

ABANT İZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES
DEPARTMENT OF CHEMISTRY



SEASONAL BIOMONITORING AND MAPPING OF AIR
POLLUTION IN THE PLAIN OF BOLU

MASTER OF SCIENCE

UĞUR SAKLANGIÇ

BOLU, MAY 2015

APPROVAL OF THE THESIS

Seasonal biomonitoring and mapping of air pollution in the plain of Bolu submitted by Uğur SAKLANGIÇ in partial fulfillment of the requirements for the degree of Master of Science in Department of Chemistry, Abant İzzet Baysal University by,

Examining Committee Members

Signature

Supervisor
Prof.Dr. Serpil YENİSOY KARAKAŞ
Abant İzzet Baysal University


.....

Member
Prof. Dr. İrfan YOLCUBAL
Kocaeli University


.....

Member
Yrd. Doç. Dr. Nazlı BALDAN PAKDİL
Abant İzzet Baysal University


.....

May 15, 2015

Prof. Dr. Duran KARAKAS

Director, Graduate School of Natural and Applied Sciences

Dedicated to my family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Uğur SAKLANGIÇ

ABSTRACT

SEASONAL BIOMONITORING AND MAPPING OF AIR POLLUTION IN THE PLAIN OF BOLU

**MSC THESIS
UĞUR SAKLANGIÇ**

**ABANT İZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES**

DEPARTMENT OF CHEMISTRY

SUPERVISOR: PROF. DR. SERPİL YENİSOY KARAKAŞ

BOLU, MAY 2015

In this study, the air quality profile of Bolu Plain was determined by applying biomonitoring methodology. Lichen and pine needle samples were collected in November 2012 and in June 2013.

The samples were analyzed by Isotope Ratio Mass Spectrometry (IR-MS) for stable carbon and nitrogen isotope ratios and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the elements. Concentrations of elements were comparable with literature data. Concentration data were used to get the pollution distribution maps of the region.

Descriptive statistics of the lichen and pine needle samples collected in summer and in winter field campaign were determined. The lowest average concentration was found for Pt as $0.3 \pm 0.2 \text{ ng g}^{-1}$ and the highest average concentration value was obtained for K as $2816 \pm 1589 \text{ } \mu\text{g g}^{-1}$ during winter field campaign for the lichen samples. The average concentration for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for lichen samples was found to be -9.72 ± 3.12 and $-22.46 \pm 0.60 \text{ } \text{‰}$, respectively, during the winter field campaign. Similar to the winter field campaign, the lowest average concentration value was found for Pt as $0.3 \pm 0.2 \text{ ng g}^{-1}$ in the summer field campaign

for lichen samples. The highest average concentration was obtained for K as $4530 \pm 1631 \mu\text{g g}^{-1}$ during the summer field campaign for lichen samples. The average concentration for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ associated with lichen samples was found to be -9.71 ± 3.89 and $-22.48 \pm 0.67 \text{‰}$, respectively, during the summer field campaign.

Elemental compositions are different for lichen and pine needle samples. Pine needle samples showed completely different composition in terms of most of the elements analyzed in the study while elemental composition was comparable for lichen species. Pine needle samples had higher accumulation capacities for Mg, K, Mn, Sr and Ca both in the summer and in the winter season.

In this study, EF_c values were calculated for winter and summer field campaigns separately. In addition, since the accumulation capacities of the lichen and pine needle samples are different, their seasonal EF_c values are also evaluated individually. EF_c values associated with Zn, Hg, Cd, Sb, As and Se were higher than 10 implying that these elements were released to the atmosphere purely from the anthropogenic sources.

Factor analysis was also performed in this study to apportion the sources affecting the chemical composition of samples collected in this study. Six-factors were retained in the winter field campaign, which are crustal, motor vehicle, coal combustion, oil combustion, biomass burning, and cement factory. All these sources account for the 81.2 % of the system variance. For the summer field campaign, five factors were resolved, which are crustal, biomass burning, coal combustion, local mixed, and motor vehicle. Five factors account for the 82.8 % of the total system variance.

Lastly, the spatial distribution of parameters for which calculated EF_c values were greater than 10 and stable carbon and nitrogen isotope ratios was evaluated in this study. The distribution of parameters is distinct from each other. However, for most of the parameters, the hot spots were close to highways and locations with higher concentrations were clustered close to the city center.

KEYWORDS: Biomonitoring, lichen, elements, isotope ratio, factor analysis.

ÖZET

BOLU OVASI'NDAKİ HAVA KİRLİLİĞİNİN MEVSİMSEL BİYOİZLEME VE HARİTALANDIRILMASI

YÜKSEK LİSANS TEZİ

UĞUR SAKLANGIÇ

ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
KİMYA ANABİLİM DALI

TEZ DANIŞMANI: PROF. DR. SERPİL YENİSOY KARAKAŞ

BOLU, MAYIS - 2015

Yaz ve kış mevsimlerinde yapılan alan çalışmasında toplanan liken ve çam iğnesi örneklerine ait istatistiksel sonuçlar incelenmiştir. Kış mevsiminde, liken örneklerinde en düşük ortalama konsantrasyon $0.3 \pm 0.2 \text{ ng g}^{-1}$ ile Pt için ve en yüksek ortalama konsantrasyon $2816 \pm 1589 \text{ } \mu\text{g g}^{-1}$ ile K için bulunmuştur. $\delta^{15}\text{N}$ ve $\delta^{13}\text{C}$ için ortalama konsantrasyon sırasıyla -9.72 ± 3.12 ve $-22.46 \pm 0.60 \text{ } \text{‰}$ olarak bulunmuştur. Kış örneklemesine benzer olarak yaz mevsiminde yapılan örneklemede liken örnekleri için en düşük ortalama konsantrasyon $0.3 \pm 0.2 \text{ ng g}^{-1}$ ile Pt için bulunmuştur. En yüksek ortalama konsantrasyon $4530 \pm 1631 \text{ } \mu\text{g g}^{-1}$ ile K için saptanmıştır. $\delta^{15}\text{N}$ ve $\delta^{13}\text{C}$ için yaz örnekleme ortalama konsantrasyonlar sırası ile -9.71 ± 3.89 ve $-22.48 \pm 0.67 \text{ } \text{‰}$ dir.

Liken ve çam iğnesi örneklerinin element bileşimleri farklıdır. Bu çalışmada, birçok elementin liken türlerinde bileşimi benzerlik gösterirken, çam iğnesinin liken türlerine göre tamamen farklı bileşim gösterdiği belirlenmiştir. Çam iğnesi örnekleri Mg, K, Mn, Sr ve Ca için hem yaz hem kış sezonlarında daha yüksek birikim kapasitesine sahiptir.

Bu çalışmada, zenginleştirme faktörü kış ve yaz örnekleme çalışmaları için ayrı ayrı hesaplanmıştır. Liken ve çam iğnesi örneklerinin birikim kapasitelerinin farklılığına ek olarak, bunların mevsimsel zenginleştirme faktörleri de birbirlerinden bağımsızdır. 10'dan büyük zenginleştirme faktörüne sahip Zn, Hg, Cd, Sb, As ve Se'un atmosfere salınımları insan kökenli kaynaklardandır.

Bu çalışmada toplanan örneklerin kimyasal kompozisyonunu etkileyen kirletici kaynaklarını bulmak amacıyla üretilen veri setine faktör analizi uygulanmıştır. Kış sezonunda toplanan örnekler için kirletici kaynakları toprak, biyo-kütle yakımı, taşıt emisyonları, kömür yakımı, petrol yakımı, çimento fabrikası olarak bulundu. Yaz mevsiminde toplanan örnekler içinse kirletici kaynakları toprak, taşıt emisyonları, biyo-kütle yakımı, kömür yakımı ve yerel karışık antropojen kaynak olarak belirlendi.

Son olarak, zenginleştirme faktörü 10'dan büyük elementlerin ve kararlı karbon ve azot izotop oranlarının alansal dağılımları değerlendirilmiştir. Dağılım parametreleri her bir element için birbirinden farklıdır. Bununla birlikte, bir çok parametre için, çok yüksek konsantrasyonlu alanlar otoyola yakın ve gözlemlediğimiz yüksek konsantrasyona sahip lokasyonlar şehir merkezine yakın yerlerde kümelenmiştir.

ANAHTAR KELİMELEER:Biyoizleme, Liken, Elementler, izotop oranı, faktör analizi

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT.....	v
ÖZET.....	vii
TABLE OF CONTENTS.....	ix
LIST OF FIGURES	xi
LIST OF TABLES	xiii
LIST OF ABBREVIATIONS AND SYMBOLS	xv
1. INTRODUCTION.....	1
1.1 Air Pollution and Biomonitoring.....	1
1.2 Advantages and Disadvantages of Biomonitoring	3
1.3 Biomonitoring Systems	4
1.3.1 Lichens.....	5
1.3.2 Lichens collected in this study.....	6
1.3.3 Pine Needle	8
1.3.4 Other Biomonitoring Species.....	8
1.4 Isotopic Composition	9
1.4.1 ¹³ C Isotopic Composition.....	11
1.4.2 Sources Determining ¹³ C Isotopic Composition.....	12
1.4.3 ¹⁵ N Isotopic Composition	13
1.4.4 Sources Determining ¹⁵ N Isotopic Composition	14
2. AIM AND SCOPE OF THE STUDY	16
3. LITERATURE REVIEW.....	17
3.1 Lichens as Biomonitors	17
3.2 Biomonitoring of Metal Pollution	19
3.3 Biomonitoring of Stable Carbon and Nitrogen Isotopes	21
4. MATERIALS AND METHODS.....	25
4.1 General Information about Bolu.....	25
4.2 Sample Collection and Sample Handling.....	25
4.3 Laboratory Studies	28
4.3.1 Sample Preparation	28
4.3.2 Elemental Analysis of Samples	29

4.3.2.1	Microwave Digestion	29
4.3.2.2	General Information about ICP-MS	30
4.3.3	Method Validation	32
4.3.4	Isotopic Composition Analysis	36
4.3.4.1	Analysis of Lichen and Pine Needle Samples by EA-IRMS	37
5.	RESULTS AND DISCUSSION.....	39
5.1	General Characteristics of Biomonitoring Data	39
5.1.1	Summary Statistics of Generated Data	39
5.1.2	Comparison of data with literature values	48
5.2	Temporal Variation of Biomonitoring Data	53
5.3	Correlations Between the Measured Parameters	57
5.4	Comparison of Bioaccumulation Capacity of Collected Species.....	66
5.5	Enrichment Factors of Biomonitoring Data	71
5.6	Factor Analysis	76
5.7	Spatial Distribution of Biomonitoring Data	80
6.	CONCLUSION	97
7.	REFERENCES	99
8.	APPENDIXES	106
9.	CURRICULUM VITAE	108

LIST OF FIGURES

	<u>Page</u>
Figure 1.1. Photos of commonly found lichen types (a) <i>Xanthoria parietina</i> (b) <i>Evernia prunastri</i> (c) <i>Parmelia sulcata</i> in Bolu Plain (Photos taken in the field)	6
Figure 4.1. Locations of samples collected during the winter field campaign.....	26
Figure 4.2. Locations of samples collected during the summer field campaign.....	27
Figure 4.3. Photos taken during the cleaning of the samples.....	28
Figure 4.4. BERGHOF Microwave oven.....	29
Figure 4.5. Heating program of microwave oven during digestion of samples	30
Figure 4.6. Perkin Elmer-ICP-MS	31
Figure 4.7. Prepared plant samples for analysis by EA-IRMS	36
Figure 4.8. EA-IRMS instrument used in the analysis of collected samples	37
Figure 4.9. The schematic diagram of the EA-IRMS instrument	38
Figure 5.1. Comparison of the stable isotope ratios found in this study with the literature values	53
Figure 5.2. Summer to winter ratios of all elements (Average $\pm$ STD values are provided)	56
Figure 5.3. Comparison of stable carbon isotope ($\delta^{13}\text{C}$) measured in the summer and in the winter.....	57
Figure 5.4. Comparison of stable nitrogen isotope ($\delta^{15}\text{N}$) measured in the summer and in the winter.....	57
Figure 5.5. Comparison of lichen and pine needle samples in terms of elemental composition in Gölcük (40.6548°N, 31.62535°E), Bolu	69
Figure 5.6. Accumulation capacities of elements calculated for the winter field campaign	69
Figure 5.7. Accumulation capacities of elements calculated for the summer field campaign	70
Figure 5.8. Crustal enrichment factor for the lichen samples collected in the winter field campaign.....	74
Figure 5.9. Crustal enrichment factor for the pine needle samples collected in the winter field campaign.....	74

Figure 5.10. Crustal enrichment factor for the lichen samples collected in the summer field campaign	75
Figure 5.11. Crustal enrichment factor for the pine needle samples collected in the winter field campaign.....	75
Figure 5.12. Spatial distribution of As and Se in summer season.....	82
Figure 5.13. Spatial distribution of Pb and Sb in summer season.....	83
Figure 5.14. Spatial distribution of Zn and Hg in summer season.....	84
Figure 5.15. Spatial distribution of K and Al in summer season	86
Figure 5.16. Spatial distribution of Mg and Ca in summer season	87
Figure 5.17. Spatial distribution of Cd in summer season	88
Figure 5.18. Spatial distribution of $\delta^{15}\text{N}$ in summer season	88
Figure 5.19. Spatial distribution of $\delta^{13}\text{C}$ in summer season.....	89
Figure 5.20. Spatial distribution of Cu and Cd in winter season	91
Figure 5.21. Spatial distribution of As and Se in winter season	92
Figure 5.22. Spatial distribution of Hg and Sb in winter season.....	93
Figure 5.23. Spatial distribution of Ca and Mg in winter season.....	94
Figure 5.25. Spatial distribution of $\delta^{15}\text{N}$ in winter season	96
Figure 5.26. Spatial distribution of $\delta^{13}\text{C}$ in winter season	96

LIST OF TABLES

	<u>Page</u>
Table 1.1. Advantages and disadvantages of biomonitoring.....	4
Table 4.1. Operating conditions for the ICP-MS	31
Table 4.2. Certified values in SRM NIST 1648a-Urban Particulate Matter and found values by ICP-MS (N=5)	33
Table 4.3. Certified values in SRM NIST 1575a-Trace Elements in Pine Needles and found values by ICP-MS (N=4)	34
Table 4.4. Certified values in SRM IAEA 336-Trace and Minor Elements in Lichen and found values by ICP-MS (N=4)	34
Table 4.4. Certified values in SRM IAEA 336-Trace and Minor Elements in Lichen and found values by ICP-MS (N=4) (Continued).....	35
Table 4.5. LOD and LOQ values of analyzed elements by ICP-MS	36
Table 4.6. Operating conditions for the EA-IRMS device.....	38
Table 4.7. Operating conditions for the EA and ConFlo IV Interface	38
Table 5.1. Summary statistics of lichen samples collected in the winter field campaign (in $\mu\text{g g}^{-1}$)	41
Table 5.1. Summary statistics of lichen samples collected in the winter field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰).....	42
Table 5.2. Summary statistics of pine needle samples collected in the winter field campaign (in $\mu\text{g g}^{-1}$).....	43
Table 5.2. Summary statistics of pine needle samples collected in the winter field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰).....	44
Table 5.3. Summary statistics of lichen samples collected in the summer field campaign (in $\mu\text{g g}^{-1}$)	45
Table 5.3. Summary statistics of lichen samples collected in the summer field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰).....	46
Table 5.4. Summary statistics of pine needle samples collected in the summer field campaign (in $\mu\text{g g}^{-1}$).....	47

Table 5.4. Summary statistics of pine needle samples collected in the summer field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰).....	48
Table 5.5. Comparison of generated data in this study with the reported values in literature (in $\mu\text{g g}^{-1}$)	51
Table 5.5. Comparison of generated data in this study with the reported values in literature (in $\mu\text{g g}^{-1}$) (Continued).....	52
Table 5.6. Binary correlation matrix of elements in summer season ($p < 0.05$)	59
Table 5.7. Binary correlation matrix of elements in winter season ($p < 0.05$).....	63
Table 5.8. Chemicals released to the atmosphere from different sources	72
Table 5.9. Varimax factor loadings for six factor solution in winter field campaign	77
Table 5.9. Varimax factor loadings for six factor solution in winter field campaign (Continued)	78
Table 5.10. Varimax factor loadings for six factor solution in summer field campaign	79
Table 5.10. Varimax factor loadings for six factor solution in summer field campaign (Continued).....	80

LIST OF ABBREVIATIONS AND SYMBOLS

AAS	: Atomic Absorption Spectroscopy
DELTA (δ)	: Isotope Fractionation
EA-IRMS	: Elemental Analyzer-Isotope Ratio Mass Spectrometry
EF_c	: Crustal Enrichment Factor
IAEA	: International Atomic Energy Agency
ICP MS	: Inductively Coupled Plasma Mass Spectrometry
LOD	: Limit of Detection
LOQ	: Limit of Quantitation
NAA	:Neutron Activation Analysis
NIST	: National Institute of Standards and Technology
PIXE	:Proton Induced X-Ray Emission
SRM	: Standard Reference Material
QA/QC	:Quality Assurance / Quality Control
XRF	:X-Ray Fluorescence

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor Prof. Dr. Serpil YENİSOY-KARAKAŞ for her supervision, guidance, advices, criticisms and encouragement throughout this research and my undergraduate research.

I want to thank my committee members, Prof. Dr. İrfan YOLCUBAL and Yrd. Doç. Dr. Nazlı BALDAN PAKDİL for their efforts and time spent in evaluating my dissertation.

I would like to thank Prof. Dr. Duran KARAKAŞ for his comments and advices and helps in the sampling of this study.

I am grateful to Yrd. Doç. Dr. Fatma ÖZTÜRK for her supports and encouragement in the experimental part of this study.

I extend my sincere thanks to my Environmental Chemistry Research Laboratory group members Elif ÖZLÜ, Hatice KARADENİZ, İlker KÖPRÜ, Yasin Çağrı KILIÇER, Eda SAĞIRLI, Özge YETGİN and Tuğçe DEMİR for their help during sampling and analysis, moral support and friendship during my hard working days.

Special thanks to Melek KELEŞ, Abdülkadir ACARCA, Berfin TANIŞ, Elif CİHAN, Nergis ÖZARSLAN, Nurseli SOY and Abdullah ÇAĞLAR for their help during sampling and analysis, encouragement, moral support and friendship.

Finally, I would like to express my deepest appreciation to my family and Ayça GÜVEN, who I have recently engaged with, for their endless support, understanding and patience not only during this study but also throughout my life.

I would like to thank Scientific and Technological Research Council of Turkey (Project No- 112Y037) for the financial support for my study.

1. INTRODUCTION

1.1 Air Pollution and Biomonitoring

Air pollution is a major environmental concern as it impacts health and quality of life in populations at an increasing trend due to growing industrialization, urbanization and increased transportation needs (Atkinson, 2000). Air pollution, which is currently a global environmental problem, can be defined as the substances that adversely influence the living organisms. These substances are present in the atmosphere in the particulate and gaseous form at such concentration, duration and frequency that above the limits. The vast majority of heavy metals, sulphur and nitrogen compounds are emitted to atmosphere from anthropogenic (man-made) sources (Pacyna and Pacyna, 2001). The natural sources that release these parameters to the atmosphere may be forest fires, volcanoes, oceans and biological decomposition (Nriagu, 1989).

Pollutants can be released to the atmosphere both from natural and anthropogenic sources. In most of the environmental studies, anthropogenic sources are much more considered than natural ones because taking precautions and management of the problem can be easy. Knowledge about the source location relative to the receptor allows calculating the concentration in exposed area. In addition, pollution type and strength of them help to recognize the effects on living organisms.

Controlling air pollutants is a very complex problem with many aspects: sources and emissions must be determined, analytical methods must be evaluated, risks must be assessed, emissions must be controlled, and economic aspects must be integrated. The necessary information on air pollutants can be obtained by field measurement of the immission (Wolterbeek and Freitas, 1999). Immission measurements need long-term sampling at large numbers of sampling sites. Such

measurements also require equipment and man power, which are associated with high costs. All of these disadvantages of active sampling can be eliminated with biomonitoring.

Biomonitoring can be defined as the use of the properties of an organism or a part of it to gather information on certain part of the biosphere. The associated information is obtained from either changes in the behavior of the monitor organism or from the concentrations of specific substances in the monitor tissues (Sloof, 1993). Alternatively, biomonitoring can be defined as the collection and analysis of biological data to assess the changes in the environment or to identify the damages to human health and environment (NAVFAC, 1997).

As long as the organism is properly selected, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wolterbeek, 2002).

Biomonitors hold quantitative information on the health of an ecosystem. A biomonitor is also a bioindicator, except that it quantifies the impact or eventual outcome on an organism or ecosystem. Bio-indicators are all organisms that provide information on the quality of environment and changes in the environment (Wittig, 1993; Markert et al., 2003).

This reliable, versatile and inexpensive method can provide information for decision-makers about health and environmental protection against potentially hazardous trace elements. Biomonitoring is very effective for tracing maps of airborne metal contamination in polluted environments providing a high density of sampling points (Monaci et al., 2000).

An ideal accumulative biomonitor should have these characteristic features (Pacyna and Otter, 1989; Markert, 1993; Kucera, 1994).

- They should be plenty of in the area of interest
- They should be able to accumulate in the environment

- They should have enough resistance against the toxicity
- They should not react with the substance to be accumulated
- They should not show any seasonality in availability and applicability
- They should not show any seasonality in accumulation capacity
- They should represent a correlation between accumulation and input to the ecosystem
- They should be independent uptake of local conditions
- They should represent average element concentration over a suitable time period
- They should not or little uptake from sources other than atmosphere, which means that they should have low background levels
- They should not lose the substance to be monitored
- They should be easily sampled and handled during sample preparation
- The accumulated matter of interest should be easily analyzed by routine analytical techniques
- A non-expert person should readily recognize the biomonitor organism in the field

1.2 Advantages and Disadvantages of Biomonitoring

The main advantage of the biomonitoring is that it can provide site-specific information about the environment. The principal drawback of the biomonitoring is that it cannot provide information about the timing, magnitude and duration of the exposure (NAVFAC, 1997). The advantages and disadvantages of bio-monitoring are summarized in Table 1.1 below:

Table1.1. Advantages and disadvantages of biomonitoring

ADVANTAGES
• It allows for interpretation of site specific exposure • It can provide evaluation of long-term exposure and impacts • Since some biological endpoints are very rapidly influenced by changing environmental conditions, bio-monitoring provide information on changing environmental conditions • Most of the biomonitoring methods are very cheap and simple as compared to chemical monitoring methods • It can provide signs of cumulative exposure and affects from contaminant mixtures
DISADVANTAGES
• It provides information on the exposure, but not the source, timing, strength or duration of the exposure • If it relies on the mobile organisms, it can reflect exposure from other areas, not a single site • Data obtained may reflect responses to all environmental stresses, not to site-specific stresses • It is required to collect large number of samples over a long period of time to identify population-level responses for some of the species

1.3 Biomonitoring Systems

Higher plants such as trees and lower organisms such as lichens, mosses and fungi are used as accumulative bioindicators of elements. Biomonitoring species are often selected on the basis of criteria such as specificity (which means that accumulation is considered to occur from the atmosphere only), accumulation ratios (Sloof, 1993) or a well-defined representation of a sampling site (Wolterbeek and Bode, 1995).

According to Conti and Cecchetti (2001), plants accumulate metals due to many factors. Element availability, the characteristics of plants (such as species, age, state of health and type of reproduction), temperature, available moisture and substratum characteristics are considered among these factors.

Lichens and mosses have the most common occurrence among the biological species used in biomonitoring. The morphology of lichens and mosses does not change with seasons; hence accumulation can occur throughout the year. Lichens and mosses usually have considerable longevity, which result in their use as long-term integrators of atmospheric deposition (Sloof, 1993). Since these lower plants do not have a root system, they are dependent on the environment to which they are exposed for the uptake of chemical substances. They have accordingly been found useful in

monitoring many heavy metals and other trace elements contributed by atmospheric deposition. Lichens were recognized as potential indicators of air pollution as early as the 1860s in Europe (Freitas, 1999). Afterwards, lichens have been widely used in air quality monitoring studies in all over the world.

1.3.1 Lichens

Lichens are symbiotic associations of a fungus and green algae or cyanobacteria. They are long-lived and slowly growing organisms. Lichen morphology does not vary with the seasons, and accumulation of pollutants can occur throughout the year (Conti and Cecchetti, 2001).

Because of these morphological features, they are widely considered for monitoring purposes. They are strongly dependent on mineral nutrients in wet and dry deposition on the plant surface. In addition, the lichen surface, structure, and roughness facilitate the interception and retention at particles (Sloof, 1993)

The body of a lichen consists of fungal filaments (hyphae) surrounding cells of green algae and/or blue-green cyano bacteria. The vegetative body of a lichen is called a thallus. Their mode of growth may be crustose (crust-forming) foliose (leaf-like) or fructose (hair-like) including various intermediates such as squamulose (pebble-like). As with bryophytes they may be epigenic (growing on soil), epilithic (growing on rocks) or epiphytic (growing on higher plants, usually trees). Lichens are classified according to the type of fungal component (Yenisoy-Karakaş, 2000).

Heavy metal content in lichen thallus is subject to change over time in phases of accumulation and subsequent release. Metal absorption in lichens is influenced by many environmental factors. The acidic precipitation, geographical variations (e.g., altitude), temporal changes (e.g., seasonal variations), soil dust, local pollution sources and long-range transport of pollution can be considered among the factors that determine the metal absorption in lichens. A rich lichen flora is an indication of improved air quality. The absence of lichens is a demonstration of poor air quality.

This situation is only qualitative. However, by comparing more than two sampling sites along a pollution gradient it is found that lichens are not either present or missing, but there is a distinct increase in lichens parallel to the increase in air quality.

Lichens may be used as bioindicators and/or biomonitors in two different ways: by mapping all species present in a specific area; through the individual sampling of lichen species and measurement of the pollutants that accumulate in the thallus, or by transplanting lichens from an uncontaminated area to a contaminated one and then measuring the morphological changes in the lichen thallus and/or evaluating the physiological parameters and/or evaluating the bioaccumulation of the pollutants (Conti and Cecchetti, 2001).

1.3.2 Lichens collected in this study

The names of the lichen species are given in two words. The first word starts with capital letter and identifies the genus; the second word starts with small letter and identifies subspecies. Lichens are important constituents of the vegetation of many ecosystems of the world.



Figure 1.1. Photos of commonly found lichen types (a) *Xanthoria parietina* (b) *Evernia prunastri* (c) *Parmelia sulcata* in Bolu Plain (Photos taken in the field)

Xanthoria parietina is a foliose, or leafy lichen. It has wide distribution, and many common names such as common orange lichen, yellow scale, maritime sun

burst lichen and shore lichen. The vegetative body of the lichen, the thallus, is foliose, and typically less than 8 centimeters wide. The lobes of the thallus are 1–4 mm in diameter, and flattened down. The upper surface is some shade of yellow, orange, or greenish yellow, while the lower surface is white, with a cortex, and with sparse pale rhizines or hapters. The majority of foliose lichens are attached to their substratum by root-like attachment organs on the lower surface, the so-called rhizines, composed of conglutinated, prosoplectenchymatous hyphae (Hannemann, 1973). Hannemann (1973) distinguished 15 different types of appendage-organs, among those rhizines and hapters. The restricted rhizines to compact, elongated hyphal strands on the lower surface of the thallus, whereas short, not elongated organs are called hapters. The vegetative reproductive structures soredia and isidia are absent in this species, however, *apothecia* are usually present.

Xanthoria parietina is a very pollution-tolerant species. In laboratory experiments, this species can tolerate exposure to air contaminants and bisulphite ions with little or no damaging effect. It is also tolerant of heavy metal contamination.

Evernia prunastri, thallus is pendulous to semi-erect, feels soft and reaches a length of 10 cm. Strap-like lobes grow to 6 mm wide and 1 mm thick. Colour varies from green-grey to yellow-grey on the upper surface; the lower surface is characteristically white. Sometimes green blotches may be seen near the ends of lobes.

Parmelia sulcata is a large genus of lichenized fungus with a global distribution, extending from the Arctic to the Antarctic continent but concentrated in temperate regions. Thallus is grey to white with brownish tips. Often develops as large rosettes, or wraps itself around twigs. Lobe tips incised. The network of veins is distinctive, being ridged and white. Soralia develops toward the centre. Underside is black with simple rhizines near margins. Apothecia are rare.

1.3.3 Pine Needle

Pine needles stand out as effective biomonitors due to the excellent uptake properties of their waxy layer (Xu et al., 2004). The needles surface is constituted by a thick cuticle (di- and trihydroxy fatty acids) and a cuticular wax (lipids, free fatty acids, n-alkanes, n-alkenes, primary alcohols) (Dolinova et al., 2004; Klánová et al., 2009). The epicuticular wax layer acts as a barrier between the plant and its environment, protecting the plant against ultraviolet radiation, desiccation and possible attacks from pathogens. Low and medium polar compounds have a high affinity towards this wax layer due to their chemical composition and, therefore, they are effectively entrapped for several years.

Needles are used to as a bioindicator of S and heavy metal pollution on many occasions (Wytenbach et al., 1997; Manninen et al., 1991). Prolonged effects of high concentrations of various pollutants have resulted in large-scale dying not only of conifers but also deciduous trees. The pollutants accumulated in the waxy surface of the needles are identified and quantified.

1.3.4 Other Biomonitoring Species

Mosses belong to a group of green plants known as bryophytes which are reproduced by spores but also they can be reproduced from new plants from small fragments of stems and leaves that are broken off. Mosses can resist curt environmental conditions as well and can be found in the Arctic, Antarctic and deserts. As regards their availability as biomonitors, mosses and lichens are often discussed together and calibration factors between some moss and lichen species have been calculated.

Mosses as indicators have the following advantages:

- many species are geographically widespread and grow under different environmental conditions, even in industrial and urban areas

- they do not have epidermis and cuticle, so metal ions easily penetrate the cell wall
- they do not have a root system, so there is no uptake of mineral substrates from other sources than the atmosphere
- some species have a layer structure and produce dead organic sediment within a year
- transport of minerals between segments is limited because of lack of vascular tissue
- mosses accumulate metals in a passive way, acting like ion exchangers
- for most metals mosses show a correlation between dry bulk amount and wet deposition concentration (Berg and Steinnes, 1997a, Berg and Steinnes, 1997b, Steinnes, 1995).

Fungi are a member of a large group of eukaryotic organisms that includes microorganisms such as yeast and molds. They accumulate only low amounts of heavy metals directly from the wet or dry deposits. Fungi are better predisposed to absorption of heavy metals present in soil than the majority of other soil organisms are (Markert, 1993).

Bark can be defined as all tissues of the tree, outside the cambium layer. The basic assumption in using bark for biomonitoring purposes is that levels of pollutants in bark should reflect the levels of environmental pollution. It is frequently used in the analysis of heavy metal deposition (Kuik and Wolterbeek, 1994).

There are many other biomonitoring organisms used for many purposes for centuries. Rabbits, pheasants, bees, mouse-form rodents, earth worms fishes and soil microorganisms are generally used as biomonitor organisms (Salanki, 1985).

1.4 Isotopic Composition

Isotopes are atoms whose nuclei contain the same number of protons but a different number of neutrons. Most of the 1500 or so naturally occurring isotopes are

unstable and undergo radioactive decay to other isotopes. A subset of isotopes, however, is not radioactive, at least within our ability to measure decay times. The science of stable isotope geochemistry is based on the fact that isotopes of the same element have slightly different thermodynamic and physical properties. As a consequence, their behaviour in chemical and physical reactions is slightly different. Isotopes therefore become separated, or fractionated, when they, or compounds which contain them, participate in chemical reactions or undergo a change of state. The resulting changes in relative isotopic abundances are often measurable and provide important information regarding the sources and the processing of atmospheric species (Emery et al., 2009).

Stable isotopic composition has been widely used in ecological and element cycling studies. The carbon and nitrogen stable isotopic compositions of plant can be affected by species, elevation, humidity, and other environmental factors (Hou et al., 2011).

Stable isotope atoms provide complementary information about the sources of hydrocarbons in the environment. Different sources have different characteristics of $\delta^{13}\text{C}$ values. The basis for this lies in differences in plant physiology and mechanisms of carbon fixation (Ballentine et al., 1996). While compounds are emitted as a result of anthropogenic activities have very little enriched values of $\delta^{13}\text{C}$, compounds are released into the atmosphere through the burning of biomass of $\delta^{13}\text{C}$ value is the same as biomass burned (Simoneit, 1997).

Similarly, $\delta^{15}\text{N}$ values are also used in the source apportionment studies. Variation in the isotopic composition of nitrogen in different ecosystem components can give information about potential sources of nitrogen and relative significance of various nitrogen cycling processes in the ecosystem (Korontzi et al., 2000).

Isotope fractionation is expressed in terms of a “delta” (δ) value. An analyzed sample having a negative delta value indicates that the analysis sample is depleted in terms of heavy isotope and having a positive delta value reveals that it’s enriched as compared to standard in terms of heavy isotope.

1.4.1 ¹³C Isotopic Composition

Carbon is the principal element of the carbohydrates, lipids and proteins that are the predominant metabolites of lichens and pine needles. Carbon has two stable isotopes, ¹²C, at a natural abundance of 98.89% and ¹³C (1.11%) (Hoefs, 1997). For two isotopes of any element, the proportion (or ratio) of one isotope relative to the other will vary slightly with chemical structure and the environment. Such small changes in the isotope ratio are referred to as fractionation. The extent of this isotope fractionation is expressed in terms of a “delta” (δ) value (Krouse and Grinenko, 1991). δ ¹³C is a measure of the change in the proportion of ¹³C to ¹²C in the sample of interest is represented by R_{sample}, relative to that in a standard substance is represented by R_{standard}. The unit of this measurement is “per mil” (‰).

