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Feasibility of using *Pleurozium schreberi* as a biomonitor to study antiozonant dispersion: A case study in Southern Quebec

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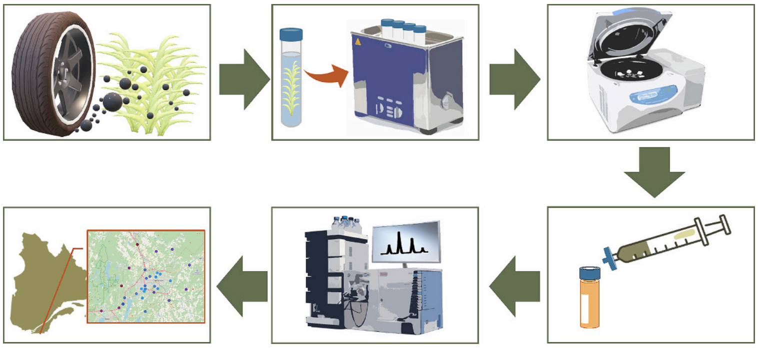
HIGHLIGHTS

- A method for the analysis of antiozonants in moss tissues was validated.
- Atmospheric dispersal of antiozonants in Quebec evaluated using moss biomonitor.
- Widespread contamination by antiozonants was observed in southern Quebec.
- Tire and road wear particles are key sources of airborne antiozonants.
- 6PPD/6PPDQ ratio shows potential as proxy for atmospheric degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

p-Phenylenediamine antioxidants (PPDs), widely used as additives in tires and rubber, are released into the environment through tire and road wear particles. These compounds undergo oxidative processes, forming quinone derivatives that pose significant environmental and health risks, particularly to aquatic organisms (EPA, 2024). While runoff has been identified as the primary transport mechanism, the atmospheric dispersion of PPDs has received less attention. Bryophytes have been widely used as biomonitors of airborne contaminants and atmospheric deposition. Using a biomonitoring approach, this study investigated the atmospheric deposition of PPD antiozonants, including 6PPD, 6PPDQ, and DPPD, across Southern Quebec (Canada), a region characterized by the highest population density and pollution levels in the province. Samples of *Pleurozium schreberi*, a common species used for biomonitoring of atmospheric deposition, were collected on three site types with varying degrees of traffic exposure: roadsides, parks/playgrounds, and non-urban areas. Our findings demonstrated atmospheric dispersion of PPDs throughout Southern Quebec with a decreasing trend in total concentrations of PPDs with increasing distance from traffic. 6PPDQ was the most frequently detected compound, ranging from <LOQ to 3.71 ng g^{-1}. DPPD, the least detected, ranged from <LOQ to 4.92 ng g^{-1}. 6PPD showed intermediate detection frequencies but the highest concentrations, up to 59.01 ng g^{-1}. This study validates that *P. schreberi* is a valuable tool for low-cost monitoring of PPD dispersal. Results underscore the spatial variability of PPDs and their

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derivatives, influenced by proximity to pollution sources, environmental conditions, and site characteristics. This study provides one of the few pieces of evidence supporting the atmospheric dispersion of PPDs, highlighting the need for further investigation.

1. Introduction

Antiozonants are additives used in rubber products to protect against oxidative damage caused by ozone, a highly reactive pollutant that degrades rubber. However, as these chemicals wear off, they are dispersed into the environment through atmospheric deposition and water runoff, accumulating in various ecosystems. The widespread use of *p*-phenylenediamine antioxidants (PPDs) in motor vehicle tires, including N-Isopropyl-*N*′-phenyl-*p*-phenylenediamine (IPPD), *N*,*N*′-Diphenyl-*p*-phenylenediamine (DPPD), and especially N-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine (6PPD), along with their transformation products, has raised significant concerns due to their adverse effects on terrestrial and aquatic ecosystems (Zeng et al., 2023; Cao et al., 2022). These effects include neurotoxicity, cardiotoxicity, and reproductive toxicity (Chen et al., 2023). Quinoid derivatives, such as IPPD-quinone (IPPDQ) and 6PPD-quinone (6PPDQ), are formed through the oxidation of PPD compounds and have shown potential for bioaccumulation and toxicity in aquatic species (EPA, 2024; Jin et al., 2023a; Jiang et al., 2024b). Their presence in dust, soil, and electronic waste samples correlated with their parent compounds, highlighting their environmental persistence and potential for transformation (Jin et al., 2023a).

Among these substances, 6PPD and its degradation product, 6PPDQ, have been identified as promising markers of tire and road wear particles in various environmental matrices (Klöckner et al., 2021a; Cao et al., 2022; Zhang et al., 2021). While 6PPD plays a critical role in protecting rubber from ozone and oxygen, its degradation product, 6PPDQ, can induce acute toxicity in aquatic species at concentrations as low as ~1 μg.L^{-1}. Notably, 40–90 % pre-spawn mortality rates in coho salmon were reported during migrations to urban and suburban streams (Tian et al., 2021; Scholz et al., 2011). Concentrations of 6PPDQ ranging from <0.3 to 19 μg.L^{-1} have been detected along the U.S. West Coast, often exceeding lethal thresholds (median lethal concentration of 0.8 ± 0.16 μg.L^{-1}) for coho salmon (Tian et al., 2021). These concerns have prompted greater regulatory scrutiny, leading to the classification of motor vehicle tires containing 6PPD as a Priority Product by the California Department of Toxic Substances Control (Department of Toxic Substances Control, 2023). This designation requires the evaluation and reduction of 6PPD in consumer products. Disruptions in glucose and lipid metabolism, as well as increases in neurotransmitter levels, have also been observed in zebrafish larvae exposed to antiozonants (Chanlin et al., 2025; Ricarte et al., 2023). Moreover, 6PPD and 6PPDQ have been detected in human urine, bloodstream, and cerebrospinal fluid samples, with notably high concentrations in pregnant women, highlighting the urgent need for research on vulnerable populations (Wan et al., 2024; Du et al., 2022). Other PPDs, such as IPPD and DPPD, also pose health and environmental risks. DPPD, an effective corrosion inhibitor, can be toxic at certain concentrations and must be used with caution (EPA, 2009; ChemRisk, Inc. and DIK, Inc., 2008). In rats, exposure to DPPD increased maternal and fetal mortality during parturition (>22 mg.kg^{-1}.day^{-1}) and pup mortality (>11 mg.kg^{-1}.day^{-1}) (Oser and Oser, 1956; Ames et al., 1956). Similarly, IPPD, a known contact allergen linked to dermatitis (Bacharewicz-Szczerbicka et al., 2021; Hartwig and MAK Commission, 2002), exhibits acute toxicity in certain fish species (OECD, 2004; Xie et al., 2024). Both compounds are classified as hazardous to the aquatic environment according to EU standards, with DPPD under ‘R50/53’ and IPPD under ‘R52/53’.

