AFLATOXIN CONTAMINATION OF MAIZE

FROM DIFFERENT STORAGE

LOCATIONS IN GHANA

By KNUST

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Declaration

I hereby declare that this submission is my own work towards the award of an M.Sc. degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

The contamination of maize by aflatoxins in Ghana is of major concern because of the health hazards associated with it. This study focused on the role played by variations in climatic factors such as relative humidity and rainfall on aflatoxin contamination of maize in different maize storage locations. The study was carried out on samples collected over a period of 10 years (1990 to 1999) in three Districts (Ejura-Sekyedumase, Afram Plains/North-Kwahu and Nkoranza) well – known for maize production in Ghana. The aim was to study the influence of storage locations on levels of aflatoxin contamination and distribution in maize. The findings indicated that significant difference exists between the aflatoxin contamination levels of samples collected from Ejura-Sekyedumase and Nkoranza (p<0.05). Also there was a significant difference between the aflatoxin contamination levels of samples collected from Ejura-Sekyedumase and North-Kwahu (p < 0.05). There was no significant difference between the contamination levels of samples from North- Kwahu and Nkoranza (p>0.05). The total aflatoxin levels in samples from Ejura-Sekyedumase, North-Kwahu and Nkoranza over the period were 120.50 ppb, 153.20 ppb and 134.17 ppb respectively. For the period 1990 to 1999 the aflatoxin distributions in the storage locations showed that Nkoranza had the highest level in 1997 and 1999 while North-Kwahu had the highest in 1990, 1991, 1993 and 1998. Similarly, Nkoranza and North-Kwahu had equal levels of 10.50 ppb in 1995. The three locations had equal levels of 9.50 ppb in 1994. On the whole, Ejura-Sekyedumase had fair distribution levels since it was the only location with its highest level far below the acceptable level of 20 ppb for humans. I hereby recommend that further research must be conducted in other districts in the country in order to create awareness of the health hazards associated with the aflatoxin contamination.

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"TO GOD BE THE GLORY".

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ABBREIATIONS

AOAC	Association of Official Analytical Chemists
APOD	Afram Plains Development Organization
B1, B2	Blue 1 & 2
BMZ	German Ministry of Economic Co-operation and Development
CEC	Commission of European Community
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
EHSO	Environmental Health Service On Line
EU	European Union
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
Fig.	Figure
G	Green
GAP	Good Agricultural Practices
Н	Hour
На	Hectare
HACCP	Hazard Analysis Critical Control Point
IARC	International Agency for Research on Cancer
IgA	Immunoglobulin A
IITA	International Institute of Tropical Agriculture
M1, M 2	Metabolites 1 & 2
Mins	Minutes
mm	Millimetre
Nm	Nanometre
Ng/ml	Nanogramme/milliliter
Ppb	Parts per billion
PPMED	Policy Planning Monitoring and Evaluation Department
SPSS	Statistical Package for Social Sciences
SRID	Statistical Research and Information Directorate
µg/kg	Microgramme/kilogramme
US	United States
USDA	United States Development Agency
WHO	World Health Organization
WFP	World Food Programme

DEDICATION

To the memory of my late maternal grandmother-"Mama Dziafa", known in private life as madam Agbetsialor Dzisa, queen mother/women's wing leader of the 1960s CPP regime of Dzita East-Anloga, Volta region.



CHAPTER ONE

1.0 INTRODUCTION

1.1. Maize production

Maize (Zea *mays*) is the third most important crop after rice and wheat cultivated in the world. Global maize prdouction increased from 200 million tonnes to 600 million tonnes from 1963 to 2003 (FAOSTAT 2006). Besides, FAOSTAT (2005) indicated that 692,034,148 tonnes of maize representing 31% of the world's cereals production was produced. In another development FAOSTAT (2005) stated in the annual cereal crop harvest report that from 2002-2004 an average of 654,907,048 tonnes of maize was produced on an average land area of 143,354,935 hectares. These figures show the importance of maize production worldwide, hence the need to adopt Good Agricultural Practices (GAP) in its production statistics based on the area cropped (ha) and the corresponding quantity produced (tonnes) from 1996 to 2006 is shown in Table 1.1

Table 1.1: The national maize production statistics from 1996 to 2006

SOURCE: [1996-1999: PPMED-MOFA, 2001-2006: SRID-MOFA].

Maize is the most widely consumed staple food in Ghana according to a nationwide survey in 1990 which revealed that 94% of all households consumed maize during an arbitrarily selected two-week period (Aldeman and Higgins, 1992). Boateng *et al.* (1990) reported that analysis based on 1987 maize consumption data in Ghana showed that maize and maize based foods accounted for 10.8% of food expenditure by the poor and 10.3% of food expenditure by all income groups. In Kumasi, maize is processed into grits and sold to Accra Breweries Limited as a domestic replacement for imported malt (Mills, 2002). Other uses of maize include baking, preparation of snack foods, animal feed, and silage, adhesives, and production of oil (Neilsen, 2003).

Maize just as any other crop can be contaminated with storage fungi, some of which may develop as by-products of mycotoxins that can be harmful to animal and human health. Mycotoxins that develop from *Aspergillus flavus*, a common post harvest fungi in maize are called aflatoxins. These toxins are hazardous to animals and human health, and constitute a factor in economic losses in food production in the world (Lubulwa and Davis, 1994). Reports on high levels of aflatoxin in maize in Ghana and health concerns have been raised in recent times (Kpodo, 1996).

1.2. Statement of the problem

Aflatoxins develop in maize in the field and during storage thus making the grains unwholesome for consumption. The predisposing factors of infection include improper drying, high relative humidity and temperature, farmers' production practicesintercropping with aflatoxin infected grains- early and delayed harvesting and poorly constructed storage structures. Maize predisposed to some of these factors has a high probability of fungal infection (*Aspergillus sp*) which may, presumably enhance the development of aflatoxins. It is therefore assumed that, since climatic conditions, especially rainfall, temperature and relative humidity as well as storage structures vary in the country, the infection of maize by fungi (*Aspergillus sp*) and the subsequent development of aflatoxin may also vary. Figure 1.1. shows a maize cob infested by fungi in the field (Kumar *et al.*, 2000).



