HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS AND AFLATOXINM1 IN COW MILKS FROM THREE LOCAL GOVERNMENT AREAS OF KANO STATE.

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DECLARATION

I Nasiru Salihu hereby declare that this work is the product of my own research effort, undertaken under the supervision of Prof. A. M. Wudil and has not been presented and will never be presented elsewhere for the award of degree or certificate. All sources have been duly acknowledged.

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Nasiru Salihu Date

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CERTIFICATION

This is to certify that the research work and subsequent preparation of this thesis by (Nasiru Salihu, SPS/17/MBC/00079) were carried out under my supervision.

Signature…… DATE…….

APPROVAL

This is to certify that the research project report has been examined and approved for the award of degree of Master OF SCIENCE in BIOCHEMISTRY (toxicology). In the department of Biochemistry, Faculty of Biomedical Science, Bayero University, Kano.

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DEDICATION

I dedicate this project work to my wonderful parents who are up and doing. May Allah bless them abundantly

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Abstract

Environmental pollution is a problem that has emerged due to increased industrialization with numerous effect on well-being of people. The current study determined the contamination of cow milk by heavy metals (Pb, Cr, Cd, Cu, Zn, Mn and Fe) and Aflatoxin M1 from three local governments (Bichi, Rano and Nassarawa) in Kano state. A total of 81 samples were collected, 27 samples from each local government, the concentrations of heavy metals were determined using atomic absorption spectrophotometer (AAS), and an Elisa kit was used for the estimation of AflatoxinM1. The results showed moderate contamination by some metals (Fe and Cr) and AflatoxinM1. The Data's obtained were used for the calculation of daily intake of metals (DIM), estimated daily intake (EDI) of Aflatoxins and total hazard index. The DIM in the three local governments was within the range of $3.4 \times 10^{-4} – 6.8 \times 10^{-3}$ µg/l. b.w/day for Cr and 0.367 – 1.55 µg/l. b.w/day for Fe. Pb was only detected in Rano local government within the range of 0.104 – 0.117 µg/l. b.w/day, whereas Zn was detected in Bichi and Rano within the range of 0.048 – 0.113 µg/l. b.w/day, the EDI of AFLM1 in the three local governments was also calculated and was within the range of 3.451 – 8.053ng/kg. b.w/day for children of age 1- 12 years. All hazard indices calculated for metals and AFLM1 were found to be below 1. Although the results of this investigation showed low risk of cancer, the variability in feeds and climatic conditions might influence contaminations, most especially AFLB1 contamination of feeds and consequently AFLM1 contamination of milk.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Heavy metals are contaminants which are found in variety of human food, they cause misfunction in human and also constitute environmental pollution, with some of them (like Cu and Fe) essential to maintain proper metabolic activity in living organisms; while others (like Pb and Cd) non-essential and have no biological role (Ayar *et al*., 2009; Qin *et al*., 2009). Milk is part of human diet that contains lot of essential elements, proteins, fats, sugars and vitamins and also constitute a major diet in infants (as a substitute for breast milk), adults in everyday life and also the elderly people. According to Enb *et al*., 2009; Qin *et al*., 2009, milk products are widely consumed by human children and adults especially elderly people around the World.

Animals are at a risk of heavy metals contaminants, this is because heavy metals contaminate plants by absorption through the roots (Gholizadeh and Ziarati, 2016), following ingestion by animals, they become contaminated. Metals that can contaminate cow's milk and other environments, such as lead, cadmium, chrome, Nickel, and cobalt, can disrupt milk at different levels and cause serious problems (Lahiji, 2016). Milk and dairy products become contaminated with heavy metals either through food stuff and water or through manufacturing and packaging processes (Anastasio *et al*., 2006; Ayar *et al.*, 2009).

Mycotoxins are a group of naturally occurring secondary metabolites which are mainly produced by the filamentous fungi (Iqbal *et al*., 2011). Among mycotoxins, aflatoxins (AFs) are the most toxic and carcinogenic class and are mainly produced by fungi *Aspergillus flavus*, *Aspergillus parasiticus*, and rarely by *Aspergillus nomius*. They can contaminate food, vegetable, fruits, cereals and cattle feed (Asi *et al*., 2012). AFs are associated with the incidence of certain types of cancer which poses a global concern over food and feed safety (Gong *et al*., 2002, 2004; Turner *et al*., 2003). There are two main ways of

aflatoxin contamination in the milk and dairy products. Firstly, when the animals in lactation period consume feeds contaminated with aflatoxin, aflatoxin B1 and B2 transform into aflatoxin M1 and M2 after being metabolized in the animals body. These metabolized toxins pass to the milk produced from the animal and the contamination occurs. Secondly, the contamination occurs when the molds synthesizing aflatoxin pass through the milk and produce aflatoxin during transport, process and storage phases after milking (Çelik *et al*., 2005).

In Kano state, studies concerning heavy metals and Aflatoxins levels in Raw cow milk are somewhat lacking, although these products are typical food stuff that are largely consumed in Kano, Nigeria. Therefore, the present study aimed at evaluating and assessing the Human health risk of heavy metals and Aflatoxins in cow's milk consumed at Bichi, Rano and Nassarawa local government areas of kano state, Nigeria. Levels of the essential metals (Cu, Zn, Mn and Fe) and nonessential metals (Pb, Cr and Cd) will also be determined.

1.1 STATEMENT OF PROBLEM

The incessant use of Cow milk in form of Fura da Nono is alarming, even though its nutritive essence has been established, Aflatoxins and Heavy Metals still remain an inevitable contaminant of great concern, and thus, this study becomes imperative to assess their accumulation effect over time.

1.2 JUSTIFICATION

Aflatoxins and heavy metals are contaminants of great concern, they cause wide varieties of diseases. Their presence in milk is always monitored in developed countries as a means of environmental pollution assessment. However in many cities of developing countries like Nigeria, very little data is found as regards to these contaminants in milk. Therefore this research is carried out to assess the levels of

aflatoxins and some heavy metals in cow's milk from Bichi, Rano and Nassarawa local government areas, Kano State.

1.3 AIM

This study is aimed at assessing the Human Health risk of Aflatoxins and Heavy metals in cow milks from three local government areas of Kano state.

1.4 OBJECTIVES

I. To Determine the levels of heavy metals in the cow milks and feeds
II. To Estimate aflatoxins in cow milk samples
III. To Evaluate the non-carcinogenic index (Chronic Daily Intake, Total Hazard Quotient) and carcinogenic index (Hazard Index) of heavy metals and Aflatoxin found in the samples

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ENVIRONMENTAL POLLUTION

Environmental pollution is a topic that emerged in the beginning of the industrial revolution which is considered as a significant global challenge, hence it changes the existence of the ecosystems (Hooke *et al.,* 2012). Pollution is defined as the accumulation of unsuitable materials and energy in the environment as a result of basic human activities. It can interfere with ambient air, soil/land resources and water resources making negative impacts on living beings. The contributors which are responsible for environmental pollution are called environmental pollutants. A pollutant can be a physical, chemical or biological material which is released into the environment that can directly or indirectly damage humans and other living organisms on earth (Khan, 2013). Heavy metals, pesticides, Aflatoxins, organic chemicals, oils and tars, fertilizers are the common pollutants in soil. Particulate matter, carbon monoxide, nitrogen oxides, sulfur dioxide and nitrates are some of common air pollutants while phosphorous and animal manure make severe deteriorations of hydrosphere. Evidences have shown that long-term exposure to ubiquitous environmental toxicants can adversely affect humans starting from their early embryonic stage and continuing throughout the postnatal life (Cao *et al*., 2016). Environmental regulations and standards implemented by international and local regulatory bodies are very important as they intervene for minimizing the adverse environmental issues and help to secure human and animal health (Liu *et al*., 2015). Although the environmental pollution cannot be completely prevented, minimization is very much needed as it has an obvious effect on economic, social and political development among societies (Bagul *et al*., 2015).

2.2. HEAVY METALS

Heavy metals referred to metal elements that have relatively high density, they include lead (Pb), mercury (Hg), copper (Cu), cadmium (Cd), zinc (Zn), arsenic (As), chromium (Cr),Manganese(Mn), iron (Fe), and

their actual density is more than 6 grams per cubic meter (Tavakoli-Hosseinabady , 2018), that are able to induce toxicity at low level of exposure (Duffus, 2002). Their wide distribution in the environment has been caused by numerous anthropogenic activities basically centralizing with rapidly expanding industrial areas and activities. Domestic disposals and technological applications have also caused for their extensive distribution while the agricultural activities play a significant role for excessive spreads of such toxic heavy metals into soil and water raising worldwide concerns over their potential influences on human health and the environment (Wuana and Okieimen, 2011). Muwanga and Barifayo, 2006; Nasiru et al., (2019) reported that heavy metals have a number of ways through which they can enter into the human body and pose alot of risk to the human; they accumulate in the environment through emissions of industries, industrial effluents, use of leaded gasoline and paints, agricultural activities, indiscriminate disposal of municipal wastes and incineration of toxic substances. These metals when ingested through food or drinking water, have a negative effect on the metabolism of living cells once the concentration of such metals exceed their maximum tolerance levels (Ojedokun and Bello, 2016). Even though their toxicity depends on several factors including the dose ingested, route of exposure as well as some factors such as age, genetics, gender and nutritional status of exposed individuals (Tchounwou *et al*., 2010). Bilandžić , (2014), associated the amount of toxicity of heavy metals with factors such as pathway, amount of use, solubility, metal oxidation status, maintenance percentage, duration of application, age, sex and the frequency of use, absorption rate and efficacy of excrement mechanisms. Heavy metals are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10ppm) in various environmental matrices (Kabata- Pendia, 2001). Their bioavailability is influenced by physical factors such as temperature, phase association, adsorption and sequestration. It is also affected by chemical factors that influence speciation at thermodynamic equilibrium, complexation kinetics, lipid solubility and octanol/water partition coefficients. (Hamelink *et al*., 1994). Biological factors such as species characteristics, trophic interactions, and biochemical/physiological adaptation, also play an important role (Verkleji, 1993).

2.2.1 Heavy metals in milk

Food safety is a worldwide concern and it has already received much attention among people. Raw milk and its products being an important food product in human diet are highly vulnerable to contaminations, and thus strict quality standards have to be implemented to ensure normal wellbeing of consumers (Qian *et al*., 2011). Milk and its products are very diverse and there are numerous elements that can be detected, many of them are essential and very primary. Some of these elements act as cofactors that enhance enzyme activity. The amount of metals in non-contaminated milk is remarkably accurate, but their content may vary considerably through the production and packaging process. Also, metals that can contaminate cows and other environments, such as lead, cadmium, chrome, Nickel, and cobalt, can disrupt milk at different levels and cause serious problems (Lahiji, 2016). Heavy metals are introduced into the plants by absorbing them through the roots (Gholizadeh and Ziarati, 2016; Lahiji, 2016). These plants are then ingested by animals through their feeds and the heavy metals will then be transferred to the animals from the plants. If the animal body is contaminated, it tends to excrete it through the mammary gland. This constitutes a great risk factor for dairy products and above all, for the health of the consumer which includes infants and the elderly. The problem of heavy metal pollution is emerging as a matter of concern at local, regional and global scales. Siddiki *et al*., 2012 reported that different agricultural activities such as irrigation with toxic metal containing water, use of drugs, pesticides and fertilizers have caused in toxic metal contamination in milk and other dairy products substantially, these metals can accumulate in human and animal tissues, especially those derived from food and water. When foodstuffs are grown on contaminated soil or irrigated with deteriorated water, these toxic heavy metals can be spread (Aslam *et al*., 2010). Farm animals are highly susceptible to the environmental pollution from heavy metals since they are often reared for milk and meat products. Recent evidences emphasizes that heavy metal residues are distinctly present in milk to an extent that exceed the maximum permissible concentrations established by international authorities (Javed *et al*., 2013). Grazing lands can be contaminated with Pb and Cd due to numerous anthropogenic activities such as continuous application of large amounts of fertilizer, disposal of industrial wastes and traffic emissions. In addition to that, packaging and other technological processes

can also increase the total concentration of heavy metals significantly in milk (De castro *et al*., 2010). Cadmium, lead and mercury are very dangerous to humans and are considered as a major threat to food in terms of industrial use. Animals use metals when grazing in the pasture and feeding with contaminated concentrations. However, in the case of cows, the transfer of minerals to milk is very variable. Pollutants are transferred to the air as a result of various industrial activities. Pollution of various industrial environments in the soil, water, food by heavy metals causes them to join the food chain and create a great threat to human and animal health.Toxins such as lead, and cadmium are typically airborne pollutants and are transported to the air due to various industrial activities.

