DETERMINATION OF AFLATOXINS IN FOOD GRAINS AND PROCESSED FOODS FROM SELECTED AREAS IN BANGLADESH WITH RISK ASSESSMENT

MOST. ZAKIYA ISLAM

REGISTRATION NO. 15-06652

DEPARTMENT OF AGRICULTURAL CHEMISTRY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY DHAKA -1207

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BY

MOST. ZAKIYA ISLAM

REGISTRATION NO.: 15-06652

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Approved By:

Dr. Noorjahan Begum Professor Department of Agricultural Chemistry SAU, Dhaka **Supervisor**	**Dr. Md. Nurul Huda Bhuiyan** Principal Scientific Officer Institute of Food Science and Technology (IFST), BCSIR **Co-supervisor**

Prof. Dr. Sheikh Shawkat Zamil
Chairman
Examination Committee

DEPARTMENT OF AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled **"DETERMINATION OF AFLATOXINS IN FOOD GRAINS AND PROCESSED FOODS FROM SELECTED AREAS IN BANGLADESH WITH RISK ASSESSMENT"** submitted to the **Department of Agricultural Chemistry**, **Faculty of Agriculture**, Sher- e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **Most. Zakiya Islam**, Registration No. **15-06652** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

December 2021

Dhaka, Bangladesh

Dr. Noorjahan Begum
Professor
Department of Agricultural Chemistry
SAU, Dhaka

Dedicated
To
My Beloved Parents

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DETERMINATION OF AFLATOXINS IN FOOD GRAINS AND PROCESSED FOODS FROM SELECTED AREAS IN BANGLADESH WITH RISK ASSESSMENT

ABSTRACT

The present study was conducted to determine the occurrence of aflatoxin residues in rice, wheat, maize, lentil, mungbean, mustard, soybean, peanut and processed food items and to identify the sources of contamination with risk assessment. To perform this experiment, 29 samples were collected from godown, 21 samples were collected from wholesale market, 10 processed food samples were collected from departmental store. So, total 60 samples were collected from different locations of Bangladesh. The study duration was March 2020 to June 2021. Collected grain samples and processed food samples were analyzed using High Performance Liquid Chromatography (HPLC) with fluorescence detector to detect residues of aflatoxin (B_1, B_2, G_1 & G_2). In this study, 8 godown samples (3 peanut samples, 2 maize samples, 3 lentil samples) and 1 wholesale market sample (rice) were detected with aflatoxin, whereas no processed food sample was detected positive for aflatoxin. The highest concentration of aflatoxin found in peanut (local) was 156.12 µg/kg and lowest concentration found in peanut (imported) was 0.5181 µg/kg. Results showed that among 9 samples, 4 samples (1 peanut sample, 2 maize samples and 1 lentil sample) exceeded the maximum residue limit (MRL) of aflatoxin B_1 (2 µg/kg). Among 4 samples, 3 samples (1 peanut sample, 2 maize samples) exceeded the maximum residue limit (MRL) of total aflatoxin B_1, B_2, G_1 & G_2 (4 µg/kg) as per Commission Regulation (EC) No. 1881/2006 for food. Human health risk assessment from aflatoxins exposure through rice, maize, lentil and peanut consumption from the godown and wholesale markets by adults showed no significant adverse health risk to humans.

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ABBREVIATIONS AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimeter
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
m^2	=	Meter squares
ml	=	Milliliter
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
^oC	=	Degree Celceous
%	=	Percentage
mg	=	Milligram
µg	=	Microgram
WHO	=	World Health Organization
LSD	=	Local Storage Depots
CSD	=	Central Storage Depots
HPLC	=	High Performance Liquid Chromatography
ND	=	Not Detected
MRL	=	Maximum Residue Limit

CHAPTER I

INTRODUCTION

Mycotoxins are toxic secondary metabolites generated by different species of fungus such as *Aspergillus, Fusarium, Penicillium,* and *Alternaria*. Aflatoxin, ochratoxin A, citrinin, patulin, fumonisins, zearalenone, trichothecenes and ergot alkaloids like ergotamine are a few examples of mycotoxins. These can be fatal to both humans and animals depending on the degree of exposure.

Among several kinds of mycotoxins, aflatoxins (AFs) are the predominant ones that affect the food quality and can also pose various threats to human health. The six primary types of aflatoxins are aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1,) and M2 (AFM2) (Quadri *et al*., 2012). Food crops such as cereals, pulses, oilseed and edible nuts include B1, B2, G1 and G2, whereas M1 (a B1 metabolite) and M2 are found in animal byproducts such as dairy products.

The International Agency for Research on Cancer has classified aflatoxins (B1, B2, G1 and G2) as category 1 human carcinogens (IARC, 2016). According to Rushing and Selim (2019), Among all of these aflatoxins, aflatoxin B1 (AFB1) is the most lethal and the most potent cancer-causing agent in nature. Aflatoxin affects mineral bioavailability and causes immunological suppression, immune system suppression, mental impairment and low birth weight depending on the extent of exposure. These poisonous metabolites pose major health risks and financial losses in Asia, Africa, and South Americas (Temba *et al*., 2017).

Aflatoxin poisoning impairs both human and animal immune systems. This is brought about by interfering with the brittleness of the immune system-stimulating cells. Aflatoxin accumulation causes liver cancer in both types of levels of aflatoxins, which can cause immediate death and economic loss as well as nutritional or immunologic impacts (Marroqun-Cardona *et al.*, 2014). Aflatoxin causes cancer by oxidizing lipids and damaging DNA (Zhang *et al*., 2015).

Aflatoxin not only causes cancer, but it also harms the liver, kidney, heart, and brain. The many epidemics in India and several African nations are also brought on by aflatoxin. Due to a lack of food regulatory laws, the outbreak's condition is worse in developing and underdeveloped countries. which are not absolutely mandated. As

decreased vaccination rates increase the danger of early illnesses, children are more susceptible to the toxicity of aflatoxins contamination.

Aflatoxin contamination in crops is a global threat that compromises the safety of food and also influences the agricultural economy and crop-dependent small-scale industries.

The predominant sources of human dietary carbohydrates are cereals. Rice is the primary supporter of the complete energy consumption in Asia (28.5%), wheat and, maize contribute similarly (30%) in Africa (FAO, 2014). With the development of an urban-industrial society, people are more likely to consume processed cereals such as breads, flours, breakfast cereals and their processed foods are frequently utilized as complementary foods for infants and young babies. 25–40% of consumed cereals are contaminated with mycotoxins consistently (El-Desouky *et al*., 2013).

At several stages of the agricultural chain, such as pre-harvest, harvest, post-harvest, processing, and transportation, cereals and cereal-based food commodities are extremely susceptible to mycotoxin contamination, particularly aflatoxin contamination. Aflatoxin contamination can occur in agricultural crops other than cereals, including lentil, mungbean, soybean, mustard, and edible nuts. Several mycotoxins can be produced when fungus infect crops throughout the processes of harvesting, storing, and shipping them. If the storage circumstances are favorable for fungal development, the post-harvest crops are more likely to be infected. Millet, sorghum, and maize collected from a storage room were contaminated with aflatoxins to a degree of roughly 92.9%, 50%, and 67.9%, respectively (Sirma *et al*., 2015).

Due to their frequent incidence and detrimental effects on crops, researchers have been studying aflatoxins for a very long time. According to Lewis *et al*. (2005), eating maize that was contaminated with aflatoxin resulted in the deaths of 125 people in Kenya in 2004 and the need for medical attention for 200 or more people. The deaths were primarily brought on by eating locally grown maize that had not been properly dried before storage or treated with fungicides. The maize may have been harvested sooner than usual at that time by farmers to avoid thefts from their fields, which may have left the grain immature and more prone to contamination during storage.

Aflatoxin contamination is caused by physical elements such as pH, moisture, light, temperature, atmospheric gases, and relative humidity. Although fungi that produce

aflatoxin may thrive in a wide pH range (1.7-9.3), the ideal pH range is (3-7) (Yoshinari *et al*., 2010). Initial pH (pH = 5) encourages the formation of AFB (Aflatoxin B), whereas higher pH (pH = 7) encourages the creation of AFG (Aflatoxin G). Aflatoxin contamination is always favored by high moisture content since wet environments are ideal for fungal proliferation and growth. Aflatoxin synthesis is best at 85% relative humidity, although it increases noticeably to a significant level at 95% relative humidity (Ding *et al*., 2015).

Production of aflatoxin is inhibited by sunlight whereas it is boosted by darkness. The formation of aflatoxins is also influenced by the availability of O_2 and CO_2. A greater CO_2 concentration and a lower O_2 concentration hinder the synthesis of aflatoxins and fungi, respectively. Aflatoxins may be produced at a variety of temperatures, although the ideal range for their formation is 25–35 °C (Siciliano *et al*., 2017).

Nations have enacted a number of rules governing the amount of these toxins in food crops in an effort to prevent the contamination of such crops with aflatoxin. The range of 2-4 ppb is used by the European Union (EU) as an interpolation for the aflatoxin concentration level in food items (Gurtler and Keller, 2019). The United States Food and Drug Administration (USFDA) has enforced severe restrictions for the aflatoxins level in impacted food commodities at 20 ppb (parts per billion), whereas 0.5 ppb in milk products. According to the Food Safety and Standards Regulations of 2011, there is a 30 µg/kg maximum limit for all food items in India.

Numerous chemical, physical, and biological techniques as well as diverse genetic engineering breeding techniques have been utilized to lower the level of aflatoxins contamination in crop plants below the advised limit. The regulation of temperature and humidity is one physical strategy for preventing the growth of mycotoxin-producing fungus or removing toxins from tainted food. Antifungal, anti-myotoxic, and anti-mycotoxin plant metabolites can also be used to eliminate mycotoxins chemically and biologically.

The Association of Official Agricultural Chemists (AOAC) offers a number of official techniques for detecting aflatoxin contamination in crop grains (Kumar *et al*., 2017). Enzyme-Linked Immunosorbent Assay (ELISA), a few chromatographic techniques including High-Performance Liquid Chromatography (HPLC), Liquid

Chromatography-Mass Spectroscopy (LCMS), and Thin Layer Chromatography (TLC), and other techniques are employed most frequently (Sulyok *et al*., 2015).

Numerous local crops and food products are contaminated by aflatoxin. Aflatoxin contamination is prevalent in foods and feed such rice, corn, dried fruits, species, figs, and nuts (Martinez-Miranda *et al*., 2019). Contamination of cereals and cereal-based foods with aflatoxins cannot be neglected because the Asian subcontinent is concerned as these are the staples of numerous areas. Researchers have been observing aflatoxins for a long time due to the widespread occurrence of those toxins and their critical impact on human health. Therefore, understanding the incidence of aflatoxins cereals, monitoring with risk characterization, and finding source of occurrence are needed to minimize health hazards and ensure sustainable food safety.

The present study is therefore aimed to determine the aflatoxin residues in selected crops (rice, wheat, maize, lentil, mungbean, mustard, soybean and groundnut) and their processed food items collected from different locations of Bangladesh by high performance liquid chromatography (HPLC). Samples were collected from three different sources (goddowns, wholesale markets and departmental stores) to identify potential sources of aflatoxin contaminations.

The research conducted to achieve the following objectives:

1. To determine the level of aflatoxin in food grains and processed foods from selected areas in Bangladesh.
2. To identify the potential sources of aflatoxin contamination with risk assessment.

CHAPTER II

REVIEW O LITERATURE

Aflatoxins are present in a number of grains, oilseeds, spices, and nuts (Iqbal *et al*., 2014).

Aflatoxin contamination of food is influenced by environmental factors, including temperature and water activity as well as the makeup of the *Aspergillus* strain (Prieto *et al*., 2007)

Most mycotoxins that endanger people and animals are produced by *Aspergillus*, *Pencillium,Fusarium,* and *Alternaria* species that frequently contaminate food and feed supplies. A major threat to food safety for field crops is the possibility of aflatoxin contamination (Dolman, 2003).

Aflatoxin rules set by the European Union for all food exports are believed to cost Africa more than 670 USD million yearly. Aflatoxin poisoning causes farmers and businesses worldwide to lose billions of dollars (Guo *et al*., 2009).

Flavonoids in plants can directly affect the economy and cause crops to lose market value. If peanuts have a lot of aflatoxins, they may not be marketed, which would cost the producer or merchant money (OBrian *et al*., 2010)

2.1 Aflatoxin residues in Food commodities

A test conducted by Chala *et al*. (2013), to measure the total amount of aflatoxins in groundnut. An ELISA test was performed to detect aflatoxin in 120 samples obtained from farmers' markets and supermarkets. Out of these, 93 were favorable, while the remaining 27 were unfavorable. The positive samples' total aflatoxin concentrations ranged from 15 µg/kg to 11,900 µg/kg.

In order to identify aflatoxin B1 (AFB1) in samples of rice, Anthony *et al.* (2014), conducted study. Five of the 15 samples he took of rice showed aflatoxin B1 in the range of 37.26-113.2 µg/kg.

370 samples of rice from six different provinces in China were examined for the presence of aflatoxins and ochratoxin A using dispersive liquid-liquid microextraction and liquid chromatography with fluorescence detection. According to the findings, AFs

and OTA were detected in 63.5% (235/370) and 4.9% (18/370) of the rice samples, respectively (Lai *et al*., 2015).

In the chosen sub-locations (408 samples), maize, sorghum, and millet were sampled from homes as well as marketplaces serving various settlements. The samples were examined for the presence of all aflatoxin. Aflatoxin levels in maize samples obtained ranged from 0.17 to 5.3 ppb in 67.90% of the cases. Aflatoxin was detected in 92.90% of millet samples, ranging from 0.1 to 6.4% ppb. 50% of the sorghum samples tested positive for aflatoxins, which have a maximum acceptable level of 10 ppb (Sirma *et al*., 2015).

According to an experiment conducted by Sserumaga *et al*. (2021), 179 samples were obtained from farmers' home stores in 18 significant groundnut-producing districts throughout seven African agro-ecological zones (AEZ).. From 0 to 1327 ppb of aflatoxin B1 were detected. Aflatoxin levels were more than the Uganda National Bureau of Standards in almost 45% of the samples (10 ppb).

The levels of AFs found in raw peanuts sold in the marketplaces in the Lusaka area were to be determined, as well as the causes of increased AF presence. Using high performance liquid chromatography, raw peanut samples from open markets and supermarkets were gathered and tested for the presence of aflatoxin (Bumbangi *et al*., 2016).

Iqbal *et al*. (2016), used HPLC with a fluorescence detector to conduct an analysis. According to the findings, 35% of the rice samples were found to be contaminated with AFs, with 19% and 24% of those samples exceeding the maximum amount for AFB 1 and total AFs allowed by the European Union (EU), respectively.

The levels of aflatoxin B1 in maize that are safe for eating by humans and animals were found by Lee *et al*. (2017), who also assessed Vietnamese citizens' attitudes toward and awareness of aflatoxins. ELISA was used to analyze a total of 2,370 samples that were gathered from six provinces. From the samples that were collected, 799 samples had concentrations over 2 µg/kg and 687 samples had concentrations above 5 µg/kg [range: below limit of detection (LOD) to 34.8 µg/kg; of the samples above LOD, mean: 13.1 µg/kg, median: 11.2 µg/kg]. 6 different provinces provided a total of 551 interviewees.

