobile and can accumulate in remote ecosystems. This highlights the need not only to track emission levels, but also to monitor their physicochemical behaviour in the atmosphere to better assess their transport, transformation and eventual deposition.

2.2.4. Roles and risks associated with trace elements

Essential and non-essential trace elements

TEs play a paradoxical role in biological systems and can be classified as essential or non-essential. Essential elements are vital for proper metabolic functions (Fe in haemoglobin for O₂ transport, Zn in DNA polymerases, Cu in cytochrome c oxidase), yet both deficiency and excess disrupt homeostasis (Islam et al., 2023). Non-essential elements (e.g., As, Cd) lack physiological function and are toxic at any detectable concentration (Soomro et al., 2024). This physiological duality underscores the narrow margin between beneficial and harmful concentrations, providing a foundation for understanding their environmental and health risks.

Direct risks

The risks associated with TEs arise from their capacity to cause acute and chronic toxicity in humans and wildlife. Inhalation of airborne TEs can lead to acute respiratory

irritation and headaches, while long-term exposure is linked to cardiovascular, neurological and developmental disorders (Bodor et al., 2021; Dahmardeh Behrooz et al., 2025).

In ecosystems, even essential elements for plants can become phytotoxic at high concentrations, affecting plant growth and health. TEs such as Cu, Zn and Cd can accumulate in soils, where they reduce plant diversity, disrupt microbial communities and alter nutrient cycling (European Environment Agency, 2020; He et al., 2005). In wildlife, sublethal exposure to TEs can disrupt physiological functions, leading to reduced fitness, impaired reproductive success and increased vulnerability. For example, birds near industrial sites often exhibit reduced body size, abnormal feather pigmentation and weakened immunity (Barton et al., 2023).

Some TEs, such as mercury, illustrate how direct toxicity can evolve into broader ecological risks. Once deposited, Hg can be converted into methylmercury, becoming not only hazardous to individual organisms but also bioavailable across trophic levels. This transformation exemplifies how TEs can generate effects that extend beyond initial exposure.

Trophic and ecological impacts

Among these indirect effects, TEs pose an insidious risk through biomagnification (Figure 6). Once released, they disperse through air, water and soil compartments: they are taken up by primary producers and progressively concentrate along the food chain. Top predators, including humans, are thus exposed to the highest levels (Chormare & Kumar, 2022; Szykowska et al., 2018).

Examples of this are Cd, which accumulates in crops grown on contaminated soils and, through diet, builds up in human tissues, causing kidney damage (European Food Safety Authority, 2012). Another example is methylmercury (among the most toxic forms of mercury to humans), which accumulates in predatory fish through biomagnification. When consumed through the diet, it builds up particularly in brain tissues and causes severe damage to the nervous system (Madgett et al., 2021).

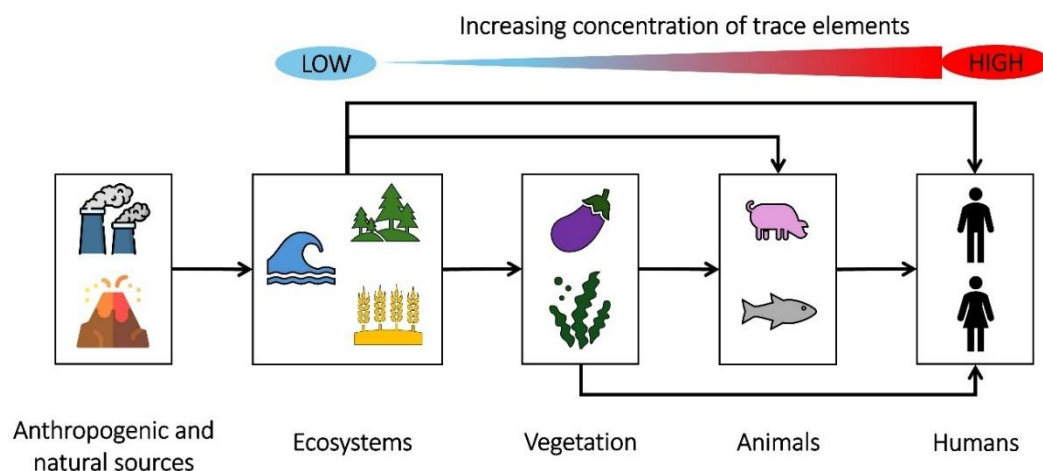


Figure 6: Simplified diagram of biomagnification of trace elements through ecosystems and living organisms.

These risks, whether direct or indirect, are not only shaped by the properties of TEs themselves, but also by the contexts and pathways through which they enter the environment. Identifying the sources and understanding the conditions under which TEs are emitted is thus a crucial step toward assessing their distribution, fate and long-term impacts.

2.2.5. Atmospheric monitoring of trace elements in Wallonia

In Wallonia, atmospheric TEs are primarily monitored through a network of fixed air quality stations that collect PM₁₀ and PM_{2.5} particles for subsequent chemical analysis. These stations, distributed across urban, industrial and some rural areas, aim to provide a representative picture of the regional air quality trends (Hooyberghs et al., 2005).

These stations operate within two delayed-measurement networks:

- The “matières lourdes” (ML) network, which collects total suspended particles and analyses them using X-ray fluorescence.
- The “éléments métalliques” (EM) network, which focuses on the chemical composition of PM₁₀ particles, which are analysed for trace elements using inductively coupled plasma mass spectrometry (ICP-MS).

Sampling involves drawing air through filters using active or passive systems, depending on the pollutant. A mobile monitoring unit also complements this network by targeting under-sampled or suspected pollution hotspots.

Currently, 16 stations are operated across the region as part of the EM network, primarily coordinated by the Walloon Air and Climate Agency (AWAC, 2023), as shown in (Figure 7). However, most are concentrated in industrial areas along the Sambre-Meuse axis (e.g., near metallurgy and urban centres), while rural and remote regions remain largely unmonitored. Moreover, the system is relatively recent (launched in 2013), limiting the availability of long term datasets and making trend analysis challenging.

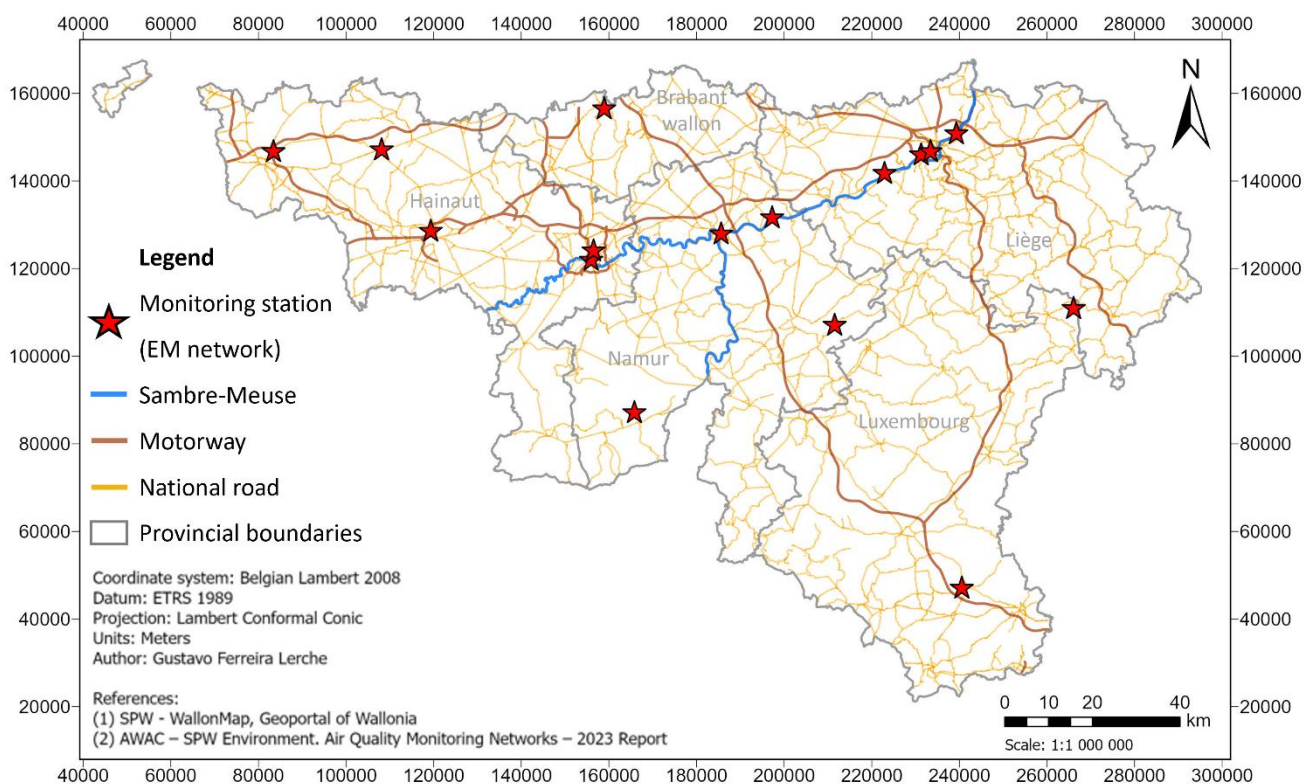


Figure 7: Spatial distribution of the 16 monitoring stations in the "éléments métalliques" (EM) network coordinated by the Walloon Air and Climate Agency (AWAC, 2023), highlighting the concentration of the stations along the Sambre-Meuse industrial axis.

While these systems offer high analytical precision, their sparse distribution, constrained by the costs of installation, maintenance and calibration (EPA, 2002), limits their capacity to reflect local pollution gradients. Point-based measurements also overlook the spatial heterogeneity of TE deposition shaped by land use, topography and microclimate. To address these limitations, biomonitoring has emerged as a valuable complementary approach.

2.3. Biomonitoring using lichens

2.3.1. Lichens: a symbiotic organism

Involved organisms

Lichens are composite organisms formed through a mutualistic symbiosis between a fungal partner (the mycobiont) and one or more photosynthetic partners (the photobionts), typically green algae, cyanobacteria or both (Honegger, 1991; Nash, 2008). In this mutualistic relationship, the photobiont performs photosynthesis, producing carbohydrates that feed the entire symbiosis. The fungal partner absorbs these compounds and, in return, builds the protective thallus, retains water and facilitates the uptake of mineral nutrients (e.g., nitrogen, phosphorus, TEs) from atmospheric sources (Kranner et al., 2009; Palmqvist, 2000; Richardson, 1999). An illustration of these exchanges is shown in Figure 8. This metabolic exchange forms the basis of a finely tuned biochemical cooperation that enables lichens to survive in nutrient-poor and highly variable environments.

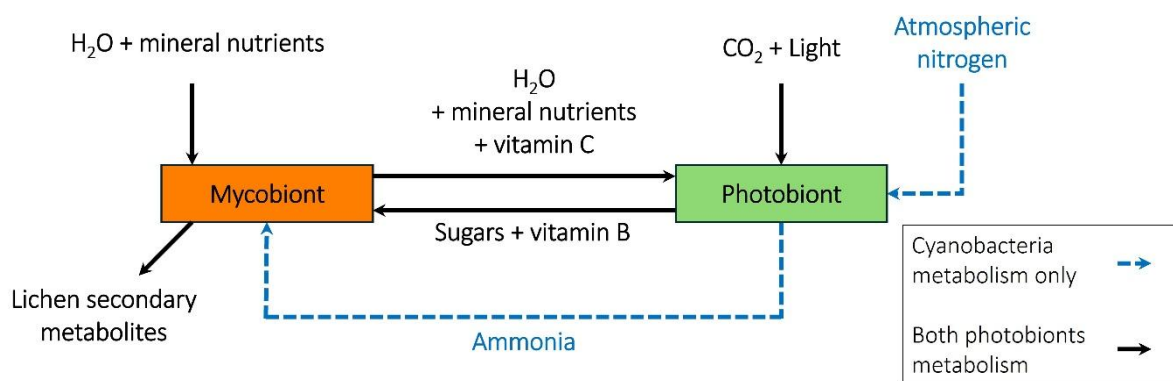


Figure 8: Illustration of the nutritional exchanges between the photobiont and the mycobiont in a lichen symbiosis. The black arrows represent metabolic processes and nutrient exchanges carried out by both photobionts (cyanobacteria and algae); the blue dashed arrows indicate processes specifically carried out by the cyanobacteria. Adapted from (Van Haluwyn et al., 2022).

In most lichens, the mycobiont is an obligate biotroph, forming about 80% of the thallus biomass and defining the species identity (Ahmadjian, 1993), whereas the photobiont may occur freely in nature and accounts for less than 20% of the symbiotic organism's biomass (Honegger, 2023). Approximately 90% of lichens host green algae, while the remaining 10% host cyanobacteria (Honegger, 1998).

Structural organisation and thallus morphology

The lichen thallus typically consists of three horizontal layers (Figure 9): a protective upper cortex formed by densely packed fungal hyphae; a medulla, composed of loosely interwoven hyphae that enclose the photobiont cells; and a lower cortex, similar in structure to the upper layer, which may bear rhizines that anchor the thallus to its substrate (Nash, 2008). This stratified architecture not only ensures mechanical support and protection but also facilitates gas exchange and the absorption of water and airborne particles.

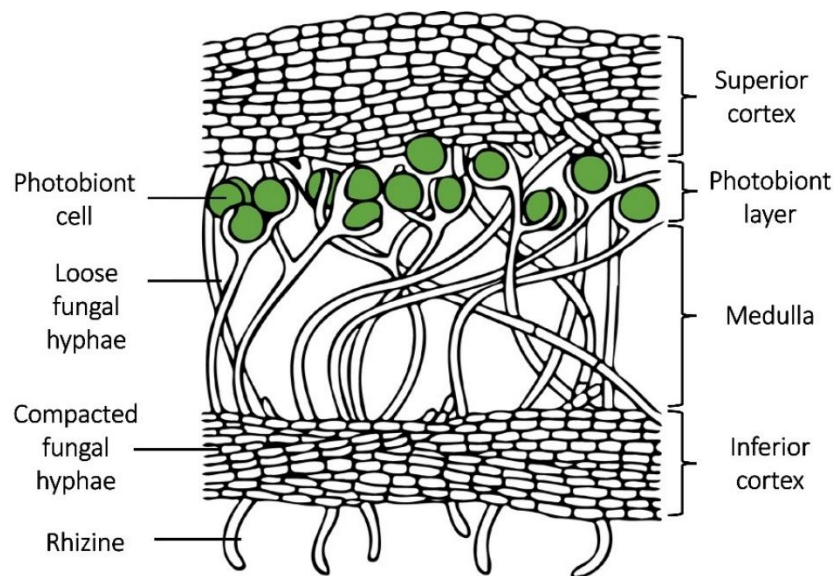


Figure 9: Illustration of the typical structure of the lichen thallus in cross-section (<https://biogeoscience.eu>).

Importantly, the upper cortex is not impermeable; its porous structure allows the penetration of nutrients, gases, water and fine particles (such as TEs). During rainfall or dew formation, particles can infiltrate the cortex either by gravitational settling or via water droplets. Once inside, the loosely structured medulla acts like a sponge, favouring the retention and accumulation of these substances (Nierboer et al., 1978). Combined with a high surface-to-volume ratio, absence of a protective cuticle and lack of stomata, these traits expose lichens more directly and intensively to atmospheric pollutants than vascular plants, rendering them especially vulnerable to air-quality changes and ideal for biomonitoring studies (Nash, 2008).

Morphologically, lichens display a wide range of thallus forms, but they are conventionally classified into four main types (Brodo et al., 2002): (1) crustose lichens form thin, tightly adhering crusts that cannot be removed from their substrate without damage; (2) foliose lichens have leaf-like lobes that are loosely attached and often secured with rhizines; (3) fruticose lichens grow in bushy or filamentous forms, either erect or pendulous, typically anchored at a single point; (4) complex lichens combine elements of multiple forms, such as a crustose base with a fruticose structure growing above.

This morphological diversity (Figure 10) influences how lichens interact with their environment and determines their capacity to accumulate and retain atmospheric pollutants.

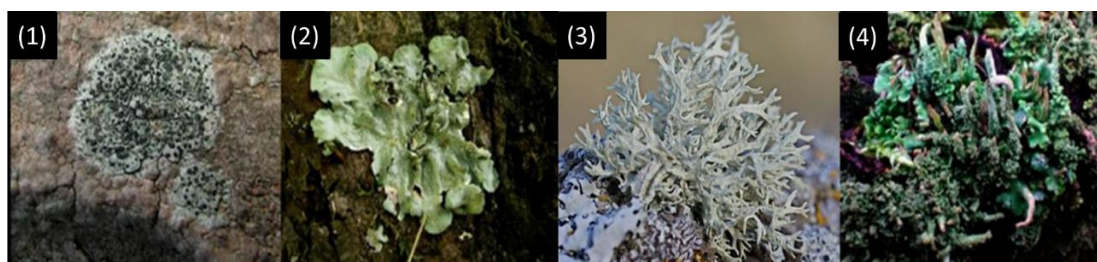


Figure 10: Examples of different morphological thalli: (1) crustose; (2) foliose; (3) fruticose; (4) complex.

Reproduction and growth

Lichens reproduce sexually (via fungal spores) and/or vegetatively (via symbiotic propagules), but successful sexual reproduction is rare due to the need for a compatible photobiont (Krishnamurthy & Upreti, 2001). In contrast, vegetative modes ensure joint dispersal through structures such as soredia and isidia (Kantelinen et al., 2022; Figure 11).

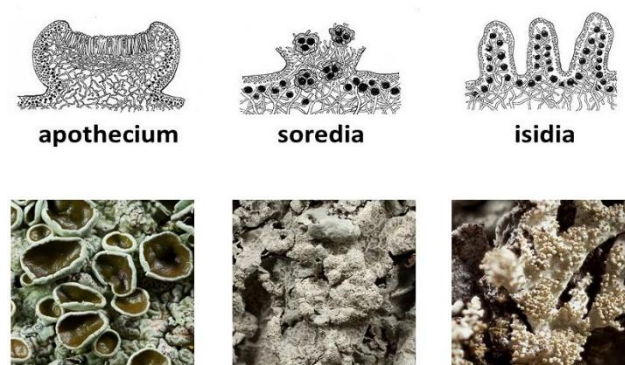


Figure 11: Illustration and images of lichen reproductive structures: sexual reproduction via apothecium (left); vegetative reproduction via soredia (middle); vegetative reproduction via isidia (right).

Once established, lichens grow slowly (only a few millimetres to centimetres per year) and can live for centuries (Armstrong & Bradwell, 2010). Their growth is limited by environmental stress, especially desiccation cycles. Some crustose species, such as *Rhizocarpon geographicum*, are even used in lichenometry to date exposed rock (Bradwell, 2009). This combination of slow growth, longevity and environmental dependence makes lichens sensitive indicators of ecological change.

2.3.2. Lichen ecology

Lichens are widely distributed across terrestrial ecosystems, with approximately 20,000 recognized species colonizing around 6% of the Earth's land surface (Brodo et al., 2002). Lichens occur in temperate and boreal forests, alpine and arctic tundras, Mediterranean rocky habitats, cold deserts, coastal cliffs and urban environments, but their distribution reflects a complex interplay of ecological preferences.

Each lichen species has distinct ecological preferences. Their occurrence depends on a range of abiotic factors such as climate, substrate type and air quality. Due to their morphological features, they are particularly sensitive to atmospheric pollutants such as SO₂, NO_x, radionuclides and TEs (Conti & Cecchetti, 2001; Nimis et al., 2002a).

Air pollution can significantly alter lichen community structure by selectively eliminating sensitive species and favouring pollution-tolerant taxa. This filtering effect leads to predictable spatial patterns in species composition, particularly along urban/rural or industrial/natural gradients. As a result, lichen diversity and assemblage shifts are widely used as indicators of air quality, both in ecological studies and pollution monitoring programs (Neitlich, 1993; Van Dobben & Ter Braak, 1998).

2.3.3. Bioindication

Ecology and sensitivity of communities

Lichen communities are also highly sensitive to changes in environmental quality, particularly air quality, which shifts the assemblages of taxa. As each species has specific ecological requirements and tolerance thresholds, the resulting community composition serves as a powerful indicator of habitat integrity. In polluted environments, sensitive taxa tend to decline or disappear, while more tolerant species proliferate. This ecological filtering results in measurable shifts in diversity and structure. As a result, lichens function as effective bioindicators for long-term or large scale atmospheric monitoring.

Application of bioindication methods to lichenology

In Europe, bioindication methods are standardised to ensure comparability across regions and are typically divided into two broad categories. Quantitative methods calculate ecological indices from the lichen community at each site. For instance, alpha diversity metrics such as species richness and Shannon's index often decline in urban or industrial areas, signalling elevated anthropogenic stress.

A widely adopted metric under the European protocol (prEN16413) is a frequency-based index that scores lichen presence across predefined grid squares on tree trunks, enabling large-scale, temporal comparisons of cumulative pollution exposure (Asta et al., 2002). This method is visually represented in Figure 12, which illustrates the application of this protocol on tree trunks to assess lichen presence and diversity. Another quantitative method, the index of atmospheric purity (IAP), combines the sensitivity of multiple species to yield a single air quality score (LeBlanc & De Sloover, 1970).

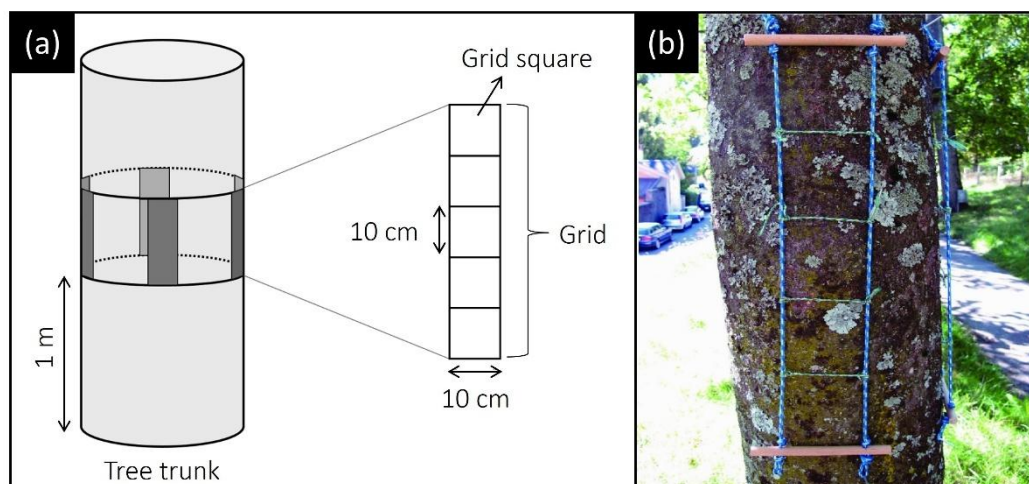


Figure 12: Illustration of the field measurement protocol for calculating the frequency based index using a 5 squares grid (10×10 cm squares) placed at 1 m height on four cardinal trunk faces, following the European prEN16413 protocol; (b) field application using a rope based grid (Truong, 2013).

Qualitative approaches rely on species sensitivity scales derived from field observations. These include, for example, species known to be sensitive to SO₂ (Hawksworth & Rose, 1970) or NH₃ (Lallemant et al., 1996). For more detailed community-level insight, Van Haluwyn & Lerond (1993) proposed a poleotolerance scale that assigns air quality scores based on the proportion of tolerant, intermediate and sensitive species at a given site. While more demanding in terms of species identification, this method offers finer ecological resolution than broader indices by directly reflecting changes in species composition along pollution gradients.

Strengths and limitations of bioindication

Lichen-based bioindication offers numerous advantages: it is non-destructive, cost-effective and integrates pollutant exposure over long periods. It can detect early ecological impacts and is especially useful in areas lacking technical infrastructure for atmospheric monitoring.

However, bioindication is also influenced by non-pollution factors such as climate, bark pH or microhabitat variability. Moreover, interpreting results requires good knowledge of lichen ecology and the use of well-established protocols to ensure reliability. As a standalone method, it provides valuable insights, but its effectiveness is enhanced when combined with quantitative approaches like bioaccumulation.

2.3.4. Bioaccumulation

Principles of lichen bioaccumulation

Conti & Cecchetti (2001) outlined key criteria for the use of living organisms as bioaccumulators. Suitable organisms must tolerate pollutant exposure and retain accumulated substances over time. They should also be widespread, sedentary, abundant and representative of the sampling area. Year-round availability, sufficient biomass for analysis and structural stability during collection and processing are equally important. Finally, ideal bioaccumulators exhibit high concentration factors, a predictable relationship between tissue content and environmental levels and consistent uptake dynamics under varying conditions.

Lichens meet these requirements. Their slow metabolism and production of protective secondary metabolites allow them to accumulate atmospheric pollutants with minimal physiological damage. Permanently anchored and non-vascular, they reflect only local atmospheric inputs. Their desiccation tolerant thalli are produced year-round and absorb pollutants passively across an unprotected surface, resulting in a linear accumulation pattern (Loppi, 2014). This leads to elevated trace element concentrations in the thallus and a close correlation with atmospheric deposition. Numerous studies have confirmed that lichen metal content reliably reflects local air quality (Calvelo et al., 1997; Cecconi et al., 2019).

Mechanisms of bioaccumulation in lichens

Lichen bioaccumulation operates through distinct yet often concurrent mechanisms (Figure 13). Particulate entrapment is a physical process in which insoluble atmospheric

particles become lodged within the porous thallus, particularly in the medulla. This accumulation is superficial and generally reversible, as particles can be removed by acid washing (Nash, 2008).

By contrast, extracellular adsorption is a chemical process in which dissolved metal ions bind to lichen surfaces via weak interactions such as ion exchange or Van der Waals forces. This process is rapid, pH dependent and primarily involves soluble pollutants (Nierboer et al., 1978).

A third, more selective pathway is intracellular uptake, where essential or metabolically active elements such as Mn^{2+} or Cu^{2+} are transported into cells through physiologically regulated mechanisms. This process, typically slower and limited to specific ions, follows Michaelis-Menten kinetics (Paul, 2005).

