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Assessing Commercial Organic and Conventionally Grown Vegetables by Monitoring Selected Heavy Metals Found in Them.

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Assessing Commercial Organic and Conventionally Grown Vegetables by Monitoring Selected
Heavy Metals Found in Them

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A thesis
presented to
the faculty of the Department of Chemistry
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Science in Chemistry

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by
Charles Kafui Dotse
May 2010

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ABSTRACT

Assessing Commercial Organic and Conventionally Grown Vegetables by Monitoring Selected
Heavy Metals Found in Them

by

Charles Kafui Dotse

Commercially available organic and conventionally grown vegetables were studied by
quantitative determination of selected metals in them and to determine if any differences found
are statistically significant. These findings can help the consumers to determine if the vegetable
products are within the recommended maximum limits as proposed by the joint FAO/WHO
Expert committee on organic foods designation. Eight edible vegetables were purchased from
local stores in both the organic and conventionally grown categories. Samples were digested
with concentrated nitric acid and the metals monitored were zinc, copper, lead, iron, cadmium,
and nickel using flame atomic absorption. The concentration range for the heavy metals found
are as follows: Zn, 2.04-69.4; Cu, 0.35-15.1; Pb, 0.00-3.99; Cd, 0.00-0.74; Fe, 2.52-319; and Ni,
39.9-53.8 µg/g. It was found that in general, conventional vegetables contain higher amounts of
most of the heavy metals studied as compared to their organic counterparts. The study also
showed that all vegetables products contain below the permissible limits for Zn, Cu, Ni, and Fe.
For Pb all vegetables exceeded the safe limit except organic cucumber and conventional
cabbage. For Cd, organic lettuce and green pepper, and conventional leafy green, green pepper,
and spinach all exceeded the limit recommended by FAO/WHO.

DEDICATION

      This work is dedicated to my mother, Mrs. Beatrice Avornyotse, and my brother, Ernest Kyekye. Both of you supported my dreams and aspirations and I do say thank you for your prayers, love, and encouragement.

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CHAPTER 1

INTRODUCTION

According to the Organic Trade Association (OTA), sales of organic foods topped $13.8 billion in 2005 representing a significant 2.5 % of the total US food sales. Compare this to 1997, when organic foods accounted for just 0.8 % of total foods sales [1]. However, there are increasing concerns about food safety and the facts that many processed foods are made from genetically modified products. Although there is no official claim of better quality for organically produced food than its conventional counterpart [2], expanding consumer demand reflects consumer perceptions of organic foods as being more environmentally friendly [3, 4], safer [5], and more nutritious and health promoting than conventional foods [6, 7]. Recent food crisis such as mad cow disease and foot-and-mouth disease have lessened consumer confidence of safety in foods in general and especially in conventionally produced foods that may use pesticides, antibiotics, and other chemicals in food production [8, 9].

One of the strongest arguments in favor of organic farming is that it supposedly does not cause pesticides and chemical run-off and the resulting contamination of watersheds and drinking water. North American studies indicate that organic farms tend to be smaller and more socially supportive, have greater diversity of crops in rotation, and reduced health risks associated with pesticides, antibiotics and nitrates [10]. One French study analyzed 12 foods and concluded that organics was ahead in terms nutritional quality and micronutrients. In organic foods one finds more micronutrients essentials for good health: vitamins A, C, E, vitamins of B group, and other elements such as zinc and calcium [11]. A recent article in the Journal of Applied Nutrition gave credence to the notion that organic foods have higher nutrient levels than non-organic food. In this study, the mineral content of organic apples, pears, potatoes, wheat, and sweet corns were compared to commercial varieties [11]. Overall, the organic foods showed much higher levels of nutrient minerals and much lower levels of hazardous heavy metals [11].

However, on February 4, 2000, ABC News correspondent John Stossel hosted a report on "20/20" that probably surprised many fans of organic foods. He made the case that organic food

is not necessarily healthier than conventional food and might actually be dangerous [12]. Manure used in organic compost may be more harmful than health risks from pesticides residues. Manure may contain *E. coli* bacterium, which can spread harmful animal-borne diseases to humans. However, the United States Development Agriculture (USDA) proposed regulations for organics that include temperature guidelines to kill any human pathogens that could be present in the manure.

On October 21, 2002, the USDA [13] provided a definition for organic food, which states that: organic food is produced by farmers who emphasize the use of renewable resources and the conservation of soil and water to enhance environmental quality for future generations. Organic meat, poultry, eggs, and dairy products come from animals that are given no antibiotics or growth hormones. Organic food is produced without using the conventional pesticides; petroleum-based fertilizers, or sewage sludge-based fertilizers, bio-engineering, or ionizing radiation. Before a product can be labeled "organic," a government-approved certifier inspects the farm where the food is grown to make sure the farm is following all the rules necessary to meet USDA organic standards. Companies that handle or processed organic food before it gets to the local supermarkets or restaurant must also be certified [13].

Organic and Conventional Practices

Despite warnings about the health effects of chemical fertilizers and pesticides, farmers abandoned the labor-intensive practices used in organic agricultures in favor of the easier to use chemicals. Before the Second World War chemicals were rarely used. But by 1995 more than 45 million tons of chemicals fertilizers and 770 million pounds of synthetic pesticides were used in U.S agriculture alone [21, 22]. Ninety-five percent of the crops in the United States are now produced using chemical fertilizers and pesticides [13], and producing crops using these chemicals have come to be known as conventional agriculture.

U.S regulations require that organic foods are grown without synthetic pesticides, growth hormones, antibiotics, modern genetic engineering techniques (including genetically modified

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crops), chemical fertilizers, or sewage sludge. Good farming practices such as crop rotation, tillage and cultivation practices, cover crops, and natural products (such as natural fertilizers) are used to enhance soil fertility. Organic farmers also employ animal and crop wastes, botanical, biological or non-synthetic pest controls and allow the use of synthetic materials that can be decomposed quickly by oxygen and sunlight. Specific methods to minimize air, soil, and water pollution are also practiced [23].

Legislation and Regulation of Organic Foods

Part of the 1990 Farm Bill introduced by the USDA was the Organic Food Production Act (OFPA). OFPA established standards for marketing organically produced products to assure consumers that organic products meet a consistent standard and to facilitate interstate commerce of organic products. The OFPA also created the National Lists of Allowed and Prohibited Substances that lists synthetic substances and ingredients allowed and natural substances and ingredients prohibited from production and handling of organic foods [23]. The establishment of National Organic Program Standards, mandated by OFPA, was announced in late 2000 [23] and was fully implemented in 2002. After the standards became effective, USDA clarified that organic certification only expressed a production philosophy, and that organic labeling did not imply a superior, safer, or healthier product than foods not labeled as organic. All foods labeled with the USDA organic seal must come from certified farm or handling operation. All products labeled as "100% Organic" must contain only organically produced ingredients; products labeled as "organic" must contain at least 95% organically produced ingredients. The other 5% of ingredients may come from the National List of Approved Substances. One hundred percent and 95% organic products may use the USDA organic seal shown in Figure 1 [23].

Figure 1. The USDA organic seal.

Products that contain at least 70% organic ingredients can be labeled "made with organic ingredients" and list up to three of those on the principal display panel. However, such products may not use the USDA organic seal. The USDA has also developed a financial assistance program, the National Organic Cost-Sharing Program, for organic farmers to assist them pay for their organic certification. The European Commission adopted new regulations on organic production. The new rule effective January 1, 2007, made it easier to be understood by both producers and consumers and was slightly flexible for the different regions in the European Union (EU). Organic products in the EU must contain at least 95% organic ingredients. Imported organic products must comply with EU standards or the country of origin must have equivalent guarantees. The United States also accepts products from countries such as the EU that have equivalent guarantees [23].

Quality and Safety Comparisons of Organic and Conventional Foods

Pesticides

According to a recent survey, 70% of consumers said they purchased organic foods to avoid pesticides [24]. Synthetic substances can be in organic foods production if they are on the National List. The use of such substances is permissible only when they do not contribute to the contamination of crops, soil, or water and when other recommended organic pest management practices prove insufficient to prevent or control pests. Among the types of synthetic substances approved for use on organic crops are water disinfectants such as calcium hypochlorite, sodium hypochlorite, and copper sulfate; and insecticides such as boric acid, lime sulfur, elemental sulfur, copper sulfate, and oils. Interestingly, though, only a small number of studies have looked at specific differences between pesticides residues on organic and conventional foods, [23].

Baker et al. [25] conducted the most comprehensive study on the relationship between pesticides residues in conventional and organic foods. The study relied on three distinct pesticide residue databases: USDA's pesticide data program (PDP), the marketplace surveillance program of the California Department of Pesticide Regulation (CDPR), and a consumer Union private residue testing program. These data are shown in Table 1. Each program differed markedly in sensitivity, analytical scope, and sample collection techniques, thus rendering comparisons of findings between the residues databases inappropriate.

Nevertheless, each individual database showed higher percentage of residues in conventional than organic produce. The findings also suggested that organic foods may be less risky than conventional foods with respect to pesticides [25, 26].

Nutrients

Many consumers have indicated that they consider organic foods to be more nutritious than conventional foods [24]. They frequently maintain that the methods commonly used to increase the yields of conventional foods such as use of pesticides and fertilizers may limit the natural ability of plants to incorporate or synthesize nutrients.

Table 1. Pesticides residues in conventional and organic produce by different monitoring programs [25, 26].

	USDA pesticide data program	CDPR market surveillance program	Consumer Union	Belgium
Conventional	73%	31%	79%	49%
Organic	23%	6.5%	27%	12%

Indirect evidence supporting this argument comes from a study [27] that compared USDA nutrient content data for 43 garden crops between 1950 (before many modern methods of agricultural production had achieved widespread adoption) and 1999. Statistically reliable values of declines were noted for 6 nutrients (protein, calcium, potassium, iron, riboflavin, and ascorbic acid) with declines ranging from 6% for protein to 38% for riboflavin. However, the authors attributed the losses to changes in cultivars (plant varieties) and not to increased pesticide or fertilizer use.

Three major review articles comparing nutritional quality of organic and conventional foods have been published. Woese et al. [28] reported an extensive literatures base of 150 comparative studies published between 1926 and 1994 that examined the quality of foods grown under different production methods. The review included foods such as cereals, potatoes, vegetables, fruits, wine, beer, bread, milk, dairy products, meat and meat products, eggs, and honey. The authors concluded that no major differences in nutrients level were found between the different production methods in some cases, while in other cases contradictory findings did not permit definitive conclusions about the influence of production methods on nutrients levels.

Worthington [19] reviewed 41 studies that compared crops produced with organic fertilizer or organic farming systems to crops produced using conventional systems. It was reported that organic crops contained 27% more vitamin C, 21.1% more iron, 29.3% more

magnesium, and 13.6% more phosphorus than conventional crops. Bourn and Prescott [14]
summarized a number of studies that compared the effect of inorganic and organic fertilizers on
the nutritional values of crops. They concluded that study designs and results were too variable
to provide any definitive conclusions about the influence of production methods on nutrients
levels.

Researchers in recent years have conducted several controlled studies to compare organic
and conventional foods with respect to nutritional composition. Some studies concluded that
organic production methods lead to increases in nutrients, particularly organic acids and
polyphenolic compounds, many of which are considered to have potential human health benefits
as antioxidants.

Two major hypotheses explaining the possible increases in organic acid and
polyphenolics in organic versus conventional foods have been proposed. One hypothesis
considers the impacts of different fertilization practices on plant metabolism. In conventional
agricultural, synthetic fertilizers frequently make nitrogen readily available for growth and
development. Therefore, plant resources are allocated for growth purposes, resulting in a
decrease in the production of plant secondary metabolites (compounds not essential to the life of
the plant) such as organic acids, polyphenolics, chlorophyll, and amino acids. The second
hypothesis considers the responses of plants to stressful environments such as attacks from
insects, weeds, and plant pathogens [23].

It has been argued that organic production methods that limit the use of insecticides,
herbicides, and fungicides to control plants pests put greater stresses on plants and may require
plants to devote greater resources toward the synthesis of their own chemical defense. Increases
in antioxidants such as plant polyphenolics have been attributed to their defense mechanisms
[29]. The same mechanisms, however, may result in the elevations of other plant secondary
metabolites that may be of toxicological rather than nutritional significance. While the two
hypothesis may explain the potential increases in nutritional compounds in organic foods relative
to conventional foods as seen in a few studies, the impact on human health of consuming greater
levels of organic acids and polyphenolics is yet to be determined.

The biggest study ever of organic foods was completed in 2007, and it found that organic fruits and vegetables contain up to 40% more antioxidants than conventional equivalents and that the figure was 60% for organic milk. The 4-year study was funded by the European Union and was the largest of its kind ever undertaken [30, 31].

There is evidence, however, that the major change in agricultural methods may not have been entirely benign from a nutritional point of view. Along with the changes in agricultural practices, there have been identified changes in the nutrient composition of fresh fruits and vegetables. Four different analyses of U.S and British nutrient content data have shown a decline in the vitamin and mineral content of fresh fruits and vegetables over the last 60 years [32-35].

Hazardous Heavy Metals

Several kinds of fertilizers contain hazardous heavy metals that enter the soil and can be absorbed by plants. Phosphate fertilizers often are contaminated with cadmium. Also, trace mineral fertilizers and liming materials derived from industrial waste can contain a number of heavy metals [36]. These heavy metals build up in the soil when these fertilizers are used year after year. As the soil becomes more contaminated, the crops grown on these soils also become more contaminated. Plants growing on these soils may absorb these heavy metals [37, 38]. Organic farmers only rarely use fertilizers and virtually never use fertilizers produced from industrial waste, which are the most contaminated [36, 39]. Thus, it might be expected that organic crops would contain lower amounts of hazardous heavy metals. However, more investigation is required to confirm this expectation.

Nitrates

While nutritional comparisons of organic and conventional foods showed variable data when possible differences in plant secondary metabolites and minerals were considered, it appears that organic production of foods does result in lower nitrate levels. Worthington [19] summarized the results of 18 studies comparing nitrate levels of organic and conventional foods and found 127 cases where nitrate levels were higher in conventional foods, 43 cases where nitrate levels of organic foods were higher, and 6 cases where no difference was observed. The

ratio of nitrate levels in conventional foods relative to organic foods ranged from 97% to 819%. A review by Woese et al. [28] also came to the same conclusion.

Nitrate levels in plants, however, are determined by a number of factors such as variety of plant, light intensity, climate, soil, and nitrogen supply. Nitrogen, specifically amount available during growth and time of application to the plant, has been considered as the source of nitrate variability in most studies comparing organically versus conventionally grown produce. In many organic fertilizers, the organically bound nitrogen is relatively insoluble and must be mineralized before it can be used by the plant. Barker [40] found that only one of five organic fertilizers resulted in significantly lower nitrate concentration (dry matter basis) in spinach compared to mineral fertilizer, and this occurred for only one of the two cultivars tested. This reduction in nitrate was due to the low mineralization rate of cow manure as opposed to the other organic fertilizers which are mineralized rapidly.

Taste

Consumers of organically grown fruits and vegetables often believe that these products taste better than conventional produce. Zhao et al. [41] conducted consumer sensory analysis of organically and conventionally grown vegetables in which they used replicated side-by-side plots to produce the vegetables for the studies. In one test, red loose leaf lettuce, spinach, arugula, and mustard greens grown organically and conventionally were evaluated for overall liking as well as for intensity of flavor and bitterness. Similarly, tomatoes, cucumbers, and onions were also grown. In general, they found no significant difference in consumer liking or consumer-perceived sensory quality. The only exception was tomatoes where the conventionally produced tomato was rated as having the significantly stronger flavor than the organically produced tomato. They indicated, however, that consumer panelists in both tests considered organic produce to be healthier (72%) and more environmentally friendly (51%) than conventional produce, while 28% considered organic produce to have better taste. Covariance analysis also indicated that consumer demographics affected sensory comparisons of organic and conventional lettuce and cucumbers.

Naturally Occurring Toxins

Hundreds of different plants' secondary metabolites have been identified and their occurrence has been comprehensively reviewed [42]. Many of these plants' secondary metabolites have not been studied for their toxicological effects, although several are considered to be of possible human health concern. For example, glycoalkaloids are naturally occurring toxins produced from plants such as potatoes and tomatoes, and they provide insect resistance. High level of exposure to these chemicals can inhibit cholinesterase enzymes in humans and other mammals. Studies have shown that glycoalkaloid levels can increase in potatoes that are damaged or exposed to light. Celery plants have been noted for their ability to synthesize linear furanocoumarins at elevated levels under stressful conditions such as fungal attack and acidic fog. Linear furanocoumarins are known for their ability to cause contract dermatitis and are considered possible human carcinogens. Breeding programs to confer pest resistance to celery plants have resulted in 10 to 15 fold increases in linear furanocoumarins levels, which can cause photophytodermatitis in grocery-store workers [23]. Mycotoxins are another example of naturally occurring toxins that could have their levels influenced by pesticides. The development of mycotoxins in food crops could be altered through the use of fungicides as well as through the use of insecticides to prevent primary insect damaged plant tissue [23]. Aflatoxins are frequently detected in several food products including corn and peanuts and can be potent mutagens, carcinogens, and teratogens. Fumonisins have been implicated epidemiologically as mycotoxins that could cause human esophageal cancer and have been shown to cause cancer and liver damage in rats, pulmonary edema in pigs, and leukoencephalomalacia in horses. Tricothecene mycotoxins frequently contaminate grain products, and low to moderate consumption of these toxins, particularly deoxynivaleonol (DON), may cause immune-system problems and gastrointestinal toxicity in animals [43].

Winter [44] published a review summarizing the influence of pesticides on the levels of naturally occurring toxins in foods and concluded that very few studies conducted directly related pesticides use to the levels of naturally occurring toxins, particularly in the case of plant secondary metabolites. The review cited examples showing reductions in mycotoxins levels in foods and fungal cultures treated with fungicides. In addition, studies of insecticides and nematicides show reductions in fungal populations on tomatoes, sunflower seeds, and decayed

fruits. A few studies showed increases in naturally occurring toxins after pesticides application. Levels of the mycotoxins nivelenol (NIV) increased after the treatment of winter wheat with fungicides, although the incidence of *Fusarium* headblights was reduced, suggesting that the fungus may itself respond to stress by increasing its synthesis of mycotoxins [45]. The application of herbicides to a variety of plants increased the production of several plant defense chemicals in broad beans, pinto beans, celery, and cotton. In these cases sublethal doses of herbicides appeared to stimulate the synthesis of certain plant secondary metabolites [46]. Results indicated that plant stress was likely related to the levels of naturally occurring toxins in foods and that pesticide may lessen plant stress, thus reducing the levels of naturally occurring toxins in some cases while increasing levels in other cases where plant stress was increased, example plants receiving sublethal doses of herbicides. In fact, a number of chemical, biological, and mechanical practices are frequently used in both organic and conventional agriculture to reduce pest pressures and plant stress, so one should not automatically assume that plants grown organically are subject to greater stresses than plants grown conventionally. In cases where naturally occurring toxin levels may differ between organic and conventional foods, the toxicological significance of such differences, if any, are yet to be determined.

Microbiological Safety

The use of animal manure as fertilizer presents potential microbiological risks if the manures have not been properly composted. While both conventional and organic agriculture frequently use animal manure for fertilization, manure use is more widespread in organic production because the use of synthetic fertilizers is not allowed in organic farming. Mukherjee et al. [47] performed one of the most comprehensive study comparing microbiological safety of organic and conventional produce. In this study 476 organic product samples and 129 conventional produce samples were collected in Minnesota and analyzed for *Escherichia coli*, *Salmonella*, and *E. coli* 0157:H7. None of the samples contained *E. coli* 0157:H7 and only 2 samples (one from organic lettuce and one from organic green peppers) contained *Salmonella*. Generic *E. coli* was detected in 9.7% of the organic samples and 1.6% of the conventional samples. In certified organic produce the rate of generic *E. coli* detection was reduced too and this amount was not statistically different from conventional produce. The corresponding generic *E. coli* detection rate for non-certified organic produce (from non-certified organic farms

that report the use of organic practices) was 11.4%. Lettuce was the produce item containing the highest rates of generic *E. coli* contamination. Certified organic lettuce did not show any generic *E. coli* in the 10 samples collected while non-certified organic lettuce had 12 positive results out of 39 samples (30.8%), and 1 of 6 conventional lettuce samples (16.7%) tested positive. The results from the study clearly indicated differences in the microbiological safety in non-certified and certified organic produce but did not demonstrate that certified organic produce was at higher risk than conventional produce in terms of microbes.

A similar study compared the microbiological safety of iceberg lettuce fertilized with inorganic fertilizer, compost, firm manure, and slurry, and the study did not indicate any differences among the various fertilizers treatments [48].

Industrial Statistics and Growth

U.S. sales of organic food and beverages had grown from $1 billion in 1990 to an estimated $20 billion in 2007, and are projected to reach nearly $23 billion in 2008. Organic food sales are anticipated to increase an average of 18% each year from 2007 to 2010. Representing approximately 2.8% of overall food and beverages sales in 2006, this continues to be a fast growing sector, 20.9% in 2006 [49]. Total U.S. organic sales, including food and non-food product, were $17.7 billion in 2006, up 21% from 2005. They were estimated to have reached $21.2 billion in 2007 and were projected to surpass $25 billion in 2008 [49].

The $13.8 billion in consumer sales of organic foods in 2005 represented 2.5% of total U.S. food sales, a 'penetration rate' that has grown from 0.8% in 1997. Organic foods have shown fairly consistent annual growth rate of 15% to 21% since 1997, when fairly comprehensive data were first available. Anecdotal data based on historical surveys and interviews with long-time participants in the organic foods business place growth estimates in a similar range of nearly 20% annually since 1990. With annual growth of total U.S. food sales in the 2%-4% range since 1997 and organic foods growth in the 15%-21% range, it is clear that

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organic foods are making continuous progress into the American mainstream, adding more than $10 billion in annual sales since 1997 [50].

Nearly 30.4 million hectares were managed organically by more than 700,000 farms in 138 countries in 2006 according to the World Organic Agriculture: Statistics and Emerging Trends 2008 report released by the international Federation of Organic Agriculture Movements (IFOAM), The Foundation Ecology and Agricultural (SOL), and the Research Institute of Organic Agriculture [51].

<div align="center">Sources and Health Effects of Heavy Metals</div>

Heavy metals are defined as a group of elements having density greater that 5.0 g/cm³. The contamination of vegetables with heavy metals poses a critical threat to society and environment as regards to the increasing concern of food safety issues, potential health risks, and detrimental effects upon soil ecosystems [52].

