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Determination of the level of contamination of cocoa beans with aflatoxins: case of three cocoa producing regions in Côte d'Ivoire (Divo, Gagnoa and Soubré)

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Abstract: *Aflatoxins are mycotoxins resulting from the secondary metabolism of molds that grow on plants in the field and during storage. They are found in coffee, cocoa, cereals and agrifood products. This study therefore proposes to determine the level of contamination of cocoa beans with aflatoxins: case of three cocoa-producing regions in Ivory Coast (Divo, Gagnoa and Soubré). A total of 30 cocoa bean samples were taken from the three major cocoa production regions. These samples were transported to the laboratory for analysis using a SCHIMADZU brand High Performance Liquid Chromatography (HPLC) line. The results of this study showed that all samples were contaminated with aflatoxins. The highest levels are found in samples from the localities of Divo and Soubré. These average AFB1 and sum AFB & G contents vary from 5.90 µg / kg to 11 µg / kg and 8.7 µg / kg to 14.72 µg / kg respectively. The levels obtained lead to a risk of exposure of the population to the toxic effects of these molecules. In general, the contaminations observed in the three localities could be due to poor agricultural practices, from the harvest to the packaging of the beans in the warehouses. Added to this is also the non-compliance with construction standards for storage warehouses and the lack of maintenance of these premises.*

Key words: *Cocoa beans, Contamination, Aflatoxins, Mycotoxins, Export crops.*

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I. Introduction

In Côte d'Ivoire, the main export crops are coffee and cocoa, which alone provide the bulk of the cash income of rural households. Cocoa plays an important role in both producing and consuming countries. The cocoa sector generates operating income and is a source of income and job creation (GASTELLU, 1989). Cocoa is an important ingredient in the confectionery and food industries, and more recently in the pharmaceutical and cosmetic industries(Gayi et al., 2016). Since 1970, the culture has grown to such an extent that Côte d'Ivoire occupies the first place in the world among cocoa-producing countries, with productions often reaching 1.2 million tons of beans per year, or nearly 40% of world production (Fowler, 1995 ; Cruz, 1988).

However, this major agricultural activity is now facing enormous difficulties, including, among others, conservation and biological contamination. The quality of cocoa beans is mainly affected by ochratoxin A and aflatoxins. Aflatoxins are naturally occurring mycotoxins produced by *Aspergillus* fungi. The four (04) main aflatoxins are aflatoxin B1 (AFB1), aflatoxin (AFG1), aflatoxin (AFB2) and aflatoxin (AFG2). Aflatoxin B1 is the best known and is the most toxic form of aflatoxin. Short-term exposure to very high concentrations of aflatoxins can cause vomiting, abdominal pain and death. Long-term exposure to higher levels of aflatoxins, especially the AFB1 form, has been associated with liver cancer and liver disease as well as inadequate growth in children. It should be noted that exposure to very high concentrations of aflatoxins is very rare in developed countries. Aflatoxins can be found in foods such as tree nuts and nut products, spices, rice, dehydrated foods, grains, coffee, and cocoa beans. During plant growth and harvest, hot, humid conditions as well as pests can promote the growth of molds resulting in contamination with aflatoxins. The main source of exposure to

aflatoxins in humans is from consumption of contaminated food, ingested directly or as ingredients (Canadian Food Inspection Agency, 2013 ; DIEME et al., 2016).

With the objective of preventing contamination of cocoa beans by aflatoxins and preserving the health of consumers, this study entitled : "Determination of the level of contamination of cocoa beans with aflatoxins : case of three cocoa-producing regions in Ivory Coast(Divo, Gagnoa and Soubré) " was developed.

II. Materials And Methods

1-Materials

The biological material for this study consists of dried cocoa beans. These beans were harvested in the three (3) regions (Divo, Gagnoa and Soubré) of the production area in the south-west of Côte d'Ivoire. The figure below shows us the biological material. The bean samples collected were analyzed in the laboratory using a SCHIMADZU brand High Performance Liquid Chromatography (HPLC) line.

