

Trace Metal Levels in Lichen Samples From Roadsides in East Black Sea Region, Turkey

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Objective To determine the metal contents of lichen species from East Black Sea region of Turkey for investigation of trace metal pollution sourced traffic. **Methods** The levels of copper, cadmium, lead, zinc, manganese, iron, chromium, nickel, cobalt, palladium in lichen samples collected from East Black Sea region of Turkey were determined by flame and graphite furnace atomic absorption spectrometry after microwave digestion method. The accuracy of the method was corrected by standard reference material (NIST SRM IAEA-336 Lichen). **Results** The contents of investigated trace metals in lichen samples were 7.19-22.4 µg/g for copper, 0.10-0.64 µg/g for cadmium, 4.03-44.6 µg/g for lead, 14.5-41.8 µg/g for zinc, 25.8-208 µg/g for manganese, 331-436 µg/g for iron, 1.20-3.01 µg/g for chromium, 1.48-3.90 µg/g for nickel, 0.20-3.55 µg/g for cobalt, 0.11-0.64 µg/g for palladium. The results were compared with the literature values. **Conclusion** Some lichen species such as *Xanthoparmelia conspersa*, *Xanthoria calcicola*, *Peltigera membranacea*, and *Physcia adscendens* are accumulated trace metals at a high ratio.

Key words: Trace metals; Lichen; Atomic absorption spectrometry; East Black Sea region-Turkey

INTRODUCTION

Air pollution is, as a rule, effectively measured or monitored instrumentally. Bioaccumulation of trace metals from atmospheric deposition is frequently evaluated by analyzing environmental biomonitors, such as mosses, plant leaves or lichens. Lichens are a result of the association among fungus, algae and, unlike plants, without roots or well developed cuticle. These morphological features emphasize the applicability for monitoring purposes^[1]. Within the last decades, however, many foliose or fruticose lichen species have been used as effective biomonitors or airborne-polluting mineral elements^[2]. Lichens are well known for bioaccumulation of heavy metals from the atmosphere and hence they have been used as bioindicators by many workers to assess heavy metal pollution^[3-4]. Lichens accumulate metals to a higher ratio than other plants because of their high surface area. Accumulation occurs by processes of particle

trapping, active uptake of anions, passive adsorption of cations, and ion exchange^[5].

Traffic is one of the sources of emission of heavy metals such as Pb, Cd, Fe, Cu, Mn, Zn, Ni, Cr, Co, Pd. Trace metal contents from traffic of various biological and environmental samples have been reported in the literature^[6-9]. Lead is a well known tracer of leaded gasoline and added to petrol as organic tetra-alkyl lead and ethyl-trimethyl lead. Motor cars release approximately 80 mg Pb km⁻¹ traveled^[10]. Such levels of emissions are liable to make the roadside environment a grossly contaminated zone. Lead is a well documented metal toxicant, exposure to which leads to many fatal diseases, including the dysfunction of renal blood and neurological systems. Cadmium is released by wearing motorcar tires. Lubricating oil often contains cadmium, copper, and zinc. Zinc and manganese may also be emitted by automobile tires. Nickel is found in car metal plating, welded plates, and tires^[11-12].

Turkey can be separated into seven geographic

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regions. One of them is the Black Sea region. The Black Sea region can be separated into three smaller geographic regions. The East Black Sea region is one of them. In this region, the climate is mild and rainy. The seasons are normally wet with mild temperatures. The region is very rich with respect to lichen species.

The main purpose of this paper was to determine quantitatively the regional atmospheric deposition of trace metals from traffic in East Black Sea region of Turkey. Traffic density is very high in sampling area. There is limited information about trace metal levels in lichen species from Eastern Black Sea region of Turkey. Therefore, the levels of trace metals in lichen samples collected from roadside were determined by flame and graphite furnace AAS after microwave digestion.

MATERIALS AND METHODS

Sampling

Twelve kinds of lichen sample were collected from vicinity of roadside (2-10 meters away) in East Black Sea region of Turkey during 2005. Control samples were collected from uncontaminated areas in East Black Sea region. The samples were dried at 105°C for 24 h. Dried samples were ground and homogenized using an agate homogenizer and stored in pre-cleaned polyethylene bottles until analysis.