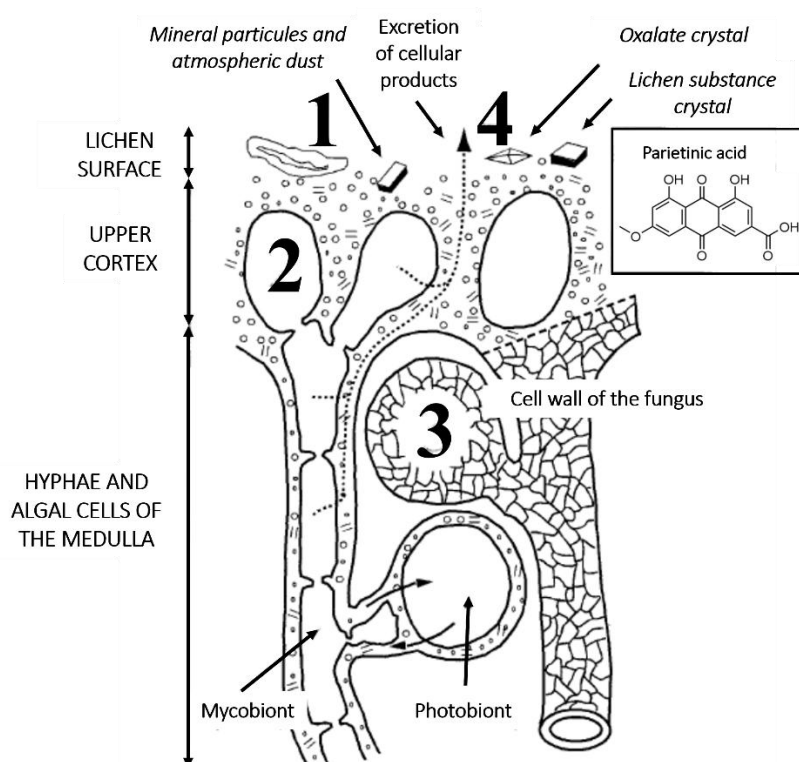


Figure 13: Possible locations and uptake mechanisms of trace elements in the lichen thallus: (1) particulate entrapment on the surface and in intercellular spaces of the fungal biont; (2) intercellular uptake of trace elements; (3) extracellular adsorption to functional groups of the fungal cell walls; (4) extracellular complexation to organic acids such as parietinic acid (Honegger, 1991).

Finally, lichens can release accumulated metals either through rainfall driven solubilisation, although its effects vary widely (Prussia & Killingbeck, 1991) or via cationic competition, where new ions displace bound metals at adsorption sites. (Paoli et al., 2018). These desorption processes create a dynamic balance between uptake and release.

Application of bioaccumulation in lichenology

Lichen bioaccumulation studies typically involve measuring concentrations of elements in thallus tissues and comparing them across sites or against reference values.

One method for bioaccumulation analysis was proposed by Nimis et al. (1993), who compiled concentration data from dozens of lichen species across unpolluted sites in La Spezia (Italy) to establish, for each element, a background range (5th-95th percentile), as shown in Table 1. Values above the 95th percentile were flagged as altered, indicating enrichment (Nimis et al., 1993).

Table 1: Scale for interpreting trace-metal concentrations (expressed in $\mu\text{g}\cdot\text{g}^{-1}$ of dry weight) in lichens, reflecting environmental alterations and deviations from natural background levels (Nimis et al., 1993).

Category	Al	As	Ba	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Very high naturality	<350	<0.2	<3.3	<0.2	<1.2	<7	<290	<20	<1	<1	<0.6	<30
High naturality	600	0.6	6	2.2	2.2	10	500	25	2	2	1.7	40
Middle naturality	1000	1.2	10	0.8	0.4	15	800	35	3	3	3.1	65
Low naturality	1600	1.9	18	1.6	2	25	1200	60	5	5	5.1	94
Middle alteration	2500	2.4	25	2	2	34	1500	90	8	8	6.7	115
High alteration	3200	3	35	2.6	2.6	53	1800	100	10	10	9.3	155
Very high alteration	>3200	>3	>35	>2.6	>2.6	>53	>1800	>140	>10	>10	>9.3	>155
Max Italy	8390	5.53	78.7	9.04	60.5	161	4276	685	34.4	494	15	358

Another common approach is by calculating enrichment indexes, which normalise each element's concentration to that of a reference element (usually Al or Fe) and then compares this ratio to natural background levels to estimate anthropogenic enrichment (Bergamaschi et al., 2002; Cecconi et al., 2021; Varrica et al., 2022).

In addition to these enrichment indices, some studies calculate the ratio of element concentration in the thallus to its background level and combine these ratios into a multiplicative composite index which provide an overall synthetic estimate of metal pollution (Boamponsem et al., 2010; Boonpeng et al., 2018).

Using these methods, some studies have assessed pollution across broad spatial and temporal scales (Agnan et al., 2015; Wu et al., 2021), while others have focused on specific time periods or point sources to determine risk element levels (Bergamaschi et al., 2002; Kováčik et al., 2023; Varrica et al., 2022).

Taken together, These indices and methods provide quantitative insights into the accumulation of pollutants in lichens and serve as practical tools for passive pollution assessment, although each approach has its limitations.

Strengths and limitations of bioaccumulation

Using lichens for bioaccumulation offers several advantages. They integrate pollutant inputs over time and require no active infrastructure, making them particularly suitable for remote or data-poor regions. Their widespread and sedentary nature improves spatial resolution and allows for fine-scale mapping of urban pollution, complementing conventional monitoring stations.

Nonetheless, some limitations must be acknowledged. Accumulation rates differ between species, complicating interspecies comparisons (Conti & Cecchetti, 2001). In the absence of reliable background levels, interpreting absolute concentrations and attributing pollution sources remains uncertain. Additionally, external factors such as leaching from rainfall, ion exchange processes or site-specific retention dynamics can alter metal content and obscure analytical interpretation (Prussia & Killingbeck, 1991). As a result, lichen bioaccumulation should ideally be combined with other ecological, chemical or statistical indicators to enhance the reliability of air pollution assessments.

2.3.5. Coupling biomonitoring methods

Lichen bioindication and bioaccumulation provide complementary insights into atmospheric pollution: the former captures ecological responses at the community level, while the latter quantifies specific TE concentrations within thallus tissues. Yet, these methods are often used in isolation, reducing their effectiveness in assessing the full spatial and functional complexity of atmospheric contamination.

Some studies have sought to quantify lichen species' sensitivity to specific atmospheric pollutants. Van Dobben & Ter Braak (1998) developed the NH_x index by linking lichen community composition to nitrogen levels using statistical analyses. This allowed researchers to assign NH_x sensitivity scores to species and estimate site level nitrogen deposition from their abundances. Their framework, validated through extensive data on nitrogen deposition, provided a robust method to convert ecological patterns into quantitative estimates.

Despite this success with nitrogen, comparable scoring systems for TE sensitivity are still lacking. Aside from (Agnan et al., 2017), who proposed an approach in forested contexts, few attempts have been made to define species-specific responses to metal exposure. Urban environments, characterised by complex and overlapping sources of TE pollution, demand distinct yet analogous methodological approaches.

This results in a persistent gap: species specific responses to trace elements remain poorly characterised in urban environments, where stressors differ markedly from forest conditions. This hampers the development of rapid, indicator-based assessment tools and

reinforces reliance on laboratory-based bioaccumulation analyses, despite their cost and lower spatial resolution compared to field-based bioindication.

3. Objectives of the study

Air pollution from trace elements remains a major environmental and societal concern. In Wallonia, air quality monitoring relies primarily on fixed stations located in predefined zones, which limits spatial resolution and leaves regional-scale data on atmospheric trace element concentrations incomplete.

Lichen-based biomonitoring offers a complementary tool to address this gap. Bioindication, based on species presence and abundance and bioaccumulation, based on the chemical composition of lichen thalli, are established methods. However, their integration is still limited, particularly in urban environments where species-specific responses to trace element exposure remain insufficiently characterised.

This study aims to construct a lichen sensitivity scale for trace element pollution using species frequency across sites with contrasting contamination levels. The objective is to estimate local pollution intensity from species composition, especially where chemical analyses are not feasible.

To achieve this, we combine ecological and chemical data. Community surveys provide species distribution patterns for bioindication, while concentrations of trace elements in *Xanthoria parietina* thalli are measured to calibrate and validate these patterns for bioaccumulation. Sites were selected across urban, agricultural and rural environments in Wallonia.

This combined approach provides the basis for developing a robust and operational scale of species sensitivity to trace element pollution.

4. Materials and methods

4.1. Study area

4.1.1. Geographical, climatic and historical context

Wallonia occupies the southern half of Belgium, stretching from the Rhine-Meuse valley in the north to the Ardennes plateau in the south. The region's topography is characterized by three major geomorphological units: the loamy lowlands of the northern plains, the intermediate karstic zones and the higher-altitude forested plateaux of the Ardennes (SPW, 2016).

The local climate is classified as temperate oceanic, characterized by mild winters, moderately warm summers and evenly distributed precipitation throughout the year. Between 1991 and 2020, it was recorded that the region's average annual temperature was 11.0°C, with 831 mm of precipitation per year spread over approximately 190 days of rainfall (IRM, 2020).

Wallonia played a central role in Belgium's industrialization, drawing on its coal and iron resources to become a major European industrial region in the 19th and 20th centuries. The Sambre-Meuse corridor, with cities like Liège and Charleroi, was shaped by coal mining, steelmaking and zinc smelting. Although many industries declined after the 1970s, their environmental legacy remains visible in the region's landscapes, soils and air quality, making Wallonia an important area for studying the impact of industrial pollution (European Commission, 2023).

4.1.2. Sampling sites

We selected 41 sampling sites distributed across nine municipalities to capture today's highly urban and industrial, peri-urban and rural contexts. These municipalities included:

- Charleroi (the largest city in Wallonia by population) and Liège (the second-largest city in Wallonia), both large, densely populated cities with historic and still-active industrial zones.
- Namur, a predominantly residential city (third largest in Wallonia) with some moderate industrial activities in its surrounding areas.
- Arlon and Marche-en-Famenne, mid-sized urban centres featuring mixed land use (residential, commercial and light industry).
- Orp-Jauche and Honnelles, smaller, low-density communes characterized primarily by agricultural landscapes.
- Vielsalm, a site dominated by extensive forested surroundings.

For each sampling site, we compiled municipal-scale data: density of population (in inhabitants per km²) and percentage of land use areas (artificialized, agricultural and tree cover), drawn from the *Institut Wallon de l'Évaluation, de la Prospective et de la Statistique* (IWEPS, 2025) to characterize the municipal context (Appendix G.2). We also generated a ternary plot for each municipality to visualize gradients in land-use composition across the region (Figure 14).

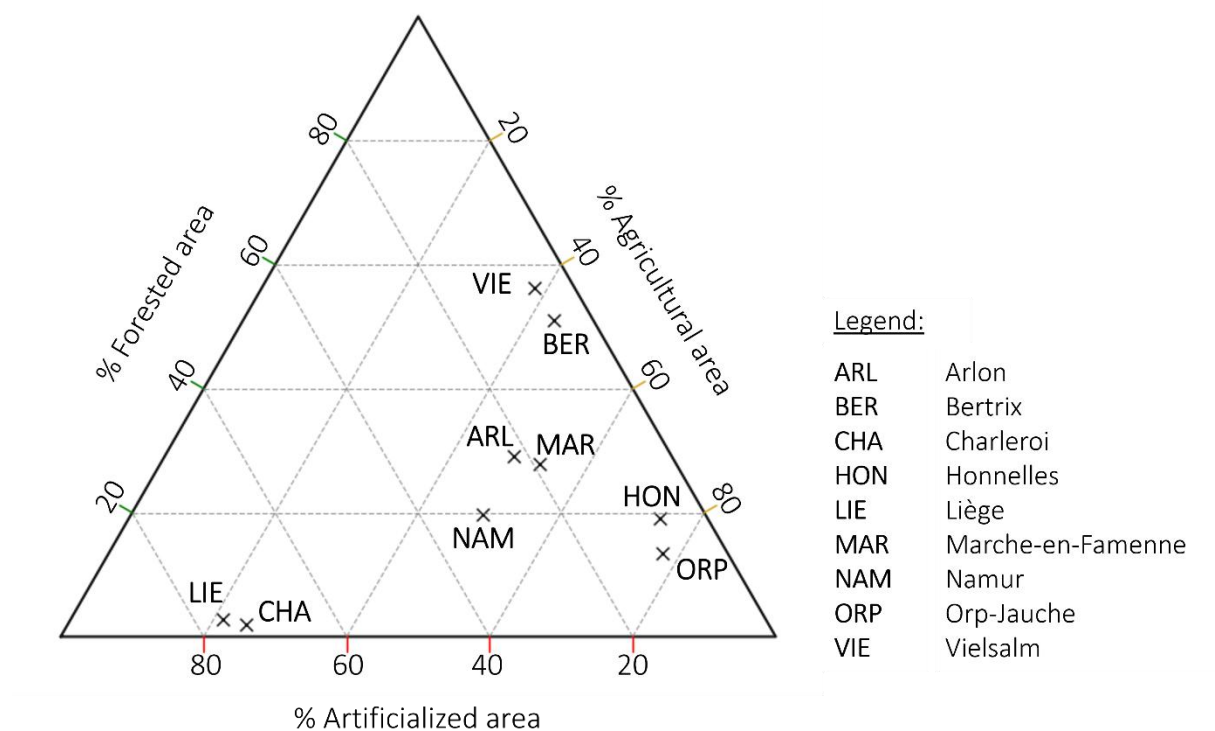


Figure 14: Ternary plot illustrating the landscape structure (percentage of artificialized, agricultural and forested areas) for every municipality, highlighting the contrasting gradients of land use.

The different municipalities in the study area can be classified as following:

- Charleroi and Liège are the most urban dominated cities with high percentage (> 60%) of artificialized area in the municipality.
- Arlon, Marche-en-Famenne and Namur being transitional cities between urban and rural settings reflected by a mix of artificial, agricultural and forested areas.
- Honnelles and Orp-Jauche being rural cities dominated by agricultural areas (> 70%).
- Bertrix and Vielsalm are the forest dominated rural areas (> 50% of tree cover).

By placing sampling points in each of these nine municipalities, we ensured full spatial coverage of highly urbanised districts, active industrial areas, suburban neighbourhoods, farmlands and forested zones (Figure 15), thereby capturing a heterogeneous regional context.

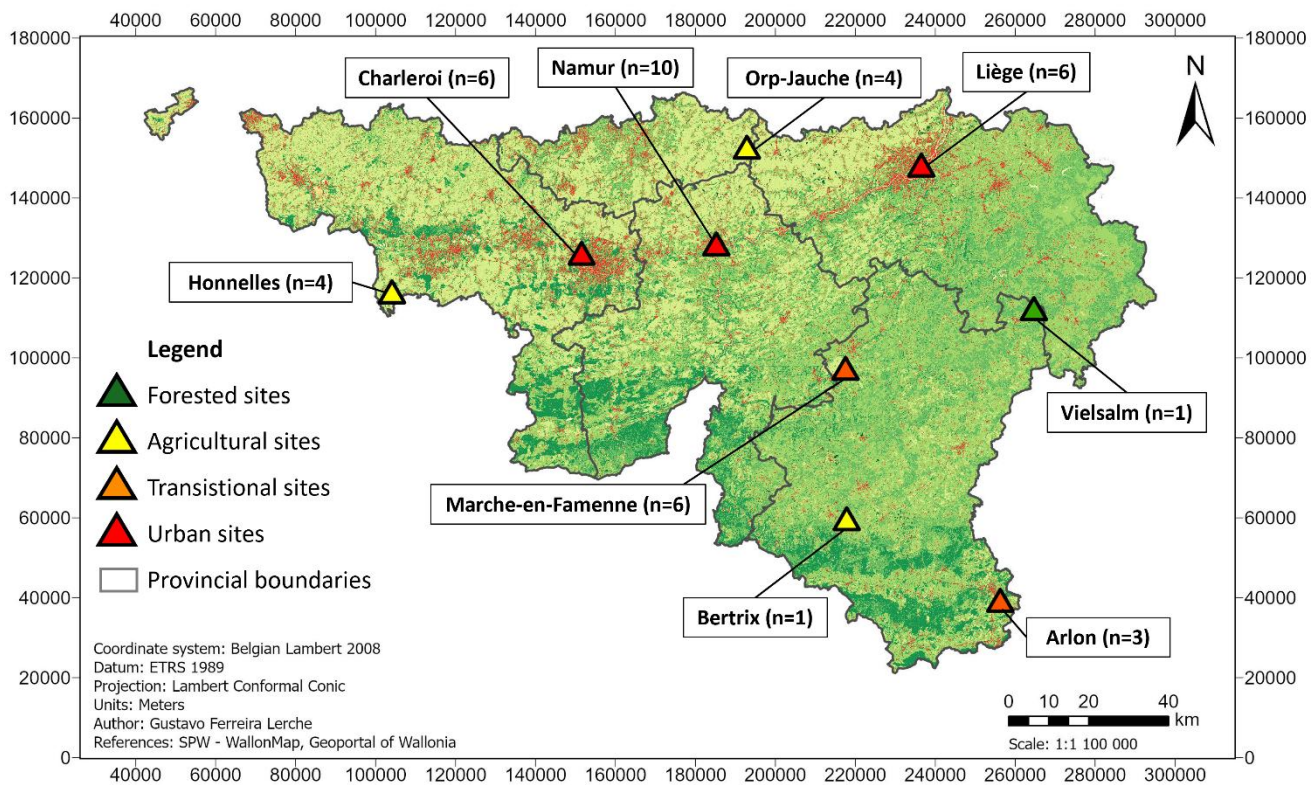


Figure 15: Spatial distribution of the 41 sampling sites in Wallonia categorized by land use type: forested site (green), agricultural sites (yellow), transitional sites (orange), and urban sites (red). The number of sites (n) at each location is indicated.

4.2. Bioindication methods

Our bioindication method follows the European standardized protocol (CEN, 2014). This standard prescribes site selection, tree selection, sampling height and frame size to ensure comparability across studies.

4.2.1. Choice of sites and trees

To minimise the influence of factors unrelated to pollution, the EU standard defines specific selection criteria for both sites and individual trees. In our study, only linden trees (*Tilia sp.*) were chosen to avoid species-related biases (Nascimbene et al., 2007). At each site, four mature lindens were identified based on their common presence in both urban and rural areas as well as their exposure to open environments under similar conditions. Trees were spaced no more than 50 m apart to ensure comparable microenvironments without overlap.

All selected trees had straight boles with diameters between 50 and 250 cm (measured at 1.3 m above ground), trunk inclinations of less than 20° in each cardinal direction, no branches below 2 m, no visible damage or significant bark flaking and less than 10% moss cover on the trunk surface. These conditions ensure comparable exposure to sunlight and atmospheric deposition while minimising bark-related interference with lichen colonisation.

4.2.2. Lichen identification and sampling

The survey is conducted on the four cardinal-facing sides of each tree using a 10 × 50 cm observation grid, with the bottom of the grid positioned at 1 m above ground level (Figure 16). Each grid consists of five squares, each measuring 10 × 10 cm. Lichens present in each square are observed using lens with 10 times magnification. For each orientation, we assign each species a score from 1 to 5 corresponding to the number of squares in which it is present (e.g., a score of 3 indicates that the species was found in three of the five squares on that side). A frequency score can then be calculated for each face (ranging from 0 to 5), per tree (0 to 20) and for each site (0 to 80). Additional information recorded during the survey includes trunk circumference (measured at 1.3 m above ground level) and geographical coordinates.

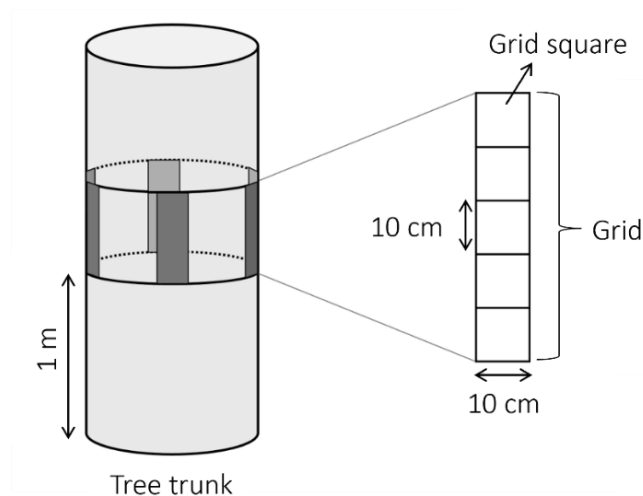


Figure 16: Schematic representation of the lichen bioindication sampling method. A 10 × 50 cm grid, composed of five 10 × 10 cm squares, is placed at 1 m above ground level on each cardinal side of the tree trunk. Observations are conducted within each square.

Species identification was carried out mainly in the field using standard reference identification keys with support from reference works (Martellos, 2018; Smith et al., 1992). When specimens proved difficult to separate morphologically, a small sample was collected for laboratory analysis (microscopy and chemical tests).

Certain abundant species difficult to distinguish in the field were grouped: *Punctelia subrudecta* with *P. borrieri* and *Physcia tenella* with *P. adscendens*, following recommendations of G  rault (2025) and Lendemer & Hodkinson (2010). Following the same logic, *Candelariella xanthostigma* and *C. reflexa* were combined as *Candelariella sp.* due to overlapping morphological traits, preventing reliable separation during surveys. All species from the genus *Lepraria* were recorded as *Lepraria sp.* for the same reason.

4.3. Bioaccumulation methods

4.3.1. Sampling

To assess TEs deposition, we selected the foliose lichen *Xanthoria parietina*, a widely distributed, pollution-tolerant species that reliably reflects atmospheric metal loads and that has been widely used in bioaccumulation studies (Cecconi et al., 2019; Kováčik et al., 2023; Rizzio et al., 2001; Vannini et al., 2016a). At each bioindication site, we collected three independent replicates (one per tree), following the protocol of Bargagli & Nimis (2002).

Sampling was always performed between 1 and 2 m above ground to limit contamination from soil particles or animal activity. Whenever possible, these three replicates came from the same trees used for bioindication. When *X. parietina* was absent on the designated bioindication trees (< 15% of cases), we sampled in adjacent linden trees. Entire lichen thalli were collected from around the tree using a ceramic knife (to prevent metal contamination) and stored in clean plastic bags.

4.3.2. Samples preparation and acid digestion

Sample preparation

In the laboratory, samples stored in plastic bags were air-dried at room temperature. The lichen samples were then sorted using non-metallic tools (plastic tweezers and ceramic knives), previously cleaned with distilled water. This step involved removing bark fragments and other epiphytes. Finally, the samples were grinded in liquid nitrogen with the use of a ceramic mortar to obtain a fine and homogeneous powder.

Acid digestion and dilutions

For the analysis, 100 mg of dry, finely ground lichen powder was weighed directly into a 7 mL Teflon container (Savillex Purillex® PFA Vial). At least two blanks samples (with no lichen powder) and two certified reference samples (lichen IAEA-336) were included in each batch. To quantify TE concentrations, an acid digestion step is required to remove organic matter and yield a clear solution, this process is composed of four main steps.

First, 1 mL of hydrogen peroxide (H_2O_2) was added to each vial in three portions. The vials were then placed in an ultrasonic bath for 3×3 minutes to ensure homogenisation. Immediately after sonication, they were left with caps partially open at room temperature for several hours to allow degassing. Evaporation was then carried out at 40°C, initially with the caps partially open for about half a day, then with the caps fully removed until the residues were completely dry.

In the second step, 0.5 mL of nitric acid (HNO_3 , 65%) and 0.5 mL of hydrofluoric acid (HF, 40%) were added to each Savillex vial. After sonication for 3×3 minutes, the vials were left partially open at room temperature for half a day to degas. The solutions were then heated

at 90°C for two days. Once heating was complete, they were evaporated at 40°C until only a minimal volume remained.

Next, 1 mL of freshly prepared aqua regia (0.8 mL hydrochloric acid, HCl 37% and 0.2 mL HNO₃ 65%) was added to each vial. The contents were sonicated for 3 × 3 minutes, then heated at 90°C for one day. This was followed by evaporation at 40°C until the samples were nearly dry. Finally, 1 mL of HNO₃ (65%) was added to each residue, followed by sonication for 3 × 3 minutes and a final heating step at 90°C for one day to complete the digestion.

Prior to the analysis of TE concentrations, the 1 mL digest was transferred into a 15 mL plastic tube and brought to 12 mL with 2% HNO₃; an additional 1:10 dilution was then performed before injection, yielding a final dilution factor of 1 200.

4.3.3. Chemical analysis

Inductively coupled plasma mass spectrometry

An inductively coupled plasma mass spectrometer (ICP-MS) was used to quantify the concentrations of the TEs of interest, expressed in micrograms per litre (µg·L⁻¹). We selected 14 TEs to focus on the rest of this study: Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn. These elements were chosen for their relevance to both anthropogenic and geogenic deposition sources, their ecological and toxicological significance and their widespread use in lichen biomonitoring to assess industrial and traffic related pollution.

Limits of detection (LOD) and quantification (LOQ), calculated from instrumental sensitivity and background equivalent concentrations (BEC) provided by the ICP-MS software, ranged from 0.000 to 0.663 µg·L⁻¹ and 0.001 to 2.208 µg·L⁻¹, respectively. All lichen concentrations exceeded these thresholds, though values approaching the LOD for Ni were interpreted with caution due to possible background interference.

4.3.4. Quality control

Blank samples analysis

To assess data quality and estimate analytical background noise, blank samples were included in each digestion batch. The ratio between the concentration measured in the blanks and the average concentration in the samples was calculated for each element (Table 2: Noise [%] of analytical blanks (n=3)Table 2).

Table 2: Noise [%] of analytical blanks (n=3)

Element	Noise [%]													
	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Sn	Pb
Sample	0.08	0.13	0.16	0.27	0.34	0.39	0.4	0	0.51	1.1	2.51	0.21	0.64	0

Results show that noise levels remained below 1% for most analysed elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Cd, Sn and Pb). Only As (2.51%) and Zn (1.1%) showed noise values above 1%. Negative or non-significant values were replaced with zero, in accordance with standard practices in trace element analysis.

