

“Spatial And Temporal Assessment of Atmospheric Nitrogen Using Hypnum Cupressiforme Mosses and Kjeldahl Analysis”

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ملخص الدراسة:

هدفت هذه الدراسة إلى التعرف إلى خبرات القيادات النسائية CALPUFF تمت دراسة محتوى النيتروجين في الحزازيات في منطقة شبه ريفية في لبنان. واستُخدم نموذج انتشار الملوثات لتقدير متوسط التركيزات السنوية لأكاسيد النيتروجين والأمونيا. أجريت ثلاث حملات لأخذ العينات على مدار عام واحد، جُمعت خلالها 33 عينة من الحزازية المدروسة خلال كل حملة. استُخدمت طريقة كيدال لتحديد المحتوى الكلي للنيتروجين في العينات المجمعة. تم تقييم محتوى النيتروجين في الحزازيات مكانياً وزمنياً كما تم تقييم العلاقات الإحصائية بين محتوى النيتروجين وتركيزات أكاسيد النيتروجين والأمونيا المقدرة بواسطة النموذج، باستخدام معامل ارتباط سبيرمان. أظهرت الأنماط المكانية بوضوح أعلى نسب للنيتروجين في الحزازيات في الأجزاء الغربية من منطقة الدراسة، بالقرب من مصادر التلوث الثابتة، والتي ارتبطت بأعلى التركيزات المقدرة لأكاسيد النيتروجين والأمونيا. في المقابل، لوحظ أدنى محتوى للنيتروجين في الحزازيات في الجزء الشرقي من منطقة الدراسة، الذي تميز بأدنى تركيزات أكاسيد النيتروجين والأمونيا. وأظهر التحليل الزمني ارتفاعاً في نسب النيتروجين خلال الموسم الرطب. كما أظهر معامل ارتباط سبيرمان علاقات ارتباط أقوى خلال المواسم الجافة.

Abstract:

This study aimed to explore the experiences of Palestinian Nitrogen content in mosses was investigated in a semi-rural area in Lebanon. CALPUFF dispersion model was used to estimate the annual average NO_x and NH_3 concentrations. Three sampling campaigns were conducted over the course of a year where 33 samples of the moss *Hypnum cupressiforme* were collected during each campaign. Kjeldahl method was used to determine the total nitrogen content in the collected samples. N content in mosses was spatially and temporally assessed. Statistical relationships between N content and the modeled NO_x and NH_3 concentrations was evaluated using spearman correlation coefficient. Spatial patterns clearly show the highest N percentages in the western parts of the study area near the stationary pollution sources associated to the highest NO_x and NH_3 modeled concentrations and the lowest N content was observed in the eastern part characterized by the lowest NO_x and NH_3 concentrations. Temporal analysis shows an increase in N percentages during the wet season. Spearman coefficient shows higher relationships during the dry seasons.

Keywords:

Biomonitoring · Mosses · Nitrogen · Kjeldahl analysis · Correlation · spatial distribution.

الكلمات المفتاحية: الرصد الحيوي · الحزازيات · النيتروجين · تحليل
 كيدال · الارتباط · التوزيع المكاني.

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Introduction:

The nitrogen cycle is an essential biogeochemical process that plays a significant role in the movement and transformation of nitrogen within the environment (Galloway, 2005).

Anthropogenic activities (agriculture, livestock farming, industrial activities, energy production, and transportation) have disrupted the nitrogen cycle over the years and have led to an increase in bioavailable nitrogen levels in ecosystems (Galloway et al., 2003; Varela et al., 2016). This increase in nitrogen concentrations constitutes a major threat to human health such as respiratory and cardiovascular problems, and can lead to adverse environmental consequences such as imbalance in the composition of air, soil and surface waters, acidification effects on vegetation, ecosystems and buildings, the formation of tropospheric ozone and fine particles and a decrease in biodiversity (Townsend et al., 2003). Nitrogen is deposited through dry or wet deposition, or as droplets from clouds and fog. NO_x and NH₃ are considered the most significant reactive species. The former originates mainly from combustion, industry, road traffic, and households. The latter originates mainly from agriculture (Vallero, 2007). Implementing monitoring programs is thus imperative to effectively assess the effects of atmospheric nitrogen deposition.

Numerous techniques are used for assessing air quality; conventional air quality analysis devices are the most widely used globally, yet they present several challenges. They fail to provide information on the transfer of pollutants to final receiving receptor - such as water, soil, and plants - and they're known for their high costs that can limit the number of sampling sites. These techniques require complex sampling procedures (such as the continuous need for personnel to replace filters) and sophisticated equipment (Markert et al., 2003). This raises issues regarding the spatial representativeness of such monitoring systems.

The analysis of contaminants present in atmospheric deposition is facilitated by the bioaccumulation approach. Developed in the 1960s, plant-based air quality biomonitoring offers a strong alternative through the use of biological indicators (Markert et al., 1996). Its primary objective is to assess pollutant quantities and dispersion within selected plant species. Consequently, it provides qualitative or semi-quantitative spatio-temporal data over extended periods at low cost and without the need for complex equipment or sampling procedures (Conti, 2008).

Lebanon lacks a truly functional measurement network due to a multitude of problems, particularly financial ones. But air quality monitoring is a must due to its direct influence on human health. In this case biomonitoring is a less expensive alternative that can be used to study air pollution.

