Research article

Lichenized fungi *Stereocaulon foliolosum* Nyl. (Stereocaulaceae, Ascomycota), indicator of ambient air metal deposition in a temperate habitat of Kumaun, central Himalaya, India

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Abstract: Himalayan habitats in the past three decades have undergone a tremendous change in the land use pattern. The increase in urbanization and associated vehicular movement had increased the heavy metal influx in the ambient air of Himalayan ecosystems. Bioindicators such as lichens due to their slow growth, longer lifespan and maximum absorption of nutrients through atmospheric deposition, can be used as an appropriate indicator of long-term heavy metal deposition in the Himalaya. In the current study, a terricolous lichen *Stereocaulon foliolosum* was studied in the temperate habitat of Munsyari, Pithoragarh district, Uttarakhand, central Himalaya, for its ability as a bioindicator of ambient air heavy metal deposition along with land use linked vehicular exhaust gradient. In the current study, *Stereocaulon foliolosum* was analyzed for lichen diversity and seven heavy metals- (Mg, Zn, Cu, Pb, Mn, Ni, Cd) deposition in five plots in Khaliya bugyal-Munsyari landscape along gradients of land use and elevation. The maximum heavy metal deposition was recorded in plots along roads which minimized in Khaliya bugyal. The total heavy metal load (ΣM_7) was found indicative of land use gradient followed by Mg, Zn, Cu and Ni accumulation in lichen thallus. The study concluded that the diversity of *Stereocaulon foliolosum* was negatively correlated to the vehicular density which was maximum at moderate elevations exhibiting intense land use and maximum vehicular movement. The study thus established that change in lichen diversity can be an appropriate indicator of land use linked pollution gradients in temperate habitat in Kumaun, central Himalaya.

Keywords: Diversity - Heavy metal - Terricolous lichen - Vehicular density.

INTRODUCTION

The Lichens, a self-supporting mutualistic association of one or two (Spribille *et al.* 2016) fungus and a green and/or blue-green algae, are by far known as one of the most successful symbiosis in nature (Honegger 1991, Galloway 1992). The peculiar morpho-anatomy of lichen thallus, characterized by the absence of root and cuticle, slow growth, long-life, the large-scale absorbance of the nutrients directly from the atmosphere makes them highly sensitive to environmental changes and allow them to accumulate pollutants in their tissue (Purvis 2000). The variation in diversity of lichen species has been an important marker of the pollution gradient in various habitats (Loppi *et al.* 2004, Pinho *et al.* 2012, Giordani *et al.* 2012).

Himalayan habitats in the recent past have undergone a drastic change in the land use pattern, due to the population growth and related zoo-anthropogenic activities (Rao & Pant 2001, Tiwari 2008). The Kumaun Himalaya along with western Nepal constitutes the central Himalayan botanical region (Singh & Singh 1987).

The topography of Kumaun Himalaya is mountainous with gentle slopes and vegetation mainly dominated by species of *Quercus* (Oak-Banj). Lichens, in the Himalaya, have been reported as bioindicators, of all sought of natural, as well as zoo-anthropogenic perturbations ranging from habitat heterogeneity, grazing, trampling to air pollution (Bergamaschi *et al.* 2002, Rai *et al.* 2012a, b, Kumar *et al.* 2014, Gupta *et al.* 2016, 2017, 2018, Yadav *et al.* 2018).

In this study, we sampled a common terricolous lichen *Stereocaulon foliolosum* Nyl. from a temperate habitat of Kumaun Himalaya and assess the bioindicator capability of the lichen for land use coupled pollution gradients along an elevation gradient.

MATERIALS & METHODS

Study area

The study was conducted in and around a semi-urban township of Munsyari, (N 30º 04′37.58″ – 30º 03′46.00″; E 80º 13′34.07″ – 80º 14′53.70″) situated in Pithoragarh district of Uttarakhand, Kumaun, central Himalaya. The area included the Khaliya bugyal (a state government notified medicinal plant conservation area-MPCA) and the Munsyari town. The township exhibits various land use ranging from natural conserved managed forests to roadside scrublands and agricultural land. The climate of the region is temperate with temperature ranging from 25ºC to -12ºC. Precipitation occurs in the form of rain, soot or snowfall. Maximum precipitation occurs through the south-west monsoon in months of July–August. Snowfall occurs in the months of Feb–March. The vegetation of the region can be classified as *Quercus-Rhododendron* mixed forests with isolated stands of *Abies* spp. in the higher elevation of conserved forests.

