T.R.N.C.
NEAR EAST UNIVERSITY
HEALTH SCIENCES INSTITUTE

EVALUATION AND MONITORING OF HEAVY METALS IN GEMIKONAĞI REGION OF CYPRUS

Jerry Tochukwu ALLOYS (20158158)

TOXICOLOGY MASTER OF SCIENCES

Advisor
Prof. Dr. Şahan SAYGI
Co-Advisor
Assoc. Prof. Dr. Dilek BATTAL

Nicosia, TRNC, 2018

The Directorate of Health Sciences Institute

This study, entitled “Evaluation and Monitoring of Heavy Metals in Gemikonagi Region of Cyprus” has been accepted by thesis committee for the degree of Master of Science in Toxicology.

Thesis Committee

Chair of Committee: Prof. Dr. Şahan SAYGI

Near East University: Faculty of Pharmacy

Member: Prof. Dr. Semra ŞARDAŞ

Marmara University: Faculty of Pharmacy

Member: Assoc. Prof. Dr. Dilek BATTAL

Mersin University: Faculty of Pharmacy

Approval:

According to the relevant articles of the Near East University Postgraduate Study – Education and Examination Regulations, this thesis has been approved by the members of the Thesis Committee and the decision of the Board of Directors of the Institute.

Prof. Dr. Kemal Hüsnü Can BAŞER

Director of Institute of Health Sciences.

ACKNOWLEDGEMENT

I will like to use this opportunity to acknowledge the effort of my parent, Mr. and Mrs. Alloys NWANKWO for all their encouragement, financial assistance and prayers during the period of my Master's degree program and to appreciate my ever-willing Advisor (Prof. Dr. Şahan SAYGI) who was always there for me whenever I needed him, the good and thorough guidance he gave to me throughout the period of my Master's program and the supervision of my thesis work. Not forgetting my Co Advisor (Assoc. Prof. Dr. Dilek BATTAL) who gave me constant support, guidance and feedback during the research work in Mersin University to enable me to complete my thesis successfully. I would like to extend my profound gratitude to Prof. Dr. Semra ŞARDAŞ for her time and effort, spent to nurture me and to make sure I acquire much knowledge in the field of Toxicology. My sincere gratitude goes to my friend Fehmi ALKAS and Kumsal KOCADAL who were always there to encourage, strengthen and support me academically with their knowledge in Toxicology. My sincere appreciation also goes to the Advanced Technology Education, Research and Application Center laboratory at the Mersin University, Turkey for their assistance in the analysis of my experimental samples.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Pages

ABSTRACT

Heavy metals are naturally present in the environment and their availability is mostly because of various anthropogenic activities such as industrial and mining activities, use of agrochemicals such as fertilizer and pesticides for agricultural purposes and urbanization could also be a contributing factor. The presence of heavy metals in various environmental matrices have become a significant problem as they have the tendency to cause severe toxicity to the environment and human health even at a very low exposure rate. Study was carried out in Gemikonagi region of North Cyprus because of the presence of an abandoned Copper mines site which left heaps of very toxic substances exposed in the environment. Five different locations were selected for the collection of samples of seawater and soil. A total of 10 fish (*Boops boops*) were collected from the Mediterranean seawater. Based on the results obtained, heavy metals concentration in seawater decreased in a sequential order from titanium> iron > chromium > vanadium > manganese > barium > zinc > copper > nickel > molybdenum. For seawater sample, titanium recorded the highest concentration with its highest concentration in site 1 (6.848mg/L) and the lowest concentration was recorded as barium in site 2 (0.06176mg/L). The concentration of copper with level of 3349.00±1559.37mg/Kg in the soil exceeded the WHO 1996 permissible limit (36mg/Kg). The mean concentrations of chromium, manganese, iron, copper, zinc and arsenic showed varying levels of 13.91±1.60, 2.44±0.29, 24.10±4.90, 8.72±1.05, 324.59±33.58, 85.69±45.54mg/Kg respectively.

Key Words: Gemikonagi, Heavy Metal, Environmental Pollutions and ICP-MS.

1. INTRODUCTION

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by heavy metals. Furthermore, human exposure has risen dramatically because of an exponential increase of heavy metal use in several industrial, agricultural, domestic and technological applications (Bradly, 2002). Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluent and atmospheric sources (Zl *et al.,* 2005). Most of these heavy metals are mined in several industries around the world but before the discharge of the toxic material, they do not undergo proper treatment process. Heavy metals that are usually left untreated in the environment, accumulate in the Earth`s surface and these are hazardous to both human health and the environment. The presence of heavy metals in various environmental matrices such as sediment and water have become a significant problem as they have the tendency to cause sever toxicity to the environment and human health even at a very low exposure rate (Tchounwou *et al.,* 2012). Heavy metal comprises of about 40 known elements in the periodic table, they possess a density that is greater than five. Although the main physiological feature of heavy metal is that they are important for growth, but they are very toxic to living or biological cells because they can alter or denature protein molecules.

1.1 Heavy Metals

Heavy metals are naturally present in the environment and their presence in the soil is mostly because of various anthropogenic activities such as industrial and mining activities, use of agrochemicals such as fertilizer and pesticides for agricultural purposes and urbanization could also be a contributing factor (Zhiyuan *et al.,* 2014). However, the utilization of fertilizers and pesticides for agricultural purposes cannot be eliminated because these agrochemicals provide an adequate amount of nutrient that is required for a successful harvest, but long-term application can be hazardous (Rui *et al.,* 2015).

Heavy metals are metallic chemical element with a relatively high density, having the potential to cause toxicity to the environment at a very low concentration. Wang *et al.,* 2011 reported that these metals possess atomic weight between 63.5 and 200.6 with a specific gravity of above 5.0. Heavy metals are well known to be persistent in the Earth crust and they tend to bioaccumulate in the environment, mainly because they can neither by degraded or destroyed.

Wood (1974) classified heavy metals according to three different groups based on their toxicity and availability: Non-critical accessible, toxic but very insoluble/very rare and very toxic/relatively accessible.

- Non-Critical Accessible: Examples are Aluminium, bromine, carbon, chlorine, fluorine, iron, hydrogen, potassium, lithium, magnesium, nitrogen, sodium, oxygen, phosphorus, rubidium, Sulphur, strontium and silicon.
- Toxic but Very Insoluble and very Rare: Examples are barium, cesium, gallium, hafnium, iridium, lanthanum, niobium, rhenium, rhodium, ruthenium, tantalum, titanium, zirconium and tungsten.
- Very Toxic and Relatively Accessible: Examples are silver, arsenic, gold, beryllium, bismuth, cadmium, cobalt, copper, mercury, nickel, lead, palladium, platinum, antimony, selenium, thallium, tellurium and zinc.

According to Rajeswari *et al.,* 2014, heavy metals are grouped under environmental pollutants because they are harmful to plant, animal and humans. Natural and anthropogenic activities contribute to the source of heavy metal toxicity in soil and other environmental matrices. The concurrent mixtures of heavy metals in the ecosystem is detrimental to aquatic life forms which constitute a threat to aquatic plant and animals (fishes), which is a major source of protein in food diet for mankind (Rajeswari *et al.*, 2014). Heavy metals are essential in the biological processes of plants and animals and sometimes the order of their chemical composition and properties of oxidation reduction gives them an added advantage to escape control mechanisms such as homeostasis, transport and cell binding. These elements bind to protein sites that they don't suppose to by removing the main primary metals from their natural binding sites, which could result to cell malfunction and ultimately toxicity (Jaishankar *et al.*, 2014). According to the report of Flora *et al.,* 2008, biological macromolecules undergo oxidative deterioration mainly due to binding of heavy metal to DNA and protein molecules. Environmental contamination with heavy metals can cause atmospheric degradation, toxicity to aquatic flora and fauna. Heavy metals gain entrance into the food chain through metal uptake mechanism exhibited by plants whereas animals get infected with these toxic materials after consumption (Zhiyuan *et al.,* 2014). Over the past few decades, heavy metals have been a major concern to both the environmental and public health sector due to the level of toxic effect that they pose to humans which could be genotoxic or carcinogenic (Tchounwou *et al.,* 2012). Heavy metals are known to bioaccumulate in the food chain and in the body and can be stored in soft (kidney) and hard (bone) tissue. They gain entrance into the body either by ingestion or inhalation. The severity of adverse health effects is related to the type of heavy metal and its chemical form and is also time and dose dependent. Several studies have shown that toxic metals exposure causes long term health problems in human populations. Although the acute and chronic effects are known for some metals, little is known about the health impact of mixtures of toxic elements. Recent reports have pointed out that these toxic elements may interfere metabolically with nutritionally essential metals such as iron, calcium, copper and zinc (Alonso M. *et al.*, 2004 & Abdulla M. *et al.*, 1990). The U.S. Agency for Toxic Substance and Disease Registry reported that Lead (Pb), Mercury (Hg), Cadmium (Cd) and inorganic Arsenic (As) are the most toxic of all the list of heavy metals, commonly detected in toxic waste sites and can cause potential toxicity to the human health at low exposure rate.

Over the years, there has been a numerical increase in the expansion of mining, textile, tanneries and battery industries worldwide and these resulted in a significant increase in the direct or indirect discharge of heavy metals contaminants into the environment which is most common in developing countries (Fenglian *et al.*, 2011). Tchounwou *et al.*, 2012 reports that environmental contamination can be brought about by deposition of metals in the atmosphere, metal decomposition, erosion of metal ions present in the soil and leaching of heavy metals and sediment resuspension. Natural occurrences such as weathering process and volcanic eruptions have also contributed to heavy metal pollution.

Some important nutrient present in specific metals such as zinc, copper, cobalt, iron, manganese, chromium, magnesium and nickel are necessary for biological organism and a deficiency in their availability can result in a variety of diseases (Tchounwou *et al.*, 2011). In plant and animals, sometimes the properties of oxidation-reduction and chemical coordination gives heavy metal the advantage of enabling them escape control mechanisms such as homeostasis, transport, compartmentalization and binding to required cell components. The mechanism for toxicity is such that these heavy metals binds with protein site that isn't made for them by replacing real metals from the natural binding sites and as a result, this leads to malfunctioning of cells. Jaishankar *et al.,* 2014, reported that the decline in the oxidation of biological macromolecules is majorly due to heavy metal binding to nuclear protein and DNA.

The various resistance mechanisms that allows microorganism to survive in very polluted heavy metal environment includes; exclusion of metals by permeable barriers, active transport of the metals away from the cell organisms, intracellular sequestration of the metal by protein binding, enzymatic action by detoxification of metals and decrease in the response of metals to target cells. The mechanism of enzymatic detoxification of metals may vary depending on the microorganism involved (Nies and Silver, 1995). Most microorganisms have resistance genes that enable them to resist these toxic metals, these genes are present on their plasmid or on chromosomes. (Nies, 1999). Absorption of Lead at a very low concentration level shows no toxicity to human health but when ingested at a very high concentration, it poses great danger or threaten the normal functioning of the nervous, circulatory, enzymatic, skeletal, endocrine and immune system (Zhiyuan *et al.*, 2014). Chronic exposure of biological organisms to cadmium can result to various forms of toxicity such as kidney dysfunction, lung cancer, prostatic proliferate lesions and bone fracture, whereas a chronic exposure to Arsenic, causes dermal lesions, skin cancer and peripheral neuropathy. Depending on environmental conditions, these heavy metals in the soil are mostly absorbed into plant and thereafter can gain entrance into the food chain (Rui *et al.*, 2015). The accumulation of heavy metals in the soil threatens the progressive growth of plant, quality of food and microbial activities (Voila *et al.*, 2012). Before an analysis can be carried out in a soil sample, some factors are taken into consideration of which include, soil mineral composition, soil origin, method of digestion and the element of interest. (Voila *et al.*, 2012). Soil is known for its inherent heterogeneity and variable mineral composition leading to analytical complexity.

Microorganisms encounter metals and their derivatives by interacting with a wide range of heavy metals in their immediate surrounding area, whether the interaction is beneficial or detrimental. Some metals such as calcium, cobalt, chromium, copper, iron, magnesium, manganese, nickel and zinc are very important as micronutrients and redox processes to stabilize molecules through electrostatic interaction and for the regulation of osmotic pressure (Bruins *et al.,* 2000). Most metals when ingested in lower concentration are not lethal to biological organisms but at a high concentration, however, they form complex compounds, leading to toxic effect. Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, the concentration of most heavy metals in the soil of both rural and urban environment could exceed the normal rate and therefore can be toxic to living organisms. The accumulation of these elements becomes toxic in the soil because their level of production is faster when compared to natural ones. Mining and milling of metal ores coupled with industries have bequeathed many countries, the legacy of wide distribution of metal contaminations in the soil. During mining, heavier and larger particles of heavy metal particles settle at the bottom of floatation cells and are directly discharged into natural depressions, including onsite wetlands resulting in elevated concentrations. Large-scale mining and smelting of lead and zinc ores have resulted in the environmental contamination with heavy metals. The production of heavy metals such as copper ores in mining industry if left untreated, can be washed down through run-offs from the mining site to other surrounding areas where the human population are at a very high risk of exposure. The concentration of toxic heavy metals in disposed products are relatively high compared to those in the receiving environment and the bioavailability of metals in the environment can increase due to the nature of their chemical forms.

1.1.1 Arsenic

These toxic heavy metal compounds are very hazardous to human health and they tend to bioaccumulate in the environment at elevated concentrations (Hettich *et al.,* 2015). Anthropogenic activities such as mining, smelting, combustion of fossil fuels, application of arsenic containing pesticides and herbicides and naturally activities such as volcanic emissions and weathering of arsenic bearing minerals have made it possible for contaminated environmental sites to be located worldwide (Wang *et al.*, 2015). On-going investigations on the toxicity of heavy metals and their detrimental effects to human health are aimed at assessing the potential physiochemical properties and the toxic manner of the toxic elements in biological organisms. The United States Environment Protection Agency (EPA) superfund list published that arsenic ranks first in the US environs and is considered as the "KING OF POISONS" (Wang *et al.*, 2015). Arsenic is known to be the 20th most abundant element on earth (Jaishankar *et al.,* 2014). Arsenic is a very toxic element that is widely distributed in the soil, fresh and marine water environment with no significant biological function (Wang *et al.,* 2015). Arsenic is extremely toxic and carcinogenic. It is available in the environment in the form of both oxides or sulphides or as iron salt, sodium salt, calcium salt etc. (Jaishankar *et al.*, 2014).

