DETECTION AND QUANTIFICATION OF AFLATOXIN B_1 IN CULTURED NILE TILAPIA IN MVOMERO AND MBARALI DISTRICTS OF TANZANIA

GRAYSON APOLLO KISSAI

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ABSTRACT

This study was carried out to assess farmers' awareness as well as to detect and quantify the levels of Aflatoxin B1 in Nile tilapia (*Oreochromis niloticus*) farms in Mbarali and Mvomero districts. A total of 36 farmers and extension workers were involved in a questionnaire survey. Ninety two samples, tilapia (n=62), fish feeds (n=10), organic manure (n=10) and pond water (n=10) were collected in February 2013 and assessed for Aflatoxin B1 by a competitive ELISA technique. About 44.4% of the respondents were aware of Aflatoxin B1. This toxin was found in all samples collected although the levels between the samples differed significantly ($P < 0.05$). Out of 92 samples collected, 7.6% showed Aflatoxin B1 concentration greater than 5.0 µg/kg. Also, out of 62 fish samples collected from both Districts, 98% were contaminated with Aflatoxin B1 while the remaining fish samples had Aflatoxin B1 concentration greater than maximum tolerable limit of 5.0 µg/kg set by TBS/FAO/WHO. The concentration of Aflatoxin B1 in manure and fish samples was significantly higher in contamination ($p= 0.016$, and 0.001, respectively at 95% C.I) in samples collected in Mvomero compared with those from Mbarali District. In conclusion, most farmers were not aware of aflatoxins or of measures to control the toxins in the field, which begins with good agricultural and handling practices (GAP/GHP). Aflatoxin B1 levels in fish were below the internationally acceptable limits for human consumption. The detection of small quantities suggest a need for extensive mycotoxin awareness creation and education program as well as the adoption of good agricultural practices that include proper storage and handling of feeds in Mvomero and Mbarali Districts.

DECLARATION

I, Grayson Apollo Kissai, do here by declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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Grayson Apollo Kissai, Date

(MSc. in PH and FS candidate)

……..... .....……...…….....

Prof. R. Mdegela Date

(Supervisor)

……..... .....……

Prof. S. Chenyambuga Date

(Supervisor)

……..... .....……

Dr. N. Madalla Date

(Supervisor)

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DEDICATION

I would like to dedicate this work to my family, especially my wife Clara, my son Collins and daughter Carleen for their patience throughout this research work.

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LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
CAC	Codex Alimentarius Commission
CAST	Council for Agricultural Science and Technology
EEZ	Exclusive Economic Zone
FAO	Food and Agricultural Organization of the United Nations
GDP	Gross Domestic Product
ICMSF	International Commission on Microbiological Specifications for Food
IITA	International Institute of Tropical Agriculture
LSD	Least Significance Difference
MoAFC	Ministry of Agriculture, Food Security and Cooperatives
MLDF	Ministry of Livestock Development and Fisheries
MNRT	Ministry of Natural Resources and Tourism
MTL	Maximum Tolerable Limit
NBS	National Bureau of Statistics
RK-MFD	Republic of Kenya, Ministry of Fisheries Development
SUA	Sokoine University of Agriculture
TAFIRI	Tanzania Fisheries Research Institute
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
URT	United Republic of Tanzania
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The United Republic of Tanzania is one of the developing countries with an estimated population of about 45 million which grows at a rate of 3% per annum. Tanzania has Gross Domestic Product (GDP) per capita of US$ 280 (NBS, 2012). The country relies heavily on agricultural sector, which contributes about 27.6% of the Gross Domestic Product (GDP). The country has a total area of 945,040 square kilometres (URT, 2003) and well-endowed with numerous water bodies from marine to fresh water that contribute significantly to the fishery sector.

The country's total freshwater area is estimated to be about 54,337 square kilometres, while the marine territorial waters cover 64,000 square kilometres (MNRT, 1997) with a coastal-line stretching for more than 1,450 kilometres long. Its continental shelf is narrow and lacks favourable oceanographic conditions such as the upwelling phenomenon (MNRT, 1997). The Exclusive Economic Zone (EEZ) is estimated to be 223,000 square kilometres (MNRT, 1997).

1.2 Background to the Fisheries Production

The fishing activities in Tanzania are dominated by small-scale fisheries which are generally open access in nature with no limitations on entry (Mgawe, 2005). This has led to the steady increase in the number of fishers over the years giving rise to excessive levels of fishing and fierce competition for the fisheries resources which are threatened by over exploitation.

Many small-scale fishers have resorted to the use of poisons, explosives and highly destructive fishing gears, methods and techniques. The use of chemicals (poisons) in mass harvesting of fish, does not only affect targeted fish but also many other untargeted organisms and their environment (Sheela *et al*, 2014).

Generally, unauthorized fishing in small scale marine and inland capture fisheries has serious negative biological, economic and environmental implications for the continued availability of fish to support the communities and populations which are critically dependent on fishing and allied activities. The role of small-scale fisheries to the socio-economic advancement of Tanzania as a country is also threatened (FAO, 2000).

The available statistics data (Figure 1) suggest that fish landing from capture fishery has reached a plateau at 300,000 to 400,000 tonnes per annum while the human population is growing at about 3% per annum (Yoshida, 2010). This means fish supply per capita will continue to decline as the time goes on, unless effective efforts are made to promote aquaculture to augment the growing deficit in fish supply (Mgawe, 2005).

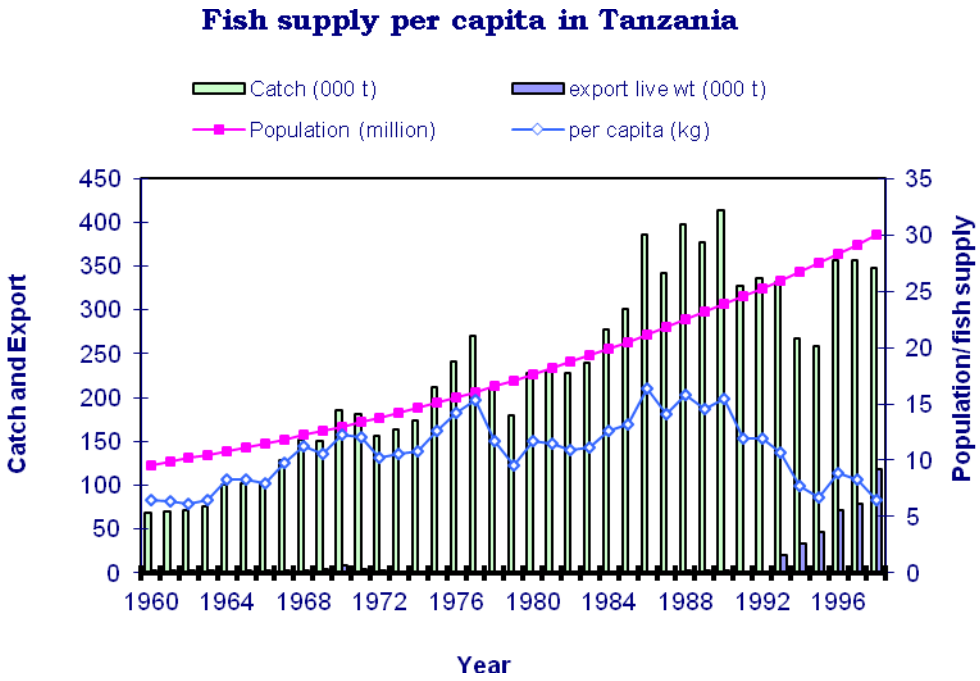

Figure 1: The relationship between fish supply per capita and population growth in Tanzania (Mgawe, 2005).

Aquaculture is the farming of freshwater and saltwater living thing including aquatic organisms such as fish, crustaceans, molluscs and plants under controlled conditions (FAO, 2008). Small-scale aquaculture offers another possible base for expanded fish production, though significant technical obstacles will have to be overcome to increase its adoption and productivity. Nile tilapia (*Oreochromis niloticus)* is the main species cultured in Tanzania because of its fast growth rate, tolerance to harsh environment and ease of culture (FAO, 2005). Thus, tilapia offers the possibility of commercial and home-grown protein sources to augment dwindling catch from capture fisheries which have been depleted (Yoshida, 2010).

1.3 Problem Statement and Justification

Aquaculture production in Tanzania is dominated by Tilapia which is largely dependent on quality of aquatic environment. They are usually raised in green water ponds, where special ponds (with water inlets and outlets) are dug, filled with water, fertilized and stocked. Feeding and day to day pond management are done aiming at improving fish growth and hence production. In these dugout ponds, natural food growth is enhanced by addition of organic manure from cattle, goats, poultry and pigs. These manures applied in a ponds may be contaminated with pathogenic and spoilage microorganisms.

Routine aquaculture practices such as feeding and fertilization increase the risks of pathogenic bacteria and fungal infections (Awoyemi, 1991 and Ogbondeminu, 1991). These may result into fish safety hazards; many remain latent and embedded in consumer. Amongst the important fungal groups of microorganisms are *Mucor, Aspergillus, Candida, Trichophyton* and *Cryptococcus*. Some of these produce mycotoxins with potential to affect humans (Hatai, 1989; Brown, 1993). *Aspergillus flavus* produces exclusively Aflatoxin B1, AFB1 (Reddy *et al*., 2005). Amongst the aflatoxins, Aflatoxin B1 is the most toxic form for mammals and presents hepatotoxic, teratogenic and mutagenic properties, causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Speijers and Speijers, 2004). It has been graded as a class one human carcinogen by the International Agency for Research on Cancer (IARC, 1993). Several disease outbreaks of aflatoxicosis in humans and animals have been reported due to the consumption of aflatoxin contaminated food and feeds (Reddy and Raghavender, 2007).