Fig.1.1: A. flavus infested maize. Source: Kumar et al. (2000)

Figure1.2. shows maize kernels infested by fungi in storage (Kumar *et al.*, 2000). Aflatoxins in the world are known to have health hazards on both humans and animals. Aflatoxins M1 and M2, for instance were first isolated from the milk of lactating animals (mammals) fed aflatoxin contaminated feeds, hence the M designation.



Fig.1.2: Maize kernels infested by fungi in storage. Kumar et al. (2000)

They are basically hydrogenated metabolites of aflatoxin B1 and B2 (Bankole and Adebanjo, 2003). Kpodo (1996) has done some work on aflatoxin contamination in maize for storage sites in Ghana. But the study was limited to the Accra Metropolis only hence the need to get credible data on aflatoxin occurrence at different locations in Ghana where maize production is prevalent. To study the levels of aflatoxin contamination in maize over a long period data collected from 1990 to 1999 in different environments was used for this research.

1. 3. Benefits of the study

The benefits of the study are as follows:

- 1. creation of awareness among the public about the aflatoxin problem.
- providing vital information for policy formulation to minimize health risks of aflatoxin contamination in agricultural commodities (oil seeds and maize as the most common).

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1.4 Objectives of this study

The objectives of this study are:

- i). to analyse the aflatoxin levels and distribution in maize samples from different storage location and years.
- ii). to assess the effect of relative humidity and rainfall on the distribution of aflatoxin in maize samples from different storage locations and years.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. The genus Aspergillus.

The genus *Aspergillus* belongs to the Deuteromycetes (Fungi Imperfecti; Hyphomycetes); their teleomorphs can be found in the Ascomycetes. The fungi find many commodities as good substrate for growth because of the large number of enzymes which they can use for their development (Hell, 1997). Pelczar *et al.* (1993) indicated that the Ascomycete produce sexual spores (ascospores) endogenously in a well-differentiated ascocarp but the Asccomycetes and the Deuteromycetes reproduce vegetatively by conidia. Hell (1997) stated that aflatoxins are only produced by two related species: *Aspergillus flavus* and *Aspergillus parasiticus*, with the latter species producing specifically the G type of aflatoxin.

2.2. Aflatoxins

Aflatoxin is a very powerful hepatocarcinogen, and naturally occurring mixtures of aflatoxins have been classified as a class 1 human carcinogen (IARC, 1993). The IARC (1993) also concluded that there was inadequate evidence for the carcinogenicity of aflatoxin M1. EHSO (2005) explained that aflatoxins are toxic carcinogenic by-products of the moulds *Aspergillus flavus* and *Aspergillus parasiticus*. The name aflatoxin was derived from a toxin producing fungus which caused a disease referred to as "Turkey X disease" in England in 1960 which resulted in the death of 100,000 young turkeys. The fungus was identified as *Aspergillus flavus* in 1961 and the toxin was named aflatoxin due to its origin (A*flavis-Afla*). EHSO indicated further that *Aspergillus flavus* is

common and widespread in nature. The mould is found in the soil, decaying vegetation and grains undergoing microbial deterioration. Keller *et al.* (1994) reported that scientists made the attempt to isolate genes associated with aflatoxin biosynthesis through cloning of genes in order to understand the enzymes regulating the biosynthesis. Furthermore, the information gained on the regulation of the genes in the path way could help to develop control strategies through inhibition of these controlling genes.

2.3. Chemical structures of aflatoxins.

The chemical structures of some aflatoxins are shown in fig. 2.1 (Cole and Cox, 1981).



Fig. 2.1: Chemical structure of aflatoxins

2.4. Properties of aflatoxins

Hell (1997) stated that four major groups of aflatoxins are identified: B1, B2, G1 and G2 as shown in the Table 2.1. These abbreviations are indicative of the colours these exhibit/fluorescence under the ultraviolet light (385 nm); thus B is for blue and G is for yellow-green, The M is a hydroxylated metabolic product of B (Bankole and Adebanjo, 2003).

	Aflatox				
B1	B2	G1	G2	M1	
$C_{17}H_{12}O_8$	C ₁₇ H ₁₄ O ₈	C ₁₇ H ₁₂ O ₇	$C_{17}H_{14}O_7$	$C_{17}H_{12}O_7$	
312	314	328	330	328	
268-269 (D) ¹	287-289 (D)	244-249 (D)	230	299 (D)	
Chloroform	chloroform	Chloroform	Ethyl acetate	Methanol	
425 nm	425 nm	450 nm	425 nm	425 nm	
	B1 C ₁₇ H ₁₂ O ₈ 312 t 268-269 (D) ¹ Chloroform 425 nm	Aflatox B1 B2 C17H12O8 C17H14O8 312 314 268-269 (D)1 287-289 (D)1 Chloroform chloroform 425 nm 425 nm	Aflatoxins B1 B2 G1 C17H12O8 C17H14O8 C17H12O7 312 314 328 268-269 (D)1 287-289 (D) 244-249 (D) Chloroform chloroform Chloroform 425 nm 425 nm 450 nm	Aflatoxins B1 B2 G1 G2 C17H12O8 C17H14O8 C17H12O7 C17H14O7 312 314 328 330 C68-269 (D)1 287-289 (D) 244-249 (D) 230 Chloroform chloroform Chloroform Ethyl acetate 425 nm 425 nm 450 nm 425 nm	

Table 2.1	The properties of aflatoxins	

 D^{1} = Decomposition, Source: Cole and Cox (1981).

2.5. Aspergillus flavus contamination of standing maize

Aspergillus flavus contaminates maize in the field in three stages. Initially, air-borne or insect transmitted conidia contaminate the silk and grow into the developing ear. Subsequently, the kernels in the ear, presumably damaged by insects or birds become infested with *A. flavus* and contaminated with aflatoxins. Stress to the plant resulting from climatic and cultural factors such as drought and nutrient deficiency make the plant more susceptible to fungal contamination (Wicklow, 1998). Marsh and Payne (1984)

also observed that kernel infection was possible through the scar left by silk detachment. Fennel *et al.* (1993) reported that records on *A. flavus* sporulation of maize planted indicated 68% for the kernels, 48% at the tip and 12% through the endosperm. Mycock *et al.* (1992) stated that maize grains internally infected with *A. flavus*, when left to germinate could develop into plants that were internally infected with the same fungus. It has been concluded that the fungi may be seed-borne, but (Cotty, *et al.*, 1994; Saito *et al.*, 1989) suggested that *A. flavus* was soil-borne and that it survived for long periods in the form of sclerotia.

