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Study on accumulation ability of two lichen species *Hypogymnia physodes* and *Usnea hirta* at iron-steel factory site, Turkey

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Abstract

The use of biological responses to contaminant exposure by lichen species has become a useful tool in environmental quality evaluation and risk assesment. Lichen *Hypogymnia physodes* and *Usnea hirta* samples were collected in 2006 from 10 sites around iron-steel factory in Karabük, Turkey. *H. physodes* and *U. hirta* samples from Yenice forest were used as a control. The aim of present study was to evaluate the bioaccumulation ability and to determine the environmental impact of an iron-steel factory in Karabük. Seven elements (Zn, Cu, Mn, Fe, Pb, Ni, Cr and Cd) were analysed by atomic absorption spectrometry (AAS). The analytical results were compared statistically by using SPSS. As expected, the study area (Yenice forest, Karabük) chosen as control site (site no 11) showed significantly lower impact in comparison to other site (site no 1-10). Compared with the two lichen species, *H. physodes showed* highest metal accumulating capacity while *U. hirta* showed lowest. These criteria attested the best suitability for *H. physodes,* followed by *U. hirta.*

Key words

Air pollution, Biomonitoring, Heavy metals, Iron-steel factory, Lichen

Introduction

Lichens were recognized as potential indicators of air pollution as early as the 1860s in Europe and elsewhere (Garty, 1993; Loppi 1996; Hamada and Miyawaki, 1998; Garty *et al*., 2003; Yenisoy-Karataþ and Tuncel, 2004).

There have been a number of studies indicating the significant increase in heavy metal content in plants, from the areas close to iron-steel factories, to busy highways. In addition, the exposure time was recorded as an important parameter for the increase in heavy metal level. A number of researchers (Conti and Cecchetti, 2001; Wolterbeek, 2002; Sczepaniak and Biziuk, 2003; Bermudez *et al*., 2009; Giardano *et al*., 2005; Adamo *et al*., 2003), displayed the importance of distance from the source of pollution. In their studies with different lichen species, the concentrations of cadmium (Cd), manganese (Mn), chromium (Cr) and nickel (Ni) elements were found higher in the samples which are close to an iron-steel factory than the samples far from the origin of pollution. Also, transplant studies manifested the differential increase in heavy metal content with regard to the variations in the time of exposure. In the investigations about Pb contamination conducted close to the highways Ward (1989); Al-Chalabi and Hawker (2000) reported the gradual increase in Pb pollution with a gradual decrease in distance from the highway in their soil analysis. Garty *et al*. (1977), Maquas *et al*. (1990), Kapu *et al.* (1991) and many other researchers displayed the significance of the closeness to highways in Pb pollution. In addition to these studies, many investigations have been carried out showing the absorption and accumulation of atmospheric heavy metals by lichens in urban areas (Mendil *et al*., 2009; Conti *et al*., 2004; Villarini *et al*., 2009).

With regard to the absorption of metals in lichens, three mechanisms have been proposed (Richardson, 1995): (i) intracellular absorption through an exchange process; (ii) intracellular accumulation; and (iii) entrapment of particles that contain metals.

Metals are classifield among the most dangerous groups of antropogenic environmental pollutants due to their toxicity and persistence in the environment (Nyarko *et al*., 2008). Consequently, the evaluation of the levels of metal deposition is of vital importance for the assessment of human exposure (Carreras *et al*., 2009). Long-term exposure to particulate matter air pollution has been associated with increased mortality from respiratory and cardiovascular diseases and from lung cancer (Pope *et al*., 2002). Copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are plant micronutrients. These elements are essential at low concentrations, but are toxic at higher levels (Divrikli *et al*., 2003; Soylak *et al*., 2004; Jamali *et al.*, 2008). Accumulation of heavy metals causes chronic damage to ecosystems and must be carefully monitored taking into account up-take movement and effects of the contaminants on both the environment and its biota. Industrial heavy metal pollution is a serious environmental problem all over the world in recent years. There has been a rapid growth in pollution in Turkey and other developing countries (Environmental Foundation of Turkey, 1999).

