REVIEW ARTICLE

# A Review on Bryophytes as Key Bio-indicators to Monitor Heavy Metals in the Atmosphere

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## Abstract

Bryophytes are one of the simplest autotrophic cryptogams invading land and characterized either by simple thalloid or erect habit lacking true leaves, root and stem within the plant body. Although they are ubiquitous in distribution, yet becomes sensitive to certain environmental conditions that can be natural or induced due to anthropogenic activity. Due to their versatile tolerance and resistance capability they can be categorically used as potential bioindicators for monitoring pollution. Bryophytes can be utilized as 'environmental specimen bank' due to their unique capacity of indicating the presence of metal and their concentration gradient in the substratum. Apart from their utility in pharmaceutical products, horticulture, household purposes they are also ecologically important. As multidimensional applications of the flora are being increasingly standardized universally, their potential in the biomapping of atmospheric pollution as well as ecological biodegradation is also enormous. Currently, a change in global climate is intensified that affected Earth's biomes and vegetation zones redistribution. At higher altitudes, this alteration is more promising with rapid consequences. Elevated temperatures are expected to produce a drier environment that affect site water balance and cause shifts in the distribution of ecosystems on a universal scale. These effects are rather evident in such ecosystems as peatlands which are sensitive to both climate and water level fluctuations. Decrease in epiphytic bryophytes because of gaseous and particulate pollutants as well as the greenhouse gases is also a serious problem. Besides this, very specific and unique responses are generated by the bryophytes; studies have proven that they act as potential monitoring bio-agents for heavy metal pollution. In the present paper, an attempt is done to do a comprehensive study on bryophytes that reflects their role as promising indicators in monitoring pollution.

**Keywords:** Bioindicators, Bryophytes, Heavy metals, Pollution.

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## Introduction

Since the advent of civilization, the environment has received various types of pollutants in one form or the other. The first record of anthropogenic pollution may well be traced back with the discovery of fire by humans that initiated adding up of toxic oxides of carbon, nitrogen, sulphur in the atmosphere. Gradually as the civilization and time proceeded, earth was contaminated by a wide array of pollutants with anthropogenic wastes which were mostly non-biodegradable. Records of pollution can be very well documented from the industrial era where ambitious human activities have led to emission of greenhouse gases resulting in the change in climate (Perera, 2018). However, pollution started well before the industrial era and it is documented there are a number of ancient civilizations which were largely responsible for build-up of anthropogenic pollution. Among various pollutants, heavy metal contamination is one of the earliest which is documented in historical records.

Detection of lead and copper from the ice cores recovered from Greenland indicates that their concentration was greater 2500 to 1700 years ago (500BC to 300AD). This is possibly due to lead and silver mining and smelting activities by ancient Greeks and Romans which were primarily used for weapons, artefacts and others. This eventually resulted in pollution of the troposphere of northern hemisphere and is probably the first record of this type due to anthropogenic activity. These metals consequently deposited in the ice sheets of Greenland (Hong *et al*., 1994; McConnel *et al*., 2018). In southern hemisphere, the earliest evidence of atmospheric metal emission was detected in sediment cores of lakes located downwind of major metallurgical centers in Peru and Bolivia (Abbott *et al*., 2003; Cooke *et al*., 2007; Cooke *et al*., 2009). Contamination of South America by metals began as early as 1800BC in Peru and Bolivia and also in the Inca Empire between 15$^{th}$ and 16$^{th}$ century which resulted in dissemination of metal pollutants across the Andes (Lechtman, 1980; Brown, 2012). Later on, the Spanish conquistadors added more pollution in the subcontinent through mining of silver and associated use of mercury amalgam (Robins and Hagan, 2012). Europe also has a long history of metal pollution starting from the Bronze Age to the modern times. The industrial revolution

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acted as a catalyst in the European continent and added a thrust to the metal pollution (Longman *et al*., 2018).

Presently rapid progress of industrialization accompanied by emission from automobiles has been adding up metals in the environment. This has resulted in a number of heavy metal related ecological disasters and harmful human diseases amongst which the Minamata disease tops the list (Harada, 1995). Thus, monitoring of heavy metal contamination is of extreme relevance to the present day society. Biological entities have so far being proven as a suitable candidate for monitoring heavy metal pollution. This is because most of the metals are toxic to the organisms and bring about marked changes at morphological, physiological as well as genetic level, which can be very well observed and documented. Among the biological organisms, plants have proven to be very handy in monitoring and detecting heavy metals and this is due to their capability of absorbence of heavy metals within their body (Azab and Hegazy, 2020; Cooper *et al*., 2020).

Among the plants, bryophytes were the first to inhabit and colonize the land surface (Kenrick *et al*., 2012). At present they are cosmopolitan in distribution and dwells in different habitats with different growth forms ranging from mats, cushions, pendents, turfs to dendroid forms. This group of plants prefer to grow in moist and shady places where water is an essential constituent for successful fertilisation; hence they are aptly termed as the 'amphibian group'. They lack true leaves, roots and vascular systems. The simple body consists of rhizioids which perform the absorption from soil as well as anchorage to them. Bryophytes are widespread in distribution and absorb the water and minerals directly from the whole plant surface thus many of the unwanted substances are also taken into the plant and making them to be good accumulator of heavy metals and thus qualify as an ideal heavy metal biomonitoring system (Tremper *et al*., 2004).