Bryophytes are highly sensitive bioindicators for a wide range of atmospheric pollutants; they respond sensitively to human-induced environmental changes, and this can be effectively utilized for monitoring purposes. They offer valuable advantages for the assessment of organic xenobiotics, as they can accumulate large quantities of these compounds in their tissues. They can be used to integrate deposition over a considerable period depending on the species and sampling methods (Gworek & Dmuchowski, 2023).

Mosses are used in monitoring programs as indicators of nitrogen air pollution. They constitute a relevant indicator of atmospheric nitrogen levels (Deluca, 2007; Díaz-álvarez et al., 2018). This is due to several characteristics: mosses largely rely on the atmosphere for their nutrients because they do not possess a true vascular system. Their cell walls are easily penetrable due to the absence of an epidermis and cuticle, resulting in an increased surface-to-volume ratio (Glime, 2017;

Solga & Frahm, 2006). In addition, moss species have the ability to live in various natural habitats and to grow on various substrates such as rock, soil and decomposing organic matter using rhizoids for anchorage (Markert et al., 2003).

Two types of biomonitoring studies are generally presented; studies that focus on the general spatial distribution of pollutants in a vast area and those that study pollution derived from a stationary source of emission such as industrial facilities (Markert, 2007).

The aim of this study was to determine if the nitrogen content in *Hypnum cupressiforme* mosses reflects the spatial dispersion of modelled atmospheric nitrogen concentrations around a stationary pollution source in 2 different seasons (dry and rainy) and over the course of a year.

The hypotheses of the study are: a. decrease in N content in moss samples with increasing distance from stationary pollution sources and b. Higher N content in moss samples during the rainy season due to higher water availability.

Study area

The study area is a semi-rural area located southeast of the capital, Beirut (Fig.1). This region is home to the oldest thermal power plant in Lebanon, located on the coast, 28 kilometers south of Beirut. This plant is fueled by heavy fuel oil (HFO), which generates considerable emissions of air pollutants, especially nitrogen oxides, the emissions of which exceed established air quality standards (Apave, 2017). The region also hosts private diesel generators scattered as community-initiated solutions as informal and decentralized energy systems to compensate for the power grid outage. The cement plant located southeast of the thermal power plant and road transport, especially the highway along the coast, are additional sources of nitrogen in the region.

The study area is characterized by a mediterranean climate with cold windy winters and hot and dry summers. The prevailing winds originating from the southwest and blowing from the Mediterranean Sea influence the region throughout the year. The area is characterized by a daily alternation of sea and land breezes (Chebbo et al., 2026).

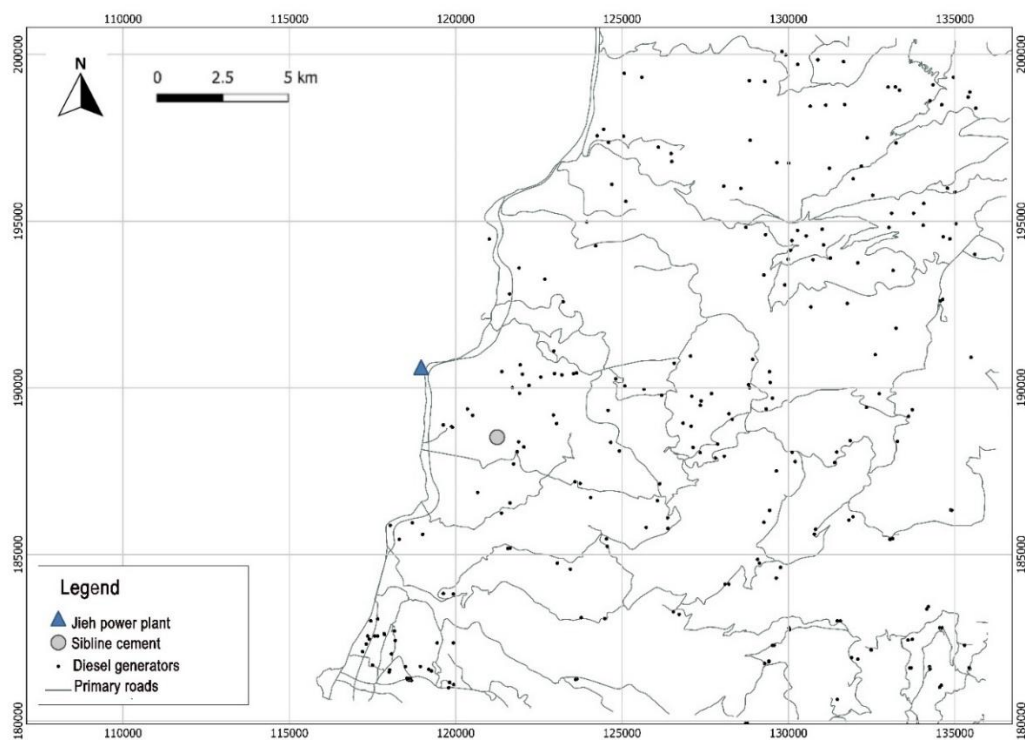


Figure 1 Sources of pollution in the study area

Methodology

Modeling air quality and selecting sampling sites

There is lack of air quality measurement networks in the study area. Thus, the pollution dispersion patterns were predicted by using dispersion models (Hao et al., 2007). Data collection campaigns were launched to develop an emission inventory of NO_x and NH_3 emissions in the study area.