Figure 1 : Dried cocoa beans

2- Methods

2.1- Sampling of beans

Sampling took place in the three regions mentioned above. In each department, in agreement with the Café Cacao Council, 10 cooperatives and buyers were chosen on the basis of tonnage. About thirty (30) samples were collected from all 3 regions at a rate of 10 samples per locality. Elementary samples were taken from different places of the bags (ends and center) and grouped together to constitute the global sample of a few kilograms (10 kg) according to the quantity available in the warehouses in accordance with Regulation **(EC) N ° 401 / 2006** of the commission of February 23, 2006.

2.2- Method of analysis of Aflatoxin

The analysis of aflatoxins in cocoa beans generally goes through several stages. These steps are summarized by the determination of the analytical parameters, the extraction protocol, the purification protocol and the detection of aflatoxins by HPLC.

2.2.1- Dosage of Aflatoxins

Aflatoxin analysis was carried out at the Central Laboratory of Agrochemistry and Ecotoxicology (LCAE). A quantity of twenty grams (20 ± 0.01g) of ground material is taken into a 200ml flask. 100ml of a mixture of an alcoholic methanol / double-distilled water solution (80/20) is added. This mixture is homogenized and protected from light for 24 hours.

2.2.2-Extraction and purification of aflatoxins

The extraction and purification were carried out according to the **ISO NF EN 14123** test DN 160 standard, adapted to laboratory conditions.

After attack, we carried out filtration on filter paper (Whatman) in 100 ml Erlenmeyer flasks. To 50ml of this filtrate is added 40ml of clarification reagents composed of 5g of hydrated phosphotungstic acid and 15g of zinc sulfate. This mixture was left to stand for 15 minutes. Then, a second filtration is carried out. The filtrate obtained is collected in a separating funnel. The aflatoxin was collected 3 times in a ground-glass glue-in NS29 / 32 balloon, each time adding 10ml of chloroform. All the filtrates obtained are placed in a 500ml decanting flask to be evaporated to dryness at 40 ° C. The residue will be attacked with 400µl of hydrochloric acid and 4.6ml of double-distilled water. The residue is collected in a vial and ready for reading on HPLC. During aflatoxin analysis, daylight should be avoided as much as possible, as aflatoxin is gradually broken down under the influence of ultraviolet light.

2.2.3- Quantification of Aflatoxins

The quantification of aflatoxin in cocoa beans was carried out by a SCHIMADZU brand HPLC line under the analytical conditions mentioned below, including the determination of certain analytical parameters :

- Mobile phase : consisting of a mixture of Acetonitrile and Methanol by volume (65 : 35), double-distilled water
- Volume injected : 20µl
- Emission : 450nm
- Excitation : 350nm
- Rinsing solvent : the mobile phase
- Analysis time : 15mn
- Flow rate : 1.5ml / min
- Detection limit : 0.034 µg / kg
- Limit of quantification : 0.1 µg / kg

III. Results And Discussion

3.1-Results

Determination of the levels of aflatoxins in cocoa beans revealed the presence of these mycotoxins in a number of samples. The average contents obtained in the three localities show that those of Divo and Soubré are the highest (Table II).

Table II : Average aflatoxin content by cocoa production localities

Sampling localities	Average aflatoxin content	
	AFB1 /µg/kg	∑ AF B&G /µg/kg
DIVO	5.90	8.70
GAGNOA	1.90	1.05
SOUBRE	11.32	1.05

The different levels of aflatoxins have been grouped according to the localities where these beans come from.

➢ **Location of Divo**

The average contents obtained in this locality are 5.90µg / kg, with a minimum of 1.20 and a maximum of 25.92µg / kg for AFB1, and 8.7µg / kg with a minimum of 1.9 and a maximum of 40.85µg / kg for the sum AF B&G.