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{‰}$$

Stable carbon isotopes (¹³C and ¹²C) have been proved to be useful geochemical markers and these isotopes were first applied to the studies of air pollution in the 1980s. Carbon isotopes were used to investigate emissions from biomass burning. The data on the stable carbon isotope ratio (δ ¹³C) can provide important information regarding the sources and the processing of atmospheric organic carbon species (Fisseha et al., 2009)

Carbon isotope ratios (δ ¹³C) of plant tissues are primarily controlled by three main factors:

1. the photosynthetic pathway of the plants, C₃, C₄ or CAM;

(Carbon dioxide is captured in a cycle of reactions known as the C₃ cycle. About 95% of the plants on earth are C₃ plants. Those plants that utilize just the C₃ cycle for carbon fixation are known as C₃ plants. C₃ plant is a plant utilizes the C₃ carbon fixation pathway as the sole mechanism to convert CO₂ into an organic compound. C₄ plant is a plant that utilizes the C₄ carbon fixation pathway in which the CO₂ is first bound to a phosphoenolpyruvate in mesophyll cell resulting in the formation of four-carbon compound (oxaloacetate) that is shuttled to the bundle sheath cell where it will be decarboxylated to liberate the CO₂ to be

utilized in the C3 pathway. C4 plants live in hot moist or arid and nonsaline habitats. It occurs in grasses, sugar cane, maize, sorghum. C4 plants are more efficient in photosynthesis than the C3 plants. Crassulacean Acid Metabolism (CAM) is a plant that utilizes the CAM as an adaptation for arid conditions. CO₂ entering the stomata during the night is converted into organic acids, which release CO₂ for the Calvin cycle during the day, when the stomata are closed.)

2. the carbon isotopic composition of atmospheric CO₂;
3. environmental factors

The carbon isotopic composition of terrestrial plant matter ($\delta^{13}\text{C}_{\text{TPM}}$) has been used to distinguish types of vegetation and to provide information on palaeoenvironment. There is no conclusive evidence of the presence of C4 plant matter in pre-Cenozoic time. Hence, the pre-Cenozoic $\delta^{13}\text{C}$ values for terrestrial plant matter represent the $\delta^{13}\text{C}$ values of C3 plants. Under the constant $\delta^{13}\text{C}$ value of atmospheric CO₂, variations in the $\delta^{13}\text{C}$ values of C3 plants depend on environmental factors such as water availability, canopy effect, osmotic stress, temperature, and difference in pCO₂ with altitude. Among these, water availability is considered the most important in causing variations of $\delta^{13}\text{C}$ in C3 plants. Plants with higher water-use efficiency are known to have more positive $\delta^{13}\text{C}$ values. In addition, it has been reported that $\delta^{13}\text{C}$ values of C3 plants depend on the mean annual rainfall. Therefore, the pre-Cenozoic $\delta^{13}\text{C}$ values of terrestrial plant matter can be used to infer palaeoclimatic conditions (Hong et al., 2011).

1.4.2 Sources Determining ^{13}C Isotopic Composition

Over geologic time scales (long-term cycle), the residence time and the magnitude of carbon fluxes have not been constant through time, as a result of perturbations inside the cycle. For example, human activities, including fossil-fuel combustion and land-use changes, have resulted in rapid changes in the carbon cycle by transferring carbon stored in sedimentary rocks and soils to the atmosphere. This process results in the increased atmospheric CO₂ concentrations, which accordingly causes to elevated mean global temperature. This modern alteration of the carbon cycle serves as a modern analog to explain how these changes could be recorded

globally and, then, allow terrestrial and marine sequences to be correlated with the help of carbon isotope ratios (Agnihotri, 2011)

During the combustion of fossil fuels a preferential enrichment of ^{12}C in atmospheric CO_2 occurs because of the more negative isotopic composition (^{12}C -enriched) of fossil fuels, thus generating an isotopic signature for this process. For example, the isotopic composition of oceans has progressively become more ^{12}C -enriched since the Industrial Revolution as it has been observed in marine carbonates (Lange and Ziegler, 1986).

Periods of unusual carbon transfer between compartments associated with increasing volcanic activity, land plant evolution, and mass extinctions of primary producers have been documented in the geologic past, and all of them are associated with changes in the isotopic composition of both marine and terrestrial carbon. Marine carbonates record this partitioning of isotopes because such secular changes affect the functioning of the marine carbon pumps, which in turn affect the distribution of the dissolved inorganic carbon (DIC) and ultimately $\delta^{13}\text{C}_{\text{Carbonate}}$ values. These isotopic values, in turn, are dependent on the carbon chemistry of the oceans (Ortiz, 2007).

As summarized by Hoefs (1997) in relation to biomolecules synthesized in nature, the ^{13}C content of a lichen or pine needle in the natural setting will be dictated by the ^{13}C content of the carbon source, isotope effects associated with the assimilation of carbon, metabolism and biosynthesis and the ultimate cellular carbon budget. Light and moisture are important factors influencing the $^{13}\text{C}/^{12}\text{C}$ ratio in the plant samples.

1.4.3 ^{15}N Isotopic Composition

Different isotopic composition of naturally occurring and man-made N compounds has a distinct $^{15}\text{N}/^{14}\text{N}$ ratio and could potentially be distinguished by using variations in ^{15}N natural abundance (expressed as $\delta^{15}\text{N}$) (Heaton, 1986). Thus,

stable isotope of ^{15}N is a useful indicator for investigating biogeochemical mechanisms that govern ecosystem N processes and it has been widely applied to elucidate research questions in environmental and ecological fields (Robinson, 2001). $\delta^{15}\text{N}$ is a measure of the change in the proportion of ^{15}N to ^{14}N in the sample of interest is represented R_{sample} , relative to that in a standard substance is represented by R_{standard} . The unit of this measurement is “per mil” (‰).

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ ‰}$$

The natural nitrogen isotope composition ($\delta^{15}\text{N}$) of ecosystem compartments reflects ^{15}N inputs and soil N processes, the latter being affected by losses of ^{15}N depleted compounds leading to progressive ^{15}N enrichment of soils and plants. N uptake and assimilation by plants rarely exert an isotope effect, and plants are therefore expected to reflect the isotopic composition of the plant-available soil N pool. ^{15}N enrichment between bulk soil and plants is correlated with the intensity of N cycling, and long-term changes in plant and soil $\delta^{15}\text{N}$ indicate changes in the nitrogen cycle, which is recorded in wood as well as in leaves (Hietz et al., 2010).

In the particular case of an artificially drained watershed have shown significant N retention, which could not be explained by in-stream denitrification and was therefore assumed to occur between the lower limit of the root-zone of cultivated plateau areas and the river edges, in spite of the presence of tile drains (Billy et al., 2008).

1.4.4 Sources Determining ^{15}N Isotopic Composition

The major anthropogenic nitrogen sources of atmospheric deposition are coal combustion and vehicle exhausts. It is a very useful method to use $\delta^{15}\text{N}$ measurements to trace anthropogenic pollutant plumes and identify the sources and fates of N that human activities are adding to forests and lakes. Moreover, during

combustion in an engine, oxygen atoms dissociate at high temperatures and start the chain reaction which is the oxidation of nitrogen to NO and NO₂ (Fry, 2006).

Nitrogen dynamics are strongly controlled by biologically mediated reactions such as nitrification, denitrification, nitrogen fixation and assimilation. These processes can result in isotopic fractionation of the nitrogen containing compound, generally observed as an increase in the $\delta^{15}\text{N}$ of the substrate and a decrease of $\delta^{15}\text{N}$ in the product. The isotopic composition of these species at any given time or place is dependent on the value of the fractionation factor and size of the remaining reactants. Many biological processes consist of a number steps, and more than one of these processes is often acting on the same nitrogen species pool. The overall fractionation for the reaction highly dependent on many environmental conditions including: the number and type of intermediate steps, the amount and concentration of available compounds, and the biological species acting on the compounds. However, many processes involve a rate-determining step that will control the amount of fractionation in the accumulated species pools. Non-rate determining steps generally involve a small pool of a compound that is rapidly converted from reactant to product. This results in no isotopic fractionation because the products are consumed as soon as they are produced (Liu et al., 2007).

2. AIM AND SCOPE OF THE STUDY

Lichen and pine needle samples collected November 2012 and June 2013 in the framework of this study. The collected samples were analyzed with one of the state-of-art instruments so called Isotope Ratio Mass Spectrometry (IRMS) in terms of stable carbon and nitrogen isotope ratios and Inductively Coupled Plasma Mass Spectrometry (ICP MS) in terms of metals. This study is unique in Turkey since stable carbon and nitrogen isotope ratios were determined in the lichen and pine needles to monitor the air quality as the first time. In addition, to our best knowledge, biomonitoring methodology was applied as the first time in Bolu plain to determine the air quality profile of the city in this study.

The major objectives of this study can be summarized as follows:

- To determine the element composition, carbon and nitrogen isotope ratios of collected lichen and pine needle samples
- To determine whether there is a seasonality in the measured concentrations of parameters of concern during a year
- To determine the spatial distribution of measured parameters over Bolu plain. Pollution maps of each measured parameter will be plotted and hence “hot spots” of Bolu in terms of air pollution will be highlighted
- To determine whether correlation exists among the measured parameters
- To compare the accumulation capacities of lichens and pine needles

3. LITERATURE REVIEW

3.1 Lichens as Biomonitors

Lichens are slow-growing associations of fungi and green algae or cyanobacteria found on trees, rocks, in soils. They are able to live in difficult conditions for many years. The use of lichens as biomonitors comes as a result of the ability of lichens to respond to air pollutants at different levels and to indicate the presence and the concentration of these pollutants.

With lichen biomonitoring and bioindication general, sampling protocols and techniques may have to be modified to accommodate site specific differences (*e.g.*, a lack of suitable substrates). As with many other biomonitors, it is often difficult to determine the variable(s) affecting a population. This problem is common in urban areas where many contaminants may be present (Kranner et al., 2002). A combination of several contaminants or habitat variables may be affecting the lichen population resulting in a lack of correlation between lichen monitoring data and a specific contaminant.

Biomonitors are being used in many aspects of environmental researches. First steps in this field were made by mapping to get to know pollution levels all over many countries. Szczepaniak and Biziuk (2003) showed that mosses and lichens, in spite of all disadvantages, are good tools for air pollution monitoring, but best results could be achieved while using both of them together, because of differences in their metal uptake and retention. In the case of mapping, local variability plays an important role and specific data mining could also help solve a lot of problems.

Despite the documented usefulness of lichen population measures, chemical analysis and lichen thalli damage assessment in assessing air pollution, lichen

population measures are the most widely used. When applied they can provide a non-destructive, preliminary assessment of air quality over a large area and the data collected can be correlated with instrumental data (van Dobben and ter Braak, 2007). Lichen population data can be analyzed using GIS, which is a multi-scale technology that can be used to detect changes in lichen abundance data (Brabynet al., 2005). It is being used in an increasing number of studies in place of conventional mapping techniques.

Slovenia is one of the Europe's most highly forested countries that a lichen survey was performed in this country in 641 plots with a grid density of 4 x 4 km and *H. physodes* was collected on a 16 x 16 km bioindication grid and analyzed for S, N, As, Br, Ce, Cd, Cr, K, La, Mo, Rb, Sb, Th, U and Zn contents over the period from the end of August to end of September 2000. Jeran et al., (2007) investigated the relationship between foliose epiphytic lichen cover and selected trace element, S and N concentrations in *H. physodes*. No relationship was found between Hypogymnia trace element, N and S concentrations and foliose epiphytic lichen cover.

Garty et al., (2003) collected the lichen *R. lacera*, growing on twigs of carob trees were applied as biomonitors of air pollution to determine the environmental impact of a coal-fired power station. For the measurement of the Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, S, Sr, V and Zn content of the lichen that was determined by inductively coupled plasma (ICP)- atomic emission spectrometry. The results indicated the accuracy of the information obtained by biomonitoring methods concerning the deposition of mineral elements. *R. lacera* was found to be an outstanding accumulator, as the elements tend to concentrate in noticeable amounts in this lichen, especially Ca, S, K, Al, Fe, P and Mg.

Van der Wat and Forbes (2015) discussed the use to age of lichens as biomonitors for air pollutants and they review the sample preparation and analytical techniques employed for such biomonitoring. As a result of this application, Lichens are useful biomonitors for Semi-volatile organic compounds as air pollutants. There has been a recent global trend in environmental analytical research to utilize lichens in this manner.

3.2 Biomonitoring of Metal Pollution

Lichens have been widely used worldwide for monitoring atmospheric pollution in local and regional studies because they are widespread and dependent largely on the atmosphere for their nutrient supply.

Nimis et al., (2000) measured the concentrations of 13 elements in peripheral parts of the epiphytic lichen *Xanthoria parietina* at 200 stations of Veneto (NE Italy). For each element, two maps were presented, showing, respectively, geographic patterns and deviations from background levels. The results were summarized by a map, showing the joint distribution of elements with concentrations close to Italian backgrounds, and of those strongly deviating from background levels. The results allowed selection of high-risk areas for instrumental monitoring.

In the northern part of Pisa Province (Tuscany, Italy), Scerbo et al., (2002) described the use of lichens as both airborne trace element biomonitors and air quality bioindicators. The following elements were analysed in *Xanthoria parietina*: As, Cd, Cr, Hg, Ni, Pb, V and Zn and the results are compared with previous study in Livorno Province using the same lichen species. The results identify spots of different environmental metal contamination and air quality. The air quality in Pisa Province is better than in Livorno Province, even if the different climatic and orographic features of the two areas may influence the presence of lichen species and thus an assessment of air quality.

Trees can be used as sensitive biomonitors to record local and global environmental change. Padilla and Anderson (2002) used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to examine trace element concentration in tree rings. *Pinus ponderosa* tree rings were analyzed for element concentration and evaluated versus local and global historical events. The elements tested did not all display the same time versus concentration patterns. The largest temporal difference in element concentrations in the tree-rings of the *Pinus ponderosa* pine cannot be correlated with any known local events. Small temporal differences for some elements (copper) can be associated with several local fire events. The largest

temporal differences in element tree-ring concentrations can be associated to global environmental changes, specifically volcanic eruptions. Unlike many tree-ring studies, their trend of declining element concentration occurs for 150 years and cannot be explained by exposure to anthropogenic acid rain.

Lichen samples from different parts of the world have been known to accumulate elements to a greater degree than higher plants, if they are exposed to these elements from the atmosphere or from water and sediments. It has been hypothesized that lichens can be used to monitor air pollution around point and area emission sources. Local variation of element concentrations would not be large enough to affect the concentration patterns in large areas. Yenisoy-Karakaş and Tuncel (2004) tested this hypothesis in the Aegean region of Turkey, which is very urbanized and industrialized. No such study has been conducted before in this part of the country. A total of 234 samples of the lichen *Xanthoria parietina* were collected from a 51800-km² area. Samples were washed and analyzed by INAA and ICP-AES for 35 elements. The range of the concentrations for most of the elements on a local scale was an order of magnitude lower than for the element concentrations on a regional scale. The mean local coefficient of variance (CV) was found to be 15, providing that the local variation did not affect the concentration of elements in the sampling region. According to cluster analysis, 8 (As, Hg, Pb, Sb, Fe, Mn, Na and K) elements were indicative of important local pollution locations and their zone of impact in the region. By mapping the concentrations of eight indicative elements in lichen *Xanthoria parietina* of the Aegean region, it was possible to relate deposition to the existence of known sources of pollution in certain areas. Location of pollution sources such as iron-steel plants, and coal burning in the cities, industrial activity and two important coal-fired power plants generally corresponded with locations of highest element accumulations in the lichens.

Al-Alawi and Mandiwana (2007) tested the needles of Aleppo pine as a possible biomonitor of heavy metal pollution in Amman City, Jordan. Unwashed and washed needles collected from different sites of the city determined in terms of concentrations of Pb, Cd, Cu and Zn. The results of the investigation indicated that the industrial site had high levels of the metal pollutants except for Pb and Cu which

was much higher on samples from the roadside. As a result, *P. halepensis* L. needles are useful biomonitors of the heavy metals in the arid environment.

In Aqaba City (Jordan), Al-Khlaifat and Al-Khashman (2007) evaluated the leaves of date palms as biomonitors of heavy metal contamination. The concentrations of Fe, Pb, Zn, Cu, Ni and Cr were determined using a flame atomic absorption spectrophotometer. Trace metals are currently of much environmental concern. The results of this study showed that the highest metal concentrations were found in the heavy traffic sites, while the lowest metal concentrations were reported in the rural and control sites. Industrial and traffic emissions were the main source of metal pollution in the atmosphere of Aqaba.

Airborne metal deposition in the major urban and the industrial districts of Kocaeli was monitored using *Xanthoria parietina* lichen specimen as a biomonitoring organism. Lichen samples were analyzed for Al, As, Co, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Ti, Tl, V and Zn contents to determine the relationship between the potential pollutant sources in the region and the degree of air borne metal deposition (Demiray et al., 2012). The results of biomonitoring survey revealed that the degree of metal deposition was severe especially in the heavily industrialized district of Kocaeli. Metals such as Al, Fe, Mn, Cd, Pb and Zn were among the most prominent elements exhibiting high concentration within the Dilovası lichen samples.

3.3 Biomonitoring of Stable Carbon and Nitrogen Isotopes

Lichens and mosses have been recognized as useful tools for biomonitoring atmospheric deposition and assessing air pollution of isotopes monitoring.

Liu et al. (2007) performed a biomonitoring study by using same type of moss with identical regional atmospheric deposition while under different growing environments. Similar variations in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were found between mosses collected from five habitats, with an increasing sequence from mosses under canopies to epilithic mosses, indicating that habitats were potentially

regulating $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses. Dryer habitats (lower water availability) and input of more aerosol N were the main reasons for higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses at open sites (especially for epilithic species), while more negative values of mosses under canopies were attributed to their wetter habitats and less uptake of aerosol N. Consequently, isotopic data of mosses under different environments could not be compared for atmospheric deposition research with each other even collected at the same site. Moss $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were affected not only by regional atmospheric N sources but also by their growing environments. $\delta^{15}\text{N}$ of epilithic *Haplocladium microphyllum* at open sites can be taken as confident bio-indicator of atmospheric N deposition, which would deepen the application of stable nitrogen isotope of bryophytes in atmosphere-plant system study. As a result of this study, Dryer habitats and direct exposure to dry deposition are mainly responsible for higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses in open fields.

In a study performed in Republic of Korea, Kwak et al., (2009) collected samples of needle, bark, litter, organic soil, and inorganic soil from red pine to evaluate the potential adequacy of carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$, expressed as $\delta^{13}\text{C}$), nitrogen isotope ratio ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$), N concentration, and Ca-to-Al ratio of forest samples as proxies for atmospheric pollution. *Pinus densiflora* stands located in industrial (polluted) and rural (background and regarded as relatively unpolluted) areas, and their chemical and isotopic compositions were compared. The $\delta^{13}\text{C}$ of needles and bark samples from the industrial area were significantly ($P < 0.05$) lower than those from the rural area by more than 1.1‰, with a greater difference in the needle samples (more than 2‰); which is reflective of ^{13}C -depleted CO_2 emissions from fossil fuel combustion in industrial areas. The $\delta^{15}\text{N}$ of all the samples from the industrial area were significantly ($P < 0.05$) lower than those from the rural area, except for mineral soil, and the differences were between 0.9‰ (litter) and 2.9‰ (organic soil). Such differences could be primarily attributed to a higher deposition of ^{15}N -depleted atmospheric N in the industrial areas. However, no significant differences were noted with regard to the total N concentrations of the samples from different areas; this was probably due to different indigenous soil N availability. Combining all the samples and isotopic and chemical variables, the $\delta^{13}\text{C}$,

$\delta^{15}\text{N}$, and Ca-to-Al ratios of the needle samples were identified as the most reliable proxies for atmospheric pollution in the study areas. Further study will be necessary to develop quantitative criteria for the practical use of each indicator in identifying forest ecosystems that are susceptible to atmospheric pollution. This study shows that needle samples are more sensitive to atmospheric pollution than other forest samples, including bark, litter, organic soil and mineral soil samples.

Another study was performed in China. Liu et al., (2010) characterized epilithic mosses by insulation from substratum N and hence meet their N demand only by deposited N. This study investigated tissue C, total Chlorophyll and $\delta^{13}\text{C}$ of epilithic mosses along 2 transects across Guiyang urban (SW China), aiming at testing their responses to N deposition. Tissue C and total Chlorophyll decreased from the urban to rural, but $\delta^{13}\text{C}$ moss became less negative. With measurements of atmospheric CO_2 and $\delta^{13}\text{CO}_2$, elevated N deposition was inferred as a primary factor for changes in moss C and isotopic signatures. Correlations between total Chlorophyll, tissue C and N signals indicated a nutritional effect on C fixation of epilithic mosses, but the response of $\delta^{13}\text{C}$ moss to N deposition could not be clearly differentiated from effects of other factors. Collective evidences suggest that C signals of epilithic mosses are useful proxies for N deposition but further works on physiological mechanisms are still needed.

Cao et al., (2011) determined stable carbon isotope ratios for the OC and EC fractions of $\text{PM}_{2.5}$ collected from 14 Chinese cities in January and June/July of 2003. Comparisons with isotopic signatures of putative sources suggested that the carbonaceous $\text{PM}_{2.5}$ was mainly from fossil fuels, especially coal combustion and motor vehicle emissions. According to study, the urban air in seven northern cities was strongly impacted by coal burned for residential heating during winter.

In France, Gueguen et al., (2012) documented the harmful effect of man-made particles on natural processes and human health by a large number studies showing a positive correlation between particulate matter concentration and health effects. They showed that tree bark biomonitoring with Pb, Sr and Nd isotope ratios

as tracers allowed a thorough analysis of the impacts of industrial and other anthropogenic emissions on the urban environment. The isotope maps including Sr, Nd and Pb emphasize that isotope tree bark biomonitoring allows for a precise analysis of traffic and industry-derived pollution in the urban environment.

4. MATERIALS AND METHODS

4.1 General Information about Bolu

Bolu is a city in the western Black Sea Region surrounded by Zonguldak, Karabük, Çankırı, Ankara, Bilecik, Eskişehir, Düzce and Sakarya. It has 9 districts and 511 villages. It is between the 30°32' and 32° 36' east longitudes; and 40° 06' and 41° 01' north latitudes. The city has an average 1000 m altitude while the city center has a 725 m altitude. The total area of that city is 845.800 hectare. 149.664 hectare of this area is used for agriculture. Approximately 60% of the total area is covered with forests and mountains. The area used for the agriculture is 17.7% of the total area. Despite the small agricultural area usage, this city is important in agriculture for Turkey (DPT, 2002).

Bolu is one of the cities suffering from frequent air pollution episodes, particularly in the winter season, due to its peculiar geography and poor control of emissions, which are released from domestic heating processes and motor vehicles. Distribution of natural gas through the city was started in 2010. It has been planned that the distribution to whole network would be completed at the end of 2015. The main industrial sector in the city is based on the broiler houses, from which significant contribution to the air pollution is not expected. However, TEM and D100 highways passing through the city are thought to be the main sources affecting the air quality and these line sources cannot be controlled. The samples were collected in this study across the Bolu Plain, which has a total size of 12.175 hectare.

4.2 Sample Collection and Sample Handling

Samples were collected in 1 x 1 km grids in winter field campaign and 3 x 3 km grids in summer field campaign since lichen species could not find in every 1 x 1

km grid in winter field campaign, coarser grid size (3 x 3 km) was decided during the summer field campaign. Since *Xanthoria parietina* is the most commonly found lichen species in the region, the samples of this species were collected from grids. However, different types of lichen species and pine needles were collected to see whether there is any difference in terms of the isotope ratios and metal accumulations among these species. In some of the grids, no samples could be collected because there were no trees or because the trees had no lichens.

125 samples were collected during the winter sampling period, in November 2012. The locations of the sampling points in the winter field campaign were depicted in Figure 4.1. The detailed sampling information was provided in Appendix I. The samples of *Xanthoria parietina* (n=102), pine needles (n=13) and different types of lichen species including *Parmelia sulcata* and *Evernia prunastri* (n=10) were collected during the winter field campaign.

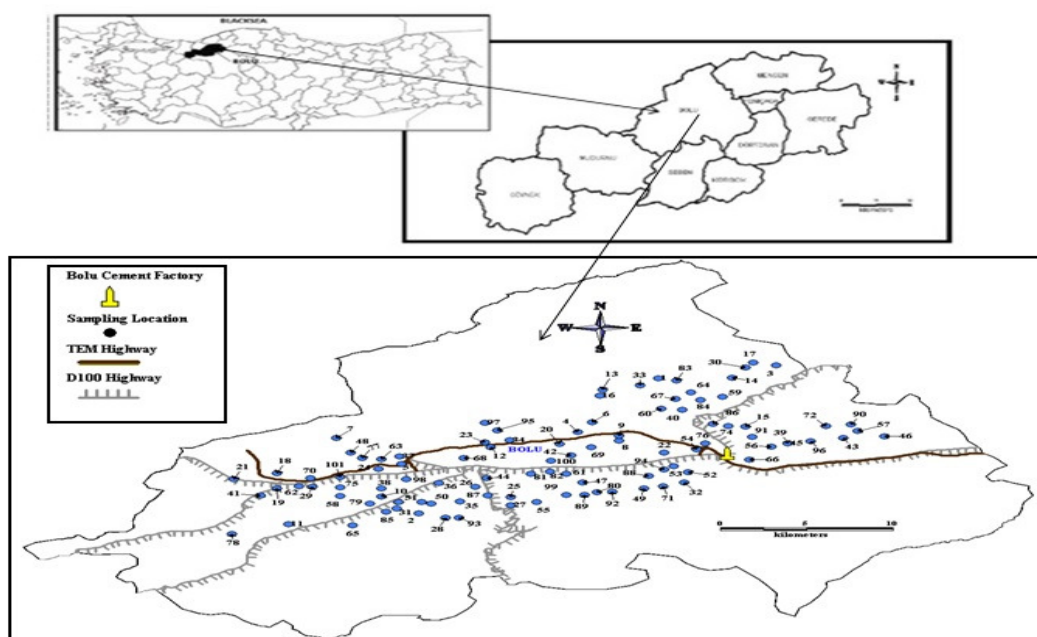


Figure 4.1. Locations of samples collected during the winter field campaign

120 samples were collected during the summer sampling period, in June 2013. The locations of the sampling points were illustrated in Figure 4.2. The detailed sampling information was provided in Appendix II. *Xanthoria parietina*

(n=64), pine needles (n=49) and different types of lichen species including *Parmelia sulcata*, *Evernia prunastri* and *Usnea subfloridana* (n=7) were the types of samples collected during the summer field campaign.

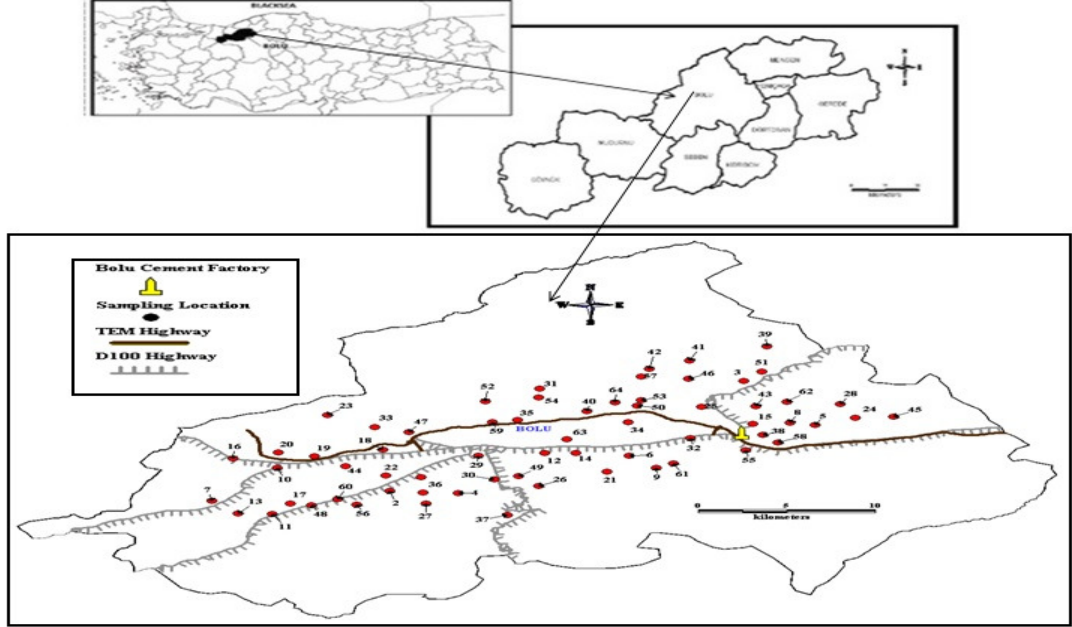


Figure 4.2. Locations of samples collected during the summer field campaign

Various lichen thalli with different sizes were obtained between 1 and 2 m in height from the ground and all around the tree. If enough lichen samples on the branches or trunks of the trees could not be found, they were chosen to be collected from the tree base. When no lichen samples were obtained during the field campaign, pine needle samples were able to be collected. Sampling in the grid was performed from many points to provide a representative sample of the grid. After the identification of the sample (coordinates, elevation, directions, tree types and date, etc.), it was put in a polyethylene bag, labeled and sealed. Materials from the same tree and from 3 to 7 nearby trees were combined in a grid to have one representative sample of this point. Polyethylene gloves were worn during the sampling, sample handling and preparation in order to avoid sample contamination.

4.3 Laboratory Studies

4.3.1 Sample Preparation

The preparation process of the samples, especially for *Xanthoria parietina*, for the analyses was challenging since it was very difficult to differentiate lichen thallus from the bark substrate without carrying out a moisturizing process. In order to separate the lichen thallus from the substrate, the samples were rinsed through deionized water.

The lichen thallus along with the bark substrate was floated in deionized water in beaker and stirred for approximately 30 seconds. Debris and soil particles settled down. After a rinsing process for 30 seconds, they could be separated from the substrate with nylon tweezers. As a matter of fact, rinsing can be required for removing soil particles without leaching out metals as well. They were sorted to remove dead tissue and external materials (adhering bark, mosses, other lichen species, soil particles, etc.) as shown in Figure 4.3. After the separation process, they were put in folded filter papers and air-dried in clean glass box in the clean room for approximately one day at room temperature. The dried samples were then kept in the labelled polyethylene bags (Yenisoy-Karakaş, 2000).



Figure 4.3. Photos taken during the cleaning of the samples

4.3.2 Elemental Analysis of Samples

4.3.2.1 Microwave Digestion

All materials used for sample preparation and extraction were stored in 30% HNO_3 (65%-Merck) bath for one day. Digestion process was done by BERGHOF Microwave Oven as shown in Figure 4.4. Samples were placed into teflon digestion vessel with a clean teflon forceps. 0.5 mL suprapure 48% HF (Merck) and 5 mL 60% ultrapure HNO_3 (distilled by subboiling) and 1 mL 31% H_2O_2 were added into the samples. Vessels were placed into the BERGHOF Microwave Oven and heated with the program shown in Figure 4.5.

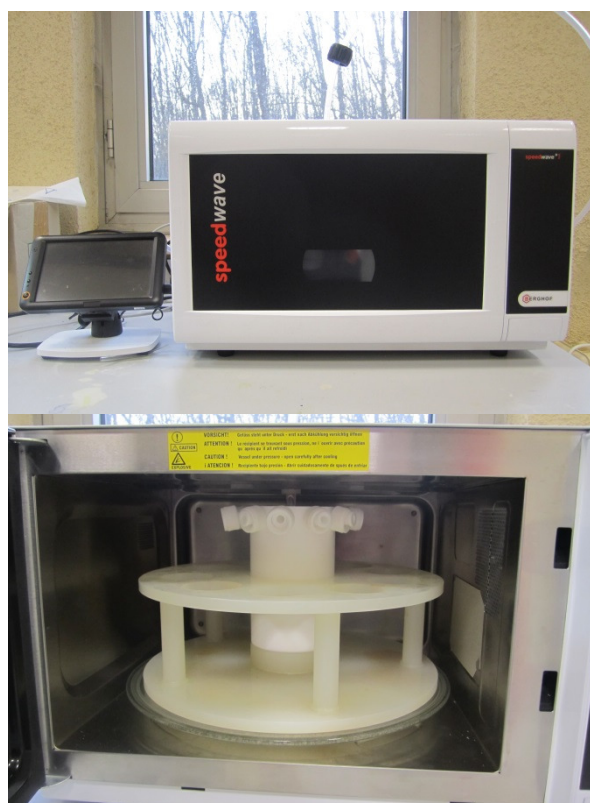


Figure 4.4. BERGHOF Microwave oven

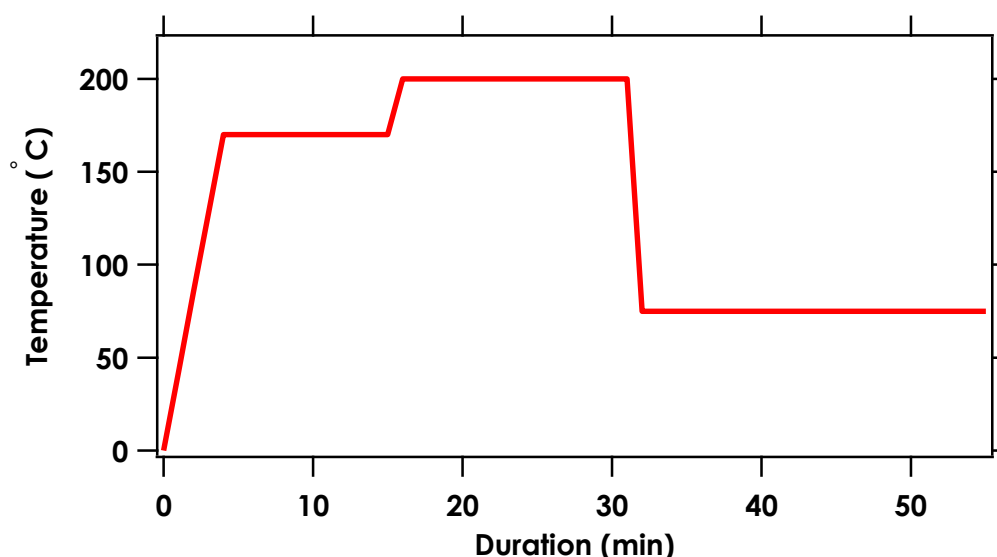


Figure 4.5. Heating program of microwave oven during digestion of samples

Microwave oven has 10 vessels with a capacity of 50 mL. In each set, 9 samples and 1 acid blank were digested. After microwave digestion process, the vessels were cooled to room temperature. Then, the lids of the vessels were opened under hood. The content of the vessels were transferred in to polypropylene centrifuge tubes and diluted to 50 mL with 18.3 μ S ultra-pure deionized water. Following the acid digestions, samples were kept in the refrigerator till ICP-MS analysis.

