DETERMINATION OF SOME ANIONS AND HEAVY METALS IN SELECTED VEGETABLES CONSUMED IN DELTA STATE, NIGERIA

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CERTIFICATION

This is to certify that this is the original research work carried out by Iyedoh Blessing Ese under the supervision of Dr (Mrs.) P.O. Agbaire in partial fulfillment of the requirements for the award of Master of Science (M.Sc) degree in Chemistry.

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DEDICATION

This Project work is dedicated to God almighty from whom all blessings flow.

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ABSTRACT

Synthetic nitrogen fertilizers are used by vegetable farmers to enhance growth performance. These fertilizers if used in excess may contain some toxic anions which bioaccumulate along the food chain. Moreover, the uptake of these toxic anions by vegetables is governed by their availability and concentration in the soil. Therefore such vegetables may accumulate these anions in excessive amounts in their leaves; this may ultimately and adversely affect humans and other species that depend on these vegetables for food, hence the need to evaluate the pollutant levels in vegetable samples in such farms. Five vegetable samples; *Talinum* triangulare(Water leaf), *Telfairia occidentalis* (Pumpkin leaf), *Amaranthus hybridus* (Green leaf), *Ocimum grattissimum* (Scent leaf), *Veronia amygdalina* (Bitter leaf) and soil from three farms in Delta state were collected. Concentration levels of some anions (nitrate, nitrite, phosphate, sulphate and chloride) were determined using Hach direct reading 2000 Spectrophotometer. The concentrations of heavy metals (zinc, copper, cadmium, iron, and lead) were determined using Atomic Absorption Spectroscopy (AAS). The results obtained from the analysis revealed that cadmium (Cd) and iron (Fe) showed the highest concentrations while copper (Cu) and lead (Pb) showed the lowest levels in the whole vegetable samples studied. The concentrations of the anions ranged between 75.00-450 µg/g nitrate, 17.00-175.00 µg/g nitrite, 96.00-380 µg/g phosphate, 94.00-282.41 µg/g sulphate and 36.30-105 µg/g chloride. *O. grattissimum* (Scent leaf) contained the highest concentration of anions when compared to the other vegetables. The concentrations of the anions were within the permissible limits set by European Commissions Scientific Committee for Food (ECSCF) and the FAO/WHO Expert Committee on Food Additives while the heavy metal concentration obtain were higher than the set limits. Thus, the high values of these metals in the samples could put the consumers of these vegetables at health risk.

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CHAPTER ONE- INTRODUCTION

1.1 Vegetables

Vegetables may be defined as any plant with edible parts especially leafy or fleshy parts that are used mainly for soups or salads or eaten as part of a main meal (Vaino *et al.,* 2003) .Vegetables can be eaten either raw or cooked and they play very vital roles in human nutrition, they are mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber. A lot of vegetables have low calorie concentration but are bulky and filling (Terry 2011). They are good sources of dietary fibre, essential vitamins, minerals, vital trace elements and important antioxidants. Vegetables are generally accepted as food worldwide. Some are staple foods but most are accessory food stuffs, adding variety to meals with their unique flavor and at the same time, adding nutrients necessary for good health.

When vegetables are regularly included in diets, there is a reduction in incidences of chronic health challenges such as cancer, stroke and cardiovascular diseases (Gruda, 2005). Research has shown that individuals that eat more than five servings of vegetables per day have an approximately 20% lower risk of developing coronary heart disease or stroke , unlike those who eat less than three servings of fruits and vegetables per day (Terry, 2011).

Vegetables make up an important part of the human diet because they contain carbohydrates, proteins, fats, as well as vitamins, minerals and bioactive compounds (Mepha *et al.,* 2007).in this era of the green revolution; consumers demand for richer green leafy vegetables is rapidly increasing among the urban community (Itanna, 2002). This is due to increased awareness from researches that have exposed their nutritive and herbal (medicinal) values. The practice of growing vegetables especially those ones that have beneficial medicinal uses all year round is rapidly increasing among the urban communities. The practice may not be safe and sustainable in long term if abused especially with the use of inorganic fertilizers. These vegetables are harvested at all stages of growth and fed fresh to livestock while they are consumed by man either as processed, semi-processed or raw (Aja *et al.,* 2007). The perception of what is accepted as a better quality is however subjective. Some consumers consider colour, size and shape as characteristics of good quality leafy vegetables. However, physical attributes of leafy vegetables are not enough to guarantee that they are free of toxic metals and other dangerous contaminants.

The consumption of vegetables as food provides a fast and less expensive means of obtaining adequate vitamin supply, minerals and fiber. Vegetables eaten as food include those used in making soup or served as salads with meals. Each plant species has its nutritive requirements different from others. Thus different plant species grown in the same farm soil will contain varying concentrations of minor and macro elements. Leafy vegetables occupy a very important place in human and live stock diet, but unfortunately constitute a group of foods which contributes maximally to nitrate, nitrite other toxic anions as well as poisonous metal consumption.

1.2 Anions (Nitrate and Nitrite, Sulphate, Phosphate and Chloride)

Nitrate and Nitrite: Nitrate is a polyatomic ion with the molecular formula NO_3^- and a molecular mass of 62.005 g/mol. The nitrate anion is the conjugate base of nitric acid, it is composed of one central nitrogen atom surrounded by three identically bonded oxygen atoms in a trigonal planar arrangement.

The nitrite ion, which has the chemical formula NO_2^-, is a symmetric anion with equal N–O bond lengths and an O–N–O bond angle of approximately 120°.

The nitrite ion has an asymmetrical structure with both N–O bonds having equal length. In valence bond theory, it is described as a resonance hybrid with unequal contributions from two canonical forms that are reverse images of each other. In molecular orbital theory, there is a sigma bond between each oxygen atom and the nitrogen atom, and a delocalized pi bond made from the p orbital's on nitrogen and oxygen atoms which is perpendicular to the plane of the molecule. Its negative charge cloud is uniformly spread on the two oxygen atoms. The nitrogen and oxygen atoms both have a lone pair of electrons. Therefore, the nitrite ion is a Lewis base. Moreover, it can behave as an ambidentate ligand towards a metal ion, contributing a pair of electrons from either nitrogen or oxygen atoms

The nitrate ion has a formal charge of -1. Almost all inorganic nitrate salts can dissolve in water at standard temperature and pressure. Green leafy vegetables are a rich source of nitrites and nitrate to humans and animals. Nitrate salts, particularly nitratine which is a major source of sodium nitrate occur naturally on earth as large deposits. They are also found in man-made fertilizers. Nitrates are the main ingredients used as fertilizers in agriculture because they are highly soluble and biodegradable. Nitrate toxicosis occurs through enterohepatic degradation of nitrate to nitrite (Addiscott *et al*., 2008). Nitrites oxidize the iron atoms in hemoglobin from ferrous iron (2+) to ferric iron (3+), making it unable to carry oxygen (kim *et al.,* 2005). This process can lead to a general lack of oxygen in organ tissue and a dangerous condition called methaemoglobinaemia. Although nitrite converts to ammonia, if there is more nitrite than can be converted, the animal slowly suffers from a lack of oxygen (Stoltnow *et al.*, 2008).

Some major symptoms of nitrate poisoning are increased heart rate and respiration; in severe cases, blood and tissue may turn a blue or brown color. Humans are susceptible to nitrate toxicity, with infants being especially vulnerable to methaemoglobinaemia, this is because nitrate metabolizing triglycerides are present at higher concentrations at infancy than at other stages of development. Methaemoglobinaemia in infants is known as blue baby syndrome. Some adults can be more prone to suffer from the effects of nitrate poisoning than others. This is because methemoglobin reductase enzyme may be under-produced or absent in some humans that have an inherited mutation condition (Avery 1999).Such individuals cannot break down methemoglobin as rapidly as those that do have the enzyme, leading to increased circulating levels of methemoglobin in their system and as a result their blood is not as oxygen-rich. Nitrate exposure may happen as a result of consuming vegetables with high levels of nitrate. Unregulated use of nitrate fertilizers during cultivation contributes to elevated levels of nitrate in the harvested vegetables (Marschner, 1999).

Phosphate, Sulphate and Chloride: The phosphate ion is a polyatomic ion with the empirical formula PO_4^{3-}. It has a molar mass of 94.97 g/mol and is composed of one central phosphorus atom surrounded by four oxygen atoms in a tetrahedral pattern. The phosphate ion bears a negative-three formal charge and is the conjugate base of the hydrogen phosphate ion. Phosphorus exists naturally as phosphate rocks or deposits. These deposits can contain significant levels of naturally occurring toxic heavy metals. Mining operations on phosphate rocks can yield tailings or piles containing elevated levels of cadmium, lead, chromium, nickel, copper and uranium. These waste by products can find their way into nearby water bodies if care is not taken to properly manage them. When these substances are absorbed by plants or ingested by marine life it can result in food products having elevated concentrations of toxic heavy metals (Gnandil *et al.,* 2006).

The sulphate ion is a polyatomic anion, it has the empirical formula SO_4^2. Sulfates exist widely in everyday life as salts of sulfuric acid and many are prepared from that acid.

The anion consists of a central sulfur atom surrounded by four equivalent oxygen atoms in a tetrahedral shape. The sulfur atom is in the +6 oxidation state while the four oxygen atoms are each in the −2 state. The sulfate ion bears a negative two charge, it is the conjugate base of the bisulfate (or hydrogen sulfate) ion, HSO_4^-, which in turn is the conjugate base of H_2SO_4, sulfuric acid.

The chloride anion is formed when chlorine which is a halogen gains an electron or when a compound such as hydrogen chloride is dissolved in water or any other polar solvent. Chloride salts such as sodium chloride are highly soluble in water (Green *et al.,* 2001). It is an vital electrolyte found in all body fluids and is responsible for maintaining and regulating acid/base equilibrium, transmitting nerve impulses and regulating fluid in cells. The word *chloride* is employed in the naming of chemical compounds in which one or more chlorine atoms are covalently bonded (Green *et al.,* 2001).

1.3 Heavy Metals

The term "heavy metal" is employed to describe metallic chemical elements having relatively high density and are toxic or poisonous at very low concentrations especially in environmental contexts. Toxic heavy metals exist naturally in the earth's surface and become concentrated over time as a result human activities. Some common heavy metals include lead, zinc, cadmium, chromium, copper, iron e.t.c. however, some elements regarded as toxic heavy metals are essential in small quantities for human health and development.

Most recently, the term "heavy metal" has been used as a general term for those metals and semimetals with potential human, animal or environmental toxicity (Tchounwou *et al.*, 2012).

Regardless of how one decides to define the category, heavy metal toxicity is an uncommon diagnosis. Emergency physicians will rarely be alerted to the possibility of metal exposure unless it involves exceptional cases of intentional or unintentional ingestion and suspected lead exposure and acute iron toxicity. Never the less, if inappropriately identified, diagnosed and treated, heavy metal exposure can lead to significant morbidity and mortality.