Figure 1.1: Arsenic

The inorganic forms of arsenic are arsenite and arsenate and they are very toxic to human health. Arsenate and arsenite tend to accumulate in the soil, crops and water for a long period of time. Arsenic residues are also present in tobacco smoke. A high concentration of arsenic compound is found in fertilizers used for soil treatment. On the other hand, humans and animals absorb these arsenic compounds into their body system when they ingest food materials containing arsenic. A high concentration of arsenic compound is present in fodder additives used for inhibiting parasite infestation in poultry farms. A large concentration of the non-hazardous form of organic arsenic such as arsenobetaine and arsenocholine can be found in fish. Other examples of organic arsenic compounds include arsenosugar and arsenolipids. Although, Meyer *et al.,* 2014, established that arsenolipids show very high toxicity. The major route for arsenic absorption is the gastrointestinal and respiratory tract. Solenkova *et al.,* 2014 proposed in his research findings that approximately 40 to 60% of inhaled and 95% of ingested arsenic is absorbed by living organisms. Arsenic is a cellular poison that affect sulfhydryl group of cells causing malfunction in cellular respiration, enzymatic cell and mitotic cell division. The major characteristics of arsenic compounds is that they are odourless and flavourless. Human toxicity with arsenic could result from drinking water poisoned with arsenic. Symptoms of acute arsenic poisoning includes swelling of eyelid and limbs, vomiting, diarrhoea and cramps whereas chronic arsenic poisoning also known as Arsenism has symptoms such as keratosis of the palms and soles, white lines on the fingernails, and peripheral neuropathy. Wang *et al.,* 2015 reported that water pollution by arsenic from regions surrounded by mining industries resulted in a high incidence of "Arsenicosis" in countries such as Bangladesh, India, Chile, Argentina and China. Some organic arsenic compounds have been found to be less toxic in comparison to inorganic arsenic compounds. The antidote for arsenic poisoning is Dimercaprol. Jamieson (2014) reported that approximately 237,000 tons of Arsenic trioxide (As_2O_3) has been the major source of contamination of the local terrestrial and aquatic environment because of its accumulation in the underground compartment of the Giant mining industry located in the Northwest territory of Canada. These environmental contaminants resulted in an increase in health risk concerns among the people regarding the potential introduction and circulation of these hazardous metals into the food chain and the resulting health hazards. Niyogi *et al.,* 2017 reported that small mammals such as snowshoe hares are more susceptible to accumulation of arsenic and other trace amount of metals directly from their natural environment because of their limited home range and soil eating behaviour. Studies also showed that a high concentration of arsenic compounds are present in the soil, underground and surface water (Jamieson 2014). Niyogi *et al.,* 2017 reported that in the surrounding areas of Yellowknife (Canada), there is limited information regarding the susceptibility of wildlife species to the environmental bioaccumulation of arsenic and cadmium, their exposure rate and the resulting health effects. Till date no investigation has been made on the biochemical effects and the functional changes in biological tissues associated with the exposure of wildlife to the increased concentration of arsenic and other trace amount of metals in the environment (S. Niyogi *et al.,* 2017). Accumulation of a high concentration of arsenic compounds and other trace metals can be discharged into the environment, bringing an increase in contaminant burden in exposed population, resulting to the development of cancer (S. Niyogi *et al.,* 2017).

1.1.2 Mercury

Mercury is the third most toxic heavy metal that have been found to bioaccumulate in the environment. It has a characteristic silver white colouring and becomes a colourless and odourless gas after heating. Most studies carried out on mercury toxicity are aimed at the toxicity of marine life forms because of its adverse effect in the marine environment. The major source of metallic mercury toxicity in the environment include various anthropogenic activities such as industrial and mining activities, use of fertilizer and pesticides for agricultural purposes, municipal wastewater discharge, and discharge of industrial wastewater (Zhiyuan *et al.,* 2014 and Chen *et al.,* 2012). The major route for mercury absorption is by inhalation or ingestion. Mercury exist in three major forms (elemental, organic and inorganic), with each having its own specific toxicity and bioavailability (Tchounwou *et al.,* 2012). Inorganic mercury is less toxic in comparison to the organic form (methyl mercury) which is highly toxic. The intake of inorganic mercury by aquatic organism such as fish, enables this compound to be biotransformed into methyl mercury and biomagnified in the tissues of these organisms. Fish protein binds more than 90% of the consumed methylmercury in ways that even thorough or vigorous cooking methods such as deep-frying, boiling, baking and pan frying cannot remove it. Methylmercury affects the nervous system and foetal development of aquatic organisms. Consumption of infected aquatic organism is the major route of human exposure to methyl mercury. Mercury poisoning can result from mercury vapor inhalation, mercury injection and absorption of mercury through the skin (Tchounwou *et al.*, 2012). About 2 to 3% of inorganic mercury is absorbed through the skin. Organic mercury is eliminated from the body by a process of demethylation to inorganic mercury or of L-cysteine complex degradation in the bile. Approximately 10% 0f organic mercury is excreted through the urine. Selenium, vitamin C and E can decrease the toxic effect of mercury by multiple mechanism. Urine mercury is used for assessing exposure to inorganic mercury. During the day, the level of urine mercury may vary depending on individuals. Current occupational safety and health administration recommendations require urinary mercury levels not to exceed 35µg mercury per gram of creatinine.

Figure 1.2: Mercury

Jaishankar *et al.,* 2014 stated that mercury can also exist as a cation with oxidation state of +1 (mercurous) or +2 (mercuric). Mercury is widely used in the preparation of amalgams for dental purposes (Jaishankar *et al.,* 2012 and Berlin M. *et al.,* 2007). For centuries it was used for some medical purposes as diuretics, antibacterial and antiseptic agents and in the late 18th century, antisyphilitic agents contained mercury. Previous reports established that many beauty products, laxatives and infant products are contaminated with mercury (Plaza M. *et al.,* 2008). Previously methylmercury compound such as thimerosal has been used as preservative agent in paediatric and adult vaccines. The toxic effects of mercury poisoning to the nervous system include oscillatory tremors and mercury poisoning could result in mercury induced cognitive impairment such as hallucinosis and unwanted excitement. The well-known Minamata disease that plagued the residents around Minamata Bay in Japan with tremors, sensory loss, ataxia and visual field constriction is a typical example of organic mercury poisoning. In Minamata Bay, organic mercury was discharged into the water and it got methylated by bacteria and thereafter was ingested by fish. Local villagers ate these contaminated fish and began to show symptoms of neurologic damage such as visual loss, numbness, deafness and ataxia. Babies were exposed to methylmercury through the breast milk of the mothers (David *et al.,* 2017).

1.1.3 Chromium

Chromium is a naturally occurring element in the earth crust. Chromium occurs in different states such as trivalent Cr(iii) and hexavalent Cr(vi) state which is toxic to plants, animals and even humans (Tchounwou *et al.,* 2012). The trivalent and hexavalent state of chromium are the most stable forms of chromium compounds and of utmost interest to humans (Zhitkovich, 2005). The major routes of chromium exposure to the human body are through inhalation, ingestion and dermal absorption. Occupational exposure generally occurs through inhalation and dermal contact, whereas the general population is exposed mostly by ingestion through chromium residue present in soil, food, and water while chromate worker gain exposure to chromium via inhalation. The presence and accumulation of chromium residue in plant beyond the permissible limit show toxicity in plant such as reduced root growth, chlorosis of leaf, inhibition of seed germination (Ghani, 2011 and Jaishankar *et al.,* 2014).

The major source of chromium in the environment results from industrial activities such as oil and coal burning, production of petroleum from ferro-chromate refractory material, fertilizer processing, oil well drilling and tannery process of metal plating, production of paint and pigments, wood preservation, chemical production, pulp and paper production. These industrial activities constitute a major role in environmental toxicity of chromium with its hazards predominantly focused on the biological and agricultural species (Ghani 2011). Examples of hexavalent compounds of chromium that are highly toxic and carcinogenic are calcium chromate, zinc chromate, strontium chromate and lead chromates, whereas trivalent compound of chromium plays a role in the metabolism of glucose and it is an essential nutritional supplement for animals and humans. Exposure to Chromium (vi) is mostly by inhalation at a rate faster than Chromium (iii). Chromium (iii) in the presence of excess oxygen is immobile and insoluble in water whereas in the presence of oxygen, Chromium (vi) becomes highly water soluble and mobile (Wolinska *et al.,* 2013). Previous reports on chromium toxicity stated that 390g/kg of chromium is contained is cigarettes but there are no concluded facts on the amount of chromium inhaled through smoking. Exposure to high amount of chromium can cause ulcer formation in the nasal septum (mostly common among chromate workers), inhibition of erythrocyte glutathione reductase and it induces DNA damage leading to DNA adduct formation, chromosomal aberrations and alteration of DNA replication and transcription (O'Brien *et al.,* 2001 and Matsumoto *et al.,* 2006). It was reported by Jaishankar *et al.,* 2014, that in August 1975, an underground water in Tokyo containing waste matters of Chromium (vi) had a 2000 times higher limits than the permissible limit while in India, the chromium level has been witnessed to be more than 12mg/L and 550-1,500ppm/L.

Figure 1.3: Chromium

1.1.4 Cadmium

Cadmium is a soft bluish white metal and it is known to be a very toxic form of heavy metals which is of environmental and occupational concern. Galunin *et al.*, 2014 reported that anthropogenic, agricultural and industrial activities such as mining, fungicides, plastics caused a rise in the level of the cadmium concentration in air, water and soil. Cadmium is a by-product of zinc production. A considerable amount of cadmium is present in sediments such as soil and rocks, coal mineral and fertilizer. Human exposure to cadmium is majorly through inhalation or ingestion. Henson and Chedrese 2004, reported that the International Agency for Research on Cancer (IARC), classified cadmium among the Group one (1) human carcinogenic compound. Human activities are the major source for the discharge of toxic cadmium compound into the environment.

Cadmium is widely distributed in the earth crust with an average concentration of 0.1mg/kg. The Agency for Toxic Substance and Disease Registry (ATSDR) grouped cadmium among the seventh most toxic heavy metal found in the earth crust (Jaishankar *et al.,* 2014). If ingested into the body of a living organism, it can be accumulated for a long period of time or for a complete life time. The Environmental Protection Agency (EPA) states that the oxidation state of cadmium is +2. Environmental factors that affects or influences cadmium retention time and mobility are pH, temperature, ionic strength, cation exchange capacity (CEC), surface area, concentration of the ligands and surface charge (Galunin *et al.,* 2014). Two major characteristics of Cadmium is that it possesses the same chemical similarity to zinc and similarity in oxidation state of +2 and lower melting point (321.1^0C). Cadmium is mostly used in electric batteries and electronic components and nuclear reactors (Jaishankar *et al.*, 2014).

Figure 1.4: Cadmium

Cadmium causes both acute and chronic toxicity to humans and their environment (Chakraborty *et al.,* 2013). Cadmium causes nephrotoxicity and accumulates in higher concentrations in the cells of the proximal tubules. Cadmium causes bone mineralization either through bone damage and kidney failure. Exposure to cadmium causes skeletal damage (osteoporosis) and disruption of calcium metabolism, formation of kidney stone and the condition of elevated calcium in urine (hypercalciuria). An increase in the absorption of cadmium via inhalation can cause lung damage. Chronic low-level cadmium exposure enables cadmium to accumulate in the kidney, thereby causing kidney disease, weak bones and damage of lungs (Bernard, 2008). Mudgal *et al.*, 2010, reported that smokers are more susceptible to cadmium intoxication than non-smokers. The major source of cadmium absorption in smokers is tobacco which results from tobacco plants. Tobacco plants can accumulate cadmium from the soil. Non-smokers are exposed to cadmium via food and some other pathways, yet cadmium uptake through other pathways is much lower. Premature birth and reduced birth weights are the issues that arise if cadmium exposure is high during human pregnancy (Henson and Chedrese, 2004). Cadmium toxicity is common among cigarette smokers. Absorption of cadmium is through the gastrointestinal tract. After absorption, cadmium binds to protein through red blood cells or albumin and undergo hepatic conjugation to metallothionein (Solenkova *et al.*, 2014). The maximum cadmium level of concentration in freshwater as established by the Brazilian Council for the Environment (CONAMA) is 10µg/L for Cadmium class of 2 and 3, whereas in the United States, the Environmental Protection Agency (EPA) established it to be 2.0µg/L in freshwater (Galunin *et al.*, 2014).

1.1.5 Lead

Lead is a very toxic metal and it is widely distributed in the environment. When released into the environment, lead gets accumulated for a very long period. Lead disintegrates in the presence of oxygen causing toxicity which endangers living organisms in the environment. Humans get exposed to Lead poisoning from several sources which include paints, water distribution system, food ingestion, metal plating and finishing operations, fertilizers and pesticides, smelting of ores, factory chimneys, additives from gasoline and pigments, wastes from battery industries, soil wastes and exhaust from automobiles (Sharma and Dubey, 2005). Inhalation and ingestion are the major pathways to Lead exposure. Exposure to Lead could result from elevated dose or a progressive sequel of repeated high or low level of exposure. Absorption is primarily through the gastrointestinal tract. 99% of absorbed lead binds with the erythrocytes (red blood cells) and 1% remains in the serum (Skerfving *et al.*, 2007). The half-life of lead in the bloodstream is approximately 36 days whereas it falls between 20 to 30 years in the bones. Excretion from the body is through the urine, sweat, hair and nails (Abadin H. *et al.,* 2007).

The International Agency for Research on Cancer (IARC) in 2006, classified inorganic compounds of Lead as Probably Carcinogenic to humans (Group 2A). Mitchell *et al.*, 2014 stated that soil in urban areas often have a high concentration of Lead which may have resulted from historical anthropogenic and industrial activities such as waste incineration, coal and oil combustion and the production and use of Lead containing paint and gasoline. Human activities such as Gardening and other related activities could lead to Lead exposure from soil contaminants through incidental soil ingestion, soil resuspension, and chicken egg consumption (Zahran *et al.,* 2013). Interest in the determination of Lead is very high and still increasing as they are known to cause toxicity in the environment, food and other biological samples because of their potential to cause acute toxicity to human and animal health (Cigden *et al.,* 2013). Lead is grouped in the class of toxic heavy metals because it is of great health concern. Cigden *et al.,* 2013 established that toxicity of Lead exposure to human health results in blood enzyme changes, anaemia, hyperactivity, neurological disorders and pathological changes in organ systems such as the central nervous system, renal system, hematopoietic and immune system. Also, it has been discovered that Lead has the tendency to induce chronic damage to the development of intelligence in juveniles.

