

**Original Research**

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**Assessment of lichens as bioindicator of ambient air heavy metal pollution in far-west Nepal**

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**Abstract**

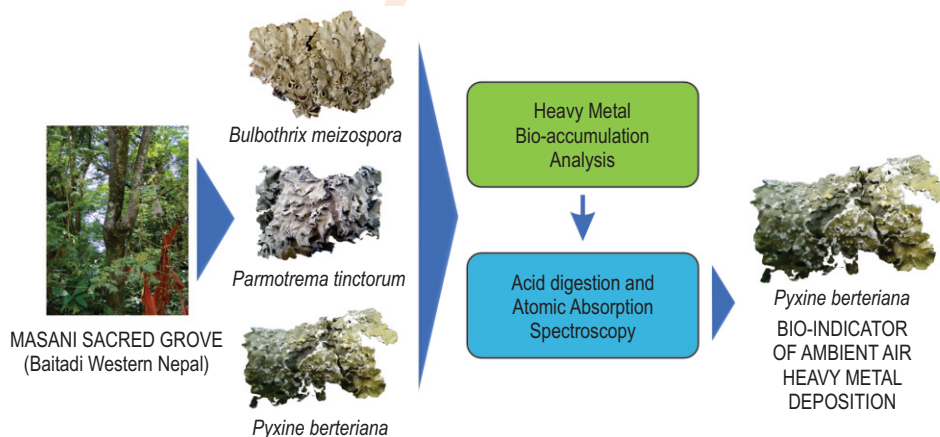
**Aim:** The aim of this research was to identify a suitable lichen species as a bio-indicator for heavy metals in the Himalayan habitats.

**Methodology:** The study was conducted in a sacred grove located in Western Nepal. The grove was situated alongside the busy Mahakali National highway. Lichen samples were collected from different areas of the grove, ranging from the edge adjacent to the highway to the center. Standard protocols were employed to identify the collected lichen species. The heavy metal content in the samples were analyzed by subjecting them to acid digestion followed by AAS estimation.

**Results:** Three lichen species were discovered: *Bulbothrix meizospora*, *Parmotrema tinctorum*, and *Pyxine berteriana*. *Pyxine berteriana* was collected near the highway, while *Bulbothrix meizospora* and *Parmotrema tinctorum* were collected away from it. The heavy metal bioaccumulation pattern observed was as follows: Fe>Al>Mg>Zn>Cu>Pb>Mn>Ni>Cd>Cr. Notably, *Pyxine berteriana* exhibited the highest metal concentrations among the three species.

**Interpretation:** Based on the findings, it can be concluded that the Physcioid lichen *Pyxine berteriana* is the most resilient species in the region and can serve as an appropriate bio-indicator for studying the deposition of heavy metals in the ambient air.

**Key words:** Physcioid lichens, *Pyxine berteriana*, *Parmotrema tinctorum*, Lichens, Sacred Grove



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## Introduction

Lichens are a symbiotic relationship between a dominant fungus (mycobiont) and a green (phycobiont) and/or blue-green algae (cyanobiont) (Rai *et al.*, 2014). They typically thrive in diverse environmental conditions, but they are highly susceptible to atmospheric pollutants such as sulfur and nitrogen. Air pollution also contributes to the reduction of lichen population, making them valuable as indicator organisms. As lichens have no root apparatus, their mineral nutrition depends mainly on atmospheric inputs and owing to the lack of protective cuticle and stomata, thalli can accumulate mineral elements, including heavy metals, at levels exceeding their metabolic requirements. Lichens are widely recognized as an excellent indicators of heavy metal pollution in the environment. Due to their unique characteristics and sensitivity to atmospheric pollutants, lichens can accumulate and retain heavy metals from the air, this is the reason why lichens are considered valuable as indicators for heavy metals.

