KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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LEVELS OF SOME HEAVY METALS IN OMEGA-3 FOOD SUPPLEMENTS.

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANALYTICAL CHEMISTRY.

BY

ADOLF OTI-BOAKYE

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DECLARATION

It is hereby declared that this thesis is the outcome of research work undertaken by the author. Any assistance obtained has been duly acknowledged. It is neither in part nor whole been presented for another degree elsewhere.

HEAD OF DEPARTMENT SUPERVISOR

…… …….

(DATE:) (DATE:)

DR. J.A.M AWDZA MR. JOSEPH APAU

CANDIDATE

……..

(DATE)

ADOLF OTI-BOAKYE

DEDICATION

I dedicate this project to my parents, Mr. & Mrs. Oti, as well as my sweetheart Angela for their help in diverse ways towards the success of my studies. May the good Lord grant them long life and bless them in abundance to enjoy the good fruit of their labour. Amen.

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ABSTRACT

Omega-3 fatty acids also called essential fatty acids are known to have numerous benefits from pregnancy to old age. The presence of heavy metals in the fishes which are the main sources of the omega-3 acids however poses a serious health threat. Levels of six heavy metals consisting of four essential ones (Iron, Manganese, Zinc and Copper) and two toxic ones (Mercury and Cadmium) were determined in one hundred (100) samples consisting of ten (10) different brands of omega-3 food supplements available on the Ghanaian market using Cold Vapour Atomic Absorption Spectrometry for mercury and Flame Atomic Absorption Spectrometry for iron, manganese, cadmium, zinc and copper. The amount of the metals consumed daily in µg/g in each brand was calculated based on the mean concentrations of the metals. The calculated daily intake of mercury in µg/g ranged from 0.021 for Cardioace brand to 0.818 for Dr. Chris brand with Iron ranging from 6.844 µg/g for Artic Sea brand to 1845.756 µg/g for Joint Care. Manganese ranged from 0.835 µg/g for Alaska brand to 488.475 µg/g for Cardioace brand and Cadmium ranged from 0.531 µg/g for Deep Sea brand to 4.712 µg/g for Seven Seas brand. The calculated daily intake of Zinc ranged from 0.812 µg/g for High Sea brand to1816.380 µg/g for Cardioace brand whilst that of Copper ranged from 0.073 µg/g for Artic Sea brand to 732.825 µg/g for Cardioace brand. Except cardioace brand, which recorded zinc as the highest metal, iron was the highest metal recorded for all the omega-3 brands. Also except the Seven Seas, Artic Sea, and Alaska brands which had copper as the lowest metal, mercury was the lowest metal recorded for all the brands.The calculated daily intakes determined for the metals were below the daily intakes recommended by the Joint FAO/WHO expert committee on food supplements and vitamins showing that the

omega-3 food supplements analysed in this study are not likely to pose any health risk due to metal level to the public through consumption for both the toxic metals and the essential metals determined.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

1.0 INTRODUCTION

1.1 STATEMENT OF PROBLEM

The first notion that omega-3 fatty acids can be beneficial was realized back in the early 1970s when Hans Olaf Bang and Jorn Dyerberg (Dyeberg and Bang, 1972) searched for the reason behind the known rarity of thrombotic diseases, especially ischaemic heart disease, in Greenland Eskimos and found that fish-based Eskimo diet, rich in docosahexaenoic acid (DHA) and another omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) were suggested to be responsible (Stillwell and Wassall, 2003). Since that time many research have been conducted on the omega-3s and have revealed a wide range of health benefits which have been divided into six categories: heart disease, cancer, immune problems, neuronal functions, aging and "other" hard to categorize problems such as migraine headaches, malaria and sperm fertility (Weber *et al.*, 2006).

As one might expect, the reputed health properties and apparent versatility of the Omega -3 has not escaped the keen eyes of the pharmaceutical industry. Each year new applications employing the omega-3 fatty acids become commercially available. Generally, the omega-3 containing products are of three types; as a supplement, as a component of infant formulas and as a component of parenteral (intravenous) and enteral (feeding tube) nutrition.

Historically the primary source of omega-3 has been the oils of cold-water fish, such as tuna, salmon, trout, herring, sardines, bass, swordfish and mackerel (Weber *et al.*, 2006). These fishes do not actually make the omega-3s themselves, but get them from feeding on the algae, which are the ultimate biological source.

Omega-3 fatty acids belong to a class of fatty acids that are called essential fatty acids (EFAs). They are so called because the body cannot produce them and thus must be obtained from the diet (Weber *et al.*, 2006). Three major nutritionally important omega-3 fatty acids that are ingested in foods and used by the body are; Alpha-linolenic acid (ALA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) with Alpha-linolenic acid (ALA) being the primary omega-3 fatty acid (Weber *et al.*, 1986). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been receiving a lot of attention lately because of their cardio-protective and other so called "pleiotropic" effects (Weber *et al.*, 1986).

Minerals (inorganic nutrients) are materials found in foods that are essential for growth and health and do not contain the element carbon. These include water, sodium, potassium, chloride, calcium, phosphate, sulphate, magnesium, iron, copper, zinc, manganese, iodine, selenium, and molybdenum etc. They are classified as either micro or macro nutrients. The micro elements mostly fall under heavy metals and are needed by the body in smaller amounts. Examples of such micronutrients are cobalt, copper, zinc, manganese iron etc. The macronutrients are however required in greater amounts by the body. Examples include potassium, magnesium and calcium (Odell and Sunde, 1997).

Much as we need these minerals for a healthy living, some of these minerals in higher or lower quantities than required may be damaging to health. Living organisms require trace amounts of some heavy metals, such as cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc, but excessive levels can be detrimental to the organism. These are termed as beneficial heavy metals. These elements, or some forms of them, are commonly found naturally in foodstuffs, in fruits, vegetables and animals, and in commercially available multivitamin products or food supplements (Schrauzer, 1984) and as well as in the environment such as the soil and water. Other heavy metals such as mercury, plutonium, cadmium and lead are toxic metals and have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of animals can cause serious health effects (Boeing, 2000).

There is a strong link between micronutrient uptake by fish and their products, and the impact of contaminations on humans (De Leonardis *et al.*, 2000; Yuzbasi *et al.*, 2003). Some toxic elements under certain conditions can be beneficial. Examples include vanadium, tungsten, and even cadmium (Lane and Morel, 2000; Lane *et al.*, 2005).

In medical usage, heavy metals are loosely defined and includes all toxic metals irrespective of their atomic weight and "heavy metal poisoning" includes excessive amounts of iron, manganese, aluminium, or even beryllium (the fourth lightest element) (Duffus, 2002).

Some of these metals are naturally found in the body and are essential to human health. Iron, for example, prevents anaemia, and zinc is a cofactor in over 100 enzyme reactions.

However, high levels of zinc can result in a deficiency of copper, another metal required by the body.

Trace metals therefore, are metals in extremely small quantities, that are found in animal and plant cells and tissues. They are a necessary part of good nutrition, although they can be toxic if ingested in excess quantities (Underwood, 1977).

Trace metals are depleted through the expenditure of energy by living organisms. They are replenished in animals by eating plants, and replenished in plants through the uptake of nutrients from the soil in which the plant grows. Human vitamin pills (food supplements) and plant fertilizers both contain trace metals as additional sources for trace metals (Schrauzer, 1984).

Heavy or trace metals have positive and negative roles in human life (Adriano, 1984; Divrikli *et al*., 2003). Some of the heavy metals such as iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are considered essential but can become harmful above certain levels. Some metals like cadmium (Cd) and mercury (Hg) are very toxic even at very low levels.

Copper acts as a catalyst in the formation of haemoglobin, the oxygen-carrying blood component (Lahey, 1975). The highest concentrations in the body tissue are found in the liver and certain areas of the central nervous system, particularly, the brain.

The presence of copper in the body helps in the oxidization of glucose and release energy, absorption of iron, secretion and balance of hormones by the thyroid gland, and assists in supplying the body's tissues with oxygen. It is also needed for the functioning of the amino acid, tyrosine, synthesis of red blood cells and the hormone, adrenaline.

Physical symptoms of low copper include, low oxygen in the cells, lowered levels of HDL cholesterol, skin problems, swollen ankles, anaemia, cells suffocation and oxygen loss as well as low enkephalins produced in the brain. Psychological symptoms of low copper include auditory hallucinations and depression.

Copper levels are more often too high than being too low. High copper concentrations can be toxic. It causes headaches, hypoglycemia, increased heart rate, nausea, inhibits urine production, anaemia, hair loss in women, damage to the kidneys, brain and liver (Nolan, 1983). High copper levels in children is associated with hyperactive behaviour, learning disorders such as dyslexia, and ear infections. High copper levels interferes with zinc, which is needed to manufacture digestive enzymes. Many people with high copper levels dislike proteins and are drawn to high-carbohydrate diets because they have difficulty digesting protein foods (Narang *et al*., 1991).

Manganese is a trace mineral in human bodies. About 20 milligrams of manganese is found in the body and mostly concentrated in bones, kidneys, liver and pancreas (ATSDR, 2008b). Manganese, which has antioxidant, free-radical-fighting properties, is important for proper food digestion and for normal bone structure. Manganese helps reduce fatigue, prevents the incidence and severity of osteoporosis, and even improves memory. Manganese also helps the body to convert protein and fat to energy. It promotes normal bone growth, helps maintain healthy reproductive, nervous, and immune systems, and is involved in blood sugar regulation. In addition, manganese is involved in blood

clotting and the formation of cartilage and lubricating fluid in the joints (ATSDR, 2008b).

Manganese levels have also been shown to vary with the menstrual or estrus cycle in humans and animals, and low manganese intakes are associated with disruption of reproduction in animals (Penland and Johnson, 1993). Manganese deficiency has been linked to bone malformation, weakness, seizures, atherosclerosis, confusion, convulsions, eye problems, hearing problems, heart disorders, high cholesterol levels, hypertension, irritability, memory loss, muscle contractions, pancreatic damage, profuse perspiration, rapid pulse, tooth-grinding, tremors, and osteoporosis. Manganese is also linked to decreased superoxide dismutase activity in white blood cells, which leaves the body more vulnerable to the damaging effects of free-radicals (ATSDR, 2008b).

Zinc is an essential mineral present in nearly every cell of the body. In addition, the body requires zinc for immunity, wound healing and maintaining senses of taste and smell.

Zinc stimulates the work of about 100 enzymes that keep the normal body function and performs both structural and catalytic functions in many different enzymes as well as different metabolic processes such as the synthesis of the nucleic acids RNA and DNA (ATSDR, 2005). It is required for the transport of vitamin A from the liver, and as part of superoxide dismutase, helps protect cells from free radicals. Zinc is also required for normal growth and development, reproductive development and function, and to support the immune system, where it has been shown to increase T-lymphocytes and enhance

other white blood cell functions. However, because of its effect on increasing white blood counts, higher intake of zinc (unless low) is contraindicated with leukaemia.

Low levels of zinc causes lung cancer, neural tube defects, slow growth, and anorexia. A study involving cancer showed maternal levels of zinc were decreased in women who bore children with neural tube defects (Hansen, 1983).

Iron is essential for the formation of haemoglobin, the chemical in the blood that carries oxygen to the cells. Low levels of iron cause anaemia (Lahey, 1975). Severe cases of iron deficiency results in children becoming flabby with abnormal growth. Milder cases of iron deficiency may not produce any physical symptoms, but children may learn at a slower pace than children with a proper amount of iron in their diet. The combination of rice, beans, and meat consumed with fresh citrus fruit provides an excellent source of absorbable iron (Gillooly *et al*., 1983).

Ingestion accounts for most of the toxic effects of iron because iron is absorbed rapidly in the gastrointestinal tract. The corrosive nature of iron seems to further increase the absorption (Hallberg *et al*., 1997). Most overdoses appear to be the result of children mistaking red-coated ferrous sulphate tablets or adult multivitamin preparations for candy. Other sources of iron are drinking water, iron pipes, and cooking ware. Target organs are the liver, cardiovascular system, and kidneys.

Cadmium is a by-product of the mining and smelting of lead and zinc. It is used in nickel-cadmium batteries, PVC plastics, and paint pigments. It can be found in soils because they are found in insecticides, fungicides, sludge, and commercial fertilizers that are used in agriculture. Cadmium may be found in reservoirs containing shellfish. Cigarettes also contain cadmium. Lesser-known sources of exposure are dental alloys, electroplating, motor oil, and exhaust. Inhalation accounts for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Target organs are the liver, placenta, kidneys, lungs, brain, and bones (ATSDR, 2008a).

Mercury (Hg) is one of the most toxic heavy metals in our environment including the lithosphere, hydrosphere, atmosphere and biosphere (Zhang and Wong, 2007).

Human activities release mercury into the air, water and soil. Mercury emissions into the environment can be from volcanic emissions, degassing from the earth's crust and oceans cycling of geological bound mercury, laboratories, municipal waste, combustors in industries, chlor-alkali plants, agricultural activities, commercial and industrial boilers, gold mining and hosts of others.

Micro bacteria convert the inorganic mercury in the water and sediments into highly toxic methylmercury. Through bioconcentration and bioaccumulation mercury can be deposited into the aquatic system and eventually threaten human health by fish consumption (Boeing, 2000).

The general population is most commonly exposed to mercury primarily from two sources:

- eating fish and marine mammals (e.g., whales, seals) that may contain some methylmercury in their tissues or
- the release of elemental mercury from the dental amalgam because it may dissolve in saliva and be ingested (Sallsten *et al*., 1996).

Mercury has no known biological function and is highly toxic to human (William *et al*., 1999). Depending on the chemical form and the dose received, mercury can be toxic to both human and wildlife. In people toxic doses can cause developmental effects in the foetus, as well as on the kidney and the nervous system in children and adults (US EPA, 1984). The nervous system is one of the most sensitive targets following an exposure to mercury and may cause cognitive, personality, sensory or motor disturbances (WHO, 1989a). Mercury poisonings have been caused by consumption of fish and fish products such as in the case of Minamata disease due to local villagers eating fishes containing high levels of methylmercury. The fishes were found from a body of water in Japan that was polluted by industrial effluent containing high concentration of methylmercury. The local villagers began to exhibit signs of neurologic effects such as visual loss, extreme numbness, hearing loss, and ataxia. Even babies exposed to the methylmercury in the uterus were the most severely affected members of the village. The exposure continued even after birth because it was discovered that methylmercury was found in the breast milk of the mothers (Yoshino *et al*., 1966).

1.3 JUSTIFICATION OF OBJECTIVES

People are exposed to heavy metals primarily by consuming fish and fish products. Heavy metal toxicity and their dangers to people is well established in scientific literature and several cases of heavy metal and particularly mercury toxicity in the environment have been reported (ATSDR, 2006). The most serious example is the case in Minamata Bay area of Japan from1953-1960, where mercury released from manufacturing plants contaminated fish and shellfish. Mercury levels of 5-20ppm were found in seafood eaten by 111people diagnosed with "Minamata disease" of which 45 people died due to the poisoning (Yoshino *et al*., 1966). Monitoring levels of heavy metals in fish and fish products has therefore become a matter of great importance in the world since then.

Thus, the benefits of micronutrients may be completely reversed if present at either higher or lower levels than the required levels. Therefore, the World Health Organisation (WHO) has established levels for metals in food above which, it should not be consumed. For this reason, levels of trace metals in whatever consumed should be of much importance and concern.

The main source of omega-3 has been the oils of coldwater fish such as tuna, salmon, trout, herring, sardines, bass, swordfish and mackerel which acquire the omega-3s from feeding the algae, which are the ultimate biological source (Weber *et al*., 1986). Studies have revealed that since these coldwater fishes are known to have high levels of heavy metals in them, there is the potential of metal poisoning in the course of people taking the omega-3s (Mahaffey, 2004).

Pharmaceutical industries in their quest to explore the numerous benefits of omega-3 fatty acids have made several preparations of omega-3 fatty acids commercially available.

Studies assessing the combined benefits of omega-3 fatty acids and the adverse effects of heavy metal exposure are not available in Ghana and that people taking omega-3s might end up exposing themselves to the effects associated with toxic metals. Due to lack of research and inadequate data on the levels of heavy metals in Omega-3 food supplements available on the Ghanaian market, this research seeks to find whether the omega-3 food supplements on the Ghanaian market is safe for human consumption.

1.1 RESEARCH OBJECTIVES

This research seeks to determine the level of some selected heavy metals in Omega -3 food supplements available on the Ghanaian market in order to ascertain their safety for human consumption. The following parameters shall be the indices of quality assessment and their respective determinations shall be the specific objectives of the study;

- Determination of the selected heavy metals levels
- To find out if the levels of the metals are of potential human health concern.

CHAPTER TWO

2.0 LIERATURE REVIEW

2.1 FACTS ABOUT OMEGA-3 FATTY ACIDS

Omega-3 fatty acids belong to a class of fatty acid called polyunsaturated fatty acids (PUFAs) found in certain foods. Omega-3 fatty acids cannot be made in the body and must be obtained from food. They are therefore known as essential fatty acids.

Polyunsaturated fats can be further divided into two groups based on the position of the first double bond: omega-3 fatty acids and omega-6 fatty acids. These numbers refer to the location of the first double bond in these unsaturated fatty acids, counting from the carbon on the methyl (omega) end of the compound. Alpha-linolenic acid (ALA) is the primary omega-3 Essential Fatty Acid (EFA) whilst linoleic acid (LA) is the primary omega-6 EFA (Weber *et al*., 1986).

2.2 SOURCES OF OMEGA 3 FATTY ACIDS

The long-chain omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be acquired mainly from sea foods in oily cold-water fish, such as tuna, salmon, trout, herring, sardines, bass, swordfish, and mackerel. With the exception of seaweed, most aquatic plants do not contain EPA or DHA. EPA and DHA are synthesized mainly by both unicellular and multicellular marine plants such as phytoplankton and algae (Pigott *et al*., 1987). They are eventually transfered through the

food chain and are incorporated into lipids of aquatic species such as fish and marine mammals, particularly those living in the cold waters at low temperatures, due to the ability of these fatty acids to maintain fluidity in such environments (Holmer, 1989).

Plant sources of Omega-3 consist of flaxseed oil, hemp oil, soybeans, navy beans, walnuts and tofu (soy protein). Plant sources also include series of chain elongation and desaturation long chain fatty acid of ALA to give DHA and EPA to be utilized by the body (Sprecher *et al*., 1995). Plant sources are however considered an oblique source and may not be as efficient as fish or fish oil which is the direct source. Other sources of omega-3 fatty acids include foods such as egg, cereals, and even peanut butter (Sprecher *et al*., 1995).

2.3 IMPORTANCE OF OMEGA- 3 FATTY ACIDS

Omega-3 fatty acids are nutrients that perform key functions in our bodies. They are highly concentrated in the brain and appear to be particularly important for cognitive (brain memory and performance) and behavioral function (Weber *et al.,* 2006). For example, they determine membrane fluidity and reactivity, oxidation rate, metabolic rate, and energy production. In addition, they are a factor in maintaining body temperature, insulating nerves, and cushioning body tissue (Weber *et al.,* 2006). They are precursors to prostaglandins, hormone-like substances that are critical to the body's overall health maintenance (Gibson *et al*., 1994). Omega-3 fatty acids regulate blood pressure, blood clotting, stimulation of the immune system, and general regulation of heart, kidneys,

liver, lungs, and brain (Weber *et al.*, 2006). They inhibit thickening of the arteries by decreasing endothelial cells' production of a platelet-derived growth factor (the lining of the arteries is composed of endothelial cells). Omega-3s also increase the activity of another chemical derived from endothelial cells (endothelium-derived nitric oxide), which causes arteries to relax and dilate (Gibson *et al*., 1994).

Omega-3 fatty acids also reduce the production of messenger chemicals called cytokines, which are involved in the inflammatory response associated with atherosclerosis (Manku, 1983). They also reduce the risk of becoming obese and improve the body's ability to respond to insulin by stimulating the secretion of leptin, a hormone that helps regulate food intake, body weight and metabolism, and is expressed primarily by adipocytes (fat cells). Omega -3s also help to prevent cancer cell growth (Stillwell and Wassall, 2003).

2.4 OTHER ESSENTIAL FATTY ACIDS

The other types of the EFAs are the omega-6 fatty acids which have the first double bond in their structures located on the sixth carbon counting from the carbon on the methyl (omega) end of the compound. EFAs are fatty acids with a double bond before the 9th position from the omega. The human body cannot form carbon to carbon double bonds before the 9th carbon from the omega position hence the inability of the human body to produce the EFAs.

It has been estimated that the present Western diet is "deficient" in omega-3 fatty acids with a ratio of omega-6 to omega-3 of 15–20:1, instead of the expected 1:1 (Crawford, 1968). Many experts agree that it is important to maintain a healthy balance between omega-3 fatty acids and omega-6 fatty acids. Studies have revealed that over-consumption of omega-6 fatty acids poses a lot of problems since some of the beneficial effects gained from omega-3 fatty acids consumption are negated by an over-consumption of omega-6 fatty acids (Geleijnse *et al*., 2002). For example, omega-3 fatty acids have anti-inflammatory properties, whereas omega-6 fatty acids tend to promote inflammation. Cereals, whole grain bread, margarine, and vegetable oils, such as corn, peanut, and sunflower oil, are examples of sources of omega-6 fatty acids. In addition, people consume a lot of omega-6 fatty acid simply by eating the meat of animals that were fed with grains rich in omega-6. Some experts suggest that eating one to four times more omega-6 fatty acids than omega-3 fatty acids is a reasonable ratio. In other words, as dietitians often say, the key to a healthy diet is moderation and balance (Geleijnse *et al*., 2002).

