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ORIGINAL PAPER

Toxinogenicity and cytotoxicity of *Alternaria, Aspergillus* and *Penicillium* moulds isolated from working environments

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Abstract There is currently limited research available on the secondary metabolites of moulds in workplaces. The aim of this study was to determine the mould contamination in museums ($N = 4$), composting plants ($N = 4$) and tanneries ($N = 4$) and the secondary metabolite profiles of *Alternaria, Aspergillus* and *Penicillium* isolates from these workplaces. *Alternaria, Aspergillus* and *Penicillium* species were identified using the ITS1/2 sequence of the rDNA region. Mould metabolites were quantitatively analysed on standard laboratory medium and mineral medium containing materials specific to each workplace using liquid chromatography-mass spectrometry. We also examined the cytotoxicity of the moulds using MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assays. Air microbiological contamination analyses showed a number of microorganisms, ranging from 2.4×10^3 CFU m^{-3} (composting plants) to 6.8×10^4 CFU m^{-3} (tanneries). We identified high percentages of *Alternaria, Aspergillus* and *Penicillium* moulds (air 57–59%, surfaces 10–65%) in all workplaces. The following moulds were the most cytotoxic (>90%): *Alternaria alternata*, *A. limoniasperae*, *Aspergillus flavus*, *Penicillium biourgeianum*, *P. commune* and *P. spinulosum*. The same mould species isolated from different working environments exhibited varying toxigenic and cytotoxic properties. Modifying the culture medium to simulate environmental conditions most often resulted in the inhibition of secondary metabolite production. Moulds isolated from the working environments produced the following mycotoxins (ng g^{-1}): chanoclavines (0.28–204), cyclopiazonic acid (27.1–1045), fumigaclavines (0.33–10,640,000), meleagrin (0.57–13,393), roquefortins (0.01–16,660), rugulovasines (112–220), viridicatin (0.12–957), viridicatol (4.23–2753) and quinocitrinines (0.07–1104), which may have a negative impact on human health.

Keywords Cytotoxicity · Moulds · Secondary metabolite · Workplaces

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Introduction

Occupational groups that are exposed to moulds include farmers, gardeners, grain elevator workers, food, beer, feed and herb industry workers, cheese producers, forestry workers, carpenters, and individuals who deal with the storage and processing of municipal solid waste and other plant or animal materials contaminated with microorganisms (Schlosser et al. 2009; Oluwafemi et al. 2012). Some data are available on mould exposure and its health implications for workers employed in museums, composting plants and tanneries (Wiszniewska et al. 2009; Persoons et al. 2010; Skóra et al. 2014).

The most widespread moulds in work environments belong to the *Aspergillus, Penicillium* and *Alternaria* genera. *Aspergillus* and *Penicillium* species can adapt to water activity (a_w) values below 0.8 and thus very frequently grow inside buildings (Nielsen 2002). The genus *Penicillium* contains approximately 100 toxigenic species, and the diversity of mycotoxins is much higher than for

other genera. Citrinin, ochratoxin A, patulin, penitrem A, penicillic acid, roquefortine C and viridicatin are the most characteristic extrolites produced by *Penicillium* species (Frisvad and Samson 2004). Ochratoxin A can also be produced by *Aspergillus* strains, in addition to aflatoxins, cyclopiazonic acid, fumonisins, tryptoquivaline, trypacidin, sterigmatocystin, gliotoxins and other mycotoxins (Fischer and Dott 2003; Nielsen et al. 2009). The *Alternaria* genus is a known producer of alternariol, alternariol monomethyl ether, altenuene, altertoxins I, II, and III, tenuazonic acid and other less toxic metabolites, which are present in human food and animal feed (Ostry 2008).

Many working environments contain bioaerosols that have mycotoxins responsible for cytotoxicity. Mycotoxins are present in the air as spores or mycelium fragments as well as dust particles and are rarely emitted in the form of volatile compounds (Sorenson 1999; Halstensen et al. 2006). Inhalation exposure to mycotoxins in work environments is poorly understood. The main reason for this is the inability to clearly identify direct causal relationships between exposure to mycotoxins and disease occurrence (Robbins et al. 2000; Brera et al. 2002; Fischer and Dott 2003). However, it is estimated that the inhalation of mycotoxins can have a tenfold more toxic effect compared to other routes of exposure (dermal, oral and intraperitoneal), as the inhaled toxins can easily penetrate the pulmonary alveoli (Petzinger and Ziegler 2000). Several cases of pulmonary cancers in oil-press workers, probably resulting from aflatoxin B1 inhalation and exposure to contaminated peanut meal, have been described (Hayes et al. 1984). During respiratory exposure, mycotoxins such as ochratoxin A and tremorgenic mycotoxin can pass into the sera to respectively induce systemic effects such as acute renal failure and neurological syndrome (Di Paolo et al. 1993; Gordon et al. 1993). In a previous study, Autrup et al. (1993) showed that the sera of exposed workers in an animal feed production plant had a significantly higher level of aflatoxin albumin adducts compared to a non-exposed control group. This could possibly explain the increased risk of liver cancer in workers from the animal feed processing industry.

It is well known that the mycotoxins produced by *Alternaria*, *Aspergillus* and *Penicillium* species are strongly cytotoxic. Bünger et al. (2004) found that extracts of *Penicillium* and *Aspergillus* moulds were highly toxic to lung, liver and nervous system cell lines.

Based on the above studies, occupational health hazards of workers can be related to the presence of moulds in the workplace and to the ability of these organisms to produce mycotoxins that induce cytotoxicity, allergenicity and mycoses. There is currently limited research on the toxinogenicity and cytotoxicity of moulds isolated from the working environments of museums, composting plants and tanneries. Therefore, our aim was to determine the mould contamination, secondary metabolite profiles and cytotoxicity of *Alternaria, Aspergillus* and *Penicillium* strains isolated from these workplaces. In addition, we compared the extrolites produced by the moulds on standard laboratory medium and mineral medium in the presence of extracts from materials from these workplaces.

Materials and methods

Description of the workplaces

Mycological contamination was analysed at four museums, four tanneries and four compost plants (a total of 40 locations) during the 2012–2013 winter season. The temperature and humidity of the air were determined using a PWT-401 hygrometer (Elmetron, Poland). Descriptions of the workplaces are given in Table 1.

Mycological analysis of workplaces

Mycological contamination of the air was determined using an MAS-100 Eco Air Sampler (Merck, Germany). Air samples (50 and 100 L) were collected on MEA medium (Malt Extract Agar, Merck, Germany) with chloramphenicol (0.1%) and DG18 medium (Dichloran 18% Glycerol Agar, Oxoid) to determine the total number of fungi (including hydrophilic and xerophilic strains). Six to eight samples were collected in each room. Samples were also collected from surfaces such as books, furniture, walls, equipment, production machines, wet-blue leather and finished leather at each site. The samples from these surfaces were collected using RODAC Envirocheck® plates with Sabouraud medium (Merck, Germany). For areas with high levels of surface contamination, the traditional swab method was used for the tests. This consisted of collecting samples using swabs containing saline solution (0.85% NaCl) on metal frames with a surface area of 0.0025 m^{-2} and inoculating them into media (MEA, DG18). Samples were collected from various surfaces in each room, with 6–10 replicates taken for each surface. The samples were incubated at 27 ± 2 °C for 7 days. After incubation, the colonies were counted and the results were expressed in units of CFU m^{-3} or CFU m^{-2} after taking into account the volume of air or the surface area, as applicable. The final results were calculated as the arithmetic means of all replicate samples. The percentage and frequency of isolation of the tested species (the percentage of positive samples) were also determined for each working environment.

