INSTITUTO POLITÉCNICO DE LISBOA

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

INTERNSHIP IN ENVIRONMENTAL MICROBIOLOGY: FROM THE FIELD TO THE LABORATORY

BRUNA MARTINS RIESENBERGER

ORIENTADORA: DOUTORA CARLA SOFIA VIEGAS – ESCOLA SUPERIOR DE TECNOLOGIAS DA SAÚDE DE LISBOA

Mestrado em Tecnologias Clinico-Laboratoriais

Lisboa, 2024

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ORIENTADORA: DOUTORA CARLA SOFIA VIEGAS – ESCOLA SUPERIOR DE TECNOLOGIA DA SÁUDE DE LISBOA

JÚRI

PRESIDENTE: DOUTORA EDNA SORAIA RIBEIRO – ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

ARGUENTE: DOUTORA SANDRA ISABEL CABO VERDE – INSTITUTO TECNOLÓGICO E NUCLEAR DO INSTITUTO SUPERIOR TÉCNICO

Mestrado em Tecnologias Clinico-Laboratoriais

Lisboa, 2024

Conseguimos! Portugal, Lisboa, esperávamos, desejávamos, conseguimos, vitória!

Sousa et al., 2019

ACKNOWLEDGMENTS

Firstly, I would like to thank to H&TRC for giving me this internship at their organization. Overall, this has been a very rich learning experience, as I have acquired a lot of knowledge about my subject of study.

I am also grateful to Professor Carla Viegas and my “super” supervisors, Renata Cervantes, Pedro Pena, Marta Dias and Bianca Gomes, for their support and contribution throughout this process. I owe a lot to their support, guidance and indications, which have helped not only in the preparation of this report, but also in my professional and personal growth. I'll always carry a bit of you with me.

My sincere thanks go to my parents and my sister, who have always been a pillar of support for me, especially during this final phase. Their unwavering belief in me and their constant encouragement have been my greatest source of strength. I would also like to thank all my friends for their support, especially Margarida Rodriguez and Liliana Marques, who were patient with me and always encouraged me beyond my internship.

I am also gratefully acknowledging the FCT/MCTES national support through the UIDB/05608/2020; UIDP/05608/2020. This work is also supported by national funds through FCT/MCTES/FSE/UE, 2023.01366.BD; UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); UI/BD/151431/2021 (https://doi.org/10.54499/UI/BD/151431/2021); and Instituto Politécnico de Lisboa, national support through IPL/2022/InChildhealth/BI/12M; IPL/IDI&CA2023/FoodAIIEU_ESTeSL; IPL/IDI&CA2023/ASPRisk_ESTeSL; IPL/IDI&CA2023/ ARAFSawmills_ESTeSL Partly funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Swiss State Secretariat for Education, Research and Innovation (SERI), or the United Kingdom Research and Innovation (UKRI), or the Australian National Health & Medical Research Council (NHMRC). Neither the European Union nor the granting authorities can be held responsible for them.

Every moment I spent with you was fun and memorable. To all of you, thank you very much.

RESUMO

No segundo ano do Mestrado Tecnologias Clínico-Laboratoriais, está integrada a unidade curricular Projeto/Tese/Estágio, em que se pode escolher a tipologia de estágio, apresentando no fim um relatório sobre as atividades desenvolvidas. O estágio realizado foi de natureza profissional e decorreu entre 11 de setembro de 2023 a 20 de Junho de 2024, no laboratório de Microbiologia Ambiental e Ocupacional, pertencente ao Health & Technology Research Center.

Durante este tempo, decorreram 3 projetos de doutoramento, com o objetivo de avaliar a exposição ocupacional/ambiental a agentes microbiológicos, nomeadamente fungos e bactérias, em diferentes ambientes. Foram realizadas campanhas de amostragem ambiental e as atividades de laboratório inerentes à preparação e análise das amostras obtidas. O tratamento de amostras, consistiu na aplicação técnicas de microbiologia clássica, e na utilização de métodos de biologia molecular como complemento.

O estágio permitiu consolidação dos conhecimentos adquiridos ao longo do primeiro ano curricular e, ainda, adquirir outros conhecimentos, novas competências práticas relacionadas com a área da microbiologia ambiental e realizar produção científica (artigos, resumos para conferências e posters científicos) e refletir sobre outros possíveis projetos de estudo.

PALAVRAS-CHAVE

Exposição ocupacional; Bioaerossóis; Trabalho laboratorial; Microbiologia ambiental e ocupacional

ABSTRACT

The second year of the master's in clinical-Laboratory Technologies includes the curricular unit Project/Thesis/Internship, in which you can choose the type of internship, presenting a report on the activities carried out at the end. The internship was of a professional nature and took place between 11 September 2023 and 20 June 2024 in the Environmental and Occupational Microbiology laboratory at the Health & Technology Research Centre.

During this time, 3 PhD projects were carried out with the aim of assessing occupational/environmental exposure to microbiological agents, namely fungi and bacteria, in different environments. Environmental sampling campaigns were carried out as well as the laboratory activities involved in preparing and analysing the samples obtained. Sample processing consisted of applying classical microbiology techniques and using molecular biology methods as a complement.

The internship allowed me to consolidate the knowledge acquired during the first year of the course and to acquire other knowledge, new practical skills related to the area of environmental microbiology and to produce scientific work (articles, abstracts for conferences and scientific posters) and to reflect on other possible study projects.

KEY WORDS

Occupational exposure; Bioaerosols; Laboratory work; Environmental and occupational microbiology.

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ABBREVIATIONS

B – Bathroom

BS – Button Aerosol Sampler

BZ – Bench Zone

C – Classroom

Ca – Canteen

CA-MRSA – Community-Associated MRSA

cDNA – Complementary DNA

CFU – Colony-forming unit

DG18 – Dichloran Glycerol Agar

E – Exterior

EDC – Electrostatic Dust fall Collectors

EDCT – Electrostatic Dust fall Collectors of clothing

ESTeSL – Escola Superior de Tecnologias da Saúde de Lisboa

G – Gym

H&TRC – Health & Technology Research Center

HA-MRSA – Health-Associated MRSA

HPLC – High-Performed Liquid Chromatography

IAQ – Indoor Air Quality

ICH – InChildHealth

ITRA – Itraconazole

L – Library

MAC – MacConkey Agar

MEA – Malt Extract Agar

MM – MasterMix

MRSA – Methicilin-Resistetant Staphylococcus aureus

MZ – Machine Zone

OD – Optical Density

Of – Office

P – Probe

PBS – Phosphate buffered Saline

PF – Primer Forward

POSA – Posaconazole

PR – Primer Reverse

qPCR – Quantitative Polymerase Chain Reaction

S – Surface Swab

SAB – Sabouraud Dextrose Agar

SD – Settled Dust

SHO – International Symposium Occupational Safety and Hygiene

TSA – Tryptic Soy Agar

VORI – Voriconazole

VRBA – Violet Red Bile Agar

1. INTRODUCTION

During the second year of the master's degree in clinical-laboratory technologies at Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), an internship and a written report are treated as mandatory parts of the curriculum. These components enable students to build practical skills and learn how to apply theoretical knowledge in real-world settings, as well as develop their research and analytical skills through the presentation of a finished report.

Because of the nature of this internship, the format and organization of this report are not strictly conventional for a traditional report. These changes were included to present the specificities of this type of internship more clearly and to give a clearer and more complete picture of the experience.

1.1. Internship Objective

The internship was conducted based on the definition of specific objectives, established according to the possibilities offered by the location and in alignment with learning of the theoretical knowledge before and during the practical experience.

The primary aim was to carry out investigation in different indoor/occupational settings, focusing on microbial contamination exposure assessment. This encompassed the determination of the specific indoor environment to be assessed, exploration of the most effective sampling techniques and the choice of appropriate methods for microbial analysis, such as fungal identification and resistance profiling. This experience granted me my initial contact to research in the field of occupational and environmental health.

Critically evaluating real-life situations in the laboratory context, with a need for appropriate responses to challenges and obstacles encountered throughout the work, contributed to the promotion of safe and responsible practices.

1.2. Justification and Relevance of the Internship

This opportunity had a significant impact on the academic and professional career, as it was possible to apply some of the theoretical knowledge acquired during classes in the first year of the master's degree, in research projects that are still ongoing and which, in turn, are inserted within the area of study.

With the support and supervision of experienced researchers, it was possible to gain more experience, in the field of sample collection, in laboratory practice, and in scientific writing.

The real-life experience in a research lab allowed us to develop new soft skills, critical thinking, and a mind-set focused on solving problems in real time.

1.3. Description of the Internship Site

The internship lasted 600 hours and took place at ESTeSL (figure 1), more specifically at the Health and Technology Research Centre (H&TRC), in the Environmental and Occupational Microbiology Laboratory, from 11 September 2023 to 19 April 2024, under the guidance of Professor Carla Viegas and the supervision of her PhD students Renata Cervantes, Marta Dias, Bianca Gomes and Pedro Pena. I was subsequently given the opportunity to continue my internship on a voluntary basis, extending my internship until 20 June 2024. The attendance sheets are included in the appendixes (Appendix 1).

The H&TRC (Figure 2) has modern and well-equipped facilities and laboratories. The possibility of doing the internship component at this institution allows for permanent updating of knowledge, reinforcing scientific skills and specialisation in various areas of health. The pedagogical and scientific rigor is supported by the qualified staff with teach quality assurance. Due to its national and international recognition, its students are also always offered practical components in different professional environments from the beginning (*Apresentação | Escola Superior de Tecnologia Da Saúde de Lisboa*, n.d.).

In addition to the teaching aspect, there is also a research dimension, supported by the H&TRC and collaborations with renowned national and international research centers. The institution actively participates in international and national academic and professional networks, stimulating research activities. The H&TRC was created in 2016 to promote academic training, community service, research and the transfer of scientific and technological knowledge in a multidisciplinary environment. Its objectives are to develop new health technologies with the potential to improve diagnostic and therapeutic techniques currently used. (*H&TRC | Escola Superior de Tecnologia Da Saúde de Lisboa*, n.d.).

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Figure 2: ESTeSL logo

H&TRC
Health and Technology Research Center

Figure 1: H&TRC logo

2. BACKGROUND

2.1. Microbiologic agents

The Microbiologic agents are microorganisms including bacteria, viruses, fungi, and parasites that operate important functions in health, disease and the community. These agents can be pathogenic in nature and can result to a variety of infections and diseases in human beings, animals and plants (Sánchez et al., 2022). Knowledge of these agents is required to find suitable treatment approaches, prevention and protection strategies, and risk evaluation, as well as to progress in the fields of medical and environmental microbiology (CDC, 2003; Chiquito-Contreras et al., 2024; Kelsic et al., 2015).

Indoor airborne microorganisms have the potential of circulate in most of our living space (Hernandez & Martinez, 2018). The indoor exposure to elevated concentrations of microorganisms has been identified as main transmission routes for infectious diseases (Eames et al., 2009), resulting in a variety of health impacts via inhalation and ingestion. Moreover, human exposure to bioaerosols is correlated with a diverse range of respiratory health issues, originating from exposure in various occupational environments. In terms of the health implications of bioaerosols, fungi play a significant role. Elevated levels of fungal particles are discovered in a range of occupational settings including animal production and waste industries, among others (Douwes, 2005; Eduard et al., 2012; Heederik & von Mutius, 2012), thus indicating an increased risk of respiratory conditions among workers (Sabino et al., 2019).

Standardizing techniques are essential to ensure consistent and comparable results, enabling a more robust risk assessment. In Portugal, the current legislation, such as Decree-Law No. 102/2009, addresses occupational health and safety in general but does not establish specific limits for bioaerosols (Governo de Portugal, 2009).

The lack of regulatory thresholds complicates the assessment of acceptable fungal concentrations in indoor and professional environments. This highlights the importance of international guidelines and recommendations, such as those from the World Health Organisation (WHO), which can serve as a reference (WHO, 2022). Advances in molecular techniques, such as quantitative polymerase chain reaction

(qPCR), provide better sensitivity and specificity in the detection of fungi (Cox et al., 2017; Shorter et al., 2016; Taylor et al., 2019). However, clear detection thresholds adapted to Portuguese professional scenarios are needed, particularly in high-risks sectors, to support effective risk management strategies. By addressing the gaps, it becomes possible to enhance fungal monitoring practices, ensuring better protection for workers and general population.

Numerous research works has been evaluating the fungal load in various occupational settings to identify risk factors associated with adverse health impacts especially among the immune-compromised people (Eduard et al., 2012; Heederik & von Mutius, 2012; Sabino et al., 2019). However, our understanding of the impact of fungal exposure remains limited because the techniques for sampling and analysing fungi have not been standardized. Also, there is no threshold for fungal concentrations in indoor and occupational environments (Eduard et al., 2012; Heederik & von Mutius, 2012; Sabino et al., 2019).

Some of the important fungi in ecosystems are species capable of causing diseases like *Aspergillus fumigatus, Aspergillus flavus, Candida auris*, Mucorales, among others (Borman et al., 2021; Caetano et al., 2017; Revie et al., 2020; Sabino et al., 2019; WHO, 2022). Some fungi are producers of mycotoxins that, depending on the level of exposure, can lead, from nausea and vomiting, to immune deficiency and cancer, especially in humans (Alshammari, 2023; Haque et al., 2020; Heederik & von Mutius, 2012; S. Viegas et al., 2018, 2020). Infection caused by fungal bioaerosols is significant concern in working environment where the levels of these organisms are higher such as poultries, sawmills, wastewater treatment plants and others occupational environments that usually relate to polluted organic matter and are ideal for the growth of fungi. Also, there has been a rising danger of these infections related with the climate change impacts causing an increase of temperature and humidity, interfering with thermal resistance and availability of fungal species (Eduard et al., 2012; Lee et al., 2019; Seidel et al., 2024; Xiao et al., 2022). Consequently, fungal spores can disperse easily, leading to an increased concentration of bioaerosols in the atmosphere. Climatic factors promote the growth and spread of fungi across various regions, posing a significant threat not only in occupational scenarios but also to the general population's well-being. The conventional occupational exposure coupled with climate change that is fast accelerating increases the need to enhance surveillance and management of fungal loads indoor. By knowing these factors, it can be easy to protect people from the risk

of fungal infections (Eduard et al., 2012; Lee et al., 2019; Seidel et al., 2024; Xiao et al., 2022).

To provide necessary prevention and control measures, it is important to embrace the knowledge that deals with the subject of fungal contamination and its effect on health. In 2022, WHO published a list of priority fungal species stating the most critical fungal pathogens of public health concern such as Aspergillus fumigatus, Mucorales, between others that have been identified to be present in various environments and that cause significant harm to human health (WHO, 2022).

In the work performed by the research team I was working with, there is a particular emphasis on Aspergillus species due to its significant impact on public health.

2.1.1. Aspergillus sp.

Aspergillus can cause a range of respiratory illnesses upon aspiration of fungal spores especially in immunocompromised individuals. Although awareness about aspergillosis continues to grow, the mortality rates have not come down significantly — and this is so even today despite advancements in identifying the condition and providing effective treatment. As with any fungus, *Aspergillus fumigatus* is known to be the most frequent aetiological agent of aspergillosis, but other species are on the rise (Cadena et al., 2021).

More recently in March 2022, *Aspergillus fumigatus* was included among the 19 most threatening fungi prioritized by WHO which categorizes it into the critical category together with *Cryptococcus neoformans*, *Candida auris*, and *Candida albicans*. Amphotericin-B and other antifungal drugs resistance is today a growing problem globally and up to 20% of *Aspergillus* isolates show primary resistance to these drugs (WHO, 2022)

2.2. Exposure Assessment

Exposure assessment is crucial to determine the possible risks and consequences about human health because of various environmental factors. This process integrates the data from hazard identification, dose-response assessment, and exposure to determine the potential risks of human or

environmental health hazards to exposure (Chiquito-Contreras et al., 2024; Kelsic et al., 2015).

In this context, quantitative assessment of exposure is important to factor exposure and to assess exposure pathways as well as routes such as inhalation, ingestion and/or dermal. For instance, relating to microbial activities mainly the fungi such as *Aspergillus* spp. To this end, there are certain factors that should be clearly defined including the density of spores within the environment and the time and frequency of exposure to the substance. Synthesising data on the toxicity and pathogenicity of these fungi enables the comparative assessment of the risks posed by certain diseases, including allergic bronchopulmonary aspergillosis or invasive aspergillosis (Cadena et al., 2021; El-Baba et al., 2020; Ioakeim et al., 2024; C. Viegas et al., 2021).

Furthermore, exposure assessment primarily offers insights into the temporal and spatial fluctuations of exposure, thereby elucidating the variability of risk over time and across different locations. Such data is integral to risk assessment as it delineates the demographics susceptible to risk, the manner, timing, and routes of exposure. This knowledge is particularly critical for vulnerable populations such as children and the elderly, who may face heightened risks from these exposures. Additionally, uncertainty analysis is conducted to address data variability and assumptions, ensuring a more robust risk characterization. This comprehensive approach enables effective risk management strategies, guiding actions to mitigate potential health impacts (Cadena et al., 2021; El-Baba et al., 2020; Ioakeim et al., 2024; C. Viegas et al., 2021).

Indoor air quality sampling for exposure assessments requires precise data collection procedures that can sustain their accuracy (Cox et al., 2017). Sampling methods for the indoor air normally employs mechanical equipment like filters, pumps and air samplers to trap the airborne particulate and microorganisms. These methods can quantify the level of pollutants such as the fungal spores for instance the *Aspergillus* spp., within indoor spaces. Some aspects to quantify include the location of the sampling points, the sampling period and the sampling interval (Whitby et al., 2022). Effective air sampling techniques are, therefore, important to ensure that accurate samples are collected that show the true status of the indoor air quality and health risk implications required. It forms the baseline for creating exposure assessment that can be used to help in the management of risks (Cervantes et al., 2022; Cox et al., 2017; Whitby et al., 2022).

2.2.1. Sampling Methods

Passive methods can be used for prolonged sampling time without using any kind of powered equipment, making them easy to operate and portable. These approaches can include settled dust, filters, Electrostatic Dust fall Collectors (EDCs), surface swabs and others, which are mostly small volume and low biomass (Cervantes et al., 2022; Whitby et al., 2022).

During the sampling campaigns, an **EDC** is placed in each study area and will subsequently be replaced every 30 days. Two volunteers are also asked to wear an EDC on their clothing (**EDCT**). These EDCTs are collected at the end of the sampling campaign (Cervantes et al., 2022; C. Viegas et al., 2022a; Whitby et al., 2022).

To collect settled dust (SD), a vacuum cleaner with a coffee filter in the mouth of the vacuum cleaner is used to vacuum a small area to collect most of the dust and dirt in the area. This is considered a passive sampling method with respect to bioaerosols (Cervantes et al., 2022; Manibusan & Mainelis, 2022; Whitby et al., 2022).

At least three surface **swabs** (S) (figure 3) are used in each study area. Two are swiped on the floor and the door, respectively. The third is wiped over the workbench in the study area (Cervantes et al., 2022; Whitby et al., 2022).

Figure 3: Demonstration of surface swab sampling.

Taking samples of different environmental matrices, for example pieces of mop or mechanical protection gloves, is also part of passive methods (C. Viegas et al., 2022a).

In contrast, **active methods** require mechanical gears, which make them suitable for environments where low outbreaks of microorganisms are likely, as they provide high volumes and biomass yields (Whitby et al., 2022).

The active sampling methods normally used are the Andersen Six Stages, the Millipore air sampler, also known as the MAS-100 (Cervantes et al., 2022) and Button Aerosol Sampler.

The six-stage Andersen sampler (figure 4): designed to replicate the sampling of various regions of the human lung. It is valuable because it provides a detailed size distribution of airborne particles, which is essential for assessing health risks and environmental impacts (Cervantes et al., 2022; Xu & Yao, 2013).

Figure 4: Andersen Six-Stages (Anderson Six Stage Bioaerosol Impactor Used for Air Sampling, n.d.).

The **MAS-100** (figure 5) is used to collect airborne particles such as microorganisms, dust and other contaminants. It is commonly used in environmental monitoring, industrial hygiene and cleanroom environments to ensure indoor air quality and safety (Cervantes et al., 2022; Xu & Yao, 2013).

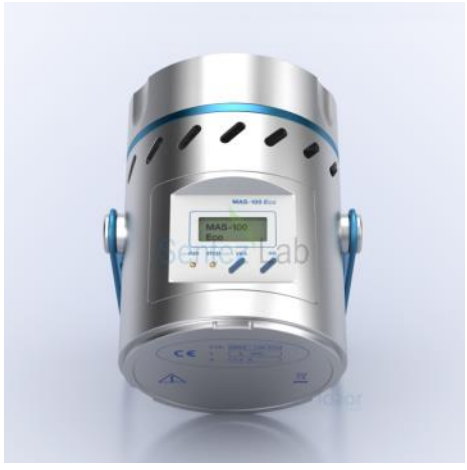

Figure 5: MAS – 100 (Microbial Air System – 100, n.d.).

Button Aerosol Sampler or Button Sampler (BS): is the device in figure 6, is used for the collection of airborne particulate matter for subsequent analysis. It is a versatile tool for collecting airborne particulate matter in various environments. Its design and operation ensure reliable and representative

sampling of particles for subsequent analysis, supporting a wide range of scientific, regulatory, and health-related applications (Whitby et al., 2022).

Figure 6: Button Aerosol Sampler

The **Particulate Matter** (figure 7) refers to solid particles in the air which may be bio-aerosols or non-bio-aerosols including dust, soot, mist etc. The identification of type and concentration, including bioaerosols, is important in determining risk to public health, setting allowable exposure limits, and determining proper air change and cleanliness to minimize the health effects to those exposed to these airborne particulates (Whitby et al., 2022).

Figure 7: Lighthouse Handheld Airborne Particle Counters (admin, 2023).

2.2.2. Sample Extraction

Sample extraction is a critical process in environmental science and is a process used to isolate and concentrate specific components or contaminants from various environmental media, such as soil, water, air, and dust, swabs, etc. The goal of this process is to prepare the samples for further analysis. The extractions performed on most of the collected passive samples are preformed into a liquid buffer (NaCl 0,9% + Tween 80 0,05%) which allows for the efficient

extraction of contaminants from dust particles to then be inoculated into the media plates (Caetano et al., 2017; Madsen et al., 2012; C. Viegas et al., 2020).

The **NaCl 0. 9% + Tween 80 0 A 5% buffer** is very commonly used in scientific research for the purpose of carrying out their characterization and sampling of microbial and particulate materials. The Sodium Chloride (NaCl) at 0,9% concentration for example enables water isotonicity a critical requirement for the extraction of microorganisms or particles without damage. The Tween 80 at 0,05 % concentration of it behaves as a wetting agent that helps to decrease the surface tension which in turn helps in proper dispersion/solubilization of particles in the solution. Thus, the use of NaCl and Tween 80 provides an appropriate environment for count and characterization of microbial loads and efficacy of sampling methods to isolate bioaerosol components from different samples for valid analysis and successful research studies (Caetano et al., 2017; Madsen et al., 2012; C. Viegas et al., 2020).

2.2.3. Assays

In microbiology, traditional methods primarily rely on cultivation techniques using different media to enable selective and differential growth, support enrichment, and facilitate biochemical identification of microorganisms as well as quantification of colony-forming units (CFU's), recover isolates, and classify specific microorganisms. For many years these procedures were employed extensively, and they continue to be used nowadays as a "gold standard" (Franco-Duarte et al., 2019).

2.2.2.1. Culture based methods

The preparation and handling of culture media must be precise to ensure the reliability and accuracy of experimental results. These media are essential for the growth and maintenance of microbial cultures, requiring an exact combination of nutrients and sterilization techniques to create the ideal environment for fungi and bacteria growth.

The next table contains the measurements for each medium that was used during the procedures.

Table 1: Measurements of each culture media.

Type of Culture media	Mass (g)	Glycerol Volume (ml)	H2O Volume (ml)	Total Volume (ml)
Tryptic Soy Agar (TSA)	**38**	-	950	950
Violet Red Bile Agar (VRBA)	**38.48**	-		
Malt Extract Agar (MEA)	**29.71**	2.23		
MacConkey Agar (MAC)	**47.5**	-		
Dichloran Glycerol Agar (DG18)	**23.63**	135	750	

Tryptic Soy Agar (TSA) can be used to culture and isolated bacteria from various origin including environmental samples. This technique is a normal practice for counting the number of CFU in each sample (Goh et al., 2022).

The Violet Red Bile Agar (VRBA) is used for selective cultivation of coliform that are suspected to be caused by fecal contamination. Bile salts and crystal violet present in the medium suppress the growth of gram-positive organisms while selectively encouraging the growth of certain gram-negative coliform bacteria. However, not all gram-negative are supported by this medium, as it is specifically designed to favor coliforms within broader group (Hervert et al., 2016; C. Viegas et al., 2023).

MacConkey Agar or **MAC** is a selective and differential culture medium that is used in the isolation and differentiation of the Gram-negative bacteria especially the members of the Enterobacteriaceae (Güley et al., 2022; Na et al., 2015).

Malt Extract Agar (MEA) is widely used to grow fungal cultures and for fungal maintenance on laboratories (Evangelista et al., 2021).

The Dichloran Glycerol Agar (DG18) is a selective and enumerative culture medium for xerophilic fungi, isolated from a low water activity substratum (Beuchat & Mann, 2016; Kujović et al., 2024).

MEA and DG18 are used to assess fungi, while TSA, VRBA and MAC are used for bacterial quantification (Güley et al., 2022; Na et al., 2015; C. Viegas et al., 2022b) The DG18 enables the growth of xerophilic fungi, i.e. those that have the ability to grow rapidly in unfavourable environments (C. Viegas et al., 2022b).

In general, azoles inhibit fungal development and decrease the population count of fungi (Kortei et al., 2023; Krishnan et al., 2023). Relying on them in agriculture, including the specific quantities listed in table 2, has been shown to cause environmental selection for resistance in pathogenic microorganisms, such as the saprophytic organism such as *Aspergillus fumigatus* (Toyotome, 2019). This resistance can lead to a decrease in the effectiveness of medical azoles in treating fungal infections (Revie et al., 2020; C. Viegas et al., 2023).

Table 2: Measurement of each azol.

Type of Culture media	Mass (g)	H2O Volume (ml)	Type of Azole	Azole Volume (ml)	Total Volume (ml)
Sabouraud Dextrose Agar (SAB)	61.75	950	-	-	950
			Itraconazole (ITRA)	7.5	
			Voriconazole (VORI)	3.8	
			Posaconazole (POSA)	0.95	

Sabouraud Dextrose Agar (SAB) is a culture medium used mainly for the isolation, growth and preservation of fungi such as yeasts and molds (Smithee et al., 2014).

According to EUCAST guidelines, **itraconazole** (ITRA), **voriconazole** (VORI), and **posaconazole** (POSA) are clinically and research-relevant azoles for *Aspergillus fumigatus*. These guidelines allow identification of fungal minimum inhibitory concentrations (MICs) and define breakpoints that help categorize isolates to improve treatment plans. All these antifungals are vital because of the difference in their substantially and their efficacy in combating *Aspergillus* species. Exposure to these drugs under EUCAST standards increases the understanding of resistance patterns, dosage, and impacts in invasive aspergillosis to obtain best-case patients' therapeutic success while preserving suitable levels of accuracy and credibility in terms of clinical and research applications (EUCAST, 2022; Mroczyńska et al., 2020).

