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Perspective Article

Mythbusters: Unravelling the pollutant uptake processes in mosses for air quality biomonitoring

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A B S T R A C T

Terrestrial mosses have been used for more than 50 years to monitor air pollution. We argue, however, that their value as biomonitors is based on two widespread but partially erroneous assumptions concerning their morphological structure (the **structural myth**) and physicochemical characteristics (the **physicochemical myth**). The structural myth consists of the oversimplification of the moss morphological structure. The physicochemical myth recognizes their high cation exchange capacity (CEC) as the only pathway for pollutant uptake. Here, we gather and discuss the evidence demonstrating that these assumptions are misleading and focus the discussion on the aspects that make mosses good biomonitors of air pollution. First, we show that these plants (i) do have a cuticle, whose structure and composition differs among species, (ii) can have epidermal cells, that differ in shape and thickness from other cell types, (iii) have a vascular system, whose degree of development is lineage- and species-specific, and (iv) have rhizoids, that can absorb water, nutrients and pollutants. The effect of these traits in the pollutant uptake processes has been understudied in biomonitoring studies. Second, we show that mosses (i) do not concentrate as much pollutants as they could according to their high CEC, (ii) can retain large amounts of particles containing atmospheric pollutants in their surfaces, and (iii) in many contexts, the spatial structure of the concentrations of pollutants in mosses depicts the pattern expected for atmospherically deposited particles. Thus, the quality of these organisms as biomonitors of air pollution also lies in their high capacity to retain particles. We do call for more research on how moss structural and physiological traits affect pollutant uptake dynamics and recommend researchers using mosses as air quality biomonitors to face critically to inaccurate or insufficiently demonstrated assumptions in this context.

1. Introduction

Terrestrial mosses have been used for more than 50 years to monitor air pollution. The seminal paper by Rühling & Tyler (1968) established the grounds for the development of what is generally known as the "*moss biomonitoring technique*". Since then, nearly 1500 publications, including scientific articles and books or book chapters, reported on the use of different moss species to assess air quality. Also, an international cooperative programme was established in 1987 to work on biomonitoring of air pollution and its effects on vegetation across Europe using, among other plants, terrestrial mosses (ICP Vegetation; https://icpvegetation.ceh.ac.uk/). All this work has been translated into multiple improvements to the practical implementation of the technique (Fernández et al., 2015). Still, the biology of these plants, including aspects related to their morphological structure and physiological characteristics, may have been overlooked in biomonitoring studies. This can complicate the biological and environmental interpretation of the concentrations of pollutants obtained from moss tissues (Boquete et al., 2017).

The value of mosses as biomonitors of air pollution is based on the idea that wet and dry (i.e. bulk) atmospheric deposition constitute the main sources of nutrients, and consequently pollutants, to these plants (UNECE - ICP Vegetation, 2020). It has been repeatedly stated that mosses integrate the elements that they receive from the atmosphere (Berg et al., 1995; Harmens et al., 2008) and that the soil contribution to their elemental concentrations is usually negligible. According to these

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ideas, the concentrations of pollutants in their tissues should provide an accurate picture of the atmospheric deposition of pollutants in their native environments. Over the years, however, multiple evidence has been provided highlighting that different aspects of moss biology strongly influence the concentrations of pollutants found in their tissues (e.g. Aboal et al., 2010; Boquete et al., 2017, 2013). Thus, the general perception of mosses as good biomonitors of air pollution may be based on two widespread, though partially erroneous assumptions, hereafter referred to as "*the two myths*" of the "*moss biomonitoring technique*": the **structural myth**, and the **physicochemical myth**.

The **structural myth** is rooted in the oversimplification of the moss morphological structure. Biomonitoring studies frequently highlight the lack of cuticle, epidermis, vascularization, and a typical water and nutrient root absorbing system as structural characteristics of mosses. Consequently, their cells would be directly exposed to the pollutants present in the atmospheric deposition and there would be no transport of pollutants throughout the plant. These statements persist in the current biomonitoring literature despite the evidence showing otherwise (e.g. Spagnuolo et al., 2017; Favas et al., 2018; Martinez-Swatson et al., 2020; Petschinger et al., 2021).

The **physicochemical myth** recognizes the high cation exchange capacity (CEC) of mosses as the only determinant of their quality in air pollution biomonitoring (e.g. Zinicovscaia et al., 2017; Jiang et al., 2018; Xu et al., 2021a). Yet, the CEC only accounts for the positively charged pollutant loads (e.g. heavy metals) which bind the negatively charged compounds available on the moss cell walls and plasma membranes, and hinder the uptake of lipophilic (e.g., polycyclic aromatic hydrocarbons, PAHs) and negatively charged pollutants (e.g., F, and compounds containing S and N). Also, most air pollutants are associated with suspended particulate matter, especially in urban and industrial environments where emission sources release large amounts of particles (usually fine particles; <2.5 μm) from different combustion processes (e.g., Córdoba et al., 2012; Weinbruch et al., 2014). Pollutants associated to these particles are expected to be deposited in particulate form on the moss surfaces (Bargagli, 1998) and would not be accounted for by the CEC.

Here, we show evidence to challenge some of the traditional assumptions that may be misleading to interpret the results obtained in moss biomonitoring studies, and open the discussion on the aspects that make mosses good biomonitors of air pollution.

