

A comparison of metallic contents in lichen *Pyxine subcinerea*, its substratum and soil

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Received: 29 November 2011 / Revised: 13 March 2012 / Accepted: 22 April 2012 / Published online: 12 May 2012
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Abstract Chromium, copper, cadmium, lead, nickel, iron and zinc contents of a lichen species (*Pyxine subcinerea* Stirton) and mango bark collected from 12 sites in Haridwar city (Uttarakhand) were compared with soil, sampled from beneath the tree from which lichens were collected. The metal contents in lichen, bark and soil ranged from 1,573 to 18,793, 256 to 590 and 684 to 801 $\mu\text{g g}^{-1}$, respectively. This clearly indicates that lichens accumulated higher amounts of metal compared to bark or soil. Statistical analysis revealed that metal concentration in lichens did not show significant linear correlation with the bark or soil. Pearsons correlation coefficients revealed negative correlation of Pb ($r = -0.2245$) and Ni ($r = -0.0480$) content between lichen and soil, which indicate direct atmospheric input of metals from ambient environment. Quantification and comparison of elemental concentration in lichens, its substratum and soil can provide valuable information about air quality in the collection area.

Keywords Atmospheric input · Bioaccumulation · Elemental content · Lichen · Statistical analysis

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Introduction

Lichens are slow-growing organism and form symbiotic associations between fungi, especially Ascomycota (mycobionts) and photoautotrophic organisms (photobionts), mainly green algae (Trebouxiophyceae and Ulvophyceae) and/or cyanobacteria. Lichens occur in nearly all terrestrial ecosystems, such as tropical, temperate, boreal climatic regions and frequently dominate the landscape in extreme environments, such as Arctic, Antarctic and Alpine climatic regions (Green et al. 2011). Lichens are commonly used to assess environmental disorder, help to biomonitor air quality, forest age and health, and climate change (Bjerke 2011; Crespo et al. 2004; McCune 2000; Nimis et al. 2002).

Lichens present thalloid structure and they lack roots or waxy cuticles and mainly rely on the atmospheric input of mineral nutrients (Wolterbeek et al. 2003; Paoli and Loppi 2008). Lichens take up nutrients from (a) from the substratum on which it is attached (bark, as in the case of epiphytic lichens) and (b) from the metal-enriched ambient atmosphere (particulates and dissolved ions) (Nieboer and Richardson 1981). The concentrations of trace elements in lichen thalli may be directly correlated with environmental levels of these elements (Loppi et al. 1999; Purvis et al. 2008; Shukla and Upreti 2007a). Therefore, lichen bio-monitoring can be applied to assess the air quality of an area. However bioaccumulation is dependent on various factors, one of them being the contribution of the substratum on which lichen colonizes (Lodenus et al. 2010; Thormann 2006; Baker 1983; Markert et al. 2003; St. Clair et al. 2002).

Lichens are employed worldwide for air quality assessment either by mapping lichen diversity (LDV and IAP studies) or by carrying out transplant studies in



polluted area and measuring bioaccumulation of contaminants within the thallus (Pfeiffer and Barclay-Estrup; 1992, Garty et al. 2003; Loppi and Frati 2006; Pinho et al. 2004; Shukla et al. 2010). Re- or neo-colonization of lichens has also been used to assess the air quality especially in metropolitan cities e.g. London, Madrid (Hawksworth and McManus 1989; Crespo et al. 2004).

Pyxine subcinerea Stirton is widely distributed in sub temperate regions of India. Earlier studies have been carried out to investigate the physiological response and concentration of polycyclic aromatic hydrocarbons in the species (Shukla and Upreti 2008; Shukla et al. 2011) but in order to propose it as an effective and reliable biomonitor for wider applicability, contribution of the substratum and the ambient atmosphere to the total metal concentration in the lichen, *P. subcinerea* is required to be investigated.

Thus, the aim of the present study was to discover, any significant correlation between the elemental composition of lichens and that of the bark or soil exists and hence to validate lichen, *P. subcinerea* Stirton as biomonitors of air pollution.