The importance of **ITRA**, particularly in the treatment of *Aspergillus fumigatus*, is greater due to its ability to inhibit the P-450 enzyme crucial for the demethylation of sterol 14a. This action interferes with the synthesis of ergosterol,

a component of the fungal cell membrane. This study is important because there is emerging drug resistance that appears to be related to in vivo results measured in isolates with high MICs from patients with invasive aspergillosis. Studies like this help to understand pathogenicity and formulate better treatment plans (Denning et al., 1997; C. Viegas et al., 2023; Xie et al., 2024).

The first-line therapy is **VORI** for invasive aspergillosis brought about by the *Aspergillus* fungus particularly in immunocompromised patients (Maertens et al., 2021; Peng et al., 2018; Ries et al., 2021). The safety of VORI has been studied widely, and the results have shown that the drug is well tolerated in patient's despite of some side effects including visual changes. Because of plasma variability, therapeutic drug monitoring is advised for medication to improve dosing and control of toxicity, if any. Thus, VORI's long-term effectiveness and safety demonstrated in several clinical studies allows to consider it as one of the key drugs in the treatment of invasive aspergillosis, thus providing essential therapeutic option for patients with an effective antifungal treatment needed (Maertens et al., 2021).

POSA is majorly prescribed for the management of invasive aspergillosis in patients at high risk, including patients who have received an organ transplant, those undergoing intensive chemotherapy for diseases like acute myeloid leukaemia or myelodysplastic syndromes. This is only allowed in the situations where other treatments could not yield any result and used to treat invasive aspergillosis. Although this formulation of POSA appeared to be effective as a rescue treatment, it is not entirely clear how effective it is when used as a first-line therapy for invasive aspergillosis. Given that POSA was less effective than VORI in a case series of patients with invasive aspergillosis encountered in clinical practice, the two drugs are compared in randomised, equal-group sampling non-inferiority dose-ranging trials to investigate whether POSA has equal efficacy to VORI as the first-line therapy. The findings of the experiment showed that POSA has shown itself to be as effective and tolerated as well as VORI to be a first-line antifungal medication for invasive aspergillosis (Maertens et al., 2021; Stemler et al., 2023)

After preparing the culture medium and placing it on plates, the medium was allowed to solidify before inoculating the samples. Each sample is then inoculated once into each medium, as shown in figure 8.

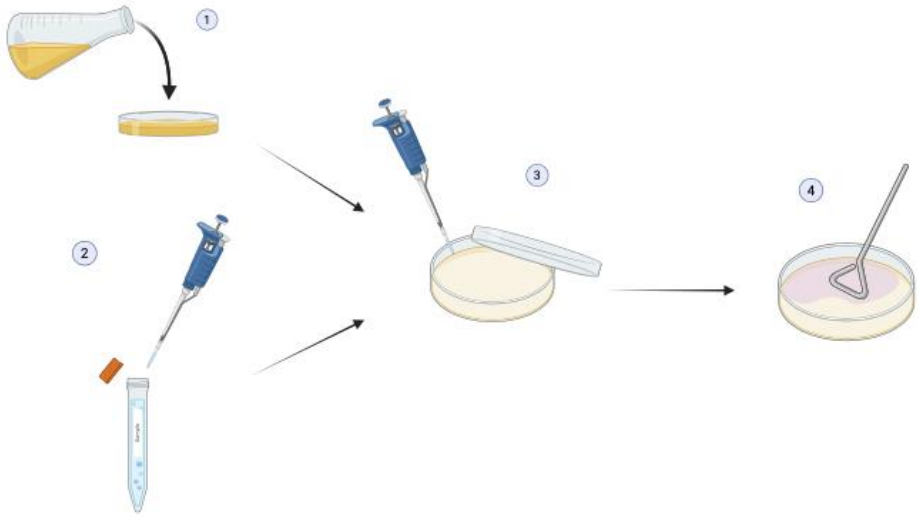

Figure 8: Inoculation.

The plates for evaluating fungal contamination (MEA and DG18) are incubated for 5 to 7 days, kept at 27ºC and for evaluating fungal pathogenicity others DG18 are incubated at 37ºC. Then, the different CFU's on each plate are counted and microscopically identified using lactophenol blue as a stain (C. Viegas et al., 2022b) as shown in figure 9.

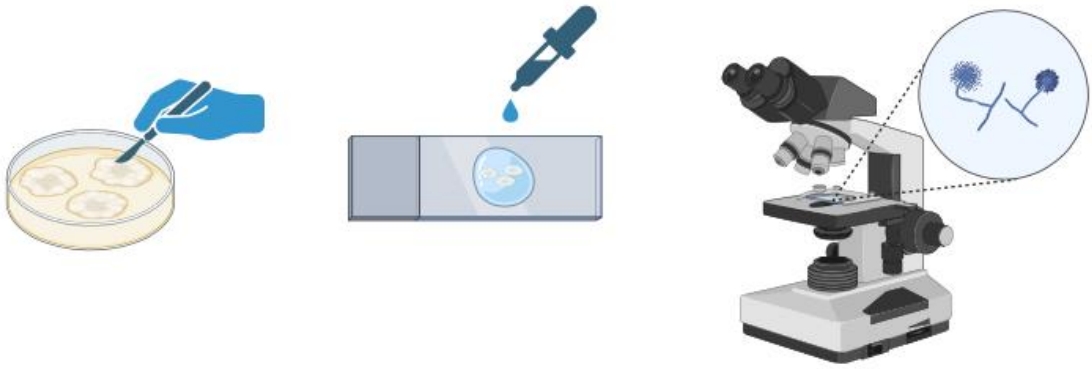

Figure 9: Fungal identification procedure.

For the bacteria contamination assessment (TSA) and Gram-negative bacteria contamination assessment (VRBA), are incubated at 30ºC and 37ºC, respectively, for 7 days (C. Viegas et al., 2022b), and MAC at 35ºC for 18-24h. Then all the colonies are counted.

Isolate recovery

The recovery of the fungal isolates has the following important responsibilities. First, it will enable the identification of susceptibilities of these isolates whereby it is possible to establish which antifungal drugs are effective in combating a

particular type of fungus; thus, providing clinicians with a tool to use in the right type of treatment against patients. Fungi contain data in how they react to various antifungal agents and from these guidelines it is possible to treat fungal infections and to keep databases of the alterations in antifungal resistiveness over time. This constant surveillance assists in establishing when fungi manage to develop resistance to certain drugs and allows alteration of the used treatment schemes. Further, it offers information on the level of toxicity of fungi, as well as its impact on lung and kidney epithelial cells, which allows us to better understand the harm that such fungi can cause. It also enables the identification of genera of fungi that pose potential threats, thereby leading to the formulation of preventive strategies in health facilities for staff and clients. Also, it guarantees that the healthcare facilities meet the legal norm in Portugal of maximum allowed concentration of fungal in health structures thus meeting the general health standards and reduce the probabilities of contamination. Finally, the ultimate vision is to perfect targeting therapy intervention to the fungi characteristics; thereby avoiding issues that arise from wrong techniques such as lack of efficacy or acquisition of resistance. Additionally, the management strategy of this approach is not only effective in enhancing a patient's health condition but also significant in guarding the health of the general public as it is sensitive to changes in threats posed by fungi in a healthcare setting (Borman et al., 2021; C. Viegas et al., 2021a).

To recover the isolates, the target fungi are first recognized and isolated on the medium plate. After that, Eppendorf tubes are made, with data of the *Aspergillus* spp. isolates collected in duplicate. Each Eppendorf is prepared with one ml of **phosphate buffered saline** (PBS), as shown in figure 10. With a loop, a piece of the described fungi is placed in the respective Eppendorf tube. After that, 250 µl of **glycerol** is added to each tube, and the isolates are archived at -80ºC.

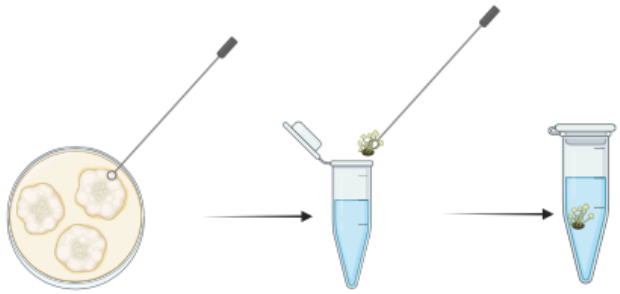

Figure 10: Isolate recovery.

The **PBS** is important in the preservation and study of fungal isolates, as it allows preserving the health and quality of isolates during storage and testing, as

it provides controlled conditions, increasing consistency and comparability in performances. In addition, it plays a crucial role in identifying susceptibility to antifungal agents and contributing to the evaluation of drug efficacy (Borman et al., 2021).

Adding **glycerol** to recovered isolates preserves their viability and integrity during ultra-low temperature storage, ensuring stability and reliability for future research and testing (Borman et al., 2021; C. Viegas et al., 2021a)

Screening

Screening helps identify which antifungal drugs are most effective against specific fungi, aiding in selecting the best treatment for patients. This allows researchers to understand how different fungi respond to various antifungal drugs, aiding in the development of treatment guidelines for fungal infections. By tracking isolates, scientists can gather data on antifungal resistance patterns, which is crucial for determining when a fungus is resistant to a specific drug. Regular screening helps monitor changes in antifungal resistance over time, allowing adjustments to treatment strategies to combat emerging resistance. This aids in personalized medicine by tailoring treatment plans based on the unique characteristics and susceptibility of isolated fungi, optimizing patient care and minimizing problems such as treatment failures or drug resistance (Borman et al., 2021; Sabino et al., 2014; C. Viegas et al., 2021a).

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA is resistant to any antibiotic that belongs to methicillin group or to any antibiotic that possesses the mecA gene. They lead to skin and other related diseases such as pneumonia and blood stream infections that are transmitted by hand-to-hand contact in healthcare or communal environments. Otherwise, it is called healthcare-associated MRSA (HA-MRSA) in hospitals; community-associated MRSA (CA-MRSA), which causes skin infections (Ribeiro, 2019). Management of infection, hand hygiene, screening, and compromising a patient's isolation are parts of infection control. Patients are given antibiotics including vancomycin for MRSA, linezolid or daptomycin for vancomycin resistant enterococci and ceftaroline for community acquired pneumonia. MRSA is worrisome due to its relation to hospital-acquired infections and community associated transmission, problems in eradication and health implications. It has good hygiene, wound care, cleanliness, and all aspects that relate to curbing new infections and spread of the diseases such as hand washing and isolation.

Therefore, effective management entails infection prevention, timely and correct diagnosis, as well as appropriate and rational utilization of antibiotics against this hard-nosed pathogen. Some researchers have investigated the ad hoc occurrence of MRSA on contact surfaces in a bid to determine the likelihood of transmission. Implementing the proper control measures, and proper use of antibiotics are some of the ways that can overcome these challenges (Gao et al., 2018; Parsons et al., 2023; Ribeiro, 2019; Xu et al., 2016)

Mycotoxins:

Mycotoxins are toxic compounds produced by certain types of fungi that can contaminate food and feed crops, posing significant health risks to humans and animals (Haque et al., 2020). Exposure to mycotoxins can occur through the ingestion of contaminated food, inhalation of airborne spores, or dermal contact with mouldy surfaces. Acute exposure may lead to symptoms such as nausea, vomiting, and acute liver damage, while chronic exposure has been linked to more severe conditions, including immune deficiency, cancer, and developmental issues (Alshammari, 2023). The impact of mycotoxin exposure is particularly concerning in regions with inadequate food storage and handling practices, where contamination rates tend to be higher (González-Curbelo & Kabak, 2023). Efforts to mitigate these risks include stringent agricultural and storage practices, regular monitoring of food products, and the development of mycotoxin detoxification techniques (González-Curbelo & Kabak, 2023).

The adverse health effects of human exposure to mycotoxins have been documented for certain mycotoxins, particularly when ingestion of contaminated food occurs. However, in the context of occupational exposure to mycotoxins, various exposure routes, including inhalation and dermal absorption, must be considered. Despite this, the health impacts following inhalation or skin contact with mycotoxins remain inadequately described (S. Viegas et al., 2018, 2020). Therefore, it is particularly relevant to properly characterize occupational exposure through the identification of the mycotoxins present, their levels, duration and main routes of exposure related to the specific occupational settings to understand the main determinants that may impact in workers´ exposure (S. Viegas et al., 2020).

For this assay, samples were extracted and will be analysed by partners (Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza) using high-performance liquid chromatography (HPLC) to detect 38 different mycotoxins. The analysis will use

scheduled multiple reaction monitoring in both negative and positive polarities for each sample.

Endotoxicity

Endotoxins are part of the outer membrane of the bacteria, specifically the Gram-negative bacteria. They are incorporated into the lipopolysaccharide embedded in the outer membrane of the bacteria. They can be released when the bacteria dies and the cell walls break apart, or during bacterial growth. Knowledge about endotoxins is vital especially today since managing and reducing their effects is a decisive factor influencing patient condition and the safety of administered drugs (Yang et al., 2022).

Cytotoxicity

Cytotoxins are molecules capable of harming or killing cells and are particularly applied in pharmacological research to neutralize specific toxins; in other words, cytotoxins are potent toxins. These molecules include such things as products of bacterial metabolism, plant related products, substances from higher animals and from chemical synthesis. Cytotoxins act by interfering with normal cellular processes of the target cell, and this often results in various forms of cell death including cytolysis, apoptosis or necrosis. To determine the extent and impact of cytotoxins researchers employ several cytotoxicity assays (Wood et al., 2023).

A cytotoxicity assay is functional micro test that is used to determine the extent to which a given compound is toxic to cells. These assays are essential in many branches of science such as pharmacology, toxicology, immunology, and clinical biochemistry. The information obtained from cytotoxicity assays is essential in forming new pharmacological agents, comprehending the toxic action and estimation of the toxic effects of chemicals and drugs (C. Viegas et al., 2022b; Wood et al., 2023).

In environmental science, these assays help in identifying the presence of toxics and in evaluating the effects of the pollutants on biological organisms. Therefore, cytotoxins are numerous compounds, which can promote cell death based on different pathways, and cytotoxicity tests represent valuable resources for evaluating these effects, they contribute to the progress of pharmacology, toxicology, and medical diagnostics as they help determine the safety of new substances (Galarneau et al., 2016; C. Viegas et al., 2022b).

2.2.2.2. Molecular Tools

DNA Extraction

DNA extraction is also performed for all samples, using commercial kits (the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) the use of commercial kits which are designed to simplify and standardize the process of isolating DNA from various environmental samples, ensures reliable and consistent isolation of genetic material, including optimized protocols and reagents that ensure efficient recovery of DNA, even from complex matrices containing inhibitors or low biomass, which is crucial for obtaining complementary data in environmental assessments (Ojo-Okunola et al., 2020; C. Viegas et al., 2022b)

Quantitative Polymerase Chain Reaction (qPCR)

Quantitative Polymerase Chain Reaction (qPCR) is a key tool in environmental science for accurately quantifying specific DNA or RNA sequences derived from environmental samples. This technique targets the DNA of organisms, such as fungi, amplifying specific sequences using highly specific primers and a thermostable DNA polymerase. During the amplification process, fluorescent signals increase in real-time, directly correlating with the initial amount of target DNA present in the sample. This real-time quantification capability makes qPCR an excellent tool for environmental samples, where it can be used to monitor microbial populations, detect pathogens, quantify pollutants, and track genetic markers of environmental health (Cox et al., 2017; Shorter et al., 2016; Taylor et al., 2019).

Flowchart of the entire process

I created a flowchart exemplifies all the steps involved in the analysis, from the preparation of the culture media and sample collection to the analyses. It shows what needs to be done to ensure that the collection, processing and analysis of samples is correct right from the sampling process and produces accurate and reliable results. The flowchart can be found in the appendices (appendix 2).

3. PROJECTS ONGOING

3.1. Projects Description

The Environmental and Occupational Microbiology Lab has several ongoing research projects such as:

InChildHealth (ICH): Identification of body burdens resulting from multipollutant (real-life scenario) indoor exposures and associated health effects, with specific focus on vulnerable population groups and sensitive life stages.

This project addresses the call HORIZON-HLTH-2021-ENVLTH-02-02: Indoor air quality and health. There are numerous and frequently ignored problems related to indoor air quality (IAQ) and its consequences for the health of children that can be solved with the help of ICH. IAQ is a significant concern for the development of health complications among children since they are more at risk than adults especially with respect to infectious diseases, allergies, and respiratory infections and are also more susceptible to damage on their brains leading to cognitive impairment. To avoid them, ICH is set to organize its intervention strategies at places where children spend most of their time including schools, homes and sports facilities. Also, the integration of the project indicators in terms of climate change pollutants guarantees its aim at new challenges in the environment.

Through its use of holistic IAQ solutions, easy-to-adopt guidelines, cheap IAQ monitoring technologies, and citizens science approach, ICH has the potential not just provide better IAQ but also create sharper public awareness of IAQ and better management and eventually improve public health by reducing costs of healthcare associated illnesses resulting from poor IAQ.

Guidance for Microbial Occupational Exposure Assessment in Sawmills.

The main objective of this project is to fill an existing gap in knowledge about microbial exposure in sawmills, that is, to increase guidelines for the assessment of occupational microbial exposure in sawmills. This involves detailed environmental monitoring to characterize this exposure to particulate matter, fungi, bacteria and organic metabolites and identify factors that influence these external exposures.

The project is essential because it seeks to protect the health of sawmill workers by assessing their exposure to microbial agents, contributing to effective

risk management in the timber industry. The project will provide essential guidelines for appropriate protective measures, supported by comprehensive data collection by understanding variations in exposure arising from the types of wood and biocides used. Their results will also play a solid foundation for future research, helping to promote broader understanding and improved safety practices in similar occupational environments.

The impact of animals bedding material on the sustainability of an industrial Portuguese poultry farm through a one health perspective.

The purposes of the current project include the evaluation of microbial exposure, and the risks associated with the use of animal bedding material on industrial poultry farms in Portugal as well as the investigation of stakeholders' knowledge and attitudes toward microbial threats and their mitigation.
Therefore, as the poultry farming affects animal welfare, human health, environment, and occupational health of the workers in the project, the project acquires very high importance.

From now on I'll refer to the projects as ICH project, Sawmills project and Poultries project, respectively.

3.2. Activities Developed

During the internship, my tasks ranged from helping to prepare, organize and participate in sampling campaigns and in the analysis of samples. The following sections provide a detailed account of the methodologies employed, samples collected, and laboratory assays performed for each project.

3.2.1. Sampling Campaign Preparation

Before each sampling campaign, thorough preparations were essential. The day prior, we ensured all equipment's batteries were fully charged and confirmed the availability of sufficient culture media needed for active sampling methods. A detailed checklist was created to avoid oversights, ensuring essentials such as sterile water for swab moistening, an adequate supply of EDCs for study sites and individuals, and sufficient NaCl 0,9% + Tween 80 0,05% for sample extraction.

The EDCs, along with the coffee filters for SD collection and the filters used in the personal BS, were sterilized in a *BioWizard Silver Line biosafety laminar flow*

hood under UV lights for 30 minutes. Subsequently, three EDCs were stapled to each booklet. The number of booklets, coffee filters for SD and the filters for BS prepared correspond to the number of sampling sites.

3.2.2. Sampling Campaign

All projects included two sampling campaigns, one during cold weather and one during warm weather, and all sampling methods were performed, including particulate matter.

In the **sawmills project**, I was only able to participate in the coldest season, due to the time when I started my internship. All sampling methods were employed at the following locations: bench area (BZ), machine area (MZ), exterior (E) and in the office (O), as figure 11 show.

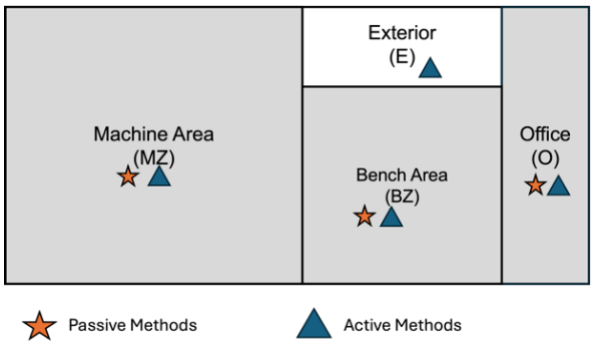

Figure 11: Sampling site of Sawmills project.

In the sampling campaign, an EDC was placed at each sampling site and replaced every 30 days. Two volunteers were asked to wear an EDCT, which was collected at the end of the campaign, and two others to wear the BS for about two hours. These volunteers had to be from different work areas. It was not always possible to get volunteers from completely different sampling sites, since in some sawmill's workers had multiple functions at different sites, or there was no distinction between sites.

Only one surface swab was used on the floor of each sampling site and the SD was performed at all sampling sites.

The active sampling methods used in this project were the same as those mentioned previously. The Andersen six-stage sampler was deployed five times at each sampling site. Although four different media were used, the DG18 was duplicated and subsequently incubated at 37 °C to estimate the pathogenic

potencial of the fungi. The MAS-100 sampler was used four times at each sampling site, once for each culture medium.

On the exterior of the sawmills, only MAS-100 and The Light House were performed.

In the **project ICH** I was able to participate in both seasons and carry out all active and passive methods. These were carried out in classrooms (C), in the canteen (Ca), in the library (L), in exterior areas (E), in bathrooms (B) and in gyms (G) if these were available, as figure 12 show. In addition to the sampling campaigns, this project also carried out citizen science campaigns.

In this project, the sampling sites were different because the structure of the school was not the same for all the presented above types. For instance, one of the schools which I visited did not have a gymnasium, the other had only one class, while the third school, had a gymnasium as well as four classes, though I did not get a chance to go this one.

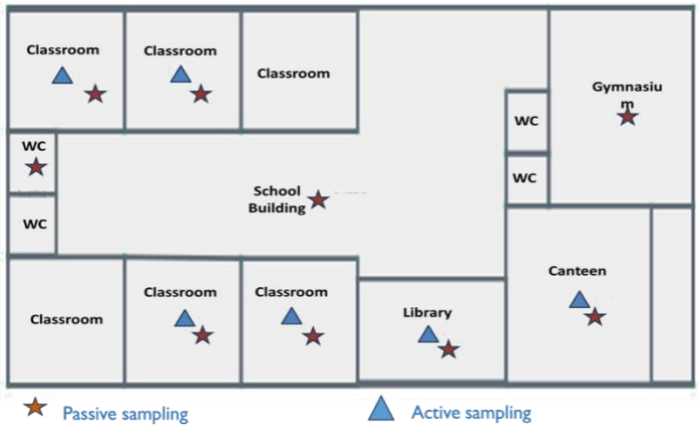

Figure 12: Sampling sites layout of ICH.

However, as in the Sawmills project, all the above sampling methods were used. At each sampling location, an information booklet with three different EDCs was left and replaced every 30 days, as figure 13 show. Regarding the selected students, in each of the two classrooms of each school I visited, one student was asked to wear an EDCT on their clothes, which was removed from the student at the end of the campaign. In addition, one teacher and one assistant were also asked to wear the BS for two hours.

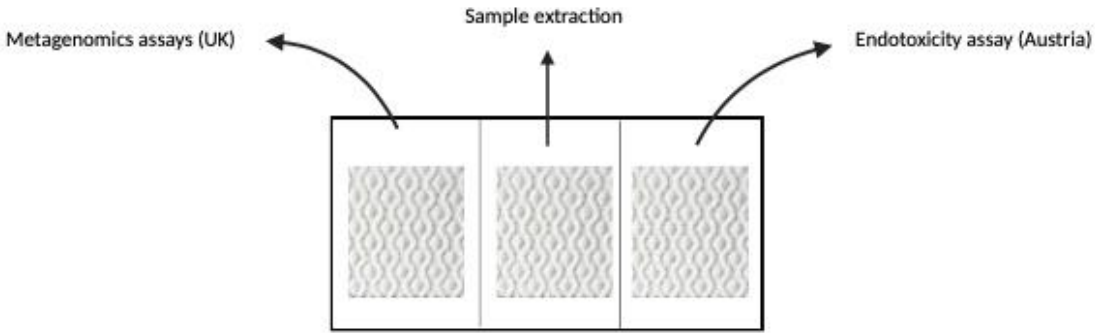

Figure 13: EDC booklet.

For each sampling site, three surface swabs were collected, except for the canteen. Regarding the application of swabs during lunchtime, one was used on the floor of the canteen and the other was used on one of the available tables while students were eating. At the other sampling sites, one swab was applied at the door while the other was applied on the floor near the door. The third swab, in the classroom, was collected from a student's desk and the other from the door of a cubicle, in the case of the bathroom.

SD was performed at all sampling sites except the bathrooms.

On the outside of the schools, only MAS-100 and The Light House were performed.

In this project, a piece of mop (figure 14) corresponding to each classroom was also collected for analysis. In the two schools I went to, both used the same mop to clean classrooms, bathrooms, canteens and libraries.

Figure 14: Bit of mop.

The active sampling methods employed in this project were identical to those previously described. The Andersen six-stage sampler was used five times at each sampling site. While four different media were utilized, DG18 was duplicated and incubated at 37°C to assess pathogenic fungi. The MAS-100

sampler was deployed four times at each sampling site, once for each culture medium.

All samples extraction were performed immediately after sampling to preserve sample integrity

Since the sampling campaigns for the **poultries project** was implemented in Madeira Island, I have only had the opportunity to participate in the laboratory assays.

The protocols for all sampling methods and sampling extraction are included in the attachments (attachment 1).

3.2.3. Citizen Science

Citizen science refers to actively involving members of the public, often referred to as citizen scientists, in scientific research processes (Guerrini & Contreras, 2020). The Project ICH used citizen science to involve the school community and children in studying air quality and health. Citizen scientists collect local data through observations and measurements, gaining hands-on skills and knowledge. This participation helps improve air quality by encouraging new behaviours and habits. Workshops and technology integration provide training and tools for making informed decisions and acting. By working together in a continuous feedback loop, these activities ensure that its solutions are effective and relevant to community needs (Cigarini et al., 2021; Poslad et al., 2022; Walsh et al., 2024).

Citizen science is one of the most differentiating parts of the ICH project. I was given the chance to actively participate in the citizen science activities, performing all the tasks involved.

In these activities, stations were set up with different tasks to be completed by the groups of students. The tasks consisted of sample extraction demonstrations, observing fungi under a microscope, filling out a child-friendly survey on indoor air quality in the school and demonstrating surface swab sampling. I engaged in all the activities, helping to bring them to life and supporting the students' learning process.

At the sample extraction station, the children were taught not only how to extract the samples from the EDCs, but also why and how all the steps of the

procedure are important and how we can extract data regarding IAQ from this sampling method. At the microscope identification station, they were able to observe fungal species and explained that although they are not visible to the naked eye, they are present in their environment. They were shown photographs of various fungi that had been identified from the school samples collected during the sampling campaign, in addition to the ones shown through the microscope, as figure 15 show.

At the sampling station with the surface swabs, it was explained that microbes can also be present on surfaces such as tables, chairs, blackboards, floors and so on. The students were asked to dip the swab in water and then pass it over a surface of their choice, after that they would inoculate the sample directly on a petri dish with culture media and allow the microorganisms to grow in their classroom for 1 week after which they would take photos and send them to Pedro and Renata for future activities regarding the project. The survey station was used to get a sense of the students' knowledge of the importance of IAQ and to raise awareness to the many factor that can influence the quality of the air in their classroom and their school in general, as shown in the following image.

