Karabük Demir Çelik Fabrikası etrafından toplanan beş biyomonitor liken türünün ağır metal akümülasyonu ve karşılaştırmalı analizi

Heavy metal accumulation of five biomonitor lichen species in the vicinity of the Karabük Iron and Steel Factory in Karabük, Turkey and their comparative analysis

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ÖZET

Amaç: Beş biyomonitor liken türünün (*Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina pollinaria*, *Usnea hirta*) ağır metal biriktirebilme yetisini incelemek amacıyla Karabük Demir Çelik fabrikası ile Yenice Ormanı arasındaki 10 istasyondan farklı beş liken türüne ait 10 örnek araştırılmıştır.

Yöntem: Beş biyomonitor liken örneğinin her biri Karabük Demir Çelik fabrikası ile Yenice Ormanı arasındaki alandan her beş km'de bir alınmıştır. Çalışılan beş farklı liken türüne ait 10 liken örneğinde Atomik Absorpsiyon Spektrofotometre (AAS) cihazı kullanılarak sekiz ağır metal; Cd, Cr, Cu, Fe, Mn, Ni, Pb ve Zn analiz edilmiştir.

Bulgular: Karabük Demir Çelik Fabrikası etrafından toplanan beş biyomonitor liken örneğinde atmosferdeki iz elementlerin akümülasyon kapasitesi karşılaştırılmıştır. İstasyon 1, 2, 7 ve 10 insan yoğunluğunun ve trafiğin fazla olduğu şehir merkezine en yakın olan yerdir. Atomik Absorpsiyon Spektrofotometresi ile yapılan analiz sonuçlarına göre, Zn elementi için *P. furfuracea* liken türünde istasyon yedi (40,628 µg/g) ve 10 (53,802 µg/g)'da çalışılan diğer liken örneklerine göre daha fazla ağır metal

ABSTRACT

Objective: To investigate the suitability of five biomonitor lichen species (*Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina pollinaria* and *Usnea hirta*) that were collected from Yenice Forest to the Karabük Iron and Steel Factory in Karabük, Turkey, from 10 sites.

Method: Each of the five biomonitor lichen species was collected from every 5 kms starting from Yenice forest to iron steel factory. Accumulation of eight heavy metals Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in examined lichen species were analyzed by Atomic Absorption Spectrophotometer (AAS).

Results: We have compared the capacity of five biomonitor lichen species to accumulate trace elements from the atmosphere which were collected from around the Karabük Iron and Steel Factory in Karabük, Turkey. Sites 1, 2, 7 and 10 were in the central parts of the city where human activities and density of traffic are very intense. Analytical studies by AAS demonstrated that the heavy metal accumulation capacity of *P. furfuracea* was significantly higher than other examined lichen species at sites 7 (40.628 µg/g) and 10 (53.802 µg/g)

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akümülasyonu olduğu gözlenmiştir. Sekizinci istasyonda *H. physodes* (4,56 μg/g) ve *E. prunastri* (4,65 μg/g) liken türlerinde Cr konsantrasyonu benzer miktarlarda tespit edilmiştir.

Sonuç: Çalışmamızın sonuçları açığa çıkarmıştır ki; *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina pollinaria* ve *Usnea hirta* liken türleri incelenen tüm elementleri ciddi oranda biriktirme eğilimi gösterdiğini açığa çıkarmıştır. Bu çalışma ile seçilen liken türlerinin ağır metal biriktirebilmede ne kadar önemli oldukları gösterilmiştir.

Anahtar Sözcükler: Hava kirliliği, ağır metal akümülasyonu, liken

by considering Zn accumulation. At the site no 8, Cr concentrations of *H. physodes* (4.56 μg/g) and *E. prunastri* (4.65 μg/g) were observed at similar levels.

Conclusion: Our results revealed that, *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina pollinaria* and *Usnea hirta* lichen species showed severe accumulation of all elements. This study demonstrated the importance of heavy metal accumulation in the selected lichen species.

Key Words: Air pollution, heavy metal accumulation, lichen

INTRODUCTION

According to the Environmental Protection Agency (USA), air pollution is a mixture of solid particles and gases in the air. Car emissions, chemicals from factories, dust, pollen and mold spores may be suspended as particles in the air (1). Some air pollutants are poisonous and inhaling them can increase human health problems. Air pollution represents a serious threat to both the environment and living organisms. Millions tons of toxic pollutants are released into the air each year. The following activities are major reasons of air pollution; vehicles (cars, buses, trucks, etc.) and industrial sources (factories, refineries, power plants, etc.) (2). Coal is recognized as the primary source of energy in Turkey, and its utilization in power generation is emerging as the biggest environmental problem as it emits fly ash, acid precursors, green house gases, non-combustible hydrocarbons, heavy metals and particulates. These pollutants can be carried a long distance by wind and ultimately have a negative impact on both biotic and abiotic environments (3).