2.2.2 Occurrence, entry route, mechanisms of toxicity and health effects

The routes of entry of heavy metals into the human body are majorly through ingestion, inhalation and dermal contact depending on the metal. Their occurrences also vary from one metal to the other, even though their sources are majorly anthropogenic activities and geological sources. Automobile emission is directly related to metal contamination at the neighboring roadside ecosystem. Metal contaminants like Pb, Cd, Zn and Cu are mobile in nature and are found in engines, tiers and petrol of vehicles. Toxic metals are accumulated in top soils and vegetation near the road ecosystem. These heavy metals do bioaccumulate in living organisms and the human body through various processes causing adverse effects. In the human body, these heavy metals are transported 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, cause mutation, mimic

hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer (Jarup, 2003). Below are some of the mechanisms of heavy metals toxicity and their health effects

2.2.2.1 Iron

Iron is a useful heavy metal in the human body as it is a constituent of certain biological molecules like the hemoglobin and involved in various physiological activities. However, in its free state, iron is one of the heavy metals generally known to generate hydroxyl radical (OH•) as shown below by the Fenton reaction.

$$Fe^{3+} + O^{2-} \rightarrow Fe^{2+} + O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \text{ (Fenton reaction)} \quad (2)$$

Net reaction (Haber-Weiss reaction):

$$O^{2-} + H_2O_2 \rightarrow OH^{-} + OH^{\bullet} + O_2 \quad (3)$$

In addition to the above reactions, the following reactions below can also occur:

$$OH^{\bullet} + H_2O_2 \rightarrow H_2O + H^{+} + O^{2\bullet-} \quad (4)$$

$$OH^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^{-} \quad (5)$$

$$LOOH + Fe^{2+} \rightarrow Fe^{3+} + LO^{-} + OH^{\bullet} \quad (6)$$

Hydroxyl radical (OH•) is the most common free radical generated by the oxidation of iron. OH• is capable of reacting with biological molecules such as proteins, lipids and DNA damaging them. When OH• reacts with guanine, a nitrogenous base of nucleic acids, it leads to the generation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxo-dG) and 2,6-diamino-5-formamido-4-hydroxypyrimidine (FAPy-G), in which the former is a good marker for oxidative damage (Valko *et al*., 2004). It is well documented that metal-induced generation of oxygen reactive species can attack polyunsaturated fatty acid such as phospholipids. The first of such observation was first presented by Bucher *et al*. (1983) who showed that

iron generated OH• can oxidize lipid membranes through a process known as lipid peroxidation. Following his experimental observations, he proposed the following mechanism:

Steps of lipid peroxidation:

Initiation: $\text{Lipid} + R^{\bullet}/OH^{\bullet} \rightarrow \text{Lipid}^{\bullet}$ (7)

Propagation: $\text{Lipid}^{\bullet} + O_2 \rightarrow \text{Lipid} - OO^{\bullet}$ (8)

$\text{Lipid} - OO + \text{Lipid}^{\bullet} \rightarrow \text{Lipid} - OOH + \text{Lipid}^{\bullet}$ (9)

Termination: $\text{Lipid}^{\bullet} + \text{Lipid}^{\bullet} \rightarrow \text{Lipid} - \text{Lipid}$ (10)

$\text{Lipid} - OO\bullet + \text{Lipid}\bullet \rightarrow \text{Lipid} - OO - \text{Lipid}$ (11)

At the initiation stage, the radical (R•)/OH• attacks the lipid membrane to form a radical lipid. This radical lipid further propagates the formation of peroxyl lipid radical by reacting with dioxygen molecule or with a lipid. This reaction further promotes damage of the lipid molecule. At the termination stage, two radical lipid molecules and/or with a peroxyl lipid radical reacts to form a stable lipid molecule.

The major aldehyde product of lipid peroxidation is malondialdehyde and it serves as a marker for lipid peroxidation. Generally, proteins are not easily damaged by H_2O_2 and other simple oxidants unless transition metals are present. Thus, protein damaged are usually metal catalyzed and involves oxidative scission, bityrosine cross links, loss of histidine residues, the introduction of carbonyl groups, and the formation of proteincentered alkyl (R•), alkoxyl (RO•) and alkylperoxyl (ROO•) radicals (Eaton and Qian, 2002).

2.2.2.2 Copper

Copper ions have been identified to participate in the formation of reactive oxygen species (ROS) as cupric (Cu^{2+}) and cuprous (Cu^{1+}) which can participate in oxidation and reduction reactions. The Cu^{2+} in the presence of biological reductants such as glutathione (GSH) or ascorbic acid can be reduced to Cu^{+} which is capable of catalyzing the decomposition of H_2O_2 to form OH• *via* the Fenton reaction (Lloyd *et al*., 1997), as shown below.

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH\bullet + OH^{-} \qquad (12)$$

The OH• radical formed is capable of reacting with several biomolecules. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases *via* oxygen free radicals (Brezova *et al*., 2003). Though *in vivo* studies have not revealed copper-induced oxidation of low density lipoprotein (LDL), *in vitro* studies clearly demonstrated LDL oxidation induced by copper (Burkitt, 2001).

2.2.2.3 Manganese

Manganese is known to accumulate in the mitochondria of neurons, astrocytes and oligodendrocytes cells and disrupts ATP synthesis (Milatovic *et al*., 2017) by inhibiting the F1/ F0 ATP synthase (Milatovic *et al*., 2017) or complex 1 (NADH dehydrogenase) of the mitochondrial respiration chain (Chen *et al*., 2001). More so, it has recently been shown that manganese inhibits ATP synthesis at two sites in the brain mitochondria which are either the glutamate/ aspartate exchanger or the complex II (succinate dehydrogenase) depending on the mitochondrial energy source(Gunter *et al*., 2010). The disruption of ATP synthesis by manganese leads to decreased intracellular ATP levels and generation of free radicals thereby increasing oxidative stress (Milatovic *et al*., 2009) which may contribute to manganese cellular

toxicity (Gunter *et al*., 2006). Furthermore, manganese can oxidize dopamine (DA) to react with quinone species thereby disrupting the dopaminergic system (Paris and Segura-Aguilar, 2011). This has been shown in animal studies were manganese exposure has led to specific deficits in the dopaminergic system (Burton and Guilarte, 2009.) The DA reactive species are taken up by the dopamine transporter (DAT1) thus causing dopaminergic neurotoxicity (Benedetto *et al*., 2010).

2.2.2.4 Cadmium

Cadmium is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. The highest level of cadmium compounds in the environment is accumulated in sedimentary rocks, and marine phosphates contain about 15 mg cadmium/kg (WHO, 1987). Its industrial applications also include the production of alloys, pigments, and batteries (Wilson, 1988).

Potential for Human Exposure

The main routes of exposure to cadmium are via inhalation or cigarette smoke, and ingestion of food. Skin absorption is rare. Human exposure to cadmium is possible through a number of several sources including employment in primary metal industries, eating contaminated food, smoking cigarettes, and working in cadmium-contaminated work places, with smoking being a major contributor (Paschal *et al.,* 2000). Other sources of cadmium include emissions from industrial activities, including mining, smelting, and manufacturing of batteries, pigments, stabilizers, and alloys (ATSDR, 2008). Cadmium is also present in trace amounts in certain foods such as leafy vegetables, potatoes, grains and seeds, liver and kidney, and crustaceans and mollusks (Satarug *et al*., 2003). In addition, foodstuffs that are rich in cadmium can greatly increase the cadmium concentration in human bodies. Examples are liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed. An important distribution route is the circulatory system whereas blood vessels are considered to be main stream organs of cadmium toxicity. Chronic inhalation exposure to cadmium particulates is generally associated with changes in pulmonary function and chest radiographs that are consistent with emphysema (Davison *et al.,* 1988). Workplace

exposure to airborne cadmium particulates has been associated with decreases in olfactory function (Mascagni *et al*., 2003). Several epidemiologic studies have documented an association of chronic low-level cadmium exposure with decreases in bone mineral density and osteoporosis (Åkesson *et al*., 2006; Gallagher *et al*., 2008).Because of continuing use of cadmium in industrial applications, the environmental contamination and human exposure to cadmium have dramatically increased during the past century(Elinder and Järup, 1996).

Molecular Mechanisms of Toxicity and Carcinogenicity

Cadmium is a severe pulmonary and gastrointestinal irritant, which can be fatal if inhaled or ingested. After acute ingestion, symptoms such as abdominal pain, burning sensation, nausea, vomiting, salivation, muscle cramps, vertigo, shock, loss of consciousness and convulsions usually appear within 15 to 30 min (Baselt and Cravey, 1995). Acute cadmium ingestion can also cause gastrointestinal tract erosion, pulmonary, hepatic or renal injury and coma, depending on the route of poisoning (Baselt, 2000). Chronic exposure to cadmium has a depressive effect on levels of norepinephrine, serotonin, and acetylcholine (Singhal *et al.,* 1976). Rodent studies have shown that chronic inhalation of cadmium causes pulmonary adenocarcinomas (Waalkes and Berthan, 1995; Waalkes, *et al*., 1996). It can also cause prostatic proliferative lesions including adenocarcinomas, after systemic or direct exposure (Waalkes and Rehm , 1992). Although the mechanisms of cadmium toxicity are poorly understood, it has been speculated that cadmium causes damage to cells primarily through the generation of ROS (Stohs, 1995) which causes single-strand DNA damage and disrupts the synthesis of nucleic acids and proteins (Mitra, 1984). Previous reports have indicated that cadmium affects signal transduction pathways; inducing inositol polyphosphate formation, increasing cytosolic free calcium levels in various cell types (Th'evenod and Jones, 1992), and blocking calcium channels (Dally and Hartwig, 1997). At lower concentrations (1–100 μM), cadmium binds to proteins, decreases DNA repair (Abshire *et al*., 1996), activates protein degradation, up-regulates cytokines and proto-oncogenes such as c-*fos*, c*jun*, and c-*myc* (Durnam and

Palmiter, 1981), and induces expression of several genes including metallothioneins (Hwua and Yang, 1998), heme oxygenases, glutathione transferases, heat-shock proteins, acute-phase reactants, and DNA polymerase β (Landolph, 1994).

Cadmium compounds are classified as human carcinogens by several regulatory agencies. The International Agency for Research on Cancer (IARC, 1993) and the U.S. National Toxicology Program have concluded that there is adequate evidence that cadmium is a human carcinogen. This designation as a human carcinogen is based primarily on repeated findings of an association between occupational cadmium exposure and lung cancer, as well as on very strong rodent data showing the pulmonary system as a target site (IARC, 1993). Thus, the lung is the most definitively established site of human carcinogenesis from cadmium exposure. Other target tissues of cadmium carcinogenesis in animals include injection sites, adrenals, testes, and the hemopoietic system (IARC, 1993; Walkes, 1996). In some studies, occupational or environmental cadmium exposure has also been associated with development of cancers of the prostate, kidney, liver, hematopoietic system and stomach (Walkes, 1996). Carcinogenic metals including arsenic, cadmium, chromium, and nickel have all been associated with DNA damage through base pair mutation, deletion, or oxygen radical attack on DNA (Landolph, 1994). Animal studies have demonstrated reproductive and teratogenic effects. Small epidemiologic studies have noted an inverse relationship between cadmium in cord blood, maternal blood or maternal urine and birth weight and length at birth (Nishijo *et al*., 2004; Zhang *et al*., 2004).

2.2.2.5 Chromium

Chromium (Cr) is a naturally occurring element present in the earth's crust, with oxidation states (or valence states) ranging from chromium (II) to chromium (VI) (jacobs and Testa, 2005). Chromium compounds are stable in the trivalent [Cr (III)] form and occur in nature in this state in ores, such as ferrochromite. The hexavalent [Cr (VI)] form is the second most stable state (Patlolla *et al*., 2009). Elemental chromium [Cr (0)] does not occur naturally. Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest

release coming from industrial establishments. Industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production. The increase in the environmental concentrations of chromium has been linked to air and wastewater release of chromium, mainly from metallurgical, refractory, and chemical industries. Chromium released into the environment from anthropogenic activity occurs mainly in the hexavalent form [Cr (VI)].chromium [Cr (VI)] is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies (IARC., 1990, U.S. EPA, 1992)

Potential for Human Exposure

In humans and animals, [Cr (III)] is an essential nutrient that plays a role in glucose, fat and protein metabolism by potentiating the action of insulin (Goyer, 2001). However, occupational exposure has been a major concern because of the high risk of Cr-induced diseases in industrial workers occupationally exposed to Cr (VI) (Guertin, 2005). Also, the general human population and some wildlife may also be at risk. It is estimated that 33 tons of total Cr are released annually into the environment .The U.S. Occupational Safety and Health Administration (OSHA) recently set a "safe" level of 5µg/m3, for an 8-hr time-weighted average, even though this revised level may still pose a carcinogenic risk (OSHA, 2006). For the general human population, atmospheric levels range from 1 to 100 ng/cm3 (Singh *et al*., 1999), but can exceed this range in areas that are close to Cr manufacturing. Non occupational exposure occurs via ingestion of chromium containing food and water whereas occupational exposure occurs via inhalation (Langård and Vigander, 1983).

Mechanisms of Toxicity and Carcinogenicity

Major factors governing the toxicity of chromium compounds are oxidation state and solubility. Cr (VI) compounds, which are powerful oxidizing agents and thus tend to be irritating and corrosive, appear to be much more toxic systemically than Cr (III) compounds, given similar amount and solubility (De Flora *et*

al., 1990). Although the mechanisms of biological interaction are uncertain, the variation in toxicity may be related to the ease with which Cr (VI) can pass through cell membranes and its subsequent intracellular reduction to reactive intermediates. Since Cr (III) is poorly absorbed by any route, the toxicity of chromium is mainly attributable to the Cr (VI) form. It can be absorbed by the lung and gastrointestinal tract, and even to a certain extent by intact skin. The reduction of Cr (VI) is considered as being a detoxification process when it occurs at a distance from the target site for toxic or genotoxic effect while reduction of Cr (VI) may serve to activate chromium toxicity if it takes place in or near the cell nucleus of target organs (Dayan and Paine, 2001). If Cr (VI) is reduced to Cr (III) extracellularly, this form of the metal is not readily transported into cells and so toxicity is not observed. The balance that exists between extracellular Cr (VI) and intracellular Cr (III) is what ultimately dictates the amount and rate at which Cr(VI) can enter cells and impart its toxic effects (Cohen *et al*., 1993). Cr(VI) enters many types of cells and under physiological conditions can be reduced by hydrogen peroxide (H2O2), glutathione (GSH) reductase, ascorbic acid, and GSH to produce reactive intermediates, including Cr(V), Cr(IV), thiylradicals, hydroxyl radicals, and ultimately, Cr(III). Any of these species could attack DNA, proteins, and membrane lipids, thereby disrupting cellular integrity and functions (De Mattia *et al*., 2004).