Bryophytes also have the potential to grow on substrate containing specific metals and thus can be used as bioindicators of specific metals. Moreover, the simple make up of these plants make them suitable candidates to study morphological and genetic changes which takes place due to metal toxicity or stress (Stanković *et al*., 2018). The ability of bryophytes to retain potentially toxic element has led to their use as an indicator of air pollution (Rühling and Tyler, 2004). Bryophytes consist of both sensitive and tolerant species and respond to pollution in either of the following ways. Firstly, many of them are highly sensitive to even a slight alteration in pollutant level at the surrounding and it's manifested in form of visible symptoms of injury in the protonema and thalli. They serve as an excellent indicator of pollution or contamination. Secondly, the other group has the capacity to absorb and retain pollutants in quantities much higher than their immediate neighbouring plants; they entrap and prevent the recycling of the metal pollutants in the environment (Govindapyari *et al*., 2010). This paper is an attempt to review the role and use of bryophyte as an effective tool to monitor metal pollution in the environment.

## Sources of Heavy Metals

At present industrial and anthropogenic activities are the major sources of heavy metal pollution on the earth. During last hundred years, industrialization has increased at a rapid rate and thus demands for increased exploitation of natural resources of Earth. This is accompanied by careless and unplanned management of earth's natural resources which have led to the pollution in various spheres of the atmosphere (Briffa *et al*., 2020). Presently, our environment is thus polluted by a number of pollutants such as heavy metals, inorganic ions, organic compounds, radioactive isotopes, gaseous pollutants and nanoparticles (Walker *et al*., 2012).

In this section various sources of heavy metal pollution would be discussed in brief. Heavy metals may be defined as those metals which have a high atomic weight and a density at least 5 times than that of water (Tchounwou *et al*., 2012). At present, there is an increasing ecological and global public health concern related to environmental pollution by these heavy metals. In addition to it, exposure of humans has also increased due to indiscriminate use of these heavy metals in industry, agriculture and domestic spheres (Bradl, 2005). Heavy metals are grossly emitted from solid fuel combustion, vehicular emissions and in industrial processes. Table 1 represents sources of selected heavy metal contamination in the environment.

## Merits of Bio-Agent against other Agents

Use of bioindicators utilize the biota to evaluate the cumulative impacts of both chemical pollutants and habitat alterations over time, in comparison to the traditionally conducted chemical assays and directly measured physical parameters of the environment (e.g., ambient temperature, salinity, nutrients, pollutants, available light and gas levels). Moreover, the use of bioindicators is fundamentally different from classic measures of environmental quality and offers several merits: (i) Firstly, they add a temporal component corresponding to the life span or residence time of an organism in a particular system, allowing the integration of current, past, or future environmental conditions. In contrast, many chemical and physical measurements only characterize conditions at the time of sampling, increasing the probability of missing sporadic pulses of pollutants. (ii) Secondly, they possess the ability to indicate or determine indirect biotic effects of pollutants that other physical or chemical measurements cannot. (iii) And thirdly, bioindicator signal is masked by an excessive number of divergent species' responses (e.g., some species may increase while others decrease) where they integrate all direct and indirect effects that focus only on subset of the biota or single species which is biologically relevant and cost-effective (Holt and Miller, 2011).

## Use of Bryophyte System as Bioindicators

Plants are widely used as bioindicators for their peculiar and distinct responses, in which bryophytes play a very crucial and specific role due to extreme sensitivity of several bryophytes to pollutants in the immediate substratum. They exhibit visible injury symptoms even in the presence of very minute traces of pollutants. Such species serve as good bioindicators and act as a "warning giver" to the environment. Bryophytes also possess the capacity to absorb and retain pollutants in concentrations much higher than those absorbed and retained by the higher plants growing in the same habitat (Jiang *et al*., 2018).

Uptake mechanisms of elements in vascular plants and bryophytes are vastly different and this also makes a visible difference. It is observed that vascular plants mainly meet their nutritional requirements by absorbing them from the soil through their developed root system (Dinneny, 2019) and their foliar systems also help in the uptake of gases (e.g. $NO_2$, $NH_3$

**Table 1:** Heavy metals and their potential sources of contamination

| *Metal* | *Symbol* | *Source* | *Reference* |
|---|---|---|---|
| Cadmium | Cd | Smelting industry | Liu, 2003 |
| | | Fertilizer | Dharma-Wardana, 2018 |
| | | Sewage sludge | Agoro *et al*., 2020 |
| Chromium | Cr | Smelting industry | Deakin *et al*., 2001 |
| | | Cement production | Isikli *et al*., 2003 |
| | | Waste incineration | Astrup *et al*., 2005 |
| | | Chromite mining industry | Dhakate *et al*., 2008 |
| | | Coal combustion | López-Antón *et al*., 2008 |
| | | Oil combustion | Cheng *et al*., 2014 |
| | | Iron and steel production | Järvelä *et al*., 2016 |
| | | Leather industry | Andleeb *et al*., 2019 |
| Copper | Cu | Smelting | Klumpp *et al*., 2003 |
| | | Industrial effluents | Singh and Chandel, 2006 |
| | | Mining | Pandey *et al*., 2007 |
| | | Electronic equipment processing | Gu *et al*., 2017 |
| Iron | Fe | Mining | Kessarkar *et al*., 2015 |
| | | Smelting | Hu *et al*., 2019 |
| Lead | Pb | Metallurgy | Ettler *et al*., 2004 |
| | | Lead paint | Clark *et al*., 2005; Lin *et al*., 2009 |
| | | Mining | Nikolaidis *et al*., 2010 |
| | | Electronic waste | Yang *et al*., 2013 |
| | | Traditional medicines | Mikulski *et al*., 2017 |
| | | Battery manufacture | Gottesfeld *et al*., 2018 |
| Manganese | Mn | Combustion of gasoline | Zayed *et al*., 1999 |
| | | Fungicides | Costa-Silva *et al*., 2018 |
| Mercury | Hg | Chlor-alkali industry | Gibicar *et al*., 2009 |
| | | Oil refining | Urgun-Demirtas *et al*., 2013 |
| | | Non-ferrous metals production | Wu *et al*., 2016 |
| | | Artisanal and small-scale gold mining | Esdaile and Chalker, 2018 |
| | | Dental amalgam | Tibau and Grube, 2019 |
| | | Cement production | Chen *et al*., 2020 |
| Nickel | Ni | Industrial waste | Krishna and Govil, 2007 |
| | | Municipal Solid waste burning | Van Praagh and Persson, 2008 |
| | | Household fuel burning | Matawle *et al*., 2017 |
| Uranium | U | Phosphate fertilization | Rufyikiri *et al*., 2006 |
| | | Gold mining | Mandeng *et al*., 2019 |
| Zinc | Zn | Municipal solid waste incineration | Struis *et al*., 2004 |
| | | Mining | Jabłońska-Czapla *et al*., 2016 |
| | | Coal combustion | Li *et al*., 2017 |

