

GENERAL ARTICLE

Bryophytes: indicators and monitoring agents of pollution**H. Govindaparyari, M. Leleeka, M. Nivedita and P. L. Uniyal**

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ABSTRACT

Bryophyte proves to be a potential bio-indicator of air pollution. The habitat diversity, structural simplicity, totipotency, rapid rate of multiplication and high metal accumulation capacity make bryophytes an ideal organism for pollution studies. The decline and absence of bryophyte populations especially epiphytes is a phenomenon primarily induced by air pollution caused by gaseous and particulate pollutants. Bryophytes are reliable indicators and monitors of air pollution as they are easy to handle and show a vast range of specific sensitivity and visible symptoms to pollutants greatly exceeding that of higher plants.

KEY WORDS: Bryophyte, bio-indicator, air pollution, pollutants.

Bryophytes are green land plants which lack a vascular system and are simple both morphologically and anatomically. The growth potential in bryophytes is not as highly polarized as vascular plants. Bryophytes grow in a variety of habitats especially in moist places on soil, rocks, trunks and branches of trees and fallen log. They obtain nutrients directly from substances dissolved in ambient moisture. Some substances are probably absorbed directly from the substrate by diffusion through the cells of the gametophyte. Bryophytes are used as reliable indicators of air pollution (Le Blanc & Rao, 1975). They are exploited as bryometers instrument for measuring phytotoxic air pollution. They either independently or together with lichens can be valuable organisms in developing an index of atmospheric purity (IAP) which is based on the number, frequency-coverage and resistance factor of species. This index can provide a fair picture of the long-range effects of pollution in a given area (Rao, 1982). There are two categories of bryophytes in response to pollution:

- which are very sensitive to pollution and show visible symptoms of injury even in the presence of minute quantities of pollutants. This serve as good indicators of the degree of pollution and also of the nature of pollutant.

- which have the capacity to absorb and retain pollutants in quantities much higher than those absorbed by other plant groups growing in the same habitat. These plants trap and prevent recycling of such pollutants in the ecosystem for different periods of time. Analysis of such plants gives a fair idea about the degree of metal pollution.

Pollutants

Pollutants may be gaseous such as carbon monoxide (CO), fluorides, hydrocarbons (HC), hydrogen sulphide (H₂S), nitrogen oxides (NO), Ozone (O₃), sulphur dioxide (SO₂), aldehydes, lead and automobile exhaust fumes. NO_x and NH₃ are primary gaseous pollutants which are strongly phototoxic. SO₂ and NO₂ are readily converted to strong acids by oxidation and solution in atmosphere as water droplets to form acid rain. The smoke and SO₂ is produced by the combustion of traditional fuel of automobiles, together with various metals released into atmosphere by smelting and other heavy metal industries. Nitrogen oxides have also greatly increased in the cities with the rise of use of automobiles. Particulate pollutants are dust, particles of metallic oxides, coal, soot and fly ash, cement, liquid particles, heavy metal and

radioactive materials. Ozone (O_3) is a secondary pollutant formed by the action of sunlight on nitrogen dioxide and on certain hydrocarbons. It is more phytotoxic than the primary pollutants. NH_3 a pollutant is also being released by intensive animal rearing. Other pollutants are agricultural pesticides and fertilizers and various forms of aquatic pollution. The air pollutant either in a gaseous state mixed with air or in a liquid state affected by dew, rain, or snow, will be noxious to bryophytes attached to the bark.

Effect of pollutants on bryophytes

There is an impoverishment of bryophytic communities in and around cities and industrial areas (Gilbert, 1968). Urban areas comprise a series of habitats with a variety of substrates and moisture regimes and are subject to varying levels of pollution. Bryophytes occupying certain substrates appear to more sensitive to air pollution than others. Bryophytes have been disappearing from urban industrial environments because of their sensitivity to polluted air. Species diversity in a polluted area varies not only with the distance from the source of pollution but also with the type of substrate.