The local population of Munsyari is mainly involved in agriculture, animal husbandry and occasional collection of rare Himalayan medicinal herbs. The township is also a tourist hub which acts as the base camp for alpine glacier treks to Mila, Ralam, and Namik. The tourism and other activities in the area involve the movement of automobiles which along with domestic and commercial exhaust contribute to pollutants (heavy metals and polycyclic aromatic hydrocarbons) in ambient air.

The climate, topography and habitat heterogeneity of the region make it suitable for the flourishing of lichen communities having diverse substrate, growth-form, and photobiont preferences. *Stereocaulon foliolosum* due to its morphological tolerance to harsher climatic conditions, grazing/ footfall induced trampling and biochemical defense against herbivore grow vigorously on the ground or on soil accumulated on rocky outcrops in Himalaya (Rai *et al.* 2012a, b). The study was conducted on samples of *Stereocaulon foliolosum*, collected from five plots situated in Khaliya bugyal (on the outskirts of Munsyari) and in the township of Munsyari (Fig. 1).
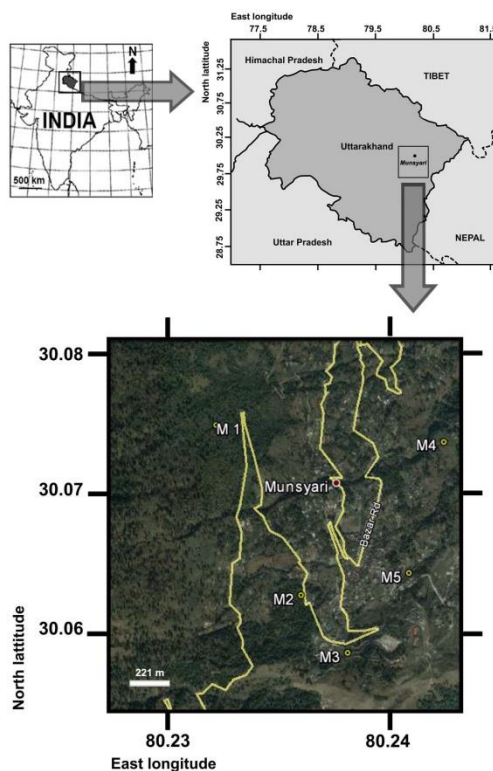

Figure 1. Location map of the study site and plot laid in the Khaliya bugyal-Munsyari landscape of Pithoragarh district, Uttarakhand, central Himalaya (Prepared using Google Earth 2018, processed in SURFER version 11.0.62).

Field methods and data recording

The sampling of *Stereocaulon foliolosum* was done employing a two-way random stratified sampling method where the land use was the primary stratifying variable along the elevation gradient of the study area (Greig-Smith 1983, Krebs 1989, Holt *et al.* 2007, Rai *et al.* 2012b) (Table 1). In order to maximize the sampling efficiency and clipping related difficulties a mixed quadrat sampling approach was undertaken (KakaÏ *et al.* 2008). Five circular plots (24 m in diam.) were randomly selected in the study landscape and lichen diversity was recorded using a standardized probabilistic method with three 10×50 cm narrow frequency grids subdivided into five sampling units of 10×10 cm, laid randomly *i.e.* fifteen, 10×10 cm sampling units were laid in each plot (Scheidegger *et al.* 2002, Rai *et al.* 2012b, Gasparyan *et al.* 2018). Four land use types were recorded in the five sampling plots (Table 1).

Table 1. Geo-attributes, land-use and vehicular pollution gradient recorded in *Stereocaulon foliolosum* Nyl. in the five study sites of Munsyari, Pithoragarh, Uttarakhand, western Himalaya.

Site abbreviation	Elevation (m)	Geo-coordinate	Land use	Lichen diversity	Vehicular frequency
M1	2350	N 30º 04′28.27″ E 80º 13′48.62″	Open canopy old grown forest	66.67	0
M2	2255	N 30º 03′56.86″ E 80º 14′06.99″	Roadside scrublands	20	15
M3	2240	N 30º 03′46.27″ E 80º 14′16.99″	Roadside scrublands	13.33	18
M4	1989	N 30º 04′25.15″ E 80º 14′37.72″	Terraced agricultural field	33.33	8
M5	2128	N 30º 04′00.93″ E 80º 14′30.15″	Secondary rural scrublands	26.67	4

Note: Lichen diversity as % Quadrat frequency, Vehicular frequency as Number of vehicle hour^{-1}.

