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Moss Bags as Active Biomonitors of Air Pollution: Current State of Understanding, Applications and Concerns

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ABSTRACT

Dual concerns involving the rise in airborne pollutant levels and bulging need to protect-preserve human health have propelled the search for innovative means for air quality monitoring to aid in evidence-based decision-making (pollution prevention-mitigation). In this regard, *moss bags* have gathered a great deal of attention as *active* biomonitors. In this reflective discourse, we systematically review the world literature to present a bird's eye view of moss bag applications and advances while highlighting potential concerns. We begin with a brief note on mosses as biomonitors, highlighting the advantages of moss bags over the *passive* technique (native moss), other living organisms (lichens, vascular plants), and instrument-based measurements. A major strand of moss bag research involves urban ecosystem sustainability studies (e.g., street tunnels and canyons, parks), while others include event-specific monitoring and change detection (e.g., SARS-CoV-2 Lockdown), indoor-outdoor air quality assessment, and change detection in land use patterns. Recent advances include biomagnetic studies, radioisotopic investigations, and mobile applications. Efforts are currently underway to couple moss bag results with a suite of indicators [e.g., relative accumulation factor (RAF), contamination factor (CF), pollution load index (PLI), enrichment factor (EF)] and spatially map the results for holistic appraisal of environmental quality (*hot spot* detection). However, while moss bag innovations and applications continue to grow over time, we point to fundamental concerns/uncertainties (e.g., lack of concordance in operational procedures and parameterization, ideal species selection, moss vitality) that still need to be addressed by targeted case studies, before the moss results could be considered in regulatory interventions.

INTRODUCTION

Worldwide surges in air quality-induced public health disorder in the recent past have prompted research to devise novel air quality monitoring techniques (Kumar & Chaudhuri 2022a, b, Kumar and Chaudhuri, 2021) to aid in more informed regulatory decision-making (pollution prevention-mitigation). In the process, an emerging strand of research is devoted to the exploration of various biomonitor species, including vascular plants (trees) (Chaudhuri & Kumar 2022), lichens (Malaspina et al. 2018), and mosses (Vuković et al. 2014, Capozzi et al. 2019, Ştefănuţ et al. 2019, Chaudhuri & Roy 2023). Biomonitoring, with the strategic use of living organisms as natural markers of environmental conditions, has been a growing interest among the world research community (Guarino et al. 2021). In this regard, mosses as biomonitors of ambient air quality have attracted a great deal of attention (Zechmeister et al. 2006, Świsłowski et al. 2022).

Moss-based biomonitoring comes under two main types: passive and active. While the former involves natively occurring species (Chaudhuri & Roy 2023), the latter uses moss transplant in specially constructed bags, exposed over a certain period for natural interception of airborne pollutants (Urošević et al. 2017). Moss bags have been used in a variety of applications involving metals and metalloids (Shvetsova et al. 2019; Zechmeister et al. 2006), polycyclic aromatic hydrocarbons (PAHs) (Di Nicola et al. 2013), atmospheric microfibers (Bertrim & Aherne 2023), and radionuclides (Isidora et al. 2015). In this reflective article, we systematically scan the moss bag literature, using a universally recognized method -the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Parakh & Chaudhuri 2023, Adithyalakshmanan et al. 2022), to present a summary view of the experiences and advances using moss bags alongside the concerns and potential future research directions.

We initiate the article by reflecting on the advantages of mosses as biomonitors, followed by a comparative overview of moss bags as against naturally occurring moss (passive techniques) and other organisms (lichens, trees), as well as traditional, instrument-based air quality measurements. Next, we highlight applications of moss bags in various domains of air quality monitoring and assessment. However, an implicit idea in envisioning this study was also to highlight the uncertainties/ambiguities around moss bag research. To that end, in the final sections, we point to certain areas that will require targeted research in days ahead for moss bags to be included in formal regulatory mechanisms. Overall, an implicit motive to present this narrative was to invite process-level discourses to discover newer avenues to expand the reach of moss bags as active biomonitors of ambient air quality. An additional motivation to imagine this research came from our belief that observations made in this narrative may even contribute to the growing research areas of several of the UN sustainable Development goals (UN SDGs) tied to air quality - Good Health and Well Being (SDG 3), Sustainable Cities (SDG 11), Life on Land (SDG 15), Partnership building (SDG 17), to name a few. Collectively, we expect, strategic and well-informed use of moss bags may become a widely adopted regulatory tool to align with climate combat interventions (SDG 13), especially for nations challenged by multidimensional financial-infrastructural shortcomings to install traditional sensor-based monitoring networks.

MATERIALS AND METHODS

We identified relevant literature using the multi-stage PRISMA methodology *(Preferred Reporting Items for Systematic Reviews and Meta-analyses)* (Chaudhuri et al. 2021). The PRISMA literature search and selection methodology comprises four main stages:

I. **Initial Document Identification:** The search is initiated by using the search words/phrases (listed below) in a variety of search engines, including SCOPUS, PubMed, Science Direct, Springer Lin, Blackwell, and Social Citation Index, Web of Science (WoS), EconLit, JSTOR, and complemented with Google Scholar. This stage of PRISMA identified 84 documents.

II. **Screening/Exclusion:** Documents selected by the previous stage were subjected to further checks using certain pre-established rules (or norms), such as duplication of results, abstract-only documents, and documents published in foreign languages, to name a few. 21 documents were excluded from the initial selection.

III. **Eligibility Test:** To further refine and eliminate articles, 18 documents did not meet the eligibility criteria, such as objectives not aligning adequately with the present study, too many confounding variables, research methodology not clearly outlined, lack of process-level interpretations, no discussion on limitations and/or on future research directions.

