

Heavy metal accumulation in *Pyrrrosia flocculosa* (D. Don) Ching growing in sites located along a vehicular disturbance gradient

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Received: 22 June 2016 / Accepted: 24 August 2016 / Published online: 3 September 2016
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Abstract Monitoring of environment is a key contemporary issue that has necessitated search for bio-indicators. The very fact that epiphytes do not have a direct contact with soil and absorb nutrients from the environment puts them among the best indicators of environmental conditions. We, therefore, selected *Pyrrrosia flocculosa* (D. Don) Ching—an epiphytic fern that commonly occurs in the Himalaya for this study. The study focused on analyzing heavy metal concentrations in the fronds of *P. flocculosa* growing along a disturbance gradient. For this, three sites representing different levels of disturbance viz., least disturbed, moderately disturbed, and highly disturbed, were identified in Kangra district of Himachal Pradesh. From each site, fronds of *P. flocculosa* were collected, categorized into three growth stages (juvenile, young, and mature), and brought to the laboratory for analyses. After drying and powdering, the samples were analyzed for Pb, Cd, Fe, Ni, Cu, Mn, and Zn using atomic absorption spectrophotometer. The results obtained were statistically compared using the software package Statistica. As expected, concentration of the metals varied among the sites

and also among the identified growth stages of the species. In general, concentration of the metals was in the order Fe (639.28 ± 81.63) > Ni (56.03 ± 4.97) > Mn (7.54 ± 0.69) > Zn (6.51 ± 0.36) > Cd (4.01 ± 0.86) > Cu (1.93 ± 0.74). Barring Mn, concentration of all the metals increased with disturbance and was positively correlated to it. However, except for Cd and Fe, none of the metals reported higher than threshold values. Effective monitoring of the environment can thus be done using *P. flocculosa*.

Keywords Epiphyte · Heavy metals · Bio monitoring · Himalaya · Environment

Introduction

Environmental pollution has considerably risen across the globe (Hauck 2009; Bajpai et al. 2014). Today, it is a major problem that has serious ecological, economical, and health ramifications (Gajananda et al. 2005; Sharma et al. 2011). This, therefore, has necessitated initiatives for monitoring the environment (Samecka-Cymerman et al. 2010; Loppi 2014). Monitoring by setting up of environmental stations is costly and labor intensive and is therefore well suited for limited areas. On the other hand, plants are easily available, widely distributed, and relatively inexpensive to monitor. Due to their high tolerance capacity to varied pollutants and their availability in remote areas, sampling of plants is relatively easy. Thus, they are well suited for this purpose (Kono and Tomiyasu 2009; Ashraf et al. 2010; Ojo et al. 2012;

Electronic supplementary material The online version of this article (doi:10.1007/s10661-016-5561-3) contains supplementary material, which is available to authorized users.

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Ozturk et al. 2015). Consequently, the focus is now on identifying plants that can be used for monitoring and assessing the status of environment (Ozturk et al. 2008). Species such as *Salvinia herzogii*, *S. minima*, and *S. natans* have been used as indicators of water pollution (Wolff et al. 2012). At the same time *Athyrium yokoscense*, *Pteris vittata*, and *Pityrogramma calomelanos* are commonly used for monitoring heavy metal contamination in soil (Nishizono et al. 1987; Mandal et al. 2014). On the other hand, *Diplotaxis tenuifolia*, *Phaeophyscia hispidula*, *Opeas pumilum*, *Lecanora conizaeoides*, and *Tillandsia caput-medusae* have been used to monitor air pollution (Brighigna et al. 1997; Shukla and Upreti 2007; Ojo et al. 2012; Ozturk et al. 2013; Loppi 2014; Bajpai et al. 2014).

During the past few years, pollution due to vehicles has also increased in the Himalayan region (Meena et al. 2012; Ganguly and Thapa 2016). Even the interior areas of Himalaya have reported a sharp increase in vehicle density (Shukla and Upreti 2007; Uniyal and Singh 2012; Ganguly and Thapa 2016). The fumes resulting from gasoline combustion in the vehicles leads to addition of lead (Pb) to the environment while vehicle wear and tear adds iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), etc. to the air (Ojo et al. 2012; Aksoy et al. 1999). These heavy metals constitute toxic pollutants and their monitoring using native plant species is desired (Kono et al. 2012; Ojo et al. 2012). We, therefore, selected *Pyrrhosia flocculosa* (D. Don) Ching (family Polypodiaceae) for analyzing heavy metal concentration along a vehicle disturbance gradient in the Himalayan region. *P. flocculosa* is an epiphytic fern that commonly occurs in the Himalaya and has a wide altitudinal gradient (Hovenkamp 1986). Being an epiphyte, major source of nutrients for the species is air (Brighigna et al. 1997; Bhatt et al. 2015). The very fact that epiphytes do not have a direct contact with soil puts them among the best indicators of vehicular air pollution (Brighigna et al. 1997). The species, thus, meets the requirements of an ideal air quality monitoring plant (Aksoy et al. 1999; Wolterbeek 2002).

The study specifically targeted (1) analyzing heavy metal concentrations in the fronds of *P. flocculosa* growing along a disturbance gradient, i.e., at least disturbed, moderately disturbed, and highly disturbed sites; (2) analyzing metal concentration in different growth stages of the fronds; and 3) correlating heavy metal concentration with disturbance.

