FILIPE DA SILVA DE OLIVEIRA

# BIOLOGICAL RISK ASSESSMENT IN COFFEE STORAGE AND HANDLING WAREHOUSES

LAVRAS – MG
2024

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia Agrícola, área de concentração em Processamento de Produtos Agrícolas, para a obtenção do título de Doutor.

Prof. Dr. Ednilton Tavares de Andrade
Orientador

Profa. Dra. Susana Viegas
Coorientadora

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APROVADA em 27 de maio de 2024.

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|---|---|
| Dr. Ednilton Tavares de Andrade | UFLA |
| Dr. Giovanni Francisco Rabelo | UFLA |
| Dra. Luana Elis de Ramos e Paula | UFLA |
| Dr. Luís César da Silva | UFV |
| Dra. Susana Viegas | ENSP/NOVA |

Prof. Dr. Ednilton Tavares de Andrade
Orientador

Profa. Dra. Susana Viegas
Coorientadora

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## RESUMO

A presença de múltiplos gêneros fúngicos e micotoxinas em unidades de rebeneficiamento de café representa um risco à saúde dos trabalhadores. O objetivo deste trabalho foi de avaliar a presença de fungos e, devido à alta contaminação microbiológica encontrada, avaliar também os metabólitos secundários presentes em unidades de rebeneficiamento de café no estado de Minas Gerais. Este estudo investigou a presença de fungos e micotoxinas em diferentes matrizes: poeira decantada (n=40), equipamentos de proteção respiratória (n=40), panos de poeira eletrostáticos (EDC) (n=70) e grãos de café beneficiados (GCB) (n=36) coletados em indústrias de rebeneficiamento de café no Brasil. Os microrganismos foram detectados nas amostras através de crescimento em meio sólido e qPCR, as micotoxinas foram detectadas por HPLC/MS. Ao todo foram duas indústrias avaliadas com um total de 46 trabalhadores expostos por turno. Dentre os gêneros ocupacionalmente mais relevantes encontrados foram *Aspergillus* e *Fusarium*, sendo relatadas a presença de seções como: *Nigri*, *Circumdati*, *Flavi* e *Fumigati*. Quanto às micotoxinas, as mais recorrentes foram ácido micofenólico (MPA) e ocratoxina A (OTA). Os resultados destacam a importância da qualidade da matéria-prima e das atividades realizadas na exposição ocupacional a fungos e micotoxinas, com implicações para a saúde dos trabalhadores. Estratégias simples, como o uso de respiradores e sistemas de captação de poeira, podem contribuir para a segurança dos trabalhadores. No entanto, são necessários estudos adicionais para entender melhor o cenário de exposição ocupacional e implementar medidas de prevenção adequadas.



**Palavras-chave:** micotoxinas; fungos; poeira; exposição ocupacional; risco biológico.

## ABSTRACT

The presence of multiple fungal genera and mycotoxins in coffee processing plants poses a health risk to workers. This study aimed to assess the presence of fungi and, due to the high microbiological contamination found, also evaluate the secondary metabolites present in coffee processing plants in the state of Minas Gerais, Brazil. This study investigated the presence of fungi and mycotoxins in different matrices: settled dust (n=40), respiratory protective equipment (n=40), Electrostatic Dust Cloth (EDC) (n=70), and green coffee beans (GCB) (n=36) . Microorganisms were detected in samples through growth on solid medium and qPCR, while mycotoxins were detected by HPLC/MS. A total of two industries were evaluated with a total of 46 workers exposed per shift. Among the occupationally most relevant genera found were *Aspergillus* and *Fusarium*, with sections such as: *Nigri*, *Circumdati*, *Flavi,* and *Fumigati* reported. Regarding mycotoxins, the most recurrent were mycophenolic acid (MPA) and ochratoxin A (OTA). The results highlight the importance of raw material quality and activities performed in occupational exposure to fungi and mycotoxins, with implications for worker health. Simple strategies, such as the use of respirators and dust collection systems, can contribute to worker safety. However, further studies are needed to better understand the occupational exposure scenario and implement appropriate prevention measures.



**Keywords:** mycotoxins; fungi; dust; occupational exposure; biological risk.

## IMPACTOS SOCIAIS, TECNOLÓGICOS, ECONÔMICOS E CULTURAIS

O estudo investigou os impactos sociais, tecnológicos, econômicos e culturais da presença de múltiplos gêneros fúngicos e micotoxinas em unidades de rebeneficiamento de café em Minas Gerais, focando especialmente nos aspectos de saúde e trabalho. Socialmente, identificou-se que 46 trabalhadores por turno estão expostos a altos níveis de contaminação microbiológica, destacando os gêneros ocupacionalmente relevantes Aspergillus e Fusarium. Essa exposição direta afeta negativamente a saúde dos trabalhadores, sublinhando a necessidade de medidas preventivas urgentes para garantir condições laborais seguras e saudáveis. Tecnologicamente, o estudo utilizou métodos avançados como qPCR e HPLC/MS para detecção de microrganismos e micotoxinas, contribuindo significativamente para o desenvolvimento de técnicas mais eficazes de monitoramento e controle desses agentes contaminantes na indústria do café. Economicamente, a implementação de estratégias de controle de qualidade da matéria-prima e segurança ocupacional não apenas melhora a saúde dos trabalhadores, mas também reduz custos relacionados a absenteísmo e tratamento médico, promovendo um ambiente de trabalho mais produtivo e sustentável. Culturalmente, o estudo promove uma cultura organizacional voltada para a segurança e bem-estar dos trabalhadores, enfatizando a importância de práticas de trabalho seguras como um valor essencial. Alinhado com os Objetivos de Desenvolvimento Sustentável da ONU, especialmente os relacionados à saúde (ODS 3) e trabalho decente e crescimento econômico (ODS 8), este trabalho destaca Minas Gerais como um território impactado por desafios significativos, onde intervenções eficazes são essenciais para garantir a manutenção da saúde e integridade física de milhares de trabalhadores.

## SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

The study investigated the social, technological, economic, and cultural impacts of the presence of multiple fungal genera and mycotoxins in coffee reprocessing units in Minas Gerais, focusing particularly on health and labor aspects. Socially, it was identified that 46 workers per shift are exposed to high levels of microbiological contamination, with occupationally relevant genera such as Aspergillus and Fusarium prominently featured. This direct exposure adversely affects worker health, emphasizing the urgent need for preventive measures to ensure safe and healthy working conditions. Technologically, the study employed advanced methods like qPCR and HPLC/MS for detecting microorganisms and mycotoxins, significantly contributing to the development of more effective techniques for monitoring and controlling these contaminants in the coffee industry. Economically, implementing strategies for raw material quality control and occupational safety not only enhances worker health but also reduces costs associated with absenteeism and medical treatment, promoting a more productive and sustainable work environment. Culturally, the study advocates for an organizational culture focused on worker safety and well-being, underscoring the importance of safe work practices as a core value. Aligned with the UN Sustainable Development Goals, particularly those related to health (SDG 3) and decent work and economic growth (SDG 8), this work highlights Minas Gerais as a region impacted by significant challenges, where effective interventions are essential to ensure the health and physical integrity of thousands of workers.

## TABLE OF CONTENTS

## LISTA DE SIGLAS

| | |
|---|---|
| **AFB2** | aflatoxin B2 |
| **CFU** | Colony Formation Unit |
| **EDC** | electrostatic dust cloth |
| **EPI** | Personal Protective Equipment |
| **FB1** | fumonisin B1 |
| **FRPD** | Filtering Respiratory Protection Device |
| **GCB** | Green Coffee Beans |
| **HPLC** | High Pressure Liquid Cromatography |
| **LOD** | Limit of Detection |
| **LOQ** | Limit of Quantification |
| **MPA** | mycophenolic acid |
| **OTA** | ocratoxin A |
| **OTB** | ocratoxin B |
| **qPCR** | Quantitative Polymerase Chain Reaction |
| **TLVs** | Threshold Limit Values |
| **ZEN** | zearalenone |

# PART I

# 1 INTRODUCTION

The coffee is one of the most important beverages worldwide (Czarniecka-Skubina *et al.*, 2021). According to the latest publication from the International Coffee Organization (ICO, 2023a), the total production of all exporting countries during the 2022/2023 crop year was approximately 10.1 million tonnes of coffee beans. Brazilian production in the same year was nearly 3.5 million tonnes, representing over a third of global production. During the same season, Brazil exported over 2.6 million tonnes, accounting for almost 70% of its total production (ICO, 2023b).

As reported by the General Register of Employed and Unemployed Persons (Brasil, 2023), in December 2023, there were 85,249 workers in the coffee industry in Brazil, with 68,180 employed in general warehouses and 17,069 in roasting companies. In other words, thousands of Brazilian workers are engaged in tasks involving the handling of coffee beans at different processing stages.

Post-harvest coffee processes include various stages ranging from washing and separating the fruits to drying, processing, and reprocessing the beans before storage. Coffee storage and handling warehouses are responsible for granulometric classification, enhancing the type and sensory characteristics of green coffee beans (GCB) (Borém, 2023). In summary, the dry milling of GCB in a company involves the following processes: reception, cleaning, sorting, selection, blending, and dispatch. In all these processes mentioned, the handling of GCB causes the rupture of these beans, forming dust (Kanageswari; Tabil; Sokhansanj, 2022).

Dry milling is a process involving the receipt, cleaning, hulling, polishing, and grading of GCB, classifying the hulled beans by size, density, and color (Rotta *et al.*, 2021). This process can produce a significant amount of dust, implying worker exposure if adequate risk management measures are not implemented, which can pose health risks to workers. The amount of dust produced in a coffee storage and handling warehouse depends on factors such as the type of milling equipment used, the size of the operation, and the condition of the coffee beans being processed. The dust is mainly composed of small particles of dried coffee fruits and other organic materials such as fungi and mycotoxins. These dust particles can vary in size, from very fine particles to larger particles that are visible to the naked eye (Zhao *et al.*, 2020). Smaller particles can be inhaled into the lungs and potentially reach the

alveoli, while larger particles become trapped in the upper respiratory tract (Vanka *et al.*, 2022).

Occupational exposure due to airborne biological agents has been reported in the literature in different workplaces (Bosson-Rieutort *et al.*, 2020; Burzoni*et al.*, 2020). Bioaerosols are defined as airborne particles, including fungal spores and hyphae, bacteria, endotoxins, β(1→3) glucans, mycotoxins, or high molecular weight allergens and organic dust in general composed of or derived from biological matter (Oppliger, 2014).

Several studies in the literature have reported the presence of fungal genera in green coffee beans, including the genera Aspergillus (Barcelo; Barcelo, 2018; Viegas *et al.*, 2017), Penicillium (Silva *et al.*, 2020), Fusarium (Abaya *et al.*, 2020), among others (Alvindia; Guzman, 2016; Culliao; Barcelo, 2015). Additionally, other authors have verified the presence of different mycotoxins in green coffee beans, such as aflatoxins (Magnoli *et al.*, 2008; Al-Ghouti*et al.*, 2020), ochratoxins (Sousa *et al.*, 2018; Twarużek*et al.*, 2020; Maman *et al.*, 2021), and others (Yassin *et al.*, 2015; Bessaire *et al.*, 2019). Therefore, since fungi and mycotoxins are commonly found in GCB, it is predictable that these agents may also be present in the workplace. However, there are few reports in the literature on occupational exposure to fungi and mycotoxins in the coffee industry.

Mycotoxins are metabolites produced by specific genera of fungi, which can cause various health effects (Huttunen; Korkalainen, 2017). These compounds can be transported by air when attached to spores, fragments, and particulate matter (Taubel; Hyvarinen, 2016). Therefore, it is of fundamental importance to study occupational exposure to mycotoxins in environments such as the coffee industry, where exposure to organic dust may occur in different tasks such as storage, loading, or handling of GCB materials (Viegas; Viegas; Oppliger, 2018).

# 2 BACKGROUND

## 2.1 Production of coffee in Brazil

Currently, Brazil is the world's largest coffee producer, accounting for approximately one-third of global production (ICO, 2023). Although coffee cultivation is widespread across the country, with a total of 1.9 million hectares (CONAB, 2023), coffee production is primarily concentrated in six states: Minas Gerais, Espírito Santo, São Paulo, Paraná, Bahia, and Rondônia (Bliska *et al.*, 2009).

Among the Brazilian states, Minas Gerais is the largest producer of *Coffea arabica*. According to the Brazilian Institute of Geography and Statistics (IBGE) (IBGE, 2022), Minas Gerais produced over 1.4 million tonnes of the bean in the year 2022.

### 2.1.1 Specialty and commodity coffees

The Specialty Coffee Association (SCA) (SCA, 2021) defines specialty coffee as coffee recognized for its distinctive attributes, which, due to these attributes, has significant added value in the market. For green coffee beans (GCB) to achieve this quality distinction, a combination of factors throughout the coffee production chain must be considered. The quality of the final product is influenced by various factors such as physical characteristics, chemical composition, geographical origin, climate, species, harvest methods, processing techniques, and storage conditions (Borém, 2023). These elements can drastically impact the final product's quality. Consequently, within the same occupational task, exposure levels may vary over time, depending on the quality of the manipulated products (Viegas, Viegas; Oppliger, 2018), including coffee beans.

Although some stages of the specialty coffee production process are common to commodity coffee processes, there are relevant differences regarding the classification and sensory characteristics of the product. The main stages of the production process are planting, harvesting, cleaning, sorting, and yarding, mechanical drying and storage, processing and storage, classification, and dry milling (Borém, 2023). According to Sanchez (2007), one of the most relevant points during harvesting, which differentiates specialty coffees from commodity coffees, is

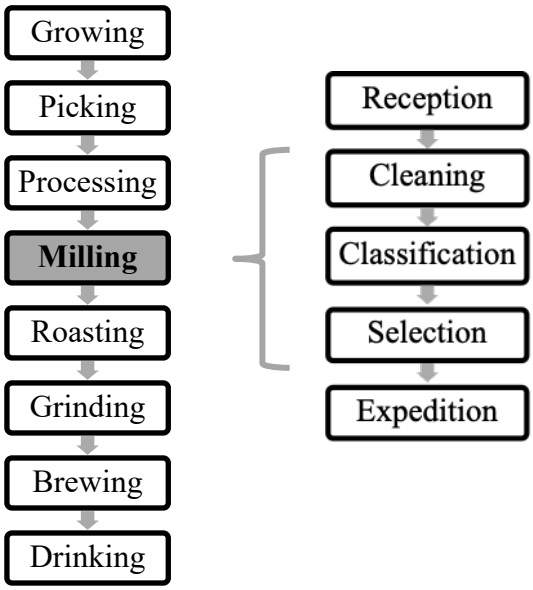
that for commodity coffees, stripping is commonly done directly on the ground, while for specialty coffees, stripping is done on cloth sheets placed under the coffee trees. In other words, specialty coffee beans do not remain in direct contact with the ground, which is the habitat of various toxigenic fungal genera.

To ascertain that the prolonged stay of coffee beans on the ground provides high concentrations of microorganisms and mycotoxins in the beans, Campos *et al.* (2009) verified that after a certain period of prolonged exposure of the beans to the ground, there was a high incidence of *Aspergillus* spp. and ochratoxin A. In the case of beans dried on the mother plant, the presence of ochratoxin A was not detected. Additionally, Batista and Chalfoun (2007) found that the earth yard increases the risk of contamination with ochratoxin A in coffee beans. The authors suggest that sweeping coffees already arrive at the yard for drying with a degree of toxigenic fungal infection or even ochratoxin A. In other words, the fungi and mycotoxins were carried between the links of the coffee production chain.

### 2.1.2 Supply Chain: storage and handling warehouses

A coffee industry involves different processes, from cultivation to preparation for consumption. Typically, the main stages include cultivation, harvesting, processing, dry milling, roasting, packaging, transportation, grinding, and preparation (FIGURE 1). Normally in Brazil, coffee storage and handling warehouses are the final stage before exportation. In Figure 1, it can be seen that the common production flow, including all stages that allow the obtaining of the final product in the coffee production chain.

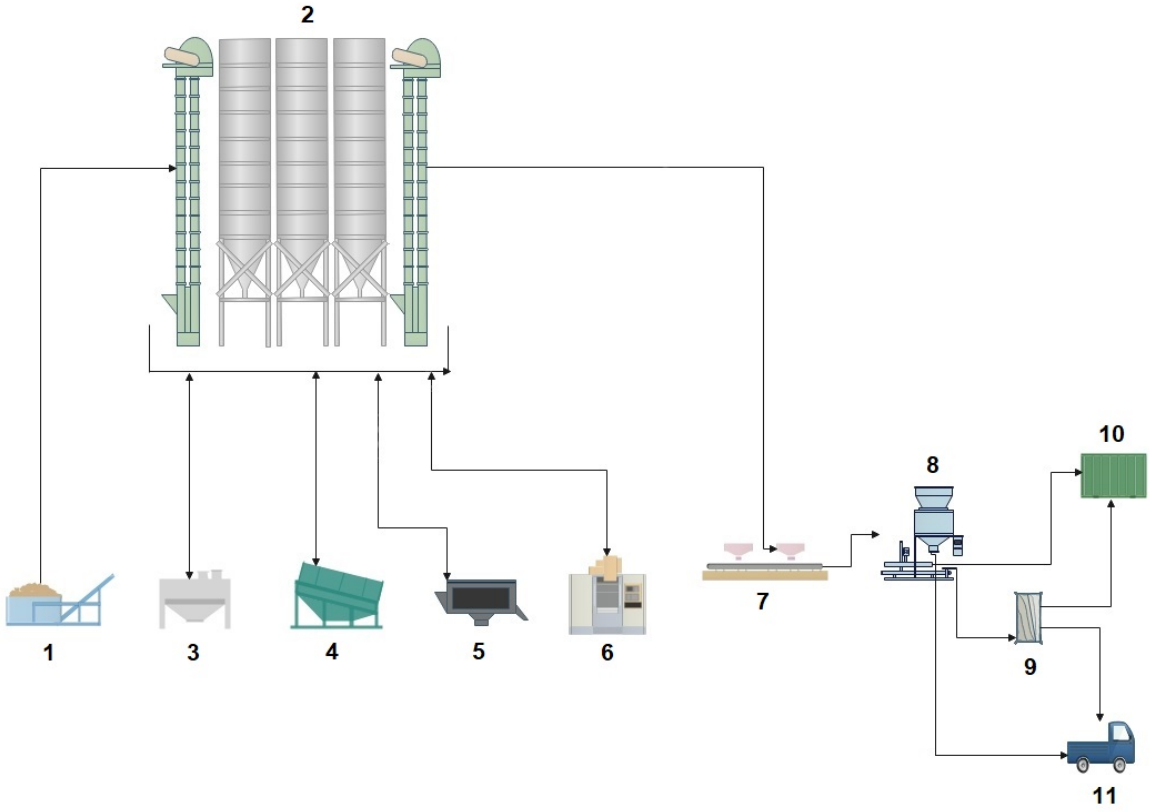
Figure 1 – Coffee production flow.