2.6. Maize contamination by aflatoxin

Maize has been documented by several authors as an excellent substrate for mould growth and aflatoxin contamination in some West African countries. In substantiation of this statement, Setamou *et al.* (1997) reported that preharvest maize samples contaminated with aflatoxin in Benin was 42.5% in 1994 and 30% in 1995. Bouraima *et al.* (1993) also found that aflatoxin levels in stored maize in Benin were 14 ppb for B1 and 58 ppb for G1. Udoh *et al.* (2000) reported that 33% of maize samples from different ecological zones in Nigeria were contaminated with aflatoxin. Hell *et al.* (2000a) reported that 9.9% to 32.2% of maize samples of different ecozones in Benin prepared for storage had aflatoxin levels more than 5 ppb and the levels increased to 15% and 32.2% after six months of storage. Kpodo (1996) reported that maize samples from silos and warehouses in Ghana contained aflatoxin levels in the range of 20 to 355 ppb; while fermented maize dough collected from major processing sites contained aflatoxin levels of 0.7 to 313 ppb.

Insects have also been reported as playing a role in the spread of *A. flavus* and increase in aflatoxin contamination. Setamou *et al.* (1998) found that the percentage of grains infected with *A. flavus* and samples contaminated with aflatoxin including the mean aflatoxin content of samples increased correspondingly with increased insect damage in preharvest maize in Benin. Invariably, Hell *et al.* (2000b) found out that maize free of insect damage had no aflatoxin contamination, but maize with 70% of the cobs damaged by insects had 30.3% of the cobs contaminated with aflatoxin. Payne (1992) indicated that preharvest aflatoxin production in maize depended on weather conditions during crop maturations. In addition, the risk of aflatoxin contamination before harvest is highest when there is moisture stress coupled with elevated temperatures (Payne, 1992).

2.7. Factors influencing fungi infection and aflatoxin developm0ent in maize

Diener *et al.* (1987) reported that the factors that influence the growth of *A. flavus* and the formation of aflatoxins can be classified into three categories: climatic factors agronomic factors and biotic factors.

2.7.1. Climatic factors

In respect of rainfall, studies in Thailand revealed that moisture content of maize samples varied from 16% to 30.7% depending on the time of harvest. Maize that was harvested early (113days) had the highest levels of aflatoxins (Kawasugi *et al.* 1988). Moisture content of less than 17% showed no infection with *A. flavus*. On the accounts of relative humidity; as there is an increase above 85%, the growth of *A. flavus* increases dramatically. In this range, even a small increase in moisture can be very influential in

terms of increasing the risk of aflatoxin contamination (Christensen and Mirocha, 1976). Aflatoxins can be formed at a relative humidity of 88, 90 and 99% (Lillehoj, 1983), humidities that are common in the Southern parts of West Africa (Hell, 1997). Furthermore, Hell (1997) indicated that commodities stored at humidity between 75 and 85% are susceptible to fungal attack within the normal storage time.

Development of storage fungi in a post harvest commodity is influenced by the length of time in storage (Lillehoj and Zuber, 1986). Ahmad (1993) observed that aflatoxin contamination in storage was dependent on the storage system. Levels of aflatoxin were lower in closed metal bins which restricted air exchange and reduced oxygen levels, than in gunny bags that allowed air to flow through stored blackgram seeds. Bhatti *et al* (1990) reported that in Pakistan seed samples stored for 8 to 12 months at higher moisture content (17%) and those collected from mud plastered store had a higher incidence of aflatoxin producing *A. flavus* strains. Aflatoxin contamination of maize stored in traditional storage structures in Bihar, India was highest in the "*kothi*" made out of mud and rice husk, as compared to the "*mora*" made from paddy hay ropes wound in a container, gunny bags or iron bins (Prasad *et al.*, 1987).

2.7.2. Other factors

The other factors that influence the aflatoxin contamination of maize are agronomic and biotic factors. If maize was grown with a crop that was also susceptible to aflatoxin development there will be an increased risk of toxin metabolism (Cotty, 1994). Cole *et al.* (1982) investigated the effect of peanut, maize, soyabean crop rotation on aflatoxin development. They found more aflatoxin if the maize was planted after groundnut.

Griffin *et al.* (1981) also observed that when maize was cropped in rotation with groundnut there was *A. flavus* population build – up in the soil over the years. Barry *et al.* (1992) showed that maize cultivars that had a resistance to ear- infesting insects also produced less aflatoxin in pre- harvest grains. The incidence of *A. flavus* fungi and aflatoxin contamination was comparatively higher in insect-damaged maize samples from different localities in India than in insect free samples (Sinha and Sinha, 1992). Zuber *et al.* (1986) reported that insects that feed on maize ears in the field predispose kernels to *A. flavus* infection through the physical damage caused by their feeding.

2.8. Aflatoxins and their effects on human and animal health

There is a widespread exposure to aflatoxin in West Africa probably starting in the utero and blood tests have shown that very high percentage of West Africans are exposed to aflatoxins (Bankole *et al.*, 2003; In: Wild, 1996). Concretely, studies carried in the Gambia, Guinea, Nigeria and Senegal indicated that over 98% of respondents tested positive to aflatoxin markers (Bankole *et al.*, 2003; In: Wild, 1996). The economic and health importance of aflatoxins need much attention because of their ability to contaminate human foods and animal feeds, especially cereals (Keller *et al.*, 1994). Besides, losses due to livestock and poultry producers resulting from aflatoxin contaminated feeds include death, immune system suppression, reduced growth rates and losses in feed efficiency. In humans the effects are acute aflatoxicosis, liver cell cancer and Hepatitis B.

Li *et al.* (2001) in a study in China found that the levels of aflatoxins B_1 , B_2 , and G_1 were quite high in maize from the high incidence area for human hepatocellular carcinoma.

They also indicated that the average daily intake of aflatoxin B_1 from the high risk area was 184.1µg/ml. Hepatocellular carcinoma is the fifth leading cause of cancer mortality in the world and it accounts for about 70% of cancer deaths in certain parts of Asia and Africa (Farombi, 2006). Aflatoxin positive "kwashiorkor" children in Togo and Benin showed much greater severity of edema, increased number of infections, lower haemoglobin levels and longer duration of hospital stay than aflatoxin-negative "kwashiorkor" children (Adhikari *et al.*, 1994; Ramjee, 1996). Presumably, the protein deficiency characteristic symptom of "kwashiorkor" reduces the capacity of the liver to detoxify aflatoxins. It is hereby, asserted that aflatoxin may be a contributory factor in increasing the morbidity of children suffering from the disease (Ramjee, 1996).