Many of the elements that can be regarded as heavy metals have no known documented benefit for human physiology. Mercury, lead and cadmium are very important examples of such "toxic metals." Yet, other metals are vital for human biochemical processes. For example, zinc is an necessary cofactor for a number of enzymatic reactions in the human body system, vitamin B-12 has a cobalt atom at its core, and one important element in hemoglobin is iron. Likewise, copper, manganese, selenium, chromium, and molybdenum are all trace elements and are important in the human diet (Saunders *et al.,* 2013)

Heavy metal toxicity depends on a number of factors also specific symptomatology varies depending on the metal in question, the dosage absorbed, and the degree of exposure either acute or chronic. The age of the person can also influence toxicity. Young children for example are more prone to suffer from the effects of lead exposure because they absorb the metal easily compared to adults and because their brains are not fully developed and even brief exposures may influence their developmental processes (Rathnayake *et al.,* 2013)

Exposure to metal poisoning may occur through the diet, from medications, from the environment, or in the course of work or play. Where heavy metal toxicity or poisoning is suspected, time taken to perform a thorough dietary investigation, occupational, and recreational history review is time well spent, because identification, separation and removal of the source of exposure is usually the most effective therapy required for a cure.

Metals may be contaminants in dietary supplements, may be absorbed into plants from contaminated soils or they may leech into food and drink stored in metal containers like lead decanters.

Lead: Lead, symbol Pb, is a dense, bluish-gray metallic element that was one of the first known metals. The atomic number of lead is 82; the element is in group 14 of the periodic table. If lead is taken or absorbed in any of its forms is highly toxic; the effects and symptoms of lead poisoning usually become noticeable after it has accumulated in the body over a period of time. Some symptoms of lead poisoning are anemia, body weakness, digestive constipation, colic, palsy, and often paralysis of the wrists and ankles. Flaking lead-based paints and toys that are produced from lead compounds are considered as serious health hazards for children. Children are especially susceptible to hazard from lead, even at levels once thought safe. Lead can hinder intelligence, delay motor skills development, impair memory, and cause hearing problems and troubles in balance. In adults, one prominent lead hazard at levels once thought safe is increased blood pressure.

Cadmium: Cadmium, symbol Cd is a silvery-white metallic element that can be readily shaped into any form. The atomic number of cadmium is 48; it is transition elements in group twelve of the periodic table.

Cadmium and solutions of its compounds are highly poisonous, with cumulative effects and symptoms similar to those of mercury poisoning. Some major effects of cadmium poisoning include lung cancer, osteomalacia and pneumonitis.

Iron: The principal ore of iron is hematite. Pure iron has a melting point of about 1538 °C (about 2800 F), and a boiling point of about 2861 °C (5182 F), and has a specific gravity of 7.87 and an atomic weight of 55.845. It is soft, malleable, and ductile. Iron is easily adopt magnetic properties at ordinary temperatures, it is difficult to magnetize when heated, and at about 790 °C (about 1450 F) the magnetic property disappears. Iron is a very active metal. It combines easily with the halogens (fluorine, chlorine, bromine, iodine, and astatine), sulfur, phosphorus, carbon, and silicon. It can displace hydrogen from most dilute acids. It burns in oxygen to form ferroso ferric oxide, Fe_3O_4. When exposed to moist air, iron becomes corroded, forming a reddish-brown, flaky, hydrated ferric oxide commonly referred to as rust. Rust formation is an electrochemical process in which the impurities present in iron form an electrical "couple" with the iron metal. It is found in meteorites, usually alloyed with nickel. In chemical compounds the metal is widely distributed and is the fourth most abundant among all the elements in earth's crust; next to aluminum it is the most abundant of all metals**.**

Zinc: Pure zinc is a crystalline metal, it cannot dissolve in hot and cold water but is soluble in alcohol, acids, and alkalis. It is extremely brittle at ordinary temperatures, but can be drawn into wires between 120 °C and 150 °C (248F and 302F) and may be rolled into sheets between heated rollers. Zinc is relatively stable in dry air; in moist air it is oxidized and becomes coated with a carbonate film that protects it from further corrosion. Zinc melts at about 420 °C (about 788 F), boils at about 907 °C (about 1665 F), and has a specific gravity of 7.14. The atomic weight of zinc is 65.39.

Copper: Copper melts at about 1085 °C (1985 F), it has a boiling point of about 2562 °C (about 4644 F), and has a specific gravity of 8.95. Copper has an atomic weight of 63.546. Copper is used in a wide range of applications because of its many desirable properties, it is a good conductor of electricity and heat, it has a high resistance to corrosion, it is very malleability and ductility, and has great beauty. Copper is principally used in electrical processes because it has an extremely high conductivity, which is second only to that of silver. Because copper is very ductile, it can be drawn into wires of any diameter from about 0.025 mm (about 0.001 in) upward. The tensile strength of drawn copper wire is about 4200 kg/sq cm. and can be used in outdoor cables, as well as in house wiring, lamp cords, and electrical machinery such as motors, generators, controllers, electromagnets, signaling devices, and communications equipment.

1.4 Statement of Problem

An ever increasing rise in the use of synthetic nitrogen in fertilizers and the resultant increase in nitrate content in vegetables which is totally undesirable has prompted scientists and researchers to monitor nitrate concentrations in vegetables and to ensure that it does not exceed the maximum limits as set by various bodies prominent among which is the European commission regulation (ECR). This research work attempts to show whether or not vegetables eaten in Delta State contain elevated levels of anions (nitrate, nitrite, sulphate, phosphate, chloride) and heavy metals (lead, copper, zinc, iron and cadmium) that may pose serious threat to the health and wellbeing of consumers.

1.5 Aim:

The aim of this work is to determine the concentrations of some anions and heavy metals in five vegetables consumed in Delta State in order to ascertain if they are within the allowable levels.

1.6 Objectives

The objectives of this study are to;

i. determine the presence and concentration of some anions (nitrate, nitrite, phosphate, sulphate, chloride) and heavy metals (lead, copper, iron, zinc and cadmium) in some commonly consumed vegetables and the farm soils from which they were harvested in Delta State.

ii. investigate seasonal variations in the concentration of these anions and heavy metals of interest.

iii. determine the transfer factor between the vegetable properties and the soil properties.

iv. compare results obtained with standard values.

1.7 Significance of the Study

This research work is important because the demand for better, richer and green leafy vegetables is rapidly increasing due to increased awareness on their nutritive and herbal values, to meet up this demand, farmers now practice growing vegetables especially those ones that have beneficial medicinal values all year round with the help of organic fertilizers. This practice may not be safe and sustainable in long term if the application of fertilizers is abused. The excessive application of nitrogen and other inorganic fertilizers to these vegetables can cause high levels of nitrate, nitrite, sulphate, phosphate, chloride other anions as well as heavy metals to accumulate in them, consequently, their consumption by humans and animals can lead to serious health challenges.

1.8 Scope and Delimitation of the Study

The study is restricted to determining the concentrations of anions (nitrate, nitrites, phosphate, sulphate, chloride) and heavy metals (lead, iron, zinc, cadmium and copper) in five vegetables commonly consumed in Delta State and the soils in which they are grown so as to determine the transfer factor and seasonal variations in their concentrations.

CHAPTER TWO- LITERATURE REVIEW

2.0 Anions

Anions are radicals (group of atoms) or atoms that have gained electrons. Since they now possess more electrons than protons, anions bear a negative charge e.g nitrate NO_3^-, nitrite NO_2^-, Sulphate SO_4^{2-}, phosphate PO_4^{3-} and chloride Cl^-.

2.1 Nitrates and Nitrites in Vegetables

Vegetables are the primary source of nitrate, they provide over 85% of the average human dietary intake (Liang *et al.,* 2005). Nitrate is a naturally occurring form of nitrogen and is an integral part of the nitrogen cycle in the environment. In the natural nitrogen cycle, bacteria convert nitrogen to nitrate which is absorbed by plants and incorporated into tissues and organs.

The over application of nitrogen and other inorganic fertilizers and inorganic manures to vegetable farms can cause the vegetables to accumulate high levels of nitrate, nitrite and other anions as well as toxic heavy metals which when consumed by humans and animals can cause serious health challenges. The range of nitrate conversion is 5-7% for average individuals and 20% for individuals with a faster rate of conversion (JECFA, 2002). Nitrate has been listed as a primary culprit leading to a variety of long term health effects. Nitrate may also react with secondary or tertiary amines to yield N-nitroso derivatives (Uhegbu *et al.,* 1995) .Certain N- nitroso compounds have been proven to cause cancers in a wide range of laboratory animals (Zhong *et al*., 2002). That nitrate is a known precursor of toxic and carcinogenic N- nitrosamines has been documented (Bassir *et al.,* 1978; Uhegbu, 1997) and induce cancer in experimental animals (Mirvish, 1995; Sen *et al.,* 1997).

Due to the increased and unregulated application of synthetic nitrogen fertilizers and livestock manure in intensive plant farming, vegetables of today may contain higher concentrations of nitrate and nitrite and other anions than in the past. The concentrations of nitrate in vegetables varies enormously, ranging from 1.0 to 10000µg/gm fresh weight (Raquel *et al.,* 2003).

There are several factors influencing NO_3 absorbtion and accumulation in vegetable tissues, they include light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity , genetics, harvesting time, size of the vegetable unit, storage time and source of nitrogen. Of all the factors investigated, nitrogen fertilization and light intensity have been pointed out as the primary factors that influence nitrate concentration in vegetables. The activity of nitrate reductase enzyme during the day can reduce nitrate. It is therefore advisable to harvest vegetables in the cool of the evening rather than any other time of the day.

The concentration and level of nitrate in vegetables is a function of the type of vegetable, the species, the atmospheric temperature at which it is grown, the sunlight intensity, soil moisture levels and the concentration of natural nitrogen in the soil. The unconventional and unmonitored agronomic practice of applying large amounts of nitrogenous fertilizers to obtain heavier yields and improper and unregulated disposal of human and animal waste may result to nitrate, nitrite and other anions accumulating in food plants (Walker, 1990). The concentration of nitrate in vegetables can vary considerably, reaching sometimes as much as 3-4g/kg fresh weight and these levels could have potential hazardous health impacts (Chung *et al.,* 2003)

Nitrate and nitrite are ubiquitous within environmental, food, industrial and physiological systems. The nitrate anion is an important analyte for solving environmental problems and its detection and quantification is essential for human health.

High nitrate levels in edible vegetables and dangerous levels of nitrate may be produced as a byproduct of microbial activity in the gastrointestinal tract of the consumers of the vegetables (Tanaka *et al.,* 1982). One major chronic effect of nitrate is those resulting from the nitrite formed by its reduction by bacterial enzymes. The most documented effect of nitrite is its tendency to combine with blood haemoglobin (OxyHb) to produce methaemoglobin (metHb) and nitrate.