The emission data was intruded into the California Puff Model (CALPUFF). CALPUFF is a certificated software recommended by environmental agencies for studying pollutant dispersion (Rola et al., 2016). As described by Lee et al., 2014, CALPUFF is a non-steady state, Gaussian puff dispersion model, recommended by the Environmental Protection Agency (EPA) for simulating long distance transport. It operates using the Lagrangian coordinate system and takes into account the geophysical data, as well as the temporal and spatial variability of meteorological conditions in three dimensions. Using meteorology that varies in time and space and is based on similarity equations, this multi-layer model is intended to study the dispersion of gases and particles.

The model results provide the annual average ground level NO_x and NH_3 concentration in a 1 km resolution grid. The degree of representativeness of the predictions was ascertained by comparing the model predictions with observed air quality data from ambient air quality stations located in the study area as well as with the measurements reported by the Aeroqual Series 500 (S500) portable air quality monitor utilized during sampling. Additionally, a model sensitivity test was conducted. Based on these results, 12 sampling sites were selected for moss material collection.

Sampling

The sampling of mosses followed the guidelines of the International Cooperative Programme on the Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation, 2020). Three sampling campaigns were conducted at each site over the course of a year to analyze the nitrogen content in the mosses. 33 samples per campaign of the moss *Hypnum cupressiforme* were collected in May and November 2023, symbolizing the dry and wet seasons respectively, and a further collection in May 2024 to assess the seasonal and annual variability of nitrogen content in the mosses. Samples were taken from a control site located in the Masser El Chouf nature reserve, situated outside the study area and far from any source of pollution. Sampling points were maintained in the same locations throughout the three campaigns. Each campaign took place over the shortest possible timeframe.

Each sample consists of 3 to 6 composite samples. Sampling took place in open areas away from the canopy; in wooded habitats, sampling was located at a distance of at least twice the height of the nearest tree. Sampling was avoided near bushes and slopes and was conducted at least 300 meters from main roads and 100 meters from smaller roads. Sandy cushions were avoided. Coarse contamination of samples by litter and dead matter was eliminated in the field. Samples were collected using disposable, talc-free plastic gloves with a plastic spatula and transported in polyethylene bags.

Sample preparation and Kjeldahl analysis

Samples were handled on clean glass plates using talc-free disposable gloves. Metal tools were avoided. The samples were cleared of dead material and attached debris. Only the green moss shoots were analyzed. The green shoots were dried without washing at 40 °C until constant weight, homogenized in an agate mortar, then sieved.

Kjeldahl method was used to determine the total N content in mosses. 0.5 g of moss samples were placed in a Kjeldahl tube with one Kjeldahl tablet-catalyst. Then 10 mL of concentrated H_2SO_4 (Merck, Germany) were added. Kjeldahl tube was placed then in the digestion unit. Samples were left to cool to room temperature after digestion. The sample is then diluted with 40 mL of demineralized water added slowly to avoid crystallization and then transferred to the distillation unit. 30 mL of NaOH (33%, m/V) were added in the Kjeldahl tube. The distillate was collected in a flask containing 30 mL of boric acid H_3BO_3 (4%, m/V). An acid-base titration is then carried out using a 0.1 mol. L^{-1} hydrochloric acid HCl standard solution until the solution has a slightly violet color.

To ensure the quality control certified reference material and laboratory standards were used with one reference sample analyzed for every ten moss samples. Samples were analyzed in duplicates. The values reported are averages of these measurements. With each batch of prepared samples, a blank sample was created using demineralized water instead of the actual sample. The recovery percentage for all samples ranged from 99.5% to 100%. Nitrogen concentrations are expressed as a percentage based on dry weight.

Statistical analysis and mapping

To study the pattern and strength of the statistical relationships between N content in *Hypnum cupressiforme* and the modeled NO_x and NH_3 concentrations IBM SPSS software was used to calculate bivariate correlation coefficients. Spearman rank correlation coefficient (r_s) was used for the evaluation. Correlations between variables were classified according to Schober et al. (2018); the correlation coefficient values are quite low when $r_s < |0.1|$, low ranging from $|0.1|$ to $|0.39|$, moderate between $|0.4|$ and $|0.69|$, high from $|0.7|$ to $|0.89|$ and very high when $r_s > |0.9|$.

The spatial distribution of N content in mosses is presented using a geographic information system (QGIS desktop application). The basic grid cell size for maps presenting the annual average concentrations of NO_x and NH_3 was 1 km x 1 km.

Results

NO_x and NH_3 modeled maps

Figures 2 and 3 show the spatial dispersion of modeled NO_x and NH_3 concentrations in the study area.

The modeled concentrations range between a maximum of $42.44 \mu\text{g.m}^{-3}$ and a minimum of $3.4 \mu\text{g.m}^{-3}$ for NO_x (Fig.2) and between $4.7 \mu\text{g.m}^{-3}$ and of $0.06 \mu\text{g.m}^{-3}$ for NH_3 (Fig.3). These concentrations are represented by a color gradient scale from dark red (heavily polluted areas) to dark green (less polluted areas).

