MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

INVESTIGATION OF GENETIC DIVERSITY, HEAVY METAL AND MINERAL NUTRIENT STATUS OF *Robinia pseudoacacia* L. PLANTS COLLECTED FROM URBAN ECOSYSTEMS

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Ph.D. THESIS
Department of Biology

Thesis Supervisor
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M. Emin URAS, a Doctor of Philosophy student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended his thesis entitled "**INVESTIGATION OF GENETIC DIVERSITY, HEAVY METAL AND MINERAL NUTRIENT STATUS OF *Robinia pseudoacacia* L. PLANTS COLLECTED FROM URBAN ECOSYSTEMS**", on July 20, 2017 and has been found to be satisfactory by the jury members.

Jury Members

Prof. Dr. Ibrahim Ilker OZYIGIT Marmara University	(Advisor)	
Prof. Dr. Nermin GOZUKIRMIZI Istanbul University	(Jury Member)	
Prof. Dr. Celal YARCI Marmara University	(Jury Member)	
Prof. Dr. Goksel DEMİR Kirklareli University	(Jury Member)	
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APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that M. Emin URAS be granted the degree of Doctor of Philosophy in Department of Biology, Biology Program on 07.08.2017 (Resolution no: 2017/18-04

Director of the Institute

Prof. Dr. Uğur YAHŞİ
Müdür

ACKNOWLEDGMENT

I would like to thank Dr. Ibrahim Ilker Ozyigit for his patience, wisdom and genuine friendship. He was a great mentor with his advices and experiences. He spent great effort to advance me in academic life. Again I would like to thank him for supporting attitude, endurance and patience. I would also like to thank my co-advisor Dr. Ertugrul Filiz. He has been a great mentor and a teacher to me. I consider it a chance to be a student of Dr. Ozyigit and Dr. Filiz. I want to become a great teacher and adviser like them.

I would like to thank Dr. Celal Yarci, Dr. Nermin Gozukirmizi, Dr. Göksel Demir, Dr. Bahattin Yalcin, Dr. Filiz Vardar, Dr. Cenk Sesal and Dr. Zeki Severoglu for their great support and guidance. I thank to all of my friends in our research group especially Dr. Recep Vatansever, Ibrahim Ertugrul Yalcin, Ahmet Yilmaz and Ugur Sen for suppoting me throughout my studies during PhD thesis.

I want to express my gratitude to my family especially my wife and children for their endless patience during my PhD thesis.

This thesis was supported by Marmara University BAPKO division with project number of FEN-C-DRP-050614-0241.

In addition, following conference papers have been presented from this thesis;

1. I. I. Ozyigit, M. E. Uras, U. Sen, I. E. Yalcin, R. Vatansever, S. Karadeniz, F. Tabanli, E. Filiz, G Demir, Utilization of a well-known biomonitor plant *Robinia pseudoacacia* L. to evaluate heavy metal pollution levels in Istanbul/Turkey, International Conference on Environment and Natural Science (ICENS) Singapore April 2, 2016, (Oral Presentation).

2. M. E. Uras, E. Filiz, I. I. Ozyigit, U. Sen, Assessment of Genetic Diversity and Phylogenetic Analyses of *Robinia pseudoacacia* L. Collected from Urban Ecosystem, XIII. Congress of Ecology and Environment with International Participation, UKECEK-2017.

July, 2017 **M. Emin URAS**

TABLE OF CONTENTS

ABSTRACT

INVESTIGATION OF GENETIC DIVERSITY, HEAVY METAL AND MINERAL NUTRIENT STATUS OF *Robinia pseudoacacia* L. PLANTS COLLECTED FROM URBAN ECOSYSTEMS

Fabaceae family member *Robinia pseudoacacia* L. is a deciduous tree, which is native to North America and has been widely planted in many parts of the world, especially in Europe, Southern Africa and Asia. Although it is considered as an invasive plant species, it has been widely used for shelter belt and land reclamation purposes. Also, this plant species is accepted as a biomonitor plant. In this study, heavy metal pollution levels of Istanbul and Kocaeli provinces were measured by using this biomonitor plant species that were collected in four different seasons, and the effects of pollution on mineral nutrient status of this plant were determined. For this purpose, fresh leaf, branch and bark samples of *R. pseudoacacia* plants and their co-located soils were collected from Prince Island (control), Bagdat Avenue, Barbaros Boulevard, TEM highway (dense traffic) and Dilovasi District (industrial). Determination of some mineral elements and heavy metals (B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) were conducted by using ICP-OES. Photosynthetic pigment and total protein contents were determined as well. Additionally, some genetic analysis were performed to reveal phylogenetic relations and genetic similarity among studied *R. pseudoacacia* genotypes. ITS1 and *trnL* - *trnF* intergenic spacer sequences, and ISSR band data were employed for genetic analysis. DNA isolation was done by using CTAB method with some modifications.

Two different types of seasonal variations on element content were observed. According to this, B, Ca, Cd, Cr, Cu, Fe, Mg, Mn and Pb concentrations were grouped within the same pattern with an increase in spring and autumn, and a decrease in summer and winter. On the other hand, K, Na, Ni and Zn grouped in another pattern with a decrease from summer to winter, and an increase in spring after winter. Total protein concentrations were observed as the highest in autumn, while relatively lower in spring and summer. Additionally, there were some fluctuations in photosynthetic pigment concentrations in leaf samples collected from different stations in three different seasons.

ITS1 and *trnL* - *trnF* intergenic spacer sequences analysis showed that *R. pseudoacacia* genotypes have a high level of interspecific genetic similarity. They were also included as a subgroup in the same clade with other *Fabaceae* member genotypes when compared with other species. According to the ISSR based Principal Component Analysis test, three subplots were obtained. While one comprised the genotypes collected from Bagdat Avenue, Barbaros Boulevard, Prince Island and Dilovası Disctrict, one other comprised only genotypes of TEM Highway and the third one comprised four genotypes of Dilovası district.

According to the results, it can be proposed that ISSR molecular markers, nuclear ITS1, and chloroplast *trnL* - *trnF* intergenic spacer sequences are effective genetic tools to analyze *R. pseudoacacia* genotypes in genetic studies.

Keywords: Biomonitoring, pollution, soil, heavy metal, mineral nutrient, molecular markers, phylogeny, ITS1, *trnL* - *trnF* intergenic spacer

July, 2017 **Mehmet Emin URAS**

ÖZET

KENTSEL EKOSİSTEMLERDEN TOPLANAN *Robinia pseudoacacia* L. BİTKİLERİNİN GENETİK ÇEŞİTLİLİĞİNİN, AĞIR METAL VE MİNERAL ELEMENT DURUMUNUN İNCELENMESİ.

Fabaceae familyasının bir üyesi olan *Robinia pseudoacacia* L., Kuzey Amerika'ya özgüdür ve dünyanın birçok yerinde, özellikle Avrupa, Güney Afrika ve Asya'da dikimi yapılan yaprak döken bir ağaçtır. Bir istilacı bitki türü olarak kabul edilmesine rağmen, erozyondan koruma ve arazi ıslahı amacıyla yaygın bir şekilde kullanılmaktadır. Ayrıca, bu bitki türünün biyomonitor olarak kullanılabileceği kabul edilmiştir. Bu çalışmada, İstanbul ve Kocaeli illerinin ağır metal kirlilik düzeyleri, dört ayrı mevsimde toplanan bu biyomonitor bitki örnekleri kullanılarak ölçülmüş ve kirliliğin bu bitkinin mineral besin durumu üzerine olan etkileri saptanmıştır. Bu amaçla Büyük ada (kontrol), Bağdat Caddesi, Barbaros Bulvarı, TEM karayolu (yoğun trafik) ve Dilovasi (endüstriyel) bölgelerinden *R. pseudoacacia* bitkisinin taze yaprak, dal ve kabuk örnekleri ile bitkilerin yetiştiği bölge toprakları toplandı. Bazı mineral element ve ağır metallerin (B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb ve Zn) miktarının belirlenmesi ICP-OES cihazı kullanılarak gerçekleştirildi. Fotosentetik pigment ve toplam protein içeriği de tespit edildi. Ayrıca, incelenen *R. pseudoacacia* genotipleri arasındaki filogenetik ilişkileri ve genetik benzerlik oranını ortaya çıkarmak için bazı genetik analizler de yapılmıştır. Genetik analizlerde, ITS1 ve *trnL-trnF* intergenik ara bölgesi dizileri ve ISSR bant verileri kullanılmıştır. DNA izolasyonu, bazı değişiklikler yapılarak CTAB yöntemi kullanılarak gerçekleştirilmiştir.

Bitki mineral element içeriklerinde iki farklı mevsimsel varyasyon gözlemlenmiştir. Buna sonuçlara göre, B, Ca, Cd, Cr, Cu, Fe, Mg, Mn ve Pb konsantrasyonları, benzer şekilde ilkbahar ve sonbaharda artarak, yaz ve kış mevsiminde ise azalarak aynı grupta toplanmıştır. Öte yandan, K, Na, Ni ve Zn, yaz mevsiminden kış mevsimine düşüş, kıştan sonra ilkbaharda bir artış göstererek başka bir grup oluşturmuştur. Toplam protein konsantrasyonlarının sonbaharda en yüksek seviyeye ulaştığı, bahar ve yaz aylarında ise göreceli olarak daha düşük seviyede olduğu tespit edilmiştir. Ayrıca, farklı istasyonlardan üç mevsimde toplanan yaprak örneklerinin fotosentetik pigment konsantrasyonlarında bir miktar dalgalanma tespit edilmiştir.

ITS1 ve *trnL-trnF* intergenik ara bölgesi dizilerinin analizi, *R. pseudoacacia* genotiplerinin, türlerarası genetik benzerlik düzeyinin yüksek olduğunu göstermiştir. Diğer bazı türler ile karşılaştırıldığında, filogenetik ağaçta *Fabaceae* familyasının bir alt grubu olarak, familyanın diğer üyeleriyle aynı grup içinde yer almıştır. ISSR verilerine dayanan Temel Bileşen Analizi (Principal Component Analysis-PCA) testine göre, üç subplot elde edilmiştir. Biri Bağdat Caddesi, Barbaros Bulvarı, Büyük Ada ve Dilovası'ndan toplanan genotiplerden ibaretken, diğeri yalnızca TEM Otoyolu genotiplerinden ve üçüncüsü de Dilovası bölgesinin dört genotipinden oluşmaktadır.

Elde edilen sonuçlara göre, ISSR moleküler işaretleyicisi, çekirdek ITS1 dizisi ve kloroplast *trnL - trnF* intergenik ara bölgesi dizisi, genetik çalışmalarda *R. pseudoacacia* genotiplerini analiz etmek için etkili genetik araçlar olarak önerilebilir.

Anahtar kelimeler: Biomonitör, kirlilik, toprak, ağır metal, mineral besin, moleküler işaretleyici, filogeni, ITS1, *trnL – trnF* intergenik ara bölgesi

Temmuz, 2017 **Mehmet Emin URAS**

CLAIM FOR ORIGINALTY

In this study, some parts (leaves, branch and barks) of *Robinia pseudoacacia* L. trees, which have already been proven as a biomonitor plant species by different researches were used for monitoring seasonal pollution levels of Istanbul, which is one of the biggest metropolitans of the world, during 2014-2015 vegetation periods. In addition to measurement of heavy metals in both plant and soil samples, mineral nutrient status of tree parts were also measured to compare the effects of accumulated heavy metals in different stations in different pollution levels. Although only one season, especially summer was preferred in most of the previous studies, in this study, samplings were done during all four seasons. Additionally, some pigment concentrations of the leaves were measured to see the effects of the pollution in different stations.

Also, plant samples were collected from 5 different stations as individuals of tree populations of these stations, and filogenetic relations and genetic diversity of these populations were studied in molecular levels.

Therefore, this study conducts the similar studies in conjunction with its obtained original and new data. Hereby, we declare that this study comprises our original work. Any material in this study has never been previously published by another person or research group. Additionally, we further declare that this study does not contain any materials, which have been submitted for any degrees or diplomas, or other qualifications at another university.

July, 2017 **Mehmet Emin URAS**

SYMBOLS

%	: Percentage
µl	: Microliter
µM	: Micromolar
cm	: Millimeter
g	: Gram
g/cm^3	: Gram per cubic centimeter
M	: Molarity
ml	: Aluminum
mm	: Centimeter
^{o}C	: Celsius
rpm	: Revolutions per minute
V	: Voltage
v	: Volume
w	: Weight

ABBREVIATIONS

AFLP	: Amplified
Ag	: Silver
Al	: Aluminum
As	: Arsenic
ATP	: Adenosintriphosphat
B	: Boron
Ca	: Calcium
Cd	: Cadmium
Cl	: Chloride
Co	: Cobalt
Cu	: Copper
DNA	: Deoxyribonucleic acid
DOIZ	: Dilovasi Organised Industrial Zone
DW	: Dry Weight
Fe	: Iron
GPS	: Global Positioning System
Hg	: Mercury
In	: Indium
ISSR	: Inter simple sequence repeat
ITS	: Internal transcribed spacer
K	: Potassium
MEHMA	: Mineral Element and Heavy metal Analysis
Mg	: Magnesium
ML	: Maximum Likelihood
Mn	: Manganese
Mo	: Molybdenum
Na	: Sodium
Ni	: Nickel
P	: Phosphorus
PCR	: Polymerase chain reaction
PEP	: Phosphoenolpyruvate

PPA : Photosynthesis pigment analysis

RAPD : Random amplified polymorphic DNA

RNA : Ribonucleic acid

ROS : Reactive oxygen species

RuBP : Ribulose 1,5-bisphosphate

Se : Selenium

TPA : Total protein analysis

Zn : Zinc

TPA : Total protein analysis

NCBI : National Center of Biotechnology Information

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1 Plant Mineral Nutrition, Heavy Metals and Environmental Pollution

Some elements have great importance in plant life cycle while some others are toxic, and exposure to these elements can be potentially harmful for plants. Plants uptake all the essential elements from the environment where they grow. Therefore it is a necessity to protect the environment. Mineral elements and heavy metals are natural components of the environment whose levels can be affected by athropogenic activities. It should also be noted that any problem in an environment could also affect every living member of that ecosystem. For instance, any threat to plants not only affects the plants but also decomposers, animals and eventually humans. Environmental pollution is one of the serious threats in every ecosystem. Thus, environmental studies like pollution monitoring, and recreational or bioremediation studies are important in terms of environmental sustainability. Bioindicator organisms could be effectively used in monitoring the pollution levels in an environment in addition to analytical methods and instruments such as chemical or physical detectors, and electrical and nonelectrical equipment.

1.1.1 Environmental pollution

Environmental pollution is a major problem threatening the future of humanity. Pollution and pollution-borne diseases have sharply increased since the beginning of this century, and this situation not only has been a threat to public health but also to plants, animals, microorganisms and to all ecosystems. Pollution can be defined as undesired changes in biological, chemical and physical composition of soil, water and air (Kilinc and Kutbay, 2008). Heavy metal exposure is regarded as one of the most dangerous pollution types in terms of consequences on biological life. In soil, heavy metals occur as natural components but due to anthropogenic activities, heavy metal levels has been significantly increased in all ecosystems (Das et. al. 2014). Thus, their deposition has led to the formation of many serious problems. For example, it has been reported by several authors that increased level of heavy metals can cause severe diseases like cancer or poisoning (Järup, 2003). Heavy metals and other pollutants may enter enter food chains from lower levels and then be transferred to the upper levels, jeopardizing all food chains. In most cases, the main way of exposure to toxic elements has been through dietary intake (Bo et. al., 2009; Das et. al. 2014).

Although there has not been a precise definition for heavy metal, they are generally defined by their density. The most frequently used definition for heavy metals is that “heavy metals are metals or metalloids which have density more than 5g/cm^3”. (Järup et. al. 2003) Some heavy metals are either essential such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) or relatively harmless such as silver (Ag) and indium (In). But most heavy metals have detrimental effects on living organisms. The most deleterious heavy metals are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) (Järup et. al. 2003; Wuana and Okieimen 2011; Tchounwou et. al. 2012).

Heavy metals can be divided into three groups; (i) essential for plant growth and development like Fe, Cu, Zn, Mn and Mo, (ii) beneficial for plant growth such as Ni and Si, and (iii) directly toxic to plants like As, Pb, Cd, Cr and Hg (Ozyigit et al., 2013).

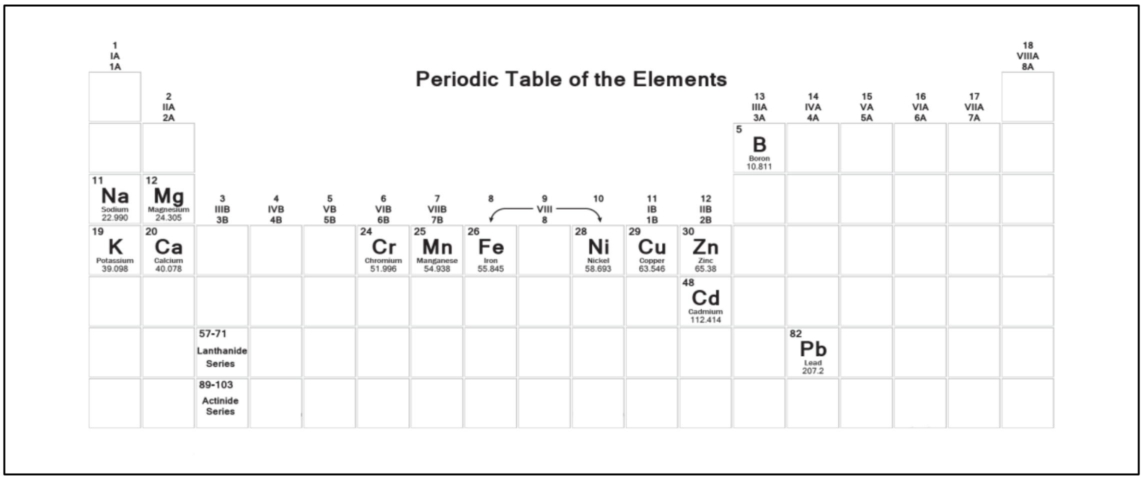


Figure 1.1. Places of studied mineral nutrient elements and heavy metals on periodic table.

Heavy metals have been widely used in various applications for almost 5000 years. For example, lead pipes were used in water transportation in ancient Rome; Hg was used as remedy for healing syphilis; Cd was used as chemical component in dyes; and As was used in wood preservation. Although hazards of heavy metals have been known from ancient times, first methodological and scientific studies were published in late 1800s (Järup et. al. 2003).

Rapid industrialization, population growth, increased consumption and environmental negligence are main reasons of pollution (Das et. al. 2014). Heavy metals can easily spread throughout the environment and can even be detected in indoor dust forms at homes, schools and offices (Kurt-Karakus, 2012). Emissions of heavy metals could be in various ways such as mine tailings, electronic and metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Wuana and Okieimen 2011). Some potential heavy metal sources are shown in Table 1.1. Although emissions and deposition of heavy metals have accelerated throughout the 20th century, it started to decrease in developed countries in last decades (Järup et. al. 2003).

Table 1.1. Some potential heavy metal sources (Markert, 1993; Yasar, 2009)

1. Smoke and particles	• Vehicle (Cd, Pb and Mo) • Fossil fuels (As, Cd, Cr, Cu, Mn, Ni, V, U, Pb, Sr, Zn and Ti) • City and industrial waste (Cd, Cu, Pb, Sn, Hg and V)
2. Industry	• Production of plastic goods (Co, Cr, Cd and Hg) • Textile (Zn, Al, Ti and Sn) • Wood preservation/processing (Cu, Cr and As) • Refinery (Pb, Ni and Cr) • Production of home gadgets (Cu, Ni, Cd, Zn and Sb)
3. Metal and mine industry	• Iron and steel production (Zn, Cu, Ni, Cr and Cd) • Metal processing (Zn, Cu, Ni, Cr, Cd, Hg, Pb and As) • Smelting metals (As, Cd, Hg, Pb, Sb and Se)
4. Agriculture	• Irrigation (Cd, Pb and Zn) • Natural and artificial fertilizers (As, Cd, Cu, Mn, Zn, U and V) • Liming (As and Pb) • Metal corrosion (Fe, Pb and Zn)
5. Wastes	• Sewage (Cd, Cr, Cu, Hg, Mn, Mo, Ni, V, Pb and Zn) • Excavation and drilling (As, Cd, Fe and Pb) • Ash (Cu and Pb)

1.1.2 Biomonitors and biomonitoring

Since the beginning of 1900s, lichens have been used in investigation of air pollution and which is later followed by many other organisms. In this context, a biomonitor has been described as an organism (or part of it) which is able to give information about the quality of environment. Biomonitoring is the continuous observation of relevant environment by employing bioindicators. Biomonitor organisms usually show resistance to accumulation of pollutants at high levels (Figueiredo et al., 2007). They are mainly selected based on criteria such as; (i) they should be distributed all over the relevant area in large quantities, (ii) they could discriminate the airborne and soil-borne pollutants, and (iii) they should easily be recognized and sampled (Aksoy et al., 1999). Fungi, lichens, tree barks and rings, plant leaves, mosses, mollusks and animals have been used for biomonitoring purposes so far. Biomonitoring is a cost effective and environmentally friendly approach (Aksoy, 2008). Aksoy et al. (2000) and Çelik et al. (2005) used *R. pseudoacacia* as a bioindicator for monitoring the heavy metal pollutions in different provinces of Turkey.

1.1.3 Studied mineral nutrient elements and heavy metals

Cd is a heavy metal with 48 atomic number, 112.4 atomic weight, and 8.65 g/cm^3 density (Wuana and Okieimen, 2011). It is naturally present in earth crust with Zn, Pb and Cu. It is a non-essential element and also one of the major three toxic heavy metals which has detrimental effects on all organisms thereby has detrimental effects on all organisms (Järup, 2003; Wuana and Okieimen, 2011; Das et. al. 2014). Cd is used in production of PVC, color pigments, anticorrosion agents, several alloys and Ni-Cd rechargeable batteries. It is also present in detergent, pesticides, phosphate fertilizers and biosolids (sewage sludge) of farmlands. Uptake of Cd rises with lower pH (Järup, 2003; Wuana and Okieimen, 2011). It is a very persistent element and remains in soil for many years. Acute exposure to Cd results with lung inflammation, while chronic exposure results in lung cancer, proteinuria; possible kidney damage and softening of bones. Itai-itai disease, which occurred in the middle of 20th century in Jintsu River Valley, near Fuchu in Japan, is the most dramatic example of Cd poisoning. (Wuana and Okieimen, 2011).

Chromium (Cr) is a heavy metal its accumulation in the environment could threaten ecological life. It has atomic number of 24, atomic weight of 51.996 and density of 7.19 g/cm^3 [61]. There are many oxidation states of Cr, among these Cr (III) ions are considered non-toxic and a biologically active trace element for humans while Cr (IV) and Cr (VI) is highly toxic and carcinogenic [61-67]. Cr (VI) are forceful oxidants at neutral/low pH value [66]. Acute exposure to Cr results in gastrointestinal bleeding, acute renal failure and hemolysis however chronic exposure results with allergic dermatitis, pulmonary fibrosis (lung scarring) and lung cancer ([68]; Wuana and Okieimen, 2011). Chromium is a desirable metal due to its high hardness and resistance to corrosion. It is used in tanning process which causes high amounts of Cr to be released in environment with waste water. In addition there are many more areas that Cr is widely used such as alloys, electroplating, oxidizing agents, pigments for textile glass, ceramic manufacturing, and photography (Yadav, 2010; Wuana and Okieimen, 2011; Das et. al. 2014). Its excess amounts are toxic to plants; causes nutrient imbalances, wilting of tops, inhibition of growth and chlorophyll production and root damages. Accordingly, plants react against toxic Cr with reactive oxygen species (ROS) compounds (Yadav, 2010).

A certain amount of some heavy metals are essential for plants and animals. They are mostly constituents of several key enzymes and structural basic materials. One of the essential heavy metals is Cu which has atomic number of 29, atomic weight of 63.5 and density of 8.96 g/cm^3 (Wuana and Okieimen, 2011). Cu exerts structural, biochemical and physiological functions. Cu serves as a cofactor for oxidative stress-related enzymes including catalase, superoxide dismutase and peroxidase. It also serves as a component of metalloenzymes that take part in hemoglobin production and carbonhydrate metabolism. Cu is also involved in CO_2 assimilation, ATP synthesis, water regulation as well as being an essential component of various compounds in photosynthetic system and respiratory electron transport chain (Yadav, 2010; Wuana and Okieimen, 2011; Tchounwou et. al. 2012). Cu cycles between oxidized Cu (II) and reduced Cu (I) states with redox reactions. During cycle transitions between states the superoxide and hydroxyl radicals are generated. Also, excessive exposure to Cu can cause Wilson disease, anemia, liver and kidney damage, and stomach and intestinal irritation in humans (Wuana and Okieimen, 2011; Tchounwou et. al. 2012).

Fe is also an essential element for plants, animals and microorganisms. It has atomic number of 26, atomic weight of 55.845 and density of 7.87 g/cm^3. Fe is a crucial mineral nutrient element which has a key role in energy transformation reactions and other life processes in cells (Kabata-Pendias and Mukherjee 2007). Iron is a component of hemoglobin and myoglobin, and it is required to make some hormones and connective tissue in humans and animals. It functions in important processes in plants such as photosynthesis, respiration and chlorophyll biosynthesis, as well as being a structural component in heme, Fe-sulfur cluster and other Fe-binding sites (Kobayashi and Nishizawa, 2012). Romheld and Marschner (1991) reported that 400-1000 μg/g of Fe in DW is toxic for plants. Its excess amounts can cause damages in plants due to ROS-derived oxidative stress (Becana, Moran & Iturbe-Ormaetxe 1998). ROS irreversibly deteriorate cellular structures, and damage biological membranes, DNA and proteins. Fe interferes with ion absorption; multiple nutritional disorder of K, P, Ca, Zn and Mg with excessive Fe uptake (Quinet et al. 2012).

Manganese (Mn) is a transition metal and a member of iron family. It has atomic number of 25, atomic weight of 54.938 and density of 7.21 g/cm^3. Mn as ethylcyclopentadienyl manganese tricarbonyl (MMT) is used as gasoline additive replacing lead. It functions as a cofactor for different enzymes such as oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrins. Especially arginase, phosphotransferase, reverse transcriptase and Mn-superoxidedismutase (Mn-SOD) are the best known enzymes including Mn (Law et al., 1998). In addition, Mn can also substitute Mg in various enzymes. There are also some relationships between Mn and N assimilation, photosynthesis and chloroplast structure (Kabata-Pendias and Mukherjee 2007). It is required by all organisms as trace metal but it has also some dangerous aspects as neurotoxin which can cause irreversible damage to mammal's nervous system. Chronic exposure to excessive Mn levels in humans can result in psychiatric and motor disturbances, referred to as manganism which shows symptoms similar to Parkinson's disease and causes death of dopaminergic neuron (Yin et al., 2010).

Nickel (Ni) is also a transition metal with 28 atomic number, 58.693 atomic weight and 8.908 g/cm^3 density. It is an essential trace element required by humans and animals. However, it could be dangerous when maximum tolerable amounts are exceeded (Wuana and Okieimen, 2011; Das et al. 2014). Ni is commonly used in metal industry as ingredient of stainless steels and other metals. Ni alloys which have corrosion and high-temperature resistance are used in aircraft and plating industries, production of magnetic components, electrical equipments and Ni-Cd batteries. In addition, Ni is a common catalyst for hydrogenation and oxidation of various organic compounds (Kabata-Pendias and Mukherjee 2007; Wuana and Okieimen, 2011). It plays a vital role in plants since it is a component of enzyme. The major sources of Ni as a pollutant are metal plating, combustion of fossil fuels and nickel mining. It is discharged into air by power plants and trash incinerators, and remains in air for a long period of time (Wuana and Okieimen, 2011). Ni exposure generally occurs with oral consumption. For instance, dishes containing Ni pigments or made from Ni alloys may release Ni into food. In addition, Ni can be absorbed directly by inhaling cigarette smoke, contact of skin with jewelry, shampoos, detergent and coins. Chronic exposure to Ni may be toxic even carcinogenic (Butticѐ, 2015). Moreover, Ni is one of the most allergic compounds (Thyssen et al. 2007).

Pb is considered as the most hazardous heavy metal for its toxicity. However, it is a useful metal which has a wide variety of applications in different areas (Kabata-Pendias and Mukherjee 2007). It has an atomic number of 82, atomic weight of 207.2 and density of 11.40 g/cm^3 (Wuana and Okieimen, 2011). One of the most important features of Pb is its persistence in environment. Leaded gasoline was the major source of Pb in 1970-80s due to gasoline additives tetraethyl and tetramethyl lead. Later, leaded gasoline was prohibited in developed countries. This prohibition was helped in reducing atmospheric Pb pollution and human blood Pb levels (Becker et al., 2013). Nowadays, Pb emission is caused by production of Pb storage batteries, solders, bearings, cable covers, ammunition, plumbing, paper/pulp, gasoline, caulking, sewage sludge, mining-smelting activities, Pb containing paints and explosives (Kabata-Pendias and Mukherjee 2007; Yadav, 2010; Wuana and Okieimen, 2011).

Lead exposure occurs via inhalation and ingestion. Gastrointestinal system, kidneys and central nervous system are the most effected systems from Pb exposure. Acute exposure to high level of Pb may cause headache, nervousness, abdominal pain, encephalopathy, acute psychosis, confusion, reduced consciousness and proximal renal tubular damage. Long term exposure and bioaccumulation of Pb may cause some effects such as damage to nervous systems, memory deterioration, prolonged reaction time, reduced ability to understand, inhibition of heme formation and synthesis, anemia, kidney damage, impaired mental development of young children, carcinogenicity and genotoxicity, impaired reproductivity, impaired growth, hyperactivity, mental deterioration and lower IQ for children (Järup, 2003; Kabata-Pendias and Mukherjee 2007; Wuana and Okieimen, 2011). In addition, Pb also affects plants via inducing ROS production, morphological and membrane deteriorations, inhibition of enzymes that regulate growth and photosynthetic processes, and water imbalance and disturbance on mineral nutrition (Yadav, 2010).

Zn is a heavy metal which has atomic number of 30, atomic weight of 65.38 and density of 7.14 g/cm^{-3}, and it is chemically similar to Mg. It naturally exists in soil but its levels rise due to anthropogenic and industrial activities such as mining, coal, waste combustion, sewage sludge, urban composts, excessive fertilizers and steel processing. Zinc is a component of various alloys, batteries, automotive equipment, pipes and household devices, and it is widely used as catalyst in production of rubber, pigments, plastic, lubricants, and pesticides. Drinking water stored in metal tanks may also contain high amount of zinc (Kabata-Pendias and Mukherjee 2007; Wuana and Okieimen, 2011). Zn is required by living organisms in small amounts but excessive concentrations could be harmful (Wuana and Okieimen, 2011). Plants utilize Zn in various metabolic functions such as being a co-factor of approximately 300 proteins related to carbohydrate, protein, and phosphate metabolism, and also in auxins, RNA and ribosome formations, and in transcription factors (Kabata-Pendias and Mukherjee 2007; Ricachenevsky et al., 2015). Utilization of zinc fertilizers in long term can affect the plants and cause poisoning. For instance, excessive zinc inhibits photosynthesis, and causes retarded growth, chlorosis in younger leaves and senescence. Excessive zinc gives rise to Mn and Cu deficiency in plant shoots (Kabata-Pendias and Mukherjee 2007). Zinc is also essential for humans. It has structural and catalytic functions in approximately 10% of all human proteins, and takes part in inter/intracellular signaling (Clemens et. al., 2014). It has also important role in human immune system and brain development of fetus (Hafeez et al., 2013).

Boron is a micronutrient for plants, which has an atomic number of 5, atomic weight of 10.81 and density of 2.34 g/cm^3. B has various usages in different areas such as production of fiberglass, borosilicate glass, flame retardant tools, textiles, agricultural fertilizers, pesticides, cosmetics, antiseptics and laundry products. B emissions can occur from natural and artificial sources, which are mainly derived from industrial activities such as mining operations, glass and ceramics industries, chemicals production, and coal fired power plants. It spreads in water resources with sewage outfalls, especially with detergent products, leaching salt deposits and B-fertilizers. (Kabata-Pendias and

Mukherjee 2007). B is an essential microelement for vascular and some aquatic plants. It is involved in carbohydrate metabolism, movement of sugars and other materials, nitrogen fixation, cell division, maintaining the cell wall structure, differentiation, maturation, development and growth. In addition, B is also required in reproduction, pollen tube growth and pollen germination (Blevins and Lukaszewski 1998; Kabata-Pendias and Mukherjee 2007; Gupta and Solanki 2013). B influences calcium metabolism, affects bone growth and central nervous system functions, alleviates arthritic symptoms, facilitates hormone action and helps reduce the risk of some cancer types (Nielsen, 2014). B is an essential element but its high amounts could be toxic. When excessive amount of B is ingested, such symptoms like nausea, vomiting, diarrhea and dermatitis could appear (Kabata-Pendias and Mukherjee 2007).

Ca is an important component of cell as an essential macroelement. It has atomic number of 20, atomic weight of 40.078 and density of 1.55 g/cm^3. Ca is used in various industrial areas such as production of cement, mortar, lime, glass, toothpaste, insecticides and many other products. Ca compound, calcium hydroxide solution [$Ca(OH)_2$] is used as an indicator of CO_2 (Lide, 2005). It serves as a secondary signaling molecule in both animal and plant signaling pathways. As a major structural element, Ca makes up the bones, teeth and shells. Thus, calcium is the most abundant mineral element in mass of most animals (Lide, 2005; Hawkesford et. al., 2012; Steinhorst and Kudla, 2014). Besides, Ca contributes to cell wall and plasma membrane integrity, prevents and reduces detrimental effects of salinity, regulates ion transport, selectivity, cation-anion balance and osmoregulation, controls ion-exchange behavior and enzyme activities (Hawkesford et. al., 2012; Tuna et al., 2007). In addition, Ca plays a vital role in neurotransmitter release from neurons, contraction of all muscle types, and fertilization, serve as a cofactor, and takes part in blood-clotting cascade. Elevated blood Ca levels may result in formation of kidneys stones.

K is a mobile macroelement involved in important metabolic functions. It is an alkali metal which has atomic number of 19, atomic weight of 39.098 and density of 0.862 g/cm^3. Cytosol includes many cations among which potassium is the most abundant one and regulates osmotic potential of the cell with other ions. Among major roles of K are regulations of membrane potential, plant-water interactions, plant movements, cell extension, CO_2 fixation in photosynthesis, and maintaining charge balance and enzyme activation (especially in protein and starch synthesis, as well as in respiratory and photosynthetic metabolism) (Hawkesford et. al., 2012; Benito et al., 2014). K is used in production of agricultural fertilizers containing potassium. Potassium influences multiple physiological processes in humans and animals, including resting cellular-membrane potential, propagation of action potentials in neural, muscular and cardiac tissue, hormone secretion and action, systemic blood pressure control, and mineralocorticoid action (He and MacGregor 2008).

Mg, which is a main structural element in chlorophyll, is a plant macronutrient. It has atomic number of 12, atomic weight of 24.305 and density of 1.738 g/cm^3. Its functions are mainly related to capacity to interact with strongly nucleophilic ligands. Mg interacts with proteins as direct connection or catalytic effect, both makes Mg one of the important homeostatic regulative elements. Chlorophyll is an essential molecule which makes photosynthesis possible, contains magnesium in its molecule center. Furthermore, Mg functions as a cofactor in structure of many important enzymes including glutathione synthase, ATPase, RuBP carboxylase and PEP carboxylase (Hawkesford et. al., 2012). Its excessive amounts cause ROS formation and deficiency of some other elements competing with Mg uptake in plants. Plants over-exposed to Mg demonstrate symptoms of leaf chlorosis, dark inclusions and/or crinkling (Fernando and Lynch 2015). Mg is also essential for humans. It interacts with polyphosphate compounds such as ATP, DNA and RNA. It takes role in enzymatic reactions as a cofactor of hundreds of enzymes. In addition, its involved in bone formation, has roles in nerve and muscle functions, and transcription, and adjusts blood sugar, pressure levels and protein synthesis (Gibson, 2012; Rude, 2012; Volpe, 2012).

Na is an alkali metal belonging group 1 and has atomic number of 11, atomic weight of 22.989 and density of 0.968 g/cm^3. Na is a highly reactive metal and sodium compounds are highly water-soluble, thus Na is one of the most common dissolved elements by weight in oceans. Salinity is a major problem in agricultural areas. Na is a non-essential element for terrestrial plants but it can be beneficial or nutritious for some species. For instance, sodium is a growth promoter for halophytic plants (Maathuis, 2014). Low level of Na can be beneficial at lower K concentrations for plants due to similar atomic structure of K and Na (Amtmann and Sanders, 1999; Maathuis, 2014). Interestingly, low soil sodium makes some crops more tasty (Maathuis, 2014). In humans and animals, Na is essential to maintain and regulate blood volume, blood pressure, osmotic equilibrium and pH. In addition, limited Na increase in fodder plants can help to prevent Na-deficiency in livestock (Maathuis, 2014). Excessive amount of sodium and chlorine induce to osmotic stress by reducing the water potential and production of ROS also can be seen in this condition. (Miller et al., 2010; Maathuis, 2014).

According to literature, scientific researches and publications increase the public awareness and accordingly responsible authorities. Precautions and regulations for avoiding this type of pollution have given favorable results but this situation must improve.

1.1.4 *Robinia pseudoacacia* L.

R. pseudoacacia L. is a perennial tree from *Fabaceae* family. In different languages, it is known as beyaz cicekli yalanci akasya (Turkish), black locust, yellow locust, or false acacia (English), gewöhnliche robinie (German), robinier faux-acacia (French), maruga (Italian), and yáng huái (Chinese). It has a wide distribution range around the world. It can grow in many soil types but particularly well on moist, loamy soils or those of limestone origin. It can survive in various types of climates, particularly preferring humid environment. The main habitat of black locust is North America but due to its invasive and adaptive features it has spread across the world naturally or by humans.

R. pseudoacacia belongs to *Fabaceae* (*Leguminosae*) family which is the third largest family in the world. This family has approximately 760 genera and 19000 species, and includes many economically important species. Along with the *Poaceae* (*Gramineae*) family, *Fabaceae* family is the most important staple food source and agricultural species in the world. *Fabaceae* family comprises three subfamilies such as *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae* (*Faboideae*). Caesalpinioids range in size from shrubs to large trees naturally found in tropical regions and includes approximately 2250 species. The second largest group of legumes is Mimosoids with approximately 3270 species. This group also ranges in size from shrubs to large trees. Mimosoids also have much wider distribution on earth than Caesalipinioids and have vital ecological role in pantropical regions.

Papilionoids form the biggest group of *Fabaceae* family with approximately 13,800 species and they are the most studied group of this family due to their ecological and economic importance (Schwarz et. al., 2015). *Astragalus* (over 2,400 species), *Acacia* (over 950 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species), and *Mimosa* (around 500 species) are largest generas in Papilionoids. The most important agricultural species in this family are *Glycine max* (soybean), *Phaseolus vulgaris* (bean), *Pisum sativum* (pea), *Cicer arietinum* (chickpea), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), *Glycyrrhiza glabra* (liquorice) (Rahman and Parvin, 2014).

Various chemical compounds have been identified in *Fabaceae* family members including several types of alkaloids, non-protein amino acids, amines, flavonoids, isoflavonoids, coumarins, phenylpropanoids, anthraquinones, di-, sesqui- and triterpenes, cyanogenic glycosides, protease inhibitors and lectins (Wink and Mohamed 2003). The subfamily *Papilionoideae* harbors the genus *Robinia*, including species *R. hispida*, *R. luxurians*, *R. neomexicana*, *R. viscosa* and *R. pseudoacacia.* In addition, other major tribes in *Papilionoideae* subfamily includes the *Swartzieae, Sophoreae, Dalbergieae, Amorpheae, Thephrosieae, Indigofereae, Phaseoleae, Desmodieae, Psoraleae, Loteae, Galegeae, Trifolieae, Podalyrieae, Liparieae, Bossiaeae, Crotalarieae, Thermopsideae,* and *Genisteae*

Table 1.2. Classification of *Robinia pseudoacacia* L.

Rank	**Scientific and Common Name**
Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Magnoliopsida* - Dicotyledons
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae/Leguminosae* - Pea family
Subfamily	*Faboideae/ Papilionoideae*
Genus	*Robinia* L. - locust
Species	*Robinia pseudoacacia* L. - black locust

R. pseudoacacia develops extensive radial root systems, about 1-1.5 times the width of its crown. Roots can spread among gullies, which are caused by erosion. It grows to become a medium-sized tree, generally up to 12-18 m in height. Tree barks are smooth and brown during development and become thick later, deeply furrowed, scaly and dark brown. Young branches are thorny (Stone, 2009).

Figure 1.2. General view of *R. pseudoacacia*

R. pseudoacacia has an invasive feature, due to its high growth ability it can spread at very fast rate. It can generate monodominant forests. Wood of *R. pseudoacacia* is durable and solid. So it has numerous usages in different fields. It can be used as timber, fuelwood and in paper production (USDA, NRCS 2016). Wood of *R. pseudoacacia* were reported to be used in ship building due to its water-durable and resistance feature (Veitch et. al. 2010).

R. pseudoacacia can be propagated through seeds and it can be propagated vegetatively by using root or branch cuttings additionally in-vitro propagation can be applied for mass production (David and Keathley 1992).

Figure 1.3. Bark of *R. pseudoacacia*

Moreover, it can be used in soil enrichment studies due to residing endophytic nitrogen binding bacteria within its roots. Also *R. pseudoacacia* can be used for erosion control (USDA, NRCS 2016). Flowers of *R. pseudoacacia* are fragrant and contain rich nectar. Bees visit these flowers and make high quality monofloral honey (Veitch et. al. 2010). *R. pseudoacacia* has also been reported to contain various chemical substances. For example, "robinin (a kaempferol 3,7-di-O-glycoside)" was obtained by Zwenger and Dronke in 1861 (Zwenger and Dronke, 1861; Veitch et. al. 2010). In other studies, different bioactive molecules with antifungal and antimicrobial activities have been identified from *R. pseudoacacia* such as robinlin (antimicrobial), D-pinitol (antifungal), robetrin, myricetin, tannins, flavonoids, flavanonols, polyphenols, dihidrobin, robinetin and quercetin (Tian et al., 2001; Chen and Dai, 2014; Marinas et al., 2014). They were reported to provide protection against pathogens and other biotic stresses. Some plant parts especially bark contains poisonous substances for animals and humans (Veitch et. al. 2010). Furthermore, it is also used for ornamental, shelterbelt, land reclamation and melliferous purposes, and stabilizing abraded fields (Marinas et al., 2014). It has some substances that have allelopathic effects against other species. Barks of *R. pseudoacacia* also have exceptional endurance for deterioration because of substances such as dihidrobin and robinetin.

Figure 1.4. Flowers of *R. pseudoacacia*

Flowers of *R. pseudoacacia* are used in alternative medicine for antispasmodic, anti-gastric acid, sedative and relaxing heat burn purposes, and contents of flowers have antioxidant substances (Marinas et al., 2014). In a previous study, seed proteins of *R. psudoacacia* were reported to have antimicrobial activity against some bacteria including *Corynebacterium michiganense, Staphylococcus aureus, Bacillus subtilis, Erwinia carotovora* subsp. *carotovora, Pseudomonas syringae* pv *syringae* and *Xanthomonas campestris* pv *campestris* (Talas-Oğras et al., 2005).

In general, *R. pseudoacacia* has 2n=20 chromosome numbers but some tetraploid *R. pseudoacacia* trees (4n=40) are also widely cultivated in China (Meng et al., 2014). Its blooming season is spring. Fruits and seeds are persistent and moderately abundant on tree. Flowers of *R. pseudoacacia* are being pollinated by hummingbirds and insects especially honeybees (Stone, 2009). Fruits and seeds begin at spring and end at summer. It can be propagated by seeds, cuttings and bare roots but it has moderate spread with seed (USDA, NRCS 2016). Black locust begins producing seeds at about 6-year-old and seeds disperse with gravity and potentially by birds. Seeds can endure for long periods of time and require scarification and mineral soil for successful germination. Seedlings are intolerant to shade (Stone, 2009). In most cases *R. pseudoacacia* grow fast but it has a short life span, living approximately 90 years but it is recorded that a *R. pseudoacacia* tree planted at 1759 in Kew Royal Botanic Garden, London, England is still alive (Figure 1.5.).

Figure 1.5 Fruit of *R. pseudoacacia*

The assessment of accumulated heavy metals and mineral nutrient elements in plant and soil samples can indicate the pollution status of environment. For this purpose *R. pseudoacacia* is commonly used in heavy metal pollution assessments as a biomonitor plant. Various heavy metals especially Cd and Pb are accumulated by *R. pseudoacacia* in plant parts. Different studies have reported that *R. pseudoacacia* is a good biomonitor organism and can be efficiently used in bioaccumulation, bioremediation and pollution studies (Filipović-Trajković, et. al. 2012; Kaya et. al. 2010; Aksoy et. al. 2000).

Figure 1.6 *R. pseudoacacia*, planted in 1759, near Elizabeth Gate at Kew Royal Botanic Garden, London, England [65]

1.2 Assessment of Genetic Similarities and Phylogenetic Relationships

1.2.1 DNA barcoding and phylogenetics

The identification and distinguishing of species have scientifically begun in 1750s and later it produced a discipline today called “Taxonomy”. At earlier times, taxonomic distinction was mainly based on the morphological and anatomical features of species but over time it has become inefficient/insufficient. Thus, it has been necessary to support the taxonomical tools with other equipment, methods and techniques. The invention of PCR and DNA sequencing technologies has been revolutionary events for not only molecular biologist but also all other researchers who are dealing with all subfields of biology including taxonomy and phylogeny. PCR and DNA sequencing techniques could help identify a particular gene or whole genome sequence, which can be further used to reveal the phylogenetic relationships between species. The logic of DNA barcoding was born from this idea. DNA barcoding lies on the sequencing of some DNA regions in species as an identification tool. Microgenomic identification systems lie on sequencing small segments of DNA which permits the species discrimination. It stands as an extremely promising approach to understand the biological diversity. This approach gains acceptance in identification of protists, bacteria and viruses, which are morphologically located at the very least distinguishable group (Hebert et. al., 2003). DNA sequencing has many advantages upon other conventional PCR-based DNA markers from aspects of reproducibility, reliability, stability and simplicity. There have been many available regions/genes (coding and noncoding sequences) used for these purposes like nuclear internal transcribed spacer (ITS), mitochondrial gene cytochrome c oxidase 1 (*CO1),* plastid sequences; *atpF-atpH* spacer, *matK* gene, *rbcL* gene, *rpoB* gene, *rpoC1* gene, *psbK–psbI* spacer, *trnL-trnF* spacer/genes and *trnH-psbA* spacer. However, selected region of DNA to be sequenced must have three properties such as (i) universality, (ii) sequence quality/coverage and (iii) discrimination power. It must be found in genome of the subject species -universality-, be easily identifiable from both two directions -sequence quality and coverage- and be able to distinguish specimens from each other -discrimination power- (CBOL Group, 2009). For example, ribosomal RNA genes (rRNA) can be used to reveal the ancient relationships between species since these genes have small changes over time. However, genes which change rapidly like mitochondrial and plastid genomes can reveal the divergences between closely related species (Hebert et. al., 2004). Unlike plants, animals have some standard marker like mitochondrial gene cytochrome c oxidase 1 (*CO1*) to understand the phylogenetic relationship. A few candidate genes or regions have been offered as a possible standard marker but none of these were widely accepted by the taxonomic community (CBOL Group, 2009). Besides, nuclear ribosomal DNA (nrDNA) ITS region has been frequently used in determination of phylogenetic relationships between animals, plants, fungi and other life forms. The ITS region has been recently proposed as universal barcode for all fungi by Schoch et al. 2012. In addition, chloroplastic *trnL* - *trnF* spacer was also used to reveal the relationship in plants and other organisms.

1.2.2 Internal transcribed spacer (ITS) as a genetic marker

Ribosomes are very crucial component of cells due to their function to catalyze the synthesis of proteins. Catalytic center of ribosomes primarily consists of ribosomal RNAs (rRNA) and this region is highly conserved from structural and functional aspects. Therefore, rRNA regions have strong primary sequence conservation interspersed with variable regions although that ITS and IGS regions show great divergence between closely related species. The exons of rRNA genes could discriminate the distantly related species while intron regions such as ITS1 and ITS2 can be used in discrimination of closely related species. These characteristics make the rRNA cistrons an ideal molecule to investigate the phylogenetic relations between organisms. Genes which encode rRNA molecules are typically arranged into an operon, with an internally transcribed spacer (ITS) that is also used to discriminate the closely related organisms (Lee et. al., 2009; Porras-Alfaro et. al., 2014). rRNA cistrons are used to reveal the phylogenetic relations in eukaryotes such as animals, plants and fungi, and prokaryotes such as bacteria, Cyanobacteria and archea (Hebert et. al., 2004; Gillespie et. al., 2006; CBOL Group, 2009; Lee et. al., 2009). Ribosomal RNA (rRNA) cistrons are organized in Nucleolus Organizer Region (NOR) in eukaryotes. NOR region contains the tandem repeats of ribosomal genes. The eukaryotic rRNA cistron contains 18S, ITS1, 5.8S, ITS2 and 28S rRNA sequences. Among rRNA cistron these ITS1, ITS2 introns and 5.8S rRNA gene are called ITS region. Following transcription, two of ITS segments (ITS1, and ITS2) are removed via RNA splicing process (Schoch et. al., 2012).

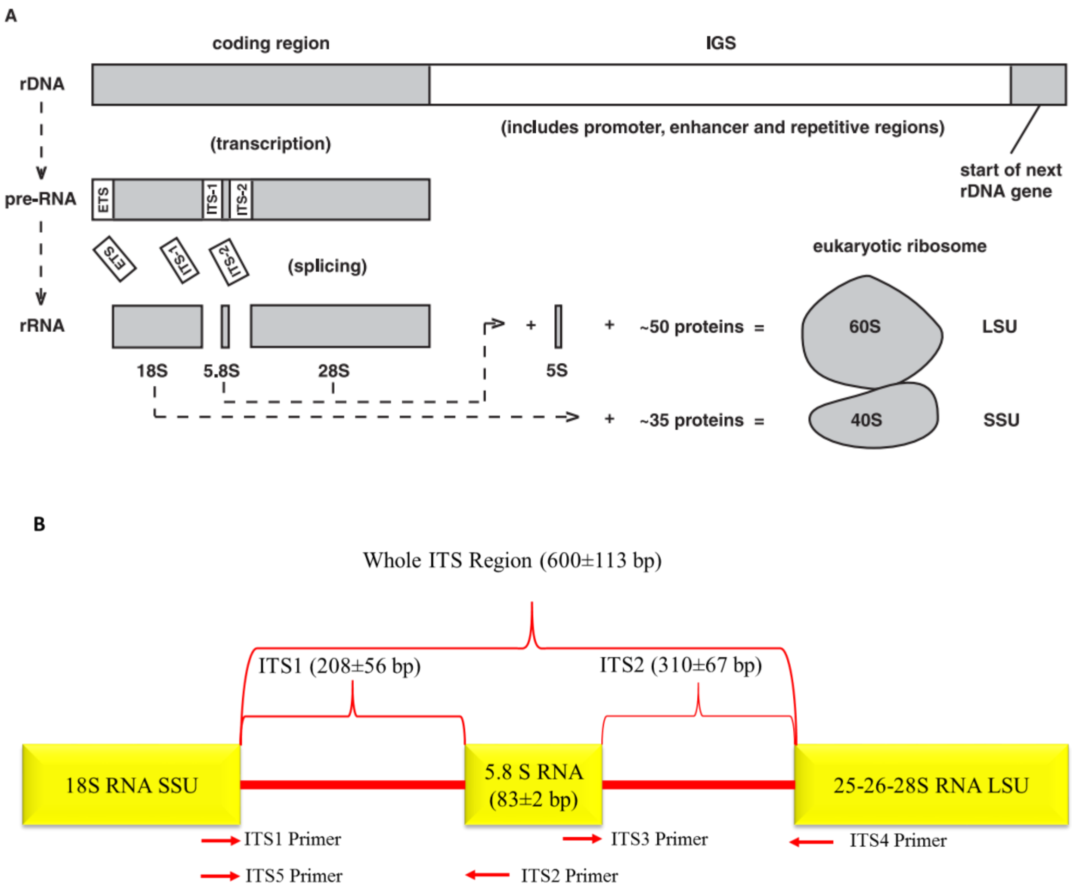


Figure 1.7 (A) Typical organization of nuclear rRNA genes in eukaryotes. (B) Structure of Internal Transcribed Spacer (ITS) region on nuclear DNA ITS. (IGS, Intergenic Spacer; ETS, Externally Transcribed Spacer; SSU, Small Subunit; LSU, Large Subunit.) Figure A and B respectively are modified from Gillespie et al. (2006) and Porras-Alfaro et al. (2014).

1.2.3 Chloroplast *trnL* - *trnF* spacer as phylogenetic marker

DNA sequencing has dramatically changed the world of molecular biology in fields of genome mapping, gene annotation, comparative genome analysis, mutation analysis, phylogenetic analysis *etc.* After the completion of first genome sequencing by Fleischmann et al., (1995), various other organisms have been sequenced subsequently.

The main function of chloroplasts is to fulfill the photosynthesis in presence of sunlight for synthesis of glucose, fatty acids (Stumpf, 2014), pigments (Back et al., 2016), starch and amino acids (Niehaus et al., 2014). The chloroplastic genes could be divided into three categories according to their roles such as photosynthetic genes, ribosomal protein genes (rRNA, tRNA) and genes that are involved in other functions (Xu et. al., 2015). The chloroplast genomes (cpDNA) have remained better conserved than nuclear genomes. The plastid genomes (plastome) consist of a quadripartite structure that contains large single copy region (LSC), a small single copy region (SSC) and two large inverted repeats (IR). They are also highly conserved with respect to their size, gene order and structural organization as well as relatively free of large deletions, insertions, transpositions, inversions and SNPs (single nucleotide polymorphism). Thus, chloroplast genome is useful in phylogenetic studies. All chloroplasts exhibit genome polyploidy thereby chloroplast DNA is abundant; could be present in one chloroplast with 50 copies and taking into account that approximately 50 chloroplasts are present in a plant cell which makes 2500 cpDNA copies per plant cell (Alzohairy et. al., 2015; Schwarz et. al., 2015). This number could be even more in some other species. As of 2016, there have been 890 land plant chloroplast genome sequences (cpDNA, plastome) available on NCBI genome database (ncbi.nlm.nih.gov/genome). Schwarz et. al., (2015) reported that most of land plant plastomes range in size from 110 kb to 170 kb with an average of 154 kb. In addition plastome size of *R. pseudoacacia* is 154,835 bp, size of LSC, SSC and both of IR regions are 86,172, 19,005 and 24,829 bp respectively, and plastome has varied protein, rRNA and tRNA genes with noncoding DNA regions. Besides, it includes 76 protein-coding, 30 tRNA and 4 rRNA genes. Moreover *R. pseudoacacia* plastome has 17 genes with introns, 35.9% with GC content, 56.3% with protein coding regions. The chloroplastic genome of *R. pseudoacacia* has been recently sequenced and annotated by Sabir et al. (2016) but this data has not been published (Genbank accession number: KJ468102).

The chloroplastic DNA genes and noncoding regions have been frequently used in molecular taxonomic and phylogenetic studies, particularly analysis for basal clades due to low mutation rate compared with nuclear genes. The cpDNA is generally inherited uniparentally (maternally in Angiosperms and paternally in Gymnosperms) as a single copy and it is nonrecombinant with contast to nuclear genes which exist with at least two copies, these copies may demonstrate with recombination and gene conversion (Soltis and Soltis, 1998; Small et. al., 1998; Guo et. al., 2007).