Table 1 Characteristics of the examined workplaces

Work environment samples	C: Cubature [m^3] T: Temperature ± SD [°C] RH: Air relative humidity ± SD [%]	Description of workplaces (designation)
Composting plant C1–C4 ($N = 4$) Production halls ($n = 12$)	C: 500–25,317 T: 14.3 ± 6.5 RH: 51.3 ± 17.7	—Production of substrate for mushrooms ($N = 2$, $n = 5$), (C1–C2) —Municipal plants—compost production from green waste ($N = 2$, $n = 7$), (C3–C4) Used materials: green waste, wheat and rye straw, poultry and horse manure; production and sale of substrate for mushroom production; sorting of green waste prior to composting, raw material stockpiling, forming and turning of compost heaps in composting boxes
Tanneries T1–T4 ($N = 4$) Production halls ($n = 14$)	C: 250–27,000 T: 19.6 ± 4.7 RH: 49.5 ± 8.5	—Processing of raw leather ($N = 2$, $n = 8$) (T1–T2) —Processing of wet-blue hides (chrome-tanned) ($N = 2$, $n = 6$) (T3–T4) (movement and segregation of raw material, storage of salted raw hides, preliminary and main soaking, rinsing of hides, fleshing, liming, bating, pickling, cutting retannage and finishing of wet-blue leather, storage of wet-blue and tanned hides)
Museums M1–M4 ($N = 4$) Warehouses ($n = 14$)	C: 52–3055 T: 18.4 ± 3.7 RH: 38.7 ± 2.9	—Museum of Textile ($n = 4$) (M1); Museum of Independence Tradition ($n = 3$) (M2); Museum of Archeology and Ethnography ($n = 3$) (M3); National Museum ($n = 4$) (M4) Collection of paintings on gantries, canvas and boards, weapons: swords, rifles, pistols; flags, banners, gathered in wooden dressers; wood and iron made material, clothes, fibres, textiles

SD standard deviation, *N* number of studied institutions/plants, *n* number of studied locations, C1–C4; T1–T4; M1–M4 particular composting plants, tanneries, museums, respectively

Identification of moulds

All isolated moulds were identified using taxonomic keys (Klich 2002; Frisvad and Samson 2004; Pitt and Hocking 2009; Bensch et al. 2010; Houbraken et al. 2012; Jurjevic et al. 2012) based on macroscopic and microscopic observations following culture on CYA medium (Czapek Yeast Extract Agar, Difco, USA) and YES medium (yeast extract with supplements according to Samson et al. 1996).

Mould isolates belonging to the *Alternaria, Aspergillus* and *Penicillium* genera were confirmed using molecular methods based on a sequence analysis of the ITS1/2 region. Genomic DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. Fragments of approximately 500 bp were amplified using the universal primer set ITS1 and ITS4 according to the method reported by White et al. (1990). The PCR mixture contained 40 pmol of each primer, 1.5 U of RedTaq ReadyMix DNA polymerase (Sigma-Aldrich, St. Louis, MO, USA) and 20 ng of template DNA in a final reaction volume of 50 µL. The amplification was performed in the MJ Mini Gradient Thermal Cycler (Bio-Rad, Hercules, CA, USA). The nucleotide sequences of the ITS1/2 region were obtained using the BigDye Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and were analysed using an Applied Biosystems model 3730 genetic analyser. The nucleotide sequences were assembled and compared with sequences available in The National Center for Biotechnology Information (NCBI) database using the blastn algorithm (BLASTN 2.2.32+) (Zhang et al. 2000).

Cultivation of moulds

The toxinogenicity and cytotoxicity of the moulds were determined using Sabouraud media (Merck, Germany) with 2% agar (as a medium rich in organic compounds) and mineral M0 medium (5 g glucose, $MgSO_4 \times 5$ g $7H_2O$, 3 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 10 g yeast extract, distilled water to 1000 mL, pH 7.0) containing material from the workplaces tested (50 g cellulose, 500 mL compost extract, 50 g fragmented chrome-tanned leather—wet-blue leather shavings). Compost extracts were prepared by suspending 10 g of finished compost in 100 mL of distilled water, then shaking for 30 min, followed by vacuum filtration. The material added was based on the working environment where the strain was isolated (strains isolated from museums were grown on a medium containing cellulose, isolates from tanneries on medium with leather and strains from composting plants on medium with compost extract). Using mineral M0 medium with the above additives allowed us to determine the impact of workplace-specific compounds on the toxigenic and cytotoxic

properties of the moulds. The samples were incubated at 27 ± 2 °C for 7 days.

Mould extracts for analyses of the cytotoxicity and secondary metabolites

Three pieces (12 mm in diameter) of Sabouraud and M0 media containing mould (after 5 days of culture; 25 ± 2 °C) were cut and suspended in 5 mL of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). Samples (agarose piece in the extraction buffer) were extracted for 90 min and used for the cytotoxicity and secondary metabolite analyses.

Cytotoxicity analysis

Cell culture and treatment

The LLC-PK1 pig kidney cell line (CLS, Germany, lot no. 607264; from the 36th passage) was used in this study. This cell line is often used as a model for mycotoxin toxicity testing (Gniadek et al. 2010; Nowak et al. 2015). The cells were cultured in T75 Roux flasks (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) as a monolayer in Dulbecco's modified Eagle's medium/Ham's nutrient mixture F12 (DMEM/Ham's F12, Sigma-Aldrich, St. Louis, USA) with the addition of 10% heat-inactivated foetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific), 200 mM L-glutamine (Sigma-Aldrich, St. Louis, USA) and 25 mM HEPES (Sigma-Aldrich, St. Louis, USA). The cells were incubated in a CO_2 incubator at 37 °C under 5% CO_2 for 7–10 days. After reaching confluence, the cells were subcultured every week. The medium was changed every 3–4 days.

LLC-PK1 cells were detached with TrypLE™ Express (Gibco, Thermo Fisher Scientific) for 15–20 min and gently shaken off the plastic flask. The reaction does not need to be terminated with FBS, as this enzyme is of plant origin. After being detached, the cells were suspended in PBS (pH 7.2) and transferred into a 15-mL Falcon tube, centrifuged (182×g, 5 min), decanted and resuspended in fresh DMEM/Ham's F12 medium. After determining the cell number and viability by trypan blue exclusion (min 90%), the cells were ready to use for experiments.

