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REVIEW ARTICLE

Pyxine cocoes (Sw.) Nyl. as an ideal lichen species for biomonitoring studies: A systematic review

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Abstract

The lichens are self-sustaining, long-living symbiotic systems that result from the symbiosis between mycobiont and photobiont. The lichen biodiversity is sensitive to environmental circumstances since certain species are more indulgent in environmental influences. The lichens accumulate both inorganic and organic pollutants present in the air and soil. The lichens are utilized in biomonitoring studies as bioindicators of environmental changes such as air pollution and climate change. Among the diverse lichen taxa, genera *Dirinaria, Heterodermia, Physcia,* and *Pyxine* are well-known groups having toxitolerant nature. Several studies utilizing *Pyxine cocoes* are available throughout the world. Thus, the present review aims to consolidate various methods adopted to monitor the quality of the environment utilizing the lichen *P. cocoes* in various parts of the world. To get insight into current practices, developments, and difficulties, a total of 25 prior studies over the preceding 12 years were examined in this review.

Based on the research area and scope, content analysis was used to categorize and comprehensively characterize the available biomonitoring studies using lichen into several groups. Two basic techniques of biomonitoring using lichen *P. cocoes* involving different scopes and types of parameters are also discussed. The pollutants can be introduced into the environment by either geogenic or anthropogenic emissions viz., soil, rocky dust, burning of fossil fuels and waste, roadsides dust, agricultural practices including the use of pesticides and chemical fertilizers, transportation, construction, and urban waste. Accumulation of various metals viz., As, Al, Fe, Ni, Pb, Zn, Mn, Cu, Co, Cr, Cd, F, Hg, K, Ca, Mg and Na in the thalli of *P. cocoes* is also presented. The EDX spectra studies together with FTIR analysis showed 41.1 to 53.53% elemental composition in *P. cocoes* and the presence of various anionic sites (e.g., hydroxyl, amine, carboxyl groups) where metal binds on the lichen thalli. Thus, the review indicates that *P. cocoes* is a potential biomonitor, bioindicator, and bioaccumulator.

Keywords: Air pollution, Biodiversity, Bioindicator, Environmental quality, Lichens.

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Introduction

In recent years, air pollution has grown into one of the most significant environmental issues facing the globe today due to the rapid emergence of industrialization and anthropogenic activities. As a result, effective and sustainable technologies for detecting and reducing air pollution are required. As an alternative to conventional physico-chemical methods, biomonitoring of air pollution using plants including lichens has gained popularity in recent years due to its affordability, sustainability, and environmental friendliness. A common method used to diagnose air pollution damage to plants is monitoring for harmful levels of air pollutants in the presence of plants (Ram *et al.* 2015). Biomonitoring is commonly described as the systematic use of living organism or their response to determine the status or changes in the environment (Thomas 1961). Four concepts are included in the field of biomonitoring; the utilization of biomarkers, bioindication, biointegration, and bioaccumulation (Badamasi 2017). Whereas, living organisms

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such as plants, animals, and bacteria are called bioindicators, and they are used to monitor the state of the environment's natural ecosystem. They are employed to evaluate the health of the environment and biogeographic changes that are occurring in nature (Parmar *et al.* 2016). The selection of good biomonitors/bioindicators has several characteristics such as good indicator ability, well-studied and documented, abundance, and distribution in an area (Figure 1).

Lichens being cosmopolitan organism of a biological system indicates their surroundings and reacts quickly to environmental changes. It is a key biomarker for determining the quality of air as well as an indicator of air pollution. The lichens are well-known symbiotic organisms and are ubiquitous, self-sustaining biological organisms (Honegger 1991). This mutual relationship is mainly due to the symbiotic association between mycobiont (fungi) and photobiont (algae) partners. The mycobiont in a lichen thallus primarily provides adequate living and growing conditions for the photobiont and the photobiont offers food for the mycobiont by converting ambient carbon dioxide into organic sugars (Nash 2008). The fungal-algal alliance is an adaptable combination that can survive in a wide array of environmental conditions, including elevation, humidity, and temperature. The lichens are employed as pollution bioindicators and the health of the ecosystem because they give a pertinent, quantitative measure for long-term environmental surveillance (Singh *et al.* 2019). Lichen communities growing on bark, rocks, and walls show alterations in response to pollutants of air, particularly sulfur dioxide (SO_2), fluoro-compounds (F), nitrogen compounds deposition, and ozone (O_3). Lichens are very useful for detecting pollution loads over long periods (Badamasi 2017). Some toxitolerant lichen species can trap pollutants in a mesh of fungal filaments and can endure for longer durations. The heavy metals released from geogenic and anthropogenic activities pose a concern to both humans and the environment due to their negative impacts on the ecosystems by contaminating air, water, and soil. The sensitivity and tolerance are essential characteristics that enable lichen (e.g. *P. cocoes*) to be utilized as a bioindicator as well as biomonitor species (Savillo 2010). Here, the study aims to give an account of *P. cocoes* and their application in pollution monitoring, metal accumulation, and other aspects.

General characteristics and distribution of *P. cocoes*

The lichen *Pyxine cocoes* (Sw.) Nyl. was originally described as *Lichen cocoes* by Swedish botanist Olof Swartz. Later the taxonomic position as species was updated by Nylander (1857) as *P. cocoes*. It is a foliose lichen with green photobiont, pale greyish-green thallus and radiating lobes that are normally less than 1 mm broad and closely attached to the substratum. The lobes bear granular soredia that protrude through the cortex in irregularly shaped patches termed soralia. The thallus medulla is white, apothecia are rounded, 1-5 mm in diameter, flat with brown-black disc. The tissue at the base apothecia's brownish-red. Ascospores are 15-22 x 6-8 µm in size.