Study area

Haridwar city is an important holy pilgrimage and industrial hub of India, located in the southwestern part of Uttarakhand state (Lat. 29.96 °N, Long. 78.16 °E). The city is 249.7 m above sea level between the Shivalik Hills to the North and Northeast, and the Ganga River to the South. Temperatures range from 15 to 39.8 °C in the summer and 6 to 16.6 °C in the winter. The Haridwar district has a population of about 2,95,213 (2001 India census). Haridwar is growing fast as a major industrial center since Uttarakhand became a new state. The State Infrastructure & Industrial Development Corporation (SIDCUL) along with Bharat Heavy Electricals Limited (BHEL) controls large manufacturing plants, [Heavy Electrical Equipment Plant (HEEP) and Central Foundry Forge Plant (CFFP)]. These are located on the outskirts of the city. BHEL is the largest engineering and manufacturing enterprise in India and manufacturers, turbo generators, AC and DC motors, gas turbines, steel castings as well as steel forgings.

Tourism related to religious centre and industry is a major anthropogenic source of elevated metal levels in the ambient air of Haridwar city.

Air quality status in Haridwar city

The oxides of sulfur and nitrogen gases in Haridwar city are under prescribed limits set by the Central Pollution Control Board (CPCB), New Delhi. The ambient air concentration of SO₂ recorded from Shivalik Nagar close to BHEL Township and SIDCUL area ranged between 8.64

and 12.30 µg m⁻³ and 13.33 and 20.12 µg m⁻³, while NO_x gases were 13.12–19.40 µg m⁻³ and 18.33–24.40 µg m⁻³, respectively. However, the SPM and PM₁₀ exceed limits set by CPCB. The PM₁₀ at the two sites in Haridwar city ranged from 98.47 to 141.28 µg m⁻³ and 160.80 to 175.78 µg m⁻³ and the SPM ranged from 357.12 to 413.33 µg m⁻³ and 497.12 to 599.36 µg m⁻³ (Chauhan et al. 2010).

Materials and methods

The area in and around Haridwar city was surveyed for lichens in July, 2009. Lichen samples were collected from 12 sampling sites in the city (Fig. 1). At each site, samples were taken from ten isolated Mango trees (*Mangifera indica*). Lichen specimens were collected from trees which fulfilled standard criteria of (a) trunk more than 35 cm in

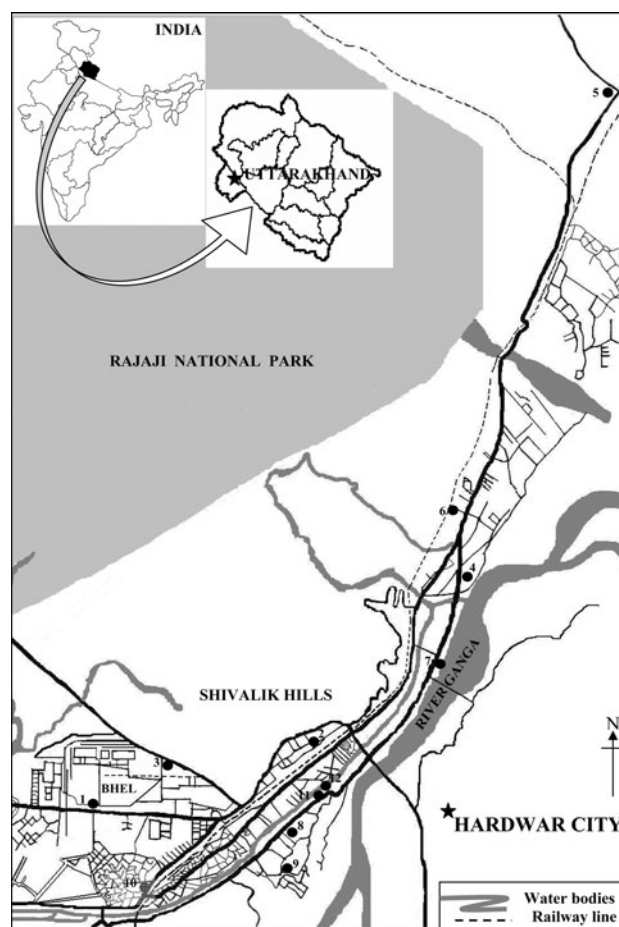


Fig. 1 Map showing different collection sites in and around Haridwar city, Uttarakhand, India. 1 BHEL (CFFP), 2 Roshnawala, SIDCUL, 3 BHEL, (HEEP), 4 Motichur, 5 Raiwala, 6 Motichur railway station, 7 Har ki Pauri, 8 Kankhal, Punjabi Khetra, 9 Kankhal, 10 Jwalapur, 11 Shankaracharya Chowk West, 12 Shankaracharya Chowk North

diameter, (b) trunk inclination less than 75° (15° deviation from vertical), (c) apparently healthy and (d) an available trunk height between 1.5 and 2 m above the ground. From each tree, 5–10 whole thalli of *P. subcinerea* were collected. In the laboratory, composite samples from each site were removed from the bark with sharp knife and sorted to remove extraneous material. The cleaned material was air-dried. The substratum (bark) and a soil sample (collected from beneath the tree) from which lichen was also collected and processed so that their elemental composition could be determined.

