COMPARATIVE STUDY OF LEAD AND ARSENIC LEVELS IN COMMERCIAL SAMPLES OF LOCALLY PRODUCED AND IMPORTED RICE VARIETIES.

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TITLE PAGE

COMPARATIVE STUDY OF LEAD AND ARSENIC LEVELS IN COMMERCIAL SAMPLES OF LOCALLY PRODUCED AND IMPORTED RICE VARIETIES.

A PROJECT WORK SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN ENVIRONMENTAL BIOCHEMISTRY

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FEBUARY, 2020

CERTIFICATION

This is to certify that Eze, George Peter a postgraduate student with registration number PG/M.Sc./17/01109 in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, has satisfactorily completed the requirement of the research work for the award of Master of Science (M.Sc) degree in Biochemistry (Environmental Biochemistry). The work embodied in this report is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

…….....

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PROF. H. A. ONWUBIKO
(Head of Department)

External Examiner

DEDICATION

This work is dedicated to my mother ,Mrs.Eunice Eze and My students .

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My gratitude goes to the Almighty God who teaches me wisdom and directs my path by his immeasurable grace even in the course of this project work.

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.

ABSTRACT

The level of heavy metals, lead (Pb) and arsenic (As) were determined in nine brands of locally produced (Nigerian) rice and nine imported rice brands from Thailand, India and China. The heavy metal analysis was conducted using atomic absorption spectrometry of digested, parboiled rice samples. The findings showed combined mean values of lead in local and imported rice brands as follows: 0.19 mg/kg and 0.11 mg/kg. All the values of lead concentration in both local and imported rice brands fall within the baseline of the maximum allowable concentration (MAC) value, 6.0 mg/kg specified by JECFA/EPSA for heavy metal (lead) in cereals and vegetables. The mean values of arsenic observed in local and imported rice brands was 4.42 mg/kg and 3.71 mg/kg respectively, which is far above maximum allowable concentration (MAC) value, 1.4 mg/kg stipulated by JECFA/EPSA for heavy metal (arsenic) in cereals and vegetables. This result shows that there might be serious risk of arsenic poising (acute or chronic) when these rice brands are consumed at the national average per capita level of 24.8 kg per annum, especially in children who are more susceptible to arsenic toxicity.

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CHAPTER ONE

INTRODUCTION

Rice is a major food staple and a mainstay for the rural population and their food security. It is the second most cultivated cereal crop worldwide and is central to the lives of billions of people around the world (Nguyen and Ferrero, 2006). Rice belongs to the Plant Division Magnoliophyta and Species, *Oryza sativa* (Table 1) with subspecies which are called Japonica and Indica. Japonica is a sticky, short grained type of rice, usually cultivated in dry fields. Indica is non-sticky and long-grained type of rice and is mostly grown in submerged fields. Both types have many different varieties. It is usually grown as an annual plant, but in the tropics it can be grown as a perennial. Rice prefers a tropical or warm climate, with a lot of rainfall. But if irrigation water is available, rice can also be grown in drier areas or during dry season. Rice is usually a self-pollinating cereal, but cross pollination by wind is possible. Rice plants are usually 1 and 1.8 meter tall depending on the variety and soil. Rice is usually grown on heavier soils that have a good water holding capacity.

Rice (*Oryza sativa)* cultivation started at the 15th century in South East Asia and spread to India, China and Japan. Rice was first domesticated in the region of the Yangtze River valley in China, however rice was found in its wild form in India. Rice goes through a series of processing before finally reaching the dinner table. Rice cultivation involves seed selection followed by land preparation that is aimed to place the soil in the best physical condition for crop growth and to ensure that soil surface is level. Seeds are sown in seed bed. Further, crop establishment involves transplanting, where pre-germinated seedlings are transferred from seed bed to wet field. Transplantation is done manually by hand after a period of 20-40 days. Water management is a crucial step where the field is maintained in a flooded condition to conserve water sufficient for the crop.

Rice is grown from seed. A rice plant starts as a seedling but because of tillering during the vegetative stage, the plant will eventually produce many tillers which each can produce a panicle with seeds. At the tip of the stem the rice plant develops a panicle, which may contain up to 200 flowers, each producing a rice seed. As the plant has many tillers, one plant can produce thousands of seeds. Rice grains are usually 5 to 12 mm long and 2 to 3 mm wide, depending on the variety. Some short duration rice varieties can mature in 100 days. But medium and long-

duration varieties require 130 to 150 days. 20x20 cm is the distance that seedlings are transplanted (often 2 or 3 seedlings together).

Rice is the predominant dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. It provides 20% of the world's dietary energy supply (FAO, 2014). The nutrition value of rice depends on the strain of rice, nutrient quality of the soil grown in, its processing and preparation before cooking.

India stands second in rice production with an annual production of 152 million tons (FAO, 2014). Rice cultivation takes place in all states of India, but West Bengal, Uttar Pradesh, Madhya Pradesh, Punjab, Orissa and Bihar are the major rice producing states. In India, rice production has increased gradually from 137 to 155 million tons. Developing countries account for 95% of the total production, with China and India alone responsible for nearly half of the world output (FAO, 2014). The major rice importing countries of India's rice are Nigeria, Philippines, Saudi Arabia, Iraq, Iran, Malaysia, Brazil, Persian Gulf countries, Europe, South Africa, Bangladesh and Indonesia (Viraktamath and Rani, 2008). Today, majority of all rice produced comes from China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Japan. Asian farmers still account for 92% of the world's total rice production.

Table 1 Scientific classification of Rice .

Kingdom	**Plantae**
Division	Magnoliophya
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	Oryza
Species	*Oryza sativa*

Source; Khush *et al*., 1990.

1.1 Heavy Metals in Human Nutrition.

Metals are natural constituents that exist in the ecosystem. They are substances with high electrical conductivity which readily donate their electrons to form ions (cations). Metals are found all over the earth including the atmosphere, earth crust, water bodies, and can also accumulate in biological organisms including plants and animals. Among the 35 natural existing metals, 23 possess high specific density above 5 g/cm^3 with atomic weight greater than 40.04 and are referred to as heavy metals (Duffus, 2002; Li *et al*., 2017). Heavy metals include: antimony, tellurium, bismuth, tin, thallium, gold, arsenic, cerium, gallium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, nickel, platinum, silver, uranium, vanadium, and zinc (Duffus, 2002; Li *et al*., 2017) . Heavy metals have not only been known for their high electrical conductivity but most importantly for their adverse effects on the ecosystem and living organisms (Bradl, 2002). Some of these heavy metals such as cobalt, chromium, copper, magnesium, iron, molybdenum, manganese, selenium, nickel and zinc are essential nutrients that are required for various physiological and biochemical functions in the body and may result to deficiency diseases or syndromes if not in adequate amounts (WHO/FAO/IAEA, 1996) but in large doses they may cause acute or chronic toxicities. These heavy metals are distributed in the environment through several natural processes such as volcanic eruptions, spring waters, erosion, and bacterial activity, and also through anthropogenic activities which include fossil fuel combustion, industrial processes, agricultural activities (Florea *et al*., 2004). In the earth crust, these heavy metals are present in ores which are recovered during mining activities as minerals. In most ores heavy metals such as arsenic, iron, lead, zinc, gold, nickel, silver and cobalt exist as sulfides while others such as manganese, aluminum, selenium gold, and antimony exist as oxides. Certain heavy metals such as copper, iron and cobalt can exist both as sulfide and oxide ores. Some sulfides may contain two or more heavy metals together such as chalcopyrite, ($CuFeS_2$) which contain both copper and iron. During these mining activities, heavy metals are released from the ore and scattered in open in the environment; left in the soil, transported by air and water to other areas. Furthermore, when these heavy metals are used in the industries for various industrial purposes, some of these elements are released into the air during combustion or into the soil or water bodies as effluents. More so, the industrial products such as paints,

cosmetics, pesticides, and herbicides also serve as sources of heavy metals. Heavy metals may be transported through erosion, run-off or acid rain to different locations on soils and water bodies. These heavy metals do bio-accumulate in living organisms and the human body through various processes causing adverse effects. In the human body, these heavy metals are transported through food and compartmentalized into body cells and tissues binding to proteins, nucleic acids destroying these macromolecules and disrupting their cellular functions. As such, heavy metal toxicity can have several consequences in the human body. It can affect the central nervous function leading to mental disorder, damage the blood constituents and may damage the lungs, liver, kidneys and other vital organs promoting several disease conditions (Monisha *et al*., 2014). Also, long term accumulation of heavy metals in the body may result in slowing the progression of physical, muscular and neurological degenerative processes that mimic certain diseases such as Parkinson's disease and Alzheimer's disease (Monisha *et al*., 2014). More so, repeated long-term contact with some heavy metals or their compounds may even damage nucleic acids in figure 1, cause mutation, mimic hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer (Jarup, 2003).