Uriah *et al.* (2001) in their study in Nigeria found that blood and semen aflatoxin levels ranged from 700 to 1393 µg/ml and 60 to 148 µg/ml respectively in infertile men and were significantly higher than that in fertile men. Gong *et al.* (2002) reported that children in Togo and Benin who ate foods contaminated with aflatoxins had the syndrome of stunted growth and underweight, which are normally associated with malnutrition. Aflatoxins have also been shown to be immunotoxic to both livestock and humans. Turner *et al.* (2003) detected aflatoxin albumin adducts in 93% of sampled children (6-9 years) in Gambia and provided evidence that immuoglobin A (IgA) in saliva may be reduced because of high dietary levels of aflatoxin exposure. The study confirmed that children in rural areas of Gambia are frequently exposed to high levels of aflatoxin. In the US, the FDA uses an action level of 20 ppb as the maximum residue limit allowed in food for human consumption, except for milk (FAO, 1996). The European Union, as a sanitary precaution measure has enacted in 1998 very severe aflatoxin tolerance standards of 2 ppb aflatoxin B_1 and 4 ppb total aflatoxins for nuts and cereals for human consumption (CEC, 1998), which came into effect from January, 2001 (Dimanchie, 2001).

Increased awareness has now been created in consumers in the developed world of the carcinogenic effect of aflatoxins, who will thus not accept or purchase a product from any supplier that has aflatoxin beyond the acceptance level. In line with this, exports of agricultural products particularly groundnuts from developing countries have dropped considerably in recent years resulting in major economic losses to producing countries (Bhat and Vashanti, 1999; Otzuki *et al.*, 2001). The World Bank indicates that the policy change by the EU was to reduce by 64% imports of cereals, dried fruits, and nuts from nine African countries: Chad, Egypt, Gambia, Mali, Nigeria, Senegal, South Africa, Sudan and Zimbabwe, and this was to cost African countries about US \$670 million in trade per year (Kellerhals, 2000). However, the new EU rule has been criticized as excessively too rigorous, because the difference between the EU limits and the Codex limits would only save two lives for every one billion people (WHO, 2000).

Lewis *et al.* (2005) reported that one of the largest aflatoxicosis outbreaks in Kenya in April 2004 with 317 cases claimed 125 lives (39.4%). Besides, they indicated that home grown maize was the source of the outbreak. In their findings 55% of maize products had aflatoxin levels greater than the Kenyan regulatory limits of 20 ppb, 35% had levels exceeding 100 ppb and 7% had levels above 1000 ppb. Furthermore, they stated that the outbreak was one of the largest and the most severe of acute aflatoxicosis documented worldwide. Invariably, Nyamong and Okioma (2005) reported that in April to September 2004 and in April 2005 the largest outbreaks of aflatoxins occurred in a large

geographical area (40,149km²) and claimed over 123 lives in Eastern Kenya. They also indicated that descriptive epidemiological studies showed a relationship between the outbreak and the local methods of harvesting, storing and processing of maize. In another development, Sharif (2004) reported that 40 people in Makueni and Kitui Districts of Kenya died out of aflatoxin poisoning resulting from maize meal. Furthermore, others on admission due to the consumption of the meal suffered from jaundice, leg edema and hepatomegaly. Besides, deaths were reported of animals and poultry fed on the maize meal and the maize samples showed high levels of aflatoxin. Aflatoxin is a very powerful hepatocarcinogen and diets contaminated with it are linked with high incidence of liver cancer (Bankole et al., 2003). The presence of the toxin in the autopsy brain tissue of some Nigerian children (Oyelami et al., 1996) has reflected in the findings of Bankole et al. (2003). From another perspective, Turner et al. (2000) stated that aflatoxin synergies other agents such as Hepatitis B in the causation of liver cancer. Ankrah et al. (1994a) studied the relationship between aflatoxin contaminated diet and liver inflammation. Maize meals (as "kenkey" and "akple") were used for the Serum, urine and faecal samples were monitored for aflatoxins or their study. metabolites with the results that 35% of the subjects studied had B1 or its metabolites which is indicative of contamination of the food items by A. parasiticus not A. flavus. The results were suggestive of liver inflammation, not liver cancer, they concluded. Other studies reported of significant correlation between the primary liver mortality rates and aflatoxin intake in the local foods in five villages in China (Yu et al, 1989).

2.9. Storage structures, environment/location and aflatoxin contamination of maize

Hell (1997) in a study of stored maize and aflatoxin contamination in four agroecological zones in Benin came out with the findings outlined below. In a zone with high relative humidity (>80%) and rainfall range of 1300-1500 mm, 20% of the maize samples tested positive for aflatoxin. Maize stored in bags for six months had the highest aflatoxin content with a mean of 250 ppb. The "Ago" and "Ebliva" storage structures which had maize being aflatoxin positive had means of 71.1ppb and 32.0 ppb respectively. Hell (1997) stated further that high level of aflatoxin contamination (40%) was observed in stores in a zone with annual rainfall range of 1000-1300 mm, with the clay stores being the highest. The crib showed the highest mean of 394.7 ppb of which at least one sample gave 2500 ppb. Baskets, floor and platform stores showed low mean aflatoxin levels. There was more aflatoxin in maize stored in bags than baskets, conical stores and the "Ago" storage structure. One other zone with annual rainfall range of 900-1300 mm had aflatoxin contents of maize stored in baskets being 133.9 ppb, on platform and in bags being 75.2 ppb and 67.7 ppb respectively. The mean contamination levels were lower than that in the zone with annual rainfall range of 1000-1300 mm, however, 52.1% of the sample tested positive for aflatoxin. The last zone with annual rainfall less than 1000 mm and with a relative humidity of 40% - 60% had the highest contamination level in the clay stores (116.4 ppb) and the "Secco" (98.8 ppb). In this zone more than 35.5% of the stores were contaminated (Hell, 1997).

