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DETERMINATION OF THE LIKELY EXPOSURE OF METALS ORIGINATED FROM URBAN AND INDUSTRIAL AIR POLLUTION IN THE URINE SAMPLES OF CHILDREN

MOHANAD BASIM KADHIM ALGBURI

DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

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This thesis, prepared by Mohanad ALGBURI, namely "DETERMINATION OF THE LIKELY EXPOSURE OF METALS ORIGINATED FROM URBAN AND INDUSTRIAL AIR POLLUTION IN THE URINE SAMPLES OF CHILDREN" is accepted by the following jury with consensus as a MASTER'S THESIS in the Department of Pharmaceutical Toxicology Gazi University.

Supervisor: Prof. Dr. Gonca ÇAKMAK
Department of Pharmaceutical Toxicology, Gazi University
I approve that this thesis is a Master's Thesis in scope and quality.

President: Prof. Dr. Sema BURGAZ
Department of Pharmaceutical Toxicology, Gazi University
I approve that this thesis is a Master's Thesis in scope and quality.

Member: Prof. Dr. İsmet ÇOK
Department of Pharmaceutical Toxicology, Gazi University
I approve that this thesis is a Master's Thesis in scope and quality.

Member: Prof. Dr. Emine Eftade GAGA
Department of Environmental Engineering, Eskişehir Technical University
I approve that this thesis is a Master's Thesis in scope and quality.

Member: Doç. Dr. Orkun ALP
Department of Analytical Chemistry, Gazi University
I approve that this thesis is a Master's Thesis in scope and quality.

Thesis Defense Date: 21/01/2020

This thesis, which was approved by the jury as a Master's Thesis, was confirmed by the Board of Directors of Gazi University Institute of Health Sciences.

....

Prof. Dr. Mustafa ASLAN
Director of Health Sciences Institute

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KENTSEL VE ENDÜSTRIYEL HAVA KİRLİLİĞİNDEN KAYNAKLANAN METALLERE OLASI MARUZİYETİN ÇOCUKLARIN İDRAR ÖRNEKLERİNDE BELİRLENMESİ

(Yüksek Lisans Tezi)

Mohanad Basim Kadhim ALGBURI

GAZİ ÜNİVERSİTESİ
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ÖZET

Toksik metallere çevresel maruziyet önemli sağlık sorunudur. Çalışmanın amacı, Kütahya kent sınırları içinde yaşayan çocukların idrar örneklerinde, 17 farklı metali analiz ederek çevresel hava kirliliği maruziyetini araştırmaktır. Seçilen metaller çevresel maruziyetler açısından en çok araştırılan ve en çok idrarda saptanan metallerdir. Çocukların sabah spot idrar örnekleri toplanmış, endüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) ile örneklerde arsenik (As), baryum (Ba), berilyum (Be), kadmiyum (Cd), krom (Cr), kobalt (Co), bakır (Cu), demir (Fe), kurşun (Pb), manganez (Mn), cıva (Hg), molibden (Mo), nikel (Ni), selenyum (Se), kalay (Sn), vanadyum (V) ve çinko (Zn) düzeyleri saptanmış ve sonuçlar kreatinin ve spesifik gravite ile düzeltilmiştir. Kütahya'da; kentsel alan olarak trafiğin yoğun olduğu Kütahya Şehir Merkezi (KC) ve kırsal/endüstriyel alan olarak Tunçbilek ve Seyitömer Termik Santrali'nin yakınındaki Tunçbilek Bölgesi (TR) seçilmiştir. Yaş ortalaması, 8.64 ± 0.621 (ortalama ± SS) olan 160 çocuk; KC'de bir ilkokuldan (n = 72) (yaş, 8.51 ± 0.435) ve TR'de 3 ilkokuldan (n = 88) (yaş, 8.75 ± 0.725) biraraya getirilmiştir. Kütahya için, düzeltilmiş ve düzeltilmemiş idrar metal düzeyleri birlikte göz önünde bulundurulduğunda, As ve Ni 'nin kırsal/endüstriyel bölgeyi, V, Mn, Fe ve Pb'nin ise trafik yoğun kentsel bölgeyi temsil ettiği bulunmuştur. Kütahya'da yaşayan çocuklar, diğer ülkelerden benzer şekilde tasarlanmış diğer çalışmalar arasında değerlendirildiğinde, metaller için değişken sonuçlar gözlenmiştir. Ayrıca çocukların üriner metal düzeyleri arasında pozitif korelasyonlar belirlenmiştir ($p<0.05$). Düzeltilmiş ve düzeltilmemiş metal düzeyleri arasında da pozitif korelasyon vardır ($p<0.05$). Sonuç olarak, Kütahya ilinde yaşayan çocukların, bölge ve kaynağa göre farklılık gösteren yüksek çevresel metal maruziyetine sahip oldukları varsayılmaktadır. Çocuklarda 17 çevresel metal düzeyini araştıran Kütahya Çalışması, Türkiye'deki ilk çevresel moleküler epidemiyoloji çalışması olarak ulusal bilimsel literatüre ve ayrıca ek bilgi olarak uluslararası bilimsel literatüre katkıda bulunmaktadır.

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DETERMINATION OF THE LIKELY EXPOSURE OF METALS ORIGINATED FROM URBAN AND INDUSTRIAL AIR POLLUTION IN THE URINE SAMPLES OF CHILDREN

(M.Sc. Thesis)

Mohanad Basim Kadhim ALGBURI

GAZİ UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

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ABSTRACT

Environmental toxic metal exposure is a crucial human health concern. The aim of the study is to investigate the environmental air pollution by analyzing 17 metals in urine samples of children living in Kütahya Province. The chosen metals were the most studied and the most likely present in urine with regards to the environmental exposures. First-spot morning urine samples are collected, arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), vanadium (V), and zinc (Zn) in the samples are determined by inductively coupled plasma mass spectrometer (ICP-MS), and the results were adjusted with creatinine and specific gravity. In Kütahya; traffic dense city center of Kütahya (KC) is chosen as urban site and Tunçbilek Region (TC), located nearby to Tunçbilek and Seyitömer Thermal Power Plants is chosen as rural/industrial site. The total population of 160 children with the average age of 8.64 ± 0.621(mean ± SD) is recruited from one primary school (n= 72) (age, 8.51 ± 0.435) located in KC and from 3 primary schools (n= 88) (age, 8.75 ± 0.725) located in TR. When adjusted and non-adjusted urinary metal levels are considered altogether for Kütahya; it is found that As and Ni levels are characterizing the rural/industrial region whereas V, Mn, Fe, and Pb are characterizing traffic intense city center. Children living in Kütahya are manifested variable urinary metal levels when evaluated among other similarly designed studies from other countries. Furthermore, positive correlations among the urinary metal levels of children are determined ($p<0.05$). As well as each adjusted and non-adjusted metal levels are positively correlated ($p<0.05$). In conclusion, children living in Kütahya are assumed to have high environmental metal exposure, which is showing differences due to the region and sources. Kütahya Study, investigating 17 environmental metal levels of children, is contributing to national scientific literature as the first environmental molecular epidemiology study in Turkey and also to international scientific literature as additional information.

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SYMBOLS AND ABBREVIATIONS

The abbreviations used in this study are presented below with their explanations.

Symbol	Explanation
%	Percent
µg L^{-1}	Microgram/Liter
µg g^{-1}	Microgram/Gram
ng mL^{-1}	Nanogram/Milliliter
PPb	Part per billion
mL	Milliliter
µL	Microliter
mg	Milligram
g	Gram
nm	Nanogram

Abbreviations	Explanation
A	Atatürk primary school
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
AUSRP	Anadolu University Scientific Research Project
Ba	Barium
Be	Beryllium
BMI	Body mass index
Cd	Cadmium
CDC	Centers for Disease Control and Prevention
Co	Cobalt
Cr	Chromium
CR	Creatinine
CR-UM	Creatinine adjusted urinary metal levels
Cu	Copper
EPA	Environmental Protection Agency

Abbreviations	Explanation
Fe	Iron
FEF%25-75	Forced Expiratory Flow between 25 and 75% expired volumes
FEV1	Forced Expiratory Volume in 1 s
FVC	Forced vital capacity
G	Gürağaç primary school
Ga	Gallium
HCL	Hydrochloric acid
Hg	Mercury
HNO_3	Nitric acid
I	Işık primary school
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometer
IS	Internal standards
ISAAC	International Study of Asthma and Allergies in Childhood
KC	Kütahya City Center
L	Linyit primary school
Li	Lithium
Mn	Manganese
Mo	Molybdenum
NaOH	Sodium hydroxide
Ni	Nickel
NO	Nitrogen oxide
NO_2	Nitrogen dioxide
O_3	Ozone
OECD	Organization for Economic Co-operation and Development
Pb	Lead
PEF	Peak Expiratory Flow
Rb	Rubidium
Rh	Rhodium

Abbreviations	**Explanation**
R-UM	Urinary metal level
Se	Selenium
SG	Specific gravity
SG-UM	Specific gravity adjusted urinary metal level
Sn	Tin
SO_2	Sulfur dioxide
TR	Tunçbilek/ Gürağaç region
U	Uranium
UM	Urinary metal levels of children
V	Vanadium
VOC	Volatile organic compound
WHO	World Health Organization
Zn	Zinc

1. INTRODUCTION

According to the World Health Organization (WHO), ambient air pollution is causing approximately 4.2 million deaths annually (Internet a). While when combined with indoor (household) air pollution approximately 7 million people die every year worldwide, and about nine out of ten people inhale high levels of pollutants (Internet b).

Among the ambient air pollution main components, there are particulate matter (PM), ozone (O_3), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) (Internet a). Exposure to toxic metals and metalloids in ambient air pollution is also a serious problem and an important health concern (Roca et al., 2016; Elinder et al., 1994). Among them, there are carcinogenic ones such as As, Be, Fe, Ni, Cr, and Cd (IARC, 2012a; IARC, 2012b). The sources and intensity of air pollution, including metals in it, are depending on many conditions. In urban areas; traffic, unplanned urbanization, commercial and domestic fuels, household fuel burning, natural dust and salt, and industrial activities (Internet c; Uchiyama et al., 2015; Szczurek et al., 2015), and in rural areas; pesticides in agriculture, burning of paddy straw and wheat conceder, cooking, and heating, using coal, charcoal, wood, crop wastes, and cow dung, (Internet p; Sathya et al., 2018) are among the pollution sources. On the other hand, industrial air pollution takes considerable place in environmental air contamination including metal processing, wood processing, chemical processing, mining and industrial waste products (Internet d; Internet o, 2018; Y. Joseph, 2008).

Environmental metal exposure have the accumulative capacity and can harm health severely even at low doses depending on the level and the duration of exposure, especially for children, elderly people and pregnant women as they are the most vulnerable groups of population (Gil et al, 2009; Rodríguez-Barranco et al., 2013).

Children are considered as a vulnerable population because of the fact that their organs and biological systems are under development so that the biological systems linked with absorption, metabolism, distribution, and elimination of chemicals are less advanced when compared to the adults (Ferguson et al., 2017). Early exposure to air pollutants may cause alteration in children’s lung function easier than adults (Esposito et al., 2014) In addition, they inhale much more air per body weight than adults (Salvi, 2007). Also, children related activities, including playing outdoors, crawling, hand-to-mouth ingestion, etc., differ and increase their exposure possibilities to contaminants within their environments (Ferguson et al., 2017). Moreover, some children have poor conditions of socioeconomic status, diet, quality of healthcare, and even working (Ericson et al., 2009), which might cause worse health problems addition to the environmental exposures in their living environment. Therefore, children are at high risk of environmental exposures due to their unique and different exposure pathways, dynamic developmental physiology, and their longer life expectancy (“World Health Organization,” 2008). Longer life expectancy of children and their differences than that of adults in many means trigger the conduction of biomonitoring studies to have children specific environmental exposure characterizations.

Quantitative measure of the different changes in a biological system can provide relevant information on the extent and source of exposure to environmental pollutants; such as metal levels in biological samples (Dagnino et al., 2008). Metals can be determined in hair, blood, and urine (Roca et al., 2016; Elinder et al., 1994). A appropriate non-invasive method, urine is easy to collect and easy to handle in the analytical determination.

The number of molecular epidemiology studies on children evaluating the environmental exposure to metals are increasing in the last two decades (Wang et al., 2019; Roca et al., 2016; Molina-Villalba et al., 2015; Sughis et al., 2014; Aguilera et al., 2010; Moreno et al., 2010; Heitland et al., 2006). Studies on children, conducted to understand the regional effects of environmental air pollution are advantageous since children do not smoke, consume alcohol, work, and move a lot (only between home and school) (Nakayama et al., 2019; Gajski et al., 2013). Therefore, the outcome of this kind of studies can be attributed to mainly their living environment conditions. Importantly, the outcome from those studies is valuable to identify the exposure related likely health effects.

Kütahya Province is one of the highest polluted city of Turkey. Although domestic heating and industrial activities have partially switched to natural gas, it is known that high pollutant levels still measured in Kütahya Province. The city center of Kütahya also has been identified with its dense traffic. There are two coal-fired thermal power plants of Seyitömer and Tunçbilek within the provincial borders, the third thermal power plant is in the process of being established, and coal is still used for heating purposes in winter months (Altug, et al., 2015). Kütahya has been chosen as a region of study for environmental air pollution characterization in recent two comprehensive interdisciplinary projects (TÜBİTAK-112Y305, Anadolu University Scientific Research Project-AUSRP-1407F398). Accordingly, the present study formed a part within AUSRP, has the goal of determining personal exposure to air pollutants and related health effects on school children living in Kütahya Province.

The aim of the present study is to analyze metals in first-spot morning urine samples of children living in Kütahya Province to quantify environmental metal exposure.

In the preliminary studies of AUSRP, two different areas of Kütahya Province are chosen as urban and rural/industrial sites. Urban site is located in the traffic dense city center of Kütahya (Kütahya Center, KC). Tunçbilek Region (Tunçbilek, TR), in the provincial borders of Kütahya, included as a rural/industrial site which is under the influence of two coal-fired active thermal power plants. In the urban and rural/industrial sites, four schools are selected. Children living in KC are chosen from one school and the children from rest of the schools (3 schools) are recruited from TR. Children in these schools are visited at specific time points (3 times, in January, February, and May of 2016) before the biomonitoring period (4^{th} visit, June 2016) of the present study. In this last sampling period of AUSRP (June 2016), urine samples of the present study are collected. Pollutant levels in the region, pollutant and wind maps, ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) levels in children, respiratory function test data [forced expiratory volume in 1s (FEV1), forced vital capacity (FVC), peak expiratory flow (PEF), forced expiratory flow between 25 and 75% expired volumes (FEF25-75), FEV1/FVC], body mass indexes (BMI), and blood pressure parameters are determined in each visit of the AUSRP.

The works conducted within the scope of the thesis are mentioned as below:

- First-spot morning urine samples of children are collected, specific gravity measurements of them are immediately done by refractometer, and samples are transferred to the laboratory in cold chain.
- According to the scientific literature, 17 metals (Cu, Co, Mn, Mo, V, Zn, As, Ba, Be, Cd, Ni, Pb, Se, Sn, Fe, Cr, and Hg) are decided to analyze by inductively coupled plasma mass spectrometry (ICP-MS) according to the most studied ones and the most likely present in urine with regards to the environmental exposures.

- The results are adjusted with two methods, specific gravity (SG) and creatinine. The statistical analyses are carried out for raw data, SG adjusted data, and creatinine adjusted data of urine metal levels of children.
- The urinary metal levels are statistically analyzed using comparison and correlation methods within the whole demographic data of children from the AUSRP.
- The buccal epithelial cells in parallel to urine samples of the same children are collected to carry out buccal epithelial micronucleus assay (BE-MN), which was the subject of another thesis (Özata, 2019). BE-MN results of the same children as a biomarker of effect are used to evaluate the relationships in the statistical analysis.
- The output is also evaluated in comparison to the studies with similar aim and designs in scientific literature.
- This study is assumed to contribute both national and international scientific literature on environmental metal exposures of children.

2. GENERAL INFORMATION

2.1. Air Pollution

Air pollution is a serious health problem. Some of the probable causes and sources of air pollution are unplanned urbanization, traffic, commercial and domestic fuels, power generation plants, industrialization, and increasing population (Lawrence et al., 2014) (Mraihi et al., 2015). Analysis of the air pollutants is important in formulating the necessary policies that can prevent pollution and promote the life and welfare of the society (Mraihi et al., 2015).

According to the World Health Organization (2019), approximately 4.2 million people die annually due to exposure to outdoor air pollution. The fine particles in such polluted environments lead to different diseases, including heart conditions, stroke, lung cancer, respiratory infections chronic obstructive pulmonary diseases and pneumonia (Internet a). The International Agency for Research on Cancer (IARC) stated that the outdoor air pollution is carcinogenic to humans (Group 1) (IARC, 2016).

2.2. Urban Air Pollution

The main sources of urban air pollution are traffic, household fuel burning, combustion and agriculture, natural dust and salt, and industrial activities (Internet c; Internet d). Traffic density causes increasing in the emissions of gases and particles to the atmosphere, which increases the levels of human exposure to chemicals, especially to the people who live near to main roadways (Brugge et al., 2007). People in urban areas spend a considerable proportion of their time in indoor environments, such as at work, home, in public, and private places (restaurants, schools, and hospitals) (Uchiyama et al., 2015). Indoor air pollution results from two sources: substances within the domestic environment (construction materials, cooking fuels, and toxic gas emissions from household equipment) and outdoor pollutants that find their way to the indoor environment (Szczurek et al., 2015). There are certain pollutants whose contribution from outside sources has significant effect on the indoor levels, such as the outdoor conditions (e.g. meteorology), interactions between a building and its surrounding (e.g. via infiltration), individual properties of the building (structure, materials, construction), heating, air conditioning and ventilation systems, indoor space arrangement (space organization, furnishing, appliances and equipment), sources of heat and pollutants emission (Szczurek et al., 2015). The contribution of the mentioned sources depends on the location of the building within the urban environment. For instance, buildings located close to industrial parks or heavy foot traffic have their effect in indoor air quality (Demirel et al., 2014).

2.3. Rural Air Pollution

People who live in rural areas suffer from both outdoor and indoor air pollution. The primary sources of outdoor air pollution are: randomly use of pesticides and insecticide sprays, also the burning of paddy straw and wheat conceder as a source of rural outdoor air pollution (Internet p). Even people in rural areas feel that they are safe and free from air pollution, the indoor environment of rural households can be polluted from different daily indoor activities, such as cooking, heating, and cleaning, those environments are characterized by a lack of efficient ventilation systems (Sathya et al., 2018). Coal, charcoal, wood, crop wastes, and cow dung are still being used in many rural environments and constitute the largest source of indoor air pollution (Sathya et al., 2018).

2.4. Industrial Air Pollution

A significant proportion of industrial air pollution comes from manufacturing activities, including metal processing, wood processing, and chemical processing, among other industrial activities (Internet d). Each of these industrial facilities produces the intended product (energy supply, metal (ferrous and non-ferrous) production, non-metallic minerals production, extractive industries, chemicals, and other manufacturing) as well as waste products that include air pollutants (Internet d). One of the causes of industrial air pollution is the burning of fossil fuels such as coal and petroleum, resulting in emission of different gases, such as sulfur dioxide, carbon dioxide, and nitrogen dioxide which can also be emitted from vehicles and trucks (Internet o). The other source of industrial pollution is agricultural activities. These include the activities to manufacture chemicals or spray the pesticides, fertilizers, and insecticides in the agricultural fields and these agricultural activities emit harmful chemicals (Internet o). It is important to note that industrial pollutants are stationary.

Also, the process of mining as an industrial activity results in the release of dust and chemicals and massive air pollution (Joseph Y., 2008).

The high concentration of air pollutants from industrial sources affects air quality; this also negatively affects the health and welfare of people living close to industrial plants or industrial cities (Ericson et al., 2009). Each of the industrial facilities emits consistent quality and quantity of pollutants. At the same time, changes in emission occur only when there is a significant improvement in production systems to reduce the level of pollution (Internet d).

2.5. Air Pollution Components

2.5.1. Organic compounds

According to EPA, Volatile organic compounds (VOC) means ‘any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, which participates in atmospheric photochemical reactions’ (Internet e). VOCs are chemicals made up of carbon and other elements. More than 1700 VOCs are reported (e.g., Acetone, Acetic Acid, Carbon Disulfide, Ethanol, Alcohol, Formaldehyde, and Methylene Chloride) (Dudley et al., 2010). There are many household products, such as paint, varnish, wax, and fresheners, also the burning of fossil fuels, dry cleaners, lawn mowers, automobiles, and landscaping equipment emit VOCs to the atmosphere ((Bloemen, et al., 1995).

VOCs lead to the formation of ground-level ozone, which has negative health consequences of interaction with VOCs such as irritation to the eyes, throat, and nose, asthma as lung diseases (Bloemen, et al., 1995).

2.5.2. Inorganic compounds

<u>Metals and metalloids</u>

Arsenic (As)

Arsenic is a metalloid found in large quantities in the earth's crust, in the atmosphere from volcanic activity, traces of As in the rocks and water bodies, and deep-drilled wells of drinking water (Garelick et al., 2008). According to the World Health Organization (WHO), the main exposure source to As is through drinking water, including the oral intake of food and beverages made from drinking water (WHO, 2011). There are different industrial processes, such as smelting, mining, and generation of power using coal that increase the concentration of As in the air. Environmental contamination by As also occurs because it is used in the manufacture of wood preservation products and pesticides. As is transferred through different means. For instance, As in soil or weathered rock is moved by water or wind. Many of As compounds bind to the soil and can move short distances when water passes through the soil (Garelick et al., 2008).

Airborne As may be inhaled by humans. The amount of inhaled arsenic that gets absorbed into the bloodstream depends on the size of the particles and how soluble the particles are in the blood. In most instances, As is converted and eliminated from the human body through urine. Blood samples, nails, and hair can also be used to determine the concentration of As in the human body. The inhalation or ingestion of As may cause different disorders, including respiratory problems, skin lesions, different types of cancers, and problems with the nervous system (Chung et al., 2014). Exposure to As may result in different health complications, including shock, dermatitis, hemolytic anemia, cardiac arrhythmias, hepatic and renal damage, black foot disease, hearing loss, diabetes mellitus, and developmental effects (Abernathy et al., 2003). Also, As cause skin, lung, liver, bladder, kidney, and prostate cancer (Hong et al., 2014; Mohammed Abdul et al., 2015). Arsenic and inorganic arsenic compounds are in Group 1 according to the IARC classification (IARC, 2012a).

Barium (Ba)

Barium is used as an alloy together with nickel to develop alloys that are used in spark plugs. Ba is also used as an oxygen-removing agent. The other uses of Ba are in fluorescent lamps and the oil and gas industry in the process of making drilling mud. Ba compounds are also used in the chemical processes for the manufacture of bricks, paints, rubber, and glass (Bhoelan et al., 2014).

Ba is abundant in the environment. High concentrations of Ba can be found in soils and different foods, including fish, seaweed, nuts, and certain plants. Due to their extensive use in different applications, there is a significant concentration of Ba in many locations of the globe (Kravchenko et al., 2014).

Ba enters the air mainly through mining activities. It also enters the air during the production of Ba compounds. There are also traces of Ba that are produced during oil and gas combustion (Bhoelan et al., 2014).

Most of the health risks of Ba originate from the inhalation of Ba compounds within their working environments. Some of the health effects associated with Ba inhalation include increased blood pressure, difficulties in breathing, muscle weakness, stomach irritation, swelling of the liver, brain and heart damage, among other complications (Kravchenko et al., 2014).

Beryllium (Be)

Beryllium is an industrial metal that has unique properties in the sense that it is lighter than aluminum while stronger than steel. It is due to this property that Be is alloying with other metals, such as copper. Be is used in the electronics and aerospace industries (Taylor et al., 2003).

Be is emitted to the atmosphere through volcanic activity and windblown dust. There are also anthropogenic sources or Be emission, including the combustion of fossil fuels that release particulate matter and fly ash that contains beryllium compounds (Bruce et al., 2011).

The other sources of Be in the environment include metal fabrication and ore processing. Be enters waterways through the weathering of soils and rocks; this includes industrial and surface waters from runoff or Be related activities (Bruce et al., 2011). The respiratory tract, especially the lung, is the main target of Be exposure. This shows that increased exposure to Be mainly affects the lungs and leads to lung complications. Beryllium and beryllium compounds are in Group 1 according to the IARC classification (IARC, 2012a).

Cadmium (Cd)

Small traces of cadmium are found in air, soil, water, and human food. For most people, food is the main source of Cd exposure. The main reason is that foods, especially plants, tend to absorb Cd (Lemos et al., 2010). Also tobacco smoking is one of the main sources of exposure to cadmium (Laamech et al., 2014).

In nature, there are only traces of Cd present. The chemical properties of Cd are similar to those of Zn. This is because Cd is obtained as a by-product of Zn refinement. Transport of Cd to the environment takes place in either water or air. Cd in air exists mainly in particulate form. This shows that Cd is at very low concentrations in air. However, it is still a chemical of concern because of its high toxicity, even at low concentration levels (Yu et al., 2010).

The largest sources of Cd in the environment include the steel industry and waste incineration. Other sources are Zn production and volcanic activity. Disposal of waste results in the largest deposition of Cd in land. Cadmium then finds its way in the body of plants and animals, which are then consumed by humans as food (Lemos et al., 2010).

Exposure to Cd may result in different health complications, including bone problems, lung conditions, and formation of stones in the kidney. Cd may also affect fetuses meaning that pregnant women should be kept away from Cd sources (Fort et al., 2014). Cadmium and cadmium compounds are in Group 1 according to the IARC classification (IARC, 2012a).

Chromium (Cr)

Chromium is (an essential trace element) associated with carbohydrate metabolism (Yoshida, 2012).

Cr is used mainly in metal alloys, such as stainless steel and metal ceramics. This metal is highly valuable in the industrial world because it can be polished to provide shinny and rust-resistant coating (Moreno et al., 2010). The availability of Cr in the environment is due to the different industrial activities, such as textile, leather, and steel manufacturing. The inhalation of Cr may have negative effects on the health and welfare of humans (Oliveira et al., 2012).

Cr may find itself in water bodies through the weathering of rocks containing Cr, leaching of soils, and discharge from industrial operations, among others (Oliveira et al., 2012). The water may then be consumed by plants and animals which are later consumed by humans (Oliveira et al., 2012).

Cr VI is categorized as the most toxic form of chromium among the other forms. However, increased exposure to Cr may result in different health complications, including ulcers, nose irritations, allergic reactions, weakened immune system, liver and kidney damage, and alteration of genetic composition. These effects may result in the death of the person (Oliveira et al., 2012). Chromium (VI) compounds are in Group 1 (IARC, 2012a), while Chromium metallic form is in Group 3 according to the IARC classification, (IARC, 1990).

Cobalt (Co)

Cobalt is essential to humans in the form of cobalamin (vitamin B12) (Lison, 2007). Co is a naturally occurring and element, which has properties that are similar to Ni and Fe. Small amounts of Co are found in soils, rocks, animals, plants, and water. Co is also found in the environment when combined with As, air, or sulfur. Co may be mixed with other metals to form alloys. These alloys are harder and more resistant to corrosion and wear (Internet f).

Co may enter the environment through natural means such as volcanic eruptions, forest fires, spraying, and windblown dust and human activities. Furthermore, there are small amounts of Co that may be released to the atmosphere through the vehicular exhaust, industrial activities, and coal-powered power plants. One of the properties of Co is that it cannot degrade in the environment (Internet f).

Co has serious effects on the lungs as it can cause difficulty in breathing, wheezing, pneumonia, and asthma (Cheyns et al., 2014). Co may result in hair loss, vomiting, bleeding, coma, diarrhea, and sterility. In some extreme conditions, prolonged exposure to Co may result in death (Cheyns et al., 2014). Co is in Group 2B according to the IARC classification (IARC, 1991).

Copper (Cu)

Copper is an essential metal performing a primary role in human metabolism, basically as a cofactor of many metalloenzymes (Pavelková et al., 2018). Copper is a reddish-brown metal that is mainly used in the manufacture of electrical conductors used for industrial activities and power distribution and also in the manufacture of alloys, including brass and bronze which are used in the manufacture of guns (ATSDR, 2004). Cu is a common substance that is naturally occurring in the environment. Industrial activities have resulted in the emission of copper-contaminated water. These are washed by rivers and streams. This water is consumed by plants and animals and directly or indirectly by humans. In addition, Cu enters the air through fossil fuel combustion (ATSDR, 2004). Cu can enter the air through both natural and human activities. Some of the natural activities include decaying vegetation, windblown dust, sea spray, and forest fires. Some of the human activities include metal production, wood production, and mining (ATSDR, 2004).

Long-term exposure to Cu may result in different health effects, including irritation of the nose, eyes and mouth, dizziness, stomach aches, and headaches, diarrhea and vomiting. High intake of Cu may cause kidney and liver problems and in extreme and dangerous cases result in death. Industrial exposure to copper fumes may cause Wilson’s disease (ATSDR, 2004). Cu is in Group 3 according to the IARC classification (IARC, 1977).

Iron (Fe)

Iron is an essential metal that presents in all body cells. It is a carrier of oxygen in the muscles and blood (as iron is a component of hemoglobin and myoglobin).

The iron industry has a significant impact on pollution in the global environment. The production of steel consumes huge quantities of minerals and energy. There are also lots of wastes that are generated from the Fe and steel industry. The implication of this is that there are significant air pollutants, residues, and solid by-products that are generated from the Fe industry. Pollution from the Fe industry takes different forms, and its impacts do not affect one geographical area as they could spread to regional and even global regions. The air pollution from the Fe industry is due to the emission of particulate matter containing Fe and iron oxide. The pollution may also contain other metals, including Cr, Cd, Zn, Cu, Pb, and As though these are metals are in trace form (Tiwari et al., 2016).

There are certain Fe plants that contain both organic and inorganic compounds. This means that the Fe industry is also a source of carbon emissions during the process of steel making when iron is mixed with carbon. In addition, the Fe industry is also a source of particulate matter which affects the visibility of persons living within the affected environments (Tiwari et al., 2016). Iron and steel founding (occupational exposure) are in Group 1 according to the IARC classification (IARC, 2012b).

Lead (Pb)

The global concentration of lead has reduced significantly over the last decades. The reason for this is that many nations have removed tetraethyl lead from gasoline. However, there is still a possibility of human exposure to Pb due to different sources, such as dust, salt, and beverages that are used and consumed daily by humans. In addition, cigarette smoke can increase the volume of Pb ingested daily (Laamech et al., 2014). Also, Pb may enter the environment through the combustion of fossil fuels, the use of Pb compounds (e.g., batteries), production (including smelting and mining), disposal, and recycling if Pb compounds (Komárek et al., 2008; Ahlberg et al., 2006). Foods, such as fruits and vegetables, meats, seafood, grains, wine, and soft drinks may also contain traces of lead if such plants and animals were raised in environments that were contaminated with Pb (Lemos et al, 2010).