In the coffee production chain in Brazil, the process that follows the beneficiation is the dry milling. According to data provided by the National Registry of Employed and Unemployed Persons (CAGED) in December 2023 (Brasil, 2023), over 100,000 workers are involved in the coffee production chain in Brazil. Three groups related to coffee are registered in CAGED: coffee cultivation, roasting and grinding, and general warehousing. In the coffee cultivation group, 86,665 workers are associated, while in the roasting and grinding group, 17,069 workers are registered. In the general warehousing group, which may contain other grains besides coffee, there are currently 68,180 workers in Brazil. In other words, thousands of Brazilian workers are engaged in tasks involving the handling of coffee beans at different processing stages. Thus, it can be observed that many workers are potentially exposed to such risks that have not yet been measured.

In coffee storage and handling warehouse, there is a great conveyance of beans by different machinery. In Figure 2, the machinery present in a real coffee storage and handling warehouse of the state of Minas Gerais can be seen. In all these mentioned processes, the conveyance of beans causes their rupture, forming dust.

Figure 02 – Illustrative sequence of machinery in coffee storage and handling warehouses.




Subtitle: (1) Reception; (2) bins; (3) stone picker; (4) ranked table; (5) density table; (6) electronic table; (7) pneumatic treadmill; (8) shipping scale; (9) bags (conventional or big bags); (10) shipping in containers; and (11) shipment by truck.
Source: Author (2024).

### 2.1.3 Industrial Processes and Dust Formation

Industrial processes in coffee production often trigger the formation of dust, an inevitable byproduct that poses occupational challenges (Banti; Abraham, 2021). Inhalation of airborne particles can affect the respiratory health of workers (Harvey *et al.*, 2020). Control strategies, such as implementing effective ventilation systems and using personal protective equipment, are crucial to mitigate the risks associated with dust exposure during industrial processes (Viegas *et al.*, 2020; Szulc *et al.*, 2022).

Dust particles usually originate from larger masses of the same material, undergoing mechanical disintegration processes such as vibration, grinding, cutting, drilling, crushing, or intense friction between specific materials, such as grains (WHO, 1999). In other words, dust is formed whenever processes involve free fall or

handling of such materials, such as transfer, filling (bagging), or emptying of bags or containers, material transfer from a hopper to a weighing station, weighing, mixing/separation, transportation, as well as air currents over granular materials (Dorman, 2014).

Organic dust originates from various work processes, such as wood dust produced during sawing and sanding (Pałubicki; Hlásková; Rogozinski, 2020), cotton dust in ginning processes (Kumie *et al.*, 2020), wool dust in sheep shearing (Ravichandran *et al.*, 2020), dust in pig units (Viegas *et al.*, 2019), and vegetable dust in grain post-harvest units (Abaya *et al.*, 2020), among others. The dust generation rate is related to the energy associated with the process; for example, applying higher vibrations during the coffee dry milling process may produce a larger amount of dust than subjecting the raw material to lower vibrations.

To reduce dust emissions from these operations, it is important to understand the mechanisms of its generation and release. Studies on dust generation by falling powders have shown that how the dust is handled can be as important as the dust-generating capacity of the bulk material, in terms of resulting exposure (Anlimah*et al.*, 2023; Asif *et al.* 2022; Collinet; Haldin; Schall, 2021). Therefore, Ogden (1999) highlights different strategies that can be used concurrently to control suspended particulate matter in grain processing industries. For example, source elimination, enclosure and ventilation, work practices, and personal protective measures.

## 2.2 The Occupational Hazards Present During Post-Harvest Processes

The management of occupational risks is essential in the coffee production chain, with special attention to biological risks. Workers at storage and handling warehouses are subjected to exposure to microbiological agents and their secondary metabolites (Abaya *et al.*, 2020; Viegas *et al.*, 2022). This exposure can have serious health implications, highlighting the need for rigorous safety practices and hygiene protocols to mitigate the biological risks associated with this stage of coffee production.

In Annex I of Regulatory Standard 01 (NR 01) - General Provisions and Occupational Risk Management (Brasil, 2022), occupational risks are defined as:

> The combination of the probability of injury or harm to health caused by a hazardous event, exposure to harmful agents, or demands of the work activity, and the severity of such injury or harm to health.

According to NR 01 (Brasil, 2022), harmful agents are defined in three categories: physical agents, chemical agents, and biological agents. Physical agents are any form of energy that, due to their nature, intensity, and exposure, can cause injury or harm to the worker's health. Chemical agents are chemical substances, either alone or in mixtures, whether in their natural state or produced, used, or generated in the work process, which, due to their nature, concentration, and exposure, can cause injury or harm to the worker's health. Biological agents, including microorganisms, parasites, or materials from organisms, can cause harm to workers' health due to their nature and type of exposure.

Thus, occupational risks result from exposure to harmful agents. It has been reported in grain storage and handling warehouses the exposure to noise (Bellochio; Coradi, 2022), dust (Viegas *et al.*, 2022), and pesticides (Moreira, 2022).

The organic dust originating in coffee storage and handling warehouses may contain chemical and biological agents. Such agents are carried from previous links in the production unit, for example, through contact with the soil or application of chemical products.

As seen above, physical, and chemical agents are already widely known in this link of the coffee production chain, but there are few studies that prove occupational exposure to biological agents present in coffee storage and handling warehouses worldwide. Therefore, it is necessary to understand which biological agents are present in these occupational environments.

### 2.2.1 Biological and Chemical Hazards

According to the American Conference of Governmental Industrial Hygienists (ACGIH) (2021), biological agents include bacteria, fungi, viruses, algae, and parasites. Furthermore, the term biological agent refers to a substance of biological origin capable of producing adverse health effects, being ubiquitous in nature, and may also contain or release mycotoxins (in the case of fungi) due to metabolic activity. In other words, exposure to fungi present in coffee storage and handling warehouses falls under this occupational risk.

Mycotoxins are secondary metabolites of fungi known to exert a wide range of toxicities in humans and animals. Depending on the type of mycotoxins, nephrotoxicity, cancer, liver toxicity, impaired immune functions, and growth retardation have been reported among their adverse health effects (Rocha *et al.*, 2014). Mycotoxins contaminate many of the most consumed foods and feeds worldwide, including cereals, nuts, dried fruits, and spices. Ingestion of contaminated food results in foodborne exposure for the general population, while high exposure to organic dust can be a source of exposure for workers through inhalation or dermal contact. Studies have shown that the number of mycotoxins found in dust can be more than ten times higher than that found in raw materials. In fact, mycotoxins are widely present on the surface of raw materials and tend to be adsorbed into dust during handling (Jargot; Melin, 2013; Straumfors *et al.*, 2015).

An important characteristic of occupational exposure to mycotoxins is the route of exposure. While the most important exposure for the general population is oral ingestion of contaminated food, in the workplace, inhalation and dermal contact are typical routes. However, knowledge about occupational exposure to mycotoxins lags behind knowledge about dietary exposure. This is caused by significant uncertainties regarding the transfer of contaminated material into the air (mycotoxin concentration) and/or the fraction of toxin absorbed through dermal contact or after inhalation (Degen 2008; Mayer *et al.*, 2008).

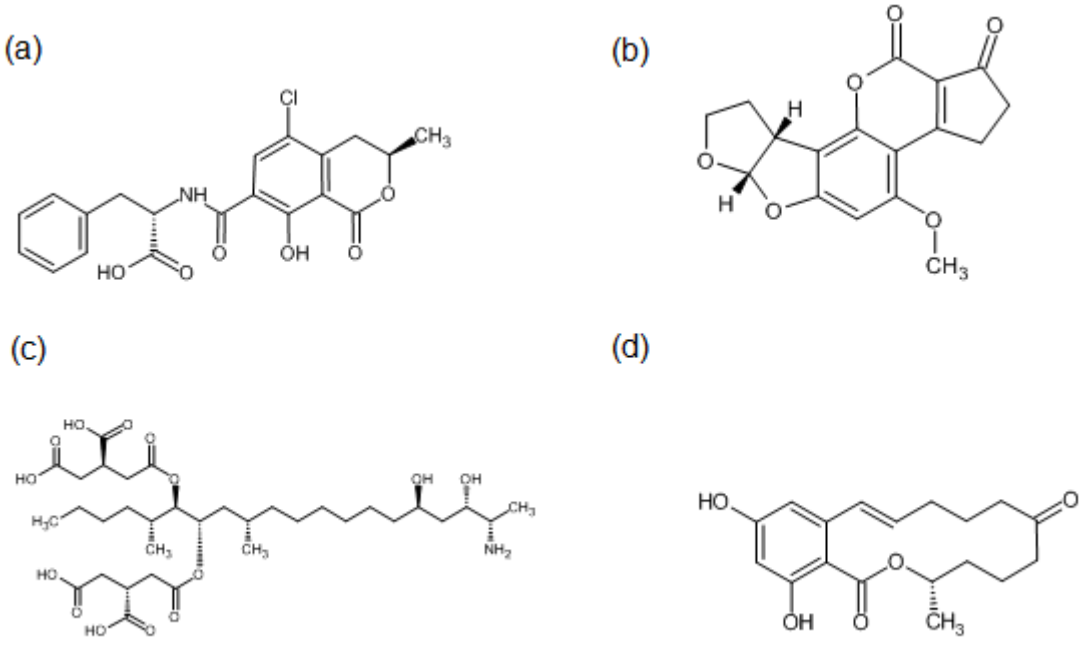
Another characteristic of occupational exposure is the handling of products and materials on an industrial scale. For example, when a moldy orange has to be discarded in a private kitchen, exposure to mold and mycotoxins will be limited, whereas in wholesale workplaces, tonnes of oranges must be disposed of in case of mold infestation, which can result in much higher exposure. Due to the large quantities of products and materials handled in occupational environments, large amounts of dust are often generated. Furthermore, mycotoxins can be enriched in dust. This probably occurs because the concentration of mycotoxins is generally higher in the outer layer of the grain. This is well demonstrated for different grains (Halstensen *et al.*, 2008; Krysinska-Traczyk; Perkowski; Dutkiewicz, 2007; Sorensen *et al.*, 1981). Jargot and Melin (2013) observed a concentration of mycotoxins up to 15 times higher in airborne dust with bulk material.

The main types of mycotoxins reported in coffee beans are: ochratoxin A (Oeung; Songsermsakul; Porasuphatana, 2022), aflatoxins (Ouakhssase; Fatini;

AitAddi, 2021), fumonisins (Bessaire *et al.*, 2019), and zearalenone (Oueslati *et al.*, 2022). The chemical structures of these secondary metabolites are presented in Figure 3.

According to the European Agency for Safety and Health at Work (EU-OSHA), biological risks in agricultural workers are characterized as emerging risks. Emerging risks are related to newly created or identified risks, increasing risks, or risks that have become widely known or established. Furthermore, according to the EU-OSHA, agricultural workers are clearly exposed to the risk of infection through unintentional exposure to bacteria, fungi, parasites, and organic dust, which is a mixture of biological agents and/or their secondary metabolites, toxins, viruses, allergenic agents, and substances generated by arthropods, bacteria, and plant material (EU-OSHA, 2020).

**Figure 3 –** Chemical Structures of the Main Secondary Metabolites Present in Coffee Beans: (a) ochratoxin A[1], (b) aflatoxin B2[2], (c) fumonisin B2[3], and (d) zearalenone[4].




Sources : El Khoury; Atoui (2010)[1]; Caceres et al. (2020)[2]; Beier; Stanker (1997)[3]; Ropekjo; Twarużek (2021)[4].

Occupational exposure to fungi and mycotoxins in coffee industriesis sparsely studied in terms of investigating the concentration of these biological risks in these locations. Thomas *et al.* (1992) found that among the sampled areas in the coffee

storage, roasting, and extraction industry, the area with the highest amount of dust present was the warehouse with 3.2 mg $m^{-3}$, and 8.14 x $10^3$ CFU $m^{-3}$ of fungi were found in the grain unloading area. Iavicoli*et al.* (2002) concluded that ochratoxin A, along with other potentially present mycotoxins in the workplace environment, represent a form of occupational exposure in coffee processing units in Italy.

Although biological agents have been reported in grain handling industries, preventive and mitigating measures that protect workers involved in these establishments satisfactorily control the workers involved. Engineering control measures, administrative measures, and proper use of personal protective equipment are examples of such applications.

Therefore, due to the lack of previous studies in coffee storage and handling warehouses regarding occupational exposure to biological risks in Brazil, it is important to conduct an exploratory study to evaluate the main species of fungi and bacteria present in the environment and the main types of mycotoxins.

## 3 FINAL CONSIDERATIONS

The present study contributes scientific evidence on the occupational exposure of workers in coffee reprocessing units in the state of Minas Gerais. The main question to be addressed through this thesis was: *are Brazilian workers exposed to biological risks present in coffee reprocessing units?* The aim was to study the presence of fungi and mycotoxins in these occupational environments.

According to the results obtained in this study, workers are exposed to fungi present in the various activities involved in coffee bean reprocessing. The main genera found were *Aspergillus*, *Cladosporium*, and *Fusarium*.

Additionally, the presence of mycotoxins was observed in different matrices collected from specialty and commodity coffee industries. In both industries, it was possible to quantify the presence of mycophenolic acid and ochratoxin A through high-pressure liquid chromatography with mass spectrometry. Furthermore, in some matrices, it was also possible to identify aflatoxins B2, fumonisin B2, zearalenone, fumonisin B1, and ochratoxin B.

Future studies should be conducted to verify occupational exposure in other links of the coffee production chain.

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# PART II - PAPERS

# PAPER I - OCCUPATIONAL EXPOSURE TO FUNGI AND MYCOTOXINS AT POST-HARVEST COFFEE INDUSTRIES: A LITERATURE REVIEW

# Occupational exposure to fungi and mycotoxins at post-harvest coffee industries: a literature review

**Filipe da Silva de Oliveira[1,*], Ednilton Tavares de Andrade[1], Carla Viegas[2], Giovanni Francisco Rabelo[3], Jéssica Raquel Sales Carvalho de Souza[4] and Susana Viegas[5]**

[1] Department of Agricultural Engineering, School of Engineering, Universidade Federal de Lavras - UFLA (Federal University of Lavras), Lavras, MG, Brazil; e-mail: filipe.oliveira2@estudante.ufla.br
[2] H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa; carla.viegas@estesl.ipl.pt
[3] Department of Control and Automation Engineering, School of Engineering, Universidade Federal de Lavras – UFLA, Lavras, MG, Brazil
[4] Department of Food Science, School of Agricultural Sciences, Universidade Federal de Lavras - UFLA, Lavras, MG, Brazil
[5] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health ResearchCenter, CHRC, NOVA University Lisbon, Lisbon, Portugal;

* Corresponding author: filipe.oliveira2@estudante.ufla.br

**Abstract:** Several studies have reported fungi and mycotoxins in coffee beans, but few address occupational exposure to these agents in industrial coffee processing. This review aimed to analyze such studies. A systematic literature search using PRISMA methodology was conducted for articles published between 1991 and 2023 in PubMed, Scopus, and Web of Science, focusing on occupational exposure to fungi and mycotoxins in the coffee industry. Five studies met the inclusion criteria. Four of the five studies selected were dedicated to green coffee beans, and the other focused on coffee roasting activities. In the papers, different environmental matrices were considered in evaluating occupational exposure, but the most used matrix was airborne dust (4 of the 5 studies). Airborne fungi were sampled using active (4 of the 5 studies) and passive sampling. Only the most recent of the studies [2022] identified microorganismsby their genera and species, and only two groups of mycotoxins were analyzed in the studies considered, namely Ochratoxin A and Aflatoxins. None of the studies reported data on both fungi and mycotoxins. Considering the possible effects related to exposure to fungi and mycotoxins and the number of workers involved in this type of industry in the world, more studies should be developed.

**Keywords:** post-harvest coffee industries**,** occupational exposure, fungi, mycotoxins, dust

## 1. Introduction

Occupational exposure to airborne biological agents has been reported in the literature at different workplaces [1,2]. Bioaerosols are defined as airborne particles, including fungal spores and hyphae, bacteria, endotoxins, β(1→3) glucans, mycotoxins, or high-molecular-weight allergens, and organic dusts in general, composed of or derived from biological matter [3].

Coffee is one of the most important beverages in the world [4]. According to the International Coffee Organization [5], the total production by all exporting countries during the 2019/2020 crop year was approximately 9.9 million tonnes of green coffee beans. Brazilian production for that same year was nearly 3.5 milliontonnes, representing more than a third of world production. Also in that crop year, Brazil exported more than 2.4 million tonnes, which is nearly 70% of its total production [6].

As reported in the General Register of Employed and Unemployed Persons [7], in September 2021, there were 78,106 workers in coffee industries in Brazil, with 60,362 working in general warehouses and 17,744 in grinding and roasting companies. Coffee post-harvest processes include steps ranging from washing and separating the fruit; drying, processing, and reprocessing the beans until they are stored; to roasting the beans. Coffee dry milling and storage industries are responsible for grading and classifying coffee beans, improving the type and sensory characteristics of the green coffee beans (GCB) [8]. Meanwhile, coffee roasting industries are responsible for receiving GCB, roasting, grinding, and packaging. In summary, in a GCB dry milling company, the following processes take place: receiving, cleaning, sorting, selecting, blending, and dispatching. In all these processes, handling GCB ruptures some beans, forming dust [9].

Dry milling is a process that involves receiving, cleaning, hulling, polishing, and sorting the GCB; the hulled beans are sorted by size, density, and color [10]. This process can produce a significant amount of dust to which workers are exposed if adequate risk management measures are not in place; the dust can pose health hazards to workers. The amount of dust produced in a coffee dry milling unit depends on factors such as the type of milling equipment used, the size of the operation, and the condition of the coffee beans being processed. The dust is mainly made up of

small particles of dried coffee fruit and other material such fungi and mycotoxins. These dust particles can range in size from very fine particles to larger particles visible to the naked eye [11]. The smaller particles can be inhaled deep into the lungs and potentially reach the alveoli, while the larger particles are trapped in the upper respiratory tract [12].

Several studies in the literature have reported the presence of fungal genera in green coffee beans, including the genera *Aspergillus* [13-14], *Penicillium* [15], and *Fusarium* [16], among others [17-18]. In addition, other authors have confirmed the presence of different mycotoxins in green coffee beans, such as aflatoxins [19-20], ochratoxins [21-23], and others [24-25]. Therefore, since fungi and mycotoxins are commonly found in the GCB, the presence of these agents in the workplace environment where the GCB are handled is foreseeable. However, there are few reports in the literature on occupational exposure to fungi and mycotoxins in the coffee industry.

Exposure to fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* can pose health risks, including respiratory issues and allergic reactions, as these molds are known to produce mycotoxins that may adversely affect human health [26-28]. Mycotoxins are metabolites produced by specific fungal genera, which may cause a diversity of health effects [29]. These compounds can become airborne when attached to spores, fragments, and dust [30]. Therefore, it is of pivotal importance to study occupational exposure to mycotoxins in settings like the coffee industry, where exposure to organic dust can occur in different tasks such as storage, loading, or handling GCB materials [31].