2.5 CONVERSIONS

The conversion of ALA which is the primary omega-3 to EPA is of much interest because the cardioprotective effects of omega-3 have been most rigorously studied and closely associated with EPA. This conversion may explain, in part or in whole, ALA's potential benefit. Isotope-labeled ALA feeding trials have shown the conversion of ALA

to EPA to vary between 0.2% and 21% and that of ALA to DHA to vary between 0% and 9% (Boden, 2005).

Most feeding studies that measure internal changes in membrane fatty acid composition show that ALA feeding will lead to an increase in EPA but has a null effect or slight decrease in DHA levels (Boden, 2005). These studies, however, are somewhat limited because the conversion of ALA to EPA and DHA is likely influenced by multiple factors including, sex, competitive inhibition of desaturase by linoleic acid, negative feedback inhibition of desaturase by EPA and DHA, and timing of the sample collection. Studies reveal that ALA's cardioprotective properties were inversely related to EPA and DHA intake and that ALA's cardioprotective properties are contingent on conversion to EPA and DHA which is inhibited by EPA and DHA intake (Mozaffarian *et al*., 2005).

2.6 HEALTH BENEFITS OF OMEGA- 3 FATTY ACIDS

Clinical studies suggest that omega-3 fatty acids may be helpful in treating a variety of health conditions. The evidence is strongest for heart disease and problems that contribute to heart disease, but the range of possible uses for omega-3 fatty acids include:

2.6.1 High cholesterol control

Those who follow a Mediterranean-style diet tend to have higher levels of high density lipoprotein (HDL or "good") cholesterol levels. Similar to those who follow a

Mediterranean diet, Inuit Eskimos, who consume high amounts of omega-3 fatty acids from fatty fish, also tend to have increased HDL cholesterol and decreased triglycerides (fatty material that circulates in the blood) (National Cholesterol Education Program, 2002). In addition, fish oil supplements containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported in several large clinical studies to reduce low density lipoprotein (LDL or "bad") cholesterol and triglyceride levels (Institute of Medicine, 2002). Finally, walnuts (which are rich in alpha linolenic acid or ALA) have been reported to lower total cholesterol and triglycerides in individuals with high cholesterol levels.

2.6.2 High blood pressure

Several clinical studies suggest that diets or supplements rich in omega-3 fatty acids lower blood pressure significantly in individuals with hypertension (Geleijnse *et al*., 2002). An analysis of 17 clinical studies using fish oil supplements revealed that supplementation with 3 or more grams of fish oil daily can lead to significant reductions in blood pressure in individuals with untreated hypertension. A meta analysis of 36 randomized trials found that fish-oil intake (median dose 3.7 g/day EPA plus DHA) reduced systolic blood pressure by 2.1mm Hg ($P < 0.01$) and diastolic blood pressure by 1.6mm Hg ($P < 0.01$) (Geleijnse *et al*., 2002). At least two mechanisms could account for this effect. First, incorporation of EPA and DHA into membrane phospholipids could increase systemic arterial compliance (Nestel *et al*., 2002). Second, EPA and DHA could improve endothelial function (Chin *et al.,* 1995). This is consistent with the observation

that the antihypertensive effect of fish oil may be greater in populations with arterial stiffness and/or microvascular dysfunction, i.e. populations with hypertension and older populations (Geleijnse, 2002). In addition, a meta analysis of 30 randomized trials found that fish-oil intake (median dose 3.5 g/day EPA plus DHA) reduced heart rate by 1.6 bpm compared with placebo (P = 0.002) (Mozaffarian *et al*., 2005).

2.6.3 Heart disease

Omega-3 fatty acids have been shown to exert cardioprotective effects in both primary and secondary coronary heart disease (CHD) prevention trials (Mozaffarian and Rimm, 2006). One of the best ways to help prevent and treat heart disease is to eat a low-fat diet and to replace foods rich in saturated and trans-fat with those that are rich in monounsaturated and polyunsaturated fats (including omega-3 fatty acids). Clinical evidence suggests that EPA and DHA found in fish oil help reduce risk factors for heart disease including high cholesterol and high blood pressure (Calder, 2004). There is also strong evidence that these substances can help prevent and treat atherosclerosis by inhibiting the development of plaque and blood clots, each of which tends to clog arteries (Calder, 2004). Clinical studies of heart attack survivors have found that daily omega-3 fatty acid supplements dramatically reduce the risk of death, subsequent heart attacks, and stroke. Similarly, people who eat an ALA-rich diet are less likely to suffer a fatal heart attack (Calder, 2004).

2.6.4 Diabetes

Individuals with diabetes tend to have high triglyceride and low HDL levels. Omega-3 fatty acids from fish oil can help lower triglycerides and apoproteins (markers of diabetes), and raise HDL, so people with diabetes may benefit from eating foods or taking supplements that contain DHA and EPA (American Diabetes Association, 2007). ALA (from flaxseed, for example) may not have the same benefit as DHA and EPA because some people with diabetes lack the ability to efficiently convert ALA to a form of omega-3 fatty acids that the body can use readily. There have been reported cases of decrease in blood sugar levels in patients with type 2 diabetes while taking fish oil supplements (American Diabetes Association, 2007).

2.6.5 Weight Loss

Appetite control is one of the most important factors involved in the success of dietary treatment of obesity. Due to its relation with energy balance, the consumption of omega-3s have a role in appetite control and hence the prevention of obesity (Abete *et al.*, 2006). Several nutritional approaches concerning energy restriction, nutrient distribution and subject characteristics have been investigated in order to achieve weight loss and maintenance in people with excess body weight (Goyenechea *et al.*, 2006). Different dietary strategies have been under research with the aim to modify nutritionally the perception of satiety and hunger after eating. An increased protein intake (Leidy *et al.*, 2007), diet-related ketosis (Boden *et al.*, 2005) and low glycemic index food consumption (Kaplan and Greenwood, 2002) have been suggested to decrease appetite.

2.6.6 Arthritis

Most clinical studies investigating the use of omega-3 fatty acid supplements for inflammatory joint conditions have focused almost entirely on rheumatoid arthritis. Several articles reviewing the research in this area concluded that omega-3 fatty acid supplements reduce tenderness in joints, decrease morning stiffness, and allow for a reduction in the amount of medication needed for people with rheumatoid arthritis (Kremer, 2000).

In addition, laboratory studies suggest that diets rich in omega-3 fatty acids (and low in the inflammatory omega-6 fatty acids) may benefit people with other inflammatory disorders, such as osteoarthritis (Danao-Camara and Shintani, 1999). In fact, several test tube studies of cartilage-containing cells have found that omega-3 fatty acids decrease inflammation and reduce the activity of enzymes that destroy cartilage (Curtis *et al.*, 2000). Similarly, New Zealand green lipped mussel (*Perna canaliculus*), another potential source of omega-3 fatty acids, has been reported to reduce joint stiffness and pain, increase grip strength, and enhance walking pace in a small group of people with osteoarthritis (Connor *et al.*, 1997). In some participants, symptoms worsened before they improved.

2.6.7 Osteoporosis

Clinical studies suggest that omega-3 fatty acids such as EPA help increase levels of calcium in the body, deposit calcium in the bones, and improve bone strength (Kruger *et al.*, 1998). In addition, studies also suggest that people who are deficient in certain essential fatty acids (particularly EPA and gamma-linolenic acid [GLA], an omega-6

fatty acid) are more likely to suffer from bone loss than those with normal levels of these fatty acids (Horrobin *et al.,* 1999).

2.6.8 Depression

People who do not get enough omega-3 fatty acids or do not maintain a healthy balance of omega-3 to omega-6 fatty acids in their diet may be at an increased risk for depression (Hibbeln *et al.,* 1995). The omega-3 fatty acids are important components of nerve cell membranes. They help nerve cells communicate with each other, which is an essential step in maintaining good mental health (Horrobin *et al*., 1999). In particular, DHA is involved in a variety of nerve cell processes (Edwards *et al.,* 1998).

2.6.9 Bipolar disorder

In a clinical study of 30 people with bipolar disorder, those who were treated with EPA and DHA (in combination with their usual mood stabilizing medications) for four months experienced fewer mood swings and recurrence of either depression or mania than those who received placebo (Stoll *et al*., 1999). Another four months long clinical study treating individuals with bipolar depression and rapid cycling bipolar disorder did not find evidence of efficacy for the use of EPA in these patients.

2.6.10 Schizophrenia

Preliminary clinical evidence suggests that people with schizophrenia experience an improvement in symptoms when given omega-3 fatty acids (Fenton *et al*., 2001). However, a recent well-designed study concluded that EPA supplements are no better than placebo in improving symptoms of this condition (Laugharne *et al*., 1996). The conflicting results suggest that more research is needed before conclusions can be drawn about the benefit of omega-3 fatty acids for schizophrenia. Similar to diabetes, individuals with schizophrenia may not be able to convert ALA to EPA or DHA efficiently (Fenton *et al*., 2001).

2.6.11 Attention deficit/hyperactivity disorder (ADHD)

Children with attention deficit/hyperactivity disorder (ADHD) may have low levels of certain essential fatty acids (including EPA and DHA) in their bodies (Arnold *et al*., 1994). In a clinical study of nearly 100 boys, those with lower levels of omega-3 fatty acids demonstrated more learning and behavioral problems (such as temper tantrums and sleep disturbances) than boys with normal omega-3 fatty acid levels (Stevens *et al*., 1996). Clinical studies that examine the ability of omega-3 supplements to improve symptoms of ADHD are still needed. At this point in time, eating foods high in omega-3 fatty acids is a reasonable approach for someone with ADHD (Baumgaertel, 1999). A clinical study used omega-3 and omega-6 fatty acid supplementation in 117 children with ADHD. The study found significant improvements in reading, spelling, and behavior in the children over the 3 months of therapy. Another clinical study found that omega-3

fatty acid supplementation helped to decrease physical aggression in school children with ADHD (Puri *et al*., 2000).

2.6.12 Burns

Essential fatty acids have been used to reduce inflammation and promote wound healing in burn victims. Animal research indicates that omega-3 fatty acids help promote a healthy balance of proteins in the body. Protein balance is important for recovery after sustaining a burn (De-Souza *et al*., 1998). Further research is necessary to determine whether omega-3 benefit people in the same way.

2.6.13 Skin disorders

In one clinical study, 13 people with a particular sensitivity to the sun known as photo dermatitis showed significantly less sensitivity to UV rays after taking fish oil supplements (Hayashi *et al*., 1999). Research indicates that topical sunscreens are however much better at protecting the skin from damaging effects of the sun than omega-3 fatty acids. In another study of 40 people with psoriasis, those who were treated with medications and EPA supplements did better than those treated with the medications alone (Boelsma *et al*., 2001). In addition, many clinicians believe that flaxseed (which contains omega-3 fatty acids) is helpful for treating acne.

2.6.14 Inflammatory bowel disease (IBD)

When added to medication, such as sulfasalazine (a standard medication for IBD), omega-3 fatty acids may reduce symptoms of Crohn's disease and ulcerative colitis (the two types of IBD) (Belluzzi *et al*., 2000).

2.6.15 Asthma

Clinical research suggests that omega-3 fatty acid supplements (in the form of perilla seed oil, which is rich in ALA) may decrease inflammation and improve lung function in adults with asthma (Okamoto *et al*., 2000). Omega-6 fatty acids have the opposite effect: they tend to increase inflammation and worsen respiratory function (Okamoto *et al*., 2000). In a small, well-designed clinical study of 29 children with asthma, those who took fish oil supplements rich in EPA and DHA for 10 months had improvement in their symptoms compared to children who took a placebo pill. Asthma is a mediator driven inflammatory process in the lungs and the most common chronic condition in childhood.

The leukotrienes and prostaglandins are implicated in the inflammatory cascade that occurs in asthmatic airways. There is evidence of airway inflammation even in newly diagnosed asthma patients within 2–12 months after their first symptoms (Laitinen *et al*., 1993). Among the cells involved in asthma are mast cells, macrophages, eosinophils, and lymphocytes. The inflammatory mediators include cytokines and growth factors (peptide mediators) as well as eicosanoids, which are the products of ALA metabolism, which are important mediators in the underlying inflammatory mechanisms of asthma (Laitinen *et al*., 1993).

2.6.16 Macular Degeneration

A clinical study confirms that EPA and DHA from fish, four or more times per week may reduce the risk of developing macular degeneration (Cho *et al*., 2001). Notably, however, this same study suggests that ALA may actually increase the risk of this eye condition.

2.6.17 Cancer

Consuming significant amounts of foods rich in omega-3 fatty acids appears to reduce the risk of colorectal cancer. For example, Eskimos, who tend to follow a high-fat diet but eat significant amounts of fish rich in omega-3 fatty acids, have a low rate of colorectal cancer (Calviello *et al*., 2004). Clinical studies have reported that low levels of omega-3 fatty acids in the body are a marker for an increased risk of colon cancer. Although not all experts agree, women who regularly consume foods rich in omega-3 fatty acids over many years may be less likely to develop breast cancer (Connolly *et al*., 1999). In addition, the risk of dying from breast cancer may be significantly less for those who eat large quantities of omega-3 from fish and brown kelp seaweed (common in Japan) (Deckere, 1999). A population based clinical studies of groups of men suggest that a low-fat diet with the addition of omega-3 fatty acids from fish or fish oil help prevent the development of prostate cancer (Aronson *et al*., 2001).

2.6.18 Other

Although further research is needed, preliminary evidence suggests that omega-3 fatty acids may also prove helpful in protecting against certain infections and treating a variety of conditions, including autism, ulcers, migraine headaches, preterm labor, emphysema, psoriasis, glaucoma, Lyme disease, systemic lupus erythmatosus (lupus), irregular heartbeats (arrhythmias), multiple sclerosis, and panic attacks (Horrocks *et al.*, 1999). Omega-3 fatty acid supplementation may also help to reduce stress and the effects it has on the body.

2.7 REVIEW ON THE SELECTED HEAVY METALS

2.7.1.0 MERCURY

Mercury (Hg) is one of the most toxic heavy metals in our environment. A series of complex chemical transformations allow mercury to occur in three different forms in the environment. These forms are elemental mercury, inorganic mercury and organic mercury (Clarkson *et al.*, 1984). The different forms of mercury have their intrinsic toxic properties and applications in industries, agriculture and medicine (Zhang and Wong, 2007). The elemental mercury which has zero oxidation state constitutes about 98% of mercury in the environment (Zhang and Wong, 2007). The inorganic mercury constitutes the simple inorganic salts of mercury such as chlorides, nitrates, and the sulphates of either Hg^{+} or Hg^{2+}. The salts of Hg^{2+} ion form an important class of organometallic compounds by attaching itself to either one or two carbon atoms to form complex of the type RHgX and RHgR'; (where R and R' represent the organic moiety). These forms of

mercury constitute the organic mercury. The organic moiety may be an alkyl, phenyl, or methoxyethyl radical. Methylmercury is the most important form of mercury in terms of toxicity and health effects from environmental exposures (Goyer, 1997).

Mercury occurs naturally in the environment as well as through industrial and man-made (anthropogenic) processes (ATSDR, 2007). The major natural sources of mercury in the environment are degassing from the earth's crust, emissions from volcanoes and evaporation from water bodies (IPCS, 1990). Mercury ore is found in all classes of rocks, including limestone, calcareous shells, sandstone, serpentine, chert, andesite, basalt, and rhyolite (ATSDR, 2007). The normal concentration of mercury in igneous and sedimentary rocks and minerals appears to be 10-50ng/g (Andersson, 1979); however, the mineral cinnabar (mercuric sulfide) contains 86.2% mercury (Stockinger, 1981).

The main man-made source of mercury is through mining as a result of dumping of mine tailings and direct discharges to the atmosphere. Other important man-made sources are the combustion of fossil fuels, the smelting of metal sulfide ores, the production of cement, and refuse incineration. Mercury also enters the environment from fertilizers, fungicides and from solid waste i.e. thermometers or electrical switches (DEFRA and EA, 2002; IPCS, 1990). A recent study suggests that total mercury levels in the atmosphere have tripled as a result of anthropogenic activities (WHO, 2008).

2.7.1.1 ROUTE OF EXPOSURE OF MERCURY TO MAN

Potential sources of general population exposure to mercury include inhalation of mercury vapour in ambient air, ingestion of drinking water and foodstuffs contaminated with mercury, and exposure to mercury through dental and medical treatment (ATSDR, 2007; IPCS, 1990). Naturally occurring elemental mercury in both ground and surface water is less than 0.5ng L^{-1}. Mercury in drinking water is therefore not considered a major source of exposure except when significant pollution occurs (WHO, 2008).

The major source of human exposure to mercury is through the diet, more specifically from the consumption of fish products. Most of the mercury consumed in fish or other seafood is the highly absorbable methylmercury form (IPCS, 1990; WHO, 2008).

Occupational exposure to mercury may be a major source of exposure. Individuals working in the production of electrical equipment, thermometers or barometers, those working in chemical processing plants or individual living in buildings where mercury-containing latex paints were used may all be exposed to elemental mercury vapour via inhalation of inorganic mercury (ATSDR, 2007).

2.7.1.2 LEVEL OF MERCURY IN THE ENVIRONMENT

2.7.1.2.1 Water

The earth's crust is an important source of mercury for bodies of natural water. Some of this mercury is undoubtedly of natural origin, but some may have been deposited from

the atmosphere as a result of human activities (Lindquist *et al*., 1984). Natural weathering of mercury-bearing rocks is estimated to release about 800 metric tonnes of mercury per year directly to surface waters of the earth (GESAMP, 1985). Representative values for dissolved total mercury are; open ocean, 0.5-3 ng/litre; coastal sea water, 2-15 ng/litre; freshwater rivers and lakes, 1-3 ng/litre (Lindquist *et al*., 1984).

Atmospheric deposition of elemental mercury from both natural and anthropogenic sources has been identified as an indirect source of mercury to surface waters (WHO, 2008). Concentrations of mercury in rainwater and fresh snow are generally below 200 ng/L (ATSDR, 2007).

Mercury associated with soils can be directly washed into surface waters during rain events. Surface runoff is an important mechanism for transporting mercury from soil into surface waters, particularly for soils with high humid content (WHO, 2008).

2.7.1.2.2 Soil

In a review of the mercury content of virgin and cultivated surface soils from a number of countries, it was found that the average concentrations ranged from 20 to 625 ng/g (0.020 to 0.625 ppm) (Andersson, 1979). Atmospheric deposition of mercury from both natural and anthropogenic sources has been identified as an indirect source of mercury to soil and sediments (WHO, 2008; ATSDR, 2007). Wisconsin lakes contained higher mercury levels of 0.09-0.24 ppm at the top 15cm of sediments relative to the lower levels recorded

for the lower sediments. Surface sediment samples from the Upper Columbia River in Washington State were found to contain up to 2.7 µg/g (ppm) mercury (Johnson and Eaton, 1980).

Mercury is released to cultivated soils through the direct application of inorganic and organic fertilizers (e.g., sewage sludge and compost), lime and fungicides containing mercury (Andersson, 1979). Cappon, (1987) studied the contamination of sludge-amended soil with inorganic and methylmercury and the emission of this mercury contamination into the atmosphere. The author reported that, routine application of municipal sewage sludge to crop land significantly increased the concentration of both total mercury and methylmercury in surface soil from 80 to 1000 µg/kg (ppb) and 0.3-8.3µg/kg (ppb), respectively.

2.7.1.3 ENVIRONMENTAL TRANSPORT AND DISTRIBUTION OF MERCURY

The natural global bio-geochemical cycle of mercury, involves degassing of mineral mercury from the lithosphere and hydrosphere, long-range transport in the atmosphere, removal by wet and dry deposition to land and surface water, sorption to soil and sediment particulates, revolatilization from land and surface water, and bioaccumulation in both terrestrial and aquatic food chains. This emission, deposition, and revolatilization make it difficult to trace movement of mercury to its sources (WHO, 2008).

About 98% of the mercury found in the atmosphere is gaseous mercury (Hg^0), the form involved in long-range (global) transport of the element. Residence time in the atmosphere has been estimated to range from 6 days to 2 years (ATSDR, 2007; IPCS, 1990). This makes transport on a hemispherical scale possible and emissions in any continent can thus contribute to the deposition in other continents (IPCS, 1990).

In soils and surface waters, mercury can exist in the mercuric (Hg^{2+}) and mercurous (Hg^{+}) states as a number of complexes with varying water solubilities. Mercuric mercury, present as complexes and chelates with ligands, is probably the predominant form of mercury present in surface waters. Vaporization of mercury from soils may be controlled by temperature, with emissions from contaminated soils being greater in warmer weather where soil microbial reduction of Hg^{2+} to the more volatile elemental mercury is greatest (WHO, 2008). The bottom sediment of the oceans is thought to be the ultimate sink where mercury is deposited in the form of the highly insoluble mercuric sulfide (IPCS, 1990). The process of methylation of inorganic mercury (Hg [II]) to methylmercury by microorganisms, which is highly bio-available, is an important key to the fate of mercury in the environment (ATSDR, 2007). Methylation is usually greatest at the sediment water interface, but also occurs in the water column (WHO, 2008).

Methylmercury in surface waters is rapidly accumulated by aquatic organisms; concentrations in carnivorous fish such as large tuna, swordfish, shark, and mackerel at the top of both freshwater and marine food chains. Humans absorb methylmercury easily by consumption of contaminated fish (IPCS, 1990).

2.7.1.4 MERCURY TOXICITY

The high concentration of mercury found in water and fish tissues results from the formation of soluble monomethylmercury ion and dimethylmercury by anaerobic bacteria in sediments. The methylation agent by which inorganic mercury is converted to methylmercury compounds is methylcobalamin, a vitamin B analog. The bacteria that synthesize methane in anaerobic decay produces methylcobalamin as an intermediate in the synthesis; hence water and sediments in which anaerobic decay occurs provide the condition under which methylmercury production occurs (IPCS, 1990).

Toxicity from eating contaminated fish is one of the main sources of mercury poisoning and one which is attracting a great deal of recent attention (ATSDR, 2007).