The Pakistan Food Authority and European Union Regulations were followed in the quantitative study of AFTs in pulses and spices. Sialkot, Narowal, Gujranwala, and Gujrat were just a few of the Punjabi locations from where the samples were drawn. Through Thin Layer Chromatography, the AFTs were found in 120 samples, above the regulatory authorities' stipulated detection threshold of 50 ppb for spices and 4 ppb for pulses (Nazir *et al*., 2019).

Aflatoxins (AFs) B1, B2, G1 and G2 concentrations in 380 samples of Serbian maize were measured by Kos *et al*. (2013). The presence of aflatoxins was assessed using the direct competitive ELISA technique on 180 samples of maize that were examined between 2009 and 2011. However, in 2012, weather changes led to the presence of AFs in 137 (68.5%) of the samples, with a concentration range of 1.01 to 86.1 µg/kg and a mean level of 36.3 µg/kg.

Al-Wadai *et al*. (2013), conducted an experiment to find aflatoxins in samples of wheat. AFB1 was identified in samples at 2.3 µg/kg, AFB2 at 2.6 µg/kg, AFG1 at 1.3 µg/kg, and AFG2 at 0.5 µg/kg, according to the study.

In 63.5% of the maize fields analyzed in 2009 and 2010, Aspergillus flavus isolates were discovered. ELISA, HPLC-FL, HPLC-MS analyses, and SOS-Chromotest tests revealed that 18.8% of these isolates were able to create aflatoxins over 5 µg/kg on maize kernels (Dobolyi *et al*., 2013)

In a research, 67 raw cereals in total (55 maize and 12 sorghum) were gathered from the Togo market. Aflatoxin B1 was found in 38% of the maize samples, with the highest contamination levels reaching 256 µg/kg, and 25% of the sorghum samples contamination levels ranged from 6 to 16 µg/kg. Aflatoxin levels in maize were high and, in some cases, exceeded the EU's maximum statutory limits for unprocessed corn that is put on the market (Hanvi *et al*., 2019).

2.2 Health Hazards of Aflatoxin residues

The considerable economic losses linked with mycotoxin assaults' effects on human health, animal production, and commerce have drawn attention on a global scale (Fandohan *et al*., 2005).

Acute aflatoxicosis, which manifests as hepatoxicity or, in severe instances, fulminant liver failure, develops quickly after ingesting a high dose of aflatoxin (Fung and Clark, 2004).

Aflatoxins are known to be hepatotoxic, carcinogenic, and teratogenic. A link has been shown between eating foods contaminated with aflatoxins and an increase in liver cancer cases globally (Adhikari *et al*., 1994).

Long-term exposure to low concentrations of toxins can cause chronic aflatoxicosis, which can cause stunting in children and delayed food conversion (Gong *et al*., 2004) and cancer, immune suppression, and a shorter life span (Farombi, 2006).

A key risk factor for hepatocellular carcinoma is chronic dietary exposure to aflatoxins, especially in regions with an endemic hepatitis B virus infection. When large levels of aflatoxin are ingested, acute aflatoxicosis develops quickly and manifests as hepatotoxicity or, in extreme situations, fulminant liver failure (Fung and Clark, 2004).

Aflatoxicosis is the poisoning that happens when someone consumes foods or feed that have been infected with aflatoxins. It is characterized by severe damage that can result in jaundice, hepatitis, mutagenic disorders of the brain system, and, in the worst cases, death (Williams *et al*., 2004).

Over five billion individuals in underdeveloped nations worldwide are at danger of chronic aflatoxin exposure, according to Williams *et al.* (2004). Aflatoxin has also been linked to a number of diseases, such as liver cancer, jaundice, and even mortality (Jolly *et al*., 2007).

Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Hell *et al*., 2008).

Aspergillus flavus and *Aspergillus paraciticus* strains create a poisonous and cancer-causing chemical called aflatoxin. The majority of known mycotoxins are aflatoxins, which are generated by *Aspergillus flavus* and *A. paraciticus* (Gnonlonfin *et al.*, 2012). If it is determined that peanuts and peanut products contain at least 20 ppb of aflatoxins, the Food and Drug Administration (FDA) will take legal action (US FDA 2000).

The main aflatoxins are B1, B2, G1, and G2, which can harm the body through mucous membranes, the skin, or the lungs and cause an excessive inflammatory reaction (Romani, 2004).

Aflatoxin consequences include hepatotoxicity, teratogenicity, and immunotoxicity can pose substantial risks to both human and animal health (Roze *et al*., 2013).

In India's states of Gujrat and Rajasthan, a significant outbreak of hepatitis caused by aflatoxin was documented in 1974. It is believed that 106 people died as a result of the outbreak (Krishnamachari *et al*., 1975). The 2 month-long pandemic was limited to tribal populations, whose primary source of food, maize, was later shown to contain aflatoxins.

Aflatoxin chronic toxicity includes immunosuppressive and cancer-causing effects. Male F344 rats' splenic lymphocyte morphologies and inflammatory cytokine expression have been examined in relation to the effects of AFT-B1 (Qian *et al*., 2014).

According to Mehrzad *et al*. (2014), AFT-B1 disrupts the capacity of porcine dendritic cells to deliver antigens, suggesting that this may be one of the substance's immunotoxin mechanisms.

AFTs-M1 also causes liver damage, decreased milk production, inhibition of the immune system, and decreased oxygen delivery to tissues resulting to anemia (Aydin *et al*., 2008).

Numerous studies have demonstrated the harmful effects of aflatoxins exposure on the epididymis, kidney, heart, liver, and kidney (Sharmila Banu *et al*., 2009), as well as other organs (Gupta and Sharma, 2011).

Oedema, hemorrhagic, necrosis of the liver, and extreme lethargy are among of the immediate signs of aflatoxicosis, whereas cancer, immune system suppression, and growth retardation are its long-term repercussions (Cotty and Jaime-Garcia, 2007).

Later, the International Agency for Research on Cancer (IARC) classified AFB1 as a category I human carcinogen (Seo *et al*., 2011).

2.3 Determination of Aflatoxin residues by Chromatographic method

Thin layer chromatography (TLC) is among one of the oldest techniques used for aflatoxin detection (Fallah *et al*., 2011).

The Association of Official Analytical Chemists (AOAC) provides information on a variety of official techniques for identifying aflatoxin contamination in agricultural plants (Kumar *et al*., 2017).

Enzyme-Linked Immunosorbent Assay (ELISA), a few chromatographic techniques including High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectroscopy (LCMS), and Thin Layer Chromatography (TLC), and other techniques are employed most frequently (Sulyok *et al*., 2015).

A highly specific sandwich ELISA with a minimum detection limit of 1 g/mL for both *A. flavus* and *A. parasiicus* was developed by Wang Li *et al*. (2017). The extensiveness, high technical skill need, and time requirement of the aforementioned procedures are only a few of their unfortunate drawbacks.

Instant results are provided via the Polymerase Chain Reaction (PCR), Fluorescence/Near-Infrared Spectroscopy (FS/NIRS), and Hyper Spectral Imaging (HSI). *A. flavus,* a fungus that produces aflatoxins, is often detected using the PCR method (Tao *et al*., 2018).

As a result of improvements in analytical methods, aflatoxins and other harmful substances may now be detected simultaneously. Time-Resolved Fluorescence Immuno-Chromatographic Assay (TRFICA) was used to concurrently detect aflatoxins and zearalenone (Tang *et al*., 2017).

Enzyme-Linked immunosorbent Assay (ELISA) procedures are the most widely used serological tests for aflatoxin analysis due to their simplicity, adaptability, and sensitivity (ICRISAT, 2007).

Based on a calculation of the blood content of AfB1-lysine, a metabolite of the AFB1 toxin, ELISA can be used to detect aflatoxins. The test specifically identifies AfB1 levels in blood as low as 5 pg/mg albumin, making it an affordable tool for routine monitoring that may also be used for hepatitis B virus identification. Food mycology frequently uses room temperature phosphorescence (RTP) in aflatoxigenic strains

cultured on medium. Absence or presence of oxygen and heavy atoms might cause RTP when aflatoxins are immobilized on resin beads (Costa-Fernandez and Sanz-Medel, 2000). In order to assure the safety of the food, several biosensors and immunoassays have been developed to detect ultra-traces of aflatoxins.

A novel approach uses Color-encoded Lateral Flow Immuno-Assay (CLFIA) to detect aflatoxins and fumonisins, a class of mycotoxins generated from *Fusarium s*pp (Di Nardo *et al*., 2019).

To detect various aflatoxins in agricultural plants, specific nanoparticle-based methods that include quantum dots (QD), carbon (CBNs), and Au/Ag are also used (Xue *et al*., 2019).

CHAPTER III

MATERIALS AND METHODS

3.1 Study area and duration

The research work was conducted in the Institute of Food Science and Technology (IFST) at Bangladesh Council of Scientific and Industrial Research (BCSIR). Samples were collected from three different sources i.e. godown, wholesale market and departmental store. Grain samples were collected from different goddowns of Dhaka, Narayanganj, Panchagarh, Thakurgaon, Dinajpur, Gaibandha, Bogura, Natore and Ishwardi and from wholesale markets of Dhaka (Mirpur, Banani, New market). Processed food samples were collected from departmental stores of Newmarket, during the period of March, 2020 to June, 2021.

3.2 Sample Collection

A total of 60 samples (50 grain samples and 10 processed food samples) were collected from goddown, wholesale market and departmental sources from different places in Bangladesh. Samples were collected during March, 2020 to June, 2021.

Table 1. A total 29 samples of different crops were collected from different goddowns

	Sample No.	Sample ID	Crop Name	Collection Area
Samples Collected from Godown	1	PIsLL	Peanut (Local)	Ishwardi godown (left)
	2	PIsLR	Peanut (Local)	Ishwardi godown (Right)
	3	PIsIL	Peanut (Import)	Ishwardi godown (left)
	4	PIsIR	Peanut (Import)	Ishwardi godown (Right)
	5	MPnL	Maize	Panchagarh godown (Left)
	6	MPnR	Maize	Panchagarh godown (Right)
	7	RDkIL	Rice (Import)	Dhaka CSD (Left)
	8	RDkIR	Rice (Import)	Dhaka CSD (Right)
	9	RDjLL	Rice (Local)	Dinajpur LSD (Left)
	10	RDjLR	Rice (Local)	Dinajpur LSD (Right)
	11	RGbLL	Rice (Local)	Gaibandha LSD (Left)
	12	RGbLR	Rice (Local)	Gaibandha LSD (Right)
	13	RNgChL	Chinigura Rice	Narayanganj godown (Left)
	14	RNgChR	Chinigura Rice	Narayanganj godown (Right)
	15	RNgBkL	Boiled Katari	Narayanganj godown (Left)
	16	RNgBkR	Boiled Katari	Narayanganj godown (Right)
	17	LNaL	Lentil	Natore godown (Left)
	18	LNaL	Lentil	Natore godown (Right)
	19	LCdNg	Canadian Lentil	Narayanganj godown
	20	LAusNg	Australian Lentil	Narayanganj godown
	21	MdBogL	Mustard	Bogura godown (Left)
	22	MdBogR	Mustard	Bogura godown (Right)
	23	MbNg	Mungbean(Import)	Narayanganj godown
	24	MbIsL	Mungbean	Ishwardi godown (left)
	25	MbIsR	Mungbean	Ishwardi godown (Right)
	26	WDjIL	Wheat (Import)	Dinajpur LSD(Left)
	27	WGbIL	Wheat (Import)	Gaibandha LSD(Left)
	28	WTgL	Wheat (Local)	Thakurgaon godown (Left)
	29	WTgL	Wheat (Local)	Thakurgaon godown (Right)

Table 2. A total 21 samples of different crops were collected from different wholesale markets

	Sample No.	Sample ID	Crop Name	Collection Area
Samples Collected from Wholesale Market	30	RMkMp	Rice	Mirpur Market
	31	RMkBn	Rice	Banani Market
	32	LMkMp	Lentil	Mirpur Market
	33	LMkBn	Lentil	Banani Market
	34	MbMkMp	Mungbean	Mirpur Market
	35	MbMkBn	Mungbean	Banani Market
	36	MdMkMp	Mustard	Mirpur Market
	37	MdMkBn	Mustard	Banani Market
	38	MzMkMp	Maize	Mirpur Market
	39	MzMkBn	Maize	Banani Market
	40	PMkMp	Peanut	Mirpur Market
	41	PMkBn	Peanut	Banani Market
	42	WMkMp	Wheat	Mirpur Market
	43	RMkNm	Rice	Newmarket
	44	LMkNm	Lentil	Newmarket
	45	MbMkNm	Mungbean	Newmarket
	46	MdMkNm	Mustard	Newmarket
	47	WMkNm	Wheat	Newmarket
	48	MzMkNm	Maize	Newmarket
	49	PMkNm	Peanut	Newmarket
	50	SbMkNm	Soybean	Newmarket

Table 3. A total 10 samples of different processed food items were collected from departmental store

	Sample No.	Sample ID	Crop Name	Collection Area
Processed Food Samples from Departmental Store	51	RpNm	Rice flour	Newmarket
	52	WpNm	Wheat flour	Newmarket
	53	MpNm	Popcorn(raw)	Newmarket
	54	LpNm	Lentil beshon	Newmarket
	55	SopNm	Soya nuggets	Newmarket
	56	WheatpNm	Macoroni	Newmarket
	57	MdpNm	kashundi	Newmarket
	58	MbpNm	Dal vaja	Newmarket
	59	BdpNm	Badamvaja	Newmarket
	60	PcNm	Popcorn(fried)	Newmarket

3.3 Sample preservation

Each crop sample was collected in each zipper bag on the sampling site and instant marking and tagging was done All the samples were transported from the spot to the Institute of Food Science and Technology (IFST), BCSIR, Dhaka and each sample was separated to one another with valid identification number were preserved in the 4° C refrigerator for further procedure.

3.4 Sample preparation:

First of all, collected crop samples were grinded and again stored in clean zipper bag with a proper labeling.

3.5 Formation of Slurry of the sample and Extraction Procedure:

From the 200 g of each raw samples, 100 g samples were taken for grinding for the mycotoxin analysis,

Equipments:

a. Spatula
b. Blender
c. Electric Balance
d. Rotary Shaker
e. Funnel
f. 24 cm Whatman No. 1 filter paper

Reagents:

a. Acetone
b. Distilled Water

Procedure:

1. 25 g of each sample was measured by electric balance machine and taken into a conical flask
2. 50 ml water was added to the sample in the ration of 1:2 (sample: water) and shaken for 30 minutes in a rotator shaker for slurry formation and slurry should be homogeneous.
3. Slurry was weighted by Electric balance and took in 500ml flask.

4. The volume of acetone required to give 4:1 ratio to acetone to water. So the calculation was done by following formula:

$$\text{Volume of Acetone} = \frac{\text{Volume of slurry} \times \text{water ratio} \times 4}{1 + \text{water ratio}}$$

5. After calculating, the volume of acetone for each sample was added to the slurry, the flask was sealed with flask lid and secured with maskin tape.
6. The flask was gently shaken manually in an up and down motion for 20 seconds
7. The flask was fixed into the flask shaker and shaken for 30 minutes.
8. After shaking the flask in rotator, filtration of the extracts through a 24 cm Whatman No. 1 filter paper with the help of funnel into a 250 ml ground joint conical flask in a fume cupboard was done.

3.6 Rectification using the Phenyl bonded-phase method

This rectification procedure had been found to be the method of choice for preparing extracts suitable for quantification by High Performance Liquid chromatography (HPLC) with fluorescence detector.

