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Determination of Trace Elements of Some *Cladonia* Species from Turkey by ICP-MS

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Abstract

Aim of study: Lichens are biological indicators of environmental pollution of both natural and artificial origin in terms of various elements. In 9 samples (*Cladonia coniocraea*, *C. fimbriata*, *C. firma*, *C. foliacea*, *C. furcate*, *C. pocillum*, *C. pyxidata*, *C. rangiformis* and *C. subulata*) belonging to the genus *Cladonia* collected from different regions of Turkey; Aluminum, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Arsenic, Selenium, Strontium, Cadmium, Tin, Mercury and Lead element amounts were investigated.

Area of study: Lichen samples belonging to the same genus were collected in field studies conducted in 7 different cities in Turkey.

Material and methods: All samples were analyzed independently after solubilization in acid medium in microwave system and in triplicate. The accuracy of the results has been confirmed by analysis of 1547 Peach Leaf certified reference material.

Main results: Sources of metals, metal accumulation mechanisms and how much they are absorbed by lichens were determined. The highest Al, Cr, Ni concentrations in *Cladonia firma* are 429.3, 3.04, 13.53 µg/g, respectively. The highest Co, Sr, Cu, Se, Hg concentrations in *Cladonia subulata* are 0.81, 33.59, 13.16, 0.158, 0.060 µg/g, respectively. The highest Pb, Cd, Sn concentrations in *Cladonia pyxidata* are 5.90, 0.30, 6.50 µg/g, respectively. The highest Fe, Mn, Zn, As concentrations were 228.7, 17.8, 26.44 and 1.335 µg/g, respect.

Highlights: The results showed that metal accumulation in lichens was directly related to the environment in which lichens were collected.

Keywords: ICP-MS, Lichens, *Cladonia*, Trace element.

Türkiye'deki Bazı *Cladonia* Türlerindeki Eser Elementlerin ICP-MS ile Belirlenmesi

Öz

Çalışmanın amacı: Likenler, çeşitli elementler açısından hem doğal hem de yapay kaynaklı çevresel kirliliğin biyolojik göstergeleridir. Türkiye'nin farklı bölgelerinden toplanmış *Cladonia* cinsine ait 9 örnekte (*Cladonia coniocraea*, *C. fimbriata*, *C. firma*, *C. foliacea*, *C. furcate*, *C. pocillum*, *C. pyxidata*, *C. rangiformis* and *C. subulata*) Alüminyum, Krom, Mangan, Demir, Kobalt, Nikel, Bakır, Çinko, Arsenik, Selenyum, Stronsiyum, Kadmiyum, Kalay, Cıva ve Kurşun element miktarları araştırılmıştır.

Çalışma alanı: Türkiye'de 7 farklı ilde yapılmış arazi çalışmalarında aynı cinse ait liken örnekleri toplanmıştır.

Materyal ve yöntem: Tüm örnekler bağımsız olarak mikrodalga sistemde asit ortamında çözünürleştirildikten sonra ve üç paralel halinde analiz edilmiştir. Sonuçların doğruluğu, 1.547 Şeftali Yaprağı sertifikalı referans materyalin analiziyle onaylanmıştır.

Temel sonuçlar: Metallerin kaynakları, metal birikim mekanizmaları ve likenler tarafından ne kadar absorblandıkları belirlenmiştir. *Cladonia firma*'da en yüksek Al, Cr, Ni konsantrasyonları sırasıyla 429.3, 3.04, 13.53 µg/g'dır. *Cladonia subulata*'da en yüksek Co, Sr, Cu, Se, Hg konsantrasyonları sırasıyla 0.81, 33.59, 13.16, 0.158, 0.060 µg/g'dır. *Cladonia pyxidata*'da en yüksek Pb, Cd, Sn konsantrasyonları sırasıyla 5.90, 0.30, 6.50 µg/g'dır. En yüksek Fe, Mn, Zn, As konsantrasyonları sırasıyla 228.7, 17.8, 26.44, 1.335 µg/g'dır.

