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Microbiological assessment of a contaminated library

Implications for book preservation and users health

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ABSTRACT

In the last years, a new kind of fungal contamination interested several Italian library and archive repositories, especially those with controlled climate conditions (18-20 °C, 50-60% relative humidity). Stored in Compactus® shelves, thousands of books were found with a spread fungal growth on their covers due to a particular xerophilic fungal species, *Eurotium halophilicum*, able to germinate and develop preferentially in air-stagnation microenvironments. There is a lack in the ecological/physiological/metabolic knowledge about this fungus and this present dissertation is dedicated to understand the entire biodegradation phenomena, in terms of the preservation of books collections and the possible relative implications for the health of workers and students. As a case study, a contaminated repository of the Library of Humanities (*Biblioteca di Area UManistica*, BAUM), Ca' Foscari University of Venice (Italy) was selected. Three samplings, prior and after a massive book disinfection, were performed within two years, which have permitted to characterize the indoor environment microbiologically and chemically. *Eurotium halophilicum* was recognized as the main fungus grown on the books, after isolation by sterile swabs, onto specific low water activity medium. Its presence was well-documented by microscopically investigations and growth condition trials were performed for its characterization. Moreover, more than 120 fungal species were detected by morphological and molecular (DNA) analyses, mainly belonging to *Aspergillus*, *Cladosporium* and *Penicillium* genera. *A. creber* and *A. jensenii*, belonging to recent revised group *Aspergillus* section *Versicolores*, were isolated for the first time from library's environments. Chemical investigations of microbial volatile organic compounds and secondary metabolite abilities of the most frequent isolated fungal species were performed by gas chromatography – mass spectrometry and liquid chromatography – tandem mass spectrometry, respectively. The fungal production was confirmed for the well-known fungal species (*i.e. A. penicillioides*, *A. vitricola*, *C. cladosporium*, *E. chevalieri*, *P. breviciompactum* and *P. chrysogenum*) while new information were obtained for the less known species (*i.e*. *A. creber*, *A. jensenii*, *A. protuberus*, *E. halophilicum*). The results were then correlated to "real" case studies, as indoor air samples, contaminated books and settled dust, highlighting similarities between all the substrates. The principal microbial volatile organic compounds, *i.e.* 1,4-pentadiene, isopropyl alcohol, acetone and 2-butanone, were identified as general chemical markers. The secondary metabolite analysis of dust samples has improved the knowledge about the indoor microbiological community, detecting markers belonging to species not isolated by aerobiological sampling. The combination of all the analyses has furnished all the information necessary to characterize the *status quo* of the repository environment, recognising the benefits obtained from the disinfection and identifying the remained weak points for a correct preservation.

SINTESI

Negli ultimi anni, una nuova tipologia di contaminazione fungina è stata riscontrata all'interno di numerose biblioteche ed archivi italiani, specialmente quelli in cui le condizioni climatiche erano ben controllate (18-20 °C e 50-60% di umidità relativa). Conservati all'interno di scaffalatura Compactus®, migliaia di libri sono risultati soggetti ad evidente crescita di tipo miceliare in corrispondenza delle copertine, ad opera di una particolare specie xerofila, *l'Eurotium halophilicum*. Questo fungo, infatti, è capace di germinare e svilupparsi preferenzialmente in corrispondenza di micro-ambienti con situazioni di ristagno d'aria. A causa della scarsità di informazioni concernenti le caratteristiche ecologiche, fisiologiche e metaboliche di questo fungo, la presente tesi è stata incentrata sull'approfondimento relativo al fenomeno di biodeterioramento, sia dal punto di vista della conservazione del materiale librario che da quello relativo all'aspetto salutistico dei lavoratori e degli studenti. Come caso studio è stato considerato un deposito contaminato della Biblioteca di Area UManistica (BAUM) dell'Università Ca' Foscari di Venezia. In totale sono stati eseguiti tre campionamenti, sia prima che dopo il massivo intervento di disinfezione dei libri, distribuiti nell'arco di due anni, che hanno permesso di caratterizzare l'ambiente *indoor* dal punto di vista microbiologico e chimico. *Eurotium halophilicum* è stato riconosciuto come il principale fungo cresciuto sui libri, grazie all'isolamento con tamponi sterili, su idoneo terreno di coltura per la crescita selettiva di specie xerofile. La sua presenza è stata documentata grazie a specifiche osservazioni al microscopio elettronico a scansione e le sue ideali condizioni di crescita sono state testate. Inoltre, più di 120 specie fungine sono state isolate dal deposito librario e identificate con analisi microbiologiche classiche associate ad analisi molecolari (DNA). I principali generi fungini riconosciuti sono stati *Aspergillus*, *Cladosporium* e *Penicillium*. Le specie fungine *A. creber* e *A. jensenii*, appartenenti al gruppo *Aspergillus* section *Versicolores* recentemente revisionato, sono state isolate per la prima volta da ambienti di conservazione di materiale librario. Le principali specie fungine isolate dal deposito contaminato sono state, inoltre, soggette ad indagini chimiche relative alla loro produzione di composti organici volatili e ad analisi dei metaboliti secondari tramite le tecniche di gascromatografia-spettrometria di massa e cromatografia liquida ad alte prestazioni accoppiata alla spettrometria di massa tandem. I risultati hanno confermato le attività metaboliche dei funghi più noti (come *A. penicillioides*, *A. vitricola*, *C. cladosporium*, *E. chevalieri*, *P. breviciompactum* e *P. chrysogenum*), incrementando allo stesso tempo le informazioni relative alle specie meno conosciute (come *A. creber*, *A. jensenii*, *A. protuberus* e *E. halophilicum*). Tali informazioni, poi, sono state correlate con reali casi studio, come libri contaminati, campioni d'aria e polveri sedimentate. Considerando tutti i diversi substrati, varie similarità sono state riscontrate. In particolare, sostanze come 1,4-pentadiene, alcool isopropilico, acetone e 2-butanone sono state evidenziate come potenziali marker chimici di derivazione fungina. L'analisi dei metaboliti secondari delle polveri ha contribuito all'incremento della comunità microbica del deposito, identificando sostanze metaboliche appartenenti a specie non isolate dai diversi campionamenti microbiologici eseguiti. La combinazione di tutte queste analisi ha permesso di identificare lo *status quo* conservativo del deposito, riconoscendo gli effetti positivi dell'opera di disinfezione eseguita ed evidenziando i punti deboli che ancora inficiano una corretta conservazione del patrimonio librario.

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ABBREVIATIONS

ACGIH: American Conference of Governmental Industrial Hygienist

AIHA: American Industrial Hygiene Association

ARPAV: Agenzia Regionale per la Prevenzione e la Protezione Ambientale del Veneto

AU: Arbitrary Unit

a_w: Water Activity

BAUM: Biblioteca di Area UManistica (Library of Humanities)

BOD: Biological Oxygen Demand

BRI: Building Related Illness

BTEX: Benzene, Toluene, Ethylbenzene and Xylene compounds

CBS-KNAW: Centraalbureau voor Schimmelcultures – Koninklijke Nederlandse Akademie van Wetenschappen

CFU: Colony Forming Unit

CREA: Creatine Sucrose Agar

CTAB: Cetyl Trimethyl Ammonium Bromide

CYA: Czapek Yeast Agar

CY20S: Czapek Yeast Agar 20% sucrose

Cz20: Czapek 20% sucrose agar

Cz40: Czapek 40% sucrose agar

Cz70: Czapek 70% sucrose agar

DG18: Dichloran-glycerol agar base

DNA: Deoxyribonucleic Acid

EBSD: Electron BackScatter Diffraction

EC: European Community

EDX: Energy Dispersive X-ray analysis

EI: Electron Ionization

ELISA: Enzyme-Linked ImmunoSorbent Assay

EPA: Environmental Protection Agency

ESI-MS/MS: Electron Spray Ionization tandem Mass Spectrometry

Et-OH: Ethanol

FDA: Food and Drug Administration

GC-MS: Gas Chromatography – Mass Spectrometry

G25N: 25% Glycerol Nitrate Agar

HP-SPME: HeadSpace Solid-Phase MicroExtraction

HPLC: High Performance Liquid Chromatography

HVAC: Heating, Ventilation and Air Conditioning

IAQ: Indoor Air Quality

ICRCPAL: Istituto Centrale per il Restauro e la Conservazione del Patrimonio Archivistico e Librario

IMA: Index of Microbial Air contamination

ITS: Internal Transcribed Spacers

LC-MS/MS: Liquid Chromatography-tandem Mass Spectrometry

MEA: Malt Extract Agar

MEA15%: Malt Extract Agar 15% NaCl

MIBAC: Ministero per I Beni e le Attività Culturali

MRM: Multiple Reaction Monitoring

MS/MS: Tandem Mass Spectometry

MUT: Mycotheca Universitatis Taurinensis

MVOCs: Microbial Organic Volatile Compounds

MY40G: Malt Extract Yeast Extract 40% Glucose

MY50G: Malt Extract Yeast Extract 50% Glucose

MY60G: Malt Extract Yeast Extract 60% Glucose

MY70GF: Malt Extract Yeast Extract 70% Glucose-Fructose

NA: Nutrient Agar

NCBI: National Center for Biotechnology Information

NIST: National Institute of Standards and Technologies

PCA: Plate Count Agar

PCR: Polymerase Chain Reaction

PDA: Potato Dextrose Agar

PDA20%: Potato Dextrose Agar 20% NaCl

PDMS: Polydimethylsiloxane

RH%: Relative Humidity

RT: Retention Time

SAS: Surface Air System

SBS: Sick Building Syndrome

SEM: Scanning Electron Microscope

SPME: Solid Phase Micro Extraction

STD: Standard deviation

TIC: Total Ion Count

TLC: Thin Layer Chromatography

UHPLC: Ultra High Performance Liquid Chromatography

UNI: Ente Nazionale Italiano di Unificazione

UV: Ultraviolet light

VOCs: Volatile Organic Compounds

WHO: World Health Organization

YES: Yeast Extract Agar

YEA15%: Yeast Extract Agar 15% NaCl

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1. INTRODUCTION

1.1 Cultural Heritage and biodeterioration

Since their formation, all art collections are subjected to natural aging, caused by physicochemical processes, such as oxidation, hydrolysis, photo-deterioration, deformations, corrosions, loss of materials, but also by biological processes, called *biodeterioration*. Hueck (1965) defined biodeterioration as *"any undesirable change in the properties of a material caused by the vital activities of organisms*". Indeed, biodeterioration processes could be caused by microorganisms, such as bacteria, yeasts and moulds, or by organisms, as insects, birds, and rodents. Focusing on microorganisms, their settlement depends on both, different ecological factors and use of substrates (*i.e.* the single cultural heritage) for their growth as support (particularly the autotrophs, such as most of bacteria, algae, lichens and so on) and/or as principal nutriment for their growth (especially the heterotrophs, such as some bacteria and fungi).

The presence of autotrophs or heterotrophs depends on the composition of the substrate, which can be inorganic (*e.g*. stone sculpture) or organic (*e.g.* wood sculpture). However, it is not possible to consider a single microbial community, because biodeterioration phenomena involve consortia of microorganisms that coexist and act together, with a continue evolution in their equilibrium (Pinna and Salvadori, 2005). To classify the materials by their ability to being colonized by living organisms, Guillitte (1995) defined the term of "bioreceptivity", initially, only for stone materials, but then this term has been extended to all the artefacts. Chemical composition, physical characteristics, such as roughness and porosity, state of conservation, as well as (micro-) environmental conditions of the conservative status are parameters that influence the development of microorganisms.

The conservation environment, both indoor and outdoor, of Cultural Heritage is fundamental for the influence of degradation processes. Buildings, monuments, statues sited or conserved in outdoor environments are subjected to different factors depending on the climate regions, the geographic and topographic locations, as well as the specific seasonal meteorological conditions, such as rain, wind and solar irradiation and pollutants (Caneva *et al*., 2005). Artefacts in indoor environments, instead, are often preserved under more stable and controlled conditions. In Italy, this have been recently improved with the regulation of environmental parameters for the preservation of Cultural Heritage by Italian Ministry (MIBAC, 2001) and the development of new technologies for the conservation in museums and libraries (Baglioni *et al*., 2015; De Nuntiis *et al.*, 2003; Ioannides *et al*., 2012). However, other factors can still invalidate the correct preservation and in most of the cases the principal causes are related to the human operations or negligence (Manente *et al*., 2012; Schieweck and Salthammer, 2001).

1.1.1 The case of indoor environments

The buildings that preserve Cultural Heritage, such as museums, archives and libraries, could be defined as confined spaces, generally characterized by a limited air exchange between the in- and out-side. The indoor environment depends also on the type of building: a) constructed *ad hoc* and equipped with air conditioning systems; b) constructed *ad hoc* without air conditioning systems; c) buildings adapted as museums, libraries, archives, originally constructed for other purposes (Gallo *et al*., 2003).

Other distinction depends on the type of collections: museums preserve normally a wide range of different materials, both organic and inorganic, but also in archives and libraries different materials with vegetable origin (*e.g*. cellulose, starch, resins) and animal origin (*e.g*. organic glue, casein, albumin, collagen) materials, or plastics (*e.g*. cellulose acetate, polyesthers) are stored. The environmental conditions (temperature and relative humidity) together with the typologies of furniture and the cleaning conditions are the parameters that have to be frequently monitored for the correct preservation of art collections (Pasquariello *et al*., 2005; Gallo *et al.,* 2003).

Unfortunately, several cases of microbial contaminations in indoor environments were documented and not all of them were consequences of exceptional events, as floods or earthquakes (Maggi *et al*., 2000; Manente *et al*., 2012). Inside libraries and archives, the management of increasing fungal contaminations due to inadequate conservations, such as malfunction of air conditioning systems, lack of cleanliness, exchange of contaminated collections or materials, has become a complex and expensive problem for book conservation, but also for the health of workers and students (Flannigan and Miller, 2011; Zielińska-Jankiewicz *et al.*, 2008). The spread of microbial contamination can interested old book collections inside libraries (Michaelsen *et al*., 2010; Micheluz *et al*., 2015a; Montanari *et al*., 2012), but also archival materials (Manente *et al*., 2012; Mesquita *et al.,* 2009), photograph materials (Borrego *et al*., 2010; Gallo, 1993; Scocchi *et al*., 2003) as well as the furniture (*e.g* wood shelves, glass, mirrors) (Flannigan and Miller, 2011; Schabereiter-Gurtner *et al*., 2001; Sterflinger, 2010).

The Italian Ministry for Cultural Heritage provides guidelines for the correct environmental conditions for the preservation of book and paper products, in particular air temperature of 19-24 °C and 50-60% of relative humidity (MIBAC, 2001; UNI 10586, 1997; UNI 10829, 1999; UNI 10969, 2002).

However, it turned out that these conditions are suitable for the development and growth of several microfungi and bacteria, which need little free water (measured as water activity, a_w) and can colonise materials with a very low water activity (Borrego *et al.*, 2012; Hocking, 1993; Micheluz *et al*., 2015a; Slonczewski *et al.*, 2010). The water activity (a_w) is defined as $\mathbf{a_w = p/p_0}$, where p = the partial pressure of water above the material and p_0 = the partial pressure of pure water at the same temperature in equilibrium. Fungi can grow at a lower a_w than bacteria, as shown in the Fig. 1.1. Samson *et al*. (2010) reported that the common indoor fungi can grow in a water activity range of $0.70 < a_w < 0.94$ and some xerophilic species can also develop in material with lower a_w (Christensen *et al*., 1959).

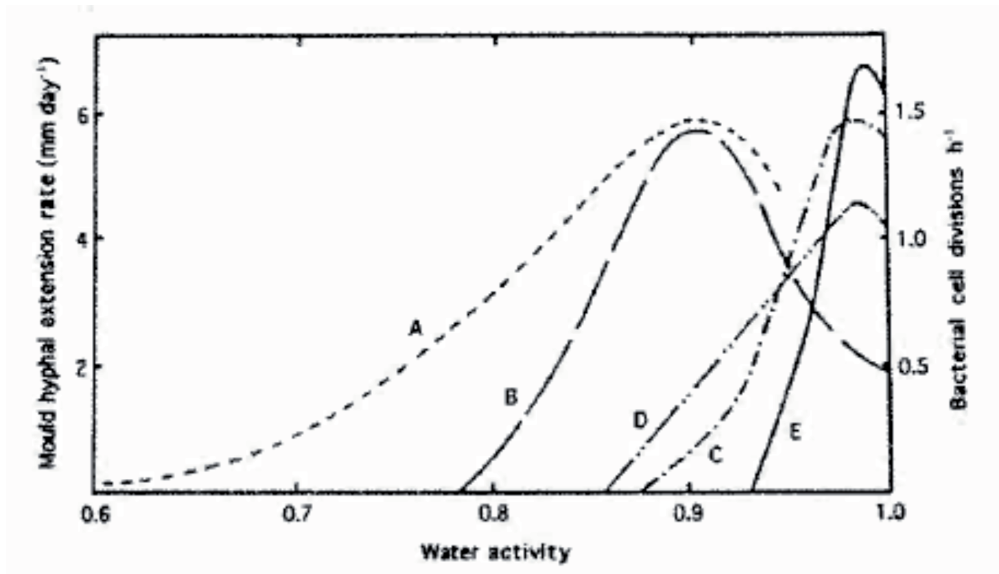


Figure 1.1 Effect of water activity (a_w) on growth of different microorganisms (from Flannigan and Miller, 2011). A: *Xeromyces bisporus*; B: *Eurotium herbariorum* (*A. glaucus*); C: *Aspergillus niger*; D: *Staphylococcus aureus*; E: *Salmonella* sp.

Referring to paper materials, hygroscopicity and the water content of individual components are important factors to consider for the biological risk evaluation. As reported in Fig. 1.2, paper's water content ≥ 8% indicates the possible microbial germination on the material (Nyushka, 1979).

In order to prevent fungal contamination, conservators and librarians should regularly check the heating, ventilation and air conditioning system (HVAC), maintaining the optimal standard climatic values and the air circulation, as well as the cleaning of the environments with frequent visual inspection of the artefacts (Montanari *et al.,* 2012).

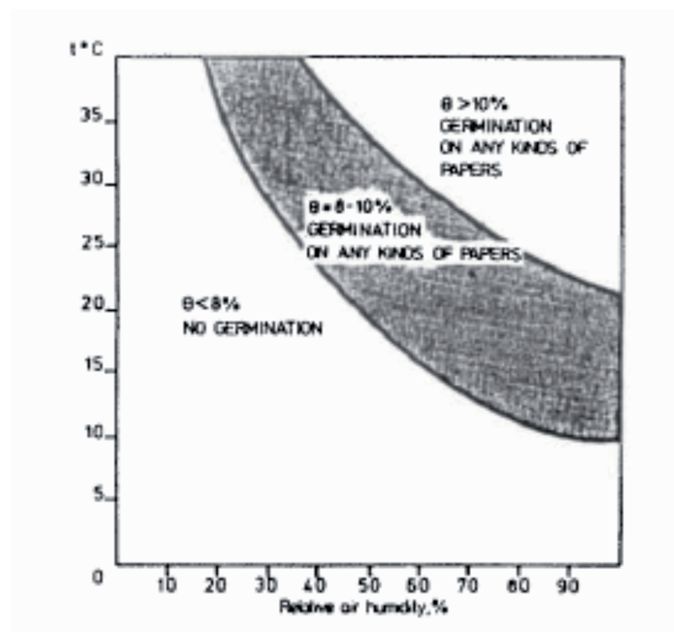


Figure 1.2 Relation between relative water (in %) content of paper and germination of fungal spores (Gallo *et al.,* 2003; Nyushka, 1979).

1.2 Microorganisms involved in biodeterioration of book collections

Autotrophic and heterotrophic microorganisms can use different substrates, including the artefacts, for their developments or as nutrients. Inside libraries and archives, different kind of bacteria damaging paper, photos, and parchment are already known. They mainly belong to the genera *Bacillus*, *Cellfalcicula*, *Cellvibrio*, *Clostridium*, *Cytophaga, Nocardia, Pseudomonas, Serratia* and *Sprocytophaga* (Borrego *et al*., 2010; Gallo, 1993; Michaelsen *et al.,* 2010).

Several studies investigated the fungal contamination of book collections, reporting a wide range of species isolated from different worldwide damaged materials. The most often detected were:

- on paper and cellulose textiles: the genera *Alternaria*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Eurotium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus, Stachybotrys*, *Stemphylium*, *Trichoderma* and *Ulocladium*;
- on parchment: the genera *Cladosporium*, *Epicoccum*, *Phlebiopsis*, *Penicillium* and *Thanatephorus*;
- on keratinous substrates: the genera *Absidia*, *Acremonium*, *Alternaria, Aspergillus*, *Aureobasidium*, *Chaetomium*, *Chrysosporium*, *Cladosporium,* *Coniosporium*, *Cunninghamella*, *Emericella,* *Epicoccum*, *Geotricum*, *Mucor*, *Penicillium*, *Phoma*, *Rhyzopus, Scopulariopsis* and *Stachybotrys* (Borrego *et al*., 2010; Gallo, 1993; Kraková *et al*., 2012; Mesquita *et al*., 2009; Michaelsen *et al*., 2010; Montemartini Corte *et al*., 2003; Nyuksha, 1994; Sterflinger, 2010; Zyska, 1997).

The major parts affected by infections are the first and the last pages, the external edges and the bindings, because of their direct contact with the environment. Moreover, the start of the infection is often due to some ubiquitous species, as *Aspergillus chevalieri* and *A. echinulatus*, which are able to develop at a low relative humidity (Kowalik, 1980). After their settlement and after the release of their metabolism products, an increase of the water content of the materials can be established, followed by a secondary microorganism attack (Gallo, 1993). In the recent years, several widespread fungal contamination records were reported from Italian archives and libraries. In all cases, common characteristics of the contamination were emerged, being summed up in the following case study's description.

1.2.1 The case of Eurotium halophilicum

In the recent years, first studies have described a peculiar fungal colonization associated with books in several Italian archives and libraries (Pinzari and Montanari, 2011; Montanari *et al*., 2012). In all cases, a widespread irregular white, mycelial spots of variable diameter were observed on the damaged books, mainly stored inside movable shelves of the Compactus® type (Fig. 1.3). These closed metal cabinets, which are sliding on guide rails, can be compacted and minimizing the space required for book storage. They are considered as suitable for preserving books from light degradation and dust deposits. However, without an efficient climate control system, particular microenvironments can be established between the closed shelves, favouring the development of particular fungal species on the books (Montanari *et al*., 2012).

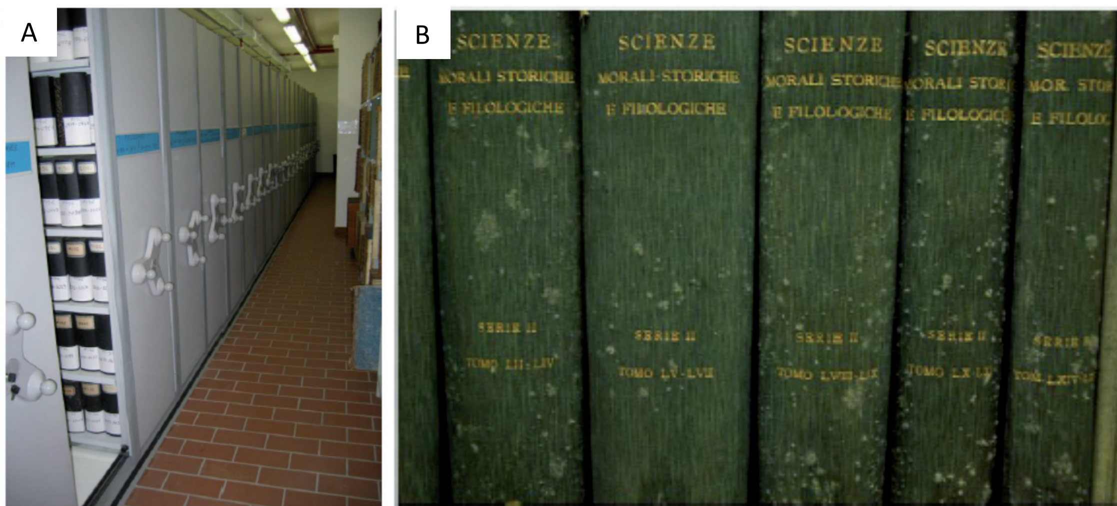


Figure 1.3 Fungal contamination related to Compactus® shelves: A: typology of Compactus® shelves (from Pinzari and Montanari, 2011); B: widespread white mycelial growth on books stored in Compactus® shelves (from Montanari *et al.,* 2012).

The main responsible for this type of fungal contamination has been identified as *Eurotium halophilicum* C.M. Chr. Papav. & C.R. Benj. (anamorph *Aspergillus halophilicus*), a xerophilic fungus with a high tolerance to water stress. The minimum water activity observed for germination and growth of this species is 0.675 (Christensen *et al.,* 1959). Because of its particular requirements, this fungus has been isolated from dry food and indoor dust in association with *Aspergillus penicillioides* and dust mites (Abdel-Hafez *et al*., 1990; Aiqing *et al*., 2011; Christensen *et al*., 1959; Hocking and Pitt, 1988; Samson and Lustgraaf, 1978). It has been recently associated with book and paper biodeterioration, especially in correspondence to very particular micro-niches inside museums, libraries or archives. This ability makes the fungus capable of developing also when the overall environment conditions are perfect for the conservation of Cultural Heritage. These niches are characterized by scarce ventilation and the presence of a water vapour gradient can lead to condensation on some materials after a sudden drop of temperature or night-day thermo hygrometric cycles. These peculiar, very often local, conditions together with soaking events in usually dry environments were suggested to favour the development of osmophilic and xerophilic fungal species (Michaelsen *et al.,* 2010; Montanari *et al*., 2012; Pinzari and Montanari, 2011; Sterflingler, 2010). Similar patterns and microscopic features observed in Italian case studies suggest that *E. halophilicum* might have a large distribution in repositories with Compactus® shelves, but its detection may be largely underestimated. Inadequacy of sampling procedures and the very slow growth of the fungus are probably the main reasons for previous underestimations (Micheluz *et al*., 2015a; Montanari *et al*., 2012).

Like all *Eurotium* species, *E. halophilicum* is characterized by developing cleistothecia structures, in general after 2-3 weeks on Malt Extract Agar (MEA) or Czapek agar with additional sucrose or NaCl concentrations. Conidial (asexual) state, usually absent during early development, appears after cleistothecia (sexual state's morphological structures) have been formed (Samson and Lustgraaf, 1978) (Fig. 1.4).

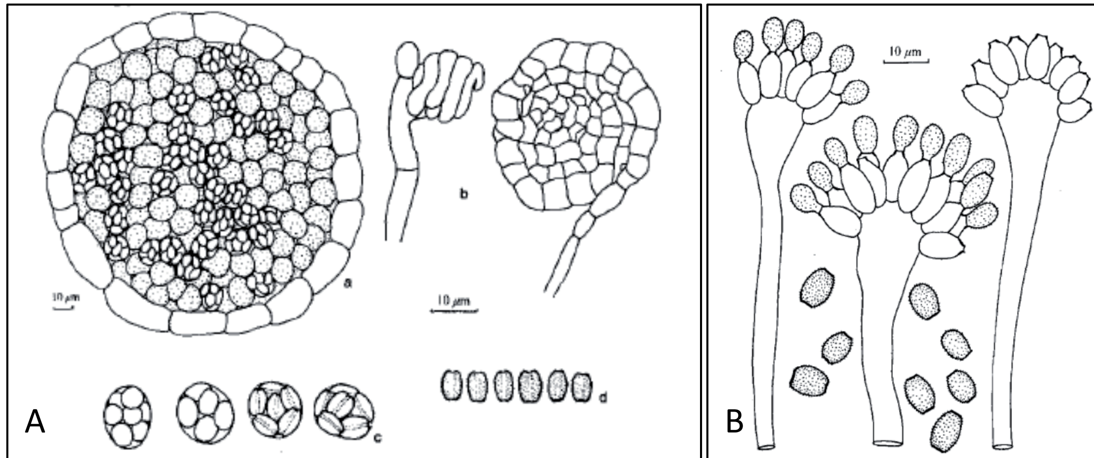


Figure 1.4 Typical *E. halophilicum* structures: A: sexual stage with a. cleistothecium, b. ascoma initials, c. asci, d. ascospores; B: asexual stage, conidiophores and conidia of *Aspergillus halophilius* (Samson and Lustgraaf, 1978).

This species presents also characteristics similar to *Aspergillus restrictus* series of Thom and Raper (1945), in particular for the cleistothecia wall, the sterile mycelium lacking of the bright yellow and orange pigments, the ascospore's size called "small-spored" and "large-spored" groups, its requirements of high concentrations of sugar or salt in the medium and its conidial state (*Aspergillus halophilicus*) (Christensen *et al*., 1959) (Fig. 1.5). In literature, several descriptions of this particular fungus are given and all information is reported in the following Table 1.1, with all the recommended references.

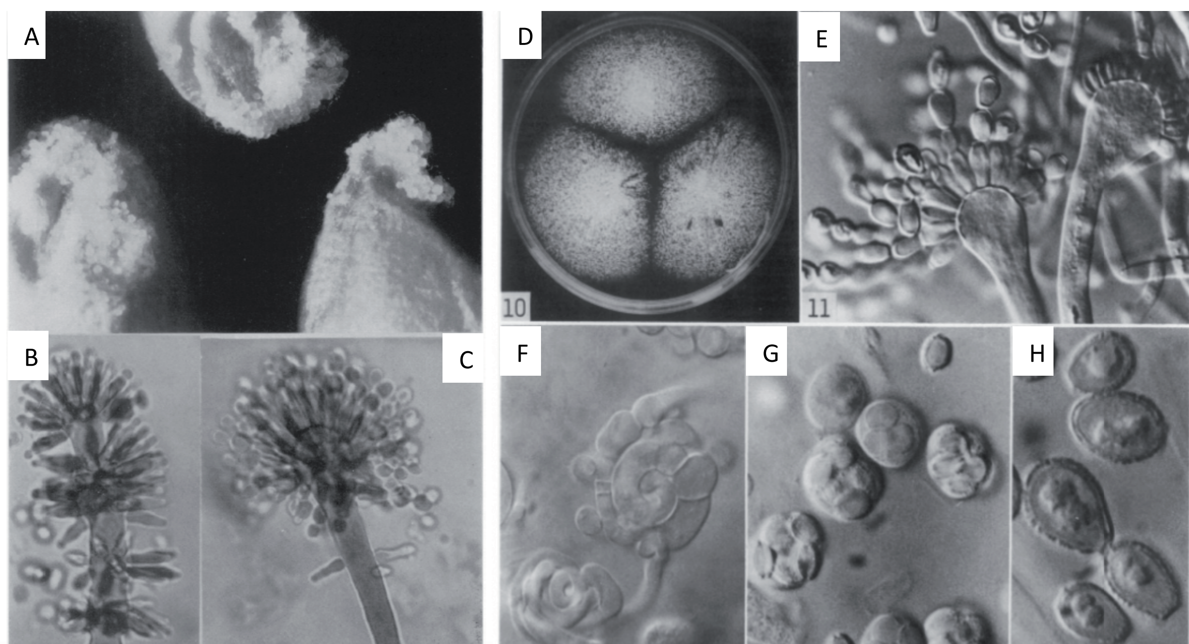


Figure 1.5 Micrographs of morphological *E. halophilcum*'s structures: A: masses of white perithecia on wheat seeds cultured on malt agar with 15% of sodium chloride; B: sporophore of *A. halophilicus* with sterigmata growing from enlarged portions below the terminal head on Czapek's agar with 55% of sucrose; C: normal *A. halophilcus'* head on Czapeck's agar with 55% of sucrose; D: *E. halophilicum* colonies on Malt Extract Yeast Extract 50% Glucose (MY50G) agar, 4 weeks; E: *Aspergillus* head on Malt Extract Yeast Extract 70% Glucose-Fructose (MY70GF) agar, 8 weeks, x750; F: Cleistothecia initials on Malt Extract Yeast Extract 40% Glucose (MY40G) agar, 3 weeks, x950; G: Asci and ascospores from MY50G agar, 4 weeks, x1200; H: conidia on MY70GF, 12 weeks, x1875 (pictures A, B, C from Christensen *et al*., 1979; pictures D, E, F, G, H from Hocking and Pitt, 1988).

Table 1.1 Overview of published information of *Eurotium halophilicum* species.

Fungal strain	Culture media or other supports	Morphological description	References
E. halophilicum NRRL 2739 Samples of wheat from terminal elevators in Nebraska, Pennsylvania and Chicago	Czapeck agar added with 70% of sucrose incubated for 45 days at 25 °C Tested several culture media, with best results on: - Malt agar with 15-20% of NaCl; - PDA with 15-20% of NaCl; - Czapek agar with 55-70% of sucrose	Perfect stage on Czapek70%: perithecia white to maize yellow, becoming buff yellow with age, spherical to subspherical or irregular, mostly 150-180 µm or 125-240 µm in diam.; asci 9-14 µm in diam., spherical; ascospores hyaline or very light green, lenticular, with equatorial surface rough, with furrow shallow and bordered by low ridges, mostly 4-5 x 5.5-7.5 µm and occasionally 7 x 9 µm in size. On PDA20% of NaCl: heads pea green to sage green arising from white, pressed mycelium; spore mass globose when young, loosely radiate when mature, 70-130 µm or 175 µm in diam.; conidiophores 300-550 µm, occasionally 750 µm long, 6-10 µm in diam., hyaline or light green, smooth or with faint wavy markings, wall 0.5-0.7 µm thick; vesicle globose to subglobose or club-shaped, occasionally with one or two enlargements below the main or terminal vesicle; sterigmata in one series, 3-6 µm wide, 6-14 µm long, covering only a portion of the top or the upper half or three-fourths of the vesicle; conidia globose, elliptical, pyriform, or irregular, spiny, hyaline to light blue-green, 4.0-6.5 x 6.0-11.0 µm or up to 8 x 15 µm	Christensen *et al.*, 1959
E. halophilicum forma A (absence of conidial state). Isolated in 1964 from a laboratory contaminant	Malt Extract Agar supplemented with 15% of sodium chloride (hypertonic medium)	Dormant spores are lenticular, having equatorial furrow, which are shallow but prominent and bordered by low ridges. Walls of ascospores are rough and measured 5.5-6.0 x 4.5 µm in diam.	Imai, 1967
E. halophilicum CBS 645.77 and CBS 644.77 House dust of crew's quartes of the oceansteamer "Balong" (Nedlloyd, Rotterdam, Captain J.W. Koning); mattress dust, Institute Bayard, Groesbeek, The Netherlands	No growth on MEA or Czapek. Colonies on MEA or Czapek agar with 64% sucrose (a_w = 0.82) are 14 and 5 mm in diameter respectively after 2 weeks incubation at 25 °C.	Cleistothecia globose, white to cream, 150-200 µm in diam., wall formed by more or less uniform flattened cells. Asci globose to obvoidal, 9-14 µm in diam., born on croziers, evanescent. Ascospores oblate, rough-walled, with a shallow furrow and low ridges, hyaline, 4-5 x 5.5-7.5 µm. Conidial state yellow green, grey-green or colourless. Conidiophores smoothwalled, hyaline, 50-300 µm in length. Heads radiate, 100-200 µm in diam., uniseriate. Vescicle globose to subglobose, 12-15 µm in diam. Phialides flask-shaped, 3-6 x 6-14 µm. Conidia globose to ellipsoidal with truncate ends, green or hyaline, echinulate or smoothwalled, 4-6.5 x 6-11 µm.	Samson and Lustgraaf, 1978
E. halophilcum FRR 2471 and FRR 2739 Isolated in 1982 from cardamom seeds	No growth after 14 days on CYA, CY20S and MEA at 25°C. On G25N, 25 °C germination after 7 days	On MY50G, 25 °C, 14 days, colonies 15-20 mm in diam., low and sparse, with white to translucent cleistotechia surrounded by a thin Weft of white mycelium; reverse pale at the margins to pale yellow centrally; margins fimbriate, subsurface. On MY60G, 25 °C, 28 days, colonies 30-40 mm in diam., with a central tuft of aerial mycelium bearing sparse *Aspergillus* heads. Cleistothecia initiated from a tight hyphal coil; subglobose, 90-200 µm in long axis, cream colored, maturing in 2-3 weeks on MY50G and MY60G. Asci globose, born singly or in short chains from ascogenous hyphae; ascospores ellipsoidal 6-0-7.5(-8.0) x 4.5-6.0(-7.0) µm, with a lenticular furrow bordered by low ridges; walls rugose. *Aspergillus* heads formed only at low water activities (below 0.85 aw). Conidia pale green, stipes smooth walled, hyaline or pale brown, 500-1250 x 8-12 µm, enlarging to hemispherical vesicles 15-30 µm diam. Bearing phialides only; phyalides enlarging gradually from the base, then narrowing abruptly to very short necks, 8-15 x 5-7 µm; conidia pyriform, often with a	Hocking and Pitt, 1988

		truncate base, or cylindroidal with rounded ends, or ellipsoidal, (6-)8-11 x 5-6 µm, with walls finely roughened, born in disordered chains.	
E. halophilicum, from dust samples that settled on the roofs of houses and stationary cars in Egypt	Colonies isolated on 10% and 20% sodium chloride-Czapek Dox agar at 28 °C. No growth on media composed by glucose, cellulose or 50% sucrose Czapek Dox agar		Abdel-Hafez *et al*., 1990
E. halophilicum FN394525, FN304527, FN394531, FN394535 Clones from contaminated Italian manuscript			Michaelsen *et al.,* 2010
E. halophilicum NRRL 2739 Colony isolated in Fuzhuan brick-tea.	Colony isolated on M40Y		Aiqing *et al*., 2011
E. halophilicum JN839940, JN839949 – JN839958 From contaminated books in historical library in Rome (Italy)	Colonies growth on DG18 observed under SEM	Presence of hypertrophic hyphae, slightly covered with bare and short hairs. Ascomata appeared spherical to subspherical, mostly 100-150 µm in diam. and spherical asci 10-15 µm in diam. Ascospores by SEM appeared lenticular, with rough surface and sharp furrow bordered by ridges, 5 x 7 µm. The ascomata with smooth surface and semi-globose prominent structures.	Montanari *et al.,* 2012
E. halophilicum KM502179 Isolated from contaminated book in university's library, Venice (Italy)	Colonies growth on MEA15% and observation of Fungi-Tape^{TM} under SEM	Uniseriate radiate to columnar conidial heads and ellipsoidal conidia 5-7.5 x 5-9 µm. The shape, ornamentation and dimensions of conidia and conidiophores corresponded to the anomorphous state namely *Aspergillus halophilicus*. Short bare "hairs" on hyphae visible at 200x magnification.	Micheluz *et al*., 2015a

1.3 Indoor air quality and human health

The indoor air quality became an important health aspect worldwide after several studies have focused the attention of indoor pollution levels found in dwellings, offices, schools, hospitals and libraries. Particular attention was given to the interaction between indoor pollution and population groups with an elevated risk, like children, elderly people and sick people, because of the duration of the exposure (Bellante De Martiis *et al*., 1992). According to the *World Health Organization* (WHO), the European population spend 90% of their lifetime indoors, and up to 95% during wintertime, so healthy indoor environments have a very high importance. Different environmental local indicators are associated with a low indoor air quality, such as:

- alteration of microclimatic parameters and thermo-hygrometric comfort;
- quantitative alteration of normal constituent of air, *e.g.* increase in expiratory carbon dioxide and in consumption of oxygen;
- presence of different kind of pollution (Micali *et al*., 2003).

The source of pollution can be distinguished between external and internal pollution. External sources could derive from soil, car traffic, industrial plants, thermal power stations and incinerators. The external pollutants can enter inside buildings because of the low degree of insulation of the buildings or with transfer of people. Internal sources include a wide range of emissions from building materials, furniture, equipment, occupiers and human activities (Després *et al*., 2012).

The effects of pollution on human health include well-known diseases with specific aetiological factor and non-specific diseases, where the direct relationship with certain pollution is not evident. These wide-spectrum effects can be acute or chronic, depending on preferential explosion route, concentration of pollutants, individual sensibility, and frequency of exposition time. Among the short and medium-term effects, two syndromes emerged in the last years: Building Related Illness (BRI) and the Sick Building Syndrome (SBS). Diseases with clearly identifiable causal factors are typical for BRI.

SBS is a syndrome with non-specific aetiology that affects the occupants of so-called “sick” buildings. Typical SBS symptoms are drowsiness, headaches, fatigue and concentration problems, often related to the upper respiratory tract (nose congestion, parched throat), to the eyes (dryness of mucous membrane, smarting) and to the skin (erythema, dryness) (Redlich *et al*., 1997). Several studies have identified many factors that could implied the SBS, such as microclimatic and ventilation factors, artificial lighting, noise, the presence of particular materials, as carpeting, cleaning products and furniture (Righi *et al*., 2001; Redlich *et al*., 1997) and also biological agents (Apetrei *et al*., 2009; Cabral, 2010; Cooley *et al*., 1998).

In the following Table 1.2 is reported a list of different effects on human health in association with indoor pollution.

Table 1.2 Effects on human health because of indoor pollution depending on different kind of causal agent (from Bellante *et al*., 1992; Micali *et al.,* 2003).

Effects on human health	Causal and concasual agents			
	Psycological	**Biological**	**Physical**	**Chemical**
IMMEDIATE				
Headache			X	X
Irritation of eye/upper respiratory tract			X	X
Nausea/discomfort			XX	XX
Allergy		X		X
Mental confusion/irritation	XX			X
SHORT-TERM				
Airborne infection		X	XX	
B.R.I.:		X	XX	
- Legionnaires' disease/Pontiac's fever				
MEDIUM-TERM				
B.R.I.:				
- humidifier fever		X	XX	XX
- allergic alveolitis		X	XX	XX
Asthma		X		X
S.B.S	X	X	X	X
LONG-TERM				
Chronic bronchopneumopathy		X	XX	X
Mutagenic and carcinogenic activity			X	X

X: Causes; XX: concauses.

Among all types of potential pollutants, biological agents need also to be better evaluated, with concern about their potential as infecting agents, sensitising or aeroallergenic antigens or toxic agents, as both individual (or part of itself) microorganism and its metabolism (sub)products (Després *et al*., 2012). Biopollutants are present as nuclei of droplets, particular matter or airborne particles. Viruses, bacteria, *Actinomycetes*, fungal spores and mycelial fragments, excrements of *Arthropoda,* pollen and dust mites are the common allergenic structures and organisms. The most allergenic fungi species belong to the genera *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Penicillium* and *Rhyzopus*. Fungal spores are known to cause allergic skin and respiratory symptoms that may appear continuously or with a seasonal rhythm. Moreover, some fungal species are producer of a wide range of *microbial volatile organic compounds* (MVOCs) and/or mycotoxins (see Par. 1.3.2 and Par. 1.3.3, respectively for a more detailed description).

The presence of moulds in indoor air can be connected with diverse health aspects:

- mould's proteins causing allergies (Gravesen *et al*., 1994);
- fungal structural elements, including β-1,3-glucans that may trigger inflammatory reactions related to symptoms, observed after exposure to endotoxin (Chew *et al*., 2001), and melanins that can activate a part of the immune system (the complement system) (Rosas *et al*., 2002);
- microbial volatile organic compounds (MVOCs) produced during growth (Pasanen *et al.,* 1998);
  mycotoxins and in addition other secondary metabolites released from fungal spores and colony fragments after inhalation, especially when their concentration in indoor air is higher than outside (building with moisture problems) (Nielsen, 2003).

Moulds growing on building materials can be divided into three groups, based on their water activity (a_w) requirements on laboratory substrates, in particular:

- primary colonizers, or storage moulds, capable to grow at $a_w < 0.8$: *Aspergillus fumigatus*, *A. niger, A. sydowii*, *A. ustus*, *A. versicolor,* several *Eurotium* species, *Paelomyces variotii, Penicillium brevicompactum, P. chrysogenum, P. commune*, *P. corylophilum*, *P. palitans* and *Wallemia sebi*;
- secondary colonizers, or phylloplane fungi, requiring a minimal a_w between 0.8 and 0.9: this group comprises species of *Alternaria*, *Cladosporium*, *Phoma* and *Ulocladium*;
- tertiary colonizers, or water-damage moulds, which need $a_w > 0.9$: they include many of the most toxic species such as *Chaetomium globosum*, *Memnoniella echinata*, *Stachybotrys chartarum*, *Trichoderma atroviride*, *T. citrinoviride*, *T. harzianum* and *T. longibrachiatum* (Nielsen, 2003).

1.3.1 The biological aerosol

Aerobiologia is the analysis of biological parts of airborne particles. This discipline includes all the airborne biological particles that are suspended in the air and that can be accumulated in dust and re-suspended in the atmosphere. Most of them are fungal and bacterial spores, *Bryophita*, *Pteridophyta*, lichen propagules, algal cells, pollen grains, protozoan cyst and viruses. The particle size distributions of typical airborne particles are summarized in Table 1.3.

Table 1.3 Airborne particles and their size (De Nuntiis *et al*., 2003).

Types	Diameters (µm)
Smoke	0.001 – 0.1
Condensation nuclei	0.1 – 20
Dust	0.1 – 100
Viruses	0.001 – 1
Bacteria	0.1 – 10
Fungal spores	2 – 50
Algae	1 – 1000
Lichen propagules	10 - 1000
Protozoa	2 – 200
Moss spores	5 – 20
Fern spores	20 – 60
Pollen grains	10 – 100
Vegetal and animal fragments, seeds, insects	> 100

Sampling and analysis of airborne particles can be quantitative and qualitative. Quantitative analysis consists of the collection of air samples followed by the determination of the viable component, while qualitative analysis is focused on the identification of the biological agents that have been collected. Passive and active sampling methods are performed in order to acquire the highest number of microorganisms. Passive sampling uses gravity to sample viable particles directly on Petri dishes, containing suitable media. In order to estimate the *Index of Microbial Air Contamination* (IMA), expressed as number of colonies appeared in a fixed area after a time-determined air sampling ($CFUdm^{-2} h^{-1}$). For this, 9-cm Petri

dishes have to be exposed to air particles for 1 h, 1 m above the floor and at a distance of about 1 m to obstacles (Pasquariello *et al.,* 2000). The aim of this kind of sampling is to find out which type of settled particles are present and how they vary in the atmosphere.

Active sampling approaches are used to measure the variability of the airborne microbial community in a specific air volume. There are different sampling techniques and the most applied are those that directly transfer the viable particles onto specific culture medium, already suitable for the development of microorganisms. The particles may be captured on a semi-solid medium (volumetric air sampler at different stages, *e.g.* Andersen cascade sampler, surface air system sampler) and subsequently placed in a thermostat at a suitable incubation temperature, or they can be collected through a filter (filter sampler) or in a liquid medium (liquid impinger sampler) and subsequently transferred onto culture medium. Regarding to many agar samplers, this last strategy offers the possibility of increased collection efficiency (Crook, 1995).

However, all these sampling techniques have an important biological limit. Studies investigating cell viability suggested that the majority of environmental microbiota is non-culturable even when viable (Amman *et al.,* 1995; Colwell, 2000; Després *et al.,* 2011; Wainwright *et al.,* 2004). Studies suggested that in indoor air the ratio estimation of total fungi with viable fungi is 100:1 (Toivola *et al.,* 2002) and only 17% of known fungal species can be cultivated *in vitro* (Bridge and Spooner, 2001). For bacteria, the fraction is less then 10% (Lighthart, 2000). In the following Table 1.4, all aerosol-sampling techniques are reassumed.

Biological investigations in indoor environments should also consider the sampling of surfaces, like objects, furniture, and floors. Different techniques can be used for this purpose, as summarised in Table 1.5. Both passive and active sampling devices can be chosen, depending of the investigation target. Generally, the combination of two or more methodologies is the best strategy for the detection of a wide range of microbial communities.

The sampling equipment must be selected for each kind of specific environment and for detecting the total microbial charge different culture media should be applied. Following basic factors have to be considered for a complete sampling planning:

- sites of sampler installation, with the number and position of samplers, both inside and outside of the studied environment;
- duration of sampling;
- specificity of the sampling, which depends of the aim of sampling (*e.g*. microbial targets, rapidity of results, checking microbial viability, target of size of particles);
- thermo-hydrometrical variations;
- counting and identification techniques of the isolated material (De Nuntiis *et al.*, 2003; Heinsohn, 2007).

Table 1.4 Types of aerosol sampler characterized in terms of collection efficiency (De Nuntiis *et al*., 2003 modified)

Type of sampler	Description	Sampling characteristics
Andersen Microbial Sampler	Static, cascade impactor. Flow rate 28.3 L min^{-1}. Collection on different stages	Show the particle size distributions based on the development of colonies. Management of high quantities of Petri dishes (6 for each sampling)
Surfaces Air System (SAS)	Static/portable one-stage impactor. Flow rate 180 L min^{-1}. Collection on agar plate	Based on the development of colonies, sampler easy to overload. Not efficiency for particles ≤ 5 µm
Sampl'air Lite™ (Biomérieux)	Static/portable one-stage impactor. Sampling volume adjustable from 0.05 to 10 m^3,. Collection on agar plate	Based on the development of colonies Management of less Petri dishes in comparison to Andersen sampler (1 for each sampling)
Casella Slit Sampler	Static one-stage impactor. Flow rate 30 or 700 L min^{-1}. Collection on revolving agar plate	Based on the development of colonies
Air Sampler RCS (Biotest Hycon)	Static/portable one-stage impactor. Flow rate 40 L min^{-1}. Collection on agar strips	Based on the development of colonies
Spore Trap (Lanzoni, Burkard)	Static one-stage impactor. Flow rate 10 L min^{-1}. Collection on slide or plastic tape	Based on counting of numbers of pollen and fungal spores by microscopically observation
Aerojet-General Cyclone	Static. Flow rate up to 1000 L min^{-1}. Collection in liquid	Various sampling methods are possible
Impingers	Static. Flow rate 12 L min^{-1}. Collection in liquid	Various sampling methods are possible
Glass Multistage Liquid Impingers	Static. Flow rate 10, 20 or 55 L min^{-1}. Collection on sintered glass disc sand in liquid, 3 stages	Shows distribution of particles sizes. Various sampling methods are possible

Table 1.5 Surface sampling methods for detection on indoor fungi (Samson *et al.,* 2010, modified).

Methods	Description	Characteristics
Mycometer® Surface	Enzymatical analytical tool that reveals the presence of spore, hyphae and fungal fragments on surfaces	Information about quantitative fungal load in short time No species identification
Cello tape preparation (scotch tape or transparent adhesive tape, Fungi-Tape™)	Direct fungal structure sampling from surfaces for microscopically observation	Low tech in short identification time. Collection of living and dead fungal structures. No identification of unknown fungal spores or structures
Material and dust sampling	Direct inoculation of small pieces of materials (as building materials) or dust samples onto an agar or liquid medium	Quantitative method with "long" response time (min. 5 days). Use of different media, with possibility of dilution. Collection of only living structures
Swab sampling (cotton swabs)	Direct sampling by dipping onto surfaces followed by inoculation on agar or liquid medium	Quantitative method with "long" response time (min. 5 days). .Use of different media, with possibility of dilution Collection of only living structures
Rodac® strips or contact plates	Direct sampling by dipping onto surfaces followed by inoculation on agar medium	Fast and easy method with "long" response time (min. 5 days) Collection of only living structures in fixed media
Non-volumetric air sampling	Passive sampling by Petri dishes exposed to the air for a given period of time onto different surfaces	Low tech, easy and cheap to perform, only for preliminary or qualitative information. Collection of only living structures with "long" response time (min. 5 days)
Volumetric air sampling	Active sampling performed closely to interested surfaces	Quantitative measure for living fungal structures with "long" response time (min. 5 days)

When aerobiological monitoring is carried out for health purposes, it is necessary to know the inhalable fraction and the use of personal sampler that reproduces the person's mouth. In agreement with the *American Conference of Governmental in industrial Hygienists* (ACGIH), the European Union has identified three airborne fractions with specific human health relevance:

- inhalable fraction, that consists of the settled particles in the extra-thoracic area and, if re-expelled through nose or mouth, can be swallowed and become a risk due to absorption in the gastro-intestinal tract;
- thoracic fraction, that consists of tracheobronchial settled particles, removed by mucociliary purification mechanisms and may swallowed;
- respirable fraction, that consists of particles settled in alveolar region and can be removed through it or by lymphatic system.

The fraction of airborne particles that is inhaled by a person depends on the particle properties, on the direction and speed of the wind around the individual, on the individual's respiratory rate and the duration of the exposure (ACGIH, 1989; UNI-EN 481, 1994).

1.3.2 The Microbial Volatile Organic Compounds (MVOCs)

Indoor air contains many volatile organic compounds (VOCs) and most of them originate from anthropogenic sources inside buildings, such as furniture, varnish or cleaning products. Furthermore, the exchange with outdoor air is a additional source of VOCs inside buildings and several of these compounds are also indoor pollutants. In addition, a small fraction of these compounds is considered to have microbial origin (MVOCs), which are low molecular weight chemicals originating from the metabolic actions of bacteria and fungi (Ryan, 2011).

MVOCs are carbon-based compounds that enter the gas phase by vaporizing at 0.01 kPa and a temperature of 20 °C. They include acids, alcohols, aldehydes, amines, aromatics, chlorinated hydrocarbons, esters, ethers, sulphur-containing compounds and terpenes (Korpi *et al.*, 1997). These compounds play an important role in fungal development, *e.g*. defence, protection against stress, communication and phatogenicity against other organisms, and some of them could be used as biomarkers for species identification (Roze *et al*., 2012).

The relationships between MVOC production with non-volatiles and mycotoxins could be explained by analysing the fungal metabolic pathway in Fig. 1.6:

- ethanol is produced under anaerobic conditions;
- alcohols derive from amino acid via the Erlich pathway;
- 1-octen-3-ol together with other 8/10 carbon compounds are synthesised by oxidation of linoleic acid;

- free fatty acids derive by the breakdown of lipids. Then they are oxidised to β-keto acids and decarboxylased to methyl ketones;
- lactones are produced from γ-keto acids;
- esters derive from enzyme catalysed reaction between acyl-CoA compounds and alcohols;
- condensation reaction between acetoin and ammonia produces pyrazines;
- methionine produces dimethylsulfide;
- terpenes derive from the mevalonic acid pathway (Magan and Evans, 2000).

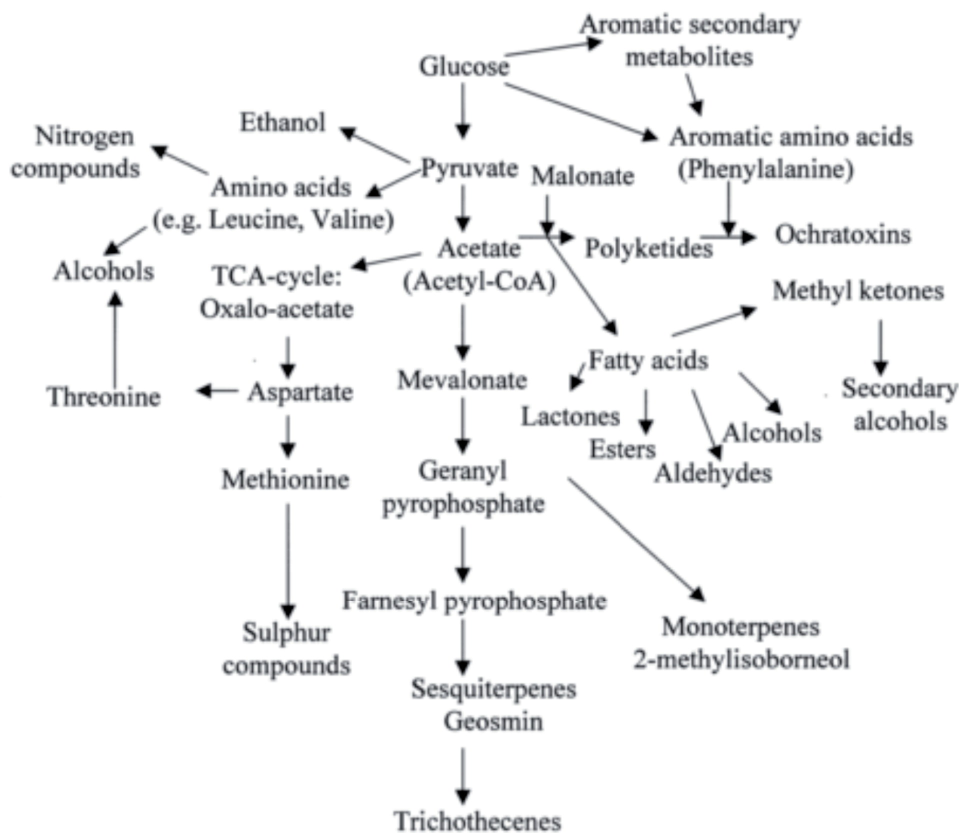


Figure 1.6 The pathways involved in different secondary metabolite productions (Magan and Evans, 2000).

The interest in MVOCs raised in the last three decades, because of the general conviction that MVOCs together with mycotoxins could be possible sources of indoor air quality (IAQ) problems caused by fungal growth. Researchers suggested that they can have adverse effects on the respiratory, blood vessel and nerve systems, and could be potentially carcinogenic (Fischer and Dott, 2003). Moreover, they can cause mental and cognitive distractions of the exposed subjects, which results in reduced performance, especially if the odour is perceived as unpleasant or unrecognizable, and all of them are symptoms linked with SBS (Elke *et al*., 1999; Flannigan *et al*., 1991; Polizzi *et al*., 2009; WHO, 2009).

Different aspects of MVOCs were studied, as their ability of diffusing from enclosed spaces (*e.g.* through vinyl wallpaper and vapour barriers or through HVAC filters), in order to use them as indicators of

latent mould growth (Ahearn *et al.,* 1997; Elke *et al.,* 1999). Other studies focused on the identification of unique MVOC profiles from pure fungal cultures with the aim of predicting species-specific mould presence (Fischer *et al.,* 2000b; Fiedler *et al*., 2001; Schuchardt and Kruse, 2009). However, it has to be mentioned that the use of MVOCs for mould detection has also disadvantages. Other studies have detected quite low emission rates of the area-specific emission of MVOCs per square meter and per hour, with the problem of their full detection (Pasanen *et al*., 1998), as well as the possibility of the presence of other sources for almost all MVOCs, as cigarette smoke, cooking, baking, frying, plant soil, compost bins and so on (Schlebinger *et al*., 2008).

Since the work of Wessén and Schoeps (1996), an early MVOC list is available in literature (Table 1.6.). The highlighted compounds are those that the American Industrial Hygiene Association (AIHA) (2005) considered as currently more frequently detected.

Table 1.6 Commonly accepted MVOCs (re-elaborated from AIHA, 2005; Ryan, 2011, modified; Wessén and Schoeps 1996). The marked compounds are the chemicals considered more current detected by AIHA (2005).

MVOCs	MW	Molecular formula	Boiling point °C
1-Butanol	74.12	$C_4H_{10}O$	117.2
2-Methyl-1-propanol	74.12	$C_4H_{10}O$	108
3-Methylfuran	82.1	C_5H_6O	65.5
3-Methyl-1-butanol	88.15	$C_5H_{12}O$	128.5
3-Methyl-2-butanol	88.15	$C_5H_{12}O$	112
2-Methyl-2-butanol	88.15	$C_5H_{12}O$	102
2-Pentanol	88.15	$C_5H_{12}O$	118-121
Dimethyl disulphide	94.19	$C_2H_6S_2$	109.7
2-Hexanone*	100.16	$C_6H_{12}O_2$	128
2-Heptanone	114.2	$C_7H_{14}O$	151.5
Ethyl isobutyrate	116.16	$C_6H_{12}O_2$	120
3-Octanone	128.22	$C_8H_{16}O$	167
1-Octen-3-ol	128.22	$C_8H_{16}O$	174
2-Octen-1-ol	128.22	$C_8H_{16}O$	87
4-Methyl-3-heptanone	128.22	$C_8H_{16}O$	163-167
3-Octanol	130.23	$C_8H_{18}O$	174-176
2-Pentylfuran	138.1	$C_9H_{14}O$	64-66
2-Nonanone	142.24	$C_9H_{18}O$	195.3
Karveol	152.2	$C_{10}H_{16}O$	226-227
Fenchone	152.24	$C_{10}H_{16}O$	193-194
Borneol	154.25	$C_{10}H_{18}O$	213
Endoborneol	154.26	$C_{10}H_{18}O$	212
Terpineol	154.3	$C_{10}H_{18}O$	210-218
1-10-Dimethyl-*trans*-9-decalol (geosmin)	182.31	$C_{12}H_{22}O$	270-271
Thujiopsene	204.2	$C_{15}H_{24}$	256-257

From the successive studies focused on pure fungal cultures, it was possible to determine a wide spectrum of MVOCs, with between 25 and 196 compounds, produced by microorganism's growth on enriched media or directly on building materials. It was demonstrated that the presence of other microbes, as well as plant extracts, other growth medium ingredients, water activity, pH, temperature, light and stress can help express usually silent secondary metabolite gene clusters (Frisvad, 2012). In this way, another MVOC list can be considered, specified for selected fungal species and culture medium (Table 1.7).

These results are useful for the single fungal species production *in vitro*, which are often strain-depending (Schlebinger *et al*., 2008). The report of Schuchardt and Kruse (2009) is one of a few studies that have considered the possible interaction of MVOCs from mixed fungal cultures, as reproduction of *in situ* situations.

A typical characteristic of MVOCs is their smell, easily detected by the nose. Since the beginning of studies concerning fungal contamination of food and feeds, the most commonly method was based on the human olfactory system (Schnürer *et al*., 1999). The most characteristic "mouldy" odour is produced by geosmin, an earthy smell often linked to sesquiterpenes (Kirivanta *et al.*, 1998). As reported by Ström *et al.* (1994) and Ryan (2011), other typical smelling MVOCs are:

- 2-methyl-iso-borneol (earthy odour);
- 1-octen-3-ol (mushroom-like odour);
- 2-octen-1-ol (musty odour);
- 3-octanol;
- 3-octanone.

The determination of MVOCs is usually done by gas chromatography-mass spectrometry (GC-MS) because of its powerful separation capability and highly sensitive detection performance (Matysik *et al.*, 2009). Different sampling methods have been commonly used for sampling MVOCs, as canister collection (EPA-TO15, 1999) or passive devices, as headspace solid-phase microextraction (HP-SPME) and Tenax desorption tubes (Tenax® TA), both with subsequent thermo-desorption. These passive techniques are economic and rapid tools able to determine very low concentrations of wide range of analytes (Fiedler *et al*., 2001; Matysik *et al.,* 2008; Schuchardt and Kruse, 2009), but their exposure is not typically accomplished volumetrically and results may be limited to a qualitative response (Ryan, 2011).

New passive devices have been developed recently, because of specific long-term requirements of the studies, as adsorbents based on activated charcoal (Matysik *et al*., 2009) and polydimethylsiloxane (PDMS) strips (Gibson *et al*., 2012). Other kinds of techniques are base on the MVOC detection by sensor-based devices, as polymer sensors responsive to variation in electrical conductivity due to VOCs adsorption (Joblin *et al*., 2010), or by electron noses (*e-noses*), based on rapid detection and identification of a pre-settled range of volatile compounds (Pinzari *et al*., 2004). In these last studies, the authors have analysed the implications of fungal MVOCs related to Cultural Heritage, especially paper, linen, rabbit skin glue, Lascaux clay and fibrous timber. Based on the evaluation of specie-specific fungal fingerprint, the results have demonstrated the device's efficacies to identify the presence of fungal growth, even in the early stages and their applicability to artefact's biodeterioration monitoring.

Table 1.7 MVOCs produced by selected fungal species on common culture media and building materials (Ryan 2011, modified).

Growing substrates	Species	MVOC produced after growing on controlled substrates																
		2-alkanones	C2-C5 alcohols	2-heptanol	2-methyl-1-propanol	2-methyl-1-butanol	3-methyl-1-butanol	2-ethyl-1-hexanol	3-methyl--furan	Dimethyl disulfide	1-butanol	Methyl alkanones	C8 complex	Limonene	Geosmin	2-penthyl--furan	Terpineol	α-pinene
MEA	*Acremonium strictum*	n	n		n	n	n		n			n	n	n				
	Alternaria alternata	n	n		n	n	n		n					n				
	Aspergillus versicolor	a, n	n	a	c	c, n	c, n		c	c			b, n	n				
	A. fumigatus	n	n		d, n	d, n	d, n		n			n	b, d, n					
	Aureobasidium pullulans	n			n	n	n		n			n		n				
	Chaetomium globosum	n	n		n	n	n		n			n	n	n				
	Cladosporium cladosporioides				c	c	c		c	c								
	C. herbarum	n			n				n			n	n					
	Fusarium solani	n	n			n	n		n					n				
	Paecilomyces variotiii				c	c	c		c	c								
	Phialophora fastigiata				c	c	c		c	c								
	Penicillium brevicompactus				d	d	d						b, d					
	P. chrysogenum	a, n	n	a		n	n					n	b, n					
	P. commune	a		a	c	c	c		c	c								
	P. expansum	n	n			n	n						n	n				
	Stachybotrys chartarum	n	n				n		n			n	n	n				
	Ulocladium atrum	n	n		n	n			n			n	n					
YES	*A. versicolor*	a		a	e, f	e, f	e, f						e, f	e, f				
	A. fumigatus				e, f	e, f	e, f							e, f				
	Paecilomyces variotiii				e, f	e, f	e, f							e, f				
	P. brevicompactus				e, f	e, f	e, f						e, f	e, f				
	P. chrysogenum	a		a														
	P. commune	a		a														
DG18	*Aspergillus versicolor*				c	c	c		c	c								
	A. fumigatus				d	d	d						d					
	Cladosporium cladosporioides				c	c	c		c	c								
	Paecilomyces variotiii				c	c	c		c	c								
	Phialophora fastigiata				c	c	c		c	c								
	Penicillium brevicompactus				d	d	d				d		d					
	P. commune				c	c	c		c	c								

Wall-paper	*A. strictum*	n	n						n					n				
	A. alternata	n	n						n					n				
	A. versicolor	n	g, n		g		g	n					g	n				n
	A. fumigatus	n	n					n	n									n
	A. pullulans	n	n					n	n					n				
	C. globosum	n	n					n	n					n				
	C. herbarum	n						n	n									
	F. solani	n	n					n	n					n				n
	P. chrysogenum	n	g, n		g		g	n					g					n
	P. expansum	n	n											n				n
	S. chartarum	n	n						n					n				
	U. atrum	n	n					n	n									
Card-board	*P. chrysogenum*		h										h					
	Stachybotrys chartarum		h		h													
Gypsum board, plasticizers	*A. versicolor*	i			h, i		i			i			i			i	i	
	A. flavus	i			i		i			i			i			i	i	
	A. fumigatus	i			i		i			i			i			i	i	
	A. niger	i			i		i			i			i			i	i	
	A. sydowii	i			i		i			i			i			i	i	
	P. chrysogenum				h							h	h					
	P. commune	l			l	l	l								l			
	S. chartarum	j	j		h	j					k		j					
	Streptomyces albidoflavus				m			m				m			m			

Key: a- Wilkins and Larsen (1995); b- Fiedler *et al*. (2001); c-Sunesson *et al.* (1995); d- Kiviranta *et al*. (1998); e- Fischer *et al.* (1999); f- Fischer *et al*. (2000a); g- Larsen and Frisvad (1994); h- Wilkins *et al.* (2000); i- Gao *et al.* (2002); j- Wady *et al*. (2003); k- Gao and Martin (2002); L- Sunesson *et al*. (1996); MEA: Malt Extract Agar; YES: Yeast Extract Sucrose agar; DG18: Dichloran 18% glycerolagar.

1.3.3 The secondary metabolite compounds

Based on several studies (Karbowska-Berent *et al*., 2011; Zielińska-Jankiewicz *et al*., 2008; Zyska, 1997), the fungal genera accounting to mycological contamination of library and archive collections include: *Alternaria, Aspergillus*, *Botrytis*, *Chaetomiun*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Stachybotrys*, *Trichoderma* spp. These genera include several species, which are already known for their allergenic and/or toxigenic properties (Nielsen, 2003).

Mycotoxins are low-molecular-weight secondary metabolites (< 1500 Da) typically produced by fungi that play a crucial role in their natural habitants against bacteria, other fungi, plants, animals and humans (Nielsen, 2003; Miller and McMullin, 2014). Mycotoxin production depends on the type of substrate available, temperature, humidity and the presence of certain trace elements or other microorganisms (Gutarowska *et al*., 2014). These metabolites are excreted into the substrate or can be present onto fungal cells and they can become airborne attached to dust or inside conidia or spores fragments (Fischer and Dott, 2003). If airborne fungal spores are inhaled, they will be lysed and the human body is thereby exposed to primary and secondary metabolites. Inhalation exposures had been suggested to cause acute kidney failure (ochratoxin), central nervous system damage (tremorgenic mycotoxins) and damage the upper respiratory tract (*Stachybotrys chartarum*) (Fischer and Dott, 2003; Miller, 1994). For these reasons, mycotoxins were recognised as a potential hazard, especially for the occupational and indoor hygiene (Fisher *et al*., 2000, 2003; Flanning and Miller, 2011).

There are several reports concerning the effects of mycotoxins to the human and animal health (Hayes, 1980; Robbins *et al*., 2000). Their presence in food and fee, *e.g.* the potential exposure through ingestions, are well and strictly regulated (European Commission, EC, 2006; Food and Drug Administration, FDA, 2004), but there is a lack in the regulation regarding the inhalation exposure (WHO, 2009). However, a few studies have examined this aspect, concluding that mycotoxins could be toxic also when exposure occurs by inhalation (Etzel *et al.*, 1998; Hodgson *et al*., 1998; Nikulin *et al*., 1996).

Exposure to airborne mycotoxins in agricultural workplaces was studied during the 1980s (Burg and Shotwell, 1984; Sorenson *et al*., 1987) and its relevance for occupational medicine was determined in the early 1990s (Hendry and Cole, 1993; Miller, 1994). In the beginning of 2000s, only trichothecenes of *Stachybotrys chartarum*, aflatoxins of *Aspergillus flavus* and metabolites of *A. fumigatus* were detected in airborne dust and bioaerosols (Fischer and Dott, 2003). Nowadays, more than hundreds of secondary metabolites with airborne bacterial and fungal origins were identified (Malachová *et al*., 2014; Nielsen and Smedsgaard, 2003). Occupational buildings with severe moisture damages are the most studied environments (Polizzi *et al.*, 2009; Slack *et al.,* 2009; Täubel *et al*., 2011), and only recently other environments, such as those that preserve Cultural Heritage, were taken into account (Gutarowska *et al*., 2013; Micheluz *et al*., 2015b). However, epidemiological studies are still missing (Fischer and Dott, 2003), as well as limits regarding the indoor biological agent exposure (WHO, 2009). Only few mycotoxins are well

described in toxicological terms and have threshold limit values: aflatoxins, ergot alkaloids, fumonisins, ochratoxin A, patulin, trichothecenes and zearalenone (Samson *et al.,* 2010). Links to health effects identified among occupants, together with mechanisms and toxicological data on inhalation exposure, need to be investigated to assess the risk related to indoor exposure and microbial toxins (Täubel *et al*., 2011). A list with the principal secondary metabolites produced by common indoor fungi is reported in Table 1.8.

Table 1.8 Overview of the most common metabolites produced by some common indoor fungi (from Abbott, 2002; Fisher and Dott, 2003; Miller and McCullin, 2014).

Fungal species	**Metabolites**
Acremonium	Citrinin
Alternaria tenuissima	Altenuene, altenusin, alternariol, alternariol monomethyl ether, altertoxin I, tentoxin, tenuazonic acid
Aspergillus	
A. amstelodami (anamorph: *Eurotium amstelodami*)	Auroglaucin, echinulin, epiheveadride, flavoglaucin, isotetrahydroauroglaucin, neoechinulin A and B
A. calidostus	Alkaloids, drimane sesquiterpenes, methyl isoquinoline, TMC-120 A-C
A. flavus	Aflatoxin B1, aspergillic acid, cyclopiazonic acid, kojic acid, 3-nitropropionic acid
A. fumigatus	Fumigaclavines, fumitoxins, fumitremorgens, gliotoxins, helvolic acid, tryptoquivalins, verruculogen
A. glaucus (anamorph: *E. herbariorum*)	Auroglucin, cladosporin, echinulin, epiheveadride, isotetrahydroauroglaucin, neoechinulin A and B
A. insuetus	Alkaloids, drimane sesquiterpenes, methyl isoquinoline, TMC-120 A-C
A. niger	Naphto-γ-pyrones, nigragillin, ochratoxin A, orlandin, tetracyclic compounds
A. ochraceus	Ochratoxin A, penicillic acid, xanthomegnin, viomellein, vioxanthin
A. ruber (anamorph: *R. rubrum*)	Auroglucin, cladosporin, echinulin, epiheveadride, isotetrahydroauroglaucin, neoechinulin A and B
A. ustus	Austalides, austamides, austdiols, austocystins, versicolorin C
A. versicolor	5-methoxy-sterigmatocystin, sterigmatocystin, versicolins, versicolorins
Bipolaris	Cytochalasin, sporidesmin, sterygmatocystin
Chaetomium globosum	Chaetoglobosin, chetomin, chaetochromin, chaetosin, cochliodinol, sterygmatocystin
Cladosporium	Cladosporic acid
Fusarium	Fumonisin, fusaric acid, fusarin, fusarochromanone, moniliformin, trichothecenes (deoxynivalino, T2 toxin), zearlenol, zearalenone
Memmoniella echinata	Components related to mycophenolic acid, dechlorogriseofulvins, epidechlorogriseofulvin, griseofulvin, spirocyclic drimanes, trichothecenes (trichodermin and trichodermol)
Myrothecium	Trichothecenes (roridin, verrucarin)
Paecilomyces	Patulin, viriditoxin
Penicillium	
P. brevicompactum	Asperphenamate, brevianamides, botryodiploidin, mycophenolic acid, Raistrick phenols, tanzawaid acid analogue
P. chrysogenum	Chrysogine, meleagrin, pyrovoylaminobenzanides, roquefortine C, secalonic acid D, xanthocillin X, ω-hydroxyemodine
P. expansum	Chaetoglobosins, citrinin, communesins, patulin, roquefortine C
P. polonicum	Anacine, cyclopenins,glycopeptides, 3-methoxy-viridicatin, penicillic acid, verrucofortine, verrucosidins, viridicatins
Phoma	Brefeldin, cytochalasin, secalonic acid, tenuazonic acid
Rhizopus	Rhizonin
Stachybotrys	Atranones, disabosquals, cyclosporin analogue, griseofulvin, spyrocyclic dromanes, stachybotryamide, stachybotrylactames, stachylysin, trichorithecenes (isosatratoxin, roridin L-2, several roridin E epimers, hydroxyroridin E, satratoxin H, G, F and iso-F, trichodermin, trichodermol, trichoverrol, verrucarin J and B)
Trichoderma	Gliotoxin, harzianum A, koninginin, trichodermin, trichodermol, viridin
Trichotecium	Roseotoxin, trichothecenes (trichothecin)
Wallamia sebi	Wallemidione, walleminol, walleminone
Zygosporium	Cytochalasin

The study of mycotoxins related to indoor human health problems due to building materials is considered as a difficult task because of the complexity of the matrix. In most of the cases, building materials are composed by various combinations of materials (*e.g*. paints, dust, wallpapers) that may interfere with the analytical method (Nielsen, 2003). Sample collection is also a crucial step for the analyses. Nielsen (2002) recommends surface sampling by ultra-clean swabs or vacuuming (*e.g*. onto 0.1 µm pore size Teflon filters), instead of the collection of pieces of building material. Lost of materials with biomass during handling of materials, collections of components, which are not necessary for the analysis (with the necessity of several clean-up steps) and the difficulty to sample only the mycotoxin-containing particle that can become airborne, are the limits of this kind of sampling (Nielsen, 2003).

During the last decade, the analysis of mycotoxins has been performed by high-performance liquid chromatography (HPLC or UHPLC) coupled with tandem mass spectrometry (MS/MS) because of the high selective, sensitive and accurate methodology. Other techniques are also commonly used in order to cover a wider range of fungal metabolites, as those based on thin layer chromatography (TLC), HPLC coupled with ultraviolet (UV) or fluorescence detectors in combination with time consuming purification step or immunochemical methods (ELISA) (Krska *et al*., 2008; Nielsen and Smedsgaard, 2003). Today, there is a trend towards the use of multi-mycotoxin methods for the simultaneous mycotoxin analysis (Krska *et al*., 2008). The liquid chromatography-tandem mass-spectrometry (LC-MS/MS) based multi-mycotoxin method developed by Sulyok *et al*. (2007) with the successive updates of Vishwanath *et al*. (2009) and Malachová *et al.* (2014) is one of the most used methods for the simultaneous detection of hundreds of microbial metabolites. This multi-method does not need sophisticated clean-up and/or multiple analytical techniques and it is generally applied for the analysis of food and feed matrix (Blandino *et al*., 2015; Chala *et al*., 2014; Ezekiel *et al*., 2012; Streit *et al.,* 2013), especially because of the necessity to cover the toxins addressed by Commission Regulation 1881/2006 (aflatoxin B1, B2, G1, G2 and M1, ochratoxin A, patulin, deoxynivalenol, zearalenone, fumonisin B1 and B2, HT-2 and T-2 toxin) with a single method (Krska *et al*., 2028). However, this method can be applied also to other kind of matrix, as dust samples (Täubel *et al*., 2011; Vishwanath *et al.,* 2011) and pure fungal cultures (Gutarowska *et al*., 2014; Micheluz *et al*., 2015b), and, as a consequence, used also for human health studies.

2. RESEARCH OBJECTIVES

The overall aim of this thesis is to increase our knowledge about the biodeterioration phenomena of Cultural Heritage, especially of book collections, focusing on the related indoor contaminations and potential health risks of workers and visitors. For this aim, a contaminated repository of the Library of Humanities (Biblioteca di Area UManistica, BAUM) of Ca' Foscari University of Venice (Italy) was selected as case study, because of the wide spreading fungal infection, which interested more than 27,000 books belonging to the university's historical collection. This thesis is focused on four major aspects: 1) microbiological investigations of indoor contaminated environment, 2) characterization of the fungal species *Eurotium halophilicum*, 3) MVOC production of the major fungi as potential fingerprints, and 4) the investigation of the ability of these major fungi to produce mycotoxins.