Metal analysis

The dried lichen and bark samples (three replicates) were ground to a powder in mortar; ≈ 1.0 g each was digested in 5 ml mixture of concentrated HNO_3 and HClO_4 (v/v 9:1) (beakers covered with watch glasses) for 1 h and then heated until close to dryness. Soil samples (weight ≈ 1.0 g each) were digested with conc. HNO_3 (5 ml) for 8 h. Residues were dissolved with 1 % HNO_3 (2 ml) followed by filtration through Whatman Filter paper No. 42 and diluted to 25 ml with 1 % HNO_3 . Analysis was done with Flame Atomic Absorption Spectrophotometer (Avanta- Σ , GBC, Australia). Stock standards were used from Merck India, traceable to NIST (National Institute of Standards Technology), to establish calibration curves that covered the region from 1, 2 and 3 $\mu\text{g g}^{-1}$ levels. Sensitivity of atomic absorption was 0.1 $\mu\text{g g}^{-1}$ for all the metals detected.

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) to evaluate the probable correlation pattern between the metal content from different sites using statistical program INDOSTAT (Hyderabad, India). Linear regressions and Pearsons correlations were obtained using MS EXCEL.

Results and discussion

Multi elemental contents in the lichen *P. subcinerea*, Mango bark and soil were compared, which revealed that total metal content in lichen, bark and soil ranged from 1,573 to 18,793, 256 to 590 and 684 to 801 $\mu\text{g g}^{-1}$, respectively (Table 1). The possible source of elements may be deduced from the pattern of elements in the sampled lichen (Table 2). Concentration of Pb and Ni was highest at the city centre highly influenced by the traffic activity followed by industrial sites. Pirintsos et al. (2006) concluded that enhanced levels of Cu and Zn, Fe and Zn,

Cd and Fe, Cd and Zn, and Pb and Ni indicated motor vehicles as the possible origin and the same was found in the present study (Table 3, significant at $P < 0.05$). Vehicular activity is reported to be the main source of atmospheric Cr, Cu and Pb (Loppi et al. 1998; Tuba and Csintalan 1993).

Cadmium is a constituent of lubricating motor oil, tyres and galvanized parts of vehicles, for example, concentrations range from 0.07 to 0.10 ppm in diesel oils and from 0.20 to 0.026 ppm in lubricating oils, while in automobile tyres levels range from 20 to 90 ppm (Kannan 1997). In the present study, the cadmium concentration was quite high in the lichen samples (4.2–51.45 $\mu\text{g g}^{-1}$) compared with its concentration in bark (0.45–2.97 $\mu\text{g g}^{-1}$) or soil (0.3–2.19 $\mu\text{g g}^{-1}$). This indicates an atmospheric origin for this metal. Competition between the metals to bind with sites holding the cations affects the uptake of metal (Nieboer and Richardson 1981). Hardiman and Jacoby (1984) observed that absorption of Cd by plant root was reduced in the presence of other cations of increasing valency or ionic radii.

Zinc is a component of automobile tyres and brake pads while nickel is found in car metal plating in welds and in tyres (Ward 1989; Sadiq et al. 1989). The current investigation (Table 3) suggests that there is a significant positive correlation between Cu and Zn ($r = 0.5841$) in lichens of Haridwar city, which indicates an anthropogenic origin.