Air pollution inhibits gametangial formation and sexual reproduction in bryophytes. They also reduce photosynthesis by degrading chlorophyll and growth of plants and eventually cause their death. Metals and metalloid are accumulated by bryophytes from the substratum, wind-blown or in wet deposition. When the metal enters the cell, it inhibits the photosynthetic activity. Enzymes and membrane are poisoned when a heavy metal gains access to the cell interiors. Mercury is particularly toxic low concentration greatly inhibited photosynthesis, temporarily increased respiration, reduced chlorophyll levels and caused loss of intracellular K^+ from *Rhytidiadelphus squarrosus* (Brown & Whitehead, 1986). It is evidenced that when the pollution level goes down, the percentage frequency of species goes up, which subsequently increases the fertility percentage. This situation varies from species to species depending on the prevailing climatic conditions in the area. Bryophytes die within a short period of time depending on the level of pollution, when transferred along with their substrates from unpolluted to polluted areas in a city or around a factory. The pollutants can affect

organisms either individually or in combination of two or more. The common symptoms of injury are plasmolysis and chlorophyll degradation in the leaf cells. SO_2 exposed plants showed brownish spots on the chloroplasts and plasmolysis in cells of leaves which contributed to the ultimate death of the plants. When plants are exposed to SO_2 , it get absorbed in the plant tissue which causes degradation of chlorophyll *a* by increasing the concentration of free H^+ ions which subsequently displace the Mg^{2+} ions from the chlorophyll molecule, converting it into phaeophytin *a*. The typical response of mosses to SO_2 pollution begin as a loss of colour at the tips of the more exposed leaves and this gradually extends down the leaves and down the shoots until they have lost all the chlorophyll. Chronic injuries such as growth retardation are seen. Low exposure of plants to hydrogen fluoride (HF) results in the minimal injury and accumulation of fluoride to this plant is low. Once hydrogen fluoride (HF) is adsorbed on the plant surface, it moves towards the tip of leaves causing a distinct pattern of injury which remain proportional to the exposure. Ozone uptake by the plant species often results in acute injury, premature ageing and senescence. Bryophytes show impairment of photosynthesis or increased membrane leakage when subjected to an acute (150 p.p.b) ozone exposure. Several species of *Sphagnum* species were found to be chronic to O_3 exposure. Ammonia (NH_3) is highly phytotoxic (Greven, 1992). Leaf tip chlorosis is followed by necrosis when plants were exposed to NH_3 . Acid rain has two distinct effects like wet deposited acidity and wet atmospheric deposition of the nutrients such as nitrogen and sulphur. Acidification may damage cell membranes, solubilize potentially toxic metals like Al^{3+} and worsen the impact of other pollutants like SO_2 (Farmer *et al.*, 1992). Acid rain produces drastic changes in the chemical properties of both epiphytes and their bark substrates by reducing the pH of stem flow, increasing the proportion of toxic bisulphate (HSO_3^{2-}) ions in stem flow, decreasing the buffer capacity of the bark, decreasing the internal pH of the epiphytes and increasing the metal concentration and chlorophyll loss in epiphytes. Lead (Pb) pollution is caused by anti-knock compounds consisting of tetramethyl lead. Pb contents in the plant correspond to the respective traffic intensities and without significant correlation to the lead content of the soil. Aquatic bryophytes

accumulate heavy metals from contaminated water to a much greater extent than vascular plants as the metal uptake is less seasonal and partly because they can absorb over their entire surface. Uptake of mercury by *Jungermannia vulcanicola* and *Scapania undulata* involved the formation of crystals of HgS in the cell walls of these taxa (Satake *et al.*, 1990). Chlorinated hydrocarbons may be accumulated by adsorption on to aquatic bryophytes (Mouvet *et al.*, 1993). *Funaria* leaves showed apical necrosis disintegration of chloroplasts and plasmolysis in their cells and other cellular abnormalities. Radioactive isotopes get accumulated in bryophytes from the fallout from nuclear weapons testing (Burton, 1968). Bryophytes behave as perfect sinks for atmospheric deposition of isotopes (Svensson & Liden, 1965). The ionizing radiations activate plant cell molecules and thus quicken chemical reactions. Injury results when the chemically altered molecules are essential components of protoplasm. Bryophytes and other non-vascular plants are less sensitive to radiation than are the vascular plants.