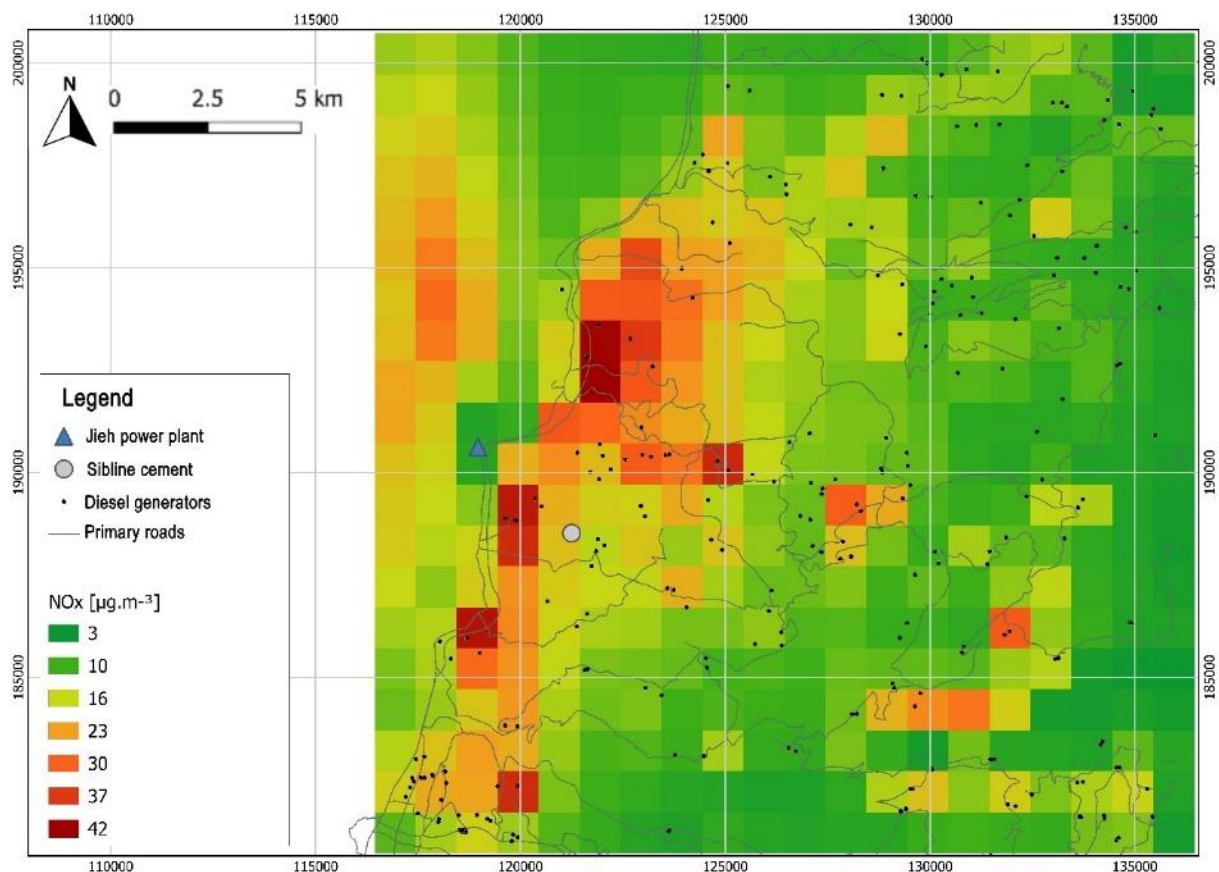


Figure 2 Spatial dispersion of modeled annual average NO_x concentrations

The highest concentrations (areas in dark red) are located in the west and southwest part of the study area ($[\text{NO}_x] \geq 37 \mu\text{g.m}^{-3}$ and $[\text{NH}_3] > 3 \mu\text{g.m}^{-3}$). These areas are characterized by the presence of the power plant, the cement industry and the highway that extends along the coastal strip.

In the central region of the study area high to moderate concentrations of pollution foci are visible in a more dispersed way: the predicted concentrations fluctuate between $16 \mu\text{g.m}^{-3}$ and $36 \mu\text{g.m}^{-3}$ for NO_x and between $1 \mu\text{g.m}^{-3}$ and $3 \mu\text{g.m}^{-3}$ for NH_3 . Although these areas and some areas located in the southeast and northeast are further from the main sources of pollution, they show localized peaks of pollution.

