

UNIVERSITY OF GHANA MEDICAL SCHOOL

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DEPARTMENT OF CHEMICAL PATHOLOGY



**ASSESSMENT OF CO-EXPOSURE OF AFLATOXIN, LEAD AND
CADMIUM IN CHILDREN AT EJURA SEKYEDUMASE DISTRICT,
ASHANTI REGION OF GHANA.**

BY

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA
LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE
AWARD OF A MASTER OF PHILOSOPHY CHEMICAL PATHOLOGY
DEGREE**

DECLARATION

I, MIRIAM ODAMTTEN hereby declare that with the exception of the references cited to other people 's work which has been duly acknowledged, this work is the result of my own research work done under supervision and has neither in part nor whole been presented elsewhere for the award of another degree.

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DEDICATION

I dedicate this thesis to God almighty, But for His help and abundant grace this journey wouldn't have been possible. I also dedicate this to Isarel and Kharis, my desire to inspire them to reach greater heights gave me strength to keep going. Finally, to Mr. Nii Sowah Kakai whose encouragement and support have been my greatest source of strength, for that, I am deeply grateful.



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ABSTRACT

Background

Major public health risks in Ghana are caused by aflatoxins and heavy metals, and children are more vulnerable because of their developing bodies and greater rates of consuming contaminated food and water. Aflatoxins are produced by *Aspergillus* fungus, which are common in staple foods like groundnuts and maize. This toxin when it exceeds safety thresholds, can cause immunological suppression, liver damage, and stunted growth. Soil, water, and food are most times contaminated by heavy metals like lead and cadmium. And this is as a result of mining, industrial processes, and inappropriate e-waste disposal. These toxic metals can cause severe health problems including neurodevelopmental disorders, kidney damage and cancer.

Aim

To assess the effects of aflatoxins and heavy metals on the kidneys and liver biomarkers of children from Ejura Sekyedumase District in the Ashanti Region of Ghana.

Method

The study design was a cross-sectional one. One hundred and sixty-two (162) participants between the ages of 3 and 9 were recruited from the Ejura-Sekyedumase district for this study. Blood, (5mLs) was taken from the antecubital fossa vein in the upper arm of all participants. The blood was divided into two, 2 mLs into EDTA and 3 mL into serum gel tube labeled with participant's name and identification number. Whole blood specimen (EDTA tube) was used to analyze reduced glutathione and full blood count using KX Sysmex 21 (Sysmex Corporation, Japan). Serum samples were used to analyze heavy metals using Graphite Furnace Atomic Absorption Spectrometer (GFAAS; PinAAcle 900H), liver and kidney bioarkers using KX Sysmex chemistry automated analyzer (Sysmex Corporation, Japan). Spot urine was collected

from participants and used to run aflatoxin levels using Shimadzu HPLC system with fluorescence detection (Shimadzu Corporation, Japan). All analyses of data obtained were performed in R (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment.

Results

In this study, there were elevated concentrations of aflatoxins in 26 males and 24 females' participants. No sex-specific differences in Cd, Pb and AFM1 levels between males and females. Hematological parameters demonstrated significant positive correlations, particularly between AFM1 and neutrophil count and between Pb and albumin levels. Although the antioxidant biomarker GSH showed negative correlations with all three exposures, none of the correlations were statistically significant. In the overall sample, none of the exposures (Cd, Pb, or AFM1) demonstrated significant associations with glutathione (GSH), haemoglobin, or most haematological parameters. The combined exposure to cadmium, lead, and aflatoxin M1 showed generally non-significant associations with most haematological and biochemical biomarkers protein, sodium, while AFM1 strongly influenced ALT and monocytes, and lead contributed most to chloride and AST.

Conclusion

This study revealed a high concentration of aflatoxin M1 (AFM1) in the urine of participants. AFM1 showed a negative correlation with monocytes and positively correlated with urea which can result in immunosuppression and kidney disease respectively. Lead showed positive correlation with chlorine whereas cadmium had a positive association with potassium which can cause metabolic dysfunction in the future. The findings from this study emphasize the need to put in measures to prevent aflatoxin and heavy metals from contaminating our foods.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Fungal species such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* produce polyketides, which are the source of highly toxic secondary metabolites known as aflatoxins (Shabeer et al., 2022). These fungi typically affect crops like wheat, maize, millet, sorghum, peanuts, and tree nuts (Mahato et al., 2019). Consumption of crops contaminated by this toxin leads to adverse health effects, including hepatotoxicity, teratogenicity, and immunotoxicity. This poses significant risks to both human and animal health (Kumar et al., 2017). Aflatoxins are classified into several types, the major ones are aflatoxin B1, B2, G1, G2 (Ramadan & Al-Ameri, 2022), with aflatoxin B1 being the most toxic and prevalence (Rushing & Selim, 2019). Aflatoxin B and G are total aflatoxin found in food. In the blood, aflatoxin B1 is bound to albumin to form an adduct (Lalah et al., 2020). Aflatoxin M1 and M2 are also types of aflatoxin, but are metabolites of B1 and B2 found in urine and milk (Bianco et al., 2012).

Aflatoxin contamination is a significant concern in food safety, and despite extensive research on improving food processing methods, normal cooking procedures cannot eliminate aflatoxins totally from contaminated food (Kumar et al., 2021). Innovations such as Good Manufacturing Practices (GMP) and Hazard Analysis of Critical Control Points (HACCP) have been implemented to ensure food safety (Gehring & Kirkpatrick, 2020). Additionally, physical,

chemical, and biological methods have been developed to either partially or completely remove these toxins from food products to reduce their health risks (Gehring & Kirkpatrick, 2020).

Heavy metals (HMs) are naturally occurring chemical elements with high atomic numbers and densities exceeding 5 g/cm³, but their distribution varies geographically (Bommarez et al., 2021). When present in elevated concentrations, heavy metals readily enter food chains and contaminate crops, water sources, and other food products, posing serious health risks to consumers (Rai et al., 2019). Their harmful effects on human health include neurotoxicity, organ damage, and interference with essential minerals (Shetty et al., 2023). Heavy metals such as lead and cadmium are of particular concern due to their toxicity (Balali-Mood et al., 2021). Children are particularly vulnerable, with exposure pathways such as breastfeeding and behaviors like hand-to-mouth activities contributing to their susceptibility to heavy metal poisoning (Balali-Mood et al., 2021).

Both aflatoxins and heavy metals enter the food chain through plant uptake or contamination of farm produce. The co-occurrence of these toxins in food products is inevitable in regions with environmental contamination, and their combined presence increases the risk of toxic effects in humans and animals (Mahato et al., 2019). This co-exposure is especially relevant in regions like the Ejura Sekyedumase District, where agricultural practices, environmental conditions, and limited regulations increase the risk of both aflatoxin and heavy metal contamination. Their combined toxic effects are likely to exacerbate oxidative stress, leading to more severe damage to vital organs, including the liver and kidneys, particularly in vulnerable populations like children.

In the Ejura-Sekyedumase district, studies have documented substantial aflatoxin contamination in crops and locally produced complementary foods, with reported levels in infant food blends ranging from 7.9 to 500 ppb and, in some maize products, reaching

extremely high concentrations that far exceed the permissible levels set by global regulatory agencies (Opoku et al., 2018). Data from risk assessments of cereals and legumes in markets in Ghana, example Tamale Aboabo market however, indicate that lead and cadmium levels are often below detection limits or within regulatory safety margins when assessed in food matrices, suggesting relatively low ingestion risks from these sources (Adam et al., 2022). However, not all environmental media show such low levels; areas affected by industrial activities or e-waste recycling have reported elevated concentrations of lead and cadmium in soil, water, and dust (Assunção, 2017). Although these findings are robust, they cannot be directly extrapolated to Ejura-Sekyedumase without localized data. The risk from heavy metal ingestion through local foods may appear low, based on available evidence but the possibility of additional exposure through inhalation or dermal contact with contaminated dust and water should not be overlooked (Domfeh, 2021). Hence the need to evaluate the levels of heavy metals and aflatoxins and access the effect of these toxins on the liver and kidneys of these children.

A powerful antioxidant in the body called glutathione (GSH) detoxify the body from toxins, and protects cells from oxidative stress and damage (Averill-Bates, 2023). It does this by binding to toxins, heavy metals and xenobiotics to make them water-soluble for excretion (Adwas et al., 2019). But when the body is exposed to high levels of toxins such as aflatoxins and heavy metals, GSH becomes heavily involved in detoxification (Adwas et al., 2019). This excess exposure can overwhelm or deplete glutathione, leading to impaired detoxification of the liver, increased oxidative damage to cells and organ dysfunction especially among children (Raj Rai et al., 2021).

1.2 PROBLEM STATEMENT

Food is a primary source of exposure to many environmental hazards, with placental transfer, breastfeeding, and complementary feeding acting as key routes for contaminants during gestation and early postnatal life (Rather et al., 2017). In Ghana, cereals such as maize and groundnuts are staple foods, making it difficult to reduce their consumption (Achaglinkame et al., 2017). The Ejura Sekyedumase District is a major agricultural hub, particularly known for the cultivation of maize, beans, and groundnuts. These crops are often used to prepare infant foods following weaning (Ansah et al., 2014)). Unfortunately, these foods are frequently contaminated with both heavy metals and aflatoxins (Kortei et al., 2021).

This raises concerns that infants and young children consuming these staple foods may be exposed to harmful levels of aflatoxins and heavy metals, leading to adverse health effects (Ismail et al., 2021). The co-occurrence of these pollutants in the food chain poses significant health risks, especially for children. The ingested toxins form adducts with albumin, which affects nutrient transport and other compounds needed by the body, kidneys of these children will get damaged, they register low haemoglobin levels due to this kidney damage resulting in stunted growth. Liver damage and cancer formation will be the end results of these toxins exposure (Pratap et al., 2022). When these happen, there is going to be frequent visit to the hospital by these children. The finances and productivity of their parents are going to be affected. Which will gradually affect the country's economy as a whole.

Therefore, it is important to assess the health risk and threat of these two contaminants in children who consume especially cereal based foods in areas where agriculture remains the primary source of occupation. This is very important contribution to public health surveillance of food safety and security. The toxicity of aflatoxin and heavy metals, as well as their joint impact on children's health, have the potential to affect the primary health care outcomes in

Ghana. The findings of the study will help to shape future prevention strategies of aflatoxin and heavy metals exposure at a younger age before it poses a health risk to child development.

1.3 JUSTIFICATION

This study seeks to address the scarcity of data by providing evidence-based research to assess the level of combined exposure of aflatoxin and heavy metals in children from Ejura Sekyedumase. Individual exposure to these toxins has been documented in some parts of Ghana (Kortei et al., 2022), but there is no available data on their co-exposure and combined impact in children at this district. Understanding these risks is crucial for developing strategies to mitigate exposure and prevent further developmental impairment in this vulnerable population. Aflatoxins and heavy metals have been detected in a wide range of food items, including cereals, nuts, dried fruits, spices, infant formula, milk, and dairy products, often at levels exceeding the maximum permitted limits (Mahato et al., 2019). The presence of aflatoxin-metabolizing products in the blood and urine of newborns and young children highlights continuous exposure through contaminated breast milk and complementary food. With increasing scientific interest in aflatoxins' impact on children's health, recent data have been published emphasizing the urgency of addressing this issue (Benkerroum & Ismail, 2022)

Assessing the health risks posed by aflatoxins and heavy metals to children in the Ejura Sekyedumase District, where agriculture is the primary economic activity, is crucial. This region produces large amounts of maize and groundnuts, which are staple foods often used in infant diets. Evaluating the co-exposure to aflatoxins and heavy metals in this context is a critical step in public health monitoring related to food safety. Understanding the extent of these contaminants' effects on children's health may significantly influence primary healthcare outcomes in Ghana.

The findings of this study will provide policymakers, including the Food and Drug Administration (FDA) and public health authorities, with valuable data on the level of exposure to these toxic substances. This will enable the formulation of strategies to minimize children's exposure to aflatoxins and heavy metals. Furthermore, the study will equip healthcare professionals with evidence on the prevalence of heavy metal poisoning and aflatoxin exposure in the region, aiding them in the diagnosis and management of these conditions.

1.4 GENERAL AIM

To assess the effects of aflatoxins and heavy metals on the kidneys and liver biomarkers of children from Ejura Sekyedumase District in the Ashanti Region of Ghana.

1.4.1 SPECIFIC OBJECTIVES

1. To evaluate the levels of heavy metals and aflatoxins in the blood and urine of participants.
2. To assess the effect of aflatoxin and heavy metals on liver and kidney function biomarkers in serum of participants.
3. To determine the association between aflatoxin, heavy metals exposure and glutathione levels in study participants.

1.5 HYPOTHESIS

The co-exposure of aflatoxin and heavy metals may not affect organ damage. (Kidneys and Liver).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Aflatoxins

Aflatoxins (AFs) are regarded as the most significant mycotoxins in human foods and animal feedstuffs because of their high toxicity, adverse effect on human and animal health, widespread distribution, and substantial economic impact (Coppock et al., 2018). **In the late 1950s and early 1960s, AFs were identified as the cause of the mysterious turkey “X” disease in Great Britain** (Allcroft & Lewis, 1963). Since these early discoveries, intensive research has reported the risk of exposure to animal and human health globally (Wangia et al., 2019). Aflatoxin linked adverse health effects occur in livestock, poultry, aquatic animals **and** humans. Aflatoxins (AF) are potent hepatotoxins, mutagens, carcinogens and immunosuppressant. They have adverse epigenetic effects, and are linked with reproductive dysfunctions, and stunted growth (Rasheed et al., 2021). AF contamination causes economic losses of corn, cottonseed, peanuts, sorghum, wheat, rice, nuts, spices, other commodities and processed foods (Awuchi et al., 2021). Because of their prevalence and toxicity, AFs are important worldwide in public health, with concerns centered on both primary poisoning from AFs in commodities, food, and feedstuffs, and relay poisoning from AFs in milk and other animal products (Coppock et al., 2018). Aflatoxicosis in the human population, especially in areas stricken by poverty, drought, and other adverse growing conditions for plants, is an important public health problem (Duchenne-Moutien & Neetoo, 2021).

2.1.1 Aflatoxigenic Fungi and Aflatoxin Production

Fungi which have the ability to produce aflatoxins are aflatoxigenic fungi (Varga et al., 2009). The most commonly recognized aflatoxigenic fungi are *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*; other species of *Aspergillus* have also been reported to produce AFs (Liao et al., 2020). Aflatoxigenic *Aspergillus* species thrive on plant debris in the soil and can also proliferate in feedlot manure (Setsetse, 2019). Insects and wind currents spread the spores of aflatoxigenic fungi to plants and these fungi commonly colonize floral organs and areas of insect damage (DeLeon, 2023). Infection can take place via silks in corn and subterranean flowers of peanuts can also be infected without insect injury (Mbata et al., 2024). In addition to plant residues, spores, mycelia, or sclerotia of aflatoxigenic fungi are commonly found in soil, commodity storage areas, processing facilities, and in the distribution systems for manufactured products (Awuchi et al., 2021). Strains of *A. flavus* can vary in AF toxicity production from nontoxic to highly toxigenic and are more likely to produce more aflatoxin B₁ (AFB₁) than aflatoxin G₁ (AFG₁) (Rao et al., 2020). *A. flavus* and other species can also produce cyclopiazonic acid (Chalivendra et al., 2017). Strains of *A. parasiticus* generally have less variation in toxigenicity and generally produce AFB₁ and varying amounts of AFB₂, AFG₁, and AFG₂ (Møller et al., 2021). The AF profile produced by *A. nomius* is similar to *A. parasiticus*, both do not produce cyclopiazonic acid. Aflatoxigenic strains of *Aspergillus* can also produce sterigmatocystin (Zingales et al., 2020).