The phylogenetic studies have showed that noncoding regions are more useful in determination of lower taxonomic ranks for their lack of functional constraints.

Therefore, phylogenetically noncoding regions can be more informative and have distinctive quality (Small et. al., 1998). During the translation mechanism in protein synthesis, tRNAs take role in transferring the information encoded in genome to structural or/and functional proteins. tRNA molecule can recognize the specific codons on messenger RNA and then carry the particular amino acids into the protein-building machinery according to DNA code written into mRNA. So, tRNAs have to be produced in large quantities and be coordinately controlled in response to need in protein synthesis. tRNA genes (*trn* genes) are highly conserved regions on the cpDNA. These preserved coding sequences like chloroplastic *trn* genes, introns and intergenic spacers are the most frequently used regions in phylogenetic studies. The *trnL* (UAA) - *trnF* (GAA) and *trnT (UGU)* - *trnL* (UAA) spacers were first characterized by Taberlet et al. (1991). Chloroplast *trnL* gene encodes the tRNA molecule that is the carrier of leucine amino acid meantime *trnF* gene encodes phenylalanine amino acid carrying tRNA molecule. Chloroplast *trnL* (UAA) - *trnF* (GAA) genes, their intron and intergenic spacer have been reported to be frequently used in phylogenetic studies (Bohle et al., 1994; Gielly and Taberlet, 1994; Ham et al., 1994; Mes and Hart, 1994: Sang et. al., 1997).

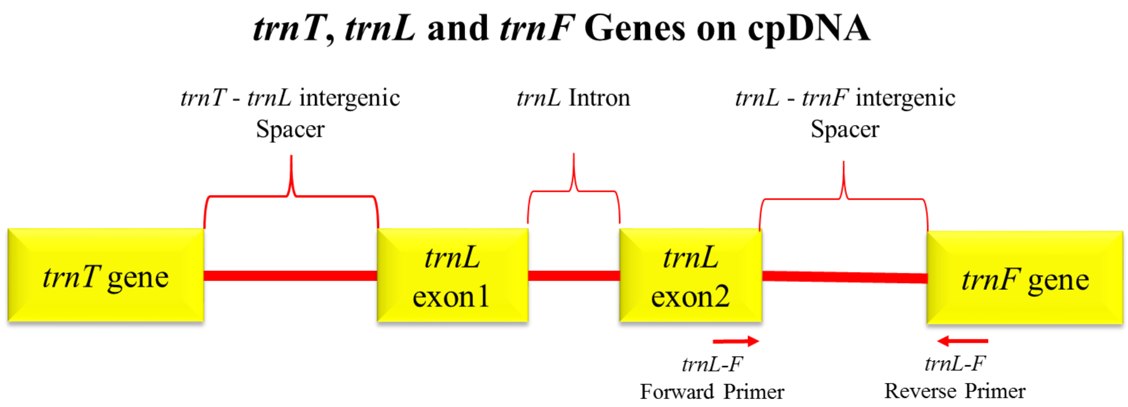


Figure 1.8 The schematic representation of *trnT*, *trnL* and *trnF* genes on cpDNA

trnT, *trnL* and *trnF* genes are separated by two intergenic spacers, and *trnL* intron is located within the first and second exon of *trnL* (UAA) gene. The *trnL-F* (GAA) intergenic spacer separates the second exon of *trnL* (UAA) gene from that of *trnF* (Poczai and Hyvönen 2011). Taberlet et al. (1991) has reported that non-coding regions display the highest genetic divergence thereby the amplification and sequencing of these regions have crucial importance in phylogenetic studies as intraspecific genetic markers. In other words, noncoding regions are genetically more variable due to the absence of gene rearrangements and high mutation levels. Thus, it has a potential to discriminate the close species in lower taxa.

1.2.4 Molecular markers and DNA fingerprinting

Molecular markers -also known as genetic markers- can be defined as a fragment of DNA that matches with a certain sequence within the genome. These markers are often used in molecular biology to identify and amplify a particular sequence on DNA in a full or partial genome. Molecular studies employ the comparative methods with direct and indirect approaches. For instance, the comparison of genome or gene sequencing is a direct method while comparison of amplified fragments of DNA according to their amplicon size is an indirect comparison method. Both methods gained ground in scientific community (Avise, 2012). Molecular markers have a great diversity but overall they can be divided into three classes according to their conception such as protein variants (allozymes), DNA sequence polymorphism and DNA repeat variation. These markers are employed in many areas such as genome mapping, population genetics, gene tagging, molecular linkage maps, map-based gene cloning, marker aided selection, genetic diversity analysis, phylogenetic reconstruction, evolutionary studies, paternity testing and forensic applications (Maheswaran, 2004; Schlötterer, 2004). Allozymes which are accepted as first molecular markers began to be used in middle of 1960s, later various markers have been invented. The information obtained from patterns of protein gel electrophoresis was used in different fields of molecular biology. Proteins (allozymes and isozymes) were used as molecular markers, showing the DNA variations indirectly with different patterns in gel electrophoresis. Later, mid 70’s are the years when DNA-based molecular markers and first DNA sequences were introduced to scientific community. In this context, RFPL were the first molecular marker, and after that various molecular markers have been developed for different purposes (Schlötterer, 2004). In other words, first generation DNA molecular markers started with RFLP. Table 1.3 shows the first generation DNA molecular markers, which are mostly derivate of RFLP markers (Maheswaran, 2004).

Table 1.3 First Generation DNA Markers (Maheswaran, 2004)

YEAR	Acronym	Reference
1974	RFLP	Grodzicker et al. (1974)
1985	VTNR	Jeffreys et al. (1985)
1986	ASO	Saiki et al. (1986)
1988	AS-PCR	Landergren et al. (1988)
1988	OP	Beckmann (1988)
1989	SSCP	Orita et al. (1989)
1989	STS	Olsen et al. (1989)

The second generation DNA molecular markers and their references are shown in Table 1.4. These molecular markers are primarily based on the micro satellites and relied on PCR technology. Micro satellites are arrays of tandemly repeated 2 - 5 nucleotide DNA sequences which dispersed all along the genomes of all eukaryotic organisms. It must be pointed out that one of the most commonly used molecular marker "Random Amplified Polymorphic DNA (RAPD)" has been published by Williams et al. (1990) in this time line. RAPD primers can attach multiple sites in the genome and produce large numbers of DNA fragments per reaction. RAPD does not require prior knowledge about primer sequences in the target species (Maheswaran, 2004; Schlötterer, 2004).

Table 1.4 Second Generation DNA Markers (Maheswaran, 2004).

Year	Acronym	Reference
1990	RAPD	Williams et al. (1990)
1990	AP-PCR	Welsh and McClelland (1990)
1990	STMS	Backman and Soller (1990)
1991	RLGS	Hatada et al. (1991)
1992	CAPS	Akopyanz et al. (1992)
1992	DOP-PCR	Telenius (1992)
1992	SSR	Akkaya et al. (1992)
1993	MAAP	Caetano-Anollés et al. (1993)
1993	SCAR	Paran and Michelmore (1993)

New generation DNA molecular markers (Table 1.5) are effective with high-throughput performance, reliable results, reproducibility, ease of application and lower cost. Inter Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism

(AFLP) came forward and are the most used markers all of new generation DNA molecular markers. PCR amplification of both markers yields multiple bands that show a presence or absence variation between individuals. New generation DNA molecular markers contain DNA sequencing technologies, genome targeting and new functional markers as well (Poczai et al., 2013).

Poczai et al., (2013) has reported that in addition to new generation DNA markers there are some developed new kind of markers such as Conserved DNA and Gene Family Based Markers (CDDP, PBA, TBP and ITP), Transposable Element Based Markers (IRAP, REMAP, ISAP, IPBS and SSAP), Resistance-Gene Based Markers (RGAP and NBS profiling), RNA-Based Markers (iSNAP, cDNA-AFLP, cDNA-RFLP and EST-SSR) and Targeted Fingerprinting Markers (DALP, PAAP, SRAP, TRAP, CoRAP and SCoT). Those markers are relatively new and also have their own advantages and weaknesses depending on the case/marker. Poczai et al., (2013) also mentioned that usage ratio of all of these new markers is 11% while RAPD, ISSR and AFLP and derivative of these markers total usage ratio is 89% until 2012. Additionally there are some new marker systems also developed like Restriction site–associated DNA sequencing (RAD-seq) (Baird et al., 2008) and InDel markers.

According to Poczai et al., (2013), most commonly used DNA Markers such as RAPD, ISSR and AFLP have some weaknesses like (i) the co-movement of same size fragments from independent loci in different samples, (ii) the co-movement of paralogous bands instead of orthologous, (iii) the nested priming, causing to amplicons from overlapped fragments, (iv) the formation of heteroduplex, alternate allelic sequences and/or similar duplicate loci generate the products, (v) the collision, two or more different fragments at equal size become in a single lane, (vi) the non-independence, due to codominancy or nested priming a band is estimated more than one, and (vii) the artifactual segregation distortions, brought about undetected codominancy, worse gel resolution or false loci scoring.

Table 1.5 New Generation DNA Markers (Maheswaran, 2004)

Year	Acronym	Reference
1994	ISSR	Zietkiewicz et al. (1994)
1994	SAMPL	Morgante and Vogel, (1994)
1994	SNP	Jordan and Humphries (1994)
1995	AFLP (SRFA)	Vos et al. (1995)
1995	ASAP	Gu et al. (1995)
1996	CFLP	Brow et al. (1996)
1996	ISTR	Rhode (1996)
1997	DAMD-PCR	Bebeli et al. (1997)
1997	S-SAP	Waugh et al. (1997)
1998	RBIP	Flavell et al. (1998)
1999	IRAP	Kalendar et al. (1999)
1999	REMAP	Kalendar et al. (1999)
1999	MSAP	--------------
2000	MITE	Casa et al. (2000)
2000	TE-AFLP	van der Wurff et al. (2000)
2001	IMP	Chang et al. (2001)
2001	SRAP	Li and Quiros (2001)

Genetic diversity is very important and critical for survival of the species in ever-changing environmental conditions. The species can deal, maintain the homeostasis and adapt to new conditions owing to genetic diversity for long term. Moreover, information obtained from genetic diversity studies could be used in development of efficient conservation, breeding and genetic resource management strategies. Molecular markers have the capacity to develop genetically improved varieties as a complementary application for phenotypic selection. Along with phenotypic traits, molecular markers can quickly resolve which variety is worth to be cultivated on a large scale (Badfar-Chaleshtori et. al., 2012; [30]).

Molecular markers and DNA fingerprinting techniques have been developed to measure the genetic variability and cultivar identification. Among DNA-based molecular markers, Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) have found significant grounds in genetic relationship studies. However, these methods are expensive and not easy in application and which need expertise. Besides, Random Amplified Polymorphic DNA (RAPD) and Inter Simple

Sequence Repeats (ISSR) markers are easy in application as well as they are cheap and require no radioactive labelling; only small amounts of DNA is sufficient especially for ISSR markers which are very reproducible, and highly polymorphic and informative (Zietkiewicz et al., 1994; Gajera et. al., 2010; Sözen 2010).

Microsatellites are DNA motifs within the range of 2-5 base pairs and are repeated 5-50 times as a single locus, and these loci disperse throughout the eukaryotic genomes with thousands copies. ISSR is a PCR-based method, which includes the amplification of DNA segments between two identical microsatellite regions oriented in opposite direction. Primers of ISSR are designed from microsatellites and adjusted to anchor from both 5' and 3' as forward and reverse directions of template DNA sequences. In this technique, a single primer can target multiple genomic loci to amplify in PCR reaction. There is no need for prior genomic sequence information for ISSR primer design. This feature makes the ISSR a very advantageous application (Reddy et. al., 2002; Huang et. al., 2012).

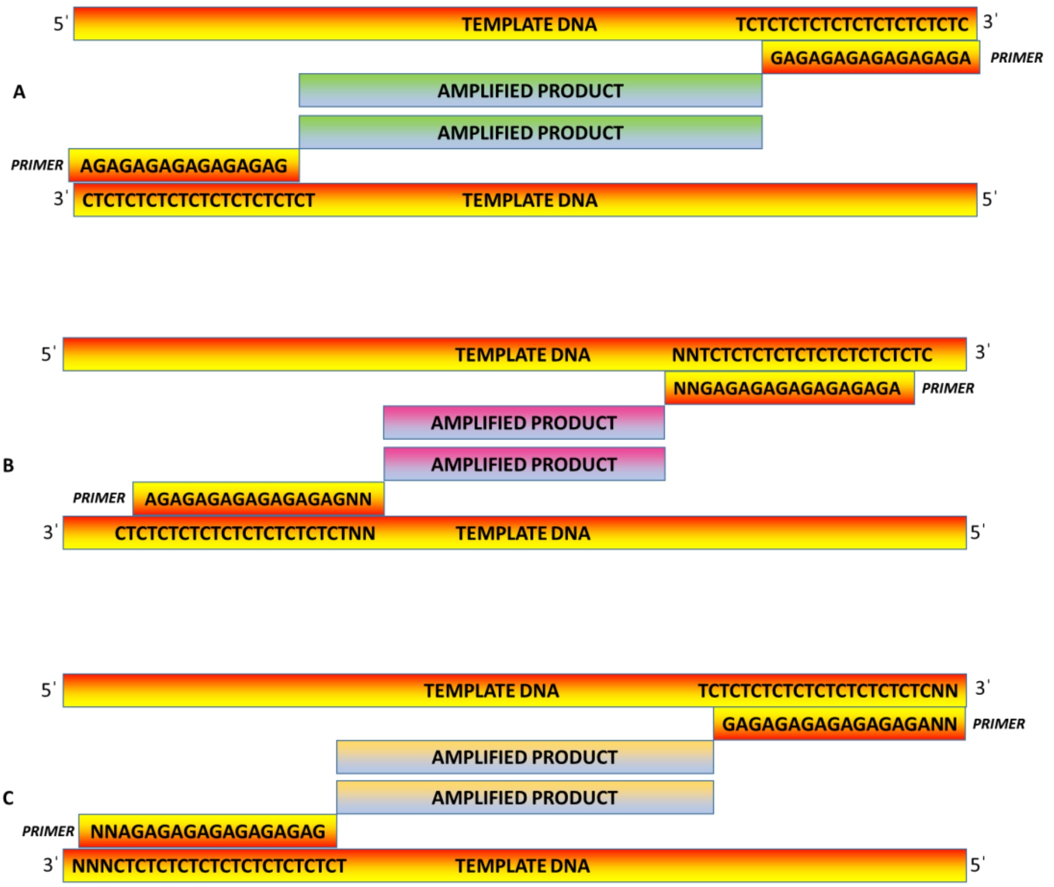


Figure 1.9 Possible matches between primers and template DNA. Unanchored (A), 3'-anchored (B) and 5'-anchored (C). (Modified from Reddy et. al., 2002)

ISSR primers can be used unanchored or (more usually) anchored with 1 to 4 bases extended into the flanking sequences either. Unanchored primers consist of only di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide of microsatellite sequences. These primers can be attached everywhere in microsatellite site of template DNA, leading to slippage and smear formation of bands (Figure 1.10. A). Anchored primers consist of microsatellite sequences along with 2-5 nucleotide flanking sequences. Anchored primers anneal specific region on the template DNA from both 3ˈ and 5ˈ directions and generate more clear bands (Figure 1.10 B and C). The produced bands of ISSR markers have a length of between 200-2000 bp. (Reddy et al**.**, 2002).

ISSR is a simple, highly polymorphic and quick method which carries the benefits of microsatellites (SSRs), amplified fragment length polymorphism (AFLP) and universality of random amplified polymorphic DNA (RAPD). ISSR markers can be used for genomic fingerprinting, genetic diversity and phylogenetic analysis, genome mapping and determining the SSR motif frequency (Reddy et. al**.**, 2002).

Having many molecular markers available, among these ISSR markers possess some advantages on others. These can be listed as (i) no need for prior or length information (ii) relatively low cost, (iii) ease in application, (iv) universality, ISSR markers can attach to multiple different loci of genome due to microsatellite sequences being abundant and dispersed throughout the eukaryotic genome, (v) diversity and discrimination power, microsatellites shows high differentiation rate compared to other parts of DNA, and (vi) reproducibility, ISSR primers (especially anchored primer) produce same bands at different applications in same PCR conditions. (Zietkiewicz et al., 1994; Reddy et. al**.**, 2002; Cao et. al., 2006).

1.2.5. Aim of this study

In this genetic context, investigation/assessment of i) heavy metal and mineral element status of *R. pseudoacacia* plants distributed in urban ecosystem in terms of seasonal changes, ii) effects of accumulated heavy metals on mineral element status in different stations and levels, iii) photosynthetic pigment and total protein levels under heavy metal pollution, iv) genetic similarity of *R. pseudoacacia* genotype groups by using ISSR band data v) phylogenetic relationships of *R. pseudoacacia* genotype groups was aimed.

2. MATERIAL AND METHODS

In Istanbul and Kocaeli provinces, *R. pseudoacacia* has been often used for ornamental and recreational purposes in parks, gardens and road sides as well as planted in industrial sites that emit heavy metals and other pollutants to the environment. Thus, *R. pseudoacacia* plants have been significantly exposed to the traffic and industrial derived heavy metal pollutions. In this work, we initially aimed to determine the effects of pollution on plant-soil interactions in terms of mineral nutrient element and heavy metal uptakes, and some other physiological parameters. Samplings were done at all four seasons, covering the whole year to determine the seasonal variations of tested metal levels in soil and plant samples. In addition, it has been known that exposure to heavy metals can also cause mutagenesis in plant genomes. Therefore, this study also attempted to investigate the mutations in plant genomes using ISSR-PCR method. Finally, this work investigated the genetic relationships between *R. pseudoacacia* plants by using ITS, *trnL-trnF* intergenic spacer sequences and ISSR-PCR. For this, plant and soil samples were collected during all four seasons namely summer (July 2014), autumn (October 2014), winter (January 2015) and spring (April 2015). Mineral nutrient elements and heavy metals in soil, bark, washed/unwashed leaves and branch samples were analyzed using ICP-OES. As physiological parameters, photosynthetic pigment and total protein analyses were done in summer, autumn and spring samples. Genetic analyses were done using summer leaf samples.

2.1. Study Areas

Plant samples were collected from four stations in Istanbul and one station in Kocaeli province. Istanbul stations have been selected from heavy traffic sites such as Bağdad, Barbaros and TEM, and as control Prince Islands due to absence of traffic, whereas Kocaeli station was from a heavy industrial area, Dilovasi. Istanbul is the biggest and most crowded city of Turkey with approximately 14.7 million inhabitants according to TUIK 2015 data. According to TUIK 2016 report, there are over 3.5 million motor vehicles registered in Istanbul as of March 2016. Thus, city has to deal with some unfavorable situations such as traffic congestion, environmental pollution, informal settlement, rapid motorization, industrial and municipal wastes (OECD, 2008).

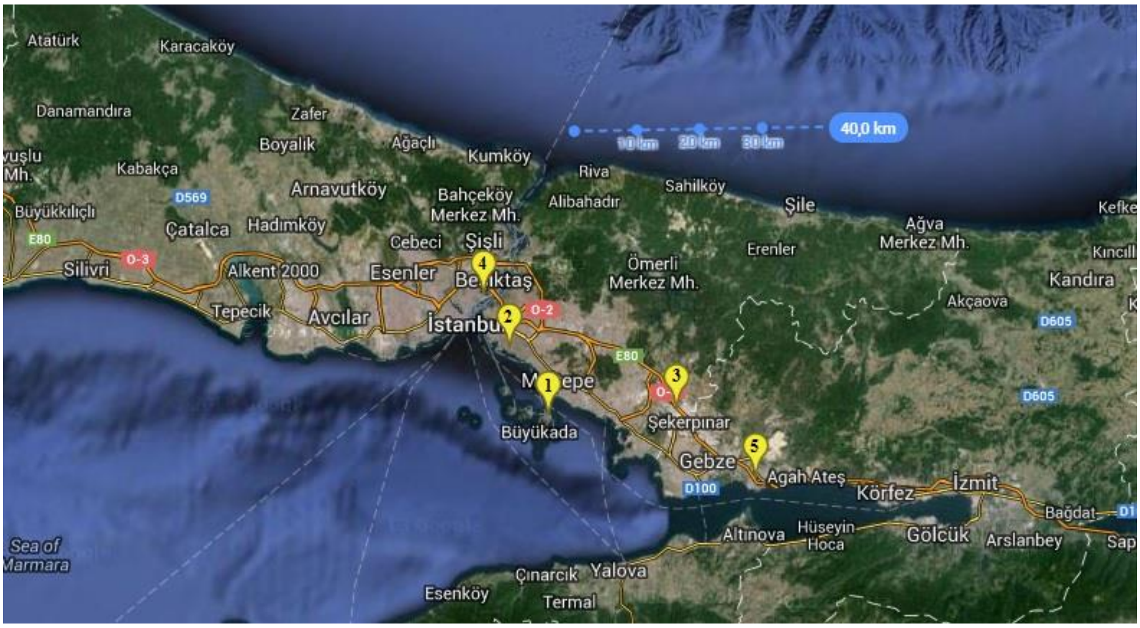


Figure 2.1 Stations Prince Island (1), Bağdat Avenue (2), TEM Highway (3), Barbaros Boulevard (4), Dilovasi District (5).

Prince Islands, Bağdad Avenue, Barbaros Boulevard and TEM highway are the stations from Istanbul province. Prince Islands are located in Marmara Sea, Bagdat Avenue is a shopping, residence and entertainment area at Asian side and TEM highway is very important route which connects Istanbul to other regions of Turkey. These tree stations are located at Anatolian side (Asian Side) of Istanbul while Barbaros Boulevard is located at European side. Barbaros Boulevard is one of the main roads connecting two central areas (Beşiktaş to Levent) of main city, therefore it has a very heavy traffic load.

Prince Islands consist of seven individual islands namely Buyukada (Prince Island), Heybeliada (Saddlebag Island), Burgazada (Fortress Island), Kınalıada (Henna Island), Kasık Adası (Spoon Island), Sedef Adası (Mother-of-Pearl Island) and Sivriada (Sharp Island). Prince Islands have been very popular recreational areas for Istanbulites as well as for tourists. Its population is estimated to raise up to 140.000 with daily visitors. The largest island is Prince Island (Buyukada) which has approximately 8.000 habitants in winter and 30.000 in summer, and it was selected as control station because of its conserved nature [63-64]. Transportation is carried out mostly with horse-drawn carriages or electric motorcycle/bicycle in Prince Islands. Only a few motorized vehicles belonging to the governmental agencies are used in islands and there are not any industrial facilities on islands. Mainland shore is Maltepe district which is 2.3 km far. This distance inhibits the transportation of pollutants from mainland to islands, thus islands are relatively conserved areas from traffic and industrial pollutions.

Figure 2.2 Prince Island

Bagdat Avenue is located between Kadikoy and Maltepe. It is a leading shopping and residential area of Asian Side of Istanbul. The avenue has heavy traffic load especially during rush hours. Barbaros Boulevard is one of the main routes extensively used by motor vehicles at downtown. The traffic load of boulevard is intense at anytime of weekdays. TEM highway also has an intense traffic but is more propitious than Barbaros Boulevard in terms of vehicle speed and geographic conditions.

Figure 2.3 Bagdat Avenue

Figure 2.4 TEM Highway

Kocaeli Province is one the leading industrialized cities of Turkey, which is located at 90 km east of Istanbul. The province has many industrial investments because of proximity to highways, sea and railway networks, and to the major cities such as Istanbul, Bursa and Ankara. Kocaeli has 12 organized industrial zones and the biggest one is the Dilovasi Organized Industrial Zone (DOIZ). DOIZ is located right in the center of Dilovası district (Hamza et al., 2011). It was established in 2002 and has 900 ha land area with 209 operational companies (Mert and Akman 2011; [64]). Major industrial branches in DOIZ are iron-steel industry, chemical industry, petrochemical industry, pharmaceutical industry, wood products industry, energy industry (coal-fired electric power plant) and non-ferrous metal industry (Durukal et al., 2008; Mert and Akman 2011; Yaylalı-Abanuz, G. 2011; Bingöl et al., 2013). Inhabitants of Dilovası district are seriously affected from pollutions caused by DOIZ. Hamza et al., (2011) reported that death rates by cancer are three times higher in Dilovasi compared to the national and global records. Lung, gastrointestinal and hematopoietic cancers as well as other respiratory disease incidences rose up in last two decades (Tuncer 2009; Hamza et al., 2011).

2.2. Sampling

Samples have been collected from five different stations namely Buyukada, Bağdad Avenue, Barbaros Boulevard, TEM Highway and Dilovası district. Initially, 61 individuals were selected, plant, soil samples collected from 5 stations however some individuals were cut or died due to construction or landscaping purposes. Thus samples of all individuals couldn’t be obtained for analysis. All individuals are shown in Tables 2.1-2.5 according to collected stations.

Table 2.1 GPS coordinates and performed analyses in Prince Island samples (MEHMA, Mineral Element and Heavy Metal Analysis; PPA, Photosynthetic Pigment Analysis; TPA, Total Protein Analysis)

Individuals	Coordinates		MEHMA	PPA	TPA	Genetic	
	North	East				ISSR	Phylogeny
Ada1	40° 52.454'	29° 07.665'	x	x	x	x	x
Ada2	40° 52.414'	29° 07.688'	x	x	x	x	-
Ada3	40° 52.409'	29° 07.701'	x	x	x	x	-
Ada4	40° 52.399'	29° 07.700'	-	-	-	x	x
Ada5	40° 52.398'	29° 07.721'	-	-	-	-	-
Ada6	40° 52.392'	29° 07.740'	-	x	x	x	-
Ada7	40° 52.396'	29° 07.815'	-	-	-	x	-
Ada8	40° 52.377'	29° 07.934'	x	x	x	x	-
Ada9	40° 52.361'	29° 07.964'	x	x	x	x	-
Ada10	40° 52.330'	29° 08.056'	x	x	x	x	x
Ada11	40° 52.258'	29° 08.170'	x	x	x	x	-
Ada12	40° 52.338'	29° 08.167'	x	x	x	x	-

Figure 2.5 Sampling at Prince Island

Table 2.2 GPS coordinates and performed analyses in Bagdat Avenue samples (MEHMA, Mineral Element and Heavy Metal Analysis; PPA, Photosynthetic Pigment Analysis; TPA, Total Protein Analysis

Individuals	**Coordinates**		**MEHMA**	**PPA**	**TPA**	**Genetic Studies**	
	North	**East**				**ISSR**	**Phylogeny**
Bag1	40° 58,420’	29° 03,255’	-	-	-	x	-
Bag2	40° 58,329’	29° 03,682’	x	x	x	x	-
Bag3	40° 58,350’	29° 03,678’	-	x	x	x	x
Bag4	40° 58,174’	29° 03,974’	x	x	x	x	x
Bag5	40° 58,087’	29° 03,992’	x	x	x	x	-
Bag6	40° 58,084’	29° 03,989’	-	-	-	x	-
Bag7	40° 57,967’	29° 04,222’	-	-	-	x	-
Bag8	40° 57,676’	29° 04,663’	x	x	x	x	x
Bag9	40° 57,658’	29° 04,711’	x	x	x	x	-
Bag10	40° 57,691’	29° 04,742’	x	x	x	x	-
Bag 11	40° 57,482’	29° 05,143’	x	x	x	x	-
Bag 12	40° 57,431’	29° 05,205’	x	x	x	x	-

Figure 2.6. Sampling at Bagdat Avenue

Table 2.3 GPS coordinates and performed analyses in Barbaros Boulevard samples (MEHMA, Mineral Element and Heavy Metal Analysis; PPA, Photosynthetic Pigment Analysis; TPA, Total Protein Analysis)

Individuals	**Coordinates**		**MEHMA**	**PPA**	**TPA**	**Genetic Studies**	
	North	**East**				**ISSR**	**Phylogeny**
Bar1	41° 03,223’	29° 00,571’	x	x	x	x	-
Bar2	41° 03,227’	29° 00,578’	x	x	x	x	-
Bar 3	41° 3.256’	29° 00,671’	-	-	x	x	-
Bar 4	41° 02,882’	29° 00,498’	x	x	x	x	-
Bar 5	41° 02,852’	29° 00,493’	x	x	x	x	-
Bar 6	41° 02,843’	29° 00,494’	x	x	x	x	-
Bar 7	41° 02,771’	29° 00,470’	-	-	x	x	-
Bar 8	41° 02,771’	29° 00,470’	x	x	x	x	-
Bar 9	41° 02,770’	29° 00,467’	-	-	x	x	-
Bar 10	41° 02,770’	29° 00,467’	-	-	x	x	x
Bar11	41° 02,665’	29° 00,452’	x	x	x	x	x
Bar12	41° 02,666’	29° 00,454’	x	x	x	x	x

Figure 2.7. Sampling at Barbaros Boulevard

Table 2.4 GPS coordinates and performed analyses in Dilovasi District samples (MEHMA, Mineral Element and Heavy Metal Analysis; PPA, Photosynthetic Pigment Analysis; TPA, Total Protein Analysis)

Individuals	Coordinates		MEHMA	PPA	TPA	Genetic Studies	
	North	East				ISSR	Phylogeny
Dil1	40° 46,951’	29° 31,793’	x	x	x	x	-
Dil2	40° 46,941’	29° 31,802’	x	x	x	x	-
Dil3	40° 46,944’	29° 31,800’	-	x	x	x	-
Dil4	40° 46,987’	29° 31,789’	x	x	x	-	-
Dil5	40° 47,015’	29° 31,786’	x	x	x	x	-
Dil6	40° 47,068’	29° 31,784’	-	x	x	x	-
Dil7	40° 47,086’	29° 31,791’	x	x	x	x	x
Dil8	40° 47,109’	29° 31,784’	x	x	x	x	x
Dil9	40° 47,233’	29° 32,070’	-	x	x	x	x
Dil10	40° 47,251’	29° 32,062’	-	x	x	x	-
Dil11	40° 47,508’	29° 31,870’	x	x	x	x	-
Dil12	40° 47,269’	29° 31,768’	x	x	x	-	-

Figure 2.8 A pollution scene in Dilovasi Organized Industrial Zone (DOIZ)

Figure 2.9 Sampling at Dilovası district

Table 2.5 GPS coordinates and performed analyses in TEM highway samples (MEHMA, Mineral Element and Heavy Metal Analysis; PPA, Photosynthetic Pigment Analysis; TPA, Total Protein Analysis)

Individuals	Coordinates		MEHMA	PPA	TPA	Genetic Studies	
	North	East				ISSR	Phylogeny
TEM16	40° 52,940’	29° 22,775’	x	x	x	x	-
TEM18	40° 52,947’	29° 22,773’	-	x	x	x	-
TEM19	40° 52,952’	29° 22,773’	-	x	x	x	x
TEM20	40° 52,960’	29° 22,758’	x	x	x	x	-
TEM21	40° 52,970’	29° 22,748’	-	x	x	x	-
TEM22	40° 52,975’	29° 22,743’	x	x	x	x	-
TEM23	40° 52,980’	29° 22,738’	-	x	x	x	-
TEM24	40° 52,982’	29° 22,731’	x	x	x	x	-
TEM25	40° 52,989’	29° 22,725’	-	x	x	x	x
TEM26	40° 53,020’	29° 22,688’	x	x	x	x	x
TEM27	40° 53,048’	29° 22,663’	x	x	x	x	-
TEM28	40° 53,052’	29° 22,659’	x	x	x	x	-
TEM29	40° 53,062’	29° 22,653’	x	x	x	x	-

Figure 2.10 Sampling at TEM Highway

2.4 Analysis of Mineral Nutrient Elements and Heavy Metals

2.4.1 Sample preparation

Samplings were conducted in summer (July 2014), autumn (October 2014), winter (January 2015) and spring (April 2015). Samples were processed each time for avoiding contamination, decay and confusion. Soil samples (about 500 g) were taken from a depth of 10 cm by using a stainless steel shovel, and were dried at 80^{o}C in an oven for 48 hrs and then passed through a 2-mm sieve. The sieves were washed with distilled water and 96% ethanol each time to avoid contamination. 0.3 g weighted soil samples, and 9 mL 65% (v/v) HNO_3, 3 mL 37% (v/v) HCl and 2 mL 48% (v/v) HF (Merck) were added into Teflon vessels. Bark, branch and leaf samples were collected from each individual and they have not been immediately subjected to any processes. They were stored in envelops from blotting papers and then labelled according to the individuals and stations. Leaf samples were allocated for physiological studies (photosynthetic pigment and total protein analyses), mineral nutrient element and heavy metal analysis, and molecular studies. Leaves which were allocated for molecular and physiological studies were packed and put into dryice, then transferred to laboratory. These samples were preserved at -80^{o}C Lexicon II ULT deep freezer (Esco Micro Pte. Ltd. Singapore) until analysis.

Figure 2.11 -80 Ultra deep freezer.

The allocated leaf samples for mineral element and heavy metal analysis were separated into two subgroups; i) one was washed with distilled water to remove the dust particles in a standardized procedure, and ii) other was put in an envelope as unwashed leaf samples.

Figure 2.12 Plant parts samples (washed and unwashed leaves, branch, and bark) in oven.

Bark, branch, washed and unwashed leaf samples, which are used for mineral element and heavy metal analysis, were kept in 80°C M 6040 P oven (Electro-mag, Istanbul, Turkey) for 48 hrs. Then, samples were ground by using mortar and pestle. 0.2 g weighed samples, and 8 ml %65 HNO_3 (Merck) were transferred into Teflon vessels. All plant and soil samples were mineralized in a microwave oven (Berghof-MWS2) according to the wet ashing procedure. Procedure was applied as 5 min at 145°C, 5 min at 165°C and 20 min at 175°C. The samples were filtered by using Whatman filters with 1-2 μm pore diameter in average. Last volume was adjusted to 50 ml with ultrapure water in volumetric flasks and then stored in falcon tubes until ICP-OES application. Stock solutions were prepared as 10, 50, 100, 250 and 500 mg/L by using multi-element stock solutions 1000 mg/L (Merck). Calibration curves were drawn by using stock solutions.

Figure 2.13 Berghoff microwave oven and Teflon vessels.

2.4.2 ICP- OES and element measurements

The mineral nutrient elements and heavy metals were analyzed by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). ICP consists of two parts, one is plasma generator (ICP) and other is spectrometer. Mineralized sample solution sprays on to plasma that is generated from argon (Ar) gas by using an intense electromagnetic field. Elements becomes induced and emits a unique radiation (uV and visible light) at the characteristic wavelengths. An optical spectrometer detects the emission of radiation. The intensity of light emissions indicates the concentration of elements within solution.

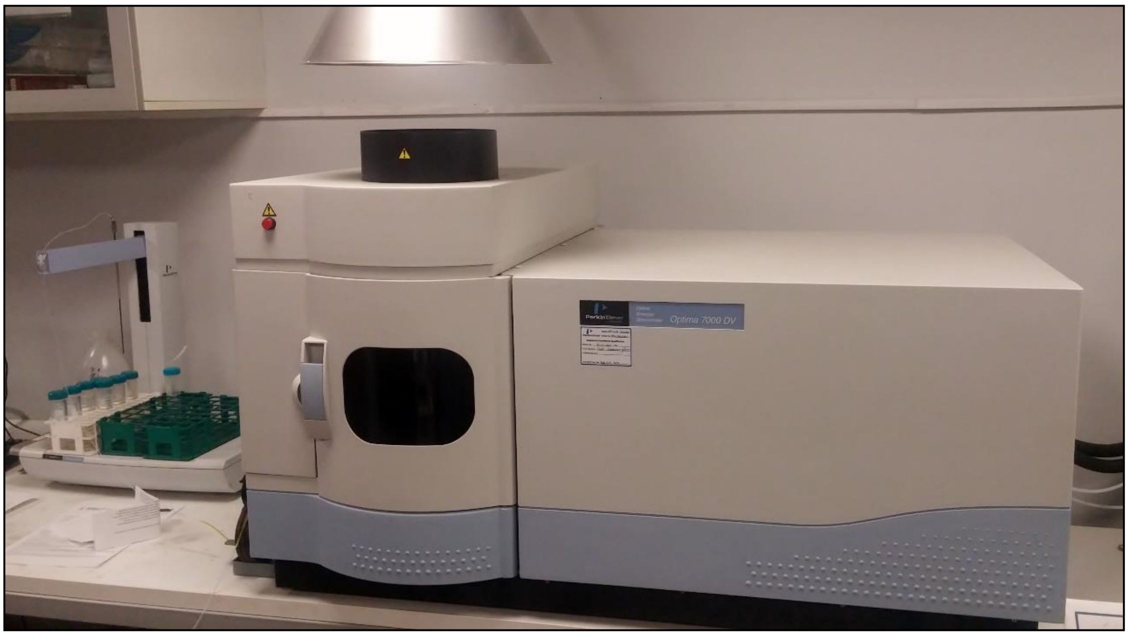


Figure 2.14 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

All samples were analyzed by using ICP-OES Optima 7000 DV (Perkin Elmer Corp., Massachusetts, USA) at Bahcesehir University, Faculty of Engineering and Natural Sciences, Laboratories of Department of Environmental Engineering. The concentrations of elements such as B, Cu, Ca, Cd, Cr, Fe, K, Mn, Na, Ni, Mg, Pb and Zn were measured in mg/kg dry weight (DW).

2.5 Photosynthetic pigment analysis

Chlorophyll *a*, *b*, *a/b*, and total chlorophyll, and carotenoids (*Cx+c*) are important photosynthetic pigments in plants. The stress factors usually affect the plant metabolism and they cause the fluctuation on levels of these compounds. Thus, photosynthetic pigments are some good indicators showing the plant situation under stress conditions.

The control and other plant leaf samples were weighed as 0.5 g and homogenized with 15 ml %80 acetone in a falcon tube by using WiseTis Homogenizer (Wisd - Witeg Laboratory Equipment, Wertheim, Germany). Homogenized leaves were centrifuged at 3000g and +4^{o}C for 10 min. After centrifuge, volume of supernatant was measured and noted. Then, the absorbance of supernatant was measured at 470, 645 and 663 nm wavelength using T60 UV Visible Spectrophotometer (PG Instruments, Leicestershire, United Kingdom). Photosynthetic pigment quantities were calculated from measured absorbance values according to Arnon (1949).

$$C_a = [12.7 \text{ x } D_{663} - 2.69 \text{ x } D_{645}] \text{ x } \frac{ml}{1000}$$

$$C_b = [22.9 \text{ x } D_{645} - 4.68 \text{ x } D_{663}] \text{ x } \frac{ml}{1000}$$

$$\text{Total C} = [20.2 \text{ x } D_{645} + 8.02 \text{ x } D_{663}] \text{ x } \frac{ml}{1000}$$

$$C_{x+c} = \frac{1000\,A_{470} - 1.90\,C_a - 63.14\,C_b}{214} \text{ x } \frac{ml}{1000}$$

Figure 2.15 Homogenizer and UV-Visible Spectrophotometer.

Figure 2.16 Mortar and pestle for sample grinding in photosynthetic pigment analysis**.**

2.6 Total Protein Analysis

The total protein concentrations were measured by using UV-VIS spectrophotometry. Initially, total protein isolation was performed and then phosphate buffer was prepared to apply the isolated protein solution. Phosphate buffer was prepared by mixing stock A and B.

Stock A: 2.76 g $NaH_2PO_4.2H_2O$ was taken and put into an erlenmeyer, and completed with distilled water up to 100 ml for 0.2 M solution.

Stock B: 3.56 g $Na_2HPO_4.2H_2O$ was taken and put into an erlenmeyer, and completed with distilled water up to 100 ml for 0.2 M solution.

Phosphate buffer: 6.4 ml Stock A and 43.6 ml Stock B were mixed and completed up to 100 ml by adding 50 ml distilled water. Final molarity of solution was adjusted to 0.1 M and pH was adjusted at 7.7.

<u>Applied Protocol</u>:

1. 0.4 g leaf tissue was weighed and put into cold mortar with 2 ml phosphate buffer (0.1 M, pH 7.7). Tissue was homogenized with a pestle on ice.
2. Homogenized samples were put into 2 ml microcentrifuge tubes and labelled.
3. Samples were centrifuged at +4^{o}C and 12.000 rpm for 20 min.
4. Supernatant was transferred into a clean tube as protein source. The exposure of samples to high temperature and light was avoided during processing.
5. 1.5 µl supernatant was measured by using OPTIZEN NANO Q Spectrophotometer (Mecasys Corp., Gyeonggi-do, Republic of Korea) at protein measurement mode for determination of protein concentration ($mg.ml^{-1}$)

Figure 2.17 Optizen NANO Q Spectrophotometer. dsDNA and protein measurement modes were shown in the figure.

2.7 Genetic and Phylogenetic Studies

There have been several PCR-based methods employed herein in detection of genetic similarities, phylogenetic relationships and mutations.

2.7.1 DNA isolation

The extraction and purification of nucleic acids are the first step in genetic studies. Because, quality and quantity of nucleic acids directly influence further studies. For instance, contaminants could affect the performance of PCR reactions or cloning studies (Somma and Querci, 2004). A variety of DNA isolation methods have been available but the most convenient method depends on such essential criteria; (i) target nucleic acid, (ii) source organism, (iii) source tissue, (iv) desired results (e.g., yield, purity), and (v) further studies (e.g., PCR, RT-PCR, cloning, cDNA synthesis). Extraction methods mainly require the lysis of cell, inactivation of cellular nucleases and separation of nucleic acids from remaining cellular components.

In this study, several isolation methods have been applied to obtain sufficient amount of DNA with good quality. These methods included the manual methods and commercial kits. Only summer samples (collected at July 2014) were used for genetic studies.

There are some manual methods for nucleic acids isolation like CTAB or SDS methods. CTAB was preferred in this study. This method is suitable for extraction and purification of DNA from plants, and it is good at polysaccharides and polyphenolic compounds

(Somma and Querci, 2004). CTAB was applied according to Doyle (1987) and Doyle (1990) with some modifications.

Table 2.6 Reagents for preparing 250ml of CTAB extraction buffer

Reagent	Amount
CTAB	5g (2% w/v)
Tris-HCl pH 8.0 (1 M)	25ml
EDTA pH 8.0 (0.5 M)	10ml
β-mercaptoethanol	% 0.2 (v/v) added just before beginning of process into extraction buffer
NaCl (5 M)	70ml
Nuclease Free Water	165 ml (or up to 250 ml)
PVP	0.05 g for 0.5 g of sample tissue (added during grinding)
Total	250 ml CTAB extraction buffer

Frozen leaves were used for DNA isolation. Before the isolation procedure, extraction buffer was prepared by using reagents in Table 2.6. For this purpose, 5g CTAB (cetyltrimethylammonium bromide), 5M 70 ml NaCl, 0.5M 10ml EDTA and 1M 25ml Tris-HCl (pH8) were mixed and completed with distilled water up to 250ml. CTAB buffer was preheated to 65°C and % 0.2 (v/v) β-mercaptoethanol was added just before the isolation. CTAB method includes the following steps:

1. 0.05-0.1g leaf tissue was homogenized by using Mixer Mill MM 400 homogenizer (Retsch GmbH., Düsseldorf, Germany) or mortar and pestle.
2. Homogenized leaf tissue was transferred in a 2ml microcentrifuge tube and 800µl CTAB extraction buffer and 0.002g PVP were added into tube. The tube was inverted several times.
3. The mixture was incubated at 65ºC for 45 min.
4. 800µl of 24:1 Chloroform/Octanol was added to mixture and inverted several times.
5. Mixture was centrifuged at 13.000g for 10 min.
6. Approximately 600µl supernatant was transferred into a clean tube.
7. 300µl 5M NaCl and 600 µl cooled %96 isopropanol were added to mixture.
8. The mixture was kept at 4-6ºC until DNA strands becomes visible.

9. After mixture was centrifuged at 10.000g, supernatant was decanted.
10. 70% ethanol was added onto pellet and tapped kindly to solve DNA.
11. Centrifuged at 10.000g for 3 min.
12. Steps 10 and 11 were repeated several times.
13. After last centrifugation at 13.000g for 3 min, supernatant was decanted.
14. Remained DNA pellet was cleaned from ethanol with air drying at fume hood.
15. 100µl of TE or ultrapure water was added on to DNA for re-hydration.
16. DNA concentration was measured using OPTIZEN NANO Q Spectrophotometer (Mecasys Corp., Gyeonggi-do, Republic of Korea) at dsDNA measurement mode.
17. After measurement of DNA concentration, final concentration was adjusted to 20-50 ng/ml with adding ultrapure water or TBE solution.

2.7.2 PCR reactions

PCR reactions are carried out for amplification of various DNA parts. PCR applications are used in many areas including diagnosis of genetic diseases, DNA cloning and sequencing, phylogeny studies, gene function diagnosis, forensic sciences, paternity testing, and detection of pathogens (Bartlett and Stirling 2003). Aeris Thermal Cycler Model G96 (Esco Micro Pte. Ltd., Singapore) below was used for amplifications in this study. ITS1 region, *trnL*$_{UAA}$-*trnF*$_{\mathrm{GAA}}$ Intergenic Spacer and Microsatellite Regions (with ISSR primers) were amplified.

Figure 2.18 Esco Aeris Thermal Cycler Model G96,

PCR reactions requires optimization of mixture of compounds at relevant conditions. Template DNA, reaction buffer, $MgCl_2$, dNTP mix, Taq polymerase, primer(s) and ultrapure water were the substances used in PCR reactions (Table 2.7).

Table 2.7 PCR reaction compounds and their total volumes

PCR mixture component	Concentration		Volume (µl)
	Recommendation	Final	
10X PCR buffer	1-1.5X	1X	2.5
25 mM $MgCl_2$	2-4mM	3mM	3
10 mM dNTP mix	0.2-0.5mM (each)	0.2mM	2
Primer (From 10µM stock)	0.2-1µM	0.5µM	1.25
Nuclease free sterile water	adjusted according to volume of other components and desired final volume		15
Template DNA (50 ng/µl)	20-50ng/µl	50ng/µl	1
Taq Polymerase	1-5U	1.25U	0.25
	Total		25 µl

PCR conditions affect the quality and quantity of DNA yield. Thus, these conditions should be carefully optimized. Reactions for ITS1, $trnL_{UAA}$-$trnF_{GAA}$ Intergenic Spacer regions and ISSR-PCR were performed according to the conditions given in Figure 2.19. Annealing temperature, length and other characteristics of primers are changed according to the design and use purposes. A total of 19 primers were herein used in PCR reactions, two for ITS, two for *trnL*$_{UAA}$-*trnF*$_{GAA}$ Intergenic Spacer and 15 for ISSR.

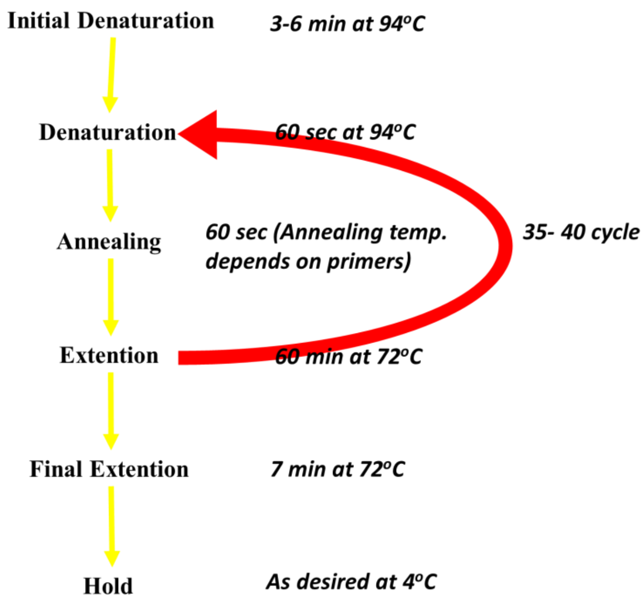


Figure 2.19 The schematic representation of PCR reactions steps

Two primers were used in amplification of ITS (Internal Transcribed Spacer). These are universal ITS1 and ITS2 primers given in Table 2.7. As mentioned above, ITS region consists of ITS1, 5.8S rRNA and ITS2 parts. In this study, ITS1 part of ITS region was amplified. ITS primers were annealed at 48^{o}C during amplification.

Table 2.8 The sequences of ITS primers (White et al., 1990)

Primer	Sequence	Annealing Temperature
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	48^{o}C
ITS2	5'-GCTGCGTTCTTCATCGATGC-3'	

Amplification of *trnL*$_{UAA}$-*trnF*$_{GAA}$ intergenic spacer region was performed at similar conditions with ITS reactions. Annealing temperature was adjusted to 48^{o}C. Primers were designed by Sang et at., (1997) similar to Taberlet et al., 1990. Primers were designed to be corresponding to nucleotide *trnL*$_{UAA}$ and *trnF*$_{GAA}$ positions of cpDNA of tobacco (Shinozaki et al., 1996).

Table 2.9 The primer sequences of *trnL - trnF* intergenic genic spacer (Sang et al., 1997)

Primer	Sequence	Annealing Temperature
trnL-F Forward	5'-AAAATCGTGAAGGTTCAAGTC-3'	48^oC
trnL-F Reverse	5'-GATTTGAACTGGTGACACGAG-3'	

Table 2.10 is showing the ISSR primers which were selected to amplify the microsatellite sites to reveal the genetic similarity and small changes on genome of *R. pseudoacacia*.

Table 2.10 The sequences of ISSR primers (Nagaoka and Ogihara 1997)

Primer	UBC Code	Sequence	Annealing Temperature
ISSR1	UBC807	$(AG)_8T$	48^oC
ISSR2	UBC811	$(GA)_8C$	53^oC
ISSR3	UBC817	$(CA)_8A$	50^oC
ISSR4	UBC818	$(CA)_8G$	53^oC
ISSR5	UBC820	$(GT)_8C$	53^oC
ISSR6	UBC823	$(TC)_8C$	53^oC
ISSR7	UBC827	$(AC)_8G$	53^oC
ISSR8	UBC825	$(AC)_8T$	54^oC
ISSR9	UBC848	$(CA)_8RG$	56^oC
ISSR10	UBC849	$(GT)_8YA$	54^oC
ISSR11	UBC855	$(AC)_8YT$	54^oC
ISSR12	UBC842	$(GA)_8YG$	56^oC
ISSR13	UBC875	$(CTAG)_4$	59^oC
ISSR14	UBC829	$(TG)_8C$	49^oC
ISSR15	UBC844	$(CT)_8RC$	56^oC

2.7.3 Gel electrophoresis

Electrophoretic methods have found very important grounds in many areas such as genetics, biochemistry, medical diagnostic and forensic sciences. Gel electrophoresis is used to discriminate and visualize the amplicons. Herein, CS-300V omniPAC MIDI Power Supply (Cleaver Scientific Ltd., Warwickshire United Kingdom) was used as power supply. Agarose (peqGOLD Universal Agarose, VWR International, Darmstadt, Germany) was used in gel electrophoresis as 1.2g for 100ml 1X TBE (Fisher Scientific, Massachusetts, USA) solution or 3g for 250ml 1X TBE solution [1.2% (m/v)] for ITS1 and trnL-trnF intergenic spacer, and as 1.6g for 100ml 1X TBE solution or 4g for 250ml 1X TBE Solution [1.6% (m/v)]. Ethidium bromide was used (MP Biomedicals, California, USA) as 2µ for 100ml or 5µ for 250 ml.

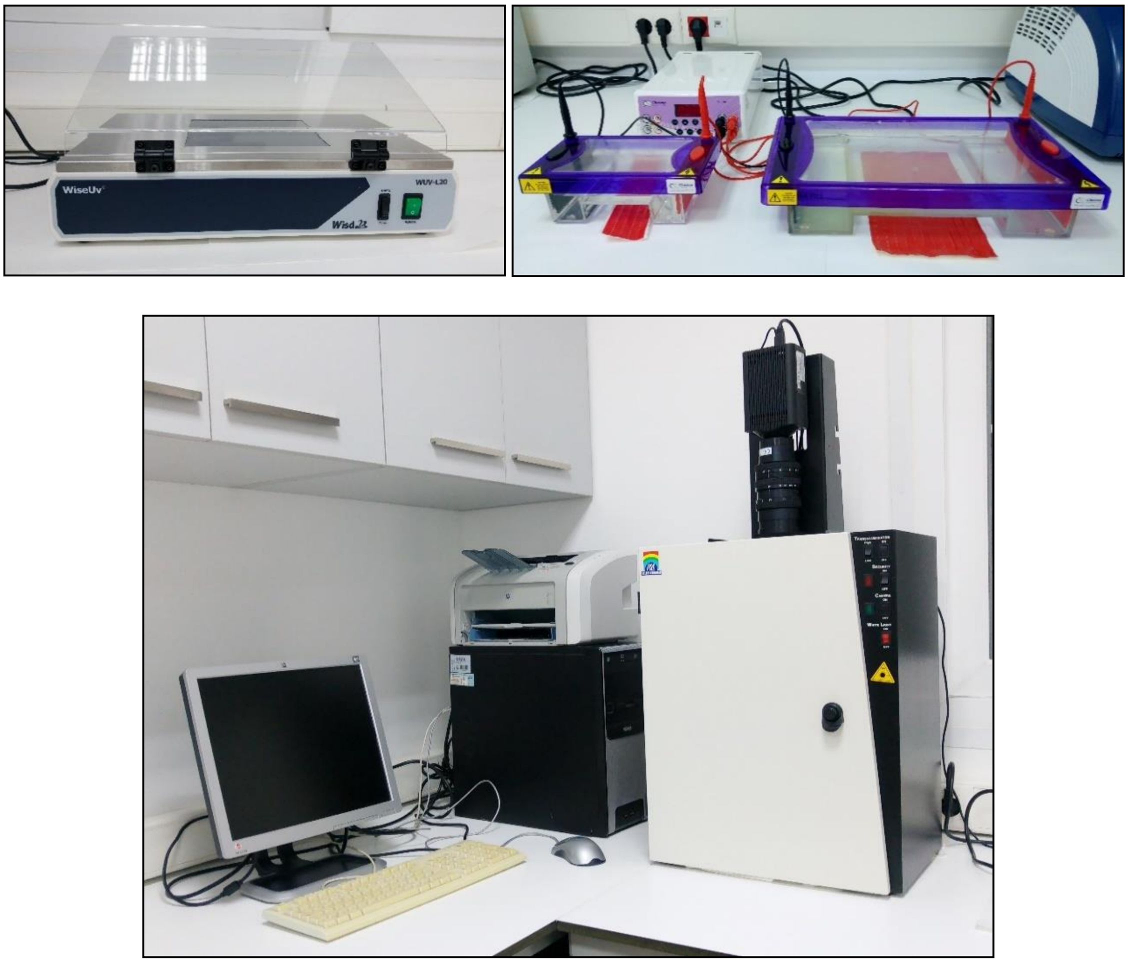


Figure 2.20 Electrophoresis equipment **(**A) and UV transilluminator (B)

Amplicons were mixed with 6X DNA loading dye which contains bromophenol blue (Termo Fisher Scientific, Massachusetts, USA) as 3µ for 12µ PCR product. Electrophorese conditions were set up as 50 V and 60 min for 100 ml small size gel or 80 V and 120 min for 250 ml large size gel. After gel electrophoresis, DNA bands were visualized by using UV transilluminator (Vilber Lourmat, Collégien, France) and WiseUV WUV-L20 UV-Transilluminator (Wisd - Witeg Laboratory Equipment, Wertheim, Germany). Amplicons of ITS and *trnL - trnF* Intergenic spacer were formed as single band while ISSR amplicons with multi bands.

