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Heavy Metals Uptake in Lichens: A Comprehensive Review

Mamta Bhat[1*], Priya Darshni[2] and Anamika Srivastava[3]

[1]*Department of Environmental Sciences, Centre for Biodiversity Studies, School of Biosciences and Biotechnology, BGSB University, Jammu and Kashmir, India*

[2]*Department of Environmental Sciences, Govt. College for Women, Parade Ground, Jammu and Kashmir, India*

[3]*Department of Environmental Sciences, Amity Institute of Environmental Sciences, Amity University, Noida, India*

Abstract

Heavy metals are regarded as one of the most significant environmental contaminants. They can easily get entered in food chain and eventually exert toxic effects through contaminated soils, crops and water, thereby becoming a serious global issue of concern today. There are numerous ways to remove them from the environment, but majority of which are expensive and difficult to employ, effectively. In order to eliminate inert metals and metal contaminants from contaminated air, soil and water, bioremediation is regarded as the most efficient and cost-effective technical approach available. However, the potential of lichens for bioremediation of heavy metals has also been recognized lately as they can accumulate heavy metals by physico-chemical and biological mechanisms including extracellular binding. This is in consequence of a series of morphological and physiological properties exhibited by their thallus such as lack of any protective, conductive and assimilatory tissues such as epidermis, xylem, phloem and roots. The present review is therefore, attempted to discuss a wide range of investigations carried out on uptake of heavy metals by lichens and their effects as well as evaluation of their potential in future bioremediation studies.

Keywords: Heavy metals • Lichens • Indicators • Ecosystem

Introduction

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration [1]. As earth's crust is the storehouse of heavy metals, the heavy metals get leached into atmosphere and water bodies from it [2]. Some heavy metals are required for optimum functioning of organisms. These heavy metals in excess or in deficient amounts can have detrimental effects on living organisms [2,3]. Some other heavy metals like Hg, Pb, Al and Cd are harmful even at very small concentrations [3]. However, heavy metals have been in use since thousands of years [4]. Humans started using metals in everyday life around 8,000 B.C. Copper was the earliest metal known to man, the discovery of which enabled many civilizations to prosper. This was followed by Lead, Zinc and Mercury. In spite of all their uses, increased use of heavy metals due to urbanization and industrialization has altered the distribution and geochemical cycles of heavy metals [5]. As a result of this, the concentration of heavy metals has increased considerably in areas where they were earlier present in smaller concentrations [2].

Heavy metals are particularly significant as pollutants [6]. Once entered into the environment, they are difficult to remove. Moreover, they have a tendency to accumulate in plants and other organisms through food chains [7].

As environmental pollution is one of the major challenges in the modern human society [8], the environmental contamination and pollution by heavy metals is a serious threat to the environment which requires attention [9,10]. Chronic exposure to heavy metals in the environment is a real threat to living organisms [11]. Therefore, the increasing concentration of heavy metals and its persistence in atmosphere calls for a continuous monitoring of their concentrations in the environment as well as study of their effects on varied ecosystems [12].

***Address for Correspondence:** Mamta Bhat, Department of Environmental Sciences, Centre for Biodiversity Studies, School of Biosciences and Biotechnology, BGSB University, Rajour, Jammu and Kashmir, Tel: 9419170597; E-mail: mamtabhat12oct@gmail.com*

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Materials and Methods

Lichens

Lichens have been described by the international association of lichenology as the association of a fungus and an alga which results in a stable thallus with specific structure and hence form a symbiotic association that represents different kingdoms (fungi and green algae, fungi and cyanobacteria or all three) and yet they behave as a composite organism [13]. Lichens are among the most significant indicators of air pollution and ecosystem health [14].

Lichens lack a vascular system and are simple both morphologically and anatomically. They grow in a variety of habitats such as soil, rocks, trunks and branches of trees and even fallen log and obtain nutrients directly from substances dissolved in ambient moisture. Some substances are probably absorbed directly from the substrate by diffusion through the lichen thallus. The various environmental factors affect the absorption and uptake of heavy metal in lichens (Figure 1).

