



Lichens as Bioindicators and Biomonitoring Agents

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Abstract

The over-exploitation of natural resources, exponential growth of human population and advancement in the technology over a short period of time has changed the major biogeochemical cycles. This has also led to irreversible loss and even the extinction of genetically distinct species. Use of technology and modernization has led to socio-economic development. However, these have also led to a variety of harmful side effects. Various harmful pollutants are liberated into the atmosphere from different sources in the form of pesticides, herbicides, nitrogen oxides, sulphur oxides, heavy metals and particulate matter. These pollutants not only threaten the human and animal health, reproduction of insect and birds, but also cause plant diseases and disturb the structure of the ecosystem. Significant primary information about the diversity, quantity and quality of pollutants present in the atmosphere can be easily studied by the use of bio monitors. This information could be very useful as an early warning signs for the identification of alterations in the environment. Different organisms have been used as bioindicators for monitoring of the atmospheric pollution. Among these organisms, lichens were very early designated as “bio-indicator” to obtain information about variation in pollution at local and regional level. This review explores the suitability of lichens as bio monitors/bioindicators and various methods which can be used for biomonitoring.

Introduction

Lichens are slow growing organisms having symbiotic association between at least two organisms, fungus and a photosynthetic partner such as cyanobacterium or an alga. They form a symbiotic association in a way wherein algae present within the thallus, manufacture food and supply it to the fungus and in return, fungus provides protection to the alga. The main body of lichen or the thallus does not resemble either the fungal or the algal partner. They are perennial and maintain a uniform morphology over time. Lichens are thalloid and do not have roots, stems and leaves so they mainly depend on the atmospheric input of mineral nutrients (Nash III, 1996). Uniquely, lichens do not shed their parts during growth so particles of various elements absorbed during the growth of the thallus become embedded in the main body of the lichen. There are

generally, three main morphological types (foliose, fruticose and crustose) of lichens available in Indian Himalayan regions (Figure 1).



Figure 1: Diversity of Lichens in Indian Himalayas, A: *Dermatocarpon velereum* (foliose); B: *Hypotrachyna physcioides* (foliose); C: *Leptogium* sp. (foliose); D: *Palmariathomsonii* (foliose); E: *Melanotrema* sp. (foliose); F: *Tephromelakhatiensis* (crustose); G: *Peltiger* sp. (foliose); H: *Dimelaena* sp. (crustose); I: *Rosella* sp. (crustose); J: *Tephromela* sp. (crustose); K: *Manegezzia* sp. (crustose); L: *Phyllospor* sp. (crustose); M: *Laprocaulon* sp. (fruticose); N: *Usnea himalayana* (fruticose) (Photographs: Courtesy Prof. P. L. Uniyal, Department of Botany, University of Delhi, India).

The absence of epidermis, stomata and cuticle makes these organisms incapable of gaseous exchange and allows absorption of micronutrients and minerals through whole surface of the body from the atmosphere. These produce a great number of secondary metabolites that

participate in ecological interactions and respond to environmental changes (Biłłoński and Dayan, 2005). In *Xanthoriaparietina*, *Physconiagrisea* and *Physciaadscendens*, heavy metals such as Cd, Pb and Zn induce the biosynthesis of phytochelatins by the photobiont partner (*Trebouxia* sp.) only. These lichens may have ecological advantage as they are capable to counter heavy metal stress with prompt phytochelatin synthesis (Pawlik-Skowronska et al., 2002).

In India, lichens form a major component of its biodiversity with approximately 2300 species (Nayaka et al., 2011). Singh and Sinha (1997) have divided India into eight lichen geographic regions on the basis of 10 dominant families and 10 largest genera of lichen which is found in India. Eastern Himalayas has the maximum number of species (1141) followed by the Western Ghats and Western Himalayas with approximately 1130 and 780 species (Singh and Sinha, 1997).