Air pollution constitutes a significant environmental problem in a number of Turkish cities. The primary source of the pollution is the usage of coal, as a main fuel for residential and industrial heating during winters. The study area included a control site in Yenice, northeast of Karabük, and 10 sites located around the Karabük iron-steel factory in Karabük, Turkey. The eastern part of Yenice river and the southern part of Yenice have the maximum species diversity of undisturbed woody species anywhere in Turkey (National Geographic Magazine, 2005). The lowest concentrations of heavy metals in control site must be of natural origin since there would not be a human activity that could cause such heavy metal input into the system. The forest is among the 100 forested areas in Turkey which must be urgently protected according to World Wildlife Fund (WWF, National Geographic Magazine, 2005). The area is classified as "Hot Spot of European Forests" and considered as one of the most valuable areas in terms of biological diversity in the region. Yenice forest is noted for its humid and rainy climate. The annual mean temperature is 8.8°C, relative humidity is 76.2% and total precipitation is about 1200 mm (Cansaran-Duman *et al*., 2009).

In Karabük, study on the multielemental composition of the environment by means of bioindicators has mainly been undertaken using lichens *Pseudevernia furfuracea* (Cansaran-Duman *et al*., 2009). But *P. furfuracea* species is not adequate enough to come to a conclusion on heavy metal accumulation in Karabük. For practical purposes, the suitability of various lichen species to monitoring heavy metal air pollution has become of special interest to determine which species is the most suitable as a biomonitor of an environmental condition (Cercasov *et al.*, 2002; Minganti *et al*., 2003; Bergamaschi *et al*., 2007).

The aim of the present study was to evaluate the accumulation of heavy metals by *Hypogymnia physodes* (L.) Nyl and *Usnea hirta* (L.) Weber ex F.H. Wigg from iron-steel factory in Karabük, Turkey. These lichen species were used to assess the atmospheric heavy metal deposition in the iron-steel factory, Karabük.

Materials and Methods

Study area: The study area is located between 44°62'18'' N, and 45°73'56'' E in the western part of the Black sea region, and belongs to Yenice district in the province of Karabük (Fig. 1). From Yenice forest to Karabük iron-steel factory, ten samples (site no 1-10) each of *Hypogymnia physodes* and *Usnea hirta* were collected from every 5 km. Control sample (site no 11) was taken from the south of Karabük, 30 km away from any source of pollution. Yenice forest area was specifically chosen because of the species abundance and therefore the collection of samples caused a very low impact on the natural population density.

Lichen sampling and preparation: Lichen sampling and preparation were made according to protocol given by Cansaran-Duman *et al*., (2009). *H. physodes* and *U. hirta* samples were collected from Yenice forests near the village of Yenice in Karabük province and from around Karabük iron-steel factory (44°62'18'' N, and 45°73'56'', Anatolia, Turkey, leg.-det. D. Cansaran-Duman), approximately 400 m above sea level. Lichen samples were collected in July, 2006 and stored at University of Ankara Herbarium.

H. physodes and *U. hirta* samples were air-dried and carefully cleaned with plastic tweezers under a binocular microscope (Olympus) to remove dead and as much extraneous material (adhering bark, mosses, soil and rock particles *etc.*) as possible. For the analysis, only the outermost parts of the thallus were used. These were pulverized and homogenized with an agate mortar and pestle. Aliquots of about 500 mg of lichen were kept in the laboratory for analyzing metals. The solutions and standards were prepared using double-deionized water. All the reagents used were of analytical grade (Merck).

Determination of metals: Determination of element content was performed according to the protocol defined by Cansaran-Duman *et al*., (2009). Analysis were conducted after extraction with a mixture of 2.0 ml HNO_3 (63%) and 1.0 ml H_2O_2 was added to 50 mg lichen sample and melted in teflon- coated pots in a milestone-mark microwave oven. Deionized water (5.0 ml) was added to the melted solution and distilled through blue band paper. The final volume was made upto 10 ml with deionized water.

Calibration curves of Zn, Cu, Mn, Fe, Pb, Ni, Cr and Cd metals were obtained with samples of various concentration (0.25, 0.50, 1.00, 2.00 and 4.00 ppm) using linear regression analysis. Calibration curves of Cd and Cr metals were obtained with samples of various concentration (10, 25, 40, 60 and 80 ppm) using linear regression analyses. Heavy metal concentration in these materials was determined using flame atomic absorption spectroscopy-Intrument (FAAS, PM Avarta model) and electrothermal atomic absorption spectroscopy (ETAAS).

Statistical analysis: The results of the chemical analyses were evaluated by a one-way ANOVA, to display the effects of the factory on the bioaccumulation status of the *H. physodes* and *U. hirta*.