Methodology

Site selection

Three sites representing different levels of disturbance viz., least disturbed, moderately disturbed, and highly disturbed were identified in Kangra district of Himachal Pradesh (Fig. 1). Categorization of the sites was done based on proximity to road and the frequency of vehicles plying on it (Aksoy et al. 1999; Shukla and Upreti 2007). Highly disturbed site represents a state highway that connects Baijnath to Mahakal. Owing to presence of famous Shiv temples, vehicle movement is relatively higher in this site. On the other hand, moderately disturbed site represents a village road that has limited vehicular movement. The least disturbed site lies in the interiors and is a bridle path. An estimate of vehicle density was generated by simultaneously recording the number of vehicles plying in each of these sites. Recordings were done on a single pre-determined date and at three time periods (morning, midday, and evening). Each recording session lasted for 30 min (Table 1). At the macro level, all the three sites are similar and lie between 900 and 930 m asl. The coordinates of the sites were recorded using a Trimble Juno SC global positioning system (Fig. 1).

Sample collection

At each site, three common phorophytes were identified. From these, fronds of *P. flocculosa* were collected on a day when there had been no rainfall during the preceding 15 days. This was done to avoid the affect of leaching (Markert 1994). The samples were collected from tree trunks 1.5 to 3 m above the ground level (Kono and Tomiyasu 2009; Shukla and Upreti 2007; Khairudin et al. 2014). Based on frond characteristics, the collected samples were categorized into juvenile, young, and mature growth stages (Mehlreter 2010). We define juvenile fronds as the ones that were smaller in size, curled, and soft in texture. Fronds that had attained full dimensions and had a fully open lamina were categorized as young, while the fronds that also had spores were classified as mature (Mehlreter 2010). A total of 27 samples

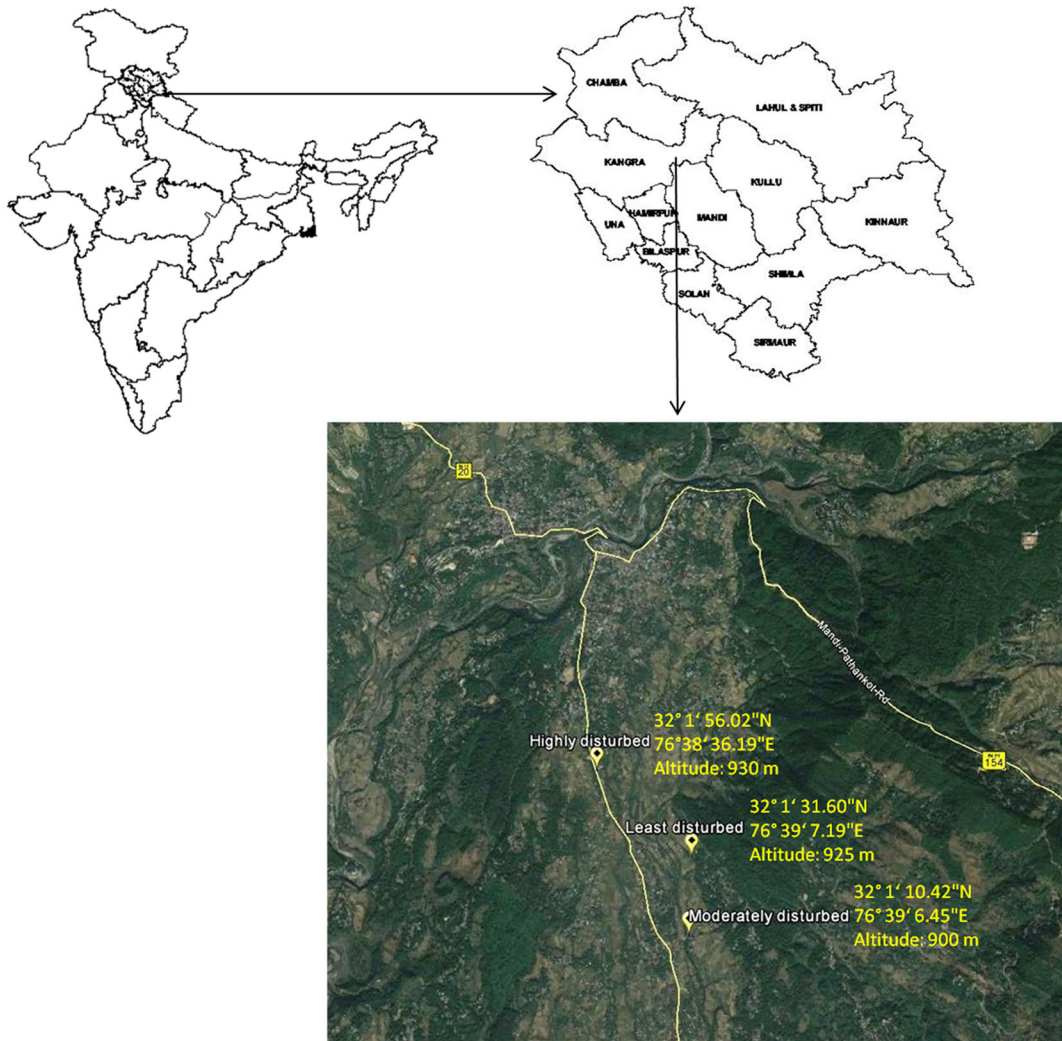


Fig. 1 Location of the study site in the west Himalayan state of Himachal Pradesh

(3 sites × 3 phorophytes × 3 growth stages) were collected. Each sample was placed separately in a brown paper bag, labeled, and brought to the laboratory for analyses.