4.3.2.2 General Information about ICP-MS

Various methods have been improved for elemental analysis of samples including proton induced X-ray emission (PIXE), atomic absorption spectroscopy (AAS), neutron activation analysis (NAA), inductively coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence (XRF). Because of having lower detection limits and large dynamic linear working range and ability to analyze large suite of elements with a very good precision within a very short period of time, ICP-MS was selected in this study to analyze the collected samples.

Analysis of lichen and pine needle samples was performed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Kocaeli University, Geological Engineering Department. Samples were analyzed for a total of 55 elements (Li, Be, Na, K, Mg, S, Al, P, Ca, Ba, Ti, V, Cr, Sc, Mn, Fe, Co, Ni, Cu, Zn, Ge, As, Se, Rb, Sr, Y, Mo, Cd, Sn, Sb, Cs, La, Ce, Pr, Nd, Eu, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, W, Pt, Au, Pb, Hg, Bi, Tl, Th and U). ICP-MS used in this study shown in Figure 4.6 and the corresponding operating conditions were summarized in Table 4.1.



Figure 4.6. Perkin Elmer-ICP-MS

Table 4.1. Operating conditions for the ICP-MS

Parameter	Value
RF power (W)	1300
Plasma gas flow (L min^{-1})	15
Auxiliary gas flow (L min^{-1})	1.2
Nebulizer	Gemtip crossflow
Nebulizer gas flow (L min^{-1})	0.96
Sampling cone(orifice dia. mm)	Platinum (1.1)
Skimmer cone(orifice dia. mm)	Platinum (0.9)
Injector	Alumina
Mass range (m/z)	5-270 amu
Spray chamber	Ryton Scott
Scanning mode	Both (analog and pulse)
Number of Scans (sweeps/reading) for each isotope	20
Number of repetitions	3
Rinse time (s)	45
Sample Uptake time (flush) (s)	35

4.3.3 Method Validation

Quality Assurance/ Quality Control (QA/QC) is one of the most important steps in analytical analysis. In this study, well-known QA/QC procedures were also followed. All the chemicals are used in ultra-pure grade. Acid blank samples were also analyzed along with the lichen and pine needle samples using ICP-MS, and contents were closely monitored. Moreover, certified reference materials including SRM 1648a-Urban Particulate Matter (NIST), SRM 1575a-Trace Elements in Pine Needles (NIST) and Reference Material IAEA-336 Trace and Minor Elements in Lichen (IAEA) were analyzed to check the accuracy of ICP-MS analysis. Prior to ICP-MS analysis, the powder form of these standards were digested under the same conditions with the samples. Results of these analyses were given in Table 4.2, 4.3 and 4.4, respectively.

The recoveries for most of the elements studied from SRM NIST 1648a were greater than 70 % or better. The lowest recovery values were obtained for Hf (47 %) and Se (65 %), for which information and reference values were provided in the certificate sheet of the standard. With the exception of Se, Cd and Zn, all the recovery values obtained in SRM NIST 1575a were greater than 80 %. The recoveries for most of the elements studied from SRM IAEA-336 were greater than 70 % or better except for Hg, Sb and Zn.

Table 4.2. Certified values in SRM NIST 1648a-Urban Particulate Matter and found values by ICP-MS (N=5)

	Certified Value ($\mu\text{g}\cdot\text{g}^{-1}$)		Found Concentration ($\mu\text{g}\cdot\text{g}^{-1}$)		Recovery (%)	
	Average	$\pm U(k=2,$ 95% CL)	Average	$\pm U(k=2,$ 95% CL)	Average	$\pm U(k=2,$ 95% CL)
Al	34300	1300	33910	1565	99	6
As	115.5	3.9	99	5	86	5
Ca	58400	1900	66304	4250	114	8
Cd	73.7	2.3	54	2	74	3
Ce	54.6	2.2	42	3	77	6
Co	17.93	0.68	27	1	148	9
Cr	402	13	351	46	87	12
Cs ^a	3.4	0.2	3.2	0.1	94	6
Cu	610	70	545	39	89	12
Fe	39200	2100	40508	1171	103	6
Hf ^b	5.2		2.4	0.1	47	1
K	10560	490	11629	227	110	6
La ^a	39	3	28	3	72	9
Mg	8130	120	8609	334	106	4
Mn	790	44	850	26	108	7
Na	4240	60	4728	111	112	3
Ni	81.1	6.8	85	3	104	9
Pb	6550	330	5377	120	82	5
Rb	51	1.5	53	2	104	5
S	55100	3600	55578	1621	101	7
Sb	45.4	1.4	31.6	1.4	70	4
Sc	6-120		24.4	4.0		
Se ^a	28.4	1.1	18.4	0.5	65	3
Sm ^a	4.3	0.3	3.5	0.3	81	10
Sr	215	17	255	8	119	10
Th	7-107		4.9	1.5		
Ti	4021	86	4487	134	112	4
V	127	11	145	4	114	10
W ^a	4.6	0.3	5.7	0.4	123	12
Zn	4800	270	3182	194	66	5

^a reference value, ^b information value, U: expanded uncertainty

Table 4.3. Certified values in SRM NIST 1575a-Trace Elements in Pine Needles and found values by ICP-MS (N=4)

	Certified Value ($\mu\text{g}\cdot\text{g}^{-1}$)		Found Concentration ($\mu\text{g}\cdot\text{g}^{-1}$)		Recovery (%)	
	Average	$\pm U(k=2, 95\% \text{ CL})$	Average	$\pm U(k=2, 95\% \text{ CL})$	Average	$\pm U(k=2, 95\% \text{ CL})$
Al	580	30	645	50	111	10
As ^a	0.039	0.002	0.032	0.002	83	6
Ca	2500	100	2953	193	118	9
Cd	0.233	0.004	0.181	0.003	78	2
Cs ^a	0.283	0.009	0.278	0.007	98	4
Cu	2.8	0.2	3.0	0.3	107	13
Fe	46	2	56	4	121	10
K	4170	70	4669	305	112	8
Mg ^a	1060	170	1122	82	106	19
Mn ^a	488	12	499	18	102	4
Na ^a	63	1	74	3	118	5
Ni ^a	1.47	0.1	1.52	0.05	103	8
P	1070	80	1147	71	107	10
Pb	0.167	0.015	0.134	0.005	80	8
Rb	16.5	0.9	17.0	0.5	103	6
Se ^a	0.099	0.004	0.047	0.007	47	8
Zn	38	2	25	1	66	5

^a reference value, U: expanded uncertainty

Table 4.4. Certified values in SRM IAEA 336-Trace and Minor Elements in Lichen and found values by ICP-MS (N=4)

	Certified Value ($\mu\text{g}\cdot\text{g}^{-1}$)		Found Concentration ($\mu\text{g}\cdot\text{g}^{-1}$)		Recovery (%)	
	Average		Average	$\pm U(k=2, 95\% \text{ CL})$	Average	$\pm U(k=2, 95\% \text{ CL})$
Al ^a	680		698	52	103	8
As	0.63		0.52	0.02	83	4
Ba	6.4		7.1	0.9	111	14
Cd ^a	0.117		0.089	0.002	76	2
Ce	1.28		1.09	0.02	85	1
Co	0.29		0.41	0.01	142	4
Cr ^a	1.06		1.48	0.12	140	12
Cs	0.11		0.11	0.00	97	1
Cu	3.6		3.2	0.1	90	2
Eu ^a	0.023		0.023	0.001	98	3
Fe	430		467	10	109	2
Hg	0.2		0.10	0.07	49	37
K	1840		1894	115	103	6
La	0.66		0.52	0.01	79	1
Lu ^a	0.0066		0.0045	0.0001	68	2

Table 4.4. Certified values in SRM IAEA 336-Trace and Minor Elements in Lichen and found values by ICP-MS (N=4) (Continued)

	Certified Value ($\mu\text{g}\cdot\text{g}^{-1}$)	Found Concentration ($\mu\text{g}\cdot\text{g}^{-1}$)		Recovery (%)	
	Average	Average	$\pm U(k=2, 95\% \text{ CL})$	Average	$\pm U(k=2, 95\% \text{ CL})$
Mn	63	70	2	110	3
Na	320	356	8	111	3
Nd^a	0.6	0.50	0.01	84	1
P^a	610	541	16	89	3
Pb^a	4.9	4.0	0.1	82	3
Rb^a	1.76	1.74	0.03	99	2
Sb	0.073	0.042	0.004	57	5
Se	0.22	0.20	0.01	89	6
Sm	0.106	0.099	0.002	93	2
Sr	9.3	9.8	0.2	105	2
Tb^a	0.014	0.013	0.000	91	1
Th	0.14	0.113	0.004	81	3
V^a	1.47	1.7	0.1	118	8
Yb^a	0.037	0.032	0.001	86	3
Zn	30.4	20.0	0.3	66	1

^a information value

Limit of detection (LOD) is defined as the lowest amount of analyte in a sample that can be detected and reported with 99% confidence that the analyte concentration is greater than zero. Various calculation methods of detection limit are available in literature (Javier del RioBocio et al., 2003; Gatari et al., 2005).

In this study, limit of detection (LOD) and limit of Quantification (LOQ) values were also calculated. Lopes et al. (2006) defined the LOD and LOQ as concentration value corresponding to 3 and 10 times the standard deviation of blank analysis, respectively. LOD and LOQ values calculated by the following this definition and results were summarized in Table 4.5. The calculated LOD values were ranged from $1 \mu\text{g L}^{-1}$ for Pr and Eu to 294 mg L^{-1} for Al.

Table 4.5. LOD and LOQ values of analyzed elements by ICP-MS

Element	LOD	LOQ	Element	LOD	LOQ
Al	294	979	Na	41	138
As	0.021	0.071	Nd	0.005	0.016
Ba	8	27	Ni	0.755	2.518
Bi	0.033	0.111	P	43	142
Ca	109	362	Pb	0.037	0.123
Ce	0.013	0.043	Pr	0.001	0.004
Co	0.019	0.064	Rb	0.028	0.095
Cr	1.73	5.78	S	79	265
Cs	0.003	0.008	Sc	0.273	0.910
Cu	0.135	0.449	Se	0.103	0.342
Dy	0.002	0.007	Sm	0.002	0.006
Er	0.001	0.004	Sn	0.131	0.437
Eu	0.002	0.006	Sr	0.270	0.900
Fe	52	172	Th	0.003	0.011
Gd	0.003	0.010	Ti	2.32	7.75
Hf	0.007	0.023	Tl	0.005	0.017
Hg	0.004	0.012	U	0.002	0.008
K	29	97	V	0.052	0.173
La	0.008	0.027	W	0.311	1.037
Li	0.029	0.096	Y	0.004	0.015
Mg	5.21	17.37	Yb	0.003	0.01
Mn	0.198	0.660	Zn	3.74	12.46
Mo	1.02	3.40			

All values given $\mu\text{g.L}^{-1}$ unit except the ones shown in bold and italic, which are in terms of mg.L^{-1} .

4.3.3 Isotopic Composition Analysis

Dried lichen and pine needle samples were prepared for isotopic composition analysis in the laboratory. Samples were powdered by mortar and pestle. Then the powdered samples were kept in the labelled Eppendorf tubes as shown in Figure 4.7.

**Figure 4.7.** Prepared plant samples for analysis by EA-IRMS

4.3.3.1 Analysis of Lichen and Pine Needle Samples by EA-IRMS

The cleaned lichen and pine needle samples were sent to the Turkey Atomic Energy Agency Sarayköy Nuclear Research Center to specify the contents of stable carbon and nitrogen isotopes with the EA-IRMS device as shown in Figure 4.8. The schematic diagram of the instrument was shown in Figure 4.9. During the analysis of samples, especially during the stable nitrogen isotope measuring process, there was a problem with repeatability caused by the samples being non-homogeneous. For this reason, collected samples sent for analysis were freeze-dried in a liquid nitrogen tank and thereafter were homogenized through a process of pulverization. After that, 0.1 mg was obtained from these samples and prepared for the stable nitrogen isotope. In addition, 2.0 mg for the carbon isotope was weighed and placed in tin capsules and then analyzed.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ rates were analyzed by using the Thermo Finnigan Deltaplus Xp Continuous Flow Isotope Ratio Mass Spectrometer which was connected to a Thermo-Electron Flash EA 1112 Model Elemental Analyzer with Thermo Finnigan ConFlo IV interface.



Figure 4.8. EA-IRMS instrument used in the analysis of collected samples

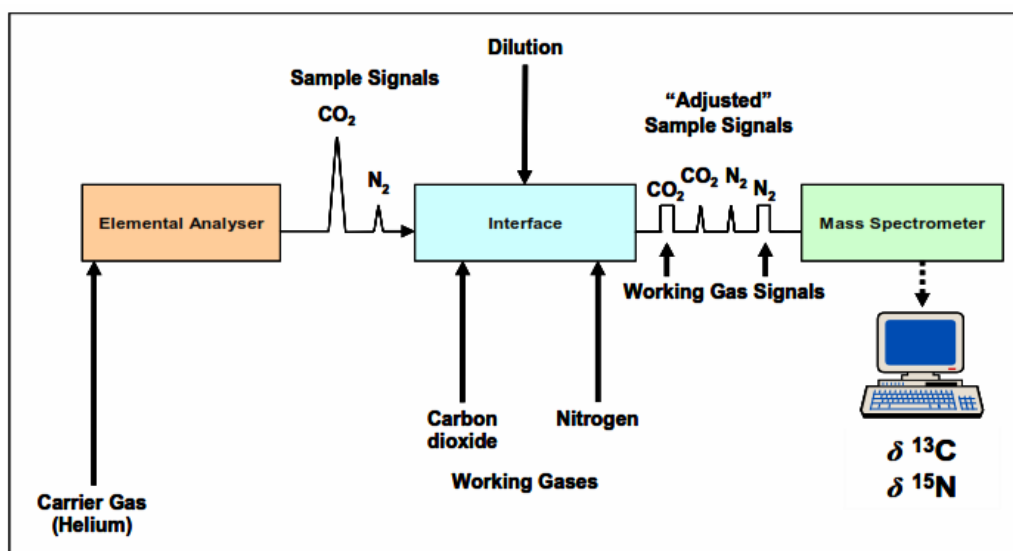


Figure 4.9. The schematic diagram of the EA-IRMS instrument

The operation conditions for EA-IRMS are provided in Table 4.6. In the EA-IRMS device, the Thermo Finnigan ConFlo IV was used as the interface, as well as an Elemental Analyser (EA) Thermo-Electron Flash EA-1112. The operational parameters for both sections are shown in Table 4.7.

Table 4.6. Operating conditions for the EA-IRMS device

$\delta^{13}\text{C}$ Analysis		$\delta^{15}\text{N}$ Analysis	
High Voltage	2.996 (kV)	High Voltage	3.000 (kV)
Magnet Step	12051	Magnet Step	9307
Ion Source- Container	0.7 mA	Ion Source- Container	0.7 mA
Ion Source Trap	0.8 mA	Ion Source Trap	0.8 mA
Vacuum	1.3×10^{-6}	Vacuum	1.3×10^{-6}
Emission	1.5 mA	Emission	1.5 mA
Electron Energy	124.000 eV	Electron Energy	124.000 eV
Reference Gas	CO_2	Reference Gas	N_2

Table 4.7. Operating conditions for the EA and ConFlo IV Interface

ConFlo IV Interface		EA	
He	1.2 bar	Reactor Temperature	1020°C
CO_2	1.2 bar	GC Colon Temperature	45°C
N_2	2.4 bar	Reference Gas	Helium (250 ml.min ⁻¹)
		Transporter Gas	Helium (100 ml.min ⁻¹)
		O_2	250 ml.min ⁻¹

5. RESULTS AND DISCUSSION

4.4 General Characteristics of Biomonitoring Data

4.4.1 Summary Statistics of Generated Data

Biomonitoring study was conducted over Bolu Plain in November 2012 and in June 2013. *Xanthoria parietina* is the most commonly found species among the other collected biomonitoring species. After analysis of collected samples in terms of metals and stable carbon and nitrogen isotopes, the generated data were evaluated in this chapter.

Summary statistics of the lichen and pine needle samples collected in winter field campaign is provided in Table 5.1 and 5.2, respectively, which includes the number of samples (N), arithmetic mean, standard deviation, minimum, maximum, median and geometric mean of the obtained concentrations. Except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are expressed as ‰, all the concentrations are expressed as $\mu\text{g g}^{-1}$. The lowest average concentration was found for Pt as $0.3\pm0.2 \text{ ng g}^{-1}$ and the highest average concentration value was obtained for K as $2816\pm1589 \text{ as } \mu\text{g g}^{-1}$ during winter field campaign for lichen samples (Table 5.1). The average concentrations of other metals range between these lowest and the highest concentrations. The average concentration for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for lichen samples was found as -9.72 ± 3.12 and -22.46 ± 0.60 ‰, respectively, during the winter field campaign.

The lowest average concentration was found for Lu as $1.2\pm1.6 \text{ ng g}^{-1}$ and the highest average concentration value was obtained for K as $7298\pm2271 \text{ as } \mu\text{g g}^{-1}$ during winter field campaign for pine needle samples (Table 5.2). The average concentrations of other metals range between these lowest and the highest concentrations. The average concentration for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for pine needle samples

was found as -3.30 ± 3.43 and -27.22 ± 2.06 ‰, respectively, during the winter field campaign.

Summary statistics of lichen and pine needle samples collected in the summer field campaign are given in Table 5.3 and 5.4. Similar to the winter field campaign, the lowest average concentration value was found for Pt as 0.3 ± 0.2 ng g⁻¹ in the summer field campaign for lichen samples. The highest average concentration was obtained for K as 4530 ± 1631 µg g⁻¹ during the summer field campaign for lichen samples. Potassium was followed by Ca and Al, for which the average concentrations were obtained as 2156 ± 2197 µg g⁻¹ and 1471 ± 777 µg g⁻¹, respectively, in lichen samples during summer season. The average concentration for δ¹⁵N and δ¹³C associated with lichen samples was found as -9.71 ± 3.89 and -22.48 ± 0.67 ‰, respectively, during the summer field campaign.

The lowest average concentration was found for Lu as 0.6 ± 0.4 ng g⁻¹ and the highest average concentration value was obtained for Ca as 6764 ± 2357 µg g⁻¹ during summer field campaign for pine needle samples (Table 5.4). The average concentrations of other metals range between the lowest and the highest concentrations. The average concentration for δ¹⁵N and δ¹³C for pine needle samples was found as 0.03 ± 2.85 and -28.09 ± 1.35 ‰, respectively, during the summer field campaign.

Table 5.1. Summary statistics of lichen samples collected in the winter field campaign (in $\mu\text{g g}^{-1}$)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Al	101	1530	626	4280	521	1411	1427
As	101	1.47	0.78	5.83	0.51	1.29	1.33
Ba	101	11.6	4.5	25.2	2.3	10.5	10.7
Be	101	0.03	0.014	0.088	0.004	0.028	0.028
Bi	101	0.015	0.008	0.049	0.004	0.013	0.014
Ca	101	2278	1428	7782	768	1756	1971
Cd	101	0.098	0.053	0.41	0.031	0.087	0.088
Ce	101	1.22	0.44	3.26	0.35	1.16	1.15
Co	101	0.557	0.22	1.3	0.17	0.51	0.52
Cr	101	3.35	1.33	8.34	1.09	3.15	3.12
Cs	101	0.18	0.085	0.51	0.051	0.16	0.16
Cu	101	5.38	3.4	34.3	2.4	4.9	4.97
Dy	101	0.078	0.028	0.21	0.026	0.073	0.073
Er	101	0.045	0.016	0.12	0.015	0.042	0.042
Eu	101	0.024	0.009	0.06	0.007	0.022	0.022
Fe	101	976	362	2270	340	908	917
Gd	101	0.094	0.034	0.25	0.029	0.091	0.089
Ge	101	0.21	0.075	0.5	0.079	0.2	0.2
Hf	101	0.068	0.027	0.21	0.024	0.064	0.063
Hg	101	0.024	0.016	0.092	0.005	0.021	0.019
Ho	101	0.015	0.006	0.040	0.005	0.014	0.014
K	101	2816	1589	7898	629	2465	2432
La	101	0.62	0.22	1.66	0.18	0.59	0.59
Li	101	0.98	0.39	2.69	0.26	0.93	0.91
Lu	101	0.0061	0.0023	0.016	0.0021	0.0057	0.0057
Mg	101	992	275	1833	516	954	956
Mn	101	29	10	73	11	27	28
Mo	101	0.095	0.074	0.59	0.036	0.076	0.084
Na	101	189	103	916	43	168	171
Nd	101	0.55	0.200	1.41	0.16	0.53	0.52
Ni	101	1.97	0.83	5.62	0.57	1.77	1.82
P	101	1240	387	2380	505	1218	1181
Pb	101	1.21	0.45	3.68	0.62	1.13	1.15
Pr	101	0.14	0.052	0.37	0.042	0.14	0.14

Table 5.1. Summary statistics of lichen samples collected in the winter field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Pt	41	0.0003	0.0002	0.0011	0.0001	0.0002	0.0003
Rb	101	5.45	4.08	24.28	0.80	4.16	4.50
Sb	101	0.15	0.13	1.16	0.049	0.123	0.13
Sc	101	0.79	0.31	1.92	0.311	0.741	0.73
Se	99	0.043	0.027	0.17	0.000	0.037	0.033
Sm	101	0.096	0.034	0.24	0.031	0.090	0.090
Sn	101	0.13	0.075	0.74	0.052	0.109	0.12
Sr	101	8.34	3.82	23.4	3.45	7.75	7.60
Tb	101	0.013	0.005	0.035	0.004	0.013	0.013
Th	101	0.17	0.064	0.49	0.055	0.161	0.16
Ti	101	108	43	307	23	98	101
Tl	101	0.058	0.066	0.39	0.008	0.035	0.040
Tm	101	0.0063	0.0023	0.017	0.0022	0.0059	0.0060
U	101	0.065	0.025	0.18	0.021	0.059	0.060
V	101	3.04	1.29	7.70	0.96	2.77	2.81
W	101	0.038	0.018	0.12	0.001	0.035	0.034
Y	101	0.46	0.17	1.22	0.15	0.43	0.44
Yb	101	0.041	0.015	0.11	0.014	0.039	0.039
Zn	101	19	8	64	6	18	18
$\delta^{15}\text{N}$	101	-9.72	3.12	0.40	-15.90	-9.64	
$\delta^{13}\text{C}$	101	-22.46	0.60	-20.77	-24.16	-22.41	

Table 5.2. Summary statistics of pine needle samples collected in the winter field campaign (in $\mu\text{g g}^{-1}$)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Al	14	362	410	1600	71	210	238
As	14	0.27	0.42	1.69	0.05	0.13	0.15
Ba	14	4.8	3.6	14.8	1.2	3.7	3.8
Be	7	0.010	0.011	0.032	0.000	0.008	0.004
Bi	14	0.004	0.004	0.014	0.001	0.003	0.003
Ca	14	6605	2552	11914	3785	6067	6187
Cd	14	0.019	0.029	0.092	0.003	0.007	0.010
Ce	14	0.26	0.28	1.06	0.05	0.15	0.18
Co	14	0.45	0.22	0.85	0.18	0.37	0.40
Cr	14	1.18	0.74	3.23	0.59	0.86	1.02
Cs	14	0.047	0.030	0.099	0.011	0.033	0.038
Cu	14	3.26	1.47	7.67	1.94	2.84	3.04
Dy	14	0.017	0.020	0.075	0.003	0.010	0.011
Er	14	0.010	0.011	0.044	0.002	0.005	0.006
Eu	14	0.006	0.006	0.024	0.001	0.004	0.004
Fe	14	241	278	1123	69	133	169
Gd	14	0.023	0.025	0.096	0.005	0.013	0.015
Ge	14	0.055	0.054	0.22	0.016	0.035	0.041
Hf	14	0.015	0.016	0.063	0.003	0.009	0.010
Hg	14	0.013	0.009	0.039	0.004	0.011	0.011
Ho	14	0.003	0.004	0.014	0.000	0.002	0.002
K	14	7298	2272	10220	2244	7928	6856
La	14	0.14	0.14	0.54	0.027	0.077	0.091
Li	14	0.34	0.26	0.93	0.055	0.23	0.26
Lu	14	0.0012	0.0016	0.0059	0.0001	0.0006	0.0007
Mg	14	1749	380	2324	862	1778	1703
Mn	14	57	53	209	8	38	38
Mo	14	0.040	0.046	0.16	0.004	0.021	0.025
Na	14	62	73	270	8	31	38
Nd	14	0.12	0.12	0.47	0.023	0.068	0.077
Ni	14	0.95	0.80	3.27	0.27	0.68	0.74
P	14	1206	231	1582	854	1172	1185
Pb	14	0.40	0.37	1.39	0.09	0.26	0.30
Pr	14	0.030	0.032	0.12	0.006	0.017	0.020

Table 5.2. Summary statistics of pine needle samples collected in the winter field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Rb	14	3.79	2.15	7.75	1.60	3.26	3.27
Sb	14	0.049	0.078	0.31	0.007	0.025	0.026
Sc	14	0.27	0.22	0.78	0.064	0.18	0.200
Se	6	0.019	0.022	0.061	0.003	0.010	0.011
Sm	14	0.021	0.023	0.087	0.004	0.011	0.014
Sn	14	0.052	0.079	0.31	0.006	0.026	0.029
Sr	14	13.89	10.31	37.69	1.79	8.65	10.78
Tb	14	0.003	0.003	0.013	0.001	0.002	0.002
Th	14	0.032	0.034	0.13	0.006	0.018	0.021
Ti	14	23	28	108	4	12	15
Tl	14	0.014	0.012	0.038	0.002	0.012	0.009
Tm	14	0.0014	0.0016	0.0061	0.0002	0.0008	0.0009
U	14	0.018	0.023	0.089	0.004	0.009	0.011
V	14	0.72	0.95	3.68	0.12	0.36	0.43
W	7	0.014	0.015	0.044	0.000	0.012	0.005
Y	14	0.10	0.11	0.42	0.02	0.05	0.07
Yb	14	0.009	0.011	0.041	0.001	0.005	0.006
Zn	14	23	7	34	9	22	22
$\delta^{15}\text{N}$	14	-27.22	2.06	-21.99	-29.12	-28.16	
$\delta^{13}\text{C}$	14	-3.30	3.43	3.29	-9.66	-2.79	

Table 5.3. Summary statistics of lichen samples collected in the summer field campaign (in $\mu\text{g g}^{-1}$)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Al	64	1471	777	3615	315	1295	1272
As	64	1.19	0.66	2.92	0.28	1.04	1.03
Ba	64	10.6	4.5	23.8	3.6	9.6	9.7
Be	64	0.030	0.014	0.074	0.008	0.026	0.027
Bi	64	0.018	0.026	0.20	0.003	0.012	0.013
Ca	64	2156	2097	14198	650	1497	1720
Cd	64	0.084	0.051	0.29	0.026	0.070	0.073
Ce	64	1.18	0.51	2.74	0.39	1.04	1.08
Co	64	0.565	0.30	1.49	0.18	0.48	0.50
Cr	64	3.85	2.08	8.89	1.10	3.19	3.36
Cs	64	0.179	0.128	0.81	0.052	0.15	0.15
Cu	64	4.51	1.38	9.32	2.16	4.29	4.33
Dy	64	0.079	0.035	0.180	0.027	0.070	0.072
Er	64	0.045	0.020	0.102	0.016	0.039	0.041
Eu	64	0.024	0.011	0.057	0.008	0.023	0.022
Fe	64	1020	490	2592	362	868	917
Gd	64	0.096	0.043	0.23	0.032	0.089	0.088
Ge	64	0.235	0.093	0.49	0.097	0.21	0.22
Hf	64	0.072	0.028	0.14	0.028	0.065	0.067
Hg	64	0.088	0.025	0.15	0.037	0.083	0.085
Ho	64	0.015	0.007	0.034	0.005	0.013	0.014
K	64	4530	1631	8711	472	4417	4149
La	64	0.59	0.262	1.46	0.19	0.52	0.54
Li	64	1.060	0.47	2.46	0.39	0.93	0.97
Lu	64	0.0060	0.0027	0.014	0.0020	0.0055	0.0055
Mg	64	1155	450	3062	321	1111	1067
Mn	64	28	9	55	12	25	26
Mo	64	0.12	0.11	0.65	0.041	0.096	0.10
Na	64	202	102	546	77	161	182
Nd	64	0.53	0.23	1.273	0.17	0.47	0.48
Ni	64	1.97	1.01	4.68	0.67	1.68	1.76
P	64	127	363	2688	303	1236	1211
Pb	64	1.12	0.51	2.63	0.29	0.98	1.01
Pr	64	0.14	0.061	0.33	0.045	0.12	0.13
Pt	32	0.0003	0.0002	0.0007	0.0001	0.0002	0.0003
Rb	64	7.00	3.93	16.62	2.10	5.65	6.02

Table 5.3. Summary statistics of lichen samples collected in the summer field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Sb	64	0.14	0.12	0.97	0.039	0.112	0.11
Sc	64	0.92	0.43	2.21	0.31	0.79	0.83
Se	61	0.037	0.016	0.074	0.001	0.035	0.032
Sm	64	0.098	0.044	0.23	0.032	0.089	0.089
Sn	64	0.13	0.056	0.34	0.057	0.12	0.12
Sr	64	7.80	3.95	20.92	2.80	6.65	7.03
Tb	64	0.014	0.006	0.031	0.005	0.012	0.012
Th	64	0.15	0.064	0.32	0.030	0.14	0.14
Ti	64	115	54	307	40	93	105
Tl	64	0.053	0.064	0.36	0.008	0.032	0.037
Tm	64	0.0063	0.0028	0.014	0.0024	0.0056	0.0058
U	64	0.059	0.024	0.12	0.019	0.055	0.054
V	64	3.01	1.46	7.79	1.03	2.52	2.71
W	64	0.041	0.022	0.12	0.013	0.033	0.036
Y	64	0.46	0.21	1.12	0.16	0.42	0.41
Yb	64	0.041	0.018	0.094	0.015	0.036	0.037
Zn	64	19	13	106	9	16	17
$\delta^{15}\text{N}$	64	-9.71	3.89	1.42	-18.14	-10.24	
$\delta^{13}\text{C}$	64	-22.48	0.67	-20.99	-23.96	-22.47	

Table 5.4. Summary statistics of pine needle samples collected in the summer field campaign (in $\mu\text{g g}^{-1}$)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Al	49	191	99	513	31	162	167
As	49	0.15	0.14	0.80	0.03	0.10	0.11
Ba	49	3.7	2.1	8.5	1.1	3.3	3.1
Be	24	0.002	0.002	0.007	0.000	0.002	0.002
Bi	49	0.003	0.003	0.014	0.000	0.002	0.002
Ca	49	6764	2357	11937	2272	6429	6328
Cd	49	0.010	0.014	0.082	0.001	0.005	0.006
Ce	49	0.16	0.07	0.36	0.04	0.16	0.15
Co	49	0.40	0.15	0.80	0.13	0.40	0.37
Cr	49	1.00	0.44	2.99	0.29	0.92	0.92
Cs	49	0.037	0.048	0.30	0.008	0.026	0.026
Cu	49	2.27	0.63	5.79	1.26	2.24	2.21
Dy	49	0.010	0.005	0.024	0.003	0.009	0.009
Er	49	0.005	0.003	0.014	0.001	0.005	0.005
Eu	49	0.004	0.002	0.008	0.001	0.004	0.003
Fe	49	147	67	343	31	131	132
Gd	49	0.014	0.007	0.031	0.003	0.013	0.012
Ge	49	0.044	0.019	0.096	0.012	0.040	0.040
Hf	49	0.009	0.005	0.023	0.002	0.008	0.008
Hg	49	0.037	0.013	0.070	0.014	0.037	0.035
Ho	49	0.002	0.001	0.004	0.000	0.002	0.002
K	49	5616	1434	9194	2459	5614	5439
La	49	0.083	0.038	0.18	0.021	0.081	0.075
Li	49	0.370	0.2	1.58	0.033	0.32	0.29
Lu	47	0.0006	0.0004	0.0019	0.0001	0.0005	0.0005
Mg	49	1394	435	2668	743	1316	1331
Mn	49	52	79	477	6	28	31
Mo	48	0.074	0.17	1.10	0.000	0.023	0.025
Na	49	82	113	683	4	50	42
Nd	49	0.073	0.034	0.16	0.017	0.074	0.065
Ni	49	0.63	0.26	1.54	0.16	0.60	0.58
P	49	867	189	1565	632	825	850
Pb	49	0.21	0.10	0.43	0.06	0.18	0.19
Pr	49	0.019	0.009	0.041	0.005	0.019	0.017
Rb	49	1.70	2.03	14.03	0.44	1.08	1.27

Table 5.4. Summary statistics of pine needle samples collected in the summer field campaign (Continued) (all in $\mu\text{g g}^{-1}$ except for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which are in ‰)

	N	Arithmetic Mean	Std	Max.	Min.	Median	Geo. Mean
Sb	49	0.028	0.021	0.089	0.003	0.022	0.021
Sc	49	0.20	0.094	0.45	0.032	0.18	0.18
Se	33	0.033	0.031	0.14	0.002	0.027	0.021
Sm	49	0.013	0.006	0.029	0.003	0.012	0.011
Sn	49	0.033	0.019	0.10	0.007	0.028	0.028
Sr	49	20.10	15.17	67.88	1.06	17.76	14.62
Tb	49	0.002	0.001	0.004	0.000	0.002	0.002
Th	46	0.017	0.011	0.053	0.000	0.013	0.012
Ti	49	14	7	36	2	11	12
Tl	49	0.016	0.024	0.14	0.000	0.008	0.008
Tm	49	0.0008	0.0004	0.0019	0.0002	0.0006	0.0007
U	49	0.010	0.006	0.031	0.002	0.009	0.009
V	49	0.38	0.21	1.06	0.06	0.31	0.32
W	32	0.010	0.017	0.073	0.001	0.004	0.005
Y	49	0.06	0.03	0.15	0.01	0.05	0.05
Yb	49	0.005	0.003	0.013	0.001	0.004	0.004
Zn	49	14	7	33	4	12	12
$\delta^{15}\text{N}$	49	0.03	2.85	6.24	-7.92	0.67	
$\delta^{13}\text{C}$	49	-28.09	1.35	-24.91	-30.56	-28.19	

4.4.2 Comparison of data with literature values

In order to show the pollution level of the study area, the data generated in this study was compared with the values reported in the literature. Though very extensive comparison could be very informative, we were confined the comparison to the parameters reported in the literature. More than 50 elements and stable carbon and nitrogen isotope ratios were measured in the collected samples in this study. There are only a few reports on lichen research that has used the natural abundance of ^{15}N and ^{13}C and integrated them with N and C monitoring to identify various sources in field experiments. It is also worth to mention that the comparison provided in Table 5.5 was performed by considering only the concentration values obtained for *Xanthoria parietina*. In other words, the results associated with pine needle samples were excluded from the comparison.