Reagents

All reagents were of analytical grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. HNO₃ and H₂O₂ were of suprapure quality (E. Merck, Darmstadt). All the plastic and glassware were cleaned by soaking in dilute HNO₃ (1+9) and rinsed with distilled water prior to use. The element

standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element supplied from Sigma.

Apparatus

A Perkin Elmer AAnalyst 700 model AAS with deuterium background corrector was used in this study. Cd, Co, and Pd in samples were determined by HGA graphite furnace using argon as inert gas. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak height. Other measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set as recommended by the manufacturer.

Microwave Digestion

Milestone Ethos D microwave closed system was used in this study. One g of sample was digested with 6 mL of HNO₃ (65%) and 2 mL of H₂O₂ (30%) in microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively. In order to validate the accuracy and precision of the method, certified reference material SRM (IAEA-336 Lichen) was analyzed for each element. The results are shown in Table 1.

Statistical Analysis

The whole data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated heavy metal concentrations of the samples. Student's *t*-test was employed to estimate the significance of values.

TABLE 1

Observed and Certified Values (µg/g) for Elemental Concentrations in SRM ($\bar{x} \pm s$)

Element	Certified Value	Observed Value	Recovery (%)
Cu	3.55	3.48±0.15	98
Cd	0.117	0.112±0.010	96
Pb	(5) ^a	4.85±0.25	97
Zn	31.6	31.3±1.9	99
Mn	64	62.7±4.5	98
Fe	426	408.9±10.7	96
Cr	(1.03) ^a	1.05±0.08	102
Co	0.287	0.278±0.02	97

Note. ^aThe values in the parentheses are not certified.

RESULTS

All metal concentrations were determined on a dry weight basis as $\mu\text{g/g}$. The relative standard deviations were less than 10% for all elements. T-test was used in this study ($P < 0.05$). The accuracy of the method was evaluated by determining trace metals in standard reference material (SRM). The achieved results were in good agreement with certified values. The results from the analysis of SRM were all within the 95% confidence limit. Lead, cadmium, iron, copper, manganese, zinc, chromium, nickel, cobalt, and palladium were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution. Lichen species and sampling locations are given in Table 2. Heavy metal levels in the analyzed samples are given in Table 3. The contents of investigated trace metals in lichen samples were 7.19-22.4 $\mu\text{g/g}$ for copper, 0.10-0.64 $\mu\text{g/g}$ for cadmium, 4.03-44.6 $\mu\text{g/g}$ for lead, 14.5-41.8 $\mu\text{g/g}$ for zinc, 25.8-208 $\mu\text{g/g}$ for manganese, 331-436 $\mu\text{g/g}$ for iron, 1.20-3.01 $\mu\text{g/g}$ for chromium, 1.48-3.90 $\mu\text{g/g}$ for nickel, 0.20-3.55 $\mu\text{g/g}$ for cobalt, 0.11-0.64 $\mu\text{g/g}$ for palladium. All metal concentrations were higher than those of control samples.

DISCUSSION

The lowest and highest copper levels in lichen species were 7.19 $\mu\text{g/g}$ in *Ramalina pollinaria* and 22.4 $\mu\text{g/g}$ in *Xanthoria calcicola*, respectively. Copper contents in lichen samples have been reported to be 3.8-14.0 $\mu\text{g/g}$ ^[4], 11.4-96 $\mu\text{g/g}$ ^[3], 7.0-13.3 $\mu\text{g/g}$ ^[13], 9.1 $\mu\text{g/g}$ ^[14]. Our copper values are in agreement with the reported values. Copper from traffic comes from corrosion of metallic parts of cars.