Monitoring air pollution is a complex process because of the high number of potentially dangerous substances, the difficulty of estimating their synergistic or antagonistic effects, the large spatial and temporal variation of pollution phenomena, the high cost of recording instruments, and hence the low sampling density of a purely instrumental approach. For these reasons it is hard to establish a region-wide monitoring system to reveal environmental risk assessment levels. Increasing awareness of the potential hazards of large scale contamination of ecosystems by pollutants has highlighted the need for continuous monitoring of the levels of contaminants in the environment (4).

A large number of pollution studies are available in which lichens are used as bioindicators (5-7). Due to their peculiar anatomical, morphological and physiological characteristics lichens are one of the most valuable biomonitors of atmospheric pollution. They can be used as sensitive indicators to estimate the biological effects of pollutants by recording changes at the community and as accumulative monitors of persistent pollutants, which can be estimated by assaying their trace element contents (7). The epiphytic lichens have been used extensively to monitor air quality around urban areas, industrial sites and to document spatial distribution and accumulation of air borne pollutants (8-10). Lichens are used as passive pollution monitors because they accumulate a variety of pollutants in their thalli at levels well above environmental concentrations and their own physiological needs. They lack a root system and therefore intercept only allogenic atmospheric matter included in wet precipitations, dry depositions and gaseous emissions (10). The use of lichens as biomonitors of geothermal air pollution was initiated by Bargagli-Petrucci who reported the absolute absence of lichens in the geothermal area of Italy around 5 km vicinity (11). A lot of passive as well as active (transplant) biomonitoring studies using lichen have been carried out in India by several studies in different climatic regions of the country against various pollution sources (12-19).

The suitability of various lichen species in 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 (20-21). Recently, many papers have been published on heavy metal monitoring using lichens in Turkey (22-31).

The main objective of the present study is to determine the most suitable lichen species among *Evernia prunastri* (L.) *Ach.*, *Hypogymnia physodes* (L.) Nyl., *Pseudevernia furfuracea* (L.) Zopf, *Ramalina pollinaria* (Westr.) Ach. and *Usnea hirta* (L.) Weber ex F.H.Wigg. species for different air pollution sources by comparing heavy metal accumulations from Karabük Iron and Steel Factory in Karabük, Turkey.

MATERIALS and METHODS

Study area

The study area is located between 44.6218° N, and 45.7356° 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 the Karabük Iron and Steel Factory, ten samples (site no 1-10)

Figure 1. Regional map of the study area.

each of *Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, Ramalina pollinaria* 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 preparations were conducted according to the protocol given by 32. *Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, Ramalina pollinaria* and *Usnea hirta* samples were collected from Yenice Forests near the village of Yenice in Karabük province and from around the Karabük Iron and Steel Factory in Karabük (44.6218° N and 45.7356° E, Anatolia, Turkey, leg.-det. D. Cansaran-Duman), approximately 400 m above sea level. Lichen samples were collected in July 2006 and all samples are stored at University of Ankara Herbarium.

All five biomonitor lichen species (*Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, Ramalina pollinaria* and *Usnea hirta*) were air-dried and carefully cleaned with plastic tweezers under a binocular microscope (Olympus) to remove dead and as much extraneous materials (adhering bark, mosses, soil and rock particles, etc.) as possible. For the analysis, only the outermost parts of the tallus 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 heavy metal concentration

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 63% HNO3, and 1.0 ml H2O2 was added on 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 solution and distilled through blue band paper. It was completed with deionized water until the final volume was 10.0 ml.

Atomic absorption spectrophotometry (AAS) was used for analyzing heavy metals. Calibration curves of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn metals were obtained with samples of various concentration (0.25; 0.50; 1.00; 2.00; 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; 80 ppm) using linear regression analyses. Heavy metal concentration in these materials was determined using FAAS (flame atomic absorption spectroscopy- Instrument PM Avarta model Atomic absorption spectrometry) and ETAAS (electro thermal atomic absorption spectroscopy).

Statistical analysis

Statistical analyses were based on the mean value of determinations performed on each sampling point. The samples were studied three replicates. Results were given standart deviation.

RESULTS

The trace element concentrations measured in the lichen samples are given in Table 1. Accumulation of eight heavy metals Zinc (Zn), Copper (Cu), Manganase (Mn), Iron (Fe), Lead (Pb), Nickel (Ni), Chromium (Cr) and Cadmium (Cd) in thalli of five biomonitor species were evaluated by using AAS. The diversity of examined lichen species (*Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, Ramalina pollinaria* and *Usnea hirta*) in 10 monitoring sites around iron steel factory is presented in Figure 2.

Table 1. Heavy metal concentration of *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *R. pollinaria* and *Usnea hirta* (4, 22).