Given the toxicity and persistence of PPD compounds in the environment, particularly 6PPDQ (Hiki et al., 2021; Hu et al., 2023), there is an urgent need for effective monitoring strategies. The presence of PPD compounds in water bodies is well documented (Johannessen et al., 2022a; Jiang et al., 2024b; Chen et al., 2023), and road runoff has been identified as an important source of PPDs in aquatic systems (Jaeger et al., 2024; Werbowski et al., 2021). The atmospheric dispersal of PPDs has received less attention, even though fine particles from tire wear and other sources could be an important vector for wind dispersal (Zeng et al., 2023; Johannessen et al., 2022b). Biomonitoring using mosses represents a relatively low-cost and efficient approach for high-spatial-resolution monitoring of contaminants in atmospheric depositions. While semi-quantitative, it allows for rapid assessment of environmental contamination and evaluation of contaminants dispersal. The effectiveness of pleurocarpous moss species, such as *Pleurozium schreberi*, for the monitoring of contaminants in atmospheric depositions has been demonstrated in many biomonitoring initiatives (Baczewska-Dąbrowska et al., 2023; Fernández et al., 2015), including the long-standing ICP-Vegetation program in Europe (Manual, 2015). Although the potential of moss to adsorb and retain antiozonants remains unexplored, their high specific surface area and ability to passively capture and retain particulate-bound deposition (Fernández et al., 2015) make them worthy biomonitor candidates.

This research evaluated the ability of mosses to capture antiozonant residues, with a primary focus on 6PPD and its derivative 6PPDQ. Specifically, the study aimed to (1) validate a method for the analysis of PPDs in moss tissues, (2) determine whether mosses can effectively adsorb and retain antiozonant compounds and their derivatives, and (3) map the dispersal of these compounds across Southern Quebec, considering site characteristics and vehicular activity. We selected *P. schreberi*, a ubiquitous moss species in Quebec, as a biomonitor. Because antiozonants are widely used in rubber-based products, we hypothesized that environmental contamination by atmospheric dispersal was widespread. To test this hypothesis, we sampled mosses in non-urban locations throughout southern Quebec (in Eastern Canada) to estimate the current state of PPDs dispersal. PPDs from non-traffic sources, such as industrial waste or product degradation, primarily enter soil and water via leaching and runoff rather than direct atmospheric dispersion (Unice et al., 2015). Thus, we further hypothesized that bioaccumulation in moss primarily reflects traffic-related emissions, mainly from tire wear and road dust resuspension. We tested the link between traffic load and bioaccumulation of PPDs in *P. schreberi* by collecting moss samples in various locations with contrasted exposure to road traffic, including roadsides, city parks/playgrounds, and non-urban areas away from major roads in the city of Sherbrooke (Quebec). On each site type (roadside, city park, and non-urban sites), we quantified PPD concentrations, especially 6PPD, 6PPDQ, and their ratio as markers of tire and road wear particles and environmental degradation (Klöckner et al., 2021a; Cao et al., 2022; Zhang et al., 2021). We predict that antiozonant concentrations will correlate with the intensity of traffic-road and that the ratio of 6PPD/6PPDQ will decrease with the distance from pollution sources (i.e., from roadside to non-urban sites), reflecting the greater transformation of the parent compound over longer dispersal. Because we expect particulate dispersal to be an important source of PPDs in moss, we evaluated the effect of precipitation and washing on PPDs concentration in moss tissues to inform on the impact of sampling (i.e., after a rain event) and sample preparation (i.e., washing) practices on PPDs quantification. By establishing a baseline for antiozonant accumulation in mosses, this study seeks to advance our understanding of their environmental fate and distribution.

2. Material and method

2.1. Sampling

Moss samples (*Pleurozium schreberi)* were collected from non-urban areas over two successive years (2022–2023) between May and June as part of a biomonitoring project. Sampling was conducted on an approximate 20 km grid, covering the most populated region of Quebec ($n = 38$). This sampling aimed to evaluate diffuse contamination that does not originate from a specific point source, such as roads in the case of PPDs, in southern Quebec. Following the guidelines in the ICP-Vegetation Manual (2015), a minimum distance of at least 50 m from secondary roads and 100 m from primary roads was considered an effective distance to minimize direct road influence. Accordingly, samples were collected from openings to avoid forest canopy interference, which can affect deposition dynamics. At each site, a composite sample, collected within 10 m × 10 m plots, was created by aggregating moss patches, providing a representative sample of each location. These samples were carefully placed in paper bags and transported to the laboratory under conditions that protected them from high temperatures and UV light, ensuring minimal contamination and degradation. Additional sampling in the city of Sherbrooke was conducted between June and August 2024 to collect samples exposed to varying levels of vehicular traffic, namely roadsides and city parks/playgrounds. This sampling aimed to evaluate the effect of anthropogenic activity on moss PPD content (parent compounds and derivatives). Road samples ($n = 22$) were collected as close as possible to roads, from upland areas and elevated surfaces, to minimize the influence of surface runoff or leaching, and were characterized by various traffic flows (Fig. 1). Urban park samples were collected from 16 locations throughout the city. All sampling procedures adhered to environmental and ethical guidelines to maintain the study's integrity and ensure reproducibility.

2.2. Chemicals and reagents

All chemicals used were of analytical grade. Solvents, including acetonitrile (ACN) and methylene chloride (DCM), were Optima® grade for LC/MS, supplied by Fisher Scientific (Ottawa, ON, Canada). Standards were procured as follows: Diphenylamine-d10 (solid) from Toronto Research Chemicals (Toronto, ON, Canada); DPPD (solid, 97 %) from Thermo Scientific Chemicals (Burlington, ON, Canada); 6PPD (solid) from Sigma-Aldrich (Oakville, ON, Canada); IPPD-Quinone from LGC (New York, NY, USA); 6PPDQ-d5 (98%) and 6PPDQ (95 %) solutions from Cambridge Isotope Laboratories (Tewksbury, MA, USA).

2.3. Sample preparation

Moss samples were washed with deionized water (DW) to remove surface particles and loosely bound compounds. The washed samples were then oven-dried at 37 °C for 72 h to ensure complete dehydration. Once dried, the samples were ground to a fine powder using a solvent-washed mortar and pestle with liquid nitrogen to minimize thermal degradation during the grinding process.