1) Microbiological investigations included the characterization of the indoor air and contaminated books stored inside the studied repository. The analyses were performed three times every six months over a period from March 2013 to May 2014. This period is from particular interest because it covers also the disinfection of the repository in April 2013. The aims of these analyses were to determine the microbiological level before and after the disinfection procedure, and therefore the evaluation of indoor microbiological quality of the studied environment.
2) In a preliminary step, *Eurotium halophilicum*. C.M. Chr., Papav. & C.R. Benj. was identified as the main responsible fungal species for the contamination inside the repository. This fungus is from particular interest because little is known about it. As a consequence, a detailed investigation of this xerophilic fungal species was included in this thesis. The microbiological study included microscope analyses with optical microscope and scanning electron microscope (SEM-EDX) investigations. Moreover, growth condition trials were performed to determine its favourite growth requirements. This knowledge may help the librarians to protect the repository from future contamination by this specific fungus.
3) MVOC analyses of the main fungal species isolated from the contaminated repository were performed by GC-MS, in order to identify specie-specific fingerprints using as possible fungal markers in indoor contaminated environments. This investigation was then applied to contaminated books and sampled indoor air.
4) Secondary metabolite investigations of the principal isolated fungal species and settled dust samples from the contaminated repository were performed by LC-MS/MS. The aim of this analysis was to determine the potential fungal ability and the mycotoxin presences inside collected dust sample for an evaluation of possible health human risks.

3. EXPERIMENTAL SECTION

The thesis was based on the evaluation of risk assessment concerning a library's repository affected by mould contamination. Two different aspects were considered: the potential problem for books' preservation and for human health of workers and student. The general overview of all investigations performed is shown in the followed flow chart (Fig. 3.1).

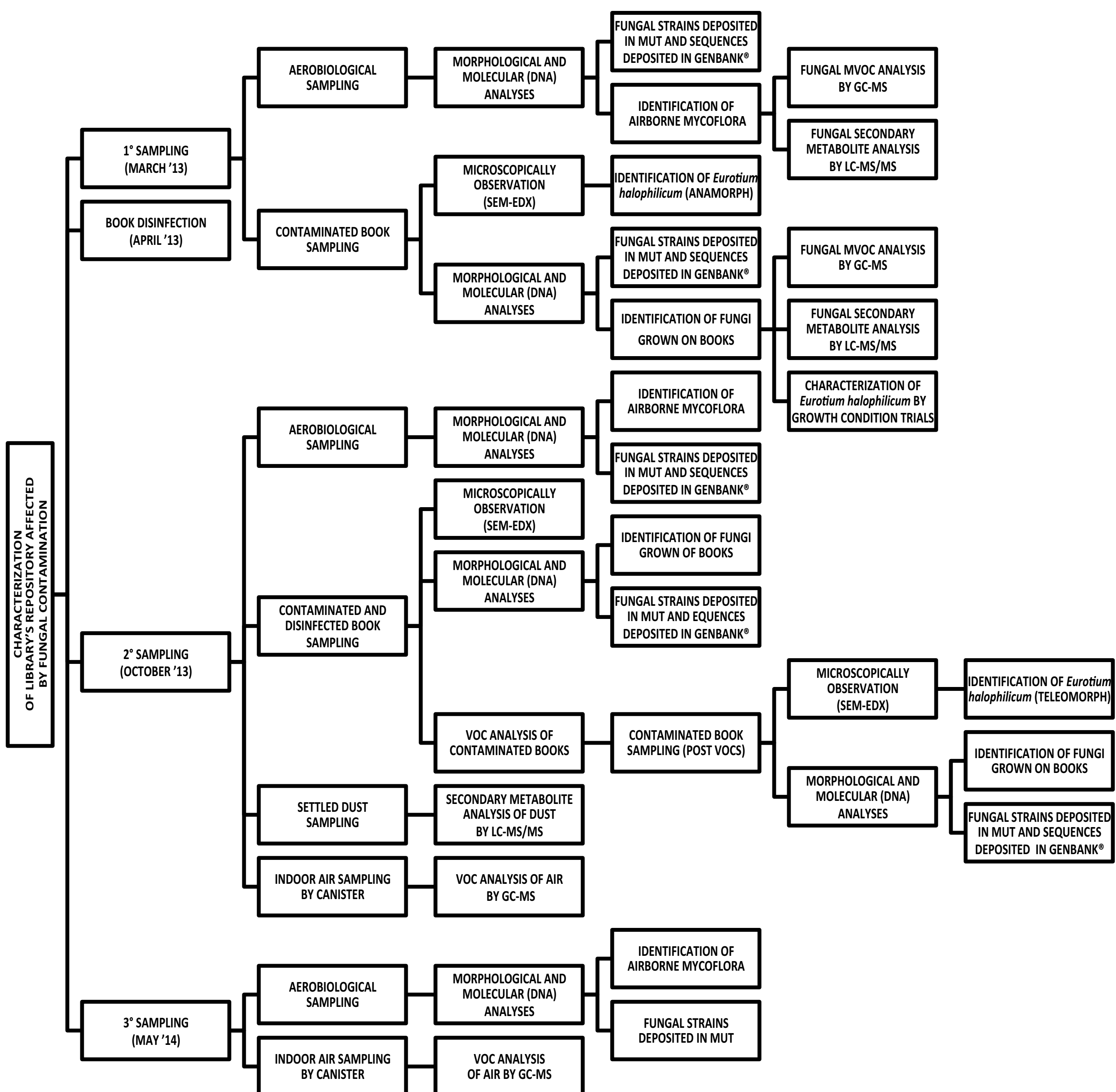


Figure 3.1 Flowchart of the all developments about the thesis project.

3.1 Microbiological investigations

This thesis is focused on the microbiological characterization on a specific indoor environment of a University's library in Venice (Italy). The implications resulting from the fungal contamination found inside the building were particularly interesting for the book preservation, students and workers that frequent that environment and highlighting the potential *status* of *Sick Building Syndrome* (SBS).

3.1.1 Study site: the Library of Humanities, Ca' Foscari University of Venice

From the fusion of the Department Libraries of Art, Archaeology, Classical Studies, Philosophy, Italian Study and History, in 2006 the Library of Humanities (*Biblioteca di Area UManistica*, BAUM) of Ca' Foscari University of Venice was born, located at *Malcanton Marcorà* Palace, in the center of Venice (Italy) (Fig. 3.2). Belonging to the Department of Humanities (Ca' Foscari University), the complex covers a total surface of 2500 m^2 on four floors, where about of 300,000 books, 3,651 journals and 600 electronic journals are available on the University network. The library collections cover the following disciplinary areas: Art, History of Art, Italian Studies, Philology, Philosophy and Social Science. The daily utilization is about 530 people (up to 700 users at peak periods) with circa 20 workers (*e.g.* librarians, employees and interns).

The library has two reading rooms and two underground floors. Most of the stored books and all the journals are open-shelf. The second underground floor has a separated repository, where only the librarians and the workers have access, because of the high value of the ancient books. Several *Cinquecentine*, manuscripts and old books from the XVI-XIX centuries belonging to the historical collection of Ca' Foscari University are stored there in about fifty Compactus® shelves.

In the beginning of 2013, a widespread fungal contamination was discovered inside the separated repository of the BAUM. More than 27,000 ancient books belonging to the historical collection were found with white spotted fungal growth.

After an initial inspection, the origin of the fungal colonization was identified in the right corner far from the entrance (correspondent to area 1 in Fig. 3.3), where the shelves had been kept closed for several months, because of damaged sliding tracks (personal communication of library director), so avoiding a minimum air re-circling or fluctuation. In several months, the fungal growth had spread around the repository, contaminating eight neighbouring Compactus® shelves and some isolated volumes stored in five shelves in the second row (correspondent to area 3 in Fig. 3.3).

The contamination level differed between the Compactus® units, with a high concentration in area 1, but also between different shelves of the same cabinet, resulting prevalent for books stored at lower areas than those positioned on the top.

Figure 3.2 *Malcanton Marcorà* Palace, Library of Humanities, Ca' Foscari University of Venice (Italy).

The fungal contamination was spread only on the book' covers, especially those with leather and textile bindings, while bound volumes and bindings made from cardboard and paperbound volumes were found to be free of moulds, as also reported by other Italian case studies (Pinzari and Montanari, 2011; Montanari *et al*., 2012). The colonies were mainly in white colour, with soft woolly consistence and in different diameters, from few mm^2 up to 2 cm^2, depending on the age of mycelia and the space of their growths (Fig. 3.2).

The climate conditions inside the repository were maintained at around 23 °C air temperature and 56% relative humidity. The air circulation system sucks the air from outdoor and, after previous filtration, dehumidification and thermal adaptation, pumps it through 12 suction ports positioned on the repository's ceiling (Fig. 3.3 – B). However, these ports are exactly in correspondence of the upper parts of the shelving blocks. If they are closed, a sufficient air-circulation is not guaranteed. This increases the risk of a creation of particular ecological niches, potentially dangerous for books' preservation.

The climate parameters, continuously monitored by two dataloggers fixed on the repository's walls, were recognised as suitable for these kinds of conservation environments, as recommended by Italian Ministry for Cultural Heritages (19-24 °C and 50-60%, MIBAC, 2001). However, a lack of ventilation inside the metal shelves and the condensation of water on the books during winter, due to lowered temperatures, had probably promoted the germination of fungal spores and allowed contamination to spread (Micheluz *et al*., 2015a; Montanari *et al*., 2012).

Figure 3.3 A: Compactus® shelves with contaminated books belonging to the historical collection of Ca' Foscari University of Venice (Italy); B: air suction port positioned over the shelves; C: spread fungal contamination on the books: D: single contaminated book between no infected volumes.

3.1.2 Sampling

Microbiological sampling was performed three times (March 2013 – October 2013 – May 2014) in the repository, in order to study and monitor the microbial indoor contamination before and after the disinfection made by a private company [1] during the April of 2013. Each sampling had different characteristics, because of different aims. The sampling protocols are described in Appendix A1 – A.

<u>1st sampling (March 2013)</u>

After a first inspection, during which the origin of the fungal infection was identified in the most distance corner from the entrance (marked as area 1, Fig. 3.1), the 1st sampling was performed in the end of March 2013, one week before the disinfection. A sampling format was prepared and filled with all repository

[1] *TiGiemme Company* performed the cleaning and the disinfection of the contaminated books with the financial funding of Ca' Foscari University of Venice. New DES 50 at 0.5% in ethanol was applied by spray on the book's covers.

characteristics (see Appendix A1 - B). The major aim of the investigation was to isolate the fungal community from the environment and contaminated books, composed mainly by xerophilic and halophilic fungi (Montanari *et al*., 2012; Pinzari and Montanari, 2011). For this reason, only selective low water activity media were used (Christensen *et al.*, 1959; Samson and Lustgraaf, 1978).

2nd sampling (October 2013)

The 2nd sampling was performed with the aim to monitor the repository six month after the disinfection. In this case, a complete aerobiological investigation was applied, testing several culture media and analysing 10 cleaned books and 10 contaminated books[2]. A sampling format was prepared and filled for each sampled book (Appendix A1 - C). In addition, indoor air was sampled by canisters for MVOC analysis (in correspondence to area 1-2-3-4-5-6) and settled dust samples (in correspondence to area 1-2-3-4) were collected, as reported in Par. 3.3.3 and 3.4.2, respectively.

3rd sampling (May 2014)

The third sampling completed the repository's monitoring, after circa 1 year from the beginning of the study. In this case, an aerobiological investigation as for the 2nd sampling was performed (without books' sampling).

3.1.2.1 Climatic monitoring

The environmental parameters, temperature (°C) and relative humidity (RH%), were measured by a hygro-thermometer HD2101.1 (Delta OHM, Padua, Italy) positioned in the middle of the repository at 1 m above the floor. Climatic measurements were recorded every 5 min during all the samplings. The recorded data were compared with two digital data logger 176 H1 (Testo, Milan, Italy) presented inside the repository.

3.1.2.2 Microbial air sampling

The air inside and outside the repository was analysed in five (1st sampling) and six (2nd and 3rd sampling) sampling areas, according to a centrifugal scheme related to the primary colonization area and in respect to the single point of entrance/exit (*i.e.* the point of air exchange; Fig. 3.4). Three replicates were made for each location. Active sampling was performed with a *Sampl'air Lite* sampler (Biomérieux, Florence, Italy) with 9-cm Petri dishes, flow rate 100 L min^{-1} and sample volume of 100 L. The sampler was placed 1.5 m above the floor to represent the breathing zone of a standing person. The total numbers of

[2] The disinfection was not applied to all contaminated books, because of an agreement between Ca' Foscari University and *TiGiemme* company for the cleaning of a fixed amount of contaminated books. Unfortunately, the operation was not done immediately and the fungal contamination spread in the separated repository (pers.comm. from the responsible of the Historical collection, Dr. Antonella Sattin).

colony-forming units (CFU) were calculated according to the conversion table provided by the supplier and the results are presented as CFU for cubic meter of air ($CFUm^{-3}$) (Peto and Powell, 1970).

Figure 3.4 Map of the sampling areas inside the separated repository of Library of Humanities (BAUM), Ca' Foscari University of Venice, (Italy). Areas 1, 2, 3 and 4 are inside the repository, area 5 is in indoor position outside the repository and area 6 is outside of the Library (garden).

For passive sampling, 9-cm Petri dishes were exposed to airborne particles for 1 h, 1 m above the floor and at a distance of about 1 m to any obstacles, in accordance to the *Index of Microbial Air Contamination* (IMA), expressed as CFU $dm^{-2} h^{-1}$ (Pasquarella *et al*., 2000).

For all samplings, different culture media were selected to capture a wide range of microbial airborne particles, considering also different incubation temperatures, as reported in Table 3.2 (Pitt and Hocking, 1997; Samson *et al*., 2004). Detailed compositions of all culture media are reported in the Appendix A2. After sampling, the dishes were closed, labelled, and transferred to the microbiological laboratory at the Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, and incubated for 7-14 days.

The species diversity inside the repository was calculated using the Simpson Diversity Index, according to the formula: $1 - D = 1 - \Sigma (n/N)^2$, where n is the number of colonies per morphological fungal species and N is the total frequency of the corresponding colony in the population (Magurran, 1998; Nunes *et al*., 2013).

3.1.2.3 Book sampling

Several books from the historical collection of Ca' Foscari University of Venice were investigated by microbiological analysis, before and after the disinfection. In particular, 5 contaminated books were analysed during the first sampling (March 2013), while 20 books, 10 cleaned and 10 still contaminated, were studied during the second monitoring phase (October 2013). The list of the books is reported in the Table 3.1. Sampling was performed using the following techniques:

1. sterile cotton swabs (Cultiplast, LP Italiana Spa, Milan, Italy) were wiped over the spots which were on the spine of bindings and then inoculated in 9-cm Petri dishes;
2. sterile nitrocellulose membranes (Advantec MFS, Inc., Dublin, CA, USA; 0.45 m pore size, 47 mm diameter) were gently pressed on the visible spots on the covers for 10 s and then transferred to Petri dishes;
3. pieces (6 x 2 cm) of Fungi-Tape^{TM} (Scientific Device Laboratory, Des Plaines, IL, USA; 1 mm thick, no. 745) were pressed over the spots to collect fungal structures and then deposited on sterile glass slide for microscopic observation;
4. active sampling with Sampl'air Lite (Biomérieux, Florence, Italy) with an air sample volume of 100 L (flow rate 100 L min^{-1}) was performed directly from the book covers to collect fungal structures directly into Petri dishes.

For all samplings, different culture media were selected to capture of a wide range of microbial particles (see Appendix A2). After sampling, the dishes were closed, labelled, and transferred to the microbiological laboratory at the Department of Molecular Sciences and Nanosystems and incubated for 7-30 days.

Table 3.1 Descriptions of investigated books of Ca' Foscari historical collection preserved at BAUM.

Sample number	Book	Position
1st sampling		
1	De Petris "Discorsi Parlamentari", n° 65, Biblioteca Unive Raccolta Ferrara	Compactus n° 3, third shelf from the top, second block
2	"Opuscoli", dono De Prosperi 95125	Compactus n° 3, sixth shelf from the top, first block
3	Journals des Economites Raccolt, BiblioR Scuola Superiore di Commercio VE Periodici C-390	Compactus n° 5, second block
4	Manier "L'aveniz Economic", II volume	Compactus n° 8, fourth shelf from the top, first block
5	Enciclopedia Popolare Italiana, Atlante, Bibl. Ist. Uni. Di Econ. E Com. Lingue e let. Stran. Ca' Foscari, dono G. Luzzati	Compactus n° 8, eighth shelf from the top, second block
2nd sampling		
L1	Biblioteca Difendiamo la Vita 102/1299, Scuola di Servizi Sociali (VE)	Isolation shelf*
L2	Biblioteca Difendiamo la Vita 102/1872, 1964, Scuola di Servizi Sociali (VE)	Isolation shelf
L3	Unità Sanitaria, anno XX luglio-dicembre	Compactus n° 48, fifth shelf from the bottom, second block
L4	Biblioteca 298/2554, Spreco nella Sicilia Occidentale, Scuola di Servizi Sociali (VE)	Isolation shelf
L5	G. D'Annunzio "Il martirio di S. Sebastiano", Università di Studi Venezia, CAM sec. XIX 1388 (1911)	Compactus n° 45B, second shelf from the bottom, first block
L6	F.D. Guerrazzi "Lettere a cura di G. Carducci", Università di Studi Venezia, CAM sec. XIX 876 (1880)	Compactus n° 45, fifth shelf from the bottom, first block
L7	Biblioteca Enciclopedica Italiana, vol. 33 (1834)	Compactus n° 45, second shelf from the bottom, third block
L8	De Sanctis "Opere XV, Il mezzogiorno e lo Stato Unitario", Università di Studi Venezia, CAM sec. XIX 95	Compactus n° 45A, third shelf from the bottom, third block
L9	G. Cumberlage "The Works of Shakespeare", Università di Studi Venezia, CAM inglese sec. XVI 29 (1954)	Compactus n° 43B, second shelf from the bottom, second block
L10	A. Dumas "Le deux Diane", Università di Studi Venezia, C FR XIX D22	Compactus n° 42B, fourth shelf from the bottom, third block
L11	Opuscoli, Fondo Storico di Ateneo, dono De Prosperi	Compactus n° 3, first shelf from the bottom
L12	Revue Brittanique, Fondo Storico di Ateneo, raccolta di Francesco Ferrara (1877)	Compactus n° 5, third shelf from the bottom, first block
L13	R. IS. Ven. Sc. Lett. Arti, Atti 9, Fondo Storico di Ateneo (1863)	Compactus n° 5, fifth shelf from the bottom, third block
L14	T. Gautier, V. Hugo "Raccolta Antonio Fradeletto 324/2830, Fondo Storico di Ateneo (1902)	Compactus n° 7, third shelf from the bottom, third block
L15	A. Montanari "Economia Politica", Fondo Storico di Ateneo (1881)	Compactus n° 8, fourth shelf from the bottom, first block
L16	H. De Graffigny "Les Moteurs Légers", Fondo Storico di Ateneo (1899)	Compactus n° 9, fifth shelf from the bottom, first block
L17	Carrau "Theorie de l'Evolution", Fondo Storico di Ateneo (1967)	Compactus n° 10, fourth shelf from the bottom, fourth block
L18	Nannucci "Manuale della letteratura vol. II", Fondo Storico di Ateneo (1858)	Compactus n° 13, fifth shelf from the bottom, fourth block
L19	Massa "Aritmetica", Fondo Storico di Ateneo (1896)	Compactus n° 15, fifth shelf from the bottom, first block
L20	Università di Catania, annali XX, Fondo Storico di Ateneo (1974)	Compactus n° 16, first shelf from the bottom, first block

*The isolation shelf, an open metallic shelf, was established by the library workers in an isolated part of the repository.

Only during the 2nd sampling (October 2013), the 20 books were analysed also for their water content by *Aqua-Boy* (ENERCORP Instruments Ltd, Toronto, Canada). The data are water content meters, based on the measurement of the electrical conductivity of materials. All changes in resistance within the relevant measuring ranges are sufficiently pronounced to ensure a high degree of accuracy for the

readings. The electrical (reading value) accuracy of the Aqua-Boy is +/- 0.1%, while reproducibility is +/- 0.2% in relation to the absolute readings on the meter (Pinzari and Montanari, 2011).

A general overview of all samplings performed during the three seasonal investigations, interested both the indoor air and the book collection, is reported in the followed Table 3.2. For further details about the protocols and culture media, see Appendixes A1 and A2.

Table 3.2 Overview of seasonal sampling performed at Library of Humanities (BAUM). The used culture media were: Malt Extract Agar with 15% of NaCl (MEA15%); Malt Extract Agar (MEA), Dichloran-glycerol Agar (DG18); Plate Count Agar (PCA).

Sampling	Air sampling		Book sampling	Settled dust sampling	MVOC analysis
	Active	Passive			
March 2013	5 sampled areas (1-5), volume 100 L. Medium: - MEA15% (*)	5 sampled areas (1-5), gravitational deposition Medium: - MEA15% (*)	5 books sampled by: - sterile cotton swabs - sterile membranes - Fungi-Tape^{TM} - active sampling Medium: - MEA15% (*)	Not performed	Not performed
October 2013	6 sampled areas (1-6), volume 100 L. Media: - MEA (**) - MEA15% (*) - DG18 (*) - PCA (**)	Not performed	20 books (10 disinfected and 10 contaminated books) sampled by: - sterile cotton swabs - Fungi-Tape^{TM} - Aqua-boy (water content) Media: - MEA (*) - MEA15% (*) - DG18 (*)	4 areas (1-4), (1.5 – 4.3 m^2) by household vacuum cleaner	6 areas (1-6) sampled by 6 canisters (3 – 6 L of volume)
May 2014	6 sampled areas (1-6), volume 100 L. Media: - MEA (**) - MEA15% (*) - DG18 (*) - PCA (**)	Not performed	Not performed	Not performed	6 areas (1-6) sampled by canisters (3 – 6 L of volume)

(*) The incubation was performed at 25 °C.
(**) The incubation was performed both at 25 °C and at 37 °C.

3.1.3 Microbiological analysis

3.1.3.1 Medium selection and cultivation

Aerobiological and book sampling have permitted to isolate mixed fungal or bacteria colonies on Petri dishes. In order to obtain separated colonies of each sampled fungal species, isolation was performed by taking a small quantity of mycelia by sterilized loop and transferring onto new 6-cm Petri dishes. Generally, MEA medium was used as generic culture media and MEA15% was used for the most xerophilic species. All the isolates were then labelled and incubated at 25 °C for 7-30 days, depending of the different fungal growth.

3.1.3.2 Morphological and molecular identification

Isolated fungi were identified with a polyphasic approach, which includes morpho-physiological features and molecular studies. The first step of fungal identification is to observe all the macroscopic and microscopic features. In general, the colony is studied after 7 days of incubation and several data, as diameter size, colour, shape, presence of exudates, are taken. A fresh glass slide with a small part of mycelia is analysed under optical microscope (Axio plan, Zeiss, Germany) at different magnitude (10x, 20, 40x) and microscopically characteristics of the colony, as *e.g*. dimension and shape of spores, presence of conidiophores, hyphae with or without septa are recorded in according with specific manuals (Raper and Fennel, 1965; Pitt, 1979; Pitt and Hocking, 1009; Klich, 2002; Samson *et al*., 2004). For specific genera, as *Penicillium* spp., isolation on special media was performed as recommended in previous studies of selected genus monograph for species identification (Samson and Frisvad, 2004) (Appendix A2).

Molecular identification was performed by extraction, amplification and sequencing of internal transcribed spacers (ITS), β-tubulin and actin genes (White *et al*., 1990; Gardes and Brunes, 1993; Carbone and Kohn, 1999; Bensch *et al*., 2012). For the protocols see Appendix A3. The resulting sequences were cleaned by Sequencer® 5.1 software (Gene Codes Corporations, MI, US) and then compared with reference sequences in online databases provided by the CBS-KNAW *Fungal Biodiversity Centre* (The Netherlands) and the NCBI, *National Center for Biotechnology Information* (US).

The majority of isolated fungi are preserved at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and System Biology, University of Turin (Italy). The MUT and GenBank® accession numbers are reported in Appendix B1-B.

3.2 Characterization of *Eurotium halophilicum*

3.2.1 Microscopically investigations

The strips of adhesive (Fungi-Tape™) used for sampling the book bindings were analysed under an optical microscope (Axio plan, Zeiss, Germany) at 200x and 400x magnification. Micrographs were acquired with a digital camera AxioCam ERc5s (Zeiss, Germany) connected to a PC with ZEN 2011 software (Zeiss, Germany). Fragments of tapes were also analysed under EVO 50 (Zeiss, Germany) scanning electron microscope (SEM) at *Istituto Centrale per il Restauro e la Conservazione del Patrimonio Archivistico e Librario* (ICRCPAL), Rome (Italy). The protocol of sample preparation is reported at Appendix A4. Statistical analysis of the elemental composition obtained by Energy Dispersive X-ray analysis (EDX) of SEM was performed by XLSTAT vers. 2015.1.03.15448.

3.2.2 Growth condition trials

The fungal species *E. halophilicum* is a xerophilic fungus characterised by slow growth and with particular culture requirements. As suggested by Christensen *et al*. (1959) and Samson and Lustgraaf (1978), low water activity media must be used for its isolation and cultivation *in vitro*. For this reason, a specific study was performed in order to determine the best growth condition of this fungus, selecting different culture media and incubation temperatures.

The first experiment was executed inoculating three replicates of 20 different *E. halophilicum* strains belonging to MUT collection (University of Turin, Italy), onto the following culture media:

- Czapeck Agar added with 20% of sucrose (Cz20), estimated a_w = 0.97 (Samson and Lustgraaf, 1978);
- Czapeck Agar added with 40% of sucrose (Cz40), estimated a_w = 0.93 (Samson and Lustgraaf, 1978);
- Czapeck Agar added with 70% of sucrose (Cz70), estimated a_w < 0.82 (Samson and Lustgraaf, 1978);
- Dichloran –glycerol agar base (DG18), estimated a_w = 0.95 (Hocking and Pitt, 1989);
- Malt Extract Agar added with 15% of NaCl (MEA15%), estimated a_w = 0.88 (Gunde-Cimmerman *et al.*, 2003).

The second experiment tested the 20 different strains for 4 weeks at different incubation temperature:

- 17 °C and 22 °C as optimal range for the book preservation inside libraries and archives;
- 25 °C because of the optimal temperature for fungal growth;
- 28 °C as temperature limit that could be inside preservation environment during summer season in temperate areas.

The protocols with the list of *E. halophilicum* species are reported in Appendix A5. The results of this study of the best culture medium and optimal incubation temperature are necessary to improve the knowledge of growing conditions of this fungus and were applied to the following chemical investigations.

3.3 MVOC analysis (GC-MS)

MVOC analysis was carried out from October 2013 to October 2014 at the instrumental laboratory of *Air section* of *Agenzia Regionale per la Prevenzione e protezione Ambientale del Veneto* (ARPAV), Padua (Italy). The analysis was performed by GC-MS (Perkin Elmer Autosystem XL and Perkin Elmer Mass-Gold), following the EPA TO15 (1999) method for the determination of volatile organic compounds (VOCs) in air collected in specially-prepared canisters and analysed by GC-MS. For the instrumental parameters, see the Appendix A7.

3.3.1 Biological Oxygen Demand (BOD) analysis

In parallel to MVOC analysis from pure fungal cultures, sample bottles closed with pressure sensor OxiTop®-C system (Weilheim, Germany) were filled with suitable agar medium. Each fungal strain was inoculated inside these bottles (Table 3.4-B) and incubated at 25 °C for 25-30 days (Fig. 3.4). The final result reported an alternative Biological Oxygen Demand (BOD) response about the consumption of internal O_2 during the fungal growth. This variation is principally related to the fungal growth velocity. For further details, see Appendix A6.

3.3.2 MVOC analysis of fungal cultures

The investigation was focused on seven different fungal species isolated from the contaminated repository of BAUM (Table 3.3). The fungal strains were chosen from the most frequent fungal species isolated during the first sampling from the air and the book covers. Only *C. cladoporioides* strains (MUT 5536) were during the second sampling. The selection has taken into consideration fungi with already known MVOC production, as *C. cladosporioides*, *E. chevalieri*, *P. brevicompactum* and *P. chrysogenum*, and fungi with unknown MVOC production, *i.e. A. creber*, *A. penicillioides* and *E. halophilicum*.

A specific culture system was design for the MVOC collection: a 1 L sampling bottle closed with a cap was directly connected to the pre-concentrator system (Entech. Instrument, Inc.) as shown in Fig. 3.5. The bottles were filled with 100 mL of culture medium, inoculated with an agar disk with mycelium (1 cm in diameter) and incubated at 25 °C. The investigations were done in three replicates from 25 to 61 days, depending on fungal species. The GC-MS analyses were performed ones per week.

Fungal MVOC correlations between the selected fungal species were analysed by Ward's hierarchical agglomerative clustering method with R (version 3.2.2) (Murtagh *et al.*, 2014). The similarities were calculated basing on the compound's presence/absence for each fungal species at different time of incubation.

Table 3.3 Fungal species analysed by GC-MS for their MVOC production.

Fungal species	**MUT number**	**GenBank number**	**Origin**
Aspergillus creber Jurievic, S.W. Peterson & B.W. Horn	MUT 470	KU179486	Indoor air (1st sampling)
A. penicillioides Spegazzini	MUT 481	KU179489	Book cover (1st sampling)
Cladosporium cladosporioides (Fresen) G.A. de Vries	MUT 5536	-	Indoor air (2nd sampling)
Eurotium chevalieri L. Mangin	MUT 472	-	Book cover (1st sampling)
E. halophilicum C,M, Chr., Papav. & C.R. Benj.	MUT 482	KM502179	Book cover (1st sampling)
Penicillium brevicompactum Dierckx	MUT 536	KM502183	Indoor air (1st sampling)
P. chrysogenum Thom	MUT 5493	KM502200	Indoor air (1st sampling)

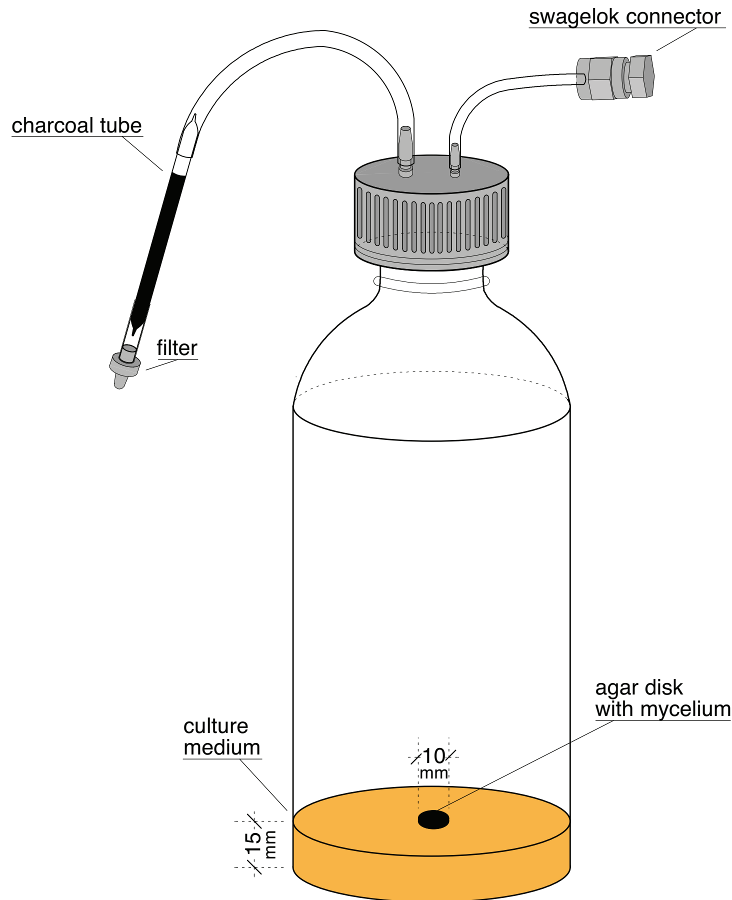


Figure 3.5 Sample bottle for MVOC analysis of fungal culture (picture created with Vectorworks vers. 2008 by E. Torresan, to whom we give our thanks).

3.3.3 MVOC analysis from contaminated books

Two contaminated books were investigated for their MVOC emissions. Specific sampling chambers were installed, composed by two empty desiccators (10 L of internal volume), furnished on the top with a ground glass joint (Fig. 3.5 - C). The connection with the pre-concentrator system is guaranteed by a direct tube from the top of each desiccator, normally being closed with a Mohr's pinchcock clamp. A second tube, equipped with activated charcoal and filter (0.2 µm), is present on the top for the re-establishment of the internal pressure after each analysis. The books were positioned into the desiccators with a beaker filled with 100 mL of MilliQ water in order to guarantee a saturated humidity inside the closed environment for all the investigations. The samples were kept at 24 °C. The GC-MS analyses were performed weekly for three months (November 2013 – January 2014).

3.3.4 MVOC analysis of indoor air from contaminated repository

The indoor air of the contaminated repository was collected during the 2nd and the 3rd sampling by six stainless steel canisters (3 – 6 L). The sampled areas (1, 2, 3 and 4) were the same as for the

aerobiological investigation. Pre-treated evacuated canisters were positioned in correspondence of each sampling area and their valves were opened allowing the filling to atmospheric pressure in less than 30 s. All canisters were closed, transported to the Laboratory of Air Quality of ARPAV, Padua (Italy) and subsequently analysed by GC-MS (Fig. 3.6-D). The preparation of the canisters is described in Appendix A7.

Figure 3.6 Sampling techniques: A: Sample bottle for BOD analysis; B: Sampling bottle for MVOC analysis from pure fungal colony; C: Sampling chamber for MVOC analysis from contaminated book; D: Air sampling by canister between shelves.

3.4 Secondary metabolites analysis (LC-MS/MS)

The fungal secondary metabolite extraction was performed in January 2015 at the Laboratory of Molecular Science and Nanosystems Department, Ca' Foscari University of Venice. The extraction solvent was acetonitrile/water/acetic acid mixture (79:20:1 v/v/v) and the samples were shaken for 90 min. The LC-MS/MS analysis were carried out in February - March 2015 at the Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (Austria).

Secondary metabolite separation and detection was performed by liquid chromatography (1290 Series HPLC System, Agilent, Waldbronn, Germany) coupled to a tandem mass spectrometer (QTrap 5500 MS/MS, Applied Biosystems, Foster City, CA) equipped with TurbolonSpray electronspray ionization (ESI) source (Fig. 3.6). The analysis followed the multi-mycotoxin method developed by Malachová *et al*. (2012). The extraction protocol and the instrumental parameters are described in Appendix A8.

3.4.1 Secondary metabolites from fungal cultures

Following fungal strains of the same species analysed by GC-MS were selected due to their high abundance inside the studied repository (see Appendix B1):

- *Aspergillus creber* (*n* = 5);
- *A. jensenii* (*n* = 1);
- *A. penicillioides* (*n* = 8);
- *A. protuberus* (*n* = 2);
- *A. vitricola* (*n* = 5*);*
- *Cladosporium cladosporioides* (*n* = 3);
- *Eurotium chevalieri* (*n* = 1);
- *E. halophilicum* (*n* = 15);
- *Penicillium brevicompactum* (*n* = 1);
- *P. chrysogenum* (*n* = 2) (Table 3.4).

For *E. halophilicum*, 3 strains came from the repository, while the other 12 were the "external" strains used in the previous growth condition characterization (see Par. 3.2.2, Appendix A5). All the strains were inoculated onto two specific culture media to promote their secondary metabolite production: Yeast Extract Agar (YES) and Czapek Agar (CYA) (Samson *et al*., 2004). For the most xerophilic fungi (*A. penicillioides*, *A. vitricola* and *E. halophilicum*) other media were used: Yeast Extract Agar added with 15% of NaCl (YES15%) and Malt Extract Agar added with 15% of NaCl (MEA15%). For some strains, colonies with different ages were analysed, in order to point out the differences of secondary metabolite production between young and old colonies.

Table 3.4 Fungal species selected for their secondary metabolite-ability production analysed by LC-MS/MS.

Fungal species	Strain	Colony age (days)	MUT no.	GenBank no.	Source
Aspergillus creber Jurievic, S.W. Peterson & B.W. Horn	40	7, 14	MUT 470	KU179486	BAUM, book cover (VE)
	41	7, 14	MUT 5691	KU179487	BAUM, book cover (VE)
	42	7, 14	MUT 5527	KU179488	BAUM, book cover (VE)
	43	7, 14	MUT 5690	-	BAUM, indoor air (VE)
	44	7, 14	MUT 5689	-	BAUM, indoor air (VE)
A. jensenii Jurievic, S.W. Peterson & B.W. Horn	47	7, 14	MUT 480	KM502178	BAUM, book cover (VE)
A. protuberus Munt.-Cvetk.	45	7, 14	MUT 5693	KU179494	BAUM, indoor air (VE)
	46	7, 14	MUT 5487	KM502193	BAUM, Indoor air (VE)
A. penicillioides Spegazzini	27	14	MUT 481	Ku179489	BAUM, book cover (VE)
	28	14	MUT 5694	KU179490	BAUM, indoor air (VE)
	29	14	MUT 5525	KU179491	BAUM, book cover (VE)
	30	14	MUT 5537	KU179492	BAUM, book cover (VE)
	31	14	-	-	BAUM, book cover (VE)
	32	14	MUT 5699	-	BAUM, indoor air (VE)
	33	14	MUT 5700	-	BAUM, book cover (VE)
	34	14	MUT 5697	KU179493	BAUM, book cover (VE)
A. vitricola Ohtsuki	35	14	-	-	BAUM, book cover (VE)
	36	14	MUT 5696	-	BAUM, book cover (VE)
	37	14	MUT 5692	-	BAUM, book cover (VE)
	38	14	MUT 5698	-	BAUM, book cover (VE)
	39	14	MUT 5695	-	BAUM, book cover (VE)
Cladosporium cladosporioides (Fresen) G.A. de Vries	51	7, 14	MUT 5536	-	BAUM, indoor air (VE)
	52	7, 14	MUT 527	KU179495	BAUM, indoor air (VE)
	53	7, 14	-	-	BAUM, indoor air (VE)
Eurotium chevalieri L. Mangin	26	7, 14	MUT 472	-	BAUM, indoor air (VE)
E. halophilicum C.M. Chr., Papav. & C.R. Benj.	2	35, 588	MUT 1922	-	Tavola Valdese archive (TO)
	3	35, 58, 596	MUT1899	-	Tavola Valdese archive (TO)
	5	58, 596	MUT1906	-	Tavola Valdese archive (TO)
	7	35, 58, 596	MUT 798	-	Stampati Vaticano archive (ROMA)
	8	596, 58, 35	MUT 1314	-	Corte costituzionale library (ROMA)
	10	35, 596	MUT 1294	-	Stampati Vaticano archive (ROMA)
	11	35, 58, 588	MUT 1306	-	Casa Madre archive (AN)
	12	35, 596	MUT 1316	-	Corte costituzionale archive (ROMA)
	15	35, 58, 596	MUT 1298	-	Corte costituzionale archive (ROMA)
	17	35, 596	MUT 1322	-	Corte costituzionale library (ROMA)
	20	35, 289	MUT 1315	-	Corte costituzionale library (ROMA)
	22	35, 58, 588	MUT 1304	-	Gregorian library (ROMA)
	23	35, 80, 132	MUT 482	KM502179	BAUM, book cover (VE)
	24	35, 58, 373	MUT 5534	KU179496	BAUM, book cover (VE)
	25	35, 58, 360	MUT 5535	KU179497	BAUM, book cover (VE)
Penicillium brevicompactum Dierckx	50	7, 14	MUT 536	KM502183	BAUM, book cover (VE)
P. chrysogenum Thom	48	7, 14	MUT 5493	KM502200	BAUM, indoor air (VE)
	49	7, 14	MUT 5492	KM502204	BAUM, indoor air (VE)

3.4.2 Secondary metabolites from settled dust samples

Settled floor dust samples were collected in the four areas inside the repository, corresponding to the sampling areas 1-4, by a conventional portable household vacuum cleaner (Dustbuster® VH780, Black & Decker, Milan, Italy) with a power level of at least 780 W and new dust bags (VHF70 Filter, Black & Decker, Milan, Italy) (Table 3.5). After 2 min m^{-2} of sampling, the dust bags were closed by plastic tape, transferred to the laboratory and stored at -20 °C until the extraction. The sampling areas varied from 1.5 to 4.5 m^2. The extraction and the LC-MS/MS were performed as for fungal samples (Fig. 3.7). The volumes of extraction solution differed between samples (4 – 6 mL), depending on dust quantities. The mycotoxin similarity among sampling areas was valuated with the Bray-Curtis Index by XLSTAT 2015.1.03. A significant dissimilarity level of 0.95 was applied.

Table 3.5 Description of dust samples collected at BAUM's repository during 2nd sampling.

Sample	Sampling location	Sampling surface (m^2)	Quantity (g)	Volume of extraction solution used (mL)
Area 1	Right corner far from the entrance between shelves	1.5	0.30	4
Area 2	Corridor close to area 1	4.3	0.04	4
Area 3	Left corner far from the entrance, between shelves	3.8	0.85	6
Area 4	Entrance of the repository	4.3	0.75	8

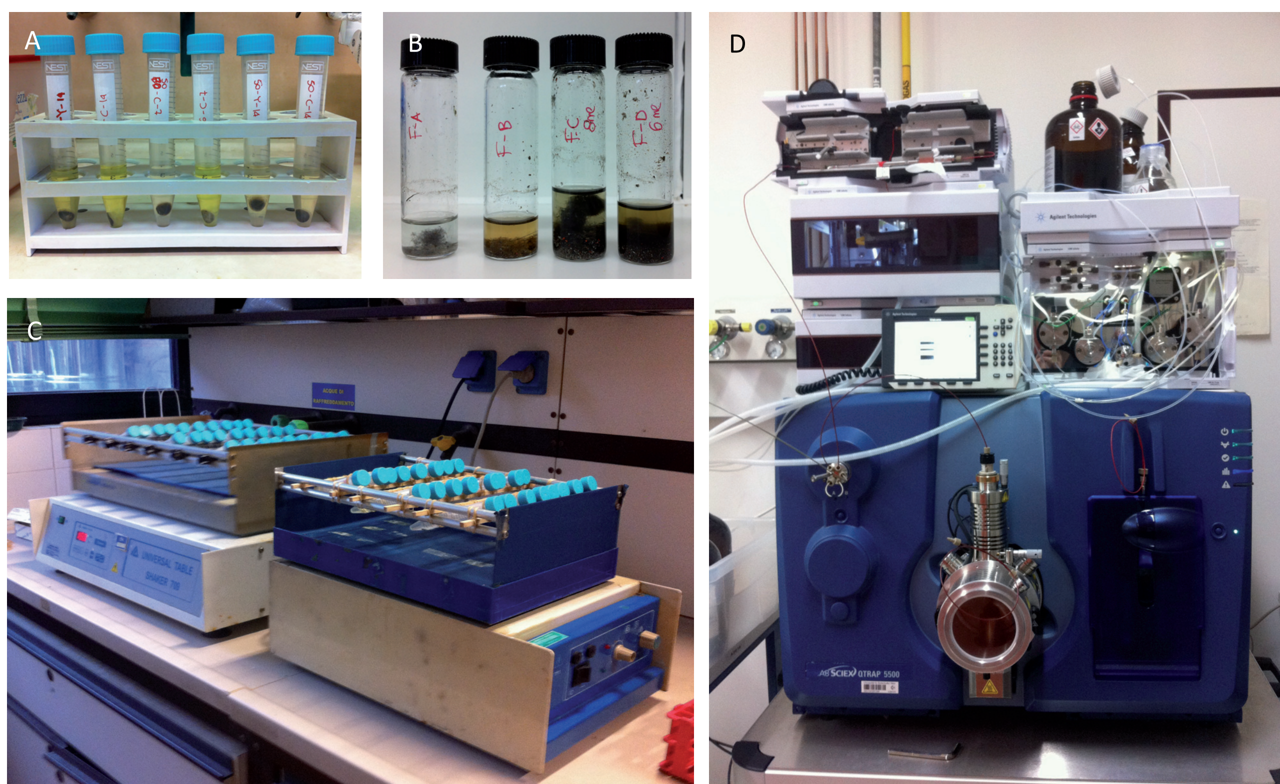


Figure 3.7 Secondary metabolite analysis: A: fungal agar disk immersed in the extraction solution (4 mL); B: settled dust samples immersed in different amount of extraction solution (4 – 8 mL); C: extraction phase performed with shaker for 90 min.; D: QTrap 5500 LC-MS/MS System (Applied Biosystems) connected with a 1290 Series HPLS System (Agilent).

4. RESULTS AND DISCUSSION

4.1 Microbiological investigations

4.1.1 Climatic monitoring

During all the three sampling periods at the BAUM's repository, thermo-hygrometrical parameters were monitored and compared with those obtained from the permanent data loggers inside the indoor environment (Table 4.1). During sampling, the indoor environmental conditions were almost in line with the recommendations of the *Italian Ministry for Cultural Heritage* for the preservation of books and paper products (19-24 °C air temperature and 50-60% relative humidity; MIBAC, 2001; UNI 10586, 1997).

Table 4.1 Thermo-hygrometrical parameters recorded during all the samplings performed at BAUM's repository.

Thermo-hygrometrical parameters	**1st sampling (March '13)**	**2nd sampling (October '13)**	**3rd sampling (May '14)**
Temperature (°C)			
Max	19 °C	23.5 °C	23.5 °C
Min	19 °C	22.4 °C	22.5 °C
Average	19 °C	22.7 °C	22.7 °C
Relative Humidity %			
Max	45%	55.8%	64.1%
Min	46%	52.4%	59.6%
Average	45.2 %	54.7%	62.4%
Data loggers inside the repository			
Temperature (°C)	19.5 °C	23 °C	22.4 °C
Relative Humidity %	46.6%	56.3%	64%

4.1.2 Aerobiological results

The 1st microbial air sampling (March 2013, 1° S) performed inside the contaminated environment was focused on the detection of xerophilic fungal species. For this purpose, specific low water activity media were used (*e.g.* MEA15%, see Par. 3.1.2 and Appendix 2 for specific and detailed information). The aerobiological results demonstrated a gradually decreasing contamination gradient from area 1 towards the exit of the repository (see Par. 3.1.2.2). As reported by Micheluz *et al.* (2015a), the source of this contamination was identified in area 1 with the most massive colonization compared to all the sample sites (2000 CFU m^{-3}), followed by the neighbouring area 2 (1620 CFU m^{-3}). These loads were ten times higher compared to the other sampling areas, which ranged between 29 and 101 CFU m^{-3}. The data from passive sampling during the same period demonstrated a lower yield compared to active sampling (data not shown)[1].

[1] The data of passive sampling are not reported because of their low information contribution.

After the disinfection and cleaning operation that interested almost all contaminated books, the 2nd (October 2013, 2° S) and the 3rd (May 2014, 3° S) samplings were carried out using different culture media and different incubation temperature (see Par. 3.1.2). The data in Table 4.2 show fungal loads of 120-710 CFU m^{-3} and 17-317 CFU m^{-3} for the 2nd and the 3rd samplings, respectively. The bacterial loads range between 23 and 160 CFU m^{-3} for the 2nd sampling, and between 117 and 803 CFU m^{-3} for the 3rd sampling. Considering the total microbial load as the sum of fungal and bacterial loads, we obtained a total load ranging between 163 and 807 CFU m^{-3} for the 2nd sampling and 130 - 866 CFU m^{-3} for the 3rd sampling.

Table 4.2 Fungal and bacterial loads and total microbial charge recorded for all samplings into BAUM's book repository.

Sampling areas	Sampling	Fungal load (CFU m^{-3})	Fungal ratio in/out	Bacterial load (CFU m^{-3})	Bacterial ratio in/out	Total microbial load (CFU m^{-3})
Area 1	1° S	2000 ± 610	69.0*	/**	/	/
	2° S	240 ± 53	0.3	77 ± 64	0.8	317 ± 83
	3° S	36 ± 20	0.1	750 ± 550	4.9	786 ± 550
Area 2	1° S	1620 ± 80	55.8*	/	/	/
	2° S	437 ± 31	0.6	160 ± 56	1.6	597 ± 64
	3° S	63 ± 32	0.2	803 ± 168	5.2	866 ± 171
Area 3	1° S	81 ± 6	2.8*	/	/	/
	2° S	120 ± 10	0.2	43 ± 32	0.4	163 ± 34
	3° S	17 ± 10	0.1	240 ± 125	1.6	257 ± 125
Area 4	1° S	101 ± 11	3.5*	/	/	/
	2° S	197 ± 32	0.3	23±15	0.2	220±35
	3° S	13 ± 9	<0.1	117±67	0.8	130±68
Area 5	1° S	29 ± 12		/	/	/
	2° S	160 ± 52	0.2	33 ± 6	0.3	193 ± 52
	3° S	68 ± 35	0.2	137 ± 64	0.9	205 ± 73
Area 6 (outdoor)	1° S	/		/		/
	2° S	710 ± 142		97 ± 90		807 ± 168
	3° S	317 ± 169		153 ± 55		470 ± 178

(*): ratio with area 5, considered "blank" for the 1st sampling because of its position outside of the contaminated repository.

/**: sampling not performed.

As highlighted in Fig. 4.1, the concentrations found in areas 1 and 2 during the 1st sampling could be classified as highly contaminanted (> 1500 CFU m^{-3}, Pacini *et al.*, 2005), and were higher compared to similar studies (Karbowska-Berent *et al.*, 2011; Nunes *et al.*, 2013; Pasquarella *et al.*, 2012). Moreover, the results demonstrated the efficacy of disinfection, with a decrease of fungal load in the indoor air: in correspondence of the source of contamination (area 1), the fungal load decreased from 2000 CFU m^{-3} to 240 CFU m^{-3} (2nd sampling) to a final load of 36 CFU m^{-3} (3rd sampling). The evidence of the strong contamination inside the repository before the disinfection is also confirmed by the comparison with the outside values (area 6) recorded during the samplings: the total fungal load inside during the 1st sampling (2000 CFU m^{-3}) was even higher than the total microbial load outside (407-807 CFU m^{-3}). Generally, the fungal load ratio indoor/outdoor should be <1. Ratios of ≥1 demonstrate a contamination from an indoor microbial source (Micali *et al.*, 2003). Considering the bacteria loads, area 2 in the 2nd sampling and area 1, 2 and 3 in the 3rd sampling have a ratio indoor/outdoor >1. These could be due to the human presence by speaking, coughing, sneezing or by the elimination of skin flakes with microorganisms sticking to them

(Micali *et al*., 2003). However, these results are similar to those observed in similar studies (Cappitelli *et al*., 2009; Pasquarella *et al*., 2012; Skóra *et al*., 2015).

Comparing to the recommendations of the *Italian Ministry guidelines* for the preservation of book collections (MIBAC, 2001, Fig. 4.1.), the fungal limit of 150 CFU m^{-3} is far exceeded by the data of the 1st sampling, mildly exceeded by the data of the 2nd sampling and not exceeded by those of the 3rd sampling. Different trends were recorded for bacterial load. The data was close to bacterial limit of 750 CFU m^{-3} (MIBAC, 2001), especially for the areas 1 and 2. However, the total microbial charge resulted generally under the maximal contamination level of 1000 CFU m^{-3}, as indicated by previous studies (Borrego *et al*., 2010; Nunes *et al*., 2013).

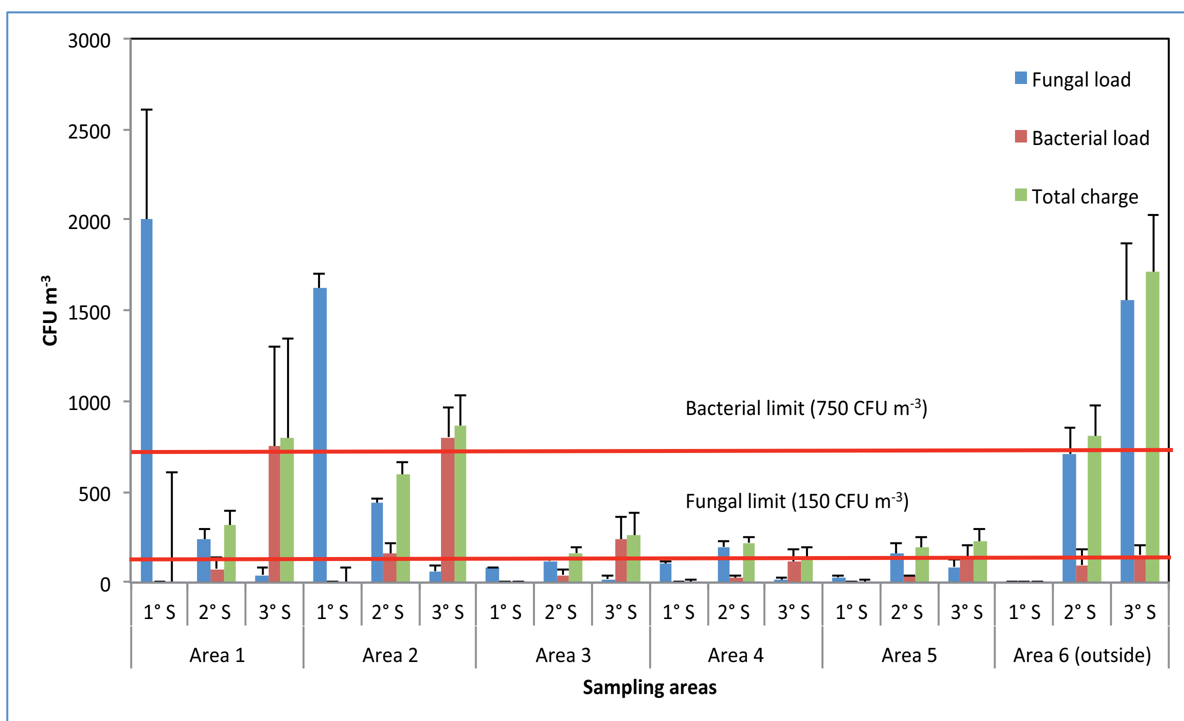


Figure 4.1 Microbial distribution among all the three samplings performed at BAUM's repository. Fungal, bacterial loads and total charge (fungal + bacterial loads) are reported, as well as the Italian regulation limits in red lines (MIBAC, 2001).

If we consider the data from each culture medium used during all samplings (see Table 4.3) we can get a general idea about the high microorganism variability of the indoor repository: generic fungi on MEA with incubation at 25 °C and mesophilic fungi on MEA at 37 °C (as potential humane pathogens), xerophilic fungi growth on MEA15% and DG18, as well as generic bacteria on PCA at 25 °C and mesophilic bacteria at 37 °C (also potential humane pathogens).

Apart of area 6, the growth on MEA at 25 °C was higher than the recommended limit of 150 CFU m^{-3} for the 2nd sampling (120-437 CFU m^{-3}), but they decreased in the 3rd sampling (13-87 CFU m^{-3}). The incubation of Petri dishes with the same culture medium at 37 °C allowed to isolate a lower number of microorganisms (3-33 CFUm^{-3}), comparable with those collected on DG18 medium (0-50 CFUm^{-3}).

Table 4.3 Overview of aerobiological data collected during all samplings in each culture medium. See Appendix A2 for details about the specific culture media.

Sampling area	Sampling	FUNGAL MEDIA				BACTERIAL MEDIA	
		MEA 25 °C ($CFUm^{-3}$)	MEA 37 °C ($CFUm^{-3}$)	MEA15% 25 °C ($CFUm^{-3}$)	DG18 25 °C ($CFUm^{-3}$)	PCA 25 °C ($CFUm^{-3}$)	PCA 37 °C ($CFUm^{-3}$)
Area 1	Mar '13	/(*)	/	2000 ± 610	/	/	/
	Oct '13	240 ± 53	10 ± 17	20 ± 10	17 ± 29	77 ± 64	70 ± 30
	May '14	36 ± 20	-	3 ± 6	38 ± 17	750 ± 550	313 ± 40
Area 2	Mar '13	/	/	1620 ± 80	/	/	/
	Oct '13	437 ± 31	3 ± 6	53 ± 6	17 ± 12	160 ± 56	87 ± 21
	May '14	63 ± 32	3 ± 6	17 ± 15	23 ± 6	803 ± 168	573 ± 187
Area 3	Mar '13	/	/	81 ± 6	/	/	/
	Oct '13	120 ± 10	7 ± 12	20 ± 21	16 ± 12	43 ± 32	133 ± 55
	May '14	17 ± 10	-	12 ± 17	23 ± 12	240 ± 125	193 ± 12
Area 4	Mar '13	/	/	101 ± 11	/	/	/
	Oct '13	197 ± 32	13 ± 6	37 ± 10	13 ± 6	23 ± 15	33 ± 32
	May '14	13 ± 9	-	6 ± 12	7 ± 6	117 ± 67	100 ± 26
Area 5	Mar '13	/	/	29 ± 12	/	/	/
	Oct '13	160 ± 52	17 ± 15	16 ± 23	6 ± 0	33 ± 6	33 ± 21
	May '14	68 ± 35	3 ± 6	25 ± 10	13 ± 23	137 ± 64	187 ± 90
Area 6 (outdoor)	Mar '13	/	/	/	/	/	/
	Oct '13	710 ± 142	67 ± 35	295 ± 57	70 ± 87	97 ± 90	83 ± 23
	May '14	317 ± 169	40 ± 31	276 ± 155	760 ± 168	153 ± 55	153 ± 81

(*)/: sampling not performed.

For xerophilic fungi isolated on MEA15% medium, the total amount decreased from the 1st to the 3rd sampling, in particular in correspondence to area 1 (100%, total decrease) and 2 (99%, almost total decrease), as pointed out in Fig. 4.2.

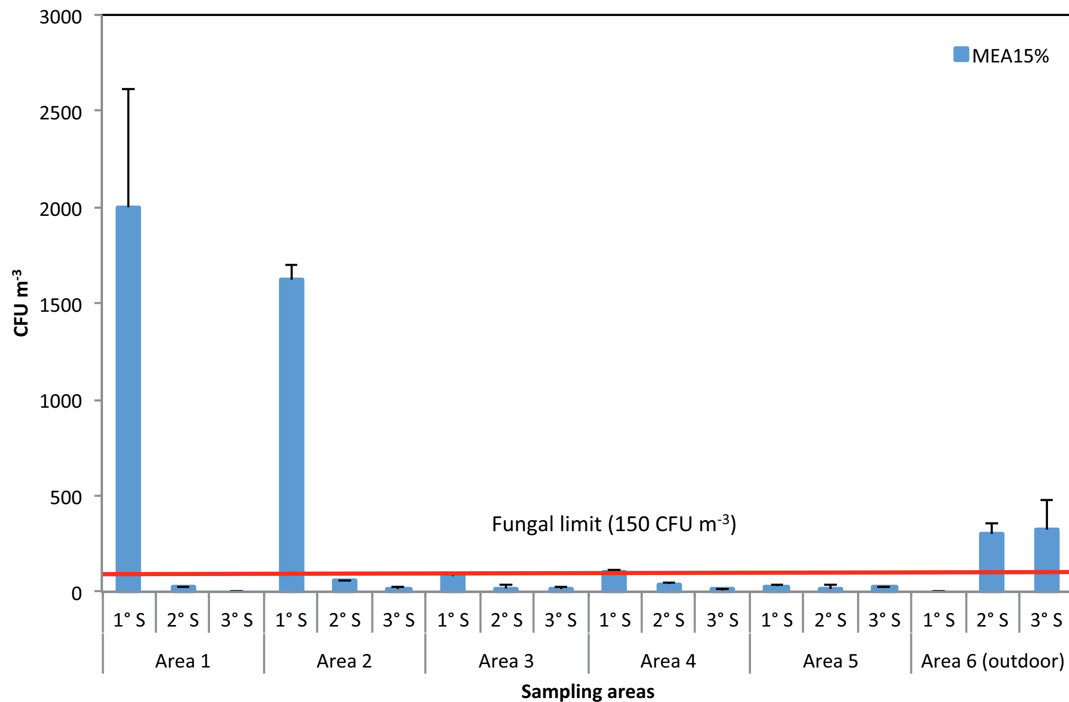


Figure 4.2 Xerophilic fungal loads on MEA15% in all samplings performed at BAUM's repository.

4.1.3 Air fungal characterization

The three aerobiological investigations performed inside the BAUM's repository at different seasons gave a wide range of results, highlighting the variety of mycological species in a quite small space (150 m^2). A first overview of fungal variability is reported in Table 4.4, where the number of taxa and the *Simpson Diversity Index* (see Par. 3.1.2.2) are expressed for each sampling area and for the different culture media.

Considering only the indoor areas, the use of MEA15% with incubation at 25 °C as culture medium during the 1st sampling permitted to isolated the highest number of species (22 taxa), followed by the 2nd and the 3rd sampling with the use of MEA with incubation at 25 °C (15 taxa and 12 taxa, respectively). The data of cultures on MEA with incubation at 37 °C correspond to the lowest number of taxa collected, especially during the 3rd sampling (1 taxa).

The *Simpson Diversity Index* varied between the culture media and the three samplings. The passage areas (areas 3, 4 and 5) had a higher index than those far from the entrance (areas 1 and 2). In particular during the 1st sampling with MEA15% as medium, area 4 had the highest fungal diversity (0.92), perhaps because of the continuous passage of workers and students, and book's crossing. During the same sampling, area 1 had the lowest value of fungal variability (0.52), perhaps due to the poor movement of the old books and the particular environmental conditions between shelves with very low air exchange rates. For the MEA with incubation at 37 °C it was not possible to calculate the entire Index because of the low quantity collected on the Petri dishes.

Table 4.4 List of numbers of taxa and fungal variability expressed as Simpson's Diversity Index for each sampling, sampling area and culture medium.

Sampling area	**Sampling**	**Fungal media**							
		MEA 25 °C		**MEA 37 °C**		**MEA15%**		**DG18**	
		N° taxa	Simpson's Index	N° taxa	Simpson's Index	N° taxa	Simpson's Index	N° taxa	Simpson's Index
Area 1	1° S	-	-	-	-	5	0.52	-	-
	2° S	10	0.63	2	0.42	3	0.61	3	0.63
	3° S	6	0.76	-	-	1	0	10	0.89
Area 2	1° S	-	-	-	-	8	0.63	-	-
	2° S	14	0.67	1	0	6	0.78	4	0.72
	3° S	12	0.84	1	0	5	0.79	6	0.79
Area 3	1° S	-	-	-	-	12	0.81	-	-
	2° S	15	0.88	1	0	2	0.25	3	0.54
	3° S	5	0.80	-	-	4	0.75	8	0.86
Area 4	1° S	-	-	-	-	21	0.92	-	-
	2° S	15	0.84	3	0.60	7	0.84	2	0.37
	3° S	4	0.72	-	-	2	0.50	3	0.67
Area 5	1° S	-	-	-	-	7	0.72	-	-
	2° S	7	0.66	3	0.54	3	0.54	2	0.50
	3° S	8	0.82	1	0	7	0.85	4	0.75
Area 6 (outdoor)	1° S	-	-	-	-	-	-	-	-
	2° S	24	0.85	4	0.68	10	0.62	2	0.09
	3° S	20	0.26	5	0.75	11	0.80	20	0.74

Morphological and molecular analyses identified 98 fungal taxa, belonging to 43 genera. The complete list of fungal species isolated by aerobiological sampling is reported in Appendix B1. Different fungal pattern were emerged depending on the culture medium used. A total of 47 fungal taxa were recorded from MEA15%, belonging to 16 fungal genera (Fig. 4.3). The 1st sampling was characterized by the prevalence of *Aspergillus* genera, especially in correspondence to areas 1, 2 and 3, followed by *Penicillium* and *Cladosporium*. The most abundant species were *A. creber*, *A. protuberus* and *P. chrysogenum*. The 2nd and the 3rd sampling recorded similar fungal compositions as the first sampling but with a much lower fungal load. In these cases, the most frequently fungi were *A. flavus*, *A. penicillioides*, *A. sydowii*, *Cladosporium halotolerans* and *P. brevicompactum*. The data measured outside (area 6) were characterized by the presence of *Cladosporium* spp., *Penicillium* spp. and sterile mycelia.

The use of low water activity medium, as MEA15%, allowed pointing out the selective presence of a wide pool of xerophilic fungal species. *A. creber* and *A. protuberus* were already detected in indoor environment (Jurjevic *et al*., 2012), but this is the first study that reported these species inside a library environment (Micheluz *et al.,* 2015a). Also for *C. halotolerans*, normally detected in hypersaline aqueous habitants (Zalar *et al*., 2007), this is the first report from an indoor environment. *P. chrysogenum* and *P. brevicompactum* instead have been already associated with the biodeterioration of art collections and were often isolated from contaminated indoor environments (Gallo, 1993; Montemartini Corte *et al*., 2003; Sterflinger, 2010; Zyska, 1997).

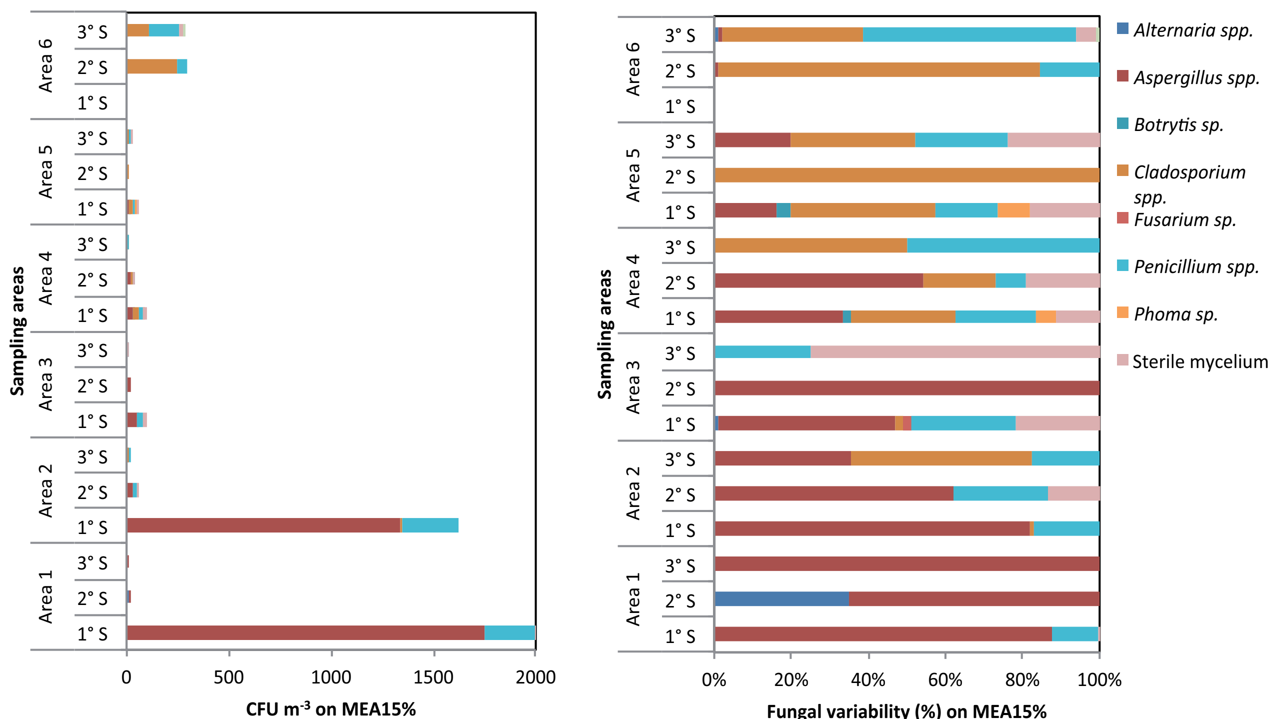


Figure 4.3 Fungal species collected on MEA15% with incubation at 25 °C during all samplings. The graph on the left reports the diversity specified as CFU m^{-3}, while the graph on the right reports the diversity %.

High fungal variability was detected from the MEA medium incubated at 25 °C. From the 2nd and the 3rd sampling we obtained a total of 68 different fungal species belonging to 32 fungal genera as shown in Fig. 4.4. The results in correspondence to indoor areas were characterized by a high yeast presence, followed by *Cladosporium* spp. and several sterile mycelia. The most frequent isolated species were *Bulleromyces albus*, *C. halotolerans*, *Merulopsis corium*, *Sporodiobolus pararoseus* for the 2nd sampling and *Cryptococcus carnescens*, *C. cladosporioides*, *P. brevicompactum* and *Rhodotorula mugilaginosa* for the 3rd sampling. The outside fungal variability was generally composed by the prevalence of *Cladosporium* spp. and *Alternaria* spp.

The detection of the above mentioned major fungal species, especially those grown on MEA at 25 °C, is in line with results reported from previous studies (Gutarowska *et al*., 2014; Karbowska-Berent *et al*., 2011). Skóra *et al*. (2015) also detected several yeasts in similar environments, in particular *Cryptococcus* spp. and *Rhodotorula* spp.

Cultures grown on MEA incubated at 37 °C were mainly characterized by *Aspergillus* spp. and sterile mycelia (Fig. 4.5). In particular, a few colonies of *Aspergillus flavus*, *A. fumigatus* and *A. versicolor* were detected inside the repository. Even if their detection was sporadic, they have the capacity to grow at high temperatures and are potentially harmful for humans.

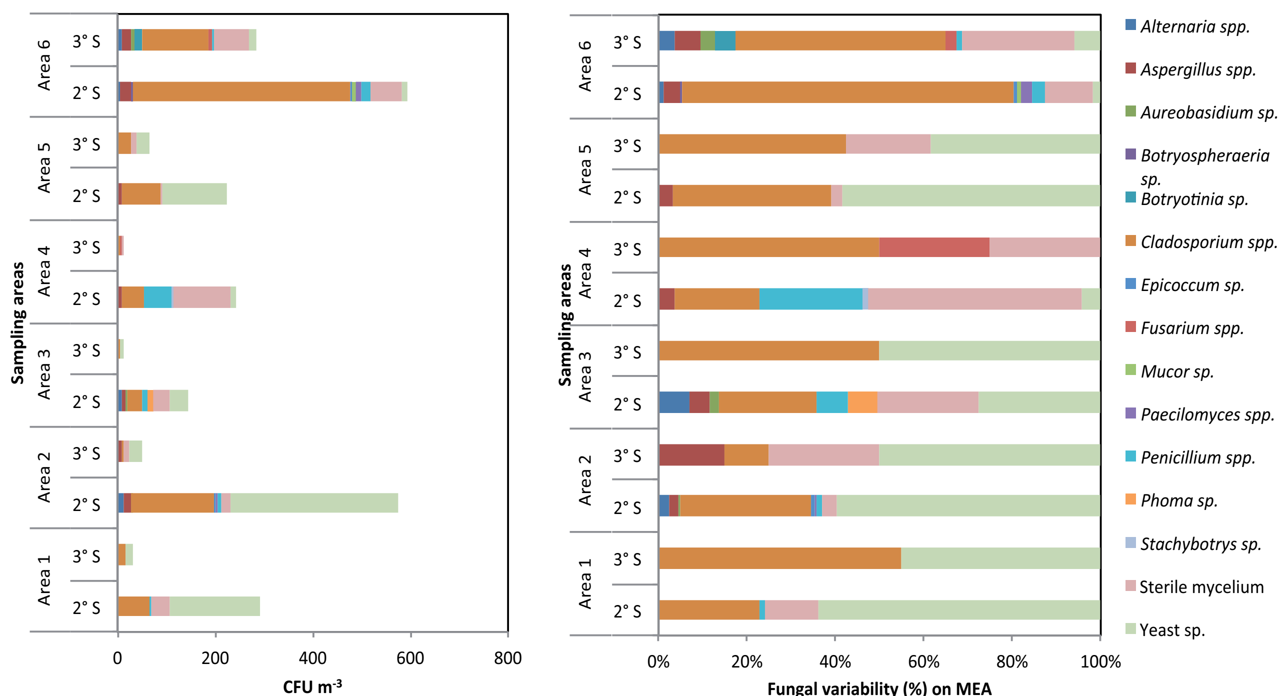


Figure 4.4 Fungal species collected on MEA (25 °C) during the 2nd and the 3rd samplings. The graph on the left reports the diversity specified as CFU m^{-3} (note the difference in the x-scale), while the graph on the right reports the diversity %.

A. flavus is a halo-tolerant fungus, known for being able to grow at high temperatures, as mycotoxin producer (*i.e*. aflatoxin B, cyclopiazonic acid and 3-nitropropionic acid) and also as opportunistic human pathogen (Samson *et al*., 2010).

A. fumigatus is the most important human pathogen causing fungal ball in lungs (hazard group = 2). For this reason its presence is worldwide regulated (ACGIH, 1997; D.Lgs 81/2008; European Commission 2000/54/EC). Moreover, it is a thermotolerant species and mycotoxin producer (*i.e*. gliotoxin, fumigaclavines, fumitremorgins and verruculogen) (Samson *et al*., 2010).

Finally, *A. versicolor* is a common worldwide indoor fungal species, mycotoxin-producer (*i.e.* sterigmatocystin) and able to grow in a wide temperature range (Raper and Fennell, 1965).

The results obtained from DG18 medium were composed mainly by *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. This selective medium was used for the collection of xerophilic fungal species like MEA15%, but only 16 fungal species (47% of the total for DG18 and 34% of the total for MEA15%) were found on both media. For this reason, the use of both culture media is recommended for the detection of a wide range of xerophilic moulds. For additional information about fungal variability see Appendix B1.

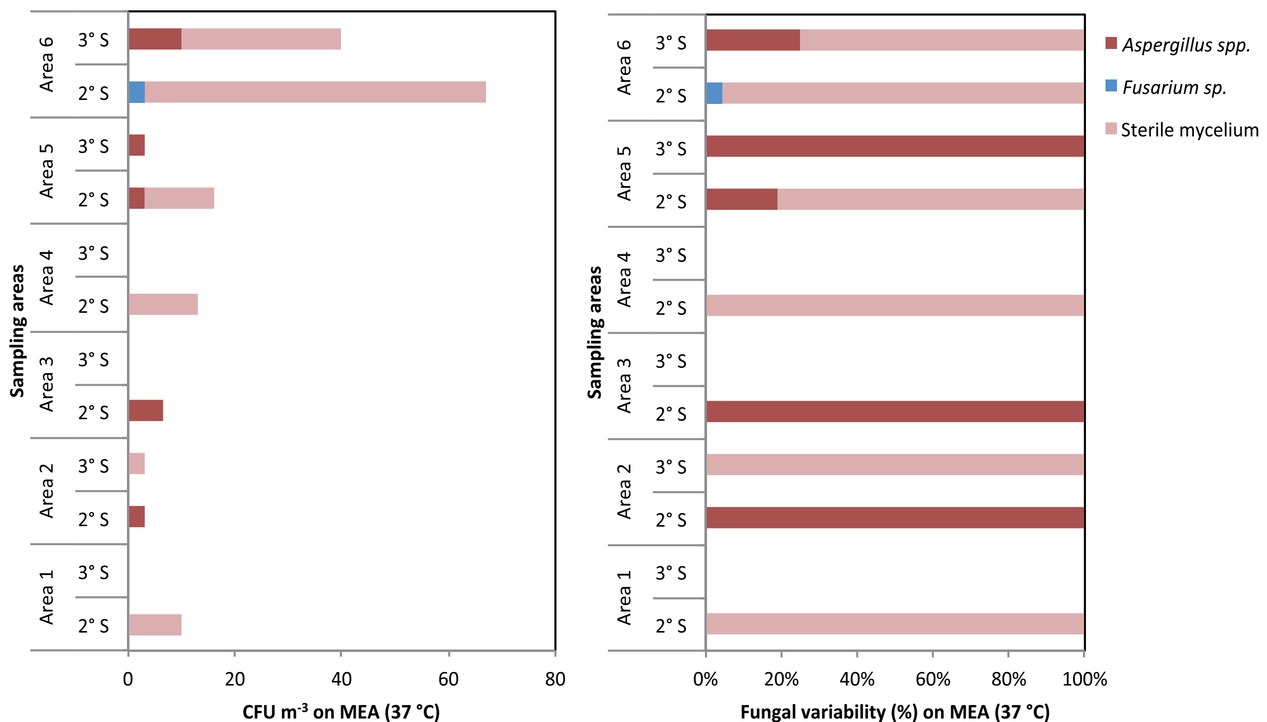


Figure 4.5 Fungal species collected on MEA (37 °C) during the 2nd and the 3rd samplings. The graph on the left reports the diversity specified as CFU m^{-3}, while the graph on the right reports the diversity %.

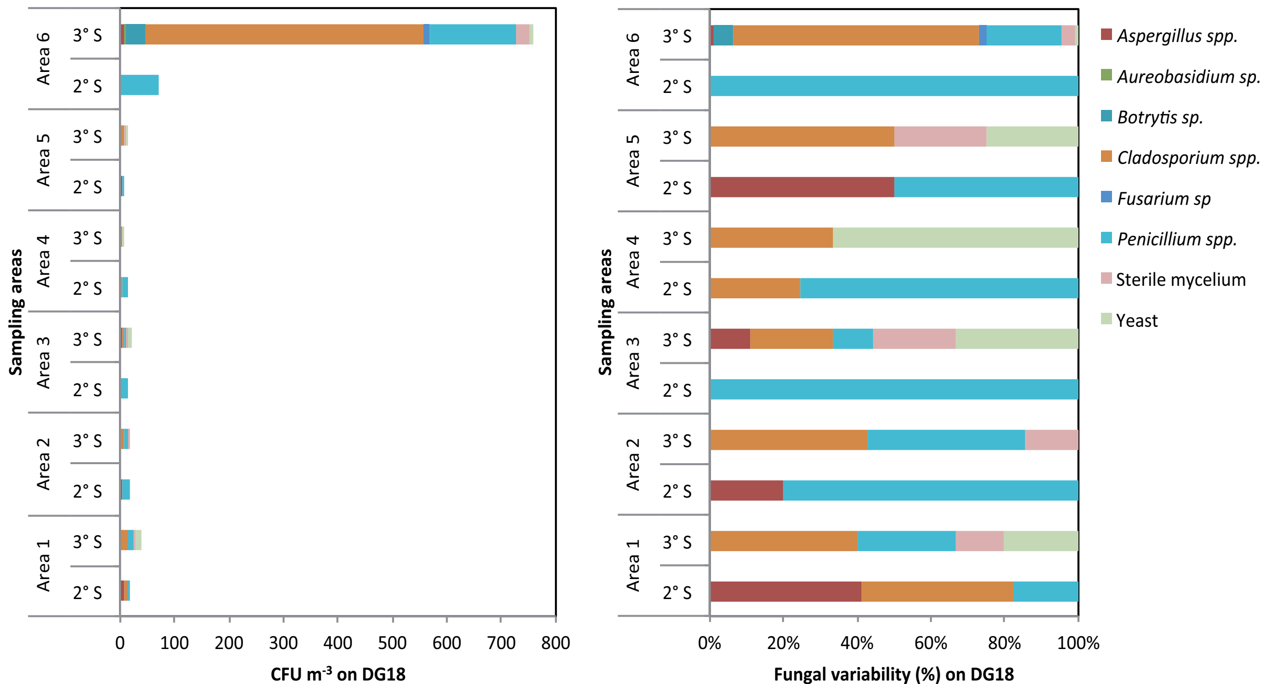


Figure 4.6 Fungal species collected on DG18 during the 2nd and the 3rd samplings. The graph on the left reports the diversity specified as CFU m^{-3}, while the graph on the right reports the diversity %.