Several authors have proposed that the risk of developing stomach cancer positively linked with three factors: (1) the nitrate concentration of drinking water,

(2) the urinary excretion and disposal of nitrate and (3) the occurrence of atrophic gastritis. Epidemiological studies have not provided any proof that there is an elevated risk of cancer related to high nitrate intake from other sources than vegetables

A lot of literature has been published in recent times concerning nitrate determination in vegetables using different methods and techniques. This has become necessary and quite important due to the important roles vegetables play in human diet worldwide and the need to ensure that vegetables grown locally or imported into a geographic zone contains as little nitrate and heavy metals as possible so that consumers do not exceed the allowed daily intake (ADI).

Several methods have been researched and reported for the determination of nitrate in plants and biological materials including ion chromatography method (Liang *et al,.* 2005), high performance liquid chromatography, capillary electrophoresis, ion exchange chromatography methods e.t.c.

The principle of spectrophotometric method is based on a diazotization of various aromatic amines of nitrate in acidic medium and on a subsequent coupling of the diazonium ions with N-(1- naphthyl) ethylenediamine. This method has been traditionally used but it is prone to interferences from other species present and time consuming besides, a heavy investment in reagents is necessary for each sample being determined.

Nidal *et al.*, (1999) reported a sensitive and direct spectrophotometric method for the determination of nitrite and nitrate in vegetables using phosphomolybdenum blue complex. This method is based on the reduction of phosphomolybdenum blue complex by the sodium sulphide. Beijaarset *et al.* (1994) reported a determination of nitrate in vegetables by continuous flow procedure and subsequent reaction of nitrate extracted with sulfanilamide and N-1-naphthyethylenediamide to form a reddish azo dye which was determined and estimated colorimetrically @ 530nm. Basset *et al.* (1978) reported a titrimetric method for the nitrate determination which could be highly erroneous if applied to vegetables. Tanaka *et al.* (1982) reported a sensitive and direct spectrophotometric method that can be used for the determination of nitrate in vegetables by making use of 2-sec- butyl phenol. The basic for the method is that 2-sec butylphenol reacts quantitatively with nitrate in acidic solution. Gaya *et al.* (2008) researched and reported a spectrophotometric method used for the determination of nitrates in vegetable plants using phenol. The principle of this method is hinged on the nitration of phenol resulting to the formation of the corresponding sodium salt. The action is quantitative and analogous to that reported by Tanaka *et al.,* (1982).

Cataldo *et al.,* (1975) developed and described a technique for the rapid determination of nitrate-N in plant and soil extracts. The complex formed by nitration of salicylic acid under highly controlled acidic conditions absorbs maximally at 410 nm in basic (pH>12) solutions. Absorbance of the chromophore is directly proportional to the amount of nitrate-N present. Ammonium, nitrite and chloride ions do not interfere

Parviz *et al.,* (2012) worked to determine nitrate concentration in eleven vegetable samples in Varzeghan city, north-western Iran, making comparism of its nitrate concentration using the cataldo method and found out that nitrate concentrations were below the values of others reported in different countries and that the mean concentration of nitrates in all vegetables in autumn were significantly higher than values obtained for spring.

In their study; "nitrate accumulation in vegetables", Maynard *et al.,* (1976) reported that leafy vegetables like spinach, lettuce and celery contained nitrate at significant levels. They observed that plants that develop fruits or storage organs like potato and tomato usually have low concentration of nitrate. Accordingly, they observed that vegetables that do not develop storage organs or fruits have a different pattern of nitrate accumulation wherein nitrate often continues to accumulate as the plants ages. The observations of Anjana *et al.,* (2006) were similar, showing that nitrate concentrations in spinach were higher at later stages of the plant growth. Hunt *et al.,* (1994) stated that nitrite content in vegetables is usually very low compared to nitrate. Stopes *et al.,* (1988) compared nitrate content in organic and conventional produced vegetable samples during winter in Great Britain. They observed that the peak nitrate content was lower in inorganically cultivated vegetables although there was considerable variation.

Uwah *et al.,* (2009) researched the concentration of nitrate and nitrite in soils and some leafy vegetables obtained in Maiduguri, Nigeria. He observed that the results obtained were higher than the published maximum permissible contents of nitrate and nitrite in some vegetables and fruits. Therefore, consumption of these vegetables as food may pose possible health hazards to consumers at the time of the study.

2.2 Anions (phosphate, sulphate and chloride) In Soils and Vegetables.

Salts of various kinds are found in most farm soils and many are essential to plant growth. However, some soils contain excessive concentrations of certain salts, which can be detrimental to plant health. Such soils are referred to as saline soils. High concentrations of such salts as chloride of sodium and boron can be toxic to some plants. Phosphate, sulphates, chlorides, and organic matter have found multiple use in the evolution of the soil medium for crop growth and need to be available and in sufficient quantities for the best crop yield to be obtained therefore, their concentrations and levels in the soil must be monitored on a regularly.

Phosphates, nitrates and sulphates are macronutrients, while chlorides are micronutrient. The total concentration of these ions in the soil is not necessarily a reliable parameter in determining its availability to the plants (Johnson *et al.*, 1997). Phosphorus (P) is an essential macronutrient and, in soils it may exist in many different forms (i.e., solution P, active P and fixed P) (Uzu *et al.*, 1975; Busman *et al.*, 2002) and is thought to be relatively immobile in the soil solution (Brady and Weil, 1999) and this immobility or fixation is related to the presence of aluminum silicates, hydrous oxides of iron and aluminum and alkaline or strongly acidic soil. The concentration of phosphorus in irrigated soil horizons below plough layers tend to reduce gradually although some deviations from this pattern may be observed.

In the soil solution, chlorine exists predominantly as Chloride Ion (Cl^-). It is an essential micronutrient for vegetables. Natural sources of chloride to soils come mainly from rainwater, dust and air pollution. In addition, human activities like irrigation and fertilization, contribute significantly to chloride deposition in the soil. The Cl^- does not form complexes easily and exhibits little affinity in its adsorption to soil components. Thus, Cl^- movement within the soil is largely dependent on the water flow or movement in the area (White *et a.,* 2001).

Sulphur exists as both organic and inorganic forms in soil. Sulphate is the most important form of inorganic sulphur in soils and is the most readily accessible form to plants (Alves *et al.,* 2004). The fate of sulphate in the soil is influenced by number chemical, biological and physical factors. Therefore, the concentration of sulphate in natural farm soils may fluctuate in both space and time depending on the prevailing environmental condition (Schnug *et al.,* 1998; Bloem *et al.*, 2001)

Modern high intensity agricultural practices often lead to loss or reduction in soil fertility and can produce negative long term environmental implications. An increase in contaminant emission may have a substantial implication on local agriculture, because heavy metal and other chemical material may be deposited and even accumulate in agricultural soils through agricultural activities and atmospheric deposition, which could elevate the risk of chemical contamination of the food chains (Li *et al*., 2008).It is therefore important to assess the environmental quality in any farming area.

2.3 Heavy metals in vegetables:

Although the nutrient content of wastes makes them attractive as fertilizers, when untreated wastes are used in plant propagation, consumers risk contacting diseases like cholera and hepatitis or suffer from effects of heavy metal contamination (Drechsel *et al.,* 1999). Heavy metals and non biodegradable materials can accumulate in soils to dangerous concentrations that can affect plant and animal life. Contamination of soils by heavy metals can be caused by a number of factors such as metal enriched parent materials, mining or industrial activities, non point source of metals especially automotive emission and use of metal enriched materials such as chemical fertilizers, farm manures, sewage sludge and waste water irrigation (Fredman *et al.,* 1981). However, soil contamination by heavy metals and toxic elements as a result of parent materials or point sources often occurs on a limited area and is easy to identified and controlled.

Soil is an important environmental component as it plays an important role as a medium for plant growth where it can recycle the nutrient needed by plant. Soils are the major sinks for heavy metals released into the environment by anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation, and their total concentration in soils persists for a long time after their introduction (Wuana *et al.,* 2011). Accumulation of heavy metals in the environment becomes a health threat because of their persistence, bioaccumulation and toxicity to plants, animals and human beings. Vegetables are exposed to heavy metals contamination when polluted soils are used to cultivate them. Heavy metals that are dissolved in soil water and soil particles will be absorbed by plant roots and accumulated in vegetables and plant organs (Aweng *et al*., 1998). Although some heavy metals such as Copper, Zinc, Manganese, and iron are essential in plant nutrition, many of them do not play any important role in the plant physiology. The uptake of these heavy metals by plants especially leafy vegetables is an avenue of their access into the human food chain with hurtful consequences on human and animal health (Abdulrahman *et al.*, 2009)

A lot of studies have shown that increasing the heavy metals content in the soil increases the uptake by plants. When these vegetables are consumed, heavy metals enter the food chain and if consumed in large quantities may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of biochemical processes resulting to cardiovascular, nervous, kidney and bone disease (Krijger *et al.,* 1999)

Leafy vegetables have greater potential of accumulating heavy metals in their edible parts than grain or fruit crops because they are largely loaded with xylem and heavy metals are generally very mobile in the xylem (Krijger *et al.,* 1999). Contamination of human food chain by heavy metals is not a direct function of the plants total uptake but rather it is influenced by the concentration in their edible parts that are directly consumed (Bieleski *et al.,* 1983). As reported by Lee *et al., (*1996) sensitivity of animals to heavy metals toxicity depends on heavy metal accumulation rate in the edible parts of the plants and the rate at which they are consumed, amongst other factors. Heavy metals rank high among the major contaminants of leaf vegetables (Mapanda *et al*., 2005). Heavy metals become toxic when they are not broken down and digested by the body and instead that accumulate in the soft tissues and organs (Ukpabi *et al.,* 2012).

Emissions of heavy metals from industries and vehicles may be deposited on the vegetable surfaces during their harvesting, transport and marketing and in their storage locations. Al Jassir *et al.,* (2005) have reported increasing levels of heavy metals in vegetables sold in the markets at Riyadh city in Saudi Arabia as a result of atmospheric deposition. Recently, Sharma et al., (2008b) reported that atmospheric deposition can contribute significantly to elevated levels of heavy metals in vegetables commonly sold in the markets of Varanasi, India. The prolonged and frequent consumption of unsafe levels of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (WHO, 1992 and Jarup, 2003). Some heavy metals such as Copper, Zinc, Manganese, Cobolt and Molybdenum act as micronutrients and are required in trace amounts for the growth of animals and human beings, whereas others such as Cadmium, Arsenic, and Chromium act as carcinogens (Feig *et al.*, 1994 and Trichopoulos, 1997). The contamination of vegetables with heavy metals as a result of soil and atmospheric contamination poses a great threat to its quality and safety. Dietary intake of heavy metals also poses risk to animals and human health. Heavy metals such as Cadmium and lead have been shown to have carcinogenic effects. Trichopoulos (1997) and Fernando *et al.,* (2011), worked on heavy metals in vegetables and potential risk for human health, they found out that ingestion of vegetables containing heavy metals is one of the ways in which these elements enter the human body. Once entered, they are deposited in bones and fat tissues, overlapping noble minerals and slowly released into the body causing an array of health challenges. They investigated the concentration of cadmium, nickel, lead, cobolt and chromium in the most frequently consumed foodstuff in Sao Paulo State, Brazil and compared the heavy metal concentrations with the permissible limits established by the Brazilian legislation. They also estimated the potential risk to the general health condition of humans.