The particulate nature of airborne heavy metal is made evident by parameters referring to the entrapment of heavy-metal containing particles by thallus of lichens. The rate of absorption and accumulation of heavy metals are dependent on the morphological features of lichen thalli. Lichen possess distinct morpho-anatomy and physiology, including the absence of cuticle and roots, longer life span, slow growth, and nutrient absorption solely through the surface. Consequently, it has become one of the most widely employed biomonitors for detecting ambient air metal pollution (Thakur and Chander, 2018; Singh *et al.*, 2018; Sujetovienė *et al.*, 2019; Salleh *et al.*, 2023). Lichens have the capacity to accumulate pollutants from the atmosphere over extended periods, encompassing the entire range of deposition variations within the study timeframe (Benítez *et al.*, 2019; Huang *et al.*, 2019; Lyanguzova *et al.*, 2019; Rola, 2020; Elrhzaoui *et al.*, 2020; Budzyńska-Lipka *et al.*, 2022; Carrillo *et al.*, 2022).

The accumulation of heavy metals in lichens may differ among species, as it is greatly influenced by land use gradients within a particular habitat (Bozkurt, 2017; Demková *et al.*, 2017a, b; Parviainen *et al.*, 2019; Popova, 2019; Vitali *et al.*, 2019; İşik and Yildiz, 2022; Mohamed *et al.*, 2023; Saib *et al.*, 2023). Among the lichen-rich regions in the Indian subcontinent, the temperate habitats of the Himalayas stand out (Upreti, 1998). Nepal, located in the western and central Himalayas, boasts a significant abundance and diversity of lichens, comprising 1078 species from 233 genera and 66 families (Baniya and Tamang, 2020). By analyzing the heavy metal content within lichens, researchers can assess the extent of environmental pollution and identify areas with elevated metal concentrations. Lichens have been widely used as bioindicators in environmental monitoring programs due to their ability to accumulate heavy metals and their sensitivity to atmospheric pollutants. Their widespread distribution and ease of sampling make them valuable tools for assessing the impact of heavy metal pollution on ecosystems. Heavy metals are defined as those elements having an atomic number greater than 20 and atomic density above 5 g cm<sup>-3</sup> and

must exhibit the properties of metal. Following are the metals found hazardous and act as pollutants when are present in higher concentrations- Iron (Fe), Aluminium (Al), Magnesium (Mg), Zinc (Zn), Copper (Cu), Lead (Pb), Manganese (Mn), Nickel (Ni) Cadmium (Cd), and Chromium (Cr).

## Materials and Methods

**Study area:** Located in the Panjunaya village, Baitadi district in western Nepal, the study was carried out in Masani sacred groove, situated on the Mahakali National Highway. Spanning an area of approximately 14,000 m<sup>2</sup>, this sacred grove comprises a diverse mix of natural forest dominated by Quercus trees and associated valley vegetation, including *Myrica* spp. Nestled within a valley, it boasts an average elevation of 800 m and is situated along a tributary of the Sharda/Kali River, adjacent to the Mahakali National Highway-H14.

**Field techniques and Data collection:** Lichen samples were randomly collected within the sacred groove, ranging from the area adjacent to the Mahakali National Highway to the center of the grove.

**Identification of lichens:** The collected lichens were identified at the Lichenology Laboratory of CSIR-NBRI, situated in Lucknow, Uttar Pradesh, India. The identification process included the examination of morpho-anatomical features, thallus chemistry using spot tests and Thin layer chromatography, and UV light reaction fluorescence studies. Standard taxonomical keys and chemistry manuals of Awasthi (2007); Orange *et al.* (2010) and Elix (2014) were utilized for accurate identification of lichens. For preservation purposes, voucher samples were meticulously archived at the Lichen Herbarium (LWG) of the CSIR-National Botanical Research Institute, Lucknow. The CSIR-NBRI functions as a national repository for Indian flora, adhering to the regulations of the Biological Diversity Act of 2002 (Sec. 6, 12) of the Ministry of Environment, Forest, and Climate Change, India, ensuring the authenticity and conservation of the specimens.

**Estimation of heavy metals:** Samples of air-dried lichen thallus (three replicates) were ground into a powder and acid digested. The resulting solution was filtered, diluted, and analyzed on atomic absorption spectrophotometer to estimate the concentrations of following heavy metals: Iron, Aluminium, Magnesium, Zinc, Copper, Lead, Manganese, Nickel, Cadmium and Chromium. The analysis was conducted in triplicate, and the concentrations were recorded in units of µg/g dry weight.