4.1.4 Book fungal characterization

Several fungal specias were isolated during the monitoring of contaminated and successively disinfected books. Most of them were also detected in the air samples. In the following Table 4.5 the list of fungal species isolated from all books is reported. The results are divided between contaminated books (*n* = 15, where 5 are from the 1st sampling and 10 from the 2nd sampling), disinfected books (*n* = 10, 2nd sampling) and books studied for MVOC production (*n* = 2).

We identified 24 species belonging to 12 genera and the genus *Aspergillus* was the major one with almost ten different species. At least five species are belonging to the recently revised *Aspergillus* section *Versicolores* group (Jurievic *et al*., 2012), with *A. creber* as the most frequent species. Most of the detected species are well-known indoor fungi (Gallo, 1993; Manente *et al*., 2012; Samson *et al*., 2004) often isolated from art collections (Kraková *et al.*, 2012; Michaelsen *et al*., 2010; Zyska, 1997). Most of them are xerophilic fungi, with low water activity requirement for their growth, as *A. penicillioides*, *Eurotium* spp. and *P. brevicompactus* (Hocking and Pitt, 1988).

From all detected fungal species, *E. halophilicum* was the most difficult species to isolate, because of its characteristic slow growth (Christensen *et al*., 1979). Its presence was determined after 20 days-incubation onto MEA15% medium at 25 °C. Recent studies (Montanari *et al*., 2012; Pinzari and Montanari, 2011) demonstrated that the white spotted fungal growth on book covers in climate-controlled environment is principally due to *E. halophilicum*'s growth and development, consistent with its ecological performances.

Table 4.5 Fungal diversity from various sampling procedures from all sampled books. In the column *Methods of isolation*: S = sterile swab; A = active sampling; M = nitrocellulose membrane. In the brackets are specified the book samples (L6 and L9) used for MVOC characterization.

Fungal species	**Quantity of contaminated books (n = 5) 1st sampling**	**Quantity of contaminated books (n = 10) 2nd sampling**	**Quantity of disinfected books (n = 10)**	**Quantity of books for MVOC studies (n = 2)**	**Methods of isolation**
Acremonium fusidioides (Nicot) W. Gams	-	1	-		S
Alternaria alternata (Fr.) Keissl.	-	-	1		S
Aspergillus carneus (Tiegh.) Blochwitz	-	-	-	1 (L9)	S
Aspergillus creber Jurjevic, S.W. Peterson & B.W. Horn	4	-	-	2	S, A, M
Aspergillus jensenii Jurjevic, S.W. Peterson & B.W. Horn	1	-	-	1 (L9)	M, A
Aspergillus penicillioides Speg.	3	1	3	2	M, S, A
Aspergillus protuberus Munt.-Cvetk.	2	-	-		S, A
Aspergillus sydowii (Bainer & Sartory) Thom & Church	-	-	-	1 (L6)	M
Aspergillus tubigensis Mosseray		2 (L9)			S
Aspergillus versicolor (Vuill.) Tirab.	1	-	-		M
Aspergillus vitricola Ohtsuki		1	-	2	M, S
Aspergillus flavus Link		1	-		S
Aspergillus spp.	-	1	3	2	M, S
Chaetomiun funicola Cooke	-	-	-	1 (L6)	S
Chaetomiun subaffine Sergeeva		1	-		S
Cladosporium halotolerans Zalar, de Hoog & Gunde-Cim.	-	2	1		S
Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun	1	-	-		M
Epicoccum nigrum Link	1	-	-		M
Eurotium amstelodami L. Mangin	-	-	-	2	M
Eurotium chevalieri L. Mangin	1	-	-		M
Eurotium halophilicum C.M. Chr. Papav. & C.R. Benj.	1	-	-	2	M, S, A
Gloeophillum abietinum (Bull.) P. Karst.	1	-	-		M
Lecanicillium kalimantanense Kurihara & Sukarno	1	-	-		A
Penicillium brevicompactum Dierckx	3	-	-		S, A, M
Penicillium catenatum D.B. Scott			1		S
Penicillium chrysogenum Thom	1	3 (L6)	3	2	M, S
Penicillium rubens Biourge	-	-	-	1 (L9)	S
Penicillium steckii K.M. Zaleski		1 (L6)			
Penicillium sp.	-	1	4	2	M, S
Phaeospheria typharum (Desm.) L. Holm	1	1	-		M
Scopulariopsis brevicaulis (Sacc.) Bainier	-	1	-		S
Sterile mycelium	-	1	-		S

(*) Fungal species that have only been identified using morphological approach.

Although its presence was visible on all books, its isolation was successfully performed only by sterile swab, obtaining few colonies *in vitro*. After initial large-scale expansion, perhaps due to the availability of water on the books, *E. halophilicum* stops growing and its mycelium acts passively as a trap and/or a substrate for

the growth of airborne fungal spores and propagules (Micheluz *et al*., 2015a). This may explain failure detection of *E. halophilicum* and the consequent detection of other fungal diversity in a previous survey (Montanari *et al*., 2012). However, its presence was recently documented on paper materials by molecular analysis and SEM imaging (Michaelsen *et al*., 2010), even if it is not considered a cellulolytic fungus (Abdel-Hafez *et al*., 1990).

Colonies of *A. penicillioides* were also frequently detected on the book samples. Already in 1978, Samson and Lustgraaf (1978) associated this fungus with *E. halophilicum* as cohabiting in house dust, emphasizing the potential origin of human and animal allergens. This species was isolated from dry food and indoor environments, such as museum, archives, shelving and carpeting (Pitt and Hocking, 1997; Samson and Lustgraaf, 1978; Samson and Hoekstra, 1994), and also from contaminated books (Michaelsen *et al.,* 2010; Montanari *et al*., 2012; Pinzari and Montanari, 2008).

From the disinfected books, only a few fungal colonies were isolated. Fungal growth was not visible on their covers, so probably only deposited fungal spores or propagules were sampled. In particular, *Alternaria alternata*, *A. penicillioides*, *C. halotolerans* and *Penicillium* spp, including *P. chrysogenum* were the only fungi identified, probably components of the biological part of indoor dust (Abdel-Hafez *et al.*, 1990; Maggi *et a*l., 2000; Samson and Lustgraaf, 1978).

We obtained following results from the two books (L6 and L9 called samples) sampled after their incubation (3 months; see Par. 3.3.2 and Appendix A7) for MVOC studies. Prior to the incubation, only colonies of *P. chrysogenum* and *Aspergillus* section *nigri* were isolated, but after incubation 13 different fungal species were detected. Several *Aspergillus* and *Penicillium* species were identified, already detected from previous book sampling (see Table 4.5). Moreover, *A. carneus*, *A. sydowii*, *C. funicola*, *E. amstelodami* and *P. rubens* were isolated for the first time from book samples. As highlighted by SEM investigations (see the followed Section 4.1.5), the main fungal growth recognizable from the books was characterized by white spotted circles, typical for *E. halophilicum*'s cleistothecia (Fig. 4.7). In this case, this fungus isolation was easier than during the first sampling, because of its isolation during its mycelium growth phase. Also several colonies of *A. penicillioides* were detected, as well as its similar species *A. vitricola*, another xerophilic fungus isolated for the first time from binocular lens (Ohtsuki, 1962) (Fig. 4.5).

The analysis of the relative water content using the Aqua-Boy device have highlighted a general content of 10% of water from the book's cover of contaminated (L1-10) and disinfected (L11-20) books (Fig. 4.8). The values collected from internal pages were 8.7% and 9.4% for contaminated and disinfected books, respectively. These results are similar to those reported in other studies (Pinzari and Montanari, 2011).

Considering the values of 8-10% reported in Par. 1.1.1, Fig. 1.2 (Gallo *et al*., 2003; Nyushka, 1979), all the data are in the critical range of possible microbial germination on any kind of papers. The slightly higher values of disinfected books could be due to the application of disinfection product by spray (see Par.

3.1.2.2). These results have to be taken into account to plan systematic monitoring of the book collection and the repository environment in order to avoid further contaminations.

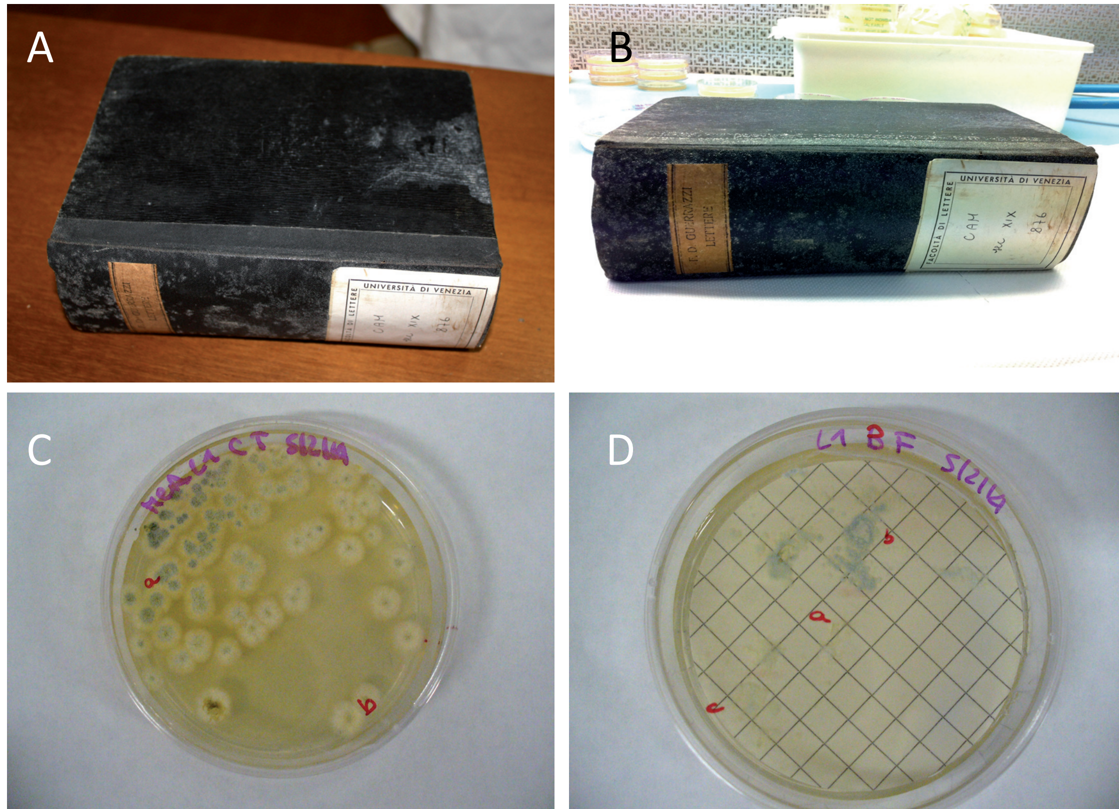


Figure 4.7 Sample L6: A) contaminated book at 1st sampling; B) contaminated book after incubation (MVOC studies) with new fungal growth; C) fungal colonies isolated from L6 by sterile swab (mainly *Aspergillus* spp.); D) fungal colonies isolated from L6 by sterile membranes (mainly blue colonies of *A. penicillioides* and white colonies of *E. halophilicum*).

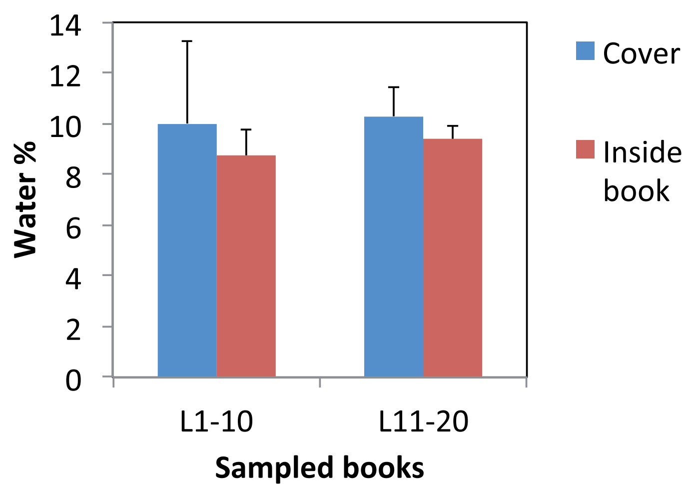


Figure 4.8 The water content differences in books with (L1-10) and without fungal contamination (L11-20).

4.1.5 Summary

The microbiological investigations inside the repository highlighted a wide range of microbial diversity among all the sampling periods (March 2013 – May 2014). A total of 98 different fungal entities, belonging to 43 genera, were identified by aerobiological sampling. The most frequent fungal species were *Penicillium* spp. (17%, *e.g. P. brevicompactum*, *P. chrysogenum* and *P. glabrum*), *Aspergillus* spp. (14%, *e.g. A. creber*, *A. flavus* and *A. versicolor*) and *Cladosporium* spp. (11%, *e.g. C. cladosporioides*, *C. halotolerans* and *C. pseudocladosporioides*). All these species are known as indoor fungi, often isolated from dry substrates (Samson *et al*., 2004). Their detections reflected the controlled environmental conditions of the repository (19 °C and 55% RH), excluding the occurrence of a moisture-building problem. Typical species linked with this kind of problem, as *Stachybotrys* spp*.* (Bloom *et al*., 2007; Nielsen *et al*., 1999), were only occasionally revealed. Furthermore, the use of both MEA15% and DG18 as selective media for xerophilic microorganisms have permitted to isolated a wider range of fungal species.

Also relative high amounts of different kind of yeasts (*e.g. Cryptococcus carnescens*, *C. diffluens*, *Rhodotorula mugilaginosa* and *Sporodiobolus pararoseus*) and bacteria were sampled inside the repository, especially during the 2nd and the 3rd sampling, because of the adoption of several culture media, *i.e.* MEA and PCA.

The investigation of mesophile microorganisms by incubation at 37 °C have highlighted the presence of both bacteria and a few (but significant) fungal species, mainly belonging to *Aspergillus* spp. In particular, *A. flavus*, *A. fumigatus* and *A. versicolor* were isolated, all well-known mycotoxin producers and human pathogens (with the exception of *A. versicolor*).

The microbial indoor variability had only 39% of the total fungal species in common with the microbial variability found outside the library. This data suggests that the remaining 61% of fungal presence may originate from indoor sources.

Several fungal species belonging to *Aspergillus* section *Versicolores* were detected during all the three sampling periods. During the 1st sampling, *A. creber* and *A. protuberus* were the most isolated fungal species, especially from the highest contaminated areas (area 1 and 2) inside the repository. Their detection was observed also during the subsequent aerobiological samplings, but with lower concentration than during the 1st sampling. In the 2nd and in the 3rd sampling, a general decrease of microbial detection was observed, mainly because of the disinfection treatment (performed after the 1st sampling) that interested the contaminated books.

Several *Aspergillus* spp., (*e.g. A. creber, A. flavus*, *A. penicillioides*, *A. protuberus*, *A. sydowii*, *A. tubigensis*, *A. versicolor* and *A. vitricola*), *Cladosporium* spp. (*e.g. C. halotolerans* and *C. ramotenellum*) and *Penicillium* spp. (*e.g. P. brevicompactum*, *P. chrysogenum*) were detected on the book covers. However, only 57% of the total fungal species isolated from the contaminated books were detected in the indoor environment and no similarity was observed between books and outdoor sampling. This suggests that the

microbial diversity found on the books could be due both to the actual indoor conditions and to the past settled microbial deposits. From the disinfected books, all the isolated fungal species were also detected by aerobiological indoor investigations.

Among all the species isolated only from the contaminated books, *Eurotium halophilicum* was recognized as the dominating fungus grown on the covers. The confirmation of its principal presence derived only by sterile swab-isolation from contaminated books onto specific low water activity medium (MEA15%) followed by identification with both classical and molecular biology techniques, and by microscopically observations of its fungal structures, sampled by Fungi-Tape™ directly from the books' covers. The cause of its sporulation on the books could be attributed to the favourite environmental conditions between the Compactus® shelves, which were closed for several months. Low air-exchange rate, even with optimal indoor climate conditions, may have created particular ecological niches that promoted its germination and consecutive proliferation. After initial large-scale expansion, *E. halophilicum* could be considered as a primary colonizer, acting as a trap and/or a substrate for the growth of airborne fungal spore.

No records of *E. halophilicum* were observed from the disinfected books, but a few colonies were isolated from the still contaminated books stored in the same repository. Planned supervision, frequent dusting, effective ventilation, and rapid diagnoses should all be ensured to prevent further fungal contamination of materials, furniture and indoor air, also to reduce potential health risks for workers and students.

4.2 Investigations of *Eurotium halophilicum*

4.2.1 Microscopically investigations

Direct observation of Fungi-Tape™ samples collected from book covers by optical microscope showed various fungal structures, such as mycelium, conidiophores and conidia, mainly belonging to *Aspergillus* spp. Uniseriate radiate to columnar conidial heads and ellipsoidal conidia of quite variable sizes (5-7.5 x 5-9 µm) were observed. Shape, ornamentation and dimension of these conidia were similar with those of the anamorphous state of *E. halophilicum*, namely *Aspergillus halophilicus* (Samson and Lustgraaf, 1978).

Moreover, characteristic short bare "hairs" on the hyphae with less than 1 nm in diameter were visible by SEM observations. They could be associated to the extracellular mycofibrils of *Aspergillus amstelodami*, observed on fox spots from a 145 year-old book paper after SEM investigation by Florian and Manning (2000). In that case, the diameter of mycofibrils ranged from 10-50 nm to close to 1 µm with a length up to 25 µm. The authors found β-glucans with a small component of protein similar to the composition of collagen. The same authors suggested that their presence could be a response to an aerial growth or a generic taxonomic feature and the amount may vary due to the physiological conditions, *e.g.* during water stress conditions (Florian, 2002).

In correspondence to the spotted mycelia, it was possible to observe the growth of crystals, shown in Fig. 4.7. Different morphologies could be documented by SEM, possibly due to a different chemical compositions or a different growth pattern.

The two books (samples L6 and L9) used for MVOC emission measurements (see Par. 3.3.3) were subjected to adhesive tape sampling before and after incubation in controlled environments. The observation of Fungi-Tape™ samples obtained from the book covers after 3 month-incubation showed the presence of fungal structures belonging to *Eurotium* genera and several cleistothecia were documented. These spherical fruiting bodies contained spherical asci, which in turn each enclose four or eight colourless ascospores, easily recognizable by the presence of equatorial rings (Fig. 4.8).

Generally, cleistothecia structures appear after 2-3 weeks of incubation, while conidial state, usually absent during the early development, appears after cleistothecia formation (Samson and Lustgraaf, 1978). Together with ascoma, also several uniseriate conidiophores with roughly ornamented conidia were recognizable, as shown in Fig. 4.7.

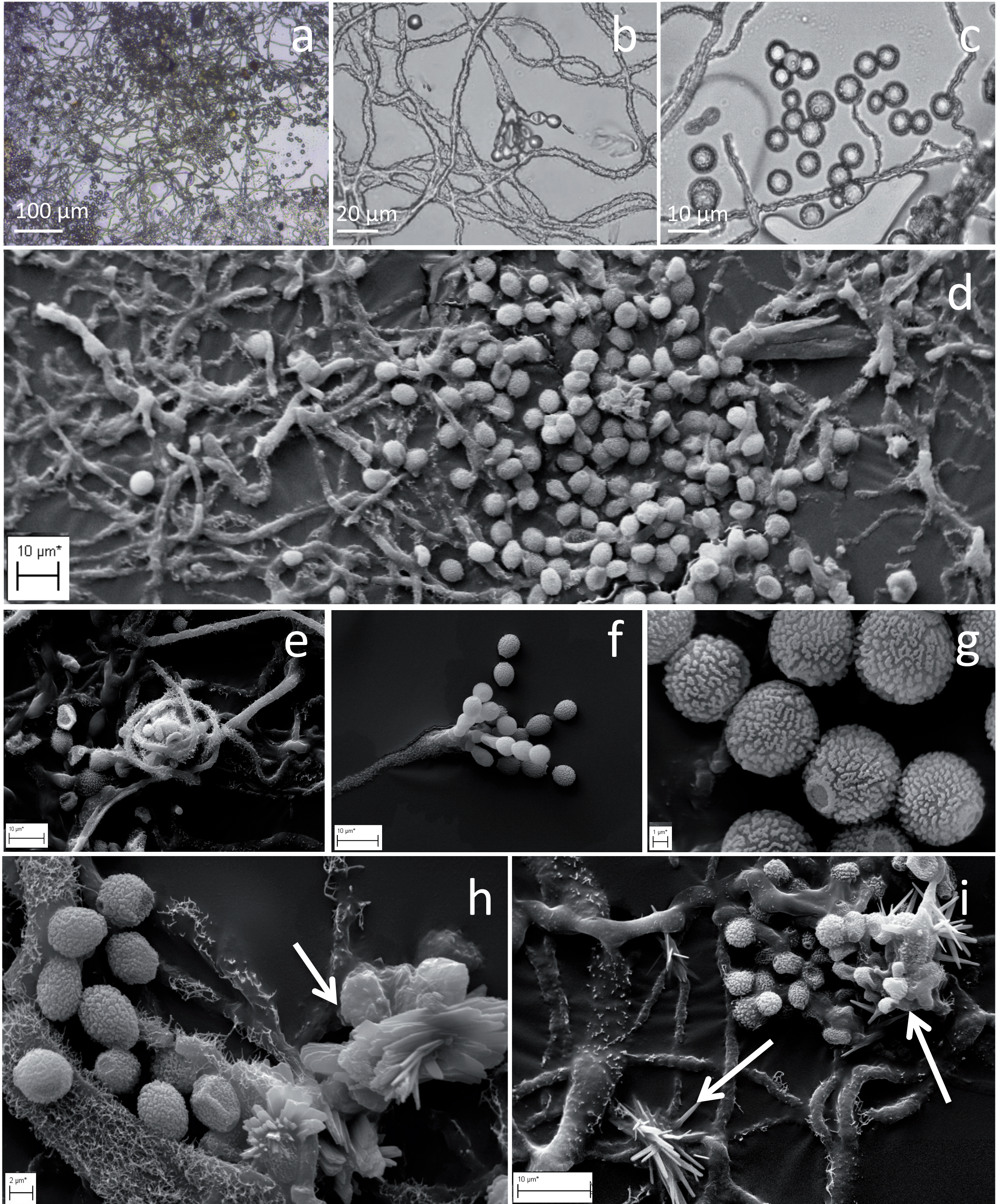


Figure 4.9 Optical microscope and SEM images of fungal structures sampled from book L9 with adhesive strip Fungi-Tape™. a: optical bright field microscopy imaging of fungal mycelium; b and c: optical bright field microscopy imagings of *E. halophilicum* hyphae, conidiophore, and conidia; d: HV SE SEM image on gold sputtered samples of *E.halophilicum* mycelium and conidia; e: HV SE SEM image on gold sputtered samples of *E. halophilicum*; detail of ascoma initial; f: HV SE SEM image on gold sputtered samples of *Aspergillus halophilicus'* conidiophore; g: HV SE SEM image on gold sputtered samples of *E. halophilicum* rough conidia; h and i: HV SE SEM image on gold sputtered samples of *E. halophilicum*; detail of plane and sharp shape secondary formation crystals emerged from hyphae (arrows).

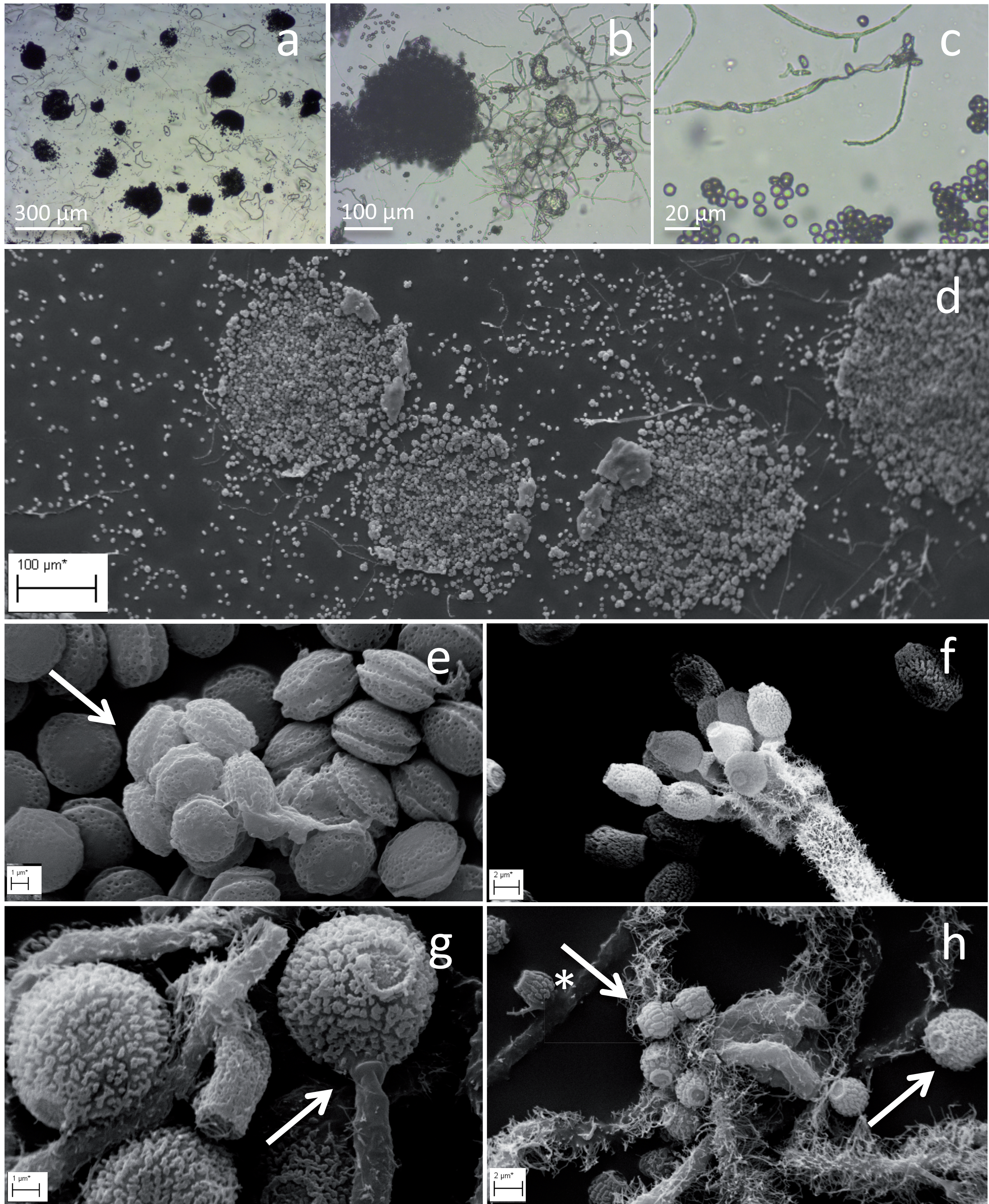
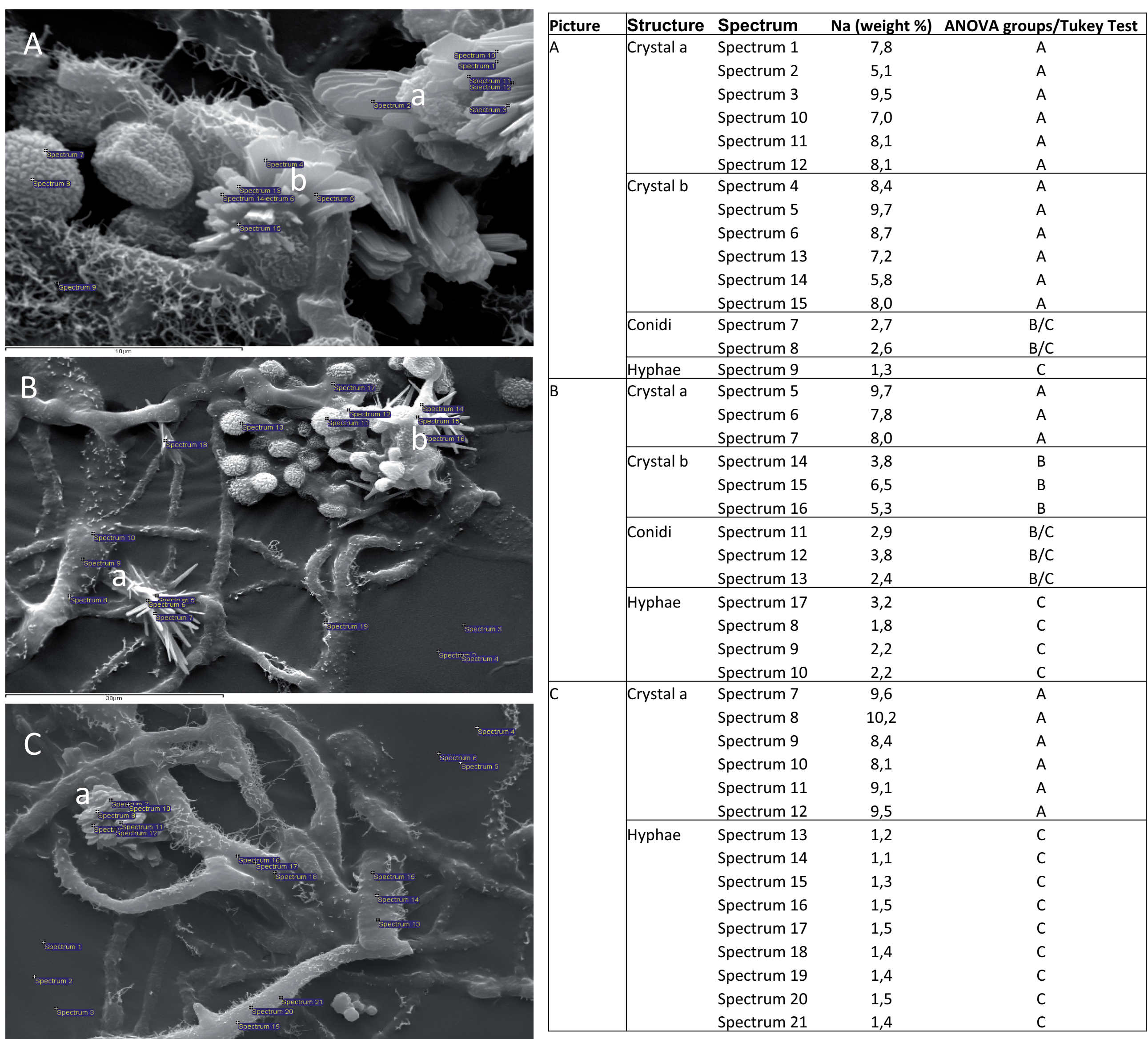


Figure 4.10 SEM and optical microscope images of fungal structures sampled from book L9 with adhesive strip Fungi-Tape™. a: optical bright field microscopy imaging of broken *E. halophilicum* cleitothecia (dark, globose masses). b: optical bright field microscopy imaging of cleistothecia and ascoma initials (bright globose masses). c: optical bright field microscopy imaging of *E.halophilicum* conidiophore, conidia and asci. d: HV SE SEM image on gold sputtered samples of *E. halophilicum* globose cleistothecia. e: HV SE SEM image on gold sputtered samples of *E. halophilicum;* detail of lenticular, rough, with furrow shallow and bordered by low ridges ascospores, assembled in an ascum (arrow). f: HV SE SEM image on gold sputtered samples of *E. halophilicum;* detail of conidiophore head. g: HV SE SEM image on gold sputtered samples of a germinating conidium of *E. halophilicum*. Detail of a germinative tube (arrow). h: HV SE SEM image on gold sputtered samples of *E. halophilicum* haired hyphae with trapped conidia belonging to two different species of fungi, and characterised by different dimensions, shapes and ornamentations (simple arrow = *E. halophilicum*, arrow with asterisk = a different fungal species).

In combination with SEM observations, EDX analysis allows analysing the chemical composition of the crystals grown on fungal conidia and hyphal mat. In the samples from book L9, EDX data, obtained on a topographical basis, allowed to characterize qualitatively the elemental composition of the main structures visible in the micrographs obtained by SEM imaging (Fig. 4.9). C, O and Na resulted as the main elements in conidia and hyphae. The crystals found on fungal hyphae are consisting of C, O, Na and also S, Ca and Si (plus several other elements in lower concentration like Mg, Al, P and Cl). All the spectra are reported in Appendix B2.



Picture	Structure	Spectrum	Na (weight %)	ANOVA groups/Tukey Test
A	Crystal a	Spectrum 1	7,8	A
		Spectrum 2	5,1	A
		Spectrum 3	9,5	A
		Spectrum 10	7,0	A
		Spectrum 11	8,1	A
		Spectrum 12	8,1	A
	Crystal b	Spectrum 4	8,4	A
		Spectrum 5	9,7	A
		Spectrum 6	8,7	A
		Spectrum 13	7,2	A
		Spectrum 14	5,8	A
		Spectrum 15	8,0	A
	Conidi	Spectrum 7	2,7	B/C
		Spectrum 8	2,6	B/C
	Hyphae	Spectrum 9	1,3	C
B	Crystal a	Spectrum 5	9,7	A
		Spectrum 6	7,8	A
		Spectrum 7	8,0	A
	Crystal b	Spectrum 14	3,8	B
		Spectrum 15	6,5	B
		Spectrum 16	5,3	B
	Conidi	Spectrum 11	2,9	B/C
		Spectrum 12	3,8	B/C
		Spectrum 13	2,4	B/C
	Hyphae	Spectrum 17	3,2	C
		Spectrum 8	1,8	C
		Spectrum 9	2,2	C
		Spectrum 10	2,2	C
C	Crystal a	Spectrum 7	9,6	A
		Spectrum 8	10,2	A
		Spectrum 9	8,4	A
		Spectrum 10	8,1	A
		Spectrum 11	9,1	A
		Spectrum 12	9,5	A
	Hyphae	Spectrum 13	1,2	C
		Spectrum 14	1,1	C
		Spectrum 15	1,3	C
		Spectrum 16	1,5	C
		Spectrum 17	1,5	C
		Spectrum 18	1,4	C
		Spectrum 19	1,4	C
		Spectrum 20	1,5	C
		Spectrum 21	1,4	C

Figure 4.11 SEM micrographs of mycelia collected from book L9. Overview of all EDX topographical analyses performed pointing the detector to the different structures: crystals, conidia and hyphae. In the table, the elementary presence of Na, expressed as weight% in each spectrum is reported, as well as the results of one-way ANOVA test, multiple comparisons Tuckey test. Three different groups are explicated: A, B and C.

If we focuse on the major element Na, because of its importance in fungal biology (Benito *et al.*, 2011) and as mineral component (Buck *et al.*, 2006), statistical analysis distinguished three groups: 1)

conidia, 2) hyphae and 3) crystals (one-way ANOVA test, followed by multiple comparisons Tuckey test). In particular, all the crystals are in the same group A (highest Na weight%: 5.1-10.2%), with the exception of crystal Bb (group B, Na weight%: 3.8-6.5%) that seems to be closer to Na concentrations inside spores (group B/C, Na weight%: 2.4-3.8%) than those inside hyphae (group C, Na weight%: 1.1-3.2%).

The elemental composition of the crystals is C, O, Na, S and Ca, with a sporadic presence of Si, Mg, Al, P, Cl, and Cu (Table 4.6). The detection of the elements Al and Si could be due to the mineral fraction of settled dust on the book cover. If we consider the abundance of the major elements (without C and O, because of their massive presence in organic substrates) it is possible to identify their potential stechiometric ratio, resulting Na:2, S:2 and Ca:1, comparable with sodium/calcium sulphate. The possible presence of glauberite ($Na_2Ca(SO_4)_2$,) or the hydration version eugsterite ($Na_4Ca(SO_4)_3*2H_2O$), need to be confirmed by further investigation (*i.e.* electron backscattered diffraction, EBSD, coupled with SEM).

Table 4.6 Elemental compositions of the crystals emerged from fungal structures. The data are reported as averages (%) of their weights with standard deviations (STD).

Element	**Picture A**				**Picture B**				**Picture C**	
	Crystal a (weight %)		**Crystal b (weight %)**		**Crystal a (weight %)**		**Crystal b (weight %)**		**Crystal a (weight %)**	
	Average	**STD**	**Average**	**STD**	**Average**	**STD**	**Average**	**STD**	**Average**	**STD**
C	36.9	3.2	37.4	3.9	40.8	1.4	52.5	0.9	36.0	2.0
N	11.5	1.2	11.9	1.9	9.8	1.4	18.3	2.8	10.1	0.7
O	31.2	2.5	33.8	2.5	30.4	1.9	18.5	1.8	31.7	5.3
Na	7.6	1.5	8.0	1.5	8.5	1.0	5.2	1.4	9.2	0.8
S	7.2	1.2	5.3	2.6	6.7	1.8	2.7	1.7	8.5	2.4
Ca	4.4	0.6	3.0	1.3	3.8	0.9	1.9	0.9	4.6	1.6
Si	0.7	0.2	0.3	0.1						
Mg	0.4	0.2					0.5	0.1		
Al	1.2	0.2								
P	0.3	-								
Cl	0.3	-					0.3	0.2		
Cu							1.3	-		

The formation of these crystals may depend on the chemical concentrations of some elements by bioaccumulation in a specific compartment inside the hyphae, or by the presence of a catalyser. Their presence suggests that the fungus can bioaccumulate and translocate mineral salts from the environment, probably as a nutritional requirement (as food storage) or for its osmotic bio-regulation system [personal communication of Dr. F. Pinzari]. Further investigations, as sampling the inorganic fractions in the indoor air as well as on contaminated books, could help to identify the origin of these crystal components, especially of sodium and sulphur, typically components of marine spray (Masiol *et al*., 2012) and salt efflorescence in porous construction materials (Rodriguez-Navarro *et al*., 2000).

4.2.2 Growth condition trials

In a first step we investigated the best growth conditions of the xerophilic fungus *E. halophilicum* on different low water activity (a_w) media. The results of the growth performances of the 20 different strains of *E. halophilicum* belonging to the MUT collection (see Par. 3.2.2) at the end of the monitored period (four weeks) are reported in Figure 4.12. All the data are reported in Appendix B3 – A.

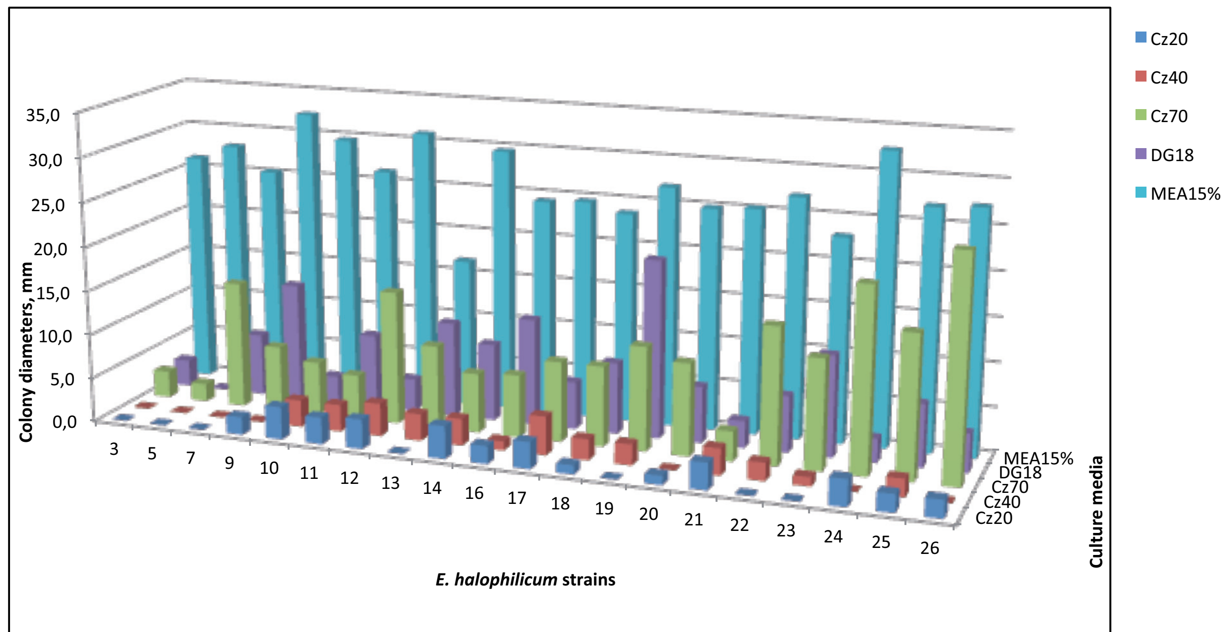


Figure 4.12 Overview of maximum growth of *E. halophilicum* strains in 4 weeks at 25 °C, on different culture media (MEA15%, e.e. MEA with 15% NaCl; DG18; Cz70, Czapeck with 70% sucrose; Cz40, Czapeck with 40% sucrose; Cz20, Czapeck with 20% sucrose).

The diagram shows that MEA15% (a_w = 0.88) resulted as the best culture medium for all the strains of *E. halophilicum* (average monthly growth = 26.6 mm in diameter) followed by Czapeck added with 70% of sucrose (Cz70, a_w < 0.82; average monthly growth = 10.4 mm in diameter) and DG18 (a_w = 0.95; average monthly growth = 7.2 mm in diameter). These results are similar to previous studies (Hocking and Pitt, 1988; Montanari *et al.*, 2012). The authors observed that the optimum a_w range for germination in glucose-fructose was 0.88-0.84 a_w, with fastest growth between about 0.85 and 0.80 a_w and the 80% of its maximal growth was observed over the range 0.88-0.77 a_w. The minimum a_w for germination and growth was 0.675 a_w, recorded in the short time period of 38 days. Over a longer time scale, Andrews and Pitt (1987) observed its growth also in medium with less than 0.675 a_w after 100 days of incubation.

Lacking growths were observed for Czapeck added with 40% (Cz40, a_w = 0.93; average monthly growth = 1.6 mm in diameter) and 20% of sucrose (Cz20, a_w = 0.97; average monthly growth = 1.6 mm in diameter). Past studies observed that its germination did not occur above 0.935 a_w, equivalent to 50% (w/w) sucrose, 39% (w/w) glucose or 10% (w/w) NaCl (Andrews and Pitt, 1987; Hocking and Pitt, 1988).

Moreover, MEA15% permitted similar growth for all the strains, with exception of strain 13, while the other media resulted in a quite variable growth trend. For these reasons, MEA15% was the favourite culture medium selected for the next *E. halophilicum* investigations.

The best growth temperature of *E. halophilicum* was determined by incubation at different temperatures. The results demonstrated that the incubation temperature of 25 °C is the favourite temperature for the growth of *E. halophilicum* (average monthly growth = 26.4 mm in diameter) (Fig. 4.13). Moderate growths were measured for incubations at 22 °C (average monthly growth = 12.1 mm in diameter) and 17 °C (average monthly growth = 7.6 mm in diameter). Among all the strains, only 5 strains were able to growth moderately at 28 °C (average monthly growth = 4.6 mm in diameter), but in general this temperature was considered as not appropriate. The growth at 17 °C demonstrated its psicro-tollerant ability, which was not reported in previous studies. This information is also important for the book preservation, because this circumstance confirmed its ability to germinate and growth in well climate controlled environments. All the data are reported in Appendix B3 – B.

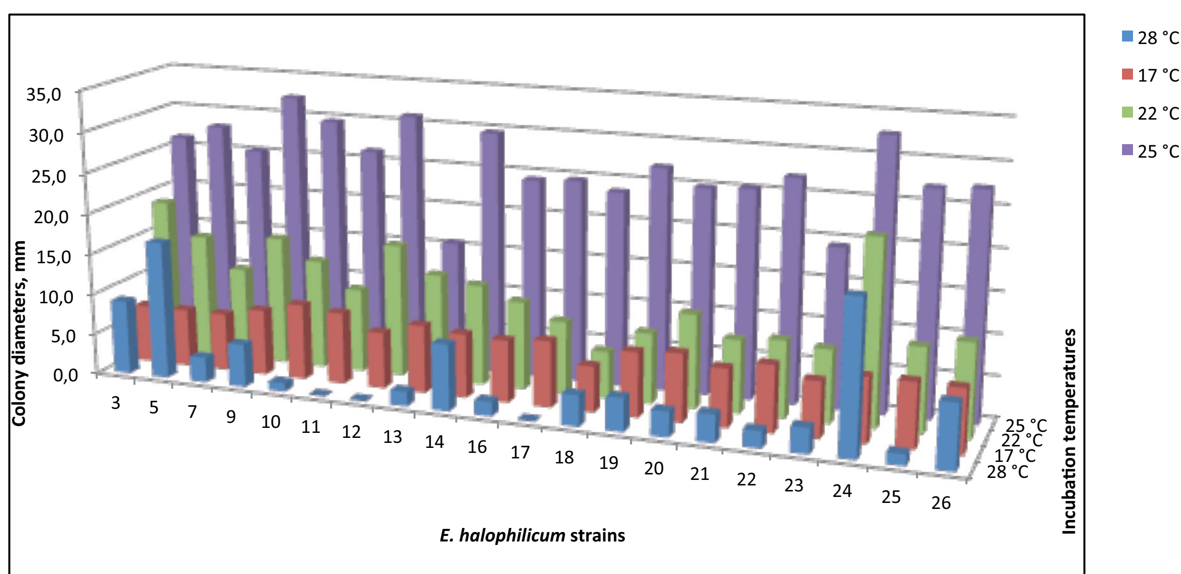


Figure 4.13 Overview of *E. halophilicum* strains growth on different incubation temperature on MEA15% in 4 weeks.

4.2.3 Summary

SEM investigations of the cello tape samples after direct sampling from the book covers have proved the univocal presence of fungal structures attributable to the xerophilic fungal species *Eurotium halophilicum*. The samples obtained from the books L6 and L9 before MVOC investigation were characterized by the presence of conidial state's structures, *i.e.* uniseriate radiate to columnar conidial heads, ellipsoidal conidia and nanometric short "hairs" on the hyphae, typical of those of the anamorphous state of the fungus, namely *Aspergillus halophilicus*.

The sample of cello tape collected after 3 month-incubation presented several fungal structures belonging to the teleomorph state, *i.e.* cleistothecia with spherical asci and ascospores. As reported by Samson and Lustgraaf (1978), the presence of cleistothecia structures are related to young colonies, while the detection of the conidial state is characteristic for old colonies. This may confirm the quite old fungal contamination (several months) of the books stored in the repository.

Together with conidial state structures, several crystals were detected in corresponding of fungal hyphae. EDX analyses have permitted to identify their potential chemical composition related to sodium-calcium sulphate (*i.e.* glauberite, $Na_2Ca(SO_4)_2$). Further investigation could help to identify the crystals, as microstructural-crystallographic characterization (SEM-EBSD). These results have highlighted the adaptation capacity of this fungal species to bioaccumulate and translocate elements from the environment in specific hyphae's compartment as a nutritional requirement or osmotic bio-regulation.

The growth requirement tests have confirmed its capacity to develop better onto low water activity ($a_w < 0.9$). In particular, highest growths were recorded on MEA15% (average monthly growth = 26.2 mm in diameter), followed by Cz70, DG18, Cz40 and Cz20. The trials performed at different temperature has identified its best growth performance at 25 °C, highlighting its versatility also in relation to a quite wide temperature range (17 – 28 °C).

All our results have significantly improved the lack of knowledge about this fungal species, in particular:

- it prefers as environmental requirements for a good growth - where it is possible to find being related to Cultural Heritage - a temperate climate environment with 19-25 °C and 55% RH, preferentially in low air-exchange conditions, as between Compactus® shelves;
- the fungal structures that it is possible to find on materials ascribable to *E. halophilicum* are the followings: conidial state structures especially for old colonies, *i.e.* uniseriate radiate to columnar conidial heads, ellipsoidal conidia, nanometric short "hairs" on the hyphae, also with the possible presence of sulphate crystals by element bioaccumulation, and teleomorph structures, *i.e.* globose cleistothecia with spherical asci and ascospores for young colonies;
- the typologies of substrates where it can grow (in a library) are the followings: leather, parchment or textile materials. Because of its poor food and low water activity substrate requirements, *E. halophilicum* could be considered as a primary colonizer, promoting the successive establishment of other airborne fungal species. No cellulolytic activity was observed;
- the best way to monitor *E. halophilicum*, *i.e.* the most efficient typology to sample it, is preferentially by sterile swab pressed onto contaminated surfaces; then, in order to isolate it is more advisable to inoculate onto MEA15% and incubate at 25 °C for at least 20 days.

4.3 MVOC analysis

4.3.1 BOD analysis of fungal cultures

As explained in Par. 3.3.1, BOD automatic device has been used for alternative BOD analysis to obtain information about different growth velocities among the fungal species selected for the MVOC analysis. In all cases, the fungi expanded their mycelia inside a close environment, so the BOD data monitored the time laps of their growth, starting from their first development until the total oxygen consumption. In the following graph (Fig. 4.14), all the O_2 consumption trends are reported, while all the data for each fungus and for the blanks (MEA and MEA15% substrates) are reported in Appendix B4.

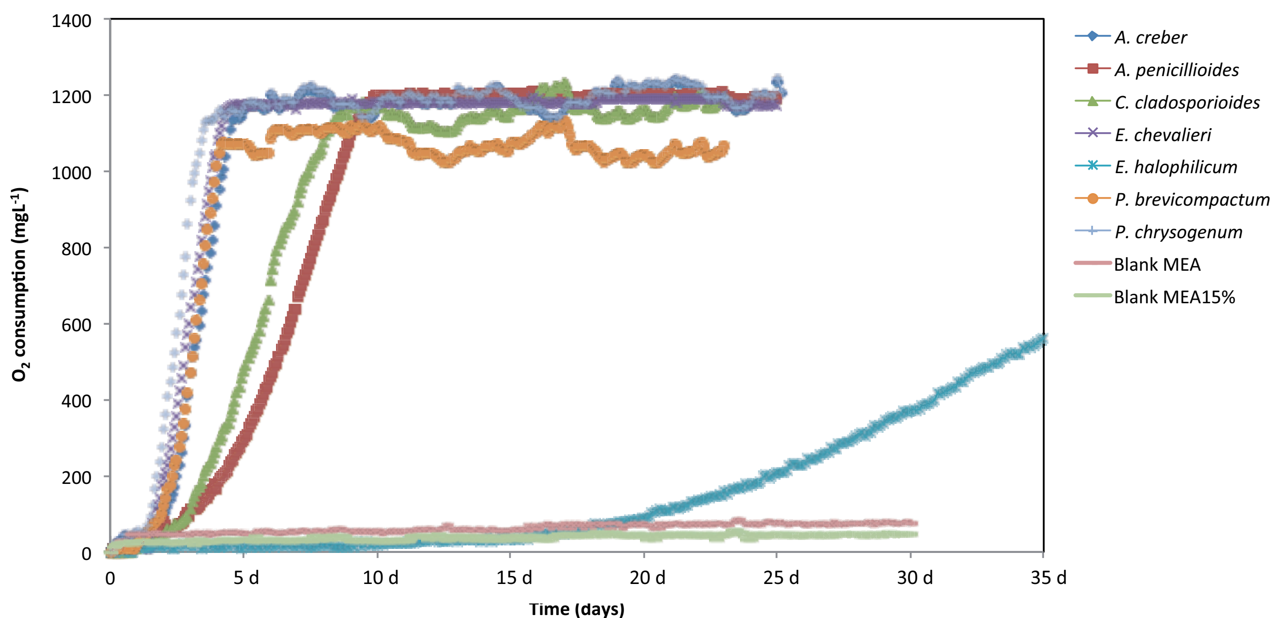


Figure 4.14 Overview of O_2 consumption trends of all selected fungal species. The monitoring period was about 25 -35 days.

In the graph it is possible to distinguish three main groups:

1) fungi with fast growth, *e.g.*, *P. chrysogenum*, *E. chevalieri*, *P. brevicompactum* and *A. creber*;

2) fungi with moderate growth velocity, *e.g.*, *C. cladosporioides* and *A. penicillioides*;

3) fungus with slow growth, *e.g. E. halophilicum*.

Based on the data, it was possible to determine for each fungus the beginning of its growth and the plateau, *i.e*. the maximum O_2 consumption as well as the maximum growth capability in the same conditions, which are summarized in the following Table 4.7.

These data shows that the *E. chevalieri* strain started to grow first after almost 1 day of inoculum, while *E. halophilicum* took almost 17 days. *P. chrysogenum* started growing after 1 1/2 day, but it reached its plateau first only after three and half days. Similar behaviour was observed for moderate growth velocity

fungi: *A. penicillioides* and *C. cladosporioides,* which started their growth after about 2 days and reached their plateau after around 9 days.

Table 4.7 Overview of BOD investigation data for the selected fungal species.

Fungal species	Starting growth point after inoculum (days)	Plateau point (total consumption of O_2, days)	Maximal O_2 consumption (mgL^{-1})
Aspergillus creber	1.5	4.5	1110
A. penicillioides	2.2	9.4	1155
Cladosporium cladosporioides	1.9	8.5	1144
Eurotium chevalieri	1.1	4.2	1138
E. halophilicum	17.2	-*	-*
Penicillium brevicompactum	1.3	4.2	1070
P. chrysogenum	1.3	3.6	1127

*Not detected.

Unfortunately, the 35 day-monitoring of *E. halophilicum* was not long enough to follow completely its development: the analysis stopped at the exponential growth phase, because of the slow growth of the fungus. However, the recorded data were used for comparison with its MVOC production.

In summary the BOD method demonstrated that through the O_2 consumption it was possible to compare the growth of different fungal species in relation with their metabolisms. Faster O_2 consumption could be related to a higher capability to activate enzymatic pattern and massive growth. Moreover, the ongoing decrease of O_2 forced the fungi to adapt to low O_2 conditions until fermentation process. All these differend conditions were monitored in parallel with MVOC analysis by GC-MS, so that each growth step corresponded to a detection of specific MVOC pattern.

4.4.2 MVOC analysis from fungal cultures

Seven different fungal species, already selected for the BOD analysis, *i.e. Aspergillus creber*, *A. pencillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum*, have been inoculated into an individual close environment. This was done in order to capture their self-synthesized volatile organic compounds (MVOCs) without external interferences. The MVOC production during the process of fungal growth (initial growth, older age, sporulation) was monitored weekly with temporary variations depending on each fungal species: from 2 up to 32 days of inoculation, especially for fungi inoculated on MEA. For the species inoculated on MEA15%, instead, the monitoring was started after 6 days, because of their slow growth and weekly analyses were performed for 28 days for *A. penicillioides* and for 60 days for *E. halophilicum* (Samson and Lustgraaf, 1978; Micheluz *et al*., 2015a). During the incubation period, we were able to follow the occurrence and the concentration trends of many compounds.

More than 100 chromatographic output signals were detected, even if only 20 up to 50 could be identified for each fungal species. The most significant signals with highest abundance were taken under

consideration. Substances were registered only in TIC mode by GC-MS (see Par. 3.3 and Appendix A7) and only distinct signals with peak areas exceeded 10^6 AU were considered (Matysik *et al.,* 2009, 2008). After the subtraction of blank samples (MEA and MEA 15% media), about 72 MVOCs emitted by the fungal cultures were chemically classified. For each fungal strain cultivated on MEA medium the detected compounds and the increase or decrease of the corresponding peak areas over the entire period of growth are reported in Table 4.8, while the results of the fungi cultivated on MEA 15% are reported in Table 4.9. For direct comparison, the peak areas from the 2nd to the 5th measurements are divided by the peak area of the first appearance of each compound, in order to operate a normalization. The resulting Area/$Area_1$ ratio reflects the relative change of the concentration.

The results demonstrated different spectra of MVOCs, which are mainly related to the different fungal species and their growth substrates (Matysik *et al*., 2009, 2008; Moularat *et al*., 2008). Following compounds were emitted by all fungal species grown on MEA medium:

- 2,4-dimethylheptane,
- ethanol,
- isopropyl alcohol,
- 3-methyl-1-butanol,
- 2-butanone.

The fungi grown on MEA15% medium all emitted instead, *i.e*.:

- 1,4-pentadiene,
- isopropyl alcohol,
- 1-butanol,
- 3-methyl-1-butanol,
- acetone,
- 2-butanone,
- cyclopentanone,
- tetrahydrofuran,
- 2-methylfuran,
- toluene,
- ethylbenzene,
- o-xylene,
- p-xylene,
- styrene.

Table 4.8 Overview of weekly MVOC production by selected fungal species grown on MEA as substrate. X: compounds with distinct signal (TIC > 10^6). Peak area/peak area$_1$ >1: +, >10: ++, >100: +++, =1: =, <1:-.

Substances	RT (min)	*A. creber*					*C. cladosporium*					*E. chevalieri*					*P. brevicompactum*					*P. chrysogenum*				
		Days after incubation					Days after incubation					Days after incubation					Days after incubation					Days after incubation				
		3	6	13	19	25	2	6	9	16	22	5	12	19	27	32	2	6	9	16	25	3	6	13	19	25
Acetamide-2-cyano	6.34							X	-				X	-							X					
Ethanol	6.48		X		-	-	X	+	+	+	+	X	++	+++	+++	+++	X	+	+	+	+	X	++	++	++	++
Acetone	6.77						X	+	++	++	++	X	+	+	+	+	X	-	+	+	+	X	+	+	+	+
Isopropyl alcohol	6.96		X	+	+	+		X	+	+	+			X	+	+	X		+	+	+		X	+	+	+
Furan	7.06	X	+				X	+	-	-	-	X	+	++	++	++	X	+	+	+	+	X	-	-	-	
Pentane	7.20												X	+	+	+			X	+	+					
1,4-Pentadiene	7.32	X	++	++	++	++						X	+	+	-	-		X	-	-	-	X	+	-	-	-
Cyclopropylcarbinol	7.38		X	+	+	+		X	-	+	+														X	-
Methyl iodide	7.47		X	-	-					X								X	+	+	+	X	+	+	+	+
Dimethyl sulfide (DMS)	7.72											X	=	+	+	+										
1-Propanol	8.57																	X	+	=	-					
2-Butanone	9.21	X	+	+	+	+			X	+	-	X	+	+	+	+	X	+	++	++	++	X	+	+	+	+
1-Hexene	9.50		X	-	-	-																				
2-Butanol, (R)	9.55																		X							
2-Methylfuran	9.75		X									X	+	++	++	++		X	++	++	++	X	+	-	-	-
Trichloromethane	9.95		X				X	+	+	+	-	X	+	+	=	+						X	-	-	-	
3-Methylfuran	10.02																X	+	+	+	+					
Tetrahydrofuran	10.39		X									X	-	-		+		X	-	+	+					X
2-Methyl-1-propanol	10.46																X		+	+	+					
1-Butanol	11.37																			X					X	-
Benzene	11.43							X										X	-	-						
2-Pentanone	11.87								X	+	-							X	=	+	+					
1,3-Dimethylcyclopentane	12.45	X	+	+	+	-											X	=	-	-	-	X	=	-	=	-
2,5-Dimethylfuran	12.73									X	-									X	+			X	+	+
Heptane	12.75									X	-									X	-					
2-Heptene	12.89																					X	+	=	-	-

3-Methyl-1-butanol	13.43	X	+	+	+	-	X	+	+	+	+	X	+	+	+	+	X	+	+	+	+		X	+	-	-
2-Methyl-1-butanol	13.54												X	+	+	+	X	+	+	++	+		X	+	+	+
Dimethyl disulfide (DMDS)	13.64									X	-	X	-													
2,5-Dimethylhesane	13.76											X	-													
2,3,4-Trimethylpentane	14.25											X														
Propanoic acid, 2-methyl-ethyl ester	14.25											X						X	+	+	+					
Acetic acid, butyl ester	14.31																			X						
Toluene	14.38																X		-	+						
2,3-Dimethylhexane	14.63											X	+	+	+	+										
2-Octene	15.09		X	=	+					X	-															
1-Octene	15.10						X	+	+	+	+															
Octane	15.36				X	+																				
2,3,5-Trimethylfuran	15.49																			X	+					
Tetrachloroethylene	15.62							X						X	+	+		X								
1,3-Octadiene	15.78		X	-								X	+	=	+	+										
2,4-Dimethylheptane	15.95				X	+				X	=	X	-	-	-	-				X				X	=	+
Acetic acid, trichloro, ethyl esther	16.41											X	-	-	-	+										
1-Butanol-3-methyl, acetate	16.79																	X	+	+	+					
p-Xylene	16.92											X	-	=	=	+										
Styrene	17.35									X							X	+		+	=		X	+	+	+
2-Butoxyethanol	17.50											X		+		-	X									
1,5-Dimethyl-1,4-cyclohexadiene	17.60					X																				
2-Octen-1-ol	18.93	X	+			-				X		X		=		+										
3-Octanone	18.99		X	-	+					X		X														
2,2,6-Trimethyloctane	19.25											X	-	-	-	-										
Decane	19.56											X	-		-	-	X									
Limonene	20.21											X	+	+	=	+		X								
2,6,10- Trimethyldocedane	20.75											X	-	-	-	-										

Table 4.9 Overview of weekly MVOC production by selected fungal species grown on MEA15% as substrate. X: compounds with distinct signal (TIC > 10^6). Peak area/peak area$_1$ >1: +, >10: ++, >100: +++, =1: =, <1: -.

Substances	**RT (min)**	***A. penicillioides***				***E. halophilicum***								
		Days from incubation				**Days from incubation**								
		8	**14**	**21**	**28**	**5**	**12**	**19**	**26**	**33**	**40**	**47**	**54**	**61**
Acetamide,2-cyano	6.43					X	+	-	-	-				
Ethanol	6.48					X	+	=	=	=	=	=	+	+
Acetone	6.77	X	+	+	+		X	+	+	++	++	++	++	++
Isopropyl alcohol	6.93	X	+	+	+	X	+	+	+	+	+	+	+	+++
Furan	7.06	X	-											
Pentane	7.20		X	-										
1,4-Pentadiene	7.32	X	+	+	+	X	+	+	+	++	++	++	++	++
Methyl iodide	7.47													X
2-Butanone	9.21	X	+	+	+		X	++	+++	+++	+++	+++	+++	+++
2-Butanol, (R)	9.55								X	+	+	+	+	+
2-Methylfuran	9.75	X	+						X	+	+	+	+	+
Ethyl acetate	9.88										X	+	+	+
Tetrahydrofuran	10.39	X	+	+	+	X	+	+	+	+	+	+	+	+
3-Methyl-2-butanone	11.17	X	+	-	-									
1-Butanol	11.37	X	-			X	+	+	+	+	+	+	+	+
Heptane	12.75		X											
3-Methyl-3-buten-1-ol	13.30	X	+	+										
3-Methyl-1-butanol	13.43	X	+	+	-	X	+	+	+	+	+	+	+	+
2-Methyl-1-butanol	13.54	X	+	+	+									X
1-Pentanol	14.25	X	+	=										
2,3,4-Trimethylpentane	14.25	X	+	=										
Propanoic acid, 2-methyl-, ethyl ester	14.25	X	+	-										
Acetic acid, butyl ester	14.31	X	++	-	-									
Toluene	14.38	X	+	+	+					X	+	-	+	+
Cyclopentanone	14.60	X	+	+	+					X	+	=	+	+
2,3-Dimethylpentane	14.63				X									
2-Penten-1-ol	14.69				X									
1-Octene	15.10	X	+	-										
Tetrachloroethylene	15.62					X	+	+						
1,3-Octadiene	15.78	X	+	-	-									
Ethylbenzene	16.74		X	+		X	++	=	+	+	=	=	+	++
1-Butanol,3-methyl, acetate	16.79													X
p-Xylene	16.92		X	-			X	-	-	-				
Styrene	17.35		X	=			X			-				
o-Xylene	17.47		X	-			X		-	-				
2-Butoxyethanol	17.50													X
2-Octen-1-ol	18.93	X	-	-										
3-Octanone	18.99	X	+	+	-									

We observed mainly aldehydes in the monitored blank substrates (MEA and MEA15%) (Fig. 4.13). The medium MEA was characterized by 79% of aldehydes, mainly butanal-3-methyl (50%), butanal-2-methyl (16%) and propanal-2-methyl (14%). Low quantities of ethers, *i.e.* furan (6%), ketones (*i.e.* acetone, 7%) and haloalkane (*i.e*. trichloromethane, 2%) were also detected.

The composition of the medium MEA15% was quite similar in comparison to the medium MEA. It was also characterized by mainly aldehydes (71%), with butanal-3-methyl (44%), propanal-2-methyl (16%) and butanal-2-methyl (9%). Ketones were also detected (16%, *i.e.* acetone, 9% and 2-butanone, 1%), as well as haloalkane (tetrachloroetilene, 5%), nitrile compound (acetonitrile, 4%), ether (furan, 2%) and several other compounds with quantities <1%.

The main difference between the two blanks is the total amount of MVOC production: MEA15% medium produced less than the half amount of volatile compounds respect to MEA medium (Fig. 4.15), probably because of the presence of 15% of sodium chloride in the medium. The salt, considered as a part of solute in a complex semi-solid water solution (agar medium), could be responsible for the total vapour pressure lowering and, as a consequence, the general decrease of gas quantities at the same temperature. The results for each fungal species will be discussed in the following paragraphs. All chromatograms as well as the concentration data for each fungus are reported in Appendix B5.

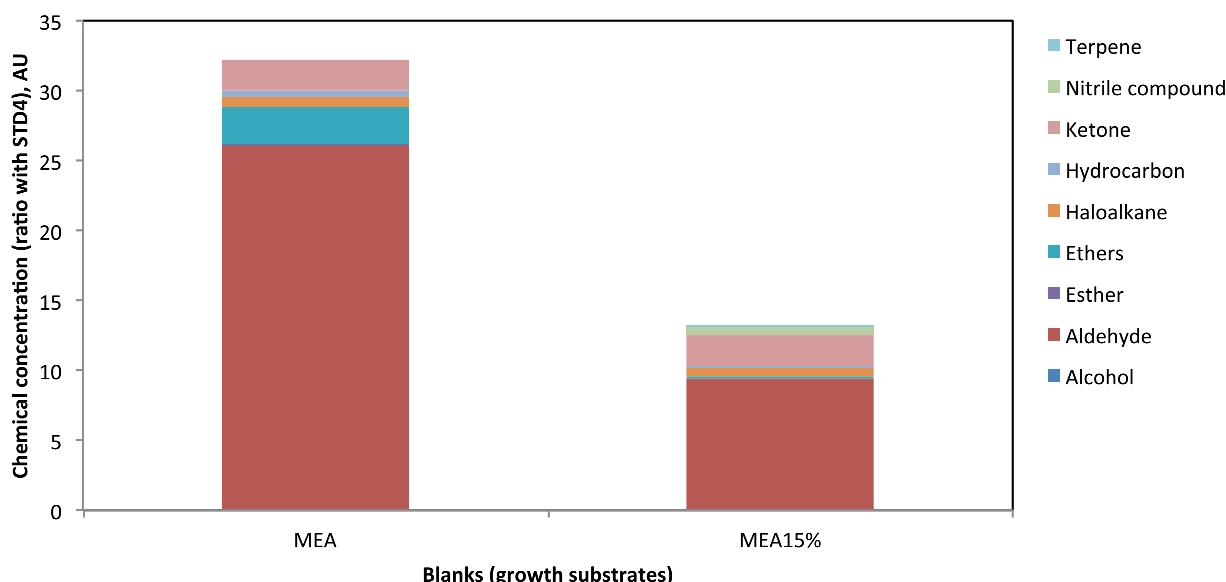


Figure 4.15 Chromatograms of blanks MEA and MEA15%, with their volatile organic compound composition, specified by types of chemical compounds.

4.3.2.1 Aspergillus creber

In the first days of incubation (3° day of incubation, corresponding to exponential fungal growth) the strain of *Aspergillus creber* produced only a few compounds, mainly ether (furan, 72%), hydrocarbons (1,3-dimethylcyclopentane, 10% and 1,4-pentadiene, 5%) and alcohols (3-methyl-1-butanol, 7%, and 2-octen-1-ol, 6%) (Fig. 4.16).

After six days of incubation, which corresponds to the plateau of growth curve and therefore to the oxygen limitation phase, the fungus produced the highest variety of chemical compounds and the highest amounts of MVOCs. In particular alcohols, such as ethanol (35%), isopropyl alcohol (3%), 3-methyl-1-butanol (2%), 2-octen-1-ol (2%) and cyclopropylcarbinol (1%) were produced, followed by ethers, as furan (23%), tetrahydrofuran (2%) and 2-methylfuran (1%). The increase of hydrocarbons was also recorded, especially for 1,4-pentadiene (11%) and the production of 1,3-octadiene (5%). The presence of haloalkanes, *i.e.* trichloromethane (4%) and methyl iodide (2%), was detected, as well as ketones (2-butanone, 1% and 3-octanone, 1%).

In the following analyses, performed at 13, 19 and 25 days after *A. creber* incubation, we observed the disappearance of ethers and the general increase of hydrocarbons (47-56%), especially 1,4-pentadiene (up to 32%) and 1,3-octadiene (up to 10%), and alcohols (34-53%), in particular ethanol (up to 28%) and isopropyl alcohol (18%). Also the ketones increased (2-butanone 9% and 3-octanone 6%), but they disappeared in the last analysis, as well as the haloalkanes.

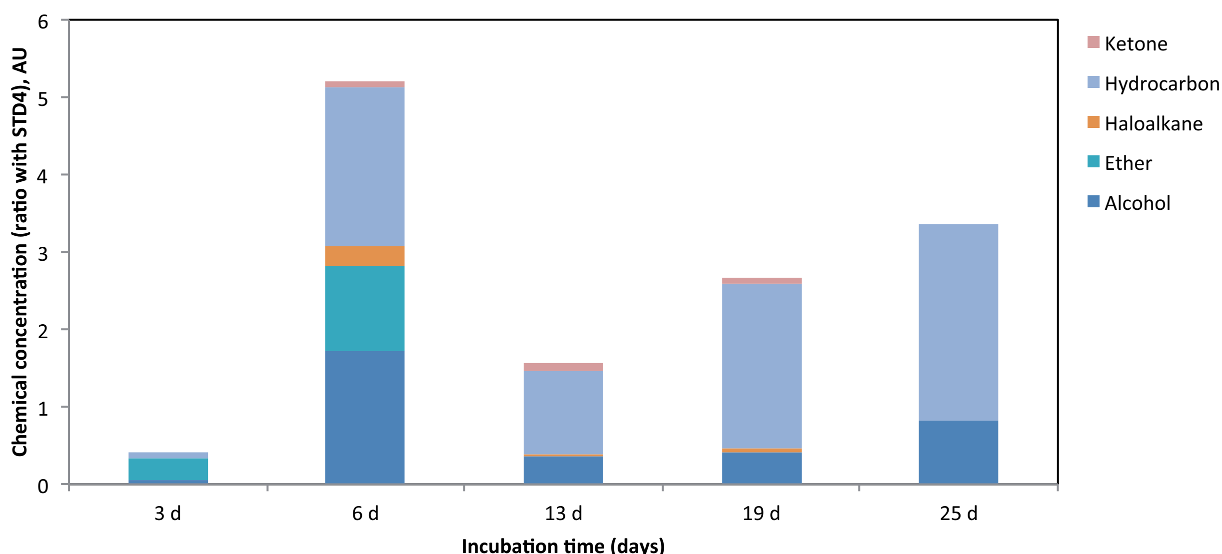


Figure 4.16 MVOC production by *A. creber* explicated as type of chemical compounds during the monitoring period.

If we look to the major MVOCs produced by *A. creber*, such as 1,4-pentadiene, 1,3-octadiene, ethanol, isopropyl alcohol, 3-methyl-1-butanol, 2-octen-1-ol, 2-butanone, furan and 2-methylfuran, we can observe a similar trend as the graph explicated for chemical groups (Fig. 4.16): an increase of the compounds was

detected until the 2nd day of sampling (after 6 days of incubation), subsequently followed by a general decrease (Fig. 4.17). This trend could also be associated with the BOD trend, because of the rise of compounds as ethanol, isopropyl alcohol and 2-butanone in the followed sampling days as indicators of anaerobic fermentation.

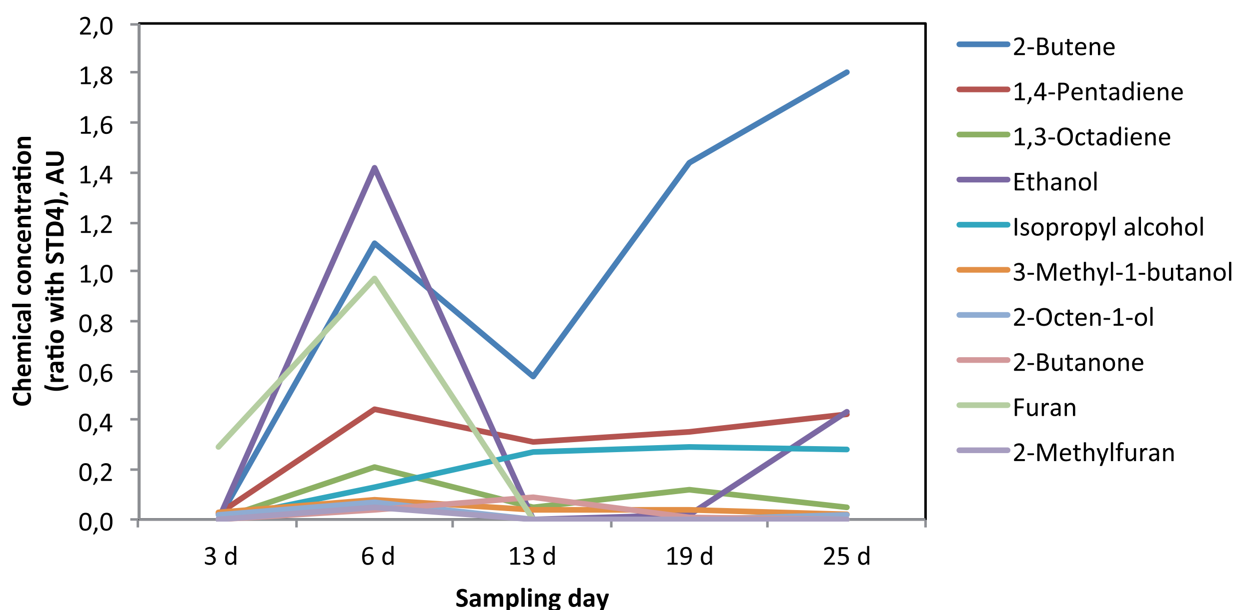


Figure 4.17 Temporal trends of principal MVOCs emitted by *A. creber*.

Due to the similarity to the known species *Aspergillus versicolor*, both belonging to *Aspergillus* section *Versicolores*, the MVOC production of *A. creber* was compared to literature data. Several studies investigated the MVOC production of *A. versicolor* grown on MEA medium (Fiedler *et al*., 2001; Polizzi *et al*., 2012; Schuchardt and Kruse, 2009; Sunesson *et al.,* 1995). They reported similar compounds compared to our results with, *e.g.* 2-octen-1 ol and 3-octanone. From DG18 medium, Sunesson *et al*. (1995) detected 1,3-pentadiene, 1-octene, 3-methyl-1-butanol and 3-methylfuran, similar to our findings. Fiedler *et al*. (2001) and Polizzi *et al.* (2012) reported compounds that were not detected in this study, *i.e*. heptane, undecane, acetone, 4-methyl-1-hepten-3-one, toluene, p-xylene, several terpenes and sesquiterpenes. In our analyses, terpenes and sesquiterpenes were not detected, because the limitations of the applied method. Direct aspiration techniques are capable to detect compounds up to C12, while with solid phase micro extraction (SPME) device and subsequently thermal adsorption it is possible to detect compounds with a higher molecular weight, as applied in the previous studies by Polizzi *et al.* (2012). Also 1,3-dimethoxybenzene, recognized as a marker of this species, was not detected in this study. However, also Fiedler *et al*. (2001) did not detect this compound in any of their analysed cultures of *A. versicolores*, so it is possible that the MVOC production, especially for some compounds, depends on the strain.

4.3.2.2 Aspergillus penicillioides

Due to its slower growth in comparison with other fungal species, the *A. penicillioides* strain started to being monitored after 8 days. This first analysis corresponded to the exponential growth phase (Par. 4.3.1), and several compounds were present in high amounts (*e.g*. 1,3-octadiene, 2-octen-1-ol), which tented to decrease in the following analyses.

Initially, half of the MVOC production was composed by ketones, especially acetone and 2-butanone that increased during the 28 days of monitoring (32-73% and 5-15%, respectively) (Fig. 4.18). Elevated production of 3-octanone was also observed, with the highest values after 14 days of incubation (21%). Several alcohols were produced, in particular isopropyl alcohol that increased with the incubation time (0.7-2.5%) or 2-octen-1-ol that decreased until the end of the observations (20-0%). Similar to the behaviour of 1,3-octadiene, found in high percentage at the beginning (13%) and disappeared until the end (0.2%), as well as the ethers (i.e. furan, 2-0%, 2-methyl furan, 0.8-0% and tetrahydrofuran, 0.3-0.2%) or the haloalkane trichloromethane (5-0%). Overall, the quantities of ketones increased, while those of alcohols, hydrocarbons, ethers and haloalkane decreased.

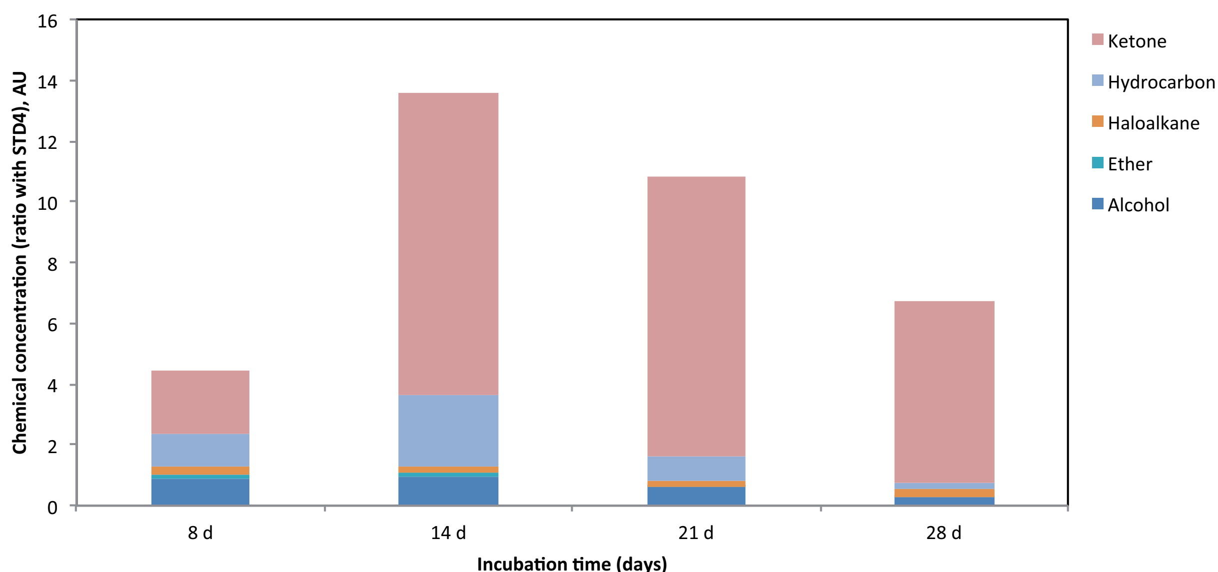


Figure 4.18 MVOC production explicated as type of chemical compounds by *A. penicillioides* during the monitoring period.