Lower concentrations of some of the elements (As, Cu, Fe, Mn, Ni, Pb, Tl, V and Zn) were obtained in this study as compared to the ones reported in the Kocaeli (Demiray et al., 2012). Kocaeli is one of the heavily industrialized cities in Turkey. Having the leading sectors in industry including chemicals and chemical products, basic metal industries and metal products industry, the share of industry in the Gross National Product (GNP) of Kocaeli is approximately 73 % (Hamzaoglu et al., 2011). Pollution levels are still elevated in the region though significant measures were taken to reduce and control the pollution (Karademir, 2006). Consequently, it is expected to observe relatively higher concentrations of anthropogenic related metals in Kocaeli as compared to ones obtained for Bolu plain.

Gür and Yaprak (2011) reported the highest concentrations of As, Cd, Cr, Cu, Fe, Ni, Se, Th, V, Zn and U among the Turkish stations used in the comparison in Table 5.5. It is very interesting to note that comparable concentrations of Hg was obtained in Soma (Gür and Yaprak, 2011), İzmir (Yenisoy-Karakaş and Tuncel, 2008) and in the summer field campaign in Bolu. Mercury is released to the atmosphere from the various natural (outgassing of the crustal material, water bodies, vegetation surfaces, wild fires, volcanoes and geothermal sources) and the anthropogenic (coal combustion, waste incineration, metal smelting, refining and manufacturing) sources (Schroeder and Munthe, 1998). The average Hg concentration in the summer and the winter field campaigns in Bolu were found as 0.088 ± 0.025 and $0.024 \pm 0.016 \mu\text{g g}^{-1}$, respectively. Since coal combustion due to domestic heating almost ceased in the summer season, this elevated concentration of Hg in Bolu in the summer can be attributed to natural emissions.

Yenisoy- Karakaş and Tuncel (2008) reported the highest concentrations of Yb, Ti, Sm, Sb, Rb, Na, Mo, Mg, Lu, La, Hf, Ba and Ce among the stations used in the comparison. Except for Sb and Mo, the other metals can be considered as pollutants released to the atmosphere mainly from earth crust.

Brunialti and Frati (2007) reported the lowest concentrations of Al and Fe among the stations used in the comparison as shown Table 5.5. The relationship of two terrigenous macro-elements (Al and Fe) confirmed the presence of

anthropogenic influence on their distribution in lichen material. Copper and Zn concentrations are higher than this study. A relationship between Cu and Zn is rather common in lichens and it may be due to the anthropogenic sources or metabolic interactions, since these two elements are essential for lichen metabolism.

Achotegui-Castells et al. (2013) collected lichen samples in Vilanova (Spain). The highest average concentration of Co, Cr and Ni reported in this study as compared to the ones presented in Table 5.5. On the other hand, As and Cd concentrations reported by Achotegui-Castells et al. (2013) were the lowest values among the others used in comparison. Another study of Ander Achotegui-Castells was performed in the Prades Mountains (Catalonia). These two studies were almost same concentrations as shown in Table 5.5. except Al element. Aluminum has the highest concentrations of all studies in the comparison table (Table 5.5).

Stable carbon and nitrogen isotope ratios measured in this study were also compared with the values reported in the literature and related comparison was provided in Figure 5.1. In terms of stable carbon isotope ratios, Bolu is characterized by the widest carbon isotope range as compared to other studies used in the comparison. In addition, ^{13}C is the most depleted in samples collected in Bolu both in summer and winter season. $\delta^{13}\text{C}$ values decrease with increasing pollution concentrations. Acid precipitation has been shown to result in a more negative $\delta^{13}\text{C}$ in plant tissue. Polluted area showed ^{13}C depletion signature as compared with the samples collected from the unpolluted area. Similar to stable carbon isotope ratio, samples collected in Bolu has the widest nitrogen isotope ratio. In addition, it can be also inferred from Figure 5.1 that samples collected in Bolu is more depleted in terms of ^{15}N as compared to samples collected in other studies given in Figure 5.1.

Table 5.5. Comparison of generated data in this study with the reported values in literature (in $\mu\text{g g}^{-1}$)

	This Study				Turkey						Italy		Spain			
	Summer(N=64)		Winter(N=101)		Kocaeli ^a		Soma ^b		Izmir ^c		Marche ^d		Vilanova ^e		Prades ^f	
	Arith. mean	Std	Arith. Mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std
Al	1471	777	1530	626	1513	787			3160	3720	1172	562	4222	269	4225	172
As	1.2	0.7	1.5	0.8	1.63	0.73	14.2	6.5	4.5	3			0.359	0.114	0.623	0.137
Ba	10.6	4.5	11.6	4.5					39	53						
Cd	0.084	0.051	0.098	0.053	0.5	0.29	0.46	0.26	0.3	0.62	0.2	0.17	0.052	0.002	0.114	0.052
Ce	1.2	0.5	1.2	0.4					2.6	3.1						
Co	0.565	0.296	0.557	0.224	1.06	0.54			0.94	1.1			2.25	0.706	0.584	0.091
Cr	3.8	2.1	3.3	1.3			19	8.3	9.9	29	2.7	1	132	44.8	14.3	4.82
Cu	4.5	1.4	5.4	3.4	15.26	5.27	17.57	7.58			9.04	4.05	4.21	0.986	7.36	1.07
Fe	1020	490	976	362	2828	1404	7531	2925	2360	5020	634	311				
Hf	0.072	0.028	0.068	0.027					0.28	0.36						
Hg	0.088	0.025	0.024	0.016	0.13	0.05	0.134	0.026	0.11	0.2						
K	4530	1631	2816	1589	4320	1380			4870	1780						
La	0.589	0.262	0.618	0.221					1.9	2.2						
Lu	0.006	0.003	0.006	0.002					0.023	0.027						
Mg	1155	450	992	275					1650	730						
Mn	27.7	9.0	29.3	9.8	88	52			60	150						
Mo	0.124	0.114	0.095	0.074					0.54	0.94						
Na	202	102	189	103					360	490						
Ni	2.0	1.0	2.0	0.8	6.03	2.82	15.7	7.5			3.05	2.83	70	23	8.28	2.57
Pb	1.1	0.5	1.2	0.5	41.18	35.33	23.99	9.7			2.43	1.85	1.99	0.624	2.57	0.21

Table 5.5. Comparison of generated data in this study with the reported values in literature (in $\mu\text{g g}^{-1}$) (Continued)

	This Study				Turkey						Italy		Spain			
	Summer (N=64)		Winter (N=101)		Kocaeli ^a		Soma ^b		Izmir ^c		Marche ^d		Vilanova ^e		Prades ^f	
	Arith. mean	Std	Arith. Mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std	Arith. mean	Std
Rb	7.0	3.9	5.4	4.1					13	12						
Sb	0.136	0.123	0.151	0.133					0.45	1.3			0.039	0.008	0.035	0.014
Sc	0.92	0.43	0.79	0.31					0.61	0.72						
Se	0.037	0.016	0.043	0.027			0.6	0.1	0.44	0.36						
Sm	0.098	0.044	0.096	0.034					0.28	0.3						
Th	0.152	0.064	0.169	0.064			1.68	1.51								
Ti	115	54	108	43	41	23			170	240			27.1	4.21	27.4	3.31
Tl	0.053	0.064	0.058	0.066	0.12	0.11										
U	0.059	0.024	0.065	0.025			2.24	1.62	0.23	0.34						
V	3.0	1.5	3.0	1.3	8	3.82	28	14	6.4	8.7	1.81	0.89	3.33	0.768	3.08	0.247
Yb	0.041	0.018	0.041	0.015					0.13	0.15						
Zn	18.9	13.0	19.0	7.9	167	80	115.8	52.9	100	410	47.8	26.9	29.8	3.22	26.8	1.82

^aDemiray et al. (2012), N=16^bGür and Yaprak (2011), N=44^cYenisoy-Karakaş and Tuncel (2008), N=120^dBrunialti and Frati (2007), N=51^eAchotegui-Castells et al. (2013), N=18-30 (no data provided in the article)^fAchotegui-Castells et al. (2013), N=18-30 (no data provided in the article)

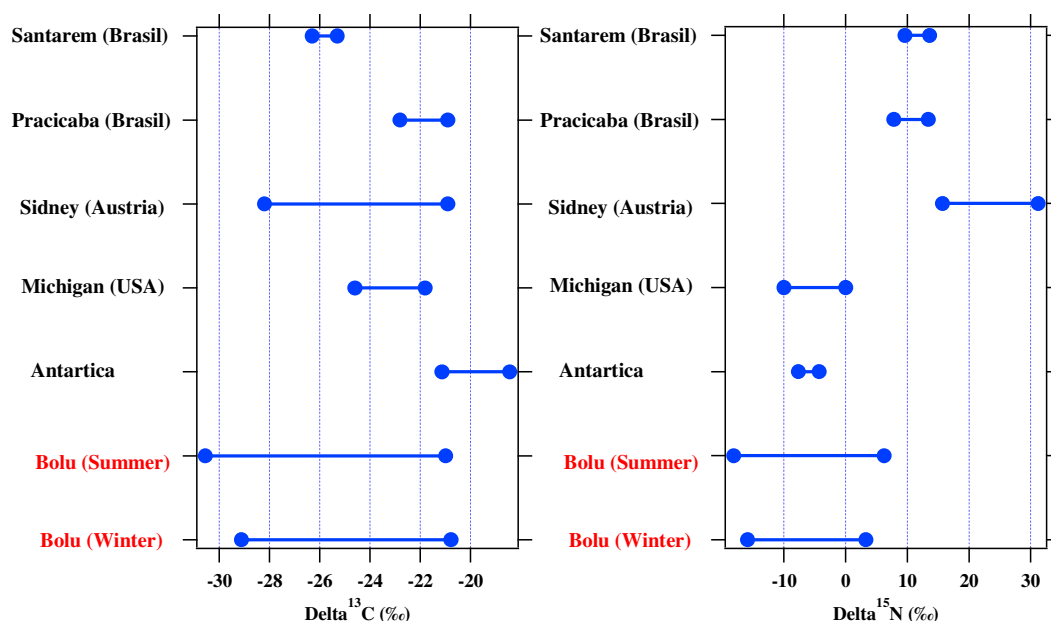


Figure 5.1. Comparison of the stable isotope ratios found in this study with the literature values

4.5 Temporal Variation of Biomonitoring Data

Performing biomonitoring study in two different seasons (winter and summer) enables us to evaluate the seasonal variation of pollutants associated with lichen and pine needle samples. Summer to winter ratios of parameters measured in this study was provided in Figure 5.2. The elements for which calculated summer to winter ratios are greater than one are Hg (2.91 ± 2.42), Sr (1.51 ± 1.96), K (1.49 ± 1.12), Ca (1.26 ± 1.91), Mo (1.25 ± 2.01), Mg (1.21 ± 0.65) and Mn (1.11 ± 1.72). For the rest of the metals measured in this study the calculated ratio is smaller than one. Potassium, Mn, Mo, Sr, Ca and Mg have mixed sources in the atmosphere. In other words, they are released to the atmosphere from anthropogenic and crustal sources (Öztürk et al., 2012). Consequently, higher summer concentrations of these three elements can be partly explained by their crustal sources. During summer, the soil is dry and these

elements are transported to the atmosphere on the soil particles by the action of wind. Mercury is released to the atmosphere from the various natural (outgassing of the crustal material, water bodies, vegetation surfaces, wild fires, volcanoes and geothermal sources) and the anthropogenic (coal combustion, waste incineration, metal smelting, refining and manufacturing) sources (Schroeder and Munthe, 1998). Higher summer concentration of Hg relative to winter concentration is an interesting finding in this study and this pattern observed for Hg could be either attributed to the natural source of Hg in summer season or the long range transport of particle bound Hg to the study area.

Summer to winter ratios less than one for the anthropogenic elements such as As, Sb, Ba, Cu and Pb is also an expected result. The origin of As in the atmosphere is coal combustion (Xie et al., 2006; Gür and Yaprak, 2011; Güllü et al., 1998; Karakaş et al., 2004; Yenisoy-Karakaş and Tuncel, 2008). Consequently, low summer concentration of this metal can be related to decrease of coal combustion for domestic heating in the summer season, which emits less As to the atmosphere. Antimony, Cu and Pb are released to the atmosphere from traffic emissions (Voutsas et al., 2002). Gietl et al. (2010) has already shown that Ba is one of the metals associated with brake wear and hence this metal has already traffic related source to the atmosphere. Since traffic is a mobile source in Bolu and its intensity does not depend on the season, the lower summer concentrations of this traffic related parameters cannot be explained by the seasonality of the source. The lower mixing layer height during winter season probably results in the accumulation of pollutants below the boundary layer, which could be a good explanation of high winter concentrations of these anthropogenic elements.

Summer and winter ratios for stable carbon and nitrogen isotope ratios were also evaluated in this study and the results are depicted in Figures 5.3 and 5.4, respectively. In both figures, the mean stable isotope ratio value was shown with the red star. Higher winter mean value for the stable carbon isotope ratio is due to the increased emissions from domestic heating and decreased rate of photosynthesis in winter season. Since emissions from domestic heating increase the carbon dioxide concentration and uptake of carbon dioxide by plants through photosynthesis is

slower in winter season, the ^{13}C enriches in the atmosphere during winter time. In contrast to stable carbon isotope ratio, the mean stable nitrogen isotope ratio is higher in summer season. Currently, 500 different broiler houses have been operated in Bolu and waste from these facilities have been applied over the land without taken any measures as fertilizer. Volatilization rate of ammonia from manure is expected to increase during summer season with increased temperature, which increases the nitrogen concentration in the atmosphere. As a result, the enriched ^{15}N during summer season can be attributed to the increase rate of nitrogen emissions from manure application over the land.

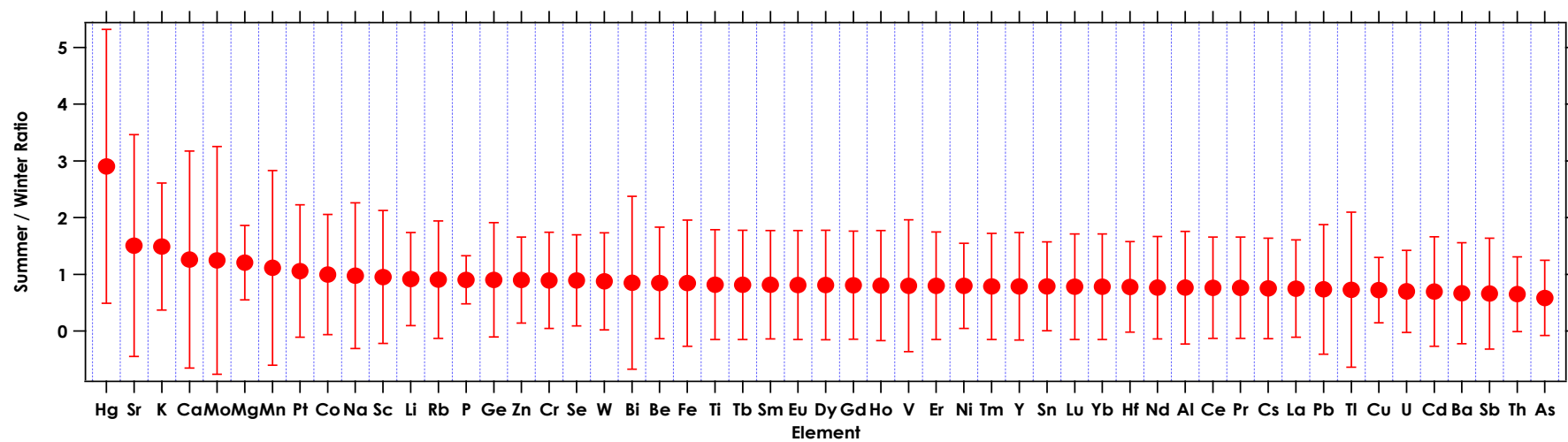


Figure 5.2. Summer to winter ratios of all elements (Average $\pm$ STD values are provided)

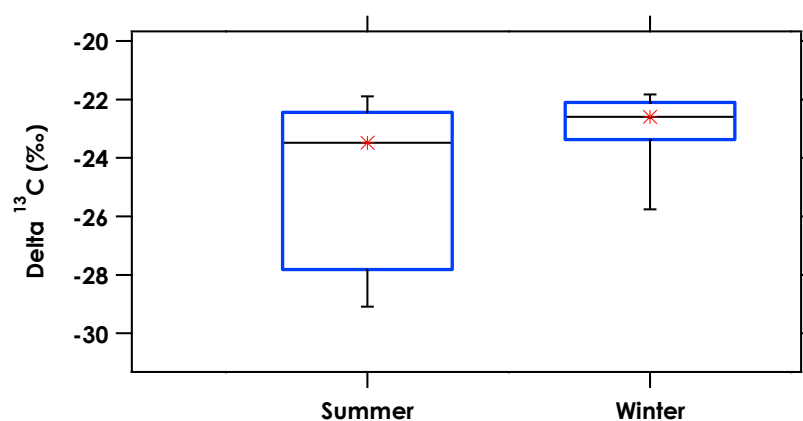


Figure 5.3. Comparison of stable carbon isotope ($\delta^{13}\text{C}$) measured in the summer and in the winter

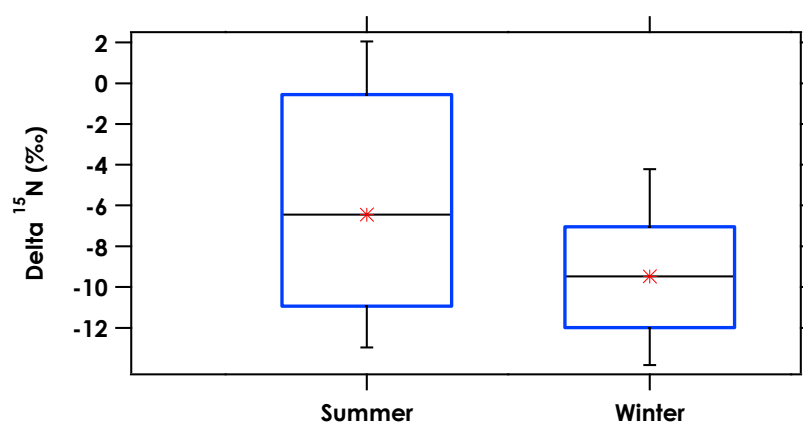


Figure 5.4. Comparison of stable nitrogen isotope ($\delta^{15}\text{N}$) measured in the summer and in the winter

4.6 Correlations Between the Measured Parameters

Correlation between the measured parameters can give very rough information about the sources of pollutants since the strong correlations between the pollutants either indicate that they are released from the same source or they have similar transport patterns in the atmosphere before reaching to the receptor site.

Binary correlation coefficients of the measured parameters both for summer and winter season were also calculated in this study and the results were presented in Table 5.6. and Table 5.7., respectively.

As expected crustal elements (Al, Ti, Cr, Fe, Sc and rare earth elements such as Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) were strongly correlated with each other ($r > 0.9$), implying that they were all crustal in origin. Correlations of V, Ni and Cr with purely crustal elements were also very strong ($r > 0.8$), which indicated that they had also a crustal source. Though Ca is also known as crust originated element, it had very low correlation with pure crustal elements and had moderate correlation ($r > 0.4$) with Mn, Co, Sr and Pb.

Poor correlation was obtained between Pb and other anthropogenic elements like As, Se, Sn and Cd. However, correlation of Pb with Co, Ni and Sr was relatively high, $r > 0.40$. In addition, Ca, Sc, Ti, V and Cr had correlation coefficients greater than 0.4 with Pb. The rare earth elements had also very good correlation with Pb, which implied that Pb transported to atmosphere on the soil particles.

It is interesting to note that anthropogenic elements such as As and Se, have no correlations at all though both of them are the well-known coal combustion markers (Shah et al., 2007; Li et al., 2014). On the other hand, As had moderate correlations with La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, W, and Ge. Contrary to this, Se had no correlation with these elements. Moderate correlation of As with rare earth elements (REE) (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Ho etc.) suggested that As was transported to the atmosphere on soil particles.

Table 5.6. Binary correlation matrix of elements in summer season ($p < 0.05$)

	Li	Be	Na	Mg	Al	P	K	Ca	Sc	Ti	V
Li	1.00										
Be	0.92	1.00									
Na	0.73	0.68	1.00								
Mg	0.15	0.14	0.31	1.00							
Al	0.90	0.90	0.84	0.30	1.00						
P	0.29	0.26	0.14	0.14	0.28	1.00					
K	-0.36	-0.32	-0.23	0.27	-0.36	0.32	1.00				
Ca	0.07	0.22	0.19	0.39	0.16	-0.35	-0.11	1.00			
Sc	0.88	0.89	0.87	0.32	0.98	0.21	-0.35	0.21	1.00		
Ti	0.94	0.96	0.81	0.18	0.97	0.32	-0.37	0.11	0.96	1.00	
V	0.83	0.82	0.90	0.34	0.96	0.22	-0.33	0.16	0.98	0.92	1.00
Cr	0.80	0.76	0.57	0.02	0.77	0.35	-0.35	0.00	0.73	0.81	0.68
Fe	0.86	0.84	0.89	0.31	0.98	0.24	-0.35	0.13	0.98	0.95	1.00
Mn	0.01	0.05	0.13	0.14	0.10	-0.07	-0.02	0.23	0.12	0.05	0.14
Co	0.72	0.79	0.78	0.41	0.85	0.05	-0.31	0.60	0.89	0.82	0.86
Ni	0.90	0.91	0.66	0.12	0.89	0.30	-0.40	0.21	0.85	0.93	0.80
Cu	0.77	0.75	0.69	0.09	0.82	0.50	-0.32	-0.08	0.78	0.83	0.80
Zn	0.55	0.62	0.53	0.24	0.60	0.37	-0.14	0.20	0.61	0.62	0.62
Ge	0.89	0.89	0.87	0.27	0.98	0.30	-0.34	0.11	0.98	0.97	0.98
As	0.69	0.56	0.43	-0.14	0.61	0.38	-0.37	-0.22	0.55	0.67	0.51
Se	0.15	0.04	0.10	-0.17	0.16	-0.10	-0.26	0.20	0.16	0.15	0.16
Rb	0.41	0.46	0.30	0.03	0.43	0.44	0.05	-0.24	0.40	0.48	0.37
Sr	0.23	0.30	0.24	0.36	0.26	-0.22	0.01	0.79	0.30	0.24	0.23
Y	0.91	0.92	0.79	0.19	0.97	0.28	-0.39	0.17	0.96	0.96	0.92
Mo	0.22	0.22	0.16	0.07	0.20	0.18	-0.02	0.11	0.19	0.23	0.17
Cd	0.67	0.74	0.64	0.06	0.76	0.15	-0.37	0.25	0.78	0.77	0.74
Sn	0.84	0.80	0.68	0.04	0.83	0.41	-0.40	-0.01	0.80	0.88	0.78
Sb	0.52	0.36	0.36	-0.02	0.46	0.28	-0.27	-0.13	0.43	0.51	0.42
Cs	0.57	0.56	0.40	-0.03	0.58	0.26	-0.27	-0.06	0.56	0.60	0.50
Ba	0.85	0.90	0.66	0.14	0.86	0.34	-0.30	0.24	0.83	0.88	0.76
La	0.90	0.96	0.69	0.09	0.92	0.28	-0.39	0.19	0.89	0.95	0.82
Ce	0.91	0.97	0.70	0.10	0.93	0.28	-0.39	0.21	0.90	0.95	0.83
Pr	0.91	0.97	0.71	0.11	0.93	0.27	-0.39	0.21	0.91	0.96	0.84
Nd	0.91	0.96	0.72	0.12	0.94	0.27	-0.39	0.22	0.92	0.96	0.85
Sm	0.92	0.96	0.75	0.13	0.95	0.27	-0.40	0.19	0.93	0.97	0.88
Eu	0.91	0.96	0.78	0.17	0.96	0.27	-0.39	0.22	0.95	0.97	0.90
Gd	0.91	0.95	0.76	0.15	0.96	0.26	-0.40	0.22	0.94	0.97	0.88
Tb	0.91	0.95	0.78	0.16	0.97	0.28	-0.40	0.19	0.95	0.97	0.90
Dy	0.91	0.94	0.79	0.17	0.97	0.28	-0.40	0.18	0.96	0.97	0.92
Ho	0.91	0.93	0.80	0.18	0.98	0.28	-0.39	0.17	0.96	0.97	0.93
Er	0.91	0.93	0.81	0.18	0.98	0.28	-0.39	0.16	0.97	0.97	0.93
Tm	0.91	0.92	0.81	0.18	0.98	0.29	-0.39	0.14	0.97	0.97	0.94
Yb	0.92	0.92	0.81	0.18	0.98	0.29	-0.39	0.13	0.96	0.98	0.93
Lu	0.92	0.92	0.81	0.18	0.98	0.29	-0.40	0.12	0.96	0.97	0.93
Hf	0.92	0.93	0.63	0.01	0.86	0.42	-0.38	-0.04	0.82	0.94	0.77
W	0.75	0.78	0.50	0.06	0.75	0.28	-0.30	0.08	0.71	0.79	0.66
Pt	0.76	0.88	0.46	0.25	0.66	-0.01	-0.25	0.60	0.62	0.76	0.50
Th	0.85	0.75	0.62	-0.02	0.82	0.40	-0.39	-0.26	0.76	0.85	0.73
Bi	0.49	0.37	0.35	-0.01	0.44	0.43	-0.23	-0.02	0.41	0.48	0.41
Hg	0.42	0.28	0.21	-0.20	0.34	0.31	-0.23	-0.26	0.31	0.42	0.30
Tl	0.30	0.21	0.18	-0.10	0.27	0.07	-0.24	-0.05	0.25	0.28	0.21
Pb	0.77	0.85	0.79	0.27	0.90	0.11	-0.35	0.43	0.93	0.88	0.90
U	0.90	0.95	0.66	0.10	0.89	0.39	-0.38	0.13	0.85	0.94	0.80

Table 5.6. Binary correlation matrix of elements in summer season ($p < 0.05$)

(Continued)

	Cr	Fe	Mn	Co	Ni	Cu	Zn	Ge	As	Se	Rb
Cr	1.00										
Fe	0.73	1.00									
Mn	-0.02	0.12	1.00								
Co	0.60	0.85	0.23	1.00							
Ni	0.87	0.83	0.09	0.78	1.00						
Cu	0.80	0.82	0.02	0.64	0.79	1.00					
Zn	0.45	0.62	0.11	0.61	0.55	0.58	1.00				
Ge	0.77	0.99	0.10	0.84	0.86	0.84	0.63	1.00			
As	0.78	0.56	-0.11	0.34	0.72	0.72	0.32	0.61	1.00		
Se	0.17	0.16	-0.08	0.29	0.17	0.13	0.11	0.17	0.22	1.00	
Rb	0.46	0.39	-0.08	0.25	0.43	0.49	0.23	0.45	0.46	-0.03	1.00
Sr	0.10	0.21	0.10	0.58	0.29	0.00	0.27	0.21	-0.09	0.22	-0.13
Y	0.81	0.94	0.06	0.84	0.91	0.83	0.57	0.96	0.67	0.19	0.44
Mo	0.17	0.19	-0.11	0.19	0.24	0.17	0.35	0.20	0.17	0.15	0.11
Cd	0.66	0.75	0.13	0.76	0.71	0.65	0.48	0.77	0.56	0.22	0.50
Sn	0.80	0.81	-0.04	0.64	0.85	0.86	0.52	0.84	0.73	0.23	0.42
Sb	0.52	0.44	-0.08	0.29	0.58	0.52	0.23	0.46	0.64	0.08	0.25
Cs	0.86	0.55	0.04	0.45	0.64	0.65	0.32	0.59	0.68	0.18	0.55
Ba	0.80	0.78	0.03	0.78	0.88	0.77	0.54	0.83	0.62	0.17	0.44
La	0.81	0.84	0.02	0.79	0.93	0.77	0.53	0.88	0.68	0.20	0.48
Ce	0.80	0.86	0.03	0.81	0.93	0.77	0.55	0.89	0.66	0.21	0.47
Pr	0.81	0.86	0.03	0.82	0.93	0.77	0.56	0.90	0.66	0.22	0.47
Nd	0.81	0.87	0.04	0.83	0.93	0.78	0.57	0.91	0.66	0.22	0.47
Sm	0.82	0.90	0.04	0.83	0.93	0.80	0.56	0.93	0.67	0.21	0.46
Eu	0.81	0.92	0.06	0.85	0.93	0.81	0.58	0.94	0.65	0.21	0.45
Gd	0.81	0.91	0.05	0.85	0.93	0.80	0.57	0.93	0.66	0.22	0.46
Tb	0.82	0.92	0.05	0.84	0.93	0.81	0.58	0.95	0.66	0.21	0.46
Dy	0.82	0.94	0.06	0.85	0.92	0.82	0.59	0.96	0.66	0.21	0.45
Ho	0.81	0.95	0.06	0.84	0.92	0.83	0.59	0.97	0.66	0.20	0.45
Er	0.81	0.95	0.06	0.84	0.92	0.83	0.59	0.97	0.67	0.20	0.45
Tm	0.82	0.96	0.06	0.83	0.91	0.84	0.59	0.97	0.67	0.19	0.45
Yb	0.82	0.95	0.06	0.83	0.92	0.84	0.58	0.97	0.68	0.18	0.45
Lu	0.82	0.95	0.10	0.82	0.91	0.84	0.59	0.97	0.68	0.21	0.45
Hf	0.85	0.81	-0.05	0.65	0.92	0.81	0.54	0.86	0.76	0.14	0.55
W	0.69	0.68	0.05	0.58	0.80	0.65	0.43	0.73	0.59	0.07	0.41
Pt	0.74	0.52	0.40	0.69	0.85	0.50	0.58	0.58	0.28	0.15	0.21
Th	0.81	0.78	-0.02	0.48	0.81	0.80	0.38	0.82	0.81	0.11	0.50
Bi	0.58	0.44	-0.01	0.34	0.49	0.55	0.69	0.48	0.52	0.20	0.13
Hg	0.50	0.32	-0.15	0.16	0.43	0.45	0.12	0.40	0.56	0.27	0.52
Tl	0.65	0.25	0.03	0.20	0.36	0.35	0.07	0.27	0.52	0.16	0.22
Pb	0.68	0.89	0.12	0.95	0.81	0.71	0.59	0.90	0.45	0.35	0.35
U	0.84	0.82	-0.01	0.76	0.94	0.82	0.55	0.88	0.73	0.21	0.50

Table 5.6. Binary correlation matrix of elements in summer season ($p < 0.05$)

(Continued)

	Sr	Y	Mo	Cd	Sn	Sb	Cs	Ba	La	Ce	Pr
Sr	1.00										
Y	0.27	1.00									
Mo	0.15	0.22	1.00								
Cd	0.30	0.81	0.13	1.00							
Sn	0.10	0.85	0.27	0.65	1.00						
Sb	-0.05	0.47	0.15	0.34	0.60	1.00					
Cs	0.02	0.64	0.08	0.62	0.59	0.33	1.00				
Ba	0.38	0.89	0.19	0.72	0.80	0.43	0.61	1.00			
La	0.32	0.96	0.23	0.80	0.85	0.47	0.63	0.92	1.00		
Ce	0.34	0.97	0.23	0.81	0.84	0.46	0.62	0.93	1.00	1.00	
Pr	0.34	0.97	0.23	0.81	0.84	0.46	0.63	0.93	1.00	1.00	1.00
Nd	0.34	0.97	0.23	0.81	0.84	0.46	0.63	0.92	1.00	1.00	1.00
Sm	0.31	0.99	0.23	0.81	0.86	0.47	0.63	0.92	0.99	0.99	1.00
Eu	0.33	0.99	0.22	0.82	0.85	0.46	0.62	0.93	0.98	0.99	0.99
Gd	0.33	0.99	0.22	0.82	0.85	0.46	0.63	0.92	0.99	0.99	0.99
Tb	0.30	0.99	0.22	0.82	0.86	0.47	0.63	0.91	0.98	0.98	0.99
Dy	0.28	1.00	0.22	0.82	0.86	0.47	0.63	0.90	0.97	0.97	0.98
Ho	0.27	1.00	0.22	0.81	0.86	0.47	0.63	0.89	0.96	0.97	0.97
Er	0.26	1.00	0.22	0.81	0.86	0.47	0.63	0.89	0.96	0.97	0.97
Tm	0.24	1.00	0.22	0.80	0.87	0.48	0.63	0.88	0.95	0.96	0.96
Yb	0.24	1.00	0.21	0.80	0.87	0.49	0.63	0.88	0.95	0.96	0.96
Lu	0.23	0.99	0.21	0.79	0.87	0.49	0.65	0.88	0.95	0.95	0.96
Hf	0.12	0.89	0.24	0.68	0.89	0.56	0.65	0.88	0.93	0.93	0.92
W	0.15	0.76	0.17	0.55	0.74	0.40	0.51	0.74	0.79	0.79	0.79
Pt	0.73	0.69	0.05	0.62	0.69	0.17	0.44	0.85	0.84	0.84	0.84
Th	-0.09	0.84	0.21	0.57	0.85	0.53	0.65	0.74	0.82	0.81	0.81
Bi	0.06	0.45	0.28	0.31	0.51	0.24	0.47	0.46	0.41	0.42	0.43
Hg	-0.13	0.43	0.11	0.42	0.53	0.28	0.50	0.42	0.46	0.45	0.45
Tl	-0.02	0.35	0.00	0.37	0.32	0.15	0.82	0.37	0.34	0.32	0.33
Pb	0.45	0.91	0.20	0.85	0.74	0.34	0.55	0.83	0.88	0.89	0.90
U	0.25	0.92	0.23	0.73	0.88	0.53	0.65	0.92	0.95	0.95	0.95