Results and Discussion

Around the Karabük iron-steel factory, the highest levels of Zn in the *H. physodes* were found in site 4 (33.1 μg g^{-1}), site 8 (30.2 μg g^{-1}) and site 5 (30.1 μg g^{-1}), respectively (Table 1). Sites 6, 7, 10 and 1 were determined close to each other value in the *H. physodes* species. Also, the highest levels of Zn in the *U. hirta* were

Table - 1: Average concentration values (±SE) of 5 samples along with the results of variance analysis (ANOVA) of chemical parameters in *H. physodes.*

Site	Zn	Cu	Mn	Fe	Pb	Ni	Cr	Cd
1	28.432±0.158	3.075±0.292	51.511±1.870	1258.600±15.176	1.80±0.16	6.07±0.11	2.86±0.04	0.854±0.002
2	26.168±0.192	2.482±0.054	195.880±7.041	1371.400±5.713	4.08±0.06	4.95±0.10	3.07±0.01	0.616±0.004
3	28.102±0.420	2.893±0.047	98.433±3.383	1679.000±305.583	3.38±0.11	10.81±0.29	3.26±0.04	0.769±0.002
4	33.155±0.271	2.966±0.038	202.730±0.606	272.966±24.999	3.39±0.06	3.92±0.15	3.79±0.03	0.692±0.013
5	30.151±0.105	2.769±0.029	183.029±5.389	1287.700±4.269	2.42±0.15	3.71±0.10	2.60±0.02	0.742±0.008
6	29.404±0.121	2.964±0.050	168.602±1.289	1505.900±31.823	2.02±0.40	4.34±0.19	2.95±0.02	0.773±0.007
7	29.975±0.844	2.895±0.162	110.977±6.409	2576.400±15.664	2.68±0.25	5.64±0.29	3.86±0.08	0.669±0.005
8	30.255±0.045	3.948±0.051	161.922±3.630	3173.400±18.381	3.37±0.27	6.27±0.15	4.56±0.04	0.843±0.010
9	22.366±0.012	2.792±0.058	106.100±1.243	2587.700±33.849	4.16±0.60	4.91±0.19	3.58±0.02	0.626±0.002
10	28.896±0.327	2.449±0.029	154.840±0.157	1823.700±16.467	2.94±0.22	3.74±0.34	3.31±0.04	0.875±0.002
11	18.375±0.675	1.590±0.022	44.805±0.134	1337.500±50.019	1.76±0.10	4.83±0.17	2.37±0.02	0.733±0.078
				ANOVA				
F ratio	0.173	0.743	0.090	0.283	0.0196	0.0021	0.0006	0.0150

Table - 2: Average concentration values (±SE) of 5 samples along with the results of variance analysis (ANOVA) of chemical parameters in *U. hirta.*

Site	Zn	Cu	Mn	Fe	Pb	Ni	Cr	Cd
1	21.126±0.049	1.939±0.044	24.267±2.272	552.004±7.740	8.780±0.105	6.169±0.056	2.050±0.015	0.472±0.026
2	10.938±0.111	1.364±0.133	66.608±0.535	916.673±43.417	1.397±0.028	2.464±0.046	1.970±0.023	0.494±0.034
3	19.008±0.188	1.669±0.016	195.926±1.550	574.742±1.709	7.675±0.089	1.566±0.065	2.067±0.006	0.526±0.007
4	18.807±0.624	1.776±0.007	150.305±1.851	649.227±4.303	2.542±0.015	5.250±0.026	2.019±0.008	0.612±0.007
5	21.427±0.163	2.123±0.064	45.561±0.156	653.943±2.960	6.234±0.178	1.695±0.220	6.751±0.057	0.386±0.008
6	15.764±0.108	1.863±0.031	124.556±0.165	583.804±14.403	3.702±0.115	1.602±0.093	4.189±0.103	0.535±0.010
7	21.258±0.298	3.116±0.033	92.839±0.331	1568.400±17.330	2.248±0.061	1.919±0.056	3.154±0.048	0.492±0.012
8	14.986±2.041	1.780±0.005	37.934±0.998	582.886±0.300	5.780±0.159	4.516±0.210	2.178±0.011	0.303±0.018
9	20.677±0.480	1.676±0.024	22.954±0.242	541.560±17.666	2.042±0.054	8.668±0.053	2.168±0.014	0.500±0.031
10	18.848±0.185	1.983±0.113	92.311±1.196	589.875±0.630	4.666±0.009	3.728±0.08	2.066±0.005	0.435±0.052
11	10.710±0.482	1.594±0.022	19.323±0.970	495.356±1.762	1.323±0.006	1.162±0.011	1.968±0.010	0.171±0.015
				ANOVA				
F ratio	0.006	0.004	0.000	0.001	0.002	0.003	0.001	0.007