Equipments:

a. 250 ml measuring cylinder
b. Spatula
c. 5ml Vials
d. Vacuum manifold and taps
e. Vacuum line
f. Phenyl bonded- phase purification column
g. Reservoirs and adaptors
h. Absoluters
i. Dispenser
j. Syringe filter
k. Sample vortex
l. HPLC Systems (Agilent 1100 series)
m. HPLC Column: C_{18}, 250 mm×10mm

Reagents:

a. Filter Aid (Celite 545)
b. Lead Acetate (10%)
c. Sodium sulphate (Na_2SO_4)
d. Florisil
e. Chloroform
f. Distilled Water
g. Methanol (HPLC grade and AR grade)
h. Acetonitrile (HPLC)
i. Mobile phase= Acetone: Methanol: Water = 22.5: 22.5: 55

Procedure:

1. Each 10 ml filtrate sample was taken in a measuring cylinder
2. In each measuring cylinder, 2ml of lead acetate (10%) solution was added.
3. Then 10 ml of methanol was added to each solution and final volume was made up to 150 ml by adding distilled water.
4. The required number of phenyl column with 70ml reservoirs was filled and the column then fitted into the tap fittings on a vacuum manifold after labeling of the column.
5. A little amount of filter aid was added to the reservoirs and washed with 10ml methanol then 15ml distilled water was passed.
6. Then 150ml of each sample solution was passed through the reservoirs (70ml)
7. After passing the sample solution, washed with 10ml distilled water and dried it for 5 to 7 minutes.
8. Fit a 25ml reservoir above the phenyl column using adaptors and below the phenyl column, a florisil column (300g) followed by a sodium sulphate column (300g) were fitted.
9. Suitable labeled and positioned 7ml glass vials were placed into rack and then kept inside the vacuum and was checked carefully that the vials are placed correctly to receive the eluent.
10. Five ml of chloroform was provided to the reservoir using the dispenser. The column tapes were opened and gently increased the vacuum to above 3 inch

mercury not more than 5inch mercury. The vacuum can release such that the elution takes 5 to 8 minutes.

11. The vials were transferred to a sample concentration supply with dry nitrogen and set at 45℃ to dry the vials
12. 1ml mobile phase was added to each dried vial
13. Then filtrated by syringe filter
14. The sample containing vials were vortex for 1-2 minutes each.
15. Then the vials were ready for HPLC analysis.

HPLC Condition:

a. Mobile phase= Acetone: Methanol: Water = 22.5: 22.5: 55
b. Column: LC- 18,250 mm×10 mm (10µm packing)
c. Flow rate: 1.5ml/min
d. Column Temperature: 30˚C
e. Injection volume: 20 µl
f. Detector: Fluorescence Detector (Agilent, G1321A)
g. Excitation Wavelength: 365nm
h. Emission Wavelength: 418 nm
i. Software: Agilent ChemStation for LC 3D Systems, Rev. A. 10.02 (1757)

3.7 Limitation of the study

First of all, there was extreme lack of resources. I collected 150 samples of different crops but due to lack of resources only 60 samples were allowed to carry out my experimental analysis.

Aflatoxin analysis

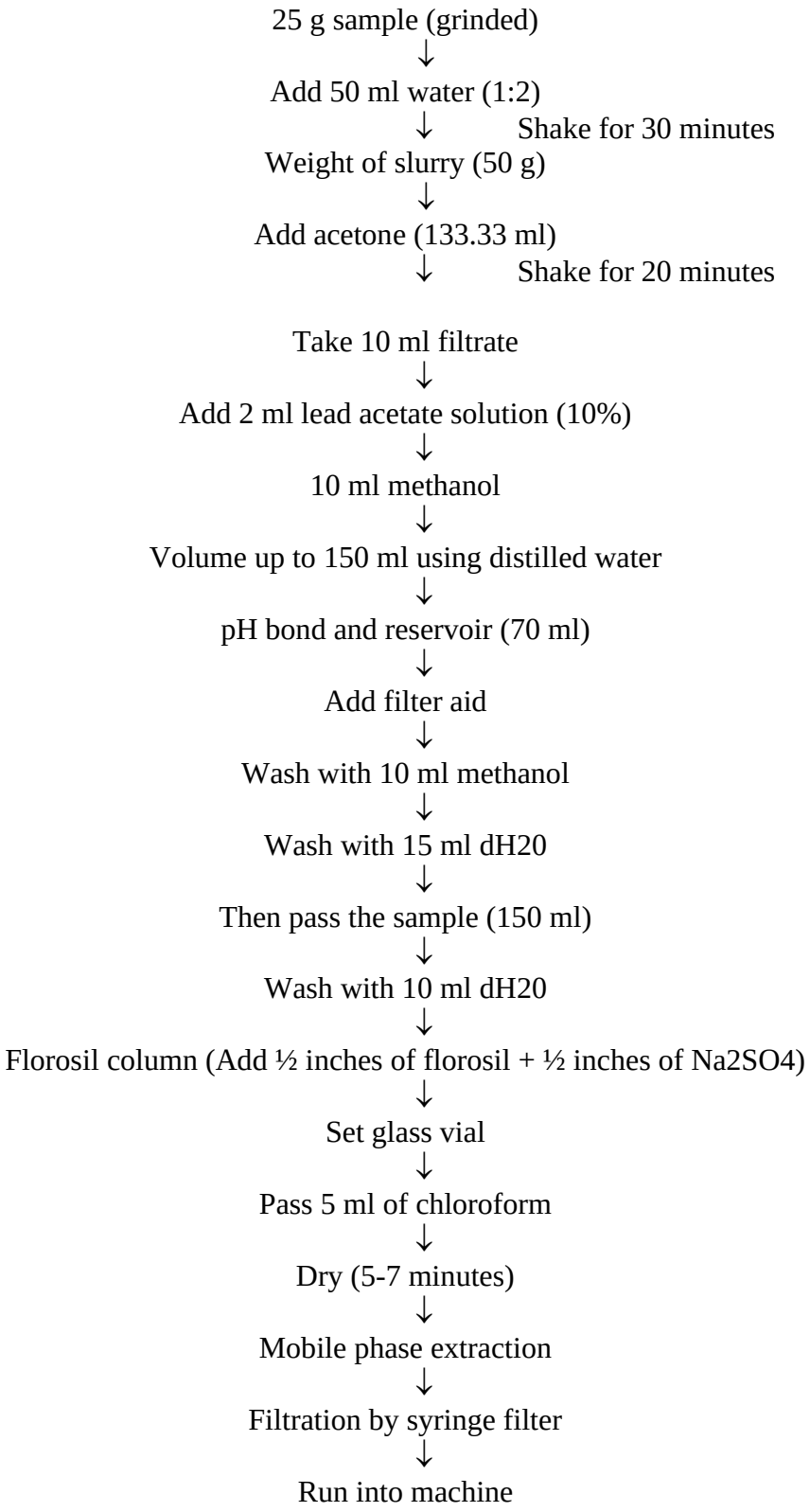
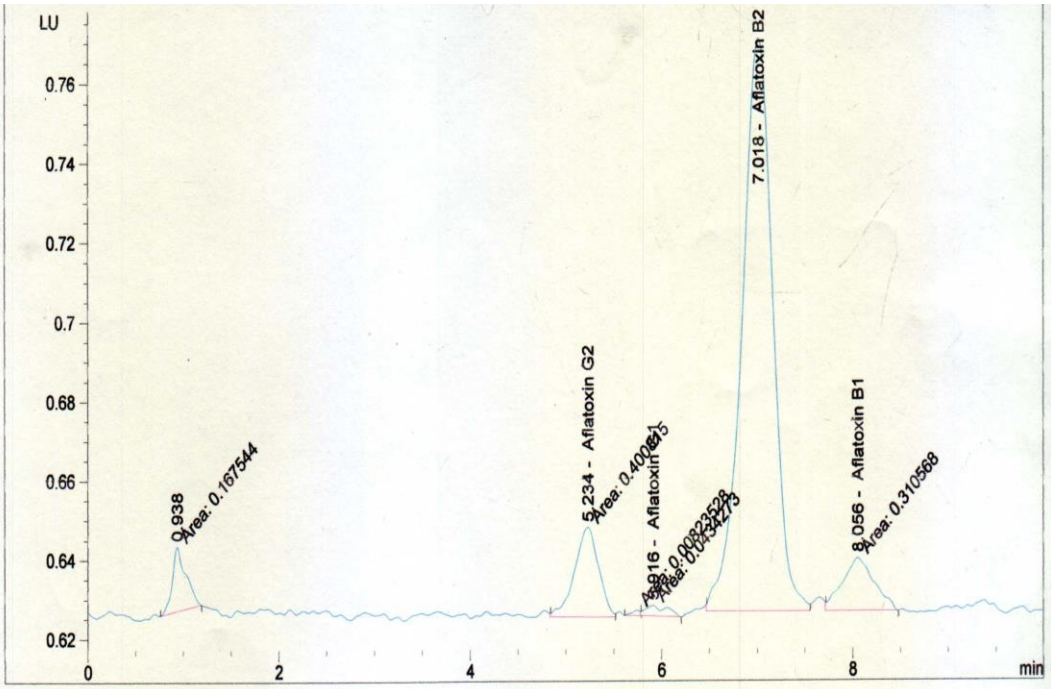


Figure 1. Flow diagram of the procedure of mycotoxin analysis by HPLC method



External Standard Report

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Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
5.234	MF	4.00815e-1	2.27452	9.11662e-1			Aflatoxin G2
5.916	FM	4.34273e-2	117.39063	5.09796			Aflatoxin G1
7.018	BV	3.24874	2.95957e-1	9.61488e-1			Aflatoxin B2
8.056	FM	3.10568e-1	15.29700	4.75076			Aflatoxin B1

Totals : 11.72187

Results obtained with enhanced integrator!

*** End of Report ***

Figure 2. Chromatogram of the standard solution

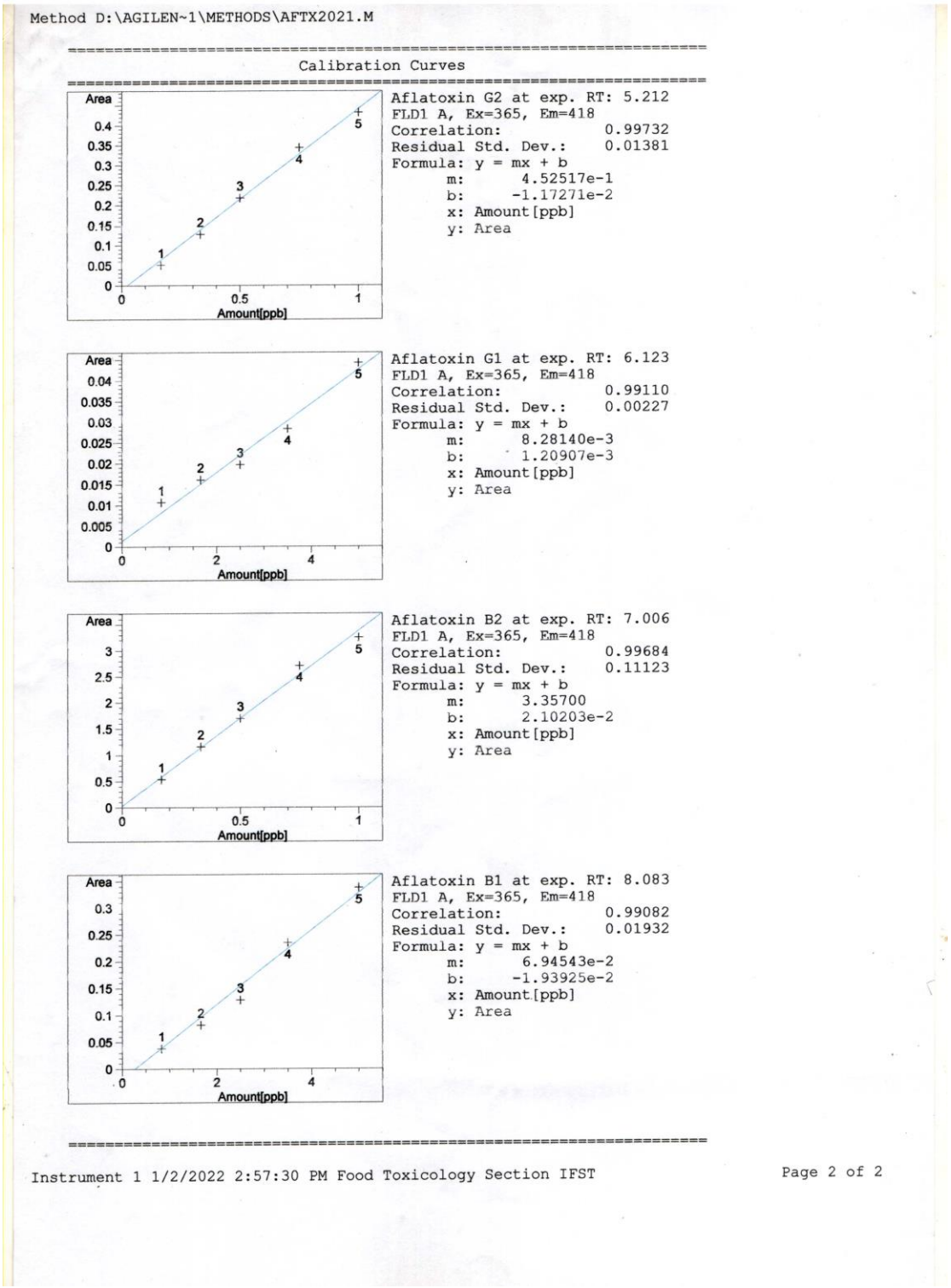


Figure 3. Calibration curves of aflatoxin B1, B2, G1 & G2

CHAPTER IV

RESULTS AND DISCUSSION

Cereals (rice, wheat and maize), pulses (lentil, mungbean), oilseed crops (mustard, soybean) and groundnut are mostly grown crops for consumption purposes in Bangladesh. A total of 60 samples were collected, of these 29 samples were from goddown, 21 samples from wholesale market and 10 process food items collected from different locations of Bangladesh. Among these samples, 14 rice samples (of these 10 samples were from goddown, 3 samples from wholesale market and 1 processed food sample namely rice flour) ,9 wheat samples (4 samples from goddown, 4 samples from goddown 3 samples from wholesale market and 1 processed food sample namely wheat flour), 7 samples of maize (2 samples from goddown, 3 samples of wholesale market and 2 processed samples namely raw popcorn and fried popcorn), 8 samples of lentil(4 samples from goddown, 3 samples from wholesale market and 1 processed food item namely lentil beshon), 6 samples of mung beans (2 samples from goddown, 3 samples from wholesale market and 1 processed food sample namely dal vaja),

After analysis evident amounts of aflatoxin residues were found in a number of collected goddown and wholesale market samples used for the current study. But no processed food sample were found to be positive for aflatoxin residues. Results are presented in tabular forms which are obtained from the chromatogram of the analyzed samples.

4.1 Determination of aflatoxin residues in collected samples

In case of 29 goddown samples, 8 samples were detected aflatoxin positive where detection limit was 0.5 µg/kg as per Commission Regulation (EC) No. 1881/2006 for Food. Samples of peanut, maize and lentil were detected positive.