In Uganda with an annual rainfall range of 500-2000 mm and an average temperature of 25°C, conditions are conducive for the growth of *A.flavus* and the subsequent production of aflatoxin (Kaaya and Warren, 2005). Thus, Kayaa and Warren (2005) in a review report of aflatoxin contamination of maize in Uganda mentioned that maize stored by

traders (majority in woven polypropylene bags) for six to seven months had mean aflatoxin levels of 107 ppb which is an indication that the grains were not suitable for the export and local markets. They also reported that in an earlier study of fungi and aflatoxin in maize grains in five districts of Uganda (Kampala, Mpigi, Mubende, Luwero and Mukono), maize samples monitored from shops and markets for five months gave aflatoxin levels in the range of 0-50 ppb; with seven out of the eight samples contaminated by the B-group. Coupled with this, over 30% of the samples had aflatoxin levels above 20 ppb; while 50% contained up to 10 ppb. Besides, they explained further that the high aflatoxin levels resulted from high moisture content as 48% of the maize samples had moisture content favourable for mould growth, that is greater than 14% and the development of aflatoxin. Invariably, farmers in Uganda who stored unshelled maize in heaps on the floor and under the verandah had 100% aflatoxin contamination. Conversely, Kaaya and Warren (2005) observed that one of the methods that protected maize against aflatoxin contamination was storage above fire racks which was unsuitable for larger quantities of grains.



2.10. Strategies for aflatoxin control and prevention

Aflatoxin control programmes if well organised will result in economic gains as well as health improvement in the West African sub- region. It is now realized by many developing countries that reducing mycotoxin levels in foods will confer international trade advantages as well as offer long-term health benefit to the local population. The new European standards of aflatoxin level in groundnuts and cereals means that effective control must be found by developing countries for them to continue to export to the attractive European Union markets. Bankole and Adebanjo (2003) have proposed many solutions against aflatoxin production in food, and some of the strategies which may be applicable in West Africa are discussed under the followings headings:

2.10.1. Education and Extension

The problem posed to the health and economy by mycotoxins is not known to a larger percentage of the populace including even the educated ones. It is therefore necessary that the National agency in each country responsible for food safety, should embrace the task of creating awareness in the populace about the need to consume pathogen-free or good quality food. Private non-governmental organizations could also be roped in to spread information especially to the most remote villages. There should be regular programmes on radio and television on mycotoxin hazards and discussion on the issue should also feature regularly in daily newspapers and magazines. Appropriately, the cradle of the education should be the farmers or producers, whom the extension staff of the Ministry of Food and Agriculture (MOFA), in Ghana for example, should educate on the need to adopt Good Agricultural Practices (GAP) to produce food free of hazards. Hazard Analysis Critical Control Point (HACCP), a food safety control system based on a systematic identification and assessment of hazards in food and the identification of their control is useful in this situation. In an ideal HACCP-based system mycotoxins would be minimised at every phase of production, harvesting, processing and distribution.

2.10.2. Mechanical drying

Among the recommendations for solving the mycotoxin problem, rapid drying of agricultural products to a low moisture content is often emphasized, because all scenarios leading to mycotoxin contamination relate to non-maintenance of stored products at safe moisture content. Drying harvested maize to 15.5% moisture content or lower within 24 to 48 hours will reduce the risk of fungus growth and consequent aflatoxin production (Hamilton, 2000). Siriacha et al (1989) found that if shelled grain was immediately sundried the chance of contamination was reduced as compared with that of undried shelled In Africa, most farmers sun-dry their harvests which often require longer maize. durations for the product to attain 'safe' moisture level especially in times of cloudy weather. The grains are spread out on polyethylene sheets spread on the floor, and the stirring or turning is done manually till the product is dry. Due to the high rainfall at the time of harvest, farmers take some steps such as stacking the products to shield it from rain, drying grains over the fire and mixing of moist and dry grains (Amyot, 1983; Begun, 1991). The effectiveness of drying was demonstrated in the report of Awuah and Ellis (2002) when groundnut kernels with 6.6% moisture were free of fungi regardless of the storage protectant used for 6 months, whereas at 12% moisture, only jute bags with *S*. *aromaticum* effectively suppressed the cross infection of healthy kernels.

Since sundrying may be a difficult task due to the high rainfall at the time of harvest, a lot of work has been done on the design of solar and mechanical dryers for use by farmers in the tropics (Axtell and Bush, 1991; Carruthers and Rodriquez, 1992). However, these dryers are not in use by farmers because large capital investment is involved. Mechanical dryers could be set up in strategic locations, which farmers can utilize if sundrying is proven difficult. In Ghana, such facilities exist in some parts of the country, Techiman and Ejura in the Brong-Ahafo and Ashanti Regions respectively, but there is the need for a countrywide coverage.

2.10.3. The use of improved storage structures

Traditional storage structures used by farmers for on the farm storage include containers made of plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet. Essentially the stores are constructed to prevent insect and rodent attack and to prevent moisture from getting into the grains. The adoption of high yielding varieties which most often do not withstand long periods of storage by farmers has made the traditional storage systems to become inadequate. However, it has been very difficult to promote new storage systems such as the use of metal bins to small-scale farmers due to their high cost. Research is needed to develop and refine suitable storage systems that are not capital intensive.

2.10.4. Smoking

Smoking is also an efficient method of protecting maize against infestation by fungi. The efficiency of smoking in protecting against insect infestation was found to be comparable to that of the chemical Actellics (primiphos–methyl) (Daramola, 1986). About 3.6-12% of farmers in various ecological zones in Nigeria used smoke to preserve their grains, and this practise was found to decrease aflatoxin levels in farmers' stores. The efficiency of smoking was also confirmed by Hell *et al.* (2000a) in the survey conducted in Benin. However, the problem with smoking is that if not carefully applied, it may discolour the product and change the taste.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. The study areas

Three districts of Ghana were selected for the study. They are Ejura-Sekyedumase in Ashanti, Nkoranza in the Brong-Ahafo and North-Kwahu/Afram Plains in the Eastern Region. These are renowned maize producing areas in the country. These three regions account for 60% of the maize produced in Ghana (FAO/WFP, 2002). The area cropped and the corresponding quantites of maize produced in tonnes from 1996 to 2006 in the regions and districts of the study are shown in Appendices II and III respectively.