In their work “concentration levels of heavy metals in soils at vegetable areas in Kota Bharu, Kelantan, Malaysia”. Halazi *et al.,* (2014) observed that the results obtained from the study in both villages were below the limits or standards proposed by various agencies. Although the concentration observed in KubangEdang was high, the farm located in the village with virgin forest and very few houses was much lower compared to results obtained from Tokkambing which is fairly industrialized and exposed to external pollution from other sources besides fertilizers and pesticides, nevertheless, results obtained was still below the limits proposed for soil.

The absorption of heavy metals by vegetables are influenced by a number of factors including climate, atmospheric depositions, the concentrations of heavy metals in the farm soil, the nature of the farm soil on which the vegetables are grown and the age and maturity of the plants at the time of harvest (Scott *et al.,* 1996).Several studies have shown that the presence of poisonous heavy metals in biological systems can lead to oxidative stress which promotes the formation of reactive oxygen species that damage cells. Plants scavenge and dispose of these reactive molecules by use of antioxidant and defense system present in several sub-cellular components. The major antioxidant species in plant are ascorbate, reduced glutathione, tocophenol and carotenoids; polyamines and flavonoids are known to provide some protection from free radical injury. The medical importance of oxidative stress has become increasingly recognized to the point that it is now thought to be a component of nearly every disease process including aging, coronary heart diseases, inflammation stroke, diabetes mellitus and cancer. The beneficial health effects of consuming vegetables have been partially attributed, to antioxidant phytochemical compounds present in them (Graziani *et al.,* 2005) and their ability to chelate some heavy metals and prevent their absorption from ingested food.

Akan *et al* (2009) worked on heavy metals and anion levels in some samples of vegetables grown within the vicinity of Challawa Industrial Area in Kanu State. Their findings indicated that all the vegetable samples analyzed had elevated levels of heavy metals and anions. Heavy metal levels were higher than those recommended by Food and Agricultural Oganization (FAO) and the WHO/EU joint limits. The high levels of these toxic metals and anions put the consumers of these and other vegetable crops grown within the vicinity of their sampling area at health risk with time unless very urgent step is taken by relevant authorities to address the issue.

Ukpabi *et al.,(*2012) worked on the phytochemical and heavy metal composition of *telfairia occidentialis* and *talinium triangulare* grown in Aba Nigeria and their environmental health implication, they concluded that both vegetables contain certain phytochemical compounds and accumulate heavy metals. Potential public health hazards especially From Cd and Pb were low but Fe, Zn, Cu, Ni, Mo, and Co was potentially hazardous.

Oliver, *et al.,* (1996) reported that soil and vegetables contaminated with Pb and Cd in Copsa Mica and Baia Mare, Romania, significantly contributed to decreasing human life expectancy (9-10 years) within the affected study areas.

From health point of view, ingestion of vegetables grown in soils contaminated with heavy metals poses a possible health risk to human and wildlife (Ismail *et al.,* 1988) Exposure to heavy metals is normally chronic due to their cumulative behavior and toxicity, heavy metals are potentially hazardous not only to crop, but also to human health through food consumption and accumulation in food crops (Haliza*., et al 2015*). Among the major effects of using pesticides are endocrine disorders, reproductive damage and development, neurotoxic, increase in morbidity and so on. Pesticides are classified as endocrine disruptors which exhibit their adverse effects by mimicking natural hormones in the body and are linked to human health challenges such as immune suppression, hormonal imbalance, reproductive abnormalities and cancer (Aktar *et al* .,2009). The excessive intake of foods contaminated with metals may lead to a number of diseases such as kidney damage and/or skeletal damage, and both metals acts as human carcinogen in the body (Hasmah *et al.,* 2011).

Potentially harmful metal contents in soils may come not only from the bedrock itself, but also from known anthropogenic sources like solid or liquid waste deposits, agricultural inputs, and fallout of industrial and urban emissions (Wilson *et al.,* 2007). Excessive accumulation in farm soils may result not only in soil contamination, but also has consequences for food quality and safety. It is therefore important to monitor food quality, given that plant absorption is one of the main channels through which heavy metals enter the food chain (Antonious *et al.,* 2009). Elevated levels of heavy metals (Cu, Cd and Pb) in fruits and vegetables have been linked to high prevalence of upper gastrointestinal cancer (Turkdogan *et al.,* 2002)

Vegetables absorb heavy metals and store them in their edible and non-edible parts at concentrations high enough to cause clinical problems to both animals and human beings. As an example, the consumption of contaminated food can seriously deplete some essential nutrients in the body leading to a decrease of immunological defenses, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer.

Lead (Pb) is the most common and prevalent toxic heavy metal contaminant (Di maio *et al.,* 2001), the adverse effect of lead is well documented. Acute exposure (usually a day or less) leads to brain dysfunction, nausea and vomiting while chronic exposure (often months or years) may cause anemia, palsy and kidney disease.

In terms of environmental pollution, toxic signs of lead poisoning manifest quicker and are more readily observed than any other substance (Baird, 2002).The toxic effects of lead focus on several organs, such as liver, kidneys, spleen and lung, producing a variety of biochemical defects. The nervous system of infants and children is particularly affected by the toxicity of this heavy metal. Adults exposed occupationally or accidentally to excessive levels of Pb exhibit neuropathology. There is association between lead in human body and the increase of blood pressure in adults (Maihara *et al.,* 2006). Others elements such as Chromium, Cobolt and Nickel, although essential for men, at concentrations higher than those recommended, may cause metabolic disorders. Moreover, an increasing knowledge of the importance of vegetables and fruits to human diet suggests that the monitoring of heavy metal concentrations in food crops should be done on a regular basis.

Cadmium is also present as a pollutant in phosphate fertilizers. Natural as well as anthropogenic sources of cadmium from industrial emissions and the application of fertilizer and sewage sludge to farm land may result to contamination of soils and to increased cadmium absorption by crops and vegetables grown for human consumption (Jarup 2003). Acute exposure to cadmium may cause lung inflammation while chronic exposure results in lung cancer, osteomalacia, excess protein in the urine and possible kidney damage. Cadmium also causes a rise of the copper kidney concentration. The effect of cadmium largely depends on its dose and exposure time and concentration as well as the nutritional state of the subject and on the dietary contents of iron, zinc and copper. Cadmium and Pb are the most toxic elements to man (Volpe *et al.,* 2009).Cadmium is a dangerous element because it can be absorbed through the alimentary tract, penetrate through placenta during pregnancy, and destroy membranes and DNA. Once in the human body, it may remain in the metabolism for 16 to 33 years and is linked to a number of health problems including renal damages and abnormal urinary excretion of proteins. Decrease in bone calcium concentrations and increase of urinary excretion of calcium have also been linkede to exposure to Cd, eventually causing death. It also affects reproduction and endocrine systems of women (WHO, 2004). Vegetables may contribute to about 70 % of the cadmium intake by humans, varying according to the level of consumption (Wagner, 1993).

Adverse effects of chromium include haemolysis, acute renal failure, pulmonary fibrosis and lung cancer.

Iron contamination of air water and soil is quite common especially places where iron is produced. Vegetables cultivated on iron rich or iron contaminated soils may contain elevated levels of iron. Physical health challenges linked with iron poisoning include diabetes, hypertension, and kidney problems. Iron is abundant in some vegetables but is generally less well absorbed because of the presence of phylates, oxalates, tannin and other substances that interfere with iron absorption. The detrimental effects of chronic iron poisoning are due in part to iron accumulation in various organs including the heart, liver, brain pancreas and joints.

Iron toxicity is commonly associated with personality characteristics such as strong ego, rigidity, hostility, stubbornness and irritability. Iron is normally stored in the liver, an excess iron accumulation results in liver damage. Iron accumulation in the liver and other tissues can result in extreme fatigue or tiredness. (Passwater *et al* 1983).

Zinc is an essential trace metal with very low toxicity in humans (Ciubotariu *et al.,* 2015). Zinc, Iron and Copper deficiency leads to lead toxicity through considerable enhancement of lead absorption from intestinal tract, producing greater degree of anemia as well as decreasing of metalloenzyme activity. The main disturbances in iron metabolism under conditions of exposure to Cadmium comprise of reduction of iron intestinal absorption, iron live Concentrations and hematological indices. Cadmium displaces zinc from biological essential structures e.g nucleic acids. This induces the synthesis of new molecules and produce changes in zinc distribution in the tissues resulting to its accumulation in the liver and kidney and a decrease in its concentration in the bones.

Zinc toxicity is a medical condition involving an overdose on, or toxic over exposure to zinc. Such toxicity levels have been seen to occur at ingestion of greater than 225mg of zinc (Fosmire, 1990). Excessive absorption of zinc can suppress copper and iron absorption. The free zinc ion in solution is highly toxic to bacteria, plants invertebrates and even vertebrate fish (Rout *et al* 2003).

2.4. Acceptable Daily Intake:

The concept of Acceptable Daily Intake (ADI) is defined by the Joint Expert Committee of the Food and Agriculture Organization of the United Nations World Health Organization (JECFA/WHO) for substances intentionally introduced to food or for contaminants (pesticides, herbicides and fertilizers) as the highest daily intake level of a chemical that, if continued over the whole life of a person, is considered to pose no health risk.

The Acceptable Daily Intake of nitrate and nitrite proposed by the European Commission's scientific committee on Food established in 1995 to monitor vegetable production practices is 3.7mg/kg body weight and 0.06mg/kg body weight respectively (Zhong *et al.*, 2002) for a person weighing 60kg. Permissible limits of heavy metals concentration set by FAO/WHO are 0.3mg/kg Fe, 0.1mg/kgPb,0.1mg/kg Cu, 0.1mg/kg Zn, 0.02mg/kg Cd. However, the World Health Organization (WHO) has said that the criteria values of nitrate content in the same kind of vegetable may vary broadly from country to country as a result of differences in vegetable consumption and vegetable production practices.