**Sample Preparation:** Three replicates of approximately 1 gram of air-dried lichen thallus were taken. The thallus samples were ground into a fine powder to increase the surface area for analysis. To remove all organic carbon, acid digestion was performed. The powder was treated with a 5 ml mixture of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (in a 9:1 v/v ratio) for 1 hour. The mixture was heated until near dryness, resulting in the formation of deposits.

The deposits were dissolved in 1% HNO<sub>3</sub>. The resulting solution was filtered through Whatman filter paper No. 42 to remove any solid particles. The filtered solution was further diluted with 1% HNO<sub>3</sub>. The concentrations of heavy metals (Fe, Al, Mg, Zn, Cu, Pb, Mn, Ni, Cd, and Cr) were determined using an atomic absorption spectrophotometer (Avanta-R, GBC, Australia). Stock standards of heavy metals, traceable to NIST (National Institute of Standards Technology), were procured from Merck India. These standards were used to establish calibration curves covering concentrations ranging from 1 to 3 µg g<sup>-1</sup>. The atomic absorption spectrophotometer had a sensitivity of 0.1 µg g<sup>-1</sup> for all the detected heavy metals. The heavy metal analysis was performed in triplicate, and the concentrations were recorded in µg g<sup>-1</sup> dry weight.

### Results and Discussion

The study conducted a thorough examination of lichen species present in the study area, identifying three distinct genera: *Bulbothrix meizospora* (Nyl.) Hale, *Parmotrema tinctorum*

(Despr. ex Nyl.) Hale, and *Pyxine berteriana* (Fée) Imsh, belonging to families Parmeliaceae and Physciaceae, respectively (Table 1; Fig. 1). Notably, *Pyxine berteriana* was primarily observed in close proximity to the highway, indicating its preference for such environments, whereas the other two species, *Bulbothrix meizospora* and *Parmotrema tinctorum* were predominantly found in the deeper regions of sacred grove, away from the influence of highway. The heavy metal concentration in the thalli of the analyzed lichens ranged between 1057.88 to 708.63 µg g<sup>-1</sup> d.wt. In all three species the concentration of Fe was higher followed by of Al>Mg>Zn>Cu>Pb>Mn>Ni>Cd>Cr. Heavy metals analysis revealed the absence of Cd and Cr in *Bulbothrix meizospora* while other metals were found in the following sequence: Fe>Al>Mg>Zn>Cu>Pb>Mn>Ni. The total metal load ΣM<sub>10</sub> was maximum for *Pyxine berteriana* followed by *Parmotrema tinctorum* and *Bulbothrix meizospora*, respectively.

The distribution pattern of *Bulbothrix meizospora* (Nyl.) Hale, *Parmotrema tinctorum* (Despr. ex Nyl.) Hale, and *Pyxine berteriana* (Fée) Imsh species provide interesting insights into