Figure 15: Citizen Sciense

All these activities gave me the opportunity not only to learn more about the project and about indoor air quality, but also made me realise the importance of education directed to science from the early ages and how important can be to a project to involve the targeted communities.

3.2.4. Laboratory assays

In the lab, I was involved in practically every assay of each project, from sample extraction, DNA extraction and qPCR analysis, to inoculation, colony counting, cutting and recovery of properly identified isolates. These experiences were essential in deepening my understanding of the entire experimental process, from initial sample handling to final data analysis. I also had the unique opportunity to participate in the screening of *Aspergillus fumigatus*, for the Guidance for Microbial Occupational Exposure Assessment in Sawmills.

Samples Extraction

I had the opportunity to carry out sample extraction for the Sawmills and ICH projects, which I explain below, along with some differences in certain procedures.

In the **sawmills** and **ICH projects**, the sample extraction processes were nearly identical. Specifically, each EDC and/or EDCT was cut into two equal pieces. One piece was stored in a sterilized bag at -20ºC for mycotoxin assays. The other piece was placed in a 50 mL Falcon tube containing 20 mL of 0,9% NaCl and 0,05% Tween 80, which was then shaken at 250 rpm for 30 minutes.

After shaking, the piece was squeezed with tweezers to recover the maximum volume of liquid. The EDC was discarded, and 5 mL of the liquid was transferred to a new 15 mL Falcon tube and stored at -80ºC for the cytotoxicity assay. The remaining liquid in the 50 mL Falcon tube had 1,25 mL of glycerol added and was also stored at -80ºC for future inoculation. This whole process is shown in the figure below (figure 16).

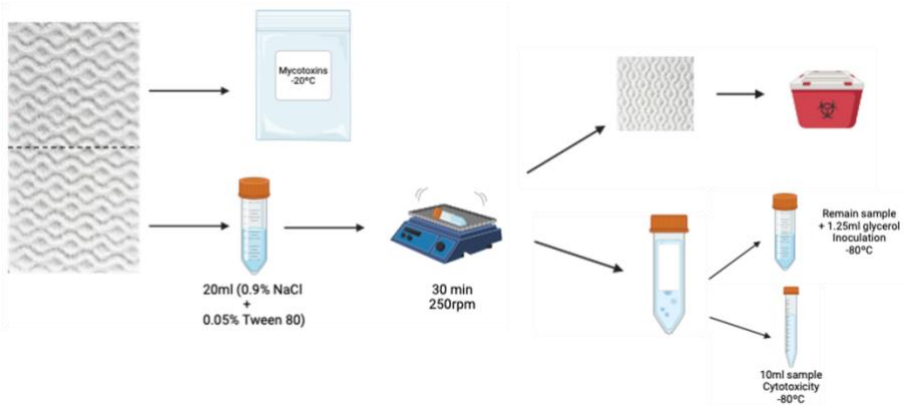

Figure 16: EDC or EDCT extraction procedure.

The collection process for the booklets in the ICH project was almost identical to that for the EDCs and EDCTs. Two EDCs from each booklet were stored in sterile bags to be sent to project partners for assays. The remaining EDC from the booklet was extracted following the same procedure as previously described.

Swab extraction involved preparing 2 mL Eppendorf tubes for each sample, each containing 1 mL of 0,9% NaCl and 0,05% Tween 80. The swabs were cut into the Eppendorf tubes and shaken at 250 rpm for 30 minutes. After shaking, the swabs were squeezed with tweezers to recover as much liquid as possible and then discarded. Finally, 0,25 mL of glycerol was added, and the samples were stored at -80ºC for later inoculation.

The SD was extracted in the same way as the coffee filter used to harvest it. Since the SD filter extraction was carried out in both projects but in a slightly different way, I will first explain the SD extraction from the sawmills project, then from the ICH project. Finally, I will detail the filter extraction, which was similar in both.

In the sawmills project, an empty 50 mL Falcon tube was weighed first, then reweighed with the SD inside. Then, 1 g of SD was weighed into a new 50 mL Falcon tube and stored at −20 °C for mycotoxin screening. Then, 1 g of SD was weighed twice more into separate 50 mL Falcon tubes, and 9,1 mL of 0,9% NaCl + 0,05% Tween 80 was added for each gram of SD, respectively. These tubes were shaken at 250 rpm for 30 min. One tube was then stored at −80 °C for cytotoxicity assays, and to the other, 2,3 mL of glycerol was added before storing at −80 °C for later inoculations. The remaining SD was stored at −20 °C for DNA extractions.

For the ICH project, a 50 mL Falcon tube was first weighed empty and then with the collected SD, and this weight was recorded. The Falcon tube was stored at -20ºC and later sent to a partner in the UK, for a metagenomic analysis.

Filter extraction involved cutting the filter into four pieces using disinfected scissors. Two pieces were stored in separate sterile bags for mycotoxin and endotoxin assays. The remaining two pieces were placed in separate 50 mL Falcon tubes, each containing 5 mL of 0,9% NaCl + 0,05% Tween 80. These tubes were shaken at 250 rpm for 30 minutes. After shaking, one tube was stored at -20ºC for cytotoxicity assays, while the other filter piece was removed with tweezers. After this, 1,25 mL of glycerol was added before storing at -80ºC for inoculations.

The extraction of the BS filter involved placing 15 mL of 0,9% NaCl + 0,05% Tween 80 in a 50 mL Falcon tube along with the filter. The mixture was shaken for one hour at 250 rpm. After shaking, the filter was removed and discarded using disinfected tweezers. Finally, 1,25 mL of glycerol was added to the solution, which was then stored at -80ºC for later inoculation.

As previously mentioned, in the ICH project, a small piece of mop was also collected. The extraction process involved cutting this piece into four similar pieces using disinfected scissors. Two of these pieces were stored separately in sterilized bags for mycotoxin and endotoxin assays. The bag for mycotoxin assays was stored at -20ºC, while the one for endotoxin assays was stored at -80ºC.

The remaining two pieces were each placed in a 15 mL Falcon tube prepared with 5 mL of 0.9% NaCl + 0.05% Tween 80. These Falcon tubes were shaken for 30 minutes at 250 rpm. Subsequently, one Falcon tube was stored at -20ºC for cytotoxicity assays. The other piece was squeezed and discarded using disinfected tweezers. Then, 1.25 mL of glycerol was added to the remaining solution, which was stored at -80ºC for inoculations.

Inoculation samples, Colony counting and identification

The Inoculation samples, Colony counting, and identification were done in the same way for all projects, so they are explained in general terms.

To assess microbial contamination, liquid samples were inoculated into each of the selected media: TSA, VRBA, MAC, MEA, and DG18. Using a 200 µL micropipette, 150 µL of the liquid sample was placed in each medium and spread carefully, without damaging the culture medium, with the help of a cell spreader.

To assess bacterial contamination, TSA plates were incubated at 30ºC. VRBA plates, intended for the evaluation of Gram-negative bacteria, were incubated at 37ºC for 7 days, while MAC plates were placed in an incubator set at 35ºC ± 2ºC for 18 to 24 hours. Colonies were counted after the respective incubation periods to determine the level of contamination.

Plates designated for fungal contamination assessment (MEA and DG18) were placed in 27°C incubators. Additionally, DG18 plates intended for assessing fungal pathogenic potential were specifically incubated at 37°C. After an incubation period of 5 to 7 days, colonies on the plates were counted, as it is shown in Figure 17. Subsequently, macroscopic identification of colonies and

their sections was performed. After colony counting and macroscopic identification, a small portion of the fungus was extracted from each colony. Using

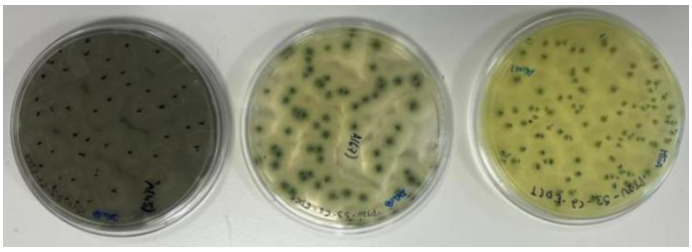

Figure 17: Assessment of fungal contamination at DG18 (27ºC and 37ºC) and MEA at the same sampling site.

a sterile scalpel and lactophenol blue cotton on a slide, the fungus was trimmed as closely as possible in preparation for subsequent microscopic identification. After microscopic examination, *Aspergillus* spp. and others fungal colonies, like Mucorales, were identified, isolated, and recovered.

To assess azole resistance, the extract was inoculated onto four types of media: standard SAB and SAB supplemented with ITRA (4 mg/ml), VORI (2 mg/ml) and POSA (0.5 mg/ml). This approach aimed to assess how the fungi responded to these antifungal agents. The samples were inoculated onto the plates in the same manner as previously described. They were then incubated at 27°C for up to 48 hours. During this incubation period, fungal colonies developed, providing insights into their ability to resist azole treatments. After incubation, the colonies were carefully examined and identified as on the media for fungal contamination. This microscopic analysis allowed the characterization of fungal species and the determination of any resistance patterns against azole compounds.

The use of different growth media is important for microbial evaluation due to the variation of nutrient needs of microorganisms. Adjusting growth media makes it possible to provide conducive growth environment for all the microorganisms and thus determine the true densities of microbial colonies. It also excludes the overgrowth or competition of one microbial group over the others all the time.

The Figure 18 shows a complete workflow of a methodical procedure. Starting from the sample extraction and up to the observation and identification process. This procedure takes an important role in getting right results and conclusions.

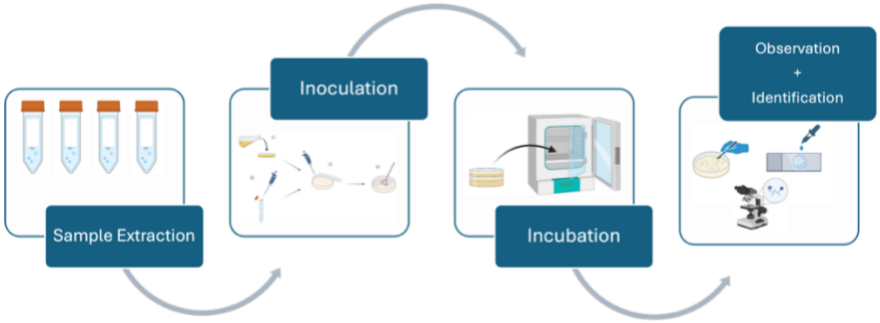

Figure 18: Procedure from sample extraction to observation and identification.

MRSA

As mentioned previously, bacterial quantification mainly targeted total bacteria, except in the ICH project, where special tests targeting MRSA were performed.

These tests consisted of inoculating extracted samples (as mentioned above) onto CHROMagar MRSA and incubating at 37°C for 24 to 48 hours. Colonies were observed at 24 and 48 hours. Colonies that grew pink/red were considered suspicious for MRSA and recorded, as figure 19 show.

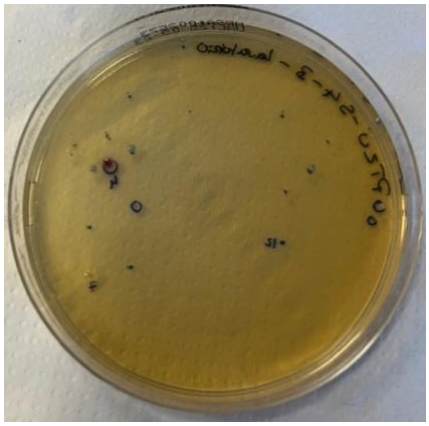

Figure 19: CHROMagar MRSA with two colonies.

CHROMagarTM MRSA was therefore the world's initial chromogenic media for the isolation of MRSA. Its colourless substrate is cleaved by an enzyme present in *S. aureas*, to yield a mauve-coloured product in MRSA positive colonies. Suspected cases are incubated at 37°C for 18-24 h, If positive a colony that ranges from pink to mauve colour is obtained.

Isolate Recovery

As in the previous topic, I had the opportunity to participate in the isolate recovery and it was done in the same way for all projects.

First, identify the isolates on the media plate that require recovery. Match each Eppendorf tube with the corresponding sample to be collected, ensuring duplicates are prepared for *Aspergillus* spp. Isolates (figure 20). Add 1 mL of PBS to each Eppendorf tube to preserve the fungal samples. Using an inoculation loop, transfer a sample of the identified fungi from the media plate into the designated Eppendorf tubes. To maintain viability, add 250 µL of glycerol to each

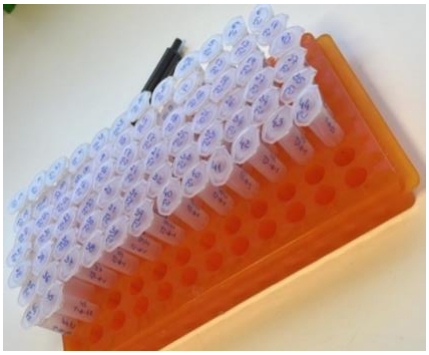

Figure 20: Preparation of Eppendorf's tubes for isolate recovery.

tube. Finally, store all isolates at -80ºC for future use and analysis.

DNA Extraction

As well as the two methods before, the DNA extraction method was also common for all projects.

Once the samples were thawed, they underwent centrifugation at 3,500 rpm for 30 minutes. For samples in Eppendorf tubes, centrifugation was adjusted to speed 10 for 5 minutes. The supernatant was then partially discarded, leaving the pellet intact. The pellet was resuspended with the remaining supernatant, and 200 µl of each sample was transferred into *ZR Bashing bead lysis tubes*. Following this, 750 µl of *bashing bead buffer* was added, and the tubes were vortexed at speed 4 for 10 minutes. Subsequently, the tubes were centrifuged at speed 10 for 1 minute in a microcentrifuge. Next, 600 µl of the supernatant was carefully transferred to *Zymo-Spin III filter columns* placed in collection tubes. The columns were centrifuged at speed 8 for 1 minute, after which they were discarded. To the filtrate from before, 1200 µl of *DNA Binding buffer* was added and thoroughly mixed. Then, 875 µl of the mixture was transferred *to Zymo-Spin 1L columns* in collection tubes and centrifuged at speed 10 for 1 minute. After discarding the liquid, the process was repeated. Following this, 200 µl of *DNA pre-wash buffer* was added to the columns and centrifuged at speed 10 for 1 minute, followed by the addition of 500 µl of *DNA wash buffer* and centrifugation

at the same speed for another minute. The columns were then transferred to Eppendorf tubes, and 200 µl of *DNA elution buffer* was added directly into the column matrix. After a 2-3 minutes incubation period, the tubes were spun at speed 10 for 30 seconds. This elution step was repeated, and the final 200 µl was divided into two additional Eppendorf tubes (100 µl and two 50 µl aliquots) for backup samples, all of which were stored at -20ºC.

The complete method details can be found in the attached documentation (attachment 2).

qPCR

I was able to conduct several qPCRs only for the sawmills project on *Aspergillus*, specifically the toxigenic portions encompassing the *circundati*, *flavus, nidulantes*, and *fumigati* areas. Prior to the allelic discrimination step, one had to establish the right concentration of supermix, primers, and the probe. Precautions to be followed before the procedure: All clothing, tools and work surfaces were required to be cleaned using 70% ethanol.

The qPCR procedure had 3 parts. In part I prepared the Primers, the Probe and the supermix. Each time, I wore gloves and cleaned the work surface, equipment and materials with 70% ethanol. I had to label four 1.5 mL Eppendorf tubes labelled for Primer Forward (PF), Primer Reverse (PR), Probe (P) and for MasterMix (MM). The primers were prepared by mixing primer and water in a 1:10 ratio (for 110 samples + 15% excesses: 10 µL of primer + 90 µL of water). For the probe, mix the probe and water (for 110 samples + 15% excess): Third, the volumes of probe and distilled water are 3 µL of probe and 27 µL of distilled water were used. Then, add the prepared probe, primers and supermix to the MM Eppendorf and shake for 15 seconds to mix the solution.

The second part consisted of preparing the PCR plate. I used a micropipette, to pipette 16 µL of MM into all the corresponding wells. For the samples I used 4 µL of each sample in each well respectively. In the positive control I used the same volume of a positive control, while in the negative control I added water. Then I sealed the reaction plate with a film and put the plate in the centrifuge for 30 seconds at 400 g. All reactions were performed in duplicates, therefore, the results obtained were always an average of the two measurements.

The final part was based on preparing the equipment, i.e. I started the qPCR machine and placed the PCR plate inside it. Then I proceeded to select the

parameters for the plate configuration: Detector (FAM), Quencher (None), Passive Reference (None), Run Mode (Standard) and Sample Volume (20 µL). I chose the respective cycling protocol and then started the PCR run. The result was exported after the cycle was completed.

This method was used to get an accurate confirmation of presence of *Aspergillus* on the samples.

***Aspergillus fumigatus* Screening for Azole Resistance and Antifungal Susceptibility Testing**

As a follow-up to the extraction and qPCR confirmation of Aspergillus spp. in the isolates, the antifungal susceptibility test was carried out as follows. In the first stage, the isolates were sown on DG18 culture medium and incubated at 27°C for 2-5 days to obtain individual colonies. For each isolate, a suspension was made by adding 250 µL of 0,9% NaCl with half of the pure colony. Then 200 µL of this solution was transferred to a new Eppendorf tube.

To measure the Optical Density (OD) at 600 nm, 100 µL of the solution was combined with 900 µL of 0,9% NaCl in a cuvette (figure 21). The OD was adjusted to 0,6 using the formula: (0,06 x 100)/OD.

A new Eppendorf tube containing the adjusted OD was prepared.

Next, 400 µL of the Aspergillus purified colonies were inoculated into RPMI 1640 medium with 2% dextrose. The E-test method was employed for testing ITRA, VORI, POSA, and Amphotericin. The cultures were incubated at 35°C, and the MIC was determined at 24 and 48 hours (figure 22).

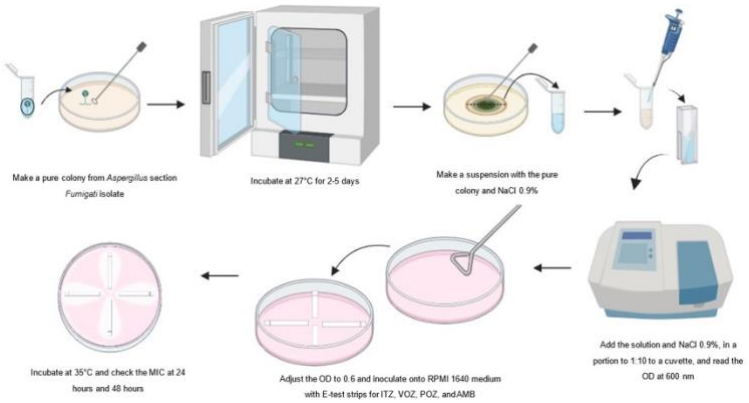

Figure 21: Screening procedure.

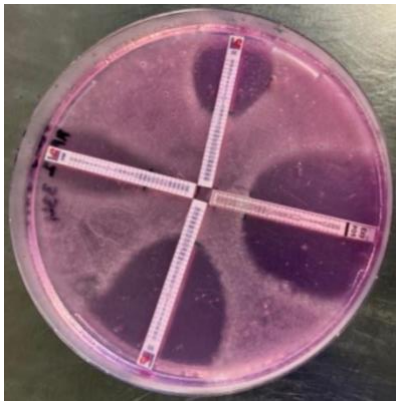

Figure 22: Screening result example.

4. SCIENTIFIC PRODUCTION

Throughout the internship I had the opportunity to take part in various tasks necessary for scientific research. From writing a scientific paper to taking part in conferences.

4.1. Scope Review on Wastewater Treatment Plants (WWTP)

Besides laboratory work, together with two other colleagues, I had the opportunity to write an article with the aim of carrying out a scoping review to provide a comprehensive overview of the current methods used to assess exposure to microbial contamination in wastewater treatment plants (WWTPs) named *Filling the Knowledge Gap Regarding Microbial Occupational Exposure Assessment in Wastewater Treatment Plants: A Scoping Review.* The study aimed to identify common protocols, standardized information retrieval and suitable microbial indicators to assess harmful microbial exposure, thus addressing the existing gaps in the assessment of occupational exposure in WWTPs (**Riesenberger** et al., 2024). The full reference for this paper is as follows:

***Riesenberger B**, Rodriguez M, Marques L, Cervantes R, Gomes B, Dias M, Pena P, Ribeiro E, Viegas C. Filling the Knowledge Gap Regarding Microbial Occupational Exposure Assessment in Wastewater Treatment Plants: A Scoping Review. Microorganisms. 2024 Jun 4;12(6):1144. https://doi.org/10.3390/microorganisms12061144 . (Appendix 7)*

The paper follows the PRISMA guidelines and criteria for review and considered data from the year 2010 to 2023 on the microscopic organisms present in WWTPs. Using bacteria, fungi, viruses as the key terms was accompanied by including only the studies published in English language, as well as the terms exposure and sampling were used as additional search terms. The electronic databases used were PubMed, Scopus, and Web of Science; converted articles were selected with the Rayyan tool. Screening involved three rounds: titles, abstracts, and manuscripts and included the evaluation of titles and abstracts only. Subthemes and data extracted for analysis involved database description, country, sampling and/or selection methods, major findings, and references, where differences were discussed within the team during coding (Hutton et al., 2015; Moher et al., 2009).

To contribute to the paper, I initially used the terms "Wastewater Treatment Plants", "bacteria", "fungi", "viruses", "exposure" and "sampling", with inclusion criteria limited to studies in English, in the Scopus database, excluding all articles that did not meet our criteria. The Rayyan-Intelligent systematic review tool was then used to select articles, speeding up the screening and selection process for academics involved in systematic reviews. This screening involved three rounds: first we assessed the titles to detect duplicates or irrelevant articles, then we went on to review the abstracts and, finally, we assessed the full article. In the case of articles that raised doubts, these were resolved through team discussion and consensus.

As the articles were analysed, a table was filled in with data on the articles, which was then used to write the paper and subsequent discussions. Afterwards, we moved on to writing the paper where, as well as being involved in writing the introduction, together with my colleagues, I assessed the risks of bias in each article seen, focusing mainly on the sampling methods and assays described in them. This analysis made it possible to guarantee the robustness and reliability of the results obtained.

4.2. Conference of International Symposium Occupational Safety and Hygiene

Beside the article I also had the opportunity to participate at the conference of International Symposium Occupational Safety and Hygiene (SHO). This conference is organized by the Sociedade Portuguesa de Segurança e Higiene Ocupacionais embraces the practice of creating awareness, training, and sharing on the set standards concerning workplace safety. Its main activities are the promotion of research and development in occupational health and safety, cooperation with governments, and sharing of contacts with other similar institutions.

It took place in Porto, and I had the opportunity to give a presentation on *Understanding and addressing the risks of exposure to fungi in primary schools: Implications for children's health and well-being*, which consisted of raising awareness of the importance of indoor air quality in schools and its monitoring, in favour of the health of children, who are more vulnerable to illness, as it is showed in the following figure.

Figure 23: Presentation at SHO.

I also contributed to the preparation of other oral communications and posters that were also part of this conference, as mentioned below.

- **Oral Communications:**

*Cervantes, R.; Pena, P.; Gomes, B.; Dias, M.**;* ***Riesenberger, B****.; Margarida, R.; Marques, L.; Viegas, C. (2024) Understanding and Addressing Fungal Exposure Risks in Primary Schools: Implications for Children's Health and Wellbeing. International conference of Occupational safety and Hygiene (SHO 2024*). Awarded as the best proceeding paper. (Appendix 8)

Dias, M.; Gomes, B.; Pena, P.; Cervantes, R.; Rodriguez, M.; ***Riesenberger, B.****; Marques, L.; Ribeiro, E.; Viegas, C. (2024) Sampling protocol to assess Aspergillus section Fumigati in woodworking environments. International conference of Occupational safety and Hygiene (SHO 2024).*

Gomes, B.; Dias, M.; Pena, P.; Cervantes, R.; Rodriguez, M.; Marques, L.; ***Riesenberger B.****; Viegas, C. (2024) Levels of fungi in the air of poultry farms following different stages of birds´ growth cycle. International conference of Occupational safety and Hygiene (SHO 2024).*

Pena, P.; Cervantes, R.; Dias, M.; Gomes, B.; ***Riesenberger, B.****; Marques, L.; Rodriguez, M.; Viegas, C. (2024). Preliminary results concerning school staff personal exposure to microbial load – Worry to be considered? International conference of Occupational safety and Hygiene (SHO 2024).*

- **Posters:**

Dias, M.; Rodriguez, M.; Vasques, C.; ***Riesenberger, B****.; Marques, L.; Gomes, B.; Pena, P.; Cervantes, R.; Viegas, S.; Viegas, C. (2024) Budget-friendly protocol for TR34/L98H and TR46/Y121F/T289A mutation detection in Aspergillus section Fumigati isolates. International conference of Occupational safety and Hygiene (SHO 2024).* Awarded the honourable mention for the abstract (Appendix 11).

Gomes, B.; Dias, M.; Pena, P.; Cervantes, R.; Rodriguez, M.; Marques, L.; ***Riesenberger, B.****; Viegas, C. (2024) A multi-approach sampling strategy to assess exposure to microbiologic agents in poultries. International conference of Occupational safety and Hygiene (SHO 2024)* (Appendix 10).

Cervantes, R.; Pena, P.; ***Riesenberger, B.****; Marques, L.; Rodriguez, M.; Gomes, B.; Dias, M.; Viegas, C. (2024) Fungal Contamination in Lisbon's Primary Schools - Sampling Insights and Analytical Approaches. International conference of Occupational safety and Hygiene (SHO 2024)* (Appendix 9).

The certificate of participation in the conference can be found in the appendices (Appendix 12)

4.3. VI H&TRC BootCamp 2024

The aim of this scientific meeting was to introduce researchers to ongoing projects, promote collaborations between them and define the strategic lines/projects to be developed in the coming year.

It was therefore proposed that all Integrated Members and Collaborators submit an abstract to the VI H&TRC BootCamp 2024 to present the results of ongoing H&TRC projects.

For this bootcamp I submitted an abstract on the article on WWTPs and participated in the abstracts submitted on the ICH, Sawmills and Poultries projects. They are all included in the appendices (appendixes 13, 14, 15 and 16).

I was also able to help prepare an abstract that was submitted to the EPICOH conference (Appendix 17).

5. ACTIVITIES TIMELINE

Throughout the report I have seen all the steps, responsibilities and tasks that have been proposed to me, which have allowed me to hone important skills and grow professionally. As described above, these activities ranged from laboratory work, which involved experiments and tests to gain practical experience, to reading and writing scientific literature, and I was also extensively involved in fieldwork activities, such as sampling campaigns and citizen science programmes, which helped me grow personally and professionally, i.e. not only did they increase my practical experience and skills, but I was also exposed to challenges that took me out of my comfort zone.

The following chronogram outlines the key activities achieved throughout my internship.