Bryophytes - rapid and inexpensive organisms for surveying heavy metal deposition in the terrestrial ecosystem

Metals are non-degradable and once released into the environment become an integral part of the habitat. Bryophytes are able to concentrate heavy metals in large amounts than that of vascular plants. Heavy metals are absorbed either from the atmosphere or from the substrate or from both the sources. The older tissues of the plant have higher concentrations of the metallic ions as compared to the younger portions. The ability of mosses to accumulate heavy metals depends upon the total leaf surface and the number of thin walled parenchymatous cells. *Atrichum undulatum* is highly sensitive to air pollution and proves best as a bio-indicator. *Ceratodon purpureus* is not a good indicator because its leaves have a small surface and contains many thick walled cells. Carpet forming bryophytes has proved to be rapid and inexpensive method for surveying heavy metal deposition in the terrestrial ecosystem (Ruhling & Tyler, 1970). The concentration of airborne material decreases in the plant tissues with distance from the source of pollution and there is a wide variation in metal accumulation from species to species and from

habitat to habitat under different microclimate conditions. Among mosses the profusely branched and ramifying pleurocarps and the densely packed acrocarps are more efficient entrappers and absorbers of metal particles than the unbranched and erect acrocarps. Accumulation of mercury is found greater in *Dicranum scoparium*. Bryophytes are able to concentrate heavy metals in large amounts, greatly surpassing the absorbing capacity of vascular plants. The gametophytes of moss can accumulate iron 5-10 times more readily than the vascular plants. The concentration of Al, Ba, Cr, Cu, Fe, Ga, Ni, Pb, Ag, Ti, Vi, Zn and Zr were higher in bryophytes than those in angiosperms. The bryophytes are able to concentrate rare earth elements. Elements which are rarely found in other plants were found in bryophytes. Bi were found in the thallose liverworts like *Conocephalum conicum* and *Marchantia polymorpha*, Sn in the saxicolous mosses *Grimmia laevigata* and *Hedwigia ciliata* and Ag in *Atrichum angustatum* and *Polytrichum commune*. Cu, Pb and B are found in the substrate of *Mielichhoferia*. Mosses of family Neckeraceae contained a high percentage of Ca and those of family Grimmiaceae, Polytrichaceae and Dicranaceae had a low percentage of it.

The moss gametophytes readily entangle and retain particulate contaminants amongst the leaves and they possess remarkable ion-exchange properties. *Sphagnum* when dry absorbs and retains rain water to a considerable extent which facilitates ionic exchange between the soluble metal and moss cell walls (Clymo, 1963). The sorption and retention of the heavy metals are in the order: Cu, Pb>Ni>Co>Zn, Mn (Ruhling and Tyler, 1970). Accumulation of mercury (Hg) is found greater in *Dicranum scoparium* than *Polytrichum commune* which is due to the differences in their life forms. The surface cuticle affects the absorption and accumulation rates of dissolved materials. The ectohydric moss *Dicranella varia* which lack cuticle and absorbs water over the whole of its surface, accumulates higher contents of heavy metals than that of myxohydric moss *Philonotis fontana* which has a more or less continuous cuticle and absorption taking place towards the base of the gametophytes. Ectohydric mosses have the ability to accumulate heavy metals and serve as indirect indices of air pollution. Because of their small nuc-

lei, the bryophytes are more resistant to ionizing radiation than higher plants.