Recovery rates

To assess the accuracy of the digestion and measurement protocol, certified reference material (lichen IAEA-336) was included in each digestion batch. Mean recovery rates were calculated for the 14 studied TEs by comparing the measured concentrations in the reference samples to the certified values.

$$\text{Recovery rate [\%]} = \left(\frac{\text{Measured concentration}_{\text{IAEA336}}}{\text{Reference concentration}_{\text{IAEA336}}} \right) \cdot 100$$

For most elements, mean recovery rates fell within the commonly accepted tolerance range of 80% to 120%, indicating satisfactory analytical accuracy. These results confirm that the acid digestion and ICP-MS measurement protocol yielded reliable and consistent data.

However, the recovery rate for Sn is less reliable, as it is based on only two measurements reported by external laboratories. As for Ni, the low recovery value may reflect either partial loss during sample preparation or interferences during measurement and should therefore be interpreted with caution.

Table 3: Mean and standard deviation recovery rates values [%] of the 14 studied elements in the IAEA336 samples (n=3)

Element	Recovery rates [%]													
	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Sn	Pb
Mean	116	117	95	99	104	105	93	53	89	99	119	85	3	90
± standard deviation	± 10	± 9	± 7	± 6	± 7	± 5	± 3	± 3	± 2	± 4	± 7	± 1	± 2	± 3

Limits of detection

Limits of detection (LOD) and limits of quantification (LOQ) for each element were calculated from instrumental sensitivity and background equivalent concentrations (BEC) provided by the ICP-MS software. For our 14 targets, LODs ranged from 0.000 to 0.663 ppb and LOQs from 0.001 to 2.208 ppb, demonstrating strong analytical sensitivity. All lichen concentrations exceeded these LODs. Although Ni's LOD was very low. Values close to it were treated with caution due to possible background noise.

4.4. Data treatment and statistical analysis

4.4.1. Bioindication indices

Lichen diversity value

The lichen diversity value (LDV) is an index used to assess air quality based on the presence and frequency of epiphytic lichen species on trees. It is calculated by summing the number of lichen species observed in standardized grids (usually divided into quadrants or faces) on several trees at a site. A high LDV indicates high species richness and typically better air quality, while a low LDV suggests poor air quality due to lichen sensitivity to pollutants (Llop et al., 2012; Pinho et al., 2004).

$$LDV = \frac{1}{4} \sum_{n=1}^4 \sum_{m=1}^4 \sum_{i=1}^S F_{imn}$$

Where:

- i : Index of the observed lichen species (between 1 and the total number of species S)
- m : Index of the orientation of observation (between 1 and 4 for each cardinal direction)
- n : Index of the tree where the observation is made (between 1 and 4)
- F_{imn} : Frequency of the species i on the orientation m of the tree n (between 1 and 5)

Shannon's diversity index

Shannon's diversity index (H') quantifies the entropy of species abundances within a community by combining both species richness (total number of species) and evenness (how evenly individuals are distributed among those species). In concrete terms, H' measures the unpredictability of randomly drawing an individual from the community and not knowing its species in advance.

$$H' = - \sum_{i=1}^S p_i \cdot \ln(p_i)$$

Where:

- i : Index of the observed lichen species (between 1 and the total number of species S)
- p_i : Proportion of the species i divided by the total number of species in the community

When all species are equally abundant ($p_i = 1/S$), the uncertainty (entropy) of picking a random individual is maximal (high H'). If one or a few species dominate (p_i close to 1), entropy is lower (low H').

4.4.2. Bioaccumulation metrics

Enrichment factor

The enrichment factor (EF) was used to assess the potential anthropogenic origin of TEs in lichen samples. It compares the ratio of the concentrations of each element (X) to a reference element (E_{ref}) in the sample to the same ratio in a geochemical background.

$$EF = \frac{\left(X/E_{ref} \right)_{sample}}{\left(X/E_{ref} \right)_{UCC}}$$

Where:

- X : Considered element
- E_{ref} : Normalizing element

We used Ti as the normalizing element due to its stability and predominantly crustal origin and calculated EF values using average upper continental crust (UCC) concentrations as background. UCC values used were proposed by Taylor & McLennan (1995).

Contamination factor and pollution load index

The contamination factor (CF) quantifies the level of pollution for a given element by comparing its concentration in the sample (C_{sample}) to a reference geochemical background concentration with minimal anthropogenic influence ($C_{background}$). The CF is calculated as follows:

$$CF = \frac{C_{sample}}{C_{background}}$$

Where:

- C_{sample} : Concentration of the considered element in the lichen sample
- $C_{background}$: Estimated background concentration for the considered element

Geochemical background values were estimated using a mirror distribution function method, assuming a symmetrical distribution up to the median for the natural variability. Further methodological details are provided in Appendix A.3 .

The pollution load index (PLI) integrates CF values from multiple elements into a single metric that reflects the overall pollution load. We only Ni, Cr, Pb, Sn, As, Cu, Zn and Cd in the PLI. It is computed as follows:

$$PLI = \left(\prod_{i=1}^n CF_i \right)^{1/n}$$

Where:

- n : Number of studied elements

- CF_i : Contamination factor for the considered element i

Interpretation thresholds for CF and PLI are provided in Appendix E.6.

4.4.3. Statistical analysis

All statistical analyses were conducted using the open-source software R 4.4.2. Data visualization was performed with the package *ggplot2* (Wickham, 2009).

Statistical tests

Normality of TE concentration data was assessed using the Shapiro-Wilk test ($\alpha = 0.05$). Spearman's rank correlation coefficient (ρ) was used to evaluate correlations between variables, as it does not assume normal distribution ($\alpha = 0.05$). The significance of statistical results was categorized as follows: significant (*) for $p < 0.05$, very significant (**) for $p < 0.01$ and highly significant (***) for $p < 0.001$.

Non-metric multidimensional scaling

A non-metric multidimensional scaling (NMDS) was used to visualize how lichen community composition differs among sampling sites. First, the Bray-Curtis dissimilarity distances were calculated on the species abundance matrix using *vegdist* function from the *vegan* package (Oksanen et al., 2015). For any two sites i and j with species counts x_{ik} and x_{jk} , the Bray-Curtis distance is:

$$BC_{ij} = \frac{\sum_k |x_{ik} - x_{jk}|}{\sum_k (x_{ik} + x_{jk})}$$

Where:

- x_{ik} : abundance of species k at site i
- x_{jk} : abundance of species k at site j
- $\sum_k$: the summation over all species k observed across the two sites

The numerator sums absolute differences across all species, representing the total “compositional gap” between sites. The denominator is simply the combined summed abundance at both sites. By dividing the gap by the total abundance, this metric expresses dissimilarity as a proportion of overall abundance. Values range from 0 (identical composition) to 1 (no shared species).

Using the resulting distance matrix, NMDS ordination was performed with *vegan*'s *metaMDS* function. The NMDS iteratively places each site in a two dimensional space so that their Bray-Curtis dissimilarity ranks are reflected by inter-point distances. In the final plot, each point represents a site and the proximity between points reflects similarity in lichen species composition.

Principal component analysis

A principal component analysis (PCA) was performed on centered log ratio (CLR) transformed (required for compositional data) TE data to explore relationships among elements and sampling sites, using the *FactoMineR* package (Husson et al., 2008) in R. The CLR transformation was applied via the *composition* package (Van den Boogaart & Delgado, 2008) and *factoextra* (Kassambara & Mundt, 2020) was used for result visualization.

Canonical correspondence analysis

A canonical correspondence analysis (CCA) was performed to examine how environmental variables shape lichen community composition. This constrained ordination method helps identify the main environmental gradients influencing species distribution. The analysis was conducted using the *vegan* package in R.

We also performed a permutation based ANOVA (999 permutations) on the CCA to test the significance of (1) the overall model and (2) each environmental predictor. This nonparametric pseudo-F test requires no assumption of normal residuals and yields empirical p-values, ensuring that any detected relationships are unlikely due to chance (Legendre & Legendre, 2012).

Finally, we performed a partial CCA controlling for all other variables to account for correlations among predictors and isolate each variable's unique effect. This approach reveals each geochemical index's independent contribution to community variation (ter Braak & Šmilauer, 2002).

4.5. Complementary data

All geographic information system (GIS) analyses were performed using ArcGIS Pro 3.1.0.

Land use data and local contrast in artificialisation

To assess the fine-scale influence of land use on lichen communities and trace metal accumulation, we developed an indicator called the local contrast in artificialisation (LCA). This index was specifically designed to quantify the contrast in land artificialisation between the immediate surroundings of a sampled tree and its broader landscape context.

Using CORINE Land Cover 2018 data, we calculated the percentage of artificial surfaces (e.g., buildings, roads, impervious areas) within two buffers around each sampled tree: a 50 m buffer (local environment) and a 200 m buffer (broader landscape matrix). LCA is defined as:

$$LCA = 1 - \frac{\%Artificialized_{200m}}{\%Artificialized_{50m}}$$

Where:

- $\%Artificialised_{50m}$: Percentage of artificialised area in a 50 m buffer of the tree
- $\%Artificialised_{200m}$: Percentage of artificialised area in a 200 m buffer of the tree

This unitless ratio allows us to distinguish between different spatial configurations of green and artificial land:

- $LCA < 0$ indicates that the tree is in a green enclave within a more urbanised area. This suggests a local refuge potentially buffered from broader urban stressors.
- $LCA > 0$ reveals that the tree is embedded in a concrete dominated environment, even if the larger landscape may be less artificialised. This reflects potential hotspots of local urban pressure.
- $LCA \approx 0$ means that the artificialisation is similar at both scales, suggesting spatial homogeneity in land use intensity.

By comparing local and surrounding land-use intensity, LCA helps contextualise environmental pressures at the scale most relevant to epiphytic lichens and atmospheric deposition. It is particularly valuable for exploring how micro-scale land use configurations affect bioindication patterns and metal accumulation in lichens, two processes highly sensitive to both immediate exposure and landscape-level structure. Detailed explanations and distribution of values for the LCA index are in Appendix A.1.

Air pollutants concentration data

We used 10 m resolution raster layers from the *Belgian Interregional Environment Agency (IRCEL - CELINE)* platform for NO_2 , PM_{10} and O_3 concentrations that used the ATMO-Street (RIO-IFDM-OSPM) models. Pollutant values (in $\mu\text{g}\cdot\text{m}^{-3}$) were extracted at each GPS point corresponding to bioaccumulation samples. Site level concentrations were then calculated as the mean of the values at all points within the site, allowing us to test for potential effects of atmospheric pollution on lichen species frequencies.

5. Results and discussions

5.1. Bioindication method

5.1.1. Lichen diversity and abundance differences across contrasted cities

To assess how lichen diversity responds to urbanisation and environmental conditions, we analysed three complementary metrics across the studied cities: species richness, which reflects the total number of taxa observed; Shannon’s diversity index, which integrates both species abundance and evenness; and the LDV, which accounts for the spatial distribution and consistency of lichen communities across the sampling sites. High richness and evenness typically indicate favourable, low-stress environments, while a high LDV points to stable habitat quality across space. As shown in Table 4, these indicators help detect general contrasts between sites (Meier, 2024; Nimis et al., 2002a). Alpha diversity metrics for all sites can be found in Appendix D.1.

Table 4: Mean and standard deviation of calculated lichen diversity indices, with corresponding municipal surface area and population density. Data from the municipalities were recovered from *Institut wallon de l’évaluation, de la prospective et de la statistique* (IWEPS, 2025).

City	Number of sites	Specific richness	Shannon’s Index	LDV	Area of municipality	Population density
	[-]	[-]	[-]	[-]	[km ²]	[habitants·km ⁻²]
Arlon	3	28 ± 1	4.07 ± 0.1	128 ± 17.2	119.1	263
Bertrix	1	22	3.82	97	138.5	65.1
Charleroi	6	21.2 ± 1.8	3.64 ± 0.1	126 ± 4.9	102.9	1984.7
Honnelles	4	23.5 ± 2.7	3.85 ± 0.2	139 ± 16.6	44.3	116.9
Liège	6	19.3 ± 2.5	3.43 ± 0.2	108 ± 14.8	68.7	2851.8
Marche-en-Famenne	6	24.3 ± 1.9	3.85 ± 0.1	132 ± 6.6	122.1	147.2
Namur	10	20.1 ± 1.2	3.49 ± 0.1	112 ± 7.2	175.9	648.8
Orp-Jauche	4	16.7 ± 0.9	3.36 ± 0.1	109 ± 10.3	50.8	179
Vielsalm	1	26	4.01	113	140.7	56.4

Arlon stands out for its well-structured and resilient lichen community. Its high Shannon's index value (4.07 ± 0.1) reflects a balanced composition with no species dominance, typical of ecologically stable, low-disturbance environments (Magurran, 2004). Its LDV (128 ± 17.2) also suggests spatially consistent conditions across sampled trees. Similar patterns appear in Marche-en-Famenne and Honnelles, with elevated Shannon indices (3.85) and LDV values (132 and 139, respectively), pointing to favourable ecological conditions. These results align with land cover patterns. Marche-en-Famenne combines 41.4% agriculture and 37% forest, while Arlon, although more urbanised, retains 25.9% forest and only 19.5% artificial land. Such landscapes support ecological continuity and microhabitat diversity, favouring lichen development (Nascimbene et al., 2007). Honnelles, while largely agricultural (79.5%) and sparsely forested (8.3%), exhibits the highest LDV in the dataset. Its low urban pressure appears sufficient to sustain diverse and evenly distributed lichen communities, underscoring the importance of considering multiple environmental gradients, not just forest extent, when interpreting diversity patterns. In areas like Honnelles, further analysis of indicator species could clarify the ecological drivers of these patterns (Rocha et al., 2022).

By contrast, Liège, Namur and Orp-Jauche show lower Shannon's index value and LDV values, suggesting less diverse and less evenly structured lichen communities, likely due to degraded habitat quality. Notably, Orp-Jauche, although 82.2% of its land is agricultural, it exhibits diversity levels comparable to more urbanised centres. The comparison between Orp-Jauche and Honnelles is striking despite similar surface areas and percentages of agricultural land (> 75%), Honnelles supports richer and more consistent lichen communities. This difference may stem from its more extensive, though fragmented, forest cover (8.3% vs. 3.5%), which likely facilitates ecological continuity. In addition, agricultural practices differ: Orp-Jauche is dominated by intensive crop production (81% of farms) with little permanent grassland (6%), a system typically linked to high ammonia emissions (de Ruijter & Huijsmans, 2019). Honnelles, by contrast, has a more diversified farming structure, with 22% grassland, which likely contributes to lower nitrogen input and more favourable lichen habitats (Lättman et al., 2014).

Charleroi shows a contrasting pattern: a relatively low Shannon's index value (3.64 ± 0.1) but a high LDV (126 ± 4.9). This suggests frequent lichen presence, yet low evenness, likely due to a few pollution-tolerant species dominating the community, a common feature in urban environments (Ramdani et al., 2019). Finally, data from cities with only one sampling site, such as Bertrix (LDV = 97, Shannon's index = 3.82) and Vielsalm (LDV = 113, Shannon's index = 4.01), should be interpreted with caution due to limited sample size.

To contextualise our findings, we compared our LDV and species richness values with those reported in other European regions using the same standardised bioindication protocol (Asta, Erhardt, et al., 2002; CEN, 2014), as shown in Table 5. This allows us to assess whether the Walloon region falls within or beyond the expected range.

Table 5: Comparison of biodiversity indices (specific richness and lichen diversity value) across different regions, with values reported in other studies.

Region of interest	Area of region	Specific richness	LDV	Reference
	[km ²]	[-]	[-]	
Sines, Portugal	203	28	33 (19-48)	Llop et al., 2012
Šalek, Slovenia	197	58	55 (28- 75)	Policnik et al. (2008)
Bou Arriridj, Algeria	3920	62	32 (12-52)	Messaoud et al. (2019)
Bohemia, Czech Republic	40	54	41 (18-65)	Svoboda (2007)
Province of Genova, Italy	1838	54	62 (0-161)	Giordani (2007)
Antwerp, Belgium	204	9	47	Rocha et al. (2022)
Wallonia, Belgium	16 901	78	116 (67-165)	This study

The diversity values observed in Wallonia are markedly higher than those reported elsewhere in Europe. LDV values range from 67 to 165 (mean = 116), compared to 62 in Genoa, 55 in the Šalek Valley, 41 in Central Bohemia and just 47 in Antwerp (Rocha et al., 2022). Similarly, our mean species richness (78 taxa) far exceeds that of Sines (28), Bourdj Bou Arréridj (62) and especially Antwerp (9).

However, such comparisons must be made with caution. Study areas vary greatly in size (from 40 km² in Central Bohemia to nearly 4,000 km² in Algeria), which affects habitat diversity. Here, species richness was calculated per site and averaged across 41 sites, limiting area related bias and allowing more robust comparisons.

Climatic context also plays a critical role. Regions such as Sines (Portugal), Genoa (Italy) and Bourdj Bou Arréridj (Algeria) have Mediterranean or semi-arid climates, where lower rainfall and higher temperatures tend to limit lichen development (Giordani, 2007). In contrast, Wallonia's temperate oceanic climate, with regular precipitation, likely favours the establishment of mesophilous and hygrophilous species.

The wide range of LDV values within Wallonia (CV = 24.7%) highlights the importance of local-scale drivers such as proximity to roads, industrial activity, landscape connectivity and forest cover in shaping lichen communities (Johansson et al., 2009; Llop et al., 2017; Paltto et al., 2010). Overall, LDV and Shannon's indices offer valuable insights into the general structure and frequency of lichen communities, but they are shaped by complex interactions between climate, land use, spatial scale and local environmental conditions. While useful as a first approximation of habitat quality, these metrics are insufficient to distinguish between cities in

a detailed ecological sense. Finer-scale analyses of species composition, ecological traits and site-level characteristics are necessary to uncover more nuanced pollution patterns.

5.1.2. Species occurrences and distribution across sites

Across the study area, lichen species exhibited diverse thallus morphologies. Foliose forms were most common (40 species), followed by crustose (33), with three fruticose species (*Evernia prunastri*, *Ramalina farinacea*, *Ramalina fastigiata*), one squamulose (*Normandina pulchella*) and one with a complex thallus (*Cladonia chlorophaea* s.l.). This morphological diversity raises the question of how species are distributed across space.

To explore this, we computed two frequency metrics from the bioindication data: (1) the number of sites where each species was observed (out of 41), indicating regional distribution and (2) its mean frequency across grid squares within each site (out of 80 standardised observation units), serving as a proxy for local abundance. As all sites were surveyed using the same protocol, both metrics are directly comparable across sites. This dual approach helps distinguish species that are widespread and locally dominant from those that are either restricted or rare (Figure 17).

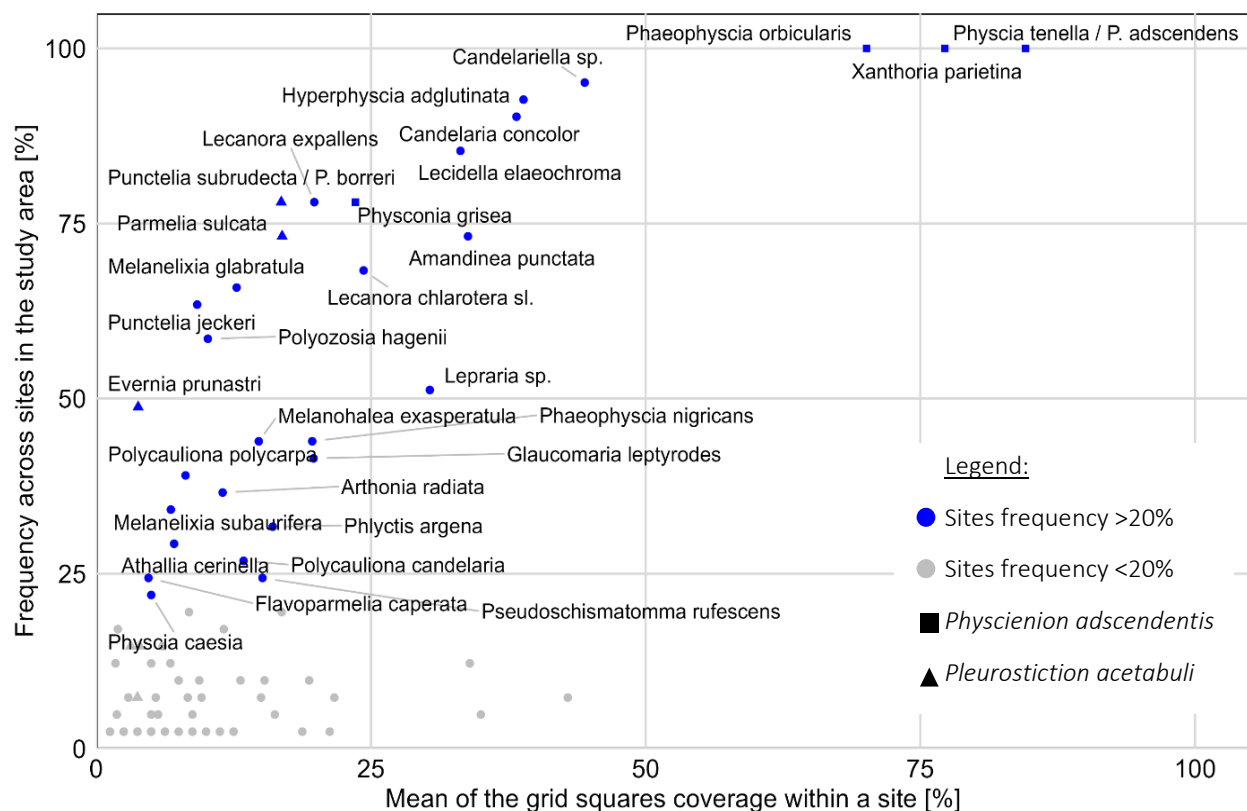


Figure 17: Frequency across sites (n = 41 sites) is plotted against the percentage of grid squares occupied by each species within a site (n = 80 grid squares per site). Species present in at least 20% of the sites are shown in blue, while those present in fewer than 20% are shown in grey. Squares and triangles represent species within the two suballiances of *Xanthorion parietinae*: the *Physcienion adscendentis* (squares) and *Pleurostiction acetabuli* (triangles).

Based on Figure 17, the species observed across the study area can be grouped according to their intersite abundance and intrasite frequency. Among the most frequently recorded taxa (> 70% of intersite frequency), several share similar ecological traits. They all score in the high end of nitrophily and heliophily and moderate tolerance to aridity and prefer neutral bark pH³. These species are *Candelariella concolor*, *Candelariella* sp., *Hyperphyscia adglutinata*, *Physconia grisea*, *Parmelia sulcata*, *Lecidella elaeochroma*, *Punctelia subrudecta*/*P.borreri*, *Amandinea punctata*. Within this group, three species (*Physcia tenella*/*P. adscendens*, *Xanthoria parietina* and *Phaeophyscia orbicularis*) stand out for their presence at all 41 sites and high local abundance (> 50% mean site coverage). Their dominance likely reflects their high tolerance to nitrogen enrichment, strong sunlight and trace metal exposure, allowing them to outcompete more sensitive taxa in anthropised environments (Brunialti & Frati, 2007; Fabiszewski & Szczepańska, 2010; Gombert et al., 2003; Jovan, 2008). These species are consistently reported as dominant in urban and peri-urban areas across Europe, including in Brussels (Van den Broek, 2012), confirming their role as a resilient core of nitrophilous, heliophilous lichens typical of urban trees. Their prevalence has likely been reinforced by the long-term decline in sulphur dioxide emissions (Adamska, 2011; Gauslaa, 2024; Manninen et al., 2023).

The second group of Figure 17 includes lichens with moderate overall frequency (20-75 %) and generally lower local abundance (mean site coverage < 50), though some may be locally abundant. Compared to the dominant taxa, these species tend to be less pollution-tolerant and less dependent on high light, explaining their lower occurrence in urban sites. Often described as ecological generalists (Lackovičová et al., 2013; Mežaka et al., 2012), they are useful indicators of intermediate levels of anthropogenic pressure. Their relative frequencies across sites can reveal subtle responses to gradients of pollution, nitrogen deposition or habitat disturbance. For example, *Melanohalea exasperatula*, near the lower end of this group, shows acidophilic tendencies that contrast with the nitrophilous species dominating disturbed environments. Its presence may signal less impacted, more rural or forested conditions, making this group particularly relevant for gradient-based bioindication.

At the other end of the spectrum, the third group includes species with low overall frequency (< 20%) and limited site coverage (< 50% in sites where they are present). All of the points coloured as grey in Figure 17 fall into this category. Their rarity and restricted distribution make them unsuitable for large-scale bioaccumulation studies. More generally, their low occurrence across the study area limits the ability to assess their sensitivity to environmental pressures, reducing their value as bioindicators in this context.

In summary, the epiphytic lichen communities observed largely align with classical lichenosociological classifications (Van Haluwyn et al., 2022). Most taxa belong to the *Xanthorion parietinae* alliance, particularly the *Physcienion adscendentis* sub-alliance, typical of nutrient-rich, sun-exposed habitats found in urban and peri-urban areas. The sporadic presence of species from the *Pleurostiction acetabuli* alliance suggests occasional forest-like or buffered conditions. Overall, community composition is shaped by high nitrogen input and

³ Some studies have shown that bark pH is not as significant as other parameters (Spier et al., 2010).

strong light exposure, favouring nitrophilous and heliophilous species in anthropised environments. Building on this overview, we now examine how community composition varies between sites to detect spatial contrasts and environmental influences.

5.1.3. Community contrasts per sites: NMDS based approach

The NMDS allowed us to identify patterns of variation in lichen community composition across sites, based on Bray-Curtis dissimilarity. It revealed contrasts in species assemblages that are not captured by diversity indices alone, offering insights into underlying ecological gradients and species turnover. Species recorded at only one site (singletons) were excluded to reduce noise. Resulting site groupings based on species composition are shown in Figure 18. Figure 18a displays the ordination with sites colour coded by land use type and Figure 18b shows the species distribution with their corresponding three-letter codes listed in Appendix C.1. One clear group includes urban sites from Liège (LIE5, LIE16) and Namur (NAM5), located at the positive end of NMDS2. These sites are characterised by high frequencies of *Candelariella vitellina* and *Protoparmeliopsis muralis*, saxicolous lichens adapted to polluted substrates. Their dominance suggests high urban pressure from NO_x, particulate matter or substrate alterations (Lisowska, 2011; Uluozlu et al., 2007)

A second, looser cluster appears along the negative NMDS2 and positive NMDS1 axes, comprising a mix of agricultural (e.g., HON3, ORP4), transitional and one forested site (e.g., MAR2, NAM15, VIE4). Despite their diversity, these sites share elevated frequencies of moderately nitrophilic and nitrotolerant species like *Glaucomaria leptyroides*, *Lecanora chlarotera* and *Polycauliona polycarpa*. While their individual tolerances vary, they are generally considered nitrophilic or nitrotolerant, tend to prefer drier bark conditions and are moderately resistant to urban pressure (Greaver et al., 2023; Llop et al., 2012). This suggests that the positioning of these sites in the NMDS ordination is primarily driven by agricultural pressure, particularly the combination of open, sunlit environments and elevated ammonium (NH₄⁺) deposition typical of farming landscapes (Van Dobben & Ter Braak, 1998).