IV. **Final Inclusion:** At the final stage of PRISMS, 46 documents were retained to be considered for review in the main body of the research

Search words/phrases were divided into 5 categories:

Category I: ‘active biomonitor*,’ ‘moss bag.’

Category II: ‘species,’ ‘Sphagnum,’ ‘Hypnum,’ ‘Pleurozium.’

Category III: ‘trace metal,’ ‘heavy metal,’ ‘PAH,’ ‘polycyclic *,’ ‘pollutant*.’

Category IV: ‘clean*’, ‘wash*’, ‘dry*’, ‘shape’, ‘mesh *’, ‘pack*’, ‘exposure *’

Category V: ‘standardize*’, ‘meteorological *’

For each category between II and IV, the term moss bag and/or active biomonitoring was added as a suffix or prefix. The asterisk symbol (‘*’) was used as a wildcard to expand the search horizon. In the final step, 104 documents were retained to be included in the review (Chaudhuri et al. 2020).

General Observations

Why moss: A confluence of physiological traits makes mosses ideal air quality sensors (Chakrabortty & Paratkar 2006, Chaudhuri & Roy 2023), including their ubiquitous geographic occurrence lack of real root system (only supporting pseudo roots), which means that nutrient uptake by mosses is not from mineral substrates (soil) but solely from ambient air; high surface-to-volume ratio; high cation exchange capacity (mainly due to presence of large number of organic functional groups) that increases pollutant interception efficiency. Pollutant uptake/interception by mosses occurs via three broad mechanisms: (a) as an aqueous solution, gas, or solid particles and can adhere to the moss cells’ surfaces *(intercellular fraction)*; (b) outer walls (via ion exchange process) *(extracellular fraction)*, and/or (c) be included into cells (via passive or active transport) *(intracellular fraction)* (Popovic et al. 2010, Fernández et al. 2013).

Moreover, the lack of waxy cuticles on moss leaves (single-layered) further facilitates *direct* pollutant interaction through dorsal and ventral surfaces. Another unique attribute is their slow and long growth cycle coupled with a low degree of differentiation (Gao et al. 2023). The growth points at the tips of stems and branches usually stimulate the continuous and periodic division of cell groups in the lower parts of mosses, which make them evergreen and, in turn, suitable for seasonal assessment of pollutants (Kromar et al. 2007).

Certain studies even identify mosses as better biomarkers of air quality than trees (vascular plants) and/or lichens (Di Nicola et al. 2013). In a study to assess heavy metal (Fe, Zn, Mn, Cu, Ni, Cd, Pb) pollution due to industrial activities (Harjavalta Cu-Ni smelter, Finland), Saleema et al. (2004) observed the highest metal concentrations in mosses, followed by lichens and lowest in vascular plants. In another comparative assessment between the epiphytic moss *Scorpiurum circinatum* and the epiphytic lichen *Pseudevernia furfuracea*, involving a suite of heavy metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, V, and Zn), Basile et al. (2008) found that the Scorpiurum sp. had the highest bioaccumulation capacity for all metals and showed a more constant and linear accumulation trend than the lichen. In another study involving 14 different moss and lichen species near the Sivas-Tokat motorway, Turkey, Mendil et al. (2009) found higher bioaccumulation capacity in mosses for several trace and heavy metals (Fe, Mn, Pb, Ni, Cr, Cu, and Cd). Moreover, in comparison with lichens, mosses are more geographically extensive in their distribution, more commonly occur in urban areas, and are more convenient to use by employing passive and active accumulators (Sun et al. 2009, Jiang et al. 2018).

Moreover, unlike traditional instrument-based sensors, mosses can offer an integrated profiling of the ambient element pollution over a certain period (Urošević et al. 2017). Due to their continuous accumulation of elements, mosses offer information about the sources of pollution long after the pollution episode itself took place (Golubev et al. 2005). Other advantages of moss-based biomonitoring include uniformity of receptor surface and exposure period, year-round availability, well-defined background concentrations of contaminants, non-destructive sampling, and greater collection efficiency of most elements (Shotyk et al. 2015).

Why moss bags: Compared to the passive technique (natively growing moss), moss bags appear more controllable as most parameters are known and could be conveniently pre-set as per the research requirements (Ares et al. 2012). Moss bags reduce the high degree of variability in the uptake of contaminants, which is a common case with native moss (Giordano et al. 2009, Adamo et al. 2007).

A unique advantage of moss bags is locational flexibility, which means they can be installed anywhere and everywhere, allowing a dense monitoring network with wider area coverage (Limo et al. 2018, Capozzi et al. 2016). This improves pollutant monitoring efficacy, for example, by generating high-resolution spatial maps to facilitate improved regulatory decision-making (Kosior et al. 2015, Aničić et al. 2009). The locational advantage is particularly advantageous for point-source pollution monitoring (e.g., industrial emissions) (Makholm & Mladenoff 2005) by placing the bags at strategic locations as per the research requirement. Moreover, as moss bags can be placed at different elevations, they help in vertical profiling of pollutant species (atmospheric stratification patterns) (Ares et al. 2012). This is useful in pollution dispersion studies (e.g., long-range pollutant transport mechanisms).

