

Determination of Trace Element Level in Some Lichens from Mount Ida (Çanakkale, Turkey) By ICP-MS

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Abstract
Pollution effects are indeed many and wide-ranging. There is no doubt that excessive levels of pollution are causing a lot of damage to human and animal health, plants, trees including tropical rainforests, as well as the wider environment. Air pollution; density, shelf life and frequency can be defined as the presence of airborne substances that adversely affect the health of organisms and cause harm to the environment. Since the last fifty years, more developed countries have become more interested in air pollution problems. There are many pollutants in the air such as sulfur oxides (SOx), nitrogen oxides (NOx), carbon monoxide (CO), ozone (O_3), different types of particles, heavy metals and various types of volatile organic compounds. The primary source of air pollution is the consumption of fossil fuels to produce energy, various industrial operations and transportation activities. Lichens have been used as bioindicators in various atmospheric pollution assessments in several countries and Turkey. In this study four lichen species were collected from Bayramic and Ayvacık (Canakkale, Turkey) province in January 2018. Prepared sample is cold leached with nitric acid then digested in a hot water bath. After cooling a modified Aqua Regia solution of equal parts concentrated HCl, HNO_3 and Deionized (DI) water are added to each sample to leach in a heating block of hot water bath. Sample is made up to volume with dilute HCl then filtered. Sample splits of 1g (VG101) can be analyzed. The concentrations of some trace metals were determined by Inductive Couple Plasm-Mass Spectrophotometry (ICP-MS). The accuracy of method was confirmed using standard reference material (SRM).

Keywords: Lichens, trace metals, Mount Ida, ICP-MS, SRM.

1. INTRODUCTION

Since the last fifty years, countries have become more interested in pollution problems. Environmental contamination by toxic metals is a serious problem due to their incremental accumulation in the food chain and continued persistence in the ecosystem.

There are many pollutants in the air such as sulfur oxides (SOx), nitrogen oxides (NOx), carbon monoxide (CO), ozone (O_3), different types of particles, heavy metals and various types of volatile organic compounds. Heavy metals are discharged in small quantities into environment through numerous industrial activities. Such as chromium, copper, lead and nickel in wastewater are hazardous to the environment and health. The primary source of air pollution is the consumption of fossil fuels to produce energy, various industrial operations and transportation activities (Figure 1).

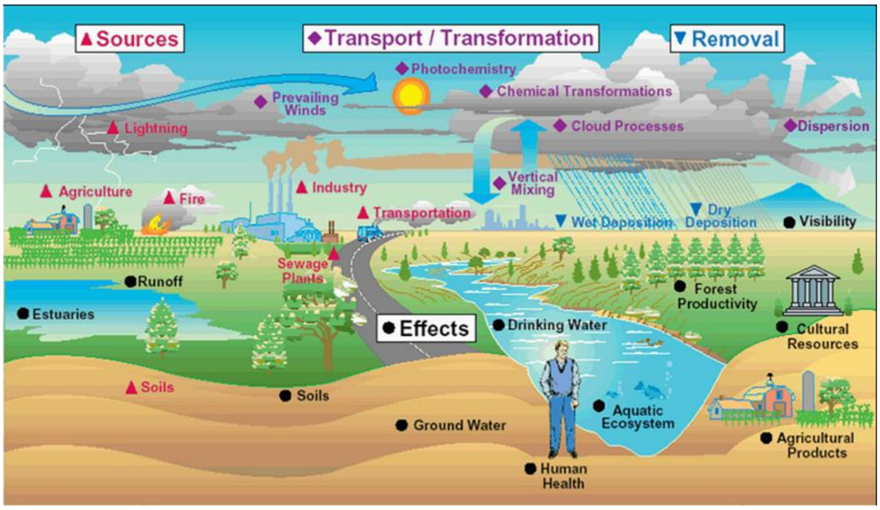

Figure 1. Various industrial operations and transportation activities (https://socratic.org/questions/how-much-pollution-goes-into-the-air-each-day)

Demonstration of environmental pollutants by living organisms ("bio-monitoring" or "bio-indicatiors") in connection with "indicator" organisms and other elements that make up the work must have some features. Large amount of organism, wide geographical distribution, identification and easy sampling, not affected by seasonal changes and have for all bio-indicator groups to be workable throughout the whole year some of the common features that are desired. In this sense, the use of bio-indicators (or bio-monitors) in pollution studies offers many advantages, but the fact that these organisms cannot give exact quantitative values and that they can not be fully standardized in their application processes are their major disadvantages.