The general growth conditions for aflatoxigenic species are described as requiring moisture contents in equilibrium with 80% - 85% or more and temperatures of 13°C - 20°C with optimum growth at 25°C - 35°C (Norlia et al., 2020). Zinc is also important for growth. The critical moisture content for growth of *A. flavus* in starchy cereal grains is 17%-18%, soybeans 17%-17.5% and for peanuts is 9%-10.5% (Rosentrater, 2022). The upper limit of moisture for growth of *A. flavus* and production of AFs is about 30%. *A. flavus* will grow

slowly below 13°C, and most rapidly at 37°C, but does not produce AFs at temperatures below 13°C or above 42°C (Hassane et al., 2017). In addition to temperature and equilibrium relative humidity, other factors that influence AF production are carbon, nitrogen, plant metabolites, and sugars in substrates (Bogati & Walczak, 2022). High AF production generally occurs when the fungus grows in the seed embryo. Aflatoxigenic *A. flavus* isolates vary from 40% to 70% of the total *A. flavus* population. The proportion of aflatoxigenic isolates may differ between ingredient and finished feeds, and aflatoxin production are primary high in finished feeds that are spoilt (Coppock et al., 2018). Seeds that are damaged during harvest or handling within commodity systems are more prone to infestation by *Aspergillus* species and other fungi. The growth of aflatoxigenic fungi and the production of aflatoxins can quickly develop in damage seeds with high moisture content (Hussein, 2023). High levels of AF production can occur in maize before a 0.5% loss in dry matter occurs. High levels of AFs in corn are not linked to the production of kojic acid (black light fluorescence) (Ichinomiya et al., 2022).

2.1.2 Sources and Mode of Contamination of Aflatoxin

Fungi that produce aflatoxin thrive in warm and humid environments, conditions that are prevalent in many parts of Ghana (Shabeer et al., 2022). The contamination of food with aflatoxins can occur at several stages, including pre-harvest, harvest, post-harvest, processing, and storage. High temperatures and humidity during the pre-harvest phase facilitate the growth of *Aspergillus* species on crops. These fungi are naturally found in soil and plant debris, and they have the potential to contaminate crops in the field (Kumar et al., 2021).

Poor harvesting practices during and after harvest can mechanically harm crops, increasing their vulnerability to fungal invasion. Postponing harvesting, particularly in damp weather, can also raise the possibility of mold development (Michailides et al., 2009).

Inadequate post-harvest drying of crops is a major concern in the processing and storage stages because moisture content above a specific threshold encourages fungus growth. Conventional storage techniques, like the use of sacks or inadequately ventilated rooms, can raise moisture content and encourage the formation of mold (Ariong et al., 2023). Furthermore, infestation by rodents and insects can harm stored crops, increasing their susceptibility to fungal contamination. Food preparation and consumption habits are also important; in rural regions, traditional food preparation techniques might not be sufficient to lower aflatoxin levels.

Techniques like fermentation and sun-drying might not be able to get rid of the toxin (Sipos et al., 2021). Children in Ghana frequently eat staple foods including sorghum, groundnuts, and maize, which can get contaminated with aflatoxin and cause chronic exposure if consumed over time.

2.1.3 Aflatoxin Metabolism

The toxicity of aflatoxins is associated with their metabolic processing in exposed organisms (Groopman & Kensler, 2005). The complex interaction between bioactivation and detoxification pathways ultimately dictates the toxicological consequences of aflatoxin exposure. Understanding of these metabolic processes is crucial for elucidating carcinogenic mechanisms and for formulating effective intervention and detoxification strategies (Bbosa et al., 2013).

I Metabolism: Bioactivation Through Cytochrome P450 Enzymes

The phase I metabolism of aflatoxins, especially aflatoxin B1 (AFB1), begins in the liver with cytochromeP450 (CYP450) enzymes. These enzymes catalyse oxidative biotransformation, which changes the parent compound into reactive metabolites (Dohnal et al., 2014b). CYP1A2, CYP3A4, and CYP3A7 are three important enzymes that work together to epoxidize AFB1 and make the highly reactive AFB1-8,9-epoxide (AFBO) (Dohnal et al., 2014b). This epoxide is the most important bioactivated intermediate that causes most of AFB1's toxic and cancer-causing effects by covalently binding to DNA and proteins in cells. This leads to the formation of DNA adducts like AFB1-N7-guanine (Bbosa et al., 2013). Research has shown that the production of AFBO is dose-dependent and can change based on the concentration of the substrate. Different CYP450 isoforms have different kinetic properties, ranging from Michaelis-Menten to Hill kinetics (Deng et al., 2018). Besides AFBO, CYP450 enzymes also create metabolites like AFM1, AFQ1, and AFP1 through other oxidative pathways. Each of these can add to the overall toxicity of aflatoxins (Deng et al., 2018). The interaction between these oxidising enzymes, their expression in specific tissues, and the resulting metabolite profiles are essential factors influencing aflatoxin-induced genotoxicity and carcinogenesis (McLean & Dutton, 1995).

The Creation of DNA Adducts and Molecular Foundation of Carcinogenicity.

Once formed, the reactive AFB1-8,9-epoxide tends to bind covalently to nucleophilic sites in DNA, especially at the N7 position of guanine. This creates AFB1–DNA adducts that can cause mutations and eventually lead to cancer (Deng et al., 2018). This mechanism is especially clear in the TP53 gene at codon 249, where G:C to T:A transversions happen a lot. This is a common mutation in cases of aflatoxin-associated HCC (Rushing & Selim, 2019). The reactive epoxide can bind to proteins, like serum albumin, in addition to DNA. This makes stable protein adducts that can be used as biomarkers of exposure and show

how aflatoxin metabolites are spread throughout the body (Popescu et al., 2022) . The stability of these adducts, along with their persistence in tissues, constitutes the molecular foundation for the prolonged carcinogenic risk associated with chronic aflatoxin exposure (Bbosa et al., 2013; O'Brien et al., 1983)

Phase II Metabolism: Detoxification Through Conjugation Reactions.

Phase II conjugating enzymes are mainly responsible for detoxifying AFB1 and its reactive intermediates. Glutathione S-transferases (GSTs) are the most important of these enzymes because they speed up the process of conjugating the reactive epoxide to reduced glutathione (GSH) (Deng et al., 2018). This conjugation produces AFB1–GSH adducts that exhibit increased water solubility and facilitate excretion, thereby reducing the likelihood of DNA binding and subsequent mutagenesis (Deng et al., 2018).). Besides GST-mediated reactions, other enzymes like microsomal epoxide hydrolase (mEH) help detoxify by breaking down AFBO into AFB1-dihydrodiol, which is a less reactive metabolite (Deng et al., 2018).). Aldehyde reductases transform dihydrodiol intermediates into dialcohols that do not bind to proteins, which lowers the cytotoxic effects of the reactive metabolites (Deng et al., 2018). The overall effectiveness of these detoxification pathways, which can be changed by chemoprotective agents, is very important for finding the right balance between bioactivation (which causes toxicity) and detoxification (which stops cellular damage)(Dohnal et al., 2014a).

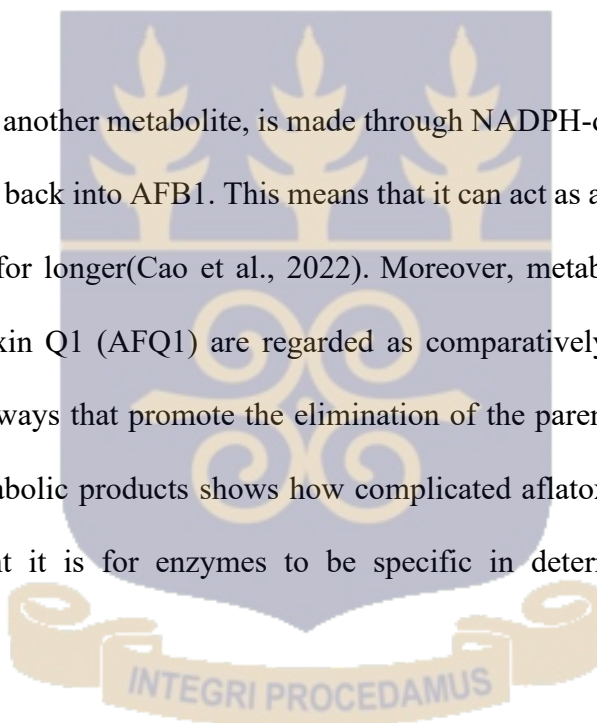
Nuclear Receptors and Chemoprotective Agents Modulate.

Nuclear receptors like the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), and the aryl hydrocarbon receptor (AhR) control the expression of both phase I and phase II enzymes that are involved in aflatoxin metabolism(Mohamed et al., 2024). On the

other hand, some foods and chemoprotective compounds can stop CYP450 activity or start phase II detoxification enzymes. This moves the balance towards detoxification and stops harmful DNA adducts from forming (Wu et al., 2017).). For example, flavonoids and sulforaphane, which are found in cruciferous vegetables, have been shown to change the expression and activity of GSTs and CYP450 enzymes. This protects against aflatoxin-induced mutagenesis and carcinogenesis (Rasouli et al., 2022) .

Creation of More Metabolites In addition to the formation of the highly reactive epoxide.

Aflatoxicol (AFL), another metabolite, is made through NADPH-dependent reduction and may be able to turn back into AFB1. This means that it can act as a reservoir that keeps the toxin in the body for longer(Cao et al., 2022). Moreover, metabolites like aflatoxin P1 (AFP1) and aflatoxin Q1 (AFQ1) are regarded as comparatively less toxic and signify detoxification pathways that promote the elimination of the parent toxin (Su, 2019). The wide range of metabolic products shows how complicated aflatoxin biotransformation is and how important it is for enzymes to be specific in determining overall toxicity (Arimboor, 2024).



Variations in Aflatoxin Metabolism Among Species

A significant characteristic of aflatoxin metabolism is the pronounced interspecies variability in the expression and activity of the enzymes responsible for both bioactivation and detoxification. For instance, poultry species like turkeys and ducks possess elevated levels of CYP450 enzymes that effectively produce reactive metabolites, alongside comparatively low glutathione S-transferase (GST) activity, making them highly susceptible to aflatoxin-induced toxicity (Bbosa et al., 2013).Conversely, rodents,

including mice, exhibit elevated levels of GST enzymes, particularly in the alpha-class, which improves their ability to detoxify genotoxic metabolites and provides a degree of resistance to aflatoxin toxicity (Deng et al., 2018). In humans, variations in the expression of CYP1A2 and CYP3A4, shaped by genetic polymorphisms and environmental influences, lead to disparities in susceptibility to aflatoxin-induced liver cancer and other toxic effects (Eaton et al., 2025). These interspecies comparisons are essential for risk assessment, as they elucidate the metabolic factors that contribute to varying sensitivities and guide the selection of suitable animal models for toxicological research (Clewell & Fuchsman, 2024).

Strategies for detoxification using enzymes and microbes

New methods for lowering aflatoxin toxicity are based on both enzymatic and microbial detoxification strategies. The induction of phase II enzymes, primarily through the administration of chemoprotective agents such as oltipraz and naturally occurring compounds like sulforaphane, has been shown to shift the metabolic balance in favour of detoxification, thereby reducing DNA adduct formation and the risk of subsequent carcinogenesis (Yates, 2010).). At the same time, biological detoxification that uses microbial enzymes to break down aflatoxins has gotten a lot of attention. Some fungal enzymes and bacterial species, especially those from *Lactobacillus* and *Saccharomyces*, have been shown to be able to bind to or break down aflatoxins in contaminated feed, making them less available and toxic (Guan et al., 2021). These strategies present promising opportunities for practical intervention in both human and veterinary contexts, as they facilitate the potential for preharvest and postharvest reduction of aflatoxin levels in food commodities (Guan et al., 2021).

2.1.4 Impact of Aflatoxins on the Liver and Kidneys

Long-term exposure to aflatoxins, especially in kids, has been linked to liver diseases like cirrhosis and acute liver failure (Mekuria et al., 2020). Aflatoxins are more likely to hurt kids' livers because their bodies aren't as good at getting rid of toxins and they take in more toxins for their body weight (Adaku Chilaka & Mally, 2020).). Aflatoxins, especially aflatoxin B1, are very toxic to the liver and hurt it in a number of ways (Benkerroum, 2020). The principal mechanism of hepatic injury involves the formation of DNA adducts, wherein aflatoxin metabolites attach to DNA, resulting in mutations that may precipitate hepatocellular carcinoma (liver cancer) (McCullough & Lloyd, 2019). Moreover, aflatoxins provoke oxidative stress by producing reactive oxygen species (ROS), resulting in lipid peroxidation and subsequent damage to cellular membranes, proteins, and DNA in hepatocytes (Marin & Taranu, 2012).). Exposure to aflatoxin impacts particular liver enzymes and biomarkers, notably alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are frequently elevated in instances of liver injury (Saad-Hussein et al., 2021). Elevated concentrations of these enzymes in the bloodstream signify hepatocellular injury, as they escape from compromised liver cells (Malakouti et al., 2017). Histopathology encompasses bile duct proliferation, hepatocyte necrosis, and the initial stages of liver fibrosis (Ferrell, 2000).). Aflatoxins primarily impact the liver, but they can also adversely affect kidney function (Saleem et al., 2021).). Aflatoxins can damage the glomeruli, which are the parts of the kidneys that filter blood, and this can make the glomerular filtration rate (GFR) lower (Ráduly et al., 2021). Over time, exposure to aflatoxins can lead to chronic kidney disease (CKD) because they cause oxidative damage to renal tissues and make it harder for the body to get rid of toxins (Wang et al., 2022). Serum creatinine and blood urea nitrogen (BUN) are two signs of kidney damage that tend to go up in people who have been exposed to high levels of aflatoxins. This means that their kidneys are not working as well as they should (Ráduly et al., 2023). In paediatric

populations, nephrotoxicity is particularly alarming due to children's heightened vulnerability to toxin accumulation and enduring renal impairment. Research indicates a link between aflatoxin exposure and renal outcomes in children, such as diminished renal function and an increased susceptibility to kidney disease, particularly in areas with significant aflatoxin food contamination (Chen et al., 2018).

Depletion of Glutathione and Intensification of Oxidative Stress.

A significant characteristic of AFB1 toxicity is the reduction of glutathione reserves. The excessive production of reactive oxygen species (ROS) during AFB1 metabolism rapidly depletes glutathione (GSH), diminishing the liver's and kidneys' ability to mitigate oxidative stress (Cao et al., 2022). This reduction in GSH levels results in an inadequate detoxification of reactive species, thereby promoting lipid peroxidation, DNA damage, and the activation of apoptosis (Liu et al., 2022). Moreover, reduced GSH levels hinder the activity of GSH-dependent enzymes, exacerbating oxidative injury and facilitating the advancement of tissue damage (Vairetti et al., 2021).

2.1.5 Aflatoxin Toxicity Among Children in Ghana and The Ejura Sekyedumase District

Studies conducted on crops like maize, millet, and groundnut in Ghana demonstrate aflatoxin contamination (Kortei et al., 2021). There are many things that cause aflatoxin contamination to happen a lot in Ghana. Some farming methods can make it more likely for mould to grow on crops, such as waiting too long to harvest or drying them the wrong way. Traditional ways of storing things make the problem worse because they often don't stop moisture from building up and instead encourage fungi to grow (Kaaya et al., 2006). Economic limitations are also a factor, as numerous farmers do not possess the means to adopt more efficient drying and storage methods. Children in the Ejura Sekyedumase District are more likely to get aflatoxin poisoning because they eat more foods that are

contaminated with aflatoxins and their liver enzymes are not fully developed, which makes it harder for them to get rid of aflatoxins (Kumi et al., 2014).