Sample processing

Samples were weighed on a Mettler Toledo ME104 electronic balance and oven dried at 60 °C till constant

Table 1 Number of vehicles recorded at the three time periods in the identified sites and the general characteristics of the sites

Site	Morning (8:30–9 a.m.)	Midday (1:30–2 p.m.)	Evening (6:30–7 p.m.)	General characteristics
Least disturbed	01	0	01	Interior area close to narrow bridle path
Moderately disturbed	26	22	9	Area away from the road with limited vehicle movement
Highly disturbed	185	90	125	At a distance of 5 m from the state highway. Relatively heavy vehicular movement.

weight was achieved (Aksoy et al. 1999; Shukla and Upreti 2007). Once dry, the samples were powdered and sieved through a 1-mm sieve.

Heavy metal analyses

The samples were analyzed for total Pb, Cd, Fe, Ni, Cu, Mn, and Zn. For this, the dried samples were weighed (1 g each) and digested in a mixture of HNO₃ (15 ml) and HClO₄ (3 ml). Digested samples were filtered through Whatman filter paper no 42. To the filtrate, distilled water was added to make 100 ml volume. The samples were then analyzed using atomic absorption spectrophotometer (AAS), AA-6300, Shimadzu, Japan. The detection limits for Pb, Cd, Fe, Cu, Zn, Mn, and Ni were 0.05, 0.005, 0.025, 0.008, 0.002, 0.01, and 0.02 ppm, respectively.

Statistical analyses

The results so obtained were compared using ANOVA and correlated using Pearson's correlation. To test for significant differences between the groups, Tukey's post hoc test was performed. The data was subjected to discriminant analysis to discriminate sites based on the concentration of heavy metals. All statistical analyses have been done using the software package Statistica (StatSoft 2004).

Results

Trend of increasing heavy metals across the disturbance gradient is clearly evident in the present study. As expected, the three identified sites differed with respect to vehicular movement. Vehicle movement was higher in the highly disturbed site as compared to the moderately and least disturbed sites (Table 1). Similarly, heavy metal concentration in *P. flocculosa* also varied among the sites. For majority of the metals, their concentration was higher in the samples collected from the highly disturbed sites and lower in the samples collected from the relatively less disturbed sites. Overall concentration of the metals was in the order Fe (639.28 ± 81.63) > Ni (56.03 ± 4.97) > Mn (7.54 ± 0.69) > Zn (6.51 ± 0.36) > Cd (4.01 ± 0.86) > Cu (1.93 ± 0.74). Lead was not detected in any of the samples.

Concentration of Cd varied among the sites and ranged from below detection limit (BDL) to 10.96 ppm (Table 2). It was not detected in the samples collected from the least disturbed site. Mean concentration of Cd was significantly greater in the highly disturbed site (8.35 ± 0.84, $p < 0.05$) compared to the moderately disturbed site. Samples from the moderately disturbed site had 56.05 % less Cd with respect to samples from the highly disturbed site. The coefficient of variation (CV (%)) for Cd in *P. flocculosa* was 104.41 (Table 2). A significant positive correlation was found between Cd and Fe ($r = 0.57$, $p < 0.05$) and Cd and Ni ($r = 0.57$, $p < 0.05$). However, in case of Cd and Mn ($r = -0.75$, $p < 0.05$), this relationship was significantly negative (Fig. 2). A non-significant weak positive relationship with

Table 2 Metal concentration (ppm) in *Pyrrrosia flocculosa* collected from different sites

	Cd	Fe	Cu	Zn	Mn	Ni
Highly disturbed	4.38–10.96 (8.35 ± 0.84)	757.89–1471.34 (1165.21 ± 68.91)	BDL–18.67 (3.74 ± 1.96)	5.26–9.79 (7.30 ± 0.45)	4.02–6.72 (5.39 ± 0.34)	11.32–104.64 (72.73 ± 10.17)
Moderately disturbed	BDL–10.35 (3.67 ± 1.48)	123.21–926.97 (447.18 ± 81.82)	BDL–7.24 (2.06 ± 0.84)	3.65–7.37 (5.71 ± 0.43)	2.05–13.58 (7.60 ± 1.48)	30.91–90.57 (55.99 ± 7.30)
Least disturbed	BDL	208.51–414.36 (305.44 ± 22.30)	BDL	3.18–10.14 (6.53 ± 0.86)	5.98–14.29 (9.63 ± 1.08)	26.72–57.51 (39.36 ± 3.83)
CV (%)	104.41	72.10	96.96	12.23	28.12	29.78
One-way ANOVA						
<i>p</i> value	1.6E–0.5*	0.12	0.44	0.21	0.03*	0.02*

Values outside parentheses represent the range, while those in parentheses are the means