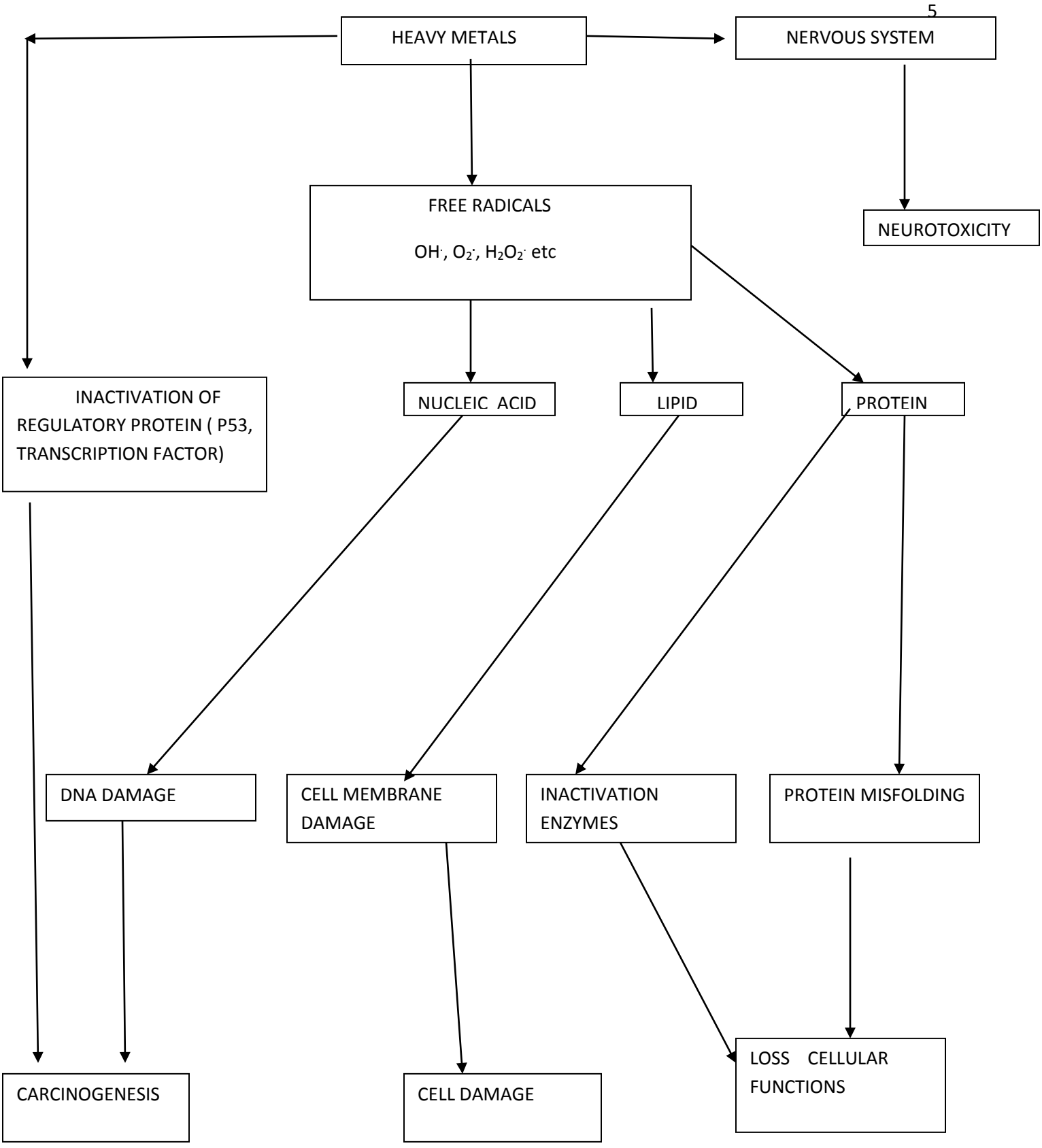

Figure 1: sketch of heavy metals exposure to humans (Ghani, 2011).

1.2 ARSENIC

Arsenic the twentieth most abundant element in the earth's crust found in a trace amount in all human tissues, probably bound to proteins. It can exist in three oxidation states as 0 (metalloid), -3 or +3 (trivalent), and +5 (pentavalent). Over the centuries, arsenic has been used as pesticides, fungicides, cotton desiccants, and wood preservatives, etc. In the tradio-medicinal such as Ayurveda or Chinese or Homeopathic, medicinal formulations are reported to contain arsenic (Kew *et al*., 1993).

1.2.1 Arsenic Toxicity in Human.

Arsenic affects almost all organs during its acute or chronic exposure. Liver has been reported as target organ of arsenic toxicity (Clarkson, 1991). The early symptoms of arsenic poisoning include headache, dizziness, insomnia, weakness, nightmare, and numbness in the extremities, anemia, palpitation, and fatigue. Many studies confirmed the generation of free radicals during arsenic metabolism in cells. Arsenic induced generation of free radicals can cause cell damage (Fig.2) and death through activation of oxidative sensitive signaling pathways (Bernstam and Nriagu, 2000).

1.2.2 Mechanism of Arsenic toxicity in Human.

Mechanism of arsenic toxicity involves arsenic biotransformation in figure 2, harmful inorganic arsenic compounds get methylated in humans to give monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA). In this biotransformation process, these inorganic arsenic species (iAs) are converted enzymetically to methylated arsenicals which are the end metabolites and the biomarker of chronic arsenic exposure. Biomethylation is a detoxification process and end products are methylated inorganic arsenic such as MMA(V) and DMA(V) in figure 2, which excreted through urine are bio-indication of chronic arsenic exposure. However MMA (III) is not excreted and remains inside the cell as an intermediate product. Monomethylarsonic acid (MMA III), an intermediate product, is found to be highly toxic compared to other arsenicals, potentially accountable for arsenic-induced carcinogenesis (Singh *et al*., 2007).

Studies conducted recently to explore the mechanism of arsenic induced toxicity showed that arsenic toxicity causes Alteration in biogenic amine levels, peripheral neuropathy, sensory neuropathy, mental confusion, anxiety, low IQ scores and verbal comprehens, Loss of capillary integrity, proteinuria, hematouria, leukocyturia and glycouria, oliguria and anuria , Myocardial depolarization, cardiac arrhythmias Blackfoot disease, atherosclerosis and hypertension, decreased activity of Protein kinase C, Hepatic fibrosis, increased activity of liver enzymes in serum, changes in cellular morphology, apoptosis, Hyperpigmentation, melanosis, hyperkartosis, warts and skin cancer (Bowen disease, squamous cell carcinomas, basal cell carcinomas), Brittle nails, (transverse bands on nails, Anemia, absolute neutropenia, leucopenia, thrombocytopenia, relative eosinophilia, basophilic stippling, increased bone marrow vascularity and rouleau formation, karyorrhexis, pyknosis of the thromboblast (Lin *et al*,.1999).

Signs and symptoms showing arsenic induced toxicity suggest that the mechanism of action predominantly depends upon the two oxidation states of arsenic.

Trivalent inorganic arseninate react with molecules containing sulfhydryl groups such as glutathione (GSH), δ-aminolevulinic acid dehydratase (ALAD), and form strong complex with vicinal thiols groups thereby inhibiting the activity of molecules. Inhibits PDH activity, binds to the lipoic acid moiety, Potent inhibitors of GSH reductase (Styblo *et al*,. 1997), and thioredoxin reductase (Lin *et al*,. 1999).

Pentavalent inorganic arsenate acts by reduction to form arsenite , which Mimic phosphate in human system thereby replacing phosphate in the sodium pump and the anion exchange transport system, form esters with glucose and gluconate resulting in glucose-6- arsenate and 6-arsenogluconate, respectively. These compounds resemble glucose-6-phosphate and 6-phosphogluconate thus they inhibit activity of hexokinase. As well, Uncouples oxidative phosphorylation known as arsenolysis.

The major mechanisms by which arsenic may damage cells are direct binding to cellular molecules, induction of conformational changes, replacement of physiologic metals from their binding sites (Qian *et al*,. 2003), or inhibition of DNA repair functions. Thus, they may act as catalysts for the redox reactions that produce reactive oxygen species (ROS) and create a condition of oxidative stress. Exact mechanism behind arsenic induced carcinogenic effects is

still not clear however, oxidative stress is supposed to be a new theory behind it as it is in accordance with the various reports obtained earlier (Flora,1999). Oxidative stress is an imbalance between pro-oxidant and antioxidant status. During normal cellular respiration oxygen is reduced to water and highly reactive free radicals.

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench them and protect the body from NH-their deleterious effects. An overproduction of free radicals either through environmental factors or due to some toxicants can increase the pro-oxidant and antioxidant balance leading to oxidative stress. The first oxidative stress theory of arsenic carcinogenesis that included a detailed arsenic metabolic pathway was presented by (Yamanaka *et al,*. 1990). They reported formation of dimethylarsenic radical $(CH3)_2As$, and dimethylarsenic peroxyl radical $(CH3)_2AsOO$, which may lead to DNA single strand breaks. Chemical species with an unpaired electron that can be anionic, neutral or cationic, e.g. Super oxide anion , Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH), atomic oxygen (O), alkoxyl radical (RO'), peroxyl radicals (ROO'), Nitric oxide (NO), Peroxynitrite (ONOO), One striking advantage to the oxidative stress theory is arsenic's ability to cause human cancers at high rates in the lungs, bladder, and skin because high partial pressure of oxygen is found in lungs. Human bladder is susceptible to arsenic carcinogenesis because high concentration of arsenic metabolites (DMA and MMA) is stored in the lumen of the bladder. Skin seems unusually sensitive to both arsenic's toxicity and carcinogenic effects because it localizes and stores arsenic due to its high keratin content (Kitchin, 2001). During normal cell function ROS are neutralized by self-defense mechanism, which includes antioxidant enzymes and glutathione cycle. Lipid peroxidation is one of the mechanism of arsenic toxicity in female rats together with a concomitant decrease in cellular reduced glutathione (GSH) concentration which is inversely correlated with lipid peroxidation in the liver but not in other tissues (Ramos *et a*l,. 2001). Lipid peroxidation involves fragmentation of polyunsaturated fatty acids. One of the important product of lipid peroxidation is 4-hydroxy-2-nonenal (HNE), which is highly reactive α, β unsaturated aldehyde. HNE reacts with nucleophilic side chains of nucleic acids and protein via Michael addition to form HNE-protein species. HNE irreversibly alkylates the protein and introduces a carbonyl group, which results in protein degradation (Ramos *et a*l., 2001).

