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Monitoring Metal Containing Particulates Distribution on a College Campus Using Dandelion (*Taraxacum officinale*) Leaves as Receptors.

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Monitoring Metal Containing Particulates Distribution on a College Campus Using Dandelion (*Taraxacum Officinale*) Leaves as Receptors

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by

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ABSTRACT

Monitoring Metal Containing Particulates Distribution on a College Campus Using Dandelion (*Taraxacum Officinale*) Leaves as Receptors

by

Suresh Chandra Regmi

This study aims to determine the distribution of particulates carrying heavy metals at selected sites on a college campus using dandelion leaves as collectors. As a comparison, sites far away from the campus surrounding Bristol Motor Speedway Car Racing Stadium were also monitored. To reduce the probability of memory effects from the long-term atmospheric deposition or absorption of metals from soil a seasonal plant, dandelion, was used to monitor the metal contents. The metals monitored are cadmium, calcium, copper, chromium, iron, lead, and zinc. Fourteen sites were monitored and samples were collected once a week initially (growing time of the plant), and later at 4-week interval from 28th March to 31st August of 2007. The metal contents of the nitric acid digested and appropriately diluted samples were determined by flame atomic absorption spectrometry using the regular standard calibration curve and also the standard addition method. From the results obtained, and the careful log of the weather and human activities at the different sites, it is concluded that human activities played a major role in the distribution of metal-laden particulates. Also dandelion leaves were proven to be viable collectors of these particulates without memory effects and as indicators of current particulates generated rather than a long-term cumulative one.

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CHAPTER 1

INTRODUCTION

An environment is the combination of the various physical and biological elements that affect the life of an organism. We commonly refer to the environment as a whole but actually there are large numbers of environments. They may have impact of their own kind that may change with time and place, but all are intimately linked and in combination constituting the whole earth/atmosphere system. Similar is the case with their size, which may vary from microscopic to global, and may be subdivided according to their nature and the effect they produce. For example, the aquatic environment, the terrestrial environment, and so on. Human activities can build the environment such as a city or a park [1].

Environmental pollution is defined in different ways by different authors and the people of different fields. Here, I want to present some of the definitions. The American Heritage dictionary definition of pollution is "act or process of polluting or the state of being polluted, especially the contamination of soil, water or the atmosphere by the discharge of harmful substances" [2]. Pierce et al. have defined environmental pollution as the contamination of air, water, or food in such a manner as to cause real or potential harm to human health or well-being or to damage or harm on human nature without justification [3].

The ecologists consider pollution as any man-made addition that is not ecologically compatible to the existing environment. However, engineers consider these additions as pollution only if they precipitate an immediate adverse effect [4]. The environment dictionary considers environmental pollution as the contamination of the physical and biological components to the earth/ atmosphere system to such an extent that normal environmental processes are adversely affected [5].

The federal government of the United States of America has defined a pollutant as any substance in water, soil, or air that degrades the natural quality of the environment; offends the senses of sight, taste, or smell or causes a health hazard. The usefulness of natural resources is usually altered by the presence of pollutants and contaminants [6]. From some of the documents, it can be understood that the federal government is very sensitive about environmental issues. This congressional declaration of national environmental policy proves this fact – "The congress, recognizing the profound impact of man's activity on the interrelation of all components of the natural environment, particularly the profound influences of population growth, high density urbanization, industrial expansion, resource exploitation and new and expanding technological advances and recognizing further the critical importance of restoring and maintaining environmental quality to the overall welfare and development of man, declares that it is the continuing policy of the federal government, in cooperation with state and local governments and other concerned public and private organizations to use all practicable means and measures, including financial and technical assistance, in a manner calculated to foster and promote the general welfare, to create and maintain conditions under which man and nature can exist in productive harmony, and fulfill the social, economic and other requirements of present and future generations of Americans" [7].

Modern technological development normally means the consumption of more fuel. Although it has made our life more comfortable, our environment is affected by this development. Heavy metal is one harmful component present in the waste gas produced of fuel consumption. Due to industrial activities and automobile traffic, the environment is being contaminated by heavy metals [8].

Biological tissues have natural tendency to absorb heavy metals, and they generally release these heavy metals very slowly. This is also true for the environment. In total, heavy metals are persistent. Once in a system, they remain for relatively long periods [9].

The ecosystem has connected all the living and nonliving beings of the planet via the food chain. Once water, soils, and sediments are contaminated, the toxicity of heavy metals reaches plants, animals, and humans via the food chain. Soils near urban or industrial settlements have often been polluted by particulates carrying heavy metals, mainly through atmospheric transport. The dissolution and phase transformations of these particulates over time influence the bioavailability of the metals in the soil and the transport into the groundwater [10].

Water, sediment, and soil quality criteria for metals in most countries are based on total concentrations. But, it is now becoming more evident for regulatory authorities to understand that it is the actual metal species that determines mobility, bioavailability, and toxicity of a metal. Again, the metal speciation depends on the site-specific seasonal and spatial variations existing in a particular water, sediment, or soil system [11]. A study has shown that the concentration of heavy metals in the environment changes with the weather or the season. Heavy metal levels in mollusks are shown to change with seasons. It was found to be higher in autumn and winter compared with spring and summer [12].

Metals like cadmium, lead, and aluminum are toxic and may interact metabolically with nutritionally essential metals. Iron deficiency increases absorption of cadmium, lead, and aluminum. Lead interacts with calcium in the nervous system to impair cognitive development. Cadmium and aluminum interact with calcium in the skeletal system to produce osteodystrophies. Lead replaces zinc in heme enzymes and cadmium replaces zinc in metallothionein. Aluminum interacts with calcium in bone and kidneys, resulting in aluminum osteodystrophy. Calcium deficiency along with low dietary magnesium may contribute to aluminum-induced degenerative nervous disease [13].

A study of water birds shows that environmental contaminants such as heavy metals and other persistent chemicals are expected to exert their toxic effects on the developing embryo rather than on adults [14]. Hence, to understand actual influence of heavy metal pollution, more information on mortality rates and developmental anomalies in embryos is required. But there is so far little research going on in this field.

Effects of Heavy Metals on Human Health

Cadmium

Cadmium is a soft ductile metal, its usual valency is 2+, and its chemistry is similar to that of zinc. Cadmium is found as greenockite (CdS), but it is produced commercially as a by-product of production of other metals (zinc, lead, and copper) from sulfide ores. The major use of cadmium is electroplating metals to inhibit corrosion. Some compounds (CdS and CdSeS) are used in pigments, primarily for plastics, and as plastic heat stabilizers. A number of alloys of cadmium are used in soldering and bracing electrical contacts and other purposes. Cadmium is also used in batteries and fungicides and has other minor uses [15]. Although cadmium is a naturally occurring element, it is rarely found as a pure metal in nature. It is generally associated with oxygen, chlorides, sulfates, and sulfides. Carbonaceous shale, coal, and other fossil fuels are also sources of Cd. Volcanism is the largest natural source of Cd [16]. Anthropogenic sources of Cd in the soil and groundwater include the use of commercially available fertilizers and the disposal of sewage sledges as soil amendments [17].

The Agency for Toxic Substances and Disease Registry (ATSDR) reported that the average American ingests about 30 mg Cd per day. However, only about 1/10 of this amount is actually absorbed into the tissues. Intake of Cd can double if one smokes cigarettes because each cigarette contains about 2 mg Cd. Acute doses (10-30 mg/kg/day) of cadmium can cause severe gastrointestinal irritation, vomiting, diarrhea, and excessive salivation, and doses of 25 mg Cd/kg body weight can cause death. Low-level chronic exposure to Cd can cause adverse health effects including gastrointestinal, hematological, musculoskeletal, renal, neurological, and reproductive effects. The main target organ for Cd following chronic oral exposure is the kidney [17], where it tends to accumulate [18]. There is insufficient evidence to determine whether oral exposure to Cd increases the risk for cancer. However, the United States Department of Health and Human Services (DHHS) has stated that cadmium compounds may be carcinogenic [17].

In recent years the amount of cadmium in the external layers of the earth, in water, and in air has increased significantly due to technological developments. Cadmium is quite soluble, hence, plants collect it well. It contaminates the biosphere as well as food, which has a major influence on its presence in the human body. Cigarettes are another source of cadmium acquisition in human body. Lungs are the main way of cadmium absorption in the industrial regions. It induces DNA damage so it can be toxic even in small concentrations. Usually, cadmium concentration is estimated postmortem in kidney, liver, and lungs in patients from polluted regions. There is no particular organ acknowledged as the best indicator of cadmium in the human body. The methodology of cadmium analysis in blood is difficult due to a very low concentration (about 100 times lower than lead) and high chance of contamination during the blood collection. Therefore, adenoids seem to be useful material for monitoring levels of cadmium and other elements in children [19].

Lead

Lead is the most abundant heavy metal in the earth's crust. It occurs at an average concentration of 12.5 mg/kg in igneous rock, 7 mg/kg in sandstones, and 20 mg/kg in shells. The decay of uranium and thorium through geologic times has produced about one-third of the lead in the earth's crust today. Although the average concentration of lead in uncontaminated soils is approximately 17 mg/kg, levels of well over 10% have been found in ore bodies. Lead exists in several valency states (0, I, II, and IV). All of these, with the possible exception of Pb (I), are of environmental significance [20].

Lead is produced by smelting. Secondary lead is recovered from scrap, mostly from automobile storage batteries. Most lead is now used as the metal and the sulfate in storage batteries, gasoline additives, and pigments. Alloys are used as cable sheaths, as solder, as type metal, as bearings, as bullets, as radiation shielding, and in other products [21]. Early Egyptians used it to glaze pottery in 7000-5000 B.C., and the earliest known figurine predates 3800 B.C. Through recorded history, lead has been used in writing, warfare, aqueducts, water pipes, ornaments, and cooking utensils. Using lead in cooking utensils is responsible for endemic lead poisoning in a number of civilizations [20].

Lead is mobilized in the environment through natural weathering and leaching, the discharge of effluent from mines and other industries, release of solid wastes, and atmospheric emission. Although the amount of lead discharged from anthropogenic sources has gradually increased over the last 4000 years, worldwide dissemination of airborne lead did not become pervasive until the industrial revolution. Lead enters the environment from both anthropogenic and natural sources. Global emissions to the atmosphere amount to approximately 475×10^3 metric tons per year, of which 450×10^3 metric tons are from manmade sources [20].

Although lead is a poisonous metal, most human and animal tissues contain significant amount of lead. On exposure to lead, the human body absorbs lead slowly and incompletely from both the gastrointestinal tract and the respiratory tract. Because lead is excreted slower than it is absorbed, the metal is accumulated inside the human body. There are different permissible limits of lead in different tissues of human body. Total permissible limit of lead in skeleton is 145 mg, the highest among all parts of the human body. The smaller organs like kidney and heart have permissible limit of less than one milligram. Although above 80 micrograms per 100 mL of blood is considered as critical blood level, 60-80 micrograms per 100 mL indicates abnormal absorption of lead. Ninety percent of blood lead is found in erythrocytes. Symptoms of lead poisoning are due to circulating lead and not due to that in fixed deposit [22].

Plants also collect lead when exposed to excessive amount of it. A study on paddy plants grown in lead contaminated soil showed that increase in lead load in soil had variable effects on plant dry biomass and lead concentration in rice plants. The concentration of Pb was shown to increase significantly with increasing Pb level in soil, but its concentration in different plant parts was different, root> stalk> leaf> grain. The study showed that repeated use of metal containing chemicals, fertilizers, waste waters, and so on, causes soil pollution and contamination of agricultural soil that can produce long-term environmental and health implications. When environmental lead pollution increases, it finally goes to the human body through the food chain [23].

A study on the vehicle derived metal pollution in the UK showed that the risk of metal pollution on human health is higher than was understood. The risk is higher in complex mixture of metals than individual ones alone. Such is the case for lead. It is a neurotoxin and it is found to be more risky even at lower than initially considered safe levels; particularly with regard to brain and kidney damage, hearing impairment, and diminished cognitive development in children [24].

As with other heavy metals, release of lead to the environment is continually increasing with the industrial and modern technological development. Due to good conductivity, resistance to corrosion, and the reversible reaction between lead oxide and sulphuric acid, lead is widely used in batteries. A recent study showed that waste water from lead battery producers had pH ranging from 1.6 to 2.9 and soluble lead concentration in the range of 5 to 15 mg/L. There is a need for new technologies that can reduce heavy metals concentration to the environmentally acceptable level at affordable costs [25]. Most nations of the world have decided not to use leaded fuel; this decision has reduced environmental lead pollution dramatically. It can be observed directly by comparing the data obtained before and after the decision was made. Lead metal pollution is still high in those countries still using leaded fuel.

Zinc

Zinc is a bluish-white lustrous metal. It is brittle at room temperature but is malleable at 100 to 150^0 C. It is a reasonable conductor of electricity. It burns in air when heated strongly producing a white cloud of zinc oxide. Commercially zinc metal is available in many forms including dust, foil, granules, powder, pieces, nano-size activated powder, shot, and mossy form. Zinc is more resistant to moist air than Iron; hence, plating thin layers of zinc on iron or steel known as galvanizing helps to protect the iron from corrosion [26].

Zinc is found in highest amount near zinc mines and smelters, iron and steel foundries, electroplating shops, and city streets due to braking and tire-wear [24]. Release to surface and ground water are the greatest sources of zinc in the environment. Zinc is normally deposited on sediments from discharge of industrial waste water; the highest contamination is normally in the immediate area of emission.

Zinc is not particularly toxic and is an essential element in animals and plants growth. Hence, slightly higher amount of zinc intake may not be harmful. Large doses of zinc chloride are corrosive to the skin and can damage gasteroenteric mucous membranes. Growth retardation, faulty reproduction, anemia, and pancreatic fibrosis are some symptoms of chronic zinc toxicity. Acute zinc toxicity causes lassitude, bloody enteritis, diarrhea, and depression of the central nervous system which may result in paralysis in extreme case. Inhalation of zinc compounds causes metal fume fever and neumonities [27].

The zinc content of a normal 70 kg man ranges from 1.4 to 2.3 g. Different tissues contain zinc in different amounts. Certain portions of the eye, the male sex gland's excretions, brain, and bones contain higher amount of zinc than other tissues and fluids. The normal human zinc intake is about 10-15 mg per day, most of which is excreted in stool. Some zinc is excreted with urine and is independent of intake [28].

Zinc is an essential metal; it is abundant in eukaryotic cells and serves as one of the major regulatory ions for growth and differentiation. Zinc is an essential element because it is an important factor in the biosynthesis of enzymes and some proteins. High concentration of zinc in plants causes the loss of food production while low concentration may cause deformation on leaves and roots. The level of zinc in plant samples was found to decrease with decrease in automobile traffic density [29].

Zinc is one of the metals that can alter the lead induced toxicity. Zinc and lead compete for similar binding sites on a metallo-thionin like transport proteins. Hence, presence of zinc reduces absorption of lead from the gastrointestinal tract. In this way zinc influences both tissue accumulation of lead and susceptibility to lead toxicity [30]. A study on fish showed that zinc can bioaccumulate in liver. It has the ability to bind to metallothioneins, although copper has a greater affinity for proteins and is able to displace zinc. This can explain the low zinc content in liver as compared to copper. In slightly basic, anoxic marsh sediment environments, zinc is effectively immobilized and not bioavailable. A very high level of soluble zinc is present under well oxidized conditions and at pH 5 to 6.5. However, low abundances of soluble zinc are found at pH 8 under all redox conditions and at pH 5 to 6.5 under moderately and strongly reducing conditions [31]. Similar effects may be observed in human beings also.

A study on atmospheric emissions by steel plants showed very high emission of zinc into the atmosphere. Zinc is mainly associated with two phases. The first phase is insoluble, identified as Zn-Fe oxide similar to franklinite. The second one is easily soluble, contains calcium, and probably is associated with carbonates and is highly harmful to the environment and human health. Zinc metal in its soluble forms induces inflammatory response in lungs [32].

Calcium

Calcium is one of the alkaline earth metals, a group that includes soft and reactive metals. In pure form, it is a silvery white metal that is highly reactive and, hence, never found in its pure state in nature. It occurs in various compounds and in these forms calcium makes up 3.64% of all igneous rock, making it the fifth most abundant element in the earth's crust. Among metals, calcium is the third most abundant and is found in every region of the earth [33].

Calcium is an element critical to many body functions including bone growth and maintenance, muscle and nerve control, blood clotting, and blood pressure regulation. The total calcium content of human body is one kg and about 99% of it is localized in the skeleton as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. About two grams of calcium are present in extra- cellular fluid and one gram in intracellular fluid. Half of the calcium present in extra-cellular fluid is found in plasma. In plasma 40% of calcium is protein bound and of the remaining 60% about 46% is free (ionized) and 14% is associated or complexed with other ions. The ionized fraction, being the biologically active calcium fraction, involves in many physiological functions. Bone mass is built until the age of 29 years or so, after that one cannot build more bone by increasing calcium intake but a good intake of calcium and vitamin D and regular exercise can prevent bone loss.

Calcium is an important part of the human diet, and most populations get about half of their dietary calcium from milk and other dairy products. Soils in humid regions generally have less calcium than those in dry regions, and calcium is often added to the soil in humid regions to reduce acidity. Calcium levels in soil do not seem to affect human nutrition because the amount of calcium taken up by a plant depends much more on the nature of the plant species than it does on the calcium content of the soil. The amount of calcium in one's diet depends on what types of plants one eats but not where they were grown and what kinds of plants are eaten by the cows whose milk one drinks.

Most of the calcium is absorbed into the body from the intestines. Most of the calcium absorbed is excreted with the urine and minor parts are lost via epithelium, hair and nails. Body calcium requirement increases during growth and lactation; hence, efficiency of calcium absorption can increase more than 60% compared with the adult steady state conditions. The intestinal absorption of calcium is influenced by many factors that can ultimately affect the availability of calcium for body function as well as mechanisms of absorption. The ions like oxalate or phosphate can bind calcium within the intestinal canal; hence, high intake of phosphate can restrict calcium absorption [34].

Chromium

Chromium is the seventh most abundant metal in the earth's crust. In nature it occurs as chromite which is a compound formed by the combination of chromium with oxygen and iron. Chromium concentration in non-polluted water varies from 1-10 µM in fresh water and from 0.03 - 1 µM in oceanic waters. But due to anthropogenic sources the level of chromium is very high; for example, levels of chromium have been reported is as high as 1.5 and 48 mM for paper mill effluents and tanning waste waters, respectively [35].

Chromium is a toxic heavy metal. It usually comes in waste streams from various industries such as mining, metal cleaning, plating, dyeing, and metal processing. According to the World Health Organization (WHO) drinking water guidelines, the maximum allowable limit for total chromium is 0.05 mg/L. Normally the natural water contains chromium in the trivalent form, Cr(III), but due to human activities water is contaminated with hexavalent chromium (Cr(VI)), which is an acute carcinogen and more mobile, soluble, and toxic [36]). The concentration ratio of Cr^{3+} and Cr^{6+} depends on pH; hence, by adjusting the pH and using a reducing agent like activated charcoal the concentration of highly toxic hexavalent chromium can be reduced from the environment [37].

A study [38] on the effect of heavy metals on small mammals near a landfill site showed that this metal leached rapidly and was often organically complexed in the effluents becoming more bioavailable and mobile through the soil. According to the study, the highest concentration of chromium was found in the liver and kidney of small mammals near the landfill site. The toxic metals alter the immune response of animals as well as humans. In addition to the well documented and numerous toxic effect of chromium on various target organs, a number of studies show that acute and chronic exposure to inorganic chromium may result in impairment of immune functions in the experimental subjects. Actually, immuno-suppression appears to be a more subtle effect of exposure to heavy metals [39].

Chromated copper arsenate (CCA), an inorganic waterborne wood preservative, was widely used in many outdoor residential and commercial constructions, such as decks, telephone and electric poles, docks, picnic tables, playground structures, railway tracks, and landscape timbers. But its use was stopped in United State and Canada in 2003. However, existing structures built from CCA-treated wood are not affected by this action, and CCA-treated wood can still be used for industrial construction. Due to its mobility, chromium easily enters the food chain. Soil microorganisms and other fauna are impacted by the leaching of metals from CCA-treated wood structures into the soil. Most of the leached metals are expected to adsorb quickly to soil particle surfaces but may be desorbed into the soil solution after rainfall or irrigation events. Soluble metals are readily assimilated through the cellular membranes of many soil organism in concentrations that can lead to acute or chronic toxicity. When soil is ingested, it is absorbed by soil fauna, and metals on soil particle surfaces can be desorbed and assimilated in the animal's digestive tract [40]. Soil is a multi-component mixture forming an open biochemical system where physical, chemical, and biological reactions may occur. In this system, both matter and energy can also be exchanged with the surroundings.

Nutrient cycling occurs as a consequence of microbial activity and is especially important in the ecosystems where input of nutrients is low.

The annual chromate discharge worldwide is 239,000 tons as a result of numerous industrial activities such as the preservation of wood, leather tanning, and metal finishing. Hexavalent chromium emitted from these industries is transported to the environment, becoming a source of toxicity in the ecosystem. Many studies have shown that hexavalent chromium can be reduced to the trivalent form by organic and inorganic ions (e.g. elemental iron, divalent iron, sodium bisulfite) and humic substances, thereby reducing its toxicity due to a decrease in its concentration [41].

Accumulation of chromium in fish is not a serious problem because the fish are able to eliminate it from the body. In fish, chromium readily penetrates gill membranes by passive diffusion and concentrates at high levels in various organs and tissues. The site of toxic action during lethal chromium exposure to fish varies with different pH values [42]. This fact may be helpful to define the effect of chromium in human body.

Copper

Archaeological evidence suggests that people have been using copper for at least 11,000 years. Relatively easy to mine and refine, people discovered methods for extracting copper from its ores at least 7000 years ago. The Roman Empire obtained most of its copper from the island of Cyprus, from where copper's name originated [43].

Copper is a reddish brown heavy metal, highly malleable and ductile in nature. It is used in large amounts by the electrical industries in the form of wire. Copper is second only to silver in electrical conductance. Because it resists corrosion from the air, moisture, and seawater, copper has been widely used in coins [44].

Copper is an essential trace nutrient to all high plants and animals. In animals, including humans, it is found primarily in the bloodstream, as a cofactor in various enzymes, and in copper-based pigments. However, in excess amounts, copper can be poisonous and even fatal to organisms [45]. A study on marine organisms shows that copper is essential for various physiological processes. But at high concentrations, copper may enhance the formation of reactive oxygen species and subsequently initiate oxidative damage because it is a redox active transition metal [46]. High concentration of copper may increase oxidative damage to lipids, proteins, and DNA not only in marine animals but also in other animals including humans. The source of copper in street pollution is due to corrosion of the metallic parts of cars like engine wear, thrust bearing, and brush wear [29].

Heavy metals normally occurring in nature are not harmful to the environment because they play an essential role in tissue metabolism and growth of plants and animals like copper does. However, severe metal imbalances are toxic and marginal imbalances contribute to deformities and impede health. Metals of major interest in bioavailability studies, as listed by the US Environmental Protection Agency (EPA), are aluminium, berylium, arsenic, cadmium, chromium, copper, mercury, nickel, lead, selenium, and antimony. These metals were selected because of their potential for human exposure and increased health risk. Hence, although copper is one of the essential metals for animals and humans, it also is one of the harmful metal listed by EPA.

A study of the effect of heavy metal pollution in aquatic ecosystems using fish showed the competitive behavior of copper and calcium. Maximum concentration of heavy metals tend to be found in gills because they are central in the uptake of dissolved substances from the water. The highest level of copper was found in the liver which can be attributed to the binding of copper to metallothioneins (MT), which serves as detoxification mechanisms. The researchers found that the main environmental factor that affects copper toxicity in water is calcium concentration. This is a result of the competition that occurs between these two ions for binding sites in the tissue of the gills and other organs. The study showed that the sites with low levels of copper had high calcium levels. Copper in the alkaline conditions precipitates as the carbonate and is non toxic [42].