Figure 1.5: Lead

1.2: Mechanism of Heavy Metal Toxicity

Heavy metals are essential environmental contaminants and their toxicity is by far a problem of increasing significance for ecological, dietary and environmental reasons. (Jaishankar *et al.,* 2013). Metals are very essential in the biological functioning of both plants and animals but sometimes their chemical coordination and oxidation reduction properties gives them an added advantage in that they can escape control mechanisms such as homeostasis, transport and binding to target cells. Generally heavy metals binds with protein sites and cause cell malfunction and thereafter toxicity may occur. For example, *Flora et al.,* 2008, carried out a study that proved that heavy metal bound to DNA and nuclear proteins results in the oxidative deterioration of biological macromolecule. Generally, the mechanism for heavy metal toxicity is primarily because these elements have the potential to interact with proteins and DNA, causing oxidative breakdown of biological macromolecule. Because of the level of environmental hazards caused by these metals, it is necessary to measure the various level of heavy metal concentration in the environment.

1.2.1 Arsenic

There are several factors upon which the toxicity of arsenic depends, these are exposure dose, length of time, biological species, gender and age, individual vulnerability, as well as genetic and nutritional factors. Studies shows that the inorganic form of Arsenic is highly toxic to humans. Inorganic trivalent arsenic compounds are known to be more toxic than pentavalent arsenic compounds. The mechanism responsible for arsenic toxicity on organ system results from the binding of thiol or sulfhydryl groups on proteins. Arsenic is known to inactivate several classes of enzymes. Studies shows that trivalent arsenic have been used for enzyme inhibition. Examples of enzymes that are inhibited by arsenic includes glutathione reductase, glutathione S-transferase, glutathione peroxidase, thioredoxin reductase, thioredoxin peroxidase, DNA ligases, trypanothione reductase, pyruvate kinase galectin, protein tyrosine phosphatase, JNK phosphatase and E3 ligases c-CBL and SIAH1 (Shengwen *et al.,* 2013). Arsenic can also exert toxicity through weakening of cellular respiration by inhibiting several mitochondrial enzymes and the dissociation of oxidative phosphorylation. Arsenate show toxicity by interacting with sulfhydryl groups of proteins and enzymes of arsenate results mostly from the interaction and substitution of phosphorus in many biochemical reactions.

Another mechanism involved in Arsenic toxicity is methylation of inorganic arsenic compound by biological organisms (bacteria algae, fungi and humans) which results in monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMA). Biomethylation is the major metabolic pathway for inorganic arsenic in biological organisms. Arsenic trioxide (AS_2O_3) is methylated into two major metabolites through a non-enzymatic process to monomethyl arsenic acid, this is further methylated enzymatically to dimethyl arsenic acid before excretion into the urine and these compounds are bioindications of chronic exposure of arsenics. Monomethyl arsenic acid is highly carcinogenic and toxic in comparison to other known arsenic compounds. This compound remains within the cells of biological organisms as an intermediate product (Singh *et al.,* 2007). Other arsenic compound such as Inorganic arsenic undergo an enzymatic conversion to methylated arsenic, the end metabolites and the biological marker for the exposure of arsenic compounds. The toxicity of arsenic compounds is dependent on the valency and methylation state of the compound.

1.2.2 Mercury

Mercury belong to a class of highly toxic heavy metal, with reported cases of over 3,596 in 1997 by the American Association of Poison Control Centers (Jaishankar *et al.,* 2014). Mercury is a neurotoxin which causes microtubule damage, destruction of mitochondrial, oxidative degradation of lipids and the accumulation of neurotoxins such as serotonin, aspartate and glutamate (Jaishankar *et al.,* 2014). Ferrara *et al.,* 2000, reported that approximately 2,200 metric tons of mercury is released into the environment annually. According to the Environmental Protection Agency (EPA) and the National Academy of Science, an estimate of approximately 8 to 10% of American women accumulated amount of mercury in their body system that can result in neurological disorders (Jaishankar *et al.,* 2014). Ashe *et al.,* 1953 reported that when animals are exposed to certain degree of toxic mercury, they begin to show or exhibit some behavioural and neurological changes. For examples, Ashe *et al.,* 1953, reported that rabbits have shown pathological abnormalities, marked deterioration of cellular tissues and brain necrosis. The brain is known to be the target organ for mercury toxicity and, yet it can damage and lead to the malfunction of other body organs. The brain cause reversal potential and interrupt the intracellular calcium homeostasis. Mercury attaches to free sulfhydryl groups under stable conditions (Patrick, 2002). In humans, approximately 80% of inhaled mercury vapor is absorbed through the respiratory tract. Inhaled mercury vapor causes respiratory and kidney problems, bronchitis, tremors, and even asthma. Mercury plays a key role in the damage of protein structure and alters cellular functions. The mechanism of mercury toxicity is based on its chemical activity and biological characteristics which suggest that oxidative stress is involved in its toxicity. Inorganic mercury and methyl mercury forms covalent bond with cysteine residues of proteins and deplete antioxidants. The interaction of mercury compounds suggests the production of oxidative damage through the accumulation of reactive oxygen species which would normally be eliminated by cellular antioxidants. Mercury binds freely to available thiols which undergo a reaction with methyl mercury and disrupts cellular structure. It is also involved with the process of cell transcription and translation resulting in the disappearance of the ribosome and obliteration of the endoplasmic reticulum and the activity of natural killer (NK) cells.

1.2.3 Chromium

In the environment, trivalent chromium [Cr (III)] is generally harmless due to its weak membrane permeability while hexavalent chromium [Cr (VI)] on the other hand has a strong membrane permeability. Chromium (VI) penetrates cells through passages for isoelectric and isostructural anions such as SO_4^{2-} and HPO_4^{2-} passage and these chromates are ingested through the process of Phagocytosis. There are two major factors governing the toxicity of chromium, which are oxidative and solubility state. When given similar amounts and solubility, chromium (VI) tends to be more corrosive and systemically toxic than chromium (III). Chromium (VI) due to its solubility rate, it tends to pass through cell membranes (Tchounwou et al., 2014). The reduction of chromium (VI) can serve in the process of detoxification and activation, when its reduction occurs some distance from the target site for toxic or genotoxic effect (detoxification process) and it can serve to activate chromium toxicity if it takes place near the cell nucleus of the target organ (Tchounwou *et al.,* 2014). If the chromium (VI) is reduced to chromium (III) extracellularly, toxicity in metal mobility intracellularly cannot be observed. The amount and rate to which extracellular chromium (VI) and chromium enters the cells and exert its toxic effects depends on the balance or stability that exist between the both toxic elements (Cr III and VI). The production of reactive oxygen species such as superoxide ion, hydrogen peroxide and hydroxyl radical occur because of the reaction between thiols and ascorbate (biological reductants) and chromium (VI), these processes could lead to DNA and protein damage. Chromium (VI) has been found to be more hazardous than chromium (III) and is finally reduced to chromium (III). The International Agency for the Research on Cancer classified Chromium (VI) as a group one carcinogenic compound because of its mutagenic properties (Jaishankar *et al.*,2014).

1.2.4 Cadmium

Although the mechanism of cadmium toxicity is not fully understood but its effects on cells are clearly known. Cadmium causes cell damage basically through the generation of reactive Oxygen species (ROS), leading to damage of single stranded DNA and the disruption of nucleic acid and protein synthesis (Patrick, 2003). Cadmium concentration in biological organisms increases when it is bound to metallothionein. In the liver, cysteine-metallothionein complexes causes liver damage, thereafter cysteine-metallothionein circulates to the kidney and accumulates in renal tissue causing kidney damage. Cadmium also binds with cysteine, glutamate, histidine and aspartate ligands, all of which results in iron deficiency in biological organisms (Castagnetto *et al.,* 2002. Cadmium share similar oxidation state with zinc and it can substitute the presence of Zinc in metallothionein, thereby inhibiting it from acting as a free radical scavenger within the cell. At lower concentrations of (1-100µM), cadmium binds to proteins, decreases DNA repair (Abshire *et al.,* 1996), activates protein degradation, up-regulates cytokines (Yang J, 1998).

1.2.5 Lead

Lead causes toxicity in cells of living organisms, this is achieved by ionic mechanism and oxidative stress. Oxidative stress in biological tissues results from the imbalance between the generation free radicals and antioxidants to either detoxify the reactive intermediate or to repair cell damage. The presence of glutathione in the cell functions to protect it from free radicals such as H_2O_2. Lead metal controls the increase in the concentration of ROS and the decrease in the level of antioxidants. The reduced form of glutathione gives its reducing equivalents ($H^+ + e^-$) from its thiol group of cysteine to ROS to keep them in a stable state. In the presence of the enzyme glutathione peroxidase, glutathione reduces disulphide bonds formed within cytoplasmic proteins and cysteines and in the process, donates an electron. In the process, glutathione is converted to its oxidized form glutathione disulphide. Under condition of oxidative stress, the level of glutathione disulphide surpass the level of glutathione (Jaishankar *et al.,* 2014). Another mechanism by which lead exert toxicity is through its interaction with proteins and biochemical processes, i.e. its ability to either mimic or inhibit the activity of calcium (Tchounwou *et al.,* 2014). Lead binds to certain groups of enzymes (sulfhydryl and amide groups), thereby altering their structure or composition and inhibiting their activities. Lead competes with important metallic cations for binding site, inhibiting enzyme activity or altering the transport of calcium (Flora *et al.*, 2007). Lead can cause damage of biological cells brought about by the formation of reactive oxygen species (ROS) (Tchounwou *et al.*, 2014). Another biomarker for Lead mechanism of toxicity is that it acts by interfering with the calcium action related to neuronal and message transduction intracellularly. Lead disrupts intracellular calcium cycling and signaling, it alters the release of organelle stores (a process also known as respiration) such as the nucleus and Golgi apparatus. In some cases, Lead inhibits calcium dependent events, including calcium dependent release of several neurotransmitters and receptor coupled ionophores in glutamatergic neurons. In other cases, Lead appears to augment calcium dependent events such as protein Kinase C and calmodulin (Tchounwou *et al.*, 2014).

2. BIOAVAILABILTY OF HEAVY METALS IN THE ENVIRONMENT

Since the industrial revolution, there has been an increase in the discharge of potential hazardous elements which may alone or in combination with other toxic substances cause harm to the ecosystem or human health. Most of the elements are well accumulated extensively in the soil (Desaules, 2012). The primary source of environmental contamination is anthropogenic, in combination with industrial discharges of toxic elements such as cadmium, chromium, lead, and mercury, agricultural and animal discharge, and pesticide application such fungicides or irrigation with wastewater (Mousavi *et al.,* 2013). There are also various strategies used by plant to increase the bioavailability of heavy metal concentration in the soil. For example, the secretion of plant iron carriers (Phyto-siderophores), carboxylates and rhizosphere acidification to ease the chelation of metal accumulated in the soil.

Several researchers attempted a definition for bioavailability based on its measurement of operation which are aimed reflecting its operational measurement. Warrington and Skogley (1997) defined bioavailability in terms of content i.e. measure or amount of heavy metal present in the soil that plants can absorb during their life cycle. Bioavailability on the other hand was defined by Taghon *et al.*, 1999 and Shor and Kosson, 2000 in terms of flux (transport rate) i.e. the rate at which metals are transported from the soil to the organism. Bioavailability describes the degree and amount of uptake (absorption) for a toxic substance (xenobiotic) which enters systemic circulation in the parent form from the site of exposure. Bioavailability in terms of processes involves the physical, chemical and biological interactions that determines the exposure of plants and animals to chemicals associated with soil and other sediments. The processes involved are; the discharge of toxic substances from solid phase, transport of the discharged toxic substances, transport of the bound toxicants to the organism membrane, transport across physiological membrane barrier and the integration into a biological system or organism through metabolic processes. Generally, bioavailability is the proportion of the total amount of toxic substances (heavy metals, pesticides etc.), accumulated in a specific or given environment that at a period, is made available for uptake by microorganisms directly from the environment. Generally, the bioavailability of heavy metals in the environment determines the physiological effects and the level of toxicity heavy metals pose to biological system. Environmental toxicity of heavy metals can be determined by the measurability of the sum of all metals after digestion with strong acid using an acceptable and standard analytical technique. In soil, the fate and behavior of heavy metal is determined by the various concentration levels of heavy metal physicochemical processes or sediment systems. Also, the presence the biloavaility and chemical reactivity (sorption/desorption, precipitation/dissolution) towards other components of a student. Kim *et al.,* 2013, mentioned that the term Bio-accessibility can be used interchangeably with external bioavailability, which correlates with the physicochemical controlled desorption process and the physiological controlled uptake process of metals. Caussy *et al.,* 2003, mentioned that bioavailability can be divided into 2; internal and external bioavailability. External bioavailability is determined by the ability for an element to be dissolved or discharged from a media such as soil and water whereas Internal bioavailability depends on the ability of metals to be absorbed or induce toxic effects on target organs subsequently. Bioavailability is considered as a compound dynamic process actively controlled by the organism type, exposure type and speciation of metal type (Kim *et al.,* 2013). Kim *et al.,* 2015, suggested that bioavailability should be considered as a dynamic process which includes three definite steps:

- Physicochemical controlled desorption process (environmental availability).
- Physiological controlled uptake process (environmental availability)
- Physiological induced effect or accumulation within the organism (toxicological availability).