Other advantages of using mosses include simplicity in operations, lack of the need for a power supply, or regular maintenance (calibration-validation, repair-replace). Moss bags offer better opportunities for temporal assessment of air pollutant patterns as the initial concentrations of target pollutants within moss tissues are known, and the *exposure periods* are fixed (Zechmeister et al. 2006, Aničić et al. 2009). In general, moss literature recommends the use of the passive technique for long-term studies (e.g., decadal assessment, as observed in the European Moss Survey (EMS; 1990-present) (Chaudhuri et al. 2023) with larger spatial dimensions (e.g., national to transnational), while moss bags serve better for micro-environments (e.g., urban habitats) over shorter periods (Gribacheva et al. 2021). However, an often-overlooked aspect of the moss bag approach is, as Zechmeister et al. (2020) pointed out, relatively small increments of pollutant accumulation over time, as compared to the pre-exposure concentrations. Moreover, mosses for the bag should be collected from background areas (unpolluted), which, in recent times, has become quite hard to find (Meier et al. 2015). Therefore, increments in pre-exposure concentrations can only be observed after long exposure periods or at high pollution levels. However, there could be several issues of bag wear and tear when exposed over a prolonged period (rain, storm, human intervention)

Applications and Experiences

Urban sustainability studies: A prime focus of moss bag applications is urban sustainability studies (Table 1). Moss bags are deemed particularly useful for urban/per-urban habitats where natively growing mosses are rare, where occurrences of native mosses are low due to lack of natural environment and rather the predominance of built-up surfaces (paved, walled, artificially landscaped) (Adamo et al. 2007, Urošević et al. 2017, Milicevic et al. 2017, Sorrentino et al. 2021a). To that end, a rich body of research explores moss bag applications in urban street canyons (Vuković et al. 2015, De Nicola et al. 2013, Zechmeister et al. 2006), parks (Shvetsova et al. 2019), and/or parking garages (Vuković et al. 2014). Along that line, moss bags have been used in more specialized urban applications to meet regulatory decision making. For example, In Belgrade, Siberia, Urošević et al.

Table 1: Selected applications of moss bags in urban air quality monitoring and assessment. (Authors' own compilation from various moss bag literature).

Reference	Experimental Design	Observations
Isidora et al. (2015)	• Location: Belgrade • Target Pollutant (s): Lead radioisotopes (^{204P}b, ^{206}Pb, ^{207}Pb, ^{208}Pb) • Environment: Street canyon • Moss: *Sphagnum. girgensohnii*	• Generation of Kohonen self-organizing map (SOM) based on Pb-isotope concentrations in moss tissues • Identification of distinct spatial clusters to aid in regulatory decision-making: *delineating pollution hot spots* • Detection of small differences in samples that apparently are very similar
Di Nicola et al. (2013)	• Place: Naples, Italy • Target Pollutant(s): 11 Metals and 15 Polycyclic Aromatic Hydrocarbons (PAHs) • Environment: Urban street canyon • Moss: *Hypnum cupressiforme*	• Efficient markers for metals and PAHs at a fine spatial scale • Process-level insights: • Poorer air quality was on one side of the canyon due to (i) variations in wind directions and (ii) localized air circulation resulting in differential exposure to vehicular emissions • Moss bag outputs corroborated well with a numerical prediction model *(Atmospheric Dispersion Modelling System (ADMS)-Road)*
Zechmeister et al. (2006)	• Place: Vienna, Austria • Target Pollutant(s): 16 PAHs and heavy metals (Al, Ba, Ca, Co, Cr, Cu, Fe, Ni, Mo, Pb, V, Hg, Cd, S, Sb, Zn) • Environment: Street tunnel • Moss: *Hylocomium splendens*	• Significantly higher pollutant levels in urban street tunnels (negative health implications) • Process-level insights: • Various vehicular processes (e.g., abrasion, diesel combustion) • No dilution effect (rain and/or wind) due to localized air circulation within closed tunnel space • Efficient multi-element air quality monitoring
Salo et al. (2016)	• Place: Turku, Finland • Target Pollutant(s): Al, Cr, Fe, Na, Ni, Pb, Cd, As, Zn, V, Hg • Environment: urban parks, courtyards, kindergarten, school yards, streets • Moss: *Sphagnum papillosum*	• Ability to distinguish seasonal air quality changes (*road dust* period and summer) • Lower pollutant loadings in parks/courtyards than in urban streets • Spatially representative dataset for air quality monitoring and modeling investigations
Rivera et al. (2011)	• Place: Girona, Spain • Target Pollutant(s): NO_2 and metals (Al, As, Cd, Cr, Cu, Mo, Pb, Sb, Sn, Zn) • Environment: High-trafficked urban street • Moss: *Hylocomium splendens*	• Negative impacts of vehicular emissions on air quality • Process-level insights: • Metal concentrations were influenced by traffic intensity *(e.g., the number of bus lines in the nearest street)* • NO_2 levels were influenced by a combination of local emissions *(street traffic)* and pollutant transport/dispersion from neighboring areas • Metal levels were more spatially variable than NO_2.
Bertrim & Aherne (2023)	• Location: Southern Ontario, Canada • Target Pollutant(s): Microplastics (<5 mm) • Environment: Street canyon • Moss Species: *Pleurozium schreberi*	• Efficient tools for microplastic detection and characterization in the urban atmosphere *(fiber types, dimensions)* • A strong influence of urban intensity (population density) on microplastic levels • Identification of spatial changes in microplastic types and concentrations
Van Lateen et al. (20202)	• Location: Gena, Central Germany • Target Pollutant(s): Al, Ca, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, Ag, As, B, Ba, Cd, Co, Cr, Cs, Cu, La, Li, Mo, Nb, Ni, Pb • Environment: Railroad lines, motorway • Species: *Hypnum cupressiforme*	• Efficient multi-element pollutant detection and assessment • Ability to distinguish between natural and anthropogenic sources • Suitable for long-term air quality monitoring and assessment over larger spatial dimensions
Swisłowski et al. (2021a)	• Location: Poland • Target Pollutant(s): Polycyclic Aromatic • Hydrocarbons (PAHs) • Moss Species: *P. schreberi, S. fallax, D. polysetum*	• Efficient natural markers of PAH distribution patterns in urban environments • Ability to characterize seasonal variations • Comparable efficiency with instrument-based monitoring • Potential threats of environmental stressors on long-term moss vitality (physiological functions)

(2017) investigated the applicability of moss bags (*Hypnum cupressiforme*) to establish the baseline air quality, taking a botanical garden for control (lower traffic activity). Results indicated significantly different trace mental signatures for the garden (low metal concentrations) in comparison to adjoining streets. However, despite lower values, metal concentrations in the garden appeared significantly higher, posing health threats.