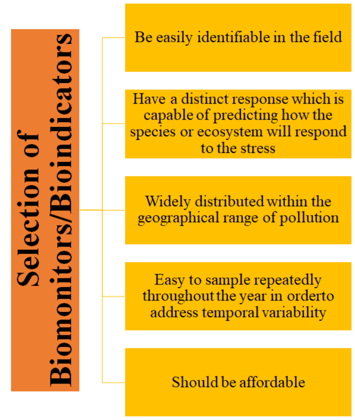

Figure 1: Criteria for the selection of biomonitors/bioindicators

P. cocoes medulla and soredia react negatively with reagents such as potassium hydroxide (K), calcium hypochlorite (C), and ethanol solution of para-phenylenediamine (P). Under short-wave ultraviolet light (200-280 nm), the thallus emits bright yellow fluorescence. The lichen substance lichexanthone and traces of unknown terpenes (skyrine and zeorin) can be detected in thin layer chromatography. *P. cocoes* grow very commonly on bark and rocks and are termed corticolous and saxicolous lichen respectively. According to Rogers (1986), *P. cocoes* could be nitrophilic in nature as they flourish in environments with enriched nutrient accessibility. The natural habitats include alkaline substrates rich in minerals as well as nitrogen-rich inputs such as rocks and tree branches. It prefers to grow on habitats such as hardwood substrates in peri-urbanized regions or agricultural fields where ammonia from animal waste or volatilized fertilizers promote the growth of lichen species.

P. cocoes is a cosmopolitan species and it has been reported from sixty-four countries or areas viz., Australia, Bangladesh, Brazil, Burundi, Cabo Verde, China, Colombia, Costa Rica, Cuba, Curacao, Democratic Republic Congo, Dominica, Ecuador, El Salvador, Ethiopia, Fiji, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, India, Indonesia, Italy, Jamaica, Japan, Kenya, Kiribati, Madagascar, Malaysia, Marshall Islands, Mauritius, Mexico, Namibia, New Zealand, Nicaragua, Norway, Panama, Papua New Guinea, Paraguay, Peru, Philippines, Portugal, Saint Lucia, Saint Vincent and the Grenadines, Seychelles, Singapore, Somalia, South Africa, Spain, Sri Lanka, Sudan, Suriname, Tanzania, Thailand, Tonga, Trinidad and Tobago, Uganda, United States of America, Venezuela, Vietnam and Zimbabwe (www.gbif.org) (Figure 2).

In India, *P. cocoes* thrive in abundance in both temperate and tropical climates. *P. cocoes* are found in different states

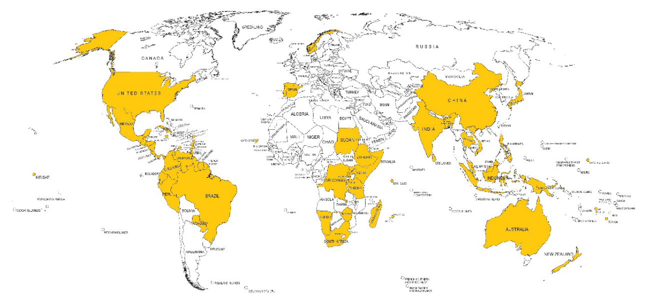

Figure 2: Worldwide occurrence of lichen species *P. cocoes*

viz., Andhra Pradesh, Assam, Goa, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Odisha, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh, and West Bengal (Figure 3).

P. cocoes have the excellent ability to uptake several pollutants from polluted environments, thus serve as excellent bioindicators of environmental contamination. It also grows on bark, twigs, and branches on lower heights and accumulates higher concentrations of metals and trace elements that do not disperse too high.

In today's world, air pollution is a major environmental issue that is posing a threat to human and environmental health. Numerous technologies have been developed to assess elements that contribute to air pollution, but one of the most frequent approaches for estimating environmental deterioration is to use living organisms. Among the lichen taxa utilized worldwide for biomonitoring studies *P. cocoes* is found to be more suitable, adaptive, and toxitolerant for monitoring various metals viz., As, Cd, Fe, Al, Cu, Cr, Zn, Pb, Ni, and Hg as given in Table 1.

P. cocoes as hyperaccumulator

The concentrations of contaminants in the environment have an impact on the chemical composition of lichens. The lichen *P. cocoes* in particular has a large surface-to-mass proportion which allows to effectively gather metals, metalloids, trace

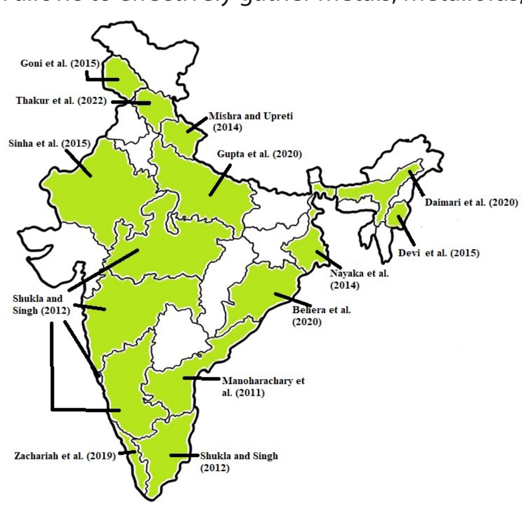

Figure 3: Map of India showing the state-wise occurrence of lichen species *P. cocoes*

elements, and radionuclides (Seaward 1988, Nimis 1993). The metal ions are also observed to be tightly linked to the lichen thallus, making it unremovable. The metal ions that collect on the lichen thalli are then connected to the cytoplasmic layer via a mechanism known as active translocation (Subbotina and Timofeeff 1961). Most macro-lichens are recognized to be sensitive to metal pollution, but certain species thrive in a metal-abundant atmosphere and are known to be a hyperaccumulator of several metals (Shukla and Upreti 2008). The other hyperaccumulator species are *Dirinaria pappillulifera, Hypogymnia physodes, Parmelia sulcata, Phaeophyscia hispidula, Phaeophyscia orbicularis, Pseudevernia furfuracea,* and *Pyxine subcinerea*.