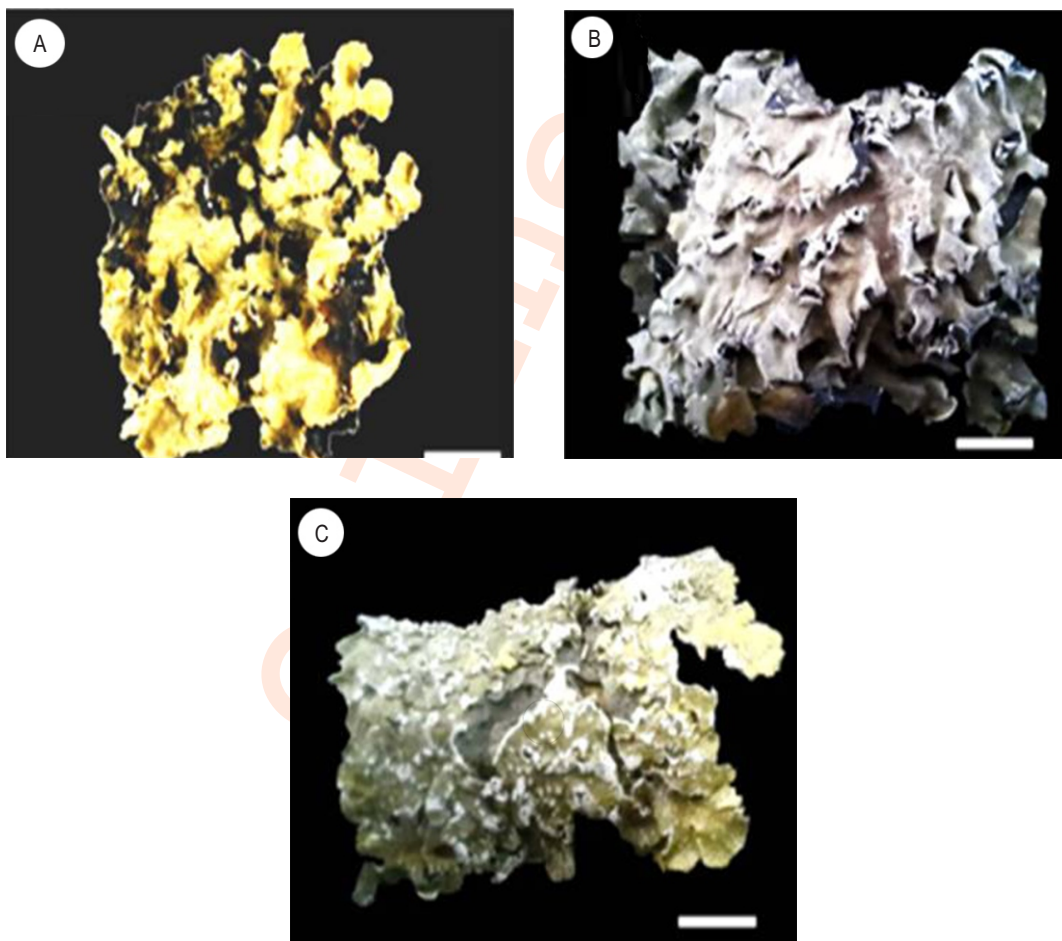


Fig. 1: Morphology of (A) *Bulbothrix meizospora* (Nyl.) Hale; (B) *Parmotrema tinctorum* (Despr. ex Nyl.) Hale and (C) *Pyxine berteriana* (Fée) Imsh.

**Table 1:** Concentration of heavy metals detected in the lichen samples collected from Masani Sacred grove, Panjunaya village, Baitadi district, Far-western Nepal.

Lichen Genus	Family	Distance from Mahakali National Highway-H14 (m)	Heavy metals ( $\mu\text{g g}^{-1}$ d.wt.*)										
			Fe	Al	Mg	Zn	Cu	Pb	Mn	Ni	Cd	Cr	$\Sigma M_{10}$
<i>Bulbothrix meizospora</i> (Nyl.) Hale	Parmeliaceae	250	201.3 $\pm 0.12$	111.11 $\pm 0.41$	43.21 $\pm 0.03$	28.02 $\pm 0.02$	15.22 $\pm 0.22$	22.22 $\pm 0.08$	12.06 $\pm 0.01$	1.52 $\pm 0.03$	-	-	434.65
<i>Parmotrema tinctorum</i> (Despr. ex Nyl.) Hale	Parmeliaceae	40	411.1 $\pm 0.11$	109.26 $\pm 0.43$	64.12 $\pm 0.01$	58.24 $\pm 0.03$	20.14 $\pm 0.24$	24.20 $\pm 0.04$	19.22 $\pm 0.01$	2.24 $\pm 0.03$	0.10 $\pm 0.01$	0.01 $\pm 0.01$	708.63
<i>Pyxine berteriana</i> (Fée) Imsh.	Physciaceae	0.5	621.1 $\pm 0.10$	226.38 $\pm 0.48$	84.24 $\pm 0.02$	38.24 $\pm 0.02$	28.26 $\pm 0.29$	28.09 $\pm 0.08$	28.04 $\pm 0.02$	3.42 $\pm 0.05$	0.10 $\pm 0.02$	0.02 $\pm 0.01$	1057.88

\*Values are mean  $\pm$  S.D.

