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# Moss, Lichens and Phytobenthos Bioindicators of Pollution

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**Mini Review**

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## Abstract

The use of lichens, mosses and phytobenthos as biomonitors of air and water pollution by heavy metals is discussed on the basis of the literature and the author's own experience. The usefulness of the available monitoring techniques is critically evaluated. Moss and lichens are considered very useful biodindicators especially for large-scale studies of heavy-metal deposition from the atmosphere. National and international organization standardized and shared monitoring protocols. We analysed the recent literature from 2019 to April 2020 and selected some significant case studies that contribute to an improvement of the analytical methods and to a development of new tools. A wide literature reports monitoring of air pollution with moss and lichens, both natural and transplanted. The use of transplanted moss as bioindicator of water pollution is less represented in the available literature. Phytobenthos represents a new frontier in the aquatic ecosystem monitoring and even if a standardized method has not yet been finalized, it represents a potential very useful biomonitor of metals, emergent pollutants and also microplastics in aquatic environment. New tools, new technologies are emerging from recent literature and the relationship between environment and human health starts to be studied from a different point of view.

**Keywords:** Pollution; Bioindicators; Moss; Lichens; Phytobenthos; As; Hg; Heavy Metal

## Introduction

Biomonitoring is a complementary tool to measure pollutants in the environment at low concentration in large geographical areas. National and international monitoring programs tried to harmonize methodology protocols and data elaboration to compare the results. The interpretative scales are fundamental to the assessment of the magnitude of pollution phenomena [1]. Despite the large number of publications, further study is needed. Only between 2019 and April 2020 about 300 papers on Web of Science were published, of which about sixty in 2020. The papers range from wide programs monitoring, specific sites monitoring, different pollutants, and methodology modifications, natural and transplanted bioindicators, new develops in biomonitoring. Among this wide literature, we choose only few papers that, in our opinion contribute to an improvement of methods, to the development of new tools and new methodologies for general use. Many papers presents interesting researches carried out in specific polluted sites and detailed studies to monitor environment perturbation. The aim of our mini-review is to treat in particular detail the new approaches in biomonitoring and to link the air monitoring with the water monitoring. In recent papers [2-4] metal dispersion has been linked to the particulate matter (PM) (wet or dry depositions). It is now well known its impact in the human health [5] and its relationship with anthropic activities and with pollutants. Often, the atmosphere, the aquatic and the terrestrial ecosystems have been studied separately, also for the necessity to reduce the studied variables. From our point of view, a multy-ecosystemic approach could be more effective and the biosphere should be considered globally in pollution studies. Moss and lichens are studied mainly for the biomonitoring of air quality, fewer publication on biomonitoring of aquatic environment are present. The use of phytobenthos or biofilm to monitor pollution in aquatic ecosystems (metals, microplastics, emergent pollutants) represent a known monitoring system [6] that could be developed in a new context [7].

### Moss and lichens Bioindicators for Air Pollution

Mosses are also considered to be very useful biomonitors especially for large-scale studies of heavy-metal deposition from the atmosphere and the use of moss bag monitoring has spread all over the world. Despite this, the standardization of a method is still open. To overcome the use of natural moss the FP7 EU MossClone (www.mossclone.eu) proposed the use of a cloned moss, *Sphagnum palustre*. To contribute to method standardization the capability to adsorb Pb of *S. palustre* was compared to that of the field–grown moss [8]. Mosses were analysed before and after pre-treatment by EDTA washing and oven devitalization. No difference between the mosses was observed. The results suggested that oven heating devitalization appears the best strategy for biomonitoring applications and that the EDTA treatment is not significant and could be avoided.