Of the 10 samples analyzed for AFB1, only 3 have a content of less than 2µg / kg (the maximum residue limit of FA indicated by the EU for cocoa). Regarding the sum AFB & G, 6 samples have a content lower than the standard of 4µg / kg.

Fig. 3, 4 and 5 shows the histograms of the aflatoxin concentrations of the Divo samples compared to the various international standards.

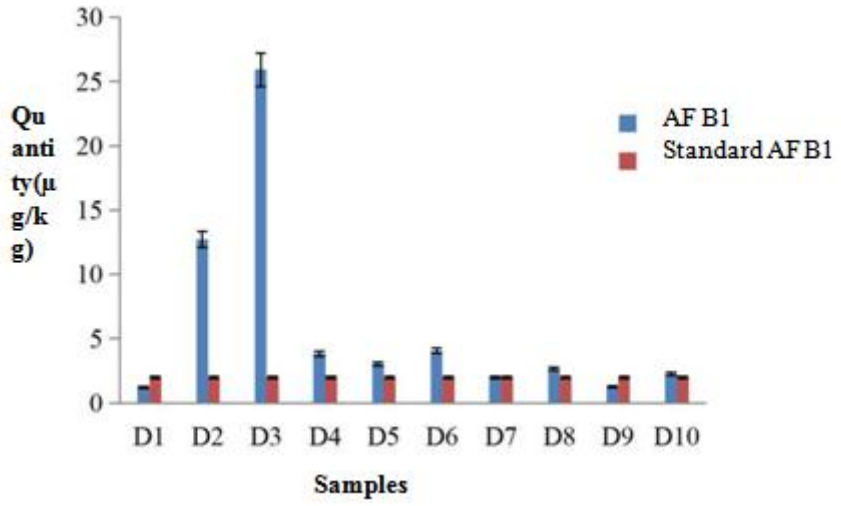

Figure 3 : AF B1 concentrations compared to the maximum residue limit (MRL).

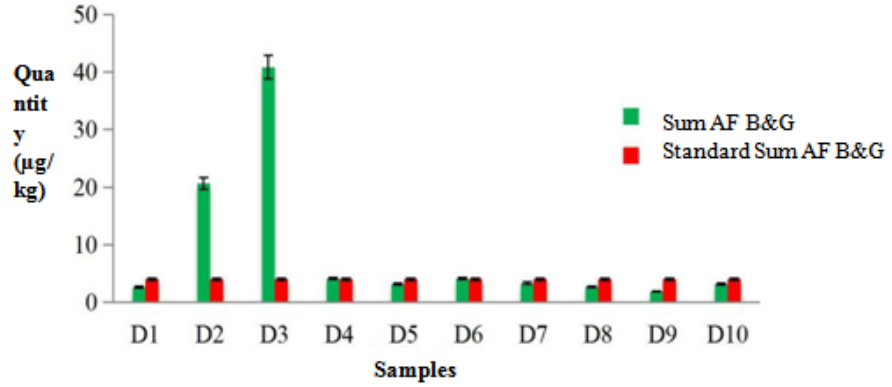

Figure 4 : Concentrations of AF B&G (sum AF B&G) compared to the maximum residue limit.

We find that only 30% of samples are accepted based on the 2µg / kg standard for AF B1 and 70% of lots are rejected. The results are acceptable for the sum of AF B&G with a probability of rejection in the order of 40% and a probability of acceptable batches in the order of 60% based on the standard of 4µg / kg.