2. The structural myth

Bryophytes consist of three different evolutionary lineages: hornworts (250 species), liverworts (7,000) and mosses (12,000). All three lineages share a common life cycle where the green photosynthetic gametophyte prevails over the attached and dependent sporophyte. This characteristic clearly separates bryophytes from tracheophytes (the ensemble of pteridophytes and seed plants), in which a structurally complex sporophyte provided with "true" roots, stems and leaves is dominant over a small, frequently microscopic, gametophyte. In addition, the large tracheophyte sporophytes have complex vascular tissues and are able to regulate their internal water content (homeohydry), whereas the small bryophyte gametophytes have a limited vascular system, being unable to regulate internal water (poikilohydry). However, bryophytes show a general desiccation tolerance that increases their competitiveness and leads to a noticeable ecological success.

The three bryophyte lineages differ in the structure of both the sporophyte and gametophyte stages. Regarding the gametophyte, the stage used for biomonitoring purposes, it is thalloid in hornworts, leafy in mosses, and either thalloid or leafy in liverworts (Fig. 1A-G). In mosses, the most used bryophytes in air quality biomonitoring, the mature gametophyte shoots (gametophores) consist of a stem (caulidium) with attached leaves (phyllidia) and rhizoids (Fig. 1D, H-L). These organs have a much simpler structure than the corresponding organs of tracheophytes. The structure of the bryophyte gametophore may have been oversimplified in the field of biomonitoring (the structural myth). In the following sections, we focus on how this oversimplification might have prevented a full understanding of pollutant uptake processes, affecting the interpretation of the results of biomonitoring studies.

2.1. The cuticle

In tracheophytes, the cuticle is a hydrophobic layer covering aerial organs at the interface between the plant and the environment (Salminen et al., 2018; Bhanot et al., 2021; Xu et al., 2021b). This structure can be divided into (i) the lower "cuticle layer", located on the surface of epidermal cell walls and composed of a cross-linked matrix of the polyester cutin covalently linked to polysaccharides; and (ii) the overlying "cuticle proper", containing waxes and fibrous polysaccharides embedded in a cutin matrix (Niklas et al., 2017; Bhanot et al., 2021). Waxes can also be epicuticular, forming either a film or crystals above the cuticle proper. The thickness of the overall structure ranges from nanometers to micrometers.

It is generally admitted that the development of a cuticle was a key evolutionary innovation to allow plants to colonize the terrestrial environment 450 million years ago. This structure would have provided protection against (mainly) water loss and solar radiation (Buda et al., 2013). Phylogenomic evidence suggests that cuticles originated in the last common ancestor of embryophytes (the ensemble of bryophytes, pteridophytes, and seed plants), and that the machinery and regulation involved in cuticle biosynthesis is ancient and highly conserved across land plants (Niklas et al., 2017; Kong et al., 2020; Xu et al., 2021b).

In bryophytes, the presence of a cuticle has been revealed using histochemical and electron microscopy techniques in the gametophores and sporophytes of mosses, liverworts and hornworts (Schönherr & Ziegler, 1975; Moore et al., 1982; Schofield & Hébant, 1984; Cook & Graham, 1998; Buda et al., 2013; Busta et al., 2016; Li et al., 2018; Salminen et al., 2018; Budke, 2019; Resemann et al., 2019; Kong et al., 2020; Glime, 2021; Kriegshauser et al., 2021). Certain characteristics of tracheophyte cuticles, such as the presence of cuticular waxes and the differentiation between cuticle layer and cuticle proper, have also been identified in bryophytes (Budke et al., 2011; Buda et al., 2013; Busta et al., 2016; Matos et al., 2021a,b). The cuticle of the leafy gametophore of the model moss *Physcomitrium patens* is, indeed, structurally and chemically similar to that of tracheophytes, although with higher proportions of phenolic acids (Buda et al., 2013; Renault et al., 2017; Li et al., 2018). In general, bryophytes have lower contents of cutin, cuticular waxes, and other compounds than tracheophytes (Kong et al., 2020), which would contribute to a comparatively lower hydrophobicity and moisture retention capacity.

The functional significance of the cuticle in bryophytes may be similar to that in tracheophytes. Cuticle can limit water loss, as demonstrated for mutants of *Physcomitrella patens* Bruch & W.P. Schimper, with defective cuticles. These mutants showed increased permeability, rate of water loss, and dehydration stress, compared to the wild type (Buda et al., 2013; Li et al., 2018). Other functions are to separate organs during development, to prevent liquid water on bryophyte surfaces from interfering with gas exchange (as in the spaces between leaf lamellae in polytrichaceous mosses: Fig. 1D, J, M), and to provide rigidity to the phyllidia and the whole gametophore of mosses (Proctor, 1984; Schofield & Hébant, 1984; Malcolm & Malcolm, 2006; Renault et al., 2017; Glime, 2021). The role of the bryophyte cuticle in the protection against high light, UV, and pathogens remains to be experimentally demonstrated.

Overall, the bryophyte cuticle shows different levels of organization, depending on evolutionary, developmental, and environmental factors, such as the lineage, the species, the life cycle stage, and the gametophore age (Kong et al., 2020; Li & Chang, 2021). The fact that ectohydric mosses (those conducting water externally through capillary movement) have less developed cuticles than endohydric mosses (those conducting water internally through vascular tissues) can be relevant in the context