2.7.4 ISSR analysis

Fragments amplified with ISSR molecular markers were treated as a character and scored as binary code (1 for presence and 0 for absence). Only visibly clear and unambiguous bands were considered for scoring. POPGENE version 1.32 (Yeh et al., 1999) and MVSP 3.22 (Multi-Variate Statistical Package) were used in analysis. Jaccard similarity index was used for collection of matrix of similarities between individuals. Genetic parameters calculated herein included the percentage of polymorphic loci (P), mean number of observed (Na) alleles, effective (Ne) alleles per locus, (Kimura, 1964) Nei's gene diversity (h) and Shannon's information index (I). Graphical representation of ISSR relationships among individuals were provided using a principal component analysis (PCA) test, which was demonstrated with variance-covariance matrix calculated from marker data using MVSP 3.2 program. Jaccard's similarity coefficients were used to generate a dendrogram by using unweight pair group method with calculating the arithmetic average (UPGMA) by MVSP 3.2.

2.7.5 Phylogenetic analysis

ITS and trnL-F Intergenic Spacer primers generated single monomorphic bands as expected. 3µ of PCR product and 1µ of DNA loading dye were mixed and loaded into agarose gel (1.2%) to display the relevant bands in expected size. The remaining 20-22µ of PCR products were used for sequencing applications. PCR products were sequenced by Iontek Molecular Diagnostics (IMD - Maslak, Istanbul, TURKEY).

Sequencing processes were performed for ITS1 and trnL-F Intergenic spacer regions by using three same individuals from each location. Obtained sequences were analyzed and short or incorrect DNA sequences were eliminated and if necessary the sequencing process were repeated. DNA sequences of other species were obtained from Nucleotide data base of GenBank/NCBI (ncbi.nlm.nih.gov/genbank/).

Obtained and our DNA sequences were aligned by using ClustalW in BioEdit 7.2.5 (Hall, 1999; Larkin et al., 2007) with default parameters. Phylogenetic tree was constructed by using MEGA 6 with Maximum-Likelihood (ML) method (Tamura et al., 2013) according to the analysis options given in Figure 3x. Bootstrap tests were applied using MEGA 6 with 1000 replicates. The evolutionary distances were calculated by using maximum composite likelihood method. Kimura 2-parameter nucleotide substitution model and complete deletion option were used to obtain the datasets. Sequence identity matrix, G+C content (%), Tajima's test of neutrality (Tajima, 1989), and conserved regions were calculated with BioEdit 7.2.5.

```
Analysis
  Analysis ------------------------ Phylogeny Reconstruction
  Statistical Method -------------- Maximum Likelihood
Phylogeny Test
  Test of Phylogeny --------------- Bootstrap method
  No. of Bootstrap Replications --- 1000
Substitution Model
  Substitutions Type -------------- Nucleotide
  Model/Method -------------------- Tamura-Nei model
Rates and Patterns
  Rates among Sites --------------- Uniform rates
Data Subset to Use
  Gaps/Missing Data Treatment ----- Complete deletion
Tree Inference Options
  ML Heuristic Method ------------- Nearest-Neighbor-Interchange (NNI)
  Initial Tree for ML ------------- Make initial tree automatically (Maximum Parsimony)
  Branch Swap Filter -------------- Very Strong
System Resource Usage
  Codons Included ----------------- 1st+2nd+3rd+Non-Coding
```

Figure 2.21 The adopted parameters for phylogenetic analysis

2.8 Statistical analysis

All element calculations were done based on the dry weight of samples. The protein concentrations were analyzed using software IBM SPSS Statistics 20 with One Way Analyses of Variance (ANOVA) with Tukey's post-hoc HSD (Table xy). Statistical significance was showed as $^{**}P<0.01$ and $^{*}P<0.05$. Tukey's post-hoc tests were employed for localities and terms.

The chlorophyll concentrations were analyzed using software IBM SPSS Statistics 20 with Multivariate Analysis of Variance (MANOVA) with Tukey's post-hoc HSD. Statistical significance was showed as $^{**}P<0.01$ and $^{*}P<0.05$. Tukey's post-hoc tests were employed for chlorophyll concentrations and terms.

The element concentrations were analyzed using software IBM SPSS Statistics 20 with Repeated Measures MANOVA with Tukey's post-hoc HSD and Pearson correlation (Table zz, Table ww and Tablo qq).). Statistical significance was shown as $^{**}P<0.01$ and $^{*}P<0.05$ (2-tailed).

3. RESULTS AND DISCUSSION

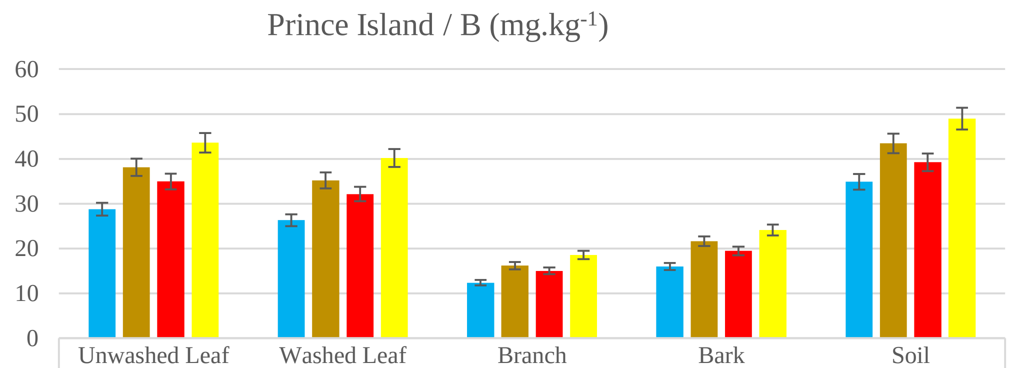
3.1 Mineral Elements and Heavy metals

In this study B, Ca, Cd, Cr, Mg, Cu, Fe, K, Mn, Na, Ni, Pb, and Zn elements were determined by using ICP-OES. Unwashed leaves (UwL), washed leaves (WL), bark (B) and branch (S) samples as plant parts and soil samples were analyzed for determination of heavy metal and mineral nutrient element levels. In addition removal rates were calculated by using element amounts in unwashed and washed leaf samples. All detected all heavy metal and mineral element concentrations were shown in supplementary section Tables 1-13.

It is aimed to reveal heavy metal levels and effects of these heavy metals on mineral nutrition status of *R. pseudoacacia* plants. *R. pseudoacacia* plants were chosen from different stations which have different levels of vehicle traffic and industrial sourced heavy metal pollution.

3.1.1 Boron (B)

Boron is a micronutrient for plants. It functions in metabolic processes and has importance as basic structural material for cell wall. But excess amount of B can be harmful (Kabata-Pendias and Mukherjee 2007). According to results, B content in plant parts ranged between 2.800±0.067 mg.kg^{-1} (Branch/Dilovasi District) and 43.604±0.927 mg.kg^{-1} (Unwashed leaves/Prince Island). B concentration of soil ranged between 17.497±0.357 mg.kg^{-1} (Dilovasi District) and 48.959±1.139 mg.kg^{-1} (Prince Island). Prince Island samples contained the highest B concentrations both in plant and soil. Dilovasi District has the lowest B concentrations.

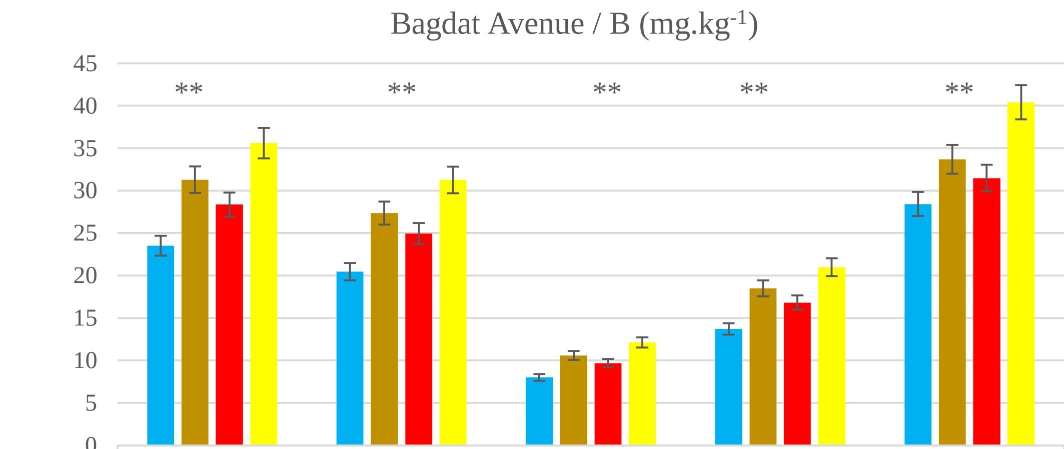


	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	28.749	26.323	12.391	16.002	34.898
Autumn	38.129	35.188	16.200	21.636	43.455
Winter	34.949	32.133	15.004	19.463	39.248
Spring	43.604	40.203	18.563	24.161	48.959

Summer Autumn Winter Spring

Figure 3.1 Average B concentrations in Prince Island

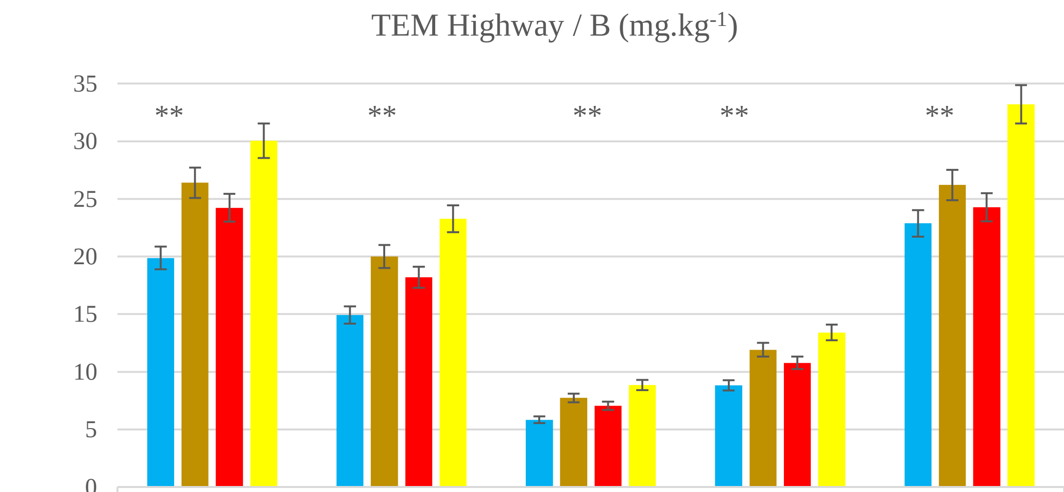
Results indicated that B concentrations in all samples for all stations tend to increase during autumn and spring, and decreasing during summer and winter. The highest B content in plant samples have accumulated at spring. Likewise the highest soil B content was also detected in spring. Whereas the lowest accumulation of B was detected in summer in both plant and soil samples of all stations.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	23.508	20.451	8.007	13.698	28.420
Autumn	31.278	27.359	10.583	18.492	33.691
Winter	28.364	24.931	9.675	16.805	31.477
Spring	35.596	31.264	12.121	20.979	40.410

Summer Autumn Winter Spring

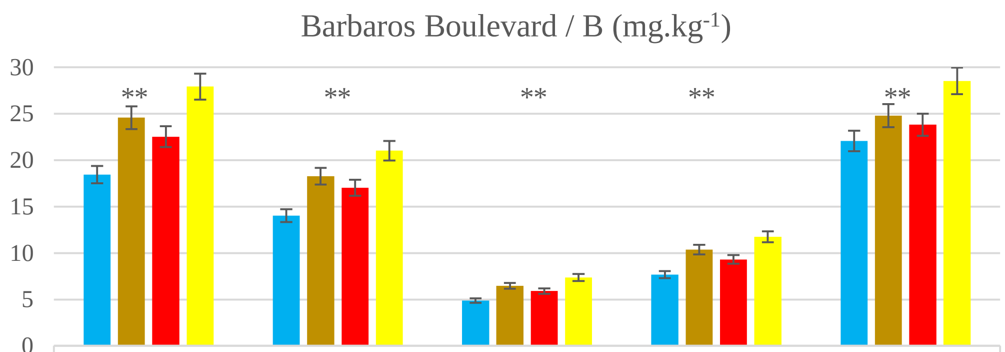
Figure 3.2 Average B concentrations in Bagdat Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	19.876	14.930	5.830	8.829	22.876
Autumn	26.395	19.994	7.730	11.907	26.206
Winter	24.231	18.191	7.046	10.776	24.268
Spring	30.041	23.287	8.863	13.413	33.213

Summer Autumn Winter Spring

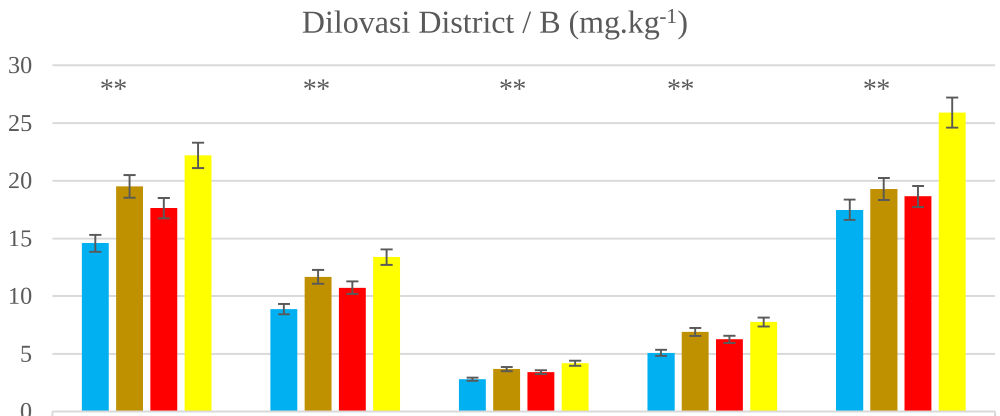
Figure 3.3 Average B concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	18.450	14.033	4.884	7.666	22.072
Autumn	24.574	18.273	6.468	10.382	24.803
Winter	22.534	17.027	5.915	9.310	23.827
Spring	27.920	21.032	7.373	11.751	28.535

Summer Autumn Winter Spring

Figure 3.4 Average B concentrations in Barbaros Boulevard.



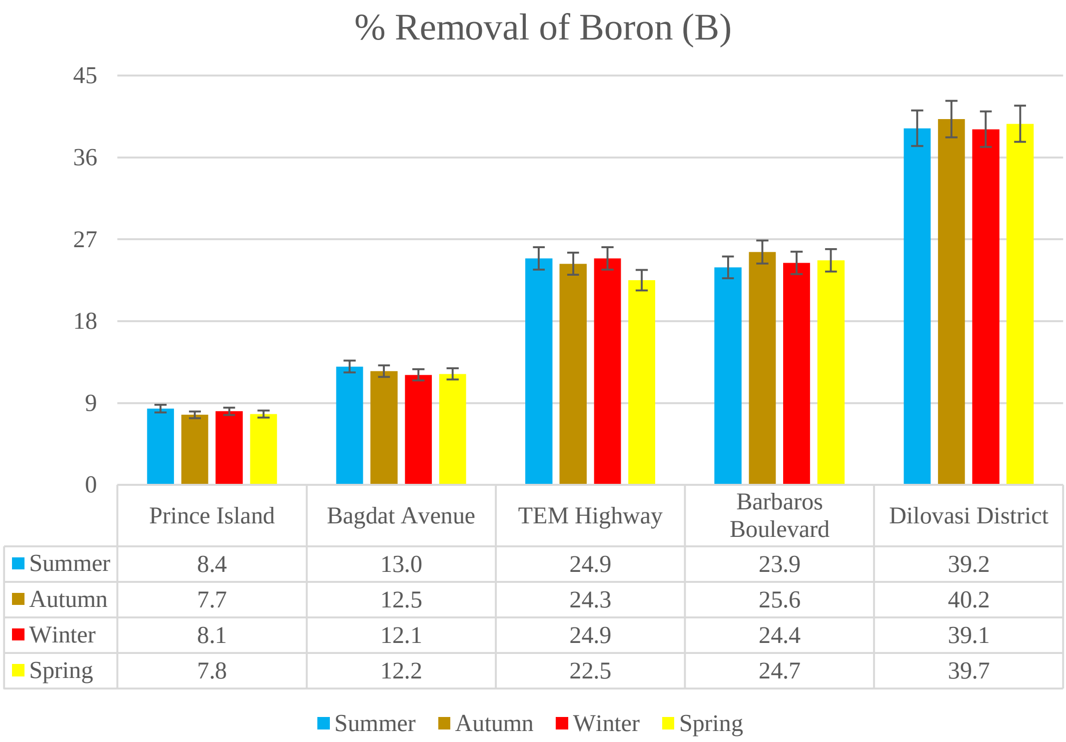
	Unwashed Leaf	Washed Leaf	Stem	Bark	Soil
Summer	14.599	8.876	2.800	5.081	17.497
Autumn	19.517	11.680	3.678	6.897	19.289
Winter	17.631	10.732	3.411	6.256	18.637
Spring	22.196	13.385	4.184	7.766	25.906

Summer Autumn Winter Spring

Figure 3.5 Average B concentrations in Dilovasi District.

The highest removal rate (Figure 3.6) was calculated in Dilovasi District for autumn samples as 40.2% and the lowest was calculated in Prince Island also for autumn samples with 7.7%. While the lowest B concentrations were detected in Dilovasi District, removal rate of B increased from Prince Island to Dilovasi District gradually. As the B concentrations in plant and soil decreased, the removal rates of B increased relatively.

Higher removal rates were determined at TEM Highway, Barbaros Boulevard and Dilovasi District (Figure 3.6). The main factor that caused increase in the removal rates may be airborne emission of B in these stations. This suggestion can be supported, since there are many sources of boron such as detergent, glass, porcelain, leather and fertilizer factories and fly ash sourced from power plants and combustion of fuel additives (Nable et al., 1997; Gan et al., 2012; Vatansever et al., 2016). Also all type of mentioned factories and power plants are present at Dilovasi District. TEM Highway and Barbaros Boulevard stations have heavy traffic flow which can emit B in to ecosystem due to combustion of fuel additives.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	8.4	13.0	24.9	23.9	39.2
Autumn	7.7	12.5	24.3	25.6	40.2
Winter	8.1	12.1	24.9	24.4	39.1
Spring	7.8	12.2	22.5	24.7	39.7

Figure 3.6 Removal rates of B

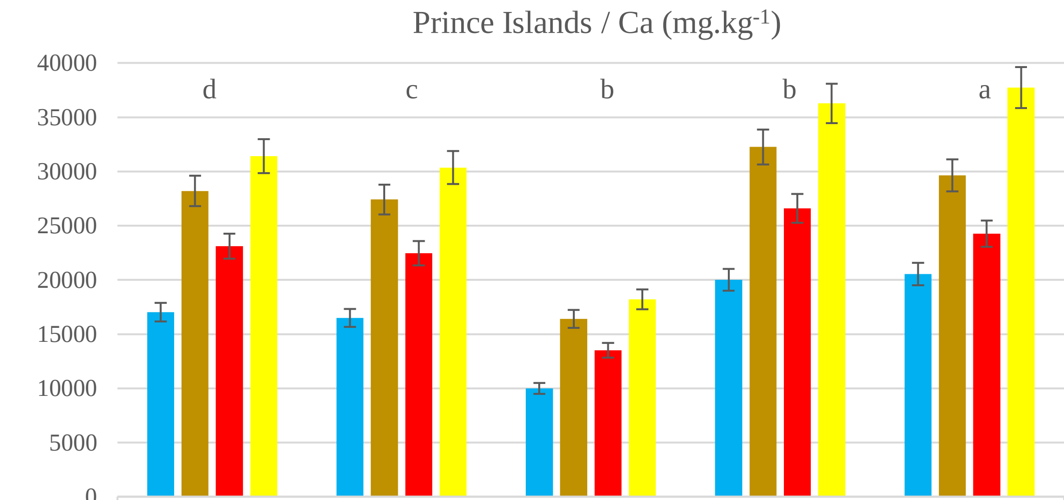
Levels of B in plant parts decreased from Prince Island to Dilovasi District in relation with decreasing levels of B in soil. B is a highly dissolving element. Coal combustion derivates fly ash contains some trace elements in large quantity (especially B) and may cause ecotoxic problems. For example fly ash sourced B is associated with reduction of plant production (Matsi and Tsadilas 2005).

Olìas et al. (2004) and Li and Zhang (2011) stated that precipitation increases the dissolving mineral elements in soil. In relation to these suggestions and removal rates, it can be suggested that especially in Dilovasi District B emissions occurred via air. Interaction between precipitation and B emission in air can increase B levels in the soil in spring season. Additionally Blevins and Lukaszewski (1998) reported that B content in plants increases in autumn and spring seasons due to taking part in the plant physiological processes.

Kacar and Katkat (2007) reported that the acceptable B levels in plant parts and soil are 10-100 and 20-200 $mg.kg^{-1}$ respectively. Highest levels in plants and soil are detected as 43.604 $mg.kg^{-1}$ (UwL/spring/Prince Island) in plants and 48.959 $mg.kg^{-1}$ (spring/ Prince Island) in soil. Lowest levels are detected as 2.801 $mg.kg^{-1}$ (branch/summer/Dilovasi) in plants and 17.497 $mg.kg^{-1}$ (summer/Dilovasi) in soil. According to these findings, plant and soil B levels are within the normal limits.

3.1.2 Calcium (Ca)

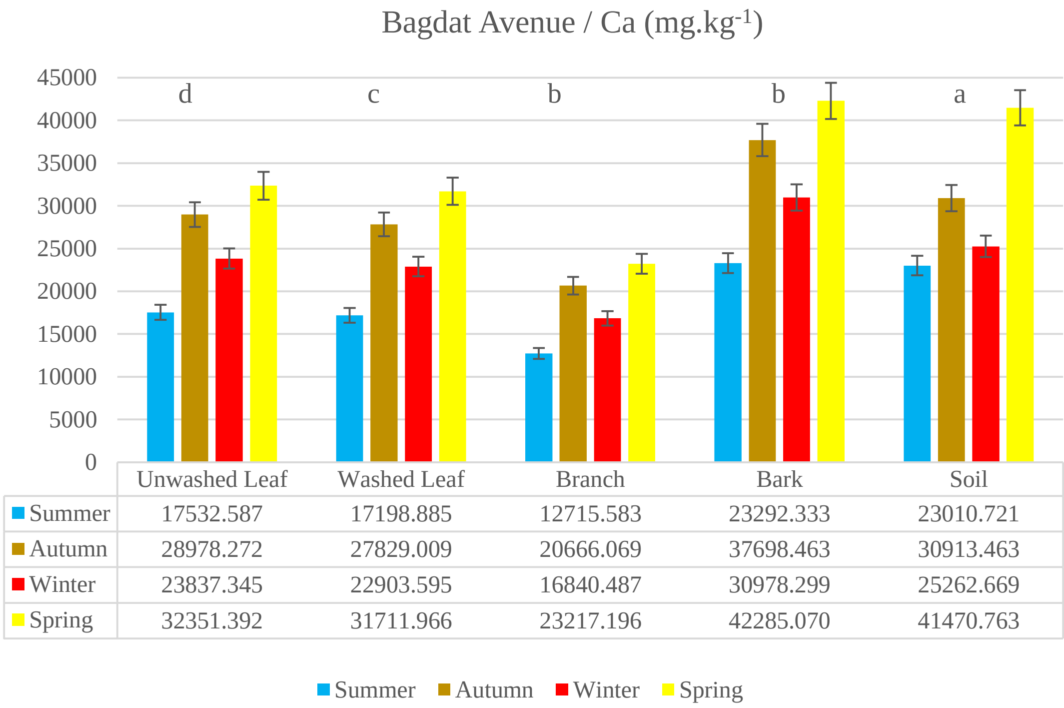
Calcium is one of the macronutrients and it serves as metabolic and structural component of cell. According to results, Ca levels of samples increased from Prince Island, which has not traffic and industrial establishment, to Dilovasi District which has heavy industrial zone. Ca concentrations of soil ranged between 20535.347±624.843 mg.kg^{-1} (Prince Island) and 55414.639±1255.819 mg.kg^{-1} (Dilovasi District). Ca concentrations in plant parts ranged between 16500.672±482.137 mg.kg^{-1} (washed leaves/Prince Island) and 54655.701±1066.560 mg.kg^{-1} (bark/Dilovasi District).



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	17032.076	16500.672	9998.307	20003.901	20535.347
Autumn	28208.951	27419.856	16398.555	32259.537	29651.258
Winter	23110.249	22451.903	13507.313	26597.678	24268.010
Spring	31414.618	30356.798	18199.914	36276.290	37728.699

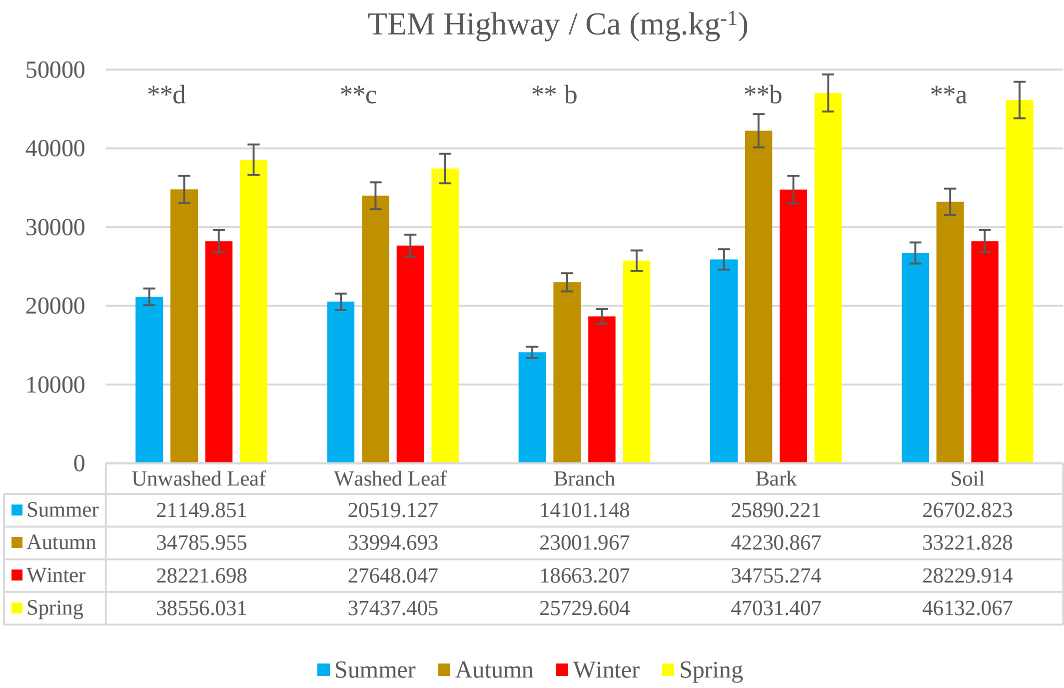
Figure 3.7 Average Ca concentrations in Prince Island.

The highest Ca content in plant samples was detected in spring among with an increase in soil samples for all stations. The lowest Ca content was detected in summer for all samples and stations. Increased Ca concentrations in autumn decreased in winter, then increased in spring.



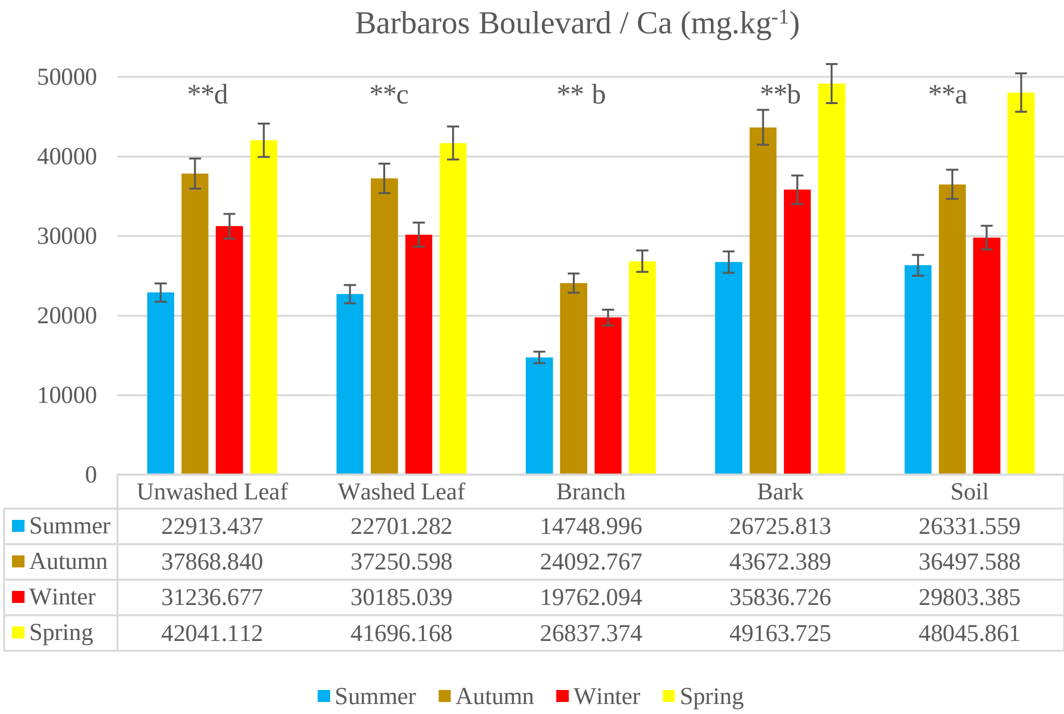
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	17532.587	17198.885	12715.583	23292.333	23010.721
Autumn	28978.272	27829.009	20666.069	37698.463	30913.463
Winter	23837.345	22903.595	16840.487	30978.299	25262.669
Spring	32351.392	31711.966	23217.196	42285.070	41470.763

Figure 3.8 Average Ca concentrations in Bagdat Avenue.



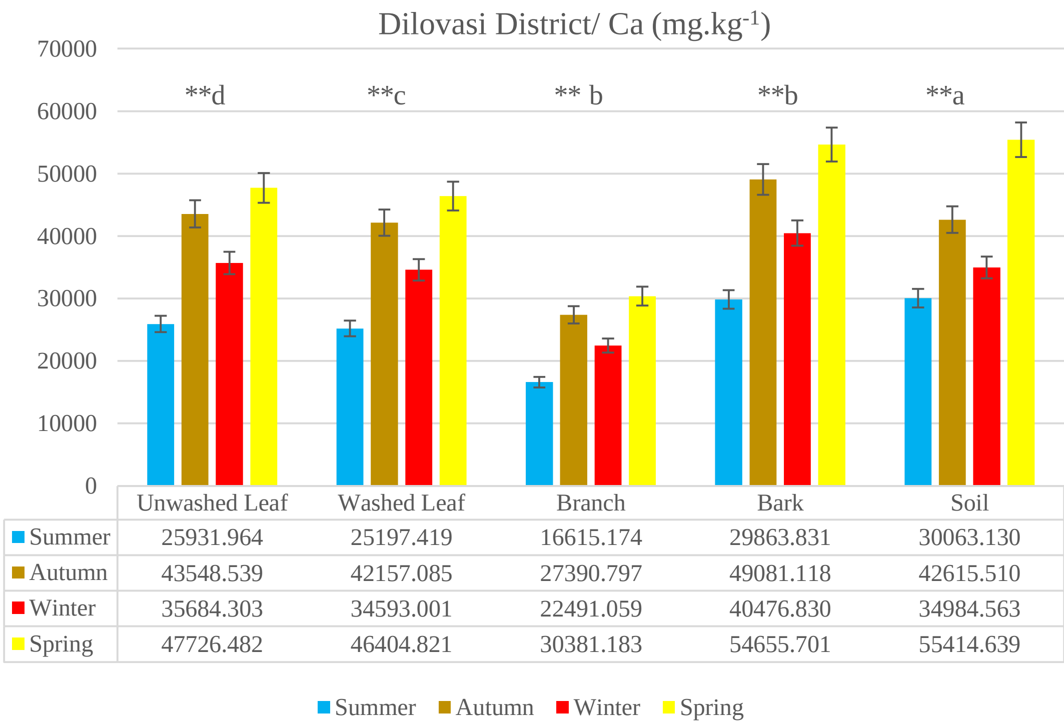
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	21149.851	20519.127	14101.148	25890.221	26702.823
Autumn	34785.955	33994.693	23001.967	42230.867	33221.828
Winter	28221.698	27648.047	18663.207	34755.274	28229.914
Spring	38556.031	37437.405	25729.604	47031.407	46132.067

Figure 3.9 Average Ca concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	22913.437	22701.282	14748.996	26725.813	26331.559
Autumn	37868.840	37250.598	24092.767	43672.389	36497.588
Winter	31236.677	30185.039	19762.094	35836.726	29803.385
Spring	42041.112	41696.168	26837.374	49163.725	48045.861

Figure 3.10 Average Ca concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	25931.964	25197.419	16615.174	29863.831	30063.130
Autumn	43548.539	42157.085	27390.797	49081.118	42615.510
Winter	35684.303	34593.001	22491.059	40476.830	34984.563
Spring	47726.482	46404.821	30381.183	54655.701	55414.639

Figure 3.11 Average Ca concentrations in Dilovasi District.

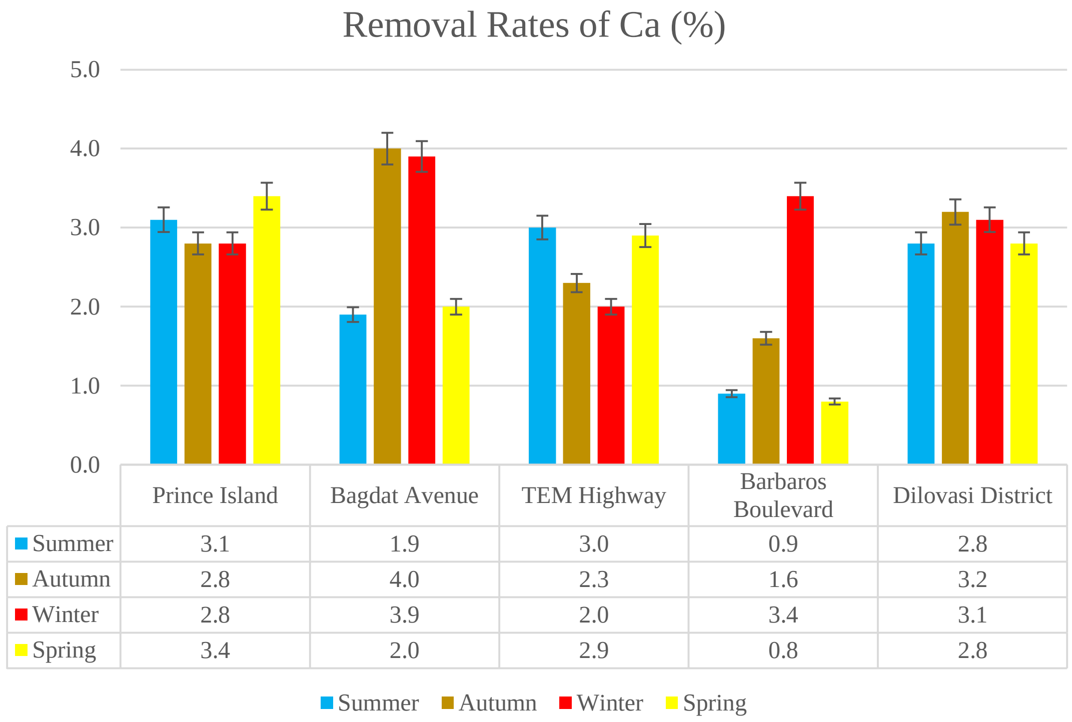


Figure 3.12 Removal rates of Ca.

Removal rates of Ca (Figure 3.12) ranged between 0.8% and 4.0% with fluctuations unrelated to stations and seasons. The highest removal rate was calculated for Bagdat Avenue in autumn samples with 4.0% and the lowest was calculated for Barbaros Boulevard in spring samples with 0.8%. Removal rate of Prince Island and Dilovasi District showed low variation in different seasons while other stations showed high variation in relation with season.

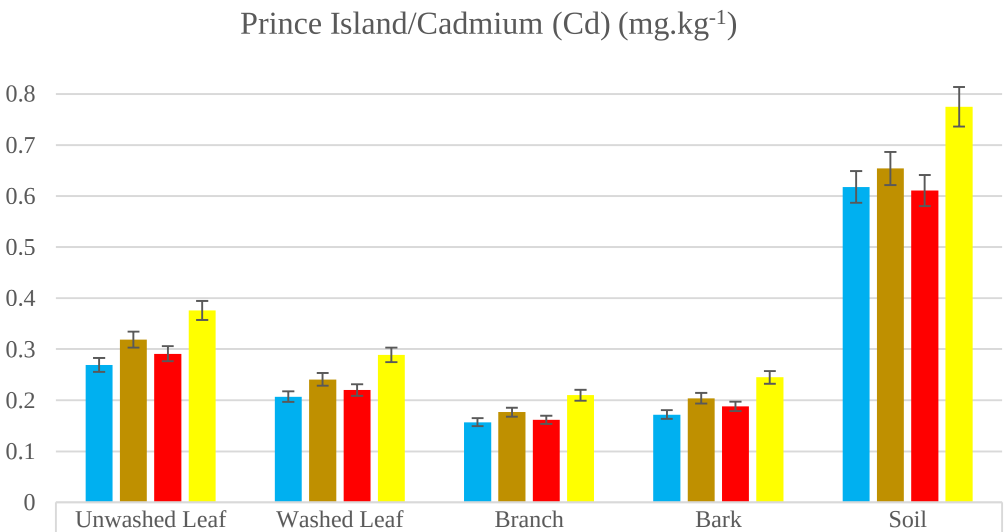
Esen et al., (2016) conducted a study for assessment of biomonitor capability of *Carpinus betulus*, *Quercus petraea* and *Tilia argentea* trees. Instrumental neutron activation analysis (INAA) was used for detecting element levels in soil, leaves and twigs. One control (Atatürk Arboretum) and 3 urban (Bahcekoy, Levent and Yildiz Park), in total four stations were selected. Ca levels in soil were detected as 1100.0, 4300.0, 27000.0 and 11700.0 mg.kg^{-1} respectively and Ca levels in leaf samples were detected between 15200.0 and 51500.0 mg.kg^{-1}. Our findings in Ca levels in soil were higher than Esen et al., (2016) while Ca levels in leaf samples were about same. Difference in soil Ca levels may be caused by study station choices and our stations has more vehicle traffic and industrial facilities. Tzvetkova and Petkova (2015) conducted a study for investigation of heavy metal accumulation by using *R. pseudoacacia* plants as biomonitor in industrial zones. According the study, Ca levels in leaves of industrial zone samples were detected between 15400.0 and 38920.0 $mg.kg^{-1}$ and for leaves of control samples between 15330.0 and 37120.0 $mg.kg^{-1}$. Leaf Ca levels of Dilovasi Disctrict as industrial zone were higher and leaf Ca levels Prince Island study as control zone were relatively lower than the results of mentioned study. Jensen et al., (2010) conducted a study for determining mineral element levels in leaves of *Fraxinus pennsylvanica* and *R. pseudoacacia* at a reclaimed mine site. According to results of the study Ca levels in *R. pseudoacacia* were determined as 12200.0 $mg.kg^{-1}$ for control site and 17800 $mg.kg^{-1}$ for reclaimed mine site. Both of control and reclaimed mine site Ca levels in leaves were lower than our control and other stations Ca level results. In a study of Rahmonov (2009), role of plant litter (*R. pseudoacacia* litter) was investigated in a sandy ecosystem by analyzing chemical composition of the plant and litter. Ca levels in leaves, shots and barks were detected as 19116.0, 9398.0 and 3478.0 $mg.kg^{-1}$ respectively. Ca Level in findings of Rahmonov (2009) were lower than our level of Ca findings. Taberi and Salehi (2009) conducted a study for investigation of effects of municipal sewage irrigation on *R. pseudoacacia* plants. Two group of plants were selected as treatment group which were artificially irrigated with sewage and control group which was irrigated with well water. Ca levels in leaves were detected as 31570.0 $mg.kg^{-1}$ for irrigated sewage water and 27480.0 $mg.kg^{-1}$ for irrigated well water. Lowest and highest leaf Ca concentrations were determined as 16500.672 in (Prince Island) and 47726.482 (Dilovasi District) $mg.kg^{-1}$ respectively. Range of these results is higher in comparison with Taberi and Salehi (2009) Ca level results. Ca concentrations in plants tissues increased from Prince Island to Dilovasi District in correlation with increasing traffic density and industrial activities. This situation may be related with increased heavy metal concentrations. Plants react to survive under heavy metal stress conditions with various protection mechanisms. Chemical similarity of Ca to Cd makes Ca a useful protector against Cd stress. Plants raise Ca levels to compete with and reduce effects of Cd (Lachman et al., 2015).

Table 3.1 Detected Ca Levels in different studies (L: leaf, B: bark, S: branch)

Reference	Plant	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/City	Method
		Control	Treatment	Control	Treatment		
Esen et al., (2016)	***Carpinus betulus***	1100	4300-27000	15400 (L)	16500-22500 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			15200 (L)	20700-36100 (L)		
	Tilia Argentea			50100 (L)	31900-51500 (L)		
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---	---	15330 (L) June	1540 (L) June	Bulgaria	AAS
		---	---	37120 (L) Sept.	38920 (L) Sept		
Jensen et al., (2010)	***R. pseudoacacia***	---		12200 (L)	17800 (L)	USA	ICP-MS
Rahmonov (2009)	***R. pseudoacacia***	---	---	19116 (L)		Poland	FAAS
		---	---	13478 (B)			
		---	---	9398 (S)			
Tabari and Salehi (2009)	***R. pseudoacacia***	20050	26290	27480 (L)	31570 (L)	Iran	AAS

3.1.3 Cadmium (Cd)

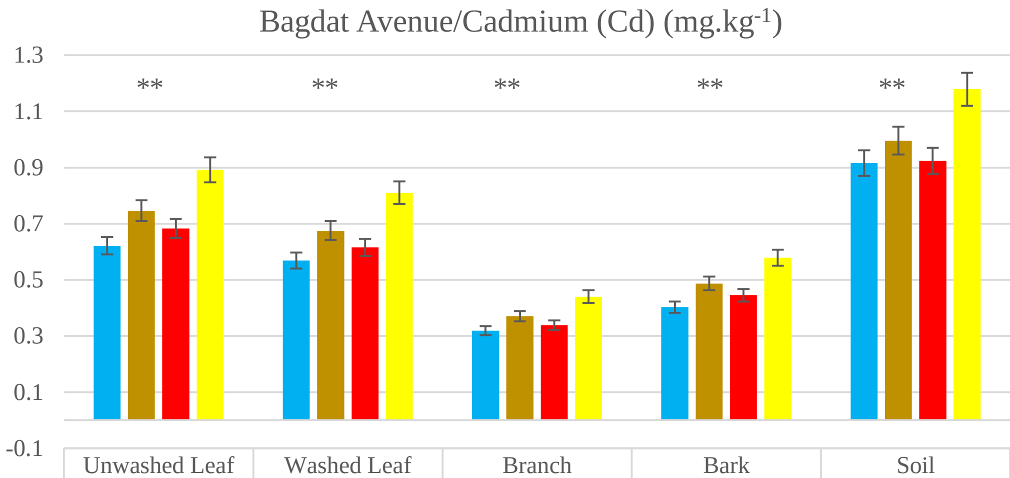
Cadmium is one of the most hazardous heavy metals, which threats all organisms. Emission of Cd is sourced mainly from anthropogenic activities and especially industrial activities (Wuana and Okieimen, 2011; Das et. al. 2014). According to results Cd concentrations in plant samples elevated in relation with soil samples from Prince Island to Dilovasi District. Lowest Cd content in plant samples was detected in Prince Island as 0.157±0.003 $mg.kg^{-1}$ in branch samples, highest Cd content was detected in Dilovasi District as 7.799±0.161 $mg.kg^{-1}$ in unwashed leaves. Soil samples Cd content ranged between 0.611± 0.014 $mg.kg^{-1}$ (Prince Island/ winter) and 8.853±0.282 $mg.kg^{-1}$ (Dilovasi District/spring).



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	0.269	0.207	0.157	0.172	0.618
Autumn	0.319	0.241	0.177	0.204	0.654
Winter	0.291	0.220	0.162	0.188	0.611
Spring	0.376	0.289	0.210	0.245	0.775

Figure 3.13 Average cadmium (Cd) concentrations in Prince Island

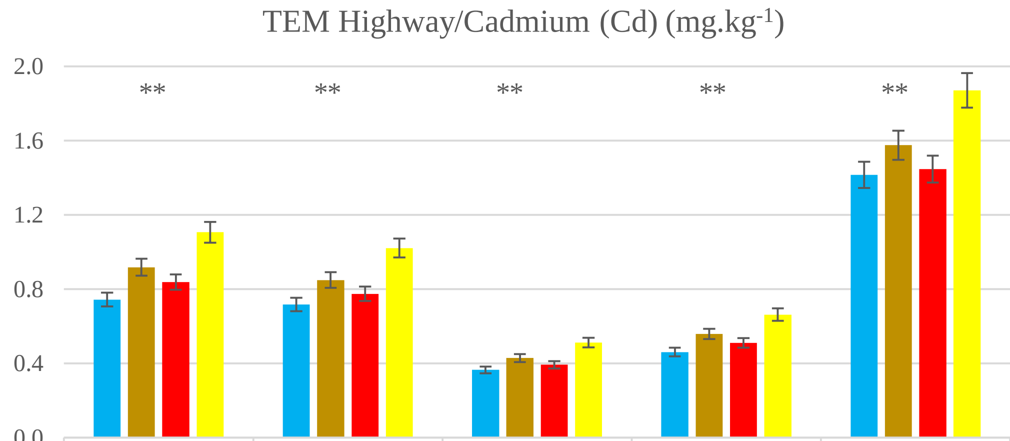
Cd content of plants and soil samples changed according to the season in all stations. The lowest Cd in plant samples were detected in summer then Cd contents increased in autumn then decreased again in winter. Finally, the highest Cd content was detected in spring. These fluctuations may be caused by various effects including dilution effect, high solubility of minerals with high precipitation and defoliation.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	0.621	0.569	0.319	0.403	0.916
Autumn	0.746	0.675	0.370	0.487	0.996
Winter	0.683	0.615	0.338	0.445	0.924
Spring	0.892	0.810	0.440	0.579	1.179

Summer Autumn Winter Spring

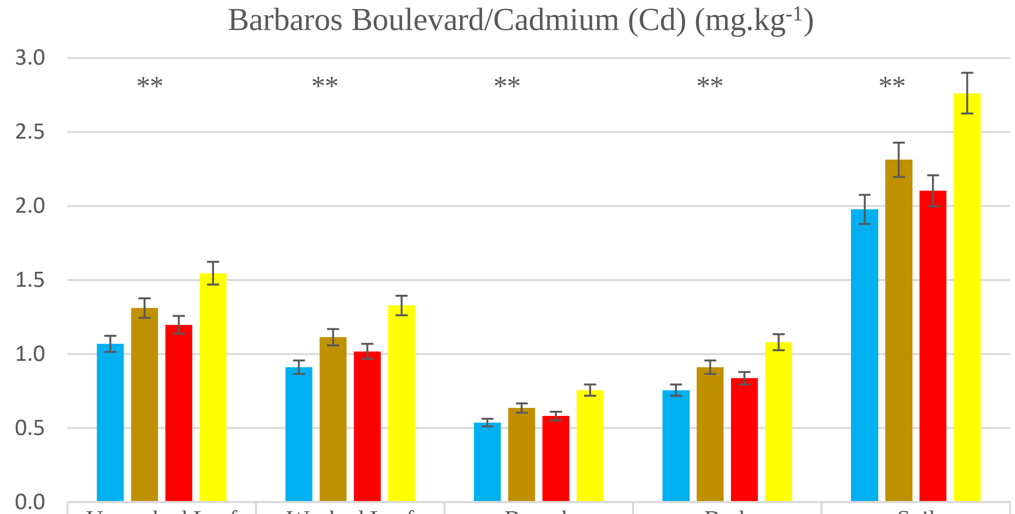
Figure 3.14 Average cadmium (Cd) concentrations in Bagdat Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	0.744	0.717	0.365	0.461	1.415
Autumn	0.918	0.849	0.429	0.559	1.575
Winter	0.838	0.775	0.393	0.511	1.446
Spring	1.106	1.021	0.512	0.663	1.870

Summer Autumn Winter Spring

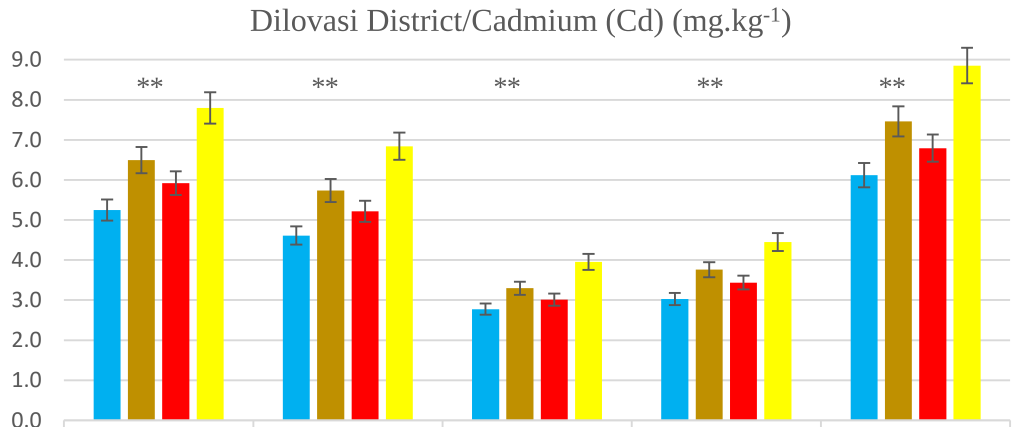
Figure 3.15 Average cadmium (Cd) concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	1.069	0.911	0.537	0.756	1.977
Autumn	1.311	1.114	0.636	0.912	2.313
Winter	1.198	1.018	0.582	0.837	2.103
Spring	1.546	1.328	0.756	1.081	2.762

Summer Autumn Winter Spring

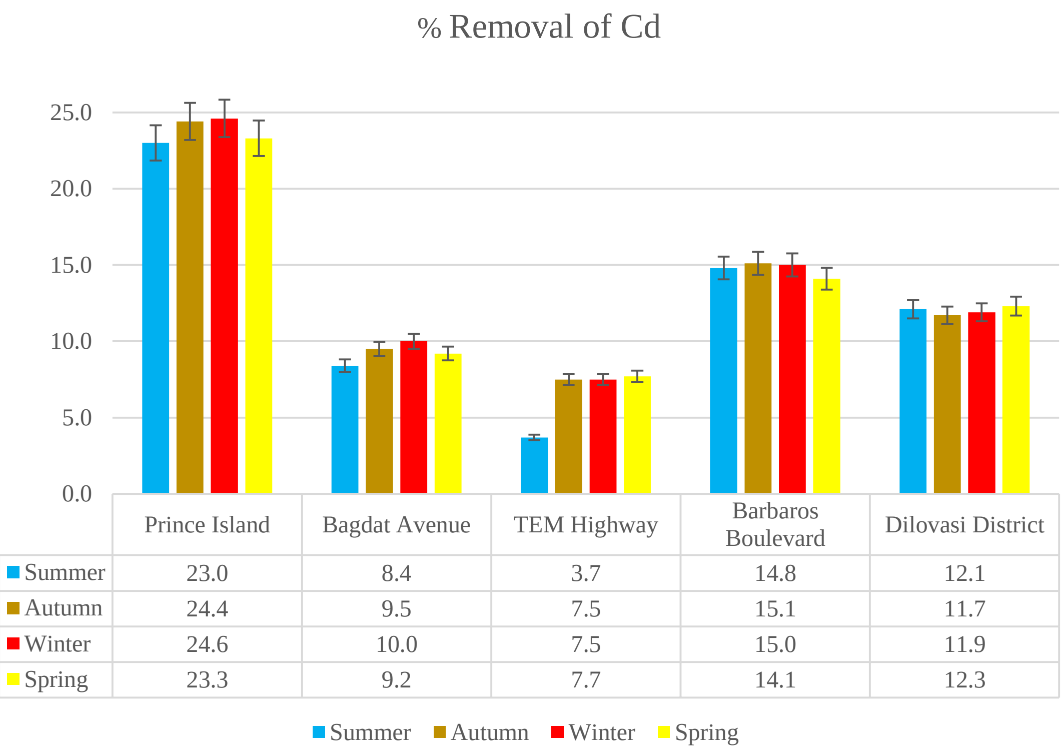
Figure 3.1.16 Average Cd concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	5.246	4.613	2.775	3.027	6.119
Autumn	6.495	5.734	3.296	3.760	7.461
Winter	5.919	5.217	3.013	3.439	6.792
Spring	7.799	6.841	3.953	4.451	8.853

Summer Autumn Winter Spring

Figure 3.1.17 Average Cd concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	23.0	8.4	3.7	14.8	12.1
Autumn	24.4	9.5	7.5	15.1	11.7
Winter	24.6	10.0	7.5	15.0	11.9
Spring	23.3	9.2	7.7	14.1	12.3

Figure 3.1.18 Removal rates of Cd between unwashed and washed leaves

The highest removal rate of Cd was calculated as 24.6 % at Prince Island. The lowest removal rate of Cd was calculated as 3.7% at TEM Highway. High removal rates in Prince Island could be sourced from too low level of Cd concentration. A small differentiation in the low values emerges as the high value of the removal rate. Additionally the high rate of removal indicates airborne emission of Cd for Prince Island. There was no significant difference between removal rates in relation with season in all stations.

Cd is a non-essential and toxic heavy metal. Cd is highly soluble and mobile in soil solutions. Thus Cd can easily penetrate plant tissues and food chains. Additionally soil Cd content effects Cd uptake and cause accumulation in plant tissues (Sarwar et al., 2010). There are a lot of studies in scientific literature for determining and assessment of Cd levels and effects on organisms. Some of them are about *R. pseudoacacia* and biomonitoring Cd levels from different countries are shown in Table 3.2. As mentioned above our detected Cd ranges are 0.611-8.853 mg.kg^{-1} for soil samples and 0.157-7.799 mg.kg^{-1} for plant samples. Expected soil Cd content usually ranged between 0.06-1.1 with 0.5 mg.kg^{-1} average (Kabata-Pendias and Mukherjee 2007). According to these values soil Cd content of TEM, Barbaros and Dilovasi stations are above the normal limits. Some results of Armaki 2016, Nadgorska-Socha et al., 2016, Celik et al., 2004, Mertens et al., 2004 and Aksoy et al., 2000 were also above the normal limits while detected soil Cd content can be considered within the normal limits.