Activities such as controlled and uncontrolled disposal of wastes, accidental and process spillages, use of agricultural fertilizers, herbicides, insecticides and pesticide, and migration of contaminants into a non-contaminated land as vapors and leacheate through soil, or as dust, or spreading of sewage sludge all contribute to contamination of the ecosystem. A wide range of materials whose contamination raise concern includes heavy metals, inorganic and organic compounds, oils and tars, toxic and explosive gases, combustible and putrescible substances, hazardous wastes, and explosives [53].

In general, vegetables are contaminated with heavy metals derived from factors such as application of fertilizer, sewage sludge, or irrigation with waste water [54-56]. Also, the uptake and bioaccumulation of heavy metals in vegetables are influenced by factors such as climate, atmospheric depositions, concentrations of heavy metals in soils, nature of the soil on which vegetables are grown, and the degree of maturity of the plants at the time of harvest [57-59].

Ingestion of vegetables grown in soils containing elevated metal concentration has been suggested as possible risk to the health of humans and wildlife [60, 61]. The health risks depend on the chemical composition of the waste material, its physical characteristics, the vegetables cultivated, and the consumption rate. Vegetables are highly recommended foods for humans. A recommended consumption of vegetables is three to five servings per day [62]. According to the USDA, the per capita consumption of total vegetables in 1972 was 338 Ibs, and in 1992 it was 387 Ibs [63]. For 1987 to 1988, total fresh vegetable consumption per person in a household was 72.5 Ibs, whereas tomato consumption was 9.72 Ibs [64].

The amount of the metal actually absorbed from the digestive tract can vary widely depending on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it distributes in tissues and organs. Excretion occurs primarily through kidney and the digestive tract. But metals tend to persist in some storage sites such as the liver, bones, and kidney for years [65]. The toxicity of the metals commonly involves the brain and the kidney, but other manifestations occur. Some metals such as arsenic are clearly capable of causing cancer. An individual with metal toxicity, even if high dose or acute, typically has very general symptoms such as weakness or headache. Chronic exposure to metals at high enough level cause chronic toxicity effects (such as hypertension in individuals exposed to lead and renal toxicity in individuals exposed to cadmium) and can also occur in individuals who have no symptoms [65].

Copper

Copper is light reddish-brown metal with face-centered cubic crystalline structure with the symbol "Cu" and atomic number 29 on the periodic table of elements. It is malleable, ductile, and an extremely good conductor of heat and electricity. It is softer than iron but harder than zinc and can be polished to a bright finish. Copper has low chemical reactivity. In moist air, it slowly forms greenish surface film called patina. This coating protects the metal from further attack [66].

Copper is released into the environment by mining, farming, and manufacturing operation and through waste water releases into rivers and lakes. Copper is also released from

natural sources like volcanoes, windblown dusts, decaying vegetations, and forest fires. Copper released into the environment usually attaches to particles of organic matter, clay, soil, or sand. Copper does not break down in the environment, buts its compounds easily breakdown to released free copper ions in air, water, and foods [67].

Humans have been using copper for nearly 10 thousand years. Since ancient times, copper has been used by itself and in combination with other metals to make weapons, tools, household items, and artwork. Copper's high conductivity made it a metal of choice in the development of electrical engineering in the 18th and 19th centuries. Copper is the third most widely consumed metal globally, after iron and aluminum. A significant amount of copper used in the United States comes from recycled scrap and scrap left over from copper production. Also, the United States pennies were made of pure copper from 1793 to 1837. In subsequent years, they were made of various copper alloys including bronze and brass. Copper sulfate, a naturally occurring and manufactured copper salt, is used as a fungicide on crops, as a pesticide to kill snails and slugs, and as water treatment to kill aquatic vegetation. Copper compounds are also used to preserve wood and as leather tanning chemicals and mordant (fixative) in the textile dyeing [68].

The routes of exposure to copper involve breathing air, drinking water, eating food, or having skin contact with copper or particulates copper or copper containing compounds attached to. Levels of copper in household drinking water would increase if the pipes are made of copper. Lakes and rivers that have been treated with copper compounds to control algae or cooling water from power plants can have high levels of copper. Soils can also have high levels of copper if they are situated near copper smelting plants. Further routes of exposure include ingesting copper containing fungicides or if one resides close to copper mine or areas where copper is processed into bronze or brass [67].

Long-term exposure to copper can cause irritation of the nose, mouth, and eyes. It can cause headaches, stomachaches, dizziness, vomiting, and diarrhoea. Scientific studies also link a decline in intelligence in young adolescence to high exposure to copper. Industrial exposure to copper fumes, dusts, or mist may result in metal fume fever with atrophic changes in nasal

mucous membranes. Chronic copper poisoning results in Wilson's disease, characterized by a hepatic cirrhosis, brain damage, demyelination, renal disease, and copper deposition in the cornea [66].

When copper ends up in the soil, it strongly attaches to organic matter and minerals. It hardly ever enters groundwater because it does not travel very far after its release in this case. In surface water copper travels great distances, either suspended on sludge particles or as free ions. Also, in soils it can accumulate in plants and animals because it does not break down easily in the environment. On copper rich soils, only a limited number of plants have the chance of survival. The activities of micro-organisms in the soil can also be influenced negatively, resulting in slow decomposition [66].

Cadmium

Cadmium is a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). Pure cadmium is a soft, silver-white, malleable metal with bluish hue found naturally in small quantities in air, water, and soil. Cadmium is not mined but it is a byproduct of the smelting of other metals such as zinc, lead, and copper. It is odorless and tasteless, and chemical analysis is required to detect it presence.

All soils and rocks including coal and mineral fertilizers contain some cadmium. Most cadmium used in the United States is extracted during the production of other metals like zinc, lead, and copper [69]. Cadmium can be released into the air when household or industrial waste, coal, or oil is burned. It is also released from car exhaust, metal processing industries, battery and paint manufacturing, and waste hauling and disposal activities. Once cadmium is in the air, it spreads with the wind and settles onto the soil or surface water as dust. Though surface water can contain some dissolved cadmium, the concentrations tend to be low because the metal is readily absorbed by marine life, especially shellfish. Because of this absorption of cadmium by aquatic life, the U.S. Environmental Protection Agency (EPA) has issued fishing advisories related to cadmium for coastal waters around New York, Connecticut, and New Jersey [70].

About three quarters of cadmium is used in Ni-Cd batteries, the remaining one-fourth is used mainly for pigment, coatings, and plating and as stabilizers for plastics. Because it is a disposable consumer product, Ni-Cd batteries also account for over half of the cadmium waste produced. This is due to the fact that their use in vented Ni-Cd batteries that are employed in aircrafts, buses, and diesel locomotives emit a significant amount of cadmium to the environment. A number of alloys of cadmium are used in soldering and bracing electrical contacts and other purposes. Cadmium is also used to make phosphor compounds that glow when bombarded with electrons, the technology that produces the image in a television tube [70].

U.S Department of Health and Human Services (DHHS) [71] estimated in 1984 that approximately 1,500,000 workers may be potentially exposed to cadmium, of which approximately 100,000 were identified with exposure to specific cadmium compounds or with industries that use cadmium (based on data from the National Occupational Hazard Survey). Cadmium exposure is encountered in industries dealing with pigment, metal plating, some plastics, and batteries. Cadmium pollution (the emissions of cadmium smelter or industry and the introduction of cadmium into sewage sludge, fertilizers, and groundwater) can result in significant human exposure to cadmium through ingestion of contaminated foodstuffs especially grains, cereals, and leafy vegetables. Airborne cadmium exposure is also a risk posed by the incineration of municipal waste containing plastics and nickel-cadmium batteries. Cigarette smoking constitutes an additional major source of cadmium exposure [65].

Breathing high levels of cadmium can severely damage the lungs. Eating food or drinking water with very high levels of cadmium severely irritates the stomach leading to vomiting and diarrhea. Furthermore, long-term exposure to lower levels of cadmium in air, food, or water leads to build up of cadmium in the kidneys and possible kidney disease. Other long-term effects are lung damage and fragile bones [69]. Cadmium damages a specific structure of the functional unit of the kidney (the proximal tubules of each nephron) in a way that is first manifested by leakage of low molecular weight proteins and essential ions such as calcium into urine, with progression over time to frank kidney failure [72]. This effect tends to be irreversible [73], and recent research suggests that the risk exists at lower levels of exposure than previously thought [74, 75]. Even without causing kidney failure, however, cadmium's effect on the kidney

can have metabolic effects with pathologic consequences. In particular, the loss of calcium caused by cadmium's effect on the kidney can be severe enough to lead to weakening of the bones. "Itai-itai" disease, an epidemic of bone fractures in Japan from gross cadmium contamination of rice stocks, has recently been shown to happen in more subtle fashion among a general community living in an area of relatively modest cadmium contamination [76]. Increased cadmium burden in this population was found to be predictive of an increased risk of bones fractures in women as well as decreased bone density and height loss (presumably from the demineralization and compression of vertebrae) in both sexes. Cadmium may also cause anemia, teeth discoloration (Cd forms CdS), and loss of smell (anosmia).

Iron

Iron is a silvery-white or grayish metal. It is ductile and malleable. Iron 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 found in considerable quantity in the sun, asteroids, and many types of star. Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport [77]. The earth's core is believed to consist largely of a metallic iron-nickel alloy.

Iron is important for the regulation of cell growth and differentiation [78, 79]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity [80]. Iron is also necessary for photosynthesis and enzyme production of plants. However, excess concentration of iron in both surface and groundwater threaten human and the environment.

Excess iron is introduced into the natural ecosystem in the liquid waste streams from many industries including: spent pickle and etch baths from plating shops and steel manufacturing [81-84], acid mine drainage from metal mines and coal mines [85], and leachates from municipal solid waste landfills. Moreover, a study on the metal pollution around landfill sites shows that iron is a redox sensitive element and hence generates hydroxides after oxygenation of leachates. In this way, the leachates becomes an important trace metal carrier

including iron that enters the plant bodies through their root and finally reaches the human body through the food chain [86].

Iron can be found in meat, whole meal products, potatoes, and vegetables. The human body absorbs iron in animal products faster than iron in plant products. Iron occurs in the hemoglobin, a molecule that carries oxygen in the lungs, and carries it to the cells. In the cells oxygen used to produce energy that body needs to survive, grow, and stay healthy.
The U.S Recommended Daily Allowance (USRDA) for iron is 18 mg. The USRDA is the amount of an element that a person needs to stay healthy. Iron may cause conjunctivitis, choroiditis, and retinitis if it contacts and remains in the tissues.

Lead

Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust. It is very soft, highly malleable, ductile, and relatively poor conductor of electricity. 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. About 10% of lead is found in out bodies but an average concentration of 17 mg/kg is also found in uncontaminated soils [87].

Lead can be found naturally in parts of the environment, but much of it comes from human activities including burning fossil fuel, mining, and manufacturing. Currently, lead is found in ore with zinc, silver, and copper and it is extracted together with these metals. The main lead mineral is galena (PbS) and there are also deposits of cerrussite and anglesite which are mined. Galena is mined in Australia, which produces 19% of the world's new lead, followed by the USA, China, Peru, and Canada. World's production of new lead is 6 million tons a year, and workable reserves total are estimated at 85 million tons, which is less than 15 years' supply [88].

The application of lead in gasoline has resulted in an unnatural lead cycle. In car engines, lead is burned, so that lead salts (chlorine, bromines, oxides) originate. These lead salts enter the

environment through the exhausts of cars. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this lead will fall back on earth when it is raining. The lead cycle caused by human production is much more extensive than the natural lead cycle [88].

Lead is used in the production of batteries, ammunition, metal products (solder and pipes), and devices to shield X-rays. Because of the health concerns, lead from paints and ceramic products, caulking, and pipes solder has been dramatically reduced in recent years. Also its use as an additive to gasoline was banned in the United States [89]. Lead is also used as a coloring element in ceramic glazes, as projectiles, and in some candles to treat the wick. It is the traditional base metal for organ pipes, and it is used as electrodes in the process of electrolysis. One of the major uses of lead is in the glass of computers and television screens where it shields the viewer from radiation.

Exposure to lead is normally through food or drinking water that contains lead. Water pipes in some older homes may contain lead solder. Lead can leach into water. In areas where lead-based paints have been used, its deterioration can result in human exposure. Moreover, deteriorating paint can also contribute to lead dust. Exposure is also possible when working in a job where lead is used or the use of health products or folks remedies that contain lead [89].

Lead can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, lead accumulation can exceed several hundreds times the threshold of maximum level permissible in human [90]. The introduction of lead into the food chain may affect human health, and thus studies concerning lead accumulation in vegetables have become important [91]. Although a maximum lead limit for human health has been establish for edible parts of crops (0.2 mg/kg) [92], soil lead thresholds for producing safe vegetables are not available.

Lead has been on intense focus of environmental health research for many decades. Studies in humans were greatly assisted by the development of methods (such as graphite

furnace atomic absorption spectroscopy) for the accurate and reliable measurement of lead in blood (measured in units of micrograms per deciliter (μg/dL), a technique that is now widely available and used for surveillance and monitoring as well as research. The general body of literature on lead toxicity indicates that depending on the dose lead exposure in children and adults can cause a wide spectrum of health problems ranging from convulsions, coma, renal failure, and death at the high end to subtle effects on metabolism and intelligence at the low end of exposures [93]. Children (and developing fetus) appear to be particularly vulnerable to the neurotoxic effects of lead. A plethora of well-designed epidemiologic studies has convincingly demonstrated that low-level lead exposure in children less than 5 years of age (with blood lead levels in the 5-25 μg/dL range) results in deficits in intellectual development as manifested by lost intelligence quotient points [94]. As a result, in the U.S. the Center for Disease Control (CDC) lowered the allowable amount of lead in a child's blood from 25 to 10 μg/dL and recommended universal blood lead screening of all children between the ages of 6 months and 5 years [95]. However, a number of issues still remain unresolved with respect to the lead toxicity in children. Among the most important is the risk posed to the fetus by mobilization of long-lived skeletal stores of lead in pregnant women [96]. Recent research has clearly demonstrated that maternal bone lead stores mobilized at an accelerated rate during pregnancy and lactation [97] and are associated with decrements in birth weight, growth rate, and mental development [98]. Because bone lead stores persist for decades, it is possible that lead can remain a threat to fetal health many years after environmental exposure had actually been curtailed [99].

In contrast to children, adults are generally allowed by regulations to be exposed to higher amounts of lead. In the U.S, for example, the Occupational Safety and Health Administration (OSHA) requires that the blood lead levels of exposed workers be maintained below 40 μg/dL as a way of preventing toxic effects to nerves, the brain, kidney, reproductive organs, and heart. This standard is probably outdated. Firstly, the standard does not protect the fetus of women who become pregnant while on the job (or even if they leave the job for several years because of issue of bone lead mobilization as discussed above). Secondly, recent epidemiology studies had linked blood lead level in the range of 7- 40 μg/dL with evidence of toxicity in adults such as neurobehavioral decrements [100] and renal impairments [101]. Thirdly, recent research studies using the newly developed technique, K-X-ray fluorescence, to

directly measure bone lead levels (as opposed to the blood lead levels) had provided evidence demonstrating that cumulative lead exposure in individuals with blood lead levels well below 40 μg/dL was a major risk factor for the development of hypertension [102 - 104], cardiac conduction delays [105], and cognitive impairments [106, 107].

Zinc

Zinc is a lustrous bluish-white metal. It is brittle and crystalline at ordinary temperature, but it becomes ductile and malleable when heated between 110 and 150 °C. It is one of the most common elements in the earth's crust. It is found in air, soil, and water and is present in all foods. Zinc combines with other elements to form zinc compounds. Common zinc compounds found at hazardous waste sites include zinc chloride, zinc oxide, zinc sulphate, and zinc sulphide [108].

Zinc is released into the environment by natural processes, but most comes from human activities like mining, steel production, and coal and waste burning. It attaches to soil, sediments, and dust particles in the air. Rain and snow remove zinc dust particles from the air. Depending on the type of soil, some zinc compounds can move into the groundwater and into lakes, streams, and rivers. Most of the zinc in soil stays bound to soil particles and does not dissolve in water. It builds up in fish and other organisms, but it does not build in plants [108].

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2 g). Skeletal muscle account for approximately 60% of the total body content, and bone mass with a zinc concentration of 1.5-3 μmol/g (100-200 μg/g), approximately 30%. The concentration of zinc in lean body mass is approximately 0.46 μmol/g (30 μg/g). Plasma zinc has a rapid turnover rate and it represents only about 0.1% of total body zinc content. The level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye (4.2 μmol/g or 274 μg/g) and in prostatic fluids (4.6 -7.7 or 300-500 mg/L) [109].

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the

metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components membranes and in this way contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the fact that zinc is essential for all life forms. Zinc plays a central role in the immune system, affecting a number of cellular and humoral immunity [110].

The dominant ore is zinc blende, also known as sphalerite. Other important zinc ores are wurtzite, smithsonite, and hemimorphite. The main zinc mining areas are Canada, Russia, Australia, USA, and Peru. World production of zinc exceeds 7 million tons a year and commercially exploitable reserves exceed 100 million tons. More that 30% of the world's need for the zinc is met by recycling [111].

Many foods contain certain concentration of zinc. Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc: concentrations in such foods are generally in the range of 25-50 mg/kg (380-760 μmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and chicken, pork, or meat with high fat content have moderate zinc content, typically between 10 and 25 mg/kg (150-380 μmol/kg). Fish, roots and tubers, green leafy vegetable and fruits are only modest sources with zinc having concentrations < 10 mg/kg (<150 μmol/kg) [112]. Saturated fats and oils, sugars, and alcohol have very low zinc contents. The use of zinc depends on the overall composition of the diet. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption [113]. Soluble organic substances of low relative molecular mass such as amino and hydroxyl acids facilitate zinc absorption. In contrast, organic compounds forming stable and poorly soluble complexes with zinc can impair absorption. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect the uptake and intestinal absorption of zinc. The risk of competitive interactions with zinc seems to be mainly related to the consumption of high doses of these ions in the form of supplements or in aqueous solutions. However, at levels present in food and at realistic fortification levels, zinc absorption appears not to be affected, for example, by iron or copper [113].

Exposure to zinc may result from drinking contaminated water or beverage that has been stored in metal containers or flows through pipes that have been coated with zinc. Other exposure sources include eating too many dietary supplements that contain zinc and working on jobs such as construction, painting, mining, smelting, wielding, and manufacture of machine parts and galvanize metals [108]. The most important information of zinc reported is its interference with copper metabolism [114, 115]. The symptoms that an acute zinc dose may provoke include tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatitis, and damage of hepatic parenchyma [116]. Although maximum zinc tolerance for humans has been established for edible parts of crops (20 mg/kg) [117], soil zinc threshold for producing safe vegetables is not available. Inhaling large amounts of zinc (as dust or fumes) can cause a specific short-term disease called metal fume fever. A long-term effect of breathing high levels of zinc is still unknown. Putting zinc acetate and zinc chloride on the skin of rabbits, guinea pigs, and mice caused skin irritation. Skin irritation will probably also occur in people [108].

The central role of zinc in cell division, protein synthesis, and growth is especially important for infants, children, adolescents, and pregnant women. These groups suffer most from an inadequate zinc intake. Zinc-responsive stunting has been identified in several studies, for example, a rapid body weight gain in malnourished children from Bangladesh was reported [118]. However, other studies have failed to show a growth promoting effect of zinc supplementation. A recent meta-analysis of 25 intervention trials comprising 1834 children less than 13 years of age, with a mean duration of approximately 7 months and mean dose of zinc of 14 mg/day (214 μmol/day), showed a small but significant positive effect of zinc supplementation on height and weight increases [119]. Zinc supplementation had a positive effect when stunting was initially present; a more pronounced effect on weight gain was associated with initial low plasma zinc concentrations. Results from zinc supplementation studies suggest that a low zinc status in children not only affects growth but is also associated with an increased risk of severe infectious diseases [120]. Episodes of acute diarrhoea were characterized by shorter duration and less severity in zinc-supplemented groups; reductions in incidence of diarrhoea were also reported. Other studies indicate that the incidence of acute lower respiratory tract infections and malaria may also be reduced by zinc supplementation. Prevention of suboptimal zinc status and zinc deficiency in children by an increased intake and

availability of zinc would consequently have a significant effect on child health in developing countries. The role of maternal zinc status on pregnancy outcome is still unclear. Positive as well as negative associations between plasma zinc concentration and fetal growth or labor and delivery complications have been reported [121]. Results of zinc supplementation studies on pregnancy also remain inconclusive [121]. Interpretation of plasma zinc concentrations in pregnancy is complicated by the effect of haemodilution and the fact that low plasma zinc levels may reflect other metabolic disturbances [122]. Zinc supplementation studies of pregnant women have been performed mainly in relatively well-nourished populations, which may be one of the reasons for the mixed results [121]. A recent study among low-income American women with plasma zinc concentration below the mean at enrolment in prenatal care showed that a zinc intake of 25 mg/day resulted in greater infant birth weights and head circumferences as well as a reduced frequency of very low-birth-weight infants among non-obese women compared with the placebo group [123].

Water is polluted with zinc due to presence of large quantities of zinc in wastewaters of industrial plants. Some fish can accumulate zinc in their bodies when they live in zinc contaminated waterways. When zinc enters the bodies of these fish it is able to bio magnify up the food chain. Only a limited number of plants have the chance of survival on zinc-rich soils. That is why there is not much plant diversity near zinc-deposited factories. Due to the effects on plants, zinc is a serious threat to the production of farmlands. Furthermore, zinc can interrupt negatively the activity of micro-organisms and earthworms. The breakdown of organic matter may seriously slow because of this [111].

Nickel

Pure nickel is a silvery-white metal. It is hard, malleable, and ductile metal. It is a fairly good conductor of heat and electricity. Nickel can combine with other metals such as iron, copper, chromium, and zinc to form alloys [124].

Nickel is found in soil, meteorites, and on the floor of ocean and also is emitted from volcanoes. Nickel and its compounds have no characteristics odor or taste. It is released into the atmosphere by oil-burning power plants, coal-burning power plants, and trash incinerators. In

the air it attaches to small particles of dust that settle to the ground or are taken out of the air in rain or snow; this usually takes many days. Nickel released in industrial waste water ends up in soil or sediment where it strongly attaches to particles containing iron or manganese. Nickel, however, does not appear to accumulate in fish or in other animals used as foods [125].

The major use of nickel is in the preparation of alloys. Nickel alloys are characterized by strength, ductility, and resistance to corrosion and heat. About 65% of nickel consumed in the Western World is used to make stainless steel, whose composition can vary but is typically iron with approximately 18% chromium and 8% nickel. About 12% of all nickel consumed goes into super alloys. The remaining 23% of consumption is divided between alloy steels, rechargeable batteries, catalysts and other chemicals, coinage, foundry products, and plating.