The lichens acquire pollutants in the intercellular spaces of the medulla by a variety of processes, including surface deposition, bio-mineralization, and physical trapping (Nash 2008). Some characteristics features that make *P. cocoes* suitable and an ideal candidate for air pollution monitoring studies are related to morphological, anatomical and chemical nature of the organism (Figure 4).

Mechanism of metal accumulation in P. cocoes

Although many lichens can withstand significant metal stress, the mechanism of tolerance is yet unknown. Out of diverse lichens growth forms, foliose lichens are the better accumulator of metals followed by crustose and squamulose forms (Paliwal *et al.* 2018). The lichens produce wide array of secondary metabolites, each with its distinct chemical profile (Nash, 1996, Shukla *et al.* 2014). In lichen thalli, secondary metabolites play a significant role in metal accumulation. The secondary chemical found in lichens have been shown to form complexes with contaminants, mostly metal ions, so protecting the fragile thallus from the detrimental effects of pollutants (Shukla *et al.* 2017).

Being a toxitolerant species, *P. cocoes* contains some chemical compounds viz., skyrine and zeorine provide strength to the lichen fight against pollution, particularly acidic gases. *P. cocoes* has pollution-tolerant behaviour specifically, both nitrogen and SO_2 pollution (Abas *et al.* 2018).

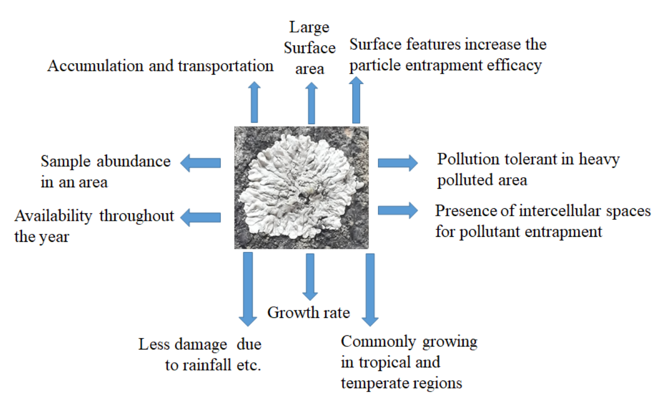

Figure 4: Characteristics of *P. cocoes* (a pollution-tolerant species)

Table 1: Utilization of *P. cocoes* for biomonitoring studies in the world

S. No.	*Countries*	*States/Regions*	*Locations*	*Techniques Used*	*References*
1	India	Assam	Cachar district	Pigment profile and chlorophyll degradation	Rout *et al.* 2010
			Nagaon Paper Mill region, Marigaon district	Heavy metal analysis	Singh *et al.* 2019
			Brahmaputra Valley plain	Metal analysis	Daimari *et al.* 2020
		Himachal Pradesh	Mandi district	Physiological analysis, biochemical analysis, and metal analysis	Thakur *et al.* 2022
		Karnataka	Bangalore city	Heavy metal analysis	Nayaka *et al.* 2003
			Bhadravathi town	Ambient air quality monitoring and pigment analysis	Danesh *et al.* 2013
		Madhya Pradesh	Katni and Rewa cities	Heavy metal analysis	Bajpai *et al.* 2011
		Uttarakhand	Dehradun city	Pigment analysis, chlorophyll degradation, Heavy metal analysis, C/N estimation	Rani *et al.* 2013
			Udham Singh Nagar	Metal analysis	Mishra and Upreti 2014
		Uttar Pradesh	Lucknow city	Pigment analysis, chlorophyll fluorescence (Fv/Fm) analysis, Electrolyte conductivity analysis, Protein, amino acids, stress hormones analysis, Metal analysis	Saxena *et al.* 2007, Bajpai *et al.* 2022
			Raebareli, NTPC	Pigment analysis, chlorophyll degradation, protein estimation, and metal analysis	Bajpai *et al.* 2010a, b
			Bachhrawan, Raebareli	Pigment analysis, chlorophyll degradation, chlorophyll fluorescence, protein estimation, arsenic estimation, antioxidative enzyme analysis	Bajpai *et al.* 2012, 2015
			Sitapur district	Physiological analysis, chlorophyll degradation, chlorophyll fluorescence, metal analysis	Karakoti *et al.* 2014
			Faizabad city	Pigment analysis, chlorophyll degradation, protein estimation and metal analysis	Gupta *et al.* 2015
			Ambedkar Nagar, Tanda TPP	Metals analysis, SEM and FTIR	Gupta *et al.* 2017a
				Pigment analysis, chlorophyll degradation and protein estimation	Gupta *et al.* 2020
			Kanpur, Panki TPP	Pigment analysis, chlorophyll degradation, protein estimation and metal analysis	Gupta *et al.* 2017b
		West Bengal	Hooghly district	Pigment analysis, chlorophyll stability index, protein estimation and Metal analysis	Bajpai and Upreti 2012
			Kolkata city	Heavy metal analysis, SEM analysis	Nayaka *et al.* 2014, Banerjee *et al.* 2022
2	Philippines	Southern Luzon	Metro Manila	Heavy metal analysis	Pabroa *et al.* 2009
3	Thailand	Lampang province	Lampang city	Atranorin analysis and SEM	Kheawsalab *et al.* 2020