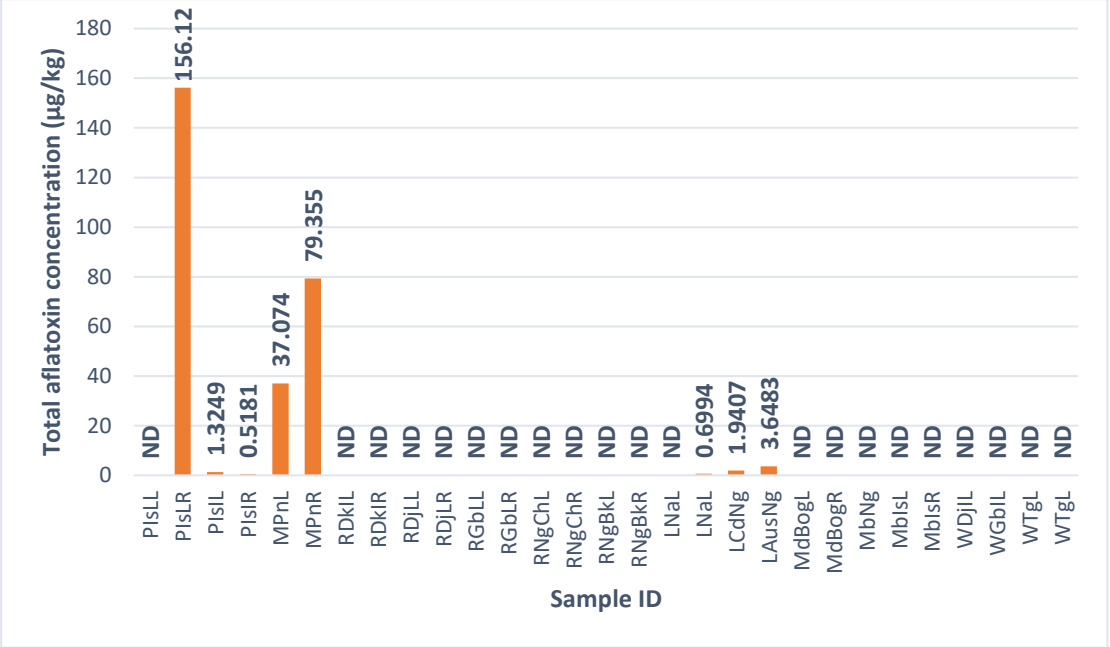


Figure 2. Detected level of total aflatoxin concentration in goddown samples

4 samples of peanut both local and imported were collected from the goddown of Ishwardi. Of these, 3 samples were detected positive for aflatoxin residues (2 imported and 1 local peanut sample). Peanut (local) sample collected from Ishwardi goddown detected with total aflatoxins 156.12 µg/kg. Peanut (imported) sample collected from Ishwardi goddown (left) detected with total aflatoxins 1.3249 µg/kg. Peanut (imported) sample collected from Ishwardi goddown (right) detected with total aflatoxins 0.5181 µg/kg. 2 samples of maize were collected from Panchagarh goddown, both were detected with aflatoxin residues.

Maize collected from Panchagarh goddown (left) and Panchagarh goddown (right) were detected with total aflatoxins 37.074 µg/kg and 79.355 µg/kg respectively. 4 samples of lentil were collected from Natore (2 sample) and Narayanganj (2 sample). Of these 3 samples of lentil were detected positive for aflatoxin residues. Lentil collected from Natore goddown was detected with total aflatoxins 0.6994 µg/kg. Lentil (Canadian) and Lentil (Australian) collected from Narayanganj goddown were detected with total aflatoxins 1.9407 µg/kg and 3.6483 µg/kg respectively.

Studies conducted by Barros *et al.* (2003), showed similar result. An average of 124.8 µg/kg of total aflatoxins was found in peanuts from Córdoba, Argentina, that were gathered in conventional peanut farming regions. In 63.5% of the maize fields analyzed in 2009 and 2010, *Aspergillus flavus* isolates were discovered. ELISA, HPLC-FL, HPLC-MS analyses, and SOS-Chromotest tests revealed that 18.8% of these isolates were able to create aflatoxins over 5 µg/kg on maize kernels (Dobolyi *et al*., 2013).

21 samples of rice, wheat, maize, lentil, mungbean, mustard, soybean and peanuts were collected from three whole sale markets namely Banani, Mirpur and Newmaket of Dhaka.

Figure 3. Detected level of total aflatoxin concentration in wholesale market samples

Of these 21 samples, only 1 rice sample from Mirpur kacha bazar, Dhaka was detected positive foraflatoxin residue with Aflatoxin B1 (AFB1) 1.4286 µg/kg . Nearly similar result was found by Lai *et al.* (2015). By combining dispersive liquid-liquid microextraction with liquid chromatography and fluorescence detection, 370 samples of rice from six different regions in China were examined for the presence of aflatoxins (AFs). 63.5% (235/370) and 4.9% (18/370) of rice samples tested positive for measurable levels of AFs, according to the findings.

10 processed food items like rice flour, wheat flour, popcorn, lentil beshon, soya nuggets, macaroni, kashundi, dal vaja, badamvaja were collected from different departmental stores of Newmarket, Dhaka. No sample was detected positive for aflatoxin in processed food items.

9 samples were detected positive for aflatoxin residues. Of which 4 samples exceeded the maximum residue limit of detection which is 2 µg/kg for Aflatoxin B1 and 4 µg/kg for Aflatoxin B1, B2, G1, G2 as per Commission Regulation (EC) No. 1881/2006 for Food.

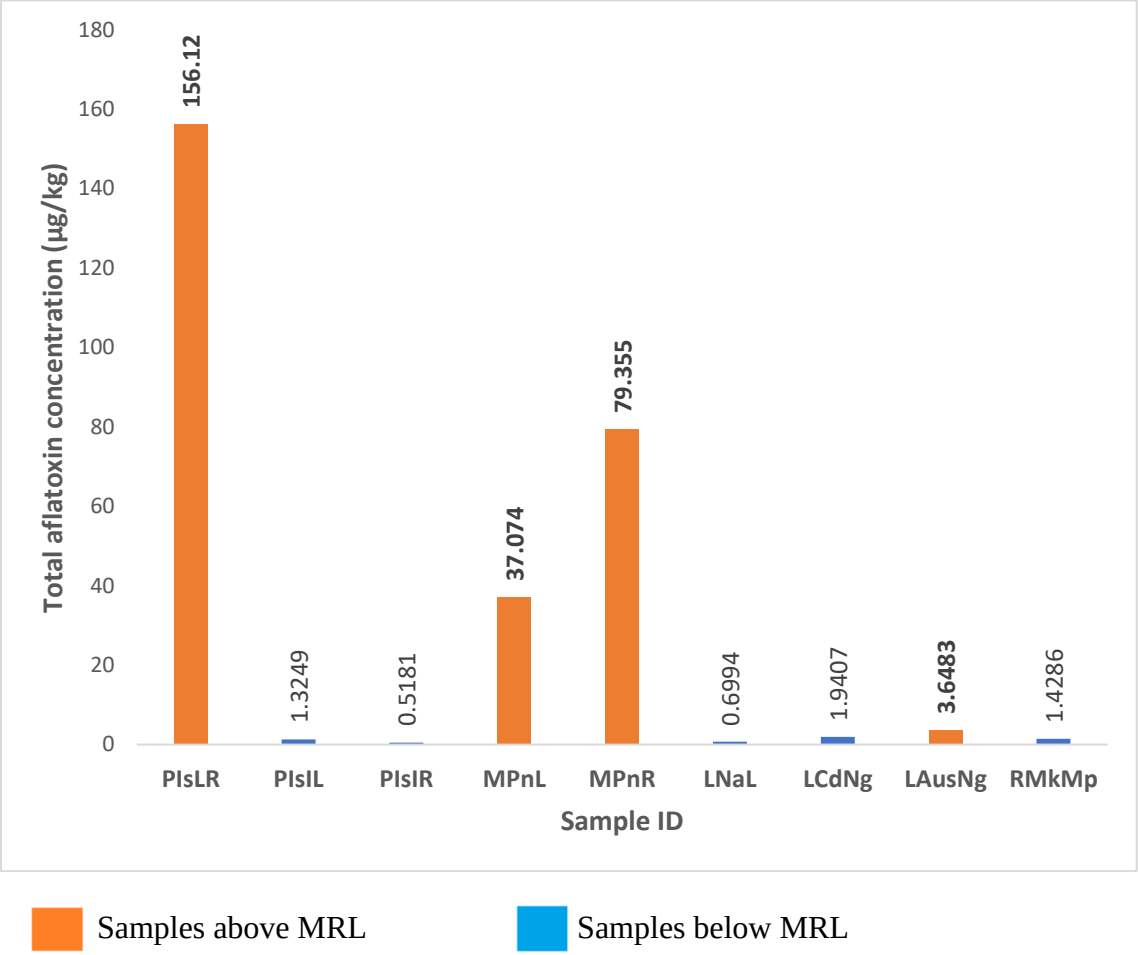


Figure 4. Detected level of total aflatoxin concentration in samples above MRL and below MRL

We can observe from the above figure that peanut (local) sample collected from Ishwardi goddown detected with Aflatoxin B1 156.1287 µg/kg and total aflatoxins 156.12 µg/kg. Maize collected from Panchagarh goddown (left) and Panchagarh goddown (right) were detected with Aflatoxin B1 37.07381 µg/kg and 79.35452 µg/kg, respectively. Lentil (Australian) sample collected from Narayanganj goddown detected with Aflatoxin B1 3.2691 µg/kg, Aflatoxin B2 0.3792 µg/kg and total Aflatoxins 3.6483 µg/kg. Similar

findings were made by Lee *et al*. (2017), who conducted research to measure the levels of aflatoxin B1 in maize suitable for human and animal consumption as well as to assess Vietnamese citizens' attitudes and understanding of aflatoxins. 2,370 samples in total were gathered from six provinces and put through an ELISA analysis. 799 samples out of the total number of samples were found to have values greater than 2 µg/kg and 5 µg/kg, respectively.

4.2 Source of contamination

A total of 60 samples were collected from different locations of Bangladesh. Of these 9 samples of peanut, maize, lentil and rice were detected positive for aflatoxin residues. It revealed that 15% samples were detected positive for aflatoxins.

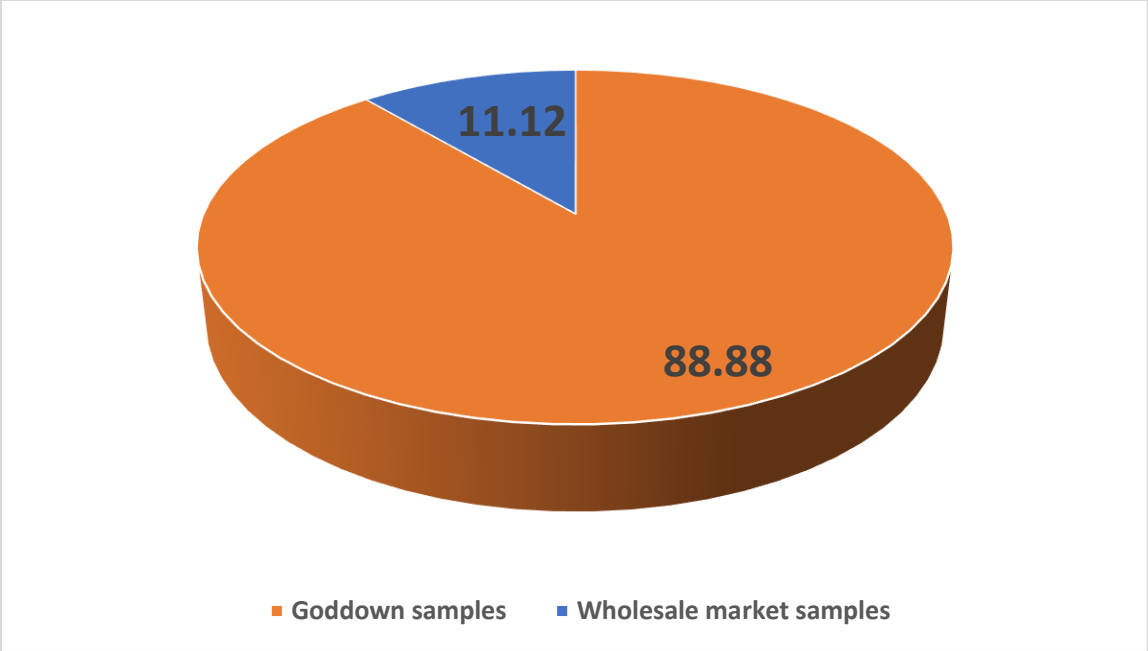


Figure 5. Showing the source of contamination

From the above figure, it is observed that among 9 positive samples, 8 samples collected from goddowns were detected positive for aflatoxins. It showed that 88.88% positive samples were from goddown. Of these, 3 samples exceeded the maximum residue limit of detection which is 2 µg/kg for Aflatoxin B1 and 4 µg/kg for Aflatoxin B1, B2, G1, G2 as per Commission Regulation (EC) No. 1881/2006 for Food. 1 sample collected from wholesale market were detected positive for aflatoxins. It revealed that 11.11% positive

samples were from wholesale market. It did not exceed the maximum residue limit. No processed food item was detected positive for aflatoxins. Studies on the naturally occurring aflatoxins in Argentinian maize have revealed a range in the levels of contamination both during harvest and storage. According to research done between 1999 and 2010, aflatoxins levels fluctuated between 6.7 and 427 µg/kg (Garrido *et al*., 2012). Insects, fungi, and aflatoxins may cause significant issues in grain storage systems when circumstances are favorable, according to Nesci *et al*. (2016).

