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RESEARCH ARTICLE

FUNGAL CONTAMINATION OF MILLING AIR: A POTENTIAL DANGER TO OUR FOOD AND TO US

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ABSTRACT

Food contaminants have many origins including soil, water and air. The latter environment may contain biological or non-biological particles that cause chronic respiratory disease and contaminate food exposed to it. Respiratory diseases rank fourth after cancer, diabetes and cardiovascular diseases worldwide in the list of priority non-communicable diseases that are the leading cause of death in the world. Among the most frequent microbiological pollutants, some moulds are responsible for respiratory diseases. Thus, man is exposed to this danger both in homes and in some of his activities. Some trades are the most exposed and also constitute cross-contamination frameworks for food. This is the case for millers and grain mills. The work presented here focused on particulate and fungal contamination of the atmosphere of cereal mills in the city of Cotonou. The fungal flora contaminating the atmosphere of the mills was assessed on direct samples by gravitational thrust and the particle size was estimated by suction on a pump equipped with a filter. The identification of fungal species is carried out morphologically and their metabolites by TLC. At the end of the work, after isolation of the different strains, the fungal flora of these mills was mainly composed of fungal particles (spores and fragments) of moulds of the genus *Rhizopus nigricans*, *Mucor spp.*, *Alternaria sp.*, *Penicillium spp.*, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus* section *Flavi*. The isolated particulate air contaminants were of different sizes but mostly ultra fine. With an inspired average load of 1.15 to 47.55 mg/min. The working conditions of millers could favour the development of chronic respiratory diseases and even certain types of cancers for sensitive subjects. The investigation of the toxinogenic nature of the *Aspergillus* of the *Flavi* section in the mill atmosphere identified 10 aflatoxin type B and 19 aflatoxin type B and 19 aflatoxin B and G producing strains and only one non-aflatoxinogen. This high fungal load could also be the basis for contamination of the cereals that are deposited in its mills.

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INTRODUCTION

Problems of indoor air quality are recognized as important risk factors for human health in both low-income, middle and high-income countries (Hänninen, 2011). Chronic respiratory diseases are chronic diseases of the respiratory tract and other lung structures. The most common include asthma, chronic obstructive pulmonary disease, respiratory allergies, occupational lung disease and pulmonary arterial hypertension (Hänninen, 2011).

Globally, chronic respiratory diseases rank fourth after cancer, diabetes and cardiovascular diseases in the list of priority non-communicable diseases that are the leading cause of death in the world (Hänninen, 2011). According to WHO estimates, 235 million people have asthma, 64 million have Chronic Obstructive Pulmonary Disease (COPD), while millions more suffer from allergic rhinitis and other chronic respiratory diseases that are often undiagnosed. In Benin, respiratory diseases represent 13% of cases frequently encountered in consultation in 2013 and not hospitalized (Houinato, 2013). In addition to exhaust emissions that could promote these respiratory diseases, many physical, chemical or microbiological agents can cause disorders such as allergies, infections, poisonings (Aleksic et al., 2015).

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Biological agents of relevance to health are widely heterogeneous, ranging from pollen and spores of plants (mainly from outdoors), to bacteria, fungi, algae and some protozoa emitted outdoors or indoors. They also include a wide variety of microbes and allergens that spreads from person to person (Hänninen, 2011; WHO, 2009). Among the most frequent microbiological pollutants, some moulds are responsible for respiratory diseases. These microorganisms are found throughout the human environment, from soil to food and agricultural products (Castegnaro et Pfohl-Leszkowicz, 2002). When conditions conducive to mold growth are present, they can proliferate, colonize various substrates and eventually end up in the ambient air. In addition, fragments of mycelium, contaminated material particles or dust containing deposited fungal particles may also be airborne (Brochard et al., 2005 and 2010). Thus, man is exposed to this danger both in homes and in some of his activities. Exposure to fungal particles (spores, fragments) or fungal metabolites can therefore be through inhalation or, to a lesser extent, through physical contact (dermal exposure) or, more rarely, through ingestion (Pfohl-Leszkowicz, 2000). The health effects of moulds on populations depending on the type and extent of exposure, nature of the agent involved and susceptibility of exposed individuals (health status, age, etc.). Exposure to moulds can cause various pathologies such as: allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis, asthma and rhinitis (Zain, 2011, Ciegler et al. 1980). Some activities are the most at risk. Among these, millers working with grain mills are highly exposed to mould contamination. Indeed, several research studies have shown high contamination of cereals with mould spores and mycotoxins (AFSSA, 2009; Bailly et al. 2008), as well as some other products ground frequently in these mills (Adjovi et al., 2015; Gnonlonfin et al., 2013a ; Gnonlonfin et al., 2013b). The aim of this study is to evaluate the exposition of mills' workers and customers and air food cross-contamination.