Exposure to nickel includes eating food containing nickel, which is the major source of exposure for most people. Other ways nickel gets into humans are drinking of nickel contaminated water, breathing nickel contaminated air, or smoking tobacco that contain small amounts of nickel. Higher exposure may occur if one works in industries that process or use nickel. An allergic reaction is the most common harmful health effect of nickel in humans. Approximately 10%-20% of the population is sensitive to nickel. People can become sensitive to nickel when jewelry or other things containing it are in direct contact with the skin for a long time. The result of direct contact is mostly skin rash at the site of contact. Less frequently, some people who are sensitive to nickel have asthma attacks following exposure to nickel [125].

Nickel fumes are respiratory irritants and may caused pneumonitis. Exposure to nickel and its compounds may result in the development of a dermatitis known as "nickel itch" in sensitized individuals. The first symptom is usually itching, which occurs up to 7 days before skin eruption occurs. The primary skin eruption is erythematous, or follicular, which may be followed by skin ulceration. Nickel sensivity, once acquired, appears to persist indefinitely [124].

CHAPTER 2

CONTAMINANTS LEVELS IN VEGETABLES AND SAMPLE PREPARATION
TECHNIQUES

Heavy Metals and Other Contaminants in Organic and Conventional Vegetables

Environmental contaminants encompass heavy metals and persistent organic pollutants (POPs). Heavy metals such as cadmium, lead, zinc, and others contaminate crops in industrialized areas as a result of air and soil pollution. POPs are more of a problem for animal products because of their bioaccumulation properties and concentration in fat. The Global Environmental Monitoring System /Food Contamination Monitoring Assessment Program of the World Health Organization (GEMS/Food) have classified heavy metals as priority food contaminants [126].

Organic farming is an alternative farming practice to reduce pesticide residues in the food chain. Baker et al. [25] have observed a better residue level in organic farming. The number of detected residues and multi-contaminated samples were lower. Still, residues of non-synthetic pesticides (sulphur, pyrethrum, bromides) could be found in organic commodities [127]. It was also highlighted that organic products were likely to be slightly contaminated throughout the environment.

Although it is clear that the emphasis of organic production is the avoidance of the use of agrochemicals and antibiotics, numerous consumers consider implicitly that their content in environmental pollutants (like metals) is lower compared to that of the conventionally produced foodstuffs [16, 17]. Such an assumption may be unfounded. For instance, in Greece, where a number of organically produced foodstuffs are not certified either because they do not fulfill the necessary conditions or because they are produced in small farms that have no interest in applying for certification, recent studies showed that additional provisions were needed.

Karavoltsos et al. [128] studied cadmium and lead content in a variety of certified and non-certified organic foodstuffs produced in Greece for which no data were available. The highest cadmium concentration were observed in cereals (21.7 ng/g) followed by leafy vegetables (15.4 ng/g). Whereas for lead the highest concentration was observed in leafy vegetables (33.4 ng/g), followed by pulses (21.4 ng/g), and alcoholic beverages (20.0 ng/g). Even though they found percentages of 64% and 61%, for cadmium and lead, respectively, for conventionally grown foodstuffs compared to the certified organically grown ones, the "uncertified" organic products contained far larger concentrations of cadmium and lead than either the certified organic or conventional foodstuffs. They contend that properly observed practices for organic agriculture could eventually lead to production of foodstuffs with lower metal content although organic agriculture as such is not able to secure low metal content in its products unless additional effort was ensured.

To assess the safety of cereals and cereal products consumed by the Belgian population, Harcz et al. [129] studied the following contaminants: mycotoxins-deoxynivanol (DON) and zearalenone (ZEA), heavy metals (Cd, Pb, and Hg), and three most frequently used post-harvest insecticides applied in cereals (chlorpyifos-methyl, dichlorvos, and pirimifos-methyl) in organic and conventionally produced cereals crops. Assuming no further change in contaminants levels during cereals processing and during the preparation of foodstuffs, conservative intakes were estimated for the consumers of cereal-based products such as flour, bread, breakfast cereals, dough, and pastry. Their results showed that for the consumers of organic foodstuffs estimated daily intakes were 0.56 μg DON, 0.03 μg ZEA, 0.19 μg Cd, 0.28 μg Pb, and 0.0006 μg Hg per kg body weight taking into account the average contaminant levels in unprocessed grains and the average cereal products consumption in Belgium.

For the consumers of conventional foodstuffs the corresponding estimated daily intakes were 0.99 μg DON, 0.06 μg ZEA, 0.17 μg Cd, 0.12 μg Pb, and 0.0007 μg per Hg kg body weight. Intakes of some post-harvest insecticides for consumers of conventional foodstuffs were taking into accounts (0.11 μg chlorpyriphos-methyl, 0.2 μg dichlorvos, and 0.24 μg piriphos-methyl kg^{-1} bw). When their results were expressed in percentages of tolerable/acceptable daily intakes (TDI/ADI), it showed intakes were high for DON, ZEA, and Cd. While the percentages

of organic cereal products showing the presence of DON was 56% and that of conventional cereal products was 99%. Similarly, the percentage of organic cereal products showing the presence of ZEA was 16% and that for conventional cereal products was 32%. Cadmium, however, was found to be present in higher percentage of organic cereal products (19%) than in conventional produced ones (17% conventional). The above studies showed that the most common pesticides (post-harvest insecticides) were regularly detected in conventional unprocessed cereals while they were not allowed in organic farming products. However, the mycotoxins such as DON and ZEA as well as the heavy metals like Cd and Pd were present in both farming systems.

Studies conducted earlier showed that, mycotoxins and particularly some *Fusarium* toxins such as trichothecenes and zearalenone (ZEA) are important contaminants of the cereal crops [130]. Trichothecene contamination starts in the field as a consequence of *Fusarium graminearum* and *F. culmorum* attacks during flowering of the cereals. Wet weather years were known to increase the contamination by DON, one of the most important trichothecenes [131]. In 2001 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established for the DON a tolerable daily intake (TDI) of 1μg/kg body weight (bw) based on a chronic toxicity study in mice [132]. The proposed limit in processed cereals is 750 μg/kg. For ZEA, the scientific committee for foods (SCF) of the European Commission established a temporary tolerable daily intake of 0.2 μg ZEA per Kg body weight on the basis of short-term study in pigs [133].

Malmauret et al. [134] also compared the level of contamination of organic and conventional raw materials in France. Fifteen kinds of products, which included meat, milk, eggs, vegetables, and cereals, were tested in their organic and conventional forms, and 192 samples in all were analyzed (98 conventional products, 94 organic ones). The samples were tested for the following contaminants: heavy metal (lead, cadmium, arsenic, and mercury), nitrates and nitrites in vegetables, mycotoxins such as DON, 3-acetyl DON, 15-acetyl DON, nivalenol (NIV), T-2 toxin, HT-2 toxin, diascetoxyscipenol, patulin, fumonizin B_1 ochratoxin A, and ZEA. In all, analysis was carried out on 3924 samples. Even though they contend that the results did not show any conclusive evidence as to whether organic products were more or less

safe than conventional products, because of their experimental design interesting observations were made based on the results obtained. The studies showed that maximum levels were exceeded for lead in organic carrots and buckwheat and in conventional wheat; for cadmium in both organic and conventional buckwheat; for nitrates in organic spinach, and for patulin in organic apples. DON was also observed in organic and conventional wheat but the level was higher in organic than in conventional wheat.

A similar study was carried out by Hoogenboom et al. [135] in the Netherlands to assess differences between organic and conventional products (plants and animals) with regards to contaminants, microorganisms, and antibiotics resistance. Most of the organic products came from the farm, while some of the conventional ones came from the stores. The categories of food sampled were organic vegetables products such as lettuce, carrots, wheat, and potatoes. Animal products such as eggs, broilers, pigs, and cows also were studied. The factors investigated included mycotoxins (DON and ZEA), nitrates, heavy metals, pesticides, and *E. coli* 0157 in vegetables and *Salmonella*, *E. coli* 0157 antibiotics-resistant bacteria, heavy metals, and veterinary drugs or their metabolic products. The results showed no differences in *Fusarium* toxins DON and ZEA in organic and conventional wheat during both dry and wet period which promoted the production of these toxins. Also, the organic products contained no elevated levels of heavy metals. However, nitrates levels in head lettuce produced organically in the open field were much lower than those in conventional products. However, Iceberg head lettuce from the greenhouse showed no detectable differences. Both the organic and conventional products contained no residues of non-polar pesticides above the legal limits, although some residues were detected in conventional lettuce. The studies concluded that in general the organic products investigated scored equally well as conventional products with reference to food safety.

Levels of Heavy Metals in Vegetables and other Foodstuffs

To determine the concentration of heavy metals in selected green vegetables grown along the banks of the Sinza and Msimbazi rivers and to estimate their contribution to the dietary daily intake of the metals, Bahemuka and Mubofu [136] analyzed four heavy metals (cadmium,

copper, lead, and zinc) in African spinach (*Amaranth sp.*), Chinese cabbage (*Brassica chinensis*), cowpea leaves (*Vigna unguiculata*), leafy cabbage (*Brassica rapa*), lettuce (*Lactuca sativa*), and pumpkin leaves (*Moschata cucurbita*). The samples were collected over a period of 6 months during the dry season (July and December) of 1994. Atomic absorption spectroscopy was used to estimate and evaluate the levels of these metals in the vegetables. The results showed the following ranges: 0.01- 0.06, 0.25-1.60, 0.19-0.66, and 1.48-4.93 in mg per100 g sample for cadmium, copper, lead, and zinc respectively. The study showed some vegetables contained higher than permissible levels given by Food and Agriculture Organization (FAO) and World Health Organization (WHO) for human consumption. When the mean levels of cadmium, copper, lead, and zinc (0.20, 7.95, 3.95, and 33.75 mg per kg sample respectively) were taken into account, the daily intake contribution of these metals were found to be 21.60 μg, 858.60 μg, 426.60 μg, and 3.65 mg for cadmium, copper, lead, and zinc respectively. The authors therefore recommended that the inhabitants of Dar es Salaam should not eat large quantities of these vegetables so as to avoid large accumulation of the heavy metals in their bodies.

Mohamed et al. [137] studied elements such as Ca, Fe, K, Mg, and Na as well as trace elements Cd, Co, Cu, Mn, Ni, Pb, and Zn in 12 different kinds of vegetables from Saudi Arabia (the Al-Taif district) using atomic absorption spectrophotometry. The vegetables studied were cucumber, vegetable marrow, tomato, potato, green pepper, eggplant, carrots, parsley, lettuce, spinach, salq, onion, leek, watercress, and cabbages. They found that the different elemental concentrations in each vegetable depended upon the selective uptake of the elements by the plant. In addition, salq and watercress were found to have higher elemental concentrations than other vegetables. All the concentrations determined were within the safety baseline levels for human consumption.

The uptake and bioaccumulation of heavy metals in vegetables are influenced by a number of factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils, the nature of soils on which the vegetables are grown, and the degree of maturity of the plants at the time of harvest [138]. Also, during transport of post-harvest vegetables, air pollution may increase the heavy metal levels in these vegetables [139]. Other anthropogenic sources of heavy metals include addition of manures, sewage sludge, fertilizers, and pesticides.

They may affect the uptake of heavy metals by modifying the physico-chemical properties of the soil such as pH, organic matter, and bioavailability of heavy metals in the soil. Whatmuff [140] and McBride [141] reported that increased concentration of heavy metals in the soil increased their uptake by crops. Larsen et al. [142] and Sanchez-Camazano et al. [143] also found that there was a positive relationship between atmospheric metal deposition and elevated concentrations of heavy metals in plants and top soils.

Sharma et al. [144] also examined the contribution of heavy metals in selected vegetables, *Brassica oleracea*, *Abelmoschus esculentus*, and *Beta vulgaris* through atmospheric deposition in Varanasi (urban India). The heavy metals studied were Cu, Pb, Zn, and Cd. They found that the concentration of Zn and Cu were highest in *B. oleracea,* that of Cd in *A. esculentus,* while Pb was highest in *B. vulgaris.* Also, heavy metal pollution index showed that *B. oleracea* was most contaminated with heavy metals, followed by *A. esculentus,* and then *B. vulgaris*. The study concluded that atmospheric deposition contributed to the increased levels of heavy metals in vegetables and therefore could elevate heavy metal levels in vegetables during marketing and posed potential health hazards to consumers.

Eslami et al. [145] investigated the levels of five different heavy metals (Cd, Pb, Zn, Cr, and As) in various vegetables including roots and leaves of radish (*Raphanus sativus* L.), leek (*Allium ampeloprasum* L.), sweet basil (*Ocimum basilicum* L.), and parsely (*Petroselium crispum*) cultivated along the bank of river passing through the city of Zanjan. The analysis was carried out using the atomic absorption spectrometry. The results of the survey in mg/kg were as follows: 3.89-32.94, 3.15-27.68, 43.61-223.10, for Pb, Cd, and Zn respectively. Chromium and arsenic were not detected. Taking the mean levels of lead and cadmium (10.65 and 9.22 mg/kg) into consideration, they calculated daily intake contribution of lead to be 2.32 mg and that of cadmium to be 2.0 mg. The values were beyond the limit given by FAO and WHO for human consumption.

In a related study, Aiwonege and Ikhuoria [146] also studied their heavy metal content of 12 vegetables samples (divided into groups on the basis of part consumed; namely root, stem, leaf, fruit, and seed) procured from local markets in Benin and Lagos using wet digestion method

and atomic absorption spectrophotometry. The metals of interest were Fe, As, Pb, Hg, Cd, Cr, Zn, and Ni. The study showed Fe with the highest concentration in all the vegetables samples with values ranging from 1.040 -13.97 µg/g. Overall, the levels of metals in the vegetables analyzed were below the safety limits.

Sample Preparation Techniques for Heavy Metal Analysis

Many analytical methods including atomic absorption spectrometry for the determination of trace elements in plant materials requires digestion of the sample [147]. Hence, the sample treatment procedure is of great importance for obtaining reliable results for the analytes [148]. The wet and dry ashing procedures are slow and difficult to carry out operate successfully. Microwave digestion method is an efficient and rapid method for preparation of samples before analysis. [149]. Atomic absorption spectrometry (AAS) is the most widely used and is the recommended technique for trace heavy metals determination due to its sensitivity, specificity, simplicity, and precision.

The major functions of the sample pretreatment are to dissolve the sample matrix and make available all metals for analysis, to concentrate or dilute the sample to bring them into a concentration range suitable for analysis, and to make possible for single or group of analytes of interest to be separated from the unwanted ones [150]. The atomic spectrometry technique was principally dedicated to analyze liquid samples and therefore all solid samples have to be brought into solution form.

Wet Digestion Procedures

Wet digestion method with oxidizing acids the most common method of sample (matrix) pre-treatment procedure. Chloric acid, the first published wet digestion reagent, was described in 1838 by Duflos [151] as well as by Fresinus and Babo [152] in 1844. The classical wet digestion reagent HNO_3-H_2SO_4, which is the most important and most versatile of the commonly known wet oxidation mixtures, was investigated in 1841 by Danger and Flandin [153] for mineralization of organic matter. Pure concentrated HNO_3 used in a closed system under high temperatures and pressure was known since 1860 from Carius [154].

Sample wet digestion is a method of converting the components of a matrix into simple chemical forms. Heat aids in the digestion process with chemical reagents such as acid or a combination of acids. Most of the wet digestion methods use a combination of oxidizing acids (HNO_3, hot concentrated $HClO_4$, and concentrated H_2SO_4) and non-oxidizing acids (HCl, HF, H_3PO_4, dilute H_2SO_4, dilute $HClO_4$), and peroxide [155]. For inorganic and organic samples wet digestion proves effective and advantageous because it destroys the matrix and helps lessen interference. From the above reagents HNO_3 is the only acid that can be used alone. It has the following advantages: it is available in high purity, nitrates are very soluble, and it may be employed over a range of temperatures. It is active at room temperature and disrupts organic materials, and almost complete mineralization can be achieved at high temperature and pressure [156].

Wet digestion is carried out at normal atmospheric temperature and pressure in an open system or at higher pressures in closed systems. When the boiling point for an acid or mixture would be exceeded, closed systems are used. Examples of closed systems are beakers made of polytetrafluorethylene (PTFE) which are covered with lid [157], flasks with long necks placed at an angle of about 45° or with narrowing for the opening, and more complicated constructions with water-cooled refluxes traps [158, 159]. The use of microwave is more advantageous because the heating takes place inside the mixture and also shorter time for digestion [160]. Hydrogen peroxide and hydrochloric acid can be used as a mixture with nitric acid to improve the quality of digestion. In the case of determining stable compounds, hydrochloric and sulfuric acids may cause interference. For samples principally containing inorganic matrices, mixtures of hydrochloric acid are used and for samples containing silicates that are insoluble in other acids a combination with hydrofluoric acid is used [155].

Boiling of organic material in concentrated nitric acid at atmospheric pressure (b.p 120°C) does not lead to complete destruction. For instance 2%-20% of the original carbon remains undestroyed after boiling under total reflux for 3 hours [161]. Wusfels et al. [162] had shown that almost complete mineralization was possible for samples heated in PTFE closed vessels with 69% HNO_3 at 180° for 3 hours. Carbohydrates also decompose at 140°C, proteins at 150°C, and lipids molecules at 160°C when heated in closed PTFE vessel in microwave oven

[163]. Karavoltsos et al. [128] in their investigation of cadmium and lead in organically produced foodstuffs from Greek markets collected foodstuffs of different origins. Five subsamples were collected each weighing 0.5-1.0 kg for vegetables, fruits, and potatoes, 0.25-1.0 kg for cereals, pulse, and eggs. The samples were rinsed on reaching the laboratory with water (18.2 MΩ cm, Milli-Q, Millipore), chopped, and homogenized. Approximately 1.0 g of wet sample was accurately weighed and placed in Teflon holders specially designed for airtight sealing. Depending on the sample size and nature, 8-12 mL of nitric acid (65% pure) were added and the sample digested overnight at 120°C. A final volume of 10 mL was prepared after gentle evaporation by addition of Milli-Q water.

Sharma et al. [144] prepared samples for heavy metals analysis in vegetables in urban India by chopping the samples into small pieces and then oven dried at 80°C till constant weight was achieved. The dried vegetables samples were powdered with stainless steel blender and passed through a 2 mm size sieve. Then 1.0 g of sample was taken into a 100-mL acid-washed beaker and 15 mL of 5:1:1 mixture (70% high purity HNO_3, 65% $HClO_4$, and 70% H_2SO_4) was added. The mixture was digested at 80°C until a transparent solution was formed. The digested sample was cooled and filtered using Whatman 42 filter paper and the filtrate diluted to 50 mL with deionized water.

In analyzing pollution caused by thermal plants, leaf samples from the sampled trees were dried for 24 hours at 105°C, while surface soil sampled at a depth of 0-30 cm were also dried at 105°C for 3 hours. Coal samples were crushed and dried at the same temperature. Then 0.5 g of each sample was digested in the microwave using $HClO_4$:HNO_3: HCl in the ratios of 1:3:5 for leaves, and 1:2:5 for soil samples and coal respectively at 140°C and for 1 hour. The samples were diluted to 100 mL after filtration with 0.1M HCl [164].

Eslami et al. [145] studied heavy metals in edible green vegetables grown along the sites of the Zanjarood River in Zanjan, Iran. Pre-treatment of samples for the above study was carried out by weighing and oven drying at 60°C for the attainment of constant weight. Each oven dried sample was grounded in mortar until it could pass through a 60 mesh sieve. Wet digestion with 2:1 HNO_3:$HClO_4$ in the conical flask for 2-3 hours in a sand bath was carried out. Then 10-mL

of HCl was added and the digested samples filtered with 0.45 μm pore size millipore cellulose nitrate membrane filter paper and made up to 100 mL with distilled water.

<u>Dry Ashing Procedures</u>

Dry ashing at elevated temperature and pressure using muffle furnace is the most commonly used method in this group. A supply of extra air to the sample [165] causes the sample to glow and reach higher temperatures than the air atmosphere in the furnace. The temperature of the sample depends on the extra air and thickness of the sample. An advantage of this procedure lies in the extra air supply that reduces ashing times critical to volatile elements such as Cd and Pb. In closed muffle furnace dry ashing, the samples' temperature is close to the temperature of the furnace even though substantial temperature gradient can exist. [166].

Dry ashing procedures have the advantage of being able to accommodating larger sample size than wet digestion methods. However, it can cause volatization of elements such as As, Cd, Pb at elevated temperature and also the possibility of reacting with the crucible materials [156]. Gorsuch, [167] showed that certain metals could be lost through volatilization or retention in the silica crucible walls when metallic standard solutions were ashed with certain chlorides. Losses of Cd in some specific sample tissue were also shown by Feinberg and Ducauze [168]. In this case the samples were ashed at 750°C with H_2SO_4 as ashing aid. To avert potential losses and to speed up the procedure, ashing aids and modification of sample matrix have been adopted. However, more often than not it results in contamination of the analyte and poorer detection limits [169].

Dry ashing is generally time consuming. It may take a day or more. But little attention to the process may be needed by the analyst. Contamination may be a problem because of long exposure of the sample to ambient air. The resulting ash may require small amount of diluents. This provides much better detection limits than wet digestion, especially when dry ashing is used with flames atomic absorption spectrometry [169].

The major drawbacks of drying ashing can be overcome to some extent by using procedures at reduced pressures (70 -100 Pa). The temperature of these systems can be as low as

100-200 °C [170]. The oxidant is activated in glass ashing chambers in which sample crucibles are placed. The low temperature prevents ash from reacting with walls of the container, hence oxygen can be in a pure state. The demerit of this procedure is the long reaction time because of the formation of crust in the surface of the sample that produces a shielding effect and reduces the rate of reaction [171].

In their studies of atmospheric heavy metal pollution in Aqaba City, Jordan, Al-khlaifat et al. [172] dried palm leaves samples at 80°C for 24 hours. The samples were milled in a pretreated micro hammer cutter and sieved. Then 2 g of the powdered samples were ashed in a muffle furnace at 480°C, followed by dissolution in 10% nitric acid. Finally, it was filtered into 25-mL polythene volumetric flask and diluted to the mark with 1% (v/v) nitric acid solution.

Bahemuka and Mubofu [173] used dry ashing procedure for sample preparation in their investigation of heavy metal content of edible green vegetables grown along the sites of the Sinza and Msimbazi Rivers in Dar es Salaam. After washing their samples with distilled water to eliminate airborne pollutants, the samples were sliced and dried on a sheet of paper to eliminate excess moisture. The samples were oven dried to constant weight and grounded in mortar until able to pass through 60 mesh sieve. Then 1.0 g was measured into a crucible with the content placed in a muffle furnace and ashed at 450°C for 12 hours. The ash was digested with 5 mL of 20% (v/v) AnalaR HCl solution. The residue was filtered into a 50-mL volumetric flask using Whatman filter paper 41 and solution was made up to the mark with deionised water.