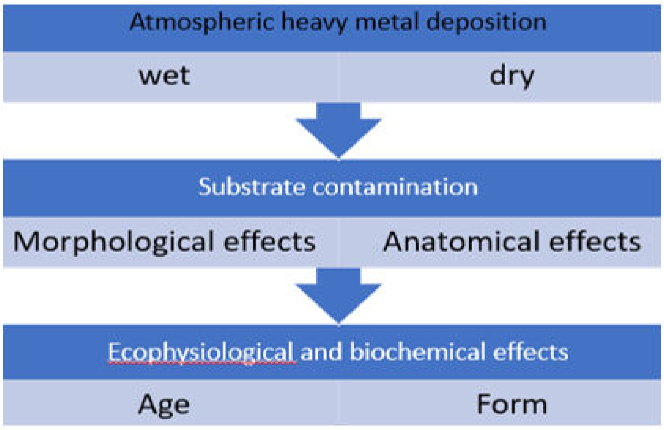

Figure 1. Factors influencing heavy metal content in lichens.

Lichens can therefore be used as reliable indicators of air pollution [15]. They either independently or together with bryophytes can be valuable organisms in developing an Index of Atmospheric Purity (IAP) which is based on the number, frequency-coverage and resistance factor of species. This index can provide a fair picture of the long-range effects of pollution in a given area [16]. The various growth forms of lichens are good indicators of the varying degree of pollution and are also used to reveal the nature of pollutants as well. Besides, they have the capacity to absorb and retain pollutants in quantities much higher than those absorbed by other plant groups growing in the same habitat. Therefore, they trap and prevent recycling of such pollutants in the ecosystem for different periods of time. Therefore, the analysis of such lichens gives a fair idea about the degree of heavy metal pollution in an area.

Lichens as biomonitors of heavy metals

Conventional methods of monitoring heavy metal pollution in environment such as electronic monitoring system are highly expensive and time consuming because of involvement of long term sampling at quite a large number of sampling sites [1]. Also, this approach does not give any correct estimation about the amount of metals being accumulated by vegetation in study sites neither their effect on biological systems. Therefore, the use of biological systems also called bioindicators or biomonitors becomes imperative [17]. The most important advantage of using a biological indicator for biomonitoring studies over the traditional methods of survey is that it can indicate vitality level of living things either as a community or as individuals [18-19]. Only empirical values are shown by traditional methods. Another important fact is that bioindicators can detect pollutants over a very wide range which can be helpful to give an estimation of past exposure and also assess susceptibility at individual level [20]. Therefore, lichens can be considered as efficient biomonitors because of their peculiarities.

Worldwide lichens are being used as pollution indicators. They are one of the most valuable biomonitor of atmospheric pollution and used as sensitive indicators to estimate the biological effects of pollutants by measuring changes at community or population level thereby acting as accumulative monitors of persistent pollutants.

A good biological indicator for monitoring should be a sensitive and an accumulative bio monitor. Sensitive bio monitors are the visual type and used as an indicator of stress caused by contaminants and act as early alarm system.

On the other hand, accumulative bio monitors have the ability to store contaminants in their tissues and are used for the measurement of the concentration of such contaminants present in the environment. Some biological indicators act as either sensitive or accumulative bio monitors but lichens can act as both.

As lichens are ubiquitous, so they occur on a variety of habitats, besides they lack defensive tissues, so they can easily absorb water, nutrients and gases straight from the environment. Owing to these physiological peculiarities, lichens are vulnerable to several anthropogenic threats, such as air emissions, climate change and deforestation.

Lichens have no root system or waxy cuticle and rely on atmospheric water and nutrients for growth and survival. These characteristics enable them to serve as great bio accumulators of atmospheric deposition. In addition, lichens can significantly absorb contaminants are absorbed over the whole thallus surface.