Mercury is present in some fish and is of considerable interest because of its potential to cause hazard to the health of people who consume them. Concentration of methylmercury in fish depends on the trophic level, age or length of fish (Zhang and Wong, 2007). Fishes such as walleye, pike swordfish, tuna and shark have high levels of methylmercury due to bioaccumulation and biomagnifications (ATSDR, 2007). Rivers, lakes and sea have abundance of fish diversity; an important number of authors have reported concentrations of mercury in some species such as tuna, carp, tench, gray mullet, ell, sual, bagrus, snakehead, and bighead, grass and common carp. Mercury levels in rivers such as River Catalonia in Spain (Cheng *et al*., 1994) and River Kahanawake in Canada (Chan *et al*., 1986), are below permissible limits (according to the international standard) therefore consumption of fishes from these rivers is considered safe for human health. However, fishes coming from Lower Stour River, UK have showed

methylmercury concentrations between 0.08 and 1.20 mg/kg wet weight (Bub *et al*., 1994), those coming from New Jersey, USA had 0.65±0.1–0.05±0.001 ppm, wet weight (Gochfeld, 2003), the ones from Mojana, Colombia had 0.346±0.171 ppm in carnivorous fish and 0.146±0.102 mg/g fresh wt (fw) in non-carnivorous fish (Marrugo-Negrete, 2008) and are therefore considered not safe for human consumption. Usero (2003) found that concentrations of mercury in three species (eel, common sole and gray mullet) of fish muscle were considerably lower than the maximum levels for human consumption in southern Atlantic coast of Spain.

2.7.1.5 HEALTH EFFECTS OF MERCURY EXPOSURE

The health effects of mercury depends on its chemical form (elemental, inorganic or organic), the route of exposure (inhalation, ingestion or skin contact), and level of exposure. Different forms of mercury have different effects in humans, because they do not all move through the body in the same way (ATSDR, 2007). The effect can be acute or chronic.

2.7.1.5.1 Acute Effects

Acute (short-term) inhalation exposure to high levels of elemental mercury in humans results in central nervous system (CNS) effects, such as hallucinations, delirium, and suicidal tendencies. Gastrointestinal effects and respiratory effects, such as chest pains, dyspnea, cough, pulmonary function impairment, and interstitial pneumonitis have also

been noted from inhalation exposure to elemental mercury (IPCS, 1990). Such effects have been reported following exposure to 1.1 – 44 mg m^{-3} elemental mercury (ATSDR, 2007). The kidneys are a major target organ following exposure to elemental mercury vapour due to the relatively high accumulation of mercury in the kidneys. High concentrations have been reported to result in acute renal failure and degeneration of the proximal convoluted tubules (ATSDR, 2007; WHO, 2008).

2.7.1.5.2 Chronic Effects

Chronic exposure to elemental mercury also affects the kidney in humans, with the development of proteinuria (ATSDR, 2007; WHO, 2008). The primary effect from chronic exposure to inorganic mercury is kidney damage, primarily due to mercury induced autoimmune glomerulonephritis (induction of an immune response to the body's kidney tissue) (WHO, 2008).

2.7.1.5.3 Reproductive / Developmental Effects

Oral exposure to methylmercury has been observed to produce significant developmental effects. Methylmercury poisoning mostly affects the nervous system and it is especially harmful to infant's developing nervous system. Maternal exposure can threaten the foetus because chemicals can be transferred to the developing foetus through the placenta (Slikker, 1994; Gochfeld, 2003); in fact, foetal brain is more susceptible than adult brain to mercury-induced damage (Clarkson *et al*., 1984). Infants born to women who ingested

high concentrations of methyl mercury exhibited CNS effects, such as retardation, ataxia, deafness and constriction of the visual field, blindness and cerebral palsy. At lower methylmercury concentration, developmental delays and abnormal reflexes were noted (ATSDR, 2007; WHO, 2008). Excessive prenatal exposure leads to delayed developmental functions including delayed walking, delayed speech development and hearing impairment.

2.7.1.6 RELATIONSHIP BETWEEN METHYLMERCURY AND OMEGA-3 FATTY ACID IN FISH

Fish and shellfish are recommended dietary constituents (USEPA, 2004) providing sufficient amount of protein, vitamins, and the essential fatty acids (EFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). In addition, fish and shellfish provide a favorable ratio of omega-6 to omega-3 fatty acids. Although many omega-3 fatty acids occur in nature, DHA and EPA are not synthesized by humans at a rate meeting metabolic needs making a dietary source necessary (Mahaffey, 2004). Over the past few years, recommendations for adequate intakes of the omega-3s have been highlighted (Stevens *et al.*, 1996; Stillwell and Wassall, 2003) as measures that help to prevent cardiovascular disease and enhance in utero development of the foetus (USEPA, 2004). Fishes rich in Omega-3s have been found to be contaminated with methylmercury which counteract the numerous benefits (Stillwell and Wassall, 2003; USEPA, 2004).The benefits of omega-3 cannot therefore be thought without considering the associated methylmercury accumulation. Studies assessing the combined impact of omega-3 status

and methylmercury exposure on in-utero neurological development suggested both adverse effects of methylmercury exposure and benefits of increasing omega-3 intakes with an overall intermediate result (USEPA, 2004). The greatest benefits of omega-3 consumption occurred when the omega-3s were obtained from fishes containing little or no methylmercury at all (Stillwell and Wassall, 2003). Consequently understanding the relative concentrations of omega-3s and methylmercury in specific fish species becomes an important consideration when formulating dietary advice (USEPA, 2004). Fishes such as tuna, salmon, trout, herring, sardines, bass, swordfish, and mackerel are much richer sources of omega-3 fatty acids (Mahaffey, 2004). There is an inconsistent relationship between the EFA content of a fish species and the mercury concentration typical of that species. For example, Domingo *et al*. (2007) demonstrated that although methylmercury concentrations increase steadily with body size in plankton and fish, EFAs show irregular retention patterns at different trophic levels.

Although both EPA and methylmercury generally increase at higher trophic levels in the planktonic food web, the magnitude of bioaccumulation is much greater for methylmercury compared with EPA. This is because aquatic organisms retain EFAs at lower rates than they retain methylmercury (Domingo *et al*., 2007).

The relationship differs for DHA and methylmercury accumulation. For EPA and methylmercury accumulation, although EPA and methylmercury increase along the planktonic food web, methylmercury bioaccumulates at higher rates than EPA (Domingo *et al*., 2007). By contrast, DHA, primarily found in phospholipids of cell membranes, is retained differently from EPA with respect to methylmercury (Domingo *et al., 2007*). Retention of dietary DHA is determined by the taxonomic composition of the planktonic

food web and independent of DHA requirements. This variability in the comparative rates of bioaccumulation for methylmercury, EPA, and DHA, helps to explain the need to determine the level of mercury in the omega-3 products (USEPA, 2004).

The amounts of seafood products consumed in the human diet is gaining prominence making it necessary to consider potential risks associated with methylmercury exposure.

2.7.2.0 CADMIUM

Cadmium occurs naturally in its elemental form in the earth's crust. Cadmium is not usually found in the environment as a metal but as a mineral combined with other element such as oxygen (Cadmium oxide), chlorine (cadmium chloride), or sulphur (cadmium sulphate, cadmium sulphide) (IPCS, 2001).

Cadmium ores are rare. Greenockite (CdS) is the only mineral of any consequence that contains cadmium (IPCS, 2001).

Cadmium does not corrode easily and has many uses in industry and consumer products, mainly in nickel-cadmium batteries, pigments used mostly in plastics, metal coatings, plastic stabilizers in polyvinyl chloride (PVC) and some metal alloys (Cook and Morrow, 1995; Thornton, 1992). Cadmium compounds are also used in printing, in textiles, in television phosphors, photography, lasers, as a neutron absorber in nuclear reactors, in photovoltaic cells, and other semi-conducting cadmium compounds in a variety of electronic applications (Elinder, 1985; OECD, 1994).

Cadmium has no essential biological function and is extremely toxic to human. It is one of the commonest environmental metal poisoning. Inhalation accounts for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Target organs are the liver, placenta, kidneys, lungs, brain, and bones (ATSDR, 2008a).

Cadmium is widely distributed in the Earth's crust (0.1-0.5 mg g^{-1}), the atmosphere (1-5 ng m^{-3}), marine sediment (~1 mg g^{-1}) and sea water (~0.1 mg g^{-1}) (IPCS, 2001). Cadmium emissions arise from two major source categories, natural sources and man-made or anthropogenic sources. Even though the average cadmium concentration in the earth's crust is generally placed between 0.1 and 0.5 ppm, much higher levels may accumulate in sedimentary rocks. Marine phosphates and phosphorities have been reported to contain levels as high as 500 ppm (Cook and Morrow, 1995; WHO, 1992) and are thus undesirable to use as fertilizers (Taylor, 1997). Weathering and erosion of parent rocks result in the transport of large quantities of cadmium to the world's oceans by rivers (OECD, 1994; WHO, 1992). Volcanic activity and forest fires have also been reported as natural sources of cadmium emission (Nriagu, 1980). Large amounts of cadmium enter the environment from human activities such as mining and smelting operations, fuel combustion, disposal of metal-containing products and application of phosphate fertilizer or sewage sludges, cement production and waste incineration (Elinder, 1985; WHO, 1992).

Estimates of cadmium emissions to the atmosphere from human and natural sources have been carried out at the world-wide, regional and national levels (IPCS, 1992). According to Nriagu (1988), about 10-15% of total airborne cadmium emission arises from natural processes, the major source being volcanic action. Atmospheric cadmium occurs mainly in the forms of cadmium oxide and cadmium chloride which are ultimately dispersed by the wind.

Cadmium emissions to water are from phosphate fertilizers, non-ferrous metals production and the iron and steel industry (OECD, 1994). Mining represent a major source of cadmium release to the aquatic environment. Contamination can arise from mine drainage water, waste water from the processing of ores, overflow from the tailing pond, and rainwater run-off from the general mine area. The release of these effluents to local water-courses can lead to extensive contamination downstream of the mining operation (IPCS, 1992). Cadmium however, is usually a minor constituent of surface and groundwater (ATSDR, 1999). The atmospheric fall-out of cadmium to fresh and marine water represents a major input of cadmium at the global level (Nriagu and Pacyna, 1988). Acidification of soils and lakes may result in enhanced mobilization of cadmium from soils and sediments and lead to increased levels in surface and ground waters (WHO, 1992).

The application of phosphate fertilizers and atmospheric deposition are significant sources of cadmium input to arable soils in some parts of the world; sewage sludge can

also be an important source at the local level (IPCS, 1992). Cadmium in soil tends to be more available when the soil pH is low.

Cadmium is taken up and retained by aquatic and terrestrial plants and is concentrated in the liver and kidney of animals that eat the plants (Elinder, 1985).

Humans normally absorb cadmium into the body either by ingestion or inhalation. Dermal exposure is generally not regarded to be of significance (Lauwerys, 1986). Human exposure to cadmium from ingestion is either from consumption of food especially grain and leafy vegetables or accidental ingestion of dust or soils contaminated with cadmium (Williams *et al.*, 1999).

The inhalation of cadmium-containing particles from ambient air, cigarette smoke, which contains cadmium taken up by tobacco or from working in areas exposed to cadmium fumes and dust constitute human exposures to cadmium (Elinder, 1985). Most drinking water contains very low levels of cadmium and is usually not an important route of exposure, although water may leach cadmium from plumbing (ATSDR, 2008a).

2.7.2.1 ENVIRONMENTAL FATE

Cadmium emitted to the atmosphere from combustion processes are usually in very small particulate forms that are subjected to long-range transport with atmospheric residence time of about 1-10 days (Keitz, 1980). Total deposition rates have been measured at numerous localities worldwide and values have generally been found to increase in the order: background < rural < urban < industrial (IPCS, 1992).

In polluted or organic-rich waters, adsorption of cadmium by humic substances and other organic complexing agents plays a dominant role in transport, partitioning, and remobilization of cadmium. Cadmium concentration in water is inversely related to the pH and the concentration of organic material in the water (Callahan *et al.*, 1979). Cadmium has a relatively long residence time in aquatic system. In Lake Michigan, a mean residence time of 4-10 years has been calculated for cadmium (Wester *et al.*, 1992).

Rivers contaminated with cadmium can contaminate surrounding land, either through irrigation for agricultural purposes, by the dumping of dredged sediments, or through flooding (Forstner, 1980; Tsuchiya, 1978). Forstner (1980) reported soil cadmium concentration of 70 mg/kg in agricultural land adjacent to Neckar River, Germany, which received dredged sediments for soil improvement and Tsuchiya (1978) reported soil contamination due to irrigation in Japan.

The most important soil factors influencing plant cadmium accumulation are soil pH and cadmium concentration (Page *et al.*, 1981). Factors such as cation exchange capacity and the contents of the hydrous oxides of manganese and iron, organic matter and calcium carbonate also influence distribution of cadmium in soil and soil solution. Increases in these parameters result in decreased availability of cadmium to plants owing to a reduction of the level of cadmium in the soil solution (IPCS, 1992). Contamination of soil by cadmium is of concern because the cadmium is taken up efficiently by plants and, therefore, enters the food chain for humans and other animals (ATSDR, 2008a ; IPCS, 1992).

2.7.2.2 HUMAN HEALTH EFFECTS

Cadmium can enter the blood by absorption from the stomach or intestines after ingestion of food or water, or by absorption from the lungs after inhalation. However, once cadmium enters the body, it is very strongly retained; therefore, even low doses may build up significant cadmium levels in the body if exposure continues for a long time. The amount of cadmium needed to cause an adverse effect in an exposed person depends on the chemical and physical form of the element. In general, cadmium compounds that dissolve easily in water (e.g., cadmium chloride), or those that can be dissolved in the body (e.g., cadmium oxide), tend to be more toxic than compounds that are very hard to dissolve (e.g. cadmium sulfide) (IPCS, 1992).

An acute intake of cadmium causes testicular damage and may affect female reproductive cycle. Within a few hours of exposure, there is necrosis and degeneration of the testes with complete loss of spermatozoa. This is thought to be due to an effect on the blood supply to these organs, reducing the blood flow (IPCS, 2001). Acute inhalation of cadmium may initially cause irritation of the upper respiratory tract, although symptoms may be delayed for 4-8hours. Dyspnea, chest pain and muscle weakness may also occur. Pulmonary oedema, bronchitis, chemical pneumonitis, respiratory failure and death may occur within days of exposure. In the long-term following exposure, progressive pulmonary fibrosis and impaired lung function may occur (WHO, 1992). Acute ingestion of cadmium produces severe gastrointestinal irritation, which is manifest as severe nausea

and vomiting, abdominal cramps and diarrhea. A lethal dose of cadmium for ingestion is estimated to be between 350 and 8900 milligrams (ATSDR, 2008a).

Chronic oral exposure to cadmium leads to renal failure, characterized by proteinuria due to renal tubular dysfunction. The accumulation of cadmium in the kidney affects renal vitamin D metabolism, which subsequently disturbs calcium balance that may lead to osteomalacia and osteoporosis (DEFRA and EA, 2002). This, as well as the increased excretion of calcium and phosphorus may result in bone disease characterized by bone and joint aches and pains, a syndrome, first described in Japan, where it was termed the itai-itai ("ouch-ouch") disease. Symptoms of this disease include weak bones that lead to deformities, especially of the spine or to more easily broken bones. It is often fatal (ATSDR, 2008a).

Long term exposure to cadmium can cause anemia, loss of sense of smell, fatigue and/or yellow staining of teeth. Chronic inhalation of cadmium causes loss of renal tubular function, leading to proteinuria and impairs lung function by causing bronchitis, obstructive lung disease and in some cases intestinal fibrosis (IPCS, 1992). Chronic obstructive airway disease has been associated with long-term high-level occupational exposure by inhalation (OECD, 1994; WHO, 1992).

Cadmium (especially cadmium oxide) is also known to be carcinogenic, and in studies has been linked with cancers in the lungs and prostate. There may be no safe level of exposure to a cancer-causing agent (Williams *et al.*, 1999).

2.7.3.0 ZINC

Zinc has a low abundance in nature (of the order 10^{-6} of the earth's crust) but has long been known because they are easily obtained from ores. It occurs widely in a number of minerals but the main source is sphaterite (ZnFeS) which occurs with Galena (PbS).

Most of the zinc is from industrial activities such as mining, coal, waste combustion, welding and steel processing. Galvanised steel in the form of wire fencing gradually dissolves in the rains and drops to the ground below and drain away into the gutters and drain pipes. Water drains from zinc roofings may also be collected and drunk resulting in zinc poisoning. Drinking water also contains certain amount of zinc which may be higher when stored in zinc tanks (Alloway *et al*., 1993). Zinc containing aerosols may settle with dust and precipitate either on vegetation or in the soil which finally enter the food chain by the uptake by roots or foliar absorption (Adriano, 1986).

Water is polluted with zinc due to the deposition of zinc-containing wastewater from industrial plants. This results in zinc polluted sludge on river banks and increase in acidity of waters and river bodies which accumulate in the bodies of fishes and finally enter the food chain (Abbasi and Soni, 1986).

Large quantities of zinc can be found in soils. When the soils of farmland are polluted with zinc, animals will absorb high levels in feeding. Water-soluble zinc that is found in soils can contaminate groundwater. Soils with high levels of zinc can be washed off into rivers and other water bodies and accumulates in the bodies of fishes (Ahlers *et al*., 1990).

High level of zinc in the soil affects the activities of microorganisms and subsequently slows down the breakdown of organic matter. Only few plants are able to survive on zinc-rich soils resulting in low plant diversity near zinc-disposing factories (Anderson and Christensen, 1988).

Zinc is also found in foods such as cereals (30-40ppm), poultry, fish and seafood, nuts, seeds, and brewer's yeast. Acid foods such as fruits stored in galvanized containers are also found to be a major source of zinc poisoning. Zinc in meat is tightly bound to myofibrils and has been speculated to influence the water binding capacity (Watanabe *et al*., 1997).

2.7.3.1 IMPORTANCE OF ZINC

Zinc is a trace element that is essential for human health to all forms of life. Zinc plays a lot of important roles in the body. Zinc appears to be one of the most biologically important metals. It is a trace element that is essential for human health such that a large number of diseases and congenital disorders have been traced to zinc deficiency. The Zn^{2+} ion is contained in several enzymes such as dehydrogenases, aldoses, and peptidases

attesting to its importance in carbohydrates, lipids and protein metabolism in virtually all organisms. These include the following;

- It is necessary for a healthy immune system (Baer *et al*., 1985)
- Zinc in the form of insoluble zinc oxide has been used in ointments in fighting skin problems such as acne, boils and sore throats (Agren, 1990).
- Zinc plays an important role in DNA synthesis and cell division, constitutes many enzymes (dehydrogenase and carbonic anhydrase) and is needed by the tissues of the hair, nail, and skin to be in their best condition (Aamodt *et al*., 1979).
- The supplementation of zinc in diets of 30-45 mol/L has been used successfully to promote the treatment of chronic ulcers (Berg and Shi, 1996).
- Zinc is further used in the growth and maintenance of muscles (Prasad, 1969).
- Children require zinc for normal growth and sexual development (Prasad, 1969).
- Zinc helps to control the oil glands, and is required for the synthesis of protein and collagen – which is good for healthy skin and wound healing (Reilly, 1985).
- In men, zinc plays an important role in the functioning of the prostrate gland and helps in the manufacture of testosterone and a shortage may induce a low sperm count, loss of libido and other emotional problems (Reilly, 1985).
- Zinc also helps in fighting infection and inflammation of the prostrate gland in older men. Sperms also need zinc to swim to the egg (Reilly, 1985).

2.7.3.2 DEFICIENCY OF ZINC

There is shortage of zinc in many people's diet since zinc is destroyed in cooking. Zinc deficiency may also be due to high intake of calcium and copper. A deficiency of zinc will result in under-performing immune system, vulnerability to infections, allergies, night blindness, loss of smell, falling hair, white spots under finger nails, skin problems, sleep disturbances etc. Men with zinc deficiency may have problems with fertility, while women may experience irregular periods (ATSDR, 2005). Children with too little zinc may have stunted growth and slow sexual maturity (Halsted, *etal*., 1974). When people have too little zinc, they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores (Apgar, 1970).

2.7.3.3 TOXICITY AND HEALTH EFFECTS OF ZINC

Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems such as stomach cramps, skin irritations (Lewis, 2004), vomiting, nausea and anaemia (Gossel and Bricker, 2001).

High levels of zinc can damage the pancreas and disturb protein metabolism, and cause arteriosclerosis. Severe cases of zinc poisoning can cause sluggishness, loss of appetite, diarrhea, impaired reproductive development and function, and impaired bone growth. Extensive exposure to zinc chloride can cause respiratory disorders. Mothers exposed to large concentrations of zinc may cause severe damage to unborn and newly born children through blood or milk (Gossel and Bricker, 2001).

Metal fume fever results from inhalation of fumes of zinc oxide produced when zinc is heated to high temperatures, such as during welding, metal cutting, or smelting zinc alloys. Victims complain of nausea and vomiting, chills and fever, muscular aches and pains, and weakness (Gossel and Bricker, 2001). There is a reciprocal relationship between plasma levels of zinc and copper, to the degree that large doses of elemental zinc result in negative copper balance in patients with Wilson's disease (Klaassen, 2001).

Acute ingestion of zinc produces gastrointestinal irritation and vomiting. Continual excessive zinc intake results in reductions in serum levels of copper (hypocupremia), sideroblastic anemia, and neutropenia (Haddad, 1998; Reynolds and Prasad, 1982).

2.7.4.0 IRON

Iron is a silvery-white or grayish metal. It makes up 5 percent 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 (Allen and Casterline, 2001). Iron, which is the chief constituent of the Earth's core, is the most abundant element in the Earth as a whole (about 35 percent) and is relatively plentiful in the Sun and other stars (Underwood, 2001).