2.4. Extraction

Using Quechers solid phase extraction technique, moss samples (400 mg) were extracted with 5 mL of DCM in Pyrex® heavy-duty glass centrifuge tubes. 9 μL of Diphenylamine-d10 (stock solution: 3740 ng mL^{-1} in ACN), as a surrogate, was added to the samples prior to extraction for quality assurance (QA) and quality control (QC) purposes, resulting in a final concentration of approximately 84.15 ng mL^{-1} in the extract. The mixture was vortexed for 1 min, followed by sonication for 15 min. All centrifugation steps were performed at 3000 rpm for 10 min at 20 °C, and the entire supernatant was collected after each centrifugation. The extraction was repeated three times, twice with 5 mL of DCM and then once with 5 mL of ACN. Purification materials (1.5 g $MgSO_4$, 0.1 g PSA, and 0.3 g C18) were subsequently added, followed by vortexing for 1 min and sonication for 15 min. After a final centrifugation, the supernatant was collected, concentrated to near dryness under nitrogen flow, and dissolved in 0.4 mL of ACN. The extract was transferred to an amber vial, spiked with 10 μL of internal standard (6PPDQ-d5: stock solution: 10,000 ng mL^{-1} in ACN), and filtered using a PTFE filter (0.22 μm) in preparation for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

2.5. UPLC-MS/MS

Extracts were analyzed using a Xevo TQ-S micro equipped with an Acquity UPLC HSS-T3 column (2.1 × 50 mm, 1.8 um, equipped with a

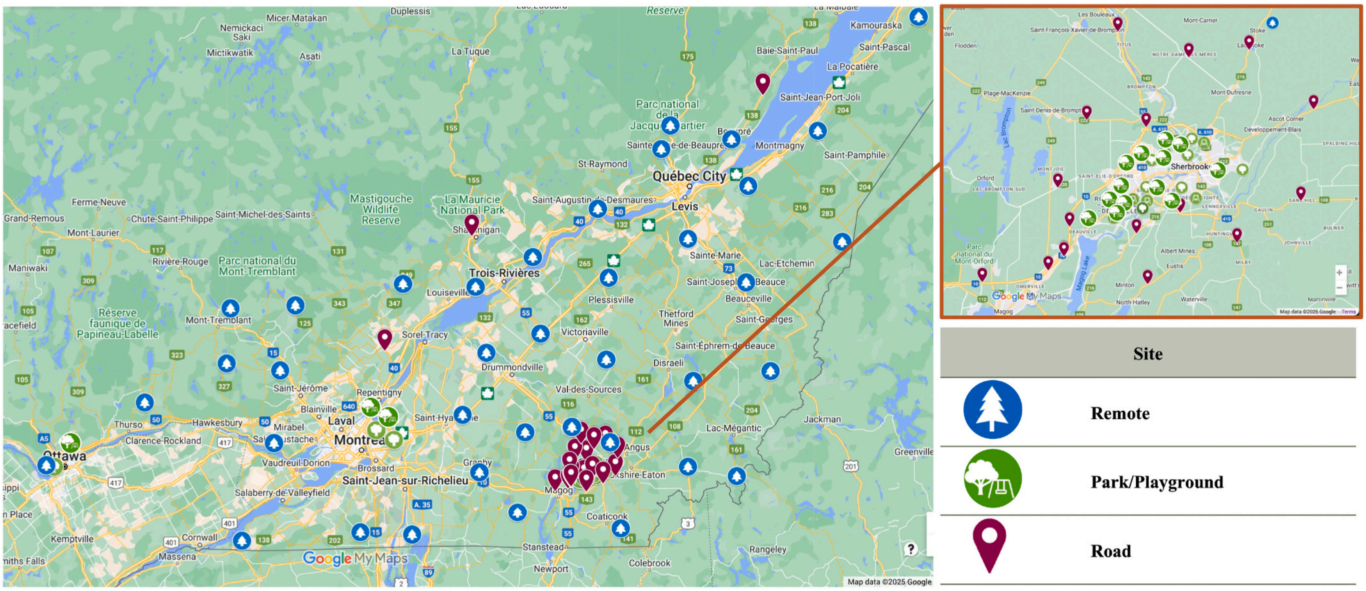

Fig. 1. Location of sampling sites in southern Quebec. Samples from non-urban areas and city parks/playgrounds are identified with blue circles and green circles, respectively. Samples from roadsides are identified with purple map markers. The insert provides a detailed view of the sampling locations around the city of Sherbrooke.

0.2 mm fritted pre-filter) (Waters Corporation, Milford, MA, USA). Mobile phases include 0.1 % formic acid in water (A), and 0.1 % formic acid in ACN (B) at a flow rate of 0.3 mL min^{-1}. The mass spectrometry analysis was performed using a positive electrospray ionization (ESI+) source in multiple reaction monitoring (MRM) mode. The elution gradient started with 2 % of eluent B for 6 min, increased to 100 % in 6 min and held for 6 min, and then back to 2 % of eluent B in 6 min with an equilibration time of 6 min. The analysis utilized an injection volume of 5 μL with the following parameters: desolvation gas flow (N_2) at 1000 L h^{-1}, desolvation temperature of 450 °C, source temperature of 150 °C, collision gas pressure (Ar) at 3.5×10^{-3} mbar, cone gas flow (N_2) at 50 L h^{-1}, and capillary voltage of 2.00 kV. Data acquisition and processing were conducted using MassLynx 4.1 software (Waters Corporation). The precursor ions and transitions used for quantification and qualification are summarized in Table 1, with detailed analytical parameters provided in Supplementary information Table S.1.

2.6. Method validation and quality assurance

The linearity concentration range, accuracy, precision, reproducibility, and matrix effect were evaluated. The limit of quantification (LOQ) was defined as the lowest point on the calibration curve where linearity was no longer maintained. To assess recovery and precision (as relative standard deviation, RSD), five samples were fortified at two levels prior to extraction: 6PPD at 171.22 ng g^{-1} and 42.80 ng g^{-1}, 6PPDQ at 73.17 ng g^{-1} and 18.29 ng g^{-1}, DPPD at 194.63 ng g^{-1} and 48.66 ng g^{-1}, IPPDQ at 73.17 ng g^{-1} and 18.29 ng g^{-1}, and the surrogate at 82.39 ng g^{-1} and 20.67 ng g^{-1}. The LOQ, mean recovery rate (±standard deviation), and RSD values are presented in Table 2, confirming acceptable accuracy and reproducibility of the quantification method. Additionally, the matrix effects (ME%) were evaluated following the method described by Hiki and Yamamoto (2022). An extract was divided into two equal sub-samples (0.2 mL each). One sub-sample was spiked with 5 μL of ACN, while the other was spiked with 5 μL of a mixed standard solution (Table S.2). The difference in analyte responses between the sub-samples was compared to the response of an external standard solution with matching analyte concentrations ($n = 5$, Sup. Info. Eq. S.1). To monitor potential background contamination introduced during sample preparation and analysis, one procedural blank was included in each extraction batch. Finally, 20 % of the environmental samples were replicated, with the results detailed in Supp. Info. Table S.3.

Table 1
The MS/MS conditions for antiozonant analysis. The parameters for quantification and qualification of antiozonants (6PPD, 6PPDQ, DPPD, and IPPDQ) and labelled compounds used as surrogate (Diphenylamine-d10) and internal standard (6PPDQ-d5). RT means retention time, and CE means collision energy.