Tolerance of bryophytes to pollution

Some bryophytes are metal tolerant and are able to withstand levels of heavy metals that are toxic to other species. *Marchantia polymorpha*, *Solenostoma crenulata*, *Ceratodon purpureus* and *Funaria hygrometrica* are some of the metal tolerant populations. Bryophytes growing on stone walls can tolerate higher levels of pollution than those of tree trunks. In case of epiphytes species growing on tree base can cope with the pollution conditions better than those of tree trunks. Shady and alkaline niches are more preferred by bryophytes. *Brachythecium rutabulum*, *Grimmia pulvinata*, *Orthotrichum diaphanum*, *Bryum capillare*, *Bryum argenteum*, *Tortula muralis*, *Rhynchostegium confertum* and *Hypnum cupressiforme* show survival and luxuriant growth in polluted environment.

The tolerance of mosses to polluted air depends to a greater extent on the ratio of metals taken up than on the absolute metal level. There is a mark difference in the tolerance levels of bryophytes for individual metals and combination of cations, for various species. Life forms of bryophytes can modify the effects of pollution. The general trends of increasing resistance to pollution among life forms are in the order of:

- rough mats, tall turfs, wefts, large cushions and leafy liverworts
- smooth mats and small cushions
- short turf and thalloid forms.

The moss protonemata, precursors of the leafy gametophores appear to be more sensitive than the mature gametophyte (Gilbert, 1969). The active metabolism and high reproductive capacity of certain bryophytes enable to colonize urban area. The reproductive potential of a species determines its degree of success in a polluted environment. The survival of *Bryum argenteum*, *Ceratodon purpureus*, *Dicranella heteromalla*, *Funaria hygrometrica*, *Leptobryum pyriforme*, *Lunularia cruciata*, *Marchantia polymorpha* and *Pohlia prolifera* lies in their high reproductive capacity and fast subsequent growth. These species produce spores or gemmae on a very large scale. Terricolous species *Dicra-*

nella heteromalla, *Pohlia nutans* and *Ceratodon purpureus* showed good growth on soils with a pH of 3.4 and in SO₂ polluted areas. *Hypnum yokohamae* and *Glyphomitrium humillimum* were able to tolerate SO₂ concentrations of 0.04 to 0.05 p.p.m. Sulphur dioxide (SO₂) resistant bryophytes show fast growth rate. The sensitive protonema usually short-lived and soon initiates protonemal buds which develop rapidly into more resistant gametophytes (Gilbert, 1970). *Pohlia nutans* and *Aulacomnium androgynum* are resistant species against fluoride pollution. *Sphagnum spp.*, *Polytrichum commune*, *Leucobryum glaucum*, *Rhytidiadelphus squarrosus* and numerous small hepatics and acrocarpous mosses can resist fluoride pollution. *Fissidens garberi* is the most resistant moss for it can survive within 10 m of a caesium source and even within 3 m in shielded habitats. There are certain bryophytes which colonize heavy metal contaminated sites which then lead to the evolution of tolerant genotypes or the development of tolerant metal ecotypes. Morphological changes in Cu and Zn stressed *Funaria hygrometrica* and *Marchantia polymorpha* are observed by Coombes and Lepp (1974). *Funaria* develops 'capsule cells' and 'brood cells' when subjected to high doses of Cu and Zn thereby reducing the surface area of the protonema in contact with Cu and Zn. *Marchantia* develops a buffering zone of outer dead cells which immobilize Cu within their cells, thus preventing toxic concentrations from reaching the inner cells. In *Sphagnum* a single mutated vegetative cell in the plant give rise to a new tolerant clone. Therefore, bog moss is good systems for studies on adaptation of plants to pollution.

Bryophytes as biomonitoring agent

The expansion and lack of environmental regulation has lead to a large input of various toxic pollutants into the atmosphere. There is a need of continuous monitoring of air quality. This is accomplished either by the construction or employment of bio-indicators/biomonitorers. Bioindicators refers to all organisms that depict the quality of the environment on the basis of changes in morphology; physiology etc. but biomonitorers provide both qualitative and quantitative information. The use of organisms as monitors of the environment is known as biomonitoring and the organisms are known as biomonitorers. The preferred organisms are those that are

able to accumulate the pollutants, be available throughout the year, easy to capture and identify, be relative sedentary and are cosmopolitan in distribution. The accumulated pollutants are easily measured by the analysis of organisms that provide the information about the level of pollutant deposition. Mosses and lichens as well, are considered as the most appropriate plant material to study the atmospheric deposition of heavy metals.