Iron is a very active metal hence rarely exists as a free metal in the earth but occurs combined in its ores. It readily combines with oxygen in moist air forming rust. The most common ores of iron are hematite, limonite, magnetite, siderite, and taconite which is a mixture of hematite and silica. The naturally occurring isotopes of iron are iron-54, iron-56, iron-57, and iron-58 (Lahey, 1975).

Man-made sources of iron are from mining and refining of other metals such as copper, that may contain iron ores, agriculture and other industrial activities such as textile, paints, ceramics,

leather, insecticide water purification and sewage treatment systems; photography, wood preservative and even in dietary supplementation (Hallberg, *et al.*, 1997).

The combination of rice, beans, and meat consumed with fresh citrus fruit provides an excellent source of absorbable iron (Gillooly *et al*., 1983).

2.7.4.1 IMPORTANCE OF IRON

Iron is one of the most important trace elements required and is widely distributed throughout the body. The total iron content of the body varies with sex, age, nutrition and state of health and species. The estimated dosage for adult males is 10mg per day and 18 mg per day for females (Allen and Casterline, 2001).

Iron is also essential element carrying oxygen and forming part of the oxygen-carrying proteins-haemoglobin in red blood cells and myoglobin in muscles. It is also a component of various enzymes and is concentrated in bone marrow, liver, kidney and spleen.

2.7.4.2 DEFICIENCY OF IRON

The deficiency of iron is based on the absorption and the rate at which it is lost. In terms of iron intake, fortified products and pharmaceutical supplements are important sources of iron in addition to the usual diet (Bothwell and Charlton, 1982). The actual amount of iron absorbed depends on the iron requirements, the presence of inhibitors and enhancers of iron absorption in the diet (Allen and Casterline, 2001). On the other hand, iron is lost in sweat and during menstruation, both of which can vary significantly among

individuals. Significant amounts of iron can also be lost due to parasitic infections such as malaria, hookworm and schistosomiasis (Allen and Casterline, 2001). Iron requirements are also increased during periods of rapid growth, such as during pregnancy, early childhood and adolescence (Allen and Casterline, 2001).

Young children and women of reproductive age especially during pregnancy have increased requirements for iron, placing them at increased risk of deficiency and related adverse consequences (Allen and Casterline, 2001). Severe iron deficiency results in anaemia, and red blood cells that have a low haemoglobin concentration. Iron deficiency in pregnant women results in premature babies or babies with low birth weight (Innis *et al*., 1997).

In young children, iron deficiency can manifest in behavioral abnormalities (including reduced attention) reduced cognitive performance and slow growth. In adults, severe iron deficiency impairs physical work capacity (Innis *et al*., 1997).

Symptoms of iron deficiency may include fatigue, poor stamina, intestinal bleeding, excessive menstrual bleeding, nervousness, heart palpitations and shortness of breath. It may also cause the mouth corners to crack, brittle hair, and difficulty in swallowing and digestive disturbances (Petersen and Parkinson, 1996).

2.7.4.3 TOXICITY AND HEALTH EFFECTS OF IRON

Iron is a heavy metal of concern, particularly because ingesting dietary iron supplements may acutely poison young children (e.g., as few as five to nine 30-mg iron tablets for a 30-lb child).

Ingestion accounts for most of the toxic effects of iron because iron is absorbed rapidly in the gastrointestinal tract. The corrosive nature of iron seems to further increase the absorption (Hallberg *et al*., 1997). Most overdoses appear to be the result of children mistaking red-coated ferrous sulphate tablets or adult multivitamin preparations for candy.

High iron content in the body has been linked to cancer and heart disease. Excessive iron intake causes genetic abnormality. Iron supplements are the leading cause of deaths in children and as little as 600 mg of iron in children can be fatal. High iron intake for a long period can result in liver and heart damage, diabetes and skin diseases (Underwood, 2001).

Large iron supplementation may also contribute to the hardening of the arteries, heart diseases and reduced zinc absorption. In infants, an increase in the daily intake of iron might saturate the lactofirein secreted into the intestines and suppress the capacity of the protein to contribute to the body's protection against infection (Bothwell and Charlton, 1982).

2.7.5.0 COPPER

Copper has a natural abundance of approximately 60 mg/kg in the earth's crust and 2.5×10^{-4} mg/litre in the sea (Lide and Frederikse, 1993).

Copper is found in a wide variety of mineral salts and organic compounds such as cuprite (Cu_2O), malachite ($Cu_2CO_3.Cu(OH)_2$), azurite ($2CuCO_3.Cu(OH)_2$), chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), and bornite (Cu_5FeS_4). The most important sources of copper are chalcocite, chalcopyrite and malachite (Weant, 1985). Copper can also be found naturally in the elemental or metallic form. The metallic form is very stable to dry air at low temperatures but undergoes a slow reaction in moist air to produce a hydroxycarbonate or hydroxysulfate that forms a greenish-grey amorphous film over the surface which protects the underlying metal from further attack (Cotton and Wilkinson, 1989). In compounds, copper usually has a valence of +2 but can exist in the metallic, +1 and +3 valence states. Copper is distributed along with other metals through the environment by precipitation into rivers which transport the particles as they flow. Depending on the flow dynamics, these particles settle out and form sedimentary deposits. An important source of copper in aquatic sediments is from dead organisms which settle out and contribute both copper and organic material. This also contributes to a significant source in the oceans.

Nriagu (1989) estimated mean worldwide emissions of copper from natural sources as follows: windblown dusts, 0.9-15 $\times 10^3$ tonnes; forest fires, 0.1-7.5 $\times 10^3$ tonnes;

volcanic particles, 0.9-18 × 10^3 tonnes; biogenic processes, 0.1-6.4 × 10^3 tonnes; sea salt spray, 0.2-6.9 × 10^3 tonnes.

Average background concentrations of copper in air in rural areas range from 5 to 50 ng/m^3. Copper levels in seawater of 0.15 µg/litre and in freshwater of 1.0-20 µg/litre are found in uncontaminated areas (Nriagu, 1979b). Background levels of copper in uncontaminated sediments range from 800 to 5000 mg/kg (dry weight), from 2 to 740 mg/kg (dry weight) in marine sediments with. median copper concentrations in uncontaminated soil reported to be in average of 30 mg Cu/kg within a range of 2-250 mg/kg (Bowen, 1985). Copper is found as a natural component of foods eaten by humans and animals (ATSDR, 2004).

Anthropogenic sources of copper include emissions from mines, smelters and foundries producing or utilizing copper, zinc, silver, gold and lead (ATSDR, 2004). Copper can also be released into the atmosphere through the burning of coal and from municipal waste incinerators. Other anthropogenic sources of copper include its use as an antifouling agent in paints, agriculture (fertilizers, algicides, feed supplements) and animal and human excreta (animal manure and human sewage sludge). Copper is also intentionally released into some water bodies to control the growth of algae (Slooff et al., 1989; ATSDR, 2004).

2.7.5.1 ENVIRONMENTAL TRANSPORT AND DISTRIBUTION

2.7.5.1.1 Water

Several processes influence the fate of copper in aquatic systems. These include complexation to inorganic and organic ligands, sorption to metal oxides, clays, and particulate organic material, bioaccumulation and exchange between sediment and water (Stiff, 1971; Callahan *et al*., 1979).

Much of the copper discharged to water is in particulate form and tends to settle out, or be adsorbed by organic matter, hydrous iron, manganese oxides and clay in the sediment or water column (Callahan *et al*., 1979).

The copper (I) ion is unstable in aqueous solution, tending to disproportionate to copper (II) and copper metal unless a stabilizing ligand is present (Callahan *et al*., 1979). The only cuprous compounds stable in water are insoluble ones such as the sulfide, cyanide and fluoride. In its copper (II) state, copper forms coordination compounds or complexes with both inorganic and organic ligands. Ammonia and chloride ions are examples of species that form stable ligands with copper. Copper also forms stable complexes with organic ligands such as humic acids. In seawater, organic matter is generally the most important complexing agent. Samples collected from the surface waters (< 200 m) of the northeast Pacific revealed that over 99.7% of the total dissolved copper was associated with organic complexes. At depths of 1000 m approximately 50-70% of the copper was in the organically complexed form. Copper complexation gave rise to very low cupric ion activities in surface waters, around 1 pg Cu^{2+}/litre. The authors reported that two

classes of copper-binding ligands were identified: an extremely strong ligand at low concentrations dominated in surface waters and a weaker class of ligand at higher concentrations was found throughout the water column (Coale and Bruland, 1988).

2.7.5.1.2 Soil

In the terrestrial environment, a number of factors influence the fate of copper in the soil. These include the nature of the soil, soil pH, organic matter content, the soil redox potential, the presence of oxides, the base status of the soil and its cation exchange capacity (CEC), the rate of litter decomposition and the proportions of clay to silt to sand particles (ATSDR, 2004). The residence time of copper in the soil is also a function of overall climate and of the vegetation present at a site.

Most copper deposited on soil are strongly adsorbed to the upper layer soil. It can be bound to the organic matter, as well as being adsorbed by carbonate minerals and hydrous iron and manganese oxides (Lehmann and Harter, 1984). Copper binds more strongly than most other metals and is less influenced by pH (Assaad and Nielsen, 1984). The greatest amount of leaching of copper occurs from sandy soils, compared with clays and peats, whereas acidic conditions favour copper leaching to the groundwater from top soil (Petruzzelli *et al*., 1988).

2.7.5.2 ENVIRONMENTAL LEVELS OF COPPER

2.7.5.2.1 Water

Copper is widely distributed in water because it is a naturally occurring element. Nriagu (1979b) reported average copper levels in seawater ranging from 0.15 µg/litre in open ocean to 1.0 µg/litre in polluted near-shore waters; levels in fresh water were 1.0-20 µg/litre. Other reports indicate that copper concentrations in seawater are highly variable, ranging from 0.005 µg/litre in the Black Sea (Haraldsson and Westerlund, 1988) to 40 µg/litre in estuaries in southwest Spain (Cabrera *et al*., 1987). Additional variation in copper concentrations is related to depth and the area in the ocean examined. Surface concentration in the North Pacific Ocean drops from 0.1 µg Cu/litre (1.2 nmol/kg) in the California Current to 0.03-0.04 µg Cu/litre (0.4-0.5 nmol/kg) in the central oceanic region, and increases to 0.24 µg Cu/litre (3 nmol/kg) in deep waters (Boyle *et al*., 1977; Bruland, 1980). In the North Atlantic Ocean surface waters display values of copper from 0.07 µg/litre (1.1 nmol/kg) to 0.11µg/litre (1.7 nmol/kg), whereas concentration of the metal increases to 0.13-0.26 µg/litre (2-4 nmol/kg) in deep waters (Moore, 1978).

2.7.5.2.2 Soil

Median copper concentrations in uncontaminated soil were reported to be 30 mg/kg (range 2-250 mg/kg) (Bowen, 1985). Shacklette and Boerngen (1984) analysed soil samples from various locations in the USA, and found that copper concentrations in soils ranged from below 1 to 700 mg/kg with an average of 25 mg/kg. Kabata-Pendias and

Pendias (1984) reviewed worldwide literature on copper in uncontaminated surface soils and reported mean concentrations ranging from 6 to 80 mg/kg (dry weight).

Copper can accumulate in soils from the long-term application of fertilizers or fungicides. Reuther and Smith (1952) analysed soils from mature Florida citrus groves and found that copper oxide levels in the topsoil increased with grove age. Copper oxide levels of 247mg/kg and 93 mg/kg (dry weight) were measured at depths of 0-8 cm and 8-15 cm, respectively. At depths of > 15 cm, copper oxide levels of 18 mg/kg were measured. Copper oxide levels in adjacent untreated soil ranged from 1 to 2 mg/kg. Christie and Beattie (1989) reported an accumulation of copper in soil from the application of pig slurry (50-200 m^3/ha per year). EDTA-extractable copper concentrations of up to 85.2 mg/kg were recorded; levels in control soils ranged from 4.4 to 5.4 mg/kg.

Paoletti *et al*. (1988) found that in Italy vineyard soil to which copper-containing fungicide had been applied contained mean copper concentrations of 89.8 mg/kg (dry weight). Soils from other locations contained mean levels ranging from 44.0 to 52.1 mg/kg. Holmgren *et al*. (1993) analysed surface soil samples from agricultural regions throughout the USA. Copper concentrations ranged from 0.3 to 495 mg/kg (dry weight). Copper levels were higher in the organic soil areas of Florida, Oregon and the Great Lakes, reflecting the use of copper fertilizers and fungicides.

Copper can also accumulate at areas near copper smelting factories. Hunter *et al*. (1987a) reported mean surface soil copper concentrations of 15.1, 543 and 11 000 mg/kg at a

control site, 1 km from a copper refinery (Merseyside, United Kingdom) and at the refinery, respectively. Almost all of the copper contamination was held at the surface of the mineral soil.

2.7.5.3 IMPORTANCE/USES OF COPPER

The unique combination of properties of copper, including durability, ductility, malleability and electrical and thermal conductivity, determine its uses in a vast range of applications. Worldwide, the largest use of copper is in electrical wire and cable and other electronic applications, which can account for as much as 65% (9.75×10^6 tonnes) of total annual copper consumption.

Rolled copper is also extensively used in architectural applications for roofing, rainwater goods and cladding, while rolled copper and brass are also used for vehicle radiators. Overall, the major industrialized countries consume over 1.5×10^6 tonnes of rolled product per year. Approximately 15% (2.25×10^6 tonnes) of copper is used annually in building and construction, including plumbing, architectural applications such as roofing, guttering and flashing, and in fixtures and fittings. Copper is also used in the manufacture of goes to transport equipment, air-conditioning and refrigeration as well as general and light engineering uses such as machine parts, and process equipment, coinage, ordnance and consumer goods, such as domestic appliances as well as production of bronze and brass alloys.

Copper is an essential element for all biota. Copper was identified in plant (Bucholtz, 1816) and animal (Harless, 1847) systems in the nineteenth century and postulated to be a biological catalyst in the early twentieth century (McHargue, 1925). Subsequent nutritional studies demonstrated that copper is necessary for optimal growth of plants and animals (McHargue, 1927a, b; Arnon and Stout, 1939; Woolhouse, 1983). Copper is also essential for the utilization of iron in the formation of haemoglobin (ATSDR, 2004); hence its involvement in anaemia prevention.

2.7.5.4 DEFICIENCY OF COPPER

The lower limit of the acceptable range of oral intake (AROI) is 20 µg/kg body weight per day. In chi;dren, this figure is 50 µg /kg body weight per day.

Characteristic clinical features of copper deficiencies in infants are anaemia refractory to iron, and low copper plasma levels (WHO, 1996). Copper deficiency has been considered to be the likely cause of the anaemia in children (Cordano *et al*., 1964). It has been shown that copper deficiency is associated with increased incidence of infection and impaired weight gain in infants recovering from malnutrition (Castillo-Duran and Uauy, 1988). Copper deficiency is associated with altered immunity in humans (Prohaska and Failla, 1993). Low copper intake has also been demonstrated to diminish glucose tolerance (Klevay *et al*., 1986) and alter cardiac rhythm (Lukaski *et al*., 1988).

The role of copper deficit in altered neurodevelopment has been postulated on the basis of the high copper content of the brain, especially of the basal ganglia. The existence of a prenatal critical phase in central nervous system (CNS) development during which copper deficiency can cause CNS damage has been suggested (Danks, 1988). This could explain the severe mental deficiency associated to prenatal tissue deficit found in Menkes disease while postnatally acquired nutritional copper deficiency is not accompanied by neurological abnormalities.

2.7.5.5 TOXICITY AND EFFECTS ON HUMANS

The upper limit of the AROI in adults is about 2-3 mg/day (ATSDR, 2004). Acute toxicity due to ingestion of copper is infrequent in humans and is usually a consequence of the contamination of beverages (including drinking-water) or from accidental or deliberate ingestion of high quantities of copper salts (ATSDR, 2004). Symptoms including vomiting, lethargy, acute haemolytic anaemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates and in some cases, coma and death have all been reported from copper toxicity (ATSDR, 2004). Other symptoms associated with copper toxicity include nausea, abdominal pain, headache, dizziness, vomiting and diarrhea (Knobeloch *etal.*, 1994).

2.7.6.0 MANGANESE

Elemental manganese was isolated in 1774, though the oxide has been used in the manufacture of glass since antiquity. In the elemental state, manganese is a white-grey, brittle, and reactive metal with a melting point of 1244°C and a boiling point of 1962°C. It can form compounds in a number of oxidation states, the most important being +2, +3, and +7.

Manganese is widely distributed in nature but does not occur as the free metal. The most abundant compounds are the oxide (in pyrolusite, brannite, manganite, and hausmannite), sulfide (in manganese blonde and hauserite), carbonate (in manganesespar), and the silicate (in tephroite, knebelite, and rhodamite). It also occurs in most iron ores in concentrations ranging from 50-350 g/kg, and in many other minerals throughout the world. A rough estimate of the average concentration of manganese in the earth's crust is about 1000 mg/kg (WHO, 2006). Manganese concentrations in igneous rock may range from about 400 mg/kg in low-calcium granitic rock to 1600 mg/kg in ultrabasic rock and sedimentary rocks. Deep sea sediments contain concentrations of about 1000 mg/kg (Turekian and Wedepohl, 1961). It has been reported that the manganese content of coal ranges from 6 to 100 mg/kg (Ruch *et al*., 1973) and that of crude oil from 0.001 to 0.15 mg/kg (Bryan, 1970).

Fumes, dust, and aerosols from metallurgical processing, mining operations, steel casting metal welding and cutting mainly in the form of manganese oxide are the principal sources of manganese. Dust from the handling of raw materials in metallurgical

processing and other manufacturing activities also contribute to the atmospheric concentration of manganese (ATSDR, 2008b).

2.7.6.1 ENVIRONMENTAL LEVELS AND EXPOSURE

2.7.6.1.1 Water

Manganese may be present in fresh water in both soluble and suspended forms. Surface waters of various American lakes were found to contain from 0.02 to 87.5 µg of manganese per litre with a mean of 3.8 µg/litre (Kleinkopf, 1960). In two other studies, the contents of large rivers in the USA ranged from below the detection limit to185 µg/litre (Durum and Haffty, 1961; Kroner and Kopp, 1965).

In studies on the manganese contents of sea water in North Sea, Northeast Atlantic, English Channel and the Indian Ocean, concentrations ranged from 0.03 to 4.0 µg/litre with mean values of 0.06 to 1.2 µg/litre. In estuarine and coastal waters of the Irish Sea and in waters along the North Sea shores of the United Kingdom, values ranging from 0.2 to 25.5 µg/litre were reported with mean values of 1.5 to 6.1 µg/litre (Jones *et al.*, 1973; Bouquiaux, 1974).

Manganese concentrations in treated drinking-water supplies in 100 large cities in the USA ranged from undetectable to 1.1 mg/litre, with a median level of 5 µg/litre; 97% of the supplies contained concentrations below 100µg/litre (Durfor and Becker, 1964).

According to a US Public Health Service survey quoted by Schroeder, (1966) manganese levels in tap water from 148 municipal supplies ranged from 0.002 to 1.0 mg/litre, with a median level of 10 µg/litre. Mean concentrations of manganese in drinking-water in the Federal Republic of Germany were reported to range from 1 to 63µg/litre (Bouquiaux, 1974).

2.7.6.1.2 Soil

The average concentration of manganese in soils is about 500-900 mg/kg (WHO, 2006). The significance of manganese levels in soils depends largely on the type of compounds present in the soil and its characteristics (Nichol *et al*., 1967).

2.7.6.1.3 Food

The edible muscle tissue of 8 common commercial species of fish in New Zealand was reported by Brooks and Rumsey (1974) to have mean concentrations of manganese ranging from 0.08 to 1.15 mg/kg wet weight. Similar values (0.03-0.2 mg/kg wet weight) were found in North Sea fish. In cod and plaice, most values were lower than 0.1 mg/kg. Scallops, oysters, and mussels dredged from Tasman Bay contained average manganese levels of 111 mg, 8 mg, and 27 mg/kg dry weight, respectively (Brooks and Rumsey, 1965).

In most human studies, the average daily intake of manganese, via food, by an adult has been reported to be between 2 and 9 mg/day. Values of about 2.3-2.4 mg/day have been reported from the Netherlands (Belz, 1960) and the USA (Schroeder *et al*., 1966). North *et al*. (1960) obtained an average daily intake of 3.7 mg for 9 American college women, and Tipton *et al*. (1969), using the duplicate portion method, reported 50-week, mean daily intakes of 3.3 and 5.5 mg, respectively, for two American adult males. Similarly, an average intake of 4.1 mg/day was reported from a Canadian composite diet (Méranger and Smith, 1972). In a study by Soman *et al*. (1969), also using the duplicate portion method, the average manganese intake for Indian adults was 8.3 mg/day, while the intake from drinking-water ranged from 0.004 to 0.24 mg/day. These results agree well with previously reported values for Indian adults on a rice diet (9.81 mg of manganese/day) and on a wheat diet (9.61 mg of manganese/day) (De, 1949).

The daily intake of manganese by bottlefed and breastfed infants is very low because of the low concentrations of manganese in cow's milk and, especially, in breast milk (McLeod and Robinson, 1972a).

2.7.6.2 TRANSPORT AND DISTRIBUTION IN ENVIRONMENTAL MEDIA

2.7.6.2.1.1 Water

All water contains manganese derived from soil and rocks. Manganese in seawater is found mostly as manganese dioxide (MnO_2), some of which is produced from manganese

salts by several species of bacteria common to soils and ocean muds. Mobilization of manganese is favoured by low pH conditions. Thus acid mine-drainage waters can give rise to high concentrations of dissolved manganese (ATSDR, 2008b). Mitchell (1971) showed that mobilization was greatly enhanced in acid, poorly drained podzolic soils and groundwaters. It was suggested by Nichol *et al*., (1967) that, in acid waterlogged soils, manganese passes freely into solution and circulates in the groundwaters and that it is precipitated on entering stream waters with pH values of 4.0 – 6.0, thus giving rise to stream sediments enriched with manganese.