Event-specific air quality monitoring and change detection: Recent advances demonstrate that moss bags could be useful for short-term air quality monitoring for change detection. In Prószków, Poland, Świsłowski et al.

(2021b) *Pleurozium schreberi* to assess short-term changes in the atmospheric aerosol pollution with selected heavy metals (Ni, Cu, Zn, Cd, Hg, and Pb), in 2019/2020 and 2020/2021. Results demonstrated the negative impacts of New Year fireworks on local air quality, realized by elevated concentrations of all metals. In addition, the authors also observed that the SARS-CoV-2 lockdown in 2020 had an ameliorating effect on air quality (lower metal concentrations). Mao et al. (2021) conducted a moss bag study (*Taxiphyllum taxirameum*) in Xinchange City, China, to monitor short-term changes in air quality (between April 2019 and 2020) involving 12 heavy metals (Al, Cr, Fe, Cu, Ni, Pb, Mn, Hg, Zn, V, As, Ba). Results indicated that due to the lockdown restrictions to stall the SARS-CoV-2 virus spread pandemic, as the vehicular emission levels were cut down alongside tourism, it led to significant drops in ambient air concentrations of several heavy metals (e.g., Pb). However, as a downside, a drop in vehicular emissions triggered atmospheric oxidation events, giving rise to various secondary particulate matter.

Indoor-outdoor air quality: While the need for robust and long-term indoor air quality monitoring has emerged as a critical need for public health experts worldwide (WHO 2018), suitable instrumental facilities are yet limited (Zechmeister et al. 2020). Moreover, with the latter, a main constraint is that these technical devices last only for a few hours to days. In this regard, a small body of literature has used moss bags to record concentrations of various trace and heavy metals (Motyka et al. 2013). Major sources of indoor air pollutants may broadly include tobacco smoke, cooking, candles, emissions from furniture, batteries, walls, and water pipes (WHO 2010). Identifying the sources and intensity of pollutant production from them is critical to devising medical interventions. Using the *Hylocomium splendens* moss variety, exposed for 49 days in an office environment (Czech Republic), Motyka et al. (2013) found that moss bags are efficient bioaccumulators of several heavy metals (Sb, Cu, Hg, Pb, Si), occurring in particulate phases.

In this regard, a rapidly evolving area of moss bag research involves the differentiation between indoor and outdoor air quality. In a cross-country tabulation, Sorrentino et al. (2021a) performed a multi-metal assay in Italy (Ca, Mg, Co, Cr, Sr, Ti, U) and Belgium (Ag, As, Cd, Mo, Pb, and Sb) at 20-paired indoor-outdoor locations, exposing using *Hypnum cupressiforme* moss bags (12 weeks exposure period). Results indicated poorer outdoor air quality. Determination of the Indoor/Outdoor ratios, computed by moss-based metal concentrations (mostly lower than 0.75), indicated indoor air quality is strongly influenced by outdoor pollution. However, certain species have typical indoor origins (e.g., Al, Ag, Cd, Co). In a similar approach, using the *Hypnum cupressiforme* moss bags in 12 coupled indoor/outdoor exposure sites, Capozzi et al. (2019) observed significantly different air quality statuses between the outdoor and the indoor environments. For example, certain species (As, B, Ca, Co, Cr, Cu, Mn, Mo, Ni, Sb, Se, Sn, Sr, V, Zn) were more abundant in the outdoor environment, while certain others (As, B, Cr, Mo, Ni, Se, V) registered elevated concentrations in the indoors as well. The latter was largely sourced to heating and cooking-related emissions, types of building material, and family lifestyle. Using chemical and magnetic analyses, the authors, however, identified that while B, Mo, and Se were enriched mostly outdoors, Ni, Cr, and V were specifically abundant indoors.

In another similar experiment, Świsłowski et al. (2022) used moss bags to assess air quality due to car workshop activities. Results indicated significantly elevated levels of Al, Cr, Fe, and Ba in the parking garage (indoors) than outdoors (traffic emissions), thus posing greater health risks to the workshop personnel (car mechanics). In a novel effort, Zechmeister et al. (2020) exposed *Pleurozium schreberi* moss bags for 8 weeks indoors and outdoors in 20 households in the city of Girona, Spain, to evaluate concentrations of Al, Cr, Cu, Zn, Sn, Cd, Pb, Mo, and Sb. Results indicated (i) different elements of metal enrichment in indoor and outdoor environments, (ii) stronger intercorrelations between metals in the outdoor environment, (iii) lower correlation for the indoors may arise due to more 'diffused' sources of metals, (iv) for certain metals, the indoor: outdoor ratio was far from unity indicating different sources and intensities (Sb, Sn, Cr, Mo) while for some others (Cd, Zn, NO2), the ratio was close to unity indicating an equilibrium state between indoor and outdoor. Overall, the study showed that moss bags could be a promising tool to assess indoor air quality.