Sensitivity of lichens to environmental changes and their use as bioindicators

Ecological conditions around the world are monitored with the help of indicators. These indicators are also meant for predicting changes in the environmental conditions with time and hence used for early warning. This is possible with the help of a relevant, sensitive and easily measurable indicator. Lichens are known to be very sensitive to environmental parameters like temperature, humidity, and pollutants of air because of the absence of vasculature. In different parts of the world it has been seen that lichen can recolonize urban areas (Rose and Hawksworth, 1981; Seaward et al., 1991; Hawksworth, 2002; Loppi et al., 2002). However, decline in abundance and spatial diversity of lichen around urban and industrial areas is still the main factor which limits their growth (Giordani et al., 2002; Gombert et al., 2004). Altered climatic conditions along with nitrogen oxides emission from vehicles also play a major role in affecting the growth and composition of lichens (Purvis et al., 2003). Data on species composition of lichen and changes in its composition can be easily used for obtaining information about changes in climate, air quality and biological processes. Effects of environmental changes are reflected effectively on the diversity, physiology, morphology, abundance and accumulation of pollutants in the plant body. The factors which affect biodiversity in general such as loss, fragmentation and degradation of habitat, over-exploitation of resources, land use patterns, pollution of air and water and changes in climatic conditions are also applicable for lichens (Scheidegger and Werth, 2009). Additionally, the growth of the lichen thallus is also easily measurable. These unique features make lichens a relevant indicator for predicting changes in the biodiversity.

Lichens obtain nutrients and heavy metals from rainfall, dust fall and underlying substrate from both natural and man-made sources. Natural sources of these nutrients are volatile metals, marine aerosols, volcanoes, leachates from foliage and bark and suspended particles derived from soil and rocks. Lichens are good accumulators and they show varying sensitivity to metals. A variety of mechanisms are employed by the lichens for the accumulation of nutrients from their environment including particulate trapping, extracellular electrolyte sorption, ion exchange, intracellular uptake and hydrolysis. Epiphytic lichens are characterized as the most

sensitive among all the ecological group of lichens. According to the reports of Sloof and Wolterbeek (1991), Reis *et al.* (1996) and Jeran *et al.* (1996), epiphytic lichens are majorly used for monitoring purpose in different countries such as Portugal, Slovenia etc. It has been observed that when the levels of air pollution increases, first fruticose lichens disappear followed by foliose lichens and the last one to disappear are the crustose lichens. Variability in the sensitive nature of lichen makes them an effective tool that can be used as biomonitors of accumulated pollutants by determining the amount of trace elements within them (Mokhtar *et al.*, 2006, Walting, 1981; Ahada and Patel, 2015; Srivastava *et al.*, 2015; Pignata *et al.*, 2007). Various studies have reported the use of lichen as active as well as passive monitors for detection of accumulated metal (Garty 2001; Jeran *et al.*, 2002). For active monitoring, lichens are transplanted on the active site whereas in passive monitoring lichens live in situ.

A detailed list of lichen which is currently being used as biomonitoring agent has been presented in table 1 (Saxena and Afreen, 2010; Lodha, 2013; Srivastava and Bhattacharya, 2015). Biological monitoring with the help of lichens has proven to be the most effective tools for detection of air pollution. In Indian cities such as Kolkata (Nayaka *et al.*, 2003) and Bengaluru (Upreti *et al.*, 2005b), loss of diversity of lichen and alterations in their communities owing to the air pollution has been reported. Additionally, uncontrolled harvesting of lichens from Himalayan and Western Ghats (Upreti *et al.*, 2005a) has also become a serious hazard for biodiversity. Lichens have the ability to trap fine metal particles of rock or soil and adsorb metal ion through ion exchange processes within their body. This feature makes them highly suitable for monitoring amount of pollutants in soil and air. It has been observed that chemical analysis of lichens growing in the premises of industrial installations can help in determination of extent and type of pollutant emissions (Negi, 2003). In Tamil Nadu (India), Krishna *et al.*, 2003 have reported very high amount of mercury in the body of lichens growing near the thermometer factory in Kodaikanal. Similarly, lichen growing in the substrates which have high arsenic content such as mining sites show high concentration of arsenic in their thallus (Bajpai *et al.*, 2009a). Chemical examination of lichen collected from Mandav district of Central India showed high concentrations of Cd, Ni, Zn and Cr. Astonishingly these metals were present in almost negligible amount in substrates which indicates that the accumulated metals were air borne (Bajpai *et al.*, 2009b). Periodic monitoring of concentration of chemicals in lichens has also proven effective in analysis of air and soil pollution over a period of time (Rani *et al.*, 2011).