Compound	RT (min)	CV (V)	Quantitative transition	CE (V)	Confirmation transition	CE (V)
6PPD	8.84	25	268.32 > 168.96	32	268.32 > 211.04	32
6PPDQ	13.21	25	299.15 > 187.00	26	299.15 > 241.04	26
DPPD	13.79	25	260.72 > 168.95	26	260.72 > 186.97	26
IPPDQ	10.31	25	257.13 > 169.99	24	257.13 > 187.06	24
Diphenylamine-d10	12.15	25	180.04 > 97.93	25		
6PPDQ-d5	13.16	25	304.15 > 192.02	26		

CV: Cone Voltage; CE: Collision Energy; Surrogate: Diphenylamine-d10; Internal Standard: 6PPDQ-d5.
Note: 6PPD produces both $[M]^{+\bullet}$ and $[M + H]^{+}$ precursor ions. $[M]^{+\bullet}$ was selected for quantification.

Table 2
Limit of quantification and recovery of the validated method. LOQ is expressed in ng.g^{-1}, and recovery rates are presented in % (±STDev).

Analyte	LOQ (ng g^{-1})	Spike level (ng g^{-1})	Mean recovery %	RSD %
6PPD	0.10	171.22	69.17 ± 3.84	5.55
		42.80	57.76 ± 8.77	15.19
DPPD	0.19	194.63	46.54 ± 1.56	3.35
		48.66	50.53 ± 1.20	2.38
6PPDQ	0.04	73.17	106.51 ± 1.85	1.73
		18.29	87.53 ± 2.41	2.75
IPPDQ	0.08	73.17	122.17 ± 2.73	2.24
		18.29	100.22 ± 2.11	2.11
Diphenylamine-d10	0.05	82.39	103.67 ± 1.59	1.53
		20.67	88.43 ± 2.18	2.46

$n = 5$.

2.7. Precipitation and washing effects

When using moss as a biomonitor, it is crucial to ensure consistency and reliability in biomonitoring results. There is no clear agreement on the necessity, or benefits, of washing samples prior analysis. The inclusion of a washing step varies between studies and the type of contaminants of interest. For particulate-borne contaminants, such as PPDs, washing samples could improve reproducibility and limit temporal variability due to the presence of particles loosely associated with the moss material. Washing moss also provides a more accurate assessment of bioaccumulation. Indeed, preliminary findings revealed significant decreases in antiozonant concentrations in moss samples collected after rain prompting an evaluation of the effects of these factors on antiozonant concentration measured in moss. To test the effect of precipitation and washing, moss samples collected from a highly trafficked roadside in Sherbrooke (52,000 cars.day^{-1}) were divided into five groups (6.5 g each) receiving one of five treatments under controlled laboratory conditions. A) Unwashed Control: samples were analyzed directly without any precipitation or washing treatments. B, C, and D) Simulated Precipitation Treatments: samples were exposed to precipitation levels of 20 mm, 50 mm, and 100 mm, respectively, using aluminum boats to simulate rainfall effects on the moss. E) Washed Control: samples were washed with deionized water to remove surface particles. All samples were dried in an oven at 37 °C for 72 h and subsequently ground to achieve uniform particle size before analysis. Each treatment was replicated three times to account for variability and enhance the reliability of the results.

2.8. Statistical analyses

All statistical analyses and data visualizations were performed using R (version 4.4.1). Antiozonant concentrations were log-transformed to reduce skewness, stabilize variance, and improve interpretability, facilitating comparisons across sampling sites (i.e., non-urban, city parks/playgrounds, and roadsides), despite not meeting the normality assumption required for parametric analysis. A Kruskal-Wallis test was performed to assess differences in concentrations among sites, followed by pairwise comparisons using Dunn's test. Additionally, values below the limit of detection (LOD) were replaced with LOD divided by the square root of two ($LOD/\sqrt{2}$), following the SAS method (Croghan and Egeghy, 2016). ANOVA followed by Tukey HSD post-hoc tests, as pairwise analysis, was conducted on absolute concentrations to evaluate the effect of washing and precipitation on antiozonant levels in moss.

3. Results

3.1. Method validation

The extraction method was optimized by testing different combinations of solvent ratios, extraction salts, and purification techniques, following an experimental setup adapted from Ji et al. (2022). Extraction conditions were selected to optimize recovery and limit matrix effects for 6PPD, 6PPDQ, DPPD, and IPPDQ. Briefly, five conditions were tested (Table S.4): A) solvent mixture: DCM (5 mL) + DCM (4 mL) + ACN (4 mL), extraction salts: 1 g NaCl and 2 g MgSO₄, purification: 1 g MgSO₄, 0.1 g primary secondary amine (PSA), and 0.2 g C18. B) Solvent mixture: DCM (5 mL) + DCM (4 mL) + ACN (4 mL), extraction salts: none, purification: 1 g MgSO₄, 0.1 g PSA, and 0.2 g C18. C) Solvent mixture: DCM (5 mL) + DCM (5 mL) + ACN (5 mL), extraction salts: none, purification: 1.5 g MgSO₄, 0.1 g PSA, and 0.3 g C18. D) Solvent mixture: DCM (5 mL) + DCM (4 mL) + ACN (4 mL), extraction salts: 1 g NaCl and 2 g MgSO₄, purification: 1 g MgSO₄, and 0.2 g C18. E) Solvent mixture: DCM (5 mL) + DCM (4 mL) + ACN (4 mL), extraction salts: 1 g NaCl and 2 g MgSO₄, purification: 1 g MgSO₄, 0.3 g C_{18}, 0.6 g Florisil, 0.6 g Alumina, 2 g Silica gel. Treatment C was selected as it provided the overall best performance for extracting the compounds of interest from *P. schreberi* samples (Fig. 2). The recovery rates and matrix effects were as follows: 62.13 % (±9.24) and 69.17 % (±3.84) for 6PPD; 81.59 % (±7.75) and 106.51 % (±1.85) for 6PPDQ; 64.91 % (±7.11) and 46.54 % (±1.56) for DPPD; 102.74 % (±11.03) and 122.17 % (±2.73) for IPPDQ. The relative standard deviations of the validated method (treatment C) (RSD%) for recovery rates and matrix effects ranged from 9.50 to 15.03 % and 1.53–5.55 %, respectively. Detailed results of other tested treatments are presented in Supp. Info. Table S.5.