Since the discovery of the aflatoxins in the 1960s, regulations have been established in many countries to protect consumers from the harmful effects of mycotoxins that may contaminate food and animal feedstuffs. Setting limits for mycotoxins such as the naturally occurring aflatoxins was focused by various factors that play a significant role in decision-making processes. These include scientific factors to evaluate risk (such as the availability of toxicological data), food consumption data, knowledge about the level and distribution of mycotoxins in foodstuffs and feeds. Economic factors, such as commercial and food security issues, also have an impact. Weighing the various factors that play a role in the decision-making process to establish mycotoxin tolerances is therefore of crucial importance (FAO, 2003).

The comprehensive overview on worldwide regulations was published as the FAO Food and Nutrition Paper 64 in 1997. At that time, 77 countries had specific regulations for mycotoxins in different foods and feeds and 13 countries had general provisions, while about 50 countries did not have data available (FAO, 2003). The previous report by the United Republic of Tanzania confirmed that Tanzanian maize is contaminated with unacceptable levels of aflatoxins (URT, 1989). Tanzania regulates maximum limits of AFB1 and total aflatoxins in food at 5.0 µg/kg and 10 µg/kg respectively (Tanzania Bureau of Standards, TBS, 2004), but magnitude of aflatoxin contamination in fish farming in Tanzania is not known. In addition, the extent of knowledge regarding this toxin is rather limited.

Therefore, this study was undertaken to assess the farmers' awareness and investigate the extent of *Aflatoxins* B_1 contamination in tissues of fish grown in ponds that receive fertilizers and feeds from various organic sources.

1.4 Research Objectives

1.4.1 Overall Objective

The overall objective of this study was to assess farmers' awareness and prevalence of Aflatoxin B1 in tilapia farms in Mbarali and Mvomero Districts.

1.4.2 Specific Objectives

The following were the specific objectives:-

1. To assess the awareness of tilapia farmers in Mvomero and Mbarali Districts on Aflatoxin B1.
2. To establish the extent of contamination of Aflatoxin B1 in tilapia farms in Mvomero and Mbarali Districts.
3. To quantify the levels of Aflatoxin B1 in feeds and cultured tilapia tissues in Mvomero and Mbarali Districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aflatoxins

Aflatoxins are ubiquitous group of mycotoxins primarily produced by the fungus *Aspergillus*. These toxins are named from the fungus producing them; that is "A" from the genus name Aspergillus, "fla" from one of the species name flavus, which is added to toxin to give the name aflatoxin (Sweets and Wrather, 2009).

Fungi are eukaryotic multi-cellular filamentous cells. All fungi are chemo-heterotrophic that synthesizes organic compounds needed for growth and energy, from pre-existing organic sources in their environment using the energy supplied from chemical reaction (Lasztity, 2002). Fungi are mostly aerobic and are natural part of the environment. The range of temperature in which fungi are able to grow is 10-40°C; a pH range of 4-8; water activity (Aw) level above 0.7 and moisture content greater than 15% (Fraizer and Westhoff, 1988).

Mycotoxins are toxic compounds which are produced in mycelia of certain filamentous fungi, and are known to accumulate toxins in specialized structures such as conidia or sclerotia as well as in the environment of the surrounding fungus (Burge, 2001; Fischer and Dott, 2003).

When plant-based ingredients as substitutes for fish protein such as oil in aquafeeds are used, the risk of contamination by mycotoxins (naturally occurring filamentous fungi or moulds produces fungal toxins) increases. Currently, several potent mycotoxins have been identified and including some are of serious concern. Based on their toxicity and ubiquity,

the most common are aflatoxin, ochratoxin A, the trichothecenes (DON, T–2 toxin), zearalenone, fumonisin, and moniliformin (Bhatnagar *et al.*, 2004).

Aflatoxin a group of toxic metabolites, is of the main concern because of its hepato-carcinogenic, tetratogenic and mutagenic, as a result of bi-sidi-hydro-furano secondary metabolites produced by fungi, but their function are not dependent on the fungi's existence (Eaton and Gallagher, 1994). These effects have been seen in several species of animals. Ingestion of aflatoxin in infected foods has been implicated in the development of liver cancer (hepatoma) to humans and thyroid cancers in hatchery fish (Bukola *et al,* 2008).

The toxins can also be produced by certain species of *Asperigillus,* especially *A. flavus, A. parasiticus* and *A. nomius* and are common crop contaminants. Of these, aflatoxin B1 (AFB1) has been found in most feeds and foods and is highly carcinogenic (Eaton and Gallagher, 1994).

Aflatoxins may be present in food as a result of the organisms growing in the food matrix or through a more indirect route for example in milk from animals which have consumed contaminated feeds (CAST, 2003). It is important that both pre-harvest and post-harvest factors such as pest attack, soil condition, type of seed planted, transport and storage conditions are carefully controlled. These factors influence not only the presence of fungi, but also that of aflatoxins. Aflatoxins are thus able to develop at any stage from farm to fork as shown in Figure 2.

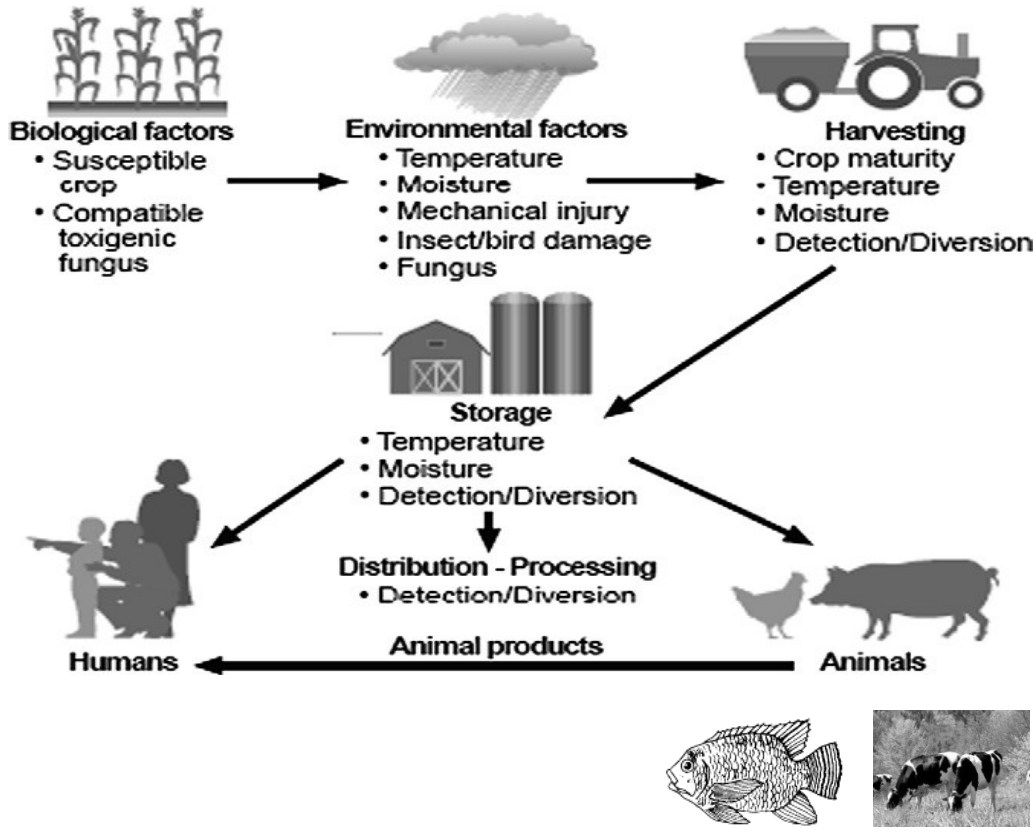

Figure 2: Factors affecting mycotoxins occurrence in the food chain (Modified from CAST, 2003).

Aflatoxin entering a cell may be metabolized in the endoplasmic reticulum to hydroxylated metabolites (Phase I bio-transformation), which may be further metabolized to glucuronide and sulphate conjugates (Phase II). The aflatoxin may alternatively be oxidized to the reactive epoxide, which undergoes hydrolysis and can bind to proteins resulting in cytotoxicity. The epoxide may then react with deoxyribonucleic acid (DNA), or protein, or be detoxified by the glutathione S-transferase enzyme (Riley and Pestka, 2005). Thus the level of metabolism determines the degree of toxicity and carcinogenicity (Patterson, 1978).

2.1.1 Undesirable health effects of Aflatoxins

Mycotoxins may affect many diverse cellular processes and have a wide spectrum of toxicological effects. This complexity is reflected in the very diverse range of responses by different animal species and it is likely that there will be a different response amongst individuals of the same race (Kuiper-Goodman, 2004).

Mycotoxin induced undesirable effects on the immune status of human have been reported (Jiang *et al*., 2005; Turner *et al*., 2003). A study by Azizz-Baumgartner *et al* (2005) reported that males are more likely to die from aflatoxicosis compared to females, in spite of eating similar quantity of contaminated food. The liver is the main target organ for aflatoxin toxicity and carcinogenicity (Abdel-Wahhab *et al*., 2007) and aflatoxins can induce liver lesions identical to those of liver cirrhosis (Amla *et al*., 1971; Shank *et al*., 1972). Aflatoxins can cross the placenta, thus affecting the foetus, and resulting in an increased incidence of still births and neonatal motality (Hendrickse, 1997; Maxwell, 1998; Wild *et al*., 1991).