In the determination of lead, cadmium, copper, iron, and zinc in foods by dry ashing the dried test portions were ashed at 450°C under gradual increase ($\leq 50°C/h$) in temperature. Then 6 M HCl was added and solution evaporated to dryness. The residue was dissolved in 0.1 M HNO_3 and the analytes determined by atomic absorption spectrometry using both the flame and graphite procedures [169].

Panigati et al. [174] studied selenium content in Italian rice by differential pulse cathodic stripping voltammetry. Sample pretreatment involved the used of dry ashing method with wet digestion during the ashing of the rice. In this process, the flour of the rice was dried at 60°C in

an oven until constant weight was obtained. To 1.0 g of the flour was added 10 mL of concentrated HNO_3 (65% w/w) and 4.0 g of $Mg(NO_3) \cdot 6H_2O$. The sample was predigested overnight at room temperature. The predigested sample was first heated to dryness using a sand bath at 373K. The temperature was then slowly raised to 473K until no fumes evolved. The beaker was heated for another 30 minutes in the muffle furnace. The residues were cooled and dissolved with 7 mL of 6 M HCl. A final heating at 373 K was conducted using sand for 10 minutes to ensure that Se (VI) reduces to Se (IV). The solution was transferred quantitatively to a 25-mL volumetric flask and then diluted to the mark.

CHAPTER 3

ANALYTICAL TECHNIQUES FOR DETERMINATION OF HEAVY METAL

Techniques for the analysis of trace elements have developed rapidly in response to the increasing need for accurate measurement of extremely low amounts in diverse matrices [175]. The motivations for developing analytical technologies capable of accurate and robust quantification of trace species are compelling. There are many areas of science and industry where the presence of elements at very low concentrations can have a significant impact on human health, the environment, or industry [176]. An example is the determination or investigation of metal levels in food products. Accurate analytical data as well as robust quality quality assurance are crucial in this area of study. This review looks at the various analytical techniques used to quantify different elements in a variety of matrices.

<u>Colorimetry</u>

Colorimetry is the measurement of amount of color of a substance. Transition metals complexes often form highly colored solutions due to electronic transitions with energy that corresponds to wavelengths in the visible region. Therefore, when white light is passed through the solution containing the metal complex, specific wavelength would be absorbed that correspond to energy of the electronic transitions [177].

In colorimetry, an incident light energy of certain wavelength and intensity is passed through a solution containing the analyte. A fraction of the incident light is absorbed and the rest transmitted. The ratio of the transmitted and incident light (better known as transmittance) can be found (the negative log of which correspond to absorbance by the analyte). A scan is made to identify the wavelength unique to the substance at which the analyte of interest absorbs maximum amount of light. The determination of concentration of the species is then made in accordance to Beer's Law [178].

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

In ICP-MS, the analyte elements can be analyzed by collisions with Ar^+, excited argon atoms or energetic electrons. This technique is expensive but offers the advantages of good sensitivity, limits of detections (LODs), throughput, and simultaneous multi-element determination. To yield the most accurate and repeatable results, the ICPMS can be used in conjunction with an isotope dilution quantitative methodology (ID-ICP-MS).

ICP-MS suffers from several atomic and molecular isobaric interferences, but these can be overcome by prudent use of collision cell or reaction cell technology, high resolution mass analyzers that can resolve elements and interferences that would have previously been co-detected [175]. Interferences such as overlapping peaks from interfering species or incomplete atomization of non-analyte species can cause positive bias on measurement results. Incomplete atomization of the target may result in negative bias when this technique is used. This may be worsen when there are matrix effects that often suppress the signal from an unknown quantity of element with the sample as compared to the same amount of element in a calibration standard [175].

Laser ablation can be use with ICP-MS which is useful in the sense that it avoids wet decomposition and risk of contamination during sample preparation and increases the power of detection. However, the introduction of sample aliquots is often more difficult to control and to assess accurately, and care must be taken to ensure that large systematic biases do not occur as a result of this type of sample introduction [175].

Instruments of this type appear to be particularly well suited for semi-quantitative analysis of samples that are difficult to decompose or dissolve such as geological materials, alloys, glasses, agricultural products, urban particulates, and soils [179].

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

This method is well suited for the determination of refractory elements with high sensitivity because very high temperatures (8000 K) are attained with plasma. It has better LODs to flame atomic absorption spectrometry (FAAS) but can detect many elements simultaneously and has a much larger linear dynamic range than AAS-based techniques. It is susceptible to interferences at low concentrations [176].

X-Ray Fluorescence (XRF)

X-ray spectroscopy is based upon the measurement of emission, absorption, scattering, fluorescence, and diffraction of electromagnetic radiation. The most used X-ray methods are the XRF and X-ray absorption for quantitative and qualitative determination of all elements in the periodic table having atomic numbers greater than that of sodium. Elements with atomic numbers in the range of 5 to 10 require special equipments [180].

X-rays are produced by the deceleration of high energy electrons or by electronic transition in the inner orbitals of atoms. Their electromagnetic radiations are of very short wavelength, which falls in the range of about 10^{-5} Å to 100 Å. Conventional X-ray spectroscopy is, however, largely confined to the region of about 0.1 to 25 Å [180].

The sensitivity of XRF depends on the energy of the incident radiation, geometry of the instrument, and efficiency of the detector. The overall precision of XRF is usually limited by the statistics of the detected photons. The LODs achievable depend on the sensitivity of the instrument and background level of the sample matrix. Modern instrumentation for XRF has precision better than 0.1%. Typical LODs of a few µg/cm² for particulate material on ambient air filters are normally achievable for a wide range of elements [181].

In total-reflection XRF (TXRF) the incoming incident radiation is normally directed on the sample at an angle less than the critical angle to cause total reflection. This geometry

produces low background that means an improvement in LODs and some of the elements can be detected at amounts as low as 2 pg with counting time of 1000 s [182].

Matrix effects do not usually play a significant role in this system because samples are often in the form of a thin film. Quantitation is usually performed by addition of a single element that is not present in the sample as an internal standard, and a calibration curve is established that is assumed valid in all matrices. Systematic errors may be relatively large due to lack of robustness in these calibration methods compared to other trace analytical techniques, and these errors can be pronounced due to very low amounts being analyzed [183].

X-ray emission may be also induced by heavy charged particles (particle-induced X-ray emission, PIXE). Calibration is often by means of thin-film standards or by using fundamental physical parameters in conjunction with an experimentally determined efficiency curve. The LODs of a few ng/cm² have been claimed for particulate material on ambient air filters for a range of elements, with repeatabilities of 1% and for an accuracy of 5% [184].

<div align="center">Neutron Activation Analysis (NAA)</div>

Activation methods rely on the measurement of radioactivity that has been induced in samples by irradiation with neutrons or charged particles such as hydrogen, deuterium, or helium ions. Three sources of neutrons are employed in activation methods: reactors, radionuclides, and accelerators. All produce highly energetic neutrons (in the MeV range) that are usually passed through a moderating material that reduces their energies to a few hundredths of electron volts. Energy loss to the moderator occurs by elastic scattering in which neutrons bounce off nuclei in the moderator material by transferring part of their kinetic energy to each nucleus they strike. The nuclei finally come to temperature equilibrium with their surroundings. Low molecular weight substances such as water, deuterium oxide, and paraffin are the most efficient moderators [185].

NAA is the most common of the activation analysis. Neutrons are used to irradiate and to activate samples. Instrumental NAA (INAA) measurement is carried out without prior chemical separation. As a consequence of a nuclear reaction between the neutron and the isotope of the element of interest, radionuclides with characteristics half-lives may be produced, emitting radiation of varying energies that may be measured by suitable detector and are characteristic of the element from which they were produced [175]. NAA technique had been applied in analysis of very pure silicon, trace determination of elements in biological samples, and the multi-element analysis of airborne particulate matter. [186]. NAA has also been used for comparative studies of complex environmental matrices. Often it shows more accurate results than ICP-MS [187].

Neutron activation methods are very sensitive and require minimal sample preparation and ease of calibration. These procedures are nondestructive, and for this reason they are applied to the analysis of art objects, coins, forensic samples, and archaeological specimens. It has the disadvantages of requiring expensive equipments and special facilities for handling and disposing of radioactive materials. It also requires a long time to complete analyses when long-lived radionuclides are used [188]. Most sources of systematic and random error (e.g. interfering nuclear reactions, overlap of spectral lines, and dead-time losses) are identifiable, because the physical principle of NAA are well understood and described. In addition, because NAA is based on principle fundamentally different from other analytical techniques, it is prone to completely different systematic biases and is therefore extremely useful in analysis of reference materials or in assessing the comparability of measurement results [175].

<u>Anodic Stripping Voltammetry (ASV)</u>

ASV is an electroanalytical technique with the lowest LOD for metals. Its LOD for solutions is in the range of 10^{-6} to 10^{-9} M [189]. The stripping voltammetry LOD can further be improved if the analyte can be concentrated. It uses a mercury drop electrode as its working electrode for the following reasons. Mercury can be exchanged and this provides a clean, reproducible surface for each determination. Mercury provides a wider range of potentials

compared to other electrodes. Finally, there is the likelihood of the element of interest to form an amalgam rather than just being absorbed onto the surface [175].

Following the concentration of large solution onto the mercury drop, the drop is isolated and equilibrated. This is necessary because if the circuit to the working electrode remained connected, more ions would be reduced. Also, metal that have been reduced enters the drop through its outer surface and it takes time for the metal atoms to diffuse and become equally distributed inside. Finally, the external solution that was stirred during the reduction-concentration step requires some times to become still [189].

In general, only a small fraction of the analyte is deposited during the electrodeposition step, hence, quantitative results depend not only upon control of electrode potential but also on the size of the electrode, length of deposition, and rate of stirring for both the sample and standard solution employed for calibration. Other materials can be used to build the microelectrode besides mercury. These are gold, silver, platinum, and carbon [190].

The hanging mercury drop electrode (HMDE) determines a metal ion by first forming a fresh hanging drop, followed by stirring, and a potential applied that is a few tenths of a volt more negative than the half-wave potential for the ion of interest. Deposition is allowed to occur in a careful manner for a measured period of time that can range from a minute or less for 10^{-7} M solutions to 30 minutes or more for 10^{-9} M solutions. These times often result in complete removal of the ions. Furthermore, the sensitivity of the electrolysis period is determined by the method employed for the completion of the analysis [190].

Other stripping techniques have been developed. For instance, platinum cathode has been used to electrodeposits a number of cations. Here, the quantity of electricity required to remove the deposit is then measured coulometrically. This method is also of merit for trace analysis. A cathodic stripping method is another that has been developed for halides. The halide ions are first deposited as mercury (I) salts on a mercury anode. Stripping is then performed by a cathodic current [190].

Adsorptive Stripping Methods

In this method the microelectrode, HMDE, is immersed in a stirred solution of the analyte for several minutes. The analyte is deposited by physical adsorption on the surface of the electrode rather than electrolytic deposition. The stirring is discontinued after enough of the analyte have built up. The deposited material is then determined by linear scan or pulsed voltammetry measurements. Quantitative information is based upon standard solutions treated in a similar manner to the samples.

Adsorption stripping can be made faster when there's effective stirring. This may required only 1 to 5 minutes to build up the analytes for analysis from 10^{-7} M solutions to 10 to 20 minutes for 10^{-9} M solutions. Adsorption method has been useful for the determination of organic and inorganic molecules at low concentrations. Detection limits of 10^{-10} to 10^{-11} M have been reported [190].

Ion Chromatography

With present method, a single separation experiment enables cations or anions to be quantified at the level of parts per billion (ppb). The retention time of the bands identify the ions and the area under the bands gives the quantity of the ion. Ions are detected by measuring the electrical conductivity of the effluent. When the bands containing ions pass through the detector, conductivity rises. This type of ion chromatography contains two exchanges. The ion exchange separates ions and the second removes background ions enabling the analyte peaks to be measured by conductimetry. The solvent dissociation produces background conductivity that determines the limit of detection. This is called suppression, and it entails the neutralization of hydroxide ions of effluents to produce water [189].

Atomic Emission Spectroscopy (AES)

In AES analyte concentration is determined by measuring the quantity of optical emission of excited atoms. The analyte atoms are sucked into the excitation region where they are desolvated, vaporized, and atomized by a flame, discharge, or plasma. Plasma source is preferred to a flame source in that they are hotter and more homogenous in temperature than flame. The energy to excite the atoms is provided by the high temperature atomization source. The excited atoms on returning to the ground state emit light that gives emission spectra because the transition occurs between distinct atomic energy levels [191].

Emission spectrometry is basically a simultaneous multi-element analysis technique. Simultaneous detection relies on polychromators, while the sequential detection relies on scanning the necessary spectral region with the sample being fed into the atomizer at the same rate. AES can be used to analyze elements with analyte content ranging from high ppm depending on the element, the matrix, and the mode of thermal excitation. But, major and trace levels is not likely to be determined simultaneously for reasons of limits of linear dynamic range of photometric instrument. Different elements show varied atomization that is dependent on their binding energies in the sample matrix [189].

Atomic Absorption Spectroscopy (AAS)

AAS is the most widely used and accepted technique capable of determining trace (μg/mL) and ultra-trace (sub μg/mL) levels of elements or metals in areas of environmental, clinical, biological, food, and geological samples with good accuracy and precision [192].

When light of a specific wavelength strikes ground state atoms, the atoms absorbed the light and become excited to a higher energy level. The intensity of this transition is dependent on the original concentration of the ground state atoms. The amount of light absorbed beside concentration depends on the atomic absorption coefficient. Absorbance, A, is related to

transmittance as the negative logarithm of transmittance. The Beer-Lambert Law also relates A to molar concentration, c, of an element as shown in the equation below.

$$A = \varepsilon_o bc$$

Where ε_o is molar absorptivity in (L/cm.mole) and b is the path length in cm, A is absorbance that has no units and typically ranges from 0.01 to 2.0. In practice it is better to work in the middle of this range (recommended 0.1 to 0.3) because precision is poorer at the extremes due to instrumental noise [192].

Flame Atomic Absorption Spectrometry (FAAS)

The FAAS consists of six components: a radiation source, flame atom cell, sample introduction unit, monochromator, detection system, and readout. The hollow cathode lamp (HCL) is the most widely used radiation source. HCL is a line source and requires each element that is to be analyzed have a specific separate lamp. But there are some lamps that can be used for multi-element analysis, for instance Ca-Mg, and Cr-Fe-Ni where the cathode is made of two or three similar elements. However, the single-atom lamps are more effective and perform better than multi-element lamps. The HCL consists of a glass envelope that is filled with an inert gas, usually neon, argon, or helium at low pressure.

The most widely used flames in AAS are air-acetylene (with air being the oxidant and acetylene, the fuel) and nitrous oxide-acetylene (nitrous oxide is the oxidant here). The flame dissociates the molecules into atoms. Air-acetylene flame can provide a temperature of 2500 K to dissociate about 50 elements on the periodic table, while nitrous oxide acetylene flame can dissociate another 10 to 20 elements at a temperature of about 3200 K [192].

The sample introduction system should be reproducible and efficient in transferring a sample to the atomizer. It should be without interference, memory, or carryover effects and should not depend on sample type. Factors such as amount of sample available, physical form of the sample, precision, sensitivity, detection limit, type of atomizer, and so on can influence the type of sample introduction system used. Solutions are introduced by pneumatic nebulizers

(PNs). The sample solution is drawn into the PN by rapid flow of oxidant past the tip of the sample capillary. Liquid breaks into fine mist as they leave the capillary. The spray is directed onto a glass bead upon which the droplets break into smaller particles [193].

The wavelength needed is isolated from the radiation by the monochromator. The AAS is highly selective and ensures that there is no interference. The monochromator typically has a resolution of 0.02 to 2 nm. The detection system used is the photomultiplier tube (PMT), while the external computers digitally display the measurement [192].

FAAS, however, is unable to determine some refractory elements with good sensitivity because flame temperature is often not hot enough to induce complete atomization. It is a relatively slow technique and is suitable for determination of a single element at a time. For large number of elements, other techniques may be substantially quicker [175].

Graphite Furnace Atomic Absorption Spectrometry (GF-AAS)

This method requires smaller sized samples (1-100 µL) compared to FAAS (minimum volume 1-2 mL) and is more sensitive than FAAS. Its also has limit of detection (LOD) that is 10-100 times better than those of FAAS. In spite of the above, it's still unable to determine refractory elements with acceptable precision [175].

The atomizer is usually a graphite tube. In the graphite furnace the analyte is confined in the optical path for several seconds, enhancing the sensitivity. Three or more steps are often required to properly atomize the samples. At each step except for the atomization step the furnace is purge with Ar or N_2 to remove volatile material. To avoid blowing away the analyte gas flow is stopped during atomization [193].

Volatility of the matrix components is increased by using chemical modifiers and this allows them to be removed at lower pyrolysis temperature. For instance, adding ammonium acetate, to a solution containing sodium chloride, NaCl, (a serious interference matrix in furnace AAS) produces a boiling point of less than 600 °C for the resulting solutions of sodium acetate

and ammonium chloride, which compared favorably to the boiling point of NaCl which is 1400 °C. NaCl can therefore be removed at the ashing stage of 600 °C [192].

Interferences in the furnace may be due to particulate matter, molecular background, and atomic background. The most widely used correcting method for background is the continuum source (usually deuterium arc). It works by alternating light from the continuum source and HCL through the atom cell. Only the background absorbs the light from the continuum source and not the elements being determined. Zeeman Effect background correction which operates on the principle that spectral line splits into its magnetic components in the presence of a magnet is often used. The Zeeman Effect is not affected by structural background and spectral interferences can be subdued if the lines are 0.2 nm apart [192].

GF-AAS method of elemental analysis is slow, typically requiring several minutes per element. It also has a low analytical range, hence it is only used when the flame and plasma atomization provides inadequate detection limits [190].

Objectives of the Research

A recent article posted on the British Broadcasting Corporation (BBC) website entitled "Organic has no Health Benefits" discussed a review conducted by United Kingdom (UK) researchers on organic and ordinary foods. The review concluded that there was little difference in nutritional value, and that there was no evidence to support the argument that eating organic foods would be of extra health benefits. According to the report, 55 out of the 162 studies that were included in the analysis show little disparity between organic and conventional foods in most nutrients. Even though the review did not consider pesticides or environmental effects, the researchers pointed out that differences between organic and conventional foods was not important due to relatively few studies [194].

A comprehensive review published in 2002 also concluded similarly for foods derived from organic and conventional systems assessed for nutritional value, sensory quality, and food

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safety. It is evident from these assessments that there are few well controlled studies that are capable of making a valid comparison. With the possible exception of nitrate content, there is no strong evidence that organic and conventional foods differ in concentrations of various nutrients. While there are reports indicating organic and conventional fruits and vegetable may differ on a variety of sensory qualities, the findings are inconsistent. Organic "certification", no matter what the rules, will not protect the consumers. They just cost more and may lessen public confidence in the safety of "ordinary" foods.

Vegetables constitute essential components of diet by contributing protein, vitamins, iron, calcium, and other nutrients that are usually in short supply [15]. They also act as buffering agents for acid substances obtained during digestion process. However, these plants contain both essential and toxic compounds over a wide range of concentrations. Chronic low levels of intake of heavy metals have ill effects on human beings and other animals, as there is no good mechanism for their elimination. Because organic farming does not employ the use of pesticides and synthetic fertilizers, one would expect produce from these farms to have lower metal contents compared to conventionally grown ones, but little research buttress this fact.

Furthermore, as organic farming becomes more common, methods to identify fraud in the industry are increasingly important because some farmers engaging in the use of synthetic nitrogen fertilizers and pesticides has the potential of increasing the heavy metals levels in organically produced vegetables and crops. Moreover, the number of studies examining the metal contents of organic foodstuffs is relatively limited [16 -19], hence there is a justifiable need to carry out this research.

There are three ways of undertaking studies to compare conventionally and organically produced foods [20]: market-orientated supply studies (samples are taken from conventional and alternative shops), surveys (samples are taken from selected farms with different forms of cultivation), and cultivation tests (samples are taken from experimental farms) [20]. A market-orientated supply study is the one employed in this research due to its relative ease to conduct and to monitor the situation of the consumer as well as the condition of the vegetable products as displayed in the market.

In view of the above, the research objectives are:

- To quantify the levels of heavy metals (Pb, Fe, Zn, Cd, Cu, Ni) contamination in commonly edible vegetables around Johnson City, Tennessee,
- Compare the levels of the above-mentioned metals of organically grown vegetables to their conventional counterparts,
- To determine if the vegetable products are within the safe levels for consumption (in the municipality based on our study) as stipulated by FAO/WHO standards.

The above heavy metals were chosen for this research because of their frequent occurrence in soils and their wide availability in fertilizer content administer to vegetables.

CHAPTER 4

MATERIALS AND METHODS

In this chapter the reagents used, where the samples were purchased, and how they were treated and prepared, and how the standard solutions were prepared are outlined. The atomic absorption instrumentation is presented and the experimental procedures for the measurements of the metals are detailed also this chapter.

Reagents and Solutions

The reagents used in this study are listed below:

1. Stock solution concentration of 1000 μg/mL each for zinc, copper, iron, nickel, lead, and cadmium were from Leeman Lab (Hudson, NH).

2. The concentrated nitric acid manufactured by Seastar Chemical from Fischer Scientific (Pittsburg, PA).

Preparation of Standard Solutions

Standard solutions were prepared from stock solution (1000 μg/mL) of each metal. Initial 10 μg/mL concentration working solutions were prepared from the stock solutions by taking 1 mL of the 1000 μg/mL into 100-mL volumetric flasks and diluting to the mark. Depending on the linear response range of the metal, the calibration standards were then prepared by appropriate dilution from the 10 μg/mL working solution. The calibration standards for each of the metal are shown in Table 2.

Table 2. Working ranges of metals examined compare to literature values.

Heavy metal	Experimental working range (µg/mL)	Working range of metal (µg/mL) [195, 196]
Zinc	0.05 - 1.0	0.1 - 2.0
Copper	0.05 - 1.0	0.5 - 5.0
Iron	0.1 - 2.0	0.5 - 5.0
Nickel	0.5 - 5.0	0.5 - 5.0
Lead	0.3 - 5.0	1.0 - 20
Cadmium	0.1 - 2.0	0.1 - 2.0

Instrumentation

The absorbance of the calibration standards and samples were measured using the Shimadzu AA 6300 model flame atomic absorption spectrophotometer manufactured by Shimadzu Corporation, analytical and measuring instruments division, Japan. Fischer Isotemp Oven and hot plates manufactured by Fischer scientific, Fair Lawn, NJ, were used to dry and gently heat vegetable samples to near dryness in digestion process respectively.