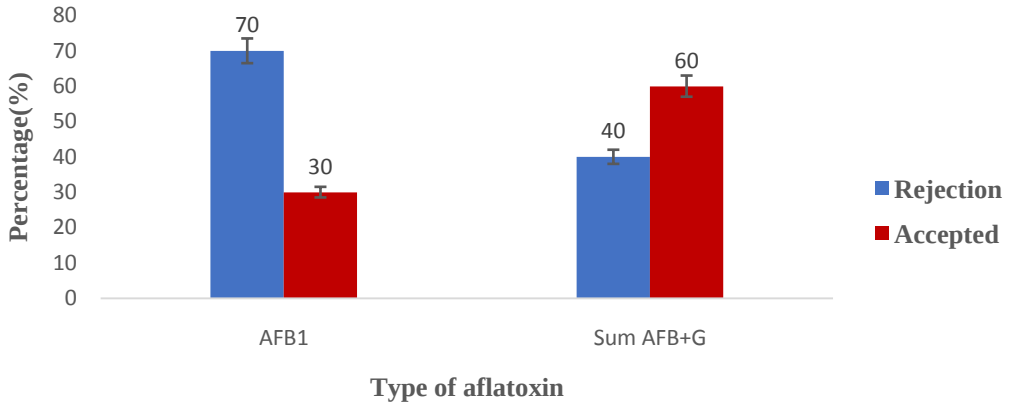

Figure 5 : Percentage releases as a function of the AFB1 and AF B&G som contents of Divo

➢ **Location of Gagnoa**

Cocoa from the Gagnoa region is also threatened by AF but at low levels. AF B1 has an average content of 1.9µg / kg, with a minimum of 0.00 and a maximum of 3.35µg / kg. The content of the sum of AF B&G is relatively low with an average of 1.05 µg / kg. The minimum value of these aflatoxins is 0.00 while the maximum is 3.75 µg / kg.

The figures below show the AFB1 concentrations compared to the maximum limit (MRL).

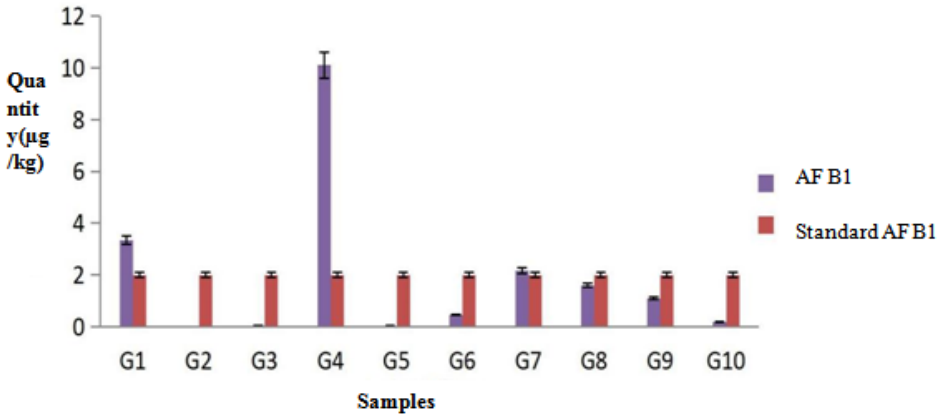

Figure 6 : AF B1 concentrations compared to the maximum residue limit (MRL)

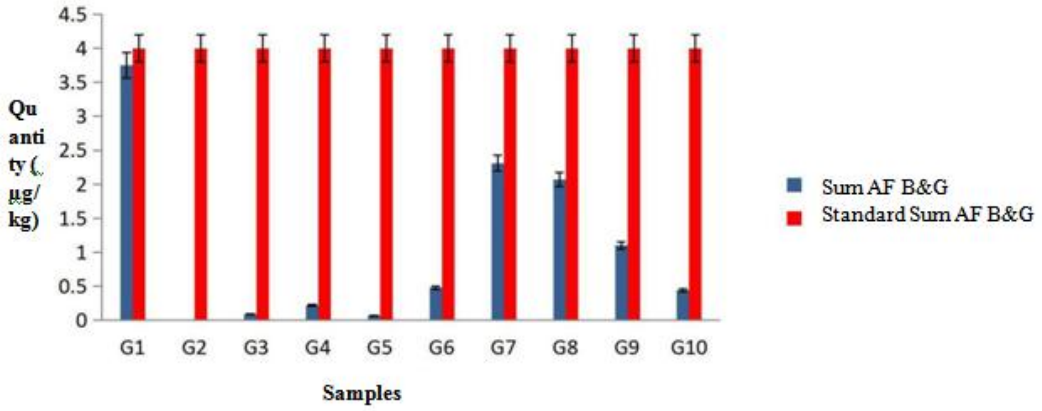

Figure 7 : Concentrations of AF B&G (sum AF B&G) compared to the maximum residue limit.