Lichens are found to be typically growing under extreme conditions of humidity, temperature, light intensity and high concentrations of heavy metals. According to Beck (1999), *Acarosporium* is one of the most metal resistant communities. Some of the secondary metabolites that are produced by lichens are considered to be stress metabolites which are synthesized in response to abiotic and biotic stimulus. These may play a significant role in protection of the thallus against the harmful toxic actions of free radicals produced by exposure to oxidative stress (Huneck and Yoshimura, 1996; Caviglia *et al.*, 2001). A few studies have reported that there are certain changes in the secondary metabolite production in lichen in response to environmental stress (Calatayud *et al.*, 2000; MacGillivray and Helleur, 2001). Since these compounds play a vital role in the adaptation of lichen to their surroundings as well as in

different ecological interactions, these qualities make lichen a suitable candidate for the detection of detrimental changes in the ecosystem (Caviglia *et al.*, 2001). Prolonged exposure to a particular stress agent can increase the production of certain metabolites or might cause a decrease in its production. A decrease in the production of the secondary metabolites or lichen acid may be attributed to their decomposition in response to stress (MacGillivray and Helleur, 2001).

Table 1: Lichen flora used as biomonitoring agents.

Level of pollution	Species of lichens used	Type of lichen thallus
High	<i>Hypogymniaphysodes</i>	Foliose
	<i>Xanthoriaparietina</i>	Foliose
	<i>Lecanoradispersa</i>	Crustose
	<i>Diploiciacanescens</i>	Crustose
	<i>Leprariaincana</i>	Crustose
	<i>Lecanoraconizaeoides</i>	Crustose
	<i>Cladoniamacilenta</i>	Fruticose
	<i>Buellia punctata</i>	Crustose
	<i>Arthopyrenianidulans</i>	Crustose
	<i>Pyxinecocoas</i>	Foliose
	<i>Phaeophyscia orbicularis</i>	Foliose
Moderate	<i>Evernia prunastri</i>	Fruticose
	<i>Foraminellaambigua</i>	Foliose
	<i>Lecanorachlarotera</i>	Crustose
	<i>Ramalinafarinacea</i>	Fruticose
	<i>Lecidellaelaechroma</i>	Crustose
	<i>Hypogymniaphysodes</i>	Foliose
	<i>Parmeliaglabratula</i>	Foliose
	<i>Parmeliasaxatilis</i>	Foliose
	<i>Plastimatia glauca</i>	Foliose
Low	<i>Parmeliacaperata</i>	Foliose
	<i>Graphis scripta</i>	Crustose
	<i>Bryoriafucescens</i>	Fruticose
	<i>Physconiadistorta</i>	Foliose
	<i>Opegraphavaria</i>	Crustose
	<i>Anaptychiaciliaris</i>	Foliose
	<i>Parmelia acetabulum</i>	Foliose
	<i>Physciaaipolia</i>	Foliose
No pollution	<i>Usneasubfloridana</i>	Fruticose
	<i>Parmeliaperlata</i>	Foliose
	<i>Degeliaplumbea</i>	Foliose
	<i>Ramalinafraxinea</i>	Fruticose
	<i>Teleoschistesflavicans</i>	Fruticose
	<i>Lobaria pulmonaria</i>	Foliose
	<i>Lobariascrobiculata</i>	Foliose
	<i>Pannariarubiginosa</i>	Foliose

