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Epiphytic Lichens: Their Usefulness as Bio-indicators of Air Pollution

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Lichens are used as effective monitors to detect low-level air pollution which may affect different flora and fauna communities. Epiphytic lichens have been widely recognized as effective eco-friendly bio-monitors for detection of metal deposition on trees, rocks and bare ground. This article is concerned with the use of lichens in bio-monitoring of air pollution, how the lichens do recognize air pollution, the mechanisms of accumulation and trapping of particulate matters, understanding the mechanisms of alterations and the methods of study and factors affecting the process.

Keywords: Air pollution, Bio-monitor, Bio-indicator, Environmental factor, Lichen, Sulfur dioxide, Epiphytic.

INTRODUCTION

Lichens have been recognized as valid tools for evaluation of air-quality especially in the environmentally challenged industrial belts, and this biological assay is now instrumental for ensuring Environmental Impact Assessment (EIA) studies. Relatively lower levels of air pollutants (especially SO_2 and F gas, and acidic compounds) adversely affect many species altering the community composition, growth rate, reproduction, physiology and morphological appearance. F-gases or Fluorinated gases are generated due to anthropogenic activities that may remain embedded in the atmosphere for hundreds of years and may act as added factors to the global greenhouse effect.

Four types of F-gases do exist, viz. Hydro-fluorocarbons (HFCs), Per-fluorocarbons (PFCs), Sulfur Hexafluoride (SF_6) and Nitrogen Tri-fluoride (NF_3). Lichens are used as effective monitors for air pollution worldwide because they have the ability to concentrate a variety of pollutants in their tissues. Under certain conditions, floristic and community analyses of lichens can be used in conjunction with measured levels of ambient or depositional pollutants accumulated by lichens to detect effects of changing air quality on vegetation.

The quality of air can be monitored by measuring the pollutants directly in the air or in deposition, by constructing models depicting the spread of pollutants, or by using bio-monitors (Markert *et al.*, 2003). The term bio-indicator / bio-monitor is used to refer to an organism or a part of it, that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations (Markert *et al.,* 1997). These can be classified as sensitive or accumulative. Sensitive bio-monitors alarm the effect of fluctuations in the environmental behavior as a result of any alterations in enzyme systems or other physiological activities. Accumulative bio-indicators are those that have the ability to store pollutants in their tissue and are used for measurement of these pollutants in the environment.

Different bio-indication methods based on epiphytic flora composition have been used in different countries. Epiphytic lichens were first recognized as useful bio-monitors of air pollutants since long. Methods varied from simply observing epiphytic lichen thallus types (Batic and Mayrhofer, 1996) to recording lichen species diversity or investigating phyto-sociological relationships between different lichen species and environmental conditions. This is partly due to the ability of lichens to accumulate metals and other pollutants like nitrogen and sulfur compounds from the atmosphere as dry or wet deposition. Epiphytic lichens have been widely used as monitors of metal deposition (Sloof and Wolterbeek, 1991; Reis *et al*., 1996; Jeran *et al*., 1996).

Decreased lichen abundance and spatial trends of lichen diversity are determined (Giordani *et al*., 2002; Gombert *et al*., 2004) around urban and industrial areas, based on the fact that anthropogenic variables are responsible for lichen decline, irrespective of natural succession of epiphytic communities (Purvis *et al*., 2003). This article is focused on pointing out the most important lines in the current state of knowledge in this

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very important field, and evaluation of the methodological applications.

WHY ARE THE LICHENS SO IMPORTANT?

Lichens are mutual associates of a fungus and an alga or *Cyanobacterium* and occur as crusty patches or bushy growths on trees, rocks and bare ground. The names given to lichens strictly refer to the fungal partner; the algae have separate names. They are very sensitive to sulfur dioxide pollution in the air. Since lichens have no roots, they absorb much of their raw materials directly from the air and moisture around them. This makes them very sensitive to air pollution and acid rain and since lichens have no way to excrete the pollutants they absorb, these materials stay inside of their cells. Since pollutants build up inside them, lichens can be used to monitor the long-term accumulation of pollutants. Scientists collect and analyze lichens near sources of pollution to determine how far the pollution has spread.