A third cluster, on the negative NMDS1 axis near the origin of NMDS2, includes transitional sites from Arlon (ARL8, ARL10) and Marche-en-Famenne (MAR5). These support more sensitive taxa such as *Physconia perisidiosa*, *Porina aenea*, *Cladonia chlorophaea s.l.* and *Ramalina farinacea*, all of which are associated with humid, low-nitrogen and low-disturbance habitats (Geiser & Neitlich, 2007; Giordani, 2007; Hauck & Wirth, 2010). These sites likely act as ecological buffers between urban and intensively farmed areas. Finally, around the NMDS origin, several sites group together due to dominance by generalist, pollution-tolerant species like *Xanthoria parietina*, *Physcia tenella*/P. *adscendens* and *Phaeophyscia orbicularis*. Their broad ecological amplitude explains the intermediate positioning of these sites across the urban-rural gradient.

The NMDS ordination reveals three main ecological gradients that structure lichen community composition.

First, a vertical gradient along NMDS2 opposes urban-tolerant and pollution-adapted communities (positive NMDS2), dominated by species such as *Candelariella vitellina* and *Protoparmeliopsis muralis*, to hygrophilous, nitrophobic species (negative NMDS2) like *Physconia perisidiosa* and *Porina aenea*, which are sensitive to urban and agricultural pressures.

Second, a horizontal gradient along NMDS1 differentiates moist, acidic and semi-natural environments (negative NMDS1) from drier, open and agriculturally influenced conditions (positive NMDS1), where moderately nitrophilic and xerophytic species such as *Glaucomaria leptyrodes* and *Polycauliona polycarpa* are more frequent.

Finally, the centre of the ordination space is occupied by generalist and ubiquitous species like *Xanthoria parietina* and *Physcia tenella*/*P. adscendens*, whose broad ecological tolerance allows them to thrive across a wide range of environmental conditions.

Together, these gradients reflect complex interactions between nitrogen inputs, bark moisture and land use intensity. Importantly, they highlight that lichen community responses are shaped more by fine-scale environmental conditions than by coarse land cover categories, confirming the value of epiphytic lichens as sensitive bioindicators of local ecological variability.

5.2. Bioaccumulation method

5.2.1. Trace element concentrations

We measured concentrations of 14 TEs in *Xanthoria parietina* collected at each site to assess spatial variations in atmospheric contamination. Figure 19 displays their distribution (in $\mu\text{g}\cdot\text{g}^{-1}$; logarithmic scale), with green and red lines indicating natural background and contamination thresholds based on Bargagli & Nimis (2002). No thresholds are available for Co and Sn. Threshold values are listed in Appendix E.5.

The median concentration levels of the elements in this study increase in the following order: $\text{Cd} < \text{Co} < \text{As} < \text{Sn} < \text{Ni} < \text{V} < \text{Pb} < \text{Cr} < \text{Cu} < \text{Mn} < \text{Zn} < \text{Ti} < \text{Fe} < \text{Al}$. This order is generally consistent with natural abundance patterns, as stated by previous studies (Appendix E.4). Al, Fe and Mn typically show the highest concentrations due to their crustal origin, while Cd, Co and As exhibit low concentrations. Although anthropogenic inputs can increase concentrations of some elements, they do not override this overall hierarchy.

However, some differences are notable: Zn ranks higher than Mn, reversing the order observed in Agnan et al. (2017) and Çobanoğlu & Kaan (2024). Cr appears unusually elevated, ranking above Pb and Ni; and Ni is among the lowest in our data, whereas it ranks much higher in Çobanoğlu & Kaan (2024), though this may partly reflect its proximity to the detection limit and associated uncertainty (see section 4.3.3). These variations may reflect site-specific inputs or differences in regional background levels.

Looking at the thresholds of naturalness (natural background level) and alteration (contaminated level) posed by Bargagli & Nimis (2002), most elements (Cd, As, Ni, V, Pb, Cu, Mn, Zn) have concentrations below the contamination threshold, indicating negligible presence. Two elements (Cr and Al) show values exceeding the threshold by up to 1.5 times the interquartile range, suggesting potential contamination at some sites. For Ti and Fe, the superior interquartile range lies above the contamination threshold, meaning a significant number of sites could exhibit elevated concentrations of these elements. However, it is important to note that Fe and Ti are generally lithogenic elements and their elevated levels may reflect natural sources rather than anthropogenic pollution (Agnan et al., 2015).

Elemental concentrations show moderate to high coefficients of variability (CV) across sites (mean CV of 66.4%). While some elements show low CVs, such as Mn (40.8%), As (42.9%) and Cu (61.6%), others display substantial variability between sites. This is particularly the case for Cd (72.3%), Cr (76.1%), Ti (76.1%) and especially Sn (120.3%), whose concentrations vary widely and suggest possible site-specific contamination. This heterogeneity likely reflects site-specific contamination sources or differences in land use, with highly variable elements such as Sn suggesting localised anthropogenic inputs.

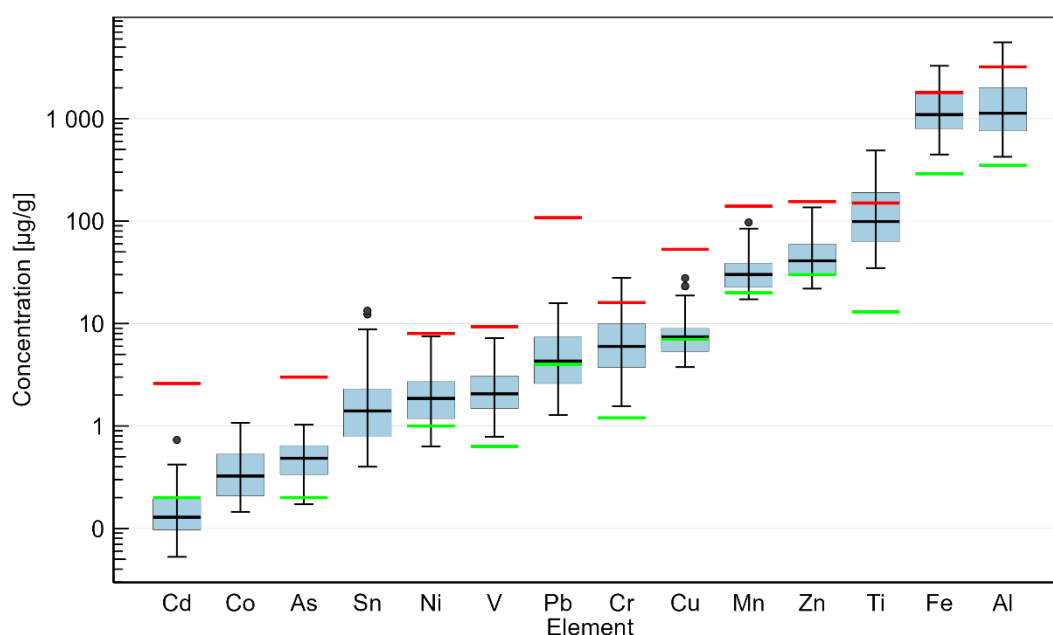


Figure 19: Distribution of trace element concentrations [$\mu\text{g}\cdot\text{g}^{-1}$] on a logarithmic scale. The natural and alteration thresholds proposed by Bargagli & Nimis (2002) are indicated in green and red, respectively.

Although this threshold-based approach offers a useful starting point for interpreting pollution levels, it comes with important limitations. The reference values proposed by Bargagli & Nimis (2002) were developed for Italian contexts and may not fully apply to Wallonia due to differing geology and background levels. Moreover, not all elements have defined thresholds, and some are naturally more abundant than others, making cross-element comparisons less meaningful. To address these limitations, we complement this analysis with more robust and context-sensitive pollution metrics.

5.2.2. Geochemical Indices for evaluating trace element contamination

Because raw concentration values are influenced by local environmental factors such as geology and background levels, other pollution metrics offer a more standardized framework for comparison by reducing site-specific biases and allowing for more meaningful spatial and inter-study assessments.

Enrichment factor

EFs help identify elements that are disproportionately elevated relative to natural background levels. This makes it easier to distinguish between geogenic and anthropogenic sources of contamination, something that raw concentrations alone cannot do, as they reflect both natural and local environmental variability.

The full distribution of EF values is shown in Figure 20, with enrichment thresholds indicated at 0.5, 2 and 10. Most lithogenic elements, such as Fe, Co, V, Mn and Ni, fall within the 0.5-2 range, which is generally interpreted as indicating natural origin with minimal anthropogenic input. Values of EF for the studied elements for each of the 41 sites can be

found in Appendix E.2. Aluminium, with a median EF slightly below 0.5, shows even stronger lithogenic dominance. These results support the assumption that these elements mainly derive from soil dust and natural background sources (Cecconi et al., 2021)

In contrast, elements such as Cr, Pb, Sn, As and Cu show median EF values between 2 and 10. This suggests moderate enrichment that may reflect diffuse anthropogenic inputs, such as traffic (brake/tire wear, historical fuel additives) or industrial emissions (metal processing, combustion) and potentially some agricultural sources (Zhao et al., 2019). For instance, Pb and Cr, known to be associated with historical combustion sources or metal processing, show values that point towards non-negligible atmospheric deposition.

The highest EF values are observed for Zn and Cd, both with medians exceeding 10, which suggest a significant anthropogenic contribution. Cd, despite low absolute concentrations, stands out due to its strong relative enrichment compared to natural background levels, a pattern often linked to smelting, mining and coal combustion (Wu et al., 2021).

The CV values provide additional insight into spatial heterogeneity. Al (11.1%) and V (33.0%) exhibit the lowest variability, indicating relatively consistent EF values across sites and reinforcing their lithogenic character. In contrast, Pb (92.3%), Ni (105%) and Cr (109%) display high inter-site variability, suggesting the presence of localised contamination hotspots, likely in the proximity of traffic, industrial or urban emission sources.

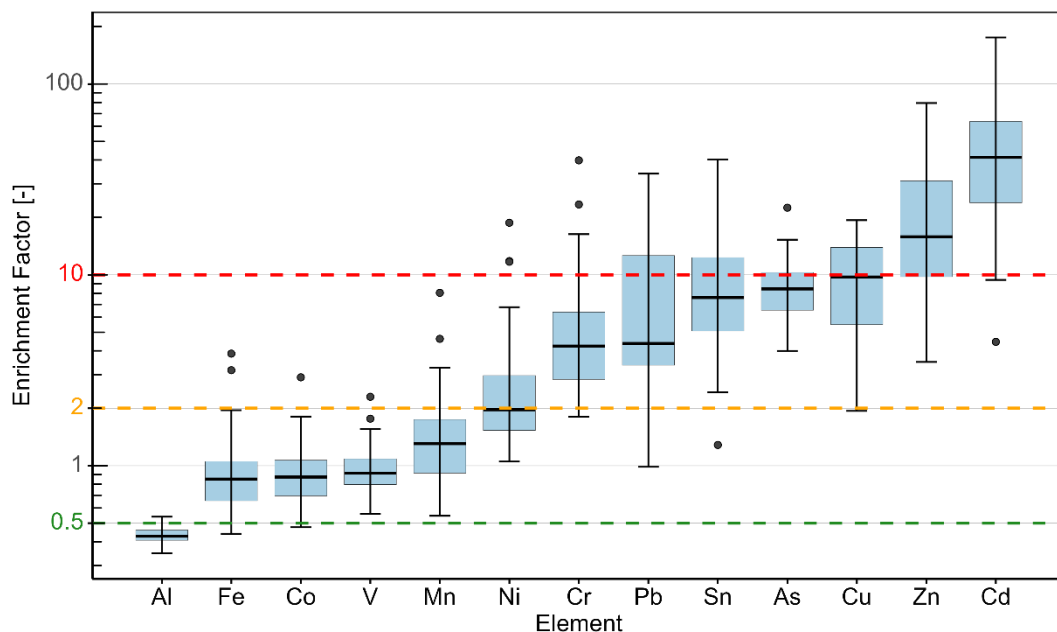


Figure 20: Distribution of enrichment factors on a logarithmic scale. Thresholds of enrichment, ranging from lowest to highest, are indicated by the green, yellow and red dashed lines, corresponding to enrichment factor values of 0.5, 2 and 10, respectively.

To contextualise our results, we compared EF values with three reference studies conducted in contrasting environmental conditions (Table 6): (1) a polluted area near a nickel smelter in Slovakia (Kováčik et al., 2023); (2) a supposedly unpolluted mountain region in China

(Liu et al., 2016); and (3) a more rural subset of the Walloon region (Matthée, 2024). While Liu et al. (2016) used *Xanthoria mandschurica*, both Kováčik and Matthée relied on *X. parietina*, as in our study, helping to minimise interspecific variation in TE accumulation.

Table 6: Comparison of the mean and standard deviation of enrichment factor values for the studied trace elements for: (a) area near a nickel smelter in Slovakia (Kováčik et al., 2023); (b) a mountain region in China (Liu et al., 2016) and; (c) the Wallon region (Matthée, 2024).

	This study	(a) Kováčik et al. (2023)	(b) Liu et al. (2016)	(c) Matthée (2024)
	Ti normalised	Al normalised	Al normalised	Ti normalised
Element	Wallonia, Belgium (n = 122)	Dolná Streda, Slovakia (n = 45)	Hebei, China (n = 40)	Wallonia, Belgium (n = 124)
Al	0.43 ± 0.05	Reference	Reference	
As	8.97 ± 3.53	27.88 ± 8.40		7,22 ± 5,76
Cd	50.90 ± 36.50	332.72 ± 738.56	123.99 ± 78.19	40,8 ± 52,2
Co	0.99 ± 0.46	7.66 ± 20.50	1.56 ± 0.53	0,77 ± 0,53
Cr	6.28 ± 6.84	55.69 ± 145.40	19.6 ± 10.79	3,14 ± 1,52
Cu	9.69 ± 4.70	13.23 ± 7.84	7.52 ± 2.04	7,91 ± 10,0
Fe	1.04 ± 0.68	3.12 ± 4.50	2.34 ± 0.78	0,71 ± 0,40
Mn	1.64 ± 1.31	3.82 ± 2.61	3.08 ± 1.06	1,60 ± 1,72
Ni	3.27 ± 3.45	19.10 ± 51.45	2.72 ± 0.86	1,94 ± 1,51
Pb	8.34 ± 7.70	29.96 ± 46.25	29.94 ± 21.29	3,63 ± 3,78
Sn	10.38 ± 8.54	5.75 ± 2.18		5,26 ± 4,38
V	1.00 ± 0.33	2.51 ± 1.15	1.81 ± 0.43	0,94 ± 0,45
Zn	23.10 ± 17.91	35.55 ± 26.20	15.47 ± 7.33	17,3 ± 27,9

The order of EF values in our study (Al < Fe < Co < V < Mn < Ni < Cr < Pb < As < Cu < Sn < Zn < Cd) closely matches that reported by Matthée (2024), who also identified Zn and Cd as the most enriched elements in Wallonia. This consistency suggests that these two elements may be persistent markers of anthropogenic pressure across the region.

However, our study shows notably higher EF values for Pb (8.34 vs. 3.63), Sn (10.38 vs. 5.26) and Cr (6.28 vs. 3.14). Both studies analysed contemporary lichen samples and used the same reference framework (UCC values and Ti normalised), which reduces methodological bias. The most likely explanation lies in the sampling context: Matthée (2024) focused primarily on rural landscapes (despite including a few urban sites), whereas our sampling design targeted a more heterogeneous set of environments. These differences likely account for the stronger enrichment patterns we observed, particularly for elements historically associated with

anthropogenic emissions such as Pb (from leaded petrol and metallurgy), Sn (from industrial and traffic sources) and Cr (linked to steel production and road traffic).

Compared to Kováčik et al. (2023), who reported exceptionally high EF values for Cd, Cr, Pb, Ni and Co in Dolná Streda, our values are significantly more moderate. This aligns with the fact that their study focused on a heavily polluted site near a former nickel smelter. These findings underscore the sensitivity of EF values to localised, point-source emissions and support the interpretation that our data more likely reflect diffuse atmospheric pollution from mixed anthropogenic activities, such as agriculture and regional transport (Nimis et al., 2002b).

Finally, Liu et al. (2016) reported EF values following a similar overall order, except for Sn, which was substantially lower than in Wallonia. Notably, this supposedly unpolluted region displayed significantly higher EF values for Cd (1.4 times higher) and Pb (2.6 times higher) compared to our study. According to the authors, this unexpected enrichment may result from a combination of long-range atmospheric transport from polluted regions and local emissions linked to past mining activities.

Overall, EF analysis confirms clear enrichment patterns: lithogenic elements remain low, while Zn and Cd stand out as the most anthropogenically influenced. Our values suggest moderate, diffuse pollution with some localised hotspots, particularly near urban and industrial areas.

To further summarise and compare pollution across sites, we introduce ΣEF , the cumulative sum of EF values for elements with $EF > 2$ (As, Cd, Cr, Cu, Ni, Pb, Sn, Zn). This metric focuses on anthropogenic contributions and allows a clearer comparison of pollution loads while excluding naturally abundant elements.

Contamination factor and Pollution load index

Unlike EF, which compares element concentrations to global background values (UCC), CF relies on background levels derived from our own lichen dataset (Appendix A.3), providing a more site specific assessment of contamination.

While EF helps distinguish between geogenic and anthropogenic sources, CF and PLI are more synthetic indices that estimate the intensity of contamination. They are especially useful for summarising cumulative pollution across multiple TEs (Boonpeng et al., 2018).

To reduce bias from naturally abundant lithogenic elements, we limited CF and PLI calculations to elements with $EF > 2$: As, Cd, Cr, Cu, Ni, Pb, Sn and Zn. This common approach in biomonitoring studies (Abdollahi et al., 2025; Varrica et al., 2022) was applied at the city scale by computing the geometric mean of values across sites, grouped by city (Figure 21), as had been done in previous studies (Boamponsem et al., 2010).

PLI values below 1 indicate unpolluted conditions, while values above 1 denote moderate pollution. Vielsalm shows the lowest PLI (0.48), indicating minimal contamination. Honnelles (0.73), Marche-en-Famenne (0.77) and Arlon (0.87) follow, suggesting background levels. Orp-Jauche (0.97) lies just below the threshold. In contrast, the highest values are found

in Namur (1.48), Liège (1.38) and Charleroi (1.32), pointing to moderate pollution levels, with Bertrix (1.1) slightly exceeding the threshold.

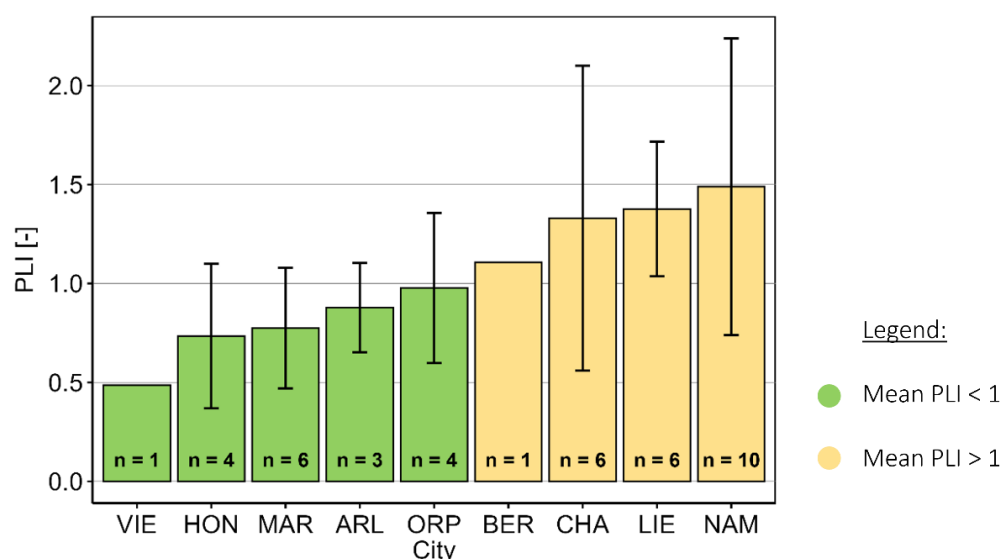


Figure 21: Pollution load index (PLI) values for the different studied cities. Pollution classes, as defined in Table 23 (see Appendix E.6), are shown in colour: green for unpolluted sites (PLI < 1) and yellow for low to moderate pollution (PLI > 1).

Table 7: Mean and standard deviation of pollution load index values for cities exceeding the threshold. A ranking of the trace elements with the highest contamination factors is shown for each city, along with the main origin of these elements.

City	PLI	Trace elements having the highest CF values	Main origin of elements Rauch & Pacyna (2009)
Bertrix	1.1	Ni > As > Cr > Cu	Mixed
Charleroi	1.32 ± 0.77	Cr > Ni > Zn > Sn	Anthropogenic
Liège	1.37 ± 0.34	Zn > Cd > Pb > Sn	Anthropogenic
Namur	1.48 ± 0.0.74	Sn >> Pb > Cu > Cr	Anthropogenic

Table 7 summarizes the mean PLI values, the dominant elements contributing to pollution and the likely origin of these elements for each city with PLI > 1. In Bertrix (BER), the relatively low PLI value (1.1) is mainly driven by elements such as Ni, As and Cr, which may reflect both natural background levels and diffuse anthropogenic inputs, suggesting a mixed origin. In contrast, cities such as Charleroi (CHA), Liège (LIE) and Namur (NAM) exhibit higher PLI values (1.32-1.48) dominated by elements like Sn, Cd, Zn, Pb and Cu. These elements are typically linked to anthropogenic sources, including traffic emissions, industrial activities and

urban infrastructure (Rauch & Pacyna, 2009). These contrasting profiles highlight distinct contamination pathways, allowing us to separate natural geochemical signals from anthropogenic pollution sources.

Comparing PLI values across studies remains challenging, as the index depends on the selection of TEs and background thresholds. However, combining PLI with ΣEF offers a more robust interpretation of contamination: while ΣEF captures the cumulative magnitude of enrichment from anthropogenic sources, PLI reflects the overall intensity of pollution and toxicological burden. Taken together, these indices help disentangle widespread diffuse contamination from more localised pollution hotspots.

To strengthen this interpretation, correlations between elements can also provide valuable insights into shared origins and accumulation processes. We now turn to these relationships to identify potential co-emission patterns and source groupings across the studied sites.

5.2.3. Exploring relationships between trace elements

The relationships between TEs were explored using PCA to identify co-occurrence patterns and potential source groupings, as shown in Figure 22. The dimensionality of the dataset was reduced to two main components: PC1 (41.2%) and PC2 (19.9%), together explaining over 60% of the total variance. PC1 opposes Co, As, V, Ti and Al (positive loadings) to Pb, Zn and Cd (negative loadings), while PC2 contrasts Ni, Cr, Mn and Fe (positive) with Sn and Cu (negative). A detailed correlation analysis between the studied elements can be found in Appendix E.1.

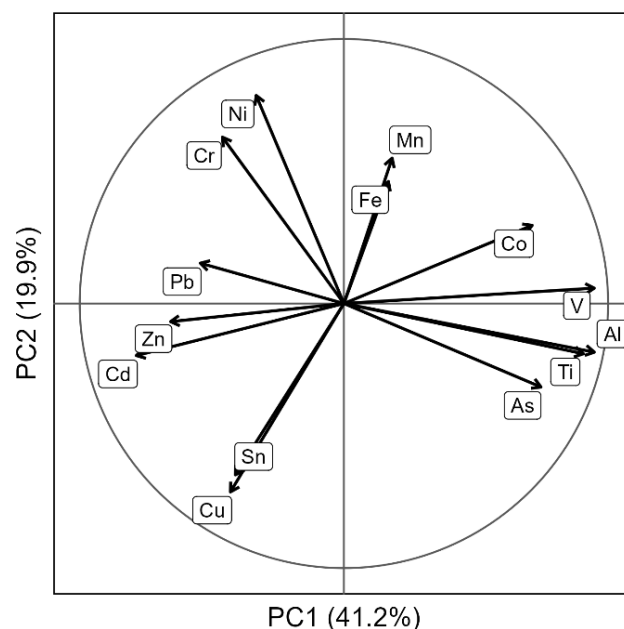


Figure 22: Principal component analysis biplot of the considered trace elements, where each arrow corresponds to a specific element.