and $SO_2$) from the atmosphere (Antunes *et al*., 2012), whereas bryophytes obtain their nutrition by absorbing the substances dissolved in air moisture from their general surface (Vats *et al*., 2010). These properties give them an edge over other vascular plants as a suitable candidate to monitor pollution. The high accumulation capacity of bryophytes for pollutants has led to their use for heavy metal monitoring.

Two categories of bryophytes are categorised according their response to pollution (Govindpyari *et al*., 2010). The first one are very sensitive to pollution illustrate observable symptoms of injury even in the presence of minute traces of pollutants and serve as good indicators of the degree of pollution and also of the nature of pollutant. The second group absorb and retain pollutants in quantities much higher than those absorbed by other plant groups growing in the similar habitat. These plants trap and prevent recycling of such pollutants in the ecosystem for different periods of time. Analysis of such plants gives us a fair idea about the degree of metal pollution in the vicinity.

Metal analysis has thus become a frequently used and dependable yardstick in the evaluation of the environmental quality of a given site (Tchounwou *et al*., 2012). The method was first used by Rűhling and Tyler (1968) to analyse lead in mosses to monitor roadside pollution in Sweden. Analysis of Pb, Cu, Zn, Ni, Cr and Cd of some mosses was done from the herbarium specimens collected during 1905-1971 from Mount Royal in Montreal, Canada. Significant increase in Zn concentration in all the mosses selected was observed in the study (Rao, 1982). A study on bio-monitoring of heavy metals due to vehicular pollution with the help of *Sphagnum* is well reported (Saxena, 2001). Bryophytes from various regions in India are studied to understand their tolerance potential for different pollutants (Chopra and Kumra, 2005, Govindapyari *et al*., 2010).

Hence, it is revealed that bryophytes are efficient accumulator of heavy metals because of their unique properties like absence of cuticle layer and true root system thereby meet the requirement of minerals through atmospheric deposition by easily permeating to water and minerals, including the gaseous pollutants and heavy metal ions; negatively charged groups present on tissues also act as efficient cation exchangers, even the dead tissues possess competence to bind ions because cell walls possess high cation exchange capacity. Bryophytes usually acquire mineral nutrition from wet and dry deposition of particles and soluble salts. Nonetheless, some bryophytes absorb metals from substrate with rising capillary water, making them less suitable for the monitoring of heavy metals (Sahu *et al*., 2007).

Consequently, this group of plants added new vistas to study the level of different types of pollution, mainly the one caused by metals. Therefore, the cosmopolitan distribution of moss species in community, measurement of their growth rates and concentration of contaminants in them are reliable aspects in biomonitoring technology (Vats *et al*., 2010). Moreover, different approaches have been revealed to indicate that bryophytes have been used as prominent pollution indicators, which can be categorized as:

## As Potential Air Pollution Indicators:

Despite lacking cuticular layer, proper rooting system they absorb heavy metals directly from the atmosphere through dry and wet atmospheric deposition over their entire surface (Liggett *et al*., 2015). They are sensitive indicators in the immediate environmental conditions. Air pollution can create moss-deserts and force many sensitive species to retreat. From long time back, bryophytes are assessed to determine the impact of environmental pollution in Japan (Taoda, 1972), Europe (Greven, 1992). One of the significant air pollutant to terrestrial and epiphytic bryophytes is considered to be $SO_2$ (Sulphur dioxide), that causes chlorophyll plasmolysis and severely affect the plant growth (Geebelen and Hoffman, 2001; Govindapyari *et al*., 2010). Bryophytes have been frequently used to monitor air pollution by analyzing the atmospheric heavy metal deposition within their thallus or plant body.