Chemical analysis is strongly dependent on the time and place of sampling. If the released pollutants are readily dispersed, their concentration in the atmosphere will fall below the detection limit and thus escape their presence whereas bryophytes have the ability to facilitate the detection of the elements present in very low concentrations. Mosses can be stored for several years without noticeable deteri-

oration and old specimens can easily be chemically analyzed. Thus bryophytes can serve as “Environmental Specimen Banks”. Bryophytes grow on a variety of habitats and obtain nutrients from the substances dissolved in ambient moisture. The substances are directly absorbed from the substrata by diffusion through the cells. Pollutants reach in the bryophyte tissues from the dry deposition in the form of gases and particles. These substances are readily accumulated in the plant and their amount exceeds from those in the surrounding environment. So bryophytes indicate the presence of element and their concentration gradient in the respective substrata. The unique qualities of bryophytes to accumulate the elements are due to their wide distribution, ability to grow on variety of habitats, large surface area, and lack of cuticle and stomata and evergreen and ectohydric nature of plants.



Figure 1. a. A tolerant species growing at a polluted site, b. Close up of a moss *Hyophila* showing no sporophyte production, c. Moss protonema in normal culture medium showing prominent growth, d. A moss bag in the water body to monitor metal content in water, e. A moss bag hangs on the tree to monitor the air metal contents of the site, f. Moss protonema in culture medium with heavy metals showing highly reduced growth in comparison to that of ‘c’.

Monitoring methods

1. Survey method

There is a gradient of meagerness or abundance of the individuals or species. These changes are directly related to the change in the level of pollutants. Surveying and comparison of communities of bryophytes of different sites indicate the quantity of pollutants or stress on the organisms of the site. Periodic surveys are made on the native bryophytes in different 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 already reported species (sensitive) and appearance of new species (tolerant) indicate the stress conditions in the sites.

2. Transplantation method

Bryophytes species are transplanted *in situ* along with their substrate from unpolluted to ecologically more or less similar but polluted or industrial site to obtain the evidence of injury caused by pollution. Transplanted species show the modified pattern of growth of shoots and branching and deposition of wax on the plant surface. The common symptoms are plasmolysis and chlorophyll degradation in the leaf cells and finally the plants lose the ability to revive or regenerate. Transplantation can be done by following three ways.

(a) Transplantation in the soil

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.

(b) Moss Bag Methods

Muslin cloth bags (20×20 cm size) are prepared in which equal amount of moss material preferably epiphytic mosses are filled. These bags are hanged in different locations of the city. Periodic analysis of rate

and ability of regeneration of these samples provides the data on the trend of pollution. Aquatic mosses filled in the bags can be kept in the water bodies. Analysis of these mosses indicates the extent of pollution in the water bodies.

(c) Bryometer

It is an impoverished box in which bryophytes are grown in moist chamber with their original substrata. The box has 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. The periodic observations on the growth and survival rate of the bryophytes indicate the trend of pollution in the site. Spore germinates pattern and protonema growth are easy to observe in this moist chamber.

3. Phytosociological method

Phytosociological changes 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. 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. Index of Atmospheric Purity (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 site.

IAP value can be determined by applying following formula

$$IAP = \frac{1}{n} \sum (Q \times f/10)$$

where

n = total no of species present at a site

f = frequency coverage of species at each site

Q = resistant factor.

4. Ecophysiological method:

In this method the bryophytes are exposed to the already known concentrations of pollutants. The exposure of pollutants can be given to the live plants in the field by fumigation or plants can be cultured in the medium having 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

Conclusion

The accumulation and retention of pollutants by bryophytes has helped in the interpretation of heavy metal emission pattern. There is a great need to extend observations on mineral location and effect to a much wider range of species. More species should be recognized to inhibit the chemical environment. The role of morphological features in trapping particulate material is an unstudied but vital research field.

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