LICHENS AS BIO-INDICATORS OF AIR POLLUTION

Lichens were recognized as potential indicators of air pollution as early as the 1860's in Britain and Europe (Hawksworth and Rose, 1976). Since then, lichens have played prominent roles in air pollution studies throughout the world because of their sensitivity to different gaseous pollutants, particularly sulfur dioxide. They have also been found to act as accumulators of elements, such as trace metals, sulfur, and radioactive elements (Stolte *et al*., 1993; Ahmadjian, 1993). The lichen species best suited as bio-monitors are foliose (having a lobed, leaf-like shape) and fruticose (having upright or pendulous branches) epiphytic lichens. The properties that make them suitable for monitoring purposes are the weakly developed cuticle and vascular bundles, absence of real roots, their slow-growing nature and long life cycle and their broad distribution (Wolterbeek *et al*., 2003).

ADVANTAGES OF LICHENS THAT MAKE THEM SUITABLE BIO-MONITORS

1. Many lichen species have large geographical ranges, allowing the study of pollution gradients over long distances.
2. Lichen morphology does not vary with the seasons, and accumulation of pollutants can occur throughout the year.
3. Lichens are usually very long lived.
4. Water and gas are exchanged over the entire lichen thallus make them sensitive to pollution.
5. Lichens lack roots and do not have access to soil nutrient pools and depend on deposition, water seeping over substrate surfaces, atmospheric and other comparatively dilute sources of nutrients. Thus, their tissue content largely reflects atmospheric sources of nutrients and contaminants.
6. They lack the protective tissues or cell types necessary to maintain constant internal water content.
7. Many lichens pass through multiple wetting and drying cycles during a day. When hydrated, nutrients and contaminants are absorbed over the entire surface of the lichen. During dehydration, nutrients and many contaminants are greatly concentrated by being converted to slow- releasing forms, i.e., absorbed to cell walls, cloistered inside organelles or crystallized between cells. During heavy rains, nutrients and pollutants are gradually leached. A dynamic equilibrium thus exists between atmospheric nutrient/pollutant accumulation and loss, which makes lichen analysis a sensitive tool for the detection of changes in air quality (Pearson, 1993).

All the lichens are not equally sensitive to air pollutants but show different sensitivity to specific atmospheric pollutants (Gries, 1996). The sensitivity usually follows a series; *crustose (flat, tightly adhered, crust-like lichens) < foliose (leafy lichens) < fruticose (shrubby lichens),* though there are exceptions to this gradation. Due to this specific response to atmospheric pollutants, lichens can be denoted as excellent markers of different emission sources and good bio-monitors of atmospheric quality (Gonzalez and Pignata, 2000).

The suitability of lichens as bio-monitors for air pollution has been strongly advocated and established by studies by Oksanen *et al*. (1991), Loppi *et al*. (1992), Seaward (1996), Hamada and Miyawaki (1998), Mulgrew and Williams (2000) and Nash and Gries (2002).

Bio-monitoring surveys by lichens have also been carried out to estimate the pollutant load in forest ecosystems (McCune, 2000; Loppi and Pirintsos, 2003; Kapusta *et al*., 2004; Gonzalez *et al*., 1996, 2003; Garty *et al*., 1997; Gonzalez and Pignata, 2000; Bates *et al*., 2001; Van Dobben *et al*., 2001; Wolterbeek, 2002 and Carreras *et al*., (2005) reported the sensitivity of lichen species to a range of concentrations of air pollutants.