If we consider the principal MVOCs emitted by this species, as 1,3-octadiene, isopropyl alcohol, 2-octen-1-ol, acetone, 2-butanone, cyclopentanone and 3-octanone, their maximum production corresponded to the 2nd sampling day (after 14 days of incubation) (Fig. 4.19). For 2-octen-1-ol, instead, a general decrease was observed, probably because it was typical for the initial growth phase of the fungus.

Wady *et al*., (2003) analysed MVOCs of *A. penicillioides* grown on DG18 and reported the detection of 2-pentanol, 2-heptanone, 3-octanone, 1-octen-3-ol and 2-methyl-1-butanol and, for heated cultures, also

methyl benzoate. In our case, most of these compounds were found together with other alcohols, ketones, ethers and hydrocarbons (see Table 4.9).

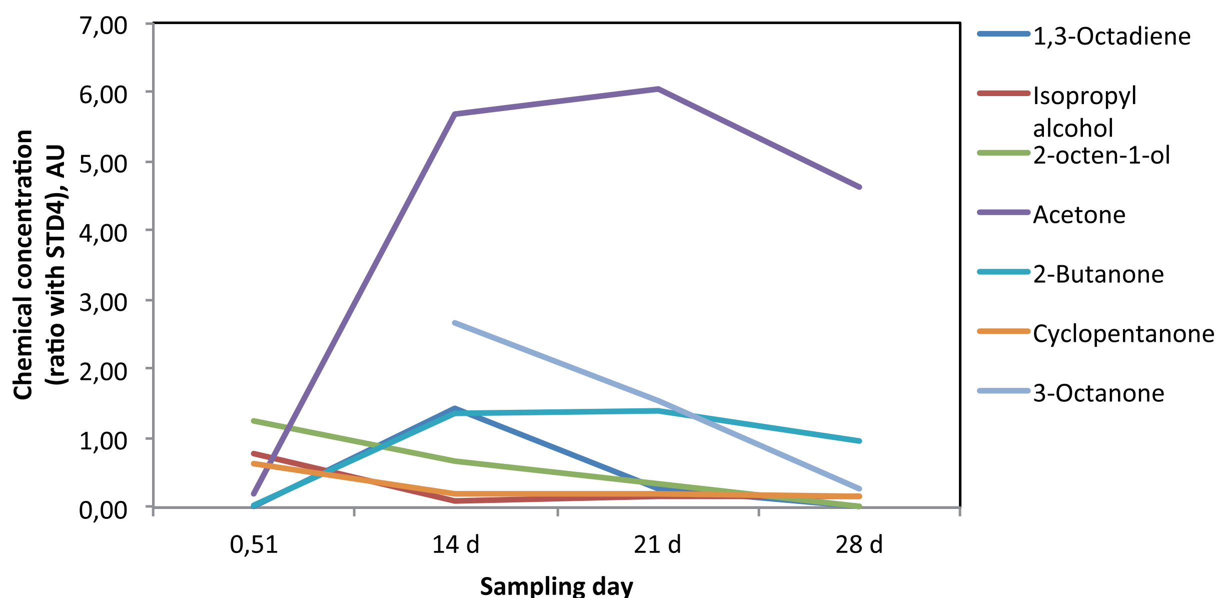


Figure 4.19 Temporal trends of principal MVOCs produced by *A. penicillioides*.

4.3.2.3 Cladosporium cladosporioides

In the beginning of MVOC monitoring the strain of *C. cladosporium* was characterized mainly by ether (furan, 58%) and alcohols (ethanol, 21% and 3-methyl-1-butanol, 4%) productions. In addition, trichloromethane (9%), acetone (6%) and 1-octene (1%) were detected in small quantities (Fig. 4.20), similar to *A. creber*. Compared to the BOD results, this analysis corresponded to the beginning of exponential growth phase of the fungus (see Fig. 4.14).

The analysis after 6 days of incubation was characterized by the occurrence of acetamide-2-cyano (11%), cyclopropylcarbinol (4%), isopropyl alcohol (2%) and tetrachloroetilene (1%). After 9 days, corresponding to the end of the exponential growth phase, the general chemical composition changed. High quantities of ketones were detected (acetone, 36%, 2-butanone, 3%), while those of ethers (*e.g.* furan, 22%), alcohols (*e.g.* ethanol, 18%), haloalkane (trichloromethane, 5%) and amide (acetamide-2-cyano, 4%) started to decrease. This similar composition characterized the analyses after the 16th and 22th day. Interestingly, there was a unique detection after 16 days of 2-octen-1-ol and 3-octanone, two well-known fungal volatile compounds (Sunesson *et al.,* 1995).

The trend of principal MVOCs emitted by *C. cladosporioides* presented acetone, ethanol and 2-butene as major compounds, which influenced the general emission trend of each related chemical compound (Fig. 4.21). Furan was stable over the entire monitored period, similar to 2-butanone which presented lower ratios between peak area/peak area STD4.

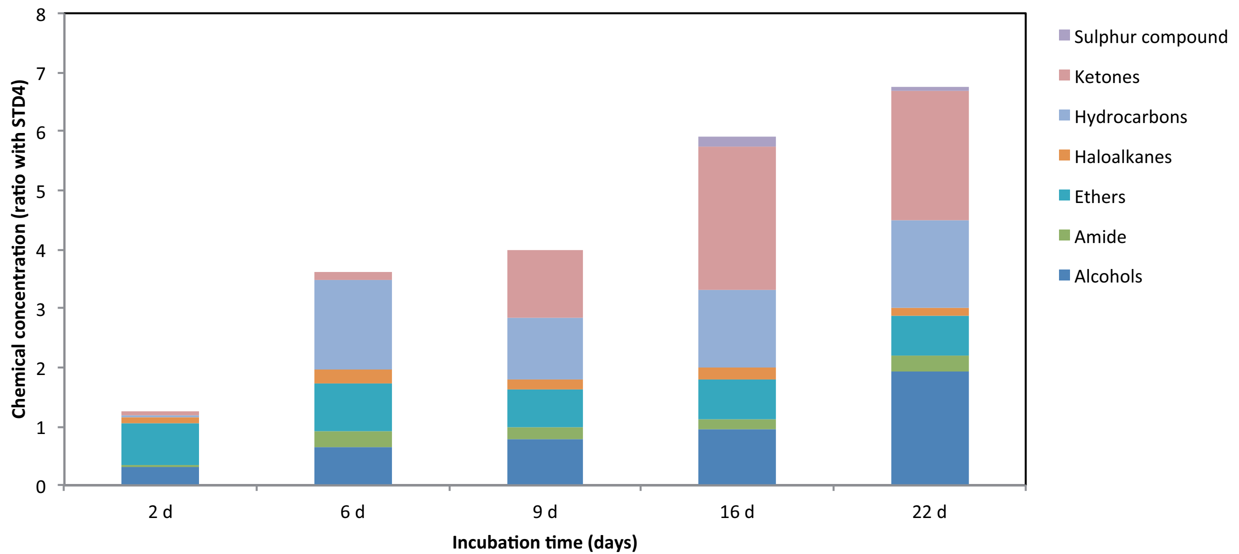


Figure 4.20 MVOC production explicated as type of chemical compounds by *C. cladosporioides* during the monitoring period.

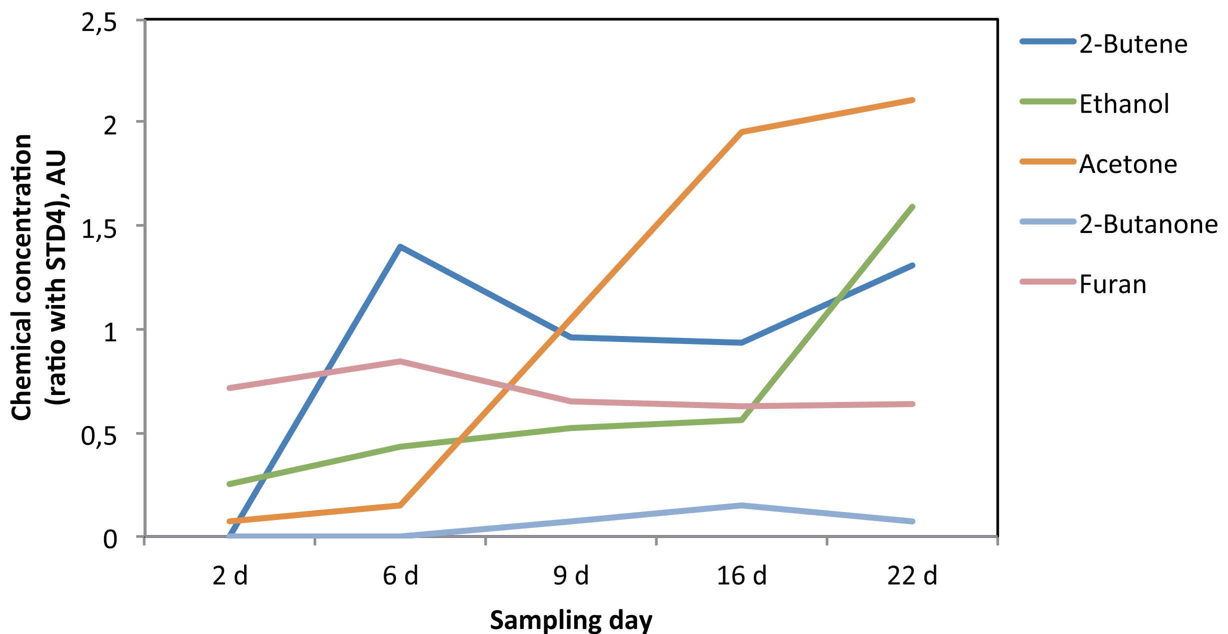


Figure 4.21 Temporal trends of principal MVOCs emitted by *C. cladosporioides*.

Because of its high frequency in mouldy indoor buildings, *C. cladosporioides* was already studied for MVOC production. However, it was generally recognised as a low volatile organic compound producer. Sunesson *et al*. (1995) did not detect any compounds on MEA and only 1-octene, 3-methylfuran and 3-pentanone on DG18. On the same medium, Matysik *et al.* (2009) found also 3-methyl-1-butanol, 1-octen-3-ol, 3-methyl-3-buten-1-ol, 2-ethyl-1-hexanol, 2-pentanone, 3-octanone, cycloexanone, 3-hydroxybutanone and ethyl acetate. Matysik *et al*. (2008) reported the occurrence of 2-pentanol, tetradecene, pentadecene, 1,3-

nonadiene, 2-pentanone, 2-heptanone, pyridine and sesquiterpenes from wallpaper. Considering the various growth conditions (in particular different culture media) in the previous studies, we suggest that 1-octene, 3-methyl-1-butanol, 2-octen-1-ol, 2-pentanone and 3-octanone may be recognised as general markers of *C. cladosporioides*.

4.3.2.4 Eurotium chevalieri

The MVOC monitoring of the strain of *E. chevalieri* started in correspondence to the end of exponential growth phase (see Fig. 4.3.1). After 5 days of incubation, high quantities of hydrocarbons (76%), in particular of 1,4-pentadiene (71%), followed by ketones (15%), with acetone (13%) and ethers (*e.g.* furan, 5%), were measured (Fig. 4.22). After 12 days the data demonstrated an increase of ketones (acetone, 25% and 2-butanone, 2%), alcohols (*e.g.* ethanol, 13%) and ethers (*e.g.* furan, 10%).

During the following analyses, a decrease of hydrocarbons, especially of 1,4-pentadiene (35-16%) was detected, but an increase of alcohols (*e.g.* ethanol, 14-24%, and isopropyl alcohol, 0.6-1.3%) and ethers (*e.g.* furan, 20-29% and 2-methyl furan, 0.8-1.4%) was observed.

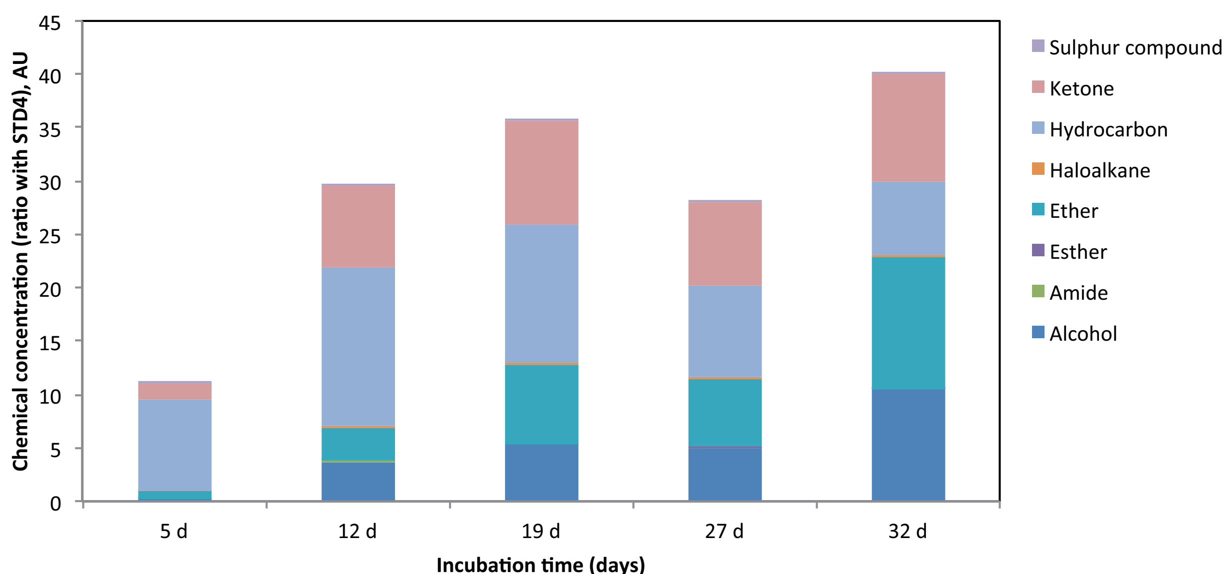
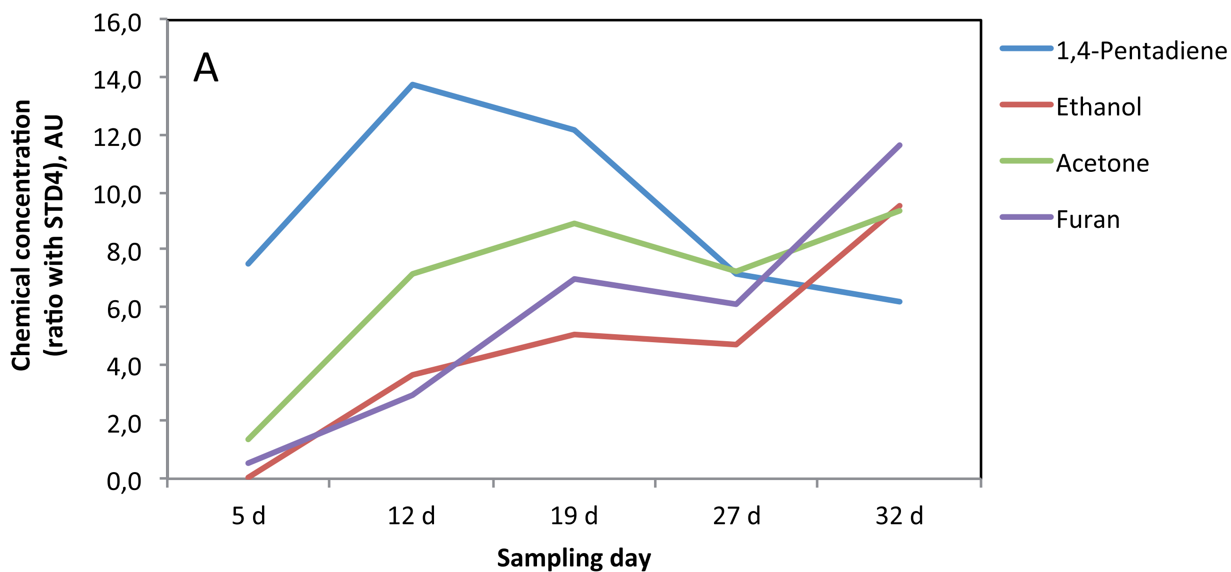


Figure 4.22 MVOC production by *E. chevalieri* explicated as type of chemical compounds during the monitoring period.

Considering the principal MVOCs emitted by *E. chevalieri*, they can be divided in two groups for better identification of all the trends in higher and lower peak area substance/peak area STD4 ratio. A similar trend was observed with a continuous increase until the last day of analysis (32 days) (Fig. 4.23). All compounds followed this trend, except of 1,4-pentadiene whit highest abundance after 12 days, followed by a slow decrease.

This fungus was identified in the group of the faster growing species, reaching the plateau already after 4 days. It means that all the production reported for this fungus corresponded to its anaerobic phase.



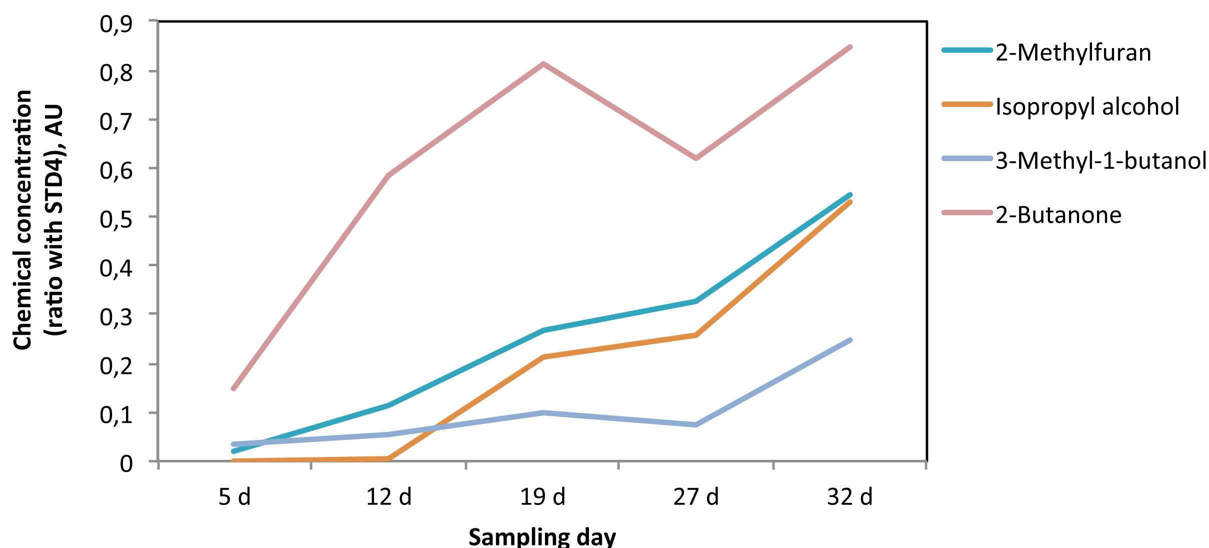


Figure 4.23 Temporal trends of principal MVOCs of *E. chevalieri* divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (0 < ratio < 15); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 0.9).

In a previous study, Elke *et al*. (1999) found a correlation between *Eurotium* species, isolated from indoor dust samples on DG18 medium, with high indoor air concentrations of 3-methylbutan-1-ol, 3-methylbutan-2-ol, heptan-2-one, hexan-2-one, octan-3-ol and thujopsene. We also found several alcohols and ketones (see Tab. 4.8) and they could be considered as possible markers of this species.

4.3.2.5 Eurotium halophilicum

It is known that *E. halophilicum* is a slow growing fungus and because of this, the monitoring was performed for 2 months (61 days). However, already during the first MVOC sampling (after 5 days of incubation) it was possible to observed already changes in the chemical composition inside the culture bottle (see chromatograms reported in Appendix B5). All the aldehydes disappeared (butanal-3-methyl and butanal-2-methyl) and the corresponding alcohol, 1-butanol-3-methyl (50%), appeared together with other alcohols, as isopropyl alcohol (13%), ethanol (5%) and 1-butanol (4%). High quantities of 1,4-pentadiene (11%), tetrahydrofuran (9%) as well as acetamide-2-cyano (5%), ethyl benzene (3%) and tetrachloroethylene (3%) were measured (Fig. 4.24).

After 12 days, the quantity of the alcohols started to decrease (72-26%), while ketones appeared, *e.g.* 2-butanone (30%) and acetone (7%). A low quantity of the hydrocarbon 1,4-pentadiene was recorded, but the increase of ethyl benzene (11%) and the appearance of other aromatic compounds, as p-xylene (8%), o-xylene (4%) and styrene (2%) was observed.

After 19 days until the end of monitoring a similar chemical compositions were observed. Ketones 2-butanone and acetone were the main compounds (83-92%), followed by alcohols (11-5%, *e.g.* 1-butanol-3-methyl, 9-1%), hydrocarbons (3-5%, *e.g.* 1,4-pentadiene, 3-2%, and ethylacetate, 2-3%), haloalkane (*e.g.* tetrachloroethylene, 2-0%) and ethers (*e.g.* tetrahydrofuran 2%-<1%).

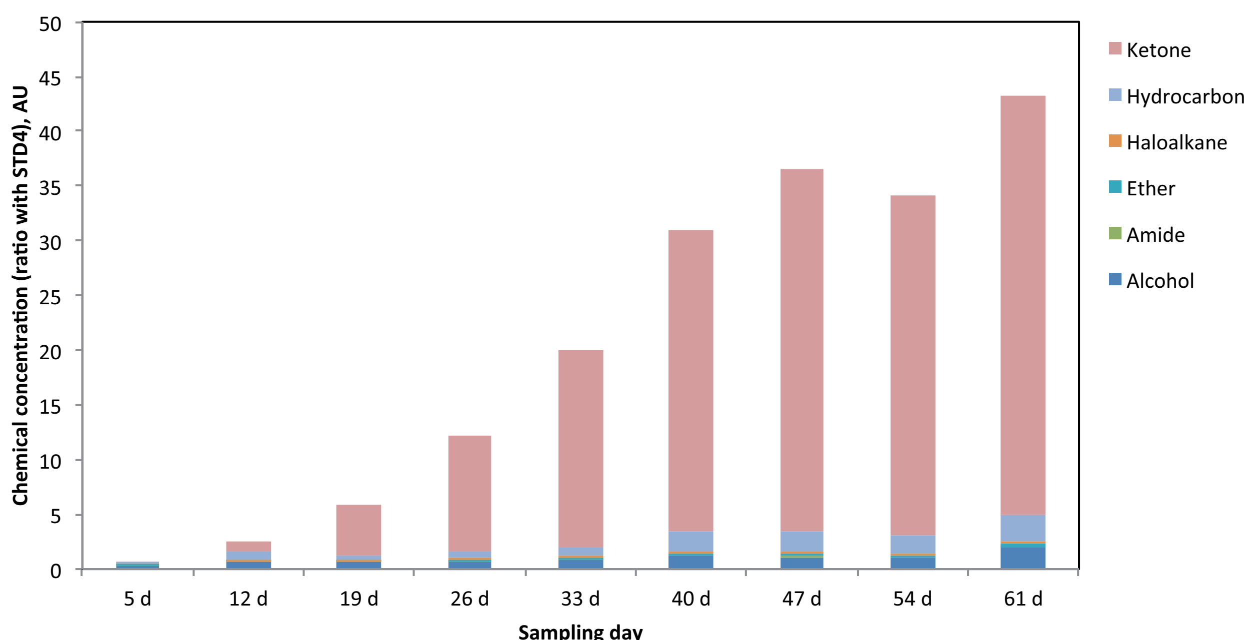


Figure 4.24 MVOC production by *E. halophilicum* explicated as type of chemical compounds during the monitoring period.

Little is known about this fungus (see Par. 1.2.1) and no data of its MVOC production is available. Our results demonstrated its capacity to produce a wide range of volatile compounds, especially ketones (acetone and 2-butanone) that clearly increased during its growth. As high relevance volatile compounds

1,4-pentadiene, ethyl acetate, 2-butanol, 3-methyl-1-butanol, tetrahydrofuran and 2-methylfuran were determind. Their trends are following the slow growth of the fungus. For 3-methyl-1-butanol and 1,4-pentadiene the highest production corresponded to 40 and 47 days of incubation, respectively, followed by a decrease. Furthermore, ethyl acetate and ethanol appeared after around 1 month of incubation and increased fast in the last part of the monitoring period (Fig. 4.25).

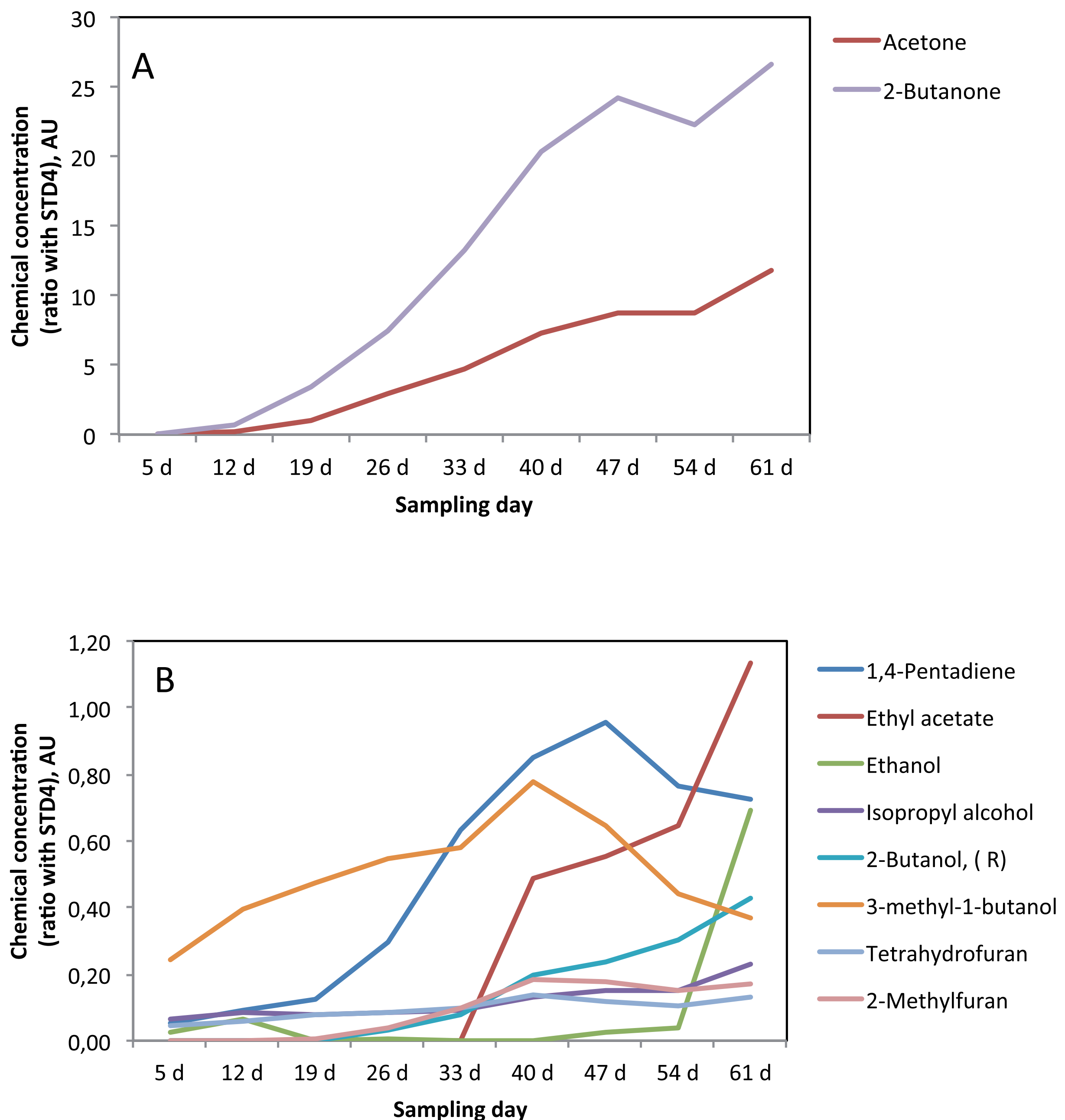


Figure 4.25 Temporal trends of principal MVOCs of *E. halophilicum* divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (0 < ratio < 30); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 1.2).

4.3.2.6 Penicillium brevicompactum

The early MVOC production (2 days of incubation) of *P. brevicompactum* was characterized by ketones production (58%), especially acetone (57%). Alcohols were present with 27% (*e.g.* ethanol, 21% and isopropyl alcohol, 2%). The remaining part was mainly composed by ethers (11%, *e.g.* furan, 9% and 3-methylfuran, 2%) and aromatic hydrocarbons (*e.g.* styrene, 4%).

After 6 days, which corresponded to the end of exponential growth phase (see Fig. 4.3.1), an increase of alcohols, especially ethanol (52%) was detected, as well as ethers (19%, *e.g.* furan, 17%), while ketones decreased (acetone, 19%). Until the end of the monitoring, high amounts of alcohols (*e.g.* ethanol, 42-13%, isopropyl alcohol, 2-3%, 3-methyl-1-butanol, 4-5%), ketones (*e.g.* acetone, 22-41%, 2-butanone, 1%) and ethers (*e.g.* furan, 20-30%) were observed, together with small amount of hydrocarbons (*e.g.* styrene, 3%) and haloalkanes (<1%) (Fig. 4.26).

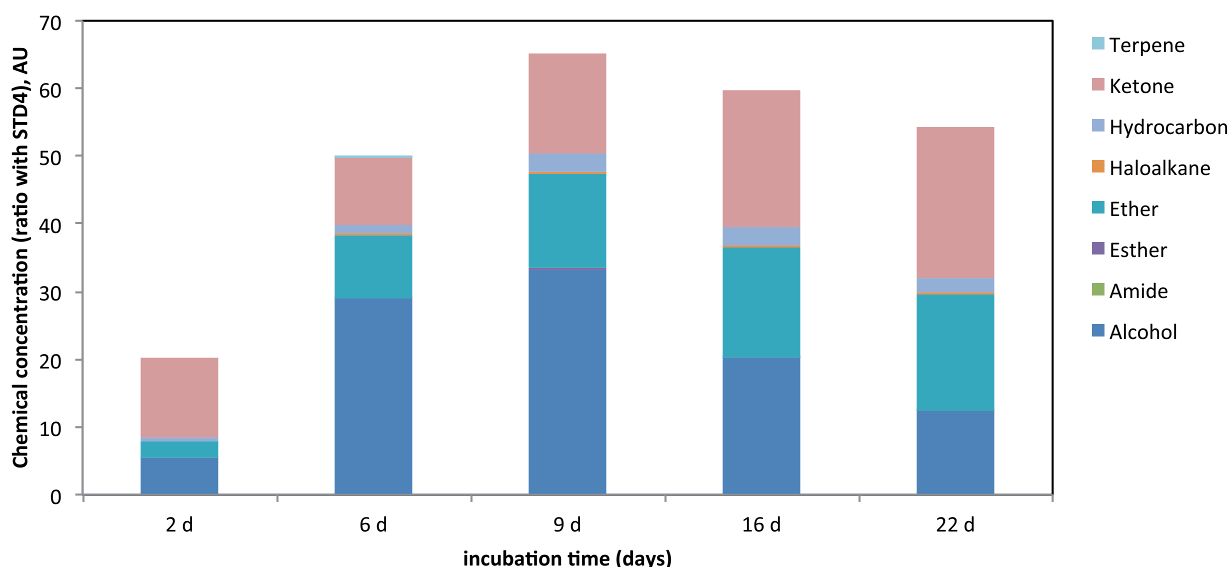


Figure 4.26 MVOC production by *P. brevicompactum* explicated as type of chemical compounds during the monitoring period.

The major MVOCs emitted by our strain of *P. brevicompactum* were: styrene, ethanol, isopropyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, acetone, 2-butanone, furan and 2-methylfuran. For these compounds it was possible to follow their temporal emission during all the monitored period (Fig. 4.27).

A previous study identified also cyclohexanone, methylisobutylketone, 1-hexene and ethyl acetate emissions from this species grown on DG18 (Maysik *et al*., 2008). On wheat, Borjessön *et al.* (1992) detected several compounds in common with our study, *e.g.* acetone, 3-methylfuran, 3-pentanon, 2-methyl-1-propanol and 2-butanone. These compounds could be considered possible markers of this species.

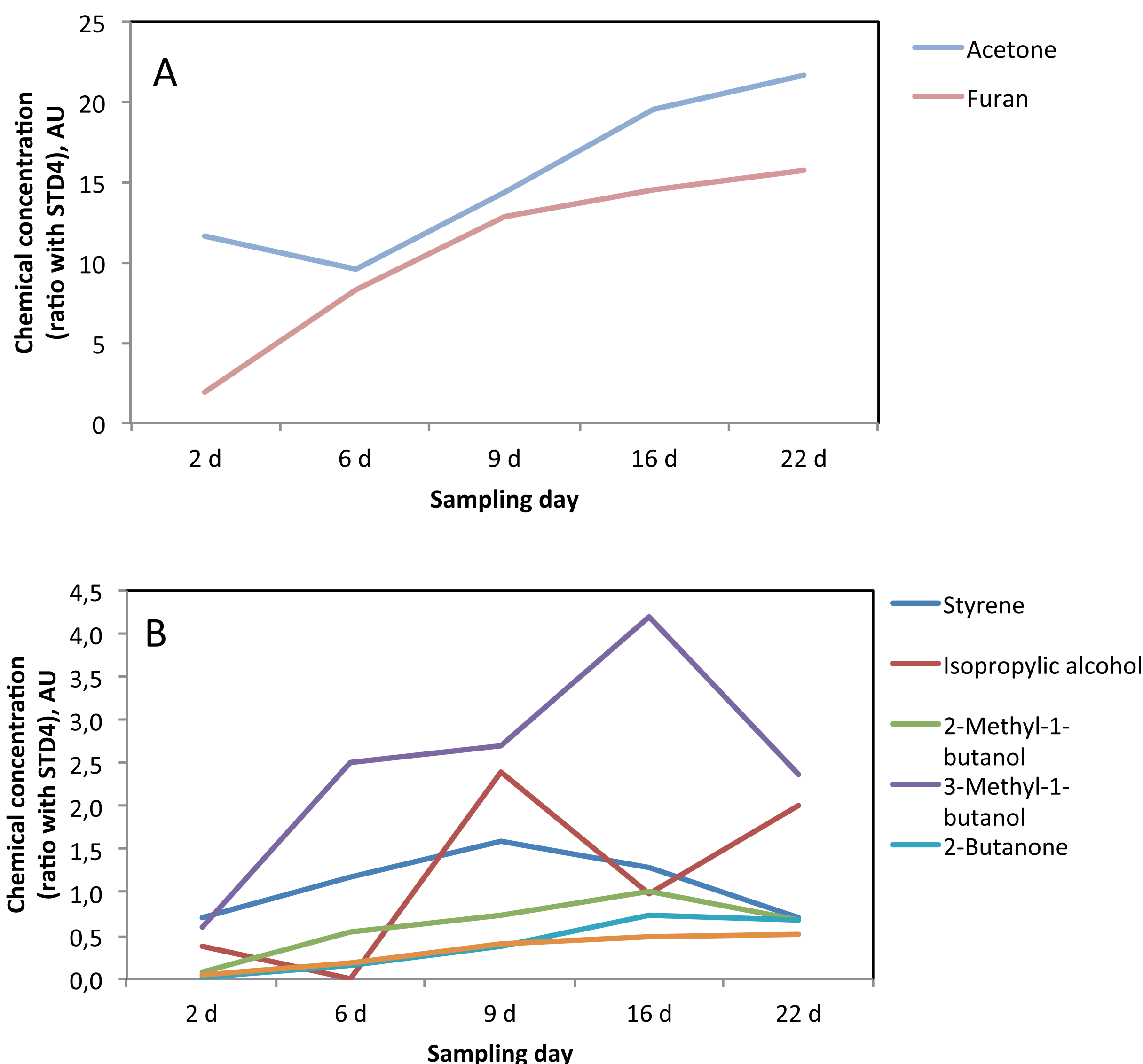


Figure 4.27 Temporal trends of principal MVOCs of *P. brevicompactum* divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (ratio > 3); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 4.5).

4.3.2.7 Penicillium chrysogenum

Similar to the previous *Penicillium* species, the early growth stage of *P. chrysogenum* was characterized by a high production of acetone (45%). Other main compounds were furan (30%), trichloromethane (13%), 1,4-pentadiene (5%) and 2-methyl furan (2%) (Fig. 4.28).

After 6 days, high quantities of alcohols (ethanol, 11% and 1-butanol-3-methyl) and hydrocarbons, especially styrene (16%), were measured. A general increase of ketones (acetone, 51-59%, and 2-butanone, 1-2%) was recorded during the subsequent measurements, as well as a stabilization of alcohols (*e.g.* ethanol, 14-16%, isopropyl alcohol, 1-3%, 1-butanol-3-methyl, 5-3%) and hydrocarbons (*e.g.* styrene, 14-19%, 1,4-pentadiene, 1%), and the disappearing of ethers (*e.g.* furan, 1-0%).

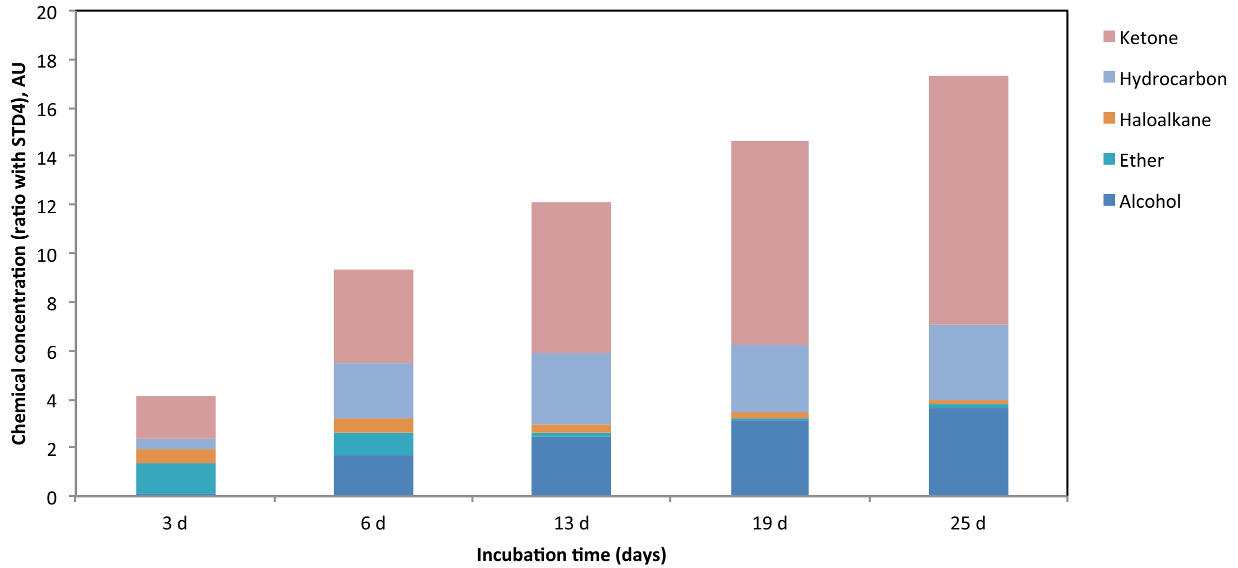
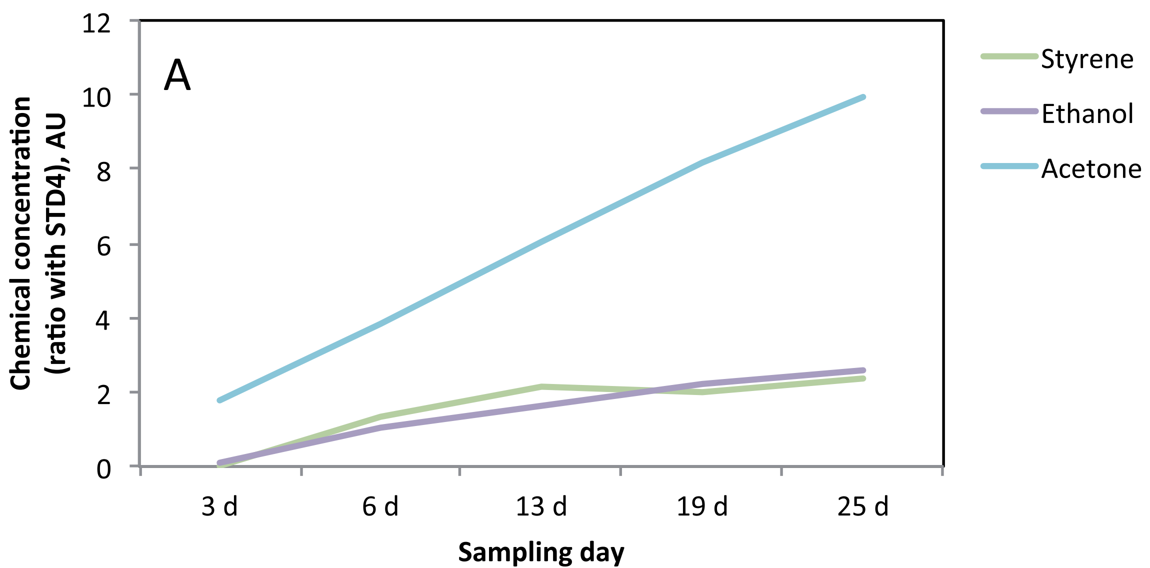


Figure 4.28 MVOC production by *P. chrysogenum* explicated as type of chemical compounds during the monitoring period.

Considering the principal emitted MVOCs, acetone demonstrated a strong increase, as well as ethanol and styrene at a lower concentration level (Fig. 4.29). In addition, isopropyl alcohol, 2-butanone, 2-methyl-1-butanol and 2,5-dimethylfuran showed a general increase and an opposite trend was observed for 1,4-pentadiene, 3-methyl-1-butanol and furan.



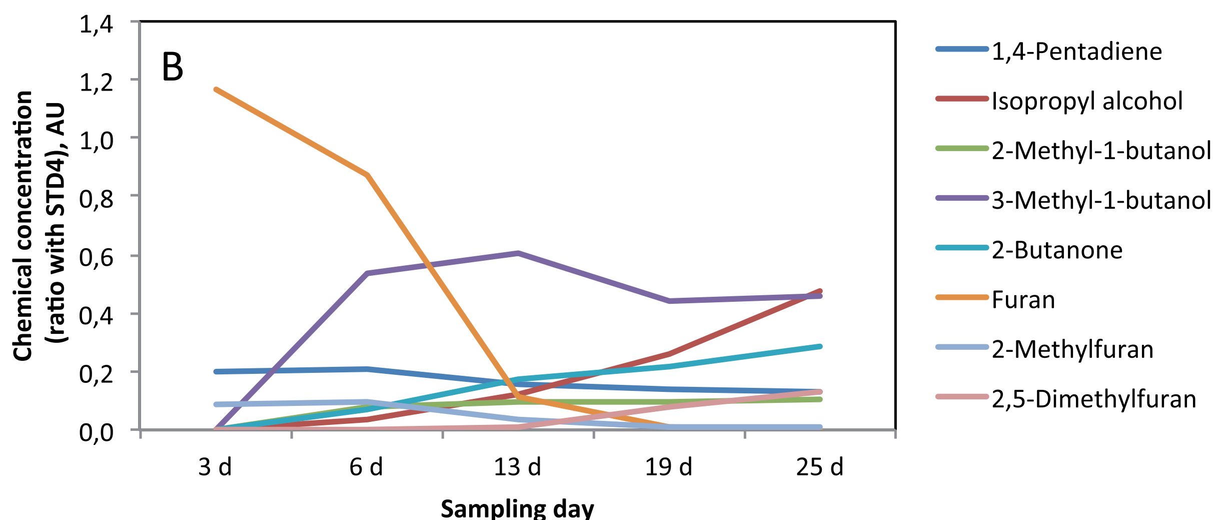


Figure 4.29 Temporal trends of principal MVOCs of *P. chrysogenum* divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (0 < ratio < 10); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 1.2).

Our results are similar to Matysik *et al.* (2008, 2009) for the emission of 3-methyl-1-butanol, 2-methyl-1-butanol and 1-heptene on DG18 and with Wilkins *et al.* (2000) that reported 1-heptene, 2-butanol for the cultivation of *P. chrysogenum* on building materials. These compounds could be considered possible markers of this species.

4.3.2.8 Statistical analysis of fungal MVOCs

The GC-MS investigations of selected fungal species have highlighted their ability to produce a wide range of MVOCs belonging to different classes. Applying the Ward's method (see Par. 3.3.2), similarities were observed between species belonging to the same genera, but these relationships often changed during the monitoring period, depending to the age of each colony.

In particular, we could considere three states called A, B and C referred to Fig. 4.30:

A. 1st analysis: 3rd day of incubation for most of the fungi, 8th day for *A. penicillioides* and 19th day for *E. halophilicum*, corresponding to their exponential grown phase (see Fig. 4.14). Unfortunately, *E. chevalieri* is not considered in this graph because of the lack of this analysis;

B. 2nd analysis: 7th day of incubation for most of the fungi, 14th day for *A. penicillioides* and 19th day for *E. halophilicum*, corresponding to the total O_2 consumptions inside the sample bottles (plateau phase, Fig. 4.14) (exponential growth phase for *E. halophilicum*);

C. *in toto* analysis: all MVOCs produced by each fungal species during the monitored periods.

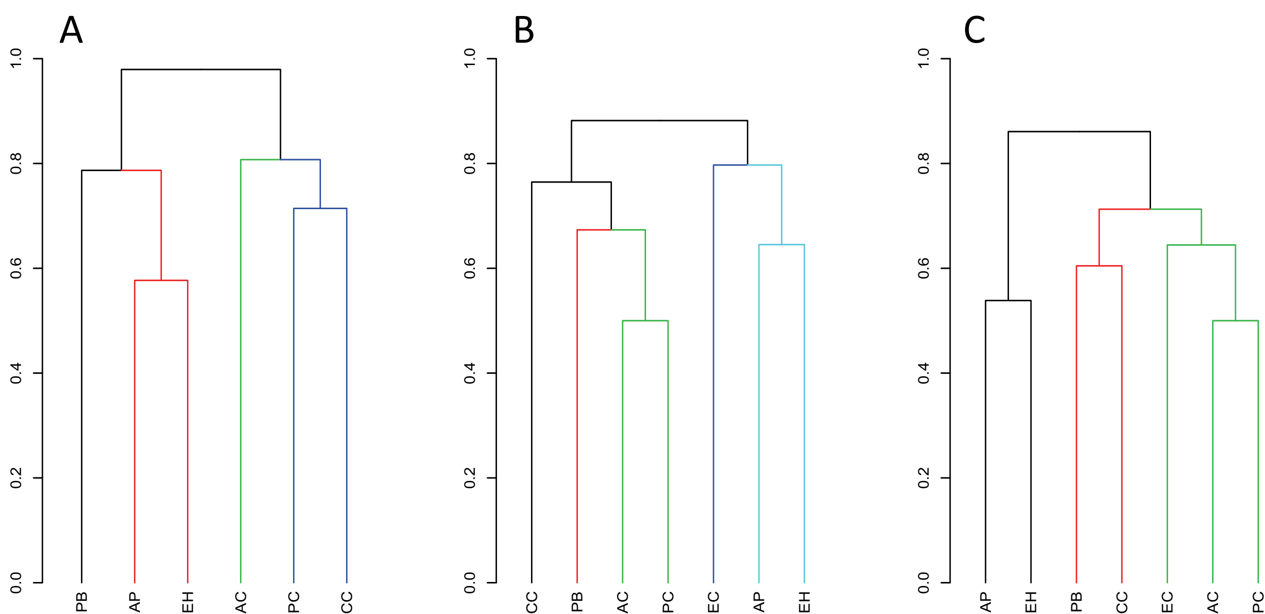


Figure 4.30 Clusters of MVOC production by selected fungal species during different incubation time: A) clustering at t_1 = 3 days of incubation (8th and 19th days for *A. penicillioides* and *E. halophilicum*); B) clustering at t_2 = 7 days of incubation (19th for *E. halophilicum*); C) clustering considering all MVOC production detected at t_{TOT} = all the monitored periods. AC: *Aspergillus creber*; AP*: A. penicillioides*; CC: *Cladosporium cladosporioides*; EC: *Eurotium chevalieri*; EH: *E. halophilicum*; PB: *Penicillium brevicompactum*; PC: *P. chrysogenum*.

Looking to the clusters, all the fungi are assembled in two main groups, which slightly changed depending on the age of the colonies. Considering the y-axes as the dissimilarity δ, with 0 < δ < 1, where 0 is minimal value and 1 is maximal value, at 1st day of analysis *P. chrysogenum* and *C. cladosporioides* formed a first group (δ = 0.7) and then a second group (δ = 0.8) with *A. creber*. On the other side, *A. penicillioides* resulted first linked with *E. halophilicum* (δ = 0.6) and then with *P. brevicompactum* (δ = 0.8). The main groups are linked together at δ = 1 that represents their total diversity.

The 2nd analysis presented differences in clustering: *A. creber* resulted more similar with *P. chrysogenum* (δ = 0.5) then with *P. brevicompactum* (δ = 0.7) and with *C. cladosporioides* (δ = 0.78). The other main group is composed by *A. penicillioides* and *E. halophilicum* (δ = 0.65) together with *E. chevalieri* (δ = 0.8). The two main groups are then linked together at δ = 0.9.

Considering the *in toto* MVOC production, *A. penicillioides* is still together with *E. halophilicum* (δ = 0.56) and it is connected with the other group at δ = 0.9. The opposite part is composed firstly by *A. creber* and *P. chrysonneum* (δ = 0.5), followed by the connection with *E. chevalieri* (δ = 0.65). In parallel, *P. brevicompactum* is linked together with *C. cladosporioides* (δ = 0.6) and then connected to *A. creber*'s group at δ = 0.7.

Analysing all the groups, close relationships resulted between *A. creber* with *P. chrysogenum* (moderate xerophilic species) and *A. penicillioides* with *E. halophilicum* (xerophilic species). These results

could match with their nutritional requirements and their common presence in the same environments or substrates (Jurjević *et al*., 2012; Micheluz *et al*., 2015a; Samson and Lustgraaf, 1978). The other species presented individual metabolic patterns, different for the quality and time of production.
However, further investigations with a higher number of strains and species, need to be done in order to establish more connection between the fungi often detected together in the same environments.

4.3.3 MVOC analysis from contaminated books

The MVOC monitoring of two contaminated books took 78 days (see Par. 3.3.3 and Appendix A7 for detailed information about the experimental design). In this period, clear pattern of volatile organic compounds had developed inside the sampling chamber, characteristic for each sampled book. As already reported in the Par. 3.1.2.3, the books were visibly contaminated by fungal growth on their covers, but the microbiologically sampling permitted to isolate only a few viable fungal species.
After incubation almost 13 different fungal species were sampled from the covers. Their development was visible recognized during the incubation period, followed by GC-MS analysis concerning MVOC emission (TIC mode was used; see Appendix B5 - C) and then confirmed by microbiological analysis (see Par. 4.1.4). Already after one month, the increase of several peaks in the chromatograms was observed, as well as the appearance of white fungal colonies on the book's covers.

4.3.3.1 Sampled book L6

The book L6, with the title "Lettere a cura di G. Carducci" of F.D. Guerrazzi dated 1880, is composed by a hardcover made with cardboard. During the first microbiologically sampling, only a few colonies of *Penicillium chrysogenum* were isolated from its cover, while after incubation, several fungal species belonging to *Aspergillus*, *Penicillium*, *Eurotium* and *Chaetomiun* spp. were detected (see Par. 4.1.4).
The GC-MS analysis demonstrated the occurrence of several MVOCs belonging to 11 chemical groups and their trends during all the monitored period are summed up in the followed graph (Fig. 4.31).
The first analysis of the book was performed at t = 0, recording the presence of several chemical compounds, especially hydrocarbons (28%, *e.g.* 2,2,4-trimethylpentane RT = 12.55 min, 6% and toluene RT = 14.43 min, 5%), alcohols (27%, *e.g.* ethanol RT = 6.24 min, 15%), ketones (21%, *e.g.* acetone RT = 6.75 min, 17%) and aldehydes (10%, *e.g.* acetaldehyde RT = 5.45 min, 6%) (Fig. 4.32). This analysis was considered as blank, so the detected signals were subtracted from the following analyses in order to recognize only the compounds their amounts directly produced during the incubation period under controlled environmental conditions.

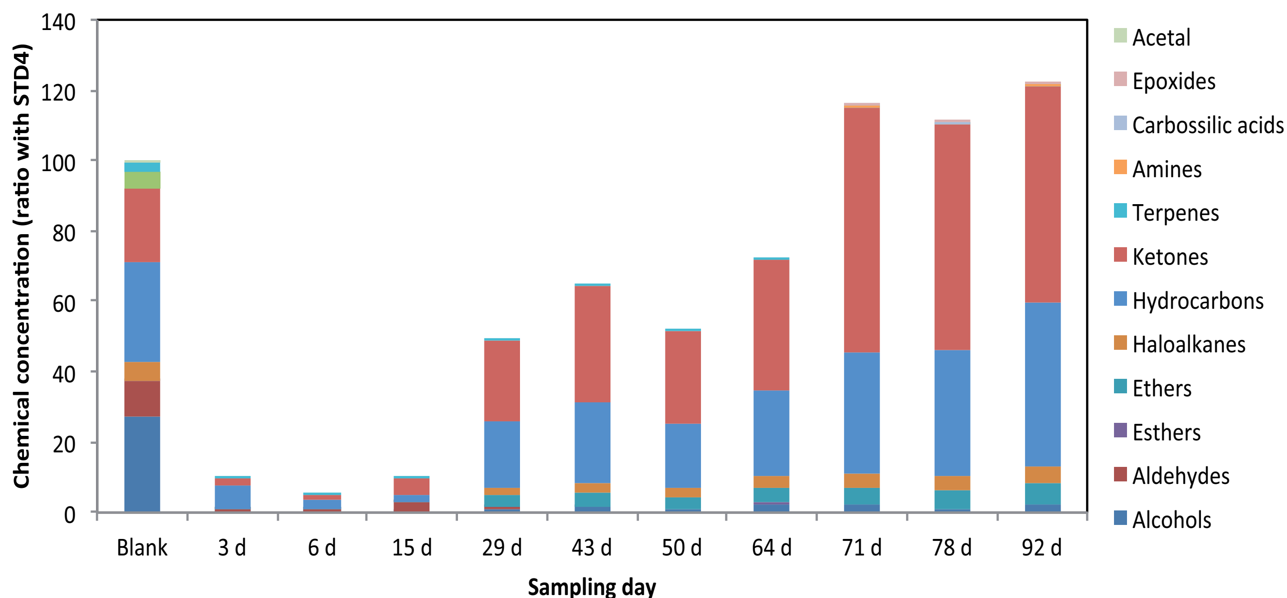


Figure 4.31 Temporal evolutions of VOCs emitted by book L6, explicated as chemical families. The "Blank" corresponds to the output's sum of the book at t = 0 and its signal was substracted from all the followed analysis.

In the beginning of the incubation an increase of hydrocarbons was observed, *e.g.* 2,2,4-trimethylpentane (22%), toluene (11%), p-xylene (5%, RT = 16.94 min), pentane (4%, RT = 7.28 min) and benzene (3%, RT = 11.49 min), while the quantity of alcohols decreased with the total disappearance of ethanol.

The analysis after 29 days corresponds to the initial of the fungal growth on the book. The visual inspection allows following the development of several white circular colonies spread on the cover. This is also evident in a change of the chromatogram, where the ketones acetone and 2-butanone (RT = 9.24 min) increased up to 34% and 11%, respectively. The concentrations of propane (RT = 5.10 min), 2-butene (RT = 5.65 min) and butane (RT = 5.75 min) also increased (19%) and other hydrocarbons appeared, as heptane (1%, RT = 12.80 min) and 1,4-pentadiene (1%, RT = 7.40 min).

Moreover, the appearance of ethers, as furan (3%, RT = 7.11 min) and 2-methylfuran (1%, RT = 9.75 min) and the haloalkane chloromethane (4%, RT = 5.34 min) was observed, and isopropyl alcohol slightly increased (1%, RT = 6.98 min).

In comparison with previous analysis, on the 50th day of incubation we observed a general increase of ketones (51%), with the additional detection of 3-methyl-2-butanone (2%, RT = 11.16 min), and ethers (7%), whereas aldehydes disappeared. The temporal evolutions of the principal emitted VOCs are reported in the following graphs (Fig. 4.33).

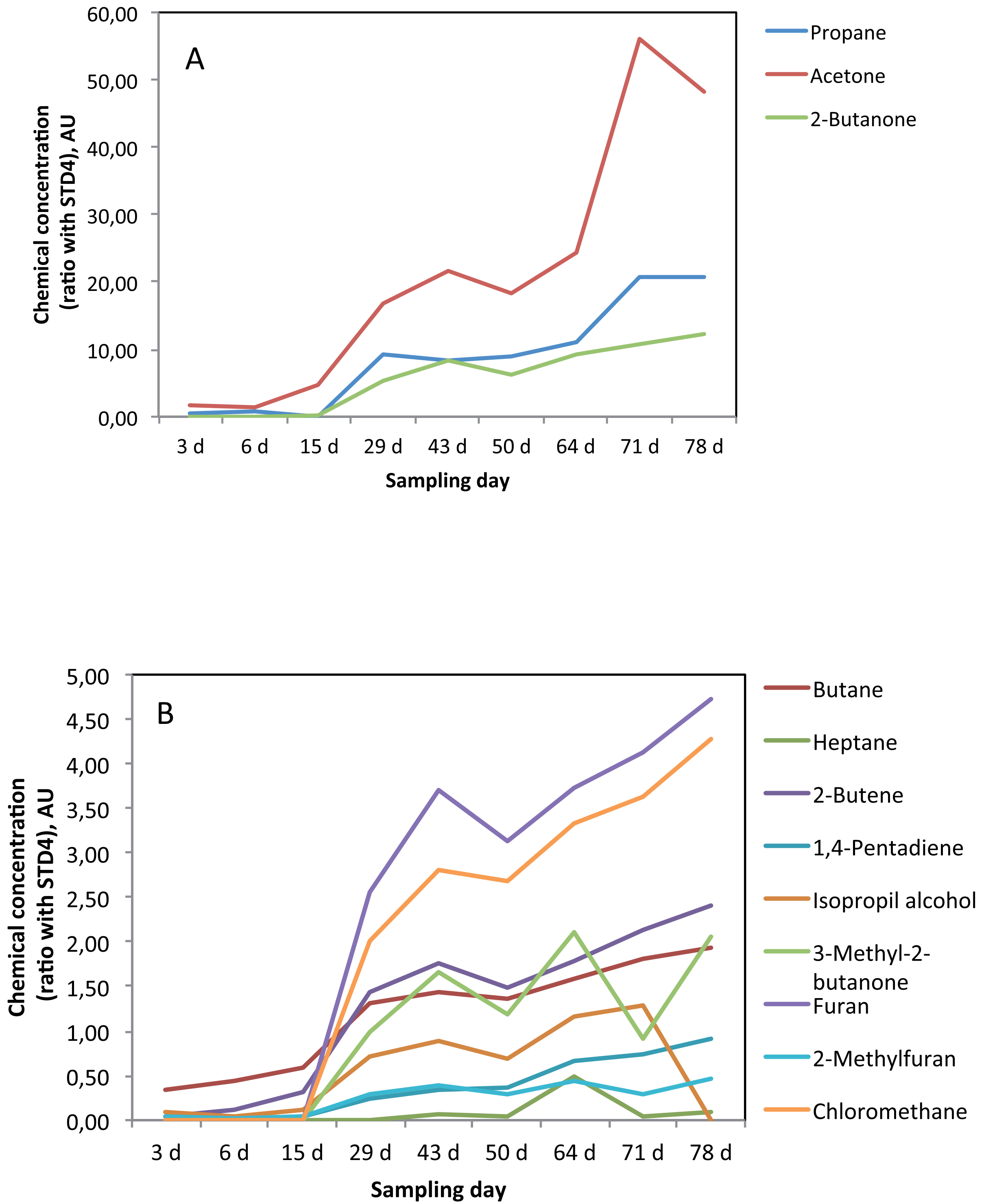
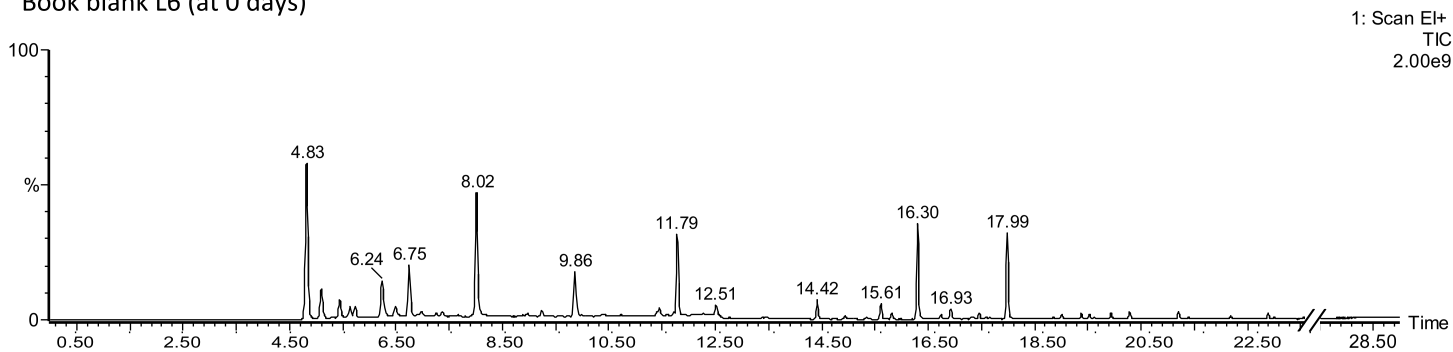
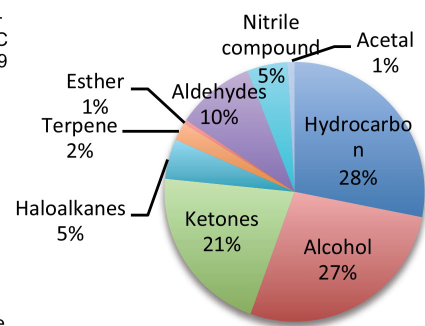


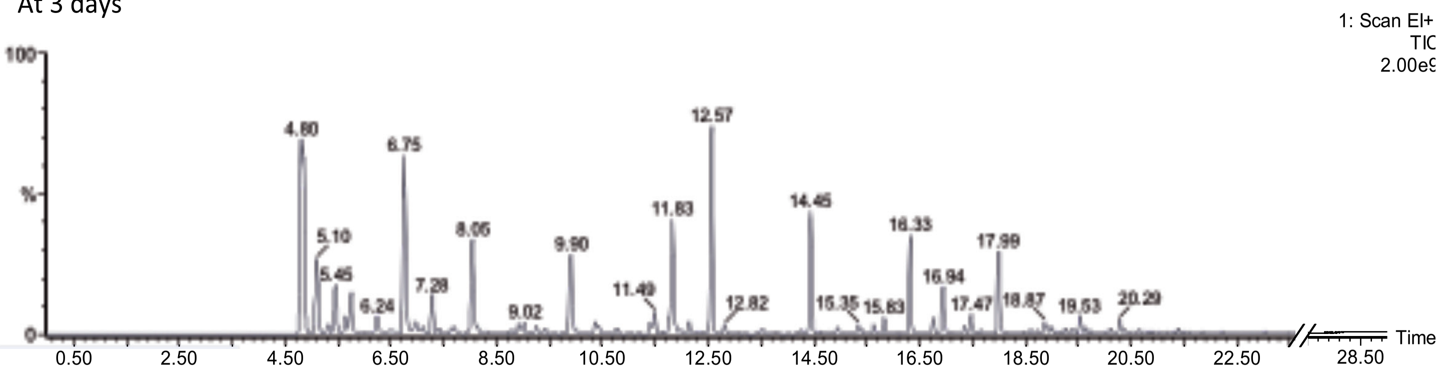
Figure 4.33 Temporal trends of principal VOCs recorded from book L6 divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (0 < ratio < 60); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 5).

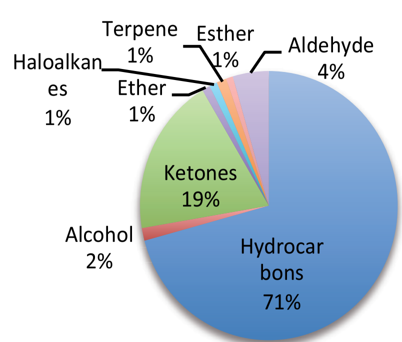
Book blank L6 (at 0 days)



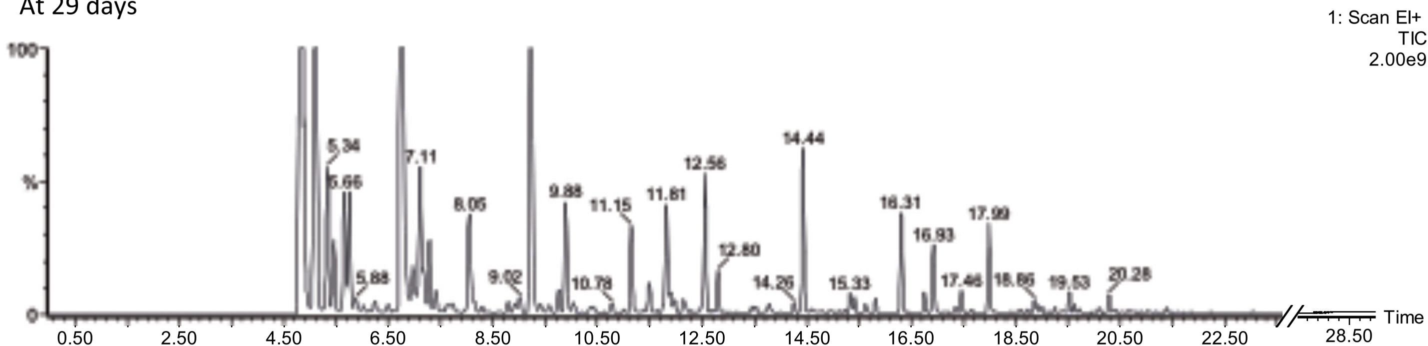


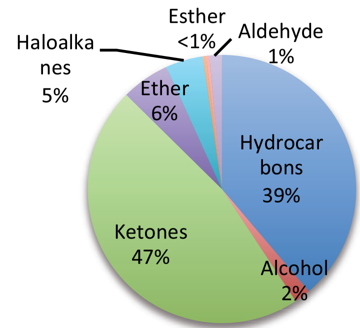
At 3 days



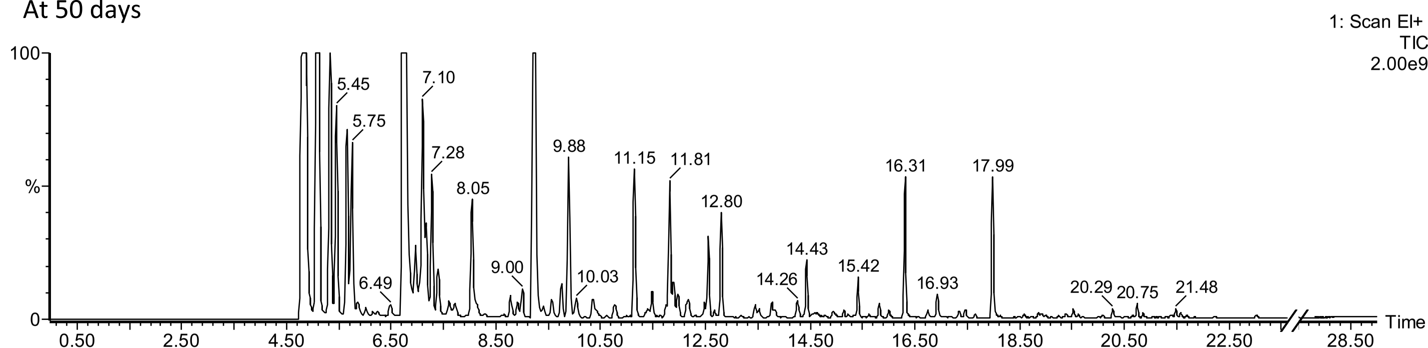


At 29 days





At 50 days



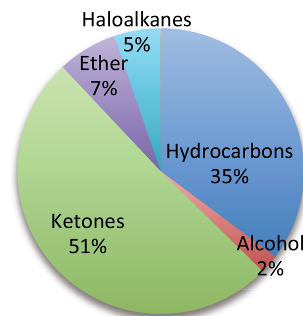


Figure 4.32 Chromatograms of VOC evolution during incubation period for book L6, with the corresponding chemical composition in percentages on the right. The analyses are recorded in TIC mode.

4.3.3.2 Sampled book L9

The book L9, with the title "The Works of Sheakspeare" of G. Cumberlage dated 1954, is composed by a hardcover made out of cardboard and fabric. During the first microbiologically sampling, only the presence of *Aspergillus tubigensis* was isolated from its cover, while after incubation several fungal species belonging to *Aspergillus*, *Penicillium* and *Eurotium* spp. were detected (see Par. 4.1.4).

Similar to the previous book, the GC-MS analyses demonstrated the occurrence of several volatile organic compounds, belonging to eight chemical families and their trends during the entire monitored period (78 days) are summed up in the graph below (Fig. 4.34).

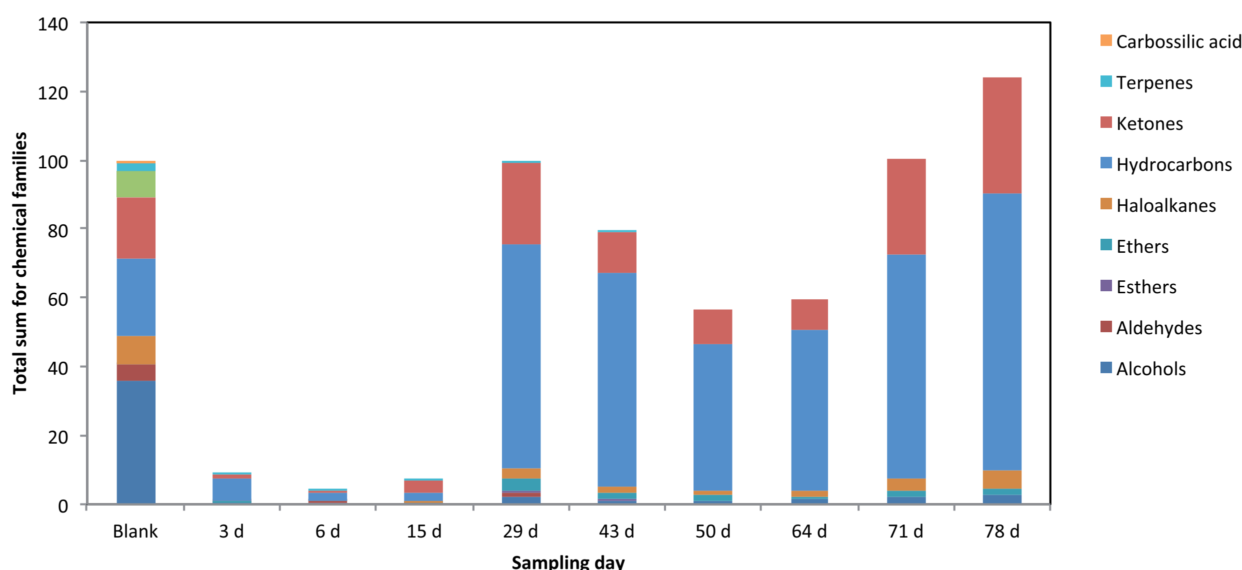


Figure 4.34 Temporal evolutions of VOCs emitted by book L9, explicated as chemical families. The "Blank" corresponds to the output's sum of the book at t = 0 and its signals were removed from all the followed analysis.

The L9 output sum, called blank, *i.e.* the analysis of the book L9 at t = 0, is quite similar to the blank of the book L6. In this case the quantity of alcohols was higher (36%, with ethanol, 27% and RT = 6.22 min and isopropyl alcohol, 3%, RT = 6.98 min), than those of hydrocarbons (22%), including toluene (5%, RT = 14.43 min), 2,2,4-trimethylpentane (3%, RT =14.25 min) and 1,3-dimethylbenzene (2.5%, RT = 16.92 min). Other detected compounds were ketones (18%), especially acetone (15%, RT = 6.75 min), haloalkanes (8%), nitrile compounds (8%), aldehydes (5%), terpenes (3%) and esthers and carbossilic acid were present in low quantities (>1%) (Fig. 4.35).

After 3 days, the chemical composition changed completely: most of the increase was attributed to hydrocarbons (74%), with 2,2,4-trimethylpentane (28%), toluene (12%), p-xylene (5%, RT = 16.92 min) and pentane (3%, RT = 7.29 min). Acetone also increased (17%), while alcohols decreased to 3%.

As for book L6, the analysis after 29 days corresponds to the initial phase of the fungal growth associated with a visual appearence of white spots on the cover. We detected in this phase p-xylene (39%), pentane

(1.7%), o-xylene (1.5%, RT = 17.47 min), isobutane (1.3%, RT = 5.46 min), 2,6-dimethylheptane (1%, RT = 16.15 min), nonane (1%, RT = 17.66 min), octane (0.9%, RT = 15.42 min), acetone (22%), 2-butanone (2%) and ethers appeared, with furan (3%, RT = 7.11 min) and 2-methylfuran (1%, RT = 9.75 min).

After 50 days, a general increased of hydrocarbons (75%), in particular p-xylene (48%), propane (4%, RT = 5.19 min), 1,1,3-trimethylcyclohexane (3%, RT = 16.47 min) and 3-ethylhexane (3%, RT = 16.81 min) was observed. The temporal evolution of the principal emitted VOCs are reported in the following graphs (Fig. 4.36).

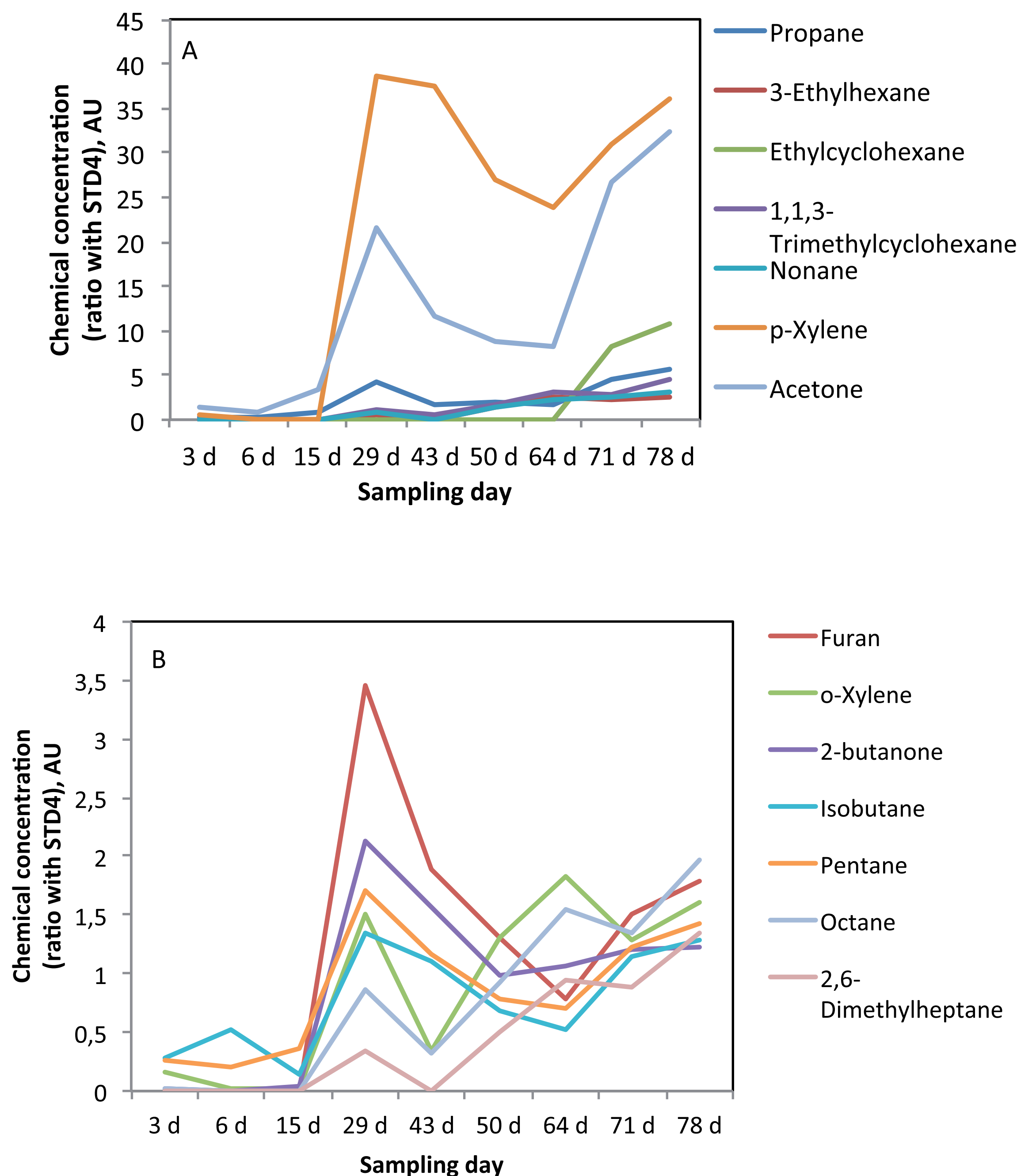
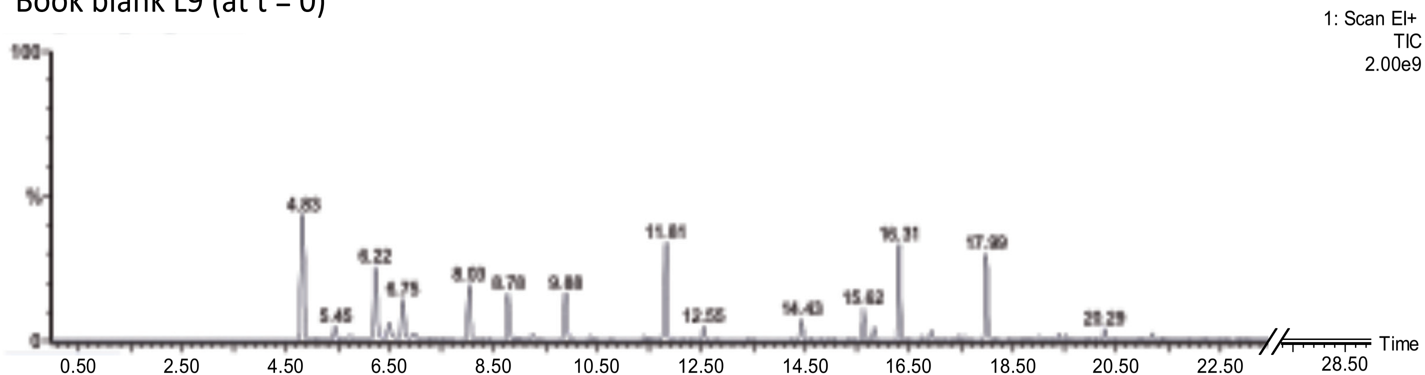
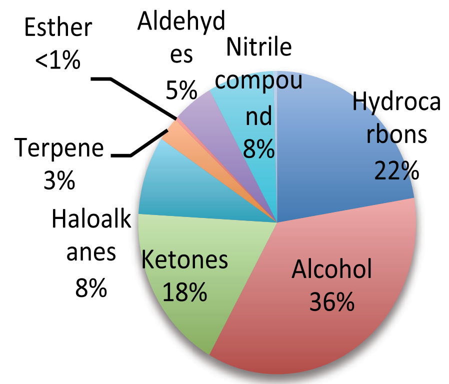


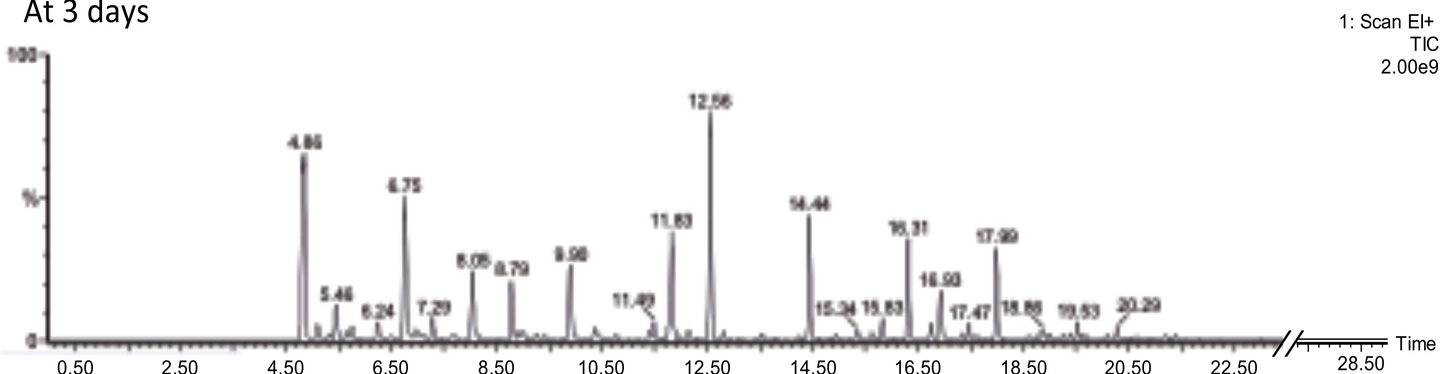
Figure 4.36 Temporal trends of principal VOCs recorded from book L) divided for quantity emission: A) chemical compounds with high ratio between peak area/peak area STD4 (0 < ratio < 45); chemical compounds with low ratio between peak area/peak area STD4 (0 < ratio < 4).