Many studies have been conducted to study the effectiveness of lichens in intercepting particles from atmosphere as well as the substrate on which they grow. These particles can then be deposited on the surface of the lichens or get trapped within the intercellular spaces of the medulla. Here they can remain unchanged for a long duration of time and can accumulate and retain many heavy metals in amounts greatly exceeding their physiological requirements.

Due to all these traits, biomonitoring of heavy metals using lichens is becoming popular world-wide. A number of studies are being conducted to study accumulation of heavy metals by lichens. Two main techniques are used to estimate the potential of lichens as accumulators of heavy metals.

- Lichen zone mapping: In this method, the luxuriant growth and abundance of lichens is used as a measure of air pollution by identifying and mapping all lichens present in an area.
- Lichen transplant technique: In this method, lichens from relatively unpolluted areas are transplanted in polluted areas and the content of heavy metals accumulated by them is estimated.

In the recent past, heavy metal accumulation by lichens has been widely studied mostly because of its relevance to biomonitoring. It is a complex process because heavy metal uptake by lichens is influenced by a number of factors. Some lichen species have the ability to accumulate conspicuous amounts of heavy metals. Acarospora rugulosa Korb. can accumulate up to 16% of Cu on a dry mass basis.

Lichens accumulate heavy metals through very dynamic processes. Some investigations have shown that if lichens are soaked in metal solutions, they accumulate metals quickly, sometimes within a few hours. The thalli of Lecidea lactea and Acarospora rugulosa growing in the region Central Scandinavia on cupriferous rock, the concentration of Cu was reported as 5% (d.m).

Copper showed maximum accumulation after 3-6 hours in active biomonitoring studies using transplantation technique where it was observed that lichens take a few months to respond to changes in atmospheric concentrations of heavy metals. Many elements in lichen thalli have a residence time of 2-5 years.

Effect of heavy metals on lichens

Changes in main anatomical features of lichens such as membranes and chlorophyll content can be an indication of heavy metal pollution. A number of such studies have been conducted in urban and industrial areas over the past decade.

In *Phaeophyscia hispidula* the chlorophyll and protein content changed after it is exposed to metallic pollutants for a period of one month. Majority of the studies carried out to study this aspect have been done under laboratory conditions and there is little information about how lichens respond to heavy metal induced stress in their actual habitat.

A variety of injury symptoms like changes in permeability of cell membrane, ultrastructure disorders and chlorophyll degradation can occur due to excess amounts of heavy metals in the environment. Exposure of lichens to conditions of heavy metal stress also causes reduction in ergosterol content which is an indicator of viability of mycobiont partner of lichen.

The trace elements that are toxic in nature induce oxidative stress that results from production of reactive oxygen species. This causes damage to various biomolecules that are of vital importance like lipids, proteins and nucleic acids.

Accumulation of heavy metals in lichens is associated with extracellular binding, intracellular accumulation and intercellular entrapment of solid particulates within the thallus. The elements getting accumulated intracellularly in excess have greatest effect on lichen metabolism.

Other effects of heavy metal stress in lichens include decrease in chlorophyll integrity, less photosynthetic efficiency and changes in the production of assimilation pigments.

An increase in lipid peroxidation, reduced glutathione and ergosterol content and diminished activity of dehydrogenase enzyme in lichens growing under conditions of heavy metal stress was observed. However, it can be emphasized here that majority of such studies were carried out under controlled laboratory environment and there have been few investigations on actual physiological response of lichens under in natural circumstances.

Among the two fungal partners, mycobiont absorbs significantly more metal than phycobiont. The algal cells are largely protected from the direct harmful effects of toxic trace elements by the mycobiont.

Although it would be normally expected that heavy metal content of lichens will increase with passage of time, the situation is actually much more complicated. As a matter of fact, it has been seen that during the study period, the contents of many elements in transplanted lichens actually rise and fall. One possible reason for this could be that physiological processes and turnover mechanisms control the content of elements under discussion.