Cytotoxicity testing using the MTT assay

In the MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole, is reduced to purple formazan in the mitochondria of living cells. The amount of formazan produced is proportional to the amount of MTT in the incubation medium. In the experiments, 5×10^4 LLC-PK1 cells were placed in each well of a 96-well plate (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), and then 100 µL of the complete culture medium was added to each well. The cells were incubated overnight at 37 °C in 5% CO_2 to allow them to attach. The following day, the medium was removed and 200 µL of the mycotoxin extract, after evaporation and dilution (composed of 1.7% ethanol, 0.3% DMSO and 98% DMEM/Ham's F12 medium without FBS), was added to each well, with eight replicate wells for each sample. The control samples consisted of cells without toxic agents. The cells were incubated in a CO_2 incubator at 37 °C in 5% CO_2 for 48 h. After incubation, the cells were washed with PBS/EDTA, 100 µL of MTT (0.5 mg/mL in PBS; Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the cells were incubated at 37 °C in 5% CO_2 for another 3 h. Following this incubation, the MTT reagent was carefully removed and formazan precipitates were solubilized by the addition of 50 µL of DMSO to each well (Sigma-Aldrich, St. Louis, MO, USA). The absorbance was measured at 550 nm using a microplate reader (ASYS UVM 340, Biogenet). The absorbance of the control sample (untreated cells) was taken to represent 100% cell viability. Cell viability (%) was calculated as follows: [(sample OD (optical density)/control OD)×100%]. Cytotoxicity (%) was calculated as: 100−cell viability (%). The results are presented as the mean ± SD (standard deviation).

Secondary metabolite extraction and analysis

Mould extracts were diluted with the same volume of solvent prior to injection (Sulyok et al. 2006). Centrifugation was not necessary because gravity produced sufficient sedimentation. The extrolites produced by the isolates on laboratory medium were quantitatively analysed using LC–MS/MS, as described by Malachova et al. (2014). Briefly, LC–MS/MS screening of target microbial metabolites was performed with the an QTrap 5500 LC–MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA).

ESI–MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode in both positive and negative polarities, with two separate chromatographic runs per sample, by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte was set to its expected retention times of ±27 and ±48 s in

the positive and negative modes, respectively. The identity of analytes was confirmed by the acquisition of two MRMs per analyte with the exception of moniliformin, which exhibited only one fragment ion. This yielded 4.0 identification points according to European Union Commission decision 2002/657 (EU 2002). The LC retention time and the intensity ratio of the two MRM transitions agreed with the values of a related authentic standard within 0.1 min and 30% rel.

The limits of detection of extrolites were as follows (ng g^{-1}): 3.000—orsellinic acid, verruculogen; 2.400—stemphylperylenol; 1.200—asperfuran; 0.900—cyclopiazonic acid; 0.600—aspterric acid, deoxybrevianamid E, pseurotin A, tenuazonic acid; 0.480—gliocladic acid, helvolic acid, tryptoquivaline F; 0.450—meleagrin; 0.420—pyripyropene A; 0.300—citreoviridin A, penicillic acid, roquefortine C; 0.240—fumonisin B1; 0.180—fumigaclavine C, heptelidic acid, viridicatol; 0.150—chlorocitreorosein, fumitremorgin A, fumitremorgin C; 0.120—cyclopenol, dehydrocyclopeptine, fumiquinazolin A, fumiquinazolin D; 0.090—tryprostatin A, viridicatin; 0.060—3-nitropropionic acid, altertoxin I, bis(de-

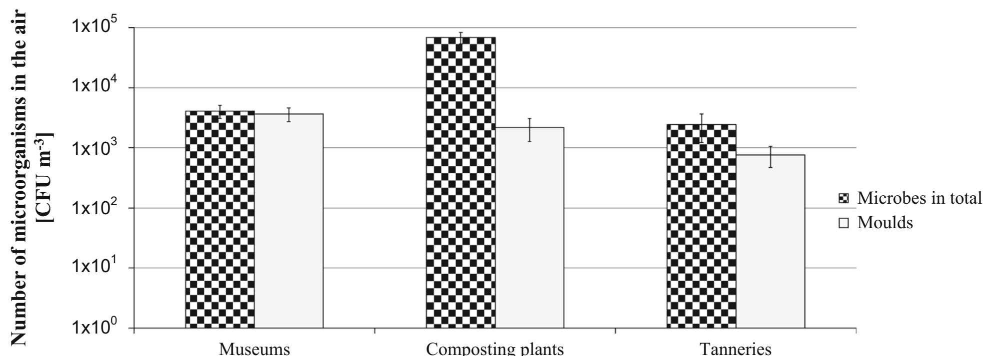

Fig. 1 Number of microorganisms in the air in tested working environments. Results are significantly different (one-way ANOVA, $p < 0.05$; Tuckey's test, $p < 0.05$)

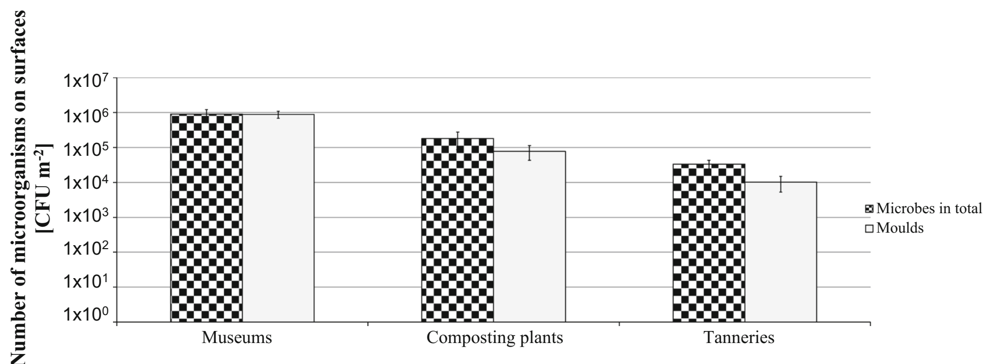

Fig. 2 Number of microorganisms on surfaces in tested working environments. Results are significantly different (one-way ANOVA, $p < 0.05$; Tuckey's test, $p < 0.05$)

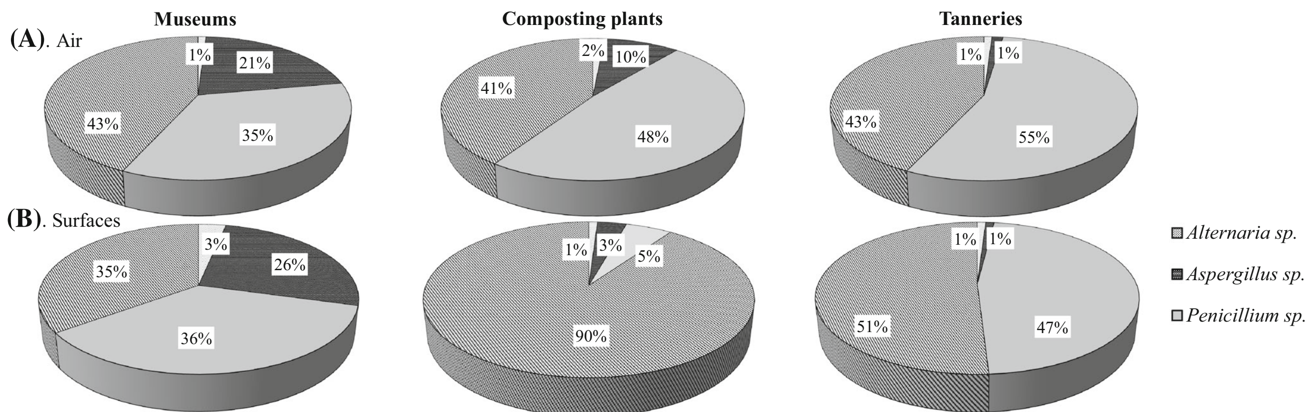

Fig. 3 Percentage of *Alternaria, Aspergillus* and *Penicillium* genera in tested working environments