Kwashiorkor, a disease in children, has also been associated with Aflatoxins, which occurs as a consequence of protein malnutrition (Adhikari *et al*., 1994). More than 5 million deaths of under 5 year old children in the developing world is due to malnutrition. Katerere *et al*. (2008) produced evidence implicating aflatoxin contamination as an important factor in the under-nutrition of infants. Some studies linked aflatoxicosis to infant growth faltering (Gong *et al.,* 2002; Gong *et al*., 2004; Polychronaki *et al*., 2007). The mechanism by which aflatoxins reduce growth rate is probably related to disturbances in protein, carbohydrate and lipid metabolism (Cheeke and Shull, 1985).

Mycotoxins have been ranked in a risk assessment field as the most important non-infectious and chronic dietary risk factor, higher than synthetic contaminants, plant toxins and food additives or pesticide residues (Kuiper- Goodman, 1998).

2.1.2 Carcinogenicity of Aflatoxin B1

The group 1 human carcinogen has been classified as Aflatoxin B1 with hepatic-cellular carcinoma as the most documented mycotoxin associated cancer. It may be diagnosed years after the symptoms appear, and persons who have been diagnosed with Hepatitis B infection and aflatoxin B1 exposure, are at a higher risk of developing hepatocellular cancer. As indicated in Figure 3, the acute symptoms following the ingestion of aflatoxin contaminated foodstuff or any other direct exposure to aflatoxins are vomiting, diarrhea, dizziness, fever, abdominal pain and seizures (Etzel, 2006; Krishnamachari *et al*., 1975).

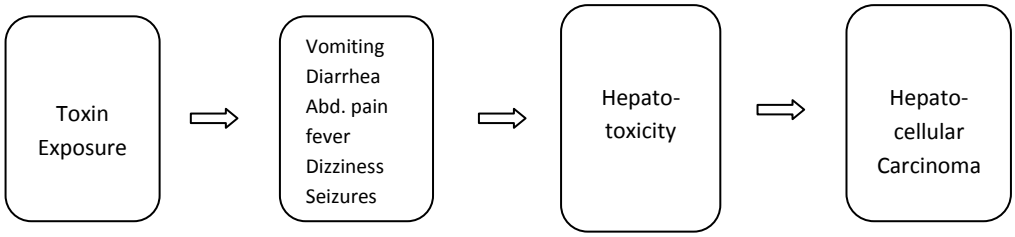

Figure 3: Time course of events preceding hepatocellular carcinoma (Etzel, 2006).

The active aflatoxin B1-8-9-epoxide (AFBO) is formed after enzymatic activation of aflatoxin B1 which is inactive form. This carcinogen may then bind with serum albumin forming a lysine adduct (Sabbioni *et al*., 1987). If, however, aflatoxin B1 binds with DNA then an AFB1-DNA adduct may form. The cell will then become an initial stage of tumour cell. This stage of tumour cell may remain dormant or become a differentiated neoplasm. As aflatoxin B1 is a full carcinogen, further biochemical changes may alter

neoplasm to a cancer, through the process of conversion and progression (Lu, 2003). Children in Taiwan and Alaska have been shown to have reduced incidence of hepatic-cellular carcinoma as a result of Universal immunization against hepatitis B (Chang *et al*., 1997; Lanier *et al*., 2003).

Also it has been documented that, the leading cause of cancer deaths in Qidong, China is hepato-cellular carcinoma, which seems to be attributed to the increased exposure of humans to aflatoxins (Wang *et al*., 1999; Ming *et al*., 2003). To lower the biological effective dose of aflatoxins, *Oltipraz,* a medicinal drug which decreases the metabolism of aflatoxin B1 to its carcinogenic form and increase the detoxification pathways of its metabolites is used (Wang *et al*., 1999).

It is estimated that 5.2 million cancer deaths occur annually with 55% of these cases being in developing countries (Pisani *et al*, 1999). It is hypothesized that early and repeated exposures to aflatoxins *in-utero* and during childhood might result in cancer of the liver, later in life (Dow, 1994). Based on the study of Miller and Marasas, (2002), the prevalence of hepatitis B and C is high in sub-Saharan Africa, and that aflatoxin consumption increases more than tenfold the risk of liver cancer.

Sub-Saharan Africa, where groundnut and maize are staple food ingredients are mostly affected by aflatoxin poisoning. Very little variation in diet occurs due to poverty and the limited availability of a variety of foodstuffs in the poorer countries especially in the rural areas. Since maize is a staple food in the African continent, the average individual is likely to consume a maize based product at every meal time. It has been reported that aflatoxin poisoning has been associated with ingesting maize which has been stored under damp

conditions (Lewis *et al*., 2005). Developing countries are undoubted at higher risk from mycotoxin contaminated food ingredients than developed countries. In developed countries, this may be attributed to both the high standards of the major food suppliers and retailers, as well as stringent regulatory enforcements deterring the importation of material exceeding the legislated permitted limits (IFST, 2009).

2.1.3 Aflatoxin B1 exposure in Tanzania

The exposure to mycotoxins may be through the ingestion of highly contaminated foodstuff or the continuous ingestion of contaminated foodstuffs at low concentration. Other routes of exposure include inhalation, direct contact and passive exposure resulting from a mycotic infection by a toxigenic fungus. As aflatoxins have been detected in the conidia of Aspergillus, it places the health of agricultural workers at risk, as they are continually exposed to the natural inhalation of dust in the fields (CAST, 2003).

It has been confirmed that food items contaminated by Aflatoxin do carry residue of the toxin to the consumers since it has a high melting point i.e. 250°C. Thus, it is certain that human beings are exposed to aflatoxin through contaminated food items among which fish is an important component (Bukola *et al.,* 2008).

The Tanzania Bureau of Standards (TBS) sets standards on many food commodities, taking into consideration the global standards as well as national production and consumption patterns. Although it is generally recognized internationally that there is no safe level of aflatoxin exposure, TBS has set the maximum acceptable limit for food at 5 ppb for Aflatoxin B1 (TBS, 2004 and FAO/WHO, 1992).

Based on previous study (TFDA and Abt, 2012), Aflatoxin contamination in maize and maize bran (main ingredient of fish feeds) as the by-product is above regulated levels for aflatoxins B1 in some parts of Tanzania. Focusing on the strain that is most toxic, aflatoxin B1 prevalence data from 2012 indicated contamination above regulated levels (5 ppb) in two zones, the Eastern zone (Morogoro) and the Western zone (Shinyanga), 43% and 40% of the maize samples had levels above 5 ppb; with average contamination of 50 ppb and 28 ppb, respectively. Studies on contamination in the Southern Highlands (Iringa, Mbeya, and Rukwa), revealed contamination above 5 ppb in only 4%; and in the Southern zone (Ruvuma), none of the samples had levels above 5 ppb (Figure 4).

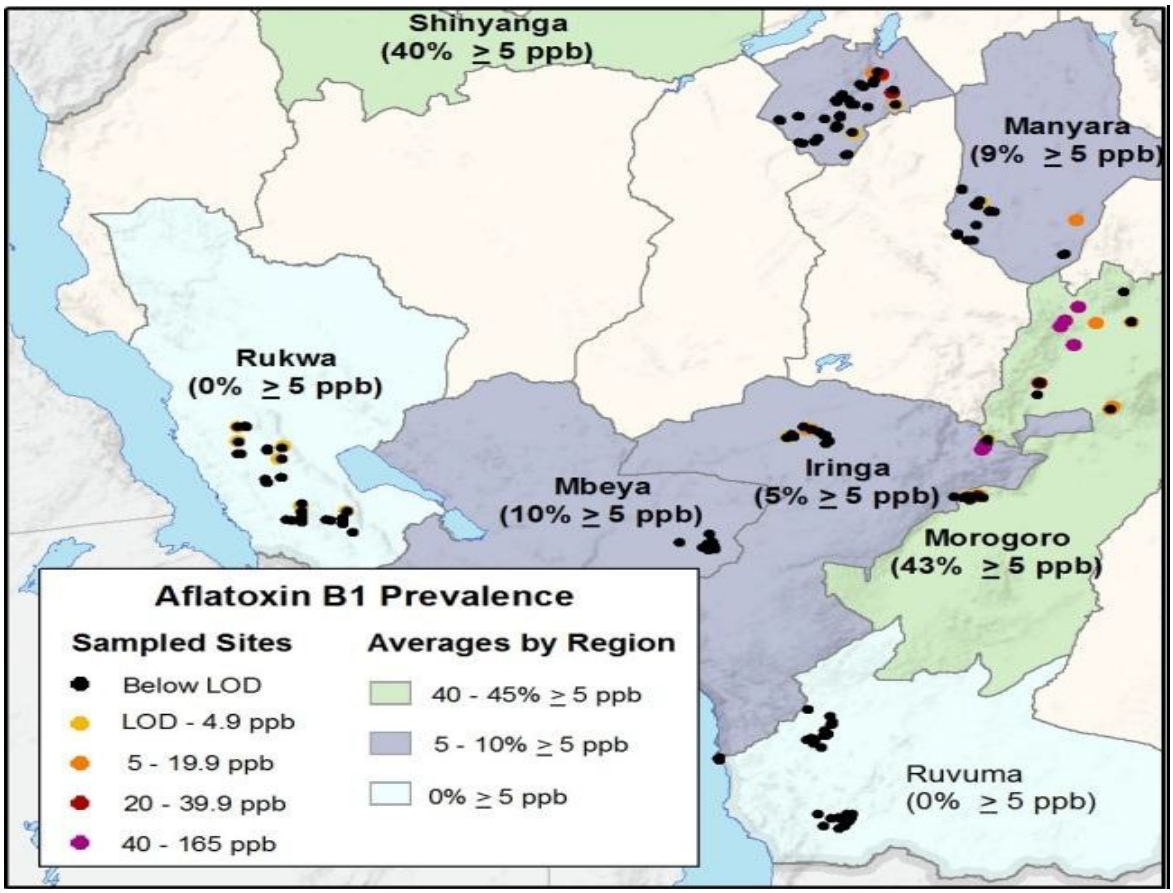

Figure 4: The prevalence of Aflatoxin B1 Contamination in Maize in Tanzania.