In aquatic sediment, the bioavailability of chemical elements to benthos doesn't depend only on form of the metal but also on the sediment geochemical properties and the different routes of exposure of the organism (Besser *et al.,* 2003, Riba *et al.,* 2004, Simpson 2005, Nobi *et al.,* 2010, Rainbow, 2007 and Simpson *et al.,* 2012b). In comparison to other element, cadmium and its compounds has high water solubility. Cadmium has a very high bioavailability and they tend to bioaccumulate in living organisms. Long-term exposure to cadmium can alter kidney function (Jaishankar *et al.*, 2014). Bioavailability is important for risk assessment which focuses on an adjustment factor that explains the ability for organism to absorb chemical elements. It is used to assess the growth of organisms and to evaluate metal absorption and toxicity. Soil properties such as soil pH, contents of organic matter, clay and iron oxide are known to alter the effects of metal loading of soil microorganism. Although soil pH is known to greatly influence metal solubility and speciation in the soil and soil solution. Therefore, as the soil pH decreases, the concentration of Zinc, Nitrogen and Cadmium in the soil solution increases rapidly. Metal bioavailability in soil solution may not be possible due to chelation by organic molecules and the occurrence of chemical forms that are been absorbed. The chemical species such as free ions are absorbed and thus toxicity results in excess.

Zhang *et al.*, 2014 reported that the three factor which influences metal bioavailability and the toxicity of sediments and water:

- The solid phase especially metal binding phases such as analysis of acid volatile sulfide (AVS), particulate organic carbon, iron and manganese oxyhydroxides (Campana et al., 2012).
- Aquatic phases i.e. water physicochemical features such as pH, redox potential. Salinity, and ligand complexes.
- Sensitivity and behaviour of the bottom dwelling organism (benthos) such as taxon, lifestyle and their previous exposure history.

Although the use of Bioassays for the measurement of bioavailability is time consuming, strenuous and expensive to carry out due to its different endpoints, time of exposure and absorption mechanism, still bioassay is the most preferred measurement for bioavailability because they give the exact measurement of biological response and bioaccumulation

(Kim *et al.,* 2015). According to Castillo *et al.,* 2013, the main sources of marine pollution in Malaga Bay (South Spain) are through industrial activities, urban encroachment and coastal development, recreation and tourism and wastewater treatment plants. Sediment and water samples were collected and measured for heavy metal concentrations. In sediments, Nitrogen was the most disturbing metal because Nitrogen concentration transcend over the effect range low (ERL), i.e. concentrations at which toxicity could be observed in 85% of the samples analyzed. The metal bioavailability decreased in the order: Cd, Ni, Pb, Cu, Cr. In the sea water samples, Cadmium and Lead were the most disturbing metals because they surpass the continuous criteria concentration of the United States Environmental Protection Agency in a 22.5% and 10% of the samples.

3. THE HISTORY OF CYPRUS MINES CORPORATION (CMC) IN GEMIKONAGI REGION OF NORTH CYPRUS.

In 1912, Charles Godfrey Gunther, an engineer from the United States, travelled to Cyprus for a survey and discovered a large amount of Copper-ore and Sulphides on the Foucassa hill of Lefke. Thereafter, Philip Wiseman and Seeley Wintersmith Mudd from the United States travelled to Cyprus to join Charles Godfrey Gunther on his expedition. They invented and operated the copper mine on the Foucassa hill which later led to the realization of the Cyprus Mining Corporation (CMC). The accomplishment of CMC brought good fortune to Cyprus as both the Greek and Turkish community gained employment to work on the mine after a permission was obtained from the British Government. Surveys were later carried out on the Southeast of Lefke (Karadag), which yielded positive results as more elements were discovered.

Further expedition resulted in the discovery of a large amount of Copper Ore in an area close to the Pendaya village. In 1922, several mining houses were constructed to meet the need of the increased inflow of workers. As the years passed by, these houses were multiplied in Skuriotissa because of a high increase of workers in the mine and in addition, a canteen was built. Equal right to operation of the canteen was given to some Turks in Lefke. This canteen was constructed because the mine workers would be able to do their shopping either in cash or credit and on the basis, that salaries can be paid to the workers through the canteen operators. In that same year (1924), a harbour was built in the West of Pendaya for the exportation of copper ores.

During the Russian Revolution in 1924, Russians from several disciplines (Medicine, Engineering etc.) relocated to Cyprus. Employment opportunities were given to many Russians to work in different facet of the corporation. Thereafter, many of the workers fell victims to the Malaria outbreak complication and this led to a construction of a hospital to meet the medical need of these victims. Eucalyptus plants were cultivated in swampy areas to prevent or reduce the further spread of malaria or the infestation of mosquitoes. In 1957, CMC faced a major problem between the both communities, the Greek Cypriots and Turkish Cypriots. It became evident that the both Communities (Greek and Turkish

Cypriots) could not work together in the mining corporation. So therefore, CMC provided Karadag mining for the Turkish workers, whereas in Foucassa, only the Greeks worked there. In the same year, the Turkish community chased the Greek Cypriots leaving in Lefke. In 1960, Cyprus gained its independence and became a republic, which led to both the Greek and Turks coming back to work together. After its independence, CMC started paying tax to the government for the development of the country.

Figure 3.1: Cyprus Mines Corporation (CMC) site and Heavy Metal Concentration.

In 1963, the political conflict between the both communities made CMC operations not to function properly. During these period, the Greek Cypriots claimed rulership over the governmental affairs of the Island and enforced the payment of heavy tax on CMC. CMC was under pressure by the Greeks and as a result, the Turkish Cypriots were deployed to Karadag mines to work because it is in the boundaries of Lefke, leaving the Greek Cypriots to work in Foucassa and Xero (Gemikonagi) areas of the island. In 1970, the Turkish Cypriots working in the Karadag mines were relieved form their duties because of the cessation in copper ore availability in the area. In 1974, the Turkish and Greek communities officially separated. The consequence of this separation was the permanent shutting down of the copper mines corporation.

Figure 3.2: Heavy Metals Concentration in Cyprus Mines Corporation

4. ASSESSMENT ON THE ENVIRONMENTAL TOXICITY OF HEAVY METALS IN GEMIKONAGI REGION OF CYPRUS

The abandonment of Cyprus Mines Corporation (CMC), left huge piles of heavy metal deposit exposed in the environment, resulting in substantial contaminations of surrounding soils and a sea shore. Toxic heavy metals residues are washed down into the Mediterranean Sea after a heavy down pour of rain. When viewed from a short distance, a discoloration, sometimes cerulean blue coloration is noticed on the sea water during its wave action. This discoloration covers a length of 2 kilometres and a width of over 600 meters. The presence of this heavy metal contaminants in the sea water can be detrimental to ocean life. Marine organism such as fish, can absorb these heavy metals which eventually gets saturated within their body system. Fishing is a major practice carried out in this surrounding area and the absorption or intake of fish from the sea, could be detrimental to the health of the residing locales. In addition to these tailing piles in the area, there are large number of earthen ponds containing tailing quantities of these contaminants. These ponds are potential source for groundwater contamination in the area. The amount of the tails of contaminants (iron, copper, Sulphur salts and other hazardous elements) in the ponds is approximately 3 million tons. There is also about 30,000 tons of cyanide tails present in the environment. About 2 million tons of copper ore and Sulphur were dumped in the surrounding of Lefke. These tons of heavy metal contaminants are carried in run offs and discharged directly into the Gemikonagi Reservoir. This reservoir serves as a source of drinking water to the resident's around Gemikonagi. During the summer, as the temperature rises from 40 to 45^0 C, pyrites piles up containing about 30 to 35% content of Sulphur which self-burns and exposes Sulphur dioxide into the air which creates air pollution.

Generally, operations carried out by CMC and her departure from the Island led to a considerable discharge of untreated contaminants to the environment which can lead to environmental toxicity. Copper, together with some other products such as $CuSO_4$, H_2SO_4 were majorly produced in this region by the Mine Corporation and when it was eventually shutdown, no preventive action or precaution was taken against environmental pollution.

Aysel *et al.,* 2000 reported that Sulphur mineralization in this region is the result of fluids loaded with heat (Hydrothermal) at the ocean bottoms. A qualitative and quantitative determination of heavy metal contamination in this region will help to ascertain the various concentration levels of heavy metal and its bioavailability in Gemikonagi region of Cyprus. The results obtained from this research will be used for future research purposes to determine if the heavy metal discharged into the seawater and the land environment, could pose a considerable health effect to the residing population. Apart from the raw ores in “Lefke” and “Karadag” region, chemical wastes in Gemikonağı Harbour, heap of metal fragments, wastes from gold production with cyanide, by-products of copper flotation process, pyrite waste ponds containing 25-30% sulphur cause not only soil, groundwater and sea pollution, but also air pollution by sulphur gases resulting from excess heating in summer time (Aysel *et al.*, 2000). Iron and sulphur with copper carried with surface waters might cause heavy metal pollution in potable and irrigation waters due to acid mine drainage. According to many research reports, there are Arsenic, Barium, Cadmium, Chromium and Selenium in high amounts at the region.

The State Laboratory Directorate of the Turkish Republic of Northern Cyprus (28th of October 1999) reported that some samples were taken from copper mines facilities in Gemikonagi. The results from the State Lab showed that Aluminium, Chromium, Copper, Iron and Manganese were detected at a very high concentration at both sites of collection. Plant sample were also taken for experimental analysis and the result obtained showed that Zinc, Chromium, Cadmium, and Lead are present in the leaf tissue of various plant species (Tangerine, Orange, bean and Cabbage). residing in this area at risk of possible These metals are known to be carcinogenic and their availability and accumulation in this region, put the health and wellbeing of the people exposure. It is well known fact that accumulation of heavy metals in plants can be transferred to humans either by ingesting or a direct consumption of the meat and milk products of animals that feed on these plants (Aysel et al., 2000).

5. TECHNIQUES USED FOR THE DETERMINATION OF HEAVY METALS IN ENVIRONMENTAL SAMPLES

Many instrumental analytical techniques are utilized to measure the various level of heavy metal concentration in the environment. Environmental samples of heavy metal from different matrixes can be analysed to obtain an accurate result using techniques such as the atomic absorption spectrometry (AAS), atomic emission spectrometry/atomic fluorescence spectrometry (AES/AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), neutron analysis (NAA), X-ray fluorescence (XRF), anodic striping voltammetry (AVS) and inductively coupled plasma mass spectrometry (ICP-MS).

The various steps involved in the analysis of heavy metal are:

- Sample collection
- Addition of chemical to the sample for its preservation.
- Transfer of sample to the laboratory
- Treatment of sample prior to analysis (extraction, pre-concentration or separation of the analytes
- Instrumental calibration
- Preparation of sample and its standard solution
- Accuracy testing for methods using Certified Reference materials.
- Statistical evaluation of results.

5.1: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Technique:

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a technique used for the determination of elements. Argon plasma is used for separation of the sample into basic atoms and ions. Inside the mass spectrometer, these ions are isolated according to their atomic mass to charge ratio by a quadrupole or magnetic sector analyser. Samples are then inoculated into the plasma torch in either gaseous or aerosol form. Liquid samples require sample nebulization whereas solid samples are dissolved before the analysis is performed or by a laser, solid samples are converted directly to aerosol. The liquid samples are pumped from a vial through a peristaltic pump and the high number of ions produced combined with very low backgrounds, provides good detection limits for most elements, normally in the parts-per-trillion (ppt) range. All elements can be analysed within a shortness of time, if the experiment is operated by a very skilled and experienced operator. This should be taken into consideration for the experiment to yield accurate results. ICP-MS has many advantages over other elemental analytical techniques such as atomic absorption spectrometry (AAS), atomic emission spectrometry/atomic fluorescence spectrometry (AES/AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma atomic emission spectrometry (ICP-AES), neutron analysis (NAA), X-ray fluorescence (XRF), graphite furnace atomic absorption spectrometry (GFAAS) and anodic striping voltammetry (AVS) because:

- Its limit of detection is excellent and more effective obtained from by GFAAS.
- It has a high throughput than GFAAS.
- It is capable of handling both simple and complex matrices with little or no matrix interference because of the high temperature of the ICP source.
- Using the same samples throughput with ICP-AES, ICP-MS has a superior detection capability.
- It has a superior ability to obtain isotopic information.

6. MATERIALS AND METHODS

6.1 Instrumentation

Ion Chromatography System Model (Dionex 3000, USA) was used for the analysis of cation and anion in water. A microwave with model CEM Mars 5 (USA) was used for the digestion process of collected samples. Prior to the analysis of samples, the ICP-MS spectrometer was calibrated using multi-elemental mixture of metal standards (Agilent Technologies, Japan). Samples were analysed using the Agilent 7500ce Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument from Agilent Technologies, Tokyo Japan, consisting of an ICP source coupled with a plasma metal shielded torch, an octapole reaction system operated in radio-frequency mode and a quadrupole mass analyser. The instrumental parameters and operating conditions used during the analytical process are as follows: purity of argon gas (99.99%), ICP Radio-frequency power (1500W), nebulizer type, concentric nebuliser, nebulizer gas (flow rate 0.9 L/min), argon auxiliary gas (flow rate 0.14 L/min), plasma gas (flow rate 15 L/min), reaction gas (helium-flow rate 0.14 L/min), spray chamber temperature of 2℃ and Integration time (flow rate 0.6 seconds).

6.2 Study Area

Gemikonagi region of North Cyprus was chosen for the collection of samples. This study area was selected for several reasons: The presence of an abandoned Cyprus Mines Corporation (CMC) facility with location of (35°08’28.80” N - 33°28’39.36” E). CMC was a major producer of copper, copper sulphate and hydro sulphuric acid for many years. After the abandonment of the copper mine, they left huge tons of tailing deposits of heavy metals exposed to the environment in large floatation ponds. The result of this exposure, led to an accumulation of a high concentration of copper, cadmium, iron, lead and other toxic elements in soil, groundwater, seawater and air (Baycu et al., 2015). The discharge of these elements was brought about by surface runoffs (rainfall) into the seashore which is visual range and subsequently distributed within the region. There have been reports on the toxic effects such as carcinogenicity caused by the accumulation of heavy metals in the region (Baycu *et al.*, 2015). Samples from 3 matrices (soil, Sea-water and fish) were collected within the region and used to assess the presence of heavy metal concentrations in these matrices.

Figure 6.1: Map showing the location of Cyprus Mines Corporationin (CMC) in Gemikonagi region of North Cyprus.