found in sites 1 (21.1 µg g^{-1}), 5 (21.4 µg g^{-1}) and 7 (21.2 µg g^{-1}), respectively. In addition to these sites, high value of Zn was found in *U. hirta.* collected from site 3 (19.0 µg g^{-1})and 9 (20.6 µg g^{-1}). Zinc concentration in the lichen samples was linearly related to the vehicle traffic, railway and activity of industrial units. Gailey and Lloyd (1986) measured the heavy metal content in *Lecanora conizaeoides* collected in Armadale (Central Scotland) and detected Zn in the range of 50-641 µg g^{-1}, depending on the distance and wind direction from a steel foundry. The authors found that only Fe and Zn were detected in the lichen collected in the peripheral sites of the town. They concluded that the steel foundry is the main source of metal pollution in this town.

Manganese is commonly used in steel production and also in alloys and batteriers (Markert, 1992). The highest levels of Mn in the *H. physodes* were found in site 2 (195.8 µg g^{-1}) and 4 (202.7 µg g^{-1}), respectively. The highest levels of Mn in the *U. hirta* were found in site 3 (195.9 µg g^{-1}) and 4 (150.3 µg g^{-1}) with a control value of 19.3 µg g^{-1}. Both samples showed high Mn concentration in site 4. Motor vehicles are known to be a source of Mn in urban areas (Monaci *et al.*, 2000) and could explain the reason of elevated Mn concentrations in site 4.

H. physodes and *U. hirta* collected from polluted and control site showed significant variations in Pb concentration, especially *U. hirta* (Table 1, 2). Sites 3, 5, 8 and especially sites 1, 10 with the highest human activities, together with high vehicular density congestion, showed the highest Pb content with the values of, 8.78 µg g^{-1} in *U. hirta* species which were significantly higher than the control site, 1.32 mg g^{-1}. It could be concluded that Pb concentration was highest in sites 1 and 10 because they are the central part of the city where human activities and density of traffic are very intense. Similar kinds of observations were made by Cansaran-Duman *et al*. (2009) while studying *Pseudevernia furfuracea* thalli as an indicator of air pollution in the same place (Karabuk), Turkey.

