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Short Communication (BioRisk)

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Abstract

According to the Lancet Commission, air pollution is one of the single largest environmental causes of premature death in the world. Particulate matter (PM), including metals, are pollutants of concern. Bryophytes, especially mosses, have been used for many decades as low-cost biomonitors of environmental deposition of heavy metals. In this exploratory investigation, we use strategically placed *Sphagnum* moss paired with portable X-ray fluorescence (pXRF) analysis as a living biomonitor for air / particulate metal pollution in and around the city of Chicago, Illinois, U.S.A. Preliminary results reveal a correlation between urbanization and some metal particulate matter, especially iron. The study warrants further investigation and supports the use of mosses and pXRF spectrometry as a rapid, low per-sample cost, and potentially accurate alternative to conventional metal pollution monitoring.

Keywords

Biological monitoring, bryophytes, heavy metals, mosses, *Sphagnum*, particulate matter, air pollution, pXRF, Chicago

Introduction

The impact of air pollution represents one of the most pressing environmental health challenges of our time and has been well documented, e.g., Manisalidis et al. (2020); Chen and Kan (2008); Sharma, S. B. et al. (2013); Fuller et al. (2022); and Shleag et al. (2024). The 2018 Lancet Commission on pollution and health identified air pollution as the single largest environmental cause of disease and premature death globally, accounting for 16% of all deaths (Landrigan et al. 2018). Recent studies have established connections between air pollution exposure and COVID-19 severity (e.g. Liang et al. 2020; Travaglio et al. 2021). Urban environments are particularly vulnerable to air pollution with cities serving as potential hotspots for air pollution-related diseases (Nieuwenhuijsen 2020). The exact extent of health effects at the city level from urban air pollution remains largely unknown (Khomenko et al. 2021). This lack of knowledge is particularly alarming in the United States, given it is among the most urbanized countries globally; in 2011 alone urbanization had sprawled 11% relative to 2001 (Bounoua et al. 2018).

Air pollution comprises a complex mixture of gaseous and particulate components, each potentially harmful to the cardiovascular and respiratory systems (Hamanaka and Mutlu 2018). Particulate matter (PM) represents a critical component in air pollution with well documented health impacts (e.g. Aryal et al. 2021). Common components of PM include nitrates, sulfates, polycyclic aromatic hydrocarbons, endotoxins, and heavy metals such as iron (Fe), copper (Cu), nickel (Ni), zinc (Zn), and vanadium (V) (Brook et al. 2010; Newby et al. 2015). We note that the term “heavy metal” has been broadly applied in the plant sciences, but not without controversy, since the term ‘‘heavy metal’’ seems to suggest that these metals (or their compounds) are toxic (Appenroth 2010). Heavy metals (HMs) specifically are emitted to the environment from common urban sources such as transportation, industry, and urbanization, as well as from fossil fuels and agriculture (Koz et al. 2008). Specifically, transportation has been shown to contribute Cu and Zn due to wear of brake linings (Liu et al. 2023).

Biomonitoring is generally defined as the systematic use of living organisms or their responses to determine the condition or changes in the environment (Thomas 1961). Biological monitoring is an important tool for evaluating the negative impacts of human activities on the atmosphere (Alam 2018). Green land plants, including bryophytes, have been effectively used as air pollution indicators for many years (Badamasi 2017). Bryophytes are of great ecological and environmental significance, playing an important role as possible indicators of climate change (Lindo et al. 2013; Ruklani et al. 2021), in nutrient cycling (Rieley et al. 1979), and, through their water retention, reducing soil nutrient loss and flooding risk (Anderson et al. 2010). Bryophytes also serve as the “macrophytes,” providing a matrix where many microscopic organisms live, including tardigrades, mites, rotifers, micro-mollusks, microalgae, microfungi, and prokaryotes (Gerson 1982; Huttunen et al. 2017).