A comparative experiment between a transplanted moss bag (*Hypnum cupressiforme*) and pseudoacacia leaves confirmed that despite the short time of exposure, moss bag accumulates large amounts of elements associated to the PM and is an optimum bioindicator of airborne pollution [9]. Despite in the last years a large number of papers have been published on the relationship between moss bag shapes (usually horizontal or round) and their uptake capacity, some questions are still open. The moss adsorption of metals carried by the wind and adsorbed in the particulate matter (PM>2.5) where monitored in Santiago (Chile), a semi-arid zone, where dry deposition is relevant and the effect of humidity and other interchange on the surface of the mosses are absent [4]. *Sphagnum*, a moss with a great capacity to accumulate pollutants for its morphological and physiological characteristics was used. Three species of *Sphagnum*: *S. falcatulum, S. fimbriatum, S. magellanicum* with different morphological characteristics were used to measure the effect of the porosity of the medium in the bag. Two shapes of moss bags, round bag (traditional shape) and semi-sphere bag, were exposed for a time of 30 days (August-November). In these conditions the bag shape was the most relevant variable. The semi-sphere bag showed a greater accumulation capability for Al, Zn, Sb, and Pb and lower accumulation for Fe, Ba and Cu. The result highlighted that in the presence of high wind speed the shape of the bag can determine the retention of PM.

Another important variable for the accumulation is the orientation of transplanted moss bag and their efficiency in contaminants retention [10]. This study was carried out exposing *Sphagnum palustre* for 14 weeks in industrial sites with high pollution. Two different systems were used, the first was attached to static poles and the second to weathervanes (free to rotate downwind). With this latter device, free to rotate, the metal uptake capacity was higher for As, Cu; Fe, Pb, Cu than for static poles. *Hylocomium splendens* was used to monitor metal pollution in Romania [11]. The monitoring time was of 4-7 months. Software for a simple monitoring tool, BioMonRo was developed to fill the gap between scientific research and decision-makers authorities. The Romanian Ministry of Environment approved the software for its simplicity as a complementary tool for the National Air Quality Monitoring Network. The results of the study confirmed the presence of pollution hotspots for Pb, Cd, Ni and As associated mainly with industrial areas in sites of historical pollution. Two common moss species, *Isothecium stoloniferum* and *Kindbergia praelonga*, were used to monitor air pollution in western Washington (USA). Metal concentrations values were compared with those measured in wet deposition [12]. The sampling period was of 12 months. The results obtained were used as screening tools to identify polluted areas. The urban samples contained large amount of metals from industrial sources, while rural moss samples were influenced by natural variability and long-range pollutant source from Asia. This work implemented the existing USFS Forest Inventory assessment lichen monitoring networks and confirmed that moss and lichens are useful tools to monitor air pollution.

Capozzi, et al. [5] carried out a study with a novel monitoring approach to evaluate indoor and outdoor air pollution. Transplanted mosses were successfully used in polluted industrial areas and urban areas. *Hypnum cupressiforme* was the utilized moss. In the case of outdoor pollution, urban sites were most impacted than rural. Indoor pollution was affected by different sources, building material, heating and cooking systems, life style of the families and traffic in the urban areas (low air quality and presence of PM). Results showed that As derived from outdoor and indoor sources; B, Mo, and Se derived mainly from outdoor sources; Ni, Cr and V derived from indoor sources.

A preliminary work, has been conducted at a polluted site in Italy, located in the Ossola valley (Italian Central Alp) closely the town of Pieve Vergonte (Vb, Italy) were investigated. The site was heavily polluted by chlor-alkali industrial activity operating since 1915. The gaseous elemental mercury (Hg0) in air and its accumulation in lichens were analysed. Results showed maximum values observed of two orders of magnitude higher than the background levels and were always measured near the chlor-alkali plant. Preliminary data on total Hg concentrations in lichens have highlighted a spatial pattern of Hg accumulation related to the wind direction [13]. In parallel moss native samples and phytobenthos were collected along the river Toce to analyse and monitoring the pollution dispersion through air and water.