3.1.1. Geographical locations and climate of the areas

3.1.1.1. EJURA-SEKYEDUMASE DISTRICT

Ejura-Sekyedumase district is located in the transition zone between the Northern and Southern zones of the country. The climatic conditions are those of savanna transition agro-ecological zone. The mean annual rainfall is 1400 mm which is received in two peaks (Dedzo, 1998), but sometimes in a unimodal pattern. Temperatures are uniformly high with the mean annual maximum value being about 32°C and the minimum around 20°C. The relative humidity values are high during the rainy season with the mean monthly values ranging between 80-88% in the morning and decreasing to 70-75% by mid day. During the dry season the value drops to around 75% in the morning and 45% by mid day (Dedzo, 1998).

3.1.1.2. NORTH-KWAHU DISTRICT

The North-Kwahu/Afram Plains district is located in the south eastern corner of the Volta river basin with a terrain, averaging 60-150 metres in elevation. The district has no historical data on relative humidity and temperature since it was recenntly created. The Afram River, the Obosome River and the Volta Lake surround it to make it look like a peninsular, and it shares boundary with the Ashanti, Brong Ahafo and Volta (Water Aid/APDO, 2004).

3.1.1.3. NKORANZA DISTRICT

The District lies within the wet semi-equatorial region of Ghana. It is part of the transitional zone between the savannah woodland of northern Ghana and the forest of the south. Thus, the Eastern part of the district is largely characterized by savanna woodland and the southern part is largely marked by forest regrowth, made up of shrubs and grasses. It has a mean annual rainfall level ranging between 800-1200 mm and relative humidity ranging from 75%-90% (GD, 2007).

3.2. Materials

Vicam Aflatest kit was used according to the Association of Official Analytical Chemists method (AOAC) for the aflatoxin analysis. Maximum weight of 1.0kg composite or aggregate samples of the second season maize was collected from each storage location yearly from 1990 to 1999. The moisture content of each sample on dry basis was determined immediately. Other materials were sodium chloride and a 500ml glass blender jar. The reagents were 70% methanol: 30% water, high-pressure liquid chromatography (HPLC) grade methanol and distilled water.

3.3. Methods

3.3.1. Sampling and Data collection

Maximum weight of 1.0 kg composite/aggregate sample was taken from each maize lot in each storage location yearly from 1990 to 1999 and made into three sub samples of about 300 g each, European Union (EU) standards (2003). Ground samples of about 25g for each sub sample were used for the aflatoxin levels analyses and the average level calculated. The second season maize which most often dry better on the farm was used for the sample collection in each storage location. The average moisture content was 12.5%.

Data on the area cropped and the quantity of maize produced from 1996 to 2006 in the three regions and districts used for the study was collected (Appendix II and III) respectively. Besides, data on the annual rrainfall, maximum and minimum relative humidities from 1990 to 1999 in the three districts used for the study was collected (Appendix I).

3.4. AFLATOXIN TEST

3.4.1. Overview

The VICAM Aflatest (Watertown, MA) of AOAC Fluorometer procedure was used. Aflatest was chosen because it is quick (10 min per test) and sensitive thus giving readings as low as 10 ppb to 2 ppb in samples. It has a wide range of readings from 0-50 ppb per sample; convenient to use in that it uses the same extract instrumentation for other mycotoxins' test; easy to use as no special skills are required and the test can be performed virtually anywhere. It is safe because it requires less toxic materials than the conventional methods and has a digital readout in ppb or can be used as a clean up for HPLC.

3.4.2. Sample extraction

Each test was performed with ground samples of the kernels weighing 25g. This was mixed with 5 g NaCl and 125 ml 70% methanol: 30% H₂O and blended in a blender jar at a high speed for 2 minutes with the blender covered. The extract was filtered with fluted filter paper and the filtrate collected in a clean vessel.

3.4.3. Extract dilution

Precisely 15ml of the filtered extract was collected with pipette and poured into a clean vessel and diluted with 30ml distilled water and mixed thoroughly. The diluted extract was filtered through a glass microfibre glass filter into glass syringe barrel using markings on the barrel to measure 15ml.

3.4.4. Column chromatography

The 15ml of the filtered extract was passed through the Aflatest column at the rate of 1.2 drops/second (15ml=1.0g sample equivalent). The column was washed with 10ml distilled water at the rate of 1-2 drops/second and repeated once until air came through the column. The Aflatest column was eluted with 1.0ml HPLC grade methanol at the rate of 1-2 drops/second and 1ml of the eluate was collected in a glass cuvette. A fresh Aflatest developer measured to 1ml was mixed thoroughly with eluate and the fluorescence measured in a calibrated fluorometer. The Aflatoxin concentration was read after 60 seconds.

3.5. Statistical analysis

Data from 1990 -1999 on aflatoxin levels for the three locations were made available for the analysis. The data was analysed with SPSS-Statistical Package of 1993 and subjected to simple descriptive statistics which looked at the range, the mean and the variance. The variance ratio test was the test statistic at p=0.05. Two study areas were compared at a time for significant difference based on the two sample 'T' tests using the 'F' distribution. This was chosen for the convenience of comparative analysis of two environments at a time.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Variations in the levels of aflatoxin contamination and/or distributions (ppb)

of maize sampled from the storage locations (I, II and III).

The variations in aflatoxin contamination (ppb) of maize sampled from Ejura-Sekyedumase (I), North-Kwahu (II) and Nkoranza districts from 1990 to 1999 are shown in Table 4.1.

Table 4.1:Variations in the levels of aflatoxin contamination (ppb) of maizesampled from storage locations (I, II and III).

Locations	Mean	Range	Standard	Standard	Variance	Total	
	Aflatoxin (ppb)	(ppb)	Deviation	Error		aflatoxin (ppb)	
Ejura-Sekyedumas	e (I) 12.05	6.50	2.73	0.86	7.469	120.50	
North-Kwahu (II)	15.32	19.80	7.37	2.33	54.380	153.20	
Nkoranza (III)	13.42	22.58	6.95	2.20	48.282	134.17	
E VALUE: 18-11	$-7.28^{*} \cdot 1.8$	III = 6.46	[*] • II & III –	1 12** Cri	tical value at 5	50/ dagraas	

F-VALUE: I & II = 7.28 ; I & III = 6.46 ; II & III = 1.13 ; Critical value at 5%, degrees of freedom (9, 9) = 3.18, p = 0.05,