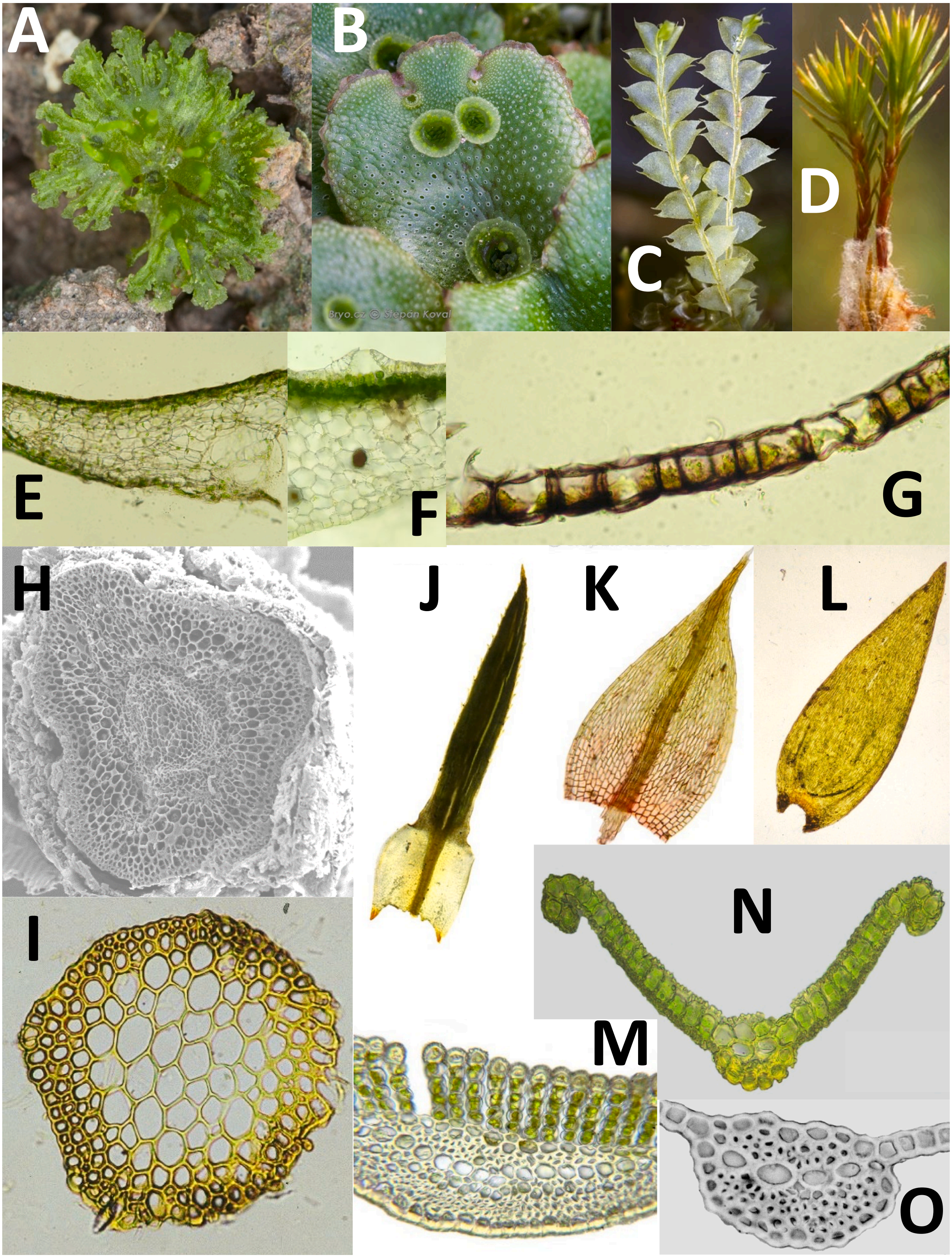

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Fig. 1. Structure of the bryophyte gametophore: A) thallus of the hornwort *Anthoceros agrestis*; B) thallus of the marchantioid liverwort *Marchantia polymorpha*, showing air pores (perforations communicating with chambers filled with photosynthetic tissues) and gemmae-cups for asexual reproduction; C) two shoots of the leafy liverwort *Chiloscyphus coadunatus* showing stems (caulidia) and two-ranked leaves (phyllidia) in each stem; D) shoots of a polytrichaceous moss showing caulidia with phyllidia and a white tomentum formed by rhizoids covering the basal part of the caulidia; E) cross-section of the thallus of the hornwort *Anthoceros punctatus*; F) cross-section of the thallus of the marchantioid liverwort *Lunularia cruciata*, showing a volcano-like air pore, dorsal and ventral monostratified epidermis, green photosynthetic tissues, and hyaline storage parenchyma; G) cross-section of the monostratified phyllidium of the liverwort *Jungermannia eucordifolia*; H) cross-section of the caulidium of the polytrichaceous orthotropic (erect) moss *Polytrichastrum alpinum* showing the structural complexity of its vascular system, with a central hydrome (water and mineral solutes conducting system) and surrounding leptome (photosynthates conducting system); I) cross-section of the caulidium of the plagiotropic (prostrate) semi-aquatic moss *Palustriella commutata* lacking central strand but showing epidermis (2–3 layers) and parenchyma; J) phyllidium of the polytrichaceous moss *Pogonatum urnigerum* showing a basal hyaline sheath and a green blade composed of parallel lamellae; K) phyllidium of the moss *Bryum ruderale* showing a central costa; L) phyllidium of the aquatic moss *Fontinalis squamosa* lacking a costa; M) cross-section of a phyllidium of the polytrichaceous moss *P. alpinum* showing structural complexity, with lamellae, surface ventral cells, a dorsal layer of stereids (in several rows), a layer of eurycists, hydroids, a ventral layer of stereids, and surface dorsal cells; N) cross-section of a phyllidium of the moss *Pseudocrossidium exiguum* showing a monostratified lamina except in the central costa; O) cross-section of the costa of the phyllidium of the moss *Didymodon cordatus* showing four central eurycists, ventral and dorsal stereids, and ventral and dorsal surface cells. Photos courtesy of Stepán Koval (A-D, J, K, M), Marc Carriquí (H), María-Jesús Cano (N) and Mayte Gallego (O). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of moss biomonitoring. Indeed, the former (*Hypnum cupressiforme*, *Pleurozium schreberi*, *Pseudoscleropodium purum*, *Hylocomium splendens*, *Sphagnum* spp., *Sanionia uncinata*) have been much more frequently used than the latter (*Pleurochaete squarrosa*, *Ceratodon purpureus*, *Bryum* spp., *Polytrichum* spp., *Dicranum scoparium*, *Tortula muralis*) (Ares et al., 2012; Harmens et al., 2013; Fernández et al., 2015; Mahapatra et al., 2019). This should be considered because their different water transport modalities and associated cuticle characteristics could affect pollutant accumulation patterns.