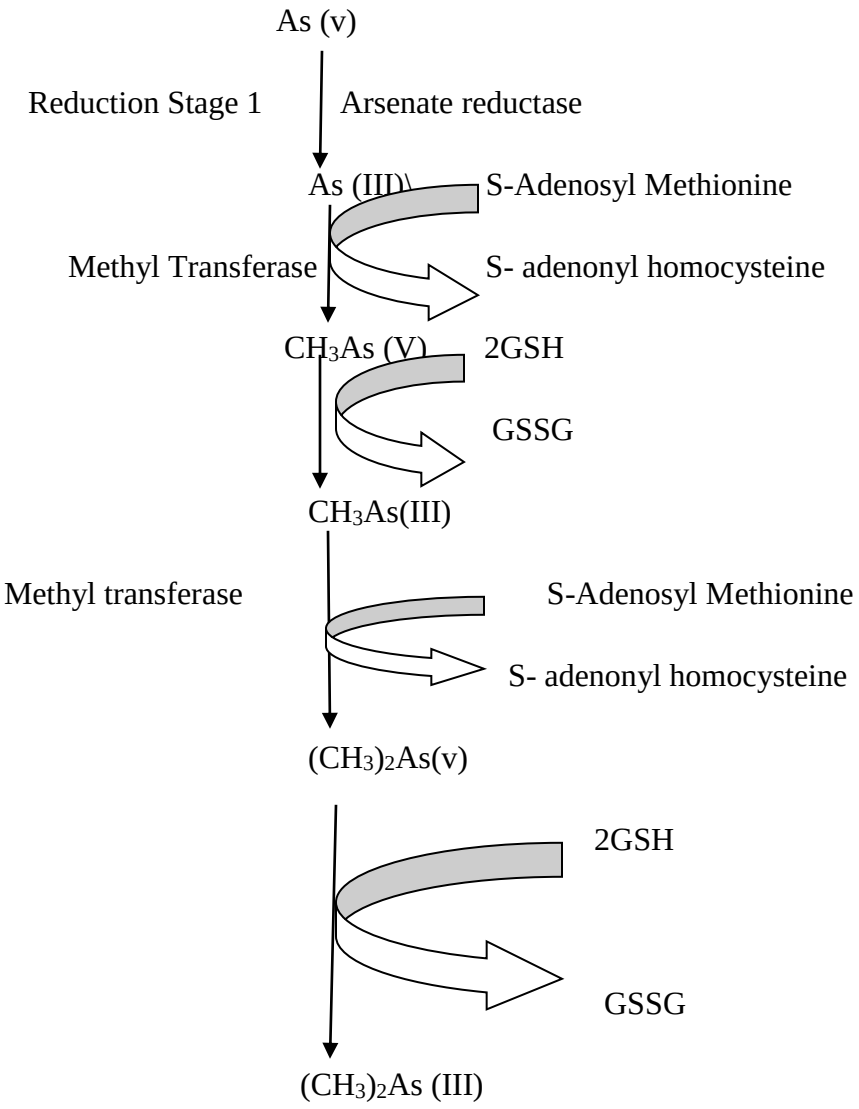

Fig 2: Biotransformation of arsenic into methylated derivative. (Source:Singh *et al*., 2007)

1.2.3 Effects of Arsenic on Insulin

Diabetes Mellitus (DM) which could results from insufficient production insulin, due to pancreatic disease or injury or inadequate utilization of insulin produced by the cells of islet in pancrease in the body (Njolstad *et al*., 2003). The major cause of type 1 diabetes mellitus

(T1DM) is auto-immune reaction to the proteins produced by pancreatic islet cells, while type 2 diabetes mellitus (T2DM) may be a result of resistance to insulin, impaired secretion, as well as lifestyle changes (Holt, 2004; Chan *et al*., 2009). Arsenic has been reported to cause injurious damages to the pancreatic beta-cells and apoptosis in Fig. 3, which may decrease insulin production, function and may result in type 1 diabetes (insulin dependent diabetes mellitus) (Johnson and Luciani, 2010)

1.2.4 Mechanisms of Arsenic Induced Hyperglycemia

Metabolism of glucose can be affected by arsenic and its metabolites (Fig. 3). Arsenite can bind covalently with sulfhydryl groups in insulin molecules and receptors, enzymes such as pyruvate dehydrogenase and alpha keto-glutarate dehydrogenase, and glucose transporters (GLU-T), which may result in insulin resistance (Tseng, 2004; Fröjdö *et al*., 2009). Arsenate is the salt or ester of arsenic acid which contain AsO3-4 ion can affect insulin secretion via adenosine triphosphate (ATP) pathway by substituting phosphate in the synthesis of ATP and other phosphate substrates necessary for glucose metabolism (Tseng, 2004). Arsenic and metabolites have also been shown to increase peripheral tissues' resistance to insulin, which might lead to hyperglycemia and subsequent diabetes (Frayn, 2001; Patel and Kalia, 2010). Studies conducted using animal models, both in vivo and in vitro on high concentration of inorganic arsenic have shown increased blood glucose and insulin levels, while in some cases decreased sensitization of insulin cells to glucose uptake was observed (Walton *et al*., 2004; Izquierdo-Vega *et al*., 2006). Transcription factors involved in insulin signal transduction and sensitivity were also altered in vitro (Paul *et al*., 2007). A number of conducted studies have indicated a strong correlation between arsenic exposure and T2DM. One of such studies was carried out o90op;kl,.n normo-glycemic and ovariectomized female mice treated with arsenic trioxide 0.5 ppm, which revealed high blood glucose levels, low plasma insulin, and impaired glucose

Arsenic toxicity interferes in Glucose Metabolism by replacing phosphate in energy transfer phosphorylation reactions, resulting in the formation of adenosine diphosphate arsenate (ADP) instead of adenosine triphosphate (ATP) (Aposhian, 1989). Non-enzymatic hydrolysis of ATP is negligible, whereas the arsenate ester of ADP-arsenate is unstable in aqueous media at neutral pH and undergoes rapid non-enzymatic hydrolysis, which is assumed to be major mechanism by

which arsenate, uncouples oxidative phosphorylation. ADP-arsenate serves as a substrate for hexokinase resulting in the formation of glucose-6-arsenate instead of glucose-6-phosphate.

Arsenic toxicity induce cells with a relatively higher concentration (200 µM) of arsenite or arsenate resulted in apoptosis (Chen *et al.,* 2002). In arsenic-induced apoptosis, it is thought that the mitochondria are most pertinent in mediating apoptosis, putatively via metal-induced reactive oxygen species (Chen, *et al*., 2001). Apoptotic cell death is the morphological aspect of the active process of cellular self destruction occurring in various physiological or pathological conditions and comprises chromatin condensation, cell shrinkage, and dissolution of the cell into apoptotic bodies. Binding of arsenic to the SH-group of glutathione (GSH) results in an accumulation of intracellular ROS leading to activation of caspases (Chen *et al*.,2002). Arsenic trioxide induces an early collapse of mitochondrial membrane potential that leads to apoptosis (Chen *et al*., 2001). The disruption of mitochondrial membrane potential causes the release of cytochrome c leading to caspase activation and apoptosis, while forced expression of bcl-2 inhibits disruption of the mitochondrial membrane potential (Larochett *et al*., 1999). Furthermore, in a cell-free system, arsenic requires the mitochondria to induce nuclear apoptosis (Larochett *et al*., 1999). Arsenic induces the opening of permeability transition pores by acting as a thiol-oxidizing agent, which initiates apoptosis (Costantini *et al*., 1996). Few researchers have investigated the role of p53 in arsenic-induced apoptosis; arsenic trioxide (0.1–100 µM) induces apoptosis through an up-regulation of p53 in human gastric cancer cells. All the above mentioned mechanisms indicate that the focal cause of arsenic induced toxic effects is excess production of free radical or reactive oxygen species.