3.2. *Effect of washing and precipitation*

For 6PPD, no statistically significant differences were observed between unwashed, precipitation treatments (20 mm, 50 mm, 100 mm), and washed samples (ANOVA: F = 1.602, $p = 0.24$; Fig. 3.A and Sup. Info. Table S.6). While not statistically significant mainly due to the high variability of the unwashed samples (all Tukey HSD $p \geq 0.28$, Sup. Info. Table S.7), a strong trend of reduced average concentration (4-fold) and variability between unwashed samples and all other treatments was observed. This suggests that water treatments (washing and simulated precipitation) removed 6PPD (particulate-borne, labile) from the moss surface. For 6PPDQ and DPPD, concentrations were significantly affected by treatments (washing and simulated precipitation) (Fig. 3B and C, ANOVA: F = 7.656, $p = 0.0043$ and F = 8.702, $p = 0.0027$, respectively, Sup. Info. Table S.6). For 6PPDQ, concentration in moss was significantly reduced by washing and precipitation treatments (50 mm, 100 mm) compared to unwashed samples (Tukey HSD: $p < 0.05$, Sup. Info. Table S.7). Precipitation treatments of 20 mm also demonstrated a marginally significant difference ($p = 0.094$) compared to unwashed samples. No difference was observed between washing and simulated precipitations and between precipitations intensities. For DPPD, washed samples showed significantly lower concentrations than unwashed ones (Tukey HSD: $p = 0.0013$, Sup. Info. Table S.7). The 50 mm precipitation treatment led to a marginally significant reduction (Tukey HSD: $p = 0.0693$, Sup. Info. Table S.7) compared to unwashed samples. Although the Washed–20 mm comparison was also marginally significant ($p = 0.0665$), and Washed–100 mm showed a moderately significant reduction ($p = 0.0258$), no significant differences were observed among other precipitation intensities. IPPDQ concentrations in moss were below the LOQ.

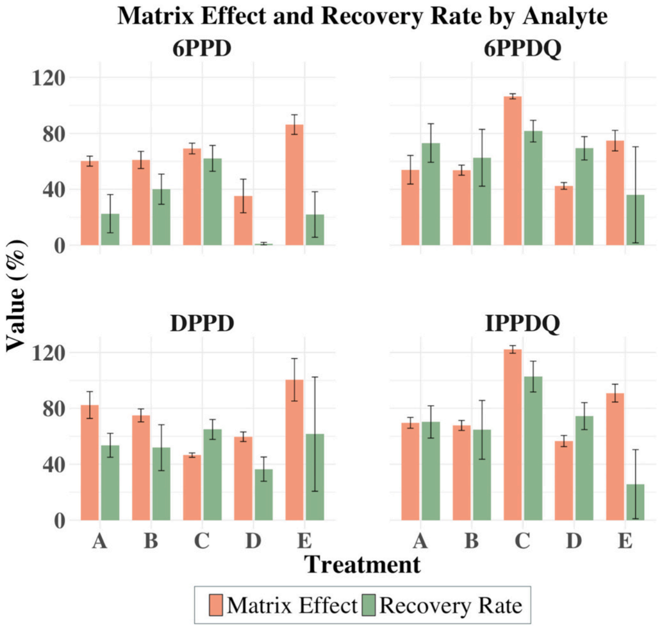

Fig. 2. Matrix effect and recovery rate of antiozonants. Matrix effects (in %) are presented in orange, and recovery rates (in %) in green. Error bars are standard errors ($n = 4$). Letters on the x-axis refer to extraction conditions: (A) DCM (5 mL) + DCM (4 mL) + ACN (4 mL), 1 g NaCl +2 g $MgSO_4$, followed by 1 g $MgSO_4$ + 0.1 g PSA + 0.2 g C_{18}; (B) DCM (5 mL) + DCM (4 mL) + ACN (4 mL), 1 g $MgSO_4$ + 0.1 g PSA + 0.2 g C_{18}; (C) DCM (5 mL) + DCM (5 mL) + ACN (5 mL), 1.5 g $MgSO_4$ + 0.1 g PSA + 0.3 g C_{18}; (D) DCM (5 mL) + DCM (4 mL) + ACN (4 mL), 1 g NaCl +2 g $MgSO_4$, followed by 1 g $MgSO_4$ + 0.2 g C_{18}; and (E) DCM (5 mL) + DCM (4 mL) + ACN (4 mL), 1 g NaCl +2 g $MgSO_4$, followed by 1 g $MgSO_4$ + 0.3 g C_{18} + 0.6 g Florisil +0.6 g Alumina +2 g Silica gel.

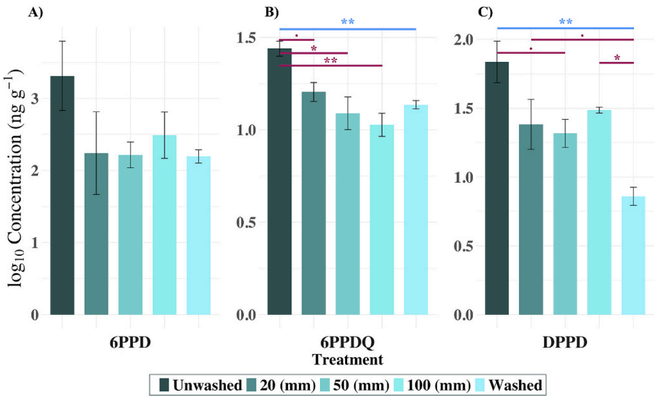

Fig. 3. Effect of simulated precipitation and washing on PPD concentrations in *P. schreberi*. Three amount of simulated precipitation was tested (20 mm, 50 mm and 100 mm). Stars indicate significant differences in PPD concentration between sites (ANOVA in blue and Post-hoc Tukey HSD in purple), with *** highly significant ($p < 0.001$), ** significant ($p < 0.01$), * moderately significant ($p < 0.05$), and · marginally significant ($p < 0.1$).

3.3. *PPDs concentration in P. schreberi in southern Quebec*

6PPD, 6PPDQ, and DPPD were detected in most samples collected throughout southern Quebec (Fig. 4). On the contrary IPPDQ concentration was below the detection limit in all samples. Significant variabilities in 6PPD (H = 51.8, $p = 5.53e^{-12}$), 6PPDQ (H = 45.9, $p = 1.07e^{-10}$), and DPPD (H = 24.7, $p = 4.34e^{-06}$) concentrations were observed between site types (non-urban, city parks/playgrounds, and roadsides) (Fig. 5, Table 3 and Sup. Info Table S.8). Concentrations of PPDs were the highest in roadside sites, with concentrations of 6PPD and 6PPDQ exceeding 40 ng g^{-1} and 3 ng g^{-1}, respectively, at multiple sampling points (Sup. Info. Fig. S.1). Urban parks and playgrounds, though varying in their proximity to major roads, are generally located farther from high-traffic corridors than roadside sites and are often covered by tree and shrub vegetation. They showed substantially lower PPD concentrations compared to road sites. Non-urban sites exhibited the lowest overall concentrations. In more detail, for 6PPD, concentrations were higher in roadside sites compared to non-urban ($p < 0.001$) and park/playground ($p = 0.0279$) sites. Concentration in park/playground were also significantly higher than in non-urban sites ($p =$