Blood is usually considered as the most representative matrix to help in the identification of Pb in the body of humans. However, the collection of blood samples is difficult in children due to children's psychological state. Urinary Pb that is released through urine considered a second alternative. This is because it is non-invasive (Cao et al., 2015). Pb is in Group 2B according to the IARC classification (IARC, 1987).

Manganese (Mn)

Manganese is an essential element. Mn involved in the activation and synthesis of numerous enzymes. Also, Mn plays a role in the regulation of the metabolism of glucose and lipids in individuals (Li et al., 2018; Heitland et al., 2006). Mn is found naturally in many types of soils and rocks. There are also trace levels of Mn in food, air, and water. The main anthropogenic sources of Mn include emissions from Mn mining, welding, alkaline battery manufacturing, and alloy production, among others. These sources interfere with the natural levels of Mn. In addition, there is also extensive use of Mn in fertilizers and fungicides in some countries, thereby increasing the concentration of Mn in the environment (Röllin et al., 2011).

Some of the health effects of Mn exposure include lung and kidney conditions, heart problems, and problems with the central nervous system (Röllin et al., 2011).

Mercury (Hg)

Mercury is a persistent and toxic chemical that finds its way into the human body through water and food. After Hg is released into the environment, it finds its way into water laid sediments. This is then converted to toxic methylmercury, which enters the food chain. Methylmercury is a significant public health concern because of the ease with which it enters the human brain (Laamech et al., 2014).

There are two main sources of Hg, including natural sources and anthropogenic sources. Natural sources of Hg include forest fires, volcanic activity, and fossil fuels. Anthropogenic sources include Hg discharge from mining, waste incineration, fuel combustion, and paper industry. Incineration of medical and municipal waste also contributes to increased levels of anthropogenic Hg (Driscoll et al., 2013).

Hg can travel several kilometers after it is released to the atmosphere before it finally deposits on the earth's surface. Hg cycles back between air and soil and takes different forms. The health concerns of Hg are related to the consumption of fish and uptake of water that has been contaminated with Hg. This can cause certain brain problems. In addition, Hg compounds may find themselves in the bloodstream of fetuses affecting the brain and overall development of the unborn fetuses (Driscoll et al., 2013). Mercury and inorganic mercury compounds are in Group 3, while Methylmercury is in Group 2B according to the IARC classification (IARC, 1993).

Molybdenum (Mo)

Molybdenum is a naturally occurring rare metal found in soil and rocks. Molybdenum is a micronutrient essential for life. Mo is part of a complex, this complex called (molybdenum cofactor), which is needed for the three mammalian enzymes sulfite oxidase (SO), xanthine oxidase (XO), and aldehyde oxidase (AO) (Sardesai, 1993; Heitland et al., 2006). Mo also found in plants and animals. Mo is applied in different functions, including the production of cast iron, stainless steel, and structural steel, among other alloys. Mo is also extensively used in the electronics industry and specialty applications. Mo is emitted from industrial and agricultural activities, e.g., as a result of fossil-fuel combustion, from mobilization and fly ash of mine wastes (Smedley et al., 2017).

Humans are exposed to Mo through food and water. In addition, inhalation of air that is contaminated or polluted with Mo is also a source of exposure. Workers in different factories or industries may also be exposed through inhalation of dust fumes that are generated during metalworking or mining (Smedley et al., 2017).

Mo is biologically inactive unless when complexed by a cofactor. This is the reason why the element is a cofactor in many enzymes. These enzymes are important in the global recycling of sulfur, carbon, and nitrogen (Smedley et al., 2017).

The toxic effect of Mo on humans and animals depends on the chemical form and animal species; this also depends on how humans are exposed to Mo, either through inhalation or ingestion. In humans, extreme exposure to Mo may result in the disruption of metabolic processes and deformation of bones (Pasieczna et al., 2017). Molybdenum trioxide is in Group 2B according to the IARC classification (IARC, 2018).

Nickel (Ni)

Nickel is an essential trace element that is important for both plant and animal health. Ni is released to the environment through mining, smelting, mining, and industrial activities. Inhalation exposure in both indoor and outdoor settings and environments is the primary route for Ni toxicity. Some of the negative health effects of toxicity include adverse effects on the immune and respiratory system (Cempel et al., 2006).

Occupational exposure to Ni is dependent mainly on industrial activities. The meaning of this is that workers in different industries are exposed to different levels of Ni in their work environments. Ni exposure results from inhalation of one of the following substances: aerosols that are developed from Ni solutions, dust, and gaseous compounds containing Ni (Cempel et al., 2006).

Exposure to Ni is mainly through oral intake of either contaminated water or food. Ni may also affect persons dealing in the stainless steel industry as Ni plating is used in stainless steel (Cempel et al., 2006). Ni, metallic and alloys are in Group 2B (IARC, 1990), while Ni compounds are in Group 1 according to the IARC classification (IARC, 2012a).

Selenium (Se)

Selenium is an essential element (non-metal). It is one of the important antioxidant enzymes components (such as glutathione peroxides and thioredoxin reductase (Bhattacharya et al., 2016).

Se is a naturally occurring trace element. Se is released to the environment through agricultural, manufacturing, petrochemical, and mining operations. It has a chemical property of bioaccumulation. The implication of this is that even low levels of Se may reach trophic levels, thereby affecting plant and animal life. From the above, it is seen that Se pollution takes place in urban, rural, and suburban environments (Staicu et al., 2017).

Exposure to Se may result in significant health problems, including heart and lung diseases, eye problems, and kidney problems, among others (Staicu et al., 2017). Selenium and selenium compounds are in Group 3 according to the IARC classification (IARC, 1975).

Tin (Sn)

Tin oxide is an insoluble compound, which also resists weathering. The implication of this is that the concentration of Sn in soil and air is relatively low when compared to other heavy metals. However, tin may be released to the atmosphere through agricultural activities, road construction, and windstorms. The other sources include volcanic emissions, continental dust flux, and forest fires that also emit Sn to the environment. Sn may also be generated from anthropogenic sources, including industrial and waste incineration and the burning of fossil fuels (Cima, 2018).

The organic compounds of Sn are more dangerous when compared to inorganic compounds. Despite the inherent dangers, tin is heavily used in different industries, including agriculture, painting, and the plastic industry. Prolonged or heavy exposure to Sn may cause stannosis, which is a disease that affects the lungs (Cima, 2018).

Vanadium (V)

Vanadium is nutritionally essential for humans and animals, as vanadium stimulated the mineralization of bones and teeth (Nielsen et al., 1990).

V is a rare element that is used in the manufacture of certain alloys These alloys are used in jet engines, air-frames, and shafts of motor vehicles. Vanadium oxide is used in the manufacture of sulfuric acid. V is released to the environment through marine aerosols, volcanic emissions, and the formation of continental dust (Fortoul et al., 2014).

V is never unbound. It found in carbon-containing deposits, such as coal, tar sands, oil shale, and crude oil. One of the main ways in which V is distributed throughout the earth’s surface is watering. The reason for this is that V is soluble in water. The uptake of V by humans occurs mainly through different foodstuffs, including eggs, apples, soya beans, buckwheat, sunflower oil, and olive oil (Fortoul et al., 2014).

V is not regarded as a serious health hazard. However, persons exposed to high levels of V may experience eye, nose, and throat irritation. Extreme exposure to V leads to lung problems such as pneumonia and bronchitis. The other effects of V exposure include bleeding of lungs and kidneys, damage to the nervous system, heart problems, headaches, nose bleeding, throat pains, and skin rashes (Fortoul et al., 2014). Vanadium pentoxide is in Group 2B according to the IARC classification (IARC, 2006).

Zinc (Zn)

Zinc is a bluish-white metal that is used in galvanizing steel. It is also used in electric batteries and in roofing gutters for building and construction. Zn is also used in the motor vehicle industry. Zinc oxide is used in the manufacture of paints and rubber. Zn is also used as a pigment in the plastic industry, wallpaper manufacture, cosmetics, pulp and paper industry (ATSDR, 2005).

Zinc is an essential nutrient for humans. It plays a role as a cofactor for many enzymes, including those involved in protein synthesis and DNA and RNA replication (McClung et al., 2005) (King, 2011). Zn occurs naturally in water, soil, and air. However, concentrations of zinc rise unnaturally due to human activities, such as manufacturing, construction, agricultural activities, and mining. This shows that persons who work in coal mines and the smelting industry have higher exposure levels of Zn. Zn emitted from many industrial plants. In addition, traffic pollutants may also have significant proportions of zinc (ATSDR, 2005).

Some of the health problems of Zn include skin irritations, stomach cramps, anemia, nausea, and vomiting. Extremely high levels of Zn may result in damage to the pancreas and affect the metabolism of proteins and respiratory disorders. Zn may also be a danger to unborn children meaning that pregnant women should not be exposed to high levels of Zn (ATSDR, 2005).

2.6. Exposure to Environmental Air Pollution

Environmental exposure to air pollution results from inefficient combustion of fuel used in cars, trucks and other human activities, such as cooking and heating. Industrial activities also generate pollutants that may harm human health other than workers (Gurjar et al., 2010). The most known and common environmental air pollutants are generating from burning activities such as traffic, heating, electricity generation, and households (Inernet g). Also tobacco smoking, construction material, fuel (e.g., Coal, charcoal, wood, crop wastes, and cow dung) used for cooking, lighting, heating, use of repellents, pesticides, and cleaning chemicals contribute to indoor air pollution and then to outdoor, environmental air pollution (Apte et al., 2016). Another massive scale contribution to air pollution is from industrial activities, such as electricity and cement production, also incineration and management of waste cause emissions of many substances. These emissions include many chemicals and materials like nitrogen oxides, sulfur oxides, dust, mercury, other toxic and heavy metals, and ammonium into the atmosphere, earth, and water (Al-Hasnawi et al., 2016). Thermal power plants are among the sources of environmental air pollutants for whom living close to these facilities (Mittal, Sharma and Singh, 2012). Thermal power plants emissions depend on the qualities of coal, operating conditions, and different combustion technologies (Mittal, Sharma and Singh, 2012). The main types of pollutants related to thermal power plant environments are carbon dioxide, carbon monoxide, sulfur dioxide, nitrogen dioxide, Particulate emissions, and trace elements like Cadmium, Lead, and Mercury (Mittal, Sharma and Singh, 2012). In all these human activities, producing air pollutants, the metals take crucial place. The toxic metals in air pollution come from industrial, traffic, domestic, agricultural, technological, and medical applications. These sources have led to the wide distribution of toxic metals in the environment (Tchounwou et al., 2012).

Also, there is contribution of indoor air pollutants to the outdoor air pollutants such as indoor heating and cooking using fuel, of dung, straws, agricultural residues, and coal. The burning of these fuels generate smoke that contains different harmful pollutants (Gurjar et al., 2010).

2.6.1. Exposure of children to air pollution

Children are not exposed to occupational pollutants, do not drink alcohol, and do not smoke. Children's exposure to air pollutants is limited to their specific living environments like their school and home environments (Gajski et al., 2013). Children are more vulnerable when exposed to air pollution when compared to adults. According to WHO, approximately 93% of the children (under the age of 15 years) in world breathe air that is polluted with different air pollutants which cause to serious health risks, including lung cancer, stroke, and heart diseases (Internet h).

Environmental air pollution is assumed to affect children and elderly people much more among the population. Children are increasingly vulnerable to these pollutants because their biological systems cannot adequately process the pollutants (Gurjar et al., 2010). Also, the fact that children have undeveloped defense mechanisms, in addition, they inhale much more air per body weight than adults (Salvi, 2007).

2.6.2. Differences between children and adults for air pollution exposure

Children are more susceptible to the toxic effects of environmental and dietary chemicals than adults (Salvi, 2007; Neri et al., 2006). The possibility of consuming more contaminated food and hand-to-mouth habits higher in children. They drink more than adults. Their body mass index (BMI) of children is higher than adults. In addition, in comparison to adults, children more exposed to indoor dust and have more contact with soil (Neri et al., 2006; Silva da Silva et al., 2015). Children are at high risk due to their unique and different exposure pathways, dynamic developmental physiology, and their longer life expectancy (Internet h).

Air pollutants have adverse health effects on both children and adults. However, children are more sensitive, as their lungs and defense mechanisms are still developing, so early exposure to air pollutants may cause alteration in lung function easier than adults (Esposito et al., 2014) In addition, they inhale much more air per body weight than adults (Salvi, 2007).

Children are exposed to air pollution especially traffic related one more than adults, because of their physical activity differences, and spending more time outside. Besides, the underdeveloped lung and immune system exacerbate the effects (Karen Huen et al., 2007). Children are mainly exposed to air pollution at home, but they spend most of their daily time at school. Therefore, they might be affected from traffic-related air pollution while they are at school and on way to school and moreover, they do physical activity in their school hours, which increase the risk of higher air pollution exposure (Mejía et al., 2011).

Table 2.1. Factors affecting children's sensitivity to air pollution*

Category	Factors
Physiological factors	• In comparison with adults, air-breathing capacity per unit body weight is high ↑ • Performance of lungs and airways in children ↓ • Amount of detoxifying enzymes ↓
Lung development and pulmonary functions	• Insufficient defence of developing airlines and alveoli • Immature immune system
Time-activity models	• Time spent outside ↑ • Ventilation rate during play and exercise ↑, mouth breathing ↑
Chronic diseases	• Prevalence of asthma ↑ • Prevalence of cystic fibrosis ↑
Acute diseases	• Frequency of acute respiratory infections ↑

*Modified from (Özata, 2019), (Silva da Silva et al., 2015) (Villarini et al., 2018))

2.7. Air Pollution Biomonitoring

2.7.1. Biomarkers in molecular epidemiology

Pollution biomonitoring biomarkers may be defined as a quantitative measure of the different changes in a biological system when compared to the healthy status of an individual in response to exposure to pollution (Dagnino et al., 2008). The cellular and molecular biomarkers can help in providing early warning health related signs to individuals and populations (Lionetto et al., 2019). The biomarkers provide relevant information on the extent of exposure to pollution and the potential impact of this exposure on the health of the persons exposed. Biomarkers can also be used to measure the underlying vulnerability of an individual. Through the biomarkers, professionals can better understand the processes through which chemicals are absorbed and used within the human body (Lionetto et al., 2019). Biomarkers can be categorized into biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (Schettino et al., 2012; Lionetto et al., 2019).

Biomarkers of exposure

Determination of human risk from pollutant exposure is done through the evaluation of biological samples, such as serum, urine, blood, fingernails, hair and saliva (Lemos et al., 2010). Continuous exposure to low levels of pollutants, such as lead and cadmium, can result in bioaccumulation of these metals within the body of humans. This results in negative health consequences (Lemos et al., 2010). Biomarkers of exposure can provide data on the route of exposure, pathway of exposure, and even sometimes give information about the source of exposure. These indicators provide information to the researchers about the sources of future exposures, in order to prevent further damage and adverse effects (Lionetto et al., 2019). The choice of the most appropriate biomarkers to be used depends on the metabolism rate and age of the patient. As has been mentioned, the use of blood as a biomarker in children raises some logistical challenges due to the state of mind of the children (Cao et al., 2015). There are two types of biomarkers of exposure; the first one is a quantitative measurement of a chemical or chemical metabolite in a biological fluid, and the second one is a measurement of an early reversible biochemical difference or change in a biological fluid that shows or reflects exposure (Lowry, 1996). Biomarkers of exposure can be used to evaluate and confirm the exposure of persons or communities to a particular substance, that can provide a connection between internal dosimetry and external exposures (Nordberg, 2010). Urinary and blood concentrations reflect to recent exposure (acute exposure), while biomarkers such as hair and nails levels reflect past exposure (chronic exposure) (Bermejo-Barrera et al., 1997).

Biomarkers of effect

A measurable physiological, behavioral, biochemical or other change or alteration within a human body that can be recognized as associated with an established or possible health disease or impairment. Biomarkers of effect can be used to document either preclinical changes or adverse health effects elicited by the absorption of a chemical and external exposure (Schettino et al., 2012; Lowry, 1996).

Moreover, the use of biomarkers of effect usually involve quantifiable alteration in physiologic or biochemical parameters such as the measurement of chromosome aberrations, cytogenetic endpoints, oxidative stress markers, sister chromatid exchange if the disease of interest is cancer (Silins et al., 2011). Genotoxicity may define as the property of chemicals to damage the genetic information within a cell that causes mutations, thereby leading to cancer. Biomarkers derived from human fluids or tissues. The source of the biomarker is therefore important to reduce the risk of cancer among other conditions on the patient. The cerebrospinal fluid is more associated with risk when compared to blood and urine (Mayeux, 2004).

Biomarkers of susceptibility

A susceptibility biomarker is one that is associated with a decreased or increased chance of developing a medical condition or disease in an individual who, from a medical or clinical point of view, does not have the risk of that condition. An example of a susceptibility biomarker is a genetic biomarker that can be used to detect the risk of cancer in an individual (Sabbioni et al., 2006; Kelly et al., 2014). Other examples of biomarkers of susceptibility are such as genetic polymorphism and metabolic phenotype (Kelly et al., 2014). Susceptibility biomarkers may be detected many years before the manifestation of clinical symptoms (Gupta, 2014).

2.8. Biomonitoring of Air Pollution by Analyzing Metals in Urine Samples of Children

Until today, many studies have conducted on air pollution and metal analysis in urine samples of children. When the scientific literature is searched, the industrial air pollution studies are found to investigate As, Ba, Be, Bi, Cr, Cd, Co, Cu, Fe, Mn, Hg, Mo, Ni, Pb, Sr, Ti, Sn, U, and Zn. (Molina-Villalba et al., 2015; Ochoa-Martinez et al., 2016; Cao et al., 2015; Moreno et al., 2010; Trejo-Acevedo et al., 2009). In case the studies about air pollution caused by traffic, the investigated metals are found to be As, Ba, Be, Cd, Co, Cu, Cs, Mn, Hg, Mo, Ni, Pb, Pt, TI, Th, U, V, Zn, Sb, and Se (Roca et al., 2016; Wilhelm et al., 2013).When the studies examining both industrial and traffic related metals investigated common metals were As, Cd, Cr, Cu, Ni, Pb, U, Bi, Be, Sn, and Tl were examined (Sughis et al., 2014; Aguilera et al., 2010). In most of the studies, the quantification of metal levels in urine samples was performed using inductively coupled plasma mass spectrometry (ICP-MS). In the rest of the studies Atomic Absorption Spectrometry (AAS) was utilized.

In a study conducted in Tunçbilek, levels of As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn in soil showed that the much higher levels of As, Cr, Hg, and Ni than that of the world soil average levels whereas the rest of the metals investigated (Cd, Cu, Pb, and Zn) were below world soil averages (Özkul, 2016). Also, in a recent study in the city center of Kütahya, (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) metal levels are measured in soils of children playgrounds. It is stated that metals such as As, Cd, Hg, Ni, Pb and Zn have been found to be high enough to cause risk in the children playgrounds (Özkul, 2019).

Below the studies in the similar design to the present study are summarized. All these air pollution evaluating studies are carried out in children analyzed the urinary metals and related to traffic and/or industrial air pollution. Some of these studies did not have control group and they compared their data either with other air pollution studies' data or international/national levels. The summary of the mentioned studies are presented in Table 5.1.

- Aguilera et al. (2010) Collected urine samples from 227 children (5-17 years old) living in Ria of Huelva (south-west Spain) to investigate the effect of industrial air pollution which includes the capital city of Huelva highly polluted because of long-term mining and industrial activities. As a control group, urine samples were collected from 196 children of the less industrialized small cities of Andalusia. **As, Cd, Cr, Cu**, and **Ni** metals were analyzed. As a result, both Huelva's Ria and other Andalusian cities in the urinary levels of the mentioned metals were in the range of regulatory values when compared to values in Europe and the US. Only Cd levels were statistically significantly increase in the industrial area States (Aguilera et al., 2010).

- Molina-Villalba and colleagues (2015) investigated the impact of air pollution in the same region of Huelva (Andalusia, Southwest Spain). They collected urine and hair samples from 261 children living in in urban, rural and metropolitan areas and did not have a control group (Molina-Villalba et al., 2015). **As, Cd, Pb, Mn**, and **Hg** metals were analyzed. They stated that **Cd** and **Hg** body burden (25-50%) of the child population living near the industrial and factory areas might have health impact but other metals were found to be in the range of regulatory values (Molina-Villalba et al., 2015).

- Ochoa-Martinez and colleagues (2016) investigated the impact of air pollution in Ciudad Juarez, Chihuahua, Mexico, which is Highly population are, close to the large manufacturing center and electronic companies (Ochoa-Martinez et al., 2016). , In urine samples of 135 children **As** and **Hg** were determined. Both As and Hg levels were higher when compared to national mean value of the US (NHANES IV 2009) (Ochoa-Martinez et al., 2016).

- Roca and colleagues conducted a study in 2016 in the region of Spain, Valencia, to investigate the effect of traffic air pollution. Urine samples were collected from 125 children (6-11 years old) from two primary schools, one from an urban area (Valencia) and another from a rural area (Alzira). **Cu, Co, Mn, Mo, V, Zn, As, Ba**, **Be, Cd, Cs, Ni, Pb, Pt, Sb, Se, Th, TI, U,** and **Hg** metals were analyzed. The average levels of **As, Hg**, and **Pb** were remarkably higher than of Huelva, Spain (Molina-Villalba et al., 2015), the reference values of USA (CDC, 2009), and Canada (HealthCanada, 2012). **Ni** and **Sb** levels were higher than those reported in Canada (HealthCanada, 2012) and the UK (Morton et al., 2014). However, **Cd** and **U** levels were similar or lower than those reported in Canada (HealthCanada, 2012), the USA (CDC, 2009), and Germany (Heitland et al., 2006). The concentrations of **Ba, Cs, Ti** found in this study were similar to those reported for children in the USA (CDC, 2009). The levels of **Co, Cu, Mn, Mo, Se, V**, and **Zn** within the range of values reported in Europe, USA, and Canada.

- Sughis and his colleagues (2014) collected urine samples from 339 (8-12 years) urban school and rural working children in the region of Lahore, Pakistan to investigate the impact of traffic and industrial air pollution. The children population of the study was chosen from 4 different sites. These are, high air pollution area (n=100), lower air pollution area (n=79), carpet weaving industry area (n=80), and brick industry area (n =80). The analysed metals were **Pb, As, Cd, U, Bi, Be, Sn,** and **T1.** . In general, the urinary concentrations of several metals (including **As, Cd, Pb,** and **U**) were higher than international reference values. In contrast, the levels of **Bi, Be, Sn, and Tl** were below detection limits. Also, the concentrations of **V, Zn, As, Cd** were significantly higher for the children working in brick kiln industry. As a result, Muhammad Sughis et al. reported that the children living in rural areas or around Lahore highly exposed to various metals such as **Pb, As** and **U** (Sughis et al., 2014).

- Wilhelm et al. (2013) conducted a study in Germany to investigate the effect of traffic air pollution. Urine samples collected from 1790 children (883 boys and 907 girls) 3–14 years old living in 150 study sites (communities or urban districts) to measure **Ni** levels. Wilhelm and his colleagues, for the first time, provided nickel levels in urine samples of children nationwide. In this study, food intake rich in nickel is suggested to increase the level of nickel in the urine. On the other hand, exposure to industrial sources may also increase urinary nickel levels (Wilhelm et al., 2013).

- Moreno et al. (2010) collected urine and blood samples from 50 children (6-11 years) living in the region of Taxco, State of Guerrero, located in Southern Mexico, to investigate the effect of industrial air pollution. Urinary concentrations of **As, Hg, Cr, Ni, Cd, Ba, Co, Cu, Zn, Mn, Mo, Si,** and **Fe** metals were determined. The results compared with the children's values of other countries. **Pb, Ni, Ba, Mn, Cd, Cr, Co, Cu, As,** and **Hg** metal levels were statistically significantly increased when compared to the reference values. **Zn** and **Mo** values were within the range of reference values (Moreno et al., 2010).

- Trejo-Acevedo et al. conducted a study in 2009 in Mexico to investigate the impact of industrial air pollution in the region. Urine and blood samples were collected from 229 (6-12 years) Mexican children living in high-risk areas (9 cities): mining, agriculture, large industries, small-scale industries, oil fields, and uncontrolled waste disposal sites. In this study, they compared their results with other studies results and reference values of the other countries. Urinary **As** and **Cd** metal levels were significantly increased when compared to the reference values. As a result, arsenic and cadmium are concerned only in some sites of mexico, while lead is a national concern (Trejo-Acevedo et al., 2009).

- Heitland and Köster conducted a study in 2006 in two different geographical areas of Germany (northern and western Germany) to indicate if population have trace element deficiencies or they exposed to higher elemental concentrations. Urine samples were collected from 87 adults and 72 (36 girl and 36 boy) children, children were divided into 3 age groups: (2–6/ 7–11/ 12–17 years). In each group there were 12 subjects. Also, they compared their results with other studies and reference values. **Li, Be, V, Cr, Mn, Ni, Co, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Rh, Pd, Ag, Cd, In, Sn, Sb, Cs, Ba, Pt,**

Au, Pb, Tl, Bi, and **U** metals were analyzed. **Cd, Pb, Tl, and U** concentrations were similar to the levels reported in the USA and European countries, Accordingly, As levels were significantly higher, while Bi levels extremely lower. In conclusion the result from this study could serve as a basis for the formulation of reference values for metals (Heitland et al., 2006).

- Wang and his colleagues (2019) collected urine samples of 214 (7.0 ±1.4 years) from rural area within two kilometers of a large coking plant (as exposure group) and from non-polluted rural area (as control group) in Shanxi Province, China to investigate the impact of industrial air pollution. The children population of the exposure group was 148, and control group was 66. The analysed metals were **Al, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, TI,** and **Zn.** In general, Urinary concentrations of most metals in the exposed group were higher than those in the control group (Z. Wang et al., 2019).

2.9. Determination of Metals and Metalloids

Determination of metals and metalloids gained special interest in recent years, since they have strong impact on the environment (Mbughuni et al., 2016), life and forensic sciences and in food, material, chemical, semiconductor and nuclear industries (Ammann, 2007). Mass spectrometry (MS) has become a powerful analytical technique with a wide range of application used in the toxicological analysis of chemicals (Mbughuni et al., 2016). Inductively coupled plasma mass spectrometer (ICP-MS) is widely used to analyze various samples because of its, high sensitivity, simultaneous monitoring deferent elements isotopes, relative salt tolerance, compound-independent element response and highest quantitation accuracy (Bahar F, 2016)**.** ICP-MS requires well-defined standards and/or reference materials for accurate calibration. Among the reference materials in the analysis might be; 1.pure substances (either essentially pure chemicals or well-characterized substances containing trace amounts of impurities); 2. Standard solutions and gas mixtures prepared from precursory pure substances; 3. Matrix reference materials (mimicking the chemical composition of the sample matrix) (Bulska & Wagner, 2016). The high ion density and the high temperature in plasma provide an ideal atomizer and element ionizer for all types of samples and matrices introduced by a variety of specialized devices (Ammann, 2007).

Inductively coupled plasma mass spectrometry (ICP-MS) is applicable to a wide variety of sample types; therefore, it is used in many different industries. To be processed efficiently in the plasma, samples must be in either gas or vapor (aerosol) form. So, while gases can be analyzed directly by the plasma, solids and liquids have to be converted to aerosol form using either a nebulizer (for liquids such as water, urine, blood samples, among others) or an ablation device (for solids) (Wilschefski et al., 2019). Solid samples and some liquid samples should be digested to decompose the organic substances. After the digestion step, samples can be directly analyzed by appropriate dilutions (Wilschefski et al., 2019).

3. MATERIALS AND METHODS

3.1. Design, Study Areas and Selection of Participants

The study area is located in Kütahya Province, one of the cities with high air pollution in Turkey. Kütahya (29°00'-30°30 ' East longitude and 38°70'-39°80' North latitude) located in Aegean Region with an area of 11,875 km^2 in the western part of Turkey (KDEU, 2015). Kütahya topography consists of mountains and hills and plains spread from northwest to southeast (Tuygun et al., 2017). According to the 2014 census, 68% of the populations live in an urban area, and the remaining 32% live in 550 different rural areas. For domestic heating, natural gas is used in some areas such as Kütahya City Center (KC), Emet, Gediz and Tavşanlı, while lignite is used for heating in rural areas (Küçükaçıl Artun et al., 2017).

There are three thermal power plants in the study region. The first one is the Seyitomer Thermal Power Plant with a capacity of 600 MWe, 20 km away from KC. The other plants are Tunçbelik Thermal Power Plant (365MWe), 50 km from the city center, and Orhaneli Thermal Power Plant (210MWe), 105 km from the city center (Küçükaçıl Artun et al., 2017; Tuygun et al., 2017). These thermal power plants use low quality lignite with a calorific value of 4800 kcal/kg and sulfur content of 2% (Tuygun et al., 2017). Also, other industrial pollution sources located around Kütahya Province include sugar, ceramics, food, transport, construction materials, boron mining, and magnesite industrial plants (Küçükaçıl Artun et al., 2017). Due to the fact that there are 12 main highways and 46 main urban streets in Kütahya, traffic is an important source of pollution. In 2014, the number of vehicles in the city was 184,661 (Küçükaçıl Artun et al., 2017) and 60% of it consist of passenger vehicles, while light-duty vehicles (minibusses, pickup trucks) and heavy-duty vehicles (buses, trucks) made up 36% and 4%, respectively (Küçükaçıl Artun et al., 2017; Tuygun et al., 2017).

Two different regions have been identified in Kütahya Province within the project (AUSRP-1407F398) in which this thesis study is a part of. The first region is Tunçbelik (TR) and represents the Tunçbelik Town and Gürağaç Village. Since Gürağaç Village belongs to Tucbilek Town, only Tunçbelik's name will be used when referring to the site. Tunçbelik is the rural area within the impact of two coal-fired active thermal power plants

(Tunçbelik Thermal Power Plant and Seyitomer Thermal Power Plant). Coal is also still being used for heating purposes in winter (Altug et al., 2015). The second study region is Kütahya City Center, which has been identified as the most dense traffic area. KC will be mentioned as the name of this region.