6.3 Sample Collection, Storage & Pre-Treatment

In Gemikonagi region of Turkish Republic of Northern Cyprus (TRNC), 5 different locations were selected close to the Cyprus Mines Corporation (CMC) site for the collection of sediment and seawater, while a total of 10 fish samples were collected from the Mediterranean Sea in the same region for quantitative determination of heavy metal concentration.

Table 6.1: Location of Study Area for Sediment Samples.

SITE	LATITUDE	LONGITUDE
1	35°08’11.9” N	32°50’05.7” E
2	35°08’20.1” N	32°49’52.6” E
3	35°08’35.0” N	32°48’46.6” E
4	35°08’20.1” N	32°49’57.1” E
5	35°08’36.9” N	32°50’25.7” E

Table 6.2: Location of Study Area for Sea-water Sample Collection.

SITE	LATITUDE	LONGITUDE
1	35°08’36.5” N	32°50’25.2” E
2	35°08’31.7” N	32°50’02.5” E
3	35°08’33.5” N	32°49’08.0” E
4	35°08’48.3” N	32°48’53.1” E
5	35°08’52.3” N	32°50’40.6” E

6.3.1 Collection of Sea-water Samples

Half litre plastic PET (polyethylene terephthalate) bottle was used for the collection of sea water sample. After collection, the sample bottle was well labelled and stored in a plastic bag. Thereafter, the samples were transported to the Toxicology Laboratory of Near East University and stored in a refrigerator at a temperature of 4℃. Sample preparation was done by sealing bottlecap with parafilm to keep sample tightly closed and secure until further analysis was carried out.

6.3.2 Collection of Soil Samples

Collection of soil sample was carried out with a pre-sanitized plastic bag with the aid of an instrument (Scoop). After collection, the instrument (Scoop) was properly decontaminated by a process of washing. The collected soil samples were stored in a well labelled plastic bag and sealed tightly. Thereafter, the samples were transported to the Toxicology Laboratory of Near East University. Thereafter, soil samples were thoroughly homogenized and sieved through a 1.0-mm-mesh sieve and weighed to an approximate of 250g. Filtration of soil sample was done to remove large particles such as rocks and to obtain finely smooth sample ready for the analytical process. Soil samples were then stored in a well labelled and tightly sealed plastic bag and transported to the Mersin University.

6.3.3 Collection of Fish Samples

The fish samples were obtained through the assistance of a local fisherman. A total of 10 samples of *Boops boops* belonging to the class actinopterygii were collected and transported to the Toxicology Laboratory and stored in a Freezer at a temperature of -20°C. Prior to testing, the length of individual fish was determined with the aid of a ruler and a laboratory balance was used to determine the mass of the fish samples. The table below shows the length and mass of the fish samples.

After the mass and length were taken, the fish samples were stored in a sealed plastic bag and transported to Mersin University in Turkey. Prior to testing using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS), the fish samples were dissected, separating the muscles and gills, dividing it into smaller pieces using a scalpel. The samples were dried in an oven, ground and sieved. The samples were then dissolved in 100% nitric acid and subjected to microwave digestion for 15 minutes. The digestion procedure for the soil and fish samples were done by (800W) microwave. Prior to the digestion process, the polytetrafluoroethylene (PTFE) vessels were properly disinfected using 9 mL of Nitric acid, heated for 10min at 180°C and rinsed with ultra-pure water. 1g of soil sample was weighed out into the PTFE vessels. 10mL of nitric acid, 6mL of

Hydrochloric acid and 1mL of Hydrogen peroxide is then added to each vessel. Vessels were then placed in the rotor and placed in the microwave. Finally, the vessels were heated to 180°C over 15 minutes and then held at 180°C for 10minutes. Before analysis, muscle, liver, gill and stomach from *Boops boops* were removed. Fish samples were homogenized in a blender and 1g of homogenate was digested.

Table 6.3: Length and Mass of Fish Samples.

NUMBER OF SAMPLES	**LENGTH (cm)**	**MASS (g)**
1	**10.0**	**33.0**
2	14.0	23.9
3	15.0	23.1
4	15.0	30.1
5	16.0	30.1
6	16.0	35.1
7	16.0	38.5
8	17.0	38.6
9	17.0	40.3
10	17.0	30.0

A microwave digestion system (Microwave model CEM Mars 5 (USA) was used to prepare the samples for analysis. In recent years, microwave digestion processes have been used in numerous studies (Machado *et al.*, 1999; Kucuksezgin *et al.*, 2001; Usero *et al.*, 2003) owing to the advantages of this technique, which include speed of digestion and less possibility of contamination during the process. Samples were mixed with 5mL nitric acid (65%) and 5 mL sulphuric acid in polytetrafluoroethylene vessels. After 10 minutes of mixing, 1mL hydrogen peroxide was added, and samples were placed in a microwave at a temperature of 105°C. After digestion, the residues were diluted to 25mL with nitric acid (0.3%). Determination of the elements in all samples was carried out by ICP–MS an Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan.

Figure 6.2: Scientific Name: *Boops boops* (Fish samples).

7. RESULTS

Heavy metals concentration in seawater samples obtained from 5 different sampling areas of Gemikonagi region of Northern Cyprus were analyzed using ICP-MS techniques. The results are given in Table 7.1. Results were compared with the WHO allowable permissible Limits for heavy metals in seawater (Table 7.2). Results shows that sample location 1,2, 3, 4 for Nickel, Copper, Zinc, Molybdenum and Barium in sample location 5 were below the detection limit.

Table 7.1: Heavy Metals Concentration in Seawater Samples.

Heavy Metals (mg/L)	**LOCATION**					**MEAN± STANDARD DEVIATION**
	LI	**L2**	**L3**	**L4**	**L5**	
Titanium	6.848	5.546	6.26	4.81	0.1776	4.73±2.66
Vanadium	0.509	0.3978	0.3748	0.3228	0.003	0.32±0.19
Chromium	1.9036	1.3484	1.3028	1.1746	0.002	1.15±0.69
Manganese	0.212	0.19812	0.16268	0.2166	0.002	0.16±0.09
Iron	3.70	2.68	2.40	2.42	0.10	2.26±1.32
Nickel	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	0.008	0.001±0.036
Copper	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	0.001	0.002±0.0004
Zinc	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	0.052	0.010±0.023
Molybdenum	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]	0.002	0.0004±0.009
Barium	0.08454	0.06176	0.07766	0.06272	<0.001[b]	0.06±0.034

Below Detection Limit (b)

Table 7.2: Heavy metals concentration in sea water samples in comparison to maximum permissible limit in water samples measured by ICP-MS.

Heavy Metals	Mean ± SD (mg/L)	Maximum Permissible Limit
Titanium	4.73±2.66	Not found
Vanadium	0.32±0.19	Not found
Chromium	1.15±0.69	0.05mg/L (WHO 2001)
Manganese	0.16±0.09	0.5mg/L (WH0 2001)
Iron	2.26±1.32	1mg/L (WHO 2001)
Nickel	0.001±0.036	0.02mg/L (WHO 2001)
Copper	0.002±0.0004	0.05mg/L (WHO 2001)
Zinc	0.010±0.023	10mg/L (WHO 2001)
Molybdenum	0.0004±0.009	Not found
Barium	0.06±0.034	Not found

Heavy Metals Concentration in Seawater Sample in Comparison to Allowable Maximum Permissible Limit in water shows the mean values for each metallic element in seawater samples investigated under the limits set by the World Health organization (WHO).

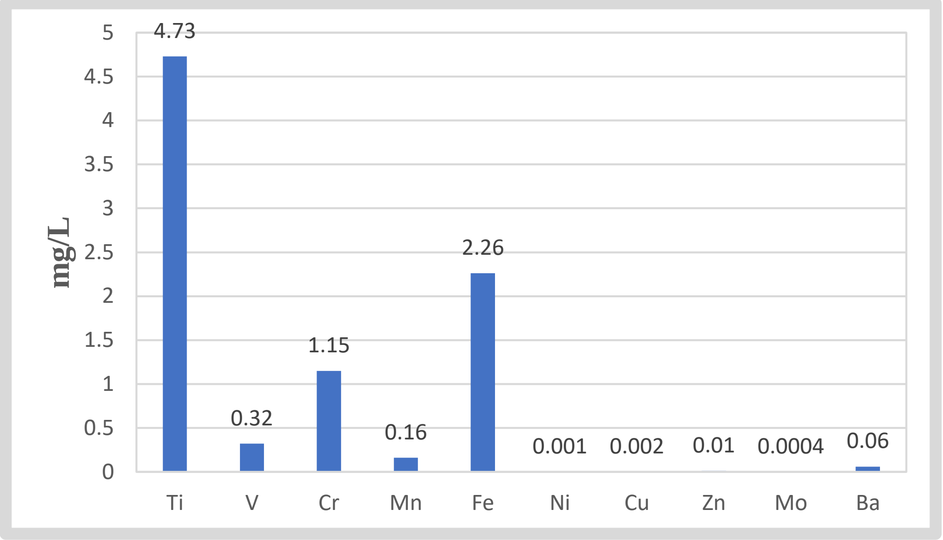

Figure 7.1: Concentration of Heavy Metals in Sea Water Samples.

Table 7.3: Heavy metals concentration in soil samples measured by ICP-MS.

Metals (mg/Kg)	**LOCATION**					**Mean ± Standard Deviation**
	L1	**L2**	**L3**	**L4**	**L5**	
Cr	665.0	782.0	803.0	946.0	548.0	748.80±150.23
Mn	5006.0	6232.0	1333.0	6103.0	1696.0	4074.00±2388.01
Fe	323953.0	339996.0	1005397.0	298250.0	818500.0	557219.20±330830.43
Co	188.0	186.0	402.0	139.0	114.0	205.80±114.12
Ni	182.0	207.0	63.0	290.0	47.0	157.80±102.16
Cu	2735.0	5729.0	2598.0	1700.0	3983.0	3349.00±1559.37
Zn	1340.0	2786.0	464.0	2172.0	617.0	1475.80±997.63
As	82.0	110.0	158.0	127.0	212.0	137.80±49.79
Mo	0.37	2.10	3.88	0.42	7.10	2.7740±2.82
Cd	7.03	6.32	5.34	4.71	2.83	5.2460±1.62
Pb	97.56	775.90	174.60	163.13	1046.50	451.5380±431.38
Ba	232.0	943.0	113.0	439.0	357.0	416.80±319.14
V	1147.0	1202.0	573.0	1182.0	1233.0	1067.40±278.14
Ti	11566.0	12073.0	4975.0	13020.0	7775.0	9881.80±3391.22

Table 7.4: Heavy Metals Concentration in Soil Sample in Comparison to the Maximum Permissible Limit in Soil.

Heavy Metals	**Mean ± Standard Deviation**	**Target Value**
Chromium	748.80±150.23	100mg/Kg (WHO 1996)
Manganese	4074.00±2388.01	500mg/Kg (WHO 1996)
Iron	557219.20±330830.43	400 (FEPA-Mohammed *et al.*, 2015)
Cobalt	205.80±114.12	70-80mg/Kg (FEPA-Mohammed *et al.*, 2015).
Nickel	157.80±102.16	38mg/Kg (WHO 1996)
Copper	3349.00±1559.37	36mg/Kg (WHO 1996)
Zinc	1475.80±997.63	50mg/kg (WHO 1996)
Arsenic	137.80±49.79	0.5mg/Kg (WHO limit, 2004)
Molybdenum	2.7740±2.82	Not found
Cadmium	5.2460±1.62	0.8mg/Kg (WHO, 1996)
Lead	451.5380±431.38	85mg/Kg (WHO 1996)
Barium	416.80±319.14	Not found
Vanadium	1067.40±278.14	Not found
Titanium	9881.80±3391.22	Not found

Table 7.4 shows that the mean values of the various heavy metals concentration in soil samples increased more than World Health Organization (WHO 1996) set standards.

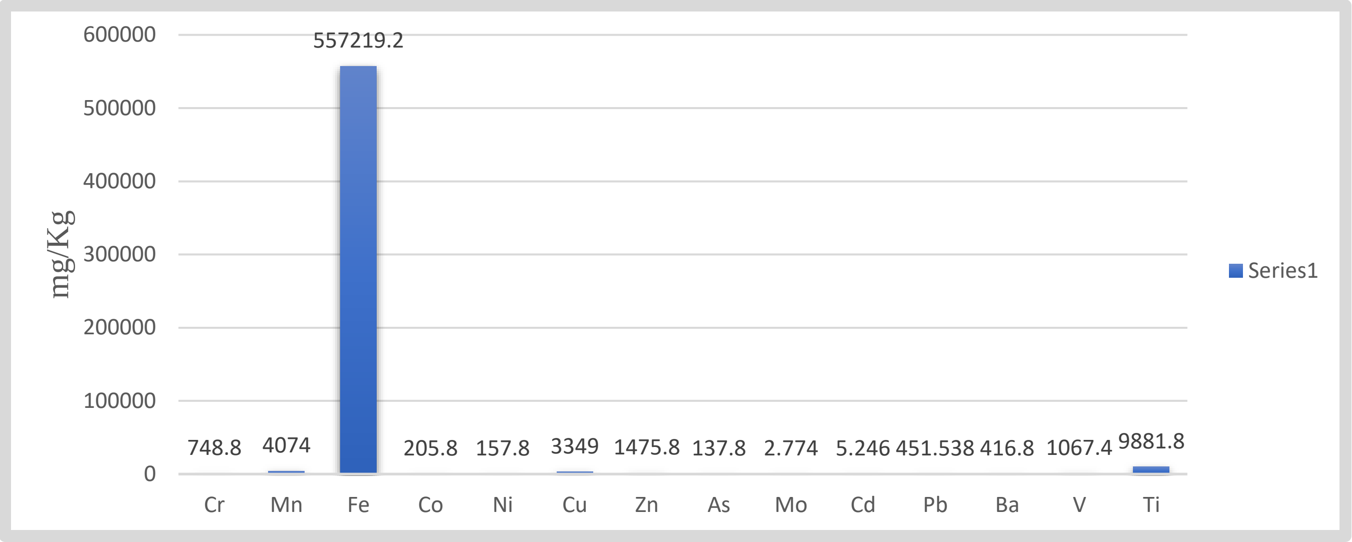

Figure 7.2: Heavy Metals Concentration in Soil Samples

Table 7.5: Heavy metals concentration in fish measured by ICP-MS.