Although, some of the lichens are exceptionally tough and grow in very uncongenial habitats, but they are equally sensitive to air pollutants, primarily sulphur dioxide and heavy metals. A common phenomenon where lichen disappears from cities forming lichen desert, was described over a hundred years ago and determined to be caused by sulphur pollution. Sensitivity of lichens for air pollutants is due to the absence of outer impermeable layer of tissue, which allows the accumulation of the pollutants. These pollutants, includes gases and particles which impair the lichen metabolism. As a consequence, the accumulation of pollutants is greater in lichens as compared to the foliage of vascular plants, which have impermeable cuticle. Although, pollution equally affects trees and other vascular plants but their response has been reported to be much slower as compared to lichens (Hawksworth and Rose, 1970). For a longer period of time lichens have the capability to retain large amounts of deposits, including heavy metals which eventually reach toxic concentrations. Lichens, thus serve as excellent biomonitors or ecological indicators. The presence or absence of sensitive species is used to determine the distribution patterns that reveal air pollutant deposition. A void in distribution often indicates that lichens in those areas have died out either due to heavy metals or sulphur dioxide pollution. Lichens which are still present in those areas and do not die out are known to accumulate trace elements and indicate pattern of deposition (Lodha, 2013).

Lichens are very widely used as biomonitors of air pollution (Nimis and Purvis, 2002). Worldwide various authors (Gombert *et al.*, 2003; McCune, 2000; Muir and McCune, 1988) have reported the use of lichen as early warning indicators of acidifying sulphur fertilizers and nitrogen-based air pollutants. In fact, the lichens are part of permanent monitoring programme for monitoring air quality in many parts of the world such as US, Switzerland, Netherlands etc (Hawksworth and Rose, 1970). In some areas of the world, often a correlation between nitrogen concentration of epiphytic lichens and traffic density of an area is done to develop a traffic index with NO_x and NH_3 (Gombert *et al.*, 2003). It has been reported that sulphur dioxide is responsible for distribution of epiphytic lichens in urban and industrial areas and qualitative study of the lichens growing on trees can be done to estimate the amount of SO_2 in the air (Hawksworth and Rose, 1970). According to a review of Seaward (1993), over the past 150 years lichens are used as biological monitors for sulphur dioxide (SO_2) pollution as almost all species of lichen are sensitive to these compounds. Many researchers have studied sensitivity of lichens to a varied range of concentrations of air pollutants (Van Dobben *et al.*, 2001; Seaward, 2004; Carreras *et al.*, 2005; Wolterbeek, 2002; González *et al.*, 2003; Giordani, 2007; Giordani *et al.*, 2001; Brunialti and Giordani, 2003; Pinho *et al.*, 2004). Several species of lichens can be used as indicator species as these species have known response to a particular concentration of the pollutant (Bates *et al.*, 2001). Interestingly, over the last decade a few scientists are developing a monitoring scheme to detect trends in status of epiphytic lichen for many protected areas of Russia and its adjacent countries that are affected by background air pollution (Insarov, 2010).

First reports of reduction in lichen diversity were made in 19th century from the city centres in Europe (Nash, 2008). Biomonitoring and bioindication, although, differ in their meaning but are often used interchangeably. Quantitative measurement of elements and

compounds accumulated on organisms or their parts is called as biomonitoring. In contrast, bioindication refers to the use of living organisms and their body parts to study the qualitative changes in richness and distribution of a species caused by environmental pollutants (Markert et al., 2003). Although, various animal and plant species are used for biomonitoring and bioindication purposes, but mosses and lichens are the most preferred ones. Bioindication and biomonitoring can be both active and passive. Active monitoring involves transplantation of species from remote and pollution free areas to target areas, while in the passive monitoring native species (naturally occurring in the target environment) are studied over a required period of time. In those cases when temporal and spatial resolution of the pollution data needs enhancement or when *in situ* species are absent in investigated area, active approach is more advantageous. However, *in situ* species can alter the deposition of pollutants level data as native species are often able to acclimatize to the ambient environmental conditions by building up detoxification mechanisms or reducing their pollutants retention capacity (Fernández & Carballeira, 2000; Boquete et al., 2013). In general, lichens are mostly used in passive studies while mosses are used for active biomonitoring.