Particulate material suspended in natural waters may contain an appreciable proportion of manganese. Preston *et al*., (1972) found that 67-84% of the total manganese in shoreline and offshore areas of the British Isles was associated with particulate matter. Levels of particulate manganese present in ocean waters are low in comparison with levels of dissolved manganese. However, much larger amounts of particulate manganese occur in estuarine and river waters, where resuspension of bottom material may occur.

In deep-sea sediments, manganese is concentrated in the form of both crustal material and coastal and shelf sediments. The composition of manganese nodules on the ocean floors is related to factors such as water composition, sedimentation rates, volcanic influences, and organic productivity. Regional variations have also been observed, especially in the Atlantic Ocean (Elderfield, 1972). On the basis of samples taken at 32 stations in the USA, Lazarus *et al*., (1970) concluded that the manganese in atmospheric precipitation was derived mainly from human activity.

2.7.6.2.2 Soil

Manganese cycles in the soil have been proposed involving di-, tri-, and tetravalent manganese. Divalent manganese is transformed through biological oxidation to the less available trivalent form and later, through dismutation, the Mn^{3+} form is biologically reduced to Mn^{2+}. The oxidizing power of higher oxides increases with acidity and thus reduction by organic matter is more likely at low pH values. Bacterial oxidation is very slow or absent in very acidic soils and Mn^{2+} predominates, thus organic matter can reduce the higher oxides of manganese.

Under conditions such as low pH and aeration, the addition of organic compounds to soil can increase the chemical reduction of manganese and its uptake by plants. Nitrogen applications consistently reduce the availability of manganese. Organic material associated with a high pH can produce organic complexes of divalent manganese leading to insufficient available manganese for susceptible plants such as peas or cereals.

2.7.6.3 ABSORPTION OF MANGANESE

The main routes of absorption of manganese are the respiratory and gastrointestinal tracts. Absorption through the skin is not considered to occur to any great extent (ATSDR, 2008b).

Not much is known about the mechanisms of absorption of manganese from the gastrointestinal tract however in vitro studies using the everted sac method, seemed that

manganese may be actively transported across the duodenal and ileal segments of the small intestine (Cikrt and Vostal, 1969). Other studies indicated that intestinal absorption of manganese takes place by diffusion in iron-overload states and by active transport in the duodenum and jejunum in iron-deficiency states (Thomson *et al*., 1971). The rate of absorption may be influenced by factors such as dietary levels of manganese and iron, the type of the manganese compound, iron deficiency, and age (WHO, 2006).

2.7.6.4 DISTRIBUTION/TRANSPORT IN HUMAN BODY

Manganese is an essential element for man and animals and thus occurs in the cells of all living organisms. Concentrations of manganese present in individual tissues, particularly in the blood, remain constant, in spite of some rapid phases in transport, indicating that such amounts may be considered characteristic for these particular organs irrespective of the animal species (ATSDR, 2008b).

The total manganese body burden of a standard man of 70 kg has been estimated to be about 10-20 mg (WHO, 2006). In general, higher manganese concentrations can be expected in tissues with high mitochondria content, with the exception of the brain which contains only low concentrations (WHO, 2006). There also appears to be a tendency towards higher concentrations in pigmented tissues such as dark hair or pigmented skin (WHO, 2006).

In a study by Kitamura *et al*., (1974) performed on 15 Japanese males and 15 females high concentrations of manganese were found in the liver, pancreas, kidney, and intestines. Comparatively high concentrations were also found in the suprarenal glands.

From birth to 6 weeks, infants have relatively higher tissue concentrations of manganese than older children, especially in tissues normally associated with low manganese levels. However, after about 6 weeks of age, no accumulation of manganese appears to take place with increasing age (Schroeder *et al*., 1966). This is in agreement with the study of Dobrynina and Davidjan (1969), who reported that manganese did not accumulate with age, and that the manganese content of the lung actually decreased with increasing age.

Absorbed manganese is concentrated in the liver and it has been suggested that it forms complexes with bile components. It has also been suggested that manganese is transported directly into the bile (WHO, 2006).

2.7.6.5 USES OF MANGANESE

Over 90% of the manganese produced in the world is used in the making of steel, either as ferromanganese, silicomanganese, or spiegeleisen. Manganese is also used in the production of nonferrous alloys, such as manganese bronze, for machinery requiring high strength and resistance to sea water, and in alloys with copper, nickel, or both in the electrical industry. In dry-cell batteries, manganese is used in the form of manganese dioxide, which is also used as an oxidizing agent in the chemical industry. Many

manganese chemicals, eg., potassium permanganate, manganese(II) sulfate, manganese dichloride, and manganese dioxide are used in fertilizers, animal feeds, pharmaceutical products, dyes, paint dryers, catalysts, wood preservatives and, in small quantities, in glass and ceramics (WHO, 2006).

2.7.6.6 METABOLIC ROLE IN THE BODY

The essential role of manganese as a trace nutrient for mammals was discovered mainly through experimental and epidemiological studies of deficiency states in animals.

Manganese has been shown to be associated with the formation of connective tissue and bone, with growth, carbohydrate and lipid metabolism, the embryonic development of the inner ear, reproductive function, and, probably, brain function (Underwood, 1971; NAS/NRC, 1973).

Manganese catalyses the formation of glucosamine-serine linkages in the synthesis of the mucopolysaccharides of cartilage. Also the mitochondrial enzyme pyruvate carboxylase is a manganese metalloenzyme, and that manganese is linked with carbohydrate metabolism (WHO, 2006).

It has also been discovered that the digestive enzymes prolidase and succinic dehydrogenase are manganese-dependent and that, *in vitro*, manganese can substitute for other metals, especially magnesium, in various biological reactions (Underwood, 1971; NAS/NRC, 1973). Lindberg and Ernster (1954) performed *in vitro* experiments on rat

liver mitochondria that demonstrated that manganese was required as a co-factor in oxidative phosphorylation.

2.7.6.7 TOXICITY AND HEALTH EFFECTS ON HUMANS

The toxicity of manganese varies according to the chemical form administered. Divalent manganese has been shown to be 2.5-3 times more toxic than the trivalent form (ATSDR, 2008b).

Signs and symptoms of extrapyramidal disorders, characteristic of manganese poisoning include symptoms such as failing memory, fatigue, increased perspiration, and tremor of the fingers, disturbed balance, adiadochokinesis, and muscular rigidity, neurological hyposexuality and manganese pneumonia (ATSDR, 2008b).

The toxic effect of manganese exposure to the liver results in the inability of the liver to clear bilirubin from the bile, reduction in bile flow, decreased serum albumen/globulin and ultrastructural alterations in the liver cells (Penland and Johnson, 1993). Hepatic congestion, central vein thrombosis, and focal necrosis with leukocyte infiltration have also been reported as a result of the toxic effect of manganese (ATSDR, 2008b).

Other effects of manganese possibly associated with the liver metabolism that have been observed include an increase in cholesterol synthesis ((ATSDR, 2008b), a disturbance in lipid and carbohydrate metabolism with lipid deposition in the liver and adrenals and an enhancement of the coagulating activity of the blood (Penland and Johnson, 1993).

Disturbances in sex function and testicular changes have been noted following exposure to potassium permanganate. Animals exposed orally or by inhalation to doses of 50 mg/kg body weight for various periods of time exhibited changes in spermatogenesis. Embryogenesis was also adversely affected (Mandzgaladze, 1967).

Processes such as genetic recombination might be affected by manganese through its influence on enzymes that control DNA structure and metabolism. Manganese can be substituted for magnesium in the binding of the two ribosomal subunits as well as in the binding of M-RNA to the whole ribosome (Buttin and Kornberg, 1966).

Studies have shown that manganese exposure may contribute to the development of a chronic lung disease such as pneumonia and bronchitis. Individuals with a history of smoking appear to be more affected than nonsmokers and there is a relationship between the degree of smoking and the prevalence of respiratory tract symptoms in the manganese-exposed workers suggesting that smoking may act synergistically with manganese (Saric and Lucic-Palaic, 1977).

Other effects of manganese exposure include alterations in immunological activity, disturbance of nitrogen metabolism and a depression by manganous chloride of the acetylcholine output (ATSDR, 2008b).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Preparation of Glassware and Sample Containers

All glassware and sample containers used were soaked in detergent solution overnight; rinsed and soaked in 10% (v/v) HNO_3 overnight. They were rinsed with tap water followed by 0.5% (w/v) $KMnO_4$, tap water again and finally rinsed with distilled water. They were then dried before use.

3.2 Apparatus/Equipment

The types of equipment used in this research are listed as follows;

- Automated Mercury Analyzer Model HG-5000 (Sanso Seisakusho Co., Ltd, Japan), equipped with mercury lamp operated at a wavelength of 253.7 nm was used for the determinations.
- SOLAAR (S Series 711239 v1.23) Flame Atomic Absorption Spectrometer
- Thick walled long neck 50 ml volumetric flasks were used as digestion apparatus.
- Hot plate with a temperature range of 150 – 350^0C.
- Analytical balance (Mettler Toledo, model B303)

3.3 Reagents and Solutions Prepared

All reagents used were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. Double distilled water was used for the preparation of all solutions.

- Mercury stock standard solution (1000 mg/L) was prepared by dissolving 0.0677 g of $HgCl_2$ in the acid mixture HNO_3: $HClO_3$ (1:1) in a 50 ml digestion flask with heating on a hot plate at a temperature between $200^0C \pm 5$ for 30 min. The solution was then diluted to 50ml with distilled water after cooling.

- Mercury standard working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution through intermediate solution using the blank solution.

- Blank solutions were also prepared in the ratio of 1:1:1 distilled water: HNO_3: $HClO_3$ bulked together for use as diluent.

- Stannous chloride solution (10% v/v) was prepared by dissolving 10 g of the salt in 100 ml 1M HCl.

- NaOH (5M) solution was prepared by dissolving 20 g NaOH in 100 ml double distilled water.

- Acidified $KMnO_4$ (0.5%) was prepared by dissolving 0.5 g $KMnO_4$ in 100 ml 0.5M H_2SO_4.

- Cadmium, Iron, Zinc, Manganese and Copper stock solutions of 1000 ppm were prepared by dissolving 0.5 g of pure metal in a small volume of 1% HCl and diluted to 500 ml with 1% HCl.

- Standard solutions for Cadmium, Iron, Zinc, Manganese and copper were prepared by diluting the appropriate amount of the stock solutions to the required volumes.

3.4 Sampling and Sample Preparation

A total of One hundred Samples of omega-3 food supplements soft gels comprising ten different brands were purchased from retail outlets on the Ghanaian Market. Samples were transported to the laboratory, coded for easy identification and then stored in clean dry place until time for analysis.

3.5 Digestion Procedure for the determination of mercury

The omega-3 samples were digested for total mercury determination by an open flask procedure developed by Akagi and Nishimura (1991) as presented in the flow chart below. In this digestion method, one soft gel sample was put in a 50 ml volumetric digestion flask, and a mixture of 1ml distilled water and 2 ml HNO_3-$HCIO_4$ (1:1) was added. The mixture was then heated at a temperature of 200 $\pm$ 5^0C for 30 min until and the solution was clear. The sample solution was then cooled and diluted to 50 ml with double distilled water.

Sample (one soft gel in 50ml digestion flask)

↓ H_2O, 1ml

HNO_3: $HClO_4$ (1:1), 2ml

Heat at $200^0C \pm 5^0C$ for 30 min

Cool to room temperature

Digested Sample

↓ H_2O, make up to 50ml

Sample Solution, 5ml

↓ 10% $SnCl_2$, 0.5ml

Analysis for Hg CVAAS – Automatic Mercury Analyzer Model HG 5000

Figure 3.0 Analytical procedure for the determination of total mercury in omega-3 samples

3.6 Digestion of Blank and Standards for mercury determination

A blank and standard solution, 25, 50 and 100 µl of 1 µg/ml standard mercury solution were subjected to the same digestion treatment as the sample to yield concentration of 25, 50 and 100 ng as standard solutions for the experimental analysis.

3.7 Determination of Mercury

Determination of mercury in all the digests were carried out by cold vapor atomic absorption spectrophotometer (CVAAS) using an automatic Mercury Analyzer Model HG-5000 (Sanso Seisakusho Co., Ltd, Japan) developed at NIMD (1991).

3.7.1 Operation of the Analyzer

- The instrument was switched on and left for 20 minutes to warm up
- The start button was pressed to purge the system for 3 minutes
- The reset button was pressed to stop purging
- A 5 ml aliquot of the sample was introduced into the reaction vessel using a micropipette.
- Then 0.5 ml of $SnCl_2.2H_2$ was added from a dispenser.

- The start button was pressed immediately the stannous chloride was dispensed. After 30 seconds the 4 way valve rotated to allow the mercury vapour generated in the reaction vessel to flow into the absorption cell so as to generate a peak.

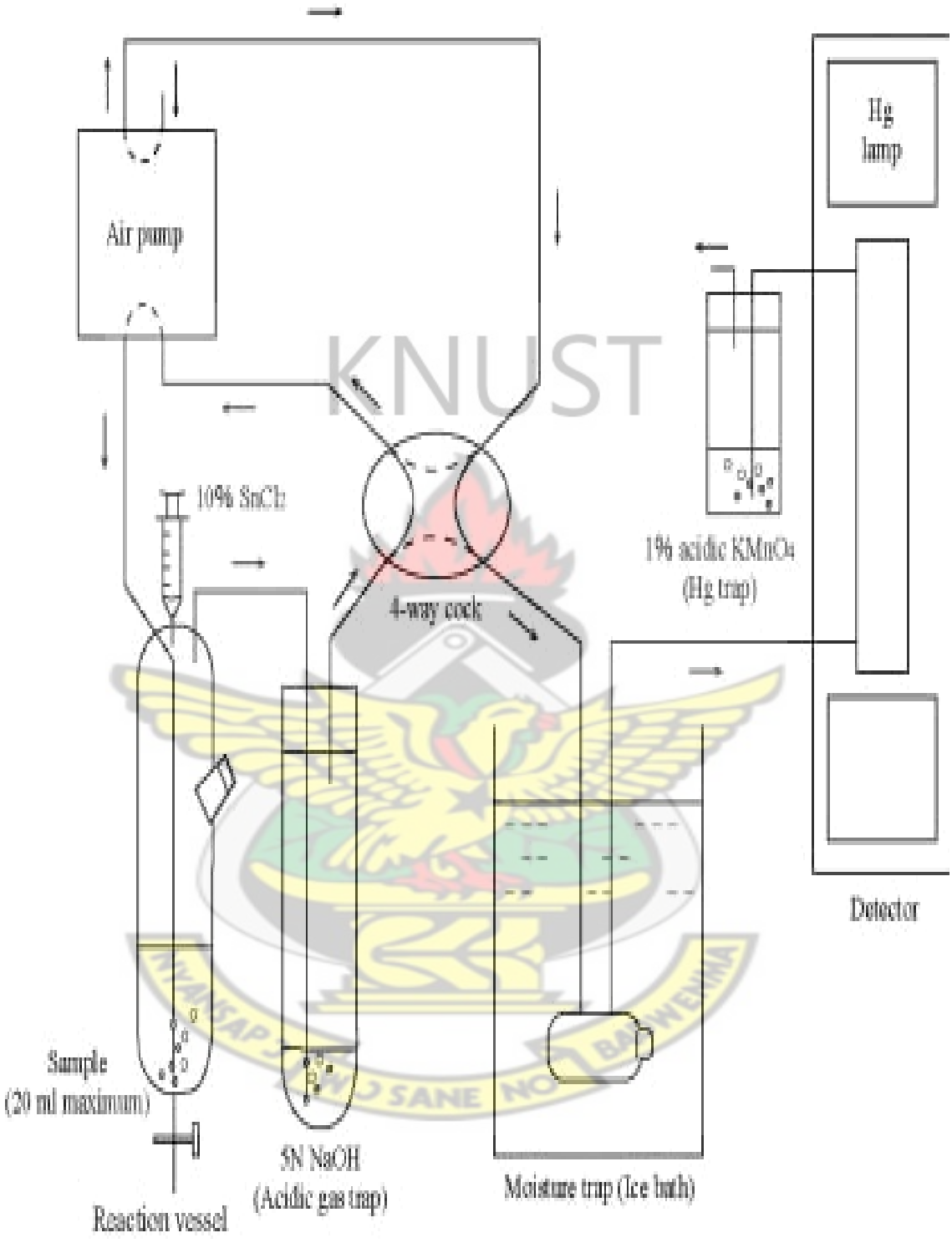

Figure 3.1 Apparatus for Mercury determination by cold vapour

3.8 Determination of Recovery

Recovery of mercury was determined by adding 25 ng and 50 ng of mercury to two different samples of omega-3. The samples were taken through the digestion procedure. The resulting solutions were analyzed for mercury concentration. Percentage recovery for the spiked solutions was then calculated.

The recovery was from 86-90% with an average of 88%. The results for recovery studies are shown in table 4.0.

3.9 Determination of other metals

The same digested sample in Section 3.5 after the mercury determination was used for the determination of copper (Cu), manganese (Mn), zinc (Zn), cadmium (Cd) and iron (Fe) using the SOLAAR (S Series 711239 v1.23) Flame Atomic Absorption Spectrometer. The wavelengths of 324.8 nm for Cu, 251.6 nm for Mn, 210.4 nm for Zn, 228.8 nm for Cd and 259.9 nm for Fe were used. The concentrations of the metals obtained were then calculated.

3.10.1 Instrument Setup

- The instrument was switched on.
- The hollow cathode lamp was set to the lamp current recommended by the manufacturer i.e. 2/3 times the maximum current. The lamp was allowed to warm for about 20 minutes.
- The wavelength and slit width were selected. By means of wavelength control, the required wavelengths were selected.
- It was ensured that the radiation path was clear and the radiation was passing through the window to the photomultiplier using a white piece of paper.
- The electronics and optics were set.
- The burner was checked to ensure that it was in such a position that it is completely clear of light path.
- The absorbance was zeroed and the exhaust fan started.
- Gas flows (air and acetylene) were adjusted and the flame ignited.
- The burner was allowed to heat up for about 15 minutes while aspirating water.
- The standard solutions were then aspirated, followed by the blank and the samples but before every measurement, the spectrometer was zeroed with distilled water.
- Each sample was aspirated three times.

Table 3.1 Operating Parameters for Solar Flame Atomic Absorption Spectrometer

Metal	Wavelength (nm)	Burner height (mm)	Fuel flow
Manganese	210.4	6.2	1.0
Iron	253.7	6.2	0.9
Copper	217.0	6.2	0.9
Zinc	228.8	6.2	0.9
Cadmium	259.9	8.6	0.9

Background Correction - D2 for all metals.

Flame type – Air-C_2H_2 for all metals.

Signal type – Continuous for all metals.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The omega-3 samples were analysed for six heavy metals. A total of hundred (100) omega-3 samples comprising ten (10) different brands obtained from Ghanaian markets were used.

Table 4.0 Recovery test for mercury analysis

Sample used: Cardioace (1.11 g) and Alaska (1.53 g)

Omega-3 Sample (g)	Hg added (ng)	Hg found (ng/g)	Recovered (ng/g)	% Recovery
1.11	0	21	0	
1.53	0	21	0	
1.11	25	43	22	88
1.53	25	43	22	88
1.11	50	66	45	90
1.53	50	64	43	86

Results for the metal concentrations in µg/g are presented in Tables 4.2 to 4.11 in Appendix I. Summary of the results showing the mean, standard deviation and the range of concentration of metals in the omega-3 brands are presented in Tables 4.1a and 4.1b. The mean concentrations of metals in µg/g consumed daily in all the omega-3 samples are also presented in Table 4.15 in Appendix I. Graphical representations of the results for metals in the omega-3 brands are shown in figures 4.1 to 4.6 in Appendix II. Graphs

comparing the level of the metals in each omega-3 brand are also shown in figures 4.7 to 4.16 in the Appendix II.

The main source of the omega-3 oils are the deep sea fishes. The level of heavy metal accumulation in fishes therefore has a direct correlation to the level of heavy metal in the omega-3 product. Heavy metal content in fish is a good indicator for human exposure to metal bioaccumulation and has been confirmed in many publications (Love, *et al,*. 2003; Goyer, 1997; Adimado and Baah, 2002). This means that the consumption of fish products could be the main source of human exposure to heavy metals. This study therefore provides the basis to the level of human exposure to the metals determined through the consumption of the omega-3 products in this work.

Aqua marine samples were analysed for the six metals. The concentrations (µg/g) of the metals ranged from 8.190 µg/g to 8.970 µg/g with a mean of 8.406 ± 0.027 for iron, from 1.470 µg/g to 1.740 µg/g with a mean of 1.593 ± 0.007 for manganese, from 0.300 µg/g to 0.450 µg/g with a mean of 0.387 ± 0.062 for cadmium, from 1.500 µg/g to1.620 µg/g with a mean of 1.548 ± 0.048 for zinc, from 0.300 µg/g to 0.510 µg/g with a mean of 0.393 ± 0.071 for copper and from 0.016 µg/g to 0.017 µg/g with a mean of 0.017 ± 0.003 for mercury.