BDL below detection limit

*Significant at 0.05

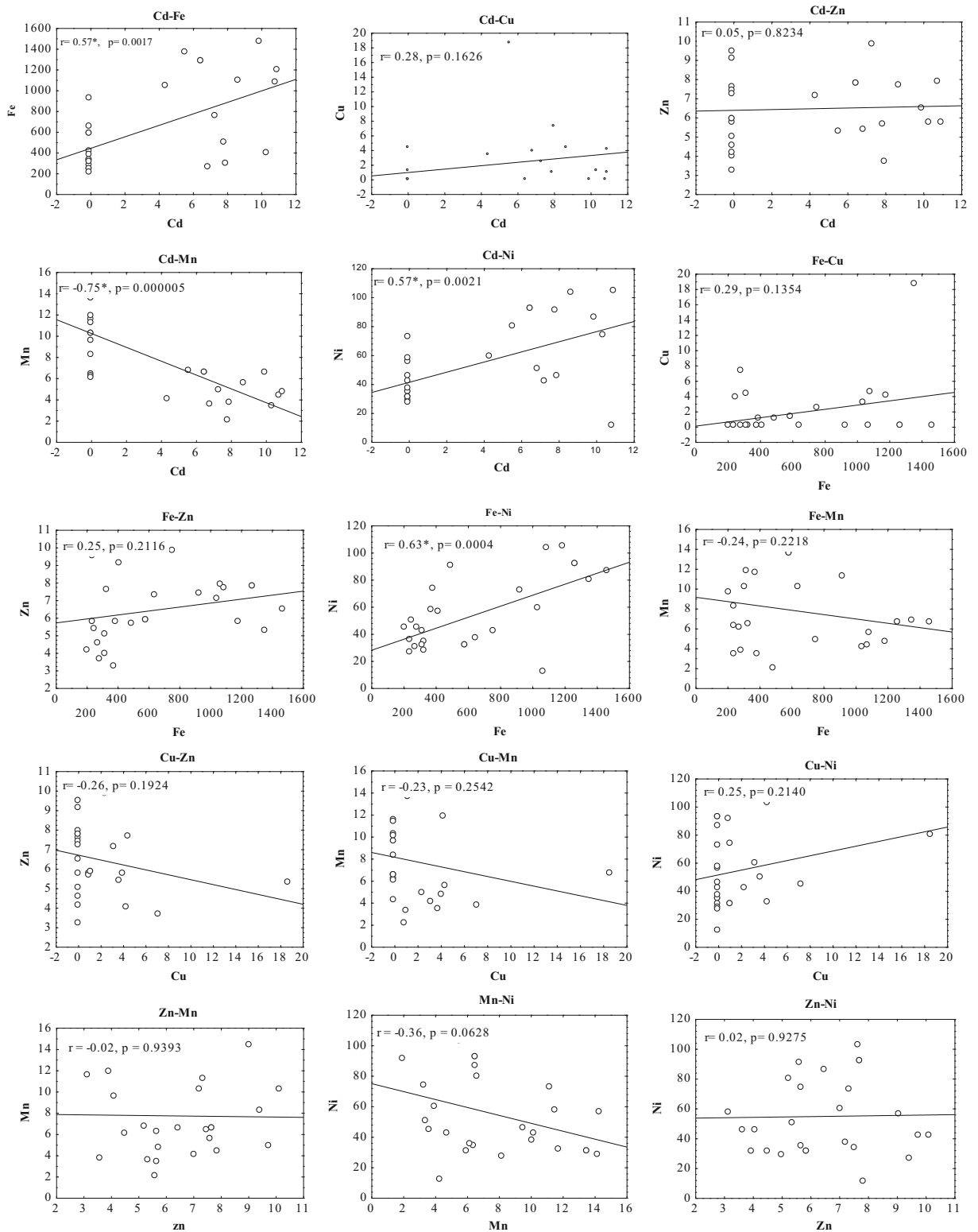


Fig. 2 Correlation among different metals found in *P. flocculosa*

Cu ($r = 0.28$) and almost no relationship with Zn ($r = 0.05$) was recorded. Mean concentration of the metal varied among the different growth stages of the fern (Table 3). As compared to the mature and young fronds, juvenile fronds reported higher concentration of Cd in both the highly and moderately disturbed sites (Fig. 3).

In case of Fe, its concentration ranged between 123.21 and 1471.34 ppm and declined with reducing disturbance (Table 2). Significantly higher concentration of Fe was recorded in samples from the highly disturbed site (1165.21 ± 68.91 , $p < 0.05$) when compared to samples from the moderately (447.18 ± 81.82) and least disturbed sites (305.44 ± 22.30) (Table 2; see supplementary material). Concentration of Fe was 61.62 % lower in the moderately disturbed site when compared to the highly disturbed site. On the other hand, least disturbed site had 31.70 % less Fe compared to the moderately disturbed site. Its reported CV (%) was 72.10 (Table 2). In addition to Cd, Fe also had a significant positive correlation with Ni ($r = 0.63$, $p < 0.05$) (Fig. 2). At the same time, weak positive relationships of Fe with Cu ($r = 0.29$) and Zn ($r = 0.25$) and a weak negative relationship with Mn ($r = -0.24$) were not significant (Fig. 2). Among the stages, the metal reported highest concentration (654.95 ± 112.96) in juvenile fronds as compared to young and mature fronds (Table 3). With respect to site and stages, in moderate and least disturbed sites, the highest concentration of Fe was in the juvenile fronds (Fig. 3). However, in the highly disturbed site, mature (1354.10 ± 68.12) followed by young (1134.36 ± 70.18) and juvenile fronds (1007.18 ± 128.91) was the order (Fig. 3).