Table 4. Results of Aflatoxin concentration found in godown, wholesale market and processed food samples

Sl. No.	Crop Name	Sample ID	Collection Area	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	Total Aflatoxins (µg/kg)
1	Peanut (Local)	PIsLL	Ishwardi godown (left)					ND
2	Peanut (Local)	PIsLR	Ishwardi godown (Right)	156.12187				156.12
3	Peanut (Import)	PIsIL	Ishwardi godown (left)		0.6635		0.6614	1.3249
4	Peanut (Import)	PIsIR	Ishwardi godown (Right)		0.5181			0.5181
5	Maize	MPnL	Panchagarh godown (Left)	37.07381				37.074
6	Maize	MPnR	Panchagarh godown (Right)	79.35452				79.355
7	Rice (Import)	RDkIL	Dhaka CSD (Left)					ND
8	Rice (Import)	RDkIR	Dhaka CSD (Right)					ND
9	Rice (Local)	RDjLL	Dinajpur LSD (Left)					ND
10	Rice (Local)	RDjLR	Dinajpur LSD Right)					ND
11	Rice (Local)	RGbLL	Gaibandha LSD (Left)					ND
12	Rice (Local)	RGbLR	Gaibandha LSD (Right)					ND
13	Chinigura Rice	RNgChL	Narayanganj godown (Left)					ND
14	Chinigura Rice	RNgChR	Narayanganj godown (Right)					ND
15	Boiled Katari	RNgBkL	Narayanganj godown (Left)					ND
16	Boiled Katari	RNgBkR	Narayanganj godown (Right)					ND
17	Lentil	LNaL	Natore godown (Left)					ND
18	Lentil	LNaL	Natore godown (Right)		0.6994			0.6994
19	Canadian Lentil	LCdNg	Narayanganj godown	1.6056	0.3352			1.9407
20	Australian Lentil	LAusNg	Narayanganj godown	3.2691	0.3792			3.6483
21	Mustard	MdBogL	Bogura godown (Left)					ND
22	Mustard	MdBogR	Bogura godown (Right)					ND
23	Mungbean (Import)	MbNg	Narayanganj godown					ND

Table 4 (cont'd)

Sl. No.	Crop Name	Sample ID	Collection Area	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	Total Aflatoxins (µg/kg)
24	Mungbean	MbIsL	Ishwardi godown (left)					ND
25	Mungbean	MbIsR	Ishwardi godown (Right)					ND
26	Wheat (Import)	WDjIL	Dinajpur LSD (Left)					ND
27	Wheat (Import)	WGbIL	Gaibandha LSD(Left)					ND
28	Wheat (Local)	WTgL	Thakurgaon godown (Left)					ND
29	Wheat (Local)	WTgL	Thakurgaon godown (Right)					ND
30	Rice	RMkMp	Mirpur Market	1.4286				1.4286
31	Rice	RMkBn	Banani Market					ND
32	Lentil	LMkMp	Mirpur Market					ND
33	Lentil	LMkBn	Banani Market					ND
34	Mungbean	MbMkMp	Mirpur Market					ND
35	Mungbean	MbMkBn	Banani Market					ND
36	Mustard	MdMkMp	Mirpur Market					ND
37	Mustard	MdMkBn	Banani Market					ND
38	Maize	MzMkMp	Mirpur Market					ND
39	Maize	MzMkBn	Banani Market					ND
40	Peanut	PMkMp	Mirpur Market					ND
41	Peanut	PMkBn	Banani Market					ND
42	Wheat	WMkMp	Mirpur Market					ND
43	Rice	RMkNm	Newmarket					ND
44	Lentil	LMkNm	Newmarket					ND
45	Mungbean	MbMkNm	Newmarket					ND

Table 4 (cont'd)

Sl. No.	Crop Name	Sample ID	Collection Area	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	Total Aflatoxins (µg/kg)
46	Mustard	MdMkNm	Newmarket					ND
47	Wheat	WMkNm	Newmarket					ND
48	Maize	MzMkNm	Newmarket					ND
49	Peanut	PMkNm	Newmarket					ND
50	Soybean	SbMkNm	Newmarket					ND
51	Rice flour	RpNm	Newmarket					ND
52	Wheat flour	WpNm	Newmarket					ND
53	Popcorn(raw)	MpNm	Newmarket					ND
54	Lentil beshon	LpNm	Newmarket					ND
55	Soya nuggets	SopNm	Newmarket					ND
56	Macoroni	WheatpNm	Newmarket					ND
57	kashundi	MdpNm	Newmarket					ND
58	Dal vaja	MbpNm	Newmarket					ND
59	Badamvaja	BdpNm	Newmarket					ND
60	Popcorn (fried)	PcNm	Newmarket					ND

Method: HPLC with fluorescence detector
Direction limit: 0.5 µg/kg
Maximum Residue Limit (MRL): a) Aflatoxin B1 : 2 µg/kg
b) Aflatoxin B1, B2, G1, G2 : 4 µg/kg
According to Commission Regulation (EC) No. 1881/2006 for Food

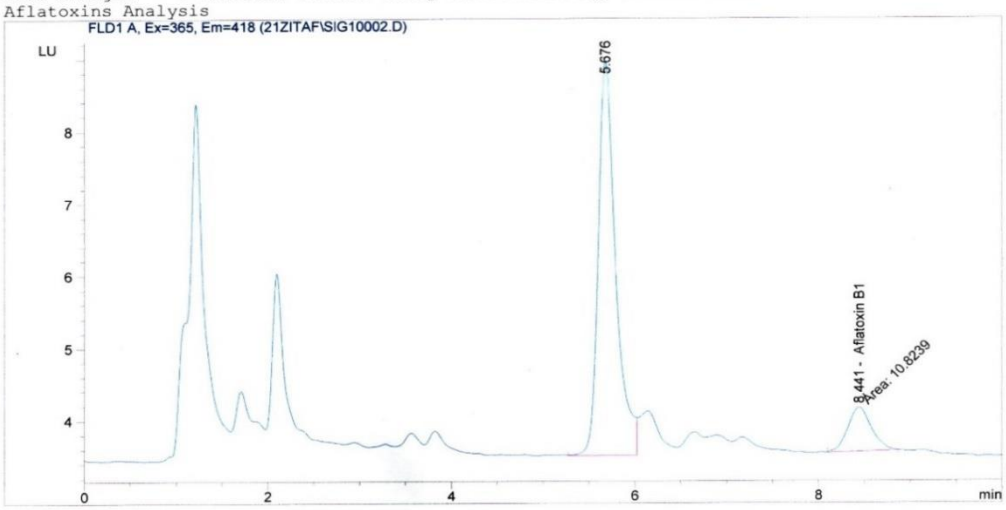
Here, ND = Not Detected

4.3 Chromatograms and calibration curves of aflatoxin analysis in positive grain samples

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10002.D Sample Name: Peanut

Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry
Sample:02.PIsLR
Food Toxicology Research Section,
IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:29.11.2021

Injection Date : 11/29/2021 1:04:59 PM
Sample Name : Peanut Location : Vial 1
Acq. Operator : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed : 11/29/2021 12:11:14 PM by Food Toxicology Section IFST
(modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed : 1/2/2022 6:25:39 PM by Food Toxicology Section IFST
Aflatoxins Analysis



External Standard Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
5.212		-	-	-			Aflatoxin G2
6.123		-	-	-			Aflatoxin G1

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10002.D Sample Name: Peanut

RetTime [min]	Type	Area LU *s	Amt/Area	Amount [ppb]	Grp	Name
7.006		-	-	-		Aflatoxin B2
8.441	MM	10.82394	14.42376	156.12187		Aflatoxin B1

Totals : 156.12187

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

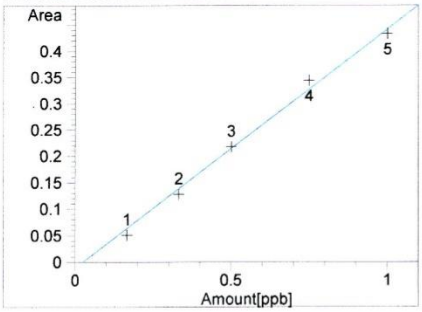
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

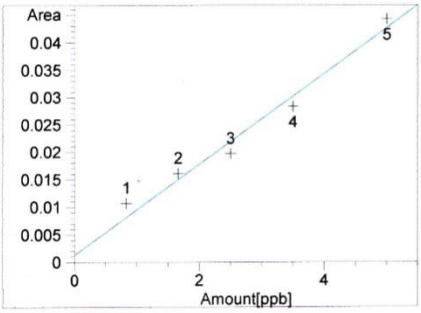
Name	Total Area LU *s	Amount [ppb]
Aflatoxin G2	0.00000	0.0000
Aflatoxin G1	0.00000	0.0000
Aflatoxin B2	0.00000	0.0000
Aflatoxin B1	10.82394	156.1219

Totals : 156.1219

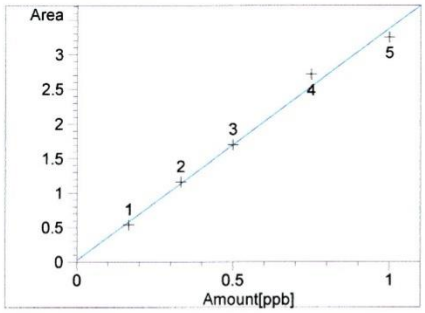
Calibration Curves



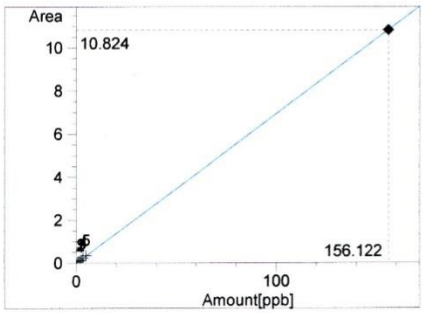
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

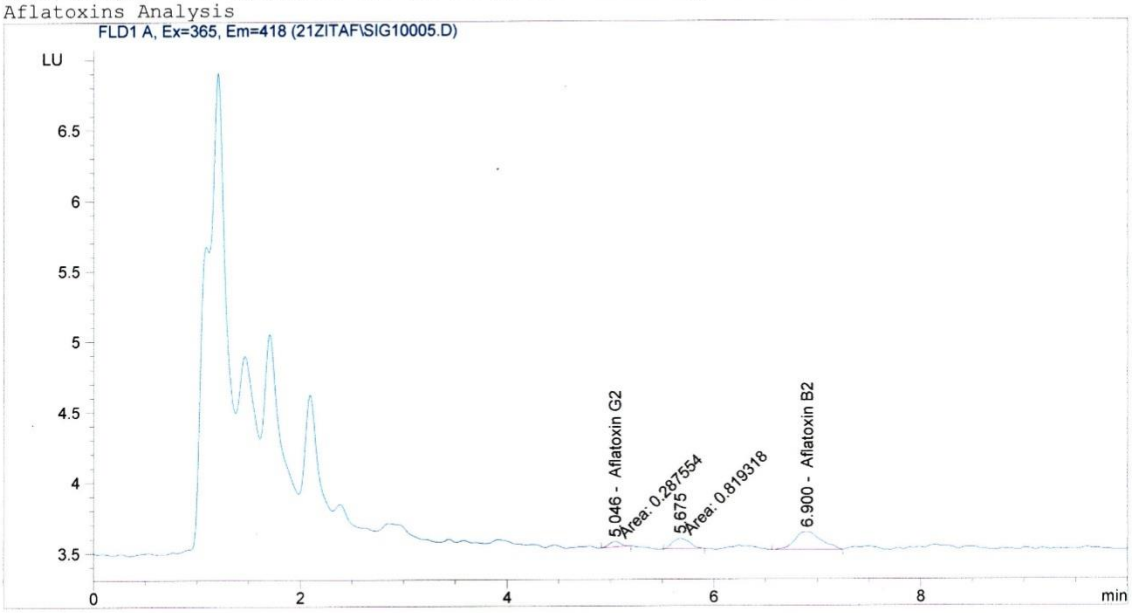
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10005.D Sample Name: Peanut

Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Sample:03.PIsIL(Repeat),
Food Toxicology Research Section,
IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:29.11.2021

```
Injection Date  : 11/29/2021 2:13:34 PM
Sample Name     : Peanut                          Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 11/29/2021 12:11:14 PM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/28/2021 4:06:39 PM by Food Toxicology Section IFST
Aflatoxins Analysis
```



External Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, March 09, 2021 11:39:25 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418
```

RetTime [min]	Type	Area LU *s	Amt/Area	Amount [ppb]	Grp	Name
5.046	MM	2.87554e-1	2.29999	6.61370e-1		Aflatoxin G2
6.023		-	-	-		Aflatoxin G1

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
6.900	VV	2.24838	2.95100e-1	6.63497e-1			Aflatoxin B2
8.083		-	-	-			Aflatoxin B1
Totals :					1.32487		

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

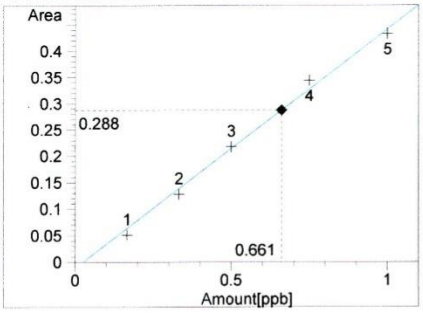
Signal 1: FLD1 A, Ex=365, Em=418

Final Summed Peaks Report

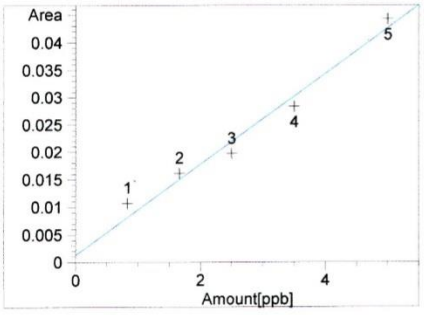
Signal 1: FLD1 A, Ex=365, Em=418

Name	Total Area LU	Area *s	Amount [ppb]
Aflatoxin G2	2.87554e-1		0.6614
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	2.24838		0.6635
Aflatoxin B1	0.00000		0.0000
Totals :	1.3249		

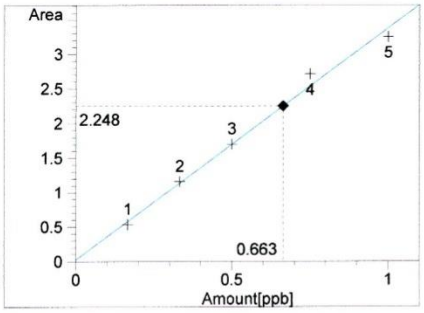
Calibration Curves



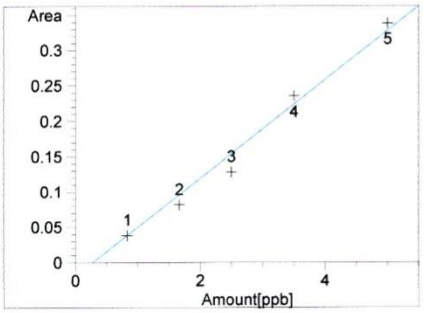
Aflatoxin G2 at exp. RT: 5.312
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.023
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

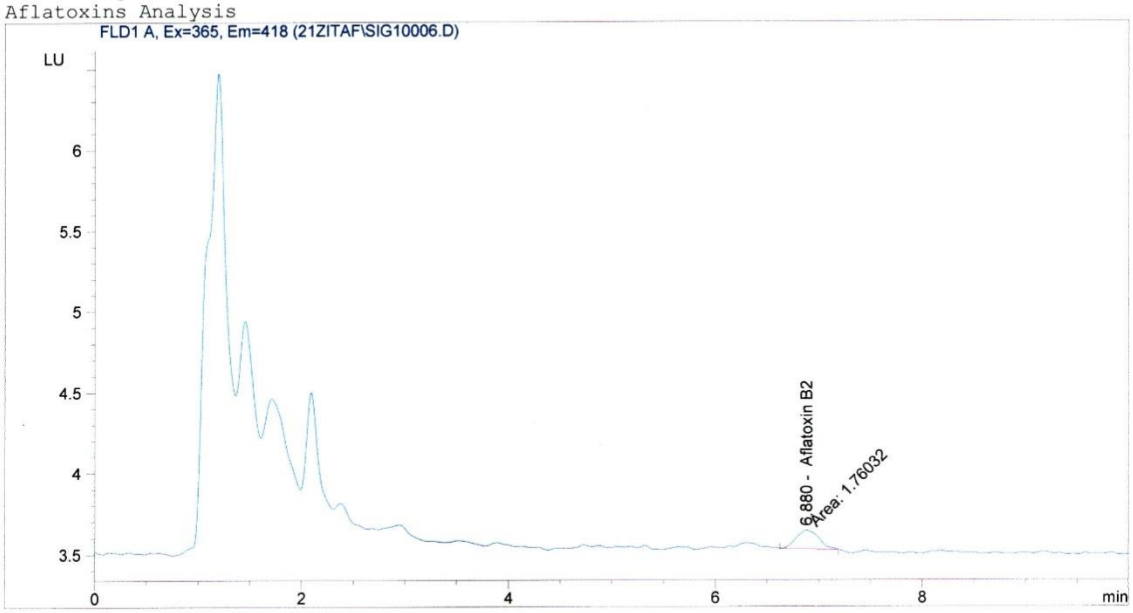
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10006.D Sample Name: Peanut

Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Sample:04.PIsIR,
Food Toxicology Research Section,
IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:29.11.2021