Araştırma vurguları: Sonuçlar likenlerde metal birikiminin doğrudan likenlerin toplandığı ortamla ilişkili olduğunu göstermiştir.

Anahtar Kelimeler: ICP-MS, Likenler, *Cladonia*, Eser element

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Introduction

Human activities and natural processes cause environmental pollution to increase with different pollutants inclusive toxic metals. Different organisms for example mosses, lichens and fungi are used to determine environmental pollution caused by metals (Chiarenzelli et al., 2001; Adamo et al., 2003; Michelot et al., 1994). Lichens are symbiotic systems consisting of a fungus and a photosynthetic partner (green algae and cyanobacteria). In some cases, it has been determined that bacteria are included in this association (Hodkinson & Lutzoni, 2009; Selbmann et al., 2010). Lichens develop in a very wide geography from deserts to poles (Brodo et al., 2001). It has a thallus without roots and cuticles, and therefore it takes minerals directly from the atmosphere. It has been one of the best biological indicators of air pollution, with its capability to collect mineral elements in excess of their wants. (Garty, 2001; Wolterbeek, 2020). It is estimated to cover about 8% of the world's terrestrial surface (Ahmadjian, 1995). Lichens have a significant impact on the conservation of terrestrial life, ecosystem function and biodiversity. (Hawkworth, 1988). Growing relatively slowly (few cm maximum per year), they are fast bare ground colonizers, some in metal-contaminated areas. (Gilbert, 1980).

Lichens are present in the structure of unique, secondary products 'stress metabolites' that enable them to survive in adverse areas. (Elix, 1996). Lichens without protective cuticles and roots absorb metals, nutrients and trace elements from dry and wet atmospheric deposition. (Bargagli, 1998). Therefore, in addition to the nutritional and toxic elements present in the atmosphere, those in the sub-strategy penetrate the lichen body. Some species can activate tremendous amounts of metal elements and are hyper accumulator (Callahan et al., 2006) Metals are directly or indirectly involved in the development of plants, fungi and lichens. Most metals, including essential elements such as Calsium, are toxic to plants and fungi when ingested in excess (Gadd, 1993). Most of the metal content of lichens originates from the atmosphere (Garty, 2001), but many studies have also shown that they retain particles from the substrate (Backor and Loppi, 2009). These particles can be stored for a long time by keeping them on the surface of lichens or in the intercellular spaces (Rinino et al., 2005). The *Cladonia* is classified in the Cladoniaceae The genus contains a large number of secondary metabolites that are systematically very important. Species of the *Cladonia* usually contain two types of thallus: a primary thallus typically in the form of horizontal scales and a vertically developed secondary thallus called podetia (Ahti et al., 2013). The formation of *Cladonia* species is often recorded in areas enriched with metal (Gilbert, 1990; Rola & Osyczka, 2014). Some species have proven to be essential epigeic colonizers of strong contaminated and disturbing areas where they play a key role in natural regeneration (Osyczka & Rola, 2013).

Lichens, besides being sensitive to air pollutants, are also good metal accumulators. Both precipitation and dust and minerals in natural and anthropological substrates (crust, soil, rock) nutrients and metals. Lead, Nickel, Mercury, Chromium, Zinc, Titanium and Vanadium are among the significant metal pollutants (Hutchinson et al., 1996). This study aimed at determining the metal content of 9 species (*Cladonia coniocraea* (Flörke) Spreng., *C. fimbriata* (L.) Fr., *C. firma* (Nyl.) Nyl., *C. foliacea* (Huds.) Willd., *C. furcata* (Huds.) Schrad., *C. pocillum* (Ach.) O.J. Rich., *C. pyxidata* (L.) Hoffm., *C. rangiformis* Hoffm., and *C. subulata* (L.) Weber ex F.H. Wigg.) belonging to the *Cladonia* genus in Turkey.