Mosses, in particular, have been used for over five decades to estimate atmospheric heavy metal deposition (Ruehling and Tyler 1968). Similarly, liverworts have been used as biomonitors of heavy metal pollution (e.g. Basile et al. 2013; Postiglione et al. 2025). Since 1990, an ongoing moss survey has had an important role in identifying spatial and temporal trends in atmospheric heavy metal pollution across Europe (Harmens et al. 2010). Recently, mosses have also been used as a biomonitor for the atmospheric deposition of anthropogenic microfibres - a ubiquitous environmental contaminant (Roblin and Aherne 2020). Bryophytes, and mosses in particular have been shown to be effective low-cost biological monitors of metal air pollution because they readily accumulate particulate matter over time, reflecting long term pollution levels (e.g. Fernández et al. 2015; Messager et al. 2021). Bryophytes have a number of morphological and physiological properties that make them ideal as bioindicators. These have been extensively reviewed by Blagnytė and Paliulis (2010), for example, their relatively small size, an ability to survive in highly polluted areas, they lack roots and obtain nutrients needed for vital processes from wet and dry deposition, and are resistant to many substances that are highly toxic to other organisms. Interestingly, mosses have also been used as possibly useful biomonitors for the atmospheric deposition of microfibers and microplastics (Roblin and Aherne 2020).

There are two well established types of biomonitoring using mosses to evaluate atmospheric contamination: 1) passive biomonitoring, using moss that grow naturally in a particular area, and 2) active biomonitoring, by transplanting moss from other locations (Ares et al. 2012). Spectroscopic techniques to determine the concentration of particulate matter in moss samples require expensive instrumentation and involve time consuming sample preparation protocols with heavy use of reagents (Messager et al. 2021). In-situ and laboratory X-ray fluorescence (XRF) spectroscopy of mosses has been presented as a rapid, low-cost, and accurate alternative to conventional metal pollution biomonitoring (e.g. Koz et al. 2008; Šoltés and Gregušková 2013; Messager et al. 2021).

Here we present an exploratory investigation into the potential application of mosses (*Sphagnum*) as a biological monitor for particulate matter in Chicago, Illinois, U.S.A. using pXRF spectrometry analysis. The aims of our study include: 1) to provide an initial evaluation of the potential use of mosses as biological indicators for air pollution, particularly heavy metals; 2) to explore if pXRF spectroscopy is an effective screening mechanism that warrants further study; and 3) to provide a preliminary investigation into different laboratory protocols and material preparations.

Materials and Methods

Early pilot studies testing feasibility

Between 2017 and 2024, we conducted a series of preliminary pilot studies to assess the feasibility of using portable X-ray fluorescence (pXRF) spectroscopy and mosses as biomonitors of atmospheric heavy metal deposition. These early efforts included in situ sampling across Chicago, testing various handheld and benchtop pXRF units, testing various species of moss, and deploying *Sphagnum* moss bags in collaboration with local volunteers. While these initial investigations were exploratory and varied in methodology, they laid the foundation for the standardized, systematic approach described in this paper. Additional details and results from these earlier efforts are included as supplemental material to recognize contributors and provide context for the evolution of the project (See https://zenodo.org/records/15097659 and data availability below).

Moss Preparation and Processing

We used two primary sources of *Sphagnum*: 1) Commercial *Sphagnum* moss (*Sphagnum squarrosum*) was purchased from Moss Acres (Honesdale, PA, USA) in June 2024 and 2) *Sphagnum fallax* from Maine, collected by Blanka Shaw in June/July 2023, 2024. Supplementary files, including experimental detail, data, and presentations are available at https://zenodo.org/records/15097659. The voucher is available at Field Museum and can be accessed here (https://collections-botany.fieldmuseum.org/catalogue/4871426). The moss was carefully cleaned to remove invertebrates, debris, and non-moss species, retaining only bright green segments. Rhizomes and pale portions were manually removed and discarded. Samples were air-dried overnight on newspaper, followed by screening through a grated mesh to remove remaining particulate matter.

Sample Washing Protocol

Three sequential washing treatments were applied using 500mL Chicago tap water in aluminum trays. Moss samples were soaked and gently agitated for 5 minutes in each tray, with excess water removed between treatments. Following the final wash, remaining debris was removed with forceps, and samples were air-dried for 48 hours.

