ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA
INSTITUTO POLITÉCNICO DE LISBOA

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Occupational exposure to fungi and mycotoxins in cork industry – An exploratory study

Susana Viegas

Bernd Osteresch, Yannick Hövelmann, Tiago Faria, Anita Quintal-Gomes, Benedikt Cramer, Carla Viegas, Hans-Ulrich Humpf

BACKGROUND – CORK INDUSTRY

Cork is:

- ✓ Impermeable material
- ✓ Resistant to fire
- ✓ Composed of suberin (hydrophobic substance)
- ✓ Used in a variety of products: the most common is wine stoppers.

Portugal is the most important producer of cork in the world and produces approximately 50% of the cork harvested annually worldwide.

Pestana and Tinoco, 2009; Gil, 2009

CORK INDUSTRY (important numbers)

Portugal produced 49% of all cork in 2016 and has 650 companies working in this production sector.

Two thirds of mundial exportation come from Portugal, being :

- ✓ 77.4% from semi-processed products;
- ✓ 82.3% from processed products from natural cork;
- ✓ 68% from agglomerate products.

Portugal's cork industry employs about ten thousands workers having 718 M euros of profit each year.

APCOR, 2016

OCCUPATIONAL HEALTH CONCERNS

The presence of the *Penicillium glabrum* complex in this industry involves the risk of respiratory diseases such as suberosis, a type of hypersensitivity pneumonitis that is one of the most prevalent diseases among cork workers.

Winck et al., 2004; Pereira et al., 2000; Serra et al., 2008; Basílio et al., 2006; Cruz, 2003; Oliveira, 2011; Pimentel and Avila 1973; Villar et al., 2009.

Epidemiologic studies had already reported an estimated prevalence between 9 to 19 % of suberosis among cork workers.

Winck, 2003

Besides *P. glabrum* complex, *Chrysonilia sítophila* was already reported as a dominant fungal species in all stages of cork production corroborating also the role in respiratory disorders in cork industry .

Danesh et al., 1997; Oliveira et al., 2003; Viegas et al., 2015

WHY STUDY OCCUPATIONAL EXPOSURE TO MYCOTOXINS?

- ✓ High exposure to organic dust already reported, particulary to particles of smaller sizes (0.3 µm and 0.5 µm).

Viegas et al., 2014

- ✓ High diversity of fungi contamination depending of many factors:
  - Crop (tree) contamination;
  - Time between collecting and processing the cork;
  - Storage conditions;
  - Cork processing (cork boiling, quick drying);
  - Cork transformation process.

Viegas et al., 2015, 2016

Videos

https://www.youtube.com/watch?v=ETsl-TiLowI

https://www.youtube.com/watch?v=NfeTxrzuf4M (Grupo Amorim)

https://en.wikipedia.org/wiki/Cork_taint - Trichoderma and Fusarium strains the responsables for the TCA

STUDY DEVELOPED

Fungi and mycotoxins occupational exposure assessment performed in one cork industry.

- 26 workers
- Process stages: Storage outside - Crushing – Autoclave/Boiling – Screening and Sorting – Finishing – Palletizing – Warehouse

STUDY DEVELOPED

Fungal burden assessment

- ✓ Air and surface samples were collected and subject to further macro and microscopic observations.

- ✓ Collected additional air samples in order to perform real-time quantitative polymerase chain reaction (PCR) amplification of genes from *Penicillium glabrum* complex (*Penicillium* section *Aspergilloides*) and *Aspergillus* section *Fumigati.*

Impaction method

Swab method

Impingir method

STUDY DEVELOPED

Mycotoxins Assessment

- ✓ Nineteen spot urine samples of cork workers were analyzed. Collected in the middle of the week.

- ✓ Workers answered a questionnaire that contained questions related with food consumption and current and previous occupational tasks developed.

- ✓ An improved "dilute and shoot" LC-MS/MS multi-mycotoxin approach was used to monitor urinary excretion of mycotoxins by the simultaneous detection of 33 biomarkers. *Gerding et al. (2014) and Hövelmann et al. (2016)*

Biomarkers: AFB1,AFB2,AFG1,AFM1,ALT,AME,AOH,BEA,CIT,DH,CIT,DON, DON—3-GlcA, EnA, EnA1, EnB, EnB1, FB1, HT-2, HT-24-GlcA, 10-OH-OTA, OTA, Otα, 2´R-OTA, T-2, ZAN, ZEN, ZEN-14-GlcA, α-ZEL, β-ZEL, α-ZEL-14-GlcA, β-ZEL-14-GlcA, TEA, allo-TEA