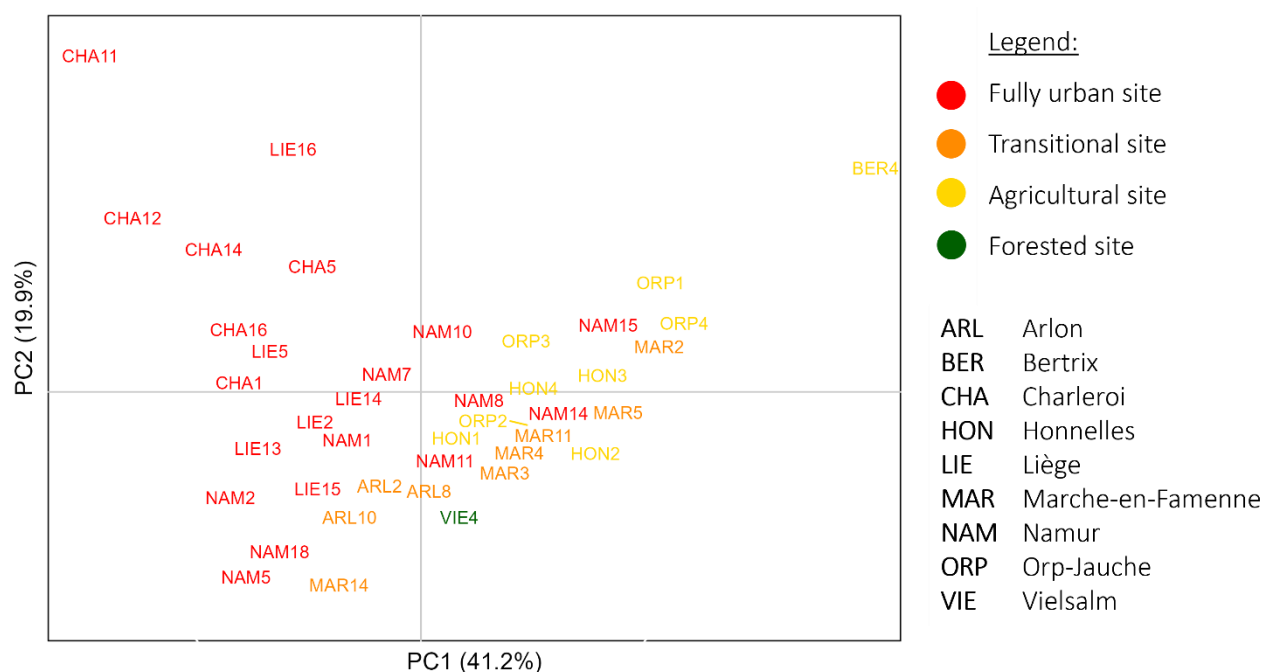


Figure 23: Scores of the studied sites projected onto the principal component analysis biplot of the trace elements. Red coloured sites are in urban areas, orange coloured sites are transitional urban-rural areas, yellow coloured sites are agricultural and the green coloured site is forested. The sites are labelled with three-letter codes.

Three distinct geochemical groups emerge from Figure 22. The elements As, V, Al, Ti and Co are clustered together with similar orientations and moderate vector lengths, suggesting a common lithogenic origin, likely linked to natural mineral sources or soil-derived particles deposited on lichens (Agnan et al., 2017; Chahloul et al., 2022; de Paula et al., 2015).

This pattern is supported by the alignment of most agricultural (yellow), forested (green) and several transitional or open urban sites (e.g. MAR5, MAR11, MAR3, MAR4; NAM15, NAM14, NAM8, NAM11) along the same vector directions in (Figure 23), indicating dominant influence of natural background inputs. While some of these are formally classified as urban, their open or vegetated surroundings with limited infrastructure likely explain their crustal signal: NAM15 and NAM14 are located near fields, NAM8 lies within a park and MAR3, though close to a railway, is situated in an open setting, possibly accumulating soil or rail-derived particles.

A second group of elements (Cu, Zn, Sn, Pb and Cd) form a distinct second cluster (Figure 22) with opposing directions to the first group of elements analysed on PC1, suggesting a different origin from the lithogenic elements. These elements are commonly associated with anthropogenic activities such as metal processing, industrial emissions, combustion of fossil fuels and abrasion from brakes and tires (Rauch & Pacyna, 2009; Taylor et al., 2015; Wu et al., 2021). Their co-alignment in the PCA biplot supports a shared pollution origin, particularly for Cu, Zn and Cd, typically linked to metallurgy, refining and smelting of ores (Piovár et al., 2017); Sn from brake abrasion from railways and vehicles (Contardo et al., 2020); and Pb from leaded petrol emissions (Chrastný et al., 2018).

The distribution of several urban (red) and peri-urban sites in Figure 23 supports this interpretation, which cluster along these vectors. NAM5, a central urban site near major roads and the Meuse River, shows strong enrichment in Cu and Sn, likely due to traffic and historical industrial pollution. Similarly, NAM18, located along the N4 motorway, shows elevated levels of the same elements. CHA1 (adjacent to the E42 motorway) and CHA16 (LCA = 0.14; an urban park enclosed by streets) are both enriched in Pb and Zn, indicating a dominant influence of road traffic and urban emissions. The spatial alignment between these sites and the anthropogenic element vectors in Figure 23 reinforces the interpretation of this cluster as a signature of pollution-linked accumulation.

A third group composed of Ni, Cr, Fe and Mn aligns more closely to the positive end of the PC2 axis (Figure 22). These elements may reflect industrial processes involving steel or alloy production, but also from natural sources, as they are commonly found in crustal materials. Their grouping likely reflects a combination of lithogenic input and localized industrial contamination (Kováčik et al., 2023; Paoli et al., 2015; Vannini et al., 2016b).

This mixed-source hypothesis is consistent with previous findings from EF and PLI analyses, which showed moderate enrichment and high variability for Ni and Cr, suggesting site specific anthropogenic influence superimposed on a natural background. This interpretation is further supported by the position of specific sites along PC2 in Figure 23. For instance, LIE16, located near the Chertal industrial zone, exhibits elevated Ni concentrations, consistent with emissions from metal processing. Similarly, CHA11, situated near the E420 motorway, shows a strong Cr signal, likely reflecting industrial dust or vehicular emissions.

Overall, the PCA patterns are consistent with the PLI-based interpretation of trace metal contamination across cities (Table 7). Cities with higher PLI values, namely Namur, Liège and Charleroi, also show strong associations with known anthropogenic elements in the PCA (Sn, Cd, Pb, Cu), as most of their sites are located in fully urban environments. Bertrix, with its low PLI and mixed geochemical signature, aligns more closely with crustal elements and rural environmental contexts. This convergence reinforces the reliability of both approaches for characterising urban pollution profiles.

The spatial separation of Groups 1 and 2 along PC1 confirms the contrast between lithogenic inputs and anthropogenic contamination, while the intermediate positioning of Group 3 on PC2 highlights the complex, overlapping origins of certain elements. These PCA results align overall with EF and PLI patterns, confirming the same division between background inputs and pollution-driven enrichment. Moreover, these spatial arrangements further reveal a gradient in trace metal composition and concentration between sites, underlining the heterogeneity of contamination patterns across the region.

By combining concentration-based indices with multivariate ordination, we gain a more nuanced understanding of contamination levels, sources and geochemical behaviour across the region. This integrated approach now sets the stage for the next section, where we examine whether species composition patterns also reflect these spatial and geochemical contrasts.

5.3. Coupling biomonitoring methods

To better understand how atmospheric TEs affect lichen communities, we combined bioindication and bioaccumulation data using multivariate analysis. This integrative approach allows us to explore the relationships between TE variables (PLI and ΣEF), atmospheric data (O_3 , NO_2 , PM_{10}), land use indicators (LCA, % artificialized area within 50 m) and community composition information (Shannon's index and LDV). It helps identify the main drivers shaping community structure and assess how different pollutants influence species composition. Alpha diversity metrics can be found in Appendix D.1, EF values in Appendix E.2 and coordinates of the sites in Appendix B).

5.3.1. Exploring correlations between lichen diversity and pollutant variables

A PCA was conducted to summarize and reduce the dimensionality of the dataset by identifying the main environmental and compositional gradients that structure variation across sampling sites (Figure 24), following the same multivariate approach commonly applied in epiphytic lichen ecology (Calvelo et al., 1997; Ongaro et al., 2022; Sheard & Jonescu, 1974).

When focusing on the relationships between variables (Figure 24), several patterns emerge: ΣEF , NO_2 and PM_{10} have a strong positive correlation ($r > 0.7$), confirming that these variables reflect a similar signal of traffic-related and urban atmospheric pollution; LDV and Shannon's index are also strongly correlated together ($r > 0.7$) and oriented in the opposite direction. This suggests that lichen diversity and evenness tend to decrease in more polluted environments, consistent with the known sensitivity of lichens to air quality (Giordani, 2007; Loppi, 2014; Van Dobben & Ter Braak, 1998). Meanwhile, LCA and % artificialisation within a 50 m radius appear only weakly correlated ($r > 0.3$), suggesting that each captures a distinct dimension of land-use structure and urbanisation intensity. The correlation matrix for the variables included in the PCA can be found in Appendix F)

Interestingly, PLI and ΣEF show a weak correlation on the PCA biplot, which is why a focused comparison was conducted to understand this (Appendix A.2). Although both derive from TE data, the divergence is not due to the geochemical baseline normalization. Recalculating PLI using UCC values showed strong correlation with the original PLI ($r > 0.7$) but not with ΣEF ($r < 0.3$). This confirms that their difference stems from their mathematical formulation: ΣEF highlights specific enriched elements due to anthropogenic inputs, while PLI reflects overall cumulative pollution.

We can then deduce that PC1 exhibits a gradient of anthropogenic pollution: it contrasts urban-related pollutants (NO_2 , PM_{10} , ΣEF) on the positive side with lichen diversity metrics (LDV and Shannon index) and O_3 on the negative side (the latter being typically higher in rural contexts). This suggests that PC1 separates highly urbanised sites with higher pollutant

levels from more natural or semi-natural ones. These latter sites show higher lichen diversity metric values.

This hypothesis is reinforced by the sites' position on Figure 25, densely urbanised sites cluster on the positive side of PC1, with higher PM₁₀, NO₂ and Σ EF and lower lichen diversity. For instance, LIE5, located in a park bordered by major roads and close to the centre of Liège, shows low LDV (86) and Shannon index (3.31), but high PM₁₀ (13.8 $\mu\text{g}\cdot\text{m}^{-3}$), NO₂ (17.02 $\mu\text{g}\cdot\text{m}^{-3}$) and Σ EF (271). Conversely, less urbanised sites score negatively on PC1, with greater diversity and lower pollutant levels. For example, MAR2, located near fields outside the village of Roy, has high LDV (157), Shannon index (4.18) and low NO₂ (2.91 $\mu\text{g}\cdot\text{m}^{-3}$), PM₁₀ (6.9 $\mu\text{g}\cdot\text{m}^{-3}$) and Σ EF (48).

PC2 represents a structural land-use gradient: it opposes lichen diversity metrics (LDV, Shannon index) on the positive side to land-use variables (LCA, %A50m) on the negative side. This suggests that PC2 captures habitat structure and fragmentation, ranging from biodiverse, vegetated areas to more artificialized and constrained environments.

Again, this hypothesis is further supported by the site's position: LIE2, in the Citadel Park of Liège, scores highly on PC2 with strong lichen diversity (LDV = 165, Shannon = 4.16), low artificialisation (%A50m = 6.91%) and a negative LCA (-3.79), reflecting extensive green space (200 m) beyond the immediate surroundings (50 m). In contrast, ORP2, located in the centre of Orp-Jauche, shows lower diversity and evenness (LDV = 98, Shannon = 3.10) but a higher %A50m (60%) and a positive LCA (0.23), indicating more artificialized immediate surroundings (%A_{50m} > %A_{200m}). This pattern suggests ecological constraints linked to urban fragmentation.

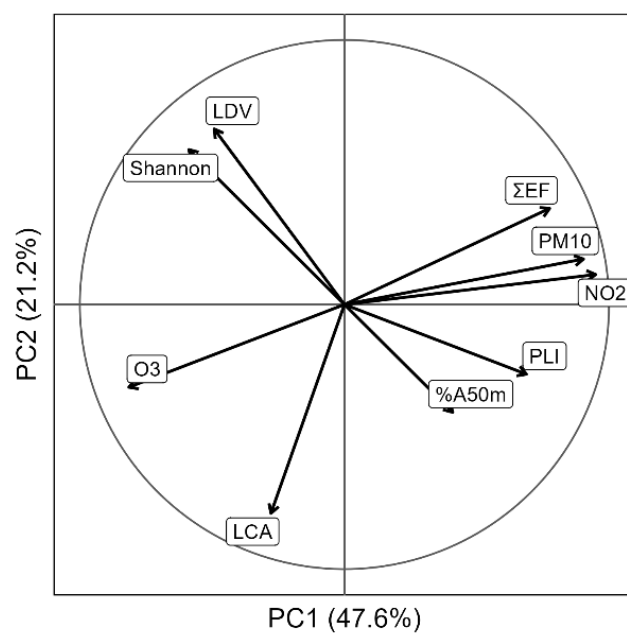


Figure 24: Principal component analysis biplot of synthetic pollution indices (Σ EF and PLI), atmospheric pollutants (NO₂, PM10 and O₃), diversity metrics (LDV and Shannon's index) and land-use variables (LCA and % artificialised in a 50 m radius).

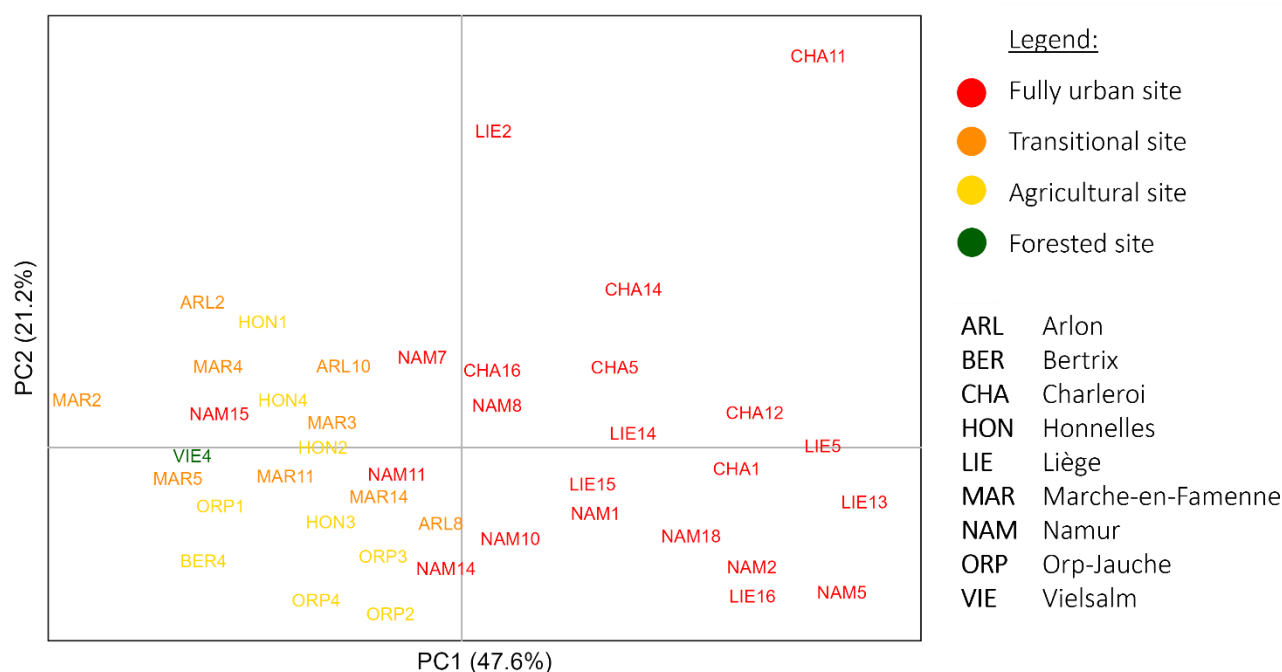


Figure 25: Scores of the studied sites projected onto the principal component analysis biplot of all variables. Red coloured sites are in urban areas, orange coloured sites are transitional urban-rural areas, yellow coloured sites are agricultural and the green coloured site is forested. The sites are labelled with three-letter codes.

The projection of sites onto the PCA biplot helps support the hypotheses of contrasted pollution levels and land-use contexts. It allows us to visualise how lichen diversity and pollution indicators vary together across sampling locations. Overall, both LDV and the Shannon index appear to be reliable indicators of anthropogenic pressure: their negative correlation with ΣEF , NO_2 and PM_{10} on PC1 and with land-cover variables on PC2 supports the idea that lichen diversity and evenness decrease in more polluted or artificialized environments. However, these indices remain indirect proxies, as they integrate the ecological response of lichen communities to a broad set of factors, including but not limited to trace metal inputs (Giordani, 2007; Sebald et al., 2022). As shown in the PCA, their weak correlation with ΣEF suggests that species diversity alone does not fully capture the specific burden of TE pollution.

This limitation is well illustrated by the case of LIE14. Despite its relatively high LDV (134.8, 11th highest) and Shannon index (3.79, 16th highest), it shows elevated levels of NO_2 (13.3 $\mu g \cdot m^{-3}$, 11th highest), PM_{10} (14.5 $\mu g \cdot m^{-3}$, 3rd highest) and ΣEF (217.7, 7th highest), suggesting notable trace metal pollution. These figures show that, even though LIE14 ranks among the sites with above-average lichen diversity ($LDV_{mean} = 119$), it remains one of the most polluted by trace metals ($\Sigma EF_{mean} = 120$). This suggests that diversity alone does not reliably indicate TE contamination, justifying a combined approach.

To address this limitation, we will examine the frequency of each lichen species along environmental gradients rather than relying solely on aggregate diversity indices. This approach is justified by the presence of structured gradients both in pollution metrics and in lichen community composition. These dual gradients provide an appropriate framework for

constrained ordination techniques like CCA, which rely on coherent variation in both species data and environmental variables to detect ecological associations.

5.3.2. Linking species diversity to pollution sensitivity

We performed a CCA to explore how specific lichen species respond to environmental gradients (Chongbang, 2018; Van Dobben & Ter Braak, 1998), using the relative frequencies of 30 lichen species occurring in at least 20% of the sampling sites (Figure 26). This threshold was set to enhance robustness and reduce the influence of rare species. Environmental predictors were Σ EF and PLI (trace-element indices), NO_2 , PM_{10} , O_3 (atmospheric pollutants) and LCA and %A50m (land-use metrics); LDV and Shannon index were excluded from the model to avoid circular reasoning, as they are derived from the species data themselves. Because Σ EF, NO_2 and PM_{10} correlate strongly ($r > 0.7$), we retained all three to compare metallic versus gaseous/particulate pollution effects and identify which gradient best explains species-composition variation.

In the CCA biplot (Figure 26), we observe that Σ EF diverges from NO_2 and PM_{10} , despite their grouping in the PCA (Figure 24). This contrast arises because PCA captures overall covariance among variables, whereas CCA focuses on how environmental predictors explain species variation.

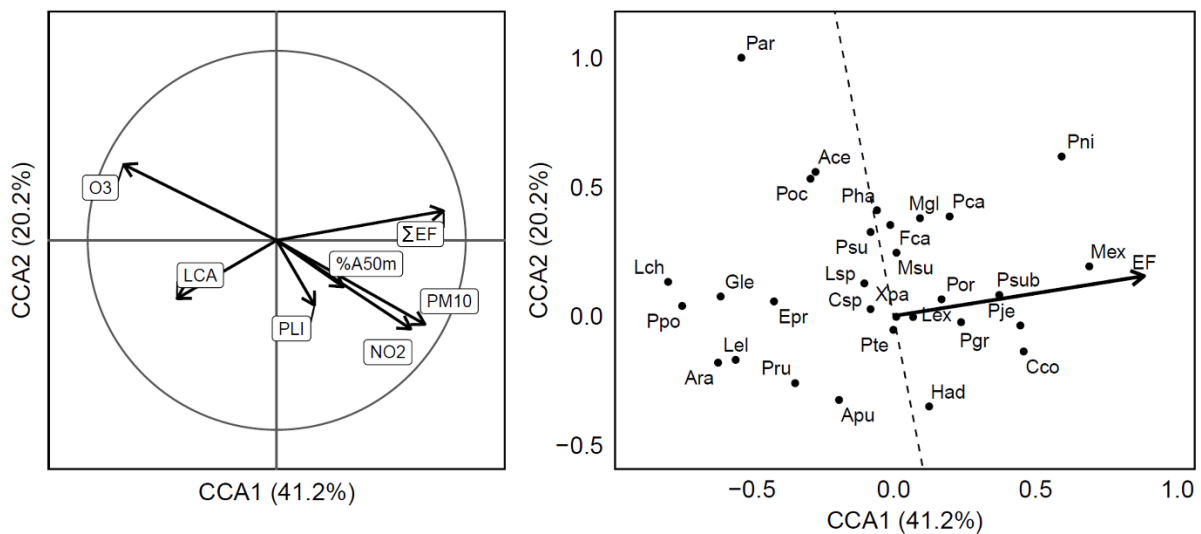


Figure 26: Canonical correspondence analysis (CCA) of lichen species composition in relation to environmental variables. The left panel shows the ordination of environmental variables, including pollution indicators (PLI, Σ EF), atmospheric pollutant concentrations (PM_{10} , NO_2 , O_3), ecological variables (LDV, Shannon diversity) and land-use proxies (%A50m, LCA). The right panel displays species scores in the CCA space, highlighting the associations between specific lichen taxa and environmental gradients.

To assess whether our seven predictors jointly explain lichen-community patterns, we first ran a permutation-based ANOVA on the full CCA. The global model was highly significant ($p < 0.01$) and only CCA1 explained meaningful variation ($p < 0.01$), so we restricted subsequent interpretation to that axis. Next, we quantified each variable's unique effect via partial CCA: for each predictor, we conditioned on all others and tested its residual contribution (Table 8).

Table 8: Permutation based ANOVA p-values for each explanatory variable in both global (all predictors included) and partial (conditioning on all other predictors) canonical correspondence analysis models. P-values < 0.05 are shown in bold.

Explanatory variable	Partial p-value
ΣEF	0.047
PLI	0.372
NO ₂	0.823
PM ₁₀	0.470
O ₃	0.329
LCA	0.522
%A50m	0.490

Only ΣEF remains significant in the partial CCA ($p = 0.047$); all other variables lose significance. This indicates that ΣEF captures a metal-contamination gradient that independently explains species variation. The non-significance of NO₂ and PM₁₀ does not imply they lack ecological impact: (1) strong collinearity with ΣEF means ΣEF absorbs most of the shared pollution signal; (2) bioaccumulation data (ΣEF) integrate local, long-term exposure, whereas ATMOSTREET NO₂ and PM₁₀ are annual averages that may obscure fine-scale gradients. Any real effects of NO₂ or PM₁₀ on lichens may be masked by methodological limitations. Additionally, they may overlap with the trace-metal gradient captured by ΣEF .

Based on these findings, ΣEF was preferred over PLI for two reasons. First, it is derived from trace element-specific enrichment factors, offering better ecological relevance and comparability with previous studies (Agnan, 2013). Second, ΣEF was the only pollution variable to show a significant effect in the partial CCA, whereas PLI was redundant.

Although ΣEF is driven mainly by Cd ($r = 0.956$), Zn ($r = 0.914$) and Pb ($r = 0.845$), it enters the CCA as a single predictor. As a result, species scores along this axis reflect the combined influence of all trace metals rather than any single element.

From the CCA ordination, we extracted the ΣEF vector ($y = 0.0439x$; Figure 27) to define the primary trace-metal gradient. We projected each species score (x, y) onto this axis to compute y' , where high y' values indicate declining frequency with increasing ΣEF (metal sensitivity) and low y' values indicate tolerance.

Based on y' , species were classified as sensitive ($y' > 0.4$), intermediate ($-0.4 \leq y' \leq 0.4$) or tolerant ($y' < -0.4$), as detailed in Table 9. Similar projection-based classifications have been used for nitrogen critical loads in lichens (Geiser et al., 2014; Glavich & Geiser, 2008) and for trace-element pollution gradients in forest ecosystems (Agnan et al., 2017).

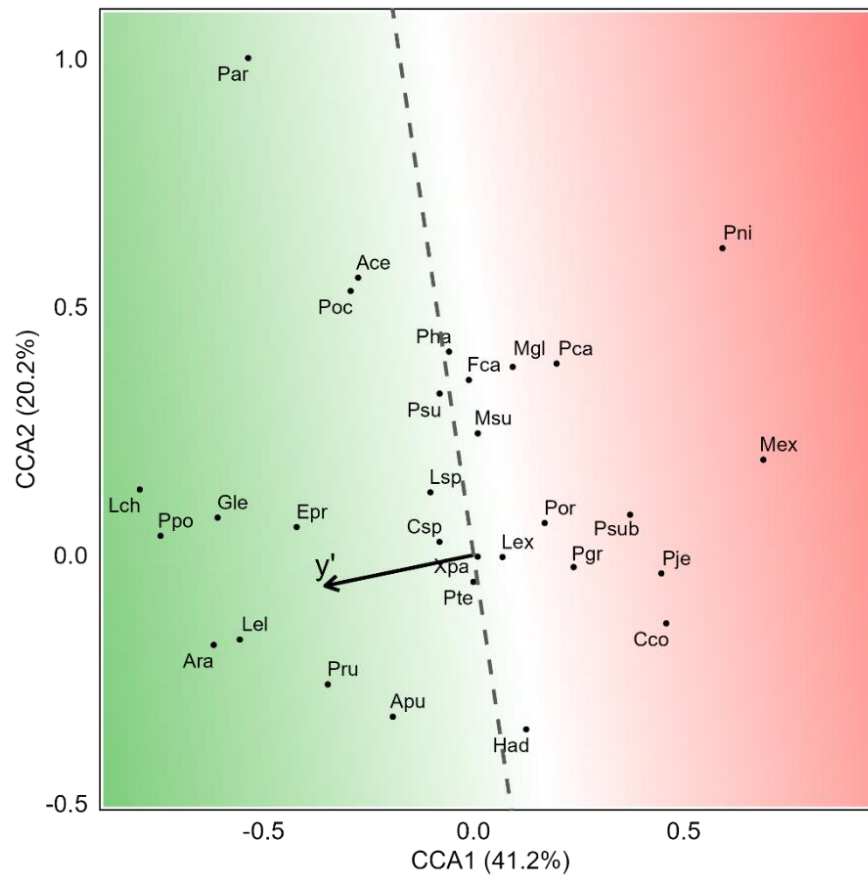


Figure 27: Canonical correspondence analysis biplot of species scores on CCA1 (x-axis) and CCA2 (y-axis). The green to red background reflects the transformed coordinates of the species onto the y' axis (green = sensitive; red = tolerant). The black arrow indicates the sensitivity vector (y') with opposed direction and magnitude to the ΣEF vector and the dashed line perpendicular to the sensitivity vector (y') separates the trace element high influence (red) from low influence (green) areas.

Table 9: List of species that are tolerant (A) with $y' < -0.4$, intermediate (B) with $-0.4 < y' < 0.4$ or sensitive (C) with $y' > 0.4$ to trace element pollution.