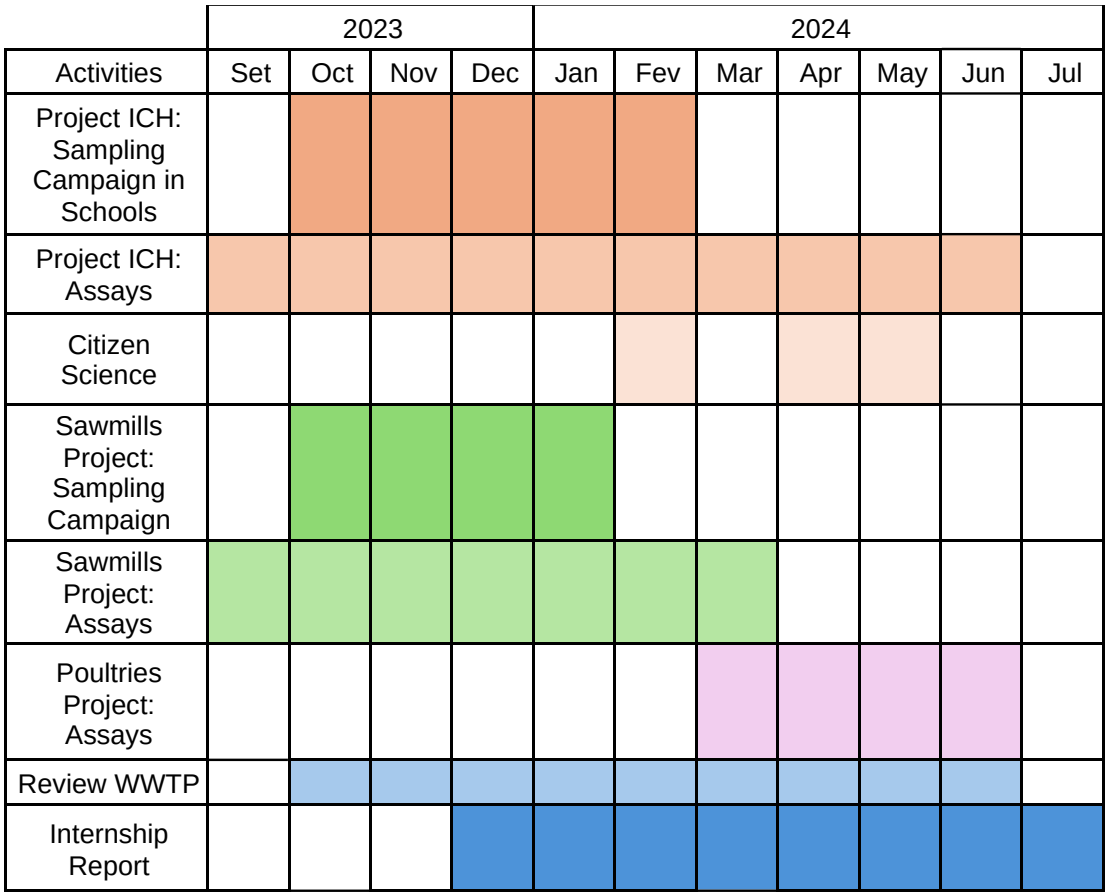

Figure 24: Activities timeline.

It should be noted that some of the scientific productions is not included in the timetable because it took place in a short space of time compared to the other activities.

6. DISCUSSION AND CRITICAL REFLECTION

As has been demonstrated, in my internship, I had the opportunity to work on three distinct projects focused on investigating indoor air quality and fungi assessment contamination. These were intended to check and enhance environmental quality in various contexts.

The Importance of Indoor Air Quality

It's important to know how good the indoor air is so that people are healthy in their homes, as well as for employers to provide healthy workstations. Poor quality air significantly affects the human health, causing big issues especially the respiratory system, the skin and other body organs. If we try to understand and control poor indoor air quality and look for ways to eliminate or reduce these health conditions, people will definitely become healthier (Cervantes et al., 2022; C. Viegas et al., 2022b; Whitby et al., 2022).

The Role of Different Sampling Methods

I was able to understand the importance of using different sampling methods because this ensures a more accurate and comprehensive assessment of occupational exposure, optimizing sampling of different environments through their advantages and reduces bias, allowing to obtain a representation of the microbial composition of the environment close to the reality (Cervantes et al., 2022; C. Viegas et al., 2022b; Whitby et al., 2022).

Passive methods are simple, inexpensive, easy to handle and maintain, are not energy-intensive or energy-dependent, allow them to be applied over long periods and thus obtain a comprehensive representation of bioaerosol levels. The active methods allow the enumeration of bioaerosol concentrations, which makes them effective in environments with low microbial levels, retaining larger volumes of air, regardless of the sizes of the units that compose it, complementing the "small details" in the research. (Cervantes et al., 2022; Whitby et al., 2022).

Different Types of Sampling Strategies

The EDCs, EDCTs and booklets are effective in collecting airborne dust and debris for a long term without the need for energy or other materials (Cervantes et al., 2022; Whitby et al., 2022). The Surface swabs are simple to handle, allowing routine assessments and providing insights into specific areas where fungal contamination is concentrated, though they are limited to surfaces, as they

may not collect airborne spores (Cervantes et al., 2022; Whitby et al., 2022). The SD collection allows for a composite sample, providing a more integrated picture of fungal contamination, and is easy to handle, but it is not effective in capturing all airborne elements, potentially underestimating fungal exposure levels (Cervantes et al., 2022; Whitby et al., 2022).

The BS are small, easy to transport and used mainly indoors. They allow for data collection of airborne elements without complex preparation but cannot capture as much sample as larger air samplers, such as the Andersen six-stage, that it separates particles by size, providing detailed data information about particle distribution within our lungs, as figure 25 show. Although is more expensive and complex. While effective for virtually all particles, it may not capture ultrafine or large particles effectively. (Cervantes et al., 2022; Whitby et al., 2022).

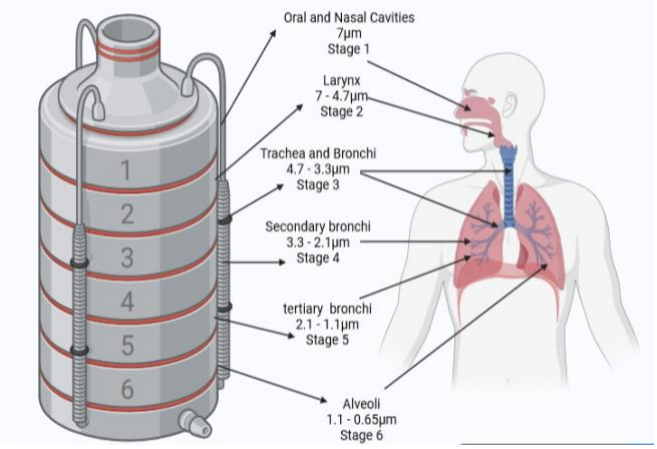

Figure 25: Diagram of Andersen Six-stages of the respiratory tract.

The MAS-100 is specifically designed for rapid collection of airborne bacteria and fungi using an impact method on the growth medium, which helps to provide accurate quantitative data on microbial contamination and allows live air to be checked for quality and presence of microbes. It is primarily used for microbial sampling, making it less efficient when sampling larger elements. The cost of purchasing and maintaining the equipment is expensive. (Cervantes et al., 2022; Whitby et al., 2022).

The Lighthouse particle monitors are very consistent when it comes to real-time tracking of particle mass per cm^3 of air across a variety of particle sizes, as well as indicating the humidity and temperature at the time of measurement. While capturing data, you can immediately check it in real time. One drawback to Lighthouse is the purchase cost, which can be quite expensive for high-end models, and maintenance and meter calibration are essential for regular readings. While useful in determining particle concentration, these monitors can

be somewhat limited in their ability to detect specific particle species or differentiate between different types of particles. (Cervantes et al., 2022; Whitby et al., 2022). A table summarising the advantages and disadvantages of all the methodologies can be found in the appendixes (appendixes 3,4 and 5).

The Importance of Using Many Culture Media for Bacteria and Fungi

I was also able to understand the importance of using more than one media for bacteria and fungi helps to separate one from the other and the result is more accurate counting of your colonies. Furthermore, the detection sensitivity is improved as each of them is created to fulfil the growth parameters of specific bacteria and fungi. Again, the use of specific growth media reduces the interference of different microbial groups in each environment, which in turn reduces contamination levels in microbial examination results of sample samples (Cervantes et al., 2022; Whitby et al., 2022).

TSA was used because allows flexibility in evaluating different bioaerosol samples for contamination and is rich in nutrients that allow the growth of a wide range of bacteria. But it is not a selective medium, which sometimes makes microbial assessments difficult. Because it is rich in nutrients, there was excessive growth and growth of other colonies, such as fungi and yeast, which made it difficult to quantify bacteria in the samples (Cervantes et al., 2022; Whitby et al., 2022).

VRBA was used in the sampling because, unlike TSA, it is selective, facilitates the growth of gram-negative bacteria and inhibits that of gram-positive bacteria, so it was possible to make a more specific assessment of contamination. This contains indicators such as lactose, neutral red and bile salts that help in the detection of coliform bacteria. This provides reliable and accurate results on bacterial contamination in the sample. However, as it is selective, it limits its versatility for the assessment of total bacterial contamination in the sample. Since it is selective for coliform bacteria, it creates the possibility of non-growth of other bacteria in the sample, "hiding" the existence of possible crucial bacteria (Cervantes et al., 2022; Whitby et al., 2022).

MAC was used because, like VRBA, it is a selective medium, but it favours the growth of only gram-negative bacteria and is an indicator of pathogenic bacteria for the health of animals and humans, such as some species of *Escherichia coli* and *Salmonella*. The problem with its use is that although it helps in the differentiation of lactose fermenting bacteria, some may exhibit atypical colonial

morphologies, making it difficult to evaluate their appearance (Cervantes et al., 2022; Whitby et al., 2022).

MEA was used because it allows the growth of a wide variety of fungi, has the right nutrients for practically all, ideal for both fast-growing and slow-growing fungi. However, it is more susceptible to contamination and since it is not selective, it allows the growth of various fungi, making their identification difficult (Cervantes et al., 2022; Whitby et al., 2022).

To complement the results of the MEA, DG18 was also used, which, in addition to allowing rapid growth of fungi, only allows the growth of xerophilic fungi and the detection of many fungi that can be potential health risks and facilitates their identification due to their characteristics when growing in this environment. But identifying fungi through their growth in the environment can be difficult, requiring some experience in distinguishing between them (Cervantes et al., 2022; Whitby et al., 2022). A table outlining the pros and cons of each growth media is available in the appendixes (appendixes 6).

The Task of CHROMagarTM MRSA in Microbial Detection

When it comes to evaluating MRSA, I have realised that this culture media considerably improves the reduction in time needed to identify the presence of methicillin-resistant *Staphylococcus aureus* and the workload, which has led to the generalisation of screening. It is highly accurate and has been reported as one of the best methods in much research works as compared to other methods of diagnosis. About 24 h of incubation are required to obtain the highest sensitivity and specificity of the method. Thus, CHROMagarTM MRSA is highly effective and reliable method for detection of MRSA (Gao et al., 2018; Ribeiro, 2019; Xu et al., 2016)

The Importance of Incubation in the Respective Temperatures

It is important to incubate the samples in the respective temperatures because is a crucial physical factor that directly affects microorganism growth by influencing their physiological functions (Heinsohn, 2005). Fungi present in the environment, including xerophilic fungi, grow at a moderate temperature, such as 25-27ºC, which corresponds to room temperature (Gordon et al., 2014; C. Viegas et al., 2021b). The ideal temperature for incubating bacteria is between 30-35ºC (Gordon et al., 2014; Gutierrez et al., 2018; C. Viegas et al., 2021b). Incubations between 35º-37º are important to check for microorganisms with

pathogenic capacity, as these temperatures are like the temperature of the human body (Gutierrez et al., 2018; C. Viegas et al., 2021b).

The Importance of *Aspergillus* sp. Isolation and Significance of Molecular Tools in Early Detection

I realized that the isolation of *Aspergillus fumigatus* as well as section *flavus* forms part of the important steps of epidemiological research and clinical approach to fungal infections. These isolates give species identification and description of the possible pathogens (Borman et al., 2021; C. Viegas et al., 2021).

Consequently, applying qPCR, it was still possible to detect *Aspergillus* sp. they may not be detectable by conventional means, thus, enabling the assessment of possible infections at an early stage, and achieving better efficiency (Cox et al., 2017; Shorter et al., 2016; Taylor et al., 2019).

With the presence of *Aspergillus* is confirmed through qPCR, it is possible to think further anti-fungal treatment approaches can be tailored based on the several species' susceptibility by the screening, thereby reducing the number of infections (Borman et al., 2021; Cox et al., 2017; Shorter et al., 2016; Taylor et al., 2019; C. Viegas et al., 2021a).

Thus, the integration of applied culture methods with molecular tools such as qPCR guarantees a well-articulated technique for the identification of *Aspergillus* sp. allowing an assessment of the exposure as close as possible to reality.

Advancements In Diagnostic Equipment and Procedures

Besides, the development of new equipment and the efforts to implement those techniques which are still only in guidelines, must go on, to solve the existing issues and to enhance the efficiency, and the speed of diagnostics. Regarding the emerging threat of partially resistant fungal infections, it is necessary to strengthen research and the training of qualified personnel that will implement effective screening programs.

7. RESEARCH WORK PROPOSAL IN THE FIELD

7.1. Introduction

As stated throughout this report, indoor air quality has been of concern due to potential health risks to all individuals, exposing them to bioaerosols, which are a source of health risks as they are capable of proliferating in humid environments, ventilation systems, water treatments or for sporting activities, being released into the air. As these are common environments in workplaces, schools and even swimming pools, among others, it is necessary to understand what the sources of contamination are and what risks they may have on our health (Eduard et al., 2012; Gollakota et al., 2021; C. Viegas et al., 2024; Whitby et al., 2022).

Having been involved in three projects on this subject, the question occurred about workers working in indoor swimming pools, considering that it is one of the most humid workplaces, with the continuous use of chemicals for water treatment, which makes it a perfect environment for the proliferation and development of bioaerosols knowing that these can be pathogenic fungi for people.
So, how do the conditions in indoor swimming pools contribute to the proliferation of bioaerosols, and what specific health risks do these poses to workers?

To obtain an answer to the question posed above, it will be necessary to evaluate the exposure to bioaerosols of workers in indoor swimming pools in Portugal. More specifically, bioaerosol levels will be quantified in different areas of the pools and compared with recommendations. Furthermore, the environmental factors that contribute to the presence and proliferation of bioaerosols would be identified and, consequently, the potential health risks associated with worker exposure would be assessed.

How does a comprehensive sampling approach assess the exposure of workers to bioaerosols in indoor swimming pools in Portugal?

7.2. State of the art

To support this hypothesis, a small and brief systematic review was carried out. The words *Swimming pools* and *bioaerosols exposure* were used, with the AND condition, in the advanced search of the *PubMed* search engine, leaving only one article in total, focusing in fitness centers, named *Assessment of Portuguese fitness centers: Bridging the knowledge gap on harmful microbial contamination with focus on fungi* (C. Viegas et al., 2024).

Unsatisfied with this research, I did an general research that has been carried out on the environments of indoor swimming pools and their characteristics, focusing on the chloramines formed from swimming pool exposure poses a great risk to the respiratory functions of the workers, such as swimming coaches and teachers (Jacobs et al., 2007; Thickett et al., 2002).

Studies carried out in Portugal, incidents in occupational environments such as schools, gyms or fitness centers with swimming pools, showed that the most frequent fungal contamination was from *Fusarium, Penicillium*, *Aspergillus fumigatus* and few others. Both *Aspergillus fumigatus* and *Penicillium* are common fungi in the environment and producers of mycotoxins (Ramos et al., 2016; C. Viegas, 2010; C. Viegas et al., 2024).

It is known that mycotoxins can cause mild or even serious and terminal illnesses (Alshammari, 2023; Haque et al., 2020; Heederik & von Mutius, 2012; S. Viegas et al., 2018, 2020). It is therefore important to detect contamination by these fungi at an early stage and to analyse the risks associated with the health of those who are constantly exposed to them, which in this case would be coaches, teachers and lifeguards.

There are studies on this subject that only take air samples to assess contamination, while others show that it is also important to take samples from the surfaces of these places (C. Viegas, 2010).

7.3. Methodology

An observational coort study will be carried out, as the workers will be observed for two periods and will have one characteristic in common, which is that they are pool workers (Fernandes & Carneiro, 2005).

As previously mentioned, the aim is to carry out the study in at least 10 swimming pools in Portugal, more specifically in the districts of Lisbon and Setubal. To this end, a collaboration protocol will be created with the institutions or companies responsible for the pools.

Desired samples are indoor air samples using a variety of methods to ensure comprehensive data collection (Cervantes et al., 2022; Gollakota et al., 2021; Whitby et al., 2022).

Air sampling will be carried out in two seasons, hot and cold, using active and passive methods. The study areas of the pools would be, pool dock, women's men's and teacher's locker room, office, lifeguard's office and outdoor area, as the following figure show, because there is some studies that showed relevant results at this sites (C. Viegas, 2010) and then the pool dock, are the most frequented places.

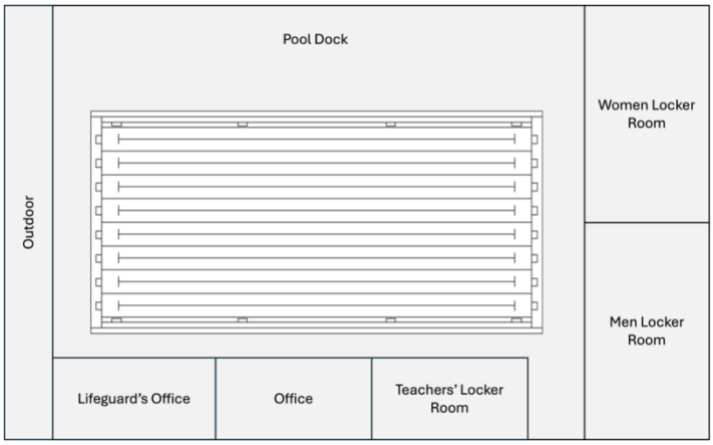

Figure 26: Sampling Sites.

For active sampling methods, devices such as the Andersen six-stage sampler, the MAS-100 and the Button aerosol sampler will be used, and for passive sampling methods, electrostatic dust collectors and surface swabs will also be used will be used to complement active methods and provide a complete assessment of indoor air quality, as it is shown in the following figure.

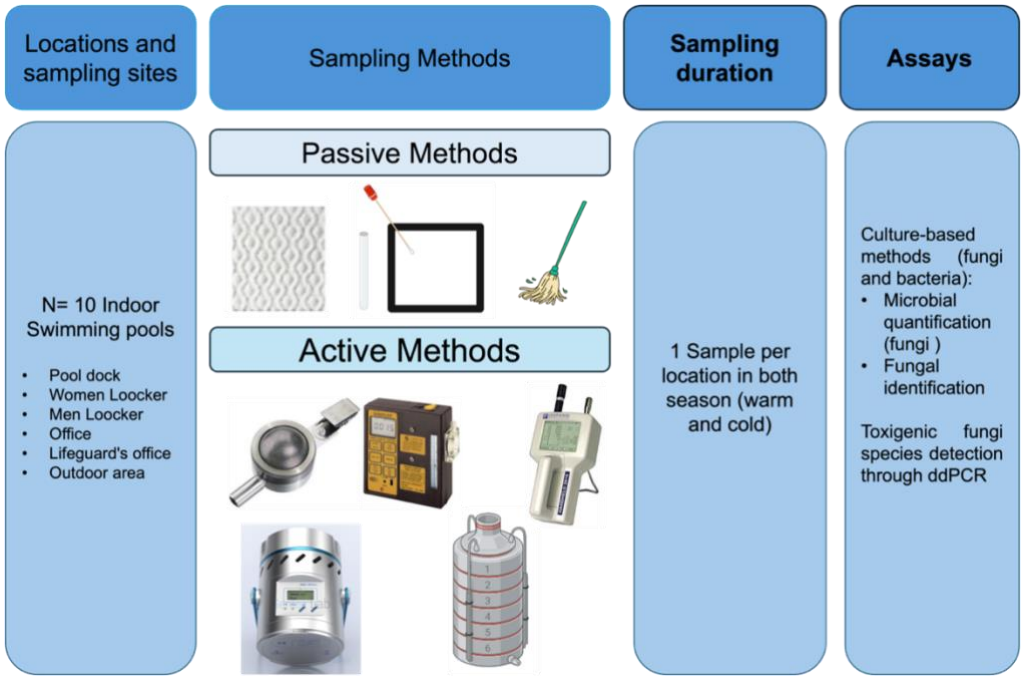

Figure 27: Sampling Strategy

All samples will be inoculated into TSA VRBA MEA MAC and DG18 (Cervantes et al., 2022; Gollakota et al., 2021; Whitby et al., 2022).

Subsequently, the samples inoculated in MEA and DG18 for 5 -7 days at 27 ºC. All samples will be repeated in DG18, to be incubated at 37ºC to understand whether there is a possibility of the development of pathogenic fungi, and in TSA at 30ºC, VRBA at 37ºC, both for 7 days and in MAC for 24hours at 35 ºC.

After this period, the bacterial colonies will only be counted and recorded, the fungal colonies will be counted and registered, identified and isolated.

With the help of statistical tools such as SPSS or Excel, graphs will be created with the quantities of bacteria and fungi and the differences between the two sampling periods will be analysed, as well as between the sampling areas of the pools.

7.4. Timeline

The activities of the project can be scheduled as shown in the following figure.

	2023				2024						
Activities	Set	Oct	Nov	Dec	Jan	Fev	Mar	Apr	May	Jun	Jul
Bibliographic Research											
Laboratory work											
1st sampling campaign											
2nd sampling campaign											
Result processing											
Scientific Production: Study article											

Figure 28: Proposal research activities timeline.

7.5. Expected Results

Relevant results can be expected on the indoor air quality of swimming pools and possible health risks can be identified, so that guidelines for good maintenance practices can be created to improve the safety and well-being of workers.

7.6. Conclusion

The aim of this study is to determine the type of contamination in indoor swimming pool air, the risks associated with it and to conclude that safety criteria are important as guidelines for better maintenance.

7.7. Limitations

As a result, there are limitations to this study that needs to be taken into consideration including the number and type of sites sampled and the changes in environmental conditions during sample collection. The main limitations include:

- The study only took 10 out of each type of pool hence maybe deriving from so many other conditions that maybe found in other part of the country or other types of facilities.
- The limitation of using different sampling techniques, for instance the active and passive devices, may not cover all the limitations presented by each device;
- The impossibility of fully controlling the circumstances under which workers were exposed during the study, which could impact on the exposure assessment to bioaerosols.

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9. ATTACHEMENTS

Attachment 1: Sampling Methods of the ICH projects

EDC sampling

For this method only a 3 EDC booklet and an envelope to transport it is needed.

The EDC should be positioned on elevated surfaces, ideally between 1.5 and 2.5 meters high, while avoiding locations with significant airflow disturbances.

Assemble 3 EDC booklets: Cut a full Swiffer into 4 pieces inside a biological safety cabinet and fold two A4 papers in half. Staple two Swiffer pieces onto adjacent pages of the folded papers, then sterilize the assembled booklet under UV light for 30 minutes. Next, staple one EDC onto one page of the folded paper and sterilize the single-page booklet using dry heat (6 hours at 160°C) to eliminate existing endotoxins. Fold and staple the two papers together, enclosing the EDC inside, and seal the three-page booklet in an envelope for transportation.

Leave the booklets at each sampling site for 30 days, ensuring to take a photo of each site. Store the envelope beneath the EDC during the sampling period. After completing the 30-day sampling period, close the booklet and return it to its envelope

EDCT Sampling

Cut a full Swiffer into 4 pieces inside a biological safety cabinet and fold two A4 papers in half. Staple two Swiffer pieces onto adjacent pages of the folded papers, then sterilize the assembled booklet under UV light for 30 minutes. Then it was stored in a sterilized bag.

Impaction method: MAS-100

For this method, are required the MAS-100 and Petri dishes with culture media x 1 for each sampling site.

After disinfecting the MAS with 70% ethanol and allowing the cotton/paper towel to dry, place a Petri dish containing culture media inside the MAS. Ensure the collection time and volume adhere to protocol before switching on the MAS. Begin sampling for the specified duration and volume (200 L at a flow rate of 100 L/min - 2 minutes per sample). Once sampling is complete, remove the Petri dish and follow the storage instructions provided below. Repeat this procedure for all designated culture media

Impaction method: Six-stages Andersen

The materials needed for performing the samples include an Anderson Cascade Impactor 6 Stage, a vacuum pump (dimensions: 22" W x 10" H x 4.5" D; weight: 8.6 lbs), and six Petri dishes with culture media for each sampling site (DG18 x2, TSA, VRBA, MEA).

After disinfecting all components of the Andersen 6 stage with 70% ethanol and drying with a cotton/paper towel, place a Petri dish containing the appropriate culture media in each stage. Activate the Andersen sampler after verifying the collection time and volume specified in the protocol (18,000 L at a flow rate of 200 L/min – 9 minutes per sampling). Upon completion of sampling, remove the Petri dish and follow the storage instructions provided below. Disinfect each stage again and repeat the procedure for all designated culture media.

Button Aerosol Sampler

Materials include pumps (SKC) and sterilized membrane filters (Polycarbonate, 0.8 um, 25 mm, [100/Pkg]).

BS sampling involves testing teachers and other school staff for a minimum of 2 hours (flow rate of 4 L/min) during a typical workday. Prepare the sterilized components of the SKC Button Sampler, including the Polycarbonate filter, before activation. Ensure the Button Sampler is started after confirming the collection time and volume align with the protocol.

Attachment 2: DNA Extraction Protocol

- Thaw the samples.
- Centrifuge at 3,5 rpm for 30 minutes.

Warning: In the case of samples in Eppendorf, centrifuge in the microcentrifuge for 5 minutes, speed 10.

- Discard most of the supernatant and keep the pellet.

Warning: In the case of Eppendorf samples, it is not necessary to discard.