MECHANISMS OF ACCUMULATION AND PARTICULATE TRAPPING

Lichens accumulate substances from their environment by a variety of mechanisms, including particulate trapping, ion exchange, extracellular electrolyte sorption, hydrolysis, and intracellular uptake (Nieboer *et al*., 1978). Lichens have a large surface area-to-volume ratio due to their thin thallus, branching, presence of projections such as isidia and phyllocladia (Tomassini, 1976). Due to the surface characteristics, various particles gets embedded in the lichen thallus under moist or dry conditions. The cell wall is mostly involved in the process of mass and charge balance.

METHODS USED TO MEASURE THE RESPONSES OF LICHENS TO AIR POLLUTION

The most widely used methods to measure these responses are fumigation and gradient studies (Stolte *et al*., 1993). The basis of undertaking gradient analysis method lies on the fact that characteristic of affected species varies according to the environmental gradients. Such studies are usually done around existing or projected sources of contaminants, with pollutant loadings expected to vary with distance from a source. Gradient studies involves observations of visible injury, such as bleaching and thallus deformation, and changes in community structure, such as species richness, abundance, or cover (Hawksworth and Rose, 1976) and physiological processes such as photosynthesis, nitrogenase activity, element uptake, membrane integrity (electrolyte leakage), pigment quantity, degradation, and fluorescence (Stolte *et al*., 1993 and Conti and Cecchetti, 2001).

Gradient methods are usually designed to monitor naturally occurring species in a region. When climate and pollution factors create unfavorable conditions for lichens, it may be impossible to identify and use a naturally occurring indicator species. Transplanting lichens is an alternative method that can be used to determine the effects of pollutants on lichens and their photobionts in polluted regions that lack a natural community of lichens. Lichens or bark discs with thalli can be attached to supports that are then placed at different distances from a pollution source (Ahmadjian, 1993).

Some difficulties may be faced when gradient studies are undertaken such as identification of species, determination suitable indicator species and proper interpretation of data showing that the observed patterns reflect pollution stress and not other biotic and abiotic factors. Gradient studies can further be classified into stages;

1. By mapping all species present in an area.
2. By transplanting lichens from uncontaminated sites to contaminated ones and measuring the bioaccumulation of pollutants.

Mapping species present in a specific area

Mapping of air quality in an area can be made by following IAP (Index of Atmospheric Purity) method (LeBlanc and De Sloover, 1970) based on number (n), frequency (F) and tolerance of the lichen present in the area under study. IAP can be determined by using the following formula:

$$IAP= \sum Fi$$

Where F is the frequency (max. 10) of every i^{th} species that is calculated as number of rectangles in the grid. The rectangle is of the dimensions 30x50 cm each in which a given species appears. The IAP values are grouped into five quality levels which are given in Table 1.

A combined study with lichen mapping and analysis of quantitative levels of trace elements is being done by Jeran *et al*. (2002) for air pollution monitoring.

Use of transplanting methods

Lichen thalli are transplanted on a suitable substrate and exposed to polluted areas. Samples are taken periodically and observed for any damage. This method is used in bioaccumulation studies in order to study absorption, retention, localization and release, tolerance and toxicity of pollutants (Garty, 1992; Garty *et al*., 1997; Sloof, 1995; Bargagali *et al*., 1997; Freitas *et al*., 1999). A comparative study of atmospheric quality in five zones of Cordoba city (Argentina) was done using transplanted *Usnea* species (Carreras *et al*., 1998).

Fumigation studies

Fumigation studies are often carried out with a known exposure of a specific pollutant under controlled environmental conditions. Fumigation studies involve measuring response variables of selected physiological processes. Sensitive processes include activity, K^+ imbalances, photosynthesis, and respiration pigment status. The results of these studies can be used to provide inputs for field studies. However, the bias may prevail due to the exposure of mixture of pollutants and dynamic nature of the environmental conditions. Fumigation field studies of lichens from areas around pollution sources have proven to be more useful in predicting the potential impact of a pollution source on various species (Ahmadjian, 1993), as they allow environmentally realistic observations of how specific physiological or morphological changes correlate with specific pollutants (Stolte *et al*., 1993).