HM	LS no	Control	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Zn	1	28.640	26.28	25.680	24.410	30.162	25.610	28.078	40.628	32.360	31.362	53.802
		±0.174	±0.158	±0.316	±0.095	±3.455	±0.063	±0.040	±0.561	±0.158	±0.119	±0.340
	2	12.543	46.698	43.359	24.139	16.532	21.895	17.891	16.215	19.055	17.860	15.526
		±0.332	±0.058	±0.772	±0.826	±0.058	±0.261	±0.029	±0.161	±0.008	±0.555	±0.316
	3	18.375	28.432	26.168	28.102	33.155	30.151	29.404	29.975	30.255	22.366	28.896
		±0.675	±0.158	±0.192	±0.420	±0.271	±0.105	±0.121	±0.844	±0.045	±0.012	±0.327
	4	10.710	21.126	10.938	19.008	18.807	21.427	15.764	21.258	14.986	20.677	18.848
		±0.482	±0.049	±0.111	±0.188	±0.624	±0.163	±0.108	±0.298	±2.041	±0.480	±0.185
	5	5.919	10.483	11.885	9.648	14.846	16.215	16.658	24.092	20.467	19.375	13.195
		±0.039	±0.025	±0.209	±0.077	±0.068	±0.047	±0.229	±0.012	±0.064	±0.084	±0.011
Cu	1	1.810	3.090	2.050	1.940	2.770	3.350	3.300	2.990	4.450	3.550	4.560
		±0.010	±0.111	±0.100	±0.032	±0.047	±0.190	±0.016	±0.551	±0.047	±0.016	±0.047
	2	1.469	3.673	3.002	1.903	1.549	1.724	1.558	2.022	2.693	1.601	1.551
		±0.032	±0.007	±0.025	±0.190	±0.007	±0.082	±0.021	±0.019	±0.014	±0.009	±0.015
	3	1.590	3.075	2.482	2.893	2.966	2.769	2.964	2.895	3.948	2.792	2.449
		±0.022	±0.292	±0.054	±0.047	±0.038	±0.029	±0.050	±0.162	±0.051	±0.058	±0.029
	4	1.594	1.939	1.364	1.669	1.776	2.123	1.863	3.116	1.780	1.676	1.983
		±0.022	±0.044	±0.133	±0.016	±0.007	±0.064	±0.031	±0.033	±0.005	±0.024	±0.113
	5	0.378	1.489	0.905	1.226	1.369	1.273	1.353	1.384	2.129	1.800	1.435
		±0.006	±0.022	±0.044	±0.003	±0.022	±0.007	±0.008	±0.042	±0.010	±0.055	±0.013
Mn	1	42.250	45.496	32.500	41.730	56.290	119.860	92.950	71.890	112.970	31.720	54.080
		±0.965	±3.197	±0.174	±2.103	±0.142	±0.380	±0.016	±1.059	±1.091	±2.087	±0.190
	2	28.830	34.425	44.185	32.201	30.428	77.026	73.773	57.955	82.773	26.333	54.663
		±0.172	±5.665	±0.641	±0.706	±2.646	±1.209	±2.687	±1.699	±0.685	±1.409	±2.812
	3	44.805	51.511	195.880	98.433	202.73	183.029	168.602	110.977	161.922	106.100	154.840
		±0.134	±1.870	±7.041	±3.383	±0.606	±5.389	±1.289	±6.409	±3.630	±1.243	±0.157
	4	19.323	24.267	66.608	195.926	150.30	45.561	124.556	92.839	37.934	22.954	92.311
		±0.970	±2.272	±0.535	±1.550	±1.851	±0.156	±0.165	±0.331	±0.998	±0.242	±1.196
	5	8.838	22.283	31.904	14.215	20.779	21.247	27.662	16.147	73.266	21.578	11.729
		±0013	±0.233	±0.849	±1.060	±1.403	±0.147	±1.061	±0.472	±0.272	±0.008	±0.577
Fe	1	918.452	2379.00	1273.03	356.460	965.25	766.350	199.030	1558.96	3016.00	1560.13	1185.60
		±7.47	±44.19	±17.0	±7.906	±15.8	±15.8	±3.162	±46.83	±13.83	±6.665	±39.84
	2	460.228	943.032	443.061	1023.90	540.79	419.541	775.832	1289.00	2187.20	786.969	827.505
		±0.30	±5.238	±0.41	±3.069	±6.83	±11.8	±8.405	±11.76	±71.98	±0.638	±32.554
	3	1337.50	1258.60	1371.40	1679.00	272.96	1287.70	1505.90	2576.40	3173.40	2587.70	1823.70
		±50.	±15.17	±5.7	±305.5	±24.9	±4.2	±31.82	±15.66	±18.38	±33.84	±16.46
	4	495.356	552.004	916.673	574.742	649.22	653.943	583.804	1568.40	582.886	541.560	589.875
		±1.76	±7.740	±43.417	±1.709	±4.30	±2.96	±14.403	±17.33	±0.300	±17.666	±0.630
	5	515.734	999.509	487.966	703.250	652.42	653.943	463.078	592.600	1932.80	1148.70	560.891
		±38.727	±19.349	±5.369	±0.537	±4.30	±10.171	±10.127	±13.934	±2.610	±16.867	±18.523

1. *P. furfuracea*, 2. *E. prunastri*, 3. *H. physodes*, 4. *U. hirta*, 5. *R. pollinaria*, HM: Heavy metal, LS: Lichen species

Table 1. Heavy metal concentration of *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *R. pollinaria* and *Usnea hirta* (4, 22).