To lower aflatoxin toxicity, the Ejura Sekyedumase District needs to improve its farming and storage methods, raise awareness among farmers and consumers, and set up effective rules (Joseph et al., 2015). Important steps that need to be taken are to promote the use of safe drying methods, improve storage facilities, and inform the public about the dangers of aflatoxin exposure (Visser et al, 2020). Farmers can be given money and tools to help them use safer methods, which will protect children's health in this area where aflatoxin pollution is a problem.

2.1.6 Heavy Metals

Heavy metals are metallic elements characterised by a comparatively high density in relation to water (Tchounwou et al., 2012). Heavy metals are naturally occurring elements found in the earth's crust. However, most of the pollution and exposure to these metals comes from human activities like mining, smelting, industrial production and use, and the use of metals and metal-containing compounds in homes and farms (He et al., 2005). Metal corrosion, atmospheric deposition, soil erosion of metal ions, leaching of heavy metals, sediment re-suspension, and metal evaporation from water resources to soil and groundwater can all lead to environmental contamination (Briffa et al., 2020). Natural events like volcanic eruptions and the weathering of rocks have also been said to be major causes of heavy metal pollution (Kapoor & Singh, 2021).

Heavy metals are especially harmful to children because their organs and systems are still growing and are more likely to be harmed. In places like the Ejura Sekyedumase District of Ghana, which are agricultural and industrial, there are many ways that people can come into contact with heavy metals. Eating and drinking contaminated food and water, touching contaminated soil, and breathing in dirty air are all examples of this. Using fertilisers and pesticides in farming in the area can put cadmium and lead into the food chain. Also, industrial activities and poor waste management can pollute the environment, putting kids at risk of being exposed to heavy metals, which can have serious health effects like neurodevelopmental delays, kidney damage, and a weakened immune system.

2.1.7 Heavy Metals in Ghanaian Children

Ghana's environment has been badly damaged by rapid industrialisation and a lot of mining. There is a lot of pollution in many places, especially mining towns, from both human and natural sources of toxic heavy metals like lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As). Artisanal and small-scale gold mining (ASGM) is a major source of these pollutants getting into water, soil, and the air (Sarpong et al., 2023).

Other sources are industrial activities, car emissions, e-waste processing, and the use of agrochemicals (Ackah, 2017). Children are especially susceptible due to their developing neurological systems, increased consumption of water and food relative to body weight, and a heightened tendency for soil and dust ingestion through hand-to-mouth actions (Gong et al., 2022).

2.1.8 Sources and Mode of Contamination of Lead

Lead (Pb) is a strong neurotoxin that has no known health benefits and can permanently harm the developing brains of children even at low levels of exposure (Ackah, 2017). In Ghana, children are exposed to lead from a diverse array of sources, marked by intricate interactions among industrial, artisanal, and unregulated activities. Numerous studies have

shown that high blood lead levels (BLLs) in children are directly linked to environmental pollution caused by mining, battery recycling, and e-waste processing activities (Desye et al., 2023). . The rules that are supposed to keep people from getting contaminated have often fallen behind, putting vulnerable groups like children at risk of lead exposure in many ways.

One of the main causes of lead pollution in many parts of Ghana is mining, especially artisanal and small-scale gold mining (ASGM). In ASGM operations, the mechanical disruption of ore and tailings can release lead into surrounding soils and water bodies; the mobilised lead then disseminates into the ecosystem (Lebbie et al., 2021).). In mining areas like Tarkwa and Obuasi, lead is released not only from the ores and mining waste, but also from mining tailings and disturbed soils, which can later be resuspended in the air (Desye et al., 2023).). Mining operations can also pollute farmland, which can cause food crops to absorb lead. This affects the nutrition of the community directly and is another way for children to be exposed to lead (Kumar et al., 2020). The environmental spread of lead through water runoff and airborne particles in mining communities spreads the pollution over a large area, which increases the risk of exposure for children (Vesilind et al., 2013). Moreover, practices like geophagy, where pregnant women eat sand and clay, which is common in some communities, also add to lead exposure because they eat soil that is already contaminated (Molale & Eze, 2023).

It has been found that both formal and informal lead battery manufacturing and recycling plants in Ghana are major sources of lead pollution in the environment. These facilities, which are often close to homes and schools, make a lot of lead-contaminated dust and soil when they break down batteries, melt them down, and throw away battery parts in the wrong way (Kinder, 2019). Research on soil near recycling plants has shown that lead levels can be very high, sometimes reaching tens of thousands of mg/kg. The contamination

only slowly goes down as you move away from the plant (Gottesfeld et al., 2018). The airborne dispersal of lead particles from these sites and the deposition of contaminated particulates onto residential soils serve as a potent source of exposure for local children who play outside (Zota et al., 2011).

Informal e-waste recycling operations constitute another critical source of lead contamination. In Ghana, sites such as Agbogbloshie in Accra have become notorious for the unsafe processing of discarded electronic components through crude methods like open burning and manual dismantling (Kyere, 2016). These practices release large quantities of lead and other heavy metals into the environment due to the unregulated emission of dust and fumes, which contaminates soil, water sources, and the ambient air (Kyere, 2016). Children residing near e-waste recycling sites, or those who participate in informal salvage activities, are exposed to lead primarily through inhalation of contaminated air as well as ingestion of contaminated dust and soil (Desye et al., 2023; Khan et al., 2019). The complexities of the e-waste sector, combined with the widespread nature of informal processing, underscore the importance of addressing these unregulated activities as part of any comprehensive lead mitigation strategy (Khan et al., 2019).

Drinking contaminated water represents an additional exposure route for lead among children in Ghana, with multiple studies indicating that water sources such as reservoirs, hand-dug wells, and boreholes contain lead levels that exceed World Health Organization (WHO) guidelines (Buba & Maina, 2020). One prominent example is the Barekese Reservoir in Kumasi, where anthropogenic activities, ranging from nearby auto mechanic operations to agricultural runoffs have led to lead concentrations above the safe limits, thereby posing exposure risks particularly to children with higher water ingestion rates relative to body weight (Akoto et al., 2020). In addition, corrosion of water distribution systems, especially those utilizing brass components with intentionally high lead content,

has been identified as a significant contributor to lead leaching into drinking water (Choucri et al., 2019).

Other sources of lead contamination in Ghana may include legacy applications such as the historical use of leaded gasoline, which has left a residual contamination in soils and urban dust even after phasing out (Edusei, 2020). Likewise, lead-containing paints and consumer products contribute to direct household exposures, although the magnitude of such contributions is often secondary to those from industrial and mining sources (Obeng-Gyasi, 2022). Moreover, the practice of geophagy, ingesting soil or clay, which is common among pregnant women and may extend to children in certain regions, can lead to direct ingestion of lead contaminated soil, thereby exacerbating exposure risks (Bonglaisin et al., 2022). The cumulative impact of these additional sources may not be as pronounced individually as the major industrial and mining contributions; however, in combination they provide a persistent backdrop of environmental lead that synergistically raises the overall exposure levels in the community (Lar et al., 2015).

In areas surrounding mining sites, battery recycling plants, and informal e-waste recycling zones, high levels of lead have been detected in topsoil and settled dust (Ohajinwa et al., 2018). Children playing in these environments inadvertently ingest soil particles and dust through hand-to-mouth contact, which results in the direct transfer of lead into the gastrointestinal tract (Gong et al., 2022). The soil to air resuspension of lead particles, especially in dry and windy climates, further increases the risk as these fine particulates are deposited in areas where children live and play (Carlsson et al., 2017).

Children residing in urban environments with heavy vehicular emissions inhale contaminated air, leading to the deposition of fine lead particles within the respiratory tract (Brugha & Grigg, 2014). The inhaled lead particles may then be absorbed into the

bloodstream either directly through the alveolar epithelium or after dissolution in the respiratory tract fluids (Dondi et al., 2023). Because children's respiratory systems are still developing and they breathe at higher relative rates compared to adults, the risk posed by inhalation of lead laden dust is considerably heightened (Taylor et al., 2019)

2.1.9 Sources and Mode of Contamination of Cadmium in Ghana

Cadmium is a non-essential heavy metal that is toxic even at low exposure levels and is known to cause adverse health effects such as kidney damage, bone demineralization, respiratory problems, and increased risk of cancers (Charkiewicz et al., 2023). In Ghana, cadmium exposure among children has emerged as a major public health concern given the country's extensive gold mining operations as well as industrial and agricultural activities that contribute to environmental pollution (Mensah et al., 2025). Children are particularly susceptible due to their higher relative exposure owing to hand-to-mouth behavior, greater food and water intake per unit of body mass, and physiological immaturity, which limits their ability to detoxify heavy metals (Ofori et al.).

In mining towns such as Obuasi and Tarkwa, heavy metals including cadmium are released into the environment during ore extraction, processing, and waste disposal activities (Obiri, 2018). Mining processes such as ore grinding, amalgamation, and smelting mobilize cadmium into the surrounding air, water, and soils (Ankush et al., 2024). Studies comparing mining regions with non-mining areas have reported up to 20-fold higher blood cadmium levels in residents, including children, from mining communities, underscoring mining's central role in environmental heavy metal exposure (Armah et al., 2012). Cadmium migration from waste rock piles and tailings, as well as dust emissions from mining sites, contribute significantly to the contamination of local soils and groundwater, establishing a basis for both direct and indirect human exposure (Madejón et al., 2021).

Artisanal small-scale gold mining (ASGM), widely practiced in rural and peri-urban areas of Ghana, often employs rudimentary methods that lack adequate pollution control measures (Takyi et al., 2021). These methods lead to the inadvertent release of cadmium along with other toxic metals, which subsequently disperse into the environment via atmospheric deposition and water runoff (Boudia et al., 2022). In towns such as Nangodi in the Upper East Region, small-scale mining processes have been directly linked to elevated cadmium concentrations in local water sources, with measured values sometimes exceeding safe thresholds by several fold (Obiri, 2018). In addition, studies conducted in the Tarkwa area, a major gold mining center, demonstrate that proximity to mining operations correlates with increased contamination of soils, which serve as important reservoirs for cadmium and other heavy metals (Hayford et al., 2009). Consequently, mining remains the dominant source of cadmium exposure in Ghana, affecting the entire ecosystem, including the food chain and water resources (Hayat et al., 2019).

Agricultural practices also play a significant role in cadmium exposure (Mubeen et al., 2023). The use of phosphate fertilizers and organic manure in crop production can introduce cadmium into agricultural soils because these inputs often contain trace amounts of cadmium as impurities (Dharma-Wardana, 2018). In mining-impacted regions such as Tarkwa and its environs, agricultural soils are frequently contaminated by the fallout from mining activities (Padhiary & Kumar, 2024). Crops grown in these soils, particularly tubers like cassava and leafy vegetables, accumulate cadmium via both root uptake and atmospheric deposition onto their surfaces (Huang et al., 2022). For instance, studies conducted in the Tarkwa area have shown that food crops exhibit detectable levels of cadmium, thereby posing dietary exposure risks, especially to children who consume large quantities of staple foods per body weight (Bortey-Sam et al., 2015). Moreover, irrigation with water contaminated by mining runoff further exacerbates cadmium uptake by crops,

amplifying the risk of chronic dietary exposure (Kuna et al., 2024). Thus, agricultural practices combined with environmental pollution from nearby mining operations form a critical route through which cadmium enters the human body (Suhani et al., 2021).

In addition to mining and agriculture, industrial processes contribute to cadmium contamination. Industries such as smelting, manufacturing, and waste incineration release cadmium into the atmosphere, which then settles on soils and water surfaces (Ankush et al., 2024). Although the mining sector is the predominant contributor in Ghana, certain urban areas have also reported elevated levels of cadmium due to industrial emissions and vehicular exhaust. Urban dust samples collected from transport stations in cities like Kumasi have revealed cadmium concentrations that pose potential carcinogenic risks, particularly to children who are prone to soil and dust ingestion during play (Rweyemamu et al., 2020). The combination of industrial discharges, urban emissions, and e-waste recycling activities thus adds to the environmental burden of cadmium, compounding exposure for nearby populations (Parvez et al., 2021).

Contaminated water sources represent a critical exposure pathway, particularly for children who rely on such water for drinking, cooking, and personal hygiene (Ritter, 2002). Aquatic organisms in polluted water bodies accumulate the metal through bioaccumulation processes (Jakimska-Nagórska et al., 2011). The consumption of fish from contaminated water bodies further increases the risk of exposure, rendering fish another important dietary source (Mozaffarian & Rimm, 2006). Given that children typically consume more food per unit body weight compared to adults, the dietary pathway is particularly concerning and underscores the need for stringent food safety monitoring and interventions (Afsana et al., 2022).

2.2.0 Mechanism of Heavy Metals Action

The absorption of metal in their elementary forms are poor when they come into contact with the body (Balali-Mood et al., 2021). Metals in liquid suspensions are available utilized when placed on the skin (Kim et al., 2015). Highly acid digestive tracts of carnivores enhance the absorption of ingested heavy metals (Gall et al., 2015). Proteins like albumin carries absorbed heavy metals in the blood stream. Heavy metals have the ability of binding to several binding sites when they get absorbed by the body (Rehman et al., 2018). Organs are damaged by heavy metals as a result of releasing free radicals through two independent mechanisms (Jan et al., 2015). These two independent mechanisms are the direct generation of reactive oxygen species (ROS) and depletion of antioxidants in cell. Heavy metals induce ROS by activating the enzyme NADPH oxidases, displacing essential cations from specific binding sites of enzymes, inhibiting antioxidative systems and changing mitochondrial membrane permeability (Wu et al., 2016)

2.2.1 Impact of Heavy Metals on the Liver, Kidney and Glutathione

Heavy metals such as lead and cadmium are known for their hepatotoxic effects, disrupting liver function through various mechanisms (Balali-Mood et al., 2021). Lead primarily affects the liver by interfering with the activities of key enzymes involved in metabolic processes (Singh et al., 2018). It generates oxidative stress by increasing the production of reactive oxygen species (ROS) and impairing antioxidant defenses, leading to lipid peroxidation, mitochondrial dysfunction, and DNA damage (Ozougwu, 2016). Cadmium, another hepatotoxic metal, accumulates in the liver and disrupts cellular function by binding to sulfhydryl groups in proteins, enzymes, and DNA, which further increases oxidative stress and inflammation (Renu et al., 2021). The interaction of heavy metals with

detoxification enzymes, including glutathione, is critical in understanding their hepatotoxic effects (Srikanth et al., 2013). Both lead and cadmium can deplete cellular levels of glutathione, an essential antioxidant in liver cells. This depletion impairs the liver's ability to detoxify reactive intermediates and neutralize oxidative stress, exacerbating liver damage (Vairetti et al., 2021). The reduction of glutathione compromises the liver's defense mechanisms, making it more susceptible to damage from toxins and other stressors (Fernandez-Checa & Kaplowitz, 2005). Epidemiological studies have shown a link between heavy metal exposure and liver function impairments, particularly in children (Rehman et al., 2018).