Concentration of Cu ranged between BDL and 18.67 ppm (Table 2) and followed a trend similar to Cd and Fe. Cu reported highest mean value (3.74 ± 1.96) in the highly disturbed site and was not detected in the samples from the least disturbed site (Table 2). Samples

from the moderately disturbed site had 44.92 % less Cu than the samples from the highly disturbed site. Though Cu had a weak positive correlation with Cd, Fe, and Ni, it had a negative correlation with Mn ($r = -0.23$) and Zn ($r = -0.26$). However, these correlations were not significant (Fig. 2). Its CV (%) along the disturbance gradient was 96.96 (Table 2). Cu reported highest mean values (3.46 ± 2.08) in mature fronds (Table 3). In all the sites, Cu had higher values in mature fronds followed by young and juvenile fronds (Fig. 3).

Zinc, on the other hand, reported values ranging between 3.18 and 10.14 ppm (Table 2). Highest mean concentration of Zn (7.30 ± 0.45) was in the highly disturbed site, while the lowest (5.71 ± 0.43) was in the moderately disturbed site (Table 2). Thus, concentration of Zn significantly varied ($p < 0.05$) between the highly and the moderately disturbed sites (see supplementary material). With respect to the highly disturbed site, 21.78 % less Zn was present in the samples from the moderately disturbed site. Samples from the least disturbed site had 14.36 % higher Zn compared to the samples from the moderately disturbed site. Zn had a weak positive and negative correlation with Fe ($r = 0.25$) and Cu ($r = -0.26$), respectively (Fig. 2). It had almost no relationship with Cd ($r = 0.05$), Mn ($r = -0.02$), and Ni ($r = 0.02$). These correlations were not significant (Fig. 2). Its CV (%) along the disturbance gradient was 12.23 (Table 2). Highest concentration of Zn (7.65 ± 0.58) was in the juvenile fronds when compared to the young and mature fronds (Table 3). In all the three sites, Zn reported highest concentration in juvenile fronds followed by young and mature fronds (Fig. 3).

Concentration of Mn ranged from 2.05 to 14.29 ppm. It reported highest mean value (9.63 ± 1.08) in the least disturbed site followed by 7.60 ± 1.48 in the moderately

Table 3 Metal concentration (ppm) in different growth stages of *Pyrrhosia flocculosa*

Metal	Juvenile	Young	Mature
Cadmium (Cd)	BDL–10.96 (5.26 ± 1.71)	BDL–8.73 (2.94 ± 1.22)	BDL–10.91 (3.82 ± 1.57)
Iron (Fe)	316.41–1188.78 (654.95 ± 112.96)	244.87–1272.18 (639.90 ± 134.23)	123.21–1471.34 (623.00 ± 184.98)
Copper (Cu)	BDL–4.05 (0.94 ± 0.47)	BDL–4.43 (1.4 ± 0.62)	BDL–18.67 (3.46 ± 2.08)
Zinc (Zn)	5.63–10.14 (7.65 ± 0.58)	3.18–9.46 (6.58 ± 0.60)	3.65–8.08 (5.30 ± 0.49)
Manganese (Mn)	2.05–14.29 (6.81 ± 1.38)	3.47–13.58 (7.69 ± 1.14)	3.7–14.26 (8.11 ± 1.12)
Nickel (Ni)	11.32–104.64 (58.39 ± 9.85)	26.72–102.6 (54.50 ± 8.97)	28.11–85.86 (55.18 ± 7.91)

Values outside parentheses represent the range, while those in parentheses are the means