All the above evidence clearly contradicts the widely admitted statement that bryophytes and specifically, mosses, have no or only a poorly developed cuticle. Consequently, moss cells would not be directly exposed to the pollutants deposited from the atmosphere, as generally assumed. Due to its position at the interface between the plant and the environment, it seems obvious that the cuticle would affect the bidirectional transport of matter, including pollutants, as demonstrated for tracheophytes (Fernández et al., 2016). Glime (2021) suggested that bryophyte cuticles could prevent the absorption of pollutants, thus playing a protective role. This has been supported by Elvira et al. (2020), demonstrating that lipophilic, wax-like substances deposited on the surface of the moss *Ptychostomum capillare* retained a significant amount of Cu-containing particles. In addition, the pollutant phenanthrene was retained and accumulated within the cuticular matrix for more than 10 days until it entered the cell cytoplasm (Wu et al., 2014). Hence, the absorption (and loss) of pollutants depend on both the type of pollutant and the characteristics of the cuticle, and we call for more research on moss cuticles and their specific roles in pollutant uptake dynamics.

2.2. The epidermis

The statement that bryophytes have no epidermis (as defined for tracheophytes, a mono- though sometimes pluristratified layer of cells covering the primary plant body) should be nuanced. Different pluristratified organs of bryophyte gametophytes can have an epidermis, such as the thallus of liverworts and hornworts, and the caulidia of mosses and leafy liverworts (Vanderpoorten & Goffinet, 2009; Frangedakis et al., 2021; Xu et al., 2021b; Fig. 1E-F, H-I). The epidermal cells of moss caulidia are often narrower than those of the underlying cortical parenchyma and their walls are usually distinctly thickened and pigmented (Fig. 1I). In some hygrophilous mosses, however, epidermal cells are thin-walled and somewhat inflated, forming the hyalodermis, which may function in external water conduction. Another peculiarity occurs in some marchantioid thalloid liverworts, which have occasionally been used as biomonitors. Their dorsal epidermis, which is in contact with the air, is perforated with pores communicating with chambers filled with photosynthetic tissues (Fig. 1B, F). Apart from thalli and caulidia, the phyllidia of mosses, although mostly monostratified, can have an epidermis covering the adaxial and/or abaxial surfaces of the pluristratified costa (also called midrib or nerve), which may be present or absent in the phyllidia, depending on the species (Fig. 1J-O). The potential role that the epidermis (and associated structures, such as air pores) in pollutant uptake processes and loads is unknown.

2.3. Vascular tissues

Although frequently stated as such, the assertion that bryophytes are non-vascular plants is inaccurate and requires detailed discussion in the context of air quality biomonitoring. Bryophytes conserve significant evidence of a vascular system whose degree of development depends on the lineage and the species. In moss gametophores, the material usually used for biomonitoring purposes, both caulidia (through a central strand, Fig. 1H) and phyllidia (through a costa when present, Fig. 1J-K, M–O) can be vascularized, showing different types of vascular cells, such as stereids, eurycists, hydroids, and leptoids (Proctor, 1984; Schofield & Hébant, 1984; Magill, 1990; Malcolm & Malcolm, 2006; Fig. 1H, M, O). In general, plagiotropic (growing horizontally prostrate on the substratum) mosses have a more reduced or lack a vascular system compared to orthotropic (growing erect) mosses (Fig. 1H-I). Curiously, plagiotropic species are usually ectohydric whereas orthotropic species are endohydric, which implies that the former have more frequently been used for biomonitoring than the latter (see above).

Bryophyte vascular tissues can transport water, mineral elements (e.g. nutrients and pollutants like heavy metals), and organic solutes, including organic pollutants. From a physiological perspective, these substances can move throughout the moss gametophores using three different routes: (i) over the gametophore surface, by capillarity; (ii) by the apoplast (cell walls and intercellular spaces) or the symplast (the protoplasm as a continuous system, bounded by the cell membranes); and (iii) using the vascular system (Proctor, 1984; Schofield & Hébant, 1984; Ligrone et al., 2000; Raven, 2003; Bates, 2008). For example, Sokolowska et al. (2017) provided evidence of the existence of symplastic and apoplastic transport of fluorescent tracers in the mosses *H. splendens* and *P. schreberi*, commonly used in biomonitoring studies. These two species also showed significant calcium uptake from the soil and subsequent apoplastic Ca movement (Bates, 2008). It has also been shown that both mineral nutrients and heavy metals can be internally recycled, i.e., translocated, from old to young bryophyte tissues (Wells & Brown, 1996; Eckstein & Karlsson, 1999; Brumelis et al., 2000; Aldous, 2002; Bates, 2008; Fernández et al., 2010; Klos et al., 2012; Thiemer et al., 2018; Liu et al., 2020). Finally, organic compounds like photosynthates can be transported from phyllidia to the basis of the caulidia, and even underground, through the symplast pathway in the moss *Grimmia laevigata* (Alpert, 1989).

Biomonitoring studies have generally overlooked the presence of vascular tissues in mosses as well as the evidence of solute transport throughout the gametophore. This transport could affect the concentrations of pollutants found in the tissues analyzed in biomonitoring studies, thus altering the relationship between exposure vs.

concentration.

