Heavy Metal Accumulation in *Pseudevernia furfuracea* (L.) Zopf from the Karabük Iron-Steel Factory in Karabük, Turkey

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Pseudevernia furfuracea (L.) Zopf lichen specimens were collected every 5 km starting from around an iron-steel factory located in the central area of Karabük province, up to Yenice Forest. Zn, Cu, Mn, Fe, Pb, Ni, Cd, Cr contents were analyzed in the samples collected from polluted and unpolluted areas. A *Pseudevernia furfuracea* (L.) Zopf sample from Yenice Forest was used as a control. The reason for this choise was the abundance of species diversity, and therefore sample collection might cause a very low impact on natural population density. The forest is among the 100 forested areas that must be urgently taken under protection according to WWF (World Wildlife Fund) researches. Results of the current study manifested significant variations among the contents of these elements between stations. As expected, the pollution sources, such as iron-steel factory, roads and railroads, industry, heavy traffic, and waste treatment plants, have major impact on the heavy metal accumulation in *P. furfuracea* (L.) Zopf, and, in accordance to their location, samples 8 and 10 displayed high element accumulation. Surprisingly, although Yenice Forest is under protection, results of our study showed that the region is becoming polluted by the influence of many pollution sources in the area. The present study also confirms the efficient metal accumulation capacity of lichens.

Key words: Lichen Monitoring, Heavy Metal, Iron-Steel Factory

Introduction

Lichens are slow-growing associations of fungi (mycobionts) and green algae or cyanobacteria (photobionts). This symbiotic association forms a common thallus that has no roots or waxy cuticles and depends mainly on the atmospheric input of mineral nutrients. These features of lichens, combined with their extraordinary capability to grow in a large geographical range and to accumulate mineral elements far above their needs, rank them among the best indicators of air pollution (Garty *et al.*, 1977).

There are many traditional studies on atmospheric contamination but most of them have been limited by problems like high costs and the difficulty of carrying out extensive sampling in terms of both time and space. There is, thus, an ever increasing interest in using indirect monitoring methods such as analysis of organisms that are bioaccumulators (Fernandez *et al.*, 2000). As far back as 1866, a study was published on epiphytic lichens used as bioindicators (Nylander, 1866). Lichens are the most studied bioindicators of air quality (Ferry *et al.*, 1973). They have been defined as ‘permanent control systems’ for air pollution assessment (Nimis *et al.*, 1992).

Numerous investigations on the interaction of air pollution and lichens performed within the last three decades revealed that lichens may be assigned to three categories in terms of their responses to air pollution. For example, during the last 50 years, many studies have stressed the possibility of using lichens as biomonitors of air quality in view of their sensitivity to various environmental factors, which can provoke changes in some of their components and/or specific parameters (Brodo, 1961; Hawksworth, 1971; Lerond, 1984; St Clair and Fields, 1986; Galun and Ronen, 1988; Nimis, 1990; Oksanen *et al*., 1991; Loppi *et al*., 1992; Seaward, 1996; Gries, 1996; Loppi, 1996; Hamada and Miyawaki, 1998). Garty *et al*. (2003) analyzed the B, Fe, Mg, Mn, Na, Pb, S, Sr and Ti concentrations in the epiphytic lichen *Ramalina lacera* (With.) in a unpolluted forest near HaZorea, transplanted in northeast Israel to 10 biomonitoring sites in the vicinity of the coal-fired power station Oroth Rabin near the town of Hadera. *R. lacera* was found to be an outstanding accumulator, as the elements tend to concentrate in noticeable amounts in this lichen. The lichen reaffirms the global applicability to biomonitoring methods.

In another study conducted in 2004 by Yenisoy-Karataş and Tuncel, a total of 234 *Xanthoria parietina* samples were collected from a 51800-km^2 area. The samples were washed and analyzed by INAA and ICP-AES for 35 elements. Locations of pollution sources such as iron-steel plants, coal burning in the cities, industrial activities and two important coal-fired power plants generally corresponded with locations of highest element accumulations in the lichens.