The copper slags and copper flotation waste generated from the copper industry are classified as “hazardous waste” according to current literature [47]. Copper flotation waste generally contains significant amounts of Cu together with trace elements of other toxic metals such as Zn, Co, and Pb. For every ton of metal production, about 2.2 tons of copper flotation waste is generated. All these copper slags and flotation waste are generally disposed without any prior solid waste treatment. The toxic metals present in these copper wastes reach underground water resources and surface waters by leaching with precipitation. In this way the highly dangerous pollutant finally affects human health and the surrounding physical environment.

Many common environmental pollutants, together with nuclear radiation, are recognized as genotoxic. Several heavy metals were found to have harmful effects on DNA. A common molecular mechanism in the toxicities of many xenobiotics is due to the production of reactive oxygen species, i.e. free radicals, which may result in a condition known as oxidative stress. Free radicals are produced in cells by ionizing reaction, by a variety of chemicals like heavy metals, and by normal metabolic processes. Copper is considered to be an important factor in the generation of free radicals and DNA damage in target cells [48].

Iron

Iron metal is a silvery, lustrous metal with important magnetic properties. It makes up 5% of the Earth's crust and is second in abundance to aluminum among the metals and fourth in abundance behind oxygen, silicon, and aluminum among the elements. It is also found in considerable quantity in the sun and many types of stars. Iron is a vital constituent of plant and animal life and is the key component of hemoglobin [49].

Little data are available on the effects on human health due to atmospheric pollution from iron and it is not considered a harmful metal in the list of the “inorganic pollutants” proposed by the World Health Organization. Some studies have reported that airborne iron can be associated with exacerbation of respiratory symptoms. Because iron from airborne particles is known to cause oxidative damage, other metals such as lead contained in particulate air pollutants could also generate reactive oxygen species leading to pulmonary injury [50]. In contrast, zinc has been known for its role in preventing free radical formation, so it could be related to protection from pulmonary injury, although zinc and iron are nearly equal in importance for human body.

A study on the metal pollution around landfill sites shows that iron is a redox sensitive element and, hence, generates hydroxide after oxygenation of leachates. In this way the leachates become an important trace metal carrier including iron that enters plant bodys through their roots and finally reaches to human body through the food chain [51].

CHAPTER 2

RESEARCH ON HEAVY METAL POLLUTION

Environmental pollution is a topic of current interest due to health issues. Hence, health organizations, research groups, research and development groups (R&D) of different industries, and governments are spending huge amounts of resources for research in this field. Heavy metal pollution is a major issue because heavy metals not only are directly related to health problems but also are genotoxic. Researchers all over the world are using a wide variety of receptors, sample treatment procedures, analytical techniques, and statistical methods to explain the results. Some recent research projects on heavy metal pollutions using different receptors and analytical techniques are summarized here.

A study on heavy metal pollution in the Czech Republic used soil samples to analyze heavy metal pollution. Not only industries affect the soil where they are active, traffic and other human activities also contribute. To assess the levels of nine heavy metals (Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and Zn) in public gardens, highways, and surroundings of the main roads accessing the city, four samplings were done per each annual season. The soil samples collected were first heated for 48 hours at 60^0C to remove the moisture before digestion with nitric acid. The sample solutions were then analyzed by flame atomic absorption spectrometry for Cu, Cr, Fe, Mn, Pb, and Zn and graphite-furnace atomic absorption spectrometry for Cd, Co, Cr, and Ni. Chemometric methods were used to analyze the data. They found that most of Fe and Co were related to the natural origin of soils, and Zn, Cd, Cu, and Pb were related to road traffic [52].

Alejandro and Jacint [51] studied the bioaccumulation of metals in animal tissues. They quantified the bioaccumulation of 10 metals and assessed several morphological and genotoxic parameters as biomarkers of pollution from the landfill of Garraf, Spain. The landfills were the main form of solid waste accumulations. Gaseous compounds like methane and carbon dioxide and some volatile organic compounds are common pollutants of landfills. The liquid effluents called leachates, produced by the decomposition of wastes or by interaction between wastes and rain water, were the sources of contamination for groundwater, adjacent soil, and even surface waters. The researchers dried the animal (white toothed shrew) tissues (liver, kidney, and blood) at 60^0 C till constant weight and digested them by nitric acid and perchloric acid. The concentration of Mg and Fe were determined by inductively coupled plasma optical spectrometer and Pb, Hg, Cd, Zn, Cu, Mn, Mo, and Cr by inductively coupled plasma mass spectrometer. The data were analyzed by three-way multivariate analysis of variance, Student's t-test, and Mann-Whitney test. By the comparison of samples from reference sites and landfill sites, they determined that there was no significant difference between males and females for any metals quantified. Specimens from the landfill site showed significantly more Pb, Cd, Zn, Mg, Cu, and Cr in the liver and Pb, Cd, and Cr in the kidney compared with reference specimens. Liver was found to be the accumulator of iron. The high metal concentrations observed in shrews from the landfill site suggested that a high exchangeability of metals from leachates produces high bioavailability of metals at levels that are potentially toxic for biota.

Sammut, Noack, and Rose conducted a study on the atmospheric emissions by a steel plant in France. Steel plants were the emitters of heavy metals to the atmosphere. Among the toxic metals present in atmospheric particulate matter, zinc is considered important and has direct relation with lung injury. They collected the dust (particulate) at the hearth cavity of the bag-house of the steel plant before the filtration system. The fraction of particulates of less than 50 µm was digested in an acid mixture (nitric acid, hydrochloric acid, and hydrofluoric acid), and the samples were analyzed by extended X-ray absorption fine structure (EXAFS) and French beam line emission (FAME). The analysis showed that the particulates were composed mainly of iron and aluminum and some alkaline elements like manganese, lead, chromium, and cadmium. They found that zinc present in the particulates was associated with two types of phases, the one containing soluble Ca and the other insoluble form close to franklinite ($Zn_xFe_{(1-x)}O_4$) where iron atoms were substituted by Zn [53].

Air borne particulate matters are derived from a wide variety of sources. Road traffic emission frequently is one of the major contributors to air-borne particulate matter concentrations. In research conducted in UK (54), samples for trace metal analysis were collected using mold. The particulates of different sizes were collected separately. Collection substrates were polypropylene-backed 0.5 µm PTFE filters. The samples were digested in aqua regia. The metals analyzed by ICPMS included Ba, Be, Bi, Ca, Cd, Ce, Cs, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Sr, and Zn. The data were analyzed by principle component analysis. In most samples Be, Ni, Se, and Cs were below detection limit. They found that none of the trace elements determined were adequate markers of road traffic emissions. The information for this research on particle size distributions is quite informative in relation to particle source types [54].

In an interesting research conducted in Poland, heavy metal pollution was analyzed by using bone as sample. Metals, as an inherent part of living organisms, can be classified as vestigial elements such as Cu, Mn, and Zn, which are indispensable for adequate metabolism, or as toxic elements including mainly Pb and Cd. Most of the metals in organisms accumulate in bones and can be liberated in some pathological conditions and during pregnancy. Bone tissue is considered to be an endogenic metal source reflecting the total metal content in an organism. In this research content of Ca, Mg, K, Fe, Zn, Cu, Pb, Cd, As, and Ag were evaluated in femoral head spongious bone of inhabitants of southern and middle Poland. All the femoral heads were acquired intraoperatively from patients submitted to the hip arthroplasty because of the coxarthrosis. The bone samples were microwave mineralized using 2 mL of pure spectrum HNO_3 and then the metals were analyzed by AAS. They found that the calcium content decreases as a function of age and there is a strong correlation between magnesium and calcium bone content. The concentration of iron in bones was found to be affected by diet. Significant geographical differences in femoral head lead and cadmium content were observed reflecting the degree of air pollution in the area [55].

Use of Vegetation as a Receptor for the Analysis of Heavy Metal Pollution

Al-Khlaifat and Al-Khashman [29] used the leaves of date palms (Phoenix dactylifera L.) to monitor the atmospheric heavy metal pollution in Aquaba, Jordan. Researchers studied the concentration of Fe, Pb, Zn, Cu, Ni, and Cr. Aquaba is a densely populated coastal city. Researchers collected sample of unwashed leaves from different parts of the city and from a rural site to use as control. They collected about 200 g of leaf from young date palms during July and August. Fifteen samples were collected from each of the industrial, urban, suburban, highway, and rural parts of the city and 8 were collected from a non polluted area about 20 km away from the city as a control. They found that the concentrations of different metals differed according to the strength and direction of wind, composition of dust, and pH values. The pH variation was from 5.88 in the industrial area to 6.32 in a suburban area near the airport. They also measured electrical conductivity of the samples. The mean metal concentration was in the order of Fe>Pb>Zn>Ni>Cu>Cr and the lowest mean concentration was in the control sample. Iron was found to have the highest concentration. Its mean value was highest (265 ppm) in industrial sites and lowest (39 ppm) at the control site. They concluded that the highest concentration of iron was due to the high industrial activity and absorption from the soil by the plant roots. The lowest lead concentration (41 ppm) they found was at the control site and highest (177 ppm) at highway sites. High lead concentration at the highway sites were mainly due to the motor-vehicle exhausts because Jordan used leaded gasoline and partly due to industrial activity. The concentration of metals in the leaves was in the proportion of industrial and human activities. They attributed the high Zn concentration in the leaves to it being an essential element for organisms. They attributed the high concentration of nickel found in their study to be due to the exhaust of motor vehicles because of its presence in gasoline and from abrasion and corrosion of vehicles. The lowest mean value of copper (6.32 ppm) was found in control samples and highest (39 ppm) was found in the samples collected from highways having heavy traffic. Corrosion of metallic parts was the reason for the high concentration. Chromium was the metal with the lowest concentration in the study area. The concentration of chromium observed was from the yellow lead chromate paint marking of the road, some industrial activities, and body erosion of automobiles.

In a study conducted in the UK [24], the researchers used paired geochemical and magnetic analyses of natural bio-monitors (curbside tree leaves) and air sample filters to examine contemporary sources of particulate pollution. They collected the leaves of the most abundant species of urban tree in that place, the birch (Betula pendula), from the road side over a 12-day dry summer period. Each sample consisted of six leaves from the outer part of the tree at a height of 1.5 m. The oldest leaves from the newest twig growth were collected so that they could get leaves of similar age and exposure time. In addition, leaf samples were taken from the same site birch trees at 30 cm intervals, from the ground to a maximum height of 2.1 m. The surface area of the leaves was calculated by digitizing their computer-scanned images. Air filter samples were obtained from the background site (200 m^3 of air sampled) and the closest possible site to the roadside (150 m^3 of air sampled, within 500 m of the roadside), using a high-volume air sampler. They found the highest pollutant values in leaves next to the uphill rather than the downhill side of the road. From this result they concluded that the major source of lead, iron, and magnetic loadings on the road side tree leaves was due to vehicle exhaust emissions rather than resuspended soil dust or tire, brake, or other vehicle wear. Analysis of leaves at different heights showed that in leaf particulates, lead and iron concentrations were highest at 0.3 m (i.e. small child height) and at 1.5–2 m (adult head height) above ground level. This result was in agreement with results of particulate study because samples collected by adjusting monitoring station collectors at 3 m above the surface significantly under-estimate curbside near-surface lead concentrations. These results indicated that vulnerable groups, especially young children, continue to be exposed to fine Pb and Fe rich vehicle-derived particulates. For the leaf leachates, Zn and Fe were found to show the highest roadside concentrations, with Mn, Ba, and Pb at lower levels, and Cd and Cr below their detection limits at all sampled sites. Background values for Fe and Pb were below detection limit.

In the Kola project (The Geological Surveys of Finland (GTK), Norway (NGU), and the Central Kola Expedition (CKE) in Russia) called Central Barent Survey, moss was used to monitor environmental heavy metal pollution. Moss is a rootless plant; hence, it takes up most of its nutrients directly from the atmosphere. Pollution monitoring programs using moss analysis started in Scandinavia in 1985. Initially, it was assumed that all elemental contributions came from the atmosphere but later on it was found that moss takes up elements from local dust sources. Hence, local site conditions could have an important effect on the reported chemical composition of a moss sample. In this research, 598 samples of terrestrial moss (*Hylocomium splendens* and *Pleurozium schreberi*) were collected from a 188,000 km^2 area of the Central Barents region (NE Norway, N Finland, NW Russia). The study analyzed moss for Al, B, Ba, Ca, K, La, Mg, Mn, Na, P, Rb, Si, Sr, Th, U, and Y concentrations. The study area included large industrial centers for Ni mining and smelting and some of the world's largest SO_2 emissions point sources. These are the Ni ore roasting plant at Zapoljarniy and the Ni smelter at Monchegorsk, which together accounted for about 300,000 t of SO_2, 1900 t of Ni, 1100 t of Cu, and 94 t of V_2O_5 emissions yearly. The sampling sites were selected by making the best use of existing knowledge of topography, earlier moss studies, geology, vegetation maps, soil maps, and regional geochemical maps. The average sampling density was 1 sample per 300 km^2. Samples were collected by thoroughly trained samplers in the three different countries and clean picked in the field using talcum-free plastic gloves. *Pleurozium schreberi* was collected where *H. splendens* could not be found. In some places, both species were collected to directly compare the results. For quality control, duplicate moss samples were collected. All samples were placed in pre-numbered, plastic-lined paper bags. The result of the study showed that the nature of the lithological substrate had a significant influence on the chemistry of moss growing on top of it. Another natural process affecting moss composition in the area was seaspray, which was most visible in the Norwegian part of the study area due to the presence of more coastal areas. The elements showing the highest influence of marine salts blown on the moss present on land mass were Na and Mg, with B, Sr, and to some extent Ca and U. The result showed a vast difference in concentration of B and K between two sides of the Finnish–Russian border. According to the authors' explanation, presence or absence of any other type of plant can influence the moss metal concentration. On the Russian side of the border the concentrations of B and K in moss were high due to the presence of high population of lichen because of lack of grazing. But the concentration of B and K was low on the Finnish side due to lower population of lichen as there was a lot of grazing activity. The study showed direct influence of anthropogenic dust and industrial emission on the moss metal concentration [56].

Plants can absorb metals in different ways. The main source of metal in the plants is the soil through roots that finally goes to the leaves. The metal deposited on the leaf surface can go inside the leaf via the stomata [57]. Many plants have the ability to accumulate heavy metal so that their metal levels are much higher than those in the air. Hence, time-averaged results can be more reliable than those obtained from direct determination of the pollutant concentrations in air for a short period. Considering this fact, Ondur and Dursun [58] decided to conduct a study on heavy metal pollution using vegetation as a receptor in Turkey. Levels of environmental pollution in some areas of Turkey were alarmingly high. Konya was one of the heaviest air-polluted cities, so they focused on Konya, which is located in central Turkey. Cedar tree (*Cedrus libani* A. Rich.) is one of the dominant tree species in the green area of Konya city. Hence, they used the needles obtained from cedar trees for their study. The needles were collected from eight sampling points in the Konya city center, four sampling points in residential areas, and three in the industrial areas. The needle samples were collected twice (December 2003 and April 2004) from young trees (about 10–15-years-old) and old trees (about 20–25-years-old). The age of the trees was determined from the documents of the Konya Municipality and the factories. The needle samples were obtained from each part of the tree and then were homogeneously mixed. During sampling wind direction was also considered. The samples were collected into clean cellulose bags separately and brought to the laboratory the same day. The results of this study showed that some heavy metals such as Pb, Cr, Co, Cu, V, and Cd were present at levels considered potentially toxic. Most of the metals studied were highest, and especially Cr, in the samples from Chrome–Magnasite Factory garden. Level of Cr, V, and Zn were between 20 - 90 ppm. These results showed that Chrome–Magnasite Factory emissions had high concentrations of metals that were directly collected in plant leaves, and indirectly from the soil polluted by the air pollution; this was the main source of heavy metal pollution in the study area.

To analyze the pollution created by a thermal power plant (TTP) in Turkey, leaves of different plants as well as soil samples were used. The Tunçbilek TPP, which was the focus point of study, is located in the Kutahya Province and has an established power of 429 MW. The main fuel used was lignite (about 7000 tons daily). Fuel oil and diesel oil were also used as auxiliary fuels. The samples were collected from locations to the east, west, north, and south of Tunçbilek TPP. One hundred forty tree leaf samples of five tree species (*Salix alba*, *Populus tremula*, *Robinia pseudoacacia*, *Quercus infectoria*, *Pinus nigra*) and 28 surface soil samples were collected during the fall leaf season of 2001. Tree leaves were representatively collected from heights of 1.5 and 2 m of the trees, and soil samples were taken at depths of 0–30 cm. Samples were collected from 28 locations, 7 locations for each principal direction. The results of the study were presented as the average of all eastern, western, and northern locations. For southern and southeastern locations, where the pollution values were highest, results were presented individually. It was found that the southern locations to the TPP, where the prevailing winds were directed, had the highest level of pollutant concentrations. Leaf and soil samples obtained from northern, eastern, and western locations of the TPP, outside the impact zone of dominant winds, had lower sulfur and heavy metal concentrations. To analyze their results, the metals were divided into three groups: Group I- non-volatile elements with high boiling points such as Mn and Fe; Group II- medium volatile elements such as, Co, Cr, Cd, Pb, and Zn; and Group III- highly volatile elements such as Hg and Se which are completely gaseous after combustion and do not condense within the furnace due to their low boiling points. The concentration of the medium volatile metals was found to increase gradually, followed by a sudden decrease in some locations of greatest accumulation. The reasons for this were that the bottom ashes were carried by wind resulting in heavy metal contamination close to the TPP. The high levels of Fe, Ni, and Cu in the region near TPP during study could be equipment and machines of workshops of the TTP [59].

Sample (Plant Tissue) Treatment Techniques

Normally, environmental samples containing trace quantities of metals can be determined by various spectroscopic or chromatographic methods. But, to use any of these techniques the sample should be in suitable form for that technique. Some of the major functions of sample preparation or sample treatment are: to dissolve all matrix and release all metals for analysis, to concentrate or dilute metals present in the sample to bring them into a concentration range suitable for analysis, and to separate a single analyte or a group of analytes from other species that might interfere in the analysis [60]. A common aim of sample treatment is the dissolution of the entire sample to produce a homogeneous solution. To fulfill all of the above goals, the simplest techniques are wet digestion in acid and dry sample ashing.

Dry Sample Ashing

Dry sample ashing is generally used for the digestion of samples containing organic matter that are being analyzed for nonvolatile metals. The most important advantage of this method is that it is good for removing organic matter and it is faster than the wet digestion methods. In open vessel dry ashing method the sample is placed in a crucible made of silica, porcelain, platinum, or pyrex glass and is ignited in a muffle furnace. The major drawbacks of the method are the possible loss of some elements by volatilization, contamination of sample by air borne dust, as it must be left open to the atmosphere, and irreversible sorption of the analytes into the wall of the vessel. But, losses from volatilization of analyte can be minimized by using minimum temperature at which ashing can take place. Dry ashing is suitable for nutrititional elements in food such as Fe, K, Ca, Mg, and Mn. If fats and oils are present in large amount, a preliminary treatment may be required before dry sample ashing [61].

In the Aquaba, Jordan study [29] palm leaf samples were dried in an oven at 80^0 C for 24 h. Then the samples were milled in a pretreated micro-hammer cutter and sieved in a 0.2 mm sieve. The samples were then stored in clean self-sealing plastic bags. Accurately weighed (2 g) powdered samples were preashed on a hot plate until all fumes disappeared. The samples were then ashed in a muffle furnace at 480^0 C. The ash was then dissolved in 10% nitric acid, filtered into 25-mL polyethylene volumetric flasks through 45 µm filters, and diluted to the mark with 1% (v/v) nitric acid solution. Metal concentrations were measured using a Perkin-Elmer Model 300 atomic absorption spectrophotometer with air-acetylene flame and hollow cathode lamps.

For the determination of selenium concentration in colored rice, researchers [62] used the dry sample ashing method. But they also combined wet digestion during ashing of the rice sample using $Mg(NO_3)_2 \cdot 6H_2O$ as ashing aid. The flour of rice samples was dried in oven at 60^0 C for about 1 day untill constant weight was obtained. One gram of the dried flour was weighed accurately then mixed with 10 mL concentrated HNO_3 (65% w/w) and 4.0 g of $Mg(NO_3)_2 \cdot 6H_2O$ in a beaker. The beaker was covered with a watch-glass and the sample allowed to predigest at room temperature overnight. The predigested material was heated at about 373 K in a sand bath and slowly evaporated to dryness. The temperature of the sand bath was then raised to 473 K until no more fumes evolved. The beaker was finally placed in a muffle furnace at 723 K for 30 min. The white residue was cooled and dissolved in 7 mL of 6 M

HCl and the beaker was then heated at 373 K in a sand bath for about 10 min to reduce Se(VI) to Se(IV). After that the solution was transferred to a 25-mL volumetric flask quantitatively and diluted to make up the volume. The solution was then analysed by HG-ICP-AES and cathodic stripping voltametry.

Wet Sample Digestion

This is the most common and simplest digestion method. Samples are digested in an open flask, pressurized sealed container, or microwave. Samples are usually digested in a strong acid. In the case of an organic matrix, an oxidizing mixture is used to destroy the entire organic matrix and solubilize the sample, which gives a clear solution containing metals. Due to the need of forming the most soluble salts, nitric acid is commonly used. Inorganic samples, soils, sediments, ores, rocks, and minerals may be digested in dilute or concentrated acids or mixtures of acids. For vigorous digestion, hydrofluoric acid is used. For even greater dissolution power, mixtures of concentrated acids with oxidizing agents or with hydrofluoric acid are used. Aqua regia, a mixture of concentrated hydrochloric acid and nitric acid, or sulphuric acid and hydrogen peroxide, are the common mixtures used. Microwave digestion has several advantages over the open container digestion method. The specially designed sealed containers used in this technique eliminate the chance of air borne dust contamination and reduce evaporation and loss of volatile metals. The electronic controls on modern microwave digesters allow very reproducible digestion conditions. Another modern technique used in wet digestion method is pressure ashing in which the sample is heated with an appropriate digesting reagent in a sealed container to a certain pressure for complete dissolution of the sample [63].

In the study conducted by Maher and co-workers [24], the leaves of birch tree were leached in 25 mL 1% HNO_3 and left in covered bottles for 72 h. All glass and plastic wares were washed with detergent, soaked in 1% HNO_3 for 24 h, and rinsed with high-purity 'MilliQ' water before use. Sample leachates were filtered through 0.2 µm filters and then analysed by ICPAES for Fe, Pb, Zn, Mn, Ba, Cd, and Cr.

In the Kola project [56], all the samples were dried in their original bags at room temperature to avoid Hg loss. For precision and accuracy, all samples were analyzed in one laboratory in Rovaniemi. The samples were inspected and fine-cleaned where necessary before milling in a centrifugal mill with Ti blades (Fritsch Pulverisette 14), to pass through a 1 mm Ti sieve. During analysis, an analytical duplicate was run at a rate of 1 in 10 samples. Certified reference material provided by the National Institute of Standards and Technology (NIST), USA (Peach Leaves CRM 1547) and by the Finnish Forest Research Institute (METLA), Finland (moss 183) were used for quality control. All the samples were digested in a microwave oven in concentrated HNO_3 and analyzed using the ICP-AES for Ca, K, La, Mg, Mn, Na, P, Si, Y, and ICP-MS for Al, B, Ba, Rb, Sr, Th, U.