P. cocoes like other lichens are poikilohydric in nature and fulfill their water needs from atmospheric water vapour which harbours many pollutants. These pollutants are absorbed/adsorbed (intracellularly/extracellularly) and entrapped in intracellular spaces of lichen thallus thus uptake of metals increases with time. The secondary metabolites such as skyrine and zeorine help in accumulating metals in thalli of *P. cocoes* to a greater extent. It accumulates trace elements, heavy metals, metalloids, and radionuclides because its secondary metabolites contain a wide range of functional groups viz., hydroxyl and carboxyl (Gupta *et al.* 2017a), which capture metal in their thalli. Being spongy in

Table 2: Categorization of biomonitoring studies related to *P. cocoes*

Techniques	*Methods used*	*References*
Active monitoring (Transplantation of lichens)	Biochemical analysis	Thakur *et al.* 2022
	Enzymatic activity	Bajpai *et al.* 2015
	Heavy metal estimation	Rani *et al.* 2013, Nayaka *et al.* 2014, Thakur *et al*. 2022
	Molecular studies	Bajpai *et al.* 2015
	Pigment and protein estimation	Rani *et al.* 2013, Bajpai *et al.* 2015, Thakur *et al.* 2022
Passive Monitoring (Through native lichens)	Ambient air quality monitoring	Danesh *et al.* 2013
	Biochemical and stress hormones analysis	Bajpai *et al.* 2022
	Chemical analysis	Bajpai *et al.* 2022
	Fourier Transform Infrared Spectroscopy (FTIR)	Gupta *et al.* 2017a
	Heavy metals estimation	Nayaka *et al.* 2003, Saxena *et al.* 2007, Pabroa *et al.* 2009, Bajpai *et al.* 2010a, b, 2011, 2012, Bajpai and Upreti 2012, Mishra and Upreti 2014, Karakoti *et al.* 2014, Gupta *et al.* 2015, 2017a, b, Singh *et al.* 2019, Daimari *et al.* 2020; Bajpai *et al.* 2022, Banerjee *et al.* 2022
	High-Performance Liquid Chromatography (HPLC)	Kheawsalab *et al.* 2020
	Pigment and protein estimation	Rout *et al.* 2010, Bajpai *et al.* 2010a, 2012, Bajpai and Upreti 2012, Danesh *et al.* 2013, Karakoti *et al.* 2014, Gupta *et al.* 2015, 2017b, 2020, Bajpai *et al.* 2022
	Scanning Electron Microscopy (SEM)	Gupta *et al.* 2017a, Kheawsalab *et al.* 2020, Banerjee *et al.* 2022

nature, due to the greater part of thalli which is made up of fungal hyphae (~90%), metals are easily translocated and accumulated in both intracellular and extracellular spaces.

Secondary metabolites are invariably fungal in origin, formed from primary metabolites but have no direct metabolic involvement, and are collected on the surface of hyphae rather than within cells. As a result, substances are referred to as extracellular chemicals (Karakoti *et al.* 2014). The three principal pathways that produce secondary metabolites are the acetyl-polymalonyl pathway, the mevalonic acid pathway, and the shikimic acid pathway (Boustie and Grube 2007).

The lichen compounds with electrons lone pair and a large number of hydroxyl groups have an intrinsic chelating capability. Metal adsorption is influenced by the chemical composition of the lichen material as well as the electrons available in metal ions for binding (Hauck and Huneck 2007). Under *in vitro* conditions, the lichen compounds have been shown to act as cation chelators, including heavy metal chelators (Purvis *et al.* 1987).

The cations as well as heavy metals have been found to attach to extracellular sites of photobiont and mycobiont cell walls. The existence of negatively charged anionic sites in the cell wall provides sites for metal accumulation (Collins and Farrar 1978). Carboxyl, amine, phosphate, and hydroxyl groups all contribute to the anionic sites. The quick release of protons is invariably followed by metal accumulation (Nieboer *et al.* 1976).

The heavy metals are retained and accumulated in lichens in an excess amount beyond their physiological requirements and may withstand high metal concentrations by sequestering metals extracellularly as oxalate crystals that interact with lichen acids. The pollutants from the atmosphere are gathered on the surface of lichen or entrapped in the medulla's intercellular gaps while the majority of the metals that are deposited are stationary. As a result, only chelation and sequestration mechanisms are used to transport it (Bačkor and Loppi 2009).

P. cocoes as bioindicator and biomonitor

The primary information (diversity, quality, and quantity of contaminants existing in the atmosphere) about pollution variations at the local and regional levels data can be assessed by the usage of "biomonitors". The information might be extremely important as an early warning indicator for detecting environmental changes. *P. cocoes* is an effective pollutant monitor, indicator, and accumulator in tropical Asian countries (Savillo 2010). The lichens are one of the first species to be classified as a "bioindicator" for obtaining information about the health of an area. The information might be obtained by utilizing various methods which may be categorized into several groups (Table 2).

Methodology

A systematic review is defined as "a review of the evidence on a specified subject that employs systematic and explicit procedures to discover, select, and critically evaluate relevant primary research, and to extract and analyze data from the studies that are included in the review". Studies that are related to *P. cocoes* have been chosen for this investigation. According to the method and site type employed for biomonitoring, articles were evaluated based on their spatial and temporal distribution as well as their contextual concerns. The present study also examined the methods used for monitoring lichens and the parameters that were recorded. To narrow down any study gaps, spatial and temporal distribution analysis is required to understand and assess where and when the impact of biomonitoring using lichens has been examined. An overview of the importance of understanding historical interpretations and studies of biomonitoring using lichen as the biological indicator is provided in terms of contextual and substantive concerns. Hence, the systematic review's final selection included 25 studies that were completely focused on biomonitoring and used lichen *P. cocoes* as the biological indicators.