Adverse health effects induced by Cr (VI) have been reported in humans. Epidemiological investigations have reported respiratory cancers in workers occupationally exposed to Cr (VI)-containing compounds (Costa, 1997; Dayan and Paine, 2001). DNA strand breaks in peripheral lymphocytes and lipid peroxidation products in urine observed in chromium-exposed workers also support the evidence of Cr (VI)-induced toxicity to humans (Gambelunghe *et al*., 2003; Goulart *et al*., 2005). Oxidative damage is considered to be the underlying cause of these genotoxic effects including chromosomal abnormalities [Wise *et al*., 2002; Wise *et al*., 2004], and DNA strand breaks (Xie *et al*., 2005). Nevertheless, recent studies indicate a biological relevance of non-oxidative mechanisms in Cr(VI) carcinogenesis(Zhitkovich *et al*., 2001). Carcinogenicity appears to be associated with the inhalation of the less soluble/insoluble Cr(VI) compounds. The toxicology of Cr(VI) does not reside with the elemental form. It varies greatly among a

wide variety of very different Cr(VI) compounds (Katz and Salem, 1993). Epidemiological evidence strongly points to Cr(VI) as the agent in carcinogenesis. Solubility and other characteristics of chromium, such as size, crystal modification, surface charge, and the ability to be phagocytized might be important in determining cancer risk (Norseth, 1981). Studies in the laboratory have indicated that chromium (VI) is cytotoxic and able to induce DNA damaging effects such as chromosomal abnormalities (Patlolla *et al*., 2008), DNA strand breaks, DNA fragmentation and oxidative stress in Sprague-Dawley rats and human liver carcinoma cells (Patlolla *et al*., 2009). Recently, laboratory studies have also demonstrated that chromium (VI) induces biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *carassius auratus* (Velma and Tchounwou, 2010).

2.2.2.6. Lead

Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations. Lead has many different industrial, agricultural and domestic applications. It is currently used in the production of lead-acid batteries, ammunitions, metal products (solder and pipes), and devices to shield X-rays. Lead in dust and soil often recontaminates cleaned houses (Farfel and Chisolm, 1991), and contributes to elevating blood lead concentrations in children who play on bare, contaminated soil (CDC, 2001). Today, the largest source of lead poisoning in children comes from dust and chips from deteriorating lead paint on interior surfaces (Lanphear *et al*., 1998). Children who live in homes with deteriorating lead paint can achieve blood lead concentrations of 20µg/dL or greater (Charney *et al*., 1980).

Potential for Human Exposure

Exposure to lead occurs mainly via inhalation of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water, and paints (ATSDR, 1999; ATSDR, 1992). Adults absorb 35 to 50% of lead through drinking water and the absorption rate for children may be greater than 50%. Lead

absorption is influenced by factors such as age and physiological status. In the human body, the greatest percentage of lead is taken into the kidney, followed by the liver and the other soft tissues such as heart and brain, however, the lead in the skeleton represents the major body fraction (Flora *et al*., 2006). The nervous system is the most vulnerable target of lead poisoning. Headache, poor attention spam, irritability, loss of memory and dullness are the early symptoms of the effects of lead exposure on the central nervous system (CDC, 2001; ATSDR, 1999). Lead is the most systemic toxicant that affects several organs in the body including the kidneys, liver, central nervous system, hematopoetic system, endocrine system, and reproductive system (ATSDR, 1999).

Molecular Mechanisms of Toxicity and Carcinogenicity

There are many published studies that have documented the adverse effects of lead in children and the adult population. In children, these studies have shown an association between blood level poisoning and diminished intelligence, lower intelligence quotient-IQ, delayed or impaired neurobehavioral development, decreased hearing acuity, speech and language handicaps, growth retardation, poor attention span, and anti-social and diligent behaviors (USEPA, 2002). In the adult population, reproductive effects, such as decreased sperm count in men and spontaneous abortions in women have been associated with high lead exposure (Hertz-Picciotto, 2000). Acute exposure to lead induces brain damage, kidney damage, and gastrointestinal diseases, while chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (Hertz-Picciotto, 2000).

One of the major mechanisms by which lead exerts its toxic effect is through biochemical processes that include lead's ability to inhibit or mimic the actions of calcium and to interact with proteins (ATSDR, 1999). Within the skeleton, lead is incorporated into the mineral in place of calcium. Lead binds to biological molecules and thereby interfering with their function by a number of mechanisms. Lead binds to sulfhydryl and amide groups of enzymes, altering their configuration and diminishing their activities.

Lead may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium (Flora *et al*., 2007). Many investigators have demonstrated that lead intoxication induces a cellular damage mediated by the formation of reactive oxygen species (ROS) (Hermes-Lima *et al*., 1991). In addition, Jiun and Hseien (1994) demonstrated that the levels of malondialdehyde (MDA) in blood strongly correlate with lead concentration in the blood of exposed workers. Other studies showed that the activities of antioxidant enzymes, including superoxide dismutase (SOD), and glutathione peroxidase in erythrocytes of workers exposed to lead are remarkably higher than that in non-exposed workers (Bechara *et al*., 1993). A series of recent studies in the laboratory demonstrated that lead-induced toxicity and apoptosis in human cancer cells involved several cellular and molecular processes including induction of cell death and oxidative stress (Yedjou *et al*., 2006), transcriptional activation of stress genes (Tchounwou *et al*., 2004), DNA damage (Yedjou and Tchounwou, 2008), externalization of phosphatidylserine and activation of caspase-3 (Yedjou *et al*., 2010).

2.3 Aflatoxins

Aflatoxins are a group of naturally occurring carcinogens that are known to contaminate different human and animal food stuffs. Aflatoxins are poisonous by-products from soil-borne fungus *Aspergillus*, which is responsible for the decomposition of plant materials (Bankole, & Adebanjo, 2003; Bennett & Klich, 2003; Gupta, 2011).The occurrence of aflatoxins in foods and food products vary with geographic location, agricultural and agronomic practices. The susceptibility of food product to fungal attack occurs during pre-harvest, transportation, storage, and processing of the foods (Wu, 2011; Thrasher, 2012). The problem of aflatoxin contamination of the food products is a common problem in tropical and subtropical regions of the world especially in the developing countries such as the sub-Saharan countries with poor practices and where the environmental conditions of warm temperatures and humidity favors the growth fungi (Wu, 2011; Thrasher, 2012). The various food products contaminated with aflatoxins include cereals like maize, sorghum, pearl millet, rice and wheat; oilseeds such as groundnut, soybean, sunflower

and cotton; spices like chillies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconut; and milk and milk products (Lopez, 2002). The aflatoxins were initially isolated and identified as the causative agent in Turkey X disease that caused necrosis of the liver in 1960 and over 100,000 turkeys died in England and USA and the death was attributed to the consumption of a mould-contaminated peanut meal (Thrasher, 2012;WHO, 2002; Otsuki, *et al*.,2002; Sudakin, 2003). Very high concentrations of aflatoxins are most often found in nutritive seeds such as maize, nuts and cereal grains in Africa and rice in China and Southeast Asia(Sudakin, 2003). Aflatoxins are a group of approximately 20 related fungal metabolites produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus* [Cortés, 2010; Reddy & Waliyar, 2012). Aflatoxins belongs to a group of difuranocoumarins that are classified into two broad groups according to their chemical structure and they include the difurocoumarocyclopentenone series (AFB1, AFB2, AFB2A, AFM1, AFM2, AFM2A and aflatoxicol) and the difurocoumarolactone series (AFG1, AFG2, AFG2A, AFGM1, AFGM2, AFGM2A and AFB3) (Cortés, 2010; Reddy & Waliyar, 2012; Salhab, 1977). The four major naturally known aflatoxins produced by the *Aspergillus* species of mold include AFB1, AFB2, AFG1 and AFG2 where the "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively. Whereas the B designation of aflatoxins B1 and B2 result from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light (Bennett, & Klich, 2003; Thrasher, 2012; WHO, 2002; Otsuki, *et al*.,2002; Sudakin, 2003). The metabolic products of aflatoxins, M1 and M2 were first isolated from milk of lactating animals fed on Moldy grains contaminated with aflatoxin hence, the M designation [Bennett, & Klich, 2003). These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Whereas, aflatoxin M1 is 4-hydroxy aflatoxin B1 and aflatoxin M2 is 4-dihy-droxy aflatoxin B2. Of the four major aflatoxins (B1, B2, G1 and G2), G2 occurs in high quantities though less toxic

while AFB1 is the most toxic of all the aflatoxin. The World Health Organization (WHO) classifies AFB1 as a class 1 carcinogen (Thrasher, 2012; WHO, 2000; Thrasher *et al*., 2009). The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB1> AFG1> AFB2> AFG2 (Cortés, 2010; Reddy & Waliyar, 2012). The extent of toxicity depends on the organ affected especially the liver. The lethal toxicity of aflatoxin B1 varies in different animals from extremely susceptible (Sheep, Rat, Dog) to resistant species (Monkey, Chicken, Mouse). However, there are no toxicity in humans though epidemiological data from studies in Africa, South Africa, South East Asia and India implicate aflatoxins in the incidence of liver cancer especially the hepatobiliary carcinoma and death of children due to malnutrition, kwashiorkor and marasmus (Peraica, 1999; Thomas, 2005). Aflatoxins have been associated with various diseases like aflatoxicosis and other health problems in humans, livestock and domestic animals globally.

2.3.1 Aflatoxins occurrence in feeds

There are six common mycotoxins that affect animals: aflatoxins, fumonisins, ochratoxins (which like aflatoxins affect liver function), trichothecenes and zearalenone. Diagnosis of aflatoxin exposure in animals is difficult, especially in large farms that use mixed feed which may contain highly varied combinations of feedstuffs. As in humans, animals exposed to high levels of aflatoxin-contaminated feed have been known to exhibit the severe form of "intoxication" which can lead to death. Usually, however, exposure in animals is of a "sub-clinical" level, which leads to liver damage, reduced weight gain and lost productivity (declines in egg and milk production) resulting in economic losses to the industry. Aflatoxins affect livestock growth, reproduction, immune functioning and ability to metabolize vaccines (Makun *et al*., 2012). Poultry and fish are most affected, but there is also a lot of concern about B1 contamination in milk, given that it is often fed to infants and young children (Galvano *et al*., 1996).

The effects of animal feed on meat and other bi-products: Consumption of aflatoxin-contaminated animal feeds varies with preparation and the type of product. USAID and Danya International (2012)

reviewed studies in which the prevalence of aflatoxins was compared across beef and edible organs that were either fresh or sundried. Consistently, organ meats (especially kidney) were more contaminated than beef, while fresh products (as opposed to sundried products) maintained higher aflatoxin contamination levels. Time of harvest has been shown to be important in influencing the occurrence and levels of aflatoxin because Aspergillus does not compete well with other molds when corn presents more than 20% moisture. Harvesting corn when moisture content is above 20% followed by rapid drying to at least 14% moisture content within 24 to 48 hours of harvest can inhibit Aspergillus growth and toxin production. Contaminated grains and their by-products are the most common sources of aflatoxin. Corn silage may also be a source of aflatoxins, because the ensiling process does not destroy toxins already present in silage (Cassel *et al.,* 2012). On the farm, more than one mold or toxin may be present in the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult. The prognosis of aflatoxicosis depends upon the severity of liver damage. Once overt symptoms are noticed the prognosis is poor. Treatment should be directed at the severely affected animals in the herd and further poisoning prevented. Aflatoxicosis is typically a herd rather than an individual cow problem. If aflatoxicosis is suspected, feed should be analyzed immediately. If aflatoxins are present, the source should be eliminated immediately. Levels of protein in feed and vitamins A, D, E, K and B should be increased as the toxin binds vitamins and affects protein synthesis. Good management practices to alleviate stress are essential to reduce the risk of secondary infections which must receive immediate attention and treatment (Cassel *et al.,* 2012).

Aflatoxin is just one of many mycotoxins that can adversely affect animal health and productivity. Care regarding animal feed must be extended not only to the nutritional and economic value, but also to food quality (Gong *et al*., 2004). The presence of molds in foodstuffs causes the appearance of flavours and odours that reduce palatability and affect feed consumption by animals as well as reduce the nutritional value of foods. Mycotoxins, in turn, affect the digestion and metabolism of nutrients in animal production, resulting in nutritional and physiological disorders, besides a negative effect on the immune system (Huff *et al*., 1975).