In the study conducted by Onder and Dursun [58] nitric acid wet digestion was used. After removing any traces of soil and other plant materials from the samples, they were carefully washed three times with demineralised water to remove adhering particles. The samples were then oven-dried at 70 °C for 48 h and finely grounded and triplicate 0.5-g samples were accurately weighed and digested with concentrated HNO_3 in a microwave system. The metals in the extracts were analyzed ICP-AES.

For the analysis of pollution caused by a thermal plant, unwashed leaf samples from all trees were dried for 24 h at 105 °C. Surface soil (0–30 cm) samples were dried for 3 h at 105 °C. Coal samples were crushed and dried at 105 °C to constant weight. The samples were then filtered using a nylon sieve (0.5 mm). Accurately weighed samples of 0.5 g each were digested in a microwave digestion unit using $HClO_4$:HNO_3:HCl acids of 1:3:5 proportions for leaves and of 1:2:5 for soil and coal, respectively, at 140 °C for 1 h. The samples were then filtered and diluted to 100 mL with 0.1 M HCl. Heavy metals were determined by flame AAS [59].

Statistical Analysis

In the Aquaba, Jordan study [29] Pearson's correlation coefficient was used to measure the correlation between logarithms of the metal concentrations. They found high correlation coefficients between Pb and Cu, Ni and Zn, and between Zn and Fe, indicating the common source of these metals. There was no meaningful correlation between Cr and Fe. Another statistical method used was one way analysis of variance (ANOVA), which showed that there were significant differences in Pb, Cu, and Ni concentrations in palm trees sample collected from different sites and no difference in the rest of the metals. Principle component analysis (PCA) indicated that Pb, Cu, Zn, and Ni had a similar source. In the Kola project, all graphics and maps were produced by using the DAS program based on exploratory data analysis (EDA) methods [56].

Onder and Dursun [58] used ANOVA to compare each heavy metal (Pb, Cu, Zn, Co, Cr, V, and Cd), each sampling site, needle age, and inter-relationship between the sampling site and needle age for both year samples separately. An F test was done in the level of 1% and 5%. For the heavy metal having significant F value, the Duncan Test was done. ANOVA and Duncan's multiple range tests were performed using the MSTAT-C. P.C. package program.

CHAPTER 3

ANALYTICAL TECHNIQUES

Flame Atomic Absorption Spectrophotometry

The modern form of AAS was developed by the Australian chemist Alan Walsh led team during 1950s. The technique typically makes use of a flame to atomize the sample, but other atomizers such as a graphite furnace have also been used. This is a technique for elemental analysis in liquids. Metallic species can be determined in both organic and inorganic samples. It is a sensitive technique that can easily determine the concentration of most elements at the parts-per-million (ppm) levels. If lower detection limits at the ultra trace levels are required, a graphite furnace is used as the atomization source (GFAAS), replacing the flame [64].

In atomic absorption, the atomic vapor of the analyte sample is obtained by the thermal energy of the flame. When the compound is introduced into a flame, it first vaporizes and partially or wholly dissociated into its elements in the gaseous form. Some of the atoms can absorb energy from the flame and become excited to a state from which they can emit characteristic radiation on returning to the lesser excited or ground state. If an external radiation source is used to supply radiation of a given wavelength some of these atoms can absorb this characteristic wavelength that corresponds to the atomic absorption spectrophotometry.

The quantity of interest in atomic absorption measurement is the amount of light at the resonance wavelength that is absorbed as the light passes through a cloud of atoms. As the number of atoms in the light path increases, the amount of light absorbed also increases.

When the resonance wavelength of intensity I_0 is focused on the flame containing ground state atoms, the initial light intensity is decreased by the amount determined by the concentration of atoms in the flame. The light is then directed to the detector where the reduced intensity, I, is measured. The amount of light absorbed is determined by comparing I and I_0. The absorbance is given by the formula- $A=Log\ (I_0/\ I)$.

According to Beer-Lambert law, $A=\epsilon bc$, "ϵ" is a constant called molar absorptivity that is characteristic of the absorbing species for the wavelength of light, "b" is the length of light path, and "c" is the molar concentration of the absorbing species. This relation shows that the absorbance and the concentration of the absorbing species are directly proportional [65]. When the absorbance of standard solutions containing known concentrations of analyte are measured and plotted against concentration, a straight line graph called a calibration curve is obtained. After such a calibration is established, the absorbance of solutions of unknown concentration may be measured and the concentration is determined from the calibration curve. In modern instrument, the calibration curve can be made within the instrument to provide a direct readout of unknown concentration.

Graphite Furnace AAS

Graphite furnace atomic absorption spectrometry (GFAAS) uses a graphite coated furnace to atomize the samples, it is also known as electrothermal atomic absorption spectrometry (ETAAS). The method is simple, quick, and applicable to a large number of metals in environmental samples. Samples are deposited in small graphite or pyrolytic carbon coated graphite tube, which can then be heated to vaporize and atomize the analyte [66].

Atomic Emission Spectroscopy

Atomic emission spectroscopy, also called optical emission spectroscopy, is a type of spectroscopy in which a sample is subjected to a high energy thermal environment to produce excited state atoms capable of emitting light. Quantitative measurement of this emission from excited analyte atoms allows its concentrations to be determined. Analyte atoms in solution are aspirated into the excitation region where they are desolvated, vaporized, and atomized by a flame, electrical discharge, or plasma. The thermal energy present in the atomization sources provides sufficient energy to promote the atoms into high energy levels. The atoms in the excited state decay to lower energy levels by emitting radiation. The emission spectrum of an element consists of a collection of the allowable emission wavelengths, commonly called emission lines because of the discrete nature of the emitted wavelengths. The spectra of samples containing many elements can be very complex, and spectral separation of nearby atomic transitions requires a high-resolution spectrometer. This emission spectrum can be used as a unique characteristic for qualitative identification of an element. Because all atoms in a sample are excited simultaneously, they can be detected simultaneously using a polychromator with multiple detectors. This ability to simultaneously measure multiple elements is a major advantage of AES compared to atomic absorption spectroscopy [66].

Emission techniques can also be used to determine how much of an element is present in a sample. For a "quantitative" analysis, the intensity of light emitted at the wavelength of the element to be determined is measured. The emission intensity at this wavelength will be greater as the number of atoms of the analyte element increases. The technique of flame photometry is an application of atomic emission for quantitative analysis. As in AAS, the sample is first treated to convert the analyte into solution. Liquid samples are generally nebulized and carried into the excitation source by a flowing gas. If the sample is in solid state the easiest way is to convert it into solution but solids can also be directly vaporized and excited by a spark between electrodes or by a laser pulse [67].

Inductively Coupled Plasma Mass Spectrometer (ICPMS)

Inductively coupled plasma mass spectrometry is an analytical technique used for elemental determinations. In this technique, an inductively coupled plasma source combines with a mass spectrometer. This technique was commercially introduced in 1983. Among the elemental analysis techniques such as atomic absorption and emission spectrometry, ICPAES etc. ICPMS is superior. Its detection limit is better than that of GFAAS and it has higher throughput than GFAAS. Due to the high temperature of ICP source, it has less matrix interferences and it can provide information about isotopes.

The inductively coupled plasma source consists of three concentric quartz tubes through which a stream of argon gas flows. The diameter of largest tube is often about 2.5 cm. At the top of this tube is a water cooled induction coil that is powered by a radio-frequency generator capable of producing 0.5 to 2 KW of power at about 27 or 41 MHz. Ionization of the flowing argon is initiated by a spark from a tesla coil. The resulting ions then interact with the fluctuating magnetic field produced by the induction coil; hence, a very high temperature plasma torch is formed by ohmic heating. Samples are carried into the torch with the help of argon flow through the central quartz tube where positively charged ions are formed. The ions from the ICP source are then focused by the electrostatic slits into the mass spectrometer. Once the ions enter the mass spectrometer, they are separated by their mass-to-charge ratio and recorded as electronic signals. This can be done by different type of mass spectrometers [68].

Colorimetry

Colorimetry is a form of spectroscopy that measures the response of an atom or molecule when exposed to an electromagnetic radiation of certain wavelength. In colorimetry only the visible region of electromagnetic radiation is used; hence, this method is applicable for those elements that can absorb radiation in visible region. Colorimetry can provide a wealth of information on colored solutions. It is a quick and non-destructive method and can be used to determine the concentration of analyte in solution accurately [69].

In colourimetry, an incident light wave of a certain wavelength and intensity is passed through the solution containing the analyte. A certain fraction of the light intensity is absorbed by the solution (only a negligible fraction is scattered) and the remaining portion is transmitted, which is measured. By comparing the incident intensity to the transmitted intensity, the absorbance, A, can be determined for that wavelength of light. A substance that transmits most of the light at a particular wavelength will have a low absorbance at that wavelength. Hence, a scan is made to obtain the spectrum to determine wavelength at which the analyte has maximum absorbance (λ_{max}), which provides qualitative information about the analyte present in the solution. Once the λ_{max} is known or the identity of the substance has been established, its concentration in solution can also be determined by colorimetry [70].

Neutron Activation Analysis (NAA)

Neutron activation analysis was discovered in 1936 by Hevesy and Levi. It is a sensitive analytical technique for both qualitative and quantitative identification of the elements present in the samples. It can be used for multi-element analysis of major, minor, and trace elements in samples.

When a neutron interacts with the target nucleus, a non-elastic collision takes place. As a result a compound nucleus forms in an excited state. The compound nucleus almost instantaneously de-excites into a more stable state by the emission of one or more characteristic gamma rays. But, during this phenomenon a radioactive nucleus may result that again de-excites (or decays) by emission of one or more characteristic delayed gamma rays but at a much slower rate. Hence, with respect to the time of measurement, NAA falls into two categories: (1) prompt gamma-ray neutron activation analysis (PGNAA), where measurements take place during irradiation, or (2) delayed gamma-ray neutron activation analysis (DGNAA), where the measurements follow radioactive decay.

The basic requirements to carry out an analysis of samples by NAA are a source of neutrons, instrumentation suitable for detecting gamma rays, and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei. Normally, solid sample is preferred for NAA and may be obtained from dilute aqueous samples by precipitation methods [60]. Different types of neutron sources: reactors, accelerators, and radio isotopic neutron emitters, are available for NAA; among them nuclear reactors producing neutrons from uranium fission offer the highest available sensitivities for most elements. The instrumentation used to measure gamma rays from radioactive samples generally consists of a semiconductor detector, associated electronics, and a computer-based multi-channel analyzer (MCA/computer). Most NAA labs operate one or more hyper pure or intrinsic germanium (HPGe) detectors that operate at liquid nitrogen temperatures (77 K) by mounting the germanium crystal in a vacuum cryostat, thermally connected to a copper rod or "cold finger" [71].

X-ray Fluorescence (XRF)

This is an analytical technique widely used in elemental analysis and chemical analysis particularly in investigation of metals, glass, and ceramics. When materials are exposed to a short wavelength x-rays or gamma rays, one or more electrons of the component atoms are ejected from the inner orbital of the atoms creating a hole. This makes the atom unstable and the electrons from the outer orbital fall into the inner orbital to fill the holes, which results in the release of energy in the form of a photon. The energy released is equal to the energy difference between the orbitals and is characteristic of the element. By measuring the energy released, elemental composition of a sample can be determined. In this technique, the word fluorescence is applied to the phenomenon in which absorption of higher energy radiation results in re-emission of lower energy radiation. Because the method is fast, non-destructive to the sample, and can be used to identify and determine the concentration of element present in solid, powdered, and liquid samples, it is the method of choice for field applications and industrial materials production [72].

For X-ray fluorescence, the solid samples may require dilution with a solid buffer material to produce less variation between samples and standards reducing the matrix effect [60]. While returning to the stable state an atom can sometime eject one of the outer electrons instead of releasing X-ray (photon). The ejected electron is called an “Auger electron”. This process is a competing process to XRF. Auger electrons are more probable in low atomic weight elements. The use of a primary X-ray beam to excite fluorescence from the sample was first proposed by Glocker and Schreiber in 1928. Since then, the technique has been modernized and now it is a major analytical technique to quantify elements heavier than sodium [73].

Anodic Stripping Voltammetry

Anodic stripping voltammetry (ASV) is an electrochemical method for trace metal analysis. In this method mercury electrode is held at a negative potential to reduce metal ions in solution and form an amalgam with the electrode. After sufficient interaction time the amalgam carries maximum concentration of analyte metal. They are re-oxidized into solution ("stripped") from the electrode by increasing the potential of the electrode. Any metal that forms a stable amalgam with mercury can be analyzed by this technique. The pre-concentration step permits analysis of very low levels of metal ions. The subsequent analysis step can be done in a number of ways, generally by linear sweep (DC) voltammetry [74]. Quantitation is achieved by standard additions. This is an example of an ultra-sensitive analysis. If all the reagents are ultrapure and the measurement is carried out with extreme care, highly accurate results can be obtained by this technique [75].

Quantitative Analysis Method

In chemical analysis, mass, time, volume, and some other parameters can be directly measured. But, chemical concentrations are determined by indirect measurements, which are based on direct measurements together with calibration. In other words, the concentration of an unknown sample is determined with the help of relationship between the content of the sample and response of assay method. In flame atomic absorption spectroscopy the calibration can be done in two ways [76].

External Standard (Calibration Curve) Method

In this method a series of standard solutions are prepared by diluting the standard stock solutions. Then the standard solutions are nebulized into atomic absorption spectrometer flame to measure the absorbance. The plot of absorbance versus concentration gives a straight line calibration curve as predicted by the Beer- Lambert's law. In the same setting the unknown samples are also nebulized and the absorbance determined. The concentration of the unknown samples can now be determined by interpolation into the calibration curve [76].

The calibration curves for different metals are given in Appendix B. For accurate determination of the concentrations of unknown samples by this method, the concentration of the unknown sample should be in the concentration range of the calibration curve. Hence, the sample solution should be diluted previously to fit into the concentration range. If the concentration of one of the standard solution is very high the calibration line may deviate towards the concentration axis, showing deviation from the Beer- Lambert law. If one of the unknown sample has a very high concentration, then for the same reason or due to interferences it may deviate from the calibration curve and accurate absorbance may not be obtained. This is the main shortcoming of external standard method.

Standard Addition Method

In the presence of interferences the calibration curve method for AAS analysis may not give accurate concentration of the unknown sample. Presence of a standard solution and an unknown sample in different matrix environment and difference in viscosity of the two type of solutions are reasons for this deviation. Standard addition method can solve this problem. In this method, known volumes of the standard solution are added to aliquots of the unknown sample solution so that the sample and the standard solutions remain in the same matrix and viscosity environment. Experimentally, at least two different volume of known concentration of the analyte standard solution are added to aliquots of the unknown sample. Another aliquot of the sample is also prepared without the addition of the analyte standard solution. All of them are nebulized in AAS and plotted for concentration added versus absorbance. When the graph is extrapolated, the point at which the straight line meets the negative concentration axis gives the exact concentration of the analyte in the unknown sample [76].

Objectives of the Research

Modern technological development has not only improved the living standard of human beings but also has made the future more secure. But there is a dark side of technological development, which is pollution. Each and every technology has generated some kind of pollution. Among all these different types, air pollution is most common. Heavy metal pollution is becoming a major concern due to their direct effect on human health as discussed in Chapter 1. Hence, to find out the most effective method of monitoring atmospheric heavy metal pollution is an important research activity. From the review of different studies discussed in Chapter 2, the most economical method of monitoring atmospheric heavy metal pollution involves the use of vegetation as receptors. Many studies associated traffic densities with metal concentrations found in vegetative species along specific highways. Use of vegetation as a receptor in environmental pollution is of great importance because of their availability and easy sample treatment process. Use of small seasonal plants like dandelion may be more significant because many of them grow readily in the wild and at many locations.

Because of their widespread applications, metals are found in our living environment where they should not. To conduct research cost is a major concern. Hence, it is important to find a receptor that is free of major and complex matrix, to determine an easy and economical way of sample treatment, and to use a cost-effective instrumental method that can analyze the collected samples with good precision and accuracy. If these ideal requirements can be achieved, the pollution can be monitored locally and a remedy found.

Considering above discussed facts, we want to achieve the following objectives in this research project.

1. To determine if the seasonal plant dandelion (Taraxacum Officinale) can be used as a receptor to monitor the atmospheric heavy metal pollution.
2. To determine if dandelion is capable of capturing the majority of particulates bearing heavy metals, such that they can be detected by atomic absorption spectrophotometer.

3. To determine the effect of weather conditions, nature of location of the site, and human activities such as use of motor vehicles, use of pesticides, and construction work on heavy metal pollution.
4. To determine the distribution pattern of the metal containing particulates around the locale chosen.

We selected the university area for our study. Our research results will show us how much metal we are inhaling when we come to the university. This is one of the exciting aspects of this research project.

CHAPTER-4

MATERIALS AND METHODS

Receptor-Dandelion

The seasonal plant dandelion was used as a receptor in this research project because of its wide availability in research sites and its good receptor characteristics in capturing particulates laden with heavy metals. Dandelion (Taraxacum Officinale) is a flowering plant. Although dandelion is found almost everywhere, it is native to Europe and Asia and favors temperate climate. It is tolerant of a variety of sites and soil. It has generally a long growing season, flowers from March through frost, and in warm climates it grows throughout the year. It has thick tap root, long leaves, and a yellow flower. The name dandelion comes from French “dent de lion” meaning lion’s tooth which represents its toothed leaves. The specific name “officinale” refers to it as a medicinal herb, but it is normally considered as weed. This plant can be eaten, cooked as soup, or as salad; it is very close to mustard green. Dandelion leaves are high in vitamin A, vitamin C, and iron [81].

Dandelions have long leaves with rough surfaces and a flowering head, thus they are capable of capturing various metal containing particulates. This plant is seasonal, so there is minimum probability of accumulating metals from soil and long-term memory effect. Samples are also collected from control sites to observe the influence of absorption of metals by the plant from soil.

Reagents Used

The reagents used in this research project are:

1. 1000 ppm standard plasma pure stock solutions of cadmium, calcium, chromium, copper, iron, lead, and zinc, Leeman Lab, Hudson, NH.

2. Trace metal grade nitric acid manufactured by Seastar Chemical from Fisher Scientific, Pittsburgh, PA.

Instrumentation

The samples were analyzed on Perkin Elmer, Model 280 atomic absorption spectrophotometer manufactured by Perkin Elmer Corp. Norwalk, CT. Fisher Isotemp Oven and hot plates manufactured by Fisher Scientific, Fair Lawn, NJ, were used to dry the dandelion leaves and heat the sample with nitric acid.

Lamp, Slit, and Wave Length Used

Hollow cathode lamps of each metal manufactured by Perkin Elmer Corp. Norwalk, CT, were used. Wave lengths and slits were used as recommended by the manufacturer. The slit used is 0.2 nm for iron and 0.7 nm for all other metals. The wave lengths of radiation used are: 422.7 nm for Ca, 228.8 nm for Cd, 357.9 nm for Cr, 324.8 nm for Cu, 248.3 nm for Fe, 289.3 nm for Pb, and 213.9 nm for Zn.

Statistical Software Used

For the analysis of data by two way ANOVA from SPSS published by SPSS, Chicago, IL was used.

Experimental Procedure

Sampling Sites

Samples were collected from March 28th to August 31st. Fourteen sampling sites were selected as given in the Table 1.

Table 1. Sampling Sites: Sites A, B, and E are from the highway, K is a control site, M and N are far from the campus at Bristol.

S.N.	Sample code	Sampling site (location)
1	A	State of Franklin four-way near Mc Donald.
2	B	State of Franklin entrance of Veterans hospital.
3	C	Parking lot of Veterans hospital.
4	D	Park of Quillen college of Medicine, ETSU.
5	E	State of Franklin four-way between Kroger and Whites.
6	F	Student parking lot, ETSU, near CPA
7	G	Brown hall courtyard.
8	H	ETSU foundation (on the side of Carillon.).
9	I	Parking lot in front of D.P. Culp center.
10	J	New Dorm construction area (governors' hall).
11	K	Water reservoir (on the side of WETS FM).
12	L	Buc Ridge.
13	M	Bristol car racing stadium (Parking lot).
14	N	Race day hotel parking lot, Bristol.

Among them, three sampling sites (A, B, E) were around a busy highway - State of Franklin, to study the effect of moving traffic on heavy metal pollution. One of the sites was selected near the veteran's hospital (C) to observe the effect of use of medicines on heavy metal pollution. Two sites were selected as a control sites to compare data, one in a green park of Quillen College of Medicine (D) and another in an elevated area and away from the motor traffic- near

WETS FM and a water reservoir (K). One of the sampling sites was in the construction site of a big dorm (Governors Hall). The site G was in the corridor of Brown Hall which houses physics, biology, and chemistry departments of ETSU. We expected to observe the effect of using different chemicals and equipment in this building. Two sampling sites were selected from the Bristol Motor Speedway stadium which is about 40 miles away from the rest of the sampling sites. All other sampling sites were selected focusing on the crowded area with a lot of human activities.

Sampling Weeks

According to our initial design, we decided to collect sample every week during the growing season of dandelion and in 4-week intervals after the growing season is over. After the sampling process started, we could not collect samples in some of the weeks due to adverse weather conditions. For easy representation the sample collection dates were expressed in terms of the weeks as shown in Table 2.

Table 2. Sample Collection Weeks: Samples were collected initially each week for a month after that in 2-week and finally in 4-week intervals.

S.N.	Date of sample collection	Week
1	28-Mar-07	1
2	6-Apr-07	2
3	22-Apr-07	4
4	2-May-07	5
5	9-May-07	6
6	31-May-07	8
7	4-Jul-07	12
8	4-Aug-07	16
9	31-Aug-07	20

Sampling Method

Most of the sampling sites are in the ETSU Campus area they are not too far apart; hence, samples were always collected from the same place of the site within a 20 square feet area. Only the portion of the plant that was above ground was collected. The plant was collected on a dry sunny day after the moisture of the morning is almost gone. Normally, clean plants were collected. After harvesting, the sample was packed in a plastic bag. All the samples were given a specific code as shown in the Table 1 to make documentation easy.

Sample Treatment

The plants were stored in plastic bags after harvesting. Then, they were transported to the laboratory. Because we are interested in adsorbed metal particulates, they were not washed. But the plants were sorted very carefully to remove all the dead tissues and soil. After that, separate samples were kept in separate beakers and dried in an air oven at 110^0C for about 12 hours. Normally, the sample collected right after rain took a few more hours to become dry. The dried plant samples were then ground with mortar and pestle. The powdered samples were then stored in clean brown glass bottles. For the digestion, the powdered sample was accurately weighed by difference in an analytical balance and directly transferred to 50 mL labeled glass beakers. The weight taken varied from 1.5 g to 2.5 g to observe the effect of concentration of the sample for metals that are present in small amounts. Each sample was weighed in triplicate, so that total number of samples to be digested was 42 for 14 sampling sites. To each beaker, 20 mL of concentrated nitric acid was added under a fume hood. The samples were then allowed to stand with nitric acid for about 12 hours. After about 12 hours the sample mixture turned to a reddish brown color with foam at the top. Then, they were slowly heated on a hot plate. Once heating started, the color of the sample slowly changed to orange and most of the solid particle dissolved in acid. During heating a glass rod was kept in each sample to avoid bumping. They were heated to near dryness, with only a small amount of residue left. The heating process took about 4 hours. The beakers containing the dried samples were allowed to cool and then about 10 mL of de-ionized water was added to each beaker to dissolve the residue. After that the samples were filtered in a suction filter flask using Whatman No- 2, 42.5 mm filter paper. The beakers containing the samples were rinsed at least three times with small portions of de-ionized water while filtering. The filtered solutions were quantitatively transferred to a 50-mL volumetric flask and diluted to the mark. After that the samples were transferred to clean and labeled plastic bottles and stored in a refrigerator. In addition to the sample, the blank was also prepared by the same process using the same amount of nitric acid without the sample.