## As Potential Water Pollution Indicators:

It is a well-documented fact that the heavy metal pollution has increased dramatically since the days of industrial revolution (Wu *et al*., 2014). These metals contaminate not only the land but also the aquatic ecosystem thereby posing threat to the aquatic life. The principal advantage is their adaptability to assimilate pollution over time and maintain a record that cannot be obtained and retain their toxic load long after death due to their slow decaying process (Onianwa, 2001). Reports supported that among *Fontinalis* sp, *Leptodictyum riparium, Platyhypnidium riparioides* and *Scapania undulate,* the later one *survived* at the low pH of 3.9 and it is a very useful accumulator for zinc, lead, and cadmium (Shacklette, 1984) in nutrient-poor water. One report states that *Fontinalis antipyretica* when transplanted in polluted water accumulated more heavy metals namely Al, Cr, Cu, Pb, V, and Zn than the native bryophytes (Samecka-Cymerman *et al*., 2005). In another study it was reported that *Fontinalis antipyretica and F. dalecarlica* possess the capacity to absorb cadmium ion in laboratory condition making both the species suitable for biomonitoring of cadmium (Bleuel *et al*., 2005). It has also been reported from a recent study that *Leptodictyum riparium* has the capacity to absorb Cd, Cr, Cu, Fe, Ni, Pb and Zn. It was further observed that the plants responded to heavy metal stress by generating reactive oxygen species. This was accompanied by changes in cellular levels and alterations in expression levels of heat shock protein 70; indicating *Lepidodictyum riparium* is a suitable candidate to monitor heavy metals in water bodies (Esposito *et al*., 2018). Another interesting study reports the accumulation of rare earth elements namely Nd, Gd, Ho, Er, Tm, Lu, La, Ce, Sm, Eu, Tb and Dy by four aquatic mosses namely *Fontinalis squamosa, Brachythecium rivulare*, *Platyhypnidium riparioides* and *Thamnobryum alopecurum*. This study indicates that aquatic bryophytes can be exploited as a promising candidate in monitoring rare Earth elements in water (Pratas *et al*., 2017). The use of *Platyhypnidium aquaticum* to monitor metal and metalloid contamination of Zamora River in the city of Loja, Peru, south America (Benítez *et al*., 2020). Accumulation of different materials differs in different parts of moss plants. Mercury sulphide (HgS) crystals in the cell walls of the aquatic bryophytes, *Jungermannia vulcaniania* Steph. *Scapania undulate* is very well illustrated (Satake *et al*., 1990). Several elemental traces such as aluminum, manganese, copper, zinc, and lead in higher concentrations are observed, 1-3 cm below growing stem tips than at tips of *Pohlia ludwigii*, but sodium, phosphorus, calcium, and iron differed little between the 1 cm tip portion and lower parts (Soma *et al.*, 1988). The higher concentration of some minerals in older parts may be due to coatings of iron and manganese oxides on leaves and stems, thus increasing adsorption of other metals (Robinson, 1981) to greater exposure time of older leaves, or to greater permeability of older leaves, providing access to interior cell-wall binding sites. Other differences may relate to the ability to transport materials from one part of the plant to another, particularly in *Sphagnum* and in other upright, emergent mosses (Clymo, 1963).

## As Potential Mineral Deposition Indicators

The Earth's crust is treasure of minerals of all the elements. Due to their unique body pattern and physiology, they easily uptake minerals from the environment and thus can be very well regarded as an indicator species. More appropriately, bryophytes are considered as bioindicators as their presence or absence in a specific area also corresponds with the occurrence of specific minerals or elements in the soil. As for example, some bryophytes act as indicators of metallic enrichments with copper in particular (Bačkor *et al.*, 2009; Sun *et al.*, 2009). A few mosses particularly grow on copper rich substrate, are often termed as copper mosses. *Scopelophila cataractae* is one such copper moss and the copper concentration in the shoots has been reported to attain a level of as high as 1-3% (Satake *et al.*, 1988). It was also reported that two fifths of the copper is bound to homogalacturonan of the cell wall pectin (Konno *et al.*, 2010). Another study revealed that *Physcomitrella patens* have the capability to grow in high copper levels (up to 100 mM Cu-EDTA) indicating that the moss is able to tolerate high concentration of copper (Sassmann *et al.*, 2010). The growth pattern of *Brachythecium rutabulum* closely relates to copper deposits in copper smelter region of Legnica in southwest Poland (Samecka-Cymerman *et al.*, 2009). Apart from this, several bryophytes were found to absorb certain metals within them. Such as, *Haplocladium angustifolium* reported from China indicates absorption of many metals within itself (Table 2). Different bryophytes studied from various regions of world has been tabulated in Table 2 that shows that bryophytes are really very good indicators.

## Methods for Monitoring Pollution Indices

There are different methods for studying the effects of pollution with the help of bryophytes. To define these indices, several parameters are noted including search for different bryophyte communities, respective cover areas and determination of their absorbance capacity mentioned as follows-