Interestingly, lichens have proven to be good bio-accumulators of radionuclides (Notter, 1988). This is an important area of research wherein fallout of radionuclides can be assessed after nuclear disaster such as Chernobyl incident (Sloof and Wolterbeek, 1992; Sawidis et al., 1997). This incident caused a significant increase in the levels of radio cesium in reindeers which majorly feeds on lichens (Jones et al 1989). This disaster caused an increase in concentration of Cs 134 and Cs 137 in vascular plants (Bretten et al., 1992). In areas close to Chernobyl *Parmeliasulcata* was used as bioindicator for the presence of radionuclides where I 129 and Cl 36 were also measured. The accumulated concentration of radionuclides was found to be positively correlated with the regional distribution pattern of the released radionuclides (Chant et al., 1996). *Xanthoriaparietina* is the best bioindicator of radioactive fallout for Cs 137 in which its biological age is 58.6 months. Sometimes, radioactive accumulation in lichens also reveals either the substratum is contaminated with radioactive particles or the origin of radioactive elements is from any accident site (Thomas and Ibrahim., 1995).

Suitability of lichens as bio monitors or bioindicators and the associated benefits

Poikilo-hydrous nature of lichens distinguishes them substantially from the higher plants (Honegger, 1993). This combined with other physiological processes makes their growth particularly susceptible to climatic variations, pollutions and other environmental factors are liable to different morphological and anatomical changes at individuals, populations and community levels (Osyczka et al., 2018). Lichens preferentially absorb mineral supplies from aerial sources (dry and wet deposition) rather than from the substrate because of the absence of a real root system, waxy waterproof cuticle and stomata (William et al., 1984, Wolterbeek, 2002). They grow very slowly and do not shed their parts during growth so whatever amounts of pollutants are absorbed by the thallus are retained within the thallus and helps in accurate determination of the amount of pollutants absorbed by the thallus. Exceptional resistance and reviviscence capabilities of the lichen are indeed the result of the special vegetal assemblage of

fungus and algal partners (Falla et al., 2000). Porous and absorbent structure of the lichens facilitates a fast penetration of sub-micronic particles within in the thallus. This structure makes them good bioaccumulators (Loppi and Nascimbene, 2010). Once the particles are absorbed by the thallus, no excretion or removal of leaf litter is possible as no organ is shed during the growth. This helps in retention of many trace elements within the thallus such as lead, iron, zinc, radium, fluorine and chlorine (Falla et al., 2000). Lichens are very sensitive to air quality since they have no outer impermeable layer to exclude gases and particles that impair their metabolism. Although higher plants are also affected by air pollutants but their response is slower than the lichens. Lichens absorb metal ions through ion exchange and trap heavy metal pollutants within their thallus. The extent and type of pollutants can be easily detected/measured by chemical analysis of lichens. They are extremely sensitive to environmental parameters such as temperature, humidity, wind and air pollutants (SO₂ and heavy metals). This is because they lack the vasculature and thus absorb nutrients and water passively from their environment. Lichens can be used as a very powerful tool to get information about changes in climate and air quality since, they respond to the environmental changes by reflecting changes in their diversity, abundance, morphology and physiology.

In remote regions, where no electricity supply is available for operation of sophisticated equipment for periodic collection of data, lichens act as a perfect choice. Bioindicators show the results of the action of pollutants on living organisms. Bioindicators can act as early warning indicators by giving rapid and sensitive responses to environmental changes. Sampling of lichen for the collection of data is usually simple. Lichens are widespread and are available for sampling year-round. Bioindicators are economically viable alternative as compared to specialized measuring systems. High surface to volume ratio of lichens supports their ability to capture pollutants from the air (Holt and Miller, 2010). Environmental stress is easily indicated by the disappearance of lichen in forests which is caused due to increase in the level of SO₂.