(A) Tolerant species	Code	y'
Melanohalea exasperatula	Mex	-0.712
Phaeophyscia nigricans	Pni	-0.690
Punctelia jeckeri	Pje	-0.433
Candelaria concolor	Cco	-0.427

(B) Intermediate species	Code	y'
Punctelia subrudecta / P. borrieri	Psub	-0.380
Physcia caesia	Pca	-0.261
Physconia grisea	Pgr	-0.230
Phaeophyscia orbicularis	Por	-0.177
Melanelixia glabratula	Mgl	-0.157
Lecanora expallens	Lex	-0.067
Hyperphyscia adglutinata	Had	-0.062
Melanelixia subaurifera	Msu	-0.052
Flavoparmelia caperata	Fca	-0.049
Polyzozia hagenii	Pha	-0.013
Xanthoria parietina	Xpa	-0.009
Physcia tenella / P. adscendens	Pte	0.009
Parmelia sulcata	Psu	0.023
Candelariella sp.	Csp	0.075
Lepraria sp.	Lsp	0.079
Athallia cerinella	Ace	0.174
Polycauliona candelaria	Poc	0.196
Amandinea punctata	Apu	0.245
Phlyctis argena	Par	0.355
Pseudoschismatomma rufescens	Pru	0.386

(C) Sensitive species	Code	y'
Evernia prunastri	Epr	0.405
Lecidella elaeochroma	Lel	0.577
Glaucumaria leptyroides	Gle	0.587
Arthonia radiata	Ara	0.640
Polycauliona polycarpa	Ppo	0.727
Lecanora chlarotera sl.	Lch	0.759

5.3.3. Assessment of the sensitivity scale to trace-element pollution

Assessment by comparison with existing literature

To validate the sensitivity scale, we cross-referenced our results with literature documenting known lichen tolerances to TE pollution.

Our classification aligns well with existing studies on TE pollution. For example, *Candelaria concolor*, one of the most tolerant species in our scale, is also recognised as tolerant in the literature. Del Guasta (2000) observed *Candelaria concolor* more frequently on the sides of *Tilia sp.* trunks facing road traffic, areas exposed to dust and metal-rich spray from vehicles. Its ability to colonise anthropised environments and its frequent use in TE biomonitoring studies further support its classification as a tolerant species (Contardo & Loppi, 2024; Sekulić, 2017).

Another example is *Melanohalea exasperatula*, ranked as the most tolerant species in our study. This species is metal-tolerant, as it synthesises phytochelatins upon exposure to TEs, particularly Cd^{2+} (and to a lesser extent Zn^{2+}), which chelate the metals, thereby reducing their toxicity (Branquinho et al., 1997).

Among intermediate species, *Xanthoria parietina* also aligns with previous studies. Sanità Di Toppi et al. (2005) demonstrated that *Xanthoria parietina* thalli can tolerate up to approximately 9 μM Cd^{2+} without any detectable loss in chlorophyll. However, once Cd^{2+} exceeds about 18 μM , the chloroplasts of the photobiont are disrupted and the chlorophyll content declines sharply. Thus, *Xanthoria parietina* behaves as an intermediate species, tolerating low to moderate Cd^{2+} exposure but suffering structural damage at higher concentrations.

At the other end of the spectrum, species classified as sensitive in our study, such as *Evernia prunastri*, align with findings in the literature. Studies show that individuals of this species exposed to high pollution levels experience significant chlorophyll loss, reflecting toxic effects from elevated atmospheric metal deposition (Cecconi et al., 2019; Conti et al., 2004). This supports its classification as sensitive to TE pollution.

Furthermore, the patterns observed in our sensitivity scale also reflect trends found in lichenosociology (see section 5.1.2). Species from the *Physcienion adscendentis* cluster (e.g., *Physconia grisea*, *Phaeophyscia orbicularis*, *Xanthoria parietina*), which are common across many sites, tend to cluster towards the tolerant end of the gradient. Their widespread presence across a variety of environments suggests that these species are more resilient to different environmental conditions, including higher pollution levels.

On the other hand, species from the *Pleurostiction acetabuli* group (e.g., *Evernia prunastri*, *Parmelia sulcata*), which are less frequent and more sparsely distributed, show a broader range of responses, from intermediate to sensitive. This variability suggests that these species have more specific habitat requirements and are less adaptable to changes in environmental conditions.

While previous studies offer useful insights, they typically examine only a handful of taxa or individual cases instead of assessing tolerance across a broader range of lichens. To strengthen our interpretation, we compared our results with Agnan et al. (2017), which applied a similar CCA-based methodology, despite differences in environmental context (i.e., forests vs open-environments), sampling methods (multiple and interspecies bioaccumulation analysis, multiple tree species used for sampling) and Σ EF composition (he included both lithogenic and anthropogenic elements, whereas we considered only anthropogenic elements). The sensitivity scale proposed by Agnan et al. (2017) can be found in Appendix C.2.

Some species classifications align across both studies, for example, *Melanohalea exasperatula* and *Evernia prunastri* are consistently identified as tolerant and sensitive, respectively. Despite differing contexts, 8 out of the 13 overlapping species fall into the same sensitivity class, suggesting broad agreement between both models.

However, several discrepancies emerge: *Arthonia radiata*, *Lecidella elaeochroma* and *Lecanora chlarotera* are classified as resistant by Agnan et al. (2017), but appear among the more sensitive species in our analysis. The discrepancies reflect contrasting ecological settings and methodological choices. Agnan et al. (2017) worked mostly in closed-canopy forests with higher microclimatic variability. In contrast, our sites include open environments, in urban and rural contexts, reducing microclimatic noise but increasing direct pollutant exposure. He sampled multiple forest types across France (introducing climatic variation); we focused solely on Wallonia, minimizing large-scale climate effects. Agnan's surveys used various host trees, while we sampled only linden trunks, removing host-related variability. He classified 44 species versus our 30, so his CCA scores spanned a broader spectrum. Finally, CCA scores are relative to each study's community composition and dataset size, affecting species' apparent sensitivity or tolerance.

To further confirm the ecological relevance of our results, we compared our species' CCA positions along the NO₂ gradient with published sensitivity classes. The resulting scale is presented in Appendix C.3.

Enhancing robustness by understanding the NO₂ influence

To assess whether our trace-element bioaccumulation ranking (Σ EF) behaves differently for another pollutant, we applied the same ordination procedure to derive NO₂ sensitivity scores for each species. In other words, we took the species' NO₂ loadings along the NO₂ axis instead of trace-element axes, producing a parallel sensitivity scale. This allows us to evaluate how the ranking shifts when we focus on NO₂ rather than on Σ EF and to gauge the extent to which NO₂ influences species frequency independently.

Comparing NO₂ sensitivity to Σ EF rankings reveals that they are similar but show some divergences: species that rank high for Σ EF do not always rank equally for NO₂ and vice versa. We plotted Σ EF sensitivity scores against NO₂ sensitivity scores (Figure 28), with points far from the regression line indicating the greatest discrepancies. These divergences confirm that each indicator captures a different facet of ecological response rather than redundantly measuring the same gradient.

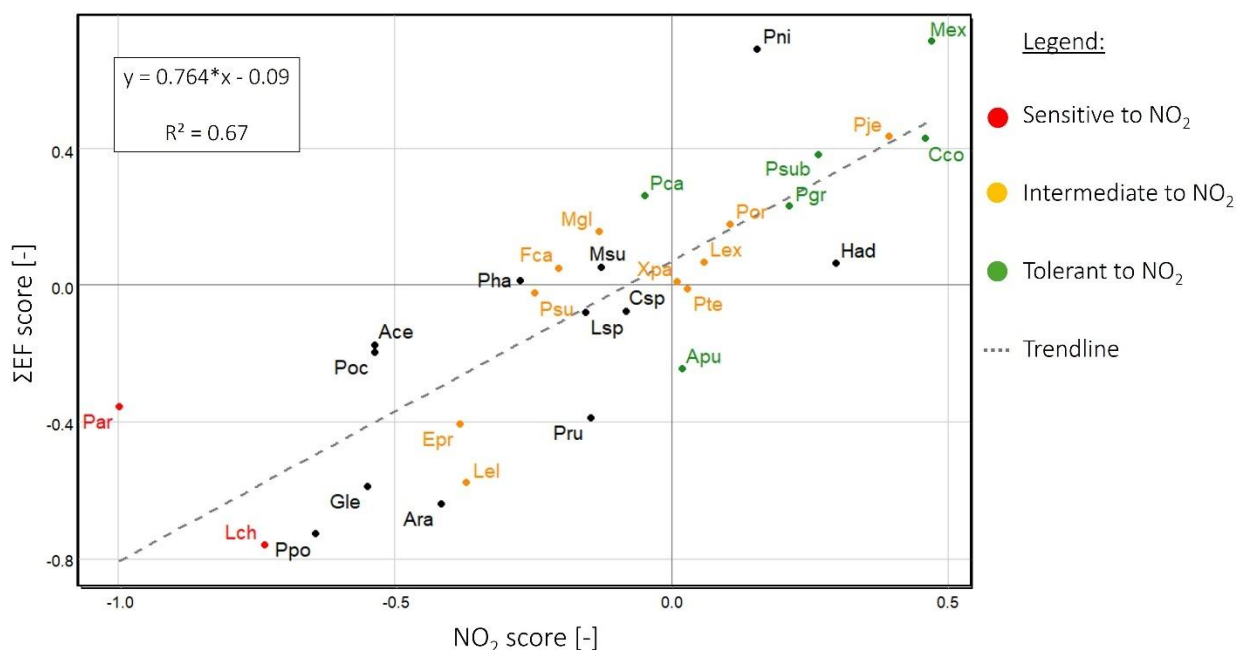


Figure 28: Scatterplot of lichen species sensitivity scores extracted from the NO₂ (x-axis) and ΣEF (y-axis) gradients, with points coloured according to literature-derived NO₂ sensitivity classes (Gombert et al., 2006). The trendline is shown with the corresponding equation and R² value.

Color-coding species by NO₂ sensitivity classes proposed in the literature (Gombert et al., 2006) generally confirms our classifications. A few examples are *Melanohalea exasperatula* that is very tolerant, *Parmelia sulcata* that is intermediate and *Lecanora chlorotera* that is sensitive. However, some mismatches appear: *Punctelia jeckeri* is tolerant in our scale and in Van Dobben & Ter Braak (1998) but listed as intermediate by Gombert et al. (2006) and *Physcia caesia* ranks intermediate here yet is labelled tolerant by Van Dobben & Ter Braak (1998).

These discrepancies stem from methodological differences: Van Dobben & Ter Braak (1998) derive sensitivity via statistical models of coarse-resolution SO₂, NO₂ and NH₃ maps and Gombert et al. (2006) combine passive NO₂/O₃/IPA measurements with CCA. Most importantly, none of these studies have measured trace metals, so their “sensitivity” scores may reflect lichen responses to metal contamination rather than (or in addition to) gaseous pollutants, introducing potential bias in sensitivity rankings.

Summary interpretation of the sensitivity scale

ΣEF proved to be a robust and specific indicator of lichen response to TEs, providing information that complements, but does not duplicate that of NO₂. Cross-validations with existing literature show that some species react specifically to TEs rather than to nitrogen oxides, underscoring the importance of integrating both parameters in bioindication studies, especially in environments where these pollutants co-vary.

Our study suggests that linking bioindication with ΣEF based bioaccumulation could potentially allow for the evaluation of TEs contamination without relying solely on laboratory analyses. In heterogeneous environments (urban, agricultural and forested), this combined

approach offers a way to disentangle TE versus gaseous effects, thereby improving maintenance strategies and air quality monitoring recommendations.

However, some limitations of this study should be considered. One limitation is the strong correlation between certain environmental variables, particularly NO₂, PM₁₀ and ΣEF. These pollutants are correlated, making it difficult to isolate and fully assess the specific contributions of each pollutant to the observed patterns in species composition.

Another limitation is the lack of validation for the sensitivity scale. While the scale was applied within Wallonia, we did not have additional data to test it. Furthermore, its applicability to other regions outside Wallonia remains unknown. This means that its ecological consistency and generalisability to different environments are uncertain.

Finally, another limitation is related to pollution source attribution. The ΣEF values used in this study are not disaggregated by individual metals, which prevents us from identifying specific pollution sources such as traffic, industry or agriculture. Additionally, PLI calculations with the estimation of geological baseline values from the lichen samples themselves could introduce bias.

6. Conclusions and perspectives

6.1. Conclusions of the study

TE pollution remains poorly characterised at the regional scale, especially in urban environments. This study combined bioindication and bioaccumulation methods to construct a species-level sensitivity scale to TEs.

Bioindication revealed that lichen diversity varies with urbanisation and land use: forested or semi-natural sites showed high species richness, LDV and Shannon's index values, while urban and intensively farmed sites showed reduced values for the same alpha diversity metrics. The NMDS analysis identified two main ecological gradients that structure lichen community composition: (1) urban vs. natural or rural environments; (2) moist and acidic vs. dry and eutrophic environments. These results confirm that lichen community composition is a reliable proxy for overall environmental quality, but it does not isolate the effect of trace metals.

Bioaccumulation data showed significant enrichment for Cd, Zn and Pb, indicating anthropogenic inputs, while Al, V and Fe exhibited low enrichment, indicating lithogenic origin. PLI identified moderate pollution in some cities, but its weak correlation with ΣEF highlights that they capture different signals: cumulative anthropogenic enrichment vs. overall intensity and potential toxic burden. A PCA of element concentrations distinguished three geochemical groups: anthropogenic, lithogenic and mixed origin.

Coupling bioindication and bioaccumulation allowed us to identify the main drivers of species composition. ΣEF , strongly correlated with NO_2 and PM_{10} , was the only variable with a significant independent effect on species frequency in the partial CCA. This enabled the derivation of a sensitivity scale to TEs distinguishing tolerant species (e.g., *Melanohalea exasperatula*), intermediate species (e.g., *Xanthoria parietina*) and sensitive species (e.g., *Evernia prunastri*). A complementary NO_2 based analysis revealed partly divergent species responses, showing that TE and nitrogen sensitivities do not fully overlap.

This integrated approach achieved the main objective: to develop an empirical sensitivity scale to trace metals based on observed lichen frequencies. The resulting sensitivity scale opens the door to evaluating metal pollution using community composition alone, but while generally consistent with the literature, classifications for some sensitive taxa remain uncertain due to the lack of TEs specific studies.

6.2. Perspectives of the study

To overcome the main limitations identified, namely the co-variation between NO₂ and TEs, the further validation of the sensitivity scale and the potential masking of source-specific signals, three directions are proposed.

To address the limitation of correlated pollutants, future studies could focus on increasing model robustness by separating the effects of pollutants like NO₂, PM₁₀ and ΣEF. One option is to install passive samplers at lichen sampling sites, enabling the collection of simultaneous data on these pollutants. Alternatively, a bioaccumulation approach could be applied at the same sites to assess the specific effects of NO₂, PM₁₀ and other pollutants. Additionally, measuring other key compounds such as SO₂ and NH₃ would help refine species specific responses and detect cross sensitivities.

To validate the sensitivity scale, two key approaches are proposed. First, applying the same protocol (inventory + CCA + ΣEF) in other regions with similar species would allow testing whether sensitive/tolerant species retain their status across biogeographical contexts. This would provide a clearer understanding of whether the scale is consistent or if regional differences affect the sensitivity classifications.

Second, it would be valuable to remain within Wallonia but extend the study to more sites outside the initial 41 sampled locations. This would allow for the collection of additional data on species frequency and metal concentrations at other sites. By applying ΣEF analysis, the relationship between metal concentrations and species frequency could be explored, particularly whether higher ΣEF values correlate with lower frequencies of sensitive species and higher frequencies of tolerant species.

Finally, to go towards source attribution, the ΣEF could be disaggregated. By isolating specific sources, such as traffic, industry or agriculture and examining metals like Pb and Cd from traffic or Zn and Cu from industrial activity, we can assess how particular species respond to different pollutants. This would allow a direct correlation between species frequency and specific metals, enhancing understanding of how pollution sources affect species.

For PLI calculation, using Wallonia's pedogeochemical background, rather than geological baselines estimated from the lichen samples, would provide a more accurate method. As the regional pedogeochemical data reflects the area's natural contamination, it would reduce bias and offer a better measure of anthropogenic contamination.

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8. Appendices

A. Supporting materials

1. Relevance of LCA index and distribution of values

i. Relevance

Land-use type, urban, agricultural or forested, reflects key environmental conditions such as microclimate, habitat structure, pollution and human influence. Indicators like the LCA index or %A50m provide valuable context for interpreting species composition and pollution levels.

Including these variables improves the ecological relevance and interpretability of multivariate analyses. As proxies for environmental pressure, they help reveal how landscape structure shapes biological responses which is a key objective in biomonitoring.

PCA is an unconstrained method (meaning it does not distinguish between explanatory and response variables in contrast to the CCA) but including land-use indicators like LCA or %A50m ensures that urbanisation gradients contribute to the main axes of variation. These variables help explain site differences alongside pollutants and diversity. Labelling or colouring sites by land-use type further clarifies how urban pressure shapes ecological patterns.

In CCA, environmental variables directly constrain species data to explain variation in community composition. Including land-use indicators like LCA or %A50m is both methodologically appropriate and ecologically meaningful. These variables quantify how quantify the extent to which urbanisation explains changes in lichen communities or trace metal accumulation. Its inclusion in CCA allows us to test how landscape gradients (e.g., green

pockets within urban matrices) shape species composition, thus offering insights into ecological resilience and/or fragmentation effects.

ii. Distribution of LCA values for the 41 sites

Figure 29 shows the distribution of LCA index values calculated for the 41 study sites. The red dashed line marks the value 0, which corresponds to a ratio of 1 between artificial surfaces in the 200 m and 50 m buffers, indicating an equal level of artificialisation at both scales. Most values are concentrated slightly above 0, around 0.5, suggesting moderate differences in artificial surface coverage between the two spatial scales.

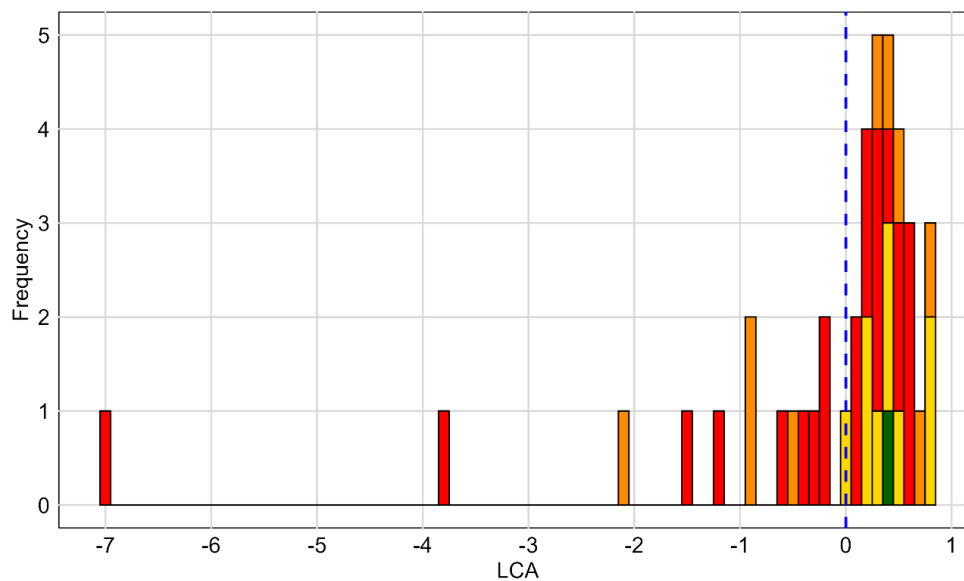


Figure 29: Distribution of LCA values for the 41 sites

Most LCA values fall between 0.3 and 0.6, indicating that the 50 m buffer is often more artificialized than the surrounding 200 m area, a pattern generally unusual in fully urbanised settings. This is consistent with the field strategy, where several trees were sampled close to roads, while the broader environment included fields, parks, or forest patches. This indicates that urban pressure is concentrated in micro-sites, rather than across large continuous urban expanses.

Conversely, a few sites show negative LCA values, meaning that the 200 m buffer is more artificialized than the immediate 50 m area. This typically reflects small green or semi-natural patches (e.g., parks, fields, tree lines) located within a larger urban matrix. In these cases, urban pressure may be lower at the micro-scale but higher at the landscape level, suggesting isolated green fragments embedded in densely built environments.

To illustrate the interpretation of the LCA index with concrete examples, as shown in Figure 30. The lowest value is observed at Charleroi (CHA11), with an LCA of -7.02 . This indicates that the 200 m buffer is substantially more artificialized than the immediate 50 m surroundings, a typical case of a green pocket (e.g., a tree or small park) embedded in a dense urban matrix.

Conversely, Marche-en-Famenne (MAR11) shows one of the highest positive values, with an LCA of 0.67. In this case, the 50 m buffer is significantly more artificialized than the 200 m zone, reflecting a site located in a small urban structure (here the road) within a more natural landscape (agricultural fields).

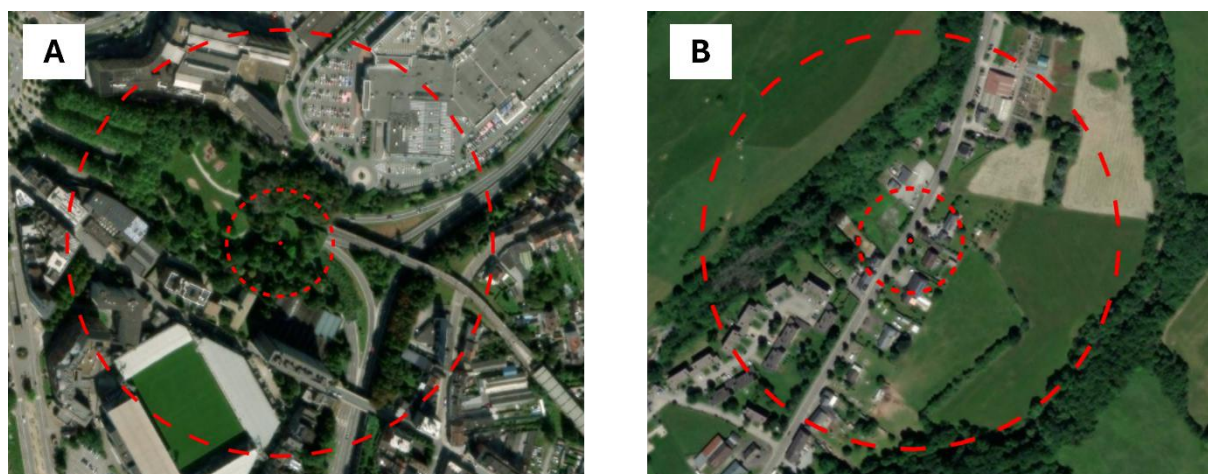


Figure 30: Visualisation of two different values of LCA : CHA11 with a green pocket in an urban environment reflected by a low LCA (A) and MAR11 with an artificial area surrounded by less artificialized area (B).

2. Understanding the differences between PLI and ΣEF

The main goal of this part is to understand the differences in the geochemical indices that were calculated. When looking at Figure 24, the arrows of ΣEF_{UCC} and PLI were weakly correlated and were adding more weight to different axis. Since one of these two indices was to be used when constructing the tolerance scale of species as the main factor influencing the frequency in the species, it is crucial to understand if they show difference is due to the normalisation method (UCC vs estimation of geochemical background values) or if it is the information conveyed that is fundamentally different.

In order to do so, PLI was recalculated using the UCC values for the 8 metals (As, Cd, Cu, Cr, Ni, Pb, Sn and Zn) having an EF over two suggesting an anthropogenic increase in the concentrations of these metals. By using the UCC values proposed by (Taylor & McLennan, 1995), the CF_{UCC} were and the PLI_{UCC} was computed and plotted against the $PLI_{estimated\ background}$ and the ΣEF_{UCC} , as shown in Figure 31.

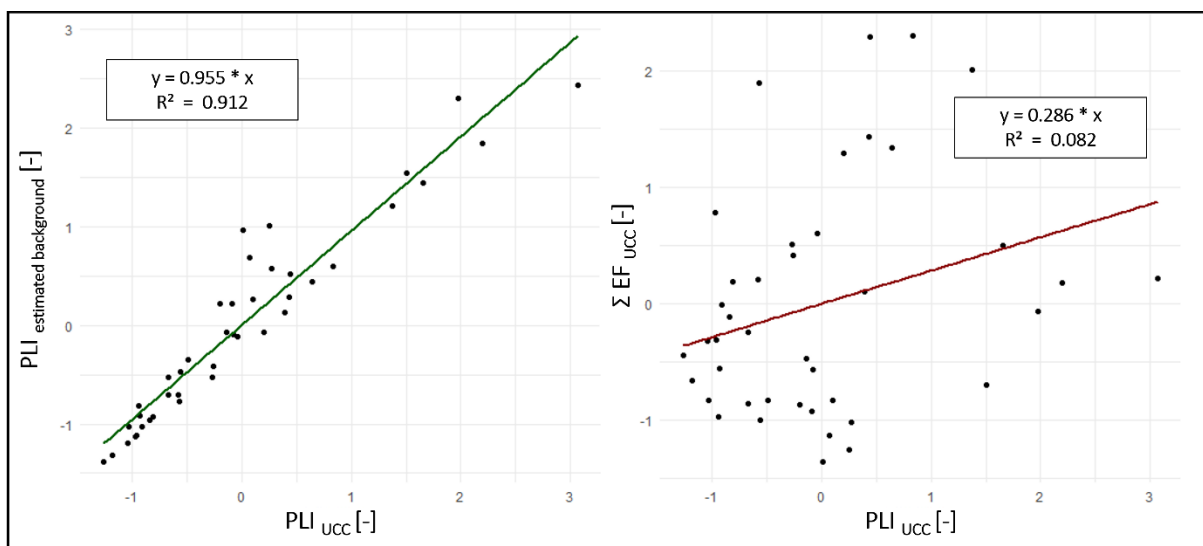


Figure 31: Comparison of the effect of normalisation (UCC versus estimation of geochemical background) for the studied geochemical indices (PLI and ΣEF)

Table 10: Correlation matrix between the studied geochemical indices (PLI_{UCC} , $PLI_{estimated\ background}$ and ΣEF_{UCC})

	PLI_{UCC}	$PLI_{estimated\ background}$	ΣEF_{UCC}
PLI_{UCC}	1		
$PLI_{estimated\ background}$	0.995	1	
ΣEF_{UCC}	0.286	0.112	1

Despite being derived from the same trace metal concentrations, ΣEF_{UCC} and PLI_{UCC} diverge significantly both statistically and ecologically. From these results, the hypothesis is confirmed, PLI_{UCC} and $PLI_{estimated\ background}$ have a high coefficient of determination ($R^2 = 0.91$) suggesting that there is a valid coherence between the information conveyed by these indices regardless of the method of normalisation. In contrast, PLI_{UCC} and ΣEF_{UCC} have a very low coefficient of determination ($R^2 = 0.08$) suggesting that they measure different aspects of pollution and give us different information. This means that the mathematical formula used to calculate both indices causes the difference in the information given.