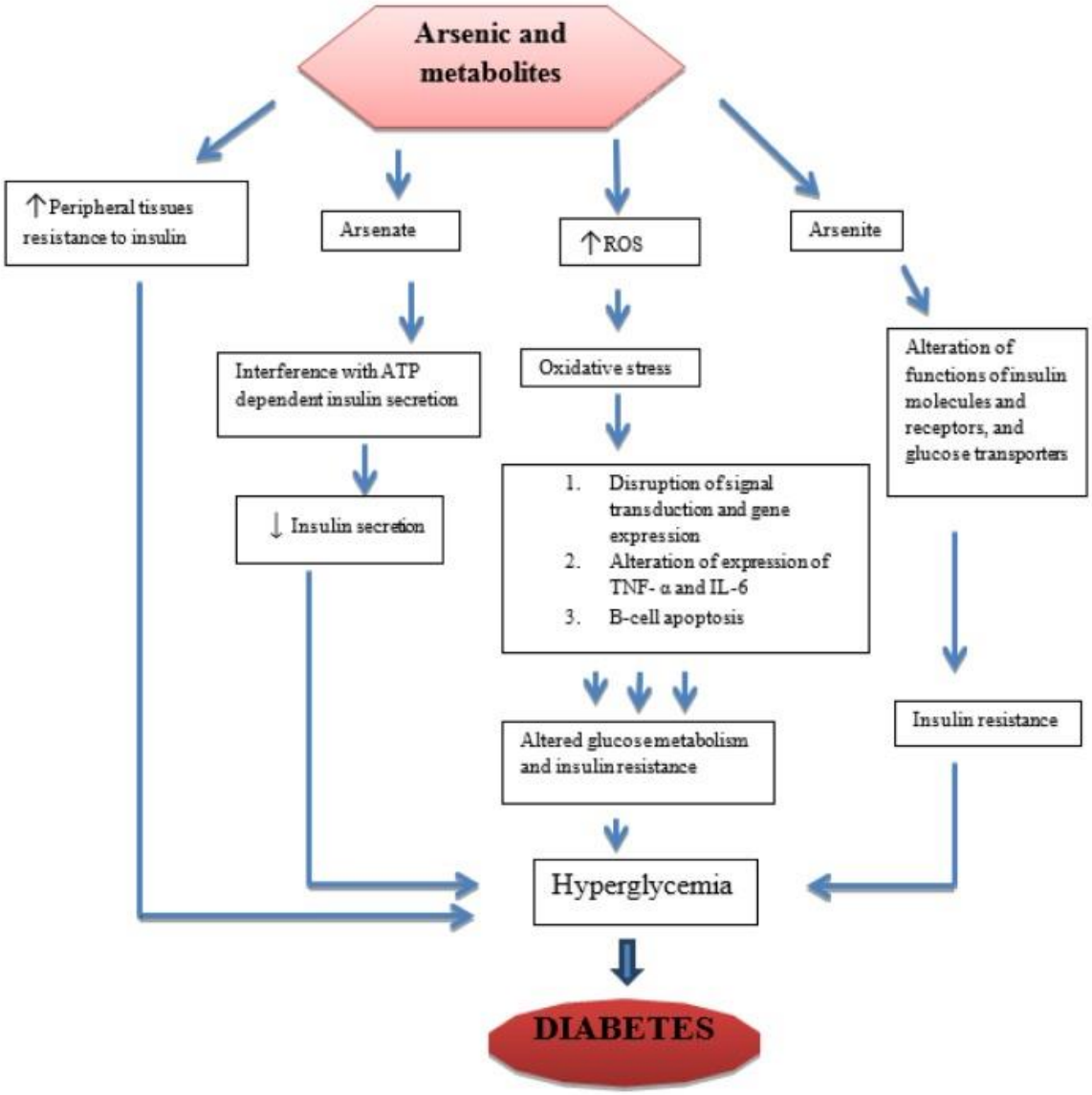

Fig. 3: Possible mechanisms of arsenic-induced diabetes Fröjdö *et al*., (2009).

1.30 Lead

Lead is a slightly bluish, bright silvery metal in a dry atmosphere. The main sources of lead exposure include drinking water, food, cigarette, industrial processes and domestic sources. The industrial sources of lead include gasoline, house paint, plumbing pipes, lead bullets, storage batteries, pewter pitchers, toys and faucets (Thurmer *et al*., 2002). Lead is released into the

atmosphere from industrial processes as well as from vehicle exhausts. Therefore, it may get into the soil and flow into water bodies which can be taken up by plants and hence human exposure of lead may also be through food or drinking water (Wan *et al*., 2015).

Fig . 4: Lead deposit , source; (Bate, 1988)

1.3.1 Mechanism of Lead Toxicity in Human

Lead toxicity in living cells follows ionic mechanism and that of oxidative stress. Many researchers have shown that oxidative stress in living cells is caused by the imbalance between the production of free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage. Antioxidants, glutathione, present in the cell protect it from free radicals such as H_2O_2. Under the influence of lead, however, the level of the ROS increases and the level of antioxidants decrease. Since glutathione exists both in reduced (GSH) and oxidized (GSSG) state, the reduced form of glutathione gives its reducing equivalents ($H^+ + e^-$) from its thiol groups of cysteine to ROS in order to make them stable. In the presence of the enzyme glutathione peroxidase, reduced glutathione readily binds with another molecule of glutathione after donating the electron and forms glutathione disulfide (GSSG). The reduced form (GSH) of glutathione accounts for 90% of the total glutathione content and the oxidized form (GSSG) accounts for 10% under normal conditions. Yet under the condition of oxidative stress, the concentration of GSSG exceeds the concentration of GSH. Another biomarker for

oxidative stress is lipid peroxidation, since the free radical collects electron from lipid molecules present inside the cell membrane, which eventually causes lipid peroxidation (Flora *et al*., 2012). At very high concentrations, ROS may cause structural damage to cells, proteins, nucleic acid, membranes and lipids, resulting in a stressed situation at cellular level (Mathew *et al.*, 2011). The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations such as Na^{+}, which ultimately disturbs the biological metabolism of the cell. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage (Flora *et al*., 2012).

Children are more vulnerable to lead exposure than adults because of the frequency of pica, hand-to-mouth activity, and a higher rate of intestinal absorption and retention. The most deleterious effects of lead are on erythropoiesis, kidney function, and the central nervous system (WHO, 1995).

1.3.2 Effect of Lead toxicity on Hemopoietic system.

The adverse effects of lead appear even with blood concentrations as low as 10 µg/dl. The best understood toxic effects of lead involve heme synthesis, as lead inhibits three important enzymes participating in the process, i.e. delta aminolevulinic acid dehydratase, delta amino-levulinic acid synthase, and ferrochelatase (Piomelli, 2002). It is suggested that the inhibition of delta aminolevulinic acid dehydrates starts at values as low as 5 µg/dl. At higher lead concentrations this inhibition is very pronounced, reaching 50% inactivation at blood lead levels of 16 µg/dl and 90% inactivation at 55 µg/dl, resulting in the accumulation of delta aminolevulinic acid in plasma and its excretion in urine. Because this enzyme is normally present in great quantities, the inhibition of its activity may pass unnoticed (Piomelli, 2002). Ferrochelatase is the enzyme that catalyzes the incorporation of iron into the porphyrin ring. If, as a result of lead toxicity, the enzyme is inhibited and its pathway is interrupted, or if adequate iron is not available, zinc is

substituted for iron, and zinc protoporphyrin concentrations increase. The critical target, however, seems to be the enzyme's heme synthesis, essential for the insertion of iron into the precursor, protoporphyrin IX (Alvares *et al*., 1976). The major consequences of this effect, which have been evaluated in both adults and children, are reduction of circulating levels of hemoglobin and the inhibition of cytochrome P 450-dependent phase I metabolism (Alvares *et al*., 1976). Lead clearly inhibits normal hemoprotein function in both respects, which results in basophilic stippling of erythrocytes related to clustering of ribosomes and microcytosis when blood lead levels are 20 µg/dl. Thus microcytic hypochromic anemia is often diagnosed in victims of lead exposure. Compared with adults, children, especially in their first year, develop certain toxic effects at lower blood lead levels, and lead induced- anemia has been related to age (WHO, 1995).

1.3.3 Effect of Lead toxicity on Nervous system.

Headaches, poor attention span, irritability, loss of memory, and dullness are the early symptoms of the effects of lead exposure on the central nervous system. The ability to think and reason is extremely sensitive to toxic metal assault. The developing nervous system of the child makes it more sensitive to lead-induced impairment. The most serious manifestation of lead poisoning is acute encephalopathy, the symptoms of which include persistent vomiting, ataxia, seizures, pappiledema, impaired consciousness, and coma. Lead encephalopathy rarely occurs at blood lead levels below 100µg/dl. Poor attentiveness, impulsiveness, inability to follow sequences or directions, decreased play activity, low intelligence quotient, and poor school performance are neurobehavioral abnormalities observed in affected children whose blood lead levels are approximately 35µg/dl. Similar abnormalities have appeared at even lower levels of lead exposure. A growing body of evidence suggests, how- ever, that the functional integrity of the central nervous system can be compromised at substantially lower levels of lead exposure, particularly in the human fetus and young child. Early post-natal neurobehavioral development is compromised by maternal or cord blood lead levels of somewhat less than approximately 10 µg/dl (a level of lead not un- common in the general population). Results of more re- cent cross-sectional and prospective studies indicate that postnatal lead exposure resulting in blood levels as low as 25 µg/dl, and probably lower, are also associated with deficits in intellectual attainment,

achievement, and affect behavior. Impaired hearing has been observed at blood concentrations of 10 to 20 µg/dl (Philip and Gerso, 1994).