Out of all the 10 batches of beans collected at Gagnoa, only two (02) have contents above the maximum limit (2 µg / kg) and eight (08) have a content respecting the maximum limit for AF B1. Contrary to the sum of AF B&G, we find that the 10 samples have contents higher than the maximum residue limit (4µg / kg). The proportions of samples rejected or accepted based on AF B1 standards and sum of AF B&G are given in Fig.8**.**

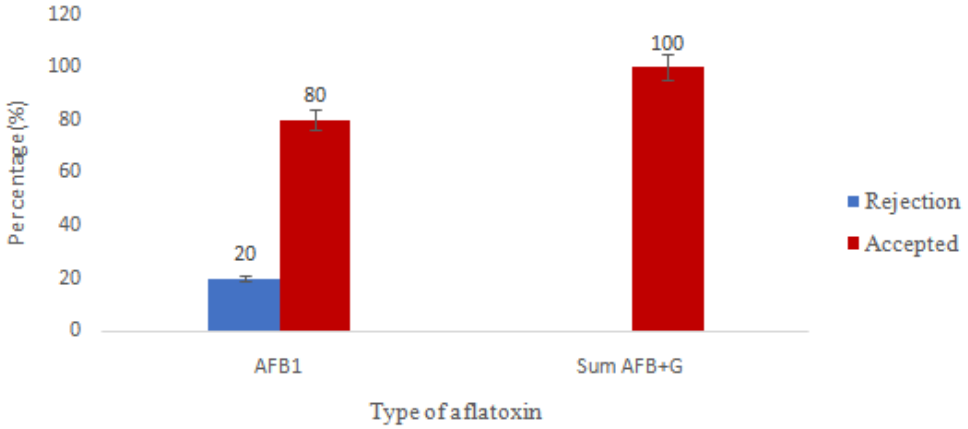

Figure 8 : Proportion of accepted or rejected contents compared to AF B1 standards and AFB&G sum of the locality of Gagnoa.

The Gagnoa samples show relatively low FA contents. For AF B1, only 20% are likely to be rejected and 80% are acceptable.
We observe that the totality (100%) of the Gagnoa samples is acceptable in view of the limit of the sums of the AF B&G.

- **Location of Soubré**

Aflatoxin contamination of cocoa beans is very high in the town of Soubré. The 4 types of aflatoxin studied within the framework of our study are found in all the samples with a strongly high average content for AFB1 (11.32µg / kg) unlike AF G2, G1 and B2, the average contents of which are respectively 0.90 ; 2.36 and 0.11µg / kg. Out of all 10 lots, only 2 lots have AFB1 contents below the maximum limit, 1 lot could not be detected because the concentration was below the LD (detection limit) and 7 have higher levels at this limit.
For the sum of these 4 types of aflatoxin, the average content is 14.72µg / kg. This concentration greatly exceeds the maximum residue limit (4µg / kg). Here, only 2 lots have contents below the maximum limit and 8 have contents higher than this limit (Fig. 9 ; 10 and 11).