The chromium concentration in non-polluted environments is reported to be quite low (Chettri et al. 1997). Cr uptake occurs as hexavalent chromate (CrO_4^{2-}) which is rapidly reduced to Cr^{3+} in soil. The trivalent form of Cr is absorbed minimally by root and its translocation from root to other parts is low (Streit and Stumm 1993). In the present study, Cr concentrations were quite low in bark ($3.4 \pm 0.33 \mu\text{g g}^{-1}$) compared with soil ($22.1 \pm 1.68 \mu\text{g g}^{-1}$) and lichens ($42.7 \pm 2.62 \mu\text{g g}^{-1}$), which reflects the fact that lichens and soil act as sinks for the pollutants. The Pb content ranged from 17.3 to 158 $\mu\text{g g}^{-1}$ which is quite low in comparison to Pb levels in other cities of Uttarakhand [Dehradun city: 66.6–12,433 $\mu\text{g g}^{-1}$ (Rani et al. 2011) and Pauri city: 231.9–425.9 $\mu\text{g g}^{-1}$ (Shukla and Upreti 2007b)]. This may be due to two factors: (i) the introduction of unleaded petrol and (ii) Haridwar is the starting point of the 'Holy voyage' and authorities encourage diesel-powered over petroleum-driven vehicles. The present observations are consistent with the findings of Ormrod (1984) and suggest that Pb, Zn, Cd, and Cu levels are linked with automobile traffic emissions.

Functional relationships were tested by applying linear regression. Scatter plots (Figs. 2, 3, 4) showed that there was no significant linear correlation between most of the metal in lichen and soil, affirming the atmospheric origin of these metals. The soil versus bark metal concentrations

Table 1 Overall mean trace element concentrations and its range ($\mu\text{g g}^{-1}$ dry weight) in lichen, bark and soil analyzed from 12 different sites in and around Haridwar city

	Cr	Cu	Pb	Cd	Ni	Fe	Zn
<i>Lichen, Pyxine subcinerea</i>							
Mean \pm SE	42.7 \pm 2.62	54.7 \pm 3.34	59.4 \pm 3.7	19.5 \pm 1.6	244 \pm 15.9	6,303 \pm 363	478 \pm 30.2
Range	16.4–94	19.7–144	17.3–158	4.2–51.45	13.5–719	795–17280	143–1,178
CV	10.64	10.56	10.89	14.59	11.28	9.99	10.97
F ratio	87.94	166.59	119.9	78.01	167.9	220.1	104.4
F prob.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<i>Bark</i>							
Mean \pm SE	3.4 \pm 0.33	4.29 \pm 0.38	4.19 \pm 0.33	1.26 \pm 0.28	2.14 \pm 0.38	338 \pm 21.3	32.5 \pm 2.59
Range	1.29–9.36	2.0–14.8	0.87–7.74	0.45–2.97	0.09–5.97	231–511	15.24–62.3
CV	16.68	15.22	13.74	38.46	30.78	10.91	13.81
F ratio	46.89	93.67	43.08	6.59	18.05	21.27	39.73
F prob.	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
<i>Soil</i>							
Mean \pm SE	22.1 \pm 1.68	25.0 \pm 1.33	15.5 \pm 1.83	0.84 \pm 0.23	28.77 \pm 2.39	555 \pm 17.1	80.2 \pm 4.87
Range	10.8–39.9	15.9–40.95	10.2–24.6	0.3–2.19	18.09–45.6	535–577	40.2–121
CV	13.15	9.24	20.45	48.15	14.39	5.33	10.51
F ratio	46.57	24.68	5.34	4.27	17.53	0.43	18.84
F prob.	0.0000	0.0000	0.0004	0.0018	0.0000	0.9274	0.0000



Table 2 Mineral element concentrations in thalli of *Pyxine subcinerea* collected from various sites of Haridwar city

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
1	6.9 ± 2.77	39.9 ± 6.05	20.4 ± 3.4	4,500 ± 224	25.50 ± 4.63	42.3 ± 2.84	224 ± 19.0
2	32.1 ± 5.05	32.1 ± 3.08	42.0 ± 2.16	7,410 ± 605	148.20 ± 4.39	102.75 ± 5.19	605 ± 19.8
3	4.2 ± 0.69	18.0 ± 2.1	19.65 ± 1.03	4,005 ± 143	187.80 ± 9.1	36.45 ± 3.23	143 ± 14.2
4	19.0 ± 2.98	16.4 ± 4.2	30.15 ± 2.79	16,185 ± 608	448.65 ± 51.8	98.25 ± 12.2	608 ± 15.7
5	21.45 ± 1.3	94.4 ± 3.8	32.40 ± 2.51	3,105 ± 158	139.65 ± 41.9	21.15 ± 3.99	158 ± 8.28
6	31.20 ± 1.7	65.9 ± 11.43	114.15 ± 6.26	5,265 ± 733	719.25 ± 28.6	157.8 ± 10.61	733 ± 4.54
7	30.45 ± 2.6	40.4 ± 2.04	43.95 ± 3.44	8,805 ± 260	284 ± 4.5	36.90 ± 1.32	260 ± 19.13
8	10.35 ± 0.6	22.9 ± 4.18	36.15 ± 2.69	4,365 ± 162	420.6 ± 17.5	67.35 ± 1.68	162 ± 19.13
9	14.10 ± 1.4	44.9 ± 5.75	36.30 ± 2.74	3,045 ± 663	13.50 ± 1.64	17.25 ± 2.36	663 ± 31.9
10	6.0 ± 0.707	17.6 ± 2.99	23.85 ± 4.26	795 ± 494	189.45 ± 11.4	47.55 ± 3.78	494 ± 19.44
11	51.45 ± 2.4	74.1 ± 4.41	113.4 ± 11.55	17,280 ± 1178	52.35 ± 2.41	43.8 ± 1.98	1178 ± 138
12	6.15 ± 0.75	45.8 ± 6.4	144.1 ± 4.90	870 ± 502	303.3 ± 13.77	41.4 ± 4.91	502 ± 11.8
<i>F</i> ratio	78.01	87.9	166.59	220.12	167.85	119.88	104.4
<i>F</i> prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Concentrations are given as $\mu\text{g g}^{-1}$ dry wt. (values expressed as mean \pm standard error) and *F* values along with *F* probability obtained by one way ANOVA analysis