Peripheral neuropathy, on the other hand, is the most common manifestation among adults with occupational lead exposure, but it is rarely seen in children except for those with sickle cell disease. Typically, the peripheral neuropathy of lead toxicity is seen as involving the extensor muscles, with minimal sensory loss. Lead-induced neuropathy on the radial and peroneal nerve in adult lead toxicity results in the characteristic "wrist drop" and "foot drop". Gastrointestinal colic is caused by high lead exposure and may be associated with lead neuropathy (Philip and Gerso, 1994) .Needleman and his associates summarized the observations of teachers that lead-exposed students exhibited behavior characterized as distractible (Needlema, *et al*., 1979) , not persistent, dependent, not organized, hyperactive, impulsive, frustrated, daydreaming, and unable to follow directions and sequences, with low overall functioning. The outcomes of four key studies of the neurobehavioral effects of low-level lead exposure in children were reviewed and analyzed by Davis, who concluded from these data that impaired performance can be caused by lead levels of 10 to 15 µg/dl or lower (Davis, 1990) . Electrophysiological changes on sensory functioning occur in children at expo- sure levels below 10 µg/dl (Lidsky and Schneider,2003) . Furthermore, changes in cortical visual-evoked potentials have been reported in children aged 3 to 12 years associated with blood lead levels from 6 to 59 µg/dl (Otto and Fox, 1993). Recently, Rothenberg and colleagues recorded the brainstem auditory-evoked response in children 5–7 years of age who had been exposed to lead prenatally and observed that a mean maternal lead level of 7.7 µg/dl at 20 weeks of pregnancy was associated with changes (Rothenberg *et al*,. 2000). Although the functional significance of these changes is not clear, it may be relevant that increases in the auditory threshold have been reported with blood lead levels ranging from 6 to 18 µg/dl (Holdstein *et al*,. 1986). There are few symptoms of chronic lead poisoning and they are mostly non-specific, involving abdominal and muscle pain, arthralgia, irritability, depres-sion, altered sleep, memory disturbances, and hyperactivity in children (Cullen *et al*., 1983). (Needleman *et al*,. 1990) also warned that childhood lead exposure is associated with deficits in central nervous system functioning that persist into adulthood. Long-term neurobehavioral effects of childhood lead poisoning were assessed in individuals 50 years after known exposure. The results of this long-term follow-up study indicate that a history of childhood plumbism is associated with cognitive dysfunction still evident in adulthood (White *et al*,. 1993). The effects

of chronic lead toxicity on psychological development were first described by Byers and Lord in 20 children who suffered lead poisoning during the first two years of life from eating paint chips. These children were re-assessed during the primary school period, and a high frequency of educational and behavioral problems was noted (Feldman and White, 1992).

The magnitude of the effects of blood lead on the IQ of young children has been estimated as an average loss of two to three points for lead levels averaging 20 µg/dl compared with lead

levels averaging 10 µg/dl. A number of studies recently reviewed by the National Research Council found an association between lead levels and intellectual functioning in children (NRC,1995). In one population, lead levels >30 µg/dl were correlated with an increase in the percentage of children with severe deficits (i.e. IQ <80), from an expected 4% to 16%, and a decrease in the percentage of children with an IQ ≥125 from the expected 5% (Needleman *et al*,. 1982). From animal models there is evidence that lead can disrupt several steps involved in neuron- al plasticity, which includes the brain's capacity to be shaped by experience, the capacity to learn and remember, and the ability to reorganize and recover after injury (Johnston, 2003). Studies using an immature animal model suggest that lead impairs neuronal growth and activity-dependent refinement of synaptic connections in the developing brain (Wilson *et al*,. 2000). Recently it has also been reported that in developing rodents, environmental enrichment can reverse the cognitive and molecular deficits induced by developmental lead exposure (Guilarte *et al*,. 2003).

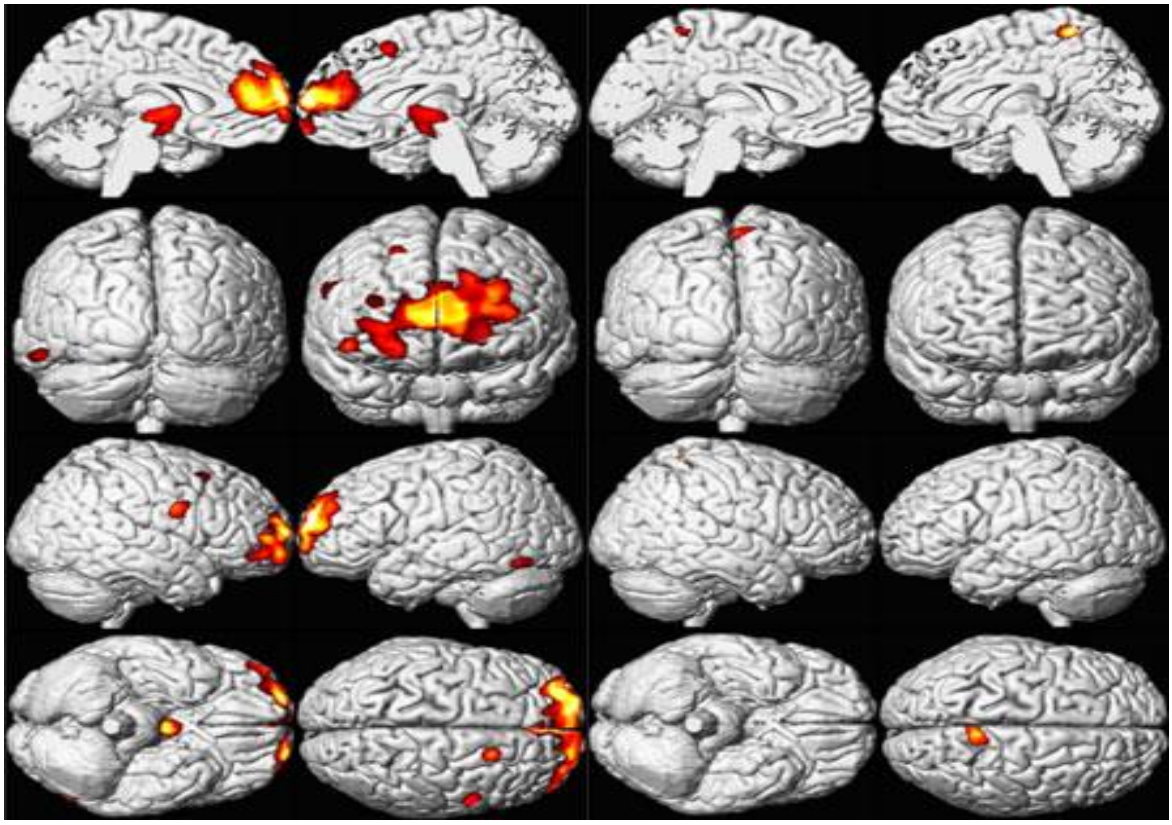

Fig. 5: Brain poisoned by lead (source; RSNA 2009)

1.3.4 Effect of Lead toxicity on Kidney.

Renal toxicity in sub-clinical lead poisoning involves inhibition in the proximal tubular lining cells and renal insufficiency. Abnormalities include aminoaciduria, glycosuria, and phosphaturia (Fanconi's syndrome). Characteristics of early or acute nephropathy include dysfunction of the proximal tubules (Fanconi's syndrome), manifesting as aminoaciduria, glycosuria, and phosphaturia with hypophosphatemia, and increased sodium and decreased uric acid excretion. These effects are reversible. Characteristics of chronic lead nephropathy include progressive interstitial fibrosis, a reduction in the glomerular filtration rate, and azothemia. These effects are irreversible. The acute form of nephropathy is most frequently reported in children, while the chronic form is mainly reported in adult (Philip and Gerso, 1994).

Evaluation of renal tubular function parameters in 134 children and young adults 8–13 years after chelating therapy for severe lead poisoning, concluded that a partial Fanconi's syndrome can persist up to 13 years after lead poisoning (Loghman-Adham ,1998).

1.4.0. Concerns about heavy metals in rice.

Heavy metal contamination has increased due to rapid industrialization and urbanization which releases these metals into irrigation water and soil and much of these metals are retained in the soil (Niu *et al*., 2013; Anjum *et al*., 2016b). Furthermore, metal processing, mining, sewage sludge, and fertilizer application is causing heavy metal and metalloid soil pollution. Heavy metal toxicity has become a major limitation to sustainable crop production, especially rice and thus, poses a serious threat to world food security. Heavy metals inhibit plant growth by disturbing the protein, lipid, thylakoid, and cellular structures (Molas, 2002; Maksymiec, 2007). Heavy metal pollution is escalating on agricultural land, especially paddy soils (Yu *et al*., 2016). Soil contamination with heavy metals is posing serious threats to ecosystems', plants', and humans' health through the food chain (Li *et al*., 2014; Gall *et al*., 2015). In a study, Du *et al*. (2013) found that B 2/3 soils of Hunan Province were contaminated with Cd (above 0.3 mg/kg) with a concentration of .0.2 mg/kg in rice grains.