Land use patterns: In Campania, southern Italy, Capozzi et al. (2016) used moss bags (*Hypnum cupressiforme* Hedw) to evaluate the rural-urban structure for ambient air quality. Investigating spatial patterns for 39 metals and 20 PAHs, the authors observed that moss bags were efficient in discriminating between the agricultural (higher metal loads) and urban sites. Main drivers at the agricultural sites included resuspension of soil dust, fertilizer applications, unregulated (and illicit) electronic waste disposal activities, and heavy-duty traffic movement. Moreover, the authors noted a significant accumulation of higher molecular weight species, such as 4-righ and 5-ring PAHs, in moss tissues. Based on the species abundance and comparing with similar studies, the authors identified potential PAH sources, such as coal and wood combustion (Dvorská et al. 2011), oil combustion (Larsen & Baker 2003), and diesel exhausts (Yunker et al. 2002).

Innovations and Opportunities

Growing concerns over air quality degradation and associated public health hazards have triggered research groups around the world to seek newer avenues of moss bag application to bolster and diversify existing air quality monitoring networks. In the following sections, we briefly highlight certain examples:

- Mobile Systems
- Bio-magnetic studies
- Radioisotope assessments
- Integrated Environmental Assessment
- Early Warning systems

Mobile air quality monitoring systems: A recent innovation came with the 'mobile' moss bag experiment, carried out by Sorrentino et al. (2021b) to distinguish between land use patterns by attaching moss bags (*Hypnum cupressiforme* species) to bicycles (Antwerp, Flanders region, northern Belgium). The study involved two experimental designs:

I. Six volunteer cyclists with three moss bags each on their bicycles through urban areas for 50 days

II. A single volunteer on four routes with three moss bags, cycling around the city for 22 days, through four routes (a heavily trafficked urban motorway; an industrial route with a non-ferrous metallurgical plant and a cement industry; a green route through agricultural and green areas; composite route (including all three above)

A combined chemical and magnetic analysis revealed that the elemental load and fluxes along the four routes followed the order: industrial > urban > composite > green, with significant Ag, As, Cd, and Pb enrichment in the industrial route. Interestingly, a comparison with 'static' moss bags revealed better efficacy of the mobile system in pollutant monitoring - elemental fluxes for As, Cu, Fe, Pb, and V for the mobile system were several folds higher than for fixed monitoring positions, which could be due to the wind effect, as maintained by Garcia-Seoane et al. (2019), which largely influences the moss metal uptake capacity/efficiency.

Moss bio-magnetic investigations: A small but growing body of moss research is involved in the biomagnetic characterization of emission-related particulate matter (Capozzi et al. 2019, Sorrentino et al. 2021a). Magnetic techniques are considered rapid and easy tools to identify spatial structures in environmental quality and changes therein (Salo & Mäkinen 2014). These studies rely on the identification of ferrimagnetic species (magnetite, maghemite, heavy metals) associated with particulate matter and gases in traffic- and/or industrial emissions. Recent studies even recommend biomagnetic monitoring of ambient air quality as a more cost-effective and non-destructive means (Hofman and Samson 2014) than the traditional methods (Li et al. 2017) to assess airborne heavy metals. In the city of Turku, Finland, Limo et al. (2018) used *Sphagnum papillosum* bags to investigate and evaluate the impacts of stop-and-go traffic emissions on air quality. Moss bags were placed alternately at the traffic light crossings (n = 19) and midway areas between the crossings (n = 29) along four street transects and one separate traffic light crossing. Coupled use of mass-specific magnetic susceptibility (χ), hysteresis parameters, and multi-elemental assays revealed that particulate matter was mainly composed of fine-grained pseudo-single-domain (PSD) magnetite and traffic light crossings have significantly higher χ, Cu, Mo, Sb concentrations than the midway street transects. The elements Ba, Cu, Fe, Mo, Ni, Pb, Sb, and Ti showed moderate to high accumulation and correlated strongly with χ and saturation magnetization (MS). Overall, the study indicated the production of low-coercivity ferrimagnetic particles (magnetite and haematite) and heavy metals due to stop-and-go traffic patterns (e.g., brake, tire, asphalt wear), which is a major source of particulate matter, especially along highly trafficked streets. However, a word of caution for adopting a moss-magnetic approach is to be cognizant of the influences of local climatic, geographical, and environmental conditions while interpreting the results as the composition and abundance of the magnetic mineral phases are largely governed by these factors (Salazar-Rojas et al. 2023).

Salo et al. (2012) deployed moss bags in an urban and industrial site in southwest Finland using magnetic profiling of heavy metals in ambient air. While vehicular emission was the main source of heavy metals at the urban site, it was emissions from the Cu-Ni smelter at the industrial site. At both sites, the ambient particulate matter chemistry revealed a distinct magnetite-like phase. Mass magnetic susceptibility (χ) profiling revealed a high enrichment of magnetic elements closer to the sources at both sites, which tapered off with increasing distance from the sources. Overall, both studies indicated that moss bags could be strategically and efficiently used to identify broad mineralogical compositions (hematite and magnetite) of anthropogenic origin (industrial and vehicular combustion products) with spatially distinct air quality patterns.

In a study in Belgrade, Vuković et al. (2015) exposed *Sphagnum girgensohnii* moss bags to distinguish pollution patterns in different urban microenvironments (street canyons, city tunnel, parking garages). Determination of the ferromagnetic particulate fraction in moss samples by Saturated Isothermal Remanent Magnetization (SIRM) revealed different metal signatures - highest SIRM in the tunnel (most polluted), minimum in the garages. Moreover, a significant correlation (R>0.90) between the moss SIRM values and traffic flow indicated vehicular emissions as the main sources. Additionally, there was a high degree of correlation between the moss SIRM values and Al, Ba, Co, Cr, Cu, Fe, Ni, and Pb concentrations. Results demonstrated that moss bags can be effectively applied for biomagnetic monitoring of the spatio-temporal distribution of road traffic and vehicle-derived pollutants in urban areas.