Lichens applied as monitors near coal-fired power stations in Portugal were found to accumulate heavy metals such as Fe, Co, Cr, and Sb originating from coal and ash particles drifting through the air and positioning on the thallus (Freitas, 1994). In his study, Freitas (1994) analyzed the comparative accumulation of Cr, Fe, Co, Zn, Se, Sb and Hg in the epiphytic lichen *Parmelia sulcata* and two vascular plants in an industrial region with a thermal coal-fired power station. The lichen was found to be the most effective bioaccumulator.

The present study was initiated with the objective to provide basic information on metal accumulation in lichen species growing in and around the Karabük Iron-Steel Factory in Karabük, Turkey. The present investigation has involved the collection of ten *Pseudevernia furfuracea* (L.) Zopf samples growing on *Pinus* sp. At 10 sites in and around the Karabük Iron-Steel Factory area. The metal contents of *P. furfuracea* (L.) Zopf samples collected from Yenice Research Forest (Yenice-Karabük) and Karabük Iron-Steel Factory were analyzed by atomic absorbtion spectrometry (AAS). The thalli of *P. furfuracea* were used to determine the levels of eight metals (Zn, Cu, Mn, Fe, Pb, Ni, Cd, Cr).

Material 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 of *P. furfuracea* (L.) Zopf were collected every 5 km. Control sites were chosen in the south of Karabük which were more than 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. The eastern part of Yenice river and the southern part of Yenice have the maximum species diversity of undisturbed woody species in Turkey (National Geographic, 2005). The forest is among the 100 forested areas in Turkey which must be urgently protected according to WWF (World Wildlife Fund; National Geographic, 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 ºC, relative humidity is 76.2%, and total precipitation is about 1200 mm.

Lichen sampling and sample collection

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 (Table I, Fig. 1). *Pseudevernia furfuracea* (L.) Zopf samples were collected from Yenice Forests near the village of Yenice in Karabük province and from around Karabük Iron-Steel Factory in Karabük, approximately 400 m above sea level. Lichen samples were collected in July 2006 and all samples are stored at University of Ankara Herbarium.

Sample preparation

Pseudevernia furfuracea (L.) Zopf samples were air-dried to constant weight and carefully cleaned with plastic tweezers under a binocular micro-

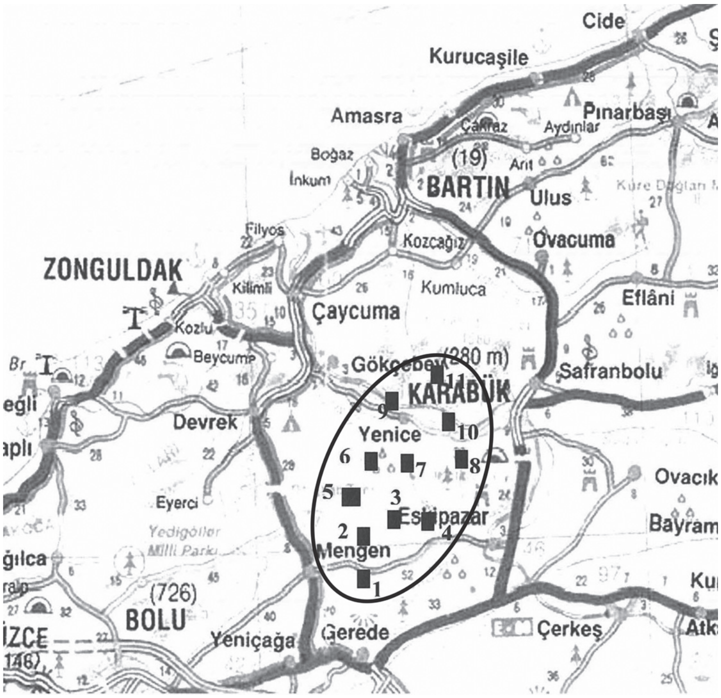

Fig. 1. Regional map of the study area.

scope (Olympus) to remove dead and as much extraneous material (adhering bark, mosses, soil and rock particles, etc.) as possible. Samples were not washed to avoid losing particles trapped on the lichen surface. For the analysis, only the outermost parts of the thalli were used. These were pulverized and homogenized with an agate mortar and pestle. Aliquots of about 500 mg of lichen were submitted to analyses.

The samples were kept in the laboratory for analyzing trace elements. The solutions and standards were prepared using double-deionized water. All the reagents used were of analytical grade (Merck).

Determination of element content

The chemical analyses of lichen samples were conducted after extraction with 2.0 ml 63% HNO_3.