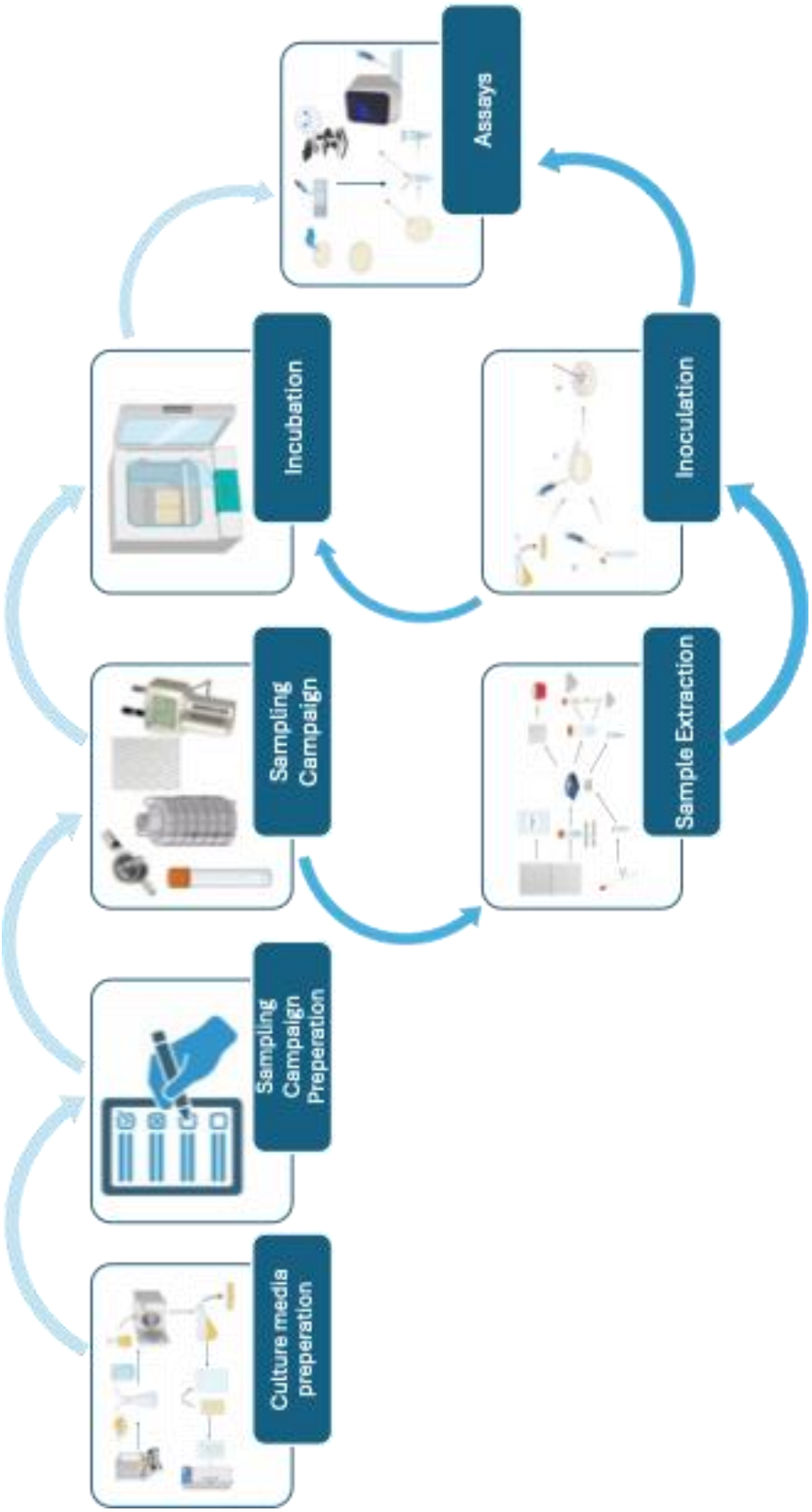
- Resuspend the pellet with the rest of the supernatant and place 200 µl of sample in the *ZR Bashing bead lysis* tube.
- Add 750 µl of *bashing bead buffer* to the tube and vortex at speed 4 for 10 minutes. Then centrifuge the tube in the microcentrifuge at speed 10 for 1 minute.
- Transfer 600 µl of the supernatant to the *Zymo-Spin III filter column* in a collection tube and centrifuge at speed 8 for 1 minute.
- Discard the column and add 1200 µl of *DNA Binding buffer* to the filtrate in the collection tube from step 6 and mix well (up and downs).
- Transfer 875 µl of the mixture from step 7 to the *Zymo-spin 1L column* in a collection tube. Centrifuge at speed 10 for 1 minute.
- Discard the liquid from the collection tube and repeat step 8.
- Add 200 µl of *DNA pre-wash buffer* to the same column in another collection tube and centrifuge at speed 10 for 1 minute.
- Add 500 µl of *DNA wash buffer* to the column in another collection tube and centrifuge at speed 10 for 1 minute.
- Transfer the column to an Eppendorf and add 200 µl of *DNA elution buffer* directly into the column matrix. Wait 2/3 minutes and spin at speed 10 for 30 seconds.
- Repeat step 12 with the same 200 µl of *DNA elution buffer* removed from the Eppendorf and add them back to the matrix.
- Finally, we divided the final 200 µl by two more Eppendorf's 100µl/50µl/50µl for backup samples and store it at -20ºC.

10. APPENDIXES

Appendix 1: Presence sheet

	2023													2024											
	Set			Oct			Nov			Dec			Jan			Fev			Mar			Apr			May			Jun		
Days	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total
1																09:00	16:00	7:00				10:00	15:00	5:00	10:00	16:00	6:00			
2				09:00	17:00	8:00	09:00	15:00	6:00													10:00	15:00	5:00	10:00	16:00	6:00	10:00	16:00	6:00
3				09:00	15:00	6:00																10:00	15:00	5:00	10:00	16:00	6:00	10:00	16:00	6:00
4				09:00	16:00	7:00							07:45	17:00	9:15				10:00	14:00	4:00	10:00	15:00	5:00				10:00	16:00	6:00
5													09:00	16:00	7:00	09:00	16:00	7:00	10:00	14:00	4:00	10:00	15:00	5:00				10:00	16:00	6:00
6				09:00	15:00	6:00	09:00	15:30	6:30							08:00	16:00	8:00	10:00	14:00	4:00				10:00	16:00	6:00	10:00	16:00	6:00
7							09:00	16:00	7:00							08:00	16:00	8:00	10:00	14:00	4:00				10:00	16:00	6:00	10:00	16:00	6:00
8							09:00	16:00	7:00	09:00	16:15	7:15	09:00	16:00	7:00	09:00	16:00	7:00	10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00			
9				09:00	14:30	5:30	09:00	15:00	6:00				09:00	16:00	7:00				10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00			
10				09:00	16:30	7:30	07:45	16:00	8:15				09:00	16:00	7:00				10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00	10:00	16:00	6:00
11	09:00	16:00	7:00	09:00	13:00	4:00							09:00	16:00	7:00				10:00	15:00	5:00	10:00	14:00	4:00				10:00	16:00	6:00
12	09:00	15:00	6:00	09:00	14:45	5:45							09:00	17:00	8:00				10:00	14:00	4:00	10:00	14:00	4:00				10:00	16:00	6:00
13	09:00	14:00	5:00	10:30	12:30	2:00													10:00	14:00	4:00				10:00	16:00	6:00	10:00	16:00	6:00
14	09:00	15:00	6:00				07:45	17:00	9:15				07:45	17:30	9:45	09:00	14:00	5:00	10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00	10:00	16:00	6:00
15							09:00	14:00	5:00				09:00	15:00	6:00							10:00	14:00	4:00	10:00	16:00	6:00			
16				09:00	14:00	5:00	09:00	15:30	6:30				09:00	16:00	7:00							10:00	14:00	4:00	10:00	16:00	6:00	10:00	16:00	6:00
17				09:00	15:30	6:30							09:00	14:30	5:30							10:00	14:00	4:00	10:00	16:00	6:00	10:00	16:00	6:00
18	09:00	14:00	5:00	07:45	17:00	9:15										09:00	15:30	6:30	10:00	14:00	4:00	10:00	14:00	4:00				10:00	16:00	6:00
19	09:00	14:00	5:00													10:00	15:30	5:30	10:00	14:00	4:00							10:00	16:00	6:00
20	09:00	15:00	6:00													10:00	15:30	5:30	10:00	14:00	4:00				10:00	16:00	6:00	10:00	16:00	6:00
21	09:00	15:00	6:00													10:00	15:30	5:30	10:00	14:00	4:00									
22	09:30	14:00	4:30										09:00	16:00	7:00	10:00	15:30	5:30	10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00			
23				09:00	13:00	4:00	09:00	15:00	6:00				09:00	15:00	6:00							10:00	14:00	4:00	10:00	16:00	6:00			
24				09:00	16:00	7:00	08:00	15:30	7:30				09:00	16:00	7:00							10:00	14:00	4:00	10:00	16:00	6:00			
25	09:00	11:30	2:30	09:00	16:00	7:00													10:00	14:00	4:00	10:00	14:00	4:00						
26	07:45	16:30	8:45	09:00	18:30	9:30										10:00	15:30	5:30	10:00	14:00	4:00	10:00	14:00	4:00						
27	09:00	14:30	5:30				10:00	16:00	6:00							10:00	15:00	5:00	10:00	14:00	4:00				10:00	16:00	6:00			
28	09:00	15:00	6:00				09:00	17:00	8:00							10:00	15:00	5:00	10:00	14:00	4:00				10:00	16:00	6:00			
29							09:00	16:00	7:00				09:00	16:00	7:00	10:00	14:00	4:00	10:00	14:00	4:00	10:00	14:00	4:00	10:00	16:00	6:00			
30				08:00	16:00	8:00	09:00	15:30	6:30				09:00	16:00	7:00							10:00	14:00	4:00	10:00	16:00	6:00			
31				09:00	15:30	6:30							09:00	16:00	7:00										10:00	16:00	6:00			

Appendix 2: Flowchart of the entire process, from the field to the laboratory



Appendix 3: Passive vs. Active Sampling Methods

Table 3: Passive vs. Active sampling methods (Cervantes et al., 2022; Whitby et al., 2022).

Sampling	Pros	Cons
Passive Methods	Are cost-effective, simple to use, and ideal for long-term monitoring. They do not require power sources, have minimal maintenance needs, and are non-intrusive. This makes them accessible for widespread environmental monitoring in remote or inaccessible locations, providing integrated data over days, weeks, or months for a comprehensive understanding of pollutant levels over time.	Have lower sensitivity and longer detection times compared to active methods, potentially missing low-concentration pollutants and delaying data collection. They are influenced by environmental conditions like temperature and humidity, limiting their range of analytes to mostly gases and vapours. Quantitative analysis with passive samplers is challenging and may be prone to contamination, impacting result reliability.
Active Methods	Quantify concentrations effectively in low microbial environments, capturing high volumes for thorough analysis. They collect diverse particle sizes and types, making them versatile in various research settings.	Have the potential to disrupt airflow and compromise accuracy. They are not cost-effective due to the need for specialized equipment and continuous operation. Depending on the device used, there may be limitations in collecting a wide range of particle sizes, impacting the representation of bioaerosols in the air sample.

Appendix 4: Pros and Cons of Passive Sampling Method

Table 4: Pros and cons of each passive sampling method (Cervantes et al., 2022; Whitby et al., 2022).

Sampling	Pros	Cons
EDC EDCT Booklet	Effectively trap dust particles without needing chemicals, making them environmentally friendly. Are versatile and safe to use on furniture, floors, electronics, and delicate items like glass.	High-quality electrostatic dust cloths have a higher initial cost than traditional cleaning cloths or disposable wipes. May not be suitable for sensitive surfaces that could be scratched or damaged by the static charge or fabric.
Surface Swabs	Are crucial for targeted sampling in environments requiring identification and monitoring of contamination. Offer consistency in sampling techniques across locations and times and can be used on various surfaces. Some allow for quantitative analysis of contamination levels.	Have limitations due to factors like surface texture, contamination levels, and sampling techniques. Improper handling of swabs can lead to contamination, affecting test accuracy. Trained professionals may be needed to interpret results and determine necessary actions. Can be time-consuming and costly, especially for frequent monitoring.
SD SD Filter	Reflects long-term indoor exposure to particles, allergens, and pollutants. Can be easily collected using simple techniques like wiping surfaces or vacuuming. Captures a wide range of particles, including dust mites and environmental pollutants, offering a comprehensive view of indoor air quality. Analysing can help identify sources of pollution, assess allergen levels, and determine health risks from prolonged exposure.	The composition can vary due to factors like indoor activities, ventilation, and cleaning practices. Is a snapshot that doesn't show real-time data on airborne particles. Methods and locations for collecting settled dust can impact sample representativeness. Expertise may be needed to interpret settled dust data accurately, as contaminant concentrations may not always directly relate to health risks.

Appendix 5: Pros and Cons of Active Sampling Methods

Table 5: Pros and cons of each active sampling method (Cervantes et al., 2022; Whitby et al., 2022).

Sampling	Pros	Cons
BS	Small, portable devices are suitable for both indoor and outdoor use. They are user-friendly and provide real-time data on airborne particles with minimal training. These samplers collect various particle sizes, making them versatile for different settings.	Limited sampling capacity hinders long-term or high-volume use. Size selectivity can cause uneven particle capture, affecting contaminant representation. Limited battery life necessitates frequent recharging or replacement, and calibration against standard methods is needed for accuracy.
Andersen Six-stages	It collects airborne particles based on aerodynamic size, dividing them into six ranges for detailed size distribution. Known for accuracy, it assesses indoor air quality, occupational exposure, and atmospheric aerosols. It provides quantitative data on particle concentration, aiding in understanding exposure and health risks. Recommended by the EPA, it is a standard method for air quality monitoring and research.	Operating and maintaining the device requires technical expertise and proper calibration for accurate results. The setup and data analysis are complex compared to simpler devices. Initial cost and maintenance expenses are relatively high, potentially posing a barrier for some organizations. It may not capture ultrafine or very large particles, limiting suitability for certain applications.
MAS-100	Efficiently captures airborne bacteria and fungi using an impact method onto growth media, providing accurate quantitative data on microbial contamination. It allows real-time monitoring of air quality and microbial levels and is easy to use for routine air sampling tasks by trained personnel in cleanroom and pharmaceutical settings	May not effectively capture particles outside the microbial size range. Initial costs, ongoing maintenance, and sampling duration can be significant, especially for facilities needing multiple units or frequent sampling. Real-time monitoring capabilities may be limited. It is best suited for microbial air sampling in controlled environments like cleanrooms, rather than broader environmental monitoring.
Particulate Matter LightHouse	Are highly accurate for real-time measurement of particulate matter concentrations across various particle sizes. They include data logging for continuous monitoring and can generate reports and graphs for analysis. Portable and versatile, they can be used indoors and outdoors, meeting diverse monitoring needs	Purchasing costs are high, especially for advanced models. Regular maintenance and calibration are needed for accuracy, increasing operational costs. Some models are complex to use and analyse, requiring trained staff. They are effective for measuring particulate matter but may have limitations in detecting specific particles or types.

Appendix 6: Pros and Cons of each growth media

Table 6: Advantages and disadvantages of each growth media (Cervantes et al., 2022; Whitby et al., 2022).

Growth Media	Pros	Cons
TSA	It is easy to prepare, allows flexibility in evaluating different bioaerosol samples for contamination, and is rich in nutrients that allow the growth of a wide range of bacteria.	It is not a selective medium, which sometimes makes microbial assessments difficult. Because it is rich in nutrients, there was excessive growth and growth of other colonies, such as fungi and yeast, which made it difficult to quantify bacteria in the samples.
VRBA	It selectively promotes the growth of gram-negative bacteria while inhibiting gram-positive bacteria, enabling specific contamination assessment. It includes indicators like lactose, neutral red, and bile salts for detecting coliform bacteria, providing reliable and accurate results on bacterial contamination in samples.	It is non-selective; it limits its versatility for evaluating the total bacterial contamination of the sample. Since it is selective for coliform bacteria, it creates the possibility of non-growth of other bacteria in the sample, "hiding" the existence of possible crucial bacteria
MAC	It is a selective medium, but it favors the growth of only gram-negative bacteria and is an indicator of pathogenic bacteria for the health of animals and humans, such as some species of Escherichia coli and Salmonella.	With its use is that although it helps in the differentiation of lactose fermenting bacteria, some may exhibit atypical colonial morphologies, making it difficult to evaluate their appearance.
MEA	It allows the growth of a wide variety of fungi, has the right nutrients for practically all, ideal for both fast-growing and slow-growing fungi.	It is more susceptible to contamination and since it is not selective, it allows the growth of various fungi, making their identification difficult.
DG18	It only allows the growth of xerophilic fungi, also allows the detection of many fungi that may be potential health risks and facilitates their identification due to their characteristics when grow in this environment.	Identifying fungi through their growth in the environment can be difficult, requiring some experience in distinguishing them.

Appendix 7: WWTP article

microorganisms

MDPI

Review

Filling the Knowledge Gap Regarding Microbial Occupational Exposure Assessment in Waste Water Treatment Plants: A Scoping Review

Bruna Riesenberger [1], Margarida Rodriguez [1], Liliana Marques [1], Renata Cervantes [1,2], Bianca Gomes [1], Marta Dias [1,2], Pedro Pena [1,2], Edna Ribeiro [1] and Carla Viegas [1,2,*]

Citation: Riesenberger, B.; Rodriguez, M.; Marques, L.; Cervantes, R.; Gomes, B.; Dias, M.; Pena, P.; Ribeiro, E.; Viegas, C. Filling the Knowledge Gap Regarding Microbial Occupational Exposure Assessment in Waste Water Treatment Plants: A Scoping Review. *Microorganisms* **2024**, *12*, 1144. https://doi.org/10.3390/microorganisms12061144

Academic Editor: Carlos A. Jerez

Received: 30 April 2024
Revised: 27 May 2024
Accepted: 30 May 2024
Published: 4 June 2024

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

[2] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, 1099-085 Lisbon, Portugal

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

Abstract: Background: Wastewater treatment plants (WWTPs) are crucial in the scope of European Commission circular economy implementation. However, bioaerosol production may be a hazard for occupational and public health. A scoping review regarding microbial contamination exposure assessment in WWTPs was performed. Methods: This study was performed through PRISMA methodology in PubMed, Scopus and Web of Science. Results: 28 papers were selected for data extraction. The WWTPs' most common sampled sites are the aeration tank (42.86%), sludge dewatering basin (21.43%) and grit chamber. Air sampling is the preferred sampling technique and culture-based methods were the most frequently employed assays. *Staphylococcus* sp. (21.43%), *Bacillus* sp. (7.14%), *Clostridium* sp. (3.57%), *Escherichia* sp. (7.14%) and *Legionella* sp. (3.57%) were the most isolated bacteria and *Aspergillus* sp. (17.86%), *Cladosporium* sp. (10.71%) and *Alternaria* sp. (10.71%) dominated the fungal presence. Conclusions: This study allowed the identification of the following needs: (a) common protocol from the field (sampling campaign) to the lab (assays to employ); (b) standardized contextual information to be retrieved allowing a proper risk control and management; (c) the selection of the most suitable microbial targets to serve as indicators of harmful microbial exposure. Filling these gaps with further studies will help to provide robust science to policy makers and stakeholders.

Keywords: wastewater treatment plants; sampling methods; assays; microbial contamination assessment; bacteria; fungi

1. Introduction

The European Commission (EC) strongly recommends circular economy implementation aiming at a zero-waste strategy, by instigating water innovations technologies for water reuse and recycling [1]. In this scope, wastewater treatment plants (WWTPs) are designed to maximize energy and water recovery, becoming of pivotal importance for the achievement of the EC's goals [2].

On WWTPs, the wastewater of domestic, hospital and industrial uses undergoes preliminary, primary, secondary, and in some cases, tertiary biological treatments [3,4]. During these treatments, bioaerosol formation is higher throughout discharging, mixing and aerating processes, as well as during the spraying of sewage [3–6]. The bioaerosols contain microorganisms, such as fungi, viruses, bacteria, and their metabolites, including endotoxins and mycotoxins, which may be potentially pathogenic to humans. Infection can occur through ingestion, dermal contact, or inhalation, and it is highly possible that due to prolonged exposure, a decline in the health status of WWTPs workers may be observed [5–7]. In fact, several negative health outcomes associated with bioaerosol occupational exposure have been reported, including respiratory and gastrointestinal effects or allergies [4,6]. In addition, WWTPs are recognized as key emission sources for the discharge of antimicrobial-resistant (AMR) bacteria and antibacterial resistance genes (ARGs) [8].

Although it is crucial to assess occupational exposure to bioaerosols in WWTPs, there is a lack of consensus regarding sampling approaches and analyses that should be performed, as well as the targets that can be used as surrogates to identify harmful microbial contamination, which is a common problem in settings where (micro)biologic agents need to be assessed. However, suggestions regarding the procedures to be employed from the field to lab have already been described in different occupational environments [9–11]. Thus, this study aims to perform a scoping review to provide a broad overview of the stateof-the-art methods (sampling and analyses) applied to perform microbial contamination assessments in WWTPs, as well as to identify the most suitable targets to be used as indicators of hazardous microbial contamination.

2. Materials and Methods

2.1. Search Strategy, Inclusion and Exclusion Criteria

This study adopted the PRISMA methodology and the Preferred Reporting Items for Systematic Review (PRISMA) checklist [12] (was completed (Supplementary Materials Table S1).

This study reports available data published between 1 January 2010 and 8 November 2023. The search aimed at selecting studies on microbiologic agents' assessment in WWTP and included the terms "Waste Water Treatment Plants", "bacteria", "fungi", "viruses", "exposure" and "sampling", with English as the chosen language. The databases chosen were PubMed, Scopus and Web of Science (WoS). Articles that did not meet the inclusion criteria were not subjected to additional review (Table 1).

Table 1. Inclusion and exclusion criteria on article selection.

Inclusion Criteria	Exclusion Criteria
Articles published from 1 January 2010 to 8 September 2023	Articles published prior to 2010
Articles published in English	Articles published in other language
Articles summarising research results from any country	Abstracts of congress, reports, reviews/state-of-the-art articles
Original scientific articles on the subject	
Articles focused to microbial occupational exposure	

2.2. Studies Selection and Information to Be Retrieved from the Papers

The articles were selected by using the Rayyan—Intelligent systematic review tool, a free online tool that significantly accelerates the process of screening and choosing papers for academics working on systematic reviews. Article selection followed three rounds: 1st: All titles were screened to identify and remove duplicated papers or those unrelated to the topic. The selected papers were uploaded to Rayyan for additional examination; 2nd: screening of all the abstracts; 3rd: Selected papers were reviewed considering the inclusion and exclusion criteria. Possible differences in the study's selection were discussed by three investigators (BR, MR and LM), and eventually decided by the remaining investigators (BG, MD, PP, RC). Data extraction was then performed by two investigators (BR, and LM), while another (MR) examined the results. The data that follows were manually extracted: Database, Title, Country, Type of WWTP, Sampling Strategies and Methods, Assays applied, Main Findings, and References.

2.3. Quality Assessment

The assessment of the risk of bias was performed by 4 investigators (BR, MR, LM, and CV). Within each research article, an evaluation of the risk of bias was performed across two parameters divided as key criteria ("Sampling methods" and "Assays"). Each parameter's risk of bias was rated as "low" "medium" "high", or "not applicable". The studies for which all the key criteria and most of the other criteria were characterized as "high" were removed.

3. Results

The workflow illustrated in Figure 1 was used for selecting studies. Initially, 191 studies were found in the database search, from which 105 abstracts were analyzed, and 40 complete texts were deemed eligible for further examination. A total of 12 papers were rejected for not satisfying the

inclusion and exclusion criteria, mostly because they did not have any information regarding microbial occupational exposure in WWTPs. Overall, the selection process yielded 28 studies on microbiologic contamination occupational exposure assessment.

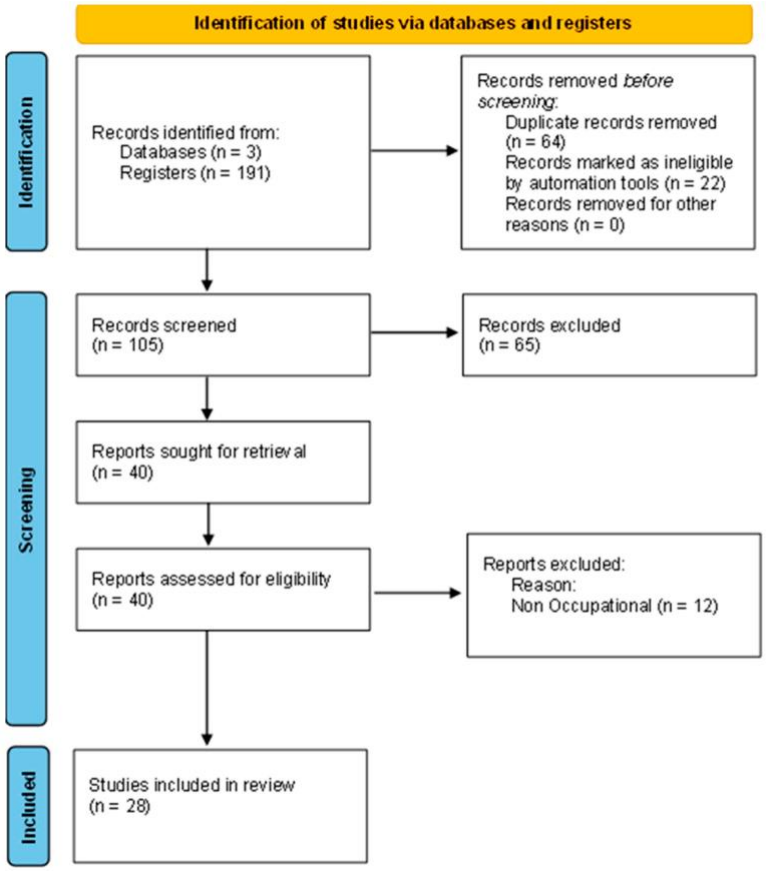


Figure 1. PRISMA methodology of selection of papers [12].

Extracted Data

After the selection of the 28 studies on microbiologic contamination occupational exposure assessment, the relevant data were extracted; the key findings are summarized in Table 2.

Among the 28 chosen studies, 9 were conducted in Europe (3 in Portugal [13–15], 2 in Poland [16,17], 2 in Denmark [18,19], 1 in Switzerland [20], and 1 in Austria [21]), 9 in Asia (specifically in China [4,5,22–28]), 6 in the Middle East (Iran [6,29–33]), and 4 in North America (3 in the USA [34–36] and 1 in Canada [37]).

Table 2.

Data extracted from the chosen papers.