Lichens are usefully used as biomonitors for air pollutants. Heavy metal accumulation is a fundamental strategy for the lichens to survive in polluted areas [14]. Lichens adsorb nutrients and toxic elements through the surface of thallus. The proportion of extra and intra cellular metal concentration depend on the lichen species and on the metals, or on the absorption competition among pollutants. Rola, et al. [14] investigated the extra- and the intra- cellular accumulation in *Cladonia cariosa,* Zn, Pb and Cd were accumulated outside cells, instead Cu and Ni inside cells. The results suggested that many factors influenced the uptake of metals in the environment, as temperature, humidity, light, wind, amount of pollutions. The element enrichment in the extracellular compartment may be a defence mechanism to survive in polluted sites and may explain the capability of colonisation of pioneer lichens.

Epiphytic lichens (*Usnea*) have been used as biomonitors of air distribution of toxics elements (Hg, As, Cd, Cr, Pb, Cu, Ni, Al, Se, Co) in Nova Scotia (Canada) [15]. Natural sources (geogenic) and anthropogenic activities (historic gold mines, mine tailings, extraction process, coal-fired power generation, waste incineration, chemical manufacturing and metal smelting) contribute to the air distribution of toxic elements in Nova Scotia. *Usnea* was chosen for its intermediate tolerance to the pollution, abundance and easy collection and identification. Analysis of the spatial pattern in lichens showed a correlation with the deposits of an old gold mine for arsenic and selenium while copper and lead showed a correlation with hot spot sources. Instead, Hg spatial mapping showed a gradient of accumulation due to the prevailing winds from southwest to northeast, supporting the hypothesis of long-range transport.

The magnetic properties of particulate matter (PM) are a recent proxy to correlate the anthropogenic fraction of PM in the lichens with the pollution of urban areas. The iron oxides, magnetite-like ferromagnetic particles, have significant magnetic properties; heavy metals are often associated to the magnetic fraction of PM incorporated in the iron oxides during combustion (industrial emission), or directly from emission source [2]. In a pilot study, a native lichen (*Xanthoria parietina*) and a transplanted lichen (*Pseudevernia furfuracea*), were analysed as bioaccumulators of airborne PM in Rome, Italy, for 4 months, in order to quantify the contribution of the human activities in cities (fraudulent fires, waste of electrical and electronic equipment). The results showed that the concentration dependent magnetic parameters were related to the abundance of Fe, Cr, Pb, Mn, Zn and Cu, but it was not possible to identify the anthropogenic source and the distribution pattern of the PM for the scarce presence of lichens in the investigated area. The experiment showed the utility of the proxy. In another paper [3], a transplanted lichen (*Evernia prunastri*), was exposed for 3 months in the city of Milan (Italy). The deposition of PM enriched in Fe, Cr, Cu, Sb determined the magnetic susceptibility of lichen samples. The results showed the main contribution of vehicular brake abrasions in the production of PM.

Ten years of literature on lichens monitoring in Italy was examined by Cecconi, et al. [1]. The authors have examined data on native lichens (*Flavoparmelia caperata, Xanthoria parietina*, foliose lichens) and transplanted lichens (*Evernia prunastri, Pseudevernia furfuracea*, fruticose lichens). They developed new interpretative scales based on the meta-analysis of bioaccumulation data from the most recent Italian literature, and they analysed the distribution of the ratios between element concentration data and species-specific background for native lichens or element concentration data and unexposed samples for transplanted lichens. Comparing previous and new scales to two case studies, the authors conclude that the latter possess a better performance. Inter specific difference were evidenced between lichens: *F. caperata* exhibited higher concentrations of As, Cd, Cu, Pb, and Zn, whereas *X. parietina* had more Al, Cr, Ni, and Ti.

