

Fungal Resistance and Public Health Implications: The Use of WWTP Sludge in Agriculture

Márcia Pimenta^{1,2}, Renata Cervantes^{1,4}, Pedro Pena^{1,4}, Raquel Sabino^{5,6,7}, Carla Viegas^{1,4}

¹ Laboratório Nacional de Referência para Infecções Parasitárias e Fúngicas, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal. ² Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal. ³ H&TRC—Health & Technology Research Center, ESTeSL—Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, Lisboa, Portugal. ⁴ NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisboa, Portugal. ⁵ Research Institute for Medicines (iMed.U.Lisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal. ⁶ Instituto de Saúde Ambiental, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal. ⁷ Laboratório Associado TERRA, Instituto Superior de Agronomia, Lisboa, Portugal

INTRODUCTION

WWTP SLUDGE & AGRICULTURE [1,2,3]

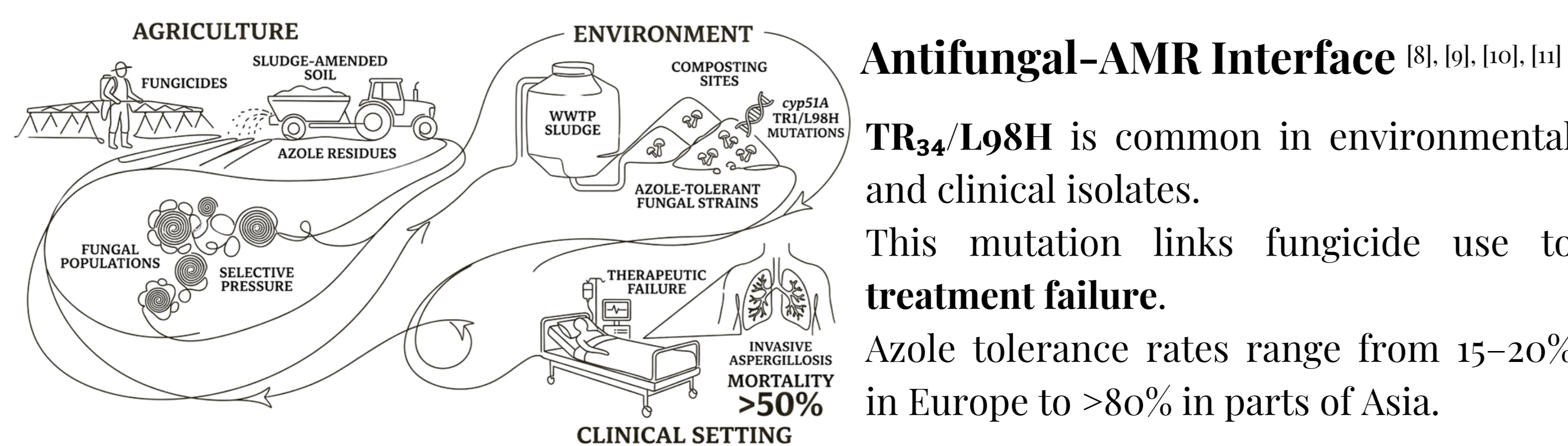
Sludge is applied to agricultural land as a N and P source.
N content: 6–8% dry weight and supports circular-economy goals.
Complex microbial communities survive conventional treatment.

FUNGAL PATHOGENS & WHO PRIORITY [4,5]

WHO 2022 classifies fungal pathogens as an emerging threat.
Aspergillus fumigatus listed as a critical priority species.
Key concerns: **triazole resistance** and **immunocompromised patients**.

AZOLE RESISTANCE: ENVIRONMENT TO CLINIC [6,7]

Azoles are used clinically and in agriculture.
Similar residues accumulate in sludge-amended soils.
Selective pressure promotes azole-tolerant fungi.
This links environmental resistance to clinical risk.



OBJECTIVES

Assess **microbial and fungal risks of commercial fertilizers and substrates** associated with **waste-derived materials** in Portugal, integrating antifungal resistance screening under a **One Health framework**.

METHODOLOGY

SAMPLING OVERVIEW

RAL-certified Sub 1, 3, 4, 5, 7, 8

Compost-based products meeting European quality standards.

⚠ May include materials derived from wastewater treatment.