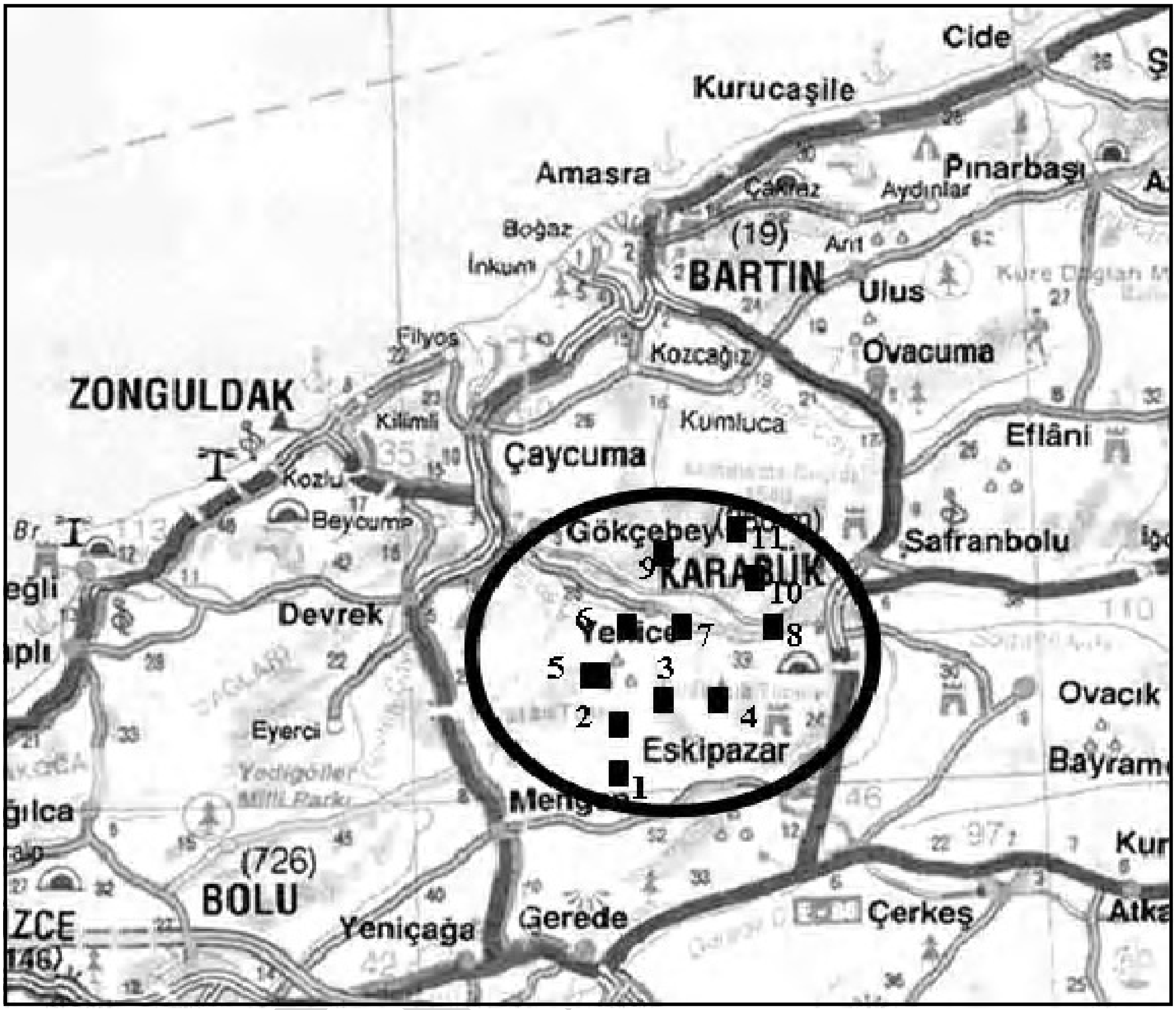

Fig. 1: Map of the study area in western part of Black sea with collection sites (1-11)

The highest level of Cr in the *H. physodes* was found in site 7 (3.86 µg g^{-1}) and 8 (4.56 µg g^{-1}), respectively (Table 1), which was slightly higher than the control value. The Cr content in sites 5 (6.75 µg g^{-1}), 6 (4.18 µg g^{-1}) and 7 (3.15 µg g^{-1}) were significantly higher than control site (1.96 µg g^{-1}) in *U. hirta* (Table 2). The most important source of Cr pollution are industrial activities like refining works and iron-steel factories.

Copper content in *H. physodes* samples ranged from 2.44 to 3.94 µg g^{-1}. Cu content in site 7 (3.11 µg g^{-1}) in *U. hirta* was significantly higher than control site (1.59 µg g^{-1}) (Table 1,2). These two species showed high Cu concentration. Nickel concentrations in site 3 was found 10.81 µg g^{-1} *in H. physodes* and 8.66 µg g^{-1} in *U. hirta collected from* site 9 (Table 1, 2).

The mean Cd concentration in sites 1 (0.85 µg g^{-1}), 8 (0.84 µg g^{-1}) and 10 (0.87 µg g^{-1}) were slightly higher than the control site (0.73 µg g^{-1}) in *H. physodes* (Table 1). All sites, especially site no 4 (0.61 µg g^{-1}), showed significantly higher Cd concentrations than control site (0.17 µg g^{-1}) in *U. hirta* (Table 2). The concentrations of Cd in *U. hirta* were significantly higher in all sites than from the control site, probably indicating the contaminants from motor vehicles, dust raised by metal business and other human activities. The most important sources of Cd pollution were regarded as fossil fuels used by the vehicles, metal business, plastics, house tools construction and sewer (Markert, 1992) and recorded the Cd levels in between 0.01 and 0.3 mg g^{-1} for unpolluted natural environments and also reported that all of the study sites were polluted except rural sites.

The presence of heavy metals in *P. furfuracea* has been reported by Cansaran-Duman *et al*., (2009). On comparing *H. physodes*, *U. hirta* with *P. furfuracea* there was no difference in Zn concentration. Especially, Zn concentration in *P. furfuracea* collected from sites 7 and 10 were significantly higher than *U. hirta* and *H. physodes*. Cansaran-Duman *et al*., (2009) reported significantly high deposition of Zn in *P. furfuracea* from sites 7 and 10 (especially, site 10 is city center), which was related to vehicular traffic, since it is the main source of air pollutants is this area.

On comparing metal concentrations in *U. hirta* and *H. physodes* species determined in this study with the levels found in iron-steel factory of other lichen species (*e.g. P. furfuracea*), it can be observed that *P. furfuracea* and *H. physodes* are in the approximate level. Data presented in this paper demonstrate that, Cd concentration in *H. physodes* and *P. furfuracea* was found in parallel, however it was found less in *U. hirta*.