Source: TFDA, MoAFC and IITA, (2012).

2.2 Aquaculture Feeds

2.2.1 Tilapia Feeds

Tilapia feed on phytoplankton and zooplankton which originate from fertilized water in ponds. Fish ponds are usually fertilized with compost made with plant and animal wastes, animal manure and plant material. For fish feeding alternative sources of feeds such as small fish, animal by-products and grain by-products are increasingly being used as ingredients for supplementary diets (De Silva *and* Anderson., 1995; El-Sayed, 1999). These types of interventions have positive effect on fish growth, health and welfare and could increase fish productivity and production.

Also subsequent use of such ingredients or contaminated diets could reduce growth and reduce survival of fish. Studies have shown that, aquafeeds can serve as carrier of a range of microbial contaminants such as moulds, mycotoxins and bacteria (Maciorowski *et al*, 2007).

2.2.2 Crops and their by-products

Cereals and oilseeds as agricultural produce (plant-based ingredients) can be infected by mycotoxins producing moulds, particularly during crop growth, harvest, storage, processing or during the storage of the manufactured compounded feed. Suitable conditions for fungal growth, in terms of warm temperature and moisture, promote mycotoxin contamination. The production of aflatoxin increases at temperatures above 27°C, humidity levels above 62 % and moisture levels above 14 % in the feed (Bhatnagar *et al.*, 2004).

The climatic factors existing in the main aquaculture producing regions of the world, notably Asia, increase the risk of Aflatoxin contamination. The extent of contamination is

affected by the type of ingredient and feed storage practices and processing methods. Furthermore, long duration of transport under poor conditions and improper storage are crucial factors favouring the growth of aflatoxin-producing moulds. Consequently, poorer aqua farmers in developing countries, where quality control of feeds may not be as high as in developed countries, are more likely to acquire contaminated feeds. Additionally, the recent increase in prices of feed ingredients is likely to drive poor farmers to look for cheaper sources and run the risk of purchasing rejected or contaminated ingredients and feeds. As the principal route of such contamination is through ingredients of plant origin, the assessment of such contamination on cultured warm-water fishes, such as tilapia, carps, milkfish and catfishes (*Pangasius* spp.) is more significant because their diets contain more plant than animal ingredients (Lim and Webster, 2001).

2.2.3 Fish meal

It is a common practice in many Asian countries to use cheap fish as aquafeed. As prices for formulated feeds increase, there is a tendency for farmers to revert to using such sources of feed. The increased frequencies of use of cheap fish exasperate water quality problems and compromises fish growth and survival. The direct infections of cultured fish through the consumption of cheap fish containing high microbial loads, particularly of the streptococcal types and moulds producing aflatoxins, are well documented (Austin, 1997; Muroga, 2001; Ghittino *et al*. 2003).

Among the most important commercial fish species used in aquaculture feeds production in East Africa is *Rastrineobola argentea* from Lake Victoria. This fishery plays a significant role in the livelihood of more than 4 million people in terms of employment, income, aquaculture feeds and provision of nutrition thereby ranking as the most

important fishery in its contribution to the local and East African regional economy. The value of *Rastrineobola argentea* fishery in the East African region is US$ 200 million on the average compared to the regional total fishery value of about US$ 600 million when both the local and export sales values are considered (RK-MFD, 2010).

The use of poor manufacturing facilities and handling practices during preparation of fish feeds of *Rastrineobola argentea* and cereal for aquaculture may resulted into heavy contamination with aflatoxigenic fungi. Contaminated feeds at all levels are not acceptable for consumption due to the presence of Mycotoxins contents and prolong intake may constitute a health risk. Since most of the moulds isolated are probably contaminants rather than originating in the fish's sample, better methods of preservation will reduce their incidence or eliminate them (Bukola *et al.,* 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The general methodology adopted in this study comprised two stages. The first stage was based on the administration of questionnaires to the fish farmers and extension workers. The second stage was the analysis that identified the presence of *Aflatoxins* in cultured Nile tilapia.

3.1 Study area

This study was conducted in Mvomero and Mbarali districts in Morogoro and Mbeya regions located in the Eastern and the Southern Highlands zones, respectively.

Mvomero District lies between latitude 5° 58" and 10° 0" to the South of the Equator and longitude 35°25" and 35°30" East. The district is characterized by two rain seasons; short and long rain seasons. The short rain which starts in October and ends in January and the long rains season which commences in Mid-February and ends in May. The annual rainfall ranges between 600-1200 mm. However, there are some areas which experience exceptional droughts.The average temperatures in the region varies according to altitude but generally the range is 18-30^{o}C (URT, 1997a).

Mbarali District lies between latitudes 7^{o} and 9^{o} South of Equator and between longitudes 33^{o} and 35^{o} East of Greenwich. The average temperature ranges between 12^{o}C and 30^{o}C annually. Mean annual rainfall ranges from 650 mm to 800 mm per year, with 3 - 4 wet months (URT, 1997b).

3.2 Stakeholders survey

A cross-sectional exploratory study was carried out whereby both qualitative and quantitative data were collected according to Kothari (2004). The survey targeted fish farmers and extension workers from 10 farms of 10 villages (1:1) among which were supplied with fingerlings from Kingolwira fish production centre through EPINAV programme.

3.2.1 Assessment of awareness on Aflatoxin B1

In assessing farmers and extension workers awareness on aflatoxin B1 in the two Districts, the questionnaires were constructed in advance and used during face to face interviews with farmers culturing Tilapia and other extension workers. Among the villages selected in Mvomero District were Mvomero, Tangeni, Mkindo, Komtonga and Langali. While the villages interviewed in Mbarali District were Rujewa (Ubaruku), Rujewa Roman Catholic Mission Centre, Mbarali Rice farm, Igawa, Chimala, Igurusi, Mambi, Mswiswi and Kongolo.

3.2.2 Fish pond parameters

In different pond management practices, the important information was obtained by observation, interview and measuring water quality parameters. The features of the pond such as depth, length and width were taken at the beginning of sampling before determination of physicochemical parameters.

In situ measurement of the ponds water pH, salinity, TDS (Total Suspended Solids), water temperature, Dissolved Oxygen (DO), conductivity were done using a portable

Conductivity meter (HACH, CO150 conductivity meter, USA). Dissolved oxygen and pH were measured using a portable (HACH DO175, USA) DO meter and Orion field pH meter (210A, Orion Laboratories, USA), respectively.

3.2. 3 Sample Collection

Ninety two samples were collected from 10 different farms. Water samples were taken randomly from ponds with a column water sampler from at least two spots in each pond at a depth of 30 cm below the water surface. The samples were mixed together in a sterile 250 mL glass container and brought to the laboratory in icebox maintaining temperature at 4°C. Fish were caught by a local fishing gear and by cast net. On average 7 Nile tilapias were caught from different points in each pond in Mvomero and Mbarali Districts. Samples of fish (62), manure (10), feed (10) and water (10) were collected and kept separately at 4°C in a cool insulated icebox filled with ice packs, before being transported to Toxicology laboratory at Faculty of Veterinary Medicine, SUA for analysis of Aflatoxin B1 concentration.

In the laboratory each fish was rinsed with de-ionized water. It was aseptically dissected and parts of the muscle were then taken for analysis. 100g of each sample, fish tissue, manure, feed samples were homogenized separately in a blender in sterile Sodium Chloride (NaCl) buffered saline of pH 7.2 and then added with 100 mL of 80% Methanol/water to the jar. The mixture was blended for 1 minute in a high speed blender then filtered separately by Whatman number 41filter paper. 5ml of the filtrate was mixed thoroughly with 20ml of distilled water, re-filtered, stored in sterile bottles and analysed for aflatoxin within 24 hours.

3.3 Detection and Quantification of Aflatoxin B1 by ELISA

The materials used for ELISA included AFB1, Aflatoxin-Horse Radish Peroxidase (HRP) Enzyme Conjugate, Rabbit anti-Aflatoxin antibody, Substrate and Stop Solution (1NHCl) and coated microtiter plates with 96 wells from Abraxis LLC, Pennsylvania, USA (2013) were used. All other chemicals were of analytical grade or chemically pure.

The Aflatoxin B1 level was established as elaborated by manufacturer Abraxis LLC, Pennsylvania, USA, (2013) where all the reagents and sample extracts were allowed to reach room temperature prior to running the test. The appropriate number of test wells was placed into a microwell holder. Then 50 µL of enzyme conjugate was dispensed into each test well. By using a pipette with disposable tips, 50 µL of calibrators and samples were added to the appropriate test wells and were run in duplicates. Again 50 µL of Antibody Solution were dispensed into each test well then incubated for 10 minutes. After washing the wells and the last of the wash removed, 100 µL of Substrate were dispensed into each well, and incubated for 10 minutes. Lastly 100 µL of stop solution was added into each test well and the absorbance of the wells was read at 450 nm using a plate reader.

The validation was carried out through the determination of recoveries and the mean variation co-efficient for samples with different concentrations of AFB1 (0, 2, 7.5, 25 and 100 µg/kg). The calibration curve for ELISA is shown in appendix 3. The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (Percentage maximum absorbance). Therefore, the zero standards were made to equal 100% and the absorbance values were quoted in percentage as shown in appendix 3. The values calculated for the standards were entered in a system of co-ordinates using Microsoft excel against the

AFB1 concentration in parts per billion (ppb). The reporting of the results from ELISA was done in units of ppb so the calibration curve and ELISA results graph were displayed in ppb units.