thio)methylthiogliotoxin, brevicompanine B, roquefortine D; 0.048—aurantiamin A, demethylsulochrin, neoechinulin A; 0.042—rugulovasine A; 0.030—alternariol, altersetin, chrysogine, cyclopeptine, neoxaline, oxaline; 0.018—mycophenolic acid, rugulusovine; 0.012—andrastin A, brevianamid F, cyclopenin, nidurufin, O-methylviridicatin; 0.006—chanoclavine, quinocitrinine A, tentoxin; 0.004—emodin; 0.003—averantin; and 0.002—alternariolmethylether, verrucofortine.

Statistical analyses

The results of the assessments of air and surface microbiological contamination, cytotoxicity levels and secondary metabolite concentrations were statistically analysed using STATISTICA 6.0 software (Statsoft, USA). The results were evaluated using one-way analysis of variance (ANOVA) at the 0.05 significance level. When a statistically significant difference was detected ($p < 0.05$), the means were compared using a post hoc Tukey's test (microbiological contamination level and secondary metabolite concentrations) and Fisher's LSD test (cytotoxicity level) at the 0.05 significance level.

Results and discussion

The quantitative analysis of microbiological air contamination showed that the number of airborne microorganisms ranged from 2.4×10^3 CFU m^{-3} (composting plants) to 6.8×10^4 CFU m^{-3} (tanneries). The number of microorganisms was significantly different ($p < 0.05$) in all workplaces tested. The concentration of moulds in the air was lowest in tanneries (7.6×10^2 CFU m^{-3}), higher in composting plants (2.2×10^3 CFU m^{-3}) and highest in museums (3.6×10^3 CFU m^{-3}) (Fig. 1). Surfaces (museum objects, walls, production surfaces, machinery and equipment) were colonized with microorganisms at concentrations between 3.3×10^4 - CFU m^{-2} (tanneries) and 8.9×10^5 CFU m^{-2} (museums), including the concentration of moulds, which ranged from 1.0×10^4 CFU m^{-2} to 8.8×10^4 CFU m^{-2}. The total microbial contamination of the surfaces was also significantly different ($p < 0.05$) depending on the workplace. It is worth highlighting that moulds dominated the microbial community in museums, accounting for 90 and 98% of the microorganisms isolated from the air and surfaces, respectively (Fig. 2).

Table 2 Tested moulds strains

Lp.	Species (working environment)	Accession number*	LOCK CPC number**
1	*Alternaria alternata* (M1)	KC456173	LOCK CPC 0610
2	*Alternaria limoniasperae* (C4)	KP341698	LOCK CPC 0612
3	*Alternaria limoniasperae* (M4)	KP341700	LOCK CPC 0614
4	*Alternaria nobilis* (T1)	KP341697	LOCK CPC 0611
5	*Aspergillus flavus* (M4)	KU561917	LOCK CPC 1097
6	*Aspergillus flavus* (C4)	KU561919	LOCK CPC 1098
7	*Aspergillus flavus* (T3)	KU561920	LOCK CPC 1099
8	*Aspergillus fumigatus* (M3)	KU561918	LOCK CPC 1100
9	*Penicillium biourgeianum* (C3)	KU561930	LOCK CPC 1101
10	*Penicillium biourgeianum* (T4)	KU561931	LOCK CPC 1102
11	*Penicillium chrysogenum* (T4)	KU561921	LOCK CPC 1103
12	*Penicillium chrysogenum* (T4)	KC 456,190	LOCK CPC 1104
13	*Penicillium commune* (T3)	KU561925	LOCK CPC 1105
14	*Penicillium commune* (T2)	KF725716	LOCK CPC 1106
15	*Penicillium commune* (T2)	KF725717	LOCK CPC 1107
16	*Penicillium cordubense* (T1)	KU561922	LOCK CPC 1108
17	*Penicillium echinulatum* (T1)	KF725718	LOCK CPC 1109
18	*Penicillium italicum* (T3)	KU561924	LOCK CPC 1110
19	*Penicillium madriti* (C1)	KU561929	LOCK CPC 1111
20	*Penicillium polonicum* (C3)	KU561923	LOCK CPC 1112
21	*Penicillium spinulosum* (T1)	KU561927	LOCK CPC 1113
22	*Penicillium spinulosum* (T3)	KU561928	LOCK CPC 1114

M*1*–M4— museums, T1–T4—tanneries; C1–C4—composting plants

* Deposited in the National Center for Biotechnology Information GenBank database

** Deposited in The Lock Collection of Pure Culture at The Institute of Fermentation Technology and Microbiology in Technical University of Lodz (LOCK CPC)

Table 3 Cytotoxicity of tested mould extracts in MTT test

No.	Species	Working environment	Frequency of isolation (%)	Cytotoxicity ± SD (%)$^{(N)}$
1	*Alternaria alternata*	M1 (a)	72	$90.7 \pm 5.6^{(6;11;15-18;20)}$
2	*Alternaria limoniasperae*	C4 (a)	64	$86.1 \pm 6.7^{(13;15-16;18)}$
3	*Alternaria limoniasperae*	M4 (s)	33	$92.7 \pm 2.9^{(6;9;11;14-21)}$
4	*Alternaria nobilis*	T1 (a)	39	$91.9 \pm 0.0^{(6;11;15-1820-21)}$
5	*Aspergillus flavus*	M4 (s)	12	$90.1 \pm 6.2^{(6-7;11;15-18;20)}$
6	*Aspergillus flavus*	C4 (a)	64	$80.4 \pm 4.9^{(1;3-7;10;13;22)}$
7	*Aspergillus flavus*	T3 (a)	7	$99.2 \pm 1.6^{(2;5-9;11-12;14-21)}$
8	*Aspergillus fumigatus*	M3 (s)	27	$84.9 \pm 3.3^{(7;10;13;15-16;18)}$
9	*Penicillium biourgeianum*	C3 (a)	80	$83.3 \pm 6.9^{(3;7;10;13;16;18;22)}$
10	*Penicillium biourgeianum*	T4 (s)	33	$94.6 \pm 2.7^{(6;8-11;14-21)}$
11	*Penicillium chrysogenum*	T4 (a)	64	$79.8 \pm 0.0^{(1;3-5;7;10;13;22)}$
12	*Penicillium chrysogenum*	T4 (s)	46	$87.1 \pm 11.2^{(7;13;15-16;18)}$
13	*Penicillium commune*	T3 (a)	86	$97.2 \pm 1.0^{(2;6;8-9;11-21)}$
14	*Penicillium commune*	T2 (s)	33	$83.7 \pm 5.9^{(3;7;10;13;16;18;22)}$
15	*Penicillium commune*	T2 (a)	86	$75.6 \pm 8.2^{(1-5;7-8;10;12-13;22)}$
16	*Penicillium corbudense*	T1 (a)	73	$72.2 \pm 13.5^{(1-5;7-10;12-14;19;21;22)}$
17	*Penicillium echinulatum*	T1 (a)	92	$78.2 \pm 6.0^{(1;3-5;7;10;13;22)}$
18	*Penicillium italicum*	T3 (a)	36	$74.0 \pm 9.6^{(1-5;7-10;12-14;19;22)}$
19	*Penicillium madriti*	C1 (a)	10	$83.7 \pm 8.8^{(3;7;10;13;16;18;22)}$
20	*Penicillium polonicum*	C3 (s)	11	$80.8 \pm 3.7^{(1;3-5;7;10;13;22)}$
21	*Penicillium spinulosum*	T1 (s)	40	$82.7 \pm 4.4^{(3;4;7;10;13;16;22)}$
22	*Penicillium spinulosum*	T3 (s)	50	$93.8 \pm 3.9^{(6;9;11;1414-21)}$