Heavy metals like lead and cadmium also exert nephrotoxic effects by damaging the kidneys through oxidative stress and apoptosis (Matović et al., 2015). Lead can disrupt kidney function by accumulating in renal tissues and inducing oxidative damage, which impairs the kidneys' filtration ability (Sanchez, 2018). It increases ROS production and interferes with cellular signaling pathways, leading to cell death (apoptosis) and tissue fibrosis (Singh et al., 2018). Cadmium, on the other hand, is toxic to the proximal tubules of the kidneys, where it accumulates and triggers inflammation, mitochondrial dysfunction, and tubular necrosis (Yan & Allen, 2021). The nephrotoxic effects of heavy metals can be assessed through renal function tests such as serum creatinine and blood urea nitrogen (BUN) (Campus & Guyana, 2020). Elevated levels of these markers indicate reduced kidney function and are often seen in individuals with chronic exposure to heavy metals (Lentini et al., 2017). Studies in children exposed to lead and cadmium have shown significant reductions in glomerular filtration rates (GFR) and increases in serum creatinine and BUN levels, reflecting impaired renal function (Fadrowski et al., 2013). Chronic exposure to these metals can lead to long-term kidney damage, including chronic kidney disease (CKD) and an increased risk of renal failure (Orr & Bridges, 2017).

2.2.2 Glutathione and Its Role in Detoxification

Glutathione is a small tripeptide composed of three amino acids: glutamine, cysteine, and glycine. Synthesized within cells, primarily in the liver, and is one of the most important antioxidants in the human body (Vašková et al., 2023). Glutathione plays a central role in maintaining cellular redox balance and protecting cells from oxidative stress by neutralizing reactive oxygen species (ROS) and free radicals (He et al., 2005). Its sulfhydryl (–SH) group on the cysteine residue is responsible for its antioxidant properties, allowing it to directly scavenge harmful oxidative molecules (Ulrich & Jakob, 2019).

It binds to toxic compounds like aflatoxins and heavy metals, forming water-soluble conjugates that are then excreted from the body through bile or urine which is a crucial mechanism in safeguarding organs like the liver and kidneys, where detoxification processes are most active (Santini & Ritieni, 2013).

Exposure to aflatoxins and heavy metals significantly depletes the body's glutathione reserves (Kidd, 1997). Aflatoxins, particularly aflatoxin B1, induce oxidative stress by generating ROS, leading to lipid peroxidation and cellular damage (Marin & Taranu, 2012).

Exposure of aflatoxin and heavy metals especially lead and cadmium place a considerable demand on glutathione for neutralization, often overwhelming the body's capacity to replenish it and is particularly detrimental to organs like the liver and kidneys, where glutathione plays a key protective role in detoxifying harmful substances (Colbert, 2011).

Children are especially vulnerable to the depletion of glutathione due to their developing systems and higher metabolic rates, their antioxidant defenses are not fully matured, making them more susceptible to the toxic effects of aflatoxins and heavy metals (Granot & Kohen, 2004). Exposure to toxins substances such as heavy metals and aflatoxins during early development can lead to long-term health consequences, including impaired growth, neurodevelopmental issues, and organ damage (Grandjean & Landrigan, 2014)

2.2.3 Effects of Aflatoxins and Heavy Metals on Glutathione Levels

The co-exposure to aflatoxins and heavy metals results in a synergistic or additive **toxic** effect, exacerbating oxidative stress and causing more severe organ damage compared to exposure to either toxic alone (Guo et al., 2020). Both aflatoxins and heavy metals induce oxidative stress by generating reactive oxygen species (ROS), but their combined presence can overwhelm the body's antioxidant defenses, particularly glutathione resulting in an increase in oxidative stress that leads to more significant cellular damage, affecting key organs like the liver and kidneys (Surai et al., 2019). Aflatoxins and heavy metals interact in ways that enhance each other's toxicity. For example, aflatoxins impair liver function and detoxification processes, reducing the body's ability to handle heavy metal toxicity (Fu et al., 2022). Conversely, heavy metals, such as cadmium and lead, deplete glutathione and other antioxidant systems, making the body more vulnerable to the damaging effects of aflatoxins (Ebrahimi et al., 2023). The synergistic effect can lead to higher levels of DNA damage, lipid peroxidation, and apoptosis, ultimately accelerating organ dysfunction (Rasouli et al., 2022). Glutathione plays a crucial role in detoxifying both aflatoxins and heavy metals, but the simultaneous exposure to these toxins places an overwhelming demand on glutathione reserves (Townsend et al., 2003). Aflatoxins deplete glutathione by inducing oxidative stress, while heavy metals such as lead and cadmium further deplete glutathione by binding to its sulfhydryl groups, reducing its availability for detoxification (Hasanein & Emamjomeh, 2019). This dual depletion weakens the body's ability to neutralize toxins, leaving cells more vulnerable to oxidative damage (Labarrere & Kassab, 2022). In children, who have lower baseline levels of glutathione compared to adults, the depletion caused by co-exposure to aflatoxins and heavy metals can be particularly detrimental (Surai et al., 2019). Reduced glutathione levels impair the detoxification process, allowing for the accumulation of toxic intermediates, which exacerbates oxidative stress and accelerates liver and kidney damage (Ballatori et al., 2009)

CHAPTER 3

3.0 METHODOLOGY

3.1 Study Design

This study employed the cross-sectional approach.

3.2 Study Area /Site

The study area was Ejura-Sekyedumase district. The major communities of this district include Ejura, Sekyedumase, Anyinasu, Hiawoanwu, Kasei, Dromankuman and Bonyon.

According to the 2010 population and housing census, the population of the district is 85,446 representing 1.8 percent of the of the region's total population. 50.2 percent constitute males and 49.8 percent represents female. Children constitute the largest proportion of the household structure accounting for 45.2 percent (Population and Housing Census, 2010). Humidity peaks at 90% in June and drops to 55% in February, while high solar radiation occurs during the dry season. Agriculture is the primary sector for employment and income, focusing on both crop production and livestock rearing. Key crops include maize, yam, beans, rice, plantain, cassava, and groundnuts, with maize and beans being cultivated for commercial purposes.

3.3 Study Population

Fifty (50) participants were recruited from the Ejura-Sekyedumase district for this study.

Children between the ages of 2 and 9 years old were selected for the study.

3.4 Variables

The variables for this study are categorized into dependent and independent. The dependent variables are

1. Glutathione levels: Which is an indicator of the children's antioxidant capacity

2. Liver function Parameters:

a. These are the enzymes Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP). These enzymes are indicators of liver damage.

According to WHO, the normal ranges for children are ALT 7–55 unit/L. AST 15-50 unit/L and ALP 130-340 unit/L.

b. Albumin and **Total Protein**: Showing liver synthetic function. Albumin ranges from 34 to 54 g/L. **Total Protein** for paediatrics 60 to 80 grams per liter (g/L)

c. Bilirubin: Reflecting liver detoxification ability. Direct (also called conjugated) bilirubin: less than 0.3 mg/dL (less than 5.1 $\mu\text{mol/L}$) Total bilirubin: 0.1 to 1.2 mg/dL (1.71 to 20.5 $\mu\text{mol/L}$).

3. kidney function Markers:

a. Creatinine and BUN: They give information about kidney filtration efficiency. Creatinine 0.3–1.2 mg/dL, BUN 6 to 24 mg/dL (2.1 to 8.5 mmol/L).

b. Glomerular Filtration Rate: It measures kidney function and that of a healthy child is from 40 to 60.

The independent variables will be

- a. Types of Aflatoxins. Example Aflatoxin M1.
- b. Types of heavy metals. Example Lead and Cadmium.
- c. Age, weight and height.

3.5 Sampling

3.5.1 Sample Size Calculation

The consent of 162 guardians or parents were sought and engaged for their ward's samples to be taken after an informed consent and a thorough explanation of what the study is about was

given. The sample size to be used were calculated using a single population proportion formula considering a 95% confidence interval and a marginal error of 0.05 as follows; $n = (Z(a/2))^2 \frac{P(1-P)}{d^2}$. Where n is the sample size to be calculated, P is the prevalence of aflatoxin contamination in maize and groundnut in major producing regions across three agroecological zones (AEZs) in Ghana which was mentioned earlier to be 12%. (0.12), d is the marginal error which is 0.05 and $Z(a/2)$ is from the Z distribution table which is 1.96. $n = (1.96)^2 \times 0.12 (1 - 0.12) / (0.05)^2$. $n = 3.841 \times 0.1056 / 0.0025$. $n = 162$ So, the sample size calculated is 162. Thus, a total of 162 samples or participants were recruited for the research

3.5.2 Inclusion Criteria

1. The study included male and female children between the ages of two and nine years.
2. Participants included children who were born and have lived in the study communities all their lives.
3. Hepatitis B negative children were included

3.5.3 Exclusion Criteria

1. Children with any form of liver injury.

3.5.4 Sampling Method and Procedure

Convenience Sampling method was used during the study.

3.5.4.1 Convenience Sampling

The researcher selected test participants from the response population at her convenience.

3.5.5 Sample Collection and Analysis

Blood

Blood, (5 mLs) was taken from the antecubital fossa vein (by venipuncture) in the upper arm of all participants by a trained phlebotomist from the **District Hospital**. The blood was divided into two, 2 mL into EDTA and 3 mL into serum gel tube labeled with participant's name and identification number. The clotted blood was centrifuged at 2000 revolution per minute for 10 minutes to get the serum. The serum was stored in aliquots in freezing vials, labeled with the participant's identification number. Whole blood and serum samples were stored at 4°C and -20°C respectively prior to transporting to Noguchi Memorial Institute **for** Medical Research (NMIMR). Whole blood and serum samples were used to assess hematology and chemistry parameters respectively. Whole blood specimen (EDTA tube) was used to analyze reduced glutathione, full blood count (white blood cells, hemoglobin level, red blood cells, and platelets) and screened for Hepatitis B viral infection as a means to control the confounding factor. Serum samples were used to analyze for aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, gamma glutamyl transferase (GGT), bilirubin, sodium, creatinine, urea, chloride and calcium.

Urine

Spot urine (20mls) samples were obtained from 50 participants by parents or guardians into a sterile specimen cup. The urine samples were frozen and transported to NMIMR in a leak free cool box. The urine was kept at -20 °C prior to analysis of aflatoxin levels at NMIMR.

Laboratory Investigations

Laboratory investigations were done at the Clinical Pathology Department (NMIMR) and Haematology department of IMaH.

Sample analysis

Full blood count

Full blood count parameters analyzed included haemoglobin level, total white blood cell count (WBC) and total red blood cell count (RBC). White blood cell count analyzed were lymphocytes, neutrophils, monocytes and platelets. These analyses were done at the International Maritime Hospital Laboratory using the KX Sysmex 21 haematology analyzer (Sysmex Corporation, Japan).

Kidney and liver biomarkers

The levels of kidney and liver biomarkers in serum were analyzed using an automated colorimetric method by Flexor E, endpoint automation, (Vital Scientific, Netherlands). Liver markers analyzed included; aminotransferase (ALT), aspartate aminotransferase (AST), albumin, gamma-glutamyl transferase (GGT), total protein and total bilirubin while kidney markers analyzed included, creatinine, urea, sodium, chloride and potassium. Serum calcium was also measured.

Urinary aflatoxin.

The levels of aflatoxin in 50 urine samples were determined using immunoaffinity column purification, AflaTest. (VICAM, USA) and HPLC-flourescence detection at 365 nm excitation and 425 nm emission wavelengths (Kumi et al.,2021).

Affinity chromatography

Each urine sample (5 mL) were acidified with 0.5 mL of 1.0 M ammonium formate (pH 4.5) and diluted with deionized water to a volume of 10 mL. The samples were then allowed to flow by gravity through the immunoaffinity columns at a rate of 1-2 drops per second. Before elution of aflatoxin with 1 mL of 80 % methanol, immunoaffinity columns were washed with 5 mL of phosphate buffer saline (PBS) and 10 mL of deionized water. The eluants were then dry with nitrogen gas before being resuspended in 1mL of methanol: 1mL of ammoniumformate (ratio 1:1) solution for analysis on a Shimadzu HPLC system with fluorescence detection (**Shimadzu Corporation, Japan**).

High performance liquid chromatography

A 250 x 4.6 mm LiCrospher RP-18EC end-capped column with a pore size of 100 Å and a particle size of 5 µm (Alltech) were used to identify aflatoxin metabolites. The mobile phase contained 22 % ethanol in deionized water, which were buffered with 20 mM ammonium formate (pH 3.0). Samples (100 µL) were injected at a rate of 1 mL/min elution rate. The detection limit was 0.5 pg AFM1/mL urine. The retention time was resolved using an aflatoxin standard which was analyzed with the samples. Aflatoxin levels were quantified using fluorescence detection procedure with 365 nm excitation and 425 nm emission wavelengths. The study of aflatoxin was carried out at Clinical Pathology Department, NMIMR.

Glutathione (GSH) measurement in whole blood

The blood samples were homogenized and diluted 200X with GSH buffer before use. The reaction mixture was 50 µL of GSH buffer (0.1M NaH₂PO₄, 5mM EDTA, pH 8.0 with 1M NaOH) with 50 µL of the blood sample and 10 µL of 0.75 mM O-Phthaldehyde (OPT). The mixture was incubated for 15 minutes at room temperature in the dark, and fluorescence

measured at 340 nm for excitation and 460 nm for emission (Paul and Russell, 1976). The experiment was carried out in triplicate. The reduced glutathione was calculated using calibration curve. Reduced glutathione obtained was expressed in terms of glutathione equivalent (mg/mL).

Pretreatment of sample containers for Lead and Cadmium Analysis

All glassware and sample containers (polyethylene bottles) and Teflon beakers were meticulously cleaned with non-ionic detergent solutions to eliminate any contaminants, followed by rinsing with tap water until devoid of detergent residues. The items were thoroughly rinsed with distilled water, thereafter, immersed in 10% (v/v) nitric acid for 48 hours, and then rinsed with distilled water before being fully dried in an oven at 120°C for 6 hours before using for sampling and analysis.

Digestion using Conventional Wet Acid Method (CDM):

Blood samples were processed using the Conventional Wet Acid Method as described by Memon et al. (2007). A volume of 0.5 mL of urine was collected into a Pyrex flask individually. About 3 mL of a freshly made mixture of concentrated nitric acid and hydrogen peroxide ($\text{HNO}_3 - \text{H}_2\text{O}_2$) in a 2:1 volume/volume ratio were allowed to stand for 10 minutes.

The flasks were covered with glass and digested at 60-70°C for 1–2 hours. The digests were then treated with 2 mL nitric acid and a few drops of H_2O_2 , heated on a hot plate at around 80°C until a clear digested solution was formed. The excess acid combination was evaporated until semi-dry, chilled, and diluted with 0.1 mL nitric acid. The mixture was then diluted with

5mL of distilled water prior to analysis. A **blank extraction (without the sample)** was performed twice through the entire operation using distilled water.

Analysis of Lead and Cadmium by Atomic absorption spectroscopy (AAS)

Instrument Calibration and Sample Analysis

The AAS machine was calibrated using a series of calibration standard solutions. A working standard solution of 100 ppm was prepared from a stock solution of 1000 ppm, from which calibration standards within the linear range were also prepared. This was achieved by diluting the required volume with 2% v/v HNO₃. The standard calibration solutions were then injected into the AAS to generate calibration data.

The concentrations of Lead and Cadmium present in pre-digested urine samples were determined using a Graphite Furnace Atomic Absorption Spectrometer (GFAAS; PinAAcle 900H) using argon gas. Acid digests of pre-digested urine samples were introduced into the spectrometer by suction through the AAS nebulizer, and the concentrations Pb and Cd were read and recorded.