BDL below detection limit

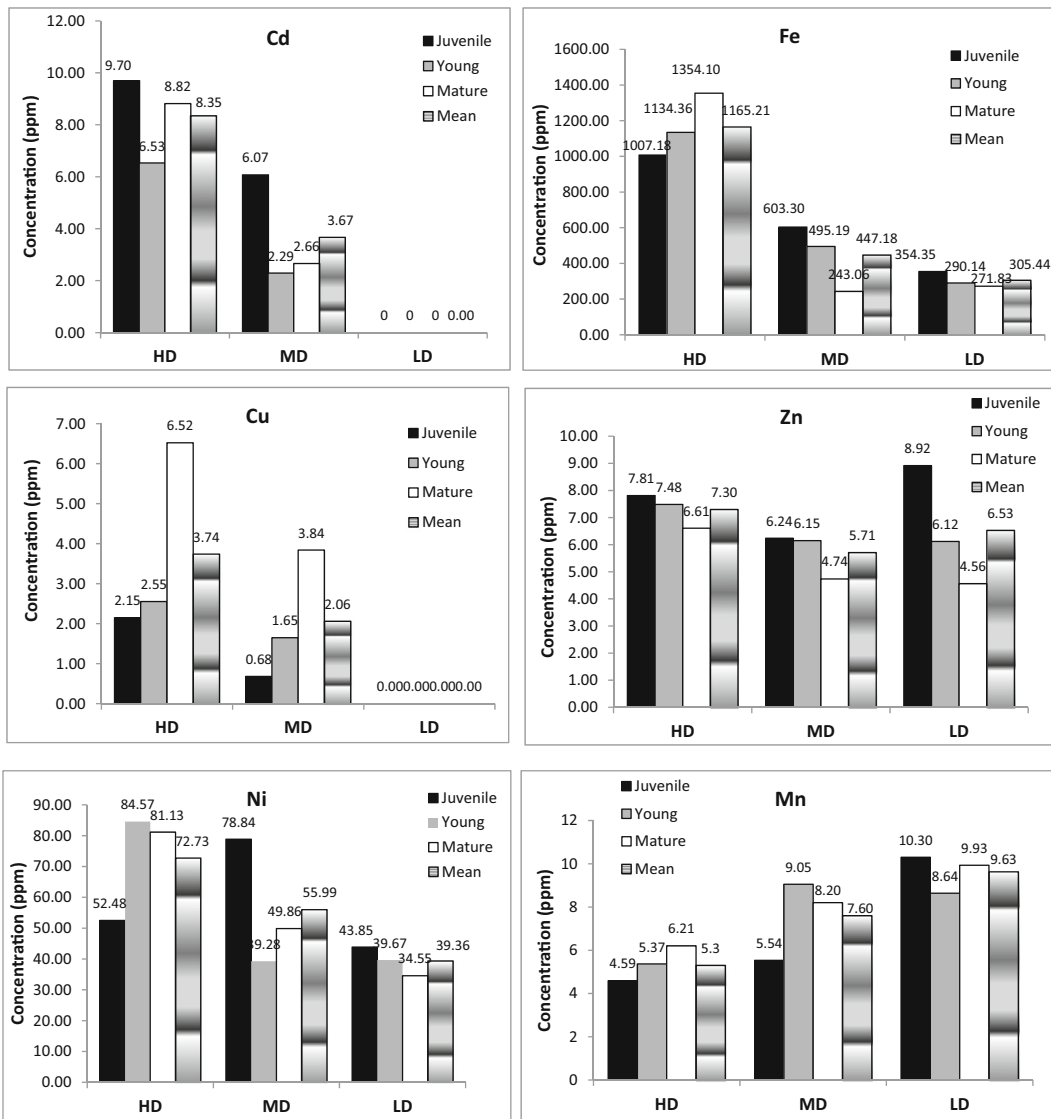


Fig. 3 Concentration of studied metals at different sites and in different growth stages (HD highly disturbed, MD moderately disturbed, and LD least disturbed)

disturbed and 5.39 ± 0.34 in the highly disturbed site (Table 2). Mn varied significantly ($p < 0.05$) between the highly and the least disturbed sites. Samples from the least disturbed site had 26.71 % higher Mn than those from the moderately disturbed site. On the other hand, samples from the moderately disturbed site had 41.00 % higher Mn compared to the samples from the highly disturbed site. The CV (%) for Mn was 28.12 (Table 2). Interestingly, Mn had a negative correlation with all the metals (Fig. 2). This relationship was strong in the case of Cd ($r = -0.75, p < 0.05$), moderate for Ni ($r = -0.36$),

and weak in the case of Fe ($r = -0.24$) and Cu ($r = -0.23$) (Fig. 2). Mn had almost no relationship with Zn ($r = -0.02$) (Fig. 2). With respect to stages, mature fronds had highest Mn (8.11 ± 1.12) when compared to juvenile and young fronds (Table 3). With respect to stages and sites, mature fronds had highest Mn concentration (6.21 ± 0.42) in the highly disturbed site, while young fronds reported highest Mn concentration (9.05 ± 2.97) in the moderately disturbed site (Fig. 3). In the least disturbed site, juvenile fronds (10.30 ± 2.27) had the highest Mn concentration (Fig. 3).

Value of Ni ranged between 11.32 and 104.64 ppm. Maximum concentration of it was recorded in the samples from the highly disturbed site (72.73 ± 10.17). In agreement to other metals, Ni declined with declining disturbance. It varied significantly ($p < 0.05$) between the highly and the least disturbed sites. Compared to highly disturbed site, 23.01 % less Ni was recorded in the samples from the moderately disturbed site. From moderately to least disturbed site, the observed decline was 29.70 %. Its reported CV (%) was 29.78 (Table 2). Ni had a strong positive relationship with Cd ($r = 0.57$, $p < 0.05$) and Fe ($r = 0.63$, $p < 0.05$) and a weak positive relationship with Cu ($r = 0.25$) (Fig. 2). Strength of this relationship was moderate with Mn ($r = -0.36$) and almost none with Zn ($r = 0.02$). These correlations were, however, not significant (Fig. 2). Highest concentration of Ni was in the juvenile fronds (58.39 ± 9.85) as compared to the young and mature fronds (Table 3). With respect to site and stage, young fronds had highest Ni concentration (84.57 ± 13.04) in the highly disturbed site while in the moderately and least disturbed sites, juvenile fronds had higher Ni concentration (Fig. 3).