Title	Type of WWTP	Microbe Assessed	Sampling Methods	Season of Sampling	Sampling Sites and Number of Samples	Assays	Main Findings	Reference
Exposure to Airborne Noroviruses and Other Bioaerosol Components at a Wastewater Treatment Plant in Denmark	no data	Noroviruses (NoVs), Adenoviruses, Endotoxin, Bacteria and Molds	Air samples: Active methods—Filtration (Personal Dust Sampling-Inhable GSP samplers with teflon filters or polycarbonate filters, average sampling period 242 min), Stationary measurements of "total dust"	no data	Personal dust sampling was carried out on 16 workers, on different wastewater processes; stationary sampling was carried out in the aeration basin at 1.5 m above the ground level	Culture-based methods (DG18 agar for cultivable moulds, nutrient agar for cultivable bacteria)	NoVs and endotoxin were detected at concentrations that could pose an occupational health risk. Positive correlations between exposure to endotoxin, bacteria, moulds and NoVs were found and indicate that the exposure to bioaerosols may be related to work tasks.	[18]
ADMS simulation and influencing factors of bioaerosol diffusion from BRT under different aeration modes in six wastewater treatment plants	Municipal WWTP	Bacteria and Intestinal Bacteria	Air samples: Active methods—Impaction (Andersen six-stage cascade impactor, flow rate = 28.3 L/min; TH-150 medium flow sampler)	Seasonal (spring)	1.5 m above aeration tanks of 6 Municipal Wastewater Treatment Plant (MWWT), 6 samples were taken at each sampling site, (n = 36)	Culture-based methods (LB medium for bacteria, and for intestinal bacteria, MAC); Ion chromatography and Illumina MiSeq high-throughtput sequencing	The concentrations of bacteria and, specifically, intestinal bacteria in the bioaerosols ranged from 389 CFU/m^3 to 1536 CFU/m^3 and 30 CFU/m^3 to 152 CFU/m^3, respectively, and the proportion of the intestinal bacteria was 8.85%. The proportion of intestinal bacteria (75.79%) produced via surface aeration by Biological Reaction Tanks (BRT) attached to large-sized bioaerosol particles was higher than that of a BRT undergoing the bottom aeration process (37.28%). The main microorganisms found in the bioaerosols included Moraxellaceae, Escherichia–Shigella, Psychrobacter, and Cyanobacteria.	[22]

Table 2. *Cont.*

Spatio-temporal variations in airborne bacteria from the municipal wastewater treatment plant: a case study in Ahvaz, Iran	Municipal WWTP	Airborne Bacteria	Air Samples: Passive methods (microbiological sampling index of microbial air contamination (1/1/1 standard))	Seasonal (warm and cold)	Grit chamber (GCh), primary sludge dewatering basin (PSDB) and at the aeration tank (AT); (n = 180)	Culture-based methods (Trwith nystatin (250 mg/L) to inhibit fungal growth); PCR-RFLP	The dominant bacterial genus included *Bacillus pumilus* (26.7%), *Staphylococcus arlettae* (23.2%), *Kocuria turfanensis* (13.6%), and *Alicycliphilus* (9.2%), and they increased with high temperatures and wind speed, and decreased with high humidity.	[6]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**
Emission level, particle size and exposure risks of airborne bacteria from theoxidation ditch for seven months observation	WWTP with orbal oxidation ditch process	Airbone Bacteria	Air samples: Active—Impaction (Andersen six-stage cascade impactor, flow rate = 28.3 L/min); Material collection (raw water in the oxidation ditch)	Seasonal (spring and summer)	ConS: Control site was set 300 m upwind from the oxidation ditch; AWS (above water surface): above water surface; AWS-0.5: above water surface 1 m; AWS-3: above water surface 3 m; ARB (above rotating brushes)-25: after the rotating brushes 25 m; ARB-55: after the rotating brushes 55 m; ARB-210: after the rotating brushes 210 m; (n = 168)	Culture-based methods (with nutrient agar for mesophilic bacteria) for air samples; Gradient gel electrophoresis for 16S rDNA; PCR	Spatial and seasonal variations in the concentrations of airborne bacteria emissions were detected. The highest concentration was observed near the rotor disc aerators (RDAs) (835 ± 91 CFU/m^3 to 8916 ± 155 CFU/m^3) during each sampling process, with the concentration decreasing by 76.70% and 79.91% as sampling distance and height increased, respectively. Most of the airborne bacteria were coarse particles that exceeded 4.7 µm in size. The dominant bacteria were *Bacillus* sp., *Lysinibacillus* sp., and *Sphingomonas* sp.	[23]

Table 2. *Cont.*

Aerosol partitioning potential of bacteria presenting antimicrobial resistance from different stages of a small decentralized septic treatment system	On-site/ decentralized WWTP	Antibiotic-Resistant Bacteria (ARB)	Air samples: Active method—Impinger (Wetted wall cyclone collectors (WWC)); Material collection (stainless steel porTable 600 mL water dipper (Grainger Industrial Supply))	Seasonal (summer and winter)	Aerosol and water samples were collected at the four tanks; 600 mL of water and 1500 L of air at each tank	Kirby–Bauer testing for antibiotic resistance, quantitative Polymerase Chain Reaction (qPCR); 16SrRNA-based Illumina sequencing	As expected, the higher concentration of bacteria was found when the lids were open; in the summer, *Legionella* was found in the water tanks 1 and 3, and in the water tank 1 *Pseudomonas* was present; in the winter, *Legionella* was also present in the water tank 1; bioaerossol samples showed a higher antimicrobial resistance of 50% (at four of the eight antbiotics tested), and the higher antimicrobial resistance of the water samples was 87.5% (resistance in the 7 of the 8 antibiotics).	[36]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**
Identification of airborne fungi's concentrations in indoor and outdoor air of municipal wastewater treatment plant	Municipal WWTP with conventional activated sludge treatment process	Fungi	Air Samples: Passive methods (microbiological sampling index of microbial air contamination (1/1/1 standard))	Seasonal (warm and cold)	Grit chamber tank, primary sludge dewatering basin, aeration tank, upstream and downstream of dominant wind blowing at the site and at the administrative building (n = 240)	Culture-based methods (SDA with chloramphenicol antibiotic (100 mg/L) to inhibit bacterial growth)	The greatest release of fungal aerosols occurred in the cold season while the minimum release occurred in the warm season; the highest concentrations of fungi were observed in the grit chamber unit; *Cladosporium* (39.23%) and *Alternaria* (19.87%) were the airborne fungal genera most common.	[29]
Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?	no data	*Aspergillus* spp.	Air samples: Active methods—Impaction (Millipore air Tester, flow rate = 140 L/min) and Impinger (Coriolis µ air sampler, flow rate = 300 L/min); Passive methods: surface samples (swabs)	1 year longitudinal study	Sampling occured at 2 Watewater Treatment Plant (WWTP); 26 air sample and 15 surface samples	Culture-based methods (MEA); Real Time PCR (RT-PCR)	At both WWTPs were found 33 different species of *Aspergillus* spp. (18 at WWTP1 and 15 at WWTP2), 7 species were only isolated in surfaces (5 in the WWTP1 and 2 at WWTP2), and 12 different Aspergillus sections were identified (6 in both WWTP).	[14]

Table 2. *Cont.*

Wastewater treatment plant workers' exposure and methods for risk evaluation of their exposure	WWTP with anaerobic–anoxic–oxic process	Airborne Bacteria, Enteric Bacteria, Endotoxins	Air Samples: Active methods—Filtration (personal and stationary GSP samplers with polycarbonate filters or with Teflon filter, flow rate = 3.5 L/min) and Impaction (Andersen six-stage cascade impactor, flow-rate 28.3 L/min)	1 year longitudinal study	Stationary samples were taken in the grid chamber house and in the aeration tank (106 personal GSP samples, 12 stationary GSP samples), and 141.5 L to 843 L of air by ASCI were taken over the year	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)	A total of 22.36% of the bacteria potentially inhaled by WWTP workers seem to be from the air around the aeration tank and 22.40% from the grid house; *Staphylococcus* (13.2%) and *Aeromonas* (11.7%) were the dominant genera at the aeration tank, while *Acinetobacter* (25.6%) was the dominant in grid house.	[19]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**
Anaerobic bacteria in wastewater treatment plant	Mechanical–biological WWTP	Anaerobic Bacteria	Air samples: Active method—Impaction (Andersen six-stage cascade impactor, flow-rate = 28.3 L/min); Material collection (sewage and sludge samples were taken directly into 50 mL sterile screwed-off Falcon tubes)	Seasonal (summer and winter)	Bar screens, containers with solids in the screens' hall, primary settling tank, sewage sludge pumping station, aeration basins incineration plant, sludge-thickening building, and at the background of WWTP (n = 22)	Culture-based methods (Schaedler agar with 5% additive of sheep blood for bacterial growth); PCR (for confirmation of *Clostridium* isolates); Biochemical API 20A test (bioMérieux)	Some of the anaerobic bacteria identified belongs to the risk group 2 (according to the EU Directive 2000/54/EC); *Actinomyces*, *Bifidobacterium*, *Clostridium* and *Propionibacterium* genera were identified in wastewater and in the air.	[16]

Table 2. *Cont.*

Bioaerosols emission characteristics from wastewater treatment aeration tanks and associated health risk exposure assessment during autumn and winter	Municipal WWTP with rotating disc aeration tank, adopted with DE oxidation ditch treatment process, and microporous aeration tank and adopted with Anaerobic–anoxic–oxic process	*Escherichia coli* and *Staphylococcus aureus*	Air samples: Active—Impaction (Andersen six-stage cascade impactor, flow-rate = 28.3 L/min); Material collection (500 mL wastewater samples were taken by a sterility water sampling bottle)	Seasonal (autumn and winter)	The sampling was carried out at 3 WWTPs, and they were located in the middle of the center corridor of the second microporous aeration tank and the first rotating disc aeration tank from north to south	Culture-based methods (for *S. aureus* MYP was used, and MAC for *E. coli*)	*Staphylococcus aureus* was about 2 times higher in winter than in autumn, while *Escherichia coli* in autumn was about 9 times higher than in winter.	[24]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**

Table 2. *Cont.*

Influence of seasons and sites on bioaerosols in indoor wastewater treatment plants and proposal for air quality indicators	Municipal WWTP with pre-, primary and secondary treatments	Bacteria and Endotoxins	Air samples: Active—Impaction (Andersen six-stage cascade impactor, flow rate = 28.3 L/min) and Filtration (37 mm cassettes (SKC) loaded with binder-free glass fiber filters, flow-rate = 2 L/min)	Seasonal (warm and cold)	Screening, degreasing/grit removal, settling tanks and biofiltration	Culture-based methods (TSA to collect total culturable aerobic bacteria and Gram-negative selective agar (GNSA) for culturable Gram-negative bacteria). In addition to total bacteria (bacteria 16S rDNA), specific qPCR was used to monitor bacteria from human flora: *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and fresh water environment: *Aeromonas hydrophila*.	The average concentration of culturable Gram-negative bacteria was approximately 100 CFU/m^3 for both seasons. Only two WWTPs showed concentrations of culturable Gram-negative bacteria higher than the recommended exposure limit (1000 CFU/m^3 according to Institut Robert Sauvé en Santé et en Sécurité du Travail (IRSST). Several values were close to the limit.	[37]
Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East	Municipal WWTP with air diffusion by fine bubble diffusers	Airborne Bacteria and Fungi	Air samples: Active method—Impaction (QuickTake 30 sample pump equipped with the Bio Stage single-stage cascade impactor, flow rate = 28.3 L/min)	1 year longitudinal study	Area adjacent to the aeration tank and secondary sedimentation units, near the tricking filter, near the sludge storage tank and sludge dewatering unit, adjacent the screening, grit chamber, and primary sedimentation unit and outside of the WWTP were the locations of the sampling; (n = 240)	Culture-based methods (TSA for airborne bacteria growth, and SDA for fungal growth)	Maximum bacterial concentration was found in the aeration tank in the summer, and the minimum was in the sludge dewatering unit during the winter; maximum and minimum fungal concentrations were in primary treatment and sludge dewatering unit in winter and summer, respectively. *Micrococcus* spp. and *Staphylococcus* spp. had the highest emission of bacteria in the winter and summer, respectively. *Cladosporium* spp., Penicillium spp., *Aspergillus* spp. and *Alternaria* spp. were the dominant fungi.	[30]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**

Table 2. *Cont.*

Characterization of the airborne bacteria community at different distances from the rotating brushes in a wastewater treatment plant by 16S rRNA gene clone libraries	Municipal WWTP with orbal oxidation ditch treatment process	Airborne Bacteria	Air samples: Active methods—Impaction (six-stage Impacting Airborne Microorganism Sampler—FA-1, 28.3 L/min) and Impinger (SKC BioSampler, flow rate = 12.5 L/min)	no data	Aerosol samples were collected at different distances from the rotating brushes in the oxidation ditch; 1.5 L for each sample	Culture-based methods; PCR; Sequencing	The majority of bacteria in the bioaerosols were *Proteobacteria* and *Bacteroidetes* around the oxidation ditch. The study concluded that the rotating brush aeration was the main source of bioaerosols in the oxidation ditch.	[25]
Genomic insight into transmission mechanisms of carbapenem-producing Citrobacter spp. isolates between the WWTP and connecting rivers	WWTP with anaerobic–anoxic–oxic treatment process	Carbapenem-Resistant *Citrobacter* spp. (CRCS)	Material collection (wastewater and sludge mixtures samples with a total volume of 1 L)	Seasonal (spring, summer, autumn and winter)	Water inlet, anaerobic tank, aerobic tank, sludge thickening tank, activated sludge tank, mud cake storage area, and water outlet; In total, 136 river water and 51 river sediment samples were collected and 189 samples were gathered from the WWTP.	Culture-based methods; PCR; 16s RNA sequencing; MALDI-TOF MS	In total, 14 CRCS were detected in 376 environmental samples, including those from the inlet (n = 7), anaerobic tank (n = 2), and rivers (n = 5). *Citrobacter braakii* (n = 6) was the dominant subtype among 14 CRCS isolates, followed by *Citrobacter freundii* (n = 5), *Citrobacter sedlakii* (n = 2), and *Citrobacter werkmanii* (n = 1). All CRCS showed resistance to the studied antibiotics.	[26]
Aspergillus flavus contamination in two Portuguese wastewater treatment plants	WWTP with primary, secondary and tertiary treatment processes	*Aspergillus* spp.	Air samples: Active methods—Impaction (Millipore, flow rate = 140 L/min) and Impinger (Coriolis µ air sampler, flow rate = 300 L/min); Passive methods: surface samples (swabs)	Seasonal (winter)	Ten sampling locations were established at the two WWTP for assessing indoor air contamination: lift station, flotation sludge, sludge dewatering, screening, co-generation, aerobic digestion (secondary treatment), canteen, operation room, grit removal, and administration room. An outdoor reference sampling was also included; air samples: 26 indoor and 2 outdoor; surface samples: 17 indoor	Culture-based methods (MEA); RT-PCR	In both WWTPs, *Aspergillus versicolor* (38%), *Aspergillus candidus* (29.1%), and *Aspergillus sydowii* (12.7%) were the most common. In the surfaces were *Aspergillus flavus* (47.3%), *Aspergillus fumigatus* (34.4%), and *Aspergillus sydowii* (10.8%). The isolates of *Aspergillus flavus* that were inoculated in coconut agar medium were not identified as toxigenic, and were not detected by RT-PCR.	[13]

Table 2. *Cont.*

Title	Type of WWTP	Microbe Assessed	Sampling Methods	Season of Sampling	Sampling Sites and Number of Samples	Assays	Main Findings	Reference
Bioaerosol emissions and detection of airbone antibiotic resistance genes from a wastewater treatment plant	Municipal WWTP with activated sludge treatment process	Culturable Bacteria and Fungi; Fluorescent Bioaerosols	Air Samples: Active method—Impaction (Reuter Centrifugal Sampler High Flow, flow-rate = 100 L/min) and Impinger (SKC Biosampler, flow-rate = 12.5 L/min; Particulate matter (Ultraviolet aerodynamic 190 particle sizer (UV-APS)	Seasonal (spring, summer, autumn, and winter)	Sludge thickening basin, biological reaction basin, screen room	Culture-based methods (with TSA and MEA for bacterial and fungal growth, respectively); PCR	Highest concentrations in sludge thickening basin (bacteria: 1697 CFU/m^3, fungi: 930 CFU/m^3). Bacterial concentrations met Chinese standards, but fungal levels exceeded World Health Organization (WHO) recommendations in some areas.	[4]
Occupational Exposure to *Staphylococcus aureus* in the Wastewater Treatment Plants Environment	Municipal WWTPs with mechanical, chemical and biological treatments processes	*Staphylococcus aureus*	Air samples: Active methods—Impaction (1-step portable air sampler made by Burkard, flow rate = 20 L/min) and Filtration (GilAir-5 pump and an open-faced aerosol sampler with a gelatin filter of a 37 mm in diameter and 3 µm pores at a flow rate of 3 L/min); Material collection (raw wastewater discharged into the wwtp and treated wastewater)	Seasonal (spring and summer)	The study was conducted in 16 WWTPs in Poland, representing different treatment technologies; a total of 286 samples were collected, including 253 air samples and 33 wastewater samples	Culture-based methods (chromogenic substrate CHROMID® S. aureus Elite agar); MALDI-TOF, and an automatic method for antibiotic resistence na alysis (WalkAway system)	The study identified *Staphylococcus aureus*, including antibiotic-resistant strains, in wastewater and air samples from WWTP. Workers engaged in mechanical treatment faced the highest health risk.	[17]

| Title | Type of WWTP | Microbe Assessed | Sampling Methods | Season of Sampling | Sampling Sites and Number of Samples | Assays | Main Findings | Reference |

Table 2. *Cont.*

COVID-19 infection risk from exposure to aerosols of wastewater treatment plants	Municipal with activated sludge treatment process	SARS-CoV-2	Air samples: Active method—Impinger (Portable pumps; flow rate = 7.5–8.5 L/min); Material collection (Grab samples—raw wastewater was colected in 250 mL in sterile glasses)	1 year longitudinal study	Pumping station and activated sludge plants; a total of 24 raw wastewater samples were collected, with 12 samples from each of the two wastewater treatment plants (WWTPs) and 15 air samples.	RT-qPCR	SARS-CoV-2 RNA was found in 37.5% of wastewater samples. Detected in 5 of 12 samples from WWTP A and 4 of 12 samples from WWTP B. The highest concentration was observed at the pumping station.	[31]
Dispersion and Risk Assessment of Bacterial Aerosols Emitted from RotatingBrush Aerator during Summer in a Wastewater Treatment Plant of Xi'an, China	WWTP with oxidation ditch process	Bacteria	Air samples: Active method—Impaction (Andersen six-stage cascade impactor, flow rate = 28.3 L/min)	Seasonal (summer)	Directly Downwind Sites: 2 m downwind 5 m downwind 10 m downwind 30 m downwind 50 m downwind 100 m downwind Lateral Sites: G1 (5 m laterally from the aerator) G2 (5 m laterally from the aerator)	Culture-based methods	Higher airborne bacteria concentrations were observed closer to the aerator.	[5]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**

Table 2. *Cont.*

Airborne bacteria in a wastewater treatment plant: Emission characterization, source analysis and health risk assessment	WWTP with anaerobic–anoxic–oxic process	Bacteria	Air samples: Active method—Impaction (Quartz membranes (90 mm, Whatman QM-A), flow-rate = 100 L/min and TH-150)	Seasonal (spring, summer and winter)	The WWTP has various treatment stages, including CS (possibly activated sludge), AGC (grit chamber), PST (primary settling tank), AnT (possibly anoxic tank), AeT (aeration tank), and SST (secondary settling tank). Indoor facilities like CS and SDH (sludge dewatering with decanter centrifuges) were compared with outdoor facilities like AGC, PST, and AeT.	High-throughput sequencing techniques	Concentrations varied by site and season. Treatment stages were significant emission sources.	[27]
Quantifying the Relationship between SARS-CoV-2 Wastewater Concentrations and Building-Level COVID-19 Prevalence at an Isolation Residence: A Passive Sampling Approach	no data	SARS-CoV-2	Passive method (tampons made from rayon with a polyester string)	Seasonal (spring)	Approximately 190 feet from the isolation residence, and the wastewater influent at this location was restricted to the isolation building	RT-qPCR	The virus was detected over 16 weeks, demonstrating its feasibility for identifying residential halls with infected individuals. The daily viral wastewater load showed a positive association with the building's COVID-19 prevalence.	[35]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**

Table 2. *Cont.*

Assessment of airborne virus contamination in wastewater treatment plants	no data	Adenovirus (AdV); Norovirus (NoV); Hepatitis E Virus (HEV)	Air samples: Active method—Impaction (3 µm pore size, 25 mm gelatine filters embedded in standard cassettes using MSA Escort Elf or SKC pocket pump 210–1002, flow rate = 4 L/min)	Seasonal (summer and winter)	Inside (Enclosed Area): One sample was collected in the enclosed area, specifically near the water inlet. The sampling point inside was close to the rake that removes large particles from incoming water. Outside (Unenclosed Area): Another sample was collected in the unenclosed area, specifically above the bubbling aeration basin; 123 air samples from 31 WWTPs.	qPCR	AdV-F was present in all WWTPs during summer and 97% during winter. Concentrations were higher in summer, reaching a maximum of 2.27 × 10^6 genome equivalent/m^3. AdV-E/D were detected in winter, only in a few samples. NoV was detected in only 3 out of 123 air samples, with concentrations below quantification limits. HEV was not detected in any of the samples.	[20]
Airborne bacteria and fungi in a wastewater treatment plant: type and characterization of bio-aerosols, emission characterization and mapping	no data	Bacteria and Fungi	Air samples: Active method—Impaction (One-Stage Andersen cascade impactor, flow rate = 28.3 L/min)	Seasonal (spring, summer and winter)	ETP (Entrance of Treatment Plant), Gch (Grit Chamber), SDB (Sludge Drying Bed), Aea tank (Aeration tank), and Lab (Laboratory Building). Within the mentioned areas, specific points were chosen for sampling, such as the pumping station, additional points in SDB, Gch, Aea tank, and the laboratory.	PCR; biochemical tests: urease, oxidative fermentative (OF), oxidase, catalase, triple sugar iron (TSI), eosin methylene blue (EMB), and Indole-Methyl red-Voges-ProskauerCitrate (IMViC) test	Various bacteria were identified (some with pathogenic potential), and fungi were present in the air of the WWTP. Bacterial concentrations exceeded the standards, as is the case of *Staphylococcus* and *Enterobacteriaceae*. Fungal concentrations varied seasonally and by location. The relationship between meteorological parameters and bio-aerosols was explored, with temperature showing significance. Particulate matter, especially PM10, correlated significantly with fungal concentrations.	[33]

Title	**Type of WWTP**	**Microbe Assessed**	**Sampling Methods**	**Season of Sampling**	**Sampling Sites and Number of Samples**	**Assays**	**Main Findings**	**Reference**

Table 2. *Cont.*

Exposure to Bioaerosol from Sewage Systems	no data	Mesophilic Bacteria; Coliform Bacteria; *Aspergillus fumigatus*	Air samples: Active methods—Impaction (MAS-100, flow rate = 100 L/min) and Impinger (SKC Biosampler, flow rate = 12.5 ± 0.1 L/min)	Seasonal (summer and winter)	At hospital sewage (K1), relief chamber of a combined sewage overflow (K2) and in the area of a city treatment plant (K3); 30 air samples	Culture-based methods (Blood agar was used for mesophilic bacteria and *Aspergillus fumigatus*, Endoagar for coliform bacteria, Coli-ID agar for *Escheriachia coli*, Hektoenagar for *Salmonella* sp., and *Camplylobacter* agar with selective supplement for *Camplylobacter* sp.)	Mesophilic Bacteria Concentrations: Location K1 had concentrations ten times higher than ambient air, attributed to the small chamber size. Location K2 exhibited concentrations comparable to ambient air, possibly due to the large size and good ventilation of the relief chamber. In the encased grit chamber (K3), mesophilic bacteria concentrations were significantly higher than in K1, K2, and ambient air. Coliform bacteria concentrations were generally low, with the highest load found in the encased grit chamber (K3). Coliform bacteria were infrequently found in aerosols of wastewater plants. *Aspergillus fumigatus* was detected at all sampling sites both indoors and outdoors.	[21]
Methicillin-Resistant *Staphylococcus aureus* (MRSA) Detected at Four U.S. Wastewater Treatment Plants	WWTP with primary, secondary and tertiary treatment processes	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Material collection (Grab Samples—Samples were collected in 1-L sterile polyethylene Nalgene® Wide Mouth Environmental Sample Bottles)	1 year longitudinal study	Mid-Atlantic WWTP1 Mid-Atlantic WWTP2 Midwest WWTP1 Midwest WWTP2; 44 grab samples were collected	Gram stain; coagulase and catalase tests; PCR	MRSA was detected in 50% of wastewater samples, at all WWTPs studied. MSSA (Methicillin-Susceptible *Staphylococcus aureus*) was also detected in 55% of wastewater samples, at all WWTPs. The occurrence of MRSA and MSSA varied across WWTPs, sampling dates, and sampling locations. MRSA isolates showed resistance to multiple antibiotics, including those approved for treating MRSA infections. MSSA isolates also exhibited antibiotic resistance patterns that varied by WWTP. In total, 93% of MRSA isolates were multidrug-resistant (MDR), while 29% of MSSA isolates were MDR.	[34]

Table 2. *Cont.*

Title	Type of WWTP	Microbe Assessed	Sampling Methods	Season of Sampling	Sampling Sites and Number of Samples	Assays	Main Findings	Reference
Characterization and source analysis of indoor/outdoor culturable airborne bacteria in a municipal wastewater treatment plant	Municipal WWTP with anaerobic–anoxic–oxic treatment process	Airborne Bacteria, Enterobacteriaceae and Opportunistic Pathogens	Air Sample: Active method—Impaction (Andersen six-stage cascade impactor, flow rate = 28.3 L/min)	Seasonal (spring, summer, autumn and winter)	Four specific sampling sites were selected within the plant: fine screens room (FS), aeration tank (AT), sludge dewatering house (SDH), and an external upwind control site; 48 air samples	Culture-based methods; Illumina MiSeq sequencing	FS had over ten times higher concentrations of culturable airborne bacteria compared to the outdoor aeration tank. Particle size distribution of culturable airborne bacteria varied between sampling sites. Enterobacteriaceae and opportunistic pathogens were detected indoors, primarily sourced from wastewater and sludge (were not detected outdoors).	[28]
Assessment of indoor airborne contamination in a wastewater treatment plant	Municipal WWTP with preliminary, primary, secondary, tertiary and sludge treatments, and deodorization processes	Bacteria and Fungi	Air Sample: Active method—Impaction (MAS 100, flow rate = 100 L/min)	Seasonal (summer, autumn and winter)	Bar Rack Chamber SEDIPAC 3D (Degritting/Degreasing/ Primary Sedimentation Facility) Secondary Sedimentation Tanks (Two Locations) Sludge Thickener Sludge Dehydration Chamber Sludge Disposal Area Outdoor Control Sampling Point	Culture-based methods (TSA for total bacteria, Mannitol salt agar and MAC for Gram-positive and Gram-negative bacteria, respectively, and DG18 for total fungi)	Out of 3 sampling campaigns, in the first one (with the highest ambient temperature) the total airborne bacteria and fungi concentrations were the highest. Gram-positive bacteria were the most dominant, and *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* were the most common fungi.	[15]
Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents	WWTPs with conventional activated sludge processes	Fecal Coliforms and Enteric Viruses	Material collection (effluent samples were collected in 1-L sterile glasses)	Seasonal (spring, summer, autumn and winter)	30 effluent samples (15 from each WWTP)	Culture-based methods	A high fecal coliform concentration was observed in the WWTPs. Enteric viruses were also detected, peaking in summer/autumn. There was a high risk for farmers (EV infection and disease burden) and risk for lettuce consumers, exceeding WHO guidelines.	[32]

Among the chosen studies, 12 (42.86%) were conducted within Municipal WWTP [4,6,15,17,22,24,25,28–31,37]. However, information regarding the type of WWTP was not explicit in 16 studies (57.14%) [5,13,14,16,18–21,23,26,27,32–36].

The most common sampled sites were the aeration tank (42.86%) [6,16,18,19,22,24,26–30,33], sludge dewatering basin (21.43%) [6,13,27–30] and grit chamber (17.86%) [6,19,27,29,30,33]. Some authors choose to perform the sampling at 1.5 m up on the aeration tanks (7.14%) [18,22]. Only one study (3.57%) [23] focused on sampling at different distances from the rotation brushes.

In terms of sampling strategy, seven papers opted to conduct sampling in two seasons (25%) [16,17,20,21,23,24,36]. Four studies (14.29%) were carried out in a single season [5,13,22,35] while another four studies covered all four seasons (14.29%) [4,26,28,32]. Furthermore, three authors conducted sampling activities across three seasons (10.71%) [15,27,33]. Five studies (17.86%) focused on a one-year longitudinal study [14,19,30,31,34]. Additionally, three studies (10.71%) differentiated sampling procedures between warm and cold seasons [6,29,37], whereas two studies did not specify the timing of their sampling activities (7.14%).