Preparation of Standard Solutions

All the standard solutions for the calibration curves were prepared by serial dilution of the 1000 ppm stock solutions. For the purpose of calibration curves the standard solutions of each metal of concentration 0.1, 0.2, 0.4, 0.6, 1, 2, and 10 ppm were prepared immediately before the analysis of the sample to avoid concentration change due to adsorption or decomposition. They were stored for 3 days after that new solutions were prepared.

Determination of Metal Concentration in the Plant Samples by Atomic Absorption Measurement

In this research project, flame atomic absorption spectrophotometry was used to determine the metal concentration in the sample solution. The metals analyzed were calcium, cadmium, copper, chromium, iron, lead, and zinc. By nebulizing the standard solutions of metal into the flame atomic absorption spectrophotometer with the hollow cathode lamp of respective metal, a calibration curve (absorbance vs. concentration) was prepared. With the help of calibration curves the concentrations of the metals in the sample solutions were determined. From preliminary observations and appropriate dilution, the concentrations of the solutions were brought within the calibration range. From the data of weight taken to prepare the sample solutions and using Excel, micrograms of metals per gram of dry samples used to prepare the solutions (ppm) were calculated. To determine the influence of interferents in the sample solution, the standard addition method was used. Only some samples of the second week and sixth week were checked for Iron, copper, and zinc by the standard addition method. For this method three, one-mL aliquots of each sample were taken in 25-ml volumetric flasks and standard solutions were added and diluted up to the mark so as to make the addition of 0, 0.2, and 0.4 ppm. The solutions were then nebulized and the concentration of the diluted sample was determined.

Statistics Used

To determine the distribution of concentration of metals at different sampling sites mean, median, and standard deviation were calculated. To avoid complex statistics, concentration of metals in the samples from Bristol were analyzed separately. The statistical software, SPSS, was used to calculate all the statistical values.

To assess the relationship of one or more factors with a dependent variable or with multiple dependent variables ANOVA was used [79]. One way ANOVA allows us to analyze mean differences between two or more groups. Similarly, two way ANOVA relates two between subjects' factors to a dependent variable. The statistical software SPSS can be used to analyze data from other analysis of variance designs also. For the analysis of distribution of heavy metals at different sites one way ANOVA was used [77], and to study the distribution of concentration of heavy metals in terms of sampling sites and sampling weeks two-way ANOVA was used.

ANOVA generally considers that data are normally distributed which is called the null hypothesis. Null hypothesis considers that all the population means are equal or there is no significant difference between the population means. If the null hypothesis is rejected, there is a significant difference in the population means. This difference may be due to the difference between the location of sampling sites or due to the week of sample collection. Normally, these two factors are treated independently and finally their interaction is observed. The significance limit (probability level) considered is 0.05 or less. F-test is one of the important tools to determine the type of distribution of data. It compares the F-values calculated with the standard F-values for the given set of degree of freedom. Degree of freedom is determined from degree of freedom from site and degree of freedom from error. If the calculated F-values is greater than the F-table value for the given degrees of freedom at the $P<0.05$ level, the difference in the mean concentration is considered as statistically significant. But, exact location of the significant difference is not indicated by this test. In SPSS, the tests recommended to determine the exact location of the significant difference are: homogeneity tests, descriptive statistics, and estimates of effect size. These tests were performed for the interpretation of data. In case of a significant difference is observed in the interaction, follow-up tests (Post Hoc) like Tukey, Dunnette's C, and FEGWQ are recommended in SPSS [78]. To avoid complex statistics only the main effects were considered from the follow-up tests.

CHAPTER 5

RESULTS AND DISCUSSION

For this research project, samples were collected from March 28 to August 31 of 2007 from 14 sampling sites. Collection of samples started when the dandelion plants were very young having no flowers. Initially it was very difficult to collect enough samples due to the small size of plants. It became easier to collect the required amount after a couple of weeks when the plants started growing faster. Towards the end of sample collection period, again the size of the plants became smaller and smaller. In some sampling sites, it became impossible to get the plant in the last few weeks. Nine samples were collected from each sampling site in the 20-week period except from Bristol. At the Bristol Speedway car racing stadium, samples were collected focusing on the car racing events on March 25-26 and August 25-26. The samples were collected immediately after the event and about 1 month before or after the event to monitor the heavy metal pollution created by the car racing events.

Following the recommended protocol, a calibration curve was made with the standard solutions of 0.1, 0.4, 0.6, 1.0, 2.0, and 10.0 ppm for each metal. An example of calibration curve is given in Figure 1. All calibration curves used in this study are presented in Appendix A.

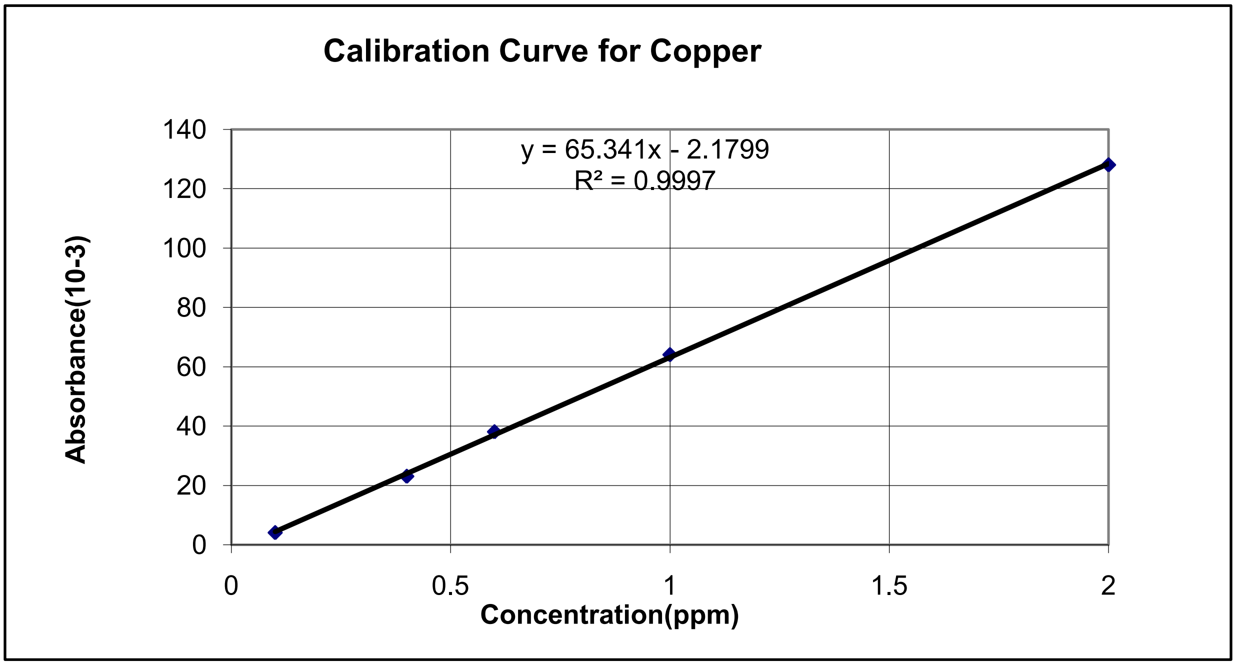

Figure 1. Calibration Curve for Copper (First Week): Concentration of metals in the sample solutions are determined with the help of this type of calibration curve.

The linearity of calibration curve shows that there is little experimental error. The calibration curve was made using the concentrations of the metal above its detection limit. For good precision and accuracy of the data, the concentration of sample solutions was made to lie between 0.4 ppm to 1.0 ppm range of the calibration curve by appropriate dilution.

To determine the influence of interferents, the concentration of the metals in some of the sample solutions was determined by the standard addition method as explained in Chapter 4. An example of the standard addition calibration curve used for the determination of concentration of metal is given in Figure 2.

Copper- spike	
A-2-a	
ppm added	absorbance
0.0	2.00
0.2	11.33
0.4	22.67
Diluted Conc.	0.0322206
Act. Conc.	0.8055152

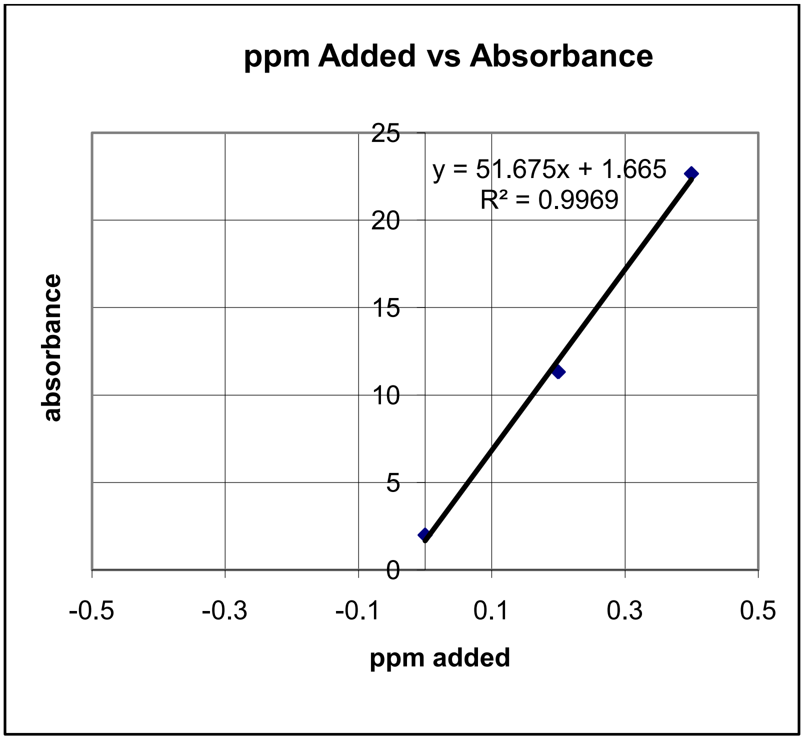

Figure 2. Determination of Metal Concentration by the Standard Addition Method: Standard addition method can determine the metal concentration present in the sample solution minimizing interference by matrix effect. The point at which the extrapolated line meets the x-axis gives the concentration of the metal in the sample solution.

Actual concentrations of metals in the samples were determined with the help of standard calibration curves as described in Chapter 3. The results of the study on the concentrations of the different metals in all the samples collected are given in Appendix B. To analyze the data statistically, ANOVA was used provided by the statistical software SPSS. The analysis was done by considering the significance of the difference [78]. Examples of a few of the statistical summary tables and the two-way ANOVA tables, respectively, are given in Tables 3 and 4, and the rest of the corresponding tables are given in Appendixes C and D.

Table 3. Statistical Values for Copper: Very close values of relative standard deviation shows the normal distribution of data. Due to the difference in weather conditions and traffic density in different sampling weeks, the relative standard deviation values are high.

			95% conf. limit		Value		
Sample code	Mean	Rel. Standard Deviation	Lower	Higher	Min	Max	Av. Dev.
A	12.11	37.00	9.24	14.98	5.83	18.78	3.68
B	10.23	35.98	7.36	13.10	4.51	16.83	2.55
C	9.70	44.02	6.83	12.57	5.55	18.64	3.23
D	5.90	69.70	3.03	8.77	0.32	14.91	2.64
E	12.11	44.67	9.24	14.98	6.29	22.50	3.68
F	6.85	37.82	3.98	9.72	3.08	10.76	1.86
G	9.35	58.06	6.48	12.22	2.40	20.69	3.82
H	7.97	53.10	5.10	10.83	2.57	15.34	3.19
I	8.05	46.70	5.18	10.92	2.42	16.17	2.45
J	9.45	29.11	6.58	12.31	5.08	14.90	1.94
K	6.20	61.29	3.33	9.07	0.00	12.38	2.77
L	10.27	59.50	7.40	13.14	4.72	24.87	3.63

Rel. = Relative, Av. Dev. = Average Deviation

Table 4. Two-Way ANOVA Table for Copper: This is the original table created by SPSS in which significance limit and partial eta square values show the distribution of data.

Tests of Between-Subjects Effects

Dependent Variable: Metal concentration.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1760.719(a)	19	92.669	17.344	.000	.789
Intercept	8776.643	1	8776.643	1642.670	.000	.949
Site	426.087	11	38.735	7.250	.000	.475
Week	1334.632	8	166.829	31.224	.000	.739
Error	470.176	88	5.343			
Total	11007.539	108				
Corrected Total	2230.896	107				

a R Squared = .789 (Adjusted R Squared = .744), df = degree of freedom, Sig. = Significance limit.

The study shows that the plants collected more particulates containing heavy metals during the growing period at the beginning of summer. This may be due to two reasons. The first reason is the presence of more particulates in atmosphere due to a long dry season immediately before the sampling period. The second reason is the fast growth of the plants and their larger size due to adequate amount of moisture in early spring [81]. Higher metal concentrations in the 4th-week samples prove this fact as shown in Table 5.

Table 5. Comparison of Metal Concentrations in ppm in the Samples at Site E: Forth week samples have higher metal concentrations than the second and fifth week samples due to the good growth of plant as a result of heavy rain in third week.

Metal	Second week	Fourth week	Fifth week
Cadmium	0.35	0.42	1.62
Calcium	84.07	477.43	233.41
Chromium	3.10	9.31	0.37
Copper	6.29	19.36	11.01
Iron	532.91	788.50	606.65
Lead	7.78	13.65	8.91
Zinc	40.95	121.61	50.69

In the overall comparison of metals, the samples from Bristol were not included in the statistical analysis by SPSS to avoid complex statistics due to different degree of freedom for different sites. Samples from Bristol were collected only for 4 weeks instead of 9 weeks from other sampling sites to focus just on the car racing event.

Our main concern in this research project was to correlate the metal concentrations found and the location of the sampling sites. Most of our results are as expected including some interesting deviations indicating specific type of influences. As expressed in the objectives of the research, the effects of weather condition and human activities were also monitored.

During the statistical analysis the concentration of metals in ppm was considered as the dependent variable and sampling sites and weeks were considered as independent variables [79]. The analysis was done by considering 'between subject factors' [78]. The concentration of different metals and their variations are discussed separately under the following headings.

Metal Concentration at Different Sites

Copper

The highest concentration of copper found during the study period is 24.87 ppm at site L (Buc Ridge) in the first week. The lowest concentration found was below the detection limit in the eighth week at the control site K (Water reservoir). The overall mean concentration of copper during the study period was 9.02 ppm. The OSHA recommended safe level of copper is 0.1 mg/m^{3}as fume and 1 mg/m^3 as dust in respiratory air. The concentration of copper in all sampling sites and weeks are given in Appendix B, Table 1. The graphical comparison of the copper concentrations is given in Appendix E, Figure 1. The statistical summary table of copper metal is in Appendix C, Table 1. The ANOVA table shows that there is significant difference in the mean concentration of copper among the main factors (both time of sampling and sites, $P<0.01$). To find out the actual location with significant differences, follow-up tests were done [77]. The follow-up (Post Hoc) tests show that the mean concentration of copper at the sampling site A is significantly higher than the mean concentration of sampling sites D ($P<0.01$), F ($P<0.01$), H ($P<0.05$), I ($P<0.05$), and K ($P<0.01$). These results may be because sampling site A

(Mc-Donald four-way) often has very high traffic density. Mean concentration of copper at site B is significantly higher than the mean concentration of sampling sites D and K which are the control sites ($P<0.05$) thus indicating that the effect of traffic is real. Similarly, the mean concentration of site C is significantly higher than the mean concentration of control site D ($P<0.05$). Being the control site, the mean concentration of copper at the site D is indeed significantly lower than the mean concentrations of sites A ($P<0.01$), B ($P<0.01$), C ($P<0.05$), E ($P<0.01$), and L ($P<0.01$). The mean concentration of copper at site F is significantly lower than the mean concentration of A and E ($P<0.01$). Sites G and J do not have significantly different mean copper concentration in comparison to other sites. Mean concentration of copper at sites H and I are significantly lower than the mean concentration at site A and E as mentioned earlier. The mean copper concentration of site K is significantly lower than the mean concentration of site L ($p< 0.05$).

The multiple comparison table of sampling weeks shows that the mean concentration of the 1st-week samples is significantly higher than that from the samples of all other weeks ($P< 0.01$). It could be due to the long dry season before the sampling week. It could also be that the dandelions had been exposed for a longer period while they were growing slowly before the weather turned warmer. The mean concentration of the 4th-week samples is again significantly higher than samples of all other weeks ($P<0.01$) except for the 1st-week samples. It could be because of the better growth and thus larger dandelion leaves due to some good rain in the 3rd week. The mean concentration of 2nd-week samples is significantly lower than the mean concentration of 1st-, 2nd-, and 16th-week samples ($P< 0.01$). It could be due to the rain before sample collection. Similarly, the mean concentration of copper on the 5th- and 6th-week samples are significantly lower than the mean concentration on the 1st-and 4th-week samples ($P<0.01$), 8th- and 20th-week samples are significantly lower than those from the 1st, 4th, and 16th weeks ($P<0.01$). Mean concentration of 12th-week samples is significantly lower than those of the 1st and 4th weeks ($p<0.01$) and also significantly lower than 16th week ($p<0.05$). There is no any specific reason for the relatively higher mean copper concentration in 16th week; but, it could be due to the heavy traffic during that period.

Iron

The highest concentration of iron noticed during the study period is 2078.2 ppm at site J (construction site) in the 20th-week samples. The lowest concentration is below the detection limit in the 8th-week samples at site K (water reservoir). The overall mean concentration of iron during the study period is 337.4 ppm with a relative standard deviation of 92.03 %. The OSHA recommended safe level of iron oxide fume is 10 mg/m^3 and that of stainless welding fume is 5 $\mu g/m^3$. The concentration of iron in all sampling sites and weeks are given in Appendix B, Table 2. The graphical comparison of concentration with sites and weeks is given in Appendix E, Table 2. The statistical summary for iron is in Appendix C, Table 2.

The correlation of concentration between sites and time of sampling shows that there is significant difference in the mean concentration of iron among the sampling sites ($p<0.01$) but not among the weeks. Hence, Post-Hoc tests were performed. The Tukey HSD test shows that the mean iron concentration of site J (construction site) is significantly higher than other sampling sites (A, G, H, I, L $p<0.05$ and C, D, F, K $p<0.01$) except sites B and E. Similarly, its concentration for samples at site E is significantly higher than the mean concentration of those at site K ($p<0.05$). The raw data also show that the iron concentration was very high in the samples of the 16th and 20th weeks at the site J. The construction work was in the final stage in those weeks so there was a lot of welding work going on which could be responsible for the very high concentration of iron and significantly different mean concentration at this sampling site. Samples at all other sampling sites have similar mean iron concentration or they are not significantly different. The comparison of concentration between weeks shows that the mean iron concentrations are not significantly different. This means that the amount of iron stayed about the same throughout the study period except when there were greater construction works or activities that involve the use of iron.

Chromium

The highest concentration of chromium found during the study period is 10.85 ppm at site E (four-way between Kroger and Whites) in the 1st-week samples. The lowest concentration found is below the detection limit in different weeks and sites mostly in 5th and 8th weeks. The overall mean concentration of chromium during the study period is 2.13 ppm and the relative standard deviation is 115.02 %. The OSHA recommended safe level of chromium in respiratory air is 1 mg/m^3 and chromium(III) compound is 0.5 mg/m^3. The concentration of chromium in all sampling sites and weeks are given in Appendix B, Table 3. The graphical comparison of the concentration with sites and weeks is given in Appendix E, Figure 3. The statistical summary for chromium is given in Appendix C, Table 3.

The correlation study shows that there is a significant difference in the mean metal concentration of chromium among the sampling sites ($p<0.01$) as well as among the sampling weeks ($p<0.01$). Hence, Post-Hoc tests were performed for chromium. The Tukey HSD test shows that the mean chromium concentration at site A is significantly higher than the mean chromium concentration at site K ($p <0.05$) which could be because site K is a control site. Site E also has significantly higher mean chromium concentration than the sites D ($p < 0.05$), I ($p < 0.05$), and K($p < 0.01$). It could be because site E has a high amount of moving traffic. Site D samples have significantly lower concentration than site E samples ($p < 0.05$) because D is also a control site. All other sites do not have significant differently high or low mean chromium concentrations.

The multiple comparison table of chromium shows that the mean metal concentration of the 1st week is significantly higher than the mean metal concentration of all other weeks ($p< 0.01$), just as the other metals do as mentioned previously. The mean chromium concentration of the 4th-week samples is significantly lower than those of the 1st week ($p<0.01$) but significantly higher than all other weeks ($p<0.01$), as are other metals mentioned before and for the same reason due to good growth of dandelion plants in this week due to sufficient moisture. Mean metal concentration of the 2nd-week samples is significantly lower than those of the 1st and 4th weeks ($p<0.01$) but higher than those of the 5th-, 8th-, and 12th-week samples ($p< 0.01$). The 5th-week mean metal concentration is also significantly lower than the mean metal concentration of 1st, 2nd, and 4th weeks ($p<0.01$). There are no neatly compelling reasons for the significant difference in mean chromium metal concentration among samples some weeks and sites. Because, the chromium concentration found is normally low; it may have been affected by random higher number of moving or stationary traffic at the time or sites.

Lead

The highest concentration of lead measured during the study period is 17.16 ppm at site G (Brown Hall court yard) in the 4th-week samples. The lowest concentration is below the detection limit for samples of several weeks at different sites. The overall mean concentration of lead during the study period is 5.25 ppm and with a relative standard deviation of 98.83 %. The OSHA recommended safe level of lead in work place air is 200 $\mu g/m^3$ as an 8-hour weighted average. The concentration of lead from all sampling sites and weeks is given in Appendix B, Table 4. The graphical comparison of concentration is given in Appendix E, Fig 4. The statistical summary for lead is in Appendix C, Table 4.

The statistical test shows that there is no significant difference in the mean metal concentration of lead among the samples from different sampling sites, but there is significant difference in the mean lead concentration among the weeks ($p<0.01$).

First week mean lead concentration is significantly lower than the samples in the 2nd and 4th weeks ($p<0.01$), actually it is against the general trend for other metals which are higher in 1st-week samples. The reason for this is unknown. Mean lead concentration in the 2nd-week samples is significantly higher than those from all other weeks except the 4th and the 5th weeks ($p<0.01$). The 4th-week samples mean lead concentration is significantly higher than all other week samples except those from the 2nd week ($p<0.01$). The reason for this high lead concentration could be sufficient moisture and good growth of the dandelion leaves because there was a lot of rain and snow on the 3rd week of sample collection. It could also be the traffic pattern during the bad weather and the way people drove then. The 5th-week samples lead concentration are significantly lower than those of 4th week ($p<0.01$) but significantly higher than the 6th- to the 20th-week samples ($p<0.01$). The 6th-week to the 20th-week samples' lead concentration are significantly lower than those of the 1st to 5th weeks ($p<0.01$), continuing the above trend. Raw data show that the difference in lead concentration in the samples from control site and sites having higher traffic density was large at the beginning and gradually reduced as the growing season of dandelion was over.

Cadmium

Cadmium is the metal having the lowest concentration in all the samples in the study area during the study period which is good for the ETSU students because it is the most harmful metal for human health. The highest concentration of cadmium found during the study period is 2.88 ppm, at site A (Mc-Donald four-way) in 5th-week samples. The lowest concentration is below the detection limit in most of the weeks and in all the sampling sites. The overall mean concentration of cadmium during the study period is 0.19 ppm and the relative standard deviation is 91.58 %. The OSHA recommended safe level of cadmium is 0.3 mg/m^3 as fume and 0.6 mg/m^3 as dust in respiratory air. The concentration of cadmium at all sampling sites and weeks is given in Appendix B, Table 5. The graphical comparison of the concentration with sites and weeks is given in Appendix E, Figure 5. The statistical summary for cadmium is found in Appendix C, Table 5.