Results and Discussion

Temporal and spatial distribution

It is clear from the Figure 5 (a) that 3 articles each have been published in the years 2010, 2014, 2020, and 2022 (12%) on physiological alterations, metal accumulation, and biomonitoring using *P. cocoes* as a bioindicator, whereas only in the years 2003, 2007, 2009, 2011, and 2019 single article each (4%) was published. Based on the number of articles published in several years, it can be stated that during 2003-09, studies related to *P. cocoes* had been initiated and the potential of lichen species was noticed by the researchers. Since the last two decades, more attention has been paid to the species, and various aspects together with special reference to biomonitoring studies were carried out in past years. It is demonstrated that lichen-based biomonitoring is still relevant and developing today even though this type of study has been carried out since the 19th century (Nylander 1866). According to Chuquimarca *et al.* 2019, there is still much to learn about lichens' enormous capacities and potential to indicate environmental quality, making lichen biomonitoring still relevant today.

It is evident from Figure 5 (b) and (c) that most of the pollution monitoring studies were conducted in Asia only. The distribution of *P. cocoes* in the world reveals the occurrence of this species on almost all continents, but so far studies related to *P. cocoes* have not been conducted on other continents. Hence, it is expected that more research work may be conducted in the future days as it has great potential to work as a biomonitor and bioindicator. Among the Asian countries 23 articles (92%) were published from India followed by a single publication (4%) each from Philippines and Thailand. Italy has strengthened its environmental policies by requiring mandatory biomonitoring as part of the environmental impact assessment (Cioffi 2009), so biomonitoring studies utilizing ubiquitous organisms such as lichens are more expected from there.

It is clear from the above observation that research on pollution monitoring with *P. cocoes* has been conducted widely in the countries like India, the Philippines, and Thailand. Thus, more studies are needed to increase the understanding of various geographic regions through various methods including biomonitoring.

Contextual issues

It is clear from the Figure 6 that the urban areas are more studied frequently with utilizing *P. cocoes* as 11 articles (44%) are available followed by power plants areas with 6 articles (24%), industrial areas with 2 articles (8%), and peri-urban, research stations, highways, hills, paper mill and valley areas are represented with single article each (4%) respectively. During the initiation of work, urban areas, industrial areas, power plant areas, highways, roadside areas, and many more have all been chosen as sites to be monitored using lichens. The urban area is the one that has had the most biomonitoring research done on it. Urban areas are frequently researched using lichen because of their propensity to gather air contaminants. The several sources that released the air pollutants were industries, traffic, and automobile exhaust. As a result, there are many different kinds of emissions in a concentrated area. In addition, 55% of the world's population resides in urban areas, making urban environmental quality an extremely sensitive issue that must

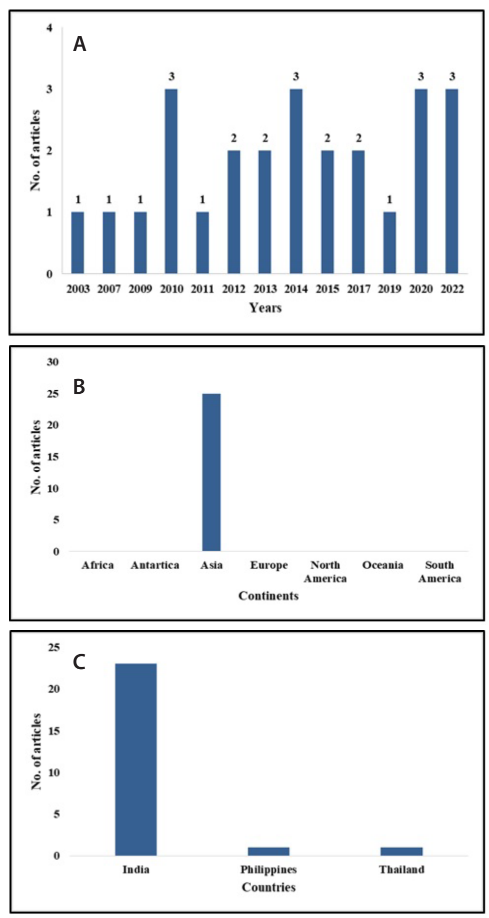

Figure 5: (a) Representation of the number of articles vs. years, (b) the number of articles vs. continents, (c) the number of articles vs. countries

be closely monitored (Owczarek *et al.* 2001; Carruthers and Ulfarsson 2003). Biomonitoring studies using *P. cocoes* have expanded from one research area to another type of area as the number of studies in other places such as hills, paper mills, and valley areas has significantly increased, indicating that these areas need to be monitored because of the air pollutant they emit.

In the past, most of biomonitoring studies limited to the source of pollution, point source, or other urban or industrial areas (Nayaka *et al.* 2003, Saxena *et al.* 2007, Pabroa *et al.* 2009, Bajpai *et al.* 2010, 2011), however, in recent years, studies employing biomonitoring techniques have been conducted in natural ecosystem type of areas, such as valley and hilly areas (Mishra and Upreti 2014, Daimari *et al.* 2020), Thus demonstrates that more regions can be studied in future using lichen biomonitoring techniques. Also, studies on different kinds of natural ecosystems such as mountain ranges, forest areas, and riverine areas are required because of the majority of pollutants released from urban or industrial areas will end up in natural ecosystems from where little information is collected merely scraping the surface. In other words, biomonitoring of natural regions will also reflect the extent to which anthropogenic activities or man-made areas may impact the diversity and distribution of an area.

Bioindicators offer a quick and cost-effective way to learn about the state of the air and the dispersion of air contaminants. Two biomonitoring techniques were used for assessing the environmental quality and alteration in the atmosphere.