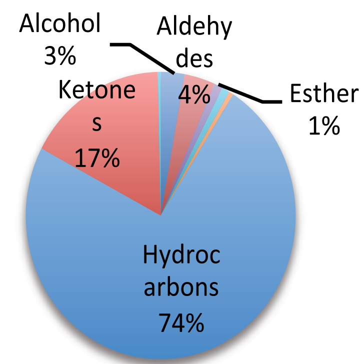
Book blank L9 (at t = 0)





At 3 days





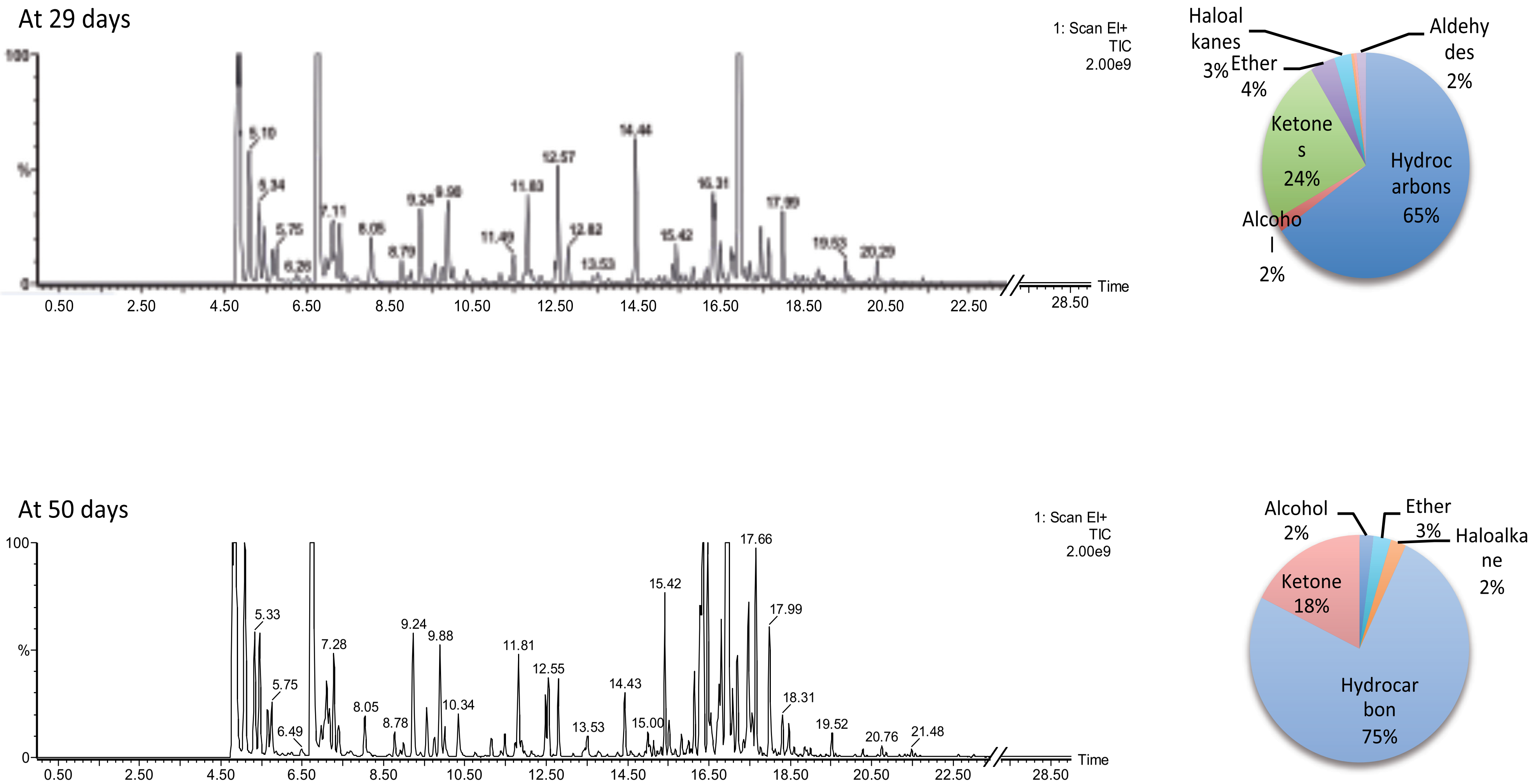


Figure 4.35 Chromatograms of VOCs evolution during incubation period for book L9, with the corresponding chemical composition in percentages on the right. The analyses are recorded in TIC mode.

The components of the books emit several volatile organic compounds, addressed as “smell of books”. Fenech *et al.* (2010) and Strlič *et al.* (2009) have addressed various aldehydes, ketones, hexadecane and 2-ethylhexanol for books with rosin, while furfural, acetic acid and hexanal were detected as a result of the degradation of cellulose, hemicellulose and lignin. Especially in the beginning of our experiment we also detected aldehydes, as acetaldehyde, 2-methylpropanal, butanal, 2-methylbutanal and hexanal together with acetic acid buthyl esther. So, these compounds could be our markers of book components.

Lattuati-Derieux *et al.* (2004) analysed VOCs with SPME method from both headspace and contact procedures and identified several volatile organic compounds. MVOCs in common with our results are: toluene, ethylbenzene, xylene, trimethylbenzene, decane and undecane. Most of these compounds are so called benzene, toluene, ethylbenzene and xylene compounds (BTEX), low molecular mass, volatile and non-polar organic compounds, which are often associated with environmental pollution. However, any external contamination was avoided, because of the isolation of the sampled chambers.

We investigated for the first time materials contaminated by fungal growth under monitored conditions (T = 20 °C and saturated moisture condition), so a mixture of VOCs, originating from the degradation of book components and fungal volatile metabolites, was expected.

4.3.3.3 Statistical analysis of fungal and books' MVOCs

The data obtained from the contaminated book monitoring can be compared to the results obtained from selected fungal species (see Par. 4.3.2) in order to highlight any potential correlation between them. The aim if this statistical analysis was to find possible fungal markers produced by the fungal species cultivated in vitro in relation to the “real” case study, as the contaminated books.

Analysing the volatile compounds production in common between contaminated books and fungal species it was possible to obtain the following graphs (Fig. 4.37). Among the 61 detected compounds from book L6, there were 30 chemicals in common with selected fungal specie, in particular:

- *P. brevicompactum*, *n* of similar VOCs = 20, with 7 chemicals considered as possible markers of this species, *i.e.* isopropyl alcohol, acetone, 2-butanone, 2-pentanone, furan, 3-methylfuran, styrene;
- *A. penicillioides*, *n* of similar VOCs = 15, with 3 chemicals considered as possible markers of this species, *i.e*. isopropyl alcohol, acetone, 2-butanone;
- *E. halophilicum*, *n* of similar VOCs = 13, with 5 chemicals considered as possible markers of this species, *i.e*. 1,4-pentadiene, isopropyl alcohol, acetone, 2-butanone, 2-methylfuran;
- *C. cladosporioides*, *n* of similar VOCs = 11, with 6 chemicals possible as possible markers of this species, *i.e*. 2-butene, 1-octene, acetone, 2-butanone, 2-pentanone, furan;
- *E. chevalieri*, *n* of similar VOCs = 11, with 6 chemicals possible as possible markers of this species, *i.e*. 1,4-pentadiene, isopropyl alcohol, acetone, 2-butanone, furan, 2-methylfuran;

- *A. creber*, *n* of similar VOCs = 9, with 7 chemicals possible as possible markers of this species, *i.e*. 2-butene, 1,4-pentadiene, isopropyl alcohol, 2-butanone, furan, 2-methylfuran;
- *P. chrysogenum*, *n* of similar VOCs = 9, with 7 chemicals possible as possible markers of this species, *i.e*. 1,4-pentadiene, isopropyl alcohol, acetone, 2-butanone, furan, 2-methylfuran, styrene.

The most detected VOCs were isopropyl alcohol and 2-butanone (emitted from 7 fungal species), followed by ethanol, acetone, 1,4-pentadiene, furan, 2-methyl furan and styrene (emitted from 6 fungal species).

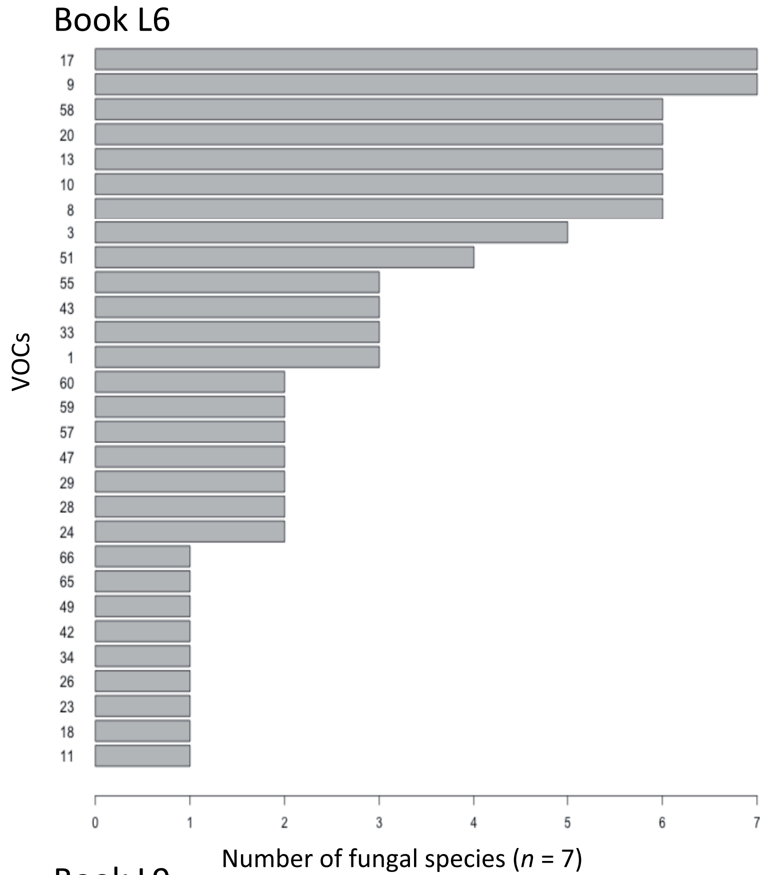
The analysis of the book L9 have permitted to identify 42 compounds, with 23 in common with selected fungal species, as specified:

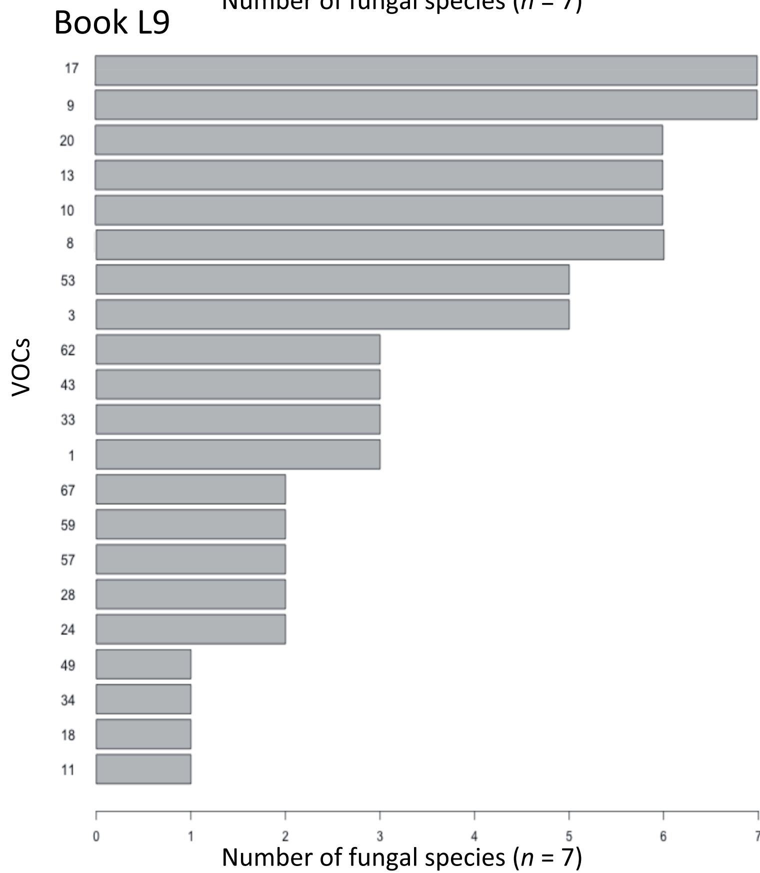
- *A. penicillioides*, *n* of similar VOCs = 14, with 4 chemicals considered as possible markers of this species, *i.e*. acetone, isopropyl alcohol, 2-butanone, 2-octen 1-ol;
- *P. brevicompactum*, *n* of similar VOCs = 13, with 4 chemicals considered as possible markers of this species, *i.e.* acetone, isopropyl alcohol, 2-butanone, furan;
- *A. creber*, *n* of similar VOCs = 10, with 7 chemicals considered as possible markers of this species, *i.e*. 2-butene, 1,4-pentadiene, isopropyl alcohol, 2-butanone, 2-octen 1-ol, furan, 2-methylfuran;
- *E. chevalieri*, *n* of similar VOCs = 11, with 6 chemicals considered as possible markers of this species (*i.e*. acetone, 1,4-pentadiene, isopropyl alcohol, 2-butanone, furan, 2-methylfuran);
- *E. halophilicum, n* = 10, with 5 chemicals considered as possible markers of this species, *i.e*. acetone, 1,4-pentadiene, isopropyl alcohol, 2-butanone, 2-methylfuran;
- *C. cladosporioides*, *n* of similar VOCs = 9, with 5 chemicals considered as possible markers of this species, *i.e.* acetone, 2-butene, 2-butanone, 2-octene-1-ol, furan;
- *P. chrysogenum*, *n* of similar VOCs = 9, with 6 chemicals considered as possible markers of this species, *i.e.* acetone, 1,4-pentadiene, isopropyl alcohol, 2-butanone, furan, 2-methylfuran.

Similar to the results for book L6, the principal detected VOCs were isopropyl alcohol and 2-butanone (fungal species = 7), followed by acetone, 1,4-pentadiene, furan and 2-methyl furan (fungal species = 6).

If we consider the most abundant compounds for the investigated books and for the studied fungal species, we obtain the short list reported in Table 4.10. These compounds could be considered as both VOCs and MVOCs. Even if these compounds are not considered as commonly accepted MVOCs (see Table 1.6, Par. 1.3.2), they were reported in several past fungal studies, as discussed in the previous paragraphs (see Par. 4.3.2.1-4.3.2.7).

The detection of these seven chemicals included all the seven selected fungal species, but only four of them (i.e. *A. creber*, *A. penicillioides*, *E. halophilicum* and *P. chrysogenum*) were isolated from the contaminated books at the end of the VOCs' monitoring period (72 days). Moreover, other species were isolated from both covers (*i.e. Aspergillus* spp., *A. vitricola*, *E. amstelodami* and *Penicillium* spp.), together with other few fungi that have characterized the books in different ways: *A. sydowii* and *Chaetomium funicola* for sampled book L6 and *A. carneus*, *A. jensenii* and *P. rubens* for sampled book L9 (see Par. 4.1.4).





Code	Compounds
1	Propane
3	2-Butene
8	Acetone
9	Isopropyl alcohol
10	Furan
11	Pentane
13	1,4-Pentadiene
17	2-Butanone
18	1-Hexene
20	2-Methylfuran
23	3-Methylfuran
24	2-Methyl-1-propanol
26	3-Methyl-2-butanone
28	Benzene
29	2-Pentanone
33	Heptane
34	1-Heptene
42	Acetic acid, butylesther
43	Toluene
47	2-Octene
49	Octane
51	Tetrachloroethylene
53	2,5-Dimethylheptane
55	Ethylbenzene
57	p-Xylene
58	Styrene
59	o-Xylene
60	2-Butoxyethanol
62	2-Octen-1-ol
65	Decane
66	Limonene
67	2,6,10-Trimethyldodecane

Figure 4.37 Graphic rappresentation about only emitted VOCs in common between each sampled book (books L6 and L9, specifically) and investigated fungal species.

Table 4.10 List of principal VOCs with relevance for both: the sampled books (books L6 and L9) and for fungal species. AC: *A. creber*; AP: *A. penicillioides*; CC: C. *cladosporioides*; EC: *E. chevalieri*; EH: *E. halophilicum*; PB: *P. brevicompactum*; PC: *P. chrysogenum*. The marked fungal species are those that were effectively isolated from the contaminated books.

Compounds	High relevance in books	High relevance for fungi and books
2-Butene	L6, L9	**AC**, CC
Acetone	L6, L9	**AP**, CC, EC, **EH**, PB, **PC**
Isopropyl alcohol	L6, L9	**AC**, **AP**, EC, **EH**, PB, **PC**
Furan	L6, L9	**AC**, CC, EC, PB, **PC**
1,4-Pentadiene	L6, L9	**AC**, EC, **EH**, **PC**
2-Butanone	L6, L9	**AC**, **AP**, CC, EC, **EH**, PB, **PC**
2-Methylfuran	L6, L9	**AC**, EC, **EH**, **PC**

Together with the different components of the books, the isolation of different fungal species from the books could be the reason for the two different VOCs pattern obtained by GC-MS analysis.

Finally, the identification of the seven high relevance compounds reported in Table 4.10 are valid for both sampled books. Further investigations of other contaminated books (from the same repository, but also from other stored environments) with these compounds as targets are needed to confirm them as valid MVOCs. Considering, only the results from *E. halophilicum* investigation, because of its predominantly growth on the book's covers, we obtain the followed list (Table 4.11).

Table 4.11 List of VOC similarities between the contaminated L6 and L9 books with *E. halophilicum*.

Compounds	Book L6 and *E. halophilicum*	Book L9 and *E. halophilicum*
Propane	X	X
Acetone	X	X
Isopropyl alcohol	X	X
1,4-Pentadiene	X	X
2-Butanone	X	X
2-Methylfuran	X	X
3-Methylfuran	X	
Toluene	X	X
Tetrachloroethylene	X	X
p-Xylene	X	X
Styrene	X	
o-Xylene	X	X
2-Butoxyethanol	X	
2,6,10-Trimethyldodecane		X

The different samples resulted similar for fourteen compounds, which means the 54% of the total for the fungus, including its high relevance MVOCs (*i.e.* 2-butanone, acetone, 1,4-pentadiene). However, none of them was only produced by this fungus. Further investigations of *E. halophilicum* grown on different substrates, *e.g.* directly on leather or textile samples, are needed to identify other markers and/or confirm these results.

4.3.4 MVOCS analysis of indoor air from contaminated repository

The indoor air sampling was performed by canister and subsequently analysed by GC-MS (for more information about air sampling and the analytical method see Par. 3.3.4 and Appendix A8). During both samplings (2nd and 3rd sampling, *i.e.* October 2013 and May 2014, respectively), a wide range of chemicals was identified (Table 4.12). The table with all the values is reported in Appendix B5 - D. If we consider only the chemical classes of the detected compounds, we obtain the graph in Fig. 4.38.

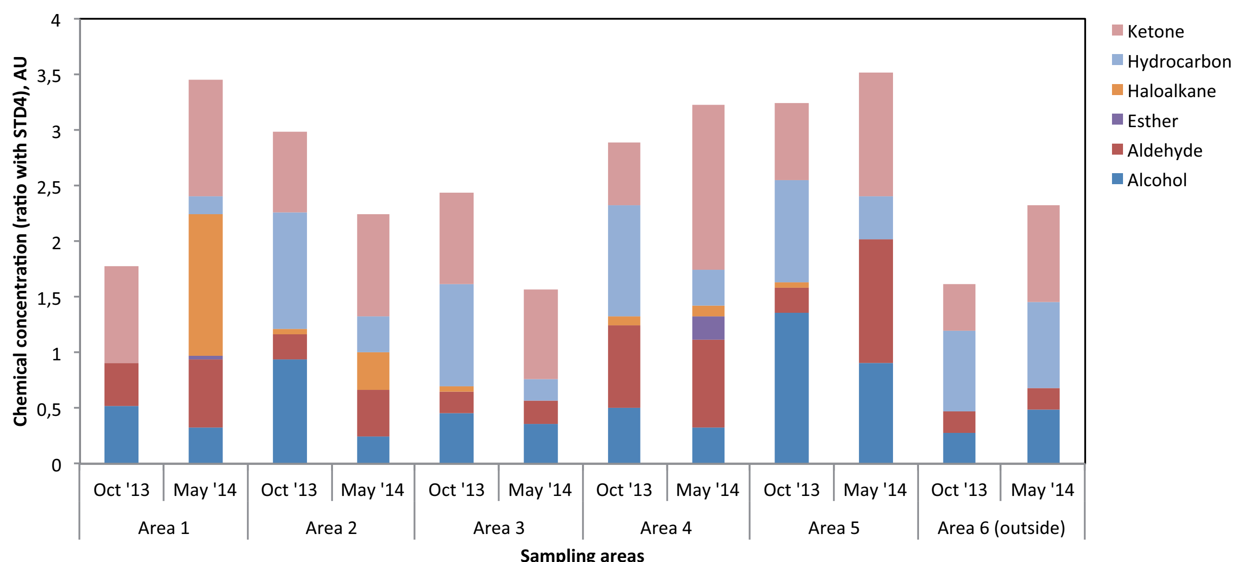


Figure 4.38 Overview of VOCs detected during the 2nd and the 3rd sampling from indoor air in the repository.

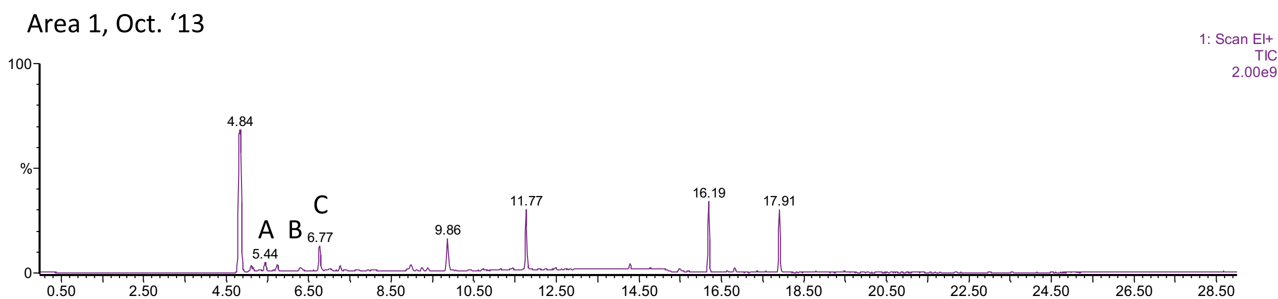
Low quantities of VOCs were detected in all the areas inside and outside the repository, probably due to the air circulation provided by the air-conditioned system. These samplings were performed after the operation of book disinfection, and as we expected, only a few fungal MVOCs (n = 11) were detected in indoor air samples. Typical compounds concerning the degradation of book materials were found, as aldehydes, especially acetaldehydes, furfural and hexanal, ketones and 2-ethylhexanol (Fenech *et al.*, 2010; Strlič *et al.,* 2009). We detected three chloride compounds, especially during the 3rd sampling in correspondence of areas 1 and 2:

- 1,2-dichloropropane;
- 1-chloro-2-propanol;
- 1-chloro-2-propanone.

Their presence might be derived from the degradation of chloride containing cleaning products used for the disinfection and their interaction with the organic volatile compounds emitted from the paper. Their presences are highlighted in the following chromatograms (Fig. 4.39).

Table 4.12 Overview of seasonal VOC detection by indoor air sampling inside the repository of BAUM, expressed for each sampling area.

Substances	RT (min)	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6 (outside)	
		Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14
Acetaldehyde	5.44	X	X	X	X	X	X	X	X	X	X	X	X
Butane	5.73	X	X	X	X	X	X	X	X	X	X	X	
Ethanol	6.24	X	X	X	X	X	X	X	X	X	X	X	X
Acetone	6.75	X	X	X	X	X	X	X	X	X	X	X	X
Isopropyl alcohol	6.98	X	X	X	X	X	X	X	X	X	X	X	X
Pentane	7.26	X		X		X		X		X		X	X
1.4-Pentadiene	7.38	X		X	X	X				X	X		
Acetic acid-cyano-1.1-dimethylethyl esther	7.60		X						X				
1-Epten-4-ol	8.91			X									
Methyl vinil ketone	8.96		X		X		X		X		X		X
2-Methylpentane	8.99	X		X		X		X		X			
m-Butanal	9.14		X		X			X	X		X		
2-Butanone	9.26	X	X	X	X	X	X	X	X	X	X		X
3-Methylpentane	9.41			X		X				X		X	
Methylcyclopentane	10.76	X		X		X				X		X	
1-Chloro-2-propanone	11.43		X		X								
Benzene	11.49			X		X				X	X	X	X
1-Chloro-2-propanol	12.13		X		X								
3-Methylhexane	12.14			X		X		X					
Pentanal	12.16										X	X	
1.2-Dichloropropane	12.32		X										
Tetramethylbutane	12.48	X			X	X		X	X	X	X		
Methylcyclohexane	13.53									X			
Cyclobutene-2-propilidene	14.39								X				
Toluene	14.44	X	X	X		X	X	X		X	X	X	
Hexanal	14.94		X					X	X	X	X		
Furfural	15.48	X											
Perchloroethylene	15.63		X	X		X		X	X	X			
Heptanal	17.30		X					X	X		X		
Benzaldehyde	18.61							X		X	X		
Octanal	19.37		X					X	X		X		
Decane	19.61	X						X					
2-Ethyl-1-hexanol	19.93							X					
Nonanal	21.20							X			X		X



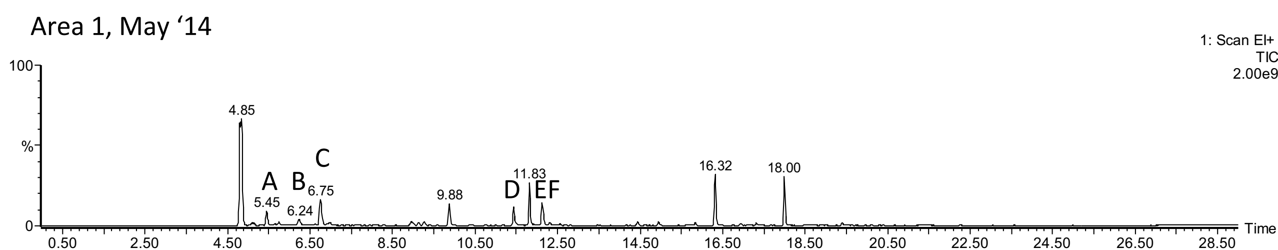


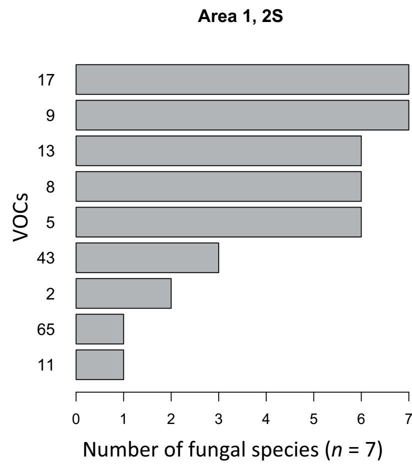
Figure 4.39 Chromatograms of indoor air sampled in correspondence to area 1 during the two samplings. A: acetaldehyde; B: ethanol; C: acetone; D: 1-chloro-2-propanone; E: 1-chloro-2-propanol; F: 1,2-dichloropropane.

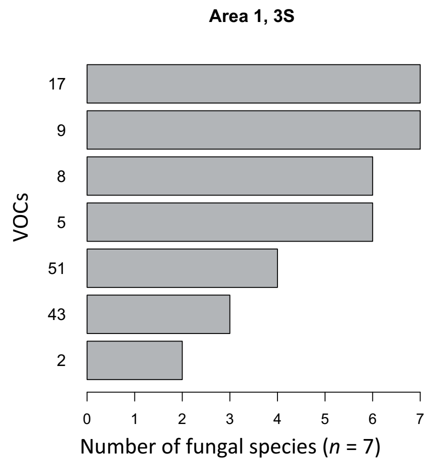
4.3.4.1 Statistical analysis of fungal and air's MVOCs

Similar to the sampled books, the data obtained from indoor air sampling can be compared to the results from the selected fungal species (see Par. 4.3.2) in order to identify possible fungal markers in both of the substrates. Analysing the chemical compound similarities we obtained the following graphs for each sampling area (areas 1, 2, 3, 4) for the 2nd and the 3rd sampling (see Par. 3.3.4) (Fig. Fig. 40).

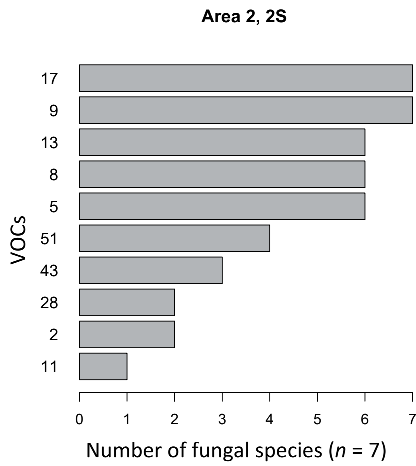
The two substrates have only 11 similar chemical compounds, which means they represent only 32% of the total compounds (n = 34) detected in the indoor air. In general, the air samples belonging to the 2nd sampling had a higher number of VOCs than those detected during the 3rd sampling. This low similarity could be explained as a direct consequence of the disinfection performed subsequently after the 1st sampling. The airborne microbial level in the highest contaminated areas (area 1 and 2, see Par. 4.1.2) was reduced of more than 70% for the 2nd sampling and more than 90% during the 3rd sampling. Probably we would have observed the same trend for VOC concentrations if we had performed the air sampling also during the 1st sampling.

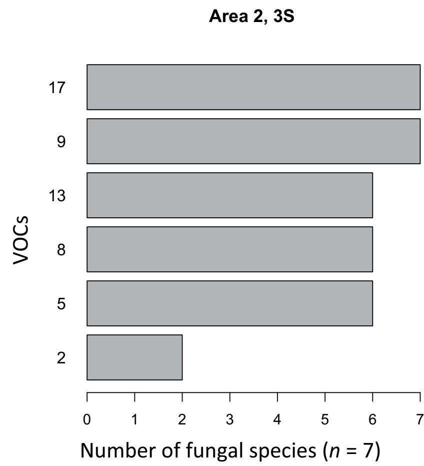
If we consider the indoor air and contaminated books' VOC production, they were similar for 16 compounds (47% of the total for indoor air), especially for the aldehyde's contribution (*e.g.* hexanal and nonanal, see Par. 4.3.3). So, VOCs originating from paper degradation contribute more to the indoor air concentration than the microbial VOCs.

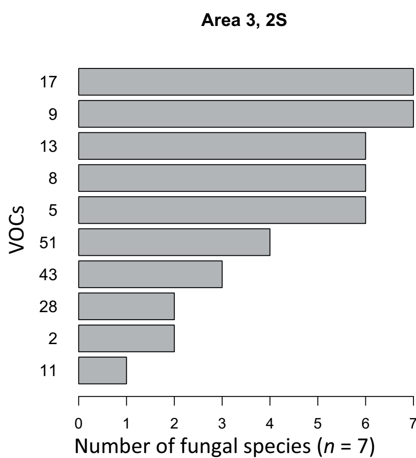


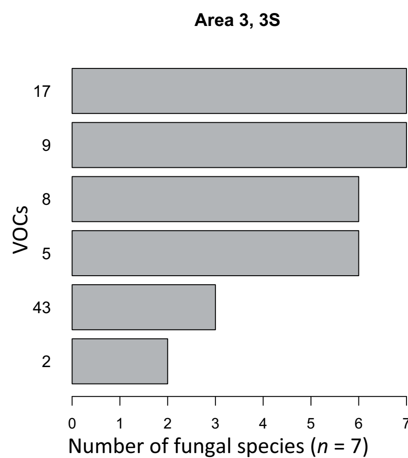


Code	Compounds
2	Acetaldehyde
5	Ethanol
8	Acetone
9	Isopropyl alcohol
11	Pentane
13	1,4-Pentadiene
17	2-Butanone
28	Benzene
43	Toluene
51	Tetrachloroethylene
65	Decane









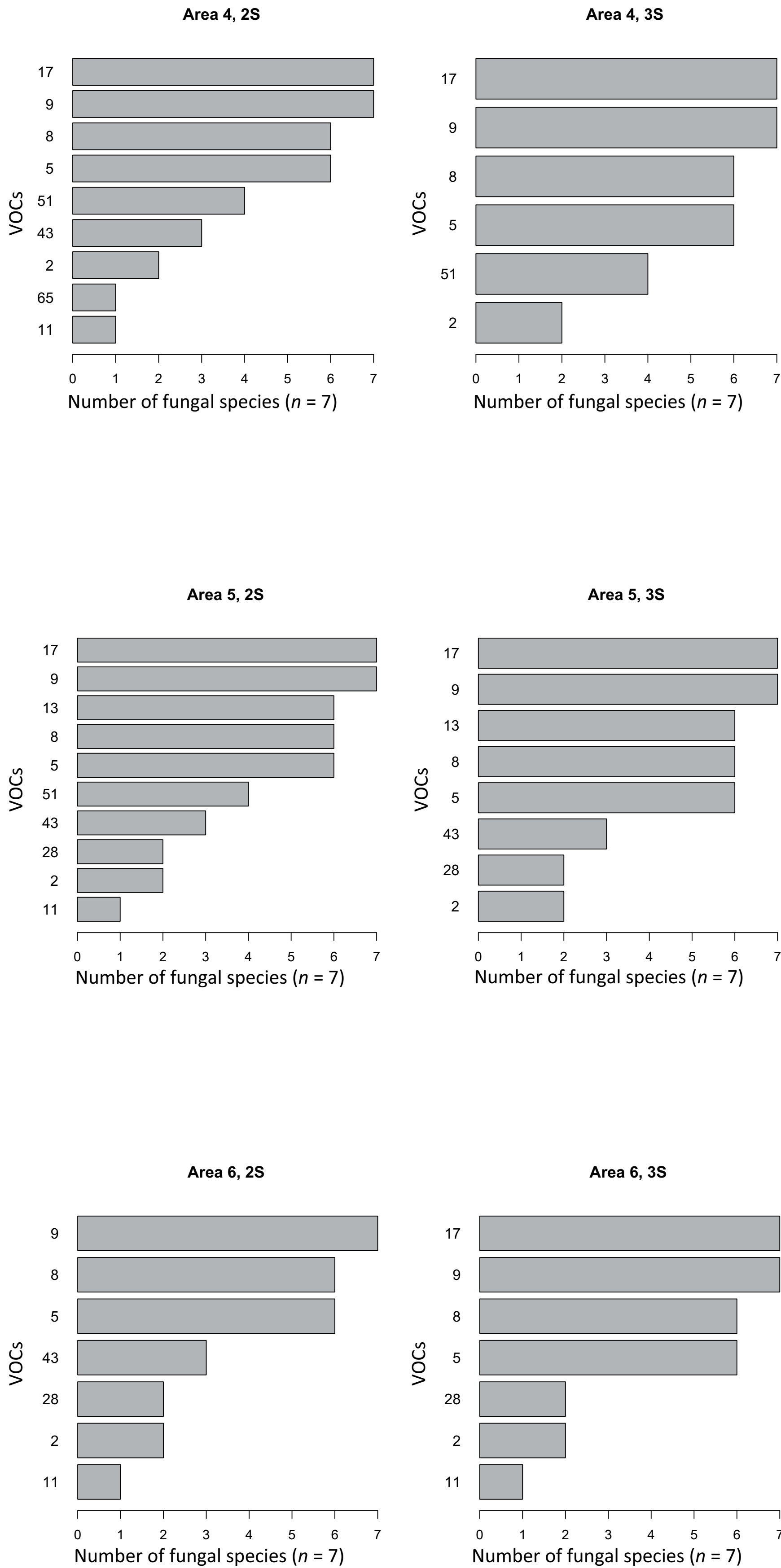


Figure 4.40 Graphic representations of emitted VOCs common between each air sampled area, considering also each sampling (2nd sampling, 2S, and 3rd sampling, 3S), and investigated fungal species.

4.3.5 Summary

The MVOC analyses of the selected fungal species isolated from the contaminated environment, from both the air and the books during the first sampling at BAUM's repository (March 2013) have highlighted the capacity to produce a total of 72 different volatile compounds in *in vitro* experiment. The fungi, cultivated inside closed sample bottles on suitable culture medium were monitored for several weeks (4-8 weeks) by GC-MS analysis together with an alternative BOD analysis that followed the total O_2 consumption inside the bottles. In this way it was possible to monitor the changes in volatile metabolite production depending on the age of the colonies and O_2 availability. Each fungal species produced a characteristic chemical compound pattern and the results are resumed in the followed Table 4.13.

Similarities between the fungal species were reported for alcohol (*e.g.* ethanol, isopropyl alcohol, 3-methyl-1-butanol), ketone (*e.g.* acetone, 2-butanone) and ether (*e.g.* furan, 2-methyl furan) productions. Other compounds were preferentially fungal-dependend, as 1-octene for *C. cladosporioides*, 2-butanol for *E. halophilicum* and 3-methylfuran for *P. brevicompactum*.

Cluster analyses have highlighted similarities in MVOC production mainly between:

- *A. creber* with *P. chrysogenum;*
- *A. penicillioides* with *E. halophilicum*.

These relationships were valid for almost all the monitored period that varied depending on the growth's velocity of each fungus. The results can be explained by the fungal similarities in terms of nutriments requirements and environmental habitats.

Table 4.12 Principal MVOCs detected for the studied fungal species considered as possible markers. Culture media: MEA (Malt Extract Agar) and MEA15% (MEA with 15% of NaCl). AC: *Aspergillus creber*; AP: *A. penicillioides*; CC: *Cladosporium cladosporioides*; EC: *Eurotium chevalieri*; EH: *E. halophilcum*; PB: *Penicillium brevicompactum*; PC: *P. chrysogenum*.

Principal MVOCs	Culture medium	Fungal species
2-Butene	MEA	AC, CC
1,4-Pentadiene	MEA	AC, EC, EH, PC
1- Octene	MEA	CC
1,3-Octadiene	MEA, MEA15%	AC, AP
Ethyl acetate	MEA15%	EH
Ethanol	MEA, MEA15%	AC, CC, EC, EH, PC
Isopropyl alcohol	MEA, MEA15%	AC, AP, EC, EH, PB, PC
2-Butanol	MEA15%	EH
2-Methyl-1-butanol	MEA	PB, PC
3-Methyl-1-butanol	MEA, MEA15%	AC, CC, EC, EH, PB, PC
2-Octen-1-ol	MEA, MEA15%	AC, AP, CC
Acetone	MEA, MEA15%	AP, CC, EC, EH, PB, PC
2-Butanone	MEA, MEA15%	AC, AP, CC, EC, EH, PB, PC
2- Pentanone	MEA	CC, PB
Cyclopentanone	MEA15%	AP
3- Octanone	MEA, MEA15%	AP, CC
Furan	MEA	AC, CC, EC, PB, PC
2-Methylfuran	MEA, MEA15%	AC, EC, EH, PC
2,5-Dimethylfuran	MEA	PC
3-Methylfuran	MEA	PB
Tetrahydrofuran	MEA15%	EH
Styrene	MEA	PB, PC

The results obtained from the fungal species may depend on the selected culture media. In order to determine potential markers independently from *in vitro* conditions, we compared the fungal VOCs with those obtained from two applied “real cases”: two contaminated books and the repository’s indoor air. The results are limited to our initial fungal selection; however, these species were the most abundant during the 1st sampling and for this reason they were considered as high relevant.

The GC-MS monitoring of the contaminated books, maintained isolated inside two desiccators at controlled environmental conditions (20 °C, saturated humidity condition), have produced two slightly different VOC patterns, characterized by typical compounds of paper degradation (*i.e.* several aldehydes) and fungal pathways. During the monitoring period (72 days) it was possible to follow the appearance and the concentration increase of several substances, especially from the 29th day of incubation. From this point on, the chemical’s increments followed the appearance of white spotted mycelial growth on the book covers. The principal books’ VOCs are reported in Table 4.14.

Table 4.13 Principal VOCs detected for the sampled books (books L6 and L9) during the monitoring period (72 days) at controlled environmental conditions.

Principal VOCs	**High relevance for book L6**	**High relevance for book L9**
Propane	X	X
Chloromethane	X	
Isobutane	X	X
2-Butene	X	X
Butane	X	
Acetone	X	X
Isopropyl alcohol	X	X
Furan	X	X
Pentane	X	X
1,4-Pentadiene	X	X
2-methylpentane	X	
2-Butanone	X	X
2-Methylfuran	X	X
3-Methylfuran	X	
2-Methyl-1-propanol	X	
3-Methyl-2-butanone	X	
Heptane	X	X
Octane	X	X
2,6-Dimethylheptane		X
Ethylcyclohexane		X
1,1,3-Trimethylcyclohexane		X
3-Ethylhexane		X
p-Xylene		X
o-Xylene		X
Nonane		X

The two books have 48% of the compounds in common. Their diversity could be due to the isolation of slightly different fungal pattern from their covers (differences in MVOC production) and their different component materials (cardboard cover for book L6 and cardboard with fabric for book L9).

Considering the contribution of *E. halophilicum* because of its massive growth on both books after 72 days of incubation, its production resulted similar to books' results for 14 compounds, including its principal MVOCs (*i.e*. 2-butanone, acetone and 1,4-pentadiene).

The canister sampling of the repository indoor air have highlighted the presence of only 34 compounds, similar to 32% of the fungal results and to 48% of the contaminated books. The low similarities might be due to the fact that the air sampling was performed after the book disinfection, thus the cleaning operation reduced the airborne microbial fraction as demonstrated by aerobiological results. For this reason, the indoor air quality resulted more influenced from the book presence, especially for aldehydes detection, than from the fungal presence. Moreover, results from the 3rd sampling suggested also the influence by chlorine cleaning products. If we consider all the similarities between VOC productions from fungi, contaminated books and indoor air samples, we obtain the following short list shown in the Table 4.15. Even if they are not included in the approved MVOC fungal list (see Par. 1.3.2, AIHA, 2005; Ryan, 2011; Wessén and Schoeps, 1996), they should be to taken into account for further investigations concerning fungal contamination inside book collection repositories.

Table 4.14 List of high relevance VOCs resulted similar in fungal, books and air investigations. AC: A. creber; AP: *A. penicillioides*; CC: *C. cladosporioides*; EC: *E. chevalieri*; EH: *E. halophilicum*; PB: *P. brevicompactum*; PC: *P. chrysogenum*. In bold type are the fungal species that were isolated from L6 and L9 books after VOC monitoring.

Substances	**High relevance for fungi and books**	**High relevance for contaminated books**	**High relevance for Indoor air**
1,4-Pentadiene	**AC,** EC, **EH, PC**	X	X
Isopropyl alcohol	**AC, AP,** EC, **EH**, PB, **PC**	X	X
Acetone	**AP,** CC, EC**, EH,** PB**, PC**	X	X
2-Butanone	**AC, AP,** CC, EC, **EH,** PB, **PC**	X	X

4.4 Secondary metabolite compounds

4.4.1 Metabolite patterns of the investigated fungal cultures

The analysis of 10 selected fungal species has permitted to identify a total of 42 metabolites. All produced metabolites, expressed for each fungal genera, are reported in Table 4.16 and related concentrations are reported in Appendix B5 - A. As already highlighted in the introduction section some of these metabolites are well-known mycotoxins.

For all strains and blanks (composed by agar disk of each culture medium used for fungal cultivation) similar production of unspecific cyclic dipeptides brevianamid F and cyclo(L-Pro-L-Tyr) was observed and subsequently excluded from the discussion. Detected metabolites differed between the genera, the strains of the same species, the culture media and the age of each colony. All these differences will be explained for each fungal genera.

Table 4.16 Overview of fungal secondary metabolites from selected fungal species.

Metabolites	***A. creber***	***A. jensenii***	***A. penicillioides***	***A. protuberus***	***A. vitricola***	***C. cladosporioides***	***E. chevalieri***	***E. halophilicum***	***P. brevicompactum***	***P. chrysogenum***
Andrastin A										X
Andrastin B										X
Aspergamid A	X*(4/5)	X								
Asperglaucide			X							
Averantin	X	X		X						
Averufanin	X	X		X						
Averufin	X	X		X						
Chaetoviridin A								X		
Chrysogin										X
Citreorosein	X*(3/5)	X	X*(4/8)	X*(1/2)					X	X*(1/2)
Demethylsulochrin										X*(1/2)
Deoxybrevianamid E	X	X	X*(1/8)					X	X	
Emodin	X	X		X		X*(1/3)	X		X	X*(1/2)
Fulvic acid										X
Meleagrin					X					X
Methoxysterigmatocystin	X	X								
Mycophenolic acid									X	
Neoechinulin A							X	X		
Neoxaline					X					X
Nidurufin	X	X		X						
Norsolorinic acid	X	X		X						
O-Methylviridicatin										
Orsellinic acid	X	X		X					X	
Oxaline										X
Pseurotin A								X*(1/3)		
Pseurotin D								X*(1/3)		
Roquefortine C										X
Roquefortine D										X

Rugulusovin								X		
Secalonic acid D										X
Stachybotriamide								X		
Sterigmatocystin	X	X		X						
Sydonic acid	X*(2/5)	X		X						
Tenellin								X*(1/3)		
Tryprostatin B	X	X	X*(1/8)					X	X	
Versicolorin A	X	X		X						
Versicolorin C	X	X		X						
Viridicatin										
Viridicatol				X*(1/2)						

*: Positive fraction metabolite production for the strains belonging to the same fungal species.

4.4.1.1 Aspergillus genera

We have analysed fungal species belonging to different *Aspergillus* genera:

- *A. creber*, *A. jensenii* and *A. protuberus* belong to *Aspergillus* section *Versicolores;*
- *A. penicillioides* and *A. vitricola* belong to *Aspergillus* section *Restricti*.

A. creber and *A. jensenii* produced similar secondary metabolite pattern as *A. versicolor* (Nielsen, 2003): methoxysterigmatocystin, sterigmatocystin and several biosynthetic precursors (averantin, averufanin, nidurufin, norsolorinic acid and versicolorin A and C) as well as orsellinic acid were detected at high levels. The strains of *A. protuberus*, also belonging to *Aspergillus* section *Versicolores*, differ from the other species by the lack of aspergamid, deoxybrevianamid E, methoxysterigmatocystin, tryprostatin B productions.

As reported by Micheluz *et al*. (2015a), *A. creber* was the most prevalent fungal species isolated by aerobiological analysis from the contaminated repository during the 1st sampling (October 2013), and because of its recent identification (Jurievic *et al.*, 2012), its mycotoxin production needs to be studied in more detail. This species, together with *A. jensenii* (26881.2 ngcm^{-2} agar) and *A. protuberus* (4662.9 ng cm^{-2} agar), showed the capacity to produce great quantity of sterigmatocystin (259.6-25841.8 ng cm^{-2} agar), in particular on YES culture media (Figg. 4.41, 4.42 and 4.43; note the different x-scale). This corroborates with previously reported values (Engelhart *et al*., 2001; Jurievic *et al*., 2013; Nielsen *et al.,* 1999). This metabolite is a biochemical precursor of aflatoxins and its toxicity, citotoxity, mutagenicity and carcinogenicity (2B carcinogen by the International Agency for Research on Cancer) both *in vitro* and *in vivo* studies was confirmed (Bloom *et al.,* 2007; Jurievic *et al*., 2013; Nielsen, 2002; Tuomi *et al*., 2000). Its carcinogenic attitude starts after activation in the liver by the cytochrome P450 mono-oxidase and operates as a strong inhibitor of tracheal ciliary movement (Cabaret *et al*., 2010; McConnell and Garner, 1994; Nielsen, 2003).

High concentrations of methoxysterigmatocystin were also detected for *A. creber* and *A. jensenii* strains, except for those of *A. protuberus*. This metabolite was measured in higher concentrations (up to 24425 ng cm^{-2} agar) than sterigmatocystin concentrations in samples cultivated on CYA medium (up to 11586 ng cm^{-2} agar), similar to the results reported by Cabaret *et al.* (2014). Further metabolites were detected which are generally produced by other fungal species, *e.g.* sydonic acid, typical of A*. sydowii* (fungal species that belongs to *Aspergillus* section *Versicolores*) and aspergamid A, typical of *A. ochraceus* (fungal species that belongs to *Aspergillus* section *Circumdati*). Their presence could be due to a secondary contamination.

The two strains of *A. protuberus* demonstrated a different behaviour: one strain (sample 46) showed the production of great quantities of sterigmatocystin, both growing on YES and CYA, while the other strain (sample 45), was lacking of these metabolites but was producing high quantities of versicolorin A. Generally, all 14 days old fungal strains presented higher secondary metabolite concentrations than those 7 days old.

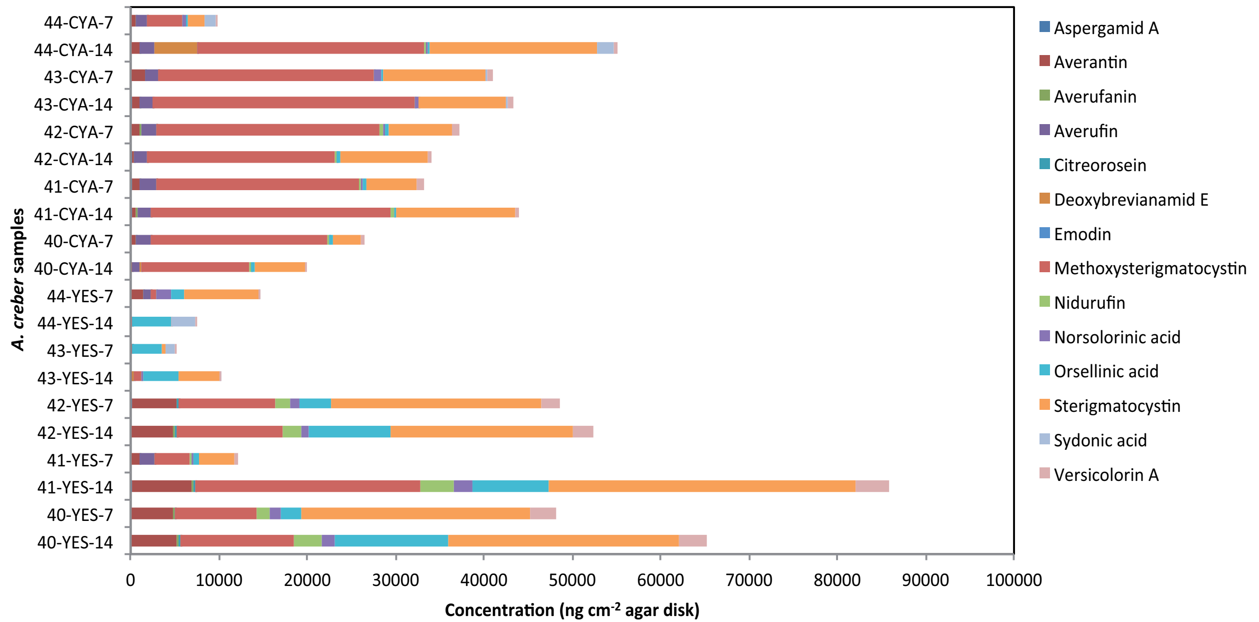


Figure 4.41 Secondary metabolites of *A. creber* strains (*n* = 5) produced on different culture media (YES and CYA) at different periods of incubation (7 and 14 days).

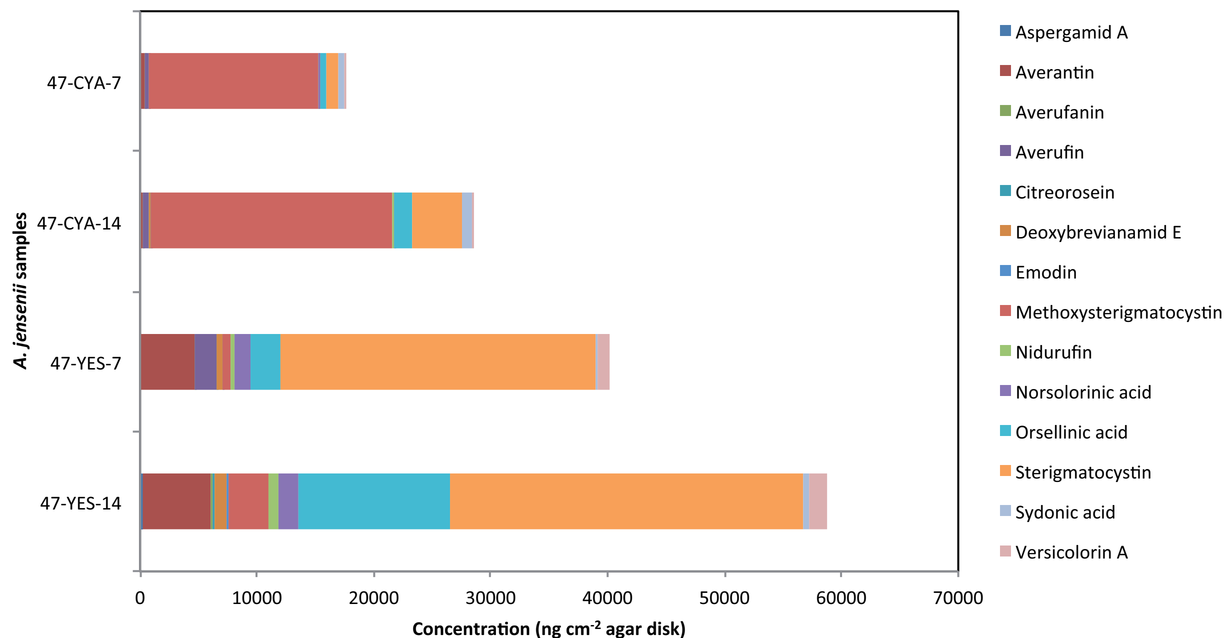


Figure 4.42 Secondary metabolites of *A. jensenii* strain (*n* = 1) produced on different culture media (YES and CYA) at different periods of incubation (7 and 14 days).

Differences in secondary metabolite production were observed for the fungal colonies grown on different culture media at different time. If we consider the species belonging to *Aspergillus* section *Versicolores*, and – specifically between the secondary metabolites - the sterigmatocystin production was generally higher for *A. creber* and *A. jensenii* that grown on YES medium in comparison with CYA medium (Fig. 4.44).

However, the opposite was observed for *A. protuberus*. Moreover, considering the different ages, the production resulted higher for the colonies grown for 14 days in comparison with those grown for 7 days.

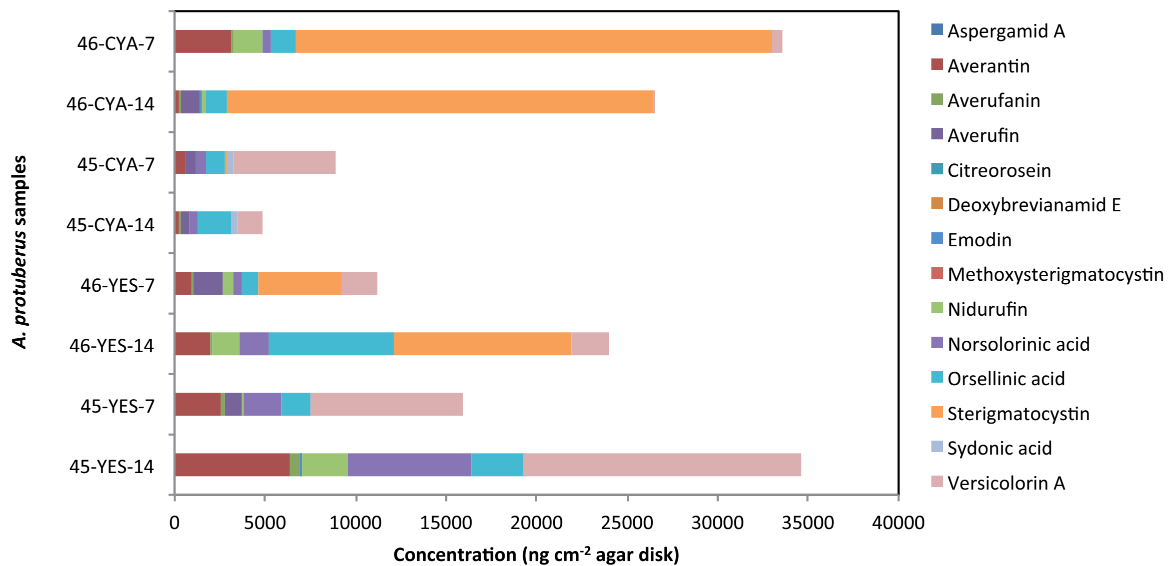


Figure 4.43 Secondary metabolites of *A. protuberus* strains (n = 2) produced on different media (YES and CYA) at different periods of incubation (7 and 14 days).

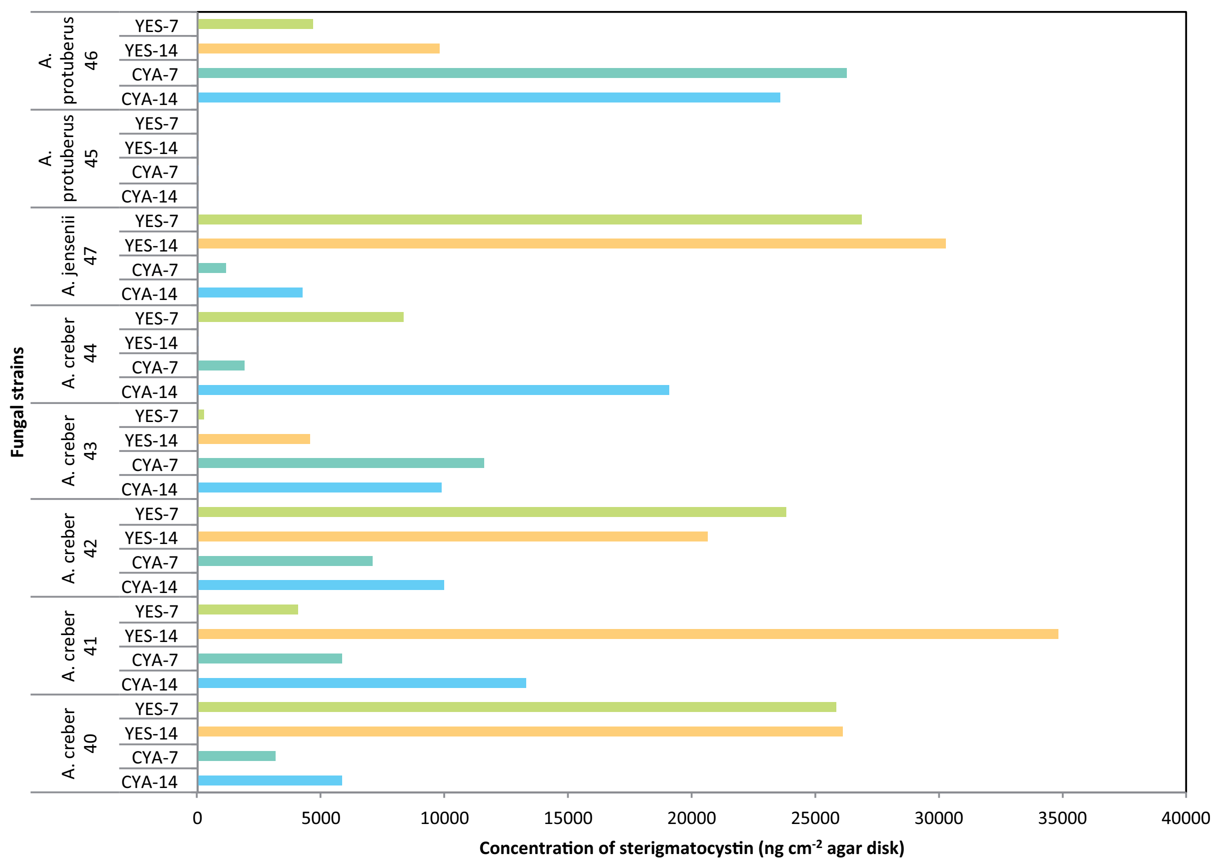


Figure 4.44 Sterigmatocystin production depending on fungal strain, culture medium and age of colony.

High concentrations of the alkaloid asperglaucide (4.9-11901.9 ng cm^{-2} agar) and a very low presence of citreorosein (5.5-25.3 ng cm^{-2} agar) and deoxibrevianamid E (22.9 ng cm^{-2} agar) characterizing the samples of *A. penicillioides* (Fig. 4.45). Other potential metabolites like cristatin A and the related arestrictin A and B that were detected in previous studies (Itabashi *et al.,* 2006) were not included in the method.

However, *A. penicillioides*, as *A. restrictus* and *A. vitricola*, is considered as stress selected species, with a poor production of secondary metabolites (Tamura *et al.,* 1999; Peterson, 2008; Frisvad, 2015). In fact, the strains of *A. vitricola* were able to produce only the unspecific metabolites brevianamid F and cyclo(L-Pro-L-Tyr).

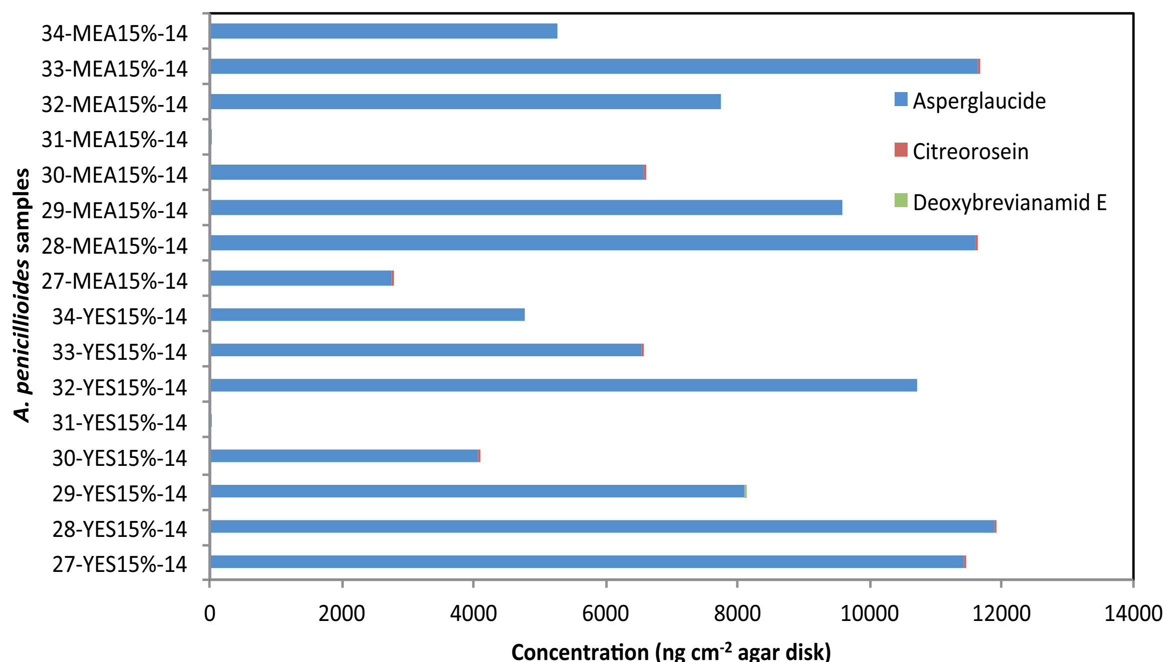


Figure 4.45 Secondary metabolites of *A. penicillioides* strains (*n* = 8) produced on different culture media (YES15% and MEA15%) after incubation of 14 days.

4.4.1.2 *Cladosporium* genera

C. cladosporioides produced only very small amounts of metabolites, with the sporadically presence of emodin (1.4 ng cm^{-2} agar) in one strain (sample 52) (Fig. 4.46). Jacyno *et al*. (1993) reported also the production of icocoumarin, cladosporin and isocladosporin, but this fungal genera is known to produce only sporadic quantities of mycotoxins (Pitt and Hocking, 1997).

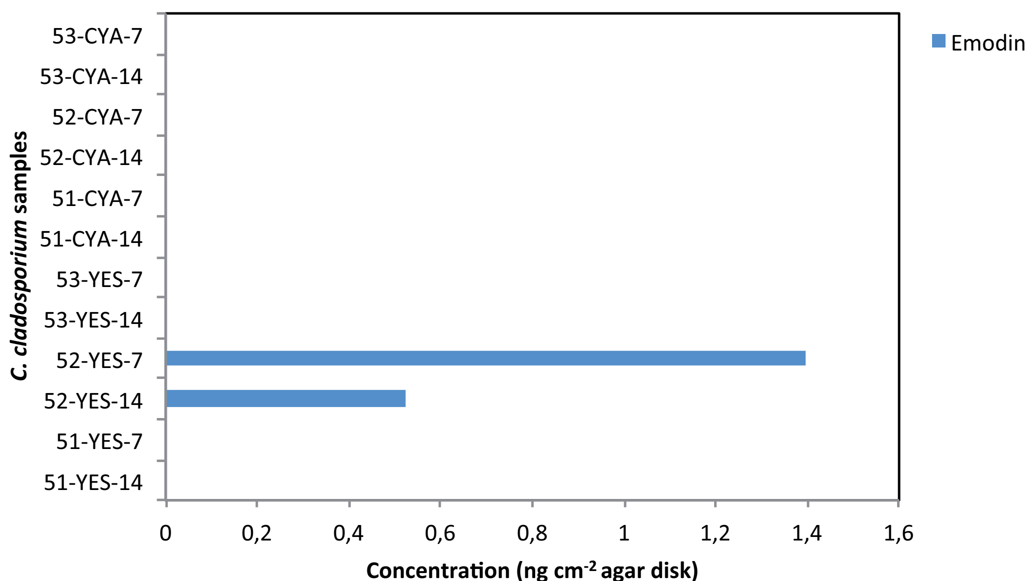


Figure 4.46 Secondary metabolite of *C. cladosporioides* strains (*n* = 3) produced on different culture media (YES and CYA) at different periods of incubation (7 and 14 days).

4.4.1.3 Eurotium genera

All the *Eurotium* strains showed the production of the typical metabolite neoechinulin A (Butinar *et al*., 2005; Slack *et al*., 2009), with values up to 3560.4 ng cm^{-2} agar and 10.7 ng cm^{-2} agar for *E. chevalieri* and *E. halophilicum*, respectively. For *E. chevalieri* the production of this secondary metabolite resulted higher on CYA medium than on YES medium, especially for younger colonies (7 days old) (Fig. 4.47). Butinar *et al*. (2005) reported several compounds which where not included in our method, *e.g.* asperentin, echinulin, neoechinulin B and C, physcion, auroglaucin, dihydroauroglaucin, tetrahydroauroglaucin among other metabolites of *E. chevalieri*, especially for strains isolated from hypersaline waters.

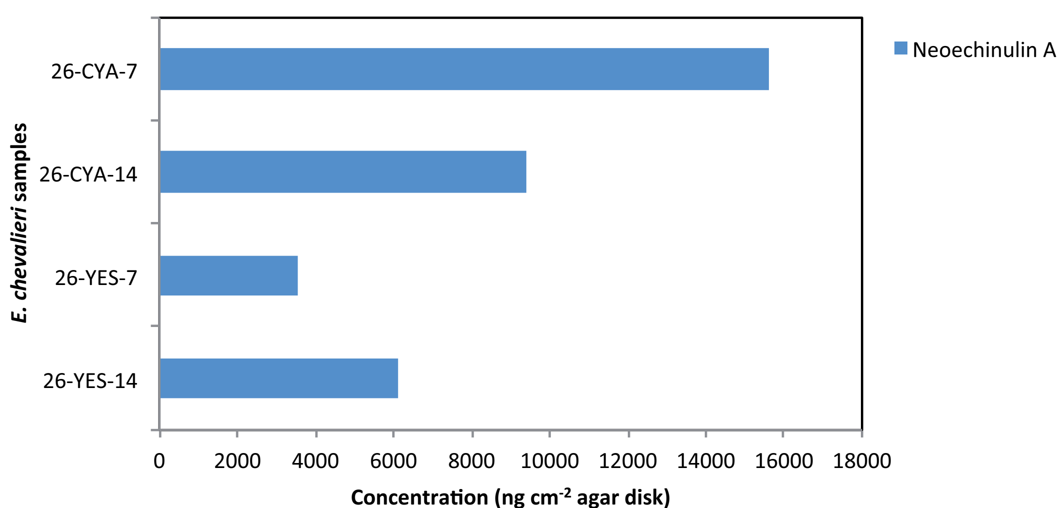


Figure 4.47 Secondary metabolite production of *E. chevalieri* strain (*n* = 1) produced on different culture media (YEA and CYA) at different periods of incubation (7 and 14 days).

Some of the *E. halophilicum* strains were characterized by the production of pseurotin A (1369.5 ng cm^{-2} agar) and pseurotin D (1046.5 ng cm^{-2} agar) (Fig. 4.48), known as typical metabolite of *A. fumigatus* (Huang *et al*., 2010).

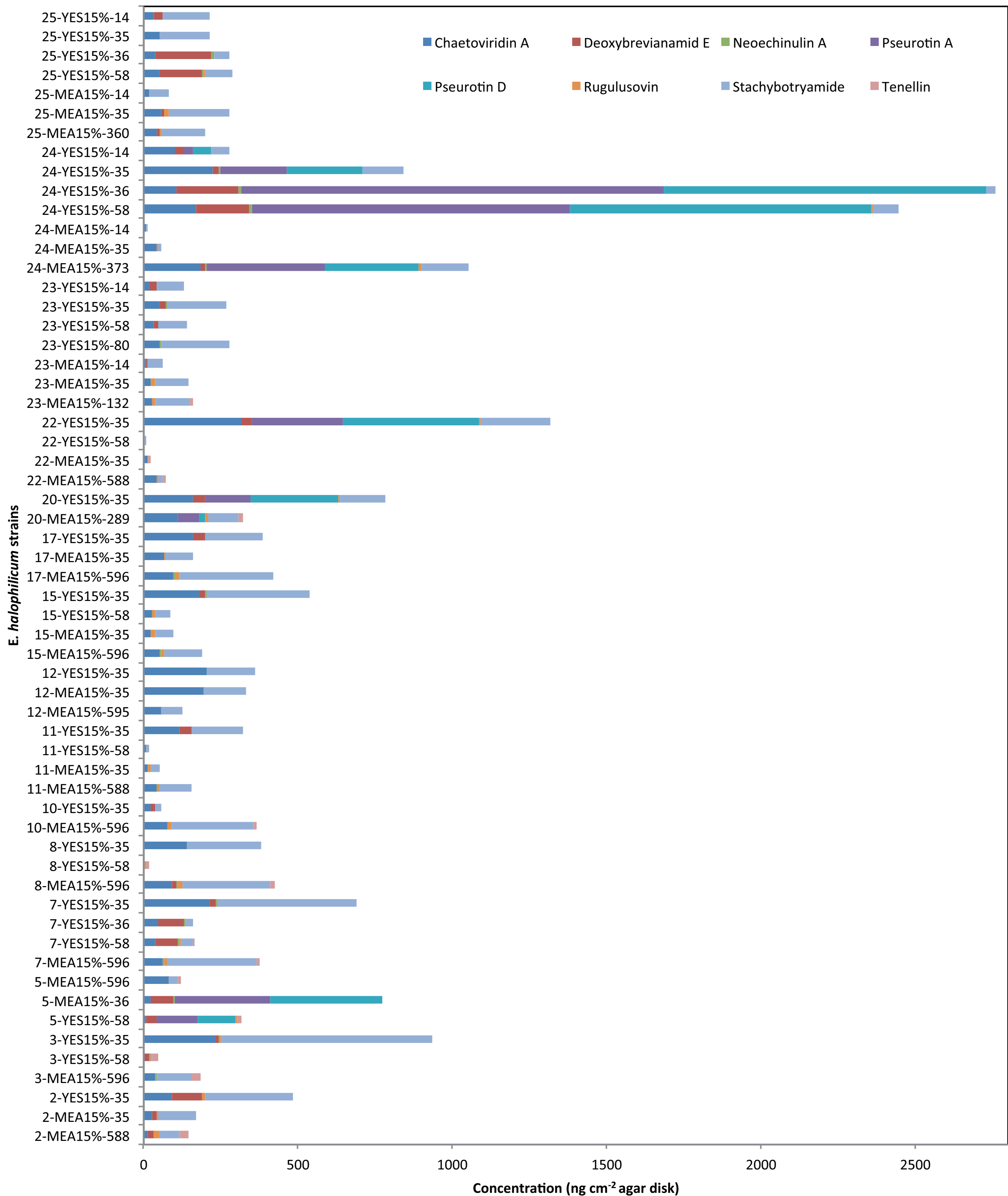


Figure 4.48 Secondary metabolites of *E. halophilicum* strains (*n* = 15) produced on different culture media (YEA15% and MEA15%) at different periods of incubation (15-596 days).

Deoxybrevianamid F, rugulosovin and tenellin were also detected as well as two compounds typical for other fungal species: chaetoviridin A from *Chaetomium* sp. and stachybotryamide from *Stachybotrys* sp. (Laatsch, 2005). For these two compounds, all related MRM transitions exhibited peaks with the correct intensity ratios and a small, but yet significant, difference in retention time compared to the authentic standards (probably isomers).

The strains isolated from the BAUM's repository (samples 23, 24 and 25) were the most productive secondary metabolite producers amoung all the *E. halophilicum* strains (Fig. 4.49). In Sample 24, the highest concentrations of pseurotin A (1369.5 ng cm^{-2} agar disk) and D (1046.5 ng cm^{-2} agar disk) were detected, especially on YES15% medium after 36-58 days of incubation, but also on MEA15% after 373 days. The dominating presence of these secondary metabolites, typically produced by *Aspergillus fumigatus*, may be due to the anamorphic status of *E. halophilicum*, namely, *A. halophilicus*. The pictures of the colony inside the Petri dishes made by stereomicroscope have demonstrated the presence of *Aspergillus* sp. conidiophores (Fig. 4.50). These typical conidial state structures were not clearly evident for the other strains. Although the species was associated with *Aspergillus restrictus* group by Raper and Fennell (1965), a recent *Aspergillus* phylogenetic study (Peterson, 2008) demonstrated how *E. halophilicum* differs from the other species belonging to this group. This might be the reason for the moderate secondary metabolite production.