Cadmium is released as combustion products in the accumulators of motor vehicles or in carburetors. Cadmium can accumulate in lichens from soil. Cadmium may accumulate in the human body and induce kidney dysfunction, skeletal damage and reproductive deficiencies. In this study, the lowest and highest cadmium levels in lichen species were 0.10 $\mu\text{g/g}$ in *Peltigera membranacea* and 0.64 $\mu\text{g/g}$ in *Xanthoparmelia conspersa*. Cadmium contents of lichen samples have been reported to be 0.24-1.4 $\mu\text{g/g}$ ^[4], 0.191 $\mu\text{g/g}$ ^[14], 0.26-2.08 $\mu\text{g/g}$ ^[13], 0.047-0.162 $\mu\text{g/g}$ ^[15], 0.97-1.26 $\mu\text{g/g}$ ^[16].

In this study, the lowest and highest lead contents in lichen species were 4.03 $\mu\text{g/g}$ in *Ramalina pollinaria*

TABLE 2

Description of Sampling Area and Lichen Species

Location	Species of Lichen	Substrate	Number of Herbarium	Altitude
Değirmenağzı Village in Keşap District of Giresun Province	<i>Cladonia furcata</i> (Huds.) Schrad. (Fruticose)	On Soil	2349	10 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Parmelina tiliaceae</i> (Hoffm.) Ach. (Foliose)	On Siliceous Rocks	2350	8 m
Değirmenağzı Village in Keşap District of Giresun Province	<i>Peltigera membranacea</i> (Ach.) Nyl. (Foliose)	On Mosses	2351	10 m
Yeniköy Village in Gülyalı District of Ordu Province	<i>Physcia adscendens</i> (Fr.) H. Olivier (Foliose)	On <i>Ficus</i> sp.	2352	5 m
Efirli Village in Perşembe District of Ordu Province	<i>Physcia tribacia</i> (Ach.) Nyl. (Foliose)	On <i>Populus</i> sp.	2353	2 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Prototrematiopsis muralis</i> (Schreb.) M. Choisy (Foliose)	On Siliceous Rocks	2354	8 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Ramalina pollinaria</i> (Westr.) Ach. (Fruticose)	On Siliceous Rocks	2355	8 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Roccella phycopsis</i> Ach. (Fruticose)	On Siliceous Rocks	2356	8 m
Değirmenağzı Village in Keşap District of Giresun Province	<i>Xanthoparmelia conspersa</i> (Ehrh. ex Ach.) Hale (Foliose)	On Siliceous Rocks	2357	3 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Xanthoparmelia somloensis</i> (Gyeln.) Hale (Foliose)	On Siliceous Rocks	2358	8 m
Koru Place in Yeşilyalı Town of Trabzon Province	<i>Xanthoria calcicola</i> Oxner (Foliose)	On Siliceous Rocks	2359	8 m
Efirli Village in Perşembe District of Ordu Province	<i>Xanthoria parietina</i> (L.) Th. Fr. (Foliose)	On <i>Populus</i> sp.	2360	2 m

TABLE 3

Levels of Trace Metals as $\mu\text{g/g}$ in Lichen Species ($\bar{x} \pm s$)