their ecological preferences and tolerance towards different environmental conditions (Boonpeng *et al.*, 2023; Kováčik *et al.*, 2023). The presence of *Pyxine berteriana* adjacent to the highway suggests its ability to withstand automobile exhaust emissions and the associated disturbances (Liu *et al.*, 2017; Salleh *et al.*, 2023). On the other hand, *Bulbothrix meizospora* and *Parmotrema tinctorum* displayed preference for relatively undisturbed and secluded interior areas of the sacred grove, indicating their affinity towards more pristine habitats with limited anthropogenic influences (Upreti, 2014). This variation in distribution highlights the adaptability and ecological versatility of lichen species in response to environmental factors and stressors. By occupying distinct microhabitats within the same ecosystem, these lichens provide regulating ecosystem services in the overall ecological functioning of sacred grove (Matos *et al.*, 2019).

Understanding the distribution and preferences of lichen species in relation to their habitat characteristics, including proximity to highways, contributes to our knowledge of their ecological significance and their potential as bio-indicators of environmental conditions (Haifeng *et al.*, 2022; Adžemović *et al.*, 2023). It provides a foundation for further investigations into the ecological interactions and responses of lichens to varying degrees of human influence and the associated implications for ecosystem health and conservation strategies. The current study significantly contributes to the existing body of knowledge by providing comprehensive insights into the bioaccumulation of heavy metals in lichens through an extensive bio-indicator analysis. The research outcomes highlight the substantial accumulation of metals in the lichen thallus, indicating the deposition of heavy metals in the region resulting from a variety of sources, including natural processes and human activities (Pandey *et al.*, 2002). The meticulous analysis of total heavy metal load, ΣM10, revealed *Pyxine berteriana* to exhibit the highest level of metal accumulation, followed by *Parmotrema tinctorum* and *Bulbothrix meizospora*. These findings consistently align with previous studies conducted by (Upreti, 2014; Singh *et al.*, 2019; Daimari *et al.*, 2020; Banerjee *et al.*, 2023; Thakur *et al.*, 2023), thereby reinforcing their validity and reliability. The remarkable tolerance demonstrated by Physcioid lichens, as confirmed by multiple studies across various Himalayan habitats Shukla and (Upreti, 2007a,b); Shukla *et al.*, 2012; 2013; Ramić *et al.*, 2019), underscores their resilience in the face of heavy metal exposure.

Furthermore, the lichen species observed adjacent to the highway exhibited a significantly higher overall accumulation of heavy metals (Abas *et al.*, 2019; Labaran, 2019). This observation emphasizes the substantial contribution of automobile exhaust emissions as a primary source of heavy metal deposition in the western regions of Nepal, a finding supported by Gupta *et al.* (2017); Nag *et al.* (2020).

While all lichen species have proven to be effective bio-indicators of ambient air heavy metals, the comprehensive analysis and observations presented in this study further validate the superior efficiency of species within the Physciaceae family in

accumulating both organic and inorganic metals compared to other lichen species. These findings enhance our understanding of the intricate dynamics of heavy metal bioaccumulation in lichens, thereby facilitating ongoing efforts in environmental monitoring and management. The study's outcomes contribute valuable knowledge that can inform future research and guide decision-making processes aimed at mitigating the adverse impacts of heavy metal pollution on ecosystems and human health.

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**Authors' contribution:** P. Nag: Formal analysis, Investigation, writing original draft; H. Rai: Formal analysis, Investigation, writing original draft; D.K. Upreti: Validation, Investigation; R.K. Gupta: Conceptualization, Data, Curation, Validation.

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### References

- Abas, A., N. Sulaiman, N.R. Adnan, S.A. Aziz and W.N.S.W. Nawang: Using lichen (*Dirinaria* sp.) as bioindicator for airborne heavy metal at selected industrial areas in Malaysia. *Environ. Asia.*, **12**, 85-90 (2019).
- Adžemović, S., S. Aliefendić, E. Mehić, A. Ranica, I. Vehab, N. Alagić, Š. Delibašić, K. Herceg, M. Karić, B. Hadžić and S. Gojak-Salimović: Estimation of atmospheric deposition utilizing lichen *Hypogymnia physodes*, moss *Hypnum cupressiforme* and soil in Bosnia and Herzegovina. *Int. J. Environ. Sci. Technol.*, **20**, 1905-1918 (2023).
- Awasthi, D.D.: A compendium of the macrolichens from India, Nepal and Sri Lank. Bishen Singh Mahendra Pal Singh, Dehra Dun, India (2007).
- Banerjee, S., S.S. Ram, A. Mukhopadhyay, N. Jana, M. Sudarshan and A. Chakraborty: Potential of epiphytic lichen *Pyxine cocoas*, as an indicator of air pollution in Kolkata, India. In: Proceedings of the National Academy of Sciences, India Section B: *Biol. Sci.*, **93**, 165-180 (2023).