There are several various methods for the definition of trace elements in lichen samples. There are various spectrometric techniques, including, neutron activation analysis (NAA) (Ila, 1988; Reis et al., 1999), X-ray fluorescence (XRF) and flame atomic absorption spectrometry (FAAS) (Herrero Fernandez et al., 2016), graphite furnace or electrothermal evaporation (GF- or ET-AAS) (Quevauviller et al., 1996), inductively coupled plasma atomic emission spectrometry (lCP-AES), Inductively Coupled Plasma-Optical Emission spectrometry (ICP-OES) (Baffi et al., 2002) and inductively coupled plasma mass spectrometry (lCP-MS) (Yayintas et al., 2018). Inductively Coupled Plasma Mass Spectrometry device has been widely used for the analyses of minor and trace elements. ICP-MS technology is a safe and efficient method in multi-element trace element analysis with higher sensitivity (Tokalıoğlu et al., 2019; Tuzen et al., 2007).

Concentrations of Aluminum, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Arsenic, Selenium, Strontium, Cadmium, Tin, Mercury and Lead elements in 9 different *Cladonia* species distributed in different regions of Turkey were determined by inductive coupling plasma mass spectrometry. The samples were solubilized in the presence of nitric acid and hydrogen peroxide using a microwave system..

Materials and Methods

Lichen Materials

Lichen samples were collected from field studies in different regions of Turkey. The names of the species were determined according to morphological and anatomical features by using diagnostic keys (Ahti & Hammer, 2002; Ahti et al., 2013). This genus is classified in the family Cladoniaceae (Lecanorales, Ascomycota), containing numerous secondary metabolites, including lichen acids and phenolic compounds Huovinen and Ahti, 1982; Miadlikowska et al., 2006; Kocakaya et al. 2021). Specimens are preserved in Yozgat Bozok University, Boğazlıyan Vocational School, Lichen herbarium. The locality information and herbarium numbers are given in below.

Turkey, Istanbul, Belgrad forests, 41°08'857"N, 28°55'683"E, 20 m., 13 Sept. 2013, (*C. coniocraea;* CLAD 77, 109). Çankırı, Ilgaz, 41°00'848"N, 33°42'495"E, 1200 m, 07 July 2014, (*C. fimbriata;* CLAD 712, 713). Çanakkale, Bayramiç, 39°55'320"N, 26°45'634"E, 220 m, 16 Sept.2013, (*C. firma;* CLAD 52). Ankara, Güdül, 40°12'55"N, 32°09'54"E, 750 m, 21 July 2014, (*C. foliacea;* CLAD 640). Rize, Kackar Mountains National Park, 40°55'592"N, 41°08'801"E, 1750 m, 16 Aug. 2014, (*C. furcate;* CLAD 488). Mersin, Anamur, 36°05'592"N, 33°04'345"E, 31 m, 19 May 2013, Mersin, Çamlıyayla, 37°11'185"N, 34°37'579"E, 1350 m, 20 May 2013, (*C. pocillum*; CLAD 1, 55). Çorum, 40°41'486"N, 34°49'277"E, 1325 m, 25 May 2013, (*C. pyxidata;* CLAD 135, 137). Çorum, 40°31'855"N, 35°04'103"E, 1186 m, 24 May 2013, (*C. rangiformis*; CLAD 53). Ordu, Çambaşı Plateau, 40°44'06"N, 37°56' 9"E, 1560 m, 24 Sept. 2014, (*C. subulata;* CLAD 998).

Sample Preparation and Digestion

Lichen samples (n=9) were washed with tap water, dried at 105 °C and ground. Approximately 100 mg of lichen sample was weighed in a teflon vessel. 2 mL of HNO_3 and 5 mL of H_2O_2 were added and waited for about 20 minutes. Separation of the samples was carried out in a microwave digestion system (Berghof application report, 2008). The solution was made up to 25 mL with double distilled water. The Berghof mws-4 microwave system (Berghof Speedwave MWS four digestion system, Germany) made of Teflon was used for microwave digestion. A blank sample was prepared for control. Each sample was analyzed 3 times.