**Table 2:** Study of metal deposition within bryophytes of specific areas

| *Study Area* | *Family* | *Plant Species* | *Metals detected* | *References* |
|---|---|---|---|---|
| Chongqing, China | Lepidoziaceae | *Bazzania yoshinagana* | Hg, Cu, Pb, Zn, Ni | Sun *et al.*, 2009 |
| Wuxi, China | Sphagnaceae | *Sphagnum junghuhnianum* | Cr, Pb, V, and Zn | Hu *et al.*, 2018 |
| Wuhan city, China | Leskeaceae | *Haplocladium angustifolium* | As,Cd,Co, Cr, Cu, Mn, Ni, Pb, V, Zn | Jiang *et al.*, 2020 |
| Taizhou, China | Leskeaceae | *Haplocladium microphyllum* | Cd, Cr, Cu, Hg,Ni, Pb, Zn | Zhou *et al.*, 2017 |
| Marmara region, Turkey | Hypnaceae | *Hypnum cupressiforme* | Pb, Cu, Cd, and Zn | Coskun *et al.*, 2011 |
| Mekrijarvi and Hameenkangas, Finland | Mniaceae | *Pohlia nutans* | Fe, Zn, Mn, Cu, Ni, Cd, Pb, Al | Salemaa *et al.*, 2004 |
| | Dicranaceae | *Dicranum sp.* | | |
| | Polytrichaceae | *Polytrichum juniperinum* | | |
| Kosovo | Brachytheciaceae | *Hypnum cupressiforme* | Cr, Ni, Pb, and Zn | Maxhuni *et al.*, 2016 |
| | Hypnaceae | *Pseudocleropodium purum* | | |
| Albania | Hypnaceae | *Hypnum cupressiforme* | As, Cd, Hg, Pb, Cu, Zn, Ni, and Cr | Qarri *et al.*, 2019 |
| Belgrade, Serbia | Brachytheciaceae | *Brachythecium rutabulum* | V, Cr, Ni, As | Anicić *et al.*, 2007 |
| | Brachytheciaceae | *Brachythecium salebrosum* | | |
| | Brachytheciaceae | *Eurhynchium hians* | | |
| | Brachytheciaceae | *Eurhynchium striatum* | | |
| Republic of Macedonia | Brachytheciaceae | *Homolothecium lutescens* | Cd, Hg, Pb | Barandovski *et al.*, 2012 |
| | Hypnaceae | *Hypnum cupressiforme* | | |
| Republic of Moldova | Hypnaceae | *Hypnum cupressiforme* | Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn | Zinicovscaia *et al.*, 2017 |
| | Orthodontiaceae | *Pleurocarpous sp* | | |
| Poland (North-East) | Hylocomiaceae | *Pleurozium schreberi* | Zn, Pb, Ni, Co, and Cd | Radziemska *et al.*, 2019 |
| Poland | Hylocomiaceae | *Pleurozium schreberi* | Cd, Cr, Ni, Pb and Zn | Zawadzki *et al.*, 2016 |
| | Polytrichaceae | *Polytrichum commune* | | |
| Poland (South West) | Polytrichaceae | *Polytrichum commune* | Cd, Co, Cr, Cu, Mo, Ni, Pb and Rb | Wojtuń *et al.*, 2018 |
| | Polytrichaceae | *Polytrichastrum formosum* | | |
| Toluca Valley, Mexico | Fabroniaceae | *Fabriona ciliaris* | Zn , Pb, Cr, Cd | Macedo-Miranda *et al.*, 2016 |
| | Leskeaceae | *Leskea angustata* | | |

### (A) Survey Method

An inclination of insufficiency regarding the abundance of the individuals or species is noted and studies revealed that the changes are directly related to the change in the level of pollutants. Surveying and comparison of various communities of specific cryptogams from different sites as well as comparing the present species occurrence and abundance with the past records indicate the quantity of pollutants or stress on the organisms of the site. Periodic surveys are made on the native bryophytes in diverse sites. The number, frequency and abundance of native species and dominance of the growth form can be compared with the past records, reports and periodic herbarium collection. Disappearance of previously reported species (sensitive) and emergence of novel species (tolerant) specified the stress conditions in the sites (Rao, 1982; Govindapyari *et al*., 2010) The method is quite competent to trace out several kinds of terrestrial bryophytes and their ability to indicate several levels of pollution.

### (B) Transplantation

In this method, specific bryophyte species along with their substrate are transplanted *in situ* from non-polluted to ecologically more or less similar but polluted or industrial site to acquire the injury caused by the pollution (Rao, 1982; Govindapyari *et al*., 2010). Transplanted species illustrate the altered pattern of growth of shoots and branching and deposition of wax on the plant surface. General symptoms are chlorophyll degradation and plasmolysis in the leaf cells and consequently, the plants lose the ability to revive or regenerate. Three ways are generally opted to study as well as determine this particular procedure:

(i) Transplantation in the soil: In this study, bryophytes grown in small plots, prepared in the ground on selected sites of pollution and some plots of non-polluted site are established as control. Extent of branching of plant parts, production of basal regenerative shoots and rate of survival are recorded periodically which give fair picture of pollution stress (Govindapyari *et al*., 2010).

(ii) Moss Bag Methods: In this study, muslin cloth bags (20×20 cm size) are prepared in which equal amounts of moss material preferably epiphytic mosses are filled. Further, these bags are hanged in different locations of the city. Periodic investigation of rate and capability of regeneration of these samples present the data on the trend of pollution. Aquatic mosses filled in the bags can be kept in the water bodies to analyze the extent of pollution in the water bodies.

(iii) Study with Bryometer: It is an instrument used for measuring the phytotoxic air pollution. In this method of study, an impoverished box in which bryophytes are grown in moist chamber with their original substrata is made. It is small, easy to handle and can be utilised throughout the year and termed as 'Bryo-meter' (Taoda, 1973). The box is with transparent sides made with thin glass with opening so that air and light can pass through the plants inside the box. The boxes are kept in different locations of the polluted area to observe the periodic growth and survival rate of the bryophytes, indicating the development of pollution at the site. Spore germinates pattern and protonemal growth are easy to observe in this moist chamber. Therefore, this method is very useful to study and monitor the pollution index.