3.7 Statistical Data analysis

Data obtained from the interview, were categorized to simplify analysis. Statistical software SPSS version 16 was used for analysis and calculations. Probability of 0.05 or less was considered significant.

From laboratory, the standard curve of % relative absorbance against concentration of the Aflatoxins B1 was used to determine the concentration of the sample. Aflatoxin B1 concentration in each sample was recorded into Microsoft Excel. Data were calculated as mean ± standard deviation (SD). Kruskal-Wallis test for non-parametric data at 95% confidence was used to compare the Aflatoxin B1 concentration between Mvomero and Mbarali Districts.

CHAPTER FOUR

4.0 RESULTS

4.1 Stakeholders Survey

4.1.1 Characteristics of the Respondents

In this study, most of the respondents were drawn from Mbarali. The results show that males were more involved in fish farming activities than females and the majority of the respondents had primary level of education (Table 1).

Table 1: Characteristics of the Respondents in Mvomero and Mbarali Districts (N =36)

	Category	**Mvomero (%)**	**Mbarali (%)**
Sex	Males	33.3	41.8
	Females	11.1	13.8
	100% (N=36)	**44.4**	**55.6**
Levels of Education	Informal	8.4	8.3
	Primary	25.2	27.6
	Secondary	2.5	5.8
	Diploma	8.3	13.9
	100% (N=36)	**44.4**	**55.6**

4.1.2 Fish pond parameters

In this study, most of the ponds were earthen type and the sources of water were rivers. Maize bran was the most common fish feed and the ponds were mostly fertilized with cattle manure. Average sizes of the ponds were 330 m^3 and 841 m^3 in Mvomero and Mbarali Districts, respectively. The values for physicochemical parameters are given in

Table 2. The levels of the physicochemical management of the ten (10) fish ponds in two Districts were determined by using standard equipment. The values of physic-chemical parameters of both Districts Mvomero (n=5) and Mbarali (n=5) respectively was between 27 ±1.3^{o}C to 25.2 ± 1.2^{o}C for temperature, 9.4 ± 4.3 mg L-1 to 6.8 ± 4.8 mg L-1 for DO, 7.6 ± 1.6 to 7.9 ± 0.7 for pH, 0.2 ± 0.08 g/l to 0.25 ± 0.04 g/l for salinity, 51.6 ± 2.5 cm to 61.5 ± 4.4 cm for transparency and 4.18 ± 1.6 µmhos/cm to 4.2 ± 0.5 µmhos/cm for electric conductivity. The essence of monitoring water quality in fish ponds, clearing ponds of rotten leaves, feacal matters and unconsumed feed to reduce organic matter which can impact adversely on water quality was also emphasized.

Table 2: The physicochemical parameters of water in fish ponds in Mvomero and Mbarali Districts

Parameter	**Mvomero** **Mean ± SD (n=5)**	**Mbarali** **Mean ± SD (n=5)**
Temperature (°C)	27 ±1.3	25.2 ± 1.2
DO (mg L-1)	9.4 ± 4.3	6.8 ± 4.8
pH	7.6 ± 1.6	7.9 ± 0.7
Salinity (g/l)	0.2 ± 0.08	0.25 ± 0.04
Transparency (cm)	51.6 ± 2.5	61.5 ± 4.4
Electric conductivity (µmhos/cm)	4.18 ± 1.6	4.2 ± 0.5

4.1.3 Pond management practices

For fertilization of pond water, a large proportion of the respondents (83 %) (N=36) used cattle manure and it was the most preferred manure. In case of fish feeds, a total of 90% (N = 36) of respondents that participated in the study used mixed feeds comprised of

cereal, fish meal and cotton/sunflower seedcake meaning any kind of feed that were available as shown in Table 3.

Table 3: Pond management technique for fish farmers used in Mvomero and Mbarali Districts (N=36)

Pond Management Techniques	Category	Percentage (%)
Sources of manure	Chicken	17
	Cow	83
Application of manure per batch	Once	6
	Twice	25
	Thrice	30
	Four times	39
Types of feeds	Maize bran	10
	Mixed feeds	90
Fish feeding per day	Once	50
	Twice	50

4.1.4 Farmers' awareness on Aflatoxin B1

Based on this study, a number of questions were used to assess awareness of farmers on AFB1. The number of ten (10) questions was categorized to simplify the analysis, where first categorization put the related questions in the three groups of assessment to farmer, (1) Occurrence of AFB1, (2) Prevention of AFB1 and (3) Consequences of AFB1. The second categorization considers the farmers' response, where two statuses namely correct and incorrect were analyzed. A proportion of the respondents 44.4 % (N=36) was aware of Aflatoxin B1. Of these, 36.1% (N=36) were women and 63.9% (N=36) were men in both Mvomero and Mbarali Districts. The overall farmers' awareness on AFB1 Figure 5:-

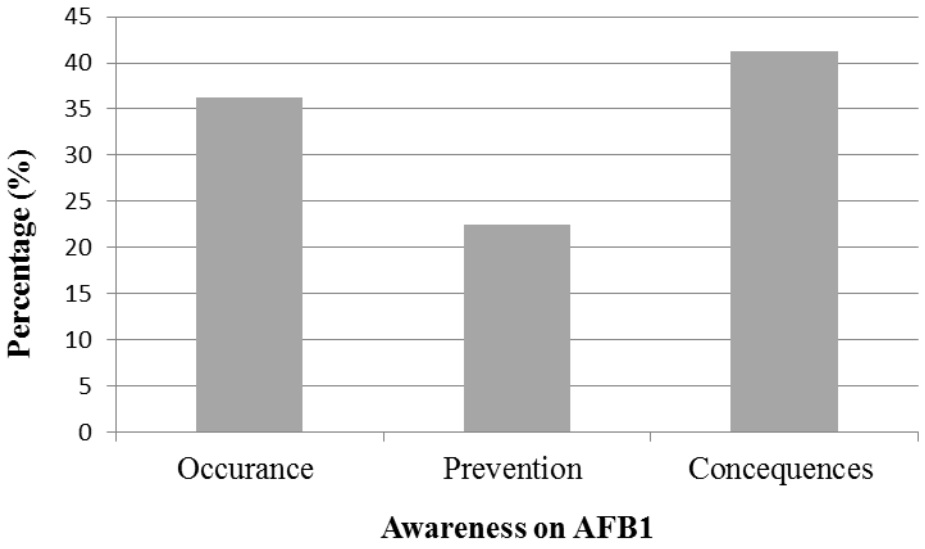

Figure 5: Response of farmers on awareness of Aflatoxin B1 in Mvomero and Mbarali Districts

4.2 Detection and Quantification of Aflatoxin B1 by ELISA

The ELISA method was used for the detection and quantification of AFB1. The percentages of contaminated samples in both Districts are shown on Table 4 below. It was found that 100% (92/92) of all samples were contaminated by aflatoxin B1. The mean Aflatoxin B1 levels together with their standard deviation for water sample, organic manure sample, fish feeds sample and fish muscles are shown on Table 5.

The intergroup analysis was done to compare the aflatoxin B1 contamination levels between Mvomero and Mbarali Districts. Kruskal-Wallis test for non-parametric data at 95% confidence interval revealed evidence of significant difference ($p=0.016$) in manure samples and ($p=0.001$) in fish samples. The fish ponds in Mvomero had higher average of aflatoxin B1 level compared to Mbarali District.

Table 4: Summary of results showing percentage of samples found positive and concentration for Aflatoxin B1 contamination from Mvomero and Mbarali Districts

Sample	N	% Positive samples	Lowest Value (ppb)	Highest Value (ppb)	% of aflatoxin B1 level > 5.0 µg/kg	
					Mvomero	Mbarali
Water	10	100	< 2	6	20	0
Organic Manures	10	100	< 2	4.8	0	0
Fish Feeds	10	100	< 2	>100	30	10
Fish Muscles	62	100	< 2	5.1	2	0

Table 5: Summary of Comparison of Aflatoxin B1 levels from Mvomero and Mbarali Districts

Sample	District	N	Mean	Std. Dev	P-Value
Water	Mvomero	5	4.3	1.9	
	Mbarali	5	3.1	0.3	0.117
Manure	Mvomero	5	3.5	1.2	
	Mbarali	5	1.4	0.6	0.016
Fish Feeds	Mvomero	5	39.3	50.4	
	Mbarali	5	2.9	2.3	0.465
Fish	Mvomero	5	2.4	1.2	
	Mbarali	5	1.4	0.8	0.001

CHAPTER FIVE

5.0 DISCUSSION

5.1 Characteristics of Respondents

The respondents involved in this study were from both Mvomero and Mbarali Districts. The overall number of men engaged in fish farming was higher than that of women, but women were involved more in feeding and pond management. The cultural practices and local customs in many communities in Tanzania make it difficult for a woman to own assets and land as these are acquired mainly through inheritance which favours men to women. This is why; men had more entrance to fish farming awareness and income adding from fish farming activities (Chenyambuga *et al*, 2014). This study was also supported by the findings of Seki and Maly (1993) that all fish ponds in Ruvuma region in Tanzania have possession of males, often the household heads.

According to this study, women has shown great participation in fish farming practices although, it is very important that women be empowered with knowledge about aflatoxin B1 contamination in feeds because they are responsible for family nutrition (Kang'ethe and Lang'a., 2009). Also the level of education attained by majority of the respondents was low. The majority of the farmers had informal education or had only attended primary school (Table 1). The low knowledge on the importance of good agriculture, handling practices and Aflatoxin B1 in Mvomero and Mbarali Districts might be because of the low level of education and exposure attained by majority farmers in both Districts, since by understanding these practices might be helpful to reduce contamination of Aflatoxins during handling of feedstuffs and feeding regime.