Heavy metal contents can transfer to rice kernels through agricultural inputs (fertilizers, pesticides, irrigation water, etc.) (Bian *et al*., 2015), causing serious health risks as these metals have a high persistence, non-degradability, and toxicity (Song *et al*., 2014). Symptoms of heavy metal toxicity appear first on young leaves with the formation of dark green ribs. Under severe toxicity, the leaves become chloratic and ultimately turn white. If Zn contamination is high in soil, rice seedlings will die after 10 days of germination (Silva *et al*., 2014). Heavy metal toxicity (Cu, Pb, and Zn) reduced the germination and seedling growth traits (seed germination, root length, total root numbers, root: shoot ratio, and shoot height of seedlings) of rice and this effect was more pronounced following the order Cu < Zn < Pb. The interactive effects of Cu, Cd, and Pb stress on rice plants unveiled that Pb stress augments Cd and Cu accumulation in shoots and roots, while Cu stress raises Cd and Pb concentrations in rice roots. However, Cd stress did not influence the Pb concentration of rice. In another study, the accumulation of high concentrations of Ni21 in rice caused chlorosis and necrosis (Samantaray *et al*., 1997). Rice grown in solution cultures with higher Ni concentrations have impaired membrane with disturbed nutrient homeostasis as Ni toxicity affects the H-ATPase activity of the plasma membrane by disturbing the lipid composition (Ramos *et al*., 2001). Lead (Pb) is one of the most hazardous heavy metals

in the food chain and it is accumulated predominantly through anthropogenic activities (Liu *et al*., 2003; Ashraf *et al*., 2015). Lead toxicity leads to changes in biochemical and physiological attributes resulting in growth impairment and yield reduction in rice (Ashraf *et al*., 2015). Cadmium is a highly mobile, persistent, and toxic environmental contaminant for living organisms (Abd-Allah *et al*., 2015; Song *et al*., 2015; Anjum *et al*., 2016a,b). Cd accumulation in high concentrations causes root and leaf ultrastructural changes and disintegrates the photosynthetic machinery (Aina *et al*., 2007). Likewise, Cu toxicity reduces rice growth, by enhancing lipid peroxidation causing oxidative stress due to ROS generation (De Vries *et al*., 2007).

1.4.1. **Uptake of lead by rice plant.**

Rice Plant features like root surface-area, root exudates, transpiration pull, mycorrhizal associations in the rhizosphere , and diversities of root varieties may affect its availability and uptake mechanism by plants (Davies 1995).There have been a significant difference of lead uptake and translocation was observed in different rice cultivars and found a decreasing trend of lead content from roots to rice grains (Liu *et al*., 2003). Roots are the first organ exposed to Pb and thus can be a major storage organ for Pb or can play an intermediary role for exporting the Pb ions from soil to the above ground plant parts (Fangmin *et al*., 2006). However, different rice genotypes show differential uptake. Some rice genotypes (Pb-tolerant) prefer roots as a major storage organ for Pb and transport a little toward shoots while others retains more in shoots (Pb-sensitive) than roots (Zeng *et al*., 2008).

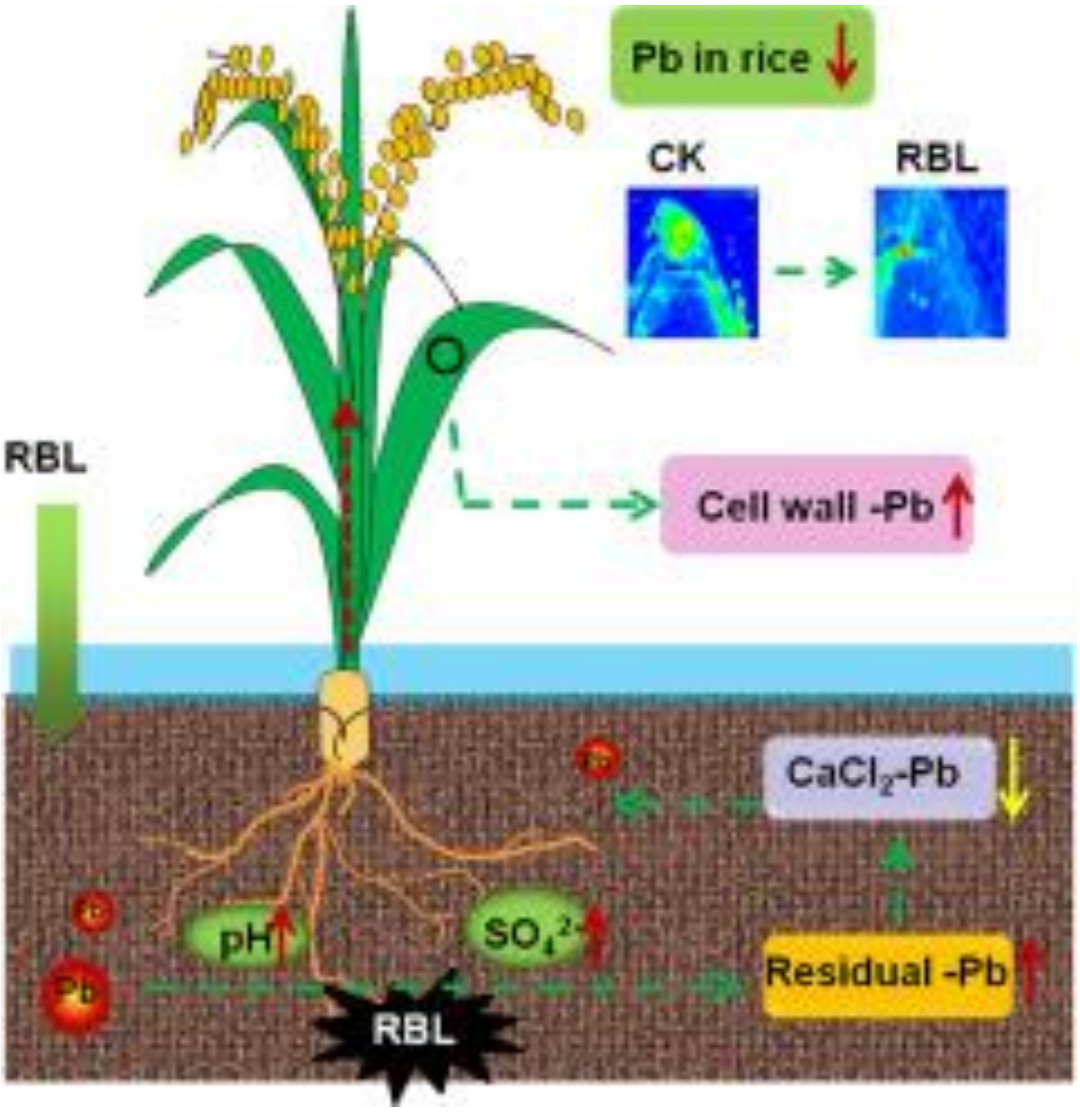

Fig .6: Translocation of lead in soil into rice grain (source; Deng *et al*., 2010).

1.4.2. **Uptake of Arsenic by rice plant.**

The biochemistry and uptake of Arsenic in the rhizosphere is complex and controlled by several factors. There are four important elements that take part in Arsenic intake in rice: Fe, P, S, and Si (Zhao *et al*., 2010).

Iron (Fe), plays an important role in the biogeochemical cycle and bioavailability of metalloid Arsenic in rice plant. Reductive Iron oxy-hydroxides in the soil and or on the plant root surface function as a strong Arsenic adsorbent. Radial Oxygen loss(ROL) is transported from the aerenchyma to the rhizosphere, it oxidize Fe2+ to Fe3+ in rhizosphere, and cause the precipitation of Arsenic in the rhizosphere soil and on the root surface (Pan *et al*., 2014). Rhizosphere interactions therefore play a key role in controlling Arsenic bioavailability in rice cultivation (Deng *et al*., 2010).

Phosphorus (P) an analogue of As- phosphate (Pi), enter the cells of rice plants through Pi transporters, such that When Phosphorus concentration increases, Arsenic uptake by rice plants decreases. Researches with different types of soil at different Phosphorus and Iron concentrations found that there is a positive correlation between available Iron and Arsenic concentrations in the grain, while the correlation is negative when soil has available Phosphorus (Jiang *et al*., 2014). Arsenic mobility in organic acid-rich soils is reduced because the acid acts as a binding agent and/or in the formation of insoluble compounds that do not allow Arsenic to be absorbed by the rice plant roots (Jiang *et al*., 2014).

Sulfur help to detoxify Arsenic through binding of As^{3+} with thio-rich peptides which help to maintain Arsenic in roots and restrict its translocation to the tillers (Rai *et al*., 2011). Maintaining enough sulfur soil fertilization can be particularly important in environments contaminated with Arsenic.

Silicon (LSi1) transporters in As uptake is particularly important because similarities already exist between silicic acid and arsenious acid; this allows arsenious acid to penetrate into the root by the silicic acid transporter. The presence of Si decreases Arsenic concentration in rice plants. Silicon fertilization can also be an effective strategy to decrease Arsenic accumulation in rice grown in As-contaminated soil (Tripathi *et al*., 2013).