The effect of rainfall on total metal content in lichen thallus is also very huge. According to Brown and Brown, rainfall by dislodging contaminant particles on the surface of thallus can remove metals also. However, instead of washing, rainfall also contributes significantly to total element content of lichens.

In active biomonitoring method involving transplantation of lichens from relatively unpolluted to polluted areas, the time of exposure can affect vitality of thalli. As a result, the process of active uptake of elements is also affected. An exposure period of 1-3 months is adequate; however, the critical exposure period is unknown.

Results and Discussion

Heavy metal uptake by lichens

Lichens have been known to accumulate Lead (Pb), Mercury (Hg), Copper (Cu), Chromium (Cr), Zinc (Zn), Arsenic (As), Cadmium (Cd), Copper (Zn), Nickel (Ni) and Iron (Fe) among their thalli.

Lichens accumulate heavy metals to a very high degree, with concentrations reflecting environmental levels. Studies conducted in various parts of world reveal that lichens can be used to monitor metal deposition both as active and passive monitors.

Hypogymnia physodes it a very good bioindicator used in the study of bioaccumulation of trace elements due to its high tolerance capacity. *Pyxine cocoes*, a foliose lichen is described it as an excellent accumulator of many heavy metals.

Arthopyrenia nidulans and foliose lichen *Phaeophyscia orbicularis* have a high capacity to accumulate heavy metals.

A high amount of mercury in lichens growing near a thermometer factory in Kodaikanal was observed. Besides, higher concentration of Arsenic was found in lichens growing on site where mining activities were performed.

In Mandav city in Central India it was reported that even though most of the metals were absent or present only in very small concentrations in soil, yet the thallus of lichen showed a high concentration of metals such as Cd, Cr, Ni and Zn among them.

Lead

Lead (Pb) is listed as a major toxic metal. Leaded petrol and diesel contain high level of lead while the unleaded petrol emission contains lead in a lesser level. The anthropogenic origins of lead are mainly attributed to traffic, vehicular emissions, brake and tyre wear and street industrial activities.

A study showed an accumulation of 34.7 μg/gm of Pb in *Hypogymnia physodes* from urban areas of Czech Republic.

A study concluded that Pb shows a high level of concentration in lichen thallus in areas associated with vehicular emission as well as coal burning. They detected Pb in the range of 5.86 ± 0.20 μg/g in the thallus of *Pyxine cocoes*.

Similarly, a study conducted revealed that a concentration of lead in the range of 9.750 ± 0.128 μg/gm is found in thallus of lichen *Pseudovernia furfuracea* growing around an iron and steel factory in Karabuk, Turkey.

Results of a study conducted showed a high concentration of Pb (102.5 μg/g) in lichen *Flavopunctelia flaventior* in the centre of the city.

Mercury

Mercury is a toxic heavy metal which is widely dispersed in nature. Mercury is a heavy metal of familiar toxicity, well known for giving rise to public health calamity in Minimata Bay, Japan and in Iraq. WHO has included Hg in the list of top ten chemicals of major public health concern.

Lichens have been found to be excellent accumulators of Hg which is released into atmosphere through various sources. In the study conducted by Bhat MA mercury levels were found to be between 7.4-20.1 ug/gm dry weight in thallus of *Hyperphyscia adglutinata*. The highest concentration of mercury was found in Muradpur area which in this study was attributed to presence of service stations, combustion of waste, alkalies and metals released in the area during repairing of vehicles.

Results of a study showed Hg contents ranging from 0.72 to 2.73 μg/gm dry weight with a median value of 1.59 μg/gm dry weight. Their study showed a gradual increase in mercury content in the direction from coast to interior.

A study concluded that marine aerosol was the main contributor of mercury contamination in lichens along with volcanic emissions.