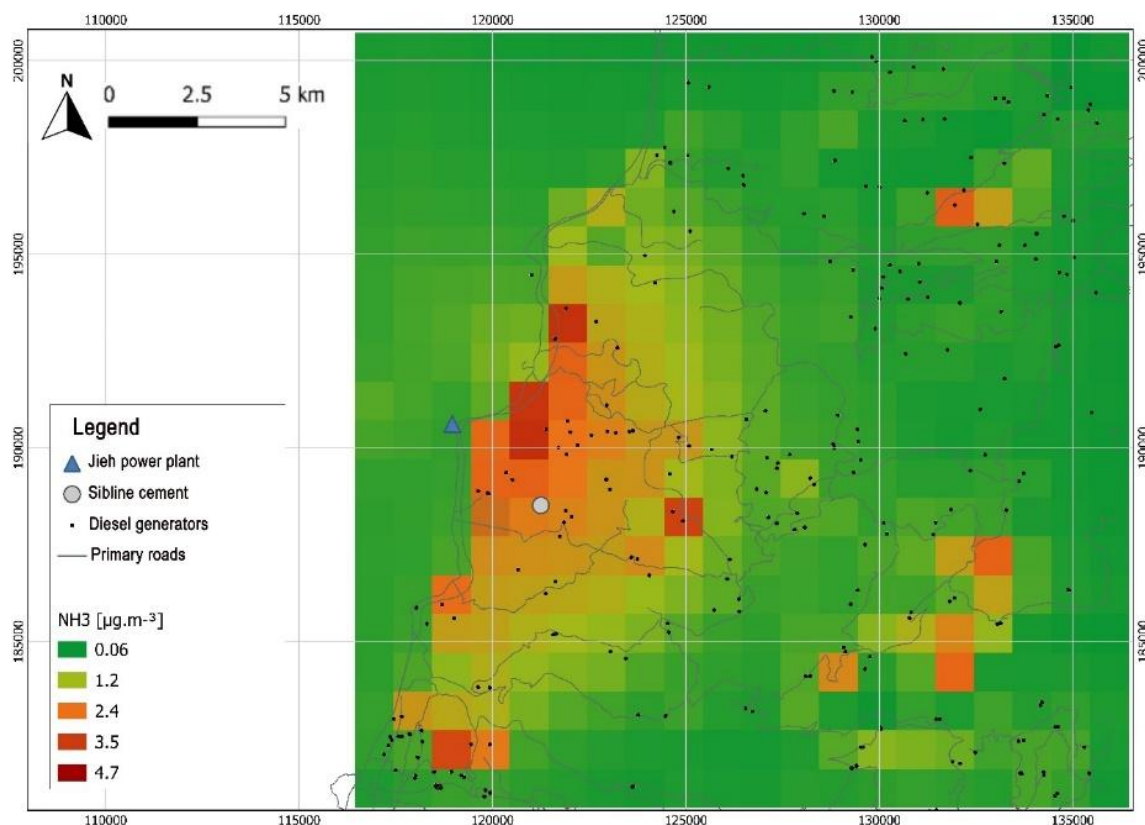


Figure 3 Spatial dispersion of modeled annual average NH_3 concentrations

This could be due to the high density of private diesel generators used frequently by the community to compensate for the power outage, especially in semi-urban areas, the presence of a dense road network with primary roads passing through some villages, or the effect of complex topography favoring the stagnation of emissions in certain valleys or enclosed areas.

The lowest concentrations of NO_x ($< 16 \mu\text{g.m}^{-3}$) and NH_3 ($< 1 \mu\text{g.m}^{-3}$) appear in the eastern part of the study area (peripheral areas in light to dark green). This is due to the rural nature of this area and the absence of major industrial sources.

Descriptive statistics

Descriptive statistics of the analysis of N percentage in samples of *Hypnum cupressiforme* collected during the three sampling campaigns are presented in Table 1.

Table 1 Descriptive statistics of the analysis of nitrogen content in moss samples during the three campaigns (results are given in %)

Campaign	N	M	Md	Min.	Max.	P10	P90	V	S	A	E
C ₁	33	1.57	1.55	0.84	2.35	1.19	2.13	0.12	0.34	0.45	0.12
C ₂	33	1.67	1.64	1.33	2.34	1.38	2.10	0.07	0.26	0.82	-0.18
C ₃	33	1.51	1.54	0.97	1.92	1.1	1.89	0.07	0.27	-0.26	-0.88

N: number of moss samples; M: mean; Md: median; Min.: minimum; Max.: maximum; P₁₀: 10 percentile; P₉₀: 90 percentile; V: variance; S: standard deviation; A: skewness; E: kurtosis.

The range of variation in nitrogen content during the dry season (C_1) is between 0.84% and 2.35%, while it is ranging from 1.33% to 2.34% during the wet season (C_2). The average nitrogen content is slightly higher during the wet season, at 1.67% and a median of 1.64%, compared to the dry season where the average content is 1.57% and the median is 1.55%. The increase in average nitrogen content between the two seasons could be due to increased nitrogen uptake by moss tissues due to high levels of water and humidity during the wet season.

Greater variability in nitrogen levels is observed during the dry season, with a standard deviation of 0.34 and a variance of 0.12, compared to 0.26 and 0.07 respectively for the wet season. This could be explained by environmental stressors or limited nutrient uptake due to reduced moisture availability.

These results reveal a seasonal influence on moss nitrogen content, with slightly higher and more homogeneous levels during the rainy season. These findings are consistent with previous studies (Fernández et al., 2015; Jovan et al., 2021; Meyer et al., 2015). The increased nitrogen content and reduced variability during the wet season indicate that periods of heavy rainfall promote atmospheric nitrogen availability and contribute to greater accumulation in moss tissues.

The annual comparison between the third campaign (C_3) and the first campaign (C_1) reflects a minimal annual decrease in nitrogen percentages: the mean and median values decrease from C_1 to C_3 from 1.57% and 1.55% to 1.51% and 1.54%, respectively, indicating a moderate decline in the central trend.

The standard deviation decreases slightly between C_1 and C_3 from 0.34 to 0.27 respectively, and the variance from 0.12 to 0.07. This decrease reflects a reduction in the variability of nitrogen percentages.

Spearman correlations

The correlation coefficient between N content in mosses (%) during the three campaigns and the modeled concentrations of NO_x and NH_3 are shown in Table 2.