Instrument and Reagents

For determination of the elements an inductively coupled plasma mass spectrometer (ICP-MS Agilent 7500a, Agilent Technologies, Tokyo, Japan) equipped with an autosampler, a Babington nebulizer, Ni cones, a Peltier cooled quartz spray chamber, a standard torchand a peristaltic sample delivery pump was employed. High purity argon gas was used to create the plasma in the ICP-MS. The pulse to analog detector factor was determined on the day of analysis. Agilent ICP-MS tuning solution of 10 µg L^{-1} (Cerium, Cobalt, Lithium, Thallium and Yttrium) was used for the tuning setting of the instrument before each experiment. All measurements were conducted using a full quantitative mode analysis. Each sample was analyzed in triplicate and three blanks were used. The optimized ICP-MS operation conditions for analysis are shown in Table 1.

High purity ICP-MS multi element standard solution obtained from Merck (Darmstadt, Germany) was used for the preparation of calibration curves in the quantitative analysis of the elements. Double distilled water (18.2 MΩ cm) was used in all solutions. A mixed internal standard solution with concentration 200 µg L^{-1} ^{45}Sc, ^{103}Rh and ^{209}Bi was used to correct changes in the sample uptake rate and plasma conditions for the ICP-MS measurements.

Table 1. Optimal ICP-MS operating conditions for analysis of samples.

Parameter	Value
Radio frequency power	1280 W
Sample depth	7.9 mm
Torch-H	-0.4 mm
Torch-V	1 mm
Carrier gas	1.23 L/min
Makeup gas	0.1 L/min
Auxiliary gas flow rate	0.9 L/min
Plasma gas flow rate	15 L/min
Nebulizer pump	0.12 rps
Spray chamber temperature	2 °C

The calibration curve was prepared in the range of 0–50 µg $L^{-1}$for Aluminum, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Arsenic, Selenium, Strontium, Cadmium, Tin, Mercury and Lead elements. In the study, the determination coefficients of the calibration curves of the elements in the concentration range of 0–50 µg L^{-1} are higher than 0.99. Standard reference material 1547 Peach Leaves (NIST) was analyzed for metallic elements utilizing the same procedure to check the effectiveness of the digestion and analytical procedure. The results are given in Table 2.

Table 2. Analysis of certified reference material (1547 Peach Leaves).

Element	Concentration (µg g^{-1}) Certified	Found	Recovey (%)
Aluminum	248.9 ± 6.5	249.2 ± 4.5	99.87
Chromium	1.020 ± 0.002	0.98 ± 0.02	104.1
Manganese	97.8 ± 1.8	99.2 ± 1.4	98.58
Iron	219.8 ± 6.8	218.7 ± 5.0	100.5
Cobalt	0.070 ± 0.01	0.068 ± 0.01	102.94
Nickel	0.689 ± 0.095	0.695 ± 0.080	99.1
Copper	3.75 ± 0.37	3.70 ± 0.25	101.3
Zinc	17.97 ± 0.53	18.00 ± 0.20	99.8
Arsenic	0.062 ± 0.014	0.064 ± 0.010	96.9
Selenium	0.120 ± 0.017	0.122 ± 0.010	98.4
Strontium	53.0 ± 5.0	52.8 ± 4.0	100.37
Cadmium	0.026 ± 0.002	0.026 ± 0.001	101.2
Mercury	0.032 ± 0.004	0.031 ± 0.002	102.9
Lead	0.87 ± 0.02	0.88 ± 0.02	99.3

Limit of detection (LOD) and limit of quantification (LOQ) are two critical performance characteristics in method validation. LOD and LOQ are terms used to express the lowest concentration of an analyte reliably measured by an analytical procedure. LOD and LOQ were calculated based on the standard deviation of blank, i.e., 3xσ and 10xσ.

The limit of detection (LOD) and limit of quantification (LOQ) of the ICP-MS were given in Table 3.