A study conducted by using lichen *Dirinaria aplanta* showed an accumulation value of 14.08 ± 1.54 μg/gm in the thallus. High mercury concentration of mercury could be due to presence of more than fifty industries, emitting various types of contaminants into the atmosphere.

Zinc

Compared to several other metal ions with similar chemical properties, zinc is relatively harmless.

Nevertheless, zinc is considered to be relatively non-toxic to humans. Anthropogenic actions including municipal waste water releases, coal burning power plants, industrial methods involving metals and atmospheric outcome are the main sources of zinc contamination.

Sites located near zinc smelters which emit large amounts of Zn containing particles showed continual entrapment of Zn-rich particles by fungal hyphae during lichen growth. In the thalli of *Cladonia cariosa*, a concentration of 960.4 ± 432.3 μg/g of Zn was found.

A study conducted by Kar showed accumulation of Zn in the range 54.6 ± 3.85 μg/g in the lichen *Parmelia caperata*. They suggested that level of Zn in atmosphere was higher in high urban traffic region with possible source being abrasion of motor vehicle tyres.

Rhzaoui selected *Evernia prunastri* for studying the bioaccumulation of heavy metals. In their study, the highest conc. of Zn (1366 μg/g) was found in an open forest area with high pollution level.

A study was conducted to show accumulation of heavy metals in lichens colonizing artificial post smelting wastes. They concluded that conc. of heavy metals in many crustose lichen samples growing in post smelting dumps were several times greater than in their host substrate.

Arsenic

Arsenic is one of the toxic compounds which poses a high risk to large human populations. Although it had been historically used as a drug to treat infections and beautification, it was also used for human murder.

In a study conducted by Osyczka and Rola, a high concentration of 12478 ± 3306 μg/g of As was found in thallus of lichen *Cladonia* spp.

Result of a study carried on by Zvernia et al., showed conc. of As in lichen *Usnea antartica* in the range 0.9-2.3 μg/gm dry weight. They also reported that contents of Arsenic in the lichen thalli decrease with increase in distance from ocean. This indicated sea spray as a potential source these metals.

Elevated levels of Arsenic in samples collected from contaminated regions were reported. A conc. of 6.4 μg/gm was found in thallus of lichen *Pseudovernia* spp.

Similarly results of a study showed As conc. in the thallus of lichen *Pyxine cocoes* in the range 77.3 ± 2.0 µg/gm. They also found that As conc. in lichen thallus exhibited an increasing trend with decreasing distance from power plant. This may be due to the fact that As is found in air in particulate form. Also the dust in the periphery of industrial area has been found to contain huge amounts of As.

Cadmium

Cadmium is a heavy metal of considerable environmental and occupational concern. It is frequently used in various industrial activities. The major industrial applications of cadmium include the production of alloys, pigments and batteries.

In a study described conc. of Cd in thallus of lichen *Cladonia cariosa* as significantly higher in town area (20.8 ± 9.7 µg/gm) as compared to control site (0.8 ± 0.2 µg/gm). The results of the experiment conducted showed that concentration levels of heavy metals in many crustose lichen samples were several times greater than in their host substrate. They found a concentration of 156 µg/gm of Cd in thallus of lichen *Lecidea fuscoatra*. The quantity of Cd bioaccumulated in lichen *Parmelia* spp. in areas of intense traffic rises by 5.7 times *vs.* the quantity found in the lichens from city park. In the central area, the quantity of Cd bioaccumulated in the lichens was as low as 0.4 µg/gm whereas in the area of intense traffic, it rose to 2.3 mg/kg.

A study conducted by Mishra and Upreti, described Cd accumulation by thalli of lichen *Pyxine cocoes* upto 3.36 µg/gm dry weight. They also reported that Cd showed a clear trend of increasing concentration in areas associated vehicular emission as well as coal burning.

Copper

Copper (Cu) is an essential element for humans and plants when present in lesser amount, while in excessive amounts it exerts detrimental effects. The key anthropogenic sources of Cu include mining, refinery, fossil fuel combustion, waste incineration, traffic, fertilizers, soil amendments etc.