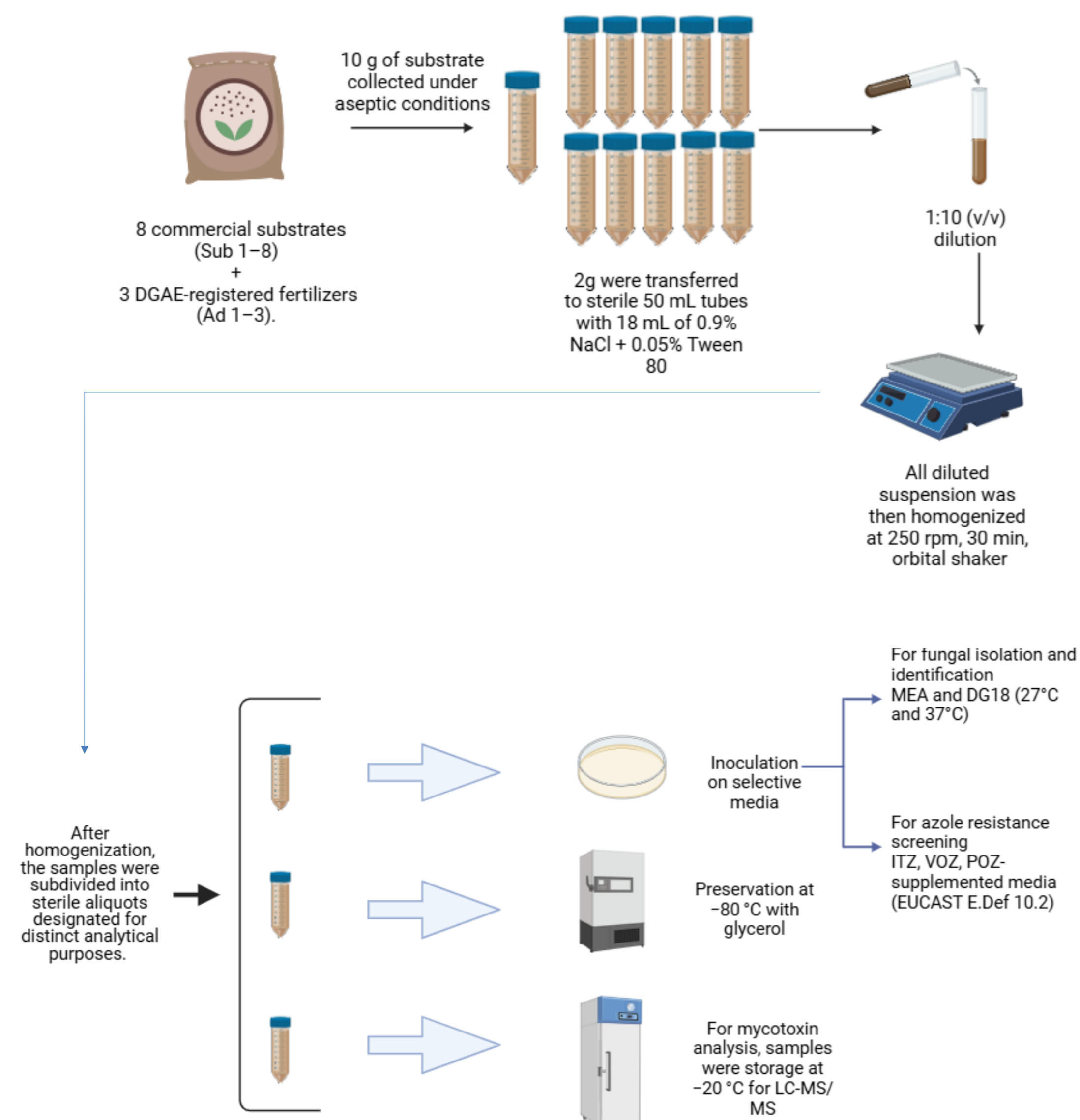
No certification Sub 2, Sub 6

No certification information was available for these substrates.

DGAE-approved Ad 1, 2, 3

Registered fertilizers approved for commercial use in Portugal.

SAMPLE PROCESSING AND LABORATORY PREPARATION



RESULTS AND DISCUSSION

FUNGAL CONTAMINATION BY PRODUCT CATEGORY

RAL-certified substrates showed the highest fungal counts and broadest taxonomic diversity (Fig. 2).

The most frequent *Aspergillus* sections were:

- *Aspergillus* section *Nigri*
- *Aspergillus* section *Circumdati*
- *Aspergillus* section *Fumigati*

Additional genera included *Penicillium*, *Fusarium*, and *Trichoderma*, contributing to taxonomic diversity in certified substrates.