2.4. Uptake of water and nutrients system

Another repeated assertion in biomonitoring studies is that bryophytes, lacking true roots, can only obtain water and nutrients (and, potentially, pollutants) from the atmosphere. Yet, bryophyte gametophores have uni- or multicellular rhizoids (Fig. 1D) that can explore a few centimeters of the substratum, from where they can absorb water and nutrients (Bates, 2008) as well as pollutants (Klos et al., 2012). Water absorption can happen by a simple capillarity process that can be enhanced through the formation of a dense tomentum of rhizoids surrounding the basal caulidium and/or the phyllidia. In contrast, nutrient absorption processes may be more complex. Despite that phyllidia may have the greatest absorption capacities in some bryophyte species (Glime, 2021), it is still unclear whether this is of general occurrence. Hence, the effect of rhizoid absorption on the results of biomonitoring studies is still unknown.

3. Physicochemical myth

The capacity of mosses to exchange cations is mainly due to the presence of de-esterified galacturonic acids in their cells, although phenolic compounds, aminoacids, silicates, and sulfate esters also contribute to a lesser extent (Richter & Dainty, 1989). These acids are synthesized in the Golgi apparatus and de-esterified (i.e., enzyme pectin methylesterase removes methyl groups) upon incorporation into the cell walls and outer layer of plasma membranes. De-esterification generates negatively charged functional groups (e.g., $–OH^-$, $–COOH^-$) that become available binding sites for elements in cationic form. Thus, the degree of methylation of galacturonic acids determines the moss CEC (Carballeira et al., 2008). Hydroxy, amine and carbonyl groups associated to other cell wall polysaccharides might also contribute to some extent to moss CEC (Świsłowski et al., 2022).

Mosses have a high amount of metal binding sites (ranging between 7.7 mmol g^{-1} for Ni and 50.4 mmol g^{-1} for Pb in *Hypnum* sp., *Sphagnum* sp., *P. purum* and *Brachythecium rutabulum*) compared to organisms like bacteria, marine and freshwater diatoms, and cyanobacteria (0.1–4 mmol g^{-1}) (González & Pokrovsky, 2014). Likewise, the maximum metal adsorption capacity of mosses is higher than that of all other organic surfaces studied (aquatic and terrestrial plants, yeasts, peat, fungi, bacteria, algae, and soil), especially for Cu and Pb (Fig. 7 in González & Pokrovsky, 2014). The high CEC reported for mosses is the result of a high density of binding sites per unit area and the high specific surface area of these plants which is mostly determined by the thickness of the phyllidia (often unistratose) and the diameter of the caulidium. These characteristics can vary significantly among species (Carballeira et al., 2008), but they can lead to rather high specific surface area values (e.g., between 10.8 ± 0.3 to 28 ± 1 m^2 g^{-1} dry weight for the moss *Sphagnum palustre*; González et al., 2016).

Despite the high CEC, evidence suggests that mosses do not concentrate as much metals as they could. For example, the maximum CEC of *H. cupressiforme* and *P. purum*, estimated after galacturonic acid de-esterification, has been established in ca. 3000 mmol g^{-1} (Carballeira et al., 2008). These authors also reported that only 10% to 70% of this capacity would be available (i.e., unmethylated) in the field. Even in the worst-case scenario (10% – 300 mmol g^{-1} - available binding sites), the concentrations of metals like Hg and Pb in *H. cupressiforme* and *P. purum* were up to eight orders of magnitude lower than they could be (Carballeira et al., 2008). Experiments in the laboratory also showed that *P. purum* accumulated only ~ 0.15 mmol g^{-1} of Hg and Pb when incubated in solutions of these two metals (Couto et al., 2004), which constitutes<0.01% of the available binding sites. Thus, even though the extremely high potential of mosses to bind cations constitutes an attractive justification for their value as air pollution biomonitors, there is no definitive scientific evidence that proves this fact. Instead, as shown below, their high capacity to retain particles also strongly supports that value.

3.1. Moss morphology supports high particle retention capacities

In line with our argument supporting the high CEC of mosses, the high specific surface area of these plants also provides them a high capacity to retain particles (Real et al., 2021). The characteristics of the phyllidia, including their surface area, thickness, and architecture (density and disposition in relation to the caulidium) determine the extent to which particles are trapped. For example, the total amount of particles accumulated in the abaxial convex surface of the phyllidia is usually lower than in the adaxial concave surface (facing towards the axis), especially at the point of insertion on the caulidium. The shape of the phyllidia when appressed towards the caulidium also determines the extent of particle retention (Adamo et al., 2008; Tretiach et al., 2011), as well as the characteristics of the surfaces (like smoothness), which allows electrostatic attraction of light particles. Bates (2000), for example, argued that mosses from the genus *Sphagnum* constitute one of the most efficient airborne particle trappers because approximately two thirds of their total dry biomass is made of phyllidia.

3.2. Mosses carry large amounts of strongly retained particles on their surface

The number of particles per gametophore surface area has been estimated in 236 ± 129 and 537 ± 171 particles mm^{-2} in samples of the moss *P. purum* from unpolluted and polluted sites respectively (Spagnuolo et al., 2013). The mean number of particles in transplants of *H. cupressiforme* ranged between 93 and 353 particles mm^{-2} with a minimum of 4 and a maximum of 838 (data extracted using WebPlotDigitizer from Fig. 8 in Tetriach et al., 2011). The majority of these particles had diameters < 2 μm and between 2.5 and 10 μm, rarely being larger than 25 μm (Adamo et al., 2008; Tetriach et al., 2011; Spagnuolo et al., 2013). From a toxicological perspective, these small particles are the most concerning, as they are associated to multiple diseases (WHO, 2003).