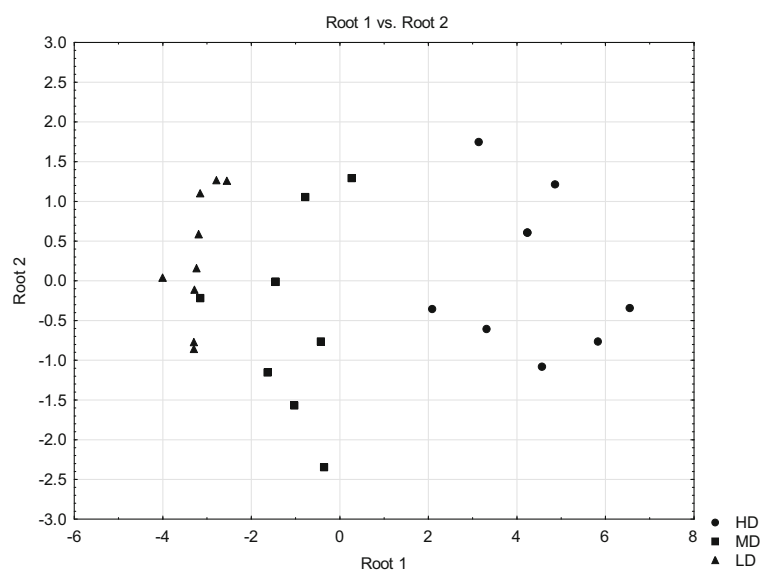
Overall, with respect to stages and sites, majority of the metals did not report significant differences (see supplementary material). However, Zn showed a significant difference ($p < 0.05$) between mature and juvenile fronds in the least disturbed site. The difference in Mn was significant between mature and juvenile fronds in the highly disturbed site only ($p < 0.05$). Ni, on the other hand, reported significant difference between juvenile

and young fronds in the moderately disturbed site only (see supplementary material). Thus, less variation is seen among the stages. Variations in metal concentration were more prominent between the sites. This is also clearly visible in the scatter plot generated using the discriminant analysis (Fig. 4). The scatter plot shows that significant and clear discrimination is possible for the highly disturbed site by the first discriminant function. It is on the right hand side of root 1. It indicates the differences between the sites depending upon vehicle flow and consequent metal concentrations (Chardi 2016).

Discussion

The study area and its surroundings are devoid of any major industry, and thus the prime sources of pollution in the area are vehicles and developmental activities. All metals, except Mn, had higher concentration in the highly disturbed site. This is expected as concentration of heavy metals is usually higher in the highly disturbed sites where human and developmental activities lead to more of their emissions (Li et al. 2008; Petrotou et al. 2012). Here, relatively higher number of vehicles in the highly disturbed site that is located in urban environs could be the reason for this (Table 1). Past studies have also reported a positive correlation between vehicle density and metal concentration (Aksoy et al. 1999; Bajpai et al. 2014). This also explains the lower concentration of heavy metals in the least disturbed site. As

Fig. 4 Scatter plot of discriminant analysis



observed by us and also reported by other workers (Demirezen and Aksoy 2004), a significant positive correlation between Cd, Fe, and Ni and their relationship with disturbance is noteworthy.

While some of the metals originate from fuel combustion, others such as Fe and Cu are a product of abrasion and wear and tear (Bargagli 1998; Thomson et al. 1997). Among the studied metals, Fe reported highest concentration at all the three sites. Alike Fe, Ni was also present in all the three sites. Concentration of both these metals decreased with increasing distance from the road. Their progressively declining values can be attributed to limited automotive exhaust and abrasion of vehicle (Akhter and Madany 1993; Garty 1993) in the least disturbed site. Higher Ni values at all the sites could be due to the use of kerosene, agriculture residue, and firewood as fuel. It has been pointed out that oil combustion in many buildings is a major source of ambient Ni (US EPA 1995, UK NAEI 2008). Furthermore, due to developmental activities in terms of construction of buildings and roads that involve welding, Ni may be higher in the area. Owing to heavy rainfall, sloping iron roofs are preferred in the area. This involves welding and thus welding shops are common.

As noted, these release Ni into the environment (Gube et al. 2013; Weiss et al. 2013).

Interestingly, Pb was not detected in any of the samples. In recent times, stringent regulations with respect to Pb emission have been put in place (Al-Khashman 2004; Kar et al. 2010). This includes lowering of lead additive limits in gasoline and ban on leaded fuel (Loppi et al. 2004). As a result of EPA’s regulatory efforts to remove lead from motor vehicle gasoline, levels of lead in the air have decreased by 89 % between 1980 and 2010 (EPA 2010). Constant improvement in automotive technologies also has a role to play in ensuring minimal Pb emissions (Kar et al. 2010). This along with relatively low traffic density in the study area, when compared to cities in lowland areas (Loppi and Corsini 2003) or religious circuits in Himalaya (Sharma et al. 2015), probably accounts for the absence of Pb in *P. flocculosa* at the present site.

While Pb was not detected in any of the samples, Cd and Cu were not detected in *P. flocculosa* samples collected from the least disturbed site. This is quite obvious as this site is the innermost and had minimal disturbance. Cd content apart from the motor vehicle emissions can vary depending upon the intensity of the