Measurement Conditions and Parameters

Hollow cathode lambs of specific wavelength manufactured by Photonics K.K., electrode tube division, Japan, was used to provide the line source needed for the maximum absorption of each selected metal. Because the absorbance of a metal in FAAS depends on the type of flame used, fuel and oxidant ratio, flow rate of fuel gas, and burner height, the measurement conditions for the Shimadzu AA 6300 model used are shown in Table 3. Also, for all metals analyzed, the flame used was Air-C_2H_2 and the burner angle and support gas flow rate were zero degree and 15.0 L/min, respectively.

Table 3. Measurement parameters ensured in the present study.

Metal	Wavelength (nm)	Flow rate of fuel gas (L/min)	Slit width (nm)	Burner height (mm)
Zinc	213.9	2.0	0.7	7.0
Copper	324.8	1.8	0.7	7.0
Iron	248.3	2.2	0.2	9.0
Nickel	232.0	1.6	0.2	7.0
Lead	217.0	2.0	0.7	7.0
Cadmium	228.8	1.8	0.7	7.0

Data Treatment and Statistics

Excel (Microsoft, Redmond, WA) was used to calculate distributive statistics for each category of vegetable. ANOVA: single factor and t-test: two sample assuming equal variances, all at $p = 0.05$ level of significance was used to statistically compare the metal levels in organic and conventional vegetables. The statistical software used was Fischer Exact Test Statisca 6 software (Stat Soft, Tulsa OK, USA).

Sample Collection

Fresh vegetables products (organic and conventional) were purchased in May-June 2009 from local supermarkets. Eight different vegetables of both organic and conventional grown were used. For each vegetable, 3 different samples were bought which total 48 fresh vegetables as shown in Table 4. The vegetables were stored in polythene bags in the refrigerator without washing.

Table 4. The number of different vegetables purchased and their fresh weight in grams.

Fresh Vegetable Products	Number of Samples Purchased	Average Weight per Sample (g)
Organic Green pepper	3	219
Conventional Green pepper	3	225
Organic Tomato	3	340
Conventional Tomato	3	299
Organic Cucumber	3	285
Conventional Cucumber	3	273
Organic Spinach	3	119
Conventional Spinach	3	127
Organic Apple[a]	3	348
Conventional Apple[b]	3	360
Organic Green Cabbage	3	184
Conventional Green Cabbage	3	183
Organic Green Leaf Lettuce	3	111
Conventional Green Leaf Lettuce	3	148
Organic Collard Greens	3	175
Conventional Collard Greens	3	181
Leafy Green vegetable[c]	1	262

[a,b]fruit sample, and [c]not purchased.

Sample Preparation and Treatment

The fresh vegetable samples were weighed using analytical balance. They were then chopped and air-dried in an oven at 110°C between 12-72 hours to constant weight to eliminate moisture. The dried samples were grounded with mortar and pestle into fine powder, sieved, and stored in plastic bottles. The dried sample of each vegetable type was stored in clean dry plastic bottles and labeled. The plastic bottles were pre-washed with concentrated nitric acid and rinsed with distilled water to be sure that it contained no metal liners that can contaminate the samples.

Sample Extraction

Exactly 1.0 g of fine powdered vegetables of each type was precisely weighed on an analytical balance and transferred quantitatively to separate 50-mL labeled beakers. Each sample was weighed in triplicate. Exactly 20 mL of concentrated nitric acid was added under fume hood and allowed to stand for 12 hours. The samples turned brownish in color with yellowish-brown foam on top of each (both organic and conventional samples) after the 12 hours. Gentle heating of the samples to near dryness for approximately 4 hours on a heating plate was carried out with glass rod kept in each sample to avoid bumping. During the heating process samples foam profusely which subsided later in the course of heating. The foam was yellowish in color. The beakers were allowed to cool after the heating and 10 mL of distilled water was added to dissolve the residues. The solutions were filtered using 42.5 mm filter paper. The sample beakers were rinsed with distilled water at least three times while filtering and the filtrate was transferred quantitatively to separate 50-mL volumetric flasks and then diluted to the mark. The test solutions were stored in a refrigerator in plastic bottles until analysis. The blank was also prepared in a similar manner without a sample.

Sample Analysis

Standard solutions were prepared from the stock solutions of each metal. The absorbances of these solutions were measured using the Shimadzu AA 6300 flame atomic absorption spectrophotometer. The samples were aspirated into the nebulizer through a capillary tube where they were converted into fine mist/ aerosol before entering the atomizer. The respective concentrations were determined from the linear calibration curve for each metal.

The standard solutions were prepared immediately before analyses to avoid adsorption of metals by the glass containers and decomposition. Also, the same instrumental conditions were used to run all the samples at a time. When the absorbance of a sample exceeded that of the highest concentration standard solution of a particular metal, appropriate dilution was made to bring the sample concentration within the linear response range.

CHAPTER 5

RESULTS, DISCUSSION, AND CONCLUSION

The present study investigates the trend of heavy metals (Zn, Cu, Fe, Ni, Pb, and Cd) in both organic and conventional vegetables. The different vegetables analyzed were green pepper, tomatoes, cucumber, spinach, cabbage, lettuce, collard greens, apple, and leafy green vegetable.

Zinc Determination

The amounts of zinc determined in the conventional vegetables were as follows: in green pepper, 24.7 µg/g; in tomatoes, 24.7 µg/g; in cucumber, 37.1 µg/g; in spinach, 69.4 µg/g; in cabbage, 32.1 µg/g; in lettuce, 20.7 µg/g; in collard greens, 13.9 µg/g; in apple, 2.21 µg/g; and in leafy greens, 29.1 µg/g. The highest amount of Zn was found in spinach while the lowest amount was recorded in apple. The organic vegetables on the other hand showed the following results for zinc: in green pepper, 22. 1 µg/g; in tomatoes, 27.5 µg/g; in cucumber, 34.6 µg/g; in spinach, 33.4 µg/g; in cabbage, 23.7 µg/g; in lettuce, 27.8 µg/g; in collard greens, 11.3 µg/g; and in apple, 2.04 µg/g as indicated in Table 5. In the organic produce, Zn was highest in cucumber and lowest in apple. Zinc content in conventional vegetables such as green pepper, cucumber, spinach, cabbage, collard greens, and apple were higher than their organic counterpart as shown in Figure 2. However, tomatoes and lettuce showed higher zinc content in organic vegetables than their conventional ones.

In spite of these variations in zinc content between the two categories of vegetables, statistical comparison of the following organic and conventional vegetables: green pepper, tomatoes, cucumber, collard greens, and apple shows no statistical differences ($p > 0.05$) between them. Meanwhile, cabbage, spinach, and lettuce showed statistically significant difference for the two categories of vegetables ($p < 0.05$).

Table 5. Zinc content in both organic and conventional vegetable products.

Samples	n^a	Mean (μg Zn g^{-1} dry wt ± SD)	Maximum (μg Zn g^{-1})	Median (μg Zn g^{-1})	Minimum (μg Zn g^{-1})	P-values[d] P(T<=t) two tail[e]
Organic Green pepper	3	22.1 ± 1.5	25.0	21.6	20.1	
Conventional Green pepper	3	24.7 ± 4.6	35.9	22.7	21.8	0.138
Organic Tomatoes	3	27.5 ± 5.2	35.0	25.7	23.1	
Conventional Tomatoes	3	24.7 ± 6.1	38.0	25.8	17.5	0.299
Organic Cucumber	3	34.6 ± 2.4	39.3	33.9	31.1	
Conventional Cucumber	3	37.1 ± 6.4	43.8	40.5	28.5	0.292
Organic Spinach	3	33.4 ± 5.0	39.5	35.1	25.6	
Conventional Spinach	3	69.4 ± 5.3	76.3	66.5	64.1	< 0.001
Organic Cabbage	3	23.7 ± 8.1	31.8	25.4	10.0	
Conventional Cabbage	3	32.1 ± 5.5	40.5	29.2	26.9	0.021
Organic Green Leaf Lettuce	3	27.8 ± 2.7	31.1	28.3	23.1	
Conv. Green Leaf Lettuce	3	20.7 ± 1.8	22.7	21.0	18.0	<0.001
Organic Collard Greens	3	11.3 ± 1.8	13.2	12.0	7.19	
Conventional Collard Greens	3	13.9 ± 3.6	21.4	14.3	9.75	0.066
Organic Apple[b]	3	2.04 ± 0.4	2.84	1.94	1.68	
Conventional Apple[b]	3	2.21 ± 0.2	2.58	2.11	1.99	0.273
Leafy Green Vegetable[c]	1	29.1 ± 4.0	33.7	27.0	26.5	-

[a] Number of different samples.
[b] Fruit sample.
[c] Random sample.
[d] ANOVA: single factor @ p = 0.05 level of significance.
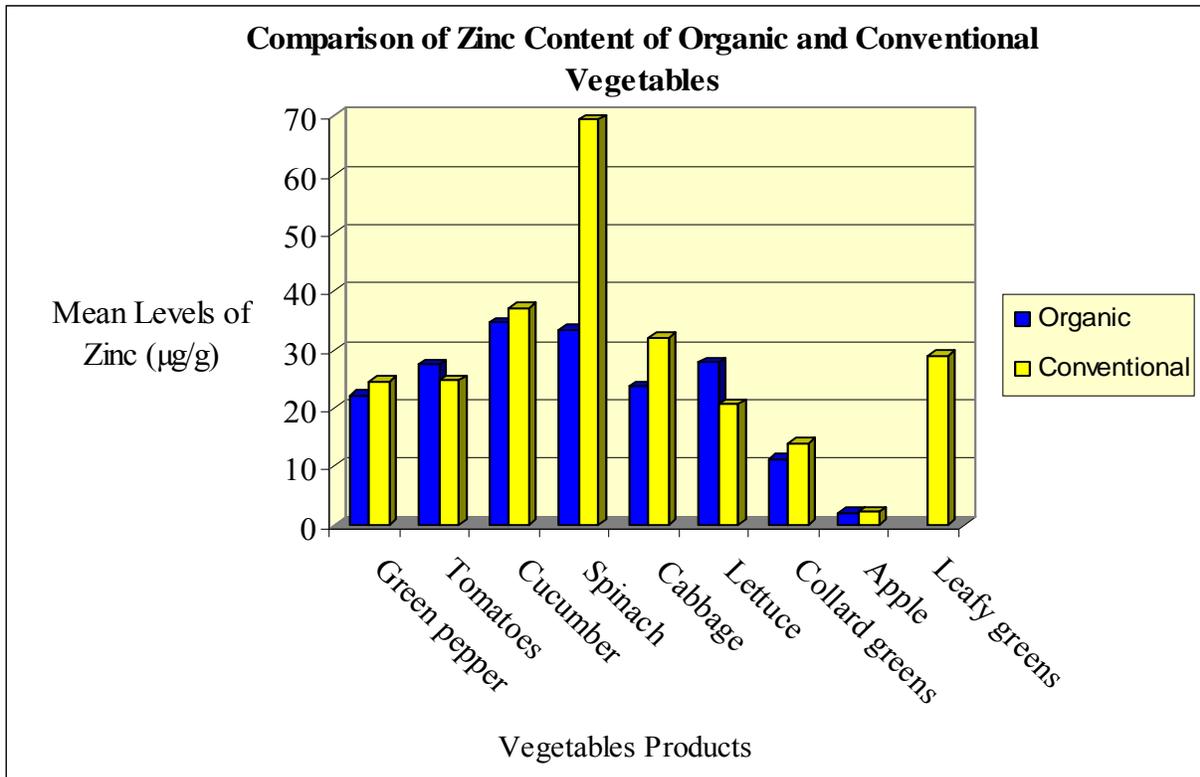[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

Figure 2. Zinc content of organic and conventional vegetables.

Apart from spinach, the other leafy vegetables (cabbage, lettuce, collard greens, and leafy greens) did not show much difference in the amounts of Zn from the other vegetables (green pepper, tomatoes, and cucumber). Both categories of vegetables were found to contain high amounts of zinc in cucumber and spinach (> 30.0 µg/g). However, more importantly the safe limit for zinc according to FAO/WHO (2001) is 100 µg/g, and so all vegetables were within the safe limit. Related studies reported similar trend of analysis. For instance, Singh et al. [199] in 2001 and Itanna [200] in 2002 analyzed six and three leafy vegetables respectively and reported higher concentration of Zn in spinach. Also, Radwan and Salama [201] in 2006 carried out a survey of various fruits and vegetables for heavy metals including Zn and concluded that cucumber and spinach were among vegetables that showed higher mean levels in Zn. Furthermore, Mohsen and Mohsen [202] investigated metal accumulation in some vegetables irrigated with waste water in Shahre Rey-Iran and its toxicological implications. They found spinach, 297 µg/g, and green pepper, 1132 µg/g, to be above the Zn permitted level according to FAO/WHO (2001).

Copper Determination

The mean values of copper found in conventional vegetables are; in green pepper 15.1 µg/g; in tomatoes, 7.15 µg/g; in cucumber, 5.94 µg/g; in spinach, 4.79 µg/g; in cabbage, 2.91 µg/g; in lettuce, 6.06 µg/g; in collard greens, 1.64 µg/g; in apple, 2.23 µg/g; and in leafy greens, 3.72 µg/g. In the organic vegetables the amount of copper found were in green pepper, 8.55 µg/g; in tomatoes, 9.90 µg/g; in cucumber, 3.78 µg/g; in spinach, 1.11 ug/g; in cabbage, 1.28 µg/g; in lettuce, 10.5 µg/g; in collard greens, 0.35 µg/g; and in apple, 1.32 µg/g as indicated in Table 6. Green pepper and collard greens showed the highest and lowest Cu content, respectively, among the conventional vegetables, while lettuce and collard greens recorded the highest and lowest Cu content, respectively, in organic vegetables. Conventional vegetables (green pepper, cucumber, spinach, cabbage, collard greens, and apple) contained higher levels of copper compared to their organic ones as shown in Figure 3. Statistical comparison also showed significant differences between all categories of vegetables ($p < 0.05$).
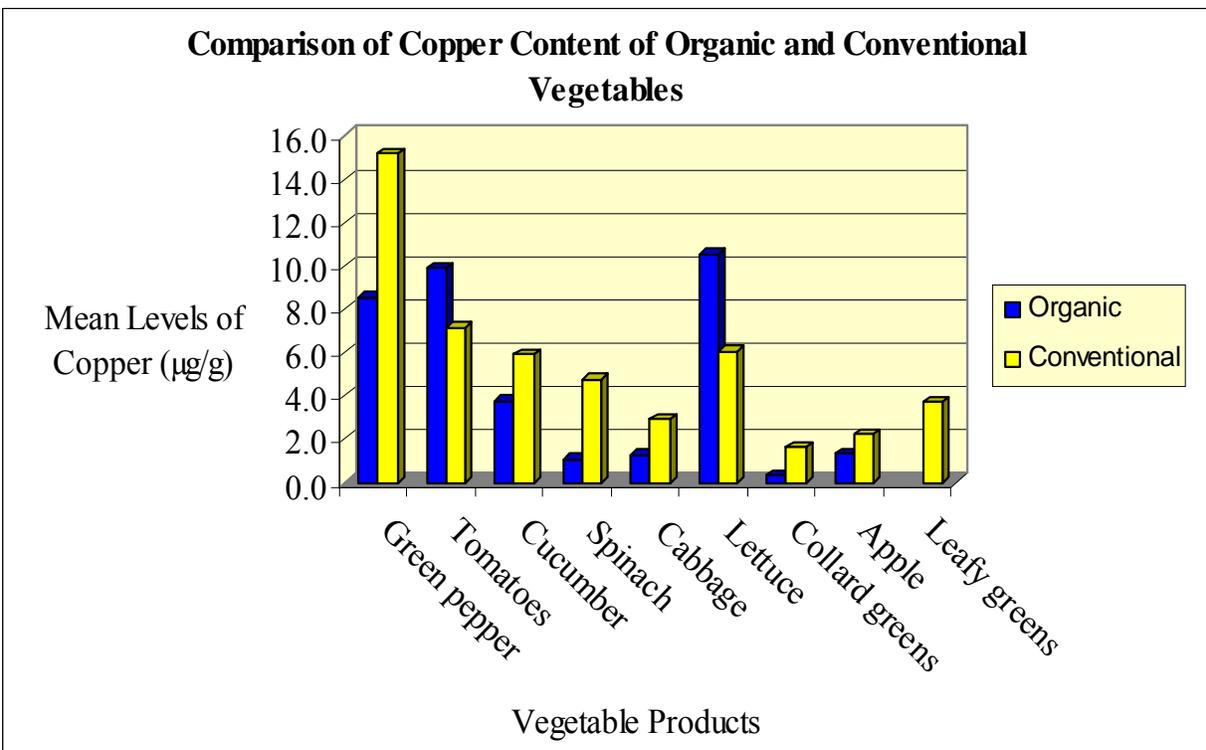

Figure 3. Copper content of organic and conventional vegetables.

Table 6. Copper content in both organic and conventional vegetable products.

Samples	n[a]	Mean (µg Cu g^{-1} dry wt ± SD)	Maximum (µg Cu g^{-1})	Median (µg Cu g^{-1})	Minimum (µg Cu g^{-1})	P-value[d] P(T<=t) two tail[e].
Organic Green pepper	3	8.55 ± 2.1	11.9	9.49	5.52	< 0.001
Conventional Green pepper	3	15.1 ± 1.4	17.9	14.5	13.8	
Organic Tomatoes	3	9.90 ± 2.2	13.9	9.11	7.50	0.006
Conventional Tomatoes	3	7.15 ± 1.3	8.90	6.91	5.31	
Organic Cucumber	3	3.78 ± 2.1	7.13	3.43	0.43	0.033
Conventional Cucumber	3	5.94 ± 1.8	8.84	5.25	3.54	
Organic Spinach	3	1.11 ± 1.2	3.00	0.48	0.00	< 0.001
Conventional Spinach	3	4.79 ± 1.1	6.65	4.93	3.59	
Organic Cabbage	3	1.28 ± 0.8	2.79	1.18	0.38	< 0.001
Conventional Cabbage	3	2.91 ± 0.8	4.39	2.79	1.55	
Organic Green Leaf Lettuce	3	10.5 ± 2.8	15.4	10.0	6.40	<0.001
Conv. Green Leaf Lettuce	3	6.06 ± 1.2	8.63	5.84	4.66	
Organic Collard Greens	3	0.35 ± 0.3	0.70	0.38	0.00	0.010
Conventional Collard Greens	3	1.64 ± 1.3	4.50	0.96	0.59	
Organic Apple[b]	3	1.32 ± 0.7	2.36	1.39	0.21	0.035
Conventional Apple[b]	3	2.23 ± 0.9	3.64	2.36	0.80	
Leafy Green Vegetable[c]	1	3.72 ± 2.8	6.97	2.47	1.71	-

[a] Number of samples.
[b] Fruit sample.
[c] Random sample
[d] ANOVA: single factor @ p = 0.05 level of significance.
[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

Copper in all the vegetables was found to be within the safe limit (40 µg/g) according to FAO/WHO (2001). The study also shows that the leafy vegetables did not contain relatively higher levels of Cu than non-leafy vegetables like tomatoes, green pepper, and cucumber. However, Demirezen and Ahmet [203] reported the levels of Cu of 22.2-76.5 µg/g in 2006, in vegetables harvested in Turkey. These were higher in leafy than non-leafy species kind. This difference might be partly due to the fact that their samples were grown in an industrial area. The condition of the area of production of purchased samples of the present study was not known, however. A similar study by Tandi et al. [204] in 2005 also found higher amount of Cu in lettuce and other leafy vegetables produced around an industrial site. Also, Mohsen and Mohsen [202] reported Cu levels in spinach, of 22.7 µg/g; in green pepper, 21.8 µg/g, and in tomatoes, 39.99 µg/g; all grown under waste water irrigation. These values were significantly higher than those in the study conducted here.

Iron Determination

Iron levels were the highest of all the heavy metals analyzed. In conventional vegetables, the amount found are in green pepper, 23.8 µg/g; in tomatoes, 34.2 µg/g; in cucumber, 35.7 µg/g; in spinach, 89.9 µg/g; in cabbage, 45.6 µg/g, in lettuce, 202 µg/g; in collard greens, 21.5 µg/g; in apple, 2.52 µg/g; and in leafy greens, 222 µg/g. In the exception of green pepper, spinach, and apple, all other organic vegetables recorded lower amounts as compared to the conventional vegetables. Iron levels found in organic vegetables were: in green pepper, 38.9 µg/g; in tomatoes, 22.2 µg/g; in cucumber, 24.6 ug/g; in spinach, 319 µg/g; in cabbage, 18.3 µg/g, in lettuce, 88.5 µg/g; in collard greens, 14.2 µg/g, and in apple, 2.82 µg/g as indicated in Table 7. Spinach and apple recorded the highest and lowest amounts of Fe respectively for organic vegetables, while leafy greens and apple recorded the highest and the least amount respectively for the conventional vegetables. Both conventional and organic vegetables of green pepper, tomatoes, cucumber, cabbage, collard greens, and apple were all found to be below 50 µg/g as shown in Figure 4. Except for collard greens and apple, the rest of the vegetables showed significant statistical differences in both categories ($p < 0.05$).

Table 7. Iron content in both organic and conventional vegetable products.

Samples	n^a	Mean (µg Fe g^{-1} dry wt ± SD)	Maximum (µg Fe g^{-1})	Median (µg Fe g^{-1})	Minimum (µg Fe g^{-1})	P-value[d] P(T<=t) two tail[e].
Organic Green pepper	3	38.9 ± 6.9	48.4	40.9	27.3	<0.001
Conventional Green pepper	3	23.8 ± 3.8	27.7	25.4	19.2	
Organic Tomatoes	3	22.2 ± 8.3	35.9	18.6	12.9	0.002
Conventional Tomatoes	3	34.2 ± 4.4	40.4	33.9	25.0	
Organic Cucumber	3	24.6 ± 6.0	38.3	23.4	18.5	0.002
Conventional Cucumber	3	35.7 ± 7.0	43.7	38.2	26.4	
Organic Spinach	3	319 ± 85	400	363	195	<0.001
Conventional Spinach	3	89.9 ± 6.3	103	88.8	81.6	
Organic Cabbage	3	18.3 ± 8.9	26.5	23.2	2.08	<0.001
Conventional Cabbage	3	45.6 ± 7.6	55.6	46.5	32.7	
Organic Green Leaf Lettuce	3	88.5 ± 23	134	81.3	63.3	<0.001
Conv. Green Leaf Lettuce	3	202 ± 55	269	204	82.9	
Organic Collard Greens	3	14.2 ± 5.1	21.5	15.8	6.70	0.289
Conventional Collard Greens	3	21.5 ± 19	69.7	14.5	7.70	
Organic Apple[b]	3	2.82 ± 2.3	6.78	2.54	0.15	0.776
Conventional Apple[b]	3	2.52 ± 2.3	7.32	2.08	0.08	
Leafy Green Vegetable[c]	1	222 ± 69	295	214	156	-

[a] Number of different samples.
[b] Fruit sample.
[c] Random sample.
[d] ANOVA: single factor @ p = 0.05 level of significance.
[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

However, both categories of vegetables were found to be below the safe limit, 425 µg/g, as proposed by the FAO/WHO joint expert committee on food additives in 2001. The amount of iron in soil and in the environment tend to be higher than other metals, so it was not surprising to find their concentration are higher in vegetables.