Table 3.2 Detected Cd Levels in different studies (L: leaf, UwL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

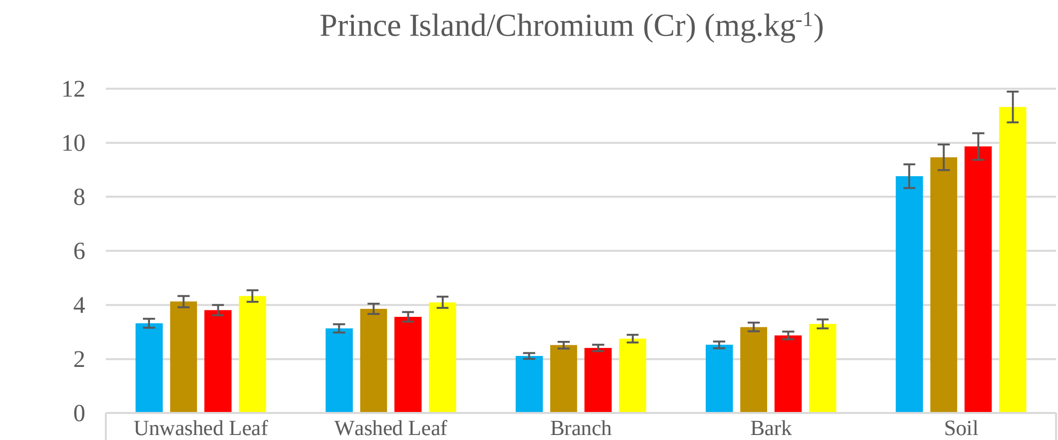
Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/city	Method
		Control	Study Site	Control	Study Site		
Armaki (2016)	***R. pseudoacacia***	3.4		2.3 (L)		Iran	ICP (OES)
				2.6 (S)			
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	1.31	3.12-31.67	0.11 (L)	0.22-3.01 (L)	Poland	Flame AAS
Palowski et al., (2016)	***R. pseudoacacia***	---	---	1.87-2.41 (L)		Poland	Flame AAS
				2.01-2.60 (B)			
Monfred et al., (2013)	***R. pseudoacacia***	---	---	11.3 (L)		Iran	ICP-OES
				9.7 (S)			
Serbula et al., (2012)	***R. pseudoacacia***	<0.5	0.5-0.86	ND	ND	Serbia	ICP-AES / AAS
Asgari and Amini (2011)	***R. pseudoacacia***					Iran	AAS
Gjorgieva et al., (2011)	***R. pseudoacacia***					Macedonia	ICP-AES
Kaya et al., (2010)	***R. pseudoacacia***	0.015-0.063	0.142-0.656	0.04	0.057-0.367	**TUR/Gaziantep**	ICP-MS
Yener and Yarci (2010)	***Alcea pallida***	0.117-1.373		0.203-1.081 (L)		**TUR/Istanbul**	AAS
				0.226-0.662 (S)			
Rahmonov (2009)	***R. pseudoacacia***	---	---	0 (L)		Poland	Flame AAS
		---	---	0 (B)			
		---	---	0 (S)			
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	0.38-0.45	0.6-1.9	0.03-0.05 (L)	0.04-1.1 (L)	Poland	Furnace AAS
				0.40-0.44 (B)	0.39-1.5 (B)		
Celik et al., (2005)	***R. pseudoacacia***	0.48	1.373-7.367	0.365 (UwL)	0.805-3.700 (UwL)	**TUR/Denizli**	Flame AAS
				0.325 (WL)	0.570-1.990 (WL)		
Mertens et al., (2004)	***R. pseudoacacia***	5.7 (Sediment)		<0.23 (L)		Belgium	Flame AAS
Aksoy et al., (2000)	***R. pseudoacacia***	0.64 (Rural)	1.20-9.88	0.47 (UwL)	0.77-3.39 (UwL)	**TUR/Kayseri**	AAS
				0.44 (WL)	0.58-1.22 (WL)		

The expected range of plant Cd contents is stated as 0.05-0.2 mg.kg^{-1} for the most of plants and above 5-30 mg.kg^{-1} is considered as toxic (Kabata-Pendias and Pendias, 2001). According to these limits, Cd contents of plant samples in Dilovasi District are in the toxic levels especially in the leaf samples. Our Cd content results are higher than the other studies which are mentioned at Table 3.2.

3.1.4 Chromium (Cr)

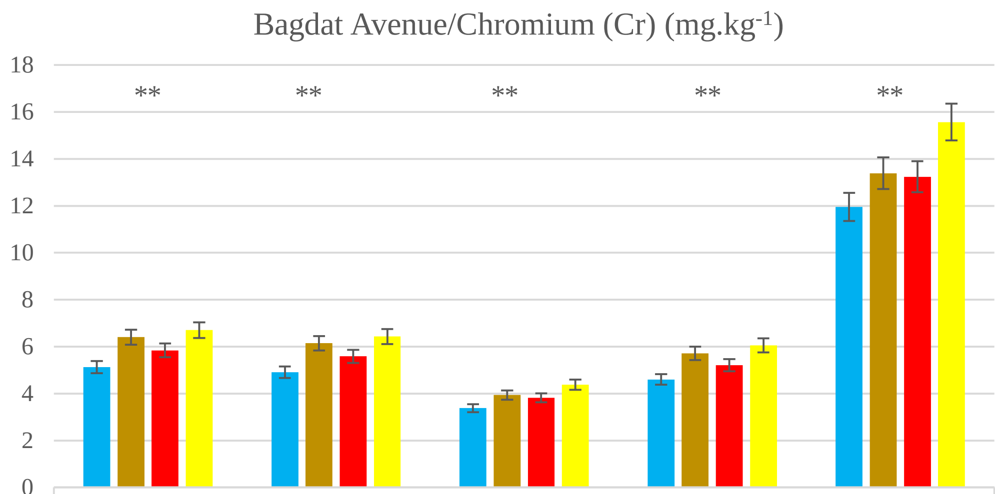
Chromium is an essential and toxic element at the same time. Some forms of Cr serve as microelement (Cr^{3+} trivalent) and some forms of it is toxic (Cr^{6+} hexavalent) to organisms depending on amount and form (Wuana and Okieimen, 2011). According to our results total Cr levels in plant samples ranged between 2.117±0.033 $mg.kg^{-1}$ (Prince Island/branch) and 21.988±0.938 $mg.kg^{-1}$ (Dilovasi District/bark). Chromium concentrations of soil ranged between 8.763±0.160 $mg.kg^{-1}$ (Prince Island) and 39.967±1.386 $mg.kg^{-1}$ (Dilovasi District).

Dilovasi District samples contained the highest Cr concentrations both in plant and soil while Prince Island has the lowest Cr concentrations. Seasonal changes in Cr contents were observed in the same way as other elements. Plant Cr contents were observed from low to high levels as follows; summer, winter, autumn, spring. Soil Cr contents also changed nearly same as plant samples while Prince Island soil Cr contents were detected in a different pattern. Soil Cr contents were observed from low to high levels as follows: summer, autumn, winter and spring.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	3.324	3.132	2.117	2.524	8.763
Autumn	4.124	3.854	2.512	3.183	9.460
Winter	3.812	3.556	2.411	2.877	9.863
Spring	4.330	4.097	2.753	3.300	11.323

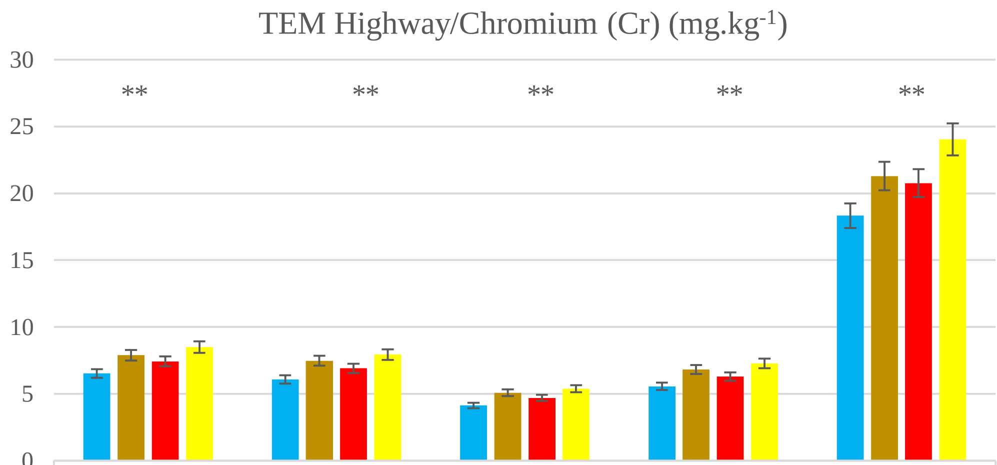
Figure 3.19 Average Cr concentrations in Prince Island



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	5.128	4.905	3.377	4.603	11.955
Autumn	6.404	6.146	3.939	5.715	13.392
Winter	5.841	5.589	3.825	5.214	13.240
Spring	6.702	6.434	4.381	6.057	15.572

Summer Autumn Winter Spring

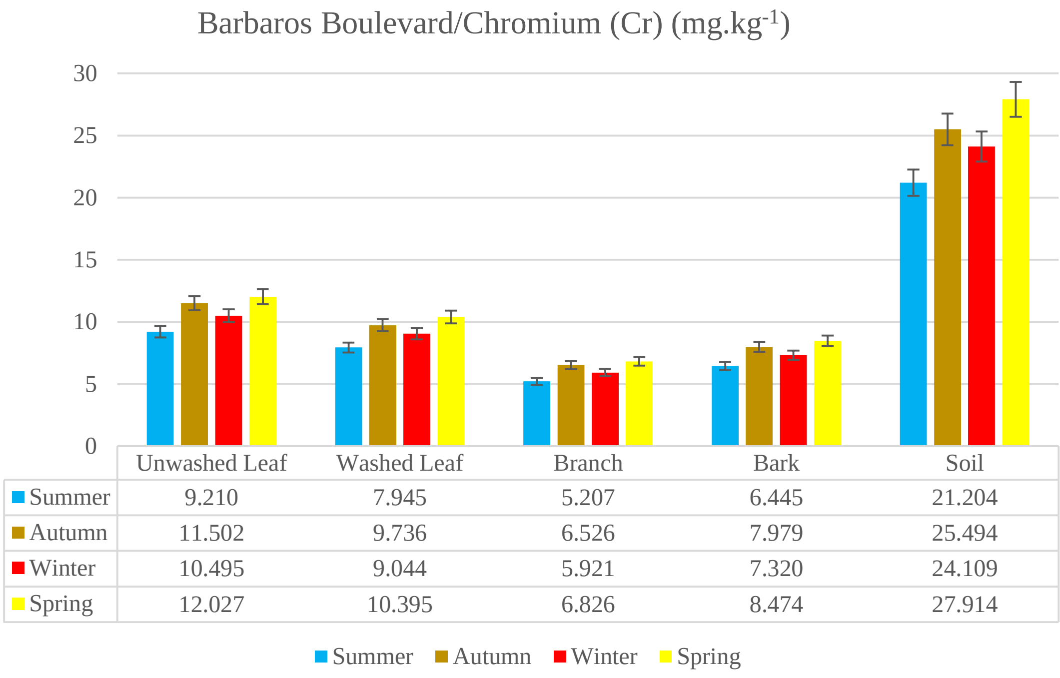
Figure 3.20 Average Cr concentrations in Bagdat Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	6.528	6.082	4.128	5.559	18.326
Autumn	7.891	7.478	5.082	6.825	21.291
Winter	7.423	6.906	4.701	6.295	20.762
Spring	8.496	7.940	5.388	7.283	24.028

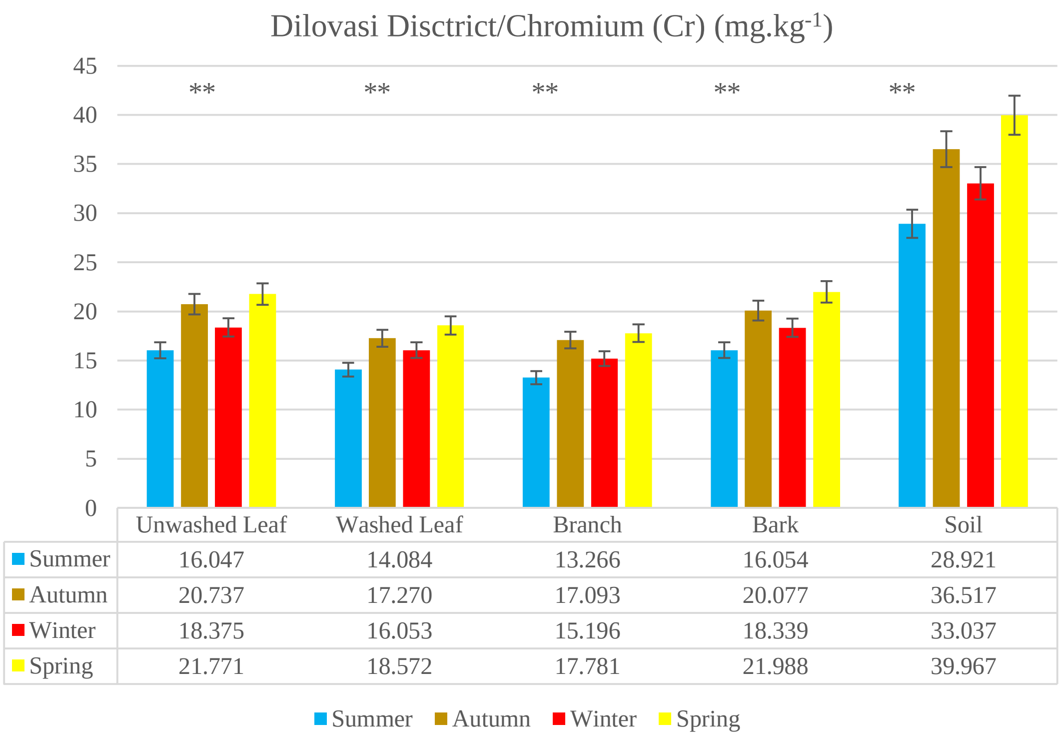
Summer Autumn Winter Spring

Figure 3.21 Average Cr concentrations in TEM Highway.



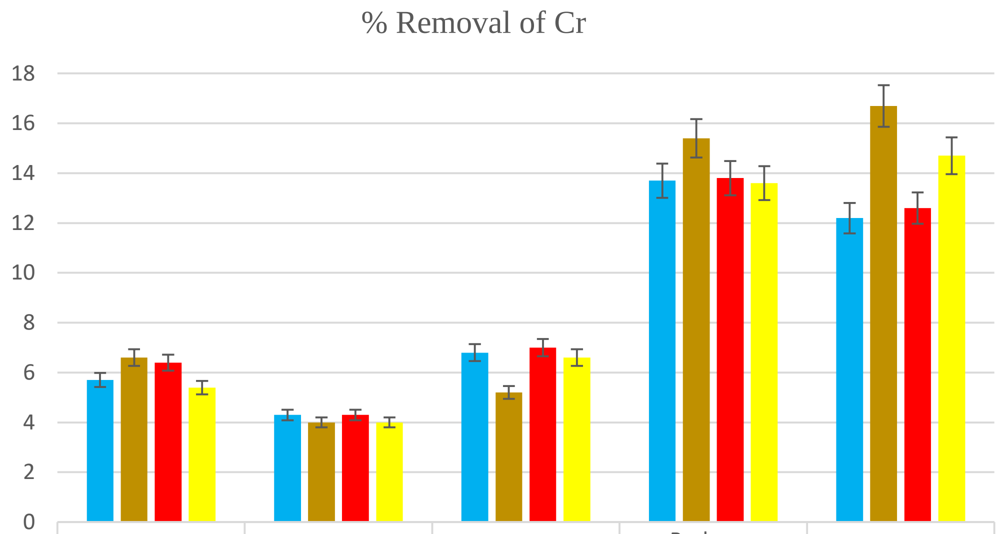
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	9.210	7.945	5.207	6.445	21.204
Autumn	11.502	9.736	6.526	7.979	25.494
Winter	10.495	9.044	5.921	7.320	24.109
Spring	12.027	10.395	6.826	8.474	27.914

Figure 3.22 Average chromium (Cr) concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	16.047	14.084	13.266	16.054	28.921
Autumn	20.737	17.270	17.093	20.077	36.517
Winter	18.375	16.053	15.196	18.339	33.037
Spring	21.771	18.572	17.781	21.988	39.967

Figure 3.1.23 Average Cr concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	5.7	4.3	6.8	13.7	12.2
Autumn	6.6	4.0	5.2	15.4	16.7
Winter	6.4	4.3	7.0	13.8	12.6
Spring	5.4	4.0	6.6	13.6	14.7

Summer Autumn Winter Spring

Figure 3.24 Removal rates of chromium (Cr)

Removal rates of Prince Island, Bagdat Avenue and TEM Highway were lower than Barbaros Boulevard and Dilovasi District. The lowest removal rate was calculated for Bagdat Avenue as 4.0% while the highest was calculated for Dilovasi District as 16.7%. The high Cr removal rate in the Dilovasi district can be attributed to intense industrial activity, while heavy traffic can cause high removal rates on Barbaros Boulevard. Additionally removal rates and seasons did not show any significant relation.

Chromium content of soil ranged from 5 to 120 $mg.kg^{-1}$ with average of 54 $mg.kg^{-1}$. According to our results soil Cr content of all stations were determined as below the world average. There are some studies in Table 3.3, conducted to determine Cr levels in soil and plant parts. In a study conducted in Istanbul by Esen et al., 2016, soil Cr content was determined as above the upper limit. Soil Cr content results of Vural 2013, Yasar et al., 2010, Samecka-Cymerman et al., 2009 and Tabari and Salehi 2009 were determined as below the average. Our results of soil Cr content were higher than the results of Vural 2013, Yasar et al., 2010, Samecka-Cymerman et al., 2009 while the results of Esen et al., 2016 and Tabari and Salehi 2009 were higher than our results.

As mentioned above, plants need Cr at low amounts to maintain metabolic processes. Kabata-Pendias and Pendias 2001 stated the plant Cr contents varies from 0.1 to 0.5 $mg.kg^{-1}$ and the toxic levels of Cr as 5-30 $mg.kg^{-1}$ for plants. According to our results plant Cr levels did not exceed the normal limits. When considering other studies, it was determined that all results are within normal limits.

Table 3.3 Cr levels (L: leaf, B: bark, S:branch)

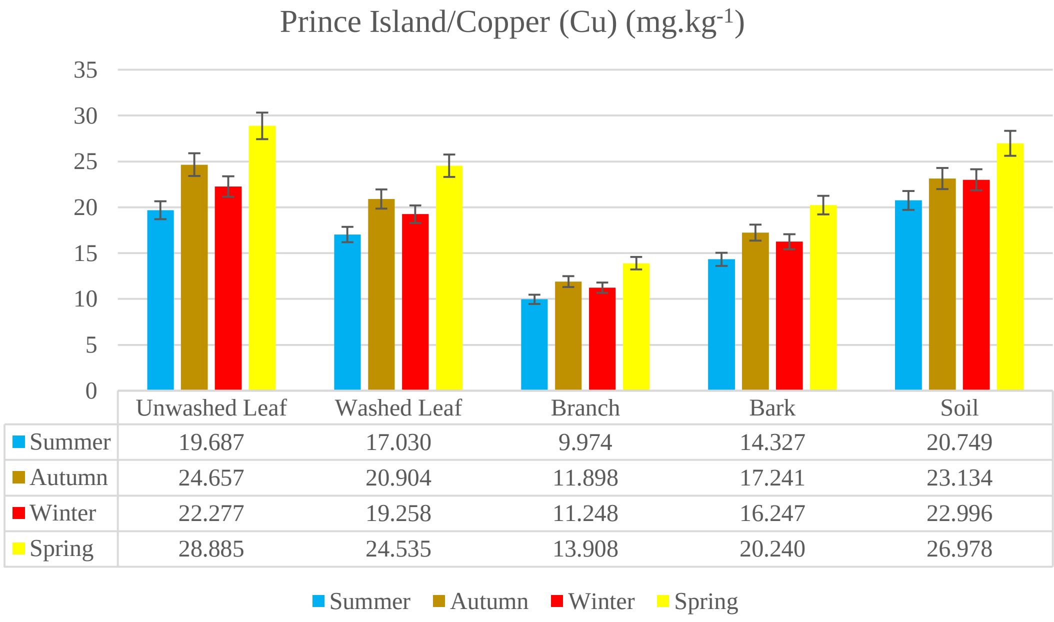
Reference	Organism	Soil ($mg.kg^{-1}$)		Plant ($mg.kg^{-1}$)		Country/city	Method
		Control	Study Site	Control	Study Site		
Esen et al., (2016)	***Carpinus betulus***	250	98.8-190	1.03 (L)	1.06-3.52 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			0.92 (L)	1.40-2.17 (L)		
	Tilia Argentea			1.24 (L)	2.11-2.99 (L)		
Vural (2013)	***R. pseudoacacia***	22-36		0.33-2.19 (S)		**TUR/Gumushane**	ICP-AES
Yasar et al., (2010)	***Cercis siliquastrum***	5.65	10.13	1.63 (L)	6.12 (B)	**TUR/Istanbul**	ICP-OES
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	7.8-8.2	10.5-17.4	0.4-0.6 (L)	0.55-1.16 (L)	Poland	ICP-MS
				4.5-4.8 (B)	4.9-7.4 (B)		
Tabari and Salehi (2009)	***R. pseudoacacia***	33.81	48.04	ND (L)	ND (L)	Iran	AAS

Our results of plant Cr content are higher than the results of other studies (Table 3.3). Esen et al., 2016 and Yasar et al., 2010 conducted Cr their studies with different plant species. The difference between the results may arise from this situation. Although Vural 2013, Samecka-Cymerman et al., 2009, Tabari and Salehi 2009 used *R. pseudoacacia* in their studies, our plant Cr results are higher than the results of these studies.

3.1.5 Copper (Cu)

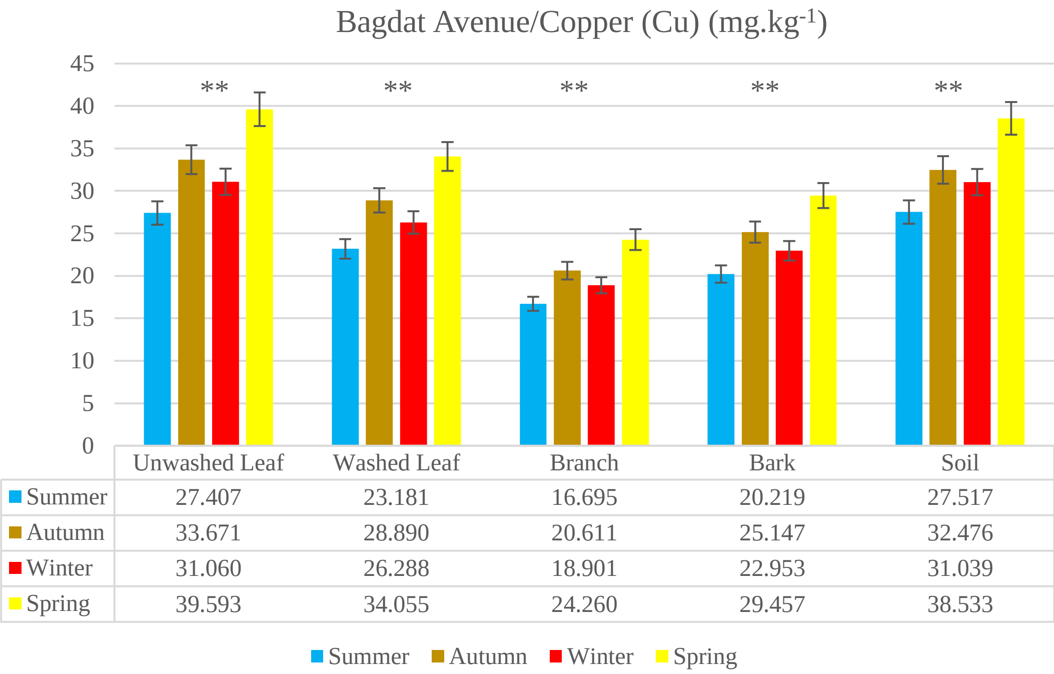
Copper (Cu) mostly functions in metabolic processes as cofactor. Cu is a microelement that must be taken up from the soil. Results showed that Cu levels in plant samples ranged between 9.974±0.196 mg.kg^{-1} (Branch/Prince Island) and 91.947±1.920 mg.kg^{-1} (Unwashed leaves/Dilovasi District). Soil Cu contents were detected between 20.749±0.411 mg.kg^{-1} (Prince Island) and 103.782±2.931 mg.kg^{-1} (Dilovasi District). Dilovasi District samples contained the highest Cu concentrations both in plant and soil while Prince Island has the lowest Cu concentrations.

In all stations, *R. pseudoacacia* has taken Cu from soil nearly as much as amount of Cu in soil. Thus Cu content in plant samples increased in relation with station soil Cu content from Prince Island to Dilovasi. Plant samples of Dilovasi District (heavy industrial zone) had accumulated Cu 3-4 times more than the Prince Island samples (no traffic, no industrial establishment) and airborne Cu was almost equal for all stations. According to results, uptake and accumulation of Cu altered in relation with the seasons. The lowest uptake and accumulation occurred in summer. The highest uptake and accumulation occurred in spring. Seasonal variation occurred same as previous elements.



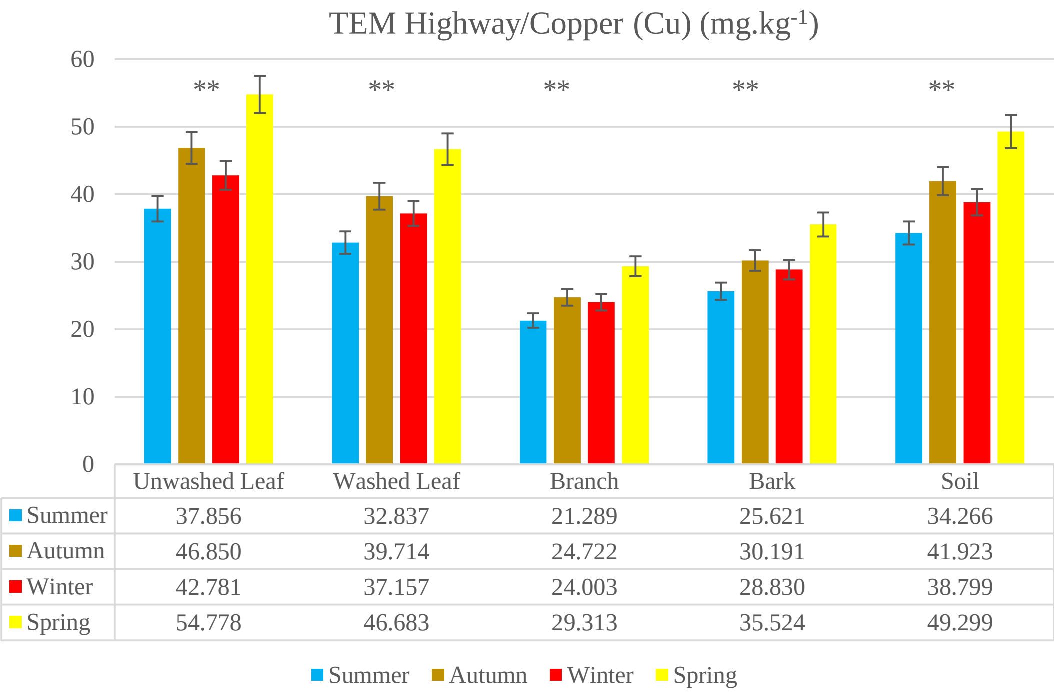
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	19.687	17.030	9.974	14.327	20.749
Autumn	24.657	20.904	11.898	17.241	23.134
Winter	22.277	19.258	11.248	16.247	22.996
Spring	28.885	24.535	13.908	20.240	26.978

Figure 3.25 Average copper (Cu) concentrations in Prince Island



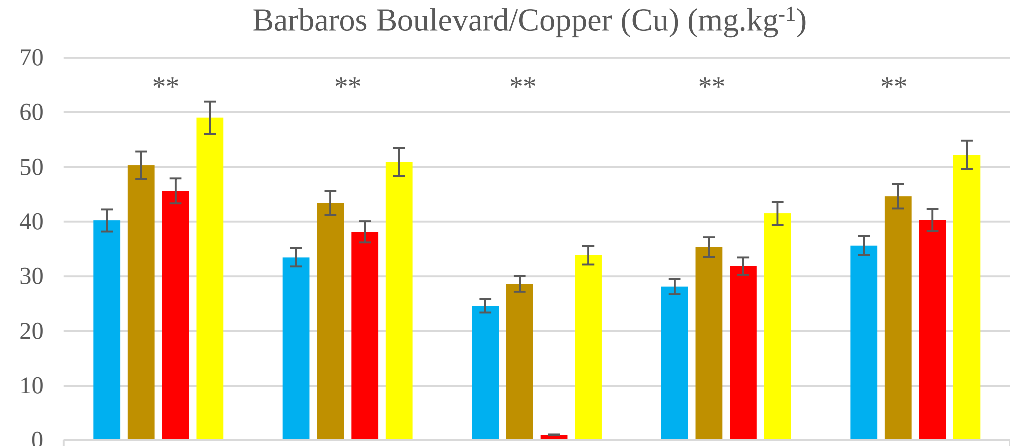
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	27.407	23.181	16.695	20.219	27.517
Autumn	33.671	28.890	20.611	25.147	32.476
Winter	31.060	26.288	18.901	22.953	31.039
Spring	39.593	34.055	24.260	29.457	38.533

Figure 3.26 Average Cu concentrations in Bagdat Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	37.856	32.837	21.289	25.621	34.266
Autumn	46.850	39.714	24.722	30.191	41.923
Winter	42.781	37.157	24.003	28.830	38.799
Spring	54.778	46.683	29.313	35.524	49.299

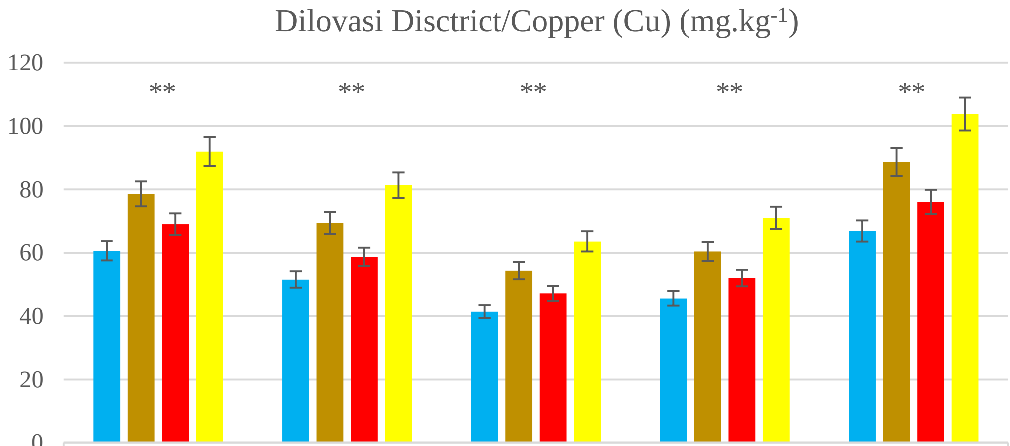
Figure 3.27 Average Cu concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	40.223	33.468	24.606	28.123	35.614
Autumn	50.316	43.376	28.604	35.357	44.635
Winter	45.627	38.131	1.000	31.865	40.320
Spring	59.021	50.898	33.874	41.520	52.186

Summer Autumn Winter Spring

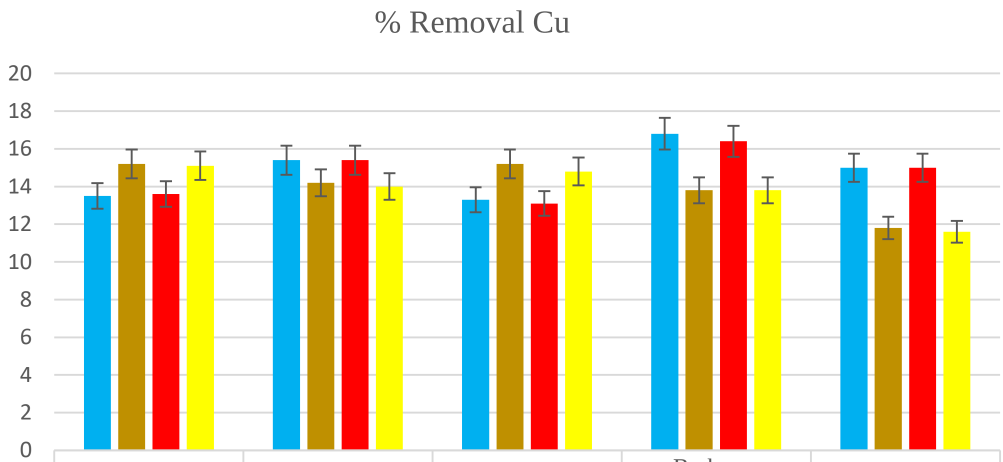
Figure 3.28 Average Cu concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	60.631	51.510	41.391	45.559	66.836
Autumn	78.592	69.350	54.328	60.383	88.641
Winter	68.995	58.635	47.135	52.015	76.063
Spring	91.947	81.312	63.543	71.032	103.782

Summer Autumn Winter Spring

Figure 3.29 Average Cu concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	13.5	15.4	13.3	16.8	15.0
Autumn	15.2	14.2	15.2	13.8	11.8
Winter	13.6	15.4	13.1	16.4	15.0
Spring	15.1	14.0	14.8	13.8	11.6

Figure 3.30 Removal rates of Cu

Differences on Cu level between unwashed and washed leaves changed slightly in terms of season and station. According to seasons, removal rate of Cu ranged between 13.3% and 16.8% for summer; 11.8% and 15.2 for autumn; 13.1% and 16.4% for winter; 11.6% and 15.1% for spring. Removal rate in terms of stations ranged between 13.5% and 15.2% for Prince Island; 14.0% and 15.4% for Bagdat Avenue; 13.1% and 15.2% for TEM Highway; 13.8% and 16.8 for Barbaros Boulevard; 11.6% and 15.0% for Dilovasi District.

Kabata Pendias and Pendias (2001) reported that Cu contents in soil usually ranged between 20-30 $mg.kg^{-1}$ and Cu contents in plant parts ranged between 5-30 $mg.kg^{-1}$. In the light of this data Cu contents in soil samples were above the normal range except Prince Island station. Cu contents in plant parts were also above the normal limits. Yener and Yarci (2010) conducted a study with *Alcea pallida* plant from 5 station in Istanbul and determined that the soil Cu contents ranged between 22.611-207.308 $mg.kg^{-1}$ (Table 3.6). Their study sites were selected stations having relatively dense traffic circulation from European side of Istanbul. These different results may be caused from station differences.

Guney at al., (2010) also conducted a study to determine impacts of heavy traffic on highways to soils of Istanbul. Their Cu contents in soil (20 cm depth) results are consistent with our results.

Table 3.6 Cu levels (L: leaf, UwL: unwashed leaf, WL: washed leaf, B:bark, S:branch)

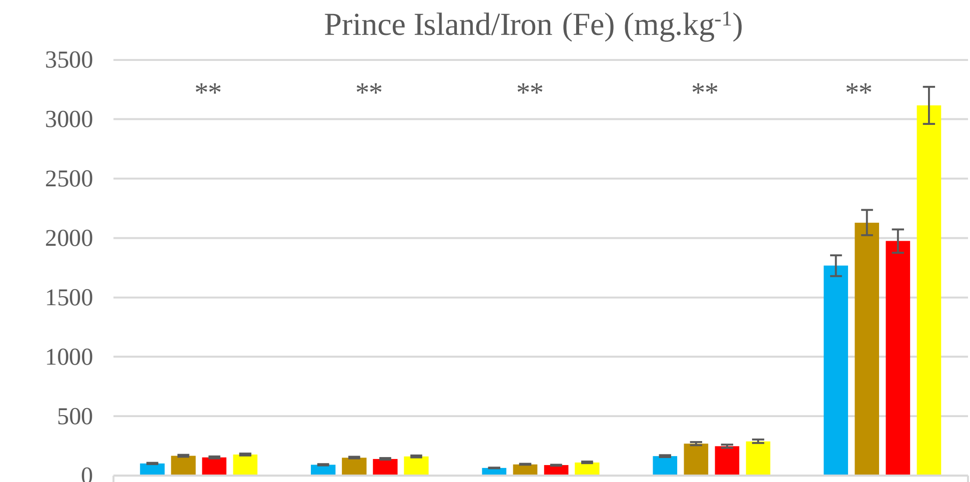
Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/city	Method
		Control	Treatment	Control	Treatment		
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	3.49	7.69-33.75	12.07 (L)	12.40 - 13.53 (L)	Poland	Flame AAS
Palowski et al., (2016)	***R. pseudoacacia***	---	---	4.69-8.59 (L)		Poland	Flame AAS
				8.32-9.69 (B)			
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---	---	17.8 (L) June	13.3 (L) June	Bulgaria	AAS
		---	---	8 (L) Sept.	17.2 (L) Sept		
Vural (2013)	***R. pseudoacacia***	8-35		2.75-34.5 (S)		**TUR/Gumushane**	ICP-AES
Serbula et al., (2012)	***R. pseudoacacia***	59.1	67.8-903.3	1.1 (WL)	0.9-236.7 (WL)	Serbia	ICP-AES / AAS
				101.2 (UwL)	38.7-286.7 (UwL)		
				110.6 (B)	27.9-6418.2 (B)		
Kaya et al., (2010)	***R. pseudoacacia***	2.0-6.9	20-38	43 (L)	6.9-9.5 (L)	**TUR/Gaziantep**	ICP-MS
Jensen et al., (2010)	***R. pseudoacacia***	---		9.32 (L)	13.0 (L)	USA	ICP-MS
Guney et al., (2010)	---	21.4-136 (Surface)		---		**TUR/Istanbul**	Flame AAS
		12.6-94.1 (20-cm depth)					
Yener and Yarci (2010)	***Alcea pallida***	22.611-207.308		17.027-23.367 (L)		**TUR/Istanbul**	AAS
				2.954- 9.641 (S)			
Tabari and Salehi (2009)	***R. pseudoacacia***	5	3	43.50 (L)	27.43 (L)	Iran	AAS
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	11.7–12.4	14–37	3.3-3.8 (L)	7.4-16.2 (L)	Poland	Flame AAS
				4.3-4.5(B)	9.1-19 (B)		
Celik et al., (2005)	***R. pseudoacacia***	8.680	17.189-69.710	5.64 (UwL)	12.22-20.81 (UwL)	**TUR/Denizli**	Flame AAS
				5.28 (WL)	8.125-10.15 (WL)		
Mertens et al., (2004)	***R. pseudoacacia***	54.2 (Sediment)		8.3 (L)		Belgium	Flame AAS
Aksoy et al., (2000)	***R. pseudoacacia***	11 (Rural)	16-79	8 (UwL)	12.96-29.12 (UwL)	**TUR/Kayseri**	AAS
				7.32 (WL)	8.96-14.04 (WL)		

Other studies in Table 3.6 were conducted by using *R. pseudoacacia* in different countries. When the results of these studies are compared with our results, our results are higher than the all results except study of Serbula et al., (2012) which was conducted at Bor/Serbia which has Cu rich mines and metal industry sites. Results of this study indicated that the area has exceptionally high Cu contents in both soil and plants.

3.1.6 Iron (Fe)

Iron is required for various metabolic processes and as a structural component thus plants and other organisms must acquire Fe to maintain their homeostasis (Kobayashi and Nishizawa, 2012). Our results showed that Fe levels in plant samples ranged between 63.565±1.188 mg.kg^{-1} (Branch/Prince Island) and 308.217±3.306 mg.kg^{-1} (Unwashed leaves/Dilovasi District). Soil Fe contents were determined between 1767.070±39.478 mg.kg^{-1} (Prince Island) and 3756.504±73.388 mg.kg^{-1} (Dilovasi District). Dilovasi District samples contained the highest Fe concentrations both in plant and soil while Prince Island has the lowest Fe concentrations. *R. pseudoacacia* has taken Fe in increasing proportions.

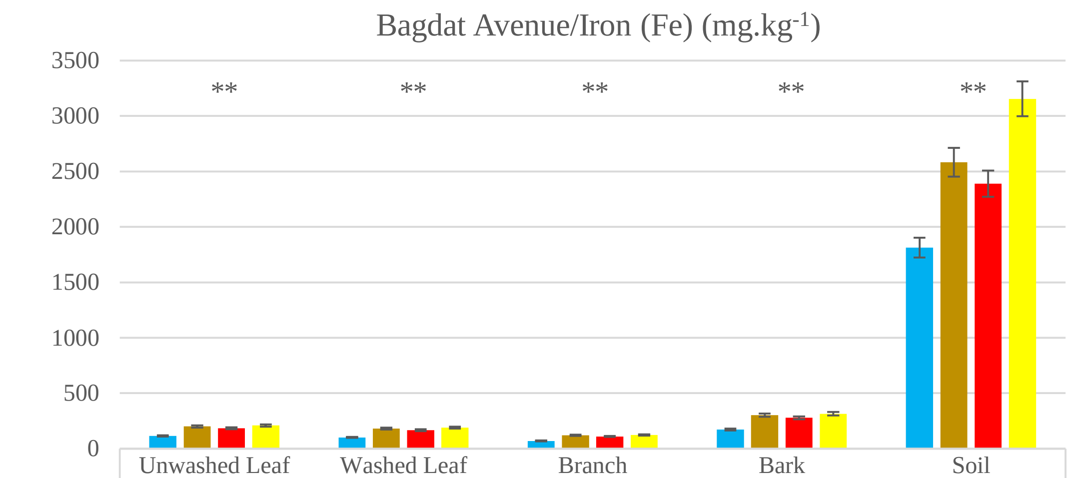
Seasonal variations in Fe contents occurred as previous elements. The highest Fe contents in soil and plant parts were detected in spring while the lowest were detected in summer. When plant parts Fe contents are compared, it was observed that bark has the highest Fe contents for all seasons and all stations.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	101.248	91.199	63.565	164.414	1767.070
Autumn	165.791	151.347	94.542	267.779	2129.075
Winter	153.073	140.018	86.928	247.105	1974.109
Spring	177.265	160.698	110.972	288.346	3117.454

Summer Autumn Winter Spring

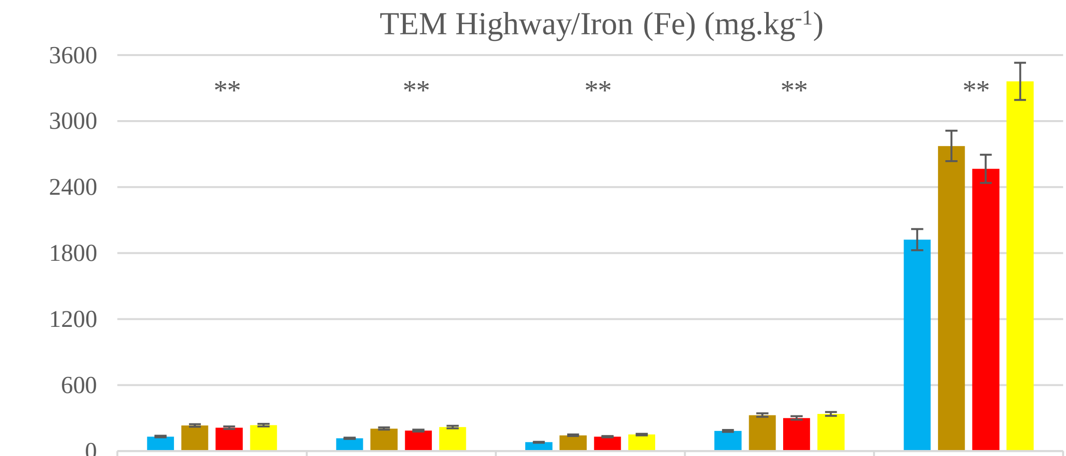
Figure 3.31 Average Fe concentrations in Prince Island.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	113.942	101.520	69.975	173.330	1813.222
Autumn	200.024	180.638	120.446	302.883	2583.754
Winter	183.665	166.178	110.427	277.740	2389.799
Spring	208.765	190.139	124.068	314.493	3154.537

Summer Autumn Winter Spring

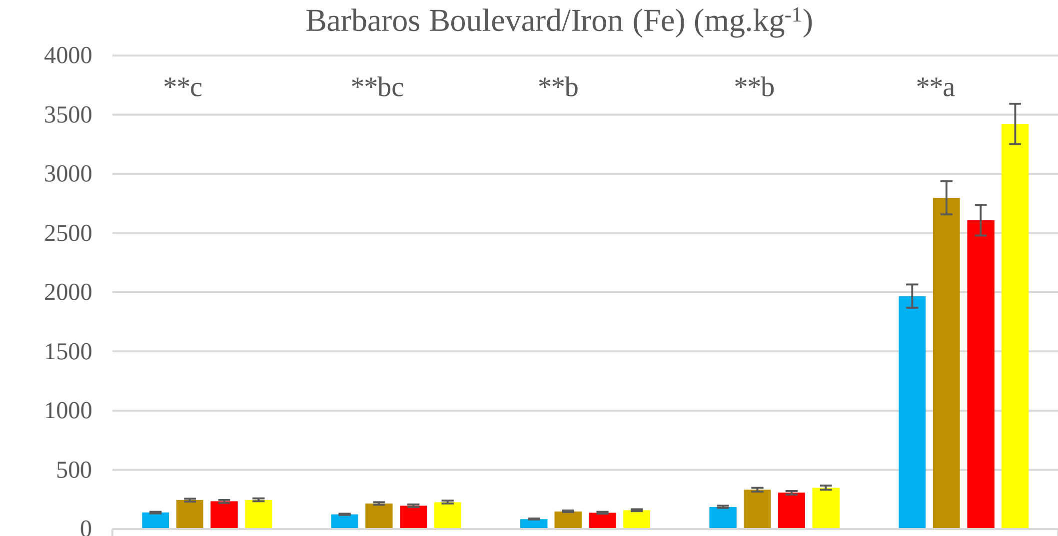
Figure 3.33 Average Fe concentrations in Bagdad Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	132.020	115.859	81.049	184.344	1924.047
Autumn	233.687	205.061	143.386	327.164	2774.603
Winter	212.866	187.341	131.868	301.452	2566.655
Spring	235.874	218.540	150.945	338.502	3362.338

Summer Autumn Winter Spring

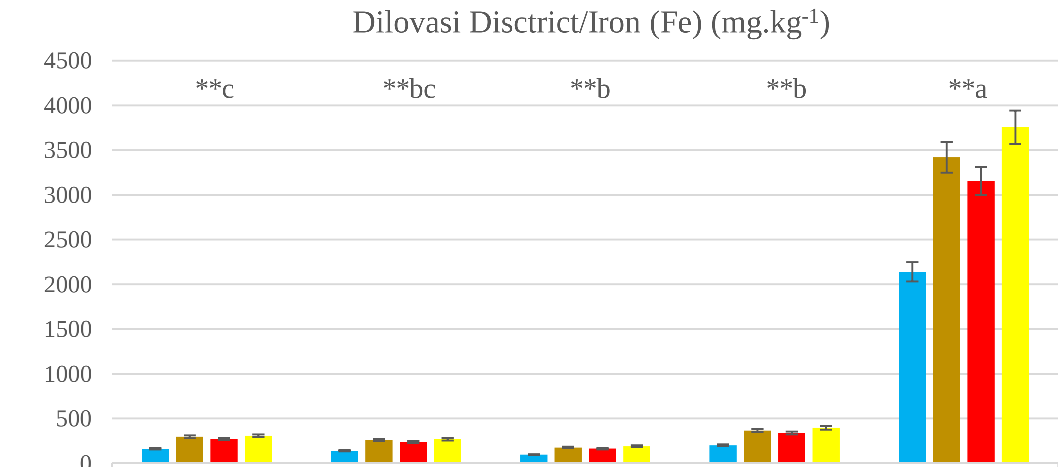
Figure 3.33 Average Fe concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	138.955	123.140	83.976	186.875	1966.721
Autumn	244.310	214.669	148.711	332.416	2797.478
Winter	233.396	196.542	137.038	306.177	2608.750
Spring	246.083	227.279	159.036	348.922	3421.278

Summer Autumn Winter Spring

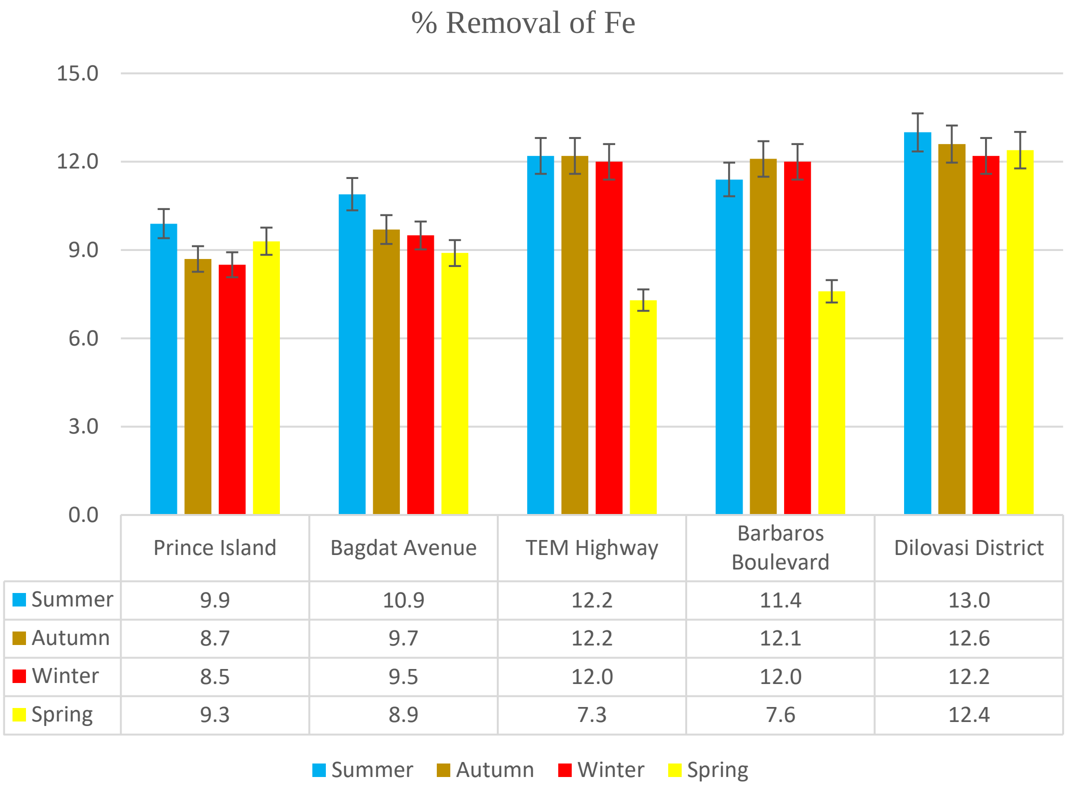
Figure 3.34 Average Fe concentrations in Barbaros Avenue.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	163.248	142.062	97.055	201.835	2139.285
Autumn	296.275	258.857	177.005	366.840	3421.369
Winter	271.371	238.235	163.736	338.942	3156.016
Spring	308.217	270.073	192.077	396.553	3756.504

Summer Autumn Winter Spring

Figure 3.34 Average Fe concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	9.9	10.9	12.2	11.4	13.0
Autumn	8.7	9.7	12.2	12.1	12.6
Winter	8.5	9.5	12.0	12.0	12.2
Spring	9.3	8.9	7.3	7.6	12.4

Figure 3.36 Removal rates of Fe

According to seasons, removal rates of Fe ranged between 9.9% and 13.0% for summer; 8.7% and 12.6% for autumn; 8.5% and 12.2% for winter; 7.3% and 12.4% for spring. Removal rate in term of stations ranged between 8.5% and 9.9% for Prince Island; 8.9% and 10.9% for Bagdat Avenue; 7.3% and 12.2% for TEM Highway; 7.6% and 12.1% for Barbaros Boulevard; 12.2% and 13.0% for Dilovasi District. The lowest removal rate was detected as 7.3% from TEM Highway in spring. The highest one was detected as 13.0% from Dilovasi District in spring. In terms of season, removal rates of Fe changed in a narrow range for Prince Island, Bagdat Avenue and Dilovasi District. TEM Highway and Barbaros Boulevard also changed within a narrow range except spring. These stations have dynamic traffic conditions when compared to the Prince Island and Dilovasi District. These dynamic conditions might have affected dispersal of Fe and changed the removal rate of Fe.

Table 3.5 Fe levels (L: leaf, uWL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

Reference	Organism	Soil ($mg.kg^{-1}$)		Plant ($mg.kg^{-1}$)		Country/city	Method
		Control	Study Site	Control	Study Site		
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	390.66	319.43-1143.32	78.28 (L)	73.19 - 106.86 (L)	Poland	Flame AAS
Palowski et al., (2016)	***R. pseudoacacia***	---	---	61.2-74.2 (Other organs)		Poland	Flame AAS
				183.9-752.1 (B)			
Esen et al., (2016)	***Carpinus betulus***	13800	28000-30000	261 (L)	272-750 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			246 (L)	413-492 (L)		
	Tilia Argentea			386 (L)	582-646 (L)		
Tzvetkova and Petkova 2015)	***R. pseudoacacia***	---	---	64.6 (L) June	68.3 (L) June	Bulgaria	AAS
		---	---	136.2 (L) Sept.	143.5 (L) Sept		
Vural (2013)	***R. pseudoacacia***	---		38.59-693.32 (S)		**TUR/Gumushane**	ICP-AES
Jensen et al., (2010)	***R. pseudoacacia***	---		98.6 (L)	116 (L)	USA	ICP-MS
Yasar et al., (2010)	***Cercis siliquastrum***	3825.42 Control - 2589.35 Urban		134.74 (uWL)-44.97 (WL)		**TUR/Istanbul**	ICP-OES
Rahmonov (2009)	***R. pseudoacacia***	---	---	258 (L)		Poland	Flame AAS
		---	---	2276 (B)			
		---	---	932 (S)			
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	847–856	655–1556	57-66 (L)	84-109 (L)	Poland	ICP-MS
				55-81 (B)	89-129 (B)		
Tabari and Salehi (2009)	***R. pseudoacacia***	19690*	23990*	91.87 (L)	110.00 (L)	Iran	AAS
Celik et al., (2005)	***R. pseudoacacia***	2695.6	2892.7-3939.3	100.2 (uWL)	255.01-3087.0 (uWL)	**TUR/Denizli**	Flame AAS
Mertens et al., (2004)	***R. pseudoacacia***	54.202 (Sediment)		---		Belgium	Flame AAS

Iron is fourth abundant element in the earth crust with 5%, however the range of Fe content in soil is between 0.1 and 10% (Kabata-Pendias and Mukherjee 2007). There are some studies conducted for determination of Fe contents in soil and plants (Table 3.5). Results of Esen et. al. (2016) and Tabari and Salehi (2009) are higher than the upper limits and our results while results of our study and other studies are within the normal limits. In addition to this, results of Celik et al. (2005) are in agreement with our results. Results of Nadgorska-Socha et al. (2016), Yasar et al. (2010), Samecka-Cymerman et al. (2009), Celik et al. (2004) and Mertens et al. (2004) are lower than our results.

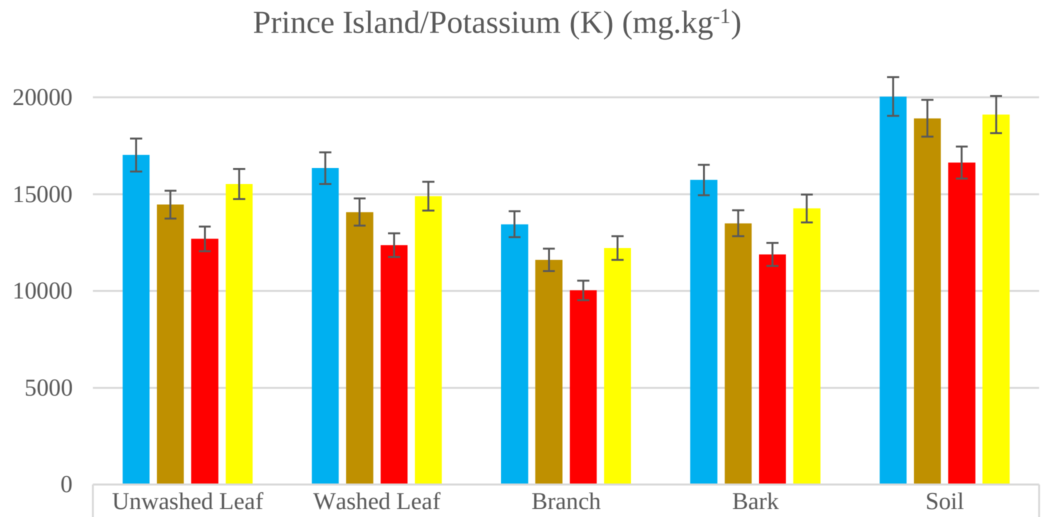
Iron content in plant parts are also determined in mentioned studies (Table 3.5). Normal limits of Fe content in plant part values are mentioned as 50-500 $mg.kg^{-1}$ (Kabata-Pendias and Mukherjee 2007; Broadley et al., 2012). According to these values, our results of Fe content are within the normal limits. Fe content in branch, unwashed and washed leaf samples ranged between 63.565 and 308.217 $mg.kg^{-1}$. Higher Fe contents were detected in bark samples (164.414 - 396.553 $mg.kg^{-1)}$ for all stations and all seasons than the other plant parts. This situation may be caused by aerial deposition and long-term accumulation of Fe in bark samples. Thus bark of *R. pseudoacacia* can be used for long term accumulation of Fe.