Herbarium No.	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni	Co	Pd
2349	8.63±0.6	0.20±0.01	7.20±0.3	16.5±1.3	26.2±2.3	367±31	BDL	1.48±0.10	0.20±0.01	0.51±0.04
2350	19.6±1.1	0.41±0.03	15.7±1.1	36.7±3.3	66.7±6.3	401±34	BDL	3.90±0.25	0.70±0.06	0.30±0.02
2351	11.4±1.0	0.08±0.006	7.18±0.6	30.9±2.5	208±18	331±29	BDL	BDL	0.20±0.01	0.26±0.02
2352	20.9±1.8	0.32±0.02	32.8±2.5	36.9±3.1	94.1±8.5	402±34	2.39±0.20	3.76±0.30	3.39±0.21	0.32±0.01
2353	15.4±1.3	0.33±0.02	17.7±1.4	40.2±2.7	44.9±2.7	413±35	BDL	1.54±0.10	1.33±0.11	0.19±0.01
2354	11.9±1.1	0.15±0.01	12.5±1.2	17.3±1.2	60.9±4.5	436±41	1.88±0.15	BDL	3.55±0.30	0.64±0.03
2355	7.19±0.6	0.25±0.02	4.03±0.3	14.5±1.3	25.8±1.7	356±28	BDL	BDL	0.25±0.02	0.11±0.01
2356	10.3±0.9	0.17±0.01	12.5±1.1	19.6±1.6	50.3±3.9	392±32	BDL	BDL	1.32±0.10	0.30±0.02
2357	11.8±1.0	0.64±0.04	44.6±4.1	29.3±1.9	45.8±4.1	398±29	3.01±0.20	1.93±0.14	1.98±0.11	0.54±0.05
2358	12.3±1.1	0.08±0.005	19.9±1.8	28.7±2.2	41.2±2.9	403±30	1.20±0.11	BDL	2.32±0.20	0.36±0.02
2359	22.4±1.9	0.17±0.01	38.1±3.3	39.8±3.5	132±11	418±33	2.53±0.24	BDL	1.70±0.11	0.19±0.01
2360	20.2±1.7	0.48±0.04	26.5±2.2	41.8±3.8	70.3±6.7	409±38	1.81±0.12	1.93±0.12	1.27±0.10	0.22±0.01
Control Samples	4.7±0.3	0.05±0.004	0.5±0.1	10.2±0.1	13.2±1.1	125±10	BDL	BDL	0.10±0.01	BDL

Note. BDL: Below detection limit.

and 44.6 $\mu\text{g/g}$ in *Xanthoparmelia conspersa*. Lead contents in lichen samples have been reported to be 27.3-50.8 $\mu\text{g/g}$ ^[16], 4.9-19.2 $\mu\text{g/g}$ ^[13], 15.9 $\mu\text{g/g}$ ^[14], 1.06-4.29 $\mu\text{g/g}$ ^[15], 4.6-12.5 $\mu\text{g/g}$ ^[3], 78-177 $\mu\text{g/g}$ ^[17]. The main source of lead from traffic is probably automobile emissions. About 75 % of the lead added to petrol is emitted through the exhaust and dispersed as an aerosol in the atmosphere^[18]. The lead contents in environmental samples from heavy traffic may be due to the exhaust of old motor vehicles because of the usage of unleaded petrol on automobiles in Turkey. Generally, lead concentrations are higher near the roadside lichen samples. Our lead values are in agreement with the reported values.

In this study, the minimum and maximum zinc contents in lichen species were 14.5 $\mu\text{g/g}$ in *Ramalina pollinaria* and 41.8 $\mu\text{g/g}$ in *Xanthoria parietina*. Zinc contents in lichen samples have been reported to be 6.48-36.9 $\mu\text{g/g}$ ^[15], 35-204 $\mu\text{g/g}$ ^[17], 37-101 $\mu\text{g/g}$ ^[13], 23.7-76.1 $\mu\text{g/g}$ ^[4].

In the present study, the lowest and highest manganese contents in lichen species were 25.8 $\mu\text{g/g}$ in *Ramalina pollinaria* and 208 $\mu\text{g/g}$ in *Peltigera membranacea*. Manganese contents in lichen samples have been reported to be 20-1021 $\mu\text{g/g}$ ^[17], 38.2 $\mu\text{g/g}$ ^[14], 57.3-104 $\mu\text{g/g}$ ^[16], 22.7-114.3 $\mu\text{g/g}$ ^[4], 12.7-67.1 $\mu\text{g/g}$ ^[3]. Manganese, one of the least toxic metals, if inhaled as MnO_2 dust, is more hazardous than ingested manganese^[19]. Toxicity limits of manganese in plants are high (400-1000 $\mu\text{g/g}$). Our values are under toxicity limits.

In our study, the lowest and highest iron contents in lichen species were 331 $\mu\text{g/g}$ in *Peltigera membranacea* and 436 $\mu\text{g/g}$ in *Prototermeliopsis muralis*. Iron contents in lichen samples have been reported to be 54.3-598.4 $\mu\text{g/g}$ ^[4], 75.1-192.1 $\mu\text{g/g}$ ^[3],

182-737 $\mu\text{g/g}$ ^[13], 1800 $\mu\text{g/g}$ ^[14], 1282-23035 $\mu\text{g/g}$ ^[17], 676-1220 $\mu\text{g/g}$ ^[16]. Our iron values are lower than the reported values.