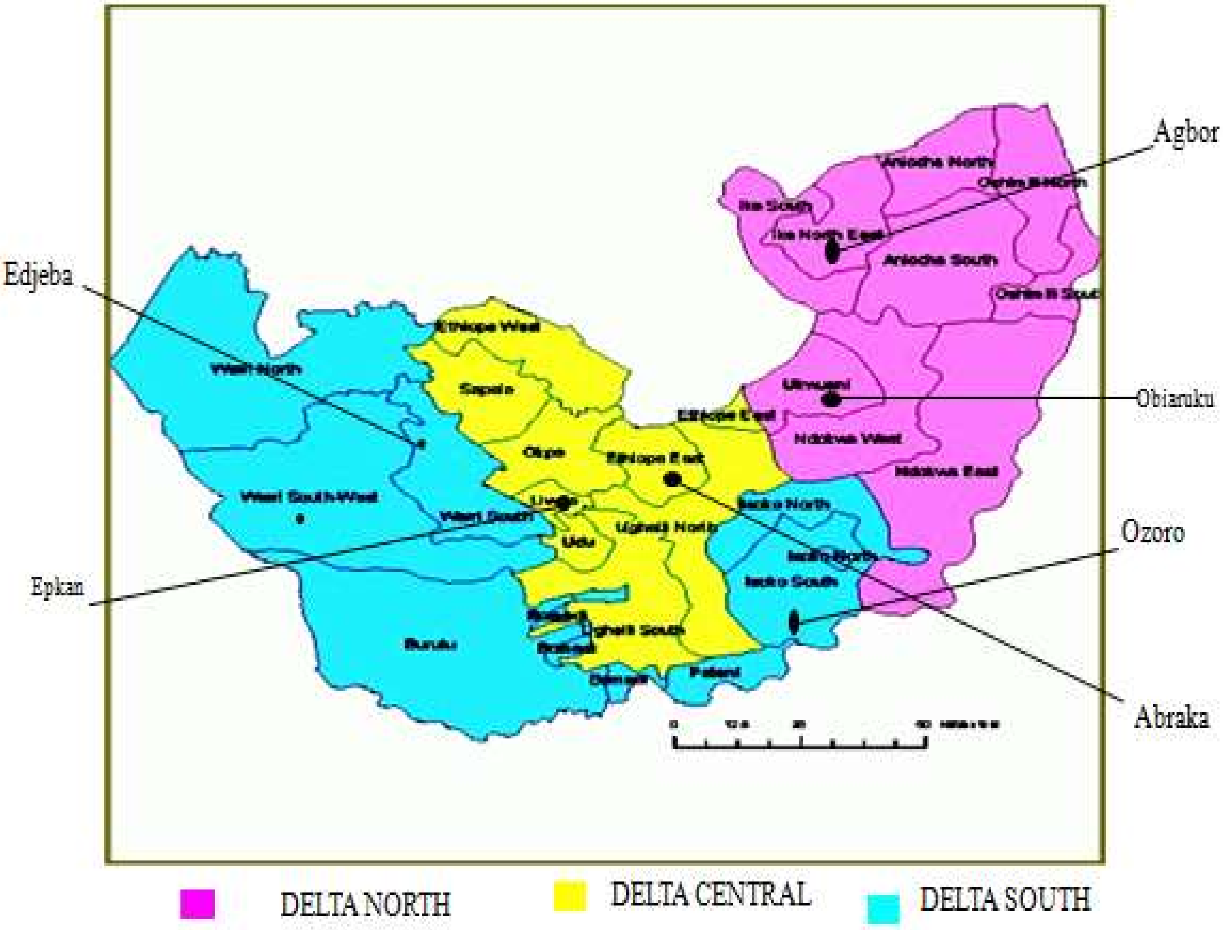
CHAPTER THREE - MATERIALS AND METHOD

3.1 Description of study area:

Delta State was created on the 27th Day of August, 1991 by the Military Administration of Gen. Ibrahim Badamonsi Babangida from the Defunct Bendel State; two States were created from the defunct Bendel State namely, Edo State retaining Benin City as her Capital city and Delta State with Asaba as her

Capital City. Delta State was made up of twelve political divisions called Local Government Area (L.G.A), which was later increased to 19 in 1996 and 25 Local Government Areas as at today.

Delta State is divided into three Senatorial Districts, they are Delta North, Delta South and Delta Central. It covers a landmass of about 18,050 Km2 of which more than 60% is land and lies approximately between Longitude 5°00 and 6°.45' East and Latitude 5°00 and 6°.30' North. It is flanked in the North by Edo State, the East by Anambra State, South-East by Bayelsa State, and on the Southern flank is the Bight of Benin covering about 160kilometres of the State's coastline. Delta State is generally low-lying without remarkable hills. It has a wide coastal belt inter-lace with rivulets and streams, which form part of the Niger-Delta.



3.2 Sampling

In this cross sectional study, 5 different types of vegetable samples (pumpkin leaf (*Telfairia occidentalis*), water leaf(*Talinum triangulare*), bitter leaf(*Verononia amygdalina*), green leaf (*Amaranthus hybridus*), and scent leaf(*Ocimum grattissimum*) and soil from farms in the three senatorial districts of Delta State were collected three times within three months in the peak of the wet and thrice within three months dry seasons in the year 2014. The vegetable sample were packaged into properly labelled paper bags and taken to the laboratory awaiting analysis. Soil samples from the farm sites were randomly sampled and bulked together to form a composite sample. In all cases soil samples were put in clean plastic bags and taken to the laboratory. Soil samples were then airs dried, crushed and passed through 2 mm mesh sieve, the samples were then put in clean plastic bags sealed and labelled.

3.3 Determination of heavy metals in soil samples:

Before digestion, air-dried soil sample were ground using a mortar and passed through a 2 mm sieve. 2g of the soil samples were weighed into acid – washed glass beaker. Soil samples were digested by adding 20 $cm^3$of aqua regia (mixture of HCL and HNO_3, ratio 3:1). The beaker was covered with a watch glass and heated over a hot plate at a temperature of 90 ^oC for two hours. The beaker wall and watch glass were washed with distilled water and the samples filtered out into flasks to separate the insoluble solid from the supernatant liquid. The volumes were adjusted to 100 cm^3 with distilled water. The collected filtrate analyzed for cadmium (Cd), copper (Cu), iron (Fe), zinc (Zn) and lead (Pb) by Atomic Absorption Spectrophotometry after calibrating the equipment with different standard concentrations.

3.4 Sample preparation and digestion of vegetables for heavy metal determination:

The vegetable samples were weighed to determine the fresh weight and dried in an oven at 80 °C for 72 hours to determine their dry weight. The dry samples were crushed in a mortar and the resulting powder digested by weighing 0.5 g of oven dried , ground and sieved (<1mm) into an acid washed porclain crucible and placed in a muffle furnance for 4 hours at 500 °C. The crucibles were removed from the furnance and cooled. 10 ml of 6M HCl were added, covered and heated on a steam bath for 15 mins. Another 1 ml of HNO_3 was added and evaporated to dryness by continuous heating for one hour to dehydrate silica and completely digest organic compounds. Finally, 5 ml of 6M HCl and 10 ml of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman no. 541 filter paper into a 50 ml volumetric flask and made up to the mark with distilled water.

3.5 Elemental Analysis of Samples:

Determination of Cu, Mn, Fe, Pb and Cd in soil and vegetable samples were made directly on each of the final solutions using Atomic Absorption Spectroscopy.

3.6 Determination Of Nitrate, Nitrite, Sulphate, Phosphate and Chloride In The Vegetable Sample

3.6.1 Vegetable samples preparations for Nitrate and Nitrite analysis.

Vegetable samples were cleaned to remove visible soil particles and then washed with tap water, thereafter with distilled water several times and then sliced into nearly uniform sizes to facilitate drying at the same rate. The sliced samples were then dried in an oven at 105 °C for 24 hours until they were brittle and crisp. At this stage no micro organism can grow and care was taken to avoid any source of contamination. The dried samples were mechanically ground into fine particles using clean mortar and pestle and sieved to obtain <2 mm fractions. A portion (1g) of each of the sieved samples were taken separately in 100 ml glass bottles, 40 ml of distilled water were added, capped and shaken for 30 minutes. The solutions were filtered and the filtrates made up to the mark in 100 ml volumetric flasks (Radojevic *et al.,* 1999).

3.6.2 Determination of nitrate and nitrite concentrations in the vegetable samples

The determination of nitrate in each of the vegetable sample solutions was carried out using spectrophotometer (model 2000) at a wavelength of 543 nm. The equipment was scrolled to select the stored programme number for nitrate (64 Nitrate-N). The result which was obtained as Nitrate-Nitrogen ($N0_{3-}$-N) was converted to ppm Nitrate (NO_{3-}) by multiplying by 4.4 (conversion factor) (LaMotte 2000). The concentration levels of nitrate (μgg^{-1}) in the samples were calculated from:

$NO_{3-}(\mu gg^{-1}) = C X V/M$

Where; C is the concentration of NO_{3-} in the sample (ppm), V is the total volume of the sample solution (100 ml) and M is the weight of the sample (1g) (Radojevic *et al.,* 1999).

Nitrite levels in the samples were similarly determined except in this case the programme number is 67 Nitrite-N.(NO_{2-} -N) was converted to ppm nitrite (NO_{2-}) by multiplying by 3.3 (conversion factor) the concentration levels of nitrite (μgkg^{-1}) in the samples were calculated from:

$NO_2^- (\mu gg^{-1}) = C X V/M$

Where; C is the concentration of NO_2^- in the sample (ppm), V is the total volume of the sample solution (100 ml) and M is the weight of the sample (1g) (Radojevic *et al*., 1999).

3.6.3 Determination Of phosphate concentrations in the vegetable samples.

Each of the vegetables samples were chopped into tiny pieces. The chopped samples were then air-dried. The air dried samples were ground and sieved with a sieve of mesh 1 mm. A known amount (1g) of each of the ground and sieved samples was weighed into acid-washed porcelain crucibles. The crucibles were labelled and 5 ml of 20 %(wv^{-1}) magnesium acetate were added and evaporated to dryness. The crucibles were then transferred into the furnace and the temperature was raised to 500 ^{o}C. The samples were ashed at this temperature for 4 hrs, removed and cooled in desiccators. 10 ml of 6M HCl were then added to each of the crucible and covered, then heated on a steam bath for 15 minutes. The content of each crucible were completely transferred into different evaporating basins and 1ml of concentrated HNO_3 was added. The heating was made to continue for 1hour to dehydrate silica. 1 ml of 6M HCl was then added, swirled and then followed by the addition of 10 ml distilled water and again heated on the steam bath to complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No. 1 filter paper into 50 ml volumetric flask with distilled water. Phosphate was determined using hach direct reading 2000 spectrophotometer.

3.6.4 Determination of Sulphate concentrations in the vegetable samples

For sulphate determination, 5 ml of magnesium nitrate solutions were added to each of the ground and sieved samples in the crucible. These were then heated to 180 ^{o}C on a hot plate. The heating process was allowed to continue until the color of the samples changed from brown to yellow. The samples were then transferred to the furnace at a temperature of 50 ^{o}C for 4 hours. Magnesium nitrate was added to prevent loss of sulphur. The content of each crucible was carefully transferred to a different evaporating basins.10 ml of conc HCl were added to each of them and covered with watch glasses. They were boiled on a steam bath for 3 mins. On cooling, 10 ml of distilled water was added to each of the basins and the content of each were filtered into 50 ml volumetric flasks and the volumes made up to the mark with distilled water. Sulphate was determined using smart spectrophotometer.