Moss Bag Preparation

Standardized 1-gram samples of prepared *Sphagnum* were distributed into 7.5×10 cm mesh bags with drawstring closures. Each bag received a unique identifier and was attached to a 20 cm hanging string with a plastic plant label secured by a binder clip.

Site Selection and Deployment

Distribution sites were selected based on accessibility and safety considerations, representing a hypothesized potential pollution sources including 1) an industrial point source (a metals recycling facility); 2) an industrial/transportation corridor (Indian Ridge Marsh); 3) a transportation hub (Des Plaines railroad station); 4) a semi-natural reference area (a Lake County natural area); and 5) a vehicle exhaust study (moss bags attached to a 2006 Ford F-350 diesel truck). Control samples were maintained at the Field Museum under both sealed (plastic bag) and exposed conditions. Deployment occurred during June-July 2024, with collection after 1, 3, and 4-week exposure periods.

pXRF Analysis

Retrieved moss samples were processed using liquid nitrogen in a clean mortar and pestle to create homogeneous powder. Powdered samples were packed into labeled pXRF sample cups and sealed with Prolene thin-film membrane to prevent contamination. Analysis was conducted using a Tracer 5 Bruker pXRF instrument with identical settings across all samples. Target elements included titanium (Ti), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn). Multiple measurements were performed on each sample to improve data reliability.

Reference samples, including those washed in different water sources and those left unwashed, were analyzed to compare the effects of the washing treatments on metal levels. Detailed settings and procedures followed to operate the pXRF unit are extensively outlined at https://zenodo.org/records/15097659 and data availability below.

Data Analysis

Results are reported as ratios of exposed sample counts to unexposed sample counts ("ratio of counts/control"). Six replicate analyses were performed on 2-3 moss bags from each location, providing 12-18 measurements per element per location. Given that there were some differences in metals content between the two sources of moss, we kept them separate, and analyzed results of exposed moss to reference samples from the same moss sources.

Results

Atmospheric Heavy Metal Capture

Twelve moss bags were deployed across four Chicagoland locations during June and July of 2024. After 25-30 days exposure, pXRF analysis revealed location-specific metal accumulation patterns compared to reference samples (Figure 1). The metals recycling facility (site 1) showed elevated levels across all measured elements, consistent with its role as a multi-metal emission source. Indian Ridge Marsh (site 2), situated adjacent to industrial and transportation facilities, demonstrated significantly increased iron and nickel concentrations. The Des Plaines railroad station (site 3) exhibited elevated iron levels, potentially reflecting particulate generation from steel rail infrastructure. Notably, even the semi-natural site in Lake County (site 4) showed elevated nickel concentrations, likely attributable to its proximity (<1 km) to a major highway, potentially demonstrating the far-reaching influence of transportation-related emissions.

Vehicle Emission Detection

Six moss bags attached to a 2006 diesel Ford F-350 truck operating in the Chicago area showed time-dependent metal accumulation patterns (Figure 2). After three days of exposure, significant increases were observed in iron, zinc, copper, titanium, and manganese compared to controls. Reduction in metal levels was observed after four days of exposure which coincided with significant rainfall in the Chicago area, suggesting potential washing effects on accumulated particulates. This observation highlights the importance of considering meteorological factors in biomonitoring protocols.

Discussion

This exploratory study demonstrates the feasibility of using standardized *Sphagnum* moss bags coupled with pXRF analysis for monitoring atmospheric particulate matter in urban environments. Our results align with a growing body of evidence supporting this technique as a cost-effective tool for preliminary environmental contamination assessments as rapid, low per-sample cost, and accurate alternatives to conventional metal pollution biomonitoring (Messager et al. 2021; Fernández et al. 2015).