2.3.2 Aflatoxins in Milk

Milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not treated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM1 are of great concern. Kiermier and meshaley (1977) reported the effect of cold treatments. They observed that detectable AFM1 decreased by 11 to 25% after 3 days at 5ºC, 40% after 4 days at 0ºC, and 80% after 6 days at 0ºC. Whereas, McKinney et al. (1973) revealed that freezing at -18ºC for 30 days resulted in an apparent loss of 14%, with 85% lost after 53 days. Stoloff et al. (1981) suggested less degradation of AFM1 at -18ºC with insignificant loss after 53 days. As to the effect of heating contradictory data have been reported. Kiermeier and Mashaley (1977), reported that various heat–time treatments caused reductions in the AFM1 concentrations of milks between 12% and 40%. Choudhary et al. (1998) studied the effect of various heat treatments on AFM1 content of cow's milk and reported that sterilization of milk at 121 °C for 15 min caused 12.21% degradation of AFM1, whereas boiling decreased AFM1 by 14.50%. They concluded that destruction of AFM1 depends on time and temperature combination of the heat treatment applied. In an investigation Conducted by Bakirci (2001), it was observed that pasteurization caused a decrease in the level of AFM1 at the rate of 7.62%. Deveci (2007) showed that pasteurization can partially reduce the amount of AFM1 in milk. However, some reports showing that aflatoxins are stable during heat-treatments such as pasteurization and sterilization (Van-Egmond *et al.*, 1977; Wiseman and Marth, 1983; Yousef and Marth, 1989; Govaris *et al*. 2001) were also published. Fluctuation in data reported in literature could be attributed to the wide range of temperature, different analytical methods, and employment of both naturally and artificially contaminated milk. AFM1 distribution in milk is not homogeneous. Cream separation can affect AFM1 distribution, since 80% is partitioned in the skim milk portion (Grant and Carlson, 1971) because of AFM1 binding to casein (Brackett, 19982). An amount of 30% of AFM1 is indeed estimated to be associated with the nonfat milk solids and in particular with casein. According to Van Egmond and Paulsch (1986) the behavior of AFM1 in processes which involve fat separation may be explained by its semipolar character,

leading to predominance in the nonfat fraction. Contradictory data have been reported on the influence of milk concentration on AFM1. Kiermeier (1973) reported no losses of AFM1, whereas some authors observed losses ranging from 60 to 75% following milk concentration (Moreau, 1976). Many authors showed that Seasonal effect influences concentration of aflatoxin M1. They reported higher concentration of AFM1 in cold seasons as compared to hot seasons (Applebaum *et al*., 1982; Blanco *et al*., 1988b; Hussain and Anwar, 2008; Tajkarimi *et al*. 2008; Fallah, 2010, Bilandzic *et al*., 2010), the reason being in winters mostly milking animals are fed with compound feeds and thus concentration of aflatoxin B1 increases which in turn enhances AFM1 concentration in milk. Moreover, temperature and moisture contents also affect the presence of aflatoxin B1 in feeds. A. flavus and A. parasiticus can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60%, furthermore, they can produce toxin (Jay, 1992). Another reason of low AFM1 level in summer may be attributed to out-pasturing of milking cattle. It is too difficult to compare the data from the literature due to wide differences between and within the countries related to feeding, animal and environmental factors, extraction and analysis procedures, and regulatory limits for aflatoxins in feeds and milk. However, in recent years the incident of AFM1 contamination seems to have been balanced on the one hand by increasing precision of extraction and analysis procedures and on the other hand the setting of stricter regulatory limits for aflatoxins in feeds and milk (Galvano *et al.,* 1996). Today the high efficiency of immuno-enzymatic extraction and the accuracy of analytical methodology and equipment, such as high pressure liquid chromatography and fluorescence detectors, allow detection limits to decrease, improving the percentage of positive samples. Furthermore, in recent years attention to the concern of aflatoxins in feeds as well as in milk has increased in most of the developed countries.

2.4 Route of entry and Absorption

Aflatoxins are highly liposoluble compounds and are readily absorbed from the site of exposure usually through the gastrointestinal tract and respiratory tract into blood stream (Agag, 2004; Larsson, & Tjalve, 2000). Human and animals get exposed to aflatoxins by two major routes (a) direct ingestion of aflatoxin-

contaminated foods or ingestion of aflatoxins carried over from feed into milk and milk products like cheese and powdered milk as well as other animal tissues mainly as AFM1 (Agag, 2004) (b) by inhalation of dust particles of aflatoxins especially AFB1 in contaminated foods in industries and factories (Coulombe, & Jr, 1994). After entering the body, the aflatoxins are absorbed across the cell membranes where they reach the blood circulation.

2.4.1 Distribution, metabolism, excretion and mechanisms of action of aflatoxins

They are distributed in blood to different tissues and to the liver, the main organ of metabolism of xenobiotics. Aflatoxins are mainly metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1 (Wild, & Montesano, 2009; Wu, & Khlangwiset, 2010). In humans and susceptible animal species, aflatoxins especially AFB1 are metabolized by cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8,9-epoxide, a reactive form that binds to DNA and to albumin in the blood serum, forming adducts and hence causing DNA damage (Wild & Montesano 2009; Wu & Khlangwiset, 2010). Various CYP450 enzymes isoforms occur in the liver and they metabolize aflatoxin into a reactive oxygen species (aflatoxin-8,9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer (Wild & Montesano 2009; Wu & Khlangwiset, 2010). The predominant human CYP450 isoforms involved in human metabolism of AFB1 are CYP3A4 and CYP1A2. Both enzymes catalyze the biotransformation of AFB1 to the highly reactive *exo*-8, 9-epoxide of AFB1 (Guengerich, 1998). CYP 1A2 is also capable of catalyzing the epoxidation of AFB1 to yield a high proportion of *endo*-epoxide and hydroxylation of AFB1 to form aflatoxin M1 (AFM1), which is a poor substrate for epoxidation (Guengerich, 1998) and less potent than AFB1 (Wild & Turner, 2002). This is generally considered as the major detoxification metabolic pathway for aflatoxins. The CYP3A4 is the major CYP450 enzyme responsible for activation of AFB1 into the epoxide form and also form AFQ1, a less toxic detoxification metabolite. The CYP3A5 metabolizes AFB1 mainly to the *exo*-epoxide and some AFQ1 (Wang, 1998). However, polymorphism

studies with CYP3A5 have indicated that, this enzyme isoform is not expressed by most people especially in Africans (Wild & Turner, 2002). Studies in Gambian children showed that aflatoxin cross the placenta and transported to the fetus and the new born where they can cause detrimental effects (Wild & Turner, 2002). The CYP3A7 is a major CYP450 enzyme isoform in human fetal liver and metabolizes AFB1 to the 8, 9- epoxide that may cause fetal defects to the developing fetus (Kitada, 1998). .

The epoxidation of AFB1 to the exo-8, 9-epoxide is a critical step in the genotoxic pathway of this carcinogen. The binding of AFB1 to DNA and DNA adduction by AFB1 exo-8,9 epoxide has been reported to cause a functional changes of DNA conformation (Raney *et al*., 1993).The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form afltoxin-N7-guanine (Guengerich, 2001). The aflatoxin-N7-guanine has been shown to be capable of forming guanine (purine) to thymine (pyrimidine) transversion mutations in DNA and hence affecting the p53 suppressor gene in the cell cycle (Bailey, 1996; Li, 1993).. The p53 gene is important in preventing cell cycle progression when there are DNA mutations, or signaling apoptosis. The mutations have been reported to affect some base pair locations more than others especially in the third base of codon 249 of the p53 gene in the region corresponding to the DNA binding do- main of the corresponding protein (Li, 1993; Sudakin, 2003) and this appears to be more susceptible to aflatoxin-mediated mutations than nearby bases (Aguilar et al., 1993). AFB1 induces the transversion of base G to base T in the third position of codon 249 and similar mutations have been observed in hepatocellular carcinoma (HCC) in high AFB1 contaminated food in regions in East Asia and Africa (Gerbes, & Caselmann, 1993; Mace, 1997).

Epoxide hydrolase and glutathione-S-transferase (GST) are both involved in hepatic detoxification of activated AFB1, but the GST-catalyzed conjugation of glutathione to AFB1-8,9-ep-oxides is thought to play the most important role in preventing epoxide binding to target macromolecules like DNA and various cell proteins (Sherratt, & Hayes, 2001). Glutathione pathway is reported to play a vital role in the detoxification of AFB1. The AFB1 8, 9 *exo* and *endo*epoxides are conjugated by glutathione to form AFB-mercapturate and the reaction is catalyzed by glutathione S-transferase (GST) (Johnson, 1997;

Farombi, & Nwaokeafor, 2005). The glutathione-aflatoxin conjugate is transported from the cells with an ATP-dependent multidrug-resistance protein through an accelerated process (Farombi, & Nwaokeafor, 2005). Despite a preference for conjugating the more mutagenic AFB1 exo-epoxide isomer, the relatively low capacity for GST-catalyzed detoxification of bio-activated AFB1 in lung may be an important factor in the susceptibility of the lung to AFB1 toxicity (Stetwart *et al*., 1996).The *exo* and *endo* epoxide can also be converted non-enzymatically to AFB1-8,9-dihydrodiol which in turn can slowly undergo a base-catalysed ring opening reaction to a dialdehyde phenolate ion (Guengerich, 1998). AFB1 dialdehyde can form Schiff bases with lysine residues in serum albumin forming aflatoxin-albumin complex (Sabbioni, &, C. 1991). Also the aflatoxin dialdehyde are reduced to a dialcoholin a NADPH-dependent catalyzed reaction by aflatoxin aldehyde reductase (AFAR) (Knight, 1999). However the guanine alkylation by aflatoxin B1 produces *exo*-8, 9-epoxide which is the reactive form and a carcinogen to the liver and the reaction is more than 2000 times more efficient in DNA than in aqueous solution (Brown, 2009).

2.5 Health effects of aflatoxins on human and animals (Aflatoxicosis)

Aflatoxicosis is a condition caused by aflatoxins in both humans and animals. It occurs in two general forms (1) the acute primary aflatoxicosisis produced when moderate to high levels of aflatoxins are consumed. Specific acute episodes of disease may include hemorrhage, acute liver damage, edema, alteration in digestion, absorption and/or metabolism of nutrients, and possibly death (Thrasher, 2012; Otsuki, *et al.,* 2002; Liu, 2012; IARC. 1972). Acute dietary exposure to AFB1 has been implicated in epidemics of acute hepatic injury (Sudakin, 2003; Farombi, 2006). Evidence of acute aflatoxicosis in humans has been reported worldwide especially in the third world countries like Taiwan, Uganda, India, Kenya and many others(USAID, 2012). (2) The chronic primary aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (USAID, 2012). The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome (WHO. 2000). The chronic forms of

aflatoxicosis include (1) teratogenic effects associated with congenital malformations (2) mutagenic effects where aflatoxins cause changes (mutations) in the genetic code, altering DNA and these changes can be chromosomal breaks, re-arrangement of chromosome pieces, gain or loss of entire chromosomes, or changes within a gene (3) the carcinogenic effect in which the carcinogenic mechanisms have been identified such as the genotoxic effect where the electrophilic carcinogens alter genes through interaction with DNA and thus becoming a potential for DNA damage and the genotoxic carcinogens that are sometimes effective after a single exposure, can act in a cumulative manner, or act with other genotoxic carcinogens which affect the same organs(Wangikar, 2005; Thrasher, & Crawley, 2012). Chronic effects of aflatoxin has been reported to impair the normal body immune function by either by reducing phagocytic activity or reduce T cell number and function as observed immunological suppression in animal model. Aflatoxins have also been reported to interfere with nutrition in a dose response relationship between exposure to aflatoxin and rate of growth in infants and children (WHO, 2000, Peraica, 1999; Wangikar, 2005; Thrasher, & Crawley, 2012). Aflatoxins also causes nutrient modification like vitamin A or D in animal models and thus making them unavailable for the normal body physiology and hence leads to nutritional deficiencies (USAID, 2012; Peraica, 1999).

The contamination of foods and feeds with aflatoxin can cause serious consequences in human and animal health. It is estimated that more than 5 billion people in developing countries worldwide are at risk of chronic aflatoxin exposure due to consumption of aflatoxin contaminated foods and of these more than 4 billion people develop aflatoxin related liver cancer especially the hepatocellular carcinoma (Strosnider, 2006, Liu, 2012; Shephard, 2008; Williams,2004). Aflatoxin exposure is mainly a problem in poor and developing countries with poor regulatory authorities in food processing and storage as well as with high levels of malnutrition. Aflatoxins have also been linked with kwashiorkor and marasmus in most of the sub-Saharan countries in children (Peraica, 1999). Many people in these countries experience chronic aflatoxicosis associated with long-term exposure to low to moderate levels of aflatoxin in the food supply chain. AFB1, AFB2 and AFM have been detected in liver, gall bladder, spleen, heart, muscle and kidney

(Murthy, 1975). Aflatoxin B1 exposure results in both steatosis and accumulation of fat and necrosis or cell death of liver cells. The amount of aflatoxins consumed contributes to the mutagenic, carcinogenic, teratogenic, and immunosuppressive health effects in the body. The adverse effect of aflatoxins in humans ranges from acute hepatic toxicity to chronic disease such as liver cancer, haemorrhages, oedema, and even immediate death. Prolonged consumption of aflatoxins has also been reported to cause impaired immune function and malnutrition and stunted growth in children and a number of disabilities and death (USAID, 2012; Barrett, 2005; Gong, *et al*., 2004) .Human studies have reported that aflatoxins cause an increase in circulating alpha tumor necrosing factor, suggesting that these mycotoxins are also immunotoxic in humans. Due to the aflatoxin body immunosuppressant, it has been associated with HIV and tuberculosis (Groopman, *et al*., 2008; Liu & Wu, 2010). Aflatoxins also pose a threat to developing fetuses and they are transferred from mother to infant in breast milk. Aflatoxins have been reported to be associated with a Reye-like Syndrome in Thailand, New Zealand, Czechoslovakia, the United States, Malaysia, Venezuela, and Europe (Thrasher & Crawley, 2012; Dvorakova, 1977).