Radioisotope Measurements

Capozzi et al. (2016) tested three species (*Sphagnum palustre, Hypnum cupressiforme,* and *Hypnum plumaeforme*) to assess airborne radiocesium activities (^{134}Cs and ^{137}Cs), eight years in the aftermath of the nuclear meltdown-fallout event in the Fukushima-Daichi Nuclear Power Plant, Japan. In Belgrade, Serbia, Popovic et al. (2010) found that *Sphagnum girgensohnii* moss bags were efficient tools for source detection: ^{137}Cs (fission products) and ^{40}K and ^{210}Pb (naturally occurring). The study detected lower levels of ^{40}K and ^{137}Cs, while high ^{210}Pb loadings in urban atmosphere, attributable to anthropogenic activities.

Integrated environmental quality: A particular strand of moss bag research involves process-level iqnterpretation of environmental changes by coupling the results with various indicators (Table 2). In Wuxi, China, Hu et al. (2018) computed the relative accumulation factor (RAF) and contamination factor (CF) based on *Sphagnum junghuhnianum* moss bags for a variety of heavy metals (Cr, Cu, Pb, V, Zn). Results indicated considerable enrichment of Cr, Cu, and V (RAF >1) in the ambient atmosphere and potential environmental health threats. Moreover, the RAF revealed a distinct seasonal pattern of air quality: higher pollutant loadings in winter than summer, most likely to be caused by lack of precipitation in the former (lower wash-off/dilution). Determination of CAF revealed a moderate degree of air pollution due to Cr, while slight contaminations due to other heavy metals. Significant correlations observed between the heavy and between the metals and traffic volume in both seasons helped in pollutant source detection: traffic intensity.

In the Donetsk region, Ukraine, Sergeeva et al. (2021) used *Ceratodon purpureus* and *Brachythecium campestre* moss bag results to compute various indicators. The RAFs helped identify significant enrichment of rare earth elements (REEs: Ce, La, Tb, Sm, Yb, Hf, Th, U, Zr) in the ambient atmosphere, while the EF indicated higher REE enrichment potential of *Ceratodon purpureus* than the Brachythecium sp. The La, Ce, Nd, Sm, Yb, Hf, and Th were sourced

Table 2: Various environmental indicators computed using information from moss bags. (Authors' own compilation from various moss bag literature).

Indicator Name	Indicator Development	Significance
Relative Accumulation Factor (RAF)	$$\mathrm{EF} = \frac{\mathrm{C_{t1}} - \mathrm{T_{t0}}}{\mathrm{C_{t0}}}$$ Ct_0: Concentration of the pollutant species after exposure period C_{t1}: Concentration of the same after exposure period	RAF > 1 = Significant Accumulation within moss tissues
Contamination Factor (CF)	$$\mathrm{CF} = \frac{\mathrm{C_i}}{\mathrm{C_B}}$$ C_i: Concentration of pollutant species within moss tissue C_B: Background concentration of the same	CF <1 = Slight Contamination CF 1-3 = Moderate Contamination CF 3–6 = Considerable Contamination CF >6 = High Contamination
Enrichment Factor (EF)	$$\mathrm{EF} = \frac{\mathrm{C_i}\ \mathrm{C_x}}{\mathrm{C_B}\ \mathrm{C_{bi}}}$$ C_i: Observed concentration of pollutant species in moss C_x = Concentration of conservative reference element in a moss sample C_B: Background metal concentration at the site C_{bi} = Concentration of conservative reference element in reference	EF $\leq$1 = No Enrichment EF 1-3 = Minor Enrichment EF 3-5 = Moderate Enrichment EF 5-10: Moderately Severe Enrichment EF $\geq$10 = Severe Enrichment
Pollution Loading Index (PLI)	$$\mathrm{PLI} = \sqrt[n]{\prod_{i=1}^{n} \mathrm{CF}}$$ *where,* CF = Contamination Factor n = Total number of pollutant species	PLI < 1 = Non Polluted PLI 1 $\leq$ PLI < 2 = Slight Polluted PLI 2 $\leq$ PLI < 3 = Moderately Polluted PLI < 3 = Highly Polluted

to anthropogenic origins (EF > 1.5), such as windblown road and/or coal mine dust. Computation of the PLI values indicated high pollution loadings. Overall, recent studies indicate that coupling moss bag outputs with established environmental indicators can not only offer a holistic view of the type/degree of air quality degradation and the overall status of environmental conditions in the region of interest but also may provide insights into the pollution sources and drivers. These indicators can be used to generate high-resolution spatial maps to identify the host spots of contamination. For example, in the Donetsk region, Sergeeva et al. (2021) were able to delineate three distinct zones with slight (CAF < 1), moderate (CF 1-3), and considerable (CF 3-6) pollution for all heavy metals.

Developing early warning systems: Using strategically placed moss bags (*Hylocomium splendens*) across Romania (142 monitoring stations), Stefanaut et al. (2019) found that ambient air concentrations of several heavy metals (Cd, As, Pb, Ni) exceeded the established standards, indicating substantial public health risks. In the process, the authors developed a specialized tool, namely BioMonRo, as an early warning system to notify the regulatory authorities about potential air quality degradation. Along that line, the tool generated high-quality spatial maps of heavy metal pollution and reports, which could be automatically disseminated among multiple parties engaged in similar research. Overall, the results demonstrated that the system could be adopted by national authorities to (i) raise general awareness regarding the risks represented by atmospheric emissions and (ii) as an efficient and cost-effective monitoring device for the long-term appraisal of airborne heavy metals.