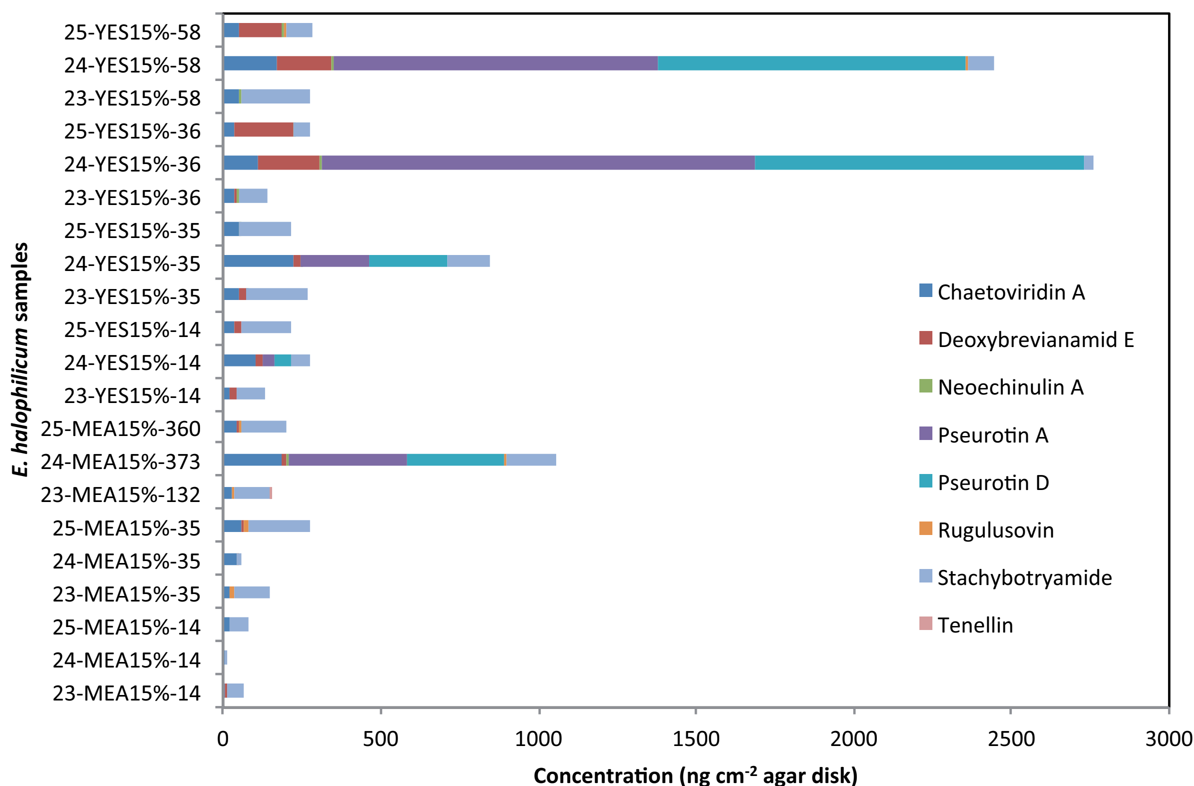


Figure 4.49 Secondary metabolites of *E. halophilicum* strains isolated from BAUM's repository (samples 23, 24 and 25) isolated on different culture media (YES15% and MEA15%) at different periods of incubation (14-373 days).

Figure 4.50 Micrograph of *E. halophilicum*, sample 24, on YES15% after 36 days of incubation. Particular of conidiophores, 15x.

4.4.1.4 Penicillium genera

Mycophenolic acid (85201 ng cm^{-2} agar), orsellinic acid (112498 ng cm^{-2} agar), citreorosein (77 ng cm^{-2} agar), deoxybrevianamid E (12 ng cm^{-2} agar) and emodin (2 ng cm^{-2} agar) were detected for *P. brevicompactum*, (Fig. 4.51). Nielsen (2003), Andersen (1991) and Frisvad *et al.* (2004) reported also botrydiploidin, asperphenamate, Raistrick phenols (2,4-dihydrosy-6-(2-oxopropyl) benzoic acid, 2,4-dihydroxy-6-(1-hydroxy-oxopropyl) benzoic acid and 2,4-dihydroxy-6-(1,2-dioxopropyl) benzoic acid) as potential metabolites of this specie. They were not detected in this study because they were not included in the list of target compounds due to lack of authentic standards.

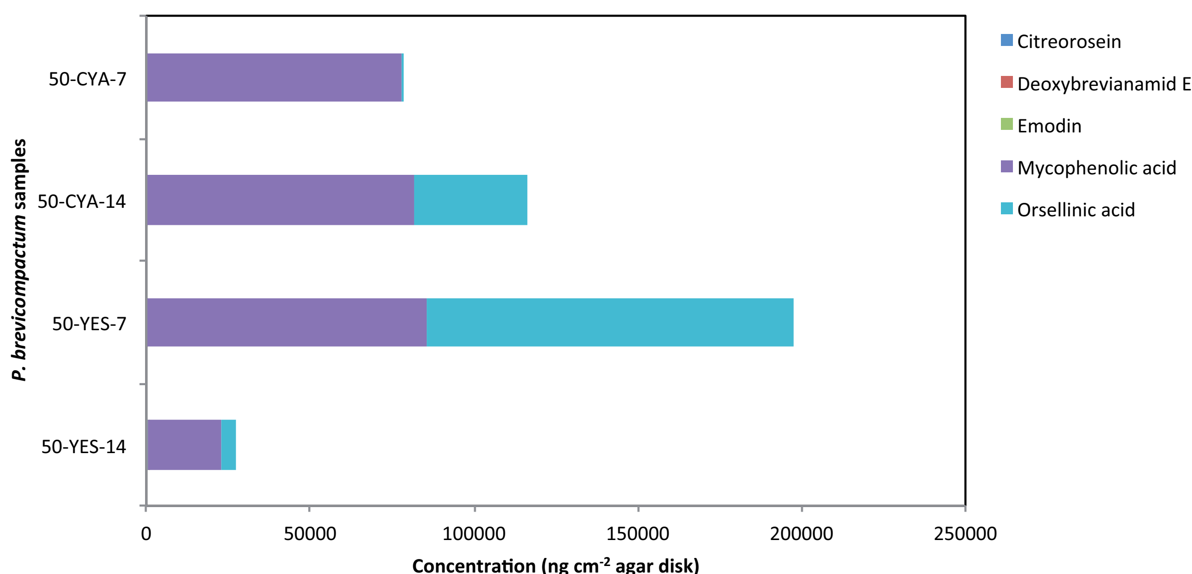


Figure 4.51 Secondary metabolites of *P. brevicompactum* strain (n = 1) produced on different culture media (YEA and CYA) at different periods of incubation (7 and 14 days).

P. chrysogenum strains produced high quantities of andrastin A (46884 - 47088 ng cm^{-2} agar) and B (873 - 1029 ng cm^{-2} agar), meleagrin (39191 - 44979 ng cm^{-2} agar), roquefortine C (2657 -13867 ng cm^{-2} agar), secalonic acid D (333 - 171905 ng cm^{-2} agar), as well as a moderate production of chrysogin (15 -73 ng cm^{-2} agar), fulvic acid (70 ng cm^{-2} agar), neoxaline (214 - 139 ng cm^{-2} agar), oxaline (3 ng cm^{-2} agar) and roquefortine D (124 -336 ng cm^{-2} agar) on YES medium (Fig. 4.52). For one strain (*P. chrysogenum* MUT4593) the additional production of citreorosein, demethyl sulochrin and emodin was detected.
The detection of these secondary metabolites is in agreement with previously reported results (Frisvad *et al*., 2004; Gutarowska *et a*l., 2014; Nielsen, 2003). Andrastin A, andrastin B, citreorosein and fulvic acid are typical for *Penicillium* spp., while oxaline was previously signalled only by *P. atramentosum, P. crustosum*, *P. glandicola* and *P. oxalicum* as well as neoxaline, which is typical for *P. atramentosum, P. coprobium* and *P. tulipae* (Frisvad *et al*., 2004; Laatsch, 2005).

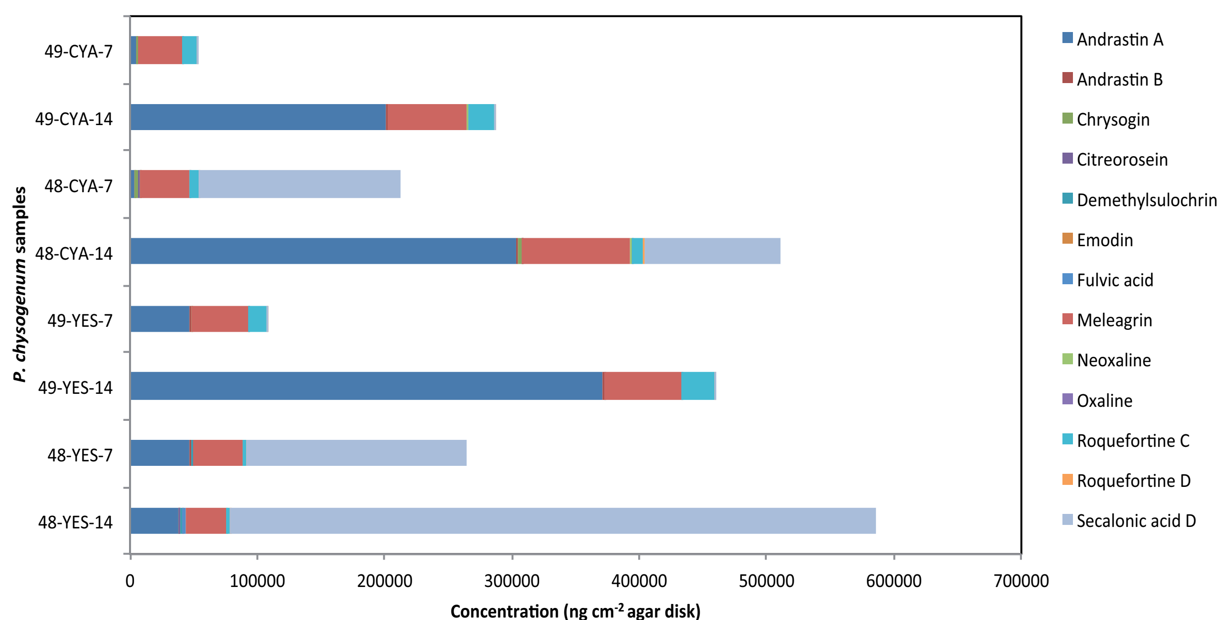


Figure 4.52 Secondary metabolites of *P. chrysogenum* strains (*n* = 2) produced on different culture media (YEA and CYA) at different periods of incubation (7 and 14 days).

4.5.2 Metabolite patterns in settled dust samples

The analysis of settled dust collected during the 2nd sampling in the repository (see Par. 3.4.2), demonstrate the occurrence of 43 different microbial metabolites, including 25 metabolites detected from the fungal cultures, 16 compounds attributed to other fungal species, 1 bacterial metabolite and 1 plant toxin (Fig. 4.53). All the secondary metabolite concentrations are reported in Appendix B5 - B. Metabolites of *Alternaria* (alternariolmethylether, altersetin, macrosporin), *Ascochyta* (ascochlorin), *Aspergillus* (asperglaucide, averufanin, averufin, neoxaline, nidurofin, metoxysterigmatocystin, physcion, sterygmatocystin, sydonic acid, terrecyclic acid), *Beaveria* (beauverucin, tenellin), *Chaetomiun* (chetomin),

Eurotium (neoechinulin A), *Fusarium* (enniatin B, ilicicolin B, monocerin), *Mycosphaerella* (usnic acid), *Penicillium* (andrastin A and B, citreorosein, cyclopenol, meleagrin, o-methylviridicatin, quinocitrinine A, roquefortine C, rugulusovin, skirin, viridicatin, viridicatol), *Stachybotrys* (stachybotryamide, stachybotrylactam), *Trichoderma* (alamethicin, chrysophanol) were detected, while emodin could be attributed to more than one genus of indoor fungi (Laatsch, 2005). As for fungal species, brevianamid F and cyclo(L-Pro-L-Tyr) were also detected in all dust samples, but they were not taken into account for the discussion (see Par. 4.4.1).

Among the total of 43 compounds, 21 metabolites were found in all the sampled areas and the most abundant metabolites were asperglaucide (58%), alamethicin (13%), andrastin A (10%), mainly belonging to *Aspergillus* spp.*,* *Trichoderma* spp. and *Penicillium* spp.

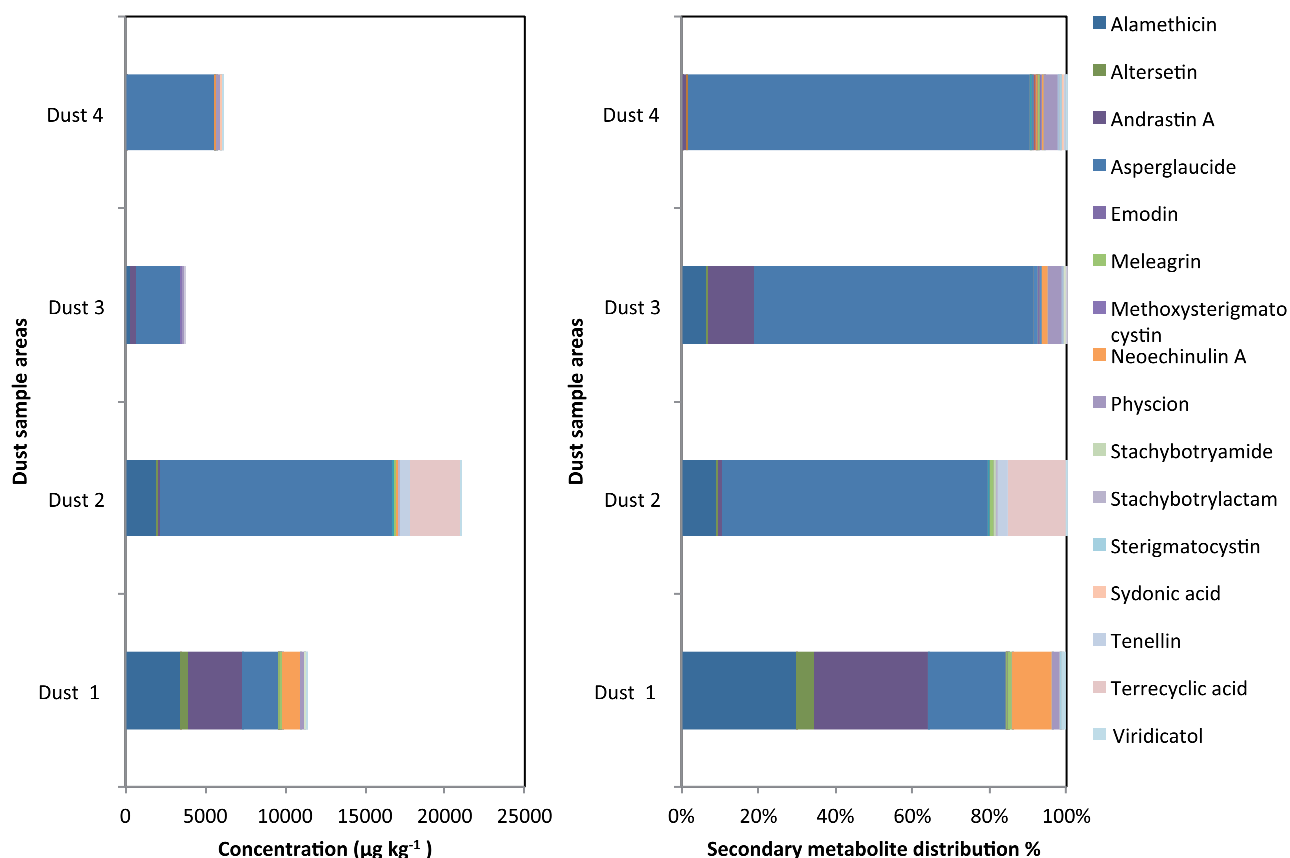


Figure 4.53 Secondary metabolite distributions found in settled dust samples collected from 4 areas inside the repository. Only the mainly frequent compounds are reported for each sample.

In the dust sample from area 1, corresponding to the highest fungal contamination during the 1st sampling (Micheluz *et al.*, 2015a, b), 32 different secondary metabolites were detected, belonging to 11 different fungal genera, with alamethicin (30%), andrastin A (30%), asperglaucide (20%) and neoechinulin A (10%) as principal products.

The dust from area 2 was dominated by asperglaucide (69%), terrecyclic acid (15%) and alamethicin (9%). In total, 24 compounds were detected, belonging to 13 different fungal species.

In area 3, 31 secondary metabolites were measured in the dust sample, belonging to 13 different fungi, with asperglaucide (72%), andrastin A (12%), alamethicin (6%) and physcion (4%) as predominant compounds.

Finally, the dust from area 4, corresponding to the entrance of the repository, presented the broadest fungal metabolite spectrum with 37 different compounds, produced by 10 different fungal genera. However, 89% of the total concentration was represented by asperglaucide.

In general, mycotoxin similarities were found among all the repository's areas. In particular, area 4 was recognised to have the lowest level (Bray-Curtis Index d^{BCS} = 0.26) if compared with area 2 and the highest level (Bray-Curtis Index d^{BCS} = 0.62) if compared with area 3.

The characterization of dust samples from the repository has emphasized the generic presence of the metabolite asperglaucide (2274 - 14563 μg kg^{-1}), potential indicative of a presence of xerophilic fungi as *A. penicillioides* and *A. restrictus*, as well as for plants (Wu *et al*., 2011). However, the particular structural characteristics of the repository do not permit an air exchange with the outside, suggesting fungal origin as the source of asperglaucide. Inside the repository, *A. penicillioides* was identified as airborne fungus and book colonizer. Already in 1978, *A. penicillioides* and *E. halophilicum* have been associated with house-dust mites, emphasizing the possibility of being responsible for allergic rhinitis and lunge disease (Samson and Lustgraaf, 1978). In several studies, this fungus was also isolated from dry food and indoor environment, including museums and archives (Pitt and Hocking, 1997; Samson *et al*., 2004). In particular, *A. penicillioides* has often been isolated from old contaminated books and manuscripts (Michaelsen *et al*., 2010; Micheluz *et al*., 2015a; Montanari *et al*., 2012).

High concentrations of *Trichoderma viride*'s peptaibol alamethicin were also detected in dust samples (4 - 3368 μg kg^{-1}), higher than those reported in similar studies of indoor environments (Täubel *et al*., 2011; Vishwanath *et al.,* 2011). Micheluz *et al*. (2015a) did not detect this species during the fungal analysis of the library, maybe due to specific low water activity medium used for the sampling, but the abundant presence of this typical metabolite in dust could be significant inside the library and for the preservation of book collections, especially because of the cellulolytic activity of *Trichoderma* sp. towards to textiles, paper and timber (Yang *et al*., 2007; Zyska, 1997). Moreover, *Trichoderma* spp. is a common indoor dust fungus, known to be implicating in damp and mouldy buildings and adverse human health effects (Larsen *et al*., 1996; Lignell *et al.,* 2008; Nielsen *et al.,* 2005).

The occurrence of typical indoor fungi *Penicillium* spp. was pointed out by high concentrations of andrastin A (67 - 3372 μg kg^{-1}) whereas other typical *Penicillium* metabolites, as cyclopenol (3 - 43 μg kg^{-1}), quinocitrinine A (0.3 – 0.5 μg kg^{-1}) and skyrin (0.6 – 4.6 μg kg^{-1}) were found (Laatsch, 2005).

Elevated concentrations of neoechinulin A were probably indicative for *Eurotium* spp., starting from 29 μg kg^{-1} in correspondence to area 4, up to 1184 μg kg^{-1} in area 1. Micheluz *et al.* (2015a) have detected their presence inside the BAUM's repository, identifyng *E. halophilicum* as the main responsible of the spread fungal colonization on the books.

In area 2 high concentrations of terrecyclic acid, a typical metabolite of *A. terreus*, were detected (3149 μg kg^{-1}). This sesquiterpene metabolite was recognized as possessor of broad-spectrum antimicrobial and antitumor activity and the fungus is normally used against plant pathogens (Hyder *et al*., 2009; Turbyville *et al.,* 2005). However, its presence was demonstrated in house-dust samples as well as deteriorated books and manuscripts (Abdel-Hafez *et al*., 1990; Michaelsen *et al*., 2010).

An additional metabolite detected in almost all areas was physcion (146 - 233 μg kg^{-1}). As chrysophanol (3 - 45 μg kg^{-1}) and emodin (11 - 21 μg kg^{-1}), this metabolite is an antraquinone derivate produced by fungi, such as *Aspergillus* spp. and *Eurotium* spp., as well as by plants (Laatsch, 2005).

The concentrations of methoxysterigmatocystin (5 – 27 μg kg^{-1}) and sterigmatocystin (2 – 17 μg kg^{-1}) in the dust samples were higher in comparison to indoor dust samples of studies of severe moisture damage/dampness problems (Bloom *et al*., 2007; Engelhart *et al*., 2002) but lower than samples from mouldy building materials (Tuomi *et al*., 2000; Vishwanath *et al*., 2011).

Low amounts of stachybotryamide (0.5 – 4.1 μg kg^{-1}) and stachybotrylactam (1 μg kg^{-1}, only for area 4), belonging to *Stachybotrys chartarum*, were detected in dust samples. The occurrence of this fungus in the dust was not detected, but it was frequently isolated from house-dust, as well as building materials (Bloom *et al*. 2007). Furthermore, *S. chartarum* is a well-known strong cellulolytic species and a possible great secondary metabolite producer (strain depending), such as highly toxic macrocyclic trichothecenes (Li *et al.,* 2005; Nielsen, 2003).

Several other metabolites belonging to fungi not analysed in this study were detected in the dust samples, but only at low concentrations. Alternariolmethylether (1.8 – 4.3 μg kg^{-1}), altersetin (3 - 524 μg kg^{-1}) and macrosporin (4.5 – 6.1 μg kg^{-1}), are *Alternaria* spp.'s metabolites, as well as enniatin B (0.1 – 0.3 μg kg^{-1}), ilicicolin B (0.5 – 16 μg kg^{-1}) and monocerin (1.6 – 5.6 μg kg^{-1}) are typical for *Fusarium* spp. and chetomin (15 – 24 μg kg^{-1}) for *Chaetomiun* spp. (Laatsch, 2005). These fungal species are common outdoor fungi but they were often isolated from indoor environments, books and documents (Gallo, 1993; Pitt and Hocking, 2009; Samson *et al*., 2004; Zyska, 1997). Metabolites as usnic acid (6.4 – 26.7 μg kg^{-1}), beaverucin (0.8 μg kg^{-1}) and ascochlorin (1.6 – 10.4 μg kg^{-1}) may originate from two outdoor organisms and fungi, such as lichens or *Mycosphaerella nawa*, *Beauveria bassiana*, and *Ascochyta vicinae,* respectively (Laatsch, 2005).

As bacterial metabolite, only chloramphenicole (1.2 – 44.4 μg kg^{-1}) was detected, especially in area 2. This metabolite probably derived from *Streptomyces* spp., Gram-positive bacteria that commonly occur in the soil and in indoor environments with heavy problems of moisture damages and dampness (Rintala *et*

al., 2004). Chloramphenicole is a well-known broad-range antibiotic and because of resistance and safety concerns has been banned for the use in food-producing animals and is restricted in human applications (Täubel *et al*., 2011).

4.4.3 Statistical analysis of fungal and dust's secondary metabolites

The results obtained from fungus analyses can be compared to the secondary metabolite content in the dust samples in order to verify their presence also as settled deposit. Contrary to the MVOCs, the secondary metabolite production is more fungal species-specific. So, it is easier to identify the presence of fungal species (alive or dead) in complex matrix, as food sample, starting from secondary metabolite pattern than from MVOC pattern.

Analysing the settled dust samples we have demonstrated the possible presence of all isolated and selected fungal species, even if not all fungal metabolite patterns were detected. Moreover, several other metabolites belonging to foreign fungi (not detected by aerobiological analyses) were detected, increasing the general fungal variability found in the repository. In the following graphs, the comparison between fungal and dust results for each samples area were reported (Fig. 4.54). The metabolite producers are also reported in the legend.

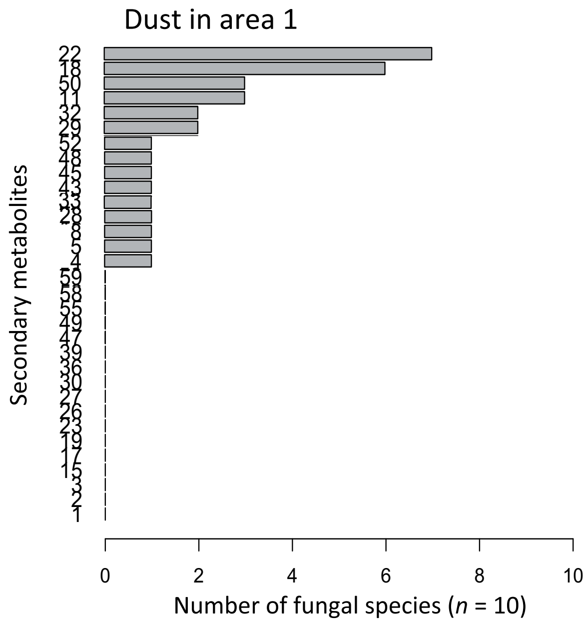
As highlighted from the bar plot, the dust collected in area 1 and selected fungal species resulted similar for 16 secondary metabolites, mainly belonging to *P. chrysogenum* (*n* = 6), *A. creber*, (*n* = 5) and *A. jensenii* (*n* = 5). The other fungal presences is composed by 4, 4, 2, 2, 2 and 1 secondary metabolites for *A. protuberus*, *E. halophilicum*, *A. penicillioides*, *E. chevalieri* and *C. cladosporioides*, respectively.

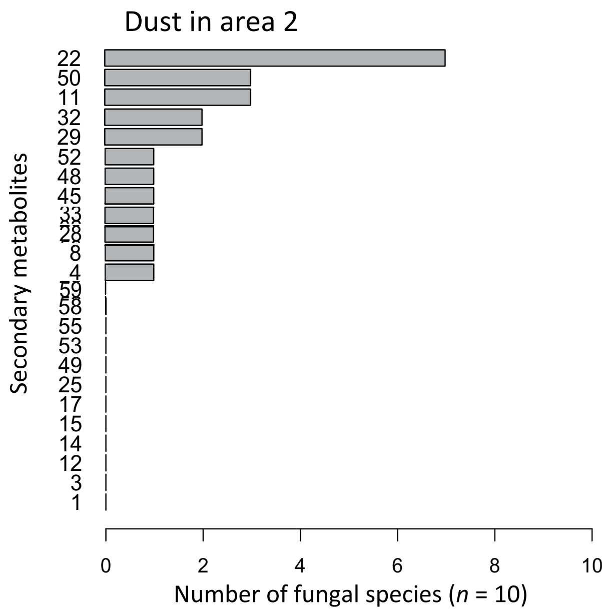
Considering the external fungal contribution, *Penicillium* sp. was present with 5 substances, followed by *Alternaria* sp. (*n* = 3), *Fusarium* sp. (*n* = 2), *Stachybotrys* sp. (*n* = 2), *Trichoderma* sp. (*n* = 2), *Aspergillus* sp. (*n* = 1), bacteria (*n* =1), *Myrothecium* sp. (*n* = 1) and plants (*n* = 1).

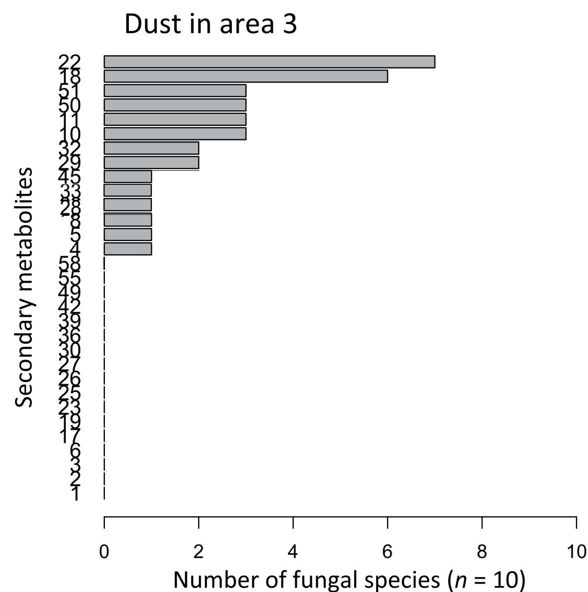
The dust from area 2 is similar to our fungi for 24 secondary metabolites, belonging to *A. creber* (n = 4), *A. jensenii* (*n* = 4), *E. halophilicum* (*n* = 4), *P. brevicompactum* (*n* = 4), *A. protuberus* (*n* = 3), *P. chrysogenum* (*n* = 3), *E. chevalieri* (*n* = 2), *A. penicillioides* (*n* = 1) and *C. cladosporioides* (*n* = 1).

The external contribution consists mainly by *Penicillium* sp. (*n* = 3), *Stachybotrys* sp. (*n* = 2), *Trichoderma* sp. (*n* = 2), *Alternaria* sp. (*n* = 1), *A. terreus* (*n* = 1), bacteria (*n* = 1), *Beauverucin* sp. (*n* =1), *Chaetomium* sp. (*n* =1), *Fusarium* sp. (*n* = 1) and *Myrothecium* sp. (*n* = 1).

In dust from area 3, 31 secondary metabolites were also detected in our fungi, in particular: *A. creber* (*n* = 7), *A. jensenii* (*n* = 7), *A. protuberus* (*n* = 6), *P. chrysogenum* (*n* = 5), *A. penicillioides* (*n* = 2), *E. chevalieri* (*n* = 2), *E. halophilicum* (*n* = 2), *P. brevicompactum* (*n* = 2) and *C. cladosporioides* (*n* = 1). The other metabolites are mainly belonging to *Penicillium* sp. (*n* = 5), *Alternaria* sp. (*n* = 3), *Fusarium* sp. (*n* = 3), *Trichoderma* sp. (*n* = 2), *Ascochyta* sp. (*n* = 1), *Aspergillus* sp. (*n* = 1), *Myrothecium* sp. (*n* = 1), plants (*n* = 1) and *Stachybotrys* sp. (*n* = 1).







Code	Secondary metabolites in settled dust	Fungal species
1	Alamethicin	TR
2	Alternariolmethylether	AL
3	Altersetin	AL
4	Andrastin A	**PC**
5	Andrastin B	**PC**
6	Ascochlorin	AS
8	Asperglaucide	**APEN**
10	Averufanin	**AC, AJ, APR**
11	Averufin	**AC, AJ, APR**
12	Beaverucin	BE
14	Chetomin	CH
15	Chlroranphenicol	BA
17	Chrysophanol	TR
18	Citreorosein	**AC, AJ, APEN, APR, PB, PC**
19	Cyclopenol	PE
22	Emodin	**AC, AJ, APR,CC, EC, PB, PC**
23	Enniatin B	FU
25	Ilicicolin B	FU
26	Lotaustralin	VEG
27	Macrosporin	AL
28	Meleagrin	PE
29	Methoxysterigmatocystin	**AC, AJ**
30	Monocerin	FU
32	Neoechinulin A	**EC, EH**
33	Neoxaline	**PC**
34	**Nidurufin**	**AC, AJ, APR**
36	O-Methylviridicatin	PE
39	Physcion	ASP
42	Quinocitrinine A	PE
43	Roquefortine C	**PC**
45	Rugulusovin	**EH**
47	Skyrin	PE
48	Stachybotryamide	**EH**, ST
49	Stachybotrylactam	ST
50	Sterigmatocystin	**AC, AJ, APR**
51	Sydonic acid	**AC, AJ, APR**
52	Tenellin	**EH**
53	Terrecyclic acid	AT
55	Usnic acid	MY
58	Viridicatin	PE
59	Viridicatol	PE

Dust in area 4

Secondary metabolites

0 2 4 6 8 10

Number of fungal species (n = 10)

Figure 4.12 Bar plots with secondary metabolite compounds detected in settled dusts samples compared to results obtained from selected fungal species (n = 10). In the legend are reported the secondary metabolites with their relative code together with their known fungal producers. AC: *A. creber*; AJ: *A. jensenii*; AL: *Alternaria* sp.; APEN: *A. penicillioides*; APR; *A. protuberus*; AS: *Ascochyta* sp.; ASP: *Aspergillus* sp.; AT: *A. terreus*; BAC: bacteria; BE: *Beauverucin* sp.; CC: *C. cladosporioides*; CH: *Chaetomium* sp.; EC: *E. chevalieri*; EH: *E. halophilicum*; FU: *Fusarium* sp.; MY: *Myrothecium* sp.; PB: *P. brevicompactum*; PC: *P. chrysogenum*; PE: *Penicillium* sp.; ST: *Stachybotrys* sp.; TR: *Trichoderma* sp.; VEG: vegetal. The marked names are for the studied fungi. *A. vitricola* is not reported because it did not produced any record.

Finally, the dust from area 4 was similar for 37 secondary metabolites to our fungal species, in particular: *A. creber* (n = 8), *A. jensenii* (n = 8), *A. protuberus* (n = 7), *E. halophilicum* (n = 4), *P. chrysogenum* (n = 4), *A. penicillioides* (n = 2), *E. chevalieri* (n = 2), *P. brevicompactum* (n = 2) and *C. cladosporioides* (n = 1). The rest of compounds, instead, were belonged to *Penicillium* sp. (n = 7), *Alternaria* sp. (n = 3), *Fusarium* sp. (n = 3), *Stachybotrys* sp. (n = 2), *Trichoderma* sp. (n = 2), *Ascochyta* sp. (n = 1), *Aspergillus* sp. (n = 1), Chaetomium sp. (n = 1), *Myrothecium* sp. (n = 1) and plants (n = 1).

Considering the microbial variability, slightly differences were observed among all the repository's areas. The dust collected in area 4 demonstrated the highest variability, with at least 11 fungal genera, bacteria and plants. Also the dust in area 2 was characterized by 11 fungal genera with only bacteria, while in dust from area 3 ten different fungal genera with plants' presence. Finally, the dust from area 1 was composed by secondary metabolites belonging to at least 9 fungal genera, bacteria and plants.
These results suggest an agreement with those obtained from the 2nd and 3rd aerobiological samplings, where the highest microbial variability corresponded to the most frequently pass area 4 (see the repository map, Par. 3.1.2.2), followed by area 2, corresponded to the corridor far from the entrance. The other areas 3 and 4, representing the situations between Compactus® shelves, could be considered as isolated areas, characterized by lower fungal load (especially in area 1 with all disinfected books) (see Par. 4.1.3).

4.4.4 Summary

The fungal species selected for the secondary metabolite analysis were demonstraded an increased number of species (and where it was possible, also of number of strains) as those from MVOC collection (see Par. 3.3.2). The aim was to study a high number of fungi isolated from both repository's indoor air and contaminated books in order to determine their secondary metabolite ability, especially for the less known fungal species, as *E. halophilicum*.

Similar metabolite products were detected for the fungal species belonging to *Aspergillus* section *Versicolores*, *i.e*. *A. creber*, *A. jensenii* and *A. protuberus*. As their closer species *A. versicolor*, they were characterized by the production of sterygmatocystin, metoxysterigmatocystin (excluded *A. protuberus*), and several biosynthetic precursors. The analysis of several fungal strains at different ages on two culture media has highlighted the highest secondary metabolite, *i.e*. sterigmatocystin, generally produced by older colonies (14 days after incubation) on YES medium, with the exception of the strains of *A. protuberus* that were more productive on CYA.

The other two *Aspergillus* species, *A. penicillioides* and *A. vitricola*, confirmed their poor secondary metabolite production. Only asperglaucide was detected for the strain s of *A. penicillioides*, while no records were observed for the other species. A similar poor productin was observered for the strains of *C. cladosporioides*, where only emodin was detected.

E. chevalieri was characterized by only neoechinulin A production, especially on CYA medium. The other *Eurotium* species, *E. halophilicum*, also produced this metabolite, but the analyses of 15 different strains on two culture media and at different ages, have highlighted the presence of several other products. Pseurotin A and D, deoxybrevianamid F, rugulosovin, tenellin together with the possible presence of chaetoviridin A and stachybotryamide characterized most of the samples, especially those grown on YES15% medium (that resulted better than MEA15%) for at least 1 month. This is the first report about secondary metabolite ability of this particular fungal species.

Finally, the analyses of *Penicillium* species confirmed the data in literature. *P. brevicompactum* was mainly characterized by mycophenolic acid, while for *P. chrysogenum* several secondary metabolites were detected (*e.g.* andrastin A and B, chrysogin, fulvic acid, meleagrin, neoxaline, oxaline roquefortine C and D and secalonic acid D) in different quantities, depending on the strains.

The results obtained from the principal fungi isolated from the indoor air and books stored in the BAUM's repository were used to confirm their presence also in the settled dust samples collected during the 2nd sampling (October 2013). However, only 58% of the total secondary metabolites found in the dust could be associated with them. Other 37% of the compounds could be attributed to other fungi, and the remaining 5% to bacteria and plants. The highest correspondence was observed the dust collected in areas 1 and 2 (both 50%) followed by the dust from area 4 (46%) and those from area 3 (45%). The other contributions derived mainly from *Penicillium* sp. (17%), *Alternaria* sp. (7%), *Fusarium* sp. (7%),

Stachybotrys sp. (5%), *Trichoderma* sp. (5%) and *A. terreus* (2.5%). These last three fungal genera and species, together with the other few possible attributions (*i.e*. *Ascochyta* sp., *Beauverucin* sp., *Myrothecium* sp.) were not emerged from aerobiological and book sampling, maybe because of a lack of their sampling or because they were not viable. Summarizing, this kind of investigation increased our knowledge about the microbial community of the repository.

Among all dust samples, the most detected compounds were asperglaucide (58%), alamethicin (13%) and andrastin A (10%), mainly belonging *A. penicillioides*, *Trichoderma* sp. and *P. chrysogenum.* The secondary metabolite characterizations of dust samples are resumed in Table 4.17.

The detection of *Trichoderma* sp. and *Stachybotrys* sp. in the floor settled dust, and so their hypothetical presence in settled dust on stored books, has to be taken into account because of their well-known cellullolytic ability and as mycotoxin producer. Moreover, the detection of high levels of asperglaucide confirmed the presence of *A. penicillioides* in the indoor environment, often isolated from this kind of matrix. Finally, we have to consider also the potential presence of other types of allergy sources in the dust, as well as suspended in the air, as dust mites, ergosterol and β-glucans.

Table 4.17 Characterization of settled dust samples collected during the 2nd sampling inside the BAUM's repository.

Dust samples	Number of secondary metabolites	Principal secondary metabolite compounds	Principal fungal presence
1	32 (16 from selected fungal species and 16 from other contributions)	alamethicin altersetin andrastin A asperglaucide neoechinulin A	*Trichoderma* sp., *Alternaria* sp. *P. chrysogenum* *A. penicillioides* *Eurotium* sp.
2	24 (12 from selected fungal species and 12 from other contributions)	alamethicin asperglaucide terrecyclic acid	*Trichoderma* sp. *A. penicillioides* *A. terreus*
3	31 (14 from selected fungal species and 17 from other contributions)	alamethicin andrastin A asperglaucide physcion	*Trichoderma* sp. *P. chrysogenum* *A. penicillioides* *Aspergillus* sp.
4	37 (17 from selected fungal species and 20 from other contributions)	asperglaucide, physcion	*A. penicillioides* *Aspergillus* sp.

5. CONCLUSIONS

The overall aim of this thesis was to increase our knowledge about the biodeterioration phenomena of Cultural Heritage, especially book collections, focusing on related indoor contamination and potential health risks of workers and visitors. A contaminated repository of the Library of Humanities (*Biblioteca di Area UManistica*, BAUM) of Ca' Foscari University of Venice (Italy) was selected as case study. The fungal contamination was spread only on the book' covers, especially those with leather and textile bindings, interesting mainly the volumes stored inside Compactus® shelvings. The typology of this contamination was recognised to be morphologically similar of other case studies found around Italy (Montanari *et al*., 2012; Pinzari and Montanari, 2011), where *Eurotium halophilicum* C.M. Chr., Papav. & C.R. Benj. was identified as the main responsible. The aims of this study were to verify if the contamination of the BAUM's repository was due to this fungus and to characterize the indoor environment from microbiological and chemical point of view in order to determine the potential risk assessment for the book collection and for the human health of workers, students and visitors. For these purposes, well-structured samplings were planned for two years, especially covering two main periods recognisable as before and after the book disinfection operated by a private company in the April of 2013.

The principal aim was determine the effects of cleaning operation and maintaining monitored the repository. Moreover, all the successive analyses can be summed up in four main topics:

1) microbiological investigations that have included the characterization of the indoor air and contaminated books stored inside the studied repository by morphological and molecular analyses;
2) characterization of *Eurotium halophilicum*, because of its principal role in the book contamination, by microscopically investigation (SEM-EDS) and growth condition trials;
3) MVOC investigation of the principal fungal species isolated from the contaminated environment by GC-MS, coupled with *in situ* experiments, *i.e.* analysis of contaminated books and indoor air samples;
4) secondary metabolite investigation of the principal fungal species isolated from the contaminated environment by LC-MS/MS, coupled with *in situ* experiment, *i.e*. analysis of settled dust samples.

<u>1) Microbiological investigations.</u>

The microbiological samplings performed during the three periods (March 2013, October 2013, May 2014) highlighted a general decrease of airborne fungi, because of the disinfection operation. During the 1st sampling the total fungal load were with 2000 CFU m^{-3} (area 1) much higher than the recommended values. The results of the samplings performed after disinfection demonstrated a significant decrease of the values

below the recommend values (36 CFU m^{-3}, areas 1, 3rd sampling), which confirmed the positive effective of the disinfection also to the airborne microbial indoor level.

Overall 98 different fungal species were isolated, belonging to 43 fungal genera. The most frequent fungal species were *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp. For the first time, *A. creber* and *A. jensenii*, two species belong to recently revised group *Aspergillus* section *Versicolores*, were reported in relation with contaminated libraries' environments. Furthermore, high amounts of different kind of yeasts (*e.g. Cryptococcus carnescens*, *C. diffluens*, *Rhodotorula mugilaginosa* and *Sporodiobolus pararoseus*) and bacteria were sampled inside the repository, with values higher then outside, probably due to worker passages.

We successfully isolated 31 different fungal species from the books, of which 57% where also found by aerobiological sampling. The most frequently detected species were *A. creber, A. flavus*, *A. penicillioides*, *A. protuberus*, *A. sydowii*, *A. tubigensis*, *A. versicolor, A. vitricola*, *C. halotolerans, C. ramotenellum*, *P. brevicompactum* and *P. chrysogenum*. However, their secondary presence were probably due to a mycelium growth of another fungal species that acted as trap and/or a substrate for their growth.

We demonstrated that the main responsible for book's contamination at BAUM's library was the xerophilic fungus ***Eurotium halophilicum***. Its isolation was successfully performed only from the book's covers by sterile swabs inoculated on MEA15% as medium, while its identification was confirmed by morphological and molecular analyses, as well as on microscopically observations by SEM-EDS. *E. halophilicum* is considered as poor susceptible to air dispersion species, which may use other spread strategies, as dust mites. Finally, the analysis by AquaBoy device demonstrated a general content of 10% of water from the all selected books. This data is in the critical range of 8-10% considered as possible microbial germination condition on any kinds of papers.

2) Characterization of *Eurotium halophilicum*

We identified two different kinds of typical *Eurotium*'s structures on contaminated books:

- the presence of conidial state's structures, *e.g.* uniseriate radiate to columnar conidial heads, ellipsoidal conidia and nanometric short "hairs" on the hyphae, typical of those of the anamorphous state of the fungus, namely *Aspergillus halophilicus;*
- fungal structures belonging to the teleomorph state, *e.g.* cleistothecia with spherical asci and ascospores.

Together with conidial state structures (anamorph), several crystals were detected in corresponding of fungal hyphae. EDX investigations have permitted to identify their potentially chemical composition, related to sodium-calcium sulphate (*i.e.* glauberite, $Na_2Ca(SO_4)_2$). These results have highlighted the adaptation capacity of this fungal species to bioaccumulate and translocate some elements from the environment in specific hyphae's compartment as a nutritional requirement or osmotic bio-regulation.

We successfully confirmed the capacity of *E. halophilicum* to develop better onto low water activity (a_w < 0.9), with highest growth on MEA15% followed by Cz70, DG18, Cz40 and Cz20. Moreover, its inoculation onto YES15% for secondary metabolite analysis has recorder better growths than on MEA15%. Furthermore, a wide temperature range between 17 – 28 °C was identified as possible growth temperature condition, with an optimum at 25 °C.

<u>3) MVOC investigations</u>

The MVOC analysis of the selected fungal species (*A. creber*, *A. penicillioides*, *C. cladosporioides*, *E. chevalieri*, *E. halophilicum*, *P. brevicompactum* and *P. chrysogenum*) demonstrated their capacity to produce a total of 72 different volatile compounds in *in vitro* experiment. Each fungal species produced a characteristic chemical compound pattern, with directly depended to the medium substrates. Among all results, similarities between the fungal species were reported for alcohol, ketones and ethers. Some compounds were preferentially detected for only fungus, as 1-octene for *C. cladosporioides*, 2-butanol for *E. halophilicum* and 3-methylfuran for *P. brevicompactum*. Furthermore, we demonstrated similarities of the total MVOC production between *A. creber* and *P. chrysogenum* and between *A. penicillioides* and *E. halophilicum*, and might be due to the fungal similarity in terms of nutriments requirements and environmental habitats.
We demonstrated that fungal contaminated books could emit typical compounds of paper degradation (*e.g.* several aldehydes) and fungal pathways. We detect several compounds that can be considered as fungal products. These compounds belong to different chemical classes, such as are alcohols, ethers, hydrocarbons and ketones. Furthermore, the presence of *E. halophilicum* on both books resulted in the production of 14 compounds, including its principal MVOCs. Our results from the BOD test suggested that *E. halophilicum* could be considered a microaerophilic species. This should explain why it could germinate and growth between Compactus® shelving without air-circulations. In addition, the analysis of indoor air samples demonstrated that indoor air quality after the disinfection was mainly influenced by the books material emissions than from fungal presence.

Finally, we have identified four compounds, *i.e.* 1,4-pentadiene, isopropyl alcohol, acetone, 2-butanone, detected from all investigated media (books, indoor air and fungi). Even if they are not included in the approved MVOC fungal lists, they should be considered in future investigations concerning fungal contamination inside book collection's repositories.

<u>4) Secondary metabolite investigations</u>

Secondary metabolite analysis of the selected fungal species (different strains of *A. creber*, *A. jensenii*, *A. penicillioides*, *A. protuberus*, *A. vitricola*, *C. cladosporioides*, *E. chevalieri*, *E. halophilicum*, *P. brevicompactum* and *P. chrysogenum*) isolated from the contaminated environment was successfully

performed. The results confirmed the production ability of well-known fungal species (*i.e*. *A. penicillioides*, *A. vitricola*, C. cladosporioides, *E. chevalier*i, *P. brevicompactum* and *P. chrysogenum*). In addition, similar metabolite production was detected for the fungal species belonging to *Aspergillus* section *Versicolores*, *i.e*. *A. creber*, *A. jensenii* and *A. protuberus*. We have successfully characterized these fungi for their production of sterygmatocystin, metoxysterigmatocystin (excluded *A. protuberus*) and several biosynthetic precursors.

In this thesis, we have investigated *E. halophilicum*'s secondary metabolite ability for the first time. From 15 different strains on two culture media and at different ages, 8 compounds in different concentrations were detected. Deoxybrevianamid F, neoechinulin A, pseurotin A and D, rugulosovin, tenellin together with the possible presence of chaetoviridin A and stachybotryamide characterized most of the samples.

Furthermore, the analysis of settled dust samples has indicated the secondary metabolite presence of all selected fungal species, but also several compounds belonging to other fungi, bacteria and plants. We suggested that these contributions mainly derived from *Alternaria* sp., *A. terreus, Fusarium* sp., *Penicillium* sp., *Stachybotrys* sp. and *Trichoderma* sp. Not all these fungal genera were emerged from aerobiological and book sampling, maybe because of a lack of their sampling or because they were not viable. This demonstrated that the secondary metabolite analysis may increased the knowledge about the microbial community of the repository. Among all dust samples, the most detected compounds were asperglaucide, alamethicin and andrastin A, mainly belonging to *A. penicillioides*, *Trichoderma* sp. and *P. chrysogenum.* Finally, the detection of secondary metabolites of *Trichoderma* sp. and *Stachybotrys* sp. in the floor settled dust suggested the potential presence of this cellullolytic and mycotoxin producer species.

The combination of the performed analyses provides all the information necessary to characterize the *status quo* of the repositor environment. The three samplings have monitored the microbiologically indoor situation, highlighting the positive effects due to the books' disinfection. After the last sampling, the climate conditions and airborne microbial load were in agreement with the Italian recommendations and no visible fungal growth was visible on the treated books.

However, some results of this study may indicate a potential risk for future contamination. The discovery of a few fungal contaminated books in the Compactus® shelving could act as a source of new fungal spreads n the future. The general water content of the books is still in the critical range for microbial germination. Moreover, we have demonstrated the presence of several airborne fungal species often related to biodeterioration of book collections as well as the presence of secondary metabolite products belonging to high cellulolityc and high mycotoxin-producer fungal species both in indoor air and settled dust samples. Finally, the lack of regular dusting could enable the development of indoor microbial communities (*i.e.* dust as a nutriment source) and increase the potential human allergy sources (*i.e.* presence of dust mites).

Concluding this thesis, we want to give few (and basic) recommendations that might help librarians and workers to prevent future contamination, and thus reduce the risk of health problems due to poor indoor quality:

- frequent visual inspection of all materials stored inside Compactus® shelving (at least ones per week);
- keep controlled the indoor climatic conditions, in order to avoid any micro-environmental anomalies;
- keep clean the repository's environment (routine maintenance) and plan dusting operation (at least one per year);
- check frequently the HVAC (heating, ventilation and air conditioning) and change often the HVAC filters (at least 2 times per year, more often in the case of contaminations);
- guarantee a minimum of air-circulation between Compactus® shelving, sliding the blocks along the tracks from time to time.

OUTLOOK

The performed analyses have highlighted several characteristics of the fungus *Eurotium halophilicum,* increasing the general knowledge about this particular xerophilic fungal species. However, further investigation need to be carry out, in order to evaluate its potential risk for book collection and humans.

We confirmed its no celluylolytic activity and we demonstrated its ability to bio-accumulate elements from the environment by the formation of crystal directly from its hyphae. Information about the exactly composition and understand the possible sources of these chemical elements could help and improve the conservation management. The presence of sulphates on book covers could become critical aspect for further chemical corrosions because of the interaction with the substances in atmosphere and a consequence chemical degradation source.

We were able to identify *E. halophilicum* only by direct sampling from contaminated materials and we hypnotised its poor susceptibility to air dispersion. More information are needed to confirm its spread strategies, *e.g.* by dust mites, and to better understand the microenvironment that could be created inside Compactus® shelvings. Confirmation of its secondary metabolite ability is needed to determine the human risk evaluation, especially if we consider that American researchers have isolated this fungus from different kind of wheat's seeds (Christensen *et al*., 1979).

Further MVOC analyses testing isolated fungal species directly on simile-Cultural Heritage-materials (also with other sampler devices, as SPME fibers) should improve the knowledge of possible chemical markers that can be used in similar a cases of contamination. Finally, we want to increase the records of *E. halophilcum* contamination in Italy, but also in Europe, in order to publicize this kind of phenomena and supply information, as a short *vademecum*, to librarians and workers for its management's treatments.

REFERENCES

Abbott, S.P., (2002). Mycotoxins and Indoor Molds. Indoor Environment Connection 3, 14-24.

Abel-Hafez, S.I.I., Moubasher, A.H., Barakat, A., (1990). Keratinophilic fungi and other moulds associated with air- dust particles from Egypt. Folia Microbiologica 35, 311-325.

Ahearn, D.G., Crow, S., Simmons, R.B., Price, D.L., Mishra, S.K., Pierson, D.L., (1997). Fungal colonization of air filters and insulation in a multi-store office buildings: production of volatile organics. Current Microbiology 35, 305-308.

AIHA, (2005). Field guide for the determination of biological contaminants in environmental samples. Hung, L.-L., Miller, J.D., Dillon, H.K., (eds.), American Industrial Hygiene Association, Fairfax, VA.

Aiqing, X., Yuanliang, W., Jieyu, W., Pin, l., Ziyin, L., Longjjun, L., (2011). Fungal community associated with fermentation and storage of Fuzhan brick-tea. International Journal of Food Microbiology 146, 14-22.

American Conference of Governamental Industrial Hygienist (ACGIH), (1989). Guidelines for the Assessment in the Indoor Environment. Cincinnati, OH.

Amman, R.I., Ludwig, W., Schleifer, K.H., (1995). Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. Microbiological Reviews 59, 143-169.

Andersen, B., (1991). Consistent production of phenolic compounds by *Penicillium brevicompactum* for chemotaxonomic characterization. Antonie van Leeuwenhoek 60, 115-123.

Andrews, S., Pitt, J.I. (1987). Further studies on the water relations of xerophilic fungi, including some halophiles. Journal of General Microbiology 133, 233-238.

Apetrei, I.C., Drăgănescu, G.E., Popescu, I.T., Carp-Cărare, C., Guguianu, E., Mihăescu, T., Ştefanache, A., Creţu, C., Patraş, X., (2009). Possible cause of allergy for the librarians: books manipulation and ventilation as sources of fungus spores spreading. Aerobiologia 25, 159-166.

Baglioni, P., Chelazzi, D., Giorgi, R., (2015). Nanotechnologies in the Conservation of Cultural Heritage. A compendium of materials and techniques. Springer, Dordrecht.

Bellante de Martiis, D., D'Arca Simonetti., A., Tarsitani, G., Vanini, G.C., (1992). L'aria indoor: la tutela della salute negli ambienti confinati. Igiene Moderna 97, 705-706.

Benito, B., Garciadeblás, B., Fraile-Escanciano, A., Rodríguez-Navarro, A., (2011). Potassium and sodium uptake systems in fungi. The transporter diversity of *Magnaporthe oryzae*. Fungal genetics and Biology 48, 812-822.

Bensch K., Braun U., Groenewald J.Z., Crous P.W., (2012). The genus *Cladosporium*. Studies in Mycology 72, 1-401.

Blandino, M., Scarpino, V., Vanara, F., Sulyok, M., Krska, R., Reyneri, A., (2015) Role of the European corn borer (Ostrinia nubilalis) on contamination of maize with 13 Fusarium mycotoxins. Food Additives & Contaminants: Part A 32, 533-543.

Bloom, E., Bal, K., Nyman, E., Must, A., Larsson, L., (2007). Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. Applied and Environmental Microbiology 73, 4211-4217.

Borjessön, T., Stöllman, U., Schnürer, J., (1992). Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Applied and Environmental Microbiology 58, 2599-2605.

Borrego, S., Guiamet, P., Gómez de Saravia, S., Batistini, P., Garcia, M., Lavin, P., Perdomo, I., (2010). The quality of air at archives and the biodeterioration of photographs. International Biodeterioration and Biodegradation 64, 139-145.

Bridge, P., Spooner, B., (2001). Soil fungi: diversity and detection. Plant soil 232, 147-154.

Buck, B.J., Wolff, K., Merkler, D.J., McMillan, N.J., (2006). Salt mineralogy of Las Vegas Wash, Nevada: morphology and subsurface evaporation. Soil Science Society of American Journal 70, 1639-1651.

Burg, W.R., Shotwell, O.L., (1984). Aflatoxin levels in airborne dust generated from contaminated corn during harvest and at elevator in 1980. Journal-Association of Official Analytical Chemists 26, 309-312.

Butinar, L., Zalar, P., Frisvad, J.C., Gunde-Cimerman, N., (2004). The genus *Eurotium* – members if indigenous fungal community in hypersaline waters of salterns. FEMS Microbiology Ecology 51, 155-166.

Cabaret, O., Puel, O., Botterel, F., Delaforge, M., Bretagne, S., (2014). Metabolic detoxification pathways for 5-methoxy-sterigmatocystin in primary tracheal epithelial cells. Xenobiotica 44, 1-9.

Cabral, J.P.S., (2010). Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. Science of Total Environment 408, 4285-4295.

Caneva, G., Nugari, M.P., Salvadori, O., (2005). La biologia vegetale per i beni culturali. Vol. I: Biodeterioramento e Conservazione. Nardini Editore, Firenze.

Cappitelli, F., Fermo, P., Vecchi, R., Piazzalunga, A., Valli, G., Zanardini, E., Sorlini, C., (2009). Chemical-physical and Microbiological Measurements for Indoor Air Quality Assessment at the Ca' Granda Historical Archive, Milan (Italy). Water, Air, and Soil Pollution 201, 109-120.

Carbone, I., Kohn, L.M., (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553-556.

CBS-KNAW Fungal Biodiversity Centre Collections (last access 10 September 2015), http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx?file=all

Chala, A., Taye, W., Ayyalew, A., Krska, R., Sulyok, M., Logrieco, A., (2014). Multimycotoxin analysis of sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) from Etiopia. Food Control 45, 29-35.

Chew, G.L., Douwes, J., Doekes, G., Higgins, K.M., van Strein, R.T., Spithoven, J., Brunekreef, B., (2001). Fungal extracellular polysaccharides, β(1-3)glucans and culturable fungi in repeated sampling of house dust. Indoor air 11, 171-178.

Christensen, C.M., Papavizas, G.C., Benjamin, C.R., (1959). A new halophilic species of *Eurotium*. Mycologia 51, 636-640.

Colwell, R., (2000). Viable but nonculturable bacteria: a survival strategy. Journal of Infection and Chemotherapy 6, 121-125.

Cooley, J.D., Wong, W.C., Jumper, C.A., Straus, D.C., (1998). Correlation between the prelevance of certain fungi and sick building syndrome. Occupational and Environmental Medicine 55, 579-584.

Crook, B., (1995). Inertial samplers: biological perspectives. In: Cox, C.S., Wathes, C.M., (eds), "Bioaerosols Handbook". Boca Raton, FL, 269-284.

D-Lgs. 9 aprile, n.81. Attuazione dell'articolo 1 della legge 3 agosto 2007, n.123, in materia di tutela della salute e della sicurezza nei luoghi di lavoro. Gazzetta Ufficiale n.101, 30 aprile 2008 – Supplemento Ordinario n.108 (ex D.L. 626/94).

De Nuntiis, P., Maggi, O., Mandrioli, P., Ranalli, G., Sorlini, C., (2003a). Monitoring the biological aerosol. In: Mandrioli, P., Caneva, G, Sabbioni, C. "Cultural heritage and Aerobiology. Methods and Measurement Techniques for Biodeterioration Monitoring". Academic Kluwer Publishers, the Netherlands, 107-144.

De Nuntiis, P., Bitelli, L., Guaraldi, C., Mandrioli, P., Salvi, A., Toschi, R., Vitali, C., (2003). Intermuseum network for conservation of the artistic heritage: MUSA PROJECT. In: Third European Symposium on Aerobiology (TESA), Worcester (UK).

Després, V.R., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, Ul., Jaenicke, R., (2011). Primary biological aerosol particles in the atmosphere; a review. Tellus B 64, 1-58.

Elke, K., Begerow, J., Oppermann, H., Krämer, U., Hermann, E., Dunemann, L., (1999). Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID-a new feasible approach for the detection of an exposure to indoor mould fungi?. J.E.M. 1, 445-452.

Engelhart, S., Loock, A., Skutlarek, D., Sagunski, H., Lommel, A., Färber, H., Exner, M., (2002). Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Applied and Environmental Microbiology 68, 3886-3890.

EPA TO-15, (1999). Compendium of methods for the determination of toxic organic compounds, Compendium method TO-15, Determination of volatile organic compounds (VOCs) in air collected in specially-prepared canister and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). EPA/625/R-96/010b, Second Edition.

Etzel, R.A., Montana, E., Sorenson, W.G., Kuliman, G.J., Allan, T.M., Deaborn, D.G., Olson, D.R., Jarvis, B.B., Miller, J.D., (1998). Acute pulmonary haemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Archives of Pediatrics and Adolescent Medicine 152, 757-762.

EUR 14988 EN (1993). Report No. 12. Biological particles in Indoor Environments. European Collaborative Action (formely COST Project 613), “Indoor air quality & its impact on man. Commission of the European Communities, Directorate General for Science, Research and Development, Joint Research Centre-Environmental Institute.

European Commission (EC) (2000) Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal of the European Union L 262, 21.45.

European Commission (EC) (2006). The commission decision, 2006/504/EC. Official Journal of the European Union, vol. L199, 21-32.

Ezekiel, C.N., Sulyok, M., Warth, B., Odebode, A.C., Krska, R., (2012). Natural occurrence of mycotoxins in peanut cake from Nigeria. Food Control 27, 338-342.

Fenech, A., Strlič, M., Kralj Cigić, I., Levart, A., Gibson, L.T., de Bruin, G., Ntanos, K., Kolar, J., Cassar, M., (2010). Volatile aldehydes in libraries and archives. Atmospheric Environment 44, 2067-2073.

Fiedler, K., Schuetz, E., Geh, S., (2001). Detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials. International Journal of Hygiene and Environmental Health 204, 111-121.

Fischer, G., Schwalbe, R., Möller, M., Ostrowski, R., Dott, W., (1999). Specie-specific production of microbial volatile organic compounds (MVOC) of airborne fungi from a compost facility. Chemosphere 39, 795-810.

Fischer, G., Müller, T., Schwalbe, R., Ostrowski, R., Dott, W., (2000a). Exposure to air-borne fungi, MVOC and mycotoxins in biowaste-handling facilities. International Journal of Hygiene and Environmental Health 203, 97-104.

Fischer, G., Müller, T., Schwalbe, R., Ostrowsky, R., Dott, W., (2000b). Species-specific profiles of mycotoxins produced in cultures and associated with conidia of airborne fungi derived from biowaste. International Journal of Hygiene and Environmental Health 203, 105-116.

Fischer, G., Dott, W., (2003). Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Archives of Microbiology 179, 75-82.

Flannigan, B., McCabe, E.M., McGarry, F., (1991). Allergenic and toxigenic micro-organisms in houses. The Journal of Applied Bacteriology 70 (Symposium supplement), 61S-73S.

Flannigan, B., Miller, J.D., (2011). Microbial growth in indoor environments. In: Flannigan, B., Samson R.A. and Miller, D. (eds), "Microorganisms in Home and Indoor Work Environments. Diversity, Health Impacts, Investigation and Control", 2nd Edition, London, CRC Press, 57-108.

Florian, M.L.E., Manning, L., (2000). SEM analysis of irregular fungal fox spots in an 1854 book: population dynamics and species identification. International Biodeterioration & Biodegradation 46, 205-220.

Florian, M.L.E., (2002). Fungal facts: solving problems in heritage collection. Archetype Publications Ltd, London, UK.

Food and Drug Administration (FDA) (2004). Compliance Guidance Manual.

Frisvad, J.C, Smedsgaard, J., Larsen, T.O., Samson, R.A., (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Studies in Mycology 49, 201-2041.

Frisvad, J.C., (2012). Media and growth conditions for induction of secondary metabolite production. In: Keller, N.P., Turner, G. "Fungal Secondary Metabolism". Springer, 47-58.

Frisvad, J.C., (2015). Taxonomy, chemodiversity, and chemoconsistency of Aspergillus, Penicillium, and Talaromyces species. Frontiers in Microbiology 5, 1-7.

Gallo, F., Pasquariello, G., Valenti, P., (2003). Libraries and archives. In Mandrioli, P., Caneva, G, Sabbioni, C. "Cultural heritage and Aerobiology. Methods and Measurement Techniques for Biodeterioration Monitoring". Academic Kluwer Publishers, the Netherlands, 175-194.

Gallo, F., (1993). Aerobiological research and problems in libraries. Aerobiologia 9, 117-130.

Gao, P., Martin, J., (2002). Volatile metabolites produced by three strains of *Stachybotrys chartarum* cultivated on rice and gypsum board. Applied Occupational and Environmental Hygiene 17, 430-436.

Gao, P., Korley, F., Martin, J., Chen, B.T., (2002). Determination of unique microbial volatile organic compounds produced by five *Aspergillus* species commonly found in problem buildings. AIHA Journal 63, 135-140.

Gardes, M., Bruns, T.D., (1993). ITS primers with enhanced specificity for basiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 1, 113-118.

Gibson, L.T., Ewlad-Ahmed, A., Knight, B., Horie, V., Mitchell, G., Robertson, C.J., (2012). Measurement of volatile organic compounds emitted in libraries and archives: an inferential indicator of paper decay?. Chemistry Central Journal 6:42.

Glass, N.L., Donaldson, G.C., (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323-1330.

Gravesen, S., Frisvad, J.C., Samson, R.A., (1994). Microfungi. Munksgard, Copenaghen.

Guillitte, O., (1995). Bioreceptivity: a new concept for building ecological study. The Science of Total Environment 167, 215-220.

Gunde-Cimmerman, N., Sonjak, S., Zalar, P., Frisvad, J.C., Diderichsen, B., Plemenitaš, A., (2003). Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Physics and Chemistry of the Earth 28, 1273-1278.

Gutarowska, B., Skora, J., Stepien, L., Twarizel, M., Blajet-Kosicka, A., Otlewska, A., Grajewski, J., (2014). Estimation of fungal contamination and mycotoxin production at workplaces in composting plants, tanneries, archives and libraries. World Mycotoxin Journal 7, 345-355.

Heinsohn, P., (2007). Conducting building mold investigations. In: Yang C.S. and Heinsohn P. (Eds), “Sampling and Analysis of Indoor Microorganisms”. New York, John Wiley, 15-50.

Hendry, K.M., Cole, E.C., (1993). A review of mycotoxins in indoor air. Journal of Toxicology and Environmental Health 38, 183-198.

Hocking, A.D., Pitt, J.I., (1988). Two new species of xerophilic fungi and a Further record of *Eurotium halophilicum*. Mycologia 80, 82-88.

Hocking, A.D., (1993). Responses in xerophilic fungi to changes in water activity. In: D.H. Jennings (ed.), “Stress Tolerance of Fungi”. Marcel Dekker, Inc., New York, 233-243.

Hodgson, M.J., Morey, P., Leung, W.Y., Morrow, L., Miller, D., Jarvis, B.B., Robbins, H., Halsey, J.F., Storey, E., (1998). Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. Journal of Occupational and Environmental Medicine, 40, 241-249

Huang, R., Zhiu, X., Xu, T., Yang, X., Liu, Y., (2010), Diketopiperazines from marine organisms. Chemistry & Biodiversity 7, 2809-2829.

Hueck, H.J., (1965). The biodeterioration of materials as a part of hylobiology. Material und Organismen 1, 5-34.

Hyder, N., Sims, J.J., Wegulo, S.N., (2009). In vitro suppression of soilborne plant pathogens by coir. HortTechnology 19, 96-100.

Imai, M., (1967). Studies on the basic physiological conditions affecting the spores germination of an obligatorily tonophilic fungus. Natural Science Report, Ochanomizu University, Vol. 18, N° 1, 21-55.

Ioannides, M., Fritsch, D., Leissner, J., Davies, R., Remondino, F., Caffo, R., (2012). Progress in Cultural Heritage Preservation. 4th International Conference, EuroMed 2012, Lemessos, Cyprus, October 29-November 3, 2012, Proceedings. Springer, Verlag Berlin Heidelberg.

Itabashi, T., Matsuishi, N., Hosoe, T., Toyazaki, N., Udagawa, S., Imai, T., Adachi, M., Kawai, K., (2006). Two new dioxopiperzine derivates, arestrictina A and B, isolated from *Aspergillus restrictus* and *Aspergillus penicillioides*. Chemical and Pharmaceutical Bulletin 54, 1639-1641.

Jacyno, J.M., Harwood, J.S., Cutler, H.G., Lee, M.K., (1993). Isocladosproin, a biologically active isomer of cladosprin from *Cladosporium cladosporioides*. Journal of Natural Products 56, 1397-401.

Jurjević, Z., Peterson, S.W., Horn, B.W., (2012). *Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 3, 59-79.

Jurjević, Z., Peterson, W.S., Solfrizzo, M., Peraica, M., (2013). Sterigmatocystin production by nine newly described *Aspergillus* species in section *Versicolores* grown on two different media. Mycotoxin Research 29, 141-145.

Karbowska-Berent, J., Górny, R.L., Strzelczyk, A.B., Wlazło, A., (2011). Airborne and dust borne microorganisms in selected polish libraries and archives. Building and Environment 46, 1872-1879.

Kirivanta, H., Tuomainen, A., Reiman, M., Laitinen, S., Liesivuori, J., Nevalainen, A., (1998). Quantitative identification of volatile metabolites from two fungi and three bacteria species cultivated on two media. Central European Journal of Public Health 6, 296-299.

Klich, M.A., (2002). Identification of common *Aspergillus* species. CBS, Utrecht, The Netherlands.

Korpi, A., Pasanen, A.-L., Pasanen, P., kalliokoski, P., (1997). Microbial Growth and Metabolism in Housed Dust. International Biodeterioration & Biodegradation 40, 19-27.

Kowalik, R., (1980). Microbiodeterioration of library materials. Part.2, chap.4 Restaurator 4, 135-219; part.2, chap.5-9 Restaurator 6, 61-115.

Kraková, L., Chovanová, K., Selim, S.A., Šimonovičová, A., Puškarová, A., Maková, A., Pangallo, D., (2012). A multiphasic approach for investigation of the microbial diversity and its biodegradative abilities in historical paper and parchment documents. International Biodeterioration & Biodegradation 70, 117-125.

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., Macdonald, S., Crews, C., (2008). Mycotoxins analysis: an update. Food additives & Contaminants 25, 152-163.

Laatsch, H., (2005). AntiBase 2005 (software). John Wiley & Sons Hoboken, New Jersey, ISBN 3-527-31721-X.

Larsen, F.O., Frisvad, J.C., (1994). Health implications of fungi in indoor environments: production of volatiles and presence of mycotoxins in conidia of common indoor penicillia and aspergilli. In: Samson, R.A., Flannigan, B., Flannigan, M.E. et al., (eds.) “Health implications of fungi in indoor environments”. Elsevier, Amsterdam, 251-279.

Larsen, F.O., Clementsen, P., Hansen, M., MaltbæK, N., Gravesen, S., Stahl Skov, P., Norn, S., (1996). The indoor microfungus *Trichoderma viride* potentiates histamine release from human bronchoalveolar cells. APMIS 104, 673-679.

Lattuati-Derieux, A., Bonnassies-Termes, S., Lavédrine, B., (2003). Identification of volatile organic compounds emitted by a naturally aged book using solid-phase microextraction/gas chromatography/mass spectrometry. Journal of Chromatography A 1026, 9-18.

Lighthart, B., (2000). Mini-review of the concentration variations found in the alfresco atmospheric bacterial pollutions. Aerobiologia 16, 7-16.

Lignell, U., Meklin, T., Rintala, H., Hyvärinen, A., Vepsalainen, A., Pekkanen, J., Nevalainen, A., (2008). Evaluation of quantitative PCR and culture methods for detection of house dust fungi and streptomycetes in relation to moisture damage of the house. Letters in Applied Microbiology 47, 303-308.

Magan, N., Evans, P., (2000). Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grains spoilage. Journal of Stored Products Research 36, 319-340.

Maggi, O., Persiani, A.M., Gallo, F., Valenti, P., Pasquariello, G., Sclocchi, M.C., Scorrano, M., (2000). Airborne fungal spores in dust present in archives: proposal for a detection methods, new for archival materials. Aerobiologia 16, 429-434.

Magurran, A.E., (1988). Ecological Diversity and Its Measurements. Princeton University Press, Princeton, NJ, 39-40.

Malachová, A., Sulyok, M., Beltrán E., Berthiller F., Krska, R., (2014). Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometry method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. Journal of Chromatography A 1362, 145-156.

Manente, S., Micheluz, A., Ganzerla, R., Ravagna, G., Gambaro, A., (2012). Chemical and biological characterization of paper: A case study using a proposed methodological approach. International Biodeterioration & Biodegradation 74, 99-108.

Masiol, M., Squizzato, S., Ceccato, D., Rampazzo, G., Pavoni, B., (2012). Determining the influence of different atmospheric circulation patterns on PM10 chemical composition in a source apportionment study. Atmospheric Environment 63, 117-124.

Matysik, S., Herbarth, O., Mueller, A., (2009). Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. Chemosphere 76, 114-119.

Matysik, S., Herbarth, O., Mueller, A., (2008). Determination of volatile metabolites originating from mould growth on wall paper and synthetic media. Journal of Microbiological Methods 75, 182-187.

McConnell, I.R., Garner, R.C., (1994). DNA adducts of aflatoxins, sterigmatocystin and other mycotoxins. IARC Scientific Publication Series 125, 49-55.

Mesquita, N., Portugal, A., Videira, S., Rodríguez-Echeberría, S., Bandeira, A.M.L., Santon, M.J.A., Freitas, H., (2009). Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. International Biodeterioration & Biodegradation 63, 626-629.

MIBAC, (2001). Atto d'indirizzo sui criteri tecnico-scientifici e sugli standard di funzionamento e sviluppo dei musei, D.Lgs. n.112/98 art.150 comma 6.

Micali, O., Montacutelli, R., Tarsitani, G., (2003). Pathogenic microorganisms and situations of risk to man. In Mandrioli, P., Caneva, G, Sabbioni, C. "Cultural heritage and Aerobiology. Methods and Measurement Techniques for Biodeterioration Monitoring". Academic Kluwer Publishers, the Netherlands, 31-46.

Michaelsen, A., Pinar, G., Pinzari, F., (2010). Molecular and microscopical investigation of the microflora inhabiting a deteriorated Italian manuscript dated from the Thrirteen Century. Microbial Ecology 60, 69-80.

Micheluz, A. Manente, S., Tigini, V., Prigione, V., Pinzari, F., Ravagnan, G., Varese, G.C., (2015a). The extreme environment of a library: Xerophilic fungi inhabiting indoor niches. International Biodeterioration & Biodegradation 99, 1-7.

Micheluz, A., Sulyok, M., Manente, S., Krska, R., Varese, G.C., Ravagnan, G., (2015b). Fungal secondary metabolite analysis applied to Cultural Heritage: the case of a contaminated library in Venice. World Mycotoxin Journal, *submitted*.

Miller, J.D., (1994). Mycotoxins. In: Rylander, R., Jacobs, R.R. (eds) "Organic dust – exposure, effects, and prevention". London, Lewis, 87-92.

Miller, J.D., McMullin, D.R., (2014). Fungal secondary metabolites as harmful indoor air contaminants: 10 years on. Applied Microbiology and Biotechnology 98, 9953-9966.

Montanari, M., Melloni, V., Pinzari, F., Innocenti, G., (2012). Fungal Biodeterioration of historical library materials stored in Compactus movable shelves. International Biodeterioration & Biodegradation 75, 83-88.

Montemartini Corte, A., Ferroni, A., Salvo, V.S., (2003). Isolation of fungal species from test samples and maps damaged by foxing, and correlation between these species and the environment. International Biodeterioration & Biodegradation 51, 167-173.

Moularat, S., Robine, E., Ramahlo, O., Oturam, M.A., (2008). Detection of fungal development in closed spaces through the determination of specific chemical targets. Chemosphere 72, 224-232.

Murtagh, F., Legendre, P., (2104). Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion?. Journal of Classification 31, 274-295.

NCBI National Center for Biotechnology Information (last access 30 September 2015), http://www.ncbi.nlm.nih.gov/

Nielsen, K.F., Gravesen, S., Nielsen, P.A., Andersen, B., Thrane, U., Frisvad, J.C., (1999). Production of mycotoxin on artificially and naturally infested building materials. Mycopathologia 145, 43-56.

Nielsen, K.F., (2002). Mould growth on Building materials. Secondary metabolites, mycotoxins and biomarkers. Ph.D. Thesis. BioCentrum-DTU, Technical University of Denmark.
Available from <http://www.biocentrum.dtu.dk/mycology/pubblications/phd-kfn.pdf>

Nielsen, K.F., (2003). Mycotoxin production by indoor moulds. Fungal Genetics and Biology 39, 103-117.

Nielsen, K.F., Smedsgaard, J., (2003). Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. Journal of Chromatography A 1002, 111-136.

Nielsen, K.F., Gräfenhan, T., Zafari, D., Thrane, U., (2005). Trichothecene production by *Trichoderma brevicompactum*. Journal of Agricultural and Food Chemistry 53, 8190-8196.

Nikulin; M., Reijula, K., Jarvis, B.B., Hintikka, E.-L., (1996). Experimental lung mycotoxicosis in mice induced by *Stachybotrys atra*. International Journal of Experimental Pathology 77, 213-218.

Nunes, I., Mesquita, N., Cabo Verde, S., Leitão Bandeira, A.M., Carolino, M.M., Portugal, A., Botelho, M.L., (2013). Characterization of an airborne microbial community: A case study in the archive of the University of Coimbra, Portugal. International Biodeterioration & Biodegradation 79, 36-41.

Nyuksha, P.Y., (1994). Biodeterioration of paper and books. In: Garg, K.L., Garg, N., Mukerji, K.G., (eds.) "Recent Advances in Biodeterioration and Biodegradation", Vol.1. Naya prokash, Calsutta, India, 1-88.

Ohtsuki, T., (1962). Studies on the glass mould: On two species of *Aspergillus* isolated from glass. Botanical Magazine Tokyo 75, 436-442.

Pacini, R., Puccinelli, G., Petrucci, M., (2005). Il controllo microbiologico ambientale nella ristorazione aziendale. Biologi Italiani 8, 62-71.

Pasanen, A.L., Korpi, A., Kasanen,, J.P., Pasanen P., (1998). Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. Environmental international 24, 703-712.

Pasquarella, C., Pitzurra, O., Savino, A., (2000). The index of microbial air contamination. The Journal of hospital infection 46, 241-256.

Pasquariello, G., Valenti, P., Maggi, O., Persiani, A.M., (2005). Problemi di biodeterioramento in relazione alla tipologia di ambienti. Biblioteche ed archive. In: Caneva, G., Nugari, M.P., Salvadori, O., "La biologia vegetale per I beni culturali. Vol. I: Biodeterioramento e Conservazione". Nardini Editore, Firenze, 171-175.

Peterson, S.W., (2008). Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100, 205-226.

Peto, S., Powel, E.O., (1970). The assessment of aerosol concentration by means of the Andersen sampler. Journal of Applied Bacteriology 33, 582-598.

Pinna, D., Salvadori, O., (2005). Meccanismi generali dei processi di biodeterioramento. In: Caneva, G., Nugari, M.P., Salvadori, O., "La biologia vegetale per I beni culturali. Vol. I: Biodeterioramento e Conservazione". Nardini Editore, Firenze, 15-34.

Pinzari, F., Fanelli., Canhoto, O., Magan, N., (2004). Electronic nose for the early detection of moulds in libraries and archives. Indoor and Buil Environment 13, 000-000.

Pinzari, F., Montanari, M., (2008). Il SEM-VP nella diagnostica di alterazioni fungine non più vitali o di difficile caratterizzazione. In: Pinzari, F., (Eds.), "Scienza e ricerca per i beni culturali. Microscopia elettronica a scansione e microanalisi". Gangemi Editore, Roma, 79-86.