M1–M4—museums; T1–T4—tanneries; C1–C4—composting plants (a—air, s—surfaces); SD—standard deviation

$^{(N)}$ No. of strains which are significantly different (one-way ANOVA, $p < 0.05$; Fisher's LSD test, $p < 0.05$)

Table 4 Metabolites produced by *Alternaria* strains

Metabolites	Concentration (ng g^{-1})							
	Alternaria alternata M1(a)		*Alternaria limoniasperae* M4(s)		*Alternaria limoniasperae* C4(a)		*Alternaria nobilis* T1(a)	
	S	M0 + cellulose	S	M0 + cellulose	S	M0 + compost	S	M0 + leather
Alternariol	1.77	1.10	231*	0.90	23.6	nd	3.60	nd
Alternariolmethylether	0.64	nd	39.3*	0.36	3.87	nd	0.56	nd
Altersetin	54.7	nd	464*	11.0	nd	nd	nd	nd
Altertoxin I	677*	222	336	411	73.7*	5.40	18.7	nd
Brevianamid F	4.77	20.1*	3.80	26.1*	3.24	13.3*	3.67	13.9*
Orsellinic acid	252	414*	nd	nd	nd	nd	nd	nd
Oxaline	6.03*	0.74	nd	nd	nd	nd	nd	nd
Rugulusovine	13.9*	0.68	nd	nd	nd	nd	nd	nd
Stemphylperylenol	nd	nd	46.3	56.0	10.0	nd	2.68	nd
Tentoxin	22.1	26.6	6.27*	0.21	nd	nd	nd	nd
Tenuazonic acid	16,420*	462	51,100*	4370	19,290*	957	1794	nd

S—Sabouraud medium; M0—mineral medium; nd—not detected; M—museums; T—tanneries; C—composting plants; a—air

* Statistically different metabolite concentrations between S and M0 medium (one-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$)

The total fungal number in all working environments did not exceed the quantitative reference thresholds specified by the Polish Committee for the Highest Permissible Concentrations and Intensities of Noxious Agents in the Workplace (Skowroń and Górny 2012), which is 5.0×10^4 CFU m^{-3}.

We confirmed the results of previous studies carried out in other museums (Gysels et al. 2004; Rojas et al. 2002), which showed microbial contamination, although we detected a higher concentration of moulds. This may be because our study was carried out in the warehouses of museums and not in the exhibition halls, which are more frequently ventilated or dusted.

The number of microorganisms detected in the work environments of composting plants was in line with previously reported mould numbers for green waste composting plants and composting facilities producing button mushroom substrates (1.3×10^3–6.8×10^4 - CFU m^{-3}) (Buczyńska et al. 2008; Persoons et al. 2010).

Microbiological contamination in tanneries has not been well explored in the literature. We found 2.4×10^3 CFU m^{-3} in the production halls and storage areas of these workplaces.

In all workplaces (composting plants, tanneries and museums), we identified a high percentage of moulds belonging to the *Aspergillus, Alternaria* and *Penicillium* genera. These accounted for 57–59% of the total number of microorganisms in the air and 10–65% on surfaces (Fig. 3). Moulds of the *Penicillium* genus predominated in most samples and were found at a high percentage (35–57%) in the air and on surfaces, except for the surfaces of composting plants where they constituted only 5% of the moulds (Fig. 3). Moulds from the *Aspergillus* genus were

Table 5 Metabolites produced by *Aspergillus* strains

Metabolites	Concentration (ng g^{-1})							
	Aspergillus flavus M4(s)		*Aspergillus flavus* C4(a)		*Aspergillus flavus* T3(a)		*Aspergillus fumigatus* M3(s)	
	S	M0 + cellulose	S	M0 + compost	S	M0 + leather	S	M0 + cellulose
3-Nitropropionic acid	1592	38,780*	750*	231	141	13,386*	nd	nd
Asperfuran	nd	nd	nd	nd	nd	nd	nd	nd
Averantin	0.48	nd	nd	nd	nd	nd	nd	nd
Bis(methylthio)gliotoxin	nd	nd	nd	nd	nd	nd	291	1168*
Brevianamid F	4.67	31.3*	6.10	12.1*	5.63	18.13	225*	114
Cyclopiazonic acid	nd	nd	nd	nd	182	1861*	nd	nd
Chanoclavine	nd	nd	nd	nd	nd	nd	22.8*	12.1
Emodin	nd	nd	nd	nd	nd	nd	6.13	0.20
Gliocladic acid	427	nd	425	nd	751	nd	nd	nd
Fumigaclavine C	nd	nd	nd	nd	nd	nd	10,640,000*	2,338,667
Fumigaclavine	nd	nd	nd	nd	nd	nd	101*	6.63
Fumiquinazolin A	nd	nd	nd	nd	nd	nd	498,667	1,144,000*
Fumiquinazolin CD	nd	nd	nd	nd	nd	nd	36,000*	23,387
Fumiquinazolin D	nd	nd	nd	nd	nd	nd	2,341,333*	1,325,333
Fumitremorgin B	nd	nd	nd	nd	nd	nd	58.33	105
Fumitremorgin C	nd	nd	nd	nd	nd	nd	1819*	485
Helvolic acid	nd	nd	nd	nd	nd	nd	701	144
Meleagrin	nd	nd	nd	nd	nd	nd	7.00	12.7*
Methylsulochrin	nd	nd	nd	nd	nd	nd	749*	1.55
Orsellinic acid	nd	nd	nd	nd	nd	nd	544	nd
Heptelidic acid	569*	31.5	467	nd	868*	69.0	nd	nd
Nidurufin	0.27	nd	nd	nd	nd	nd	nd	nd
Pyripyropene A	nd	nd	nd	nd	nd	nd	105	180
Tryprostatin A	nd	nd	nd	nd	nd	nd	1595	2960*
Tryprostatin B	nd	nd	nd	nd	nd	nd	64.3*	33.3
Tryptoquivaline F	nd	nd	nd	nd	nd	nd	73,333*	61,600
Verruculogen	nd	nd	nd	nd	nd	nd	164*	57.3

S—Sabouraud medium; M0—mineral medium; nd—not detected; M—museums; T—tanneries; C—composting plants; a—air; s—surfaces

* Statistically different metabolite concentrations between S and M0 medium (one-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$)

also very common in museums (21–26%) but were found at lower levels in composting plants (3–10%) and tanneries (1%). Strains of the *Alternaria* genus accounted for 1–3% of all isolated moulds in the tested environments (Fig. 3). A high prevalence of the *Penicillium, Aspergillus* and *Alternaria* genera in working environments was also noted in earlier studies (Fischer and Dott 2003; Wiszniewska et al. 2009) (Table 2).