Similar studies reported very high Fe content in vegetables. For instance, Mohsen and Mohsen [202] examined Fe in some vegetables irrigated with waste water and found spinach, green pepper, and tomato to contain 509 µg/g, 319 µg/g, and 180 µg/g respectively. Aiwonegbe and Ikhuoria [205] studied selected heavy metals (Fe, As, Pb, Hg, Cd, Cr, Zn, and Ni) in some Nigerian vegetables and concluded that Fe has the highest concentration in all the vegetable samples analyzed with values ranging from 1.04-13.9 µg/g.
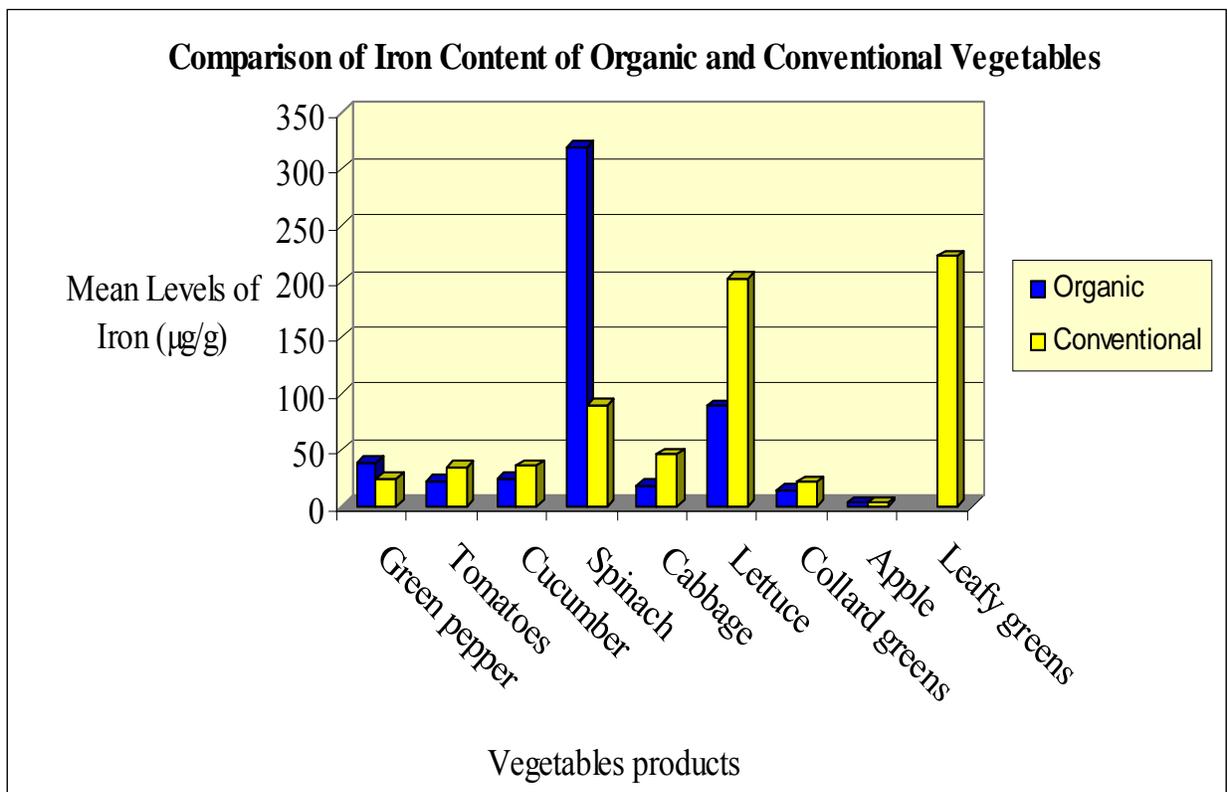

Figure 4. Iron content of organic and conventional vegetables.

Mohamed et al. in 2003 [137] assessed essential and toxic elements in leafy and non-leafy vegetables. Their study reported high levels of Fe in the following vegetables: cucumber, 83.5 µg/g; tomatoes, 60.2 µg/g; green pepper, 49.0 µg/g; lettuce, 324 µg/g; spinach, 166 µg/g; and cabbage, 76.9 µg/g. These results are comparable to the values obtained from present study in that both studies found high Fe content in spinach and lettuce. All values however were below the safe limit according to FAO/WHO (2001).

<p style="text-align:center;">Nickel Determination</p>

Nickel was the only metal whose amount in all vegetables was 40 µg/g and above. The amounts of Ni found in conventional vegetables were: in green pepper, 53.1 µg/g; in tomatoes, 45.3 µg/g; in cucumber, 46.2 µg/g; in spinach, 47.9 µg/g; in cabbage, 40.5 µg/g; in lettuce, 42.3 µg/g; in collard greens, 41.6 µg/g; in apple, 47.2 µg/g; and in leafy greens, 53.8 µg/g. In organic vegetables the results were: for green pepper, 49.9 µg/g; tomatoes, 43.9 µg/g; cucumber, 50.6 µg/g; spinach, 43.9 µg/g; cabbage, 39.9 µg/g; lettuce, 42.8 µg/g; collard greens, 40.8 µg/g; and apple, 44.4 µg/g as indicated in Table 8. The highest amount was found in the conventional leafy greens vegetable, 53.8 µg/g. There were 6 cases as shown in Figure 5 where the conventional vegetables (green pepper, tomatoes, spinach, cabbage, collard greens, and apple) showed higher levels of nickel as compared to 2 cases of organic vegetables, cucumber and lettuce, where the level of Ni was higher than found in conventional vegetables. Collard greens, lettuce, cabbage, and tomatoes showed no significant statistical differences ($p > 0.05$) in both categories while apple, spinach, cucumber, and green pepper ($p < 0.05$) did. All the reported values of Ni in this study were found to be within the safe limit of 67 µg/g according FAO/WHO (2001).

The high Ni content of apple was not surprising in that higher values have been reported in literature for apple and other fruit vegetables. Mahdavian and Somashekar [206] in 2008 analyzed Ni and other metals in fruits such as apple, grape, guavas, and others collected from K.R. market in Bangalore City, India. They found 97.7 µg/g, 44.4 µg/g, and 46.6 µg/g of Ni respectively in apple, grape, and guava. Mohamed et al. in 2003 [137] however reported lower

Table 8. Nickel content in both organic and conventional vegetable products.

Samples	n[a]	Mean (µg Ni g^{-1} dry wt ± SD)	Maximum (µg Ni g^{-1})	Median (µg Ni g^{-1})	Minimum (µg Ni g^{-1})	P-value[d] P(T<=t) two tail[e].
Organic Green pepper	3	49.9 ± 1.6	51.8	49.8	47.9	0.004
Conventional Green pepper	3	53.1 ± 2.4	57.7	52.5	49.8	
Organic Tomatoes	3	43.9 ± 2.0	45.9	44.8	40.6	0.101
Conventional Tomatoes	3	45.3 ± 1.2	47.3	45.6	43.8	
Organic Cucumber	3	50.6 ± 1.0	52.4	50.6	49.2	<0.001
Conventional Cucumber	3	46.2 ± 2.5	49.1	46.6	42.2	
Organic Spinach	3	43.9 ± 1.0	45.2	44.1	42.3	<0.001
Conventional Spinach	3	47.9 ± 1.8	50.3	48.1	45.6	
Organic Cabbage	3	39.9 ± 1.2	41.8	39.1	38.9	0.280
Conventional Cabbage	3	40.5 ±1.0	42.1	40.9	38.7	
Organic Green Leaf Lettuce	3	42.8 ± 1.2	44.4	42.4	41.3	0.302
Conv. Green Leaf Lettuce	3	42.3 ± 0.8	43.3	42.3	41.1	
Organic Collard Greens	3	40.8 ± 1.0	42.19	40.4	39.9	0.117
Conventional Collard Greens	3	41.6 ± 1.2	43.9	41.1	40.4	
Organic Apple[b]	3	44.4 ± 1.3	45.6	44.8	41.8	<0.001
Conventional Apple[b]	3	47.2 ± 1.2	48.5	47.4	44.4	
Leafy Green Vegetable[c]	1	53.8 ± 0.7	54.5	53.8	53.1	-

[a] Number of different samples.
[b] Fruit sample.
[c] Random sample.
[d] ANOVA: single factor @ p = 0.05 level of significance.
[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

mean values of Ni in cucumber, 10.9 µg/g; in tomatoes, 14.6 µg/g; and in spinach, 17.1 µg/g. But the difference may be partly because their samples were washed and also other conditions of production of the vegetables.

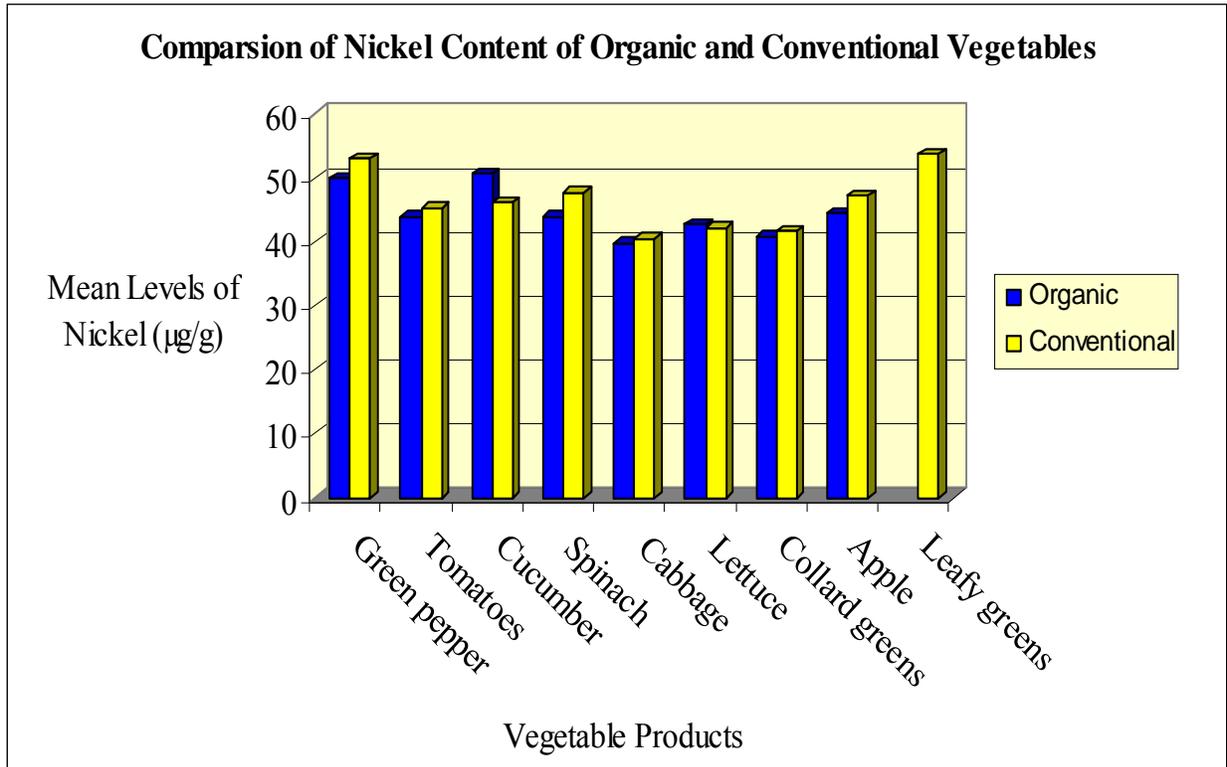Figure 5. Nickel content in organic and conventional vegetables.

The least amounts of metals detected in all the vegetables were lead and cadmium as shown in Figures 6 and 7. But these happened to be the metals that exceeded the safe limit according to FAO/WHO in some vegetables of both categories. In conventional vegetables, the mean values of Pb were as follows: in green pepper, 1.87 µg/g; in tomatoes, 2.75 µg/g; in cucumber, 0.49 µg/g; in spinach, 1.14 µg/g; in cabbage, 0.09 µg/g; in lettuce, 2.75 µg/g; in collard greens, 3.23 µg/g; in apple, 2.66 µg/g; and in leafy greens, 1.16 µg/g as indicated in Table 9.
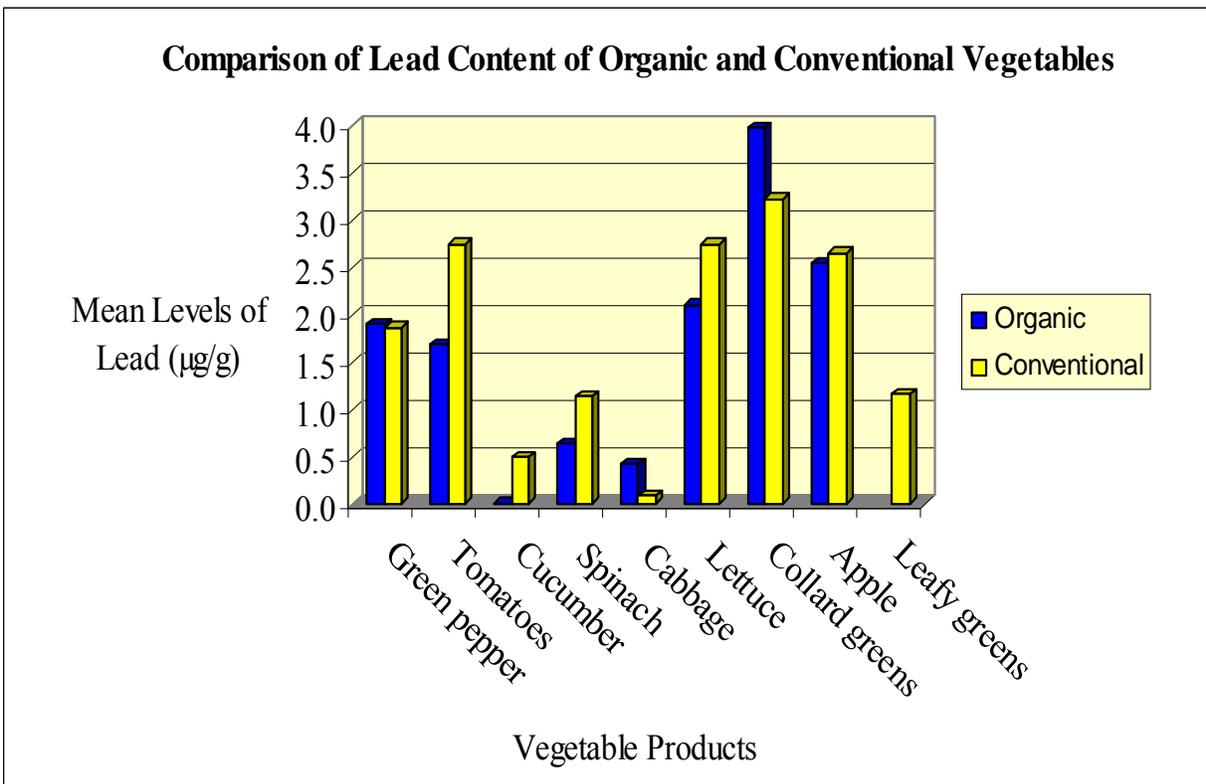

Figure 6. Lead content of organic and conventional vegetables.

The mean values of Pb in organic vegetables were: in green pepper, 1.90 µg/g; in tomatoes, 1.69 µg/g; in spinach, 0.63 µg/g, in cabbage, 0.42 µg/g; in lettuce, 2.11 µg/g; in collard greens, 3.99 µg/g; and in apple, 2.55 µg/g. No detectable amount of Pb was found in organic cucumber.

Table 9. Lead content in both organic and conventional vegetable products.

Samples	n[a]	Mean (µg Pb g^{-1} dry wt ± SD)	Maximum (µg Pb g^{-1})	Median (µg Pb g^{-1})	Minimum (µg Pb g^{-1})	P-value[d] P(T<=t) two tail[e]
Organic Green pepper	3	1.90 ± 0.9	3.36	1.21	1.08	
Conventional Green pepper	3	1.87 ± 0.7	3.09	1.75	0.81	0.941
Organic Tomatoes	3	1.69 ± 0.7	2.82	1.61	0.67	
Conventional Tomatoes	3	2.75 ± 0.7	3.76	2.69	1.61	0.006
Organic Cucumber	3	0.00 ± 0.0	0.00	0.00	0.00	
Conventional Cucumber	3	0.49 ± 0.5	1.20	0.27	0.00	0.010
Organic Spinach	3	0.63 ± 0.8	2.02	0.00	0.00	
Conventional Spinach	3	1.14 ± 0.6	2.02	1.21	0.00	0.162
Organic Cabbage	3	0.42 ± 0.6	1.61	0.13	0.00	
Conventional Cabbage	3	0.09 ± 0.2	0.40	0.00	0.00	0.117
Organic Green Leaf Lettuce	3	2.11 ± 0.8	3.76	1.88	1.08	
Conv. Green Leaf Lettuce	3	2.75 ± 0.7	3.63	2.82	1.88	0.090
Organic Collard Greens	3	3.99 ± 0.6	5.11	3.90	3.36	
Conventional Collard Greens	3	3.23 ± 0.6	3.90	3.23	2.28	0.012
Organic Apple[b]	3	2.55 ± 0.5	3.23	2.69	1.75	
Conventional Apple[b]	3	2.66 ± 0.6	3.49	2.69	1.61	0.673
Leafy Green Vegetable[c]	1	1.16 ± 0.9	2.15	0.94	0.40	-

[a] Number of different samples.
[b] Fruit sample.
[c] Random sample.
[d] ANOVA: single factor @ p = 0.05 level of significance.
[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

The range of Pb in the vegetables was 0.00-3.99 µg/g. Organic collard greens vegetables recorded the highest amount of 3.99 µg/g. Statistical comparison of the vegetables showed significant difference in both categories of vegetables for tomatoes, cucumber, lettuce, and collard greens ($p < 0.05$). The safe limit according to FAO/WHO (2001) for Pb is 0.3 µg/g. The results showed that only organic cucumber and conventional cabbage have Pb below the safe limit.

Cadmium Determination

The amount of cadmium found in this study was small in all vegetables. The amount of cadmium range from 0.00-0.80 µg/g. Green pepper, tomatoes, and lettuce all showed no statistical significant differences in organic and conventional vegetables ($p > 0.05$) but spinach ($p < 0.05$) did. Amounts of Cd found in conventional vegetables were; in green pepper, 0.29 µg/g; in tomatoes, 0.04 µg/g; in spinach, 0.74 µg/g; in lettuce, 0.17 µg/g; and in leafy greens, 0.48 µg/g. The amounts of Cd found in organic vegetables were: in green pepper, 0.24 µg/g; in tomatoes, 0.05 µg/g; and in lettuce, 0.39 µg/g. These values are indicated in Table 10. The highest amount of Cd, 0.74 µg/g, was found in conventional spinach.

Conventional vegetables (cucumber, cabbage, collard greens), organic vegetables (cucumber, spinach, cabbage, collard greens) and apple were all found to contain < 0.01 µg/g of cadmium as shown in Figure 7. These were below the safe limit for cadmium of 0.2 µg/g according to FAO/WHO. Conventional vegetables (green pepper, spinach, leafy greens) and organic lettuce however exceeded the FAO/WHO (2001) safe limit.

Related studies in literature regarding Pb and Cd gave varied results. Farooq et al. in 2008 [207] investigated Pb, Cu, Cr, Zn, and Cd in different vegetables (including spinach, lettuce, cabbage) grown in the vicinity of an industrial area found that Pb gave the highest and Cd the least concentration of the metals analyzed. Their study also showed that cadmium contents in all vegetables fell below the safe limit for FAO/WHO (2001) while Pb contents in the vegetables exceeded safe limits.

Table 10. Cadmium content in both organic and conventional vegetable products.