After curating representative lichen samples for taxonomic authentication, about 8 grams of lichen sample was pooled in from each plot and was transported to the laboratory in zip-locked airtight polyvinyl bags, for heavy metal analysis. The geotagging and elevation data recording of sampling sites were done with a hand-held GPS unit (Garmin GPSMAP® 76S^{TM}). The vehicular density at sites was recorded as vehicle frequency/ hour (Glenn *et al.* 1995, Liu *et al.* 2017).

Lichen sample authentication

The lichen samples were authenticated up to the species level at the Lichenology Laboratory of the National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh, India using standard morpho-anatomical examination, chemical spot tests, standardized thin-layer chromatography and relevant literature (Awasthi 2007, Orange *et al.* 2010, Elix 2014). The authenticated samples were deposited as voucher specimens in the lichen herbarium (LWG), NBRI.

Heavy metal estimation

The metal content of lichen samples was analyzed by inductively coupled plasma- optical emission spectrometer (ICP-OES), employing Nitric (HNO_3)-Perchloric ($HClO_4$) acid digestion method for the sample preparation (Walting 1981). Lichen samples were processed in triplicate for metal estimation.

One gram of air-dried lichen sample was ground to a fine powder using a glass mortar and pestle. The powdered lichen thallus was digested in 100 ml glass beaker with concentrated HNO_3 (20 ml) overnight. It was then mixed with 10 ml of conc. HNO_3 and $HClO_4$ (4:1) solution, followed by evaporation of the acid mixture using a hot plate (120ºC) to complete dryness (Walting 1981). The residue was treated with 10 ml of 20% HNO_3 for 6 hours, filtered through a Millipore filtration unit and made up to 20 ml using Milli-Q water for analysis in ICP-OES.

The seven heavy metals- Magnesium (Mg), Zinc (Zn), Copper (Cu), Lead (Pb), Manganese (Mn), Nickel (Ni) and Cadmium (Cd) were analysed in digested lichen samples using inductively coupled plasma- optical emission spectrometer (ICP-OES) (PerkinElmer® Optima 2100 DV^{TM}, Software-Winlab 32^{TM}). The precision and accuracy of the analytical procedure was calibrated using commercially available standards (Merck KGCA, 64271 Damstadt, Germany, ICP-Multielement standard solution IV). The heavy metal readings were reported as microgram per gram dry weight (µg g^{-1} DW).

Data analysis

The diversity of *Stereocaulon foliolosum* was estimated as % quadrat frequency in each plot (Curtis &