### (C) Phytosociological Method

These are typical changes that are directly related to the changes in the levels of air quality which show the Index of Atmospheric Purity (IAP). In this method, bryophytes growing on the tree bark are examined along a line or belt transect having pollution gradient (LeBlanc and DeSloover, 1970). Studies on the frequency and abundance of species through a number of transect radiating in all direction and with increasing distance from the source of pollution are recorded. IAP is determined on the basis of number, frequency coverage and resistance factor of species can provide a fair picture of the long range effect of pollution of a particular area concerned. Mathematically IAP is determined by the below mentioned formula:

$$\text{IAP} = \frac{1}{10}\sum_{i}^{n} Q_i \times f_i \,,$$

Where n = total number of species per the sampling plot, Qi = ecological index, fi=combined index of the coverage and the frequency. Furthermore, a modified index of atmospheric purity (IAPm) (Kondratyuk, 1994) based on a quantitative assessment of abundance and species coverage can be used to analyze the earlier parameters.

### Ecophysiological Method

Here, bryophytes are exposed to the previously known concentrations of pollutants like, fumigation of the live plants in the field or plants can be cultured in the medium of different concentrations of pollutant and heavy metals. Observation on the growth and survival rate, injury, chloroplast degradation, or other kind of unusual growth of the protonema and the mature plants indicate the toxicity of pollutants. This method is useful in determining toxicity level of pollutants and the tolerance levels of different species (Rao, 1982).

### Chlorophyll Fluorescence

The practice of chlorophyll fluorescence has become universally acceptable in plant ecophysiological studies. The basic principle of chlorophyll fluorescence analysis is comparatively straightforward. Light energy absorbed by chlorophyll molecules in a leaf can experience following outcomes. It can be used to drive photosynthesis where excess energy can be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence. These processes occur in such a manner that any increase in the efficiency of one will result in a decrease in the yield of the other two. Therefore, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained. Moreover, bryophytes are sensitive to several abiotic stresses and is considered an environmental indicator. This measurement has been used as a probe to study, evaluate and compare the tolerance power of numerous bryophytes with heavy metal stress (Tuba *et al*., 2010; Chen *et al*., 2019). Scientists are working to understand the mechanism in more details that needs more experimentation.

## Practical Applications of Bryophytes to Monitor Heavy Metal Pollution

Transplants of bryophytes have been used in a number of studies to assess heavy metal deposition rates. *Hypnum cupressiforme* transplanted in industrial area in Wales died after sometime, but it continued to accumulate heavy metals after death (Sahu *et al.*, 2007). 25 moss samples to monitor the lead pollution in various parts of Chandigarh city are revealed (Sharma and Kapila, 2007). In Japan, bryophytes have been used as a bryometer to assess the air quality (Taoda, 1973). The classic method of monitoring pollution is through the use of moss bags. It is a common active biomonitoring technique using terrestrial mosses. This technique was first introduced by Goodman and Roberts (1971) involving exposure of moss within mesh bags in order to monitor the contaminants in air. In general inorganic contaminants are monitored through this technique (Ares *et al.*, 2012). The moss bag technique is reliable and acceptable from financial point of view due to its low cost of material acquisition (Debén *et al.*, 2018). The preparatory phase includes collecting moss from clean area, removing the impurities followed by proper rinsing with water, drying and then transferring them into the bags for monitoring pollution (Temple *et al.*, 1981). Selection of moss shoot (old or young), different substrates and exposure to the atmosphere can also be considered because elemental deposition of moss on soil and rock dust is very different from ones grown on tree trunks (Adamo *et al.*, 2011). In addition, low pH increases the metal availability due to competing with the sites of theses metal ions as the hydrogen ion has a higher affinity for negative charges on the colloids, thereby, releasing the metals (Uniyal and Singh, 2018).Characteristics of a good bioindicator can be summarised as broad distribution, long life cycle, sensitive to specific pollutants, inertness and genetic uniformity in the area (Cenci, 2008). Table 3 represents use of mass bags to monitor pollution in selected cities of the world. Monitoring of heavy metals through bryophytes is not only cost-effective, but it also provides efficient way to assess the qualitative and quantitative differences in metal concentrations at distinct locations and on local and landscape scales.

## Mechanism of Accumulation Of Heavy Metals in Bryophytes

A number of mechanisms are involved in accumulation of heavy metals by bryophytes. One of the most common and primary process is the adsorption in the cell surface (González and Pokrovsky, 2014). The adsorbed metals are generally trapped as particulate matters within the cell surface. In this context the high surface to mass ratio of epigeic mosses is effective in entrapment of airborne particles (Bargagli *et al.*, 2002). They may also be dissolved in liquids of body surface or deposited in surrounding cells as intercellular fractions. It is also bound as exchangeable forms at the chelating sites of cell wall and on the outer surface of plasma membrane as extracellular fractions or