Table I. The localities of the lichen samples used in the study.

Locality No.	Date of collection	GPS co-ordinates	Name of locality	Altitude [m]
1	15.11.2005	44°62' N, 45°73' E	Karabük, Yenice, Kuzdağ district	1125
2	15.11.2005	41°15' N, 32°35' E	Karabük, Yenice, Kabaklı kaya	1140
3	15.11.2005	41°13' N, 32°28' E	Karabük, Yenice, vicinity of Hamzakıran district	1140
4	15.11.2005	41°14' N, 32°35' E	Karabük, Yenice, Dikilitaş	1125
5	15.11.2005	41°12' N, 32°25' E	Karabük, Yenice, vicinity of Kuzdere, Hamdioğlu district	1400
6	15.11.2005	41°15' N, 32°34' E	Karabük, Yenice, north of Yalnızca plateau	1200
7	15.11.2005	41°11' N, 32°27' E	Karabük, Yenice, Acısu Center	1375
8	15.11.2005	41°14' N, 32°33' E	Karabük, Yenice, Kazancıoğlu district	1750
9	15.11.2005	41°12' N, 32°29' E	Karabük, Yenice, Hacıömerler district	1380
10	15.11.2005	41°12' N, 32°29' E	Karabük, Yenice, Kızılgöz kayası	1385
11*	15.11.2005	41°10' N, 32°24' E	Karabük, Yenice, vicinity of Cami district	1100

* Control sample.

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. 5.0 ml deionized water were added to the melted mixture and distilled through blue band paper. Deionized water was added until the final volume was 10.0 ml.

Calibration curves of Mn, Zn, Fe, Pb and Cu metals were obtained with samples of various contents (0.25, 0.50, 1.00, 2.00, 4.00 ppm) using linear regression analysis. Heavy metal contents in these materials were determined using FAAS (flame atomic absorption spectrometry; Instrument PM Avarta model atomic absorption spectrometer). The accuracy of the process was checked with standard extension.

Calibration curves of Cd and Cr metals were obtained with samples of various contents (10, 25, 40, 60, 80 ppm) using linear regression analysis. Cd and Cr contents in *P. furfuracea* (L.) Zopf samples were determined using ETAAS (electrothermal atomic absorption spectrometry). The accuracy of the process was checked by standard extension.

Statistical analysis

The results of the chemical analyses were evaluated by one-way ANOVA, to display the effects of the factory on the bioaccumulation status of *Pseudevernia furfuracea* (L.) Zopf.

Results and Discussion

Metal contents in the *Pseudevernia furfuracea* (L.) Zopf species were investigated. All the stations were statistically analyzed to determine their relationships with respect to each heavy metal. Analysis of SPSS 11.5 was used to show the relationships of the stations, and all the results were shown using dendograms. Around the Karabük Iron-Steel Factory, the highest levels of zinc (Zn) were found at site 10 (53.8 mg/kg) and site 7 (40.6 mg/kg) (Fig. 2A). The Zn content in the lichen samples was linearly related to the vehicle traffic, railway and activity of industrial units. In another study conducted in an unpolluted area of Finnish Lapland, *Hypogymnia physodes* thalli samples collected from 16 sites displayed (109 ± 27) mg/kg Zn and (0.51 ± 0.28) mg/kg Cd (Sarkela and Nuorteva, 1987). However, only Zn showed a tendency to occur in higher amounts in more elevated localities. In their study area, the Zn contrent was high because of solid waste at site 7. In our study, the highest Zn contents were recorded at the stations near to Karabük Iron-Steel Factory.

Manganese (Mn) is commonly used in steel production and also in alloys and batteries (Markert, 1992). The Mn values in all of the samples ranged from 31.72 mg/kg to 119 mg/kg, with a control value of 42.25 mg/kg. The 5^{th} and 8^{th} stations, which were located close to the iron-steel factory and major motor vehicle traffic, manifested the highest level of Mn (Fig. 2B). Motor vehicles are known to be a source of Mn in urban areas (Monaci *et al.*, 2000) and could explain the elevated Mn contents in station 5.