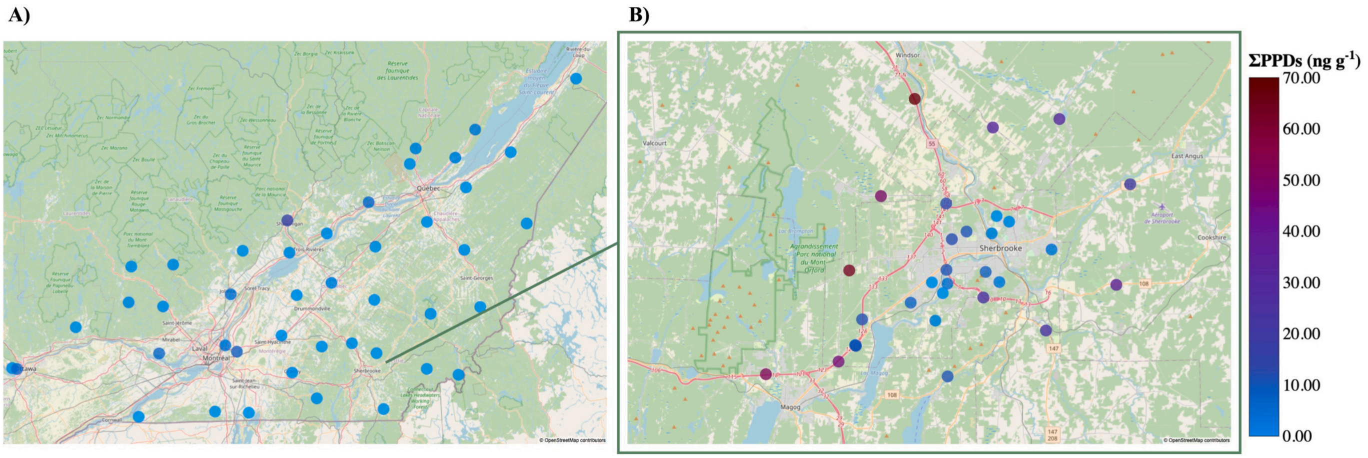

Fig. 4. Concentration of PPDs across Southern Quebec. (A) PPD concentrations at the studied sites throughout Southern Quebec. (B) PPD concentrations in urban sites (roadsides and parks/playgrounds) in the city of Sherbrooke. Each site is represented by a circle, with its color proportional to the total PPD concentration measured at that site.

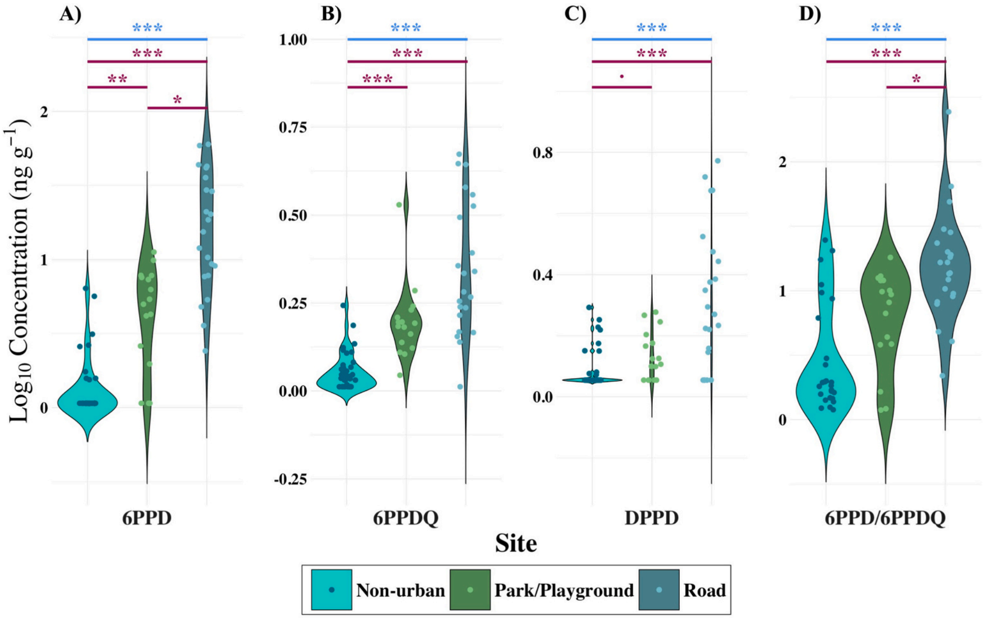

Fig. 5. Concentrations of PPDs and 6PPD/6PPDQ ratios across different site types. A) 6PPD, B) 6PPDQ, C) DPPD, and D) 6PPD/6PPDQ ratio. Data from non-urban sites are shown in light blue, park/playground in green, and roadsides in dark blue. Stars indicate significant differences in PPD concentrations and 6PPD/6PPDQ ratios between site types (Kruskal-Wallis test in blue and Dunn's test in purple), with *** highly significant ($p < 0.001$), ** significant ($p < 0.01$), * moderately significant ($p < 0.05$), and marginally significant ($p < 0.1$).

Table 3
Frequency of detection and concentration of PPDs in southern Quebec by site type. Concentrations are presented in ng g^{-1} (dw), and detection frequency in %.

PPD	Non-urban (n = 38)			Parks/Playgrounds (n = 16)			Roadsides (n = 22)		
	DF%	Median	Range (ng g^{-1})	DF%	Median	Range (ng g^{-1})	DF%	Median	Range (ng g^{-1})
6PPD	26.32	<LOQ	<LOQ-5.39	81.3	2.50	<LOQ-10.23	100	15.97	1.41–59.01
6PPDQ	65.8	0.08	<LOQ-0.75	100	0.36	0.11–2.38	95.45	1.04	<LOQ-3.71
DPPD	28.95	<LOQ	<LOQ-0.96	68.8	0.26	<LOQ-0.89	77.27	0.92	<LOQ-4.92

0.0013). For 6PPDQ, park/playground and roadside sites exhibited higher concentrations than non-urban sites ($p < 0.001$). However, no statistically significant differences were observed between roadside and park/playground sites ($p = 0.348$). For DPPD, roadside sites exhibited significantly greater concentrations than non-urban sites ($p < 0.001$), while park/playground sites showed a marginally significant difference compared to non-urban sites ($p = 0.0813$). However, no significant difference was observed between roadside and park/playground sites ($p = 0.132$). Significant differences between sites were also observed for the 6PPD/6PPDQ ratio ($H = 22.6$, p-value $= 1.26e^{-05}$). This ratio informs on the degree of oxidation of the parent compound 6PPD, with a decreasing ratio with increasing degradation. The ratio was higher at roadside sites than at non-urban ($p < 0.001$) and park/playground ($p = 0.0363$) sites. However, no significant differences were observed between park/playground and non-urban sites ($p = 0.252$). As for concentrations, the detection frequency of PPDs significantly varied with site type, with the highest frequencies observed in roadside sites (6PPD: 100 %, 6PPDQ: 95.45 %, and DPPD: 77.27 %) and the lowest in non-urban sites (6PPD: 26.32 %, 6PPDQ: 65.8 %, and DPPD: 28.95 %) (Fig. 6). Parks/playgrounds had intermediate detection frequencies for 6PPD and DPPD at 81.25 % and 68.75 %, respectively. Notably, 6PPDQ was detected in 100 % of Park/Playground samples.