Lichen is a composite organism that arises from algae or cyano bacteria living among filaments of multiple in a symbiotic relationship. Lichens come in many colors, sizes, and forms (Figure 2). The properties are sometimes plant-like, but lichens are not plants. Lichens may have tiny, leafless branches, flat leaf-like structures (foliose), flakes that lie on the surface, a powder-like appearance or other growth forms.

Figure 2. Lichens in many colors, sizes, and forms (https://education.maps.com/2017/09/lichens-everywhere)

Lichens and mosses are used as bio-indicator organisms because they don't have real roots, they only feed from the atmosphere, they have large surfaces and they don't have a protective layer. In contrary to other analysis methods, lichens and mosses does not give us momentarily information about the atmospheric pollution of the area, but a long term information because they absorb the contaminants arrived at their surface by atmospheric deposition (Puckett 1988, Nash 1996, Brown and Beckett, 1989). Lichens have different reactions to pollutants from different levels by storing air borne pollutants in their bodies. Since they do not have a root system, they exchange water and mineral substances with all surfaces and store pollutants in their bodies. For this reason, it can be determined by analyzing the heavy metals accumulated in the lichens and whether the contaminants exceed the acceptable limits and the amount in the air. There are many studies in the literature to determine pollution by means of lichens. The various heavy metals that are damaged for many other living organisms such as Pb, Zn, Cd, Ni, Cu, Hg, and Cr, can be deposited simultaneously by a lichen sample without damaging it in many cases, and most lichens are highly tolerant to these metals (Garty 1993). A large number of pollution studies are available in which lichens are used as bio-indicators.
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. Lichens are used as passive pollution monitors due to collect pollutants above their environmental concentrations and physiological needs. They are lack a root system, they collected only allogenic atmospheric substances, including wet precipitations, dry deposits and gas emissions (Policnik et al., 2008, Yıldız et al., 2017). The use of lichens as bio-monitors of geothermal air pollution was initiated by Loppi (2000) who reported the lichens indicated that trace element pollution is generally low in the investigated geothermal area of Italy. The ability of lichens to absorb and accumulate heavy metals and radionuclides at various levels against sulfur dioxide, nitrogen dioxide and ozone has made these organisms a valuable marker of pollution in urban, industrial or rural areas (Gries 1996, Garty 2001, Nimis and Purvis, 2002).
Metal accumulation studies of lichens are limited and often difficult to perform due to lichens' relatively low biomass and intimate association with the substrate (Backor and Fahselt 2004). The aim of the present study was to investigate the behavior of some lichens, representing two main types of thallus morphology, in terms of bioaccumulation capacities and accumulation patterns as their adaptation to such extreme conditions.

2. Material and Method

2.1. Sample collection and preparation

In this study, three lichen species were collected from Bayramiç and Ayvacık (Çanakkale, Turkey) province in January 2018. These species are *Cladonia pyxidata* (L.) Hoffm., *Cladonia rangiformis* Hoffm. and *Ramalina fastigiata* (Pers.) Ach. Prepared sample is cold leached with nitric acid then digested in a hot water bath. After cooling a modified Aqua regia solution of equal parts, concentrated HCl, $HNO_3$and H_2O are added to each sample to leach in a heating block of hot water bath. Sample is made up to volume with dilute HCl then filtered. Sample splits of 1g can be analyzed. The concentrations of some trace metals were determined by ICP-MS (Perkin Elmer/NexION model). The accuracy of method was confirmed using standard reference material (SRM).