Iron (Fe) is a common construction material. It is also emitted from iron-steel plants and during the combustion of fossil fuels such as coal (Fernandez *et al.*, 2000). The mean levels of Fe contents in sites 1 and 8 were very significant (Fig. 2C). At site 8, combustion of coal and other kinds of fuels rather than natural gas seem to be the reason of air pollution. The content of Fe in lichens exposed to an Fe-contaminated environment was found to depend on the length of exposure. Gailey and Lloyd (1986) investigated the accumulation of heavy metals in spherical moss bags, samplers and *Hypogymnia physodes* transplanted to a Scottish industrial town (Armadale, Central Scotland), and they detected a very high increase in mean concentrations of Fe from 1 to 10 weeks. In the current study higher metal contents were recorded for the Karabük samples compared with the study by Gailey and Lloyd (1986).

The comparison of lead (Pb) contents in the lichens from the study area with the control site displayed significant variations (Fig. 2D). Since Pb is an easily vaporized element, a higher value is expected in settling areas where small and medium size industrial plants exist (Spiro and Stigliani, 1996) which might explain the high Pb values recorded for stations 1 and 10. Site 10 with the highest human activities, together with high vehicular density congestion, showed the highest Pb levels with the value of 9.75 mg/kg which is significantly higher than that of the control site (4.00 mg/kg). In the present study, the relative high content of Pb at site 10 was traceable to the vehicular activity on the main Karabük, Safranbolu and Zonguldak roads. Takala and Olkkonen (1981) demonstrated that the average Pb content in *H. physodes* decreases as a function of the distance from the nearest road or street up to about

