T.R.N.C.

NEAR EAST UNIVERSITY

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ASSESSMENT OF CHRONIC HEAVY METAL EXPOSURE ON THE POPULATION OF GEMİKONAĞI AND TEPEBAŞI (NORTH CYPRUS)

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Heavy metals are described as metal or metalloid elements with high atomic mass and density which is 5 or more times greater than that of water. As they are found in the earth's crust naturally, a group of heavy metal(loid)s are essential for life as they exert biochemical and physiological functions in both plants and animals.Heavy metals persist in the environment for long periods of time as they lack the ability to be destroyed or degraded. The aim of this study was to investigate the heavy metal exposure levels using soil, groundwater and human blood samples from the area of Gemikonagi, Lefke where the old mining area is located in immediate vicinity and compare these results with a control area, Tepebasi, Girne located 40 km away from the mining site and devoid of any mining activities. High technology inductively coupled plasma mass spectrometry is used to analyse the samples. Arsenic levels in blood is approximately 18 times higher than the maximum allowed level in Gemikonagi and 16 times higher in Tepebasi.Arsenic, chromium and copper levels in soil are found to be above the permissible levels. Mean copper level in the soil collected from Gemikonagi is approximately 66 times more than the Earth's average. In conclusion, heavy metals act as environmental pollutants and harmful agents to biological systems. Further studies should be done to evaluate chronic effects of heavy metals in human population.

Key words: *heavy metals, icp-ms, exposure.*

1. INTRODUCTION

Heavy metals are described as metal or metalloid elements with high atomic mass and density which is 5 or more times greater than that of water (Tchounwou et al., 2012).As they are found in the earth's crust naturally, a group of heavy metal(loid)s are essential for life as they exert biochemical and physiological functions in both plants and animals. They are components of key enzymes and perform important roles in some oxidation-reduction reactions. Essential elements can be listed as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). Insufficient supply of the essential elements results in different types of deficiency syndromes or diseases (WHO, 1996).In addition to essential elements, many of them are highly toxic and a small portion is considered as precious elements.Non-essential elements that have no biological functions can be listed as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U)(Tchounwou et al., 2012).

Heavy metals persist in the environment for long periods of time as they lack the ability to be destroyed or degraded. Although they are natural constituents of earth's crust, anthropogenic activities, such as smelting and mining, industry and agriculture, are the major cause of environmental contamination and human exposure. Persistent heavy metals are able to enter the body systems via contaminated food, air and water and have a tendency to bio-accumulate (Tchounwou et al., 2012; UNEP/GPA, 2004).

Some heavy metals such as zinc (Zn), copper (Cu), selenium (Se),chromium (Cr), cobalt (Co), iodine (I), manganese(Mn), and molybdenum (Mo)are also named as trace elements as they present in trace amounts whose concentrations are not larger than 100 parts per million (ppm) (Kabata-Pendias and Pendias, 2006). Physical factors like temperature, adsorption, sequestration and phase association have an influence on the trace element bioavailability. Additionally, thermodynamic

equilibrium, complexation kinetics, lipid solubility and octanol/water coefficients are the chemical factors that affect the bioavailability of trace elements (Hamelink, 1994). Biological factors also play a role in heavy metal bioavailability (Verkleji, 1993).Heavy metals are found in their ores in nature. Mining processes allow extraction of the precious trace elements. However, during this process, some metals cannot be extracted and they left behind as tailings. These tailings can be transported by wind and water, causing an environmental problem (Habashi 1992).

The island of Cyprus which is located in the Eastern Basin of the Mediterranean Sea. The island is rich in terms of heavy metals naturally as its name comes from the copper ores. The island had given several names through the time such asKryptos, Kypros,and adjectives like chalkoessa (because of the copper veins)(North Cyprus Online, 2001). It is estimated that history of mining started around 4000 BC in Cyprus. Ancient residents in Cyprus are known to have metalworking skills. In 1914, Cyprus Mines Corporation (CMC) was founded. Skouiriotissacopper mine located 2-3 km away from the mining site in Gemikonagi region, which is thought to be the world's oldest mine in operation was also founded as a part of CMC. CMC suspended the mining processes during the World War II and started to resume in 1974. After 1974 conflict, CMC was sold to Hellenic Copper Mines Ltd. Mining operations in Gemikonagi, Lefke was subsequently abandoned(Euromines, 2012).Since then, the site has been left to its fate without sufficient governmental oversight. The passage of time has deteriorated the site structures and resulted in the release of metals in to the environment.

The aim of this study was to investigate the heavy metal exposure levels using soil, groundwater and human blood samples from the area of Gemikonagi, Lefke where the old mining area is located in immediate vicinity and compare these results with a control area, Tepebasi, Girne located 40 km away from the mining site and devoid of any mining activities. During this study, heavy metal exposure levels of human blood, soil and groundwater samples obtained from residential areas were measured by using high technology equipment,specifically inductively coupled plasma mass spectrometry (ICP-MS). Results obtained from the samples will enlighten new areas

of further studies, such as the relationship between heavy metal exposure and genotoxicity.

2. HEAVY METALS AND THEIR TOXICITIES

Heavy metals are inorganic molecules that have at least 5 times the specific gravity of water (Fergusson, 1990). The Agency for Toxic Substances and Disease Registry (2007), explained that arsenic, lead, cadmium, andmercurycauseserious health implications(Csavina et al., 2012; Sharma et al., 2014; Gupta et al., 2015a). these metals have patho-physiological significance and they can bioaccumulate in living systems and can lead to vital damages to the vital organs especially reproductive and nervous systems, gastrointestinal tract and mucous tissues (Sharma et al., 2014; Gupta et al., 2015b). Although the exact mechanism of action of the heavy metals is not fully understood, it is known that heavy metals or metalloids can induce free radical formation and cause oxidative stress (Singh et al., 2017). Figure 1 summarises the possible mechanisms of heavy metals.

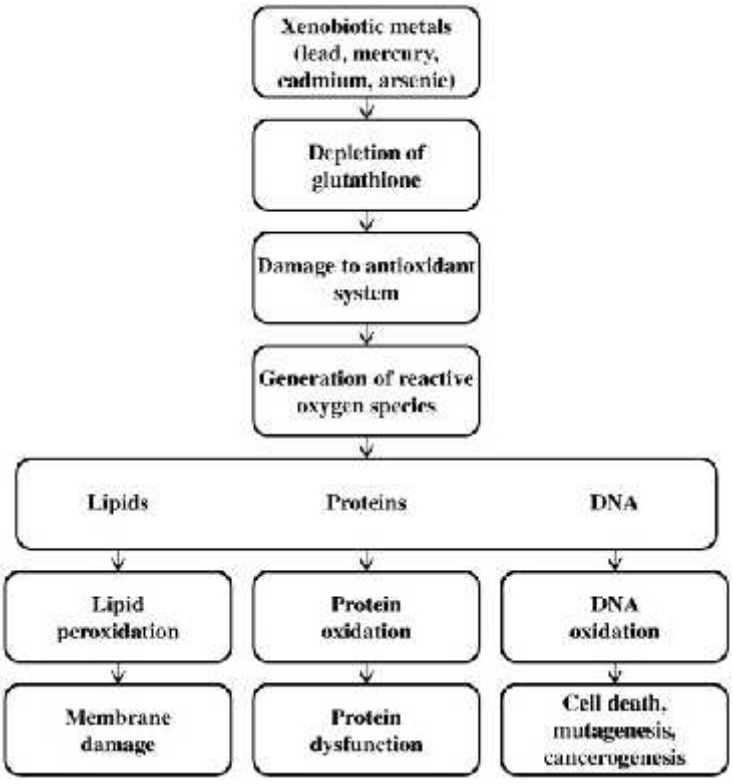

Figure 1: Route of exposure and mechanisms of action of heavy metals.(Solenkova et al., 2014)

2.1. Arsenic

Arsenic's characteristics of being ubiquitous make it possible to be detected at low concentrations in almost all environmental matrices.Arsenic pollutes the environment either by natural sources such as volcanic eruptions and soil erosion or anthropogenic activities (ATSDR, 2000). In industry, arsenic-containing compounds are manufactured and they are being used to produce products for agricultural activities. Insecticedes, herbicides, fungicides, sheep dips, wood preservatives, dye-stuffs are good examples of arsenic containing industrial products. Veterinary medicines that are used for tapeworm eradication in sheep and cattle also includes arsenic (Tchounwou, 1999). Arsenic has also medical application as it had been used in the treatment of some diseases such as syphilis, yaws, and trypanosomaiasis in the past and it is still being used in the treatment ofamoebic dysentery and African sleeping sickness (Tchounwou, 1999). Arsenic is known to induce programmed cell death (apoptosis) in leukaemia cells. Thus, FDA approved an arsenic compound, arsenic trioxide as an anticancer drug in the treatment of acute promyelocytic leukaemia (Rousselot, 1999; Yedjou 2007).

2.1.1. Potential Human Exposure and Toxicity

The ground water of several countries like India, Chile, Bangladesh, Mexico, Taiwan, and Uruguay is highly contaminated with arsenic. Millions of habitants of these countries are exposed to arsenic via ingestion, inhalation and through dermal contact in a chronic manner (ATSDR, 2000; Tchounwou, 1999) Ingestion is the primary route of exposure to arsenic as it is estimated that an average intake is approximately 50 µg per day. Exposure via inhalation and dermal contact have a less extend to occur. However, these routes of exposure are also significant in the areas under contamination. Occupational exposure to arsenic is also widespread. People who work in vineyards, ceramic, and glassmaking, mining and smelting industries, pesticide manufacturing and application are under the risk of high arsenic exposure (National Research Council, 2001). U.S. EPA proposed the inclusion of arsenic on the national priority list as it is detected at 781 sites from the 1300 hazardous waste

sites (ATSDR, 2000; National Research Council, 2001). Exposure to arsenic either acute or chronic threatens human health.

Acute arsenic poisoning is characterised by nausea, vomiting, profuse watery diarrhoea and salivation. Acute psychosis, skin rash, cardiomyopathy, and seizures are also seen in acute arsenic poisoning. Abnormalities in haematology, renal and respiratory failures and pulmonary oedema are very common. Neuropathy and encephalopathy are also seen.

Chronic arsenic exposure virtually affects all organ systems. Arsenic has a tendency to accumulate in liver, kidneys, heart, and lung and to a smaller extend in the muscles, nervous systems, gastrointestinal tract, spleen, and lungs. Chronic exposure to arsenic cause the presence of Mee's lines in the nails. Arsenic has a correlation with the malignancies and causes dermatological changes like hyperpigmentation. Chronic arsenic exposure elevates the risk of cardiovascular, peripheral vascular and respiratory diseases as well as diabetes mellitus and neutropenia (Ratnaike, 2003).