Although a large proportion of the particles adsorbed onto moss surfaces originate from soil and rock dust (Adamo et al., 2008; Giráldez et al., 2021), anthropogenic activities can constitute an important source, especially in urban and industrial environments (e.g., Córdoba et al., 2012; Weinbruch et al., 2014). As an example, a sample of the moss *P. purum* collected in the surroundings of a FeSi-smelter has so many particles (Fig. 2) that the magnetic field produced by a laboratory shaker makes the moss dance (see video in supplementary materials – SM; sample collected by Fernández et al., 2004). Finally, these particles are generally strongly retained on the moss surface as demonstrated by the difficulty in their removal even when exposed to dry washing with N_2-jet or ultrasound bath (Spagnuolo et al., 2013), or water washing (Aboal et al., 2011).

3.3. Particles retained by mosses contain atmospheric pollutants

Studies using Energy Dispersive X-ray (EDX-ray) microanalysis confirmed the presence of metals (Al, Cu, Cr, Fe, and Zn) adsorbed to particles deposited onto moss surfaces (Pérez-Llamazares et al., 2011a; Tetriach et al., 2011). Quantification of the concentrations of pollutants on these particles is crucial to estimate their contribution to the total pollutant loads in mosses. Thus, Fernández et al. (2004) determined the concentrations of metals and metalloids in particles detached during the processing of moss samples collected in the surroundings of a FeSi-smelter. These particles reached levels as high as 4 μg g^{-1} of As, 426 μg g^{-1} of Cu, and 45 991 μg g^{-1} of Fe. The contribution of particles to the total metal loads can also be estimated with the Sequential Elution Technique as the difference between the total concentration and the sum of the concentrations in the extra- and intracellular fractions (Pérez-

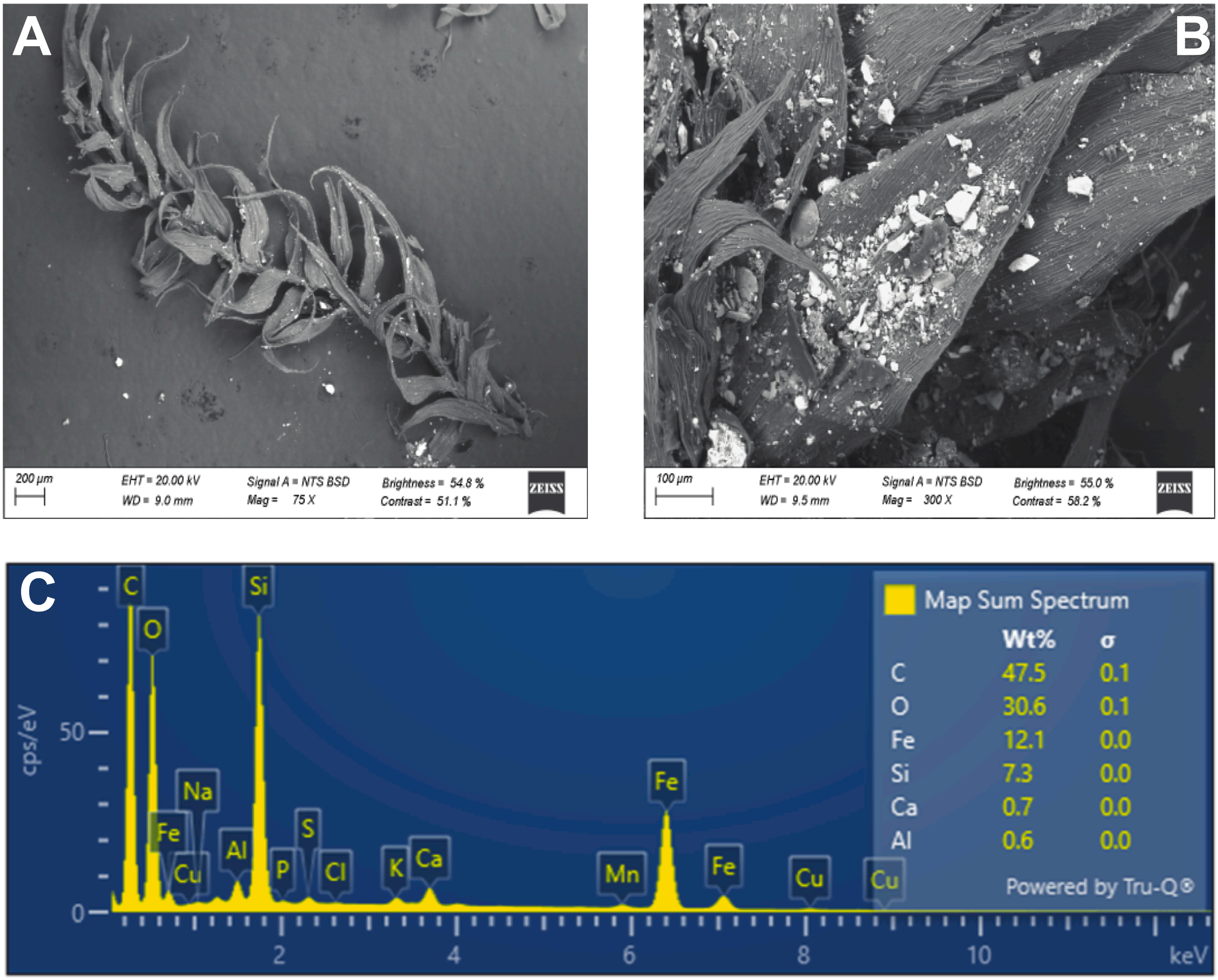

Fig. 2. Scanning electron microscope (SEM) pictures of fragments of the gametophore (A) and detailed view of the phyllidia (B) of the moss *Pseudoscleropodium purum* collected in the surroundings of a Fe-Si smelter showing the particulate matter trapped on the surface. (C) Energy Dispersive X-ray (SEM-EDX) microanalysis spectrum of the same samples showing the relative levels of different elements, including heavy metals (Fe, Cu).