Statistical test shows that there is no significant difference in the mean concentration of cadmium among the sampling sites but significantly different among the sampling weeks. The follow-up test (Tukey HSD) shows that the 1st-week samples' cadmium concentration is significantly lower than those of the 2nd, 4th and 5th weeks ($p<0.01$). This is an unusual result in comparison to the results on other metals but somewhat similar to lead. There is no specific reason for this. The 2nd- and 4th-week samples mean cadmium concentration are significantly higher than those of the 1st, and 6th to 20th week ($p<0.01$) but significantly lower than the 5th-week mean cadmium concentration ($p<0.01$). The 5th-week mean cadmium concentration is significantly higher than all other weeks ($p<0.01$). The 6th- to 20th-week mean cadmium concentration are significantly lower than those of the 2nd to 5th weeks ($p<0.01$). Actually, there is no specific reason for this variation in cadmium concentration. Because the cadmium concentration is very close to the detection limit, results may not be reproducible and meaningful.

Zinc

The highest concentration of zinc found during the study period is 409.48 ppm at site D (college of medicine park) for the 1st-week samples. The lowest concentration is below the detection limit in the 8th- and 12th-week samples of some sites. The overall mean concentration of zinc during the study period is 70.65 ppm and the relative standard deviation is 152.59 %. The OSHA recommended safe level of zinc oxide dust is 15 mg/m^3 and fume is 5 mg/m^3, zinc chloride fume is 1 mg/m^3 in respiratory air. The concentration of zinc in all sampling sites and weeks is given in Appendix B, Table 6. The graphical comparison of the concentration with sites and weeks is given in Appendix E, Figure 6. The statistical summary for zinc is given in Appendix C, Table 6.

The statistical test shows that there is no significant difference in the mean zinc concentration among the sampling sites but significantly different among the samples from different weeks. Multiple comparison table of sampling week shows that the 1st-week mean zinc concentration is significantly higher than the mean zinc concentration of all other weeks ($p<0.01$). The very high concentration of zinc metal in the 1st-week samples may be due a systematic error. Tongs having zinc coating were used to handle the beakers during digestion; it may be responsible for the very high concentration of zinc in the samples from this week. After this experience, the use of any metal apparatus was avoided. The zinc concentration for the 2nd-week samples is significantly lower than those of the 1st week ($p<0.01$) and significantly higher than those from the 12th week ($p<0.05$). Fourth-week zinc concentration is significantly lower than those of the first week ($p<0.01$) but significantly higher than those samples from the 8th, 12th and 20th weeks ($p<0.01$). The 8th-week mean zinc concentration is significantly lower than that of the 16th week ($P<0.05$). The 12th-week zinc concentration is significantly lower than those of the 1st, 4th, and 16th weeks ($p<0.01$) and that of the 2nd week ($p<0.05$). The 20th-week mean zinc concentration is significantly lower than the 1st- and 4th-week mean zinc concentrations ($p<0.01$). The concentration of zinc observed during the project shows that it does not specifically depend on the weather condition or the site and week but it depends particularly on the use of pesticides. Because our sampling sites are mostly in the human populated area and the season was either dry or rainy, the campus community used pesticides. After the use of pesticides the concentration of zinc was found to be higher for about a 5-week period.

Calcium

The highest concentration of calcium found during the study period is 2856.64 ppm at site K (water reservoir) in the 12th-week samples. The lowest concentration is below the detection limit in the 6th- and 8th-week samples in some sites. The overall mean concentration of calcium during the study period is 342.88 ppm with a relative standard deviation of 100.72 %. The OSHA recommended safe level of calcium carbonate and calcium hydroxide dust is 15 mg/m^3 and fume is 5 mg/m^3 in respiratory air. The concentration of calcium in all sampling sites and week is given in Appendix B, Table 7. The graphical comparison of the concentration with sites and weeks is given in Appendix E, Fig 7. The statistical summary table of calcium is given in Appendix C, Table 7.

Statistical test shows that there is no significant difference in the mean concentration of calcium among the sampling sites, but it is significantly different among the sampling weeks. The follow-up tests show that the mean calcium concentration of the 1st-week samples is significantly higher than the mean calcium concentration of the samples from the 8th week. The concentration of calcium is not actually higher or lower in any specific site or specific week as other metals but is random. It could be due to two effects. The first one is human activity, the higher the human activity, more dust particles go in to the air and collect on plant leaves. The second reason may be the human activity in the spring when lime is applied to the soil to reduce soil acidity.

Sample from Bristol Motor Speedway Car Racing Stadium

There are only two sampling sites around Bristol Speedway car racing stadium and the concentrations of the metals are not significantly high or low. Separate statistical analysis of metal concentration is not appropriate because of only two degrees of freedom for sites. Two-way ANOVA cannot give correct p value in such case. When they were analyzed with other data including only 4 sampling weeks, it gave similar result as explained above.

Factors Affecting Metal Concentration in Plant Sample

In this research project we tried to find out the factors that affect the concentration of metals found in plant samples. Our results show that the most important factor affecting deposition of metal containing particulates on plant leaves is the weather condition. We collected sample at least 24 hours after rain but we found that the rain in the week and sufficient humidity in air reduced the metal containing particulates in air. High metal concentration in the 1st-week samples and the sudden decrease in metal concentration in the samples of the following weeks is the proof of this fact. The wind pattern was also found to affect the metal concentration because high speed wind may carry the metal containing particulates far away instead of localize in the nearby surrounding areas. Low concentration of most of the metals in the 8th-week sample is the evidence of this fact.

The nature of plant species and the nature of leaf surface is also an important factor to determine the metal concentration. Analysis of samples from site G (Brown Hall) in the last few weeks shows lower concentration of heavy metals. This may be due to the difference in particulate adsorbing ability of the other seasonal plant that was collected from that site as dandelion was not available. Comparison of metal concentration in the samples from Bristol in the last 2 weeks also is a good explanation for this fact. Unlike higher metal concentration in the samples collected on the week of the first car racing event than 4 weeks after the event, the metal concentration in the samples collected on the week of second event was lower than the metal concentration in the samples collected 4 weeks before the event. The possible reason for this is that we had to collect leaves of other seasonal plants due to the lack of dandelion because of the drought. The plants collected instead of dandelion had smooth surface that may not be as good an adsorbent as the dandelion. Location of sampling site was also found to be a factor affecting the concentration of metal. Metal concentration in the elevated area away from the roads with traffic was found to be less (site K) and samples collected from the areas covered by trees also was found to contain lower concentration of metal (site F). This conclusion is also supported by the research conducted in UK [24].

Interferences

In atomic absorption analysis using flame, two factors affecting precision and accuracy are common. The first one is an instrumental factor, which can be removed by running the standard as well as the sample solutions in the same setting. We analyzed the samples immediately after the measurement of standard solutions for the calibration curve. Once, the instrument is turned off or the lamp taken out, a new calibration curve was made by running the standard solutions again.

The other factor in AAS is matrix interference. Literature shows that this is the most important problem with atomic absorption measurement. There can be physical or chemical interferences. The matrix interferences are most probable in normal calibration curve method because the standard solutions and the sample solutions are in different chemical environments. The standard solutions are prepared from the pure substances, but the sample solutions may contain different interfering compounds simultaneously formed during digestion [76]. To find out whether there are such interferences or not, some of the samples were analyzed by the standard-addition (spike) method for copper, iron, and zinc. The concentration of these metals in the samples determined by normal calibration curve and the standard addition method are given in Tables 6, 7, and 8.

Table 6. Concentration of Iron in the Sample Solution Determined by Calibration Curve and Standard Addition Method: The values are microgram of metal per mL of sample solution.

Sample code	Calibration curve method	Standard addition method	Difference	% Difference
A-2-a	8.37	7.02	1.35	16.09
A-2-b	6.61	8.98	-2.37	-35.88
A-2-c	8.00	9.34	-1.34	-16.74
B-2-a	8.80	10.05	-1.25	-14.24
B-2-b	7.37	9.52	-2.15	-29.15
B-2-c	6.18	5.37	0.81	13.08
C-2-a	6.71	7.78	-1.07	-15.98
C-2-b	6.81	8.24	-1.43	-21.03
C-2-c	5.12	7.00	-1.88	-36.80
D-2-a	3.39	0.15	3.24	95.58
D-2-b	2.39	2.67	-0.28	-11.58
D-2-c	3.13	4.89	-1.76	-56.33
M-2-a	9.82	14.80	-4.98	-50.65
M-2-b	9.76	11.45	-1.69	-17.34
M-2-c	9.56	11.04	-1.48	-15.49
N-2-a	1.84	1.85	-0.02	-0.82
N-2-b	2.70	3.59	-0.89	-33.11
N-2-c	3.13	2.78	0.35	11.13
M-6-a	8.13	9.84	-1.71	-20.97
M-6-b	9.39	11.36	-1.97	-20.94
M-6-c	8.30	8.63	-0.33	-3.99
N-6-a	1.84	3.08	-1.25	-67.85
N-6-b	1.67	1.83	-0.16	-9.65
N-6-c	1.57	2.55	-0.98	-62.42

Table 7. Concentration of Copper in the Sample Solution Determined by Calibration Curve and Standard Addition Method: The values are microgram of metal per mL of sample solution.

Sample code	Normal calibration curve method	Standard addition method	Difference	%difference
A-2-a	0.35	0.81	-0.46	-131.43
A-2-b	0.15	1.05	-0.90	-586.27
A-2-c	0.22	0.25	-0.03	-11.61
B-2-a	0.24	0.31	-0.07	-28.10
B-2-b	0.12	0.10	0.02	14.53
B-2-c	0.05	0.14	-0.10	-211.11
C-2-a	0.19	0.14	0.05	25.93
C-2-b	0.26	0.32	-0.06	-23.08
C-2-c	0.08	0.07	0.01	14.81
D-2-a	0.05	0.03	0.01	24.44
D-2-b	0.00	0.03	-0.03	0.00
D-2-c	0.01	0.10	-0.09	-900.00

Table 8. Concentration of Zinc in the Sample Solution Determined by Calibration Curve and Standard Addition Method: The values are microgram of metal per mL of sample solution.

Sample code	Normal calibration curve method	Standard addition method	Difference	% Difference
A-2-a	1.58	1.53	0.05	3.16
A-2-b	1.46	1.35	0.11	7.53
A-2-c	1.42	1.48	-0.06	-4.23
B-2-a	1.64	1.55	0.09	5.49
B-2-b	0.79	1.05	-0.26	-32.91
B-2-c	0.22	0.46	-0.24	-109.09
C-2-a	0.96	0.83	0.13	13.54
C-2-b	0.95	0.86	0.09	9.47
C-2-c	0.41	0.12	0.29	70.73
D-2-a	1.78	3.07	-1.29	-72.47
D-2-b	1.43	1.23	0.20	13.99
D-2-c	1.23	1.03	0.20	16.26

The data show that copper and iron have positive interference and zinc has negative interference from the matrix. Hence, the concentration of copper and iron determined by standard addition methods are slightly higher than the concentrations determined by the normal calibration curve method and the concentrations of zinc determined by the standard addition methods are slightly lower than the concentrations determined by the normal calibration curve method. The difference between the concentrations determined by normal calibration curve and standard addition method are within 20%.

Recovery Studies

To determine the validity of the data measured by the atomic absorption spectrophotometer, recovery study was also performed. As mentioned in the standard addition calibration curve method, three aliquots of samples were made, first aliquot was diluted 25 times without addition, second aliquot was diluted with 0.2 ppm addition of standard solution of the metal, and third aliquot was diluted with 0.4 ppm addition of standard solution of the metal. The recovery obtained for was within 80% to 120%. For zinc, the average recovery is 95.33% with relative standard deviation of recovery 8.16%. Data obtained from the recovery study for zinc are shown in Table 9.

Table 9. Recovery Study for Zinc: The first aliquot (eg A-2-a-1) has no addition for which the table shows zero recovery, second aliquot (eg A-2-a-2) has 0.2 ppm addition, and third aliquot (A-2-a-3) has 0.2 ppm further addition (0.4 ppm in first aliquot).

Sample code	Absorbance	Conc. (ppm)	Recovery	% Recovery
A-2-a-1	10.00	0.06	NA	NA
A-2-a-2	33.00	0.25	0.18	92.32
A-2-a-3	58.00	0.45	0.20	100.35
A-2-b-1	7.00	0.04	NA	NA
A-2-b-2	35.00	0.26	0.22	112.39
A-2-b-3	57.00	0.44	0.18	88.30
A-2-c-1	11.00	0.07	NA	NA
A-2-c-2	32.00	0.24	0.17	84.29
A-2-c-3	57.00	0.44	0.20	100.35
B-2-a-1	12.00	0.08	NA	NA
B-2-a-2	34.00	0.25	0.18	88.30
B-2-a-3	58.00	0.45	0.19	96.33
B-2-b-1	7.00	0.04	NA	NA
B-2-b-2	31.00	0.23	0.19	96.33
B-2-b-3	53.00	0.41	0.18	88.30
B-2-c-1	5.00	0.02	NA	NA
B-2-c-2	27.00	0.20	0.18	88.30
B-2-c-3	51.00	0.39	0.19	96.33
C-2-a-1	5.00	0.02	NA	NA
C-2-a-2	30.00	0.22	0.20	100.35
C-2-a-3	56.00	0.43	0.21	104.36
C-2-b-1	5.00	0.02	NA	NA
C-2-b-2	30.00	0.22	0.20	100.35
C-2-b-3	55.00	0.42	0.20	100.35
C-2-c-1	3.00	0.01	NA	NA
C-2-c-2	27.00	0.20	0.19	96.33
C-2-c-3	52.00	0.40	0.20	100.35
D-2-a-1	15.00	0.10	NA	NA
D-2-a-2	36.00	0.27	0.17	84.29
D-2-a-3	57.00	0.44	0.17	84.29
D-2-b-1	8.00	0.05	NA	NA
D-2-b-2	30.00	0.22	0.18	88.30
D-2-b-3	55.00	0.42	0.20	100.35
D-2-c-1	8.00	0.05	NA	NA
D-2-c-2	30.00	0.22	0.18	88.30
D-2-c-3	57.00	0.44	0.22	108.37

CHAPTER 6

CONCLUSION

The concentration of metals present in dandelion samples are expressed in ppm (µg/g) of dry dandelion leaf. OSHA limit expresses the maximum safe level of metals in respiratory air; hence, it may not be possible to express the concentration of metals detected in this research project are under safe limit or not. OSHA safe limit of different metals are mentioned in Chapter 5. Because dandelion plant is rich in some of the metals like iron, some of the metal concentrations may be from the the plant itself. The literatures as well as our experience show that the concentration of metals obtained by subtraction of the control site metal concentration gives the actual metal concentration collected by the plant from air in the form of particulates quite well.

The highest concentration of iron found during the study period is 2078.21 ppm at site J (construction site) in the 20th-week samples. It is an interesting result that the concentration of iron at this site is very high in the last 2 sampling weeks during which a lot of welding work were going on in the nearby building, and heavy equipment was used on those days. It shows that the human activity is responsible for the heavy metal pollution in air.

The highest concentration of copper found during the study period is 24.87 ppm at site L (Buc Ridge) in the 1st-week samples. It should be due to the idling stationary traffic because this is a very crowded place. In most of the other weeks the concentration of copper in this site is also high. The 2nd highest concentration of copper is in site E (22.5 ppm) also represents the copper metal concentration related with traffic density.

The highest concentration of chromium found during the study period is 10.85 ppm, at site E (four-way between Kroger and Whites) in the 1st-week samples. Chromium concentration is higher at this site for most other weeks indicating that atmospheric chromium concentration correlate with moving traffic or gasoline consumption. The study shows that chromium concentration is higher at sites with heavy moving traffic.

The highest concentration of lead found during the study period is 17.16 ppm at site G (Brown Hall court yard) in the 4th-week samples. This result could be due to the use of lead containing chemicals in the laboratories in Brown Hall. Both chemistry and biology labs present in Brown Hall use such chemicals. Lead concentration also is higher for this site in samples collected in other weeks. Other sites having heavy moving traffic have higher concentration of lead also showing that atmospheric lead concentration is mainly due to gasoline combustion.

The highest concentration of cadmium found during the study period is 2.88 ppm at site A (Mc-Donald four-way) in the 5th-week samples. The data show that cadmium metal concentration is very low in the study area and it is relatively higher only at sites with heavy traffic, which indicates that the atmospheric cadmium concentration could be due to the use of motor vehicles.

The highest concentration of zinc found during the study period is 409.48 ppm at site D (College of Medicine park) in the 1st-week samples. The use of pesticides around the site could be responsible. Concentration of zinc was found to increase and decrease suddenly in the same site, even in the control sites, showing the effect of human activity or the use of pesticides.

Exceptionally high concentration of zinc in the 1st week of sample collection also represents the same fact i.e. use of excessive pesticides in early part of spring.

The highest concentration of calcium found during the study period is 2856.64 ppm at site K (water reservoir) in 12th week. As discussed in Chapter 5, the concentration of calcium was found to be distributed randomly. There are mainly two sources for very high calcium concentration in the plant sample. One is the soil dust and other is the early spring liming of the soil. The highest concentration of calcium in site K may be due to the soil dust because this site is surrounded by an open grass field.

It is interesting to compare metal concentration in samples from different weeks as it can reveal the effects of weather condition on the deposition of atmospheric metal concentration. A detailed record of weather condition was made during the study period that correlates well with the average metal concentration of different weeks.

There was no rain for a long time before we started the sample collection; hence, the metal concentration is very high in 1st-week samples. After the 1st-week sample collection, there was rain in the area. On the 4th day of rain, 2nd-week samples were collected. Hence, most of the metal concentrations in the 2nd-week samples were lower than those samples collected in the 1st week. After that, there was heavy rain and snow in the 3rd week. The 4th-week samples' metal concentration is higher due to good growth of dandelion. In the 5th-week samples the metal concentration is slightly lower as samples were collected soon after a few hot sunny days. The 6th-week samples contain lower concentration of metals as the samples were collected after rain. Mostly, the concentration of metals in the 6th-week samples were lower in those sites that were in the inner part of the university as there was less human activities due to the summer vacation. The 8th-week samples were collected in a very windy day after several dry sunny days so both factors made the concentration of metal in the average range. Metal concentrations in all the other weeks were average as the weather condition was similar.

Among our sampling sites, sites A, B, and E are around the highway; therefore, the metal concentration is found to be higher. Site C is near the VA hospital parking lot, and we can see the effect of stationary traffic on the metal concentration. Site D, a park, was selected as a control site. But, in some of the sampling weeks the metal concentration is slightly higher because in those weeks the samples were collected from the road side as dandelion was not available inside the park. The metal concentration of this site shows the effect of use of pesticides. The result of site F is interesting. To observe the scattering pattern of the metal containing particulates and compare the effect of shade by trees, samples of some weeks were collected from shaded area and some week from relatively more open area. Higher concentration of metals in the 12th-, 16th-, and 20th-week samples from this site revealed this fact as samples were collected from the relatively open area. Lower concentration of metal was found at this site in comparison to other similar sites (H and I) showed that the metal containing particulates were first scattered up and then settled down to the ground. The trees can obstruct the metal containing particulates to come to the ground. Site G indicates the use of chemicals in the laboratories. Sites H and I were selected to observe the effect of stationary traffic, but it was very difficult to find dandelion in these sites. Site F was selected to observe the effect of construction works on atmospheric metal pollution. Very high concentration of iron in weeks 16 and 20 show that construction works was responsible for some types of metal pollution. Site K is the control site; most of the metals are present in lower concentration at this site. Site L shows the effects of large population and stationary traffic. It is around the dorm. Sites M and N are from Bristol; samples were collected from these sites focusing on the car racing events on March 25-26 and August 25-26, 2007. Concentrations of heavy metals are higher in site M (near stadium) and lower in N (far from stadium). Samples collected 4 weeks after of first event contain lower concentration of metals indicating the increase in heavy metal pollution due to human activity. Unlike the result of samples in the first event, the samples collected in the week of the second event were found to contain lower amount of metals than in the samples collected 4 weeks before the event. The specific reason for this anomaly is that different plant leaves were collected on the week of second event as there was no dandelion. Due to the smooth surface of the leaves of another seasonal plant, it could collect lower amount of particulates. This result shows that the metal concentration deposited in plant leaves also depends on the specific plant and the nature of its leaf surface.

Future Direction

Determination of heavy metals by using vegetation as a receptor is an economical and a reliable method. One can conduct the project by using easy-to-operate economical atomic absorption spectrophotometer. Because the method provides direct information about the metal content in vegetation, this method can be helpful to determine the amount of heavy metals present in vegetables that we use as our daily food. Similar research can be conducted to study how much heavy metals cows eat on a farm located near a road, which finally reaches human body through their milk. To study the amount of heavy metals present in vegetables like mustard greens one can grow the vegetable in a flower pot and place in the study site followed by digestion and analysis of the metals. This type of research project can be done in a way that can directly give information about the amount of heavy metals reaching the human body.

A research project similar to this can be conducted by including a whole city. Because the concentration of metal containing particulates depend on the weather condition, humidity, and traffic density, it is better to conduct the project by a group so that sample can be collected from different parts of the city at the same time of the day.

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APPENDIXES

APPENDIX A

Calibration Curve for Different Metals

Figure 1. Calibration Curve for Copper (16th week).

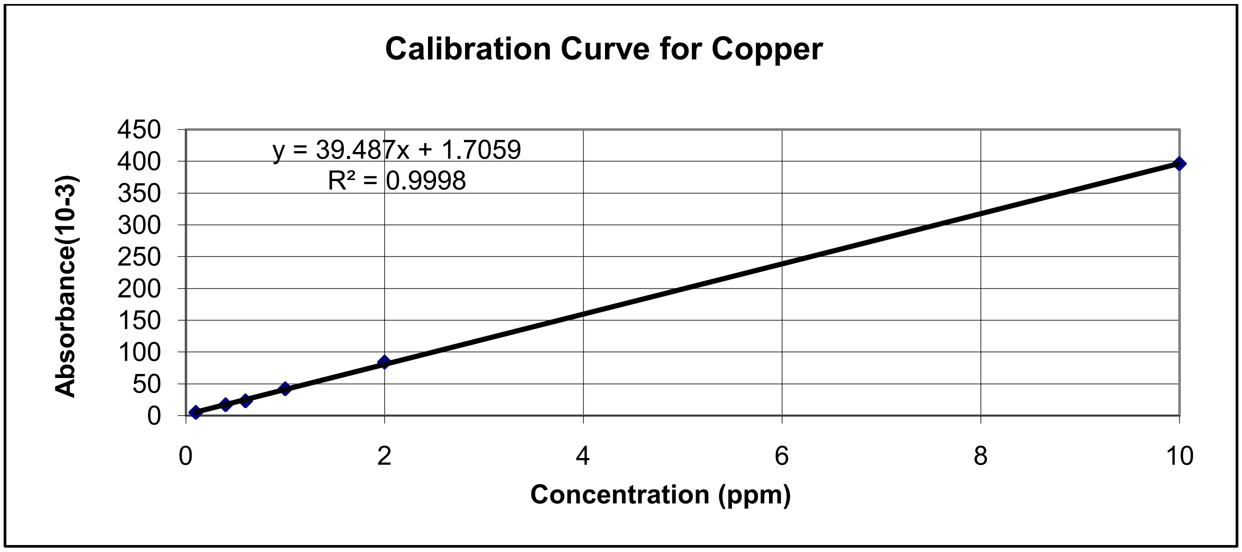


Figure 2. Calibration Curve for Iron (16th week).