Arsenic exerts its toxicity in several different ways. It inhibits important mitochondrial enzymes and uncouples oxidative phosphorylation which in turn causing the impairment of cellular respiration. Arsenic interacts with sulfhydryl groups of proteins and enzymes and substitutes phosphorous in some biochemical reactions (Wang, 1996). Methylation is the primary process in arsenic metabolisation. Two major metabolites of arsenic trioxide which are methylated through non-enzymatic routes are monomethylarsonic acid and dimethyl arsenic acid (Tchounwou, 2008; Hughes, 2002).

Arsenic compounds cause genotoxicity and it has been shown that they cause DNA repair inhibition, induction of chromosomal aberrations, sister-chromatid exchanges, and formation of micronuclei (Li, 1989; Hartmann, 1994; Patlolla, 2005). Arsenic trioxide also induces DNA damages (Anderson, 1994). Furthermore, arsenic compounds cause the induction of gene amplification, cellular arrests, inhibition of DNA repair and induction of *c-fos* gene expression and oxidative stress (Saleha, 2001; Hartmann 1994). It has been shown that arsenic compounds are highly cytotoxic and they induce the transcription of stress genes (Tchounwou, 2003).

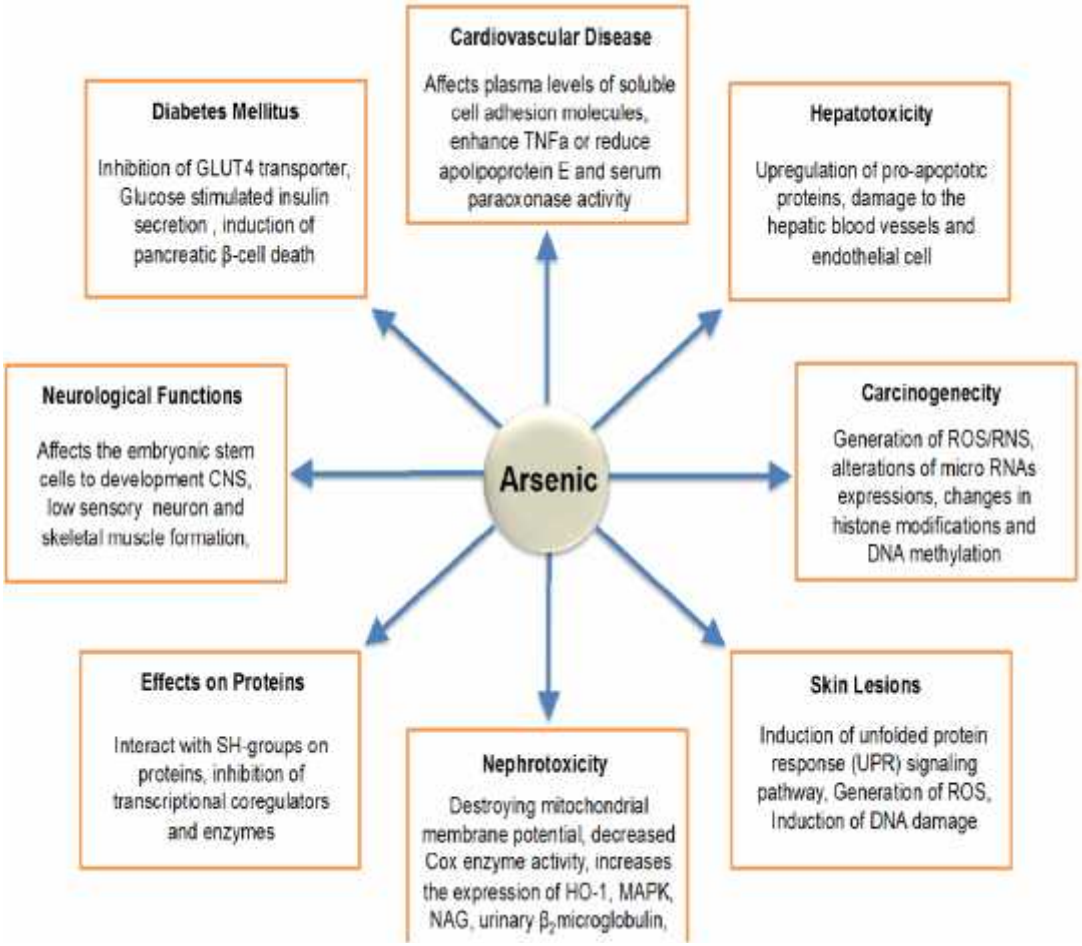

Figure 2: Schematic Diagram Representing the Toxicities of Arsenic. (Khairul et al., 2017)

According to epidemiological studies, chronic arsenic exposure causes carcinogenesis. It might induce DNA hypomethylation causing the facilitation of aberrant gene expression resulting in carcinogenesis (Zhao et al., 1997). In addition, arsenic is a known potenet stimulator of extracellular signal-regulated protein kinase Erk1 and AP-1 transactivational activity, and as well as and efficient inducer of *c-fos* and *c-jun* gene expression which is associated with JNK activation (Liu et al, 1996).

2.2. Cadmium

Cadmium (Cd) is a heavy metal wich is characterised by its soft, dusctile, silvery white with bluish colour, shimmering and electropositive properties. It is taste- and odour-less. It can form adifferent complex of organic mines, sulfur complex, chloro complexes, and chelates. In addition, Cd ions form salts of carbonates, arsenates, phosphates, and ferrocyanide which are soluble(Rafati Rahimzadeh et al., 2017).

Cadmium attracts attention as an environmental and occupational concern. The level of cadmium in the earth's crust is high with an average concentration of approximately 0.1 mg/kg (WHO, 1987). Cadmium is used industrially during the production of alloys, pigments, and batteries (Wilson, 1988). The use of cadmium batteries has shown an increasing trend. However, industrial use of cadmium has become restricted because of environmental concerns. In response to restrictions, in the USA, the daily cadmium intake about 0.4µg/kg/day, less than half of the U.S. EPA's oral reference dose(EPA, 2016).

2.2.1. Potential Human Exposure and Toxicity

The primary reasons for cadmium exposure are cigarette smoking, inhalation, and ingestion. Dermal absorption is relatively rare. Occupational exposure is high for people who work in metal industries. Mining and smelting activities, manufacture industries of batteries, pigments, stabilizers, and alloys increase the cadmium emissions (IARC, 1993; Paschal, 2000; ATSDR, 2008). Low amounts of cadmium are present in foodstuffs like potatoes, grains and seeds, liver and kidney and leafy vegetables (Satarug et al., 2003). Consuming cadmium-containing foods can highly increase the risk of cadmium accumulation in human bodies (Davison, 1988). During the past century, continuing use of industrial applications of cadmium there is and dramatic increase of environmental pollution and human exposure to cadmium (Elinder and Jarup, 1996).

Ingestion and inhalation of cadmium have very severe adverse effects like pulmonary and gastrointestinal irrigation. Acute cadmium exposure cause symptoms to start to show within 15 to 30 minutes. Abdominal pain, burning sensation, nausea, vomiting,

excessive salivation, muscle cramps, vertigo, shock, loss of consciousness and convulsions are the typical symptoms of acute cadmium poisoning (Baselt, 1995). Furthermore, long-term cadmium exposure depresses the levels of noradrenaline, 5-HT3, and acetylcholine (Singhal, 1976).The underlying mechanisms of chronic cadmium toxicity are not fully understood. However, it has been suggested that cadmium causes the generation of reactive oxygen species (ROS), single-strand DNA damages and disruption of the synthesis of nucleic acids and proteins (Stohs, 1995; Mitra, 1984). Long-time arsenic exposure is being linked to induction of hypertension (Li et al., 2013).Arsenic also has an effect on testicular tissue and may cause infertility (Saygi et al., 1991).Studies have shown that cadmium induces inositol polyphosphate formation, elevates cytosolic free calcium levels in some cell types and blocks calcium channels (Th'evenod and Jones, 1992; Suszkiw et al., 1984; Dally, 1997).

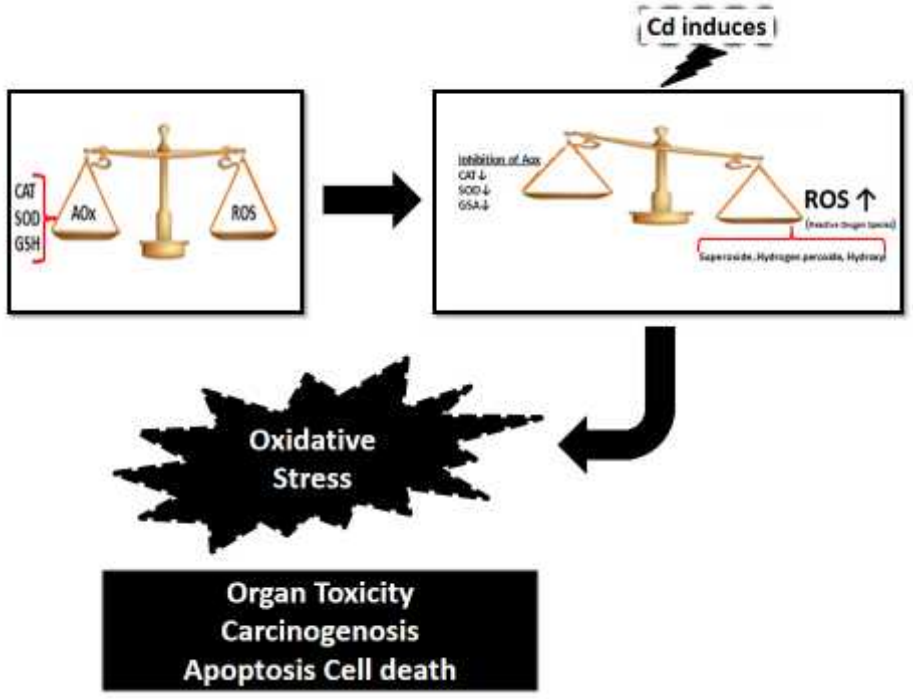

Figure 3: Cd-induced reactive oxygen species (ROS) generation in human body(Rafati Rahimzadeh et al., 2017)

Cadmium compounds are considered as human carcinogens (IARC, 1993). Cadmium can bind to proteins, decrease DNA repair, activate protein degradation, up-regulate cytokines and proto-oncogene (*c-fos, cjun*, and *c-myc*), induce the expression of

metallothionein, heme-oxygenases, glutathione transferases, heat-shock proteins, acute-phase reactants, and DNA polymerase-β at lower concentration (Abshire, 1996; Durnam and Palmiter, 1981; Hwua ,1998; Landolph, 1994). The lung is the most suspicious organ for human carcinogenesis caused by cadmium exposure (IARC, 1993). Occupational and environmental exposure to cadmium may increase the development of certain cancer types like prostate, kidney, liver, hematopoietic system and stomach (Waalkes, 1995; Waalkes, 1996).