5.2 Pond Management Practices

5.2.1 Fish pond fertilization

Based on the results of this study, a large proportion of farmers were widely using cattle manure during pond fertilization and it was the most preferred type of manure. This was due to its availability (Table 3). The use of organic fertilizers has a long tradition in semi-intensive aquaculture. The management strategies of fish ponds for the lower levels of intensification involve the use of organic fertilizers to encourage natural food productivity of fish and to improve the levels of dissolved oxygen (Solomon *et al*, 2013). The organic manure from cow, goats, poultry or pigs was used to promote the greenish of water in fish ponds, which, in turn, lifts up fish productivity. Fish yields from such techniques have been found to be higher than those from normal unfertilized pond systems (Hickling., 1962; Hepher., 1963 and Green., 1992). When fertilizer is added to the ponds, it leads to increase in fish yields through soluble and/or particulate pathways. Release of soluble nitrogen and phosphorus stimulates algal production, which in turn can be consumed by fish directly or after intermediate processing by zooplankton or microbes (detritus formation) (Green, 1992).

5.2.2 Fish feeding

Based on the findings from this study, a large proportion of the respondents were using mixed feeds comprised of maize bran, fish meal and cotton/sunflower seedcake (Table 3). Maize is a food that is part of the staple diet in Sub-Saharan Africa (Kimanya *et al*., 2008). It is consumed at a level of about 400 g per person per day (Muriuki and Siboe 1995) and its by- product maize bran is consumed directly or after processing by animals including cultured fish. It was observed that, the maize bran used for feeding fish in both Districts was wet and stored in closed plastic bags which create high temperature and

humidity which favours the growth of moulds. Most of the farmers were not applying good agricultural practices, especially in handling and storage of feeds. Such practices should be addressed so as to reduce some associated risks that may occur (Brown *et al*., 1999; Chen *et al*., 2004).

5.3 Farmers' awareness on Aflatoxin B1

The awareness of farmers on Aflatoxin B1 varied among men and women. The awareness on aflatoxin B1 was 44.4% in the two Districts (Figure 5 and 6). Awareness is important for enhancing the ability to make appropriate feed choices, improving pond management and application of good agricultural practices.

The aflatoxin information is required to alert the population on health and risks posed by aflatoxin contamination of a staple commodity such as maize. According to the previous study done by TFDA and Abt (2012); the populations in both Districts were exposed to unacceptably high levels of aflatoxin in maize grains. The level of contamination of maize samples was above regulated levels (5ppb) in the eastern (Morogoro) and the southern highlands (Iringa, Mbeya and Rukwa) zones by 43% and 4% respectively; with an average contamination of 50ppb.

The farmers awareness on aflatoxin B1 is very important among the fish farmers in these District since it helps to trigger behavioural changes if populations targeted believe in the information received and/or understand the aflatoxin fully enough to be convinced to change old habits and non-good agricultural practices. The aflatoxin awareness may alert people on the existence and nature of the Aflatoxin B1. However, public awareness on knowledge-intensive ideas, such as aflatoxin contamination, should not be regarded as

stand-alone interventions to tackle effectively the problems being addressed. Experiences in Asia underline the need for a twin approach in which information are complemented by learning in order to effectively convince millions of people to adopt certain agronomic practices (Escalada and Heong, 2004). This underlines the need to integrate such awareness with sustained efforts in local capacity building on aflatoxin management. An example in this study was a farmer participatory research to demonstrate the effect of the toxin on broilers and layers and test remedial measures to decontaminate feed from the toxin (Adda *et al.,* 2003).

5.4 Detection and Quantification of AFB1

5.4.1 Water Samples

This study showed that all of the pond water samples from Mvomero and Mbarali Districts were contaminated with aflatoxin B1. Twenty percent (n=10) of water samples were contaminated with AFB1 at levels above the Tanzania MTL of 5.0 μg/kg (Table 4). The samples which exceeded the MTL for AFB1 were from Mvomero District (TBS, 2004 and FAO/WHO, 1992). In water samples, the concentration of aflatoxin B1 from Mvomero District was comparable between the Districts ($p>0.05$) as shown in Table 5. Food contamination can be linked with various sources, like pests, domestic animals, flies, nightsoil , polluted water , unclean utensils and pots, dirty hands, and a polluted environment caused by lack of sanitation, domestic animal droppings, dust and dirt (Motarjemi *et al*., 1993). Water like feed for animals, is a vehicle for the transmission of many agents of diseases (Kirby *et al*., 2003). The source of water used in Mvomero and Mbarali Districts was a river, in which continuous fertilization and addition of artificial feeds for fish possibility may have acted as a vehicle of transmission and cannot be ruled out.

5.4.2 Manure samples

In this study, it was found that 100% (n=10) of the samples were contaminated with AFB1 at levels below the Tanzania MTL of 5.0 µg/kg compared to the standards reported by TBS, (2004) and FAO/WHO, (1992) as shown in Table 4. Fertilization of ponds involved a lot of handling, thus increasing the chance of contamination with microbes coming from the surrounding environment. Most of the sources were located not far from the animal holdings or either drainage system of the animal waste. This increased the possibility of contaminating the products as dusts and other pollutants may contaminate the manure involved in the fertilization. Also absence of clear drainage system or any other suitable means of disposing waste water in a sanitary manner might have increased the chances of contamination. The concentration of AFB1 was significantly higher in manure collected in Mvomero than in Mbarali District ($p=0.016$) as shown in Table 5. The observation suggest that, failure to control animals and pests including rodents, flies, insects or vermin infestation were likely to be sources of contamination.

Good agricultural practices and behaviour reduces the chance of contaminating the manures. However, the overall fish farming practices were unsatisfactory. Such conditions and practices are likely to cause cross contamination of organic manure. The high percentage of unsatisfactory pond parameters and the premises with low hygiene need further attention. Food borne illnesses can be prevented by good agriculture and hygiene practices (Scott, 2000). To prevent the occurrence of food bone illnesses, it is therefore important to ensure that organic manure is safe and hygienic.

5.4.3 Feed samples

This study showed that all the feed samples from Mvomero and Mbarali Districts were extremely contaminated with aflatoxin B1. Ideally, animal feeds and many other food products are expected to have pathogenic micro-organism (TBS, 2007; Kimanya 2008). According to TBS, (2004) and FAO/WHO, (1992), 40% (n=10) of the feed samples from both Districts were contaminated with AFB1 at the levels above the Tanzania MTL of 5 µg/kg as shown in Table 4. Forty percent (n=5) of samples from Mvomero exceeded by far the MTL of AFB1 with values of 80 µg/kg and >100 µg/kg. For feeds, the concentration of AFB1 in Mvomero was comparable to that of Mbarali (p=0.465) as shown in Table 5. Generally, the environment around the fish ponds was also not clean and this can lead to the contamination of the product by microbes. However, poor hygiene practices of food handlers during feed preparation and also use of contaminated raw materials might have resulted in contamination of the final product.

In various stages of production; harvesting, transportation, storage or processing, the mycotoxins may be produced (CAST, 2003; Murphy *et al*., 2006). Approximately 25 to 50% of world's agricultural crops are contaminated with mycotoxins (Betran and Isakeit, 2003, Fandohan *et al*., 2003) among which, aflatoxins (AF) and fumonisins (FB) are the most significant. The feed ingredients when stored for a long time, especially if coupled with favourable ecological factors such as high temperature and relative humidity, provide an ideal condition for fungi to develop. According to Dashti *et al*. (2009), the feed ingredients used and the seasonal effects from the area of origin of feed, are all factors which have an effect on the levels of aflatoxin contamination in feeds. Usually,

the amount between 1% and 6% of aflatoxin B1 may be present in contaminated feeds, however values as high as 6% have been reported too (Pittet, 1998).

It was observed that, most of the storage sheds used in aquaculture ponds were not adequately enclosed, ventilated and protected against the harsh elements of nature. Seasonal rains can initiate mould growth in feed ingredients which are not stored under-cover, and fungal spores may be carried by wind settling on feed ingredients resulting in possible mycotoxin development. Also the respiration of feed ingredients tends to occur due to increased environmental temperatures, thus creating hotspots which may support the growth of storage fungi with potential to produce a range of mycotoxins.

5.4.4 Fish Muscle (tissue) samples

The results for aflatoxin B1 concentration showed that, the toxins were found in all fish samples suggesting that these toxins may have originated from feeds and manure used to fertilize ponds. The finding revealed that, all fish samples (n=62) were contaminated with AFB1. Two percent (n=62) of these samples contamination exceeded the MTL of 5.0 µg/kg of AFB1 (TBS, 2004), (FAO/WHO, 1992) as shown in Table 4, this fish samples was from Mvomero District. For fish sample, the concentration of AFB1 in Mvomero District was significantly higher compared to that of Mbarali District ($p=0.001$) as shown in Table 5. It is possible that aflatoxins were found in fish feeds and subsequently transmitted to the fish through ingestion. It is important to point out that in the case of fish as in mammals, Aflatoxin B1 are accumulated fundamentally in the liver and hence Aflatoxicosis (Deng *et al*., 2010); meaning that it is in this tissue that the greatest aflatoxin concentration will be found if the animal has been exposed. The elimination of

this organ before human consumption through evisceration significantly reduces the risk from ingesting aflatoxins as a result of consuming these foods.