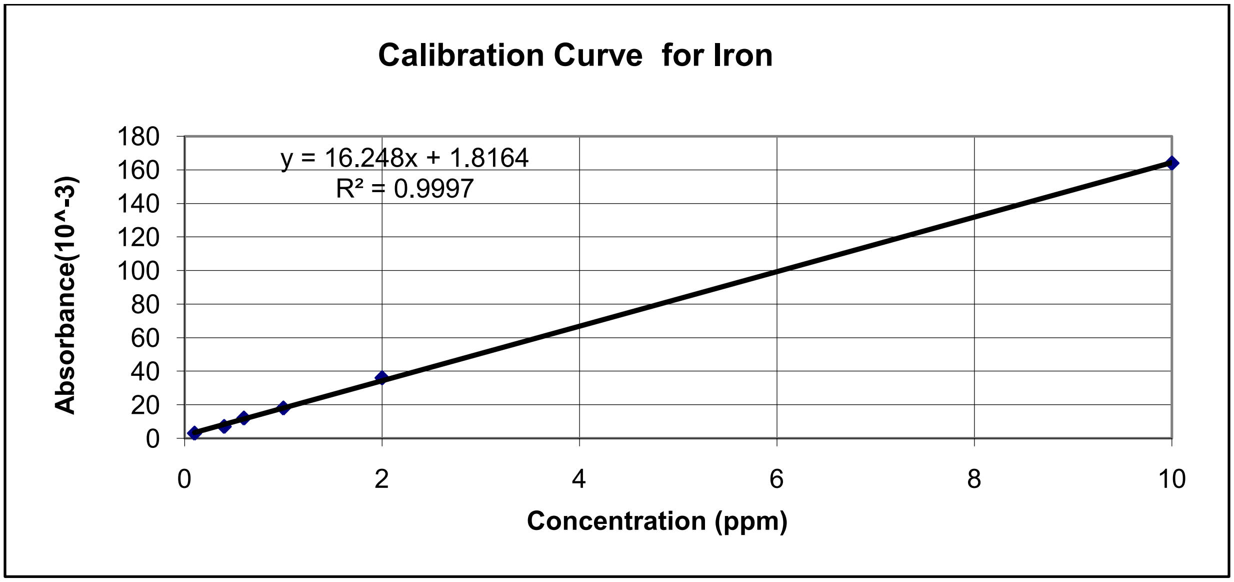


Figure 3. Calibration Curve for Chromium (16th week).

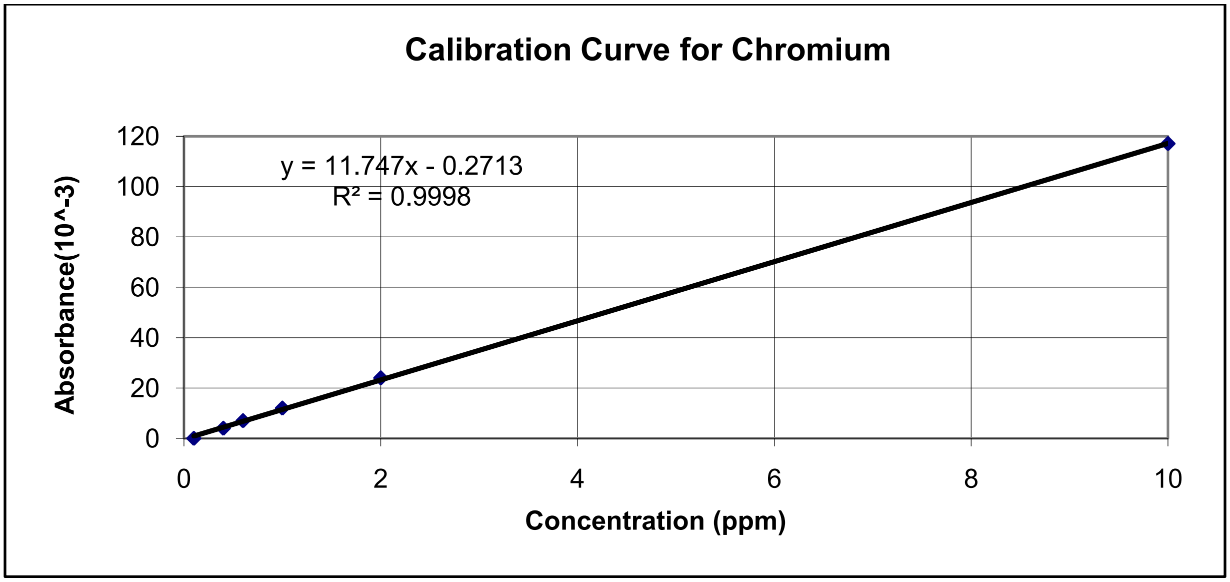


Figure 4. Calibration Curve for Lead (16th week).

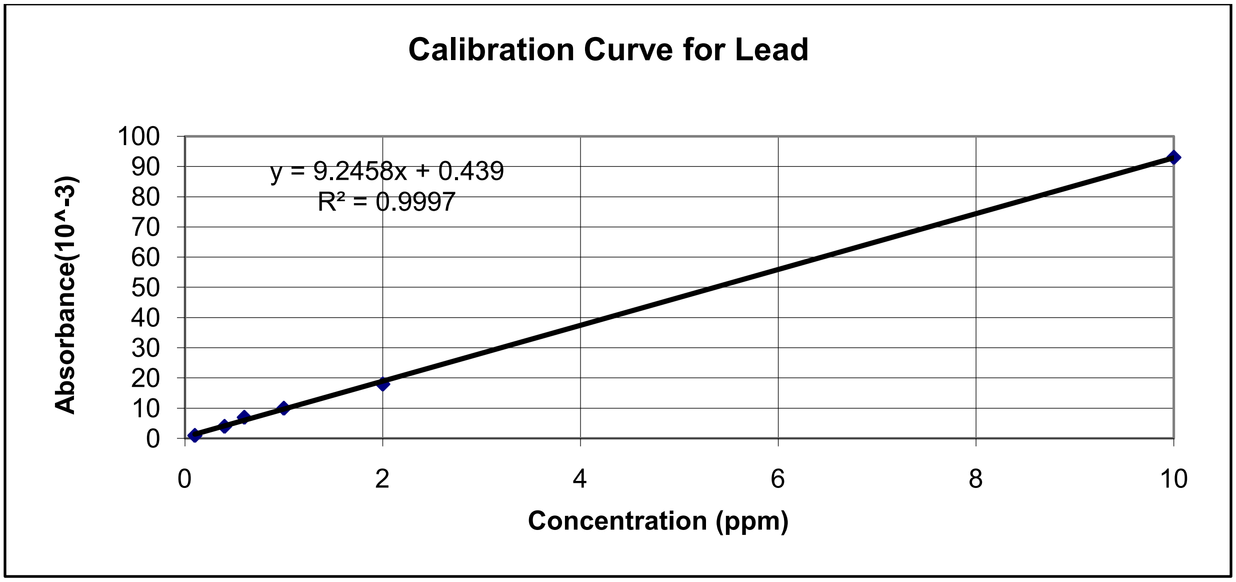


Figure 5. Calibration Curve for Cadmium (16th week).

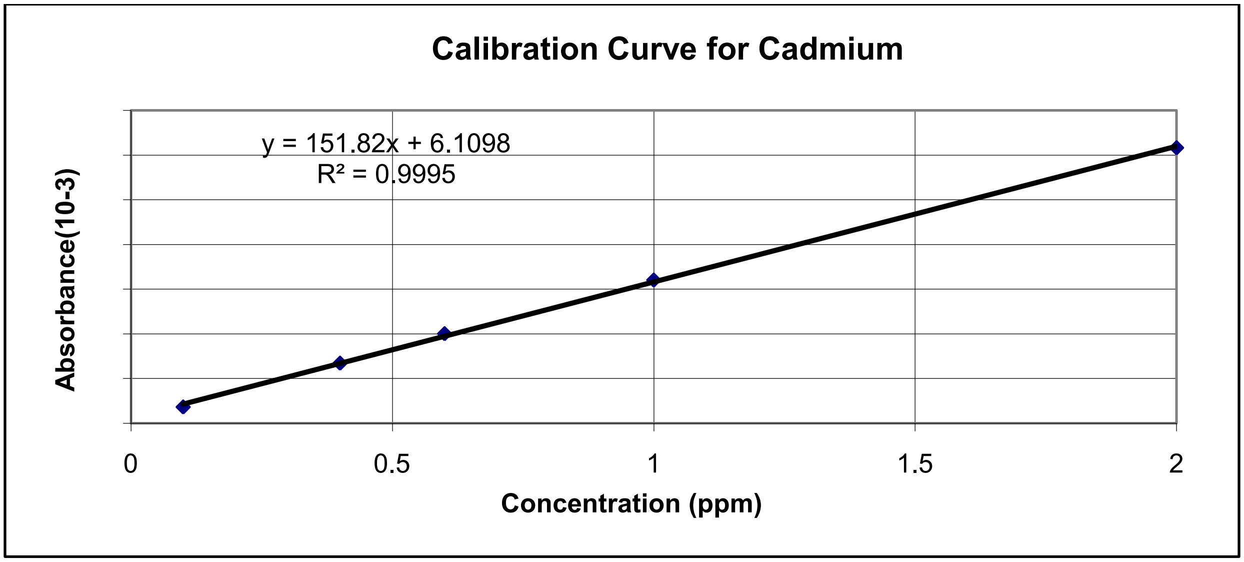


Figure 6. Calibration Curve for Zinc (16th week).

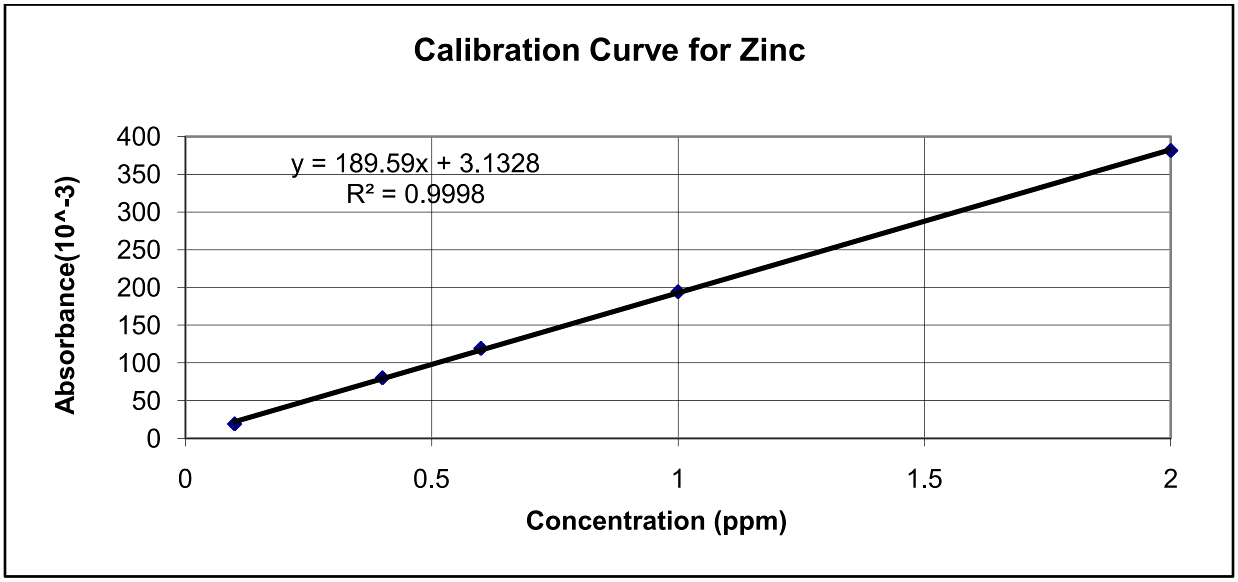
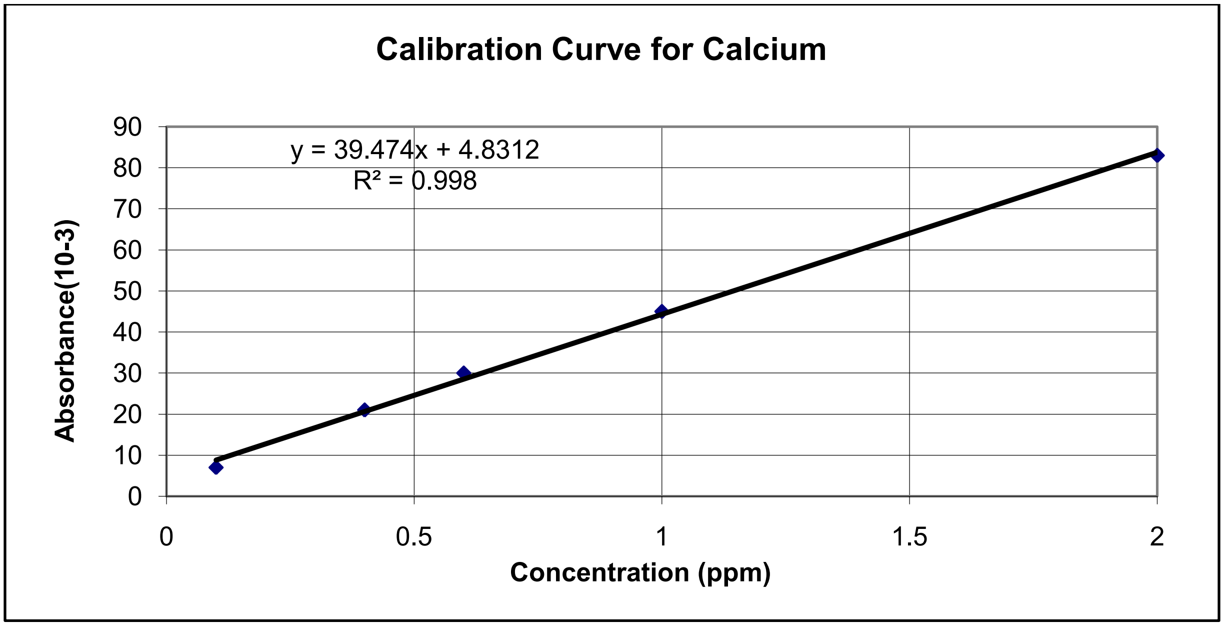


Figure 7. Calibration Curve for Calcium (16th week).



APPENDIX B

Concentration of Metals

Table 1. Concentration of Copper (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	18.78	8.54	15.92	11.62	17.28	8.42	5.83	13.27	9.32
B	16.83	4.51	13.94	9.23	8.82	8.27	9.25	12.84	8.35
C	18.64	5.88	11.74	5.64	8.68	5.55	7.73	11.39	12.06
D	14.91	0.32	6.72	4.50	2.65	5.40	5.44	8.63	4.50
E	22.50	6.29	19.36	11.01	11.68	8.15	7.72	12.01	10.29
F	10.76	3.09	7.53	5.64	6.95	3.08	7.73	9.46	7.40
G	20.69	7.28	11.83	10.41	8.23	2.40	7.73	12.22	3.38
H	15.34	3.70	11.55	5.64	6.37	6.38	7.91	12.23	2.57
I	16.17	6.28	9.68	7.72	6.78	5.96	7.54	9.91	2.42
J	14.90	8.05	11.55	9.23	5.08	7.73	8.29	9.90	10.28
K	12.38	6.08	11.04	6.54	7.38	0.00	4.54	4.62	3.22
L	24.87	10.50	14.26	8.93	6.95	4.72	6.78	8.00	7.40
M		8.69			6.49			10.74	8.04
N		4.54			6.08			4.62	4.34

Table 2. Concentration of Iron (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth weeek	Eighth week	12th week	16th week	20th week
A	687.76	273.00	356.54	348.53	336.62	427.13	84.69	378.99	194.46
B	490.80	248.88	466.83	712.31	504.95	198.51	347.51	455.41	288.13
C	713.99	206.08	149.26	125.83	646.66	133.54	103.03	71.26	176.88
D	691.68	98.63	216.22	124.40	146.69	87.63	194.62	173.77	24.20
E	666.69	532.91	788.50	606.65	842.93	427.61	371.64	276.36	206.33
F	518.41	83.42	131.49	306.31	130.97	81.17	427.16	186.42	241.46
G	392.04	123.76	329.19	452.36	676.89	74.73	365.92	378.95	12.43
H	483.36	99.76	451.42	290.33	602.30	205.56	188.41	353.66	18.29
I	303.10	260.45	224.96	242.26	416.84	94.37	457.12	597.59	59.49
J	430.71	307.21	330.10	325.94	323.13	720.77	457.12	1983.13	2078.31
K	203.85	91.06	82.40	65.87	48.46	0.00	23.61	7.06	24.17
L	726.35	341.74	298.72	335.59	563.32	139.98	261.86	186.67	212.21
M		324.20			287.31			391.78	118.11
N		85.78			56.32			122.56	59.36

Table 3. Concentration of Chromium (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	5.83	4.93	8.77	1.67	1.82	1.11	1.04	1.55	0.63
B	8.23	2.54	3.20	2.54	1.82	0.53	0.67	0.84	0.63
C	4.98	3.58	5.92	0.00	1.49	0.00	1.04	0.84	0.63
D	7.22	0.95	2.65	0.00	0.84	0.24	0.29	0.13	1.99
E	10.85	3.10	9.31	0.37	2.47	0.82	1.42	1.55	2.00
F	6.27	2.01	5.43	0.00	0.84	0.00	0.67	0.84	3.36
G	6.85	1.49	4.31	0.00	1.33	0.00	1.05	1.55	0.63
H	6.45	2.00	3.75	0.00	1.33	0.00	0.67	1.55	0.00
I	7.18	0.96	2.64	0.00	1.16	0.00	0.29	1.55	0.63
J	7.00	1.48	4.31	0.37	1.33	0.24	0.67	2.26	3.36
K	7.16	1.48	0.42	0.00	0.84	0.00	0.29	0.13	0.00
L	5.85	5.84	4.31	0.00	1.42	0.00	1.04	0.13	0.63
M		5.69			0.96			1.56	2.00
N		1.49			0.44			0.13	0.63

Table 4. Concentration of Lead (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	7.74	8.32	15.40	12.73	1.28	3.48	0.01	0.62	1.93
B	9.98	7.83	13.66	10.83	1.28	2.69	0.00	0.00	0.92
C	4.04	11.25	13.53	8.91	2.00	1.90	1.11	1.52	1.93
D	10.27	13.58	8.40	9.02	0.00	1.90	1.11	0.00	0.92
E	13.96	7.78	13.65	8.91	1.28	2.69	2.22	1.52	2.95
F	5.18	10.13	11.92	12.72	1.28	2.69	0.00	0.61	0.00
G	8.46	12.47	17.16	8.91	1.28	1.90	0.00	1.52	0.00
H	7.36	10.12	15.40	8.92	1.28	2.70	0.00	0.00	0.00
I	7.70	15.95	13.66	8.91	1.04	1.11	0.00	0.62	0.92
J	11.17	13.58	13.65	8.92	1.27	1.11	0.00	0.62	1.93
K	6.43	7.80	8.41	8.92	0.00	1.11	0.00	0.00	0.00
L	3.99	13.19	13.66	8.92	1.28	2.69	0.01	0.00	0.92
M		8.99			7.83			0.62	1.93
N		7.86			7.82			0.00	0.91

Table 5. Concentration of Cadmium (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	0.00	0.38	0.49	2.88	0.00	0.00	0.00	0.00	0.00
B	0.00	0.53	0.49	1.83	0.00	0.00	0.00	0.00	0.00
C	0.00	0.45	0.28	2.04	0.00	0.00	0.00	0.00	0.00
D	0.00	0.36	0.07	1.01	0.00	0.00	0.00	0.00	0.00
E	0.00	0.35	0.42	1.62	0.00	0.00	0.00	0.00	0.00
F	0.00	0.27	0.42	1.62	0.00	0.00	0.00	0.00	0.00
G	0.00	0.36	0.28	1.62	0.00	0.00	0.00	0.00	0.00
H	0.00	0.19	0.28	1.00	0.00	0.00	0.00	0.00	0.00
I	0.00	0.01	0.35	1.21	0.00	0.00	0.00	0.00	0.00
J	0.00	0.36	0.35	1.21	0.00	0.00	0.00	0.00	0.00
K	0.00	0.37	0.07	0.58	0.00	0.00	0.00	0.00	0.00
L	0.00	0.38	0.35	1.00	0.00	0.00	0.00	0.00	0.00
M		0.37			0.45			0.00	0.00
N		0.28			0.54			0.00	0.00

Table 6. Concentration of Zinc (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	310.85	52.40	58.85	52.17	74.33	40.00	14.53	86.38	35.56
B	459.00	29.62	32.47	52.48	45.31	35.00	13.44	77.53	25.04
C	398.70	25.57	52.55	25.15	33.76	20.00	4.76	54.57	32.06
D	409.48	49.03	42.51	29.74	29.12	7.22	3.68	36.94	13.68
E	332.08	40.95	121.61	50.69	50.47	22.52	30.78	41.34	25.08
F	365.76	25.72	54.82	27.28	27.38	9.25	0.00	34.69	24.20
G	322.92	33.88	57.69	33.14	19.88	12.33	4.76	45.73	28.56
H	356.21	22.06	41.72	21.45	7.23	9.28	15.62	44.66	21.55
I	340.57	31.71	37.01	28.14	30.49	18.45	18.89	34.77	26.84
J	370.65	30.89	45.11	35.66	14.09	0.00	0.00	52.34	19.80
K	321.12	134.61	75.92	31.68	49.31	0.00	24.30	36.95	32.06
L	394.97	37.63	88.57	33.33	23.94	13.34	0.00	31.44	18.96
M		41.78			31.00			30.35	20.69
N		34.99			48.54			16.05	18.02

Table 7. Concentration of Calcium (ppm).

Sample code	First week	Sec. week	Fourth week	Fifth week	Sixth week	Eighth week	12th week	16th week	20th week
A	602.64	139.70	353.07	531.72	618.31	0.00	106.45	1005.10	283.32
B	463.62	166.19	168.56	208.63	180.51	39.00	199.33	709.46	256.44
C	607.20	256.73	390.77	236.15	0.00	225.72	280.63	446.52	536.01
D	899.15	213.92	313.68	841.64	293.50	11.64	269.32	256.20	221.19
E	350.21	84.07	477.43	233.41	519.32	53.10	112.15	446.71	225.68
F	382.22	127.37	185.43	161.41	1055.23	59.91	0.00	419.17	190.07
G	478.42	282.94	325.00	510.17	0.00	197.99	13.42	314.21	372.04
H	523.50	117.97	183.14	603.63	0.00	0.00	514.34	282.68	415.98
I	279.45	173.93	414.36	930.39	213.93	232.87	77.00	293.49	479.34
J	514.05	89.26	160.96	641.45	0.00	0.00	0.00	720.83	207.61
K	456.90	311.09	367.72	318.02	528.70	0.00	2856.64	414.53	736.20
L	988.49	207.22	411.22	393.61	100.00	0.00	0.00	361.78	599.58
M		186.23			309.01			261.28	261.07
N		81.94			384.59			245.46	163.17

Table 8. Concentration of Metals (ppm) in Samples from Bristol.