An extensive survey conducted revealed a concentration of Cu 24.39 ± 1.85 µg/gm dry weight in the thallus of *Phaeophyscia hispidula*.

The results of a study showed Cu accumulated in the thallus of *Phaeophyscia orbicularis* in the range of 28.7-24.2 µg/gm dry weight from year 1999-2016.

Similarly, a study revealed Cu concentration 39.54 5.6 µg/gm at the dumping site in the thallus of lichen *Physcia dilatate*.

A similar study revealed that Cu concentration ranged from 10.19-223.91 µg/gm in lichen *Placidium lacinulatum*. The high concentration was attributed to high precipitation of pollutants due to petroleum and gypsum activities in these areas.

Chromium

Chromium is a naturally occurring heavy metal in industrial processes. This heavy metal finds wide use in plating and alloying industry. It is also used in tanning of animal hides, textiles, dyes, alloying etc. Due to wide anthropogenic uses of Cr, the consequent environmental contamination has increased and has become an increasing concern in the last few.

A study reported Cr content in the range of 5.75-20.07 µg/gm in lichen *Cladonia rangifera* L. The greatest accumulation was observed on the sections nearest to highways.

Similarly, in a study by Hosairin and Siregar, the maximum concentration of Cr was found in the thallus of crustose lichen *Ochrolechia tartarea* the value of which was 107.6 µg/gm.

Iron

It is one of the most abundant metals. It is an essential micronutrient that is problematic for biological systems since it is toxic as it generates free radicals by interconverting between ferrous and ferric forms. The major anthropogenic sources of Iron could be indicated as industrial activities like refining works and heavy traffic activity.

A study revealed iron concentration in the thalli of *Hypogymnia physodes* in the range of 180-1135 µg/gm dry weight. They attributed the higher values of iron concentration to such geological features as carrstones and iron-manganese nodules.

The thallus of lichen *Usnea misaminensis* accumulated Fe in the range 84.43 ± 0.04 µg/gm as shown in a study. The high concentration of Fe in their study was attributed to industrial and traffic activities.

Results of a study showed a high concentration of Fe in the thallus of *Lepraria lobificans Nyl*. in the range of 3196 ± 2.40 µg/gm dry weight. The high concentration of Fe at this site was attributed to vehicular activity, coal burning, paint and steel work. A study revealed an iron concentration of 3.0 µg/gm accumulated by lichen *Buellia disciformis*. They propounded that heavy metals accumulated are emitted into atmosphere by the wearing of engines and tyres of vehicles in traffic and may also be present in fuel composition or exhausted by vehicles.

Manganese

Concentration of heavy metal Manganese has been studied in different lichens. In the year 2002, Pandey et al., studied Mn accumulation in *Phaeophyscia hispidula* (1021 micor g/g dry weight), *Dirinaria consimilis* (271 micro g/g dry weight) and *Dirinaria applanate* (366 micro g/g dry weight). Besides, Mn accumulation in

Hypogymnia physodes and *Lenanora conizaeoides*. However, Mn accumulation in *Trebouxia jasmesii*. *Lobaria pulmonaria, Lecanora saturninum* was also reported. Mn accumulation in *Usnea antarctica*, *Collema subnigrescens* (186.30 ± 0.68b µg/g), *Physcia adcends* (71.58 ± 0.32d µg/g), *Xanthoparmelia conspersa* (70.66 ± 0.17d µg/g), *Ramalina farinacea* (33.36 ± 0.14c µg/g), *Physcia aipolia* (143.10 ± 0.14c µg/g), *Xanthoria parietina* (195.10 ± 1.20 µg/g) was also reported.