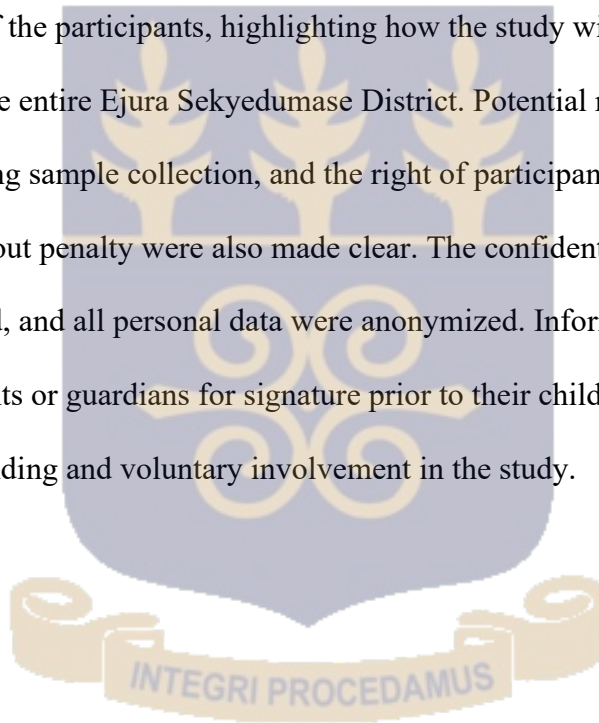
Quality Control and Validation of Analytical Data

Glassware used were soaked overnight in a solution of 10% v/v HNO₃ to avoid contamination of samples from the previous analysis. Standard solutions of known concentrations were analyzed intermittently, together with the unknown samples. A percentage metal recovery analysis was done for each metal to ensure the accuracy of analytical data using internal reference materials of known concentrations. For each parameter, triplicate measurements were

done. This is going to be used to determine the precision of analytical data expressed as relative standard deviation.

3.6 Ethical Consideration

Ethical approval was sought from the Ethical and Protocol Review Committee at the College of Health Sciences, University of Ghana, **with Protocol Identification number CHS-Et/M.11-P5.11/2024-2025**, before the commencement of the study. The purpose, significance, procedures, and benefits of the research were thoroughly explained to the parents or guardians of the participants, highlighting how the study will benefit not only the participants but also the entire Ejura Sekyedumase District. Potential risks, including any minor discomfort during sample collection, and the right of participants to withdraw from the study at any time without penalty were also made clear. The confidentiality of participants was strictly maintained, and all personal data were anonymized. Informed consent forms were provided to parents or guardians for signature prior to their child's participation, ensuring full understanding and voluntary involvement in the study.



3.7 Data Collection Approach

Samples were collected from children living in Ejura -Sekyedumase through the administration of questionnaire. The questionnaires were designed in English. However, questions were translated into local dialects for those who do not understand the English language. Parents with children of the required age for this study were approached and the whole study was then explained to them. Those who agreed to participate were given a consent form to fill and sign.

Section A and B of the questionnaires contained basic data and clinical information

respectively. Section D contained data from dietary habits. 5mls of blood samples were drawn from every participant, and sent to Noguchi Memorial Institute for Medical Research to be analyze. Fully automated Siemens Dimension EXL 200 chemistry analyzers and Atomic Absorption spectrophotometer were used to run all the samples.

3.8 Quality Control

All equipment were calibrated and controlled with commercially prepared reagents before samples were analyzed. All samples were handled according to standard laboratory practices to ensure accuracy and reliability. Data collected from both blood and urine samples were documented meticulously, ensuring participant confidentiality and adherence to ethical guidelines throughout the study.

3.9 Data Analysis

All analyses were performed in R (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment. Data distributions were examined for normality using graphical inspection and statistical tests. Descriptive statistics were reported as means with standard deviations (SD) for normally distributed variables or medians with interquartile ranges (IQR) for non-normally distributed variables. Comparisons between male and female participants were performed using independent samples t-tests for normally distributed biomarkers and the Mann–Whitney U test for skewed variables.

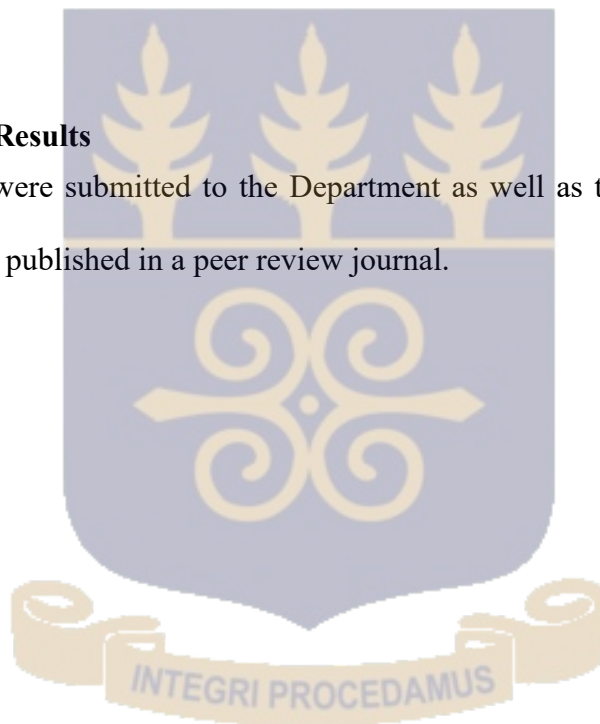
Associations between exposures (cadmium [Cd], lead [Pb], and aflatoxin M1 [AFM1]) and hematological, hepatic, renal, and electrolyte/glutathione biomarkers were assessed using correlation analysis. Pearson's correlation coefficients were used for normally distributed biomarkers, while Spearman's rank correlation coefficients were used for non-normal biomarkers. Significance levels were annotated in correlation heatmaps.

Multivariable linear regression models were then fitted to evaluate the independent associations of Cd, Pb, and AFM1 with biomarker outcomes, adjusting for age, weight, and height. Analyses were conducted in the overall population and stratified by sex. Regression coefficients (β) with 95% confidence intervals (CI) and p-values were reported. Finally, the combined effects of exposures were assessed using weighted quantile sum (WQS) regression, adjusting for age, weight, and height, with WQS weights estimated to indicate the relative contribution of each exposure to the mixture.

Two-sided $p < 0.05$ was considered statistically significant.

3.9 Dissemination of Results

Copies of the results were submitted to the Department as well as the School Library. The findings would also be published in a peer review journal.



CHAPTER 4

4.0 RESULTS

4.1 Haematological and Biochemical Parameters of Study Participants

In this study, the comparison of biochemical and hematological parameters between male (n = 26) and female (n = 24) participants showed largely similar distributions across groups. No significant differences in sex were observed in hemoglobin concentration, white blood cell count, platelet count, lymphocyte, monocyte, or neutrophil percentages (all $p > 0.05$).

Table 4.1 Comparison of Haematological and Biochemical Characteristics of Participants by Sex

Variable	Male	Female	p-value
Total	n = 26	n = 24	
Ht (m)	1.20 (1.15 - 1.30)	1.15 (1.04 - 1.24)	0.074
Hb (g/dL)	11.79 $\pm$ 0.81	11.52 $\pm$ 0.61	0.180
WBC ($10^9/L$)	7.25 $\pm$ 1.84	7.45 $\pm$ 1.36	0.652
Platelets ($10^9/L$)	312 $\pm$ 95	333 $\pm$ 69	0.383
Lymphocytes (%)	45 $\pm$ 10	45 $\pm$ 11	0.861
Monocytes (%)	8.62 $\pm$ 2.05	8.31 $\pm$ 2.10	0.608
Neutrophils (%)	38 $\pm$ 12	39 $\pm$ 10	0.752
Albumin (g/dL)	46.00 (44.00 - 47.00)	45.50 (44.00 - 47.00)	0.659
ALT (U/L)	20 $\pm$ 7	20 $\pm$ 6	0.920
AST (U/L)	28 (15 - 34)	28 (13 - 31)	0.674
GGT (U/L)	25.4 (22.1 - 28.8)	19.9 (15.1 - 23.1)	0.002
Total Bilirubin (mg/dL)	5.80 (4.90 - 8.80)	8.15 (5.85 - 9.45)	0.658
Total Protein (g/dL)	78.5 $\pm$ 5.3	75.8 $\pm$ 3.7	0.039
Urea (mg/dL)	2.96 (2.50 - 3.60)	3.28 (2.57 - 4.01)	0.943
Creatinine (mg/dL)	59.3 (55.0 - 64.0)	58.5 (54.5 - 61.6)	0.334
Sodium (mmol/L)	135.2 $\pm$ 5.8	137.1 $\pm$ 5.3	0.236
Potassium (mmol/L)	3.36 (3.15 - 3.49)	3.40 (3.21 - 3.71)	0.381
Chloride (mmol/L)	102.9 (99.6 - 108.2)	103.3 (101.4 - 106.1)	0.964
Glutathione (μ mol/L)	0.31 $\pm$ 0.08	0.28 $\pm$ 0.10	0.292
Cadmium (μ g/L)	0.006 (0.006 - 0.012)	0.006 (0.006 - 0.015)	0.403
Lead (μ g/L)	0.012 (0.006 - 0.024)	0.018 (0.009 - 0.030)	0.873
AFM1 (ng/mL)	77 (9 - 835)	130 (13 - 393)	0.289

Ht-Height (m); Hb-Haemoglobin (g/dL); WBC-White Blood Cell ($10^9/L$); PLT-Platelet ($10^9/L$); Lymph-Lymphocytes (%); Mon-Monocytes (%); Neut-Neutrophils (%); Alb-Albumin (g/dL); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transferase (U/L); GGT-Gamma Glutamate (U/L); TBil-Total

Bilirubin (mg/dL); TProtein-Total Protein (g/dL); Urea (mg/dL); Creat-Creatinine (mg/dL); Na-Sodium (mmol/L); K-Potassium (mmol/L); Cl-Chloride (mmol/L); GSH-Glutathione ($\mu\text{mol/L}$);

Serum albumin, liver enzymes (ALT, AST), total bilirubin, urea, creatinine, electrolytes (sodium, potassium, chloride), glutathione, and heavy metals (cadmium and lead) did not differ significantly by sex. However, males had higher serum Gamma-glutamyl transferase (GGT) activity compared to females (median 25.4 vs. 19.9 U/L, $p = 0.002$), while females exhibited slightly lower total protein levels than males (75.8 ± 3.7 vs. 78.5 ± 5.3 g/dL, $p = 0.039$). Overall, except for GGT and total protein, the biochemical and hematological profiles were broadly comparable between sexes (Table 4.1).

4.2 Comparison of Heavy metals and Aflatoxin by Sex

As depicted in Table 4.1, no sex-specific differences were observed for Cd, Pb and AFM1 levels between males and females (Figure 4.1).

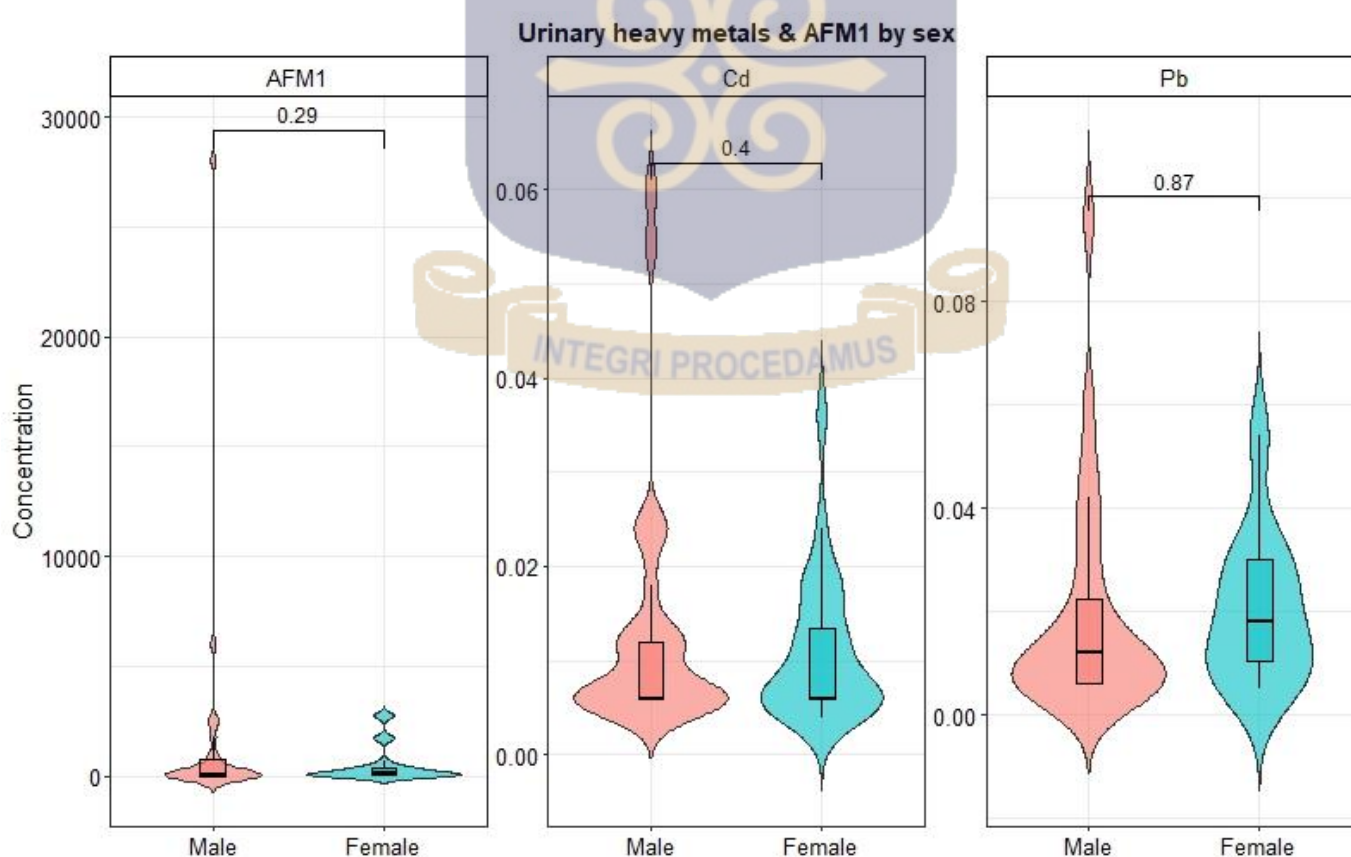
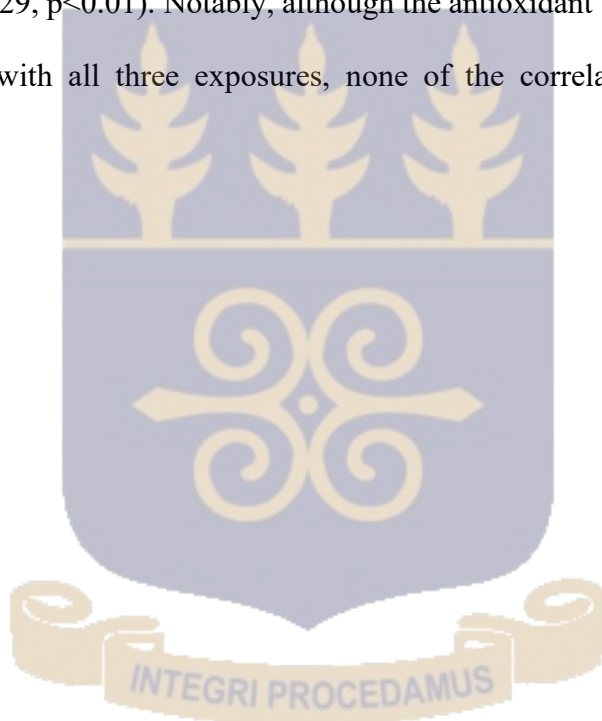


Figure 4.2 Levels of Heavy metals and AFMI Stratified by Sex

Cd-Cadmium ($\mu\text{g/L}$); Pb-Lead ($\mu\text{g/L}$); AFM1-Aflatoxin M1 (ng/mL).