Passive monitoring i.e. biomonitoring through native lichen flora approaches employed existing lichen species as the indicator species for more polluted areas, whereas active monitoring i.e. transplanted lichen is a monitoring technique that uses lichen species for pristine or remote regions to be the indication for another area. In this review, the majority of articles i.e. 84% used native lichens, while 16% of the articles employed transplanted lichen techniques as their method of assessing the environment (Figure 7). The most widely utilized bioindicators are epiphytic lichens, which are found on the branches and trunks of trees and are particularly sensitive to air pollution and environmental changes. *P. cocoes* is a potent lichen species known as epiphytic lichen that grows on trees, either on the leaf (foliicolous lichen) or the bark (corticolous lichen). When compared to other lichen types, epiphytic lichen is more diverse and is also more frequently observed growing on trees.

Almost all trees, whether those in a forest or that have been planted in urban, industrial, paper mill, or power plant regions, are susceptible to epiphytic lichen *P. cocoes* (Daimari *et al.* 2012, 2020, Gupta *et al.* 2017, 2020, Singh *et al.* 2019). Foliose lichen *P. cocoes* have been utilized frequently in the transplanted lichen approach because of its wide surface area, which boosts its capacity to absorb contaminants from the environment and makes it simpler to spot any morphological and physiological changes that occur in it. In addition, it is simple to transplant to other locations without harming their original substrate (Liu *et al.* 2016a, 2016b).

Among the various aspects of pollution monitoring studies, 80% articles were published on heavy metals analysis together with trace elements estimation followed by 52% articles on physiological changes (pigments) and protein estimation. 12% articles on Scanning Electron Microscopy (SEM) and 8% on biochemical analysis. Only 4% research on parameters, viz., ambient air quality monitoring, chemical analysis, enzymatic activity, Fourier Transform Infrared Spectroscopy (FTIR), High-Performance Liquid Chromatography (HPLC), molecular studies, and N & C elements estimation are available (Figure 8).

Substance and content analysis

P. cocoes was used as a biological indicator in 25 articles that discussed biomonitoring regarding substance and content analysis. Two primary categories of biomonitoring techniques (active and passive) have been divided in the present study (Table 2). Eight different types of parameters have been measured using the native lichen monitoring technique, whereas five different types of parameters have been measured using the transplanted lichen monitoring technique.

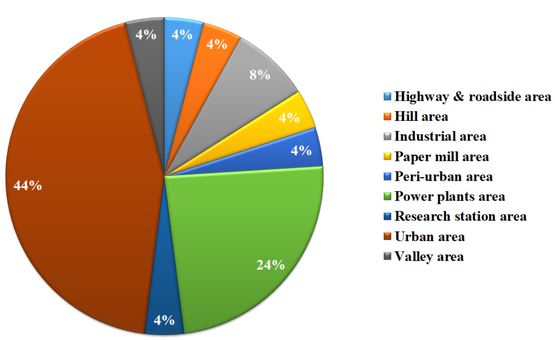

Figure 6: Representation of the article percentage vs. researched areas

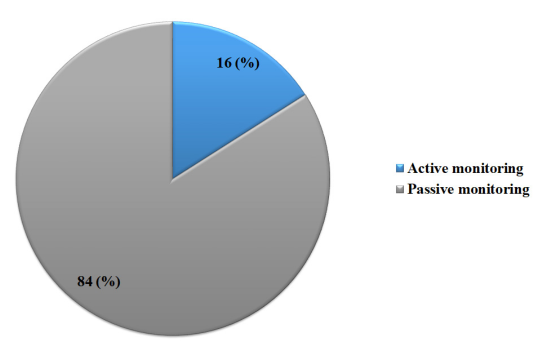

Figure 7: Representation of articles percentage vs. monitoring techniques utilized

Ambient air quality monitoring

Air quality is a significant problem, particularly in highly regulated industries, factories, and coal and oil-fired power generation plants. Normally, ambient air contains 21% oxygen and 78% nitrogen. A mixture of carbon, helium, methane, argon, and hydrogen makes up the additional 1%. A significant amount of industrial and chemical pollutants are released into the atmosphere during manufacturing processes and burning of fossil fuels, which has an immediate influence on ambient air quality. These processes release potentially harmful pollutants including particulate matter, sulfur dioxide, and oxides of nitrogen which cause respiratory diseases, lung cancer death, and chronic obstructive pulmonary disease (COPD) deaths. The ambient air quality monitoring was carried out by following standard methods of the National Ambient Air Quality Monitoring (CPCB, 2003-04) using APM-410 and APM-411 high-volume air samplers. Determination of suspended particulate matter (SPM), sulfur dioxide (SO_2), and oxides of nitrogen (NO_x) is done in the two large-scale industries (Visweswaraya Iron and Steel Plant and Paper Mill Ltd.) in addition to numerous small-scale industries and several vehicles in an industrial town, Bhadravathi, Karnataka, India. It was evident that the diversity of lichens was affected by industrial and vehicular air pollution. In the town center of Poland, Lisowska (2011) reported recolonization of the former 'lichen desert' where species richness of lichens at study sites has increased along with an improvement in the health of lichen thalli was noted and correlated with air quality improvement, mainly SO_2 decline in the last few decades and transport-related compounds, mainly NO_x and dust that have become the main pollutants in Poland.