3.6.5 Determination of chloride

Chloride was extracted by shaking 300 mg of dried vegetable sample with 30 ml of 0.01 M $CaSO_4$ solution for 30 minutes. A 0.85 g of pre-washed charcoal was added to each sample and shaking was continued for 5 additional minutes. The mixture was centrifuged for 20 minutes and filtered using Whatman No. 42. The aliquot was analyzed colorimetrically by the mercury (II) thiocyanate method. In this method, a reddish yellow-colored complex of ferric thiocyanate was formed when chloride ions sequester the mercury ions of mercuric thiocyanate (thereby freeing the thiocyanate) in the presence of excess ferric nitrate. The color was measured on a Cecil CE 2041 2000 series spectrophotometric instrument.

3.6.6 Determination of Nitrate and Nitrite in the soil Distillates:

Nitrate and nitrite concentrations in the soil distillates were then determined by using spectrophotometer at a wavelength of 543 nm, similar to those previously described for the vegetable samples.

3.7 Transfer factors (TF) for heavy metals and anions from soils to vegetables

Transfer factor (TF) is the ratio of the concentration of the anions or heavy metals in a plant sample to their concentration in the soil. TF for nitrate and nitrite were computed based on the method described by Harrison and Chirgawi, (1983) according to the following formula:

TF = Ps (µgg-1 dry wt) / St (µgg-1 dry wt) (Raiswell *et al* 1980)

Where Ps is the plant anion or heavy metal concentration originating from the soil and St is the total anion or heavy metal concentration in the soil.

3.8 Data Analysis: Data collected were subjected to statistical tools of mean and standard deviation and statistical tests of significance using the student t-test and analysis of variance (ANOVA). All statistical analyses were done by SPSS software for windows.

CHAPTER FOUR- DATA ANALYSIS, RESULTS AND DISCUSSION

4.1 **Nitrate and nitrite in the samples**.

Concentrations in µgg^{-1} of nitrate and nitrite in samples collected from farms in Delta State during the dry and rainy seasons.

Table 1	**sample**	**NO_3^- µgg^{-1}(dry)**	**NO_3^- µgg^{-1}(wet)**
Agbor	*A. hybridus* (Green leaf)	204.25±2.00	177.50±3.60
	V. amygdalina (Bitter leaf)	225.80±1.52	223.0±3.00
	T. triangulare (Water leaf)	306.00±3.05	254.95±1.02
	T. occidentalis (Pumpkin leaf)	327.05±2.51	254.30±2.00
	O. grattissimum (Scent leaf)	443.70±3.091	269.80±2.00
	Soil	450±2.45	360.10±1.76
		NO_2^-µgg^{-1} (dry)	**NO_2^-µgg^{-1} (wet)**
Agbor	*A. hybridus* (Green leaf)	58.50±4.16	54.95±2.51
	V. amygdalina (Bitter leaf)	64.80±1.02	54.35±3.02
	T. triangulare (Water leaf)	64.80±2.64	60.0±1.53
	T. occidentalis (Pumpkin leaf)	66.80±1.52	60.65±1.00
	O. grattissimum (Scent leaf)	97.70±2.52	90.75±2.67
	Soil	150.00±2.30	132.8±1.53

Table 2	**sample**	**NO_3^- µgg^{-1}(dry)**	**NO_3^- µgg^{-1}(wet)**
Abraka	*A. hybridus* (Green leaf)	75.15±1.06	76.00±5.03
	V. amygdalina (Bitter leaf)	119±2.08	142.8±6.0
	T. triangulare (Water leaf)	154.75 ± 0.02	174.1±2.30
	T. occidentalis (Pumpkin leaf)	187.25±2.64	216.8±0.49
	O. grattissimum (Scent leaf)	226±4.16	255.2±2.51
	Soil	300.25±1.43	328.5±2.64
		NO_2^-µgg^{-1}(dry)	**NO_2^-µgg^{-1}(wet)**
Abraka	*A. hybridus* (Green leaf)	23.05±1.43	17.95±2.08
	V. amygdalina (Bitter leaf)	34.25±1.52	18.40±2.73
	T. triangulare (Water leaf)	36.30±4.51	36.80±2.000
	T. occidentalis (Pumpkin leaf)	69.30±2.08	66.50±4.933
	O. grattissimum (Scent leaf)	72.50±9.07	86.6±2.5
	Soil	126.5±1.15	175.5±3.78

Table 3	**sample**	**NO_3^- µgg^{-1}(dry)**	**NO_3^- µgg^{-1}(wet)**
ozoro	*A. hybridus* (Green leaf)	85.25±0.06	96.00±5.03
	V. amygdalina (Bitter leaf)	96.1±0.12	102.8±6.0
	T. triangulare (Water leaf)	105.30±0.12	114.1±2.30
	T. occidentalis (Pumpkin leaf)	120.05±0.25	200.8±0.49
	O.grattissimum (Scent leaf)	130.25±0.14	225.2±2.51
	Soil	150.25±0.11	230.5±2.64
		NO_2^-µgg^{-1}(dry)	**NO_2^-µgg^{-1}(wet)**
ozoro	*A. hybridus* (Green leaf)	46.32±0.25	17.95±2.08
	V. amygdalina (Bitter leaf)	42.8±0.45	18.40±2.73
	T. triangulare (Water leaf)	54.86±0.45	36.80±2.000
	T. occidentalis (Pumpkin leaf)	57.3±0.4	66.50±4.933
	O. grattissimum (Scent leaf)	72.60±0.33	86.6±2.5

Soil	95.5±1.15	105.5±3.78

Concentration in µg/g of other anions in the study sample.

Table 4

sample	PO_4^{3-}	SO_4^{2-}	Cl^-	PO_4^{3-}	SO_4^{2-}	Cl^-
	(Dry season)			**(Rainy seasons)**		
(Agbor) Soil						
Scent leaf	218.00±0.33	194.52±1.23	65.20± 1.22	188.2±1.05	166.20±0.43	80.00±1.24
pumpkin	166.23±1.25	184.50±0.64	64.31±1.54	146.00±1.86	136.45±2.02	80.05±2.42
water leaf	156.82±0.26	163.82±1.58	64.20±2.34	138.20±0.86	135.05±2.86	75.56±3.04
bitter leaf	134.05±2.84	99.05±1.66	60.00±2.44	125.05±0.26	110.02±3.17	74.00±2.00
green leaf	125.00±2.24	94.00±1.46	51.02±2.00	96.00±0.28	104.00±1.46	72.01±2.01
(Abraka) Soil	170.45±1.43	155.04±2.34	74.22± 2.41	185.46±1.32	164.86±2.00	85.04±2.25
Scent leaf	164.23±0.23	143.50±2.53	50.29±0.42	176.00± 1.24	152.46±1.22	82.00±0.05
pumpkin	162.00±1.22	130.18±0.81	54.3±1.24	166.05±0.85	152.4±0.22	75.24±1.20
water leaf	151.25±1.04	126.28±0.42	53.00±0.33	154.84±0.21	130.58±0.22	70.50±2.00
bitter leaf	140.77± 0.33	120.80±1.24	45.46±1.06	152.45± 0.24	128.00±1.04	68.00±2.06
green leaf	134.22±1.02	122.20±0.04	36.3±0.14	147.00±1.04	126.00±1.22	68.00±0.04
(Ozoro)Soil	300.05±0.26	260.32±1.02	105.4±1.22	380.54±0.34	282.41±0.08	100.5±1.42
Scent leaf	257.56±0.26	255.00±1.08	85.45±1.44	343.82±0.06	265.21±0.04	88.51±1.04
Pumpkin	246.35±0.14	248.531±0.08	85.32±2.05	325.20±0.23	265.1±1.00	86.00±2.01
water leaf	246.00±2.00	241.01±0.08	65.25±0.15	333.10±1.20	243.00±2.00	86.25±3.01
bitter leaf	232.31±0.01	180.00±1.08	50.45±1.05	322.35±0.22	230.11±0.07	85.41±0.44
green leaf	205.22±0.02	135.35±2.05	50.25±2.00	250.11 ±1.13	192.14±0.04	85.42±2.10

.Concentrations in mgkg^{-1} of selected heavy metals in samples collected from Delta State during the dry and rainy seasons.

Table 5 . Sample	**Cu**	**Fe**	**Cd**	**Pb**	**Zn**
(Agbor dry) Soil	1.5±1.37	5.5±0.027	7.0±0.013	1.7±0.016	2±0.038
Scent leaf	1.02±0.017	4.40±0.024	5.0±0.084	1.52±0.014	1.45±0.01
Green	0.21±0.014	3.1±0.032	3.51±0.126	0.43±0.007	1.26±0.025
Bitter leaf	1.01±0.007	1.24±0.003	5.03±0.052	1.00±0.035	1.07±0.012
Water leaf	0.45±0.124	4.28±0.01	4.20±0.014	1.45±0.02	1.21±0.016
Pumpkin	1.32±0.037	3.05±0.02	5.21±0.016	1.31±0.038	1.45±0.001
Agbor(wet) Soil	2.03±0.008	6.54±0.011	3.50±0.027	1.25±0.017	2.00±0.12
Scent leaf	1.31±0.013	5.24±0.013	2.20±0.013	1.00±0.126	1.25±0.016
Green	1.00±0.016	4.20±0.001	2.22±0.016	0.01±0.034	1.26±0.008
Bitter leaf	1.05±0.027	4.01±0.014	1.38±0.038	1.00±0.126	1.35±0.037
Water leaf	0.20 ±0.018	3.24±0.008	1.56±0.045	0.26±0.024	1.42±0.007
Pumpkin	1.24±0.038	4.20±0.025	2.00±0.01	1.18± 0.010	1.98±0.011
Abraka(dry) Soil	2.5±0.011	6.40±0.007	4.0±0.024	2.5±0.037	3.4±0.124
Scent leaf	1.26±0.084	4.50±0.024	3.02±0.017	1.46±0.126	2.21±0.031
Green	1.31±0.001	2.01±0.012	2.31±0.011	1.31±0.017	2.00±0.019
Bitter leaf	1.05±0.004	4.51±0.035	2.43±0.010	1.20±0.062	2.00±0.032
Water leaf	1.20±0.025	2.20±0.013	1.6±0.028	1.51±0.001	0.15±0.014
Pumpkin	1.00±0.027	2.50±0.001	2.89±0.016	1.31±0.014	1.26±0.003
(Abraka wet) Soil	2.4±0.01	46.5±0.038	4.6±0.045	3.2±0.029	3.4±0.017
Scent leaf	1.05±0.024	5.1±0.017	3.4±0.011	2.5±0.024	2.41±0.001