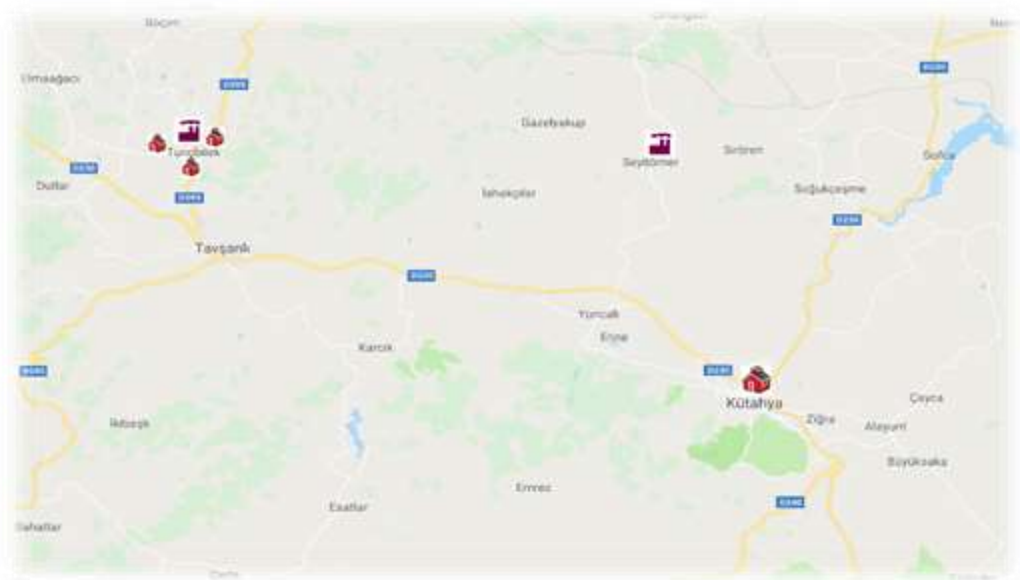
Four primary public schools in Kütahya Province are used to represent the two regions. Three of these primary schools are located in an industrial area near to thermal power plants in Tunçbelik town. 60. Yil Isık Primary School (I), is 1 km far away from Tunçbelik Thermal Power Plant and 35 km from Seyitomer Thermal Power Plant, the second one is Atatürk Primary School (A) located 2 km far away from Tunçbelik Thermal Power Plant and 35 km from Seyitomer Thermal Power Plant, and the third school is Primary School (G) located 3.5 km far away from Tunçbelik Thermal Power Plant and 39 km from Seyitomer Thermal Power Plant. The fourth school is Linyit primary school (L) located in the urban area with high traffic density in KC, which is 64 km far away from Tunçbelik Thermal Power Plant and 19.5 km from Seyitomer Thermal Power Plant (Table 3.1). The schools are chosen according to their locations and the number of students in the planned age range.

Table 3.1. The number of children in the study and the selected schools

Working area	Selected Primary School	Number of children included in the study	Teaching status*	Tunçbelik Thermal Power Plant distance	Seyitomer Thermal Power Plant distance	Altitude
	G	37	Normal education	3.5 km	39 km	853 km
TR (n: 88)	A	18	Normal education	2 km	35 km	840 km
	I	33	Normal education	1 km	35 km	836 m
KC (n: 72)	L	72	Dual education	64 km	19.5 km	937 m

*Normal education from 08:20 to 14:30, Dual education: from 7:30 to 13:20 and from 13:30 to 18:20, TR: Tunçbelik, KC: Kütahya City Center, G: Gürağaç Koyu primary schools, A: Atatürk Primary school, I: 60. Yil Isık primary school, L: Linyit primary school

Map 3.1. Kütahya Province map



Map 3.2. Kütahya Province map showing the location of Schools in Tunçbilek region and Kütahya City Center

In the scope of the AUSRP-1407F398, in January, February, May, and June of 2016, 4 field studies were conducted in the sample area and children were visited. Pollutant levels in the region where the schools are located, pollutant and wind mapping; respiratory function test data (forced expiratory volume (FEV1), forced vital capacity (FVC), peak expiratory flow rate (PEF), Forced expiratory flow (FEF25-75), Forced expiratory volume (FEV1/FVC)), body mass indexes (BMI), blood pressure and pulse parameters and individual samplers attached to the arms to determine the levels of ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) levels were determined. Also, in each field study, a detailed questionnaire was applied to children. In the survey; demographic, socioeconomic information about children and their families, possible allergic rhinitis, allergic eczema and asthma in children [The **I**nternational **S**tudy of **A**sthma and **A**llergies in **C**hildhood (ISAAC) survey, ISAAC 1998], information about the indoor environment of the child (domestic heating, smoking, pet ownership, mold and humidity) and the external environment (domestic heating, smoking, pet ownership, mold and humidity) were obtained. The present biomonitoring study is carried out during June 2016 study campaign of the AUSRP-1407F398 and from 160 children aged between 8-10 years additionally urinary samples are collected. In TR 88 children (33 children from I, 18 children from A, and 37 children from G), and in KC 72 children from L were the study population for the present thesis (Table 3.1). All the parents were informed and gave their written consents before the starting of the study.

3.2. Urine Sample Collection and Storage

First-spot morning urine samples were collected in polypropylene 100 mL containers (pretreated for 24 hours with 10% HNO_3 (v/v) followed by washing with deionized water and dried). The specific gravity of the urines was measured immediately after the collection by Refractometer UG-α. The samples transported to Gazi University Faculty of Pharmacy Toxicology Department Laboratory in cold chain. The samples were stored in -20 ^{0}C freezer until use for experiments.

3.3. Determination of Metals in Urine Samples

Seventeen metals; arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), vanadium (V), and zinc (Zn) were determined by using inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7800, Agilent Technologies, USA) (picture 3.1) equipped with a microconcentric nebulizer.

(a) (b)

Picture 3.1. a. Inductively coupled plasma mass spectrometer (Agilent 7800), b. autosampler

ICP-MS was operated in two acquisition modes: No gas and He mode. No gas mode means not using the collision cell, and He mode means when the collision cell is pressurized with pure He to overcome the polyatomic interferences. ICP-MS operating conditions were summarized in Table 3.2

Calibration solutions for determination of metals

All the reagents used were of analytical reagent grade and unless it was specified, all the solutions were prepared in deionized (DI) water with minimum resistivity of 18.2 MΩ.cm from a Milli-Q system (Millipore, Milford, MA, USA). Calibration standards were prepared by appropriate dilution of 10 mg/l mix stock solution in the range of 0.2 – 150 ng/mL whereas for Hg 0.2 – 5 ng/mL (Figure 3.1). All the calibration standards and urine samples were diluted to 5 mL with a solution that contains 2% HNO_3 and 0.5% HCl. ^{6}Li, ^{45}Sc, ^{72}Ge, ^{115}In, and ^{209}Bi were used as internal standards (IS) throughout the experiments.

Sample preparation for determination of metals

Before dilution, 2.5 mL of each urine sample were transferred to centrifugation tube, followed by centrifugation at 4000 RPM for 10 minutes. 500 µL were directly diluted in polypropylene tubes and completed to 5 mL with dilution solution (2%HNO_3 + 0.5% HCl), then followed by vigorous shaking and analyzed by ICP-MS. Each sample was prepared in triplicate.

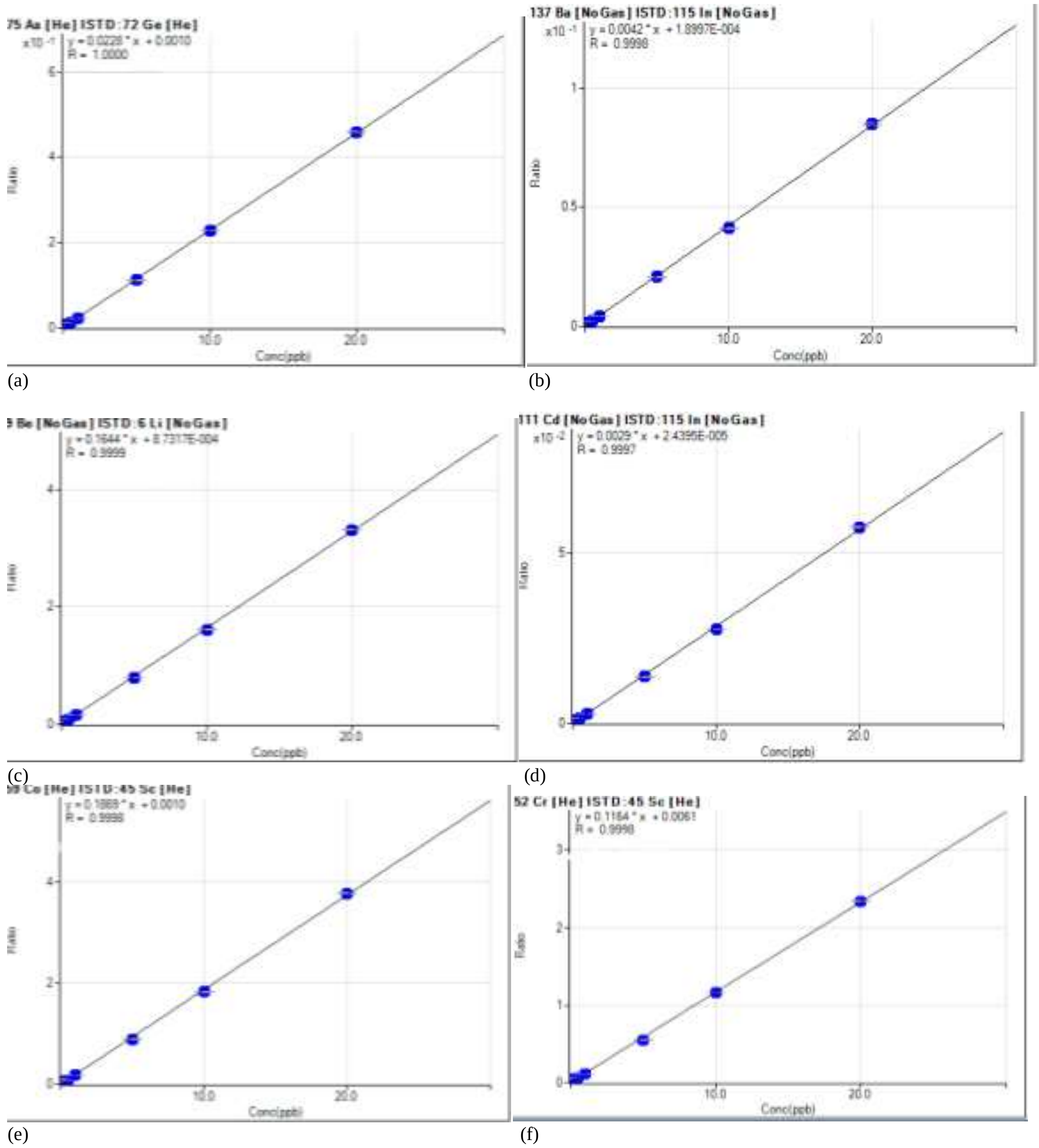
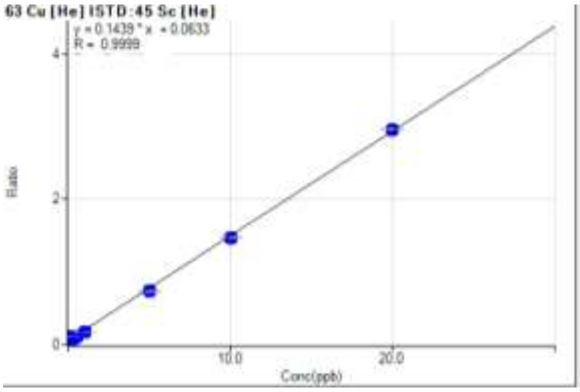
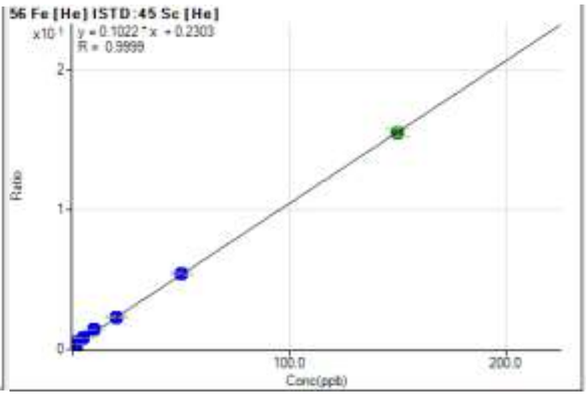


Figure 3.1. Inductively coupled plasma mass spectrometer calibration curves of (a) Arsenic, (b) barium, (c) beryllium, (d) cadmium, (e) cobalt, (f) chromium, (g) copper, (h) iron, (i) mercury, (j) manganese, (k) molybdenum, (l) nickel, (m) lead, (p) selenium, (o) tin, (p) vanadium, (q) zinc



(g)



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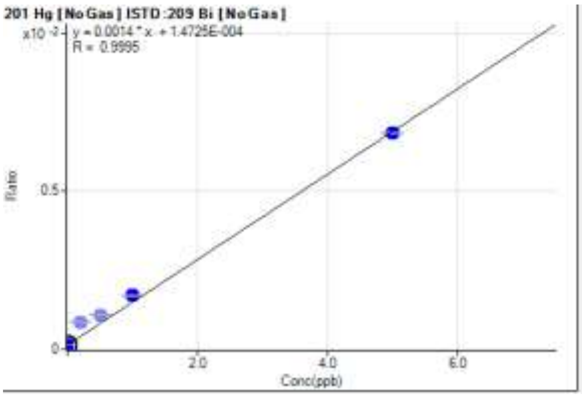
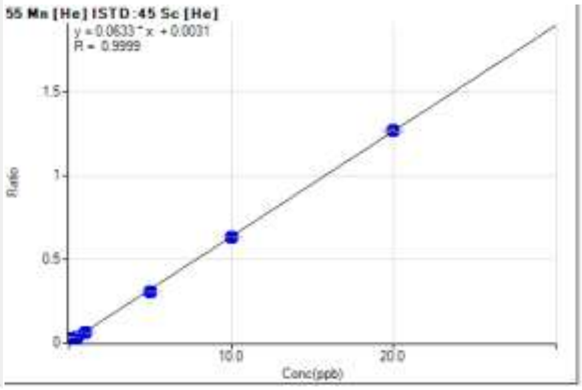


(i)



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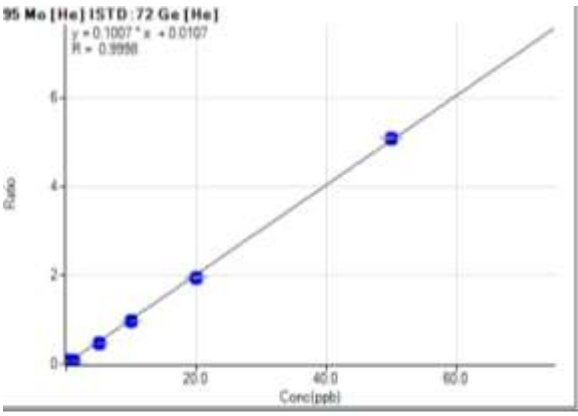
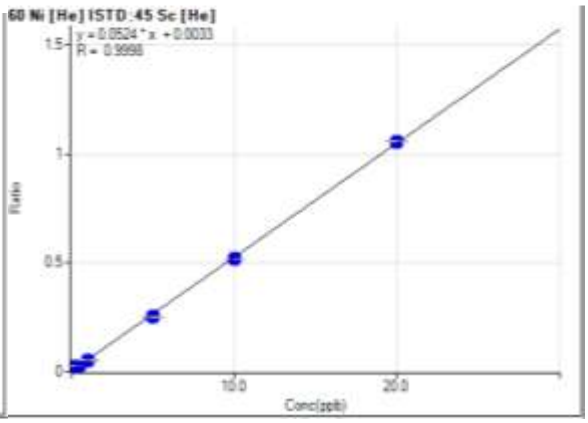
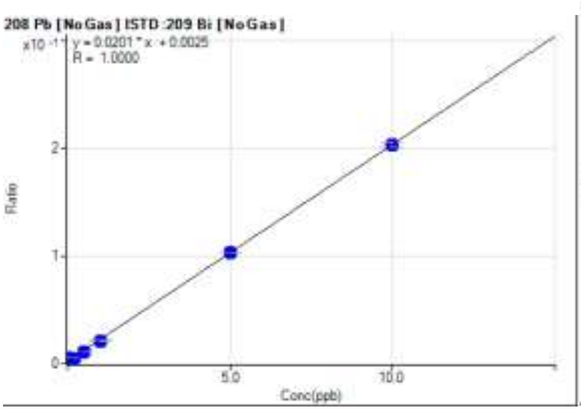


(k)

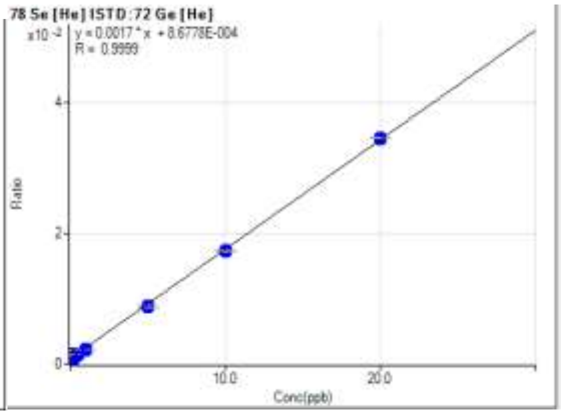


(l)

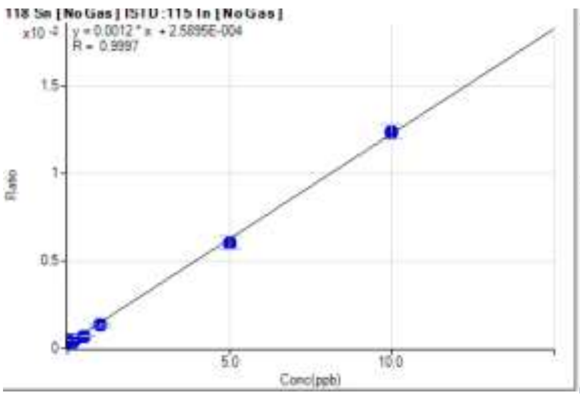
Figure 3.1. (continues) Inductively coupled plasma mass spectrometer calibration curves of (a) Arsenic, (b) barium, (c) beryllium, (d) cadmium, (e) cobalt, (f) chromium, (g) copper, (h) iron, (i) mercury, (j) manganese, (k) molybdenum, (l) nickel, (m) lead, (p) selenium, (o) tin, (p) vanadium, (q) zinc



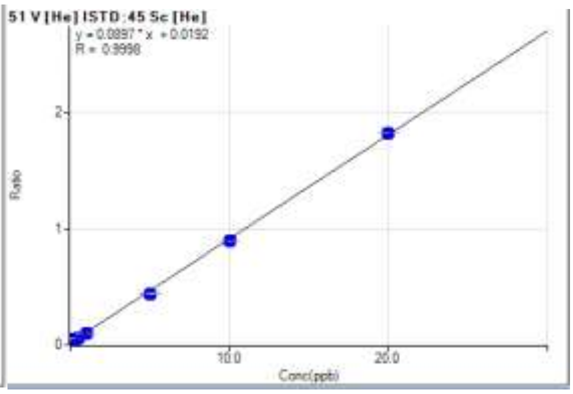
(m)



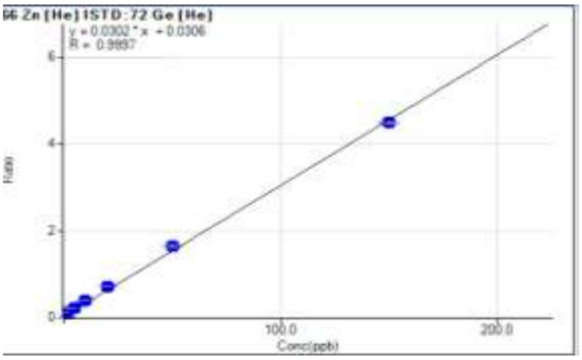
(n)



(o)



(p)



(q)

X: concentration (μg L^{-1}), Y: ratio (analyte signal/ internal standard signal)

Figure 3.1. (continues) Inductively coupled plasma mass spectrometer calibration curves of (a) Arsenic, (b) barium, (c) beryllium, (d) cadmium, (e) cobalt, (f) chromium, (g) copper, (h) iron, (i) mercury, (j) manganese, (k) molybdenum, (l) nickel, (m) lead, (p) selenium, (o) tin, (p) vanadium, (q) zinc

Table 3.2. Inductively coupled plasma mass spectrometer operating conditions

Instrument parameter	Operating condition
RF power (W)	1550
Plasma gas flow rate, L/min	15
Carrier gas flow rate, L/min	1.03
Sampling depth, mm	8
He flow rate in collision cell mL/min	4.3
Number of replicates	3
Isotopes monitored	Beryllium (^{9}Be), vanadium (^{51}V), chromium (^{52}Cr), manganese (^{55}Mn), iron (^{56}Fe), cobalt (^{59}Co), nickel (^{60}Ni), copper (^{63}Cu), zinc (^{66}Zn), arsenic (^{75}As), selenium (^{78}Se), molybdenum (^{95}Mo), cadmium (^{111}Cd), tin(^{118}Sn), barium (^{137}Ba), mercury (^{201}Hg), lead (^{208}Pb). Internal standards: ^{6}Li, ^{45}Sc, ^{72}Ge, ^{115}In, and ^{209}Bi

3.4. Creatinine Analysis of The Urine Samples

Working reagents for creatinine analysis;

- Creatinine (Sigma kat no C4255)
- Sodium hydroxide (NaOH) (Merck)
- Picric acid (Merck)
- Hydrochloric acid (HCL) (Merck)

The creatinine analysis of urine samples were carried out by using Agilent Cary 60 UV-Vis Spectrophotometer.

Analysis solutions were prepared as follows;

- 0,1 N HCl: 4,2 mL of concentrated HCl than completed to 500 mL with distilled water.
- %10 NaOH: 10 g of sodium hydroxide was weighed and then completed to 100 mL with distilled water.

- Picric acid solution: 11.75 g of picric acid was dissolved in distilled water and completed to 1 litre.
- Creatinine stock solution 0.5g Creatinine was dissolved in 0.1 N HCl, completed to 500 mL, and then kept in the fridge.
- Diluted creatinine solution: 4 ml stock creatinine solution, completed to 100 ml with 0,1 N HCl.

Creatinine analysis of urine samples were carried out as follows;

- 1 mL of urine was centrifuged at 3000 rpm.
- 0.1 mL of filtrate was taken up and completed to 10 mL with distilled water.
- 5 mL was taken to another tube and added 2.5 mL of picric acid and 0.5 mL of 10% NaOH, followed by vortex mixing then it was kept for 10 min and read at 520 nm in the spectrophotometer.
- Before the samples were read, a calibration curve (Figure 3.2) was created by preparing standards from diluted creatinine solution in the range from 0.0/ 0.2 / 0.4 / 0.6 / 0.8 / 1/2 / and 4 mg / 100 mL.

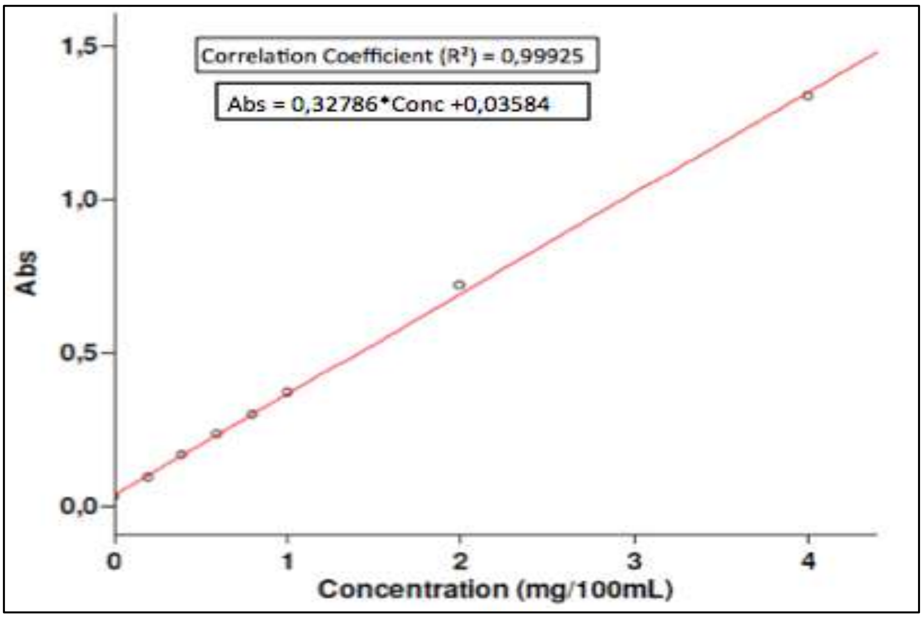


Figure 3.2. Creatinine calibration curve

3.5. Specific Gravity-Adjusted Urinary Metals (SG-UM)

Specific gravity of the urines was measured immediately after the collection of urine samples by Digital Urine Specific Gravity Refractometer UG-α (alpha). Specific gravity-adjusted urinary metal concentration (SG-UM) is calculated using the following equation (Cone et al., 2009);

$$\text{SG-UM} = \text{Metal concentration (X)} * \frac{\text{SG (average)} - 1}{\text{SG (X)} - 1}$$

(Where SG is Specific Gravity, and (X) is urine sample of child)

3.6. Statistical Analysis

A descriptive analysis is conducted, in which metal concentrations are described by using mean, geometric mean and standard deviations for continuous variables and for frequency (%) for categorical variables for regions and schools separately. Shapiro-Wilk test was used to determine whether continuous variables were compatible with normal distribution. Based on the results of this test, differences in mean metal concentrations between two groups were evaluated by using Wilcoxon Sign Test. On the other hand, for more than two groups Kruskal Wallis was carried out on metal concentrations to extract the group(s), which is significantly different from the others. Significance was considered for values of $p<0.05$. Pearson chi-square test was used to analyze the categorical data in the data set. Spearman's correlation test was used to determine whether there was a statistically significant correlation between continuous variables and also variables from other parameters of the Project-AUSRP-1407F398. All statistical analyses were performed by open source R software version 3.2.3 (Internet r).

4. RESULTS

4.1. Demographic Characteristics of Children

In the present study, metal concentrations in urine samples of 88 (56.8% girls) children from Tunçbilek region and 72 (44.4 % girls) children from Kütahya City Center were evaluated. The demographic characteristics of those children according to the region are shown in Table 4.1.

According to the study regions, there are no statistically significant differences in gender, BMI, and vaccination. But the age, smoking status of the parents and x-ray exposure are statistically significantly different in two regions (Table 4.1).

Table 4.1. Demographic characteristics of children living in Tunçbilek region and Kütahya City Center

	TR (n=88)	**KC (n=72)**	**Total (n=160)**	**p-value**
Gender (n(%))[a]				
Girl	50 (56.8%)	32 (44.4%)	82 (51.2%)	0.162
Boy	38 (43.2%)	40 (55.6%)	78 (48.8%)	
Age (year)[b,*]				
Mean (SD)	8.75 (0.725)	8.51 (0.435)	8.64 (0.621)	**0.038**
Med [Min, Max]	8.70 [7.45, 10.0]	8.51 [7.67, 9.98]	8.60 [7.45, 10.0]	
BMI[a]				
Mean (SD)	17.6 (3.58)	17.5 (3.61)	17.5 (3.58)	0.846
Med [Min, Max]	16.5 [9.76, 28.9]	16.9 [10.3, 28.2]	16.8 [9.76, 28.9]	
ETS (n(%)) [b,]**				
Yes	63 (71.6%)	35 (48.6%)	98 (61.2%)	**0.007**
No	25 (28.4%)	36 (50.0%)	61 (38.1%)	
Missing	0 (0%)	1 (1.4%)	1 (0.6%)	
Vaccination[b]				
Yes	5 (5.7%)	3 (4.2%)	8 (5.0%)	0.865
No	75 (85.2%)	67 (93.1%)	142 (88.8%)	
Missing	8 (9.1%)	2 (2.8%)	10 (6.2%)	
X-ray[b,*]				
Yes	8 (9.1%)	17 (23.6%)	25 (15.6%)	**0.021**
No	75 (85.2%)	51 (70.8%)	126 (78.8%)	
Missing	5 (5.7%)	4 (5.6%)	9 (5.6%)	

[a]Wilcox test, [b]chi square test, ETS: environmental tobacco smoke (father or mother of the children or both of them are smoking). TR: Tunçbelik Region, KC: Kütahya City Center, BMI: body mass index, SD: standard deviation, Med: median, Min: minimum value, Max: maximum value, *statistically significant difference ($p<0.05$), ** ($p<0.01$).

Daily water intake determined by the number of glasses of water daily, and children using vehicles (by car, school service, and bus) for transportation or walking between home and school are similar in two groups ($p > 0.05$). 50% of children in TR and 40.3% of children in KC go to school by walking.

The children in the present study, from 3 primary schools in TR (Gürağaç Primary School (G), 60.yil Işık Primary School (I), and Atatürk Primary School (A)), and from one school in KC (Lignite Primary School (L)) are also evaluated according to the demographic characteristics (Table 4.2). There are no significant difference between the distribution of boys and girls, BMI, smoking status of parents (mother and/or father), X ray exposure, and vaccination among the schools (>0.05, Table 4.2). Only 8 of children are declared of using vitamin supplementation. Daily water intake is similar among four schools. All the children in G are walking to the school and from the school, while the proportions in other schools are; 76.9% in A, 6.25% in I and 49.2% in L.

Table 4.2. Demographic characteristics of children according to their schools

	TR			KC
	Gürağaç Primary School (G) (n=37)	**Atatürk Primary School (A) (n=18)**	**60.yil Işık Primary School (I) (n=33)**	**Lignite Primary School (L) (n=72)**
Gender (n (%))				
Girl	27 (73.0%)	9 (50.0%)	14 (42.4%)	32 (44.4%)
Boy	10 (27.0%)	9 (50.0%)	19 (57.6%)	40 (55.6%)
Age (year)				
Mean (SD)	8.75 (0.755)	9.55 (0.252)	8.31 (0.449)	8.51 (0.435)
Med [Min, Max]	8.77 [7.45, 10.0]	9.54 [9.13, 9.92]	8.37 [7.49, 8.92]	8.51 [7.67, 9.98]
BMI				
Mean (SD)	17.8 (3.81)	16.8 (2.62)	17.7 (3.80)	17.5 (3.61)
Med [Min, Max]	18.1 [9.76, 26.1]	16.3 [11.7, 21.6]	16.1 [13.2, 28.9]	16.9 [10.3, 28.2]
ETS (n (%)) Y/N				
Yes	27 (73.0%)	14 (77.8%)	22 (66.7%)	35 (48.6%)
No	10 (27.0%)	4 (22.2%)	11 (33.3%)	36 (50.0%)
Missing	0 (0%)	0 (0%)	0 (0%)	1 (1.4%)
Vaccination				
Yes	0 (0%)	3 (16.7%)	2 (6.1%)	3 (4.2%)
No	34 (91.9%)	15 (83.3%)	26 (78.8%)	67 (93.1%)
Missing	3 (8.1%)	0 (0%)	5 (15.2%)	2 (2.8%)
X-ray				
Yes	3 (8.1%)	1 (5.6%)	4 (12.1%)	17 (23.6%)
No	32 (86.5%)	17 (94.4%)	26 (78.8%)	51 (70.8%)
Missing	2 (5.4%)	0 (0%)	3 (9.1%)	4 (5.6%)

ETS: environmental tobacco smoke (father or mother of the children or both of them are smoking). TR: Tunçbelik Region, KC: Kütahya City Center, BMI: body mass index, SD: standard deviation, Med: median, Min: minimum value, Max: maximum value

4.2. Urinary Metal Concentrations of Children

Metal concentrations in urine samples of children living in Tunçbilek region and Kütahya City Center region are shown in Table 4.3. Creatinine adjusted urinary levels of metals in urine samples of children according to region are shown in Table 4.4. While specific gravity adjusted urinary levels of metals in urine samples of children living in TR and KC are shown in Table 4.5. For the statistical comparisons of metal concentrations in these three tables median levels are used.