The objective of this study is to identify the literature available on occupational exposure to fungi and mycotoxins in the coffee processing industry and identify gaps in knowledge and the need for future research.

## 2. Materials and Methods

### 2.1 Search Strategy and Inclusion and Exclusion Criteria

This study reports on the data available on occupational exposure to fungi and mycotoxins in post-harvest coffee industries published between January 1,1991, and

February14, 2024, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [32]. The databases chosen were PubMed, Scopus, and Web of Science, and the keywords used were “occupational exposure” AND “mycotoxins” OR “fungi” AND “coffee”. Searches were carried out in English, and articles that did not meet the inclusion criteria and duplicate articles were excluded from further analysis (Table 1).

**Table 1.** Inclusion and exclusion criteria in the selected articles.

| Inclusion criteria | Exclusion criteria |
|---|---|
| Articles published in the English language | Articles published in other languages |
| Original scientific articles on the topic | Abstracts of congress, reports, reviews/state of the art articles |
| Articles related to occupational exposure in the coffee industry | Articles related to other food commodities |
| Articles related to occupational exposure | Articles dedicated only to coffee bean quality analysis |

*2.2 Selection of Studies and Data Extraction*

Two rounds of article selection were performed by three investigators (FO, JS, and GR). The first round consisted of screening all titles and abstracts. In the second round, the full texts of all potentially relevant studies were reviewed considering the inclusion and exclusion criteria. Potential divergences in the selection of the study were discussed and ultimately resolved by the remaining investigators (SV, CV, and EA). Three investigators (FO, JS, and GR) extracted data, and the other three reviewed that data. The following information was manually extracted: (1) title, (2) country, (3) occupational setting, (4) types of samples considered, (5) sampling methods, (6) analytical methods, (7) results, and (8) main conclusions.

*2.3 Quality Assessment*

The risk of bias was assessed by two investigators (CV and SV). Within each study, this risk was evaluated across four parameters divided as key criteria (type of samples considered, sampling method, microorganisms detected, and mycotoxins

found) and other criteria (incomplete data about the key criteria and conflict of interest). The risk of bias for each criterionwas evaluated as “low”, “medium”, “high”, or “not applicable”. The studies for which all the key criteria and most of the other criteria are characterized as “high” were excluded.

## 3. Results

The flow diagram for selection of studies is shown in Figure 1. The primary search on the databases yielded 32 papers. This resulted in 19 reports assessed for eligibility, and 14 of them were excluded for not meeting the inclusion criteria. Of the 14 papers excluded, 7 were unrelated to occupational exposure; 4 were not related to the coffee industry; 2 were reviews; and 1 was excluded for not being published in English. Therefore, a total of 5 papers were included in this review.

Analysis of the five papers used in this study (Table 2) included examination of the occupational environment studied, the country where the study was developed, the sampling methods used (including the matrixes used), the analytical methods applied, and the exposure data obtained. None of the papers reported on both fungi and mycotoxins, and three papers used biomonitoring approaches to assess workers’ exposure to mycotoxins besides those from settled/airborne dust [33-34,37].

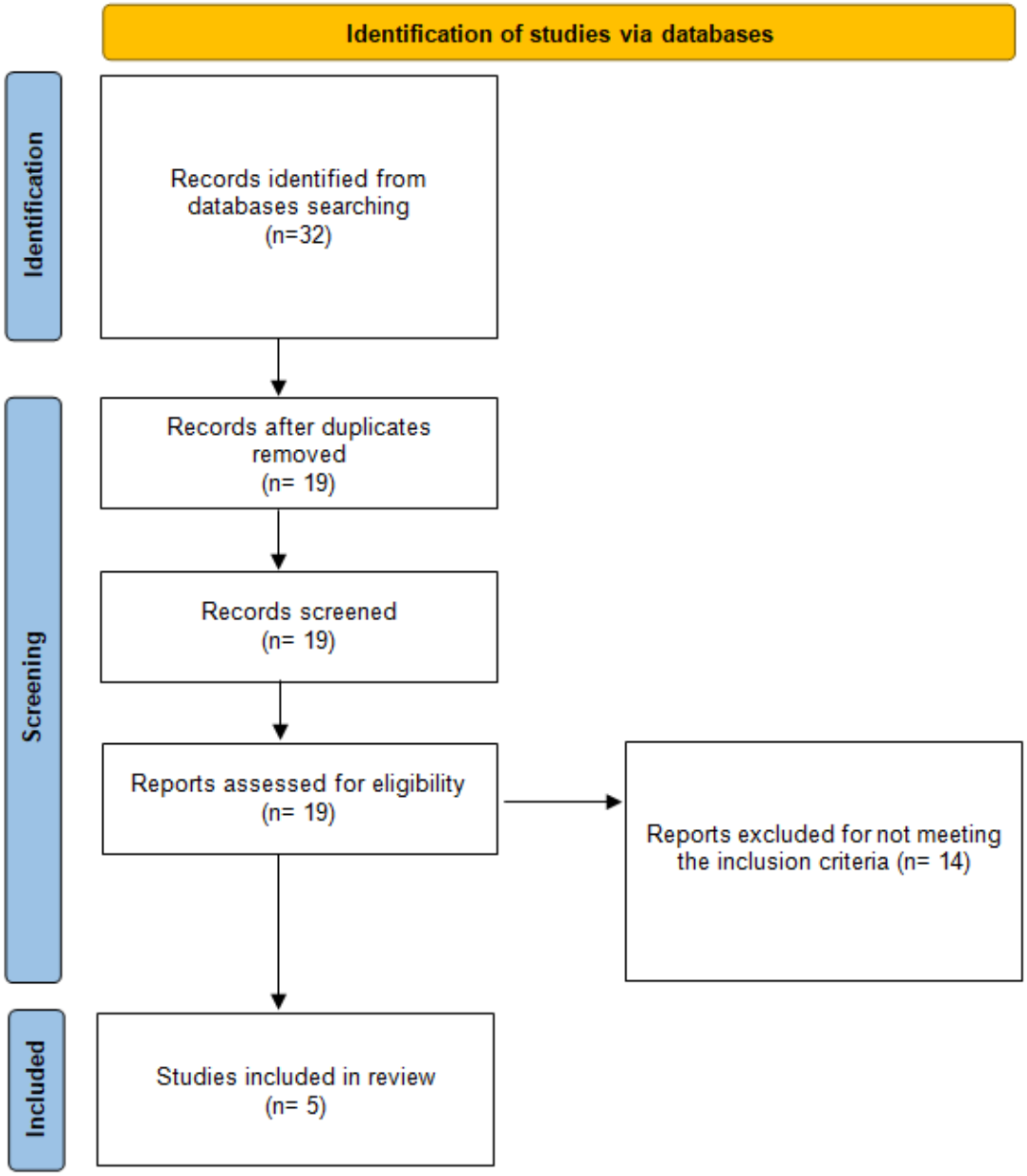


**Figure 1.** PRISMA methodology for selection of papers.

**Table 2.** Data obtained from the selected articles.

| Title | Study | Firm Sample Location | Occupational Setting | Matrices analyzed | Sampling Method | Analytical Method | | Results | | Main Conclusions |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Toxins/microorganisms studied | Laboratory method | Microorganisms identified | Mycotoxins detected | |
| Microbial Contamination in the Coffee Industry: An Occupational Menace besides a Food Safety Concern? | [35] | Brazil | Coffee dry milling firm | Dust; FRPD; GCB | EDC; settled dust | Fungi; bacteria | Plate incubation, qPCR | *Cladosporium*; *Paecilomyces*; *Aspergillus*; *Penicillium*; other fungal genera; Gram negative bacteria | | This study draws attention to the need to consider occupational exposure to mycotoxins in the dry milling stage and other stages, due to high fungal diversity and contamination. |
| Use of high-performance liquid chromatography to assess airborne mycotoxins | [36] | Spain | Coffee processing | Dust | Filtration | Mycotoxins | HPLC | | Green coffee from big bags<br>OTA: <2.0 ng $m^{-3}$<br>AFL: < 0.06 ng $m^{-3}$<br>Coffee big bags on a conveyor belt<br>OTA: <0.4 ng $m^{-3}$<br>AFL: < 0.01 ng $m^{-3}$ | The authors concluded that the concentration of mycotoxins was lower than the detection limit of the method used. However, the authors emphasize that occupational exposure limits have not been set. |
| Exposure assessment to mycotoxins in workplaces: aflatoxins and ochratoxin A occurrence in airborne dusts and human sera | [33] | Italy | Warehouse: handling and processing of coffee beans | Dust; serum | Stationary and personal filtration | Mycotoxins | HPLC | | OTA Personal: 0.007 - 0.066 ng $m^{-3}$<br>Dust: 0.006 - 0.018 ng $m^{-3}$ | This study showed a wide range of OTA levels in the samples. This could be related to the distance between the worker and the stocked raw materials and the manual tasks being developed. |

| Title | Study | Firm Sample Location | Occupational Setting | Matrices analyzed | Sampling Method | Analytical Method | Results | Main Conclusions | Title | Study |
|---|---|---|---|---|---|---|---|---|---|---|

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| External and internal dose in subjects occupationally exposed to ochratoxin A | [34] | Italy | Coffee processing warehouse | Dust; serum | Stationary and personal filtration | Mycotoxin: OTA | HPLC | | Airborne OTA: 0.051 ng $m^{-2}$<br>Serum: 2.41 ng $mL^{-1}$ | OTA represents an occupational hazard, in addition to other mycotoxins potentially present in the workplace. |
| Factors relating to the development of respiratory symptoms in coffee process workers | [37] | England | Coffee processing firm: unloading; tipping; roasting | Dust; serum | Impaction | Total fungi; total bacteria | Culture-based methods | <u>Bacteria</u><br>Container unloading: 4.12 $10^{-3}$ CFU $m^{-3}$<br>Tipping: 8.50 $10^{-3}$ CFU<br>Roasting: 1.90 $10^{-3}$ CFU$m^{-3}$<br><u>Fungi</u><br>Container unloading: 8.14 $10^{-3}$ CFU $m^{-3}$<br>Tipping: 3.40 $10^{-3}$ CFU $m^{-3}$<br>Roasting: 5.65 $10^{-3}$ CFU $m^{-3}$ | | The collected data shows that different work areas resulted in different mean concentrations of airborne dust and microorganisms. The study also suggests that respiratory symptoms are work related. |

*AFL: aflatoxins; CFU: colony forming unit; EDC: Electrostatic Dust Cloth; FRPD: Filtering Respiratory Protective Devices; GCB: Green Coffee Beans; HPLC: High Pressure Liquid Chromatography; NS: not specified; OTA: ochratoxin A; qPCR: quantitative polymerase chain reaction.*

Considering the locations of the firms of the five papers selected to this review, two are in Italy [33-34], one in Brazil [35], one in Spain [36], and one in the United Kingdom [37]. Regarding the sources of contamination in the occupational settings, four of the five papers studied GCB dust [33-36], and one of the five papers studied both GCB and roasted coffee bean dust within the same company [37]. Concerning the location of the industries, four [33, 34,36, 37] of the five locations are in Europe; only one study [35] assessed industries in a producer country. Concerning the origin of the samples used in the studies, four papers [33-36] specified the origin of the GCB being Brazil and one paper indicated Africa, South East Asia, and Central and South America [37].

While most studies [33, 34,36, 37] collected airborne dust and biological samples (workers' sera), one [35] collected only environmental samples (EDC, settled dust, GCB, and FRPD). As for the analytical methods employed, three of the five papers studied the presence of mycotoxins in dust [33, 34,36] and reported the use of HPLC for the sample analysis. Among these three papers, one analyzed different mycotoxins, namely aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, and ochratoxin A [33], while the other two papers studied only one mycotoxin – ochratoxin A [33, 34]. One study assessed only microorganisms present in dust but did not distinguish them by species or genera [37]. Another [35] reported the presence of several fungal genera present at the coffee dry milling industries from Brazil.

## 4. Discussion

European countries are mainly engaged in processing/handling coffee beans from various regions, such as Brazil, as they are not significant coffee producers. The only tasks studied in the papers selected are the storing and the roasting of the green beans [33-37]. Therefore, research is lacking not only on the exposure that occurs during those tasks, but also on occupational exposure to fungi and mycotoxins in countries that produce coffee beans, especially in industries with a considerable amount of coffee bean conveyance, e.g., in dry milling industries. Although literature is limited regarding occupational exposure to fungi and

mycotoxins in coffee industries, there is evidence of the presence of fungal and mycotoxin contamination in GCB from all over the world, such as from Africa [13,16,25], America [15,21], and Asia [14,20,23].

Airborne fungal particles found in occupational environments consist of spores, mycelium fragments, and debris present as single particles or complex aggregates [38]. In addition, their incidence depends on the natural selection that occurs in these very complex microbiological communities; several parameters are involved. From a worker exposure and health effects perspective, it is important to characterize microbial contamination in occupational environments. An example in a published study considers bakery workers exposed to a vast number of microbial species present in the air [39]. In that case, some of the flour provides essential nutrients favorable to the growth of fungi and bacteria. Coffee industries should likewise be assessed in order to better understand exposure, identify the most suitable risk management measures to prevent health effects, and understand how much of airborne exposure to mycotoxins is due to the contamination present in the handled material, in this case, GCB [40-42].

Many processes conducted in the coffee supply chain directly affect coffee microbiota and mycotoxins, such as ripening, harvesting, drying, roasting, and brewing methods. According to one of the analyzed papers [32], the ochratoxin concentrations measured were lower than the detection limits of the methods commonly used. However, it is noteworthy that the raw material used by the authors in that study came from Brazil, where the coffee beans were previously milled before export. Thus, the concentrations might represent occupational exposure at Brazilian companies where the GCB are handled and processed after harvesting (Figure 2). In fact, the mycotoxin levels tend to decrease at the end of the coffee supply chain. That is illustrated in a paper studying the effect of different roasting and brewing methods on mycotoxins. The authors reported that both roasting and brewing might reduce the presence of ochratoxins and aflatoxin A, depending on the roasting level and the brewing method used [40].

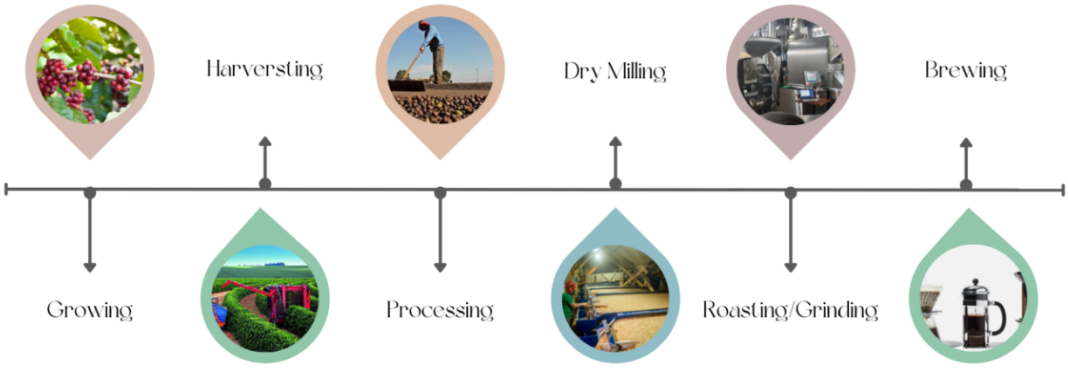


**Figure 2.** Schematic representation of the coffee supply chain.

Several approaches are available to assess occupational exposure to mycotoxins in workplace settings, such as biomonitoring [41-45] and environmental monitoring [46-47]. Different methods were applied to collect data on mycotoxin exposure in the studies selected [33-37], and the analytical methods used by the authors for measuring occupational exposure through environmental and biological monitoring gave precise and accurate results.

Electrostatic dust cloths (EDC) have recently been used in dust sample collections to collect airborne materials in different occupational environments [47-48], and they have been used to detect fungal genera in specific indoor environments [49]. Some authors [50] have compared EDC with the air impaction procedureto measure airborne fungi, and they concluded that EDC provide a more precise estimation of fungal exposure compared to a single air impaction procedure. This is because EDC are not onlymuch less tedious and time-consuming to use thanother airborne dust collection procedures [51], but also make for a longer collection time and, because of that, they better represent exposure levels. Settled dust has also been used as a matrix, and this procedure involves collecting dust that has settled at different places in the workplace environment [52-53]; it has been widely used in various occupational environments [39,54-56]. In this case as well, the exposure window considered is wider compared to single air impaction.

The type of task developed also affects the levels of workers' exposure to fungi and mycotoxins. The papers analyzed (Table 2) indicate that studying each workplace and task separately is of critical importance. The authors concluded that the warehouse was the dustiest among the areas evaluated in the factory. They came to the conclusion that there was a higher concentration of xerotolerant fungi at the unloading area than any other area in the study [37]. A paper published by Viegas et al (2018) came to the same conclusion: the dustiness of the tasks is an important determinant of fungal and mycotoxin exposure. This is due to the high intensity of coffee bean handling during the process, e.g., in mechanical sieving [31]. Differences in exposure to fungal and bacterial species have also recently been quantified in different tasks developed in a cucumber greenhouse [57]. Therefore, to better understand variations in occupational exposure to airborne fungi and mycotoxins in a coffee warehouse, tasks that include manual handling of coffee beans should be carefully evaluated, tasks such as grain reception, stone picking, bean pre-cleaning, use of densiometric tables, filling containers, and dispatch. Such evaluation will allow identification of which tasks bring about higher exposure to the different agents. This, in turn, will provide better understanding of the risk management measures to apply to mitigate exposure to the different agents present.

Coexposure to multiple mycotoxins is a common feature in workplace environments [31,48,58] Ochratoxin A has been indicated asan occupational hazard, along with other mycotoxins potentially present in occupational settings of loading, storing, and unloading the jute bags containing the GCB [34]. Even though the available data in the literature only assess one or two groups of mycotoxins (mainly aflatoxins and OTA) in coffee industries, coexposure to other mycotoxins is very likely to occur in these industries since multiple mycotoxin contamination of GCB has been reported [22,25]. Therefore, it would be more fitting to choose analytical methods that are able to measure multiple mycotoxins in samples collected from these occupational environments [58].

## 5. Conclusions

In general, our findings indicate a lack of published studies analyzing occupational exposure to fungi and mycotoxins in the coffee processing industry. The published research suggests the potential for high exposure to organic dust and its constituents in the coffee industry. Therefore, more studies should be conducted in coffee industries to better understand the hazards present in these workplaces and the related health risks. The importance of considering coexposure to several mycotoxins and fungi species and the impact this might represent for workers' health was also highlighted. Considering that data from companies outside the European continent is limited, studies should be conducted at coffee industries in coffee producing and processing countries, such as Brazil, which is the main producer and exporter in the world.

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# PAPER II - MICROBIAL CONTAMINATION IN THE COFFEE INDUSTRY: AN OCCUPATIONAL MENACE BESIDES A FOOD SAFETY CONCERN?