* Highly significant, p <0.05; ** Not siginificant, p>0.05

In Table 4.1 the variance ratio test showed that siginificant difference exists between the aflatoxin contaminations (ppb) of Ejura-Sekyedumase and North-Kwahu districts. The variances indicated that the variations were more pronounced in the North-Kwahu district than Ejura-Sekyedumase or the contamination levels in the North-Kwahu were higher

than that of Ejura-Sekyedumase. Thus the range of contamination in Ejura-Sekyedumase was 6.50 ppb and that of Afram Plains was 19.80 ppb. The total aflatoxin in Ejura was 120.50 ppb compared to that of Afram Plains of 153.20 ppb. Similarly, the levels of contamination in Ejura-Sekyedumase district were lower than that of Nkoranza district. Comparatively, there was a significant difference between the environments. The total aflatoxin in Nkoranza district was 134.17 ppb while that of Ejura-Sekyedumase district was 120.50 ppb with the ranges being 22.58 ppb and 6.50 ppb respectively. On the other hand there was no significant difference between the aflatoxin contaminations (ppb) of North-Kwahu and Nkoranza districtss; their variances differ by 6.0 ppb and the ranges were 19.80 ppb and 22.58 ppb respectively. Ejura-Sekyedumase district had a mean aflatoxin contamination of 12.05 ppb, North-Kwahu district had 15.32 ppb and Nkoranza district had 13.42 ppb. The total aflatoxin for Ejura-Sekyedumase was 120.5 ppb, and North-Kwahu had 153.20 ppb and Nkoranza had 134.17 ppb (Table 4.1). The highest level of aflatoxin contamination in Ejura-Sekyedumase district was 15.0 ppb while that of the North-Kwahu and Nkoranza districts were 26.0ppb and 29.5ppb respectively (Fig. 4.1 & Appendix IV).

An aflatoxin contamination level (ppb) in Ejura-Sekyedumase, North-Kwahu and Nkoranza districts at a glance is shown by Fig. 4.1



Fig.4.1: Aflatoxin contamination levels (ppb) in locations I, II & III.

For the period 1990 to 1999 (Appendix IV and Figure 4.1) aflatoxin distributions in the storage locations showed that Nkoranza district had the highest levels in 1997 and 1999 while North-Kwahu district had the highest levels in 1990, 1991, 1993 and 1998. The three locations had equal level of 9.50 ppb in 1994. Similarly Nkoranza and North-Kwahu districts had equal levels of 10.50 ppb in 1995. On the whole, Ejura had fair distribution levels since it was the only location with its highest level far below the acceptable level of 20 ppb for humans (FAO, 1996).

4.2 Effects of relative humidity and rainfall on the distributions of aflatoxin in the storage locations from 1990-1999.

North-Kwahu district had the highest mean aflatoxin contamination and/or distribution of 15.32 ppb followed by Nkoranza district of 13.42 ppb and Ejura-Sekyedumase with the least of 12.05 ppb. The aflatoxin distributions and contaminations in the locations may

be attributed to the high relative humidity and annual rainfall values obtained over the period of the study. The highest maximum and minimum humidity figures for Ejura-Sekyedumase district over the period were 94% and 58%, while that of North-Kwahu district were 93% and 81% and Nkoranza district had 99% and 62% (Appendix I). The highest annual rainfall value was 1401mm, 1366 mm and 1469 mm for North-Kwahu, Nkoranza and Ejura-Sekyedumase districts respectively (Appendix I). The North-Kwahu district with its special geographical location of being more or less a peninsular (Water Aid/APDO, 2004) might have enhanced its moistness and the subsequent higher levels of aflatoxin contamination and/or distribution over the period of the study. The findings of the current research tally with that of Hell (1997), who studied the aflatoxin contamination of maize in four agroecological zones in Benin. He reported that in a zone with relative humidity greater than 80% and annual rainfall range of 1300-1500mm, 20% of the maize samples tested positive for aflatoxin. In the other three zones one had an annual rainfall of 1000mm and relative humidity ranging from 40%-60% and aflatoxin contamination level of the samples being greater than 35.5%. The remaining two zones with annual rainfall ranges of 1000-1300mm and 900-1300mm had aflatoxin contamination levels of their samples being 40% and 52.2% respectively. Kayaa and Warren (2005) in a review report of aflatoxin contamination of maize in Uganda with annual rainfall range of 500-2000mm mentioned that maize stored by traders (majority in woven polypropylene bags) for six to seven months had mean aflatoxin levels of 107ppb which is an indication that the grains were not suitable for the export and local markets. They also reported that an earlier study of fungi and aflatoxin in maize grains in five districts of Uganda (Kampala, Mpigi, Mubende, Luwero and Mukono), maize samples

monitored from shops and markets for five months gave aflatoxin levels in the range of 0-50 ppb; with seven out of the eight samples contaminated by the B-group. Coupled with this, over 30% of the samples had aflatoxin levels above 20 ppb; while 50% contained up to 10 ppb. Besides, Kaaya and Warren (2005) explained that the high aflatoxin levels resulted from high moisture content as 48% of the maize samples had moisture content favourable for mould growth (>14%) and the development of aflatoxin. Invariably, farmers in Uganda who stored unshelled maize in heaps on the floor and under the verandah had 100% aflatoxin contamination. Conversely, Kaaya and Warren (2005) observed that the only method that protected maize against aflatoxin contamination was storage above fire racks which was unsuitable for larger quantities of grains. The reports of Kaaya and Warren (2005) are in support of the findings of the current research in that the preailing annual rainfall of 500-2000 mm in Uganda which influenced the aflatoxin contamination levels is similar to that in the study areas (AppendixI I).

The current research report again has the support of the findings in Thailand, which indicated that as relative humidity increases above 85% the growth of *A. flavus* increases dramatically and that in this range even a small increase in moisture can be very influential in increasing the risk of aflatoxin contamination (Chritensen and Mirocha, 1976; Sauer and Burroughs, 1986). Finally, the observations that high relative humidities of 88%, 90% and 99%, which are humidities common in West Africa, influence aflatoxin growth have reflected in the findings of Hell (1997) and are strongly in support of the current findings.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The conclusion drawn from the research findings is that, storage locations of maize influenced the levels of aflatoxin contamination and/or distributions. Thus, the North-Kwahu district with a unique geographical location (peninsular) had higher mean aflatoxin level and distributions of 15.32 ppb followed by Nkoranza district of 13.42 ppb and Ejura-Sekyedumase district from 12.05 ppb. Relative humidity also influenced the results with the highest maximum and minimum values for the locations being, 94%, 58%; 93%, 81% and 99%, 62% for Ejura –Sekyedumase, North –Kwahu and Nkoranza districts respectively. The highest annual rainfall values of 1401 mm, 1366 mm and 1469 mm for North-Kwahu, Nkoranza and Ejura respectively over the period of study also played a role in fungi development and the aflatoxin contamination of maize.