Urinary metal levels (R-UM) of of Be, V, Mn, Fe, and Pb are statistically significantly higher in KC, while UM of Cr, Co, Ni, Zn, As, and Ba are statistically significantly higher in TR than that of KC (Table 4.3) (Figure 4.1).

Table 4.3. Urinary metal concentrations (μg L^{-1}) of children living in Tunçbilek region and Kütahya City Center.

	TR (n=88)	**KC (n=72)**	**Total (n=160)**	**p-value**
Be*				
Mean (SD)	0.00825 (0.0205)	0.0154 (0.0263)	0.0115 (0.0235)	**0.029**
GM	0.00227	0.00367	0.00282	
Med [Min, Max]	0.00150 [0.00150, 0.111]	0.00150 [0.00150, 0.107]	0.00150 [0.00150, 0.111]	
V*				
Mean (SD)	1.03 (1.11)	1.53 (1.24)	1.26 (1.19)	**0.018**
GM	0.293	0.487	0.368	
Med [Min, Max]	0.589 [0.0170, 4.58]	1.57 [0.0170, 4.06]	0.940 [0.0170, 4.58]	
Cr*				
Mean (SD)	0.502 (0.322)	0.400 (0.316)	0.456 (0.322)	**0.013**
GM	0.386	0.238	0.311	
Med [Min, Max]	0.454 [0.00450, 1.92]	0.331 [0.00450, 1.75]	0.382 [0.00450, 1.92]	
Mn**				
Mean (SD)	2.36 (5.34)	2.10 (1.02)	2.24 (4.01)	**0.004**
GM	1.07	1.73	1.33	
Med [Min, Max]	1.39 [0.00650, 49.2]	2.09 [0.00650, 4.89]	1.68 [0.00650, 49.2]	
Fe*				
Mean (SD)	30.4 (71.3)	27.6 (14.1)	29.1 (53.6)	**0.016**
GM	18.4	23.8	20.7	
Med [Min, Max]	19.5 [0.200, 676]	27.1 [3.81, 72.4]	21.9 [0.200, 676]	
Co***				
Mean (SD)	1.72 (1.53)	1.14 (0.975)	1.46 (1.34)	**<0.001**
GM	1.31	0.758	1.03	
Med [Min, Max]	1.23 [0.321, 9.41]	0.845 [0.00100, 3.96]	1.00 [0.00100, 9.41]	

Table 4.3. (continues) Urinary metal concentrations (μg L^{-1}) of children living in Tunçbilek region and Kütahya City Center.

Ni* **				
Mean (SD)	7.97 (8.05)	4.78 (4.37)	6.53 (6.82)	**<0.001**
GM	6.45	3.58	4.95	
Med [Min, Max]	7.02 [1.33, 70.8]	3.80 [0.442, 29.8]	5.04 [0.442, 70.8]	
Cu				
Mean (SD)	17.1 (30.6)	12.9 (11.7)	15.2 (24.1)	0.261
GM	12.4	10.8	11.7	
Med [Min, Max]	12.2 [3.95, 276]	11.3 [0.861, 102]	11.6 [0.861, 276]	
Zn **				
Mean (SD)	711 (408)	532 (270)	630 (363)	**0.005**
GM	613	465	541	
Med [Min, Max]	613 [130, 2660]	482 [116, 1330]	559 [116, 2660]	
As* **				
Mean (SD)	46.7 (41.8)	21.1 (21.5)	35.2 (36.4)	**<0.001**
GM	36.7	16.1	25.3	
Med [Min, Max]	34.8 [11.8, 298]	17.7 [1.79, 172]	24.0 [1.79, 298]	
Se				
Mean (SD)	19.8 (9.37)	18.1 (10.5)	19.0 (9.91)	0.201
GM	17.5	15.0	16.3	
Med [Min, Max]	17.8 [4.98, 43.0]	15.5 [1.55, 45.6]	16.4 [1.55, 45.6]	
Mo				
Mean (SD)	93.7 (77.6)	82.5 (53.8)	88.7 (68.0)	0.672
GM	73.0	65.2	69.4	
Med [Min, Max]	73.5 [17.6, 533]	71.4 [5.86, 305]	71.7 [5.86, 533]	
Cd				
Mean (SD)	0.320 (0.344)	0.335 (0.384)	0.327 (0.361)	0.956
GM	0.0853	0.0655	0.0758	
Med [Min, Max]	0.216 [0.00200, 1.59]	0.284 [0.00200, 1.87]	0.252 [0.00200, 1.87]	
Sn				
Mean (SD)	0.501 (0.452)	0.532 (0.718)	0.515 (0.585)	0.99
GM	0.294	0.250	0.273	
Med [Min, Max]	0.426 [0.0275, 2.09]	0.430 [0.0275, 5.70]	0.427 [0.0275, 5.70]	
Ba **				
Mean (SD)	6.75 (9.87)	5.45 (9.70)	6.17 (9.79)	**0.002**
GM	4.82	3.57	4.21	
Med [Min, Max]	4.94 [0.946, 89.5]	3.21 [0.947, 61.6]	3.73 [0.946, 89.5]	
Hg				
Mean (SD)	0.112 (0.384)	0.0404 (0.0792)	0.0796 (0.291)	0.416
GM	0.0223	0.0177	0.0201	
Med [Min, Max]	0.0115 [0.0115, 3.25]	0.0115 [0.0115, 0.400]	0.0115 [0.0115, 3.25]	
Pb **				
Mean (SD)	1.73 (1.42)	2.56 (2.29)	2.10 (1.90)	**0.009**
GM	0.879	1.73	1.19	
Med [Min, Max]	1.33 [0.00700, 8.60]	1.99 [0.00700, 11.1]	1.68 [0.00700, 11.1]	

TR: Tunçbelik Region, KC: Kütahya City Center, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. *Statistically significant difference (p<0.05), ** (p<0.01), *** (p<0.001).

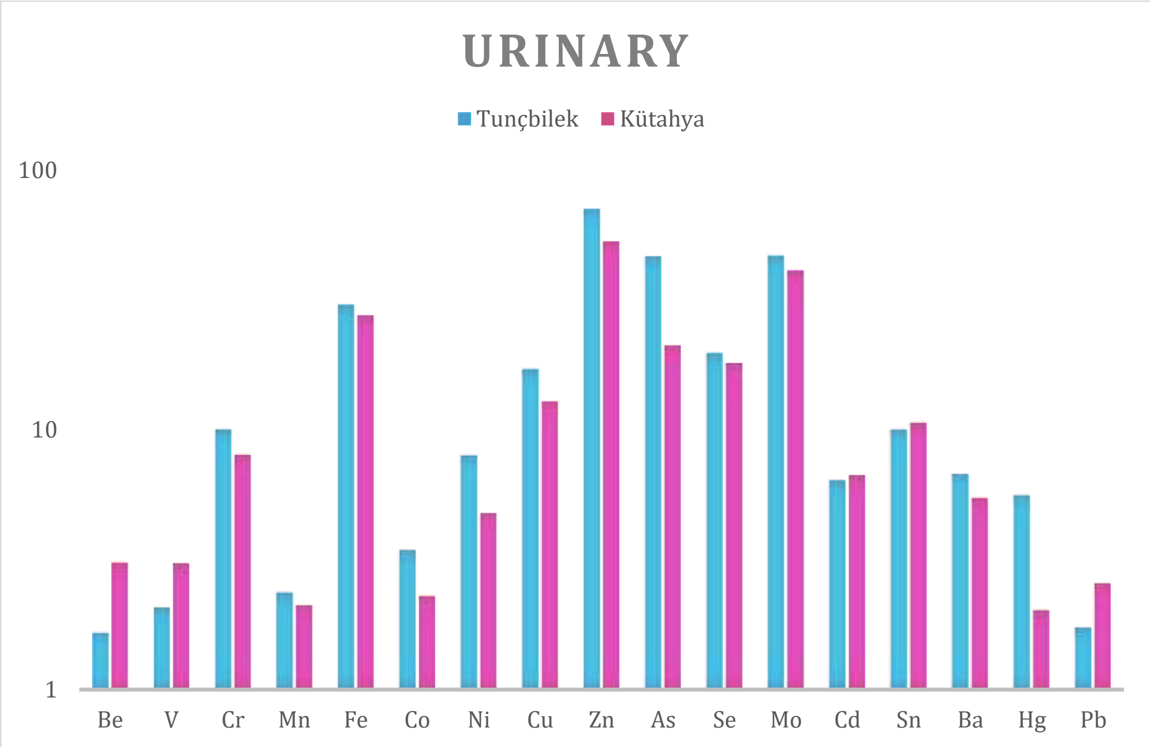


Figure 4.1. Urinary metal concentrations (μg L^{-1}) of children living in Tunçbilek region and Kütahya City Center

Creatinine-adjusted urinary metal levels (CR-UM) showed significant differences between TR and KC of Be, V, Mn, Fe, Ni, Cu, As, Se, and Pb levels. The CR-UM of Be, V, Mn, Fe, Cu, Se, and Pb are statistically significantly higher in KC, while CR-UM of Ni, and As are statistically significantly higher in TR than that of KC (Table 4.4) (Figure 4.2).

Table 4.4. Creatinine adjusted urinary metal levels (μg g^{-1}) of metals of children living in Tunçbilek region and Kütahya City Center

	TR (n=88)	**KC (n=72)**	**Total (n=160)**	**p-value**
Be_CR* **				
Mean (SD)	0.00843 (0.0247)	0.0174 (0.0296)	0.0125 (0.0273)	**<0.001**
GM	0.00190	0.00444	0.00278	
Med [Min, Max]	0.00131 [0.000484, 0.158]	0.00232 [0.000556, 0.148]	0.00150 [0.000484, 0.158]	
V_CR**				
Mean (SD)	1.10 (1.61)	2.04 (2.47)	1.52 (2.09)	**0.002**
GM	0.245	0.550	0.353	
Med [Min, Max]	0.549 [0.00586, 8.54]	1.49 [0.0100, 14.5]	0.816 [0.00586, 14.5]	
Cr_CR				
Mean (SD)	0.470 (0.475)	0.455 (0.409)	0.464 (0.445)	0.705
GM	0.323	0.269	0.298	
Med [Min, Max]	0.349 [0.00375, 3.19]	0.324 [0.00450, 2.00]	0.331 [0.00375, 3.19]	

Table 4.4. (continues) Creatinine adjusted urinary metal levels (μg g^{-1}) of metals of children living in Tunçbilek region and Kütahya City Center

Mn_CR* **				
Mean (SD)	2.77 (8.94)	3.06 (4.03)	2.90 (7.14)	**<0.001**
GM	0.892	1.96	1.27	
Med [Min, Max]	1.16 [0.00210, 81.9]	2.09 [0.00650, 30.6]	1.60 [0.00210, 81.9]	
Fe_CR* **				
Mean (SD)	27.0 (40.9)	34.9 (28.9)	30.6 (36.1)	**<0.001**
GM	15.4	26.9	19.8	
Med [Min, Max]	15.2 [0.143, 338]	26.8 [3.81, 198]	22.1 [0.143, 338]	
Co_CR				
Mean (SD)	1.40 (1.18)	1.09 (0.738)	1.26 (1.01)	0.112
GM	1.10	0.856	0.983	
Med [Min, Max]	1.14 [0.331, 8.55]	0.885 [0.0100, 3.96]	0.955 [0.0100, 8.55]	
Ni_CR* **				
Mean (SD)	6.76 (7.71)	4.88 (3.87)	5.92 (6.33)	**<0.001**
GM	5.40	4.04	4.74	
Med [Min, Max]	5.59 [1.44, 70.8]	3.88 [1.11, 27.1]	4.50 [1.11, 70.8]	
Cu_CR*				
Mean (SD)	16.6 (48.8)	14.5 (12.5)	15.6 (37.1)	**0.014**
GM	10.4	12.2	11.2	
Med [Min, Max]	9.77 [3.86, 460]	10.8 [5.02, 92.6]	10.3 [3.86, 460]	
Zn_CR				
Mean (SD)	559 (221)	585 (325)	571 (272)	0.999
GM	513	525	519	
Med [Min, Max]	527 [149, 1100]	522 [116, 2390]	524 [116, 2390]	
As_CR* **				
Mean (SD)	38.9 (31.2)	21.2 (21.5)	30.9 (28.6)	**<0.001**
GM	30.7	18.2	24.3	
Med [Min, Max]	24.9 [7.49, 135]	17.4 [7.28, 191]	20.7 [7.28, 191]	
Se_CR **				
Mean (SD)	15.3 (4.17)	17.4 (4.04)	16.2 (4.24)	**0.001**
GM	14.7	16.9	15.6	
Med [Min, Max]	15.3 [6.02, 24.6]	17.6 [4.74, 26.8]	16.2 [4.74, 26.8]	
Mo_CR				
Mean (SD)	73.2 (47.9)	85.1 (49.8)	78.6 (48.9)	0.055
GM	61.1	73.6	66.5	
Med [Min, Max]	65.4 [10.5, 333]	75.7 [19.5, 339]	70.6 [10.5, 339]	
Cd_CR				
Mean (SD)	0.331 (0.495)	0.461 (0.726)	0.389 (0.611)	0.287
GM	0.0714	0.0740	0.0726	
Med [Min, Max]	0.140 [0.000952, 2.96]	0.223 [8e-04, 3.74]	0.180 [8e-04, 3.74]	
Sn_CR				
Mean (SD)	0.392 (0.361)	0.612 (0.871)	0.491 (0.650)	0.053
GM	0.246	0.282	0.262	
Med [Min, Max]	0.309 [0.0125, 1.84]	0.459 [0.0125, 6.33]	0.359 [0.0125, 6.33]	
Ba_CR				
Mean (SD)	6.57 (15.8)	7.64 (19.0)	7.05 (17.3)	0.623
GM	4.04	4.03	4.04	
Med [Min, Max]	3.80 [0.676, 149]	3.56 [0.826, 154]	3.57 [0.676, 154]	
Hg_CR				
Mean (SD)	0.131 (0.592)	0.0455 (0.0779)	0.0923 (0.443)	0.062
GM	0.0187	0.0200	0.0193	
Med [Min, Max]	0.0110 [0.00371, 5.42]	0.0128 [0.00500, 0.445]	0.0115 [0.00371, 5.42]	

Table 4.4. (continues) Creatinine adjusted urinary metal levels (µg g^{-1}) of metals of children living in Tunçbilek region and Kütahya City Center

Pb_CR* **				
Mean (SD)	1.52 (1.39)	4.30 (11.4)	2.77 (7.78)	**<0.001**
GM	0.736	1.95	1.14	
Med [Min, Max]	1.21 [0.00350, 6.62]	1.99 [0.00778, 94.8]	1.50 [0.00350, 94.8]	

TR: Tunçbelik Region, KC: Kütahya City Center, CR: creatinie,, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. *Statistically significant difference ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

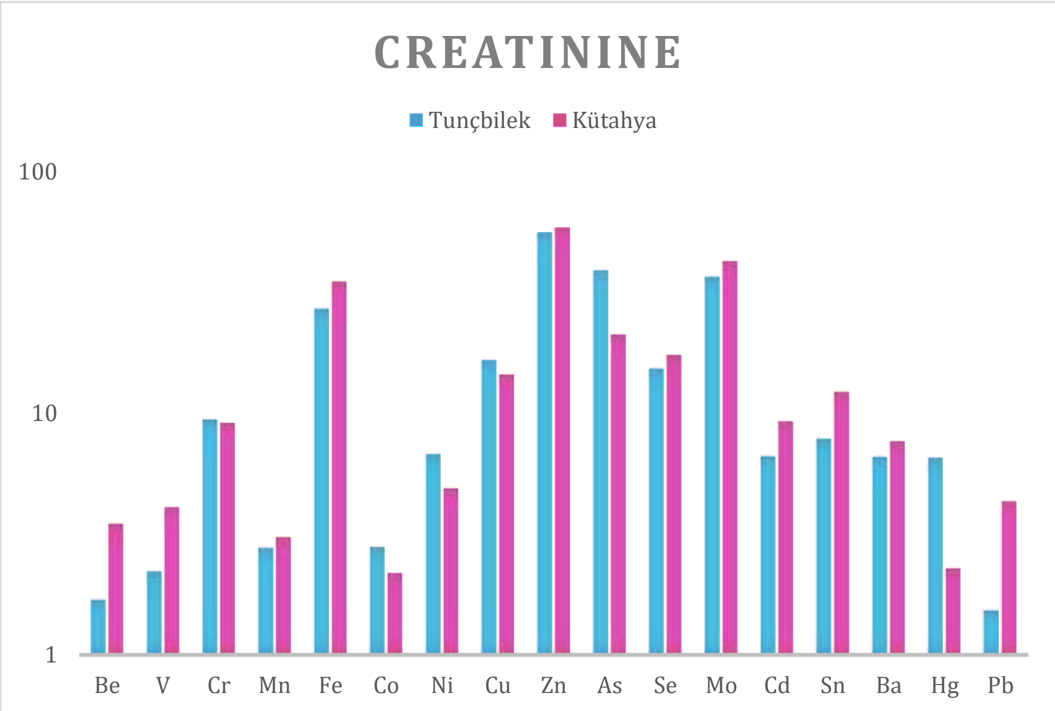


Figure 4.2. Creatinine adjusted urinary metal levels (µg g^{-1}) of metals of children living in Tunçbilek region and Kütahya City Center

Specific gravity adjusted urinary metals levels (SG-UM) showed significant differences between TR and KC of Be, V, Mn, Fe, Co, Ni, As, Hg, and Pb levels. The SG-UM of Be, V, Mn, Fe, Hg, and Pb are higher in KC ($p<0.05$). However, the SG-UM of Co, Ni, and As are higher in TR ($p<0.05$) (Table 4.5) (Figure 4.3).

Table 4.5. Specific gravity adjusted metals levels in urine samples of children living in Tunçbilek region and Kütahya City Center

	TR (n=88)	KC (n=72)	Total (n=160)	p-value
Be_SG* **				
Mean (SD)	0.00571 (0.0146)	0.0136 (0.0242)	0.00925 (0.0198)	**<0.001**
GM	0.00156	0.00315	0.00215	
Med [Min, Max]	0.00110 [0.000643, 0.0857]	0.00145 [0.000695, 0.114]	0.00120 [0.000643, 0.114]	
V_SG **				
Mean (SD)	0.836 (1.09)	1.46 (1.34)	1.12 (1.24)	**0.001**
GM	0.202	0.418	0.280	
Med [Min, Max]	0.473 [0.00729, 5.38]	1.35 [0.00868, 6.17]	0.712 [0.00729, 6.17]	
Cr_SG				
Mean (SD)	0.356 (0.311)	0.344 (0.289)	0.351 (0.301)	0.35
GM	0.266	0.205	0.236	
Med [Min, Max]	0.323 [0.00315, 2.67]	0.244 [0.00243, 1.48]	0.298 [0.00243, 2.67]	
Mn_SG* **				
Mean (SD)	1.85 (4.87)	1.93 (1.20)	1.89 (3.69)	**<0.001**
GM	0.734	1.49	1.01	
Med [Min, Max]	1.03 [0.00365, 44.3]	1.83 [0.00352, 6.17]	1.30 [0.00352, 44.3]	
Fe_SG* **				
Mean (SD)	20.8 (34.8)	24.4 (13.4)	22.4 (27.3)	**<0.001**
GM	12.7	20.4	15.7	
Med [Min, Max]	14.8 [0.0980, 315]	22.7 [2.06, 61.4]	18.2 [0.0980, 315]	
Co_SG*				
Mean (SD)	1.14 (0.890)	0.903 (0.700)	1.03 (0.816)	**0.03**
GM	0.906	0.651	0.781	
Med [Min, Max]	0.920 [0.245, 5.88]	0.653 [0.000647, 3.68]	0.752 [0.000647, 5.88]	
Ni_SG* **				
Mean (SD)	5.51 (6.63)	3.89 (3.52)	4.79 (5.50)	**<0.001**
GM	4.45	3.07	3.76	
Med [Min, Max]	4.77 [1.10, 62.6]	3.05 [0.637, 25.3]	3.90 [0.637, 62.6]	
Cu_SG				
Mean (SD)	12.2 (26.6)	11.1 (10.6)	11.8 (20.9)	0.115
GM	8.56	9.25	8.87	
Med [Min, Max]	8.25 [1.85, 249]	8.98 [1.86, 86.4]	8.53 [1.85, 249]	
Zn_SG				
Mean (SD)	464 (199)	436 (178)	451 (190)	0.39
GM	423	400	412	
Med [Min, Max]	443 [100, 1350]	430 [91.2, 1020]	433 [91.2, 1350]	
As_SG* **				
Mean (SD)	31.3 (23.9)	16.4 (13.5)	24.6 (21.2)	**<0.001**
GM	25.3	13.9	19.3	
Med [Min, Max]	19.4 [8.71, 138]	13.9 [1.55, 114]	17.7 [1.55, 138]	
Se_SG				
Mean (SD)	12.7 (3.68)	13.8 (4.72)	13.2 (4.20)	0.331
GM	12.1	12.8	12.4	
Med [Min, Max]	12.6 [2.14, 22.0]	13.2 [1.50, 26.0]	13.0 [1.50, 26.0]	
Mo_SG				
Mean (SD)	60.7 (40.7)	63.5 (32.7)	62.0 (37.2)	0.272
GM	50.3	56.0	52.8	
Med [Min, Max]	52.7 [7.53, 274]	56.9 [8.95, 209]	55.0 [7.53, 274]	

Table 4.5. (continues) Specific gravity adjusted metals levels in urine samples of children living in Tunçbilek region and Kütahya City Center

Cd_SG				
Mean (SD)	0.238 (0.303)	0.296 (0.362)	0.264 (0.331)	0.335
GM	0.0589	0.0563	0.0577	
Med [Min, Max]	0.136 [0.000909, 1.70]	0.190 [0.00108, 1.82]	0.153 [0.000909, 1.82]	
Sn_SG				
Mean (SD)	0.326 (0.280)	0.431 (0.497)	0.373 (0.395)	0.183
GM	0.202	0.215	0.208	
Med [Min, Max]	0.240 [0.0141, 1.36]	0.355 [0.0145, 3.61]	0.287 [0.0141, 3.61]	
Ba_SG				
Mean (SD)	4.77 (8.61)	5.40 (13.3)	5.05 (10.9)	0.211
GM	3.32	3.07	3.21	
Med [Min, Max]	3.18 [0.812, 80.6]	2.83 [0.888, 105]	2.92 [0.812, 105]	
Hg_SG*				
Mean (SD)	0.102 (0.491)	0.0379 (0.0735)	0.0733 (0.368)	**0.045**
GM	0.0153	0.0152	0.0152	
Med [Min, Max]	0.00846 [0.00496, 4.53]	0.0102 [0.00533, 0.352]	0.00899 [0.00496, 4.53]	
Pb_SG* **				
Mean (SD)	1.19 (1.06)	2.24 (2.12)	1.66 (1.71)	**<0.001**
GM	0.606	1.48	0.906	
Med [Min, Max]	0.941 [0.00300, 6.53]	1.39 [0.00443, 12.6]	1.25 [0.00300, 12.6]	

TR: Tunçbelik Region, KC: Kütahya City Center, SG: specific gravity, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. *Statistically significant difference (p<0.05), ** (p<0.01), *** (p<0.001).

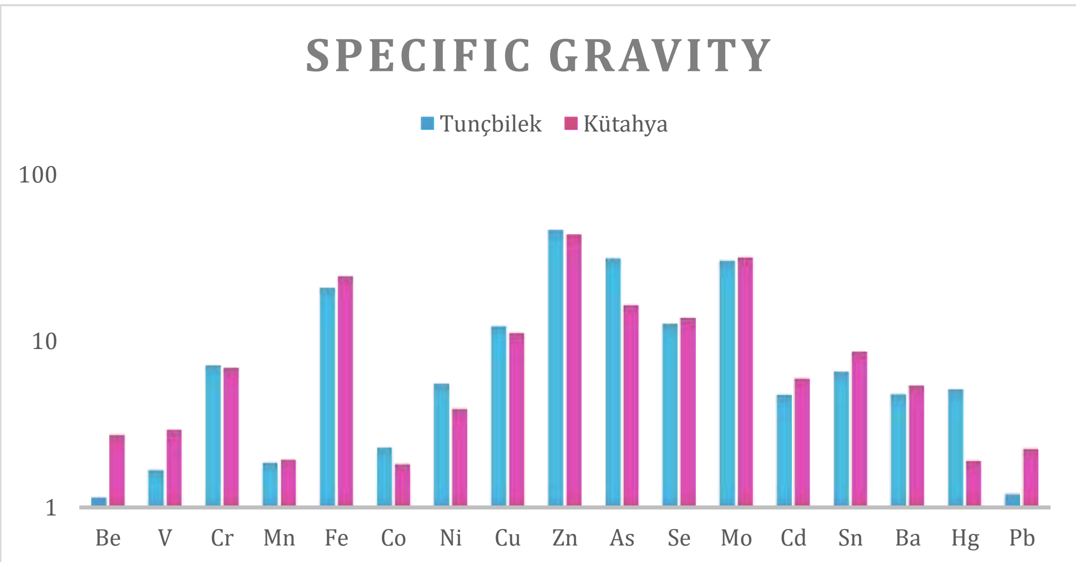


Figure 4.3. Specific gravity adjusted urinary metal levels of children living in Tunçbilek region and Kütahya City Center

Statistically significant differences are found for R-UM of Be, Cr, Mn, Fe, Co, Ni, Zn, As, Ba, and Pb among the schools. Statistically significantly higher levels of Be and Mn measured in I and L than that of G. The Cr level in A is statistically significantly higher than the levels of other schools. Fe level in L is statistically significantly higher than the level of G. The Co level in L is statistically significantly lower than the levels of A and I. Also the level of Ni in L is statistically significantly lower than the levels of G, I, and A. Zn level in L is statistically significantly lower than the level of A. As level in G is statistically significantly higher than the levels found in the other schools. Ba R-UM in I is statistically significantly higher than that of L. Pb level in G is statistically significantly lower than all schools (Table 4.6).

Statistically significant differences are found in our results between the schools in the CR-UM of Be, Mn, Fe, Ni, As, Se, Mo, and Pb. Statistically significantly higher level of Be measured in L than G and A, and higher level in I than A. Mn level in L is statistically significantly higher than the levels measured in G. Fe level in L is statistically significantly higher than the level of G and A. The levels of Ni in G and I are statistically significantly higher than the concentration of L. The level As in G is statistically significantly higher than the other schools and the level in I is higher than the levels of A and L. The level of Se in L is statistically significantly higher than G. Statistically significantly lower level of Mo metal measured in G than the other schools. Pb levels in I and L are statistically significantly higher than the level of G (Table 4.7).