```
Injection Date  : 11/29/2021 2:26:39 PM
Sample Name     : Peanut                        Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 11/29/2021 12:11:14 PM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/28/2021 4:06:39 PM by Food Toxicology Section IFST
Aflatoxins Analysis
```



External Standard Report

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Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, March 09, 2021 11:39:25 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          LU    *s                [ppb]
-------|------|---------|----------|----------|--|------------------
  5.312           -         -          -         Aflatoxin G2
  6.023           -         -          -         Aflatoxin G1
```

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10006.D Sample Name: Peanut

RetTime [min]	Type	Area LU	Amt/Area *s	Amount [ppb]	Grp	Name
6.880	MM	1.76032	2.94328e-1	5.18110e-1		Aflatoxin B2
8.083		-	-	-		Aflatoxin B1

Totals : 5.18110e-1

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

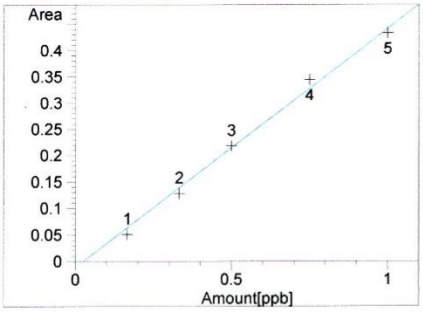
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

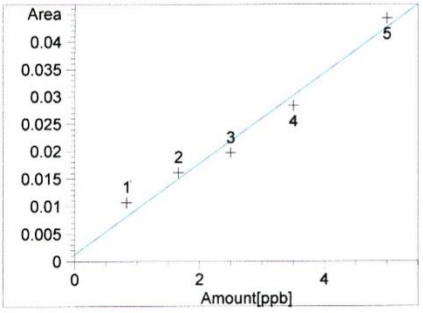
Name	Total Area LU	*s	Amount [ppb]
Aflatoxin G2	0.00000		0.0000
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	1.76032		0.5181
Aflatoxin B1	0.00000		0.0000

Totals : 5.1811e-1

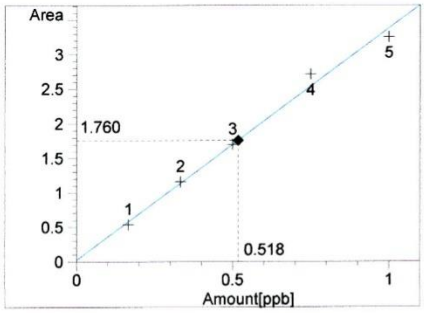
Calibration Curves



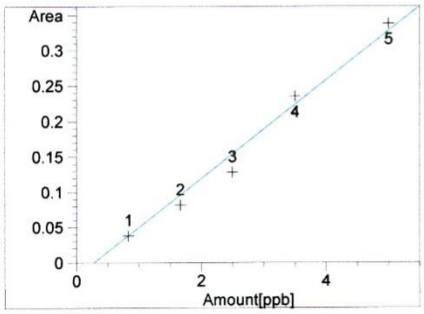
Aflatoxin G2 at exp. RT: 5.312
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.023
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

==========

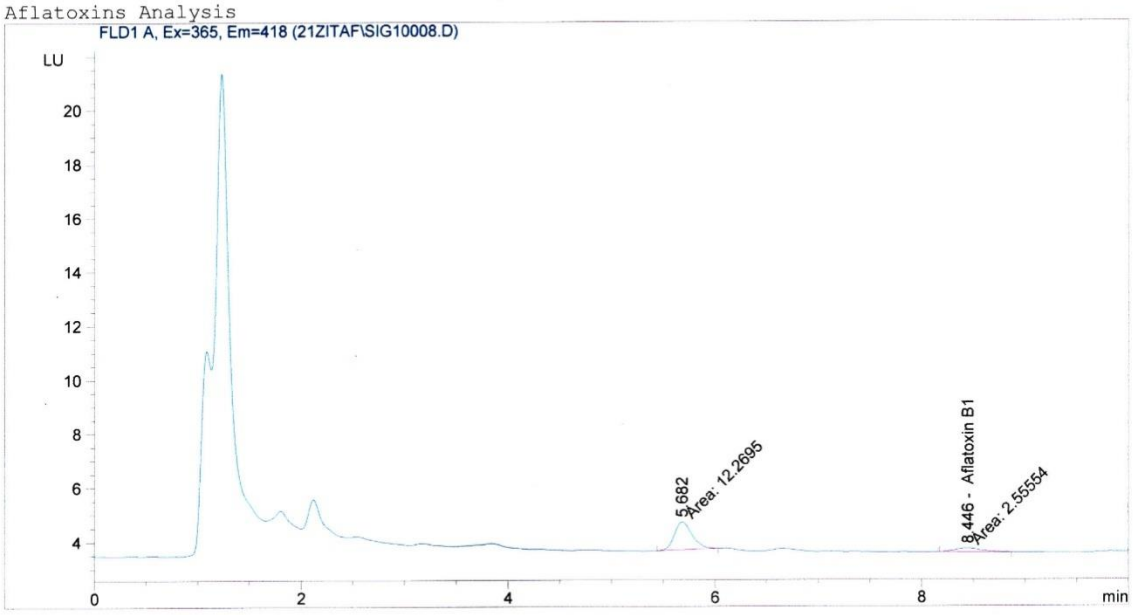
*** End of Report ***

Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Sample:05.Maize,Sample Code:MPnL,
Food Toxicology Research Section,
IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:29.11.2021

==========

Injection Date : 11/29/2021 3:10:17 PM
Sample Name : Peanut Location : Vial 1
Acq. Operator : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed : 11/29/2021 12:11:14 PM by Food Toxicology Section IFST
(modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed : 1/2/2022 2:25:26 PM by Food Toxicology Section IFST
(modified after loading)

Aflatoxins Analysis



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/2/2022 2:25:23 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10008.D Sample Name: Peanut

RetTime [min]	Type	Area LU	Amt/Area *s	Amount [ppb]	Grp	Name
5.212		-	-	-		Aflatoxin G2
6.123		-	-	-		Aflatoxin G1
7.006		-	-	-		Aflatoxin B2
8.446	MM	2.55554	14.50722	37.07381		Aflatoxin B1

Totals : 37.07381

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

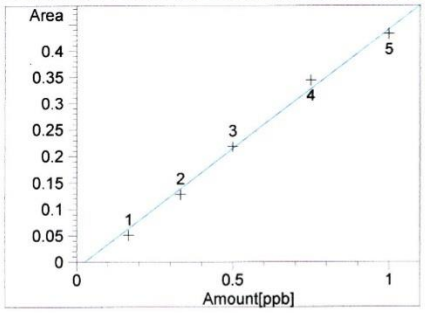
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

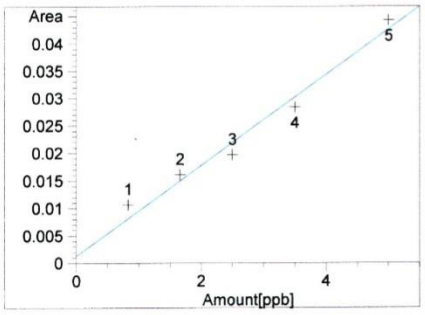
Name	Total Area LU	*s	Amount [ppb]
Aflatoxin G2	0.00000		0.0000
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	0.00000		0.0000
Aflatoxin B1	2.55554		37.0738

Totals : 37.0738

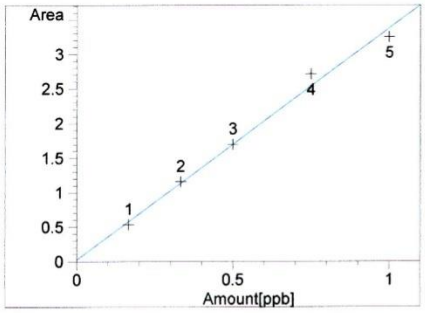
Calibration Curves



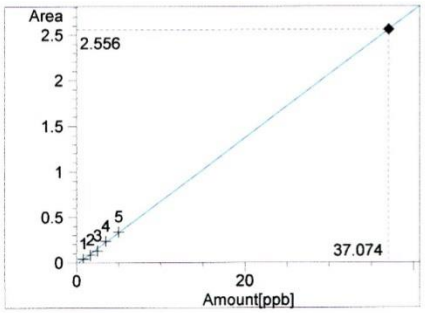
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

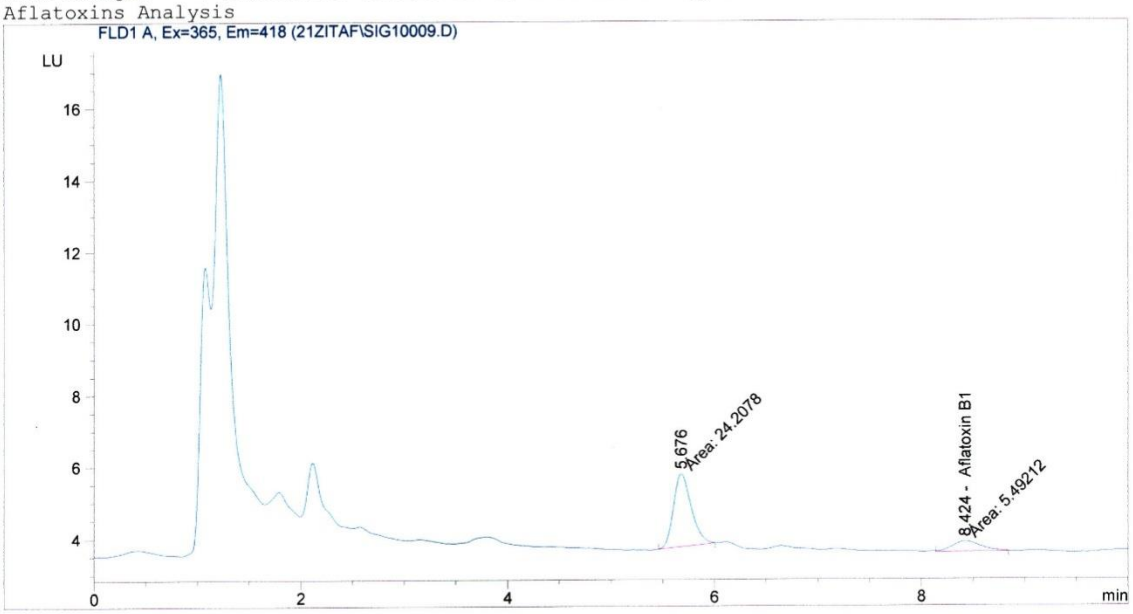
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10009.D Sample Name: Peanut

Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Sample:06.Maize,Sample Code:MPnR,
Food Toxicology Research Section,
IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:29.11.2021

```
=============================================================================
Injection Date  : 11/29/2021 3:22:31 PM
Sample Name     : Peanut                          Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 11/29/2021 12:11:14 PM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/28/2021 4:06:39 PM by Food Toxicology Section IFST
Aflatoxins Analysis
```

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=============================================================================
                        External Standard Report
=============================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, March 09, 2021 11:39:25 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         LU    *s                 [ppb]
-------|------|---------|----------|----------|--|------------------
  5.312           -          -          -          Aflatoxin G2
  6.023           -          -          -          Aflatoxin G1
```

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10009.D Sample Name: Peanut

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
7.006		-		-	-		Aflatoxin B2
8.424	MM	5.49212		14.44880	79.35452		Aflatoxin B1

Totals : 79.35452

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

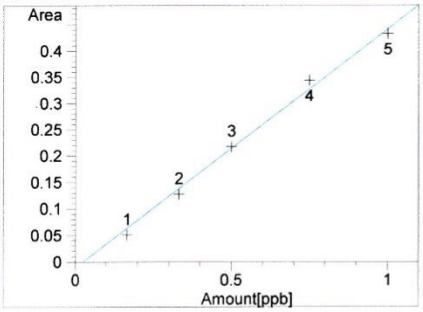
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

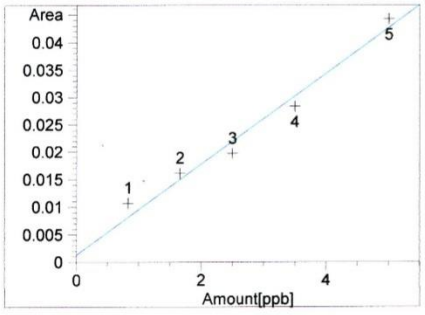
Name	Total Area LU	Total Area *s	Amount [ppb]
Aflatoxin G2	0.00000		0.0000
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	0.00000		0.0000
Aflatoxin B1	5.49212		79.3545

Totals : 79.3545

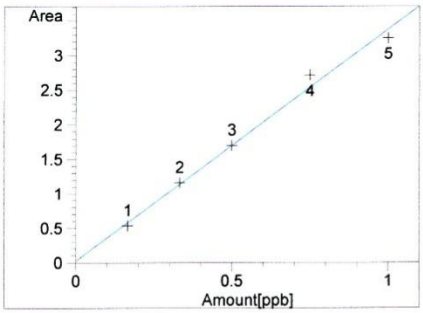
Calibration Curves



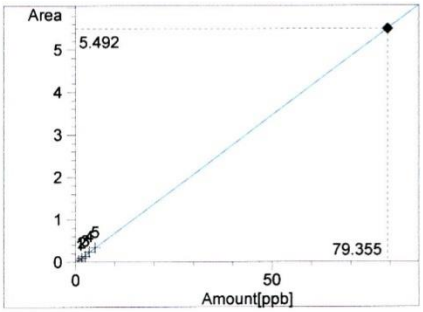
Aflatoxin G2 at exp. RT: 5.312
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.023
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

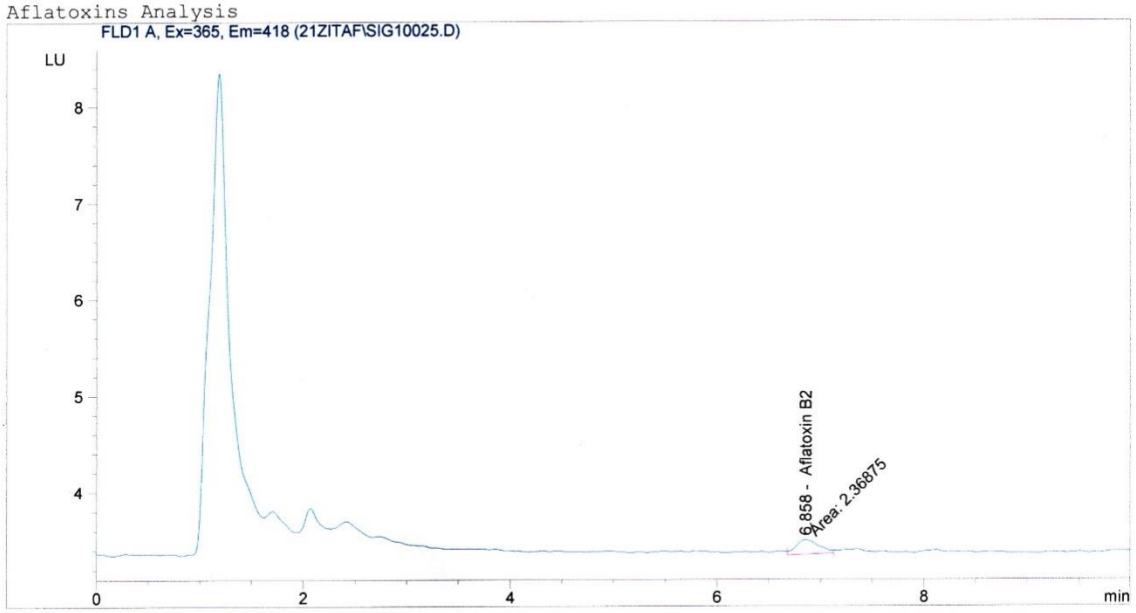
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10025.D Sample Name: Lentil

Sample:18.Lentil,Sample Code:LnaR,
Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Food Toxicology Research Section,IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:06.12.2021