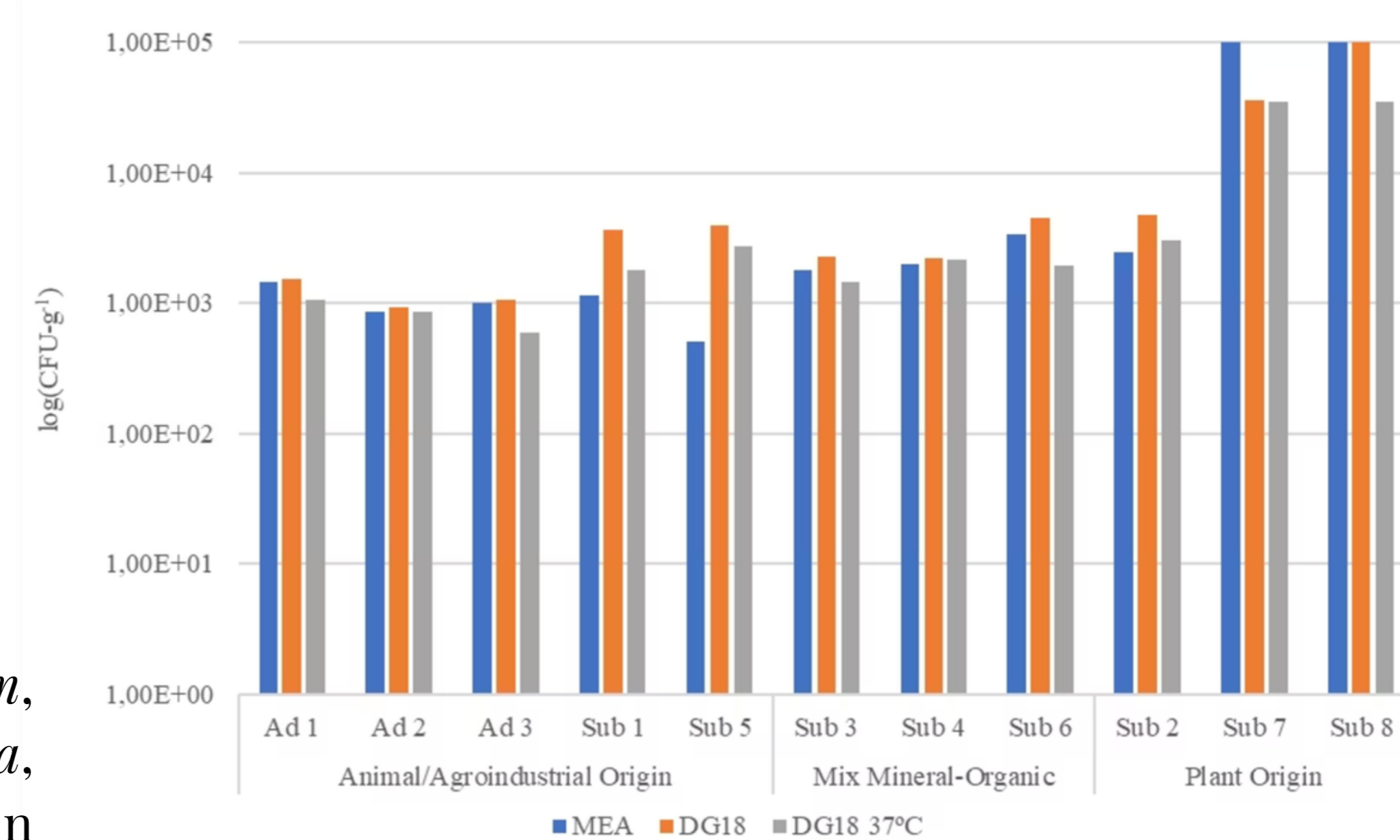


Figure 2 – Fungal counts (log CFU·g⁻¹) quantified in fertilizers and substrates from different origins (animal/agro-industrial, mixed mineral-organic, and plant origin) in three culture media (MEA, DG18, and DG18 at 37°C).