Fish Spp	**METALS (mg/Kg)**										
	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Pb
F1	24.50	3.85	12.34	2.43	18.59	0.16	0.07	6.29	316.50	103.18	0.07
F2	25.67	4.24	12.90	3.04	24.18	0.13	0.11	8.72	291.18	95.04	0.15
F3	26.37	3.99	13.60	2.69	22.43	0.14	0.08	8.56	327.63	10.11	0.13
F4	27.88	3.68	14.20	2.29	20.75	0.19	0.07	8.88	329.81	54.07	0.15
F5	24.72	4.17	13.52	2.57	22.63	0.17	0.08	8.27	367.01	54.07	0.17
F6	23.19	3.63	12.56	2.41	35.08	0.17	0.05	9.85	312.00	171.61	0.15
F7	20.89	3.52	14.59	2.09	19.94	0.14	0.07	8.93	314.02	78.32	0.10
F8	28.24	4.41	14.99	2.17	27.32	0.19	0.04	9.76	270.46	88.64	0.08
F9	21.93	3.37	12.75	2.56	21.89	0.14	0.06	9.72	387.01	137.33	0.11
F10	28.61	4.60	17.69	2.11	28.17	0.20	0.06	8.20	330.27	64.51	0.10
Mean ± SD	25.20± 2.66	3.95± 0.40	13.91± 1.60	2.44± 0.29	24.10± 4.90	0.16± 0.02	0.07± 0.02	8.72± 1.05	324.59± 33.58	85.69± 45.54	0.12± 0.03

Table 7.6: Heavy metals concentration in Gills measured by ICP-MS.

Gill	**METALS (mg/Kg)**										
	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Pb
Gill 1	561.90	19.15	50.14	101.04	1188.77	2.98	6.48	8.39	406.02	109.21	2.40
Gill 2	579.93	24.09	67.51	102.91	1246.99	3.13	7.43	9.17	418.04	115.40	2.47
Mean± SD	570.92 ±12.75	21.62 ±3.49	58.83 ±12.28	101.98 ±1.32	1217.88 ±41.17	3.06 ±0.11	6.96 ±0.67	8.78 ±0.55	412.03 ±8.50	112.31 ±4.38	2.44 ±0.05

Table 7.7: Heavy Metals Concentration in Fish Samples and Gill in comparison to Allowable Maximum Permissible Limit in Fish.

Heavy Metals (mg/Kg)	**Mean ± Standard Deviation**		**Maximum Permissible Limit**
	Fish	**Gill**	
Titanium	25.20±2.66	570.92±12.75	Not found
Vanadium	3.95±0.40	21.62±3.49	Not found
Chromium	13.91±1.60	58.83±12.28	0.15mg/Kg (WHO 1985)
Manganese	2.44±0.29	101.98±1.32	0.50mg/Kg (WHO 1985)
Iron	24.10±4.90	1217.88±41.17	0.30-10mg/Kg (WHO 1985)
Cobalt	0.16±0.02	3.06±0.11	Not found
Nickel	0.07±0.02	6.96±0.67	0.60mg/Kg (WHO 1985)
Copper	8.72±1.05	8.78±0.55	3.00mg/Kg (WHO 1985)
Zinc	324.59±33.58	412.03±8.50	10-75mg/Kg (WHO 1985)
Arsenic	85.69±45.54	112.31±4.38	0.01mg/Kg (WHO 1985)
Lead	0.12±0.03	2.44±0.05	2.00mg/Kg (WHO 1985)

Values of heavy metal concentrations in Table 7.7 are represented in Mean ±SD. The heavy metals concentration in fish and gill samples shows that in fish sample Chromium, Manganese, iron, Copper, Zinc, and Arsenic were above the standard limit for WHO 1985, whereas in gill Chromium, Manganese, iron, Nickel, Copper, Zinc, Arsenic and Lead exceeded the permissible limits.

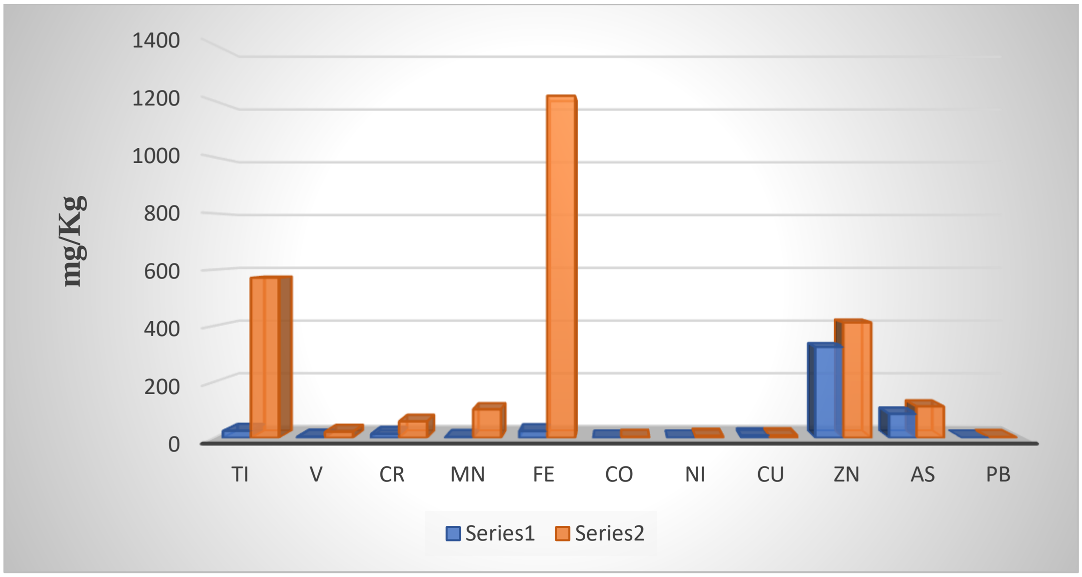

Figure 7.3: Heavy metals concentration in fish and gill measured by ICP-MS.

Series 1: *Fish samples.* ***Series 2:*** *Gill.*

The concentration of heavy metals such as iron, titanium, zinc, arsenic, manganese, chromium, and Lead are shown to have higher level compared to fish samples.

8. DISCUSSION

Heavy metals are a known cause of long-term health effects in humans. Such effects include not only nefarious effects of acute and/or chronic toxicity, but also special cases of toxicity such as carcinogenicity and genotoxicity. To the authors' knowledge, there exist 3 previous studies that investigated the presence of heavy metals in Gemikonagi region of North Cyprus (Altinbas *et al.,* 2002, Baycu *et al.,* 2015, Kontas *et al.,* 2015). Sampling was conducted in late November. This period was selected specifically because it's the winter period as it begins to rain. A heavy down pour of rain carries metal residues along in surface runoffs into the seashore located just close to the copper mine. When viewed from space during this period, a cerulean blue coloration is seen around the sea shore indicating that the sea water is heavy polluted. The soil on the other hand get saturated with residues of heavy metals due to surface runoffs into low lands and farm areas.

8.1: Seawater Samples

Seawater samples were analyzed with ICP-MS to estimate the concentration of heavy metals. A total of 10 heavy metals concentration were detected in seawater (titanium, vanadium, Chromium, manganese, iron, nickel, copper, zinc, molybdenum and barium). Heavy metals concentration decreased in a sequential order from titanium> iron > chromium > vanadium > manganese > barium > zinc > copper > nickel > molybdenum. For seawater sample, titanium recorded the highest concentration with its highest concentration in site 1 (6.848mg/L) and the lowest concentration was recorded as barium in site 2 (0.06176mg/L). Titanium had the highest mean value of (4.73±2.66mg/L) and molybdenum had the lowest mean value of (0.06±0.034mg/L). The World Health Organization (WHO), United States Environmental Protection Agency (USEPA) and European Union (EU) did not propose any limits for titanium and molybdenum, hence titanium and molybdenum could not be included in checking the seawater suitability. The mean concentration for manganese (0.16±0.09mg/Kg) exceeded the maximum permissible limit in water which is in good agreement with the investigation done by Alkas *et al.,*2017 on heavy metal concentration in Gonyeli lake, Cyprus. The accumulation of manganese in Cyprus soil could be attributed to the presence of manganese bearing minerals, such as umber and iron (Alkas *et al.,* 2017). Manganese toxicity can result in a permanent neurological disorder known as manganism with symptoms that include tremors, difficulty walking and facial muscle spasms. Exposure of manganese can result in reproductive effects such as increased sexual dysfunction and reduced sperm quality in men. Comparing the heavy metals concentration recorded at the different sampling locations with the standard maximum permissible limits suggested by WHO (2011), chromium and iron exceeded the permissible limit and could be hazardous to marine organisms. Ingestion of hexavalent chromium can result in human cancer. Iron toxicity results in neurodegenerative diseases such as Alzheimer's disease (disease of aging), arteriosclerosis, diabetes mellitus. Iron catalyzes the generation of toxic reactive oxygen species that can cause damage to most biological molecules, including DNA, protein and lipids, resulting in damage to membranes and various cellular organelles. In correlation to the high concentration of iron detected in this study, Ozturk *et al.,* 2009 carried out a research on the Avsar dam lake in Turkey, determining the concentration of six heavy metals in water. Heavy metals concentrations were decreased in the sequence of iron > copper > lead > chromium > nickel > cadmium (Ozturk *et al.,* 2009). In contrast to the low concentration of copper and zinc obtained in this study, the Eastern China sea estuary by Yang *et al.,* 2016 found copper and zinc to be much higher. According to Konijnendijk, 2015, the concentration of iron and titanium in Mediterranean Sea could be related to the fact that storm tracks over the Mediterranean, carrying Saharan dust from North Africa and sediments from the Nile river of Egypt are enriched in a few elements including titanium and iron. In contrast to the high concentration of titanium determined in this study, Sharma 2009, in his study proposed that the predicted environmental concentration (PEC) values for nano-TiO2 in water, ranged from 0.7-16µg/L which nearly equal to or higher than the predicted no effect concentration (PNEC) of <1µg/L. The values of titanium concentration obtained from this study (4.73±2.66mg/L) exceeded the predicted no effect concentration (PNEC) of <1µg/L, which indicates a possible ecotoxicity of titanium in water. Sharma in 2009 conducted a study using Daphnia magma as a test organism to determine the toxicity of nano-TiO2 on aquatic invertebrates. Results showed that low exposure levels of TiO2 caused low toxicity, however the high exposure levels caused some indications of chronic toxicity and behavioral changes.

8.2: Soil Samples

A total of 14 metals (chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, molybdenum, cadmium, Lead, barium, vanadium, and titanium) were determined in the soil samples using ICP-MS. Heavy metals concentration were decreased in the sequence of iron > titanium > manganese > copper > zinc > vanadium > chromium > lead > barium > cobalt > nickel > arsenic > cadmium > molybdenum. Iron had the highest concentration of 1005397.0mg/Kg in sampling location 3. Heavy metals concentrations obtained in the soil samples were compared with sediment quality guidelines, which showed that chromium, iron, nickel, copper, zinc, arsenic, cadmium, and lead did not exceeded the probable effect concentration (PEC) level (NOOA, 2009). Iron concentration was compared with sediment quality guidelines, which showed that iron exceeded the lowest effect level (LEL) concentration (NOOA, 2009). The mean concentration for chromium in soil was between the ranges of 748.80±150.23mg/Kg (Table 7.4), the target value recommended by WHO standard for chromium in soil is 100mg/Kg. The high value of chromium in the soil was much higher in location 4 with 946.0mg/Kg (Table 7.3). The higher concentration of chromium in the soil may be because of human activities and natural processes. In this study, the mean concentration for Zinc in soil was between the ranges of 1475.80±997.63mg/Kg (Table 7.4), the target value is 50mg/kg (Table 7.4). the mean concentration of zinc exceeded the target value for zinc in the soil. The concentration of Zinc from the soil locations varied widely between 464.0 to 2786.0mg/Kg (Table 7.3). All the concentrations exhibited very high concentration that exceeded the target values for zinc in the soil. Zinc is used for anti-corrosion coating for steel, brass and other metals. The concentration may be because of the availability of a mining facility in this region. Copper concentration in soil was between the ranges of 1700.0-5729.0mg/Kg (Table 7.3). The target values recommended by WHO is 36mg/Kg (Table 7.4). The high value of copper in the soil was much higher in location 2 with 5729.0mg/Kg (Table 7.3). Copper is known to be a micro-element that is naturally occurring in the soil. The high concentrations recorded in all the sampling locations may be because of the presence of copper mines in Gemikonagi region. Copper ore is the major element mined in Cyprus. The concentrations of iron, Chromium, Manganese, Cobalt, Nickel, Copper, Zinc, Arsenic, Cadmium and Lead exceeded the standard target values set by WHO (Table 7.4). A study was conducted by Baycu *et al.*, 2015 in Cyprus showed that Copper and Cadmium were above permissible limit in the soil and were in contaminated or heavy contaminated range. Similarly, Alkas *et al.*, 2017 conducted a study in Gonyeli lake, Cyprus which showed high concentration of Copper with concentration range of 613ppm. Kontas *et al.*, 2015 conducted a study in Cyprus and determined the concentration range of iron and nickel to be 10634-50972mg/Kg and 44-245mg/Kg respectively, these agrees to the concentration range of iron and nickel obtained in this study. In correlation to the high concentration of iron, copper, manganese, nickel and arsenic, Duman *et al.*, 2012 carried out a study on shelf sediment concentration in Northern Cyprus, which showed that iron, copper, manganese, nickel and arsenic with concentrations range of 1800-84400mg/Kg, 1.6-74mg.Kg, 141-1241mg/Kg, 6.6-114mg/Kg and 3.3-55.4mg/Kg respectively were above the USEPA permissible limit. Heavy metals are generally carcinogenic. All heavy metals analysed in soil samples exceeded the target values set by WHO 1996 and are very hazardous. One major occupation mostly practiced by the local Cypriot is Agriculture, as the entire country rely mostly on the food crops cultivated on the island.