2.3. Lead

Lead is naturally occurring bluish-grey metal element. Despite its natural occurrence, fossil fuel burning, mining, and manufacturing are the human activities which increase the release of lead to the environment at high concentrations. Lead is being used industrially, agriculturally and domestically. The most well-known uses of lead can be listed as the production of lead-acid batteries, ammunitions, metal products and in the devices to shield X-rays. Production of lead-acid batteries is the most liable source of lead release to the environment which accounts for approximately 83% (Gabby, 2006; Gabby, 2003). There is an effort to decrease the industrial use of lead from paints and ceramic products, caulking and pipe solder (CDC, 1991). However, lead contained paints are still the major cause of lead exposure for human population (CDC, 2001).

2.3.1. Potential Human Exposure and Toxicity

The major lead exposures occur as a result of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water, and paints (ATSDR, 1999; ATSDR, 1992). Depending on several factors like age and physiological condition, absorption of lead is subject to change. In human subjects, kidneys take up the greatest percentage of lead which is followed by the liver, heart, brain and the skeleton (Flora, 2006). Lead poisoning greatly affects the nervous system as a headache, poor attention spam, irritability, loss of memory and dullness are the significant symptoms of lead poisoning (CDC, 2001; ATSDR, 1999). Apart from being a nervous system toxicant, lead also effects several organs and systems including the kidneys, liver, hematopoietic, endocrine and reproductive systems (ATSDR, 1999).

In general, lead exposures occur from lead-contaminated household paints, lead-containing crystals, and ceramic, several lead-containing medicines and cosmetics. Occupational lead exposure is also widespread (CDC, 1991; ATSDR, 1992). Exposure to lead during pregnancy is one of the major concerns. Lead can be transferred to the foetus directly and causes a decrease in birth weight, early delivery and neuro-developmental deficiencies (Ong et al., 1985; Corpas et al., 1995; Andrews et al., 1994).

Figure 4: Summary of lead poisoning(The Guardian, 2016)

The most prominent effect of lead exposure is seen in children. Children who are exposed to lead in their early life tend to have diminished intelligence with low intelligence quotient-IQ, underdeveloped neurobehaviour, decreased hearing acuity, speech handicaps, growth retardation, poor attention and anti-social and diligent behaviours (U.S. EPA, 2002; Kaul et al., 1999; Litvak et al., 1998; Amodio-Cocchieri et al., 1996). High exposure to lead affects reproduction system for adults. It is correlated with low sperm counts in men and spontaneous abortions in women (Hertz-Picciotto, 2000; Apostoli et al., 1998). Acute lead exposure causes brain and kidney damages as well as gastrointestinal diseases. On the other hand, long term lead exposure negatively affects blood and central nervous systems, blood pressure, kidneys and the metabolism of vitamin D (ATSDR, 1992; ATSDR, 1999; U.S. EPA, 2002; Kaul, 1999; Litvak, 1998).

Lead has the ability to inhibit or mimic the calcium actions and interact with proteins (ATSDR, 1999). Lead acts as calcium within the skeleton. There are certain mechanisms by which lead exerts its actions. By interacting with sulfhydryl and amide groups of enzymes, lead diminish the enzymatic activities. It also exerts its action via competing with essential elements for their binding sites, inhibiting the activity of the enzymes and altering the transport of essential cations (Flora, 2006). According to the studies, lead induces the formation of reactive oxygen species (ROS), and thus, causes cellular damage (Hermes-Lima et al., 1991). People who are exposed to lead occupationallyare shown to have increased antioxidant enzyme activities, especially superoxide dismutase (SOD) and Glutathione peroxidase in their erythrocytes (Bechara et al., 1993).

Various researches found out that lead interferes with calcium-dependent processes which are interrelated to intracellular signal transductions and neuronal signalling. Lead disrupts intracellular calcium cycling and alters the release of calcium from endoplasmic reticulum and mitochondria (Goldstein, 1993; Simons, 1993). Lead can also inhibit calcium-dependent release of neurotransmitters and also, receptor-coupled ionophores in glutamatergic neurons (Vijverberg et al., 1994). In contrast, it may augment protein kinase C and calmodulin activity (Goldstein, 1993; Schanne et al., 1997). Furthermore, exposure to lead increases the likelihood of gene mutations and sister chromatid exchanges (Yang et al., 1999; Lin et al., 1994).

2.4. Chromium

Chromium (Cr) is found in the earth's crust naturally with valence states from chromium (II) to chromium (IV) (Jacobs, 2005). The most stable state of chromium is the trivalent form (Cr(III)) and it is present as ores at this valence state. Natural and anthropogenic activities allow chromium to enter theair, water,and soil. The industry is the largest cause of the chromium emissions. Metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production are the most suspected industries for chromium release to theenvironment. In this way, environmental concentrations of chromium show an increasing trend. Thus, chromium acts as an environmental pollutant of many environmental systems (Cohen et al., 1993). Commercial uses of chromium are

industrial welding, chrome plating, dyes and pigments, leather tanning, wood preservation, and as an anticorrosive in cooking systems and boilers (Norseth, 1981; Wang et al., 2006).

2.4.1. Potential Human Exposure and Toxicity

For humans and animals, chromium is one of the key essential elements as it plays a role in the metabolism of glucose, fat, and protein by potentiating the action of insulin. However, occupational exposure to chromium causes Cr-induced diseases. Thus, it has still been one of the major concerns as it threatens the wildlife and human population as well as the environment (Guertin, 2005). Occupational chromium exposure is highly widespread. It is estimated that more than 300,000 individuals are exposed to chromium occupationally worldwide. (Goyer, 2001). Annual chromium release is estimated to be 33 tons (ATSDR, 2000). Average atmospheric levels of chromium range from 1 to 100 ng/cm3. However, these levels might become higher in areas where Cr is being processed (Singh et al., 1999).

Chromium can be ingested via chromium containing food and water (Langård and Vigander, 1983). Generally, most of the fresh foods contain a few amounts of chromium. Although non-occupational chromium exposure is not low, people who exposed to chromium occupationally get into contact with chromium at least two times higher than the average (ATSDR, 2008). Inhalation is the primary route of human exposure in terms of occupational exposure. Thus, thelung is the most affected organ. Despite this fact, dermal contact with chromium is not insignificant (Costa, 1997; Shelnutt et al., 2007).

Toxicity of chromium compound depends on the valence state and solubility. Cr (VI) compounds are known to be powerful oxidising agents, so they act as irritating and corrosive agents. In comparison, Cr(VI) compounds show more toxicity than Cr(III) compounds (Connettand Wetterhahn, 1983; De Flora et al., 1990). The reason

for Cr(VI) is more toxic is that Cr(VI) can pass through cell membranes and reduced to reactive intermediates. On the other hand, Cr(III) is poorly absorbed via any route of administration. Cr(VI) can be absorbed by the lung, gastrointestinal tract, and skin. Reduction of Cr(VI) is either a detoxification process or toxic depending on the site. Reduction process which takes place away from the target site is considered as detoxification. Besides, reduction of Cr(VI) shows toxic and/or genotoxic effects if it takes place in or near cell nucleus of target organs (Dayan and Paine, 2000).

Reduction of Cr(VI) relies on several enzymes depending on the type of the cell that it entered. Hydrogen peroxide (H2O2), glutathione (GSH) reductase, ascorbic acid, and GSH are the keys enzymes during this process. As a result of reduction reactions, Cr(V), Cr(IV), thiol radicals, hydroxyl radicals, and Cr(III) are the reactive intermediates produced. These reactive intermediates can disrupt cellular integrity and functions by attacking DNA, proteins and membrane lipids (De Mattia et al., 2004; O' Brien et al., 2003).

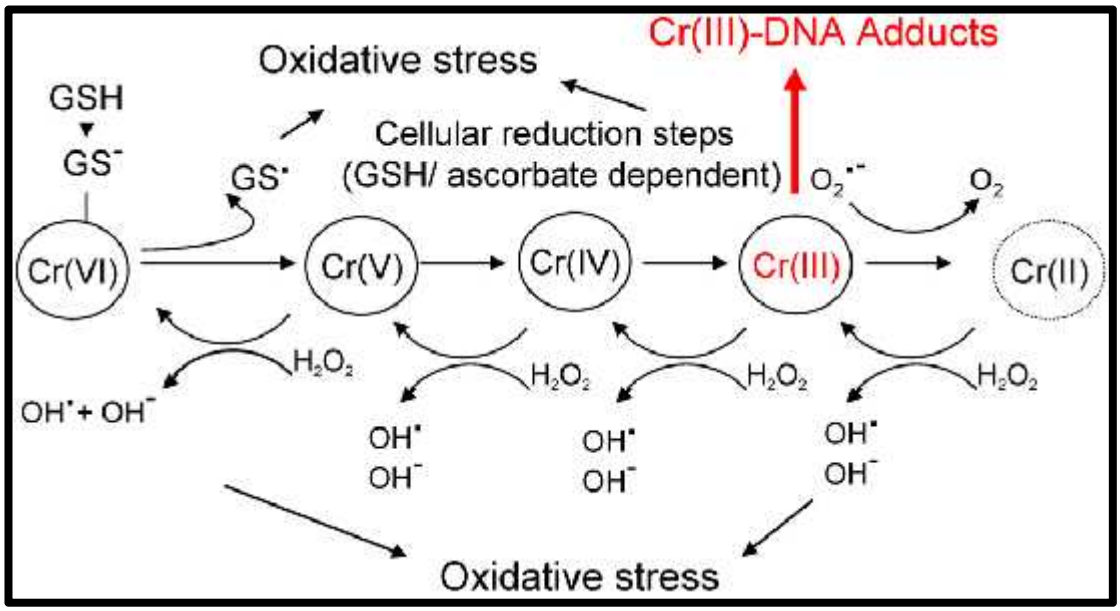

Figure 5: Pathway of chromium induced oxidative stress(Henkler, Brinkmann and Luch, 2010)

Cr (VI) induced toxicity is supported by DNA strand breaks in peripheral lymphocytes and lipid peroxidation products(Gambelunghe et al., 2003; Goulart et

al., 2005). Cr (VI) can induce genotoxicity as well as cytotoxicity, which are caused by oxidative damage such as chromosomal abnormalities and DNA strand breaks (Wise et al., 2002; Wise et al., 2004; Xie et al., 2005, Patlolla, 2008). Nevertheless, studies show that non-oxidative biological mechanisms also can induce Cr(VI) carcinogenesis(Zhitkovichet al, 2001). The risk of induction of cancer by chromium compounds depends on several factors such as solubility, size, crystal modification, thecharge of the surface and the ability to be phagocytized (Norseth, 1981). Occupational exposure to Cr(VI) compounds has a tendency to cause respiratory compounds (Dayan, 2000).