AZOLE RESISTANCE SCREENING

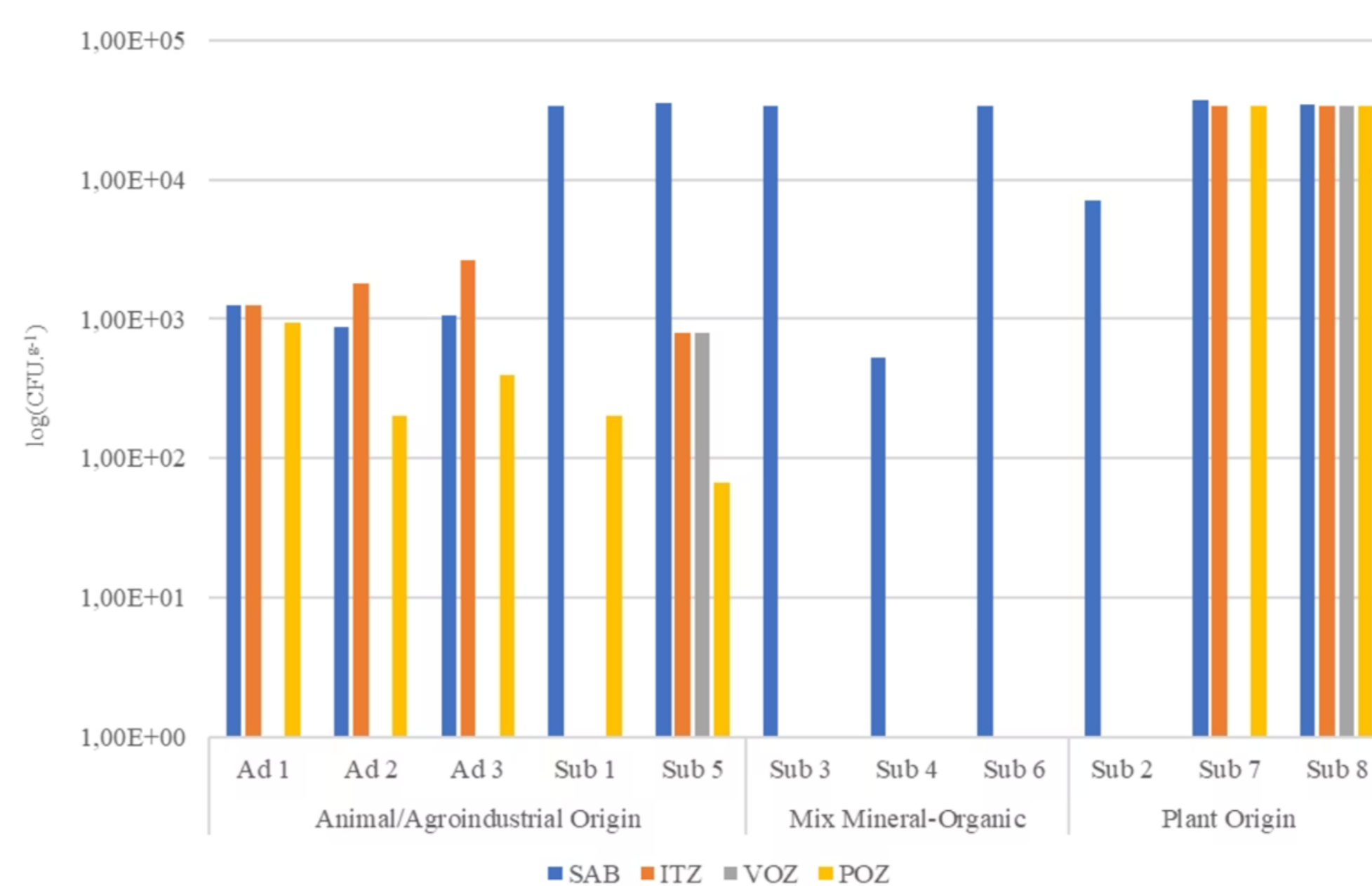


Figure 3 – Fungal counts (log CFU·g⁻¹) quantified in azole screening media (ITZ, VOZ, POZ) and control media (SAB) across different substrate categories (animal/agro-industrial, mixed mineral-organic, plant origin).