Fe content in plant parts results of Palowski et al. (2016), Esen et al. (2016), Vural (2013), Rahmonov (2009) and Celik et al. (2005) were higher than our results. Additionally Fe content in bark results of Palowski et al. 2016, branch results of Vural (2013), bark and branch results of Rahmonov (2009) and unwashed leaf samples of Celik et al. (2005) were also higher than the normal limits. The results of Nadgorska-Socha et al. (2016), Tzvetkova and Petkova (2015), Jensen et al. (2010), Yasar et al. (2010), Samecka-Cymerman et al. (2009) and Tabari and Salehi (2009) were lower than ours.

Although Fe content in soil is abundant, acquisition of Fe is difficult for plants due to low solubility of Fe in neutral and basic pH values. Plants have two mechanisms to acquire Fe from soil named strategy I and II. Dicots - including *R. pseudoacacia* - and non-graminaceous monocots acquire Fe from soil using strategy I. Soil pH value is very important for these plants. The soil pH difference may alter Fe accumulation by altering Fe solubility. For instance, despite the fact that sil soil Fe content results of Tabari and Salehi (2009) are quite higher, plant Fe content is quite low.

3.1.7 Potassium (K)

Potassium is a mobile macroelement and involved in some important metabolic functions such as regulation of osmotic balance and enzyme activation (Hawkesford et. al., 2012; Benito et al., 2014). According to our results, K content in plant samples ranged between 5593.069±95.772 mg.kg^{-1} (Branch/Dilovasi District) and 17026.936±283.127 mg.kg^{-1} (Unwashed leaves/Prince Island). Soil K contents were determined between 9229.131±231.342 mg.kg^{-1} (Winter/Dilovasi District) and 20047.094±353.103 mg.kg^{-1} (Summer/Prince Island). Prince Island samples has the highest K concentrations both in plants and soil while Dilovasi District has the lowest Fe concentrations. The highest K levels in plant and soil were detected in summer.

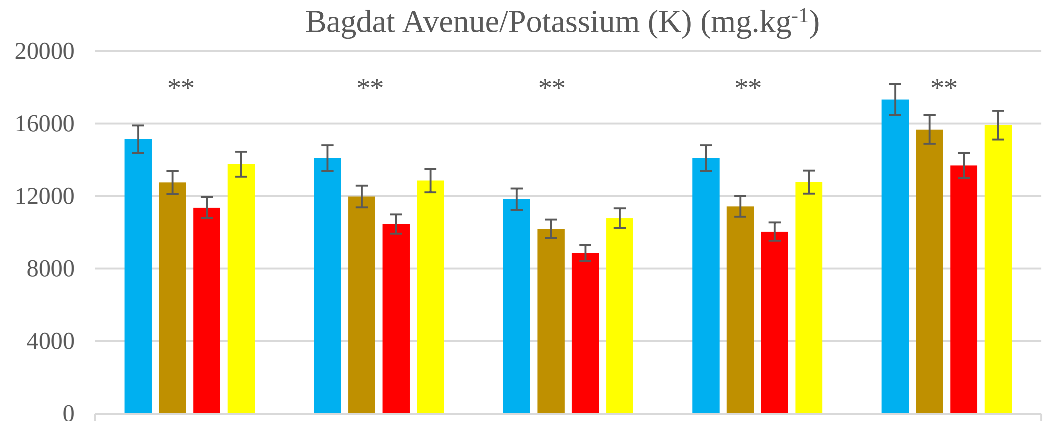


	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	17026.936	16346.990	13448.485	15736.251	20047.094
Autumn	14464.156	14074.462	11605.117	13499.323	18921.083
Winter	12694.691	12369.577	10031.097	11883.835	16634.400
Spring	15532.070	14898.904	12218.964	14261.977	19118.645

Summer Autumn Winter Spring

Figure 3.37 Average K concentrations in Prince Island**.**

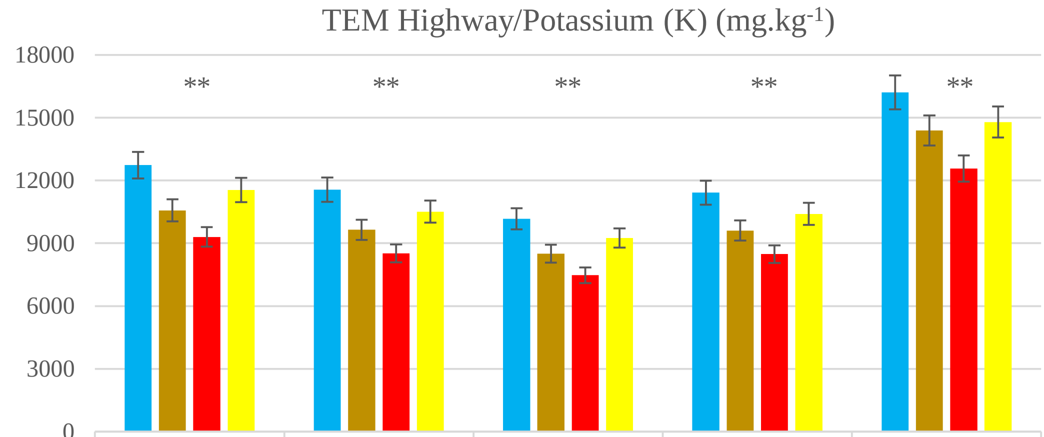
K showed different pattern of seasonal variation. The highest K content in plant parts and soil was detected in summer. After summer, K contents decreased in autumn and later the lowest K levels were detected in winter. In spring K levels were elevated above the autumn levels. As mentioned above, K is an essential macroelement for plants and all living plant cells need it at different proportions. Uptake of K is affected by various factors. Such as in rainy seasons, increased K levels in plant parts and soil may be caused by the increase of K solubility and mobility with rain.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	15136.318	14093.684	11833.591	14096.058	17326.435
Autumn	12753.531	11983.585	10201.592	11433.587	15671.699
Winter	11365.885	10461.734	8846.211	10039.375	13685.508
Spring	13763.853	12856.851	10782.160	12770.280	15906.628

Summer Autumn Winter Spring

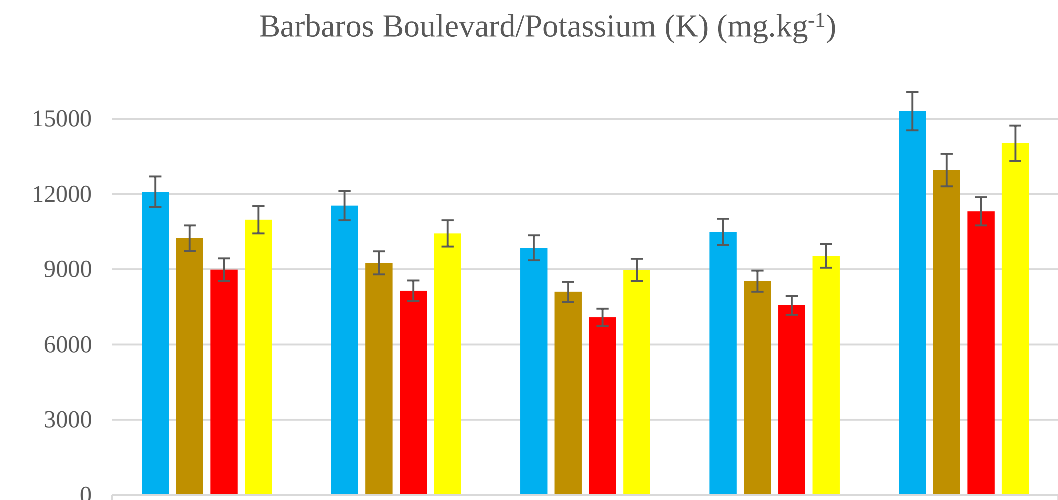
Figure 3.38 Average K concentrations in Bagdat Avenue**.**



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	12732.600	11558.949	10169.429	11417.622	16212.020
Autumn	10566.701	9646.108	8499.516	9603.999	14384.121
Winter	9301.668	8516.700	7469.894	8481.207	12563.607
Spring	11540.315	10509.546	9253.162	10404.444	14792.912

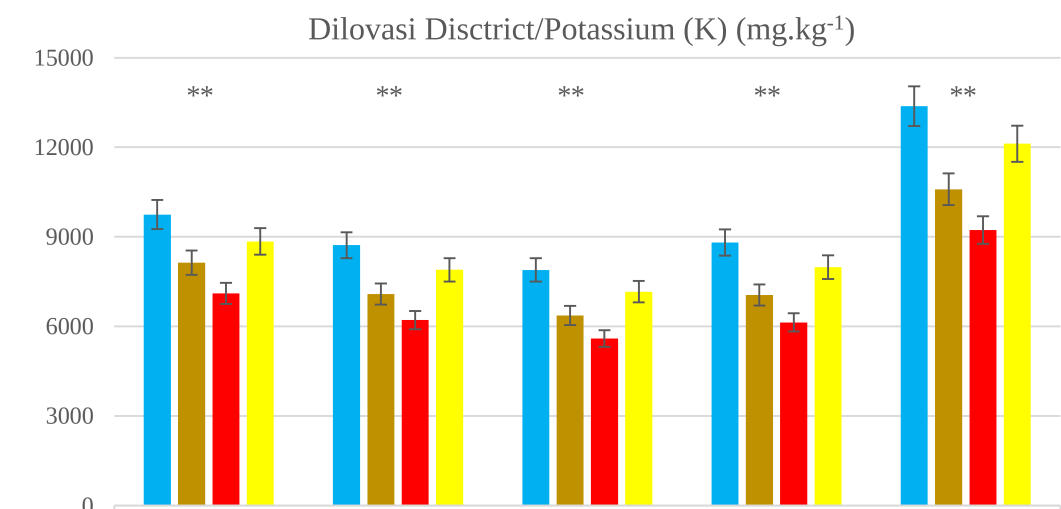
Summer Autumn Winter Spring

Figure 3.39 Average potassium (K) concentrations in TEM Highway.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	12096.288	11538.746	9857.346	10499.294	15309.631
Autumn	10241.682	9255.456	8103.143	8530.604	12962.485
Winter	8992.411	8148.890	7084.292	7569.747	11311.939
Spring	10974.806	10436.564	8980.650	9539.426	14030.700

Figure 3.40 Average K concentrations in Barbaros Boulevard.

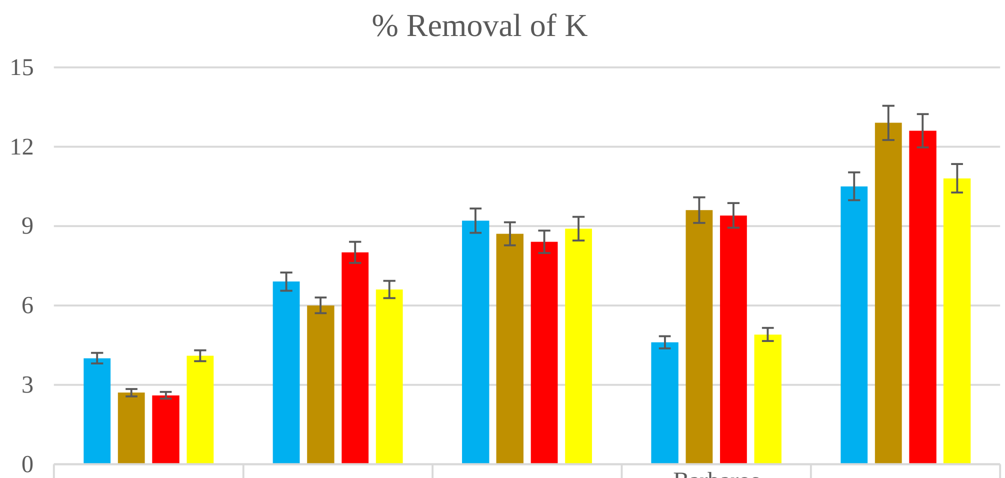


	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	9743.798	8720.331	7890.791	8809.664	13375.820
Autumn	8134.978	7086.059	6365.958	7051.373	10592.138
Winter	7100.930	6209.397	5593.069	6132.529	9229.131
Spring	8844.876	7892.570	7160.655	7985.615	12118.502

Figure 3.41 Average K concentrations in Dilovasi District.

Soil K reserve consists of three forms of K which can be defined as structural, fixed and exchangeable. Available K forms for plants include directly soluble K in soil, water and exchangeable form of K which is attached to surface of soil particles electrostatically. Directly available (0.1-0.2%) and exchangeable forms of K for plants are represented with 1-2% of total K content in soil (Moody and Bell 2006; Sardans and Peñuelas 2015). Available soil K content is affected by several factors including temperature, pH, water content and wetting-drying cycles, aeration, mineralogical/textural factors and biological processes.

Available K reserve expands with decaying of falling leaves and dead roots, rainfall, atmospheric deposition and weathering of K contained minerals. Whereas K uptake by crops, fixation between plates of clay minerals and leaching shrinks the available K reserve (Raghavendra et al., 2016). Cause of increase in K contents of soil decomposition of plant falling leaves and dead roots in spring and summer. Additionally K content in plants may be increased to maintain increased physiological functions. Increased precipitation may cause decrease in K content of soil with leaching in autumn and winter due to the high solubility of K.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	4.0	6.9	9.2	4.6	10.5
Autumn	2.7	6.0	8.7	9.6	12.9
Winter	2.6	8.0	8.4	9.4	12.6
Spring	4.1	6.6	8.9	4.9	10.8

Figure 3.42 Removal rates of K

Changes in Removal rate of K occurred in a wide range depending on locality and season. According to seasons, removal rates of K ranged between 4.0% and 10.5% for summer; 2.7% and 12.9% for autumn; 2.6% and 12.6% for winter; 4.1% and 10.8% for spring. Removal rate in terms of stations ranged between 2.6% and 4.1% for Prince Island; 6.0% and 8.0% for Bagdat Avenue; 8.4% and 9.2% for TEM Highway; 4.6% and 9.7% for Barbaros Boulevard; 10.5% and 12.9% for Dilovasi District. The highest removal rate was detected as 12.9% from Dilovasi District in autumn. The lowest was detected as 2.6% from Prince Island in spring. In terms of location, high removal rates were detected at Dilovasi District while lower results were detected in Prince Island. High removal rates of K in Dilovası and low rates in Prince Island may indicate that the source of K is industrial and combustion. The samples of Dilovasi were collected from vicinity of detergent plant which is one of the aerial sources of K.

Table 3.6 K levels (L: leaf, uWL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

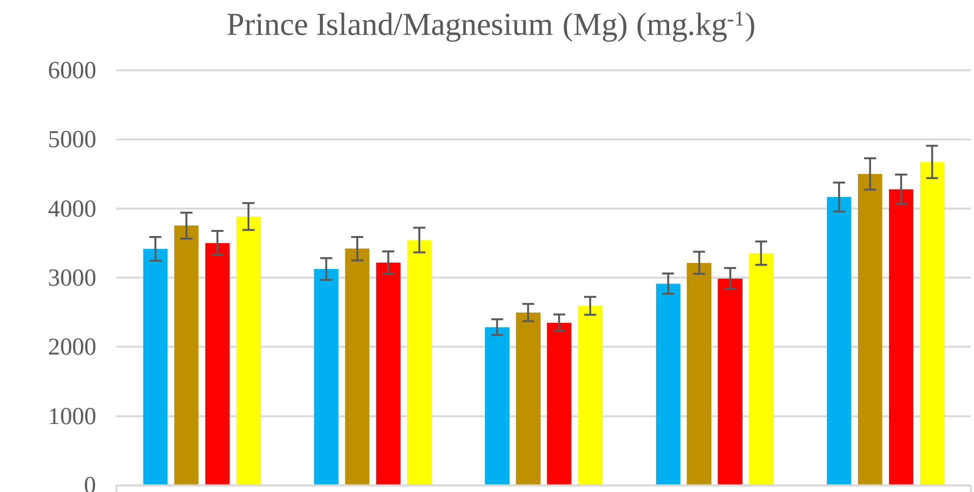
Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/city	Method
		Control	Study Site	Control	Study Site		
Esen et al., (2016)	***Carpinus betulus***	6500	11200-14000	12200 (L)	11800-12000 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			11300(L)	13000-19800 (L)		
	Tilia Argentea			15100 (L)	15100-46600 (L)		
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---		42470 (L) June	19750 (L) June	Bulgaria	AAS
				23550 (L) Sept.	14530 (L) Sept		
Jensen et al., (2010)	***R. pseudoacacia***	---		25800 (L)	24500 (L)	USA	ICP-MS
Rahmonov (2009)	***R. pseudoacacia***	---		6554 (L)		Poland	Flame AAS
				810 (B)			
				640 (S)			
Tabari and Salehi (2009)	***R. pseudoacacia***	3430	2560	5730(L)	8120 (L)	Iran	AAS
Mertens et al., (2004)	***R. pseudoacacia***	9155 (Sediment)		---		Belgium	Flame AAS

There are some K levels in soil and plant parts in table 3.6 from different studies. Soil K contents in this study is found to be higher than the previous studies with the exception of some results from Esen et al., 2016. When plant parts results are considered, results of Esen et al., 2016, Tzvetkova and Petkova 2015 and Jensen et al., 2010 were higher than this study.

3.1.8 Magnesium (Mg)

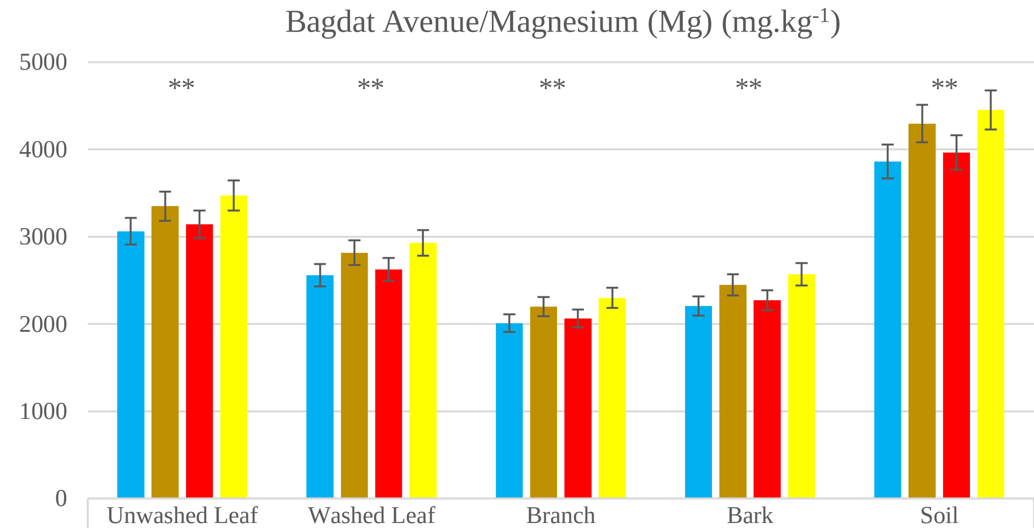
Mg, which is a plant macronutrient, functions as a main structural element in chlorophyll and as an important cofactor (Hawkesford et. al., 2012). According to results, Mg levels in plant samples ranged between 991.260±30.121 mg.kg^{-1} (Branch/Dilovasi District) and 3882.824±63.000 mg.kg^{-1} (Unwashed leaves/Prince Island). Soil Mg contents were determined between 2721.780±72.774 mg.kg^{-1} (Dilovasi District) and 4672.004±195.616 mg.kg^{-1} (Prince Island). Prince Island samples contained the highest Mg concentrations both in plant parts and soil while Dilovasi District has the lowest Mg contents.

Seasonal variation pattern of Mg remained the same for all stations. The highest Mg levels in plant and soil were detected in spring while the lowest were detected in summer for all stations. Mg concentrations in all plant and soil samples tend to increase during autumn and spring and decrease during winter and summer.



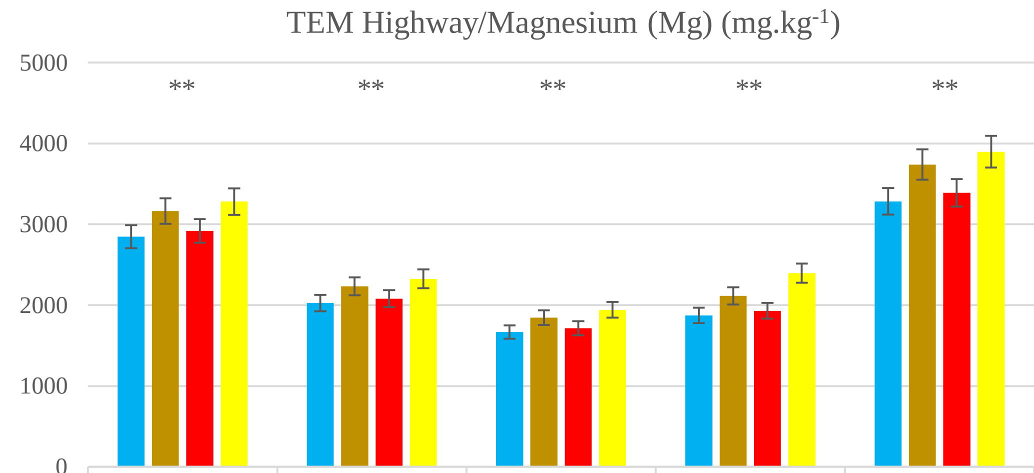
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	3417.441	3126.231	2285.519	2915.039	4166.854
Autumn	3754.495	3419.663	2496.916	3215.586	4499.405
Winter	3502.323	3218.433	2350.942	2988.458	4276.617
Spring	3882.824	3543.874	2592.717	3354.455	4672.004

Figure 3.43 Average Mg concentrations in Prince Island.



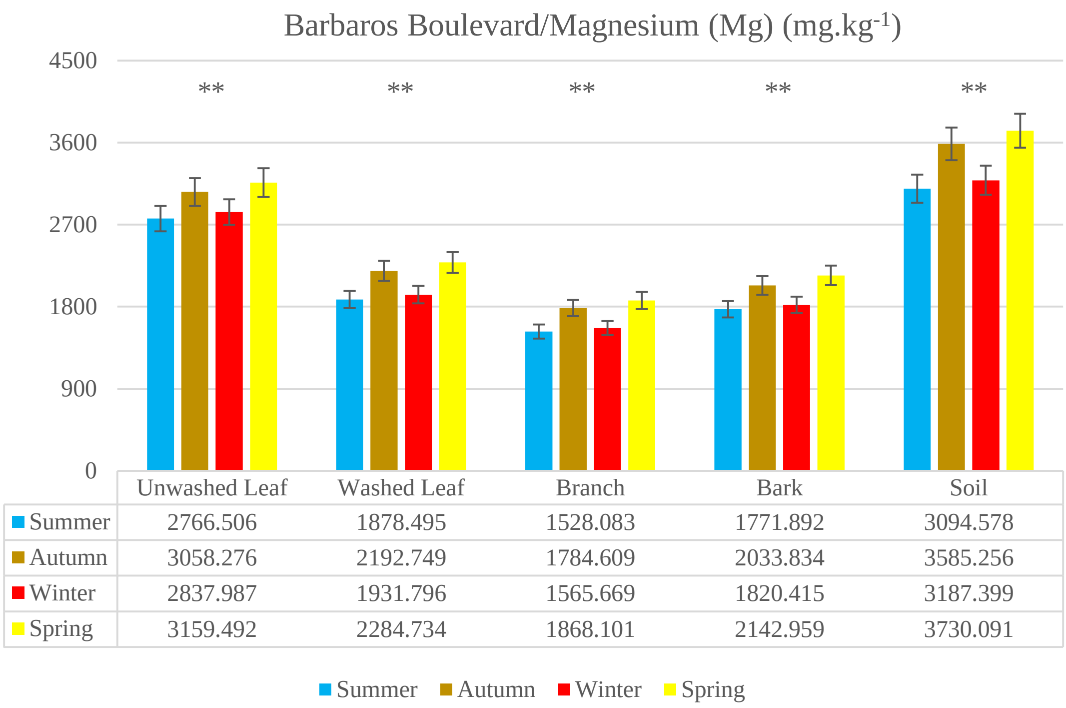
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	3062.937	2558.627	2009.780	2207.030	3862.218
Autumn	3350.931	2816.667	2199.274	2448.488	4295.518
Winter	3142.091	2625.817	2063.720	2272.823	3965.900
Spring	3473.408	2929.401	2299.267	2570.097	4452.838

Figure 3.44 Average Mg concentrations in Bagdat Avenue.



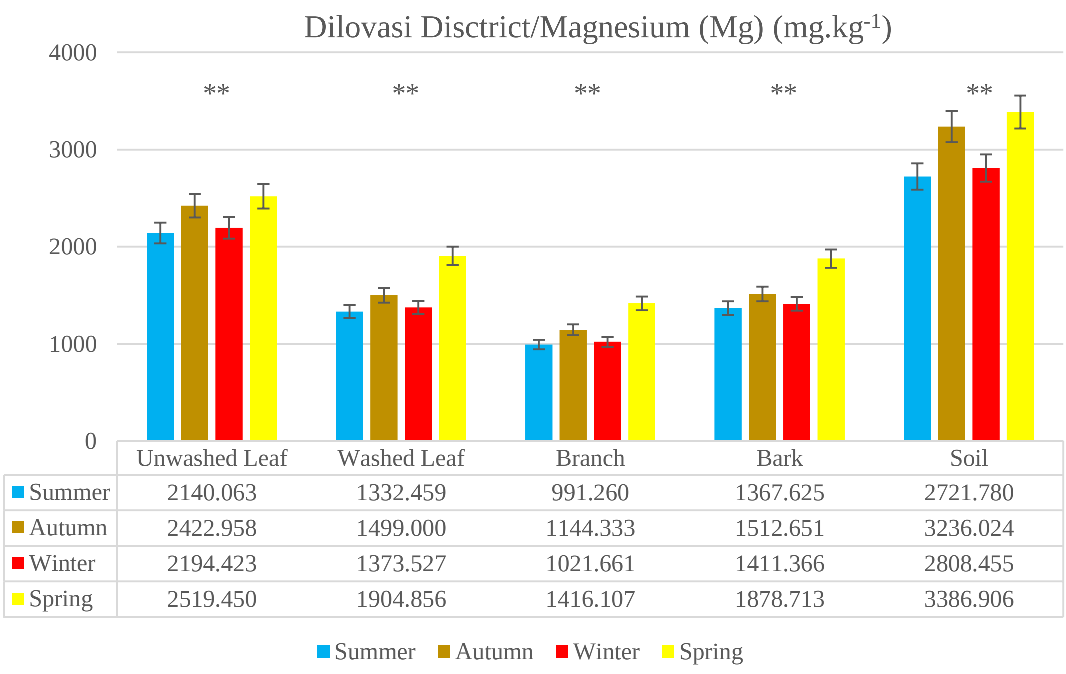
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	2846.149	2026.466	1665.637	1872.756	3283.149
Autumn	3164.919	2233.153	1844.378	2114.106	3738.563
Winter	2916.653	2080.623	1714.134	1929.533	3387.957
Spring	3280.528	2325.613	1942.074	2394.581	3896.907

Figure 3.45 Average Mg concentrations in TEM Highway.



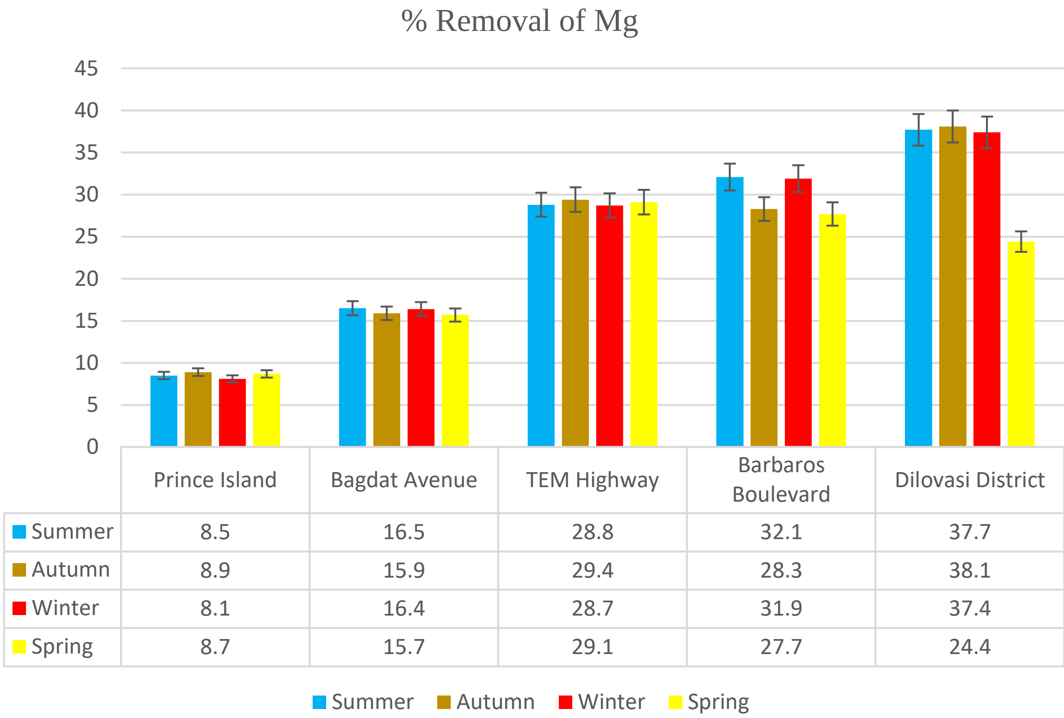
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	2766.506	1878.495	1528.083	1771.892	3094.578
Autumn	3058.276	2192.749	1784.609	2033.834	3585.256
Winter	2837.987	1931.796	1565.669	1820.415	3187.399
Spring	3159.492	2284.734	1868.101	2142.959	3730.091

Figure 3.46 Average Mg concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	2140.063	1332.459	991.260	1367.625	2721.780
Autumn	2422.958	1499.000	1144.333	1512.651	3236.024
Winter	2194.423	1373.527	1021.661	1411.366	2808.455
Spring	2519.450	1904.856	1416.107	1878.713	3386.906

Figure 3.47 Average Mg concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	8.5	16.5	28.8	32.1	37.7
Autumn	8.9	15.9	29.4	28.3	38.1
Winter	8.1	16.4	28.7	31.9	37.4
Spring	8.7	15.7	29.1	27.7	24.4

Figure 3.48 Removal rates of Mg between unwashed and washed leaves.

Changes in removal rate of Mg especially depend on locality. Removal rate levels were similar in term of season. Removal rate of Mg ranged between 8.5% and 37.7% for summer; 8.9% and 38.1% for autumn; 8.1% and 37.4% for winter; 8.7% and 29.1% for spring. Removal rate in terms of stations ranged between 8.1% and 8.9% for Prince Island; 15.7% and 16.7% for Bagdat Avenue; 28.7% and 29.4% for TEM Highway; 27.7% and 32.1% for Barbaros Boulevard; 24.4% and 38.1% for Dilovasi District. The highest removal rate was detected as 38.1% from Dilovasi District in autumn. The lowest was detected as 8.1% from Prince Island in winter.

Removal rate of Mg leaped at TEM Highway and reached the maximum level at Dilovasi District. According to these results, sources of Mg were mainly airborne particles which are generated by heavy traffic and industrial establishments in TEM, Barbaros and Dilovasi. Also decreased levels of Mg in soil might have affected the uptake of Mg by the plants. In Dilovasi District and Barbaros Boulevard, decrease in removal rates of Mg might be sourced from Mg increase in leaves at spring.

Table 3.7 Mg levels (L: leaf, uWL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/ city	Method
		Control	Treatment	Control	Treatment		
Tzvetkova and Petkova (2015)	*R. pseudoacacia*	---	---	2420 (L) June	1870 (L) June	Bulgaria	AAS
		---	---	2320 (L) Sept.	2250 (L) Sept		
Jensen et al., (2010)	*R. pseudoacacia*	---		3290 (L)	3880 (L)	USA	ICP-MS
Rahmonov (2009)	*R. pseudoacacia*	---		1618 (L)		Poland	Flame AAS
				462 (B)			
				338 (S)			
Tabari and Salehi (2009)	*R. pseudoacacia*	301	378	2380 (L)	3380 (L)	Iran	AAS

Tabari and Salehi, (2009) reported that results of Mg content in soil irrigated with municipal sewage water ranged between 301 and 378 mg.kg^{-1}. According to results of this study, Mg content in soil of control station which is Prince Island ranged between 4166.854 and 4672.004 mg.kg^{-1}. This difference in the Mg content of the soil may be due to soil type and mineral element content. Additionally Mg content in upper layer of soil ranged from 300 to 8400 mg.kg^{-1} (Merhaut, 2007). Mg content results of Tabari and Salehi, (2009) and this study are within the normal range.

Mg contents in plant parts of our study are higher than results of Tzvetkova and Petkova, (2015) and Rahmonov (2009); and consistent with the results of Jensen et al. (2010) and Tabari and Salehi (2009). Hawkesford et al., (2012) reported that plant vegetative parts Mg requirement ranges from 1500 to 3500 mg.kg^{-1}. Results of this study showed that Mg content in plant parts are within the range. But some results in Dilovasi District were lower than the normal values.

3.1.1.9 Manganese (Mn)

The main function of Mn is being a cofactor for different enzymes, thus Mn serves as a micronutrient for all organisms (Das et al. 2014). According to results, Mn levels in plant samples ranged between 24.740±0.553 $mg.kg^{-1}$ (Branch/Prince Island) and 242.712±7.392 $mg.kg^{-1}$ (Bark/Dilovasi District). Soil Mn concentrations were determined between 389.210±8.465 $mg.kg^{-1}$ (Prince Island) and 932.012±16.726 $mg.kg^{-1}$ (Dilovasi District). Prince Island samples contained the lowest Mn concentrations both in plant and soil while Dilovasi District has the highest Mn concentrations.

The highest Mn levels in plant and soil were detected in spring in all stations while the lowest were detected in summer. Results indicated that Mn concentrations in all plant and soil samples for all stations tend to be increase during autumn and spring and decrease during summer and winter. Additionally seasonal variation pattern remained the same for all stations.

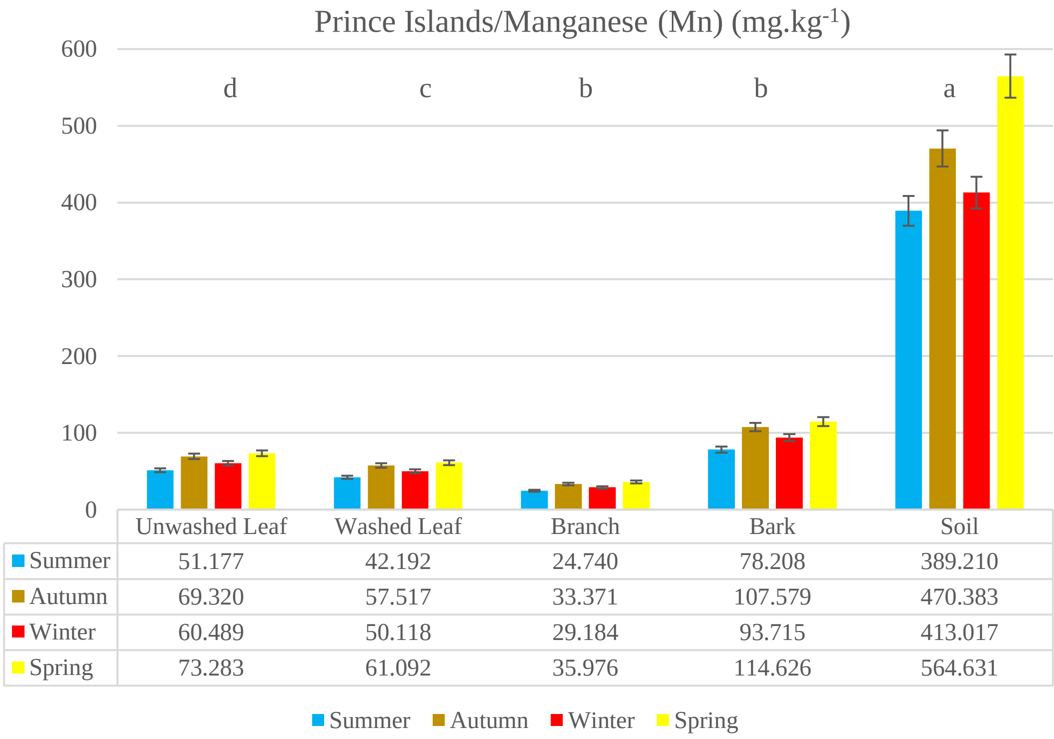
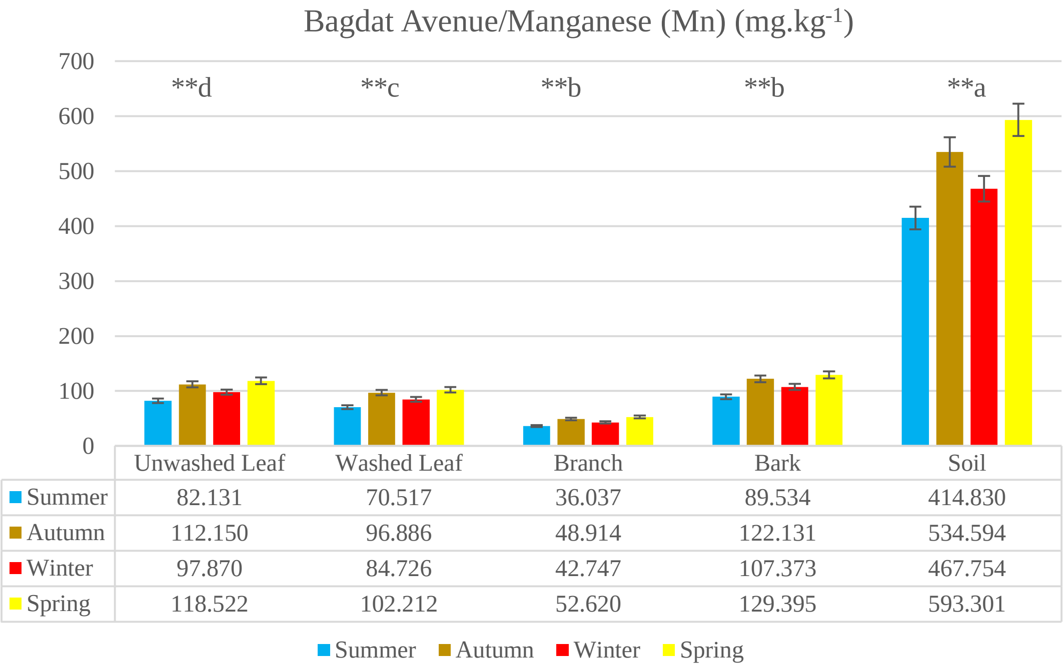
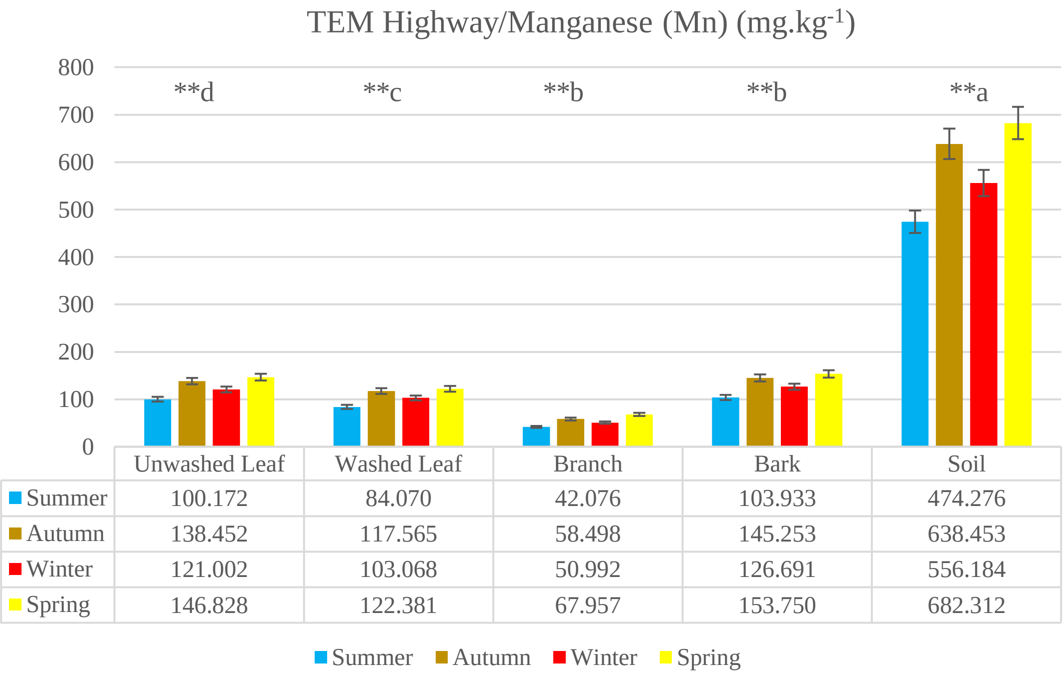


Figure 3.49 Average Mn concentrations in Prince Island



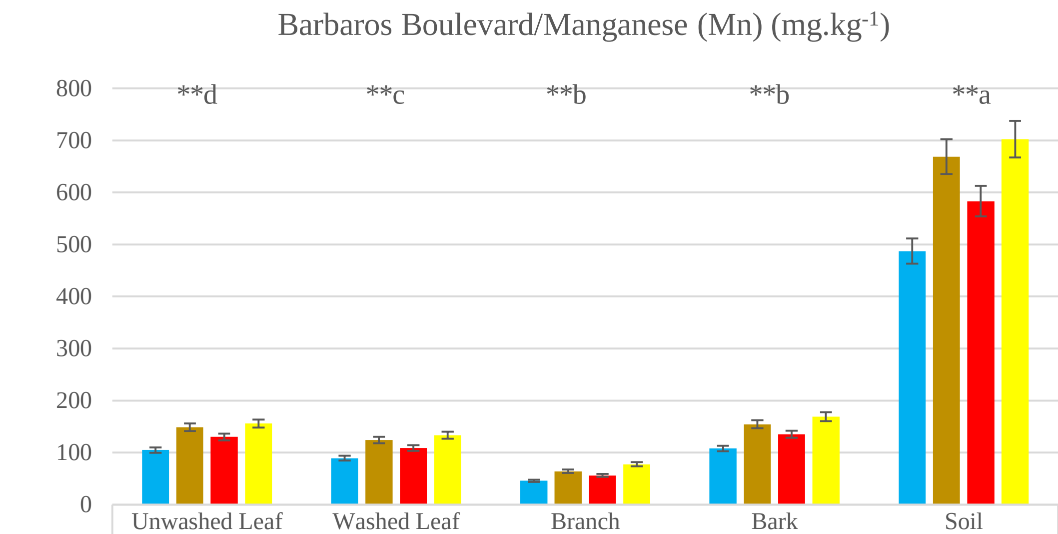
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	82.131	70.517	36.037	89.534	414.830
Autumn	112.150	96.886	48.914	122.131	534.594
Winter	97.870	84.726	42.747	107.373	467.754
Spring	118.522	102.212	52.620	129.395	593.301

Figure 3.50 Average Mn concentrations in Bagdat Avenue



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	100.172	84.070	42.076	103.933	474.276
Autumn	138.452	117.565	58.498	145.253	638.453
Winter	121.002	103.068	50.992	126.691	556.184
Spring	146.828	122.381	67.957	153.750	682.312

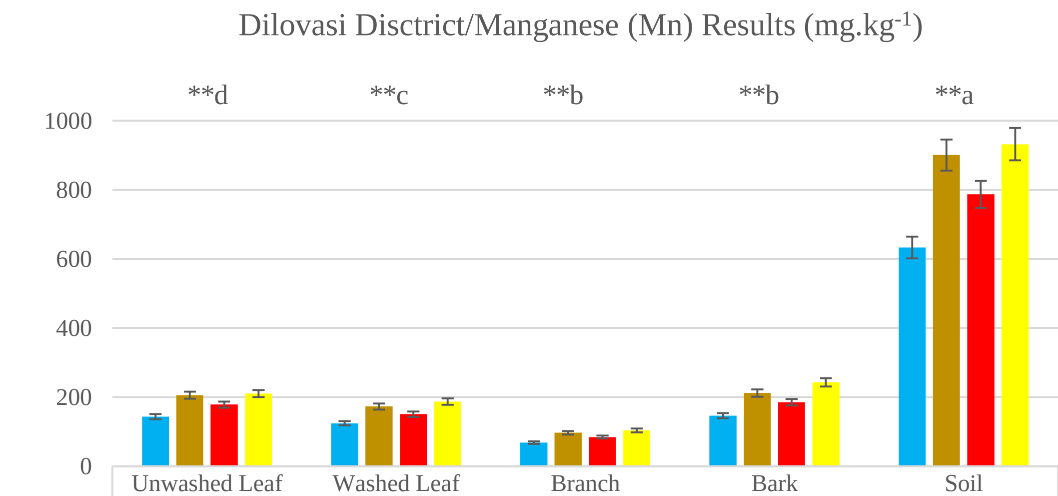
Figure 3.51 Average Mn concentrations in TEM Highway



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	105.017	89.361	45.839	107.957	487.232
Autumn	148.911	124.299	64.031	154.325	668.726
Winter	130.127	108.754	55.985	135.078	583.229
Spring	155.981	133.241	77.672	168.971	702.152

Summer Autumn Winter Spring

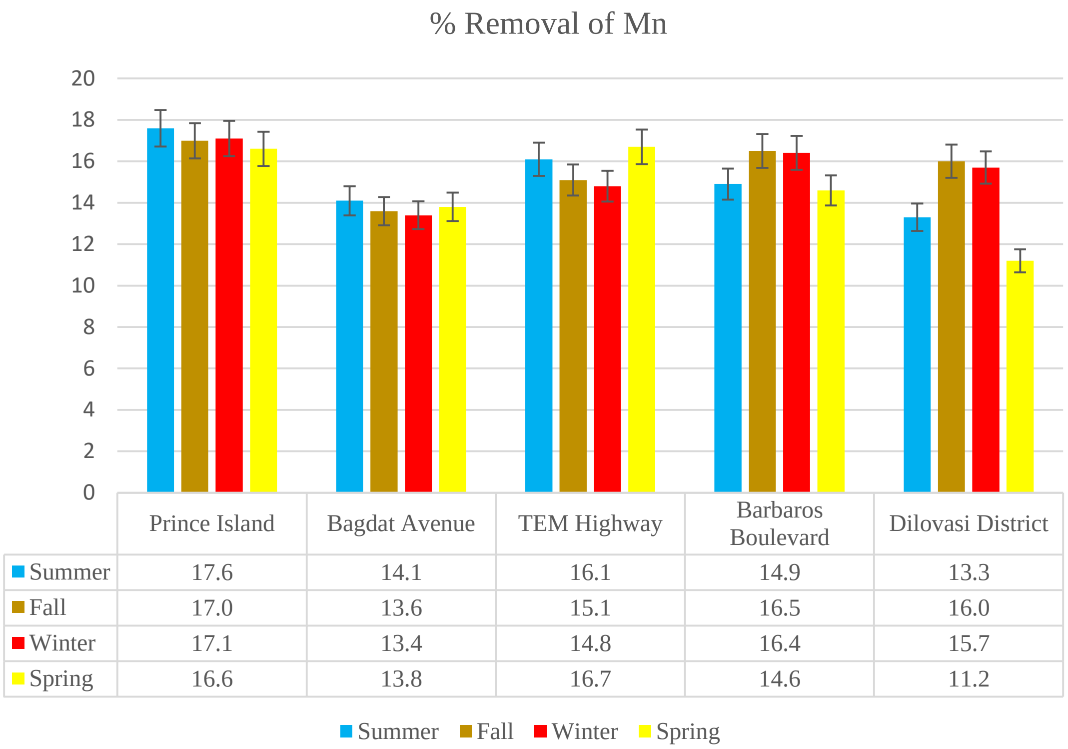
Figure 3.52 Average Mn concentrations in Barbaros Boulevard



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	143.406	124.279	68.463	146.023	633.313
Autumn	205.749	172.866	96.706	211.943	900.886
Winter	178.469	150.501	84.475	184.849	786.953
Spring	210.321	186.817	103.631	242.712	932.012

Summer Autumn Winter Spring

Figure 3.53 Average Mn concentrations in Dilovasi Disctrict



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	17.6	14.1	16.1	14.9	13.3
Fall	17.0	13.6	15.1	16.5	16.0
Winter	17.1	13.4	14.8	16.4	15.7
Spring	16.6	13.8	16.7	14.6	11.2

Figure 3.1.54 Removal rates of Mn

The highest removal rate was detected as 17.6% from Prince Island in summer. The lowest was detected as 11.2% from Dilovasi District in spring. Removal rate of Mg ranged between 13.3% and 17.6% for summer; 13.6% and 17.0% for autumn; 13.4% and 17.1% for winter; 11.2% and 16.7% for spring. Removal rate in terms of stations ranged between 16.6% and 17.6% for Prince Island; 13.4% and 14.1% for Bagdat Avenue; 14.8% and 16.7% for TEM Highway; 14.6% and 16.5% for Barbaros Boulevard; 11.2% and 16.0% for Dilovasi District. According to results, control, heavy traffic and industrial zones had similar Mn removal rates whereas removed amount of Mn with washing procedure increased from Prince Island to Dilovasi District. The main air emission sources of Mn are fossil fuel combustion, re-entrainment of Mn-rich soils and industrial emissions (Williams et al., 2012). The increase in Mn content in Bagdad, TEM and Barbaros stations may be due to heavy traffic but the highest Mn content in Dilovasi District may be sourced from industrial emission specially iron-steel production factories.

The Mn content of the plant parts has increased in parallel with the increasing Mn content in the soil. Millaleo et al., (2010) reported from Neumann and Römheld (2001) that soil mobilization of many micronutrients are affected by soil pH value, organic acid and organic matter contents. Williams et al., (2012) reported that combustion of fossil fuel which contains Mn compound additives is the main source of soil Mn. In relation with that, it is possible that differences at soil pH, texture, organic acid and organic matter ingredients may cause the increase in Mn content from Prince Island to Dilovasi District.

Table 3.8 Mn levels (L: leaf, uWL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country /City	Method
		Control	Study Site	Control	Study Site		
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	21.76	151.53 - 250.64	21.43 (L)	12.99- 21.91 (L)	Poland	Flame AAS
Esen et al., (2016)	***Carpinus betulus***	410	610-840	3840 (L)	555-970 (L)	**TUR /Istanbul**	k0-INAA
	Quercus petraea			1930 (L)	1040-1240 (L)		
	Tilia Argentea			598 (L)	272-402 (L)		
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---		28.9 (L) June	43.3 (L) June	Bulgaria	AAS
				93.0 (L) Sept.	100.7 (L) Sept		
Vural (2013)	***R. pseudoacacia***	---		3.74-14.0 (S)		**TUR /Gumushane**	ICP-AES
Jensen et al., (2010)	***R. pseudoacacia***	---		41.5 (L)	52.8 (L)	USA	ICP-MS
Rahmonov (2009)	***R. pseudoacacia***	---		110 (L)		Poland	Flame AAS
				30 (B)			
				16 (S)			
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	201-214	266-553	101-108 (L)	91-216 (L)	Poland	ICP-MS
				45-48 (B)	49-167 (B)		
Tabari and Salehi (2009)	***R. pseudoacacia***	641.91	742.36	31.56 (L)	46.56 (L)	Iran	AAS
Celik et al., (2005)	***R. pseudoacacia***	271.870	337.36-786.47	53.6 (UwL)	147.8- 349.2 (UwL)	**TUR /Denizli**	Flame AAS
				43.3 (WL)	95.4-229.2 (WL)		
Mertens et al., (2004)	***R. pseudoacacia***	683 (Sediment)		---		Belgium	Flame AAS

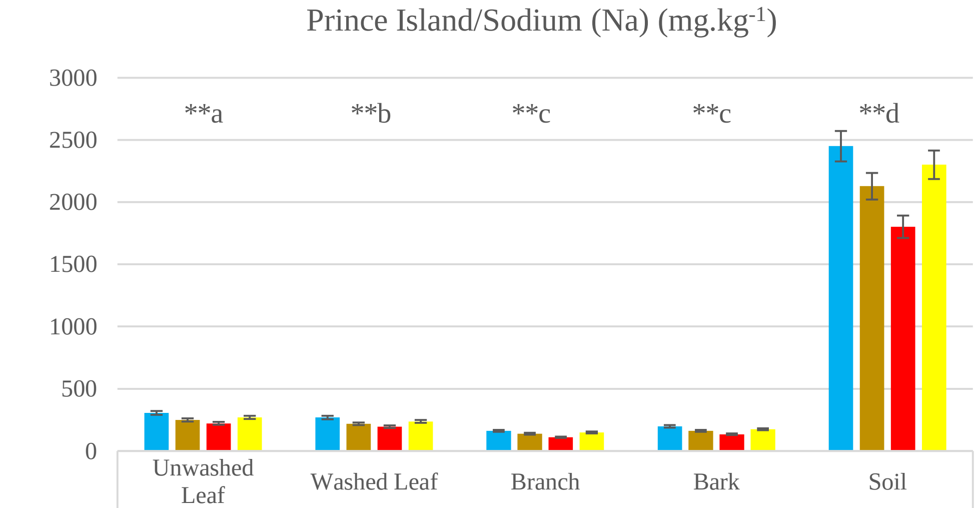
Mn contents in soil are highly variable as 10 - 9000 mg.kg^{-1} but overall average is calculated as 437 mg.kg^{-1} (Kabata-Pendias and Mukherjee, 2007). There are some studies shown in table 3.8 about determination of some mineral element levels in *R. pseudoacacia* and/or other species. There is not an extreme soil Mn content level in this study or other studies and Mn contents in soil were within the normal limits for all studies.

Plant Mn content generally ranges from 30 to 300 mg.kg^{-1} and 400-1000 mg.kg^{-1} of Mn is condsidered as excessive and toxic therefore Mn is both an essential and toxic mineral element (Kabata - Pendias and Pendias, 2001). Plant part Mn levels of this study remained within the normal limits thus it can be said that, *R. pseudoacacia* plants have acquired sufficient amount of Mn to maintain its physiological processes. When other studies were examined, Esen et al., (2016) reported that *Carpinus betulus* and *Quercus petraea* plants have accumulated Mn in high proportions than the *R. pseudoacacia* leaves as 3840 and 1930 $mg.kg^{-1}$ respectively. The authors did not mention any of the toxicity symptoms caused by Mn. The genetic and physiological differences among plants may affect mineral element accumulation like in this case. In study of Nadgorska-Socha et al., (2016) detected Mn level in plant parts is under the sublimit. Extreme levels of Mn were not detected in other studies.