ΣEF_{UCC} is calculated as an additive sum of enrichment factors ($EF > 2$), emphasizing element specific deviations from the UCC geochemical background and highlighting anthropogenic contributions. It is particularly sensitive to elevated values of individual trace elements.

PLI_{UCC} , is a geometric mean of contamination factors, integrating the overall exposure across multiple metals and reflecting cumulative pollution intensity.

From an ecological perspective, ΣEF_{UCC} is more informative for identifying pollution sources and effects from specific TEs, while PLI provides a composite measure of total contamination (all TEs together), which may relate more directly to cumulative biological stress. Both offer complementary insights depending on the focus: specific TE pressure versus integrated, overall pollution pressure.

3. Estimation of geochemical background values from lichen samples

To estimate the geochemical background concentration of TEs in lichens, we used a method based on the analysis of the cumulative distribution function of the measured concentrations.

This approach assumes that values below the median reflect the natural distribution of concentrations in the absence of significant anthropogenic influence. Values above the median, which may be affected by anthropogenic inputs, are "corrected" by mirroring the lower half of the distribution. A theoretical distribution (dark red curve) is thus reconstructed from this portion considered representative of natural background levels.

As an example, the estimation of geochemical background for Pb is shown in figure. The statistical parameters of this corrected distribution are then calculated:

- Mean (μ) = 2.6 [$\mu\text{g/g}$]
- Standard deviation (σ) = 0.88 [$\mu\text{g/g}$]

The upper limit of the geochemical background is defined as $\mu + 2\sigma$, i.e., 4.37 [$\mu\text{g/g}$]. This value was used as the reference threshold for computing geochemical indices (CF and PLI).

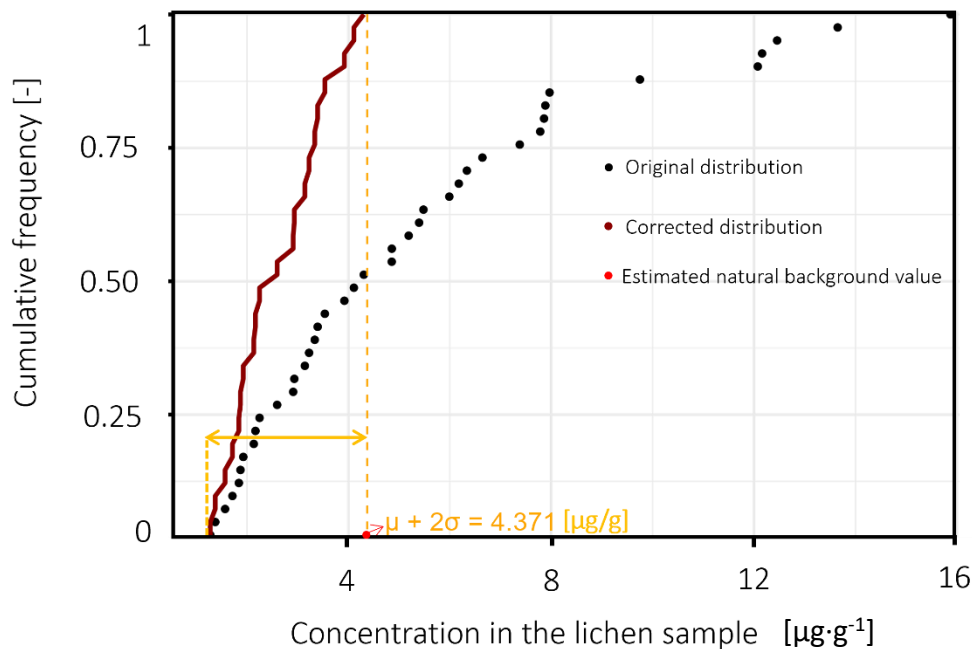


Figure 32: Estimation of the geochemical background values using the mirror method: example of Pb

B. Coordinates of the sites

Table 11: Coordinates of the 41 sampling sites in the study area

Site	Latitude	Longitude	Site	Latitude	Longitude
ARL10	49.6361	5.8157	MAR14	50.1592	5.2442
ARL2	49.6465	5.9093	MAR2	50.185	5.4002
ARL8	49.6867	5.7966	MAR3	50.1975	5.3092
BER4	49.8455	5.3122	MAR4	50.2015	5.3164
CHA1	50.4806	4.4446	MAR5	50.1818	5.3198
CHA11	50.4161	4.455	NAM1	50.4717	4.8911
CHA12	50.4267	4.4689	NAM10	50.4959	4.7969
CHA14	50.4606	4.4288	NAM11	50.4843	4.7918
CHA16	50.4363	4.3693	NAM14	50.4276	4.9181
CHA5	50.4094	4.3811	NAM15	50.451	4.9904
HON1	50.3684	3.7342	NAM18	50.4499	4.8939
HON2	50.3567	3.7675	NAM2	50.4707	4.853
HON3	50.3737	3.6985	NAM5	50.4626	4.8725
HON4	50.3325	3.6958	NAM7	50.4842	4.8376
LIE13	50.617	5.5767	NAM8	50.4383	4.8652
LIE14	50.6287	5.5444	ORP1	50.6571	4.9442
LIE15	50.6014	5.6028	ORP2	50.6943	4.9875
LIE16	50.7018	5.6722	ORP3	50.7133	4.9931
LIE2	50.6548	5.578	ORP4	50.6683	4.9775
LIE5	50.6266	5.5767	VIE4	50.3099	6.0128
MAR11	50.1778	5.2976			

C. Species information

1. Code of the species and authority

Table 12: Three letter code abbreviation of the species in our dataset and authorities associated

Species	Code	Authority
<i>Candelariella sp.</i>	Csp	Müll.Arg.
<i>Evernia prunastri</i>	Epr	(L.) Ach.
<i>Flavoparmelia caperata</i>	Fca	(L.) Hale
<i>Hyperphyscia adglutinata</i>	Had	(Flörke) H. Mayrhofer & Poelt
<i>Lecanora chlarotera sl.</i>	Lch	(Nyl.)
<i>Lecanora expallens</i>	Lex	(Ach.)
<i>Lecidella elaeochroma</i>	Lel	(Ach.) M. Choisy var. flavicans (Ach.) Hazsl.
<i>Lepraria sp.</i>	Lsp	(L.) Ach.
<i>Melanelixia glabratula</i>	Mgl	(Lamy) Sandler & Arup
<i>Melanohalea elegantula</i>	Mel	(Zahlbr.) O. Blanco, A. Crespo
<i>Parmelia sulcata</i>	Psu	Taylor
<i>Parmelina tiliacea</i>	Pti	(Hoffm.) Hale
<i>Phaeophyscia orbicularis</i>	Por	(Neck.) Moberg
<i>Phlyctis argena</i>	Par	(Spreng.) Flot.
<i>Physcia caesia</i>	Pca	(Hoffm.) Fűrnr. var. caesia
<i>Physcia tenella / P. adscendens</i>	Pte	(Scop.) DC.
<i>Physciella chloantha</i>	Pch	(Ach.) Essl.
<i>Physconia distorta</i>	Pdi	(With.) J.R. Laundon
<i>Physconia grisea</i>	Pgr	(Lam.) Poelt subsp. grisea
<i>Physconia perisidiosa</i>	Pper	(Erichsen) Moberg
<i>Pleurosticta acetabulum</i>	Pac	(Neck.) Elix & Lumbsch
<i>Polycauliona candelaria</i>	Poc	(L.) Frödén, Arup & Söchting
<i>Porina aenea</i>	Pae	(Wallr.) Zahlbr.
<i>Pseudoschismatomma rufescens</i>	Pru	(Pers.) Ertz & Tehler
<i>Punctelia jeckeri</i>	Pje	(Roum.) Kalb

<i>Punctelia subrudecta</i> / <i>P. borrieri</i>	Psub	(Sm.) Krog
<i>Xanthoria parietina</i>	Xpa	(L.) Th. Fr.
<i>Amandinea punctata</i>	Apu	(Hoffm.) Coppins & Scheid.
<i>Buellia griseovirens</i>	Bgr	(Sm.) Almb.
<i>Candelaria concolor</i>	Cco	(Dicks.) Stein
<i>Cladonia chlorophaea</i> sl.	Cch	(Sommerf.) Spreng.
<i>Glaucomaria subcarpineae</i>	Gsu	(Szatala) S.Y. Kondr., Lőkös & Farkas
<i>Lecania naegelii</i>	Lna	(Hepp) Diederich & van den Boom
<i>Lepra albescens</i>	Lal	(Huds.) Hafellner var. albescens
<i>Melanohalea exasperatula</i>	Mex	(Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch
<i>Parmelia saxatilis</i>	Psa	(L.) Ach.
<i>Polycauliona polycarpa</i>	Ppo	(Hoffm.) Frödén, Arup & Sørching
<i>Ramalina farinacea</i>	Rfa	(L.) Ach.
<i>Athallia cerinella</i>	Ace	(Nyl.) Arup, Frödén & Sørching
<i>Normandina pulchella</i>	Npu	(Borrer) Nyl.
<i>Phaeophyscia nigricans</i>	Pni	(Flörke) Moberg
<i>Physcia dubia</i>	Pdu	(Hoffm.) Lettau
<i>Polyzozia hagenii</i>	Pha	(Ach.) S.Y. Kondr., Lőkös & Farkas
<i>Hypotrachyna afrorevoluta</i>	Haf	(Krog & Swinscow) Krog & Swinscow
<i>Lecanora pulicaris</i>	Lpu	(Pers.) Ach.
<i>Physconia enteroxantha</i>	Pen	(Nyl.) Poelt
<i>Arthonia radiata</i>	Ara	(Pers.) Ach.
<i>Glaucomaria leptyroides</i>	Gle	(G.B.F. Nilsson) S.Y. Kondr., Lőkös & Farkas
<i>Lecania cyrtella</i>	Lcy	(Ach.) Th. Fr.
<i>Lecanora cenisia</i>	Lce	Ach. f. cenisia
<i>Melanelixia subaurifera</i>	Msu	(Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch
<i>Parmotrema perlatum</i>	Ppl	(Huds.) M. Choisy
<i>Flavoparmelia soredians</i>	Fso	(Nyl.) Hale
<i>Catillaria nigroclavata</i>	Cni	(Nyl.) J. Steiner

<i>Flavoplaca flavocitrina</i>	Ffl	(Nyl.) Arup, Frödén & Söchting
<i>Hypotrachyna revoluta</i>	Hre	(Flörke) Hale
<i>Melanohalea laciniatula</i>	Mla	(H. Olivier) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch
<i>Chaenotheca ferruginea</i>	Cfe	(Sm.) Mig.
<i>Diploicia canescens</i>	Dca	(Dicks.) A. Massal.
<i>Physcia leptalea</i>	Ple	(Ach.) DC.
<i>Opegrapha niveoatra</i>	Oni	(Borrer) J.R. Laundon
<i>Hypogymnia physodes</i>	Hph	(L.) Nyl.
<i>Candelariella vitellina</i>	Cvi	(Hoffm.) Müll. Arg.
<i>Protoparmeliopsis muralis</i>	Pmu	(Schreb.) M. Choisy var. muralis
<i>Athallia pyracea</i>	Apy	(Ach.) Arup, Frödén & Söchting
<i>Lecanora horiza</i>	Lho	(Ach.) Linds.
<i>Parmelina pastillifera</i>	Ppa	(Harm.) Hale
<i>Lecanora argentata</i>	Lar	(Ach.) Malme
<i>Lecanora compallens</i>	Lco	Herk & Aptroot
<i>Alyxoria varia</i>	Ava	(Pers.) Ertz & Tehler
<i>Ochrolechia turneri</i>	Otu	(Sm.) Hasselrot
<i>Pertusaria pertusa</i>	Ptr	(L.) Tuck. var. pertusa
<i>Polycauliona phlogina</i>	Pph	(Ach.) Arup, Frödén & Söchting
<i>Physcia aipolia</i>	Pai	(Humb.) Fűrnr.
<i>Ramalina fastigiata</i>	Rft	(Pers.) Ach.
<i>Phaeophyscia endophoenicea</i>	Phe	(Harm.) Moberg
<i>Lecanora symmicta</i>	Lsy	(Ach.) Ach.
<i>Fuscidea lightfootii</i>	Fli	(Sm.) Coppins & P. James

2. Trace element sensitivity scale proposed by Agnan (2017)

Table 13: Sensitivity scale proposed of species to trace elements proposed by Agnan (2017)

Species	Code	y'	Classification
<i>Lecanactis subabietina</i>	Lsu	-1.442	Tolerant
<i>Pertusaria leioplaca</i>	Pli	-1.402	Tolerant
<i>Pertusaria albescens</i>	Pal	-1.093	Tolerant
<i>Graphis scripta</i>	Gsc	-0.919	Tolerant
<i>Cladonia fimbriata</i>	Cfi	-0.893	Tolerant
<i>Melanohalea exasperatula</i>	Meu	-0.854	Tolerant
<i>Dendrographa decolorans</i>	Dde	-0.799	Tolerant
<i>Ochrolechia pallescens subsp. parella</i>	Opp	-0.781	Tolerant
<i>Ochrolechia androgyna</i>	Oan	-0.656	Tolerant
<i>Pertusaria amara</i>	Paa	-0.62	Tolerant
<i>Lepraria incana</i>	Lic	-0.615	Tolerant
<i>Lecanora allophana</i>	Lal	-0.608	Tolerant
<i>Physcia tenella</i>	Pte	-0.555	Tolerant
<i>Calicium salicinum</i>	Csa	-0.551	Tolerant
<i>Acrocordia gemmata</i>	Age	-0.537	Tolerant
<i>Schismatomma cretaceum</i>	Scr	-0.525	Tolerant
<i>Arthonia radiata</i>	Ara	-0.513	Tolerant
<i>Lecidella elaeochroma</i>	Lel	-0.494	Intermediate
<i>Chrysothrix candelaris</i>	Cca	-0.482	Intermediate
<i>Lecanora chlarotera</i>	Lch	-0.428	Intermediate
<i>Melanelixia glabratula</i>	Mgl	-0.389	Intermediate
<i>Lecanora conizaeoides</i>	Lcn	-0.284	Intermediate
<i>Lecanora expallens</i>	Lex	-0.234	Intermediate
<i>Parmelia sulcata</i>	Psi	-0.187	Intermediate
<i>Lecanora argentata</i>	Lar	-0.021	Intermediate
<i>Ochrolechia turneri</i>	Otu	-0.018	Intermediate
<i>Amandinea punctata</i>	Apu	0.066	Intermediate

<i>Lecanora barkmaniana</i>	Lba	0.08	Intermediate
<i>Buellia disciformis</i>	Bdi	0.124	Intermediate
<i>Lecanora carpinea</i>	Lca	0.17	Intermediate
<i>Parmelina carporrhizans</i>	Pca	0.204	Intermediate
<i>Xanthoria parietina</i>	Xpa	0.244	Intermediate
<i>Pleurococcus viridis</i>	Pvi	0.342	Intermediate
<i>Phlyctis argena</i>	Par	0.493	Intermediate
<i>Pleurosticta acetabulum</i>	Pac	0.519	Sensitive
<i>Caloplaca ferruginea</i>	Cfe	0.524	Sensitive
<i>Pseudevernia furfuracea</i>	Pfu	0.59	Sensitive
<i>Hypogymnia physodes</i>	Hph	0.706	Sensitive
<i>Evernia prunastri</i>	Epr	0.732	Sensitive
<i>Usnea sp.</i>	Usn	0.739	Sensitive
<i>Physcia adscendens</i>	Pad	0.771	Sensitive
<i>Ramalina farinacea</i>	Rfr	0.824	Sensitive
<i>Pertusaria coccodes</i>	Pco	1.256	Sensitive
<i>Physconia distorta</i>	Pdi	1.405	Sensitive

3. NO₂ sensitivity scale

Table 14: Sensitivity scale of the species to NO₂ derived from the canonical correspondance analysis ordination.

Species	Code	y'	Gombert et al. (2006)	Van Dobben & Ter Braak (1998)
<i>Phlyctis argena</i>	Par	-0.99891	Sensitive	Sensitive
<i>Lecanora chlarotera</i> sl.	Lch	-0.73613	Sensitive	Intermediary
<i>Polycauliona polycarpa</i>	Ppo	-0.64343		
<i>Glaucomaria leptyroides</i>	Gle	-0.55029		
<i>Polycauliona candelaria</i>	Poc	-0.53711		
<i>Athallia cerinella</i>	Ace	-0.53688		
<i>Arthonia radiata</i>	Ara	-0.41665		
<i>Evernia prunastri</i>	Epr	-0.3827	Intermediary	
<i>Lecidella elaeochroma</i>	Lel	-0.37098	Intermediary	
<i>Polyozosia hagenii</i>	Pha	-0.27423		
<i>Parmelia sulcata</i>	Psu	-0.24653	Intermediary	Tolerant
<i>Flavoparmelia caperata</i>	Fca	-0.2035	Intermediary	
<i>Lepraria</i> sp.	Lsp	-0.15543		
<i>Pseudoschismatomma rufescens</i>	Pru	-0.14622		
<i>Melanelixia glabratula</i>	Mgl	-0.13075	Intermediary	
<i>Melanelixia subaurifera</i>	Msu	-0.1264		
<i>Candelariella</i> sp.	Csp	-0.08234		
<i>Physcia caesia</i>	Pca	-0.04727		Tolerant
<i>Xanthoria parietina</i>	Xpa	0.009899	Intermediary	
<i>Amandinea punctata</i>	Apu	0.018723	Tolerant	
<i>Physcia tenella</i> / <i>P. adscendens</i>	Pte	0.029267	Intermediary	
<i>Lecanora expallens</i>	Lex	0.059613	Intermediary	Intermediary
<i>Phaeophyscia orbicularis</i>	Por	0.105705	Intermediary	Intermediary
<i>Phaeophyscia nigricans</i>	Pni	0.154577		
<i>Physconia grisea</i>	Pgr	0.212103	Tolerant	Tolerant
<i>Punctelia subrudecta</i> / <i>P. borreri</i>	Psub	0.266239	Tolerant	
<i>Hyperphyscia adglutinata</i>	Had	0.297761		
<i>Punctelia jeckeri</i>	Pje	0.393461		Intermediary
<i>Candelaria concolor</i>	Cco	0.458622	Tolerant	Tolerant
<i>Melanohalea exasperatula</i>	Mex	0.470306	Tolerant	Tolerant

D. Bioindication data

1. Diversity indices by site (species richness, LDV and Shannon's index)

Table 15: Alpha diversity metrics (species richness, lichen diversity value, Shannon's index) for the 41 sites in the study area

Site	Specific richness	Shannon	LDV
ARL10	27	3.99	122
ARL2	30	4.22	160
ARL8	27	3.99	101.3
BER4	22	3.82	97.5
CHA1	17	3.44	124.5
CHA11	24	3.73	142.8
CHA12	19	3.49	109.5
CHA14	24	3.82	116
CHA16	27	3.95	132
CHA5	16	3.4	131.5
HON1	28	4.11	164.8
HON2	21	3.74	132.5
HON3	17	3.46	94.5
HON4	28	4.09	164.3
LIE13	19	3.26	106.8
LIE14	24	3.79	134.8
LIE15	18	3.56	85.8
LIE16	10	2.46	66.8
LIE2	28	4.17	165
LIE5	17	3.32	86.5
MAR11	22	3.78	144.3

Site	Specific richness	Shannon	LDV
MAR14	21	3.48	116.8
MAR2	30	4.18	157
MAR3	18	3.47	115.5
MAR4	29	4.02	134.3
MAR5	26	4.15	125.5
NAM1	21	3.67	95.5
NAM10	17	3.36	119
NAM11	26	3.82	137.5
NAM14	19	3.36	110.5
NAM15	24	3.67	154
NAM18	19	3.24	101.8
NAM2	20	3.38	91.5
NAM5	14	2.95	78.8
NAM7	24	3.9	127.8
NAM8	17	3.54	103.5
ORP1	18	3.55	140
ORP2	17	3.1	98.8
ORP3	18	3.48	103
ORP4	14	3.32	95.8
VIE4	26	4.01	113.8

E. Bioaccumulation data

1. Spearman correlations of trace elements

Table 16: Spearman correlation between the trace elements of interest. Values of $\rho > 0.7$ are in bold.

Element	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sn	Ti	V	Zn
Al	1													
As	0.87	1												
Cd	0.06	0.57	1											
Co	0.85	0.86	0.45	1										
Cr	0.24	0.37	0.57	0.47	1									
Cu	0.54	0.76	0.6	0.45	0.52	1								
Fe	0.67	0.76	0.62	0.9	0.65	0.59	1							
Mn	0.54	0.51	0.28	0.73	0.56	0.59	0.83							
Ni	0.35	0.35	0.36	0.51	0.97	0.4	0.65	0.61						
Pb	0.58	0.51	0.71	0.43	0.46	0.54	0.83	0.44	0.46					
Sn	0.46	0.52	0.62	0.39	0.45	0.52	0.52	0.56	0.61	0.56				
Ti	0.54	0.69	0.53	0.38	0.38	0.59	0.56	0.53	0.48	0.56	0.56	1		
V	0.99	0.96	0.11	0.92	0.42	0.37	0.82	0.68	0.46	0.21	0.46	0.53	1	
Zn	0.02	0.31	0.74	0.38	0.52	0.46	0.56	0.44	0.48	0.62	0.56	0.69	0.21	1

2. Trace element enrichment factors

Table 17: Enrichment factors of the trace elements of interest for the 41 sites (first part)

Site	Al	As	Cd	Co	Cr	Cu	Fe
ARL10	0.44	12.59	57.69	0.95	5.9	19.33	1.35
ARL2	0.41	10.24	47.34	0.73	4.37	11.69	0.92
ARL8	0.35	7.63	31.35	0.68	3.79	8.98	0.85
BER4	0.42	4	4.45	0.66	1.81	1.94	0.57
CHA1	0.35	5.86	39.68	0.79	9.8	10.13	0.84
CHA11	0.41	10.3	110.87	1.68	39.81	14.79	3.16
CHA12	0.42	10.41	88.58	1.41	23.37	16.5	1.69
CHA14	0.46	14.99	111.51	1.66	16.32	15.87	1.79
CHA16	0.48	8.81	91.65	1.17	8.2	14.4	0.98

CHA5	0.46	10.29	52.69	1.01	13.17	12.97	1.04
HON1	0.47	9.35	45.71	1	3.22	8.2	0.71
HON2	0.36	5.49	19.75	0.51	1.92	4.15	0.5
HON3	0.37	5.36	18.78	0.58	2.7	3.73	0.58
HON4	0.46	7.86	27.46	0.8	2.96	7.66	0.67
LIE13	0.43	11.5	100.98	1.81	6.72	16.37	1.38
LIE14	0.52	22.47	96.42	1.73	7.01	15.51	1.95
LIE15	0.47	13.58	63.66	1.07	4.03	9.73	0.95
LIE16	0.46	15.28	127.54	2.9	11.56	13.92	3.86
LIE2	0.41	11.59	66.16	1.02	4.55	10.45	1.24
LIE5	0.38	10.06	175.32	1.5	5.99	8.5	1.74
MAR11	0.44	7.58	18.52	0.79	2.4	5.49	0.72
MAR14	0.42	8.81	41.29	0.7	3.23	12.81	0.86
MAR2	0.47	7.14	18.26	0.71	2.07	5.14	0.66
MAR3	0.48	8.44	28.86	0.87	3.38	13.35	0.83
MAR4	0.5	9.31	35.9	0.89	2.73	9.75	0.79
MAR5	0.43	7.13	17.03	0.68	2.1	4.16	0.65
NAM1	0.44	10.92	54.11	1.06	5.36	10.51	0.9
NAM10	0.42	6.52	33.91	0.83	6.38	6.46	0.65
NAM11	0.36	6.36	27.59	0.63	4.23	6.1	0.66
NAM14	0.43	6.01	23.39	0.73	3.87	5.31	0.66
NAM15	0.44	6.62	26.89	1.2	2.83	3.91	0.61
NAM18	0.45	7.68	66.54	0.93	6.63	16.54	1.15
NAM2	0.36	6.14	51.81	0.93	6.01	14.83	1.05
NAM5	0.36	6.33	54.95	0.81	4.42	14.19	0.93
NAM7	0.48	8.98	56.45	1.01	6.07	9.68	0.91
NAM8	0.54	13.89	38.81	1.17	4.36	9.97	0.94
ORP1	0.39	5.49	14.06	0.6	2.94	2.47	0.49
ORP2	0.42	6.78	23.05	0.65	2.5	5.75	0.55
ORP3	0.43	6.78	23.87	0.7	4.23	4.76	0.57
ORP4	0.35	4.53	9.41	0.48	2.22	2.07	0.44
VIE4	0.43	8.66	44.68	0.66	2.49	9.34	0.65

Table 18: Enrichment factors of the trace elements of interest for the 41 sites (second part)

Site	Mn	Ni	Pb	Sn	V	Zn
ARL10	1.97	2.54	4.97	27.07	1.28	29.33
ARL2	1.81	1.85	6.23	8.94	1.23	22.09
ARL8	1.16	1.46	3.27	16.29	0.96	12.96
BER4	0.86	1.25	0.99	1.28	0.73	3.49
CHA1	1.2	4.36	4.28	16.25	0.8	25.86
CHA11	4.62	18.75	24.16	12.32	1.56	62.26
CHA12	3.26	11.81	13.94	19.56	1.28	44.47
CHA14	2.59	11.71	33.87	9.53	1.76	49.45
CHA16	3.07	5.68	7.05	8.99	1.01	34.57
CHA5	2.47	6.77	6.83	7.6	1.06	25.09
HON1	1.43	2.16	8.24	5.13	1.08	15.95
HON2	0.66	1.05	3.24	3.22	0.68	7
HON3	0.86	1.26	2.64	3.89	0.73	6.13
HON4	1.5	1.93	4.37	3.72	1	15.72
LIE13	1.73	3.19	13.66	19.11	1	49.98
LIE14	2.45	3.95	17.57	14.93	1.5	39.84
LIE15	1.37	2.32	13.02	7.39	0.98	52.76
LIE16	8.05	5.78	30.39	8.89	2.3	79.45
LIE2	1.48	2.63	15.38	10.15	1.09	31.05
LIE5	1.43	2.89	14.59	9.4	1.06	44.67
MAR11	0.93	1.49	4.03	6.45	0.81	9.75
MAR14	1.12	1.62	3.62	18.9	0.79	46.3
MAR2	1.72	1.52	1.95	3.4	0.88	8.74
MAR3	1.3	1.9	3.42	5.19	0.91	14.61
MAR4	1.8	1.95	3.91	5.25	0.91	19.21
MAR5	0.75	1.32	3.85	5.18	0.84	10.88
NAM1	1.09	2.97	17.73	11.34	1.15	16.17
NAM10	1.22	2.82	3.81	6.58	0.82	12.13
NAM11	0.7	1.77	3.57	10.32	0.82	9
NAM14	0.78	1.66	2.68	7.17	0.81	8.96
NAM15	0.81	1.76	5.49	3.46	0.84	8.15
NAM18	1.11	2.5	4.36	40.1	0.92	14.34
NAM2	1.03	2.61	12.49	25.62	0.78	14.75

NAM5	0.9	1.97	8.7	31.11	0.76	15.82
NAM7	1.75	3.27	8.06	9.83	1.12	18.17
NAM8	1.58	2.39	12.63	5.89	1.24	14.53
ORP1	0.68	1.53	1.97	2.42	0.64	5.61
ORP2	0.96	1.45	3.26	3.29	0.71	13.08
ORP3	0.91	1.61	3.36	2.83	0.73	9.12
ORP4	0.55	1.19	1.36	2.64	0.56	3.53
VIE4	1.54	1.43	3.12	5.07	0.82	22.11

3. Pearson correlation between enrichment factors of trace elements and ΣEF

Table 19: Pearson correlation (r) between the enrichment factor of the trace elements and ΣEF

Element	As	Cd	Cr	Cu	Ni	Pb	Sn	Zn
r	0.695	0.956	0.688	0.777	0.708	0.845	0.443	0.914

4. Comparison of concentration between studies

Table 20: Comparison mean and standard deviation values of concentrations [$\mu\text{g}\cdot\text{g}^{-1}$] in trace elements for a polluted area (Çobanoğlu & Kaan, 2024), an unpolluted area (Bergamaschi et al., 2004) and a heterogeneous area (Agnan, 2017).