# MICROBIAL CONTAMINATION IN THE COFFEE INDUSTRY: AN OCCUPATIONAL MENACE BESIDES A FOOD SAFETY CONCERN?

Carla Viegas 1,2,3,* , Bianca Gomes 1 , Filipe Oliveira 4 , Marta Dias 1,3 , Renata Cervantes 1 , Pedro Pena 1 , Anita Quintal Gomes 1,5, Liliana Aranha Caetano 1,6 , Elisabete Carolino 1 , Ednilton Tavares de Andrade 4 and Susana Viegas 1,2,3

1 H & TRC—Health & Technology Research Center, ESTeSL—Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisbon, Portugal
2 Public Health Research Centre, NOVA National School of Public Health, Universidade NOVA de Lisboa, 1099-085 Lisbon, Portugal
3 Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, 1169-056 Lisbon, Portugal
4 Department of Agricultural Engineering, Faculty of Engineering, Federal University of Lavras, Lavras 37203-202, Brazil
5 Faculty of Medicine, Institute of Molecular Medicine, University of Lisbon, 1649-004 Lisbon, Portugal
6 Research Institute for Medicines (iMed.uLisboa), Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal

* Correspondence: carla.viegas@estesl.ipl.pt

## ABSTRACT

Respiratory abnormalities among workers at coffee roasting and packaging facilities have already been reported; however, little is known about microbiological contamination inside coffee production facilities. This study intends to assess the microbial contamination (fungi and bacteria) in two coffee industries from Brazil with a multi-approach protocol for sampling and for subsequent analyses using four main sources of samples: filtering respiratory protection devices (FRPD) used by workers, settled dust, electrostatic dust cloths (EDC), and coffee beans. The fungal contamination in the assessed industries was also characterized through the molecular detection of toxigenic species and antifungal resistance. Total bacteria contamination presented the highest values in FRPD collected from both industries (7.45 × 104 CFU·m$^{-2}$; 1.09 × 104 CFU·m$^{-2}$). *Aspergillus* genera was widespread in all the environmental samples collected and sections with clinical relevance (*Fumigati*) and with toxigenic potential (*Nigri* and *Circumdati*) were recovered from FRPD. *Circumdati* section was observed in 4 mg mL$^{-1}$ itraconazole. Sections *Circumdati* (EDC, coffee beans and settled dust) and *Nidulantes* (EDC, coffee beans and FRPD) were detected by qPCR. Some of the targeted *Aspergillus* sections that have been identified microscopically were not detected by qPCR and vice-versa. Overall, this study revealed that microbial contamination is a potential occupational risk in the milling stage and should be tackled when assessing exposure and performing risk assessment. In addition, a multi-sampling campaign should be the

approach to follow when assessing microbial contamination and FRPD should be included in this campaign. Occupational exposure to mycotoxins should be considered due to high fungal diversity and contamination. A One Health approach should address these issues in order to prevent consumption of coffee crops and beans infected by fungi and, more specifically, to avoid widespread azole resistance.

**Keywords:** milling stage; multi-approach for sampling and analyses; *Aspergillus*; azole resistance; One Health approach

## 1. Introduction

Coffee consumption has been increasing each year and coffee exports have amounted to 10.92 million bags in April 2021, compared with 11.24 million in April 2022. In fact, the export of coffee in the first 7 months of 2021/22 (21 October to 22 April) has increased by 0.6% [1]. However, we should bear in mind that climate change is also critically affecting the agricultural sector as plant growth is compromised but also toxigenic fungal growth, a major cause of plant death [2]. Thus, as with other crops, coffee that is one of the most traded commodities in the world is threatened by changing climate conditions and, consequently, by fungal infections [2].

Respiratory abnormalities among workers at coffee roasting and packaging facilities have already been reported [3–7] and exposure to dust, endotoxins, carbon monoxide, diacetyl, 2,3-pentanedione and other volatile organic compounds were previously assessed in coffee roasting facilities and coffees [8]. However, little is known about microbiological contamination inside coffee production facilities. In addition, since azole fungicides are largely applied in agriculture and material protection, fungi can come into contact with azoles everywhere. Thus, “Hot spots”—a habitat in which fungal species are disseminated and exposed to a fungicidally effective azole at concentrations that are high enough to select for resistant individuals potentially multiplying and spreading to other habitats—have already been identified by assessing the resistance risk in several occupational environments prioritizing those that handle food commodities. The coffee industry is one of these environments [9,10].

Mycotoxin occupational exposure should also be a concern since coffee beans are frequently contaminated with these fungal secondary metabolites. This happens due the crop infection by toxigenic fungi that commonly infect the plant during the various production stages (cultivation, processing or transport) [2,11]. As coffee requires wet conditions, the rainfall and humidity in areas for cultivation create the ideal conditions for *Aspergillus* species to grow, as these have optimal growth in warmer and humid climates [2,12]. Therefore, in an increasingly warmer world, mycotoxin production will increase, as higher temperatures and wetter climates provide perfect conditions for fungal growth and, consequently, mycotoxin production [2,12].

To our knowledge, data regarding occupational exposure to microbial contamination, obtained by a multi-approach strategy based on the use of different sampling methods and assays in coffee industries, have not been previously reported, and this omission has prevented risk management and control measures. Thus, this study intends to assess the microbial contamination (fungi and bacteria) in two coffee industries from Brazil with a multi-approach protocol for sampling and subsequent analyses using four main sources of samples: filtering respiratory protection devices used by workers, settled dust, electrostatic dust cloths and coffee beans. The fungal contamination in the assessed industries was also characterized through the molecular detection of toxigenic species and antifungal resistance.

## 2. Materials and Methods

### 2.1. Coffee Industries Characterization

The coffee industry involves different processes from the growth of the crops to the last step of being prepared for drinking. Typically, the main steps are growing, picking, processing, milling, roasting, packaging, shipping, grinding, brewing and drinking (Figure 1). Usually in Brazil, the country where samples have been collected, milling companies are the last stage before exportation. In Figure 1 we can see the common production flow, including all the steps that allow obtaining the final product on the coffee supply chain.

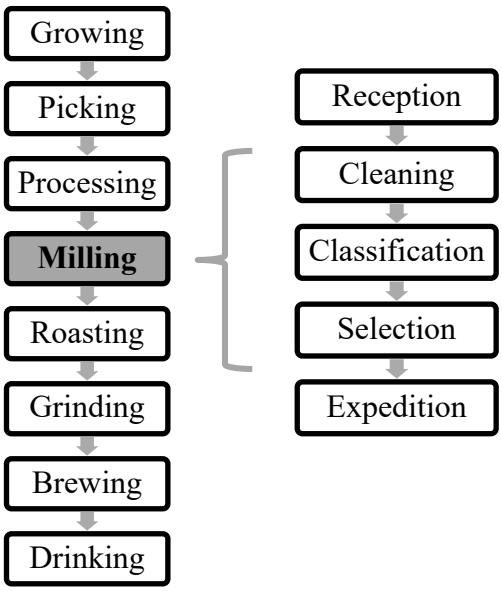


**Figure 1.** Coffee production flow.

The samples were collected at two milling industries from two different mesoregions Campo das Vertentes e Sul and Sudoeste de Minas, which are within the largest producers of the country. According to the Brazilian Geographic and Statistics Institute (IBGE) [13], both mesoregions together produced 985,577 tons of coffee in 2017, which represents 27% of the national production.

Two industries—A and B—were sampled. Industry A has 11 workers and Industry B has 35 workers per shift (44 h per week), all working in warehouses from each industry. Regarding personal protection equipment used by workers, it was reported that workers have available respiratory protection devices with FFP1 filters, with or without exhalation valves.

### 2.2. Sampling Campaign Performed

The samples have been collected during August 2021, which is coffee harvest time in Brazil. The workplaces assessed and the sampling methods used are described in Table 1. Electrostatic dust collectors (EDC), settled dust, coffee bean samples and filtering respiratory protection devices (FRPD) were used as passive sampling methods.

Table 1 presents descriptions of the processing of *C. arabica* grains, their subcategories, varieties and types of processing for the companies in each region described above. Industry A works with specific grains from each producer, and thus,

when they arrive at the processing unit, the grains are processed separately in batches. Industry B works in a single production batch with grains from different producers. This is the reason why it was not possible to distinguish, for the samples from industry B, which varieties were present no the processing used in the post-harvest.

The workplaces assessed were reception, milling, storage and expedition. In reception and expedition, the main activities carried out are weighing the grains, unloading the grains into the hoppers, sampling the product for classification and water content, loading the grains into the bags and moving the product. In milling, the main activities that take place in the sector are the movement of the product, removal of sticks, stones, leaves and other impurities, and separation of grains by size, density and color. In storage, the main activities that occur are the movement of the already bagged product and its storage.

Table 1. Workplaces assessed and sampling methods applied.

| Industry | Production | Number of workers per shift | Workplaces assessed | Sampling methods (n) | | | | Observations (Photos from the workplaces) |
|---|---|---|---|---|---|---|---|---|
| | | | | EDC | Settled dust | Coffee beans | FRPD | |
| A | | 11 | Reception/ Expedition | 8 | 2 | 1 | - |  |
| | | | Milling | 22 | 8 | 2 | 4 |  |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage | 10 | 10 | 7 | 4 |  |
| | | Total | 40 | 10 | 10 | 8 | |
| B | 35 | Reception | 2 | 1 | - | n.s |  |
| | | Storage | 2 | 2 | - | n.s |  |
| | | Expedition | 6 | 2 | - | n.s |  |
| | | Milling | 20 | 5 | 8 | n.s |  |
| | | Total | 30 | 10 | 8 | 12 | |

n.s.: not specified; FRPD: Filtering respiratory protection devices

EDC were places in the sampling areas 1.5 ± 0.5 m above the ground for 15 days. Settled dust samples were collected with a sterilized spoon to gather the accumulated dust in each workplace [14]. Green coffee beans (GCB) were places according to their subcategory using the classification from the Brazilian Official Classification for Coffee (COB) [15] (Table 2). Filtering respiratory protection devices

(FRPD) used by coffee industry workers were also collected from workers belonging to industry A [16]. All samples were kept refrigerated (0-4 °C) in sterilized bags preceding analysis.

**Table 2. Green coffee beans sample description.**

| Industry | Samples | Category | Subcategory | Variety | Processing Type |
|---|---|---|---|---|---|
| A | 1 | *Cofeea arabica* | bica corrida | n.s. | n.s. |
| | 2 | *Cofeea arabica* | n.s. | n.s. | n.s. |
| | 3 | *Cofeea arabica* | n.s. | n.s. | n.s. |
| | 4 | *Cofeea arabica* | bica corrida | Catuaí/ Catucaí | wet process |
| | 5 | *Cofeea arabica* | bica corrida | Catuaí/ Catucaí | wet process |
| | 6 | *Cofeea arabica* | mocha | Bourbon/ Catucaí | wet process |
| | 7 | *Cofeea arabica* | bica corrida | Catuaí/ Catucaí | dry process |
| | 8 | *Cofeea arabica* | large flat | Bourbon | wet process |
| | 9 | *Cofeea arabica* | large flat | Bourbon/ Catucaí | dry process |
| | 10 | *Cofeea arabica* | medium flat | Catuaí | wet process |
| B | 1 | *Cofeea arabica* | large flat | mixed | n.s. |
| | 2 | *Cofeea arabica* | large flat | mixed | n.s. |
| | 3 | *Cofeea arabica* | mocha | mixed | n.s. |
| | 4 | *Cofeea arabica* | medium flat | mixed | n.s. |
| | 5 | *Cofeea arabica* | large flat | mixed | n.s. |
| | 6 | *Cofeea arabica* | bica corrida | mixed | n.s. |
| | 7 | *Cofeea arabica* | bica corrida | mixed | n.s. |
| | 8 | *Cofeea arabica* | bica corrida | mixed | n.s. |

n.s.: not specified

### 2.3. Sample Extraction and Characterization of Viable Microbiota

Passive samples were washed with 0.1% Tween 80 saline (0.9% NaCl) solution (250 rpm, 30 min), as follows: 20 mL solution for EDC; 9.1 mL solution for 1 g of settled dust sample and coffee beans [17] and 10 mL for FRPD filters [16]. Extracts were maintained frozen (−80 °C) with glycerol (2.23 mL for each g of settled dust and coffee beans, and 1.25 for FRPD) prior analysis [14,16].

Sample extracts were inoculated (150 µL) in malt extract agar (MEA) supplemented with chloramphenicol (0.05%), dichloran–glycerol agar (DG18), tryptic soy agar (TSA) supplemented with nystatin (0.2%), and Violet Red bile agar (VRBA) were used for fungi (MEA and DG18, 27 °C, 5–7 days), mesophilic (TSA, 30 °C, 7

days) and Gram-negative (VRBA, 35 °C, 7 days) bacteria selectivity. Microbial contamination quantification was determined as colony-forming units (CFU) and CFU concentration ($CFU \cdot m^{-2} \cdot day^{-1}/g^{-1}/m^{-2}$) after plate incubation. Morphological identification of fungal species was carried out through notation of macro and microscopic characteristics [18] by a trained mycologist.

### 2.4. Antifungal Susceptibility Testing

The screening of azole-resistant fungi was firstly carried out by seeding 150 µL of the extracts of all passive samples (N = 128) on Sabouraud dextrose agar (SDA) (Frilabo, Maia, Portugal) supplemented with 4 mg/L itraconazole (ITZ), 2 mg/L voriconazole (VCZ), or 0.5 mg/L posaconazole (PSZ), adapted from EUCAST guidelines [19,20]. The controls used were *A. fumigatus* reference strain (ATCC 204305) as the negative control, and pan-azole resistant *A. fumigatus* strain as the positive control, both provided by the National Health Institute Doctor Ricardo Jorge, IP. After incubation for 2–3 days at 27°C, identification was performed as previously described for fungal assessment [14].

### 2.5. Molecular Detection of the Targeted Fungal Sections

*Aspergillus* sections were amplified by quantitative PCR (qPCR) in the 8.8 mL samples' extracts used in this study [17]. First, we isolated fungal DNA from the samples using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, CA, USA). Then we performed qPCR amplification using the CFX-Connect PCR System (Bio-Rad, Amadora, Portugal). Reactions were performed in a 20 µL final volume containing 1 × iQSupermix (Bio-Rad, Amadora, Portugal), 0.5 µM of each primer, and 0.375 µM of TaqMan probe. qPCR conditions included a three-step reaction consisting of 40 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s.

H2O was used as a negative control and DNA isolated from a reference strain was used as a positive control. The reference strains were kindly provided by the Reference Unit for Parasitic and Fungal Infections from the Department of Infectious Diseases, National Health Institute Doctor Ricardo Jorge, Lisbon, Portugal. All reference strains were sequenced for ITS, B-tubulin and Calmodulin.

### 2.6. Statistical Analysis

Data were analyzed using SPSS statistical software for windows, version 27.0. The results were considered significant at the 5% significance level. To test the normality of the data, the Shapiro–Wilk test was used. For the comparison of bacterial contamination, fungal contamination and fungal resistance, the Kruskal–Wallis test was used, since the assumption of normality was not verified and given the small size of the sample. To study the relationship between bacterial contamination, fungal contamination and fungal resistance, Spearman's correlation coefficient was used, since the assumption of normality was not verified. To assess species diversity, Simpson and Shannon indices, given by Shannon Index (H) = $\sum_{i=1}^{s} p_i \ln(p_i)$and Simpson Index (D) =$\frac{1}{\sum_{i=1}^{s} p_i^2}$, were used, where pi is the proportion ($n_i/n$) of individuals of one particular species found (ni) divided by the total number of individuals found (n).

## 3. Results

### 3.1. Viable Bacterial Contamination

In what concerns industry A, total bacteria contamination (measured by TSA) presented the highest values in FRPD (7.45 × $10^4$ CFU·$m^{-2}$), followed by grains (2.20 × $10^4$ CFU·$g^{-1}$), EDC (2.20 × $10^4$ CFU·$m^{-2}$·$day^{-1}$) and settled dust (1.17 × $10^3$ CFU·$g^{-1}$). In VRBA media, EDC evidenced the highest Gram-negative counts among the matrices (4.5 × $10^5$ CFU·$m^{-2}$·$day^{-1}$). Grains and FRPD had similar values of Gram-negative bacteria (2.52 × $10^3$ CFU·$g^{-1}$ and 2.00 × $10^3$ CFU·$m^{-2}$, respectively), while a lower value was obtained from settled dust samples (1.17 × $10^3$ CFU·$g^{-1}$).

Among all the analyzed matrices from industry B, the highest counts for total bacteria were found on FRPD (1.09 × $10^4$ CFU·$m^{-2}$), while the second highest values were obtained from EDC (1.51 × $10^4$ CFU·$m^{-2}$·$day^{-1}$). The lowest values of total bacteria were obtained from grains (6.69 × $10^2$ CFU·$g^{-1}$) and settled dust (4.40 × $10^2$ CFU·$g^{-1}$) (Figure 2). Regarding VRBA media, the highest counts of gram-negative bacteria were found in EDC (1.69 × $10^4$ CFU·$m^{-2}$·$day^{-1}$), followed by FRPD (5.5 × $10^5$ CFU·$m^{-2}$). Similar counts were obtained for settled dust and grains (4.40 × $10^2$CFU·$g^{-1}$ and 4.16 × $10^2$ CFU·$g^{-1}$, respectively).

### 3.2. Viable Fungal Contamination

FRPD from industry A have evidenced the highest fungal counts (MEA: 3.50 × $10^3$ CFU·$m^{-2}$; DG18:1.50 × $10^3$ CFU·$m^{-2}$) among all the collected samples, which was followed by EDC (MEA: 2.29 × $10^3$ CFU·$m^{-2}$·$day^{-1}$ DG18: 3.15 × $10^3$ CFU·$m^{-2}$ ·$day^{-1}$). The lowest counts were obtained on grains (MEA: 7.35 × $10^1$ CFU·$g^{-1}$; DG18: 1.41 × $10^2$ CFU·$g^{-1}$) and settled dust samples (MEA: 7 CFU·$g^{-1}$; DG18: 5.80 × $10^1$ CFU·$g^{-1}$).

Regarding samples from industry B, the highest fungal contamination numbers were observed on EDC samples on both MEA and DG18 (6.73 × $10^3$ CFU·$m^{-2}$·$day^{-1}$; DG18: 1.06 × $10^4$ CFU·$m^{-2}$·$day^{-1}$, respectively), followed by grains (MEA: 2.01 × $10^1$ CFU·$g^{-1}$; DG18: 5.34 × $10^2$ CFU·$g^{-1}$) and settled dust (MEA: 7 CFU·$g^{-1}$; DG18: 5.80 × $10^1$ CFU·$g^{-1}$). In contrast, no fungal counts were obtained in FRPD samples on MEA, only being identified on DG18 (DG18: 5.80 × $10^1$ CFU·$m^{-2}$) (Figure 2).