3.1.10 Sodium (Na)

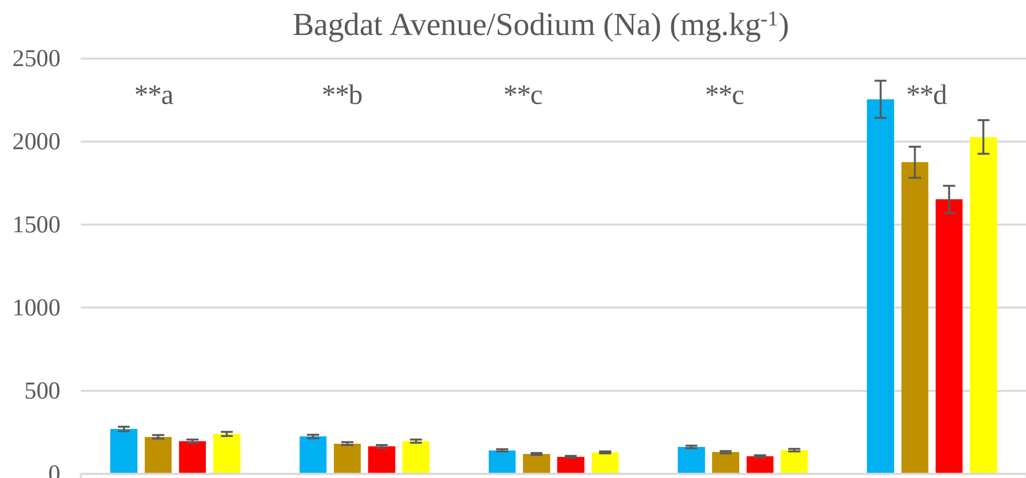
Na is a non-essential alkali metal and it is responsible for salinity in agricultural areas. According to results, Na levels in plant samples ranged between 52.325±2.448 mg.kg^{-1} (branch/winter/Dilovasi District) and 304.958±9.606 mg.kg^{-1} (unwashed leaves/summer/Prince Island). Soil Na contents were determined between 992.662±35.412 mg.kg^{-1} (winter/Dilovasi District) and 2449.783±71.939 mg.kg^{-1} (summer/Prince Island). Prince Island samples contained the highest Na concentrations both in plant and soil while Dilovasi District has the lowest Na concentrations.

The highest Na level in plants and soil was detected in summer in all stations while the lowest was detected in winter. Na contents in soil and plant varied similar to the seasonal variation of K. Seasonal variation pattern remained same for all stations for all samples. Results indicated that Na concentrations in all plant and soil samples for all stations tend to be decrease from summer to winter, followed by an increase again in spring.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	304.958	268.640	161.334	197.197	2449.783
Autumn	249.699	218.388	138.299	160.829	2128.253
Winter	221.794	195.065	110.109	134.401	1802.195
Spring	269.646	236.926	149.320	174.287	2300.677

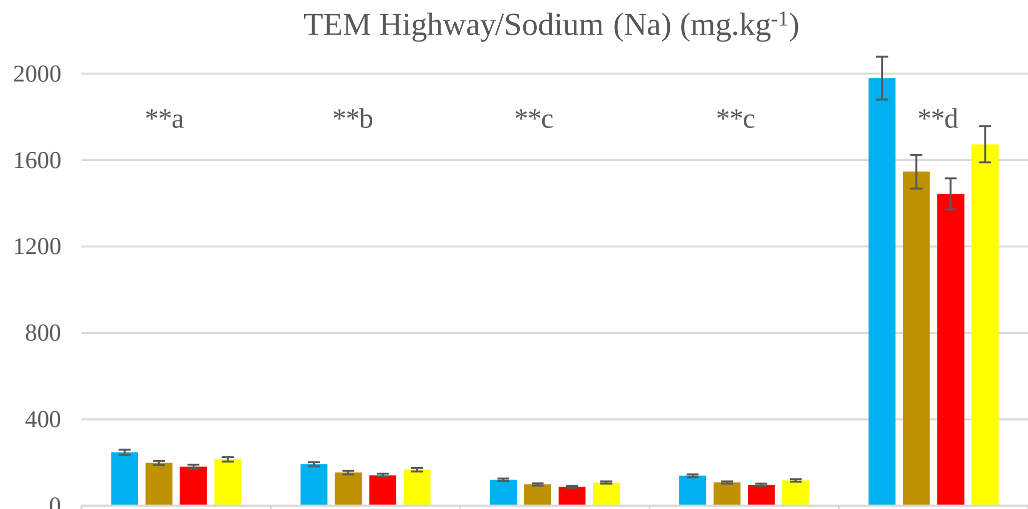
Figure 3.55 Average Na concentrations in Prince Island



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	269.722	224.322	141.094	161.851	2254.987
Autumn	221.916	181.312	118.668	130.359	1875.931
Winter	196.449	164.525	101.960	105.144	1652.175
Spring	239.783	196.308	128.554	141.932	2028.231

Summer Autumn Winter Spring

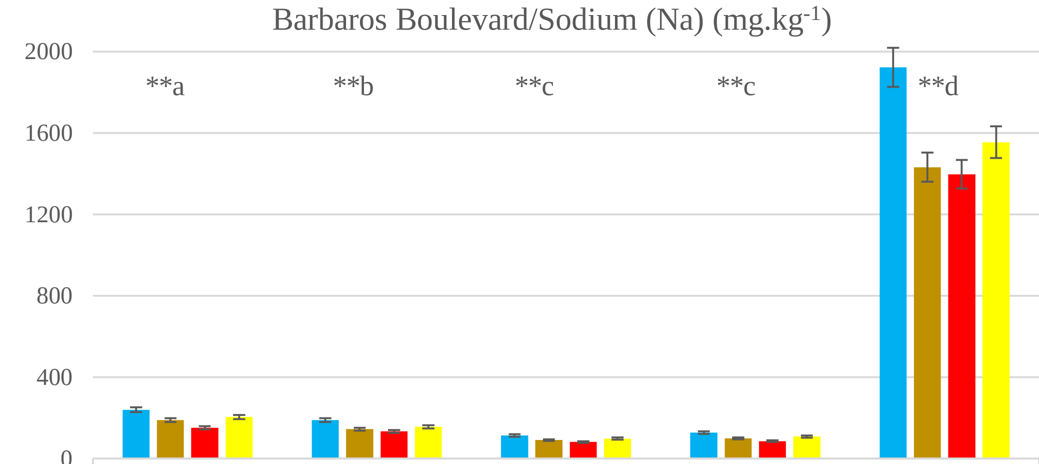
Figure 3.56 Average Na concentrations in Bagdat Avenue



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	247.226	192.413	120.433	138.642	1979.174
Autumn	197.829	153.189	98.577	107.779	1545.988
Winter	179.919	141.157	87.405	96.526	1443.375
Spring	214.391	166.655	107.008	117.429	1672.726

Summer Autumn Winter Spring

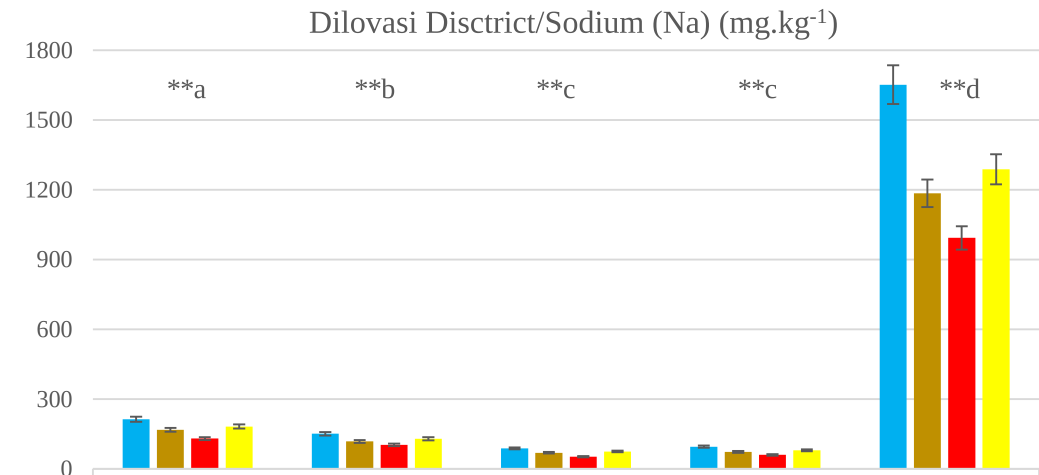
Figure 3.57 Average Na concentrations in TEM Highway



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	240.565	188.817	113.832	127.811	1922.489
Autumn	188.721	145.048	91.123	99.496	1431.980
Winter	152.179	133.674	82.165	86.003	1397.340
Spring	204.695	156.591	98.869	108.461	1554.945

Summer Autumn Winter Spring

Figure 3.58 Average Na concentrations in Barbaros Boulevard



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	213.254	150.990	87.802	95.227	1651.652
Autumn	167.708	117.981	68.812	73.101	1184.355
Winter	130.334	103.058	52.325	59.913	992.662
Spring	181.871	129.434	74.711	79.924	1287.986

Summer Autumn Winter Spring

Figure 3.59 Average Na concentrations in Dilovasi District

Increase in soil Na content is a consequence of natural events (effect of seawater, climatic conditions and rock weathering) or anthropogenic impacts (industrial and agricultural activities, irrigation, road salt, water softener, sewage. etc.). Intrusion of seawater in coastal areas or islands can increase soil Na content. (Kelly et al., 2008; Yadav et al., 2011). The higher Na content in Prince Island and Bagdat Avenue, which are the nearest stations to sea, may be a consequence of this phenomenon.

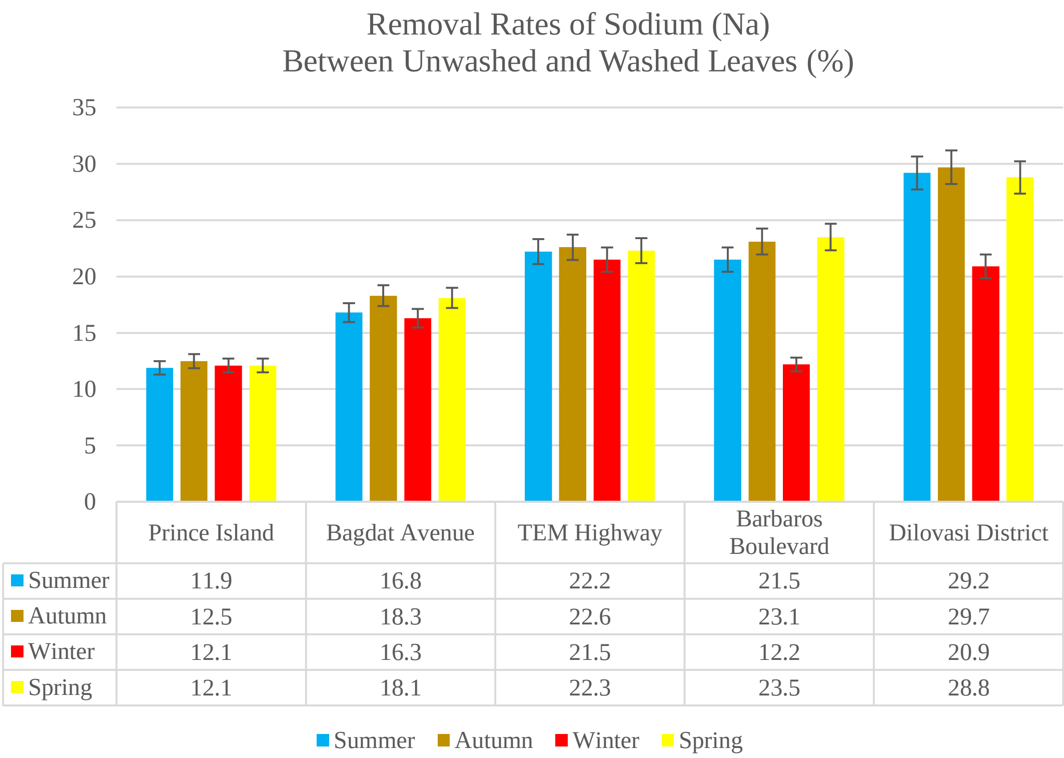


Figure 3.60 Removal rates of Na between unwashed and washed leaves

The highest removal rate of Na was detected as 29.7% from Dilovasi District in autumn. The lowest was detected as 11.9% from Prince Island in summer. Removal rate of Na ranged between 11.9% and 29.2% for summer; 12.5% and 29.7% for autumn; 12.1% and 21.5% for winter; 12.1% and 28.8% for spring. Removal rate in terms of stations ranged between 11.9% and 12.5% for Prince Island; 16.3% and 18.3% for Bagdat Avenue; 21.5% and 22.6% for TEM Highway; 12.2% and 23.5% for Barbaros Boulevard; 20.9% and 29.7% for Dilovasi District. Changes in removal rate of Na especially depend on locality.

Plants adjust the optimal ratio between K^+ and Na^+ to maintain metabolic functions, sufficient growth and yield development. In this work, it can be suggested that *R. pseudoacacia* plants adjusted Na levels in plant parts efficiently.

Table 3.9 Na levels (L: leaf, uWL: unwashed leaf, WL: washed leaf, B: bark, S: branch)

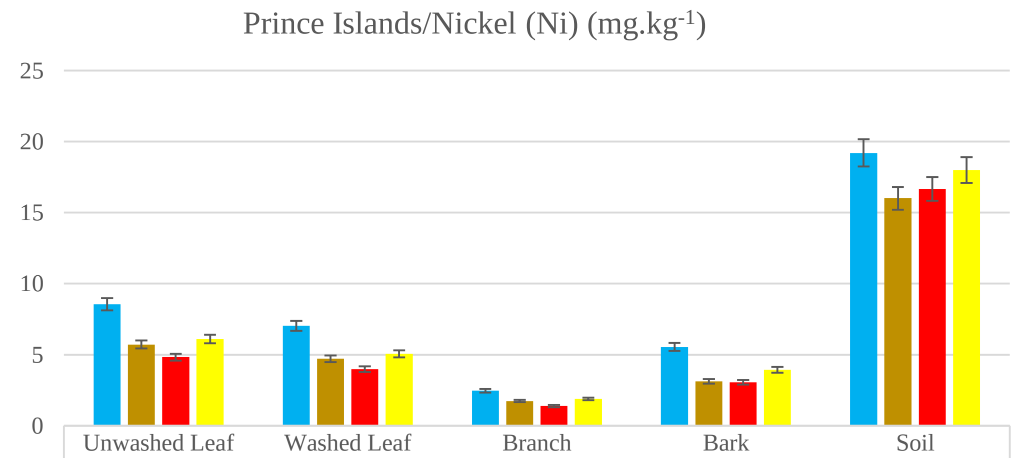
Reference	Organism	Soil ($mg.kg^{-1}$)		Plant ($mg.kg^{-1}$)		Country	Method
		Control	Treatment	Control	Treatment		
Esen et al., (2016)	***Carpinus betulus***	1600	3400-14800	411 (L)	443-1550 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			780 (L)	1000-990 (L)		
	Tilia Argentea			555 (L)	990-930 (L)		
Jensen et al., (2010)	***R. pseudoacacia***	---		17.0 (L)	43.7 (L)	USA	ICP-MS
Rahmonov (2009)	***R. pseudoacacia***	---		464 (L)		Poland	Flame AAS
				408 (B)			
				460 (S)			
Tabari and Salehi (2009)	***R. pseudoacacia***	0.887	1.09	0.887 (L)	1.09 (L)	Iran	AAS
Mertens et al., (2004)	***R. pseudoacacia***	457 (Sediment)		---		Belgium	Flame AAS

Esen et al., (2016) detected soil Na content in different stations in Istanbul between 1600 and 14800 $mg.kg^{-1}$ (Table 3.9). According to results, Na content in soil of control station is lower than the control station of this study. While Na content of other stations were higher than the Na content values of other stations in this study.

3.1.11 Nickel (Ni)

Nickel is a heavy metal and a trace element for human and animals. Ni is commonly used in metal industry as ingredient of stainless steels and other metals (Kabata-Pendias and Mukherjee 2007; Wuana and Okieimen, 2011). According to results, Ni levels in plant samples ranged between 1.380±0.065 mg.kg^{-1} (branch/autumn/Prince Island) and 27.215±1.072 mg.kg^{-1} (unwashed leaves/summer/Dilovasi District). Soil Ni contents were determined between 16.010±0.598 mg.kg^{-1} (autumn/Prince Island) and 42.754±1.619 mg.kg^{-1} (summer/Dilovasi District). Dilovasi District samples contained the highest Ni concentrations both in plant and soil while Prince Island samples have the lowest Ni concentrations.

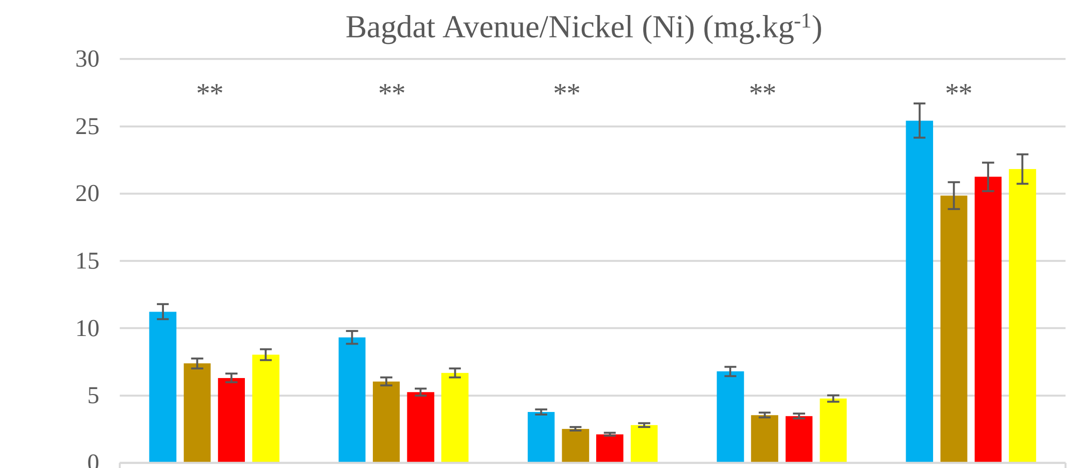
The highest Ni levels in plant and soil were detected in summer in all stations. The lowest results were detected in winter in plant samples but the lowest result of soil samples were detected in autumn in all stations. Seasonal variation pattern remained the same for all stations. Results indicated that Ni concentrations in all plants at all stations tend to decrease from summer to winter, followed by an increase again in spring.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	8.539	7.034	2.464	5.54	19.194
Autumn	5.719	4.717	1.739	3.129	16.011
Winter	4.823	3.975	1.381	3.056	16.662
Spring	6.101	5.058	1.891	3.934	17.994

Summer Autumn Winter Spring

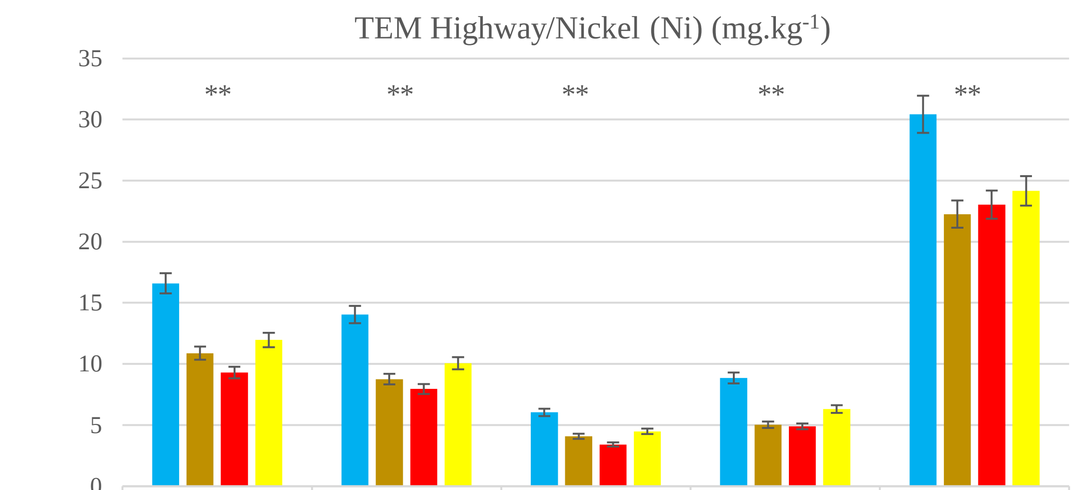
Figure 3.61 Average Ni concentrations in Prince Island



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	11.232	9.321	3.789	6.792	25.421
Autumn	7.389	6.048	2.528	3.544	19.851
Winter	6.308	5.258	2.124	3.482	21.244
Spring	8.031	6.671	2.811	4.781	21.831

Summer Autumn Winter Spring

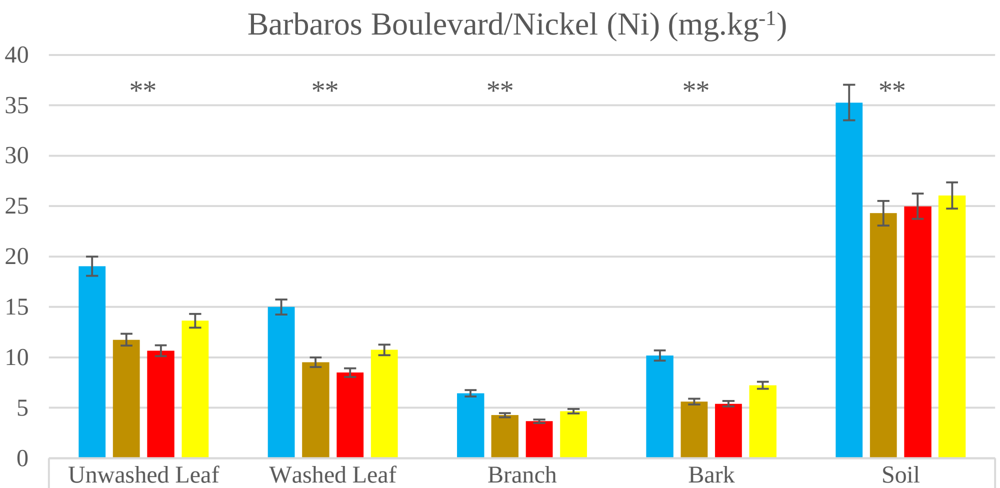
Figure 3.62 Average Ni concentrations in Avenue



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	16.596	14.041	6.041	8.861	30.439
Autumn	10.877	8.751	4.084	5.025	22.251
Winter	9.291	7.948	3.407	4.894	23.031
Spring	11.962	10.056	4.478	6.302	24.156

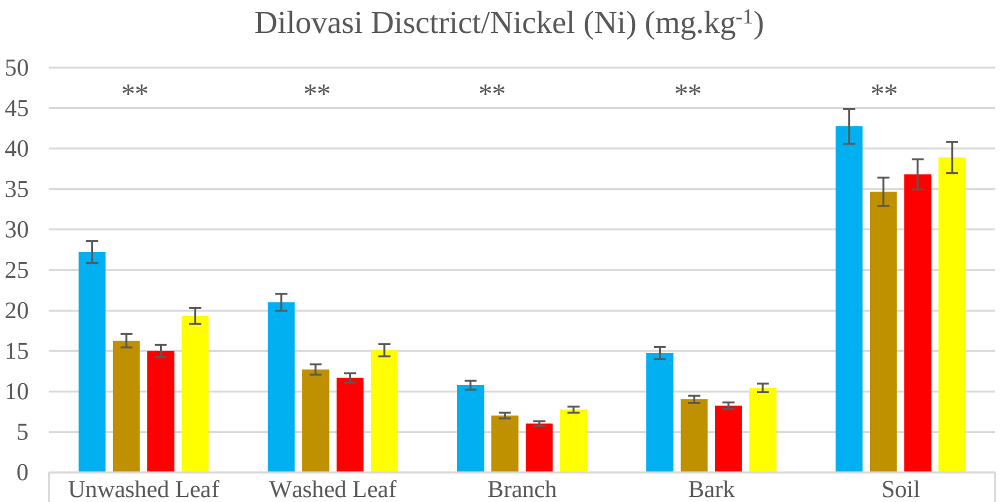
Summer Autumn Winter Spring

Figure 3.63 Average Ni concentrations in TEM Highway



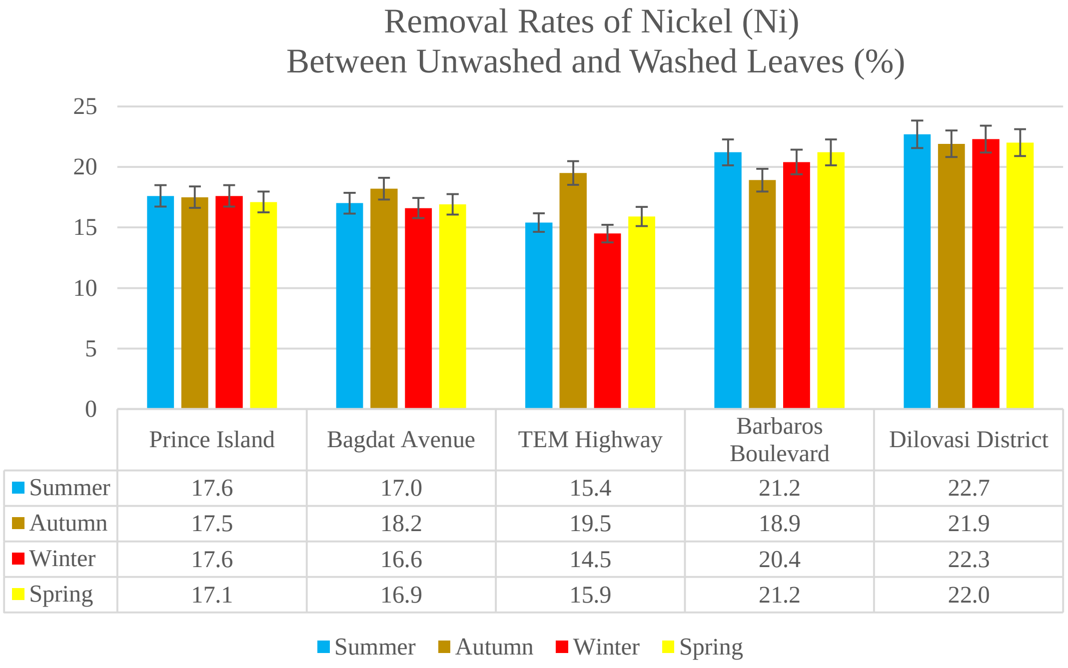
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	19.031	14.994	6.421	10.166	35.268
Autumn	11.742	9.521	4.262	5.603	24.295
Winter	10.656	8.481	3.655	5.387	24.977
Spring	13.631	10.735	4.649	7.224	26.058

Figure 3.64 Average nickel (Ni) concentrations in Barbaros Boulevard



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	27.215	21.034	10.787	14.741	42.754
Autumn	16.274	12.714	7.026	9.044	34.672
Winter	15.031	11.681	6.042	8.251	36.804
Spring	19.339	15.091	7.769	10.445	38.893

Figure 3.65 Average Ni concentrations in Dilovasi District



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	17.6	17.0	15.4	21.2	22.7
Autumn	17.5	18.2	19.5	18.9	21.9
Winter	17.6	16.6	14.5	20.4	22.3
Spring	17.1	16.9	15.9	21.2	22.0

Figure 3.66 Removal rates of nickel (Ni) between unwashed and washed leaves.

The highest removal rate of Ni was detected as 22.7% from Dilovasi District in summer. The lowest was detected as 14.5% from TEM Highway in winter. Removal rate of Ni ranged between 15.4% and 22.7% for summer; 17.5% and 21.9% for autumn; 14.5% and 22.3% for winter; 15.9% and 22.0% for spring. Removal rate in terms of stations ranged between 17.1% and 17.6% for Prince Island; 16.6% and 18.2% for Bagdat Avenue; 14.5% and 19.5% for TEM Highway; 18.9% and 21.2% for Barbaros Boulevard; 21.9% and 22.7% for Dilovasi District. Changes in removal rate of Ni mainly depend on locality. The high level of Dilovasi District Ni content indicates industrial emission of Ni.

Ni is a microelement that is involved in the function of urease and some other important enzymes. Additionally, Ni is also involved in N metabolism (Broadley et al., 2012). Kabata-Pendias and Mukherjee, (2007) reported that the average Ni concentration in soil is 19- 22 $mg.kg^{-1}$. According to these values, Ni content in soil samples of Prince Island and Bagdat Avenue are within the normal range. Soil Ni contents of Tem Highway, Barbaros Boulevard and Dilovasi District are higher than the normal range. In relation with the high levels of soil Ni content, plant Ni contents were also at excessive levels in these stations.

Table 3.10 Ni levels (L: leaf, uWL:unwashed leaf, WL:washed leaf, B: bark, S: branch)

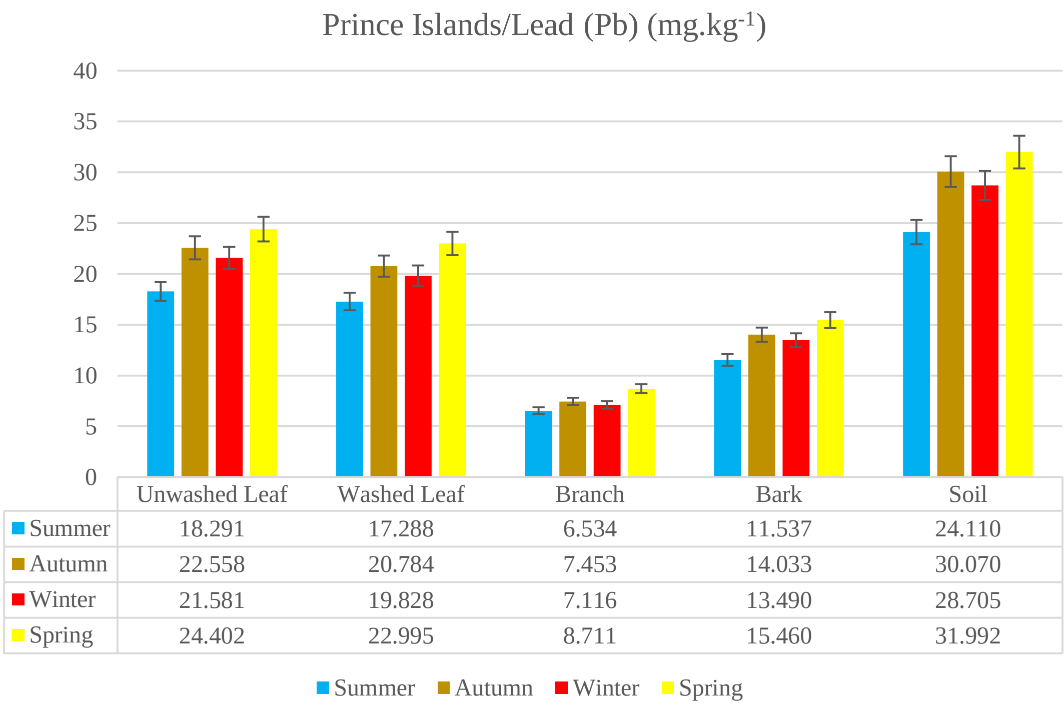
Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country	Method
		Control	Study Site	Control	Study Site		
Esen et al., (2016)	***Carpinus betulus***	16.3	40.7-53.0	8.1 (L)	4.1-4.9 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			8.3 (L)	6.9-10.1 (L)		
	Tilia Argentea			6.5 (L)	3.8-4.1 (L)		
Ozen and Yaman (2015)	***R. pseudoacacia***	---		6	3-10	**TUR/Bursa, Gaziantep**	ICP-MS
Vural (2013)	***R. pseudoacacia***	10-37		1.09-5.41 (S)		**TUR/Gumushane**	ICP-AES
Yasar et al., (2010)	***Cercis siliquastrum***	15.07 Urban roadside- 5.34 Control		4.47 (UwL) - 2.19 (WL)		**TUR/Istanbul**	ICP-OES
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	0.7-0.9	8.9-17.8	0.8-1.0 (L)	1.3-3.9 (L)	Poland	ICP-MS
				1.3-2.7 (B)	2.6-5.4 (B)		
Tabari and Salehi (2009)	***R. pseudoacacia***	27.63	38.56	ND (L)	ND (L)	Iran	AAS

Some studies are shown in Table 3.10 about Ni levels in soil and plant. Soil Ni content results of Esen et al., (2016) and Tabari and Salehi (2009) were higher than the results of this study. While plant Ni contents in all other studies were lower than the results of present study.

3.1.12 Lead (Pb)

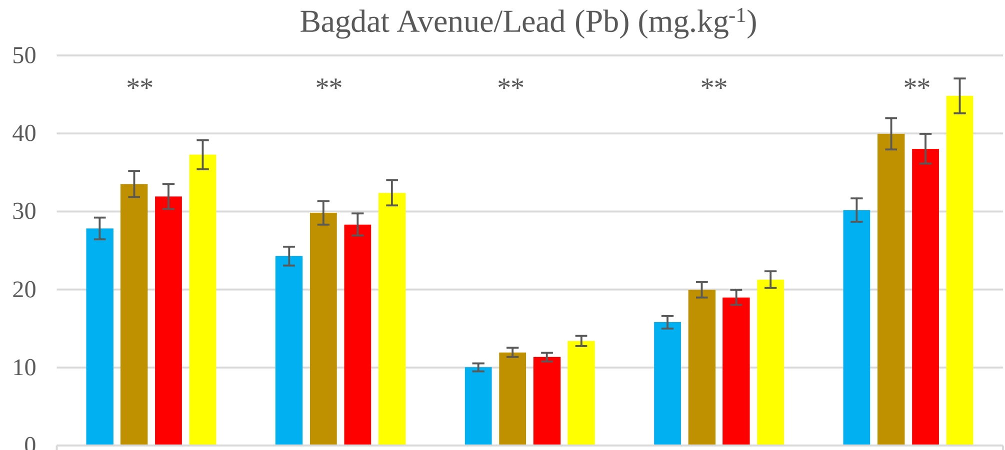
Lead (Pb) is one of the most toxic and exposed pollutant for plants. In the year 2013 Pb is regarded as most toxic and hazardous heavy metal after arsenic according to occurance, toxicity and human exposure by Agency for Toxic Substances and Disease Registry (ATSDR) (Pourrut et al., 2011). According to results, Pb levels in plant samples ranged between 6.534±0.242 $mg.kg^{-1}$ (branch/summer/Prince Island) and 103.356±3.994 $mg.kg^{-1}$ (unwashed leaves/spring/Dilovasi District). Soil Pb contents were determined between 24.110±0.738 $mg.kg^{-1}$ (summer/Prince Island) and 112.868±2.781 $mg.kg^{-1}$ (spring/Dilovasi District). Dilovasi District samples contained the highest Pb concentrations both in plant and soil samples while Prince Island samples have the lowest.

The highest Pb concentrations were detected in spring at all stations however the lowest values were detected in summer at all stations for both plant and soil samples. Pb concentrations of all samples increased in relation with increased Pb concentrations in soil. Alexander et al., 2006 reported that members of *Fabaceae* family accumulated Pb at low levels in comparison with some other important families.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	18.291	17.288	6.534	11.537	24.110
Autumn	22.558	20.784	7.453	14.033	30.070
Winter	21.581	19.828	7.116	13.490	28.705
Spring	24.402	22.995	8.711	15.460	31.992

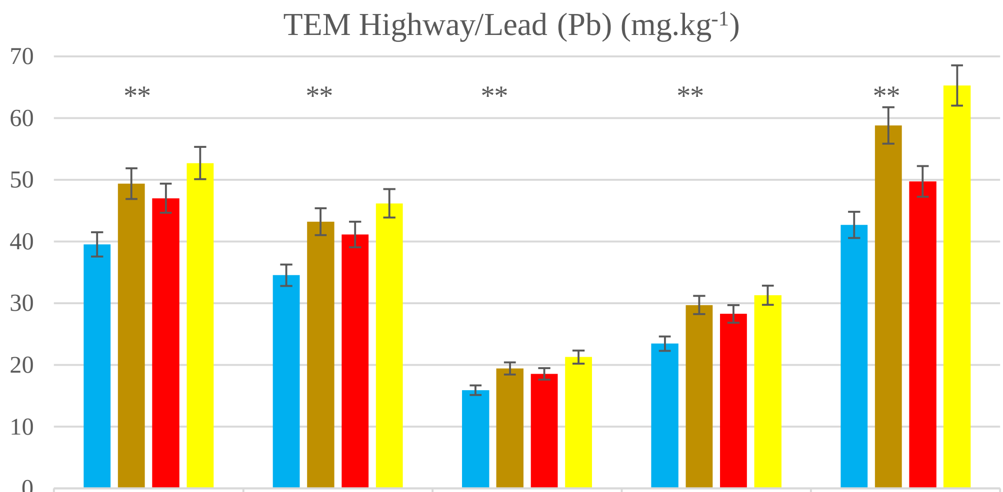
Figure 3.67 Average Pb concentrations in Prince Islands.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	27.824	24.278	10.019	15.796	30.164
Autumn	33.523	29.809	11.919	19.947	39.943
Winter	31.921	28.317	11.327	18.982	38.036
Spring	37.264	32.374	13.389	21.256	44.806

Summer Autumn Winter Spring

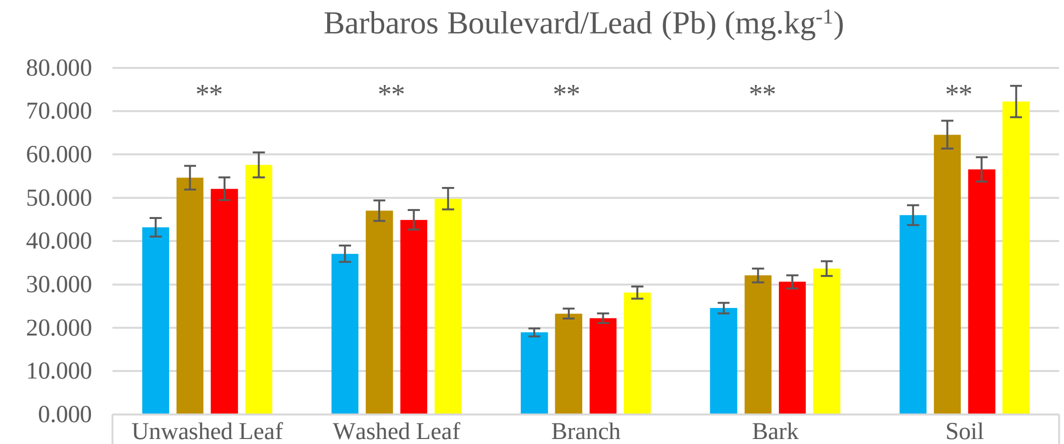
Figure 3.68 Average Pb concentrations in Bagdat Avenue



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	39.527	34.527	15.909	23.454	42.684
Autumn	49.368	43.197	19.420	29.693	58.778
Winter	46.989	41.123	18.528	28.258	49.725
Spring	52.699	46.175	21.270	31.275	65.238

Summer Autumn Winter Spring

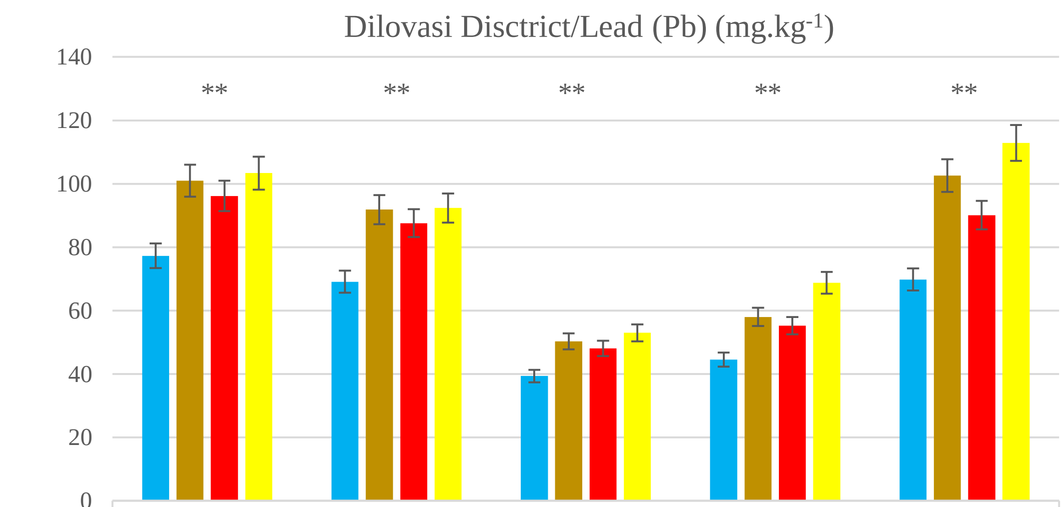
Figure 3.69 Average Pb concentrations in TEM Highway



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	43.206	37.093	18.938	24.541	46.011
Autumn	54.656	47.046	23.272	32.077	64.572
Winter	52.090	44.909	22.218	30.607	56.557
Spring	57.600	49.794	28.154	33.684	72.247

Summer Autumn Winter Spring

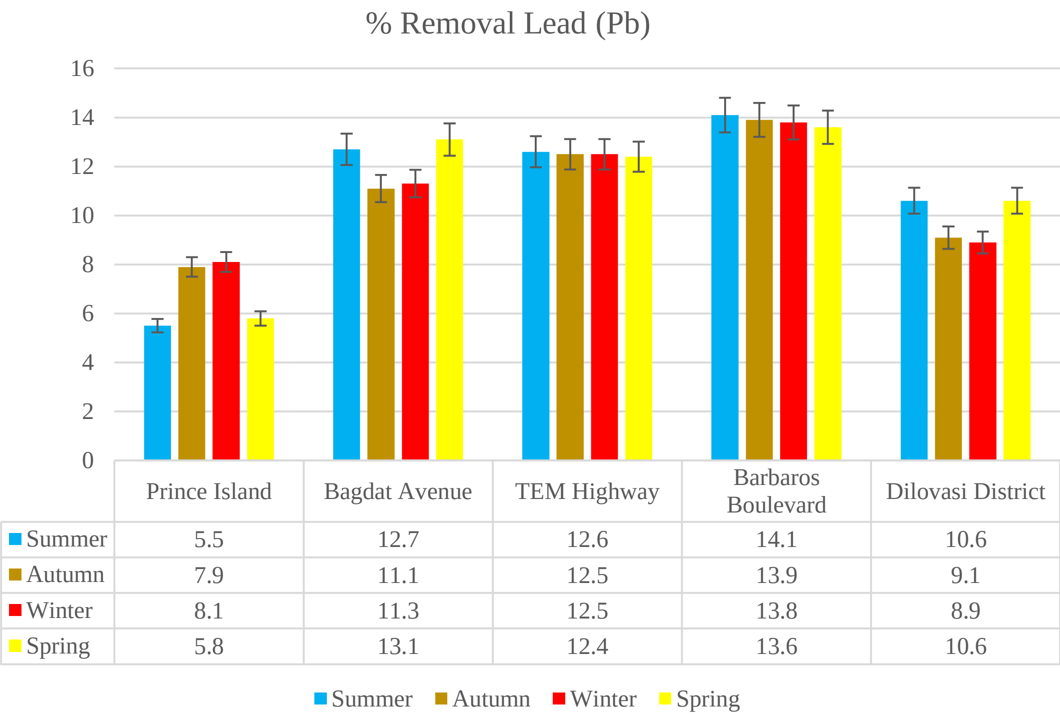
Figure 3.70 Average Pb concentrations in Barbaros Boulevard



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	77.305	69.118	39.393	44.553	69.811
Autumn	101.025	91.879	50.341	58.031	102.602
Winter	96.182	87.577	48.093	55.254	90.127
Spring	103.356	92.372	53.004	68.765	112.868

Summer Autumn Winter Spring

Figure 3.71 Average Pb concentrations in Dilovasi Disctrict



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	5.5	12.7	12.6	14.1	10.6
Autumn	7.9	11.1	12.5	13.9	9.1
Winter	8.1	11.3	12.5	13.8	8.9
Spring	5.8	13.1	12.4	13.6	10.6

Figure 3.72 Removal rates of Pb between unwashed and washed leaves

Removal rate of Pb ranged between 5.5% and 14.1% for summer; 7.9% and 13.9% for autumn; 8.1% and 13.8% for winter; 5.8% and 13.6% for spring. Removal rate in terms of stations ranged between 5.5% and 8.1% for Prince Island; 11.1% and 13.1% for Bagdat Avenue; 12.4% and 12.6% for TEM Highway; 13.8% and 14.1% for Barbaros Boulevard; 8.9% and 10.6% for Dilovasi District. The highest removal rate of Pb was detected as 14.1% from Barbaros Boulevard in summer. The lowest was detected as 5.5% from Prince Island in summer.

Changes in removal rate of Pb occurred in a narrow range and mainly dependent on locality. Removal percentage of Pb in Bagdat Avenue, TEM Highway and Barbaros Boulevard were detected to be higher than the other stations. These stations have a dense traffic flow and high level emission of Pb. This emission may be sourced from combustion of fuel. Prince Island doesn’t have traffic or industrial facilities, Dilovasi District has relatively less traffic flow than the other locations, but it has heavy industrial facilities. According to the results it can be said that aerial emission of Pb is sourced from industrial activities.

Table 3.11 Pb levels (L: leaf, uWL:unwashed leaf, WL:washed leaf, B: bark, S: branch)

Reference	Organism	Soil ($mg.kg^{-1}$)		Plant ($mg.kg^{-1}$)		Country/city	Method
		Control	Study Site	Control	Treatment		
Armaki (2016)	***R. pseudoacacia***	11.2		11.3 (S) 9.7 (S)		Iran	ICP (OES)
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	75.76	117.02-513.50	2.79 (L)	3.57-27.93 (L)	Poland	Flame AAS
Palowski et al., (2016)	***R. pseudoacacia***	---		11.3-25.6 (L) 19.3-35.0 (B)		Poland	Flame AAS
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---		14.9 (L) June 22.2 (L) Sept.	28.6 (L) June 30.7 (L) Sept	Bulgaria	AAS
Monfred et al., (2013)	***R. pseudoacacia***	---		2.3 (L) 2.6 (S)		Iran	ICP (OES)
Vural (2013)	***R. pseudoacacia***	15-747		---		**TUR/Gumushane**	ICP-AES
Serbula et al., (2012)	***R. pseudoacacia***	32.3	29.6-96.5	13.1 (WL) 23.3 (UwL) 23.5 (B)	4.9-25.7 (WL) 22.3-58.9 (UwL) 9.7-38.8 (B)	Serbia	ICP-AES / AAS
Guney et al., (2010)	---	191 (Surface Soil) 81.2 (20-cm depth soil)		---		**TUR/Istanbul**	Flame AAS
Kaya et al., (2010)	***R. pseudoacacia***	1.2-4.1	27-602	1 (L)	3-1865 (L)	**TUR/Gaziantep**	ICP-MS
Jensen et al., (2010)	***R. pseudoacacia***	---		0.0888 (L)	0.222 (L)	USA	ICP-MS
Yener and Yarci (2010)	***Alcea pallida***	11.534-61.952		2.753-7.623 (L) 0.524-2.303 (S)		**TUR/Istanbul**	AAS
Rahmonov (2009)	***R. pseudoacacia***	---		12 (L) 84 (B) 68 (S)		Poland	Flame AAS
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	27-32	30-99	1.9-2.2 (L) 1.2-1.9 (B)	7.5-39 (L) 32-93 (B)	Poland	ICP-MS
Tabari and Salehi (2009)	***R. pseudoacacia***	55.64	93.01	ND (L)	ND (L)	Iran	AAS
Mertens et al., (2004)	***R. pseudoacacia***	75.2 (Sediment)		2.3 (L)		Belgium	Flame AAS
Celik et al., (2005)	***R. pseudoacacia***	34.260	74.86-336.55	15.11 (UwL) ---	21.84- 206.2 (UwL) ---	**TUR/Denizli**	Flame AAS
Aksoy et al., (2000)	***R. pseudoacacia***	39 (Rural)	70-468	15.98 (UwL) 14.89 (WL)	26.67-176.88(UwL) 21.04-62.42 (WL)	**TUR/Kayseri**	AAS

Pb is a natural component of earth crust but its level increase due to anthropogenic effects. Pb mainly is mainly emitted from smelting, mining, combustion of leaded gasoline and Pb containing garbages. In soil of unpolluted sites, Pb levels were detected as 25 mg.kg^{-}

^{1}. Pb content in plant parts are detect as 0.1-10 mg.kg^{-1} and above 30 mg.kg^{-1} is considered as excessive or toxic for plants (Kabata - Pendias and Pendias, 2001). According to results of this study, soil Pb content of Prince Island is slightly above the normal limits while plant part Pb contents were above the expected limits. Pb content levels in soil and plant samples from the other stations were detected above the normal levels. Measured Pb levels in both soil and plant samples ranged from low to higher levels in Bagdat Avenue, TEM Highway, Barbaros Boulevard and Dilovasi District respectively.

There are some studies shown in Table 3.11 including Pb levels from different countries. Measured Pb level in soil samples of Nadgorska-Socha et al., (2016), Vural (2013), Serbula et al., (2012), Guney et al., (2010), Kaya et al., (2010), Yener and Yarci (2010), Samecka-Cymerman et al., (2009), Tabari and Salehi (2009), Mertens et al., (2004), Celik et al., (2005) and Aksoy et al., (2000) were higher than the normal range. Additionally, measured Pb levels in study of Nadgorska-Socha et al., (2016), Vural (2013), Guney et al., (2010), Kaya et al., (2010), Celik et al., (2005) and Aksoy et al., (2000) were higher than the results of this study.

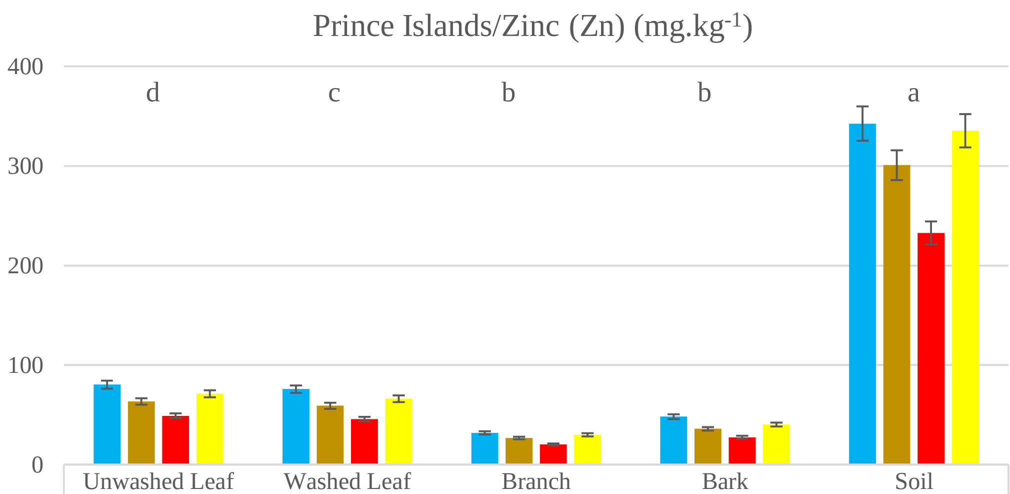
Measured Pb levels in plants samples of Nadgorska-Socha et al., (2016), Palowski et al., (2016), Tzvetkova and Petkova (2015), Serbula et al., (2012), Rahmonov (2009), Samecka-Cymerman et al., (2009), Celik et al., (2005) and Aksoy et al., (2000) were higher than the normal plant Pb content. Along with that measured Pb levels in plants samples of Kaya et al., (2010), Celik et al., (2005) and Aksoy et al., (2000) were higher than the results of this study. Pb pollution has reached the highest level in the Dilovasi Dictrict due to heavy industrial facilities.

Pourrut et al., (2011) reported that mineral nutrition status is affected by the high level of Pb. In relation with that, there are some positive correlation between Pb and Ca, Cd, Cr, Mn and Ni with .64, .91, .86, .96, .55 and .68 scores, respectively. There are not any significant negative correlation between Pb and any other element.

3.1.13 Zinc (Zn)

Zinc is an essential microelement for plants. Zn has structural, regulatory and catalytic functions. As a structural element Zn is found in structure of some proteins like proteins that have zinc-finger domains. Zn works with transcription factors for regulation of gene expression. Zn also acts as a cofactor for hundreds of enzymes which are the most important catalytic compounds of cell (Ricachenevsky et al., 2015).

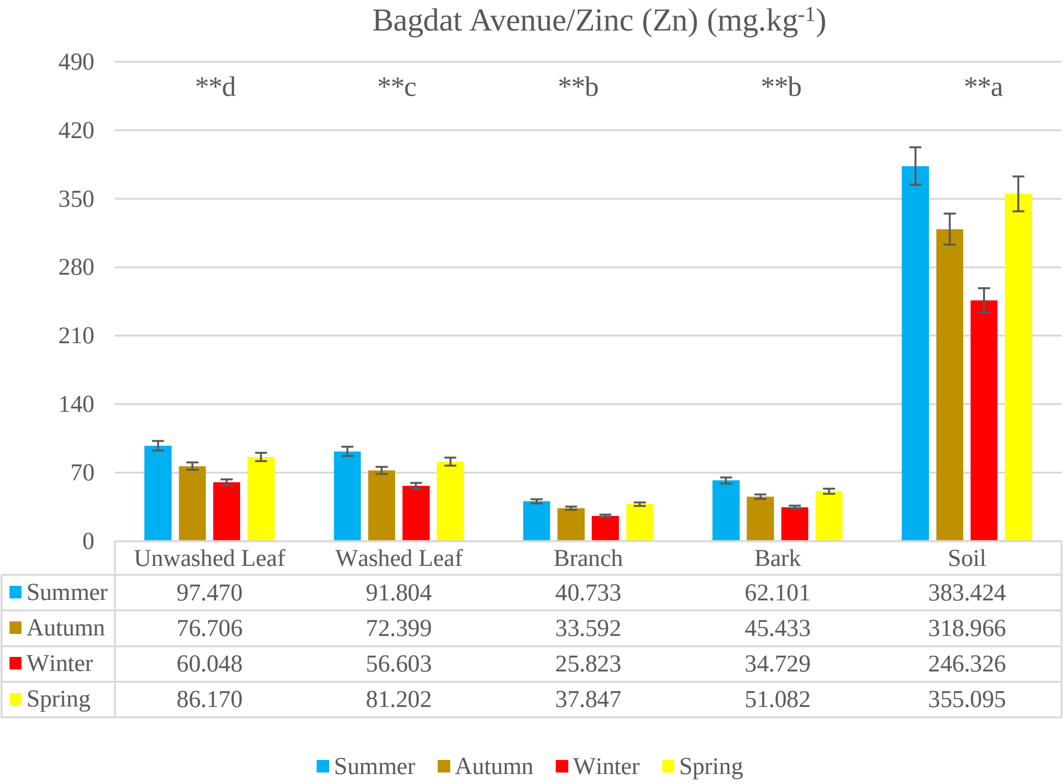
According to our findings, Zn levels in plant samples ranged between 20.207±1.066 (branch/ winter/ Prince Island) and 135.388±3.547 mg.kg^{-1} (unwashed leaves/ summer/ Dilovasi District). Soil Pb contents were determined as between 232.676±6.305 (winter/ Prince Island) and 452.105±12.177 mg.kg^{-1} (summer/ Dilovasi District). Dilovasi District samples contained the highest Zn concentrations both in plant and soil samples while Prince Island samples has the lowest. Zn levels in all samples were detected as higher in summer and lower in winter and plant part Zn levels changed in relation with the increase in soil levels.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	80.392	75.819	31.844	48.109	342.640
Autumn	63.489	59.058	26.702	35.945	300.881
Winter	48.950	45.611	20.207	27.476	232.676
Spring	71.095	66.166	29.878	40.218	335.489

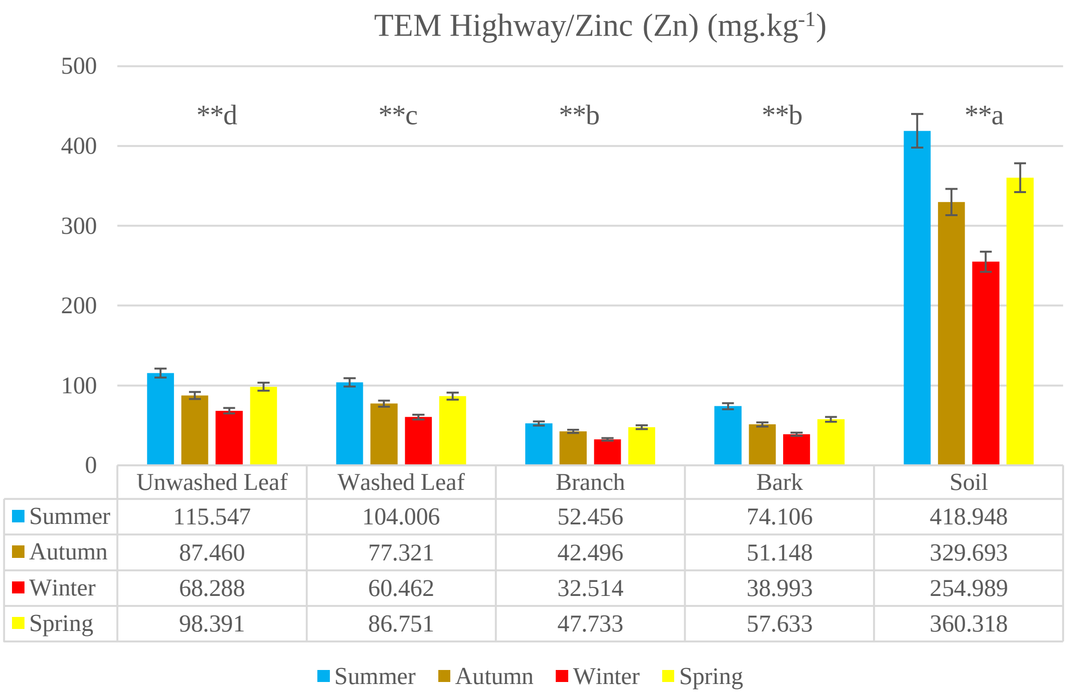
Summer Autumn Winter Spring

Figure 3.73 Average Zn concentrations in Prince Islands.