The heavy metal concentration in Valupak samples ranged from 6.975 µg/g to 8.618 µg/g with a mean of 8.078 ± 0.057 for iron, from 1.891 µg/g to 2.139 µg/g with a mean of 2.027 ± 0.076 for manganese, from 0.620 µg/g to 0.899 µg/g with a mean of 0.729 ±

0.014 for cadmium, from 1.178 µg/g to 1.302 µg/g with a mean of 1.234 ± 0.055 for zinc, from 0.186 µg/g to 0.248 µg/g with a mean of 0.217 ± 0.025 for copper and from 0.091 µg/g to 0.097 µg/g with a mean of 0.093 ± 0.002 for mercury.

Cardioace samples had heavy metal concentration ranging from 12.330 µg/g to 12.465 µg/g with a mean of 12.402 ± 0.043 for iron, from 487.350 µg/g to 489.600 µg/g with a mean of 488.475 ± 0.067 for manganese, from 0.945 µg/g to 1.080 µg/g with a mean of 0.995 ± 0.047 for cadmium, from 1815.300 µg/g to 1817.560 µg/g with a mean of 1816.380 ± 0.071 for zinc, from 732.150 µg/g to 733.500 µg/g with a mean of 732.825 ± 0.057 for copper and from 0.020 µg/g to 0.021 µg/g with a mean of 0.021 ± 0.003 for mercury. The high content of 488.475 µg/g for manganese, 1816.380 µg/g for zinc and 732.825 µg/g for copper determined in the Cardioace samples was due to the fact that 0.45mg of manganese, 1.8mg of zinc and 0.7mg of copper were added during the formulation of the product as mineral supplement (stated on label of product). This means that 38.475 µg/g of manganese, 16.380 µg/g of zinc and 32.825 µg/g of copper were the actual heavy metal levels in the Cardioace samples.

The metal concentration (µg/g) in Dr. Chris ranged from 21.976 µg/g to 22.177 µg/g with a mean of 22.056 ± 0.082 for iron, from 6.633 µg/g to 7.571 µg/g with a mean of 7.229 ± 0.040 for manganese, from 1.139 µg/g to 1.407 µg/g with a mean of 1.226 ± 0.110 for cadmium, from 1.340 µg/g to 1.608 µg/g with a mean of 1.447 ± 0.021 for zinc, from

0.938 µg/g to 1.139 µg/g with a mean of 1.025 ± 0.071 for copper and from 0.260 µg/g to 0.280 µg/g with a mean of 0.273 ± 0.005 for mercury.

The results for Seven Seas for the heavy metal concentration (µg/g) ranged from 3.729 µg/g to 3.828 µg/g with a mean of 3.772 ± 0.038 for iron, from 1.914 µg/g to 2.013 µg/g with a mean of 1.957 ± 0.063 for manganese, from 0.726 µg/g to 0.858 µg/g with a mean of 0.785 ± 0.103 for cadmium, from 0.396 µg/g to 0.475 µg/g with a mean of 0.465 ± 0.032 for zinc, from 0.032 µg/g to 0.099 µg/g with a mean of 0.063 ± 0.063 for copper and from 0.110 µg/g to 0.130 µg/g with a mean of 0.123 ± 0.004 for mercury.

The metal concentration (µg/g) in Artic Sea ranged from 6.765 µg/g to 6.930 µg/g with a mean of 6.844 ± 0.031 for iron, from 0.825 µg/g to 0.924 µg/g with a mean of 0.878 ± 0.131 for manganese, from 0.759 µg/g to 0.858 µg/g with a mean of 0.789 ± 0.014 for cadmium, from 0.792 µg/g to 0.858 µg/g with a mean of 0.822± 0.039 for zinc, from 0.033 µg/g to 0.132 µg/g with a mean of 0.073 ± 0.042 for copper and from 0.610 µg/g to 0.700 µg/g with a mean of 0.658 ± 0.001 for mercury.

Joint Care had levels of heavy metals ranging from 613.800 µg/g to 614.440 µg/g with a mean of 615.252± 0.051 for iron, from 7.260 µg/g to 7.392 µg/g with mean of 7.333 ± 0.043 for manganese, from 0.660 µg/g to 0.825 µg/g with a mean of 0.762 ± 0.030 for cadmium, from 2.013 µg/g to 2.211 µg/g with a mean of 2.122 ± 0.041 for zinc, from

0.033 µg/g to 0.099 µg/g with a mean of 0.066 ± 0.074 for copper and from 0.015 µg/g to 0.019 µg/g with a mean of 0.018 ± 0.005 for mercury.

The range of heavy metal concentration (µg/g) determined in Deep Sea samples were from 14.850 µg/g to 15.081 µg/g with a mean of 14.959 ± 0.059 for iron, from 2.310 µg/g to 2.541 µg/g with a mean of 2.455 ± 0.056 for manganese, from 0.396 µg/g to 0.660 µg/g with a mean of 0.531 ± 0.025 for cadmium, from 1.023 µg/g to 1.254 µg/g with a mean of 1.148 ± 0.029 for zinc, from 0.099 µg/g to 0.231 µg/g with a mean of 0.149 ± 0.065 for copper and from 0.023 µg/g to 0.029 µg/g with a mean = 0.027 ± 0.008 for mercury.

Alaska samples had heavy metal concentration (µg/g) ranging from 5.610 µg/g to 5.808 µg/g with a mean of 5.722 ± 0.032 for iron, from 0.759 µg/g to 0.924 µg/g with a mean of 0.835 ± 0.072 for manganese, from 0.660 µg/g to 0.858 µg/g with a mean of 0.762 ± 0.059 for cadmium, from 0.792 µg/g to 0.990 µg/g with a mean of 0.908 ± 0.035 for zinc, from 0.033 µg/g to 0.297 µg/g with a mean of 0.119 ± 0.028 for copper and from 0.410 µg/g to 0.430 µg/g with a mean of 0.428 ± 0.002 for mercury.

Finally, the results for the heavy metal concentration (µg/g) in High Sea samples ranged from 12.903 µg/g to 13.266 µg/g with a mean of 13.127 ± 0.024 for iron, from 7.062 µg/g to 7.194 µg/g with a mean of 7.112 ± 0.004 for manganese, from 0.561 µg/g to

0.660 µg/g with a mean of 0.611 ± 0.063 for cadmium, from 0.726 µg/g to 0.924 µg/g with a mean of 0.812 ± 0.044 for zinc, from 0.033 µg/g to 0.165 µg/g with a mean of 0.102 ± 0.038 for copper and from 0.027 µg/g to 0.030 µg/g with a mean of 0.029 ± 0.004 for mercury.

Table 4.1a Total metal concentration (µg/g) in omega-3 samples

OMEGA-3 SAMPLE	IRON			MANGANESE			CADMIUM		
	Mean	Std Dev	Range	Mean	Std Dev	Range	Mean	Std Dev	Range
AQUA MARINE	8.406	0.027	8.190 – 8.970	1.593	0.007	1.470 – 1.740	0.387	0.062	0.300 – 0.450
VALUPAK	8.079	0.057	6.975 – 8.618	2.027	0.076	1.891–2.139	0.729	0.014	0.620–0.899
CARDIOACE	12.402	0.043	12.330 – 12.465	488.475	0.067	487.35-489.60	0.995	0.047	0.945–1.080
DR. CHRIS	22.056	0.082	21.976 – 22.177	7.223	0.040	6.633–7.571	1.226	0.110	1.139–1.407
SEVEN SEAS	3.772	0.038	3.729 – 3.828	1.957	0.063	1.914–2.013	0.785	0.103	0.726–0.858
ARTIC SEA	6.844	0.031	6.765 – 6.930	0.878	0.131	0.825–0.924	0.788	0.014	0.759–0.858
JOINT CARE	615.252	0.051	613.80 – 616.44	7.333	0.043	7.260–7.392	0.762	0.030	0.660–0.825
DEEP SEA	14.959	0.059	14.850 – 15.081	2.455	0.056	2.310–2.541	0.531	0.025	0.396–0.660
ALASKA	5.722	0.032	5.610 – 5.808	0.835	0.072	0.759–0.924	0.762	0.035	0.660–0.858
HIGH SEA	13.127	0.024	12.903 – 13.266	7.112	0.004	7.062–7.194	0.611	0.063	0.561–0.660

Table 4.1b Total metal concentration (µg/g) in omega-3 samples contd.

OMEGA-3 SAMPLE	ZINC			COPPER			MERCURY		
	Mean	Std Dev	Range	Mean	Std Dev	Range	Mean	Std Dev	Range
AQUA MARINE	1.548	0.048	1.500 – 1.620	0.393	0.071	0.300 – 0.510	0.017	0.003	0.016– .017
VALUPAK	1.237	0.055	1.178– 1.302	0.217	0.025	0.186–0.248	0.093	0.002	0.091– .097
CARDIOACE	1816.38	0.071	1815.3-817.56	732.825	0.057	732.15–733.50	0.021	0.003	0.020– .021
DR. CHRIS	1.447	0.021	1.340 – 1.608	1.025	0.071	0.938–1.139	0.273	0.005	0.26 – 0.28
SEVEN SEAS	0.465	0.032	0.396 – 0.475	0.063	0.063	0.032 – 0.099	0.123	0.004	0.11 – 0.13
ARTIC SEA	0.822	0.039	0.792 – 0.858	0.073	0.042	0.033– 0.132	0.658	0.001	0.61 – 0.70
JOINT CARE	2.122	0.041	2.013 – 2.211	0.066	0.074	0.033– .099	0.018	0.005	0.015– .019
DEEP SEA	1.148	0.029	1.023 – 1.254	0.149	0.065	0.099– 0.231	0.026	0.008	0.023– .029
ALASKA	0.908	0.035	0.792 – 0.990	0.119	0.028	0.033– 0.297	0.428	0.002	0.41 – 0.43
HIGH SEA	0.812	0.044	0.726 – 0.924	0.102	0.038	0.033– 0.165	0.029	0.004	0.027– .030

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 (Dallman, 1986; Institute of Medicine, 2001). It is also essential for the regulation of cell growth and differentiation (Bothwell *et al.*, 1979; Andrews, 1999). A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. Iron deficiency is associated with anaemia, constipation, nausea, vomiting, and diarrhoea (Bhaskaram, 2001; Haas and Brownlie, 2001). On the other hand, excess amounts of iron can result in increased risk of free radical damage, cancer and even death (Corbett, 1995). Iron, which is an essential trace element, was determined in the omega-3 products analysed in this project. The range for iron concentration in the omega-3 products was from 3.772 µg/g in the Seven Seas sample to 615.252 µg/g in Joint Care sample.

Vanaja *et al*, 2007 determined the concentrations of trace metals in some brands of fish oil supplements and had the concentration of iron ranging from 25.8 µg/g to 161.8 µg/g. Howe, (1998) analysed levels of trace metals in some omega-3 fatty acids in Australian fishes and recorded mean metal concentration of 32.08 µg/g for iron.

The concentrations of iron found in other researches are comparable to the iron concentration obtained in the omega-3 products in this study. The high content of iron of 615.252 µg/g found in Joint Care samples may be due to the source and type of fish from which the omega-3 oil was extracted. The main source of the omega-3 oils are the deep

sea fishes. The level of heavy metal accumulation in fishes therefore has a direct correlation to the level of heavy metal in the omega-3 product. The calculated daily concentration (µg/g) of iron consumed which ranged from 6.844 µg/g for Artic Sea to 1845.756 µg/g for Joint Care was however below the daily threshold value of 15000 µg/70kg body weight for iron recommended by the Joint FAO/WHO Committee on food supplements. The general trend for the concentration (µg/g) of iron in the omega-3 products was Seven Seas < Alaska < Artic Sea < Valupak < Aqua Marine < Cardioace < High Sea < Deep Sea < Dr. Chris < Joint Care.

Manganese is the most important element in the treatment of menopausal symptoms, osteoporosis, and postpartum depression. Inadequate manganese intake has been associated with parenteral nutrition, resulting in dermatitis, changes in hair pigmentation and slowed hair growth (ASTDR, 2008a). Individuals who regularly dislocate joints (particularly knee joints) are known to have insufficient manganese levels. On the other hand, high manganese levels increase the risk for tendon/ligament tears, pneumonia, loss of sex drive and sperm damage in men. Excess manganese also interferes with the absorption of dietary iron (Blaurock-Busch, 1997; ATSDR, 2008a).

In this research, manganese levels in the omega-3 samples ranged from 0.835 µg/g for Alaska samples to 488.475µg/g for Cardioace samples. The concentrations (µg/g) of manganese recorded in this work are comparable to the work done by Vanaja *et al*, (2007) who determined the concentrations of trace metals in some brands of fish oil

supplements and Howe, (1998) who analysed levels of trace metals in some omega-3 fatty acids in Australian fishes and recorded manganese concentration (µg/g) ranging from 0.008 µg/g to 0.54 µg/g and a mean manganese concentration of 10.27 µg/g respectively. The calculated daily manganese consumption which fell within the range of 0.835 µg/g for Alaska to 488.475 µg/g for Cardioace, are however on the lower side when compared to the recommended daily threshold value of 5000 µg/70kg body weight for manganese set by the Joint FAO/WHO expert Committee on food Supplements. The order for the level of manganese determined was; Alaska < Artic Sea < Aqua Marine < Seven Seas < Valupak < Deep Sea < High Sea < Dr. Chris < Joint Care < Cardioace.

Eating food or drinking water with very high cadmium levels severely irritates the stomach, leading to vomiting and diarrhoea, hypertension, and sometimes death. Accumulation of lower levels of cadmium over a long period of time can lead to a build-up of cadmium in the kidneys and cause kidney damage (ATSDR, 2008b).

Cadmium, a toxic metal was determined in the omega-3 products. The mean heavy metal concentration (µg/g) obtained in this study ranged from 0.387 µg/g for Aqua marine samples to 1.226 µg/g for Dr. Chris samples. The mean cadmium concentrations (µg/g) recorded in this study are comparable to the concentration (µg/g) obtained by Vanaja *et al*, (2007) who determined the concentrations of trace metals in some brands of fish oil supplements and had mean cadmium concentrations ranging from 0.012 µg/g to 1.320 µg/g. Kotb, *et al.* (1991) studied the levels of some heavy metals in omega-3 fatty acids in popular species of Arabian Gulf fish and obtained mean cadmium concentration

ranging from 0.530 µg/g to 2.093 µg/g. The mean cadmium concentration ranging from 0.387 µg/g for Aqua marine samples to 1.226 µg/g for Dr. Chris samples obtained in this research is comparable to the results obtained by Kotb, *et al.*, (1991).

The different levels of cadmium in the omega-3 products may be due to the fact that the fish oils were extracted from fishes from different sources. The mean cadmium concentration (µg/g) in the products were in the order; Aqua marine < Deep sea < High sea < Valupak < Joint care = Alaska < Seven Seas <Artic Sea < Cardioace < Dr. Chris. Generally, levels of cadmium consumed daily in the omega-3 products which ranged from 0.531 µg/g for Deep Sea to 4.712 µg/g for Seven Seas are below the daily threshold value of 17 µg/70kg body weight recommended by the Joint FAO/WHO Expert committee on food supplements for cadmium. Hence consuming these products poses no health risk for the consumer.

Zinc is important during puberty, pregnancy, and menopause. If large doses of zinc (10-15 times higher than the recommended daily intake) which is 8-15 mg/day are consumed, stomach cramps, nausea, and vomiting may occur. Ingesting high levels of zinc for several months may cause anaemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol. Consuming low levels of zinc is at least as important a health problem as consuming too much zinc. Without enough zinc in the diet, people may experience loss of appetite, decreased sense of taste and smell, decreased immune function, slow wound healing, and skin sores. Too little zinc in the diet may also

cause poorly developed sex organs and retarded growth in young men. If a pregnant woman does not get enough zinc, her babies may have birth defects (ATSDR, 2005).

Zinc, which is one of the most essential heavy metals, was determined in the omega-3 products. The highest level was obtained in Cardioace samples (1816.38 µg/g) with the lowest of 0.4653 µg/g determined in Seven Seas samples.

The concentration (µg/g) obtained in this study is comparable to the studies by Howe, (1998) who obtained mean concentration of 3.02 µg/g of zinc in some omega-3 fatty acids in Australian fishes and Vanaja *et al*. (2007) who obtained zinc concentration (µg/g) ranging from 0.28 µg/g to 8.12 µg/g in some brands of fish oil supplements. The high level of zinc in Cardioace as compared to the other products is due to the addition of 1800 µg/g of zinc during formulation of the product (as indicated on label of product). The calculated daily intakes of zinc in the omega-3 samples which ranged from 0.812 µg/g for High Sea to 1816.380 µg/g for Cardioace are however below the WHO/FAO recommended daily intake of 15000 µg/70kg body weight. The zinc concentration (µg/g) obtained were in the order; Seven Seas < High Sea < Artic Sea < Alaska < Deep Sea < Valupak < Dr Chris < Aqua Marine < Joint Care < Cardioace.

The consumption of the omega-3 products is thus not likely to pose any health risk to human and is safe for consumption in terms of zinc content.

Copper helps the body to use iron. It is also important for nerve function, bone growth, helps body use sugar and protects cell membranes from being destroyed by free radicals. A wide range of cardiovascular and blood disorders may be attributed to copper

deficiency. Higher than normal levels of copper may cause nausea, vomiting, stomach cramps, or diarrhoea (ATSDR, 2004). The range of copper concentration (µg/g) determined in the omega-3 products was from 0.063 µg/g for Seven Seas to 732.825 µg/g for Cardioace which had 700 µg/g of copper added during the formulation (information on label). This means that 32.825 µg/g of copper is the level due to heavy metal accumulation.

Howe (1998) analysed levels of trace metals in some omega-3 fatty acids in Australian fishes and recorded mean metal concentration of 1.08 µg/g for copper which is comparable to the range from 0.063 µg/g to 732.825 µg/g recorded in this work. Also Vanaja, *et al*. (2007) analysed fish oil supplements and obtained copper concentration (µg/g) ranging from 0.006 µg/g to 22.025 µg/g which is comparable to the concentration (µg/g) obtained in this study.

All the omega-3 products including Cardioace samples had concentration (µg/g) of copper below the recommended daily intake of 2000 µg/70kg body weight set by joint WHO/FAO expert committee on food supplements for copper. The order of copper levels was; Seven Seas < Joint Care < Artic Sea < High Sea < Alaska < Deep Sea < Valupak< Aqua Marine < Dr. Chris < Cardioace. All the products are thus safe for consumption as far as the level of copper is concerned.

Mercury one of the most toxic metals was determined in the omega-3 samples. The mean mercury concentration (µg/g) which ranged from 0.017 µg/g for Aqua marine to 0.658 µg/g for Artic sea is comparable to the study by Kotb, *et al.* (1991) on the levels of some

heavy metals in omega-3 fatty acids in popular species of Arabian Gulf fish who obtained mean mercury concentration (µg/g) ranging from 0.04 µg/g to 1.45 µg/g. The mean mercury concentration range obtained in this study is also comparable to the result obtained by Vanaja, *et al.* (2007) who found that the concentration of mercury in some fish oil supplements ranged from 0.06µg/g to 0.012µg/g.The determined daily intakes for mercury which ranged from 0.021 µg/g for Cardioace to 0.818 µg/g for Dr. Chris were below the recommended daily intake of 16 µg/70kg body weight set by the Joint FAO/WHO expert committee on food supplements for mercury. The consumption of the omega-3 products thus poses no health risk as far as level of mercury is concerned.

The level of mercury in the omega-3 products were in the order; Aqua marine < Joint care < Cardioace < Deep sea < High sea < Valupak < Seven seas < Dr. Chris < Alaska < Artic sea. The different levels of mercury in the omega-3 products may be due to the fact that the oils were from different fishes from different sources at perhaps different trophic levels as found by Love *et al*. (2003). Formulation factors were also different for each product hence the variations in mercury levels.

Lindquist in 1991 reported that the main factors for the accumulation of heavy metals in aquatic media (fish, algae, aquatic plants) are organic matter, pH, seasonal changes, regional variations and hydrologic conditions as well as the rate of atmospheric deposition.

The low levels of metals determined in this work may be due to the fact that the omega-3 products were extracted from fishes of waters with low level of atmospheric depositions of metals.

Also human activities such as agriculture and mining that contribute to heavy metal accumulation could be minimal.

The low levels of metals obtained in this study may also be due to the fact that factors such as pH, temperature, microbial activities, and organic matter that affect the accumulation of heavy metals could be unfavourable.

Seidler (1987) found that factors such as cooking, frying, and refining processes could decrease levels of metals in fish oils to as much as 15% depending on the thermal treatment. The low levels of the metals determined in this study could also be attributed to the extraction and the refining methods used.

Comparing the values of the calculated daily intakes from taking the omega-3 product for the metals shown in Tables 4.15 to the their FAO/WHO recommended daily intakes of 15000 µg/70kg for iron, 5000 µg/70kg for manganese, 71 µg/70kg for cadmium, 15000 µg/70kg for zinc, 2000 µg/70kg for copper and 16 µg/70kg for mercury, it is observed that there is no likelihood of metal poisoning from taking the omega-3 products under this study.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

From the analysis carried out, the following conclusions may be deduced from the results obtained.

- Some metal content in some omega-3 food supplements available on the Ghanaian market were determined. All the concentrations of the metals in the omega-3 products were below the World Health Organization (WHO) limit.

- The calculated daily intakes of the metals determined in the omega-3 food supplements in microgram/gram are far below the recommended daily intakes by the FAO/WHO Joint Committee on food supplements which shows that the omega-3 food supplements analysed in this study are not likely to pose any health risk to the public through consumption for both the toxic elements and the essential micronutrients determined.

5.2 RECOMMENDATIONS

The following recommendations are suggested as a result of the outcome of this study

- Concentrations of heavy metals in other omega-3 food supplements on the Ghanaian market that are not covered by this research should be determined.

- Monitoring of levels of heavy metals in omega-3 food supplements should be encouraged.

- Other works such as dioxins and PCBs levels not covered by this research should be determined in omega-3 supplements on the Ghanaian market.