2.5. Copper

Copper is a malleable transition metal with low corrosion and alloying ability, high thermal and electrical conductivity. Thus, it is one of the most important metals for industry. It has been used in machinery, construction, transportation,and weapons (Barceloux, 1999; Winge and Mehra, 1990). In addition, it is an important constituent of white gold in thejewellery industry, dental products and cosmetics (Okereke et al., 1972; Vilaplana et al., 1991; Lucas and Lemons, 1992). In nature, Cu can be found as its elemental form and as a compound. Ions of Cu can exist in its oxidised form or cupric, reduced and cuprous state (Linder et al., 1996; Linder et al., 1998).

Figure 6: Different copper minerals(Earth Observary, 2002)

Table 1: Composition of different copper minerals(nfalliance.org, no date).

MINERAL	COMPOSITION	COPPER CONTENT (%)	APPEARANCE
Chalcopyrite	$CuFeS_2$	34	Yellow-gold
Bornite	Cu_2FeS_4	63	"Peacock ore" Irridescent
Malachite	$CuCO_2Cu(OH)_2$	57	Green, variegated
Cuprite	Cu_2O	89	Red, earthy

Cu can be emitted to the environment from Cu water pipes, Cu cookware, drinking water, birth control pills and intrauterine devices, vitamin and mineral supplements, Cu-contained fungicides and foods (Pohl et al., 2011).Variety of cells and tissues, especially hepatic cells contain high concentrations of Cu (Turnlund, 1998).

2.5.1. Potential Human Exposure and Toxicity

Depending on several intrinsic and extrinsic factors, exposure to Cu is subject to change. Depending on food choices, dietary customs,and environmental factors, Cu intake may increase or decrease.Occupational exposure to Cu is highly widespread especially people who work as plumbers, welders, machinists and people who work in Cu industry. In general, exposure to Cu is in combination with the other metal exposures such as arsenic, iron, or mercury, and chemicals such as ethanol, polychlorinated biphenyls, and pesticides (Pohl et al., 2011).

Cu is an essential element for life. However, more than the needed concentrations, Cu may cause cellular damage. In order to regulate Cu concentrations, there is a complex system of metal ion transporters and chaperones. Cu homeostasis disruptions may cause tissue damages and a number of diseases (Bleackley and Macgillivray, 2011; de Romana et al., 2011). Apart from direct interactions with essential macromolecules, Cu cause toxicity indirectly via free radical-induced oxidative damage. Cu ions induce the production of ROS that causes conformational changes to essential biomolecules (Lippard, 1999). Presence of superoxide or reducing agents like ascorbic acid or GSH allows the reduction of Cu2+ to Cu+ that

have the ability to catalyse the formation of hydroxyl radicals from hydrogen peroxide via the Haber-Weiss reaction (Bremner, 1998; Kadiiska et al., 1993).

In the human population, disruption of Cu homeostasis causes Menkes and Wilson's disease. Insufficient Cu in the body, which is known as Menkes disease, is characterised by the inactivation of key metabolic enzymes (Song et al., 2011). In contrast, accumulated Cu in the body cause Wilson disease that lead to Cu-induced oxygen radical-mediated damage (White et al., 2009; Sayre et al., 2000). People who suffer from Wilson disease have lipid peroxidation in the mitochondria of the hepatocytes and decreased antioxidant vitamin E concentration in liver and blood (Myers et al., 1993).Apart from ROS induced oxidative damage, there are more pathways and mechanisms which Cu overload exerts its mechanisms. Lipid metabolism regulation, antimicrobial defence mechanisms, and kinase-mediated signal transduction are good examples of Cu toxicity mechanisms (Hasan and Lutsenko 2012).

2.6. Iron

Iron (Fe) is a chemical element which is found in the Earth's crust naturally. In terms of mass, it is the most common element and is a constitute of Earth's outer and inner core. Iron is one of the most common elements(Meije et al., 2016). Iron compounds have many uses and they have been used since ancient times. Iron oxide, for example, is used in combination with aluminium powder create thermite reaction which is used in welding and purifying ores. Iron combines with halogens and chalcogens form binary compounds.Iron also one of the key elements in biological compounds. In haemoglobin and myoglobin, iron forms complexes with molecular oxygen and supports oxygen transport. In plants and animals, iron is found at the metal at the active site of redox enzymes which act as important enzymes in cellular respiration and oxidation and reduction reactions(Iron Disorders, 2009).

2.6.1. Potential Human Exposure and Toxicity

As iron is an essential element, it is strictly regulated in the body. During the day, only small amount of iron is excreted through mucosa and skin. Thus, the primary way of regulating iron levels in the body is to regulate the uptake (Ramzi et al. 1999). Excess intake of iron causes excessive free iron levels in the blood. High levels of free iron at ferrous (Fe^{2+}) induces free radical formation and DNA damage. Toxicity of iron depends on the iron in the cell. Excess levels of the free iron result after the saturation of the transferrin that binds to the iron. Iron commonly damages myocardiocytes and hepatocytes in the first place and causes adverse effects such as coma, metabolic acidosis, liver failure, respiratory distress and even death (Cheney et al., 1995).

2.7. Nickel

Nickel(Ni) is a naturally found element in the Earth's crust. It is the twenty-fourth most abundant natural element. Nickel's primary source is found in the earth's core. Thus, it cannot be obtained and used. However, volcanic eruptions, soils, ocean floors and ocean water act as sources of nickel (Stimola, 2007). Nickel is being used in several industries. Making stainless steel and other alloys cover 80% of the total nickel usage. Following industries are electroplating and printing inks that account for 10% and 5% of nickel usage respectively (Nickel Institute, 2006).

The nickel emission to the atmosphere is widespread during nickel mining and the industrial production of stainless steel and other nickel alloys, or by industries which are using nickel and its compounds. Moreover, oil burning power plants, coal-burning power plants,and trash incinerators increase the release of nickel into the atmosphere. Once it is in the atmosphere, it sticks to dust particulates and reach the ground or washed out from the air by rain or snow. During industrial processes, nickel is released into soil or sediments and binds to iron or manganese particles (Cameron et al., 2011).

2.7.1. Potential Human Exposure and Toxicity

Humans are exposed to nickel through inhalation and ingestion of contaminated food and water, and as well as tobacco smoking. Dermal exposure to nickel is also possible via the direct contact with the nickel containing jewellery, stainless steel,and coins. In response to nickel-producing industries, the contamination of drinking waters with nickel increases. In soil, average nickel concentration is between 4-80 ppm. However, areas around nickel producing factories have significant amounts of nickel which can be up to 9000 ppm (ATSDR, 2005). In relation to increased nickel concentration in soil, dermal contact with the contaminated soil is the usual source of exposure. On the other hand, food stuff like tea, coffee, chocolate, soybeans, nuts, oatmeal, cabbage, spinach, and potatoes are most likely to contain nickel. Thus, they are the major dietary sources of nickel exposure (Cameron et al., 2011).

Occupational exposure to nickel is usually through inhalation. People who occupationally exposed to nickel have significantly higher concentrations of nickel in their body compared to the general population (Ohio Environmental Protection Agency, 2002).Acute nickel toxicity in humans occurs in response to inhalation or absorption of nickel via the gastrointestinal tract. According to the study conducted by Das and Buchner, the main underlying mechanism of nickel toxicity is the result of the depletion of glutathione and bonding to the sulfhydryl groups of proteins (Das and Buchner, 2007, Valko et al., 2005). A study conducted by US EPA found that treatment with nickel causes irreversible lung damage abnormal pulmonary functions, renal tubular necrosis, anemia, eosinophilia, and nasal septum ulceration. Furthermore, it is also reported that physiological chemistry is altered by reducing nitrogen retention, glucosurea, phosphaturea, and urinary excretion of calcium ion and zinc ion. Inhibited ATPase activity may result in neurological disorders, convulsions,and coma. Long-time exposure may lead to disruption of oxidative phosphorylation (EPA, 1994; Nielsen, 1982).

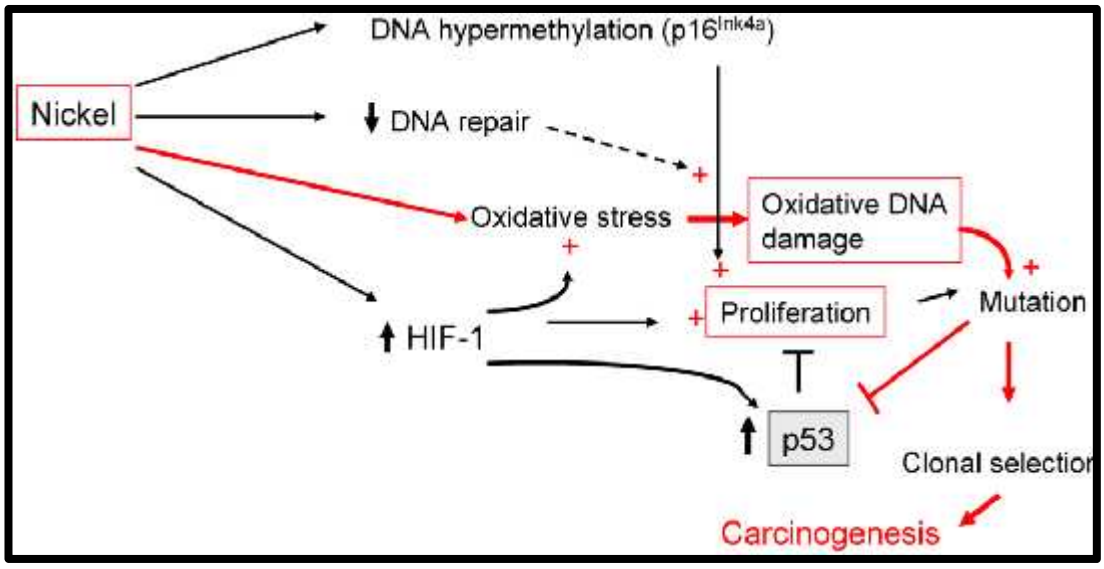

Figure 7:Summary of nickel induced toxicity(Henkler et al., 2010).

According to the study conducted by using HL-60 human leukaemia cells, chronic Ni+2 ion exposure causes DNA fragmentation, cell death, and Ros (Jia and Chen, 2008). High doses of Ni2+ is taken up by phagocytosis and may lead to genotoxicity and mutagenicity. Induction of genotoxicity by nickel compounds is thought to be as a result of thegeneration of ROS and inhibition of DNA repair (Kasprzak et al., 2003). Nickel compounds have the ability to enter the nucleus. Thus, they can readily interact with chromatin. One of the main mechanisms of nickel carcinogenesis is heterochromatinization (Ellen et al., 2009).

To sum up, nickel induced carcinogenesis results from several factors including oxidative stress, DNA damage, epigenetic effects and its effect on specific signal transduction pathways.

3. ANALYTICAL METHODS AND TECHNIQUES FOR HEAVY METALS DETERMINATION

Analytical measurements are important in determining environmental and biological pollution and harm. Thus, they should provide significant information for:

- Routine monitoring of regulated contaminants;
- Area and level of contamination for affected sites for remediation;
- Assessment of possible health effects.