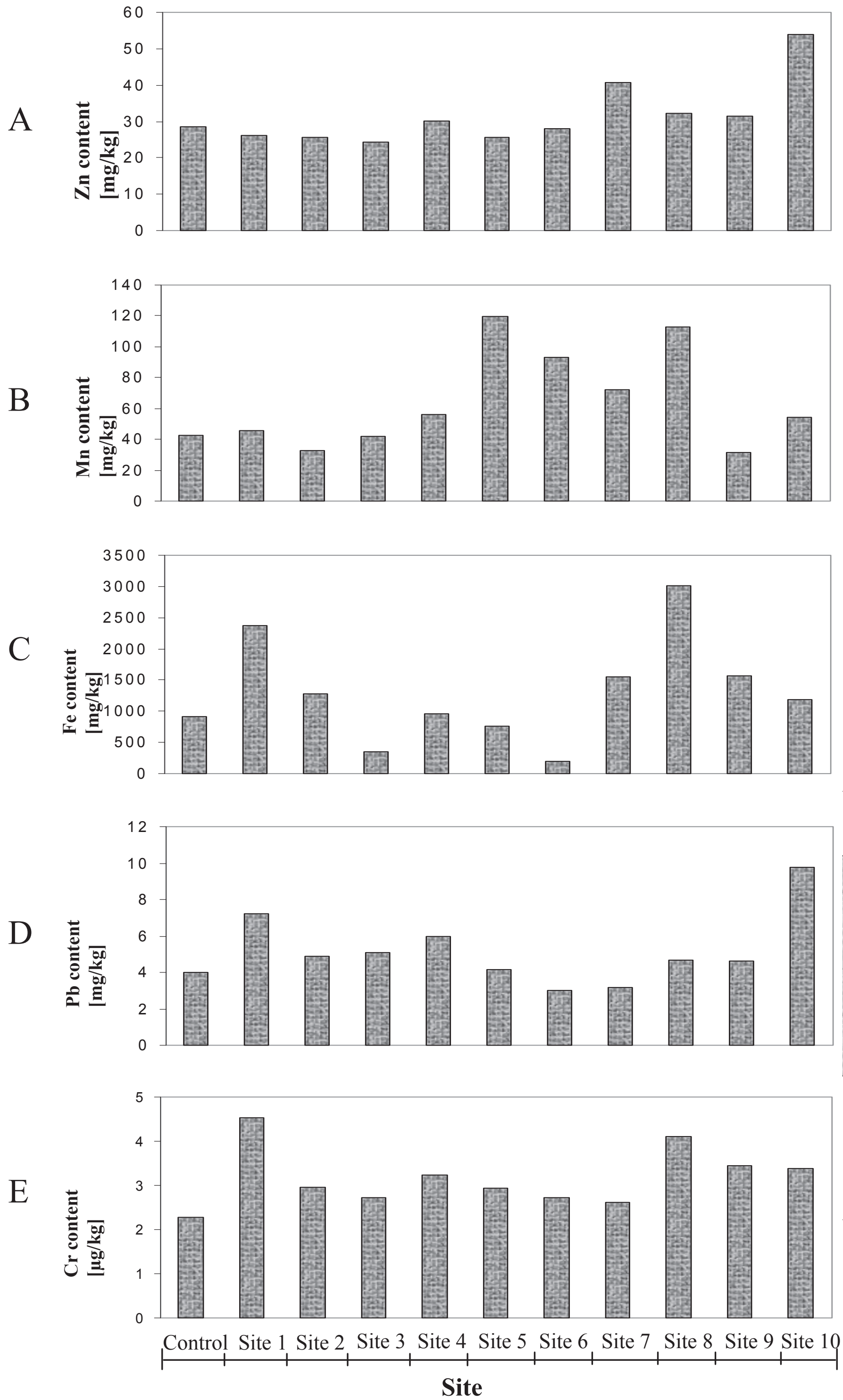

Fig. 2. Mean heavy metal contents in *Pseudevernia furfuracea* (L.) Zopf. (A) Mean Zn contents. (B) Mean Mn contents. (C) Mean Fe contents. (D) Mean Pb conctents. (E) Mean Cr contents.

100 m. In accordance with the literature, in this study we detected a decline in the metal values when moving away from the highway.

The highest nickel contents were observed at the sites 1 and 10. These two stations were close to the main roads, especially site 10 is an area exposed to heavy traffic. In another study conducted in Israel the *Pseudevernia furfuracea* samples collected from a clean area of HaZorea were transplanted to a highly polluted area. The nickel content was recorded as (29.2 ± 0.9) mg/kg in the samples exposed from February 1979 till March 1980. After one year of exposure the Ni content was recorded as (51 ± 26) mg/kg and the increase was attributed to a power station (the Maor David Power Plant) established in August 1981 (Garty and Hagemeyer, 1988). The high Ni content obtained in the present study might be explained by the railway passing close to station 1.

The chromium (Cr) concent at the sites 1 (4.54 μg/kg), 8 (4.10 μg/kg), 9 (3.44 μg/kg), and 10 (3.39 μg/kg) were slightly higher than at the sites 2, 3, 4, 5, 6, 7 and were significantly higher than at the control site (2.28 μg/kg) (Fig. 2E). The most important sources of Cr pollution are indicated as industrial activities like refining works and iron-steel factories. Pilegaard (1978) showed that *Lecanora conizaeoides* collected 0.6 km from steelworks and an iron foundry in Frederiksvaerk, Denmark, contained 121 μg/g Cr, whereas samples of the same lichen species collected at a distance of 2.3 km from this industrial area contained only 5 μg/kg Cr. In our study we found that both Karabük Iron-Steel Factory and leather waste plants at stations 4 and 8 can cause high values of Cr (Fig. 2E).

The mean cadmium (Cd) contents at sites 1 (0.73 μg/kg), 2 (0.69 μg/kg), 4 (0.71 μg/kg), 7 (0.67 µg/kg), 8 (0.67 μg/kg), 9 (0.72 μg/kg), and 10 (0.77 μg/kg) were slightly higher than at the control sites. Burning of lignite is the main source of Cd in plants. For this reason higher Cd contents were recorded in lichens growing close to thermal power plants burning lignite, and it is expected to find lower Cd contents in iron-steel factories than in thermal power plants (Bliefurt, 2002). In a study by Beckett and Brown (1984), Cd contents were detected in the range of 1.26–5.05 μg/kg and 1.56–6.40 μg/kg for *Aspicilia ciliaris* and *Lobaria pulmonaria*, respectively. These values are considered to be close to the appearance of toxicity symptoms.

Bari *et al*. (2001) found a significant correlation between several heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) accumulated in thalli of the lichen *Pseudevernia furfuracea* (L.) Zopf transplanted for one year in a rural site of northern Italy and concluded that lichen samples are a suitable tool for monitoring particulate air pollution. Similarly, the present study showed the ability of the lichen *Pseudevernia furfuracea* (L.) Zopf to be used as a bioindicator of airborne chemical elements on a regional scale and also demonstrated the suitability of the lichen samples for the detection of air pollution.

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