4. Discussion

4.1. *Moss potential for the biomonitoring of PPDs in atmospheric deposition*

Our findings demonstrate the efficiency of *P. schreberi* in capturing PPDs. However, the record of PPDs is compound dependent. 6PPD, 6PPDQ, and DPPD were present in *P. schreberi*, but the derivative IPPDQ was not observed in any samples. The rapid flushing of IPPDQ from road surfaces through precipitation has been associated with its high hydrophilicity (Jaeger et al., 2024). Therefore, the absence of detectable IPPDQ in moss tissues could result from its rapid elimination in the field through precipitation. Alternatively, *P. schreberi* could achieve very limited affinity for this compound, both in terms of surface sorption and assimilation, compared to other PPDs.

The ability of moss to capture PPDs is likely linked to their large surface-to-mass ratio, allowing them to efficiently intercept atmospheric depositions, especially particles. Studies in urbanized areas reported that mosses efficiently capture coarse particles (Clough, 1975; Taylor and Witherspoon, 1972), especially in the range of 10 to 100 μm (Haynes et al., 2019). Consistently, antiozonants such as 6PPD, DPPD, and 6PPDQ are predominantly present in particles <100 μm (Deng et al., 2022; Klöckner et al., 2021b). Specifically, over 70 % of PPDs in road and indoor parking lot dust are found in size fractions <125 μm (Deng et al., 2022). Furthermore, recent evidence suggests that 6PPD-Q is negatively correlated with clay content (particles <4 μm) in road dust (Jin et al., 2023b) and preferentially accumulates in coarse inhalable particles (9–10 μm) rather than finer ones (<5 μm) (Zhang et al., 2022), indicating size-dependent sorption behavior. Given that moss efficiently intercepts coarse airborne particle size (Haynes et al., 2019), these findings support its use as a passive sampler for field-based monitoring of PPD derivatives. The importance of particle capture in the accumulation of PPDs in moss is further supported by our finding that precipitation treatments and washing removed an average of 23 % of 6PPD, 25 % of DPPD, and 65 % of 6PPDQ, suggesting that these compounds were loosely associated with the moss as deposited particulate matter. Our washing experiment also suggests that PPDs could derive from different types of particle sizes. Washing and simulated precipitation had comparable effects on the loss of 6PPD and 6PPDQ, while washing was significantly more efficient than simulated precipitation for DPPD. The major difference between washing and simulated precipitation is that washing provides a more uniform and controlled removal of particles from the moss surface. In contrast, precipitation may result in uneven rinsing, as water distribution can vary depending on moss patch density, surface structure, and exposure to rainfall. The differences in the response of 6PPD, 6PPDQ, and DPPD to washing could stem from a combination of factors, including discrepancies in their relative particle size distribution, potential differences in adsorption strength to moss surfaces, and differences in solubility. In addition, the ionization states of these aniline-based compounds are pH-dependent (László et al., 2007), which may further influence their solubility and environmental behavior. The solubility of these compounds remains poorly characterized, with discrepancies among reported values (B.C. Ministry of Water, Land, and Resource Stewardship, 2025), highlighting the need for further research. The role of particle size on PPDs retention by *P. schreberi* will also require further dedicated research. Nonetheless, this study demonstrates the feasibility of using moss to biomonitor antiozonants and their derivatives in atmospheric deposition. Our results show that the accumulation of PPDs by *P. schreberi* correlates with exposure, an important prerequisite for biomonitoring applications. Indeed, the concentrations and frequencies of PPDs were consistent with the predicted exposure, with roadside sites being the most contaminated, non-urban sites the least contaminated, and parks/playgrounds occupying an intermediate position. The dominance of 6PPD, the antiozonant found in the highest concentration across all sites, aligns with findings from other studies demonstrating 6PPD as the most prevalent PPD in surficial sediments, run-off water, and roadside soils (Zeng et al., 2023; Cao et al., 2022). This suggests that 6PPD is a reliable marker for environmental PPDs dispersal.

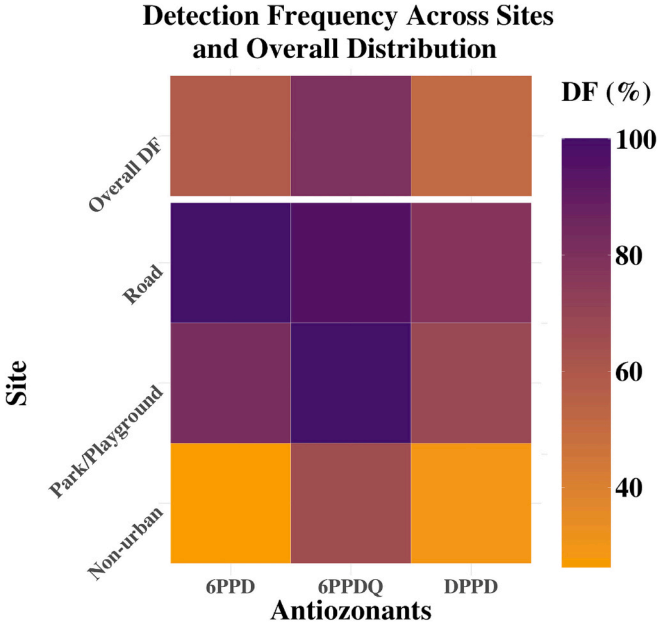

Fig. 6. Detection frequencies of antiozonants. Data are presented in % of detection across all sites (top row), roadside sites (second row), park/playground sites (third row), and non-urban sites (bottom row).