Sizing-up lichens

The size of lichens is a good indicator of air quality which depends on age and amount of sunlight. Assuming standard conditions for growth the data can be used to determine air quality. The relation between size and air quality is being given in Table 2.

RESPONSES OF LICHENS TO AIR POLLUTION

Lichens have been used often as receptor-based bio-monitors in air quality studies. Several experimental studies have been published on the effects of sulfur dioxide, nitrogen compounds, ozone, heavy metals and other atmospheric pollutants on the morphology and physiology of lichens (Garty, 2000; Nimis *et al*., 2001; Van Dobben *et al.,* 2001; Bates *et al*., 2001; Giordani *et al*., 2002; Purvis *et al*., 2003; Lalley and Viles, 2005; Lalley and Viles, 2008). A number of researchers have recorded and assessed the air-quality using lichens as instrumentals for assay (Mulgrew and Williams, 2000; Conti and Cecchetti, 2001; Nash and Gries, 2002; Nimis *et al*. 2002; Garty, 2000, 2001). Characters of lichens which are used to measure the magnitude of air pollution include morphological, physiological and population characteristics. Studies have emphasized the significance of lichen morphology and physiology in the accumulation of elements (Brown, 1991; Sloof and Wolterbeek, 1991).

Historically, lichens have been used in a qualitative way, with observations of population changes and morphological effects serving as indicators of pollutants. In the last few decades, quantitative measurements of the chemical content of lichens and sensitive physiological processes have increasingly been used to indicate pollutants. Possible responses to air pollution stress include chlorophyll degradation, changes in photosynthesis and respiration, alterations in nitrogen fixation, membrane leakage, accumulation of toxic elements, and possible changes in spectral reflectance, lichen cover, morphology, community structure and reproduction.

Microscopic and molecular effects include reduction in the number of algal cells in the thallus (Holopainen, 1984), ultra-structural changes of the thallus (Holopainen, 1984; Pearson, 1985), changes in chlorophyll fluorescence parameters (Gries *et al*., 1995), degradation of photosynthetic pigments (Kauppi, 1980a, 1980b; Garty *et al*., 1993), and altered photosynthesis and respiration rates (Sanz *et al*., 1992; Rosentreter and Ahmadjian, 1977).

HEAVY METALS

The action of rain, surface water and passive upward diffusion from the substrate likely bring dissolved minerals in contact with lichen thalli (Richardson, 1988). The amount of each type of metal ion that can be accumulated by lichen is dependent upon the uptake characteristics of that particular species and the amount and availability of metal ions in the surrounding environment. Extracellular uptake of metal ions is essentially a passive process of ion exchange determined by the character of the ligands in the fungal cell walls. Intracellular uptake is limited by the nature of the metal ion, cell membrane permeability, and the concentration of extracellular ligands with affinity for cations (Tyler, 1989).

Table 1: Quality levels of IAP values

IAP Levels	Degree of pollution
0 ≤ IAP ≤ 12.5	Very high level of pollution
12.5 < IAP ≤ 25	High level of pollution
25 < IAP ≤ 37.5	Moderate level of pollution
37.5 <IAP ≤ 50	Low level of pollution
IAP > 50	Very low level of pollution

Table 2: Lichen size and air quality

Size in cm^2	Air quality rating
0-3	Poor
4-6	Fair
7-9	Good
10-12	Excellent

Heavy metals can be absorbed by lichens by intracellular absorption, intracellular accumulation or deposition of particles that contains heavy metals. Epiphytic lichens have been widely used as monitors of metal deposition (Sloof and Wolterbeek, 1991; Reis *et al*., 1996; Jeran *et al*., 1996). Heavy metals have also been found to affect e.g., the permeability of the cell membranes of lichens (Tyler, 1989; Tarhanen *et al*., 1996; Nimis *et al*., 2001). However, the detrimental effects of heavy metals on e.g., the occurrence of lichens usually only become apparent at high heavy metal concentrations (Van Dobben *et al*., 2001). Heavy metals have also been reported to increase a lack of water in the thallus (Nieboer *et al*., 1976).