HM	LS no	Control	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Pb	1	4.000 ±0.035	7.200 ±0.158	4.900 ±0.158	5.100 ±0.152	6.000 ±0.073	4.130 ±0.095	4.130 ±0.095	3.150 ±0.080	4.700 ±0.160	4.600 ±0.158	9.750 ±0.128
	2	1.315 ±0.292	5.171 ±0.236	0.316 ±0.005	1.037 ±0.033	0.958 ±0.092	1.011 ±0.097	1.011 ±0.097	3.087 ±0.886	1.606 ±0.473	2.863 ±0.578	1.030 ±0.264
	3	1.76 ±0.10	1.80 ±0.16	4.08 ±0.06	3.38 ±0.11	3.39 ±0.06	2.42 ±0.15	2.42 ±0.15	2.68 ±0.25	3.37 ±0.27	4.16 ±0.60	2.94 ±0.22
	4	1.323 ±0.006	8.780 ±0.105	1.397 ±0.028	7.675 ±0.089	2.542 ±0.015	6.234 ±0.178	6.234 ±0.178	2.248 ±0.061	5.780 ±0.159	2.042 ±0.054	4.666 ±0.009
	5	0.833 ±0.006	1.263 ±0.011	1.038 ±0.006	1.119 ±0.009	0.881 ±0.008	0.817 ±0.009	0.817 ±0.009	0.975 ±0.006	1.733 ±0.008	1.167 ±0.009	0.656 ±0.008
Ni	1	2.100 ±0.100	5.170 ±0.079	2.090 ±0.063	1.490 ±0.063	2.350 ±0.158	1.974 ±0.052	1.450 ±0.079	1.240 ±0.159	2.490 ±0.063	1.880 ±0.063	4.190 ±0.079
	2	0.594 ±0.037	7.819 ±0.201	2.665 ±0.010	4.627 ±0.082	1.862 ±0.073	1.970 ±0.071	0.950 ±0.137	1.830 ±0.485	2.290 ±0.445	2.426 ±0.375	2.312 ±0.209
	3	4.83 ±0.17	6.07 ±0.11	4.95 ±0.10	10.81 ±0.29	3.92 ±0.15	3.71 ±0.10	4.34 ±0.19	5.64 ±0.29	6.27 ±0.15	4.91 ±0.19	3.74 ±0.34
	4	1.162 ±0.011	6.169 ±0.056	2.464 ±0.046	1.566 ±0.065	5.250 ±0.026	1.695 ±0.220	1.602 ±0.093	1.919 ±0.056	4.516 ±0.210	8.668 ±0.053	3.728 ±0.08
	5	0.010 ±0.001	2.265 ±0.006	3.039 ±0.009	1.474 ±0.007	0.260 ±0.013	0.356 ±0.011	0.568 ±0.009	2.169 ±0.006	0.561 ±0.011	0.101 ±0.009	2.888 ±0.006
Cr	1	2.280 ±0.007	4.540 ±0.047	2.950 ±0.032	2.730 ±0.031	3.242 ±0.024	2.940 ±0.016	2.730 ±0.007	2.620 ±0.032	4.100 ±0.063	3.440 ±0.063	3.390 ±0.079
	2	1.694 ±0.029	2.719 ±0.017	2.718 ±0.029	3.364 ±0.011	1.801 ±0.007	1.821 ±0.017	2.329 ±0.013	5.752 ±0.012	4.650 ±0.091	2.851 ±0.096	2.395 ±0.011
	3	2.37 ±0.02	2.86 ±0.04	3.07 ±0.01	3.26 ±0.04	3.79 ±0.03	2.60 ±0.02	2.95 ±0.02	3.86 ±0.08	4.56 ±0.04	3.58 ±0.02	3.31 ±0.04
	4	1.968 ±0.010	2.050 ±0.015	1.970 ±0.023	2.067 ±0.006	2.019 ±0.008	6.751 ±0.057	4.189 ±0.103	3.154 ±0.048	2.178 ±0.011	2.168 ±0.014	2.066 ±0.005
	5	1.748 ±0.010	1.999 ±0.030	1.740 ±0.023	1.733 ±0.011	1.706 ±0.042	1.751 ±0.059	1.757 ±0.011	1.727 ±0.010	2.728 ±0.068	2.203 ±0.043	1.672 ±0.010
Cd	1	0.630 ±0.007	0.725 ±0.002	0.690 ±0.008	0.490 ±0.019	0.706 ±0.007	0.618 ±0.005	0.632 ±0.004	0.668 ±0.002	0.671 ±0.003	0.720 ±0.006	0.770 ±0.007
	2	0.306 ±0.006	0.620 ±0.001	0.644 ±0.002	0.604 ±0.004	0.682 ±0.001	0.624 ±0.006	0.505 ±0.003	0.630 ±0.003	0.696 ±0.003	0.560 ±0.018	0.609 ±0.003
	3	0.733 ±0.078	0.854 ±0.002	0.616 ±0.004	0.769 ±0.002	0.692 ±0.013	0.742 ±0.008	0.773 ±0.007	0.669 ±0.005	0.843 ±0.010	0.626 ±0.002	0.875 ±0.002
	4	0.171 ±0.015	0.472 ±0.026	0.494 ±0.034	0.526 ±0.007	0.612 ±0.007	0.386 ±0.008	0.535 ±0.010	0.492 ±0.012	0.303 ±0.018	0.500 ±0.031	0.435 ±0.052
	5	0.062 ±0.015	0.262 ±0.026	0.048 ±0.001	0.020 ±0.001	0.390 ±0.028	0.243 ±0.004	0.265 ±0.010	0.222 ±0.012	0.403 ±0.018	0.237 ±0.008	0.207 ±0.009