4.3 Association between haematological and biochemical parameters of participants

As depicted in Figure 4.2, hematological parameters demonstrated significant positive correlations, particularly between AFM1 and neutrophil count ($r = 0.37$, $p < 0.01$) and between Pb and albumin levels ($r = 0.31$, $p < 0.001$). However, AFM1 showed a moderate negative correlation with monocyte ($r = -0.33$, $p < 0.01$). AFM1 positively correlated with urea ($r = 0.35$, $p < 0.01$). Lead showed positive correlation with Cl ($r = 0.31$, $p < 0.01$) whereas Cd correlated positively with K ($r = 0.29$, $p < 0.01$). Notably, although the antioxidant biomarker GSH showed negative correlations with all three exposures, none of the correlations were statistically significant.



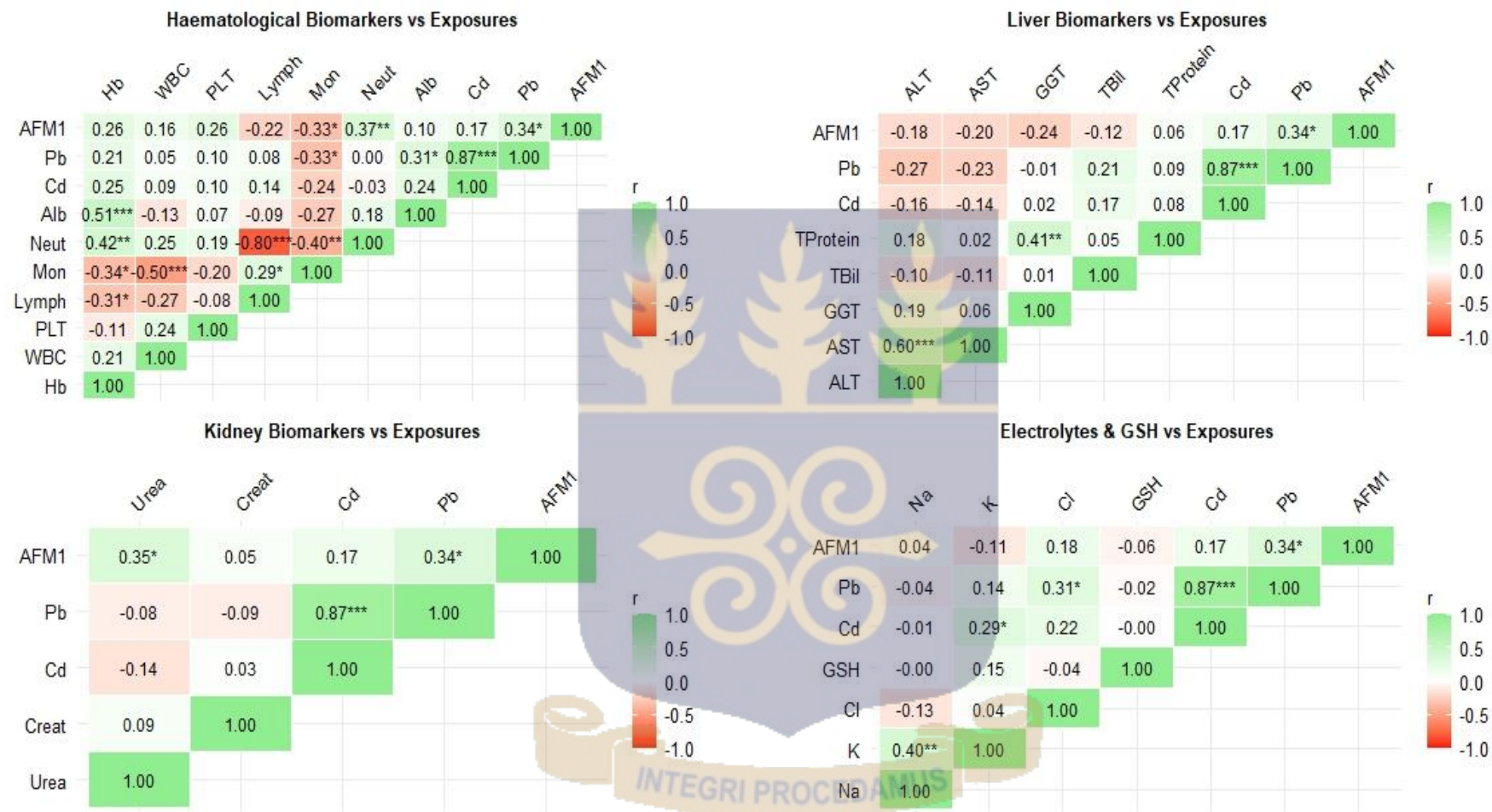


Figure 4.3 Correlation matrices depicting association between heavy metals/aflatoxin exposures and biomarkers of haematological, hepatic renal and glutathione status. Hb-Haemoglobin (g/dL); WBC-White Blood Cell ($10^9/L$); PLT-Platelet ($10^9/L$); Lymph-Lymphocytes (%); Mon-Monocytes (%); Neut-Neutrophils (%); Alb-Albumin (g/dL); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transaminase (U/L); GGT-Gamma Glutamate (U/L); TBil-Total Bilirubin (mg/dL); TPProtein-Total Protein (g/dL); Urea (mg/dL); Creat-Creatinine (mg/dL); Na-Sodium (mmol/L); K-Potassium (mmol/L); Cl-Chloride (mmol/L); GSH-Glutathione ($\mu\text{mol/L}$); Cd-Cadmium ($\mu\text{g/L}$); Pb-Lead ($\mu\text{g/L}$); AFM1-Aflatoxin M1 (ng/mL).

4.4 Multivariate analysis of haematological and biochemical parameters

In the overall sample, none of the exposures (Cd, Pb, or AFM1) demonstrated significant associations with glutathione (GSH), haemoglobin, or most haematological parameters (white blood cell, lymphocyte, monocyte, neutrophil counts) after adjusting for age, weight, and height (all $p > 0.05$). Among liver and renal biomarkers, AFM1 showed a positive association with neutrophils ($\beta = 0.001$; 95% CI: 0.000–0.002; $p = 0.006$) and urea ($\beta = 0.000$; 95% CI: 0.000–0.000; $p = 0.013$) (Table 2). Sex-stratified analyses revealed that in males, Cd and Pb were significantly associated with sodium ($\beta = 618.56$; 95% CI: 119.71–1117.42; $p = 0.018$; $\beta = -443.78$; 95% CI: -782.35 to -105.20; $p = 0.013$) and potassium ($\beta = 39.78$; 95% CI: 15.76–63.79; $p = 0.003$; $\beta = -24.19$; 95% CI: -40.49 to -7.89; $p = 0.006$) (Table 3). In females, AFM1 was negatively associated with neutrophils ($\beta = -0.006$; 95% CI: -0.012 to -0.001; $p = 0.033$) (Table 4).

Table 4.2 Multivariable Linear Regression of cadmium, lead and aflatoxinM1 with Biochemical and haematological outcomes Adjusted for age, weight and height in the general population

Variable	Estimate	95% CI	P-value
Glutathione (GSH) ($\mu\text{mol/L}$)			
Cd	0.352	-5.245, 5.949	0.900
Pb	-0.191	-3.815, 3.433	0.916
AFM1	0.000	-0.000, 0.000	0.681
Model R^2	0.015		
Hb (g/dL)			
Cd	20.419	-20.225, 61.063	0.317
Pb	-6.246	-32.560, 20.068	0.635
AFM1	0.000	-0.000, 0.000	0.110
Model R^2	0.144		
WBC ($10^9/\text{L}$)			
Cd	55.014	-38.568, 148.596	0.242
Pb	-32.718	-93.307, 27.870	0.282
AFM1	0.000	-0.000, 0.000	0.164

Model R ²	0.072		
Platelets (10⁹/L)			
Cd	-370.417	-4868.432, 4127.598	0.869
Pb	310.495	-2601.697, 3222.686	0.831
AFM1	0.005	-0.001, 0.011	0.129
Model R ²	0.200		
Lymphocytes (%)			
Cd	92.738	-478.860, 664.336	0.745
Pb	44.326	-325.749, 414.401	0.810
AFM1	-0.001	-0.001, 0.000	0.095
Model R ²	0.099		
Monocytes (%)			
Cd	7.655	-103.083, 118.394	0.890
Pb	-31.012	-102.709, 40.684	0.388
AFM1	0.000	-0.000, 0.000	0.100
Model R ²	0.200		
Neutrophils (%)			
Cd	15.627	-595.643, 626.898	0.959
Pb	-99.171	-494.932, 296.590	0.616
AFM1	0.001	0.000, 0.002	0.006
Model R ²	0.197		
Albumin (g/dL)			
Cd	-45.013	-170.524, 80.497	0.473
Pb	64.373	-16.887, 145.634	0.117
AFM1	0.000	-0.000, 0.000	0.779
Model R ²	0.135		
ALT (U/L)			
Cd	205.796	-167.916, 579.507	0.273
Pb	-207.046	-449.001, 34.910	0.092
AFM1	0.000	-0.001, 0.000	0.632
Model R ²	0.167		
AST (U/L)			
Cd	346.619	-260.144, 953.382	0.256
Pb	-316.905	-709.748, 75.937	0.111
AFM1	0.000	-0.001, 0.001	0.572
Model R ²	0.170		
GGT (U/L)			
Cd	4.815	-344.645, 354.276	0.978
Pb	23.113	-203.141, 249.368	0.838
AFM1	0.000	-0.001, 0.000	0.111
Model R ²	0.069		
Total Bilirubin (mg/dL)			

Cd	-50.102	-220.987, 120.783	0.557
Pb	78.079	-32.558, 188.717	0.162
AFM1	0.000	-0.000, 0.000	0.140
Model R ²	0.148		
Total Protein (g/dL)			
Cd	98.783	-173.740, 371.306	0.469
Pb	-37.581	-214.023, 138.861	0.670
AFM1	0.000	-0.000, 0.000	0.597
Model R ²	0.108		
Urea (mg/dL)			
Cd	-4.144	-53.752, 45.464	0.867
Pb	-9.202	-41.321, 22.916	0.566
AFM1	0.000	0.000, 0.000	0.013
Model R ²	0.198		
Creatinine (mg/dL)			
Cd	183.885	-153.271, 521.042	0.277
Pb	-144.344	-362.632, 73.944	0.189
AFM1	0.000	-0.000, 0.001	0.378
Model R ²	0.122		
Sodium (mmol/L)			
Cd	120.453	-208.961, 449.866	0.465
Pb	-86.294	-299.570, 126.981	0.419
AFM1	0.000	-0.000, 0.001	0.724
Model R ²	0.047		
Potassium (mmol/L)			
Cd	16.705	-2.810, 36.220	0.091
Pb	-5.268	-17.903, 7.367	0.405
AFM1	0.000	-0.000, 0.000	0.308
Model R ²	0.257		
Chloride (mmol/L)			
Cd	-171.398	-489.822, 147.026	0.284
Pb	196.360	-9.800, 402.520	0.061
AFM1	0.000	-0.000, 0.000	0.977
Model R ²	0.251		

Hb-Haemoglobin (g/dL); WBC-White Blood Cell ($10^9/L$); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transferase (U/L); GGT-Gamma Glutamate (U/L); Cd-Cadmium ($\mu g/L$); Pb-Lead ($\mu g/L$); AFM1-Aflatoxin M1 (ng/mL).

Table 4.3 Multi Linear Regression of Cadmium, Lead and Aflatoxin M1 with Biochemical and Heamatological Outcomes Adjusted for Age,Weight and Height among males

Variable	Estimate	95% CI	P-value
Glutathione (GSH) (μmol/L)			
Cd	-0.431	-8.441, 7.578	0.911
Pb	-0.455	-5.891, 4.981	0.863
AFM1	0.000	-0.000, 0.000	0.688
Model R ²	0.114		
Hb (g/dL)			
Cd	14.860	-53.902, 83.622	0.656
Pb	-1.789	-48.459, 44.880	0.937
AFM1	0.000	-0.000, 0.000	0.372
Model R ²	0.321		
WBC (10⁹/L)			
Cd	96.847	-80.082, 273.776	0.266
Pb	-56.386	-176.469, 63.697	0.338
AFM1	0.000	-0.000, 0.000	0.171
Model R ²	0.128		
Platelets (10⁹/L)			
Cd	2117.459	-5697.893, 9932.811	0.577
Pb	-1225.389	-6529.722, 4078.945	0.634
AFM1	0.009	-0.000, 0.018	0.058
Model R ²	0.360		
Lymphocytes (%)			
Cd	113.137	-745.824, 972.098	0.786
Pb	22.638	-560.345, 605.621	0.936
AFM1	-0.001	-0.002, 0.000	0.216
Model R ²	0.261		
Monocytes (%)			
Cd	50.639	-103.543, 204.821	0.500
Pb	-57.107	-161.751, 47.537	0.268
AFM1	0.000	-0.000, 0.000	0.171
Model R ²	0.465		
Neutrophils (%)			
Cd	176.275	-784.614, 1137.164	0.705
Pb	-223.110	-875.272, 429.052	0.483
AFM1	0.001	0.000, 0.003	0.013
Model R ²	0.432		
Albumin (g/dL)			
Cd	-162.826	-375.581, 49.929	0.126
Pb	132.629	-11.769, 277.028	0.070

AFM1	0.000	-0.000, 0.000	0.402
Model R ²	0.277		
ALT (U/L)			
Cd	220.776	-410.241, 851.793	0.473
Pb	-246.014	-674.290, 182.262	0.244
AFM1	0.000	-0.001, 0.001	0.808
Model R ²	0.291		
AST (U/L)			
Cd	313.168	-754.060, 1380.395	0.546
Pb	-302.686	-1027.020, 421.649	0.393
AFM1	0.000	-0.002, 0.001	0.515
Model R ²	0.253		
GGT (U/L)			
Cd	-55.127	-559.891, 449.637	0.822
Pb	38.389	-304.198, 380.976	0.817
AFM1	-0.001	-0.001, 0.000	0.067
Model R ²	0.243		
Total Bilirubin (mg/dL)			
Cd	-31.308	-340.072, 277.456	0.834
Pb	83.303	-126.257, 292.863	0.416
AFM1	0.000	-0.001, 0.000	0.323
Model R ²	0.267		
Total Protein (g/dL)			
Cd	27.428	-475.726, 530.582	0.910
Pb	-13.612	-355.105, 327.882	0.934
AFM1	0.000	-0.001, 0.001	0.922
Model R ²	0.160		
Urea (mg/dL)			
Cd	-14.004	-101.347, 73.339	0.741
Pb	-6.192	-65.473, 53.088	0.829
AFM1	0.000	0.000, 0.000	0.041
Model R ²	0.341		
Creatinine (mg/dL)			
Cd	184.429	-362.080, 730.938	0.489
Pb	-176.172	-547.092, 194.747	0.333
AFM1	0.000	-0.000, 0.001	0.407
Model R ²	0.358		
Sodium (mmol/L)			
Cd	618.564	119.711, 1117.417	0.018
Pb	-443.779	-782.354, -105.203	0.013
AFM1	0.001	-0.000, 0.001	0.064
Model R ²	0.310		
Potassium (mmol/L)			

Cd	39.776	15.762, 63.790	0.003
Pb	-24.188	-40.487, -7.890	0.006
AFM1	0.000	-0.000, 0.000	0.407
Model R ²	0.658		
Chloride (mmol/L)			
Cd	-101.352	-582.692, 379.988	0.664
Pb	183.973	-142.715, 510.662	0.253
AFM1	0.000	-0.001, 0.001	0.961
Model R ²	0.317		

Hb-Haemoglobin (g/dL); WBC-White Blood Cell ($10^9/L$); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transferase (U/L); GGT-Gamma Glutamate (U/L); Cd-Cadmium ($\mu g/L$); Pb-Lead ($\mu g/L$); AFM1-Aflatoxin M1 (ng/mL).