Concerns and Future Directions

Despite the myriad advantages of moss bags, and matched by a growing number of applications and advances through the recent past, there are yet concerns regarding their meaningful and strategic use to assess ambient air quality.

Ideal Species Selection

Before others, a fundamental puzzle for the regulatory for the regulatory authorities is to identify an ideal moss variety, which is keyed to the pollutant uptake capacity of the moss (Fig. 1). A major consideration in this regard is solid understanding of the ion exchange process that takes place on most surfaces – types and proportions of various reactive functional groups (e.g., phosphodiester, carboxyl, phosphoryl, amine, sulfhydryl, and polyphenols) that determine the pollutant binding affinity. However, another consideration is how the pollutant species interact with the surficial binding sites (Fernández et al. 2013). For example, using *Hypnum cupressiforme* moss bags in an oil refinery area of Sardinia (Italy), Cortis et al. (2016) observed a sequence of metal affinity by following the order: Pb > Co > Cr > Cu, Cd, Mo, Ni, V > Zn > As (Cortis et al. 2016). However, this sequence could vary by moss species and environmental conditions, which calls for more species-specific, process-level research. Along that line, some research could also be devoted to understanding the pollutant apportionment process within moss cells (as inter-, extra-, or intra-cellular fractions).

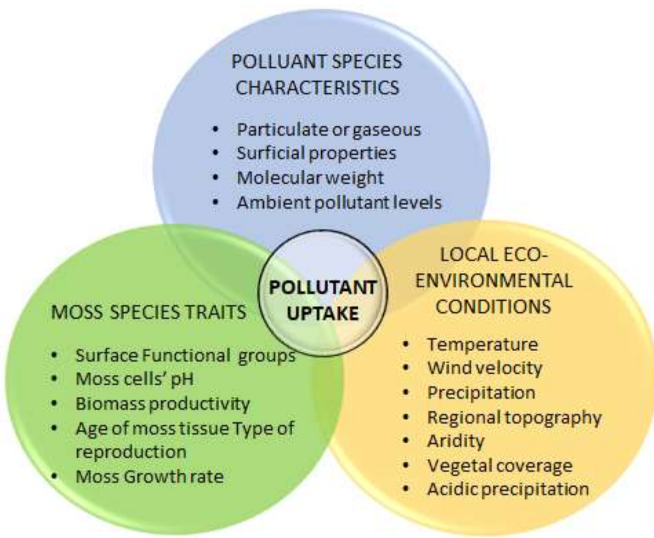

Fig. 1: Potential regulatory consideration for selecting an ideal moss variety *(Authors' own illustration)*.
[NOTE: The considerations depicted herein are of largely 'generic' nature. Besides these, the regulatory authorities might need to think about the context-specific traits (e.g. types of air pollutants, intensity of pollution, distribution of pollutants) to include others]

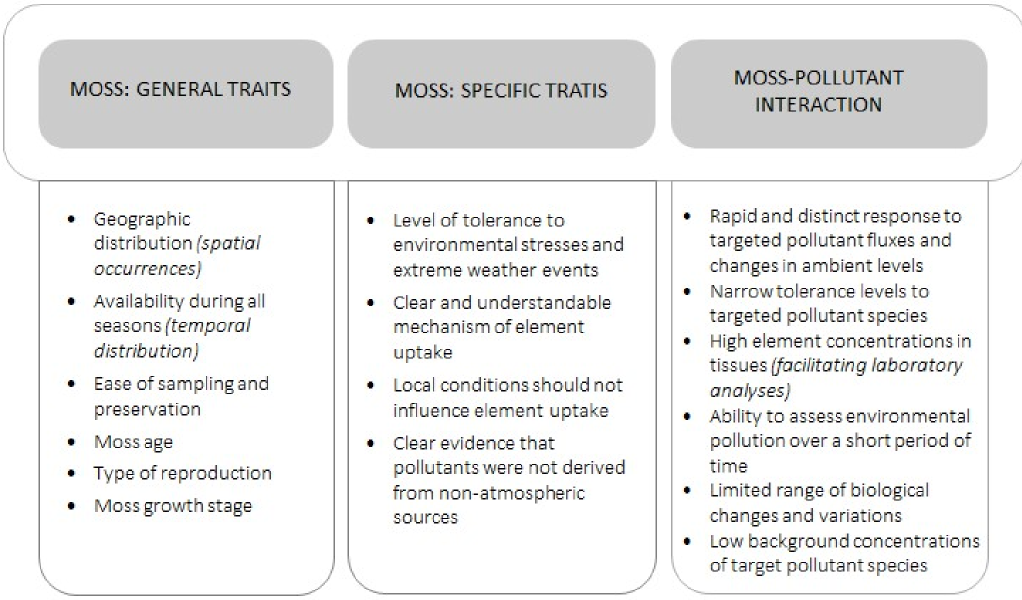

Fig. 2: Overall considerations for identifying the ideal moss variety as found in various moss bag literature *(Authors' own illustration)*.

Table 3: Process-level significance of selected moss bag parameters. [NOTE: Included are broad recommendations for the regulatory authorities, as found in from moss bag literature; Authors' own compilation].