1. *P. furfuracea*, 2. *E. prunastri*, 3. *H. physodes*, 4. *U. hirta*, 5. *R. pollinaria*, HM: Heavy metal, LS: Lichen species

Comparative heavy metal analyses of five biomonitor lichen species, maximum Zn concentration (53.80 µg/g) was reported in *P. furfuracea* in site no 10 where it takes place in the vicinity of iron steel factory (Table 1). High levels of Pb (9.75 µg/g) and Zn (53.80 µg/g) were measured in *P. furfuracea* in site no 10 (Table 1). On the other hand, minimum accumulation of Zn (10.48 µg/g) was reported for *R. pollinaria* in site no 1 and lower values of Pb (0.31 µg/g) was observed in *E. prunastri* in site no 2 (Fig. 2). The reason for higher concentration of Pb and Zn around the iron-steel factory may be due to heavy vehicular activity and other anthropogenic activities. Apart from engine emissions, Ni, Pb, Zn and Cr enter the surrounding environment due to abrasion of metallic vehicle parts. Pb mainly originates from automobile exhausts whereas Zn may be emitted by automobile tires and brake pads (33, 34). Various interactions are known to occur when plants are exposed to unfavorable concentrations of more than one trace metal. Such combination effects were categorized by Berry and Wallace as independent, additive, synergistic or antagonistic (34).

Among all the metals Ni was found to have the lowest concentration in all sites. The maximum level of Ni (10.81 µg/g) was reported in site no 3 in *H. physodes* but sites 4 (0.26 µg/g) and 9 (0.10 µg/g) had the minimum level in *R. pollinaria* (Fig 2). *H. physodes* exposed at site no 3 (10.81 µg/g) had significantly higher content of Ni, but other biomonitor lichen species are in the similar range for all other sites (Fig 2). Merely, Ni content was higher in *U. hirta* species from site no 9 (8.66 µg/g) than the other examined lichen species (Table 1). The highest value of Cu (4.56 µg/g) was measured in *P. furfuracea* thalli from site no 10 (Table 1). Wind direction may be a probable reason for dumping of this metal from outside the source. Minimum concentration of the Cu (0.90 µg/g) was reported in *R. pollinaria* in site no 2 (Table 1). Ni and Cu are both large particle metals emitted in the immediate vicinity of the station and are incapable of long-range dispersion (35). Both of the metals; Ni and Cu were accumulated to a maximum level at sites 3 and 10 in all examined lichen species (Fig. 2).

According to Fernandez et al., Cr and Fe are normally associated with the coarsest fraction of fly ash, which tends to fallout close to the source (36). In the present study the collected samples from every 5 km around iron steel factory the show higher accumulation of Cr. High Cr concentrations in *U. hirta* (6.75 µg/g) at site no 5 was recorded, although it was not found high at the samples from other sites (Table 1). But interestingly at the site no 8, Cr concentrations of *H. physodes* (4.56 µg/g) and *E. prunastri* (4.65 µg/g) revealed similar levels (Fig. 2). Fe metal at site no 8 in *H. physodes* (3173.40 µg/g) had a similar level with *P. furfuracea* species (3016.00 µg/g) from the same site (Fig. 2). The highest Fe concentration was found in *H. physodes* (3173.40 µg/g) at site no 8, while Fe concentration of *U. hirta* (582.88 µg/g) and *R. pollinaria* (592.60 µg/g) were minimum at the same site (Fig 2).

The order of magnitude of Mn accumulation at 2nd, 4th and 10th sites were; *H. physodes* > *U. hirta* > *P. furfuracea* > *E. prunastri* > *R. pollinaria*. Although *U.hirta* showed the highest levels of Mn (195.92 µg/g) at site no 3, *H. physodes* was accumulated higher amount of metal than the examined lichen species at sites 2 (195.88 µg/g) and 4 (202.73 µg/g) (Fig 2). 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 (37). The mean Cd concentration at sites 1 (0.85 µg/g), 8 (0.84 µg/g) and 10 (0.87 µg/g) were found to have the maximum level in *H. physodes* (Table 1).

In the present study *Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, R. pollinaria* and *Usnea hirta* employed, indicated that following exposure, accumulation or severe accumulation of all elements have occurred. This study demonstrated the importance of heavy metal accumulation on the selected lichen species. Heavy metals had highest concentration in the samples from around the Karabük Iron and Steel Factory in Karabük.