Depending on several factors, such as, quantitative determination for multielement analysis, determination of individual metals or metalloids and speciation of heavy metals, different analytical techniques are available. These methods and their techniques depend on the property of the analyte that can be analysed by an

appropriate equipment. Table 2 summarizes the principles of the analytical methods and techniques used in the heavy metal analysis.

For human biomonitoring, analysis of heavy metals blood and urine are the most preferable biological samples. However, other matrices, for instance, saliva, hair, nail,and teeth are also suitable for heavy metal detection.

Atomic absorption spectrometry (AAS) is one of the techniques used in the detection of heavy metals. During the process, a flame of a furnace is used to heat the specimen till the atomisation of the element. At the resonance line, the light beam is absorbed by the atoms and the attenuation intensity of the light beam is measured. In case of biological samples, nitric acid digestion is needed for the pre-treatment of the specimen (Sigel, 2013). In general, it is used for single element detection. However, up to 6 elements can be detected using this method. AAS is widely used and it is the standard method for single element analysis. One major disadvantage of the AAS is that it is hard to work with solid samples. During the analysis, one element at a time can be detected. In addition, AAS is a relatively more expensive than the than techniques.

Table 2: Summary of the principles of the analytical methods and techniques used in the heavy metal analysis(Draghici et al., 2010).

Method and technique	**Principle**	**Multielement analysis**	**Applications**	**Other observations**
Optical methods and techniques				
Atomic absorption spectrometry (AAS)	Absorption of radiant energy produced by a special radiation source (lamp), by atoms in electronic ground state	Single-element technique multielement analysis (2–6 elements)	Widely used method, standard one	Most valuable technique for environmental heavy metals analysis
Inductively coupled plasma – atomic emission spectrometry (ICP – AES)	Measures the optical emission from excited atoms to determine analyte concentration	Simultaneous multielement analysis	Widely used method for environmental trace analysis	

Inductively coupled plasma – mass spectrometry(ICP – MS)	Argon plasma is used as ion source mass analysis is the method used for separating ions based on their mass-to-charge ratio (m/z)	Simultaneous multielement analysis	Widely used also used for isotope determination	Interferences arise when a species has the same nominal m/z as the analyte
X-ray fluorescence(XRF)	Uses X-rays as primary excitation source, usually provided by X-ray tubes, or radioisotopes, which cause elements in the sample to emit secondary X-rays characteristic λ	Simultaneous determination of most elements,exception of those with atomic number below 8	Less suitable for analysis of minor and trace elements	Non-destructive analysis
Neutron activation analysis(NAA)	Based on conversion of stable nuclei of atoms into radioactive ones and subsequent measurement of characteristic nuclear radiation emitted by the radioactive nuclei	Simultaneous multielement analysis	Most elements can be determined with some limitations such as for Pb	Highly sensitive procedure
Atomic fluorescence spectrometry(AFS)	Measures the light that is reemitted after absorption	Single-element technique	Mercury, arsenic, and selenium	Complementary technique to AAS
Molecular absorption spectrometry (colorimetry)	Relationship between molecular absorption of UVVIS radiation by a solution and the concentration of the coloured species in solution	Single-element technique	Speciation analysis	Poor selectivity requires prior separation of the element to be determined
Separation methods and techniques				
Gas chromatography(GC)	Based on the different repartition of the analyte between a stationary phase and a mobile one (gas)	Simultaneous multielement analysis	Volatile or thermally stable compounds (Hg, Sn, Pb alkyl compounds)	Hyphenated techniques for metal speciation: GC-AAS, GCAES, GC-MS
Liquid chromatography(LC)	Based on the different repartition of the analyte between a stationary phase and a mobile one (liquid)	Simultaneous multielement analysis	Environmental metal speciation (Cr, As, Se, Sn, Hg, and Pb)	Hyphenated techniques for metal speciation:, HPLC-AAS, HPLC-AES, HPLC-ICPAES, HPLCICP-MS
Ion chromatography(IC)	IC is a liquid chromatography technique which uses ion-exchange resins (as stationary phase) to separate atomic/molecular ions based on their interaction with the resin	Simultaneous multielement analysis	U.S. EPA method 218.6 describes the procedure for Cr(VI) determination in water	No selectivity control hyphenated techniques for 148 IC-AAS, IC-ICP-MS IC-ICP-AES,

Capillary electrophoresis(CE)	Differential migration of charged analytes along a capillary filled with a suitable electrolyte	Simultaneous multielement analysis	Ions, organic, and inorganic compounds of the same metal	Hyphenated techniques CEMS, CE-ICPMS
Electrochemical methods and techniques				
Electrochemical methods	Controlled variable: voltage or current polarography potentiometry voltammetry, anodic stripping voltammetry (ASV)	Consecutive analysis of distinct metal ions is possible	Speciation analysis for transition metals and metalloid	ASV advantage: select between different oxidation states of the same metal
Biochemical methods Immuno-chemical methods	Relies on an antibody that is developed to have a high degree of sensitivity to the target compound	Single element technique	Applicable to any pollutant for which a suitable antibody can be generated	Highly selective and sensitive

3.1. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) is another technique used during theheavy metal determination. ICP-MS operates by heating in an argon plasma which is activated by a high-voltage field. Following this process, the atoms become ionised. Furthermore, an electric field is used to accelerate ions generated to reach the analyser of the mass spectrometer. Then, ions are separated according to their mass of specific isotopes.The technique relies on the separation of the ions depending on their mass-to-charge ratio. It allows a simultaneous multielement analysis. ICP-MS allows rapid scanning, has a large dynamic and mass range that makes it an ideal technique for heavy metal detection.

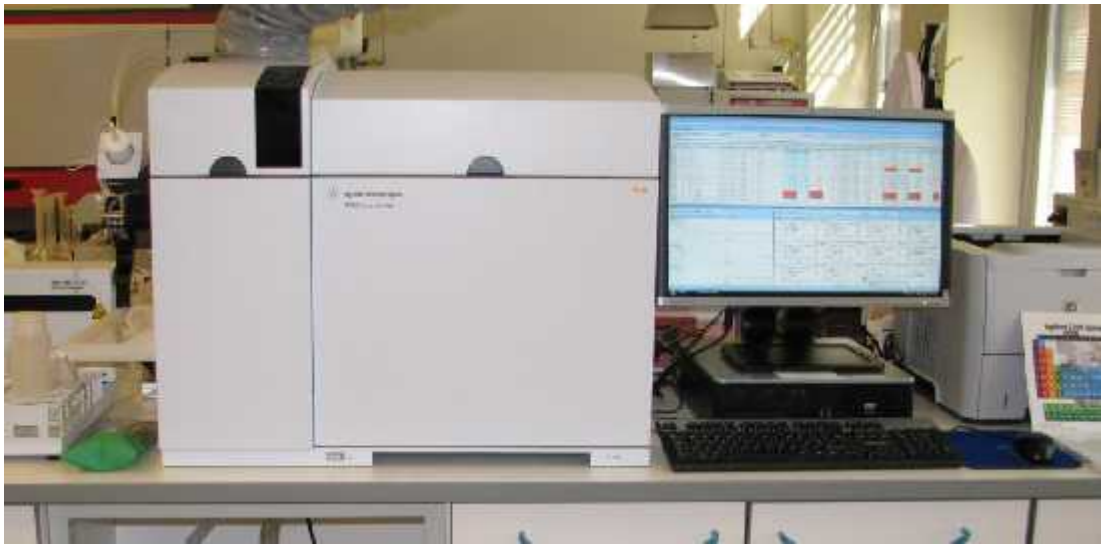

Figure 8A: Agilent 7700 ICP-MS

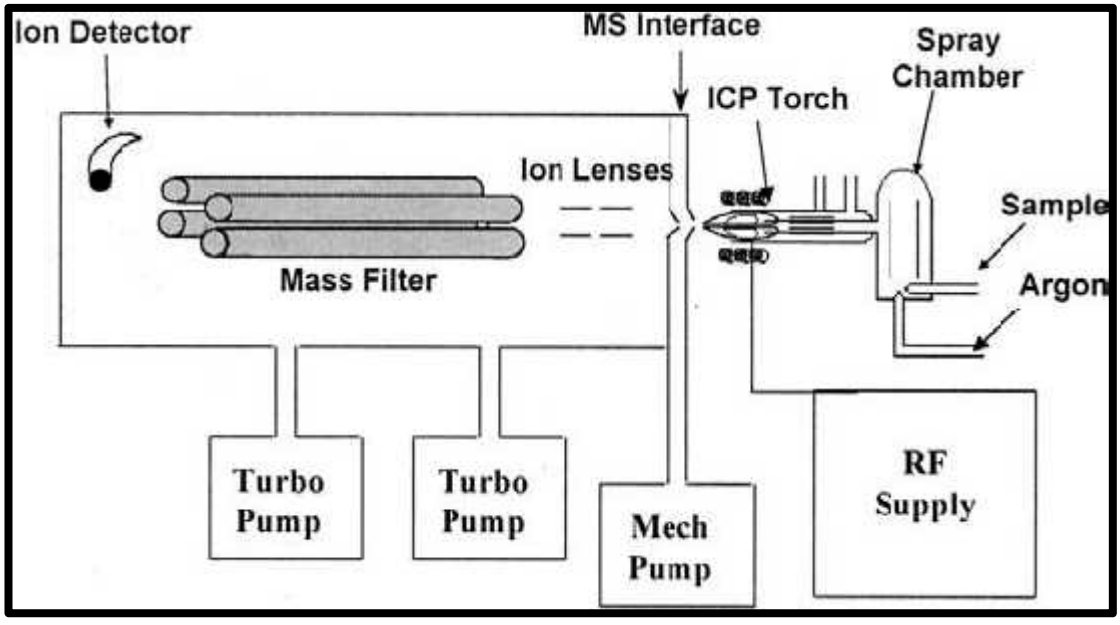

Figure 8B: Schematic diagram of a typical ICP-MS setup(biochem.pepperdine.edu, no date).

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents

Following chemicals and reagents were used during the experiment:Glacial acetic acid, purity 99.5%, nitric acid, purity 70%and hydrochloric acid, purity 37% from Fluka (Madrid,Spain); and analytical grade ammonium acetate, purity99.5%, trimethylamine, purity 99.5% and hydrogen per-oxide solution for ultra-trace analysis, purity 35% fromSigma-Aldrich (Steinheim, Germany). Water was obtained from a Milli-QTM system (Millipore, Bedford,MA, USA) and Pyrex glass digestion tubes (Foss,MN, USA).

4.2. Ethical Considerations and Survey

Prior to this study, Turkish Republic of Northern Cyprus, Ministry of Health was approved and accepted the study as suitable for ethical considerations. Ethics approval for the study was obtained from The NEU Joint-Committee of the Research and Ethics Committee (YDU/2017/46-399).After the Informed Consent Forms were signed by the participants, they were asked to fill out the study questionnaire.The questionnaire form was composed of 2 sections, demographic section (12 questions) and the study oriented section (16 questions).