Table 5.6. Binary correlation matrix of elements in summer season ($p < 0.05$)

(Continued)

	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb
Nd	1.00									
Sm	1.00	1.00								
Eu	0.99	1.00	1.00							
Gd	1.00	1.00	1.00	1.00						
Tb	0.99	1.00	1.00	1.00	1.00					
Dy	0.98	0.99	0.99	0.99	1.00	1.00				
Ho	0.98	0.99	0.99	0.99	1.00	1.00	1.00			
Er	0.97	0.99	0.99	0.99	1.00	1.00	1.00	1.00		
Tm	0.97	0.98	0.99	0.98	0.99	1.00	1.00	1.00	1.00	
Yb	0.97	0.98	0.99	0.98	0.99	1.00	1.00	1.00	1.00	1.00
Lu	0.96	0.98	0.98	0.98	0.99	0.99	1.00	1.00	1.00	1.00
Hf	0.92	0.92	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.91
W	0.78	0.79	0.78	0.79	0.78	0.78	0.77	0.77	0.77	0.78
Pt	0.82	0.80	0.79	0.79	0.76	0.73	0.70	0.70	0.68	0.69
Th	0.81	0.83	0.81	0.82	0.83	0.84	0.84	0.84	0.85	0.86
Bi	0.44	0.44	0.45	0.44	0.46	0.46	0.46	0.47	0.47	0.47
Hg	0.45	0.45	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Tl	0.33	0.33	0.33	0.34	0.34	0.34	0.33	0.34	0.34	0.34
Pb	0.90	0.91	0.92	0.92	0.92	0.92	0.92	0.91	0.91	0.90
U	0.95	0.95	0.94	0.94	0.94	0.93	0.93	0.92	0.92	0.92

	Yb	Lu	Hf	W	Pt	Th	Bi	Hg	Tl	Pb	U
Yb	1.00										
Lu	1.00	1.00									
Hf	0.91	0.90	1.00								
W	0.78	0.77	0.83	1.00							
Pt	0.69	0.68	0.88	0.79	1.00						
Th	0.86	0.87	0.90	0.71	0.39	1.00					
Bi	0.47	0.48	0.52	0.38	0.61	0.46	1.00				
Hg	0.44	0.44	0.56	0.41	-0.06	0.54	0.30	1.00			
Tl	0.34	0.36	0.35	0.23	0.20	0.41	0.39	0.41	1.00		
Pb	0.90	0.90	0.74	0.66	0.67	0.58	0.37	0.33	0.29	1.00	
U	0.92	0.92	0.96	0.82	0.88	0.82	0.50	0.52	0.36	0.83	1.00

Table 5.7. Binary correlation matrix of elements in winter season ($p < 0.05$)

	Li	Be	Na	Mg	Al	P	K	Ca	Sc	Ti	V
Li	1.00										
Be	0.82	1.00									
Na	0.64	0.68	1.00								
Mg	-0.12	-0.03	-0.12	1.00							
Al	0.93	0.86	0.79	-0.15	1.00						
P	0.24	0.17	0.17	0.52	0.22	1.00					
K	-0.24	-0.11	-0.28	0.86	-0.30	0.50	1.00				
Ca	-0.18	0.01	-0.09	0.02	-0.16	-0.28	0.07	1.00			
Sc	0.85	0.86	0.73	-0.13	0.92	0.18	-0.26	0.07	1.00		
Ti	0.92	0.87	0.80	-0.17	0.98	0.20	-0.31	-0.11	0.94	1.00	
V	0.87	0.78	0.68	-0.17	0.91	0.24	-0.29	-0.06	0.92	0.92	1.00
Cr	0.87	0.72	0.53	-0.19	0.84	0.13	-0.32	-0.13	0.80	0.83	0.80
Fe	0.93	0.79	0.73	-0.16	0.97	0.26	-0.31	-0.17	0.92	0.96	0.94
Mn	-0.04	0.17	0.00	0.25	0.00	-0.06	0.16	0.45	0.10	-0.01	0.00
Co	0.52	0.55	0.42	-0.08	0.54	-0.03	-0.13	0.65	0.70	0.57	0.62
Ni	0.84	0.74	0.57	-0.18	0.82	0.12	-0.28	0.16	0.86	0.82	0.84
Cu	0.34	0.27	0.39	-0.10	0.38	0.15	-0.17	-0.07	0.39	0.37	0.45
Zn	0.12	0.25	0.17	0.24	0.15	0.19	0.18	0.11	0.17	0.13	0.17
Ge	0.89	0.75	0.71	-0.16	0.94	0.26	-0.30	-0.14	0.92	0.94	0.93
As	0.55	0.41	0.52	-0.19	0.62	0.32	-0.28	-0.20	0.55	0.62	0.58
Se	-0.14	-0.26	-0.21	-0.05	-0.10	0.03	0.00	-0.13	-0.06	-0.08	-0.02
Rb	0.18	0.18	0.09	0.35	0.18	0.48	0.40	0.00	0.20	0.21	0.16
Sr	0.17	0.49	0.28	0.18	0.22	-0.04	0.16	0.52	0.35	0.22	0.23
Y	0.87	0.80	0.65	-0.19	0.92	0.19	-0.31	-0.25	0.88	0.91	0.89
Mo	0.24	0.17	0.21	-0.22	0.27	0.02	-0.22	-0.04	0.30	0.27	0.27
Cd	0.25	0.18	0.17	-0.33	0.27	-0.01	-0.38	0.09	0.28	0.30	0.30
Sn	0.37	0.24	0.42	-0.32	0.35	-0.01	-0.34	0.04	0.36	0.38	0.41
Sb	0.32	0.17	0.22	-0.19	0.31	0.07	-0.23	-0.04	0.33	0.35	0.40
Cs	0.50	0.51	0.27	-0.15	0.50	0.01	-0.21	0.02	0.51	0.51	0.43
Ba	0.29	0.66	0.41	-0.14	0.40	-0.06	-0.17	0.33	0.52	0.40	0.39
La	0.86	0.91	0.76	-0.24	0.94	0.13	-0.36	-0.19	0.89	0.93	0.85
Ce	0.87	0.91	0.76	-0.23	0.94	0.14	-0.36	-0.21	0.89	0.93	0.86
Pr	0.88	0.90	0.74	-0.23	0.95	0.14	-0.36	-0.21	0.89	0.94	0.86
Nd	0.88	0.89	0.73	-0.23	0.95	0.14	-0.35	-0.21	0.90	0.94	0.87
Sm	0.88	0.85	0.71	-0.23	0.94	0.15	-0.36	-0.24	0.89	0.93	0.89
Eu	0.81	0.90	0.74	-0.21	0.91	0.12	-0.33	-0.10	0.90	0.90	0.86
Gd	0.84	0.89	0.70	-0.23	0.92	0.12	-0.35	-0.15	0.90	0.91	0.88
Tb	0.87	0.84	0.70	-0.22	0.94	0.16	-0.34	-0.23	0.90	0.93	0.90
Dy	0.88	0.83	0.68	-0.21	0.94	0.17	-0.34	-0.24	0.90	0.92	0.90
Ho	0.88	0.82	0.68	-0.20	0.94	0.18	-0.33	-0.24	0.89	0.92	0.91
Er	0.88	0.81	0.67	-0.20	0.94	0.19	-0.33	-0.26	0.89	0.93	0.91
Tm	0.89	0.81	0.68	-0.18	0.94	0.20	-0.32	-0.26	0.89	0.93	0.91
Yb	0.89	0.81	0.67	-0.19	0.94	0.20	-0.32	-0.27	0.89	0.93	0.91
Lu	0.90	0.81	0.68	-0.19	0.95	0.20	-0.32	-0.23	0.91	0.94	0.92
Hf	0.90	0.82	0.76	-0.21	0.94	0.16	-0.32	-0.21	0.86	0.94	0.83
W	0.75	0.73	0.50	-0.04	0.76	0.18	-0.05	0.01	0.77	0.81	0.76
Pt	0.37	0.46	0.58	-0.02	0.55	-0.18	-0.10	0.24	0.53	0.55	0.43
Th	0.89	0.87	0.76	-0.22	0.95	0.16	-0.34	-0.25	0.89	0.95	0.86
Bi	0.44	0.42	0.28	-0.34	0.49	-0.12	-0.38	0.02	0.50	0.47	0.47
Hg	0.22	0.19	0.19	-0.30	0.19	-0.13	-0.33	0.02	0.11	0.17	0.14
Tl	0.25	0.22	0.00	-0.15	0.21	-0.09	-0.20	-0.12	0.16	0.19	0.12
Pb	0.38	0.65	0.34	-0.31	0.45	-0.21	-0.33	0.44	0.59	0.47	0.49
U	0.86	0.85	0.73	-0.26	0.93	0.15	-0.37	-0.04	0.89	0.92	0.89

Table 5.7. Binary correlation matrix of elements in winter season ($p < 0.05$)
(Continued)

	Cr	Fe	Mn	Co	Ni	Cu	Zn	Ge	As	Se	Rb
Cr	1.00										
Fe	0.86	1.00									
Mn	-0.04	-0.06	1.00								
Co	0.51	0.55	0.36	1.00							
Ni	0.85	0.84	0.15	0.73	1.00						
Cu	0.30	0.45	-0.05	0.26	0.32	1.00					
Zn	0.03	0.13	0.10	0.25	0.14	0.35	1.00				
Ge	0.82	0.98	-0.04	0.56	0.82	0.45	0.17	1.00			
As	0.53	0.62	-0.18	0.26	0.52	0.28	0.18	0.61	1.00		
Se	-0.14	-0.06	-0.12	-0.13	-0.11	-0.10	-0.05	0.04	0.00	1.00	
Rb	0.11	0.18	0.01	0.13	0.11	0.04	0.18	0.22	0.13	0.07	1.00
Sr	0.07	0.13	0.36	0.56	0.27	0.07	0.39	0.15	-0.01	-0.14	0.14
Y	0.83	0.93	-0.02	0.46	0.76	0.37	0.14	0.92	0.56	-0.02	0.16
Mo	0.31	0.29	-0.09	0.16	0.29	0.11	-0.05	0.28	0.27	-0.02	-0.03
Cd	0.31	0.29	-0.04	0.31	0.31	0.16	0.07	0.29	0.31	-0.01	0.26
Sn	0.35	0.40	-0.11	0.31	0.45	0.37	0.06	0.39	0.30	-0.15	-0.05
Sb	0.37	0.39	-0.12	0.24	0.48	0.26	0.14	0.38	0.38	0.02	-0.03
Cs	0.67	0.49	0.07	0.36	0.46	0.08	-0.09	0.47	0.37	-0.07	0.26
Ba	0.28	0.31	0.32	0.51	0.40	0.16	0.23	0.32	0.13	-0.21	0.15
La	0.81	0.89	0.02	0.48	0.76	0.32	0.14	0.87	0.58	-0.09	0.14
Ce	0.80	0.90	0.02	0.47	0.75	0.32	0.14	0.88	0.57	-0.07	0.15
Pr	0.82	0.91	0.01	0.47	0.76	0.32	0.14	0.89	0.58	-0.06	0.15
Nd	0.83	0.91	0.01	0.48	0.77	0.33	0.14	0.90	0.58	-0.06	0.15
Sm	0.83	0.92	-0.01	0.47	0.77	0.35	0.12	0.91	0.58	-0.05	0.13
Eu	0.76	0.87	0.09	0.54	0.75	0.35	0.18	0.86	0.53	-0.08	0.15
Gd	0.81	0.89	0.06	0.53	0.78	0.34	0.14	0.87	0.55	-0.08	0.13
Tb	0.83	0.93	0.00	0.48	0.78	0.36	0.12	0.91	0.57	-0.05	0.13
Dy	0.84	0.93	-0.01	0.47	0.78	0.37	0.12	0.92	0.58	-0.04	0.14
Ho	0.84	0.93	-0.02	0.48	0.78	0.37	0.12	0.92	0.58	-0.05	0.14
Er	0.85	0.94	-0.02	0.47	0.78	0.38	0.11	0.92	0.59	-0.05	0.14
Tm	0.85	0.94	-0.03	0.46	0.78	0.38	0.12	0.92	0.58	-0.05	0.15
Yb	0.85	0.95	-0.04	0.45	0.78	0.38	0.11	0.93	0.59	-0.05	0.14
Lu	0.86	0.96	-0.02	0.56	0.82	0.39	0.12	0.94	0.59	-0.05	0.17
Hf	0.79	0.90	-0.03	0.49	0.76	0.30	0.15	0.88	0.58	-0.05	0.14
W	0.76	0.77	0.00	0.52	0.81	0.19	0.17	0.75	0.47	-0.05	0.14
Pt	0.21	0.44	0.35	0.49	0.36	0.09	0.13	0.52	0.18	-0.01	0.10
Th	0.81	0.91	-0.02	0.51	0.78	0.30	0.14	0.90	0.59	-0.07	0.17
Bi	0.68	0.50	-0.03	0.38	0.47	0.14	-0.05	0.49	0.35	-0.14	-0.03
Hg	0.37	0.18	-0.06	0.15	0.24	0.07	-0.22	0.06	0.19	-0.37	-0.14
Tl	0.50	0.21	-0.03	0.05	0.15	-0.03	-0.22	0.15	0.10	-0.13	-0.04
Pb	0.42	0.39	0.29	0.66	0.54	0.15	0.15	0.40	0.15	-0.07	0.08
U	0.81	0.91	0.00	0.62	0.83	0.34	0.15	0.89	0.64	-0.13	0.10

Table 5.7. Binary correlation matrix of elements in winter season ($p < 0.05$)

(Continued)

	Sr	Y	Mo	Cd	Sn	Sb	Cs	Ba	La	Ce	Pr
Sr	1.00										
Y	0.17	1.00									
Mo	-0.04	0.27	1.00								
Cd	0.04	0.25	0.12	1.00							
Sn	0.01	0.34	0.21	0.14	1.00						
Sb	-0.05	0.31	0.24	0.19	0.42	1.00					
Cs	0.12	0.51	0.24	0.37	0.09	0.04	1.00				
Ba	0.69	0.38	0.11	0.15	0.19	0.11	0.39	1.00			
La	0.26	0.95	0.27	0.25	0.34	0.28	0.54	0.51	1.00		
Ce	0.26	0.95	0.27	0.25	0.34	0.29	0.53	0.51	1.00	1.00	
Pr	0.24	0.96	0.27	0.25	0.34	0.28	0.54	0.49	1.00	1.00	1.00
Nd	0.23	0.97	0.27	0.25	0.34	0.29	0.54	0.47	0.99	1.00	1.00
Sm	0.20	0.98	0.29	0.25	0.35	0.31	0.52	0.43	0.98	0.98	0.99
Eu	0.38	0.94	0.27	0.24	0.35	0.28	0.53	0.65	0.97	0.97	0.97
Gd	0.30	0.96	0.29	0.25	0.35	0.31	0.54	0.56	0.98	0.98	0.98
Tb	0.20	0.99	0.29	0.25	0.35	0.32	0.52	0.43	0.97	0.97	0.98
Dy	0.18	0.99	0.29	0.25	0.35	0.33	0.52	0.41	0.96	0.97	0.97
Ho	0.18	0.99	0.29	0.25	0.35	0.33	0.51	0.40	0.96	0.96	0.97
Er	0.16	0.99	0.29	0.25	0.35	0.33	0.51	0.38	0.95	0.95	0.97
Tm	0.15	0.99	0.29	0.25	0.35	0.34	0.51	0.38	0.95	0.95	0.96
Yb	0.14	0.99	0.29	0.25	0.35	0.35	0.51	0.36	0.95	0.95	0.96
Lu	0.17	0.99	0.30	0.30	0.36	0.35	0.55	0.36	0.94	0.94	0.95
Hf	0.19	0.88	0.27	0.24	0.39	0.36	0.50	0.38	0.93	0.93	0.93
W	0.21	0.72	0.23	0.17	0.31	0.57	0.41	0.34	0.75	0.75	0.75
Pt	0.43	0.52	0.14	0.16	0.30	0.01	0.08	0.27	0.59	0.61	0.60
Th	0.20	0.93	0.27	0.27	0.36	0.30	0.54	0.42	0.97	0.97	0.97
Bi	0.07	0.58	0.31	0.28	0.39	0.27	0.72	0.36	0.56	0.55	0.56
Hg	0.00	0.14	0.15	0.22	0.23	0.07	0.41	0.19	0.23	0.21	0.20
Tl	-0.09	0.27	0.18	0.19	0.02	-0.03	0.73	0.13	0.29	0.27	0.28
Pb	0.61	0.47	0.17	0.31	0.32	0.21	0.49	0.87	0.56	0.55	0.54
U	0.27	0.88	0.29	0.31	0.42	0.39	0.48	0.44	0.91	0.90	0.91

	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb
Nd	1.00									
Sm	0.99	1.00								
Eu	0.97	0.96	1.00							
Gd	0.98	0.98	0.99	1.00						
Tb	0.99	1.00	0.96	0.99	1.00					
Dy	0.98	0.99	0.95	0.98	1.00	1.00				
Ho	0.98	0.99	0.95	0.98	1.00	1.00	1.00			
Er	0.97	0.99	0.94	0.97	1.00	1.00	1.00	1.00		
Tm	0.97	0.99	0.94	0.97	0.99	1.00	1.00	1.00	1.00	
Yb	0.97	0.98	0.93	0.96	0.99	1.00	1.00	1.00	1.00	1.00
Lu	0.96	0.98	0.92	0.95	0.99	0.99	0.99	1.00	1.00	1.00
Hf	0.93	0.91	0.88	0.89	0.90	0.90	0.89	0.89	0.90	0.90
W	0.76	0.75	0.72	0.74	0.75	0.75	0.75	0.75	0.75	0.76
Pt	0.58	0.56	0.56	0.52	0.53	0.52	0.50	0.49	0.48	0.48
Th	0.97	0.96	0.91	0.93	0.95	0.94	0.94	0.94	0.94	0.94
Bi	0.56	0.58	0.58	0.60	0.58	0.58	0.58	0.57	0.57	0.56
Hg	0.20	0.22	0.21	0.25	0.20	0.19	0.19	0.19	0.18	0.19
Tl	0.28	0.29	0.25	0.29	0.27	0.28	0.28	0.28	0.27	0.28
Pb	0.54	0.51	0.67	0.62	0.51	0.50	0.49	0.47	0.46	0.45
U	0.91	0.91	0.89	0.91	0.91	0.90	0.90	0.90	0.90	0.90

Table 5.7. Binary correlation matrix of elements in winter season ($p < 0.05$)

(Continued)

	Lu	Hf	W	Pt	Th	Bi	Hg	Tl	Pb	U
Lu	1.00									
Hf	0.90	1.00								
W	0.76	0.77	1.00							
Pt	0.48	0.64	0.29	1.00						
Th	0.94	0.95	0.78	0.58	1.00					
Bi	0.56	0.51	0.45	0.08	0.52	1.00				
Hg	0.18	0.17	0.15	-0.16	0.17	0.45	1.00			
Tl	0.27	0.24	0.20	-0.17	0.26	0.70	0.48	1.00		
Pb	0.48	0.44	0.44	0.32	0.51	0.53	0.26	0.24	1.00	
U	0.91	0.91	0.78	0.51	0.91	0.55	0.25	0.23	0.53	1.00

4.7 Comparison of Bioaccumulation Capacity of Collected Species

Two well-known mechanisms by means of which lichens accumulate elements from the atmosphere are accumulation at ion exchange sites and particulate trapping. Because of having very different morphology and eco-physiology, lichen species have very different sensitivity to elements and different accumulation capacity accordingly (Malaspina et al., 2014). For example, fruticose lichens, particularly pendulous *Usnea* species, are more dependent on wet deposition for particle trapping while foliose *Parmelia* accumulates more particles from dry deposition (Bergamaschi et al., 2007). Nimis et al. (2007) have found in NE Italy that relevant differences were observed in the concentrations of Al, Fe, Cd and Zn between *Flavoparmelia caperata* and *Xanthoria parietina*, which are two foliose species with a distinct eco-physiology. On the other hand, Minganti et al. (2003) showed that specific differences present within the genus *Parmelia* in the accumulation capacity of Mn, Pb, V and Zn.

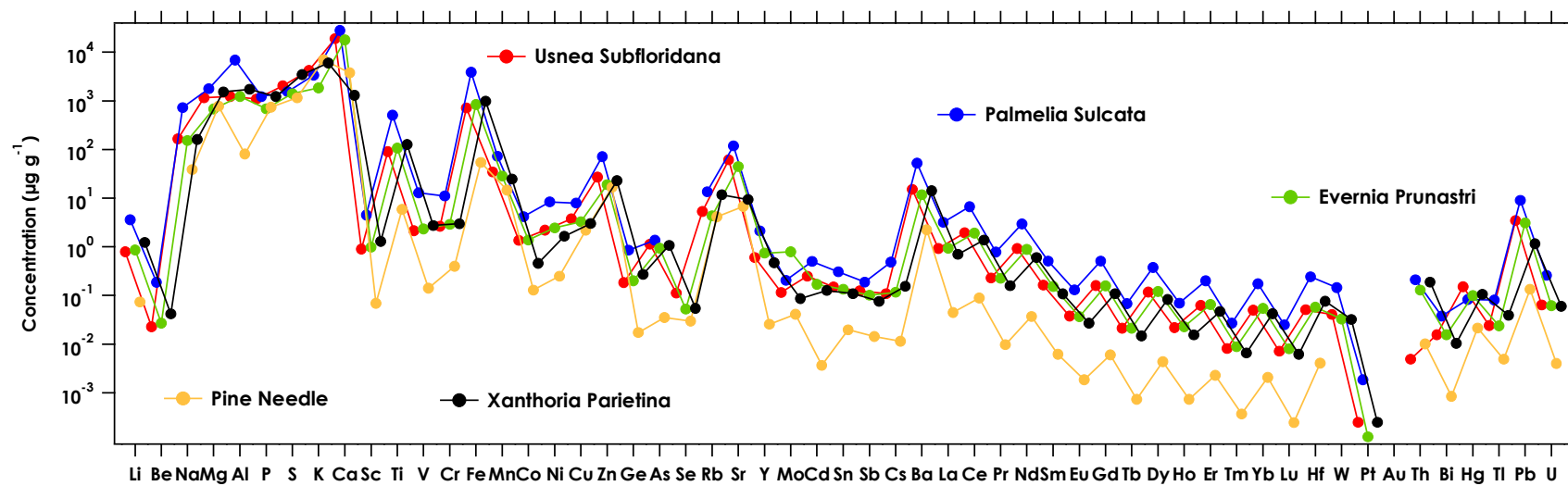
Elemental compositions of different lichen and pine needle samples collected in Gölcük (40.6548°N, 31.62535°E) were also investigated in this study and results were displayed in Figure 5.5. It is clear from Figure 5.5 that pine needle samples showed completely different composition in terms of most of the elements analyzed in the study while elemental composition was comparable for lichen species. The seasonal accumulation capacity of lichen and pine needle samples was also compared

in this study and the results were depicted in Figures 5.6 and 5.7, for winter and summer, respectively.

In order to have an idea about the respective accumulation capacity of the collected samples, the ratio of elemental composition of the lichen samples to that of pine needle samples was calculated. For winter season, the elements can be grouped into three different classes in terms of the accumulation capacity. The elements from As to Fe have the ratio between 4 and 6 in Figure 5.6., which indicated significantly higher uptake rate of this group of elements in lichen samples as compared to pine needle samples during the winter season. The elements from Cs to Ni in Figure 5.6 have lichen to pine needle ratio between 2 and 4, which again implied that the accumulation capacity of lichen samples was significantly higher as compared to pine needle samples in term of the elements in this group. For the last group of elements (Hg, Cu, Rb, Co, P, Zn, Sr, Mn, Mg, Ca and K) in Figure 5.6, the calculated lichen to pine needle ratio is between 0.38 and 2. Phosphorus, Zn, Sr, Mn, Mg, Ca and K have lichen to pine needle ratio below 1, implying that the relatively higher accumulation capacity for these elements in pine needle samples as compared to lichens. Phosphorus may come from the plant structure and it might not be related with the pollution (Pike, 1978; Motiejunaite et al., 2014).

Similar to winter season, in the summer season the significantly higher accumulation capacity was observed for lichen samples as compared to pine needle samples in terms of most of the elements analyzed in the study (Figure 5.7). Different from the winter, the relatively higher lichen to pine needle ratios were obtained in the summer season. Again, it is possible to interpret the accumulation capacities of elements for the summer season based on the calculated lichen to pine needle ratios. Beryllium and Lu were in the first group, which had lichen to pine needle ratio greater than 10. In the second group, including elements from Cd to Sc, the calculated ratio was between 5 and 10. For the last group of elements, the ratio was below 5. Magnesium, K, Mn, Sr and Ca in this last group have ratio below 1, suggesting that accumulation capacity of pine needle samples in terms of these elements was higher than that of lichen samples. Consequently, it could be concluded

that pine needle samples had higher accumulation capacities for Mg, K, Mn, Sr and Ca both in the summer and in the winter season.



69 **Figure 5.5.** Comparison of lichen and pine needle samples in terms of elemental composition in Gölcük (40.6548°N, 31.62535°E), Bolu

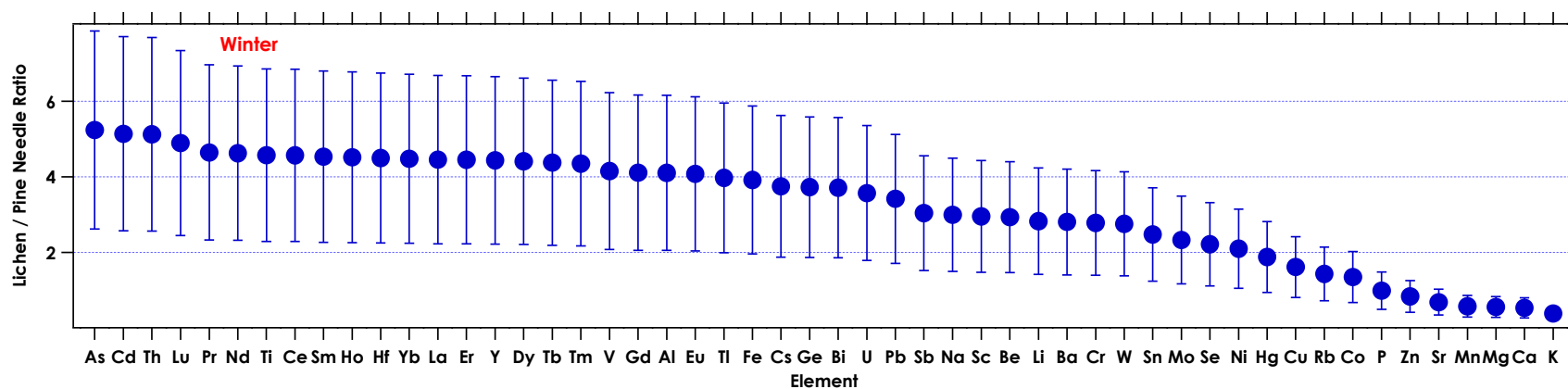


Figure 5.6. Accumulation capacities of elements calculated for the winter field campaign

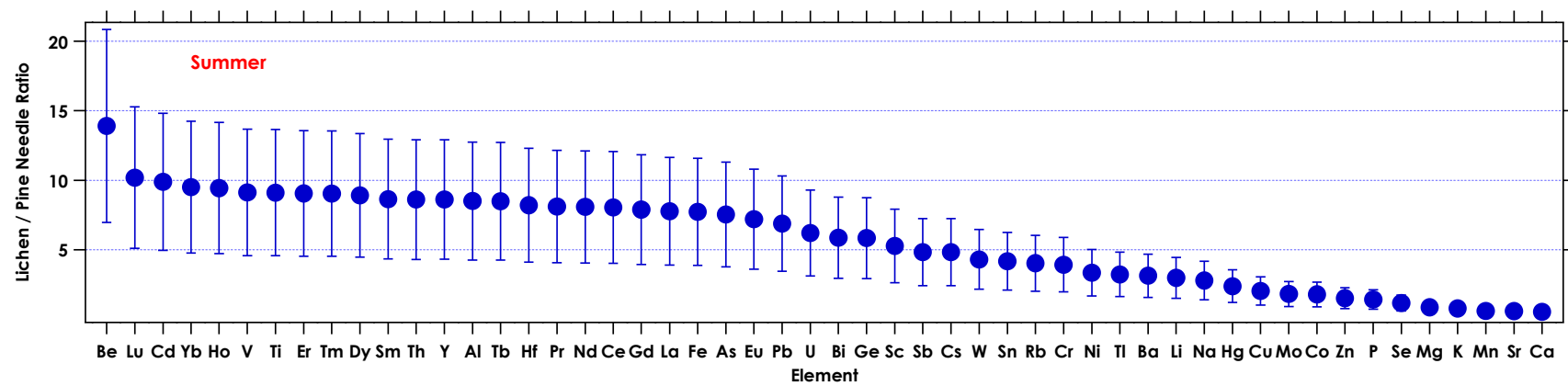


Figure 5.7. Accumulation capacities of elements calculated for the summer field campaign

4.8 Enrichment Factors of Biomonitoring Data

In order to have an idea on the contribution of anthropogenic pollutants to atmospheric concentrations, the crustal enrichment factor (EF_c) was calculated using Fe as reference element and upper crustal composition provided by Taylor and McLennan (1985). The calculation of EF_c values were performed by using the mathematical formula given in Equation 5.1.

$$EF_c = \frac{\left(\frac{C_x}{C_{Fe}}\right)_{sample}}{\left(\frac{C_x}{C_{Fe}}\right)_{crust}} \quad (Equation 5.1)$$

If calculated EF_c value close to unity, the predominant source is crustal. Calculated EF_c range from 1 to 10 revealed that they are generally from the crustal origin considering the differences in the soil structure of literature and the sampling places. If calculated $EF_c > 10$, the associated element is almost released to the atmosphere from the anthropogenic sources (Koçak et al., 2004).

Chow (1995) compiled the chemicals associated with particles emitted from different sources. Table 5.8 shows which chemicals released to the atmosphere from which sources and their corresponding abundances.

Table 5.8. Chemicals released to the atmosphere from different sources

Source Type	Dominant Particle Size	Chemical Abundance in Percent Mass			
		<0.1 %	0.1 to 1 %	1 to 10 %	> 10 %
Paved Road Dust	Coarse (2.5 to 10 µm)	Cr, Sr, Pb, Zr	SO ₄ ⁼ , Na ⁺ , K ⁺ , P, S, Cl, Mn, Zn, Ba, Ti	Elemental Carbon (EC), Al, K, Ca, Fe	Organic Carbon (OC), Si
Unpaved Road Dust	Coarse	NO ₃ ⁻ , NH ₄ ⁺ , P, Zn, Sr, Ba	SO ₄ ⁼ , Na ⁺ , K ⁺ , P, S, Cl, Mn, Ba, Ti	OC, Al, K, Ca, Fe	Si
Construction	Coarse	Cr, Mn, Zn, Sr, Ba	SO ₄ ⁼ , K ⁺ , S, Ti	OC, Al, K, Ca, Fe	Si
Agriculture Soil	Coarse	NO ₃ ⁻ , NH ₄ ⁺ , Cr, Zn, Sr	SO ₄ ⁼ , Na ⁺ , K ⁺ , S, Cl, Mn, Ba, Ti	OC, Al, K, Ca, Fe	Si
Natural Soil	Coarse	Cr, Mn, Sr, Zn, Ba	Cl ⁻ , Na ⁺ , Ec, P, S, Cl, Ti	OC, Al, Mg, K, Ca, Fe	Si
Lake Bed	Coarse	Mn, Sr, Ba	K ⁺ , Ti	SO ₄ ⁼ , Na ⁺ , OC, Al, S, Cl, K, Ca, Fe	Si
Motor Vehicle	Fine	Cr, Ni, Y, Sr, Ba	Si, Cl, Al, P, Ca, Mn, Fe, Zn, Br, Pb	Cl ⁻ , NO ₃ ⁻ , SO ₄ ⁼ , NH ₄ ⁺ , S	OC, EC
Vegetative Burning	Fine	Ca, Mn, Fe, Zn, Br, Pb, Rb	NO ₃ ⁻ , SO ₄ ⁼ , NH ₄ ⁺ , S, Na ⁺	Cl ⁻ , K ⁺ , Cl, K	OC, EC
Residual Oil Combustion	Fine	K ⁺ , OC, Cl, Ti, Cr, Co, Ga, Se	NH ₄ ⁺ , Na ⁺ , Zn, Fe, Si	V, OC, EC, Ni	S, SO ₄ ⁼
Incinerator	Fine	V, Mn, Cu, Ag, Sn	K ⁺ , Al, Ti, Zn, Hg	NO ₃ ⁻ , Na ⁺ , EC, Si, S, Ca, Fe, Br, La, Pb	SO ₄ ⁼ , NH ₄ ⁺ , OC
Coal Fired Boiler	Fine	Cl, Cr, Mn, Ga, As, Se, Br, Rb, Zr	NH ₄ ⁺ , P, K, Ti, V, Ni, Zn, Sr, Ba, Pb	SO ₄ ⁼ , OC, EC, Al, S, Ca, Fe	Si
Oil Fired Power Plant	Fine	V, Ni, Se, As, Br, Ba	Al, Si, P, K, Zn	NH ₄ ⁺ , OC, EC, Na, Ca, Pb	S, SO ₄ ⁼
Smelter Fine	Fine	V, Mn, Sb, Cr, Ti	Cd, Zn, Mg, Na, Ca, K, Se	Fe, Cu, As, Pb	S
Antimony Roaster	Fine	V, Cl, Ni, Mn	SO ₄ ⁼ , Sb, Pb	S	None reported
Marine	Fine and Coarse	Ti, V, Ni, Sr, Zr, Pd, Ag, Sn, Sb, Pb	Al, Si, K, Ca, Fe, Cu, Zn, Ba, La	NO ₃ ⁻ , SO ₄ ⁼ , OC, EC	Cl ⁻ , Na ⁺ , Na, Cl

In this study, EF_c values were calculated for winter and summer field campaigns separately. In addition, since the accumulation capacities of the lichen and pine needle samples are different, their seasonal EF_c values are also evaluated individually. Crustal enrichment factor of elements measured in lichen species in the winter season is depicted in Figure 5.8. It is clear from Figure 5.8 that elements till K had EF_c values less than 10 and hence these elements are generally thought to be crustal in origin. EF_c values associated with Zn, Hg, Cd, Sb, As and Se were higher than 10 implying that these elements were released to the atmosphere purely from the anthropogenic sources.