DISCUSSION

Recent literature indicates that lichen biomonitoring is often used as receptor based method in air quality studies. It can be useful in risk assessment for human health and it can be a powerful tool for administrators involved in environmental planning.

In the present study, heavy metal concentration results of five biomonitor species collected from around an iron steel factory were reported. According to results especially, *P. furfuracea* accumulated significantly higher levels of heavy metal than other examined lichen species at sites 7 and 10 (Fig. 2). Sites 1, 2, 7 and 10 were the central part of the city where human activities and density of traffic are very intense (Fig. 1).

According to Garty et al., the pattern of increase near the source of metal/ash content and of decrease away from the source, is relevant to the particulate nature of metals accumulated in lichen thalli (8).

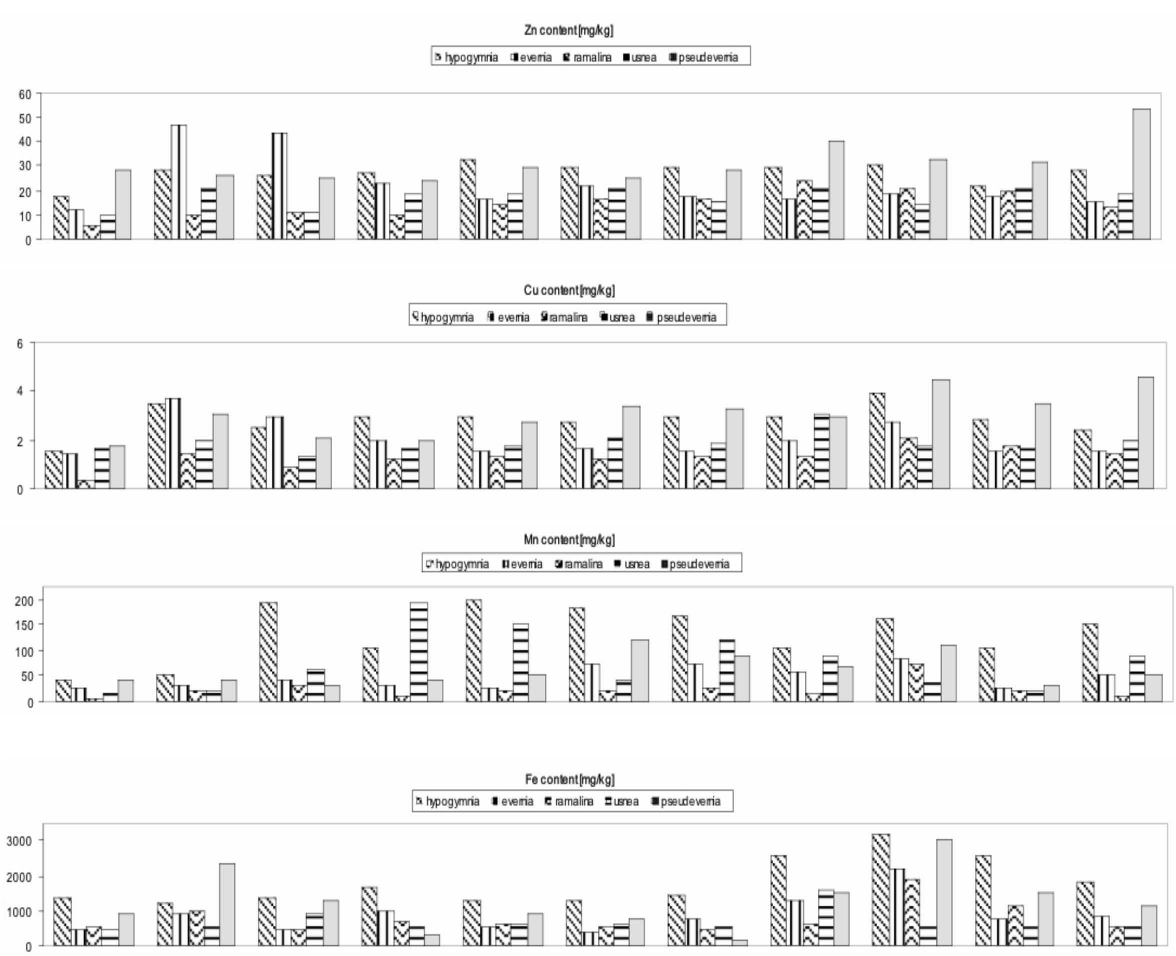

Figure 2. Comparison of *Evernia prunastri*, *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Ramalina pollinaria* and *Usnea hirta* heavy metal accumulation.

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Other studies detected a decrease of metal content at a distance of few kilometers from the source of pollution or even at a greater distance from emission points (38-40).

Cadmium has known to be associated with catalytic converters and auto exhaust (41). In the study by Uluözlü et al., the lowest and highest Cd levels in lichen species were 0.10 µg/g in *Peltigera membranacea* and 0.64 µg/g in *Xanthoparmelia conspersa* (29). Cadmium contents of other examined lichen samples have been reported to be 0.24-1.4 µg/g, 0.191 µg/g, 0.26-2.08 µg/g , 0.047-0.162 µg/g, 0.97-1.26 µg/g (42-46). Results indicate that, the maximum level of Cd (0.875 µg/g) was at site no 10 in *Hypogymnia physodes*.