2.2. Samples

Cladonia pyxidata (L.) Hoffm. This is a species of lichen belonging to the genus *Cladonia* of the order Cladoniaceae. This species is polymorphic. It adapts itself to a great variety of climates and this is certainly the main reason for its wide diffusion at all latitudes and longitudes. It prefers a pH of the substrate with intermediate values between very acid and sub neutral. It is the most widespread species of *Cladonia* fairly common along the northern part of the regions of the Alpine arctic.
Cladonia rangiformis Hoffm. This species is characterized by greyish, richly branched, commonly terminating in spiky tufts with pointed, dark apices, with groups of green algal cells on a paler background. Widespread, generally on dunes or in rather dry, neutral to basic grassland.
Ramalina fastigiata (Pers.) Ach. is a species of lichen belonging to the genus *Ramalina* of the familia Ramalinaceae. Thallus tufted, green (when wet) to grey, branches irregularly shaped, flattened; laminal and terminal apothecia usually abundant. Widespread and locally common on tree trunks and branches, often an early colonist on young, planted trees.

2.3. Research area

Species were collected from Ayazma Natural Park (Çanakkale, Turkey) from Ida Mountain as seen as the map (Figure 3). Ayazma Natural Park is known as oxygen store of Çanakkale is located near the Evciler village of Bayramiç district and park GPS coordinates are 39 ° 48'42.5844 "and 26 ° 37'16.9140".

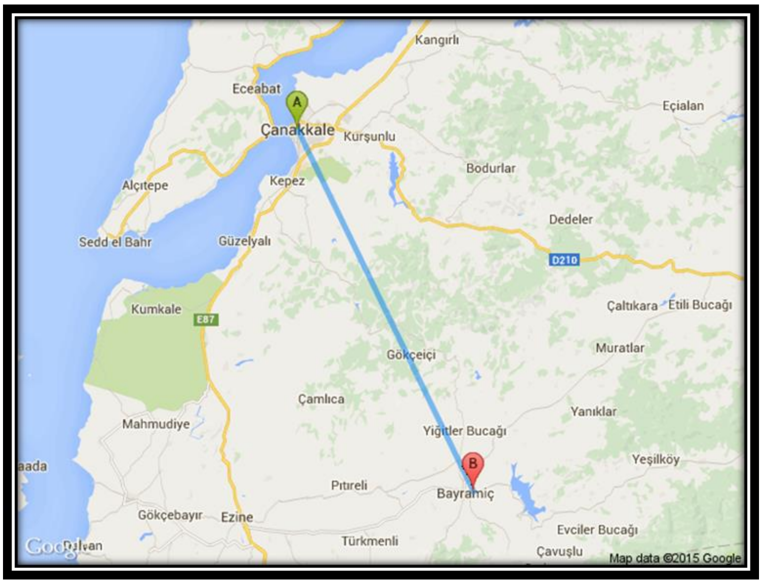

Figure 3. Research area (http://www.arasikackm.com/m/canakkale_bayramic-ayazma)

3. Results

In this study, the concentrations of some trace metals were determined by inductive couple plasm-mass spectrophotometry (ICP-MS). ICP-MS is an advanced technology analysis technique that allows fast, inexpensive, precise and accurate quantitative, quantitative or semi-quantitative measurement of multiple elements in solid and liquid samples. The ionisation of the sample by argon plasma, which has a temperature of 10000 ºK by technical electromagnetic induction; the separation of ionized elements by mass spectrometry and the measurement of elemental concentrations by an electron multiplexing detector. The concentration of all elements in the sample is measured in a relatively short period of time ranging from 1 to 2 minutes. ICP-MS The advantages ICP-MS of the other known methods can be given as lower detection limits (ppb), fast, direct and simultaneous analysis of multiple elements in solid and liquid samples. Therefore, in this study, ICP-MS preferred to determine of some trace element levels in some lichens**.** The concentration of trace elements of three species are shown on Table 1.

Table 1. The concentration of trace elements of the species

Species	Cu (ppm)	Pb (ppm)	Zn (ppm)	Ni (ppm)	Co (ppm)
Cladonia pyxidata	18.20	31.00	97.80	25.80	5.99
Cladonia rangiformis	8.04	6.52	43.30	53.60	5.24
Ramaliaa fastigiata	5.27	4.51	51.20	4.10	0.49

As a result of the analyzes, *C. pyxidata* has accumulated more heavy metals than the other species have. Also *R. fastigata* has accumulated less heavy metals as can be seen from Figure 4. The levels of trace elements were found higher than the upper limits value determined by FAO/WHO (2003) as can be seen from Table 2.