8.3: Fish Samples

A total number of 11 heavy metals concentration were obtained from the fish species *Boops boops* (Titanium, Vanadium, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Arsenic and Lead). Tuzen in 2003 monitored the concentration of heavy metals in fish samples collected from black sea (Turkey) which showed that concentrations of Lead, iron, Copper, Manganese and Zinc) were determined in the region. The result obtained from this study indicated that in fish sample, the mean concentrations of chromium, manganese, iron, copper, zinc and arsenic with levels of 13.91±1.60, 2.44±0.29, 24.10±4.90, 8.72±1.05, 324.59±33.58, 85.69±45.54mg/Kg respectively (Table 7.7) were above the WHO established limits whereas in gill, the mean concentration of chromium, manganese, iron, nickel, copper, zinc, arsenic and lead with levels of 58.83±12.28, 101.98±1.32, 1217.88±41.17, 6.96±0.67, 8.78±0.55, 412.03±8.50, 112.31±4.38 and 2.44±0.05mg/Kg exceeded the WHO permissible limit of 0.15mg/Kg, 0.50mg/Kg, 0.30-10mg/Kg, 0.60mg/Kg, 3.00mg/Kg, 10-75mg/Kg, 0.01mg/Kg and 2.00mg/Kg respectively (Table 7.7). Lead induces cell death, oxidative stress, transcriptional activation of stress genes and DNA damage. Studies shows that excessive exposure and absorption of copper can results in liver and kidney damage. However, the Lead (0.12±0.03mg/Kg) and nickel (0.07±0.02mg/Kg) concentrations in the fish sample did not exceeded the WHO permissible limit. In relation to the limit levels used in this study and those measured in Kalyoncu *et al.*, 2012, the levels of Lead, Cadmium, manganese and Chromium exceeded the standard established limit, whereas nickel was below its permissible limit in Işıklı Dam Lake. Similarly, in Karacaören Dam Lake, the levels of lead, cadmium, manganese, copper and chromium were above the standard permissible limits, whereas only nickel remained below the acceptable limit (Kalyoncu et al., 2012). Studies conducted by Alkas *et al.,* 2017 on the assessment of heavy metals concentration in Gonyeli lake, Cyprus concluded that the levels of manganese, copper, arsenic, cadmium and lead in fish samples exceeded the intentionally established limits in living organisms. The levels of metals in fish samples caught from the Mediterranean seawaters in Gemikonagi region of Cyprus can be due to heavy metal pollution resulting from the abandoned copper mines factory. Karadede *et al*., 2000 studied the concentration of some heavy metals in water, sediment and fish species from the Ataturk dam lake (Euphrates), Turkey, which showed that the concentrations of copper, iron, manganese, and zinc were detected in different tissues of fish samples, while cadmium, cobalt, mercury, nickel, and lead were undetected. The highest concentrations of heavy metals, obtained in the gill may be attributed to the fact that gill epithelia when compared with other integuments of fish has a very large surface area and actively interacts with the external environment. So, gills virtually have no protection from the toxicity of metals in fresh and sea waters generally. Studies revealed that high concentration of various metals can be observed in gills due to element complexing with the mucus, which is completely impossible to remove from between the lamella, before the fish tissue is analyzed (Heath, 1987). Studies by Tchounwou *et al.*, 2012 showed that hexavalent chromium is cytotoxic and able to induce DNA damaging effects such as chromosomal abnormalities, DNA strand breaks, DNA fragmentation and human liver carcinoma cells. Hexavalent chromium also induces biochemical, genotoxic and histopathologic effects in liver and kidney of fish (Tchounwou et al., 2012).

9. CONCLUSIONS

Heavy metal toxicity is a major problem of concern in Gemikonagi region of Northern Cyprus. The aim of this study was to determine the various concentrations and levels of heavy metals in Gemikonagi region of Northern Cyprus using the ICP-MS technique. The matrices used for this study are seawater, soil and fish. The analytical results revealed high levels of various heavy metals concentration (titanium, chromium, manganese, iron, copper, zinc, nickel, arsenic, cadmium and lead) in seawater, soil and fish samples. Potential toxicity from these heavy metals concentration ranges from carcinogenicity, oxidative stress, DNA damage, liver and kidney damage, and neurological disorder known as manganism with symptoms that include tremors, difficulty walking and facial. Based on the results obtained, this region is contaminated with varying levels of titanium, chromium, manganese, iron, copper, zinc, nickel, arsenic, cadmium and lead which shows that people are subject to significant potential health risk from possible exposure and absorption of metal availability in the soil, seawater and fish. Plant absorb metals from the soil whereas fish accumulated metals in the body system after consumption. Human are subject to metal toxicity after consumption of contaminated plant material and fish.

In summary measures should be taken into consideration to reduce or control these levels in the environment. General environmental decontamination processes should be carried out by the governmental authorities, specifically on the copper mines sites as it is one of the major cause for the spread and accumulation of heavy metals in the region in other to mitigate the imminent health risks.

10. REFERENCES

A. Mihaly-Cozmuta, L. Mihaly Cozmuta, V. Viman, GH. Vatca and C. Varga. (2005). Spectrometric Methods Used to Determine Heavy Metals and Total Cyanides in Accidental Polluted Soils. American Journal of Applied Sciences 2 (1): 358-362.

Abadin H, Ashizawa A and Stevens YW. (2007). Agency for toxic substances and disease registry (ATSDR) toxicological profiles. Toxicological profile for Lead. Atlanta (GA): U.S. Department of Health and Human services.

ABM Helaluddin, Reem Saadi Khalid, Mohamed Alaama and Syed Atif Abbas. (2016). Main Analytical Techniques Used for Elemental Analysis in Various Matrices. Tropical Journal of Pharmaceutical Research; 15 (2): 427-434.

Abshire MK, Devor DE, Diwan BA, Shaughnessy JD Jr and Waalkes MP. (1996). *In vitro* exposure to cadmium in rat L6 myoblasts can result in both enhancement and suppression of malignant progression in vivo. Carcinogenesis. 17:1349–1356.

Ademola O. Olaniran, Adhika Balgobind and Balakrishna Pillay. (2013). Bioavailability of heavy metals in soil. Impact on microbial Biodegradation of organic compounds and possible improvement strategies. Int. J. Mol. Sci; 14, 10197-10228.

Adrian A. Ammann. (2002). Speciation of heavy metals in environmental water by ion chromatography coupled to ICP–MS. Anal Bioanal Chem; 372: 448–452. DOI 10.1007/s00216-001-1115-8.

Agency for Toxic Substances and Disease Registry (ATSDR). (1999). Public Health Service. Atlanta: U.S. Department of Health and Human Services. Toxicological Profile for Lead.

Agency for toxic substances and diseases registry (ATSDR) toxicological profiles. (1999). Toxicological profile for mercury. Atlanta (GA): U.S. Department of Health and Human Services and Public Health Services.

Agency for toxic substances and diseases registry (ATSDR). (2011). The priority list of hazardous substances. U.S. Department of Health and Human Services and Public Health Services.

Alonso Castillo ML, Sanchez Trujillo I, Vereda Alonso E, Garcia de Torress and Cano Pavon JM. (2013). Bioavailability of heavy metals in water and sediments from a typical Mediterranean Bay (Málaga Bay, Region of Andalucía, Southern Spain). Mar Pollut Bull: 76 (1-2): 427-34.

Amuno S, Niyogi, S, Amuno, M. and Attitaq J. (2016). Heavy metal bioaccumulation and histopathological alterations in wild Arctic hares *(Lepus arcticus*) inhabiting a former lead-zinc mine in the Canadian high Arctic: a preliminary study. Sci. Total Environ. 556, 252–263.

As per Canadian or B. C. Health Act Safe Drinking Water Regulation BC Reg 230/92 and 120. 2001. Task Force of Canadian Council of Resources and Environ, Minister's Guideline for Canadian Drinking Water Quality. 1996. Amend. Health Canada (2006).

Aysel T. Atimtay and E. Volkan SARICICEK. (2000). Environmental effects of copper mining activities in Lefke, Gemikonagi region of Northern Cyprus. A review. Lefke Avrupa Universitesi/Subat.

Berlin M, Zalups RK and Fowler BA, Nordberg GF, Fowler BA, Nordberg M and Friberg LT. (2007). Handbook on the toxicology of metals. 3rd ed. Elsevier. P. 675-729.

Bernard A. (2008). Cadmium & its adverse effects on human health. *Indian J Med Res* **128** (4): 557–64.

C. Radalescu, I.D. Dulama, C. Stihi, I. Ionita, A. Chilain, C. Necula and Elena Daniela Chelarescu. (2014). Determination of heavy metal levels in water and therapeutic mud by atomic absorption spectrometry. Rom. Journ. Phys., Vol. 59, Nos. 9-10, P.1057-1066.

C. Voica, A. Dehelean, A. Iordache and I. Geana. (2012). Method validation for determination of metals in soils by ICP-MS. Romanian Reports in Physics, Vol. 64, No. 1, P. 221–231.

Castagnetto JM, Hennessy SW, Roberts VA, Getzoff ED, Tainer JA and Pique ME. (2002). MDB: the metalloprotein database and browser at the Scripps Research Institute. *Nucleic Acids Res* 30 (1): 379–382.

Chakraborty S, Dutta AR, Sural S, Gupta D and Sen S. (2013). Ailing bones and failing kidneys: A case of chronic cadmium toxicity. *Ann Clin Biochem* **50** (5): 492–495.

Chang Zhang, Zhi-gang Yu, Guang-Ming Zeng, Min Jiang, Zhong-Zhu Yang, Fang Cui, Meng-Ying Zhu, Liu-qing Shen, Liang Hu. (2014). Effects of sediment geochemical properties on heavy metal bioavailability. Environment international 73: 270-281.

Chen CW, Chen CF and Dong CD. (2013). Distribution and accumulation of mercury in sediments of kaoshiung River Mouth, Taiwan. APCBEE *Procedia* 1: 153-158.

Chiroma T. M, Ebwele R. O and Hymore F. K. (2012). Levels of heavy metals (Cu, Zn, Pb, Fe, and Cr) in Bushgreen and Roselle irrigated with treated and untreated urban sewage water. Int. Res. J. Environment sci 1, 50-55.

Christoph Moor, Theopisti Lymberopoulou, and Volker J. Dietrich. (2001). Determination of Heavy Metals in Soils, Sediments and Geological Materials by ICP-AES and ICP-MS. Mikrochim. Acta 136, 123-128.

Daniel Rosado, Jose Usero and Jose Morillo. (2016). Assessment of heavy metal bioavailability and toxicity towards *Vibrio fischeri* in sediment of the Huelva estuary. Chemosphere 153; 10-17.

David A. Olson and Tarakad S. Ramachandran. (2017). Mercury Toxicity.

de Jong ME, Scheiber IBR, Van den Brink NW, Braun A, Matson KD, Komdeur J and Loonen MJ. (2017). Indices of stress and immune function in Arctic barnacle goslings (Branta leucopsis) were impacted by social isolation but not a contaminated grazing environment. Sci. Total Environ. 601, 132–141.

Desaules A. (2012). Critical evaluation of soil contamination assessment methods for trace metals. The Science of the Total Environment, 426, 120–131.

E.I. Geana, A. M. Iordache, C. Voica, M. Culea and R. E. Ionete (2011). Comparison of Three Digestion Methods for Heavy Metals Determination in Soils and Sediments Materials by ICP-MS Technique. Asian Journal of Chemistry; Vol. 23, No. 12, 5213-5216.

Eisa SOLGI and Javad PARMAH. (2015). Analysis and assessment of nickel and chromium pollution in soils around Baghejar Chromite Mine of Sabzevar Ophiolite Belt, North-Eastern Iran. Vol.25, Issue 7, Pages 2380-2387.

El Bassam N. and Tietjen C. (1977). Soil Organic Matter Studies. Vienna: IAEA. Municipal sludge as organic fertilizer with special reference to the heavy metals constituents.

Erez ben-Yosef, Ron Shaar, Lisa Tauxe, Thomas E. Leavy and Vasiliki Kassianidou. (2017). The Cyprus Archaeomagnetic project (CAMP): targeting the slag deposits of Cyprus and the Eastern Mediterranean. Antiquity. A review of world archaeology. Issue 360.

FAO/WHO, 2001. Codex alimaentary commission. Food additives and contaminants. Joint FAO/WHO food standards programme, ALINORM 01/12A, pp: 1-289.

Ferrara R, Mazzolai B, Lanzillotta E, Nucaro EA and Pirrone N. (2000). Temporal trends in gaseous mercury evasion from the Mediterranean seawaters. Sci Tot Environ 259(1–3): 183–190

Flora SJS, Mittal M and Mehta A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J Med Res* 128: 501–523.

Flora SJS, Saxena G, Gautam P, Kaur P and Gill KD. (2007). Lead induced oxidative stress and alterations in biogenic amines in different rat brain regions and their response to combined administration of DMSA and MiADMSA. Chem Biol Interac. 170:209–220.

G. Baycu, D. Tolunay, H. Ozden, I. Csatari, S. Karadag, T. Agba & S. E. Rognes. (2015). An Abandoned Copper Mining Site in Cyprus and Assessment of Metal Concentrations in Plants and Soil, International Journal of Phytoremediation, 17:7, 622-631, DOI: 10.1080/15226514.2014.922929.

Geoffrey M. Gadd and Alan J. Griffiths. (1978). Microorganisms and heavy metal toxicity. Microbial toxicity 4:303-317.

Ghani A. (2011). Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of *Brassica juncea.* L. *Egyptian Acad J Biol Sci* **2**(1): 9–15.

Grant J. Batterham, Niels C. Munksgaard and David l. Parry. (1999). Determination of Trace Metals in Seawater by Inductively Coupled Plasma Mass Spectrometry After Off-line Dithiocarbamate Solvent Extraction. Journal of Analytical Atomic Spectrometry, Vol. 12 (1277–1280).

Heath A. G. (1987). Water pollution and fish physiology, CRP press, Florida. Pp. 245.

Héctor Hernández-Mendoza1, Marcos Mejuto, Ana Isabel Cardona, Avelino García-Álvarez, Rocio Millán and Abel Yllera. (2013). Optimization and Validation of a Method for Heavy Metals Quantification in Soil Samples by Inductively Coupled Plasma Sector Field Mass Spectrometry (ICP-SFMS). American Journal of Analytical Chemistry 4, 9-15.

http://www.who.int/water sanitation health/dwq/chemicals/cadmium. Pdf.