Table 3. Instrumental limit of detection and limit of quantitation

Element	LOD (µg L^{-1})	LOQ (µg L^{-1})
Al	0.105	0.315
Cr	0.006	0.020
Mn	0.016	0.050
Fe	0.014	0.042
Co	0.015	0.045
Ni	0.002	0.006
Co	0.005	0.015
Zn	0.002	0.006
As	0.004	0.012
Se	0.009	0.027
Sr	0.010	0.030
Cd	0.003	0.009
Sn	0.018	0.056
Hg	0.003	0.009
Pb	0.001	0.003

Results and Discussion

In this work the concentrations of Aluminum, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Arsenic, Selenium, Strontium, Cadmium, Tin, Mercury and Lead elements in nine lichen samples were determined by using inductive couple plasm-mass spectrophotometry (ICP-MS). The mean metal concentrations (µg/g) and standard deviations for lichen samples from the various regions of Turkey were shown in Table 4. It has been determined that the elements studied in our study are generally compatible with certified reference material values.

If we examine our work to two groups in the first group, the toxic effects of the amounts accumulated in the analyzed lichens and the spatial distribution of metals, and in the second group, what are the geographical factors that affect this spatial distribution. Today, when the comfort and convenience of modern life has become indispensable for human beings, to provide these conditions, especially the use of fossil-sourced fuels for energy production (especially motor vehicles), agricultural fields, fertilizers and chemicals in industrial activities, metals that cause environmental pollution can be counted among the sources. The metal absorption abilities of lichens are related to both their anatomical and morphological structures. It has been determined that lichens with large surface areas accumulate more metals. Other factors that increase metal intake; wide intercellular spaces, high cell permeability, high atmospheric pollutant count, and thin upper cortex or absence of upper cortex (Garty & Ammann, 1987).

If we look at element by element to summarize this classification better. Elements such as Aluminum, Iron, Magnesium and Manganese are plenty in the earth's crust. Unwashed lichen thallus has a high metal content due to dust and soil. (Rossbach et al., 1999). Aluminum makes up about 8% by weight of the Earth's crust and is the most abundant metallic element. High Al concentrations are ecologically important because Al can alter the cycling and availability of important elements such as P, organic C and some trace metals and is potentially toxic to organisms (Driscoll & Schecher, 1990). Al concentration is in the lichen samples varied lowest Al concentration is *C. pyxidata* 109.7 μg/g and the highest value of Al concentration was recorded at *C. firma* (Turkey, Çanakkale, Bayramiç, Northwest of Hacıbekirler village) 429.3 μg/g in this study.

Chromium is commonly found in rocks, freshwater and seawater (Krishnamurthy & Wilkens, 1994). The maximum Cr concentration was recorded at *C. firma* 3.04 μg/g (Turkey, Çanakkale, Bayramiç, Northwest of Hacıbekirler village). The average Cr concentration was 0.57-3.04 μg/g in the samples. Most of the metal content of lichens is of atmospheric origin (Garty, 2001), but also It has been documented in many studies that they also capture particles from the substrate (Backor and Loppi, 2009). Our results

Mn, one of the least toxic metals, if inhaled as MnO_2 dust is more hazardous than ingested manganese. (Egyed and Wood, 1996). The average manganese concentration was 5.2-10.7 μg/g in the samples. The lowest and highest manganese values are observed *C. pocillum* and *C. subulata*. Our values are lower than literature value (Mendil et al., 2005).

In terms of iron concentration is in the lichen samples varied between 48.49 and 240 mg/L. *C. furcata* (Turkey, Rize, Çamlıhemşin, Kackar Mountains National Park, North of Ayder, Kavrun plateau road) is the highest. Iron is an essential metal for most living organisms and humans. It is usually more abundant in water enviroment than other metals, due to its high occurrence on Earth. High Fe concentration has adverse effects on humans, animals and the environment (Forstner & Wittmann, 1979).

Ni concentration is similar in all samples but the sample *C. foliacea* is quite different from the others (Turkey, Ankara, Güdül, Between Güdül and Beypazarı, serpentine rocks). While normal values for nickel should be between 0.1 and 5 μg/g, in this example it is around 13 μg/g, this value is highly toxic. Ni pollution is mainly caused by industrial activities, mineral and organic fertilizers, chemical pesticides, Ni-added diesel fuels and engine oils spread by exhaust, residential areas, industry, refinery, and sewage wastes. The Ni in these wastes is dispersed in the soil and air, and its transition to plants is easy. Nickel is easily absorbed by plant roots, and it limits root growth and shoot development in concentrations (Nriagu, 1979; Mishra & Kar, 1974).