4.3. Study Area

Soil and groundwater samples were collected from Gemikonagi (35°08’28.8"N 32°49’59.8"E) and Tepebasi (35°18’22.2"N 33°03’17.6"E) located in North Cyprus. Figure 9 shows the two regions. Blood samples were obtained from the volunteers residing in Gemikonagi and Tepebasi. Gemikonagi was chosen due to its proximity to the suspected heavy metal contamination, whereas Tepebasi was chosen due to its relative isolation as well as a lack of mining operations.

Figure 9: Location of Gemikonagi and Tepebasi (North Cyprus).

4.4. Sample Collection, Storage,and Pre-treatment

4.4.1. Soil Samples

A total of 10 soil samples were collected on 16th November 2017, following the first seasonal rainfalls of Cyprus. The coordinates of the sampling sites were given in Table 3. The collection was held by pre-sanitised, PTFE-coated large spoon and collected samples were transferred to the pre-sanitised sample collection bag (500 g). After the evacuation of the air, the bag was sealed. The collected soil samples were immediately transferred to the Near East University Toxicology Laboratory and refrigerated at 4°C to minimise the bacterial colonisation until ICP-MS analysis.

Table 3: Coordinates of the sampling sites.

	Gemikonagi (coordinates)	**Tepebasi (coordinates)**
Sampling Site 1	35°13’66.8"N 32°83’49.13"E	35°30’33.26"N 33°05’74.08"E
Sampling Site 2	35°13’89.27"N 32°83’12.63"E	35°30’42.73"N 33°06’54.84"E
Sampling Site 3	35°14’30.49"N 32°81’29.42"E	35°30’93.65"N 33°06’15.54"E
Sampling Site 4	35°13’52.06"N 32°83’25.25"E	35°31’05.31"N 33°04’98.86"E
Sampling Site 5	35°14’35.75"N 32°84’04.65"E	35°29’89.40"N 33°04’56.95"E

Right before the analysis, the sediment samples left to dry at room temperature for 72 hours and ground. After these processes, 0.25 g of each sample was weighted and 5 ml of concentrated 65% nitric acid was added to each sample. Following this, the samples were heated up to 180 °C. Acid addition and heating processes were

repeated two more times (Eaton and Franson, 2005). Then, deionised water was added to the residue. The suspended material was filtered (Whatman filter Merck, 0.45 μm) and deionised water was added up to the final volume 50 mL. The analysis was carried out using ICP-MS 7500ce (Tokyo, Japan) at the Advanced Technology Education Research and Application Centre Laboratory of Mersin University, Turkey (Alkas et al 2017).

4.4.2. Water Samples

The groundwater samples were collected simultaneously with the soil samples. Two aliquots, one for anion and one for cation sampling, were collected into water sampling polyethylene bottles (250 mL) from the pumped water from deep-wells from both regions. Immediately after collection, the bottles were sealed and transported to the Near East University Toxicology Laboratory and refrigerated at 4°C. During pre-treatment, 65% nitric acid was added to one of the aliquots to obtain pH<2. Then, deionised water was added to the acidified sample up to the final volume of 50 mL.

4.4.3. Blood Samples

Blood samples were collected from people who live in Gemikonagi and Tepebasibetween the time period of 26th July 2017- 23rd August 2017. All participants ascend was confirmed through signed Informed Consent Forms. The exclusion criteria were as follows: being under 18 years old, living in outside of the selected regions, living in the selected regions less than a year, being any type of cancer sufferer or received any type of cancer treatment. Prior to the blood collection, participants were asked to complete a questionnaire that allows getting demographic and study-specific data. Blood collection was conducted in two centres. Camlibel Health Centre was chosen as it is the closest health centre to Tepebasi region and Lefke Health Centre was also chosen for being the closest centre to Gemikonagi area. Professional nurses collected blood samples from the median cubital vein found in cubital fossa of the forearm (Figure 10) by using 5 cc syringe (Set inject).

Figure 10: Site of blood drawing(anatomyatlases.org, no date).

Immediately after drawing, 2 mL blood samples were enclosed in Vacutest Clot activator tubes and transported to the Near East University Toxicology Laboratory inside an icebox and immediately refrigerated at -20 °C. Prior to analysis, samples were kept at 4°C to allow liquification. Afterwards, 1 ml of blood sample was put into a Teflon vessel using microwave digestion system. Then, 2 ml 65% of nitric acid and 0/2 ml of hydrogen peroxide were added to the specimen. Following this, thesampleswere dissolved at 800 W for five minutes(Lee et al., 2012).

Figure 11:Flowchart of Blood pre-treatment.

4.5. Heavy Metal Analysis

4.5.1. Instrumentation

In order to analyse heavy metals in different samples, the inductively coupled plasma mass spectrometry (ICP-MS) was used from Agilent 7500ce Octupole Reaction System with 99.99% helium from Agilent Technologies (Tokyo, Japan), containing an ICP source with a plasma-shielded torch (grounded metal plate), an octupole reaction system operated in radiofrequency (RF)-only mode and a quadrupole mass

analyser with a secondary electron multiplier operating in dual mode (i.e. either a pulse-counting mode or analogue mode, depending on the ion intensity). The followings were the operating conditions:nebuliser type, concentric nebuliser; nebuliser gas (argon) flow rate, 0.9 l/min; auxiliary gas (argon) flow rate, 0.14 L/min; plasma gas (argon) flow rate, 15 L/min; reaction gas (helium) flow rate, 0.14 L/min; spray chamber (S/C) temperature, 2 °C; and ICP RF power, 1500 W. The argon gas used was of spectral purity (99.998%). Validation parameters for the ICP-MS method are shown in Table 4.

Table 4: Validation parameters of the inductively coupled plasma mass spectrometry method.

	Cr	Mn	Ni	Cu	Cd	Pb	As
Calibration Range (ng/mL)	0-50	0-50	0-50	0-50	0-50	0-50	0-50
Determination Coefficient (R^2)	0.9998	0.9997	0.9999	1	1	0.9999	1
Rec.* (%)	91	99	89	95	110	93	94
RSD (%) (n=10)	6.9	7.8	10.2	9.9	10.1	11.9	12.4
LOD (µg/kg)	0.08	0.11	0.06	0.03	0.15	0.12	0.06
LOQ (µg/kg)	0.005	0.005	0.007	0.001	0.008	0.006	0.004

4.5.2. Data analysis

Statistical analysis was carried out using IBM SPSS Statistics. (IBM SPSS Statistics 21. SPSS inc., an IBM Ca. Somers, NY).During the analysis of the data, Levenee's Test is used to calculate normal distribution. For normally distributed data, t-test for independent samples was applied. For the non-parametric data, Mann Withney U-test was applied. Statistical significance was accepted at $p < 0.05$ level.

5. RESULTS

Demographic characteristics of the volunteers from Tepebasi (n=49) and Gemikonagi (n=52) were given in table 5. There is no significant difference in gender and age between the two groups as p=0.772 for age and p=0.192 for gender.

Table 5: Number of female and male participants and mean ages depending on the area of participation.

Region	Female	Male	Total	Mean Age (years) ± SD
Tepebasi	29	20	49	40.55±14.586
Gemikonagi	24	28	52	42.14±14.063

Figure 12 illustrates the smoking habits of the volunteers from two different regions. There is no statistically significant difference between two regions in terms of smoking habits (p=0.749).

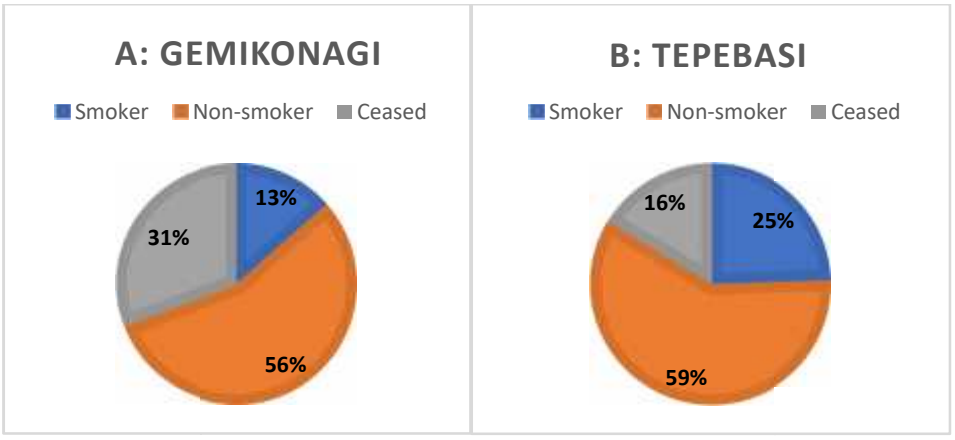

Figure 12: Percentages of smokers, non-smokers and ceased smokers.

Blood metal levels for As, Cr, Cu, Fe, and Ni obtained from ICP-MS analysis were given in Table 6 and Figure 13.

Table 6: Blood metal levels and their statistical significance.

Metal	Region	Mean ± SD	Range (min.-max)	p-value *	Legal Limits (ppm)	Reference
As	Gemikonagi	0.22881 ± 0.127324[a]	0.026 - 0. 562	0.021	0.012	Mayo Clinic (2017)
	Tepebasi	0.20335 ± 0.233831[a]	0.01 - 1.274			
Cr	Gemikonagi	3.52656 ± 4.210884[a]	0.640 - 32.294	0.257	1.4	Medlineplus.gov (2017)
	Tepebasi	2.81400 ± 1.201769[a]	0.000 - 6.824			
Cu	Gemikonagi	0.76683 ± 0.320856[b]	0.148 - 1.632	0.016	1.4	Urmc Rocherster (2017)
	Tepebasi	0.62396 ± 0.197096[b]	0.257 - 1.124			
Fe	Gemikonagi	521.929882 ± 238.668762[b]	124.908 - 1569.486	0.011	$15x10^4$	Mayoclinic (2017)
	Tepebasi	423.62417 ± 82.508225[b]	41.055 - 573.919			
Ni	Gemikonagi	0.13215 ± 0.571523[a]	0.000 - 3.890	0.017	$2x10^{-4}$	ATSDR (2017)
	Tepebasi	0.00037 ± 0.002571[a]	0.000 - 0.018			

*p<0.05 is accepted as significant.