4.2. *Atmospheric dispersal of PPDs in southern Quebec*

Results reveal the diffuse contamination of terrestrial ecosystems by PPDs throughout southern Quebec (Fig. 4 and Sup. Info Fig. S.2). Tire wear has been identified as an important source of PPDs to aquatic systems through road runoff (Jaeger et al., 2024; Werbowski et al., 2021). The consistency between exposure to traffic (roadside vs parks/playgrounds vs non-urban sites) and PPDs concentration suggests that tire wear is likely an important source of PPDs in terrestrial ecosystems as well. Wind contributes to the long-distance transport of airborne particles (Zeng et al., 2023) and is a likely mechanism for the dispersal of particulate-borne antiozonants. The 6PPD/6PPDQ ratio has been reported to decrease with atmospheric transport of antiozonants from pollution sources, with the highest ratios observed in vehicle and road dust and in urban rivers (Zeng et al., 2023; Huang et al., 2021). This suggests that this ratio is influenced by environmental transformations (exposure to light, ozone, temperature) and by the higher stability and persistence of quinone forms in the environment rather than direct emissions (Jiang et al., 2024b; Zeng et al., 2023). The 6PPD/6PPDQ ratio data support the emission of antiozonants from vehicular tire wear, followed by atmospheric wind dispersal. Roadside sites exhibited the highest ratios, followed by parks/playgrounds and non-urban sites. The 6PPD/6PPQ ratio emerges as a potential proxy for estimating the dispersal of antiozonants from anthropogenic sources. The degradation of 6PPD into 6PPDQ is facilitated by environmental factors such as ozone, sunlight, and temperature (Jiang et al., 2024b), suggesting that higher residence time in the environment associated with longer transport from point sources could result in lower 6PPD/6PPDQ ratios. Indeed, the highest ratios were observed in vehicle and road dust and in urban rivers (Zeng et al., 2023; Huang et al., 2021). The 6PPD/6PPDQ ratio measured in moss decreased with the distance from potential point sources, with the highest ratios observed in roadside sites, followed by parks/playgrounds, and with non-urban sites achieving the lowest ratios. We interpret this trend as indicative of significant emissions of antiozonants from vehicular tire wear (roadside sites), followed by atmospheric dispersal. Further research evaluating the effect of environmental factors (e.g., light, ozone) across different climatic contexts and seasons on the 6PPD degradation to 6PPDQ is required to validate observations made here and elsewhere (Zeng et al., 2023; Huang et al., 2021) suggesting that the 6PPD/6PPDQ ratio could be used as a proxy of antioxidant dispersal from point source emissions. Further research, using transects and replicated traffic intensities is required to further characterize the role of tire wear as a point source for diffuse contamination of antiozonants in southern Quebec and elsewhere. Nonetheless, the ubiquitous presence of antiozonants in moss throughout southern Quebec shows that PPDs are subject to atmospheric transport and calls for broader environmental risk assessments. Indeed, the toxicity of antiozonants to aquatic species is well documented (e.g., Tian et al., 2021; Brinkmann et al., 2022; Lo et al., 2023; Nair et al., 2023), but data for terrestrial organisms are lacking, hindering a comprehensive assessment of the effect of the observed dispersion of PPDs on terrestrial wildlife. Further investigation into the effects of environmental factors, such as meteorological parameters and elevation gradients, on the fate and dispersion of PPD particles is also recommended in future studies. Previous studies have demonstrated the influence of topography and sandstorms on the dispersion and deposition of airborne pollutants (Geng et al., 2025; Zhang et al., 2017). In addition, positive correlations between wind speed and PM2.5-bound PPDs, as well as directional influence, have also been documented, underscoring the importance of these variables in shaping contaminant patterns (Jiang et al., 2024a). Regulatory thresholds for synthetic organic compounds such as 6PPD are urgently needed. The persistence of these compounds warrants similar regulatory scrutiny to that applied to persistent organic pollutants (POPs).

4.3. Improvement and further directions

Standardizing moss sampling and analysis methodologies is essential to establish a robust framework for applying moss as a non-invasive and effective biomonitoring tool for PPDs and to ensure consistency and comparability across studies. To support future standardization efforts, a checklist of key field data parameters to guide moss sampling campaigns is provided in Table S.10. For instance, our data show that washing samples before analysis reduces variability by removing loosely bound particles on the moss surface. Future research is needed to fully assess the implications of washing on overall exposure assessment. The choice to wash samples should be made based on the study objectives, notably on the interest to account for loosely bound particles. In addition, the use of freeze-drying (lyophilization) instead of mild oven drying should be prioritized in future work to further minimize any potential degradation of sensitive PPD derivatives during sample preparation. This study used one species sampled primarily in late spring (May–June), with additional samples collected in August. Further research is required to evaluate the efficiency of different species in recording various PPD compounds and to test for seasonal variability in moss PPD concentrations. Indeed, the ability to record contaminants is often species-specific, complicating comparisons across different biomonitors (Chaos et al., 2024; Dołęgowska and Migaszewski, 2011). Seasonal variability in the biomonitoring of various contaminants is also well documented (Augusto et al., 2013; Michel et al., 2024). Year-long investigations are required to characterize such variability and guide sampling strategies. The 6PPD/6PPDQ ratio emerges as a potential proxy for environmental degradation associated with the length of dispersal. However, the degradation of 6PPD to 6PPDQ is facilitated by high temperatures and sunlight-promoting ozone reactions (Jiang et al., 2024b). Better characterizing how climate variables affect the 6PPD/6PPDQ ratio is essential to properly use this ratio as a proxy of atmospheric transport, especially when comparing data across seasons and locations with different climates. Furthermore, the concentration of contaminants in moss results from a balance between accumulation kinetics and biomass growth rates. Because exposure to contaminants and environmental factors (e.g., temperature, humidity) can affect growth, accounting for seasonal and locational differences in growth rates is important for accurately estimating depositions. The potential influence of urban vegetation on the dispersion and deposition of airborne antiozonants, as suggested for other airborne contaminants (Wei et al., 2017; Chen et al., 2016; Linden et al., 2023), warrants further investigation in future studies using quantitative vegetation data. Such investigations could provide critical insights into leveraging urban and natural green spaces as ecological barriers to mitigate environmental contamination by tire-related antiozonants and their derivatives.

5. Conclusion

This study confirms the potential of mosses as biomonitors for antiozonants in atmospheric deposition. Our findings also support the hypothesis that the 6PPD/6PPDQ ratio may serve as a marker of antiozonant dispersion, though further validation is needed to confirm its utility as a proxy for long-range transport. This study also comprehensively assesses the environmental dispersion and spatial distribution of 6PPD, 6PPDQ, DPPD, and IPPDQ across Southern Quebec. It highlights diffuse contamination by antiozonants throughout southern Quebec, with roads emerging as an important hotspot for antiozonants emission to the atmosphere. Further research is required to characterize the mechanisms underpinning antiozonant accumulation in moss and the impact of environmental variables on the quality of the records. Developing standardized sampling and analysis protocols is essential to enhance reliability and ensure comparability of data between studies.

CRediT authorship contribution statement

Shaghayegh Ramezany: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Guillaume Martinez:** Methodology. **Adrien Mugnai:** Validation, Methodology, Formal analysis. **Daniel Houle:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Jean-Phillippe Bellenger:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shaghayegh Ramezany reports financial support was provided by Quebec Research Fund Nature and Technology. Jean-Phillippe Bellenger reports financial support was provided by Natural Sciences and

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180047.

Data availability

Data will be made available on request.

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