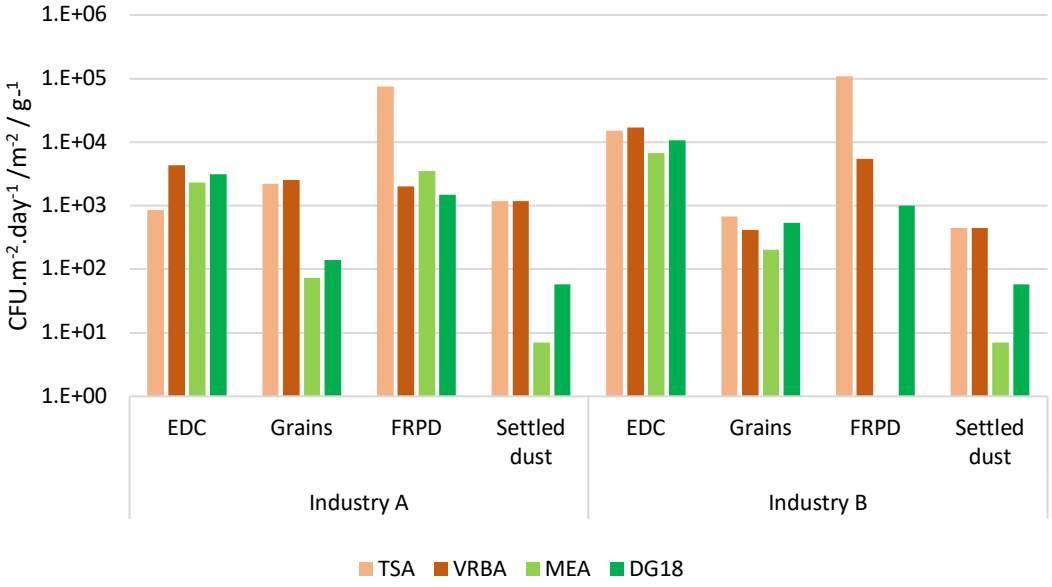


**Figure 2.** Bacterial (TSA; VRBA) and fungal (MEA; DG18) distribution among the sampled matrices (EDC: log [CFU.$m^{-2}$.$day^{-1}$]; Grains, settled dust: log [CFU.$g^{-1}$]; FRPD: log [CFU.$m^{-2}$]).

Concerning fungal distribution, the highest fungal diversity was obtained from EDC samples on both coffee companies. Grain samples had the same diversity on samples isolated from both companies (MEA: 8 species; DG18: 5 species). In settled dust from industry B, five species were identified on MEA and seven species on

DG18, while on industry A, three species were found on MEA and four species on DG18 in samples from the same matrix. Lower fungal diversity was associated with FRPD samples from industry A (MEA: five species; DG18: two species), whereas on industry B, two species were found on DG18.

Regarding species diversity on DG18, industry B was the one with higher diversity (Shannon index (H) = 1.23, Simpson index (D) = 2.83) (Table S1—Supplementary Material).

In what concerns industry A, the most common fungal genera observed in EDC samples was *Cladosporium* sp. on MEA (57.10%), and *Penicillium* sp. on DG18 (62.99%). The genera *Cladosporium* sp. was also frequent in grains on MEA (32.65), while on DG18, Mucor sp. was prevalent (92.20%). Regarding filters, *Paecilomyces* sp. and *Aspergillus* sp. were recurrent on MEA (42.86%), while on DG18, *Aspergillus* was the only genera identified (100%). In settled dust samples *Rhizopus* sp. was the dominant genera (71.43%), while on DG18, *Penicillium* sp. was prevalent (62.07%) (Table 3).

**Table 3.** Fungal distribution per sampling method EDC: log [CFU·m$^{-2}$ day$^{-1}$]; Grains, settled dust: log [CFU·mg$^{-1}$]; Filters: log [CFU·mm$^{-2}$]).

| | MEA | | | DG18 | | |
|---|---|---|---|---|---|---|
| **INDUSTRY A** | | | | | | |
| **Sample** | Fungi | CFU. m$^{-3}$ / m$^{-2}$ / g-$^{1*}$ /CFU.m$^{-2}$.day$^{-1}$ | % | Fungi | CFU. m$^{-3}$ / m$^{-2}$ / g-$^{1}$/*CFU.m$^{-2}$.day$^{-1}$ | % |
| EDC | *Cladosporium* sp. | 1309.27 | 57.10 | *Penicillium* sp. | 1981.60 | 62.99 |
| | *Mucor* sp. | 286.62 | 12.50 | *Cladosporium* sp. | 346.78 | 11.02 |
| | *Chrysosporium*sp. | 208.78 | 9.10 | *Aspergillus* sp. | 608.63 | 19.35 |
| | *Aspergillus* sp. | 120.31 | 5.25 | Other species | 208.78 | 6.64 |
| | Other species | 368.01 | 16.05 | | | |
| Grains | *Cladosporium* sp. | 24.00 | 32.65 | *Mucor* sp. | 130.00 | 92.20 |
| | *Penicillium* sp. | 21.00 | 28.57 | *Penicillium* sp. | 5.00 | 3.55 |
| | *Chrysosporium*sp. | 20.00 | 27.21 | *Aspergillus* sp. | 5.00 | 3.55 |
| | *Aspergillus* sp. | 8.00 | 10.88 | Other species | 1.00 | 0.71 |
| | Other species | 3.50 | 4.76 | | | |
| FRPD | *Paecilomyces*sp. | 1500.00 | 42.86 | *Aspergillus* sp. | 1500.00 | 100.00 |
| | *Aspergillus* sp. | 1500.00 | 42.86 | | | |
| | Other species | 500.00 | 14.29 | | | |
| Settled dust | *Rhizopus* sp. | 5.00 | 71.43 | *Penicillium* sp. | 36.00 | 62.07 |
| | *Aspergillus* sp. | 1.00 | 14.29 | *Mucor* sp. | 12.00 | 20.69 |
| | *Penicillium* sp. | 1.00 | 14.29 | *Aspergillus* sp. | 10.00 | 17.24 |
| **INDUSTRY B** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| EDC | *Penicillium* sp. | 2176.22 | 32.35 | *Penicillium* sp. | 5046.00 | 47.76 |
| | *Cladosporium* sp. | 2041.76 | 30.35 | *Aspergillus* sp. | 3825.19 | 36.20 |
| | *Aureobasidium* | 2075.73 | 15.99 | *Cladosporium* sp. | 1641.90 | 15.54 |
| | *Aspergillus* sp. | 1376.50 | 20.46 | Other species | 53.08 | 0.50 |
| | Other species | 56.62 | 0.84 | | | |
| Grains | *Cladosporium* sp. | 109.00 | 54.36 | | | |
| | *Penicillium* sp. | 66.00 | 32.92 | *Penicillium* sp. | 355.00 | 66.48 |
| | *Rhizopus* sp. | 13.50 | 6.83 | *Aspergillus* sp. | 140.00 | 26.22 |
| | *Aspergillus* sp. | 10.00 | 4.99 | *Cladosporium* sp. | 39.00 | 7.30 |
| | Other species | 2.00 | 1.00 | | | |
| FRPD | - | - | - | *Aspergillus* sp. | 1000.00 | 100.00 |
| Settled dust | *Rhizopus* sp. | 5.00 | 71.43 | *Penicillium* sp. | 36.00 | 62.07 |
| | *Aspergillus* sp. | 1.00 | 14.29 | *Mucor* sp. | 12.00 | 20.69 |
| | *Penicillium* sp. | 1.00 | 14.29 | *Aspergillus* sp. | 10.00 | 17.24 |

Among samples from industry B, *Penicillium* sp. was the most frequent fungal genera observed on EDC (32.35% MEA; 7.76% DG18). The same genus was prevalent on DG18 (66.48%) in grain samples, while on MEA, *Cladosporium* sp. was the most frequent (54.36%). *Aspergillus* sp. was the only genera found in FRPD on DG18 (100%), while *Rhizopus* sp. and *Penicillium* sp. were the most common genera identified in settled dust samples on MEA and DG18, respectively (Table 3).

Regarding *Aspergillus* sp., the highest value of this genera was obtained in samples incubated in DG18 from both industries (A: 43.83%; B: 40.92%) when compared to samples incubated with MEA (A: 27.73%; B: 20.01%). In samples from industry A, the most contaminated matrix with *Aspergillus* sp. genera were FRPD (42.86% MEA; 100.00% DG18), followed by settled dust (14.29% MEA; 17.24% DG18), grains (10.88% MEA; 3.55% DG18) and EDC (5.24% MEA; 19.35% DG18). Despite not having detected *Aspergillus* sp. on MEA, FRPD samples from industry B had the highest values of this fungal genera in DG18 (100%), followed by EDC (20.46% MEA; 36.20% DG18), settled dust (14.29% MEA; 17.24% DG18) and grains (4.99% MEA; 26.22% DG18) (Figure 3).

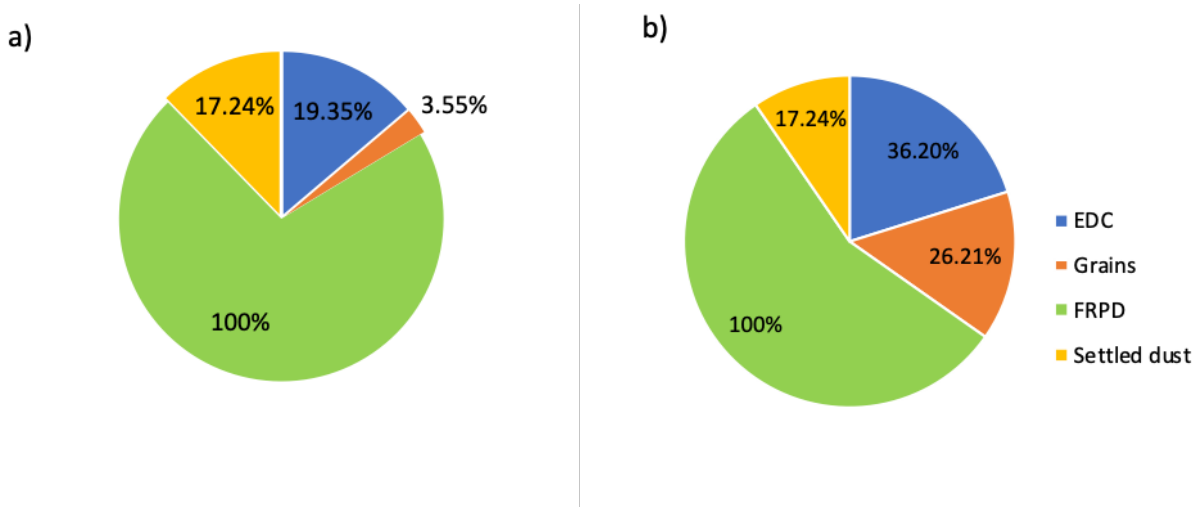


**Figure 3.***Aspergillus* sp. distribution in DG18 culture medium in samples from: (a) industry A and (b) industry B. (EDC: log [CFU·m$^{-2}$·day$^{-1}$]; Grains, settled dust: log [CFU·g$^{-1}$]; FRPD: log [CFU·m$^{-2}$]).

Concerning section distribution on EDC from industry A, four *Aspergillus* sections were detected on MEA (3.70% *Nigri*; 0.93% *Circumdati*; 0.31% *Flavi*; 0,31% *Fumigati*) and six sections on DG18 (9.67% *Nigri*; 6.64% *Circumdati*; 1.57% *Fumigati*; 0.56% *Flavi*; 0.56% *Nidulantes*; 0.34% *Aspergilli*), while on FRPD, three sections were identified using MEA (14.29% *Circumdati*; *Fumigati*; *Nidulantes*), and two using DG18 (66.67% *Nigri*; 33.33% *Circumdati*). Two sections were reported in the grain samples on MEA (5.44% *Restricti*; *Circumdati*) and on DG18 (2.13% *Circumdati*; 1.42% *Candidi*). *Aspergillus* section *Nidulantes*was dominant in the settled dust as detected on MEA (14.29%), while on DG18, two section were identified (13.79% *Nigri*; 3.45% *Circumdati*).

Similar results were obtained in EDC samples from industry B, were four *Aspergillus* sections were reported on MEA (7.00% *Nigri*; 6.58% *Fumigati*; 6.58% *Circumdati*; 0.32% *Flavi*) and six sections on DG18 (31.58% *Circumdati*; 3.01% *Nigri*; 0.74% *Nidulantes*; 0.33% *Terrei*; 0.30% *Aspergilli*; 0.23% *Flavi*). On the grains, four sections were detected on MEA (2.49% *Circumdati*; 1.50% *Nidulantes*; 0.50% *Nigri*; 0.50% *Flavi*) and three sections on DG18 (24.53% *Circumdati*; 0.94% *Nidulantes*; 0.75% *Candidi*). In contrast to the prevalence of section *Nidulantes*in MEA (100%), two *Aspergillus* sections were identified in the settled dust on DG18 (13.79% *Nigri*;

3.45% *Circumdati*). In FRPD, two sections were detected in DC18 (50.00% *Circumdati* and 50.00% *Fumigati*) (Figure 4).

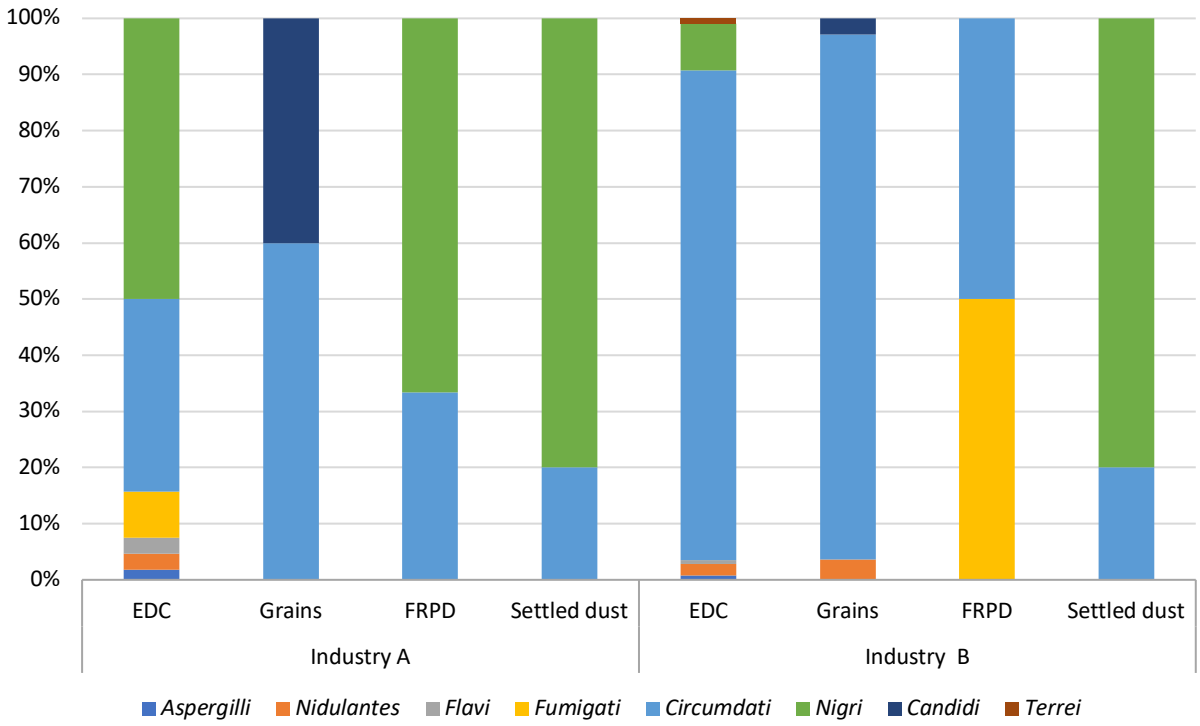


**Figure 4.** *Aspergillus* sections distribution in DG18 in both industries (EDC: log [CFU m-2 day -1]; Grains; settled dust: log [CFU g-1]; FRPD: log [CFU m-2]).

### 3.3. Fungal Diversity in Azole-Supplemented Media

The highest average fungal counts were determined in FRPD and EDC (Figure 5). The most frequent fungi present in SDA and in azole-supplemented SDA media were *Cladosporium* sp. and *Penicillium* sp., with five different *Aspergillus* sections observed in SDA (Figure 6). In azole-supplemented media, only section *Circumdati* was observed, more specifically in 4 $mgL^{-1}$ ICZ, recovered from one EDC sample in one coffee brand (Supplementary Material—Figure S1).

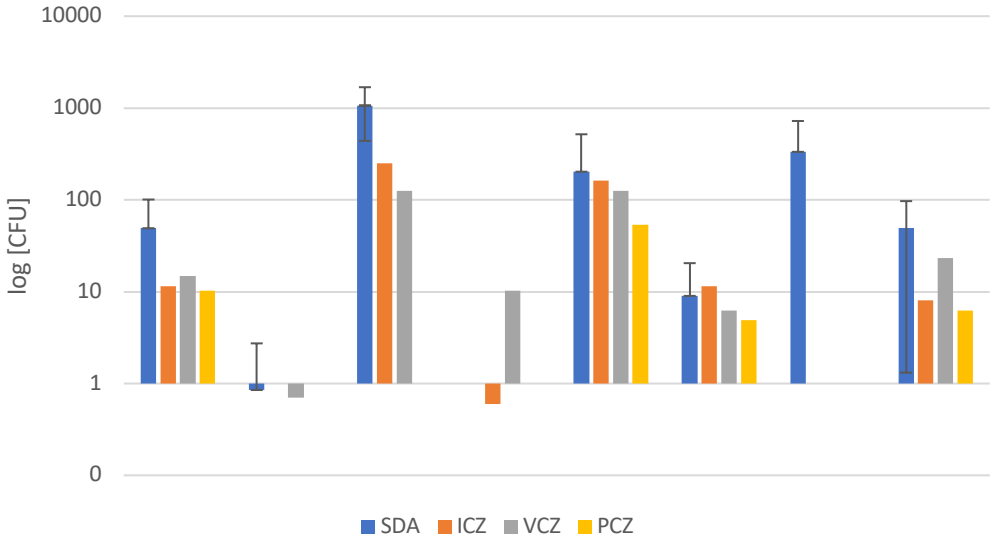


**Figure 5.** Fungal average counts, per industry and sample matrix (EDC, log CFU.m$^{-2}$.day$^{-1}$; Grains, settled dust, log CFU.g$^{-1}$; FRPD, log CFU.m$^{-2}$), by screening in azole-supplemented Sabouraud dextrose agar (SDA) media. ICZ, 4 mgmL$^{-1}$ itraconazole; VCZ, 2 mgmL$^{-1}$ voriconazole; PCZ, 0.5 mgmL$^{-1}$ Posaconazole.