Hulya Karadede, Erhan Unlu. (2000). Concentrations of some heavy metals in water, sediment and fish species from the Ataturk dam lake (Euphrates), Turkey. Chemsphere. Vol 41, Issue 9, pp. 1371-1376.

Hwua Y and Yang J. (1998). Effect of 3-aminotriazole on anchorage independence and mutagenicity in cadmium- and lead-treated diploid human fibroblasts. Carcinogenesis. 19:881–888.

J. Retka1, A. Maksymowicz and D. Karmasz. Determination of Cu, Ni, Zn, Pb, Cd by ICP-MS and Hg by AAS in Plant Samples.

Jamieson H. E. (2014). The legacy of arsenic contamination from mining and processing refractory gold ore at Giant Mine, Yellowknife, Northwest Territories, Canada. Rev. Mineral. Geochem: 79 (1): 533–551.

Jehad Abdullah Shaban. (2017). Determination of heavy metals in environmental samples by inductively coupled plasma-mass spectrometry (ICP-MS).

Jingxi Li, Li Zheng, Xiaofei Yin, Junhui Chen, Bin Han and Jiangtao Wang. (2015). Determination of Heavy Metals and other Water Quality Parameters from the South China Sea. Analytical Letters, 48: 204–212.

Julieta Griboff, Daniel A. Wanderlin and Magdalena V. Monferran. (2017). Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from 3 eutrophic reservoirs. Their consumption represents a risk for human health. Microchemical Journal 130: 236-244.

Kalyoncu, L., Kalyoncu, H., & Arslan, G. (2012). Determination of heavy metals and metals levels in five fish species from Işıklı Dam Lake and Karacaören Dam Lake (Turkey). Environmental Monitoting Assessment, 184, 2231

Kevin M. Rice, Ernest M. Walker Jr, Miaozong Wu, Chris Gillette and Eric R. Blough. Environmental Mercury and Its Toxic Effects. J Prev Med Public Health; 47:74-83.

Krishna M. Satyanarayanan and Pradip K. Govil. (2008). Assessment of heavy metal pollution in water using multivariate statistical techniques in an industrial area: A case study from Patancheru, Medak District, Andhra Pradesh, India. doi: 10.1016/j.jhazmat.12.131.

Kucuksezgin, F., Altay, O., Uluturhan, E., Kontas, A., (2001). Trace metal and organochlorine residue levels in Red Mullet (Mullus barbatus) from the Eastern Aegean, Turkey. Water Res., 35: 2327–2332.

M. Bettinellia, G.M. Beoneb, S. Speziaa, C. Baffib. (2000). Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis. Analytica Chimica Acta 424, page 289–296.

M. Öztürk, G. Özözen, O. Minareci and E. Minareci. (2009). Determination of Heavy Metals in Fish, Water and Sediments of Avsar Dam Lake in Turkey. Iran. J. Environ. Health. Sci. Eng., Vol. 6, No. 2, pp. 73-80.

M. Öztürk, G. Özözen, O. Minareci, Iran. J. (2009). DETERMINATION OF HEAVY METALS IN FISH, WATER AND SEDIMENTS OF AVSAR DAM LAKE IN TURKEY Environ. Health. Sci. Eng., Vol. 6, No. 2, pp. 73-80.

Machado, L. M., Bebianno, M. J., Boski, T., Moura, D. M., (1999). Trace metals on the Algarve Coast, II: Bioaccumulation in mussels Mytilus galloprovincialis (Lamarck, 1819). Bol. Inst. Esp. Oceanogr., 15: 465– 471.

Maria C. Hernamdez-Soriano and Jose C. Jimenez-Lopez. (2012). Effects of soil water content and organic matter addition on the speciation and bioavailability of heavy metals. Science of the total environment. 423; 55-61.

Matsumoto ST, Mantovani MS, Malaguttii MIA, Dias AL, Fonseca IC and Marin Morales MA. (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and Chromosome aberrations in onion root-tips. *Mol Biol* 29 (1): 148-158.

McGrath S, Chang A, Page A and Witter E. (1994). Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. Environ Rev. 2:108–118. doi: 10.1139/a94-006.

Mercury in canned tuna still in concern: New tests reinforce a need for some people to limit consumption. (2011). Consum Rep; 76 (1): 20-1.

Mohammed S. A and Folorunsho J. O. (2015). Heavy metals concentration in soil and *Amaranthus retroflexus* grown on irrigated farmlands in the Makera Area, Kaduna, Nigeria. Journal of geography and regional planning. Vol. 8 (8), pp. 210-217.

Monisha Jaishankar, Blessy Baby Mathew, Moshami Sailesh Shah, Krishna Murthy TP and Sangeetha Gowda KR. (2014). Biosorption of Few Heavy Metal Ions Using Agricultural Wastes. Journal of Environment Pollution and Human Health,Vol. 2, No. 1, 1-6.

Monisha Jaishankar, Tenzin Tsetsen, Naresh Anbalagan, Blessing B. Matthew, Krishna-Murthy N. Beeregowda. (2014). Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology; 7(2): 60-72.

Morgana Frena, Daiane P. C. Quadros, Ivan N. B. Castilho, Jefferson S. de Gois, Daniel L. G. Borges, Bernhard Welz and Luiz A. S. Madureira. (2014). A novel extraction-based procedure for the determination of trace elements in estuarine sediment samples by ICP-MS. Doi.org/10.1016/j.micro.05.014.

Mousavi S. M., Bahmanyar M. A and Pirdashti H. (2013). Phyto availability of some micronutrients (Zn and Cu), heavy metals (Pb, Cd), and yield of rice affected by sewage sludge perennial application. Communications in Soil Science and Plant Analysis, 44, 3246–3258.

Mudgal V, Madaan N, Mudgal A, Singh RB and Mishra S. (2010). Effect of toxic metals on human health. *Open Nutraceuticals J 3*: 94-99.

Mustafa Tuzen. (2003). Determination of heavy metals in fish samples of middle black sea (Turkey) by graphite furnace atomic absorption spectrometry. Food chemistry. Vol 80, Issue 1, pp. 119-123.

Natalia V. Solenkova, Jonathan D. Newman, Jefferey S. Berger, George Thurston, Judith S. Hochman and Gervasio A. Lamas. (2014). Metal pollutants and cardiovascular disease: Mechanisms and consequences of exposure. American Heart Journal; 168: 812-822.

NOAA (National Oceanic and Atmospheric Administration), (2009). SQUIRT, Screening Quick Reference Tables for in Sediment, http://response.restoration.noaa.gov/book_shelf/122_NEW-SQuiRTs.pdf (online update: 23.03.2009).

Nobi EP, Dilipan E, Thangaradjou T, Sivakumar K and Kannan L. (2010). Geochemical and geostatistical assessment of heavy metal concentration in the sediments of different coastal ecosystems of Andaman Islands, India. Estuarine Coast Shelf Sci; 87: 253–64.

O'Brien T, Xu J and Patierno SR (2001). Effects of glutathione on chromium-induced DNA crosslinking and DNA polymerase arrest. In Molecular Mechanisms of Metal Toxicity and Carcinogenesis (pp. 173-182).

Occupational safety and health standards. Mercury. (2007). U.S. Department of Labor, Occupational Safety and Health Administration (OSHA).

P. Raja, S. Veerasingam, G. Suresh, G. Marichamy and R. Venkatachalapathy. (2009). Heavy metals concentration in four commercially valuable marine edible fish species from Parangipettai coast, South East coast of India. International Journal of animal and veterinary advances 1(1): 10-14.

Patrick L. (2002). Mercury toxicity and antioxidants: Part 1: role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity. Altern Med Rev 7(6): 456–471.

Patrick L. (2003). Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. *Altern Med Rev* **8**(2): 106–128.

Paul B Tchounwou, Clement G Yedjou, Anita K Patlolla, and Dwayne J Sutton. (2012). Heavy Metals Toxicity and the Environment. EXS. 101: 133–164.

Rainbow PS. (2007). Trace metal bioaccumulation: Models, metabolic availability and toxicity.
Environ Int 2007; 33:576–82.

Raymond A. Wuana and Felix E. Okieimen. (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology. Article ID 402647.

Riba I, Delvalls TÁ, Forja JM and Gómez-Parra A. (2004). The influence of pH and salinity on the toxicity of heavy metals in sediment to the estuarine clam Ruditapes philippinarum. Environ Toxicol Chem. 23 (5): 1100-7.

Rog-Young Kim, Jang-Ki Yoon, Tae Seung Kim, Jae-E Yang, Gary Owens and Kwon-Rae Kim. (2015). Bioavailability of heavy metals in soils: definitions and practical implementation-a critical review. Eniron Geochem Health: 37:1041-1061.

Ronaldo A. M. Junior, Aparecida M. S. Mimura, Alex C. Divino, Roberta F. Silva, Julio C. J. Silva & Virginia S. T. Ciminelli (2014). Levels of Metallic Cations in the Surface Sediments in the Vicinity of the Três Marias Dam Lake (Brazil) Determined by ICP-MS and Microwave Sample Preparation, Soil and Sediment Contamination: An International Journal, 23:3, 257-269.

Rui M. de Carvalho Jr. & Jéssica A. dos Santos & Jessee A. S. Silva & Thiago G. do Prado & Adriel Ferreira da Fonseca & Eduardo S. Chaves & Vera L. A. Frescura (2015). Determination of metals in Brazilian soils by inductively coupled plasma mass spectrometry. Environ Monit Assess 187: 535 DOI 10.1007/s10661-015-4769-y.

S. M. Enamorado-Báez, J. M. Abril, and J. M. Gómez-Guzmán. (2013). Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples. ISRN Analytical Chemistry, Article ID 851713, page 14.

S. Meyer, J. Schulz, A. Jeibmann, M.S. Taleshi, F. Ebert, K.A. Francesconi and T. Schwerdtle. (2014). Arsenic containing hydrocarbons are toxic in the in-vivo model *Drosophila melanogaster*. Metallomics, 6.

SEPA, 2005. The Limits of Pollutants in Food. State Environmental Protection Administration, China.

Sharma P and Dubey RS. (2005). Lead toxicity in plants. Brazilian Journal of Plant Physiology 17 (1): 35-52.

Shengwen Shen, Xing Fang Li, William R. Chris, Michael Weinfield and X. Chris Le. (2013). Arsenic binding to proteins. Chem Rev. 113 (10): 7769-7792.

Simpson SL, Yverneau H, Cremazy A, Jarolimek CV, Price HL and Jolley DF. (2012). DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. Environ Sci Technol: 46:9038–46.

Simpson SL. (2005). Exposure effect model for calculating copper effect concentrations in sediments with varying copper binding properties: a synthesis. Environ Sci Technol: 39: 7089–96.

Singh N, Kumar D and Sahu A. (2007). Arsenic in the environment: effects on human health and possible prevention. *J Environ Biol* 28 (2 Suppl): 359–365.

Skerfving S, Bergdahl IA, Nordberg GF, Fowler BA and Friberg LT. (2007). Handbook on the toxicology of metals. 3rd ed. Elsevier; p. 599-643.

T. Raja Rajeswari and Namburu Sailaja. (2014). Impact of heavy metals on environmental pollution. Journal of chemical and pharmaceutical sciences.

T. Raja Rajeswari and Namburu Sailaja. (2014). Impact of heavy metals on environmental pollution. JCHPS special Issue 3:

T. V. Luong, Thailand and S. Guifan, W. Liying and S. Dianjun and P. R. China. (2004). PEOPLE-CENTRED APPROACHES TO WATER AND ENVIRONMENTAL SANITATION. Endemic Chronic Arsenic Poisoning – China. 30th WEDC International Conference, Vientiane, Lao PDR.

Tellez-Plaza M, Navas-Acien A and Crainiceanu CM. (2008). Cadmium exposure and hypertension in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). Environ Health Perspect; 116 (1): 51-6.

Tiuri Konijnendijk. (2015). 1.2 million years of climate change, globally and in the Mediterranean. Published by Faculty of Geosciences, Utrecht University, the Netherlands, in: Utrecht Studies in Earth Sciences (USES), ISSN 2211-4335.

Usero, J., Izquierdo, C., Morillo, J., Gracia, I., (2003). Heavy metals in fish (Solea vulgaris, Anguilla anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. Environ. Int., 29: 949–956.

V. Hanuman Reddy, P. M. N. Prasad, A. V. Ramana Reddy and Y. V. Rami Reddy (2012). Determination of heavy metals in surface and groundwater in and around Tirupati, Chittoor (Di), Andhra Pradesh, India. Der Pharma Chemica, 4 (6): 2442-2448.

Velma V, Tchounwou PB. Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, carassius auratus. Mutat Res. 2010;698(1–2):43–51. [PMC free article]

Vibor Roje. (2010). A fast method for multi-metal determination in soil samples by high-resolution inductively-coupled plasma-mass spectrometry (HR–ICP–MS). Chemical Speciation & Bioavailability, 22: 2, 135-139.

WHO (2004). Task group on environmental health criteria for arsenic and arsenic compounds 18, World Health Organization, Geneva, Switzerland 1-174.

Wolinska A, Stepniewska Z, Wlosek R. (2013). The influence of old Leather Tannery district on Chromium contamination of soils, water and plants. *Nat Sci* 5(2A): 253-258.

Ya Wang, Shu Wang, Pingping Xu, Cong Liu, Misha Liu, Yulan Wang, Changhai Wang, Chunhua Zhang and Ying Ge. (2015). Review of arsenic speciation, toxicity and metabolism in microalgae. Rev Environ Sci Bio Technol: 14: 427-451.

YANG Rujun, NING Yutong, ZHANG Aibin, LI Yan, and SU Han. (2016). Analysis of Cu, Co, V and Zn in Coastal Waters of the East China Sea by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ISSN 1672-5182, 15 (5): page: 825-834.

Zahran M. A, S. Laidlaw, S. P. McElmurry, G.M. Filippelli and M. Taylor (2013). Linking source and effect: resuspended soil Lead, air Lead and Children's blood Lead Levels in Derroit, Michigan. Environ. Sci. Technol., 47, pp. 2839-2845.

Zhitkovich A. (2005). Importance of Chromium-DNA adducts in mutagenicity and toxicity of chromium (VI). *Chem Res Toxicol* 18 (1): 3-11.