Air sampling emerged as the most employed technique, utilized in 24 out of 28 studies (85.71%) [4–6,13–25,27–31,33,36,37]. Active air sampling was carried out in 22 papers (78.57%) [4,5,13–25,27,28,30,31,33,36,37], and among these, the impaction method was predominant, with 19 studies (67.86%) [4,5,13–17,19–25,27,28,30,33,37] using different sampling devices such as the six-stage (32.14%) [5,16,19,22–25,28,37] and single-stage impaction (25%) [13–15,17,21,30,33]. The impingement method was employed in seven studies (25%) [4,13,14,21,25,31,36], while only five studies (17.86%) [4,13,14,21,25] utilized both impaction and impingement methods, simultaneously. Four studies used the filtration method (14.29%) [17–19,37]. Regarding passive sampling, it was employed in five studies (14.29%) [6,13,14,29,35], the 1/1/1 standard was used in two studies (in accordance with the microbiological sampling index of the air, a plate is placed at 1 m height, at 1 m distance to the (possible) source of contamination, and it is performed for a period of 1 h) (7.14%) [6,29], and surface samples were used in two papers (7.14%) [13,14]. Active and passive sampling strategies were carried out simultaneously in 2 out of the 28 studies (7.14%) [13,14]. Regarding the type of microbial contamination assessed, the majority of the studies (50%) [5,6,16,17,19,22–25,27,28,34,36,37] focused only on bacteria, while three studies (10.71%) [13,14,29] focused solely on fungi, and another three (10.71%) [20,31,35] evaluated only virus exposure. Six studies (21.43%) [4,15,18,21,30,33] included both fungi and bacteria, while one (3.57%) [18] examined bacteria, fungi, and viruses collectively, and another (3.57%) [32] assessed bacteria and viruses together.

Culture-based methods were the most frequently employed assays, utilized in 20 out of 28 studies (71.43%) [4–6,13–18,21–26,28–30,32,37]. Among the most used culture media, for fungal growth, three studies used MEA (Malt Extract Agar) (10.71%) [4,13,14], two studies Saboraud dextrose agar (SDA) (7.14%) [29,30], and one used Dichloran Glycerol agar (DG18) (3.57%) [18]. For bacteria, four studies used Tryptic Soy Agar (TSA) (14.19%) [4,15,30,37], three studies MacConkey Agar Medium (MAC) (10.71%) [15,22,24], and only one used Mannitol Egg Yolk (MYP) (3.57%) [24]. Nine of these studies only used one culture media for bacteria and/or fungi growth [4,6,13,14,16,17,23,29,30], and four used more than one culture media for bacteria growth [15,21,24,37]. In total, five studies did not mention the culture media used (17.86%) [5,25,26,28,32].

Molecular techniques were applied in 19 papers (67.86%): 13 employed Polymerase Chain Reaction (PCR) (46.43%) [4,6,13,14,20,23,25,26,31,33–35,37], and 6 used sequencing (21.43%) [22,25–28,36]. In PCR assays, to target bacterial strains, 5 out of 28 studies amplified bacterial 16S rRNA using universal primers (17.86%) [6,22,23,25,33], one amplified *Eschecrichia coli* MG1655 (3.57%) [36], and another used Chis150f and Clostlr primers for *Clostridium* sp. (3.57%) [16]. To detect bacterial pathogenic species, for *Staphylococcus aureus*, the primers used were NUC1 and NUC2 to target the NUC gene (3.57%) [34]. Another study targeted

bacterial populations from human flora, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and bacterial populations from freshwater environments such as *Aeromonas hydrophila* (3.57%) [37]. Three out of twenty-eight studies focused on antibiotic resistance profiling, one for MRSA using ECA1 and MECA2 primers for the amplification of mecA gene (3.57%) [34], one study (3.57%) used PCR coupled with gel electrophoresis to detect antibiotic resistance genes, such as sul1, sul2, sul3 for sulfonamide, tetA, tetC, tetO, tet W for tetracycline and integrons (intl1, intl2 and intl3) [4], and other amplified blaNDM, blaKPC, blaOXA-48, blaIMP, and blaVIM genes for Carbapenem-Resistant Citrobacter spp. (CRCS) (3.57%) [26]. For targeting viruses, two papers focused on SARS-CoV-2 (7.14%), one on the N1 and N2 unique genes [35] and one on RdRp, ORF-1ab, and N [31]. In one study (3.57%), three duplex qPCR were performed to target NoV180 GGII/RYMV and HEV/RYMV for RNA viruses, and AdV-40/AdV-E/D for DNA viruses [20]. For fungi, PCR was used to target *Aspergillus* sections such as *Flavi* (toxigenic strains), *Fumigati* and *Circumdati* in one paper [14], and only *Aspergillus* section *Flavi* in another [13]. Regarding sequencing methodologies, three out of six studies targeted the identification of airborne bacteria [22,27,28]; one targeted 16 rRNA to delineate the composition and similarities of microbiomes in water and air samples [36], one targeted taxonomic species of CRS [26], and one used sequencing to evaluate the positive clones of *Eschecrichia coli* [25]. In total, 11 out of 28 studies (39.29%), applied both molecular techniques and culture-based methods [4,6,13,14,16,22,23,25,26,28,37].

Among the species identified, the most prevalent Gram-positive bacteria were *Staphylococcus* sp. (21.43%) [17,19,24,30,33,34], *Bacillus* sp. (7.14%) [6,23] and *Clostridium* sp. (3.57%) [16], and the most prevalent Gram-negative were *Escherichia* sp. (7.14%) [22,24] and *Legionella* sp. (3.57%) [36]. *Aspergillus* sp. (17.86%) [13–15,21,30], *Cladosporium* sp. (10.71%) [15,29,30] and *Alternaria* sp. (10.71%) [15,29,30] dominated the fungal presence. One study focused on the dissemination of Methicillin-resistant *Staphylococcus aureus* (MRSA) [34], while another investigated the occupational exposure to *Staphylococcus aureus* in wastewater treatment plants, particularly focusing on antibiotic resistance [17].

4. Discussion

WWTPs are crucial for the implementation of the zero-waste strategy which is in the scope of the EC's circular economy management. Interestingly, the geographical distribution of the analyzed studies corroborated the urge for tackling WWTPs' pollution threat and to answer to the determined environmental goals worldwide. In agreement with previous reviews held in different settings, such as poultries [9] and sawmills [10], no standardization was observed in the sampling campaigns performed, as well as in the assays employed. Furthermore, the lack of standardized contextual information retrieved through the developed studies hinders the possibility to identify the environmental variables that contribute effectively to the occupational exposure assessment, as well as to propose suitable recommendations to avoid microbial exposure and dissemination [38]. In fact, the contextual information (e.g., implemented occupational health measures, training on safety issues related to the working tasks, cleaning practices, ventilation conditions, number of workers in each workstation, protection devices used by workers), when retrieved, should allow the identification of the most critical scenario and, thus, the selection of proper sampling sites following the "worst case scenario" approach as a first step for exposure assessment. In those sampling sites considered as the most critical, besides the environmental sampling campaign, nasopharyngeal swabs should be collected from the workers' nose to obtain additional information regarding workers' exposure. In previous studies, nasopharyngeal swabs were also taken to assess MRSA prevalence in workers from different occupational settings [39] or to corroborate the predominant fungi present in the Portuguese cork industry and, more specifically, exposure to *Penicillium* section *Aspergilloides* [40]. In addition, this approach can help occupational health services to prioritize multiple interventions in workers' education or even in personal protection device (e.g., gloves, respiratory protection devices) selection and replacement frequency.

The assessment of microbial dynamics in WWTPs is critical for ensuring public health and environmental safety. Seasonal evaluation plays a crucial role in this assessment, particularly given the influence of global warming and human activities, such as intensive agriculture, on microbial ecology [41,42]. In fact, recent studies [43,44] suggest that these factors contribute to the emergence of new fungal species, underscoring the need for comprehensive monitoring strategies. Recognizing the prevalence of research in specific regions and climatic periods is vital for contextualizing findings and understanding their implications for human health. Moreover, linking environmental exposure to health outcomes emphasizes the importance of establishing regulatory limits based on health considerations. This underscores the interconnectedness of the environment, exposure, and health outcomes, necessitating comprehensive regulatory frameworks.

Most of the selected papers (78.57%) exclusively applied active sampling methods, with impaction being the most frequently used method (67.86%). This sampling strategy is based on culture-based methods, which only allows the evaluation of culturable microorganisms, and thus microorganisms' cells that are potentially damaged due to the high velocity of the airflow are not isolated [10,37,45]. Furthermore, it is critical to emphasize that air is not uniform in place or time and that it is always subject to change based on the kind and intensity of the activities occurring there or other environmental variables (e.g., climate conditions) [36,46]. Thus, the sampling period must match the setting of the research and the work being developed in that specific environment. Passive sampling methods were applied in only a few of the analyzed studies as a stand-alone method (14.29%). However, passive sampling methods are expected to be more reliable than active sampling methods since they can collect contamination over longer periods, allowing to cover all the changes that may happen in the environment [47] such as the ventilation, environmental features [48], water infiltrations and damage [49], as well as the type of task being developed in that workplace [10,50,51]. Additionally, passive sampling methods allow the combination of different assays such as culture-based methods and molecular tools increasing the accuracy of obtained results [52]. Although only two papers (7.14%) used active and passive sampling methods together, this should be the trend to follow, since this allows each sampling methods' drawbacks to be overcome [10].

The fact that culture-based methods are primarily used for microbial characterization as standard methods for microbial assessment [53,54] might justify its frequent use among the selected papers (71.43%). This methodology is crucial to estimate health risks, since microorganisms' viability can limit microorganisms' inflammatory and/or cytotoxic potential [10,54,55]. Despite the advantages, conventional approaches may underestimate results since incubation temperatures and culture conditions may favor specific species. Plus, typical procedures may not always be effective in cultivating certain common microorganisms [53]. Furthermore, a recent study [53] highlights the importance of culture media selection and its significant impact on fungal counts and species diversity. Although some studies (17.86%) did not mention what culture media were employed, accurate culture media selection is critical for exposure assessment in different environments, particularly when targeting *Aspergillus* sp. [53]. Overall, three cultural media were employed for fungal assessment (MEA, DG18, and SDA). MEA and SDA are the most used non-selective media for fungi and yeasts, whereas DG18 is a fast-growing fungi inhibitor, allowing more diversity in the growth of fungal strains [56]. MEA and DG18 have both been used alongside and have proven to be useful in the growth of *Aspergillus* species according to the matrix, sampling method employed, and indoor environment assessed [57]. For bacterial assessment, TSA was the most non-selective media related to the growth of fastidious bacteria, while MAC was the most used selective and differential media related to the growth of Gram-negative bacteria, useful for the identification of enteric bacteria [58]. MYP allows the identification of Gram-positive bacteria as *Bacillus cereus* [59]. The use of multiple culture media is fundamental for the isolation and identification of a wider spectrum of microorganisms. Also, the integration of multiple culture media and different incubation temperatures in culturomics methods (such as MALDI-TOF) permits

a more precise identification of unknown isolates [60,61]. This approach allows accurate microbial characterization, particularly the rapid identification of potential pathogens. In fact, culturomics methods bridge the gap between culture-based methods and molecular techniques, providing a comprehensive assessment of bioaerosols [38].

Recently, culture-independent techniques such as PCR and genome sequencing have been demonstrated to be useful for various bioaerosol measurements [52]. Indeed, PCR and sequencing were frequently performed by the authors in the selected papers. These techniques enable the detection of non-viable microorganisms as well as their potentially allergenic components [52,62], providing more information regarding microbial diversity in the evaluated environment [9]. Molecular techniques along with culture-based methods were applied by some papers (39.29%). This strategy is highly supported, since both viable and non-viable microorganisms are considered, providing a wider microbial characterization [9,10,52], and a more accurate characterization of the exposure scenario [14].

Furthermore, molecular techniques development has also enabled the assessment of Antibiotic Multidrug Resistance (AMD), including resistance genes associated with bacteria contamination. Recently, the World Health Organization (WHO) released an updated Bacterial Priority Pathogens List (BPPL) 2024, in which 15 families of antibiotic-resistant bacteria were grouped into critical, high and medium categories in order to allow an effective prioritization [63]. Additionally, the European Food Safety Authority (EFSA) panel on Biological Hazards recently emitted a Scientific Opinion in which the highest priority antimicrobial-resistant bacteria (ARB) and antibiotic resistance genes (ARG) were identified in different sources, including water. Among the most relevant ARB, the panel indicated carbapenem or extended-spectrum cephalosporin and/or fluoroquinolone-resistant *Enterobacterales*, fluoroquinolone-resistant *Campylobacter* sp., Methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant *Enterococcus faecium* and *E. faecalis*. Regarding the highest priority ARGs, the panel reported *blaCTX-M*, *blaVIM*, *blaNDM*, *blaOXA-48-like*, *blaOXA-23*, *mcr*, *armA*, *vanA*, *cfr* and *optrA* genes. The EFSA report also evidenced the existence of several data gaps regarding sources and the relevance of transmission routes and diversity of ARB and ARGs [64]. The data analyzed in this review demonstrate that antibiotic resistance profiling, including MRSA, *mecA gene* [31], sulfonamide, *sul1*, *sul2*, *sul3*, tetracycline, *tetA*, *tetC*, *tetO*, *tet W*, integrons, *intl1*, *intl2 and intl3* [4], and Carbapenem-Resistant *blaNDM*, *blaKPC*, *blaOXA-48*, *blaIMP*, and *blaVIM* genes [26] is already a reality. Moreover, despite the fact that the quantitative microbial risk assessment (QMRA) of WWTPs has been classically focused on risk-based monitoring targets, it is accepted that the expansion of QMRA methodologies, to include ARG, may be key for the assessment of the relative risk of these contaminants [65]. The assessment of ARG units is crucial for the identification of relevant/high-priority sources and natural reservoirs of AMR, allowing the establishment of effective mitigation strategies in a One Health approach. Despite the fact that microbial assessment in water samples and sewage treatment plants has been carried out, the development of official monitoring strategies and effective risk assessment in sewage treatment plants is crucial. In agreement with the newly updated WHO-BPPL, which demonstrates the highly dynamic nature of AMR, increasing evidence and expert reports clearly highlight the urge to promote a comprehensive public health approach and international coordination to engage innovation and mitigation strategies [63].

On the other hand, it is important to note that ARGs identification may be influenced by the different methods employed and divergences in the measuring process from sampling to wet-lab differences, among others [66]. In addition to the multi-criteria decision analysis (MCDA) method developed by the WHO in the 2017 WHO BPPL, which is still currently applied in the 2024 WHO BPPL [63] and EFSA Panel on Biological Hazards (BIOHAZ) risk assessment monitoring (https://www.efsa.europa.eu/en/topics/topic/ biological-hazards), other international multi-disciplinary networks, such as NEREUS COST Action ES1403 [67], created to access the current challenges related to wastewater reuse and high-priority concerns regarding public health and environmental protection, concluded that scientific research and environmental management should follow systematic, quantitative, and comparable ARG datasets, and reported that the

research community should adopt "ARG copy per cell" [66]. Thus, the development of effective mitigation measures including new monitoring technologies, such as on-line sensors that are able to detect and quantify bacterial pathogens, ARB and ARG, is crucial, as is the implementation and improvement of links between research and policy [65].

The identification of the most suitable fungal indicators in WWTPs is also critical for assessing treatment efficacy, environmental impacts, and public and occupational health risks [68]. Commonly used fungal species such as *Aspergillus* sp. and *Penicillium* sp. serve as markers for organic matter removal and microbial contamination [69]. Monitoring fungal indicators enables the identification of seasonal variations, climate influences, and anthropogenic impacts on wastewater quality, essential for tailoring treatment strategies. Additionally, their presence aids in the early detection of potential health hazards, such as opportunistic pathogens or allergenic molds, ensuring the safety of both workers and the public [70]. *Aspergillus* sp. was recurrent and also the most prevalent in the selected papers; the prevalence of this genera in waste management industries has already been recognized, highlighting the need for further research regarding occupational exposure [14]. In fact, *Aspergillus* section *Fumigati* was already suggested as an indicator of harmful fungal exposure in the waste management industry [71–74] and listed by the WHO as a critical priority, considering specific criteria such as antifungal resistance, mortality, evidence-based treatment, access to diagnostics, annual incidence and complications and sequelae [75]. However, the WHO list did not consider the toxicologic potential from fungal species, neglecting the possible occupational exposure to mycotoxins, as was already reported in different occupational environments [76].

In agreement with bacteria contamination analysis, fungal assessment should also cover the resistance profile. Indeed, antifungal drug resistance is a growing global concern in both space and time. This includes newly emerging species that are resistant to multiple antifungal drugs (like the yeast *Candida auris*), as well as novel resistant variants of previously susceptible pathogens (such as the ubiquitous mold *Aspergillus fumigatus*) [77]. Because of the selection of resistant strains triggered by the growing use of triazole drugs, azole resistance in *Aspergillus fumigatus* is currently seen as an emerging hazard to global public health [78,79]. In *Aspergillus fumigatus*, azole resistance can evolve through two different pathways. First, in the setting of chronic pulmonary aspergillosis, as in individuals with cystic fibrosis, resistant strains may be chosen during or following a long-term azole therapy [79,80]. Second, the prolonged use of azole antifungals in agriculture may be connected to azole resistance [79,81–84]. Relevantly, it is reported that several antifungals cause inherent resistance in *Fumigati* cryptic species. However, selected pressure brought on by the prolonged azole therapy of patients with chronic aspergillosis or environmental selection pressures are the reasons behind the emergence of resistance acquisition in *Aspergillus fumigatus* sensu stricto. Mutations in genes engaged in the *Aspergillus fumigatus* ergosterol pathway are frequently linked to the mechanisms of azole resistance, especially in the cyp51A gene that encodes cytochrome P450 14-lanosterol demethylase, the primary target of azole antifungals [79,85,86], highlighting the relevance of using these mutations as an indicator for fungal resistance.

Considering the above, further research should be performed to select the most suitable indicators of harmful microbial contamination for this occupational setting. The lists provided by the WHO regarding fungi [86] and bacteria [87] should be considered for this endeavor, but the resistance and toxicological potential from fungi and bacteria should not be neglected.

5. Conclusions

Overall, this scope review concluded what is needed to provide robust science for the guidance of occupational exposure assessments: (a) common protocol from the field (sampling campaign) to the lab (assays to employ) when aiming to perform exposure assessment in WWTPs; (b) standardized contextual information to be retrieved, allowing a proper risk control and management; (c) the selection of the most suitable microbial targets to serve as indicators of harmful microbial exposure. Filling these gaps with further studies

will allow robust science to be provided to policy makers and stakeholders.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms12061144/s1, Table S1: PRISMA Checklist.

Author Contributions: Conceptualization, C.V.; methodology, B.R., M.R., L.M., R.C., B.G., M.D. and P.P.; formal analysis, B.R., M.R., L.M., R.C., B.G., M.D. and P.P.; investigation, B.R., M.R., L.M., R.C., B.G., M.D. and P.P.; resources, C.V.; writing—original draft preparation, B.R., M.R., L.M., R.C., B.G., M.D., P.P., E.R. and C.V.; writing—review and editing, R.C., B.G., M.D., P.P., E.R. and C.V.; supervision, E.R. and C.V.; funding acquisition, C.V. All authors have read and agreed to the published version of the manuscript.

Funding: Authors gratefully acknowledge the FCT/MCTES national support through the UIDB/ 05608/2020; UIDP/05608/2020. This research was funded by national funds through FCT/MCTES/FSE/UE, 2023.01366.BD; UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); UI/BD/151431/2021 (https://doi.org/10.54499/UI/BD/151431/2021) and Instituto Politécnico de Lisboa, national support through IPL/2022/InChildhealth/BI/12M; IPL/IDI&CA2023/FoodAIIEU_ESTeSL; IPL/IDI&CA2023/ASPRisk_ESTeSL; IPL/IDI&CA2023/ARAFSawmills_ESTeSL.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

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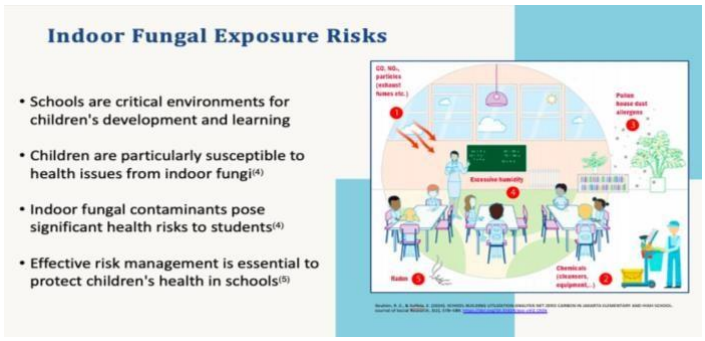
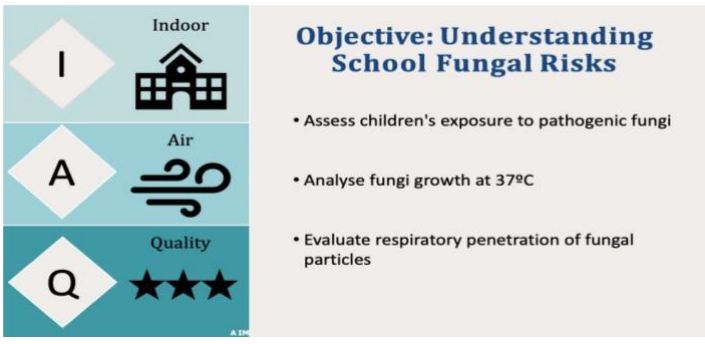
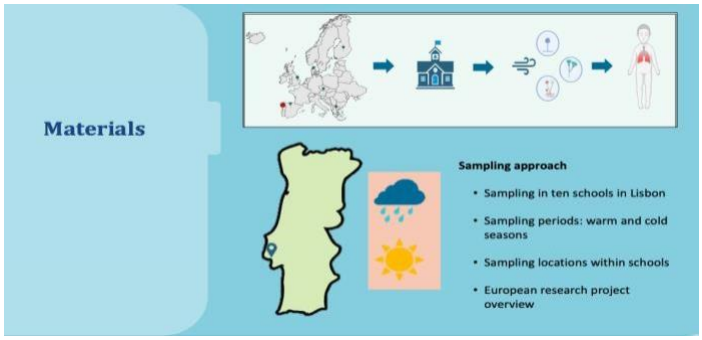
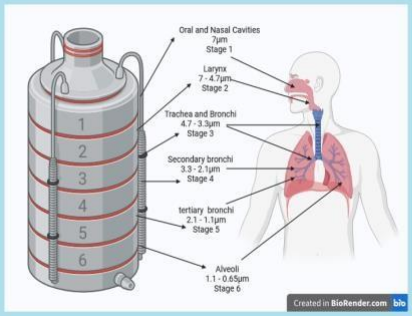
Appendix 8: Presentation in the Occupational Safety and Hygiene Symposium (SHO 2024) in Porto, The award-winning work, titled "Understanding and Addressing Fungal Exposure Risks in Primary Schools: Implications for Children's Health and Well-being,"





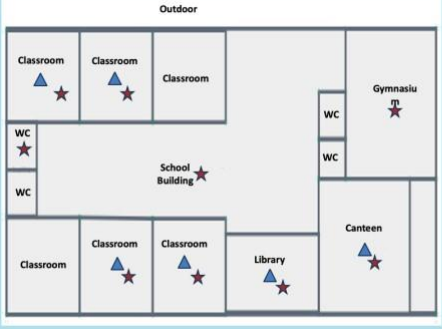




Methodology

Study Design

- Anderson six-stage air sampler
- Lighthouse for indoor temperature and humidity measurement
- Sampling media: MEA, DG18, TSA, VRBA



Methodology

Air Sampling Techniques

- Use of Anderson six-stage air sampler
- Differentiation of particle sizes
- Sampling locations within schools
- Indoor temperature and humidity measurement

Lighthouse

Anderson 6-stage

Analysis

Incubation of samples at 27ºC and 37ºC

Macro and Micro identification

Analysis

Counting colony-forming units (CFU)

Examining Data on Fungal Exposure and Impact

In Warm and Cool Seasons' Impact

In Warm and Cold Seasons' Impact

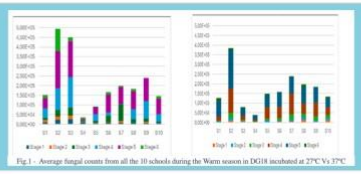
Warm Season

Initial Findings - Warm Season
- **Title:** Warm Season Results
- **Content:**
  - Fungal counts at 27ºC and 37ºC
  - Higher counts in School 3
  - Increase in stages 4 and 5 (respiratory penetration)

Cold Season

Initial Findings - Cold Season
- **Title:** Cold Season Results
- **Content:**
  - Fungal counts at 27ºC and 37ºC
  - Lower counts overall except in School 2
  - Stages 4, 5, and 6 show notable differences

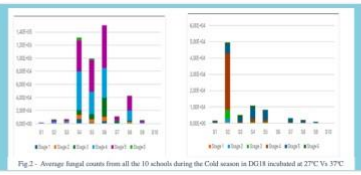
Warm Season



DG18 media at 27ºC

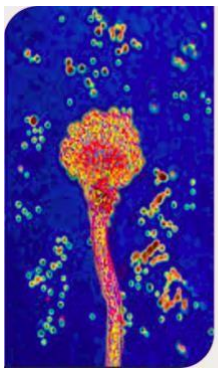
Fig.1 - Average fungal counts from all the 10 schools during the Warm season in DG18 incubated at 27ºC Vs 37ºC

Cold Season



DG18 media at 37ºC

Fig.2 - Average fungal counts from all the 10 schools during the Cold season in DG18 incubated at 27ºC Vs 37ºC



Discussion

- Health risks associated with indoor fungal exposure[6]
- Seasonal variations observed in fungal loads[1]
- Influence of climate change on fungal distribution patterns[1]
- Importance of conducting comprehensive evaluations[7]

Health Risks and Mitigation

Health Risks and Mitigation

- Vulnerability of children to respiratory issues[4]
- Need for systematic evaluations and monitoring[7]
- Importance of proactive measures in school[8]

Health Implications

Conclusions

- Necessity for comprehensive studies
- Importance of targeted mitigation strategies
- National initiatives to improve indoor air quality
- Proper ventilation and humidity control reduce fungal growth risks
- Prevent moisture with regular structure checks and maintenance
- Educate staff and students to spot fungal issues early for prompt mitigation.

Acknowledgments

Authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020; UIDP/05608/2020. This work is also supported by national funds through FCT/MCTES/FSE/UE, 2023.01366.BD; UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); UI/BD/151431/2021 (https://doi.org/10.54499/UI/BD/151431/2021); and Instituto Politécnico de Lisboa, national support through IPL/2022/InChildhealth/BI/12M; IPL/IDI&CA2023/FoodAllEU_ESTeSL; IPL/IDI&CA2023/ASPRisk_ESTeSL; IPL/IDI&CA2023/ARAFSawmills_ESTeSL

"Partly funded by the European Union. Views and opinions expressed are howe Número do Diapositivo author(s) only and do not necessarily reflect those of the European Union or the Swiss State Secretariat for Education, Research and Innovation (SERI), or the United Kingdom Research and Innovation (UKRI), or the Australian National Health & Medical Research Council (NHMRC). Neither the European Union nor the granting authorities can be held responsible for them."

Project funded by

UK Research and Innovation

Funded by the European Union

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Thank you

Open floor for discussion

Appendix 9: Participation in poster presented in SHO 2024 in Porto - Fungal Contamination in Lisbon's Primary Schools – Sampling Insights and Analytical Approaches

Fungal Contamination in Lisbon's Primary Schools - Sampling Insights and Analytical Approaches

Renata Cervantes [1,2], Pedro Pena [1,2], Bianca Gomes[1,3], Marta Dias[1,2], Bruna Riesenberger[1], Margarida Rodriguez[1], Liliana Marques[1], Carla Viegas [1,2]

[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, Comprehensive Health Research Center, NOVA National School of Public Health, CHRC, REAL, CCAL, NOVA University Lisbon, 1099-085 Lisbon, Portugal
[3] CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal

Introduction

Climate change is posing challenges for Portugal due to intense weather changes, affecting public health and causing pathogens to adapt and spread, increasing the global risk of infectious diseases [1,2].