Samples	n^a	Mean (µg Cd g^{-1} dry wt ± SD)	Maximum (µg Cd g^{-1})	Median (µg Cd g^{-1})	Minimum (µg Cd g^{-1})	P-value[d] P(T<=t) two tail[e].
Organic Green pepper	3	0.24 ± 0.2	0.55	0.15	0.00	0.544
Conventional Green pepper	3	0.29 ± 0.2	0.51	0.34	0.05	
Organic Tomatoes	3	0.05 ± 0.1	0.22	0.00	0.00	0.750
Conventional Tomatoes	3	0.04 ± 0.1	0.19	0.00	0.00	
Organic Cucumber	3	0.00 ± 0.0	0.00	0.00	0.00	-
Conventional Cucumber	3	0.00 ± 0.0	0.00	0.00	0.00	
Organic Spinach	3	0.00 ± 0.0	0.00	0.00	0.00	0.012
Conventional Spinach	3	0.74 ± 0.7	1.91	0.43	0.00	
Organic Cabbage	3	0.00 ± 0.0	0.00	0.00	0.00	-
Conventional Cabbage	3	0.00 ± 0.0	0.00	0.00	0.00	
Organic Green Leaf Lettuce	3	0.39 ± 0.7	1.86	0.00	0.00	0.363
Conv. Green Leaf Lettuce	3	0.17 ± 0.2	0.62	0.00	0.00	
Organic Collard Greens	3	0.00 ± 0.0	0.00	0.00	0.00	-
Conventional Collard Greens	3	0.00 ± 0.0	0.00	0.00	0.00	
Organic Apple[b]	3	0.00 ± 0.0	0.00	0.00	0.00	-
Conventional Apple[b]	3	0.00 ± 0.0	0.00	0.00	0.00	
Leafy Green Vegetable[c]	1	0.48 ± 0.2	0.60	0.56	0.27	

[a] Number of different samples.
[b] Fruit sample.
[c] Random sample.
[d] ANOVA: single factor @ p = 0.05 level of significance.
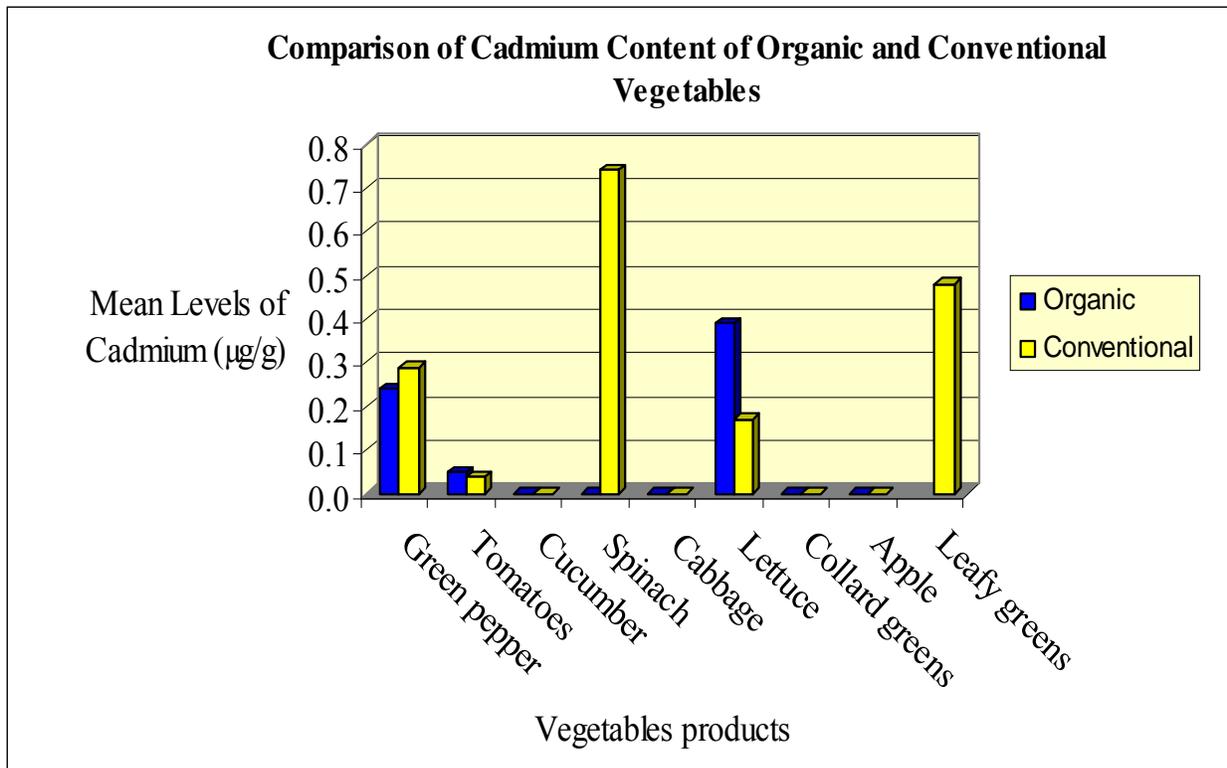[e] t-test: Two-Sample Assuming Equal Variances @ p = 0.05 level of significance.

Figure 7. Cadmium content in organic and conventional vegetables.

Mohsen and Mohsen [202] also examined different metals (including Cd and Pb) in vegetables irrigated with waste water and found 0.20 µg/g Cd in spinach and 0.01 ug/g in tomatoes. The mean values of lead found were: 2.57 µg/g in spinach, 1.56 µg/g in green pepper, and 1.94 µg/g in tomatoes. They further stated that Pb concentration in all vegetables were above the permitted level. Giordano and Mays [208] also found Cd to be more prevalent in leafy vegetables and that the highest amounts were found in lettuce, spinach, and radish. Aiwonegbe and Ikhuoria [205] reported similar values of Cd in cabbage and spinach. They however found no Cd in cabbage but showed spinach to contained 0.12 µg/g.

The variation in magnitude of the six metals analyzed in all vegetables decreases in the following order: Fe > Ni > Zn > Cu > Pb > Cd as shown in Figure 9 and Figure 11. This study shows that among the organic and conventional samples leafy vegetables contained the highest levels of metals. The organic vegetables with the highest abundance of the selected heavy metals were spinach and lettuce as shown in Figure 8. For conventional vegetables leafy greens, spinach, and lettuce gave the highest abundance as shown in Figure 10.
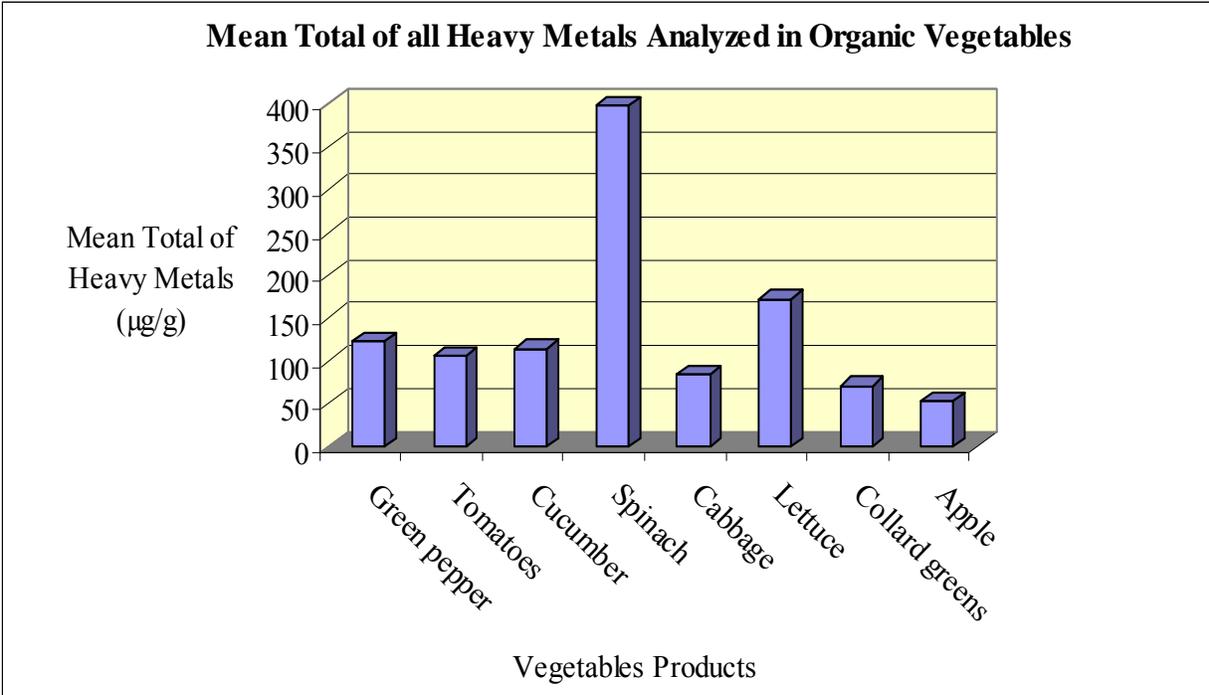
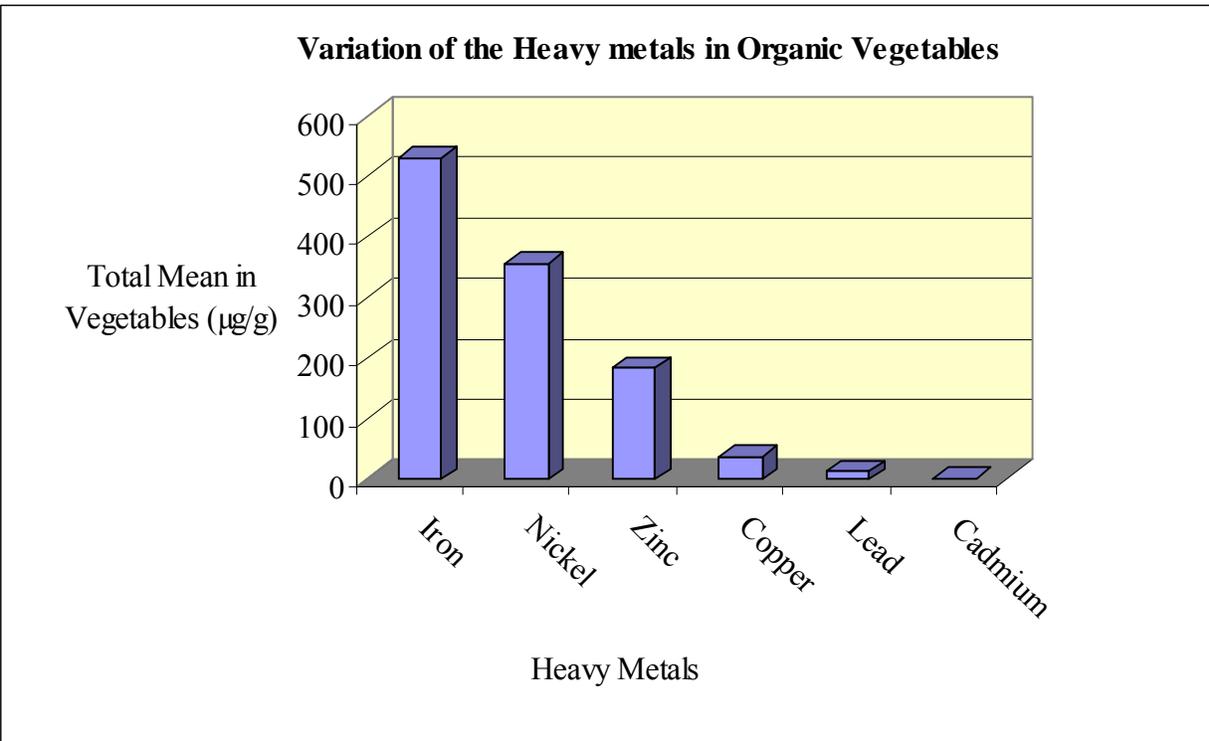Figure 8. The abundance of heavy metals in the various organic vegetables.

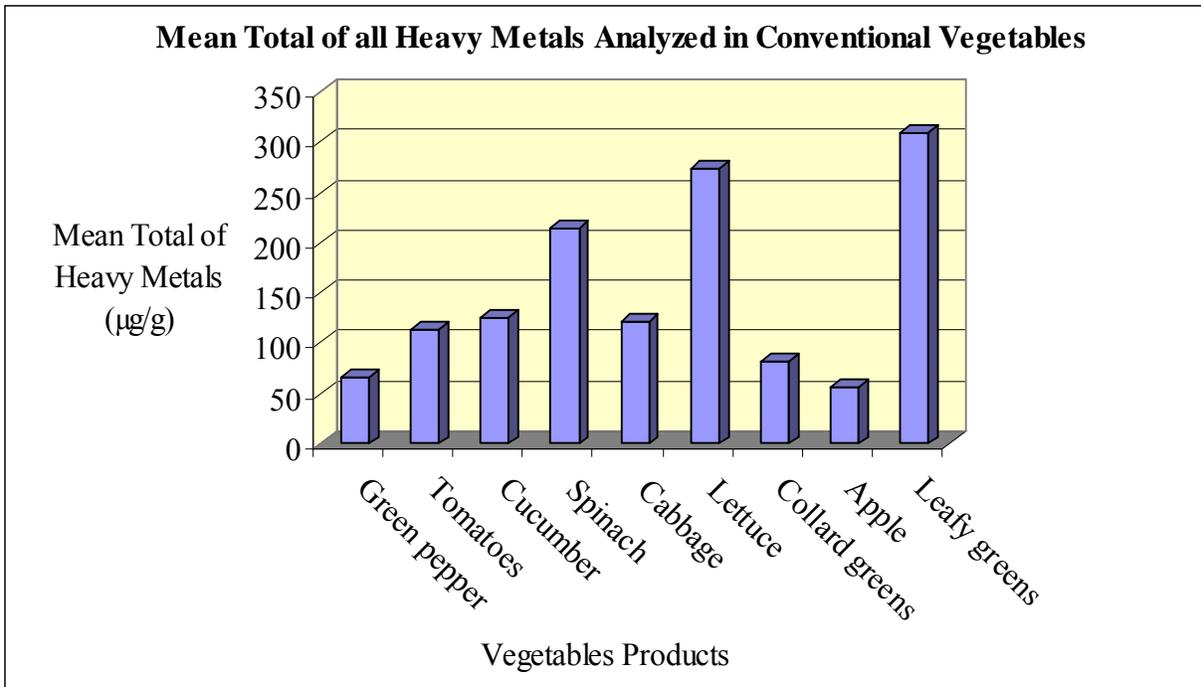Figure 9. The variation of the respective metals in organic vegetables.

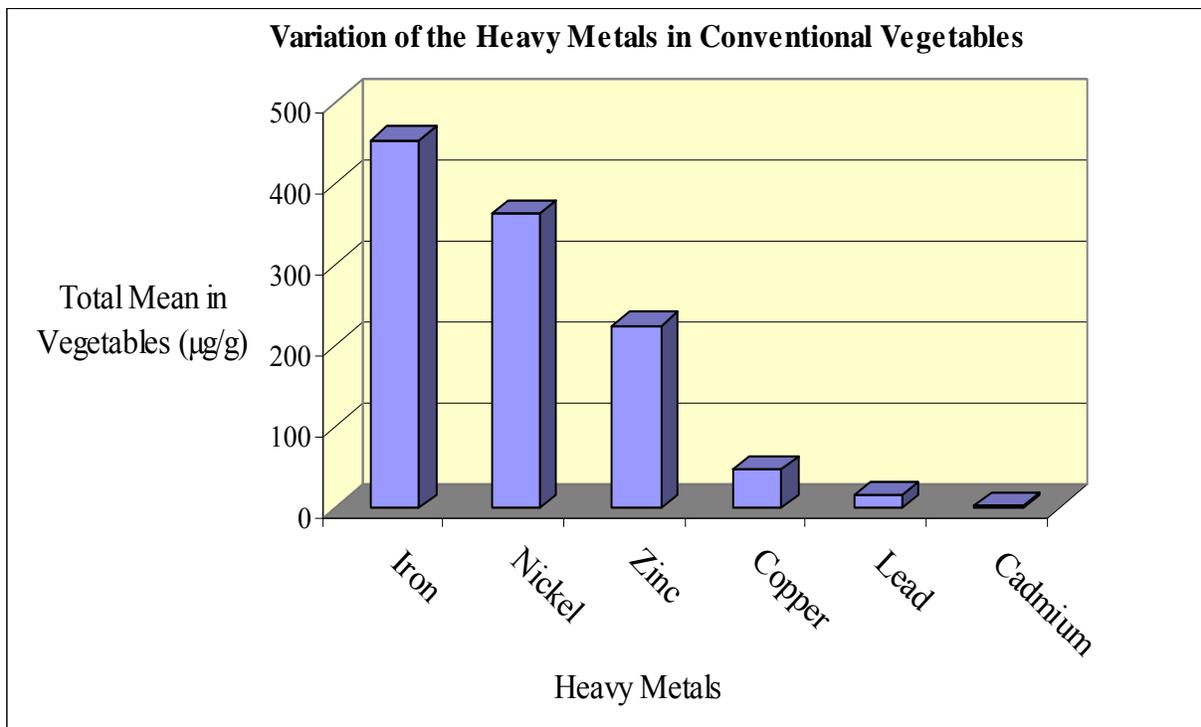Figure 10. The abundance of heavy metals in the various conventional vegetables.

Figure 11. The variation of the respective metals in conventional vegetables.

The most prevalent metal in the leafy vegetables was iron. Apple however was the sample with the least amount of metals. The study shows that heavy metals like Fe, Ni, Zn, and Cu were all within the safe limits proposed by the joint FAO/WHO expert committee on food additives and contaminants for both categories of vegetables while for some vegetables of both categories the proposed safe limits were exceeded for Pb and Cd as recommended by FAO/WHO (2001). The permissible limit in vegetables, 0.3 and 0.2 µg/g respectively, for Pb and Cd is an indicator that they occur in small amounts in food stuffs but can be toxic beyond the stated limits. Fe is also taken in and accumulated selectively by some vegetables such as spinach, while leaves accumulate metals as a result of air pollution. Mahdavian and Somashekar [206] investigated several metals including Pb and Cd in fruits and found that the abundance of the metals in the fresh fruits samples were in the order as follows: Fe > Cr > Mn > Pb > Ni > Co > Zn > Cu > Cd. Iron was the highest and Cd was the least of the metals detected in their studies. It can also be inferred from the ranking that Ni > Zn > Cu is consistent with the present study of these metals in organic and conventional vegetables. However, the amount of Pb reported was higher than the current study. They attributed the above order to the pollution of urban environment, contaminated food transport and supply chains, poor market sanitary conditions, and the use of contaminated or waste water for irrigation.

The relatively high metal levels in all the vegetables samples in this study may partly be attributed to the fact that the vegetables were not washed. This was done to obtain some information on the condition of vegetables as displayed in the market. Not washing the vegetables has the tendency of elevating the metal content of vegetables as demonstrated by Yusuf and Oluwole [197]. In their study of heavy metals (Cu, Zn, Pb) contamination of five leafy vegetables species of *Talinum triangulare*, *Celosia argentea*, *Amaranthus viridis*, *Cucurbita maxima*, and *Corchorus olitorius*, they found that both washed and unwashed vegetables showed high metal levels in the urban city, with the unwashed vegetables recording the highest amounts in statistical comparison with the washed ones ($p < 0.001$). Their study also indicated that the levels of Cu and Zn were not high enough in these vegetables as to pose serious health problems to consumers. Atmospheric deposition of metals might be the reason for the elevated metal content of vegetables. Atmospheric deposition happens to be one of the factors that account for the uptake and bioaccumulation of heavy metals in vegetables.

Also recent studies by Sharma et al. [144] were accomplished in which the heavy metals (Cu, Zn, Cd, and Pb) were studied for their role in the contamination of vegetables in Varanasi, India. They found that the results of washing revealed atmospheric deposition to have contributed to increased levels of heavy metals in vegetables. Their study concluded that atmospheric deposition can elevate the levels of heavy metals in vegetables during marketing and could pose a potential health hazard to consumers. Larsen et al. [142] also carried out field studies of heavy metals and found that there was a positive correlation between atmospheric metal deposition and concentration of heavy metals in plants and top soil. Washing of vegetables is thus very important in that it reduces metal concentrations due to aerial contamination in vegetables bought from stores.

Because this study is a market-oriented supply study, the study does not have information on the conditions under which the vegetables were produced. But from literature review it is known that other factors such as climate, concentration of heavy metals in the soil, the nature of soil on which the vegetables are grown, the degree of maturity, as well as human activities including addition of manures, sewage sludge, fertilizers, and pesticides can influence the physico-chemical parameters of the soil such as pH, organic matter and bioavailability of the soil, thereby affecting the uptake and bioaccumulation of these metals found in vegetables.

Due to the nature of this study and the results obtained, it is impossible to suggest whether organic leafy vegetables are less or more safe than their conventional counterparts. Even though in general more kinds of metals and amounts were found in conventional vegetables than organic vegetables, there were cases where the metal levels in organic leafy vegetables exceeded those of the conventional ones. For instance, it was found that the following organic vegetables: tomatoes of 27.5 µg/g, and lettuce of 27.8 µg/g, for zinc; tomatoes of 9.90 µg/g and lettuce of 10.5 µg/g, for copper; green pepper of 38.9 µg/g and spinach of 319 µg for iron; cucumber of 50.6 µg/g and lettuce of 42.8 µg/g for nickel; green pepper of 1.90 µg/g, cabbage of 0.42 µg/g and collard greens of 3.99 µg/g for lead; and tomatoes of 0.05 µg/g and lettuce of 0.39 µg/g, for cadmium; all exceeded the amounts found in their respective conventional vegetables.

There were no significant differences statistically ($p > 0.05$) for both categories of vegetables in tomatoes for zinc; lettuce for nickel; green pepper and cabbage for lead; and in tomatoes and lettuce for cadmium. However, the following vegetables did show statistically significant difference ($p < 0.05$): in lettuce for zinc; in tomatoes and lettuce for copper; in green pepper and spinach for iron; in cucumber for nickel; and in collard greens for lead. Related studies reported in the literature also showed similar trends for heavy metals analyzed in organic and conventional vegetables. Woese et al. [198] in a review deduced that there were no major differences with respect to the levels of heavy metals between vegetables from organic and conventional production. Malmauret et al. [134] studied contaminants including Pb and Cd in organic and conventional foodstuffs in France and concluded that there was no evidence whether conventional products are more or less safe than organic ones. In a comparative study of cadmium and lead in organically produced foodstuffs in Greece, Karavoltsos et al. [128] concluded in their studies that organic agriculture does not necessarily reduce cadmium and lead in organic products. These conclusions are in line with this study.

Conclusions

From the results of the present study and within the limit of experimental errors, the concentration ranges of the selected heavy metals were as follows: zinc, 2.04-69.4; copper, 0.35-15.1; lead, 0.00-3.99; cadmium, 0.00-0.74; iron, 2.52-319; and nickel, 39.9-53.8 µg/g.

The conventional vegetables generally contained higher amounts of the metals studied compare to their organic vegetables. However, the observations did not suggest that conventional vegetables were less safe than their organic counterparts because the organic vegetables in some cases indicated higher amounts of metals for some vegetables.

All vegetable products were within the permissible limits for Zn, Cu, Ni, and Fe, while for some vegetables of both categories the proposed safe limits as recommended by FAO/WHO (2001) were exceeded for Pb and Cd.

The varied nature of the metal contents in both categories of vegetables also suggests that the production method of the vegetables to a large extent might not be the only reason for higher metal content of the vegetables. Other factors such as atmospheric deposition, post-harvest handling and transport, market sanitary conditions, etc. could influence the concentration of heavy metals in vegetables. Edible vegetables products from the market should therefore be thoroughly washed before consumption because studies indicate much difference in metal content between washed and unwashed vegetables.

From this study, it was found that iron was the most prevalent metal in all the vegetables while cadmium was the least. For the conventional vegetables, spinach, lettuce, and leafy greens were found to be the vegetables with the highest metal content, while spinach and lettuce contained the highest in the case of organic vegetables. Leafy vegetables such as spinach, lettuce, cabbage, leafy greens, and collard greens were higher in metal contents than non-leafy vegetables tomatoes, green pepper, and cucumber.

Because the metal content in vegetables is accounted for by a number of factors including interferences the method of analysis by calibration curve may not give as accurate value of concentration of the metals in the vegetable samples. Also, the samples and the standard solutions occur in different matrix environment and therefore differ in viscosity. To determine the validity of the measured data in this study future work would be to carry out standard addition method of analysis to account for the contributions of the unknown effects.