**Table 3:** List of bryophytes used as promising moss bags in specific sites to monitor pollution

| *Name of the moss* | *Family* | *Location* | *Metals accumulated* | *Reference* |
|---|---|---|---|---|
| *Brotherella* sp. | Pylaisiadelphaceae | Chongqing, China | Cu , Pb , Zn, Ni | Sun *et al.*, 2009 |
| *Dicranum nipponense* | Dicranaceae | | | |
| *Haplocladium microphyllum* | Leskeaceae | Shanghai, China | S, Cu, Pb, and Zn | Cao *et al.*, 2009 |
| *Hypnum plumaeforme* | Hypnaceae | Laoyingshan, Guizhou, China | Cd | Xie *et al.*, 2014 |
| *Sphagnum sp* | Sphagnaceae | Eskisehir Hasan Polatkan Airport (Eskisehir, Turkey) | Cr, Sn, Li,Ge, Ni. Cd, Ce, Cs, Cu, Fe, La, B ,Be, Gd, Al. | Turgut *et al.*, 2019 |
| *Sphagnum girgensohnii* | Sphagnaceae | Belgrade, Serbia | V, Cu, As, and Ni. | Anicić *et al.*,2009 |
| *Sphagnum papillosum* | Sphagnaceae | Harjavalta Industrial Park in southwest Finland | As, Cd, Cu, Fe, Hg, Ni, Pb, Ti, Zn. | Salo *et al.*, 2016 |
| *Sphagnum girgensohnii* | Sphagnaceae | Nikola, Tesla International Airport, Belgrade | Zn, Na, Cr, V, Cu and Fe | Vuković *et al.*, 2017 |
| *Pleurosium* spp., | Hylocomiaceae, | Prešov city, Slovakia | Cd, Pb, Zn, Cu, Cr, Ni, Co, Mn, Fe, Hg. | Lenka *et al.*, 2017 |
| *Polytrichum* spp., | Polytrichaceae | | | |
| *Rhytidiadelphus* spp. | Hylocomiaceae | | | |
| *Rhynchostegium riparioides* | Brachytheciaceae | Vicenza, NE Italy | Pb and Cu (chronic contamination) | Cesa *et al.*, 2006 |
| | | | Cr, Zn and Ni (intermittent contamination) | |
| *Hypnum cupressiforme* | Hypnaceae | Naples (South Italy) | Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, Ti, V, Zn | Giordano *et al.*, 2009 |
| | | Sardinia (Italy) | Cr, Cu, Ni, Zn, Pb | Cortis *et al.*, 2016 |
| *Sphagnum fuscum* | Sphagnaceae | Mount Etna, Italy | Tl, Bi, Se, Cd, As, Cu, B, Na, Fe, A | Calabrese *et al.*, 2015 |

they may be transported inside the cell and retained in soluble form as intracellular fraction (Stanković *et al*., 2018). The ion exchange process is involved in extracellular accumulation of heavy metals (Breuer and Melzer, 1990). In certain cases, the heavy metals also form complexes with organic functional groups in the cell walls of bryophytes (Shakya *et al*., 2008). The polygalacturonic acid and other polymers present in the cell wall helps in the complexation process with heavy metals (Itouga *et al*., 2017). Several functional groups present in the cell wall of bryophytes acts as binding site of heavy metals. These include phosphodiester, carboxyl, phosphoryl, amine and polyphenol functional groups (González and Pokrovsky, 2014). Membrane transport proteins present in the cell membrane mediates transportation of heavy metals into the cells of the moss (Basile *et al*., 2012). The pattern of accumulation of metals on or within the moss is also an indication of the pattern of contamination. The extracellular fraction is indicative to current environmental contamination while the intracellular fraction indicates deposition or presence of contaminants for a prolonged period of time (Fernandez *et al*., 2006). Thus they can serve as an effective model system to determine the pattern of environmental contamination.

## Mechanism Of Heavy Metal Stress

The mechanism of perception and counter heavy metal stress in bryophytes is similar to higher plants. Thus in this section, the mechanistic aspect of heavy metal stress in plants would be discussed in general and it can well be assumed that this system well applies to the bryophytes as well. Similar to other stresses, signalling pathways are involved in heavy metal stress of plants. These include perception of external signal followed by transmission of signal to the downstream components and ultimately induction of appropriate biochemical responses to neutralize harmful effects of the heavy metal mediated stress (Dutta *et al*., 2018). In plants excess of heavy metals alters the calcium channel activity in plants and results in increase of calcium flux within the cell. Within the cell, calcium acts as a second messenger and stimulates calmodulin which in turn regulates uptake of heavy metals, transport and metabolism (Ghori *et al*., 2019). Lead is reported to bind to all four calcium binding sites of calmodulin resulting in improper activation (Ouyang and Vogel, 1998; Kern *et al*., 2000). It is also reported that most of the metals acts as calcium analogues and results in induction of calmodulin in signal transduction pathway (Snedden and Formm, 2001). Nitric oxide is also reported to increase cytosolic calcium in plants in response to heavy metals or other abiotic stress. On the other hand NO synthesis is induced by increased concentration of cytosolic calcium. Thus nitric NO and calcium acts synergistically to acclimatize the plan in response to abiotic stress (Malik *et al*., 2020).