Among the 22 species of moulds isolated in this study, 14 were isolated from the air and 8 from surfaces (Table 3). Their frequency of isolation from the air and surfaces varied from 7 to 92%. The most frequently isolated species were *Alternaria alternata* (72%), *A. limoniasperae* (64%), *Aspergillus flavus* (64%), *Penicillium biourgeianum* (80%), *P. chrysogenum* (64%), *P. commune* (86%), *P. echinulatum* (92%) and *P. spinulosum* (50%) (Table 3).

The general cytotoxicity of the mould strains against the LLC-PK1 swine kidney cells was high, with the calculated values ranging from 72 to 99% (Table 3). The following moulds were the most cytotoxic (calculated cytotoxicity > 90%): *Alternaria alternata* (museum M1), *A. limoniasperae* (M4), *Aspergillus flavus* (M4 and tannery T3), *Penicillium biourgeianum* (T4), *P. commune* (T3) and *P. spinulosum* (T3). We found that the same mould species isolated from different working

Table 6 Metabolites produced by *Penicillium* strains

Metabolites	Concentration (ng g^{-1})							
	P. chrysogenum T4(a)		*P. chrysogenum* T4(s)		*P. cordubense* T1(a)		*P. italicum* T3(a)	
	S	M0 + leather	S	M0 + leather	S	M0 + leather	S	M0 + leather
Aspterric acid	nd	nd	nd	nd	2567*	48.7	nd	nd
Andrastin A	730*	172	130,433	nd	nd	nd	nd	nd
Andrastin C	nd	nd	28,700	nd	nd	nd	nd	nd
Andrastin D	5927	nd	639,333	nd	nd	nd	nd	nd
Brevianamid F	7.50	17.4*	7.12	5.30	7.23	17.2*	399*	179
Brevicompanine B	nd	nd	nd	nd	43.0*	10.2	nd	nd
Chrysogine	71.0	85.7	16.9	24.5	nd	nd	nd	nd
Cyclopenin	nd	nd	6.07*	0.25	4387*	765	4.47*	1.16
Cyclopenol	nd	nd	5.03*	0.42	14,490*	2112	20.7	16.0
Cyclopeptine	nd	nd	0.43	nd	115*	7.67	0.11	nd
Cyclopiazonic acid	nd	nd	919*	27.1	nd	nd	nd	nd
Deoxybrevianamid E	nd	nd	nd	nd	nd	nd	5670*	3010
Dehydrocyclopeptine	nd	nd	nd	nd	39.7*	2.21	nd	nd
Fumigaclavine	nd	nd	1.02	nd	nd	nd	nd	0.33
Fumiquinazolin CD	nd	nd	nd	nd	14,653*	5927	nd	nd
Meleagrin	13,393*	8560	1454*	253	0.57	0.65	1.85	2.43
Mycophenolic acid	4.97	nd	nd	nd	nd	nd	nd	nd
O-Methylviridicatin	nd	nd	nd	nd	1041*	28.2	nd	nd
Orsellinic acid	nd	nd	1.65	nd	33,133*	1213	nd	nd
Neoxaline	213*	6.40	nd	nd	nd	nd	nd	nd
Oxaline	31.3*	5.37	nd	0.34	0.44	0.73	0.11	1.07*
Quinocitrinine A	1.15	nd	nd	nd	0.27	0.12	nd	0.07
Penicillic acid	nd	nd	nd	nd	4783*	954	nd	nd
Roquefortine C	6043*	1530	16,660*	6.10	0.51	58.7*	nd	nd
Roquefortine D	78.3	43.7	145*	22.4	nd	nd	nd	nd
Rugulusovine	94.7*	18.7	64.7*	5.17	271*	121	1.44	nd
Tryprostatin B	nd	nd	nd	1.03	nd	nd	9870*	5227
Verrucofortine	nd	nd	nd	nd	63.7*	14.8	nd	nd
Viridicatin	nd	nd	1.86	nd	957*	33.3	9.30*	4.33
Viridicatol	nd	nd	nd	nd	2753*	83.0	12.5*	5.37

S—Sabouraud medium; M0—mineral medium; nd—not detected; M—museums; T—tanneries; C—composting ants; a—air, s—surfaces

* Statistically different metabolite concentrations between S and M0 medium (one-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$)