Design Factors	Process-level Significance and Best practice
Bag Material	• Plastics and glass fiber are recommended • Non-plastic materials are not recommended as they may interfere with the pollutant uptake process.
Bag Mesh Net Dimension	• Inappropriate selection of the mesh size may lead to the loss of large amounts of moss due to wind and/or rain events. • Mesh size should be determined as a compromise between maximization of the interception of aerial deposition and minimization of the risk of loss of material.
Bag Shape	• The idea should be to maximize the surface area of moss exposed to the atmosphere. • The 3-D bag shape allows for uniform pollutant capture from all directions; it also allows sample collection by gravitational sedimentation.
Moss Amount and Packing	• Even though sufficient moss amounts should be placed in the bag, moss should be loosely packed in thin layers to reduce compaction. • Caution should be practiced to avoid moss layer overlapping and compression, which collectively reduces chances of even exposure to the contaminants (e.g., when shoots overlap, concentrations may decrease gradually)
Exposure Period	• Should be guided by research objective • Sufficient time to intercept pollutants • Enough time to 'equilibrate' with ambient conditions • Minimize risks of potential 'leaching' losses (rain events) and/or windblown losses (storm events)
Bag Exposure Elevation	• Should be guided by research objective • Entrap pollutant sources close to the ground (e.g., vehicular emissions, storm dust) • Potential risks involve undesirable human interventions (curious citizens, children, etc.) that may tamper with the bag.

Overall, the moss bag literature reveals preference for the Sphagnum variety (Gonzalez & Pokrovsky 2014), partly owing to their ubiquitous geographic occurrence (availability). More process-level reasons include (i) abundance of surficial proton exchange cites and thus, high cation-exchange capacity of the Sphagnum (better pollutant interception/binding capacity); (ii) large area/volume ratio (higher amount of exchange cites exposed to pollutants); (iii) high permeability of tissues to water, coupled with high water retention capacity (resistant to drought/hydric stresses) (Aničić et al. 2009, Fernández et al. 2009). However, in the absence of Sphagnum, other moss varieties are noted in the literature as well, including *Pleurozium schreberi*, *Hypnum cupressiforme*, and *Pseudoscleropodium purum*. Overall, for best outcomes, the species selection process might want to consider the following in the least

(Fig. 2): Availability, Moss Characteristics, and Moss Data.

Constructional Artefacts

A frequently raised concern about moss bags is the diversity of practices and, thus, lack of concordance in constructional details – there are yet no universally accepted protocols for bag parameterization (Ares et al. 2012, Capozzi et al, 2016). A moss bag embodies a multitude of parameters, including the bag shape (circular, flat), dimension, bag material (nylon, polythene), mesh net sizes, moss amount, moss packing within the bag, ratio between moss weight to bag volume, bag exposure period, bag elevation (Ares et al. 2014). However, moss bag literature documents differences even in the moss 'preparation' details, that is before the moss is placed within the bag. It ranges from moss sampling protocols (Capozzi et al. 2017) to washing-cleaning procedures (Ares et al. 2014), to drying (Giordano et al. 2009), and homogenizing (Milicevic et al. 2017). Such differing approaches, however, make comparisons between moss bag results difficult (Świsłowski et al. 2021d). To develop a viable moss bag construct (reliable, replicable), the first need is to understand the process-level significance of each parameter and how they may influence the chemical analysis results (Table 3). To that end, we envision more targeted action in the days ahead to standardize the moss bag protocols.

Moss Vitality

A prime issue about moss bags is the vitality and functional quality of the moss. It owes to the observation that relocation from their natural habitat entails changes in their original environmental and meteorological conditions (Świsłowski & Rajfur 2021c), which may result in hydric and photo stress, affecting the moss viability in the long run (Urošević et al. 2017). Moreover, sustained exposure to high pollutant levels in the ambient atmosphere could alter various physiological parameters in the moss, such as loss of pigmentation due to a drop in chlorophyll contents, which needs to be further researched (Sun et al. 2009). In their study in Sardinia, Italy, Cortis et al. (2016) observed that elevated concentrations of various heavy and trace metals (Cr, Cu, Ni, Zn, Cd, Pb) led to significant proteonomic changes in moss cells. For example, 15 gel spots exhibited differential expression profiles between the moss samples collected at the study site and the control site. 14 spots showed a decrease in protein expression, of which nine were associated with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and proteins of the light-harvesting complexes of photosystem (PS) II, while three were associated with protein synthesis, and three with stress-related proteins.

However, on the brighter side, strategic monitoring of such physiological/structural disorders and deformities in moss samples can also help the air quality regulators as the onset of air quality degradation events, for example, an *early warning system*, to devise pre-emptive pollution prevention and remediation mechanisms.

CONCLUSION

With the rise of air quality degradation-induced health hazards around the world, the need to expand the monitoring network has never been so imperative. In the process, moss bags have emerged as cheaper, eco-friendly, and efficient biomonitors, capable of offering denser spatial coverage than the traditional, instrument-based sensors (highly capital and infrastructure intensive) and/or lichens and/or vascular plants. In the present context, we present a bird's eye view of the current global experiences around moss bags in a variety of applications, including urban sustainability studies, bio-magnetic investigations of particulate matter, land use structure, indoor-outdoor air quality assessment, and short-term change detection, to name a few. More novel applications of moss bags include mobile systems, radioisotopic investigations, and integrative environmental quality assessment with indicator variables. Computation of various environmental indicators. Despite several advantages, however, moss bag literature reveals that much research needs to be done before moss bags can be meaningfully used for regulatory purposes (pollution control-prevention). Future areas of research may include but are not limited to, moss morphological-physiological investigations to aid in species selection, developing a standardized/harmonized protocol for moss bag preparation and parameter optimization, and improving our understanding of the potential influences of environmental-meteorological stressors on moss health.

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