Lead concentration of *P. furfuracea* (9.75 µg/g) was higher than other lichen species in site no 10. For *U. hirta* the highest concentration of Pb (8.78 µg/g) was observed at site no 1 which can be related to a selective cationic uptake as was informed (Fig 2). This finding might represent a greater affinity between Pb cations and the lichen cell wall exchange sites that are probably strongly attached to binding sites. On the other hand, lead contents in lichen samples have been reported to be 27.3-50.8 µg/g, 4.9-19.2 µg/g, 15.9 µg/g, 1.06-4.29 µg/g, 4.6-12.5 µg/g, 78-177

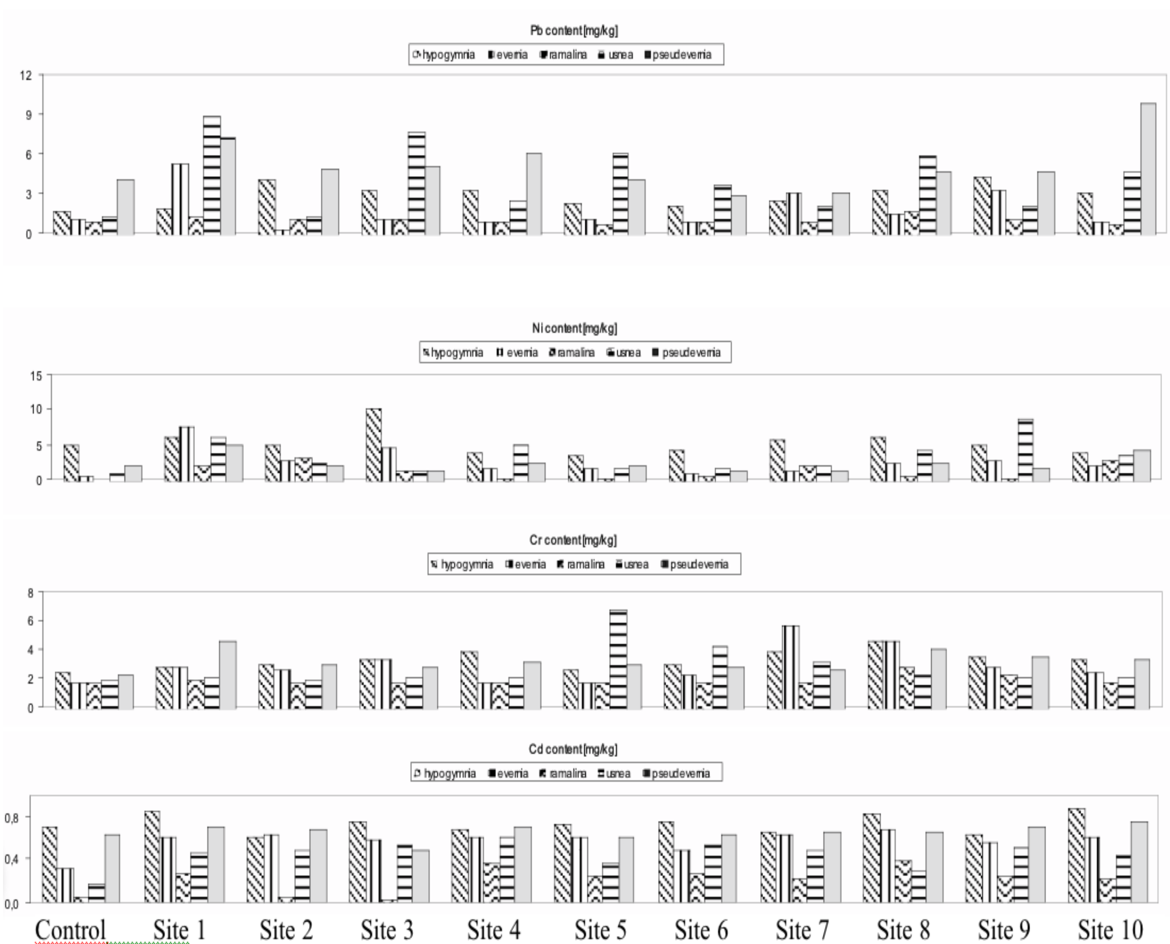

Figure 2. Comparison of *Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea, Ramalina pollinaria* and *Usnea hirta* heavy metal accumulation.

µg/g (43-48). The main source of lead from traffic is probably caused by automobile emissions. According to the Divrikli et al. about 75 % of the lead added to petrol is emitted through the exhaust and dispersed as an aerosol to the atmosphere (49). The lead contents in environmental samples from heavy traffic may be due to the exhaust of old motor vehicles because of the usage of leaded petrol in automobiles in Turkey. Generally, lead concentrations are higher in lichen samples close to the roadside (29). Our lead values in sites 1 and 10 were similar to previous studies conducted by other researchers (29, 49).