Pinzari, F., Montanari, M., (2011). Mould growth on library materials stored in compactus-type shelving units. In: Abdul-Wahab Al-Sulaiman, SA (Eds.), "Sick Building Syndrome: Public Buildings and Workplaces". Springer-Verlag, Berlin Heidelberg, 193-206.

Pitt, J.I., (1979). The genus *Penicillium* and its teleomorphic states: *Eupenicillium* and *Talaromyces*. Academic Press.

Pitt, J.I., Hocking, A.D., (1997). Fungi and food spoilage. Blackie Academic and Professional of Chapman & Hall, London, UK.

Polizzi, V., Delmulle, B., Adams, A., Moretti, A., Susca, A., Picco, A.M., Rosseel, Y., Kindt, R., Bocxlaer, J.V., De Kimpe, N., Van Peteghem, C., De Saeger, S., (2009). Fungi, mycotoxins and microbial volatile organic compounds in mouldy interiors from water-damaged buildings. Journal of Environmental Monitoring 11, 1849-1858.

Polizzi, V., Adams, A., Malysheva, S.V., De Saeger, S., Van Peteghem, C., Moretti, A., Picco, A.M., De Kimpe, N., (2012). Identification of volatile markers for indoor fungal growth and chemotaxonomic classification of *Aspergillus* species. Fungal Biology 116, 941-953.

Raper, K.B., Fennell, D.I., (1965). The genus *Aspergillus*. Willians & Wilkins, Baltimore, USA.

Redlich, C.A., Sparer, J., Cullen, M.R., (1997). Sick-building syndrome. Lancet 349, 1013-1016.

Righi, E., Aggazzotti, G., Fantuzzi, G., Ciccarese, V., Predieri, G., (2002). Air quality and well-being perception in subjects attending university libraries in Modena (Italy). The Science of the Total Environment 286, 41-50.

Rintala, H., Hyvärinen, A., Paulin, L., Nevalainen, A., (2004). Detection of Streptomyces in house dust – comparison of culture and PCR methods. Indoor Air 14, 112-119.

Robbins, C.A., Swenson, L.J., Nealley, M.L., Gots, R.E., Kelman, B.J., (2000). Health effects of mycotoxins in indoor air: a critical review. Applied Occupational and Environmental Hygiene 15, 773-784.

Rodriguez-Navarro, C., Doehne, E., Sebastian, E., (2000). How does sodium sulphate crystallize? Implications for the decay and testing of building materials. Cement and Concrete Research 30, 1527-1534.

Rosas, A.L., MacGill, R.S., Nosanchuk, J.D., Kozel, T.R., Casadevall, A., (2002). Activation of the alternative complement pathway by fungal melanins. Clinical and Diagnostic Laboratory Immunology 9, 144-148.

Roze, L.V., Beaudry, R.M., Linz, J.E., (2012). Analysis of volatile compounds emitted by filamentous fungi using solid-phase microextraction-gas chromatography/mass spectrometry. In: Keller, N.P., Turner, G., "Fungal Secondary Metabolism". Springer, 133-142.

Ryan, T.J., (2011). Microbial volatile organic compounds. In: Flannigan, B., Samson, R.A., Miller, J.D., "Microorganisms in Home and Indoor Work Environments. Diversity, health impacts, investigation and control". 2nd Edition, London, CRC Press, 279-290.

Samson. R.A., Lustgraaf, B.V.D., (1978). *Aspergillus penicilloides* and *Eurotium halophilicum* in association with house-dust mites. Mycopathologia 64, 13-16.

Samson, R., Hoekstra, E., (1994). Common fungi occurring in indoor environments. Health Implications of Fungi in Indoor Environments, Vol. 2. Amsterdam. Elsevier, 541-546.

Samson, R.A., Frisvad, J.C., (2004). *Penicillium subgenus Penicillium*: new taxonomic schemes, mycotoxins and other extrolites. Studies in Mycology 49, CBS, Utrecht, The Netherlands.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., (2004). Introduction to Food and Airborne Fungi. ASM Press, US.

Samson, R.A., Houbraken, J., Thrane, U., Frisvad, J.C., Andersen, B., (2010). Food and indoor fungi. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Schabereiter-Gurtner, C., Piñar, G., Lubitz, W., Rölleke, S., (2001). Analysis of fungal communities on historical church window glass by denaturing gradient gel electrophoresis and phylogenetic 18S rDNA sequence analysis. Journal of Microbiological Methods 47, 345-54.

Schieweck, A., Salthammer, T., (2011). Indoor air quality in passive-type museum showcases. Journal of Cultural Heritage 12, 205-213.

Schleibinger, H., Laussmann, D., Bornehag, C.-G., Eis, D., Rueden, H., (2008). Microbial volatile organic compounds in the air of moldy and mold-free indoor environments. Indoor air 18, 113-124.

Schnürer, J., Olsson, J., Börjesson, T., (1999). Fungal volatiles as indicators of food and feed spoilage. Fungal Genetics and Biology 27, 209-217.

Schuchardt, S., Kruse, H., (2009). Quantitative volatile metabolite profiling of common indoor fungi: relevancy for indoor air analysis. Journal of Basic Microbiology 49, 350-362.

Scocchi, M.C., Matè, D., Ruggiero, D., (2003). I costituenti organici dei materiali fotografici: loro suscettibilità ad attacco biologico. ATF, 35 Rivista di di Storia e Fotografia, Prato.

Skóra, J., Gutarowska, B., Pielech-Przybylska, K., Stępień, Ł., Pietrzak, K., Piotrowska, M., Pietrowski, P., (2015). Assessment of microbiological contamination in the work environments of museums, archives and libraries. Aerobiologia 31, 389-401.

Slack, G.j., Puniani, E., Frisvad, J., Samson, R.A., Miller, J.D., (2009). Secondary metabolites from *Eurotium* species, *Aspergillus calidoustus* and *A. insuetus* common in Canadian homes with a review of their chemistry and biological activities. Mycological Research 113, 480-490.

Slonczewski, J. L., Coker, J.A., DasSarma, S., (2010). Microbial growth under multiple stressors. Microbe 5, 110-116.

Sorenson, W.G., Fraser, D.G., Jarvis, B.B., Simpson, J., Robinson, V.A., (1987). Trichothecene mycotoxins in aerosized conidia of Stachybotrys atra. Applied and Environmental Microbiology 53, 1370-1375.

Sterflinger, K., (2010). Fungi: Their role in deterioration of cultural heritage. Fungal Biology Reviews 24, 47-55.

Streit, E., Schwab, C., Sulyok, M., Naehrer, K., Krska, R., Schatzmayr, G., (2013). Multi-mycotoxin screening revelas the occurrence of 139 different secondary metabolites in feed and feed ingredients. Toxins 5, 504-523.

Strlič, M., Thomas, J., Trafela, T., Cséfalvayova, L., Cigić, I.K., Kolar, J., Cassar, M. (2009). Material degradomics: on the semll of old books. Analytical Chemistry 81, 8617-8622.

Ström, G., West, J., Wessén, B., Palmgren, U., (1994). Health implications of fungi in indoor environments: quantitative analysis of microbial volatiles in damp Swedish houses. In: Samson, R.A., Flannigan, B., Flannigan, M.E. et al. (eds) “Health Implications of Fungi in Indoor Environments”. Elsevier, Amsterdam, 291-305.

Sulyok, M., Krska, R., Schumacher, R., (2007). A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. Analytical and Bioanalytical Chemistry 389, 1505-1523.

Sunesson, A.-L., Vaes, W.H.J., Nilsoon, C.-A., Blomquist, G., Andersson, B., Carlson, R., (1995). Identification of volatile metabolites from five fungal species cultivated on two media. Applied and Environmental Microbiology 61, 2911-2918.

Sunesson, A.-L., Nilsson, C.-A., Blomquist, G., Andersson, B., (1996). Volatile metabolites produced by two fungal species cultivated on building materials. The Annals of Occupational Hygiene 40, 397-410.

Tamura, M., Kawasaki, H., Sugiyama, J., (1999). Identify of the xerophilic species *Aspergillus penicillioides*: integrated analysis of the genotypic and phenotypic characters. The Journal of General and Applied Microbiology 45, 29-37.

Täubel, M., Sulyok, M., Vishwanath, V., Bloom, E., Turunen, M., Järvi, K., Kauhanen, E., Krska, R., Hyvärinen, A., Larsson, L., Nevalainen, A., (2011). Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments. Indoor air 21, 368-375.

Thom, C., Raper, K.B., (1945). A manual of the Aspergilli. Williams and Wilkins, Baltimore, Md., 373.

Toivola; M:, Alm, S., Reponen, T., Kolari, S., Nevalainen, A., (2002). Personal exposure and microenvironmental concentrations of particles and bioaerosols. Journal of Environmental Monitoring 4, 166-174.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E-L., Lindroos, O., Kalso, S., Koukila-Kähkölä, P., Mussalo-Rauhamaa, H., Gaahtela, T., (2000). Mycotoxins in crude building materials from water-damaged buildings. Applied and Environmental Microbiology 66, 1899-1904.

Turbyville, T.J., Wijeratne, E.M., Whitesell, L., Gunatilaka, A.A., (2005). The anticancer activity of the fungal metabolite terrecyclic acid A is associated with modulation of multiple cellular stress response pathways. Molecular Cancer Therapeutics 4, 1569-76.

UNI-EN 481 (1994). Atmosfera nell'ambiente di lavoro. Definizione delle frazioni granulometriche per la misurazione delle particelle aerodisperse.

UNI 10586 (1997). Condizioni climatiche per ambienti di conservazione di documenti grafici e caratteristiche degli alloggiamenti. Roma.

UNI 10829 (1999). Beni di interesse storico artistico; Condizioni ambientali di conservazione, misurazione ed analisi. Roma.

UNI 10969 (2002). Principi generali per la scelta e il controllo del microclima per la conservazione dei beni culturali in ambienti interni. Roma.

Vishwanath, V., Sulyok, M., Labuda, R., Bicker, W., Krska, R., (2009). Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatograpgy/tandem mass spectrometry, Analytical and Bioanalytical Chemistry 395, 1355-1372.

Vishwanath, V., Sulyok, M., Weingart, G., Kluger, B., Täubel, M., Mayer, S., Schuhmacher, R., Krska, R., (2011). Evaluation of settled floor dust for the presence of microbial metabolites and volatile anthropogenic chemicals in indoor environments by LC-MS/MS and GC-MS methods. Talanta 85, 2027-2038.

Wady, L., Bunte, A., Pehrson, C., Larsson, L., (2003). Use of gas chromatography-mass spectrometry/solid phase microextraction for the identification of MVOCs from mouldy building materials. Journal of Microbiological Methods 52, 325-332.

Wainwright, M., Wickramasinghe, N.C., Narlikar, J.V., Rajaratman, P., Perkins, J., (2004). Bacteria in the stratosphere-confirmation of viable but non-culturable forms. Instrumental, Methods and Missions for Astrobiology 7, 218-221.

Wessén, B., Schoeps, K.-O., (1996). Microbial volatile organic compounds-what substances can be found in sick buildings?. Analyst 121, 1203-1205.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., (Eds.), "PCR Protocols: A Guide to Methods and Applications". Academic Press, New York, 315-322.

Wilkins, K., Larsen, K., (1995). Variation of volatile organic compound patterns of mold species from damp buildings. Chemosphere 31, 3225-3236.

Wilkins, K., Larsen, K., Simkus, M., (2000). Volatile metabolites from mold growth on building materials and synthetic media. Chemosphere 41, 437-446.

WHO Regional Office for Europe (2009). Guidelines for indoor air quality: dampness and mould. World Health Organization, DK 2100, Copenhagen, Denmark.

Wu, B., Wang, K., Wu, X., (2011). A new phenolic diglycoside produced in responde to copper toxicity and a new flavan dimer from the leaves of *Viburnum ichangense* (Hemsl.) Rehd. Helvetica Chimica Acta 94, 1677-1684.

Yang, C.S., Li, D.-W., (2007). Ecology of fungi in the indoor environment. In: Yang C.S. and Heinsohn P. (Eds), "Sampling and Analysis of Indoor Microorganisms". New York, John Wiley, 191-214.

Zalar, P., de Hoog, G.S., Schroers, H.-J., Crous, P.W., Groenewald, J.Z., Gunde-Cimerma, N., (2007). Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Study in Mycology 58, 157-183.

Zielińska-Jankiewicz, K., Kozajda, A., Piotrowska, M., Szadkowska-Stańczyk, I., (2008). Microbiological contamination

with moulds in work environment in libraries and archive storage facilities. Annals of Agricultural and Environmental Medicine 15, 71-78.

Zyska, B., (1997). Fungi isolated from library materials: a review of the literature. International Biodeterioration & Biodegradation 40, 43-51

PAPERS

Paper 1: The extreme environment of a library: Xerophilic fungi inhabiting indoor niches

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The extreme environment of a library: Xerophilic fungi inhabiting indoor niches

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Compactus shelving

ABSTRACT

The use of Compactus shelves by libraries and archives is a good solution to optimize the storage space and prevent dust deposits on books. However, they are probably the cause of severe cases of fungal colonization in historical library materials. A typical phenomenon occurs as a spread of white mycelial growth forming scattered spots, mainly on volumes with leather or fabric bindings. Recent studies have identified the xerophilic fungus *Eurotium halophilicum* (anamorph *Aspergillus halophilicus*) as responsible for this kind of contamination. A similar situation was found inside the Library of Humanities (BAUM), at Ca' Foscari University, Venice (Italy). Various sampling methods, including cotton swabs and adhesive tape, were adopted to isolate fungi from books and a set of aerobiological analyses was performed to characterize the environment of the repository. The presence of *E. halophilicum* on both books and in the indoor air was confirmed by direct observation of adhesive tape samples, microscopic observations and molecular methods. Moreover, *Aspergillus creber* and *Aspergillus protuberus* belonging to the revised group *Aspergillus* section *Versicolores*, were also isolated for the first time in Italian conservation environments.

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Introduction

The proper management of particular indoor environments, such as libraries or museums, is not always adequate to preserve their contents from various kinds of degradation. The best environmental conditions for preserving books and paper products, according to the Italian recommendations (MIBAC, 2001), are 19–24 °C air temperature and 50–60% relative humidity. However, these conditions turn out to be suitable for the development and growth of several microfungi and bacteria which need little free water and which can colonise materials with a very low water activity (Hocking, 1993; Slonczewski et al. 2010; Borrego et al. 2012; Lavin et al. 2010).

Over the last few years, some authors have described a peculiar fungal colonization associated with books in several Italian archives and libraries (Pinzari and Montanari, 2011; Montanari et al. 2012). In all the cases, the damaged books were stored inside movable shelves of the Compactus type, i.e. closed metal cabinets which, sliding on guide rails, can be compacted and therefore minimize the space required for book storage. These kinds of shelves are considered suitable for preserving books from light degradation and dust deposits. However, without an efficient climate control system, movable shelves can create a micro-environment which supports the growth of particular fungal species, involving the spread of infections which damage books as white spots of mycelium appear on them. The main agent responsible for this kind of fungal contamination has been identify as *Eurotium halophilicum* C.M. Chr., Papav. & C.R. Benj. (anamorph *Aspergillus halophilicus*), a xerophilic fungus with high tolerance to water stress. The minimum water activity observed for its

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germination and growth of this species is 0.675 (Christensen et al. 1959). Because of its particular requirements, this fungus has been isolated from dry food and indoor dust in association with *Aspergillus penicillioides* and dust mites (Christensen et al. 1959; Samson and Lustgraaf, 1978; Hocking and Pitt, 1988; Abdel-Hafez et al. 1990; Aiqing et al. 2011). It has recently been associated with books and paper biodeterioration, and in particular to the presence of very particular micro-niches inside museums, libraries or archives, capable of developing also when the overall environmental conditions seem perfect for the conservation of Cultural Heritage. These niches are characterised by scarce ventilation and the presence of a water vapour gradient that can lead to the condensation on some particular materials after sudden drop of temperature or night–day thermo hygrometric cycles. These peculiar, often very local, conditions together with soaking events in usually dry environments seemed to favour the development of osmophilic and xerophilic fungal species (Michaelsen et al. 2010; Sterflinger, 2010; Pinzari and Montanari, 2011; Montanari et al. 2012). Similar colonization patterns and microscopic features observed in several Italian case studies suggest that *E. halophilicum* might have a large distribution in repositories with Compactus shelves, but its detection may be largely underestimated. Inadequacy of sampling procedures and the very slow growth of the fungus on most media are probably the main reasons for previous underestimations.

In the spring of 2013, a widespread fungal contamination was discovered inside one of the libraries of Ca' Foscari University, Venice (Italy). Stored on Compactus shelves, more than 27,000 old books were found to be spotted with a white growth similar to typical colonization by *E. halophilicum* and the aim of this study was to verify if it is indeed due to this fungus. Aerobiological analyses were carried out to characterize the repository environment in order to find a connection between airborne fungi and the observed phenomena of book deterioration.

Materials and methods

Sampling area

Sampling was performed in a repository of the Library of Humanities (*Biblioteca di Area Umanistica*, BAUM), at Ca' Foscari University, Palazzo Malcanton Marcorà, in Venice (Italy). The repository covers an area of about 150 m^2, is located in the second underground floor, and is furnished with 50 two-row Compactus shelves. After an initial inspection in early 2013, the origin of the fungal colonization was identified in a corner far from the entrance, marked Area 1 in Fig. 1a. In this area, the manuscripts and oldest books of the University collection were stored inside blocks of shelves, which had been kept closed for several months, because of the damage to the sliding tracks (pers.comm. library director).

The fungal growth had spread to eight neighbouring Compactus shelves and was also found on some isolated volumes stored in five shelves of the second row. More than 27,000 *Cinquecentine*, manuscripts and books, especially volumes with leather or fabric bindings dating to the XVI-XIX centuries and belonging to the historical collection of Ca' Foscari University, had been colonized by a white spotted growth (Fig. 1b). The environmental conditions of the repository corresponded to the recommendations of the Italian Ministry for Cultural Heritage (T = 23 °C and RH = 56.3%) (MIBAC, 2001), but apparently, a lack of ventilation inside the Compactus shelves and condensation of water on the books during winter, due to lowered environmental temperature, had probably promoted the germination of fungal spores and allowed contamination to spread.

Book sampling

Five books were sampled with various methods to isolate the fungal colonies: (1) sterile cotton swabs (Cultiplast, LP Italiana Spa, Milan, Italy) were wiped over the spots which had developed on the spines of bindings and then inoculated in 9-cm Petri dishes; (2) sterile nitrocellulose membranes (Advantec MFS, Inc., Dublin, CA, USA; 0.45 m pore size, 47 mm diameter) were gently pressed on the spots visible to the naked eye on the covers of contaminated books for 10 s and then transferred to Petri dishes; (3) pieces (6 × 2 cm) of Fungi-Tape™ (Scientific Device Laboratory, Des Plaines, IL, USA; 1 mm thick, no. 745) were pressed over the spots to collect fungal structures and then deposited on sterile glass slides for microscopic observation; (4) active sampling with Sampl'air Lite (Biomérieux, Florence, Italy) with an air sample volume of 100 L (flow rate 100 L/min) was performed directly from the book covers, fungal structures being collected directly into Petri dishes. As a result of a previous selection of low water activity media, Malt Extract Agar added with 15% NaCl (MEA 15%) (Christensen et al. 1959) and supplemented with 0.05 g/L chloramphenicol to limit bacterial development was used as the most appropriate selective culture medium. All the Petri dishes containing the inocula were then transferred to the laboratory and incubated at 25 °C for 7–14 days.

Microbial air sampling

The air inside and outside the repository was analysed, to determine the level of fungal contamination. Five sampling areas were chosen according to a centrifugal scheme related to the primary colonization area and in respect to the single point of entrance/exit (i.e., of air exchange; Fig. 1a). Three replicas were made for each location. Both active and passive sampling was carried out to collect fungi directly from the air and the hourly viable particles settling on surfaces. To verify the presence of *E. halophilicum* and, in general, of xerophilic and halophylic fungi, MEA 15% supplemented with 0.05 g/L chloramphenicol was chosen to collect airborne fungi. Active sampling was performed with a Sampl'air Lite sampler (Biomérieux, Florence, Italy) with 9-cm Petri dishes, flow rate 100 L/min and sample volume of 100 L. The sampler was placed 1.5 m above the floor, to represent the breathing zone of a standing person. The total numbers of colony-forming units (CFU) in each sample were calculated according to the conversion table provided by the supplier; results are presented as CFU per cubic meter of air (CFU/m^3) (Peto and Powel, 1970).

For passive sampling, 9-cm Petri dishes were exposed to air particles for 1 h, 1 m above the floor and at a distance of about 1 m to obstacles, to determine the Index of Microbial Air Contamination (IMA) expressed as CFU/dm^2/h (Pasquarella et al. 2000).

After samplings, the dishes were closed, labelled, transferred to the laboratory and incubated at 25 °C for 7–14 days.

Optical and scanning electronic microscopic observation of sample tapes

The strips of adhesive tape (Fungi-Tape™) used for sampling the book bindings were observed under a optical microscope (Axio plan, Zeiss, Germany) at 200× and 400× magnification. Micrographs were acquired with a digital camera (AxioCam ERc5s, Zeiss) connected to a PC with ZEN 2011 software (Zeiss).

Fragments of tapes were observed under an EVO 50 (Zeiss), scanning electron microscope (SEM). The tapes were placed in phosphate buffer (pH 7.0), fixed in glutaraldehyde buffer for 2 h, rinsed in distilled water, post-fixed in 2% OsO_4 for 12 h at 5 °C, dehydrated in an ethanol series, taken to amyl acetate, and critical point-dried in a Polaron E–3000 dryer (Quorum Technologies,

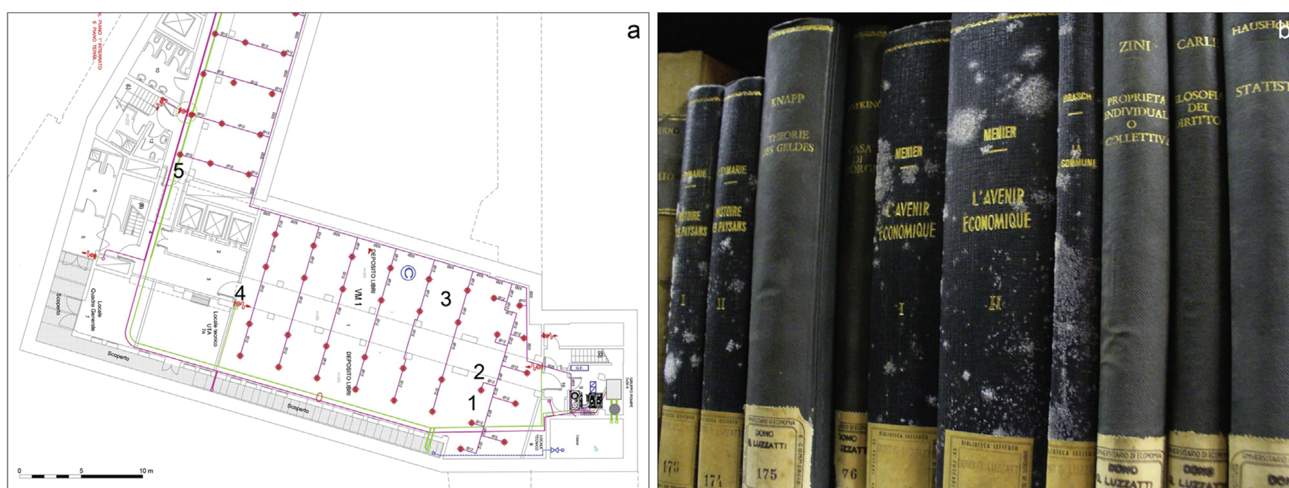

Fig. 1. a: Map of the five sampling areas inside the repository of Library of Humanities (BAUM), Ca' Foscari University, Venice (Italy). **b**: white fungal growth on book covers.

Ringmer, UK) with carbon dioxide. The samples were then coated with gold (Baltec Sputter Coater) for analysis in High Vacuum mode. Sputtering was performed under an Argon gas flow at a working distance of 50 mm, 0.05 mbar of pressure and 40 mA current for 60 s, to obtain a gold film about 15 nm thick. Some samples were gold-coated and observed by SEM without previous fixation, to evaluate any formation of artefacts and the natural degree of dehydration.

Fungal identification

Fungi were identified with a polyphasic approach which couples morpho-physiological features with molecular studies. After determination of genera according to macroscopic and microscopic features (Christensen et al. 1959; Raper and Fennell, 1965; Pitt, 1979; Pitt and Hocking, 1997; Klich, 2002; Samson et al. 2004), the fungal strains were transferred to the media recommended by the authors of selected genus monographs for species identification. Molecular identification was performed by amplification and sequencing of internal transcribed spacers (ITS), β-tubulin and actin genes (White et al. 1990; Gardes and Bruns, 1993; Carbone and Kohn, 1999; Bensch et al. 2012). The resulting sequences were compared with reference sequences in online databases provided by the CBS-KNAW Fungal Biodiversity Centre (The Netherlands) and the NCBI National Center for Biotechnology Information (US). The fungal sequences and corresponding species identifications were deposited in GenBank under accession numbers KM264279–KM264297 and KM502176–KM502208. All fungi are preserved at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and Systems Biology, University of Turin (Italy).

Statistical analyses and diversity assessment

The Bray–Curtis Index was applied to the airborne microbial population in order to describe similarity among sampling areas, with XLSTAT 2014.4.01. A significant dissimilarity level of 0.95 was applied. The Simpson Diversity Index was also calculated to explain the level of species diversity inside the repository, according to the formula: $1 - D = 1 - \sum (n/N)^2$, where n is the number of colonies per morphological fungal species and N is the total frequency of the corresponding colony in the population (Magurran, 1988; Nunes et al. 2013).

Results and discussions

Book sampling

The uniform spread of fungal colonization was visible to the naked eye on all sampled books, as white spots of varying sizes, ranging on average from 0.2 to 1 cm in diameter (Fig. 1b). Direct observation of Fungi-Tape™ samples by optical microscope showed various fungal structures, such as mycelium, conidiophores and conidia, mainly belonging to *Aspergillus* spp. Uniseriate radiate to columnar conidial heads and ellipsoidal conidia of quite variable size (5–7.5 × 5–9 μm) were observed. The shape, ornamentation and dimensions of these conidia were consistent with those of the anamorphous state of *E. halophilicum*, namely *Aspergillus halophilicus* (Fig. 2) (Samson and Lustgraaf, 1978). Short bare "hairs" on the hyphae were visible even at 200× magnification, and even clearer under SEM. These hairs were found in fixed, gold-coated and directly sputtered samples, thus confirming that they were not an artefact of preparation.

Fourteen species belonging to eight genera were identified (Table 1). Sterile swabbing, nitrocellulose membranes and active sampling favoured the isolation of five, nine and eight taxa, respectively. Although the nitrocellulose membranes isolated the highest number of species, they did not isolated *E. halophilicum*. Instead, active sampling isolated all the species isolated by sterile swabbing and also some others. Several authors have already demonstrated the effectiveness of nitrocellulose membranes for fungal isolation from varying types of substrates (e.g., Florian, 2002; Manente et al. 2012). The most frequent fungal species found in our case were *Aspergillus creber* and *Penicillium brevicompactum*. *A. creber*, belonging to the recent revisited section *Versicolores* (Jurjevic et al. 2012), was found on stored books for the first time; *P. brevicompactum* is well-known as one of the most xerophilic Penicillia (Hocking and Pitt, 1988). *E. halophilicum* was preferentially isolated by sterile swabbing and active sampling very close to the contamination on the books. Although its white growth was visible on all the book covers, its isolation was difficult and sporadic, and samples were obtained from only one volume. These results match literature reports of this species. After initial large-scale expansion, due to the sudden availability of water on the books, identifiable as mycelium sporification, *E. halophilicum* stops growing and its starving mycelium acts passively as a sort of trap for other airborne spores. A hypothesis still to be proven is that this

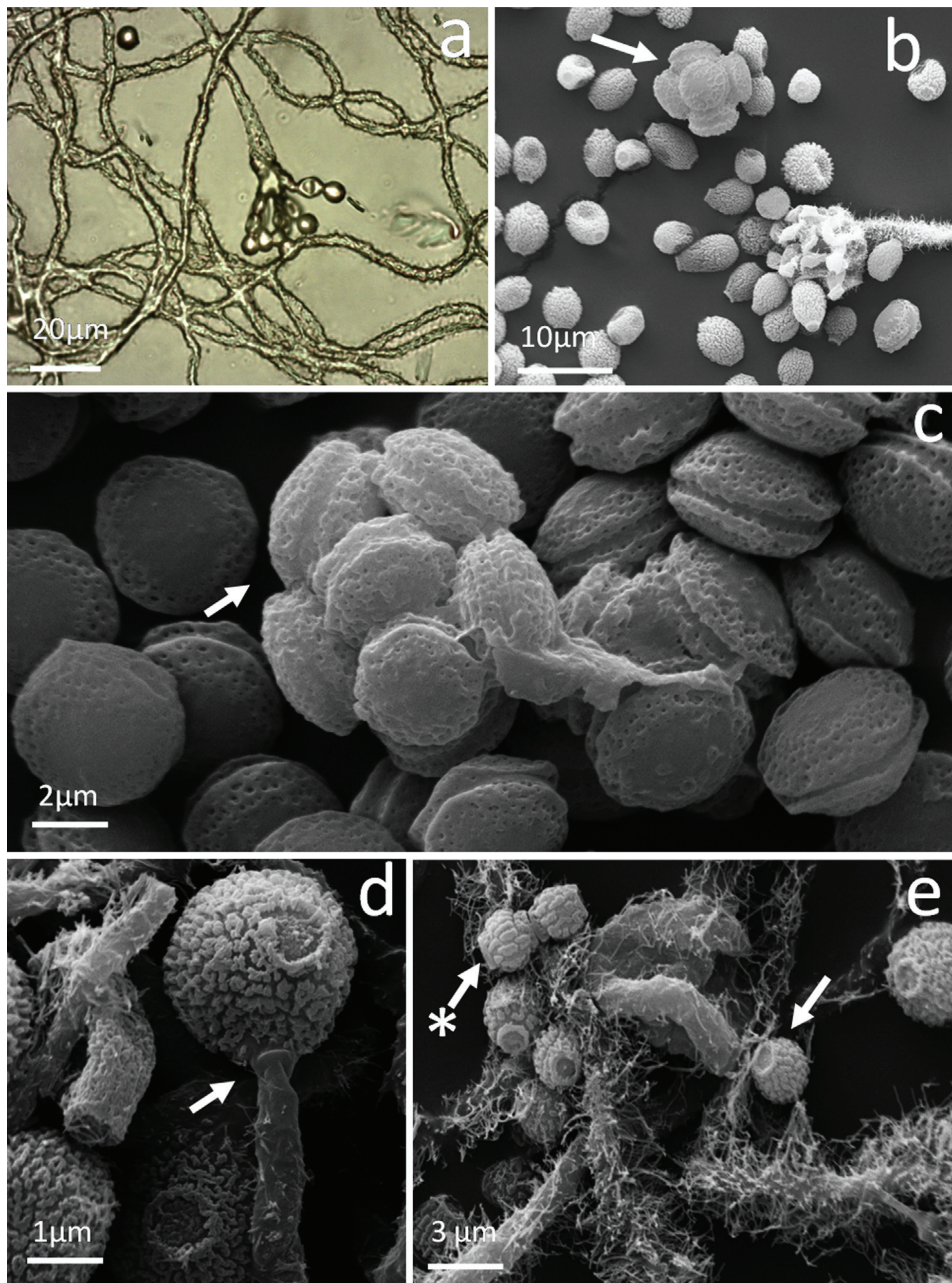

Fig. 2. SEM and optical microscope images of fungal structures sampled from books with adhesive Fungi-Tape™. **a**: Optical bright field microscopy imaging of *E. halophilicum* hyphae, conidiophore, and conidia; **b**: HV SE SEM image of gold-sputtered samples of *E.halophilicum*: details of rough lenticular ascospores still assembled in an ascum (arrow) and a typical haired conidiophore with vescicle and conidiogenous cells surrounded by many ornamented conidia. **c**: HV SE SEM image of gold-sputtered samples of *E. halophilicum;* details of rough lenticular ascospores, with shallow furrows bordered by low-ridged ascospores, assembled in an ascum (arrow). **d**: HV SE SEM image of gold-sputtered samples of a germinating conidium of *E. halophilicum*: details of a germinative tube (arrow). **e**: HV SE SEM image of gold-sputtered samples of *E. halophilicum* haired hyphae with trapped conidia belonging to two different species, characterized by different dimensions, shapes and ornamentations (simple arrow = *E. halophilicum*, arrow with asterisk = a different fungal species).

starving mycelium provides the substrate for the growth or survival of spores and propagules of other fungi. This may explain why old contaminations in libraries are due to fungal species other than the *E. halophilicum*, although its presence on materials can very often be documented by molecular methods and SEM imaging.

Colonies of another xerophilic fungus, *A. penicillioides*, were also frequently isolated from books. Already in 1978, this fungus was associated with *E. halophilicum* by Samson and Lustgraaf (1978) as cohabiting in house dust, emphasizing the possibility what it was responsible for allergic reactions and lung disease. This species was also been isolated from dry food and indoor environments, such as museums, archives, shelving and carpeting (Samson and Lustgraaf, 1978; Pitt and Hocking, 1997; Samson et al. 2004). In particular, *A. penicillioides* has often been associated with the mycoflora isolated from old contaminated books and manuscripts (Pinzari and Montanari, 2008; Michaelsen et al. 2010; Montanari et al. 2012).

Table 1
Fungal diversity from various sampling procedures on books, with GenBank accession numbers.

Fungal species	Number of books	Methods of isolation	Accession number
Aspergillus creber Jurjevic, S.W. Peterson & B.W. Horn	4	S, A, M	(*)
Penicillium brevicompactum Dierckx	3	S, A, M	KM502183
Aspergillus penicillioides Speg.	3	S, A	KM502180
Aspergillus protuberus Munt.-Cvetk.	2	S, A	(*)
Eurotium halophilicum C.M. Chr. Papav. & C.R. Benj.	1	S, A	KM502179
Aspergillus jensenii Jurjevic, S.W. Peterson & B.W. Horn	1	A	KM502178
Lecanicillium kalimantanense Kurihara & Sukarno	1	A	KM264285
Eurotium chevalieri L. Mangin	1	M	(*)
Aspergillus versicolor (Vuill.) Tirab.	1	M	KM264292
Penicillium chrysogenum Thom	1	M	(*)
Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun	1	M	KM264294
Epicoccum nigrum Link	1	M	KM264283
Phaeospheria typharum (Desm.) L. Holm	1	M	KM264282
Gloeophillum abietinum (Bull.) P. Karst.	1	M	KM264291

S = sterile swab; A = active sampling; M = nitrocellulose membrane.
(*) Fungal species that have only been identify using morphological approach.

Microbial air sampling

The results of microbial air sampling demonstrated a gradually decreasing contamination gradient from area 1 toward the exit of the repository (Table 2). The source of colonization in area 1 was the most contaminated of all the sampled sites, followed by area 2 (2000 and 1620 CFU/m^3, respectively) and they presented the highest fungal similarity ($d^{BCS} = 0.84$). These loads were ten times higher than in the other sampled areas, which ranged between 101 and 29 CFU/m^3, with low similarity indices ($0.06 < d^{BCS} < 0.4$). The total fungal loads in areas 1 and 2 were higher than those reported in similar studies (Karbowska-Berent et al. 2011; Pasquarella et al. 2012; Nunes et al. 2013). They were also higher than the 150 CFU/m^3 value recommended for Italian libraries (MIBAC, 2001), and even higher than other international recommended limits, which indicated a maximal contamination value of 1000 CFU/m^3 for archives (Borrego et al. 2010; Nunes et al. 2013). The data from passive sampling recorded a lower yield compared with active sampling, with 4–44 CFU/m^2/h. The absence of CFU from the passive sampler in the area 5 may be explained by the lack of settling microbial particles able to grow on the selective media used, or by the different ventilation system outside the repository.

Several fungal patterns were found in the sampled areas, highlighting the variety of mycological species found in a quite small space (150 m^2), and the Simpson Diversity Index ranged between 0.5 and 0.9 (Table 2). Area 4 had the highest fungal diversity, perhaps because of the continual passage of workers and students. Area 1 also had the lowest value of fungal variability, perhaps be due to the particular environmental conditions between the shelves, with very low air exchange rates.

Morphological and molecular analysis isolated 76 different fungal taxa, belonging to eleven fungal genera (Table 3). *Aspergillus* was the most frequent genus (82.4%), followed by *Penicillium* (14.7%) and *Cladosporium* (1.4%). The remaining isolates presented low frequency, between 0.8% and <0.1%. The most abundant species were *A. creber* (55.7%), *Aspergillus protuberus* (25.7%), *Penicillium chrysogenum* (11.5%) and *P. brevicompactum* (3.1%), characterizing in particular the colonization source in area 1 and the surrounding area 2. Found also on the sampled books, these species are common indoor fungi (Samson et al. 2004; Jurjevic et al. 2012). *P. chrysogenum* and *P. brevicompactum*, in particular, have also been associated with the biodeterioration of Cultural Heritage materials (Zyska, 1997; Montemartini Corte et al., 2003; Sterflinger, 2010). Conversely, the genera *Cladosporium* (1.4%) was peculiar to transition areas 4 and 5, together with other outdoor fungi such as *Pleosporales* sp., *Embellisia* sp., *Schyzophillum* sp. and *Botrytis* sp. (Pitt and Hocking, 1997; Samson et al. 2004). Area 3 showed a mixture of fungi also found in the other areas, both indoor, such as *Aspergillus* spp. and *Penicillium* spp. (Gallo, 1993; Zyska, 1997; Sterflinger, 2010) and outdoor, such as *Fusarium* sp. and *Alternaria* sp., were found (Pitt and Hocking, 1997; Samson et al. 2004).

Fungi collected with passive sampling were limited to a few colonies of *A. penicillioides*, *Aspergillus proliferans*, *Penicillium corylophilum* and *Penicillium italicum*. Active sampling may have revealed their presence, but they could not be isolated due to the high number of CFU/m^3.

The sampled air did not allow isolation of colonies of *E. halophilicum*. This result emphasizes how the presence of this fungus may easily be neglected or underestimated during air quality and conservation surveys in libraries and archives, as it is difficult to isolate from the air and grows slowly *in vitro*.

Conclusions

Fungal contamination caused by a proliferation of *E. halophilicum* was identify inside the Library of Humanities (BAUM) at Ca' Foscari University, Venice (Italy). Its presence was found on books and in the nearby surrounding indoor air, but could not be isolated by aerobiological analysis, probably because of its poor susceptibility to air dispersion. It appears that it may proliferate and

Table 2
Fungal air contamination values in book repository, active sampling (AS, CFU/m^3) and passive sampling (PS, CFU/dm^2/h) with standard deviations (STD). Total number of fungal taxa and Simpson Diversity Index presented for each sampled area and for entire environment of repository.

Sampling areas inside the deposit	AS (CFU/m^3) mean ± st. dev.	PS (CFU/dm^2/h) mean ± st. dev.	Number of fungal taxa	Simpson's index of fungal population
1: Right corner far from the entrance, between shelves	2000 ± 610	44 ± 9	5	0.5
2: Corridor close to the area 1	1620 ± 80	35 ± 8	8	0.6
3: Left corner far from the entrance, between shelves	81 ± 1	11 ± 5	12	0.8
4: Entrance	101 ± 12	4 ± 2	22	0.9
5: Storage room adjacent to the deposit studied	29 ± 13	0	7	0.7
Total	3831	94	27	0.6

Table 3
Fungal diversity results from aerobiological analysis, expressed for sampled areas as relative frequency, together with GenBank accession numbers.

Fungal species	Relative frequency (%)						Accession no.
	Area 1	Area 2	Area 3	Area 4	Area 5	Total count	
Aspergillus creber Jurjevic, S.W. Peterson & B.W. Horn	64.3% (P)	50.3% (P)	27% (P)	14.9%	–	55.7%	KM502191
Aspergillus protuberus Munt.-Cvetk	23.3% (P)	31.5% (P)	7%	6.9%	–	25.7%	KM502192
Penicillium chrysogenum Thom	9.5% (P)	13.6% (P)	20%	9.9% (P)	3.6%	11.5%	KM502200
Penicillium brevicompactum Dierckx	2.8%	3.4%	7%	1%	–	3.1%	(*)
Aspergillus spp.	–	(P)	10%	2%	10.7%	0.4%	(*)
Cladosporium pseudocladosporioides Bensch, Crous & U. Braun	(P)	(P)	(P)	5% (P)	–	0.1%	(*)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–	–	–	9.9%	46.4%	0.6%	KM264293
Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun	–	0.3%	2%	5%	14.3%	0.4%	KM264295
Aspergillus penicillioides Speg.	–	(P)	–	–	–	–	(*)
Phaeospheria herpotrichoides (De Not.) L. Holm	–	–	–	2% (P)	7.1%	0.1%	KM264281
Aspergillus niger Tiegh.	–	0.3%	–	5%	–	0.3%	KM502177
Penicillium sizovae Baghd.	–	0.3%	–	5%	–	0.3%	KM502185
Botrytis cinerea Pers.	–	–	–	2% (P)	–	0.1%	KM264280
Aspergillus sydowii (Bainier & Sartory) Thom & Church	(P)	–	1%	–	–	<0.1%	KM502191
Aspergillus proliferans G. Sm.	–	(P)	–	–	–	–	KM502181
Penicillium italicum Wehmer	–	(P)	–	–	–	–	KM502188
Penicillium corylophilum Dierckx	–	–	(P)	–	–	–	KM502184
Pleosporales sp. Luttr. Ex M.E. Barr	–	–	1%	5%	–	0.2%	(*)
Cladosporium sphaerospermum Penz.	–	–	–	5.9%	–	0.2%	KM264296
Aspergillus insuetus (Bainier) Thom & Church	–	0.3%	–	–	–	0.1%	KM502182
Cladosporium ossifragi (Rostr.) U. Braun & K. Schub.	–	–	–	5%	–	0.1%	(*)
Phoma glomerata (Corda) Wollenw. & Hochapfel	–	–	–	5%	–	0.1%	KM264279
Penicillium glabrum (Wehmer) Westling	–	–	–	4%	–	0.1%	KM502187
Eurotium chevalieri L. Mangin	–	–	–	2%	3.6%	0.1%	(*)
Fusarium oxysporum Schltdl.	–	–	2%	–	–	0.1%	KM264286
Aspergillus sclerotiorum G.A. Huber	(P)	–	1%	–	–	0	KM502176
Aspergillus flavus Link	–	–	–	1%	–	<0.1%	(*)
Embellisia abundans E.G. Simmons	–	–	–	1%	–	<0.1%	KM264288
Alternaria infectoria E.G. Simmons	–	–	1%	–	–	<0.1%	KM264290
Schyzophyllum commune Fr.	–	–	–	1%	–	<0.1%	KM264287
Sterile mycelium	0.3% (P)	(P)	21%	2%	14.3%	0.8%	(*)

(*) Fungal species that have only been identify using morphological approach.
(P) Fungal species isolated by passive sampling.

spread only during its first period of growth, after which, it is difficult to isolate, even from contaminated surfaces with visible fungal growth. This is why its spread may also be attributed to other strategies, such as dust mites. *A. penicillioides* was also frequently isolated from books in association with *E. halophilicum*, probably because of the similar behaviour and low water requirements of both these species. Aerobiological analysis revealed high fungal concentrations, exceeding Italian recommended limits for libraries, inside the repository and in particular close to the source of colonization between shelves. *A. creber*, *A. protuberus*, *P. chrysogenum* and *P. brevicompactum* were the species most frequently isolated. Moreover, *A. creber* and *A. protuberus* belonging to the *Aspergillus* section *Versicolores* group were also detected for the first time inside the Italian repository environment. The use of sampling media suitable for halophilic and xerophilic fungal species inside archives and libraries emphasizes a very peculiar ecological dynamic in these indoor environments, as unsuspected and little investigated niches are occupied.

Apart from the biodegradation of very old books, our results should be considered in relation to the effect which these poorly studied fungal species may have on human health. In theory, most of the identified species, such as *Aspergillus* and *Penicillium* spp., and their high concentrations in closed environments, may cause health problems for both library workers and visitors. Rapid diagnoses, frequent dusting and effective ventilation should all be ensured, to prevent fungal contamination of materials.

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References

Abel-Hafez, S.I.I., Moubasher, A.H., Barakat, A., 1990. Keratinophilic fungi and other moulds associated with air- dust particles from Egypt. Folia Microbiol. 35, 311–325.
Aiqing, X., Yuanliang, W., Jieyu, W., Pin, l, Ziyin, L., Longjjun, L., 2011. Fungal community associated with fermentation and storage of Fuzhan brick-tea. Int. J. Food Microbiol. 146, 14–22.
Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W., 2012. The genus *Cladosporium*. Stud. Mycol. 72, 1–401.
Borrego, S., Guiamet, P., Gómez de Saravia, S., Batistini, P., Garcia, M., Lavin, P., Perdomo, I., 2010. The quality of air at archives and the biodeterioration of photographs. Int. Biodeterior. Biodegrad. 64, 139–145.
Borrego, S., Lavin, P., Perdomo, I., Gómez de Saravia, S., Guiamet, P., 2012. Determination of indoor air quality in archives and biodeterioration of the documentary heritage. ISRN Microbiol. 2012, 10.
Carbone, I., Kohn, L.M., 1999. A methods for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.
Christensen, C.M., Papavizas, G.C., Benjamin, C.R., 1959. A new halophilic species of *Eurotium*. Mycologia 51, 636–640.
Florian, M.L.E., 2002. Fungal Facts: Solving Problems in Heritage Collection. Archetype Publications Ltd, London, UK.
Gallo, F., 1993. Aerobiological research and problems in libraries. Aerobiologia 9, 117–130.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 1, 113–118.
Hocking, A.D., Pitt, J.I., 1988. Two new species of xerophilic fungi and a further record of *Eurotium halophilicum*. Mycologia 80, 82–88.
Hocking, A.D., 1993. Responses in xerophilic fungi to changes in water activity. In: Jennings, D.H. (Ed.), Stress Tolerance of Fungi. Marcel Dekker, Inc., New York, pp. 233–243.
Jurjevic, Z., Peterson, S.W., Horn, B.W., 2012. *Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 3, 59–79.

Karbowska-Berent, J., Górny, R.L., Strzelczyk, A.B., Wlazło, A., 2011. Airborne and dust borne microorganisms in selected polish libraries and archives. Build. Environ. 46, 1872–1879.

Klich, M.A., 2002. Identification of Common *Aspergillus* Species. CBS, Utrecht, The Netherlands.

Lavin, P., Gómez de Saravia, S.G., Guiamet, P.S., 2010. An environmental assessment of biodeterioration in document repositories. Biofouling 30, 561–569.

Magurran, A.E., 1988. Ecological Diversity and its Measurements. Princeton University Press, Princeton, NJ, pp. 39–40.

Manente, S., Micheluz, A., Ganzerla, R., Ravagnan, G., Gambaro, A., 2012. Chemical and biological characterization of paper: a case study using a proposed methodological approach. Int. Biodeterior. Biodegrad. 74, 99–108.

MIBAC, 2001. Atto d'indirizzo sui criteri tecnico-scientifici e sugli standard di funzionamento e sviluppo dei musei, D.Lgs n.112/98 art.150 comma 6.

Michaelsen, A., Pinar, G., Pinzari, F., 2010. Molecular and microscopical investigation of the microflora inhabiting a deteriorated Italian manuscript dated from the thirteen century. Microb. Ecol. 60, 69–80.

Montanari, M., Melloni, V., Pinzari, F., Innocenti, G., 2012. Fungal biodeterioration of historical library materials stored in compactus movable shelves. Int. Biodeterior. Biodegrad. 75, 83–88.

Montemartini Corte, A., Ferroni, A., Salvo, V.S., 2003. Isolation of fungal species from test samples and maps damaged by foxing, and correlation between these species and the environment. Int. Biodeterior. Biodegrad. 51, 167–173.

Nunes, I., Mesquita, N., Cabo Verde, S., Leitão Bandeira, A.M., Carolino, M.M., Portugal, A., Botelho, M.L., 2013. Characterization of an airborne microbial community: a case study in the archive of the University of Coimbra, Portugal. Int. Biodeterior. Biodegrad. 79, 36–41.

Pasquarella, C., Pitzurra, O., Savino, A., 2000. The index of microbial air contamination. J. Hosp. Infect. 46, 241–256.

Pasquarella, C., Saccani, E., Sansebastiano, G.E., Ugolotti, M., Pasquariello, G., Albertini, R., 2012. Proposal for a biological environmental monitoring approach to be used in libraries and archives. Ann. Agric. Environ. Med. 19, 209–212.

Peto, S., Powel, E.O., 1970. The assessment of aerosol concentration by means of the Andersen sampler. J. Appl. Bacteriol. 33, 582–598.

Pinzari, F., Montanari, M., 2008. Il SEM-VP nella diagnostica di alterazioni fungine non più vitali o di difficile caratterizzazione. In: Pinzari, F. (Ed.), Scienza e ricerca per i beni culturali. Microscopia elettronica a scansione e microanalisi. Gangemi Editore, Roma, pp. 79–86.

Pinzari, F., Montanari, M., 2011. Mould growth on library materials stored in compactus-type shelving units. In: Abdul-Wahab Al-Sulaiman, S.A. (Ed.), Sick Building Syndrome: Public Buildings and Workplaces. Springer-Verlag, Berlin Heidelberg, pp. 193–206.

Pitt, J.I., 1979. The Genus *Penicillium* and its Teleomorphic States: Eupenicillium and *Talaromyces*. Academic Press.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage. Blackie Academic and Professional of Chapman & Hall, London, UK.

Raper, K.B., Fennell, D.I., 1965. The Genus *Aspergillus*. Williams & Wilkins, Baltimore, USA.

Samson, R.A., Lustgraaf, B.V.D., 1978. *Aspergillus penicilloides* and *Eurotium halophilicum* in association with house- dust mites. Mycopathologia 64, 13–16.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., 2004. Introduction to Food and Airborne Fungi. ASM Press, US.

Samson, R.A., Frisvad, J.C., 2004. Penicillium Subgenus Penicillium: New Taxonomic Schemes, Mycotoxins and Other Extrolites. In: Studies in Mycology, vol. 49. CBS, Utrecht, The Netherlands.

Slonczewski, J.L., Coker, A.J., DasSarma, S., 2010. Microbial growth under multiple stressors. Microbe 5, 110–116.

Sterflinger, K., 2010. Fungi: their role in deterioration of cultural heritage. Fungal Biol. Rev. 24, 47–55.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Zyska, B., 1997. Fungi isolated from library materials: a review of the literature. Int. Biodeterior. Biodegrad. 40, 43–51.

Paper 2: Fungal secondary metabolite analysis applied to Cultural Heritage: the case of a contaminated library in Venice

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Abstract

The secondary metabolite production of several fungal strains of *Aspergillus creber*, *A. jensenii*, *A. penicillioides*, *A. protuberus, A. vitricola*, *Cladosporium cladosporioides, Eurotium chevalieri*, *E. halophilicum, Penicillium brevicompactum* and *P. chrysogenum*, were characterized by LC-MS/MS. All were isolated from both air and book covers as well as settled dust from a contaminated library in Venice (Italy). For *A. creber* and *A. jensenii*, we identified sterigmatocystin, methoxysterigmatocystin, versicolorin A and related precursors and side metabolites from the biosynthetic pathway. Deoxybrevianamid E, neoechinulin A, pseurotin A and D and rugulusovin were principally detected from the strains of *E. halophilicum*, an emerging fungal contaminant implicated in book contaminations in specific indoor niches. The analysis of settled dust produced a wide range of toxic or bioactive fungal metabolites. A total of 45 different metabolites were identified in different concentrations, in particular with high contribution of asperglaucide, alamethicin, andrastin A, terrecyclic acid and neochinulin A. As bacterial metabolite, only chloramphenicole was detected. This study increased the knowledge about metabolite production of less-known fungal species and improved information about the indoor presence of further fungi not detected by aerobiological sampling. The results emphasize how routine dusting operations are necessary and essential in order to avoid any microbiological development and potential health implications of students and workers.

Keywords

Mycotoxins, indoor environment, book contamination, settled dust, *Eurotium halophilicum*

1. Introduction

The theme of microbiological hazards related to enclosed environments and indoor spaces is still a big subject of discussion worldwide. In the last years, several studies have pointed out the critical microbiological contribution towards airway infections, impaired immune function, bronchitis, asthma, recurrent airway infections and extreme fatigue (Miller and McMullin, 2014; Nielsen, 2003; WHO, 2009). Inside libraries and archives, for example, the management of increasing fungal contaminations due to inadequate conservations, such as malfunction of air conditioning systems, lack of cleanliness, exchange of contaminated collections or materials, has become a complex and expensive problem, for book conservation but also for the health of workers and students (Flannigan and Miller, 2011; Micheluz *et al.*, 2015; Zielińska-Jankiewicz *et al.*, 2008).

The directive of 2000/54/EC and the Italian regulation (D.Lgs. 9 April n.81) describe the microbiological risks at work, classifying biological agents in four risk groups according to their level of risk of infection. Many bacteria, viruses, parasites and fungi are listed, including microfungi e.g. *Aspergillus fumigatus*. However many fungal toxigenic species that may be present in the indoor environments are not included and only few workplaces have been described in terms of fungal occurrence (Gutarowska *et al.*, 2014). As concerns museum, libraries and archives, the Italian Ministry for Cultural Heritage provides guidelines for microbiological limits (750 CFU/m^3 for total bacterial load and 150 CFU/m^3 for fungal load) (MIBAC, 2001). Several studies have examined these kinds of environments by aerobiological analysis (Borrego *et al.*, 2010; Nunes *et al.*, 2013; Pasquarella *et al.*, 2012), and some of them have also considered the occurrence of microorganisms in the dust (Apetrei *et al.*, 2009; Karbowska-Berent *et al.*, 2011; Maggi *et al.*, 2000) and the indoor mycotoxin-potential (Gutarowska *et al.*, 2014).

Fungi can be present in indoor environment distributed by airborne particles, remaining in suspension for a period of time determined by air movements and their aerodynamic diameters, and they can undergo sedimentation as settled dust (Karbowska-Berent *et al.*, 2011; Maggi *et al.*, 2000). The airborne particle and dust compositions in libraries and archives depend on the building, on the seasons, on activities going on therein, on the state of collections and on the occurrence of exceptional events, as floods. They could perform potential biodeterioration activities when the microclimatic conditions and the water activity (a_w) of the preserved materials exceed the level risk (20 °C and 65% of RH, 10% water content and $a_w = 0.65$ in the materials) and allow them to germinate and growth (Maggi *et al.*, 2000).

The fungal genera accounting for mycological contamination of library and archive collections include *Alternaria, Aspergillus*, *Botrytis*, *Chaetomiun*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Stachybotris*, *Trichoderma* spp. These genera include species well-known for their being allergenic and/or toxigenic (Karbowska-Berent *et al.*, 2011; Zielińska-Jankiewicz *et al.*, 2008; Zyska, 1997). Mycotoxins are low-molecular-weight secondary metabolites produced by fungi that have been demonstrated to play a crucial role in their natural habitants against bacteria, other fungi but also plants, animals and humans (Nielsen, 2003; Miller and McMullin, 2014). Their production depends on the type of substrate available, temperature, humidity and the presence of certain trace elements or other microorganisms (Gutarowska *et al*, 2014). These metabolites are excreted into the substrate or can be present onto fungal cells, and they can become airborne being in the dust or inside conidia or spores fragments (Fischer and Dott, 2003). If airborne fungal spores are inhaled, the human body could be expose to primary and secondary metabolites, with possible health problems as acute kidney failure (ochratoxin), central nervous system damage (tremorgenic mycotoxins) and damage the upper respiratory tract (*Stachybotrys chartarum*) (Fischer and Dott, 2003). For these reasons, mycotoxins were recognised as a potential hazard, especially for the occupational and indoor hygiene (Fischer *et al.*, 2000, 2003; Flanning and Miller, 2011).

The aims of this work were to determine the potential mycotoxin occurrence inside a contaminated environment of a library's repository in Venice (Italy). The major fungal species isolated both from the air and from contaminated books were analysed for their capability to produce mycotoxins.

Moreover, mycotoxin analyses of settle dust were performed in order to estimate the potential health hazard of the librarians.

2 Materials and methods

2.1 Study site and sampling

Air and dust samplings were performed during the spring and autumn seasons of 2013 in a repository of the Library of Humanities (Biblioteca di Area Umanistica, BAUM), at Ca' Foscari University, Palazzo Malcanton Marcorà, in Venice (Italy). The repository is located in the second underground floor and covers an area of about 150 m^2. Two rows of 50 Compactus® shelves were disposed mainly for the storage of the manuscripts and oldest books of the Ca' Foscari University collection. Five sampling areas, both inside and outside of the repository, were chosen in order to characterize the indoor environment and the spread fungal contaminations on the books. The details about active air sampling are given in Micheluz *et al.* (2015). Settled floor dust samples were collected in the four areas inside the repository, corresponding to the same air sampling areas, by a conventional household vacuum cleaner with a power level at least 780 W and new dust bags (Table 1). After sampling for 2 min/cm^2, the dust bags were closed by plastic tape, transferred to the laboratory and stored at -20 °C until their extraction. The areas of deposit floor sampled varied from 1.5 to 4.5 m^2. The environmental conditions of the repository were 19.6 °C and 51.8% of RH and 22.7 °C and 54.7% of RH during the spring and autumn samplings respectively. These parameters are in accordance with the Italian recommendations (MIBAC, 2001).

Table 5 Dust sampling areas inside the contaminated repository of Library of Humanities, (BAUM), Ca' Foscari University of Venice (Italy). Sampling surfaces (m^2) are included.

Sample	Sampling location	Sampling surface (m^2)
Area 1	Right corner far from the entrance between shelves	1.5
Area 2	Corridor close to area 1	4.3
Area 3	Left corner far from the entrance, between shelves	3.8
Area 4	Entrance of the repository	4.3

2.2 Selection of relevant fungal species

The species-specific fungal characterization of the repository provided important information about the features of the fungal spreading, identifying *Eurotium halophilicum* as the main responsible of that kind of contamination, as reported by Micheluz *et al*. (2015). In the present study, different strains of the most prevalent fungal species isolated both from the air, occurring with highest colony forming unit (CFU) counts, and from book covers were investigated for their mycotoxin-production ability. In particular, *Aspergillus creber*, *A. jensenii*, *A. penicillioides, A. protuberus*, *A. vitricola, Cladosporium cladosporioides, E. chevalieri, Penicillium brevicompactum* and *P. chrysogenum*, together with strains of *E. halophilicum* were selected for this purpose. The list of fungi with their detailed information and accession numbers is reported in the Table 2. All the fungi are preserved at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and System Biology, University of Turin.

To detect a wide range of metabolites and toxins, each strain was growth on different media, depending also of its nutrient requirements: yeast extract-sucrose agar (YES) and Czapek-yeast autolysate agar (CYA) were used for *A. creber*, *A. jensenii, A. protuberus*, *C. cladosporioides, E. chevalieri*, *P brevicompactum and P. chrysogenum*, while MEA15% and YES added with 15% NaCl (YES15%) were used for *A. penicillioides, A. vitricola* and *E. halophilicum*. The media were prepared according to the literature (Christensen *et al.*, 1979; Samson *et al.*, 2004). The strains were

three-point inoculated on the media in 9-cm Petri dishes and incubated for 7 days (14 days for strains of *A. penicillioides* and A. *vitricola*, and 35 days for strains of *E. halophilicum*) at 25 °C.

Table 6 List of fungal species isolated from the contaminated repository and selected for their secondary metabolite production analysis by LC-MS/MS. MUT numbers and GenBank accession numbers are included.

Fungal species	**MUT no.**	**GenBank accession number**	**Source**
A. creber Jurievic, S.W. Peterson & B.W. Horn	MUT 470		Book cover
	MUT 5691		Book cover
	MUT 5527		Book cover
	MUT 5690		Indoor air
	MUT 5689		Indoor air
A. jensenii Jurievic, S.W. Peterson & B.W. Horn	MUT 480	KM502178	Book cover
A protuberus Munt.-Cvetk.	MUT 5693		Indoor air
	MUT 5487	KM502193	Indoor air
Aspergillus penicillioides Spegazzini	MUT 481		Book cover
	MUT 5694		Indoor air
	MUT 5525		Book cover
	MUT 5537		Book cover
	MUT 5692		Book cover
Cladosporium cladosporioides (Fresen) G.A. de Vries	MUT 5536		Indoor air
	MUT 527		Indoor air
Eurotium chevalieri L. Mangin	MUT 472		Indoor air
E. halophilicum C,M, Chr., Papav. & C.R. Benj.	MUT 482	KM502179	Book cover
	MUT 5534		Book cover
	MUT 5535		Book cover
Penicillium brevicompactum Dierckx	MUT 536	KM502183	Book cover
P. chrysogenum Thom	MUT 5493	KM502200	Indoor air
	MUT 5492	KM502204	Indoor air

2.3 Mycotoxin analysis

From each fungus on each type of medium one agar disk (1 cm in diameter taken from the marginal part of the fungal colony, including mycelium, conidia and cleistothecia for *Eurotium* spp.) were punched out using a cork borer and treated with 4 µL of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). The extraction was performed for 1.5 h using a Universal Table Shaker 709 (ASAL srl, Milan, Italy) and subsequently centrifuged for 10 min at 3000 rpm (MPW-251 centrifuge, MPW Med. Instruments, Warsaw, Poland). The extracts were transferred into glass vials, and 500 µL aliquots were diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v). After appropriate mixing, 5 µL of the diluted extract was injected into LC-MS/MS system without further pre-treatment.

In the case of dust samples, the materials were collect by sterile brush inside glass vials and then weighed. Different aliquots of extraction solution, from 4 to 8 mL were added, depending of the different dust amount. The extraction and the dilution of the samples were made as for fungal samples.

Analysis of mycotoxins was performed with the recently updated multi-toxin method, which cover 320 analytes, as described by Malachová *et al.* (2014). As mass-spectrometer, a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with TurbolonSpray electronspray ionization (ESI) source was used connected with a 1290 Series HPLC System (Agilent, Waldbronn,

Germany). Chromatographic separation was performed at 25 °C on a Gemini C18-coloum, 150 x 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 x 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US).
ESI-MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in 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 time ±27 sec and ±48 sec in the positive and the negative modes, respectively. Confirmation of positive analyte identification was obtained by the acquisition of two MRMs per analyte. The LC retention time and the intensity ratio of the two MRM transitions agreed with the related values of an authentic standard within 0.1 min and 30% rel., respectively. Quantification was performed using external calibration based on serial dilution of multi-analyte stock solution (Malachová *et al*., 2014).

2.4 Statistical analyses of dust samples
The Bray-Curtis Index was applied to the mycotoxin results of dust samples in order to describe similarity among sampling areas, with XLSTAT 2015.1.03. A significant dissimilarity level of 0.95 was applied.

3 Results

3.1 Metabolite patterns in the investigated fungal cultures
A total of 36 metabolites were detected in the strains belonging to the 10 investigated fungal species. Different metabolite were observed for each fungus and also between the strains of the some species, remarked with percentages % in Table 3.
A. creber, *A. protuberus* and *A. jensenii*, belonging to *Aspergillus* section *Versicolores*, produced a similar metabolite pattern in comparison to *A. versicolor* (Nielsen, 2003): methoxysterigmatocystin, sterigmatocystin and several biosynthetic precursors (averantin, averufanin, nidurufin, norsolorinic acid and versicolorin A and C) as well as orsellinic acid were detected at high levels. The strains of *A. protuberus* (also belonging to *Aspergillus* section *Versicolores*) differed from the other species by the lack of aspergamid, deoxybrevianamid E, methoxysterigmatocystin, tryprostatin B production.
*A. penicillioide*s' strains were characterized by the production of asperglaucide, while the strain of *A. vitricola* produced only the unspecific metabolites brevianamid F and cyclo(L-Pro-L-Tyr) (data not shown). This low production was also observed for the strains of *C. cladosporioides*, and only the presence of emodin was reported.
The species belonging to the genus *Eurotium* were characterized by the production of neoechinulin A. Moreover, for *E. halophilicum*, other metabolites were detected, such as chaetoviridin A, deoxybrevianamid E, pseurotin A, pseurotin D, rugulusovin, stachybotryamide and tryprostatin B.
The strains of *P. chrysogenum* produced andrastin A and B, meleagrin, roquefortine C, secalonic acid D, as well as chrysogin, fulvic acid, neoxaline, oxaline and roquefortine D. For one strain (*P. chrysogenum* MUT 4593) the production of citreorosein, demethyl sulochrin and emodin was recorded as well. Finally, for the strain of *P. brevicompactum*, deoxybrevianamid E, emodin, mycophenolic acid and orsenillic acid were identified.

Table 7 Secondary metabolites produced by the selected fungal species.

Metabolite	AC	AJ	APE	APR	CC	EC	EH	PB	PC
Andrastin A									X
Andrastin B									X
Aspergamid A	X* (80%)	X							
Asperglaucide			X						

Averantin	X	X		X					
Averufanin	X	X		X					
Averufin	X	X		X					
Chaetoviridin A							X		
Chrysogin									X
Citreorosein	X* (60%)	X	X* (75%)	X* (50%)				X	X* (50%)
Demethylsulochrin									X* (50%)
Deoxybrevianamid E	X	X	X* (25%)				X	X	
Emodin	X	X		X	X* (50%)	X		X	X* (50%)
Fulvic acid									X
Meleagrin									X
Methoxysterigmatocystin	X	X							
Mycophenolic acid								X	
Neoechinulin A						X	X		
Neoxaline									X
Nidurufin	X	X		X					
Norsolorinic acid	X	X		X					
Orsellinic acid	X	X		X				X	
Oxaline									X
Pseurotin A							X* (33%)		
Pseurotin D							X* (33%)		
Roquefortine C									X
Roquefortine D									X
Rugulusovin							X		
Secalonic acid D									X
Stachybotriamide							X		
Sterigmatocystin	X	X		X					
Sydonic acid	X* (40%)	X		X					
Tenellin							X* (33%)		
Tryprostatin B	X	X					X	X	
Versicolorin A	X	X		X					
Versicolorin C	X	X		X					

*: Positive fraction metabolite production for the strains of the same fungal species, (%).
AC: *A. creber*; AJ: *A. jensenii*; APE: *A. penicillioides*; APR: *A. protuberus*; AV: *A. vitricola*; CC: *C. cladosporioides*; EC: *E. chevalieri*; EH: *E. halophilicum*; PB: *P. brevicompactum*; PC: *P. chrysogenum*.

3.2 Metabolite patters in dust samples

From dust samples, 46 different microbial metabolites were detected (Table 4), including 25 metabolites recorded for the fungal cultures, 19 compounds attributed to other fungal species, one bacterial metabolite and one plant toxin. In particular, metabolites of the following microbial taxa, *Alternaria* (alternariolmethylether, altersetin, macrosporin), *Ascochyta* (ascochlorin), *Aspergillus* (aspergamid A, asperglaucide, averufanin, sterygmatocystin), *Beaveria* (beauverucin, tenellin), *Chaetomiun* (chaetomin), *Fusarium* (enniatin B, monocerin), *Mycosphaerella* (usnic acid), *Penicillium* (andrastin A and B, cyclopenol, meleagrin, skirin, o-methylviridicatin, viridicatin, viridicatol), *Stachybotrys* (stachybotryamide, stachybotrylactam), *Trichoderma* (alamethicin, chrysophanol) were detected, while the other metabolites could be attributed to more than one genus of indoor fungi (Laatsch, 2005). Only 21 metabolites were found in all the sampled areas and, overall, the most abundant were asperglaucide (58%), alamethicin (13%), andrastin A (10%), mainly belonging to *Aspergillus* spp., *Trichoderma* spp. and *Penicillium* spp. Moreover, area 2 was also intensely characterized by the presence of terrecyclic acid, typically produced by *A. terreus* (Laatsch, 2005). The sampling area 4, corresponding to the entrance of the repository, presented the broadest fungal metabolite spectrum with 39 different compounds produced by at least 12 different fungal genera, while area 1, corresponding to the highest fungal contamination (Micheluz *et al*, 2015) was characterized by 34 different metabolites, belonging to 11 different fungal genera. Finally, the samplings carried out between shelves corresponding to the areas 3 and in the corridor called area 2 had recorded 32 and 27 different metabolites respectively, and ascribable to 10 different fungal genera (Laatsch, 2005). Generally mycotoxin similarities were found among all the repository's areas. In particular, area 4 was recognised to have the lowest level, $d^{BCS} = 0.26$, if compared with areas 2 and the highest level, $d^{BCS} = 0.62$, if compared with area 3.

Table 8 Secondary microbial metabolites detected in settled dust samples from the four areas inside the contaminated repository. Quantification values are average of replicates.

Metabolite	**Area 1 µg/kg (±STD)**	**Area 2 µg/kg (±STD)**	**Area 3 µg/kg (±STD)**	**Area 4 µg/kg (±STD)**
Alamethicin	3368.0 (±273.0)	1924.1 (±417.6)	229.2 (±381.4)	3.6 (±0.7)
Alternariolmethylether	4.3 (±0.5)	-	1.8 (±0.5)	2.1 (±0.5)
Altersetin	524.4 (±90.3)	53.4 (±10.3)	32.1 (±15.6)	3.7 (±7.5)
Andrastin A	3372.4 (±338.6)	186.0 (±372.0)	436.5 (±361.4)	66.7 (±133.3)
Andrastin B	16.1 (±5.8)	-	0.2 (±0.4)	-
Ascochlorin	-	-	1.6 (±0.4)	10.4 (±4.0)
Asperglaucide	2273.7 (±292.9)	14562.7 (±48)	2688.0 (±627.3)	5404.4 (±103.2)
Averufanin	-	-	0.1 (±0.3)	0.5 (±0.3)
Averufin	2.5 (±0.3)	9.7 (±0.7)	0.9 (±0.1)	3.9 (±1.1)
Beauvericin	-	0.8 (±1.5)	-	-
Chetomin	-	23.8 (±41.2)	-	15.4 (±10.3)
Chloramphenicol	1.2 (±0.0)	44.4 (±4.6)	-	7.2 (±1.0)
Chrysophanol	2.7 (±5.5)	13.7 (±27.3)	44.6 (±23.4)	30.3 (±12.4)
Citreorosein	15.1 (±7.0)	-	4.0 (±8.0)	21.5 (±5.2)
Cyclopenol	43.2 (±7.8)	-	2.9 (±5.7)	7.3 (±3.0)
Emodin	11.0 (±2.1)	15.2 (±0.8)	20.8 (±21.2)	16.6 (±1.4)
Enniatin B	0.3 (±0.1)	0.3 (±0.2)	-	0.1 (±0.0)
Ilicicolin B	-	9.0 (±6.4)	0.5 (±1.0)	15.9 (±1.6)
Lotaustralin	3.6 (±0.6)	-	6.4 (±1.0)	5.1 (±1.4)
Macrosporin	6.1 (±1.1)	-	4.9 (±0.8)	4.5 (±0.7)
Meleagrin	102.4 (±16.3)	130.5 (±18.6)	0.6 (±1.3)	18.9 (±8.5)

Methoxysterigmatocystin	5.2 (±0.4)	4.8 (±0.0)	5.7 (±2.1)	27.0 (±1.9)
Monocerin	1.6 (±0.4)	-	2.8 (±0.3)	5.6 (±0.4)
Neoechinulin A	1184.3 (±112.8)	59.0 (±9.9)	42.5 (±14.9)	28.7 (±2.8)
Neoxaline	1.3 (±0.1)	3.2 (±0.3)	0.5 (±0.2)	0.7 (±0.5)
Nidurufin	-	-	-	1.1 (±0.3)
O-Methylviridicatin	4.3 (±0.7)	-	0.7 (±0.3)	4.7 (±0.5)
Physcion	203.9 (±145.0)	-	146.3 (±47.5)	232.5 (±105.3)
Quinocitrinine A	-	-	0.3 (±0.3)	0.5 (±0.5)
Roquefortine C	16.3 (±1.9)	-	-	-
Rugulusovin	4.7 (±1.5)	8.5 (±1.2)	8.6 (±0.9)	4.3 (±0.3)
Skyrin	4.6 (±1.2)	-	-	0.6 (±1.2)
Stachybotryamide	1.4 (±0.0)	60.5 (±5.6)	-	2.0 (±0.7)
Stachybotrylactam	1.4 (±0.3)	93.2 (±12.3)	1.0 (±0.3)	14.2 (±1.2)
Sterigmatocystin	17.4 (±0.4)	6.1 (±1.5)	2.1 (±0.2)	12.8 (±1.0)
Sydonic acid	-	-	0.9 (±1.7)	53.5 (±4.2)
Tenellin	38.1 (±11.6)	545.5 (±326.1)	-	17.3 (±2.2)
Terrecyclic acid	-	3149.3 (±241.6)	-	-
Usnic acid	13.9 (±2.0)	26.7 (±8.6)	26.7 (±10.6)	6.4 (±3.1)
Viridicatin	3.8 (±1.2)	26.7 (±14.1)	0.5 (±1.0)	3.2 (±2.0)
Viridicatol	116.3 (±24.5)	40.8 (±30.0)	-	11.1 (±22.3)

4. Discussion

The analysis of 22 strains belonging to 10 selected fungal species produced a wide range of secondary metabolites, confirming the metabolite pattern for those already well-known (Nielsen, 2003) and improving information for the less-known fungi. The cultivation onto proper media have, in fact, let them the opportunity to produce the maximal range of metabolites and the results are usefully as first step to understand their potential abilities in real environments. As reported by Micheluz *et al*. (2015), *A. creber* was the most prevalent fungal species and because of its recent identification as separated species from *A. versicolor* (Jurievic *et al*., 2012), its mycotoxin production needed to be study in more details. This species, together with *A. jensenii* and *A. protuberus*, showed the capacity to produce sterigmatocystin, comparable with results of other authors (Engelhart *et al*. 2001; Jurievic *et al*. 2013; Nielsen et al. 1999). This metabolite is a biochemical precursor in the biosynthesis of aflatoxins, showing weak toxicity, mutagenicity, carcinogenicity and citotoxity in both *in vitro* and *in vivo* studies and it has been recognized as a 2B carcinogen by the International Agency for Research on Cancer (Bloom *et al*., 2007; Jurievic *et al*., 2013; Nielsen, 2003; Tuomi *et al*., 2000). Methoxysterigmatocystin were also detected for these species, except for the strains of *A. protuberus*. This metabolite is produced by *Aspergillus* spp. known to produce sterigmatocystin, as *A. versicolor* and *Emericella nidulans*. However, very few studies have addressed methoxysterigmatocystin, mostly concerned contamination by *A. versicolor* (Nielsen *et al*., 1999). Other typical metabolites of *A. versicolor*, such as averantin, averufanin, averufin, nidurofin, norsolorinic acid and versicolorin A and C, were detected in all the three species belonging to the *Aspergillus* section *Versicolores*. Moreover, sydonic acid, typical of A. *sydowii*, aspergamid A, typical metabolite of *A. ochraceus* (fungal species that belongs to *Aspergillus* section *Circumdati*) and unspecific metabolites as citreorosein, deoxybrevianamid E and emodin were identified (Laatsch, 2005).

Only the presence of the alkaloid asperglaucide characterized the samples of. *A. penicillioides*, which, as the other species belong to *Aspergillus* section *Resctricti*, i.e. *A. restrictus* and *A. vitricola*, is considered a stress selected species, with a poor production of secondary metabolites (Frisvad, 2015; Itabashi et al., 2006; Peterson, 2008; Tamura et al., 1999). For *A. vitricola*, in fact,

no records were detected, as well as for *C. cladosporioides*, that only a sporadically presence of emodin was emerged.
All the *Eurotium* strains showed the production of the typical metabolite neoechinulin A (Butinar *et al*., 2005; Slack *et al*., 2009). About *E. halophilicum* strains, some of the samples were characterized by the production of pseurotin A and D and tryprostatin B, already known as typical metabolite of *A. fumigatus* (Huang *et al*., 2010), as well as deoxybrevianamid E, rugulosovin and tenellin. Finally, two compounds typically belonging to other fungal species were pointed out: chaetoviridin A from *Chaetomium* sp. and stachybotryamide from *Stachybotrys* sp. (Laatsch, 2005). For these two compounds, all related MRM transitions exhibited peaks with the correct intensity ratios and a small, but yet significant, difference in retention time compared to the authentic standards.
Another fungal species frequently isolated from the environment studied was *P. chrysogenum*, well known as common indoor airborne fungus (Nielsen, 2003). Typical metabolites identified were chrysogin, demethylsulochrin, meleagrin, roquefortine C, roquefortine D and secalonic acid D, as reported by many authors (Frisvad *et al.*, 2004; Gutarowska *et al.*, 2014; Nielsen, 2003). The production of andrastin A, andrastin B, citreorosein and fulvic acid are typical for *Penicillium* spp., while oxaline was previously signalled only by *P. atramentosum, P. crustosum*, *P. glandicola* and *P. oxalicum* as well as neoxaline, which is typical for *P. atramentosum, P. coprobium* and *P. tulipae* (Frisvad *et al*., 2004; Laatsch, 2005).
As considers *P. brevicompactum*, prevalent production of mycophenolic acid was detected, with lower presences of citreorosein, deoxybrevianamid E, emodin and orsellinic acid.

Several toxic fungal metabolites were detected in settled, previously airborne, dust, which indicates the real potential of their inhalation exposure inside the repository. Most of them could be attribute to the fungal species analysed, but the hypothetical presence of other fungi not isolated from the indoor environment studied were pointed out. Generally, the results have emphasized the presence of the metabolite asperglaucide (2273.7 - 14562.7 μg/kg), potential indicative of xerophilic fungi as *A. penicillioides* and *A. restrictus*, but also plant’s metabolite (Itabashi *et al*., 2006; Wu *et al*., 2011). Anyway, the particular structural characteristics of the repository do not permit a big air exchange with the outside and the source of asperglaucide is more likely of fungal origin. Inside the repository, *A. penicillioides* was identified both as airborne fungus and book colonizer. Already in 1978, *A. penicillioides,* together with *E. halophilicum,* was associated with house-dust mites, emphasizing the possibility that it was responsible for allergic rhinitis and lunge disease (Samson and Lustgraaf, 1978). In several studies, this fungus was also isolated from dry food and indoor environment, such as museums and archives (Pitt and Hocking, 1997; Samson *et al*., 2004). In particular, *A. penicillioides* has often been isolated from old contaminated books and manuscripts (Michaelsen *et al*., 2010; Micheluz *et al*., 2015; Montanari *et al*., 2012).
Elevated concentrations of *Trichoderma viride* peptaibol alamethicin were also detected in the dust samples (3.6 - 3368.0 μg/kg), higher than those reported in similar studies concerned indoor environments (Täubel *et al*., 2011; Vishwanath *et al*., 2011). Micheluz and collaborators (2015) did not detect this species during the fungal analysis of the library, maybe due to specific low water activity medium used for the sampling, but the abundant presence of this typical metabolite in dust could be significant inside the library and for the preservation of book collections, especially because of the cellulolytic activity of *Trichoderma* sp. towards to textiles, paper and timber (Yang *et al*., 2007; Zyska, 1997). Moreover, *Trichoderma* sp. is a common indoor dust fungus, well-known to be implicate with damp and mouldy buildings and adverse human health effects (Larsen *et al*., 1996; Lignell *et al*., 2008; Nielsen *et al*., 2003).
The occurrence of typical indoor fungi *Penicillium* spp. was pointed out by high concentrations of andrastin A (66.7 - 3372.4 μg/kg) whereas other typical *Penicillium* metabolites, as cyclopenol (2.9 - 43.2 μg/kg), quinocitrinine A (0.3 – 0.5 μg/kg) and skirin (0.6 – 4.6 μg/kg) (Laatsch, 2005).