1 BHEL (CFFP) (industrial), 2 Roshnawala, SIDCUL (industrial), 3 BHEL, (HEEP) (industrial), 4 Motichur (periurban), 5 Raiwala (periurban), 6 Motichur railway station (periurban), 7 Har ki Pauri, (city center) 8 Kankhal (urban), Punjabi Khetra (urban), 9 Kankhal (urban), 10 Jwalapur (urban), 11 Shankaracharya Chowk West (urban), 12 Shankaracharya Chowk North (urban)

Table 3 Values of correlation coefficient between the elements analyzed in lichen, *P. subcinerea*

	Cu	Pb	Cd	Ni	Fe	Zn
Cr	0.4531	-0.0962	0.5422	-0.0963	0.0659	0.2732
Cu		0.2764	0.4004	0.3346	0.1120	0.5841*
Pb			0.3025	0.7868**	0.2470	0.3124
Cd				0.0494	0.7148**	0.6899**
Ni					0.0555	0.0038
Fe						0.5628*

* $P < 0.05$, ** $P < 0.01$

showed a non-significant correlation. Pearsons correlation was then applied to establish the strength of the linear relationship. The Pearsons correlation (Table 4) further substantiated the atmospheric origin of the metals present at higher concentrations in the lichen samples. The Pearson correlation also showed a weak negative correlation of Pb content ($r = -0.2245$) and Ni ($r = -0.0480$) in lichen and soil, affirming the anthropogenic origin. The metals like Cu, Cr and Zn showed positive correlation between lichen and bark content (Cu: $r = 0.6932$:: Cr: $r = 0.8642$:: Zn: $r = 0.5434$), which suggests that bioaccumulation of these metals in lichens is both from direct atmospheric deposition and also through the bark of the trees when growing in contaminated soil.

The morphology and anatomy of lichen play an important role in the accumulation of metals (Goyal and Seaward 1982). Among the different foliose lichens, *P. subcinerea* accumulates higher ranges of metals than *Ramalina lacera* (With.) J.R. Laund., *Parmelia sulcata*, *Evernia prunastri*, which are utilized for biomonitoring of inorganic pollutants in other parts of the world (Garty et al. 2003; Loppi et al. 1998, 1999; Lodenius et al. 2010). Result also indicates that *Phaeophyscia hispidula*, a closely related Physciaceae member, quite similar in morphology accumulates higher concentration of metals especially iron in comparison to *P. subcinerea* (Shukla and Upreti 2007b; Rani et al. 2011). The probable reason of higher metal content may be due to presence of dense tuft of rhizinae on the lower side of thallus which act as metal reservoir (Goyal and Seaward 1982).