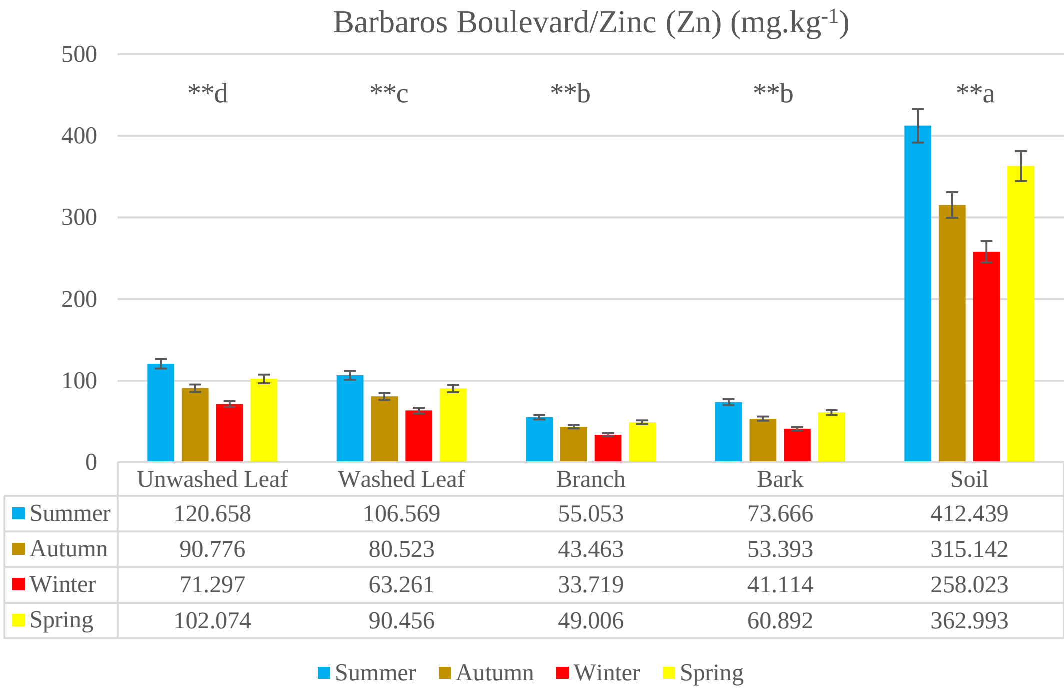
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	97.470	91.804	40.733	62.101	383.424
Autumn	76.706	72.399	33.592	45.433	318.966
Winter	60.048	56.603	25.823	34.729	246.326
Spring	86.170	81.202	37.847	51.082	355.095

Figure 3.74 Average Zn concentrations in Bagdat Avenue



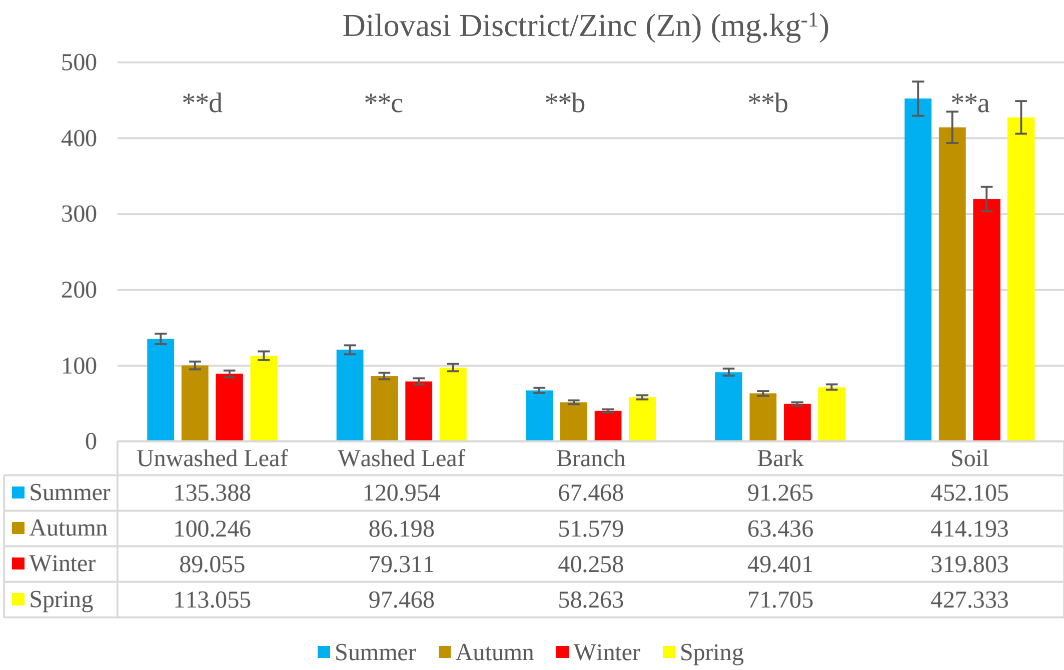
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	115.547	104.006	52.456	74.106	418.948
Autumn	87.460	77.321	42.496	51.148	329.693
Winter	68.288	60.462	32.514	38.993	254.989
Spring	98.391	86.751	47.733	57.633	360.318

Figure 3.75 Average Zn concentrations in TEM Highway.



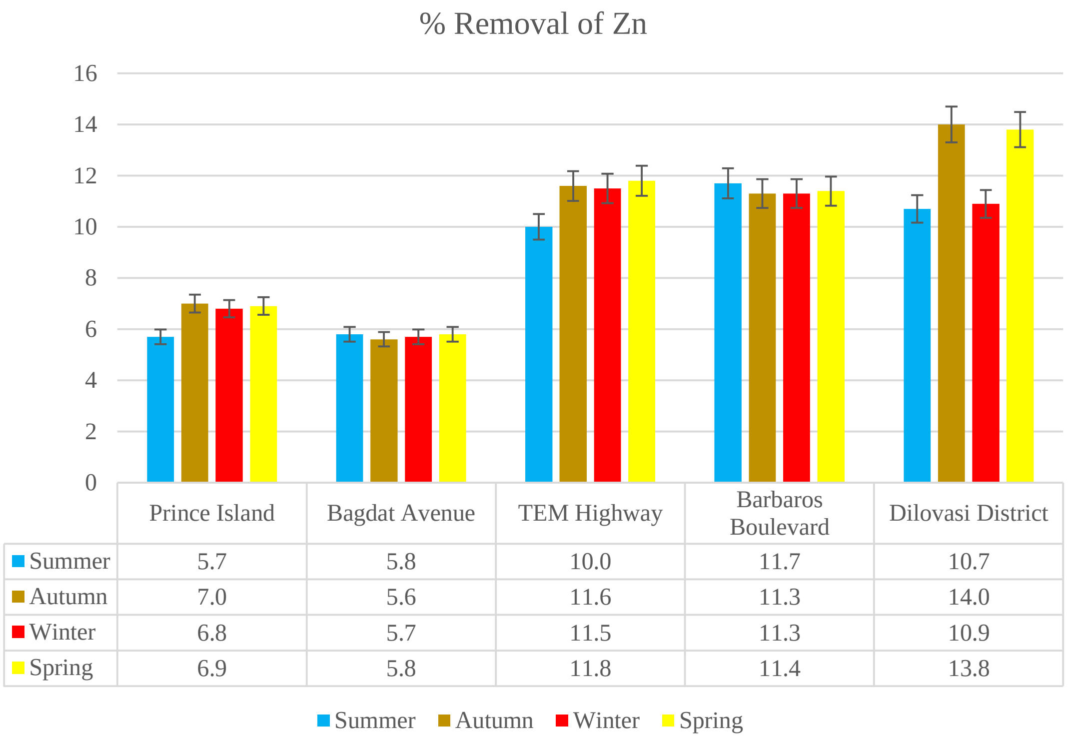
	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	120.658	106.569	55.053	73.666	412.439
Autumn	90.776	80.523	43.463	53.393	315.142
Winter	71.297	63.261	33.719	41.114	258.023
Spring	102.074	90.456	49.006	60.892	362.993

Figure 3.76 Average Zn concentrations in Barbaros Boulevard.



	Unwashed Leaf	Washed Leaf	Branch	Bark	Soil
Summer	135.388	120.954	67.468	91.265	452.105
Autumn	100.246	86.198	51.579	63.436	414.193
Winter	89.055	79.311	40.258	49.401	319.803
Spring	113.055	97.468	58.263	71.705	427.333

Figure 3.77 Average Zn concentrations in Dilovasi District.



	Prince Island	Bagdat Avenue	TEM Highway	Barbaros Boulevard	Dilovasi District
Summer	5.7	5.8	10.0	11.7	10.7
Autumn	7.0	5.6	11.6	11.3	14.0
Winter	6.8	5.7	11.5	11.3	10.9
Spring	6.9	5.8	11.8	11.4	13.8

Figure 3.78 Removal rates of Zn between unwashed and washed leaves

Removal rate of Zn ranged between 5.7% and 11.7% for summer; 5.6% and 14.0% for autumn; 5.7% and 11.5% for winter; 5.8% and 13.8% for spring. Removal rate in terms of stations ranged between 5.7% and 7.9% for Prince Island; 5.6% and 5.8% for Bagdat Avenue; 10.0% and 11.8% for TEM Highway; 11.3% and 11.71% for Barbaros Boulevard; 10.9% and 14.0% for Dilovasi District. The highest removal rate of Pb was detected as 14.0% from Dilovasi Discrict in autumn. The lowest was detected as 5.6% from Bagdat Avenue in autumn. Prince Islands and Bagdat Avenue has lower removal rate of Zn. The other stations have high removal rates of Zn. Adachi and Tainosho (2004) reported that tire and brake dust is an important source of heavy metal emission. Especially tire dust contains and emits high proportions of Zn. As the traffic rate increases, the amount of heavy metal that is emitted to the environment increases. Thus it can be said that emission of Zn is caused by traffic in TEM Highway and Barbaros Boulevard stations. Additionally in Dilovasi Disctrict, Zn emission may be sourced from industrial facilities.

As a micronutrient element, Zn is taken up from soil mainly as a diavalent cation (Zn^{+2}). Average total Zn content in agricultural soils range from 3 to 770 $mg.kg^{-1}$ with an average of 65 mg.kg^{-1} (Alloway, 2009; Storey, 2007). According to the results of this study, Zn content in soil ranged 232.676 to 452.105 mg.kg^{-1}. Thus Zn content in soils of all stations are within the expected range but above the world average.

Table 3.12 Zn levels (L: leaf, UwL:unwashed leaf, WL:washed leaf, B: bark, S: branch)

Reference	Organism	Soil (mg.kg^{-1})		Plant (mg.kg^{-1})		Country/city	Method
		Control	Treatment	Control	Treatment		
Esen et al., (2016)	***Carpinus betulus***	36	65.7-131	28.2 (L)	29.3-41.6 (L)	**TUR/Istanbul**	k0-INAA
	Quercus petraea			22.5 (L)	23.1-35.6 (L)		
	Tilia Argentea			17.7 (L)	26.5-32.2 (L)		
Nadgorska-Socha et al., (2016)	***R. pseudoacacia***	50.23	159.78-1787.40	70.06 (L)	80.16-109.64 (L)	Poland	FAAS
Palowski et al., (2016)	***R. pseudoacacia***	---		29.4-54.2 (L)		Poland	FAAS
				36.6-60.7 (B)			
Tzvetkova and Petkova (2015)	***R. pseudoacacia***	---		23.2 (L) June	30.0 (L) June	Bulgaria	AAS
				13.8 (L) Sept.	19.0 (L) Sept		
Vural (2013)	***R. pseudoacacia***	76-477		8.58-47.0 (S)		**TUR/Gumushane**	ICP-AES
Serbula et al., (2012)	***R. pseudoacacia***	130.7	130.1-330.1	43.1 (WL)	32.0-100.3 (WL)	Serbia	ICP-AES / AAS
				81 (UwL)	31.6-192.7 (UwL)		
				109.9 (B)	110.1-4699.8 (B)		
Guney et al., (2010)	---	255 (Surface Soil)		---		**TUR/Istanbul**	Flame AAS
		211 (20-cm depth soil)					
Jensen et al., (2010)	***R. pseudoacacia***	---		51.7 (L)	46.6 (L)	USA	ICP-MS
Yener and Yarci (2010)	***Alcea pallida***	34.869-118.821		21.467-56.300 (L)		**TUR/Istanbul**	AAS
				11.567-47.767 (S)			
Rahmonov (2009)	***R. pseudoacacia***	---		200 (L)		Poland	FAAS
				96 (B)			
				116 (S)			
Samecka-Cymerman et al., (2009)	***R. pseudoacacia***	61-70	132-381	25-30 (L)	33-95 (L)	Poland	ICP-MS
				12-15 (B)	41-115 (B)		
Tabari and Salehi (2009)	***R. pseudoacacia***	148.77	99.77	30.62 (L)	20.62 (L)	Iran	AAS
Celik et al., (2005)	***R. pseudoacacia***	10.670	81.23-506.43	13.02 (UwL)	33.20-139.0 (UwL)	**TUR/Denizli**	Flame AAS
				11.53 (WL)	21.01-53.05 (WL)		
Mertens et al., (2004)	***R. pseudoacacia***	358 (Sediment)		45 (L)		Belgium	Flame AAS
Aksoy et al., (2000)	***R. pseudoacacia***	63 (Rural)	106-1189	21 (UwL)	35-242 (UwL)	**TUR/Kayseri**	AAS
				19 (WL)	26-98 (WL)		

Average Zn contents in plant parts range from 27 to 150 $mg.kg^{-1}$ and therewithal 100 - 400 $mg.kg^{-1}$ of Zn content can be excessive or toxic for different plants (Kabata-Pendias and Pendias, 2001). According to results of this study, Zn content in plant parts were detected between 20.207 and 135.388 $mg.kg^{-1}$ thereby it can be said that *R. pseudoacacia* plants have acquired Zn from its soil sufficiently and within the expected range.

Detected Zn content in soil and plant parts in some other studies shown at Table 3.12. Soil Zn content in study of Nadgorska-Socha et al., (2016), Vural (2013), Celik et al., (2005) and Aksoy et al., (2000) were detected higher than soil Zn content of this study. When considering Zn contents in plant parts, results of Serbula et al., (2012), Rahmonov (2009), Celik et al., (2005) and Aksoy et al., (2000) were higher than the results of this study. According to these results, it can be proposed that *R. pseudoacacia* plants growing on different ecological conditions have accumulated Zn in different amounts.

3.1.15 Statistical Analysis

Table 2.13 Results of Repeated Measures Multivariate Tests

Multivariate Tests[a]							
Effect			**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**
Between Subjects	**Intercept**	Pillai's Trace	1.000	355872,075[b]	13.000	163.000	0.000
		Wilks' Lambda	.000	355872,075[b]	13.000	163.000	0.000
		Hotelling's Trace	28382.435	355872,075[b]	13.000	163.000	0.000
		Roy's Largest Root	28382.435	355872,075[b]	13.000	163.000	0.000
	Locality	Pillai's Trace	3.889	449.296	52.000	664.000	0.000
		Wilks' Lambda	.000	3801.577	52.000	633.407	0.000
		Hotelling's Trace	6376.298	19803.310	52.000	646.000	0.000
		Roy's Largest Root	5560.385	71001,836[c]	13.000	166.000	.000
	Plant Part	Pillai's Trace	3.485	86.368	52.000	664.000	.000
		Wilks' Lambda	.000	1039.136	52.000	633.407	0.000
		Hotelling's Trace	7157.583	22229.800	52.000	646.000	0.000
		Roy's Largest Root	6916.691	88320,829[c]	13.000	166.000	0.000
	Locality * Plant Part	Pillai's Trace	8.202	18.699	208.000	2275.000	0.000
		Wilks' Lambda	.000	84.131	208.000	1683.205	0.000
		Hotelling's Trace	478.045	370.379	208.000	2095.000	0.000
		Roy's Largest Root	264.670	2894,833[c]	16.000	175.000	.000
Within Subjects	**Seasons**	Pillai's Trace	1.000	20973,680[b]	39.000	137.000	.000
		Wilks' Lambda	.000	20973,680[b]	39.000	137.000	.000
		Hotelling's Trace	5970.610	20973,680[b]	39.000	137.000	.000
		Roy's Largest Root	5970.610	20973,680[b]	39.000	137.000	.000
	Seasons * Locality	Pillai's Trace	3.628	35.023	156.000	560.000	.000

	Wilks' Lambda	.000	246.167	156.000	548.690	0.000
	Hotelling's Trace	1481.260	1286.607	156.000	542.000	0.000
	Roy's Largest Root	1269.077	4555,661[c]	39.000	140.000	.000
Seasons * Plant Parts	Pillai's Trace	3.250	15.563	156.000	560.000	.000
	Wilks' Lambda	.000	95.266	156.000	548.690	0.000
	Hotelling's Trace	1162.460	1009.701	156.000	542.000	0.000
	Roy's Largest Root	1100.479	3950,437[c]	39.000	140.000	.000
Seasons* Locality * Plant Part	Pillai's Trace	8.597	4.526	624.000	2432.000	.000
	Wilks' Lambda	.000	12.360	624.000	2105.229	0.000
	Hotelling's Trace	300.929	65.165	624.000	2162.000	0.000
	Roy's Largest Root	191.409	746,005[c]	39.000	152.000	.000

a. Design: Intercept + locality + plantpart + locality * plantpart
Within Subjects Design: Season

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table 3.14 Pearson Correlation Matrix (R) scores.

	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Zn
B	.132**	-.184**	.003	-,109**	,431**	**,763****	**,926****	.361**	**.550****	.251**	-.006	.437**
Ca		**.510****	**.563****	**.683****	.328**	-.185**	.101**	.434**	.107**	.307**	**.638****	.249**
Cd			**.832****	**.918****	.319**	-.366**	-.195**	.470**	.102**	**.610****	**.913****	.361**
Cr				**.828****	**.713****	-.124**	.087*	**.822****	.479**	**.833****	**.860****	**.709****
Cu					.305**	-.406**	-.146**	.466**	.066	**.591****	**.956****	.342**
Fe						.436**	**.597****	**.975****	**.883****	**.787****	.408**	**.923****
K							**.844****	.334**	**.657****	.264**	-.310**	**.511****
Mg								**.519****	**.700****	.362**	-.039	**.584****
Mn									**.825****	**.858****	**.553****	**.928****
Na										**.719****	.193**	**.924****
Ni											**.677****	**.904****
Pb												.446**

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

Repeated Measures MANOVA test results are tabulated in Table 3.13. The results were obtained from analysis of plant parts and soil samples collected from same trees for 5 locations separately for all 4 seasons. All comparisons values difference is significant at the 0.01 (p) level. Homogeneous subsets (TUKEY HSD) results are also shown on the graphs. At the same time, all of these values Pearson Correlation and matrix results are shown in Table 3.14. When these results are examined; significant for B; Positive correlation between K, Mg, and Na (>0.55, >0.93), for Ca; Positive correlation between Cd, Cr, Cu, and Pb (>0.51, >0.68), for Cd; Positive correlation between Cr, Cu, Ni, and Pb (>0.61, >0.92), for Cr; Positive correlation between Cu, Fe, Mn, Ni, Pb, and Zn (>0.71, >0.86), for Cu; Positive correlation between Ni and Pb (>0.59, >0.96), for Fe; Positive correlation between Mg, Mn, Na, Ni, and Zn (>0.60, >0.98), for K; Positive correlation between Mg, Na, and Zn (>0.51, >0.84), for Mg; Positive correlation between Mn, Na, and Zn (>0.52, >0.70), for Mn; Positive correlation between Na, Ni, Pb, and Zn (>0.55, >0.93), for Na; Positive correlation between Ni and Zn (>0.72, >0.92), And finally for Ni; Positive correlation between Pb and Zn (>0.68, >0.90) have been identified.

3.2 Photosynthetic Pigment Analysis

Results of photosynthetic pigments analyzes are shown in Table 3.15. According to results photosynthetic contents ranged between 0.411 and 0.473 for *Ca*, 0.220 and 0.633 for *Cb*, 0.631 and 1.064 for Total *C*, 0.222 and 0.304 for *Cx+c* and 0.746 and 2.194 for *Ca*/*Cb*.

Table 3.15 Concentrations of *Cl* a, *Cl* b Total *Cl*, *Cx+x*, and *Cl* a/*Cl* b with statistics.

Station	Season	*Ca*	*Cb*	Total *C*	*Cx+c*	*C* a/*C* b
Prince Island	Summer	0.439^{**a}	0.527^{*a}	0.966^{*a}	0.240^{**ab}	0.867^{b}
	Autumn	0.434^{**b}	0.414^{*c}	0.848^{*c}	0.256^{**b}	1.356^{a}
	Spring	0.460^{**a}	0.357^{*b}	0.817^{*b}	0.234^{**a}	1.357^{b}
Bagdat Avenue	Summer	0.432^{**a}	0.633^{*a}	1.064^{*a}	0.239^{**ab}	0.746^{b}
	Autumn	0.422^{**b}	0.353^{*c}	0.775^{*c}	0.233^{**b}	1.579^{a}
	Spring	0.456^{**a}	0.528^{*b}	0.983^{*b}	0.304^{**a}	0.912^{b}
Barbaros Boulevard	Summer	0.439^{**a}	0.579^{*a}	1.017^{*a}	0.252^{**ab}	0.798^{b}
	Autumn	0.462^{**b}	0.343^{*c}	0.805^{*c}	0.262^{**b}	1.742^{a}
	Spring	0.429^{**a}	0.311^{*b}	0.739^{*b}	0.225^{**a}	1.440^{b}
TEM Highway	Summer	0.473^{**a}	0.440^{*a}	0.913^{*a}	0.263^{**ab}	1.146^{b}
	Autumn	0.411^{**b}	0.220^{*c}	0.631^{*c}	0.222^{**b}	2.194^{a}
	Spring	0.459^{**a}	0.399^{*b}	0.857^{*b}	0.245^{**a}	1.259^{b}
Dilovası District	Summer	0.439^{**a}	0.460^{*a}	0.899^{*a}	0.236^{**ab}	1.191^{b}
	Autumn	0.417^{**b}	0.253^{*c}	0.670^{*c}	0.229^{**b}	1.892^{a}
	Spring	0.466^{**a}	0.392^{*b}	0.858^{*b}	0.257^{**a}	1.232^{b}

Cl a: chlorophyll a, *Cl* b: chlorophyll a, Total *Cl*: Total chlorophyll, *Cx+c*: total caretinoid and *Cl* a/*Cl* b: Ratio of chlorophyll *a* to chlorophyll *b*. The mean difference is significant at **P<0.01 and *P<0.05 level by the Tukey's test and multivariate analysis of variance (MANOVA).

The highest *Ca* contents were detected at spring in Prince Island, Bagdat Avenue and Dilovasi Distcrict stations, and detected at autumn in Barbaros Boulevard and at summer in TEM Highway stations. The lowest *Cl* a contents were detected at autumn in Prince Island, Bagdat avenue, TEM Highway and Dilovasi Distcrict stations whereas at spring in Barbaros Boulevard station. Altough the lowest and highest values were determined at different seasons, the *Cl* a changed in the same narrow range in all stations. The highest *Cl* b contents were detected at summer in all stations, while the lowest content detected at spring in Prince Island and Barbaros stations and at spring in others. In contrast to *Cl* a content, *Cl* b value changed in a wider range. *Cx+c* content also changed in a narrow range like *Ca*.

Statistical analysis showed that, all the photosynthetic pigments changed in the same pattern in term of season in all stations. The *Ca* content detected in autumn was included in different homogeneous subset. It can be suggested that, *Ca* changed in a different pattern due to the physiological changes at autumn. *Cb* content changed independently at all seasons. *Cx+c* changes were found in different homogeneous subsets in spring and autumn and close to these homogeneous subsets in summer.

It can be said that changes at photosynthetic pigment contents occurred at the same pattern for all sations. Thus photosynthetic pigments of *R. pseudoacacia* plants are limitedly affected by industrial and heavy traffic pollution.

3.3 Results of Total Protein analysis

Total protein analyses were conducted for summer, autumn and spring seasons. Total protein content was detected as 27.237 mg/ml at spring in Prince Island and 78.190 mg/ml at autumn in Bagdat Avenue (Table 3.16). Total protein contents of *R. pseudoacacia* plants changed at same pattern in relation with season in all stations. The highest protein content was detected at autumn while the lowest was detected at spring. The highest protein content at autumn may be related to the physiological activities of the plant and there are not any significant changes in total protein levels between stations. Thus it can be suggested that changes at total protein contents of R. *pseudoacacia* plants occurred independently from their environmental conditions.

Table 3.16 Total protein contents with statistics.

Station	Summer	Autumn	Spring
Prince Island	45.688	61.907^{**ab}	27.237^{*b}
Bagdat Avenue	45.744	78.190^{**a}	32.493^{*ab}
Barbaros Boulevard	43.700	46.479^{**b}	35.021^{*ab}
TEM Highway	52.189	70.052^{**a}	33.038^{*ab}
Dilovası District	47.749	72.132^{**a}	42.159^{*a}

Statistical analyses such as one way analyses of variance (ANOVA) with Tukey's post-hoc HSD were performed. The mean differences are significant at $p<0.01$ (**) and $p<0.05$ (*) levels.

Results of statistical analysis revealed that, during the autumn period total protein contents of Barbaros Boulevard samples were separated into different homogeneous subset from other stations. Whereas in spring, total protein contents of Prince Island samples and Dilovasi District samples were separated from each other. These two stations were exposed to different environmental conditions. Thus this difference may be an outcome of different environmental effects on the plants. Ozyigit et. al. (2014) reported that total protein content of plants changes in relation with the heavy metal pollution as total protein content increase firstly at low levels. If heavy metal exposure on the plant increases, total protein content of plant usually decreases. Higher level of TP in Dilovasi Plants at spring may be an indicator of a high level of pollution.

3.4 Genetic Analyses

In this study, ITS1 and *trnL-trnF* intergenic spacer sequences and ISSR band data were used for analyzing phylogenetic relationships and genetic diversity analyses, respectively for *R. pseudoacacia* genotypes.

3.4.1 ISSR data

After the ISSR-PCR reactions, obtained DNA fragments were migrated in agarose gel and band profiles were scored as 1 for presence and 0 for absence. Only visible and distinguishable bands were chosen and scored. ISSR analyses conducted for 11 genotypes from Prince Islands, 12 genotypes from Bagdad Avenue, 12 genotypes from Barbaros Boulevard, 10 genotypes from Dilovasi District, 13 genotypes from TEM Highway and all genotypes at once as a single group. 15 ISSR primers were applied and 9 ISSR primers gave results with visible and distinguishable bands (Table 3.15). Total 100 loci were obtained from nine primers. These loci ranged in size from 200 to 1800 bp with an average of 11.1 bands formed per primer.

Table 3.17 Details of nine ISSR primers used in this study, including primer name, amplicon size, band numbers and polymorphism ratio

No	Primer	Name	Amplicon Size (bp)	Total number of			(n=58) Polymorphism (%)
				Amplified bands	Monomorphic Bands	Polymorphic Bands	
1	ISSR 2	UBC811	200-1100	11	0	11	100%
2	ISSR 4	UBC818	200-1100	13	0	13	
3	ISSR 5	UBC820	300-1200	8	0	8	
4	ISSR 6	UBC823	300-1200	13	0	13	
5	ISSR 7	UBC827	200-1100	10	0	10	
6	ISSR 8	UBC825	250-1800	12	0	12	
7	ISSR 10	UBC849	200-1200	12	0	12	
8	ISSR 11	UBC855	300-1200	10	0	10	
9	ISSR 12	UBC842	300-1000	11	0	11	
Total number of amplified bands:				**100**	**0**	**100**	

Table 3.18 Summary of genetic variation statistics for all loci by using diploid ISSR data set with Popgene 32 software

(n=58)	PPL (%)	na	ne	h	I	Ht	Hs	Gst
Mean	**100**	**2.0000**	**1.5325**	**0.3169**	**0.4811**	**0.3169**	**0.2220**	**0.2993**
Highest		2.0000	1.9999	0.5000	0.6931	0.4998	0.4624	0.9464
Lowest		-	1.0178	0.0515	0.1221	0.0156	0.0151	0.0181

PPL: percentage of polymorphic loci, na: Observed number of alleles, ne: Effective number of alleles [Kimura and Crow (1964)], h: Nei's (1973) gene diversity, I: Shannon's information index [Lewontin (1972)], Ht: Total gene diversity, Hs: Within population gene diversity, Gst: genetic differentiation coefficient, n: Number of genotypes.

The rate of percentage of polymorphic loci (PPL) and effective number of alleles were observed for overall as 100% and 1.5325, respectively and shown in Table 3.16. Nei's (1973) gene diversity (h) ranged from 0.0515 to 0.5000 with an average of 0.3169. Shannon's information index (I) ranged from 0.1221 to 0.6931 with an average of 0.4811. Total gene diversity (Ht), within population gene diversity (Hs) and genetic differentiation coefficient (Gst) mean values were calculated as 0.3169, 0.2220 and 0.2993, respectively.

Table 3.19 Single-Population Descriptive Statistics by using Diploid ISSR Data Set with Popgene 32 software

Prince Islands					
(n=11)	**PPL (%)**	**na**	**ne**	**h**	**I**
Mean	**73**	**1.7300**	**1.4186**	**0.2454**	**0.3693**
Highest		2.0000	1.9576	0.4990	0.6922
Lowest		1.0000	1.0000	0.0000	0.0000
Bagdad Avenue					
(n=12)	**PPL (%)**	**na**	**ne**	**h**	**I**
Mean	**58**	**1.5800**	**1.3796**	**0.2166**	**0.3200**
Highest		2.0000	2.0000	0.5000	0.6931
Lowest		1.0000	1.0000	0.0000	0.0000
Barbaros Boulevard					
(n=12)	**PPL (%)**	**na**	**ne**	**h**	**I**
Mean	**46**	**1.4600**	**1.2873**	**0.1654**	**0.2454**
Highest		2.0000	2.0000	0.5000	0.6931
Lowest		1.0000	1.0000	0.0000	0.0000
Dilovasi District					
(n=10)	**PPL (%)**	**na**	**ne**	**h**	**I**
Mean	**76**	**1.7600**	**1.4192**	**0.2511**	**0.3809**
Highest		2.0000	1.9819	0.4954	0.6886
Lowest		1.0000	1.0000	0.0000	0.0000
TEM Highway					
(n=13)	**PPL (%)**	**na**	**ne**	**h**	**I**
Mean	**59**	**1.5900**	**1.4122**	**0.2317**	**0.3388**
Highest		2.0000	1.9969	0.4992	0.6924
Lowest		1.0000	1.0000	0.0000	0.0000

PPL: percentage of polymorphic loci, na: Observed number of alleles, ne: Effective number of alleles [Kimura and Crow (1964)], h: Nei's (1973) gene diversity, I: Shannon's information index [Lewontin (1972)], n: Number of genotypes.

The rate of PPL was observed as 73% for Prince Island, 58% for Bagdad Avenue, 46% for Barbaros Boulevard, 76% for Dilovasi District and %59 for TEM Highway. According to results, the highest genetic diversity was calculated for Dilovasi District and the lowest for Barbaros Boulevard (Table 3.17). From single-population descriptive statistics, h and I values were calculated for Prince Islands, Bagdad Avenue, Barbaros Boulevard, Dilovasi District and TEM Highway as 0.2454, 0.2166, 0.1654, 0.2511 and 0.2317, respectively for h;. 0.3693, 0.3200, 0.2454, 0.3809 and 0.3388, respectively for I.

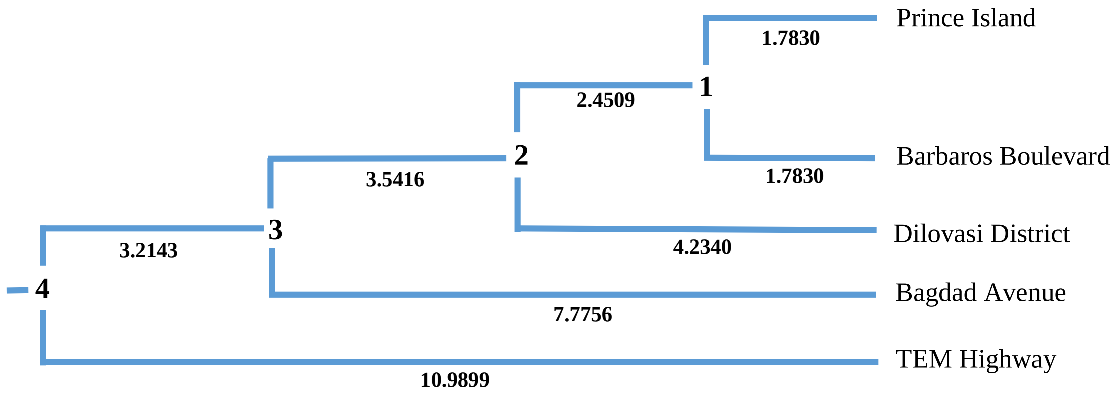


Figure 3.79 Dendrogram Based on Nei's (1978) Genetic distance by using UPGMA method with Popgene 32 software. The values on tree branches indicate the genetic distance of plant groups.

For revealing genetic distance a dendrogram constructed according to Nei's (1978) genetic distance by using unweighted pair group method with arithmetic mean (UPGMA) method in Popgene 32 software. The dendrogram is shown at Figure 3.81. Genetic distance values ranged from 1.7830 to 10.9899. According to our results, Prince Island and Barbaros Boulevard genotypes were found as genetically closest groups with 1.7830 genetic distance value. The most distant group is determined as TEM Highway genotypes with 10.9899 genetic distance value.

Table 3.20 Nei's unbiased measures of genetic identity and genetic distance (Nei 1978) [Nei's genetic identity (above diagonal) and genetic distance (below diagonal)]

Genotype Groups	Prince Islands	Bagdad Avenue	Barbaros Boulevard	Dilovasi District	TEM Highway
Prince Islands	******	0.8856	0.9650	0.9304	0.8155
Bagdad Avenue	0.1215	******	0.9032	0.7840	0.8326
Barbaros Boulevard	0.0357	0.1018	******	0.9074	0.7976
Dilovasi District	0.0722	0.2433	0.0972	******	0.7665
TEM Highway	0.2040	0.1833	0.2261	0.2659	******

The lowest and highest Nei's unbiased measures of genetic distance values ranged between 0.0357 (between Prince Island and Barbaros Boulevard) and 0.2659 (between

TEM Highway and Dilovasi District). Genetic Identity values ranged between 0.7665 (between TEM Highway and Dilovasi District) and 0.9650 (between Prince Island and Barbaros Boulevard) in agreement with genetic distance. Genetic distance can be defined as genomic diversification among populations or species and is measured by using some mathematical methods (Nei, 1987). When genetic distance value is low, it can be considered that subject genotypes or taxa are closely related. It can be suggested that, Prince Island and Barbaros Boulevard genotypes may be the most genetically similar groups. Conversely,TEM Highway and Dilovasi District were determined as the most diverse group.

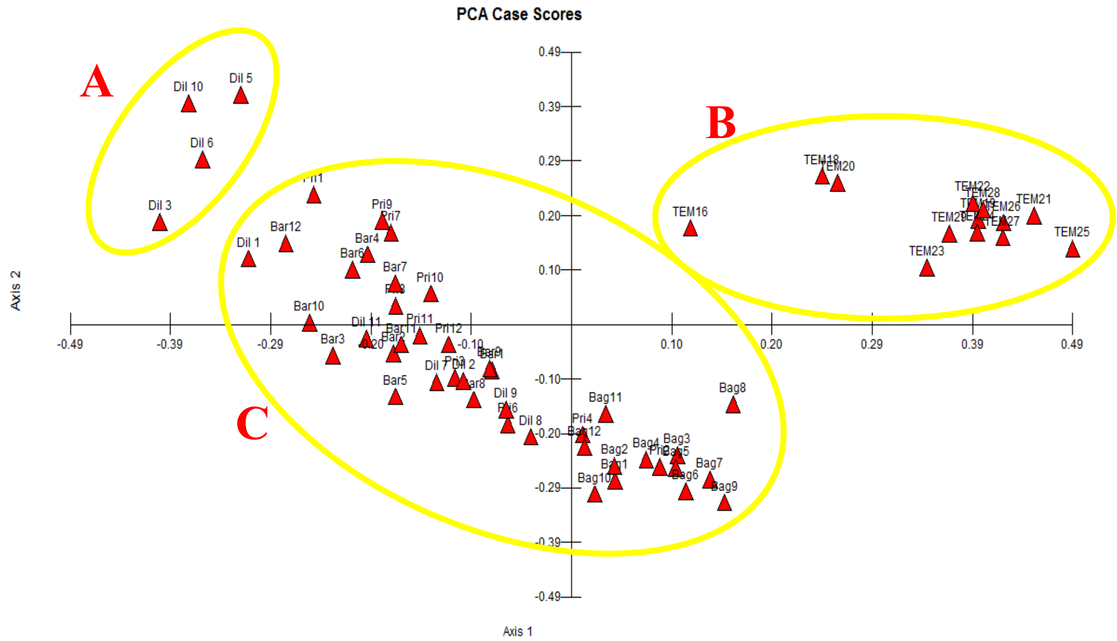


Figure 3.80 Principal Component Analysis (PCA) of *R. pseudoacacia* genotypes generated by using MVSP 3.22. The red triangles and yellow circles show the plant genotypes and major groups, respectively. The numbers on the axis1 and 2 indicate PCA scores.

Based on PCA case scores data, three major clusters were found and named as A, B and C (Figure 3.82). The group A consisted of only four genotypes from Dilovasi District. While all genotypes of TEM Highway clustered in group B, group C showed the mixture of other genotypes from other stations. Although most of Dilovasi genotypes were found in group C, some members were detected in group A, suggesting that environmental factors could be the cause of genomic alterations which resulted in divergence of these genotypes. In addition isolated group B which included all TEM genotypes can be explained by vegetative propagation of these plants by municipality activities.

3.4.2 Phylogenetic analyses

ITS1 and *trnL-trnF* intergenic spacer sequences were employed for revealing phylogenetic relationships in this study.

3.4.2.1 Internal transcribed spacer 1 (ITS1)

After PCR reactions, amplicons were migrated in 1.2% agarose gel and generated single bands in size of 250-350 bp long. After the sequencing process, raw sequences were aligned and edited. Length of edited sequenced were 239 bp and only included ITS1 sequence. Images of migrated bands are shown in Figure 3.83.

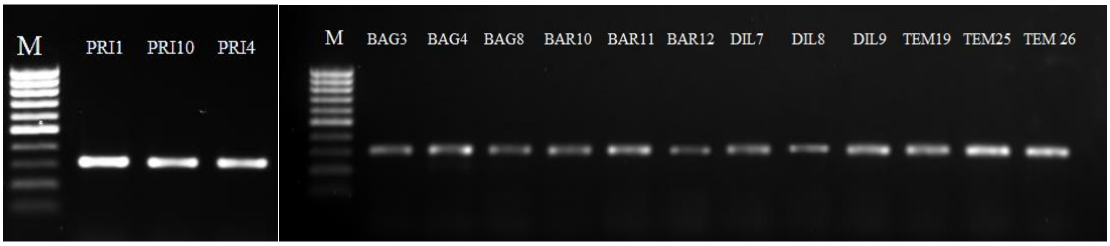


Figure 3.81 ITS1 amplicons in agarose gel. (M: 100-1000bp DNA ladder, the symbols on the wells show the genotype's names)

Sequences were submitted to NCBI GenBank database and accession numbers were acquired, which are shown in Table 3.19.

Table 3.21 NCBI GenBank accession numbers and some charasteristics of ITS1 sequences obtained from this study.

Genotype	Sequence Name	Length of sequences	GC Content (%)	NCBI GenBank Accession Number
Prince Island 1	PRI1_ITS1 (ADA1)	239	53.97	KY311818
Prince Island 10	PRI10_ITS (ADA10)		53.55	KY311819
Prince Island 4	PRI4_ITS1 (ADA4)		53.13	KY311820
Bagdad Avenue 3	BAG3_ITS1		53.56	KY311821
Bagdad Avenue 4	BAG4_ITS1		52.72	KY311822
Bagdad Avenue 8	BAG8_ITS1		53.13	KY311823
Barbaros Boulevard 10	BAR10_ITS1		53.14	KY311824
Barbaros Boulevard 11	BAR11_ITS1		53.56	KY311825
Barbaros Boulevard 12	BAR12_ITS1		53.97	KY311826
Dilovasi District 7	DIL7_ITS1		53.56	KY311827
Dilovasi District 8	DIL8_ITS1		56.56	KY311828
Dilovasi District 9	DIL9_ITS1		53.14	KY311829
TEM Highway 19	TEM19_ITS1		53.14	KY311830
TEM Highway 25	TEM25_ITS1		53.14	KY311831
TEM Highway 26	TEM26_ITS1		53.14	KY311832

ITS1 sequences were searched in nucleotide collection of NCBI to find and compare similar sequences by using NCBI MegaBlastn Suite. Top three similar sequences in results of MegaBlastn are shown in table 3.22.

Table 3.22 Details of top three ITS1 sequences similar to *R. pseudoacacia* genotypes in this study. The sequences retrieved from NCBI GenBank database.

Our Sequences	Similar Sequence retrieved from NCBI GenBank				
	Organism	Family	Accession Number	Cover (%)	Identity (%)
Prince Island 1 KY311818	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	92
			KU193707	99	92
Prince Island 10 KY311819	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	93
			KU193707	99	93
Prince Island 4 KY311820	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			AF174637	99	93
			KU193707	99	92
Bagdat 3 KY311821	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	93
			KU193707	99	93
Bagdat 4 KY311822	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	93
Bagdat 8 KY311823	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92
Barbaros 10 KY311824	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92
Barbaros 11 KY311825	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	92
			JQ007359	99	92
			AF174637	99	92
Barbaros 12 KY311826	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	93
			KU193707	99	93
Dilovasi 7 KY311827	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	93
			KU193707	99	93
Dilovasi 8 KY311828	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92
Dilovasi 9KY311829	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92
TEM 19 KY311830	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92
TEM 25 KY311831	*R. Pseudoacacia*	*Fabaceae*	JQ007359	99	93
			AF174637	99	93
			KU193707	99	93
TEM 26 KY311832	*R. Pseudoacacia*	*Fabaceae*	JQ007413	99	93
			JQ007359	99	93
			AF174637	99	92

Phylogenetic tree (Figure 3.84) was constructed by using ITS1 sequences obtained from this study. According to our results, two major groups (A and B) were observed and one genotype (Bagdat4) was separated distinctively from others.

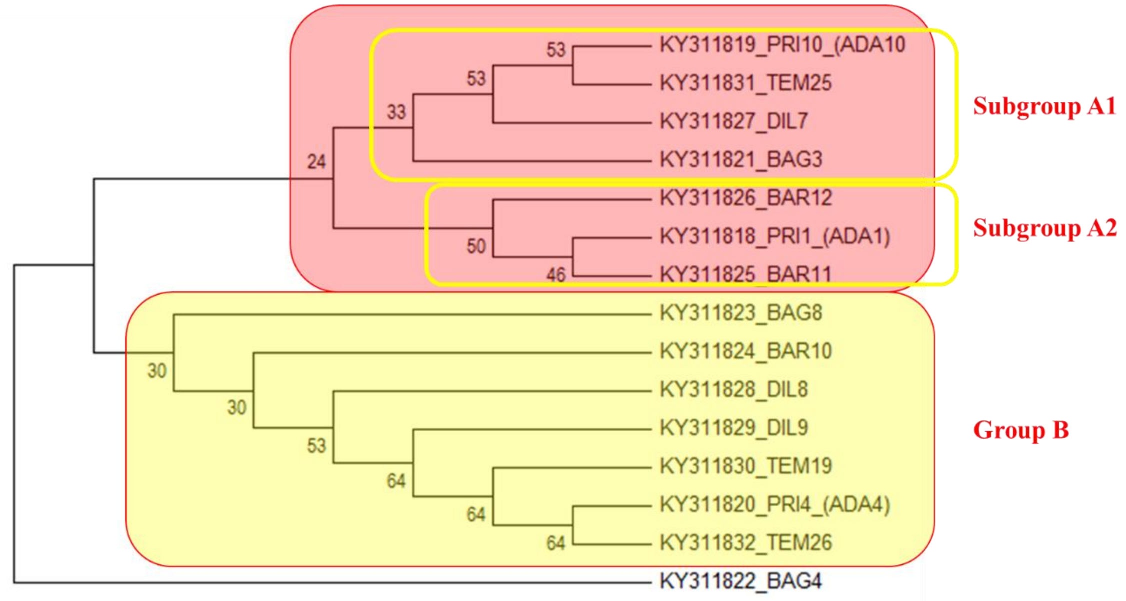


Figure 3.82 Phylogenetic distribution of *R. pseudoacacia* ITS1 sequences. Phylogeny was constructed by MEGA6 using Maximum likelihood (ML) method for 1000 bootstraps.

Members of group A were also separated into two subgroups named as A1 and A2. The genotypes were homogeneously dispersed into the groups. Subgroup A2 was observed as the most similar as subgroup A1. These differences between subgroups A1 and A2 can be explained by the genomic rearrangement and variations in their genome by genomic events. Although coverage and identity values were found to be high, lower bootstrap values in phylogenetic tree may prove the weak phylogenetic relationship. These results could be related with the genomic variabilities in our genotypes.

The joining tree (Figure 3.85) was constructed by using both ITS1 sequences obtained from this study and retrieved from NCBI GenBank database. The tree was constructed to reveal phylogenetic relationships among *R. pseudoacacia* and same/other species at intraspecific level by using ML method.

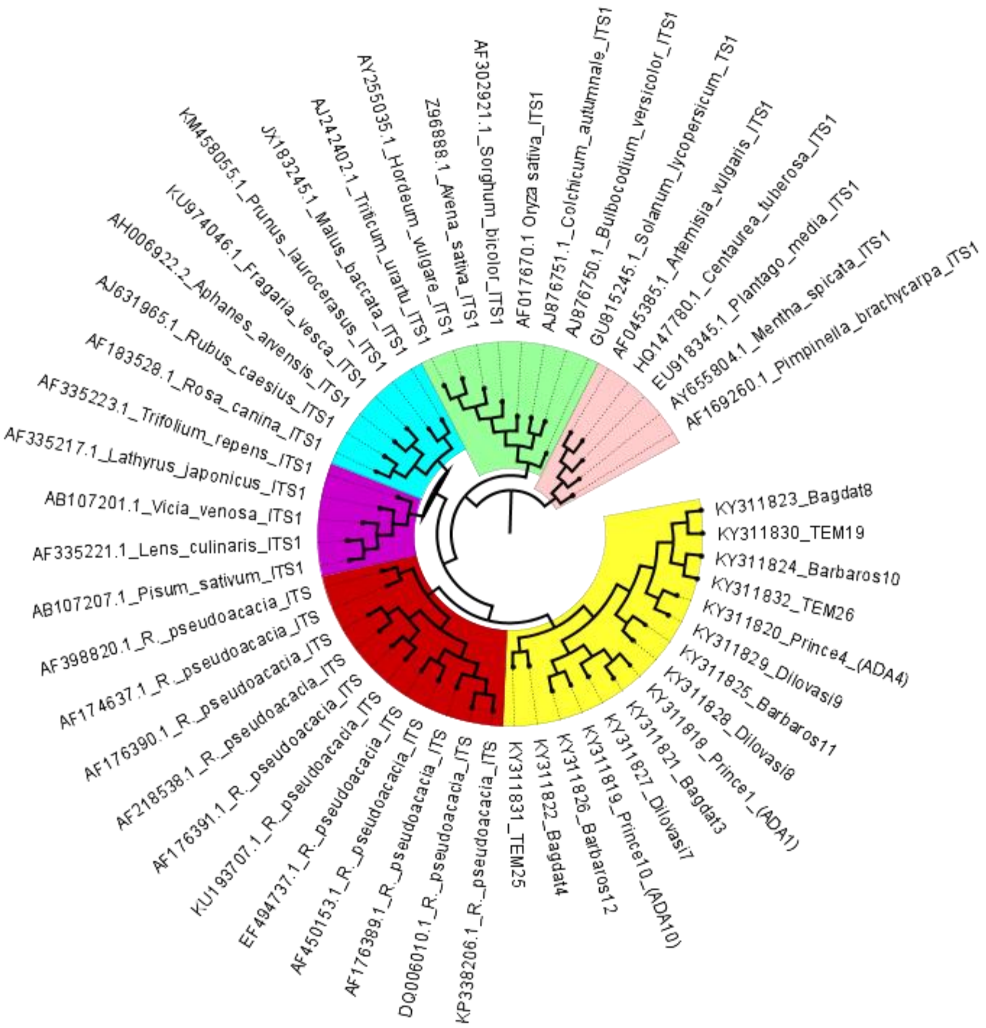


Figure 3.83 The joining phylogenetic tree of ITS and ITS1 sequences with *R. pseudoacacia* and other plant species retrieved from NCBI GenBank database. Yellow cluster shows the obtained ITS1 sequences from this study.

According to phylogenetic tree, first main group is formed from studied genotypes of this study (yellow) and other *R. pseudoacacia* plants retrieved from GenBank (red). Second major group consisted members of *Fabaceae* (purple) and *Rosaceae* family (turquoise). Members of other taxa formed a third major group.

Sequencing of ITS region is one of the most popular DNA barcoding techniques and phylogenetic markers, and its widely used in analyzing phylogenetic relationships of different taxa (Porras-Alfaro et al., 2014). In this study, ITS1 region revealed the phylogenetic relationships of *R. pseudoacacia* plants with its relatives and other taxa.

Genotypes of this study and other *R. pseudoacacia* genotypes retrieved from NCBI GenBank database were clustered together and showed closest phylogenetic relationship. Also members of *Fabaceae* family were located on near branch to *R. pseudoacacia* genotypes in joining tree. This situation may be due to the high conservation of ITS1 region in *R. pseudoacacia* and its relatives. Additionally, it can be said that ITS1 region can be used as a marker to distinguish intraspecific levels.

3.4.2.2 *trnL-trnF* intergenic spacer

trnL-trnF intergenic spacer region of cpDNA is second phylogenetic marker used in this study for revealing phylogenetic relationships. Previous procedures were applied for *trnL-trnF* intergenic spacer region as applied for ITS1. *trnL-trnF* intergenic spacer DNA regions were amplificated and 450-500 bp long amplicons were obtained. The bands formed by the *trnL-trnF* intergenic spacer amplicons in the agarose gel are shown in the Figure 3.85.

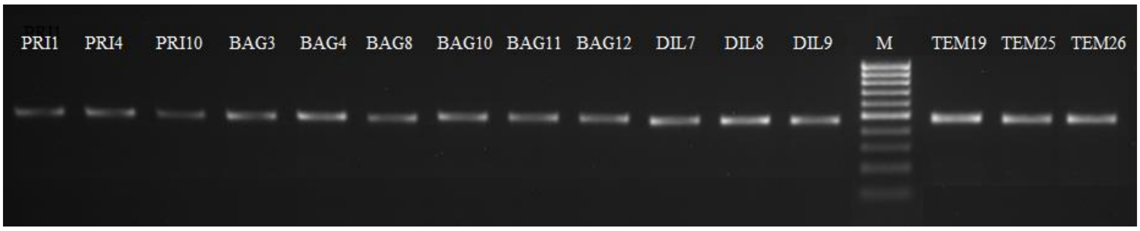


Figure 3.84 *trnL-trnF* intergenic spacer amplicons in agarose gel. (M: 100-1000bp DNA ladder, the symbols on the wells show the genotype’s names)

After the sequencing process, the raw sequences edited and final sequences submitted to NCBI GenBank database. GenBank accession numbers and some features of *trnL-trnF* intergenic spacer obtained in this study are shown in Table 3.23. Length of *trnL-trnF* intergenic spacer sequences were 453 bp.

Table 3.23 NCBI GenBank accession numbers and some charasteristics of *trnL-trnF* intergenic spacer sequences obtained from this study.

Genotype	Sequence Name	Length of Sequences	GC content (%)	NCBI GenBank Accession Number
Prince Island 1	PRI1_trnL-trnF (ADA1)		29.36	KY274204
Prince Island 10	PRI10_trnL-trnF (ADA10)		29.14	KY290233
Prince Island 4	PRI4_trnL-trnF (ADA4)		29.58	KY290234
Bagdad Avenue 3	BAG3_trnL-trnF		29.58	KY290235
Bagdad Avenue 4	BAG4_trnL-trnF		29.14	KY290236
Bagdad Avenue 8	BAG8_trnL-trnF		29.36	KY290237
Barbaros Boulevard 10	BAR10_trnL-trnF		29.80	KY290238
Barbaros Boulevard 11	BAR11_trnL-trnF	453	29.36	KY290239
Barbaros Boulevard 12	BAR12_trnL-trnF		29.36	KY290240
Dilovasi District 7	DIL7_trnL-trnF		29.80	KY290241
Dilovasi District 8	DIL8_trnL-trnF		29.58	KY290242
Dilovasi District 9	DIL9_trnL-trnF		29.14	KY290243
TEM Highway 19	TEM19_trnL-trnF		29.14	KY290244
TEM Highway 25	TEM25_trnL-trnF		29.36	KY290245
TEM Highway 26	TEM26_trnL-trnF		29.58	KY290246

The sequences were searched in nucleotide collection of NCBI GenBank database to compare with our sequences. Basic Local Alignment Search Tool (BLASTn) of NCBI was used with “Highly similar sequences (Megablast)” option. Retrieved results of top three sequences per genotype are shown in Table 3.24.