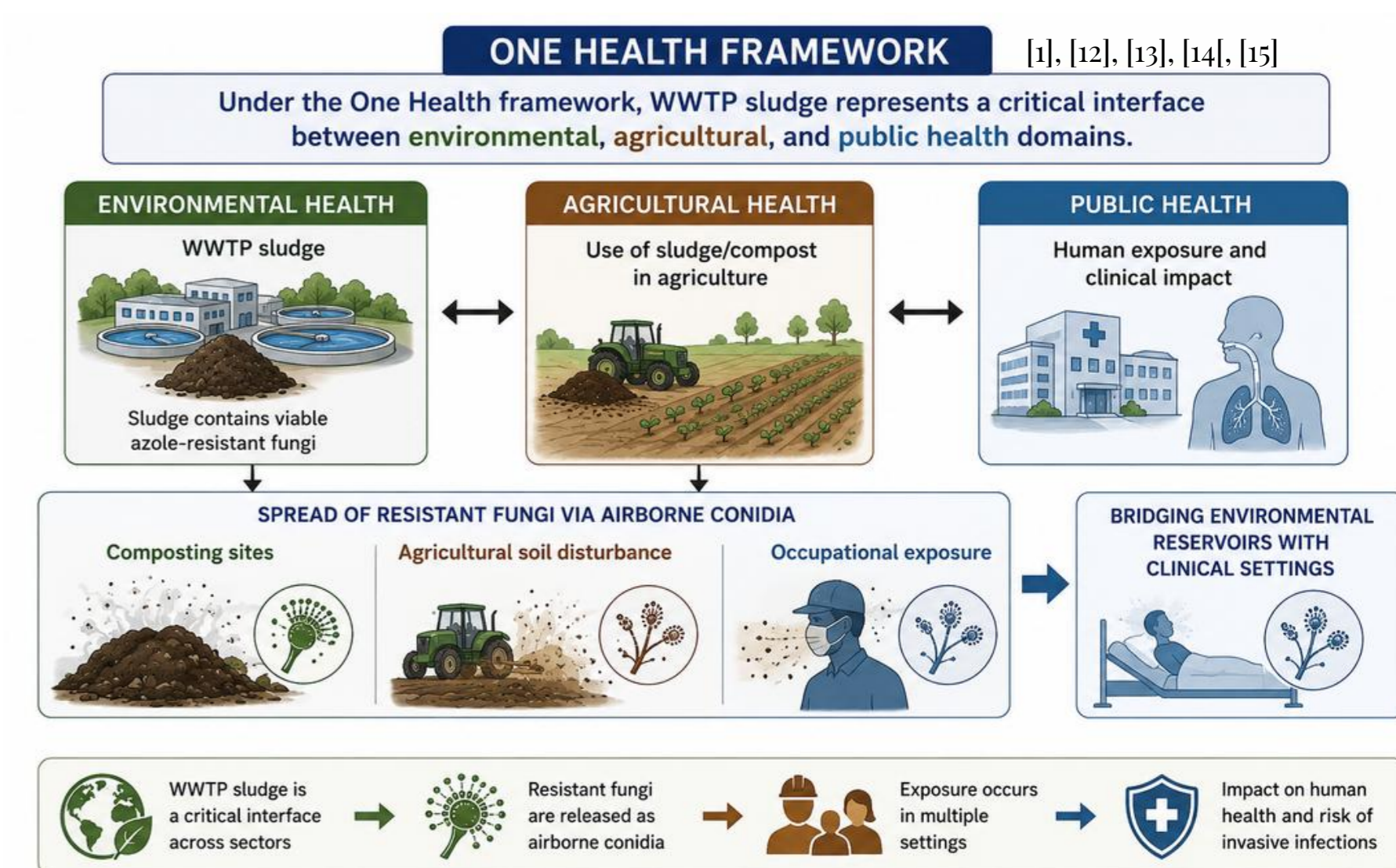
- RAL substrates showed most azole-tolerant fungi (Fig. 3).
- Demonstrates azole tolerance capacity potentially reflecting selective pressures.
- *A.* section *Circumdati* and *A.* section *Nigri* grew mainly in RAL substrates.

Product Type	Azole Tolerance	Risk Level
RAL Substrates	ITZ · VOZ · POZ	HIGH
Non-Certified	Sporadic	MEDIUM
DGAE Fertilizers	Minimal	LOW

REGULATORY GAPS AND ONE HEALTH FRAMEWORK

Parameter	Status
Heavy metals	Included*
Bacterial indicators	Included*
Fungal contamination	Not included
Mycotoxin producers	Not included
Antifungal residues	Not included
Azole-tolerant fungi	Not included

*Directive 86/278/EEC



CONCLUSIONS AND RECOMMENDATIONS

Domain	Future direction
Sampling	Expand sampling geographically and seasonally.
Methodology	Apply standardized EUCAST methods across studies.
Molecular tools	Integrate qPCR or sequencing for resistance detection.
Resistance genes	Target key resistance genes: <i>cyp51A</i> and <i>habeE</i> .
Mycotoxins	Apply multi-mycotoxin profiling (OTA, aflatoxins).
Co-occurrence	Assess whether toxigenic potential and azole tolerance co-occur in environmental isolates.

- This study provides baseline data for evidence-based risk assessment and regulatory reform.
- Also supports safe agricultural reuse of waste-derived materials.
- Future policy must integrate mandatory fungal quality criteria.
- Sludge regulations should align with One Health principles.

REFERENCES & ACKNOWLEDGEMENTS



Scan the QR code to access the references

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Figure 1 – Workflow of sample processing and microbiological preparation for substrates associated with waste-derived materials.