Cobalt is one of the most basic elements on earth. Cobalt polluting our environment as waste; It is found in small amounts on rocks, soil, plants, animals, and ocean bottoms. The formation of metamorphic rocks is based on cobalt concentrate. Cobalt is obtained as a by-product with ores of copper, nickel, silver, as well as gold, lead and zinc. In its pure form, cobalt has few applications, but its use as an alloying element and as a chemical source makes it strategically important. It has important usage areas in industrial applications and military fields. Cobalt is mainly used in the rocket industry, superalloys and special steels, as well as in rechargeable batteries of electronic devices such as mobile phones and laptops. (Richardson, 1995) The highest Co was determined in C. subulata, *C. coniocraea and C. rangiformis* species. *C. pocillum* has the lowest Co concentration.

If we examine copper, similar results were obtained in all samples. Standard values should be between 4-15 μg/g and the results in all samples remained within this range (Markert, 1993). None of the measured values for zinc are normal above the limit values to a toxic effects not. Many physical and chemical properties of Zn and cadmium is similar. Because both are II B group elements. In addition, they often coexist in mineral deposits and compete over reactions with various ligands. The reality Cd is a toxic metal and zinc is an essential element makes this combination interesting and reveals that the toxic effects of Cd can be prevented by Zn (Hinesly et al., 1984).

Zinc is a common element in the earth's crust and is released into the environment through both natural and anthropogenic origins. Emissions from anthropogenic sources are more than natural sources. Anthropogenic sources of zinc in the environment (air, water, soil) are often associated with zinc-containing mining and metallurgical processes, as well as the use of zinc-containing commercial products. The anthropogenic sources of zinc in the soil appear to originate from smelting tailings, mine tailings, coal ash dumping, and the use of commercial products such as zinc-containing fertilizers and wood preservatives. While the highest zinc concentration was 26.4 μg/g, the lowest zinc concentration was 8.12 μg/g.

As is a component of the earth's crust and is often found in the air, water and land. Studies have shown that it is highly toxic in inorganic form (Kroukamp et al., 2020). *C. fimbriata* has the highest as concentration 1.33 μg/g. The lowest as concentration is 0.63 μg/g.

Se concentration was found below the concentration detection limit in other species except *C. pocillum, C. subulata and C. fimbriata* species.

Hg is the most toxic and non-essential heavy metal of all heavy metals. Except for *C. pocillum* and *C. subulata* species, the Hg concentration was found below the detection limit in other species.

The average strontium concentrations was found 17.58-33.59 μg/g. Strontium is transferred from atmospheric air to water and soils (Vinogradov, 1962). Strontium concentration was also found well below the detection limit in other species.

Tin is one of the toxic elements like mercury and it is very dangerous to be found in nature more than average. The tin content was compatible with each other and no significant difference was found in this study when compared with the literature (Koz et al., 2010).

If we look at the cadmium contents in the samples, the distribution in all samples is homogeneous and the amounts are within the normal range. It was determined at *C. fimbriata* with the lowest 0.016 ± 0.025 μg/g value, and at *C. pyxidata* with the 0.260 ± 0.025 value. In the contents of 9 different lichen samples examined, tin content was compatible with each other, and no significant difference was found. The amounts of mercury in all the samples examined are compatible both among themselves and with appropriate values (Zahir et al., 2005).

The source of the lead (Pb) in the research area is probably factory wastes and other contaminant. The high amount of lead contamination in area Turkey, Çorum, North of Çatak Village (*C. pyxidata*) is most likely due to environmental pollution.

Yayintas et al. (2018) worked trace element levels some lichens from Mount Ida in Çanakkale by ICP-MS. In this study Cu, Pb, Zn, Ni, Co element concentrations were dedected for *C. pyxidata* species 18.20, 31.00, 97.80, 25.80, 5.99 μg/g respectively.