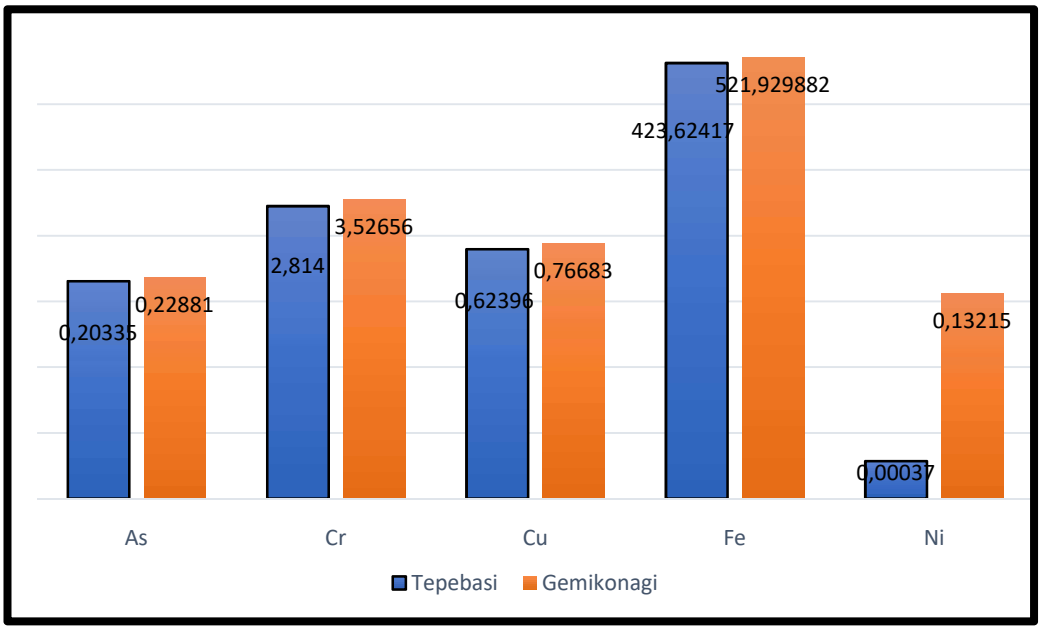

Figure 13: Blood heavy metal levels.

Soil heavy metal analysis (As, Cr, Cu, Fe, Ni, Cd and Pb) obtained from ICP-MS were shown in Table 7 and Figure 14.

Table 7: Mean heavy metal concentrations and legal limits of heavy metals in soil.

Metal	Region	Mean ± SD (ppm)	Legal Limits (ppm)	Reference
As	Gemikonagi	137.8 ± 49.792 [a]	0.39	USEPA 2001
	Tepebasi	273.200 ± 52.428[a]		
Cr	Gemikonagi	748.8 ±150.225[a]	23	MDE 2008
	Tepebasi	1342.2 ± 99.558[a]		
Cu	Gemikonagi	3349 ± 1559.371 [a]	50	ATSDR
	Tepebasi	221.600 ± 41.843 [a]		
Fe	Gemikonagi	557219.200 ± 330830.423 [a]	$55x10^4$	Bodek et al. 1988
	Tepebasi	213994 ± 13935.642[b]		
Ni	Gemikonagi	157.800 ± 102.160[b]	1000	WHO 1996
	Tepebasi	683.800 ± 260.144[b]		
Cd	Gemikonagi	5.246 ± 1.618[b]	85	USEPA 2000
	Tepebasi	12.298 ± 9.792[b]		
Pb	Gemikonagi	451.538 ± 431.383 [a]	400	USEPA 2000
	Tepebasi	285.301 ± 338.430 [b]		

[a] above the legal limits.

[b] below the legal limits.

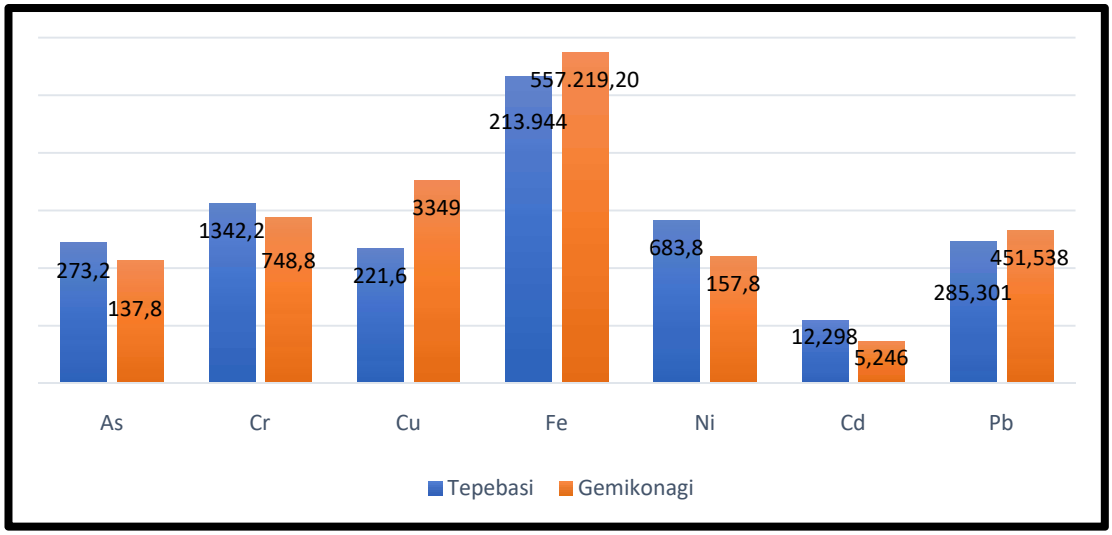

Figure 14: Soil heavy metal levels.

Groundwater metal levels for As, Cr, Cu, Fe, Ni, Cd,and Pbobtained from ICP-MS analysis were given in Table 8.

Table 8: Amount of heavy metals in groundwater and legal limits of heavy metals in water.

Metal	Region	Metal Level (ppm)[b]	Legal limit(ppm)	Reference
As	Gemikonagi	<0.001	0.01	USEPA 2001
	Tepebasi	0.002		
Cr	Gemikonagi	0.002	0.1	USEPA 2008
	Tepebasi	0.005		
Cu	Gemikonagi	0.001	1.3	USEPA 2001
	Tepebasi	0.013		
Fe	Gemikonagi	0.1	0.3	WHO 1996
	Tepebasi	0.089		
Ni	Gemikonagi	0.008	0.01	WHO 1996
	Tepebasi	<0.001		
Cd	Gemikonagi	<0.001	0.05	USEPA 2009
	Tepebasi	<0.001		
Pb	Gemikonagi	<0.001	0	EPA 2000
	Tepebasi	<0.001		

[b] Below the maximum allowed limits.

6. DISCUSSION

According to the results obtained from our study, arsenic levels in theblood is approximately 18 times higher than the maximum allowed level in Gemikonagi and 16 times higher in Tepebasi. Chromium and nickel are above the maximum limits. Although Nickel levels of blood from Tepebasi region are still higher than the legal limit, there is no significant difference between the maximum allowed level and the detected level. Thus, it can be accepted as negligible. On the other hand, copper and iron levels are below the maximum allowed levels, founding in the safe margins.

The analysis of data from the soil and water samples were also analysed. Arsenic, chromium and copper levels in soil are found to be above the permissible levels. To specify, mean copper level in the soil collected from Gemikonagi is approximately 66 times more than the Earth's average. This number is only 4 times in Tepebasi. Interestingly, lead showed thedifference between two regions. Obtained soil lead values from Gemikonagi are above the limit. On the other hand, soil lead values obtained from Tepebasi is below the limits. According to the water analysis, all heavy metals from both regions are below the maximum allowed limits. This indicates that groundwaters are safe for human use in terms of heavy metals.

Engrossing levels of arsenic were observed in Tepebasi region. It is known that the island of Cyprus naturally rich in heavy metals. However, this richness is subject to change depending on the geochemical structure of the rocks and soils. Gemikonagi is found in the mountainside of the Trodos Mountains. These mountains originated from thevolcanic structure. On the other side, Tepebasi has completely different soil and rock structure as it is geogenically and geochemically different from Gemikonagi. As some soil types are richer than in terms of arsenic and other heavy metals, this may be the cause of high arsenic levels in Tepebasi.

During the analysis, it was observed that one sample that was collected from the "zero" point from the CMC area showed an abnormal heavy metal amounts. For instance, copper level in the soil from that site is more than 100 times higher than Earth's average. Lead value of this site is above 700 ppm which is approximately 2

times higher than normal. According to these results, it can be said that CMC is still releasing toxic heavy metals to the environment.

During the study, blood is used to investigate the levels of heavy metals in human subjects. However, blood can only give acute exposure levels as heavy metals are attracted to sulfhydryl group-rich tissues (kidney, liver etc) and accumulate in that these tissues. Detecting high levels of heavy metals from blood samples resulted from acute exposure. Moreover, circulating heavy metal levels are influenced by avariety of factors making it hard to control. Due to the dynamics of heavy metal pollutants, a single blood test does not reflect the total body burden.

Recently, elevated environmental contamination with heavy metals generates an increasing ecological and public health concern globally. There is no doubt that uses of heavy metals in awide variety of applications such as agriculture, industry,and technology, cause a dramatic escalation of human exposure. Geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources still keep heavy metal emission to the environment. Areas which mining, foundries and smelters, and other metal-based industrial operations are located are under the risk of very prominent environmental pollution (Tchounwou, 2012). Heavy metal elements are persistent in the environment. According to this property, heavy metals have a high capacity to stay and pollute the environment for long periods of time.

Apart from being environmental pollution, chronic exposure to heavy metals is known to be toxic to thehuman population. Accordingly, mining areas and regions with high industrial activity are under the risk of along time heavy metal exposure. Heavy metals are capable of binding to vital components of the cell, for instance, DNA, RNA or structural proteins, and act as a genotoxin. Genotoxins are the compounds that lead to DNA damage by breaking either single-strand or double-strand (Zhang, 2017, Karakaya et al., 2005). Exposure to heavy metals environmentally, even if they are at lower concentrations, could cause DNA damages. The preliminary cause of DNA damage is the induction of ROS. Once the free radicals are formed, ROS collects electrons from primary free radicals and

converts them into secondary free radicals with a cascade of reactions. Cascade of events during the formation of secondary free radicals damage the cell in a random manner. Therefore, oxidative stress induced by heavy metal exposure may lead to DNA damages. Furthermore, environmental heavy metal exposure may also cause cellular changes. These changes may alter the balance between DNA repair mechanisms. Heavy metals favour the error-prone results during DNA repair. Thus, double-strand breaks cannot be repaired properly and increase the possibility of cancer risk (Morales et al., 2016).

To conclude, heavy metals act as environmental pollutants and harmful agents to biological systems. Thus, routinely monitoring is essential for the people who live nearby these sources or in the environmental that is naturally rich in heavy metals.
In order to evaluate chronic exposure and related toxic effects of heavy metals, genotoxicity studies will be conducted.