Cobalt

Co accumulation in *Umbilicaria muhlenbergii* (17.8 ± 1.0 micro mole/g lichen), *Collema subnigrescens* (4.27 ± 0.03b µg/g), *Physcia adcendes* (0.71 ± 0.04d µg/g), *Xanthoparmelia conspersa* (1,36 ± 0.01c µg/g), *Physcia aipolia* (1.52±0.02c µg/g), Xanthoria parietina (0.22 ± 0.04d µg/g), *Flavoparmelia caperata* (0.31 ± .01d µg/g), *Leptogium gelatinosum* (5.11 ± 0.16b µg/g) was reported.

Caesium

Cs accumulation in *Hypogymnia physodes* (180±40 Bq/kg dry mass), *Parmelia sulcate* (500-6100 Bq kg dry weight), *Xanthoria parietina* (500-6100 Bq kg dry weight, and *Lecanora conizaeoides* (500-6100 Bq kg dry weight) (Table 1).

Table 1. Accumulation of various heavy metals by lichens and their concentration.

Heavy metal	Lichen Spp.	Concentration
Cu	*Phaeophyscia hispidula*	24.39 ± 1.85 µg/gm
	Phaeophyscia orbicularis	28.7-24.2 µg/gm
	Physcia dilatate	39.54 ± 5.6 µg/gm
	Placidium lacinulatum	10.19-223.91 µg/gm
Cr	*Cladonia rangiferina*	5.75-20.07 µg/gm
	Cetrariella delisei	4.9-16.4 µg/gm
Pb	*Hypogymnia physodes*	34.7 µg/gm
	Pyxine cocoes	5.86 ± 0.20 µg/gm
	Pseudevernia furfuracea	9.750 ± 0.128 µg/gm
	Flavopunctelia flaventior	102.5 µg/gm
Hg	*Hyperphyscia adglutinata*	7.4-20.1 µg/gm
	Usnea antartica	0.72-2.73 µg/gm
	Dirinaria applanta	14.08 ± 1.54 µg/gm
Zn	*Cladonia cariosa*	960.4 ± 432.3 µg/gm
	Parmelia caperata	54.6 ± 3.85 µg/gm
	Evernia prunestri	1366 µg/gm
	Candelariella aurella	2149 µg/gm
As	*Cladonia* spp.	12478 ± 3306 µg/gm
	Usnea antartica	0.9-2.3 µg/gm
	Pseudovernia spp.	6.4 µg/gm
	Pyxine cocoes	77.3 ± 2.0 µg/gm
Cd	*Cladonia cariosa*	20.8 ± 9.7 µg/gm
	Lecidea fuscoatra	156 µg/gm
	Parmelia spp.	2.3 µg/gm
	Pyxine cocoes	3.36 µg/gm
Cu	*Phaeophyscia hispidula*	24.39 ± 1.85 µg/gm
	Phaeophyscia orbicularis	28.7-24.2 µg/gm
	Physcia dilitata	39.54 ± 5.6 µg/gm
	Placidium lacinulatum	10.19-223.19 µg/gm

Cr	*Cladonia rangiferina*	5.75-20.07 µg/gm
	Cetrariella delisei	4.9-16.4 µg/gm
	Ochrolechia tartarea	107.6 µg/gm
Fe	*Hypogymnia physodes*	180-1135 µg/gm
	Usnea misaminensis	84.43 ± 0.04 µg/gm
	Lepraria lobificans	3196 ± 2.40 µg/gm
	Buellia disciformis	3.0 µg/gm

Conclusion

The accumulation and retention of pollutants by lichens has helped in the interpretation of heavy metal absorption pattern in them. Still, there is a dire need to extend observations on mineral location and effect to a much wider range of species. However, the role of morphological features in trapping particulate material is an unstudied but vital research field which needs further investigation to understand better the relationship between lichens and heavy metals. This can open new avenues in the development of new innovations or technologies in the field of air pollution monitoring and management.

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Competing Interests

The authors declare they have no conflicts of interest or competing interests.

Authors' Contributions

All the authors contributed equally towards conceptualization, data collection and compilation of manuscript.

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Ethical Considerations

"Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors."

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