Generation of reactive oxygen species is one of the most common biochemical manifestations of plant in response to heavy metal stress. The metals induce generation of reactive oxygen species through stimulation of activity of NADPH oxidase, displacement of essential cations from specific binding sites of enzymes and inhibition of enzyme activities from their affinity of -SH groups (Shahid *et al.,* 2014). A number of studies have demonstrated the generation of reactive oxygen species in plants in response to metal stress. It is found that Cd stress results in differential regulation of reactive oxygen species metabolism, redox homeostasis by NADPH oxidase and Respiratory burst oxidase homoluges (Gupta *et al*., 2017). Cd can regulate NADPH oxidase activity and expression of putative NOX/RBOG gene (Groppa *et al*., 2012). It is also assumed that Cd stress also affects plasma membrane $H^+$-ATPase activity (Astolfi *et al*., 2003).It si also found that transcript levels of $H^+$-ATPase (CsHA2, CsHA3, CsHA4, CsHA8, and CsHA9) genes increased upon treatment with cadmium (Janicka-Russak *et al*., 2012). Some elements of brassinosteroid pathways are activated in response to cadmium stress and $H^+$-ATPase and NADPH oxidase mediate this activation (Jakubowska and Janika, 2017). In addition, lead and Nickel have been reported to induce oxidative burst and increase ROS production through activity of NOX like enzyme (Pourrut *et al*., 2008), of all reactive oxygen species, hydrogen peroxide have the ability to cross the plasma membrane and therefore it is considered to be involved in cell to cell signalling (Slesak *et al*., 2007; Niu and Liao, 2016). Hydrogen peroxide is also involved in downstream signalling cascades which functions by binding to calcium binding proteins and activation of phospholipid signalling and ultimately results in activation of MAPK pathway (Ghori *et al*., 2019). MAPKs are signalling molecules and play an important role in signal transduction pathway (Chardin *et al*., 2017). It is well documented that reactive oxygen species is responsible for activation of MAPKs (Jalmi and Sinha, 2015). Since a number of heavy metals disturbs the redox balance in plants, it can be well speculated that they are also involved in activation of MAPKs (Jonak *et al*., 2004). A wide array of other responses is also observed in plants in response to heavy metal stress. Salicylic acid is another important phenolic compound that is generated in plants in response to heavy metal stress. Under heavy metal stressed condition, salicyclic acid interacts with other plant hormones namely auxin, abscisic acid and gibberellin and promotes the generation of antioxidant compounds for counteracting the stress (Sharma *et al*., 2020). In addition to it, cadmium also trigger the synthesis of ethylene within the plant and is also presumed to have its own response in heavy metal stress (Schellingen *et al*., 2014). Proline also accumulates in the plant in response to heavy metals and is involved in neutralizing free radicals. In addition to it, proline also chelates the heavy metals (Hayat *et al*., 2012). In addition to it, heavy metals also stimulate the phenyl propanoid biosynthetic pathway by increasing the activities of phenylalanine ammonia lyase, shikimate dehydrogenase, glucose-6-phosphate dehydrogenase, cinnamyl alcohol dehydrogenase which results in production of polyphenols (Mishra and Sangwan, 2019). The phenolic compounds are involved in chelation of heavy metals on one hand and inhibiting lipid peroxidation on the other (Lavid *et al*., 2001; Kaur *et al*., 2009). It has also been reported that heavy metals increase the expression levels of genes of antioxidant enzymes namely Ascorbate peroxidase, catalase, superoxide dismutase and peroxidase and these enzyme play the role of scavenging the free radicals (El-Esawi *et al*., 2020). However, at higher concentration heavy metals result in inactivation of biomolecules either through blockage of functional groups or through displacement of essential metal atoms (Bhaduri and Fulekar, 2012). Heavy metals can inactivate catalase activity through inactivation of heme group bound to the enzyme (Malar *et al*., 2016). Thus, it is evident that heavy metal stress induces a wide array of biochemical reactions in plants in order to counter the damaging effect. Though the mechanistic aspect is discussed mostly based on the responses of the higher plants but it is quite likely that the bryophytes also follow the same pattern.

## Conclusion

With more progressive advancement of lifestyle, human has polluted the environment and making it difficult to dwell. So, proper precautions and adequate occupational hygiene should be taken in handling them.

Accretion and maintenance of various types of pollutants by bryophytic plant groups helped in the elucidation of heavy metal emission prototypes. There is a great need to extend observations on mineral location and effect to a much wider range of species. With no cuticle, no developed roots, high surface/volume ratio and high cation exchange capacity, bryophytes has increased adsorbent efficacy. They accumulate large amounts of trace elements and also lack variability in morphology throughout the growing season (Giordano *et al*., 2005). The role of morphological features in trapping particulate material is understudied but vital research field. The capability of the hepatics to accumulate heavy metal within its body is dependent on the surface area of the thallus and similarly for the moss, it is dependent on its leaf surface area and number of parenchymatous cells.

It has been observed that bioaccumulation potential of mosses for metals depends upon their tolerance to the particular metal and varies greatly (Uniyal *et al*., 2017). The pleurocarpous are cushion-like and form a mat on the substratum; hence they can be considered as an ideal indicator of the metallic pollutant in the environment. This group of plant can be utilized as bryometer and are aptly utilized as 'environmental specimen bank.' Use of bryophytes for transplantation, as biomonitoring agents and as an indicator of pollution and minerals is simple, cost effective, reliable and convenient method, the only need is to select a suitable species to carry out the study. Amalgamation of moss biomonitoring with statistical analysis of the data plays an integral role in the identification of prone areas which are at risk of high atmospheric deposition and also in the investigation of spatial deposition flux (Maxhuni *et al*., 2016). It is therefore, suggested to advocate the use of appropriate techniques with the help of relevant bryophyte species. As mentioned earlier, bryophytes act as an excellent system in monitoring of heavy metals. However, study on their physiological responses and mechanistic aspects of their heavy metal tolerance or response is yet to be documented properly. It is therefore required to study the processes of signal transduction, transcript directive, homeostasis and biosynthesis defense proteins needs to be thoroughly studied in order to understand the stress tolerance mechanism in bryophytes. In addition, exploration of bryophytes at biochemical, protein and gene level should be promoted to understand the mechanism of heavy metal stress in the plant. At a whole bryophytes have proven themselves as a very effective model system to study and monitor metal pollution and further research is extremely relevant to have a deep insight in their mechanistic activity. In-depth study in molecular level is also required to understand the mechanism of acclimatization of bryophytes in varied stressed condition and this would help us to customize the requirement of this versatile species in monitoring activity as per requirement of the situation. Thus, bryophytes have immense potential as a monitoring agent and should be used diligently to study the environmental pollution.

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