Aflatoxicosis is a disease that can affect many species of fish, as feed contaminated with aflatoxins is eaten by the fish or human (Ashley, 1970). Farmers should bear in mind that animals that feed on aflatoxin contaminated feeds may not show symptoms of aflatoxin toxicity. The risk of cancer due to various forms of aflatoxin is based on the cumulative lifetime dose (FAO/WHO, 2007). The extent of disease caused by consumption of feeds contaminated with aflatoxins depends on the dose, the age and species of the fish. Fry are more susceptible to aflatoxicosis than adults and some species of fish are more sensitive to aflatoxins than others (Royes and Yanong, 2002).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed low awareness of aflatoxin B1 among fish farmers and extension workers. The awareness level on aflatoxin is still very low in both Districts, while the use of maize bran as fish feed is very high. This implies that even small levels of aflatoxin contamination in maize bran could present a high risk of aflatoxin exposure in both Districts. Combined with the often unobservable effects of aflatoxins, makes it difficult to incentivize and inform farmers on the risks associated with aflatoxins. Most of the farmers were neither aware of aflatoxins, nor of measures to control aflatoxins in the field, which begins with good agricultural and handling practices (GAP/GHP). This study demonstrates that fish feeds are the main source of aflatoxin B1 contamination in pond water and fish cultured. In Nile tilapia samples collected in local fish ponds across the two Districts, all samples were contaminated with AFB1, although their levels were below the Tanzania maximum tolerable limits and internationally acceptable limits for human consumption.

6.2 Recommendations

- Extension services for fish farming activities should be strengthened up to the ward and village level so as to increase the understanding on good aquaculture practices so as to reduce the level of aflatoxins contamination in all possible sources.
- In view of awareness, agricultural extension workers advising farmers on good agricultural practices should be provided with a mycotoxin control package of good agricultural practices that they should advocate to farmers in the rural areas for adoption.
- Gender participatory training programmes should be conducted to improve women roles and level of involvement in fish farming activities.
- The appropriate technologies for minimizing aflatoxins in maize during farming, transport, storage and preparation for use need to be adopted by farmers and households in the rural places. These could include adoption of fungal resistant varieties and good agricultural and handling practices such as use of fertilizers in growing maize and sorting to remove mouldy maize prior to storage and use. Since the maize bran as the by-product is the main source of feed for fish farming in Tanzania.
- Other measures could include campaigns to sensitize the public on effects of mycotoxins in human health and their impact on the economy.
- More research is needed to develop improved strains of agricultural crop and better quality and fish feed rations from locally available feed resources.
- Governments and regulatory authorities should set standards in acquisition of raw materials, production procedures of animal feeds.

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Appendix 1: Aflatoxin B1 Analysis form

Date:……..

Type of the analysis required:……..

Name of the Laboratory:……

Experiment done by:……..

Position:…….

			Results for Laboratory analysis				
SN	**WELL Content**	**OD**	**Mean OD**	**SD**	**RSD**	**% BO**	**CONCENTRATION (ppb)**
1							
2							
3							
4							
5							
6							
7							
8							
9	**SAMPLE**						

Analysis done by:……

Position:……..

Name of the laboratory:……..

Date:……

Appendix 2: Questionnaire

QUESTIONNAIRES

Personal information

1. Respondent Name:……..
2. Ward Name:…….
3. Village name:……
4. Sex of respondent

   1 = Male, 2 = Female **[ ]**
5. Education status

   1 = Primary Education, 2 = Secondary Education, 3 = Vocational Education,

   4 = Diploma, 5 = University Education **[ ]**

No	Questions	Tick your answer	(*v*)
	Manure		
1	Which type of organic manure do you use for pond fertilization?	1 = Cow, 2 = Chicken, 3 = Goat, 4 = Pig, 5 = Other specify	[ ]
2	How many times do you applying manure in Tilapia farming?	1 = 0, 2 = 1, 3 = 2, 4 = 3, 5 = 4	[ ]
3	From your experience, which type of manure do you prefer mostly for pond fertilization?	1 = Cow, 2 = Chicken, 3 = Goat, 4 = Pig, 5 = Other specify	[ ]
	Feeds		
4	Which type of supplementary feeds do you use for fish?	1 = Natural food, 2 = Cereals, 3 = Trash, 4 = Mixed, 5 = Other specify	[ ]
5	How many times do you feeding Tilapia?	1 = 0, 2 = 1, 3 = 2, 4 = 3, 5 = 4	[ ]

6	From your experience, which type of feeds do you prefer mostly?	1 = Natural food, 2 = Cereals, 3 = Trash, 4 = Mixed, 5 = Other specify	[ ]
	Aflatoxins		
7	What is Aflatoxins?	1 = Toxins, 2 = Fish feeds, 3 = Chemicals, 4 = Witches, 5 = I don't know	[ ]
8	Where does Aflatoxins come from/ produced?	1 = Bacteria, 2 = Fungi, 3 = Virus, 4 = Algae, 5 = I don't know	[ ]
9	What type of food/environment favours Aflatoxins production?	1 = Cereals, 2 = Meat, 3 = Nuts, 4 = Fruits, 5 = I don't know	[ ]
10	How can you prevent Aflatoxins from contamination?	1 = Mixing foods, 2 = Separating foods, 3 = Cooking foods, 4 = Washing foods, 5 = I don't know	[ ]
11	How can you inhibit Aflatoxins production in food/ feeds? By Maintaining…….	1 = Wet environment, 2 = Moisture and humidity environment, 3 = Dry environment, 4 = All of the above, 5 = I don't know	[ ]
12	What are the effect/ infection of Aflatoxins to human health?	1 = Toxins, 2 = Poisoning, 3 = Safe, 4 = Death, 5 = I don't know	[ ]
13	Which diseases can be caused by Aflatoxins in human?	1 = Carcinogen (hepatoma), 2 = Kwashiorkor, 3 = Diarrhea, 4 = HIV-Aids, 5 = I don't know	[ ]
14	Is it possible for fish to have/ be	1 = YES, 2 = NO,	

	contaminated with Aflatoxins?	If YES, Explain……	[ ]
15	How can fish be contaminated with Aflatoxins?	1 = Feeding, 2 = Contact, 3 = Drinking water, 4 = Sunlight, 5 = I don't know	[ ]
16	Can fish be affected by Aflatoxins?	1 = YES, 2 = NO	[ ]
17	How can fish be affected by Aflatoxins?	1 = Harvesting rate, 2 = Swimming rate, 3 = Feeding rate, 4 = I don't know 5 = Production rate,	[ ]

……

……

……

Appendix 3: Standard curve

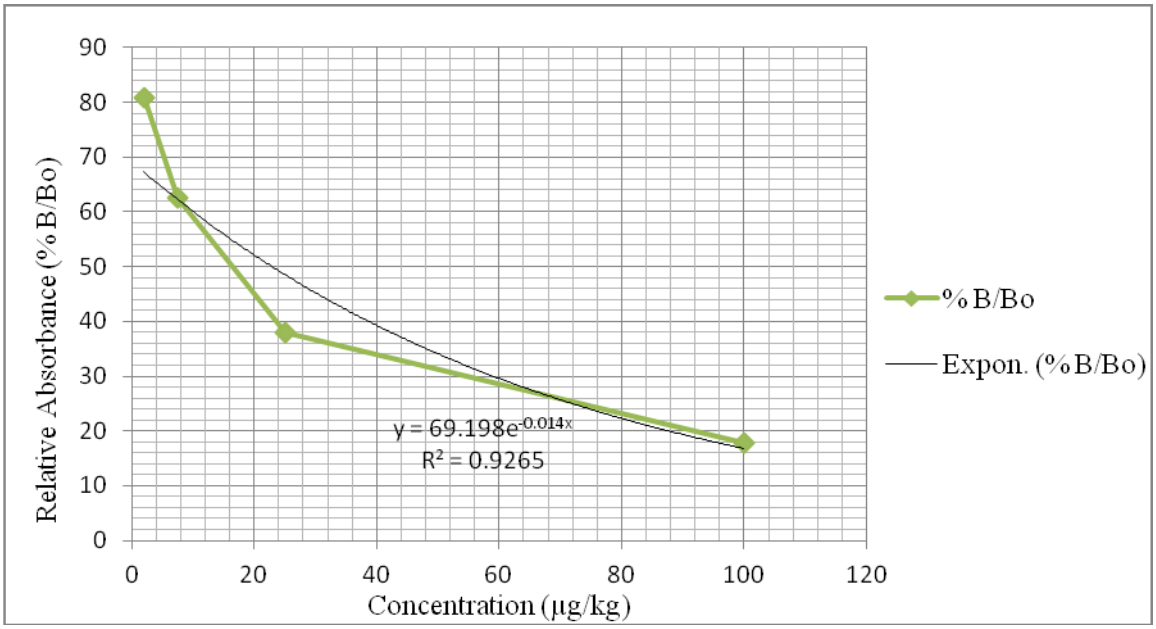

Figure 5: Graph of Relative Absorbance (%) against Concentration of Aflatoxin B1 (ppb)

A regression value of : $y = -0.5416x + 67.993$

$R^2 = 0.9265$

This value shows good agreement ($R^2 = 0.9265$) indicating that the matrix effect on the ELISA assay could result in a slightly estimation of the aflatoxin content.