McIntosh 1950, Rai *et al.* 2012b). Univariate descriptive statistics (mean and standard deviation) was used to characterize heavy metal concentrations (differential as well as total metal load-ΣM_7) in *Stereocaulon foliolosum* collected in the five plots of Khaliya bugyal-Munsyari landscape. The heavy metal concentration in the lichens thallus at different sites were visualized using a two-dimensional metal distribution contour map using SURFER version 11.0.62 (Fig. 2). Bivariate two-tailed correlation analysis was done (Pearson's correlation coefficients) to compare explanatory variables– differential as well, total heavy metal load, vehicular density, elevation and response variable–lichen diversity using IBM® SPSS® Statistics ver. 20 (Rai *et al.* 2012b, Gupta *et al.* 2017).
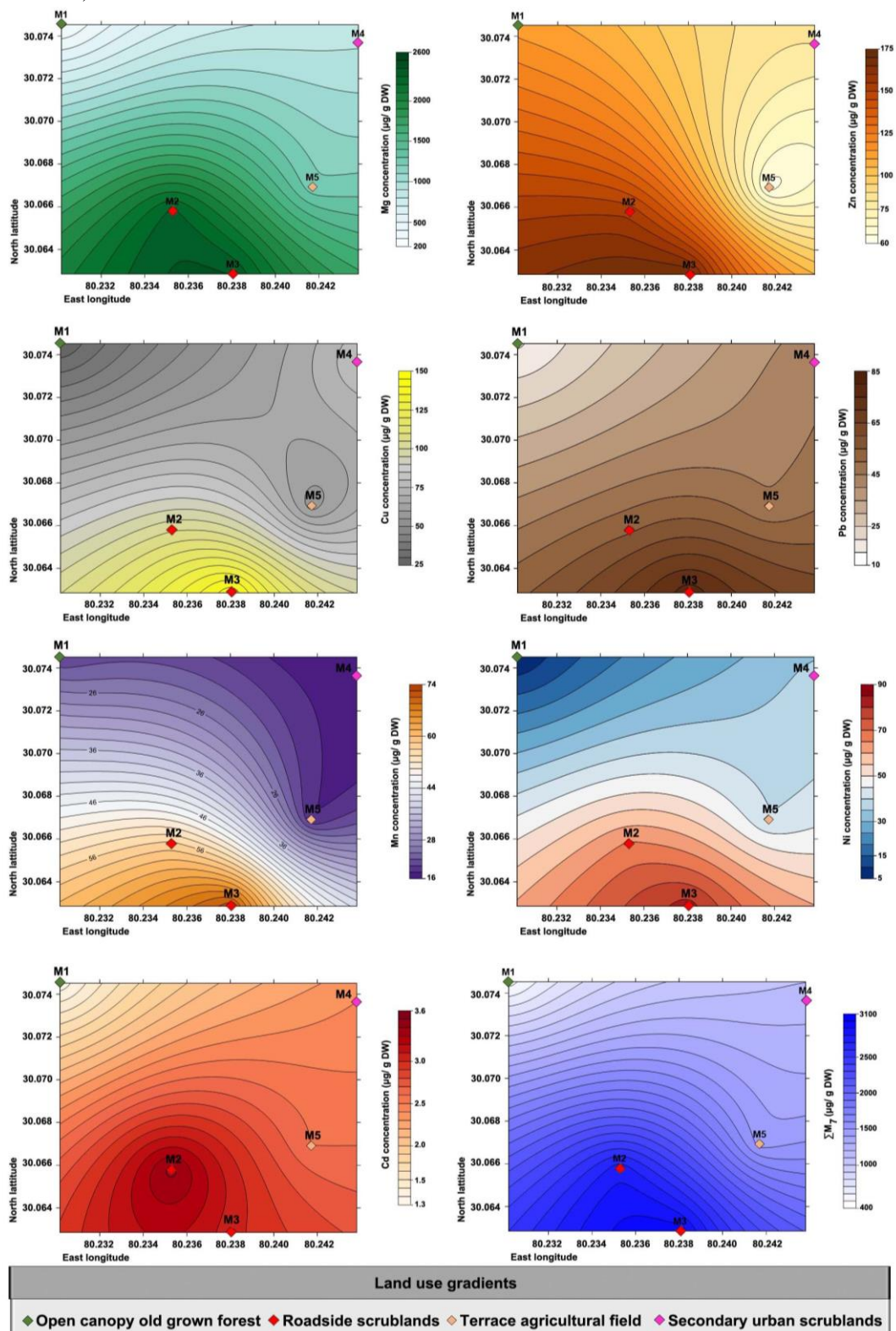

Figure 2. Concentration of heavy metals analyzed (Mg, Zn, Cu, Pb, Mn, Ni, Cd and total metal load (ΣM_7) in *Stereocaulon foliolosum* Nyl. from different plots along four land use gradient.

RESULTS

The diversity of Stereocaulon foliolosum was found varying in different sites. The diversity was maximum at site M1 in Khaliya bugyal which decreased to its lowest in sites M2, M3 and further increased in sites M4, M5 (Table 2). Though there was no significant linear relationship between lichen diversity and sampling plot elevation with vehicular density, polynomial fits the data suggesting that the diversity of *Stereocaulon foliolosum* decreases at mid-altitude (2240–2255 m) due to increased vehicular density (Table 1; Fig. 3). Further as the land-use intensity decrease at lower elevation the lichen diversity increases in accordance with decreased vehicular density (Table 1; Fig. 3).

Table 2. Pearson's correlation coefficients between quadrat frequency, vehicular density, elevation and heavy metal accumulation in *Stereocaulon foliolosum* Nyl. from Munsyari, Pithoragarh district, Uttarakhand state, India, Western Himalaya (significant correlations are tagged)

	VD	ΣM_7	Mg	Zn	Cu	Pb	Mn	Ni
ΣM_7	0.954[a]							
Mg	0.941[a]	0.999[b]						
Zn	0.768	0.729	0.702					
Cu	0.982[b]	0.925[a]	0.909[a]					
Pb	0.914[a]	0.908[a]	0.901[a]					
Mn	0.905[a]	0.914[a]	0.896[a]	0.927[a]				
Ni	0.961[b]	0.977[b]	0.973[b]	0.641	0.967[b]	0.975[b]	0.867	
Cd	0.863	0.920[a]	0.934[a]	0.455	0.822	0.847	0.688	0.914[a]