APPENDIX I

Table 4.2 LEVELS OF METALS IN AQUA MARINE (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
AM 1	8.850	1.500	0.300	1.530	0.510	0.017
AM 2	8.280	1.740	0.420	1.50	0.300	0.016
AM 3	8.190	1.620	0.390	1.50	0.330	0.016
AM 4	8.970	1.740	0.450	1.620	0.450	0.016
AM 5	8.340	1.470	0.360	1.500	0.390	0.017
AM 6	8.250	1.560	0.330	1.530	0.420	0.017
AM 7	8.370	1.650	0.420	1.590	0.330	0.016
AM 8	8.220	1.590	0.450	1.530	0.390	0.016
AM 9	8.310	1.500	0.390	1.560	0.360	0.017
AM 10	8.280	1.560	0.360	1.620	0.450	0.017

Table 4.3 LEVELS OF METALS IN VALUPAK (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
VA 1	7.471	2.046	0.620	1.178	0.217	0.096
VA 2	8.277	2.077	0.682	1.240	0.186	0.097
VA 3	6.975	1.984	0.682	1.178	0.217	0.092
VA 4	8.742	2.077	0.744	1.178	0.248	0.092
VA 5	8.525	2.139	0.713	1.240	0.186	0.091
VA 6	8.463	1.891	0.6913	1.178	0.217	0.093
VA 7	8.184	2.015	0.837	1.302	0.248	0.091
VA 8	7.564	1.922	0.620	1.302	0.217	0.094
VA 9	7.967	2.046	0.899	1.271	0.248	0.091
VA 10	8.618	2.077	0.806	1.302	0.186	0.093

Table 4.4 LEVELS OF METALS IN CARDIOACE (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
CD1	12.420	488.250	1.035	1815.300	733.050	0.021
CD 2	12.465	487.350	0.990	1816.200	733.500	0.020
CD 3	12.375	488.250	0.900	1817.550	732.150	0.020
CD 4	12.375	487.800	0.990	1816.200	732.150	0.020
CD 5	12.465	488.250	0.990	1815.300	733.500	0.021
CD 6	12.420	489.150	1.035	1816.650	732.600	0.021
CD 7	12.375	488.250	0.945	1817.100	732.150	0.021
CD 8	12.330	489.150	0.990	1816.650	732.600	0.021
CD 9	12.420	488.700	1.080	1816.650	733.050	0.020
CD 10	12.375	489.600	0.990	1816.200	733.500	0.020

Table 4.5 LEVELS OF METALS IN DR. CHRIS (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
DC1	21.976	7.504	1.407	1.340	1.072	0.277
DC 2	22.110	7.571	1.340	1.474	0.938	0.275
DC 3	21.976	7.437	1.206	1.340	1.005	0.276
DC 4	21.976	6.633	1.273	1.407	0.938	0.285
DC 5	22.177	7.437	1.139	1.608	1.072	0.276
DC 6	22.043	7.504	1.139	1.474	0.938	0.269
DC 7	21.976	6.700	1.072	1.407	1.005	0.266
DC 8	22.110	7.370	1.340	1.340	1.139	0.268
DC 9	22.177	7.504	1.139	1.474	1.072	0.270
DC 10	22.043	6.633	1.206	1.608	1.072	0.271

Table 4.6 LEVELS OF METALS IN SEVEN SEAS (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
SS1	3.729	2.013	0.825	0.429	0.099	0.112
SS 2	3.762	1.947	0.792	0.495	0.033	0.133
SS 3	3.729	1.980	0.759	0.462	0.066	0.123
SS 4	3.828	1.914	0.792	0.495	0.099	0.128
SS 5	3.762	1.947	0.858	0.396	0.066	0.119
SS 6	3.762	1.914	0.759	0.462	0.033	0.121
SS 7	3.795	2.013	0.825	0.495	0.099	0.124
SS 8	3.729	1.947	0.726	0.462	0.066	0.119
SS 9	3.795	1.980	0.792	0.462	0.033	0.121
SS 10	3.828	1.914	0.726	0.495	0.032	0.128

Table 4.7 LEVELS OF METALS IN ARTIC SEA (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
AS1	6.798	0.924	0.825	0.825	0.066	0.651
AS 2	6.93	0.858	0.759	0.825	0.033	0.635
AS 3	6.831	0.891	0.792	0.792	0.099	0.725
AS 4	6.765	0.858	0.726	0.825	0.066	0.672
AS 5	6.798	0.924	0.792	0.858	0.132	0.712
AS 6	6.897	0.825	0.759	0.792	0.033	0.625
AS 7	6.864	0.858	0.792	0.825	0.099	0.622
AS 8	6.831	0.924	0.825	0.825	0.066	0.700
AS 9	6.831	0.891	0.759	0.858	0.099	0.621
AS 10	6.897	0.825	0.858	0.792	0.033	0.618

Table 4.8 LEVELS OF METALS IN JOINT CARE (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
JC1	614.790	7.293	0.726	2.079	0.066	0.018
JC 2	613.800	7.392	0.825	2.145	0.066	0.017
JC 3	616.440	7.392	0.693	2.211	0.099	0.018
JC 4	614.460	7.260	0.825	2.046	0.033	0.017
JC 5	615.450	7.392	0.825	2.013	0.099	0.019
JC 6	614.790	7.293	0.660	2.112	0.066	0.018
JC 7	616.110	7.260	0.792	2.079	0.033	0.016
JC 8	615.120	7.392	0.726	2.145	0.066	0.019
JC 9	616.440	7.359	0.759	2.178	0.099	0.017
JC 10	615.120	7.293	0.792	2.211	0.033	0.019

Table 4.9 LEVELS OF METALS IN DEEP SEA (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
DS1	14.883	2.442	0.429	1.254	0.165	0.029
DS 2	14.949	2.508	0.561	1.056	0.132	0.027
DS 3	15.081	2.442	0.396	1.089	0.099	0.027
DS 4	14.916	2.508	0.462	1.122	0.165	0.023
DS 5	14.982	2.475	0.594	1.155	0.099	0.027
DS 6	14.850	2.409	0.528	1.023	0.132	0.024
DS 7	15.081	2.310	0.462	1.188	0.099	0.028
DS 8	14.949	2.409	0.627	1.221	0.198	0.029
DS 9	14.916	2.508	0.594	1.155	0.231	0.027
DS 10	14.982	2.541	0.660	1.221	0.165	0.025

Table 4.10 LEVELS OF METALS IN ALASKA (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
AL1	5.709	0.759	0.759	0.957	0.099	0.430
AL 2	5.808	0.825	0.726	0.891	0.132	0.434
AL 3	5.775	0.792	0.726	0.990	0.066	0.431
AL 4	5.643	0.891	0.792	0.891	0.099	0.431
AL 5	5.610	0.825	0.759	0.924	0.033	0.430
AL 6	5.676	0.924	0.825	0.924	0.033	0.414
AL 7	5.742	0.858	0.693	0.858	0.132	0.432
AL 8	5.775	0.792	0.660	0.792	0.297	0.431
AL 9	5.808	0.891	0.858	0.891	0.231	0.429
AL 10	5.676	0.792	0.825	0.957	0.066	0.419

Table 4.11 LEVELS OF METALS IN HIGH SEA (µg/g)

SAMPLE CODE	IRON	MANGANESE	CADMIUM	ZINC	COPPER	MERCURY
HS1	13.233	7.095	0.627	0.759	0.165	0.028
HS 2	13.134	7.161	0.561	0.858	0.066	0.027
HS 3	12.903	7.095	0.66	0.924	0.033	0.030
HS 4	13.233	7.062	0.561	0.759	0.066	0.029
HS 5	13.068	7.128	0.594	0.792	0.132	0.028
HS 6	13.101	7.062	0.594	0.726	0.132	0.028
HS 7	13.068	7.161	0.660	0.825	0.132	0.028
HS 8	13.134	7.062	0.627	0.792	0.132	0.029
HS 9	13.266	7.095	0.627	0.825	0.099	0.030
HS 10	13.134	7.194	0.594	0.858	0.066	0.029

Table 4.12 MEAN CONCENTRATIONS OF METALS IN SAMPLES (µg/g)

OMEGA-3 SAMPLE	MEAN CONCENTRATION OF METALS (µg/g)					
	Fe	**Mn**	**Cd**	**Zn**	**Cu**	**Hg**
AQUA MARINE	8.406	1.593	0.387	1.548	0.393	0.017
VALUPAK	8.079	2.027	0.729	1.237	0.217	0.093
CARDIOACE	12.402	488.475	0.995	1816.380	732.825	0.021
DR. CHRIS	22.056	7.223	1.226	1.447	1.025	0.273
SEVEN SEAS	3.772	1.957	0.785	0.465	0.063	0.123
ARTIC SEA	6.844	0.878	0.788	0.822	0.073	0.658
JOINT CARE	615.252	7.333	0.762	2.122	0.066	0.018
DEEP SEA	14.959	2.455	0.531	1.148	0.149	0.026
ALASKA	5.722	0.835	0.762	0.908	0.119	0.428
HIGH SEA	13.127	7.112	0.611	0.812	0.102	0.029

Table 4.13 RANGES OF CONCENTRATIONS OF METALS IN SAMPLES (µg/g)

OMEGA-3 SAMPLE	RANGES OF CONCENTRATIONS OF METALS IN SAMPLES (µg/g)					
	Fe	Mn	Cd	Zn	Cu	Hg
AQUA MARINE	8.19 – 8.97	1.47 – 1.74	0.300 – 0.450	1.50 – 1.62	0.30 – 0.51	0.016 – 0.017
VALUPAK	6.975 – 8.618	1.891 – 2.139	0.620 – 0.899	1.178– 1.302	0.186– 0.248	0.091 – 0.097
CARDIOACE	12.330 – 12.465	487.35 – 489.60	0.945 – 1.080	1815.30-1817.56	732.150– 733.50	0.020 – 0.021
DR. CHRIS	21.976 – 22.177	6.633 – 7.571	1.139 – 1.407	1.340 – 1.608	0.938– 1.139	0.260 – 0.280
SEVEN SEAS	3.729 – 3.828	1.914 – 2.013	0.726 – 0.858	0.396 – 0.475	0.032– 0.099	0.110 – 0.130
ARTIC SEA	6.765 – 6.930	0.825 – 0.924	0.759 – 0.858	0.792 – 0.858	0.033– 0.132	0.610 – 0.700
JOINT CARE	613.80 – 616.44	7.260 – 7.392	0.660 – 0.825	2.013 – 2.211	0.033– 0.099	0.015 – 0.019
DEEP SEA	14.850 – 15.081	2.310 – 2.541	0.396 – 0.660	1.023 – 1.254	0.099– 0.231	0.023 – 0.029
ALASKA	5.610 – 5.808	0.759 – 0.924	0.660 – 0.858	0.792 – 0.990	0.033– 0.297	0.410 – 0.430
HIGH SEA	12.903 – 13.266	7.062 – 7.194	0.561 – 0.660	0.726 – 0.924	0.033– 0.165	0.027 – 0.030

TABLE 4.14 STANDARD DEVIATIONS OF CONCENTRATIONS OF METALS

OMEGA-3 SAMPLE	STANDARD DEVIATION OF CONCENTRATION OF METALS (µg/g)					
	Fe	Mn	Cd	Zn	Cu	Hg
AQUA MARINE	0.027	0.007	0.062	0.048	0.071	0.003
VALUPAK	0.057	0.076	0.014	0.055	0.025	0.002
CARDIOACE	0.043	0.067	0.047	0.071	0.057	0.003
DR. CHRIS	0.082	0.040	0.110	0.021	0.071	0.005
SEVEN SEAS	0.038	0.063	0.103	0.032	0.063	0.004
ARTIC SEA	0.031	0.131	0.014	0.039	0.042	0.001
JOINT CARE	0.051	0.043	0.030	0.041	0.074	0.005
DEEP SEA	0.059	0.056	0.025	0.029	0.065	0.008
ALASKA	0.032	0.072	0.035	0.035	0.028	0.002
HIGH SEA	0.024	0.004	0.063	0.044	0.038	0.004

Table 4.15 MEAN DAILY CONSUMPTION OF METALS IN SAMPLES (µg/g)

OMEGA-3 SAMPLE	DAILY DOSAGE (CAPSULE)	Mean Daily Consumption of Metals in Samples (µg/g)					
		Fe	Mn	Cd	Zn	Cu	Hg
RDI/µg/Day		**15000**	**5000**	**71**	**15000**	**2000**	**16**
AQUA MARINE	2	16.812	3.186	0.774	3.096	0.786	0.033
VALUPAK	1	8.079	2.027	0.729	1.237	0.217	0.093
CARDIOACE	1	12.402	488.475	0.995	1816.380	732.825	0.021
DR. CHRIS	3	66.169	21.688	3.678	4.342	3.075	0.818
SEVEN SEAS	6	22.631	11.741	4.712	2.792	0.376	0.738
ARTIC SEA	1	6.844	0.878	0.789	0.822	0.073	0.658
JOINT CARE	3	1845.756	21.998	2.287	6.366	0.198	0.053
DEEP SEA	1	14.959	2.455	0.531	1.148	0.149	0.027
ALASKA	1	8.406	0.835	0.762	0.908	0.119	0.428
HIGH SEA	1	8.079	7.112	0.611	0.812	0.102	0.029

*The calculations for the maximum intake were based on recommended daily intake values from www.lenntech.com/recommended-daily-intake.htm recommended daily intake on minerals and vitamins and Joint FAO/WHO expert committee on food supplements.

*The mean daily consumptions were calculated by multiplying the calculated mean metal levels by the dosage stated on the product.