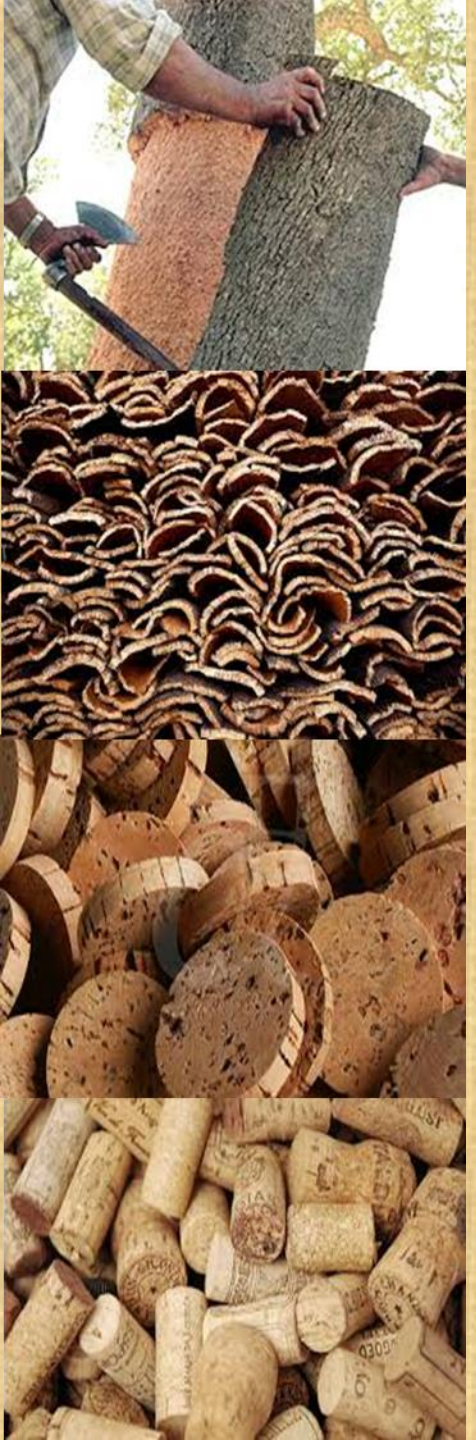
RESULTS – FUNGI

Air Samples

- ✓ **Conventional methods:** Most prevalent fungus was *Cladosporium* sp. (42.8%). *Lichtheimia* sp. (21.2%), *Penicillium* sp. (19.2%) and *Geomyces* sp. (16.8%) were also isolated.
- ✓ **Molecular analysis**: Detected *P. glabrum* and *Aspergillus* section *Fumigati*.

Surfaces samples

- ✓ *Penicilium* sp. (66.4%) was the most abundant. *Cladosporium* sp. (11.7%). *Geomyces* sp., *Chrysonilia* sp., *Alternaria* sp. and *Aureobasidium* sp. also isolated in surfaces but with much lower counts.
- ✓ *Chrysonilia* sp., *Alternaria* sp. and *Aureobasidium* sp. were only identified in surfaces.



RESULTS – MYCOTOXINS

- ✓ In most of the samples **(63.1%)** was detected **more than one mycotoxin/metabolite.**

- ✓ **9** different mycotoxins/metabolites were detected in workers urine samples: **DH-CIT, DON-3-GlcA, EnA1, EnB, EnB1, OTA, 2´R-OTA, TeA and *allo*-TeA.**

- ✓ **The most reported mycotoxin was TeA (94%>LOQ) followed by *allo*-TeA (44.4% >LOQ).** TeA (ng/ml): <LOD - 37.75/mean=7; *allo*-TeA (ng/ml): < LOQ -5.54/mean=2)

- ✓ **OTA was detected in 5 samples** (26.3%) and 2 of those samples have quantified values (median = 0.12 ng/ml) and the same samples also have quantified values of 2´R-OTA (mean=0.31 ng/ml).

- ✓ **5 Mycotoxins/metabolites <LOQ:**
  - DH-CIT detected in 4 samples; DON-3-GlcA detected 1 sample; EnA1 detected in 1 sample; EnB detected in 3 samples; EnB1 detected in 1 sample.

DISCUSSION

- ✓ This is the first study intended to assess the co-exposure to **multiple mycotoxins** of a **group of workers** from a specific occupational setting (**cork industry).**
- ✓ **Low values** - probably related predominantly with **food consumption**. The case of TeA?
- ✓ Multibiomarker approach unveil real exposure scenario: co-exposure to low vales of several mycotoxins.
- ✓ Different results were obtained in other settings where there was a higher exposure to mycotoxins related with also higher prevalence of toxigenic species (animal production, slaughterhouses, waste management).
- ✓ Besides the mycotoxins detected other should be targeted considering ***Aspergillus*** **section *Fumigati* prevalence (gliotoxin).**

CONCLUSIONS

- ✓ Need of recognizing mycotoxins as a possible occupational risk factor. Mycotoxins should be targeted for occupational biomonitoring programs.
- ✓ Considering the type of tasks developed several biomonitoring campaigns should be performed to allow an accurate exposure assessment.
- ✓ Multibiomarker approach generates important data to perform exposure assessment – Real life exposure scenario = co-exposure to several mycotoxins.
- ✓ Even at low levels can we expect negative health effects due to mycotoxins interactions?
- ✓ Challenges related with the lack of toxicokinetic data for some mycotoxins generating several additional questions to be answer to allow an accurate and adequate exposure and risk assessment. **Not a straight line!!**

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