Llamazares et al., 2011b). Fernández et al. (2010) and Pérez-Llamazares et al. (2009) applied this technique to moss samples collected in the vicinities of an aluminum- and a FeSi-smelter. They found that the particulate fraction constituted 68–73%, 4–22%, 19–26%, and 23–43% of the total levels of Fe, Zn, Hg, and Cu respectively. This contribution, however, was lower in unpolluted sites (6% for Zn and 17% for Cu; Fenández et al., 2010). Regardless of the fraction, the absolute concentrations of these elements in particles were considerably high: 7322 µg g^{-1} for Fe; 28.9 µg g^{-1} for Cu, 12.5 µg g^{-1} for Zn, and 133 ng g^{-1} for Hg.

Other studies reported that mosses are also very efficient at capturing magnetized metal particles. These particles are generated by the release of large amounts of metallic Fe by cars or the friction between the wheels of trains or trams in public transport. Eventually, they would bind to other pollutants already present in the air giving them magnetic properties (Kardel et al., 2012). For example, Salo et al. (2012) found a significant correlation between the concentrations of heavy metals in transplants of *Sphagnum papillosum* and the mass magnetic susceptibility (χ) of the samples: the highest metal content was observed near main roads, with higher χ values ($\chi = 25.6\ 10^{-8}\ m^3\ kg^{-1}$), whereas the lowest metal content was found in the vicinity of minor roads, with lower χ values ($\chi = 1.7\ 10^{-8}\ m^3\ kg^{-1}$). Similarly, the moss *Sphagnum girgensohnii* showed a high correlation between the Saturated Isothermal Remaining Magnetisation (SIRM; enrichment ranging between 0.82 and 15.02) and the concentrations of Al, Ba, Co, Cr, Cu, Fe, Ni, and Pb in microenvironments characterized by heavy traffic (e.g. street canyons, a city tunnel and car parks) (Vuković et al., 2015).

Several studies found evidence of pollutants other than heavy metals, including PAHs and negatively charged molecules like SO_2^- or NO_3^- that cannot be retained through the cation exchange sites, reaching the moss surfaces via the particulate fraction. For example, by incubating several moss species on phenanthrene solutions, Spagnuolo et al. (2017) observed micron-size particle aggregates of this compound in the incubation solution that were taken up by the mosses physically rather than chemically. Aboal et al. (2020) found significant regressions, with rather high determination coefficients, between the concentrations of intermediate and heavy PAHs (4-, 5-, and 6-rings) in transplants of *S. palustre* and in PM_{10} and bulk deposition, whereas no significant relationships were found between these matrices for lighter PAHs (2-, 3-rings). These authors argued that heavier PAHs are more frequently associated with particles whereas lighter PAHs can also be taken up in gaseous form. Taken together, these findings suggest that PAHs can reach the mosses both in gaseous and particulate forms but the relative importance of these two pathways differs between lighter and heavier PAHs.

Human activities, as well as volcanic eruptions and sea spray, release sulfur-rich particles into the environment (Robinson & Robbins, 1970; Saunders & Wood, 1973). These particles can associate with heavy metals like Pb leading to an increase in the metallic concentrations of mosses through particle trapping mechanisms (Vingiani et al., 2004). For N, even though mosses readily accumulate reduced forms of this element (e.g. Stevens et al., 2011; Varela et al., 2013), in which case cation exchange sites could have a relevant role, most N is emitted to the atmosphere in oxidized form and finally deposited on the moss as particulate-associated NO_3^- (Erisman et al., 2007; Fowler et al., 2013).

3.4. *Pollutant concentrations in mosses decrease as a result of particle detaching*

Even though complete particle removal from moss surfaces is difficult due to their recalcitrant nature to washing (Aboal et al., 2011), and that washing can alter the extracellular pollutant balance, some studies have demonstrated that particle removal reduces the concentrations of pollutants in these plants. Fernández et al. (2010) estimated this effect as the difference between the concentrations of metals and metalloids in washed and unwashed samples of *P. purum* divided by the concentrations in unwashed samples. They found that washing caused a mean concentration decrease ranging between 10 and 20% for As, Cu, Hg, and Pb, 20–30% for Cu and V, and 35–40% for Al, Fe, and Ni (maximum decrease of $<$ 20% for Hg, ca. 30% for Cu and Pb, and between ca. 50–70% for Al, As, Cr, Fe, Ni, and V). Türkan et al. (1995) obtained similar values, with concentrations decreasing between 30 and 83% for Pb, 11–28% for Cd, 34–72% for Zn, 57–66% for Cr, 51–70% for Mn, and 50–90% for Fe.