Appendix 4: AFB1 concentration in Water Sample

Well Content		OD1	OD2	Mean OD	STDEV	% RSD	% B/Bo	Conc (PPB)
0		2.08	2.025	**2.05**	0.03	1.34		
2		1.785	1.532	**1.66**	0.13	7.63	80.8	**2.0**
Std 7.5		1.388	1.182	**1.29**	0.10	8.02	62.6	**7.5**
25		0.845	0.712	**0.78**	0.07	8.54	37.9	**25**
100		0.423	0.307	**0.37**	0.06	15.89	17.9	**100**
Water Sample	**Mvomero**	1.543	1.527	**1.54**	0.01	0.52	74.8	**3.8**
		1.382	1.416	**1.40**	0.02	1.22	68.2	**6.0***
		1.449	1.502	**1.48**	0.03	1.80	71.9	**4.9**
		1.437	1.385	**1.41**	0.03	1.84	68.7	**5.5***
		1.799	1.798	**1.80**	0.00	0.03	87.6	**<2**
	Mbarali	1.631	1.563	**1.60**	0.03	2.13	77.8	**3.3**
		1.632	1.567	**1.60**	0.03	2.03	77.9	**3.2**
		1.583	1.518	**1.55**	0.03	2.10	75.5	**3.4**
		1.573	1.534	**1.55**	0.02	1.26	75.7	**2.8**
		1.597	1.483	**1.54**	0.06	3.70	75.0	**2.9**

Appendix 5: AFB1 concentration in Manure Sample

Well Content		OD1	OD2	Mean OD	STDEV	% RSD	% B/Bo	Conc (PPB)
0		2.13	2.091	**2.11**	0.02	0.92		
2		1.78	1.696	**1.74**	0.04	2.42	82.4	**2.0**
Std 7.5		1.313	1.291	**1.30**	0.01	0.84	61.7	**7.5**
25		0.747	0.793	**0.77**	0.02	2.99	36.5	**25**
100		0.362	0.36	**0.36**	0.00	0.28	17.1	**100**
Manure Sample	**Mvomero**	1.792	1.699	**1.75**	0.05	2.66	82.7	**<2**
		1.717	1.641	**1.68**	0.04	2.26	79.6	**2.7**
		1.693	1.547	**1.62**	0.07	4.51	76.8	**3.5**
		1.563	1.535	**1.55**	0.01	0.90	73.4	**4.4**
		1.598	1.438	**1.52**	0.08	5.27	71.9	**4.8**
	Mbarali	2	1.994	**2.00**	0.00	0.15	94.6	**<2**
		1.839	1.687	**1.76**	0.08	4.31	83.5	**<2**
		1.94	1.884	**1.91**	0.03	1.46	90.6	**<2**
		1.894	1.873	**1.88**	0.01	0.56	89.2	**<2**
		1.738	1.72	**1.73**	0.01	0.52	81.9	**2.1**

Appendix 6: AFB1 concentration in Feed Samples

Well Content		OD1	OD2	Mean OD	STDEV	% RSD	% B/Bo	Conc (PPB)
0		2.08	2.025	**2.05**	0.03	1.34		
2		1.785	1.532	**1.66**	0.13	7.63	80.8	**2.0**
Std 7.5		1.388	1.182	**1.29**	0.10	8.02	62.6	**7.5**
25		0.845	0.712	**0.78**	0.07	8.54	37.9	**25**
100		0.423	0.307	**0.37**	0.06	15.89	17.8	**100**
Feeds Sample	**Mvomero**	1.822	1.84	**1.83**	0.01	0.49	89.2	**<2**
		1.79	1.741	**1.77**	0.02	1.39	86.0	**<2**
		0.476	0.479	**0.48**	0.00	0.31	23.3	**80***
		1.306	1.309	**1.31**	0.00	0.11	63.7	**7.0***
		0.195	0.216	**0.21**	0.01	5.11	10.0	**>100***
	Mbarali	1.533	1.699	**1.62**	0.08	5.14	78.7	**3.0**
		1.707	1.778	**1.74**	0.04	2.04	84.9	**<2**
		1.55	1.75	**1.65**	0.10	6.06	80.4	**2.0**
		1.053	1.593	**1.32**	0.27	20.41	64.5	**6.9***
		1.764	1.825	**1.79**	0.03	1.70	87.4	**<2**

Appendix 7: AFB1 concentration in Fish Muscles (tissue) Sample in Mvomero

Well Content	OD OD1	OD2	Mean OD	STDEV	%RSD	% B/Bo	Conc (PPB)
0	2.13	2.091	**2.11**	0.02	0.92		
2	1.78	1.696	**1.74**	0.04	2.42	82.4	**2.0**
Std 7.5	1.313	1.291	**1.30**	0.01	0.84	61.7	**7.5**
25	0.747	0.793	**0.77**	0.02	2.99	36.5	**25**
100	0.362	0.36	**0.36**	0.00	0.28	17.1	**100**
	1.48	1.509	**1.49**	0.01	0.97	70.8	**5.1***
	1.649	1.551	**1.60**	0.05	3.06	75.8	**3.7**
	1.791	1.75	**1.77**	0.02	1.16	83.9	**<2**
	1.575	1.578	**1.58**	0.00	0.10	74.7	**4.0**
	1.647	1.574	**1.61**	0.04	2.27	76.3	**3.6**
	1.819	1.791	**1.81**	0.01	0.78	85.5	**<2**
	1.873	1.949	**1.91**	0.04	1.99	90.5	**<2**
	1.89	1.944	**1.92**	0.03	1.41	90.8	**<2**
	1.897	1.89	**1.89**	0.00	0.18	89.7	**<2**
	1.817	1.573	**1.70**	0.12	7.20	80.3	**2.5**
	1.572	1.598	**1.59**	0.01	0.82	75.1	**3.9**
	1.604	1.73	**1.67**	0.06	3.78	79.0	**2.9**
	1.597	1.714	**1.66**	0.06	3.53	78.4	**3.0**
	1.619	1.722	**1.67**	0.05	3.08	79.2	**2.9**
	1.619	1.732	**1.68**	0.06	3.37	79.4	**2.8**
	1.892	1.994	**1.94**	0.05	2.62	92.1	**<2**
	1.696	1.998	**1.85**	0.15	8.18	87.5	**<2**
	1.919	2.024	**1.97**	0.05	2.66	93.4	**<2**
	1.892	1.75	**1.82**	0.07	3.90	86.3	**<2**
	1.92	2.001	**1.96**	0.04	2.07	92.9	**<2**
	1.612	1.772	**1.69**	0.08	4.73	80.2	**2.6**
	1.646	1.884	**1.77**	0.12	6.74	83.6	**<2**
	1.649	1.797	**1.72**	0.07	4.29	81.6	**2.2**
	1.725	1.699	**1.71**	0.01	0.76	81.1	**2.3**
	1.714	1.715	**1.71**	0.00	0.03	81.2	**2.3**
	1.837	1.679	**1.76**	0.08	4.49	83.3	**<2**
	1.905	1.847	**1.88**	0.03	1.55	88.9	**<2**
	1.856	1.838	**1.85**	0.01	0.49	87.5	**<2**
	1.656	1.603	**1.63**	0.03	1.63	77.3	**3.4**
	1.618	1.522	**1.57**	0.05	3.06	74.4	**4.1**
	1.574	1.551	**1.56**	0.01	0.74	74.0	**4.2**

Fish Samples (Mvomero District)

Appendix 8: AFB1 concentration in Fish muscles Sample in Mbarali

Well Content	OD OD1	OD2	Mean OD	STDEV	%RSD	% B/Bo	Conc (PPB)
0	2.08	2.025	**2.05**	0.03	1.34		
2	1.785	1.532	**1.66**	0.13	7.63	80.8	**2.0**
Std 7.5	1.388	1.182	**1.29**	0.10	8.02	62.6	**7.5**
25	0.845	0.712	**0.78**	0.07	8.54	37.9	**25**
100	0.423	0.307	**0.37**	0.06	15.89	17.8	**100**
Fish Samples (Mbarali District)	1.803	1.789	**1.80**	0.01	0.39	87.5	**<2**
	1.658	1.668	**1.66**	0.01	0.30	81.0	**<2**
	1.91	1.925	**1.92**	0.01	0.39	93.4	**<2**
	1.943	1.897	**1.92**	0.02	1.20	93.5	**<2**
	1.806	1.765	**1.79**	0.02	1.15	87.0	**<2**
	1.736	1.469	**1.60**	0.13	8.33	78.1	**3.0**
	1.676	1.65	**1.66**	0.01	0.78	81.0	**<2**
	1.756	1.577	**1.67**	0.09	5.37	81.2	**<2**
	1.936	1.81	**1.87**	0.06	3.36	91.3	**<2**
	2.002	1.869	**1.94**	0.07	3.44	94.3	**<2**
	1.846	1.782	**1.81**	0.03	1.76	88.4	**<2**
	1.842	1.8	**1.82**	0.02	1.15	88.7	**<2**
	1.779	1.825	**1.80**	0.02	1.28	87.8	**<2**
	1.701	1.723	**1.71**	0.01	0.64	83.4	**<2**
	1.832	1.712	**1.77**	0.06	3.39	86.3	**<2**
	1.698	1.717	**1.71**	0.01	0.56	83.2	**<2**
	1.597	1.653	**1.63**	0.03	1.72	79.2	**3.0**
	1.936	2.095	**2.02**	0.08	3.94	98.2	**<2**
	1.954	2.215	**2.08**	0.13	6.26	101.6	**<2**
	1.807	1.954	**1.88**	0.07	3.91	91.6	**<2**
	1.838	1.96	**1.90**	0.06	3.21	92.5	**<2**
	1.809	1.972	**1.89**	0.08	4.31	92.1	**<2**
	1.797	1.895	**1.85**	0.05	2.65	89.9	**<2**
	1.768	1.979	**1.87**	0.11	5.63	91.3	**<2**
	1.781	1.81	**1.80**	0.01	0.81	87.5	**<2**
	1.776	1.734	**1.76**	0.02	1.20	85.5	**<2**
	1.752	1.918	**1.84**	0.08	4.52	89.4	**<2**
	1.707	1.778	**1.74**	0.04	2.04	84.9	**<2**
	1.56	1.75	**1.66**	0.10	5.74	80.6	**2.0**
	1.553	1.558	**1.56**	0.00	0.16	75.8	**2.9**
	1.593	1.539	**1.57**	0.03	1.72	76.3	**2.8**