### Moss Bioindicator for Freshwater Pollution

Water sporadic sampling followed by biochemical analysis of samples can lead to possible underestimation of occasional pollutants or overestimation of non-bioavailable forms. To solve this problem the WFD recommends to use also the biota as active matrix for pollutants monitoring, in order to evaluate the bioavailable fraction of pollutants in the aquatic environments. Different protocols have been developed and used in the different studies with transplanted mosses [16]. A new protocol for the use of aquatic moss and the necessity of a standardization of methods was investigated in a study carried out in industrial and mine areas with high metal contamination (Al, As, Cd, Co, Cu, Fe, Hg, Ni, Zn and Pb) located in Spain. The aquatic moss used was *Fontinalis antipyretica*. The pre-exposure washing (with or without EDTA), the ratio between moss weight and bag surface area and the depth of exposure were analysed. Authors hypothesize that the moss capability of adsorption could be influenced by the water flow. Different devices (unperforated open tubes, perforate close tube, or uncovered moss) were used to clarify this aspect. The results demonstrated that the adsorption in the uncovered moss where higher for almost all the elements and flat moss bags were the best type of support for transplanted moss. The thermal inactivation was necessary. The EDTA wash used to remove eventually present metals did not show an increase in speed and capacity of accumulation. A 7 day exposure period was sufficient to obtain quantifiable differences and it is suggested extend it to 3-4 weeks. Washing treatment was suggested for short time exposure (one week). The depth of the water column did not affected pollutant accumulation.

The literature comparison of the pollutants diffusion in air and water seems to be different; the lowest ratio for terrestrial moss was four times lower than this ratio in aquatic moss (2.84 vs 12.5 mg $cm^{-2}$). Moss clone should be developed to improve the methodology also for aquatic moss.

The chlorophyll fluorescence of an aquatic moss with large area leaves (*Taxiphyllum taxirameum*) was used to monitor heavy metal contamination in water [17]. The proposed method, that needs to be implemented, estimated visually the metal contamination using chlorophyll fluorescence images. The fluorescence can be influenced by environmental parameters like low temperature or salinity. These two parameters did not affect the significantly chlorophyll. Also, heat stress, high light or osmotic stress, may not compromise the accuracy of the method. The colour pattern remained stable with the increasing metal concentration and developed a metal-ion-specific fluorescence colour. This pattern can be observed in moss but not in higher plants. Further investigations are necessary on the metals accumulation and translocation in different part of the thallus. This method can be useful to monitor heavy metal on the surface water of mining areas. To monitor industrial sites with toxic organic and inorganic pollutants this promise method need to be develop [17].

A laboratory study was carried out to improve knowledge on moss resistance to aquatic pollutants [18]. The aquatic moss *Leptodictyum riparium* can accumulate very high concentration of toxic metals, including Cd, with a higher bioconcentration factor. This moss was proposed to monitor metal contamination and to carry out phytoremediation in polluted areas. The apparent Cd tolerance in contaminated environment might be due to an efficient intracellular detoxification process. Biochemical assays and confocal laser imaging (CLSM) of monochlorobimane-stained thiols of *L. riparium* gametophytes and optical/electron microscopy techniques were used, measuring the effects of increasing amount of Cd. The detoxification pattern in living cells often is not a linear relationship but rather a complex system of detoxification, which involve different enzyme (CAT, SOD, GSH), thiols, γ-glutamylcysteine, glutathione, phytochelatins, SH-proteins. The cellular detoxification mechanism for Cd in *L. riparium* can be attributed to the thiol peptides mediated intracellular detoxification, to the activation of GST, to a moderate contribution of phytochelatins and to a contribution of vacuolar compartmentalization.

## Phytobenthos Bioindicator for Freshwater Pollution

The Water Framework Directive (WFD) is a milestone of the European Union water policy [19]. Its objective is to protect and enhance the status of aquatic ecosystems and to promote sustainable water use [6]. Since the WFD was formally adopted, some implementations have occurred in particular in the innovation of the methodology, providing new monitoring tools (Earth Observation, genomics, citizen science). The use of satellite data for surveillance and monitoring of water colour, chlorophyll-a, cyanobacteria has a great potential. Meta-barcoding and environmental DNA are complementary tools with automated sensor technologies and flying, floating and submerged drones equipped with multisensors.