Element	Wallonia, Belgium (n = 122)	Burdur, Turkey (n = 140)	Khumbu, Nepal (n = 28)	France (n = 220)
	This study	Çobanoğlu & Kaan (2024)	Bergamaschi et al. (2004)	Agnan (2017)
Al	1523 ± 1066	1915 ± 1261		1175 ± 983
As	0.51 ± 0.22	1.60 ± 1.90	0.64 ± 0.47	0.50 ± 0.48
Cd	0.17 ± 0.12		0.04 ± 0.03	0.20 ± 0.26
Co	0.39 ± 0.21		0.82 ± 0.53	0.31 ± 0.21
Cr	7.64 ± 5.81	26.9 ± 24.1	2.50 ± 1.40	2.27 ± 2.05
Cu	8.76 ± 5.39	6.55 ± 1.00	5.90 ± 3.70	6.01 ± 2.71
Fe	1359 ± 748	2202 ± 1910	1512 ± 756	703 ± 562
Mn	33.3 ± 16.9	53.1 ± 32	55 ± 26	100 ± 126

Ni	2.19 ± 1.50	44.6 ± 52.8		1.66 ± 1.41
Pb	5.40 ± 3.69	5.66 ± 0.74	14.0 ± 3.9	5.21 ± 9.26
Sn	2.43 ± 2.93			0.49 ± 0.43
Ti	139 ± 105			87.9 ± 76.6
V	2.40 ± 1.31	5.88 ± 4.27	2.40 ± 1.20	2.36 ± 1.66
Zn	52.6 ± 31.1	32.4 ± 16.9	34 ± 13	37.1 ± 22.9

5. Thresholds proposed by Bargagli & Nimis (2002)

Table 21: Thresholds of naturalness and alteration proposed by Bargagli & Nimis (2002) in $[\mu\text{g}\cdot\text{g}^{-1}]$

Element	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Ti	V	Zn
Naturalness threshold	350	0.2	0.2	1.2	7	290	20	1	4	13	0.63	30
Alteration threshold	3200	3	2.6	16	53	1800	140	8	108	150	9.3	155

6. Thresholds for PLI and CF values

Table 22: Ranges of contamination factors (CF) and associated levels of contamination

CF Range	Contamination Level
0 - 1	No contamination
> 1 - 2	Suspected contamination
> 2 - 3.5	Slight contamination
> 3.5 - 8	Moderate contamination
> 8 - 27	Severe contamination
> 27	Extreme contamination

Table 23: Ranges of pollution load index (PLI) and associated interpretation of pollution

PLI Range	Interpretation
< 0.9	Unpolluted
~ 1	Close to natural background
1.1 - 1.5	Low pollution
1.5 - 2.0	Moderate pollution
> 2.5	Very high pollution

F. Correlations between the variables used in the CCA

Table 24: Spearman correlation between the geochemical indices (PLI and ΣEF), diversity metrics (LDV and Shannon's index), pollutants (NO_2 , O_3 , PM_{10}) and land use variables (LCA and %A50m)

	ΣEF	NO_2	PM_{10}	O_3	LDV	PLI	%A50m	LCA
ΣEF	1							
NO_2	0.745	1						
PM_{10}	0.685	0.890	1					
O_3	-0.745	-0.844	-0.742	1				
LDV	-0.156	-0.318	-0.234	0.184	1			
PLI	0.278	0.562	0.524	-0.411	-0.456	1		
%A50m	0.234	0.425	0.340	-0.293	-0.282	0.200	1	
LCA	-0.586	-0.404	-0.383	0.395	-0.087	0.107	0.217	1

G. Cartography

1. Maps

i. Σ EF map for each site

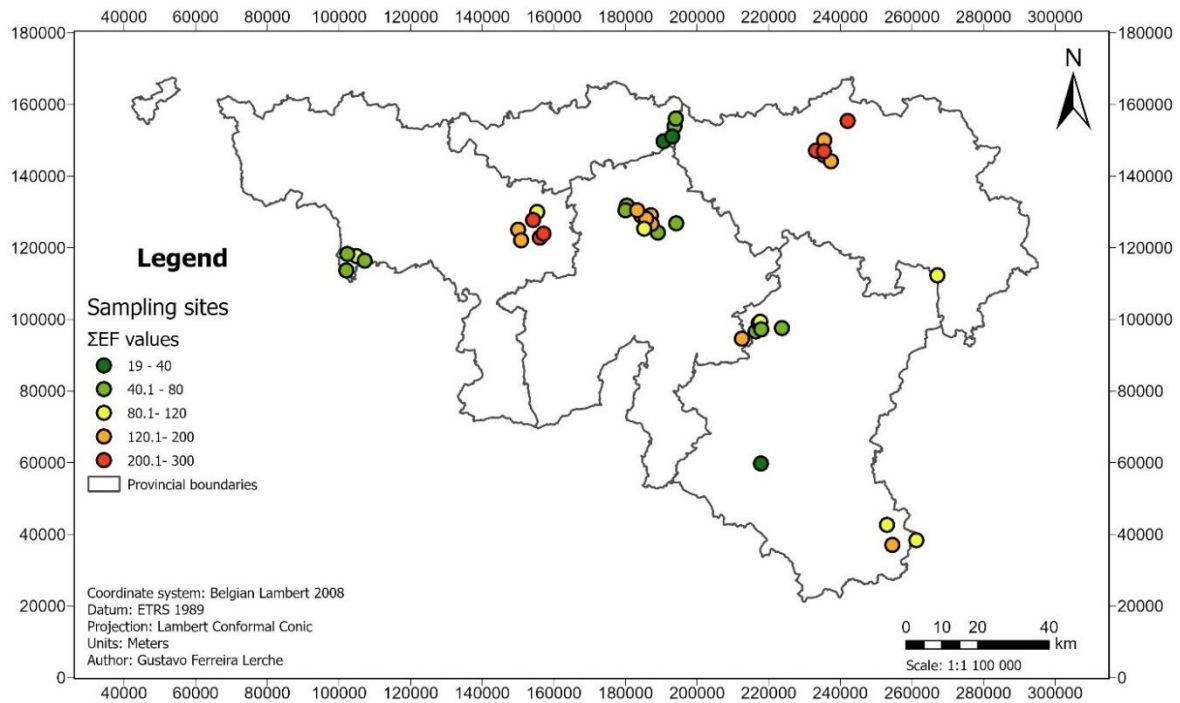


Figure 33: Spatial distribution of sampling sites and Σ EF values for each site

ii. PLI map for each site

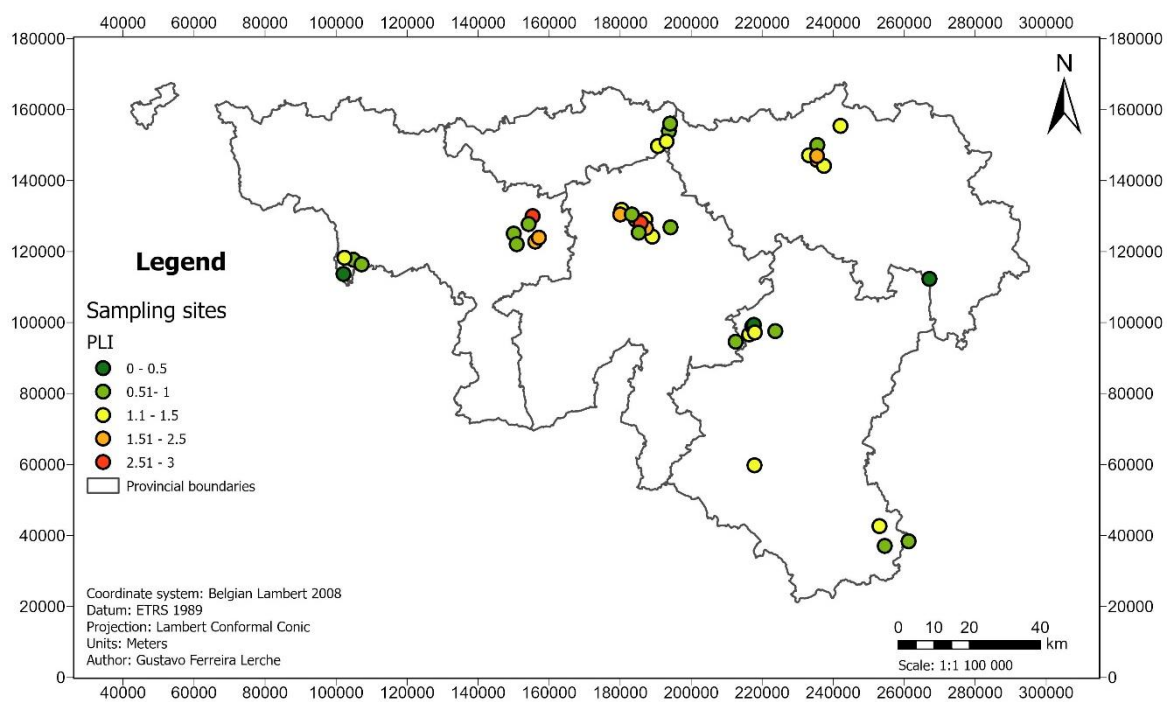


Figure 34: Spatial distribution of sampling sites and PLI values for each site

iii. NO₂ map for each site

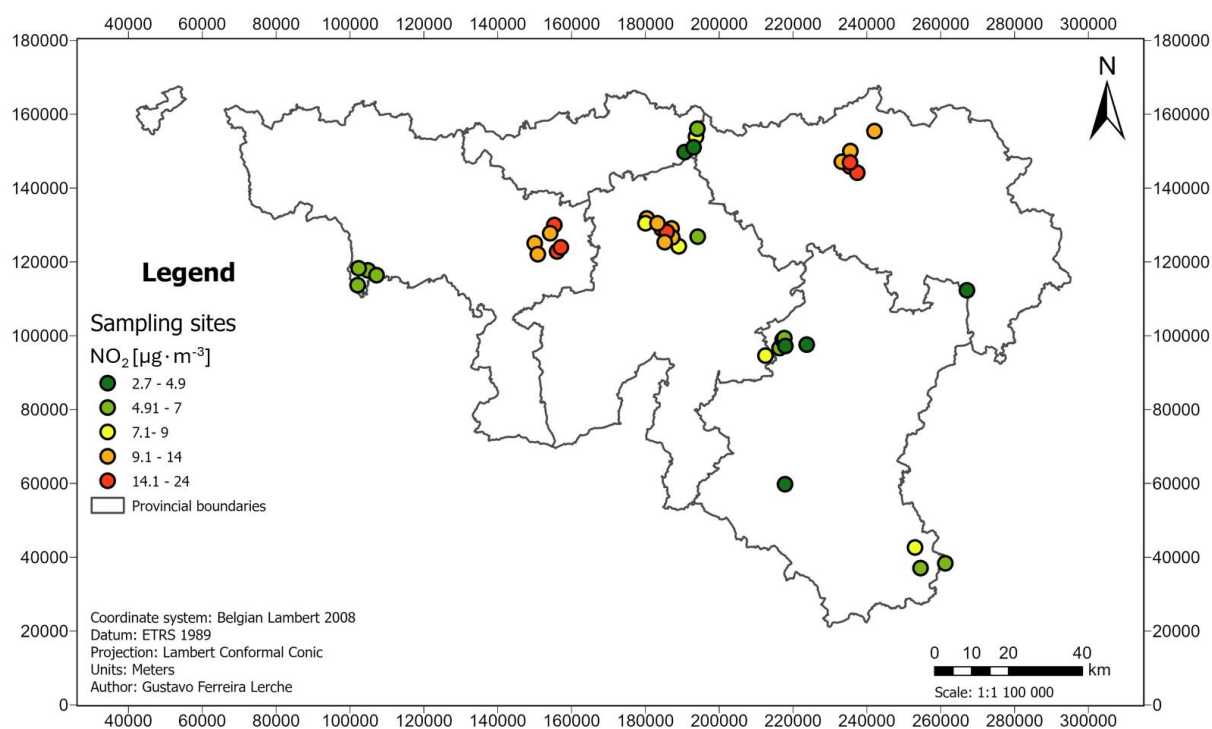


Figure 35: Spatial distribution of sampling sites and NO₂ values for each site

iv. PM₁₀ map for each site

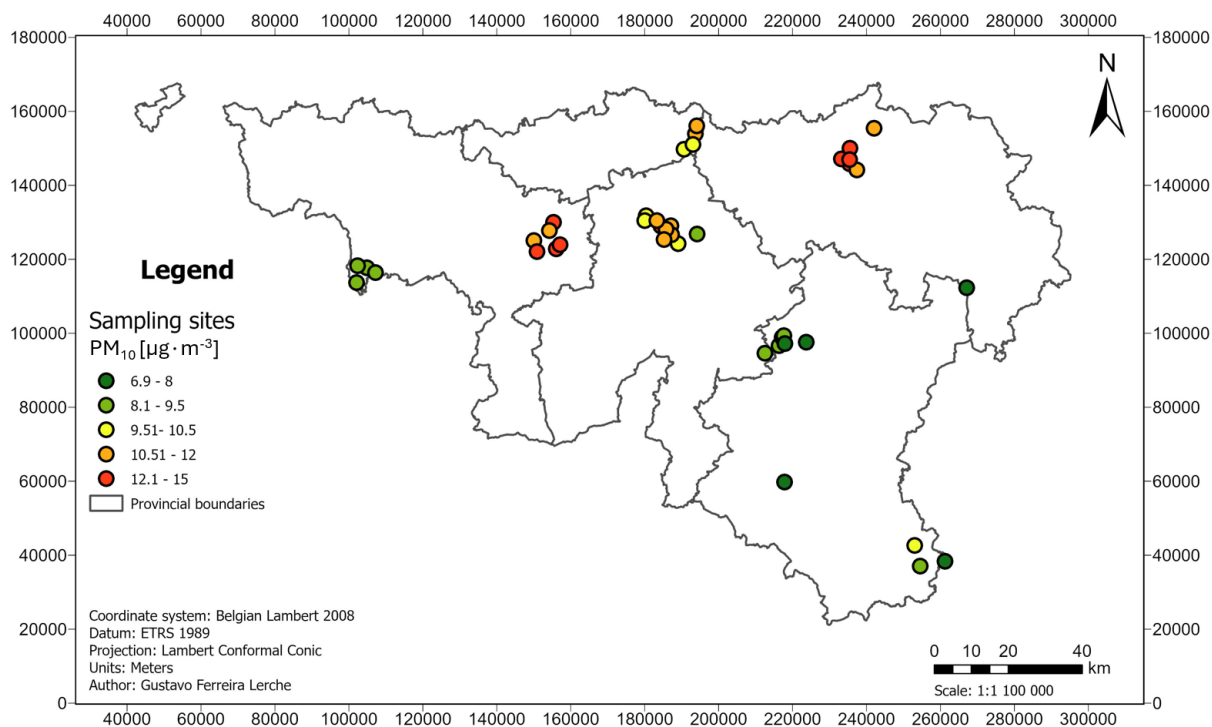


Figure 36: Spatial distribution of sampling sites and PM₁₀ values for each site

v. LDV map for each site

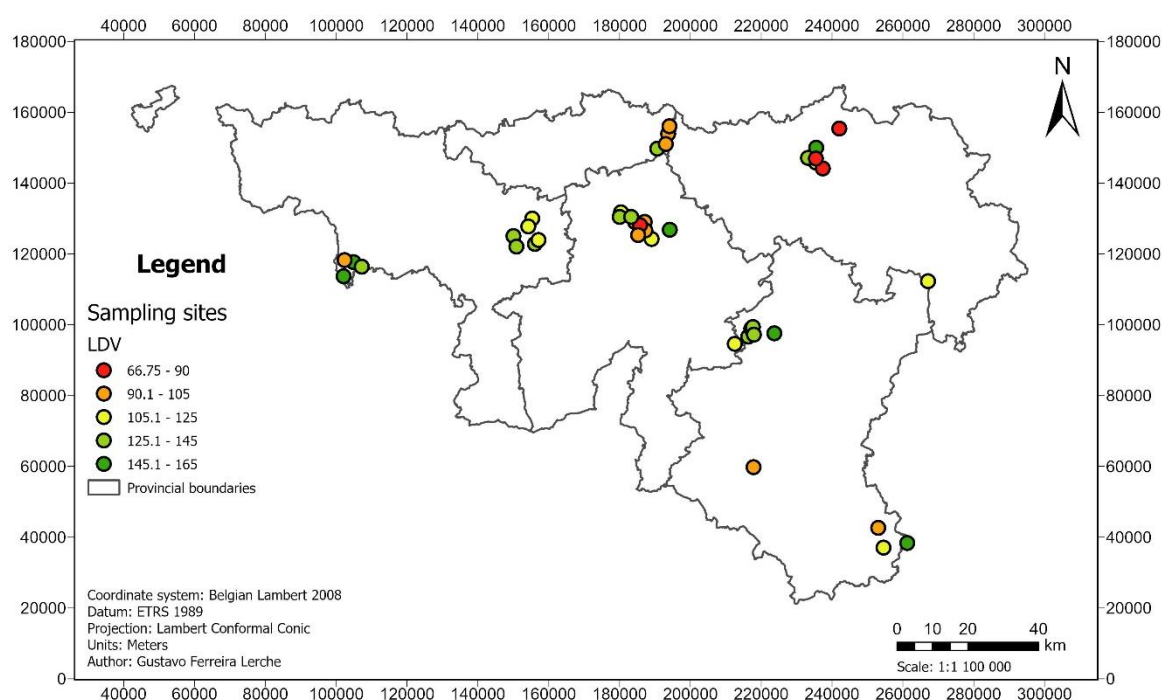


Figure 37: Spatial distribution of sampling sites and LDV values for each site

vi. Shannon's index map for each site

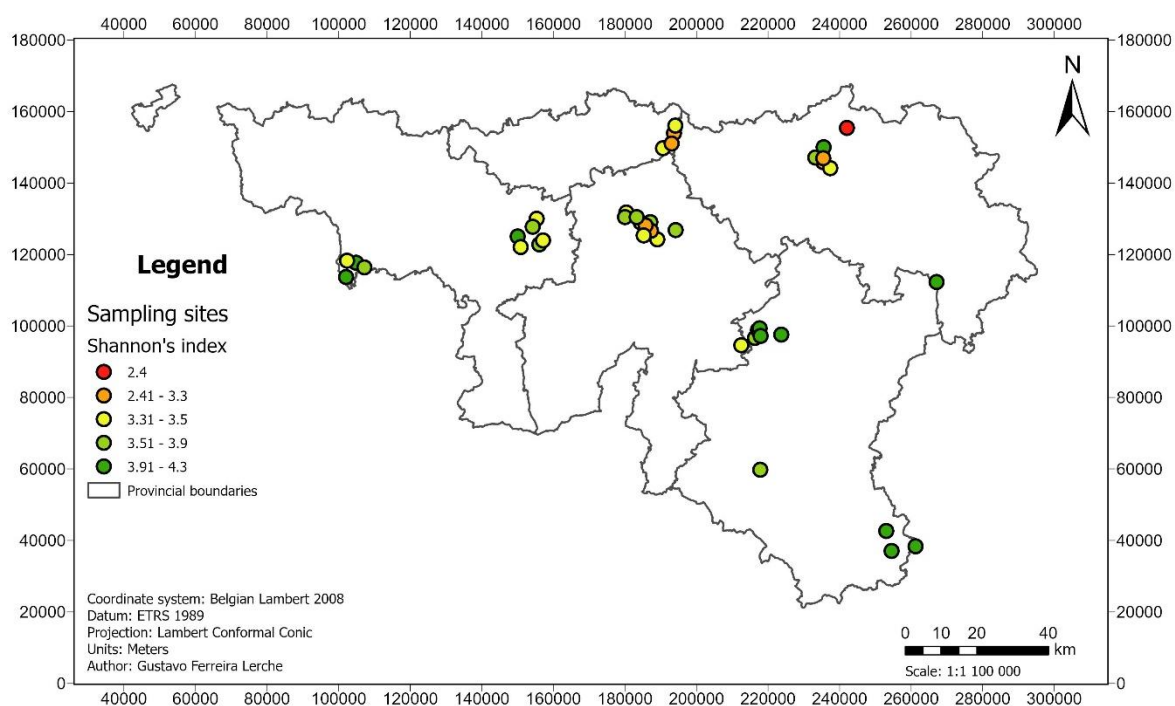


Figure 38: Spatial distribution of sampling sites and Shannon's index values for each site

2. Municipalities data

Table 25: Overview of land cover and demographics in cities within the study area

City	Artificialized [%]	Agricultural [%]	Forested [%]	Area of municipality [km ²]	Population density [hab./km ²]	Population of municipality [hab.]
Arlon	19.5	43	25.9	119.1	263	31 310
Bertrix	5	38.7	48.5	138.5	65.1	6,179
Charleroi	51.4	17.5	5.2	102.9	1984.7	204 322
Honnelles	7.3	79.5	8.3	44.3	116.9	5176
Liège	54.8	15.4	6.9	68.7	2851.8	195 778
Marche- en- Famenne	15	41.4	37	122.1	147.2	17 972
Namur	26.3	41.5	21.2	175.9	648.8	114 142
Orp-Jauche	9.8	82.2	2.9	50.8	179	9094
Vielsalm	5.1	34.6	51.6	140.7	56.4	7 940

Coupling biomonitoring methods to assess trace element deposition

A new interpretative scale of lichen sensitivity to trace element pollution

Gustavo Ferreira Lerche

Air pollution, particularly from trace elements (TEs), remains insufficiently characterised at regional scales, especially in urban contexts. Because conventional monitoring networks are limited in spatial coverage and mainly target other pollutants, complementary approaches are needed. This study uses lichens as biomonitors to evaluate TE pollution and build a species-level sensitivity scale.

The objective was to develop an empirical sensitivity scale based on the coupling of bioindication (species inventories and frequency analysis) and bioaccumulation (chemical quantification and geochemical indices). At 41 sites across Wallonia, lichen species were surveyed to calculate relative frequencies and alpha diversity indices (LDV, Shannon). In parallel, *Xanthoria parietina* thalli were sampled and analysed for 13 TEs using ICP-MS. Two geochemical indices were derived: the enrichment factor (EF) and the pollution load index (PLI).

Bioindication results showed that diversity decreased in urban and agricultural sites, while forested zones exhibited higher LDV and Shannon indices. NMDS ordination highlighted two dominant ecological gradients, influencing species assemblages. Although indicative of general environmental quality, community composition alone could not disentangle the specific role of TEs. Bioaccumulation revealed significant enrichment for Cd, Zn and Pb, pointing to anthropogenic sources, while Al and Fe had lower enrichment, consistent with lithogenic inputs. Σ EF and PLI captured different pollution signatures: Σ EF reflects cumulative anthropogenic enrichment; PLI relates more directly to toxic pressure and overall pollution. PCA confirmed the presence of three distinct geochemical groups (anthropogenic, lithogenic, mixed).

The integration of both approaches in a partial CCA demonstrated that Σ EF had a significant independent effect on species frequency, allowing the derivation of a sensitivity scale. Species were classified as tolerant intermediate or sensitive. Nitrogen-based analyses showed only partial overlap, suggesting that TE sensitivity is a distinct ecological trait. Despite limitations (co-variation with NO₂, limited specificity for some taxa and lack of seasonal replication) this study achieves its aim: establishing a robust, field-based sensitivity scale to TEs in lichen communities.

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