Figure 5.9 illustrates the crustal enrichment factor for pine needle samples collected in the winter field campaign. As it can be seen from Figure 5.9 that the calculated EF_c from Lu to Ge was in between 1 and 10, which implied that these elements were mostly emitted to the atmosphere from the earth crust. Elements from Sr to Se in Figure 5.9 had the calculated EF_c values greater than 10. Though Ca, Sr and Mg are crustal elements, they have also $EF_c > 10$, which could be attributed to the uplift of road dust to the atmosphere. The rest of the elements (Rb, Mn, Cu, Cd, As, Hg, Sb, Zn, K and Se) having $EF_c > 10$ were dominantly released to the atmosphere from the anthropogenic sources.

Crustal enrichment factor for the lichen samples collected during the summer field campaign is shown in Figure 5.10. It is clear from Figure 5.10 that K, Zn, Cd, As, Sb, Se and Hg have $EF_c > 10$ implying that these elements were released to the atmosphere from the anthropogenic sources in the summer season.

Figure 5.11 depicts the EF_c values for pine needle samples collected in the summer field campaign. The calculated EF_c values for the elements of Ge, Rb, Sr, Cu, Cd, Mo, Mn, Mg, As, Sb, Ca, Zn, K, Hg and Se were greater than 10. Consequently, it can be concluded that these parameters were associated with the anthropogenic sources in the summer season. Overall, when the calculated EF_c values were compared in terms of pine needle and lichen samples with respect to the season that they have collected, it could be concluded that As, Sb, Zn, Hg, Cd and Se were purely released to the atmosphere from anthropogenic sources.

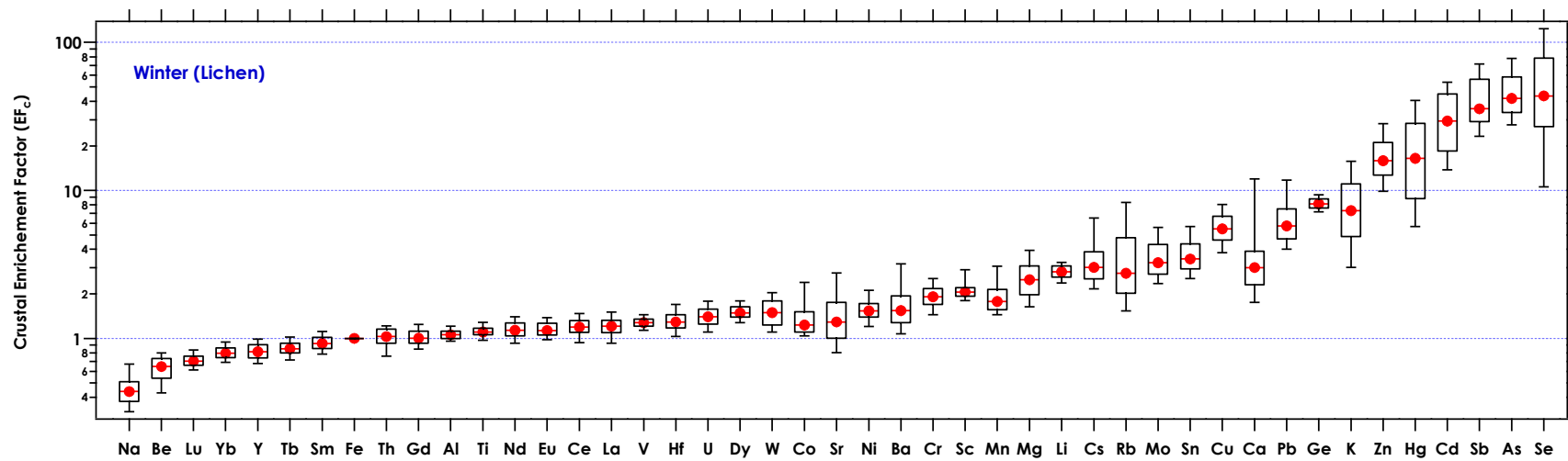


Figure 5.8. Crustal enrichment factor for the lichen samples collected in the winter field campaign

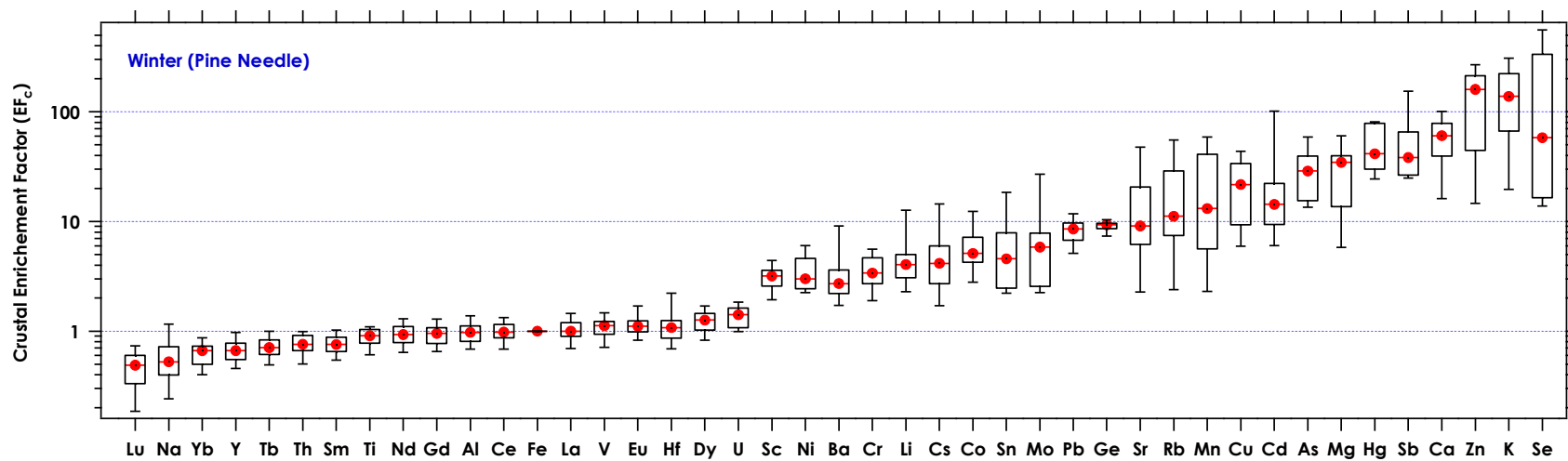


Figure 5.9. Crustal enrichment factor for the pine needle samples collected in the winter field campaign

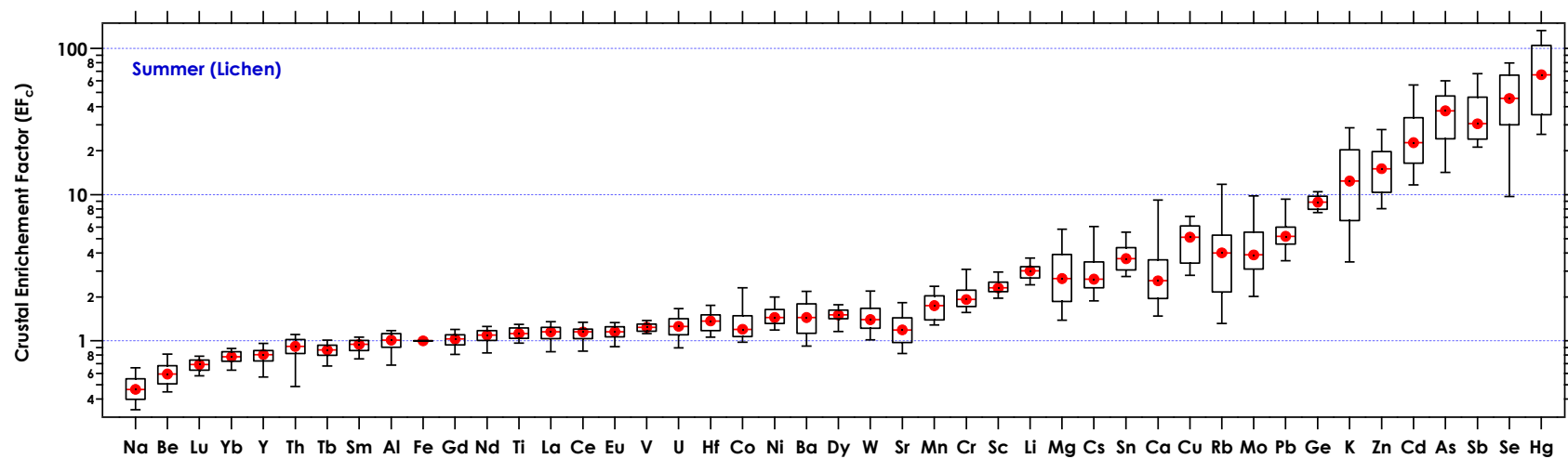


Figure 5.10. Crustal enrichment factor for the lichen samples collected in the summer field campaign

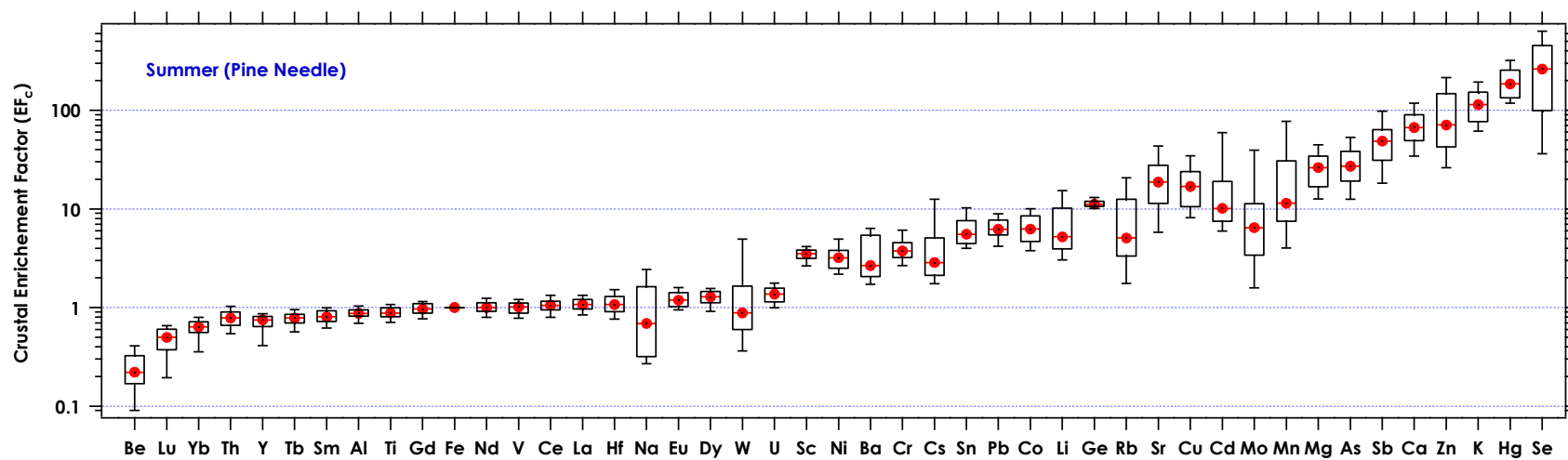


Figure 5.11. Crustal enrichment factor for the pine needle samples collected in the winter field campaign

4.9 Factor Analysis

Factor analysis has widely applied to environmental data in order to apportion the sources affecting the levels of pollutants of concern. The technique classifies the parameters whose concentrations increase and decrease together in time or space. In this study, factor analysis has also performed to find the sources of pollutants determined in collected lichen samples both in winter and summer season. Minitab software (version 15.1.0.0) was used to conduct the factor analysis. To decide the number of factors (sources) retained in the final analysis, both eigenvalues whose values are greater than one and physically meaningful results were taken into account. In some trials, eigenvalues greater than one found for the factors but the factor loadings in some factors were not greater than 0.25 for any of the parameters (pollutants) analyzed, hence, less number of factors was interpreted in the study.

Factor analysis results for winter field campaign were provided in Table 5.9. Six-factor solution was retained in the winter field campaign. Table 5.9 includes also the eigenvalues, variance, percent variance and communalities for the factors. Factor loadings were equal or greater than 0.25 were shown in Table 5.9. Factor 1 (F1) has high loading in crustal element such as Al, Be, Fe, Ti, Cr, Ni, V, and rare earth elements. Accordingly, this factor had been identified as crustal factor. Factor 2 (F2) has loadings of Mg, K and Rb, which exists in association with K^+ minerals (Drobner and Tyler, 1998). It has been well reported in the literature that K is potential inorganic marker of biomass burning emissions (e.g., Cheng et al., 2013) and these are the elements found in the plant material and biomass burning is the main source of these elements to the atmosphere. Therefore, this factor was called as biomass burning factor. Factor 3 (F3) has high loadings of Bi, Tl, Hg, Pb, Cs, Cr. The lead has the second highest loading in this factor and it is the well-known tracer from automobile emissions. Consequently, this factor has been identified as motor vehicle emission factor. Factor 4 (F4) has high loadings of Ca, Co, Cu, Zn, Sr, Cd and Sn, which are known as anthropogenic elements. The highest loading belongs to Ca, for which only known major anthropogenic source is a cement factory, hence this factor is called as cement factory. Factor 5 (F5) has the highest loading of Ni, As, Mo, Sn and Sb. Antimony is well-known tracer for oil combustion, which is why this factor was called as oil-coal combustion factor. Factor 6 (F6) has high loading of Se,

which is mainly associated with the aged particles. Consequently, this factor was called as long range transport factor. Six factors retained in the study account for the 81.2 % of the system variance in total.

Table 5.9. Varimax factor loadings for six factor solution in winter field campaign

Variable	F1	F2	F3	F4	F5	F6	Communality
Li	0.9						0.865
Be	0.897						0.885
Na	0.652						0.777
Mg		0.908					0.878
Al	0.965						0.967
P		0.845					0.778
K		0.93					0.884
Ca	0.409			0.624			0.595
Sc	0.93						0.924
Ti	0.96						0.963
V	0.863						0.864
Cr	0.766		0.462				0.876
Fe	0.934						0.95
Mn	0.718						0.548
Co	0.793			0.436			0.882
Ni	0.821				0.402		0.864
Cu				0.59			0.457
Zn				0.609			0.52
Ge	0.915						0.942
As	0.394				0.308		0.353
Se						0.749	0.607
Rb		0.801					0.681
Sr	0.691			0.416			0.747
Y	0.948						0.972
Mo					0.364		
Cd				0.491			0.308
Sn				0.267	0.471		0.46
Sb					0.86		0.786
Cs	0.334		0.807				0.811
Ba	0.646						0.621
La	0.964						0.968
Ce	0.973						0.972
Pr	0.978						0.979
Nd	0.981						0.984
Sm	0.976						0.985
Eu	0.972						0.982
Gd	0.963						0.984
Tb	0.97						0.984
Dy	0.964						0.983
Ho	0.958						0.978
Er	0.957						0.975

Table 5.9. Varimax factor loadings for six factor solution in winter field campaign
(Continued)

Variable	F1	F2	F3	F4	F5	F6	Communality
Tm	0.958						0.976
Yb	0.957						0.976
Lu	0.956						0.974
Hf	0.905						0.855
W	0.748				0.471		0.827
Th	0.965						0.951
Bi	0.309		0.767				0.78
Hg			0.507				0.689
Tl			0.889				0.833
Pb	0.624		0.473				0.742
U	0.895						0.891
Eigenvalues	30.75	3.92	2.71	1.95	1.58	1.30	
Variance	28.75	3.47	3.47	2.50	2.14	1.89	42.22
% Var	0.553	0.067	0.067	0.048	0.041	0.036	0.812

Factor analysis results for the summer field campaign were provided in Table 5.10. Five-factor solution was retained in the winter field campaign. Table 5.10 includes also the eigenvalues, variance, percent variance and communalities for the factors. Factor loadings were equal or greater than 0.25 were shown in Table 5.10. Factor 1 (F1) has high loading in crustal element such as Al, Be, Fe, Ti, Cr, Ni, V, and rare earth elements. Accordingly, this factor had been identified as crustal factor. Factor 2 (F2) has loadings of As, Cr, Se, Cd, Cs, Bi, Hg, Tl, Pb. Since As and Se are associated with the coal combustion emissions, this factor was called as coal combustion factor. Factor 3 (F3) has high loadings of Na, P, Cu, Zn, Ge, Sr, Mo, Sn and Bi. These pollutants are mainly anthropogenic in origin and released to the atmosphere from different sources. Consequently, this factor has been identified as local mixed factor. Factor 4 (F4) has the highest loading of Ca, for which only known major anthropogenic source is a cement factory, hence this factor is called as cement factory. Factor 5 (F5) has high loadings of Mg, P, K and Rb. As discussed in the factor analysis results for the winter field campaign, these are the elements found in the plant material and biomass burning is the main source of these elements to the atmosphere. Five factors retained in the study account for the 82.8 % of the system variance in total.

Table 5.10. Varimax factor loadings for six factor solution in summer field campaign

Variable	F1	F2	F3	F4	F5	Communality
Li	0.944					0.931
Be	0.926					0.876
Na	0.78		0.282			0.705
Mg				0.568	0.611	0.762
Al	0.94					0.938
P			0.645		0.507	0.726
K					0.805	0.817
Ca	0.299			0.895		0.899
Sc	0.944					0.928
Ti	0.94					0.94
V	0.909					0.947
Cr	0.596	0.606		0.283		0.844
Fe	0.937					0.963
Mn	0.816			0.406		0.895
Co	0.737			0.58		0.935
Ni	0.846			0.316		0.883
Cu	0.591		0.468	0.262		0.705
Zn			0.893			0.837
Ge	0.916		0.27			0.945
As	0.617	0.452				0.638
Se		0.395				0.38
Rb					0.77	0.643
Sr	0.728		0.349	0.356		0.797
Y	0.945					0.953
Mo			0.586			0.434
Cd		0.617				0.461
Sn	0.698		0.354			0.699
Sb	0.297					
Cs	0.256	0.861				0.849
Ba	0.752					0.675
La	0.969					0.961
Ce	0.981					0.98
Pr	0.979					0.982
Nd	0.978					0.984
Sm	0.98					0.992
Eu	0.971					0.985
Gd	0.971					0.988
Tb	0.969					0.989
Dy	0.965					0.985
Ho	0.964					0.983
Er	0.963					0.983
Tm	0.961					0.98
Yb	0.962					0.978
Lu	0.958					0.974
Hf	0.905					0.897

Table 5.10. Varimax factor loadings for six factor solution in summer field campaign (Continued)

Variable	F1	F2	F3	F4	F5	Communality
W	0.656			0.318		0.541
Th	0.958					0.949
Bi		0.301	0.809			0.821
Hg		0.436				
Tl		0.871				0.82
Pb	0.688	0.317		0.547		0.906
U	0.822			0.386		0.893
<i>Eigenvalue</i>	33.22	3.33	2.72	2.17	1.60	
<i>Variance</i>	30.11	3.67	3.63	3.24	2.39	43.04
<i>% Var</i>	0.579	0.071	0.07	0.062	0.046	0.828

4.10 Spatial Distribution of Biomonitoring Data

One of the objectives of this study is to find the spatial distribution of pollutants of interest over the Bolu plain. To reach this objective, lichens and pine needles samples were collected throughout the plain from different points in winter and summer season. Spatial distribution of the pollutants gives valuable information about extent of pollution which inhabitants has been exposed in specific areas.

Spatial distribution of parameters analyzed in collected samples was evaluated using Map Info version 8.5. TIN interpolator was used in plotting the thematic map in MapInfo. TIN interpolater works best for terrain data and points that have a linear relationship to each other across the grid. Since the concentrations of the parameters have a relationships, which means that a source in a specific grid affect the concentrations in other closer grids, we decided to use TIN interpolator in this study. Since the number of pine needle samples was not enough to make a spatial interpretation with interpolation, the data generated for lichen samples were used to construct the pollution maps. In addition, the pollutants having high EF_c values were evaluated herein to see which areas in Bolu plain are under the influence of these pollutants. Since biomonitoring study was performed both in summer and winter season, the maps were also plotted for these seasons.

Maps of the selected pollutants determined in the summer field campaign were depicted in Figures 5.12-5.19. When the maps were evaluated in terms of As and Sb, it can be concluded that highways are important sources for these pollutants (Figure 5.12). Higher concentrations were observed particularly along D100. Common hot spots for As is located along the city center, consuming coal for domestic heating. Consequently, it can be concluded that coal consumption and traffic are the important sources that release As and Se to the city atmosphere, respectively. It is well known that the largest contribution of atmospheric As comes from coal burning. Low summer concentration of this metal can be related to decrease of coal combustion for domestic heating (Markert, 1993).

The largest contribution of atmospheric Se also comes from coal burning. Concentration distribution pattern shows similarities with arsenic especially around the village sites of Bolu plain. Karga, Tekerlek, Eğreltiyazı and Yanıkköknar hills, Yukarışeyhler and Evri neighborhoods and Kovmak fountain are the places where considerably higher concentrations of Se were observed during the study period.

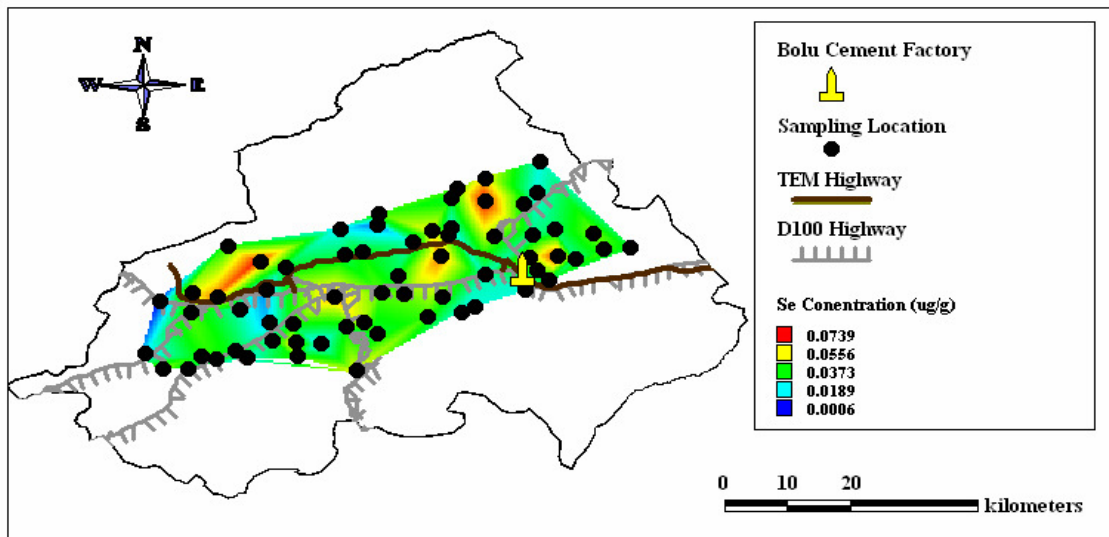
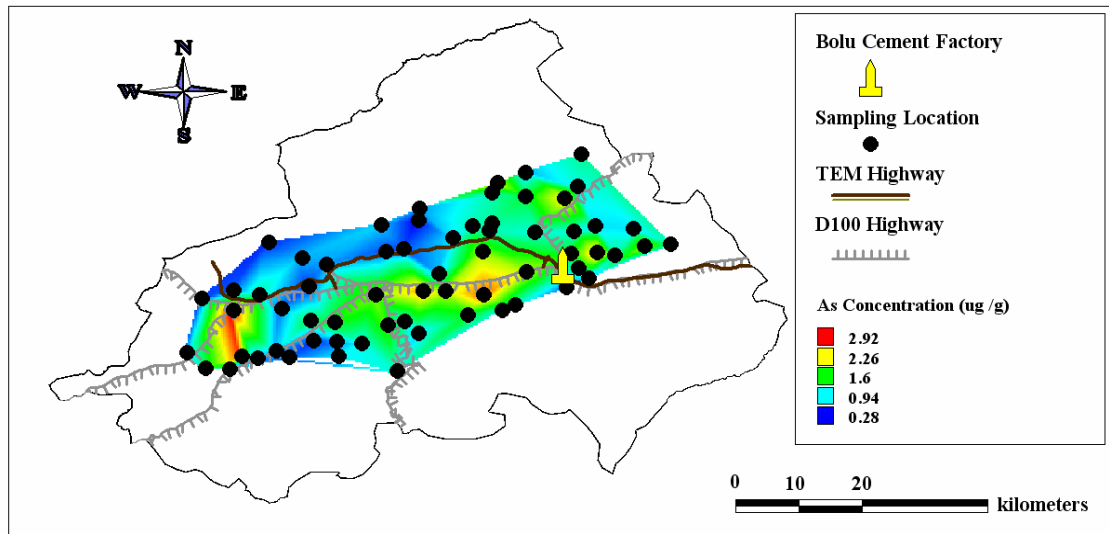


Figure 5.12. Spatial distribution of As and Se in summer season

Antimony is released to the atmosphere from traffic emissions. When the maps were evaluated in terms of Pb and Sb, it can be concluded that highways are important sources for these pollutants. Spatial distributions of Pb and Sb in summer season were illustrated in Figure 5.13. The hot spots for Pb can be listed as Kürtler and Yukarışeyhler neighborhoods and Kocaboz district and along the highways. Motor vehicle traffic is the largest emission source of metals such as As, Cd, Co, Ni, Pb, Sb, V, Zn. These elements are mainly released to the atmosphere from combustion residues and losses from fuels and engine and transmission oils, abrasion

from tires, brake linings, exhaust catalysts and road pavements and galvanized protection barrier (Wang et al., 2009).

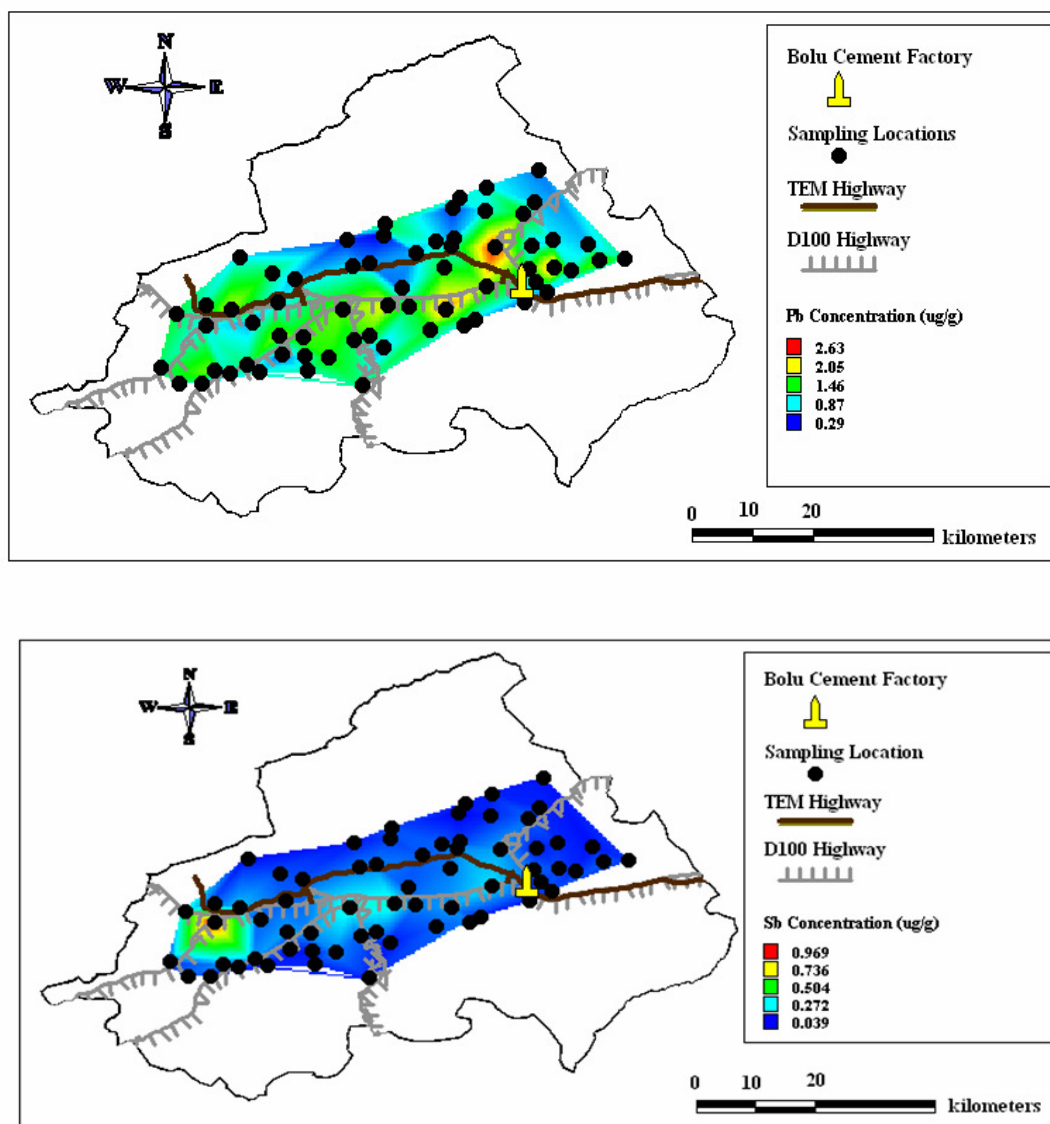


Figure 5.13. Spatial distribution of Pb and Sb in summer season

Spatial distribution of Zn and Hg were depicted in Figure 5.14. Bahçeköy and the region between two highways are the locations that we observed considerably the highest concentration of Zn during summer season. Zinc is an essential element in all organisms in nature and its most relevant anthropogenic emission sources are incinerators, traffic, pesticides, fertilizers, the rubber industry, iron and steel production corrosion protection and coal burning (Scerbo et al., 2002). Zn shows different pattern at the highway sites as lower than agricultural area. This shows that Zn emissions are related with to pesticides or fertilizers rather than traffic.

Mercury is one of the less abundant elements on earth. Coal is a source of Hg but it is also widely used in numerous sectors; steel making, paper mills, the chemical industry, chlor-alkali plants, paints and fungicides. The highest concentrations of Hg are seen around agricultural area due to usage of them as insecticide and fungicide (Scerbo et al., 2002). Karga, Tekerlek, Eğreltiyazı, Yanıkköknar and Top hils as well as Ilıcakınık location and Kovmak fountain are the source regions affecting the distribution of Hg over the city.