```
==========================================================================
Injection Date  : 12/6/2021 3:52:26 PM
Sample Name     : Lentil                          Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/6/2021 11:49:17 AM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 1/2/2022 3:58:42 PM by Food Toxicology Section IFST
                  (modified after loading)
Aflatoxins Analysis
```

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==========================================================================
                        External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      1/2/2022 2:25:23 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         LU    *s                 [ppb]
-------|------|----------|----------|----------|--|------------------
  5.212           -          -          -         Aflatoxin G2
  6.123           -          -          -         Aflatoxin G1
```

RetTime [min]	Type	Area LU	Amt/Area *s	Amount [ppb]	Grp	Name
6.858	MM	2.36875	2.95241e-1	6.99353e-1		Aflatoxin B2
8.083		-	-	-		Aflatoxin B1

Totals : 6.99353e-1

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

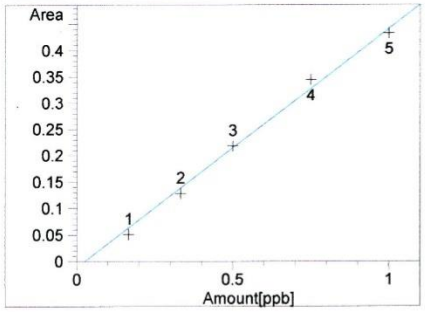
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

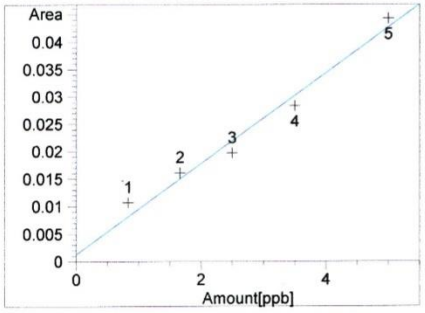
Name	Total Area LU	*s	Amount [ppb]
Aflatoxin G2	0.00000		0.0000
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	2.36875		0.6994
Aflatoxin B1	0.00000		0.0000

Totals : 6.9935e-1

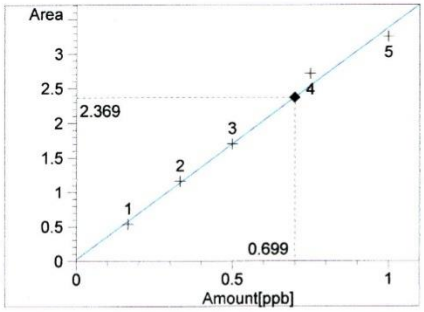
Calibration Curves



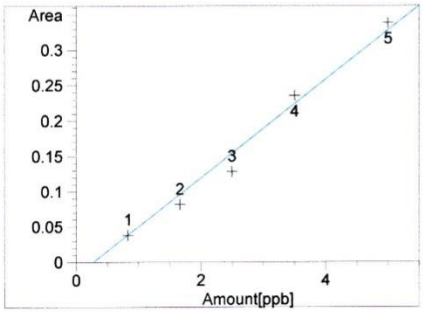
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10026.D Sample Name: Lentil

Sample:19.Lentil,Sample Code:LCdNg,
Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Food Toxicology Research Section,IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:06.12.2021

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=====================================================================
Injection Date  : 12/6/2021 4:04:59 PM
Sample Name     : Lentil                          Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/6/2021 11:49:17 AM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 1/2/2022 3:58:42 PM by Food Toxicology Section IFST
                  (modified after loading)
Aflatoxins Analysis
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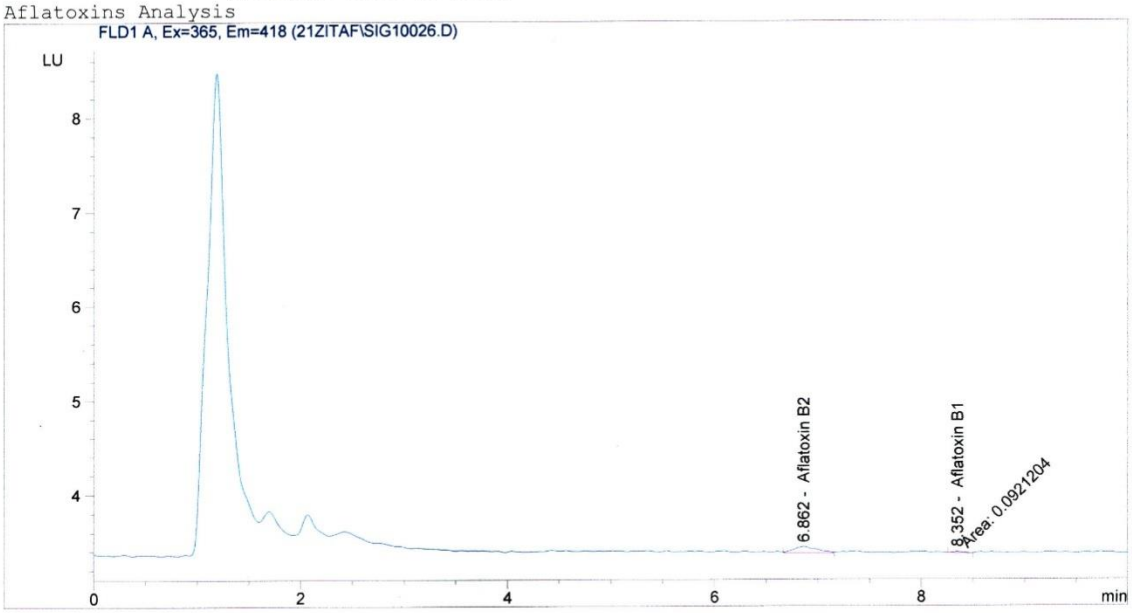


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Sorted By             :      Signal
Calib. Data Modified  :      1/2/2022 2:25:23 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         LU    *s                 [ppb]
-------|------|----------|----------|----------|--|------------------
  5.212            -          -          -          Aflatoxin G2
  6.123            -          -          -          Aflatoxin G1
```

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10026.D Sample Name: Lentil

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
6.862	VV	1.14620	2.92422e-1	3.35174e-1			Aflatoxin B2
8.352	MM	9.21204e-2	17.42891		1.60556		Aflatoxin B1
Totals :					1.94073		

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

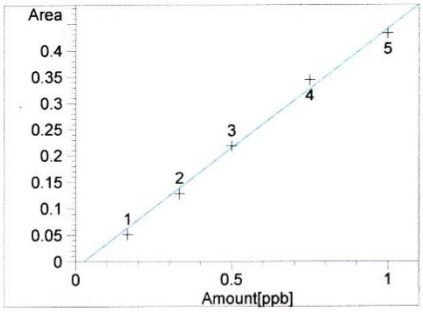
Signal 1: FLD1 A, Ex=365, Em=418

Final Summed Peaks Report

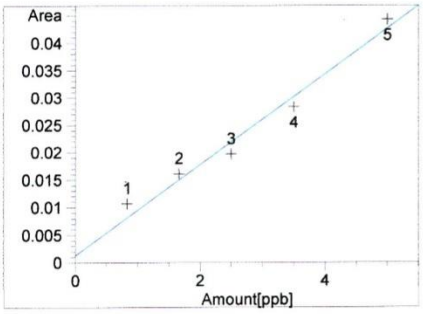
Signal 1: FLD1 A, Ex=365, Em=418

Name	Total Area LU	Area *s	Amount [ppb]
Aflatoxin G2		0.00000	0.0000
Aflatoxin G1		0.00000	0.0000
Aflatoxin B2		1.14620	0.3352
Aflatoxin B1		9.21204e-2	1.6056
Totals :			1.9407

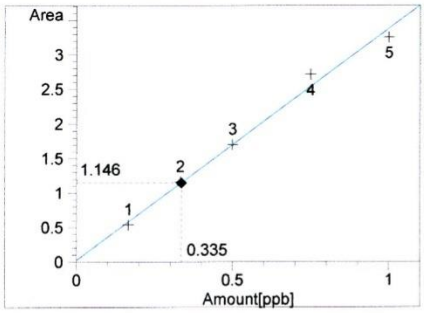
Calibration Curves



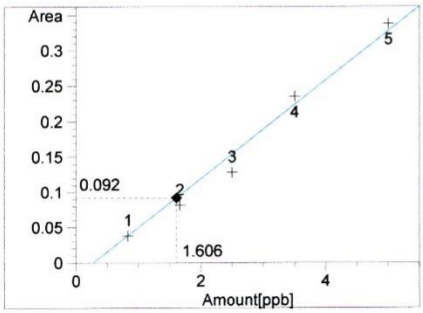
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

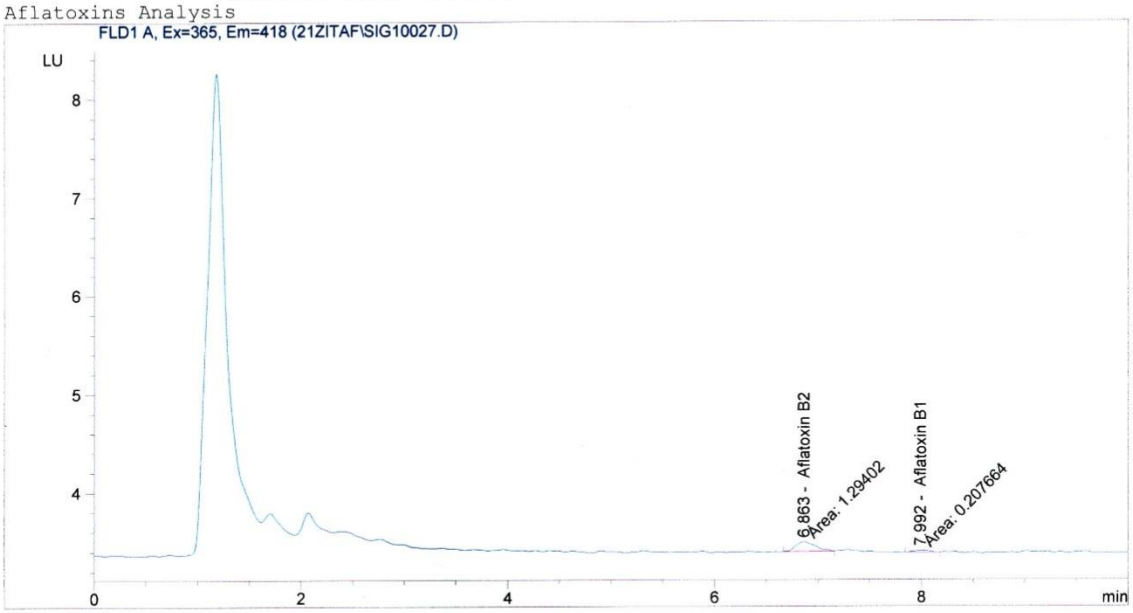
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10027.D Sample Name: Lentil

Sample:20.Australian Lentil,Sample Code:LAusNg,
Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Food Toxicology Research Section,IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:06.12.2021

```
================================================================================
Injection Date  : 12/6/2021 4:17:48 PM
Sample Name     : Lentil                              Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/6/2021 11:49:17 AM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 1/2/2022 3:58:42 PM by Food Toxicology Section IFST
                  (modified after loading)
Aflatoxins Analysis
```

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================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      1/2/2022 2:25:23 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]          LU    *s                 [ppb]
-------|------|----------|----------|----------|--|------------------
  5.212            -          -          -          Aflatoxin G2
  6.123            -          -          -          Aflatoxin G1
```

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10027.D Sample Name: Lentil

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
6.863	MM	1.29402	2.93046e-1	3.79206e-1			Aflatoxin B2
7.992	MM	2.07664e-1	15.74250		3.26914		Aflatoxin B1

Totals : 3.64835

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

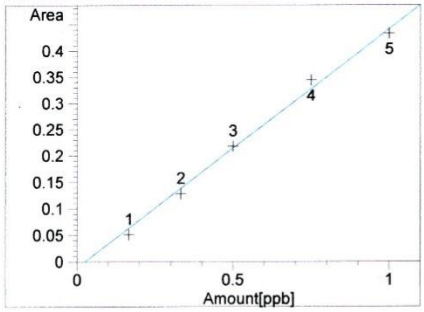
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

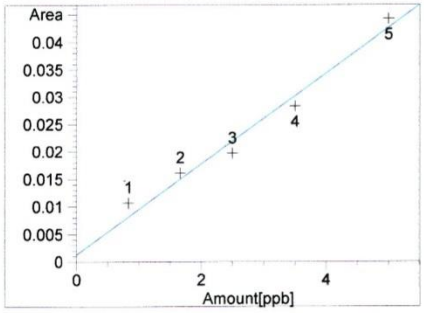
Name	Total Area LU	Total Area *s	Amount [ppb]
Aflatoxin G2		0.00000	0.0000
Aflatoxin G1		0.00000	0.0000
Aflatoxin B2		1.29402	0.3792
Aflatoxin B1		2.07664e-1	3.2691

Totals : 3.6483

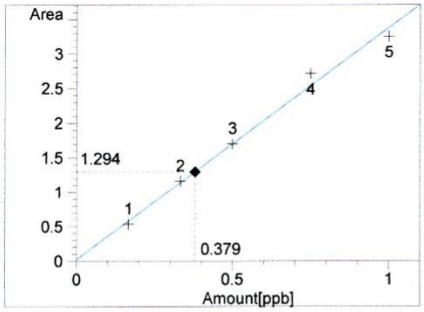
Calibration Curves



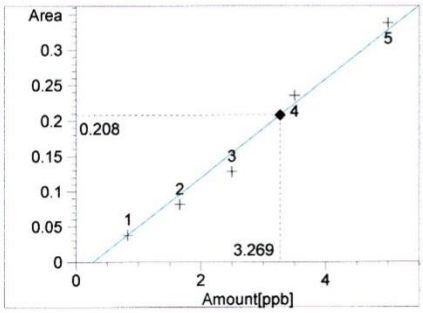
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