Green	1.21±0.001	5.05±0.014	3.02±0.008	2.4±0.124	2.26±0.037
Bitter leaf	1.95±0.025	4.15±0.084	2.81±0.039	2.32±0.017	2.32±0.012
Water leaf	0.02±0.024	2.1±0.001	2.00±0.126	1.41±0.010	1.4±0.007
Pumpkin	1.5±0.034	2.9±0.062	2.50±0.029	2.35±0.045	2.4±0.034
(Ozoro dry) Soil	3.5±0.01	10.5±0.35	5.0±0.017	3.2±0.384	4.5±0.007
Scent leaf	2.42±0.10	6.20±0.01	4.02±0.012	2.30±0.027	3.26±0.014
Green	2.5±0.15	6.00±1.28	3.59±0.007	2.00±0.013	3.50±0.037
Bitter leaf	2.52±0.05	4.21±0.57	3.03±0.024	1.00±0.038	3.0±0.084
Water leaf	1.00±0.12	3.40±0.03	2.40±0.126	1.25±0.024	2.20±0.400
Pumpkin	2.05±0.57	4.30±0.32	3.05±0.401	2.00±0.001	2.69±0.124
(Ozoro wet) Soil	4.4±1.037	11.5±0.028	12.0±0.037	4.5±0.014	5.5±0.036
Scent leaf	3.50±0.001	8.60±0.037	9.45±0.221	3.5±0.011	4.5±0.384
Green	3.25±0.017	7.50±0.022	10.05±0.054	3.02±0.054	4.39±0.084
Bitter leaf	3.25±0.007	6.52±0.300	10.50±0.031	3.26±0.043	3.50±0.400
Water leaf	2.51±0.029	5.41±0.21	8.2±0.12	2.43±0.03	2.54±0.14
Pumpkin	3.20±0.40	6.50±0.04	10.86±0.06	3.5±0.21	3.54±0.065

Transfer factor for nitrate and nitrite (µg/g): Transfer factor of the anions between the soils and the vegetables identify the efficiency of a vegetable species to accumulate a given anion. These factors were based on the root uptake of the anions.

Table 6

sample	**Nitrate µg/g**	**Nitrite**	**Nitrate**	**Nitrite**
	(Dry season)		**(Rainy seasons**	
(Agbor) Scent leaf	0.94	0.64	0.86	0.35
Green	0.43	0.51	0.58	0.42
Bitter leaf	0.48	0.42	0.91	0.43
Water leaf	0.65	0.25	0.29	0.25
Pumpkin	0.70	0.13	0.57	0.24
(Abraka) Scent leaf	0.61	0.55	0.59	0.21
Green	0.64	0.57	0.36	0.21
Bitter leaf	0.34	0.11	0.44	0.38
Water leaf	0.21	0.20	0.18	0.17
Pumpkin	0.49	0.30	0.34	0.10
(Ozoro) Scent leaf	0.94	0.77	0.73	0.65
Green	0.94	0.70	0.70	0.64
Bitter leaf	0.51	0.28	0.67	0.48
Water leaf	0.47	0.29	0.03	0.21
Pumpkin	0.53	0.32	0.35	0.25

Table 7: Transfer factor µg/g of other anions in the study sample.

sample	**PO_4^{3-}**	**SO_4^{2-}**	**Cl^-**	**PO_4^{3-}**	**SO_4^{2-}**	**Cl^-**
	(Dry season)			**(Wet season)**		
(Agbor)Scent leaf.	1.29	1.13.	1.35	1.05	0.43	1.24
Green.	1.43.	1.13.	1.54	1.86	2.02	2.42
Bitter leaf	1.38.	1.21.	2.34	0.86	2.86	3.04
Water leaf.	1.65.	0.36.	2.44	0.26	3.17	2.00
Pumpki	1.55	1.05	2.00	0.28	1.46	2.01
(Abraka) Scent leaf	0.23	2.53	0.42	1.24	1.22	0.05
Green	1.22	0.81	1.24	0.85.	0.22	1.20

	Bitter leaf	1.04	0.42	0.33	0.21	0.22	2.00
	Water leaf	0.33	1.24	1.06	0.24	1.04	2.06
	Pumpkin	1.02	0.04	0.14	1.04	1.22	0.04
(Ozoro)	Scent leaf	0.26	1.08	1.44	0.06	0.04	1.04
	Green	0.14	0.08	2.05	0.23	1.00	2.01
	Bitter leaf	2.00	0.08	0.15	1.20	2.00	3.01
	Water leaf	0.12	1.08	1.00	0.22	0.07	0.44
	Pumpkin	0.02	2.05	2.00	1.13	0.04	2.10

Transfer factor of heavy metals in the study sample

Table 8 .		**Cu**	**Fe**	**Cd**	**Pb**	**Zn**	**Cu**	**Fe**	**Cd**	**Pb**	**Zn**
		(dry)								**(wet)**	
(Agbor)	Scent leaf	0.68	0.81	0.71	0.89	0.72	0.64	0.80	0.62	0.82	0.63
	Green	0.14	0.56	0.53	0.25	0.63	0.49	0.64	0.63	0.01	0.63
	Bitter leaf	0.67	0.23	0.71	0.62	0.53	0.52	0.61	0.39	0.81	0.68
	Water leaf	0.32	0.77	0.63	0.85	0.61	0.09	0.49	0.45	0.21	0.71
	Pumpkin	0.88	0.55	0.74	0.77	0.73	0.61	0.64	0.57	0.94	0.99
(Abraka)	Scent leaf	0.52	0.70	0.76	0.58	0.65	0.43	0.78	0.74	0.78	0.71
	Green	0.52	0.31	0.58	0.52	0.59	0.50	0.78	0.67	0.75	0.66
	Bitter leaf	0.42	0.70	0.61	0.48	0.59	0.81	0.64	0.61	0.73	0.68
	Water leaf	0.48	0.34	0.42	0.60	0.04	0.01	0.32	0.43	0.44	0.41
	Pumpkin	0.43	0.39	0.72	0.52	0.37	0.61	0.41	0.54	0.73	0.71

(Ozoro)Scent leaf	0.69	0.59	0.80	0.71	0.72	0.79	0.75	0.79	0.78	0.82
Green	0.71	0.72	0.71	0.63	0.78	0.74	0.65	0.83	0.67	0.79
Bitter leaf	0.72	0.40	0.61	0.31	0.67	0.74	0.57	0.87	0.72	0.64
Water leaf	0.29	0.32	0.48	0.39	0.49	0.57	0.47	0.68	0.54	0.46
Pumpkin	0.59	0.41	0.61	0.63	0.59	0.73	0.57	0.91	0.78	0.64

Table 9: Descriptive statistics for nitrate in µg/g in the samples.

Variables	Mean	Std.Dev,	p-value
(Agbor).Dry Season	204.1167	1.476	<0.05
Rainy Season	166.0958	1.050	
(Abraka).Dry Season	80.6927	8.532	≤0.05
Rainy Season	132.9292	9.964	
(Ozoro)Dry Season	86.3817	3.676	≤0.05
Rainy Season	108.4292	7.443	

Source: SPSS Output, 2016

Table 10: Descriptive statistics for sulphate in µg/g of the samples

Variables	Mean	Std. Dev	p-value
(Agbor) Dry Season	50.7175	2.8413	<0.05
Rainy Season	47.5029	4.9107	

Abraka) Dry season	**43.7788**	**1.0906**	**≤0.05**
Rainy Season	**49.1002**	**5.7314**	
Ozoro) Dry season	**98.8739**	**3.5561**	**≤0.05**
Rainy Season	**86.1774**	**2.7052**	

Table 11: Descriptive statistics for phosphate in µg/g of the samples

Variables	**Mean**	**Std.Dev**	**p-value**
(Agbor) Dry Season	**70.2175**	**1.5403**	**<0.05**
Rainy Season	**54.1039**	**3.6117**	
Abraka) Dry season	**46.2318**	**1.2936**	**≤0.05**
Rainy Season	**52.1102**	**3.5364**	
Ozoro) Dry season	**88.3639**	**3.2041**	**≤0.05**
Rainy Season	**76.1244**	**2.2032**	

Table 12: Descriptive statistics for chloride in µg/g of the samples

Variables	**Mean**	**Std.Dev**	**p-value**
(Agbor)Dry Season	**40.3175**	**2.8413**	**<0.05**
Rainy Season	**27.2029**	**4.9107**	
Abraka) Dry season	**33.744**	**1.0906**	**≤0.05**
Rainy Season	**29.3052**	**5.73I4**	
Ozoro) Dry season	**48.0739**	**3.5561**	**≤0.05**
Rainy Season	**36.1664**	**2.7052**	

4.6 DISCUSSION

In the whole vegetable samples studied, nitrate concentration was higher than nitrite concentration. This trend is in agreement with similar study carried by Aword *et al* (1980). They reported that nitrate content was higher than nitrite content in different samples of fresh spinach. In Agbor samples, the concentration of anions in the dry season are higher than the rainy season, this may be attributed to the fact that Agbor suffers from water erosion during the heavy rains and most of the fertilizers applied are washed off into surrounding water bodies.

In Agbor samples, maximum concentration of the anions in the dry season was found in scent leaf (*Ocimum gattissimun*); nitrate (443.70 μg g^{-1}), nitrite (97.70 μg g^{-1}), phosphate (218 μg g^{-1}), sulphate (194.52 μg g^{-1}) and chloride (65.20 μg g^{-1}). Chloride anion showed similar values in pumpkin leaf (*Telfairia occidentalis)* (64.31 μg g^{-1}), and water leaf (*Talinum triangulare)* *(*64.20 μg g^{-1}).while the minimum concentration value of the anions was found in green leaf (*Amaranthus hybridus)* with values of nitrate (204.25 μg g^{-1}),nitrite (58.50 μg g^{-1}),phosphate (125 μg g^{-1}), sulphate (94 μg g^{-1}) and chloride (51 μg g^{-1}).

During the rainy season as displayed in table 1, maximum concentration of nitrate was found in scent leaf (269.80 μg g^{-1}) and the minimum in green leaf (177.5 μg g^{-1}). Water leaf and pumpkin leaf had similar concentrations of

(254.95 μg g^{-1}) and (254.30 μg g^{-1}) respectively. Minimum concentration of nitrate was found in green leaf (177.50 μg g^{-1}). Nitrite value ranged from (90.75 μg g^{-1}) in scent leaf to (54.95 μg g^{-1}) in green leaf. Water leaf and pumpkin leaf also showed similar concentrations of (60.0 μg g^{-1}) and (60.65 μg g^{-1}) respectively. From table 4 Phosphate, sulphate and chloride had maximum values of (188.2 μg g^{-1}), (166.2 μg g^{-1}) and (80.00 μg g^{-1}) respectively in scent leaf.

The concentrations of the anions in the samples are in the order of scent leaf>pumpkin leaf>water leaf>bitter leaf>green leaf.

Statistical test of significance using Analysis of Variance (ANOVA) revealed marked difference ($p<0.05$) between the anion contents of all the leafy vegetables in the dry and rainy seasons. . In table 9, descriptive statistics for nitrate and nitrite during the dry and rainy seasons in Agbor revealed that mean dry season for Agbor is 204±42.64 while the rainy season had a mean value of 66±30.33. this result suggests that in Agbor, the highest mean was recorded during the dry season for nitrate and nitrate The higher levels of anions obtained during the dry season may be attributed to the fact that since Agbor suffers from water erosion during the rainy season, most of the fertilizers applied are washed away into surrounding water bodies and are not totally available to the vegetables. Other environmental factors such as drought, day light intensity and soil type may also contribute to this observed difference.