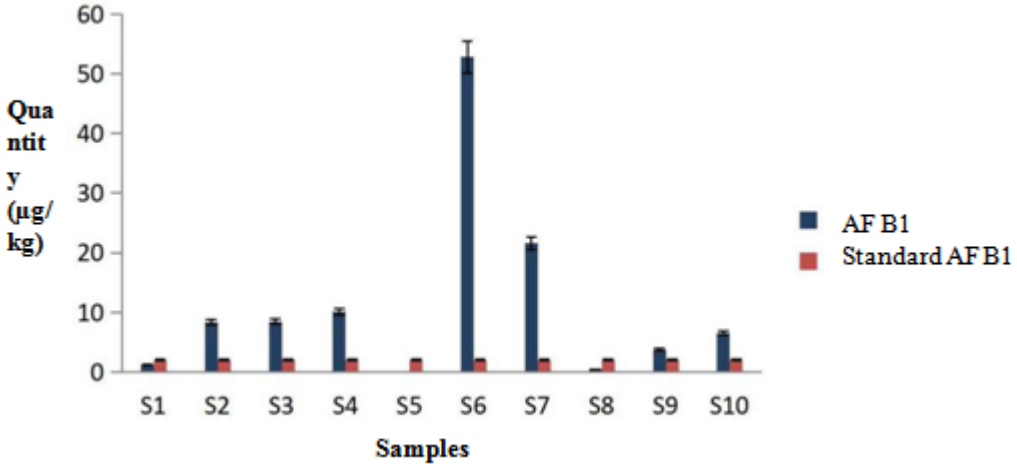

Figure 9 : AF B1 concentrations compared to the maximum residue limit (MRL).

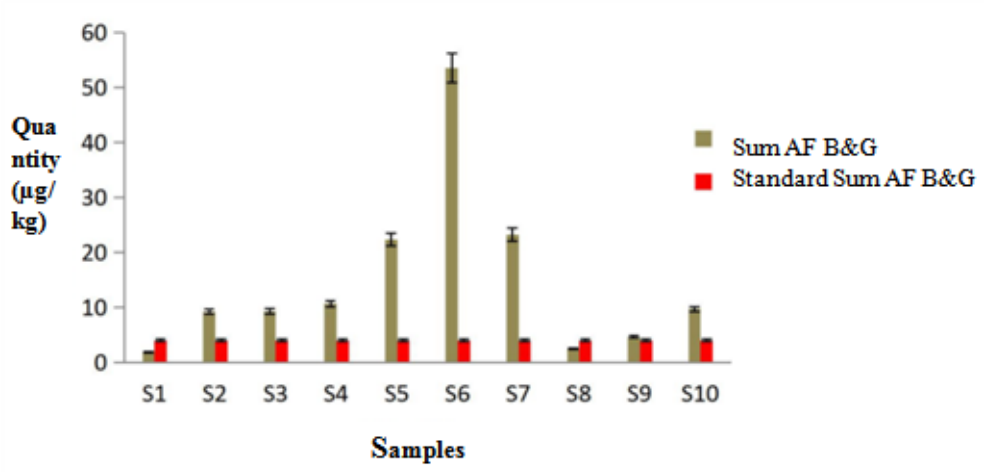

Figure 10 : Concentrations of AF B&G (sum AF B&G) compared to the maximum limit.

The proportions of bean samples rejected or accepted according to AF B1 standards and sum of AF B&G are shown in **Figure 11**.

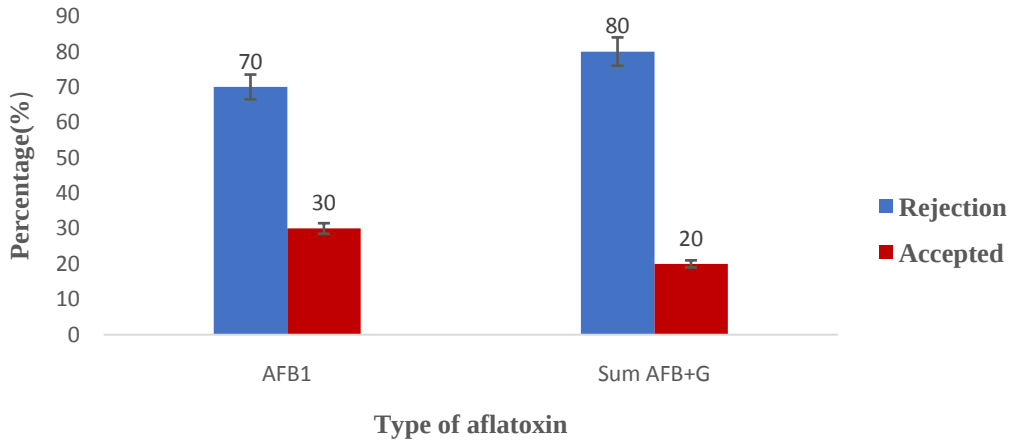

Figure 11 : Proportion of accepted or rejected contents compared to AF B1 and AF B&G de Soubré standards