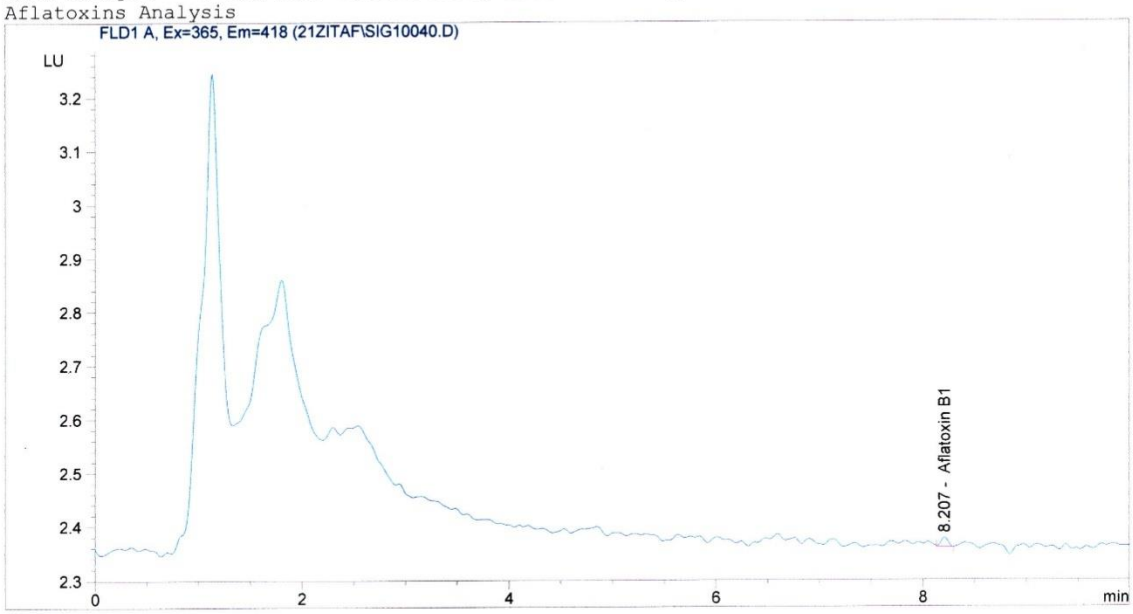
==========

*** End of Report ***

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10040.D Sample Name: Rice

Sample:30.LocalWheat,Sample Code:RMkMp(Repeat),
Thesis Title: Determination of Aflatoxins in cereals
and there Processed Food available in Bangladesh.
Name:Most.Zakiya Islam(MS Student),Session:2020-2021,
Sher-e-Bangla Agricultural Universitry,
Food Toxicology Research Section,IFST,BCSIR.Dhaka.
Aflatoxin Analysis,Date:08.12.2021

```
==========================================================================
Injection Date  : 12/8/2021 2:59:48 PM
Sample Name     : Rice                          Location : Vial 1
Acq. Operator   : Food Toxicology Section IFST
Acq. Instrument : Instrument 1
Acq. Method     : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 12/8/2021 2:51:57 PM by Food Toxicology Section IFST
                  (modified after loading)
Analysis Method : D:\AGILEN~1\METHODS\AFTX2021.M
Last changed    : 1/2/2022 6:25:39 PM by Food Toxicology Section IFST
Aflatoxins Analysis
```

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==========================================================================
                      External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      1/2/2022 2:25:23 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FLD1 A, Ex=365, Em=418

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         LU    *s                 [ppb]
-------|------|----------|----------|----------|--|------------------
  5.212           -          -          -         Aflatoxin G2
  6.123           -          -          -         Aflatoxin G1
  7.006           -          -          -         Aflatoxin B2
```

Data File C:\HPCHEM\1\DATA\21ZITAF\SIG10040.D Sample Name: Rice

RetTime [min]	Type	Area LU	Area *s	Amt/Area	Amount [ppb]	Grp	Name
8.207	VP	7.98327e-2		17.89543	1.42864		Aflatoxin B1

Totals : 1.42864

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

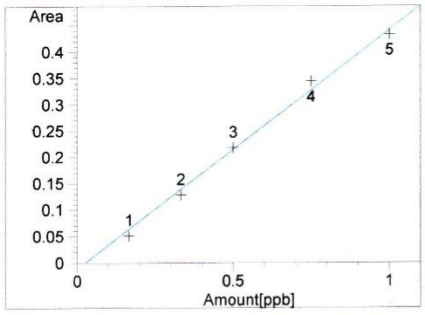
Final Summed Peaks Report

Signal 1: FLD1 A, Ex=365, Em=418

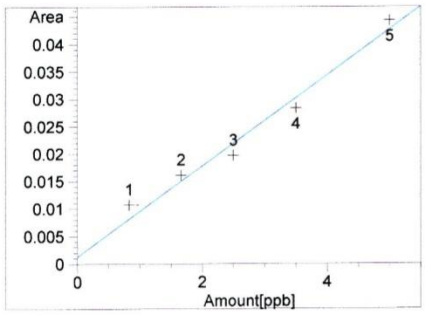
Name	Total Area LU	Total Area *s	Amount [ppb]
Aflatoxin G2	0.00000		0.0000
Aflatoxin G1	0.00000		0.0000
Aflatoxin B2	0.00000		0.0000
Aflatoxin B1	7.98327e-2		1.4286

Totals : 1.4286

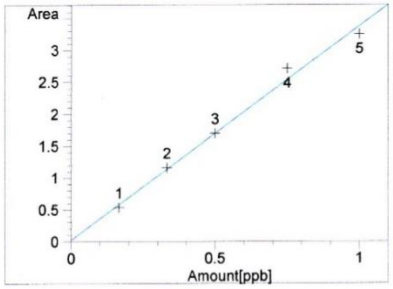
Calibration Curves



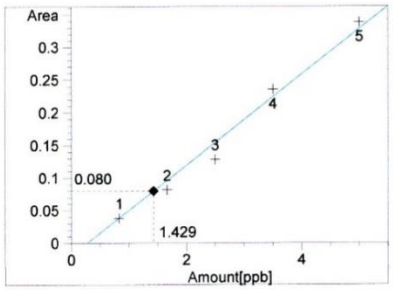
Aflatoxin G2 at exp. RT: 5.212
FLD1 A, Ex=365, Em=418
Correlation: 0.99732
Residual Std. Dev.: 0.01381
Formula: y = mx + b
m: 4.52517e-1
b: -1.17271e-2
x: Amount[ppb]
y: Area



Aflatoxin G1 at exp. RT: 6.123
FLD1 A, Ex=365, Em=418
Correlation: 0.99110
Residual Std. Dev.: 0.00227
Formula: y = mx + b
m: 8.28140e-3
b: 1.20907e-3
x: Amount[ppb]
y: Area



Aflatoxin B2 at exp. RT: 7.006
FLD1 A, Ex=365, Em=418
Correlation: 0.99684
Residual Std. Dev.: 0.11123
Formula: y = mx + b
m: 3.35700
b: 2.10203e-2
x: Amount[ppb]
y: Area



Aflatoxin B1 at exp. RT: 8.083
FLD1 A, Ex=365, Em=418
Correlation: 0.99082
Residual Std. Dev.: 0.01932
Formula: y = mx + b
m: 6.94543e-2
b: -1.93925e-2
x: Amount[ppb]
y: Area

==========

*** End of Report ***

4.4 Risk assessment

Estimated Daily Intake (EDI): Calculation of the Estimated Daily Intake (EDI) was done by using the mean level of aflatoxins obtained in positive samples, the daily intakes of the same samples and the average body weight(adult). EDI for mean aflatoxins was calculated according to the following formula and expressed in µg kg−1 of body weight/day (Dos Santos *et al*., 2013).

$$\text{EDI} = \frac{Daily\ intake\ (food) \times mean\ aflatoxin\ level}{average\ body\ weight}$$

Estimation of hazard quotient (HQ): Hazard Quotient (HQ) is referred to as the non-carcinogenic effects of the toxin. The non-carcinogenic effect of the individual toxin is designated by hazard quotient (HQ) as described by (Kortei *et al.,* 2019)

$$\text{HQ} = \frac{\text{EDI}}{\text{MRL}}$$

Maximum Residue Limit (MRL): a) Aflatoxin B1 : 2 µg/kg
b) Aflatoxin B1, B2, G1, G2 : 4 µg/kg
According to Commission Regulation (EC) No. 1881/2006 for Food

Hazard Index (HI): Hazard index (HI) used for expression of non-cancer impacts on health calculated as the summation of HQs at different locations. If the value is below 1.0, there prevails no significant adverse health risk to humans.
HI=HQ1+HQ2+HQ3+HQ4+…….

Table 5. Risk assessment for Aflatoxin B1

Crop	Daily intake (g)	Average bodyweight (kg)	EDI (µg/kg)	HQ	HI
Peanut	1.00	58	0.003	0.0015	0.008
Maize	2.00		0.002	0.001	
Lentil	18.00		0.0008	0.0004	
Rice	416		0.01	0.005	

Table 6. Risk assessment for total aflatoxins

Crop	Daily intake (g)	Average body weight (kg)	EDI (µg/kg)	HQ	HI
Peanut	1	58	0.0009	0.002	0.006
Maize	2		0.002	0.0005	
Lentil	17.92		0.0006	0.0002	
Rice	416		0.01	0.003	

From table 5 and 6, we can observe that Hazard index found below 1.0 for both aflatoxin B1 and total aflatoxins. So, human health risk assessment from aflatoxins exposure through rice, maize, lentil and peanut consumption from the godown and wholesale markets by adults showed no significant adverse health risk to humans.

CHAPTER V

SUMMARY AND CONCLUSION

A total of 60 samples were collected, of these 29 samples were from godown, 21 samples from wholesale market and 10 processed food items collected from different locations of Bangladesh.

Then the collected grain samples and their processed food samples were analyzed at Institute of Food Science and Technology Laboratory of Bangladesh Council of Scientific and Industrial Research, Dhaka. Aflatoxin residues (B1, B2, G1, G2) of collected samples were determined by using High Performance Liquid Chromatography (HPLC) system with Fluorescence detector.

In this study, 8 godown samples (3 peanut samples, 2 maize samples, 3 lentil samples) and 1 wholesale market sample (rice) were aflatoxin positive, whereas no processed food sample was aflatoxin positive. Results showed that among 9 samples, 4 samples (1 peanut sample, 2 maize samples and 1 lentil sample) exceeded the maximum residue limit (MRL) of aflatoxin B_1 (2 µg/kg). Among 4 samples, 3 samples (1 peanut sample, 2 maize samples) exceeded the maximum residue limit (MRL) of total aflatoxin B_1, B_2, G_1 & G_2 (4 µg/kg) as per Commission Regulation (EC) No. 1881/2006 for food.

From the results and findings of the present investigation it can be concluded that near about 15 % (9/60) of the samples collected from different sources of different locations of Bangladesh were contaminated with aflatoxin residues. In this experiment, 6.7% (4/60) of the sample exceeded the maximum residue limit (MRL) of aflatoxin (2 µg/kg for AFB1 and 4µg/kg for total aflatoxins) as per Commission Regulation (EC) No. 1881/2006 for Food. Again, aflatoxin residues were not detected in processed food sample. From the 9 positive samples, 8 samples were from godown sources and 1 sample was from wholesale market.

RERERENCES

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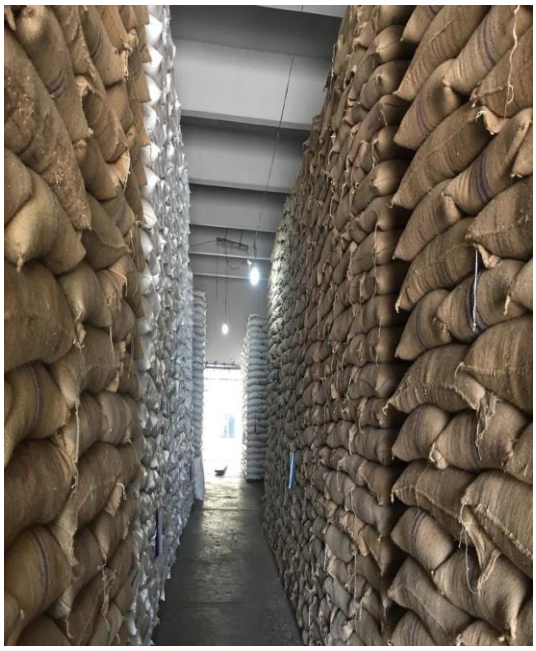
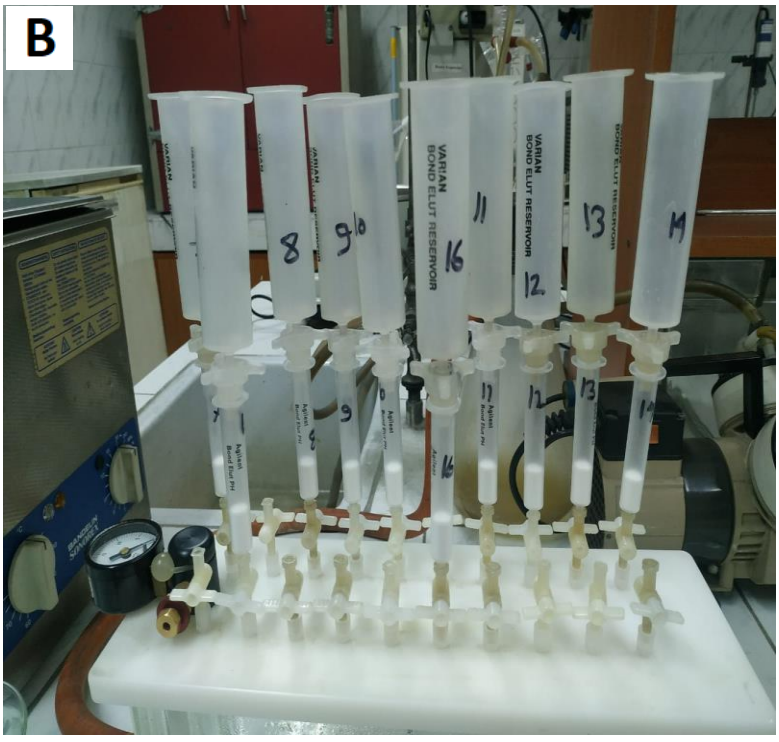
Appendix I. Pictorial representation of research work



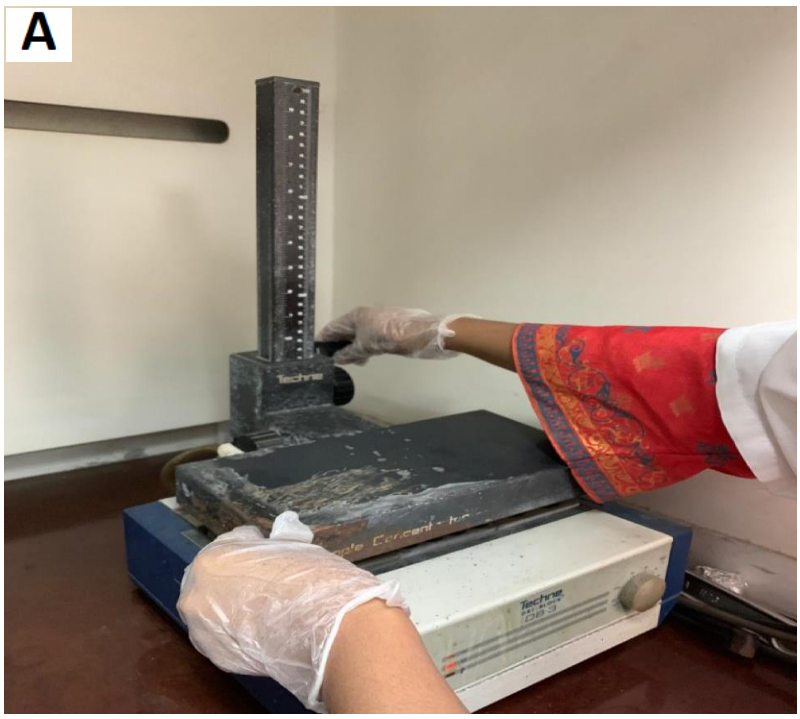
Visited different godowns for collecting samples

Labeling of grinded samples

Grinded samples were measured and taken about 25g into a conical flask (A).
Shaking into a rotary shaker for the homogeneous slurry formation (B).



Filtration of extracts done through a 24 cm What-man No.1 filter paper (A). Drain of filtrate sample extract through SPE by using pH bond elute reservoir (B).



Placing of vial in fume hood and drying by N_2 gas (A).
Vortexing the sample by vortex mixer (B).

Inject of sample solution in to HPLC