Table 3.24 Details of top three *trnL-trnF* Intergenic spacer sequences similar to *R. pseudoacacia* genotypes in this study. The sequences retrieved from NCBI GenBank database.

Our Sequences	Similar Sequence retrieved from NCBI GenBank				
	Organism	Family	Accession Number	Cover (%)	Identity (%)
Prince Island 1 KY274204	*R. pseudoacacia*	*Fabaceae*	KJ468102	98	96
	Bobgunnia fistuloides		AY232778	94	97
	Indigofera tinctoria		KJ468098	100	87
Prince Island 4 KY290234	*R. pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Lecointea peruviana		AY232779	90	88
Prince Island 10 KY290233	*R. pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	88
	Indigofera tinctoria		KJ468098	100	87
Bagdat 3 KY290235	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	96	87
Bagdat 4 KY290236	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Lecointea peruviana		AY232779	90	88
Bagdat 8 KY290237	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	87
Barbaros 10 KY290238	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	88
	Indigofera tinctoria		KJ468098	100	87
Barbaros 11 KY290239	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	87
Barbaros 12 KY290240	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	96
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	87
Dilovasi 7 KY290241	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	88
	Indigofera tinctoria		KJ468098	100	87
Dilovasi 8 KY290242	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	87
Dilovasi 9 KY290243	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	96
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	86
TEM 19 KY290244	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	96
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	92	88
TEM 25 KY290245	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	88
	Indigofera tinctoria		KJ468098	92	88
TEM 26 KY290246	*R. Pseudoacacia*	*Fabaceae*	KJ468102	98	97
	Bobgunnia fistuloides		AY232778	94	87
	Indigofera tinctoria		KJ468098	100	87

According to our results, the most similar sequences to *trnL-trnF* intergenic spacer sequences are *R. pseudoacacia*, *Bobgunnia fistuloides*, *Indigofera tinctoria* and *Lecointea peruviana* with accession numbers of KJ468102, AY232778, KJ468098 and AY232779, respectively. Phylogenetic (Figure 3.86) tree was constructed to analyse phylogenetic relationships of *R. pseudoacacia* genotypes at interspecific level.

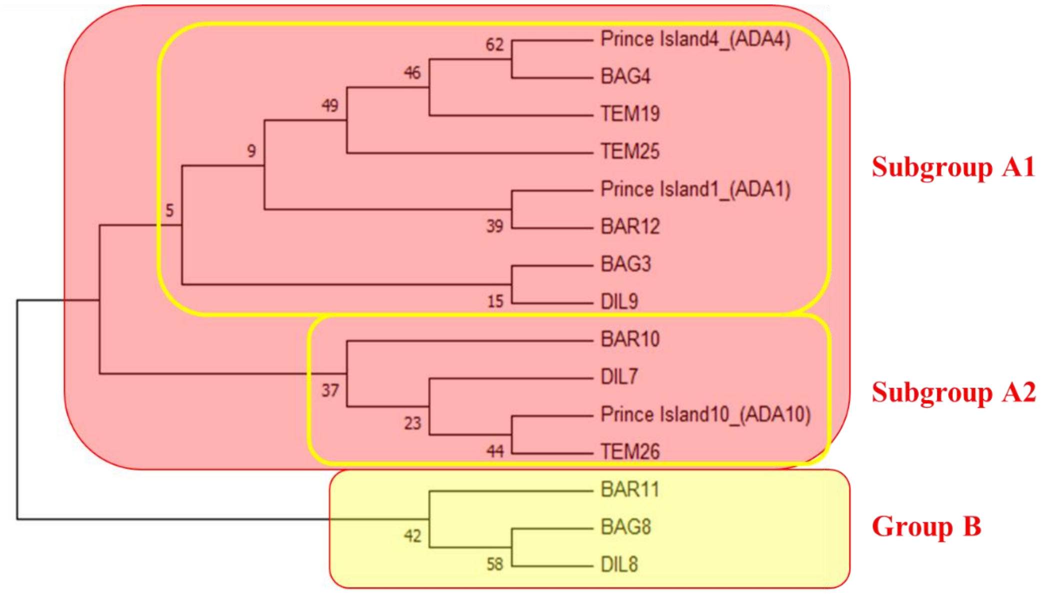


Figure 3.85 Phylogenetic distribution of *trnL-trnF* intergenic spacer sequences of *R. pseudoacacia* genotypes. Phylogeny was constructed by MEGA6 using ML method for 1000 bootstraps.

Two main group were identified in phylogenetic tree which were named as A and B. Group A further include two subgroups named as A1 and A2. Group B consists of only three *R. pseudoacacia* genotypes and show higher genetic similarty than the other subgroups (A1 and A2). When the groups are analyzed in terms of genotypes, there is a mixed distribution and lower bootsrapt values were identified in the phylogenetic tree. This situation may indicate the genetic variations in *trnL-trnF* intergenic spacer regions of *R. pseudoacacia* genotypes.

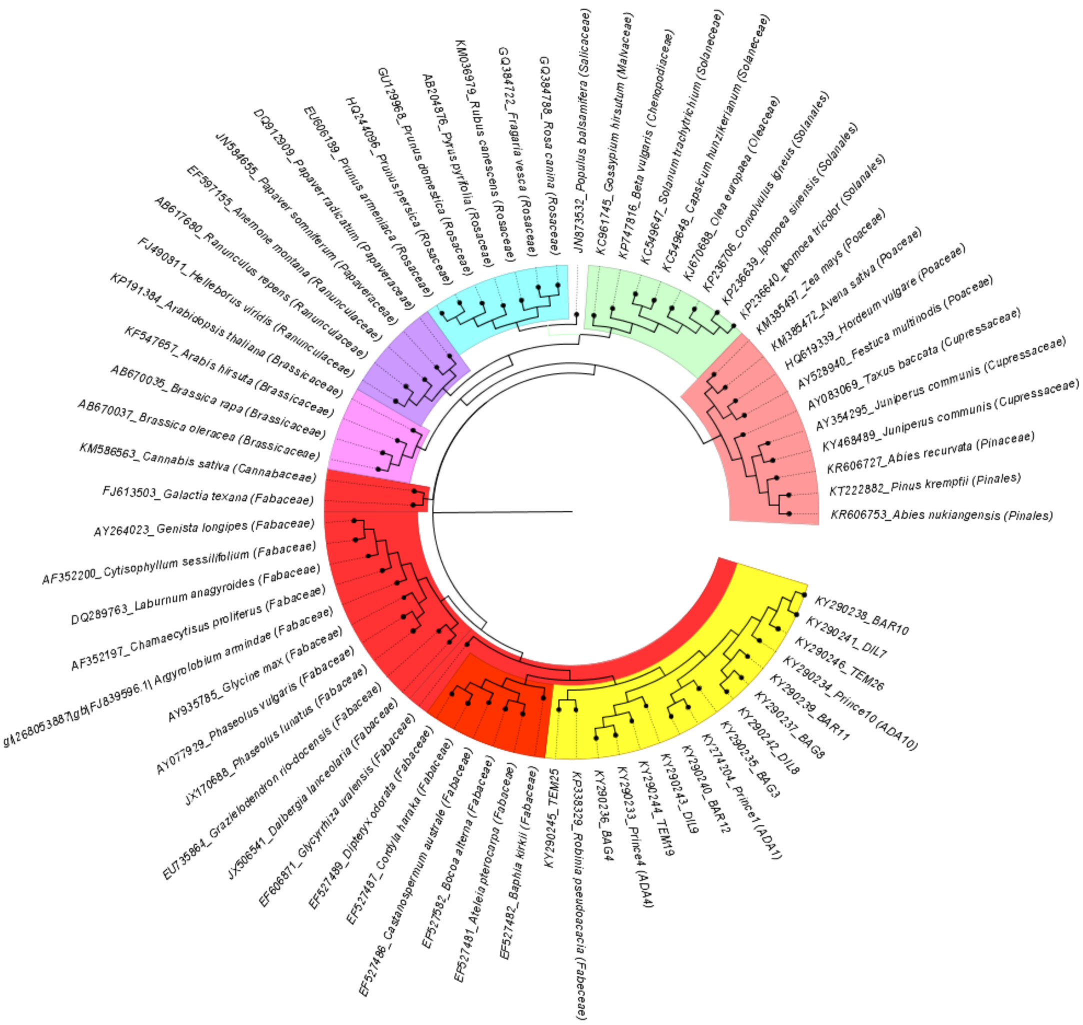


Figure 3.86 The joining phylogenetic tree of *trnL-trnF* intergenic spacer sequences with *R. pseudoacacia* and other plant species retrieved from NCBI GenBank database. Yellow cluster shows the obtained ITS1 sequences from this study.

The joining phylogenetic tree constructed by using both of *trnL-trnF* intergenic spacer sequences obtained in this study and retrieved from NCBI GenBank database. Total of 55 sequences were retrieved and selected from prominent families of vascular plants. According to results, studied *R. pseudoacacia* genotypes were clustered as yellow group with one *R. pseudoacacia* genotype (Accession Number: KP338329) retrieved from NCBI GenBank. The closest neighbor of *R. pseudoacacia* genotypes are members of *Fabaceae* family as red group. It can be suggested that *trnL-trnF* intergenic spacer sequences is an effective marker to discriminate genotypes at intraspesific level.

4. CONCLUSION

This study was conducted on two different aspects. Firstly, mineral element and heavy metal status, photosynthetic pigments and total protein content of *R. pseudoacacia* plants from different stations at all four seasons, which has different environmental conditions, has been investigated to reveal environmental pollution effects. According to the results, the conclusion and suggestions can be listed as follows;

The stations that have dense traffic (Bagdat Avenue, TEM Highway and Barbaros Boulevard) and industrial sites (Dilovasi District) are under influence of heavy metal pollution in different levels, when compered to the control station (Prince Island).

Two patterns of seasonal variations on element content were observed. All B, Ca, Cd, Cr, Cu, Fe, Mg, Mn and Pb levels show an increasing pattern in spring and autumn, and a decreasing pattern in summer and winter. Spring and winter values are relativelty high when compared to autumn and summer. K, Na, Ni and Zn elements on the contrary, tend to decrease from summer to winter.

R. pseudoacacia is a widely accepted effective biomonitor for observing enviromental pollution levels in nearly all types of areas. In this study, *R. pseudoacacia* individuals are observed to be well adapted to their environments in all stations. Additionally, airborne pollution of some elements can be easily determined by using *R. pseudoacacia* leaves. It may be suggested that *R. pseudoacacia* can be planted at polluted sites for ornamentation, reclamation and monitoring.

Second aspect aimed in this study is to reveal phylogenetic relationships and genetic diversity of *R. pseudoacacia* genotypes. According to results, the following conclusions and suggestions can be given;

In this study, genetic diversity level of *R. pseudoacacia* genotypes collected from urban ecosystem was investigated using nine ISSR markers and the obtained results were meaningful. According to the results ISSR marker systems can be applied effectively to understand genetic diversity level for *R. pseudoacacia* plants.

Based on values of genetic diversity level, Nei's values were found ranging from 0.165 to 0.251 and Shannon's values ranged from 0.245 to 0.381.

Apart from some samples collected from Dilovasi District, genotypes showed similar genetic structure in genetic analyses. The isolation of Dilovasi district can be explained with heavy industrial activities and consequently exposure of environmental pollution. In addition, the highest genetic diversity level for Nei's (0.251) and Shannon's (0.381) was found in Dilovasi District genotypes.

In phylogenetic analyses two genome regions (ITS1 from nuclear genome, trnL-trnF IGS from chloroplast genome) were used to investigate the phylogenetic relationship among genotypes. It was understood that ITS resolution power is stronger than trnL-trnF IGS region for phylogenetic analyses based on bootstrap values.

Among close relatives of *R. pseudoacacia*, *trnL-trnF* intergenic spacer region (94%) appeared to be more powerful than ITS1 region (91%) in distinguishing studied genotypes in joining phylogenetic trees.

According to results, it can be proposed that ISSR molecular markers, nuclear ITS1 region, and chloroplast *trnL-trnF* intergenic spacer region are effective genetic tools to analyze *R. pseudoacacia* genotypes in genetic studies.

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SUPPLEMENTARY

Suppl. 1 - Table 1 Boron (B) ($mg.kg^{-1}$)

Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Prince Island								
Summer	Average	28.749	26.323	2.426	8.4	12.391	16.002	34.898
	St. Deviation	0.490	0.528			0.299	0.389	0.713
Autumn	Average	38.129	35.188	2.941	7.7	16.200	21.636	43.455
	St. Deviation	1.032	0.954			0.506	1.212	1.815
Winter	Average	34.949	32.133	2.816	8.1	15.004	19.463	39.248
	St. Deviation	0.823	0.748			0.532	0.398	0.990
Spring	Average	43.604	40.203	3.402	7.8	18.563	24.161	48.959
	St. Deviation	0.927	1.089			0.485	0.696	1.139
Bagdat Avenue								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	23.508	20.451	3.058	13.0	8.007	13.698	28.420
	St. Deviation	0.571	0.345			0.164	0.244	0.601
Autumn	Average	31.278	27.359	3.919	12.5	10.583	18.492	33.691
	St. Deviation	0.837	0.745			0.265	0.787	0.723
Winter	Average	28.364	24.931	3.433	12.1	9.675	16.805	31.477
	St. Deviation	0.599	0.538			0.350	0.461	1.258
Spring	Average	35.596	31.264	4.333	12.2	12.121	20.979	40.410
	St. Deviation	0.882	0.899			0.248	0.814	1.778
TEM Highway								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	19.876	14.930	4.946	24.9	5.830	8.829	22.876
	St. Deviation	0.371	0.323			0.146	0.173	0.459
Autumn	Average	26.395	19.994	6.401	24.3	7.730	11.907	26.206
	St. Deviation	0.796	0.943			0.195	0.451	0.596
Winter	Average	24.230	18.190	6.040	24.9	7.046	10.776	24.268
	St. Deviation	0.484	0.432			0.246	0.246	1.197
Spring	Average	30.041	23.287	6.754	22.5	8.863	13.413	33.213
	St. Deviation	0.811	0.763			0.227	0.300	0.946
Barbaros Boulevard								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	18.450	14.033	4.417	23.9	4.884	7.666	22.072
	St. Deviation	0.356	0.235			0.058	0.147	0.264
Autumn	Average	24.574	18.273	6.300	25.6	6.468	10.382	24.803
	St. Deviation	0.588	0.679			0.163	0.435	0.457
Winter	Average	22.534	17.027	5.507	24.4	5.915	9.310	23.827
	St. Deviation	0.525	0.521			0.178	0.241	0.571
Spring	Average	27.920	21.032	6.888	24.7	7.373	11.751	28.535
	St. Deviation	0.678	0.574			0.163	0.445	0.920
Dilovasi District								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	14.599	8.876	5.723	39.2	2.800	5.081	17.497
	St. Deviation	0.280	0.120			0.067	0.121	0.357
Autumn	Average	19.517	11.680	7.837	40.2	3.678	6.897	19.289
	St. Deviation	0.528	0.352			0.086	0.398	0.360
Winter	Average	17.631	10.732	6.899	39.1	3.411	6.256	18.637
	St. Deviation	0.602	0.349			0.086	0.251	0.824
Spring	Average	22.196	13.385	8.811	39.7	4.184	7.766	25.906
	St. Deviation	0.561	0.328			0.107	0.302	0.559

Suppl. 2 - Table 2 Calcium (Ca) ($mg.kg^{-1}$)

Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Prince Island								
Summer	Average	17032.076	16500.672	531.404	3.1	9998.307	20003.901	20535.347
	St. Deviation	402.199	482.137			304.225	585.075	624.843
Autumn	Average	28208.951	27419.856	789.095	2.8	16398.555	32259.537	29651.258
	St. Deviation	754.250	766.106			322.177	918.291	1084.769
Winter	Average	23110.249	22451.903	658.347	2.8	13507.313	26597.678	24268.010
	St. Deviation	625.825	627.834			380.589	775.234	889.029
Spring	Average	31414.618	30356.798	1057.820	3.4	18199.914	36276.290	37728.699
	St. Deviation	781.467	860.118			569.075	1477.620	1067.989
Bagdat Avenue								
Summer	Average	17532.587	17198.885	333.702	1.9	12715.583	23292.333	23010.721
	St. Deviation	520.706	491.573			298.786	695.940	573.292
Autumn	Average	28978.272	27829.009	1149.264	4.0	20666.069	37698.463	30913.857
	St. Deviation	683.812	693.054			409.346	958.311	852.238
Winter	Average	23837.345	22903.595	933.750	3.9	16840.487	30978.299	25262.669
	St. Deviation	732.945	770.966			361.234	766.752	825.751
Spring	Average	32351.392	31711.966	639.426	2.0	23217.196	42285.070	41470.763
	St. Deviation	1071.082	970.583			640.007	1640.902	765.486
TEM Highway								
Summer	Average	21149.851	20519.127	630.724	3.0	14101.148	25890.220	26702.823
	St. Deviation	624.677	715.666			375.279	757.239	824.524
Autumn	Average	34785.955	33994.693	791.262	2.3	23001.967	42230.867	33221.828
	St. Deviation	1522.722	926.914			485.289	839.045	1453.272
Winter	Average	28221.698	27648.047	573.651	2.0	18663.207	34755.274	28229.914
	St. Deviation	1069.710	696.886			657.861	834.591	1294.465
Spring	Average	38556.030	37437.405	1118.625	2.9	25729.604	47031.407	46132.067
	St. Deviation	1174.737	1254.794			756.695	1747.681	1169.107
Barbaros Boulevard								
Summer	Average	22913.437	22701.282	212.155	0.9	14748.996	26725.813	26331.559
	St. Deviation	552.040	433.870			342.447	637.633	787.315
Autumn	Average	37868.840	37250.598	618.242	1.6	24092.767	43672.389	36497.588
	St. Deviation	726.901	1217.352			531.163	942.221	1366.897
Winter	Average	31236.677	30185.039	1051.638	3.4	19762.094	35836.726	29803.385
	St. Deviation	655.773	769.494			428.811	784.263	1129.146
Spring	Average	42041.112	41696.168	344.944	0.8	26837.374	49163.725	48045.861
	St. Deviation	999.951	881.135			911.472	1132.986	1385.040
Dilovasi District								
Summer	Average	25931.964	25197.419	734.546	2.8	16615.174	29863.831	30063.130
	St. Deviation	733.210	676.887			371.827	491.525	625.418
Autumn	Average	43548.539	42157.085	1391.454	3.2	27390.797	49081.118	42615.510
	St. Deviation	1680.228	1398.241			555.251	1192.289	1012.275
Winter	Average	35684.303	34593.001	1091.302	3.1	22491.059	40476.830	34984.563
	St. Deviation	1413.982	1256.151			520.203	905.055	1166.363
Spring	Average	47726.482	46404.821	1321.661	2.8	30381.183	54655.701	55414.639
	St. Deviation	1320.995	1265.576			688.458	1066.560	1255.819

Suppl. 3 - Table 3 Cadmium (Cd) (mg.kg^{-1})

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	0.269	0.207	0.062	23.0	0.157	0.172	0.618
	St. Deviation	0.005	0.004			0.003	0.003	0.011
Autumn	Average	0.319	0.241	0.078	24.4	0.177	0.204	0.654
	St. Deviation	0.005	0.004			0.003	0.003	0.014
Winter	Average	0.291	0.220	0.072	24.6	0.162	0.188	0.611
	St. Deviation	0.010	0.007			0.006	0.007	0.014
Spring	Average	0.376	0.289	0.088	23.3	0.210	0.245	0.775
	St. Deviation	0.019	0.006			0.007	0.005	0.032
Bagdat Avenue								
Summer	Average	0.621	0.569	0.052	8.4	0.319	0.403	0.916
	St. Deviation	0.012	0.010			0.006	0.007	0.017
Autumn	Average	0.746	0.675	0.071	9.5	0.370	0.487	0.996
	St. Deviation	0.013	0.011			0.006	0.008	0.022
Winter	Average	0.683	0.615	0.068	10.0	0.338	0.445	0.924
	St. Deviation	0.023	0.020			0.011	0.015	0.020
Spring	Average	0.892	0.810	0.082	9.2	0.440	0.579	1.179
	St. Deviation	0.021	0.017			0.014	0.020	0.052
TEM Highway								
Summer	Average	0.744	0.717	0.027	3.7	0.365	0.461	1.415
	St. Deviation	0.014	0.014			0.007	0.009	0.029
Autumn	Average	0.918	0.849	0.069	7.5	0.429	0.559	1.575
	St. Deviation	0.016	0.014			0.007	0.010	0.044
Winter	Average	0.838	0.775	0.063	7.5	0.393	0.511	1.446
	St. Deviation	0.027	0.025			0.013	0.017	0.053
Spring	Average	1.106	1.021	0.085	7.7	0.512	0.663	1.870
	St. Deviation	0.026	0.022			0.013	0.028	0.085
Barbaros Boulevard								
Summer	Average	1.069	0.911	0.158	14.8	0.537	0.756	1.977
	St. Deviation	0.020	0.017			0.010	0.014	0.039
Autumn	Average	1.311	1.114	0.197	15.1	0.636	0.912	2.313
	St. Deviation	0.022	0.019			0.011	0.016	0.060
Winter	Average	1.198	1.018	0.180	15.0	0.582	0.837	2.103
	St. Deviation	0.039	0.033			0.019	0.029	0.068
Spring	Average	1.546	1.328	0.218	14.1	0.756	1.081	2.762
	St. Deviation	0.085	0.037			0.028	0.046	0.085
Dilovasi District								
Summer	Average	5.246	4.613	0.633	12.1	2.775	3.027	6.119
	St. Deviation	0.097	0.084			0.061	0.056	0.114
Autumn	Average	6.495	5.734	0.761	11.7	3.296	3.760	7.461
	St. Deviation	0.111	0.098			0.056	0.064	0.161
Winter	Average	5.919	5.217	0.702	11.9	3.013	3.439	6.792
	St. Deviation	0.192	0.174			0.082	0.113	0.226
Spring	Average	7.799	6.841	0.957	12.3	3.953	4.451	8.853
	St. Deviation	0.161	0.188			0.083	0.203	0.282

Suppl. 4 - Table 4 Chromium (Cr) ($mg.kg^{-1}$)

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	3.324	3.132	0.191	5.7	2.117	2.524	8.763
	St. Deviation	0.054	0.050			0.033	0.040	0.160
Autumn	Average	4.124	3.854	0.271	6.6	2.512	3.183	9.460
	St. Deviation	0.080	0.075			0.049	0.062	0.238
Winter	Average	3.812	3.566	0.245	6.4	2.411	2.877	9.863
	St. Deviation	0.089	0.049			0.032	0.044	0.417
Spring	Average	4.330	4.097	0.233	5.4	2.753	3.300	11.323
	St. Deviation	0.063	0.040			0.052	0.035	0.465
Bagdat Avenue								
Summer	Average	5.128	4.905	0.222	4.3	3.377	4.603	11.955
	St. Deviation	0.081	0.082			0.055	0.074	0.207
Autumn	Average	6.404	6.146	0.258	4.0	3.939	5.715	13.392
	St. Deviation	0.125	0.120			0.077	0.111	0.340
Winter	Average	5.841	5.589	0.252	4.3	3.825	5.214	13.240
	St. Deviation	0.080	0.077			0.080	0.100	0.460
Spring	Average	6.702	6.434	0.268	4.0	4.381	6.057	15.572
	St. Deviation	0.064	0.073			0.108	0.135	0.202
TEM Highway								
Summer	Average	6.528	6.082	0.446	6.8	4.128	5.559	18.326
	St. Deviation	0.104	0.100			0.065	0.088	0.291
Autumn	Average	7.891	7.478	0.414	5.2	5.082	6.825	21.290
	St. Deviation	0.154	0.146			0.099	0.133	0.500
Winter	Average	7.423	6.906	0.517	7.0	4.701	6.295	20.762
	St. Deviation	0.107	0.100			0.064	0.115	0.410
Spring	Average	8.496	7.940	0.557	6.6	5.388	7.283	24.028
	St. Deviation	0.136	0.081			0.055	0.101	0.309
Barbaros Boulevard								
Summer	Average	9.210	7.945	1.265	13.7	5.207	6.445	21.204
	St. Deviation	0.146	0.133			0.082	0.102	0.337
Autumn	Average	11.502	9.736	1.766	15.4	6.526	7.979	25.494
	St. Deviation	0.224	0.190			0.127	0.155	0.525
Winter	Average	10.495	9.044	1.451	13.8	5.921	7.320	24.109
	St. Deviation	0.145	0.130			0.082	0.106	0.343
Spring	Average	12.027	10.395	1.632	13.6	6.826	8.474	27.914
	St. Deviation	0.119	0.100			0.095	0.178	0.621
Dilovasi Disctrict								
Summer	Average	16.047	14.084	1.963	12.2	13.266	16.054	28.921
	St. Deviation	0.282	0.235			0.210	0.254	0.456
Autumn	Average	20.737	17.270	3.467	16.7	17.093	20.077	36.517
	St. Deviation	0.404	0.336			0.333	0.391	0.707
Winter	Average	18.375	16.053	2.322	12.6	15.196	18.339	33.037
	St. Deviation	0.337	0.220			0.332	0.311	0.569
Spring	Average	21.771	18.572	3.199	14.7	17.781	21.988	39.967
	St. Deviation	0.603	0.434			0.889	0.938	1.386

Suppl. 5 - Table 5 Copper (Cu) (mg.kg^{-1})

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	19.687	17.030	2.657	13.5	9.974	14.327	20.749
	St. Deviation	0.387	0.338			0.196	0.294	0.411
Autumn	Average	24.657	20.904	3.753	15.2	11.898	17.241	23.134
	St. Deviation	0.671	0.222			0.126	0.183	0.352
Winter	Average	22.277	19.258	3.019	13.6	11.248	16.247	22.996
	St. Deviation	0.392	0.351			0.222	0.286	0.525
Spring	Average	28.885	24.535	4.349	15.1	13.908	20.240	26.978
	St. Deviation	0.866	0.388			0.315	0.317	0.772
Bagdat Avenue								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	27.407	23.181	4.225	15.4	16.695	20.219	27.517
	St. Deviation	0.590	0.465			0.332	0.400	0.598
Autumn	Average	33.671	28.890	4.781	14.2	20.611	25.147	32.476
	St. Deviation	0.357	0.307			0.219	0.267	0.863
Winter	Average	31.060	26.288	4.772	15.4	18.901	22.953	31.039
	St. Deviation	0.544	0.442			0.342	0.431	0.798
Spring	Average	39.593	34.055	5.538	14.0	24.260	29.457	38.533
	St. Deviation	0.588	0.554			0.365	0.548	0.747
TEM Highway								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	37.856	32.837	5.019	13.3	21.289	25.621	34.266
	St. Deviation	0.844	0.758			0.421	0.512	0.686
Autumn	Average	46.850	39.714	7.135	15.2	24.722	30.191	41.923
	St. Deviation	0.497	0.422			0.262	0.320	0.952
Winter	Average	42.781	37.157	5.624	13.1	24.003	28.830	38.799
	St. Deviation	0.979	0.804			0.528	0.734	0.689
Spring	Average	54.778	46.683	8.095	14.8	29.313	35.524	49.299
	St. Deviation	1.213	0.695			0.804	0.530	1.211
Barbaros Boulevard								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	40.223	33.468	6.755	16.8	24.606	28.123	35.614
	St. Deviation	0.795	0.669			0.484	0.554	0.713
Autumn	Average	50.316	43.376	6.940	13.8	28.604	35.357	44.635
	St. Deviation	0.534	0.460			0.304	0.375	1.119
Winter	Average	45.627	38.131	7.496	16.4	27.750	31.865	40.320
	St. Deviation	0.824	0.886			0.600	0.579	0.718
Spring	Average	59.021	50.898	8.122	13.8	33.874	41.520	52.186
	St. Deviation	0.976	0.820			0.834	0.640	1.553
Dilovasi District								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	60.631	51.510	9.121	15.0	41.391	45.559	66.836
	St. Deviation	1.322	1.021			0.820	0.895	1.313
Autumn	Average	78.592	69.350	9.241	11.8	54.328	60.383	88.641
	St. Deviation	0.834	0.736			0.577	0.641	2.181
Winter	Average	68.995	58.635	10.360	15.0	47.135	52.015	76.063
	St. Deviation	1.281	1.307			1.085	1.563	1.783
Spring	Average	91.947	81.312	10.635	11.6	63.543	71.032	103.782
	St. Deviation	1.920	1.391			1.363	1.055	2.931

Suppl. 6 - Table 6 Iron (Fe) (mg.kg^{-1})

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	101.248	91.199	10.049	9.9	63.565	164.414	1767.070
	St. Deviation	2.247	2.038			1.188	3.659	39.478
Autumn	Average	165.791	151.347	14.444	8.7	94.542	267.779	2129.075
	St. Deviation	2.855	2.606			1.628	4.611	68.627
Winter	Average	153.073	140.018	13.055	8.5	86.928	247.105	1974.109
	St. Deviation	3.034	2.694			2.210	5.004	75.638
Spring	Average	177.265	160.698	16.567	9.3	110.972	288.346	3117.454
	St. Deviation	4.064	2.758			3.508	6.185	72.923
Bagdat Avenue								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	113.942	101.520	12.422	10.9	69.975	173.330	1813.222
	St. Deviation	3.713	2.255			1.810	3.877	40.494
Autumn	Average	200.024	180.638	19.387	9.7	120.446	302.883	2583.754
	St. Deviation	3.445	3.111			2.074	5.216	83.558
Winter	Average	183.665	166.178	17.487	9.5	110.427	277.740	2389.799
	St. Deviation	5.163	4.065			3.471	8.611	89.744
Spring	Average	208.765	190.139	18.626	8.9	124.068	314.493	3154.537
	St. Deviation	5.303	2.947			4.437	3.430	115.742
TEM Highway								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	132.020	115.859	16.161	12.2	81.049	184.344	1924.047
	St. Deviation	2.948	2.574			1.906	4.115	42.969
Autumn	Average	233.687	205.061	28.626	12.2	143.386	327.164	2774.603
	St. Deviation	4.024	3.531			2.469	5.634	153.317
Winter	Average	212.866	187.341	25.525	12.0	131.868	301.452	2566.655
	St. Deviation	10.099	7.469			3.298	6.621	153.091
Spring	Average	235.874	218.540	17.334	7.3	150.945	338.502	3362.338
	St. Deviation	5.710	2.112			3.568	5.626	91.900
Barbaros Boulevard								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	138.955	123.140	15.814	11.4	83.976	186.875	1966.720
	St. Deviation	3.166	2.290			1.875	4.271	43.938
Autumn	Average	244.310	214.669	29.641	12.1	148.711	332.416	2797.478
	St. Deviation	4.207	3.697			2.561	5.724	75.211
Winter	Average	223.396	196.542	26.854	12.0	137.038	306.177	2608.750
	St. Deviation	8.417	6.801			2.991	6.887	105.367
Spring	Average	246.083	227.279	18.804	7.6	159.036	348.922	3421.278
	St. Deviation	6.140	2.330			2.490	3.970	126.434
Dilovasi District								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	163.248	142.062	21.186	13.0	97.055	201.835	2139.285
	St. Deviation	3.625	3.174			2.168	4.478	40.760
Autumn	Average	296.275	258.857	37.418	12.6	177.005	366.840	3421.369
	St. Deviation	5.102	4.458			3.048	6.317	57.161
Winter	Average	271.371	238.235	33.137	12.2	163.736	338.942	3156.016
	St. Deviation	9.120	5.658			3.148	6.585	73.918
Spring	Average	308.217	270.073	38.144	12.4	192.077	396.553	3756.504
	St. Deviation	3.306	4.139			3.806	4.832	73.388

Suppl. 7 - Table 7 Potassium (K) ($mg.kg^{-1}$)

Prince Islands

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	17026.936	16346.990	679.946	4.0	13448.485	15736.251	20047.094
	St. Deviation	283.127	254.054			281.323	219.801	353.103
Autumn	Average	14464.156	14074.462	389.694	2.7	11605.117	13499.323	18921.083
	St. Deviation	197.665	192.340			158.594	184.480	518.808
Winter	Average	12694.691	12369.577	325.114	2.6	10031.097	11883.835	16634.400
	St. Deviation	199.526	217.934			399.474	245.633	549.545
Spring	Average	15532.070	14898.904	633.166	4.1	12218.964	14261.977	19118.645
	St. Deviation	371.607	366.797			347.487	338.080	515.954

Bagdat Avenue

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	15136.318	14093.684	1042.634	6.9	11833.591	14096.058	17326.435
	St. Deviation	249.322	219.794			179.970	381.921	279.124
Autumn	Average	12753.530	11983.585	769.945	6.0	10201.592	11433.587	15671.699
	St. Deviation	174.288	163.766			139.414	156.250	208.469
Winter	Average	11365.885	10461.734	904.152	8.0	8846.211	10039.375	13685.508
	St. Deviation	581.922	176.476			278.295	163.298	341.443
Spring	Average	13763.853	12856.851	907.002	6.6	10782.160	12770.280	15906.628
	St. Deviation	321.231	331.129			227.984	480.761	558.736

TEM Highway

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	12732.600	11558.949	1173.650	9.2	10169.429	11417.622	16212.020
	St. Deviation	212.916	207.253			136.262	183.936	261.898
Autumn	Average	10566.701	9646.108	920.592	8.7	8499.516	9603.999	14384.121
	St. Deviation	144.403	131.822			116.153	131.247	335.329
Winter	Average	9301.668	8516.700	784.968	8.4	7469.894	8481.207	12563.607
	St. Deviation	191.261	231.625			131.531	234.683	450.224
Spring	Average	11540.315	10509.546	1030.769	8.9	9253.162	10404.444	14792.912
	St. Deviation	310.121	263.280			181.055	262.801	357.852

Barbaros Boulevard

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	12096.288	11538.746	557.542	4.6	9857.346	10499.294	15309.631
	St. Deviation	187.970	306.429			173.624	198.723	237.904
Autumn	Average	10241.682	9255.456	986.227	9.6	8103.143	8530.604	12962.485
	St. Deviation	139.961	126.484			110.736	116.578	301.405
Winter	Average	8992.411	8148.890	843.520	9.4	7084.292	7569.747	11311.939
	St. Deviation	145.748	170.567			107.323	301.475	311.356
Spring	Average	10974.806	10436.564	538.242	4.9	8980.650	9539.426	14030.700
	St. Deviation	255.991	422.472			206.520	246.458	470.012

Dilovasi District

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	9743.798	8720.331	1023.467	10.5	7890.791	8809.664	13375.820
	St. Deviation	155.862	141.709			122.619	154.549	207.854
Autumn	Average	8134.978	7086.059	1048.919	12.9	6365.958	7051.373	10592.138
	St. Deviation	111.171	96.837			86.996	96.363	209.682
Winter	Average	7100.930	6209.397	891.533	12.6	5593.069	6132.529	9229.131
	St. Deviation	121.206	90.170			95.772	149.089	231.342
Spring	Average	8844.876	7892.570	952.305	10.8	7160.655	7985.615	12118.502
	St. Deviation	204.982	150.564			166.376	211.267	294.929

Suppl. 8 - Table 8 Magnesium (Mg) (mg.kg^{-1})

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	3417.441	3126.230	291.211	8.5	2285.519	2915.039	4166.854
	St. Deviation	102.116	93.142			74.432	88.578	147.013
Autumn	Average	3754.495	3419.663	334.833	8.9	2496.916	3215.586	4499.405
	St. Deviation	58.221	53.029			38.720	49.864	179.457
Winter	Average	3502.323	3218.433	283.890	8.1	2350.942	2988.458	4276.617
	St. Deviation	113.102	114.223			91.937	100.321	166.640
Spring	Average	3882.824	3543.874	338.950	8.7	2592.717	3354.455	4672.004
	St. Deviation	63.000	56.153			45.136	86.637	195.616
Bagdat Avenue								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	3062.937	2558.627	504.310	16.5	2009.780	2207.030	3862.218
	St. Deviation	84.481	74.545			59.873	65.950	135.816
Autumn	Average	3350.931	2816.667	534.265	15.9	2199.274	2448.488	4295.518
	St. Deviation	51.963	43.678			34.104	37.969	137.171
Winter	Average	3142.091	2625.817	516.274	16.4	2063.720	2272.823	3965.900
	St. Deviation	93.617	84.608			67.725	85.672	152.705
Spring	Average	3473.408	2929.401	544.008	15.7	2299.267	2570.097	4452.838
	St. Deviation	55.335	57.866			71.070	105.273	145.815
TEM Highway								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	2846.149	2026.466	819.682	28.8	1665.637	1872.756	3283.149
	St. Deviation	84.662	52.523			43.918	55.791	99.764
Autumn	Average	3164.919	2233.153	931.766	29.4	1844.378	2114.106	3738.563
	St. Deviation	49.079	34.630			28.601	32.784	100.755
Winter	Average	2916.653	2080.623	836.030	28.7	1714.134	1929.533	3387.957
	St. Deviation	92.306	56.711			51.580	70.676	147.297
Spring	Average	3280.528	2325.613	954.916	29.1	1942.074	2394.581	3896.907
	St. Deviation	52.231	51.650			95.469	108.534	138.415
Barbaros Boulevard								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	2766.506	1878.495	888.012	32.1	1528.083	1771.892	3094.578
	St. Deviation	82.432	65.611			49.884	53.842	141.197
Autumn	Average	3058.276	2192.749	865.528	28.3	1784.609	2033.834	3585.256
	St. Deviation	47.425	34.003			27.674	31.539	189.155
Winter	Average	2837.987	1931.796	906.192	31.9	1565.669	1820.415	3187.399
	St. Deviation	91.185	84.530			56.254	65.702	185.710
Spring	Average	3159.492	2284.734	874.758	27.7	1868.101	2142.959	3730.090
	St. Deviation	54.331	53.175			63.507	109.021	218.915
Dilovasi District								
Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	2140.063	1332.459	807.604	37.7	991.260	1367.625	2721.780
	St. Deviation	63.754	34.733			30.121	33.499	72.774
Autumn	Average	2422.958	1499.000	923.958	38.1	1144.333	1512.651	3236.024
	St. Deviation	37.573	23.245			17.745	23.457	105.767
Winter	Average	2194.423	1373.527	820.896	37.4	1021.661	1411.366	2808.455
	St. Deviation	70.092	44.096			42.098	40.566	94.154
Spring	Average	2519.450	1904.856	614.594	24.4	1416.107	1878.713	3386.906
	St. Deviation	48.955	46.669			33.431	85.770	142.537

Suppl. 9 - Table 9 Manganese (Mn) ($mg.kg^{-1}$)

Prince Islands								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	51.177	42.192	8.985	17.6	24.740	78.208	389.210
	St. Deviation	1.108	0.923			0.553	1.645	8.465
Autumn	Average	69.320	57.517	11.803	17.0	33.371	107.579	470.383
	St. Deviation	1.239	1.028			0.597	1.923	12.042
Winter	Average	60.489	50.118	10.371	17.1	29.184	93.715	413.017
	St. Deviation	1.423	1.085			0.798	2.001	17.541
Spring	Average	73.283	61.092	12.191	16.6	35.976	114.626	564.631
	St. Deviation	2.437	1.895			1.401	2.013	18.922

Bagdat Avenue								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	82.131	70.517	11.614	14.1	36.037	89.534	414.830
	St. Deviation	1.808	1.527			0.788	1.957	9.082
Autumn	Average	112.150	96.886	15.264	13.6	48.914	122.131	534.594
	St. Deviation	2.005	1.732			0.874	2.183	13.697
Winter	Average	97.870	84.726	13.143	13.4	42.747	107.373	467.754
	St. Deviation	2.313	2.311			1.113	4.192	15.197
Spring	Average	118.522	102.212	16.310	13.8	52.620	129.395	593.301
	St. Deviation	3.336	3.276			2.451	3.752	20.621

TEM Highway								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	100.172	84.070	16.102	16.1	42.076	103.933	474.276
	St. Deviation	2.014	1.837			1.145	2.167	10.384
Autumn	Average	138.452	117.565	20.887	15.1	58.498	145.253	638.453
	St. Deviation	2.475	2.101			1.046	2.596	17.661
Winter	Average	121.002	103.068	17.934	14.8	50.992	126.691	556.184
	St. Deviation	3.162	3.349			1.126	2.897	19.742
Spring	Average	146.828	122.381	24.447	16.7	67.957	153.750	682.312
	St. Deviation	3.524	4.898			3.784	3.786	19.147

Barbaros Boulevard								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	105.017	89.360	15.657	14.9	45.839	107.957	487.232
	St. Deviation	2.298	1.694			1.004	2.388	10.648
Autumn	Average	148.910	124.299	24.611	16.5	64.031	154.325	668.726
	St. Deviation	2.662	2.222			1.145	2.758	16.470
Winter	Average	130.127	108.754	21.373	16.4	55.985	135.078	583.229
	St. Deviation	3.373	3.074			1.509	3.928	18.196
Spring	Average	155.981	133.241	22.740	14.6	77.672	168.970	702.152
	St. Deviation	1.572	4.555			6.579	3.850	19.475

Dilovasi District								
Season		Unwashed Leaf	Washed Leaf	Removed amount	Removal Rate (%)	Branch	Bark	Soil
Summer	Average	143.406	124.279	19.127	13.3	68.463	146.023	633.313
	St. Deviation	3.138	2.480			1.499	3.326	13.180
Autumn	Average	205.749	172.866	32.883	16.0	96.706	211.943	900.886
	St. Deviation	3.678	3.090			1.729	3.788	19.398
Winter	Average	178.469	150.500	27.970	15.7	84.475	184.849	786.953
	St. Deviation	3.624	3.130			2.132	4.214	21.841
Spring	Average	210.321	186.817	23.504	11.2	103.631	242.712	932.012
	St. Deviation	4.260	6.853			3.060	7.392	16.726

Suppl. 10- Table 10 Sodium (Na) (mg.kg^{-1})

Season		Unwashed leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	**Average**	304.958	268.640	36.319	11.9	161.334	197.197	2449.783
	St. Deviation	9.606	7.793			4.588	5.901	71.939
Autumn	**Average**	249.699	218.388	31.311	12.5	138.299	160.829	2128.253
	St. Deviation	2.044	1.788			1.132	1.317	67.990
Winter	**Average**	221.794	195.065	26.729	12.1	110.109	134.401	1802.195
	St. Deviation	9.080	5.469			3.584	3.423	64.205
Spring	**Average**	269.646	236.926	32.720	12.1	149.320	174.287	2300.677
	St. Deviation	4.908	2.139			2.785	1.830	77.978

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Bagdat Avenue								
Summer	**Average**	269.722	224.322	45.400	16.8	141.094	161.850	2254.987
	St. Deviation	7.667	6.416			4.302	4.707	70.132
Autumn	**Average**	221.916	181.312	40.604	18.3	118.668	130.359	1875.931
	St. Deviation	1.817	1.484			0.972	1.067	32.025
Winter	**Average**	196.449	164.525	31.923	16.3	101.960	105.144	1652.175
	St. Deviation	7.018	5.487			4.937	3.895	56.108
Spring	**Average**	239.783	196.308	43.474	18.1	128.554	141.932	2028.230
	St. Deviation	4.017	2.385			1.425	1.579	39.850

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
TEM Highway								
Summer	**Average**	247.226	192.413	54.813	22.2	120.433	138.642	1979.174
	St. Deviation	7.160	5.568			3.367	4.149	56.648
Autumn	**Average**	197.829	153.189	44.640	22.6	98.577	107.779	1545.988
	St. Deviation	1.620	1.254			0.807	0.882	45.584
Winter	**Average**	179.919	141.157	38.762	21.5	87.405	96.526	1443.375
	St. Deviation	6.989	4.776			3.602	4.354	50.926
Spring	**Average**	214.390	166.655	47.735	22.3	107.008	117.429	1672.726
	St. Deviation	2.239	1.638			0.923	1.466	48.336

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Barbaros Boulevard								
Summer	**Average**	240.565	188.817	51.748	21.5	113.832	127.811	1922.489
	St. Deviation	7.556	6.348			3.297	3.707	52.127
Autumn	**Average**	188.721	145.048	43.673	23.1	91.123	99.496	1431.980
	St. Deviation	1.545	1.188			0.746	0.815	58.831
Winter	**Average**	152.179	133.674	18.506	12.2	82.165	86.003	1397.340
	St. Deviation	5.795	4.370			4.510	3.614	52.062
Spring	**Average**	204.695	156.591	48.104	23.5	98.869	108.461	1554.945
	St. Deviation	1.894	2.961			0.883	1.475	62.626

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Dilovasi District								
Summer	**Average**	213.254	150.990	62.264	29.2	87.802	95.227	1651.652
	St. Deviation	6.574	5.950			2.543	3.581	50.783
Autumn	**Average**	167.708	117.981	49.727	29.7	68.812	73.101	1184.355
	St. Deviation	1.373	0.966			0.563	0.598	30.179
Winter	**Average**	130.334	103.058	27.277	20.9	52.325	59.913	992.662
	St. Deviation	3.418	2.777			2.448	3.922	35.412
Spring	**Average**	181.871	129.434	52.437	28.8	74.710	79.924	1287.986
	St. Deviation	1.721	3.849			0.640	1.658	33.360

Suppl. 11- Table 11 Nickel (Ni) ($mg.kg^{-1}$)

Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands								
Summer	Average	8.539	7.034	1.505	17.6	2.464	5.540	19.194
	St. Deviation	0.343	0.277			0.096	0.228	0.745
Autumn	Average	5.719	4.717	1.002	17.5	1.739	3.129	16.010
	St. Deviation	0.092	0.076			0.028	0.050	0.598
Winter	Average	4.823	3.975	0.848	17.6	1.380	3.056	16.662
	St. Deviation	0.217	0.179			0.065	0.261	0.542
Spring	Average	6.101	5.058	1.043	17.1	1.890	3.934	17.994
	St. Deviation	0.319	0.270			0.083	0.233	0.603
Bagdat Avenue								
Summer	Average	11.232	9.320	1.913	17.0	3.789	6.792	25.420
	St. Deviation	0.416	0.361			0.131	0.254	0.995
Autumn	Average	7.389	6.048	1.341	18.2	2.528	3.544	19.850
	St. Deviation	0.119	0.097			0.041	0.057	0.645
Winter	Average	6.308	5.258	1.050	16.6	2.124	3.482	21.244
	St. Deviation	0.286	0.227			0.096	0.388	1.302
Spring	Average	8.030	6.671	1.359	16.9	2.811	4.780	21.831
	St. Deviation	0.402	0.341			0.148	0.336	1.349
TEM Highway								
Summer	Average	16.596	14.041	2.555	15.4	6.041	8.860	30.439
	St. Deviation	0.657	0.614			0.245	0.345	1.197
Autumn	Average	10.877	8.751	2.126	19.5	4.084	5.025	22.251
	St. Deviation	0.175	0.141			0.066	0.081	0.977
Winter	Average	9.291	7.948	1.343	14.5	3.407	4.894	23.031
	St. Deviation	0.450	0.395			0.161	0.359	0.512
Spring	Average	11.962	10.056	1.906	15.9	4.478	6.302	24.156
	St. Deviation	0.710	0.540			0.160	0.337	0.445
Barbaros Boulevard								
Summer	Average	19.031	14.994	4.037	21.2	6.421	10.166	35.268
	St. Deviation	0.759	0.600			0.253	0.401	1.072
Autumn	Average	11.742	9.521	2.221	18.9	4.262	5.603	24.295
	St. Deviation	0.189	0.153			0.069	0.090	0.902
Winter	Average	10.656	8.481	2.175	20.4	3.655	5.387	24.977
	St. Deviation	0.550	0.419			0.186	0.338	0.850
Spring	Average	13.631	10.735	2.897	21.2	4.649	7.224	26.058
	St. Deviation	0.705	0.577			0.285	0.401	0.648
Dilovasi District								
Summer	Average	27.215	21.034	6.181	22.7	10.787	14.741	42.754
	St. Deviation	1.072	0.816			0.424	0.602	1.619
Autumn	Average	16.274	12.714	3.560	21.9	7.026	9.044	34.672
	St. Deviation	0.262	0.205			0.113	0.146	1.458
Winter	Average	15.030	11.681	3.349	22.3	6.042	8.250	36.804
	St. Deviation	1.156	0.700			0.301	0.405	1.099
Spring	Average	19.339	15.090	4.249	22.0	7.769	10.445	38.893
	St. Deviation	1.072	0.807			0.426	0.577	0.892

Suppl. 12- Table 12 Lead (Pb) ($mg.kg^{-1}$)

Site	Season		Unwashed Leaf	Washed Leaf	Removed Amount	Removal Rate (%)	Branch	Bark	Soil
Prince Islands									
	Summer	Average	18.291	17.288	1.003	5.5	6.534	11.537	24.110
		St. Deviation	0.564	0.562			0.242	0.244	0.738
	Autumn	Average	22.558	20.784	1.773	7.9	7.453	14.033	30.070
		St. Deviation	0.455	0.419			0.150	0.283	0.999
	Winter	Average	21.580	19.828	1.752	8.1	7.116	13.490	28.705
		St. Deviation	0.506	0.541			0.186	0.303	1.169
	Spring	Average	24.402	22.995	1.408	5.8	8.711	15.460	31.992
		St. Deviation	0.963	0.717			0.262	0.529	0.818
Bagdat Avenue									
	Summer	Average	27.824	24.278	3.546	12.7	10.019	15.796	30.164
		St. Deviation	1.001	0.809			0.287	0.486	0.994
	Autumn	Average	33.523	29.809	3.714	11.1	11.919	19.947	39.943
		St. Deviation	0.676	0.601			0.240	0.402	1.341
	Winter	Average	31.921	28.317	3.604	11.3	11.327	18.982	38.036
		St. Deviation	0.982	1.016			0.396	0.608	1.730
	Spring	Average	37.264	32.374	4.890	13.1	13.389	21.256	44.806
		St. Deviation	1.394	1.206			0.462	1.374	2.616
TEM Highway									
	Summer	Average	39.525	34.527	4.998	12.6	15.909	23.454	42.684
		St. Deviation	1.045	1.177			0.524	0.762	0.989
	Autumn	Average	49.368	43.197	6.171	12.5	19.420	29.693	58.778
		St. Deviation	0.996	0.871			0.392	0.599	1.559
	Winter	Average	46.989	41.123	5.865	12.5	18.528	28.258	49.725
		St. Deviation	1.487	1.285			0.502	0.904	3.650
	Spring	Average	52.699	46.175	6.524	12.4	21.270	31.275	65.238
		St. Deviation	1.444	2.010			0.846	1.074	2.681
Barbaros Boulevard									
	Summer	Average	43.206	37.093	6.113	14.1	18.938	24.541	46.010
		St. Deviation	1.403	1.205			0.616	0.795	1.485
	Autumn	Average	54.656	47.046	7.610	13.9	23.272	32.077	64.572
		St. Deviation	1.102	0.949			0.469	0.647	2.346
	Winter	Average	52.090	44.909	7.181	13.8	22.218	30.607	56.557
		St. Deviation	1.515	1.181			0.579	0.825	3.176
	Spring	Average	57.600	49.794	7.807	13.6	28.154	33.684	72.247
		St. Deviation	1.958	2.345			1.299	0.964	2.993
Dilovasi District									
	Summer	Average	77.305	69.118	8.188	10.6	39.393	44.553	69.810
		St. Deviation	2.521	2.144			1.249	1.447	2.214
	Autumn	Average	101.025	91.879	9.146	9.1	50.341	58.031	102.602
		St. Deviation	2.038	1.853			1.015	1.170	4.105
	Winter	Average	96.182	87.577	8.605	8.9	48.093	55.254	90.127
		St. Deviation	2.992	2.525			1.206	1.708	5.366
	Spring	Average	103.356	92.372	10.984	10.6	53.004	68.765	112.868
		St. Deviation	3.994	4.419			2.893	4.236	2.781

Suppl. 13- Table 13 Zinc (Zn) ($mg.kg^{-1}$)

Prince Islands								
Season		**Unwashed leaf**	**Washed Leaf**	**Removed Amount**	**Removal Rate (%)**	**Branch**	**Bark**	**Soil**
Summer	**Average**	80.392	75.819	4.572	5.7	31.844	48.109	342.640
	St. Deviation	2.049	1.975			0.829	1.335	9.098
Autumn	**Average**	63.489	59.058	4.431	7.0	26.702	35.945	300.881
	St. Deviation	1.658	1.536			0.694	0.935	6.971
Winter	**Average**	48.950	45.611	3.339	6.8	20.207	27.476	232.676
	St. Deviation	1.198	0.990			1.066	0.778	6.305
Spring	**Average**	71.095	66.166	4.930	6.9	29.878	40.218	335.489
	St. Deviation	2.452	2.484			1.156	1.558	12.694

Bagdat Avenue								
Season		**Unwashed leaf**	**Washed Leaf**	**Removed Amount**	**Removal Rate (%)**	**Branch**	**Bark**	**Soil**
Summer	**Average**	97.470	91.804	5.666	5.8	40.733	62.101	383.424
	St. Deviation	2.559	2.333			1.072	1.641	9.986
Autumn	**Average**	76.706	72.399	4.307	5.6	33.592	45.433	318.966
	St. Deviation	1.995	2.257			0.874	1.182	8.266
Winter	**Average**	60.048	56.603	3.445	5.7	25.823	34.729	246.326
	St. Deviation	3.056	2.378			0.522	0.981	6.443
Spring	**Average**	86.170	81.202	4.967	5.8	37.847	51.082	355.095
	St. Deviation	3.120	3.506			1.383	1.844	12.368

TEM Highway								
Season		**Unwashed leaf**	**Washed Leaf**	**Removed Amount**	**Removal Rate (%)**	**Branch**	**Bark**	**Soil**
Summer	**Average**	115.547	104.006	11.542	10.0	52.456	74.106	418.948
	St. Deviation	3.009	2.825			1.608	2.068	10.564
Autumn	**Average**	87.460	77.321	10.139	11.6	42.496	51.148	329.693
	St. Deviation	2.275	2.041			1.105	1.330	10.900
Winter	**Average**	68.288	60.462	7.826	11.5	32.514	38.993	254.989
	St. Deviation	3.024	3.555			0.860	1.332	7.266
Spring	**Average**	98.391	86.751	11.640	11.8	47.733	57.633	360.318
	St. Deviation	3.555	2.323			1.729	2.108	8.953

Barbaros Boulevard								
Season		**Unwashed leaf**	**Washed Leaf**	**Removed Amount**	**Removal Rate (%)**	**Branch**	**Bark**	**Soil**
Summer	**Average**	120.658	106.569	14.089	11.7	55.053	73.666	412.439
	St. Deviation	2.416	3.339			1.488	1.939	10.977
Autumn	**Average**	90.776	80.523	10.253	11.3	43.463	53.393	315.142
	St. Deviation	2.361	2.095			1.130	1.389	10.110
Winter	**Average**	71.297	63.261	8.035	11.3	33.719	41.114	258.023
	St. Deviation	4.235	3.824			0.989	0.813	4.466
Spring	**Average**	102.074	90.456	11.619	11.4	49.006	60.892	362.993
	St. Deviation	3.685	3.273			1.810	3.267	5.615

Dilovasi District								
Season		**Unwashed leaf**	**Washed Leaf**	**Removed Amount**	**Removal Rate (%)**	**Branch**	**Bark**	**Soil**
Summer	**Average**	135.388	120.954	14.434	10.7	67.468	91.265	452.105
	St. Deviation	3.547	3.186			1.776	2.654	12.177
Autumn	**Average**	100.246	86.198	14.048	14.0	51.579	63.436	414.193
	St. Deviation	2.607	2.242			1.341	1.650	14.933
Winter	**Average**	89.055	79.311	9.744	10.9	40.258	49.401	319.803
	St. Deviation	2.752	3.958			1.746	1.872	10.149
Spring	**Average**	113.055	97.468	15.588	13.8	58.263	71.705	427.333
	St. Deviation	4.190	3.842			2.234	2.799	8.151

RESUME

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