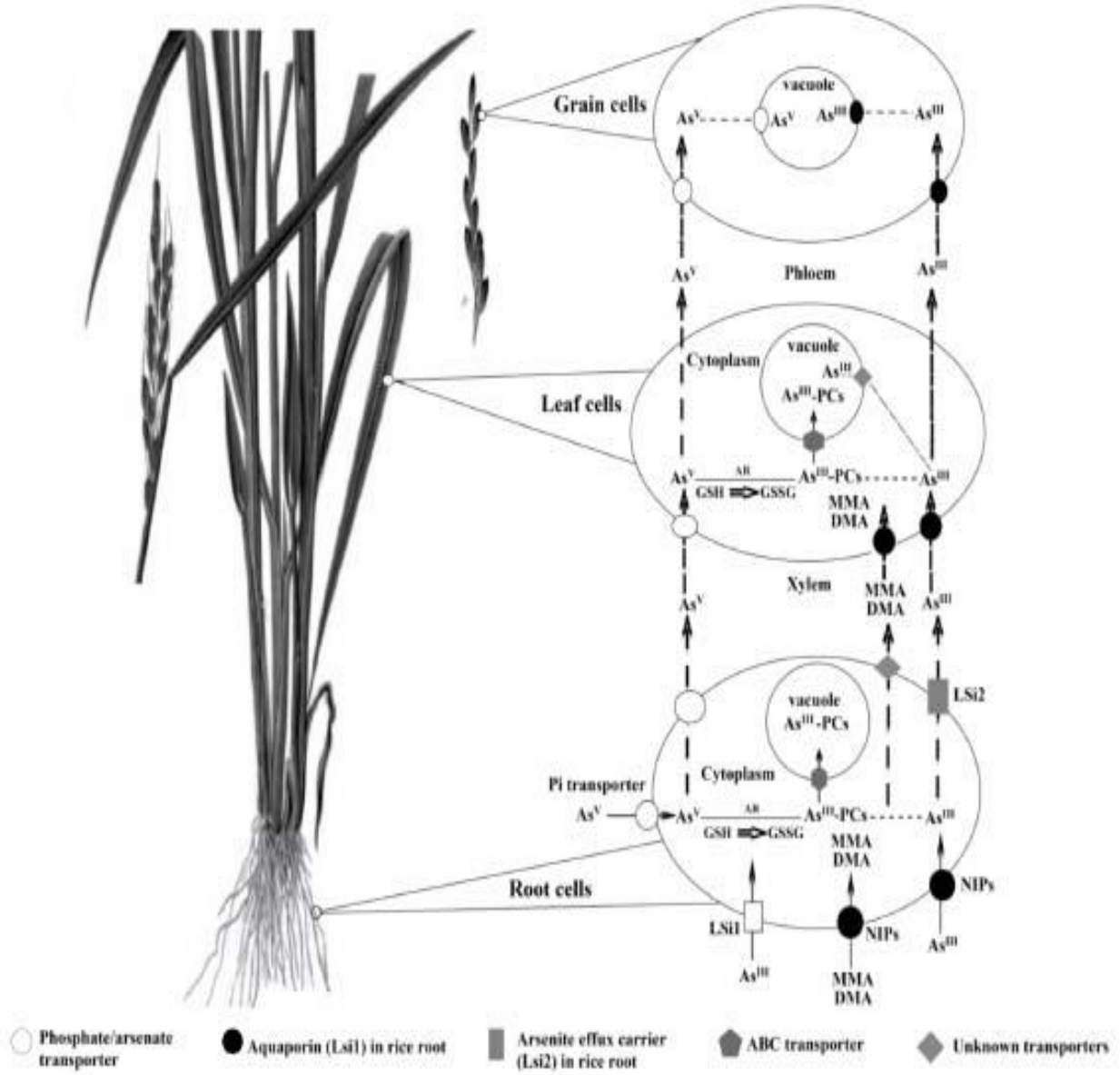

Fig . 7 : Mechanism of arsenic uptake Arsenic uptake in rice plant, (Ali *et al* ., 2009).

1.5 Rationale for the Study

The concern for food safety and consumption of rice which could contain some heavy metals, lead (Pb) and arsenic (As) that are toxic to the human body is reason for the study.

1.6 AIMS AND OBJECTIVES OF THE STUDY

1.6.1 Aim of the Study

The present study was aimed at comparing the levels of lead and arsenic in commercial samples of locally produced and imported rice varieties.

1.6.2 Specific objectives of the study

- To determine the concentrations of arsenic and lead in local and imported brands of rice.
- To compare the concentrations of arsenic and lead in local and imported brands of rice

CHAPTER TWO

MATERIALS AND METHODS

2.1 **Materials**

2.1.1 **Chemicals and Reagents**

The reagents include 30 cm^3 of aqua regia (a mixture of 3M of HNO_3 and Conc. HCl acids in the ratio of 1:3), de-ionized water, and double distilled water.

2.1.2 **Instrumentation and Apparatus**

Atomic Absorption spectrometer (model AA-700 shimadzu, Japan) were used for the analysis. Electronic weighing balance(Locosc Ningbo Precision Technology co., Ltd Zhejiang , China) and 100ml standard flask were used as well glass wares used were soaked into 3 M HNO_3 overnight and washed with de-ionized water to reduce the chances of interferences.

2.2 **Methods**

2.2.1 **Sample Collection**

Different samples of rice were purchased from different locations in Nigeria. They include both local and imported rice. The local rice samples include Uburu-rice (government), Adani rice (14-16), Adani rice (fadama), Abakaliki rice (max), BP-IDAH rice, Olam rice (Mama choice), Garina rice, Alede rice, Lafia rice. Meanwhile, the imported rice are mostly from Thailand and they include safari rice, cap–cap rice, Royal Thai rice, Royal stallion rice, Tomato Aroso rice, Thai-PB rice and sweet mama rice. The other imported rice samples were Indian Rice (Golden Eagle) and Chinese rice (Carlrose), all purchased from Ogige-market in Nsukka Senatorial Zone of Enugu State, Nigeria.

2.2.2 **Sample Preparation**

One hundred grams (100 g) of each of the rice samples was ground using mortar and pestle to obtain a fine powder. The powdered rice samples were sieved with 5nm mesh to remove debris. The powdered samples were kept in air-tight containers until analysis.

2.2.3 **Sample Digestion**

One gram (1 g) each of the samples was weighed into digestion flask and 30 cm^3 of aqua regia was added and digested in a fume-cupboard until clear solution was obtained. It was cooled, filtered and then made up to 50 ml mark in a standard volumetric flask with de-ionized water (AOAC, 2012).

2.2.2 **Statistical analysis**

The data obtained were analyzed using IBM statistical product and Service solution (SPSS) version 20.0 for windows. Results were expressed as mean ± SD and Correlation analysis (independent t-test) was used to determine and compare whether there is significant difference in concentration of lead and arsenic in the rice type at $p \leq 0.05$.

CHAPTER THREE

RESULTS

3.1 Lead Concentration of local and imported brands of rice.

Table 2 shows the lead concentrations of the 18 brands of local and imported rice. Their concentration values were found to range from 0.00 – 0.50 mg/kg, indicating that all the brands analyzed were far below the maximum allowable concentration value, 6.0 mg/kg stipulated by Joint FAO/WHO Expert Committee on Food Additive, JECFA (2010).

Table 2. Concentration of lead in local and imported rice brands .

Local rice	lead content (mg/kg)	Imported rice	lead content.(mg/kg)
14-16 Adani	0.25 ± 0.02	Cap-cap	0.00 ± 0.00
B-P Idah	0.25 ± 0.1	Safari	0.00 ± 0.00
Alede	0.50 ± 0.17	Royal stallion	0.25 ± 0.19
Fadama	0.25 ± 0.15	Carlose	0.00 ± 0.00
Government	0.00 ± 0.00	Tomato aroso	0.00 ± 0.00
Lafia	0.00 ± 0.00	Royal Thai	0.50 ± 0.20
Mama choice	0.25 ± 0.15	Golden Eagle	0.25 ± 0.11
Max	0.25 ± 0.01	Thai PB	0.00 ± 0.00
,Garina	0.00 ± 0.00	Sweeet mama	0.00 ± 0.00
Local rice	0.19 ± 0.17	Imported rice	0.11 ± 0.18

N= 18 (number of rice samples); values presented in mean ± standard deviation.

3.2 Concentration of Aresenic in Local and Imported Brands of Rice.

Table 3 shows the arsenic concentrations of the 18 brands of local and imported rice. The concentration of arsenic in all the varieties analyzed ranged from 0.68 – 10.61 mg/kg, indicating that most of the brands analyzed were above the maximum allowable concentration value of 1.40 mg/kg, stipulated by Joint FAO/WHO Expert Committee on Food Additive, JECFA (2010). Local rice (Government) and imported rice (safari) recorded highest values 10.61 mg/kg and 7.00 mg/kg, respectively. The lowest concentration was recorded in Golden Eagle and Thai-PB as 0.68 mg/kg.

Table 3 Concentration of Arsenic in local and imported brands of rice.

Local rice	Arsenic content (mg/kg)	Imported rice	Arsenic content(mg/kg)
14-16 Adani	5.42 ± 2.31	Cap-cap	3.16 ± 0.19
B-P Idah	2.71 ± 0.23	Safari	7.00 ± 3.50
Alede	2.71± 0.21	Royal stallion	3.39 ± 0.31
Fadama	2.26 ± 0.20	Carlose	1.13 ± 0.11
Government	10.61 ± 5.31	Tomato aroso	3.61 ± 0.21
Lafia	4.06 ± 1.50	Royal Thai	6.10 ± 1.53
Mama choice	3.61 ± 0.27	Golden Eagle	0.68 ± 0.10
Max	4.97 ± 1.35	Thai PB	0.68 ± 0.10
Garina	3.39 ± 0.21	Sweet mama	4.07 ± 2.31
Local rice`	4.42 ± 2.55	Imported rice	3.71 ± 1.91

N=18 (number of rice samples); Values presented in mean ± standard deviation .