Table 4.6. Urinary metal concentrations ($\mu g\ L^{-1}$) of children according to their schools

	G (n=37)	**A (n=18)**	**I (n=33)**	**L (n=72)**
Be				
Mean (SD)	0.00150 (0.00)	0.00641 (0.0146)	0.0168 (0.0299)	0.0154 (0.0263)
GM	0.00150	0.00219	0.00368	0.00367
Med[Min, Max]	**0.00150 [0.00150, 0.00150]**^{I,L}	0.00150 [0.00150, 0.0544]	0.00150 [0.00150, 0.111]	0.00150 [0.00150, 0.107]
V				
Mean (SD)	0.893 (0.977)	1.04 (1.07)	1.19 (1.28)	1.53 (1.24)
GM	0.338	0.302	0.246	0.487
Med[Min, Max]	0.562 [0.0170, 3.36]	0.829 [0.0170, 3.42]	0.572 [0.0170, 4.58]	1.57 [0.0170, 4.06]
Cr				
Mean (SD)	0.403 (0.235)	0.744 (0.297)	0.480 (0.359)	0.400 (0.316)
GM	0.295	0.695	0.378	0.238
Med[Min, Max]	**0.375 [0.00450, 1.12]**^{A}	0.683 [0.348, 1.46]	**0.372 [0.0378, 1.92]** ^{A}	**0.331 [0.00450, 1.75]** ^{A}
Mn				
Mean (SD)	2.75 (8.07)	1.99 (1.61)	2.12 (1.62)	2.10 (1.02)
GM	0.671	1.48	1.50	1.73
Med[Min, Max]	**1.09 [0.00650, 49.2]**^{I,L}	1.51 [0.231, 6.71]	1.81 [0.00650, 7.85]	2.09 [0.00650, 4.89]
Fe				
Mean (SD)	21.3 (17.1)	24.2 (16.9)	44.0 (114)	27.6 (14.1)
GM	15.6	15.6	24.2	23.8
Med[Min, Max]	**17.1 [0.200, 80.3]**^{L}	22.1 [0.200, 53.0]	20.6 [9.47, 676]	27.1 [3.81, 72.4]
Co				
Mean (SD)	1.52 (1.46)	1.75 (1.09)	1.92 (1.80)	1.14 (0.975)
GM	1.16	1.47	1.43	0.758
Med[Min, Max]	1.09 [0.321, 8.31]	1.57 [0.472, 4.64]	1.53 [0.439, 9.41]	**0.845 [0.00100, 3.96]**^{A,I}
Ni				
Mean (SD)	9.21 (12.0)	7.48 (2.46)	6.84 (3.03)	4.78 (4.37)
GM	6.47	7.03	6.14	3.58
Med[Min, Max]	5.93 [2.18, 70.8]	8.32 [2.92, 12.9]	6.47 [1.33, 15.9]	**3.80 [0.442, 29.8]**^{G,A,I}
Cu				
Mean (SD)	19.6 (43.7)	16.9 (15.5)	14.6 (16.3)	12.9 (11.7)
GM	12.2	13.8	11.9	10.8
Med[Min, Max]	12.2 [3.95, 276]	12.4 [6.61, 75.1]	10.8 [5.99, 103]	11.3 [0.861, 102]
Zn				
Mean (SD)	640 (303)	942 (582)	664 (362)	532 (270)
GM	573	802	571	465
Med[Min, Max]	610 [202, 1340]	729 [327, 2660]	516 [130, 1680]	**482 [116, 1330]**^{A}

Table 4.6. (continues) Urinary metal concentrations ($\mu g\ L^{-1}$) of children according to their schools

As				
Mean (SD)	69.6 (54.3)	28.9 (13.5)	30.7 (17.2)	21.1 (21.5)
GM	55.9	26.2	27.5	16.1
Med[Min, Max]	60.7 [19.4, 298]	**25.1 [12.1, 58.2]^{G}**	**27.4 [11.8, 105]^{G}**	**17.7 [1.79, 172]^{G,A,I}**
Se				
Mean (SD)	18.0 (9.90)	22.4 (10.1)	20.4 (8.13)	18.1 (10.5)
GM	15.5	20.0	18.6	15.0
Med[Min, Max]	14.1 [4.98, 43.0]	24.9 [8.14, 39.0]	20.9 [5.80, 35.6]	15.5 [1.55, 45.6]
Mo				
Mean (SD)	87.7 (56.1)	80.4 (75.0)	108 (97.5)	82.5 (53.8)
GM	71.9	59.6	82.9	65.2
Med[Min, Max]	74.2 [17.6, 236]	58.3 [23.5, 296]	82.9 [25.1, 533]	71.4 [5.86, 305]
Cd				
Mean (SD)	0.335 (0.362)	0.305 (0.303)	0.313 (0.353)	0.335 (0.384)
GM	0.0826	0.148	0.0655	0.0655
Med[Min, Max]	0.198 [0.00200, 1.32]	0.255 [0.00200, 1.17]	0.217 [0.00200, 1.59]	0.284 [0.00200, 1.87]
Sn				
Mean (SD)	0.533 (0.439)	0.475 (0.550)	0.480 (0.419)	0.532 (0.718)
GM	0.391	0.196	0.266	0.250
Med[Min, Max]	0.427 [0.0275, 1.87]	0.290 [0.0275, 1.87]	0.441 [0.0275, 2.09]	0.430 [0.0275, 5.70]
Ba				
Mean (SD)	7.62 (14.3)	5.65 (3.24)	6.39 (5.18)	5.45 (9.70)
GM	4.66	4.90	4.98	3.57
Med[Min, Max]	3.95 [0.946, 89.5]	4.93 [1.85, 14.5]	4.97 [1.03, 25.8]	**3.21 [0.947, 61.6]^{I}**
Hg				
Mean (SD)	0.0535 (0.0951)	0.0744 (0.168)	0.197 (0.603)	0.0404 (0.0792)
GM	0.0217	0.0203	0.0242	0.0177
Med[Min, Max]	0.0115 [0.0115, 0.461]	0.0115 [0.0115, 0.677]	0.0115 [0.0115, 3.25]	0.0115 [0.0115, 0.400]
Pb				
Mean (SD)	1.22 (1.28)	1.89 (1.20)	2.22 (1.53)	2.56 (2.29)
GM	0.377	1.60	1.63	1.73
Med[Min, Max]	**0.855 [0.00700, 5.07]^{A,I,L}**	1.44 [0.768, 4.42]	2.01 [0.00700, 8.60]	1.99 [0.00700, 11.1]

G**:** Güraǧaç Primary School, A: Atatürk Primary School, I: 60.yil Işık Primary School, L; Lignite Primary School, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. $^{\mathbf{G}}$Statistically significant increase in G, $^{\mathbf{A}}$Statistically significant increase in A, $^{\mathbf{I}}$Statistically significant increase in I, $^{\mathbf{L}}$Statistically significant increase in L against to the corresponding school(s) (p<0.05).

Table 4.7. Creatinine adjusted urinary metal levels (µg g^{-1}) of metals in urine samples of children according to their schools.

	G (n=37)	**A (n=18)**	**I (n=33)**	**L (n=72)**
Be_CR				
Mean (SD)	0.00151 (0.000880)	0.0111 (0.0329)	0.0147 (0.0314)	0.0174 (0.0296)
GM	0.00132	0.00161	0.00312	0.00444
Med[Min, Max]	**0.00136 [0.000484, 0.00500] [L]**	**0.000969 [0.000517, 0.136] [L,I]**	0.00150 [0.000714, 0.158]	0.00232 [0.000556, 0.148]
V_CR				
Mean (SD)	0.851 (1.12)	1.26 (2.08)	1.30 (1.79)	2.04 (2.47)
GM	0.298	0.222	0.208	0.550
Med[Min, Max]	0.549 [0.00944, 4.79]	0.512 [0.00586, 8.54]	0.574 [0.00810, 7.18]	1.49 [0.0100, 14.5]
Cr_CR				
Mean (SD)	0.389 (0.330)	0.656 (0.562)	0.461 (0.544)	0.455 (0.409)
GM	0.260	0.512	0.321	0.269
Med[Min, Max]	0.299 [0.00375, 1.51]	0.410 [0.158, 2.42]	0.332 [0.0252, 3.19]	0.324 [0.00450, 2.00]
Mn_CR				
Mean (SD)	3.74 (13.5)	2.29 (3.82)	1.95 (1.82)	3.06 (4.03)
GM	0.591	1.09	1.27	1.96
Med[Min, Max]	**1.06 [0.00210, 81.9][L]**	1.15 [0.169, 16.8]	1.45 [0.0130, 8.80]	2.09 [0.00650, 30.6]
Fe_CR				
Mean (SD)	21.1 (20.9)	26.9 (34.1)	33.6 (57.9)	34.9 (28.9)
GM	13.7	11.5	20.5	26.9
Med[Min, Max]	**16.8 [0.143, 118][L]**	**9.95 [0.200, 126][L]**	16.1 [4.61, 338]	26.8 [3.81, 198]
Co_CR				
Mean (SD)	1.24 (0.909)	1.33 (0.960)	1.62 (1.51)	1.09 (0.738)
GM	1.02	1.08	1.21	0.856
Med[Min, Max]	1.14 [0.401, 4.61]	0.907 [0.472, 4.25]	1.38 [0.331, 8.55]	0.885 [0.0100, 3.96]
Ni_CR				
Mean (SD)	7.90 (11.2)	5.91 (4.17)	5.94 (3.05)	4.88 (3.87)
GM	5.70	5.17	5.21	4.04
Med[Min, Max]	5.82 [1.44, 70.8]	4.31 [2.74, 21.2]	5.87 [1.47, 14.5]	**3.88 [1.11, 27.1][G,I]**
Cu_CR				
Mean (SD)	22.7 (74.1)	11.6 (7.29)	12.5 (13.7)	14.5 (12.5)
GM	10.8	10.1	10.1	12.2
Med[Min, Max]	9.63 [5.23, 460]	9.62 [4.75, 35.8]	10.4 [3.86, 85.4]	10.8 [5.02, 92.6]
Zn_CR				
Mean (SD)	542 (206)	631 (236)	539 (229)	585 (325)
GM	505	590	485	525
Med[Min, Max]	511 [188, 1100]	566 [321, 1020]	531 [149, 933]	522 [116, 2390]

Table 4.7. (continues) Creatinine adjusted urinary metal levels ($\mu g\ g^{-1}$) of metals in urine samples of children according to their schools.

As_CR				
Mean (SD)	60.0 (37.2)	20.1 (6.43)	25.6 (13.1)	21.2 (21.5)
GM	49.3	19.3	23.3	18.2
Med[Min, Max]	55.2 [16.0, 135]	**18.7 [11.7, 39.3]**[G,I]	**23.9 [7.49, 80.4]**[G]	**17.4 [7.28, 191]**[G,I]
Se_CR				
Mean (SD)	14.3 (4.27)	15.2 (3.83)	16.4 (4.09)	17.4 (4.04)
GM	13.7	14.7	15.8	16.9
Med[Min, Max]	**13.1 [6.02, 23.6]**[L]	15.1 [9.66, 21.5]	16.6 [6.04, 24.6]	17.6 [4.74, 26.8]
Mo_CR				
Mean (SD)	70.9 (30.5)	51.1 (28.0)	87.8 (65.3)	85.1 (49.8)
GM	63.3	43.9	70.3	73.6
Med[Min, Max]	71.5 [10.5, 147]	**43.1 [11.7, 102]**[G,I,A]	70.7 [12.5, 333]	75.7 [19.5, 339]
Cd_CR				
Mean (SD)	0.399 (0.658)	0.272 (0.354)	0.286 (0.319)	0.461 (0.726)
GM	0.0728	0.109	0.0555	0.0740
Med[Min, Max]	0.109 [0.00105, 2.96]	0.126 [0.000952, 1.39]	0.191 [0.000952, 1.22]	0.223 [8e-04, 3.74]
Sn_CR				
Mean (SD)	0.451 (0.393)	0.275 (0.312)	0.389 (0.341)	0.612 (0.871)
GM	0.344	0.144	0.225	0.282
Med[Min, Max]	0.327 [0.0275, 1.84]	0.146 [0.0212, 1.17]	0.316 [0.0125, 1.56]	0.459 [0.0125, 6.33]
Ba_CR				
Mean (SD)	8.84 (24.1)	4.25 (2.80)	5.29 (3.36)	7.64 (19.0)
GM	4.10	3.60	4.22	4.03
Med[Min, Max]	3.87 [0.676, 149]	3.28 [1.65, 11.0]	4.63 [0.838, 12.9]	3.56 [0.826, 154]
Hg_CR				
Mean (SD)	0.0564 (0.0936)	0.0626 (0.175)	0.251 (0.950)	0.0455 (0.0779)
GM	0.0191	0.0149	0.0205	0.0200
Med[Min, Max]	0.0115 [0.00371, 0.359]	0.00885 [0.00397, 0.752]	0.00958 [0.00523, 5.42]	0.0128 [0.00500, 0.445]
Pb_CR				
Mean (SD)	1.13 (1.47)	1.49 (0.917)	1.98 (1.41)	4.30 (11.4)
GM	0.333	1.18	1.39	1.95
Med[Min, Max]	**0.562 [0.00368, 6.34]**[I,L]	1.52 [0.349, 2.99]	1.55 [0.00350, 6.62]	1.99 [0.00778, 94.8]

G**:** Gürağaç Primary School, A: Atatürk Primary School, I: 60.yil Işık Primary School, L: Lignite Primary School, CR: creatinine SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. [G] Statistically significant increase in G, [A] Statistically significant increase in A, [I] Statistically significant increase in I, [L] Statistically significant increase in L against to the corresponding school(s) ($p<0.05$).

Table 4.8. Specific gravity adjusted metals concentration in urine samples of children according to their schools

	G (n=37)	**A (n=18)**	**I (n=33)**	**L (n=72)**
Be_SG				
Mean (SD)	0.00115 (0.000385)	0.00706 (0.0205)	0.0101 (0.0176)	0.0136 (0.0242)
GM	0.00109	0.00140	0.00249	0.00315
Med[Min, Max]	**0.00112 [0.000643, 0.00215][L]**	**0.000965 [0.000647, 0.0857] [L]**	0.00114 [0.000682, 0.0712]	0.00145 [0.000695, 0.114]
V_SG				
Mean (SD)	0.723 (0.881)	0.840 (1.29)	0.960 (1.19)	1.46 (1.34)
GM	0.246	0.193	0.166	0.418
Med[Min, Max]	0.474 [0.00729, 3.55]	0.514 [0.00734, 5.38]	0.424 [0.00773, 3.86]	1.35 [0.00868, 6.17]
Cr_SG				
Mean (SD)	0.299 (0.189)	0.478 (0.194)	0.354 (0.437)	0.344 (0.289)
GM	0.215	0.444	0.256	0.205
Med[Min, Max]	**0.254 [0.00315, 0.817][A]**	0.405 [0.190, 0.883]	**0.277 [0.0194, 2.67][A]**	**0.244 [0.00243, 1.48][A]**
Mn_SG				
Mean (SD)	2.31 (7.30)	1.58 (2.34)	1.48 (1.15)	1.93 (1.20)
GM	0.489	0.943	1.01	1.49
Med[Min, Max]	**0.839 [0.00365, 44.3][L]**	**1.12 [0.148, 10.6][L]**	1.38 [0.00760, 4.73]	1.83 [0.00352, 6.17]
Fe_SG				
Mean (SD)	16.4 (12.7)	19.1 (21.0)	26.8 (53.0)	24.4 (13.4)
GM	11.3	9.99	16.4	20.4
Med[Min, Max]	**14.6 [0.0980, 63.6] [L]**	**11.4 [0.131, 79.6] [L]**	**16.7 [4.85, 315] [L]**	22.7 [2.06, 61.4]
Co_SG				
Mean (SD)	1.05 (0.818)	1.13 (0.706)	1.24 (1.06)	0.903 (0.700)
GM	0.842	0.939	0.965	0.651
Med[Min, Max]	0.861 [0.245, 4.12]	0.875 [0.309, 2.68]	1.05 [0.254, 5.88]	0.653 [0.000647, 3.68]
Ni_SG				
Mean (SD)	6.73 (9.89)	4.88 (2.42)	4.50 (1.79)	3.89 (3.52)
GM	4.71	4.48	4.15	3.07
Med[Min, Max]	5.04 [1.10, 62.6]	4.77 [2.22, 13.4]	4.53 [1.12, 9.96]	**3.05 [0.637, 25.3][G,A,I]**
Cu_SG				
Mean (SD)	15.5 (39.6)	10.4 (8.72)	9.61 (9.70)	11.1 (10.6)
GM	8.91	8.79	8.07	9.25
Med[Min, Max]	8.79 [1.85, 249]	7.95 [5.02, 43.2]	7.81 [3.60, 61.7]	8.98 [1.86, 86.4]
Zn_SG				
Mean (SD)	451 (166)	566 (270)	423 (175)	436 (178)
GM	417	512	386	400
Med[Min, Max]	431 [100, 853]	554 [235, 1350]	414 [152, 875]	430 [91.2, 1020]

Table 4.8. (continues) Specific gravity adjusted metals concentration in urine samples of children according to their schools

As_SG				
Mean (SD)	48.0 (27.5)	17.2 (4.26)	20.2 (11.1)	16.4 (13.5)
GM	40.7	16.7	18.5	13.9
Med[Min, Max]	50.8 [15.3, 138]	**18.1 [9.84, 26.4][G]**	**18.0 [8.71, 74.7][G]**	**13.9 [1.55, 114][G, I]**
Se_SG				
Mean (SD)	12.2 (4.34)	13.1 (3.06)	13.0 (3.19)	13.8 (4.72)
GM	11.3	12.8	12.6	12.8
Med[Min, Max]	12.4 [2.14, 19.5]	13.5 [6.18, 17.5]	12.5 [6.94, 22.0]	13.2 [1.50, 26.0]
Mo_SG				
Mean (SD)	60.3 (27.1)	46.6 (31.8)	69.0 (54.4)	63.5 (32.7)
GM	52.3	38.1	56.0	56.0
Med[Min, Max]	57.8 [7.53, 117]	37.6 [11.6, 128]	55.0 [17.8, 274]	56.9 [8.95, 209]
Cd_SG				
Mean (SD)	0.266 (0.357)	0.217 (0.266)	0.217 (0.258)	0.296 (0.362)
GM	0.0603	0.0947	0.0442	0.0563
Med[Min, Max]	0.119 [0.000993, 1.70]	0.141 [0.00115, 0.921]	0.146 [0.000909, 1.21]	0.190 [0.00108, 1.82]
Sn_SG				
Mean (SD)	0.373 (0.294)	0.268 (0.317)	0.305 (0.240)	0.431 (0.497)
GM	0.284	0.125	0.180	0.215
Med[Min, Max]	0.280 [0.0243, 1.36]	0.187 [0.0176, 1.21]	0.281 [0.0141, 1.08]	0.355 [0.0145, 3.61]
Ba_SG				
Mean (SD)	5.94 (12.9)	3.57 (1.98)	4.12 (2.85)	5.40 (13.3)
GM	3.39	3.13	3.36	3.07
Med[Min, Max]	3.12 [0.812, 80.6]	2.89 [1.41, 7.41]	3.41 [0.845, 14.5]	2.83 [0.888, 105]
Hg_SG				
Mean (SD)	0.0465 (0.0889)	0.0457 (0.106)	0.195 (0.791)	0.0379 (0.0735)
GM	0.0158	0.0129	0.0161	0.0152
Med[Min, Max]	0.00926 [0.00529, 0.402]	0.00788 [0.00496, 0.436]	0.00772 [0.00523, 4.53]	0.0102 [0.00533, 0.352]
Pb_SG				
Mean (SD)	0.892 (1.03)	1.23 (0.793)	1.51 (1.16)	2.24 (2.12)
GM	0.275	1.02	1.10	1.48
Med[Min, Max]	**0.441 [0.00300, 4.20][A,I,L]**	1.13 [0.419, 3.07]	1.37 [0.00793, 6.53]	1.39 [0.00443, 12.6]

G**:** Gürağaç Primary School, A: Atatürk Primary School, I: 60.yil Işık Primary School, L; Lignite Primary School, SG: specific gravity SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. [G]Statistically significant increase in G, [A]Statistically significant increase in A, [I]Statistically significant increase in I, [L]Statistically significant increase in L against to the corresponding school(s) ($p<0.05$).

Statistically significant differences found in our results between the schools in SG-UM of Be, Cr, Mn, Fe, Ni, As, and Pb. Statistically significantly higher level of Be measured in L than G and A. The level of Cr in A is statistically significantly higher than the other school. Mn level in L is statistically significantly lower than the levels measured in G and A. Fe level in L is statistically significantly higher than the other schools. The level of Ni in L is statistically significantly lower than the levels of the other schools. The level As in G is statistically significantly higher than the other schools, and the level in I is statistically significantly higher than the level of L. Pb levels in A, I, and L are statistically significantly higher than the level of G (Table 4.8).

From this point of the results section only CR-UM data will be explained and shown in the tables. When it is needed the R-UM and SG-UM data will also be mentioned.

4.2.1. Urinary metal concentrations of children according to gender

In the total group CR-UM of Pb is statistically significantly higher in boys than that of girls ($p<0.05$).

Be, Mn, and Fe levels of girls and boys in KC are statistically significantly higher than the corresponding levels of girls and boys living in TR. Statistically significantly higher level of Ni and As of girls and boys measured in TR than the corresponding levels of girls and boys of KC. CR-UM of V and Cd of boys in KC are statistically significantly higher than the levels of boys living in TR. Se and Pb levels of girls in KC are statistically significantly higher than the levels of girls in TR. Pb level of boys in TR is statistically significantly higher than the level of girls in TR (Table 4.9).

Table 4.9. Creatinine adjusted urinary metal levels ($\mu g\ g^{-1}$) of metals in urine samples of girls and boys according to their regions

	TR		**KC**	
	Girl (n=50)	**Boy (n=38)**	**Girl (n=32)**	**Boy (n=40)**
Be_CR				
Mean (SD)	0.00665 (0.0213)	0.0108 (0.0287)	0.0215 (0.0361)	0.0141 (0.0231)
Med[Min, Max]	0.00138 **[0.000484, 0.136]** $^{g\text{-}KC}$	**0.00131 [0.000484, 0.158]** $^{b\text{-}KC}$	0.00300 [6e-04, 0.148]	0.00188 [0.000556, 0.0970]
V_CR				
Mean (SD)	1.27 (1.88)	0.879 (1.16)	2.20 (3.11)	1.91 (1.84)
Med[Min, Max]	0.550 [0.00586, 8.54]	**0.508 [0.00773, 4.22]** $^{g\text{-}KC}$	1.21 [0.0100, 14.5]	1.59 [0.0100, 8.12]

Table 4.9. (continues) Creatinine adjusted urinary metal levels (μg g^{-1}) of metals in urine samples of girls and boys according to their regions

Cr_CR				
Mean (SD)	0.453 (0.344)	0.493 (0.610)	0.423 (0.430)	0.481 (0.395)
Med[Min, Max]	0.349 [0.00375, 1.52]	0.325 [0.0252, 3.19]	0.250 [0.00563, 2.00]	0.354 [0.00450, 1.67]
Mn_CR				
Mean (SD)	3.75 (11.8)	1.47 (1.09)	2.91 (2.83)	3.19 (4.81)
Med[Min, Max]	**1.16 [0.00382, 81.9]** g-KC	**1.16 [0.00210, 4.23]** b-KC	2.08 [0.237, 13.2]	2.09 [0.00650, 30.6]
Fe_CR				
Mean (SD)	31.2 (51.7)	21.5 (18.4)	35.4 (23.3)	34.6 (32.9)
Med[Min, Max]	**16.1 [0.143, 338]** g-KC	**14.6 [0.200, 73.0]** b-KC	27.8 [6.91, 83.0]	26.5 [3.81, 198]
Co_CR				
Mean (SD)	1.32 (0.941)	1.50 (1.44)	1.03 (0.661)	1.14 (0.799)
Med[Min, Max]	1.16 [0.409, 4.61]	1.06 [0.331, 8.55]	0.824 [0.256, 3.19]	0.905 [0.0100, 3.96]
Ni_CR				
Mean (SD)	7.66 (9.87)	5.58 (2.84)	5.35 (5.04)	4.51 (2.61)
Med[Min, Max]	6.00 [1.44, 70.8]	4.90 [1.47, 14.5]	**3.99 [1.11, 27.1]** g-TR	**3.53 [1.42, 11.4]** b-TR
Cu_CR				
Mean (SD)	21.5 (64.5)	10.1 (3.27)	16.3 (17.2)	13.0 (6.73)
Med[Min, Max]	9.97 [3.89, 460]	9.65 [3.86, 18.3]	11.4 [5.47, 92.6]	10.6 [5.02, 34.8]
Zn_CR				
Mean (SD)	563 (218)	555 (228)	571 (282)	597 (359)
Med[Min, Max]	529 [188, 1100]	517 [149, 963]	515 [116, 1800]	550 [200, 2390]
As_CR				
Mean (SD)	43.0 (35.0)	33.6 (24.7)	17.1 (6.11)	24.4 (28.0)
Med[Min, Max]	25.8 [11.7, 135]	24.5 [7.49, 123]	**17.0 [7.28, 38.7]** g-TR	**18.2 [9.01, 191]** b-TR
Se_CR				
Mean (SD)	14.8 (3.84)	15.9 (4.55)	16.9 (4.46)	17.8 (3.69)
Med[Min, Max]	**14.3 [6.02, 23.6]** g-KC	16.2 [6.04, 24.6]	17.7 [4.74, 26.8]	17.6 [11.1, 26.4]
Mo_CR				
Mean (SD)	74.8 (48.8)	71.2 (47.1)	92.3 (64.6)	79.3 (33.5)
Med[Min, Max]	64.8 [10.5, 333]	66.5 [12.5, 232]	81.7 [20.0, 339]	73.1 [19.5, 155]
Cd_CR				
Mean (SD)	0.402 (0.609)	0.237 (0.259)	0.286 (0.381)	0.601 (0.894)
Med[Min, Max]	0.127 [0.000952, 2.96]	**0.179 [0.00100, 1.22]** b-KC	0.132 [8e-04, 1.42]	0.239 [0.00111, 3.74]
Sn_CR				
Mean (SD)	0.416 (0.395)	0.360 (0.312)	0.521 (0.451)	0.684 (1.10)
Med[Min, Max]	0.309 [0.0145, 1.84]	0.312 [0.0125, 1.56]	0.438 [0.0125, 1.55]	0.476 [0.0125, 6.33]
Ba_CR				
Mean (SD)	7.86 (20.7)	4.88 (3.64)	11.1 (27.9)	4.85 (4.44)
Med[Min, Max]	3.77 [0.676, 149]	3.80 [0.838, 19.5]	3.63 [0.826, 154]	3.39 [1.07, 21.7]

Table 4.9. (continues) Creatinine adjusted urinary metal levels (µg g^{-1}) of metals in urine samples of girls and boys according to their regions

Hg_CR				
Mean (SD)	0.0794 (0.172)	0.198 (0.881)	0.0474 (0.0884)	0.0440 (0.0696)
Med[Min, Max]	0.0115 [0.00371, 0.863]	0.0105 [0.00371, 5.42]	0.0154 [0.00500, 0.445]	0.0115 [0.00523, 0.281]
Pb_CR				
Mean (SD)	1.26 (1.27)	1.87 (1.49)	2.41 (2.00)	5.81 (15.0)
Med[Min, Max]	**0.895 [0.00368, 5.86]$^{b-TR, g-KC}$**	1.54 [0.00350, 6.62]	2.00 [0.0700, 8.20]	1.99 [0.00778, 94.8]

TR: Tunçbelik Region, KC: Kütahya City Center, CR: creatinine SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. $^{g-TR}$Statistically significant increase in girls of Tunçbilek region, $^{b-TR}$Statistically significant increase in boys of Tunçbilek region, $^{g-KC}$Statistically significant increase in girls of Kütahya City Center, $^{b-KC}$Statistically significant increase in boys of Kütahya City Center($p<0.05$).

4.2.2. Urinary metal concentrations according to the parents' smoking status

In the total population CR-UM of As is higher in the children with passive smoking ($p<0.05$). CR-UM of Be, V, and Mn levels of children with and without passive smoking in KC are statistically significantly higher than the corresponding passive smoking status in TR. CR-UM of Be, Fe, and Cu levels of children with passive smoking in TR are statistically significantly higher than the levels of children without passive smoking in TR. Statistically significantly higher CR-UM of Co, Ni, and As of passive smoker children are measured in TR than that of KC. As levels are significantly increased for non-smoker children in TR against to the non-smoker children in KC (Table 4.10).

Table 4.10. Creatinine adjusted urinary levels (µg g^{-1}) of children living in Tunçbilek region and Kütahya City Center according to their parents' smoking status

	TR		KC	
	Yes (n=63)	**No (n=25)**	**Yes (n=35)**	**No (n=36)**
Be_CR				
Mean (SD)	0.00928 (0.0278)	0.00628 (0.0144)	0.0177 (0.0328)	0.0171 (0.0270)
Med[Min, Max]	**0.00150 [0.000517, 0.158]$^{y-KC}$**	**0.00100 [0.000484, 0.0536]$^{y-TR, n-KC}$**	0.00188 [0.000556, 0.148]	0.00300 [6e-04, 0.118]
V_CR				
Mean (SD)	1.14 (1.63)	1.02 (1.59)	1.78 (1.87)	2.34 (2.95)
Med[Min, Max]	**0.551 [0.00586, 8.54]$^{y-KC}$**	**0.381 [0.00654, 7.18]$^{n-KC}$**	1.59 [0.0100, 9.59]	1.40 [0.0100, 14.5]
Cr_CR				
Mean (SD)	0.512 (0.521)	0.364 (0.314)	0.461 (0.411)	0.430 (0.400)
Med[Min, Max]	0.367 [0.00450, 3.19]	0.265 [0.00375, 1.49]	0.332 [0.00450, 2.00]	0.302 [0.00450, 1.67]

Table 4.10. (continues) Creatinine adjusted urinary levels (μg g^{-1}) of children living in Tunçbilek region and Kütahya City Center according to their parents' smoking status

Mn_CR				
Mean (SD)	3.31 (10.5)	1.40 (1.85)	2.47 (1.98)	2.88 (2.66)
Med[Min, Max]	**1.30 [0.00650, 81.9]** $^{y\text{-}KC}$	**0.753 [0.00210, 8.80]** $^{n\text{-}KC}$	2.00 [0.367, 10.0]	2.13 [0.00650, 13.2]
Fe_CR				
Mean (SD)	31.0 (46.0)	16.9 (21.1)	29.6 (20.0)	35.6 (22.7)
Med[Min, Max]	18.9 [0.200, 338]	**10.3 [0.143, 99.6]** $^{y\text{-}TR,\ n\text{-}KC}$	26.2 [5.99, 83.0]	31.4 [3.81, 99.0]
Co_CR				
Mean (SD)	1.38 (0.976)	1.45 (1.61)	0.984 (0.651)	1.22 (0.793)
Med[Min, Max]	1.18 [0.331, 4.61]	1.14 [0.448, 8.55]	**0.825 [0.256, 3.19]** $^{y\text{-}TR}$	1.00 [0.242, 3.96]
Ni_CR				
Mean (SD)	7.22 (8.89)	5.61 (3.07)	4.96 (4.88)	4.67 (2.54)
Med[Min, Max]	5.77 [1.44, 70.8]	5.56 [1.47, 14.5]	**3.53 [1.39, 27.1]** $^{y\text{-}TR}$	4.15 [1.11, 11.4]
Cu_CR				
Mean (SD)	19.6 (57.5)	9.07 (3.86)	15.1 (16.2)	13.4 (7.54)
Med[Min, Max]	10.4 [3.89, 460]	**8.44 [3.86, 23.2]** $^{y\text{-}TR,\ n\text{-}KC}$	10.9 [5.47, 92.6]	10.5 [5.02, 36.8]
Zn_CR				
Mean (SD)	556 (227)	566 (212)	541 (213)	578 (275)
Med[Min, Max]	508 [187, 1100]	575 [149, 1020]	558 [200, 1260]	510 [116, 1800]
As_CR				
Mean (SD)	40.8 (31.2)	34.2 (31.4)	19.4 (6.88)	22.9 (29.7)
Med[Min, Max]	25.9 [11.7, 131]	24.3 [7.49, 135]	**17.4 [9.01, 37.0]** $^{y\text{-}TR}$	**17.4 [7.28, 191]** $^{n\text{-}TR}$
Se_CR				
Mean (SD)	15.7 (4.06)	14.1 (4.33)	16.7 (3.97)	17.9 (4.01)
Med[Min, Max]	15.8 [6.02, 24.6]	**14.0 [6.04, 22.3]** $^{n\text{-}KC}$	16.3 [4.74, 24.2]	17.8 [11.5, 26.8]
Mo_CR				
Mean (SD)	72.1 (36.5)	76.0 (69.6)	75.9 (38.8)	92.6 (57.8)
Med[Min, Max]	69.0 [11.7, 232]	**58.5 [10.5, 333]** $^{n\text{-}KC}$	70.5 [25.2, 218]	82.8 [19.5, 339]
Cd_CR				
Mean (SD)	0.329 (0.492)	0.335 (0.512)	0.369 (0.414)	0.468 (0.801)
Med[Min, Max]	0.151 [0.000952, 2.96]	0.124 [0.000952, 2.50]	0.235 [0.000870, 1.89]	0.134 [8e-04, 3.74]
Sn_CR				
Mean (SD)	0.365 (0.365)	0.459 (0.349)	0.660 (1.06)	0.481 (0.406)
Med[Min, Max]	0.298 [0.0125, 1.84]	0.359 [0.0688, 1.56]	0.459 [0.0125, 6.33]	0.438 [0.0125, 1.40]
Ba_CR				
Mean (SD)	7.40 (18.6)	4.48 (2.58)	6.14 (9.20)	8.71 (25.3)
Med[Min, Max]	3.83 [0.676, 149]	3.68 [0.838, 12.3]	3.57 [0.826, 53.4]	3.45 [1.07, 154]
Hg_CrR				
Mean (SD)	0.159 (0.696)	0.0581 (0.110)	0.0532 (0.0776)	0.0362 (0.0787)
Med[Min, Max]	0.0115 [0.00397, 5.42]	0.00821 [0.00371, 0.359]	0.0144 [0.00500, 0.281]	0.0121 [0.00523, 0.445]

Table 4.10. (continues) Creatinine adjusted urinary levels (µg g^{-1}) of children living in Tunçbilek region and Kütahya City Center according to their parents' smoking status

Pb_CR				
Mean (SD)	1.65 (1.44)	1.20 (1.23)	2.74 (2.15)	3.31 (4.41)
Med[Min, Max]	**1.40 [0.00368, 6.62]** $^{y\text{-}KC}$	**0.703 [0.00350, 4.50]** $^{n\text{-}KC}$	2.08 [0.00778, 8.20]	1.48 [0.0700, 21.5]

Parents' smoking status (father and/or mother of the children are smoking). TR: Tunçbelik Region, KC: Kütahya City Center, CR: creatinine, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. $^{y\text{-}TR}$Statistically significant increase for passive smoker children in Tunçbilek region, $^{n\text{-}TR}$Statistically significant increase for non-smoker children in Tunçbilek region, $^{y\text{-}KC}$Statistically significant increase for passive smoker children in Kütahya City Center, $^{n\text{-}KC}$Statistically significant increase for non-smoker children in Kütahya City Center($p<0.05$).