- Baniya, C.B. and R. Tamang: Lichens of Nepal. Plant Diversity of Nepal. Botanical Society of Nepal, Kathmandu, pp. 55-61 (2020).
- Benítez, Á., J. Medina, C. Vásquez, T. Loaiza, Y. Luzuriaga and J. Calva: Lichens and bromeliads as bioindicators of heavy metal deposition in Ecuador. *Diversity*, **11**, 28 (2019).
- Boonpeng, C., D. Sangiamdee, S. Noikrad and K. Boonpragob: Assessing seasonal concentrations of airborne potentially toxic elements in tropical mountain areas in Thailand using the transplanted lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale. *Forests*, **14**, 611 (2023).
- Bozkurt, Z.: Determination of airborne trace elements in an urban area using lichens as biomonitor. *Environ. Monit. Assess.*, **189**, 573 (2017).
- Budzyńska-Lipka, W., P. Świsłowski and M. Rajfur: Biological monitoring using lichens as a source of information about contamination of mountain with heavy metals. *Ecol. Chem. Eng.*, **29**, 155-168 (2022).
- Carrillo, W., J. Calva and Á. Benítez: The use of bryophytes, lichens and bromeliads for evaluating air and water pollution in an Andean City. *Forests*, **13**, 1607 (2022).
- Daimari, R., P. Bhuyan, S. Hussain, S. Nayaka, M.J. Mazumder and R.R. Hoque: Biomonitoring by epiphytic lichen species—*Pyxine cocolos* (Sw.) Nyl.: Understanding characteristics of trace metal in ambient air of different landuses in Mid-Brahmaputra valley. *Environ. Monit. Assess.*, **192**, 1-11 (2020).
- Demková, L., B. Baranová, J. Oboňa, J. Árvay and T. Lošák: Assessment of air pollution by toxic elements on petrol stations using moss and lichen bag technique. *Plant Soil Environ.*, **63**, 355-361 (2017a).
- Demková, L., L. Bobul'ská, J. Árvay, T. Jezný and L. Ducsay: Biomonitoring of heavy metals contamination by mosses and lichens around Slovinky tailing pond (Slovakia). *J. Environ. Sci. Health. A Tox Hazard Subst. Environ. Eng.*, **52**, 30-36 (2017b).
- Elix, J.A.: A Catalogue of Standardized Chromatographic Data and Biosynthetic Relationships for Lichen Substances. 3<sup>rd</sup> Edn., Published by the author, Canberra (2014).
- Elrhzaoui, G., P.K. Divakar, A. Crespo, H. Tahiri, F.E. El Alaoui-Faris and R. Khellouk: Spatial mapping of heavy metals using lichen bioaccumulation capacity to assess air contamination in Morocco. *Crypto. Biodive. Assess. J.*, **4**, 19-24 (2020).
- Gupta, S., H. Rai, D.K. Upreti, R.K. Gupta and P.K. Sharma: Lichenized fungi Phaeophyscia (Physciaceae, ascomycota) as indicator of ambient air heavy metal deposition, along land use gradient in an Alpine habitat of Western Himalaya, India. *Pollut. Res.*, **36**, 150-157 (2017).
- Haifeng, D.I.N.G., Z. Zhang, W.A.N.G. Zhuhui, W.U. Qimei and W.A.N.G. Dengfu: Using mosses and lichen to study roadside pollution and the correlation of toxic elements and elevation in a mountainous area of Guizhou, China. *Taiwania*, **67**, 351-360 (2022).
- Huang, Y.P., J.T. Xiang, C.H. Wang, D. Ren and T. Xu: Lichen as a biomonitor for vehicular emission of metals: a risk assessment of lichen consumption by the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*). *Ecotoxicol. Environ. Saf.*, **180**, 679-685 (2019).
- Işık, V. and A. Yıldız: The heavy metal biomonitoring study using lichen *Xanthoria parietina* (L.) Th. Fr. in Ankara province (Turkey). *EQA-Int. J. Environ. Qual.*, **52**, 1-10 (2022).
- Kováčik, J., L. Husáková, M. Vlassa, M. Piroutková, M. Vydra, J. Patočka and M. Filip: Elemental profile identifies metallurgical pollution in epiphytic lichen *Xanthoria parietina* and (hypo) xanthine correlates with metals. *Sci. Total Environ.*, **883**, 163527 (2023).
- Labaran, I.: Determination of some concentrated atmospheric pollutants such as heavy metal (Pb, Mn, Cd, Cr And Zn) in lichens found in urban and peri-urban areas of Kaduna State. *IDOSR- J. Biol. Chemi. Pharma.*, **3**, 23-64 (2019).