5.2. Recommendations

- 1. More research should be undertaken in different districts of the regions used for the study.
- 2. Studies should also be carried out on the aflatoxin contamination of standing maize in the different storage locations used for the study.
- 3. Quality standards for maize should be developed
- 4. Maize storage structures should be designed to meet standards taking into consideration the psychometric characteristics of he wind in order to prevent moisture drift into grains in order to maintain optimum storage temperatures.

- 5. The Agricultural Extension staff of MOFA should educate farmers on the need to adopt Good Agricultural practices (GAP) in order to produce food free of hazards such as aflatoxins.
- 6. Humans should learn to consume good quality grains as well as feed their livestock and poultry with uncontaminated feeds.
- 7. The media should also educate the public on the health hazards of aflatoxins.
- 8. Hazard Analysis Critical Control Point (HACCP) should be employed in the agricultural production channel to minimize the aflatoxin contamination of foods.



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APPENDICES

Appendix I

Mean annual rainfall, maximum and minimum relative humidity of the study areas from 1990-1999.

Year			Study	y areas								
		Rainfall (mr	n)	Relative humidity (%)								
	Ejura,	North-Kwaw	vu, Nko <mark>ran</mark>	za Eju	ira	North-K	wawu	Nkora	nza			
				Max1	M1111	Maxi	Mini	Maxi	Mini			
1990	1145	1145	1131	93		51	-	89	58			
1991	1207	1401	879	92	57	13	2.	95	62			
1992	926	_*	1343	90	54		X	88	58			
1993	1332	201	1017	85	55	-	-	88	58			
1994	1043		1063	86	56	-	2.	87	54			
1995	1469	546	11 <mark>27</mark>	90	58	92	81	<mark>8</mark> 6	54			
1996	1334	623	13 <mark>66</mark>	94	58	- L	10	94	60			
1997	1434	882	815	92	58	87	69	88	57			
1998	1283	27	1196	90	56	93	68	86	56			
1999	1390	-	1238	92	58	-	-	99	58			

SOURCE: Ghana Meteorological Agency National Office, Accra, Ghana 2007. -*: Not available

Appendix II

Area cropped (ha) and the corresponding quantity of maize produced (tonnes) in the Regions of the study from 1996-2006.

Year			REGIO	ON				
	Asha	nti	Eas	tern	Brong-Ahafo			
	area	quantity	area	quantity	area	quantity		
1996	101098	166040	136500	262000	76475	159630		
1997	99010	175005	143000	2 <mark>4920</mark> 0	72300	159630		
1998	109890	183553	160000	287000	82154	143417		
1999	117649	193522	151800	273535	89432	180422		
2000	114874	191903	148400	241990	94798	166326		
2001	1194 <mark>73</mark>	170000	147744	201000	99277	168000		
2002	170120	269480	201530	322690	160730	268980		
2003	119620	1939 <mark>2</mark> 0	150600	244000	176800	265170		
2004	113639	183032	141950	241621	167900	281267		
2005	129517	161816	124900	206467	178880	358259		
2006	138793	164266	133844	209542	191691	363595		

SOURCE: (1), 1996-1999; PPMED (MOFA); (2), 2000-2006; SRID (MOFA)

Appendix III

Area cropped (ha) and corresponding quantity of maize produced (tonnes) in Districts of the study from 1996-2006.

Year

Districts

	Ejura-Sel	kyedumase	North	n-Kwawu	Nko	Nkoranza		
	area	quantity	area	quantity	area	quantity		
1996	16698	26717	19000	36600	6843	15335		
1997	13587	24457	20000	<mark>3800</mark> 0	6110	16497		
1998	14945	25408	24000	46000	8894	20456		
1999	17500	26423	22600	42940	8450	22815		
2000	15500	24360	25650	41040	8957	22280		
2001	16120	18165	27000	30600	9405	22288		
2002	32978	41499	52765	87390	23073	<mark>5089</mark> 1		
2003	16140	22550	29940	48500	28430	62830		
2004	15333	21423	27840	50112	27000	60750		
2005	14952	19319	25532	44482	26833	66583		
2006	16022	19606	27360	45144	28755	67574		

SOURCE: (1), 1996-1999; PPMED (MOFA), (2), 2000-2006; SRID (MOFA), ACCRA 2007.

Appendix IV

Aflatoxin distribution (ppb) in maize sampled from different storage locations in a particular year from 1990- 1999.

Location					Year	ſ				
	1990	0 1991	1992	1993	1994 1	.995	1996	1997	1998 1	999
Ejura (I)	9.50	14.5	10.5	15.0	9.50	8.50	14.5	14.5	14.5	9.50
Afram Plains (II)	24.0	26.0	6.20	20.50	9.50	10.50	8.0	19.50	20.0	9.0
Nkoranza (III)	20.5	6.92	7.75	9.50	9.50	10.50	10.50	29.50	14.50	15.0
Mean (ppb)	18.0	15.81	8.15	15.0	<mark>9.50</mark>	9.83	11.0	21.17	16.33	11.17
Standard Error	4.37	5.55	1.26	3.18	0.00	0.67	1.89	4.41	1.83	1.92
Standard Deviatio	n 7.57	9.61	2.17	5.50	0.00	1.16	3.28	7.84	3.18	3.33
Variance	57.25	5 92.29	4.74	30.25	0.00	1.33	10.75	58.33	10.08	11.08
Range (ppb)	14.50	19.08	4.30	11.0	0.00	2.0	6.50	15.0	5.0	6.0
Total (ppb)	54.0	47.42	24.45	45.0	28.50	29.50	33.0	63.50	49.0	33.50

Appendix V

Aflatoxin contamination levels (ppb) in Ejura-Sekyedummmase are shown in Fig 4.1.





Appendix VI



Fig. 4.2 illustrates the aflatoxin contamination levels (ppb) in North-Kwawu district

Fig.4.2: Aflatoxin contamination of maize sampled from North-Kwawu district

Appendix VII

Aflatoxin contamination levels (ppb) in Nkoranza district are shown in Fig. 4.3.



Fig.4.3: Aflatoxin contamination of maize sampled in Nkoranza district