All species of animals are susceptible to aflatoxicosis and the susceptibility of individual animals to aflatoxicosis varies considerably depending on dose, duration of exposure, species, age, sex and nutrition. AFB1, AFB2 and AFM have been detected in liver, gall bladder, spleen, heart, muscle and kidney of growing swine when protein and protein-free portions of the diet were separately fed (Murthy, 1975). Chronic exposure of aflatoxins to animals causes immunosuppression and also interferes with protein metabolism and multiple micronutrients that are critical to health due to adduct formation. These adduct are responsible for mutations, cancer, immunosuppression, lung injury and birth defects (Wallace, 1997). In animals, the aflatoxins cause liver damage, decreased milk production, reduced reproductively and suppressed immunity in animals consuming low dietary concentrations. The aflatoxicosis syndrome in animals may also be characterized by vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart. In dairy and beef cattle, the signs of acute toxicosis include anorexia, depression, dramatic drop in milk production, weight

loss, lethargy, gastrointestinal dysfunctions such as ascitis, icterus, tenesmus, abdominal pain, bloody diarrhoea, decreased feed intake and efficiency; weight loss, jaundice, abortion, hepatoencephalopathy, blindness, walking in circles, ear twitching, frothy mouth, photosensitization, bleeding and death [Thrasher, 2012; Agag, 2004; Fapohunda, 2007)In poultry, beside inappetance, weight loss, decreased egg production, leg and bone problems, poor pigmentation, fatty liver, kidney dysfunction, bruising and death, suppression to natural immunity and susceptibility to parasitic, bacterial and viral infections can occur (Agag, 2004; Thrasher, 2012).

2.6 Permitted levels of aflatoxin

Some countries have set permitted levels of aflatoxins in food in order to control and reduce detrimental effects of these toxins. These levels are variable and depend on economic and developing status of the countries(Gonçalez *et al*., 2004)In US, Food and Drug Administration (FDA) has permitted a total amount of 20ng/g in livestock feed and 0.5g/kg or 50ng/l in milk (Ellis, 1995). In European countries, permitted levels of aflatoxin M1 in milk, milk products and baby food are 0.005mg/kg (Creppy, 2002). Also, different countries have set different regulations for permitted levels of aflatoxin in livestock feed. For instance, European Union (EU) has set permitted levels of aflatoxin from 0.05 to 0.5µg/kg. Factors such as weather conditions are also effective in determining permitted levels of aflatoxin. Permitted levels of this toxin in tropical countries are higher compared to mild and cold countries (Van Egmond, 1989).

2.7 Control strategies of Aflatoxins

Aflatoxins mitigation could be done during pre-harvest, post- harvest and also during storage, many of the pre-harvest solutions currently available are based on Good Agricultural Practices, which typically include use of insect resistant crops, good tillage and weeding practices, appropriate use of fertilizers, irrigation and crop rotation (Milićević *et al*., 2010). In addition to GAP, practices such as treating soil with lime and farmyard manure have proven successful at reducing aflatoxin contamination levels (Waliyar *et al*., 2005). The post-harvest solution include varieties of new technologies as follows

Physical treatment: Heat and Irradiation

Physical means of removing AFB1 from foods are most commonly heating and irradiation using gamma (γ) rays. Aflatoxins are well known to be stable at high temperatures, so harsh heating is needed to effectively remove amounts. Recent studies have shown that temperatures of 150-200oC can remove significant amounts of AFB1 (an average of 79% reduction), which is most effective at high humidity (Arzandeh and Jinap, 2011; Hwang and Lee, 2006; Lee *et al*., 2015; Park *et al.,* 2005; Park and Kim, 2006; Raters and Matissek, 2008; Soliman, 2002; Yazdanpanah *et al*., 2005; Zheng *et al*., 2015). One of the challenges of this strategy is ensuring the integrity of product after the heating/roasting is complete. This sometimes limits the maximum temperature that can be used, which may result in only a partial removal of AFB.

However, this technique can be carried out easily, with low cost, and can be performed in 2 hours or less giving it logistical advantages. The other most commonly reported physical decontamination method is γ radiation. Studies have reported the use of γ radiation on a number of different food substrates including groundnuts, grains, and animal feed. This technique involves irradiating food products with a γ-ray source (such as 60Co) until a certain amount of ionizing radiation is achieved which has ranged from 6-60 kGy in recent studies. This technique is moderately effective with an average percent reduction of 65% across all of the reviewed studies (Di Stefano *et al*., 2014a, 2014b; Ghanem *et al*., 2008; Herzallah *et al*., 2008; Iqbal *et al*., 2013; Jalili *et al*., 2012, 2010; Mohamed *et al*., 2015). The logistical issues of using powerful radiation, particularly safety, may make the implementation of this technique into many developing countries difficult.

Biological treatment: organism, enzyme, extracts

Biological based interventions have also been investigated for their potential in reducing AFB1 levels in contaminated foodstuffs. One strategy is to inoculate food substrates with strains of bacteria which then reduce AFB1 presumably through metabolism or by physically binding AFB1 directly. Several genera of bacteria have been investigated such as *lactobacillus, saccharomyces, cellulosimicrobium* and others.

Fungal inoculation has also been investigated as potential detoxification method as seen by Branà *et al*. who used *Pleurotus eryngii* to degrade AFB1. Treatment times using this approach however are very long, usually requiring several days to carry out. AFB1 degradation using these methods however is typically high, averaging approximately 86% across recent studies (Branà *et al.,* 2017; Farzaneh *et al*., 2012; Hamad *et al*., 2017; Haskard *et al.,* 2001; Liu *et al*., 2017; Oluwafemi *et al*., 2010). Another approach to biological degradation of AFB1 is to use botanical extracts. These studies have used aqueous extracts of various plant species to dissolve AFB1 and determine percent degradation after incubating the toxin in this mixture for a given amount of time (24-72 hours). Although this also requires long treatment times, this method has been shown to be highly effective, particularly extracts from *Adhatoda vasica Ness* and *Corymbia citriodora,* which both achieved >95% degradation of AFB1 (Iram *et al.,* 2016a, 2016b, 2015; Velazhahan *et al.,* 2010; Vijayanandraj *et al*., 2014). Further studies need to be done to determine the efficacy of these methods when used on various food substrates. Additionally, the identification of the active components responsible for this degradation could prove useful in increasing the efficiency of this process.

Lastly, the use of purified enzymes from various biological sources has been investigated for AFB1 degradation potential. Recently, these have included laccases, manganese peroxidase, and the recently identified Bacillus aflatoxin-degrading enzyme. The efficacy of these approaches has been high, but they also have not been tested on food substrates, so the efficacy on food products is still unknown. As is the case with all the biological control methods, the time of treatment is high, taking several days to complete which may not be feasible in large scale applications (Alberts *et al*., 2009; Loi *et al.,* 2016; Xu *et al*., 2017; Yehia, 2014). Additionally, the degradation products in many of these studies were not identified. Without characterizing the end products of the treatments, the safety of treated foods cannot be fully determined.

Chemical treatment: acidification, ammoniation, ozonation

The use of chemical additives on contaminated foods has also become a popular choice particularly if the additives themselves are already used in the food industry. Acidification of AFB1 contaminated foods has been shown to be highly effective when citric, lactic, tartaric, and hydrochloric acid are used, however other acids such as succinic, acetic, ascorbic, and formic have only been marginally successful. These methods simply involve soaking contaminated foods in acidic solutions for a given amount of time. Even when carried out at room temperature, high AFB1 degradation can be observed in 24 hours or less (Lee *et al*., 2015; Rushing and Selim, 2016; Safara *et al*., 2010). Additionally, the detoxification product of AFB1 in acid has been wellcharacterized as AFB2a. As described earlier, AFB2a has been shown to be far less toxic than AFB1, making this method an attractive option. Another benefit is the simplicity of these methods, so the need for specialized equipment or specific skills is not required.

Conversely, ammoniation has been used to break down AFB1 in an alkaline environment. Although these studies haven't been performed recently, the technique is still referenced to this day due to its efficacy. This technique involves treating contaminated foods with either gaseous or liquid ammonia (usually 1.5-2%). If carried out at room temperature, this process can take a very long time, ranging from 24 hours to 15 days. The extent of degradation of this technique is high, sometimes reaching above 99% (Bagley, 1979; Galil and Naguib, 1997; Jorgensen and Price, 1981; Moerck *et al*., 1980; Weng *et al.,* 1994). The degradation product due to ammoniation –aflatoxin D1 (AFD1) – is also well characterized. Formed due to a hydrolysis and decarboxylation, AFD1 has been shown to be far less mutagenic than AFB1, although a reversion back into AFB1 can occur if the extract is acidified (Grove *et al*., 1984; Lee and Cucullu, 1978; Schroeder *et al*., 1985). A disadvantage to this technique however, is the requirement for complex infrastructures to perform the ammoniation which has prevented the widespread use of this technique worldwide.

Finally, ozonation is another commonly used chemical control method. Ozonolysis at a concentration of 6-90 mg/L has been shown to be effective at degrading AFB1 especially considering the relatively short treatment times. In as little as 20 min, El-Desouky *et al*. observed an 86.75% reduction in

AFB1 levels in wheat. Other recent studies have used treatment times of 30-180 min and have seen >65% reductions. Longer treatment times have also been employed, with some studies going up to 96 hours of treatment time. Additionally, the variety of food substrates that have been investigated with ozone is very high, indicating it can be effective on many different foodstuffs (Akbas and Ozdemir, 2006; Chen *et al*., 2014; de Alencar *et al*., 2012; Diao *et al*., 2013; El-Desouky *et al*., 2012; Inan *et al*., 2007; Luo *et al*., 2014a, 2014b; Zorlugenç *et al*., 2008). The breakdown products of AFB1 after ozonolysis have been identified by Diao et al. Thirteen oxidation products were identified and based on the chemical structures, the moieties responsible for mutagenicity disappeared, indicating that these products are likely less toxic, although it has not been verified using mutagenicity assays (Diao *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Equipment and Apparatus

Analytical balance (Mttlerto leadop, model AG 204, Switzerland) with precision of + 0.001g, 100 ml round bottomed flasks, Borosilicate volumetric flasks (25, 50 ml, 100 ml & 1000 ml), measuring cylinders (Duran, Germany), pipettes (Pyrex. USA), micropipettes (Dragonmed 1- 10 ml, 100-1000 ml, Shangao, China) Muffle furnace, Digestion tubes, flame atomic absorption spectrophotomers (buck scientific model 210VGP AAS, USA) equipped with deuterium arc back ground correctors and lead, cadmium and chromium hollow cathode lamp with air-acetylene flame used to conduct spectral analysis of the concentration of Pb (II), Cr (VI) and Cd (II) at equilibrium time. Filter paper whatmann No 1 and other Glass ware were used.

Reagents and Chemicals

Reagents and chemicals used for the laboratory work were all of analytical grade and purchased from reputable companies.

3.2 Methodology

Collection of Samples

A total of 81 samples of cow milk and obtained samples of feed were collected. All samples were analysed for heavy metals and only the milk samples were analysed for AFLM1, the samples were collected from three senatorial regions of Kano; Bichi in Kano North, Rano in Kano South and Nassarawa in Kano central. In each local government, samples were collected from “Ruga”. At each “Ruga” nine (9) samples were collected from three different locations. In Bichi Ruga point 1(BR1),

nine samples were taken, so as in point 2(BR2) and 3(BR3), Making 27 samples from each local government. Coupled to these samples were also the available feeds found at each "Ruga".

Determination of Heavy metals

Preparation of series of standard solutions of the respective metal ion

100ml of standard stock solutions of chromium, cupper, iron, lead, Manganese and Zinc and 20ml of Cadmium were added to 1000ml volumetric flasks and then diluted with deionized water to 1000ml to form a secondary stock solution 1, 100ml of this stock solution is added to 1L volumetric flask, and dilute to 1000ml with deionized water to form secondary stock solution 2,these standards were then added in varying concentrations to 250ml volumetric flask each containing 2,5ml nitric acid and 25ml caesium lanthanum solution and then diluted to 250ml with deionized water.

Sample preparation and Digestion by Anastasio *et al.,* (2006).

Digestion and estimation: Digestion of sample was done as per the method described by Anastasio *et al.,* (2006). For heavy metal analysis, entire milk sample in a clean and dry conical flask was dried at 70°C on a hot plate. To dried 3gm-sample, 30cm^3 of HNO_3 (65%) and 6 cm^3 H_2O_2 (30%) were added. The conical flask containing the milk sample and the acid mixture was kept on a hot plate and digested until the content of flask turned colourless. The flask was then removed from the hot plate and kept at room temperature. After cooling, 20 cm^3 of distilled water was added into each flask (to dilute the acid content) and the contents were then filtered into a 100 cm^3 volumetric flask using Whatman filter paper no. 42. The conical flasks were rinsed and washed 3 times with distilled water and filtered into the respective volumetric flasks until their full volume was attainted (100 cm^3). The concentration of heavy metals (Fe, Cu, Zn, Cr, Mn, Pb and Cd) in digested milk samples were estimated using Atomic Absorption Spectrophotometer

Digestion of cow feeds by Sobia *et al*., (2015)

Samples under study were first digested using wet digestion method. Sample (0.2gms) were taken in 100cm^3 volumetric flask and 4 cm^3 of HNO_3 was added and solution was allowed to stand for few hours than it was carefully heated over water bath till red fumes coming from the flask completely ceased. Flask was allowed to cool at room temperature and then 4 cm^3 of perchloric acid was added and then flask was heated again over water bath to evaporate till a small portion which was than filtered through whattman filter paper no.42 and made up the volume using distilled water to 100 cm^3.