Metal	M-2	M-6	M-16	M-20	N-2	N-6	N-16	N-20
Copper	8.69	6.49	10.74	8.04	4.54	6.08	4.62	4.34
Iron	324.20	287.31	391.78	118.11	85.78	56.32	122.56	59.36
Chromium	5.69	0.96	1.56	2.00	1.49	0.44	0.13	0.63
Lead	8.99	7.83	0.62	1.93	7.86	7.82	0.00	0.91
Cadmium	0.37	0.45	0.00	0.00	0.28	0.54	0.00	0.00
Zinc	41.78	31.00	30.35	20.69	34.99	48.54	16.05	18.03
Calcium	186.23	309.01	261.28	261.07	81.94	384.59	245.46	163.17

APPENDIX C

Statistical Summary Table

Table 1. Statistical Values for Copper.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	12.109	11.62	4.48	9.24	14.98	5.83	18.78	3.68
B	10.227	9.23	3.68	7.358	13.1	4.51	16.83	2.55
C	9.7011	8.68	4.27	6.832	12.57	5.55	18.64	3.23
D	5.8967	5.4	4.11	3.028	8.766	0.32	14.91	2.64
E	12.112	11.01	5.41	9.243	14.98	6.29	22.5	3.68
F	6.8489	7.4	2.59	3.98	9.718	3.08	10.76	1.86
G	9.3522	8.23	5.43	6.483	12.22	2.4	20.69	3.82
H	7.9659	6.38	4.23	5.097	10.83	2.57	15.34	3.19
I	8.0511	7.54	3.76	5.182	10.92	2.42	16.17	2.45
J	9.4456	9.23	2.75	6.577	12.31	5.08	14.9	1.94
K	6.2	6.08	3.8	3.331	9.069	0	12.38	2.77
L	10.269	8	6.11	7.399	13.14	4.72	24.87	3.63

Table 2. Statistical Values for Iron.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	343.08	348.5	166	162.1	524.1	84.69	687.8	107
B	412.59	455.4	159	231.6	593.6	198.5	712.3	121
C	258.5	149.3	243	77.49	439.5	71.26	714	146
D	195.32	146.7	195	14.3	376.3	24.2	691.7	105
E	524.4	532.9	222	343.4	705.4	206.3	842.9	180
F	234.09	186.4	155	53.08	415.1	81.17	518.4	118
G	311.81	365.9	209	130.8	492.8	12.43	676.9	151
H	299.23	290.3	191	118.2	480.2	18.23	602.3	153
I	295.13	260.4	172	114.1	476.1	59.49	597.6	128
J	772.94	430.7	725	591.9	954	307.2	2078	439
K	60.72	48.46	62.6	120.3	241.7	0	203.8	43.1
L	340.72	298.7	189	159.7	521.7	140	726.4	130

Table 3. Statistical Values for Chromium.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	3.0389	1.67	2.81	1.383	4.695	0.63	8.77	1.89
B	2.3333	1.82	2.42	0.6775	3.989	0.53	8.23	1.54
C	2.0533	1.04	2.21	0.3975	3.709	0	5.92	1.61
D	1.59	0.84	2.29	-0.06588	3.246	0	7.22	1.35
E	3.5433	2	3.81	1.887	5.199	0.37	10.85	2.4
F	2.1578	0.84	2.35	0.5019	3.814	0	6.27	1.73
G	1.9122	1.33	2.25	0.2563	3.568	0	6.85	1.39
H	1.75	1.33	2.14	0.09412	3.406	0	6.45	1.45
I	1.6011	0.96	2.25	-0.0547	3.257	0	7.18	1.29
J	2.3356	1.48	2.22	0.6797	3.991	0.24	7	1.59
K	1.1467	0.29	2.31	-0.5092	2.803	0	7.16	1.09
L	2.1356	1.04	2.48	0.4797	3.791	0	5.85	1.85

Table 4. Statistical Values for Lead.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	5.7233	3.48	5.61	2.125	9.322	0.01	15.4	4.48
B	5.2433	2.69	5.33	1.645	8.842	0	13.66	4.46
C	5.1322	2	4.78	1.533	8.731	1.11	13.53	3.57
D	5.022	1.9	5.25	1.423	8.621	0	13.58	4.36
E	6.1067	2.95	5.13	2.508	9.705	1.28	13.96	4.07
F	4.9478	2.69	5.27	1.349	8.547	0	12.72	4.23
G	5.744	1.9	6.24	2.146	9.343	0	17.16	4.91
H	5.0867	2.7	5.58	1.488	8.685	0	15.4	4.5
I	5.5456	1.11	6.19	1.947	9.144	0	15.95	4.85
J	5.8056	1.93	5.9	2.207	9.404	0	13.69	4.92
K	3.63	1.11	4.11	0.03122	7.229	0	8.92	3.51
L	4.9622	2.69	5.52	1.363	8.561	0	13.66	4.17

Table 5. Statistical Values for Cadmium.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	0.41667	0	0.943	0.070856	0.7625	0	2.88	0.417
B	0.31669	0	0.609	-0.02914	0.6625	0	1.83	0.317
C	0.30778	0	0.67	-0.03803	0.6536	0	2.04	0.308
D	0.16	0	0.34	-0.1858	0.5058	0	1.01	0.16
E	0.26556	0	0.535	-0.0255	0.6114	0	1.62	0.266
F	0.2566	0	0.534	-0.08914	0.6025	0	1.62	0.257
G	0.2511	0	0.532	-0.0947	0.5969	0	1.62	0.251
H	0.16333	0	0.331	-1825	0.5091	0	1	0.163
I	0.17444	0	0.405	-0.1714	0.502	0	1.212	0.174
J	0.21333	0	0.404	-0.1325	0.5591	0	1.21	0.213
K	0.11333	0	0.213	-0.2325	0.4591	0	0.58	0.113
L	0.19222	0	0.342	-0.1536	0.538	0	1	0.192

Table 6. Statistical Values for Zinc.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	80.563	52.4	88.9	5.48	155.6	14.53	310.9	43.1
B	85.543	35	141	10.46	160.6	13.44	459	59.3
C	71.902	32.06	124	-3.181	147	4.76	398.7	51.6
D	69.044	29.74	129	-6.039	144.1	3.68	409.5	53.8
E	79.502	41.34	99.2	4.419	154.6	22.52	332.1	48.4
F	63.233	27.28	114	-11.85	138.3	0	365.8	47.1
G	62.099	33.14	99.1	-12.98	137.2	4.76	322.9	43.9
H	59.976	21.55	112	-15.11	135.1	7.23	356.2	45.7
I	62.986	30.49	104	-12.1	138.1	18.45	340.6	39.1
J	63.171	30.89	117	-11.91	138.3	0	370.6	52.2
K	78.439	36.95	98.8	3.356	153.5	0	321.1	54.8
L	71.353	31.44	124	-3.73	146.4	0	395	55.4

Table 7. Statistical Values for Calcium.

				95% conf. limit		Value		
Sample code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Av. Dev.
A	404.48	353.1	317	175.1	633.9	0	1005	248
B	265.75	199.3	201	36.34	495.2	39	709.5	120
C	331.08	280.6	185	101.7	560.5	0	607.2	140
D	368.92	269.3	298	139.5	598.3	11.64	899.1	183
E	278.01	233.4	178	48.6	507.4	53.1	519.3	147
F	286.76	185.4	319	57.35	516.2	0	1055	189
G	277.13	314.2	180	47.73	506.5	0	510.2	132
H	293.47	282.7	231	64.06	522.9	0	603.6	195
I	343.86	279.4	251	114.5	573.3	77	930.4	158
J	259.35	161	289	29.94	488.8	0	720.8	222
K	665.53	414.5	845	436.1	894.9	0	2857	398
L	340.21	361.8	317	110.8	569.6	0	988.5	232

APPENDIX D

Two Way ANOVA Table

Table 1. Two Way ANOVA Table for Copper.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1760.719(a)	19	92.669	17.344	.000	.789
Intercept	8776.643	1	8776.643	1642.670	.000	.949
Site	426.087	11	38.735	7.250	.000	.475
Week	1334.632	8	166.829	31.224	.000	.739
Error	470.176	88	5.343			
Total	11007.539	108				
Corrected Total	2230.896	107				

a R Squared = .789 (Adjusted R Squared = .744)

Table 2. Two Way ANOVA Table for Iron.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	4170499.945(a)	19	219499.997	3.143	.000	.404
Intercept	12292919.380	1	12292919.380	176.022	.000	.667
Site	3131046.203	11	284640.564	4.076	.000	.338
Week	1039453.742	8	129931.718	1.860	.076	.145
Error	6145677.802	88	69837.248			
Total	22609097.128	108				
Corrected Total	10316177.747	107				

a R Squared = .404 (Adjusted R Squared = .276)

Table 3. Two Way ANOVA Table for Chromium.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	529.963(a)	19	27.893	21.704	.000	.824
Intercept	491.435	1	491.435	382.395	.000	.813
Site	41.795	11	3.800	2.956	.002	.270
Week	488.168	8	61.021	47.482	.000	.812
Error	113.093	88	1.285			
Total	1134.491	108				
Corrected Total	643.056	107				

a R Squared = .824 (Adjusted R Squared = .786)

Table 4. Two Way ANOVA Table for Lead.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2609.244(a)	19	137.329	44.572	.000	.906
Intercept	2972.027	1	2972.027	964.608	.000	.916
Site	40.403	11	3.673	1.192	.304	.130
Week	2568.841	8	321.105	104.219	.000	.905
Error	271.134	88	3.081			
Total	5852.405	108				
Corrected Total	2880.378	107				

a R Squared = .906 (Adjusted R Squared = .886)

Table 5. Two Way ANOVA Table for Cadmium.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	23.139(a)	19	1.218	28.298	.000	.859
Intercept	6.011	1	6.011	139.682	.000	.613
Site	.703	11	.064	1.486	.151	.157
Week	22.436	8	2.804	65.166	.000	.856
Error	3.787	88	.043			
Total	32.938	108				
Corrected Total	26.926	107				

a R Squared = .859 (Adjusted R Squared = .829)

Table 6. Two Way ANOVA Table for Zinc.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1200826.238(a)	19	63201.381	130.047	.000	.966
Intercept	539089.173	1	539089.173	1109.259	.000	.926
Site	7384.540	11	671.322	1.381	.196	.147
Week	1193441.698	8	149180.212	306.961	.000	.965
Error	42767.169	88	485.991			
Total	1782682.580	108				
Corrected Total	1243593.407	107				

a R Squared = .966 (Adjusted R Squared = .958)

Table 7. Two Way ANOVA Table for Calcium.

Tests of Between-Subjects Effects

Dependent Variable: Metal conc.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3385824.059(a)	19	178201.266	1.672	.057	.265
Intercept	12697148.134	1	12697148.134	119.165	.000	.575
Site	1221958.170	11	111087.106	1.043	.417	.115
Week	2163865.889	8	270483.236	2.539	.016	.188
Error	9376477.345	88	106550.879			
Total	25459449.539	108				
Corrected Total	12762301.404	107				

a R Squared = .265 (Adjusted R Squared = .107)

APPENDIX E

Concentration Vs Sampling Site Graph

Figure 1. PPM of Copper at Different Locations and Weeks.

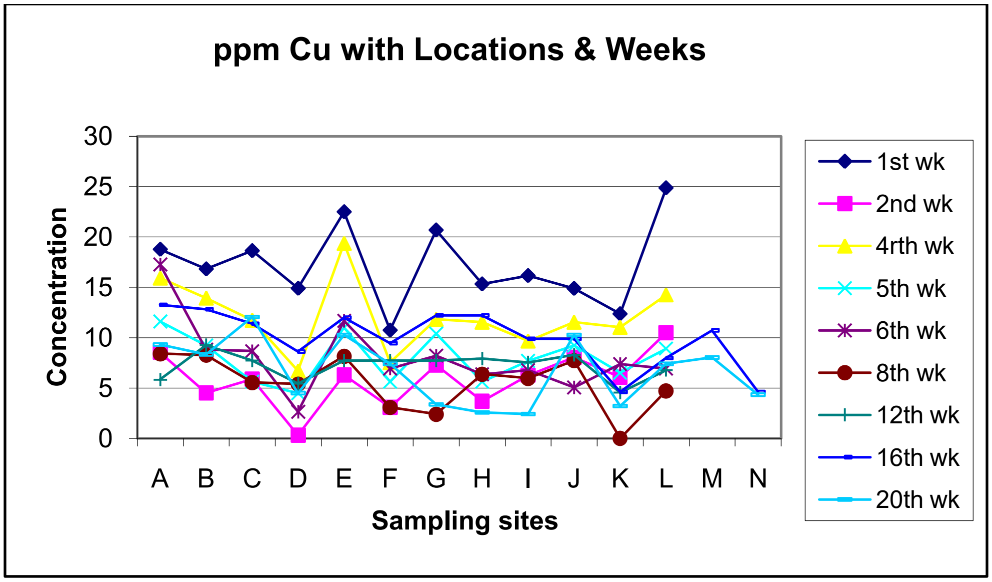


Figure 2. PPM of Iron at Different Locations and Weeks.

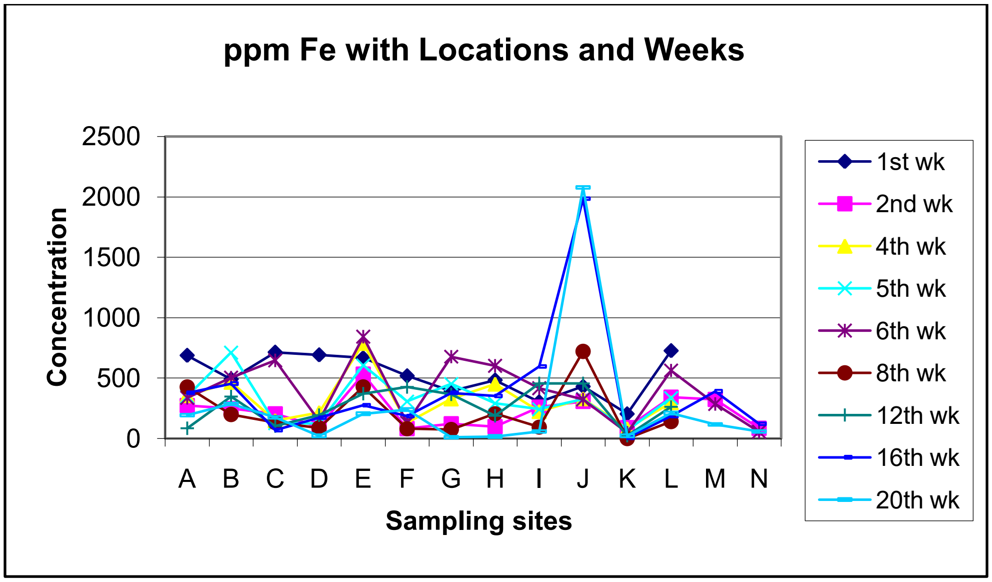


Figure 3. PPM of Chromium at Different Locations and Weeks.

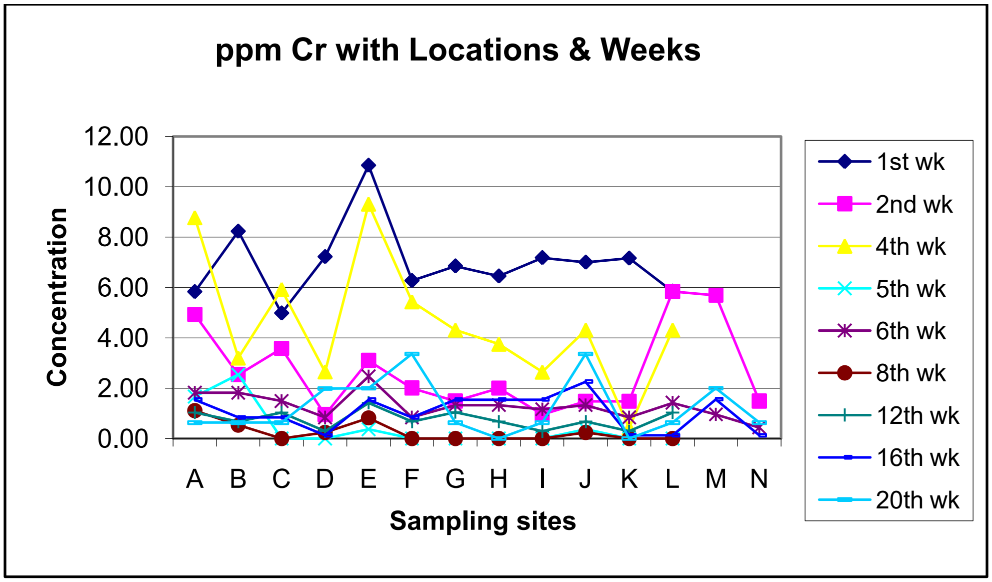


Figure 4. PPM of Lead at Different Locations and Weeks.

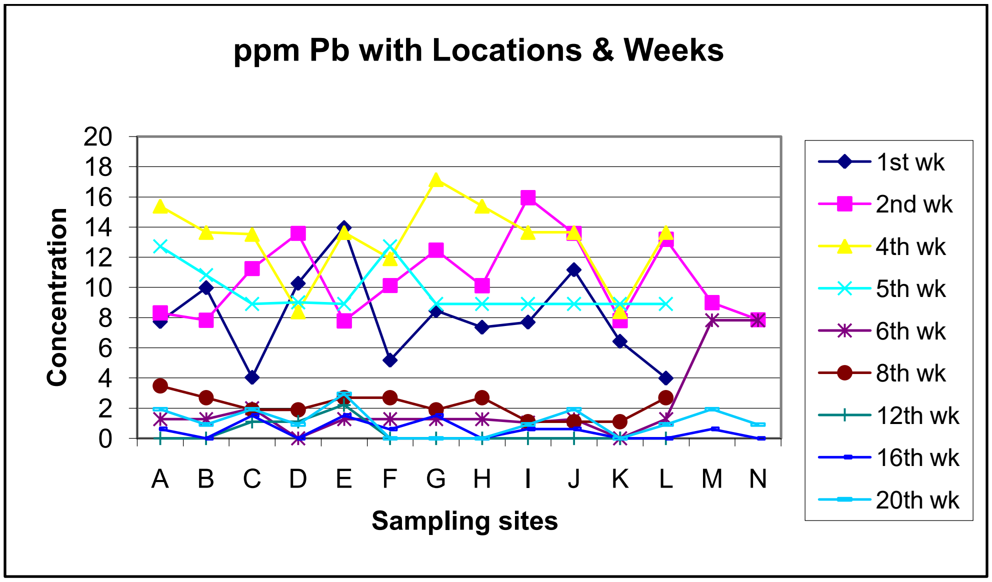


Figure 5. PPM of Cadmium at Different Locations and Weeks.

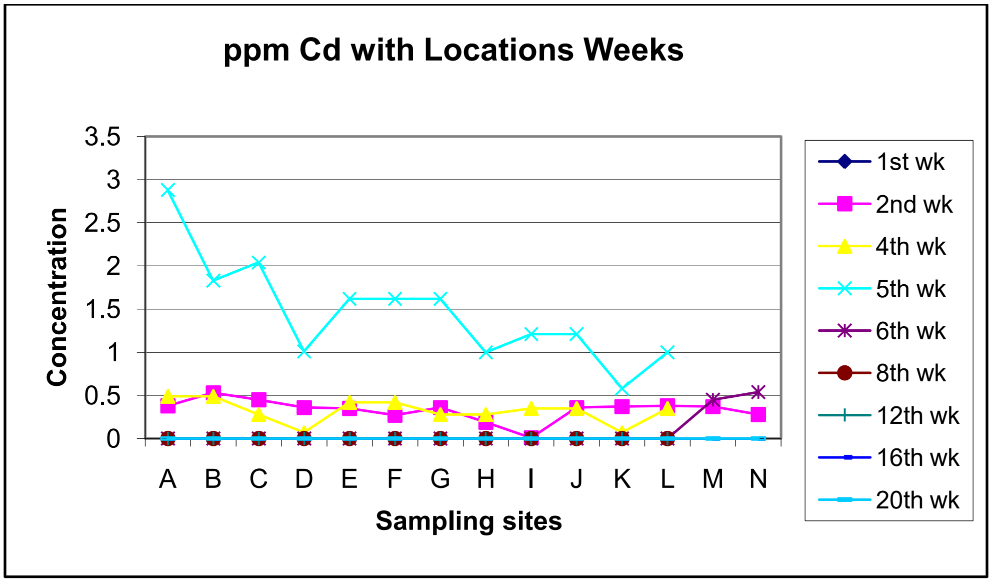


Figure 6. PPM of Zinc at Different Locations and Weeks.

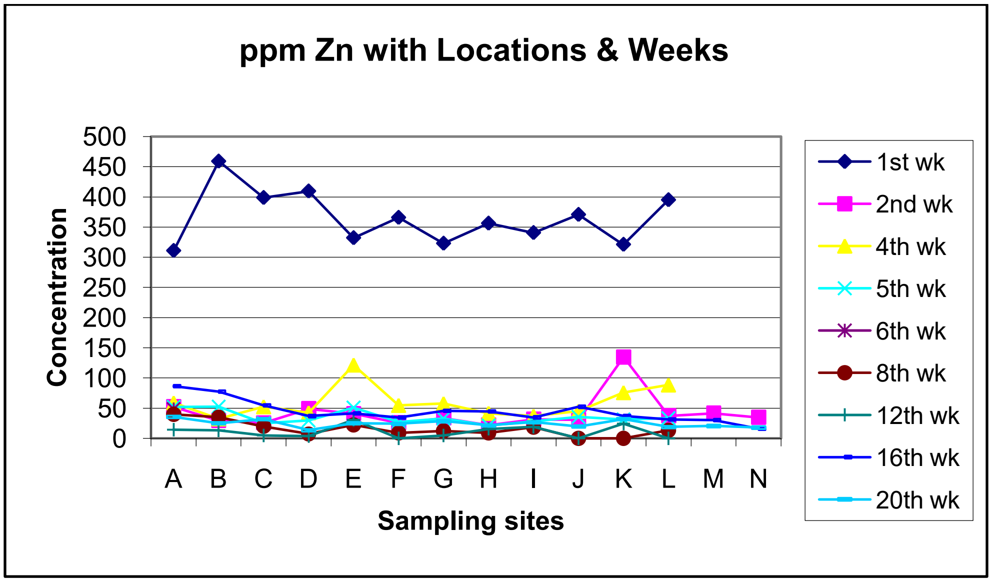


Figure 7. PPM of Calcium at Different Locations and Weeks.