Conclusion

The present study suggests that the elevated metal content of *P. subcinerea* in Haridwar city, India, is mainly due to adsorption/absorption from direct atmospheric input of anthropogenic origin. The statistical treatment of the lichen biomonitoring data helps to elucidate the sources of inorganic pollutants (substratum and/or atmosphere) and helps



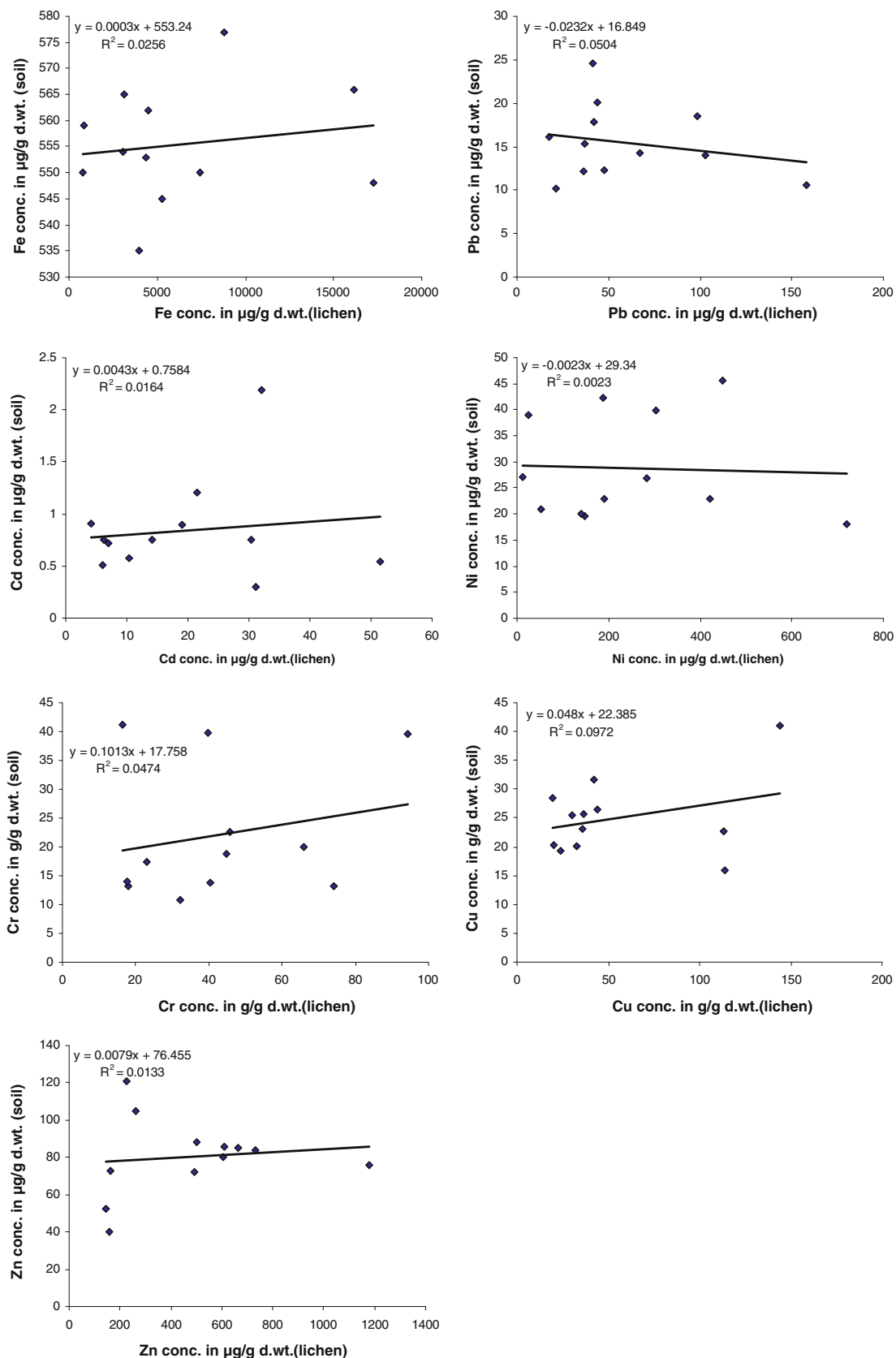


Fig. 2 Scatter plot showing the linear regression between the individual metal concentration (in $\mu\text{g/g}$) in lichen and soil at 12 sampling sites in Haridwar city