Environmental pollution with F provides an excellent proof of the importance of the particulate matter pathway in moss pollutant loads. Fluorine is the most electronegative element so in its ionic form is negatively charged. Therefore, F cannot be taken up through the cation exchange sites; rather, it will be bound to particles adsorbed on the moss surface (Real et al., 2003). Fluorine is released by aluminum smelters together with Al as $Na_5Al_3F_{14}$ and AlF_3, or as a solid agglomerated with alumina and carbon dust (Grjotheim & Welch, 1988). When emitted in gaseous form (~50% of the F released by the industry), F can also be adsorbed to carbon dust. Here, we present unpublished results of the concentrations of F and Al in washed vs. unwashed samples of the terrestrial moss *P. purum* collected twice a week for 12 months in the surroundings of an aluminum smelter in Galicia (NW Spain; see supplementary material S2 for detailed methods and results). These results showed that for Al, despite the high dispersion, the slope of the fitted regression line was very close to 1 (0.96) (Fig. S1). For F, however, the difference in the concentrations between unwashed and washed samples were always below the line of slope 1 and increased proportionally to the increase in F concentration (Fig. S1). Two non-mutually exclusive explanations are possible for the different behavior of Al and F: (i) molecules containing both elements could undergo hydrolysis releasing Al^{+3}, that could be retained by the cation exchange sites available in moss tissues, and F^-, that would be lost since negative ions cannot be retained by these sites; and (ii) an important part of F could be associated to dust particles (independently of Al); when high amounts of particles were present in the moss, those that were not directly adhered to the tissues could be more easily removed (up to 20%). This would not happen at lower amounts when most F-containing particles would be strongly attached to the moss surfaces. On the other hand, the concentrations of F, but not Al, in moss were significantly related with precipitation so that when precipitation was low (<15 mm), F concentrations increased or remained mostly unchanged between samplings; when precipitation was high, (>15 mm), the concentrations of F decreased between samplings (Fig. S2A, S2D). These results suggest that F may mainly be adsorbed onto particles deposited on the moss surface that can be partially removed by water splash or washing during strong enough precipitation events. Similar effects of particle washing from mosses by precipitation have been reported for negatively charged, sulfur-containing molecules (Vingiani et al., 2004).

3.5. *Pollutant dispersion patterns depict particle deposition processes*

Studies of the spatial distribution of pollutants in the surroundings of industries have shown that their concentrations in mosses decrease exponentially with the increase in the distance to the source, both for metals and metalloids (see as examples: Türkan et al., 1995; Brūmelis et al., 1999; Fernández et al., 2000, 2004; Pöykiö et al., 2001; Čeburnis et al., 2002; Real et al., 2003; Fernández et al., 2007; Varela et al., 2014) and organic pollutants like PAHs (e.g. Ares et al., 2009, 2011). Close to the source, the slope of the distance vs. concentration curve is steep and it decreases with increasing distance. In most cases, these pollutants were detected at distances up to 4000 m. Similarly, heavy metal concentrations in mosses tended to decrease with the distance to roads (Santelman & Gorham, 1988; Naszradi et al., 2004; Zechmeister et al., 2005, 2006). This decrease was already significant within 1000 m (Carvajal et al., 2010). This kind of spatial structure depicts the pattern expected for the atmospheric deposition of particles that are heavier than gaseous pollutants and, therefore, less dispersible.

Studies assessing the spatial distribution of the concentrations of heavy metals in regional moss sampling networks (Zn in Schröder & Nickel, 2018; Cd, Pb, and V in Boquete et al., 2009) demonstrated that most of the variance in these concentrations occurred at small spatial scales (<15 km), which also supports the predominance of medium and short distance dispersion processes, generally associated with particles. Finally, Boquete et al. (2011) showed that the concentrations of Mn in moss only varied in accordance to its concentrations in the atmospheric deposition when emitted in particulate form. It thus seems clear that mosses provide an accurate picture of atmospheric pollutant deposition patterns when these are associated with particles.

4. Conclusions

In this article, we gathered evidence from different scientific fields to demonstrate that the arguments used to justify the use of mosses as biomonitors of the atmospheric deposition of anthropogenic pollutants may be partially misleading. On one hand, bryophytes in general, and mosses in particular, have different structural characteristics that may affect pollutants uptake, such as a more or less developed cuticle, epidermal cells, a vascular system (even though they are frequently considered as non-vascular plants), and rhizoids. This means that they can absorb water, nutrients, and pollutants through different pathways and transport them throughout the gametophore, thus influencing the relationship between the concentrations of pollutants to which the plants are exposed and the concentrations found in their tissues. All these structural characteristics, which are developed to a different extent in a lineage- and species-specific manner, have frequently been obviated in biomonitoring studies, so far restricting our understanding of pollutant uptake processes, and hindering a better biological and environmental interpretation of the results of these studies. On the other hand, we demonstrated that the CEC of mosses cannot fully explain the pollutant loads found in their tissues. These plants have a great capacity to retain particles containing pollutants and their concentrations clearly depict particle deposition patterns in many circumstances. Particles can originate both from the atmosphere and from the soil, with the soil contribution being considerable in some contexts, potentially blurring the patterns of anthropogenic pollution sought with this technique.

Considering all of the above, the fact that bryophytes are good air quality biomonitors should not be based on traditionally assumed ideas that may be wrong (i.e. their structural simplicity facilitates a close relationship between external and internal pollutants concentrations; their only source of pollutants is atmospheric deposition) or insufficient (they have a high CEC). Rather, we should consider the influence of their true structural characteristics and their high capacity to retain particles (in addition to their high CEC) on the uptake, transport, and accumulation of pollutants, fostering further experimentation to elucidate how bryophyte biological characteristics affect biomonitoring processes. Experimenting with clones and mutant lines with structural or biochemical alterations in cuticles, epidermis, or vascular tissues, for example, could greatly improve our interpretation of biomonitoring results, based on a better comprehension of bryophyte physiology.

CRediT authorship contribution statement

Z. Varela: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **M.T. Boquete:** Conceptualization, Investigation, Writing – review & editing. **J.A. Fernández:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – review & editing, Supervision, Funding acquisition. **J. Martínez-Abaigar:** Conceptualization, Resources, Writing – original draft, Writing – review & editing. **E. Núñez-Olivera:** Conceptualization, Resources, Writing – original draft, Writing – review & editing. **J.R. Aboal:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110095.

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