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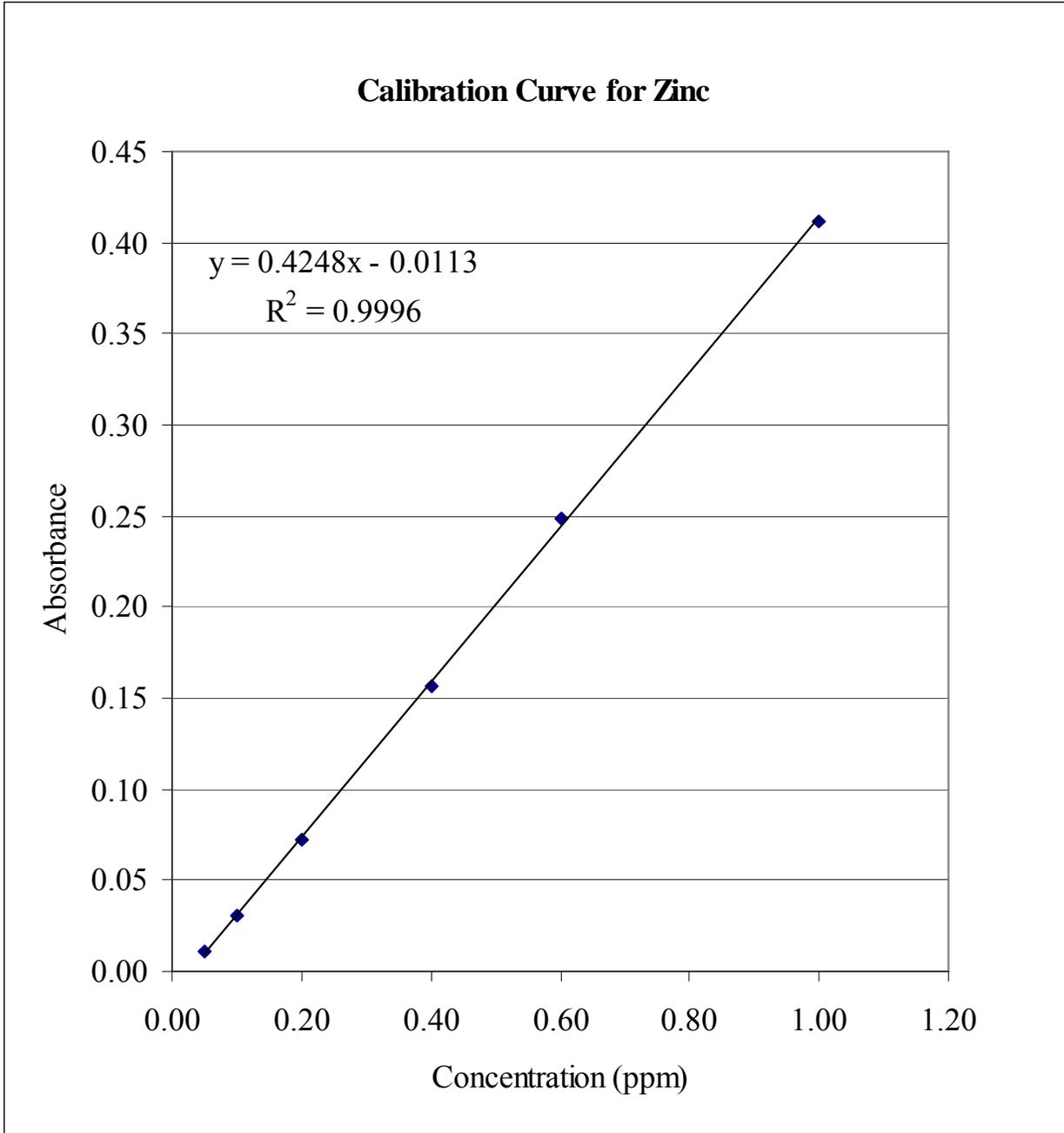
Calibration Curves for Different Metals
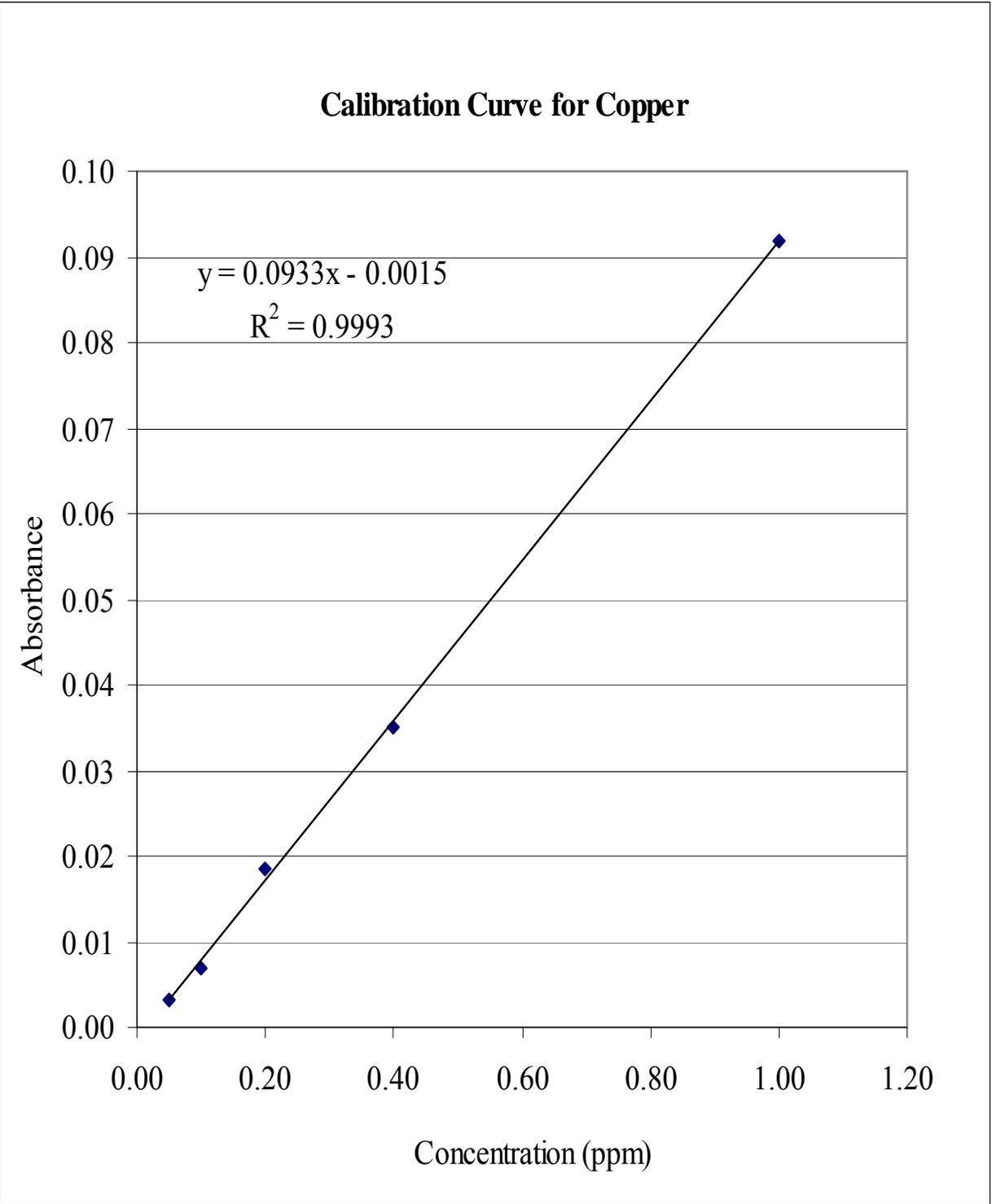

Figure 12. Calibration curve for zinc.

Calibration Curve for Copper

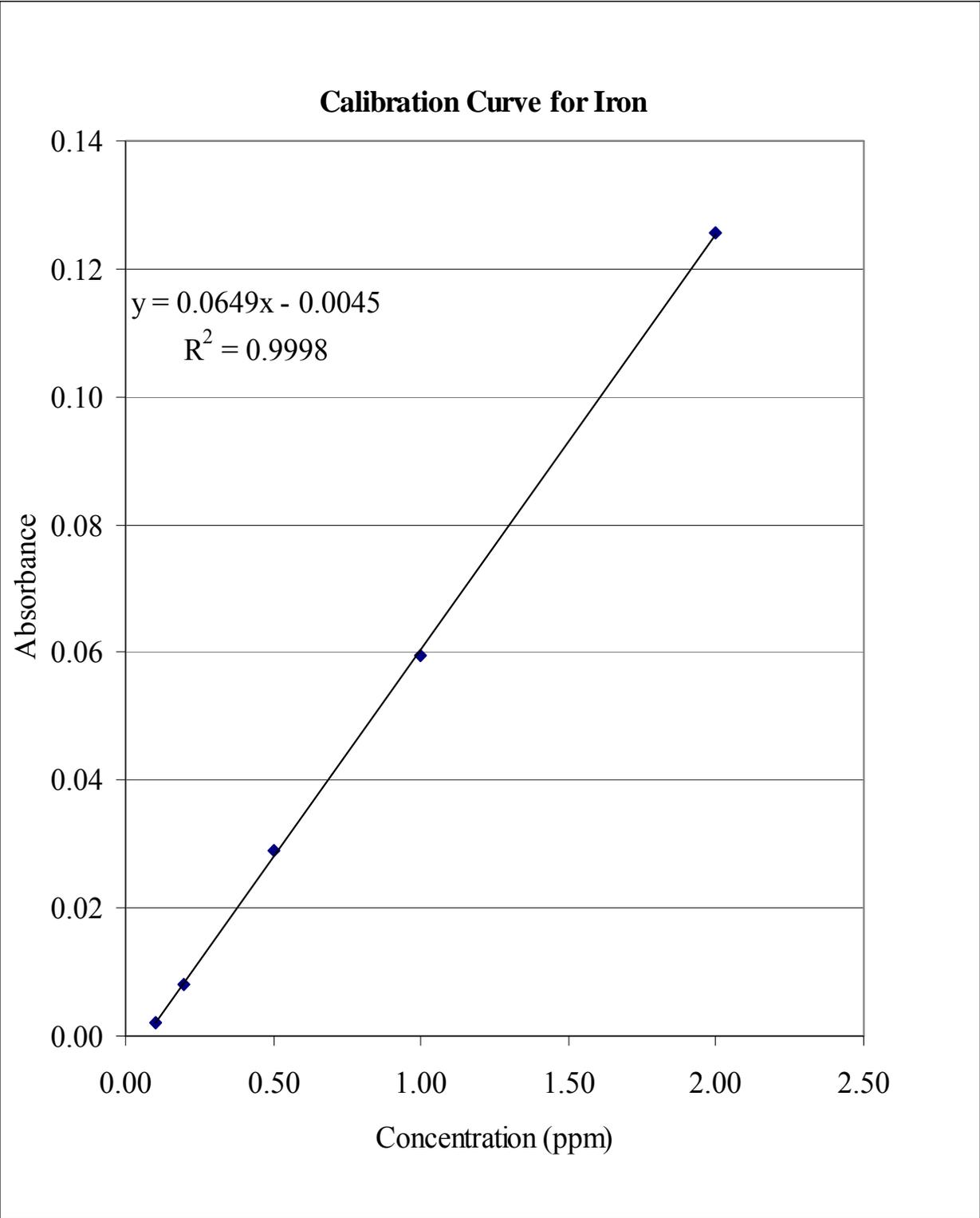$y = 0.0933x - 0.0015$

$R^2 = 0.9993$

Absorbance

Concentration (ppm)

Figure 13. Calibration curve for copper

Figure 14. Calibration curve for iron
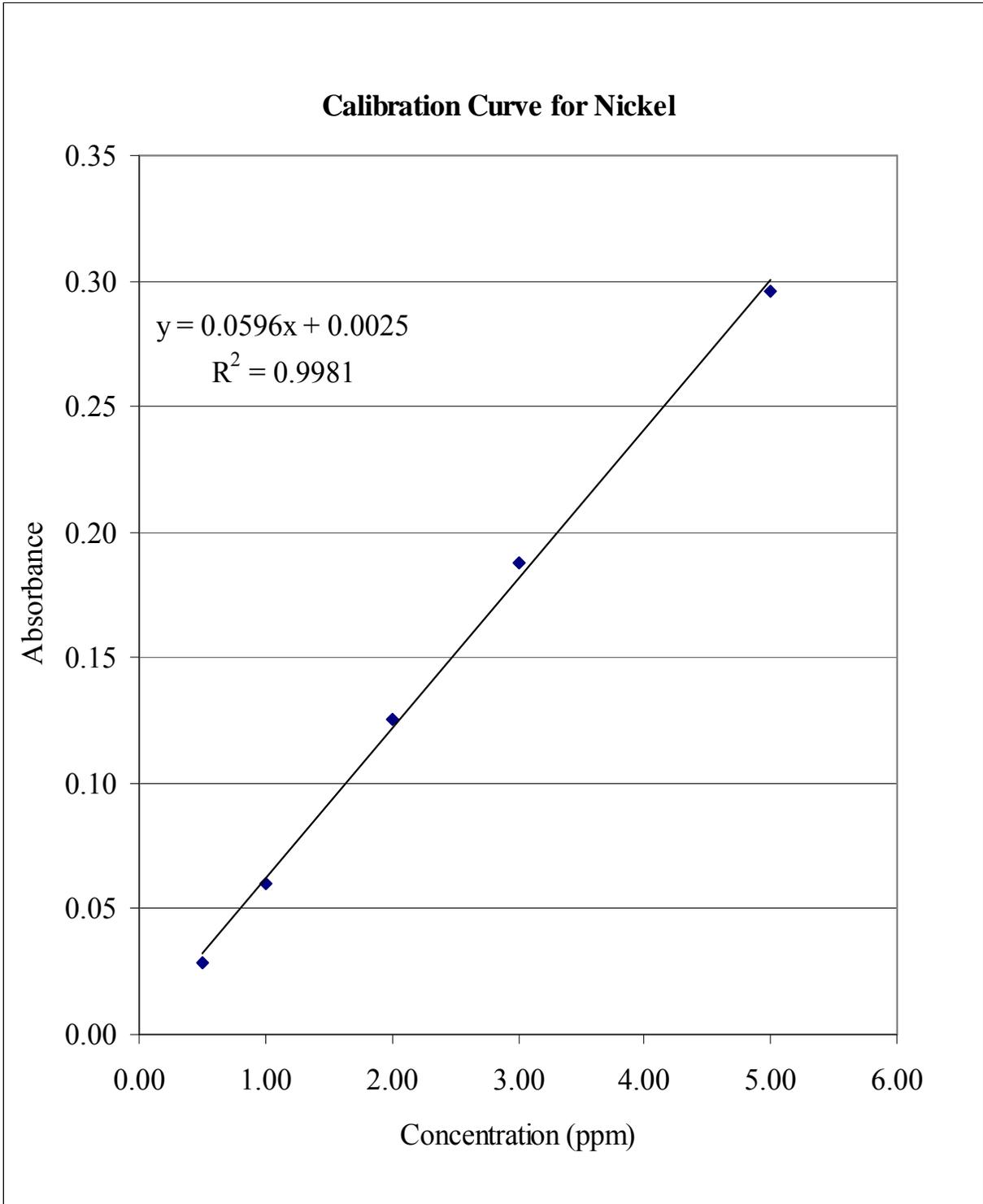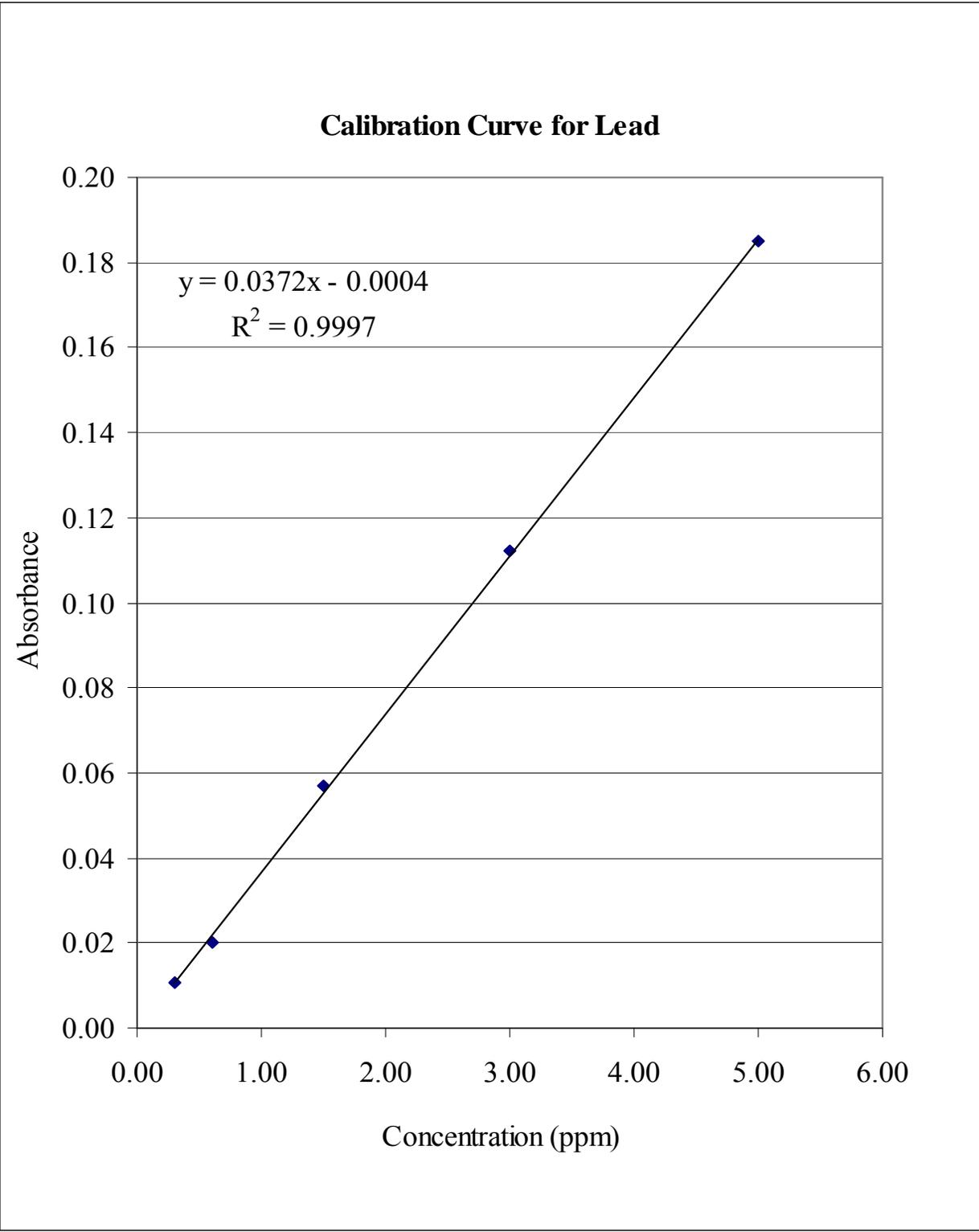
Figure 15. Calibration curve for nickel
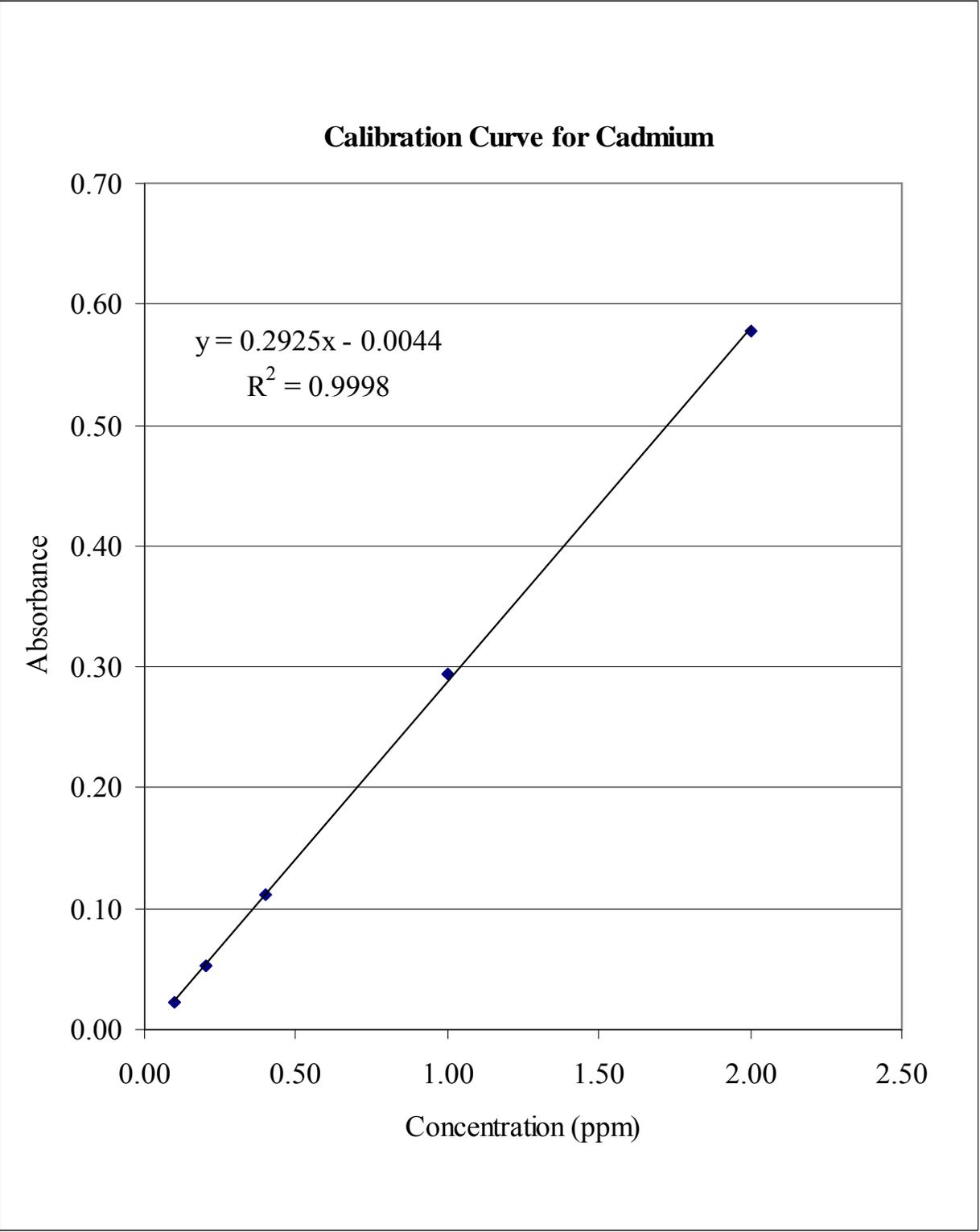
Figure 16. Calibration curve for lead.

Figure 17. Calibration curve for cadmium.

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