Biochemical and stress hormones analysis

Lichens can flourish in a variety of conditions, including air that has been contaminated with dust, particulate matter, and various pollutants including metals. Lichens produce a wide range of secondary metabolites, which most likely aids in their ability to thrive in polluted areas. Phenolic and flavonoid compounds are one of the most potent classes of antioxidants among numerous biomolecules. They serve a crucial part in the metabolism that permits the organism to thrive in polluted air with heavy metals because they can form non-toxic chelates with metals, which is one of the mechanisms responsible for the ability to tolerate toxic metals in the air. Additionally, it has been proposed that phenolics may function as indicators of metal exposure (Márquez-GarcíaBelén *et al.* 2012). A significant relationship between the polyphenolic compounds and the polluted areas in the Mandi district, Himachal Pradesh was studied by Thakur *et al.* 2022. According to Babula *et al.* 2008, plants have developed severe tolerance mechanisms, such as increased protein or other stress metabolite production

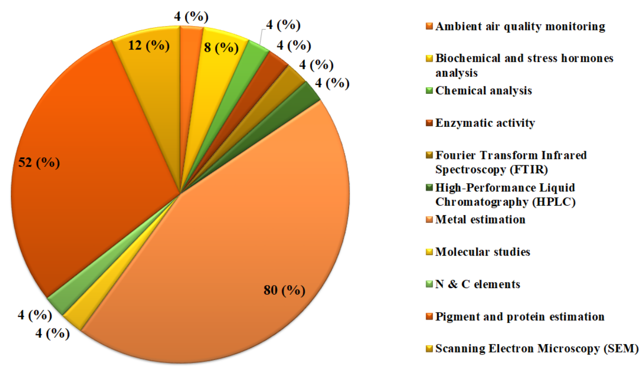

Figure 8: Representation of articles percentage vs. parameters measured

to withstand air pollution for their improvement in stress conditions. Similarly, lichens also utilize stress tolerance mechanism, which dynamically associates with the metallic contaminant, protecting the safeguards of thallus from the damaging effects of pollutants. The amino acid accumulation in lichens reduces stress and could be an indicator of sudden changes in air pollution (Bajpai *et al.* 2022). Lichens significantly accumulate proline and other amino acids under stress (Hayat *et al.* 2012). Indole-3-acetic acid (IAA), Abscisic acid (ABA), and ethylene are common phytohormones in lichen species. Studies on ethylene production overwhelmingly demonstrate that lichen species are not only capable of producing ethylene but also of converting 1-Aminocyclopropane-1-carboxylate (ACC) to ethylene (Ott *et al.* 2001). Bajpai *et al.* 2022 reported a decline in the level of the auxin IAA, no significant changes in ABA level, and upregulation of ethylene in pollution stress in thalli of lichen *P. cocoes*.

Chemical analysis

The electrical conductivity (EC) is an index of cell membrane integrity that shows the intracellular uptake susceptibility and membrane integrity in lichens. The epiphytic lichen *P. cocoes* is an effective metal-tolerant colonizer of highly disturbed and polluted sites. According to Osyczka and Rola 2019 and Paoli *et al.* 2011, intracellular heavy metal accumulation affects the level of cell membrane damage and ultimately increases electrical conductivity. Bajpai *et al.* 2022 utilized *P. cocoes* to assess the rigidity of cell plasma membrane and concluded that pollution stress damages the permeability of cell membrane in polluted regions.

Enzymatic activity

Metabolic activities such as respiration and photosynthesis result in the production of reactive oxygen species (ROS) (Kohen and Nyska 2002). During stress conditions such as limitation of nutrition and xenobiotics, and exposure cells evolved protection mechanisms including antioxidant enzymes (catalase, superoxidase dismutase (SOD), and peroxidase), thereby evading the potential damage effects of ROS (Weissan *et al.* 2005) and cause less damage to bioorganisms. Bajpai *et al.* 2015 analyzed the amino acid profile, catalase activity, superoxidase dismutase activity, and ascorbate peroxidase activity in lichen species *P. cocoes*. Among the various amino acid, proline showed a significant increase in concentration and correlated negatively with ascorbate peroxidase (APX) and catalase activity, whereas positively with superoxide dismutase (SOD) activity.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a crucial analytical technique used to obtain the infrared spectrum of a solid, liquid, or gas's absorption or emission and is concerned with the vibration of molecules. Functional groups have specific vibrations because they are composed of various atoms with various binding strengths. Each functional group has a distinct vibrational energy that can be used to identify a molecule by adding all functional groups together. It is an effective technique for assessing air quality using multiple components. The FTIR analysis carried out by Gupta *et al.* 2017a revealed the existence of several functional groups viz., amide, hydroxyl, carbonyl, carboxyl, amine, phosphodiesters, haloformyl, methoxy, octahedral (AlO_8) in *P. cocoes* and a primary amine, secondary amine hydroxyl, alkanes, carbonyl, phosphodiesters, polysaccharides, methoxy, octahedral (AlO_8) in *B. incongruens*. It was concluded that *P. cocoes* contained both primary and secondary metabolites which carried more carboxyl and hydroxyl groups while *B. incongruens* lack the secondary metabolites and had less number of bands than *P. cocoes*.

High-Performance Liquid Chromatography (HPLC)

The use of HPLC for analysis of environmental contamination levels has increased, as it provides detection and quantification of strongly polar or non-volatile chemicals in the air. Lichen secondary metabolites are organic compounds produced by mycobiont cells. Atranorin is a common secondary metabolite in depsides group of lichens has a wide range of ecological advantages, including photoprotective action (Lohézic-Le Dévéhat *et al.* 2013), antioxidant activity (Kosanicetal 2014, Rajan *et al.* 2016), and other advantages. Bialoska and Dayan 2005 found that lichens transplanted close to chemical industrial sites exhibit lowering of atranorin levels. Atranorin is susceptible to the accumulation of heavy metals and acidic inorganic sulfur compounds. Kheawsalab *et al.* 2020 studied the effect of the sulphuric acid solution on atranorin content by employing *P. cocoes*. The results revealed that the greatest average concentration of atranorin was obtained in a pH 3.0 treatment, while the lowest concentration was observed in a sample without any treatment.