Table 2 Spearman correlations between N content in mosses (%) and the modeled annual average concentrations of NO_x and NH_3

Correlation of N content (%)	r (spearman)					
	Campaign 1 (Dry season)		Campaign 2 (Rainy season)		Campaign 3 (Dry season)	
	r	p	r	p	r	p
$[\text{NO}_x]_{\text{Modeled}}$	0.86**	0.001	0.45**	0.01	0.64**	0.001
$[\text{NH}_3]_{\text{Modeled}}$	0.7**	0.001	0.49**	0.006	0.62**	0.001

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations between N content in collected mosses and the modeled annual average concentrations of NO_x are particularly high during the dry seasons ($r = 0.86$; $p < 0.01$ during C_1 and $r = 0.64$; $p < 0.01$ during C_3). These values reflect the impact of NO_x emissions on the nitrogen accumulation in mosses. These correlations are moderate during the rainy season ($r = 0.45$; $p = 0.01$).

Correlations between the modeled annual average concentrations of NH_3 and N content are high during C_1 ($r = 0.7$; $p < 0.01$), moderate to high during C_3 ($r = 0.62$; $p < 0.01$) and moderate during the rainy season ($r = 0.49$; $p = 0.006$). These results are correlated with the atmospheric dynamics that characterizes the study area that promotes the stagnation of pollutants during the dry season and promotes their dispersion during the rainy season by southwesterly winds coming mainly from the mediterranean sea and by the precipitation gradients differences between sampling sites characterized by topographic complexity which can weaken the correlations between the nitrogen content in mosses and the modeled concentrations.

Spatial and temporal analysis of N content in mosses

Figures 4, 5 and 6 show the spatial distribution of the total nitrogen (%) accumulated in *Hypnum cupressiforme* during the campaigns C_1 , C_2 and C_3 respectively.

Spatial analysis indicates a notable variability in nitrogen percentages among the 14 sampling sites. Some sites show relatively high concentrations, suggesting significant exposure to nitrogen deposition, while others show lower levels: the highest N percentages are observed in sites close to pollution sources, notably Jieh power plant and the Sibline cement plant where N percentages in moss tissues can reach up to 2.35%. These highest N percentages correspond to the highest NO_x and NH₃ modeled concentrations (Fig.4-5) where the concentrations of NO_x and NH₃ are greater than 20 µg.m⁻³ and 1 µg.m⁻³ respectively).

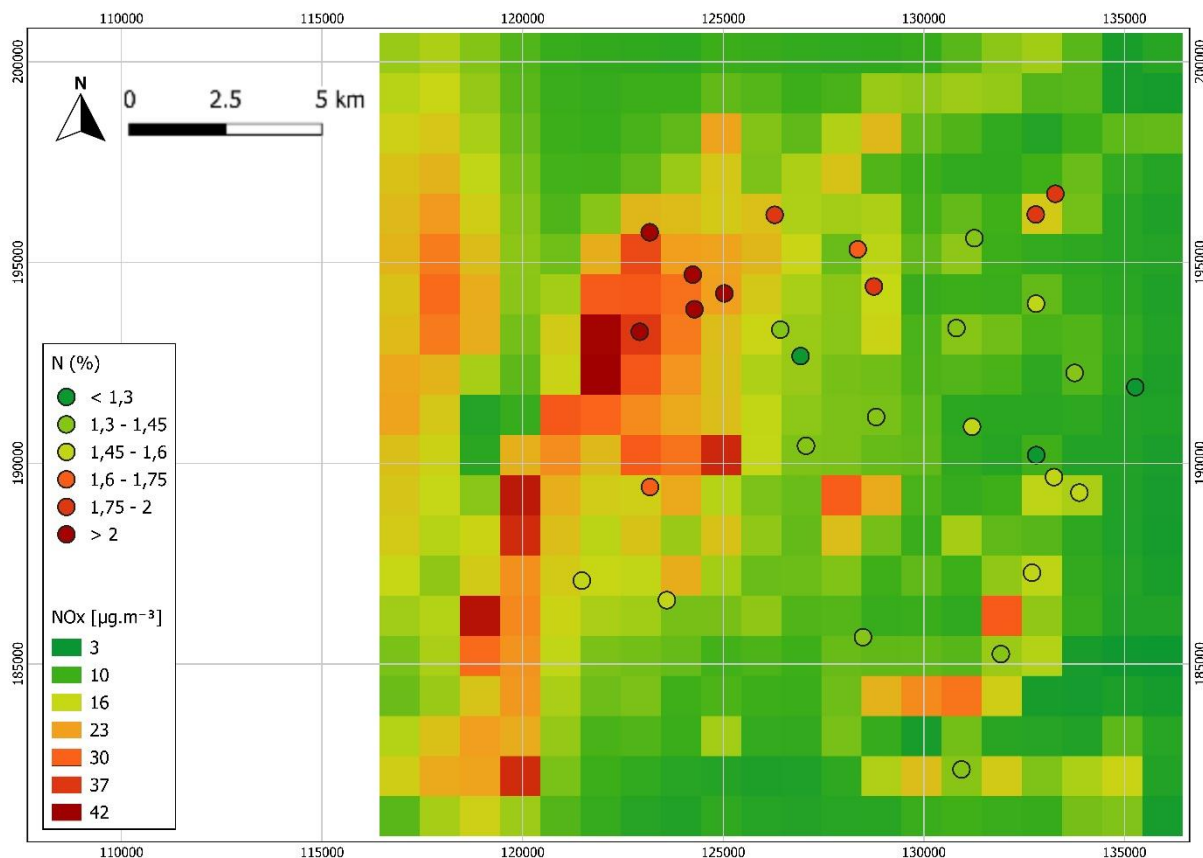


Figure 4 Spatial distribution of N content in mosses (%) during C₁

The lowest percentages are observed in the most distant sites, such as the Maasser El Chouf nature reserve, which is a control site located outside the study area and the furthest from pollution sources, where N percentages in mosses are the lowest (< 1.5%). These results are found in sites characterized by the lowest NO_x and NH₃ concentrations which confirm the modeled maps.