River ecosystems are often modified by human activities: riparian deforestation, geomorphology modification, increase catchment, introduction of effluents from wastewater treatments plants, introduction of contaminants, metals, pesticides and nutrients loads [20]. Benthic diatoms (eukaryotic algae often dominant in benthic habitats) were used to define the ecological status of aquatic ecosystems for their large species diversity and sensitivity to specific physical, chemical and biological changes. They were used as bioindicators to assess the specific index of pollution sensivity, IPS. The Water Framework Directive [6] requires the ecological assessment of surface waters and includes the benthic diatoms as bioindicators. The study of Trabert, et al. [20] suggests that diatoms in a large river like Danube reflect the effect of the hydromorphology rather than the nutrients loads and that the IPS index could be used also for the effect of land use.

The diatom communities have higher ubiquity and sensitivity to environmental perturbation; therefore, they are good bioindicators. In the study of Salmaso [21], different environmental factor (geographical, chemical and hydrological) were investigated in 34 sites located in 13 watercourses of northern Italy according to the Water Framework Directive. The normative index currently adopted in Italy (ICMi) was calculated. The results showed that the taxonomical and functional analysis of diatoms communities were associated to the altitude and geological characteristics of the studied watercourses. Moreover the data analysis revealed differences in the chemical and hydrological alteration. The ICMi in the samples ranked more than 90%, index of highest quality class water but the index could not detect the effect of anthropogenic perturbations. There is the necessity of a different approach in diatom data interpretation to reach reliable information on the ecological status of the watercourses.

The microscopic world of the freshwater biofilm is a complex association of organism [7]. Understanding the relationship between these organisms and their environment is the complicate processes of the biofilm life. It is possible to separate and identify each organism of the community, but in this way, the inter-relationship within the community is lost.

The identifications of the community is an important step but need to be supported also by other approaches like the study of the biofilm formation and evolution. The digestion of biofilms, necessary for the diatoms identification, leads to loss of information on host-epiphyte associations [7]. The three dimensional organization of the biofilm remains one of the difficult aspect to clarify. Often, during the samples collection biofilm is greatly distorted and the artificial substrate is often different in composition, compared to natural surfaces. A possible way to incorporate information on the interaction within the biofilms could be to introduce the study of "functional groups" such as "motile", "stalked", "adnate" with SEM, that could be used to understand three-dimensional structures [7]. There is the necessity to change the perception that qualitative information is less relevant than the quantitative one, and that description is less important than identification. It is necessary to change way of seeing to understand the role of biofilm in the environment.

## Conclusion

Mosses and lichens are largely used for the monitoring of metal pollution. The method of the moss bag monitoring was deeper studied but can be optimized taking in consideration moss bag shape, position, rotation, direction of the wind. Further investigation on the dynamics of the PM present in the bulk deposition, the seasonality and the mechanism of adsorption of metals need to be investigated to improve the monitoring. To improve the standardization the possibility of developing standard moss clone can be useful, but it is suggested in any case the thermal devitalization of the moss. Insights in the detoxification mechanisms of bioindicators and their relationship with the pollutants could improve the monitoring. New approaches, as chlorophyll fluorescence and image analysis, drone images, non-destructive methods could be investigated, optimized and compared to traditional methods.

The necessity to estimate the human pollutants exposure risk, indoor, in working place and in living place can be supported by moss bag monitoring. The indoor air quality monitoring could link the environmental pollution with the human health and permit the development of operative protocols for the safety. A different approach to study the biofilm and its role in the natural environment and in polluted sites could be a useful tool. Phytobenthos has a three-dimensional structure (the assembly of different organism) that allows to survive in perturbed environment. It could be interesting to investigate the possibility to use the biofilm as bioindicator of freshwater pollution thanks to its characteristics of being a natural bio-adsorbent. Moss, lichens and phytobentos could be used also to measure pollution of microplastic and other emergent pollutants.

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