Table 7 Metabolites produced by *Penicillium* strains

Metabolites	Concentration (ng g^{-1})									
	P. biourgeianum C3(a)		*P. biourgeianum* T4(s)		*P. echinulatum* T1(a)		*P. madriti* C1(a)		*P. polonicum* C3(s)	
	S	M0 + compost	S	M0 + leather	S	M0 + leather	S	M0 + compost	S	M0 + compost
Andrastin	nd	nd	nd	nd	133,600*	59,267	nd	nd	nd	nd
Andrastin C	nd	nd	nd	nd	54,800*	34,300	nd	nd	nd	nd
Andrastin D	nd	nd	nd	nd	197,200*	158,900	nd	nd	nd	nd
Aspterric acid	nd	nd	nd	nd	nd	nd	nd	nd	2567*	48.7
Aurantiamin A	nd	nd	nd	nd	1.37	nd	nd	nd	nd	nd
Brevianamid F	7.17	13.23	2.75	3.17	5.83	14.90*	8.57	19.3*	7.23	17.2*
Brevicompanine B	nd	nd	nd	nd	nd	nd	nd	nd	43.0*	10.2
Chanoclavine	nd	nd	nd	nd	16.00*	0.28	nd	nd	nd	nd
Citreorosein	3.90	nd	1.59	nd	nd	nd	nd	nd	nd	nd
Citreoviridin	nd	nd	nd	nd	nd	nd	3200*	109	nd	nd
Cyclopiazonic acid	nd	nd	nd	nd	nd	nd	1045*	102	nd	nd
Cyclopenin	nd	0.46	nd	nd	0.80	nd	nd	nd	4387*	765
Cyclopenol	nd	nd	nd	nd	nd	nd	nd	nd	14,490*	2112
Cyclopeptine	nd	nd	nd	nd	nd	nd	nd	nd	115*	7.67
Dehydrocyclopeptine	nd	nd	nd	nd	nd	nd	nd	nd	39.7*	2.21
Emodin	0.51	nd	0.45	0.17	nd	0.75	nd	nd	nd	nd
Fumigaclavine	nd	nd	0.98	nd	nd	nd	nd	nd	nd	nd
Fumigaclavine C	nd	nd	nd	nd	nd	5040	nd	nd	nd	nd
Fumiquinazolin A	nd	nd	nd	nd	nd	1094	nd	360	nd	nd
Fumiquinazolin D	nd	nd	nd	nd	nd	1288	nd	nd	nd	nd
Fumiquinazolin CD	nd	nd	nd	nd	nd	nd	nd	nd	14,653*	5927
Fumonisin B1	nd	nd	69.7	nd	nd	nd	nd	nd	nd	nd
Meleagrin	1.53	0.84	3.33*	0.57	3420*	586	9.63	111*	0.57	0.65
Mycophenolic acid	10,127*	1064	5017*	818	nd	nd	8.97	nd	nd	nd
Neoechinulin A	nd	nd	nd	nd	nd	1.75	nd	nd	nd	nd
Neoxaline	nd	nd	nd	nd	4.17*	0.74	0.05	0.37*	nd	nd
O-Methylviridicatin	nd	nd	nd	nd	1.63	nd	nd	nd	1041*	28.2
Orsellinic acid	999*	322	nd	656	nd	nd	nd	nd	33,133*	1213
Oxaline	nd	1.15	nd	nd	2880	3313*	nd	0.60	0.44	0.73
Penicillic acid	nd	nd	nd	nd	nd	nd	nd	nd	4783*	954
Pseurotin A	nd	nd	nd	nd	nd	nd	nd	nd	5.80	23.0*
Quinocitrinine A	957	604	1104*	421	2.22*	0.78	0.60*	0.09	0.27	0.12
Roquefortine C	0.01	3.08*	5.40	3.24	2760*	579	1.33	19.0*	0.51	58.7*

Table 7 continued

Metabolites	Concentration (ng g^{-1})									
	P. biourgeianum C3(a)		*P. biourgeianum* T4(s)		*P. echinulatum* T1(a)		*P. madriti* C1(a)		*P. polonicum* C3(s)	
	S	M0 + compost	S	M0 + leather	S	M0 + leather	S	M0 + compost	S	M0 + compost
Roquefortine D	nd	nd	nd	nd	82.3*	18.6	nd	nd	nd	nd
Rugulovasine A	nd	nd	nd	nd	220*	112	nd	nd	nd	nd
Rugulusovine	1.97	nd	0.84	nd	1.64	nd	nd	nd	271*	121
Verrucofortine	3.40*	0.48	nd	nd	nd	nd	nd	nd	63.7*	14.8
Viridicatin	nd	nd	nd	nd	nd	nd	nd	nd	957*	33.3
Viridicatol	nd	nd	nd	nd	nd	nd	nd	nd	2753*	83.0

S—Sabouraud medium; M0—mineral medium; nd—not detected; M—museums; T—tanneries; C—composting plants; a—air, s—surfaces

* Statistically different metabolite concentrations between S and M0 medium (one-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$)

environments exhibited varying but statistically significant ($p < 0.05$) levels of cytotoxicity (Table 3). *Aspergillus flavus* had a cytotoxicity in the range of 80–99%; the cytotoxicity of *P. biourgeianum* (strains from composting plants and tanneries) was 83–95% and that of *Penicillium spinulosum* was 83–94% (strains from two different tanneries) (Table 3). These results were in agreement with an earlier report (Gutarowska et al. 2014), which showed different levels of cytotoxicity for *Penicillium* strains depending on the place of isolation. Our study also showed that the cytotoxicity may be different for samples isolated from the air and surfaces (*P. commune*). The strains isolated from surfaces had higher cytotoxicity. To the best of our knowledge, this has not been previously reported in the literature.

The published data concerning the cytotoxicity of moulds are inconclusive and often depend on the methods used for testing. Schulz et al. (2004) studied the cytotoxicity of spore extracts of various mould species, including *Aspergillus fumigatus*, *A. ochraceus, A. niger, Penicillium expansum, P. chrysogenum, Rhizopus stolonifera* and *Paecilomyces variotii*, using different methods: MTT (methylthiazol tetrazolium), MB (methylene blue) and PTG (pollen tube growth test) assays. They showed that the MTT assay was much more sensitive than the other two methods. Moreover, despite the cytotoxic properties of spores, mycotoxins were only detected in the extracts of *A. fumigatus* (Schulz et al. 2004). In our study, we used the MTT assay with a swine kidney cell line and found that all mould extracts were cytotoxic.

The profiles of the secondary metabolites were tested for four different mould species from the *Alternaria* genus. The largest number of secondary metabolites was detected for strains isolated from museums, which included *A. alternata* and *A. limoniasperae* ($N = 11$ and $N = 9$ compounds, respectively). The number of secondary metabolites detected in isolates from composting plants and tanneries was lower ($N = 7$) (Table 4). All tested *Alternaria* strains produced high concentrations of mycotoxins on Sabouraud medium, particularly altertoxin I (5.40–677 ng g^{-1}) and tenuazonic acid (1794–51,100 ng g^{-1}) (Table 4).

We observed a lower concentration of metabolites produced by *Alternaria* moulds grown on mineral medium containing material from the working environments (cellulose, compost extract and fragmented leather), as a carbon source, than when Sabouraud medium was used for the culture ($p < 0.05$) The only exception was brevianamid F. Mikušová et al. (2014) also observed variations in the production of secondary metabolites (alternariol, alternariol methylether, altenuete, and tenuazonic acid) for 11 strains of *Alternaria* depending on the culture medium (CYA and YES).

In all of the metabolite profiles tested, 3-nitropropionic acid (141–38,780 ng g^{-1}) and brevianamid F (4.67–225 ng g^{-1}) from *A. flavus* strains were present at the highest concentrations (Table 5). It has previously been reported that 3-nitropropionic acid is characteristic of *A. flavus* species (Hedayati et al. 2007). However, brevianamid F is not specific to this species and has also been described for many others species. In addition, significant amounts of gliocladic acid (425–751 ng g^{-1}) and heptelidic acid (31.5–868 ng g^{-1}) were also detected in our study. These substances were mainly identified from *Gliocladium, Chaetomium* and *Trichoderma* moulds in a previous study (Itoh et al. 1980). In the present study, the *A. fumigatus* strains produced large amounts of fumigaclavine C, fumiquinazolin A, fumiquinazolin CD, fumiquinazolin D and tryptoquivaline F (concentrations ranging from 23,387 to 10,640,000 ng g^{-1}). In addition to the above compounds, the secondary metabolites most commonly produced by *A. fumigatus* genera include fumagillin, fumitremorgins, helvolic acid and gliotoxin (Boudra and Morgavi 2005). We also detected low concentrations of fumitremorgins and helvolic acid but could not detect fumagillin and gliotoxin in our samples.