The lowest and highest copper contents in lichen species were 0.90 µg/g in *R. pollinaria* and 4.56 µg/g *P. furfuracea*, respectively. Cu contents in lichen samples in East Black Sea Region, Turkey have been reported to be 7.19 µg/g and 22.4 µg/g (29). Copper from traffic comes from corrosion of metallic parts of cars (29). According to the heavy metal results in our study, copper values in five biomonitor lichen species are in agreement with the reported values in literature.

In the current study, zinc concentration was measured as 53.80 µg/g in *P. furfuracea* at site no 10 which is located at close vicinity of the iron and steel factory. Zn contents in lichen samples have been reported to be 6.48-36.9 µg/g, 35-204 µg/g, 37-101 µg/g, and 23.7-76.1 µg/g in another study (42, 44, 45, 48). Results obtained in this study were similar to the results of other studies (42).

Iron contents in lichen samples have been reported to be 54.3-598.4 µg/g, 75.1-192.1 µg/g, 182-737 µg/g, 1800 µg/g, 1282-23035 µg/g, and 676-1220 µg/g (42-48). Fe concentrations of five biomonitor lichen species were higher than the previously reported values (Table 1).

Chromium is an essential nutrient for plant and animal metabolism. At the same time, chromium is a major water pollutant, usually as a result of some industrial pollution in tanning factories, steel works, industrial electroplating, wood preservation, and artificial fertilizers. At high levels it can cause several disorders, including lung cancer (29). Chromium contents in lichen samples have been reported to be 2.62-6.69 µg/g, 111-244 µg/g, 3.6 µg/g, 1.6-39.3 µg/g, 1.6-4.7 µg/g (42-44, 46, 48). The highest concentrations of Cr in *U. hirta* (6.75 µg/g) was measured at site no 5.

Nickel contents in lichen samples have been reported to be 2.6-11.4 µg/g, 1.1-1.8 µg/g, 0.83-10.20 µg/g (42, 45, 50). The maximum level of Ni (10.81 µg/g) was reported at site no 3 in *H. physodes*.

Pignata et al. and Wannaz et al. demonstrated that Zn was related to urban and industrial areas and Mn to agricultural activity (51, 52). High Zn content in thallus is due to motor vehicle traffic and also industrial and agricultural activities (53, 54).

Aslan et al. reported higher concentrations of Ca, Ti, Fe and Ba elements in *H. physodes* in Ordu province (26). The higher metal concentrations in *H. physodes* may be the result of a larger intercellular space in the medulla and cortex in this species (26).

Gailey and Lloyd measured the heavy metal content in *Lecanora conizaeoides* collected from Armadale (Central Scotland) and detected Zn in the range of 50-641 µg/g, depending on the distance and wind direction from a steel foundry (55). The authors found that only Fe and Zn were detected in the lichens collected from the peripheral sites of the town. Authors emphasized that the steel foundry is the main source of metal pollution in the town (55).

Several researches have evaluated bioaccumulation of heavy metals in different lichen species (20, 21, 56-58). Results are different because responses differ among species, threats differ among metals and environmental influences. Bergamaschi et al. measured twenty-nine 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 (21). Bergamaschi et al. found that, in general elements did not exhibit well defined trends, but rather showed fluctuations, and indicated that *H. physodes, P. furfuracea, U. hirta* have a similar accumulation capacity, while that of *Parmelia sulcata* is lower.

In our study, generally *P. furfuracea* as a passive biomonitor in an iron steel factory in the province of Karabük showed higher concentrations than other examined lichen species. According to our observations, accumulation capacities of heavy metal of *P. furfuracea* have comparable to *H. physodes* lichen species. Several studies revealed that lichens may selectively accumulate extracellular elements and metabolize or eliminate those elements that enter the cell wall (39, 40). Aslan et al. mentioned the heavy metal accumulation of *P. furfuracea* species from around the Karabük Iron and Steel Factory, Karabük (22). Results obtained in Zschau et al. suggest that lichen species can be successfully used to monitor air pollution (41). However, several other factors should be considered before taking a decision on the preferred biomonitor species, such as background elemental concentration, selective uptake or detoxificant mechanisms.

Finally, in this article we have compared the capacity of five biomonitor lichen species to accumulate trace elements from the atmosphere around the Karabük Iron and Steel Factory in Karabük, Turkey. This study demonstrated the importance of heavy metal accumulation in the selected lichen species. Results revealed that heavy metal accumulation in five of the biomonitor species was the highest around the Karabük Iron and Steel Factory in Karabük (Sites 1, 7, 10). According to the results, higher heavy metal concentrations were found at the sites closer to the Karabük Iron and Steel Factory in Karabük, among the five biomonitor species (Sites 1, 7, 10). When all elements were considered, *P. furfuracea* species showed higher heavy metal concentrations than *U. hirta*. *P. furfuracea* and *H. physodes* lichen species were close quarters to heavy metal accumulation in the iron-steel factory (Fig. 2). Data obtained indicate that particularly *P. furfuracea* and *H. physodes* are the suitable lichen species for the detection of air quality. This research confirms the idea that lichen species could be successfully used to monitor air pollution.

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