APPENDIX II

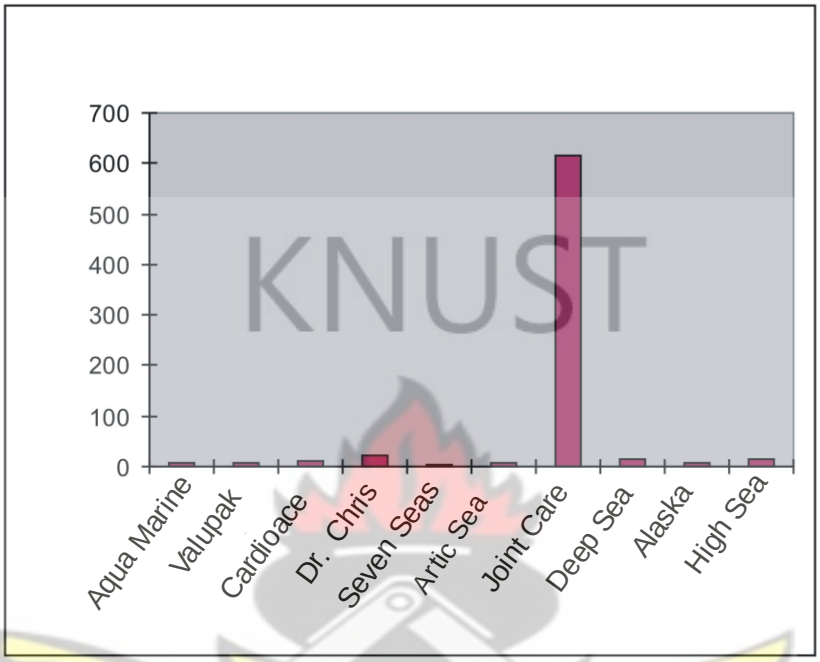

Fig.4.1: Level of iron in the omega-3 food supplements

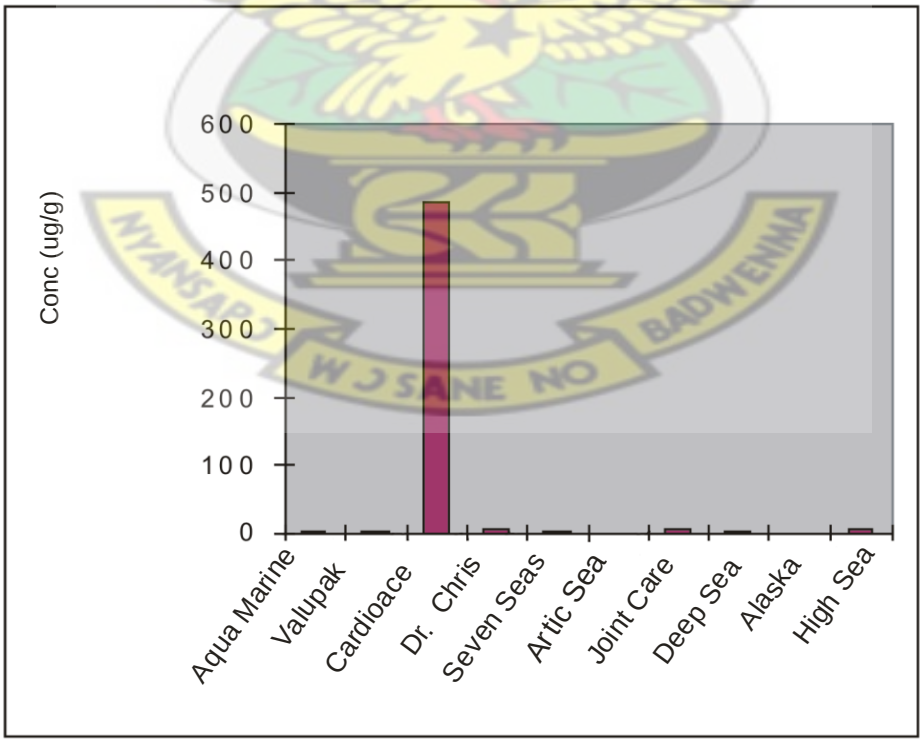

Fig.4.2: Level of manganese in the omega-3 food supplements

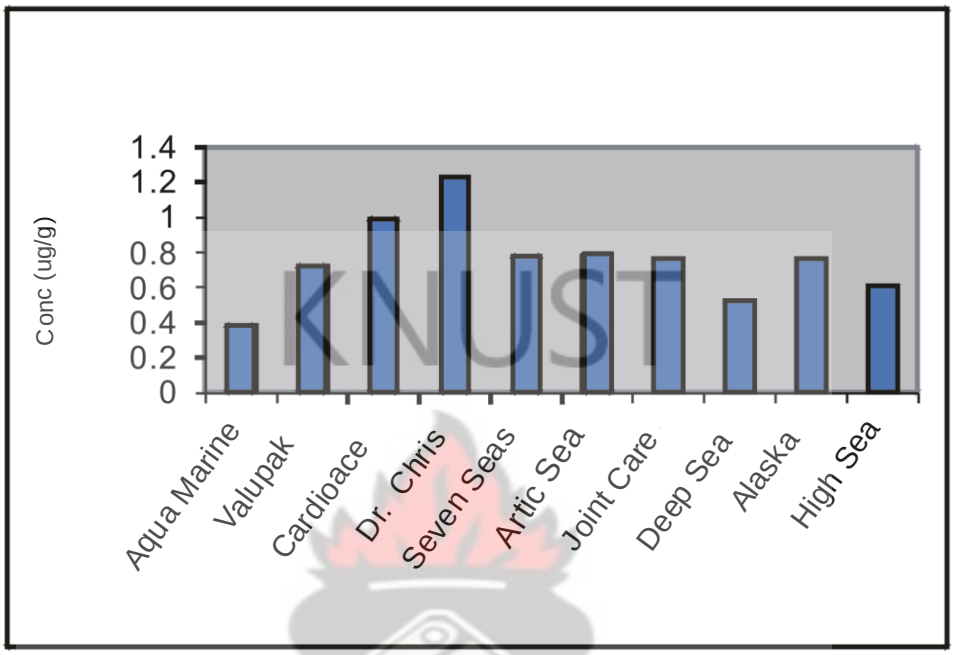

Fig 4.3: Level of cadmium in the omega-3 food supplements

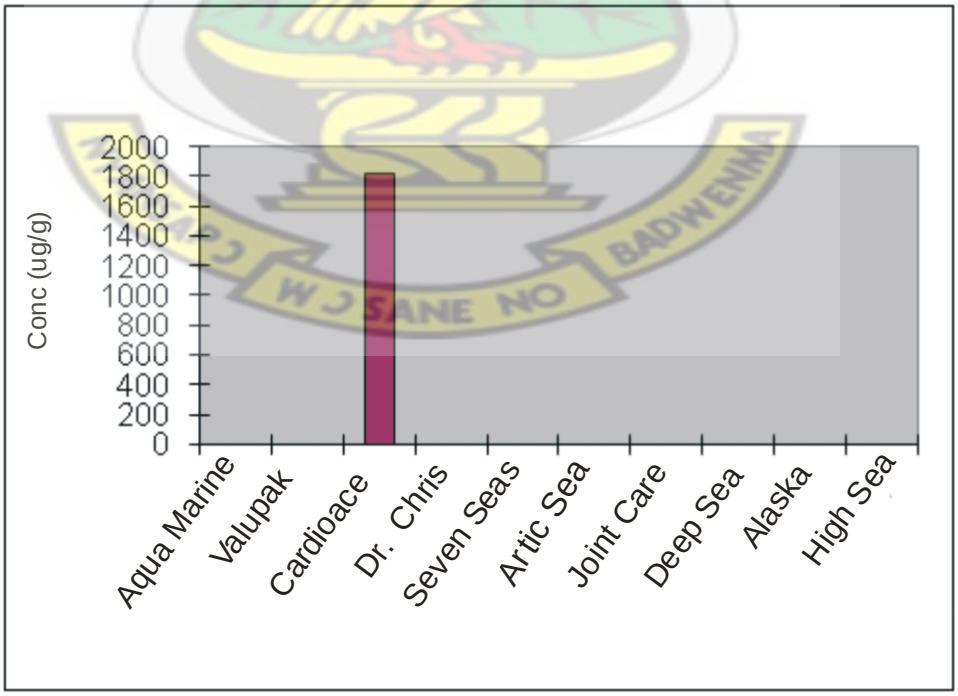

Fig 4.4: Level of zinc in the omega-3 food supplements

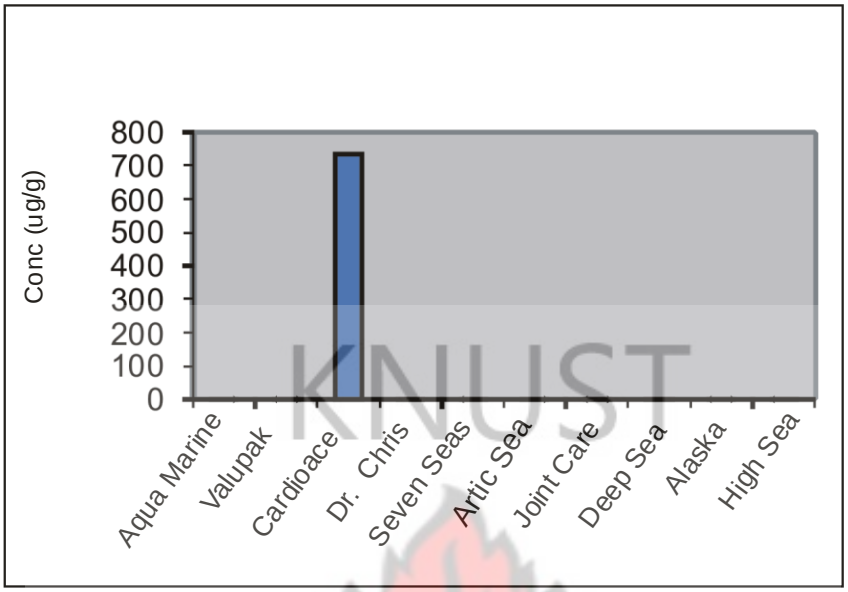

Fig 4.5: Level of copper in the omega-3 food supplements

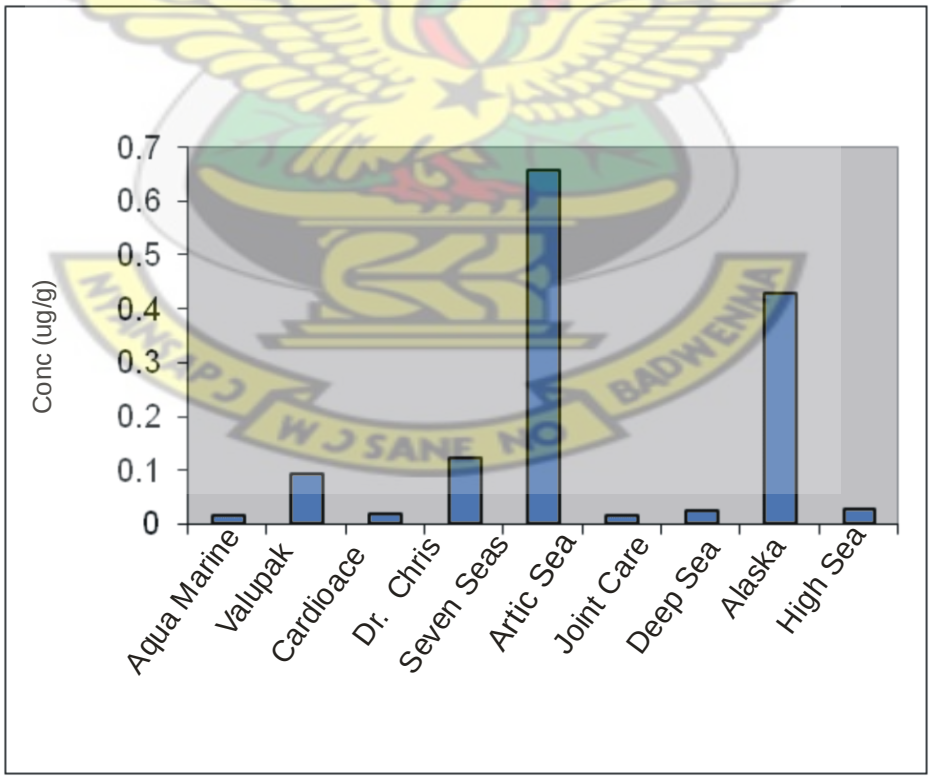

Fig 4.6: Level of mercury in the omega-3 food supplements

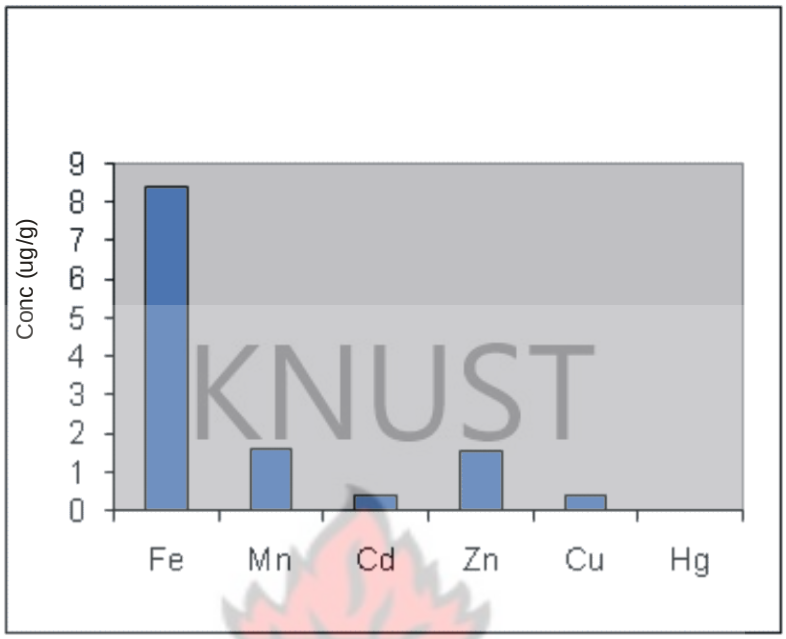

Fig 4.7: levels of the metals in Aqua Marine

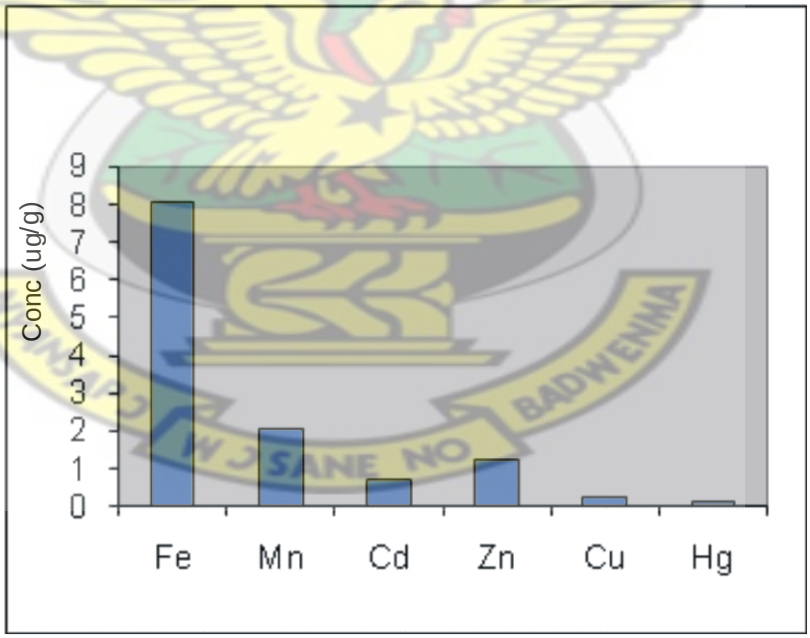

Fig 4.8: levels of the metals in Valupak

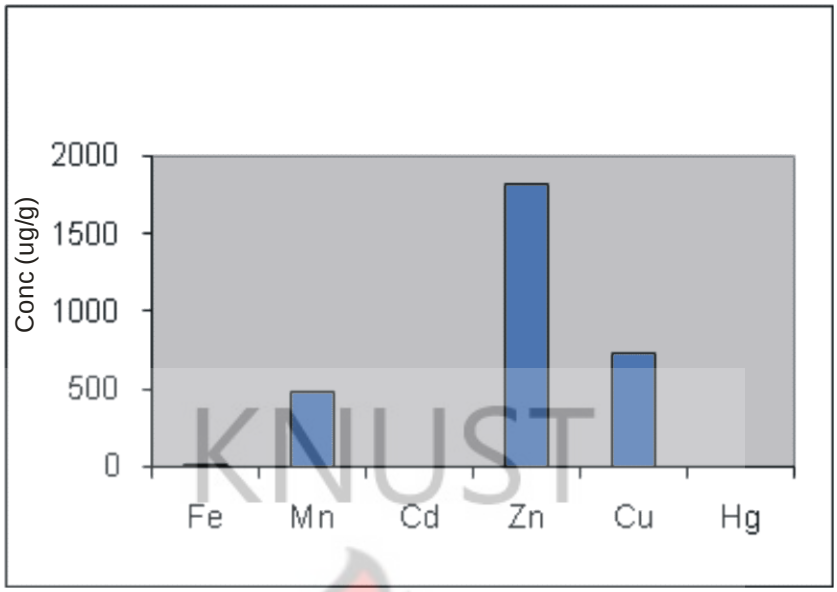

Fig 4.9: levels of the metals in Cardioace

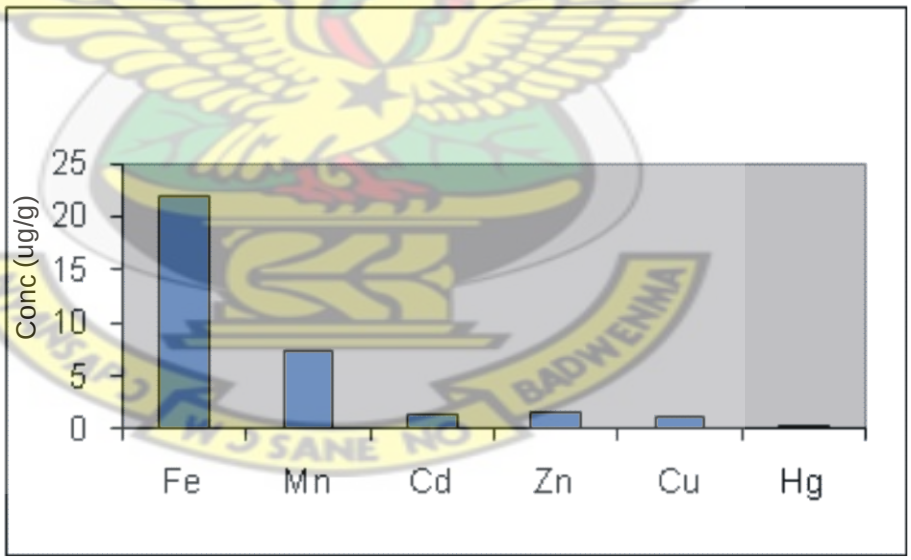

Fig 4.10: levels of the metals in Dr. Chris

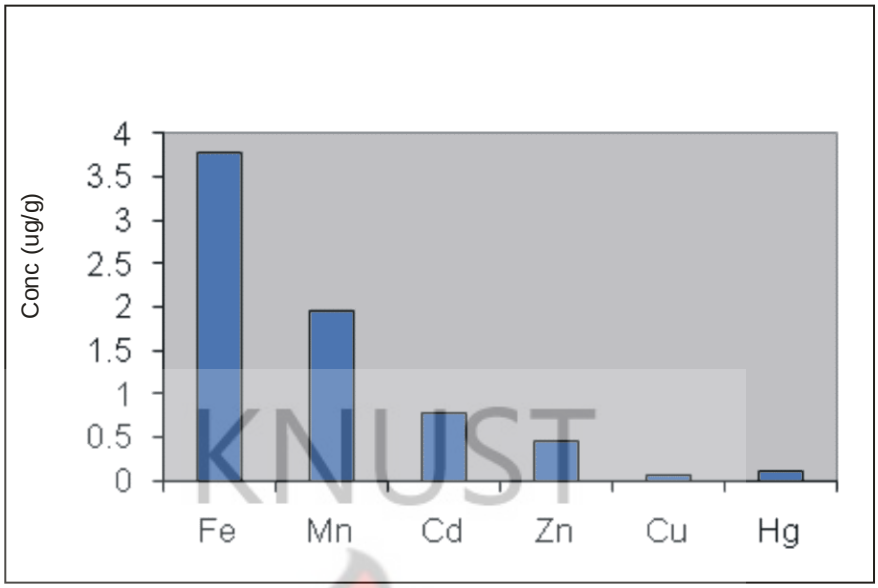

Fig 4.11: levels of the metals in Seven Seas

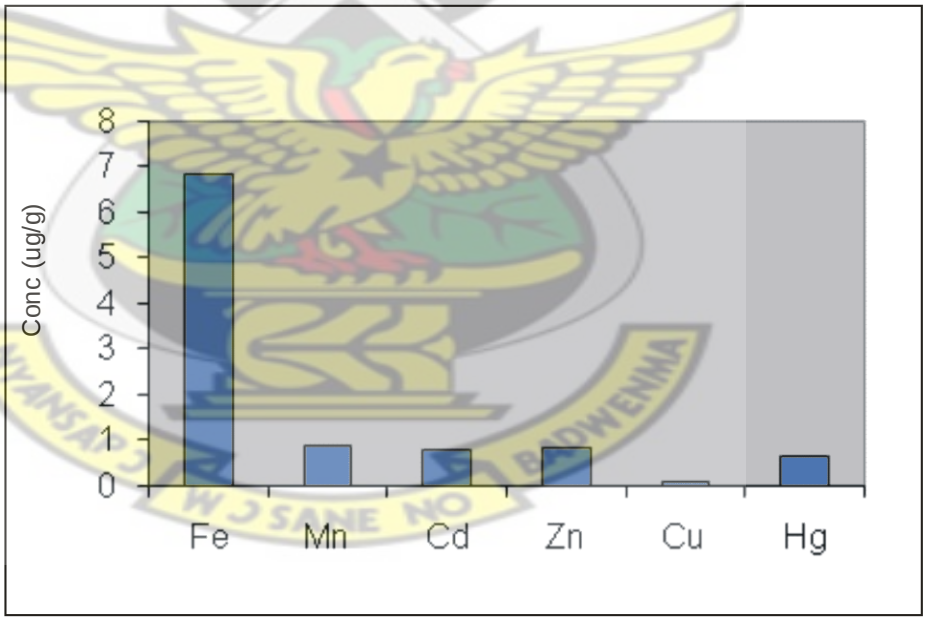

Fig 4.12: levels of the metals in Artic Sea

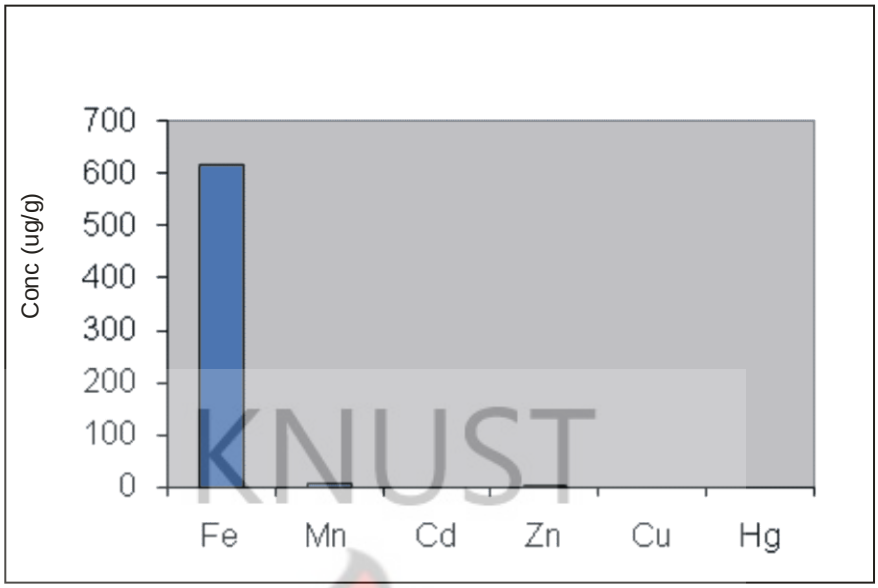

Fig 4.13: levels of the metals in Joint Care

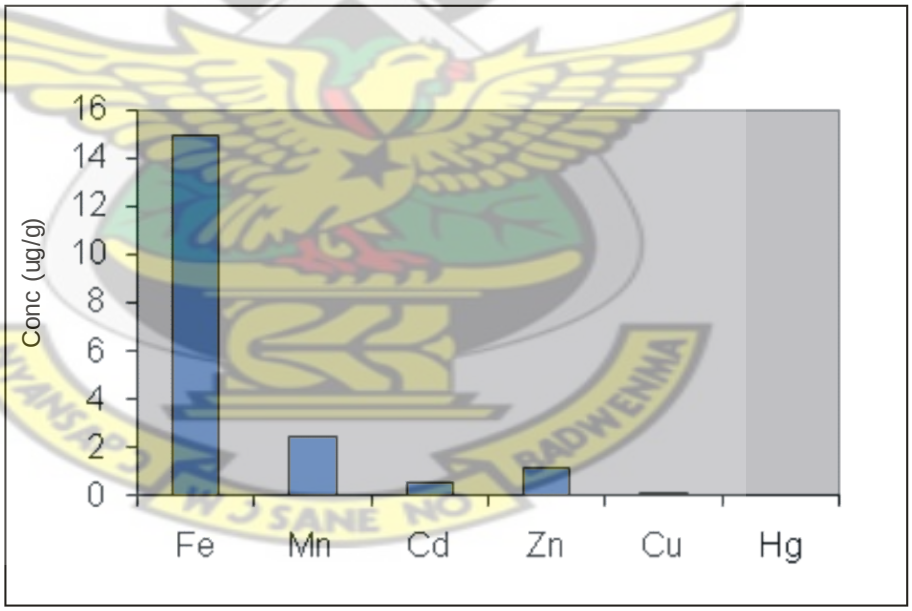

Fig 4.14: levels of the metals in Deep Sea

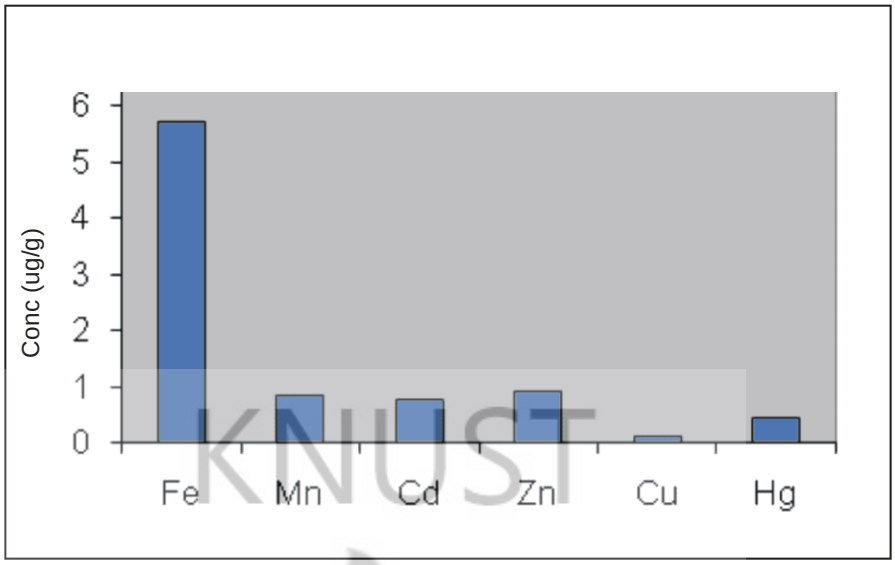

Fig 4.15: levels of the metals in Alaska

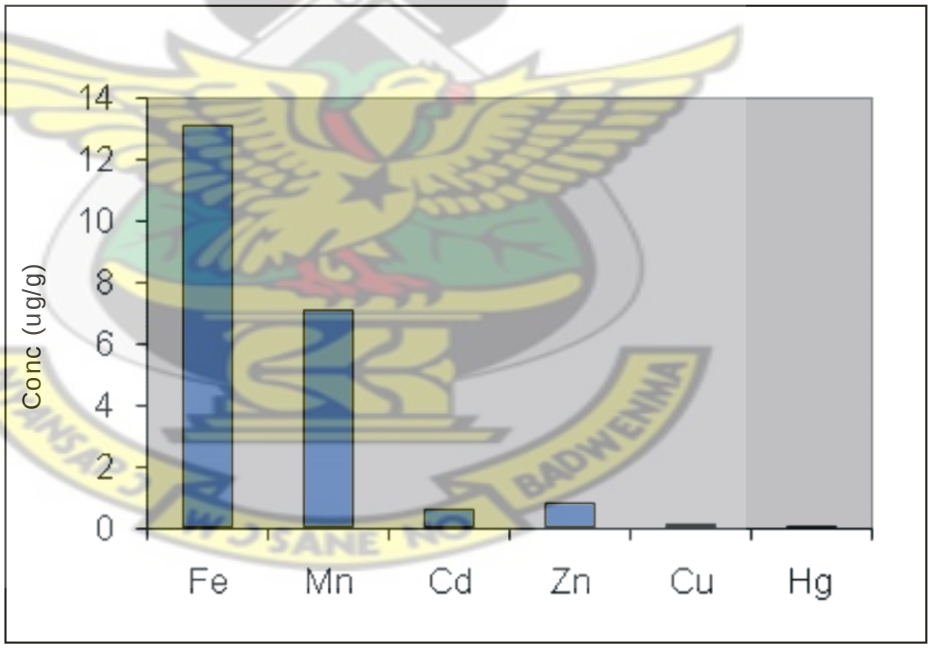

Fig 4.16: levels of the metals in High Sea

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