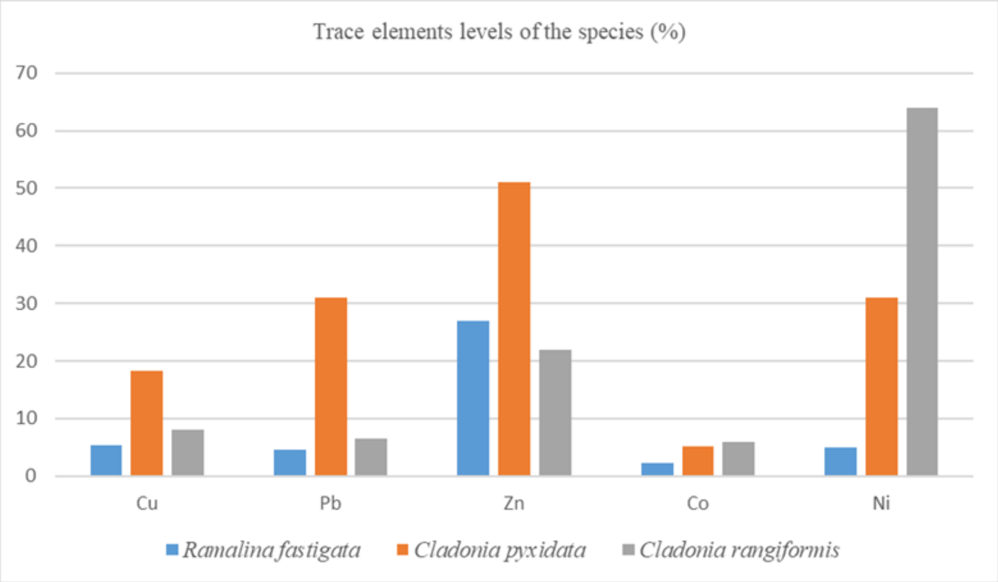

Figure 4. The graphics of trace elements of the species.

Table 2. 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	30.0
Zn	30.0

4. Discussion

The cause of excess of heavy metals in all three lichen species; fossil fuel heating, exhaust pollution from vehicles, thermal power plants used for energy production, and other pollution from anthropogenic sources. The other pollutant sources, there are absolutely atmospheric pollution spreads in four thermic power stations in Çanakkale (Cenal Karabiga thermic power station, İçdaş Bekirli thermic power station, İçdaş Biga thermic power station and Çan thermic power station).

Copper (Cu)

The source of copper (Cu) that pollutes our environment as waste: electric cables, auto radiators, electrolytic coating, copper and brass production factories are the source of copper in the waste. The alloys made of copper with zinc are generally called brass. Brass is the most widely used copper alloy and has a wide range of use in the form of plates, strip pipes and die-casting products (Seven et al., 2018). The source of copper (Cu) in the research area is the thermal power plants used for heating and energy production with fossil fuels (Yıldız et al., 2018).

Lead (Pb)

The source of lead (Pb) which pollutes our environment as waste: Road vehicles and machinery manufacturing industry; in the production of batteries and automobiles and various machines and devices,
-Build; In the construction of plating, lead pipe, installation material,
-Harp industry; Alloy for the production of bullet cores and various weapons and equipment,
-Communication industry; In the coating of cables,
- Packaging industry; Package seal lead, in the production of various packaging materials,

- Chemical industry; Lead oxide, lead chromate, lead grease, lead boroasilicate production,
- Other uses; Acid-resistant in-store coatings, anti-vibration blocks, for X-ray protection. It is also used in the production of glaze in porcelain and ceramic industry (Markert, B., 1993; Seven et al., 2018).

The source of the lead (Pb) in the research area is the thermal power plants used for heating with fossil fuels, exhaust pollution from vehicles and energy production (Yıldız et al., 2008; Yıldız et al., 2018).