For *C. rangiformis* species Cu, Pb, Zn, Ni, Co element concentrations are defined 8.04, 6.52, 43.30, 53.60,5.24 μg/g respectively. (Yayintas et al., 2018) It was observed to be less toxic when compared to the *C. pyxidata* species Cu, Pb, Zn, Ni, Co element concentrations are 11.0, 5.9, 24.3, 3.27, 0.46 μg/g in this study.

In the study conducted by Demirbaş (Demirbaş, 2004), the elemental concentrations of Al, Iron Manganese, Zinc, Copper, Lead, Nickel, Chromium, Cadmium, Mercury for the *C. furcata* species are respectively; reported as 782.4, 427.9, 53.46, 42.66, 14.06, 6.58, 1.16, 1.60, 1.02, 0.56 µg/g. Compared to our study, the results obtained are at low concentrations except for Ni, Cr and Hg elements.

In the study by Mendil et al. (2005), Iron, Manganese, Zinc, Lead, Nickel, Chromium, Copper, Cadmium elements were analyzed for the *C. rangiformis* species collected from roadsides and an industrial zone in Trabzon. The results obtained are 406.2, 103.7, 76.1, 7.1, 10.1, 4.2, 8.4, 0.89 µg/g, respectively. (Mendil et al., 2005). Pb, Zn, Cr, Cu, Cd elements were analyzed for *C. rangiformis* species collected from Balıkesir and Çanakkale by Cayır et al. The results obtained are 0.31, 5.52, 3.09, 6.90, 30.5 µg/g, respectively. (Cayir et al., 2007). As a result of comparison with other studies conducted for *C. rangiformis species*, Zn, Pb, Cr, Cd elements were found in lower concentrations in this study and Cu concentrations were higher than in other studies.

The levels of trace element limits value determined by FAO/WHO (2003) as can be seen from Table 5. As a result of the analyzes in this work, C. pyxidata has accumulated has the highest lead concentration according to the data. Cd concentrations were observed below the limit values for all species. The highest Ni and Cr concentration was determined in the *C. firma* species. In other species, the Ni concentration is below the limits. But Cr concentrations are above the limit value. Cu concentration is generally above the limit value in all species. The highest Cu was determined in C. subulata and C. furcata species. Zinc and iron concentrations are above the determined limits.

Table 4. Metal concentrations (µg/g) of *Cladonia* species samples analyzed by ICP-MS.