Regarding AF B1, 70% of the samples are rejected and 30% are accepted. Regarding the sum of AF B&G, only 20% of samples are accepted and 80% are rejected.

3.2- Discussion

The search for aflatoxins in samples of cocoa beans from southwestern Ivory Coast revealed the presence of these mycotoxins. This presence of aflatoxin in the various samples is very important in the localities of Divo and Soubré. The average AFB1 and sum AFB&G contents of these regions vary from 5.90 µg / kg to 11 8.7 µg / kg and from 8.70 µg / kg to 14.72 µg / kg respectively. These results obtained are close to those obtained by Diomandé et al, (2019) on maize, rice, cassava, peanuts and okra produced with aflatoxin B1 contents of 0.02 to 18.65 µg / kg.

All these levels obtained are above the maximum residue limits, which are 2µg / kg for AF B1 and 4µg / kg for the sum of aflatoxins.

About 70% of the samples collected in each of these two localities are rejected and only 30% are accepted for AF B1. Considering the sum of the AF B&G, 40% of the samples are rejected at Divo and 80% at Soubré. On the other hand, the locality of Gagnoa has a relatively low AFB1 contamination rate (20%), the average AF B1 and som AF B & G contents are 1.90 µg / kg and 1.05 µg / kg so no batch has been rejected. Contrary to the results obtained in the Divo and Soubré regions, the levels obtained in Gagnoa are below the maximum residue limits for AFB1 (2µg / kg) and below for the sum of aflatoxins (4µg / kg).

The high rate of contamination of beans in the town of Divo could be explained by the fact that the samples were taken in February, the end of the great campaign. At this point, there is a relaxation of good practices of husking, fermentation, drying or storage and even transport. In fact, Dembélé et al, 2006 indicated in their work that the quality of cocoa decreases as we tend towards the end of the season or the end of the main harvest. Also, during the main season, the productions being high, the producers to be faster, carry out the denting with knives which sometimes injure the beans and expose them to contamination.

For the Soubré region is located in an area of high rainfall (65 to 70 mm / month), the humidity is relatively high during the harvest, favoring the contamination of the beans by fungi. This is because cocoa beans exchange moisture with the outside ; the latter, properly dried, could absorb moisture during storage and therefore may cause contamination of fungi (Koua et al, 2017, Kouadio et al., 2015).

In general, the contaminations observed in the three localities could be due to bad practices observed during our surveys on Good Agricultural Practice (GAP), from the harvest to the packaging of the beans in the warehouses. To this is also added the non-compliance with the construction standards of storage warehouses and the lack of maintenance of these premises. Sampling in the warehouses revealed the state of health of buyers and cooperative stores, especially in the town of Divo.

IV. Conclusion

The study conducted to determine the level of contamination of cocoa beans in the production area of southwestern Ivory Coast revealed that there is an improvement in the quality of the cocoa produced, especially during the great countryside.

The results of our study revealed that cocoa in the 3 departments studied is affected by mycotoxins, mainly aflatoxin, but in varying proportions depending on the locality. Divo and Soubré have the highest contamination rates. On this basis for each of these localities, 70% of the batches sampled are rejected for the case of AF B1. For the case of the sum of AF B & G, batch rejections of 40% and 80% are recorded respectively. Only 20% rejection in Gagnoa for the case of AF B1 and no rejection for the sum of AF B&G. These levels obtained in aflatoxins lead to a risk of consumer exposure to the toxic effects of these molecules.