- Liu, H.J., J.G. Wang, Y. Xia, M.J. Yang, S.W. Liu, L.C. Zhao, X.P. Guo, Y.J. Jiang, X. Li, Q.F. Wu and S.B. Fang: Elemental compositions of lichens from Duolun County, Inner Mongolia, China: Origin, road effect and species difference. *Sci. Rep.*, **7**, 5598 (2017).
- Lyanguzova, I.V., M.S. Bondarenko, A.I. Belyaeva, M.N. Kataeva, V.S. Barkan and A.Y. Lyanguzov: Migration of heavy metals from polluted soil to plants and lichens under conditions of field experiment on the Kola Peninsula. *Russ. J. Ecol.*, **51**, 528-540 (2020).
- Matos, P., J. Vieira, B. Rocha, C. Branquinho and P. Pinho: Modeling the provision of air-quality regulation ecosystem service provided by urban green spaces using lichens as ecological indicators. *Sci. Total Environ.*, **665**, 521-530 (2019).
- Mohamed, E., M. Loudiki and A. El Gharnali: Bioaccumulation and sources identification of atmospheric metal trace elements using lichens along a rural-urban pollution gradient in the Safi-Essaouira coastal area. *Chem Ecol.*, **39**, 107-122 (2023).
- Nag, P., H. Rai, R.K. Gupta and D.K. Upreti: Lichenized fungal family Physciaceae (Ascomycota) as an indicator of ambient air heavy metal pollution in a temperate forest of Western Himalaya, India. *Shodh Sarita*, **7**, 190-193 (2020).
- Orange, A., P.W. James and F.J. White: Microchemical Methods for the Identification of Lichens. 2<sup>nd</sup> Edn., British Lichen Society (2010).
- Pandey, V., D.K. Upreti, R. Pathak and A. Pal: Heavy metal accumulation in lichens from the Hetauda industrial area Narayani zone Makwanpur district, Nepal. *Environ. Monit. Assess.*, **73**, 221-228 (2002).
- Parviainen, A., M. Casares-Porcel, C. Marchesi and C.J. Garrido: Lichens as a spatial record of metal air pollution in the industrialized city of Huelva (SW Spain). *Environ. Pollut.*, **253**, 918-929 (2019).
- Popova, E.: Accumulation of heavy metals with the lichen thalli of *Cladonia rangiferina* L. at the roadside phytocenoses of the West Siberian subarctic. In IOP Conference Series: *Earth Environ. Sci.*, **395**, 012045 (2019).
- Rai, H., R. Khare, C. Baniya, D.K. Upreti and R.K. Gupta: Elevational gradients of terricolous lichen species richness in the Western Himalaya. *Biodiver. Conserv.*, **24**, 1155-1174 (2014).
- Ramić, E., J. Huremović, T. Muhić-Šarac, S. Đug, S. Žero and A. Olovčić: Biomonitoring of air pollution in Bosnia and Herzegovina using epiphytic lichen *Hypogymnia physodes*. *Bull. Environ. Contam. Toxicol.*, **102**, 763-769 (2019).
- Rola, K.: Insight into the pattern of heavy-metal accumulation in lichen thalli. *J. Trace. Elem. Med. Biol.*, **61**, 126512 (2020).
- Saib, H., A. Yekkour, M. Toumi, B. Guedioura, M.A. Benamar, A. Zeghdaoui, A. Austruy, D. Bergé-Lefranc, M. Culcasi and S. Pietri: Lichen biomonitoring of airborne trace elements in the industrial-urbanized area of eastern Algiers (Algeria). *Atmos. Pollut. Res.*, **14**, 101643 (2023).
- Salleh S., Nur S. Amalina and A. Abas: Monitoring heavy metal concentrations using transplanted lichen in a Tourism City of Malaysia. *Sustainability*, **15**, 5885 (2023).
- Shukla, V. and D.K. Upreti: Heavy metal accumulation in *Phaeophyscia hispidula* en route to Badrinath, Uttaranchal, India. *Environ. Monit. Assess.*, **131**, 365-369 (2007a).
- Shukla, V. and D.K. Upreti: Physiological response of the lichen *Phaeophyscia hispidula* (Ach.) Essl., to the urban environment of Pauri and Srinagar (Garhwal), Himalayas, India. *Environ. Pollut.*, **150**, 295-299 (2007b).
- Shukla, V., D.K. Patel, D.K. Upreti, M. Yunus and S. Prasad: A comparison of metallic contents in lichen *Pyxine subcinerea*, its substratum and soil. *Int. J. Environ. Sci. Technol.*, **10**, 37-46 (2013).
- Shukla, V., D.K. Upreti and D.K. Patel: Physiological attributes of lichen,