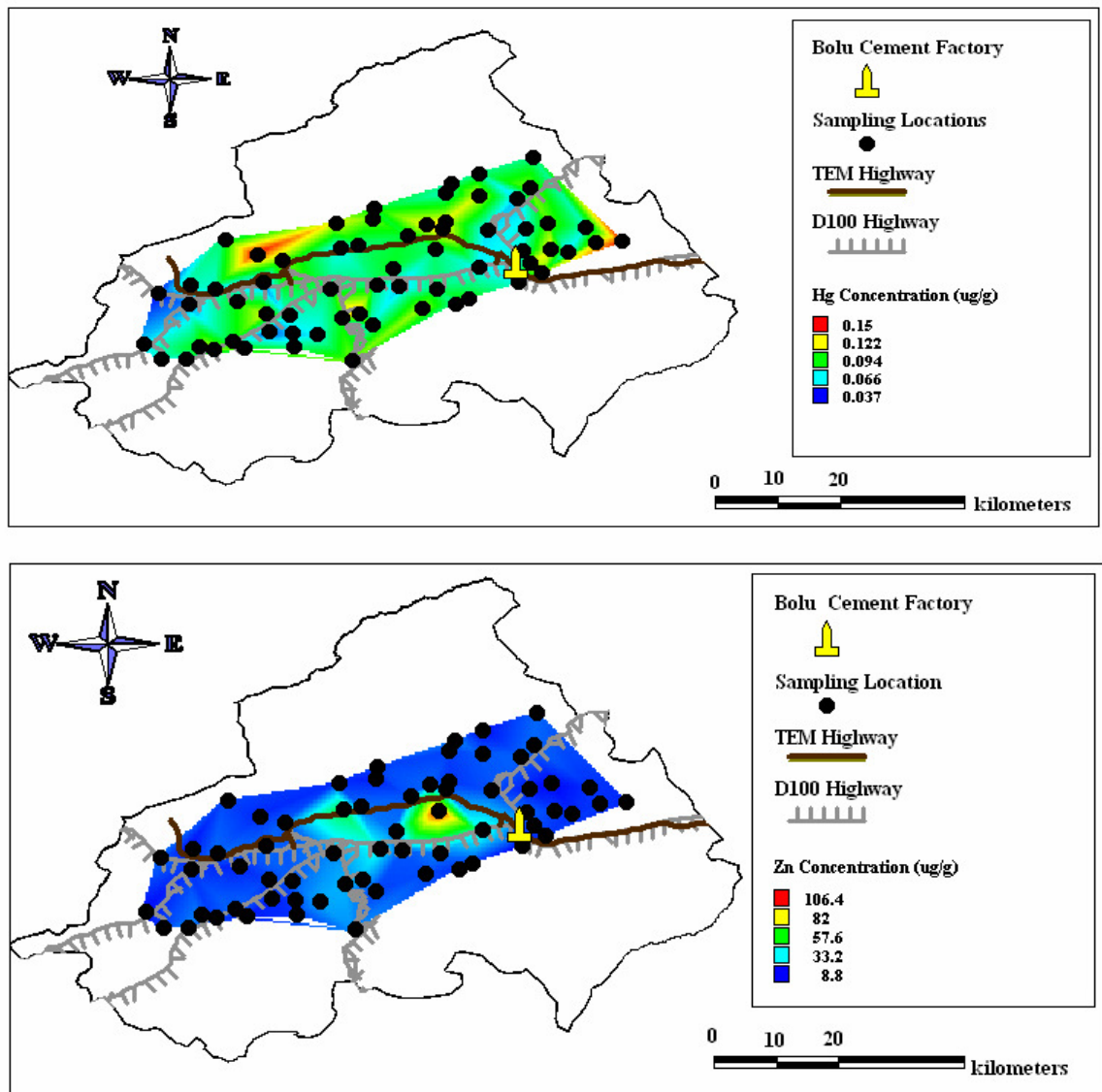


Figure 5.14. Spatial distribution of Zn and Hg in summer season

Spatial distribution of K and Al were depicted in Figure 5.15. The highest concentration for K was found where AIBU, Aktopraklık and Cevizlik districts were located. K is trace element for wood combustion, so it has higher concentration at village sites of Bolu than around the city center. The other source of K in lichens is lichen metabolism itself since it is nutrient element for lichens. Lichens also take K from leachate obtained from other dead foliar and from the atmosphere.

High aluminum levels in the samples are in eastern site of Bolu which has lots of industrial factories such as wire and cement production. In the remaining locations, the degree of environmental alteration varied from low to medium. This means that Al concentrations differ not only due to lithological differences in soil but also pollution. Kaşarkası and Beyharmanlık districts are two locations, which are approximately 1.44 km away from D100 highway, where the highest Al concentration was measured during the summer field campaign. In addition, Ovadüzü district is another place that relatively higher Al concentration was determined during the study.

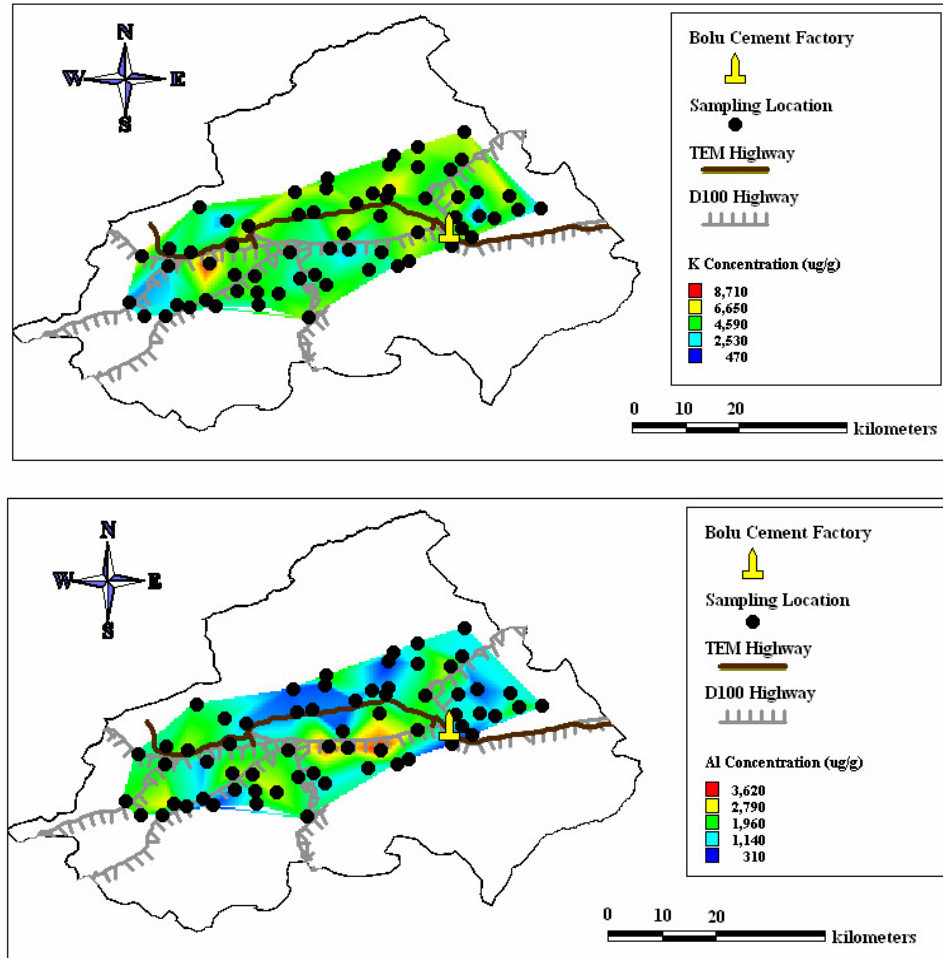


Figure 5.15. Spatial distribution of K and Al in summer season

The spatial distribution of Mg and Ca were depicted in Figure 5.16. Kürtler district, the highest concentration for Mg was determined in the summer season. Similar to Mg, highest concentration of Ca was determined at Kürtler district during summer field campaign. Calcium comes from concrete material and cement factories. Especially, around the Bolu cement factory, Ca concentration was higher than other locations of Bolu plain. An important source of Ca, Mg, K in the study area was the dry depositon of dust. Also, winds coming from the west and southwest could have carried Ca and Mg elements to the around the cement factory.

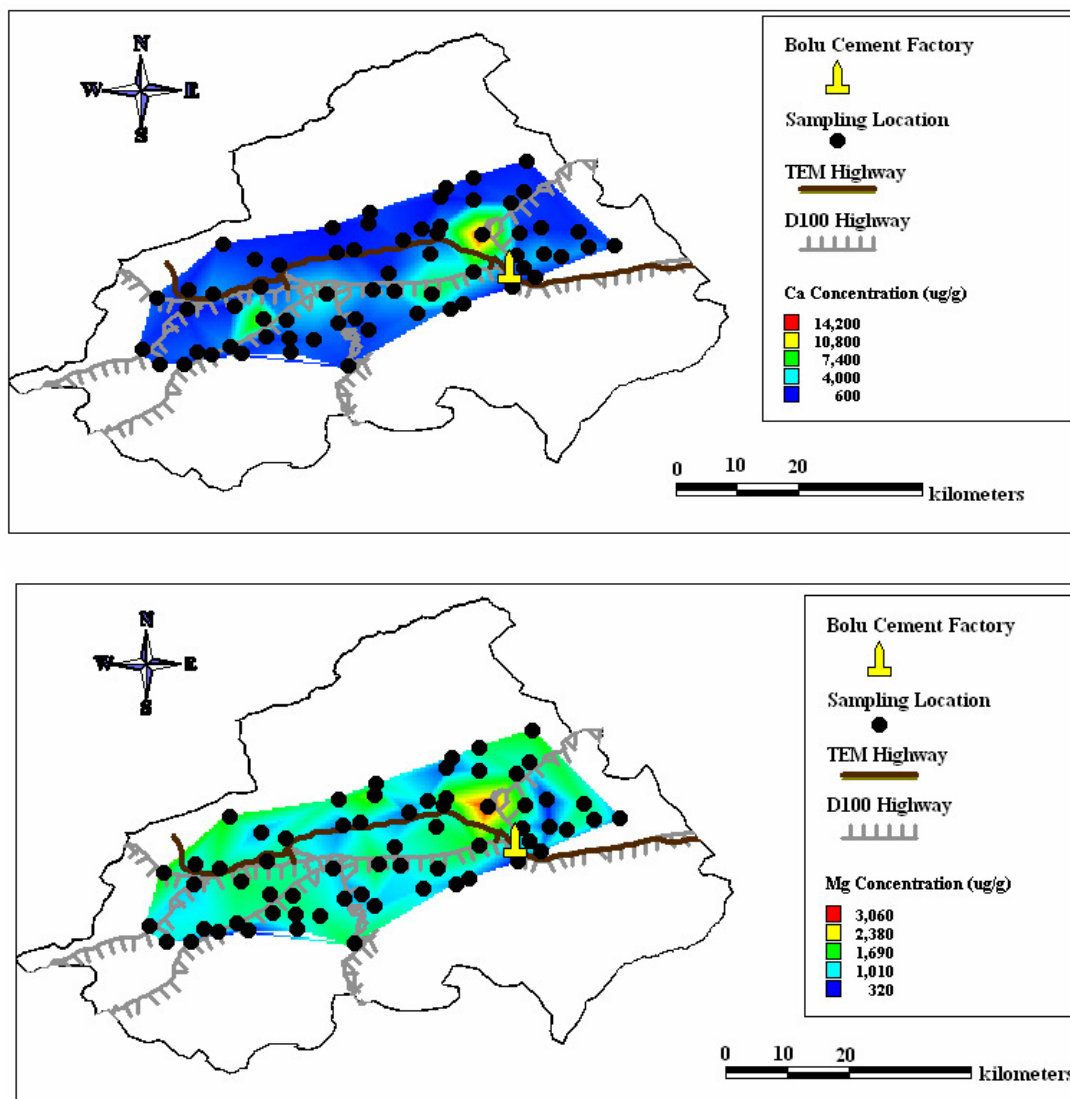


Figure 5.16. Spatial distribution of Mg and Ca in summer season

The spatial distribution of Cd was illustrated in Figure 5.17. Erenler and Sarıçam hills in addition to Davran district are the places, where the highest concentration of Cd was determined during summer field campaign. Cadmium is related to urban waste incinerators, combustion processes and far from urban centres generally derives from fertilizers. Atmospheric cadmium levels are probably enhanced by vehicle emissions. It is also released by tyre wear and fuel combustion.

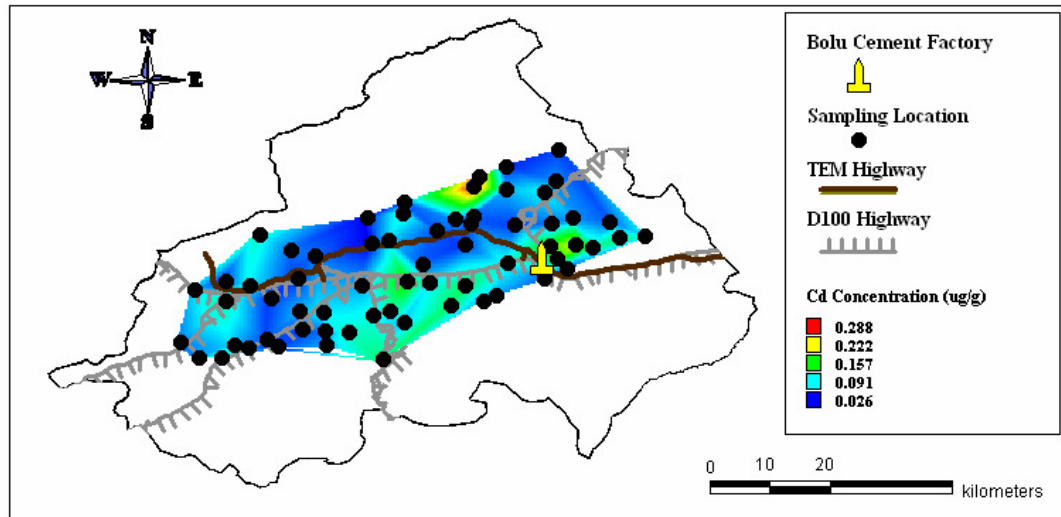


Figure 5.17. Spatial distribution of Cd in summer season

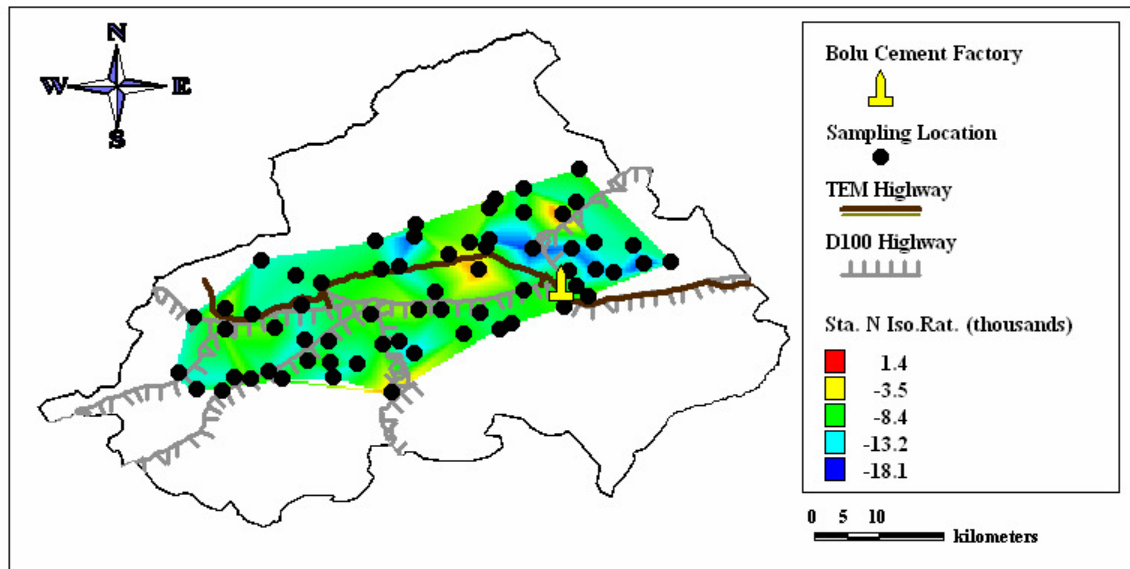


Figure 5.18. Spatial distribution of $\delta^{15}\text{N}$ in summer season

Figure 5.18 represents the spatial distribution of $\delta^{15}\text{N}$ in summer season. Ilıcabaşı district and Arap neighborhood, located 0.7 km away from D100 highway, and Bahçeköy district, between two highways, are the places that the highest stable nitrogen isotope ratio was determined during summer field season. Ilıcabaşı and Arap districts that there are lots of broiler house which have capacities from 2500 to 200000 chicken have been operated. Waste from these facilities has been applied over the land without taken any measures as fertilizer. NH_3 is mainly emitted by

agriculture and returns to ecosystems primarily in the form of dry deposition of NH_3 . The most frequently reported N isotope ratios in plants are roughly between -10 and +10 ‰. Some research that has dealt specifically with the environmental N inputs and pathways in vascular epiphytes or lichens could detect primarily $\delta^{15}\text{N}$ ratios often lower than -10 ‰, caused by a high depletion of ^{15}N . With an increase of agricultural activity, $\delta^{15}\text{N}$ signatures in lichens become highly negative. The fact that lichens in this study were more enriched in ^{15}N at higher altitudes testifies to a change in the prevailing deposited N compounds and its composition. Simultaneously, lichens were detected that with less negative $\delta^{15}\text{N}$ ratios around the city center. This, in turn, also suggests that the predominant N oxides from traffic, industrial emissions and also from surrounding metropolitan areas are responsible for the enrichment of ^{15}N and correspondingly less negative $\delta^{15}\text{N}$ ratios in samples.

Stable carbon isotopes have proven to be useful geochemical markers and these isotopes were first applied to studies of air pollution beginning in the 1980s (Chesselet et al., 1981). Light and moisture are important factors influencing the $^{13}\text{C}/^{12}\text{C}$ ratio in lichen. The spatial distribution map corresponding to stable carbon isotope ratio was depicted in Figure 5.19 below.

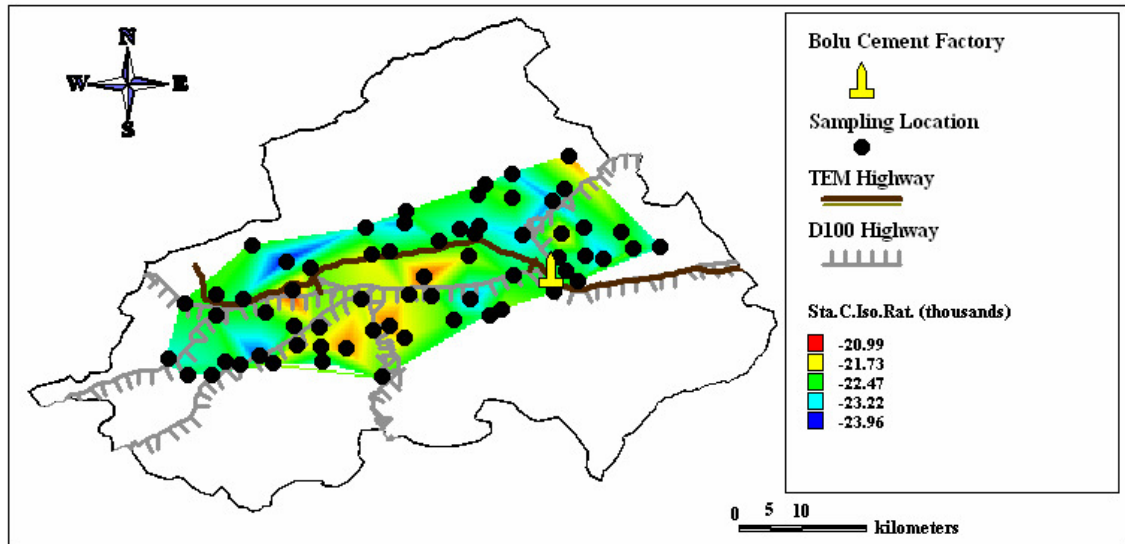


Figure 5.19. Spatial distribution of $\delta^{13}\text{C}$ in summer season

It is clear from Figure 5.19 that Karaköy, Devedamı district, Ilıcakınık and Yatakeri hills and Delikız spring are the locations where the highest $\delta^{13}\text{C}$ was

determined during summer field campaign. As summarized by Hoefs, in relation to biomolecules synthesized in nature, the ^{13}C content of a lichen in natural setting will be dictated by the ^{13}C content of the carbon source, isotope effects associated with the assimilation of carbon, metabolism and biosynthesis and the ultimate cellular carbon budget. It was found that, of natural factors, humidity and distance from the major source of pollution all tended to increase the value of $\delta^{13}\text{C}$. Altitude which the lichen was exposed tended to decrease $\delta^{13}\text{C}$ values.

Spatial distribution of anthropogenic parameters for which EF_c values calculated to be greater than ten during winter season was also investigated in this study and associated maps were given from Figures 5.20 to 5.26.

Copper is a minor trace metal in leaves and roots and is considered a microelement essential for all organisms. It is an important constituent of many enzymes of oxidation-reduction reactions. The source of copper in the street dust was ascribed to corrosion of the metallic parts of cars like engine wear, thrust bearing and brush wear. Cu is also released by fungicides or pesticides at the agricultural sites of Bolu plain. The highest concentration for Cu was determined between two highways, close to Yukarıçivril neighborhood.

Cadmium is derived from fertilizers, coal burning and urban wastes. Elevated cadmium levels were found close to city center of Bolu which is both industrialized and urbanized part. Cd is also released by tyre wear and fuel combustion. Mesçiçe village and Kansızlar neighborhood are the two places that we observed relatively higher concentration of Cd during winter season in this study.

Cadmium is toxic both for animals and plants and its high carcinogenicity has been demonstrated. However, the prevalence of natural conditions in both surveys indicates the lack of risk from this pollutant in the study area.

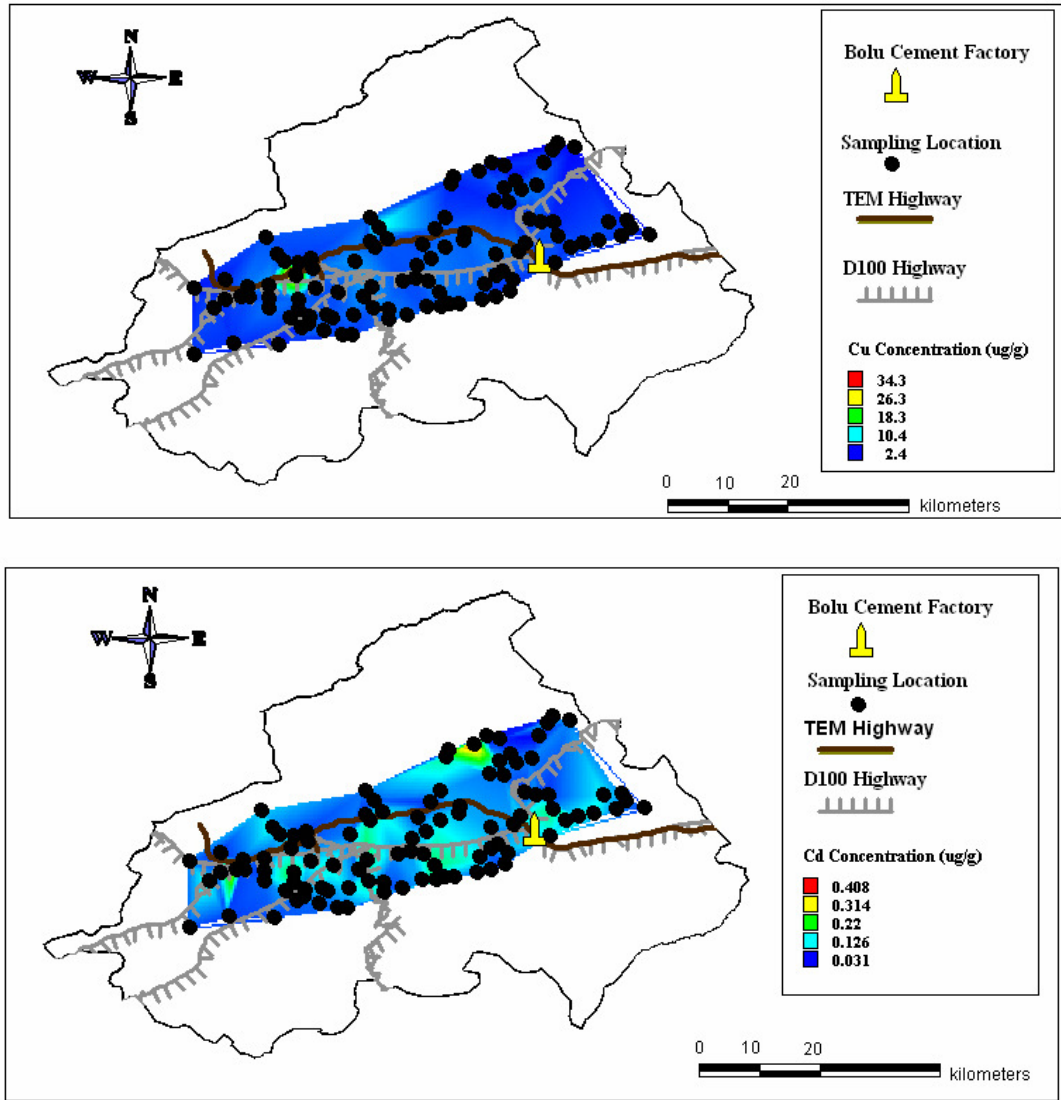


Figure 5.20. Spatial distribution of Cu and Cd in winter season

The spatial distribution of As and Se during winter season was depicted in Figure 5.21. The origin of As to the atmosphere is coal combustion. Other emission sources for As include paints, smelting of metals, the use of arsenic based pesticides, and steel production. High As concentrations in the urban lichen samples were observed at the villages of Bolu which are used coal for heating. For As, the highest concentration was observed at Tokmaklar village, which is 1.8 km away from D100 highway. The highest concentration for Se was found at Çökeklik and Kayalarbaşı districts, located 0.9 and 0.2 km away from D100 highway, respectively.

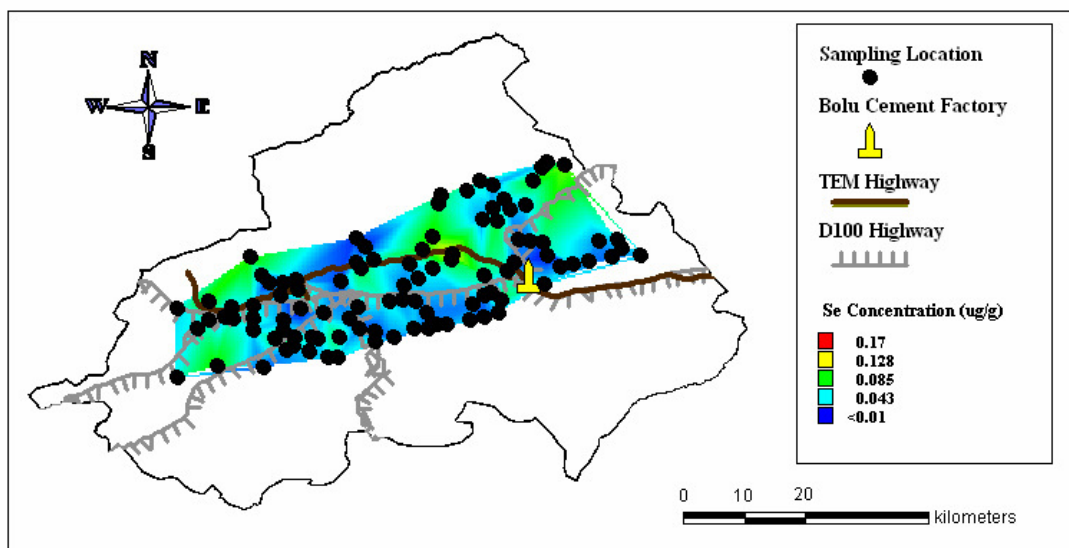
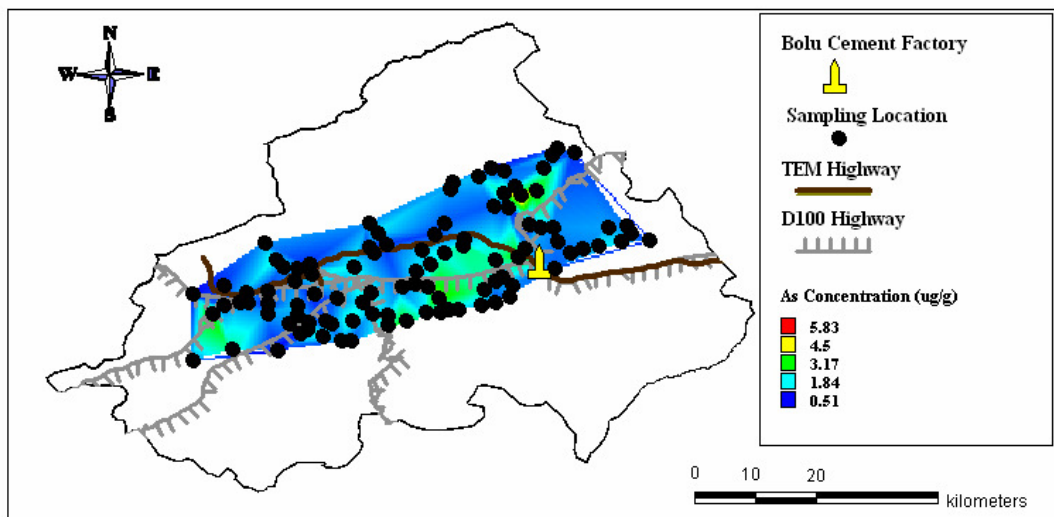


Figure 5.21. Spatial distribution of As and Se in winter season

Important potential pollution sources for Hg include chemical manufacturing, chlor-alkali plants, paints, paper mills, steel production, fabric dyeing, electronic component production and fungicide application (Jeran et al., 2007). Hg emission mainly originates from coal burning.

High mercury levels in southern zones of Bolu plain so that agricultural activities are much more than other sites. The spatial distributions of Hg and Sb were in Figure 5.22. The highest concentrations of Hg were determined at Yüvürek meadow, Değirmenözü neighborhood, Susuzkılık and Sazakkılık districts. Ömerler

village, which is only 0.4 km away from D100 highway, is the place that we observed the highest concentration of Sb during the winter field campaign in this study.

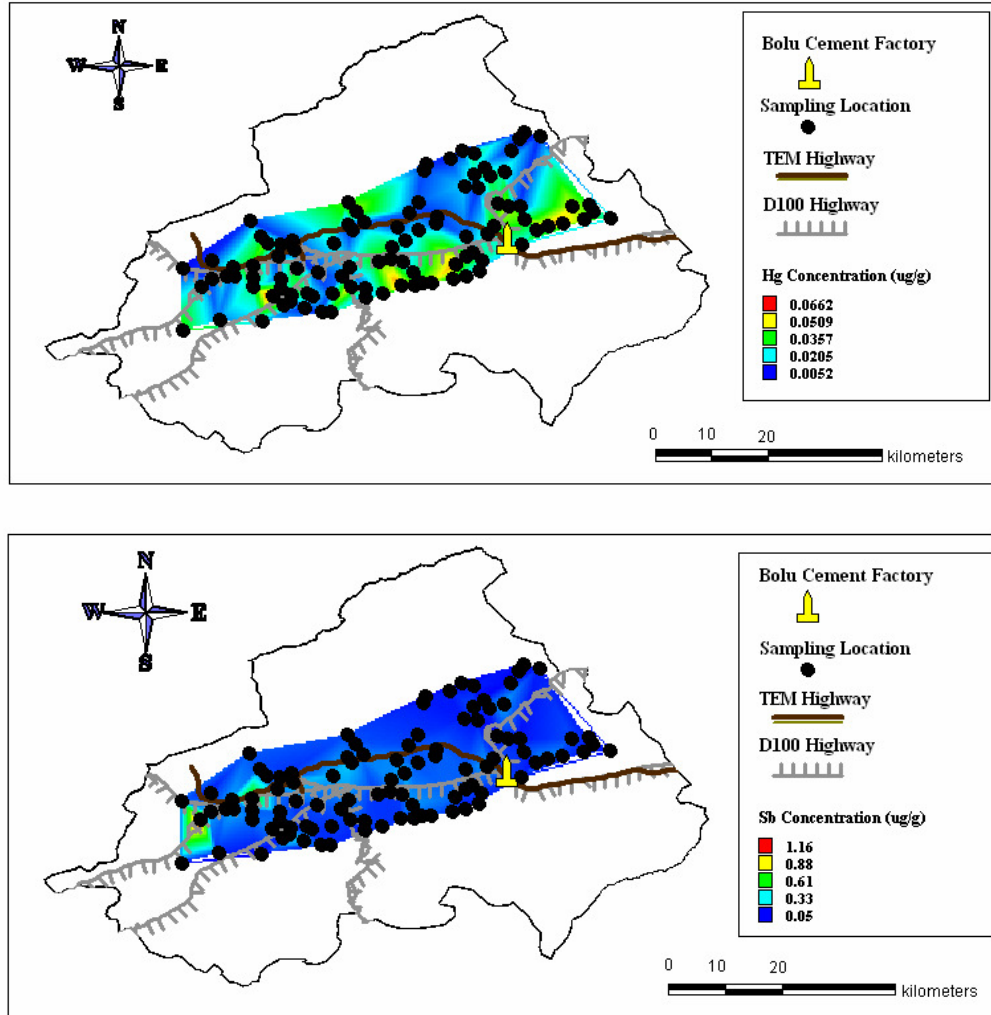


Figure 5.22. Spatial distribution of Hg and Sb in winter season

Maps showing the spatial distributions of Ca and Mg were illustrated in Figure 5.23. Arif and Bekçisayvan hills, Değirmençayırı and Kuduzlarbayırı districts are the locations that the highest concentrations of Ca were measured over the Bolu plain during winter field campaign. Kıranderuğu hill, Küçükömerler, Karca and Sümer neighborhood and the place between the Boyacılar, Yeniakçakavak and Kaynaklar districts are the locations that the highest concentration of Mg was observed during the winter season.