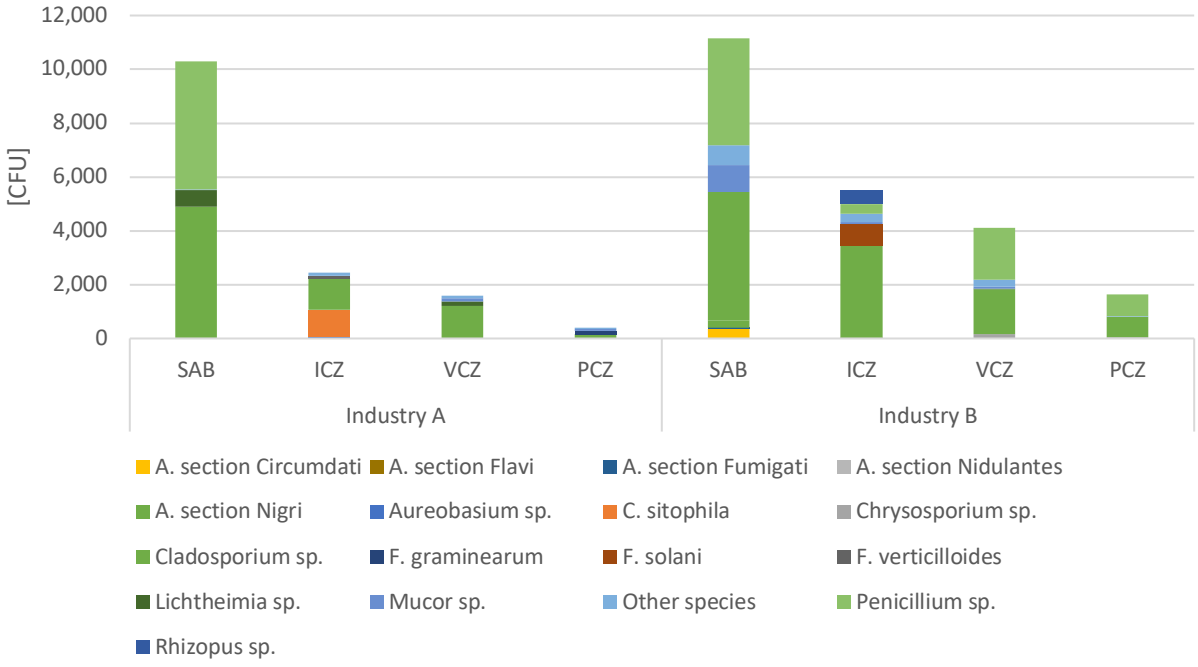


**Figure 6.** Fungal diversity, per industry, by screening in azole-supplemented Sabouraud dextrose agar (SDA) media. ICZ, 4 mg mL$^{-1}$itraconazole; VCZ, 2 mg mL$^{-1}$voriconazole; PCZ, 0.5 mgmL$^{-1}$posaconazo.

### 3.4 Detection of the Targeted Fungal Sections

*Aspergillus* section *Nidulantes* was detected in 20 out of 128 samples (15.6%), with 6 (4.7%) being present in EDC samples, 3 (2.3%) in coffee beans samples, 1 (0.8%) in FRPD samples and 10 (7.8%) in settled dust samples. Concerning *Aspergillus* section *Circumdati*, it was detected in 26 samples out of the 128 samples (20.3%), with 20 being detected in EDC samples (0.8%), 1 in coffee bean samples and 6 (4.7%) in settled dust samples (Supplementary Material—Table S2).

Despite not having detected *Aspergillus* in some samples, the genus was identified through culture-based methods in some matrices from industry A (two samples from EDC (2.5%); one from settled dust (5%) and one from FRPD (16.6%), and in industry B (one sample from EDC (1.6%); one from settled dust (5%) and one from coffee beans (6.25%)). In addition, *Aspergillus* section *Circumdati* was identified in industry A, more specifically in 14 EDC samples (17.5%), 1 settled dust sample (5%) and 3 types of coffee beans (15%). On the other hand, in industry B, *Circumdati* was observed in 11 EDC samples (18.3%), 3 settled dust samples (15%) and 7 coffee bean (4.4%) samples.

### 3.5. Comparisons and Correlation Analysis

Regarding bacterial contamination in TSA, statistically significant differences were detected between the sampling sites of the two companies ($\chi$ 2 (7) = 115.163, p = 0.000). It was found that FRPD and EDC from industry B, as well as FRPD from industry A, were the ones with the highest bacterial contamination in TSA. In VRBA, statistically significant differences were also detected between the sampling methods of the two companies ($\chi^2(7)=77.673$, p=0.000), and it was verified that the EDC of industry B were the ones that showed the highest contamination, followed by the settled dust and the grains of industry A (Supplementary material - Figure S2).

Considering the fungal contamination, statistically significant differences were detected between the sampling methods of the two companies, both in MEA ($\chi^2(7)=72.164$, p=0.000) and in DG18 ($\chi^2(7)=60.836$, p=0.000), having been verified in both media that the industry B's EDC were the ones with the highest contamination (Supplementary material - Figure S2).

With regard to fungal resistance, statistically significant differences were also detected between the sampling methods of the two companies in all the media applied, SDA ($\chi$ 2 (7) = 65.232, p = 0.000), ITZ ($\chi$ 2 (7) = 74.681, p = 0.000), VCZ ($\chi$

2 (7)=58.673, p = 0.000) and PSZ (χ 2 (7) = 42.085, p = 0.000). The sampling methods that showed the highest values were the FRPD of industry A, followed by the EDC of industry B in SDA; the EDC of industry B followed by the FRPD of industry A in ITZ; industry B's EDC were followed by industry B's settled dust in VCZ; EDC and settled dust of industry B in PSZ (Supplementary Material—Figure S2).

Regarding the relationship between bacterial and fungal contamination and fungal resistance, the following significant correlations were detected: (i) greater bacterial contamination in TSA is related to greater bacterial contamination in VRBA, higher values of fungal resistance in SDA and ITZ; (ii) higher bacterial contamination in VRBA is related to higher fungal contamination in MEA and DG18 and higher values of fungal resistance in ITZ, VCZ and PSZ; (iii) higher fungal contamination in MEA is related to higher fungal contamination in DG18 and higher values of fungal resistance in SDA, ITZ, VCZ and PSZ; (iv) fungal contamination in DG18 is related to higher values of fungal resistance in SDA, ITZ, VCZ and PSZ; (v) higher values of fungal resistance in SDA are related to higher values of fungal resistance in ITZ, VCZ and PSZ; (vi) higher values of fungal resistance in ITZ are related to higher values of fungal resistance in VCZ and PSZ; (vii) higher values of fungal resistance in VCZ are related to higher values of fungal resistance in PSZ (Table 4).

**Table 4.** Study of the relationship between bacterial and fungal contamination and fungal resistance. Results of Spearman correlation coefficient.

| | | Bacteria | Fungi | | Fungal resistance | | | |
|---|---|---|---|---|---|---|---|---|
| | | VRBA | MEA | DG18 | SDA | ITZ | VCZ | PSZ |
| Bacteria | TSA | 0.322** | 0.020 | -0.053 | 0.300** | 0.219* | 0.003 | -0.002 |
| | VRBA | | 0.357** | 0.303** | 0.038 | 0.444** | 0.391** | 0.342** |
| Fungi | MEA | | | 0.649** | 0.448** | 0.627** | 0.573** | 0.506** |
| | DG18 | | | | 0.289** | 0.592** | 0.504** | 0.382** |
| Fungal resistance | SDA | | | | | 0.551** | 0.335** | 0.339** |
| | ITZ | | | | | | 0.686** | 0.615** |
| | VCZ | | | | | | | 0.584** |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

## 4. Discussion

Several studies have previously reported both microbial [21–30] and mycotoxin contamination [25,27,30–33] in coffee grains. The later are essentially

ochratoxin A, the only mycotoxin monitored so far in coffee production (green and roasted coffee) [34]. However, in what concerns occupational health, less attention has been given to exposure assessment in the coffee industry. Based on this concern, the International Labour Organization has recently published a toolkit for action to improve occupational safety and health in the coffee supply chain where microbiologic agents are referred to as a risk factor to be considered in this industry [35]. In fact, despite the potential contamination of coffee cherries and beans on the different phases of plant development and in all the supply chain, mainly with fungi and mycotoxins, most of the studies have only reported results of exposure to organic dust and workers' respiratory health [3,36,37]. A deeper analysis concerning microbial contamination exposure assessment in coffee industry facilities has been lacking thus far.

To address this issue, in our study, a comprehensive protocol of passive sampling methods was applied in two coffee industries, A and B assessed. Concerning bacterial contamination, the same sampling method—FRPD—exhibited the highest counts in both industries, while on fungal contamination, different sampling methods presented the highest counts within each industry (FRPD on A; EDC on B). These results followed the same trend as in previous studies developed in other types of occupational environments, where sampling methods provided different results when applied to various workplaces [38–40]. Additionally, statistically significant differences were found between the different sampling methods in both industries for bacterial and fungal contamination. This situation reinforces the need to apply multiple sampling methods, thus avoiding a stand-alone sampling method approach [40]. As in previous studies performed in the waste sorting industry [41], FRPD proved to be a proper passive sampling method to be applied in comprehensive sampling campaigns to assess occupational exposure to fungal contamination. In fact, during FRPD use, suitable conditions for microorganisms' growth can be provided (water vapor, humidity, temperature, etc.) potentiating workers exposure [42]. This was confirmed by the presence, in this study, of the highest counts of *Aspergillus* sp. on FRPD.

In this work, it was also possible to validate the microbial contamination as an occupational risk factor in this specific coffee industry occupational environment. In fact, Gram-negative bacteria was common in all environmental matrices analyzed also supporting the possible exposure to endotoxins in this setting, as previously

reported in studies conducted in coffee industries [3,43]. Therefore, the exposure to bacteria in this setting, in particular, the exposure to specific species/strains and endotoxins should be seriously considered. Furthermore, it is known that bacteria viability on the analyzed protection devices (FRPD) can be increased [44], since this occupational environment is characterized by having high amounts of dust [3,36,37] allowing the transport of nutrients to be retained on the filtration material from FRPD [45,46]. In addition, fungal species with toxigenic potential [47] and clinical relevance [48] were observed and detected through all the different matrixes analyzed, emphasizing their presence in FRPD, that are in direct contact with workers´ respiratory airways, allowing a more real exposure scenario by inhalation and subsequent health effects [41]. *Aspergillus* section *Fumigati* presence on FRPD should be considered as a critical occupational risk, since inhaling its spores may cause several diseases (aspergilloma, invasive pulmonary aspergillosis and different forms of hypersensitivity diseases), depending on the immunological status of the exposed workers [49,50]. Overall, the obtained results corroborate the microbiologic contamination as an occupational risk and justify the inclusion of the FRPD in screening campaigns in order to achieve detailed exposure assessments [41].

As in previous studies performed by our team, *Aspergillus* sections were observed at higher counts on DG18 [14,17], due the feature of this media to restrict fungal species with fast-growing rates, such as those belonging to Mucorales order (*Mucor*, *Rhizopus* and *Lichtheimia* genera) [16].

In addition, the results clearly show the relevance of using different methods to detect and/or identify microorganisms in a given sample as some microorganisms that have been identified microscopically were not detected by qPCR and vice-versa. These differences between molecular and culture-based methods have been observed previously where the low growth speed of fungal species was pinpointed as the main reason for the lack of their detection in culture systems, on the one hand, and high spore prevalent fungi as being on the basis of preferential detection by molecular-based methods, on the other hand [51]. Importantly, qPCR allows the detection of toxigenic species which are not possible to distinguish microscopically [52]. The presence of the *Aspergillus* sections *Nidulantes* and *Circumdati* also identified by molecular biology tools, reveals an important contamination by potential toxigenic fungi. Their quantification would provide a better idea of the risk assessment exposure of each worker/working space. Still, it is possible, from the CT

values, to identify areas with a higher degree of contamination, as a lower CT indicates the presence of a higher microorganism contamination.

As mentioned before, the main OTA producers were observed (*Aspergillus* sections *Circumdati* and *Nigri*) in all the environmental matrices and section *Flavi* was identified in both industries. Of most relevance is the observation of section *Flavi*, the main producer of aflatoxins (e.g., aflatoxin B1), classified by IARC as carcinogenic to humans (Group 1) [53]. These findings claim attention for the need to consider mycotoxin presence in this workplace environment [54,55]. Indeed, the coffee workplace environment is the ideal setting for this phenomena due to several factors, including (a) the fact that the raw material handle is prone to fungal contamination [23,29], (b) fungal species known as mycotoxin producers are detected (e.g., *Aspergillus* species), (c) high dust contamination due to manual tasks are performed in this setting (e.g., as storage work, loading, handling or milling) promoting high exposure to organic dust [36,56] that act as carriers of mycotoxins to the lungs [57,58] promoting exposure via inhalation [59–63], but also dermal absorption due to the deposition of dust particles containing mycotoxins in the skin. In addition, work surfaces contaminated with dust particles can also be considered opportunities for further skin contact and hand to mouth contact promoting exposure also by ingestion [64,65].

The widespread use of demethylation inhibitors (DMI) as fungicides in several economic sectors, such as agriculture, medicine, animal husbandry and material preservation, has led to the reduced efficacy of medical DMI antifungals used to treat patients infected with *Aspergillus* fumigatus due to the presence of azole-resistant isolates [66–70]. Due to their high efficiency and broad-spectrum activity, the DMI fungicides (which include triazoles and imidazoles) are the most used fungicides in many disease management programs to protect crops against fungal infections that compromise production yields [71–73]. They are particularly important in the protection of cereals, fruits, vegetables, and other crops against fungal diseases, thus supporting food security.

Although in the present study, *Aspergillus* section *Fumigati* was not detected in azole supplemented media by passive sampling (only *Circumdati* section was observed in the presence of 4 mg/mL itraconazole), the surveillance of azole resistance in crops and other environments is highly recommended. The crop protection industry strongly encourages the research on the potential for specific

agricultural settings that are able to select and amplify azole-resistant A. fumigatus. The science-based, multisectoral One Health approach is of utmost importance to address this problem, by evaluating settings in which the selection of resistance mutations is plausible, and defining effective mitigation measures when necessary. In addition, it would be of relevance to identify mutations in these strains that can correlate with the azole resistance, namely in the case of *Aspergillus* fumigatus, due to its clinical relevance.

## 5. Conclusions

Overall, this study clearly suggests that the microbial contamination should be considered an occupational risk in the coffee industry (in this case, the milling stage) and should be tackled when assessing exposure and performing risk assessment. In addition, a multi-sampling campaign should be the approach to follow where FRPD analysis should be included.

The present study also draws attention to the need for considering occupational exposure to mycotoxins, in the milling stage, among others, due to high fungal diversity and contamination. Moreover, these workers are exposed simultaneously to fungi, bacteria and probably to their metabolites, an exposure scenario that brings several challenges concerning risk assessment and management.

A One Health approach applied to the coffee industry will address these issues through effective specific actions such as preventing coffee crops and beans from being infected by fungi and, more specifically, avoiding widespread azole resistance. This represents important challenges due to the climate change scenario that requires proper attention and accurate risk management measures.

**Supplementary Materials:** The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/ijerph192013488/s1, Table S1: Shannon and Simpson diversity indices in the EDC matrix; Figure S1: *Aspergillus* sections' frequencies, per industry (A, B) and sampling matrix (EDC, grains, settled dust), by screening in azole-supplemented Sabouraud dextrose agar (SDA) media. ICZ, 4 mg/mL itraconazole; VCZ, 2 mg/mL voriconazole; PCZ, 0.5 mg/mL posaconazole; Table S2: *Aspergillus* sections detection in the different matrices analyzed; Figure

S2: Comparison of bacterial, fungal contamination and fungal resistance between the sampling methods of the two industries (A and B). Results of the Kruskal Wallis test.

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# PAPER III - OCCUPATIONAL EXPOSURE TO MYCOTOXINS IN TWO BRAZILIAN COFFEE INDUSTRIES: A ONE HEALTH CONCERN?

# OCCUPATIONAL EXPOSURE TO MYCOTOXINS IN TWO BRAZILIAN COFFEE INDUSTRIES: A ONE HEALTH CONCERN?

Filipe Oliveira[1,*], Ednilton Tavares de Andrade[1], Carla Viegas[2,3,4], Magdalena Twarużek[5]; Robert Kosicki[5], Carla Martins[3], Susana Viegas[2,3,4]

[1] Department of Agricultural Engineering, Faculty of Engineering, Federal University of Lavras, Lavras 37203-202, Brazil; filipe.oliveira2@estudante.ufla.br (F.O.)

[2] H&TRC—Health & Technology Research Center, ESTeSL—Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisbon, Portugal

[3] Public Health Research Centre, NOVA National School of Public Health, Universidade NOVA de Lisboa, 1099-085 Lisboa, Portugal

[4] Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, 1169-056 Lisboa, Portugal

[5] Kazimierz Wielki University, Faculty of Natural Sciences, Institute of Experimental Biology, Department of Physiology and Toxicology, Chodkiewicza 30, 85–064 Bydgoszcz, Poland

## ABSTRACT

Occupational exposure to mycotoxins in coffee processing facilities has been scarcely reported in the literature. However, there are indications of multiple contaminants present due to the generation and suspension of dust. This study investigated the presence of mycotoxins in settled dust, filtering respiratory protection device (FRPD), and green coffee beans (GCB) in two coffee storage and handling warehouses located in Brazil. Mycophenolic acid (MPA) and ocratoxin A (OTA)were the main mycotoxins found in this occupational environment, with MPA present in 100% of GCB samples, and OTA in 60% of commodity coffee samples, and 100% of specialty coffee samples. The mycotoxins detected in settled dust, FRPD, and GCB samples were mycophenolic acid (69%), ochratoxin A (36%), aflatoxin B2 (10%), fumonisin B2 (9%), zearalenone (7%), fumonisin B1 (3%), and ochratoxin B (2%). Up to four types of mycotoxins were detected per settled dust sample. The mode of mycotoxins found per analyzed matrix was two mycotoxins for settled dust and one mycotoxin for FRPD and GCB.The results highlight how the contamination of raw materials influences the contamination of the occupational environment

and the exposure of workers. Simple strategies, such as the installation of dust collection systems and the use of respirators, can contribute to worker health. However, further studies are needed to better understand the occupational exposure scenario and implement appropriate prevention measures.

**Keywords:** dust; environmental assessment; mycotoxins co-exposure; filtering respiratory protective device.

## INTRODUCTION

Coffee is one of the most important beverages in the world[1].According to the International Coffee Organization[2], the total production by all exporting countries during the 2021/2022 crop year was approximately 10.1million of tonnes of green coffee beans. Brazil's production for the same year was nearly 3.5 million of tonnes, which represents more than a third of the world's production. During the same season, Brazil exported more than 2.6million of tonnes, which is nearly 70% of its total production[3].

As reported by the General Register of Employed and Unemployed Persons[4], in December 2023, there was 85,249 workers at coffee industry in Brazil, being 68,180 at general warehouses and 17,069 at grinding and roasting companies.

The coffee post-harvest processes include several steps that range from washing and separating the fruits, through drying, processing and reprocessing the beans until they are stored. The coffee storage and handling warehouses are responsible for granulometric classification, improving the type and sensory characteristics of the green coffee beans (GCB)[5]. In a coffee storage and

handling warehouse, several activities are performed, such as receiving, cleaning, sorting, selecting, blending, and dispatching. In these activities, grains rupture occurs forming dust[6].

Several studiespointout the presence of biological risks in different occupational environments such as fungi in sawmills[7,8], bacteria in waste sorting plant and livestock farms[9,10], endotoxins in pig farms and vegetable greenhouses[11,12] and, still underreported, mycotoxins in grain handling[13,14].