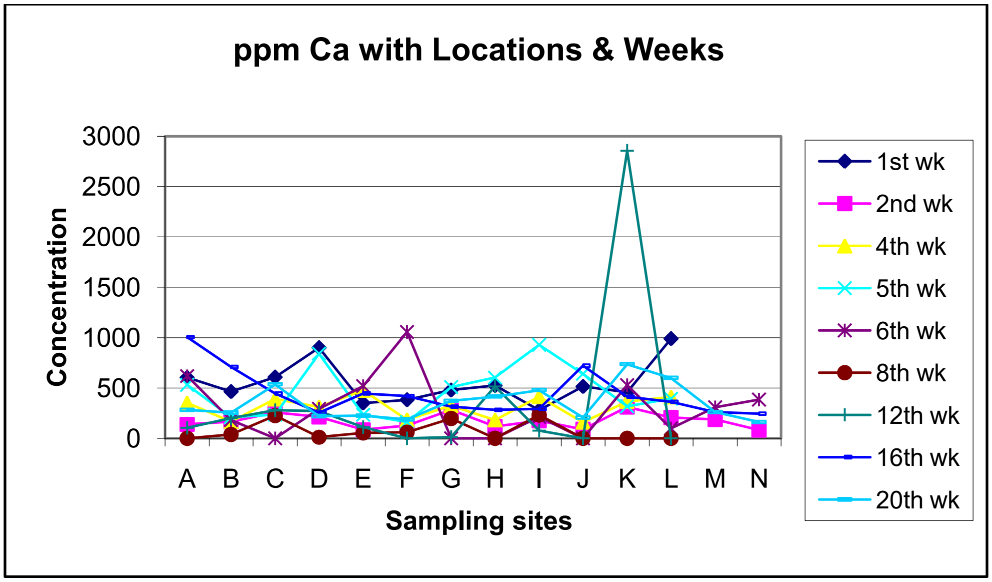
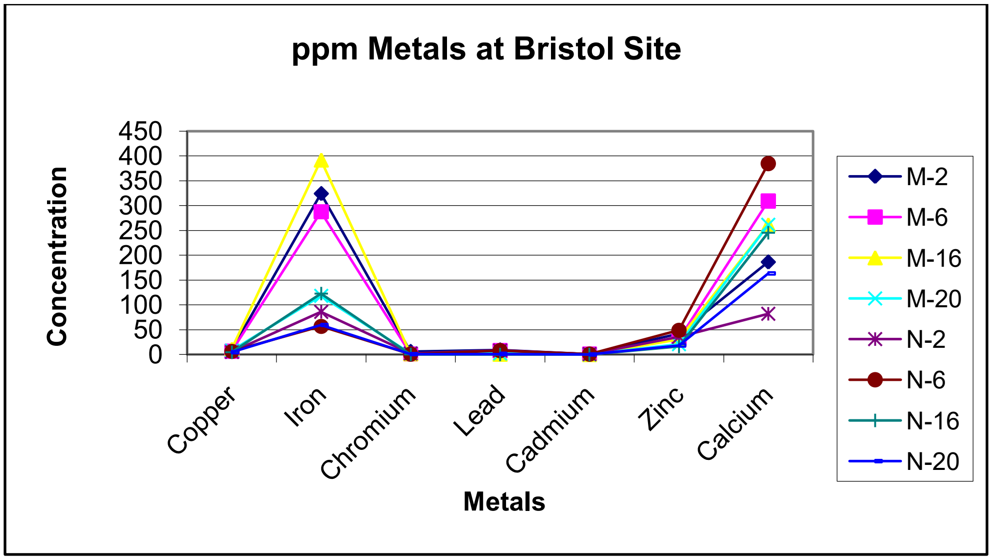


Figure 8. PPM of Different Metals at Bristol.



APPENDIX F

Concentration of Metals in Samples at Different Sites for Specific Weeks

Figure 1. Concentration of Different Metals in the First-Week Samples.

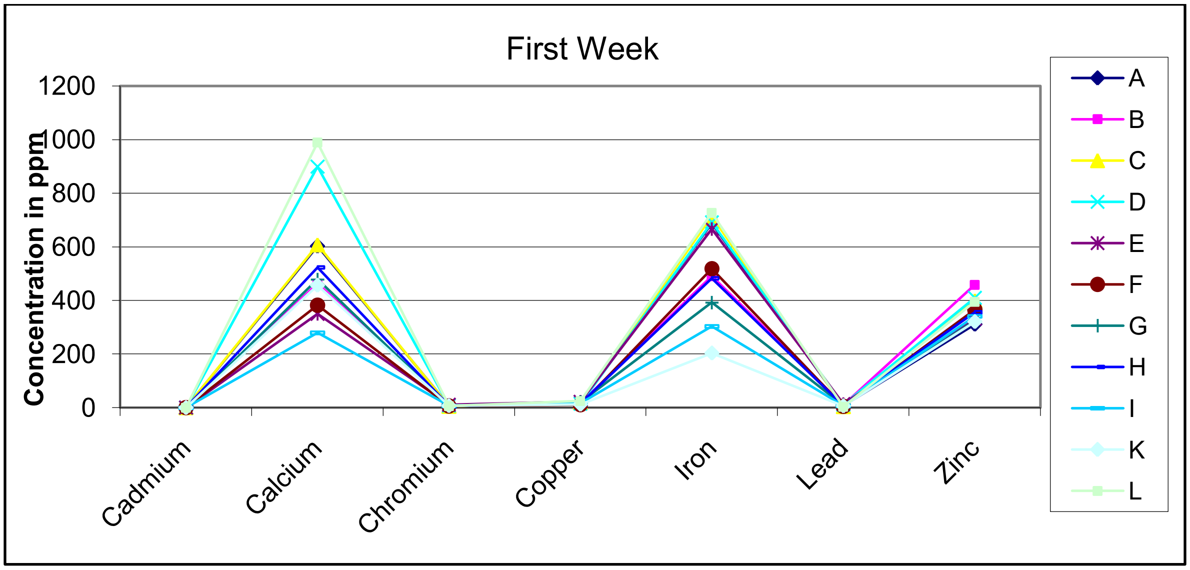


Figure 2. Concentration of Different Metals in the Second-Week Samples.

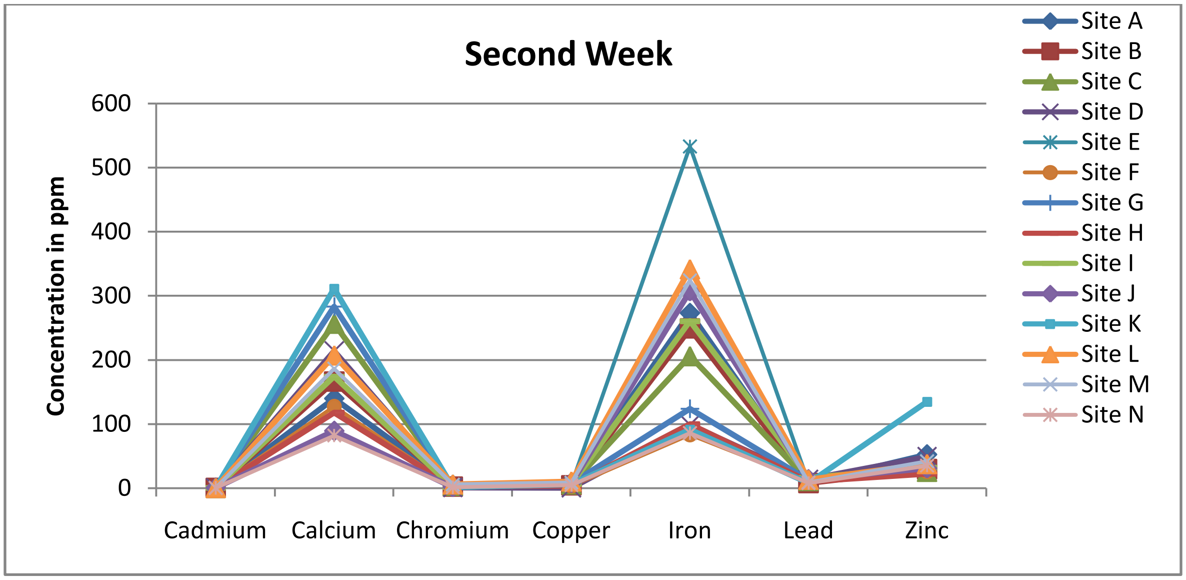


Figure 3. Concentration of Different Metals in the Fourth-Week Samples.

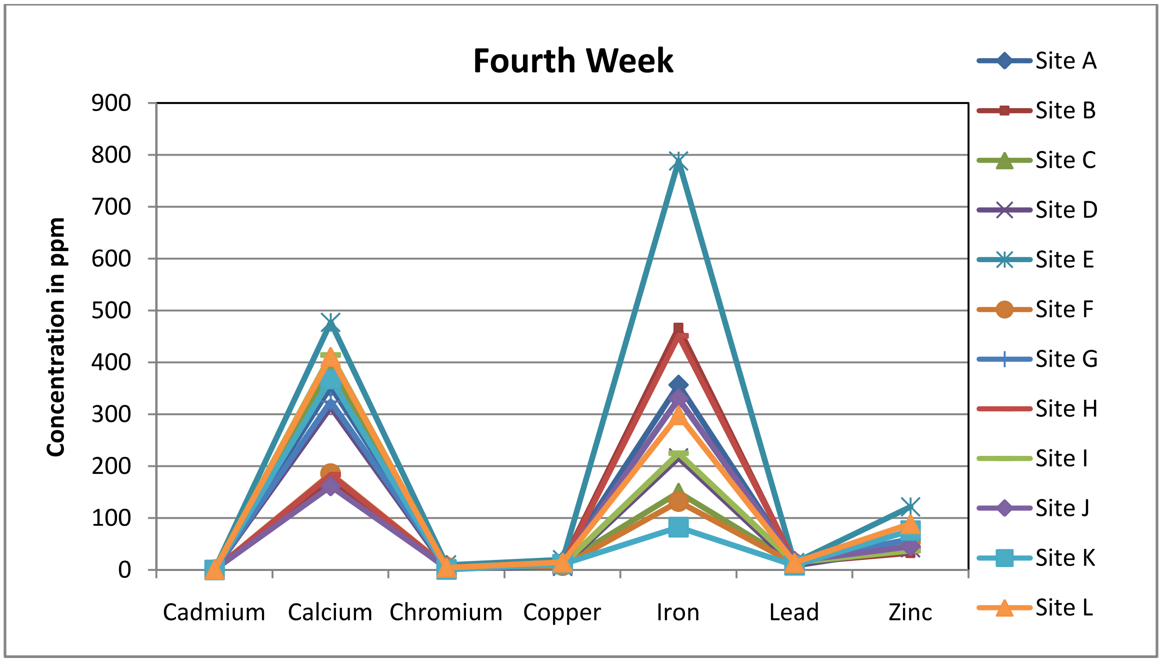


Figure 4. Concentration of Different Metals in the Fifth-Week Samples.

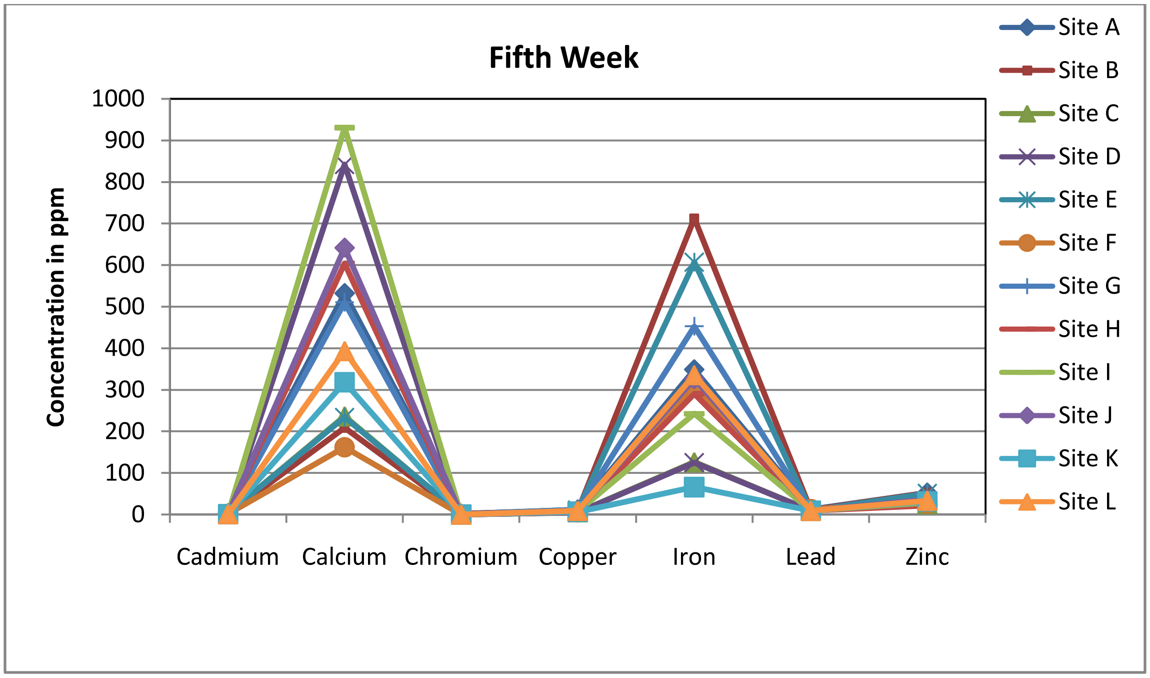


Figure 5. Concentration of Different Metals in the Sixth-Week Samples.

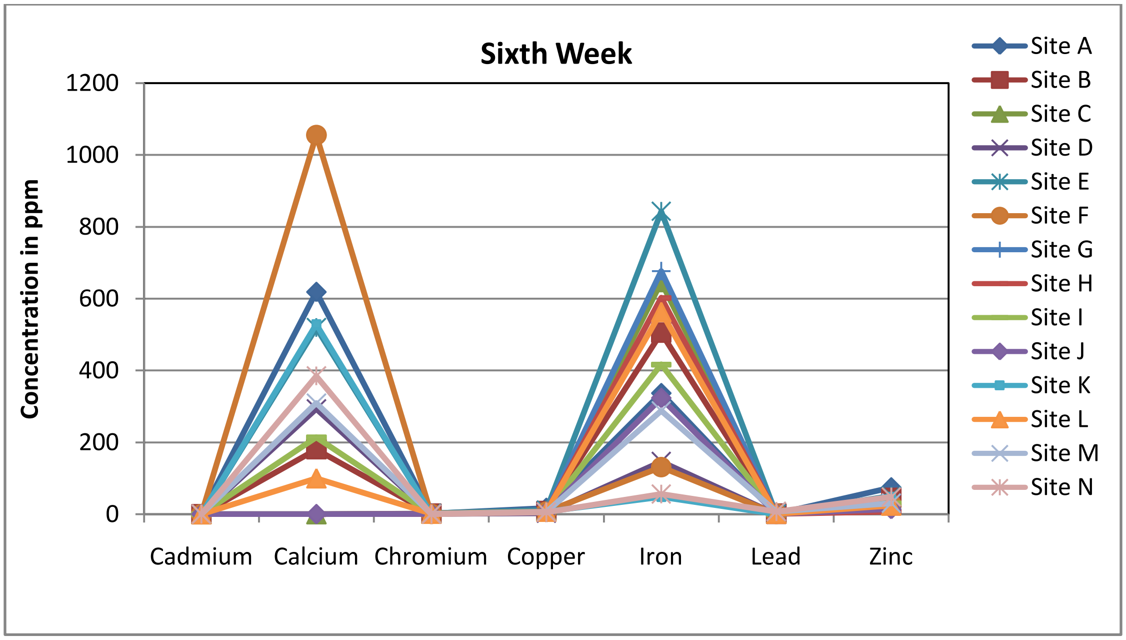


Figure 6. Concentration of Different Metals in the Eighth-Week Samples.

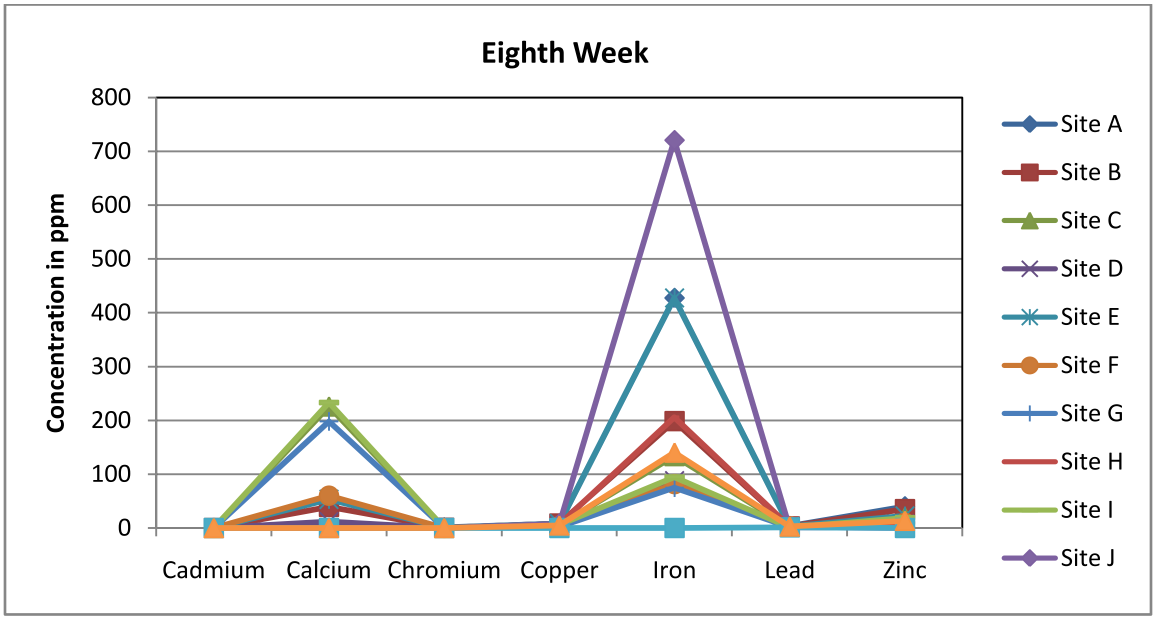


Figure 7. Concentration of Different Metals in the 12th-Week Samples.

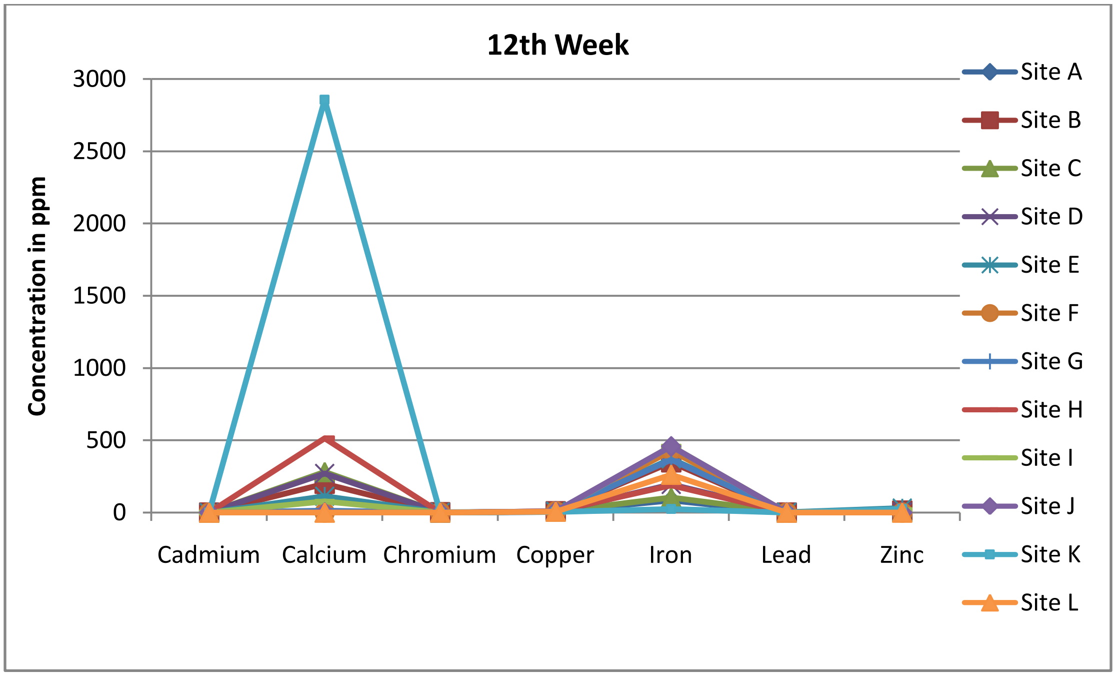


Figure 8. Concentration of Different Metals in the 16th-Week Samples.

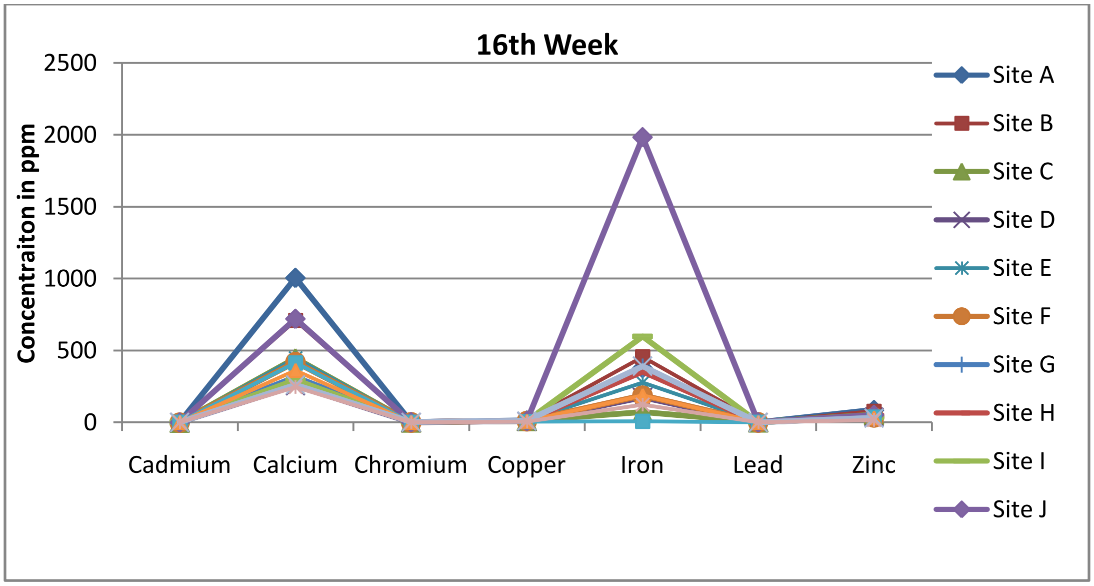
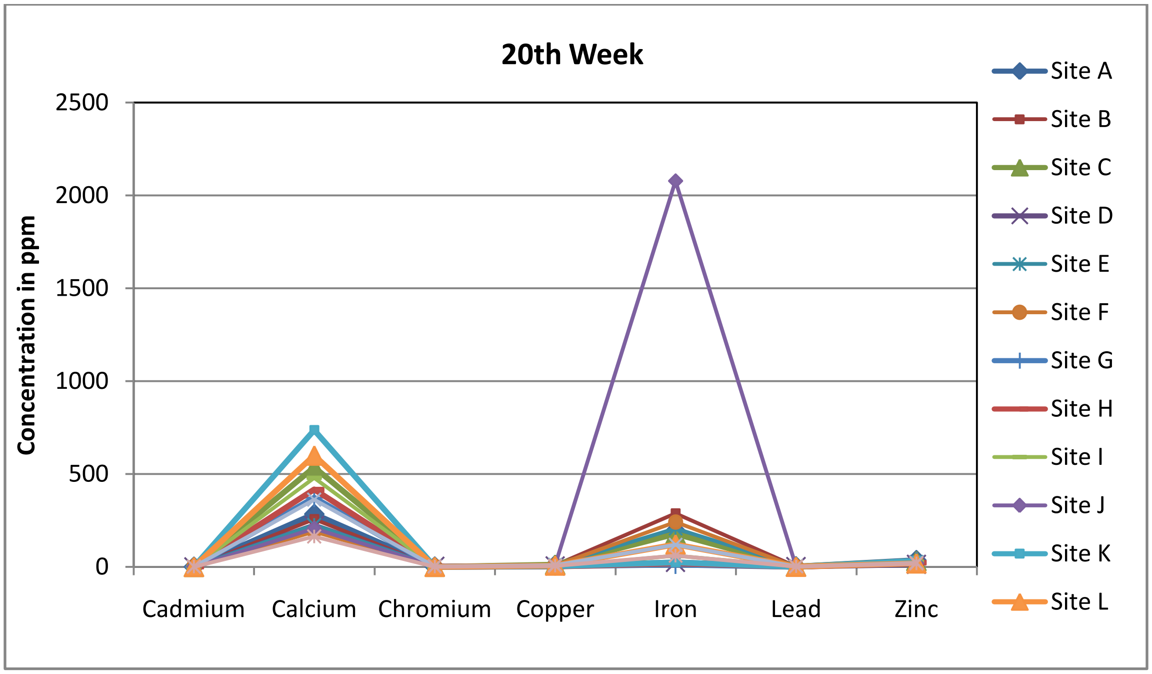


Figure 9. Concentration of Different metals in the 20th-Week Samples.



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