Table 4.4 Multivariable Linear Regression of Cadmium Lead and AlatoxinM1 with Biochemical and Haematological outcomes Adjusted for Age, Weight and Height among females

Variable	Estimate	95% CI	P-value
Glutathione (GSH) (μmol/L)			
Cd	1.499	-11.739, 14.736	0.814
Pb	1.440	-5.618, 8.498	0.672
AFM1	0.000	-0.000, 0.000	0.300
Model R ²	0.134		
Hb (g/dL)			
Cd	-7.912	-80.148, 64.324	0.820
Pb	8.547	-29.968, 47.062	0.646
AFM1	0.000	-0.001, 0.000	0.065
Model R ²	0.260		
WBC (10⁹/L)			
Cd	41.373	-132.083, 214.830	0.621
Pb	-38.191	-130.676, 54.294	0.396
AFM1	0.000	-0.001, 0.001	0.541
Model R ²	0.123		
Platelets (10⁹/L)			
Cd	642.119	-7759.920, 9044.159	0.874
Pb	-967.534	-5447.396, 3512.327	0.654
AFM1	0.000	-0.044, 0.043	0.985
Model R ²	0.217		
Lymphocytes (%)			
Cd	-149.424	-1507.649, 1208.801	0.819
Pb	124.579	-599.609, 848.767	0.721
AFM1	0.004	-0.004, 0.011	0.305
Model R ²	0.107		
Monocytes (%)			
Cd	14.749	-209.703, 239.201	0.891
Pb	-34.175	-153.850, 85.500	0.555
AFM1	0.001	-0.000, 0.002	0.053
Model R ²	0.387		
Neutrophils (%)			
Cd	614.186	-517.805, 1746.177	0.268
Pb	-300.094	-903.658, 303.469	0.309
AFM1	-0.006	-0.012, -0.001	0.033
Model R ²	0.353		
Albumin (g/dL)			
Cd	65.552	-161.347, 292.452	0.550
Pb	36.123	-84.857, 157.103	0.537

AFM1	-0.001	-0.002, 0.000	0.051
Model R ²	0.349		
ALT (U/L)			
Cd	782.112	81.735, 1482.490	0.031
Pb	-375.822	-749.254, -2.389	0.049
AFM1	-0.001	-0.005, 0.002	0.468
Model R ²	0.358		
AST (U/L)			
Cd	268.892	-1041.216, 1578.999	0.670
Pb	-216.387	-914.920, 482.146	0.522
AFM1	0.000	-0.007, 0.007	0.990
Model R ²	0.109		
GGT (U/L)			
Cd	-412.394	-1044.585, 219.797	0.187
Pb	310.717	-26.359, 647.793	0.069
AFM1	0.000	-0.004, 0.003	0.849
Model R ²	0.256		
Total Bilirubin (mg/dL)			
Cd	-125.689	-465.775, 214.397	0.446
Pb	64.648	-116.682, 245.978	0.462
AFM1	0.001	-0.001, 0.002	0.537
Model R ²	0.076		
Total Protein (g/dL)			
Cd	315.373	-50.646, 681.391	0.087
Pb	-47.903	-243.059, 147.254	0.611
AFM1	-0.002	-0.004, -0.000	0.017
Model R ²	0.482		
Urea (mg/dL)			
Cd	14.147	-76.034, 104.328	0.745
Pb	-17.338	-65.421, 30.746	0.457
AFM1	0.000	-0.000, 0.000	0.920
Model R ²	0.292		
Creatinine (mg/dL)			
Cd	-258.047	-932.019, 415.926	0.430
Pb	113.030	-246.324, 472.383	0.516
AFM1	0.000	-0.004, 0.003	0.915
Model R ²	0.095		
Sodium (mmol/L)			
Cd	-57.675	-748.622, 633.272	0.862
Pb	75.591	-292.813, 443.995	0.671
AFM1	-0.001	-0.005, 0.002	0.525
Model R ²	0.086		

Potassium (mmol/L)			
Cd	-12.930	-54.860, 28.999	0.524
Pb	16.038	-6.318, 38.395	0.149
AFM1	0.000	-0.000, 0.000	0.421
Model R ²	0.239		
Chloride (mmol/L)			
Cd	-322.997	-1064.843, 418.849	0.371
Pb	211.112	-184.431, 606.655	0.276
AFM1	-0.002	-0.006, 0.001	0.191
Model R ²	0.341		

Hb-Haemoglobin (g/dL); WBC-White Blood Cell (10⁹/L); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transaminase (U/L); GGT-Gamma Glutamate (U/L); Cd-Cadmium (µg/L); Pb-Lead (µg/L); AFM1-Aflatoxin M1 (ng/mL).

4.5 Combined effects of heavy metals on haematological and biochemical parameters

In WQS regression analyses in Table 4.5, the combined exposure to cadmium, lead, and aflatoxin M1 showed generally non-significant associations with most haematological and biochemical biomarkers. A statistically significant negative association was observed for monocyte percentage ($\beta = -0.931$, 95% CI: -1.755 to -0.108, $p = 0.036$), with AFM1 (78%) and Pb (13%) being the major contributors. Chloride concentration demonstrated a significant positive association with the mixture ($\beta = 2.953$, 95% CI: 0.915 to 4.991, $p = 0.009$), primarily driven by Pb (65%) and AFM1 (31%). Although AST showed a marginal negative association ($p = 0.075$), its primary drivers were Pb (43%) and Cd (37%). Across biomarkers, cadmium was the dominant contributor for several haematological (WBC, lymphocytes, neutrophils) and biochemical outcomes (albumin, total bilirubin, total protein, sodium), while AFM1 strongly influenced ALT and monocytes, and lead contributed most to chloride and AST.

Table 4.5 Combined effect of Cadmium, Lead and Aflatoxin M1 on Biochemical and Haematological Biomarkers using Weight Quantum Sum (WQS) Regression, adjusted for age, weight and height.

Outcome	Estimate	95% CI	P-value	Cd weight	Pb weight	AFM1 weight
Hb (g/dL)	-0.091	-0.512, 0.329	0.674	0.424	0.063	0.513
WBC (10 ⁹ /L)	0.625	-0.183, 1.434	0.142	0.723	0.001	0.276
Platelets (10 ⁹ /L)	19.606	-11.967, 51.18	0.235	0.656	0.182	0.162
Lymphocytes (%)	-1.053	-6.057, 3.951	0.683	0.695	0.069	0.236
Monocytes (%)	-0.931	-1.755, -0.108	0.036	0.091	0.129	0.780
Neutrophils (%)	-1.357	-7.279, 4.566	0.657	0.628	0.132	0.241
Albumin (g/dL)	0.917	-0.149, 1.983	0.105	0.600	0.261	0.139
ALT (U/L)	-1.37	-3.320, 0.580	0.179	0.059	0.031	0.910
AST (U/L)	-3.766	-7.759, 0.226	0.075	0.372	0.433	0.195
GGT (U/L)	-0.159	-2.811, 2.493	0.907	0.838	0.162	0.000
Total Bilirubin (mg/dL)	-0.366	-2.084, 1.351	0.680	0.831	0.078	0.091
Total Protein (g/dL)	1.037	-1.609, 3.683	0.450	0.779	0.081	0.139
Urea (mg/dL)	-0.359	-0.793, 0.075	0.119	0.221	0.505	0.275
Creatinine (mg/dL)	-0.703	-3.238, 1.833	0.592	0.671	0.225	0.104
Sodium (mmol/L)	1.752	-0.020, 3.524	0.063	0.888	0.000	0.112
Potassium (mmol/L)	0.063	0.089, 0.215	0.425	0.521	0.085	0.394
Chloride (mmol/L)	2.953	0.915, 4.991	0.009	0.043	0.650	0.307
Glutathione (μmol/L)	0.004	-0.037, 0.045	0.849	0.517	0.009	0.473

Hb-Haemoglobin (g/dL); WBC-White Blood Cell (10⁹/L); ALT-Alanine Amino Transferase (U/L); AST-Aspartate Amino Transaminase (U/L); GGT-Gamma Glutamate (U/L).



CHAPTER FIVE

5.0 DISCUSSION

5.1 This study examined the levels of blood heavy metals and urine aflatoxin (AFM1) of participants.

The relationships between cadmium (Cd), lead (Pb), and aflatoxin M1 (AFM1) exposures and a range of hematological and biochemical biomarkers in male and female participants were investigated.

5.2 Biochemical and Haematological Studies.

The biochemical and hematological profiles were largely comparable across sexes, with the exception of higher gamma-glutamyl transferase (GGT) levels in males and lower total protein concentrations in females. The higher GGT levels in male children is consistent with previous research by **Ashraf et al., (2024)** whose studies showed that children shows elevated liver enzymes including GGT when exposed to aflatoxin. The low level of total protein in female children recorded also aligns with **Ashraf et al., (2024)**, who reports exposure of aflatoxin in children can affect protein synthesis. Aflatoxin continues to be a major problem in this district as the present study and the findings of Kumi et al. (2015; 2022) reported significant aflatoxin exposure in children. This observation indicates that children in the district are exposed to contaminated aflatoxin diet.

Significant levels of heavy metals were also detected in the blood of study participants. This aligns with the study of Adu et al., (2020) on assessing metal contaminants in milled maize products in the Ghanaian market, and report that different concentrations of metals

in maize has been recorded from different parts of the country with the highest coming from Ejura Sekyedumase district.

5.3 The effect of aflatoxin and heavy metals on hematological, liver and kidney function biomarkers in serum of participants.

Correlation analysis revealed that AFM1 showed a negative correlation with monocyte. Meaning high aflatoxin levels can negatively affect monocytes count and therefore weakening the immune system. Aligning with a study carried out on effect of aflatoxin on the immune system by Saha Turna et al. (2024). The correlation analyses also revealed that AFM1 was positively associated with neutrophil counts and urea levels, while Pb demonstrated positive associations with albumin and chloride, and Cd with potassium.

Regression models further highlighted that AFM1 was independently associated with increased neutrophil counts and urea concentrations in the overall sample, aligning with evidence that aflatoxins elicit inflammatory and renal stress responses. This aligns with a study done by Lentini et al. (2017) indicating that heavy metals do have effect on the kidneys. Sex-stratified analyses revealed that in males, Cd and Pb were strongly associated with alterations in sodium and potassium levels, suggesting that heavy metal exposures may disrupt electrolyte balance more prominently in males. In contrast, AFM1 was negatively associated with neutrophils in females, indicating possible immunological responses(Thenuwara et al., 2024). Additionally, female participants demonstrated significant associations of Cd and Pb with liver enzymes (ALT) due to high absorption of metals from low iron levels and increased detoxification capacity in females(Wahlang,

2023), highlighting the link between hepatocellular damage and heavy metal exposures.(Chang et al., 2023)

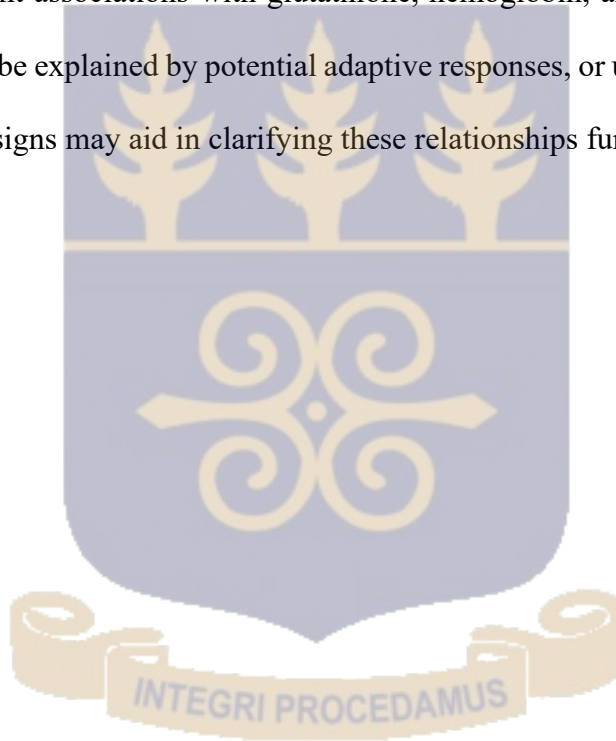
5.4 Determining the association between aflatoxin, heavy metals exposure and glutathione levels in study participants.

Glutathione, a key antioxidant defense biomarker, was not significantly associated with any exposure, despite showing a consistent trend toward negative correlations. In the present study, reduced GSH in the participants blood were lower (Table 4.1) than the acceptable ranges. The average median for male and female reduced glutathione levels for this study is 0.28 ± 0.10 - 0.31 ± 0.08 $\mu\text{mol/L}$ respectively, while the normal paediatric range is $2.0 - 6.0$ $\mu\text{mol/L}$ (Grunwell et al., 2015). Aflatoxin influences glutathione levels via decreasing reduced glutathione (GSH), principally through the induction of oxidative stress(Narasaiah et al., 2006). This occurs because the body uses GSH to neutralize the reactive oxygen species (ROS) produced by aflatoxin, leading to a depletion of GSH. However, the oxidative stress pathways potentially activated by these toxins were not adequately expressed within the cross-sectional framework of this study or may require chronic exposure levels to manifest measurable depletion.

The weighted quantile sum (WQS) regression provided insight into the combined effects of Cd, Pb, and AFM1. While most associations were non-significant, significant findings included a negative association with monocyte percentage, driven mainly by AFM1, and a positive association with chloride levels, primarily influenced by Pb (Table 4.5). These findings bring out the importance of considering mixture effects, as single exposure

analyses may underestimate the cumulative biological impact of environmental toxins. Particularly, the dominance of different exposures across biomarker categories suggests distinct mechanistic pathways: AFM1 influencing immune parameters, Pb affecting electrolyte balance, and Cd affecting protein and hepatic indices.

Taken together, the results highlight sex-specific and biomarker-specific effects of Cd, Pb, and AFM1 exposures, while also pointing to the complexity of mixture interactions. The lack of significant associations with glutathione, hemoglobin, and several hematological parameters may be explained by potential adaptive responses, or unmeasured confounders. Longitudinal designs may aid in clarifying these relationships further.



CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study demonstrates that exposures to cadmium, lead, and aflatoxin M1 are differentially associated with hematological, hepatic, renal, and electrolyte outcomes, with several notable sex-specific effects. While overall biomarker profiles were similar between males and females, AFM1 was linked to increased neutrophils and urea in the total sample, Cd and Pb were associated with electrolyte imbalances in males, and AFM1 showed a negative relationship with neutrophils in females. The combined effects analysis further revealed that mixtures of these toxicants may exert subtle but significant influences on immune and electrolyte biomarkers.