Mycotoxins are recognized as secondary metabolites, that enter the human body through ingestion[16,17]or dermal/inhalation[13,18,19]. Exposure to mycotoxins has been observed in different branches of industry as varied as waste collection facilities[20,21], livestock[22,23], and agricultural production[24-26].

While the most important mycotoxins exposure source for the general population is the ingestion of contaminated food, at workplaces inhalation and dermal contact are typical routes[27,28]. It happens because mycotoxins can become airborne by techniques and processes that generate dust from materials contaminated by these microorganisms[28,29], such as the coffee beans. Indeed, handling GCB on an industrial scale might also potentialize occupational exposure to mycotoxins, where massive doses of organic dust are constantly handled[30]. This exposure at high amounts for long periods might cause mucous membrane irritation, skin rash, nausea, immune system suppression, acute or chronic liver damage, acute or chronic central nervous system damage, endocrine effects, and cancer[31-34]. However, scarce knowledge is available about mycotoxins' exposure in industries of coffee supply chain. Thus, this study intends to assess the mycotoxins environmental

contamination of two coffee industries from Brazil dedicated to storage and handle green coffee beans.

## METHODS

This study, which is part of an enlarged exploratory study, was conducted in August 2021 in two coffee storage and handling warehouses from Brazil[35]. While being part of a larger study, in which additional microbiological contamination characterization was carried out, this paper presents the results regarding environmental samples collected by passive methods in which mycotoxins analyses was performed.

### *Samples collection*

The two-coffee storage and handling warehouses are located intwo mesoregions of Minas Gerais states in Brazil - Campo das Vertentes and Sul e Sudoeste de Minas. The industry located in Campo das Vertentes receives specialty coffee, while the one located in Sul e Sudoeste de Minas receives commodity coffee from various locations, meaning that the companies dry mill GCB of varying qualities. Figure 1 shows the sequence of machinery in which GCBs pass by during dry milling processes.

The workplaces assessed and the sampling methods used are described in Table 1, while Figure 2 shows sample collection locations on the floor plan of one of the companies assessed. GCB were collected according to their subcategory using the classification from the Brazilian Official Classification for Coffee (COB)[35]. Settled dust samples were collected by using a sterilized spoon to gather the accumulated dust in each workplace[36,37]. Filtering respiratory

protection devices (FRPD) used by coffee industry workers were collected accordingly with procedures previous published[38]. Samples were transferred to sterilized Falcon tubes and stored at -20ºC until analysis.

*Sample preparation*

Obtained settled dust samples of 0.10g each were shaken for 60 min with 3.0 mL of ACN:H2O:AcOH (79:20:1, v/v/v) solution, centrifuged for 5 min at 5000 rpm. Two milliliters of the extract was evaporated under stream of nitrogen, reconstituted in 400 µL of MeOH:H2O(20:80, v/v), and centrifuged again for 30 min at 14500 rpm . The sample dilution factor was 6.

Samples of 0.05 g each, obtained from FRPD, were shaken for 60 minutes with 2.5 mL of ACN:H2O:AcOH (79:20:1, v/v/v) solution. The sample was then centrifuged for 5 min at 5000 rpm and 2mL of the extract was evaporated under stream of nitrogen, reconstituted in 400 µL of MeOH:H2O (20:80, v/v), and centrifuged again for 30 min at 14500 rpm. The sample dilution factor was 10.

Regarding GCB, each sample (0.2 g) was shaken for 60 min with 1.0 mL of ACN:H2O:AcOH (79:20:1, v/v/v) solution. The sample was then centrifuged for 5 min at 5000 rpm. Raw extract (0.2mL) was diluted with 0.2 mL of water, mixed and centrifuged again for 30 min at 14500 rpm. The sample dilution factor was 10.

*Analytical determination*

Analytical determination was performed using a Nexera High Performance Liquid Chromatograph (HPLC) (Shimadzu, Tokyo, Japan) with an API 4000 mass spectrometry detector (Sciex, Foster City, CA, USA). Separation of the compounds was done on a Gemini NX-C18 (150x4.6 mm, 3 µm) chromatographic column (Phenomenex, Torrance, CA, USA). The mobile phases (A: 5 mM ammonium acetate in water and B: 5 mM ammonium acetate in methanol) contained a 1% addition of acetic acid. The flow rate of the mobile phase was set to 0.75 mL/min and the injection volume to 7 µL. Optimized parameters of mycotoxins ionization (MRM transitions, polarity, collision energies, declustering and cell exit potentials) were used as reported before[39]. This analytical method detects and quantifies the following mycotoxins: T-2 tetraol, diepoxy-deoxynivalenol (DOM-1), Neosolaniol (NEO), 15-acetyldeoxynivalenol (15-AcDON), 3-acetyldeoxynivalenol (3-AcDON), monoacetylscirpenol (MAS), diacetoxyscirpenol (DAS), aflatoxin $M_1$ ($AFM_1$), aflatoxin $B_1$ ($AFB_1$), aflatoxin $B_2$ ($AFB_2$), aflatoxin $G_1$ ($AFG_1$), aflatoxin $G_2$ ($AFG_2$), fumonisin $B_1$ ($FB_1$), fumonisin $B_2$ ($FB_2$), fumonisin $B_3$ ($FB_3$), gliotoxin, T-2 triol, roquefortine C (ROQ-C), griseofulvin (GRIS), toxin T-2 (T-2), toxin HT-2 (HT-2), ochratoxin A (OTA), ochratoxin B (OTB), mycophenolic acid (MPA), mevinolin (MEV), sterigmatocystin (STER), moniliformin, patulin (PAT), nivalenol (NIV), deoxynivalenol-3-glucoside (DON-3-G), deoxynivalenol (DON), fusareon-X (FUS-X), zearalanon (ZAL), zearalenone (ZEL), α-zearalanol(α-ZAL), β-zearalanol (β-ZAL),α-zearalenol (α-ZEL), β-zearalenol (β-ZEL).In-house validation of the analytical method was performed and limits of detection and

quantification, recovery rate (%), and intermediate precision (RSD, %) were determined.

*Statistical analysis*

Descriptive statistics was performed using Microsoft Excel®. Univariate descriptive statistics included frequency (n, %), average and mode.

## RESULTS

This is a first exploratory study that evaluates the presence of 38 different mycotoxins at coffee storage and handling warehouses. Results for the in-house method validation are presented in Table 2.

Seven mycotoxins were detected in all samples (Table 3). Among the different samples, settled grain dust was the ones with highest number of mycotoxins identified (4 mycotoxins) (Figure 3).

Considering all the samples and as presented in Figure 4, mycophenolic acid was detected in 40 out of 58 samples (<20.7 – 657.4µg $kg^{-1}$). Ochratoxin A was reported in 21 out of 58 samples (10.8 – 45.8µg $kg^{-1}$). Aflatoxin B2, was detected in six samples (<6.1µg $kg^{-1}$). Fumonisin B2 was detected in six samples (<19.6 – 20.4µg $kg^{-1}$). Zearalenon was detected in four samples (<5.9µg $kg^{-1}$). Fumonisin B1 was detected in two samples (<31.1µg $kg^{-1}$). Ochratoxin B was reported only once (<10.8µg $kg^{-1}$) (Figure 4).

Regarding samples from Industry A, six mycotoxins were detected, being $AFB_2$, $FB_1$,$FB_2$, MPA, OTA, and ZEA. Among samples of settled dust, MPA was quantified at all samples (195.0 – 547.9 µg $kg^{-1}$). OTA was detected. Among

samples of FRPD, OTA was detected at all samples with concentration range below LOQ. Among GCB, $AFB_2$ was detected at 30% of samples.

Similar results were obtained from Industry B. Six mycotoxins were detected, namely $AFB_2$, $FB_1$, MPA, OTA, OTB, and ZEA. Among samples of settled dust, MPA and OTA were detected at all samples (160.2 – 657.4 μg $kg^{-1}$ and <45.8 μg $kg^{-1}$, respectively). OTA was also detected at FRPD. $AFB_2$ was detected at almost 40% of samples.

Analyzing samples from matrices separately (settled dust, filters, and grain), the FRPD had a more diverse array of mycotoxins rather than settled dust or GCBs (Table 3). Among samples of settled grain dust, mycophenolic acid was detected in all the samples for both industries (160.2-657.4 μg $kg^{-1}$). $FB_1$ and ZEA were detected only for industry B. OTA was detected in 60% of samples for industry A (<12.8 μg $kg^{-1}$) and 100% of samples for Industry B (12.0 -45.8 μg $kg^{-1}$).

Regarding samples from FRPD, OTA was detected at all samples from Industry A (<4.9 μg $kg^{-1}$). $FB_1$ and $FB_2$ were only detected at Industry A, with concentration range between <11.7 μg $kg^{-1}$ and 9.5 – 20.4 μg $kg^{-1}$, respectively. Although mycophenolic acid was detected in 75% of samples for Industry A and 50% of samples for Industry B, the concentration range for Industry A is <10.5 – 10.6 μg $kg^{-1}$ and <10.5 – 150.6 μg $kg^{-1}$. ZEA was detected at only one sample for each Industry with concentration ranges lower than 4.9 μg $kg^{-1}$, at both companies.

Regarding GCB, it was only detected $FB_2$ and OTA at one sample each (Industry A) with concentration ranges lower than 32.9 and 2.6μg $kg^{-1}$, respectively. $AFB_2$ was detected at both industries with concentration ranges

lower than 2.5 μg $kg^{-1}$. Mycophenolic acid was detected at both Industry A and Industry B with concentrations varying between 11.4 – 203.3μg $kg^{-1}$ and 297.0 μg $kg^{-1}$.

## DISCUSSION

This study demonstrates the co-occurrence of mycotoxins, including MPA, OTA, $AFB_2$, $FB_2$, ZEN, $FB_1$, and OTB, in samples collected. Our findings reveal MPA and OTA as the predominant mycotoxins in this occupational setting.

The analysis of mycotoxins in settled dust reveals the prevalent occurrence of MPA and OTA in both coffee storage and handling warehouses assessed. MPA was found in 100% of samples from commodity and specialty coffee industries, while OTA was detected in 60% of commodity coffee samples and 100% of specialty coffee samples. Similar observations have been reported in coffee post-harvest facilities in other countries[40,41]. Quantifiable levels of OTA and MPA were identified in settled dust from both coffee storage and handling warehouses, with the highest reported mean concentrations at industry B being 657.4 μg $kg^{-1}$ for MPA and 45.8 μg $kg^{-1}$ for OTA. Contamination of settled dust by multiple mycotoxins, including MPA, OTA, $FB_1$, and ZEN, has also been reported in in grain storage facilities[25,13,14]. Literature reports indicate the presence of mycotoxins in coffee beans, with OTA being the most commonly found[42,53,54]. This finding highlights the occupational exposure scenario in coffee storage and handling warehouses. The extent of exposure may vary depending on the quality of received beans and the type of activities performed[43-46]. This is because toxigenic microorganisms are present in coffee crop environments,

harvesting, processing, and storage[36,47,48]. Thus, the same fungi that produce mycotoxins are responsible for undesirable alterations in coffee's organoleptic quality[49]. Therefore, it is expected that higher concentrations of microorganisms and mycotoxins are correlated with lower-quality coffees in the industry. Workers who handle lower-quality GCBs are consequently exposed to higher concentrations of fungi and mycotoxins.

Due to the continued presence of mycotoxins in dried and processed coffee fruits at the farm, these substances are expected to be present in the raw material received by the coffee storage and handling warehouse. Therefore, the transport of mycotoxins in the dust generated during the separation, classification, and transportation processes of GCBs within the industry is anticipated. Reference[50] conducted a detailed study of mycotoxin reduction in grains throughout post-harvest operations. One of the crucial processes for reducing OTA and DON in wheat grains is the separation, cleaning, and grading processes, with OTA reductions of up to 60% from the initial levels. This is due to the removal of impurities accompanying the raw material at the beginning of the process.

The multi-matrix approach (settled dust, FRPD, and GCB) applied in this study enables the recognition of a broader spectrum of mycotoxins present in the sampled occupational environments. One of the most reported methods for collecting dust samples is settled dust. Reference[37] studied the presence of mycotoxins in dust from grain elevators and feed mills in Norway through settled dust collection. The authors found a wide variety of mycotoxins in these grain dusts, such as trichothecenes, depsipeptides, and secondary metabolites

produced by fungi of the genus *Fusarium*, among others. Similarly to the present study, Reference[37] identified MPA in all analyzed samples.

Another aspect evaluated by Reference[14] indicates significant differences in the microbiota present in grain dust according to different geographical locations in Norway. This diversity may result in distinct occupational exposures depending on the industry's location. This occurs because the evaluated industries are in regions with different climatic conditions, and consequently, grains are locally produced in these distinct regions.

As per ACGIH[52], there are currently no Threshold Limit Values (TLVs) established for the airborne concentrations of total culturable or countable bioaerosols, specific culturable or countable bioaerosols, infectious agents, or detectable biological contaminants. Nonetheless, there is ongoing advancement in assay methods for certain prevalent airborne antigens and mycotoxins, with field validation also making progress. This suggests the potential for future establishment of exposure limits for some detectable airborne contaminants derived from biological sources.

The coffee storage and handling warehouse is a concerning occupational environment due to its high contamination load by toxigenic fungi, resulting from the composition of organic raw materials and the environmental conditions experienced during its production chain. However, simple measures such as the use of FRPD or the implementation of dust capture systems can contribute to the safety of grain reprocessing industry workers. As FRPD are used near workers' airways, their analysis allows for a better understanding of what workers are exposed to through inhalation[51]. For this reason, researchers have analyzed the number of mycotoxins present in the lower layers of respirators

used by workers in different occupational environments, such as waste sorting industries[21].

This preliminary work examined the concentration of mycotoxins in coffee storage and handling warehouse through different collection methods but did not use active dust sampling methodologies that simulate workers' breathing. Future studies should be developed using both active and passive collection methods, as well as identifying workers by activity and raw material used in the industry. Thus, continuous study of these occupational environments is necessary for a better understanding of the occupational exposure scenario.

It is evident that despite the mandatory distribution of FRPD (PFF1 and PFF2) by companies, it was found that many workers commonly choose not to use this personal protective equipment properly during their activities. This makes occupational exposure even more plausible. Additionally, it is worth noting that both sampled companies are equipped with dust capture systems during processes, aiming to reduce the concentration.

## CONCLUSIONS

Based on the results of this study, it is concluded that the presence of mycotoxins, especially mycophenolic acid (MPA) and ochratoxin A (OTA), is frequent in the assessed coffee storage and handling warehouses. These substances were found in all settled dust samples, indicating significant worker exposure to these toxins during the storage and handling processes. Additionally, it was observed that the quality of received grains and the type of activity performed may influence occupational exposure to mycotoxins.

As future work, further studies are suggested to evaluate the effectiveness of existing exposure control measures and identify possible gaps in worker protection. Furthermore, investigations into the presence of mycotoxins in other stages of the coffee production chain, such as harvesting and storage, may provide a more comprehensive insight into the occupational risks associated with coffee production.

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575 Table 1 - Sampling matrixes and sampling locations

| Matrix | Identification | Locations/ Functions |
|---|---|---|
| **Green CoffeeBeans** | GRSA01 | Reception in truck |
| | GRSA02 | Bagging |
| | GRSA03 | Bagging |
| | GRSA04 | Storage |
| | GRSA05 | Storage |
| | GRSA06 | Storage |
| | GRSA07 | Storage |
| | GRSA08 | Storage |
| | GRSA09 | Storage |
| | GRSA10 | Storage |
| | GRLI01 | Sieve 17 coffee grain |
| | GRLI02 | Sieve 16 coffee grain |
| | GRLI03 | Mocha coffee grain |
| | GRLI04 | Sieves 13/15 coffee grain |
| | GRLI05 | Sieve 18 coffee grain |
| | GRLI06 | Lighter coffee grain |
| | GRLI07 | Residue coffe grain |
| | GRLI08 | Bottom sieve–coffee grain |
| **SettledDust** | POSA01 | Top of the hopper near to sieve #15 |
| | POSA02 | Densimetric table separator 01 |
| | POSA03 | Densimetric table separator 02 |
| | POSA04 | Sorting separator |
| | POSA05 | Stone picker machine |
| | POSA06 | Dust collector - stone picking machine |
| | POSA07 | Stone picker machine #1 |
| | POSA08 | Above electronic densimetric table |
| | POSA09 | Bagging |
| | POSA10 | Grain reception - near to expedition |
| | POLI01 | Balance |
| | POLI02 | Storage #15 - conveyor's belt hopper |
| | POLI03 | Dust blower - dust has been stored for 3 days |
| | POLI04 | Containers' elevator |
| | POLI05 | Elevator near to balance 1 |
| | POLI06 | Floor dust - central storage |
| | POLI07 | Sieve's classificator |
| | POLI08 | Stone picker machine |
| | POLI09 | Dust collector |
| | POLI10 | Room–electronic densimetric table |
| **FRPD** | MASA01 | Storekeeper |
| | MASA02 | Forklift operator |
| | MASA03 | Storekeeper |
| | MASA04 | Production operator |

| | | |
|---|---|---|
| | MASA05 | Storekeeper |
| | MASA06 | Storekeeper |
| | MASA07 | Forklift operator |
| | MASA08 | Machine operator |
| | MALI01 | Sampling operator |
| | MALI02 | Inspector |
| | MALI03 | Sampling assistant operator |
| | MALI04 | Forklift operator |
| | MALI05 | Supervisor |
| | MALI06 | Forklift operator - machine output operator |
| | MALI07 | Machine output operator - assistant |
| | MALI08 | Eletronic densimetric table operator |
| | MALI09 | Machine operator |
| | MALI10 | Hopper assistant |
| | MALI11 | Hopper #6 assistant |
| | MALI12 | Forklift operator; reprocessing |

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577 Table 2 – Mycotoxins and their concentrations present in the analyzed samples (µg/kg).