Zinc (Zn)

The source of zinc (Zn) that pollutes our environment as a waste: Zinc is the chemical substance of the industry and it is the main ingredient of the industry. Due to its strong electro positive properties, it is used to protect other metals, especially iron and steel products against corrosion. Zinc's main application areas;

- Corrosion prevention
- Coupling description
- Plastics and alloys (Seven et al., 2018).

The source of zinc in the research area (Zn) is the exhaust pollution caused by vehicles (Yıldız et al., 2018).

Nickel (Ni)

Nickel (Ni) source that pollutes our environment as waste: Nickel passes through the metal processing and coating industry to wastewater. Nickel plating baths are usually nickel solutions containing sulphonate, chloride and fluoroborate with sulphate, borate and chloride acidic solutions (1,2,4).

The nickel (Ni) source in the research area is thermal power plants used for heating with fossil fuels, exudates from vehicles and energy production (Yıldız et al., 2018).

Cobalt (Co)

The source of cobalt (Co) which pollutes our environment as waste: It is found in small amounts in rocks, soil, plants, animals and ocean bottoms. The formation of metamorphic rocks is based on cobalt concentrate. Therefore, the level of cobalt in the metamorphic rocks is mainly based on the amount of element in the volcanic or sedimentary rock source. Cobalt is obtained as a by-product with copper, nickel, silver, as well as gold, lead and zinc ores. Cobalt is one of the most essential elements in the world. In pure form, cobalt has a very small amount of application, but its use as an alloying element and as a source of chemicals makes the cobalt strategically important. It has important usage areas in industrial applications and military fields. Cobalt is mainly used in super alloys and special steels used in rocket industry and also in rechargeable batteries of portable electronic devices such as mobile phones and laptops. Its compounds are used as a catalyst in the oil and ceramic industry, as a drying agent in pigments, inks and varnishes in paints (Seven et al., 2018).

The source of the cobalt (Co) in the research area is the natural pollution caused by natural rocks and vehicles.

Lichens bio-accumulate both essential and nonessential elements through various mechanisms including surface complexation, bio-mineralization, and physical trapping of dust and soil particulates in the intercellular spaces of the medulla (Richardson 1995; Wilson 1995; Nash III 2008a). The whole surface of the thallus is involved in the absorption, so that elements present in the atmosphere as well as those present in the substrate can penetrate into the lichens' bodies (Tyler 1989; Basile et al. 2008; Nash III 2008a). Because of lichens lack of root systems, it is widely believed that atmospheric deposition is the main source of elements in the thalli.

Lichens are devoid of an epidermis and cuticle. Leaching of some accumulated heavy metals seems a reasonable. Therefore, it has been postulated that the accumulation of a heavy metal in lichens reflects the proportion of that element in the environment, where other heavy metals in various proportions also exist.

C. pyxidata is a kind of fructicose and terricole lichen and it grows on the soil. The reason for the heavy metal elements in *C. pyxidata* species grows on the soil, not only the pollution coming from the atmosphere but also the heavy metal levels are high because of they are kept in the soil and pollution carried by the precipitation. It also helped to absorb more heavy metal by holding in the goblet of the *C. pyxidata* which is called cup liquer.

C. rangiformis is a fructicose and terricole lichen because it grows on the soil. The reason for the excessive growth of heavy metal elements in *C. rangiformis* is that this lichen species grows on the soil not only because of the pollution coming from the atmosphere but also heavy metals being held in the soil and pollution carried by the rains.

R. fastigiata is a kind of fructicose lichen. It's corticol because it grows in the tree bark. The reason for

the lower output of heavy metal elements in *R. fastigiata* is that this lichen species absorbs pollution from the atmosphere only because it grows on tree bark. Therefore, it shows atmospheric pollution at the best levels.
The results of the present experiments also support that the accumulation of heavy metals depends on the nature of the exchange site, the affinity of the species for these sides of the area. Dominant winds in our study area is the NE and SW. and our stations are dominated by winds. Therefore, all three lichen species are under the influence of this pollution. As a result, the heavy metal levels are quite high.

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