	C. pocillum	*C. subulata*	*C.pyxidata*	*C. coniocraea*	*C. foliacea*	*C. firma*	*C. furcata*	*C. fimbriata*	*C. rangiformis*
Al	119.2 ± 2.0	289.7 ± 3.0	109.7 ± 2.8	229.7 ± 3.0	290.2 ± 2.5	429.3± 3.0	384.6 ± 3.0	192. 4 ± 3.0	206.1 ± 2.6
Cr	0.57 ± 0.03	1.60 ± 0.01	1.25 ± 0.02	1.75 ± 0.03	2.45 ± 0.03	3.04 ± 0.01	2.27 ± 0.03	1.48 ± 0.03	1.66 ± 0.02
Mn	5.2 ± 2.0	10.7 ± 2.0	9.3 1.8	17.8 ± 2.0	11.4 ± 1.8	10.7 ± 1.5	4.9 ± 2.0	9.5 ± 1.6	9.6 ± 2.0
Fe	48.5 ± 3.5	90.1 ± 2.4	50.3 ± 2.0	152.3 ± 1.2	111.4 ± 1.5	228.7 ± 2.2	240.5 ± 1.6	104.3 ± 2.8	94.7 ± 6.0
Co	0.35 ± 0.01	0.81 ± 0.03	0.46 ± 0.02	0.80 ± 0.05	0.53 ± 0.07	0.52 ± 0.05	0.38 ± 0.07	0.37 ± 0.07	0.80 ± 0.03
Ni	1.67 ± 0.01	2.85 ± 0.05	3.27 ± 0.08	2.46 ± 0.04	1.94 ± 0.08	13.53 ± 0.08	1.63 ± 0.06	1.91 ± 0.02	1.93 ± 0.08
Cu	9.6 ± 0.1	13.6 ± 0.1	11.0 ± 0.2	12.2 ± 0.2	8.7 ± 0.3	7.6 ± 0.3	13.3 ± 0.1	8.7 ± 0.1	9.66 ± 0.2
Zn	26.44±0.03	22.80±0.10	24.30±0.15	13.80±0.03	10.35±0.05	11.89±0.12	8.12±0.22	18.85±0.03	13.85 ± 0.16
As	0.806 ± 0.001	0.674 ± 0.003	0.673 ± 0.002	0.741 ± 0.001	1.095 ± 0.002	1.156 ± 0.002	1.001 ± 0.002	1.335 ± 0.001	0.821 ± 0.002
Se	0.103 ± 0.001	0.158 ± 0.001	< LOD	< LOD	< LOD	< LOD	< LOD	0.101 ± 0.001	< LOD
Sr	25.02 ± 5.01	33.59 ± 5.02	24.04 ± 5.00	30.19 ± 5.01	26.14 ± 5.05	23.51 ± 5.07	18.18 ± 5.00	17.58 ± 5.00	17.36 ± 5.02
Cd	0.06 ± 0.02	0.08 ± 0.01	0.30 ± 0.01	0.11 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.03 ± 0.02
Sn	2.6 ± 1.2	5.0 ± 1.5	6.5 ± 1.3	3.0 ± 1.0	4.4 ± 1.0	4.3 ± 1.0	5.2 ± 1.0	4. 6 ± 1.5	5.6 ± 1.3
Hg	0.053 ± 0.001	0.060 ±0.001	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Pb	0.64 ± 0.01	1.37 ± 0.02	5.90 ± 0.05	1.39 ± 0.01	0.31 ± 0.08	1.18 ± 0.04	1.46 ± 0.02	0.50 ± 0.01	2.17 ± 0.01

Table 5. Upper limits value for trace elements determined by FAO/WHO (2003)

Heavy metals	Max value (mg/kg)
Pb	2.00
Cd	0.50
Ni	5.00
Cr	0.50
Cu	5.00
Fe	30.00
Zn	30.00

Conclusion

Trace metal pollution continues to be a global threat to biodiversity, people and the environment. The regulatory mechanisms in lichens need to be carefully studied for bioavailability, accumulation, toxicity, and metal detoxification. Lichens are associations formed from the symbiotic relationship between fungi and green algae or cyanobacteria. Although they are long-lived, they have a slow growth mechanism. They do not disappear from the environment with seasonal changes and therefore they are constantly exposed to pollutants over the years (Conti & Cecchetti, 2001). In studies conducted to determine the concentrations of trace elements in the tissue of lichens, it has been seen that lichens are important biological indicators in determining the levels of these elements in the environment.

This study trace element concentration in lichen species collected from various regions of Turkey aimed to evaluate the terms. As a result of the analyzes, *C. pyxidata* has accumulated more trace elements than the other species have. Also *C. coniocraea*, *C. foliacea, C. fimbriata* and *C. rangiformis* have accumulated less metals as can be seen from table 4. The results of the present experiments also support that the accumulation of trace elements depends on the nature of the exchange site, the affinity of the species for these sides of the area and also It is thought that these species findings can be useful to determine in mining researches as a clue. Metal concentrations were analyzed using an inductively coupled plasma – mass spectrometer after nitric acid digestion.

Ethics Committee Approval

N/A

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Author Contributions

Conceptualization: F.K.D; Investigation: Z.K.; Material and Methodology: F.K.D, S.K.; Z.K. and M.K.; Supervision: S.P.; Writing-Original Draft: F.K.D., S.K., Z.K., M.K. and M.Ç.; Writing-review & Editing: F.K.D., S.K., Z.K., M.K. and M.Ç.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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