4.2.3. Urinary metal concentrations according to the transportation between home and school

In total population, CR-UM of children walking or using vehicles for transportation between homes and schools are not different ($p> 0.05$).

Be, V, Mn, Fe, Se, and Pb levels in children walking between home and school in KC are significantly increased than that of in TR. Be, Se, and Pb levels in children using vehicles for transportation in TR are statistically significantly higher than that of walking children in TR. Mn and Hg levels are significantly increased in children using vehicle in KC when compared to the children using vehicles for transportation in TR. As_CR level of walking children in TR is statistically significantly higher than the vehicle transported children both in TR, KC and also than of walking children in KC (Table 4.11).

Table 4.11. Creatinine adjusted urinary levels (µg g^{-1}) in urine samples of children living in Tunçbilek region and Kütahya City Center according to transportation way between home and school

	TR		**KC**	
	Vehicle* (n=33)	**Walking (n=44)**	**Vehicle (n=30)**	**Walking (n=29)**
Be_CR				
Mean (SD)	0.0188 (0.0377)	0.00238 (0.00682)	0.0156 (0.0271)	0.0220 (0.0357)
Med[Min, Max]	0.00150 [0.000577, 0.158]	**0.00115 [0.000484, 0.0462]** $^{v\text{-}TR,\ w\text{-}KC}$	0.00214 [0.000556, 0.118]	0.00375 [0.000652, 0.148]
V_CR				
Mean (SD)	1.55 (2.18)	0.895 (1.16)	1.62 (1.47)	2.90 (3.30)
Med[Min, Max]	1.04 [0.00654, 8.54]	**0.549 [0.00586, 4.79]** $^{w\text{-}KC}$	1.28 [0.0121, 4.67]	1.85 [0.0100, 14.5]

Table 4.11. (continues) Creatinine adjusted urinary levels (μg g^{-1}) in urine samples of children living in Tunçbilek region and Kütahya City Center according to transportation way between home and school

Cr_CR				
Mean (SD)	0.479 (0.557)	0.427 (0.349)	0.503 (0.448)	0.411 (0.336)
Med[Min, Max]	0.330 [0.0252, 3.19]	0.349 [0.00375, 1.52]	0.373 [0.00450, 2.00]	0.332 [0.00450, 1.18]
Mn_CR				
Mean (SD)	2.32 (3.17)	3.44 (12.4)	2.55 (1.88)	4.06 (5.91)
Med[Min, Max]	**1.45 [0.0130, 16.8]** $^{v\text{-}KC}$	**1.09 [0.00210, 81.9]**$^{w\text{-}KC}$	2.23 [0.00650, 10.0]	2.11 [0.367, 30.6]
Fe_CR				
Mean (SD)	26.8 (26.4)	27.6 (52.3)	28.6 (17.4)	44.3 (38.8)
Med[Min, Max]	16.1 [1.68, 126]	**14.0 [0.143, 338]**$^{w\text{-}KC}$	26.2 [3.81, 82.8]	37.0 [7.86, 198]
Co_CR				
Mean (SD)	1.68 (1.58)	1.20 (0.859)	1.25 (0.858)	0.955 (0.661)
Med[Min, Max]	1.20 [0.331, 8.55]	1.06 [0.409, 4.61]	0.929 [0.331, 3.96]	0.823 [0.0100, 3.19]
Ni_CR				
Mean (SD)	6.29 (4.07)	7.30 (10.3)	5.60 (5.44)	4.50 (2.33)
Med[Min, Max]	5.45 [1.47, 21.2]	5.43 [1.44, 70.8]	3.55 [1.11, 27.1]	**4.30 [1.39, 10.5]** $^{w\text{-}TR}$
Cu_CR				
Mean (SD)	12.8 (13.8)	20.5 (68.0)	16.9 (17.6)	13.9 (7.68)
Med[Min, Max]	10.4 [3.86, 85.4]	9.65 [3.89, 460]	11.6 [6.98, 92.6]	11.3 [5.47, 36.8]
Zn_CR				
Mean (SD)	545 (237)	561 (224)	513 (175)	675 (455)
Med[Min, Max]	545 [149, 969]	512 [188, 1100]	483 [200, 838]	554 [220, 2390]
As_CR				
Mean (SD)	25.9 (12.9)	51.8 (38.3)	19.0 (7.24)	18.8 (7.26)
Med[Min, Max]	**23.9 [7.49, 80.4]**$^{w\text{-}TR}$	37.3 [12.0, 135]	**17.6 [7.28, 39.4]** $^{v\text{-}TR}$	**17.6 [10.0, 38.7]** $^{w\text{-}TR}$
Se_CR				
Mean (SD)	16.6 (4.14)	14.6 (4.20)	16.8 (3.60)	17.2 (4.59)
Med[Min, Max]	16.7 [6.04, 24.6]	**13.6 [6.02, 23.6]**$^{v\text{-}TR,\ w\text{-}KC}$	16.3 [11.0, 26.8]	17.7 [4.74, 26.4]
Mo_CR				
Mean (SD)	85.6 (66.0)	66.6 (30.8)	78.3 (32.1)	89.0 (67.0)
Med[Min, Max]	70.7 [11.7, 333]	62.6 [10.5, 147]	74.6 [33.8, 188]	71.2 [19.5, 339]
Cd_CR				
Mean (SD)	0.313 (0.370)	0.366 (0.606)	0.231 (0.299)	0.583 (0.830)
Med[Min, Max]	0.191 [0.000952, 1.39]	0.129 [0.00105, 2.96]	0.131 [8e-04, 0.948]	0.246 [0.000870, 3.44]
Sn_CR				
Mean (SD)	0.370 (0.348)	0.434 (0.396)	0.708 (1.14)	0.509 (0.729)
Med[Min, Max]	0.308 [0.0125, 1.56]	0.325 [0.0212, 1.84]	0.431 [0.0229, 6.33]	0.304 [0.0125, 3.61]
Ba_CR				
Mean (SD)	5.60 (3.39)	7.76 (22.2)	6.37 (10.1)	10.4 (28.1)
Med[Min, Max]	5.35 [0.838, 12.9]	3.52 [0.676, 149]	3.56 [1.07, 53.4]	3.34 [0.826, 154]

Table 4.11. (continues) Creatinine adjusted urinary levels (μg g^{-1}) in urine samples of children living in Tunçbilek region and Kütahya City Center according to transportation way between home and school

Hg_CR				
Mean (SD)	0.245 (0.951)	0.0660 (0.138)	0.0449 (0.0674)	0.0467 (0.0670)
Med[Min, Max]	**0.00958 [0.00442, 5.42]$^{v\text{-}KC}$**	0.0110 [0.00371, 0.752]	0.0144 [0.00605, 0.281]	0.0128 [0.00500, 0.239]
Pb_CR				
Mean (SD)	1.92 (1.46)	1.21 (1.17)	2.43 (2.08)	6.84 (17.6)
Med[Min, Max]	1.54 [0.00350, 6.62]	**0.844 [0.00368, 5.86]$^{v\text{-}TR,\ w\text{-}KC}$**	1.84 [0.00778, 8.20]	2.28 [0.0700, 94.8]

Vehicle* (include car, school service, or bus), TR: Tunçbelik Region, KC: Kütahya City Center, CR: creatinine, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. $^{v\text{-}TR}$Statistically significant increase for transportation of children by vehicle in Tunçbilek region, $^{w\text{-}TR}$Statistically significant increase for walking children in Tunçbilek region, $^{v\text{-}KC}$ Statistically significant increase for transportation of children by vehicle in Kütahya City Center, $^{w\text{-}KC}$Statistically significant increase for walking children in Kütahya City Center($p<0.05$).

4.2.4. Urinary metal concentrations of children according to the drinking water source

In total population, CR-UM of As is statistically significantly lower in children drinking carboy water than the levels of children drinking water from other sources (Tap Water, overflow water, and mixed) while CR-UM of V is higher in children drinking carboy water than that of using other sources.

CR-UM of Be, V, Mn, Co, Se, Hg, and Pb of children in KC drinking carboy water and CR-UM of Be, Se, and Mo of children in KC drinking water from other sources are statistically significantly higher than that of in TR. Ni and As levels in children drinking carboy water and in children drinking water from other sources in TR are statistically significantly higher than that of in KC. Furthermore, CR-UM As in children drinking carboy water is increased significantly against to As level in children drinking from other water sources in TR. (Table 4.12).

Table 4.12. Creatinine adjusted urinary levels (µg g^{-1}) of metals in urine samples of children living in Tunçbilek region and Kütahya City Center according to the drinking water sources

	TR		KC	
	Carboy Water (n=23)	**Others* (n=50)**	**Carboy Water (n=40)**	**Others (n=30)**
Be_CR				
Mean (SD)	0.00769 (0.0177)	0.00582 (0.0206)	0.0176 (0.0323)	0.0179 (0.0269)
Med[Min, Max]	**0.00136 [0.000517, 0.0668]**[c-KC]	**0.00120 [0.000484, 0.136]**[o-KC]	0.00232 [0.000556, 0.148]	0.00190 [6e-04, 0.0970]
V_CR				
Mean (SD)	1.05 (1.24)	1.06 (1.81)	2.44 (2.86)	1.54 (1.79)
Med[Min, Max]	**0.800 [0.00586, 3.77]** [c-KC]	0.438 [0.00810, 8.54]	1.71 [0.0100, 14.5]	1.26 [0.0100, 8.12]
Cr_CR				
Mean (SD)	0.404 (0.327)	0.476 (0.501)	0.462 (0.424)	0.439 (0.382)
Med[Min, Max]	0.291 [0.00375, 1.52]	0.351 [0.00450, 3.19]	0.285 [0.00563, 1.67]	0.329 [0.00450, 2.00]
Mn_CR				
Mean (SD)	1.17 (0.852)	3.86 (11.8)	3.44 (4.95)	2.60 (2.50)
Med[Min, Max]	**1.01 [0.00650, 2.74]** [c-KC]	1.29 [0.00210, 81.9]	2.13 [0.237, 30.6]	1.84 [0.00650, 10.7]
Fe_CR				
Mean (SD)	20.1 (16.6)	32.0 (51.8)	37.0 (32.6)	32.5 (24.4)
Med[Min, Max]	**14.0 [0.143, 66.3]**[c-KC]	16.9 [0.200, 338]	28.4 [5.99, 198]	24.4 [3.81, 99.0]
Co_CR				
Mean (SD)	1.32 (0.884)	1.35 (0.958)	1.00 (0.659)	1.20 (0.830)
Med[Min, Max]	1.04 [0.448, 3.90]	1.19 [0.331, 4.61]	0.830 [0.0100, 3.19]	0.957 [0.261, 3.96]
Ni_CR				
Mean (SD)	6.08 (2.97)	7.33 (9.91)	4.94 (4.32)	4.85 (3.39)
Med[Min, Max]	5.89 [1.60, 12.9]	5.22 [1.44, 70.8]	**3.57 [1.11, 27.1]**[c-TR]	**3.88 [1.39, 18.4]** [o-TR]
Cu_CR				
Mean (SD)	9.55 (3.21)	21.9 (64.4)	14.6 (14.0)	13.5 (10.0)
Med[Min, Max]	10.2 [4.75, 15.7]	9.65 [3.89, 460]	10.9 [5.47, 92.6]	10.6 [5.02, 59.9]
Zn_CR				
Mean (SD)	569 (185)	595 (227)	611 (403)	545 (193)
Med[Min, Max]	553 [261, 933]	580 [188, 1100]	510 [116, 2390]	540 [200, 942]
As_CR				
Mean (SD)	32.5 (25.9)	45.3 (35.5)	21.0 (28.2)	20.7 (7.30)
Med[Min, Max]	22.5 [13.8, 114]	29.0 [11.7, 135]	**15.9 [7.28, 191]**[o-KC, c-TR]	**19.2 [9.01, 39.4]** [o-TR]
Se_CR				
Mean (SD)	15.7 (3.84)	15.0 (3.94)	17.7 (4.10)	17.1 (4.07)
Med[Min, Max]	**14.2 [10.2, 23.7]** [c-KC]	**15.5 [6.02, 23.6]** [o-KC]	17.9 [11.0, 26.8]	17.2 [4.74, 26.4]
Mo_CR				
Mean (SD)	87.3 (64.5)	67.9 (39.4)	75.8 (35.6)	96.6 (64.0)
Med[Min, Max]	76.9 [11.7, 333]	**60.9 [10.5, 232]** [o-KC]	71.6 [19.5, 153]	78.9 [26.5, 339]

Table 4.12. (continues) Creatinine adjusted urinary levels (µg g^{-1}) of metals in urine samples of children living in Tunçbilek region and Kütahya City Center according to the drinking water sources

Cd_CR				
Mean (SD)	0.209 (0.274)	0.404 (0.606)	0.594 (0.920)	0.286 (0.290)
Med[Min, Max]	0.0980 [0.00105, 0.899]	0.142 [0.000952, 2.96]	0.220 [0.000909, 3.74]	0.223 [8e-04, 0.948]
Sn_CR				
Mean (SD)	0.501 (0.504)	0.370 (0.255)	0.551 (0.641)	0.672 (1.13)
Med[Min, Max]	0.393 [0.0145, 1.84]	0.335 [0.0183, 1.38]	0.451 [0.0125, 3.61]	0.446 [0.0125, 6.33]
Ba_CR				
Mean (SD)	4.98 (3.61)	7.92 (20.7)	6.25 (8.94)	4.80 (4.83)
Med[Min, Max]	4.63 [1.65, 19.5]	3.61 [0.676, 149]	3.63 [1.11, 53.4]	3.39 [0.826, 23.1]
Hg_CR				
Mean (SD)	0.0426 (0.155)	0.165 (0.767)	0.0433 (0.0835)	0.0498 (0.0738)
Med[Min, Max]	**0.00958 [0.00397, 0.752]** $^{c\text{-}KC}$	0.0115 [0.00371, 5.42]	0.0144 [0.00523, 0.445]	0.0115 [0.00500, 0.281]
Pb_CR				
Mean (SD)	1.40 (0.919)	1.73 (1.62)	5.54 (14.8)	2.10 (1.47)
Med[Min, Max]	**1.23 [0.386, 4.07]** $^{c\text{-}KC}$	1.38 [0.00368, 6.62]	1.88 [0.0700, 94.8]	1.99 [0.00778, 6.73]

Other*(tap water, overflow water, and mixed). TR: Tunçbelik Region, KC: Kütahya City Center, CR: creatinine, SD: standard deviation, Med: median, GM: Geometric mean, Min: minimum value, Max: maximum value, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. $^{c\text{-}TR}$Statistically significant increase for carboy drinking water in Tunçbilek region, $^{o\text{-}TR}$Statistically significant increase for other drinking water sources in Tunçbilek region, $^{c\text{-}KC}$ Statistically significant increase for carboy drinking water in Kütahya City Center, $^{o\text{-}KC}$Statistically significant increase for other drinking water sources in Kütahya City Center($p<0.05$).

4.3. Correlation Analysis

Spearman’s correlation analyses for every possible pair of metals are performed. R-UM results showed that the metals: Be -V, Be-Mn, Be-Fe, Be-Zn, Be-Sn, V-Cr, V-Mn, V-Fe, V-Zn, V-Mo, V-Cd, V-Sn, Cr-Mn, Cr-Fe, Cr-Co, Cr-Ni, Cr-Cu, Cr-Zn, Cr-As, Cr-Se, Cr-Cd, Cr-Ba, Cr-Hg, Mn-Fe, Mn-Cu, Mn-Cd, Mn-Pb, Fe-Cu, Co-Ni, Co-Cu, Co-Zn, Co-As, Co-Se, Co-Mo, Co-Sn, Co-Ba, Ni-Cu, Ni-Zn, Ni-As, Ni-Se, Ni-Mo, Ni-Cd, Ni-Ba, Cu-Zn, Cu-As, Cu-Se, Cu-Mo, Cu-Cd, Cu-Sn, Cu-Ba, Cu-Pb, Zn-As, Zn-Se, Zn-Mo, As-Se, As-Mo, As-Sn, As-Ba, Se-Mo, Se-Sn, Se-Ba, Se-Pb, Mo-Sn, Mo-Ba, Cd-Ba, Cd-Pb, Sn-Ba, Sn-Pb, and Hg-Pb are statistically correlated ($p < 0.05$) (Figure 4.4) (Table 4.14). Be-Zn, Be-Sn, V-Zn, V-Mo, V-Sn, and Hg-Pb are statistically negatively correlated, while all the other pairs of metals are positively correlated (Figure 4.4) (Table 4.13).

Regarding CR-UM, results showed that the metals: Be -V, Be-Cr, Be-Mn, Be-Fe, Be-Co, Be-Ni, Be-Cu, Be-Se, Be-Cd, Be-Ba, Be-Hg, Be-Pb, V-Cr, V-Mn, V-Fe, V-Co, V-Ni, V-Cu, V-Se, V-Cd, V-Sn, V-Ba, V-Hg, V-Pb, Cr-Mn, Cr-Fe, Cr-Ni, Cr-Cu, Cr-Zn, Cr-As, Cr-Cd, Cr-Ba, Cr-Hg, Cr-Pb, Mn-Fe, Mn-Co, Mn-Ni, Mn-Cu, Mn-Zn, Mn-Se, Mn-Mo, Mn-Cd, Mn-Ba, Mn-Hg, Mn-Pb, Fe-Co, Fe-Ni, Fe-Cu, Fe-Zn, Fe-Se, Fe-Mo, Fe-Cd, Fe-Ba, Fe-Hg, Fe-Pb, Co-Ni, Co-Cu, Co-As, Co-Cd, Ni-Cu, Ni-Zn, Ni-As, Ni-Mo, Ni-Cd, Ni-Ba, Ni-Hg, Ni-Pb, Cu-Zn, Cu-As, Cu-Se, Cu-Mo, Cu-Cd, Cu-Ba, Cu-Hg, Cu-Pb, Zn-Mo, Zn-Cd, Zn-Ba, Zn-Hg, Zn-Pb, As-Mo, As-Cd, Se-Mo, Se-Cd, Se-Pb, Mo-Sn, Mo-Ba, Mo-Pb, Cd-Sn, Cd-Ba, Cd-Pb, Sn-Pb, Ba-Hg, Ba-Pb, and Hg-Pb are statistically correlated ($p < 0.05$) (Figure 4.5) (Table 4.15). All the pairs of metals mentioned above are positively correlated except the correlation between V-Sn are negative (Figure 4.5) (Table 4.14).

For SG-UM: Be-V, Be-Co, V-Cr, V-Mn, V-Fe, V-Mo, V-Cd, V-Hg, Cr-Mn, Cr-Fe, Cr-Cu, Cr-Cd, Cr-Ba, Cr-Hg, Cr-Pb, Mn-Fe, Mn-Cu, Mn-Se, Mn-Cd, Mn-Ba, Co-Ni, Co-Se, Ni-Zn, Ni-As, Cu-Zn, Cu-Ba, Cu-Pb, Zn-Se, Se-Mo, and Cd-Pb are statistically correlated ($p < 0.05$) (Figure 4.6) (Table 4.16). All the pairs of metals mentioned above are positively correlated, except the correlation between V-Mo and Mn-Se is negative (Figure 4.6) (Table 4.15).

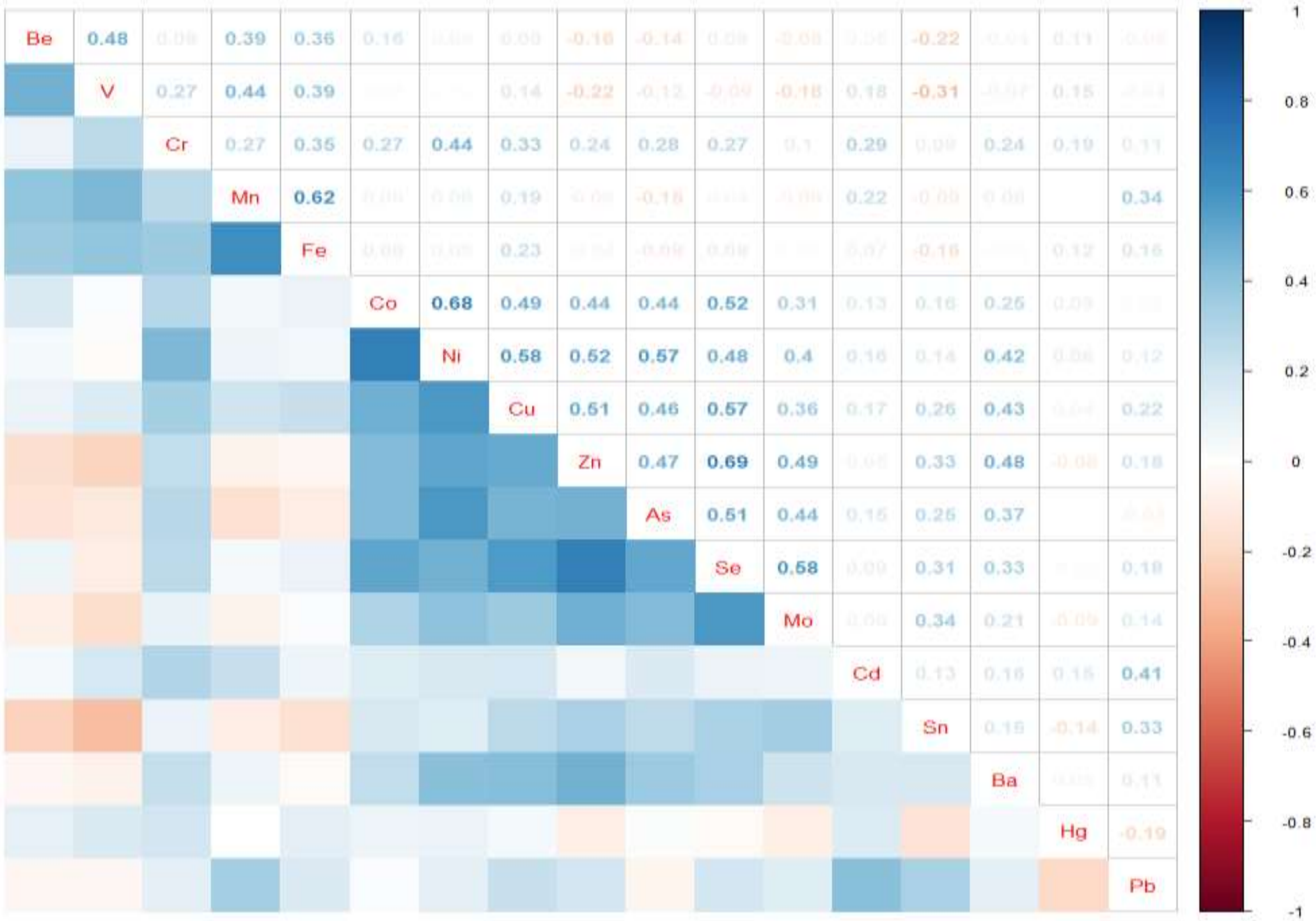


Figure 4.4. Correlation between metals

Spearman correlation test of each pair of metal was determined. Data represent correlation coefficient (rho)

Table 4.13. p-values of correlation between metals

	Be	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Ba	Hg	Pb
Be																	
V	**0.000**	NA															
Cr	0.300	**0.000**	NA														
Mn	**0.000**	**0.000**	**0.000**	NA													
Fe	**0.000**	**0.000**	**0.000**	**0.000**	NA												
Co	0.040	0.720	**0.000**	0.450	0.270	NA											
Ni	0.550	0.830	**0.000**	0.420	0.470	**0.000**	NA										
Cu	0.290	0.070	**0.000**	**0.010**	**0.000**	**0.000**	**0.000**	NA									
Zn	**0.040**	**0.010**	**0.000**	0.430	0.600	**0.000**	**0.000**	**0.000**	NA								
As	0.070	0.140	**0.000**	0.050	0.240	**0.000**	**0.000**	**0.000**	**0.000**	NA							
Se	0.340	0.250	**0.000**	0.650	0.300	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	NA						
Mo	0.310	**0.020**	0.210	0.430	0.780	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	NA					
Cd	0.530	**0.020**	**0.000**	**0.000**	0.400	0.100	**0.040**	**0.030**	0.520	0.060	0.270	0.450	NA				
Sn	**0.000**	**0.000**	0.280	0.240	0.050	**0.040**	0.090	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.100	NA			
Ba	0.610	0.390	**0.000**	0.330	0.730	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.010**	**0.040**	**0.040**	NA		
Hg	0.180	0.050	**0.020**	0.940	0.130	0.320	0.290	0.610	0.310	0.860	0.780	0.270	0.070	0.070	0.530	NA	
Pb	0.550	0.590	0.150	**0.000**	0.050	0.760	0.150	**0.000**	**0.020**	0.490	**0.020**	0.080	**0.000**	**0.000**	0.160	**0.010**	NA

As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. NA: not applicable.

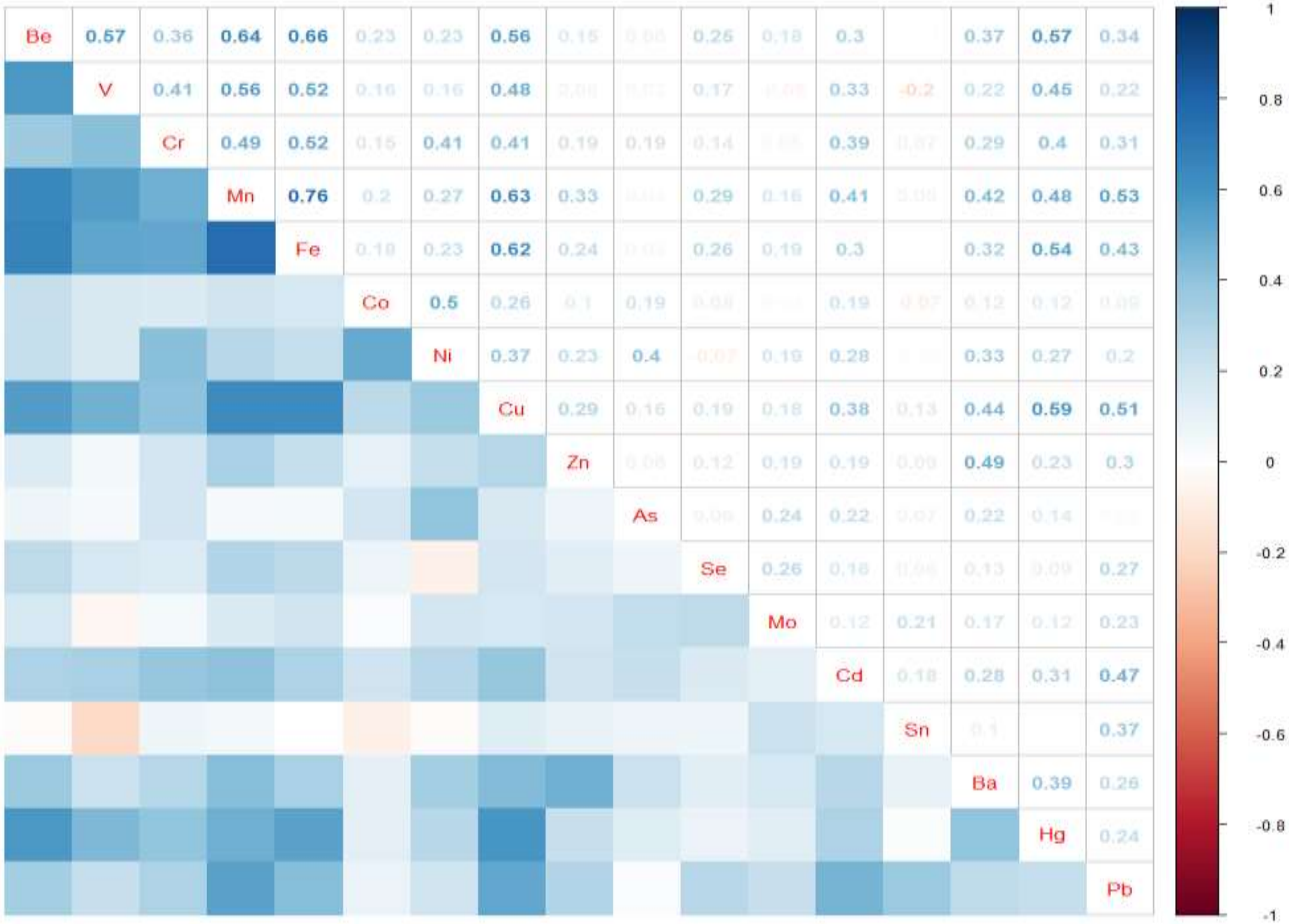


Figure 4.5. Correlations between metals adjusted with creatinine

Spearman correlation test of each pair of metal was determined. Data represent correlation coefficient (rho)

Table 4.14. p-values of correlations between creatinine adjusted metals

	Be	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Ba	Hg	Pb
Be	NA																
V	**0.000**	NA															
Cr	**0.000**	**0.000**	NA														
Mn	**0.000**	**0.000**	**0.000**	NA													
Fe	**0.000**	**0.000**	**0.000**	**0.000**	NA												
Co	**0.000**	**0.040**	0.060	**0.010**	**0.020**	NA											
Ni	**0.000**	**0.040**	**0.000**	**0.000**	**0.000**	**0.000**	NA										
Cu	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	NA									
Zn	0.070	0.490	**0.020**	**0.000**	**0.000**	0.200	**0.000**	**0.000**	NA								
As	0.420	0.670	**0.020**	0.630	0.590	**0.020**	**0.000**	**0.040**	0.430	NA							
Se	**0.000**	**0.030**	0.070	**0.000**	**0.000**	0.310	0.360	**0.020**	0.120	0.420	NA						
Mo	**0.030**	0.560	0.570	**0.040**	**0.020**	0.730	**0.020**	**0.030**	**0.020**	**0.000**	**0.000**	NA					
Cd	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.010**	**0.000**	**0.000**	**0.020**	**0.000**	**0.040**	0.130	NA				
Sn	0.860	**0.010**	0.400	0.510	0.920	0.370	0.850	0.100	0.240	0.390	0.450	**0.010**	**0.020**	NA			
Ba	**0.000**	**0.010**	**0.000**	**0.000**	**0.000**	0.140	**0.000**	**0.000**	**0.000**	0.010	0.100	**0.030**	**0.000**	0.230	NA		
Hg	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.140	**0.000**	**0.000**	**0.000**	0.090	0.260	0.120	0.000	0.830	**0.000**	NA	
Pb	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.280	**0.010**	**0.000**	**0.000**	0.770	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	NA

As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. NA: not applicable.