| Mycotoxins | Settled Dust | | | | | Filters | | | | | GCB | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LOD | LOQ | Spiking level µg/kg | Recovery % | RSD % n=3 | LOD | LOQ | Spiking level µg/kg | Recovery % | RSD % n=3 | LOD | LOQ | Spiking level µg/kg | Recovery % | RSD % n=3 |
| T2 Tetraol | 4.7 | 15.5 | 110 | 85 | 7 | 1.9 | 6.3 | 110 | 101 | 7 | 6.8 | 22.6 | 110 | 85 | 7 |
| Deepoxydeoxynivalenol | 9.5 | 31.5 | 183 | 83 | 5 | 5.3 | 17.7 | 183 | 98 | 4 | 11.9 | 39.5 | 183 | 86 | 4 |
| Neosolaniol | 4.8 | 15.9 | 104 | 77 | 7 | 2.7 | 9.0 | 104 | 89 | 7 | 1.9 | 6.2 | 104 | 85 | 7 |
| 15-Acetyldeoxynivalenol | 20.4 | 67.8 | 293 | 73 | 9 | 7.5 | 25.0 | 293 | 91 | 8 | 15.3 | 51.0 | 293 | 88 | 8 |
| 3-Acetyldeoxynivalenol | 8.9 | 29.5 | 213 | 71 | 8 | 3.6 | 12.1 | 213 | 79 | 7 | 5.4 | 17.9 | 213 | 87 | 8 |
| Monoacetoxyscirpenol | 3.7 | 12.3 | 110 | 82 | 9 | 2.5 | 8.3 | 110 | 110 | 8 | 3.3 | 11.1 | 110 | 88 | 8 |
| Diacetoxyscirpenol | 1.8 | 5.9 | 163 | 80 | 7 | 1.6 | 5.3 | 163 | 100 | 6 | 3.4 | 11.3 | 163 | 86 | 7 |
| Aflatoxin M1 | 1.0 | 3.2 | 5 | 63 | 15 | 0.9 | 3.1 | 5 | 86 | 12 | 0.8 | 2.7 | 5 | 74 | 13 |
| Aflatoxin B1 | 0.9 | 3.0 | 21 | 61 | 5 | 0.9 | 3.1 | 21 | 80 | 5 | 0.7 | 2.5 | 21 | 72 | 5 |
| Aflatoxin B2 | 1.8 | 6.1 | 5 | 66 | 7 | 0.9 | 3.1 | 5 | 83 | 6 | 0.8 | 2.5 | 5 | 74 | 7 |
| Aflatoxin G1 | 0.9 | 3.0 | 22 | 62 | 9 | 0.9 | 3.2 | 22 | 69 | 8 | 0.8 | 2.5 | 22 | 75 | 8 |
| Aflatoxin G2 | 1.8 | 5.9 | 5 | 68 | 10 | 0.9 | 3.0 | 5 | 85 | 8 | 0.8 | 2.6 | 5 | 72 | 9 |
| Fumonisin B1 | 9.3 | 31.1 | 539 | 60 | 6 | 3.5 | 11.7 | 539 | 83 | 6 | 9.9 | 32.9 | 539 | 72 | 6 |
| Fumonisin B2 | 5.9 | 19.5 | 541 | 74 | 9 | 2.8 | 9.5 | 541 | 90 | 9 | 6.2 | 20.7 | 541 | 78 | 8 |
| Fumonisin B3 | 5.9 | 19.6 | 154 | 80 | 14 | 3.8 | 12.8 | 154 | 103 | 13 | 8.0 | 26.7 | 154 | 84 | 12 |
| Gliotoxin | 10.1 | 33.5 | 160 | 69 | 10 | 6.6 | 22.0 | 160 | 86 | 10 | 5.1 | 16.9 | 160 | 73 | 10 |
| T2 Triol | 9.7 | 32.4 | 110 | 69 | 11 | 5.0 | 16.5 | 110 | 78 | 9 | 7.6 | 25.4 | 110 | 79 | 10 |
| Roquefortine C | 4.5 | 14.9 | 171 | 51 | 1 | 1.6 | 5.2 | 171 | 69 | 1 | 2.6 | 8.8 | 171 | 60 | 6 |
| Griseofulvin | 4.6 | 15.2 | 160 | 89 | 4 | 2.4 | 8.1 | 160 | 112 | 4 | 2.6 | 8.7 | 160 | 92 | 3 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T2 | 3.0 | 9.8 | 160 | 80 | 1 | 2.5 | 8.3 | 160 | 102 | 1 | 1.9 | 6.2 | 160 | 94 | 3 |
| HT2 | 6.3 | 21.0 | 160 | 68 | 6 | 3.5 | 11.7 | 160 | 91 | 6 | 4.1 | 13.7 | 160 | 82 | 6 |
| Ochratoxin A | 3.2 | 10.8 | 58 | 76 | 5 | 1.5 | 4.9 | 58 | 103 | 5 | 0.8 | 2.6 | 58 | 85 | 4 |
| Ochratoxin B | 3.6 | 12.0 | 60 | 73 | 7 | 1.9 | 6.2 | 60 | 98 | 5 | 0.7 | 2.4 | 60 | 81 | 7 |
| Mycophenolicacid | 6.2 | 20.7 | 163 | 78 | 6 | 3.2 | 10.5 | 163 | 87 | 6 | 3.4 | 11.4 | 163 | 90 | 5 |
| Mevinolin | 10.8 | 36.0 | 160 | 81 | 5 | 6.7 | 22.2 | 160 | 92 | 5 | 2.6 | 8.6 | 160 | 91 | 5 |
| Sterigmatocystin | 1.9 | 6.2 | 67 | 73 | 2 | 1.3 | 4.3 | 67 | 85 | 2 | 0.8 | 2.6 | 67 | 90 | 2 |
| Moniliformin | 9.6 | 31.9 | 534 | 79 | 6 | 3.2 | 10.8 | 534 | 110 | 6 | 2.6 | 8.8 | 534 | 92 | 5 |
| Patulin | 18.2 | 60.7 | 162 | 71 | 5 | 8.5 | 28.3 | 162 | 85 | 5 | 17.6 | 58.8 | 162 | 78 | 4 |
| Nivalenol | 5.9 | 19.8 | 320 | 78 | 8 | 4.1 | 13.6 | 320 | 94 | 6 | 8.3 | 27.5 | 320 | 93 | 7 |
| Deoxynivalenol-3-Glucoside | 8.0 | 26.5 | 185 | 87 | 3 | 5.1 | 16.8 | 185 | 98 | 3 | 7.8 | 25.9 | 185 | 91 | 3 |
| Deoxynivalenol | 11.3 | 37.6 | 320 | 74 | 5 | 8.1 | 26.8 | 320 | 89 | 5 | 10.1 | 33.7 | 320 | 90 | 5 |
| Fusarenon X | 12.6 | 42.0 | 214 | 69 | 7 | 10.1 | 33.5 | 214 | 84 | 7 | 10.9 | 36.4 | 214 | 83 | 7 |
| Zearalanon | 1.8 | 5.9 | 31 | 93 | 4 | 1.5 | 5.0 | 31 | 107 | 3 | 1.8 | 6.0 | 31 | 93 | 4 |
| Zearalenon | 1.9 | 6.4 | 159 | 84 | 3 | 1.2 | 4.0 | 159 | 102 | 3 | 0.8 | 2.6 | 159 | 96 | 4 |
| aZearalanol | 3.8 | 12.8 | 31 | 82 | 6 | 2.5 | 8.3 | 31 | 90 | 5 | 1.8 | 6.0 | 31 | 90 | 6 |
| bZearalanol | 4.1 | 13.5 | 31 | 82 | 2 | 3.2 | 10.6 | 31 | 107 | 2 | 2.5 | 8.4 | 31 | 94 | 3 |
| bZearalenol | 3.0 | 10.1 | 32 | 99 | 5 | 2.4 | 8.1 | 32 | 109 | 5 | 1.8 | 6.1 | 32 | 101 | 5 |
| aZearalenol | 2.7 | 9.0 | 31 | 76 | 9 | 1.9 | 6.2 | 31 | 96 | 8 | 0.8 | 2.5 | 31 | 91 | 9 |

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584 Table 3: Presence of mycotoxins in samples from industry A and B.

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| | | Aflatoxin B2 | | Fumonisin B1 | | Fumonisin B2 | | Mycophenolic acid | | Ochratoxin A | | Ochratoxin B | | Zearalenon | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of Samples with Detectable Values | Concent. Range (µg kg-1) | Number of Samples with Detectable Values | Concent. Range (µg kg-1) | Number of Samples with Detectable Values | Concent. Range (µg kg-1) | Number of Samples with Detectable Values | Concent. Range (µg $kg^{-1}$) | Number of Samples with Detectable Values | Concent. Range (µg kg-1) | Number of Samples with Detectable Values | Concent. Range (µg kg-1) | Number of Samples with Detectable Values | Concent. Range (µg kg-1) |
| Industry A | SD | 0 | ND | 0 | ND | 0 | ND | 10 (100%) | 195.0-547.9 | 6 (60%) | <LOQ | 0 | ND | 0 | ND |
| | FRPD | 0 | ND | 1 (12.5%) | <LOQ | 4 (50%) | <LOQ-20.4 | 6 (75%) | <LOQ-10.6 | 10 (100%) | <LOQ | 0 | ND | 1 (12.5%) | <LOQ |
| | GCB | 3 (30%) | <LOQ | 0 | ND | 1 (10%) | <LOQ | 2 (20%) | <LOQ-203.3 | 1 (10%) | <LOQ | 0 | ND | 0 | ND |
| Industry B | SD | 0 | ND | 1(10%) | <LOQ | 0 | ND | 10 (100%) | 160.2-657.4 | 10(100%) | <LOQ-45.8 | 1(10%) | <LOQ | 2 (20%) | <LOQ |
| | FRPD | 0 | ND | 0 | ND | 0 | ND | 6 (50%) | <LOQ-150.6 | 3 (25%) | <LOQ-7.3 | 0 | ND | 2 (8.3%) | <LOQ |
| | GCB | 3 (37.5%) | <LOQ | 0 | ND | 0 | ND | 6 (75%) | <LOQ-297.0 | 0 | ND | 0 | ND | 0 | ND |

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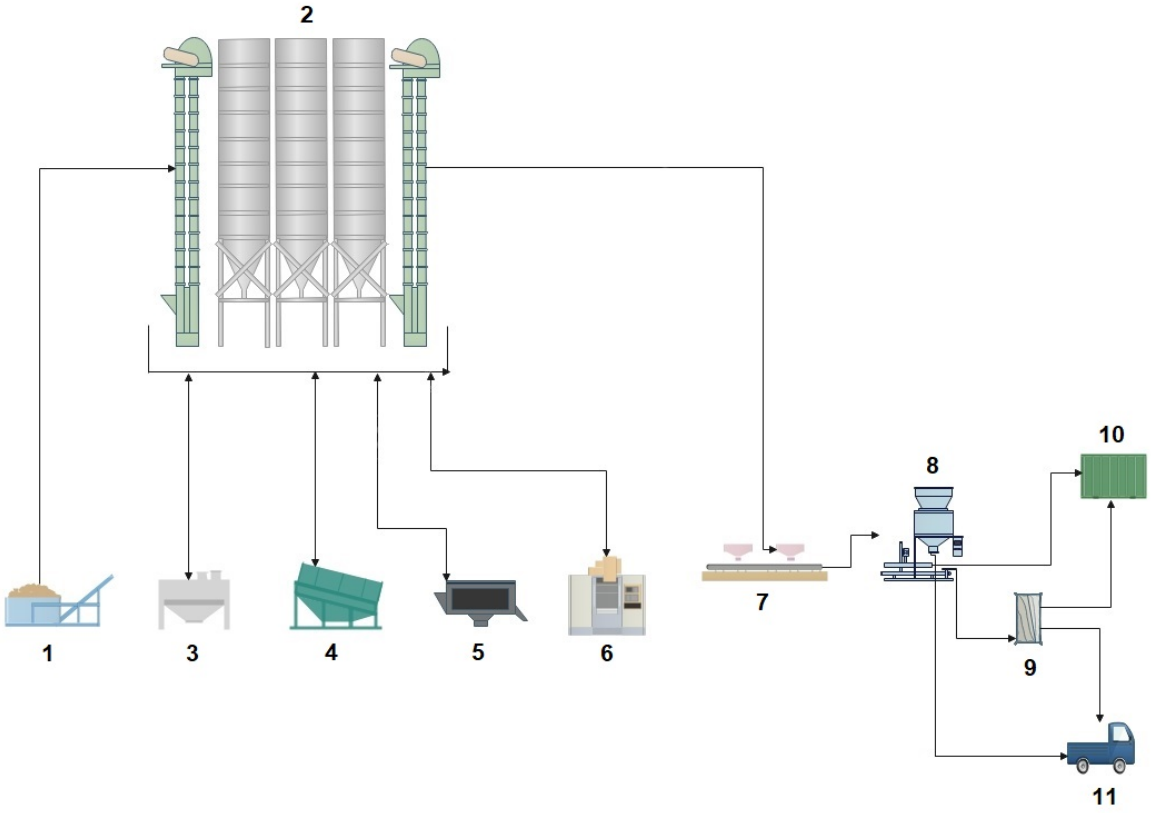


Subtitle: (1) Reception; (2) bins; (3) stone picker; (4) ranked table; (5) density table; (6) electronic table; (7) pneumatic treadmill; (8) shipping scale; (9) bags (30 or 60kg); (10) shipping in containers; and (11) shipment by truck.
Source: Authors (2022).

Figure 01 – Illustrative sequence of machinery in coffee storage and handling warehouse.

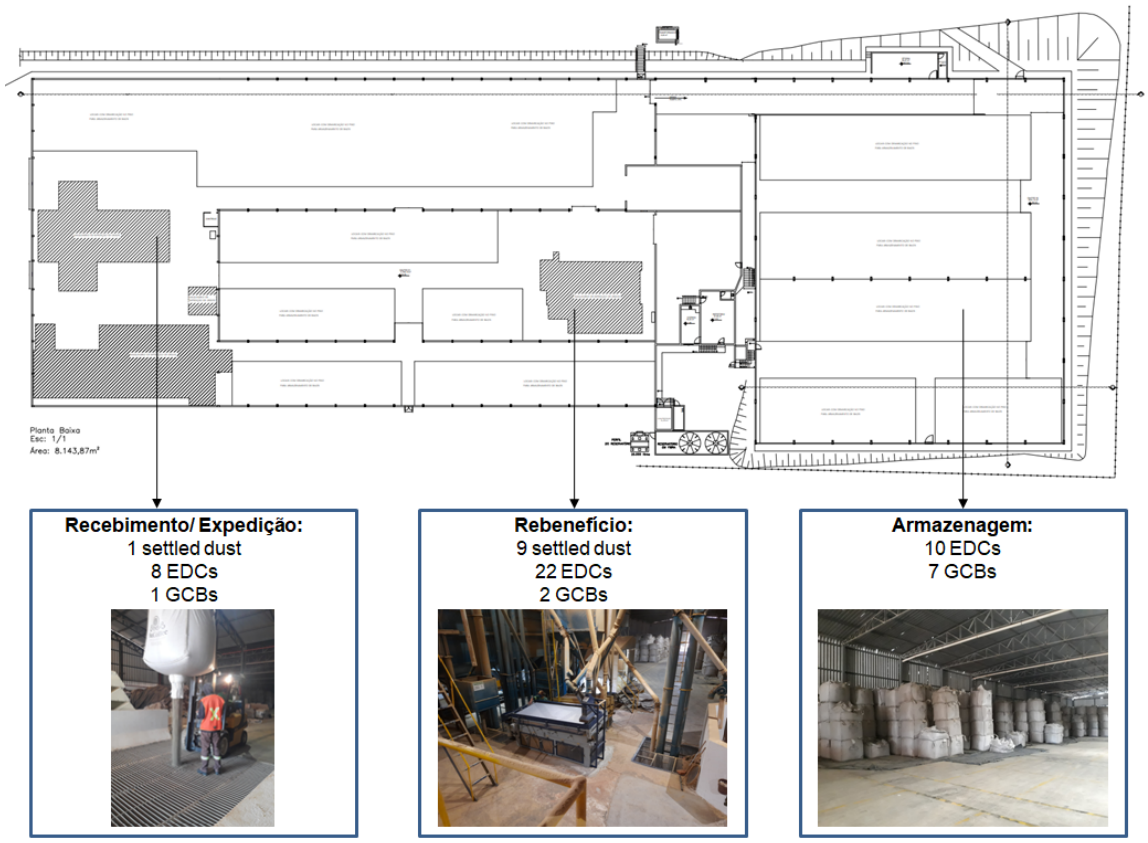


Figure 2 – Sample collection locations available on the floor plan of one of the companies.

Source: Authors (2022).

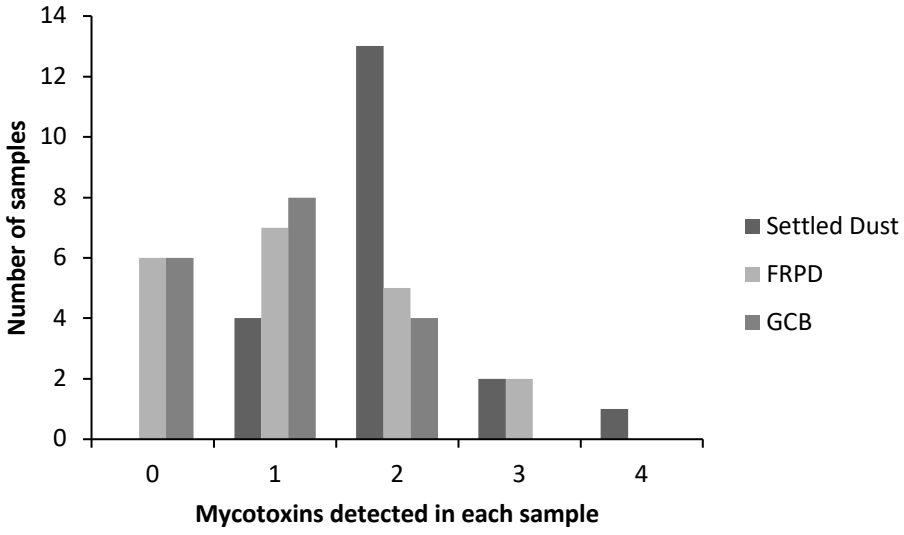


Figure 3 - Number of mycotoxins detected (>LOD) in each analyzed sample.

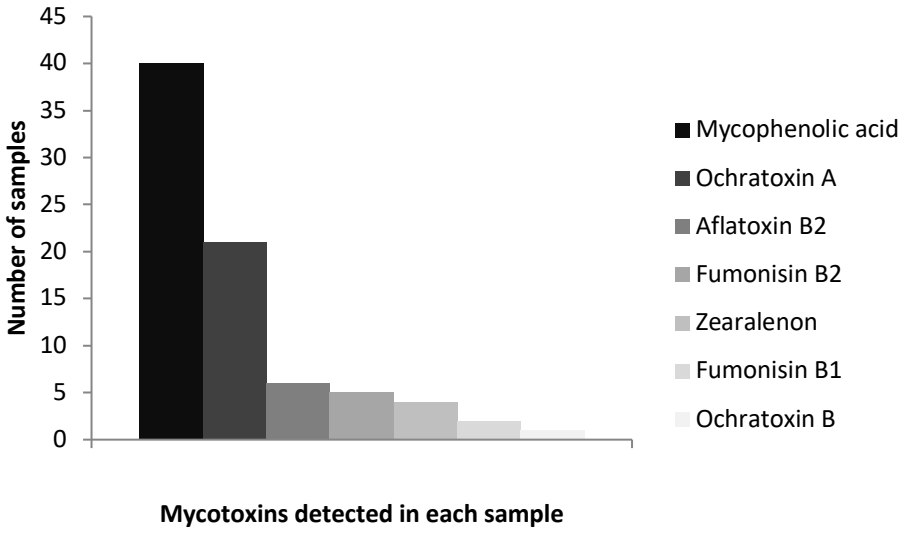


Figure 4 - Number of samples presenting detectable levels of mycotoxins (>LOD).