Note: VD- Vehicular density at sites, Mg- Magnesium concentration in the thallus, Zn- Zinc concentration in the thallus, Cu- Copper concentration in the thallus, Pb- Lead concentration in the thallus, Mn- Manganese concentration in the thallus, Ni- Nickel concentration in the thallus, Cd- Nickel concentration in the thallus, ΣM_7- Total accumulation of the seven heavy metals analysed.
[a]Correlation is significant at the 0.05 level (2-tailed), [b]Correlation is significant at the 0.01 level (2-tailed).
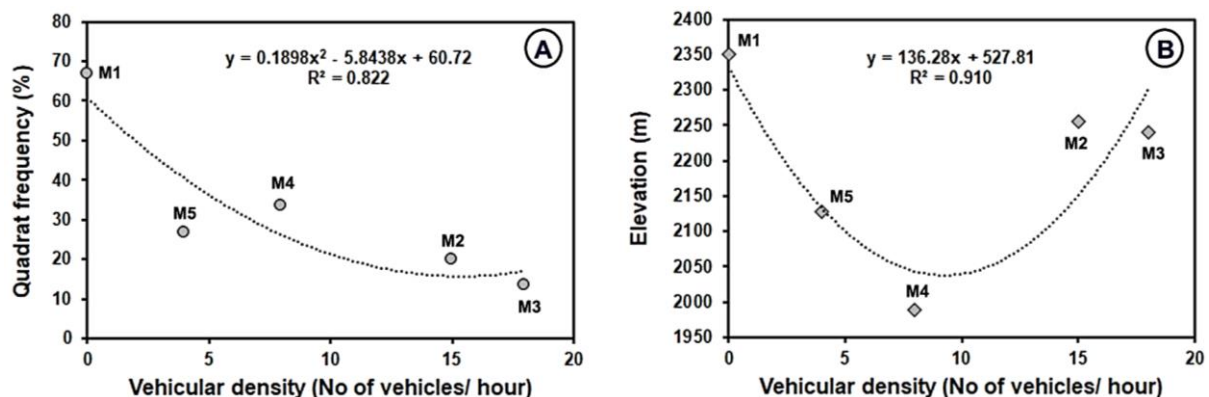

Figure 3. Diversity and elevation of *Stereocaulon foliolosum* Nyl. plotted against vehicular density: **A**, Diversity (% quadrat frequency); **B**, Elevation of sampling plot elevation of all sites. [The dashed line is a best-fit trend polynomial; See table 1 for abbreviations]

The bivariate two-tailed correlation analysis concludes that the vehicular density was positively correlated with total metal load (ΣM_7) and ambient air deposition of metals (Mg, Cu, Pb, Mn, and Ni) (Table 2). Among the seven heavy metal analyzed significant positive correlation was found different metals *i.e.* Mg-Cu-Pb-Mn-Ni-Cd, Zn-Mn, Cu-Ni, Pb-Ni, Ni-Cd suggesting a synergistic accumulation of heavy metals in Stereocaulon foliolosum thallus at the five sites (Table 2).

DISCUSSION AND CONCLUSION

The lichen communities in Himalaya are highly sensitive to zoo-anthropogenic pressures (Rai *et al.* 2012a, b). The higher lichen diversity of *Stereocaulon foliolosum* at high elevation plots with least vehicular density and the further decrease of diversity along decreasing elevational gradient can be attributed to increased vehicular density in the study landscape of Munsyari (Mendil *et al.* 2009, Gupta *et al.* 2017). The positive significant correlation of vehicular density at plots and the thallus heavy metal concentration (Table 2) in *Stereocaulon foliolosum* indicate that vehicular movement is a major source of pollution in and around Munsyari township (Gupta *et al.* 2017, Liu *et al.* 2017). The synergistic accumulation of heavy metals in *Stereocaulon foliolosum* observed between different estimated metals can be attributed to their higher deposition through vehicular exhaust (Ng *et al.* 2006, Liu *et al.* 2017).

The perpetual distribution of *Stereocaulon foliolosum* throughout the study landscape and correlative accumulation of heavy metals along different land use validates the bioindicator capabilities of Stereocaulon foliolosum in the temperate habitats of Himalaya. The study further highlights that lichens having such larger distribution can be used for long-term biomonitoring studies in the Himalayan habitats.

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