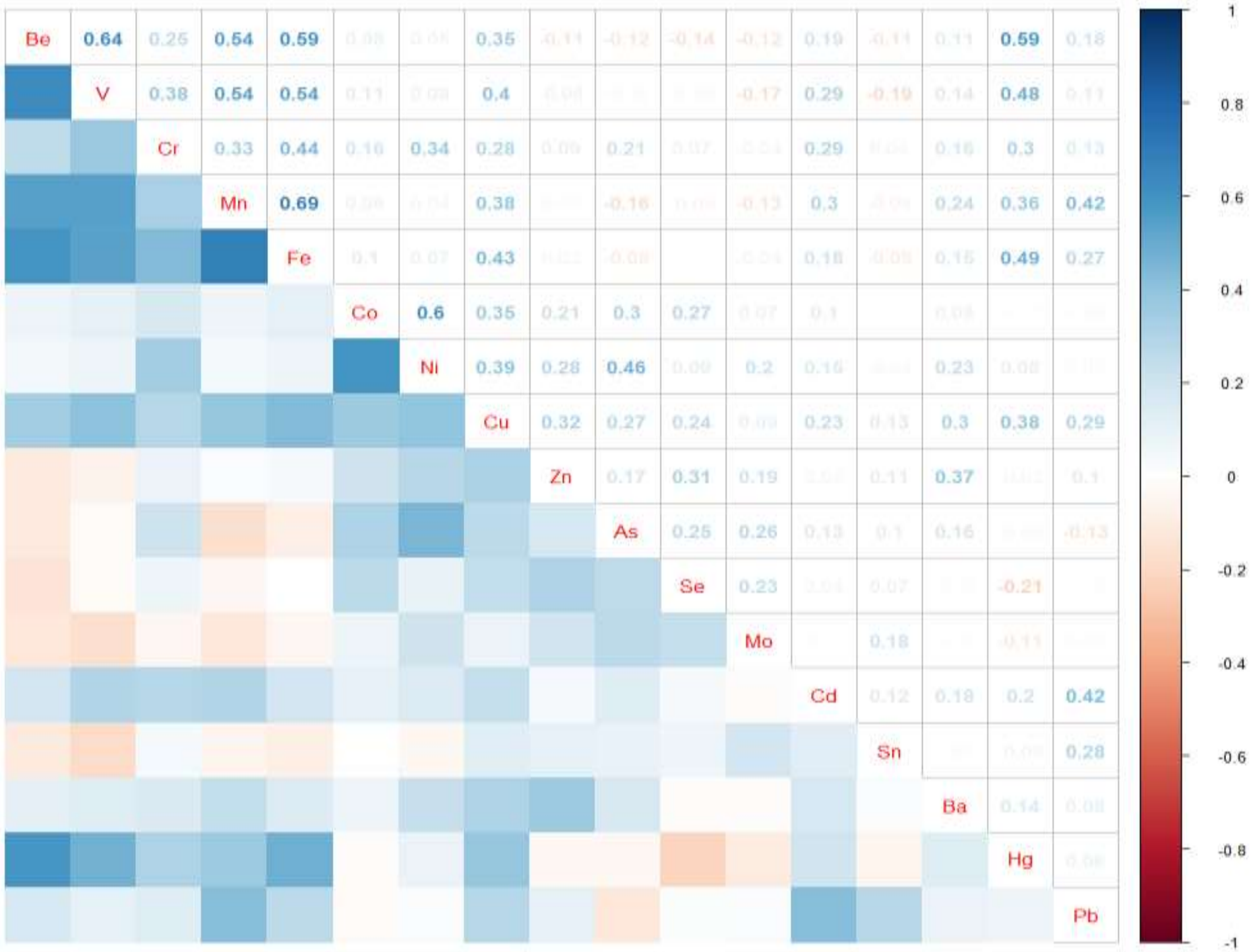


Figure 4.6. Correlation between specific gravity adjusted metals

Spearman correlation test of each pair of metal was determined. Data represent correlation coefficient (rho)

Table 4.15. p-values of correlation between specific gravity adjusted metals

	Be	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sn	Ba	Hg	Pb
Be	NA																
V	**0.000**	NA															
Cr	0.480	**0.000**	NA														
Mn	0.170	**0.000**	**0.000**	NA													
Fe	0.050	**0.000**	**0.000**	**0.000**	NA												
Co	**0.000**	0.270	0.280	1.000	0.890	NA											
Ni	0.880	0.830	0.120	0.340	0.800	**0.000**	NA										
Cu	0.780	0.190	**0.010**	**0.000**	0.080	0.830	0.050	NA									
Zn	0.350	0.880	0.090	0.390	0.470	0.190	**0.010**	0.030	NA								
As	0.060	0.360	0.700	0.230	0.530	0.110	**0.020**	0.150	0.400	NA							
Se	0.240	0.440	0.260	**0.040**	0.800	**0.020**	0.870	0.190	**0.000**	0.080	NA						
Mo	0.980	**0.040**	0.370	0.350	0.710	0.050	0.280	0.590	0.250	0.180	**0.020**	NA					
Cd	0.550	**0.000**	**0.000**	**0.020**	0.280	0.350	0.900	0.130	0.870	0.100	0.180	0.840	NA				
Sn	0.820	0.210	0.610	0.580	0.460	0.210	0.290	0.750	0.670	0.770	0.940	0.140	0.640	NA			
Ba	0.640	0.720	**0.010**	**0.000**	0.400	0.310	0.100	**0.000**	0.080	0.500	0.030	0.610	0.600	0.460	NA		
Hg	0.820	**0.010**	**0.000**	0.790	0.240	0.290	0.910	0.950	0.410	1.000	0.280	0.640	0.490	0.300	0.830	NA	
Pb	0.580	0.060	**0.040**	0.080	0.210	0.400	0.740	**0.010**	0.790	0.210	0.210	0.990	**0.000**	0.170	0.050	0.980	NA

As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc. NA: not applicable.

In general, there is a positive correlation between R-UM and SG-UM of each metal ($p<0.001$) where r values are as following $r_{Ba} = 0.66$, $r_V = 0.937$, $r_{Cr} = 0.856$, $r_{Mn} = 0.864$, $r_{Fe} = 0.835$, $r_{Co} = 0.881$, $r_{Ni}= 0.834$, $r_{Cu} = 0.594$, $r_{Zn} = 0.707$, $r_{As} = 0.823$, $r_{Se} = 0.769$, $r_{Mo} = 0.842$, $r_{Cd} = 0.948$, $r_{Sn} = 0.916$, $r_{Ba} = 0.97$, $r_{Hg} = 0.679$, and $r_{Pb} = 0.893$. At the same time, there is a positive correlation between R-UM and CR-UM of each measured metal ($p<0.001$), except Cu ($r_{Cu}= 0.128$, $p= 0.106$) shows no statistically correlation between R-UM and SG-UM where r values are as following $r_{Ba} = 0.658$, $r_V = 0.907$, $r_{Cr} = 0.696$, $r_{Mn} = 0.781$, $r_{Fe} = 0.704$, $r_{Co} = 0.718$, $r_{Ni}= 0.578$, $r_{Zn} = 0.322$, $r_{As} = 0.624$, $r_{Se} = 0.314$, $r_{Mo} = 0.588$, $r_{Cd} = 0.923$, $r_{Sn} = 0.83$, $r_{Ba} = 0.602$, $r_{Hg} = 0.674$, and $r_{Pb} = 0.81$. Finally, there is a positive correlation between CR-UM and SG-UM of each metal ($p<0.001$) where r values are as following $r_{Ba} = 0.828$, $r_V = 0.962$, $r_{Cr} = 0.857$, $r_{Mn} = 0.919$, $r_{Fe} = 0.884$, $r_{Co} = 0.845$, $r_{Ni}= 0.769$, $r_{Cu} = 0.607$, $r_{Zn} = 0.647$, $r_{As} = 0.764$, $r_{Se} = 0.45$, $r_{Mo} = 0.761$, $r_{Cd} = 0.967$, $r_{Sn} = 0.912$, $r_{Ba} = 0.819$, $r_{Hg} = 0.861$, and $r_{Pb} = 0.95$.

The data below is the correlations with the other parameters measured in the project, which the present thesis is part of. Buccal Epithelial Micronucleus Assay of the same children was the research of another thesis study (Özata, 2019). As well as, pulmonary function tests and O_3, NO_2 and SO_2 levels from personal samplers of the same children were available (Özata, 2019) and they are used herewith for correlation analysis.

According to the pulmonary function test parameters of children and CR-UM results, there is a negative correlation between Forced Expiratory Volume (FEV1) and Cr (r= -0.1951, p= 0.0200), FEV1 and Fe (r= -0.2018, p= 0.0160), FEV1 and Ni (r= -0.1838, p= 0.02286), FEV1 and Cu (r= -0.3469, p= 0.00002), FEV1 and Mo (r= -0.1998, p= 0.0171), FEV1 and Hg (r= -0.2041, p= 0.0148), and FEV1 and Pb (r= -0.1986, p= 0.0178). There is a negative correlation between Forced Expiratory Volume in 1s/ Forced Vital Capacity (FEV_1/FVC) and Mn (r= -0.2063, p= 0.0138).

The R-UM result shows positive correlation between micronucleus (MN) frequencies and Ni (r= 0.2116, p= 0.0106), As (r= 0.3001, p= 0.0002), and Mo (r= 0.1995, p= 0.0161). The CR-UM result shows positive correlation between MN and As (r= 0.3001, p= 0.0002). There is negative correlation between MN and Be (r= -0.1700, p= 0.041), Mn (r= -0.2003, p= 0.0157), and Fe (r= -0.1838, p= 0.0269). The SG-UM result shows a positive correlation between MN and Ni (r= 0.2530, p= 0.0021), As (r= 0.3194, p= 0.0001), and Mo (r= 0.2535, p= 0.0021).

The CR-UM result shows negative correlation between O_3 and Be (r= -0.1637, p= 0.0432). There is positive correlation between O_3 and Ni (r= 0.2245, p= 0.0053), and As (r= 0.3616, p= 0.000004). There is negative correlation between NO_2 and As (r= -0.3008, p= 0.0001). There is positive correlation between NO_2 and Be (r= 0.1881, p= 0.0191), Mn (r= 0.1604, p=0.0462), and Fe (r= 0.2555, p=0.0013). On the other hand, there is positive correlation between SO_2 and As (r= 0.3642, p=0.000003), and Ni (r= 0.1952, p=0.0149).

The number of subjects below LOD, above LOD, and LOD values of all urinary metals levels are shown in Table 4.16

Table 4.16. Limit of detection values of urinary metal levels

Metal	LOD values (ug/L)	Number of subjects below LOD (%)	Number of subjects above LOD (%)
As	0.011	0 (0%)	160 (100%)
Ba	0.013	0 (0%)	160 (100%)
Be	0.003	132 (82.5%)	28 (17.5%)
Cd	0.004	48 (30%)	112 (70%)
Cr	0.009	9 (5.6%)	151 (94.4%)
Co	0.002	1 (0.6%)	159 (99.4%)
Cu	0.04	0 (0%)	160 (100%)
Fe	0.4	2 (1.25%)	158 (98.75%)
Pb	0.014	11 (6.9%)	149 (93.1%)
Mn	0.013	7 (4.4%)	153 (95.6%)
Hg	0.023	129 (80.6%)	31 (19.4%)
Mo	0.04	0 (0%)	160 (100%)
Ni	0.006	0 (0%)	160 (100%)
Se	0.022	0 (0%)	160 (100%)
Sn	0.055	35 (21.88%)	125 (78.12%)
V	0.034	50 (30.12%)	110 (68.8%)
Zn	0.2	0 (0%)	160 (100%)

LOD: Limit of detection, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc

5. DISCUSSION

In the last few decades, Turkey, as a developing country, has experienced industrial growth, enlargement of cities, and increased numbers of vehicles and population. For example, the vehicle numbers in Turkey is increased to 22,462,346 in March 2018 from 6,626,885 vehicles in May 1999 (Internet i, 2018). Moreover, the population of Turkey is growing very fast and in 20 years changed from 62.243.779 (year 1999) to 80.810.525 (year 2018) (Internet j). Unfortunately, this growth has been accompanied by a crucial negative impact on environment, especially because of traffic and heating emissions and industrial activities.

Metals and especially the toxic ones are important air pollution components and are prone to increase in traffic intense and industrialized cities and regions (Brugge et al., 2007; Internet o). Air pollution components including toxic metals cause adverse health effects, especially on children as one of the vulnerable groups of the population (Gil et al. 2009; Rodríguez-Barranco et al., 2013; Internet d). Metals might have toxic effects from hypersensitivity (Be, Ni, Co, Cu, Cr, and Hg) (Wang, Y. et al., 2012), to genotoxic effects (Cd, Cu, Cr, Ni, Zn, Pb, As, and Hg) (Jadoon et al., 2017; Montvydienė et al., 1999) and also might have a contribution to the diseases of a wide range such as asthma (Cr, Se, Mo, Cd, and Ni) (Huang et al., 2016; Nemery, 1990) and cancer (Chung et al., 2014; IARC, 2016) and As, Be, Fe, Ni, Cr, Cd are classified as human carcinogens by IARC (IARC, 2012a; IARC, 2012b). Biomonitoring studies are a traditional approach to assess the amount of environmental and occupational exposure to metals (Markosian et al., 2019), which is also the aim of the present study as the environmental biomonitoring of children. Exposure data in the present molecular epidemiology study is complementary to the great number of findings of the two connected comprehensive projects for monitoring the air pollution of the Kütahya Province (TÜBİTAK-112Y305, AUSRP, No: 1407F398). According to the preliminary data of those projects, Kütahya Province is characterized with its high air pollution among the cities of Turkey from traffic, heating and industrial sources (Küçükaçıl Artun et al., 2017; Tuygun et al., 2017). The strong impact of thermal power plants on industrial air pollution especially in the rural area of Tunçbilek (TR) region and the impact of traffic in the city center of Kütahya (KC) are presented (Küçükaçıl Artun et al., 2017; Tuygun et al., 2017).

Here, we present and discuss our findings of urinary 17 metal levels of 160 primary school children from 8 to 10 years old in 4 different schools of TR and KC regions of Kütahya. The demographic and socioeconomic properties of children are found to be similar in the regions. The molecular epidemiology studies of children to find out the regional effects of environmental air pollution are advantageous since children do not; smoke, drink alcohol, work, and move (only home to school) (Nakayama et al., 2019; Gajski et al., 2013). Therefore, the outcome of this kind of studies can be attributed to mainly their living environment conditions.

Essentially, in the biomonitoring studies using urine as a biomarker, the ideal method is to collect the whole day (24 hours) urine samples for exposure assessment (Lermen et al., 2019). However, it is not practical and an obstacle for field study conditions, especially when the study population is children. It is found to be more efficient and easy to collect spot urine samples, but then the results might show high variability depending on liquid consumption, kidney function, and metabolism (L. Huang et al., 2013). So that, generally it is preferred to collect the first morning (spot) samples, which have less variability and more concentrated than other spot samples (L. Huang et al., 2013). The general procedure to overcome the variations is, the adjustment of the first spot urine samples with some procedures such as creatinine and specific gravity adjustments (L. Huang et al, 2013). Creatinine adjustment for urinary analysis is the most common procedure to eliminate the first-spot urine variability (Aguilera et al., 2010) Roca et al., 2016; Heitland et al., 2004). On the other hand, even the use of specific gravity adjustment is rare, it is recommended in children biomonitoring studies as an inexpensive, easy method, does not generate chemical waste, and to overcome the changes in creatinine levels parallel to the age dependent development of muscle mass (Pearson et al., 2009). In the similarly designed studies to the present study, non-adjusted urinary metal levels (R-UM) or/and creatinine adjusted urinary levels (CR-UM) but not specific gravity adjusted urinary metal levels (SG-UM) of children are reported. In the present study, SG-UM of children is also available, and this parameter is found to be correlated with both R-UM and CR-UM of children for each metal evaluated. Therefore, as one of the outcomes of the study, specific gravity adjustment can be suggested as an alternative method for children biomonitoring studies.

Briefly, all the metals analyzed in the urine of children for this study are found to be abundant but with differing levels in both of the regions characterized by industrial or traffic related air pollution of Kütahya. The main reason for this abundance might be either industrial and traffic related pollution or geology and natural occurrence of the metals in this environment. Among them, only Be is generally lower than the limit of detection in the urine of most of the children. In our study in the rural/industrial region, particularly nearby the coke fired power plants, statistically significantly higher R-UM of As, Co, Ni Cr, Zn, Ba, CR-UM of Ni and As, and SG-UM of Co, As, Ni are found when compared to traffic intense city center region. Among all these metals As and Ni are common ones in both non-adjusted and adjusted urinary levels. Despite the efficient control systems in the power plants, during the coal combustion, generated fly ash can be released to the surrounding environment. In varying ranges and levels, the released fly ash is shown to contain toxic metals (Soni et al., 2000; Ghosh, 2010). In a study, which is conducted to measure Pb, Cd, Cr, As, Cu, Zn, Ni, and Fe accumulation in soils around a coal fired power plant in India, high levels of Pb, Cd, Cr, and As are reported (Ghosh, 2010), which are also metals found in high levels in the urine of children living in power plant region of the present study. Karayigit et al. reported that Seyitomer, Tuncbilek, and Soma Thermal Power plants have coals containing high levels of Cr, Cs, Mo, Ni, Rb, Th, U, and V and the levels of As, Co, Cu, Ga, Sc, Sn, Mn, Li, Ta, Tl are very high those exceeding the ranges for most of the coals all over the world (Karayigit et al., 2000). Karayigit et al. study is in the same industrial area of the present study and confirming the high levels of some of these metals are found in the urine of the children living in this region. In a study conducted in Tunçbilek, levels of As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn in soil showed that the much higher levels of As, Cr, Hg, and Ni than that of the world soil average levels whereas the rest of the metals investigated (Cd, Cu, Pb, and Zn) were below world soil averages (Özkul, 2016).

Children living in the urban region of Kütahya are manifested significantly higher R-UM of Be, V, Mn, Fe, and Pb, CR-UM of Be, V, Mn, Fe, Cu, Se, and Pb, and SG-UM of Be, V, Mn, Fe, Hg, and Pb when compared to rural/industrial region and among them Be, V, Mn, Fe, and Pb are the common ones for both adjusted and non-adjusted levels. Traffic is assumed to be the source of Pb and Mn in urban regions which are causing an increase in the levels and contamination of atmosphere during the combustion process of gasoline in vehicles are causing (Joselow et al., 1978; Gulson et al., 2006; Parekh et al., 2002). Also, it is known that the brake linings and tires of the vehicles are sources of some metals such as Cu, Cd, and Zn in the urban regions (M. Wang et al., 2018). Rastegari and his colleagues are introduced two main sources of metals in soils as urban activities (Cu, Pb, and Zn); and industrial activities (mainly As, Cd, Zn, and Pb) (Rastegari Mehr et al., 2017). In a recent study in the city center of Kütahya, (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) metal levels are measured in soils of children playgrounds. It is stated that metals such as As, Cd, Hg, Ni, Pb and Zn have been found to be high enough to cause risk in the children playgrounds (Özkul, 2019).

After this point, the urinary metal levels (◌UM) of children will be representative for all of the urinary metal levels of R-UM and CR-UM and SG-UM. By the way, in case of overview to and comparisons with other studies, only UM will be mentioned while all the comparisons are made with the corresponding adjusted or non-adjusted urinary metal levels.

There are very few human biomonitoring studies assessing metal levels in the urine of children carried out in industrial and traffic intense urban regions. Our study is contributing and giving additional data to the air pollution biomonitoring studies with its children population and measuring 17 urinary metal in Kütahya Province, with high air pollution in Turkey.

Below, the present study data is discussed among others with similar children study design analyzing urinary metals likely present in environmental air pollution (Table 5.1, Table 5.2). However, there are possible differences among them because of the study population in; size, age range, demographic, lifestyle and socioeconomic properties, and study area in; geology, characteristics, and sources of the air pollutants.

Accordingly, there are four studies carried out on children affected by both industrial and traffic related air pollution. These are Huelva Study I and Huelva Study II, which are located in Spain, Ria of Huelva (south-west Spain), including the capital city of Huelva with discharge of large amounts of pollutants from mining and industrial activities and since it is the capital region, traffic is also an important cause of air pollution (Aguilera et al., 2010; Molina-Villalba et al., 2015). The third study is Lahore Study, in the region of Lahore, Pakistan, investigating the impact of traffic and industrial air pollution on children (Sughis et al., 2014). The fourth study is Ciudad Study, a highly populated city of Ciudad

Juarez, Chihuahua, Mexico, involving large manufacturing centers, electronic companies (Ochoa-Martinez et al., 2016).

When only industrial air pollution effects on children are considered, there is 'Taxco Study', which is carried out in Taxco, State of Guerrero, located in Southern Mexico. It is mentioned as one of the largest Mexican metal producing regions of gold and silver, among other metals (Moreno et al., 2010). The second study is 'Mexico Study', involving different Mexican cities associated with mining, agriculture, major industry, small-scale industry, oil fields, and non-controlled waste disposal sites (Trejo-Acevedo et al., 2009). The third study is 'China Study' located in Shanxi Province, China, investigating the impact of industrial/rural area in children as an exposed group and comparing the results with the results of non-polluted rural area (Z. Wang et al., 2019).

There is one study focused on only traffic related air pollution, which is called herewith as 'Valencia Study' and carried out in urban and agricultural areas of Valencia Region, Spain (Roca et al., 2016).

On the other hand, UM results of current study also compared to the reference data from the United States and Germany which are mentioned as 'USA Reference Study (RV-USA)' (CDC, 2009) and 'Germany Reference Study (RV-G)' (Heitland et al., 2006), which are designed to find out the reference values of average urinary metal levels in children for these countries. Briefly, in Kütahya Study, the UM of As, Ba, Co, and Pb of children are higher, whereas V is lower than the values of both of the reference studies (CDC, 2009; Heitland et al, 2006) (Table 5.2). Cd and Mo levels are within the range of the RV-USA; Cu, and Sn metals are in line with the RV-G. The levels of Cr, Mn, Mo, Ni, Se, and Zn reported in the current study are higher, and the Cd level is lower than the RV-G. Higher levels of Hg metal reported in the RV-USA when compared to the present study (CDC, 2009) (Table 5.2).

When the population size and the age range of the biomonitoring studies mentioned above (Table 5.1) are compared to the present study with 160 children and age of 8 to10, it can be considered as a high number of population with a narrow age range. The highest population size is in Lahore Study with 339 children (Sughis et al., 2014) which is followed by Huelva Study II with 261 (6-9 years old) (Molina-Villalba et al., 2015), Mexico Study with 229 (6-12 years old) (Trejo-Acevedo et al., 2009), Huelva Study I with 227 (5-17 years old) (Aguilera et al., 2010) and China Study with 214 (7.0 ±1.4 year) children (Z. Wang et al., 2019). The rest of the studies have lower population size than the present study. They are Ciudad Study with 135 children (6-12 years old) (Ochoa-Martinez et al., 2016), Valencia Study with 125 (6-11year) (Roca et al., 2016), and Taxco Study with 50 children (6-11 year) (Moreno et al., 2010)

Below, UM of children of each metal in Kütahya Study is discussed among other studies. The tables of (Table 5.1 and Table 5.2) are summarizing and showing the place of Kütahya Study among other biomonitoring studies.

- Arsenic

Arsenic, as a human carcinogen (IARC, 2012b), is among the most investigated elements both in environmental and occupational biomonitoring studies. Mainly drinking water (especially groundwater) and then food (mainly cereals like rice and pulses) are the sources of As in environmental exposures (CDC, 2009; Roca et al., 2016). In the present study, UM of As is taking attention. As levels in Kütahya Province (total) and especially in the Tunçbilek region are higher than the levels measured in Huelva Studies I and II (Aguilera et al., 2010; Molina-Villalba et al., 2015), Ciudad and Taxco Studies (Ochoa-Martinez et al., 2016; Moreno et al., 2010) while lower levels are seen when compared to Valencia and Lahore Studies (Roca et al., 2016; Sughis et al., 2014) (Table 5.2). In Kütahya Province, the highest As levels are seen in Gürağaç children in the region of power plants (Table 4.7). From previous studies in Kütahya (Şener & Karakuş, 2017), the drinking water As levels are found to exceed 10 μg/L of the permissible limit value of As in drinking water (Internet k). In the questionnaires of Gürağaç children, the drinking water source has been declared mostly as spring water that can be an additional source to industrial contamination of the region. This might explain the highest As levels in Gürağaç. Similarly, Molina et al. are stated in Huelva II Study that the highest urinary As concentrations are shown in children drinking spring or well water (Molina-Villalba et al., 2015).

➢ Lead

The best Pb biomarker is blood, as the blood lead is more reliable (most common and accurate biomarker) than urinary lead biomonitoring. However, in specific population subgroups such as children, the use of non-invasive biological sampling is crucial therefore urinary lead analysis can also provide useful information in children biomonitoring studies (CDC, 2009; Sughis et al., 2014; Roca et al., 2016; Lionetto et al., 2019).

Pb level in Kütahya Province (total) is elevated in comparison to the levels reported in Huelva Study II (Molina-Villalba et al., 2015), and Valencia Study (Roca et al., 2016). However, much higher levels in Lahore Study (urban high air pollution area) (Sughis et al., 2014), and China Study (Z. Wang et al., 2019) than that of Kütahya are reported (Table 5.2). In the present study, Pb level is highest in the traffic intense city center while children in the rural area with no traffic (Gürağaç), where all children are going school by foot, are shown the lowest Pb UM (Table 4.7). Lead is a traditional marker of air pollution caused by traffic (Parekh et al., 2002; Sughis et al., 2014; Gulson et al., 2006), which is also confirmed with Kütahya Province results. Laamech et al. is also found that urinary Pb levels in children living in the urban area are higher than the levels in children living in rural or industrial areas (Laamech et al., 2014).

➢ Manganese

Similar to Pb data, KC has higher Mn UM in Kütahya Province (Table 4.4). Mn urinary levels are clearly higher in children living in KC characterized by traffic intensity comparing the Gürağaç region children, which does not have traffic related exposures (Table 4.7). Kütahya Province is found to have higher UM when compared to children in Spain based studies of Valencia Study (Roca et al., 2016), Huelva II Study (Molina-Villalba et al., 2015), whereas higher levels of Mn UM measured in Asian studies of Lahore Study, in both highly and low polluted city regions (Sughis et al., 2014), and in China Study (Z. Wang et al., 2019) (Table 5.2). As manganese enters in composition of gasoline for its anti-knock properties as methylcyclopentadienyl manganese tricarbonyl (MMT) and marketed to around 30 countries after 2003, unavoidably high levels might be emitted to the atmosphere through the combustion process in vehicles, and become a pollutant of city-environment (Joselow et al., 1978; Gulson et al., 2006). Therefore, the levels of Mn can be assumed as depend on the traffic intensity, and our study data is also confirming this possibility.

- Mercury

Among all elements analyzed in the present study, Hg levels in Kütahya Province (total) are found to be lower among other studies (Roca et al., 2016; Molina-Villalba et al., 2015; Moreno et al., 2010; Ochoa-Martinez et al., 2016; Wang et al., 2019) (Table 5.2). Also, in the two region of Kütahya Province there is no difference for Hg (Table 4.4).

Hg is one of the most toxic metals that possibly impair the neurodevelopment of the children (Molina-Villalba et al., 2015). The main source of exposure to Hg is by consumption of fish in methylmercury form (Castaño et al., 2012; Roca et al., 2016; Molina-Villalba et al., 2015). Although using blood levels of Hg is better and more suitable to investigate the Hg exposure, researchers stated that high dietary fish can also induce higher excretion of the inorganic Hg in urine (Castaño et al., 2012; Roca et al., 2016; Molina-Villalba et al., 2015). Also, the presence of Hg in dental amalgams is one of the factors associated with the increase of urinary concentrations of Hg (Roca et al., 2016; Castaño et al., 2012). Unfortunately, in the current study, fish consumption and dental amalgam information are not questioned in detail. However, the diet of the population living in the Kütahya Province is not depending on fish consumption. As well as, EUROFISH stated that the fish consumption of Turkish people is relatively lower than European countries (Internet l).

- Barium

The levels of Ba in Kütahya province children (total) are higher than the levels observed in children of Valencia Study (Roca et al., 2016) and Lahore Study (Sughis et al., 2014). However, higher levels are measured in Taxco Study (Moreno et al., 2010) (Table 5.2). The urinary levels of Ba in both TR and KC regions are similar (Table 4.4). Sughis et al. stated that the levels of barium in urine reflect recent exposure of mainly amounts present in drinking water and food (Sughis et al., 2014).

- Beryllium

In general, Be levels are found to be lower than the limit of detection for all biomonitoring studies as well as in Kütahya Study (Table 5.2). Only a few children have very low levels of Be in their urine. The reason for these low levels is that environmental exposure of Be is negligible besides occupational exposures. Very low levels can occur through food ingestion, drinking water, or breathing air containing the metal. The main exposure source of Be occurs near some hazardous waste sites and in the workplaces (CDC, 2009).

- Nickel

Higher urinary levels of Ni is measured in Kütahya Province when compared to Huelva Study I (Aguilera et al., 2010), Lahore Study (Sughis et al., 2014), and Valencia Study (Roca et al., 2016) (Table 5.2). However, the levels are remarkably lower than the levels in Taxco Study (Moreno et al., 2010) and China Study (Z. Wang et al., 2019). The urinary Ni levels of children living in TR are increased compared to the levels of children living in the city center (Table 4.4).