These findings highlight the importance of accounting for both individual and combined toxicant exposures when assessing human health risks. Moreover, the observed sex differences suggest that risk assessments should incorporate biological sex as a modifying factor in exposure-outcome relationships. Although many associations were non-significant, the identified patterns highlight the potential for even low-level environmental exposures to influence key physiological systems.

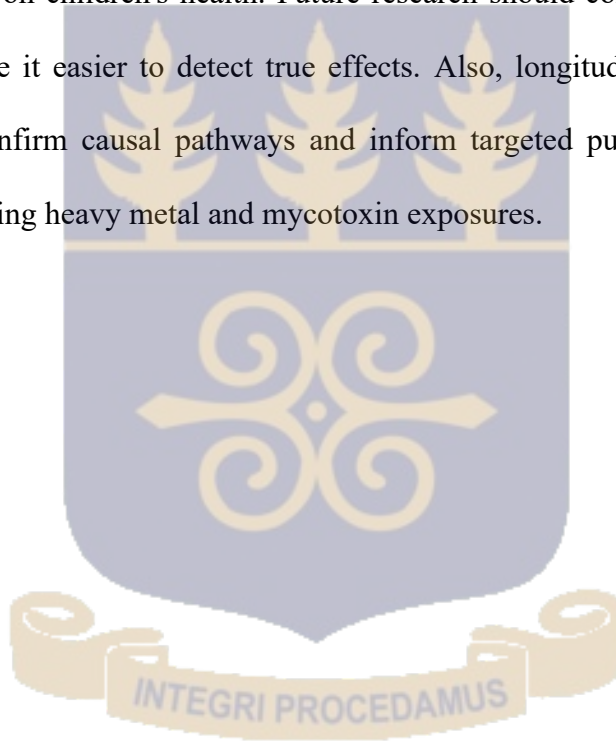
6.2 LIMITATIONS

Small sample size.

Cross section study design is a one-point data collection hence there is no follow up period to measure long term situation and new cases overtime.

6.3 RECCOMENDATION

Mothers should be educated on aflatoxin and heavy metals exposure and the developmental risk it can have on children's health. Future research should consider large sample size, which will make it easier to detect true effects. Also, longitudinal approach should be employed to confirm causal pathways and inform targeted public health interventions aimed at mitigating heavy metal and mycotoxin exposures.



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7.0 APPENDIX

7.1 APPENDIX I

7.1.1 INFORMATION SHEET

Dear Parent/Guardian,

I am Miriam Odamtten, an MPhil student of Chemical Pathology, University of Ghana. I kindly request your cooperation for your child to participate in a research study titled ASSESSMENT OF CO-EXPOSURE OF AFLATOXIN, LEAD AND CADMIUM IN CHILDREN FROM EJURA SEKYEDUMASE DISTRICT, ASHANTI REGION OF GHANA.

This study aims to assess the levels of aflatoxin and heavy metals in children, as well as their impact on glutathione, liver, and kidney functions. The information obtained will help identify health risks and develop strategies to prevent further health complications in children from the district. As part of the study, 5 mls of venous blood and a urine sample will be taken from your child for laboratory analysis. The blood will be analyzed to assess the level of damage to kidney, liver and glutathione levels, while the urine will be tested for the presence of heavy metals and aflatoxin. Additionally, you may be required to provide information regarding your child's dietary habits and socio-demographic data. Please answer honestly and to the best of your knowledge.

Participation: Your child's participation in this study is entirely voluntary. You may choose to withdraw your child from the study at any time without any consequences.

7.1.2 INFORMED CONSENT DOCUMENTS

Risk/Benefits

Children participating in this study were provided with valuable information on exposure to aflatoxins and heavy metals in the district. The risk associated with this study was minimal. However, children experienced slight discomfort during the collection of blood samples.

Confidentiality

All information obtained in this study was kept strictly confidential. Child's identity was not disclosed in any reports or publications resulting from the study. Officials from the ethics and protocol review committee of the College of Health Sciences, University of Ghana was however given access to some of the records as part of our duty to conduct this research efficiently.

CONSENT FORM.

I, _____, have read and understood the invitation for my child to participate in the research study titled Assessment of co-exposure of Aflatoxin, Lead and Cadmium in Children from Ejura Sekyedumase District, Ashanti Region of Ghana

I have received adequate information regarding the nature of the study, the procedures involved, and the potential risks and benefits. I am fully aware of my child's right to withdraw from the study at any point without any penalties or consequences.

I hereby give consent for my child to participate in this study.

Parent/Guardian's Signature: _____ Date: _____

Researcher's Signature: _____

Date:

7.1.3 CHILD ASSENT FORM (AGES 3–9 YEARS)

Study Title: Assessment of co-exposure of aflatoxin, lead and cadmium in children from Ejura Sekyedumase District, Ashanti region of Ghana.

Principal Investigator: Miriam Odamtten

Institution: University Of Ghana, College of health Sciences.

Why are we talking to you?

We are doing a research study and this is to learn how some substances like aflatoxin and heavy metals might affect children's health, especially the liver, kidneys, and certain chemicals in the body. We are talking to you to invited you to take part in it.

What will happen if I say yes?

If you say yes and want to be in the study:

- We will ask for a small amount of your blood and urine.
- A researcher, doctor or nurse will take the sample.
- This will take only a few minutes
- We will also ask you a few questions about your health and what you eat.

Will it hurt?

You might feel a small pinch when we take your blood, like a quick mosquito bite. Some kids say it doesn't hurt much. You may feel okay afterward, and we will help you stay comfortable.

Do I have to be in the study?

No. You do not have to be in the study if you don't want to. No one will be upset with you if you say no. Even if you say yes now, you can change your mind and stop at any time.

Will anything good or bad happen to me?

You won't get anything from this study but what we learn could help other children in the future. We don't think anything will happen to you, but if anything does, we will take care of you.

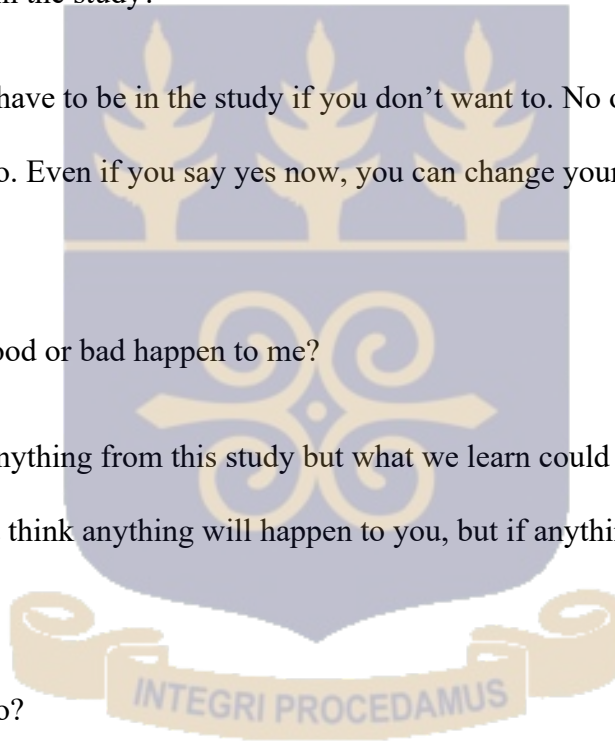
Who can I talk to?

If you have questions, you can talk to:

Miriam Odamtten: 0244774780, mijoy1983@gmail.com

You can also talk to your parents or someone you trust.

Do you want to be in this study?



Please circle one answer:

YES, I want to be in this study

NO, I do not want to be in this study

Your Name (Child): _____

Your Signature (or mark): _____

Date: _____

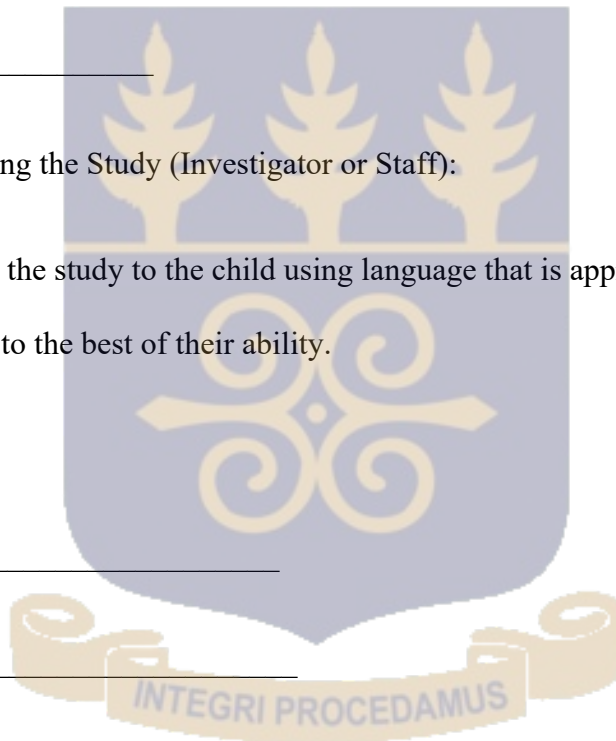
Person Explaining the Study (Investigator or Staff):

I have explained the study to the child using language that is appropriate and ensured they understood to the best of their ability.

Signature: _____

Name: _____

Date: _____

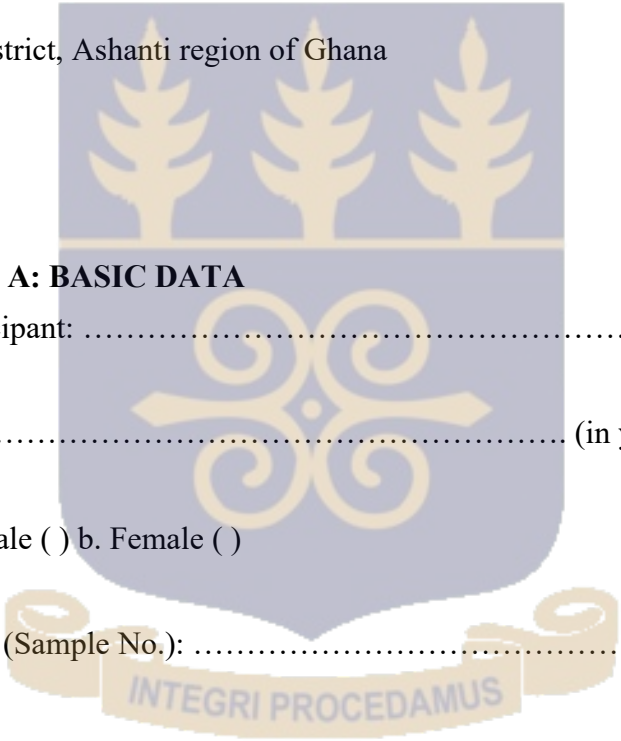


7.2 APPENDIX II

7.2.1 STUDY QUESTIONNAIRE

The assessment of co-exposure of aflatoxins, lead and cadmium in children from Ejura
Sekyedumase district, Ashanti region of Ghana

7.2.2 SECTION A: BASIC DATA

- 
- i. Name of Participant:
- ii. Age: (in years)
- iii. Gender: a. Male () b. Female ()
- iv. Study ID No. (Sample No.):
- v. Place of Birth:
- vi. Participant's Educational Background: a. Basic () b. Secondary () c. Tertiary ()
- vii. Place of Residence:

7.2.3 SECTION B: CLINICAL INFORMATION

Condition Yes [] No []

1. History of Sickle Cell Disease

2. History of G6PD Deficiency

3. History of Liver Disease

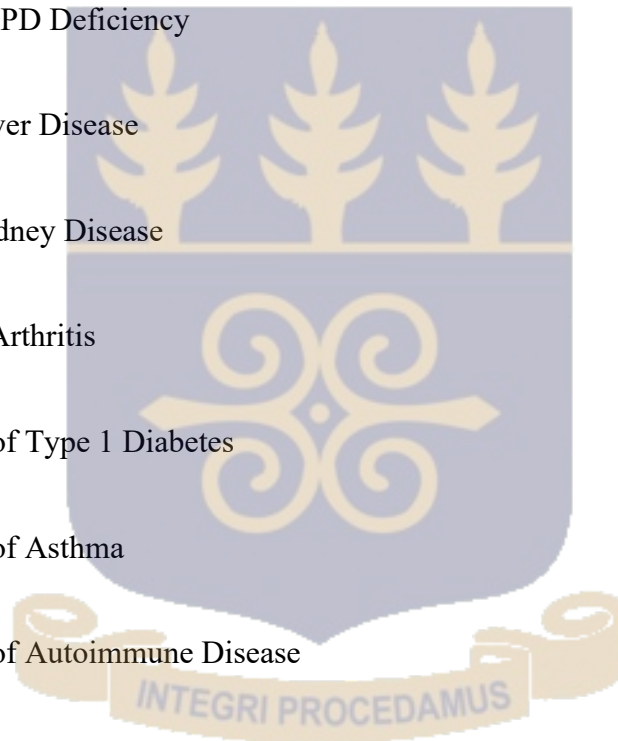
4. History of Kidney Disease

5. Rheumatoid Arthritis

6. Any History of Type 1 Diabetes

7. Any History of Asthma

8. Any History of Autoimmune Disease



7.2.4 SECTION C: ENVIRONMENTAL EXPOSURE

Instructions: Please indicate whether your child has been exposed to the following conditions.

Exposure Yes [] No []

1. Consumption of moldy or spoiled food?

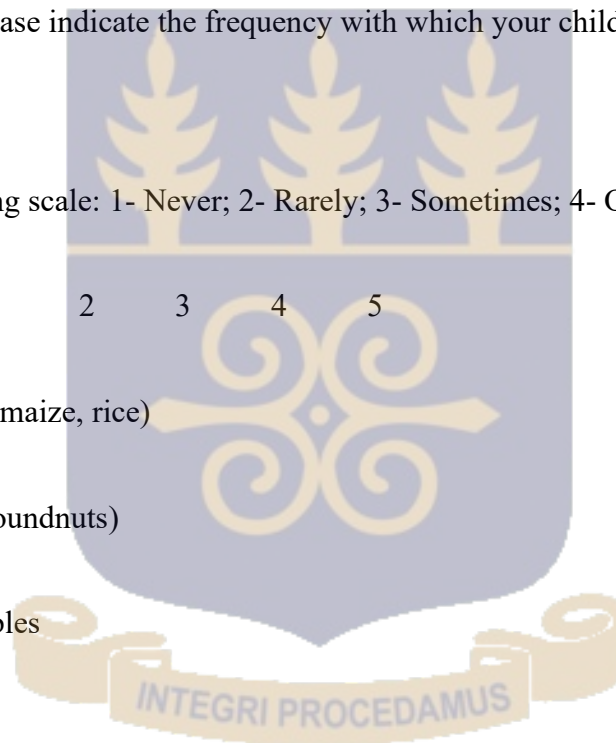
2. Drinking water from natural sources?
3. Living near agricultural fields?
4. Exposure to industrial waste?
5. Use of contaminated cooking or washing water?

7.2.5 SECTION D: DIETARY HABITS

Instructions: Please indicate the frequency with which your child consumes the following foods.

Use the following scale: 1- Never; 2- Rarely; 3- Sometimes; 4- Often; 5- Always

Food Item	1	2	3	4	5
1. Cereals (e.g., maize, rice)					
2. Nuts (e.g., groundnuts)					
3. Leafy vegetables					
4. Meat and fish					
5. Fruits (e.g., bananas, oranges)					



7.2.7 SECTION E: HEALTH STATUS

Instructions: Please provide information about your child's health status.

Health Condition Yes [] No []

1. Has your child experienced unusual fatigue or weakness?
2. Has your child shown signs of jaundice (yellowing of skin)?
3. Has your child had any recent infections?