Metal estimation

Metal and heavy metals are well-known environmental contaminants due to their toxicity, long environmental persistence, and bioaccumulation capacity. While trace metals typically exist in the environment at very low concentrations. Some elements are essential for living organisms, but they cause severe risks to the ecosystem and can be dangerous to a human being if present in an excess amount in the environment. By utilizing an active monitoring technique (transplantation of lichens), metals such as Fe, Hg, Cd, Mn, Cr, Ni, Cu, Zn, and Pb were analyzed in *P. cocoes* transplanted at 12 sites of Dehradun, Uttarakhand, India (Rani *et al.* 2013) and at ten sites in Kolkata, West Bengal, India (Nayaka *et al.* 2014). Meanwhile, As, Cd, Pb, Cu, and Ni were analyzed in *P. cocoes* from urban areas in Himachal Pradesh (Thakur *et al.* 2022). Several studies on passive monitoring techniques conducted by Nayaka *et al.* 2003, Saxena *et al.* 2007, Pabroa *et al.* 2009, Bajpai *et al.* 2010a, b, 2011, 2012, Bajpai and Upreti 2012, Mishra and Upreti 2014, Karakoti *et al.* 2014, Gupta *et al.* 2015, 2017a, b, Singh *et al.* 2019, Daimari *et al.* 2020; Banerjee *et al.* 2022; Bajpai *et al.* 2022 on *P. cocoes* from urban, coal-fired power plants, hilly, industrial and valley areas are available. Distribution of various elements viz., Al, As, Br, Ca, Cd, Co, Cr, Cu, F, Fe, Hg, K, Mg, Na, Ni, Pb, S, and Zn were analyzed in the environment to monitor the environmental changes. The sources of these elements are generally vehicular emissions, construction, industries, factories, and coal-based power plants.

Molecular studies

The most widely used methods for molecular systematics are Inter Simple Sequence Repeat (ISSR)-PCR and Internal Transcribed Spacer (ITS). A method known as inter simple sequence repeat (ISSR)-PCR uses microsatellite sequences as primers in a polymerase chain reaction to produce multi-locus markers, whereas the ITS region has been suggested as the standard fungal barcode sequence since it is the DNA region of fungi that has been most thoroughly sequenced in terms of molecular ecology. Bajpai *et al.* 2015 analyzed genotoxicity generated by chromium-treated *P. cocoes* collected from Bachhrawan, Raebareily district, Uttar Pradesh, India, and found no significant relation in ITS data.

Nitrogen (N) & Carbon (C) elements

Numerous substances, including ammonium nitrate (NH_3), nitrogen dioxide (NO_2), and carbon monoxide (CO) are composed of N & C elements. Carbon (C) and nitrogen (N), among other environmental elements, are essential for plant growth and their fundamental functions (Zheng 2009). Recent studies have demonstrated that lichen can assess N and C elements using monitoring methods. A study by Rani *et al.* 2013 utilized the lichen species *P. cocoes* to assess the level of C & N and concluded that air pollution causes a reduction in lichens photosynthetic and respiration rates.

Pigment and protein estimation

To monitor the lichen physiological changes caused by air pollution, the lichen anatomy including its membrane, chlorophyll, and protein content was observed and examined by many of the researchers in the review. Active monitoring by using the lichen transplant method was conducted by several researchers (Rani *et al.* 2013, Bajpai *et al.* 2015, Thakur *et al.* 2022) in India, where they concluded that epiphytic lichen species *P. cocoes* is a pollution-tolerant species. In metropolitan areas of India, this study revealed alterations in the protein and chlorophyll contents after the transplantation of thalli of lichens and their exposure to metallic pollution. Numerous studies using native lichen flora were conducted by several researchers (Rout *et al.* 2010, Bajpai *et al.* 2010a, 2012, 2022, Bajpai and Upreti 2012, Danesh *et al.* 2013, Karakoti *et al.* 2014, Gupta *et al.* 2015, 2017b, 2020) in India. These studies mainly include urban, industrial, traffic, and coal-fired power plants region, where pigment and protein contents were directly proportional to the pollution load in an area. Bajpai *et al.* 2022 also utilized the chlorophyll stability index (CSI) in studying the effect of pollution stress on the physiology of *P. cocoes* and demonstrated that the CSI score is a straightforward, simple, and accurate way to recognize lichen under stress brought on by sudden environmental changes. It was also studied that sudden fluctuations in the level of pollution change the chlorophyll pigment regulation fluorescence in *P. cocoes*. Thus, it is very important to know about and comprehend physiological changes that occur in lichen to better understand how air pollution could harm human physiological processes of the human being (Paoli *et al.* 2019b).

Scanning Electron Microscopy (SEM)

SEM has been widely used mainly for the determination of the possible existence, nature, and extent of pollution in a given area. Measurement and observation of the morphology of the solid particles present in the air can also be described by the instruments. Gupta *et al.* 2017, Khewasalab *et al.* 2020 and Banerjee *et al.* 2022 have widely used this technique to visualize the image of the surface of *P. cocoes*.

Conclusion

It is clear from the review that *P. cocoes* has gained widespread utilization as a biological indicator and monitor. The review also revealed the peculiarities in the biomonitoring studies using lichen, as well as several topic areas that might be further investigated. The number of studies on biomonitoring using lichen has increased and most of the studies were focused on areas such as urban, industrial, and thermal power plants. Two biomonitoring techniques were used by the researchers employing native lichen and transplanted lichen. The review also confirms that there are numerous limitations and lacunas in the biomonitoring study using lichen. The information about biomonitoring studies using *P. cocoes* from other countries is still lacking. To identify environmental changes in an area, details on a variety of locations including forests, remote zones, and mountain ranges are also required. It is also suggested to explore some areas like air-borne pollutants-related comprehensive study, molecular studies, spectral signatures, and other applied aspects related to its symbionts. Although the research of biomonitoring has been going on for decades, there is still a need to define a paradigm or framework for employing lichen as a biological indicator for air pollution.

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