Nickel is widely distributed in the environment, and Ni is released to the environment through mining, smelting, and industrial activities. Occupational exposure is one of the main sources of exposure. However, in general population, Ni exposure routes are many, such as inhalation exposure in both indoor and outdoor environments, and oral intake of either contaminated water or food (Wilhelm et al., 2013; Cempel et al., 2006).

- Cadmium

The level of Cd metal in our study is lower than levels reported in Huelva Study I and II (Aguilera et al., 2010; Molina-Villalba et al., 2015), Valencia Study (Roca et al., 2016), Lahore Study (Sughis et al., 2014), China Study (Z. Wang et al., 2019), and Taxco Study (Moreno et al., 2010) (Table 5.2). The concentrations obtained for Cd in both TR and KC are similar (Table 4.4). Also, Sughis et al. stated similar results, that there are no statistically significant differences between urban and rural children or between school-children from low and high pollution areas in their study (Sughis et al., 2014).

Cd is a toxic metal distributed widely at low levels in the environment. In non-smoker people, food (especially cereals and vegetables) is the main source of exposure. For cigarette smoke the most studied metal is Cd, as smoking is the primary source of Cd (Pappas, 2011; Bernhard et al., 2005) and children can be exposed to Cd via secondhand tobacco smoke (Hays et al., 2008; Molina-Villalba et al., 2015; Roca et al., 2016). Urinary Cd is proportional to the body burden, so it is also a good biomarker of chronic exposure (an indicator of lifetime exposure) (Hays et al., 2008; Roca et al., 2016; Molina-Villalba et al., 2015). Therefore, Cd levels in urine may also represent past exposure (prenatal period and during childhood) (Molina-Villalba et al., 2015).

- Tin

The concentration of Sn in Kütahya province is similar to the reference values reported in Germany (Heitland et al., 2004). While, Sughis et al. reported levels of Sn below the limit of detection in Lahore, Pakistan (Sughis et al., 2014). Higher levels measured in China Study (Z. Wang et al., 2019) (Table 5.2). Urinary levels of Sn show no statistically significant differences in TR and KC (table 4.4). Also, results show no differences between the schools (Table 4.7).

The implication of these low levels is that the concentration of Sn in soil and air is relatively low when compared to other heavy metals. However, tin may be released to the atmosphere through agricultural activities, road construction, and windstorms. Sn may also be generated from anthropogenic sources, including industrial and waste incineration and the burning of fossil fuels (Cima, 2018).

- Iron

Fe levels reported in this study lower than the levels of Fe reported in Taxco Study (Moreno et al., 2010) and China Study (Z. Wang et al., 2019) (Table 5.2). The concentration levels of Fe are highly influenced by traffic vehicles in addition to the industrial sources (Sekhavatjou et al., 2010). Based on the results of our study, Fe levels in the urban area (KC) higher than the levels of rural/industrial area (TR) (Table 4.4). Moreover, the levels of L school are higher than the levels of G and A schools (Table 4.7).

- Molybdenum

Urinary levels of Mo obtained in our study of Kütahya Province fell within the range of values reported for Valencia Study (Roca et al., 2016), while higher levels reported in Lahore Study (Sughis et al., 2014), and China Study (Z. Wang et al., 2019). However, the levels of TR, KC, and total are higher than the levels reported in Taxco Study (Moreno et al., 2010) (Table 5.2). Urinary levels of Mo in both TR and KC regions are similar (Table 4.4).

Mo is emitted from industrial activities, e.g., as a result of fossil-fuel combustion, from mobilization and fly ash of mine wastes. Humans are exposed to Mo through food and water, and inhalation (Smedley et al., 2017). CDC stated that the main source of Mo is coal combustion emissions (Internet n).

- Selenium

Se concentrations measured in Kütahya Province (total) are lower than the concentrations measured in Valencia Study (Roca et al., 2016), Lahore Study (Sughis et al., 2014), and China Study (Z. Wang et al., 2019) (Table 5.2). Our urinary Se levels reported in the urban region (KC) are higher than the levels reported in the rural/industrial area (TR) (Table 4.4). Higher concentrations measured in L school than the concentrations of G school (Table 4.7). Also, Valencia Study is showed that urinary Se levels in children living in the urban area higher than the levels in children living in rural or industrial areas (Roca et al., 2016; Sughis et al., 2014).

Humans expose to Se by ingestion of water or food containing selenium, inhalation of air containing selenium, or by dermal contact. It can enter the environment through agricultural, manufacturing, petrochemical, and mining operations (Staicu, et al., 2017).

- Vanadium

The levels of V in Kütahya Province children (total) are higher than the levels observed in children of Valencia Study (Roca et al., 2016). However, Lahore Study reported levels of V higher to the levels reported in the current study (Sughis et al., 2014) (Table 5.2). Statistically significant differences were also obtained between the two geographical regions for V, with higher levels in children living in KC than children in TR (Table 4.4), but not between the schools (Table 4.7).The emissions of V to the atmosphere are mainly from fuel combustion and coal combustion. The levels of Vanadium in urban areas might be higher than the other areas (Nielsen, 1987). Also, the air contaminated by V caused by industrial plants less than that caused by heating equipment, power stations, and petroleum refineries (Visschedijk et al., 2013; Nielsen, 1987).

- Zinc

The urinary levels of Zn in Kütahya Study are similar to the levels reported in Valencia Study (Roca et al., 2016) and Taxco Study (Moreno et al., 2010), but higher than Lahore Study (Sughis et al., 2014) (Table 5.2). Besides, similar concentration measured in both TR and KC (Table 4.4) without differences between schools (Table 4.7). Zn and its compounds are released to the environment from fumes and dust of mining, coal and fuel combustion, Zn production plants, brass works, and steel and iron production (Al-Sultani et al., 2012).

- Chromium

UM of Cr in children is found to be lower than the levels of Huelva Study I (Aguilera et al., 2010), Lahore Study (Sughis et al., 2014), and China Study (Z. Wang et al., 2019). Moreover, the levels reported in Taxco Study are remarkably higher than the levels of Kütahya Province (Moreno et al., 2010) (Table 5.2). According to Chromium levels of children there are no regional and school-based differences in Kütahya Study. (Table 4.4, Table 4.7). Chromium chemicals production, steel production, coal and oil combustion, cement production, cooling towers, asbestos mining and milling, and coke ovens are the main sources of chromium emissions to atmosphere (Yap et al., 2017).

- Cobalt

Co levels in Kütahya Study is higher than Lahore Study (Sughis et al., 2014) and lower than Valencia Study (Roca et al., 2016) and Taxco Study (Moreno et al., 2010), while similar levels reported in China Study (Z. Wang et al., 2019) (Table 5.2). With regards to Co levels of children, there are no regional and school-based differences in Kütahya Study

(Table 4.4, Table 4.7). In addition to the natural source of Co, It is also can be released to the atmosphere from anthropogenic activities such as burning oil and coal, from the exhausts of truck, car, and airplane, and the use of metal or its compounds in industrial processes (Internet m).

- Copper

The values of Cu in this study are similar to the levels of Huelva Study (Aguilera et al., 2010), and of Lahore Study (Sughis et al., 2014) and lower than Valencia Study (Roca et al., 2016), Taxco Study (Moreno et al., 2010), and China Study (Z. Wang et al., 2019) (Table 5.2). The level of Copper in the city center of Kütahya is found to be higher than Tuçbilek Region (Table 4.4), and there is no difference among children from different schools (Table 4.7).

Actually, the main releases of copper are from coal-fired power stations, metal production, waste incinerators, sewage treatment processes, and from the application of agricultural chemicals. Smaller amounts are also released naturally from the earth's crust (ATSDR, 2004). In Kütahya Study, Cu source is seemed not from industrial activities. Also, in the other studies both it is not distinguishable whether the source is industry or traffic.

Table 5.1. Human biomonitoring studies in urine samples of children population

Air pollution exposure source and/or site/region	**Exposure biomarker / analyzed metals** Urine=U Blood=B Hair=H	**Other biomarkers and analysis/notes**	**Study population** -Total number (n for girls / boys) -Age range for years - Number of study group/ control group	**Reference**
“Huelva Study I” **(1)** The “polluted” area corresponds to the Ria of Huelva, which includes the capital city of Huelva and seven municipalities also located close to the industrial sites. (2) The “reference” area, formed by the remaining capital cities of the Andalusian provinces (small or non-existing industrial (Spain)	**U /** As, Cd, Cr, Cu, and Ni	-No other biomarkers -Comparison between Ria of Huelva (1), and reference area (2). -Comparison with other study results and regulatory values	-423 [(1) (110/117)] [(2) (91/105)] -5-17	(Aguilera et al., 2010)
“Huelva Study II” **Industrial** (near mining and industrial areas) **Huelva (South West Spain)** one of the most polluted estuaries in the world owing to the discharge of mining and industrial related pollutants in their proximity	**U** / As, Cd, Pb, Mn and Hg **H** / As, Cd, Pb, Mn and Hg	-No other biomarkers -Comparison with other study results and regulatory values	-261 (126/135) -6-9 - No control group	(Molina-Villalba et al., 2015)

Table 5.1. (continues) Human biomonitoring studies in urine samples of children population

"Ciudad Study" **Ciudad Juarez, Chihuahua, Mexico** (A major manufacturing center in Mexico)	**B** / Pb **U** / As and Hg	-No other biomarkers -PBDEs, PCBs, and total DDT in blood -1-hydroxypyrene levels in urine -Comparison with other study results and regulatory values	-135 Boys %50 -6-12 -No control group	(Ochoa-Martinez et al., 2016)
"Valencia Study" **Valencia Region, Spain** (Agricultural and an urban area)	**U** / Cu, Co, Mn, Mo, V, Zn, As, Ba, Be, Cd, Cs, Ni, Pb, Pt, Sb, Se, Th, TI, U and Hg	-No other biomarkers -Two primary schools, one from an urban area (Valencia) and another one from a rural area (Alzira) -Comparison with other study results and regulatory values	-125 -6-11 - No control group	(Roca et al., 2016)
"Lahore Study" **Lahore, Pakistan** (Urban school children and rural working children) traffic intensity in Lahore & children working in carpet weaving or the brick industry outside Lahore.	**U** / Pb, As, Cd, U, Ba, Bi, Be, Sn, Se, Sb, Tl, Al, V, Cr, Cu, Mn, Mo, Co, Ni, and Zn.	-No other biomarkers.	-339 (47% g) -8-12 -100/79/80/80 (High air pollution area/ Lower air pollution/ Carpet weaving industry area/ Brick industry area)	(Sughis et al., 2014)
Germany 150 study locations (communities or urban districts)	**U** / Ni	- Comparison with other study results and regulatory values	-1790 (907/ 883) -3-14 -No control group	(Wilhelm et al., 2013)
(1) The "**polluted**" area corresponds to the **Ria of Huelva**, which includes the capital city of Huelva and seven municipalities also located close to the industrial sites. (2) The "**reference**" area, formed by the remaining capital cities of the Andalusian provinces (small or non-existing industrial (Spain)	**As, Cd, Cr, Cu, Ni**	Comparison between **Huelva** results, **Reference** group, and the other studies	-423 [**227** (117 b +110 g) living in the **Ria of Huelva** + **196** (105 b + 91 g) (Reference group) in other capital cities of Andalusia] -5-17	Aguilera I et al., 2010 ☺☺☺
"Taxco Study" Mine tailings zone in Southern **Mexico** (The Taxco mining district in Southern Mexico was one of the largest producers of metals, such as Ag, Au, Cu, Pb, and Zn)	**B** / Pb **U** / Ar, Hg, Cr, Ni, Cd, Ba, Co, Cu , Zn, Mn, Mo, Sr, and Fe	- Comparison with other study results and regulatory values	-50 (26/24) -6-11 -No control group	(Moreno et al., 2010)
"Mexico Study" **Mexican** children living in high-risk areas: mining, agriculture, major industry, small-scale industry, oil fields, and non-controlled waste disposal sites. **(In 9 cities)**	**B** / Pb **U** / As, Cd	- Comparison with other study results and regulatory values	-229 -6-12 -No control group	(Trejo-Acevedo et al., 2009)
Germany "RV-G" Areas of **Aachen** and **Erkelenz** in **western Germany** and in **Bremen** in **northern Germany**	**U** / Li, Be, V, Cr, Mn, Ni, Co, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Rh, Pd, Ag, Cd, In, Sn, Sb, Cs, Ba, Pt, Au, Pb, Tl, Bi, and U	- 87 urine samples were collected from adults	- 72 - The ratio male / female is 1/1	(Heitland et al., 2006)
"China Study" **Shanxi province, China Tow study areas** **1.Exposed Group:** a rural area within two kilometers of a large coking plant	**U** / Al, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, TI, and Zn	-For exposed group: 142 urine samples were collected from elderly -For Control group: 142 urine samples were collected from 96 elderly - -Comparison between	-148 (84/64) -7.0 ±1.4 Control group: -66	(Z. Wang et al., 2019)

Table 5.1. (continues) Human biomonitoring studies in urine samples of children population

2..Control Group: a non-polluted rural area 70 Km away.		Group 1 and 2 - S-phenylmercapturic acid (S-PMA) levels in urine	-7.0 ±1.4	
“China Study” **Shanxi province, China Tow study areas** **1.Exposed Group:** a rural area within two kilometers of a large coking plant **2..Control Group:** a non-polluted rural area 70 Km away.	**U /** Al, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, TI, and Zn	-For exposed group: 142 urine samples were collected from elderly -For Control group: 142 urine samples were collected from 96 elderly - -Comparison between Group 1 and 2 - S-phenylmercapturic acid (S-PMA) levels in urine	-148 (84/64) -7.0 ±1.4 Control group: -66 -7.0 ±1.4	(Z. Wang et al., 2019)

As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: Chromium, Co: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc, Sr: strontium, Cs: caesium, Pt: platinum, Th: thallium, U: uranium, H: hair, B: blood, U: urine, G: girl, B: boy, Y: year.

Table 5.2. Comparison of urinary concentrations (µg L^{-1}, µg g^{-1} creatinine, geometric mean) of toxic metals obtained in our study and in other HBM studies in children populations

Metal µg L^{-1} µg g^{-1}	Current study			Reference values		Other studies									
	Total	TR	KC	USA[a]	Germany[b]	Spain[c]	Spain[d]	Spain[e]	Pakistan[f]		Mexico*,[g]	Mexico*,[h]	Mexico*,[I]	China*,[j]	
									Low Urban	High Urban				Exposed group	Control group
As	25.3 24.3	36.7 30.7	16.1 18.2	7.08 8.25	9.3 8.1	1.36 1.60	0.701 2.438	- 33.3	67.6 -	55.5 -	- 19.5(30.9)	16.5 (35.2) -	- 22.35 (30.9)		
Ba	4.21 4.04	4.82 4.04	3.57 4.03	2.21 2.58	1.3 1.2			- 1.86	3.65 -	3.65 -		18.4 (6.17) -			
Be	<LOD-0.00282 <LOD-0.00278	0.0022 0.0019	0.0036 0.0027	<LOD <LOD	<LOD <LOD			- <LOD	<LOD -	<LOD -					
Cd	0.076 0.073	0.085 0.071	0.065 0.074	0.077 0.09	0.12 0.12	0.35 0.41	0.215 0.747	- 0.176	0.43 -	0.45 -		4(0.327) -	- 0.78 (0.39)	- 0.90 (0.39)	- 0.77
Cr	0.311 0.298	0.386 0.323	0.238 0.269		0.22 0.19	0.39 0.43			0.46 -	0.39 -		15 (0.456) -		- 25.9 (0.46)	- 20.2
Co	1.03 0.983	1.31 1.10	0.758 0.856	0.454 0.529	0.61 0.53			- 1.41	0.78 -	0.65 -		18 (1.46) -		- 1.38 (1.26)	- 1.05
Cu	11.7 11.2	12.4 10.4	10.8 12.2		12 10.5	10.02 11.46		- 35.0	15.8 -	14.8 -		29.6 (15.2) -		- 28.3 (15.6)	- 21.5
Fe	20.7 19.8	18.4 15.4	23.8 26.9									25 (29.1) -		- 202 (30.6)	- 185
Pb	1.19 1.14	0.879 0.736	1.73 1.95	0.795 0.926	0.8 0.7		0.830 -	- 1.16	5.32 -	11.1 -				- 63.8 (2.8)	- 62.3
Mn	1.33 1.27	1.07 0.892	1.73 1.96		<LOD <LOD		0.120 -	- 0.430	1.59 -	1.73 -		5.2 (2.24) -		- 4.98 (2.90)	- 6.47
Hg	0.020 0.019	0.022 0.019	0.018 0.02	0.254 0.297			0.304 1.059	- 0.730			2.10(0.08) -	0.7 (0.08) -		- 4.48 (0.09)	- 3.83
Mo	69.4 66.5	73 61.1	65.2 73.6	62.2 72.5	64 40			- 62.58	142 -	127 -		52 (88.7) -		- 180 (78.6)	- 112
Ni	4.95 4.74	6.45 5.4	3.58 4.04		1.9 1.7	1.44 1.61		- 4.27	4.22 -	3.74 -		75.4 (6.53) -		- 8.70 (5.92)	- 6.31
Se	16.3 15.6	17.5 14.7	15.0 16.9		13.6 11.8			- 55.7	28.6 -	28.2 -				- 43.1 (16.2)	- 26.6
Sn	0.273 0.287	0.294 0.246	0.250 0.282		0.28 0.29				<LOD -	<LOD -				- 2.20 (0.49)	- 13.7
V	0.368 0.353	0.293 0.245	0.487 0.55		<LOD <LOD			- 0.223	0.42 -	0.56 -					
Zn	541 519	613 513	465 525		359 313			- 515	352 -	320 -		628 (630) -		- 926 (571)	- 858

[a] Reference values of the **USA "RV-USA"** (CDC, 2009), [b] Reference values of **Germany "RV-G"** (Heitland et al., 2006), [c] **"Huelva Study I"** (Aguilera et al., 2010), [d] **Huelva Study II** (Molina-Villalba et al., 2015), [e] **Valencia Study** (Roca et al., 2016), [f] **"Lahore Study"** (Sughis et al., 2014), [g] **"Ciudad Study"** (Ochoa-Martinez et al., 2016), [h] **"Taxco Study"** (Moreno et al., 2010)**,** [I] **"Mexico Study"** (Trejo-Acevedo et al., 2009), [J] **"China Study"** (Z. Wang et al., 2019) TR: Tunçbilek region, KC: Kütahya city center, GM: geometric mean, As: arsenic, Ba: barium, Be: beryllium, Cd: cadmium, Cr: Chromium, Co: cobalt, Cu: copper, Fe: iron, Pb: lead, Mn: manganese, Hg: mercury, Mo: molybdenum, Ni: nickel, Se: selenium, Sn: tin, V: vanadium, Zn: zinc.*mean value of metals in urine in the studies of g, h, i, and j are available. In the parenthesis the mean value of the urinary metal levels of Kütahya province is presented.

6. CONCLUSION

Complex characteristics, due to natural, environmental, and industrial sources of the study areas in addition to the population-based variations in age, lifestyle and socioeconomic properties, are challenges of environmental biomonitoring studies. There are few molecular epidemiology researches concerning the impact of environmental metal exposures in children.

Why it is important and needed to have information on children is a crucial question with the following answering points:

- Children are considered to be more sensitive to adverse health effects of metals than adults.
- Children are assumed to be a better representative of the determined study area to find out the environmental metal exposure levels where they are living.
- Any children-based metal exposure data is valuable to review related toxicities and toxicity mechanisms and revise the regulatory limit values of environmental pollutants precisely.

Kütahya Study is contributing;

- to the mentioned rare children studies with analyzing 17 urinary metal levels of 160 children as the first study in Turkey,
- to the national and international scientific literature with exposure biomarker data specific to children on rural/industrial and urban air pollution,
- to possible awareness on the levels of air pollution and relationship with health outcomes, and pollution sources,
- to reinforce the control and regulate the potential contamination sources,
- and moreover, to provide information on spot urine adjustment methods for future studies.

The disadvantages of the study are not having diet based detailed questionnaire, whereas food intake is also a main exposure route to the metals. Additionally, the inconsistent information of parents on drinking water and passive smoking status are avoiding to make conclusions on these items. The study is carried out in June so that the effects of heating sources are negligible.

As a brief and general outcome, urinary As and Ni levels of children in the industrial region and Mn, Fe, V and Pb levels in traffic dense region are taking attention for Kütahya Province. When the levels of the metals are compared to the other regions all over the world, especially As levels are among higher and Hg levels are among lower ones. $O_{3,}$ and SO_2 personal levels of children correlated with As and Ni as industrial representatives whereas NO_2 levels with Mn and Fe as traffic intensity representatives. The correlations of buccal epithelial micronucleus frequencies with As and Ni are also highlighting.

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APPENDIX

APPENDIX-1. Ethics committee approval

GAZİ ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU
GİRİŞİMSEL OLMAYAN ARAŞTIRMALAR KARAR FORMU

ETİK KURUL BİLGİLERİ	ETİK KURULUNUN ADI	Gazi Üniversitesi Klinik Araştırmalar Etik Kurulu
	AÇIK ADRES	Gazi Üniversitesi Tıp Fakültesi Dekanlık Binası 06500 Beşevler/Ankara
	TELEFON	0312 202 69 58
	FAKS	0312 202 46 73
	E-POSTA	tipetikkurulu@gazi.edu.tr

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	Endüstriyel ve Kentsel Hava Kirleticilerin Çocuklarda Maruziyet Etki ve Duyarlılık Biyogöstergeleri ile İrdelenmesi
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI ÜNVANI/ADI/SOYADI	Doç.Dr.Gonca ÇAKMAK DEMİRCİGİL
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI /UZMANLIK ALANI/ BULUNDUĞU MERKEZ	Gazi Üniversitesi Eczacılık Fakültesi / Toksikoloji AD.
	DESTEKLEYİCİ (Varsa)	
	ARAŞTIRMANIN TÜRÜ	Diğer: Girişimsel olmayan yollarla alınan biyolojik öneklerde (idrar,tükürük,yanak sürüntüsü) moleküler epidemiyoloji çalışması- Bireysel Araştırma Projesi
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒ / ÇOK MERKEZLİ ☐ / ULUSAL ☒ / ULUSLARARASI ☐

	Belge Adı	Tarihi	Ver.No	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ	31.10.2014	2	Türkçe ☒ İngilizce ☐ Diğer ☐
	AYDINLATILMIŞ ONAM FORMU	31.10.2014	2	Türkçe ☒ İngilizce ☐ Diğer ☐

	Belge Adı		Açıklama
DEĞERLENDİRİLEN DİĞER BELGELER	ARAŞTIRMA BÜTÇESİ 31.10.2014 Ver. 2	☒	
	BİYOLOJİK MATERYAL TRANSFER FORMU	☐	
	DİĞER	☐	

KARAR BİLGİLERİ

Karar No: 512 **Toplantı tarihi: 10.11.2014**

Yukarıda bilgileri verilen başvuru dosyası ile ilgili belgeler araştırmanın gerekçe amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve "bütçesi dışında" uygun bulunmuş olup araştırmanın dosyasında belirtilen merkez/merkezlerde gerçekleştirilmesinde etik ve bilimsel sakınca bulunmadığına G.Ü.Klinik Araştırmalar Etik Kurulu üyelerinin oybirliği ile karar verilmiştir. Ancak araştırma kapsamında, solunum fonksiyon testi normalden farklı çıkan çocukların semptomu olan/ olmasın bir Çocuk Allerji yada Göğüs Hastalıkları uzmanına yönlendirileceği bilgisinin hem protokole eklenmesi hem de yazılı olarak etik kurulumuza sunulması gerekmektedir.

Etik Kurulun kararı, projenin bütçesi TÜBİTAK tarafından kabul edildiği takdirde yürürlüğe girecek olup, TÜBİTAK kararının Kurulumuza bildirilmesi gerekmektedir.

GAZİ ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU

ETİK KURULUN ÇALIŞMA ESASI	Klinik Araştırmalar Hakkında Yönetmelik (13.04.2013), İyi Klinik Uygulamaları Kılavuzu
BAŞKANIN UNVANI / ADI / SOYADI:	Prof.Dr.Canan ULUOĞLU

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırma ile İlişki		Katılım *		İmza
Prof.Dr.Canan ULUOĞLU BAŞKAN	Tıbbi Farmakoloji A.D	G.Ü.T.F	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	

APPENDIX-1. (continues) Ethics committee approval

Prof.Dr.Arzu BAKIRTAŞ BAŞKAN YARD.	Çocuk Sağlığı ve Hast.A.D	G.Ü.T.F	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Gonca AKBULUT RAPORTÖR	Fizyoloji A.D	G.Ü.T.F	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Bülent BOYACI ÜYE	Kardiyoloji A.D	G.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☐	H ☒	Katılmadı
Prof.Dr.Sefer AYCAN ÜYE	Halk Sağlığı A.D	G.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Mehmet Akif ÖZTÜRK ÜYE	İç Hastalıkları A.D	G.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Elvan İŞERİ ÜYE	Çocuk Psikiyatrisi A.D	G.Ü.T.F	E ☐	K ☒	E ☐	H ☒	E ☐	H ☒	Katılmadı
Prof.Dr.Nesrin ÇOBANOĞLU ÜYE	Tıp Tarihi ve Etiği A.D	G.Ü.T.F	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Prof.Dr.Sercan AKSOY ÜYE	İç Hastalıkları AD.	H.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Doç.Dr.Hakan KAYIR ÜYE	Tıbbi Farmakoloji A.D	G.A.T.A	E ☒	K ☐	E ☐	H ☒	E ☐	H ☒	Katılmadı
Doç.Dr.Mustafa ARSLAN ÜYE	Anesteziyoloji ve Reanimasyon A.D	G.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Doç.Dr.Murat AKIN ÜYE	Genel Cerrahi A.D	G.Ü.T.F	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Av.Arzu BUZKIRAN KAYA ÜYE	Avukat	G.Ü.	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Emine ŞEKER ÜYE	Sivil Temsilci	-	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	

* :Araştırma ile İlişki
** :Toplantıda Bulunma

APPENDIX-2. Gönüllü beyan formu

GÖNÜLLÜ BEYAN FORMU

Yaşadığınız bölgede olası hava kirliliğinin çocuklar üzerinde oluşturabileceği sağlık etkilerini ve bunların önlenme yollarını değerlendirmek üzere hazırlanan çalışmaya çocuğumun katılmasını gönüllü olarak onaylıyorum. Solunum fonksiyonlarına ve tansiyonlarına bakılmasını, tükrük, idrar ve yanak süründüsü örneklerinin projedeki çalışmalarda kullanılmasını kabul ediyorum.

Velinin Adı:

Soyad :

Telefon no:

İmza: Tarih:

APPENDIX-3. Questionnaire forms

Questionnaire

Anket tarihi (gün/ay/yıl): ..../.............../................

1.Çocuğun Adı Soyadı:

2.Doğum tarihi (gün/ay/yıl) ..../.............../................

3.Okul Adı:

4.Son altı aydır ev değiştirdiniz mi ? Evet……. Hayır…….

5.Cevabınız evet ise aşağıdaki soruları yanıtlar mısınız? Cevabınız hayır ise 6. Soruya geçebilirsiniz)

-Adresiniz:

-Telefon numaranız:

-Çocuğunuz okula nasıl gidiyor?

	Yürüyerek	Bisiklet	Otomobil	Servis
Süre:	……….dk.	………dk.	……….dk.	………dk.

-Okul ve ev arasındaki mesafe ne kadar? …….km.

6.İçme suyunuz;

Şebeke suyu ( ) Damacana ( ) Karışık( ) Taşıma su ( ) Taşıma su ise nereden ? ………….

7.Günde kaç bardak su içiyor? …………bardak

8.Son bir yılda herhangi bir aşı oldu mu? Evet □ Hayır □
Cevabınız evet ise ne aşısı oldu?

9.Son 3 ay içinde röntgen çektirdi mi ?(Diş ve diğer) Hangisi?

APPENDIX-3. (Continues) Questionnaire forms

10. Anne ya da babanın sigara içme alışkanlığı var mı ?

Anne
Evet………… Hayır……….

Baba
Evet……….. Hayır……

-Cevabınız evet ise aşağıdaki soruları cevaplayınız. Cevabınız hayır ise 11. Soruya geçebilirsiniz.

Son altı ayda annenin içtiği sigara sayısında artış oldu mu ? Evet…… Hayır…….

Son altı ayda babanın içtiği sigara sayısında artış oldu mu ? Evet…… Hayır…….

-Cevabınız evet ise aşağıdaki soruları cevaplayınız.

Anne

Günde kaç adet sigara içiyor? Çocuğun yanında içiyor mu?

Baba

Günde kaç adet sigara içiyor? Çocuğun yanında içiyor mu?

CURRICULUM VITAE

Personal Information

Surname, first name	: ALGBURI, Mohanad Basim Kadhim
Nationality	: Republic of Iraq
Birth date and place	: 27.07.1989 -Iraq
Marital status	: Married
Phone number	: 0090-535-622-72-60
e-mail	: aljbory.mohanad@gmail.com

Education

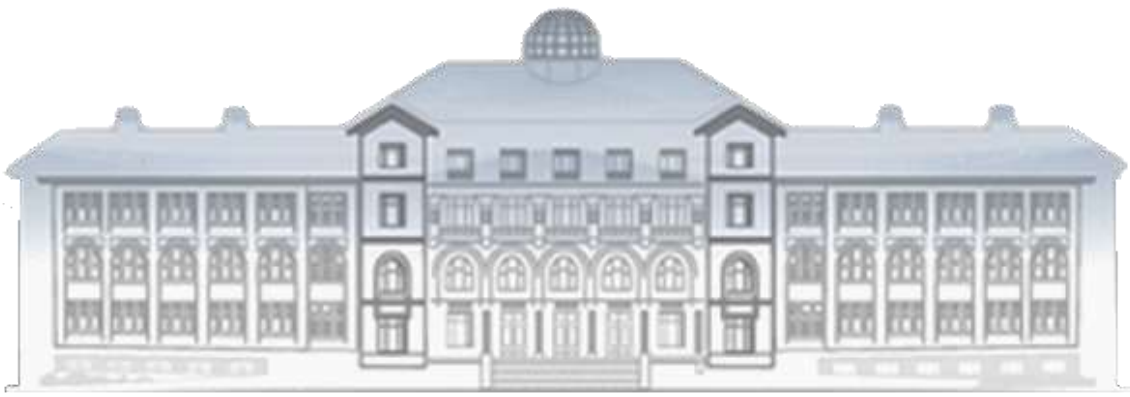
Degree	Education	Graduation date
Master's Degree	Gazi University / Faculty of Pharmacy / Department of Pharmacutical Toxicology	Continues
Bachelor's degree	MSA University	2013
High school	Almarkziya high school	2007

Languages

Arabic, Turkish, English

Hobbies

Traveling, listening to music, swimming, and reading



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