MATERIALS AND METHODS

Sampling: This research is transversal and descriptive. As a first step, an awareness campaign on the dangers of cereal dust contaminated with moulds and mycotoxins was carried out. Then, a reasoned sampling of flour mills in markets and neighbourhoods was carried out according to the importance of the number of visitors to the site *in situ* observation and discussion with miller. In average 15 per day. This study covers 13 mills in Cotonou city, the economic capital of Benin (figure 1). Meanwhile, ten (10) mills results were objectively significant: Fidjrosse, Agla, Fifadji, Vedoko, Midombo, Hinde, St-Michel, Tanto, Wologuede and Zongo.

Collection of atmospheric molds samples: Spores and viable fungal elements are collected by direct deposition of atmospheric particles on an open agar medium box (PDA and MEA) for a period of 15 min. At the same time, all microbial elements are collected by direct deposition of atmospheric particles on an empty Petri dish. (Kanaani et al., 2008)

In each mill three samples were taken:

- next to the miller;
- towards the entrance of the mill and
- next to the mill.

Airborne particle collection: Three daily samples were taken between June 2 and 5, 2016 with a filter change in the morning. The three pumping operations were carried out with an average flow rate of 10 l/min. The filter pumping device was installed in the same location as the particle counter and at a height of 1.70 metres. It corresponds to the inhalation height. A staplex pump with a 4" TFA41 filter (10.16 cm) Diameter is used for this purpose.

Processing of mycological samples

Isolation and identification: The sampling agars are incubated at 30°C. After 4 days of cultivation, the fungal colonies are transplanted onto PDA or MEA. Identification of isolated strains is done by microscopy, based on the distinctive characteristics of the fungal species, described by Pitt and Hocking (2009).

Toxinogenic nature: Moulds of the genus *Aspergillus Flavi* section are transplanted onto different selective media, namely: Yeast Extract Sucrose (YES), *Aspergillus Flavus Parasiticus* Agar (AFPA), Oat Agar (OA). The toxinogenic nature of the isolated strains is assessed, after extraction with chloroform, by thin layer chromatography in the presence of mycotoxin standards as described by Adjovi et al., 2019.

RESULTS

Particles contamination: The filter used retains particles between 10-0.01 μ with a particle collection efficiency of 1 μ , 95% and 58% for 0.3 μ particles. The quantities of particulate matter absorbed after sampling are presented in the following Table 1. The particles quantities obtained by the pump with a debit of 80m³/min, was between 2 and 48 mg / min. The average of inspired particle is 147 μ g /m³ (particles between 10 μ and 0.01 μ) ranged from 14 μ g/m³ to 594 μ g/m³.

Fungal atmospheric contamination of flour mills: Various agricultural products are ground by millers in Benin. In order of importance, there are cereals such as maize, millet, sorghum and rice; tubers and roots such as yam and manioc chips; oilseeds such as soya and groundnuts; and legumes such as beans, Bambara groundnut and dried chilli. Previous works has shown the high contamination of its foodstuffs by fungal spores and their mycotoxins. Atmospheric samples taken during this work revealed the importance and diversity of viable fungal particles in the air of millers in Cotonou. Figure 2 shows the main species encountered at the three sampling points. In general, high contamination by mucoral species (*Mucor* spp. and *Rhizopus* spp.), as well as the genus *Alternaria* spp. and species of the genus *Aspergillus* (*A. flavus*, *A. niger* and *A. fumigatus*) is noted. *Aspergillus flavus* was the most important species in the mill air with 29 different strains isolated (Figure 2). As for the other species, samples taken at the entrance to the mills showed a predominance of viable *Aspergillus niger* particles followed by *A. fumigatus* and *Rhizopus nigricans*. The air in the immediate vicinity of millers also shows high contamination by *A. niger* and mucorales (Figure 2). On the third sample, the same order of predominance as before is followed. The distribution of species in the milling rooms is not significantly different. Fröhlich-Nowoisky et al., 2009 showed in a study that several allergens and pathogens were frequently found in both fine and coarse particle samples

Table 1. Aerial particles charge

Samples	m (mg)	Duration (min)	Debit (m ³ /min)	Particles (mg/min)	Quantity (mg/m ³ /min)
Fifadji (market)	281	20	80	14.05	0.176
Agla (street)	146	20	80	7.3	0.091
St Michel (street)	40	20	80	2	0.025
Wologuede (market)	960	20	80	48	0.600
Midombo (market)	212	20	80	10.6	0.133
Midombo (street)	233	20	80	11.65	0.146
Vedoko (market)	183	20	80	9.15	0.114
Zongo (market)	72	20	80	3.6	0.045
Hinde (market)	43	20	80	2.15	0.027
Wologuede (market)	23	20	80	1.15	0.014
Fidjrosse (street 1)	94	20	80	4.7	0.059
Fidjrosse (street 2)	40	20	80	2	0.025
Fidjrosse (street 3)	35	20	80	1.75	0.022
Tanto (street)	951	20	80	47.55	0.594