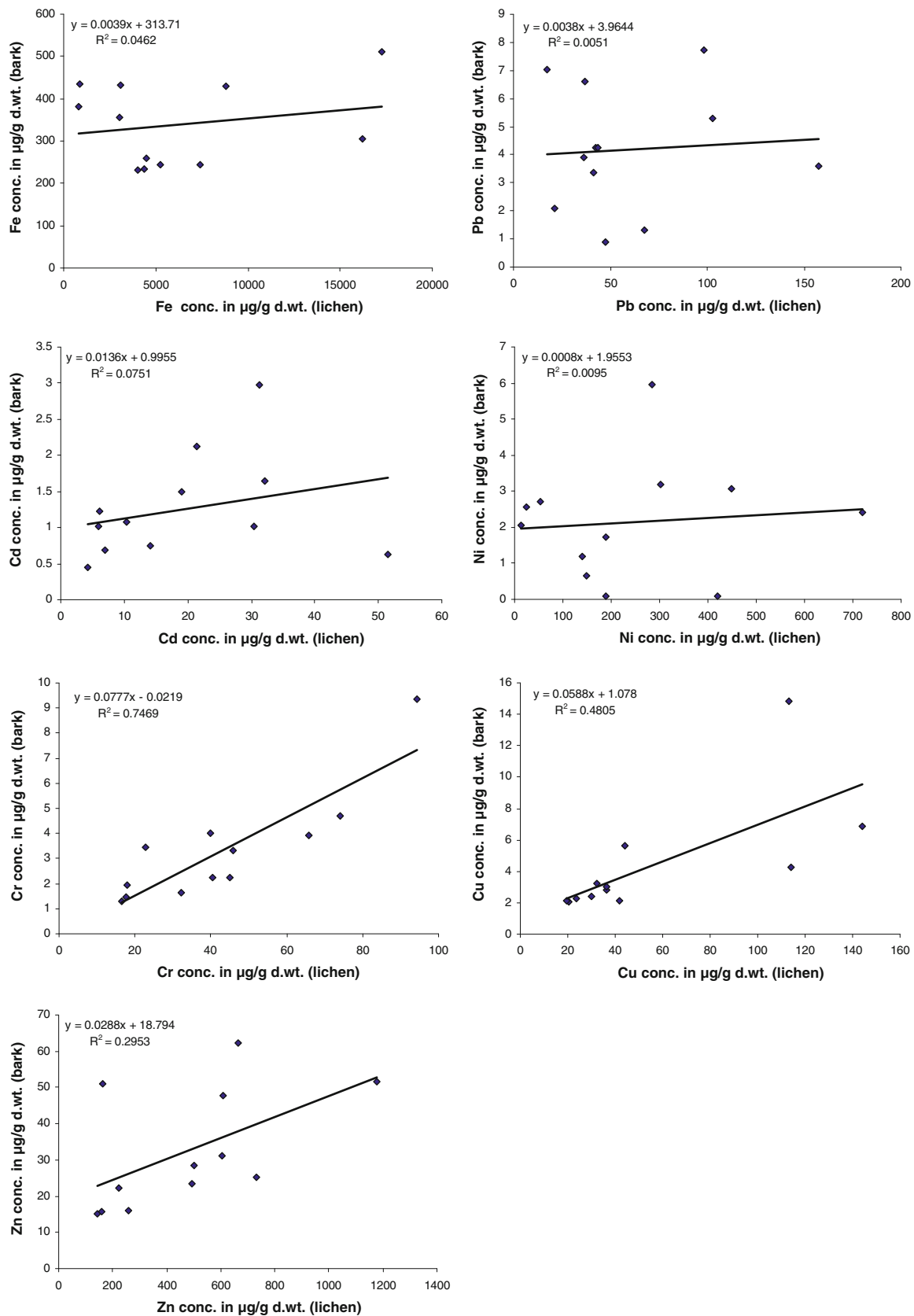


Fig. 3 Scatter plot showing the linear regression of individual metal concentration (in $\mu\text{g/g}$) in lichen and bark samples at 12 sampling sites in Haridwar city