Heavy Metal Sources and Patterns

The metal accumulation patterns observed in our study are consistent with known urban emission sources. Elevated iron levels across multiple sites likely reflect its widespread presence in urban particulate matter, predominantly originating from vehicle exhaust, brake wear, rail transport, and industrial processes (e.g., Abbasi et al. 2013; Mohsen et al. 2018; Mayer et al. 2024). The high iron recorded near railroad infrastructure supports previous studies linking rail transport with significant metal particulate emissions (e.g., Abbasi et al. 2013; Mohsen et al. 2018). Copper and zinc accumulation in mosses exposed to vehicle emissions reinforces established associations of these metals with transportation sources, particularly from brake lining abrasion and tire wear (Pearson et al. 2000; Mayer et al. 2024). Elevated nickel concentrations detected at the semi-natural Lake County site site suggest the possibility of regional atmospheric metal dispersion potentially linked to transportation-related emissions, a pattern observed in European moss monitoring studies (ICP Vegetation 2020; Godzik 2020). Interestingly, recent μ-SRXRF analyses provided insights into how heavy metals distribute at the microscale within moss tissues, enhancing our understanding of accumulation dynamics observed in *Sphagnum* bags (Weinberger et al. 2025). Similarly, moss biomonitoring has been successfully applied to map detailed spatial patterns of metal contamination in urban environments, underscoring the utility of moss bags in pollution assessments (Urošević et al. 2023).

Methodological Considerations

pXRF spectroscopy proved valuable for rapid, cost-effective screening of metal concentrations in moss samples. The ratio-based analytical approach effectively normalized variations in the moss sources, providing meaningful comparisons across sites and exposure periods. While our washing protocol appeared adequate in removing surface contamination and maintaining moss bioaccumulation capacity, future standardization of sample preparation, particularly washing duration, water quality specifications, and drying protocols, would enhance reproducibility and comparability of results (Fernández et al. 2015; Messager et al. 2021). Temporal dynamics observed in vehicle-mounted samples emphasize the need for standardized exposure durations and incorporation of meteorological data. The observed decrease in accumulated metals after rainfall underscores the significant impact weather conditions have on biomonitoring outcomes and developing a bag design to circumnavigate this.

Broader Implications

Our study addresses a notable research gap in North American bryophyte biomonitoring, complementing the established European moss survey network (ICP Vegetation 2020). Demonstrating the effective application of moss biomonitoring in Chicago indicates strong potential for broader adoption across other US urban regions. Moss bag biomonitoring offers a cost-effective, community-accessible alternative to laboratory-based monitoring, enabling resource-limited communities disproportionately affected by pollution to document environmental contamination and advocate for remediation. Combining moss biomonitoring with portable XRF technology can facilitate community-based environmental monitoring, enabling frequent and widespread pollution assessments at significantly reduced costs (Messager et al. 2021).

Study Limitations

Several limitations must be recognized. Our study's exploratory nature involved limited sample sizes and temporal coverage. Although weather impacts were noted, systematic meteorological evaluations were not conducted. Additionally, commercially sourced Sphagnum moss, while facilitating standardization, may introduce baseline contamination variability compared to locally sourced material (Fernández et al. 2015). Other potential sources of contamination include the water used to wash the mosses, and the newspaper on which they were dried. Furthermore, pXRF provides semi-quantitative results, and may have detection limits unsuitable for low-level contamination scenarios (Šoltés and Gregušková 2013; Messager et al. 2021).

Future Research Directions

This preliminary study suggests multiple avenues for future research. Replication of established moss biomonitoring protocols would enhance methodological consistency, allowing broader comparative analyses (ICP Vegetation 2020; Fernández et al. 2015). Expanding this approach to additional urban settings and pollution sources could elucidate broader regional pollution patterns and facilitate comprehensive air quality assessments.

Integrating meteorological data and examining seasonal variations would improve understanding of temporal dynamics in metal accumulation. Direct comparisons between active (transplanted) and passive (native) moss monitoring techniques could further optimize deployment strategies to achieve specific environmental monitoring objectives (Ares et al. 2012).

Utilizing historical herbarium moss specimens for retrospective pXRF analyses presents a compelling opportunity for reconstructing historical pollution profiles, evaluating long-term environmental changes, and assessing remediation effectiveness (Belloeil et al. 2021). Emphasizing community-based biomonitoring initiatives could significantly enhance environmental justice efforts by providing accessible tools for marginalized communities facing disproportionate pollution exposure (Fuller et al. 2022).