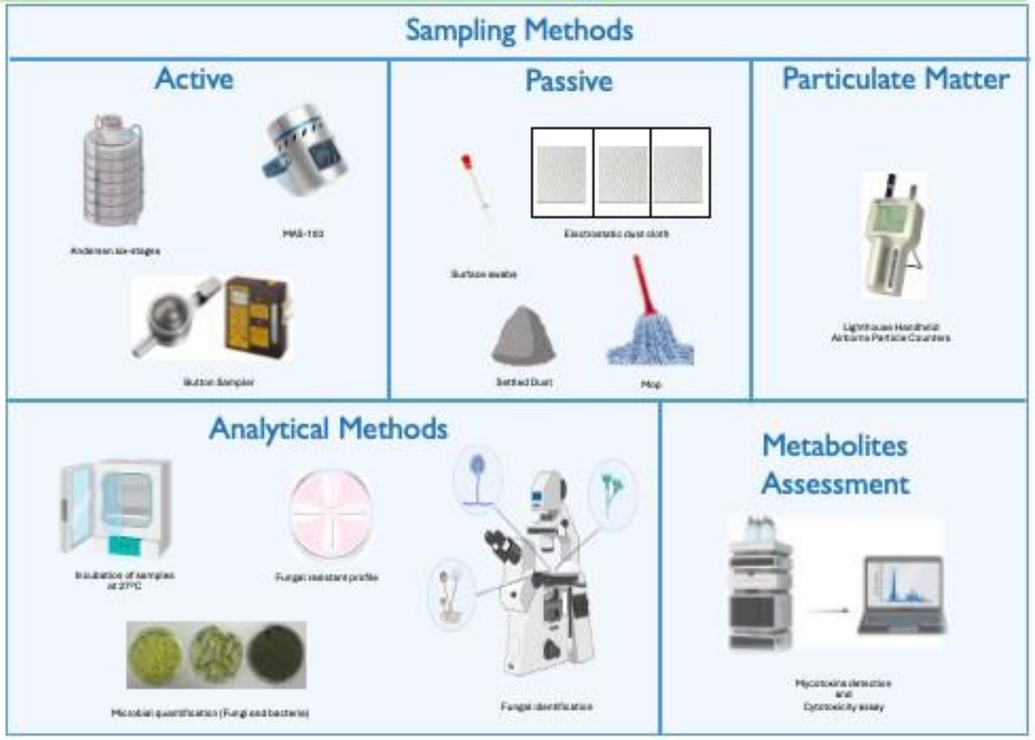
Azole fungicides are less effective against resistant fungi, raising concerns for children [1,2].

Warm and humid conditions promote the growth of pathogenic fungi and the production of mycotoxins, impacting health by causing gastrointestinal problems, organ damage and chronic diseases. Even after fungi removal, mycotoxins continue to pose risks [3,4,5].

Objectives

- Identifying fungal species present in indoor environments.
- Assessing spatial distribution and concentration levels within classrooms and other areas.
- Investigating factors influencing fungal proliferation, such as building characteristics and seasonal variations.
- Evaluating the effectiveness of existing cleaning protocols and providing insights into proactive management strategies to protect students' and staff members' health and well-being.

Methodology



Results and discussion

The expected results are that seasonal variations in fungal load show complex environmental interactions[1,2].

Examining fungal load distribution in DG18 media at 27°C and 37°C helps assess growth preferences at different temperatures[6].

Methods used to assess azole resistance and mycotoxin provide essential insights into the resilience and potential harm of fungal species under varying environmental conditions[3,4,5].

Addressing fungal exposure risks requires a comprehensive approach for an accurate risk assessment and to target mitigation strategies on educational environments [6].

Conclusions

- **Standardized protocols** need to be defined and implemented for **effective risk assessment.**
- it is essential to consider **climate changes and seasonal influences into health policies** to mitigate the risks associated with fungal exposure.

Acknowledgements

This project was supported by FCT/MCTES UIDP/05608/2020 (https://doi.org/10.54499/UIDP/05608/2020) and UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020. This work is also supported by national funds through FCT/MCTES/PSE/UE, 2023.01366.BD; UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); UI/BD/151431/2021 (https://doi.org/10.54499/UI/BD/151431/2021); and Instituto Politécnico de Lisboa, national support through IPL/2022/InChildhealth/BI12M; IPL/IDI&CA2023/FoodAIEU ESTeSL; IPL/IDI&CA2023/ASPRisk ESTeSL; IPL/IDI&CA2023/ARAFSawmills ESTeSL. This project was partly funded by EU Horizon 2021 grant no. 101056883 and co-funding from author's organizations and/or Ministries. Funding from Swiss SERI grant 22.00324, UKRI grant 10040524, and NHMRC grant APP2017786 and APP2008813. Views expressed are of the author(s) and do not necessarily reflect those of EU, Swiss SERI, UKRI, or NHMRC

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Appendix 10: Participations in poster presented in SHO 2024 in Porto - A multi-approach sampling strategy to assess exposure to microbiologic agents in poultries.

A multi-approach sampling strategy to assess exposure to microbiologic agents in poultries

Bianca Gomes [1,2,*], Marta Dias [2,3], Pedro Pena [2,3], Renata Cervantes [2,3], Margarida Rodriguez [2], Liliana Marques, [2], Bruna Riesenberger [2], Carla Viegas [2,3]

[1] Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal, bianca.gomes@estesl.ipl.pt
[2] H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Lisboa; pedro_migpena@hotmail.com; renata.cervantes@estesl.ipl.pt;
[3] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal; martasfd@gmail.com; carla.viegas@estesl.ipl.pt

Introduction

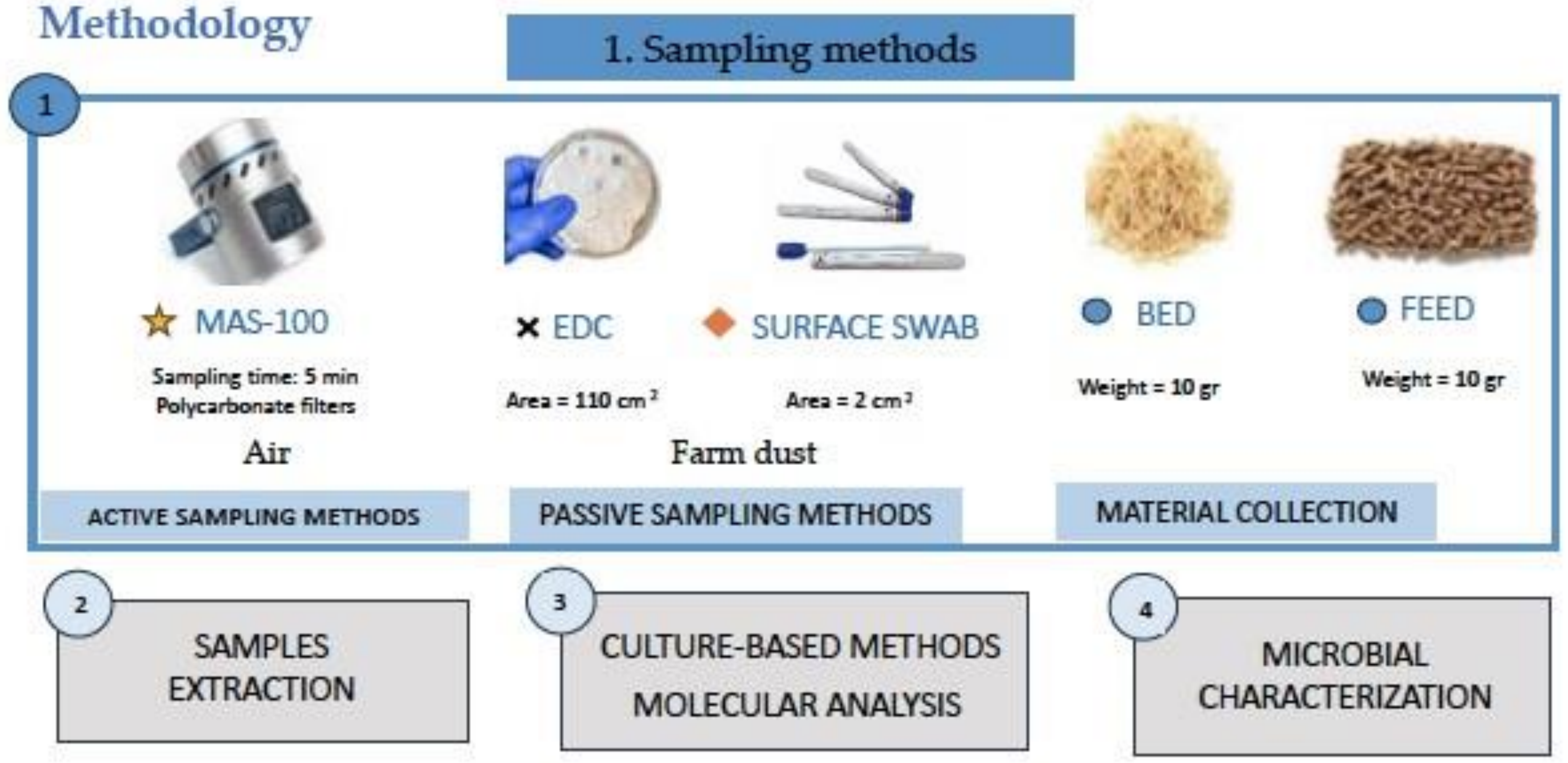
A reasonable number of studies focusing on **microbiological contamination** associated with the poultry industry evidence various health concerns [1,2]

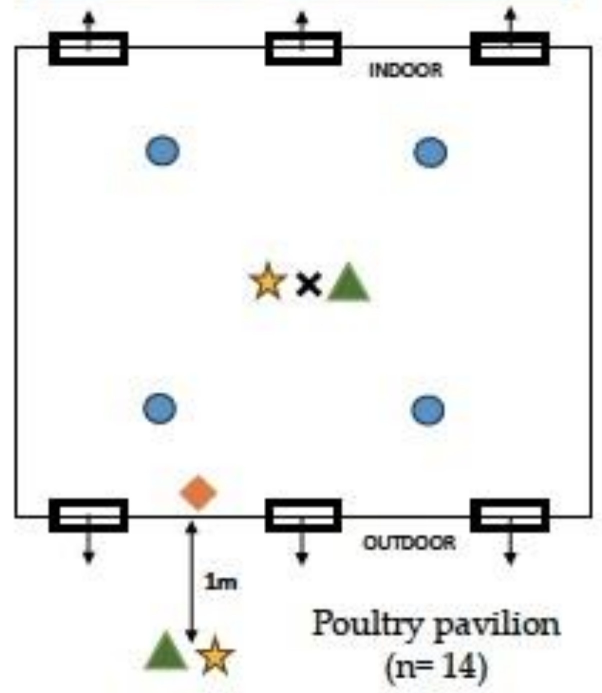
In occupational studies focusing on microbiological contamination in poultry farms, air sampling is typically the only sampling method used [3]

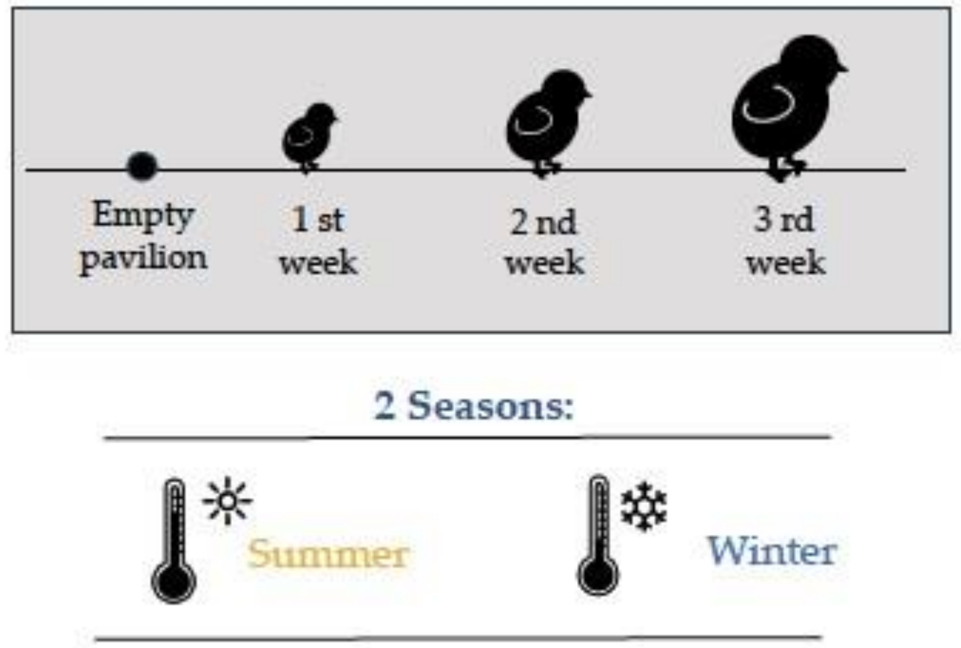
To better understand the relationship between factors influencing microbial contamination and adverse health effects, data regarding the amount, composition, and risk category of the common microorganisms are needed [4].

This study intends to apply a multi-approach sampling protocol and corroborate the importance of its application for a wider microbial characterization

Methodology







Results and discussion

Culture based-methods: Fungal prevalence in indoor air samples was highest during: the 3rd week (35%), followed by 2nd week (33%) and 1st week (10%).

Molecular analysis: Fungal detection in indoor air samples was highest during: the 3rd week (69%), followed by 2nd week (64%) and 1st week (43%).

Culture based-methods offer the advantage of enabling identification and quantification of viable microorganisms which is essential to estimate health risks since microorganisms' viability can restrain microorganisms' pathogenic potential. Culture-based methods, on the other hand, may understimate the results since incubation temperature and culture conditions may favor specific species [3].

PCRbased techniques have been widely used in detection of microorganisms from environmental samples to determine accurately and quantitatively, the composition of microbial communities [3]. These methods allow the detection of non-viable microorganisms which, may justify the differences between in the obtained results from conventional and molecular methods.

Conclusions

- **Both methods have advantages and limitations** when applied to characterize occupational exposure to biological agents in different settings. The results highlight the importance of using a multi-approach sampling strategy and laboratory assays including culture-based methods along with molecular tools [3].
- The multi-approach sampling strategy and assays will **enhance data findings, enabling a more accurate intervention** in order to propose strategies to improve poultry environment, enhance workers and animal safety while reducing environmental impact.

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Acknowledgements
This project was supported by FCT/MCTES UIDP/05608/2020 and UIDB/05608/2020. This work is also supported by national funds through FCT/MCTES/PIE/UE, 2023.01366.BD; UI/BD/153746/2022 and CHRC unit UIDB/00329/2020; UI/BD/151431/2021; and Instituto Politécnico de Lisboa, national support through IPL/2022/...

Appendix 11: Participations in poster presented in SHO 2024 in Porto – Budget-friendly protocol for TR34-L98H and TR46/Y121FT289A mutation detection in *Aspergillus* section *Fumigati* isolates

Budget-friendly protocol for TR34/L98H and TR46/Y121FT289A mutation detection in *Aspergillus* section *Fumigati* isolates

M. Dias[a,b], M. Rodriguez[b], C. Vasques[b], B. Riesenberger[b], L. Marques[b], B. Gomes[b,c], P. Pena[a,b], R. Cervantes[a,b], S. Viegas[a,b], C. Viegas[a,b]

[a] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal (msf.dias@ensp.unl.pt)
[b] H&TRC- Health & Technology Research Center, ESTeSL- Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Portugal (marta.dias@estesl.ipl.pt)
[c] CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal

Introduction

Aspergillus section *Fumigati* is one of the **most common sections**, in the environment [1].

It has been found in **different occupational environments, such as sawmills and waste sorting** [1,2].

Its **cryptic species show intrinsic resistance** to several antifungals [3].

Resistance in *A. fumigatus* is emerging due to selective pressure caused by the prolonged use of azoles.

It is often associated **with mutations in the Cyp51A gene** [3].

The **fungal priority pathogens list (WHO)**, includes ***A. fumigatus* with critical priority** [4].

Further analysis to identify potential resistance mechanisms and mutations is needed.

Objective

This evaluation aims to offer a protocol for mutation detection in *Aspergillus* section *Fumigati* isolates,

It will contribute for the development of guidance that can support future occupational exposure assessments.

Methodology

Hypotheses were determined based on the advantages and disadvantages of each suggested method, including its cost.

	Whole genome sequencing	Sequencing (x2) of all isolates	Sequencing (x2) of resistant isolates	Sequencing + qPCR	Incubation + RT-PCR
👍	All information with one analysis	All the necessary information	Information regarding the section and the presence of TR and point mutations	Information regarding the presence of TR and point mutations	All the necessary information
👎	Additional unnecessary information for this analysis	↑	Loss of information regarding point mutations in the CYP51A	Cryptic species not identified	↑ Loss of information regarding point mutations in the Cyp51A

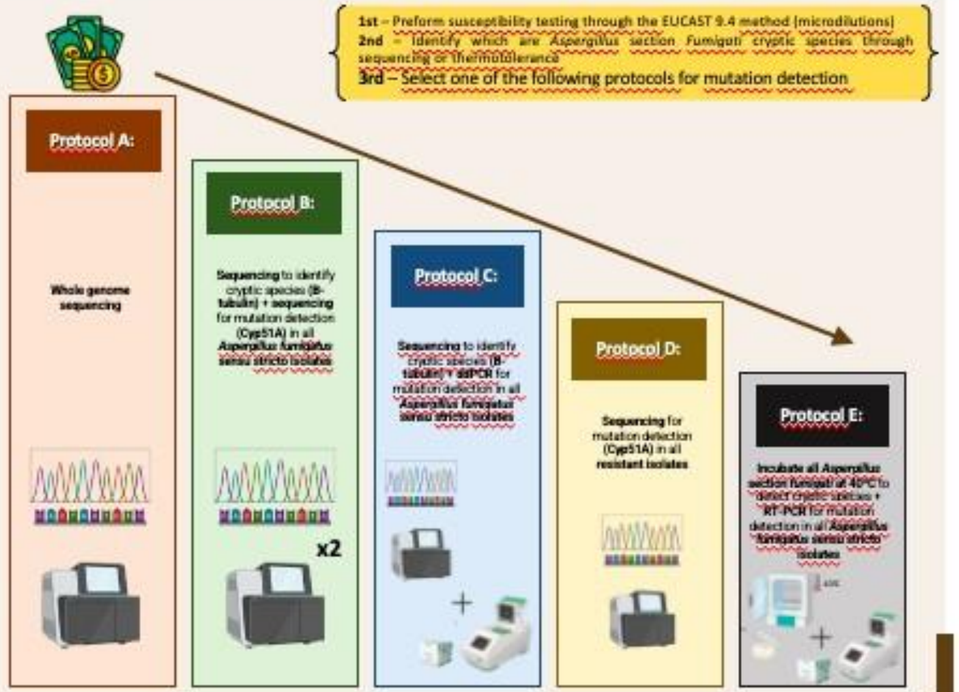
*Tandem repeat

Conclusions

This study allowed determining several ways to detect mutation in *Aspergillus* section *Fumigati* isolates. It **provided the necessary tools to perform an accurate occupational exposure assessment to *Aspergillus* section *Fumigati*** and allowed a **more detailed risk assessment** while overcoming cost issues at the same time.

Results and discussion

- Azole resistance is mostly caused by **particular mutations in CYP51A** [5].
- **Wild-type CYP51A-resistant** isolates question the effectiveness of the available methods [5].
- **Whole-genome sequencing is** becoming increasingly common to address these issues [5].



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Aknowledgements

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Appendix 12: Certificate of participation in SHO 2024

International Symposium

sho 24

Occupational Safety and Hygiene

CERTIFICATE OF PARTICIPATION

This is to certify that

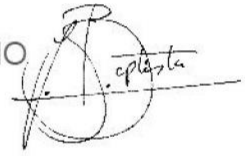
BRUNA RIESENBERGER

[redacted]

has participated in SHO 2024 – International Symposium on OCCUPATIONAL SAFETY AND HYGIENE, hybrid event held on the 4th and 5th July, 2024.

The President of SPOSHO

João Santos Baptista



SPOSHO

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Guimarães

University of Porto
Porto

University of Lisbon
Lisboa

Appendix 13: Abstract submitted for VI H&TRC BootCamp 2024

Microbial Exposure Assessment in Waste Water Treatment Plants

Bruna Riesenberger [1], Margarida Rodriguez [1], Liliana Marques [1], Renata Cervantes* [1,2], Pedro Pena [1,2], Bianca Gomes [1,3], Marta Dias [1,2], Carla Viegas [1,2]

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, Comprehensive Health Research Center, NOVA National School of Public Health, CHRC, REAL, CCAL, NOVA University Lisbon, 1099-085 Lisbon, Portugal

3 CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal

*Autor da apresentação: brunarayzen@gmail.com

Background: The European Commission's circular economy strategy depends on wastewater treatment plants (WWTPs) [1]. However occupational exposure to bioaerosols in these facilities should be consider [1,2]. This study aims to perform a scoping review to provide a broad overview of the state-of-the-art methods (sampling and analyses) applied to perform microbial contamination assessments in WWTPs **Methods:** Using the PRISMA methodology, a comprehensive search was conducted in PubMed, Scopus, and Web of Science [3,4], including the terms "Waste Water Treatment Plants", "bacteria", "fungi", "viruses", "exposure" and "sampling", with English as the chosen language. The study includes data published from 1 January 2010 to 8 November 2023. **Results:** The scope resulted in the selection of 28 relevant articles. The most frequently sampled sites in WWTPs were the aeration tank (42.86%), sludge de-watering basin (21.43%), and grit chamber (17.86%). Air sampling emerged as the preferred technique (24 out of 28 studies), with culture-based methods being the most commonly used assays (20 out of 28 studies). The predominant bacterial isolates included *Staphylococcus* sp. (21.43%), *Bacillus* sp. (7.14%), *Clostridium* sp. (3.57%), *Escherichia* sp. (7.14%), and *Legionella* sp. (3.57%). Fungal isolates were dominated by *Aspergillus* sp. (17.86%), *Cladosporium* sp. (10.71%), and *Alternaria* sp. (10.71%). **Conclusions:** The review identified critical needs for future research and policy development, including: (a) a standardized protocol from sampling in the field to laboratory assays for exposure assessment in WWTPs; (b) the collection of standardized contextual information to ensure effective risk control and management; (c) the identification and use of suitable microbial indicators for harmful exposure [5]. Addressing these gaps will enhance the scientific foundation available to policymakers and stakeholders,

facilitating informed decision-making and improved public health safeguards [6]. Future research should target more specific qualitative and quantitative analysis of microbial aerosols in WWTPs to improve the existing understanding and provide recommendations concerning the air quality in WWTPs.

Acknowledgements
This project was supported by FCT/MCTES UIDP/05608/2020 (https://doi.org/10.54499/UIDP/05608/2020) and UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020. This work is also supported by national funds through FCT/MCTES/FSE/UE, 2023.01366.BD; UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); UI/BD/151431/2021 (https://doi.org/10.54499/UI/BD/151431/2021); and Instituto Politécnico de Lisboa, national support through IPL/2022/InChildhealth/BI/12M; IPL/IDI&CA2023/FoodAIIEU_ESTeSL; IPL/IDI&CA2023/ASPRisk_ESTeSL; IPL/IDI&CA2023/ARAFSawmills_ESTeSL
This project was partly funded by EU Horizon 2021 grant no. 101056883 and co-funding from author's organizations and/or Ministries. Funding from Swiss SERI grant 22.00324, UKRI grant 10040524, and NHMRC grant APP2017786 and APP2008813. Views expressed are of the author(s) and do not necessarily reflect those of EU, Swiss SERI, UKRI, or NHMRC

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Informações adicionais

A. Identificar a linha de investigação do H&TRC em que se insere o trabalho

Occupational Health

B. Identificar qual o Objetivo de Desenvolvimento Sustentável (ODS) em que se insere o trabalho

3; 6; 8;11; 14

Appendix14: Abstract “The Power of Citizen Science: Insights and Achievements from the InChildHealth Project” submitted for VI H&TRC BootCamp 2024

The Power of Citizen Science: Insights and Achievements from the InChildHealth Project

Renata Cervantes* [1,2], Pedro Pena [1,2], Bruna Riesenberger [1], Margarida Rodriguez [1], Liliana Marques [1], Bianca Gomes [1,3], Marta Dias [1,2], Carla Viegas [1,2]

[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, Comprehensive Health Research Center, NOVA National School of Public Health, CHRC, REAL, CCAL, NOVA University Lisbon, 1099-085 Lisbon, Portugal

[3] CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisbon, Portugal

*Autor da apresentação: r.w.cervantes@gmail.com

Appendix 15: Abstract “Indoor microbial levels in poultry pavilions” submitted for VI H&TRC BootCamp 2024

Indoor microbial levels in poultry pavilions

Bianca Gomes[1,2*], Marta Dias [2,3], Renata Cervantes [2,3], Pedro Pena [2,3], Margarida Rodriguez [1], Liliana Marques [1], Bruna Riesenberger [1], Carla Viegas [2,3]

[1]H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Lisboa;

[2] CE3C- Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa;

[3] NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal

*Autor da apresentação: bianca.gomes@estesl.ipl.pt

Appendix 16: Abstract "Protocol to detect CYP51A mutations in *Aspergillus* section *Fumigati* isolates" submitted for VI H&TRC BootCamp 2024

PROTOCOL TO DETECT CYP51A MUTATIONS IN *ASPERGILLUS* SECTION *FUMIGATI* ISOLATES

Marta Dias[1,2], Margarida Rodriguez[2], Catarina Vasques[2], Bruna Riesenberger[2], Liliana Marques[2], Bianca Gomes[2,3], Pedro Pena[1,2], Renata Cervantes[1,2], Susana Viegas[1,2], Carla Viegas[1,2]

[1]NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal

[2]H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa; carla.viegas@estesl.ipl.pt

[3]CE3C—Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências,Universidade de Lisboa, 1749-016 Lisbon, Portugal

·Autor da apresentação: marta.dias@estesl.ipl.pt

Appendix 17: Abstracts submitted for EPICOH 2024

Epidemiology in Occupational Health (EPICOH) 2024

Mitigating Health Risks in Wastewater Treatment Plants: Identifying Key Microbial Contaminants and Protocol Needs

Renata Cervantes[1,2]; Bruna Riesenberger [1], Margarida Rodriguez [1], Liliana Marques [1], Bianca Gomes [1,3], Marta Dias [1,2], Pedro Pena [1,2], Edna Ribeiro [1] and Carla Viegas[1,2]

Analysis protocol to assess occupational exposure to microbial contamination in woodworking environments

Marta Dias[1,2]; Bianca Gomes[2,3]; Pedro Pena[1,2]; Renata Cervantes[1,2]; Sara Gonçalves[2]; Margarida Rodriguez[2], Bruna Riesenberger[2], Liliana Marques[2] Susana Viegas[1,2]; Carla Viegas[1,2]