3.3 Correlation analysis of rice type (Imported and local) on the heavy metal content.

Table 4 shows the correlation of the concentration of lead and arsenic in local and imported brands of rice at ($P > 0.05$). The correlated value were greater than P- value of 0.05, indicating that no significant difference between the lead and arsenic concentrations of local and imported rice type.

Table 4 Correlation between rice origin (imported and Local) on heavy metal content.

Rice type	lead (mg/kg)	Arsenic (mg/kg)
Local rice	0.326	0.518
Imported rice	0.326	0.519

CHAPTER FOUR

DISCUSION

Heavy metals contaminant and bio-accumulation in foods and its antecedent health implications, have been extensively investigated. In the present research, the levels of two heavy metal (As and Pb) in commercial samples of locally produced and imported brands of rice were investigated.

For all the rice samples assayed in this study using atomic mass spectrometry, the concentration of lead (Pb) ranged from 0.00 to 0.50 mg/kg (Table 3.1) with mean value of 0.19 mg/kg and 0.11 mg/kg for local and imported brands of rice respectively, with all the rice brands far below the maximum allowable concentration level, 6.0 mg/kg recommended by JECFA (2010). Comparable results were reported by Ugochukwu *et al.,* (2017) with means values of 0.23 mg/kg and 0.28 mg/kg for local and imported brands rice respectively. The highest concentration of lead was recorded in local rice brand (Alede) and Imported rice brand (Royal Thai) while the concentrations of lead in local brands of rice (Government, lafia , garina) and imported brands of rice (cap-cap, safari, carlose, tomato aroso, Thai PB,sweet mama), were below detectable concentration of heavy metals at <0.001 mg/kg which could be as a result of rice genotype that are lead tolerant preferring roots as a major lead storage organ for lead (Zeng *et al*., 2008).

The arsenic concentrations of local and imported rice brands were assayed using atomic mass spectrometry and the values ranged from 0.68 to 10.61 mg/kg (Table 3.2) with mean value of 4.42 mg/kg and 3.71 mg/kg for local and imported brands of rice respectively. The highest concentration was recorded in local rice (Government) which could be the result ofgrowing the rice plant under flooded condition (Duxbury *et a*l., 2007) and inadequate knowledge on the use of herbicides and pesticides (Wilfred, 2006) while imported rice brands (Golden eagle and Thai-PB) had least concentration values. The arsenic concentration in some rice samples (Table 3.2) was far than the maximum allowable concentration value of 1.4 mg/kg recommended by (JECFA 2010), which might be due to the frequent use of agro-chemicals such as pesticide, herbicide, fungicides, chemo-fertilizer that contain some mineral elements (iron and phosphorus) on agricultural soils of rice plants that enhance arsenic precipitation (Pan *et al*., 2014), the genetic make-up of rice plant, aquaporins (Kamiya *et al*.,2009; Xu *et al*.,2015), silicon influx transporter

Lsi1 (Ma *et al*., 2006, 2007, 2008) that triggers uptake of arsenic by rice plant and invariably increases arsenic concentration in the rice grains. Some previous studies demonstrated that the concentration and chemical form of arsenic applied has significant effects on the arsenic uptake and translocation by rice plants in pot experiment (Ma *et al*., 2006) and distribution of arsenic compounds into different rice tissues were also strongly dependent on rice strains (Rahman *et al*., 2011). The arsenic concentration of Carlrose rice, Golden eagle and Thai-PB falls below Maximum Allowable Concentration of 1.4 mg/kg could be as a results of the use of some fertilizers such as silicon that shown to reduce the total amount of arsenic in rice grain (Li *et al*., 2009) by inhibitory effect of silicic acid on arsenic uptake by rice plant (Bodgan and Schenk, 2008), *Oryza sativa* C-type ATP-binding cassette transporter 1 (OsABCC1) present in the upper nodes of rice plant detoxify and reduce the amount of arsenic by restricting its distribution to the grain through sequestering it in the vascular of the phloem companion cells of diffuse vascular bundles directly connected to the grain (Song *et al*., 2014), strong oxygen release characteristics (Mei *et al*., 2009), presences of soil microbes (Chibuike and Obiora, 2014; Niaz *et al*., 2016) such as *Rhobdococcus, Comamonas, Serratice and Streptomyces* species which are capable of reducing the arsenic activity in plant root (Aafi *et al*., 2012; Yan *et al*., 2012;) and Bacterial strain *Brevibacillus species* that posses genes which are capable of reducing and oxidizing arsenic (Banerjee *et al*., 2013). The high in arsenic concentration of local rice brands and some imported rice brands analyzed could be due to the absence or lack in aforementioned factors, and it implies that there might be a real danger of arsenic poisoning in consuming in such rice brands, since the amount is far greater than the recommended arsenic concentration by JECFA (2010) while Carlrose rice, Golden eagle and Thai-PB are good for consumptions since they fall within the baseline of Maximum Allowable Concentration of lead and arsenic as stipulated by (JECFA, 2010).

The comparative study of the concentrations of lead and arsenic conducted at ($P > 0.05$) correlated using independent t- test showed that there is no significant difference between the lead and arsenic concentrations of locally produced and imported brands of rice analyzed.

4.2 Conclusion

WHO recommended that the maximum concentration of lead and arsenic should not exceed 6.0 mg/kg and 1.4 mg/kg, respectively. Species of rice that is above the threshold may not be good

for consumption and no discrepancy should comes in between imported and locally produced rice brands since both stands chance of arsenic poisoning. Human activities that leads to solubilization of heavy metals in soils should be discouraged since rice plant is a potent adsorbent of heavy metals (Pb and As).

4.3 Suggestions for Further Studies

1 Arsenic and Lead Content in cooked rice foods

2 Roles and Mitigation of Abiotic factors in assimilation of heavy metals (Arsenic and Lead) by rice type

3 Comparative Study of Arsenic and Lead content in Local and Imported Brands of cooked rice

4 Study of Arsenic and Lead content of rice type cultivated in organo-rich soils

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APPENDICES

Appendix 1: Mean ± standard deviation of lead and arsenic concentrations of local and imported rice brands.

	Group	N	Mean	Std. Deviation	Std. Error Mean
Lead	**Local rice**	**9**	**.1944**	**.16667**	**.05556**
	Imported rice	9	.1111	.18162	.06054
Arsenic	Local rice	9	4.4158	2.54996	.84999
	Imported rice	9	3.7132	1.91309	.63770

Appendix 2: Independent T-test for lead and arsenic concentrations of locally and imported rice brands

.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Lead	Equal variances assumed	.180	.677	1.014	16	.326	.08333	.08217	-.09085	.25752
	Equal variances not assumed			1.014	15.883	.326	.08333	.08217	-.09096	.25763
Arsenic	Equal variances assumed	.277	.606	.661	16	.518	.70252	1.06261	-1.55012	2.95516
	Equal variances not assumed			.661	14.839	.519	.70252	1.06261	-1.56453	2.96957

Appendix 3. Prevalence of heavy metals in imported and locally available rice brands, using lead alone (MAC ≤ 6.0 mg/kg).

Lead

Rice type	Number	Percentage (%)	N
Local	-	-	9
Imported	-	-	9

Appendix 4. Prevalence of heavy metals in imported and locally available rice brands , using arsenic alone (MAC ≤ 1.4 mg/kg).

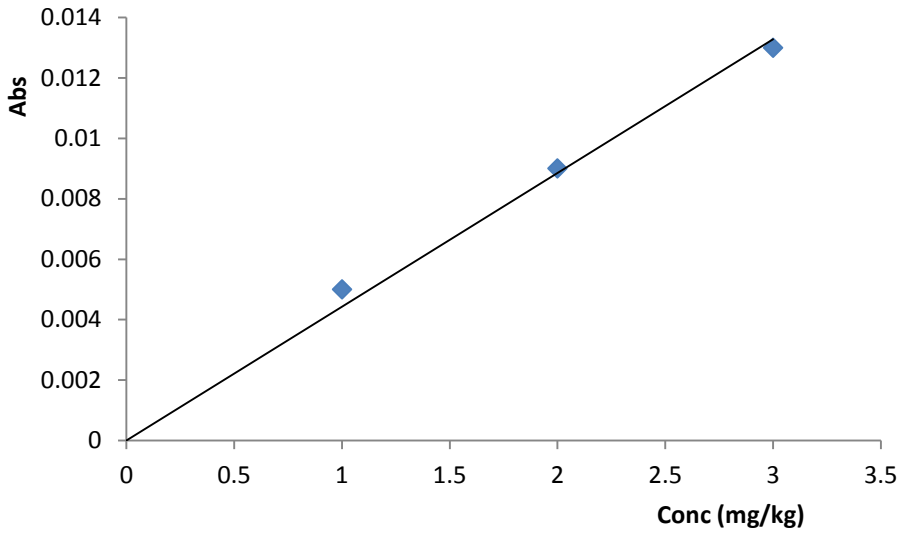
Arsenic

Rice type	Number	Percentage (%)	N
Local	9	100	9
Imported	5	56	9

Appendix 5. Prevalence of heavy metal in Imported and locally available rice brands using both Arsenic and Lead.

	Local	Imported	N
Normal As and Normal Pb	-	3	18
High As and Normal Pb	9 (50%)	6 (33%)	18
High Pb and Normal As	-	-	18
High Pb and High A	-	-	18

Appendix 6. Lead Calibration curve.



Appendix 7. Arsenic calibration curve