Lichens have been proven to be good accumulators of heavy metals, and that the concentrations correlate well with the concentrations measured in deposition. The concentrations have been very high near the emission sources, and they have decreased exponentially with increasing distance from the emission sources (Nieboer *et al*., 1972, Pilegaard, 1979).

Elevation in the content of heavy metals in the thallus has also been documented in many cases (Garty, 2001; Addison and Puckett, 1980; Carlberg *et al*., 1983; Gailey and Lloyd, 1986; Gough and Erdman, 1977), but it is not always easy to establish what specific effect these elevated levels will have on lichen condition or viability. Tolerance to metals may be phenotypically acquired, but the sensitivity of lichens to elevated tissue concentrations of metals varies greatly among species, populations, and elements (Tyler, 1989). The toxicity of metal ions in lichen tissue is the result of three main mechanisms: the blocking, modification, or displacement of ions or molecules essential for plant function.

Metal toxicity in lichens is evidenced by adverse effects on cell membrane integrity, chlorophyll content and integrity, photosynthesis and respiration, potential quantum yield of photo-system II, stress ethylene production, microstructure, spectral reflectance responses, drought resistance, and synthesis of various enzymes, secondary metabolites, and energy transfer molecules (Garty, 2001). Ikingura and Akagi (2002) studied the bio-monitoring activity of lichens in gold mining areas of Tanzania.

In Italy, lichens are being used to study Pb concentration in the atmosphere due to vehicular pollution. Pb is bound to insoluble anionic sites, accumulated extracellular, and concentrated in the medulla. Once bound, Pb is not easily removed by rain or wind. The toxic effects of Pb on lichens are minimal. Some species can accumulate up to about 2000 ppm, after which concentrations do not increase, indicating a degree of physiological turnover. Surveys of Pb content have proved useful in establishing background (preindustrial) values which can be referenced to present levels (Lawrey and Hale, 1981). The accumulative effect of Pb, Cu and Zn on water loss from lichen thalii is being documented by Chettri and Sawidis (1997). Nieboer *et al*. (1979) studied the toxicity of Cd to lichens. Concentration intervals of 1.26-5.05 and 1.56-6.40 µg g^{-1} have been found for *A. ciliaris* and *L. pulmonaria* respectively. At these concentrations, toxicity symptoms get started (Conti and Cecchetti, 2001).

Cd is having a highly negative correlation with protein and reducing sugar content. The cationic concentrations in lichens can be used as an index of acid precipitation. The variation in Mg^{++} concentration in lichens can be considered as a good indicator of acid rain (Hyvarinen and Crittenden, 1996). Accumulation of heavy metals in some selected species in some geographical areas is given in Table-3.

SULFUR COMPOUNDS

The effect of sulfur compounds on lichens has been studied by many coworkers. Most work has been carried out on the effects of SO_2, either directly from the atmosphere or via the substrate, on the occurrence and abundance of epiphytic lichen, (Gilbert, 1986; Farmer *et al.*, 1991; Van Dobben *et al.*, 2001). Many studies show a positive correlation between sulfur content of lichens and SO_2 present in the atmosphere (Rope and Pearson, 1990; Silberstein *et al*., 1996). According to the Guidelines for Studies Intended for Regulatory and Management Purposes by USDA Forest Service sensitive species are damaged or killed by annual average levels of sulfur dioxide as low as 8-30 µg/m^3 (0.003-0.012 ppm) and very few lichens can tolerate levels exceeding 125 µg/m^3. Lichens are adversely affected by short-term exposure to nitrogen oxides as low as 564 µg/m^3 (Holopainen and Kärenlampi, 1984) and by peak ozone concentrations as low as 20-60 µg/m^3 (Egger *et al*., 1994; Eversman and Sigal, 1987). Sensitivity towards SO_2 varies according to species (Insarova *et al*., 1992).