Determination of metal content by AAS

Principle of AAS Analysis

This is a physical process involving the absorption of free atom of an element of light at wavelength specific to that element. On absorbing the energy, the atoms become excited and the extent of absorption of atoms is dependent on the number of atoms in the ground state in the path of the radiation.

The principle of its operation is based on the fact that metallic compound or metallic salts, when aspirated through the flame produce a vapour that contains atoms of the metal, some of these gaseous metal atom may be excited, but much larger number of gaseous metal will normally remain in the ground state.

The ground state atoms are capable of their own/specific resonance at a particular wavelength. Hence, if light of the resonance wavelength is passed through the flame containing the atom, that part of the light will be absorbed. The extent of absorption is proportional to the number of ground state atoms present in the flame.

For the heavy metal analysis of the digested samples, the amount of lead, cadmium, Chromium, Zinc and Iron in the prepared samples were determined using Air/acetylene flame.

Procedure:

The instrument was switched on and allowed to warm for twenty minutes. The blank solution was first aspirated into the flame of the machine to set the instrument at its reference on zero (0).the light was

passed through the flame atomizer into which the same solution were sprayed. The flame dissolved the sample mist and decomposed the sample solution into atom of the analyte. The analyte atoms then absorb the radiation at characteristic wavelength isolated by monochromator and decrease the amount of light that reach the detector.

When the sample atoms were present in the flame, this was a measure of the concentration of the analyte atoms.The linear relationship between the measured absorbance and the concentration of analyte atoms in the flame which is proportional to the concentration of atoms in the solutions was obtained.

Health Risk assessment (CDI, HRI) by Guo *et al*., 2016.

$$DIM = \frac{C\ \text{metal} \times C\ \text{factor} \times D\ \text{food intake}}{B\ \text{average weight}} \quad (1)$$

, where Cmetal = concentration of metal in samples

Cfactor = conversion Factor

Dfood intake = Average daily intake of milk

and B average weight = average body weight

the values used are conversion factor (0.085) (Jan *et al*., 2010). Average daily intake of milk (0.14 kg per person per day) (Abdi et al., 2015), and average body weight (15.0 kg for children and 70.0 kg for adult), respectively (Omar *et al*., 2013; Tang *et al*., 2015).

The health risk index (HRI) for the population of study area through the consumption of cow's milk was assessed in accordance with equation 2 (Khan et al., 2008; Jan et al., 2010; Guo *et al*., 2016):

$$HRI = \frac{\text{DIM}}{\text{RfD}} \quad (2)$$

, where DIM and RfD are daily intake of metal and oral reference dose of metal, respectively. The RfD for Cd, Cr, Cu, Pb, Fe and Zn were 1.0, 150, 40.0, 3.50, 700 and 330.0 µg/kg/day, respectively. Here, if the HRI < 1, is assumed the exposed populations to be safe (Liang *et al* 2015; Guo *et al*., 2016).

The total HRI (THRI) of heavy metals for the milk was calculated according to equation 3 (Saha and Zaman, 2013):

$$\text{THRI} = \text{HRI (toxicant 1)} + \text{HRI (toxicant 3)} + \ldots + \text{HRI (toxicant } n) \quad (3)$$

Toxicants as used in equation are Pd, Cd, Cr, Mn, Zn, Fe and Cu

Quantitative Determination of Aflatoxins

Sample collection and lyphophilization

Samples were collected in well cleaned and autocleaved rubber containers and were stored in freezer to freeze-dry prior to experiment to prevent increase in moisture content.

Extraction of AflM1

The extraction of AFM1 from milk was carried out by the validated method (Asi et al., 2012) with some modifications, each milk sample was transferred into plastic centrifuge tube, and Samples were defatted through centrifugation for 10 min at 3000 × g. The upper layer of fat was removed carefully by using a spatula. Defatted milk samples were used for the determination of AFM1 using ELISA kits

Analysis of AFM 1

ELISA assay protocol

ELISA kits were purchased from Solarbio inc, China, and the analysis for AFM1 was performed according to the guidelines provided in the kit manual. All reagents and samples were brought to room temperature (20-25^0 C) before use, Defatted samples and standard solutions (100 µL each) were added

in duplicate into the corresponding microwells of the Eliza plates, 50ul of diluted HRP(horse radish peroxidase) conjugate(prepared just before use) was added immediately to each well, the microplate was then covered with new adhesive foil and briefly shakened for seconds after which it was incubated for 30minutes at room temperature protected from light, each well was then aspirated and washed four times, washing was done by filling each well with 300ul of wash buffer(1x) and then left to stand for 30seconds , 100ul of the mixture of substrate solution A and B(prepared just before use in 1: 1) was then dispensed into each well, and then incubated for 10minutes at room temperature, 50ul of stop solution was then pipetted into each well and the optical density of each well was then recorded using a microplate reader set at 450nm and 630 nm

Health Risk assessment (EDI) by Shephard *et al.,* 2007. And HQ by Kuiper-Goodman (1990)

$$\text{EDI (ng/kg b.w. /day)} = \frac{\text{DMI} \times \text{C.}}{\text{BW}} \qquad (4)$$

Where, EDI is Estimated Daily Intake (ng/kg/bw/day); DMI, daily milk intake (ml/d); C, mean AFM1 concentration (ng/l); and BW as mean body weight (kg)

Estimated hazard index (HI)

Estimated HI. The carcinogenic risk was estimated with the HI, which was calculated using the EDI and the TD50, i.e., the dose at which 50% of test animals would have malignant tumors, with a safety factor of 50,000, which has been suggested as 100 ng/kgbw/day (Brera *et al*., 2015; EFSA, 2011; Marin *et al*., 2018, Fakhri *et al*., 2019). IF HI higher than 1 value indicated that consumers are in the considerable liver carcinogenic risk (Kuiper-Goodman, 1990, Fakhri *et al*., 2019).

$$\text{HI} = \frac{\text{EDI (ng/kg b.w. /day)}}{\text{100ng/kg b.w}} \qquad (5)$$

Statistical analysis

Statistical analyses were performed using SPSS statistical package (version 20; SPSS, Chicago, IL). The one-way analysis of variance (ANOVA) was used to verify significant differences in samples from all locations.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Concentrations of heavy metals from Bichi

The mean concentrations of heavy metals from the "Ruga" of Bichi local government were presented below, all samples collected were contaminated with some heavy metals and some heavy metals were totally below the detection limit. The differences observed in concentrations of heavy metals were not statistically significant (at p= 0.05). Zn was only detected in 50% of samples in this location, with a maximum of 0.665mg/l at BR1 and a minimum of 0.285mg/l at BR3, this concentration is below the permissible limit and tends not to pose a risk to the consumers except for cumulative and/ or synergistic effect, Mn was detected in feed samples, with a negligible concentration not even enough to facilitate or take part in reactions. Cu, Pb and Cd were totally not detected in the samples, this might be as a result of the "Ruga" being far away from the city and the area has no industrial activities that might cause the contamination of water and soil and subsequently the animal feed, which can then be found in the milk of the grazing animals in the site. Cr was detected in 100% of the samples with a minimum concentration of 0.003mg/L and a maximum concentration of 0.012mg/L, these concentrations detected are below the permissible limits set for Cr. Fe contributed to the highest contamination in the site, it was also detected in all the samples in the range of 5.400 - 2.163mg/L(Table 4.11).

Table 4.11: Heavy metal concentrations (mg/L) of the milk samples from Bichi LGA

Samples	Concentrations(ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
BR1	0.665±0.262	BDL	BDL	BDL	BDL	0.012±0.004	3.990±1.342
BR2	BDL	BDL	BDL	BDL	BDL	0.003±0.001	4.867±1.359
BR3	0.285±0.403	BDL	BDL	BDL	BDL	0.004±0.001	5.400±1.198
BRfeed	BDL	0.06±0.00	BDL	BDL	BDL	0.008±0.001	2.163±1.430

BR1, BR2, BR3: Bichi "Ruga" point 1, 2, 3 BRfeed: Bichi ruga animal feed

4.12 Concentrations of heavy metals from Rano

Table 4.12 presents the mean concentration of heavy metals in the raw milk samples of Rano local government area, significant difference were observed in RR1 and RR2, RR1 and RR3, RR1 and RRfeed in Cr and Fe concentrations in all samples. Fe had elevated level ranging from 0.830 – 9.100 mg/L, this concentration is above permissible limit and will tend to pose a risk. Cr was also detected in all samples but the concentrations were low (ranging from 0.003 – 0.016mg/L), these concentrations are below permissible limits. Pb was only observed in this local government, it was observed in almost 30% of the samples with the range of 0.610 to 0.690 mg/L. this concentration is above the permissible limit of lead and will tend to pose a risk, but considering the number of positive samples, generalization would be inappropriate. Cd and Cu were all below detectable limits in all samples.

Table 4.12: Heavy metal concentrations (mg/ L) of the milk samples from Rano LGA

Samples	Concentrations(ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
RR1	0.115±0.162	BDL	BDL	0.610±0.00	BDL	0.004±0.014 [a,b,c]	9.100±3.111 [a,b,c]
RR2	BDL	BDL	BDL	0.690±0.00	BDL	0.016±0.002[a]	3.780±0.645 [a]
RR3	BDL	BDL	BDL	BDL	BDL	0.015±0.005[b]	3.640±1.063 [b]
RRfeed	BDL	1.14±0.00	BDL	BDL	BDL	0.003±0.001[c]	0.830±0.142[c]

RR1, RR2, RR3: Rano "ruga" point 1, 2, 3 RRfeed: Rano ruga animal feed

Superscripts letters (a, b, c) indicate significant difference along the column

4.13 Concentrations of heavy metals from Nassarawa

The mean concentrations of heavy metals from Nassarawa local government was recorded in Table 4.13, Significant difference were recorded between NR1, NR2 and NR3, and also between NR3 and NRfeed in Cr concentrations. NRfeed, NR3 and NR2 also had significant difference in Fe concentrations in samples analyzed. Cd, Cu, Mn and Zn were all below detectable limits in all samples. This local government also had elevated level of Fe (1.620 – 4.986mg/L) but lowest as compared to Rano and Bichi.

Table 4.13: Heavy metal concentrations (mg/ L) of the milk samples from Nassarawa LGA

Samples	Concentrations(ppm)						
	Zn	Mn	Cu	Pb	Cd	Cr	Fe
NR1	BDL	BDL	BDL	BDL	BDL	0.002±0.000 [a]	3.87±0.440
NR2	BDL	BDL	BDL	BDL	BDL	0.002±0.000 [b]	3.64±0.501 [a]
NR3	BDL	BDL	BDL	BDL	BDL	0.020±0.004 [a, b, c]	4.986±0.997 [b]
NRfeed	BDL	BDL	BDL	BDL	BDL	0.006±0.000 [c]	1.620±0.740 [a, b]

NR1, NR2, and NR3: Nassarawa "ruga" point 1, 2, 3 NRfeed: Nassarawa ruga animal feed

Superscripts letters (a, b, c) indicate significant difference along the column

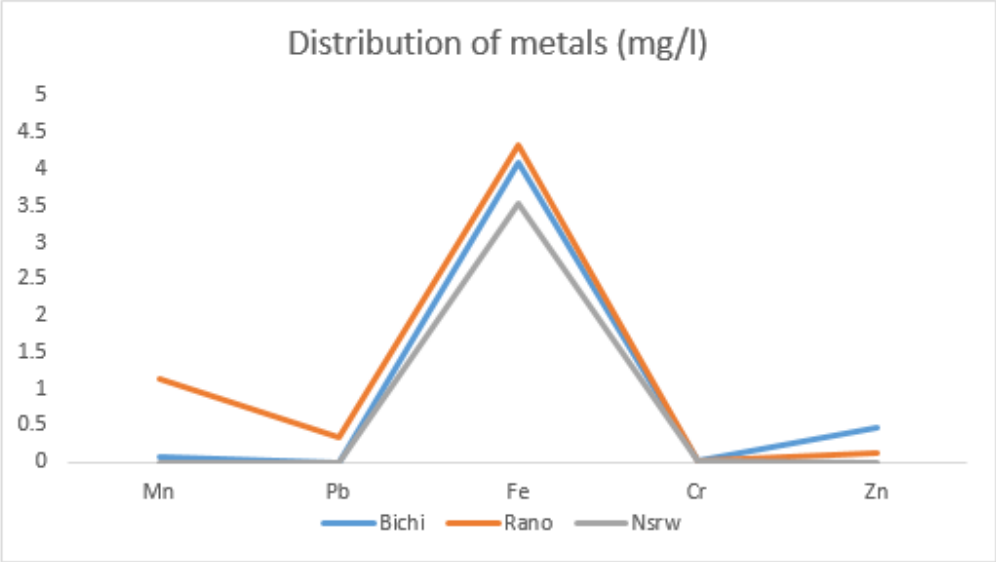

Fig 4.11: distribution of metals in the three local governments

The figure 4.11 shows the distribution of heavy metals in the three local governments, the concentrations of Mn, Pb, Fe, Cr and Zn were compared in the three local government, Difference in the level of the heavy metals between local governments were not statistically significant (at p= 0.05). Fe recorded the highest concentration in all samples, and Cr with lowest concentration even though it was present in all analyzed samples.