Table 2 displays nitrate and nitrite concentration in samples from vegetable farm in Abraka, Delta State sampled in the dry and rainy season respectively. From table 2, mean maximum concentration value of nitrate was found in scent leaf (255.2µg g^{-1}) and the minimum concentration in green leaf (76 µg g^{-1}). Bitter leaf and pumpkin leaf had mean concentrations of (142.8 µg g^{-1}) and (216.8 µg g^{-1}) respectively. Nitrite concentration ranged from (17.95-86.6) µg g^{-1}. The output from the descriptive statistics result for nitrate and nitrite during the dry and rainy seasons in Abraka suggests that the highest mean and standard error mean for nitrate and nitrite was recorded during the rainy season. From table 4, Mean phosphate concentration ranged from (147.00 µg g^{-1}) green leaf to (176.00 µg g^{-1}) scent leaf. Sulphate showed maximum concentration in scent leaf and pumpkin leaf with values of (152.46 µg g^{-1}) and (152.40 µg g^{-1}) respectively. Minimum sulphate concentration was obtained in green leaf (126.00 µg g^{-1}) while chloride ion had equal minimum concentration value in bitter leaf and green leaf; (68.00 µg g^{-1}) each and a maximum concentration in scent leaf (82.00 µg g^{-1}). These values are higher when they are compared to results obtained during the dry season. Mean nitrate value ranged from (75.15-226) µg g^{-1}. The minimum concentration of nitrate was found in green leaf (75.15 µg g^{-1}) and maximum concentration in scent leaf (226 µg g^{-1}). Results from bitter leaf, water leaf and scent leaf are (119 µg g^{-1}), (154.75 µg g^{-1}) and (187.25 µg g^{-1}) respectively. Nitrite concentration ranged from (23.05 µg g^{-1}) green leaf to (72.50 µg g^{-1}) scent leaf. Mean maximum concentration of phosphate anion is (164.25µg g^{-1}) scent leaf and minimum value (134.22 µg g^{-1}) green leaf. Sulphate anion had a lower value of (122.20 µg g^{-1}) green leaf, (120.8 µg g^{-1}) bitter leaf. Maximum sulphate concentration was (143.50 µg g^{-1}) scent leaf.

The t-test from SPSS Output 2016 was used to statistically compare the values of the means from the two seasons, it revealed a marked significant difference of $p \leq 0.05$.

Table 3 displays anion (nitrate and nitrite) concentration in samples from vegetable farm in Ozoro, Delta State sampled in the dry and rainy season respectively. Maximum concentration of nitrate was found in scent leaf (*Ocimum grattissimum) (*130.25 µg g^{-1}) and the minimum value in green leaf (85.25 µg g^{-1}). Nitrite had a maximum concentration value in scent leaf (72.60 µg g^{-1}) and minimum value in bitter leaf (42.8 µg g^{-1}). From table 4, Phosphate concentration ranged from (257.56 µg g^{-1}) scent leaf to (205.22 µg g^{-1}) green leaf. Pumpkin leaf and water leaf had concentration values of (246.35 µg g^{-1}) and (246.00 µg g^{-1}) respectively. Sulphate anion showed maximum concentration value in scent leaf (255.00 µg g^{-1}) and minimum value in green leaf (135.35 µg g^{-1}). The concentration of chloride ion was approximately equal in scent leaf (85.45 µg g^{-1}) and pumpkin leaf (85.32 µg g^{-1}). Green leaf and bitter leaf had values of (50.25 µg g^{-1}) and (50.45 µg g^{-1}) respectively.

Results obtained from the samples in the rainy season were higher than in the dry season. Maximum nitrate concentration was found in scent leaf (225.2 µg g^{-1}) while the minimum concentration value was found in green leaf (96.00 µg g^{-1}). Nitrite concentration ranged from (86.60 µg g^{-1}) scent leaf to (17.95 µg g^{-1}) green leaf. Phosphate concentration had maximum value in scent leaf (343.82 µg g^{-1}) and minimum concentration in green leaf (250.11 µg g^{-1}). Sulphate ion had maximum value in scent leaf (265.21 µg g^{-1}) and green leaf (265.1 µg g^{-1}) respectively and minimum concentration was found in green leaf (192.14 µg g^{-1}). Chloride ion showed maximum value in scent leaf (88.51 µg g^{-1}), pumpkin leaf and water leaf had concentrations of (86.00 µg g^{-1}) and (86.25 µg g^{-1}) respectively. Minimum concentration was found in bitter leaf (85.41 µg g^{-1}) and green leaf (85.42 µg g^{-1}).

From table 9, descriptive statistics for nitrate and nitrite during both seasons in Ozoro suggests that the highest mean and standard error mean was recorded during the rainy season.

Result of Analysis of Variance (ANOVA) showed that variation between vegetables from the two seasons were statistically significant ($p \leq 0.05$).

Transfer factor is used to identify the efficiency of a vegetable type to accumulate a given anion or metal from the soil, this factor is based on the root uptake of the vegetable and is dependent on a number of factors such as the species of the particular vegetable and the xylem structure of the plant. The value for the transfer factor for nitrate and nitrite is shown in table 6. In Agbor samples, scent leaf had the highest value for transfer factor of 0.94 µg/g while green leaf had the lowest value during the dry season, bitter leaf had the highest value during the rainy season. The value for the transfer factor of the other anions is shown in table 7. It is important to note that the values for transfer factor cannot be controlled as different species have different uptake capabilities.

The toxicity of nitrate is thought to be due to its reduction to nitrite and conversion to nitrosamines and nitrosamides through reaction with amines and amides, whose carcinogenic action is well known. (Walker, 1990). In a joint report by the WHO and FAO of the United Nation, the Acceptable Daily Intake (ADI) of nitrate was 3.65 mg NO_3 Kg^{-1} body weight while nitrite was put at 0.2 mg NO_2 Kg^{1} body weight (Zhong *et al.,* 2002). From the results of this analysis, the concentration of nitrate and nitrite in the entire vegetable samples did not exceed the WHO regulation limit. The low levels of nitrate and nitrite might be ascribed to regulated application of fertilizer and low absorption of this nitrogenous fertilizer by the roots of vegetables.

Excessive phosphorus intake typical of modern diet has been reported to be associated with the cause of hyperphosphatemia, hypocalcaemia, osteoporosis and soft tissue calcinosis (Draper *et al*., 1979). Observations have however shown that every individual has different tolerance level to the amount of dietary phosphate and sulphate which when exceeded could result to behavioral problems, skin disorder, susceptibility to allergies or may even lead to several disorders simultaneously in a highly sensitive person. The Acceptable Daily Intake of dietary phosphate, sulphate and chloride is 70.0, 90.0 and 230 mg kg^{-1} body weight (WHO 1987). From the results of this analysis, the concentration of phosphate and sulphate in the entire vegetables were below limit set by WHO.

The permissible limit set by FAO/WHO for heavy metals are 0.3 mg/kg iron (Fe), 0.1 mg/kg lead (Pb) ,0.1 mg/kg copper (Cu), 0.1 mg/kg zinc (Zn) and 0.02 mg/kg cadmium (Cd). Table 5 indicates that the heavy metal concentration in the vegetable samples are higher than the permissible limits. This is because vegetable farming in the selected sampling sites includes application of manure and chemical fertilizers which have been reported to be a source of metals in foods and vegetables (Allowa 1995). At the same time, most of the vegetable farms are located along highways which might be receiving deposits of metals from vehicle emissions, runoff water from mechanic workshops also find their way to vegetable farms. Heavy metals are toxic because they react with bodies biomolecules, clog up receptor sites, break and bend sulphur bonds in important enzyme such as insulin and damage the DNA (Abdul 2012). For instance, most of the accumulated Pb in a body is sequestered in the skeleton where it will have a half life of 20 to 30 years. The high gastrointestinal uptake and the permeable blood barrier make children more susceptible to Pb exposure than adults. Cd is carcinogenic even in low concentrations and renal effects may also occur due to sub chronic consumption of Cd. (Mico et al 2006). Zn, Cu and Fe are considered essential components of biological activities in the body, however, in excess are reported to cause problems to humans (Luo *et al.*, 2009).

Transfer factor is used to identify the efficiency of a vegetable type to accumulate a given anion or metal from the soil, this factor is based on the root uptake of the vegetable and is dependent on a number of factors such as the species of the particular vegetable and the xylem structure of the plant. The value for the transfer factor for nitrate and nitrite is shown in table 6. In Agbor samples, scent leaf had the highest value for transfer factor of 0.94 µg/g while green leaf had the lowest value during the dry season, bitter leaf had the highest value during the rainy season. The value for the transfer factor of the other anions is shown in table 7. It is important to note that the values for transfer factor cannot be controlled as different species have different uptake capabilities.

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CHAPTER FIVE- CONCLUSION

At the end of this research work the following conclusions were made

1. The concentration of the anions (nitrate, nitrite, sulphate, phosphate and chloride) investigated is within the range recommended by Joint Expert Committee on Food Additives (JEFA) and FAO/WHO (Food and Agriculture Organization.

2. The heavy metal concentration (lead, cadmium, copper, zinc and iron) in the vegetable samples are higher than the permissible limits

3. In Agbor samples, Statistical test of significance using Analysis of Variance (ANOVA) revealed marked difference ($p<0.05$) between the anion contents of all the leafy vegetables in the dry and rainy seasons.

4. Analysis of Variance (ANOVA) of Abraka samples revealed a marked significant difference of $p \leq 0.05$ between the two seasons.

5. Result of Analysis of Variance (ANOVA) of Ozoro samples showed that variation between vegetables from the two seasons were statistically significant ($p \leq 0.05$).

5.2 RECOMMENDATION

Based on my findings in this research work, I would like to make the following recommendations:

1. The use of fertilizers in vegetable farms should be properly regulated and monitored to avoid over use.
2. The government should organize seminars that will educate vegetable farmers on proper procedures for the application of fertilizers in their farms and ensure that the procedures are adhered to strictly.
3. Vegetable farmers should be educated on the adverse post consumption effects that eating of over fertilized vegetables will have on their customer's health and general wellbeing. This knowledge may help to checkmate excesses.
4. As much as possible, vegetable farms should be sited away from factories and industrialized places as this will help reduce exposure to heavy metal contamination.
5. Analysis on anion and heavy metal concentration on other vegetables should be carried out so as to provide more balanced information on a wider range of vegetables.

5.3 Contribution to knowledge

This research work has provided information on anion and heavy metal concentrations in some vegetables consumed in Delta State and have shown that anion concentrations in the samples were within the allowed limit even as their heavy metal content exceeded the limit set by the WHO. Therefore efforts should be geared towards providing a remedy for this discovery.

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