Lobaria pulmonaria is considered to be one of the most sensitive species and *Hypogymnia physodes* is a species that is resistant to SO_2. It has been observed that exposure of this species to a highly acidic condition does not produce any effect (Garty *et al*., 1995).

Table-3: Accumulation of heavy metals (mg / kg^{-1} dry weight) in some selected species in some geographical areas

Species	Site	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Anaptychia ciliaris	Southern Greece	3.09	-	4.06	2153	43.87	-	8.60	-	31.22
Lobaria pulmonaria	Southern Greece	3.42	-	6.85	1103	65.32	-	9.76	-	28.16
Ramalina duriaei	Israel	-	-	-	-	-	14.3	-	23.1	-
Evernia prunastri	Rome	-	14.05	13.37	-	-	-	40	5.2	57.45
Hypogymnnia physodes	Slovenia	1.05	5.78	-	1253	-	-	-	-	90.2
Parmelia caperata	Travele-Radicondoli (Central Italy)	0.329	4.51	10.8	1019	85.8	4.41	6.3	-	43

Source: Adapted from Conti, M. E. and Cecchetti, G. (2001). *Environmental Pollution*, 114: 471-492.

Table 4: General Mechanisms of SO_2 toxicity

Type of reaction	Observed / expected response or injury	References
Enzyme deactivation: (e.g. sulfitolysis) -Chemical modification -Binding to metal centers (Vitamin B_{12}; Fe) -Inhibition	Reduced metabolic activity; loss of membrane integrity, membrane function and cell osmolality, (competition with HCO_3^-, $H_2PO_4^-$)	Zeigler, 1975, Nieboer *et al.*, 1976, Khan and Malhotra, 1982, Gunnison *et al.,* 1981, Mansfield and Freer-Smith, 1981, Alscher-Herman, 1982.
Stimulation of enzyme systems: (Often in response to low pollution levels) -Glucose-6-phosphate-dehydrogenase, -Increases in glutathione and total protein SH	Use of increases in enzyme activity as a bio-indicator for non-visible injury and detoxification of metabolism absorbed SO_2	Rabe and Kreeb, 1980, Miszalski and Zeigler, 1979, Grill *et al.,* 1980.
Reaction with reactive bio-molecules: -Chemical (bisulfite adducts) -Redox (acts as electron acceptor / donor at pH 7)	Modification of metabolic precursors and products; interference with electron flow in photosynthetic and respiratory electron-transport chains	Puckett *et al.,* 1973; Ziegler, 1975; Gunnison, 1981, Nieboer *et al*., 1976; Shapiro, Petering and Shih, 1975.

Source: Hutchinson *et al.*, 2006, Online document *http://www.fs.fed.us/r6/aq/lichen/almanac.htm#Physiological%20Responses.*

It is being interpreted that chronic SO_2 fumigation may cause interference in the flow of carbohydrates causing symbiotic damage. Protein biosynthesis may be interrupted due to damage of membrane protein due to SO_2. As lichens have a tendency to lose moisture under highly polluted conditions the evaluation of dry weight to fresh weight ratio can be used as bio-monitoring purpose. Production of ethylene is another indicator of stress. These solutions increase the solubility of dust bearing heavy metals within the hyhpae.

Besides, this acidic environment alters the substrate chemistry, thereby affecting species diversity and composition at the community level. Bark pH is found to have a profound effect on species (Farmer, *et al*., 1992). It has been noticed that bark pH is a critical factor in the establishment of acidophilus species. Bark texture and moisture, as well as air quality appeared to influence the structure and composition of these lichen communities. The lichen communities on the urban trees appeared to be affected by the region's air pollution both in terms of diversity and species composition (Gary, 2010). The general mechanism of SO_2 toxicity is described in the Table-4.