4.14 Daily intake of metals and health risk index of metals from Bichi, Rano and Nassarawa

Daily intake of metals was calculated for all local governments, Results recorded from bichi showed a minimum daily intake of Cr at 0.00051 and a maximum of 0.00204µg/l, these were slightly lower than DIM of Cr determined from Rano, which were in the range of (0.00068 – 0.0068) but higher than that of Nassarawa which was in the range of (0.00034 – 0.0034), Fe has the highest daily intake irrespective of location, because all the locations had a very high concentration, even though Rano has the highest range (0.619 – 1.55), and Bichi has the lowest range (0.367 – 0.827),the DIM of Zn is very low and was only for the two locations where it was detected. Lead having the lowest occurrence has an approximate DIM of 0.1 but with the highest health risk index as shown in Table 4.14.

Table 4.14; Daily intake of metals and health risk index of metals from Bichi, Rano and Nsrw (µg/l.b.w/ day)

Locations	metals	DIM	HRI
		(µg/l.b.w/ day)	
BR1		2.04×10^{-3}	1.94×10^{-3}
BR2		5.1×10^{-4}	3.4×10^{-4}
BR3		6.8×10^{-4}	4.53×10^{-4}
RR1	Cr	6.8×10^{-4}	4.5×10^{-4}
RR2		5.1×10^{-3}	1.81×10^{-3}
RR3		6.8×10^{-3}	1.70×10^{-6}
NR1		3.40×10^{-4}	2.20×10^{-4}
NR2		3.40×10^{-4}	2.20×10^{-4}
NR3		3.40×10^{-3}	2.20×10^{-3}
BR1		0.678	0.96
BR2		0.827	1.18
BR3		0.367	1.30
RR1		1.55	2.20
RR2	Fe	0.643	0.92
RR3		0.619	0.88
NR1		0.658	0.94
NR2		0.619	0.88
NR3		0.848	1.20
BR1	Zn	0.113	0.437
BR3		0.048	0.162
RR1		0.0196	0.0653
RR1	Pb	0.104	26.0
RR2		0.117	29.3

BR1, BR2, BR3: Bichi "Ruga" point 1, 2, 3 BRfeed: Bichi ruga animal feed
RR1, RR2, RR3: Rano "ruga" point 1, 2, 3 RRfeed: Rano ruga animal feed
NR1, NR2, NR3: Nassarawa "ruga" point 1, 2, 3 NRfeed: Nassarawa ruga animal feed
DIM: Daily intake of metals
HRI: Hazard risk index

Fig. 4.12 indicates the total hazard index (THI) of the detected metals (Fe, Cr and Zn) in the three local governments, Fe has the maximum THI and Cr has the lowest, and hazard was more in Rano local government as compared to the other two (Bichi and Nassarawa). But all THI values calculated are below 1, which implies that risk is not highly pronounce/ or might not have obvious effect.

Total Hazard risk Index

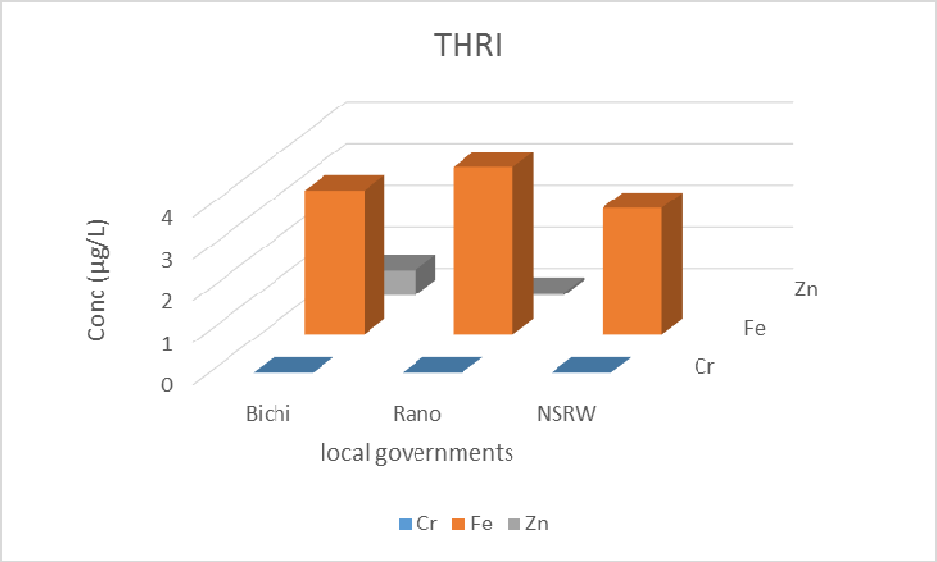

Fig4.12: The total hazard risk index of the most prevalent heavy metals (µg/L)

4.2 Concentration and Exposure Assessment of AflatoxinM1

AflM1 were detected in all local governments understudy, Significant differences were recorded between the mean value of AflM1 in all local governments and the Nafdac reference value, so also significant difference were observed between AflM1 concentrations in Rano and Nassarawa local governments.

Table 4.21: The occurrence of AFM1 in three different locations of Kano.

				Concentration of AFM (μg/kg)				
Location	N	positives (%)	Range (min. - max.)	Mean ± Sd	MPL	t	p	Above MPL(%)
Bichi	27	66.67	0.117 – 0.291	0.211 ± 0.066*	0.5	-12.35	<0.01	none
Rano	27	44.44	0.259 – 0.287	0.275 ± 0.007	0.5	-93.66	<0.01	none
Nassarawa	27	88.89	0.095 – 0.283	0.193 ± 0.057*	0.5	-15.00	<0.01	none

One sample t-test was used to check significant difference between values and the maximum permissible limits of Aflatoxinm1 in milk Samples

Values with * are statistically significant at $p < 0.05$

4.22 Exposure Assessment of AflatoxinM1

This study focuses more on exposure effects to children rather than adult, this is because Infancy is a critical period of human life and a stage of exponential growth rate, and also because of the higher growth rate and lower body weight of infants, detoxification rates are lower than those of adults (Eaton and Groopman, 1994; Parkinson, 2007). Therefore, infants are more susceptible than adults to mycotoxins such as AFM1 (World Health Organization, 2006). And also because reliable data's on daily consumption varies widely.

The estimation of Hazard Index (HI) is based on proposal of Kuiper-Goodman, 1990 which was reported also by Shundo *et al*. (2009). In more details, estimated daily intake (EDI) was computed using mean concentration of AFM1 residues in positive samples and the daily consumption of milk. The consumption of milk was roughly estimated at 250 ml for the age of 1, 400 ml for the ages of 3, 5 and 7 and 800 ml for the age of 12 (according to municipal day care centers, elementary schools and pediatric clinics; Tsakiris *et al*., 2013). The exposure assessment scenarios were developed for the ages of 1, 3, 5, 7 and 12 and body weights of 10, 14, 19, 24 and 37 kg respectively (based on Greek pediatric development normograms).EDI calculated for the three local government shows high concentration in 3year children across the local government and lowest concentration in children of 7years.the concentration appears in ranks as 3yrs> 1yr> 12yrs > 5yrs> 7yrs. With Rano contributing the highest concebtration in all years among the three local governments as shown in Table 4.22

Table 4.22: EDI (µg/kg body weight/ day) of Children (1-12years) from Bichi, Rano and Nassarawa LGA.

	EDI (ng/kg body weight/ day)					
Locations	1	3	5	7	12	(years)
Bichi	5.486	6.179	4.553	3.604	4.680	
Rano	7.150	8.053	5.934	4.697	6.095	
Nassarawa	5.252	5.915	4.359	3.451	4.476	

Fig 4.22 depicts the HI values calculated from AFLM1 consumption in raw milks, the HI values ranks as Rano > Bichi > Nassarawa local government wise, so also with respect to ages, the rank follows the order 3yrs> 1yr> 12yrs > 5yrs> 7yrs. These values although less than one will tend to contribute to the risk of liver cancer. The values showed the highest risk in age 3 irrespective of local governments.

HAZARD INDEX AFLATOXINM1

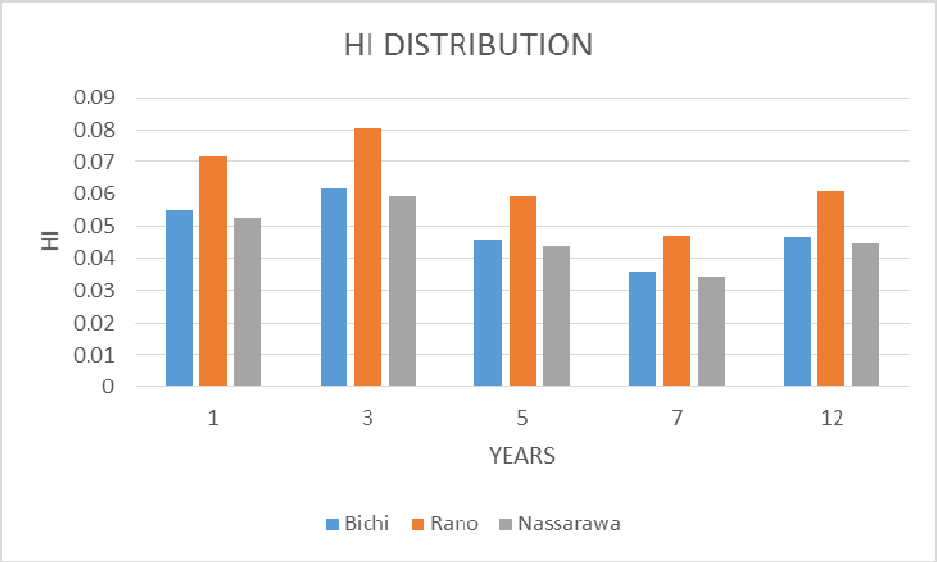

Fig 4.22: Hazard index distribution

4.3 relationship between heavy metals detected and Aflatoxinm1 levels

Below is a Scatter plot of the correlation between Aflatoxinm1 levels and the heavy metals Cr and Fe found in the milk samples. The plots showed little or no correlation between the two variables with r value of 0.310 and 0.104 respectively.

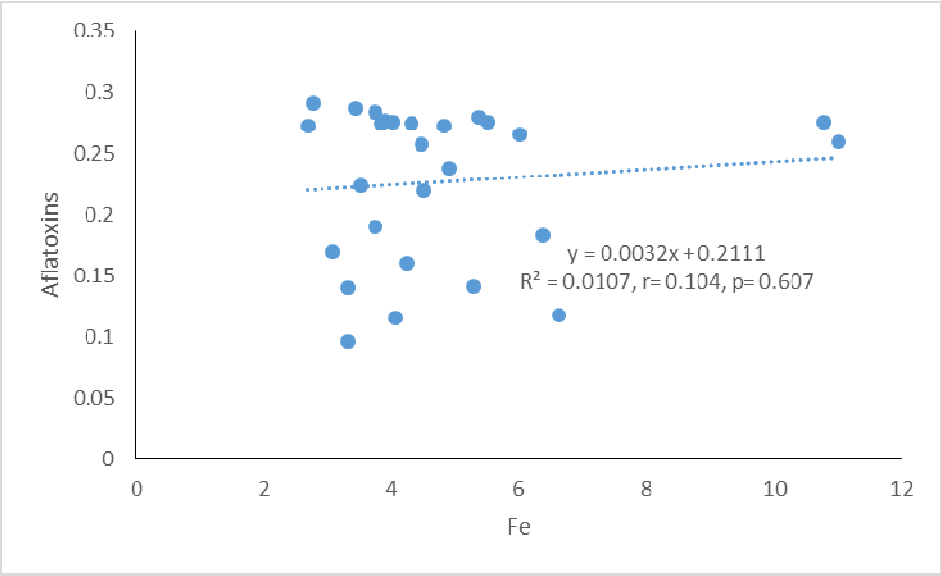

Fig 4.31: showing the correlation of Aflatoxin levels to Fe concentration in milk samples, Y is aflatoxin concentration, x is the Fe concentration, R^2 is the variance, r is the Pearson's correlation constant and p is the significance level of the correlation.

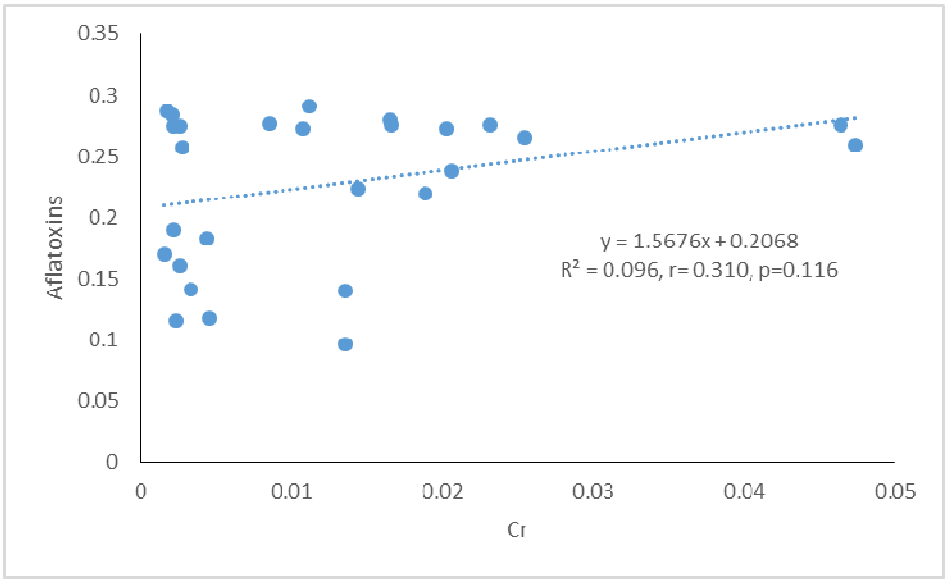

Fig 4.32: showing the correlation of Aflatoxin levels to Cr concentration in milk samples, Y is aflatoxin concentration, x is the Cr concentration, R^2 is the variance, r is the Pearson's correlation constant and p is the significance level of the correlation.