However, there are certain drawbacks associated with the use of lichen as bio monitors or bioindicators. Usually, an increase has been observed in the abundance of nitrophilic lichens due to the increase in bark pH caused by an increased ammonia concentration in the environment (Sparrius, 2007; Frati et al., 2008). However, an increase in bark pH is also a result of dust deposition and dry conditions, which complicates the detection of the effects of nitrogen compounds (Frati et al., 2008). A specific training is required for obtaining information about the air quality using lichens. Changes can only be measured when the damage has occurred at species level or community level. So, for the detection of early stress symptoms attempts have been made to measure ecophysiological changes such as chlorophyll degradation, chlorophyll fluorescence and ethylene concentration (Gartyet al., 2002; Piccotto et al., 2011). Lichens are present in limited climatic conditions and their diversity is sensitive to various abiotic factors at both micro (light, water, nutrients etc.) and macro climatic (temperature, precipitation, soil chemistry etc.) conditions. Due to limited climatic conditions, disturbance at local level leads to habitat fragmentation and loss in lichen biodiversity (Pinho et al., 2003).

Materials and Methods

Lichens are perennial and sensitive as well. Any change in environmental conditions can be easily studied on them. Once the lichens are exposed to high pollution levels the phycobiont (algal) partner first gets affected and the rate of photosynthesis and carbohydrate formation decreases. This results into the death of fungal partner (mycobiont) and finally the whole lichen thallus gets disrupted. The regular monitoring of an area can tell about the loss in diversity of lichens. There are various methods for monitoring of lichens such as survey, phytosociological and ecophysiological methods. These methods have been explained in the following paragraphs.

Survey method

Changes in the gradient of abundance of a species or individuals at a site are directly related to the changes in the level of pollutants. Regular survey and comparison of the communities of lichen at different study sites might indicate the quantity and quality of pollutants or stress that a particular lichen species is facing. Periodic surveys can be made on the native lichen species at different study sites. The abundance, number and frequency of native species at a particular site 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 (Govindaparyari *et al.*, 2010).

Phytosociological method

As the bryophytes are used for finding Index of Atmospheric purity (IAP), Lichens can also be used for the same. Atmospheric purity will change the biodiversity of lichens, which is directly linked to the changes in the levels of air quality. This method is predominantly used for bryophytes but can be extended for the lichens as well. In this method the species of interest is examined along a belt transect or a line having the pollution gradient. The frequency and abundance of species is recorded through a number of transects which are radiating in all directions from the line of transect and with increasing distance from the source of pollution. IAP is determined on the basis of number of frequency and resistance factor of species. This provides a fair picture of the long-range effect of pollution at a site (Brunialti *et al.*, 2010; Govindaparyari *et al.*, 2010).

IAP value can be determined by using following formula:

$$IAP = \Sigma(Q \times f/10)/n$$

(where n = total no of species present at a site, f = frequency coverage of species at each site, Q = resistant factor)

Ecophysiological method

This method is also well adapted for bryophytes but will be equally beneficial for lichens also. In this method the species of interest is exposed to already known concentrations of

pollutants. The live plant can be exposed to different concentrations of pollutants or heavy metals in the field itself by fumigation or by culturing them in the medium. Physiological parameters such as growth and survival rate, injury, chloroplast content and degradation (Kardish et al., 1987; Garty et al., 1988) or other kind of unusual growth of the lichen, photosynthesis (Ronen et al., 1984), decrease of ATPs, changes in respiration levels (Kardish et al., 1987), variation in level of endogenous auxins and ethylene production (Epstein et al., 1986, Garty et al., 1993) are used to evaluate the environmental damage to lichens. This method helps in determining toxicity levels of pollutants and the tolerance levels of different species (Govindaparyi et al., 2010).

Conclusion

The studies regarding accumulation and retention of pollutants, heavy metals and harmful compounds by lichens have helped in elucidation of patterns of air pollution across the globe. However, at this age of industrial development, it has become mandatory to extend these observations across the world and on a wider range of species. There is a need to recognize more species of interest which can adapt to the ongoing chemical changes in our environment. There is a further need to understand role of morphological features of different lichen species in trapping particulate material which till now is an unexplored but vital research field. In the present world, many fold advantages of lichen as bioindicators have outweighed their restrictions as these are helpful, objective, straightforward and reproducible. One more quality which makes them highly usable is the fact that these can be utilized at various scales for assessment of changes taking place in a specific biological community. Therefore, because of qualities that were mentioned in the text, use of lichens as bio monitors and bioindicators seems to be a potential method for studying the effect of external factors on ecosystem and differentiating polluted and unpolluted areas.

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