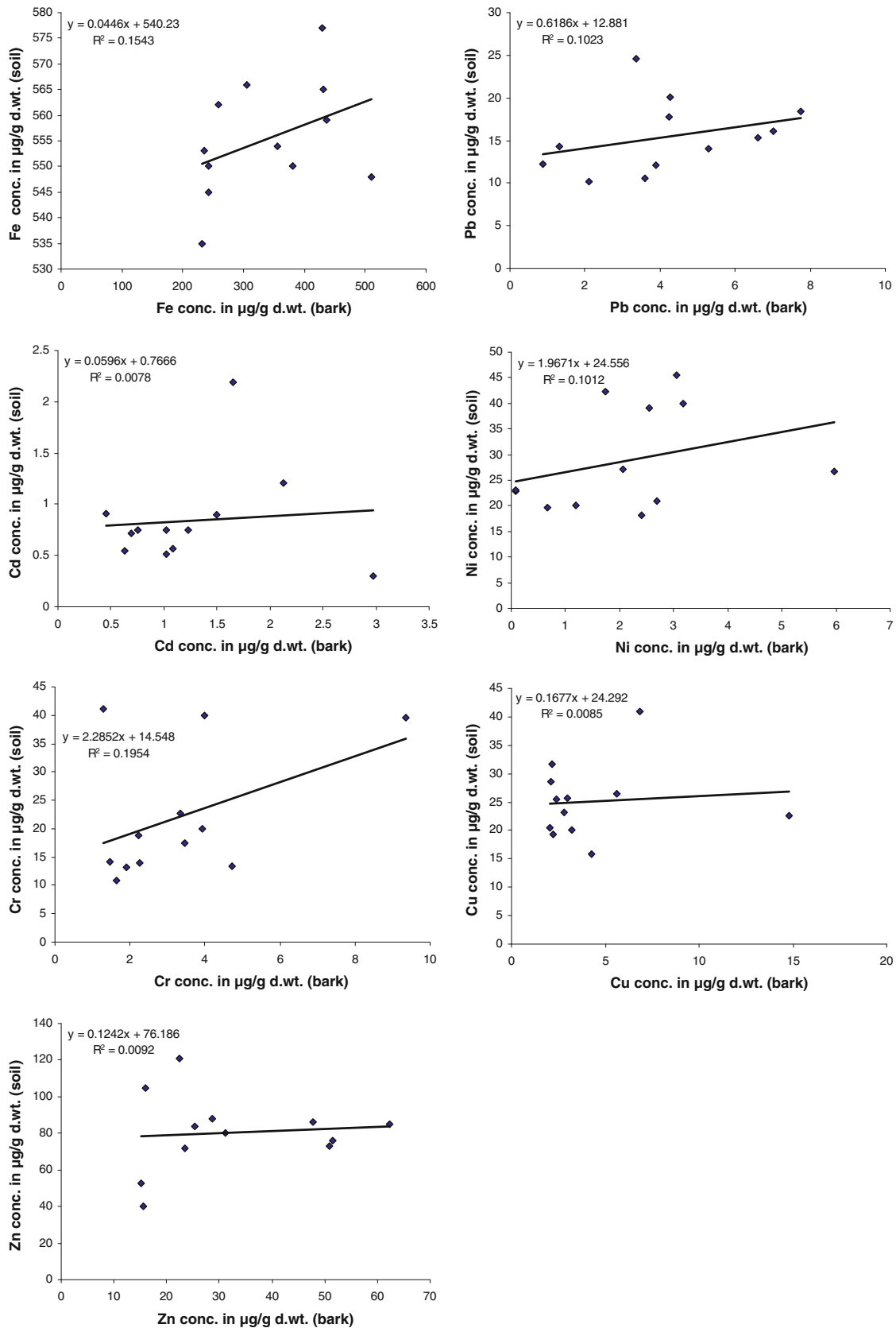


Fig. 4 Scatter plot showing the linear regression of individual metal concentration (in µg/g) in soil and bark samples at 12 sampling sites in Haridwar city



Table 4 Pearsons correlation between individual elemental concentration in lichen with bark and soil and between bark and soil

	Lichen versus bark	Lichen versus soil	Bark versus soil
Cr	0.8642**	0.2178	0.4419
Cu	0.6932**	0.3118	0.0923
Pb	0.0712	−0.2245	0.3198
Cd	0.2741	0.1282	0.0885
Ni	0.0977	−0.0480	0.3181
Fe	0.2151	0.1601	0.3927
Zn	0.5434**	0.1154	0.0960

* $P < 0.05$, ** $P < 0.01$

to distinguish between the contribution of the substratum and atmospheric inputs of metals.

Thus the present study establishes value of the lichen *P. subcinerea* Stirton, as a biomonitor for inorganic pollutants when assessing air quality of urban/industrial area in the Indian subcontinent.

Acknowledgments Authors (M.Y. and V.S.) are thankful to the Vice Chancellor, Babasaheb Bhimrao Ambedkar (Central) University, Lucknow for providing Laboratory facilities. V.S. wishes to thank Scientific and Engineering Research Council Division, Department of Science and Technology, New Delhi (SR/FT/LS-028/2008) for the financial support. Authors wish to thank Dr. Sudhir Shukla for statistical analysis of the data and Ms Anu Rastogi for valuable help.

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