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ARAŞTIRMA AMAÇLI ÇALIŞMA İÇİN AYDINLATILMIŞ ONAM FORMU

(Araştırmacının Açıklaması)

Kronik ağır metal maruziyeti ve genotoksisite ile ilgili yeni bir araştırma yapmaktayız. Araştırmanın ismi "Kronik Ağır Metal Maruziyeti ve Buna Bağlı Olası Genotoksik Etkilerinin Gemikonağı ve Tepebaşı (KKTC) Populasyonunda Değerlendirilmesi"dir.

Sizin de bu araştırmaya katılmanızı öneriyoruz. Bu araştırmaya katılıp katılmamakta serbestsiniz. Çalışmaya katılım gönüllülük esasına dayalıdır. Kararınızdan önce araştırma hakkında sizi bilgilendirmek istiyoruz. Bu bilgileri okuyup anladıktan sonra araştırmaya katılmak isterseniz formu imzalayınız.

Bu araştırmayı yapmak istememizin nedeni, olası kronik ağır metal maruziyeti olduğu düşünülen çevrede yaşayan insanların maruziyet düzeyini belirlemek (biyoizlem) ve bu maruziyetin aynı popülasyon üzerinde olası genotoksik etkilerini araştırmaktır. Yakın Doğu Üniversitesi Eczacılık Fakültesi Toksikoloji Anabilim Dalları'nın ortak katılımı ile gerçekleştirilecek bu çalışmaya katılımınız araştırmanın başarısı için önemlidir.

Eğer araştırmaya katılmayı kabul ederseniz, sizden 10 ml kan örneği alınacak ve bir araştırma formu doldurmanız istenecektir.

Bu çalışmaya katılmanız için sizden herhangi bir ücret istenmeyecektir. Çalışmaya katıldığınız için size ek bir ödeme de yapılmayacaktır.

Sizinle ilgili tıbbi bilgiler gizli tutulacak, ancak çalışmanın kalitesini denetleyen görevliler, etik kurullar ya da resmi makamlarca gereği halinde incelenebilecektir.

Bu çalışmaya katılmayı reddedebilirsiniz. Bu araştırmaya katılmak tamamen isteğe bağlıdır ve reddettiğiniz takdirde size uygulanan tedavide herhangi bir değişiklik olmayacaktır. Yine çalışmanın herhangi bir aşamasında onayınızı çekmek hakkına da sahipsiniz.

Katılımcı	**Görüşme tanığı**	**Araştırmacı**
Adı, soyadı:	Adı, soyadı:	Adı soyadı, unvanı:
Adres:	Adres:	Adres:
Tel.	Tel.	Tel.
İmza	İmza:	İmza

ARAŞTIRMA AMAÇLI ÇALIŞMA İÇİN AYDINLATILMIŞ ONAM FORMU

(Katılımcının / Hastanın Beyanı)

Sayın Prof. Dr. Şahan Saygıtarafından Yakın Doğu Üniversitesi Toksikoloji Anabilim DalındaKronik Ağır Metal Maruziyeti ve Buna Bağlı Olası Genotoksik Etkilerinin Gemikonağı ve Tepebaşı (KKTC) Populasyonunda Değerlendirilmesi" konusunda bir araştırma yapılacağı belirtilerek bu araştırma ile ilgili yukarıdaki bilgiler bana aktarıldı. Bu bilgilerden sonra böyle bir araştırmaya "katılımcı" olarak davet edildim.

Eğer bu araştırmaya katılırsam araştırmacı ile aramda kalması gereken bana ait bilgilerin gizliliğine bu araştırma sırasında da büyük özen ve saygı ile yaklaşılacağına inanıyorum. Araştırma sonuçlarının eğitim ve bilimsel amaçlarla kullanımı sırasında kişisel bilgilerimin ihtimamla korunacağı konusunda bana yeterli güvence verildi.

Projenin yürütülmesi sırasında herhangi bir sebep göstermeden araştırmadan çekilebilirim. (Ancak araştırmacıları zor durumda bırakmamak için araştırmadan çekileceğimi önceden bildirmemim uygun olacağının bilincindeyim) Ayrıca tıbbi durumuma herhangi bir zarar verilmemesi koşuluyla araştırmacı tarafından araştırma dışı tutulabilirim.

Araştırma için yapılacak harcamalarla ilgili herhangi bir parasal sorumluluk altına girmiyorum. Bana da bir ödeme yapılmayacaktır.

İster doğrudan, ister dolaylı olsun araştırma uygulamasından kaynaklanan nedenlerle meydana gelebilecek herhangi bir sağlık sorunumun ortaya çıkması halinde, her türlü tıbbi müdahalenin sağlanacağı konusunda gerekli güvence verildi. (Bu tıbbi müdahalelerle ilgili olarak da parasal bir yük altına girmeyeceğim).

Araştırma sırasında bir sağlık sorunu ile karşılaştığımda; herhangi bir saatte, Prof. Dr. Şahan Saygı'yı 05338297333 (cep) telefonundan adresinden arayabileceğimi biliyorum. Bu araştırmaya katılmak zorunda değilim ve katılmayabilirim. Araştırmaya katılmam konusunda zorlayıcı bir davranışla karşılaşmış değilim. Eğer katılmayı reddedersem, bu durumun tıbbi bakımıma ve hekim ile olan ilişkime herhangi bir zarar getirmeyeceğini de biliyorum.

Bana yapılan tüm açıklamaları ayrıntılarıyla anlamış bulunmaktayım. Kendi başıma belli bir düşünme süresi sonunda adı geçen bu araştırma projesinde "katılımcı" olarak yer alma kararını aldım. Bu konuda yapılan daveti kabul ediyorum.

İmzalı bu form kâğıdının bir kopyası bana verilecektir.

Katılımcı	**Görüşme tanığı**	**Araştırmacı**
Adı, soyadı:	Adı, soyadı:	Adı soyadı, unvanı:
Adres:	Adres:	Adres:
Tel.	Tel.	Tel.
İmza	İmza:	İmza

YAKIN DOĞU ÜNİVERSİTESİ

SAĞLIK BİLİMLERİ ENSTİTÜSÜ-TOKSİKOLOJİ ANABİLİM DALI, LEFKOŞA-KKTC

"KRONİK AĞIR METAL MARUZİYETİNİN DNA HASARI İLE İLİŞKİSİNİN ARAŞTIRILMASI"

ANKET FORMU

Katılımcı no._____ Tarih ___/___/_____

Değerli katılımcı;

Bu çalışma, Yakın Doğu Üniversitesi, Toksikoloji Anabilim dalı tarafından kronik ağır metal maruziyeti ve buna bağlı olası genotoksik etkilerinin değerlendirilmesi amacı ile başlatılmıştır. Verilecek olan tüm cevaplar araştırma için kullanılacak olup, katılımcı kimlikleri gizli tutulacaktır.

Proje Yöneticisi: Prof. Dr. Şahan SAYGI, E-mail: sahan.saygi@neu.edu.tr

Araştırmacı: Ar. Gör. Kumsal KOCADAL

Yukarıdaki bilgileri okudum ve araştırmaya katılmayı kabul ediyorum. ☐

I. DEMOGRAFİK BİLGİLER

1.

YAŞ	
CİNSİYET	

2. Eğitim Düzeyiniz nedir? Lütfen uygun kutuyu işaretleyiniz.

İlkokul	
Ortaokul	
Lise	

Üniversite	
Yüksek Lisans	
Doktora	

3. Nerede çalışıyorsunuz?

4. Göreviniz nedir?

5. Kaç yıldır bu pozisyonda çalışıyorsunuz?

6. Daha once CMC'de çalıştınız mı?

Evet: Hayır:

Yanıtınız Evet ise soru 7 ye geçiniz

Yanıtınız Hayır ise soru 9 a geçinizNe zaman ayrıldınız?

7. Kaç yıl çalıştınız?

8. Ne zaman ayrıldınız?

9. Aylık geliriniz nedir?

	<1000 TL
	1000-3000 TL
	3001-5000 TL
	5001-7000 TL
	≥ 7001 TL

10. Nerede yaşıyorsunuz?

11. Kaç yıldır aynı adreste yaşamaktasınız?

12. Daha önce nerede yaşamaktaydınız?

II. ARAŞTIRMAYA ÖZGÜ BİLGİLER

13. Sigara içiyor musunuz?

Hiç içmedim		
Bıraktım		Soru 14'ü yanıtlayınız
Kullanıyorum		Soru 15 ve 16'yı yanıtlayınız

14. Bıraktınız ise ne zaman bıraktınız?

15. Hala içiyorsanız kaç sigara içiyorsunuz?

16. Ne kadar zamandır içiyorsunuz?

17. Sigara içilen ortamlarda sıkça bulunur musunuz?

Evet	
Hayır	

18. Alkol kullanır mısınız?

Evet		Soru 19'a gidiniz
Hayır		

19. Ne Sıklıkta alkol tüketirsiniz?

Nadiren	
Haftada 1 kez	
Haftada 2-3 kez	
Haftada 4-5 kez	
Haftada 6-7 kez	

20. Evinizde saksı bitki yetiştiriyor musunuz?

Evet	
Hayır	

Cevabınız evet ise lütfen kullandığınız toprak türünü belirtiniz.

Hazır Toprak	
Yerel Toprak	
Taşıma Toprak	

21. Bahçeniz var mı? Bahçenizde bitki yetiştiriyor musunuz?

Evet	
Hayır	

22. Daha önce Gemikonağı bölgesinde yaşadınız mı ya da bu bölge ile herhangi bir bağlantınız var mı?

23. CMC hakkında bir bilgiye sahip misiniz?

24. Evinizde kullandığınız kullanım suyu hakkında bilgi veriniz.

Hazır Su	
Şebeke Suyu	
Arıtılmış Su	
Kuyu Suyu	

25. Son 1 yıl içerisinde sürekli veya düzenli olarak kullandığınız ilaç, vitamin veya antioksidan var mı?

26. Son 1 yıl içerisinde röntgen, bilgisayarlı tomografi (CT) veya sintillografi gibi radyasyon içeren testlere tabii tutuldunuz mu?

Evet	
Hayır	

Cevabınız evet ise lütfen bilgi veriniz.

27. Sizde veya ailenizde genetik bir hastalık mevcut mu?

Evet	
Hayır	

Cevabınız evet ise lütfen bilgi veriniz.

28. Aileniz içerisinde kanser, organ yetmezliği, diyabet, nörodejeneratif hastalık (Alzheimer, Parkinson, Mültipl Skleroz, Erken Bunama) gibi hastalıklardan muzdarip veya vefat etmiş kişi veya kişiler var mı?

Evet	
Hayır	

Cevabınız evet ise lütfen bilgi veriniz.

29. Son 1 yıl içerisinde herhangi bir hormon tedavisi (tiroid, insulin, doğum kontrol ilacı) gördünüz mü?

Evet	
Hayır	

Tedavi görmüşseniz lütfen bilgi veriniz.

KATKI KOYDUĞUNUZ İÇİN TEŞEKKÜR EDERİZ.