The temporal analysis shows moderate to high percentages of nitrogen at the majority of sites in the first campaign (Fig. 4) with sites characterized by percentages greater than 2% in the most polluted areas, especially in the western part near the main stationary sources of pollution (dark red).

During the wet season (campaign 2), a significant increase in the number of sites with high nitrogen percentages (> 1.75%) was observed (Fig. 5), including the areas less exposed during the first campaign. This increase is explained by a rise in the solubility of nitrogen compounds in the atmosphere and their facilitated absorption by mosses due to increased water availability during the wet season through rainfall or leaching.

These results are consistent with previous studies that have identified rainfall as a significant factor causing increased pollutant concentrations in moss tissues (Fernández et al., 2015; Ross, 1990; Steinnnes, 1989).

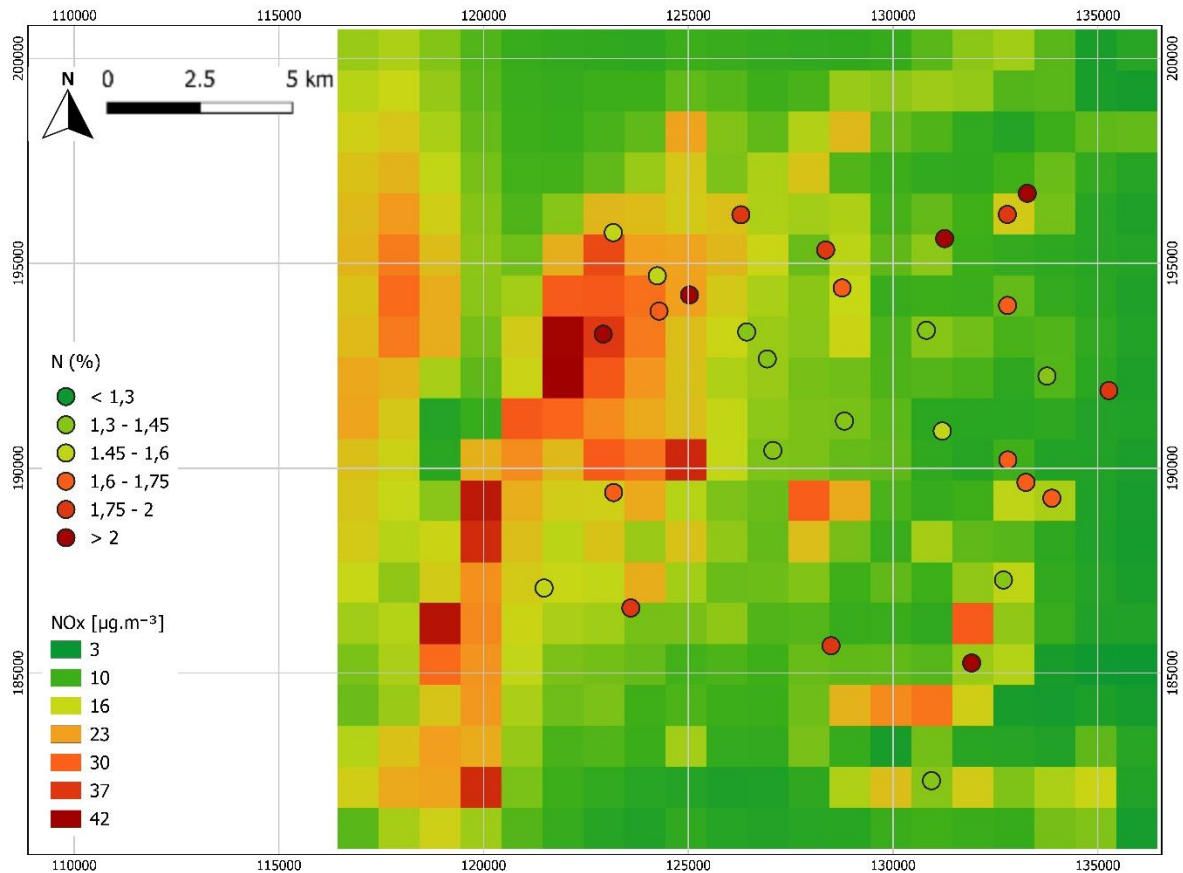


Figure 5 Spatial distribution of N content in mosses (%) during C₂

Furthermore, the distribution of N values during C₂ appears more heterogeneous than during C₁, where the nitrogen percentages are distributed in a less structured way, whereas during C₁ a decreasing percentage gradient is observed with increasing distance from the pollution sources. This difference can be attributed either to the difference in precipitation gradients between sites, which can influence the canopy drip and deposition of atmospheric nitrogen between sites, or to the prevailing wind patterns during the wet season, which can modify the dispersion of atmospheric nitrogen. These factors explain the moderate correlations of nitrogen content with NO_x and NH₃ during the wet season (Table 2).