Conclusion

This study highlights the practicality and effectiveness of using Sphagnum moss coupled with pXRF analysis for urban atmospheric pollution biomonitoring. The approach offers significant promise as a cost-effective, accessible environmental monitoring tool. Future research, including broader geographical application and methodological standardization, will strengthen its utility in addressing environmental justice and promoting sustainable urban air quality management.

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Data availability:

All data and procedures used in the preparation of this paper are available at: https://doi.org/10.5281/zenodo.15097659

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Figure 1: Ratio of pXRF counts for exposed samples to pXRF counts for unexposed samples for six metals. The horizontal line represents a ratio of 1, i.e., no net accumulation of that metal in the exposed samples. Error bars represent the standard deviation of the ratio of the measurements of the exposed samples to the unexposed samples.

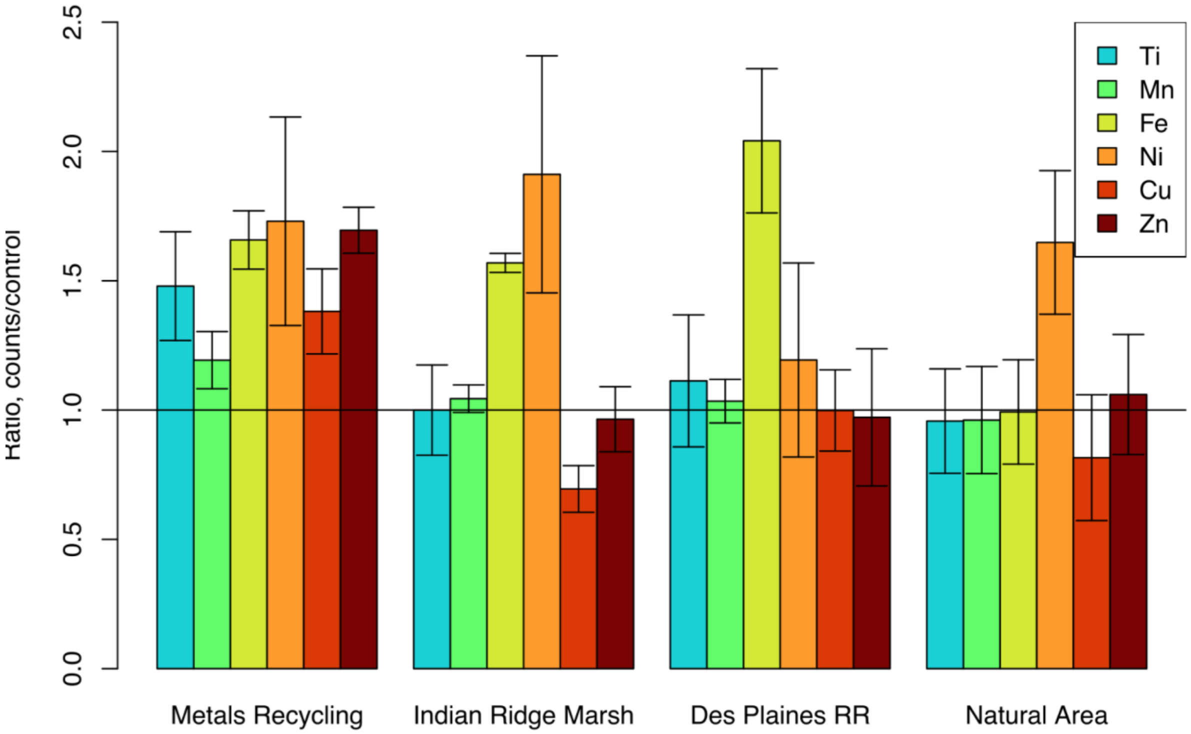

Figure 2: Ratio of pXRF counts for exposed samples to pXRF counts for unexposed samples for six metals, versus exposure time of the samples.