- Phaeophyscia hispidula* in heavy metal rich sites of Dehra Dun, India. *J. Environ. Biol.*, **33**, 1051 (2012).
- Singh, P., P.K. Singh, P.K. Tondon and K.P. Singh: Heavy metals accumulation by epiphytic foliose lichens as biomonitors of air quality in Srinagar city of Garhwal hills, Western Himalaya (India). *Curr. Res. Environ. Appl. Mycol. J. Fungal Biol.*, **8**, 282-289 (2018).
- Singh, P.K., P. Bujarbarua, K.P. Singh and P.K. Tandon: Report on the bioaccumulation of heavy metals by foliose lichen (*Pyxine cocoes*) from air polluted area near Nagaon Paper Mill in Marigaon, Assam, North-east India. *J. New Biol. Rep.*, **8**, 15-21 (2019).
- Sujetovienė, G., P. Smilgaitis, R. Dagiliūtė and J. Žaltauskaitė: Metal accumulation and physiological response of the lichens transplanted near a landfill in central Lithuania. *Waste Manag.*, **85**, 60-65 (2019).
- Thakur, M. and H. Chander: Bioindicator lichens of Sikandra hills of North west Himalaya. *Asian J. Adv. Basic Sci.*, **6**, 35-37 (2018).
- Thakur, M., B. Kapoor, D. Kapoor and H. Chander: Physiological alterations and heavy metal accumulation in the lichen *Pyxine cocoes* (Sw.) Nyl. of Mandi District, Himachal Pradesh. *J. Plant Growth Regul.*, **42**, 415-422 (2023).
- Upreti, D.K.: Diversity of lichens in India. Perspectives in Environment (Eds.: A. Jain, S.K. Agarwal, J.P. Kalishik and K.K. Koul). APH Publishing Corporation, New Delhi, pp.71-79 (1998).
- Upreti, D.K.: Multidimensional Approaches in the Study of Lichens. Plant Taxonomy and Biosystematics Classical and Modern Methods (Eds.: T.S. Rana, K.N. Nair and D.K. Upreti). New India Publishing Agency, New Delhi, pp. 73-99 (2014).
- Vitali, M., A. Antonucci, M. Owczarek, M. Guidotti, M.L. Astolfi, M. Manigrasso, P. Avino, B. Bhattacharya and C. Protano: Air quality assessment in different environmental scenarios by the determination of typical heavy metals and persistent organic pollutants in native lichen *Xanthoria parietina*. *Environ. Pollut.*, **254**, 113013 (2019).