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, etc. and artificial fertilizers. At high levels it can cause several disorders, including lung cancer. In this study, the lowest and highest chromium contents in lichen species were 1.20 $\mu\text{g/g}$ in *Xanthoparmelia somloensis* and 3.01 $\mu\text{g/g}$ in *Xanthoparmelia conspersa*. Chromium contents in lichen samples have been reported to be 2.62-6.69 $\mu\text{g/g}$ ^[16], 111-244 $\mu\text{g/g}$ ^[17], 3.6 $\mu\text{g/g}$ ^[14], 1.6-39.3 $\mu\text{g/g}$ ^[13], 1.6-4.7 $\mu\text{g/g}$ ^[4]. Chromium levels in many analyzed lichen samples were below the detection limit of flame AAS. Our chromium levels are lower than the reported values.

In the present study, the lowest and highest nickel contents in lichen species were 1.48 $\mu\text{g/g}$ in *Cladonia furcata* and 3.90 $\mu\text{g/g}$ in *Parmelina tiliaceae*. Nickel contents in lichen samples have been reported to be 2.6-11.4 $\mu\text{g/g}$ ^[4], 1.1-1.8 $\mu\text{g/g}$ ^[15], 0.83-10.20 $\mu\text{g/g}$ ^[20]. Nickel levels in many analyzed lichen samples were below the detection limit of flame AAS.

The minimum and maximum cobalt contents in lichen species were 0.20 $\mu\text{g/g}$ in *Cladonia furcata* and 3.55 $\mu\text{g/g}$ in *Prototermeliopsis muralis*. Cobalt contents in lichen samples have been reported to be 0.28-0.55 $\mu\text{g/g}$ ^[16], 0.284 $\mu\text{g/g}$ ^[14].

The lowest and highest palladium contents in lichen species were 0.11 $\mu\text{g/g}$ in *Ramalina pollinaria* and 0.64 $\mu\text{g/g}$ in *Prototermeliopsis muralis*. There is limited information about palladium contents in lichen samples. Palladium contents in lichen samples

are sourced from catalytic converters in cars. Palladium occurs in the environment in ultra trace amounts. The use of catalytic converters has led to an increase of palladium concentrations in different environmental matrices such as soil, water, vegetation and lichen in areas near intensive vehicle traffic, because a part of this element leaves the catalyst surface during the lifetime of a catalyst and is transferred into the environment^[21].

The concentration of trace metals in samples

depends on lichen species. Some species such as *Xanthoparmelia conspersa*, *Xanthoria calcicola*, *Peltigera membranacea*, *Physcia adscendens* are accumulated trace metals at a high ratio.

A linear regression correlation test was performed to investigate correlations between metal concentrations. The values of correlation coefficients between metal concentrations are given in Table 4. There are positive correlations between metal concentrations in general.

TABLE 4

Correlation Between Metal Concentrations in Lichen Samples (r=95%)

	Cu	Cd	Pb	Zn	Mn	Fe	Cr	Ni	Co	Pd
Cu	1.000									
Cd	0.284	1.000								
Pb	0.612	0.577	1.000							
Zn	0.871	0.385	0.592	1.000						
Mn	0.336	-0.295	0.084	0.396	1.000					
Fe	0.530	0.224	0.510	0.334	-0.321	1.000				
Cr	0.201	0.607	0.841	0.193	0.355	-0.204	1.000			
Ni	0.694	0.001	0.128	0.334	0.775	0.225	-0.019	1.000		
Co	0.326	0.028	0.496	0.122	-0.081	0.733	-0.083	0.421	1.000	
Pd	-0.288	0.070	0.098	-0.411	-0.233	0.277	0.095	-0.188	0.460	1.000

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(Received October 10, 2006 Accepted April 4, 2007)