Table 4 Comparative account of the present study with studies done elsewhere

Taxa, study site	Cd (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Ni (ppm)	Source
<i>Brachythecium plumosum</i> , China	1.44 ± 0.3	6159 ± 1240	36.6 ± 4.2	169 ± 34	156 ± 27	15.4 ± 6.4	Chen et al. (2010)
<i>Eurhynchium laxirete</i> , China	0.97 ± 0.2	4648 ± 937	19.5 ± 3.6	114 ± 21	111 ± 19	11.9 ± 2.8	Chen et al. (2010)
<i>Taxiphyllum taxirameum</i> , China	1.21 ± 0.3	7590 ± 1788	139 ± 27	229 ± 33	170 ± 33	16.4 ± 5.4	Chen et al. (2010)
<i>Hygroamblystegium strictulum</i> , China	1.22 ± 0.4	4088 ± 651	34.9 ± 5.2	124 ± 47	109 ± 26	11.5 ± 4.3	Chen et al. (2010)
Lichens, New York	–	–	9–24	20–60	–	–	Bowen (1979)
<i>Pseudevernia furfuracea</i> , Italy	0.46	–	5.42	99	–	–	Adamo et al. (2003)
<i>Parmelia hypogymnia</i> , Italy	<0.1–0.3	–	04–10	20–90	–	–	Adamo et al. (2003)
<i>Evernia prunastri</i> , Italy	0.05–0.09	–	1.94–4.45	20.3–53.2	–	–	Conti et al. (2004)
<i>Dirinaria picta</i> , Singapore	0.14–0.28	–	11.75– 45.13	44.17– 83.15	–	–	Ng et al. (2006)
<i>Cladonia rangiformis</i> , Argentina	0.14–0.69	–	1.06–5.29	9.15–47.6	–	–	Cayir et al. (2007)
<i>Capsella bursa-pastoris</i> , Yorkshire, UK	0.45–1.07	–	9–26	53–200	–	–	Ross (1994)
<i>Poa annua</i> , Yorkshire, UK	0.27–0.84	–	9–26	50–151	–	–	Ross (1994)
<i>Phaeophyscia hispidula</i> , Uttarakhand	–	4505–10,923	24.02– 35.76	84.99– 141.8	–	54–67.9	Shukla and Upreti (2007)
<i>Pyrosia flocculosa</i> , Himachal Pradesh	BDL –10.96	123.21– 1471.34	BDL–18.67	3.18–10.14	2.05– 14.29	11.32– 104.64	Present study

BDL below detection limit

dust emission containing this element (Abollino et al. 2002; Li et al. 2008; Petrotou et al. 2012). Similarly, though main source of Cu is combustion of coal (Bargagli 1998; Anagnostatou 2008), variations in Cu have also been related to diesel engines and excessive use of vehicle brake pads (Anagnostatou 2008). Potholes in the roads are a common sight in the study area. This calls for frequent use of brakes that subsequently appear to be a source of the same, as has also been reported by other workers (Dietl et al. 1997; Fujiwara et al. 2011). Zinc is also a prime metal released by vehicle brake shoes (Davis and Williams 1975). Thus, its highest concentration in the highly disturbed site is justified. Higher value of Zn in the least disturbed site, as compared to moderately disturbed site, can be linked to the use of pesticides and fossil fuel combustion in the village areas. Studies done elsewhere (Adamo et al. 2003; Kar et al. 2010; Kord and Kord 2011; Loppi and Pirintzos 2003) have also reported similar patterns.

The trend of increasing Mn with decreasing disturbance can be explained on the basis of land use and not on the basis of vehicular emission. Our least disturbed site is near agricultural fields, where use of fertilizers and pesticides is a common practice. Use of fertilizers and pesticides may have resulted in higher concentration of Mn in the least disturbed site. Pignata et al. (2002) also observed Mn enrichment in areas around agricultural field and attributed the same to the use of fertilizers and pesticides. Similar pattern has also been reported by Bermudez et al. (2009). In another study, contamination factor for Mn was found to be positively correlated with agriculture (Wannaz et al. 2006). High value of coefficient of variation reveals particulate nature of these metals and hence their low dispersion. High CV values for heavy metals have also been reported by Shukla and Upreti 2007. It indicates nature of the element accumulated and entrapped by plants.

On the other hand, higher concentration of most of the metals in juvenile stage can be linked to higher metal sequestering capacity of the young growing tissues. Actively developing tissues accumulate higher quantity of elements (Pyatt et al. 1999). On the other hand, mature plants have a temporal advantage. There are evidences of translocation of elements to older parts of the plant which results in their higher concentration in older plants (Pyatt et al. 1999; Nishizono et al. 1987). In *A. yokoscense*, significantly higher concentration of Zn and Cu was found in dead leaves as compared to live ones (Nishizono et al. 1987).

A comparative account of the present study with studies done elsewhere, using plants as bio-indicators, is presented in Table 4. In the present study, concentrations of Cd and Ni are on the higher side and those of Fe, Cu, Zn, and Mn are on the lower side (Table 4). However, only Cd and Fe reported values that are higher than the threshold limits (Allen 1989).

Conclusions

The study is among the pioneers from the west Himalayan state of Himachal Pradesh where such studies are meager and much desired. To our knowledge, the present study is the first to demonstrate use of *P. flocculosa* as an indicator of heavy metal pollution. In addition to vehicular movement, land use practices also play a role in guiding distribution of heavy metals. While increased levels of Cd, Fe, Cu, Zn, and Ni were found to be correlated to vehicular emission and developmental activities, distribution of Mn could be explained on the basis of agriculture land use. Of all the metals analyzed, only Cd and Fe reported values higher than the threshold limits. Thus, *P. flocculosa* can be effectively used for bio-monitoring of the environment.

Acknowledgments The authors are thankful to the Director CSIR-IHBT Palampur for the facilities and encouragement. Head and members of the High Altitude Biology Division are acknowledged for their valuable help and support. Special thanks are due to Dr. Yogesh Pakade and Mr. Ramdeen Prasad for helping us in metal analyses. We are thankful to the editor and two anonymous reviewers whose comments and suggestions helped us in improving the earlier version of the manuscript. We thank Department of Biotechnology, Government of India for financial assistance via project GAP 0126 and Ministry of Environment, Forests and Climate Change via project GAP 0199. This is IHBT communication number 3919.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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