High concentrations of Pb in *U. hirta* and *P. furfuracea* was found in site 1. But in site 10, Pb concentration of *P. furfuracea* was highest than *U. hirta* and *H. physodes*. Thus, in *H. physodes* and *U. hirta* the highest concentration of Pb can be related to selective cation uptake as reported previously by Cansaran-Duman *et al*., (2009). The authors attributed this finding to a greater affinity between Pb cations and the lichen cell wall exchange sites that are probably strongly attached to binding sites.

Iron acumulation in *H. physodes* collected from site 8 was similar to those obtained by Cansaran-Duman *et al*., (2009) in *P. furfuracea* species from same site of iron-steel factory in Karabük. Highest Fe concentration was found in *P. furfuracea* collected from site 1, while low Fe concentration was found in *U. hirta* and *H. physodes*.

The order of magnitude of Mn accumulation at 2, 4 and 10 sites were *H. physodes* > *U. hirta* > *P. furfuracea*. Although *U. hirta* showed the highest levels of Mn in site 3, *H. physodes* was highest accumulator than *P. furfuracea* and *U. hirta at* sites 5 and 9. Mn could be tracer of both eolic dust particles as well as vehicular traffic, since this element has recently been used as a substitute for Pb in additives (Ardeleanu *et al*., 1999).

H. physodes exposed in site 3 showed significantly higher content of Ni, but *U. hirta* and *P. furfuracea* showed similar range at all other sites. In the lichen *U. hirta*, significant higher values of Cr element were measured in site 5. As it was observed in *U. hirta*, the levels of Pb were also higher in site 5. Regarding Cr, *U. hirta* and *H. physodes* species showed a significant heavy metal accumulation. Previous studies which used *P. furfuracea* as a passive biomonitor in the province of Karabük showed similar concentrations of Cr.

Our findings are consistent with data reported by Cansaran-Duman *et al*. (2009), who monitored *P. furfuracea* species in the same district. Heavy metals were found in high concentration around the iron-steel factory in Karabuk, which is in accordance with previous data (Cansaran-Duman *et al*., 2009).

Many researches have evaluated bioaccumulation of heavy metals in different lichen species (Bergamaschi *et al*., 2007; Cercasov *et al*., 2002; Conti *et al*., 2009; Bermudez *et al*., 2009; Mendil *et al*., 2009). Results are different because responses differ among species, threats differ among metals and environmental influences. Bergamaschi *et al*. (2007) measured 29 elements (Al, As, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Fe, Hg, I, K, La, Mg, Mn, Ni, Pb, Rb, Sb, Sc, Se, Sm, Th, Ti, V and Zn) in *H. physodes*, *P. furfuracea, U. hirta* and *P. sulcata* in Italy. Bergamaschi *et al*. (2007) reported in general elements do not exhibit well defined trends, but rather showed fluctuations, and indicated that *H. physodes*, *P. furfuracea, U. hirta* have a similar accumulation capacity, while *Parmelia sulcata* has lower.

Generally, previous studies that used *P. furfuracea* as a passive biomonitor in a iron-steel factory in the province of Karabük showed concentrations higher than *U. hirta* species found in the present study. *P. furfuracea* and *H. physodes* were close quarters to heavy metal accumulation in iron-steel factory.

The presence of heavy metals in *P. furfuracea* was already reported in Cansaran-Duman *et al*., (2009). Moreover, results of the present paper are comparison with *U. hirta* and *H. physodes* species. It was observed that the accumulation of metals in *P. furfuracea* was similar to the one observed in *H. physodes*, with significantly higher values of all elements in samples exposed in the Karabük. Moreover, in previous study *P. furfuracea* and in the current study *H. physodes* proved to be almost similar bioaccumulator than *U. hirta* species. Several studies revealed that lichens may selectively accumulate extracellular elements and metabolize or eliminate those elements that enter the cell wall (Branquinho *et al*., 1997; Chettri *et al*., 1997). According to Carreras *et al*. (2009), the results obtained suggest that lichen species can be successfully used to monitor air pollution. However, several other factors should be considered before taking a decision on the preferred biomonitor species, such as selective uptake or detoxificant mechanisms.

Finally, in this article we have compared the capacity of two lichen species to accumulate trace elements from the atmosphere around the iron-steel factory in Karabuk, Turkey. The results obtained indicate that particularly *H. physodes* demonstrated the suitability of the lichen samples for the detection of air quality.

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