4.4 DISCUSSIONS

4.41 Heavy Metals from Bichi, Rano and Nassarawa local governments

Table 4.11 results show that all the metals determined had concentrations below 1mg/dl but Fe had elevated concentration even up to 5mg/dl, though Fe is required by the body in some concentrations to mediate metabolic reaction, it also tends to be harmful in concentrations greater than permissible limits, and these concentrations detected greatly exceeds or violates the limit set for Fe and might lead to damaged cells in the heart, liver and elsewhere, which can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long term organ damage, and even death (Cheney *et al*., 1995).These contamination might be from different sources which includes the feeds the animals eat, the water they drink and may be other routes.

Results from Table 4.12; is the mean concentration of metals from Rano, the "Ruga" from which these milk samples were taken is far away from the city and had rocks within it, with wide bush and grasses. Zn was detected only at a single location, presenting 25% of contamination, this concentration is far below the concentration needed by the body for metabolic reaction and also very low as compared to the level detected in Bichi local government. Mn was not detected in the milk samples but a low concentration was detected in the feed, which signifies that the concentration wasn't even enough to be carried over in the milk. Cu and Cd were below the detection limits in all the samples. While these two metals were not detected just like in Bichi local government, Pb was an exception, a concentration ranging from 0.610 – 0.690 mg/L was detected, this contamination was principally from other sources not from the feed, it might be from their drinking water or it was inhaled by the animals from the surrounding, because lead could be found in variety of sources like the use of leaded gasoline, lead painting which might

decompose in water, recycling of lead battery and many other ways. The concentration of lead detected was above the permissible limit and might cause toxicity which will cause renal impairment or neurobehavioral decrements (Schwartz *et al.,* 2001). Cr was detected in all samples with a minimum concentration of 0.003mg/L detected in the feeds and a maximum concentration of 0.016mg/L detected in location two, these concentrations are minute and negligible as detected in Bichi local government and would not pose a risk. Fe has the highest concentration detected just as it was detected in Bichi, ranging from 0.830 – 9.10 mg/L , these concentrations have exceedingly violate the permissible limit and might pose a health risk to consumers.

Table 4.13 is the results of heavy metal contaminations from Nassarawa local government. This local government has lower contamination as compared to the other two local governments, only two of the seven heavy metals were detected in all the samples. Zn, Mn, Cu, Pb and Cd were below detectable limits. Cr had a concentration ranging from 0.002 – 0.02 mg/L, these concentrations were below the concentrations detected from the other two local governments, and these concentrations are also below permissible limit and will have no effect on health. Fe was determined in this local government with values ranging from 1.620 – 4.986mg/L, these concentrations are also below those determined from the other two local governments.

Fig 4.11 compares the average concentrations of metals in the three locations, with Fe having the highest contributing concentration, within the range of 3.529 – 4.33 mg/L, this concentrations between the three locations were not statistically significant (at P= 0.05), this concentration is slightly lower than the average (5.987412mg/L) observed by Ogabiela *et al*. in 2011 in raw milk samples from Challawa, Kano State, and a little bit higher than the average(3.661 µg/kg) detected by Abdallah (2011),and was in agreement with the average detected by Garba *et al.,*

(2018), at “Kasuwan Shanu” in Maiduguri but very much less than what he detected from University of Maiduguri farm. Report has it that, Fe can present a problem in dairy technology because of its catalytic effect on oxidation of lipids with development of unpleasant smell, bounding preferably to proteins and membrane lipoproteins of milk fatty globule (Rao and Murthy, 2017). Mn was within the range of 0.06 – 1.14 mg/l, this concentration was very much low as compared to the work done by Muhib *et al*., (2016), but very much close to the range(0.170 to 1.965) detected by Garba *et al.,* (2018) at Auno village of Maiduguri, and also in agreement to the work by Ogabiela et al.(2011). The level observed in this study is higher than what was reported in cow milk of Borena Zone, Ethiopia (Malhat *et al*., 2012). However, the element could not be detected in cow's milk samples from Dodoma Urban District, Tanzania (Wedler, 1994), just like how it wasn’t observed or detected in Nassarawa local government in the present study. Manganese is a naturally occurring element found in rock, soil, water, and food. In humans and animals, manganese is an essential nutrient that plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and formation of glycosaminoglycans (CAC, 2003). Although the element is an essential nutrient required by both plants and animals, exposure to high levels via inhalation or ingestion may cause some adverse health effects. It has been suggested that these adverse health effects, especially neurologic effects, are occurring on a ―continuum of dysfunction that is dose-related (Bandani *et al.,* 2016). Pb was only detected in Rano with 50% abundance, Cr was detected in all locations but having a very low concentration even less than the average concentration detected from Challawa and also the average detected at Zaria by Ogabiella *et al*. (2016) even though the present study has concentrations within the permissible limits. The concentration of Cr detected was very low, ranging from 0.002 – 0.016mg/l, but

difference in Cr concentrations were statistically significant (P= 0.017) between Rano and Nassarawa and also very significant (p= 0.004) between Rano and Bichi, these concentration will cause no harm to the consumers.

Figure 4.12 compares the total Hazard risk index of Zn, Fe and Cr, and the values obtain were in the sequence of Fe > Zn > Cr, all the values obtain were below one(1), except for Fe, therefore Fe toxicity is highly expected from these regions, but threat due to the other heavy metals in the milk of these areas is very low, but not withstanding, toxicity might still occur, due to the fact that milk is not the only source of heavy metal exposure, other sources include water, foods such as cereals and others and some other times, contamination might occur due to inhalation from the air .

4.42 Aflatoxins in Milk

Table 4.21 presents the mean concentration of Aflatoxins detected from Bichi, Rano and Nassarawa local governments areas as 0.211, 0.275 and 0.202 μg/kg respectively, these concentrations are in the order of Rano > Bichi > Nassarawa.they appear to be greater than the maximum permissible limit set by European union as 0.05 μg/kg, but were below the limit set by National agency of Food and drugs administration agency (NAFDAC) as 0.5 μg/kg. Therefore with respect to the Nigerian standard, all samples have tolerable concentrations of AFlM1. The percent abundance varies with different local government but averaged at 66.67%. the contamination level and the concentration are almost close but a little bit lower than work done by Oyeyipo et al., (2017) in milks from south western Nigeria, he found the concentration range between 0.05 – 0.48 μg/kg, A similar study carried out by Makun *et al*., (2016) found AFLM1 contamination level of 80% in cow milks from Minna, Niger State. Studies also carried out in member states like Lagos, Ogun, Osun, Oyo and Ekiti have AFLM1 concentrations in milk

higher than the present study as pointed out by Oyeyipo et al., (2017). Shahzad and Muhammad, (2013) from Punjab, Pakistan, reported almost same mean concentration (212.2 ±11.9 ng/l) as this study, so also the results are comparable to the contamination of AFM1 in milk from other parts of Pakistan (Hussain and Anwar, 2008). It's well documented that fungi growth varies with ecological condition and climate, just like how Makun *et al*., 2010 maintains that hot and humid conditions of South-West are more favourable for fungal growth and mycotoxins production than the cold condition in the South-South of Nigeria, in his comparative assumptions, but this study proves otherwise, because the northern part of Nigeria is hotter with higher temperatures as compared to the south. Therefore the findings of this study might also be attributed to variation in feed type, aseptic conditions during sampling and more inclusively climatic condition during the study period.But since contamination of milk and other dairy products by aflatoxin M1 is still a major concern for the producer, consumer and regulatory bodies (Rahmani *et al.,* 2018), the producer and personnels analyzing its presence should take note of that weak or even lack of GMP (Good Manufacturing Practices), can cause an increase in the probability of contamination of feed by mycotoxin producer fungi (e.g., *Aspergillus flavus*, *Aspergillus parasiticus,* and *Aspergillus nomius*) which consequently led to contamination by AFB1 and furthermore by AFM1. However, the topographic factors (topographic wetness index and relief position), and weather conditions (humidity, temperature, and rainfall) can affect the occurrence of different mycotoxins particularly AFM1.

Table 4.22 is an estimate of the daily intake of toddlers in the ages stated provided the assumed quantity of milk is ingested per day, The EDI for Rano is the highest in the range of 4.697 – 8.053 ng ingestion per day, with children within 1-3 years having the highest daily intake, The EDI values for infants decreased with increasing body weight. Bichi's EDI also ranges between

3.604 - 6.179,and Nassarawa having close range to Bichi, between 3.451 – 5.915, in all these children, at the age of 1-3 years the highest contamination is recorded.A study by Fakhri *et al*.,(2019) calculated EDI for male and female infants as 0.02 to 5.57 and 0.02 to 3.68 ng/kgbw/day similar to the present study,so also EDI values for AFM1 in human breast milk from Spain, Argentina, Thailand, Brazil, and Pakistan were 0.018 to 5.45 ng/kgbw/day, also similar to our findings (Alonso *et al*., 2010; Cano-Sancho *et al*., 2010; Ishikawa *et al*., 2016; Ismail *et al*., 2016; Ruangwises *et al*., 2011). Due to the fact that aflatoxins are carcinogenic international expert commitees (JECFA) did not specify a tolerable daily intake (TDI) for these substances and concluded that daily exposure as low as < 1ng/kg b.w contributed to the risk of liver cancer, it was therefore recommended that levels should be reduced to as low as reasonably achievable (Joice sifuentes *et al*., 2015).however the present study have EDI even upto 8ng/kg b.w. which translates to high contamination to the consumers and in essence predisposition to liver cancer.

From fig. 4.22, The rank order of age of inhabitants based on EDI and HI values is 3yrs > 1yr > 12yrs > 5yrs > years, these ranks irrespective of local governments are the same, indicating a higher carcinogenic risk for younger infants from AFM1 in cow milk. The highest carcinogenic risk in children due to consumption of raw milk was observed in Rano. However, the consumers of cow milk in Nassarawa are not as exposed as Rano to a risk for carcinogenesis of AFM1, even though risk tends not to be so high, reduction plans are required to be implemented for reducing the concentration of AFM1 in cow milk.

The study of the relationship between aflatoxinM1 quantity to heavy metals was found to be low (16% variance) and the Pearson correlation was insignificant, these indicate that aflatoxinM1 quantities do not depend on heavy metals concentration and vice versa.

CHAPTER 5

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

Environmental pollution is a great challenge of the 21st century brought about by increase in industrialization, several environmental pollutants exist that contaminates the soil, thereby taken up by plants through their roots, and then transferred to plant consumers (animals) or run off into water bodies which are also consumed by animals. These pollutants affects human health greatly, prompting developed countries and developing countries to monitor their levels in different consumed commodities (e.g. water, milk. Etc.). The current study is a qualitative and quantitative study that assessed the health risk of aflatoxinM1 and heavy metals in cow milks from Rano, Bichi and Nassarawa local government areas of Kano state, the assessment was done on 81 samples of cow milk gotten from the three local governments. Preliminarily, the quantity of heavy metals and aflatoxins were determined, their carcinogenic index and non-carcinogenic index were then modelled to obtain their probabilistic or predictive risk. Determination of heavy metals was carried out using wet digestion and then by the use of atomic absorption spectrophotometer, while determination of AflatoxinM1 was carried out using Elisa kits. The mean concentrations of heavy metals from the "Ruga" of Bichi local government were determined, some samples were contaminated with some heavy metals (Zn, Cr, and Fe) and some heavy metals (Mn, Cu, Pb, Cd) were totally below the detection limit. The Ruga in Rano local government had Cr, Fe, Pb and Zn present in most of the milk samples whereas Mn, Cd and Cu were totally absent from all the samples. The samples from Nassarawa local government had only Fe and Cr in all samples and all the other metals were absent. Risk calculated showed that all consumers from the three local government might suffer from iron toxicity due to its high

hazard index. AflatoxinM1 concentration in the three local governments were in the order of Rano > Bichi > Nassarawa, with 44.44, 66.67 and 88.89% sample abundance respectively. The concentrations were below the maximum permissible limits set by NAFDAC. On calculating the total hazard index, Rano had the highest hazard index in all local governments and risk was higher in children of 3years. There the current study identify risk in Fe toxicity and also established safety as regards to the other metals and AflM1 in the aforementioned local governments.

5.2 Conclusion

Heavy metals contamination of milk from the study areas were found to be low, except for Fe. Therefore consumers might suffer Fe toxicity. Aflatoxin M1 was also a source of risk, due to its elevated hazard index, therefore consumers are to take caution, and concern agencies should take proper action in reducing or eliminating these risks.the study of the correlation of Aflatoxin levels to heavy metal level also revealed that there is little or no correlation between the two variables.

5.3 Recommendations

- Research should also be carried out in other local governments in Kano, to check for heavy metals and Aflatoxins contamination
- There is also a need to check for heavy metals concentration and Aflatoxins from the market sources, because storage and transport might also increase their levels
- Subsequent studies should also be carried out in these areas in order to model the ratios of people at risk.

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