Table 2. *Aspergillus* of section *Flavi* isolated

	Small sclerotia	Medium sclerotia	Big sclerotia	Without sclerotia	Aspergillie production acid	Total
Aflatoxins B	3	0	0	6	9	9
Aflatoxins B & G	7	4	2	7	18	20
TOTAL	10	4	2	13	27	29

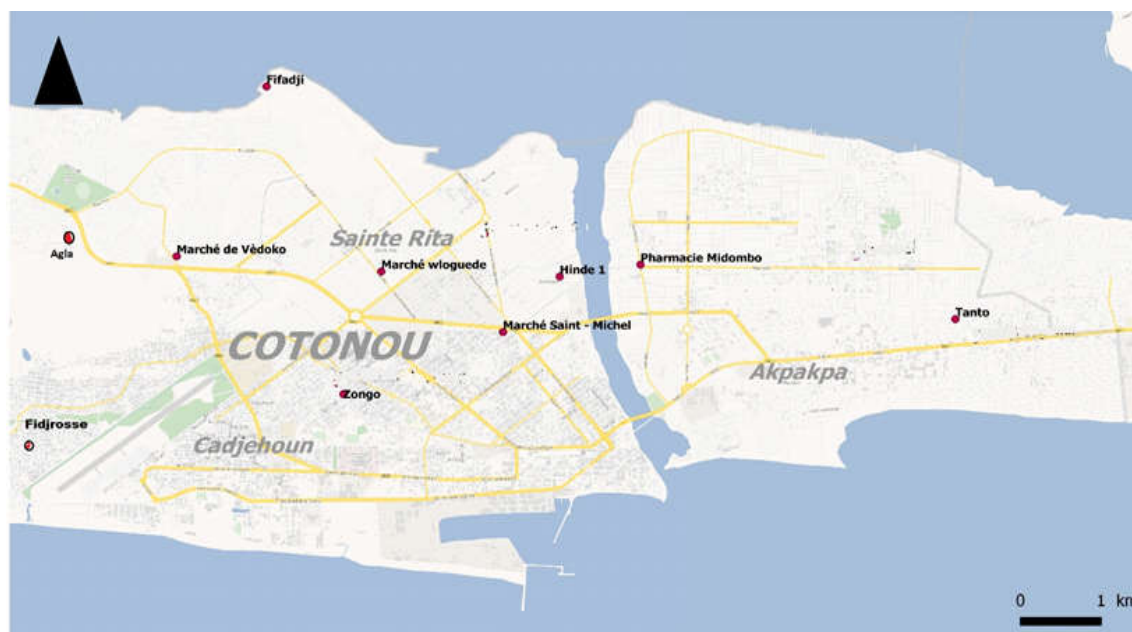


Figure 1. Sampling



Figure 2. Petri dish with fungal aerial contamination

(e.g., *Cladosporium* sp., *Alternaria* spp., *Penicillium* spp., and *Aspergillus* spp.).

DISCUSSION

Working in a mill is not an easy thing for the miller who works there 24 hours a day, 6 days a week. This study showed the level of exposure of millers to airborne particles from ground grains. According to WHO (2009), the range of health effects of particulate matter is wide and the entire population is affected, but the sensitivity in pollution can show variations according to health status and age. It has been shown that the risk increases with exposure for various diseases and there is no evidence to suggest that there is a threshold below which no adverse health effects could be expected. Epidemiological data show adverse effects of particulate matter following short and long-term exposures (WHO, 2009). The lower limit of the margin of concentrations at which adverse effects have been identified is not much higher than the maximum concentration which, for particles below 2.5 μm (PM_{2.5}) is estimated to be 3 to 5 $\mu\text{g}/\text{m}^3$ in the United States of America and Western Europe. The limit in Benin from which particulate pollution is evident is 230 $\mu\text{g}/\text{m}^3$ for 24H (or 3.83 $\mu\text{g}/\text{m}^3$ per minute) for particles below 10 μm (Conseil des Ministres Benin, 2001). However, according to our results, the minimum particulate content in flour mills is 14 $\mu\text{g}/\text{m}^3$, which is well above the environmental standards in force in Benin and four times higher than the estimates with health effects. These mills are not ventilated to provide confined atmospheres. The results also showed increasing deposition rates with increasing ventilation rates, for all particles under investigation.