NITROGEN COMPOUNDS

Lichens don't directly respond to the nitrogen levels found in the environment. Their reaction depends on the pH levels of the substrate relating to higher levels of ammonia in the atmosphere (Van Dobben and Ter Braak, 1998). *C. portentosa*, *H. physodes* has also been proposed as a bio-indicator of nitrogen pollution (Sochting, 1995). There are number of observations reporting that high NO_x concentrations from road traffic negatively affect lichen presence (Bates *et al*., 2001; Giordani *et al*., 2002; Purvis *et al*., 2003; Fuentes and Rowe, 1998), and may presently be the main limiting factor for lichen colonization in urban areas (Van Dobben *et al*., 2001).

OTHER ATMOSPHERIC POLLUTANTS

Conti and Cecchetti (2001) described the bio-monitoring of fluorides, polychlorinated dibenzodioxins and polychlorinated dibenzofurans and radio-nuclides as follows.

Effects of Fluorine

The effect of fluorine includes decrease in respiration and photosynthesis, an increase in membrane and thallus permeability with a concomitant loss of ions, and changes in cellular ultra-structure. Damage to lichens begins at levels of 50-70 ppm (Gilbert, 1971). Above 80 ppm, fluorine concentration chlorosis was observed. The ability of lichens to accumulate F is a function of relative humidity, which determines the moisture conditions of the thallus.

Effects of photochemical toxins

The effect of photochemical oxidants like Ozone and PAN includes decreases in photosynthesis, decrease in species distribution, morphological and ultra-structural changes.

Photochemical toxins may have synergistic effects when combined with other pollutants, under low pH conditions (Farmer *et al*., 1992). Fumigated lichen shows bleaching and discoloration at 300 ppm ozone exposure for 34 days (Ruoss and Vonarburg, 1995).

Factors affecting the sensitivity of lichens to air pollutants

There are a considerable number of factors which may affect the concentrations of pollutants in lichens (Brown, 1991; Garty, 2000; Jurga, 2007). These factors include the type of pollutant input, nature and composition, climatic factors such as composition of precipitation, temperature, wind, drought, and local environmental factors such as vegetation, quality of the substrate and altitude of area. According to a study carried out by Giordani (2007), the diversity of epiphytic lichens was strictly correlated with mean annual rainfall and mean annual temperature. Different variables were found to affect the lichen diversity in urban vs. forested areas.

In urban areas, air pollutants, mainly SO_2, are still the main limiting factor, even if under ameliorating conditions this relationship becomes less significant. In forested areas, harvesting and forest fires showed a predominant effect. Lenka *et al*. (2006) in his research focuses on understanding of environment influences on sensitive epiphytic lichens and their abundance and distribution in Irish broadleaf woodlands. Caceres *et al*. (2007) concluded that community formation of understory lichens subtly correlates with two main environmental factor complexes i.e., bark characteristics and microclimate.

DISCUSSION

Bio-monitoring of air-pollution is the procedure to evaluate the concentrations of trace elements in aerosols and their deposition. The efficiency of the bio-monitor on the environmental impact is considered as resulting in changes in the dose-response relationships. Elemental atmospheric-availability is detected from lichens, and from this monitoring derives the relevance of the presumed impact of the pollutants on both ecosystem performance and human health; their source distribution is also regarded as a very important parameter for purposes of emission regulatory management.

Lichens play a prominent role in air pollution studies throughout the world because of their sensitivity to different gaseous pollutants. Due to its specific response to atmospheric pollutants lichens can be denoted as excellent markers of different emission sources and good bio-monitors of atmospheric quality. As all the lichens are not equally sensitive to every contaminants care should be taken regarding the identification of species, determination suitable indicator species and proper interpretation of data showing that the observed patterns reflect pollution stress and not other biotic and abiotic factors. Appropriate technique should be developed especially in developing countries to use lichens as bio-monitoring of air pollution.

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