References

[1]. **GASTELLU JM. (1989).** Riches paysans de Côte d'Ivoire. Paris : L'Harmattan, 178 p. (Alternatives Paysannes). ISBN 2-7384-0411-1.

[2]. **Gayi K.S. et TsowouK. (2016).** L'industrie du cacao : intégrer les petits exploitants dans la chaîne de valeur mondiale. Conférence des Nations Unies sur le commerce et le développement (CNUCED, New York et Genève, 2016).

[3]. **Fowler MS. 1995.** La qualité des fèves de cacao pour les fabricants de chocolat. In : Rencontres cacao, Les différents aspects de la qualité /CIRAD-CP, Montpellier : CIRAD, pp.41-48; 147-153.

[4]. **Cruz J.F., Troude F., Griffon D., Hebert JP.(1988).** Conservation des grains en régions chaudes : Caractéristiques des grains. Techniques rurales en Afrique, 2è éd. Paris, pp 3-15.

[5]. **Agence Canadienne d'Inspection des aliments. 2013.** Aflatoxines dans les produits du maïs, les noix, les produits de noix, les raisins secs, la poudre de cacao, la poudre de chili et le paprika – 1 avril 2012 au 31 mars 2013.

[6]. **DIEMEE., FALLR., SARRI., Fallou SARR, TRAORED. et SEYDIM. (2016).** Contamination des céréales par l'aflatoxine en Afrique : revue des méthodes de lutte existantes. International Journal of Biological and Chemical Sciences, 10(5): 2285-2299.

[7]. **Commission des Communautés Européennes (2006).** Règlement (CE) N° 401/2006 de la commission du 23 février 2006 portant fixation des modes de prélèvement d'échantillons et des méthodes d'analyse pour le contrôle officiel des teneurs en mycotoxines des denrées alimentaires.

[8]. **MOCÁKJ., JANIGAI.,RÁBAROVÁ E. (2009).** Evaluation of iupac limit of detection and iso minimum detectable value - electrochemical determination of lead.Nova Biotechnologica 9-1.

[9]. **Cofrac (2010).** Recommandations d'accréditation en plombémie. Document sh ref 20, p 4.
[10]. **ISO NF EN 14123. (2016).** Sécurité des machines- Réduction des risques pour la santé résultant de substances dangereuses émises par des machines -Partie 2 : Méthodologie menant à des procédures de vérification.
[11]. **Commission des Communautés Européennes (2006).** RÈGLEMENT (CE) No1881/2006 DE LA COMMISSION du 19 décembre 2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires.
[12]. **DIOMANDEA. F., KOUAKOUK. JM.,DIEMELEOUC. A, TRAOREK. S. et DEMBELEA. (2019).** Exposition alimentaire aux mycotoxines cancérogènes dans le département de Séguéla (Nord-Ouest de la Cote d'Ivoire): cas de l'aflatoxine B1,International Journal of Biological and Chemical Sciences 13(2): 937-949.
[13]. **DEMBELEA., COULIBALYA., TRAORÉK.S., KONEM., SILUEN.&TOURE A. A.(2009).** Détermination du niveau de contamination de l'Ochratoxine A (OTA) dans les fèves de cacao à l'exportation. In Tropicultural, (27), pp 26-30.
[14]. **KOUAK.B., EKOUNP.M.,GBAHA P. (2017).** Séchage des fèves de cacao dans un séchoir solaire indirect à circulation forcée d'air. Revues du CAMESSciences Appliquées et de l'Ingénieur, 2(2):15-19.
[15]. **KOUADIOA. K.A., AWS. , ASSIDJON. E., and KOUAMEL. P.(2015).**Etude de la qualité physico-chimique et mycologique du cacao (T*heobroma cacao*) Produit dans les zones de Yamoussoukro et Soubre (Côte d'Ivoire).International Journal of Innovation and Scientific Research,13(1) :330-340.

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