A great diversity of microorganisms has been noted in cultures. According to (Rahav et al., 2016) the diversity and viability of airborne microbes depends on the aerosol's route prior deposition. The most important ones are mucorales and the genera *Aspergillus* and *Alternaria*. In another study, Kanaani et al., 2008 have showed that the deposition rates of fungal spores (*Aspergillus niger* and *Penicillium* spp.) were similar, especially at very low air exchange rates, to the other aerosols (canola oil and talcum powder) as cereals fine powder. Our results are similar to Janine et al 2008 study which found frequently in tropical forests several allergens and pathogens in both fine and coarse particle samples (*Cladosporium* sp., *Alternaria* spp., *Penicillium* spp., and *Aspergillus* spp.). This study noted high contamination by mucoral species (*Mucor* spp and *Rhizopus* spp), as well as the genus *Alternaria* spp. these fungi are responsible for various respiratory diseases (disease references). *Mucor* species are from a fungi group called Filamentous Basidiomycetes (FBM) which are known for causing chronic lung disease and allergic sensitization in patients with chronic respiratory diseases and have been incriminated in intracavitary pulmonary fungal ball and fungal pneumonia. FBM are better known as allergens than agents of invasive disease, and their non-invasive respiratory manifestations include colonization, allergen sensitization, and allergic sinopulmonary mycoses (Chowdhary, 2016). *Alternaria* spp are Pleosporales implicated in respiratory infections (Bush and Prochnau, 2004), are associated with allergic fungal rhinosinusitis (AFRS), bronchial asthma, hypersensitivity pneumonitis, allergic bronchopulmonary mycosis (ABPM), and invasive lung disease (Chowdhary et al., 2014). AFRS is a form of polypoid chronic rhinosinusitis caused by type I hypersensitivity to fungal antigens. *A. alternata* is known to produce a severe form of asthma through

outdoor allergen sensitization. As for the species of the genus *Aspergillus* (*A. flavus* and *A. fumigatus*), they are known to be responsible for aspergillosis (Su et al., 2019). Invasive pulmonary aspergillosis are the most prevalent opportunistic fungal infections (Su et al., 2019; Dasbach et al., 2000) and *Aspergillus* spp. remains the first etiology. It is responsible for various clinical pictures, different pathophysiological mechanisms. These are aspergilloma by colonization of pre-existing pulmonary cavities, invasive pulmonary aspergillosis, an infection that is often fatal in immunocompromised patients, and chronic necrotizing pulmonary aspergillosis (Germaud et al., 2009). *Aspergillus* spp. are considered to be the major culprit of severe asthma with fungal sensitization (SAFS), although a range of other fungi, such as *Alternaria* and *Cladosporium* spp. are also involved (Chowdhary et al., 2014). Millers who spend a lot of time in this atmosphere loaded with fungal particles and cereal and metal dust from the mill are highly exposed to respiratory diseases. In addition, these particles and fungal debris in the atmosphere of flour mills constitute additional charges to the various agricultural products that are deposited there for milling and that are already ground there. Air throughout the facility serve effectively as vectors for dispersion of fungi (Snyder et al., 2018). These conditions for cereal flour production are also a means of contaminating foodstuffs. These studies have shown that by atmospheric deposition, after 15 minutes, which represents the minimum waiting time in a flour mill, the fungal load deposited is varied and significant. Lack of air pressure controls has been associated with fungal spoilage (Wang et al., 2015).

Filamentous fungi have proved particularly well suited to dispersion and cross-contamination within the food processing infrastructure. Because of these diverse structural features and survival mechanisms, fungal species are well adapted to ecological niches and are able to contaminate and spoil processed foods and storage foods. According to Wyatt et al (2013), *Aspergillus*, *Cladosporium*, and *Penicillium* spores can be found in every cubic meter of air. The density of spores in an area varies seasonally and is highest during spring and summer (Snyder et al, 2018) and is more important in tropical region. These different fungal contaminants, depending on storage conditions, can produce mycotoxins in contaminated products.

Conclusion

This descriptive study shows on the one hand the impact of the milling activity on millers and on the other hand the risks of cross-contamination in the food products that are ground there. The air of these mills is highly charged with fungal particles (cell debris and spores) of different species. To better understand all the risks associated with this activity, this research must be continued with the evaluation of the state of health of millers and the importance of the load that contaminates food during their passage through the mill. The study area can be extended, and the target contaminated population can be raised, to complement confirm the result of this study.

Key-points

- The atmosphere of the mills and highly charged with dust and fungal particles.
- The fungal species encountered are responsible for lung disease.

- The mills are places where agricultural products can be contaminated by fungal particles, particularly *Aspergillus flavus* aflatoxinogens

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