Campaign 3 (Fig.6) shows a general decrease in nitrogen percentages in moss tissues, with a slight decrease in the number of sites highly enriched in nitrogen (>2%), reflecting a decrease in atmospheric nitrogen pollutant concentrations. This could be explained by a decrease in industrial emissions in the study area or by interannual variability in deposition conditions. Another explanation for this decrease in C₃ percentages is the reduction in the number of private diesel generators scattered throughout the study area, considered a significant source of NO_x emissions. In recent years, Lebanon has seen a growing adoption of domestic photovoltaic systems due to rising fuel costs. Consequently, a significant portion of the population has turned to these systems as a sustainable alternative. This could explain the decrease in nitrogen bioaccumulated by mosses during the third campaign. These seasonal differences demonstrate the sensitivity of mosses to temporal variations in nitrogen atmospheric pollution, and reinforce their bioindicator character and suggest an ability of tissues to adapt to environmental conditions.

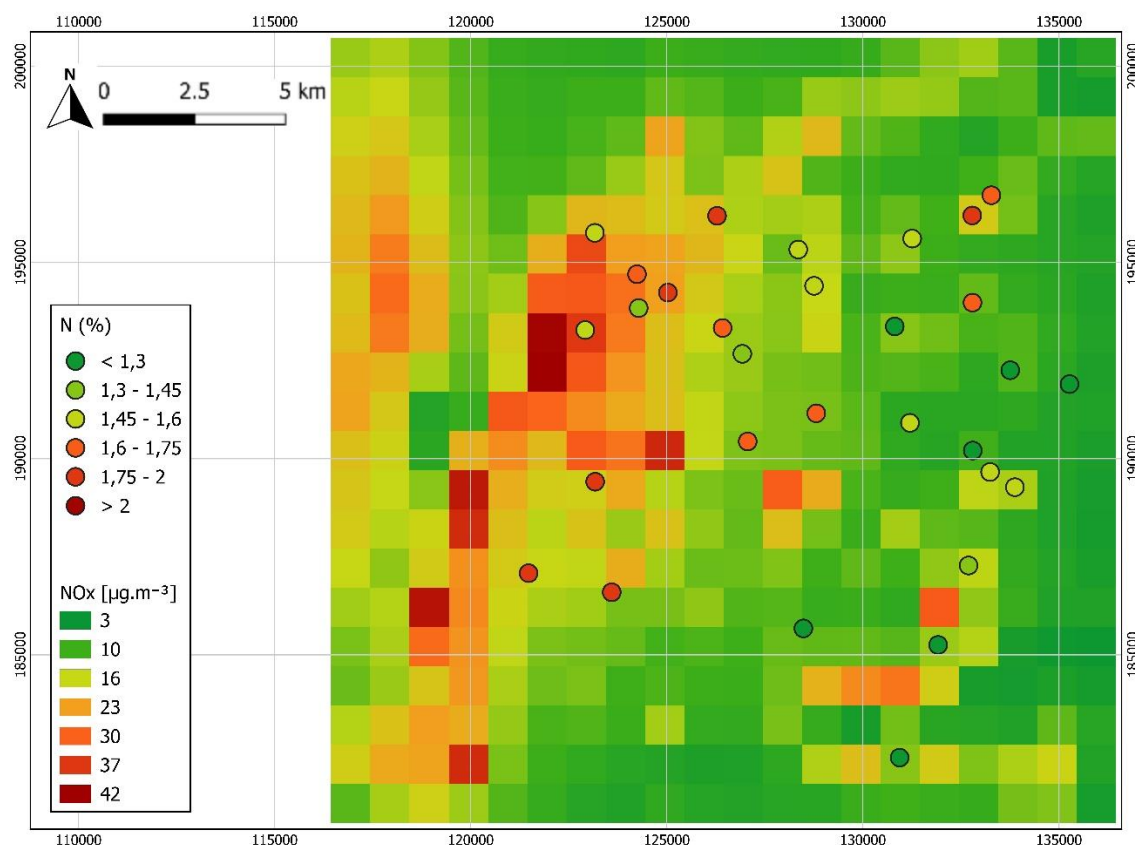


Figure 6 spatial distribution of N content in mosses (%) during C₃
Limitation of the study

Studies generally indicate the importance of avoiding coastal areas during biomonitoring surveys due to specific effects associated with sea salts, which can influence the pollutant accumulation capacity of bioindicators. The specific nature of this study is dictated by the location of the main pollution sources - particularly the thermal power plant and the highway - near the coast. Consequently, the combined impact of coastal pollution sources and marine influences can affect the spatial distribution and accumulation of pollutants in moss tissues.

Conclusion

In this study *Hypnum cupressiforme* mosses were studied as biomonitors of atmospheric nitrogen. Moss samples were collected in May and November 2023 to analyze seasonal influence on N content in mosses and then in May 2024 to analyze the annual influence.

The spatial distribution of N content in mosses was higher in mosses collected from sampling sites near the stationary pollution sources (power plant and cement plant) and lower in the distant sites confirming the modeled NO_x and NH₃ dispersion maps. Different precipitation gradients and wind patterns during the rainy season affected the N content in mosses which appears more heterogenous. Relationships are the strongest during the dry seasons and moderate during the rainy season.

The highest N percentage in mosses was found in sampling sites with highest NO_x and NH₃ concentrations during the dry season C₁. During C₂, an increase of number of moss samples with high N percentage was observed due to increased water availability and humidity. The lowest N percentages were found in the reserve nature far from all sources of pollution during the three campaigns.

These findings demonstrate the capability of the moss *Hypnum cupressiforme* to show the spatial dispersion patterns of the total atmospheric nitrogen in different seasons and confirm the use of these species as a low-cost alternative for detecting and analyzing air pollution.

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