We observed statistically significant differences in the quantitative and qualitative production of secondary metabolites by the *Aspergillus* genus, which depended on the composition of the culture medium. Most compounds (averantin, gliocladic acid, heptelidic acid, nidurufin, chanoclavin, fumigaclavine C, fumigaclavine, fumiquinazolin CD, fumitremorgin C, helvolic acid, methylsulochrin, orsellinic acid, tryprostatin B, tryptoquivaline F and verruculogen) were produced at higher concentrations ($p < 0.05$) on Sabouraud medium than on the medium simulating the location where the strain was isolated. This dependence did not apply to bis(methylthio)gliotoxin, brevianamid F, cyclopiazonic acid, or 3-nitropropionic acid for *A. flavus,* or fumiquinazolin A, fumitremorgin B, meleagrin or tryprostatin A for *A. fumigatus*.

The profiles of the secondary metabolites for 14 strains belonging to the *Penicillium* genus, isolated from the air and surfaces of composting plants and tanneries, were also tested. All *Penicillium* isolates produced varying amounts

Table 8 Metabolites produced by *Penicillium* strains

Metabolites	Concentration (ng g^{-1})									
	P. commune T3(a)		*P. commune* T2(s)		*P. commune* T2(a)		*P. spinulosum* T1(s)		*P. spinulosum* T3(s)	
	S	M0 + leather	S	M0 + leather	S	M0 + leather	S	M0 + leather	S	M0 + leather
Andrastin A	nd	nd	39.7	nd	86.0*	21.9	360	nd	nd	nd
Brevianamid F	20.2*	6.87	5.47	14.6*	6.03	8.27*	5.00	20.0*	4.07	18.0*
Chanoclavine	40.7	204*	187*	30.0	nd	nd	nd	nd	nd	nd
Chrysogine	nd	nd	5.27	nd	nd	nd	nd	nd	27.3	379*
Cyclopenin	0.60	0.65	0.60	nd	3304*	717	0.74	nd	nd	nd
Cyclopenol	7.30	nd	nd	nd	2070	3791	nd	nd	nd	nd
Cyclopeptine	nd	nd	nd	nd	252*	62.0	nd	nd	nd	nd
Cyclopiazonic acid	463	8358*	8003*	996	nd	nd	nd	nd	nd	nd
Fumigaclavine	910	2007*	2660*	945	nd	nd	nd	nd	nd	nd
Meleagrin	2.07*	0.77	2450*	7.60	nd	nd	2.77	67.3*	5410	5760
O-Methylviridicatin	nd	2.57	1.85	nd	nd	nd	nd	nd	nd	nd
Orsellinic acid	nd	0.31	nd	nd	nd	nd	nd	nd	nd	nd
Neoxaline	nd	nd	7.27	nd	nd	nd	nd	nd	7.70	nd
Oxaline	nd	nd	2.13	nd	nd	0.31	nd	nd	3.77	3.04
Roquefortine C	2.21	7.33*	66.7*	2.70	54.0*	2.86	19.1	15.3	6390*	3173
Roquefortine D	nd	nd	12.8	nd	nd	nd	nd	nd	54.7	42.7
Rugulusovine	nd	1.89	3.63	nd	nd	nd	nd	nd	1.51	nd
Tryprostatin B	5.03	nd	nd	nd	nd	nd	nd	nd	nd	nd
Viridicatin	2.54	1.79	nd	nd	647*	189	0.62	nd	0.12	nd
Viridicatol	4.23	nd	nd	nd	72.3	212*	nd	nd	nd	nd

S—Sabouraud medium; M0—mineral medium; nd—not detected; M—museums; T—tanneries; C—composting plants

* Statistically different metabolite concentrations between S and M0 medium (one-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$)

of compounds that are characteristic secondary metabolites for this genus including andrastin A, andrastin D, brevianamid F, cyclopenin, cyclopenol, fumiquinazolin CD, meleagrin, penicillic acid and roquefortine C (Tables 6, 7, 8). These metabolites have been previously described in the literature (Frisvad et al. 2004; Frisvad and Samson 2004; Kozlovsky et al. 2013).

We also found qualitative and quantitative differences in the profiles of secondary metabolites from the same species [*P. biourgeianum, P. chrysogenum, P. commune* and *P. spinulosum* (Tables 6, 7, 8)] when they were isolated from different locations. For instance, although there were many common compounds produced by both strains of *P. biourgeianum,* the strain that was isolated from the composting plant produced orsellinic acid and verrucofortine, while the strain from the tannery produced fumigaclavine and fumonisin B1 (Table 7).

The amounts of secondary metabolites from *Penicillium* strains also depended on the culture medium. Modifying the culture medium to simulate the environmental conditions most often inhibited secondary metabolite production. The exceptions were brevianamid F, chrysogine, meleagrin, neoxaline, O-methylviridicatin, orsellinic acid, oxaline, pseurotin A, roquefortine C and rugulusovine, which were produced at higher concentrations ($p < 0.05$) on the medium containing leather and compost than on Sabouraud medium (Tables 6, 7, 8).

All moulds belonging to the *Aspergillus, Alternaria* and *Penicillium* genera showed variations in their secondary metabolite profiles depending on the species and culture medium. The production of mycotoxins can be affected by many factors, including the chemical composition of the media, the presence of certain trace elements, temperature, humidity, the presence of accompanying microorganisms and the strain genotype (Jarvis et al. 2000).

We detected various mycotoxins produced by *Aspergillus, Penicillium* and *Alternaria* that may have a negative impact on human health. The identified substances affect the central and peripheral nervous systems (rugulovasines, cyclopiazonic acid, fumigaclavines and chanoclavines), can cause neurohumoural and antibiotic activity (roquefortins, meleagrin, quinocitrinines rugulovasines, viridicatin, viridicatol) and are nephrotoxic (cyclopiazonic acid) (Kozlovsky et al. 2013).

Conclusion

A quantitative analysis of microbiological air contamination showed that the number of microorganisms present ranged from 2.4×10^3 CFU m^{-3} (composting plants) to 6.8×10^4 CFU m^{-3} (tanneries). In all workplaces, we identified high percentages of *Alternaria, Aspergillus* and *Penicillium* moulds (57–59% in the air, 10–65% on the surfaces). The general cytotoxicity of the mould strains against swine kidney cells was high, with the calculated values ranging from 72 to 99%. The highest cytotoxicity (>90%) was found for the following moulds: *Alternaria alternata*, *A. limoniasperae*, *Aspergillus flavus*, *Penicillium biourgeianum*, *P. commune* and P. *spinulosum*. The same mould species isolated from different working environments exhibited varying levels of cytotoxicity. We also found qualitative and quantitative differences in the secondary metabolite profiles of *Alternaria*, *Aspergillus* and *Penicillium* moulds, which depended on the culture medium. The modification of the culture medium to simulate environmental conditions most often inhibited secondary metabolite production.

Aspergillus, Alternaria and *Penicillium* produced mycotoxins that may have a negative impact on human health. These included chanoclavines, cyclopiazonic acid, fumigaclavines, meleagrin, roquefortins, rugulovasines, viridicatin, viridicatol and quinocitrinines. Due to varying cytotoxicity and toxinogenicity of the isolates, future studies should assess their health hazards to workers.

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