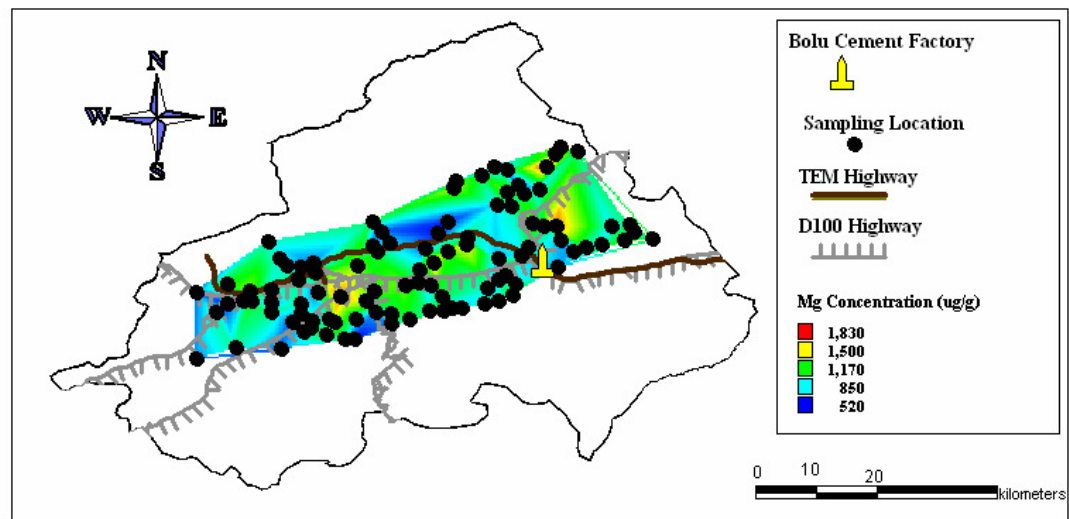
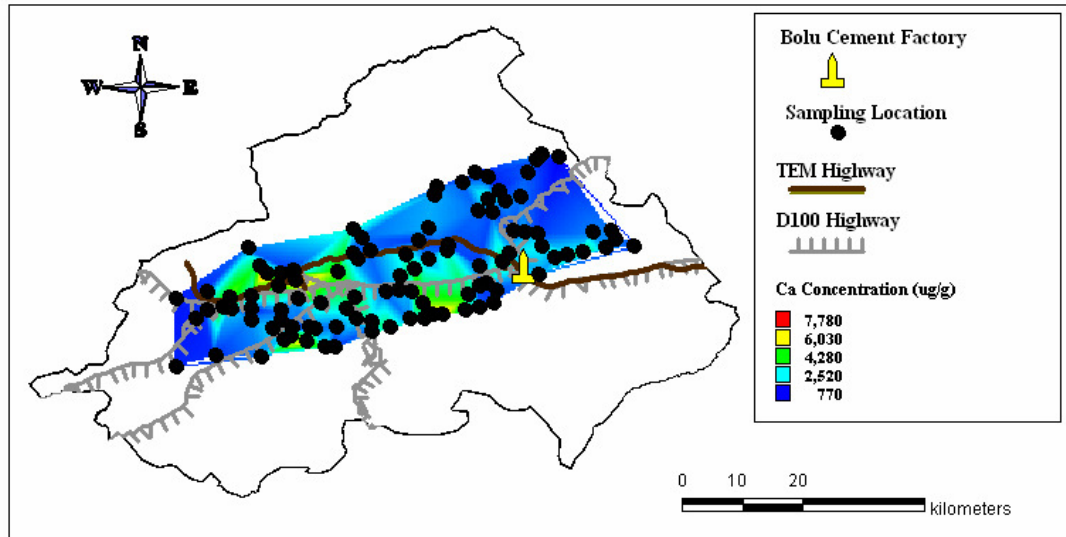


Figure 5.23. Spatial distribution of Ca and Mg in winter season

The spatial distributions corresponding to K and Zn were depicted in Figure 5.24. Its anthropogenic emission sources are incinerators, traffic, pesticides, fertilizers, the rubber industry, coal burning and combustion plants such as power plants. Being a ubiquitous pollutant in nature, its distribution in the atmosphere does not allow localizing its contamination sources. In the study area, the highest Zn concentrations were found around cultivated areas of Bolu plain. The highest concentration for Zn was measured at Dıbekbaşı district, which is about 0.44 km and 0.74 km away from TEM and D100 highways, respectively.

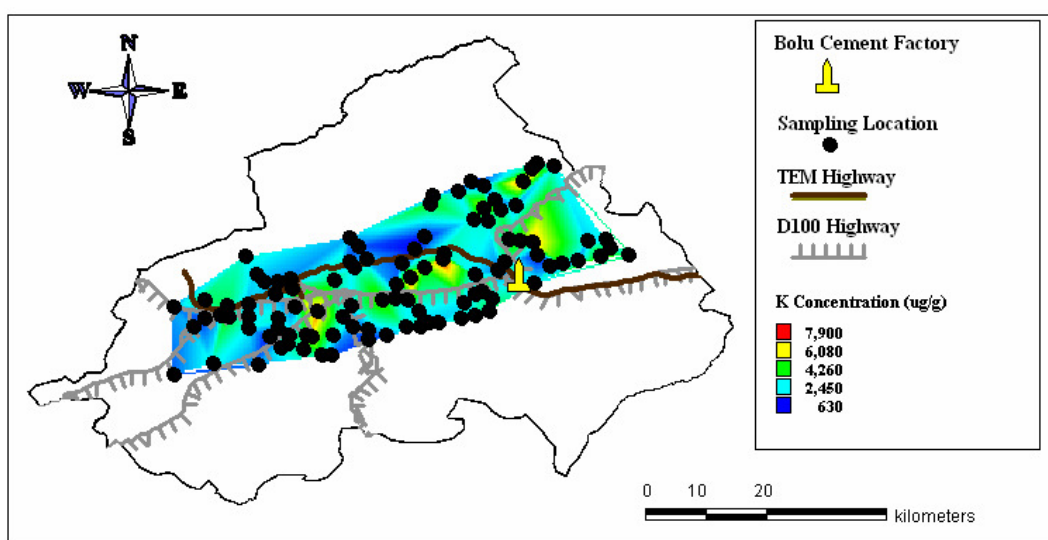
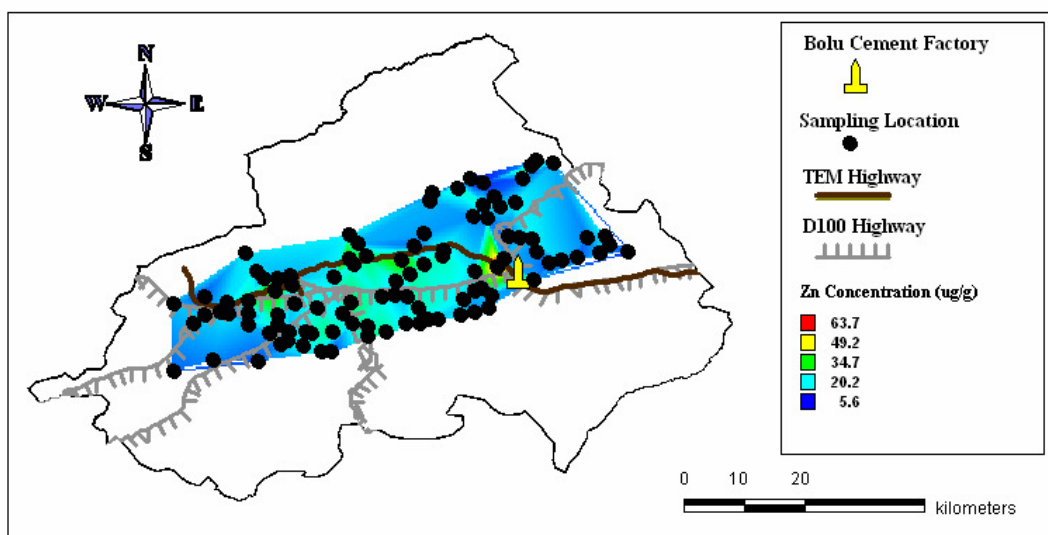


Figure 5.24. Spatial distribution of K and Zn in winter season

The spatial distribution of stable nitrogen isotope ratio was illustrated in Figure 5.25. The highest concentration corresponding to this parameter was found at Tuzla hill, which is only 0.54 km away from the TEM highway. In addition, Top hill and Musluklar are other locations that we observed relatively higher concentration of stable nitrogen isotope ratio over the Bolu plain in winter season during this study.

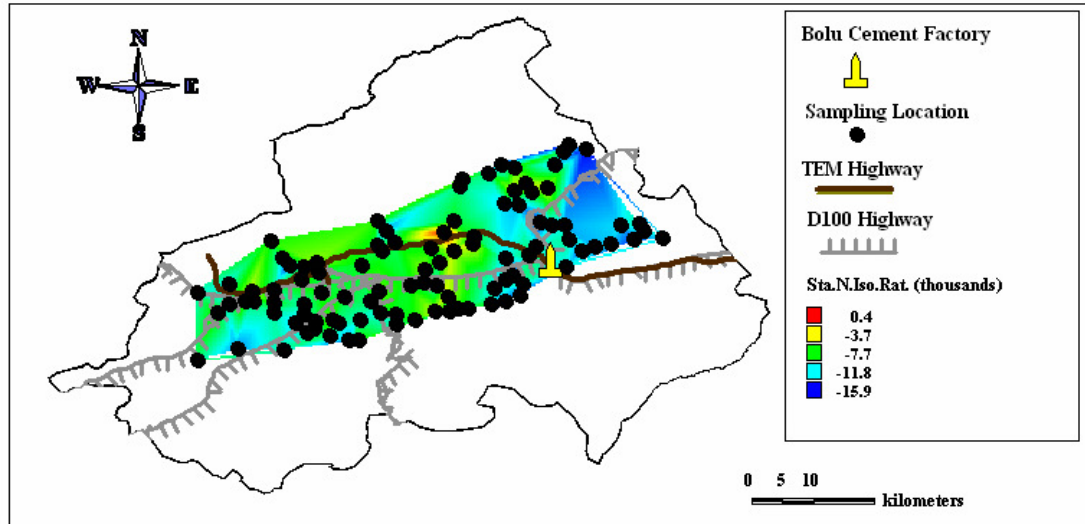


Figure 5.25. Spatial distribution of $\delta^{15}\text{N}$ in winter season

Map showing the spatial distribution of stable carbon isotope ratio was depicted in Figure 5.26. Vakıfakçakavak neighborhood, located between D100 and TEM highways, is the place at which the highest concentration of stable carbon isotope ratio was determined in winter field campaign. In addition, Kocaboz and Sakızlık districts are other locations that relatively higher concentration of stable carbon isotope ratio was measured in this study during winter field campaign.

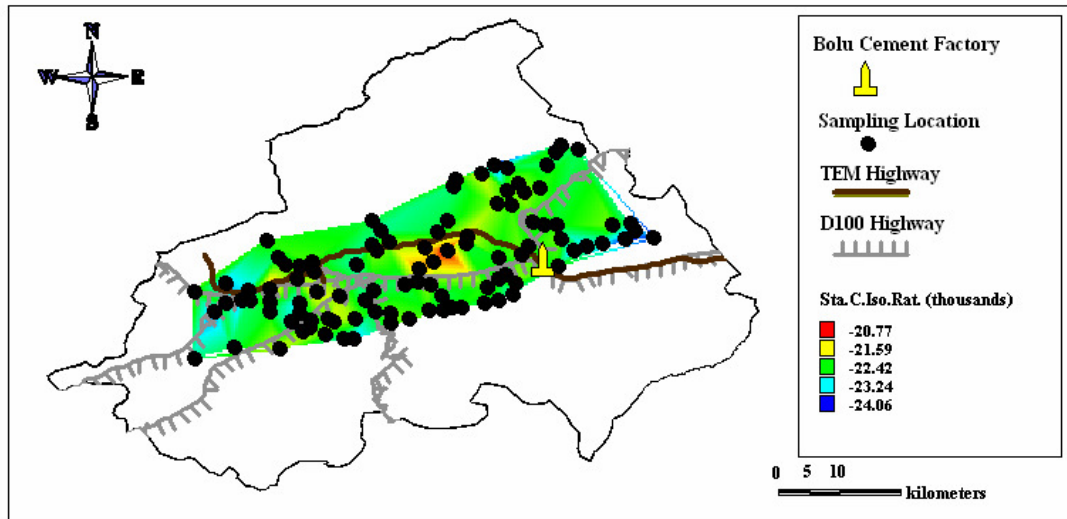


Figure 5.26. Spatial distribution of $\delta^{13}\text{C}$ in winter season

5. CONCLUSION

Biomonitoring study was conducted over Bolu Plain in November 2012 and in June 2013. In addition to lichen samples, mainly *Xanthoria parietina*, and pine needle samples were collected during these periods and collected samples were analyzed in terms of elements and stable carbon ($\delta^{15}\text{N}$) and nitrogen isotope ratios ($\delta^{13}\text{C}$).

For some of the elements such as As, Cu, Fe, Mn, Ni, Pb, Tl, V and Zn, lower concentrations were measured in this study as compared to ones reported at Kocaeli. In addition, comparable concentrations of Hg were obtained during summer field campaign in Bolu and Izmir and Soma. This is an unexpected result since Kocaeli, Izmir and Soma are the places, where many industrial facilities are located.

Temporal variations of measured parameters were also evaluated in this study. Mercury, Sr, K, Ca, Mo, Mg, and Mn are the elements for which summer to winter ratio calculated was greater than one. Relatively higher concentration of Hg during summer season as compared to winter is attributed to the natural source of Hg in summer season or long range transport of particle bound Hg to the Bolu plain. The elements such as As, Sb, Ba, Cu and Pb have summer to winter ratio less than one, which was explained by the increased fossil fuel combustion and lower mixing layer height during winter season.

Correlation between the measured parameters was also investigated in this study. Purely crustal elements such as Al, Ti, Cr, Fe and Sc and rare earth elements were strongly correlated. Lead and other anthropogenic elements such as As, Se, Sn and Cd showed poor correlation in this study.

Accumulation capacities corresponding to lichen and pine needle samples were also evaluated in this study. For both of the seasons, significantly higher

accumulation capacity was observed for lichen samples as compared to pine needle samples in terms of most of the elements analyzed.

The crustal enrichment factor (EF_c) was calculated to find the contribution of anthropogenic pollutants to atmospheric concentrations. The calculated EF_c values were greater than 10 for As, Zn, Hg, Cd, Sb, and Se both in lichen and pine needle samples implying that these elements were released to the atmosphere from anthropogenic sources.

Factor analysis was also performed in this study to apportion the sources affecting the chemical composition of samples collected in this study. Six-factors were retained in the winter field campaign, which are crustal, motor vehicle, coal combustion, oil combustion, biomass burning, and cement factory. All these sources account for the 81.2 % of the system variance. For the summer field campaign, five factors were resolved, which are crustal, biomass burning, coal combustion, local mixed, and motor vehicle. Five factors account for the 82.8 % of the total system variance.

Lastly, the spatial distribution of parameters for which calculated EF_c values were greater than 10 and stable carbon and nitrogen isotope ratios was evaluated in this study. The distribution of parameters is distinct from each other. However, for most of the parameters, the hot spots were close to highways and locations that we observe higher concentrations are clustered close to the city center. Also, coal and wood combustion of domestic heating increased As and Se elements concentrations around the village and city center of Bolu.

6. REFERENCES

- Agnihotri R, Mandal TK, Karapurkar SG, Naja M, Gadi R, Ahammed YN, Kumar A, Saud T, Saxena M (2011) "Stable carbon and nitrogen isotopic composition of bulk aerosols over India and northern Indian Ocean", *Atmospheric Environment*, 45:2828-2835.
- Al-Alawi MM and Mandiwana KL (2007) "The use of Aleppo pine needles as a biomonitor of heavy metals in the atmosphere", *Journal of Hazardous Materials*, 148:43-46.
- Al-Khlaifat Al and Al-Khashman OA (2007) "Atmospheric heavy metal pollution in Aqaba city, Jordan, using *Phoenix dactylifera* L. Leaves", *Atmospheric Environment*, 41:8891-8897.
- Atkinson R (2000) "Atmospheric Chemistry of VOCs and NO_x", *Atmospheric Environment*, 34:2063-2101.
- Ballentine DC, Macko SA, Turekian VC, Gilhooly WP, Martincigh B (1996) "Compound specific isotope analysis of fatty acids and polycyclic aromatic hydrocarbons in aerosols: implications for biomass burning", *Organic Geochemistry*, 25:97-104.
- Berg T and Steinnes E (1997) "Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute deposition values", *Environmental Pollution*, 98:61-71.
- Bergamaschi L, Rizzio E, Giaveri G, Loppi S, Gallorini M (2007) "Comparison between the accumulation capacity of four lichen species transplanted to a urban site", *Environmental Pollution*, 148:468-476.
- Billy C, Billen G, Sebilo M, Birgand F, Tournebise J (2008) "Nitrogen isotopic composition of leached nitrate and soil organic matter as an indicator of denitrification in a sloping drained agricultural plot and adjacent uncultivated riparian buffer strips", *Universite' Pierre et Marie Curie, Paris, France*.
- Cao J, Chow JC, Tao J, Lee S, Watson JG, Ho K, Wang G, Zhu C, Han Y (2011) "Stable carbon isotopes in aerosols from Chinese cities: Influence of fossil fuels", *Atmospheric Environment*, 45:1359-1363.
- Cheng Y, Engling G, He KB, Duan FK, Ma YL, Du ZY, Liu JM, Zheng M, Weber RJ (2013) "Biomass burning contribution to Beijing aerosol", *Atmospheric Chemistry and Physics*, 13; 7765-7781.

Chow JC (1995) "Measurement Methods to Determine Compliance with Ambient Air Quality Standards for Suspended Particles", Journal of Air & Waste Management Association, 45: 320–382.

Conti ME and Cecchetti G (2001) "Biological Monitoring: Lichens as Bioindicators of Air Pollution Assessment-a Review", Environmental Pollution, 114:471–492.

Demiray DA, Yolcubal I, Akyol NH, Çobanoğlu G (2012) "Biomonitoring of airborne metals using the Lichen *Xanthoria parietina* in Kocaeli Province, Turkey", Ecological Indicators.

Dolinova J, Klanova J, Klan P, Holoubek I (2004) "Photodegradation of organic pollutants on the spruce needle wax surface under laboratory conditions", Chemosphere, 57:1399-1407.

Drobner U and Tyler G (1998) "Conditions controlling relative uptake of potassium and rubidium by plants from soils", Plant and Soil, 201 (2); 285-293.

Emery D, Robinson A, Aplin A (2009) "Inorganic Geochemistry: Applications to Petroleum Geology", Hoboken, NJ, USA, Wiley-Blackwell, 73.

Fisseha R, Saurer M, Jaggi M, Siegwolf RTW, Dommen J, Szidat S, Samburova V, Baltensperger U (2009) "Determination of primary and secondary sources of organic acids and carbonaceous aerosols using stable carbon isotopes", Atmospheric Environment, 43:431-437.

Freitas MC, Reis MA, Alves LC, Wolterbeek HTh (1999) "Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*", Environmental Pollutant, 106:229 –235.

Fry B (2006) "Stable Isotope Ecology", Springer, USA.

Garty J, Tomer S, Levin T, Lehr H (2003) "Lichens as biomonitors around a coal-fired power station in Israel", Environmental Research, 91:186-198.7

Gietl JK, Lawrence R, Thorge AJ, Harrison RM (2010) "Identification of brake wear particles and derivation of a quantitative tracer for brake dust at a major road", Atmospheric Environment, 44:141-146.

Gueguen F, Stille P, Geagea ML, Perrone T, Chabaux F (2012) "Atmospheric pollution in an urban environment by tree bark biomonitoring – Part II: Sr, Nd and Pb isotopic tracing", Chemosphere, 86:641-647.

Hamzaoglu O, Etiler N, Yavuz CI, Çağlayan Ç (2011) "The causes of deaths in an industry-dense area: example of Dilovası (Kocaeli)", Turk J Med. Science, 41(3): 369-375.

Hannemann B (1973) “Anhangsorgane der Flechten: ihre Strukturen und ihre systematische Verteilung”, *Bibliotheca Lichenologica*, 1:1-123.

Heaton THE (1986) “Isotopes studies of nitrogen pollution in the hydrosphere and atmosphere: a review”, *Chemical Geology*, 59:87-102.

Hietz P, Dünisch O, Wanek W, (2010) “Long-Term Trends in Nitrogen Isotope Composition and Nitrogen Concentration in Brazilian Rainforest Trees Suggest Changes in Nitrogen Cycle”, University of Vienna.

Hoefs J (1997) “Stable Isotope Geochemistry”, Springer-Verlag, Berlin

Hong SK, Lee YI, Yi S, (2011) “Carbon isotopic composition of terrestrial plant matter in the Upper Cretaceous Geoncheonri Formation, Gyeongsang Basin, Korea: Implications for Late Cretaceous palaeoclimate on the East Asian continental margin”, *Republic of Korea*, 305-350.

Hou W, Lian B, Dong H, Jiang H, Wu X (2011) “Distinguishing ectomycorrhizal and saprophytic fungi using carbon and nitrogen isotopic compositions”, *China University of Geosciences, Geoscience Frontiers*, 1-6.

Jackson BP and Bertsch PM (2001) “Determination of Arsenic Speciation in Poultry Wastes by IC-ICP-MS” *Environ. Sci. Technol.*, 35 (24): 4868–4873.

Jeran Z, Mtrak T, Jacimovic R, Batic F, Kastelec D, Mavsar R, Simoncic P (2007) “Epiphytic lichens as biomonitors of atmospheric pollution in Slovenian forests”, *Environmental Pollution*, 146:324-331.

Karademir A (2006) “Evaluation of the potential air pollution from fuel combustion in industrial boilers in Kocaeli, Turkey”, *Fuel*, 85:1894-1903.

Klanova J, Cupr P, Barakova D, Seda Z, Andel P, Holoubek I (2009) “Can pine needles indicate trends in the air pollution levels at remote sites?” *Environmental Pollution*, :157:3248-3254.

Korontzi S, Macko SA, Anderson IC, Poth MA, (2000) “A stable isotopic study to determine carbon and nitrogen cycling in a disturbed southern Californian forest ecosystem”, *Global Biogeochemical Cycles*, 14(1):177-188.

Kranner I, Beckett RP, Varma AK (2002) “Protocols in Lichenology”, Springer-Verlag Berlin Heidelberg, Germany.

Kucera J (1994) “International Atomic Energy Agency: Applied research on air pollution using nuclear-related analytical techniques”, Report on the first research coordination meeting, Vienna, Austria, 30 March- 2 April 1993, 265-274.

Kuik P and Wolterbeek HTh (1994) “Factor analysis of trace element data from tree-bark samples in the Netherlands”, *Environmental Monitoring and Assessment*, 32:207.

Krouse HR (1997) "Sulphur isotope tracing of the fate of emissions from sour gas proceedings in Alberta, Canada. In: Krouse HR and Grinenko VA (Eds.) "Stable Isotopes in the Assessment of Natural and Anthropogenic Sulphur", Wiley, London, England, pp.309-326.

Kwak JH, Choi WJ, Lim SS, Arshad MA (2009) " $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, N concentration and Ca-to-Al ratios of forest samples from *Pinus densiflora* stands in rural and industrial areas", *Chemical Geology*, 264:385-393.

Lange OL and Ziegler H (1986) "Different limiting processes of photosynthesis in Lichens", *Martinus Nijhoff Publishers, Dordrecht*, pp.147-161.

Li X, Dai S, Zhang W, Li T, Zheng X, Chen W (2014) "Determination of As and Se in coal and coal combustion products using closed vessel microwave digestion and collision/reaction cell technology (CCT) of inductively coupled plasma mass spectrometry (ICP-MS)", *International Journal of Coal Geology*, 124:1-4.

Liu XY, Xiao HY, Liu CQ, Li YY (2007) " $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of moss *Haplocladum microphyllum* (Hedw.) Broth. for indicating growing environment variation and canopy retention on atmospheric nitrogen deposition", *Atmospheric Environment*, 41:4897-4907.

Liu XY, Xiao HY, Liu CQ, Li YY, Xiao HW, Wang YL (2010) "Response of stable carbon isotope in epilithic mosses to atmospheric nitrogen deposition", *Environmental Pollution*, 158:2273-2281.

Malaspina P, Giordani P, Modenesi P, Abolmoschi ML, Magi E, Soggia F (2014) "Bioaccumulation capacity of two chemical varieties of the lichen *Pseudevernia furfuracea*", *Ecological Indicators*, 45:605-610.

Markert B (1993) "Plants as biomonitors- indicators for heavy metals in the terrestrial environment", *VCH Publishers: Weinheim*.

Manninen S, Huttunen S, Torvela H (1991) "Needle and lichen sulphur analyses on two industrial gradients", *Water, Air and Soil Pollution*, 59:153-163.

Minganti V, Capelli R, Drava G, De Pellegrini R, Brunialti G, Giordani P, Modenesi P (2003) "Biomonitoring of trace metals by different species of lichens (*Parmelia*) in North-West Italy", *Journal of Atmospheric Chemistry*, 45: 219-229.

Monaci F, Moni F, Lanciotti E, Grechi D, Bargagli R (2000) "Biomonitoring of airborne metals in urban environments: new tracers of vehicle emission, in place of lead", *Environmental Pollution*, 107:321-327.

NAVFAC (2007) "Biomonitoring: Guide for the Use of Biological Endpoints in monitoring species, habitats, and projects, by NAVFAC Risk Assessment Workgroup and Argonne Laboratory POC: Amy Hawkins", *Technical Report, TR-2284-ENV*.

Nimis PL, Lazzarin G, Lazzarin A, Skert N (2000) “Biomonitoring of trace elements with in Veneto (NE Italy)”, *The Science of Total Environment*, 255:97-111.

Nimis PL, Andreussi S, Pittao E (2001) “The performance of two lichen species as bioaccumulators of trace metals”, *Science of the Total Environment*, 275: 43-51.

Nriagu JO (1989) “A global assessment of natural sources of atmospheric trace metals”, *Nature*, 338:47-49.

Ortiz HC (2007) “Carbon isotopic composition of plant-derived organic matter in tropical sedimentary sequences as a recorder of Late Cretaceous-Early Paleogene changes in the carbon cycle”, Iowa State University, Ames, Iowa.

Öztürk F, Zararsız A, Dutkiewicz VA, Husain L, Hopke PK, Tuncel G (2012) “Temporal variations and sources of Eastern Mediterranean aerosols based on a 9-year observation”, *Atmospheric Environment*, 61:463-475.

Pacyna JM and Pacyna EG (2001) “An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide”, *Environmental Reviews*, 9:269-298.

Pacyna JM and Otter B (1989) “Control and fate of atmospheric trace metals: NATO ASI Series: Series C: Mathematical and Physical Sciences, Kluwer Academic Publishers in Netherlands, 268:321-338.

Padilla KL and Anderson KA (2002) “Trace element concentration in tree-rings biomonitoring centuries of environmental change”, *Chemosphere*, 49:575-585.

Robinson D (2001) “ $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle”, *Trend in Ecology and Evolution*, 16:153-162.

Salanki J (1985) “Biological monitoring of the state of the environment: Bioindicators: An overview of the IUBS programme on bioindicators”, IUBS Monograph Series No.1, IRL pres in UK.

Scerbo R, Ristori T, Possenti L, Lampugnani L, Barale R, Barghigiani C (2002) “Lichen (*Xanthoria parietina*) biomonitoring of trace element contamination and air quality assessment in Pisa Province (Tuscany, Italy)”, *The Science of the Total Environment*, 286:27-40.

Schroeder W and Munthe J (1998) “Atmospheric Mercury- an overview”, *Atmospheric Environment*, 32(5):809-822.

Shah P, Strezov V, Stevanov C, Nelson PF (2007) “Speciation of Arsenic and Selenium in coal combustion products”, *Energy and Fuels*, 21:-506-512.

Simoneit BRT (1997) "Compound-specific carbon isotope analyses of individual long-chain alkanes and alkanolic acids in Harmattan aerosols", *Atmospheric Environment*, 31(15):2225-33.

Sloof JE (1993) Environmental lichenology biomonitoring trace element air pollution, Ph.D. Thesis, Delft University of Technology, The Netherlands.

Steinnes E (1995) "A critical evaluation of the use of naturally growing moss to monitor the deposition of atmospheric metals", *The Science of the Total Environment*, 160/161:243-249.

Szczepaniak K and Biziuk M (2003) "Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution", *Environmental Research*, 93:221-230.

Van Der Wat L and Forbes PBC (2015) "Lichens as biomonitors for organic air pollutants", *Trends in Analytical Chemistry*, 64:165-172.

Van Dobben HF and Ter Braak CJF (2007) "Ranking of Epiphytic Lichen Sensitivity to Air Pollution Using Survey Data: A Comparison of Indicator Scales", *The Lichenologist*, 31:27-39.

Voutsas D, Samara C, Kouimtzis TH, Ochsenkühn K (2002) "Elemental composition of airborne particulate matter in the multi-impacted urban area of Thessaloniki, Greece", *Atmospheric Environment*, 36:4453-4462.

Wang LK, Chen JP, Hung YT, Shammes NK (2009) *Heavy Metals in the Environment*, CRS Press, Taylor and Francis Group.

Wittig R (1993) "General aspects of biomonitoring heavy metals by plants. In: Markert B (ed) *Plants as biomonitors - Indicators for heavy metals in the terrestrial environment*", VHC, Weinheim, 3-27.

Wolterbeek B (2002) "Biomonitoring of trace element air pollution: principles, possibilities and perspectives", *Environmental Pollution*, 120:11-21.

Wolterbeek HT, Bode P (1995) "Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution", *Science of the Total Environment*, 176:33-43.

Wolterbeek HT and Freitas MC (1999) "Preface", *Science of the Total Environment*, 232:1-2.

Wytenbach A, Bajo S, Furrer V, Langenauer M, Tobler L (1997) "The accumulation of arsenic, bromine and iodine in needles of Norway spruce (*Picea Abies* [L.] Karst.) at sites with low pollution", *Water, Air, and Soil Pollution*, 94:417-430.

Xie R, Seip HM, Wibetoe G, Nori S, Mcleod CW (2006) "Heavy coal combustion as the dominant source of particulate pollution in Taiyuan, China, corroborated by high concentration of Arsenic and Selenium in PM₁₀", Science of the Total Environment, 370:409-415.

Yenisoy-Karakaş S (2000) Biomonitoring of atmospheric pollutants at western anatolia, Doctor of Philosophy thesis, the department of chemistry, Middle East Technical University, Ankara.

Yenisoy-Karakaş S and Tuncel SG (2004) "Geographic patterns of elemental deposition in the Aegean region of Turkey indicated by the lichen, *Xanthoria parietina* (L.)", Th. Fr. Sci. Total Environment, 329:43-60.

7. APPENDIXES

APPENDIX I: Sampling information for winter field campaign

Sampling Point	Latitude	Longitude	Sampling Point	Latitude	Longitude
1	40,85	31,73	52	40,73	31,75
2	40,68	31,57	53	40,74	31,74
3	40,86	31,82	54	40,76	31,76
4	40,78	31,68	55	40,70	31,65
5	40,74	31,56	56	40,76	31,81
6	40,79	31,69	57	40,78	31,87
7	40,77	31,51	58	40,70	31,52
8	40,77	31,71	59	40,82	31,78
9	40,78	31,71	60	40,81	31,74
10	40,70	31,54	61	40,73	31,67
11	40,67	31,48	62	40,72	31,49
12	40,76	31,62	63	40,75	31,54
13	40,83	31,70	64	40,83	31,76
14	40,85	31,79	65	40,67	31,52
15	40,79	31,79	66	40,75	31,80
16	40,83	31,69	67	40,82	31,75
17	40,87	31,80	68	40,75	31,60
18	40,73	31,47	69	40,76	31,69
19	40,71	31,47	70	40,73	31,49
20	40,77	31,67	71	40,71	31,74
21	40,72	31,44	72	40,79	31,85
22	40,76	31,74	73	40,70	31,57
23	40,77	31,61	74	40,79	31,78
24	40,74	31,54	75	40,71	31,52
25	40,70	31,63	76	40,77	31,77
26	40,71	31,61	77	40,75	31,53
27	40,69	31,63	78	40,66	31,44
28	40,68	31,59	79	40,69	31,54
29	40,71	31,50	80	40,71	31,69
30	40,86	31,79	81	40,73	31,65
31	40,69	31,55	82	40,73	31,66
32	40,72	31,75	83	40,84	31,75
33	40,84	31,72	84	40,82	31,76
34	40,77	31,63	85	40,68	31,55
35	40,70	31,60	86	40,79	31,77
36	40,72	31,58	87	40,70	31,62
37	40,75	31,56	88	40,73	31,73
38	40,71	31,54	89	40,70	31,68
39	40,77	31,82	90	40,79	31,87
40	40,81	31,75	91	40,78	31,80
41	40,70	31,46	92	40,71	31,70
42	40,75	31,67	93	40,68	31,60
43	40,77	31,86	94	40,74	31,74
44	40,72	31,62	95	40,78	31,62
45	40,77	31,82	96	40,77	31,84
46	40,78	31,89	97	40,79	31,62
47	40,72	31,68	98	40,72	31,56
48	40,76	31,52	99	40,70	31,67
49	40,71	31,72	100	40,75	31,66
50	40,69	31,58	101	40,73	31,52
51	40,70	31,56			

APPENDIX II: Sampling information for summer field campaign

Sampling Point	Latitude	Longitude	Sampling Point	Latitude	Longitude
1	40,70	31,57	33	40,76	31,54
2	40,68	31,55	34	40,77	31,71
3	40,82	31,78	35	40,77	31,63
4	40,68	31,59	36	40,68	31,57
5	40,77	31,83	37	40,65	31,63
6	40,73	31,71	38	40,75	31,80
7	40,67	31,43	39	40,86	31,80
8	40,77	31,81	40	40,78	31,68
9	40,71	31,72	41	40,84	31,75
10	40,71	31,47	42	40,83	31,72
11	40,66	31,47	43	40,79	31,79
12	40,73	31,65	44	40,71	31,52
13	40,66	31,44	45	40,78	31,88
14	40,73	31,67	46	40,82	31,75
15	40,77	31,79	47	40,76	31,56
16	40,72	31,44	48	40,67	31,49
17	40,67	31,48	49	40,70	31,63
18	40,73	31,54	50	40,79	31,71
19	40,73	31,50	51	40,83	31,79
20	40,73	31,47	52	40,79	31,61
21	40,71	31,69	53	40,80	31,71
22	40,70	31,54	54	40,80	31,65
23	40,78	31,50	55	40,73	31,78
24	40,77	31,86	56	40,67	31,52
25	40,79	31,76	57	40,82	31,71
26	40,69	31,65	58	40,74	31,81
27	40,67	31,57	59	40,77	31,61
28	40,79	31,85	60	40,67	31,51
29	40,73	31,61	61	40,72	31,74
30	40,70	31,62	62	40,79	31,81
31	40,81	31,65	63	40,75	31,66
32	40,75	31,75	64	40,79	31,70

8. CURRICULUM VITAE

Name SURNAME : Uğur SAKLANGIÇ

Place and Date of Birth : Edremit, 04.01.1988

Universities Bachelor's Degree : Abant İzzet Baysal University, Department of
Chemistry

E-mail : ugursaklangic@gmail.com

Address : Tuzcumurat Mahallesi, 121. Sokak, No:38
Edremit / Balıkesir, Türkiye