High concentrations of neochinulin A were probably indicative for the presence of *Eurotium* spp., with concentration of 28.7 µg/kg in area 4 and up to 1184.3 µg/kg in area 1. This data corroborates with the previous findings of Micheluz *et al.* (2015).
Specifically related to area 2, high concentrations of terrecyclic acid, a typical metabolite of *A. terreus*, were detected (3149.3 µg/kg). This sesquiterpene metabolite was recognized as possessor of broad-spectrum antimicrobial and antitumor activity, and the fungus is normally used against plant pathogens (Hyder *et al*., 2009; Turbyville *et al*., 2005). Its presence was pointed out also in house-dust samples as well as deteriorated books and manuscripts (Abdel-Hafez *et al*., 1990; Michaelsen *et al*., 2010) but not by Micheluz *et al*. (2015). Another metabolite found in all the areas with the exception of area 2 was physcion (146.3 - 232.5 µg/kg). As chrysophanol (2.7 - 44.6 µg/kg) and emodin (11.0 - 20.8 µg/kg), this metabolite is an antraquinone derivate, produced by fungi, such as *Aspergillus* spp. and *Eurotium* spp., as well as derived from plants (Laatsch, 2005).
The concentrations of methoxysterigmatocystin (4.8 – 27.0 µg/kg) and sterigmatocystin (2.1 – 17.4 µg/kg) in the dust samples were comparable with studies concerned severe moisture damage/dampness problems (Bloom *et al*., 2007; Engelhart *et al*., 2002), while small amounts of stachybotryamide (0.5 – 4.1 µg/kg) and stachybotrylactam (1.1 µg/kg, only for area 4), belonging to *Stachybotrys chartarum*, were detected in dust samples. The occurrence of this fungus in the dust, not detected by Micheluz *et al*. (2015), could be possible because it was frequently isolated from house-dust, as well as building materials. Furthermore, *S. chartarum* is well-known as strong cellulolytic species and great secondary metabolite producer, such as highly toxic macrocyclic trichothecenes (Bloom *et al*., 2007; Li *et al*., 2005; Nielsen, 2003).
Several other metabolites belonging to fungi not analysed in this study were detected in the dust samples, even if in low concentrations. Alternariolmethylether (1.8 – 4.3 µg/kg), altersetin (3.7 - 524.4 µg/kg) and macrosporin (4.5 – 6.1 µg/kg), for example, are *Alternaria* spp.'s metabolites, while enniatin B (0.1 – 0.3 µg/kg), ilicicolin B (0.5 – 15.9 µg/kg) and monocerin (1.6 – 5.6 µg/kg) are typical for *Fusarium* spp. and chetomin (15.4 – 23.8 µg/kg) for *Chaetomiun* spp. (Laatsch, 2005). These fungal species are common outdoor fungi but they were often isolated also from indoor environments on books and documents (Gallo, 1993; Pitt and Hocking, 2009; Samson *et al*., 2004; Zyska, 1997). Metabolites as usnic acid (6.4 – 26.7 µg/kg), beaverucin (0.8 µg/kg) and ascochlorin (1.6 – 10.4 µg/kg) may come from to outdoor organisms and fungi, such as lichens or *Mycosphaerella nawa*, *Beauveria bassiana*, and *Ascochyta vicinae* respectively (Laatsch, 2005).
As bacterial metabolite, only chloramphenicole (1.2 – 44.4 µg/kg) was detected, especially in corresponding of area 2. This metabolite probably derives from *Streptomyces* spp., Gram-positive bacteria that commonly occur in the soil, but also in indoor environments with strong problems of moisture damages and dampness (Rintala *et al*., 2004).
The analysis of settled dust samples increased the knowledge about the microbial community inside the repository and, consequently, given more information to conservators about proper preservation and management of book collections.
Finally, it remains to determine the level of mycotoxins in native bioaerosol in order to estimate the real health hazards for librarians. Regular dusting operation and frequent inspections should be ensured, to prevent microbiological contamination and ensuring healthy and safe working environments.

Supplementary material

Table S1 Secondary metabolite concentrations produced by selected fungal strains on Yeast Extract-Sucrose agar (YES), Czapeck-Yeast Autolysate agar (CYA), Yeast Extract-Sucrose agar with 15% of NaCl (YES15%) and Malt Extract Agar with 15% of NaCl (MEA15%). Results are reported as ng/cm^2 of agar disk.

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References

Abdel-Hafez, S.I.I., Moubasher, A.H. and Barakat, A., 1990. Keratinophilic fungi and other moulds associated with air-dust particles from Egypt. Folia Microbiologica 35: 311-325.

Apetrei, I.C., Drăgănescu, G.E., Popescu, I.T., Carp-Cărare, C., Guguianu, E., Mihăescu, T., Ştefanache, A., Creţu, C. and Patraş, X., 2009. Possible cause of allergy for the librarians: books manipulation and ventilation as sources of fungus spores spreading. Aerobiologia 25: 159-166.

Bloom, E., Bal, K., Nyman, E., Must, A. and Larsson, L., 2007. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. Applied and Environmental Microbiology 73: 4211-4217.

Borrego, S., Guiamet, P., Gomez de Saravia, S., Batistini, P., Garcia, M., Lavin, P. and Perdomo, I., 2010. The quality of air at archives and the biodeterioration of photographs. International Biodeterioration & Biodegradation 64: 139-145.

Butinar, L., Zalar, P., Frisvad, J.C. and Gunde-Cimerman, N., 2004. The genus *Eurotium* – members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiology Ecology 51: 155-166.

Christensen, C.M., Papavizas, G.C. and Benjamin, C.R., 1959. A new halophilic species of *Eurotium*. Mycologia 51: 636-640.

D-Lgs. 9 aprile, n.81. Attuazione dell'articolo 1 della legge 3 agosto 2007, n.123, in materia di tutela della salute e della sicurezza nei luoghi di lavoro. Gazzetta Ufficiale n.101, 30 aprile 2008 – Supplemento Ordinario n.108 (ex D.L. 626/94).

Engelhart, S., Loock, A., Skutlarek, D., Sagunski, H., Lommel, A., Färber, H. and Exner, M., 2002. Occurrence of toxigenic *Aspergillus versicolor* tsolates and sterigmatocystin in carpet dust from damp indoor environments. Applied and Environmental Microbiology 68: 3886-3890.

European Commission (EC), 2000. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal of the European Union L 262, 21.45.

Fischer, G., Müller, T., Schwalbe, R., Ostrowsky, R. and Dott, W. (2000) Species-specific profiles of mycotoxins produced in cultures and associated with conidia of airborne fungi derived from biowaste, *Int. J. Hyg. Envir. Heal.*, 203, 105-116.

Fischer, G. and Dott, W., 2003. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Archives of Microbiology 179: 75-82.

Flannigan, B. and Miller, J.D., 2011. Microbial growth in indoor environments. In: Flannigan, B., Samson R.A. and Miller, D. (eds) Microorganisms in Home and Indoor Work Environments. Diversity, Health Impacts, Investigation and Control, 2th Edition, CRC Press, London, UK, pp. 57-108.

Frisvad, J.C, Smedsgaard, J., Larsen, T.O. and Samson, R.A., 2004. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Studies in Mycology 49: 201-2041.

Frisvad, J.C., 2015. Taxonomy, chemodisversity and chemoconsistency of Aspergillus, Penicillium, and Talaromyces species. Frontiers in Microbiology 5: 1-7.

Gallo, F., 1993. Aerobiological research and problems in libraries. Aerobiologia 9: 117-130.

Gutarowska, B., Skora, J., Stepien, L., Twarizel, M., Blajet-Kosicka, A., Otlewska, A. and Grajewski, J., 2014. Estimation of fungal contamination and mycotoxin production at workplaces in composting plants, tanneries, archives and libraries. World Mycotoxin Journal 7: 345-355.

Huang, R., Zhiu, X., Xu, T., Yang, X. and Liu, Y., 2010. Diketopiperazines from marine organisms. Chemistry & Biodiversity 7: 2809-2829.

Hyder, N., Sims, J.J. and Wegulo, S.N. (2009) In vitro suppression of soilborne plant pathogens by coir, *HortTechnology*, 19, 96-100.

Itabashi, T., Matsuishi, N., Hosoe, T., Toyazaki, N., Udagawa, S., Imai, T., Adachi, M. and Kawai, K., 2006. Two new dioxopiperzine derivates, arestrictina A and B, isolated from *Aspergillus restrictus* and *Aspergillus penicillioides*. Chemical and Pharmaceutical Bulletin 54: 1639-1641.

Jurjevic, Z., Peterson, S.W. and Horn, B.W., 2012. *Aspergillus* section *Versicolores*: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 3: 59-79.

Jurievic, Z., Peterson, W.S., Solfrizzo, M. and Peraica, M., 2013. Sterigmatocystin production by nine newly described *Aspergillus* species in section *Versicolores* grown on two different media. Mycotoxin Research 29: 141-145.

Karbowska-Berent, J., Górny, R.L., Strzelczyk, A.B. and Wlazlo, A., 2011. Airborne and dust borne microorganisms in selected Polish libraries and archives. Building and Environment 46: 1872-1879.

Laatsch, H., 2005. AntiBase 2005 (software). John Wiley & Sons Hoboken, New Jersey, ISBN 3-527-31721-X.

Larsen, F.O., Clementsen, P., Hansen, M., MaltbæK, N., Gravesen, S., Stahl Skov, P. and Norn, S., 1996. The indoor microfungus *Trichoderma viride* potentiates histamine release from human bronchoalveolar cells. APMIS 104: 673-679.

Li, D.-W. and Yang, C.S., 2005. Taxonomic history and current status of *Stachybotrys chartarum* and related species. Indoor Air 15: 5-10.

Lignell, U., Meklin, T., Rintala, H., Hyvärinen, A., Vepsalainen, A., Pekkanen, J. and Nevalainen, A. (2008) Evaluation of quantitative PCR and culture methods for detection of house dust fungi and streptomycetes in relation to moisture damage of the house, *Lett. Appl. Microbiol.*, 47, 303-308.

Maggi, O., Persiani, A.M., Gallo, F., Valenti, P., Pasquariello, G., Sclocchi, M.C. and Scorrano, M., 2000. Airborne fungal spores in dust present in archives: proposal for a detection methods, new for archival materials. Aerobiologia 16: 429-434.

Malachová, A., Sulyok, M., Beltrán E., Berthiller F. and Krska, R., 2014. Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometry method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. Journal of Chromatography A 1362: 145-156.

MIBAC, 2001. Atto d'indirizzo sui criteri tecnico-scientifici e sugli standard di funzionamento e sviluppo dei musei, D.Lgs n.112/98 art.150 comma 6.

Michaelsen, A., Pinar, G. and Pinzari., F., 2010. Molecular and microscopical investigation of the microflora inhabiting a deteriorated Italian manuscript dated from the thirteen century. Microbial Ecology 60: 69-80.

Micheluz, A. Manente, S., Tigini, V., Prigione, V., Pinzari, F., Ravagnan, G. and Varese, G.C., 2015. The extreme environment of a library: Xerophilic fungi inhabiting indoor niches. International Biodeterioration & Biodegradation 99: 1-7.

Miller, J.D. and McMullin, D.R., 2014. Fungal secondary metabolites as harmful indoor air contaminants: 10 years on. Applied Microbiology and Biotechnology 98: 9953-9966.

Montanari, M., Melloni, V., Pinzari, F. and Innocenti, G., 2012. Fungal biodeterioration of historical library materials stored in compactus movable shelves. International Biodeterioration & Biodegradation 75: 83-88.

Nielsen, K.F., 2003. Mycotoxin production by indoor moulds. Fungal Genetics and Biology 39: 103-117.

Nielsen, F.K., Gravesen, S., Nielsen, P.A., Andersen, B., Thrane, U. and Frisvad, J.C., 1999. Production of mycotoxin on artificially and naturally infested building materials. Mycopathologia 145: 43-56.

Nunes, I., Mesquita, N., Cabo Verde, S., Leitão Bandeira, A.M., Carolino, M.M., Portugal, A. and Botelho, M.L.. 2013. Characterization of an airborne microbial community: A case study in the archive of the University of Coimbra, Portugal. International Biodeterioration & Biodegradation 79: 36-41.

Pasquarella, C., Saccani., Sansebastiano, G.E., Ugolotti, M., Pasquariello, G. and Albertini, R., 2012. Proposal for a biological environmental monitoring approach to be used in libraries and archives. Annals of Agricultural and Environmental Medicine 19: 209-212.

Peterson, S.W., 2008. Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. Mycologia 100: 205-226.

Pitt, J.I. and Hocking, A.D., 2009. Fungi and food spoilage. Springer. New York, US.

Rintala, H., Hyvärinen, A., Paulin, L. and Nevalainen, A., 2004. Detection of Streptomyces in house dust – comparison of culture and PCR methods. Indoor Air 14: 112-119.

Samson. R.A. and Lustgraaf, B.V.D., 1978. *Aspergillus penicilloides* and *Eurotium halophilicum* in association with house- dust mites. Mycopathologia 64: 13-16.

Samson, R.A., Hoekstra, E.S. and Frisvad, J.C., 2004. Introduction to Food and Airborne Fungi. ASM Press, US.

Slack, G.j., Puniani, E., Frisvad, J., Samson, R.A. and Miller, J.D., 2009. Secondary metabolites from *Eurotium* species, *Aspergillus calidoustus* and *A. insuetus* common in Canadian homes with a review of their chemistry and biological activities. Mycological Research 113: 480-490.

Tamura, M., Kawasaki, H. and Sugiyama, J., 1999. Identify of the xerophilic species Aspergillus penicillioides: integrated analysis of the genotypic and phenotypic characters. The journal of general and Applied Microbiology 45: 29-37.

Täubel, M., Sulyok, M., Vishwanath, V., Bloom, E., Turunen, M., Järvi, K., Kauhanen, E., Krska, R., Hyvärinen, A., Larsson, L. and Nevalainen, A., 2011. Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments. Indoor air 21: 368-375.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E-L., Lindroos, O., Kalso, S., Koukila-Kähkölä, P., Mussalo-Rauhamaa, H. and Gaahtela, T., 2000. Mycotoxins in crude building materials from water-damaged buildings. Applied and Environmental Microbiology 66: 1899-1904.

Turbyville, T.J., Wijeratne, E.M., Whitesell, L. and Gunatilaka, A.A., 2005. The anticancer activity of the fungal metabolite terrecyclic acid A is associated with modulation of multiple cellular stress response pathways. Molecular Cancer Therapeutics 4: 1569-76.

Vishwanath, V., Sulyok, M., Weingart, G., Kluger, B., Täubel, M., Mayer, S., Schuhmacher, R. and Krska, R., 2011. Evaluation of settled floor dust for the presence of microbial metabolites and volatile anthropogenic chemicals in indoor environments by LC-MS/MS and GC-MS methods. Talanta 85: 2027-2038.

WHO Regional Office for Europe, 2009. WHO Guidelines for indoor air quality: dampness and mould. World Regional Office for Europe, Copenhagen, Denmark.

Wu, B., Wang, K. and Wu, X., 2011. A new phenolic diglycoside produced in responde to copper toxicity and a new flavan dimer from the leaves of *Viburnum ichangense* (Hemsl.) Rehd. Helvetica Chimica Acta 94: 1677-1684.

Yang, C.S. and Li, D.-W., 2007. Ecology of fungi in the indoor environment. In: Yang C.S. and Heinsohn P. (eds), Sampling and Analysis of Indoor Microorganisms, John Wiley, New York, US, pp. 191-214.

Zielińska-Jankiewicz, K., Kozajda, A., Piotrowska, M. and Szadkowska-Stańczyk, I., 2008. Microbiological contamination with moulds in work environment in libraries and archive storage facilities. Annals of Agriccultural and Environmental Medicine 15: 71-78.

Zyska, B., 1997. Fungi isolated from Library Materials: a Review of the Literature. International Biodeterioration & Biodegradation 40: 43-51.

Supplementary material

Fungal secondary metabolite analysis applied to Cultural Heritage: the case of a contaminated library in Venice

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Table S1. Secondary metabolite concentrations produced by selected fungal strains on Yeast Extract-Sucrose agar (YES), Czapeck-Yeast Autolysate agar (CYA), Yeast Extract-Sucrose agar with 15% of NaCl (YES15%) and Malt Extract Agar with 15% of NaCl (MEA15%). Results are reported as ng/cm^2 of agar disk.

Fungal species*	**Fungal strain**	**Metabolites on YES (ng/cm^2 agar)**	**Metabolites on CYA (ng/cm^2 agar)**
Aspergillus creber	MUT 470	ASP-A (46.2), AVE1 (4805.6), AVE2 (88.5), CIT (48.0), EMO (7.0), MET (9213.8), NID (1474.5), NOR (1342.0), ORS (2259.1), STE (25841.8), VER-A (2937.8)	AVE1 (549.8), AVE2 (28.1), AVE3 (1700.7), DEO (44.1), EMO (1.3), MET (19972.4), NID (167.3), NOR (104.0), ORS (408.5), STE (3154.8), VER-A (425.4)
A. creber	MUT (41)**	AVE1 (1029.2), AVE2 (38.2), AVE3 (1631.4), CIT (10.0), EMO (3.3), MET (3999.6), NID (186.0), NOR (185.5), ORS (627.5), STE (4056.6), VER-A (307.6)	ASP-A (84.4), AVE1 (961.9), AVE2 (34.0), AVE3 (1735.4), DEO (38.7), EMO (1.3), MET (22988.6), NID (262.1), NOR (165.5), ORS (327.3), STE (5836.8), VER-A (680.9)
A. creber	MUT 5527	ASP-A (72.5), AVE1 (5081.7), AVE2 (111.4), CIT (63.4), DEO-E (29.1), EMO (10.8), MET (10984.8), NID (1726.2), NOR (1039.4), ORS (3472.8), STE (23814.0), VER-A (2251.0)	AVE1 (1065.9), AVE2 (42.9), AVE3 (1776.1), DEO (51.9), EMO (1.5), MET (5291.6), NID (383.6), NOR (152.6), ORS (488.2), STE (7077.0), VER-A (861.9)
A. creber	MUT (43)**	AVE1 (102.7), AVE2 (2.4), AVE3 (46.6), MET (18.6), NID (1.2), NOR (43.9), ORS (3373.9), STE (259.6), SYD (1153.5), VER-A (2.7)	AVE1 (1617.2), AVE2 (29.4), AVE3 (1438.8), DEO-E (9.5), EMO (0.6), MET (24425.4), NID (86.5), NOR (777.0), ORS (138.8), STE (11586.0), SYD (278.9), VER-A (606.1)
A. creber	MUT (44)**	AVE1 (1315.5), AVE2 (16.3), AVE3 (840.4), DEO-E (50.7), EMO (1.1), MET (623.4), NID (53.6), NOR (1741.5), ORS (1441.9), STE (8325.2), SYD (58.9), VER-A (180.0)	AVE1 (659.5), AVE2 (14.1), AVE3 (1110.7), DEO (61.4), EMO (0.4), MET (4033.2), NID (41.6), NOR (261.3), ORS (278.7), STE (1891.3), SYD (1293.1), VER-A (248.8)
A. jensenii	MUT 480	AVE1 (4659.9), AVE2 (61.8), AVE3 (1773.1), CIT (80.2), DEO-E (474.3), EMO (12.2), MET (748.4), NID (268.2), NOR (1423.5), ORS (2569.9), STE (26881.2), SYD (212.1), VER-A (980.2)	AVE1 (329.7), AVE2 (8.9), AVE3 (347.7), DEO-E (26.2), EMO (1.0), MET (14581.9), NID (27.6), NOR (64.1), ORS (487.3), STE (1143.3), SYD (412.3), VER-A (111.8)
A. protuberus	MUT (45)**	AVE1 (2573.0), AVE2 (163.0), AVE3 (917.5), CIT (14.0), EMO (3.1), NID (119.6), NOR (2087.9), ORS (1599.8), SYD (56.1), VER-A (8419.0)	AVE1 (560.8), AVE2 (56.2), AVE3 (568.5), EMO (0.8), NID (25.9), NOR (516.4), ORS (1104.6), STE (29.2), SYD (337.9), VER-A (5744.1)

A. protuberus	MUT 5487	AVE1 (951.8), AVE2 (63.5), AVE3 (1632.4), NID (625.8), NOR (429.8), ORS (924.6), STE (4662.9), VER-A (1892.3)	AVE1 (3088.6), AVE2 (114.6), EMO (87.1), NID (1512.2), NOR (559.4), ORS (1325.7), STE (26269.8), SYD (6.5), VER-A (606.1)
Cladosporium cladosporioides	MUT 527	EMO (1.4)	-
Eurotium chevalieri	MUT 472	NEO-A (3560.4)	NEO-A (15631.5)
P. brevicompactum	MUT 536	CIT (76.7), DEO (11.6), EMO (2.0), MYC (85208.8), ORS (112497.6)	CIT (59.1), EMO (0.8), MYC (78045.2), ORS (361.2)
Penicillium chrysogenum	MUT 5493	AND-A (47088.0), AND-B (1029.2), CHR (72.6), CIT (474.0), DEM (561.6), EMO (133.5), FUL (70.2), MEL (39190.7), NEO (214.4), ROQ-C (2656.5), ROQ-D (336.4), SEC (171905.3)	AND-A (3987.3), AND-B (95.0), CHR (2774.7), CIT (309.3), DEM (183.1), EMO (126.2), MEL (39425.1), NEO (264.3), ROQ-C (6026.4), ROQ-D (145.6), SEC (159269.7)
P. chrysogenum	MUT 5492	AND-A (46884.2), AND-B (873.4), CHR (15.2), MEL (44978.7), NEO (139.0), OXA (2.8), ROQ-C (13868.6), ROQ-D (124.1), SEC (332.5)	AND-A (5247.9), AND-B (90.8), CHR (243.2), MEL (35593.7), NEO (45.4), OXA (4.1), ROQ-C (10882.9), ROQ-D (50.3), SEC (114.2)
Fungal species	**Fungal strain**	**Metabolites on YESNaCl (ng/cm^2 agar)**	**Metabolites on MEANaCl (ng/cm^2 agar)**
A. penicillioides	MUT 481	ASP (11412.8), CIT (24.2)	ASP (2767.6), CIT (24.2)
A. penicillioides	MUT 5694	ASP (11901.9), CIT (5.5)	ASP (11616.6), CIT (19.9)
A. penicillioides	MUT 5525	ASP (8118.4), DEO-E (22.9)	ASP (9585.7)
A. penicillioides	MUT 5537	ASP (4048.5), CIT (25.3)	ASP (6565.4), CIT (24.8)
E. halophilicum	MUT 482	DEO-E (20.38), NEO-A (2.8)	RUG (12.8)
E. halophilicum	MUT 5534	DEO-E (198.4), NEO-A (10.7), PSE-A (1369.5), PSE-D (1046.5)	RUG (5.5)
E. halophilicum	MUT 5535	DEO-E (183.6), NEO-A (6.2)	DEO-E (10.7), RUG (16.4)

*: Reported results are related only on positive secondary metabolite producers, therefore some *C. cladosporium* strains as well as all *A. vitricola* strain are not mentioned in the table.

**: Fungal strains that are still in acquisition in MUT collections.

AND-A: andrastin A; AND-B: andrastin B; ASP: asperglaucide; ASP-A: aspergamid A; AVE1: averantin; AVE2: averufanin; AVE3: averufin; CHR: chrysogin; CIT: citreorosein; DEM: demethylsulochrin; DEO-E: deoxybrevianamid E; EMO: emodin; FUL: fulvic acid; MEL: meleagrin; MET: methoxysterigmatocystin; MYC: mycophenolic acid; NEO: neoxaline; NEO-A: neoechinulin A; NID: nidurofin; NOR: norsolorinic acid; ORS: orsellinic acid; OXA: oxaline; PSE-A: pseurotin A; PSE-D; ROQ-C: roquefortine C; ROQ-D: roquefortine D; RUG: rugulusovin; SEC-D: secalonic acid D; STE: sterygmatocystin; SYD: sydonic acid; VER-A: versicolorin A; VIR1: viridicatin; VIR2: viridicatol.

APPENDIX

APPENDIX A

- Protocols, materials and methods -

Appendix A1

A - Protocols of indoor sampling

Protocol of the 1st sampling

The sampling is composed of two parts:

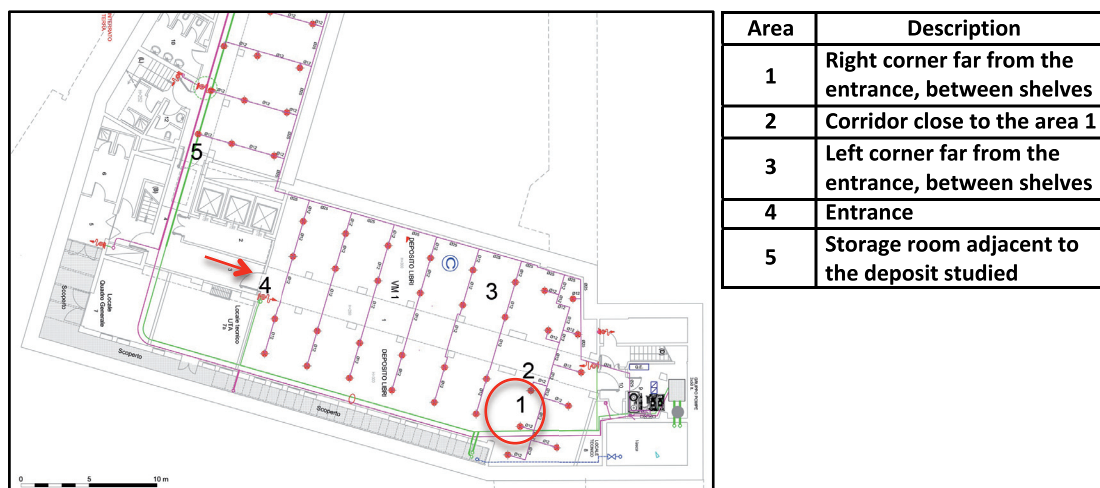
1) aerobiological sampling;
2) contaminated book's sampling.

1) Aerobiological sampling

- Make an inspection of the environment and fill the sampling environment form (Appendix A1 - B).
- Consider the right number of sampled areas representative of the *status quo* of the environment, both inside and outside of the repository. As reported in Fig. A1, 5 sampled are considered.
- Consider a selective culture medium, in order to capture mainly the xerophilic fraction of airborne microorganisms: Malt Extract Agar added with 15% of NaCl (MEA15%). The medium is added with 0.05 gL^{-1} of chloramphenicol in order to avoid bacterial growth.
- Perform the air sampling both in active and in passive mode.

  Carry out the active sampling by Sampl'air Lite using 9-cm Petri dishes in three replicates for each medium flow rate 100 Lmin-1 and sampled volumes 100 L and 500 L. Place the sampler 1.5 m above the floor, to represent the breathing zone of a standing person. Calculate the total numbers of colony-forming units (CFU) according to the conversion table provided by the supplier and present the results as CFU per cubic meter of air $CFUm^{-3}$.

  For passive sampling expose 9-cm Petri dishes to air particles for 1 h, 1 m above the floor and at a distance of about 1 m to obstacles to determine the *Index of Microbial Air Contamination* (IMA) in $CFUdm^{-2}h^{-1}$.



Area	Description
1	Right corner far from the entrance, between shelves
2	Corridor close to the area 1
3	Left corner far from the entrance, between shelves
4	Entrance
5	Storage room adjacent to the deposit studied

Figure A1 Sampled areas inside the repository. The row indicates the entrance while the circle is in correspondence of the high contaminated area 1, far from the entrance.

2) Book's sampling

- Select the contaminated books by visual inspection and fill the book sampling forms (Appendix A1 - C).
- Sample the contaminated books by:
- sterile cotton swab wiped over the spots which had developed on the spine of bindings and then inoculated in 9-cm petri dishes;
- sterile nitrocellulose membranes, gently pressed on the spots visible to the naked eye on the book's covers for 10 s and then transferred to Petri dishes;
- pieces (6 x 2 cm) of Fungi-Tape^{TM}, pressed over the spots to collect fungal structures and then deposited on sterile glass slide for microscopic observation;

- active sampling with Sampl'air Lite, with an air sampled volume of 100 L (flow rate 100 L min^{-1}), performed directly from the books' covers.
- As culture media test different low water activity media:
  - Malt Extract Agar added with 15% of NaCl (MEA15%);
  - Czapeck 70% sucrose agar (Cz70);
  - Potato dextrose added with 20% of NaCl agar (PDA20%).
- Label all Petri dishes and transfer them to the laboratory.
- Incubate all samples at 25 °C for 7-14 days.

Protocol of the 2nd sampling

The sampling is composed of four parts:

1) air sampling by canister;
2) aerobiological sampling;
3) contaminated book's sampling;
4) settled dust sampling.

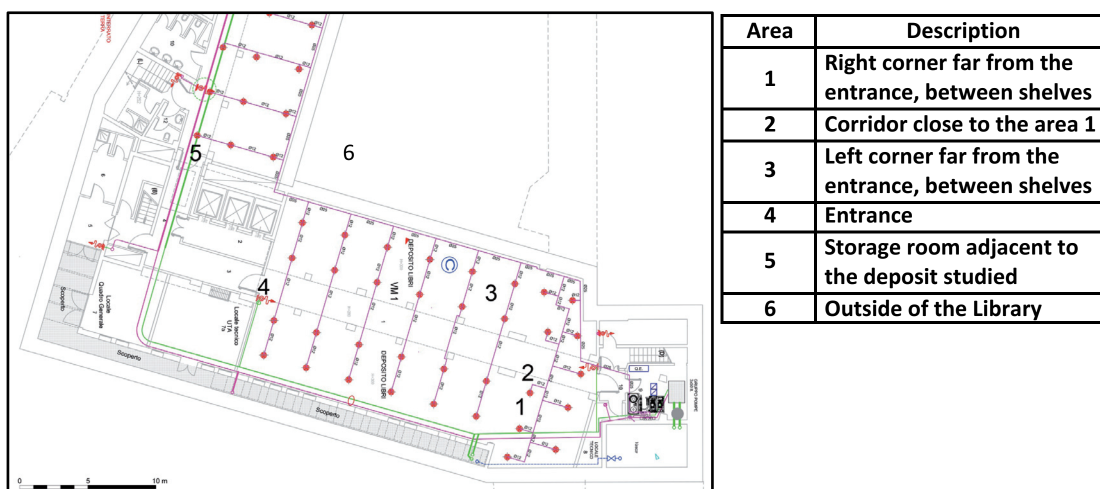
1) Air sampling by canister

- Collect the samples in correspondence of the six sampled areas (Fig. A2), opening the valves of the canister (sampled volume 3-6 L) until the inside pressure is arrived at 1 atm.
- Close the canister and transfer them to the laboratory for the GC-MS analysis.

2) Aerobiological sampling

- After a first sampling, consider 6 sampled areas inside and outside of the repository as reported in Fig. A2.
- Use several culture media, in order to capture all kind of airborne microorganisms, in particular:
  - Malt Extract Agar (MEA), incubated at 25 °C and 37 °C;
  - Malt Extract Agar added with 15% of NaCl (MEA15%);
  - Dichloran -glycerol agar base (DG18);
  - Plate Count Agar (PCA), incubated at 25 °C and 37 °C.
- All media are added with 0.05 gL^{-1} of chloramphenicol and 0.015 gL^{-1} of streptomycin in order to avoid bacterial growth with an exception of PCA medium that is added with 2% of miconazole in order to promote the only bacterial growth.
- Perform the air sampling only in active mode.

  Carry out the active sampling by Sampl'air Lite using 9-cm Petri dishes in three replicates for each medium, flow rate 100 $Lmin^{-1}$ and sampled volume 100 L. Place the sampler 1.5 m above the floor to represent the breathing zone of a standing person. Calculate the total numbers of colony-forming units (CFU) according to the conversion table provided by the supplier and present the results as CFU per cubic meter of air ($CFUm^{-3}$).



Area	Description
1	Right corner far from the entrance, between shelves
2	Corridor close to the area 1
3	Left corner far from the entrance, between shelves
4	Entrance
5	Storage room adjacent to the deposit studied
6	Outside of the Library

Figure A2 Sampled areas inside and outside the repository. The external area 6 is added to the other 5 sampling areas.

3) Book's sampling

- Select 10 contaminated books and 10 disinfected books and fill the book sampling forms (Appendix A2 - C).
- Sample all the books by:
  - sterile cotton swab, wiped over the spots which had developed on the spine of bindings and then inoculated in 9-cm petri dishes;
  - pieces (6 x 2 cm) of Fungi-Tape^{TM}, pressed over the spots to collect fungal structures and then deposited on sterile glass slide for microscopic observation.
- Test different cultivate media:
  - Malt Extract Agar (MEA);
  - Malt Extract Agar added with 15% of NaCl (MEA15%).
- Test each book for its water content by Aqua-Boy device, positioning the sensor on the book cover and between the pages in the middle of the book. Consider 3 records for each analysis.
- Label all Petri dishes and transfer then to the laboratory.
- Incubate all samples at 25 °C and 37 °C for 7-14 days.

4) Settled dust sampling

- Consider the right representative number of sampling areas. In this case, they correspond to areas 1, 2, 3 and 4 of air sampling (see Fig. A1).
- Sample the settled dust by a conventional households vacuum cleaner with a power level at least 780 W and new dust bags, for 2 $minm^{-2}$.
- Close the dust bags by plastic tape and transfer them to the laboratory.
- Store the dust samples at -20 °C until the extraction.

Protocol of the 3rd sampling

The sampling is composed of two parts:

1) air sampling by canister;
2) aerobiological sampling.

For the descriptions of the samplings, consult the points 1 and 2 of the previous protocol (Protocol of the 2nd sampling).

B - Format for indoor sampling

Ca' Foscari University of Venice

Sampling form for indoor environment characterization

DATE:……
POSITION:………..
………
BUILDING:………..
AIM OF SAMPLING:………..
………
DURATION OF SAMPLING:……………………………………………………………………………………………………
………
PRINCIPAL ACTIVITIES INSIDE THE ENVIRONMENT: ……………………………………………………………………
………
………
TIME OPENING: ……
………
TOTAL OR PARTIAL SAMPLING: ……………………………………………………………………………………………
………
ENVIRONMENT SIZE: ………
………
ARCHITECTURAL STRUCTURES: ……………………………………………………………………………………………
………
AREA ADJACENT TO OUSIDE: ………………………………………………………………………………………………
………
PRESENCE OF DOORS/WINDOWS: …………………………………………………………………………………………
………
PRESENCE OF TRANSITION AREAS: ………………………………………………………………………………………
………
CLIMATE SYSTEMS: ……
………
LIGHT: ………..
………
NOTE: ……
………
………
……….
………
………
………
………

C- Format for book sampling

Ca’ Foscari University of Venice

Sampling form for microbial characterization of book sample

REFERENCE:…………………………………………………………………….
OPERATOR: ……………………………………………………………………
DATE: ……………………………………………………………………………
LIBRARY: ………………………………………………………………………
DEPOSIT: ………………………………………………………………………
……………………………………………………………………………………….
BOOK TITLE: …………………………………………………………………
……………………………………………………………………………………….
BOOK DESCRIPTION: …………………………………………………….
……………………………………………………………………………………….
CATALOGING: ……………………………………………………………….
……………………………………………………………………………………….
SAMPLING AREAS: ………………………………………………………..
SAMPLING TECNIQUES:……………………………………………….
CULTURE MEDIUM: ………………………………………………………
INCUBATION TEMPERATURE: ……………………………………….
FUNGI-TAPE™: ……………………………………………………………..
AQUA-BOY: …………………………………………………………………

SKETCH

PETRI DISHES N°		CULTURE MEDIUM			FUNGI-TAPE™
Sampling	Colony	Number	Macro charact.	Identification	Description

Note: ………
……….
……….
……….
……….
……….

Appendix A2

Culture media used during the thesis project

Media for aerobiological sampling (used acronym in the text)

Malt Extract Agar (MEA). generic media for fungal growth

Glucose (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Malt extract (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Peptone (Fluka, Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Malt Extract Agar added with 15% NaCl (MEA15%)

Glucose (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Malt extract (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Peptone (Fluka, Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
Sodium chloride (MicroBiol, Cagliari, Italy)	150 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Nutrient Agar (NA) (Fluka, Sigma-Aldrich, Milan, Italy), generic medium for bacterial growth

Meat extract	1 g L^{-1}
Peptone	5 g L^{-1}
Sodium chloride	5 g L^{-1}
Yeast extract	2 g L^{-1}
Agar	15 g L^{-1}

Plate Count agar (PCA) (MicroBiol, Cagliari, Italy), generic medium for the total bacterial count

Glucose	1 g L^{-1}
Triptone	5 g L^{-1}
Yeast extract	2.5 g L^{-1}
Agar	15 g L^{-1}

Specific media for *Penicillium* identification (Samson and Frisvad, 2004).

Creatine sucrose agar (CREA)

Creatine*1H_2O (Sigma-Aldrich, Milan, Italy)	3 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	30 g L^{-1}
K_3PO_4*7H_2O (Sigma-Aldrich, Milan, Italy)	1.6 g L^{-1}
$MgSO_4$*7H_2O (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
KCl (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$FeSO_4$*7H_2O (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
$CuSO_4$*5H_2O (Sigma-Aldrich, Milan, Italy)	0.005 g L^{-1}
$ZnSO_4$*7H_2O (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Bromocresol purple (Fluka, Sigma-Aldrich, Milan, Italy)	0.05 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	15 g L^{-1}
Distilled water	
Final pH	8.0 ± 0.2

Czapeck Yeast Autolysate agar (CYA) (inoculated at 25 °C and 30 °C)

$NaNO_3$ (Sigma-Aldrich, Milan, Italy)	3 g L^{-1}
Yeast extract (Fluka, Sigma-Aldrich, Milan, Italy)	5 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	30 g L^{-1}
K_2HPO_4*3H_2O (Sigma-Aldrich, Milan, Italy)	1.3 g L^{-1}

$MgSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
KCl (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$FeSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
$CuSO_4*5H_2O$ (Sigma-Aldrich, Milan, Italy)	0.005 g L^{-1}
$ZnSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	15 g L^{-1}
Distilled water	
Final pH	6.3 ± 0.2

Malt Extract Autolysate agar (MEA Blakeslee)

Malt extract (Fluka, Sigma-Aldrich, Milan, Italy)	30 g L^{-1}
Bacterial peptone (Fluka, Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
Glucose (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
$CuSO_4*5H_2O$ (Sigma-Aldrich, Milan, Italy)	0.005 g L^{-1}
$ZnSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Distilled water	
Final pH	5.3 ± 0.3

Yeast extract sucrose agar (YES)

Yeast extract (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	150 g L^{-1}
$MgSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$CuSO_4*5H_2O$ (Sigma-Aldrich, Milan, Italy)	0.005 g L^{-1}
$ZnSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Specific media for *Eurotium halophilicum*

Czapeck added with 20% of sucrose (Cz20)

$NaNO_3$ (Sigma-Aldrich, Milan, Italy)	3 g L^{-1}
KH_2PO_4 (Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
$MgSO_4$ (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
KCl (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$FeSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	200 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Czapeck added with 40% of sucrose (Cz40)

$NaNO_3$ (Sigma-Aldrich, Milan, Italy)	3 g L^{-1}
KH_2PO_4 (Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
$MgSO_4$ (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
KCl (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$FeSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	400 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Czapeck added with 70% sucrose (Cz70)

$NaNO_3$ (Sigma-Aldrich, Milan, Italy)	3 g L^{-1}
KH_2PO_4 (Sigma-Aldrich, Milan, Italy)	1 g L^{-1}
$MgSO_4$ (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
KCl (Sigma-Aldrich, Milan, Italy)	0.5 g L^{-1}
$FeSO_4*7H_2O$ (Sigma-Aldrich, Milan, Italy)	0.01 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	700 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Malt Extract Agar added with 15% NaCl (MEA15%)*
See the previous receipt

Dichloran –glycerol agar base (DG18) (OXOID LTD, Basingstoke, Hampshire, England)

Peptone	5 g L^{-1}
Glucose	10 g L^{-1}
KH_2PO_4	1 g L^{-1}
$MgSO_4$	0.5 g L^{-1}
Dichloran	0.002 g L^{-1}
Agar	15 g L^{-1}

Added 15.75 g in 500 mL and 110 g of Glycerol (ACEF, Piacenza, Italy) in 500 mL.

Specific media for secondary metabolite fungal compounds

Czapek dox agar (modified) (CYA) (OXOID LTD, Basingstoke, Hampshire, England)

$NaNO_3$	2 g L^{-1}
KCl	0.5 g L^{-1}
$C_3H_7MgO_6P$	0.5 g L^{-1}
$FeSO_4$	0.01 g L^{-1}
KSO_4	0.35 g L^{-1}
Sucrose	30 g L^{-1}
Agar	12 g L^{-1}
Yeast extract*	5 g L^{-1}

*Yeast extract (Fluka, Sigma-Aldrich, Milan, Italy)

Malt Extract Agar added with 15% NaCl (MEA15%)*
See the previous receipt

Yeast Extract agar (YES)

Yeast extract (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	150 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy)	20 g L^{-1}

Yeast Extract agar added with 15% of NaCl (YES15%)

Yeast extract (Fluka, Sigma-Aldrich, Milan, Italy),	20 g L^{-1}
Sucrose (ACEF, Piacenza, Italy)	150 g L^{-1}
Sodium chloride (MicroBiol, Cagliari, Italy)	150 g L^{-1}
Agar (Fluka, Sigma-Aldrich, Milan, Italy),	20 g L^{-1}

Appendix 3

Protocols of fungal DNA extractions

Protocol 1: traditional DNA extraction from fungal strains (modified CTAB)

1) Take 50-200 mg mycelium and place the sample into 1.5 mL micro centrifuge tube.
2) Add 700 µL of CTAB (Sigma-Aldrich, Milan, Italy) 1% solution.
3) Add 1% of β-mercaptoethanol (Sigma-Aldrich, Milan, Italy) and homogenize the fungal fragments. Incubate at 65 °C for 1 h. Every 15 min invert the tubs twice (take preferentially mycelia instead of spores or conidia).
4) Centrifuge at 14000 rpm for 10 min.
5) Collect the clear flow through and transfer into a new 1.5 mL micro centrifuge tube (circa 600 µL).
6) Add the same volume (600 µL) of phenol (Sigma-Aldrich, Milan, Italy): chloroform (Sigma-Aldrich, Milan, Italy): isoamyl alcohol (Sigma-Aldrich, Milan, Italy) solution (25:24:1) and mix thoroughly.
7) Centrifuge at 14000 rpm for 10 min.
8) Collect the water phase, typically 400-500 µL.
9) Add the same amount of phenol: chloroform: isoamyl alcohol (or just chloroform) (25:24:1) and mix thoroughly.
10) Centrifuge at 14000 rpm for 10 min.
11) Collect only the water phase and transfer into a new 1.5 mL micro centrifuge tube (typically 300 µL).
12) Add 2/3 of the collected volume of isopropanol micro centrifuge tube and incubate for 30 min at 4 °C (200 µL).
13) Centrifuge at 14000 rpm for 10 min (4 °C)
14) Discard the supernatant onto a piece of paper (the DNA remain at the bottom of the micro centrifuge tube).
15) Add 300 µL of sterile H_2O MilliQ 30 µL of Sodium Acetate (Sigma-Aldrich, Milan, Italy) 3M pH 5.2 and 660 µL of absolute Et-OH (100%) (Sigma-Aldrich, Milan, Italy).
16) Incubate for 1 h at -80 °C or Over Night a -20 °C.
17) Centrifuge at 14000 rpm for 10 min (4 °C).
18) Discard the supernatant.
19) Add 500 µL of Et-OH 70%.
20) Incubate for 20 min at 4 °C.
21) Centrifuge at 14000 rpm for 10 min at 4 °C.
22) Discard the supernatant and let the pellet drying (open micro centrifuge tube or use heater at 45 °C).
23) Re-suspend the pellet in 100 µL of sterile H_2O MilliQ.

Preserve at -20 °C until the amplification.

Protocol 2: genomic DNA from fungi (NucleoSpin® Plant II, Macherey-Nagel, Düren, Germany) modified by MUT

1 - 2 Homogenize sample and cell lysis

Take 50-200 mg mycelium (fresh weight) or material from a fruiting body or macro fungi obtained from a liquid culture or scarped off (with or without) from the surface of a solid medium.

Place the sample into 1.5 mL microcentrifuge tube. Add 2 stainless steal spheres and 200 µL Buffer PL1. Homogenize sample using a micro pistil and vortex regularly. Add additional 200 µL Buffer PL1 and continue to homogenise the sample.

Add 10 µL RNase A solution and mix thoroughly.

Incubate for 60 min at 65 °C.

3 Filtration/Clarification of crude lysate

Place a NucleoSpin® Filter (violet ring) into a new Collection Tube (2 mL) and load the lysate onto the column. Centrifuge for 2 min at 11.000 x g (13.500 rpm). Collect the clear flow through and discard the NucleoSpin® Filter.

If not all liquid has passed the filter. Repeat the centrifugation step.

If a pellet is visible in the flow-through, transfer the clear supernatant to a new 1.5 mL micro centrifuge tube.

4 Adjust DNA binding conditions

Add 450 µL Buffer PC and mix thoroughly by pipetting up and down (5 times) or by vortexing.

5 Bind DNA

Place a NucleoSpin® Plant II Column (green ring) into a new Collection Tube (2 mL) and load a maximum of 700 µL of the sample.

Centrifuge for 1 min at 11.000 x g (13.500 rpm) and discard the flow-through.

The maximum loading capacity of the NucleoSpin® Plant II Column is 700 µL. For higher volumes repeat the loading step.

6 Wash and dry silica membrane

1st wash

Add 400 µL Buffer PW1 to the NucleoSpin® Plant II Column. Centrifuge for 1 min at 11.000 x g (13.500 rpm) and discard flow-through.

2nd wash

Add 700 µL Buffer PW2 to the NucleoSpin® Plant II Column. Centrifuge for 1 min at 11.000 x g (13.500 rpm) and discard flow-through.

3rd wash

Add another 200 µL Buffer PW2 to the NucleoSpin® Plant II Column. Centrifuge for 2 min at 11.000 x g (13.500 rpm) in order to remove wash buffer and discard flow-through.

Finally. Centrifuge for 1 min at 11.000 x g (13.500 rpm) in order to dry the silica membrane completely.

7 Eluate DNA

Place the NucleoSpin® Plant II Column into a new 1.5 mL micro centrifuge tube.

Pipette 50 µL Buffer PE (65 °C) onto the membrane. Incubate the NucleoSpin® Plant II Column for 5 min at 65 °C. Centrifuge for 1 min at 11.000 x g (13.500 rpm) to elute the DNA.

Store the elute DNA at -20 °C until amplification.

PCR conditions

Table A1 PCR conditions for selected primer used.

Primers		**Initial denaturation**	**PCR cycle**				**Final elongation**	**References**
			Denaturation	**Annealing**	**Elongation**	**N° of repetition**		
ACT-512F	**Temp**	94 °C	94 °C	61 °C	72 °C		72 °C	Carbone & Kohn, 1999
ACT-783R	**Time***	8'	15''	20''	40''	34	10'	
Bt2a	**Temp**	94 °C	94 °C	58 °C	72 °C		72 °C	Glass & Donaldson, 1995
Bt2b	**Time**	4'	35''	35''	49''	39	5'	
ITS1	**Temp**	95 °C	95 °C	55 °C	72 °C		72 °C	White *et al.*, 1990
ITS4	**Time**	5'	40''	50''	50''	34	8'	

*: ‘ = minutes; ‘’ = seconds.

For *Aspergillus* sec. *Nigri* use BT at 65 °C annealing temperature.

Appendix A4

Sample preparation for scanning electron microscopically observations

- Place the sample tapes in phosphate buffer (pH 7.0).
- Fixe them with glutaraldehyde buffer for 2 h.
- Rinse them in distilled water.
- Post-fixe them in 2% OsO_4 for 12 h at 5 °C.
- Dehydrate them in an ethanol series.
- Take them to amyl acetate and critical point-dried in a Polaron E-3000 dryer (Quorum Techonologies, Ringmer, UK) with carbon dioxide.
- Coat them with gold (Baltec Sputter Coater) for analysis in High Vacuum mode. Sputtering is performed under an Argon gas flow at a working distance of 50 mm, 0.05 mbar of pressure and 40 mA current for 60 s to obtain a gold film about 15 nm thick.
- Observe the samples by SEM without previous fixation to evaluate any formation of artefacts and the natural degree of dehydration.

Appendix A5

Study of growth condition of *Eurotium halophilicum*

In order to understand the best growth condition of the xerophilic fungal species *Eurotium halophilicum* two different studies are performed:

1) fungal growth on different culture media;
2) fungal growth on the best culture medium at different temperature.

For this study 22 different strains of *E. halophilicum* are considered (Tab. A2). All the strains are deposited and preserved at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and System Biology, University of Turin (Italy).

Table A2 Descriptions of 22 *E. halophilicum* strains.

N°	Original name	N° MUT	Depositor	Origin
3	3	MUT 1916	V. Tigini	Archivio Tavola valdese (Torre Pellice)
5	5	MUT 1922	V. Tigini	Archivio Tavola valdese (Torre Pellice)
6	6	MUT 1899	V. Tigini	Archivio Tavola valdese (Torre Pellice)
7	7	MUT 1896	V. Tigini	Archivio Tavola valdese (Torre Pellice)
8	8	MUT 1906	V. Tigini	Archivio Tavola valdese (Torre Pellice)
9	MM 443	MUT 1309	M. Montanari	Archivio Tavola valdese (Torre Pellice)
10	MM 374	MUT 798	M. Montanari	Archivio Stampati Vaticano (Roma)
11	Corte cost n°8	MUT 1314	M. Montanari	Biblioteca Corte costituzionale (Roma)
12	MM 442	MUT 1307	M. Montanari	Archivio Tavola valdese (Torre Pellice)
13	MM 378	MUT 1294	M. Montanari	Archivio Stampati Vaticano (Roma)
14	MM 441	MUT 1306	M. Montanari	Archivio della Casa Madre (Loreto)
16	Corte Costo. N°3	MUT 1316	M. Montanari	Archivio Corte Costituzionale (Roma)
17	MM 405	MUT 1303	M. Montanari	Archivio Corte Costituzionale (Roma)
18	Corte cost n°2	MUT 1313	M. Montanari	Biblioteca Corte costituzionale (Roma)
19	MM 401	MUT 1298	M. Montanari	Archivio Corte Costituzionale (Roma)
20	Bibl GE. (A).	MUT 1311	M. Montanari	Biblioteca Universitaria (Genova)
21	Corte cost n°4	MUT 1322	M. Montanari	Biblioteca Corte costituzionale (Roma)
22	MM 440	MUT 1305	M. Montanari	Archivio della Casa Madre (Loreto)
23	MM 377	MUT 1293	M. Montanari	Archivio Stampati Vaticano (Roma)
24	Corte cost n°6	MUT 1315	M. Montanari	Biblioteca Corte costituzionale (Roma)
25	MM 402	MUT 1300	M. Montanari	Archivio Stampati Vaticano (Roma)
26	MM 439	MUT 1304	M. Montanari	Biblioteca Gregoriana (Roma)

Protocol 1: testing of different low water activity media.

22 different strains of *E. halophilicum* are inoculated on 5 low water activity media[*]:

- ✓ Czapeck Agar added with 20% of sucrose (Cz20);
- ✓ Czapeck Agar added with 40% of sucrose (Cz40);
- ✓ Czapeck Agar added with 70% of sucrose (Cz70);
- ✓ Dichloran -glycerol agar base (DG18);
- ✓ Malt Extract Agar added with 15% of NaCl (MEA15%).

- From each strain, cut one agar disk 5 mm in diameter from the youngest part of the colony (border part), with a cork borer.
- Transfer the sample in the middle of 6-cm Petri dishes.

[*] For the media receipts, consult the Appendix A2.

- For each medium, consider 3 replicates.
- Incubate all samples at 25 °C for 4 weeks.
- Take the fungal colony diameters every 7 days for 4 weeks.

Protocol 2: testing the growth of selected medium (MEA15%) at different incubation temperatures.

As result of a previous test MEA15% is the best culture medium for *E. halophilicum*'s growth. For this investigation 22 different strains of *E. halophilicum* are considered for the incubation at:

- ✓ 7 °C;
- ✓ 14 °C;
- ✓ 21 °C;
- ✓ 25 °C;
- ✓ 28 °C.

- From each strain, cut one agar disk 5 mm in diameter from the youngest part of the colony (border part), with a cork borer.
- Transfer the sample in the middle of 6-cm Petri dishes with MEA15% as culture medium.
- For each temperature, consider 3 replicates.
- Incubate all the samples for 4 weeks.
- Take the fungal colony diameters every 7 days for 4 week.

Appendix A6

BOD analysis with OxiTop® control system

- The inoculate the fungal strains reported in Table A3 in 1 L septum sealed nozzle bottles with 100 mL of medium, MEA15% for *Aspergillus penicillioides* and *Eurotium halophilicum* and MEA for the other fungal species (for details about culture media, see Appendix A2).
- From each fungal species, cut one agar disk of 10 mm in diameter from the youngest part of the colony (border part), including mycelia, conidia and cleistothecia for *Eurotium* spp. using a cork borer.
- Transfer the sample inside the BOD bottle.
- Close all BOD bottles with a pressure sensor OxiTop®-C system (Weilheim. Germany) mounted on the top.
- Refill the arms of BOD bottles with a few droplets of NaOH (Carlo Erba Reagents, Milan, Italy).
- Activate the BOD sensor and incubated for 25 – 35 days at 25 °C.

The carbon dioxide evolved during aerobic respiration is quantitative adsorbed using droplets of NaOH inside the sealed nozzle septum of the bottles. The pressure drop detected (every 100 min) in the bottle is proportional to the amount of oxygen used by fungi.

<u>Sensor setting</u>
Operation mode: BOD routine.
Measuring time: 25 days for *Aspergillus penicillioides*; 35 days for *Eurotium halophilicum*; 30 days for all other strains.
Filled volume: 97 ml.

Table A3 Fungal species selected for BOD and MVOCs analysis.

Fungal species	**MUT number**
Aspergillus creber	MUT 470
Aspergillus penicillioides	MUT 481
Cladosporium cladosporioides	MUT 5536
Eurotium chevalieri	MUT 472
Eurotium halophilicum	MUT 482
Penicillium brevicompactum	MUT 536
Penicillium chrysogenum	MUT 5493

Appendix A7

Microbial Volatile Organic Compound (MVOC) analysis

MVOC sampling from pure fungal cultures

- Cultivate the selected fungal species reported in Table A3 inside 1 L glass bottles with an area of approximately 64 cm^2 and 100 mL of medium (MEA15% for *Aspergillus penicillioides* and *Eurotium halophilicum* and MEA for the other strains).
- Consider three replicas of each fungal strain
- Close each bottle with a cap furnished of three thermoresistent plastic tubes: the first tube as the direct connection with GC-MS instrument, normally closed with Mohr's pinchcock clamp, while the second tube with activated charcoal (Standard Charcoal tubes ORBO™, 32, Supelco) and 0.2 µm filter (IC Millex®-LG, Millipore Corp., Carrigtwohill, Co.Cork, Ireland) to permit the regulation of the inside pressure and the third tube as security, normally kept closed.
- Store the sample bottles in a dark place at 25 °C for 1-2 months.

VOCs from books

- Position the book samples (L6 and L9) in standing position inside two empty desiccators of 10 L as internal volume, together with a beaker filled with 100 mL of MilliQ water in order to guarantee a closed saturated humidity.
- Position the ground glass joint on the top, furnished with 2 tubes: one as direct connection with the pre-concentrator system, normally closed with a Mohr's clamp, while the other one is equipped with an activated charcoal (Standard Charcoal tubes ORBO™, 32, Supelco) and a 0.2 µm filter (IC Millex®-LG, Millipore Corp., Carrigtwohill, Co.Cork, Ireland) to permit the re-establishment of the internal pressure after each analysis.
- Maintain the desiccators close by Parafilm M® (Bemis NA, WI, US).
- Store the desiccators for 3 months at 24 °C.

VOCs from indoor air by canisters

- Evacuated the canister to 0.05 mm Hg, as recommended by the Compendium Method EPA TO-15 (1999), in preparation for sub atmospheric sample collection.
- Sample the indoor air opening the canisters in correspondence of each sampling areas (see Fig. A2). (When the canister is opened to the atmospheric containing the VOCs to be sampled, the differential pressure causes the sample to flow into the canister).
- Generally, grab samples are collected in 10 – 30 seconds.

Before and after the sample collections, the canister must be clean and free of any contaminants. For the cleaning and certification program, refer to Compendium Method EPA TO-15 (see section 8.4)

VOC pre-concentration

- Connect a direct tube from the sampling bottles to the microscale *purge & trap* Entech 7100 system (Entech Instrument. Inc), in order to collect the fungal VOC productions.
- For each analysis, the sample and 100 mL of internal standards (i.s.) spiking mixture at 20 μgm^{-3} (1.4-bromofluorobenzene, 1.4-difluorobenzene, bromochloromethane and chlorobenzene-d_5) are suctioned separately with a pump, controlled by a Mass Flow Controller (Entech Instrument. Inc) and injected together inside the pre-concentrator system in order to quantified the unknown compounds using the internal standard method. The i.s. is prepared from 1 mgm^{-3} certificated mixture and the compounds are selected because of their non-interfering behaviour with environmental and fungal VOCs.
- The concentration of the sample is carried out in successive stages, starting inside module 1 and consisting of a trap with glass microspheres cooled to -150 °C with liquid nitrogen. The VOCs with water and carbon dioxide are quantitatively blocked, while the main air constituent, oxygen, nitrogen and argon, are discharged. Then, the module 1 is heated to 10 °C and the VOCs plus water are transferred to a module 2 by helium stream.

- The module 2 is a Tenax trap cooled to -30 °C. In this passage, carbon dioxide is discharged, while water remains in the module 1. The VOCs, instead, are adsorbed in the Tenax^{R} resin because of their high affinity. By heating at 180 °C, the VOCs are thermally desorbed from module 2 and transferred to the module 3.
- The module 3 is constituted by a cryofocuser cooled to -150 °C with liquid nitrogen. Finally, the module 3 is quickly heated and the analytes are transferred the gas chromatographic column.

Gas chromatography-mass spectrometry (GC-MS)

Analysis is carried out with Autosystem XL gas chromatographic system equipped with Mass-Gold quadrupole mass spectrometer (both Perkin Elmer Inc. USA) and Turbo Mass vers. 5.4.2 acquisition software. For separation, an Equity 1 capillary (Supelco. 60 m. 0.32 mm i.d.. 1 µm df) with helium as carrier gas is used.
The GC oven program is chosen according to the following scheme: 40 °C for 6 min. 10 °C/min up to 250 °C and 250 °C for 5 min.
For MS detection electron ionization (EI) with 70 eV is applied and mass fragments are detected between 33 and 270 m/z in the total ion current mode (TIC). A reference standard library (NIST MS Search 2.0. National Institute of Standards and Technology) is used to identify the VOCs.
The sampled volume was 40 mL for fungal cultures, 400 mL for both the sampled books and for the indoor air by canister sampling.

Appendix A8

Secondary metabolite compound analysis by LC-MS/MS

Chemicals

LC gradient grade methanol and acetonitrile, MS grade ammonium acetate and glacial acetic acid are purchased from Sigma-Aldrich (Vienna. Austria). A Purelab Ultra system (ELGA LabWater, Celle, Germany) is used for purification of reverse osmosis water.

Extraction

1) Cute one agar disk 10 mm in diameter from each species on each type of medium, from the youngest part of each fungal colony (border part), including mycelium, conidia and cleistothecia for *Eurotium* spp. using a cork borer.
2) Transfer the samples to 15 mL polypropylene falcon tubes with sterile loop.
3) Add 4 mL of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) to each sample.
4) Shake the samples for 90 min using a Universal table Shaker 709 (ASAL srl, Milan, Italy).
5) Centrifuge the samples for 10 min at 3000 rpm on MPW-251 centrifuge (MPW Med. Instruments, Warsaw, Poland).
6) Transfer the extracts into glass vials and dilute 500 µL aliquots with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1. v/v/v).
7) After appropriate mixing, inject 5 µL of the diluted extract into LC-MS/MS system.
8) In the case of dust samples, collect the materials by sterile brush inside 24 mL glass vials.
9) Weigh the dust samples.
10) Add different aliquots of extraction solution depending of the different dust amount (4-8 mL). The extraction and the dilution of the samples are made as for fungal samples.

LC-MS/MS analysis

Analysis is performed with a mass-spectrometer QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with TurbolonSpray electronspray ionization (ESI) source, connected with a 1290 Series HPLC System (Agilent, Waldbronn, Germany).
Chromatographic separation is performed at 25 °C on a Gemini C18-coloum, 150 x 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 x 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US).
ESI-MS/MS is performed in the time-scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte is set to its expected retention time ±27 s and ±48 s in the positive and the negative modes, respectively.
Confirmation of positive analyte identification is obtained by the acquisition of two MRMs per analyte. The LC retention time and the intensity ratio of the two MRM transitions agree with the related values of an authentic standard within 0.1 min and 30% rel., respectively.
Quantification is performed using external calibration based on serial dilution of multi-analyte stock solution (Malachová *et al*., 2014).
The method is based on the multi-mycotoxin analysis developed by Malachová *et al.* (2014).

APPENDIX B

- Data -

Appendix B1 - A

Mycological results of aerobiological indoor sampling

Table B1 Overview of fungal species detected by aerobiological indoor sampling, expressed for each sampling area and culture medium, in particular: A: MEA at 25 °C; B: MEA at 37 °C; C: MEA15%; D: DG18. The data are expressed as CFUm^{-3}.

Fungal species	Area 1			Area 2			Area 3			Area 4			Area 5			Area 6	
	1° S	2° S	3° S	1° S	2° S	3° S	1° S	2° S	3° S	1° S	2° S	3° S	1° S	2° S	3° S	2° S	3° S
Alternaria alternata (Fr.) Keissl.	-	7.0 (C)	-	-	13.0 (A)	-	-	10.0 (A)	-	-	-	-	-	-	-	6.7 (A)	3.3 (A)
Alternaria infectoria E.G. Simmons	-	-	-	-	-	-	1.0 (C)	-	-	-	-	-	-	-	-	-	6.7 (A)
Alternaria seleniiphila Wangeline & E.G. Simmons	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (A)
Antrodia serialis (Fr.) Donk	-	-	-	-	-	-	-	-	3.3 (A) 3.0 (C)	-	-	2.5 (A)	-	-	-	-	13.3 (B)
Antrodia sinuosa (Fr.) P. Karst.	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-	-	7.0 (A)	-	10.0 (A)	-
Arthrinium arundinis (Corda) Dyko & B. Sutton	-	-	-	-	-	-	-	-	2.5 (D)	-	-	-	-	-	-	-	10.0 (A)
Aspergillus creber Jurjevic, S.W. Peterson & B.W. Horn	1285.0 (C)	-	-	815.0 (C)	3.0 (C)	-	27.0 (C)	-	-	15.0 (C)	3.0 (A)	-	-	-	-	3.0 (A)	-
Aspergillus flavus Link	-		-	-	10.0 (C) 3.0 (B)	-	-	17.0 (C)	-	1.0 (C)	-	-	-	3.0 (B)	-	6.0 (A)	-
Aspergillus fumigatus Fresen.	-	-	-	-	3.0 (A)	-	-	6.7 (B)	-	-	-	-	-	-	-	-	10.0 (B)
Aspergillus hortae (Langeron) C.W. Dodge	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-
Aspergillus insuetus (Bainer) Thom & Chrurch	-	-	-	5.0 (C)	-	2.5 (A)	-	-	-	-	-	-	-	-	-	-	-
Aspergillus niger Tiegh.	-	-	-	5.0 (C)	-	3.0 (C)	-	-	-	5.0 (C)	-	-	5.0 (C)	-	-	-	-
Aspergillus penicillioides Spegazzini	-	3.0 (C)	-	-	17.0 (C)	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-
Aspergillus protuberus Munt.-Cvetk.	46.0 (C)	-	-	510.0 (C)	-	-	7.0 (C)	-	-	7.0 (C)	-	-	-	-	-	-	-
Aspergillus section *Nigri*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (A)

Aspergillus sclerotiorum G.A. Huber	-	-	-	-	10.0 (A)	-	1.0 (C)	7.0 (A)	-	-	3.0 (A)	-	-	-	-	-	-
Aspergillus section *versicolores*	-	-	-	-	3.3 (D)	2.5 (A)	-	-	-	-	-	-	-	-	-	-	3.3 (A)
Aspergillus sydowii (Bainer & Sartory) Thom & Church	-	10.0 (C) 7.0 (D)	-	-	-	-	1.0 (C)	3.0 (C)	-	-	-	-	-	3.0 (D)	-	-	-
Aspergillus tubingensis Mosseray	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-	-	-	-	-	-
Aspergillus versicolor (Vuill.) Tirab.	-	3.0 (A)	-	-	3.0 (C)	5.0 (A)	-	-	-	-	3.0 (C)	-	-	-	5.0 (A) 3.3 (B)	-	3.3 (D)
Aspergillus vitricola Ohtsuki	-	-	3.0 (C)	-	-	-	-	-	-	-	7.0 (C)	-	-	-	5.0 (C)	-	-
Aspergillus spp.	-	-	-	-	-	-	10.0 (C)	-	2.5 (D)	2.0 (C)	7.0 (C)	-	3.0 (C)	-	-	3.0 (C)	3.0 (C) 3.3 (D)
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	-	-	-	-	3.0 (A)	-	-	3.0 (A)	-	-	-	-	-	-	-	-	10.0 (A)
Aureobasidium pullulans var. pullulans (de Bary & Löwenthal) G. Arnaud	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (D)
Bjerkandera adusta (Willd.) P. Karst.	-	-	-	-	-	-	-	-	-	-	3.0 (B)	-	-	-	-	-	-
Botryosphaeria stevensii Shoemaker	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-
Botrytis cinerea Pers.	-	-	-	-	-	-	-	-	-	2.0 (C)	-	-	2.0 (C)	-	-	-	13.3 (A) 36.8 (D)
Bulleromyces albus Boekhout & Á. Fonseca	-	57.0 (A)	-	-	140.0 (A)	-	-	10.0 (A)	-	-	-	-	-	60.0 (A)	-	3.0 (A)	-
Cladosporium allicinum (Fr.) Bensch, U. Braun & Crous	-	-	2.5 (D)	-	-	-	-	-	3.3 (A)	-	-	3.0 (C)	-	-	3.3 (D)	-	-
Cladosporium cladosporioides (Fresen.) G.A. de Vries	-	3.0 (A)	13.0 (A) 5.0 (D)	-	10.0 (A)	-	-	3.0 (A)	3.3 (A)	10.0 (C)	23.0 (A)	5.0 (A)	13.0 (C)	3.0 (C)	17.5 (A) 3.3 (D) 3.0 (C)	113.0 (A) 7.0 (C)	303.0 (D) 50.0 (C)
Cladosporium coralloides W. Yamam.	-	-	-	-	-	-	-	3.0 (A)	-	-	-	-	-	-	-	-	-
Cladosporium halotolerans Zalar, de Hoog & Gunde-Cim.	-	7.0 (A) 7.0 (D)	3.3 (A) 2.5 (D)	-	10.0 (A)	5.0 (D) 5.0 (C)	-	13.0 (A)	-	-	10.0 (A) 3.3 (D) 7.0 (C)	2.5 (D)	-	10.0 (A) 3.0 (C)	-	210.0 (A) 160.0 (C)	3.3 (D)
Cladosporium ossifragi	-	-	-	5.0 (C)	-	-	-	-	-	-	-	-	-	-	-	-	-

(Rostr.) U. Braun & K. Schub.																	
Cladosporium oxysporum Berk. & M.A. Curtis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5 (A)	-	-
Cladosporium perangustum Bensch, Crous & U. Braun	-	-	-	-	-	2.5 (A)	-	-	2.5 (D)	-	-	-	-	7.0 (A)	-	-	3.3 (D)
Cladosporium pseudocladosporioides Bensch, Crous & U. Braun	-	-	-	-	10.0 (A)	2.5 (A) 2.5 (D)	-	3.0 (A)	-	5.0 (C)	13.0 (A)	-	-	3.0 (A)	2.5 (A)	120.0 (A)	23.0 (A) 10.0 (D)
Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun	-	-	-	5.0 (C)	-	-	2.0 (C)	-	-	5.0 (C)	-	-	4.0 (C)	-	-	-	-
Cladosporium sphaerospermum Penz.	-	-	-	-	-	3.0 (C)	-	-	-	6.0 (C)	-	-	5.9 (C)	10.0 (C)	-	80.0 (C)	13.0 (C)
Cladosporium spp.	-	-	5.0 (D)	-	-	2.5 (M)	-	-	2.5 (D)	-	-	-	-	-	2.5 (A) 5.0 (C)	-	113.0 (A) 37.0 (C) 190.0 (D)
Coriolopsis gallica (Fr.) Ryvarden	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (B)
Cryptococcus carnescens (Verona & Luchetti) M. Takash., Sugita, Shinoda & Nakase	-	-	3.3 (A)	-	-	5.0 (A)	-	-	3.3 (A)	-	-	-	-	-	7.5 (A)	-	16.7 (A)
*Cryptococcus diffluens (*Zach) Lodder & Kreger-van Rij	-	-	2.5 (D)	-	-	-	-	-	2.5 (D)	-	-	2.5 (D)	-	-	-	-	3.0 (C)
Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij	-	-	2.5 (D)	-	-	2.5 (D)	-	-	-	-	-	-	-	-	-	-	3.3 (D)
Discostroma tricellulare Okane, Nakagiri & Tad. Ito	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (D)
Dyaporthe endophytica R.R. Gomes, C. Glienke & Crous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (A)
*Dyaporthe phaseolorum (*Cooke & Ellis) Sacc.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (D)
Didymella graminicola Punith.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (A)
Ellurema indica (Punith.) Nag Raj & W.B. Kendr.	-	-	-	-	-	-	-	-	-	-	-	2.5 (A)	-	-	-	-	-

Embellisia abundans E.G. Simmons	-	-	-	-	-	-	-	-	-	1.0 (C)	-	-	1.0 (C)	-	-	-	-
Emericella heterotallica	-	-	-	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-	-	-
Emericella stella-maris Zalar, Frisvad & Samson	-	-	-	-	-	2.5 (A)	-	-	-	-	-	-	-	-	-	-	-
Engyodontium album (Limber) de Hoog	-	-	-	-	-	2.5 (A)	-	-	3.0 (C)	-	-	-	-	-	-	-	-
Epicoccum nigrum Link	-	-	-	-	3.0 (A)	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-
Eurotium chevalieri L. Mangin	-	-	-	-	-	-	-	-	-	2.0 (C)	-	-	2.0 (C)	-	-	-	-
Funalia trogii (Berk.) Bondartsev & Singer	-	7.0 (B)	-	-	-	-	-	-	-	-	-	-	-	-	-	20.0 (B)	3.3 (B)
Fusarium equiseti (Corda) Sacc.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.0 (A)	-
Fusarium oxysporum E.F. Sm. & Swingle	-	-	-	-	-	-	2.0 (C)	-	-	-	-	-	-	-	-	3.0 (B)	10.0 (A)
Fusarium tricinctum (Corda) Sacc.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.7 (A) 13.3 (D)
Gloeophyllum sepiarium (Wulfen) P. Karst.	-	10.0 (A)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gloeophyllum subferrugineum (Berk.) Bondartsev & Singer	-	-	-	-	-	-	-	-	-	-	3.0 (B)	-	-	-	-	-	-
Gnomonia petiolorum (Schwein.) Cooke	-	-	-	-	-	-	-	3.0 (A)	-	-	-	-	-	-	-	3.0 (A)	-
Hexagonia nitida Durieu & Mont.	-	3.0 (B)	-	-	-	-	-	-	-	-	-	-	-	3.0 (B)	-	27.0 (B)	-
Leptosphaeria microscopica P. Karst.	-	-	-	-	-	-	-	-	-	-	10.0 (A)	-	-	-	-	-	-
Meruliopsis corium (Pers.) Ginns	-	3.0 (CA)	-	-	-	-	-	7.0 (A)	-	-	47.0 (A)	-	-	-	-	7.0 (A)	-
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0 (A)	-
Paecilomyces farinosus (Holmsk.) A.H.S. Br. & G. Sm.	-	-	-	-	7.0 (A)	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-
Paecilomyces lilacinus (Thom) Samson	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (A)
Paraconiothyrium fuckelii (Sacc.) Verkley & Gruyter	-	-	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-	-	-	-
Penicillium aurantiogriseum Dierckx	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73.0 (C)

Penicillium brevicompactum Dierckx	55.0 (C)	3.0 (A)	-	55.0 (C)	7.0 (A) 13.0 (C)	10.0 (A) 7.5 (D)	7.0 (C)	-	-	1.0 (C)	3.0 (A) 10.0 (D)	2.5 (A) 3.0 (C)	-	-	-	97.0 (A) 20.0 (C) 66.7 (D)	23.3 (A) 77.0 (C) 137.0 (D)
Penicillium caseifulvum F. Lund, Filt. & Frisvad	-	-	-	-	-	-	-	3.0 (D)	-	-	-	-	-	-	-	-	-
Penicillium chrysogenum Thom	190.0 (C)	-	3.3 (A)	220.0 (C)	-	5.0 (D)	20.0 (C)	-	-	10.0 (C)	-	-	1.0 (C)	-	-	13.0 (C)	-
Penicillium citreonigrum Dierckx	-	3.0 (D)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium citrinum Thom	-	-	-	-	3.3 (D)	-	-	-	-	-	-	-	-	3.0 (D)	-	-	-
Penicillium commune Thom	-	-	-	-	-	-	-	-	2.5 (D)	-	-	-	-	-	-	-	-
Penicillium crustosum Thom	-	-	5.0 (D)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium decumbens Thom	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3 (A)
Penicillium digitatum (Pers.) Sacc.	-	-	3.3 (A) 5.0 (D)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium diversum Raper & Fennell	-	3.0 (A)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium expansum Link	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0 (A)	-
Penicillium giganteum R.Y. Roy & G.N. Singh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (C)	-	-
Penicillium glabrum (Wehmer) Westling	-	-	-	-	6.7 (D)	-	-	10.0 (D)	-	4.0 (C)	-	-	4.0 (C)	-	-	3.0 (A) 3.0 (C)	-
Penicillium ingelheimense J.F.H. Beyma	-	-	-	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-	-	-
Penicillium olsonii Bainier & Sartory	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (C)	-
Penicillium pancosmium Houbraken, Frisvad & Samson																	6.7 (D)
Penicillium sclerotigenum T. Yamam.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.7 (D)
Penicillium sizovae Baghd.	-	-	-	5.0 (C)	-	-	-	-	-	5.0 (C)	-	-	5.0 (C)	-	-	-	-
Penicillium spinulosum Thom	-	-	-	-	-	-	-	-	-	-	3.0 (C)	-	-	-	-	3.0 (C)	-
Penicillium terrigenum	-	-	-	-	3.3 (D)	-	-	-	-	-	-	-	-	-	-	3.0 (A)	3.3 (A)

Houbraken, Frisvad & Samson																3.0 (C)	6.6 (D)
Penicillium turbatum Westling	-	-	-	-	-	-	-	3.0 (D)	-	-	-	-	-	-	-	-	-
Penicillium spp.	-	-	-	-	-	3.0 (C)	-	-	-	-	-	-	-	-	3.0 (C)	3.3 (D)	3.0 (C)
Peniophora lycii (Pers.) Höhn. & Litsch.	-	-	-	-	-	-	-	7.0 (A)	-	-	-	-	-	-	-	13.0 (A)	-
Peniophora tamaricicola Boidin & Malençon	-	-	-	-	-	-	-	-	-	-	7.0 (A)	-	-	-	-	-	-
Phaeosphaeria herpotrichoides (De Not.) L. Holm	-	-	-	-	-	-	-	-	-	2.0 (C)	10.0 (A)	-	2.0 (C)	-	-	-	33.3 (A)
Phoma glomerata (Corda) Wollenw. & Hochapfel	-	-	-	-	-	-	-	-	-	5.0 (C)	-	-	5.0 (C)	-	-	-	-
Phoma negriana Thüm.	-	-	-	-	-	-	-	10.0 (A)	-	-	-	-	-	-	-	-	-
Pleosporales sp.	-	-	-	-	-	-	1.0 (C)	-	-	5.0 (C)	-	-	5.0 (C)	-	3.0 (C)	-	7.0 (C)
Pseudotaeniolina globosa De Leo, Urzì & De Hoog	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-	-	-
Rhodotorula mucilaginosa (A. Jörg.) F.C. Harrison	-	-	10.0 (A) 5.0 (D)	-	-	20.0 (A) 10.0 (D)	-	-	3.3 (A) 5.0 (D)	-	-	2.5 (D)	-	-	17.5 (M) 3.3 (D)	-	6.7 (D)
Schizophyllum commune Fr.	-	-	-	-	3.0 (A)	-	-	-	-	1.0 (C)	-	-	1.0 (C)	-	-	-	-
Sporidiobolus pararoseus Fell & Tallman	-	130.0 (A)	-	-	203.0 (A)	-	-	30.0 (A)	-	-	7.0 (A)	-	-	70.0 (A)	-	7.0 (A)	-
Stachybotrys chartarum (Ehrenb.) S. Hughes	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-	-	-	-	-	-
Stereum gausapatum (Fr.) Fr.	-	20.0 (A)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterile mycelium	5.0 (C)	-	2.5 (D)	-	7.0 (C) 13.0 (A)	5.0 (A) 33.0 (B)	21.0 (C)	7.0 (A)	2.5 (D)	2.0 (C)	7.0 (C) 50.0 (A) 7.0 (B)	-	2.0 (C)	10.0 (B)	3.0 (C) 7.5 (A) 3.3 (D)	33.0 (M) 17.0 (B)	7.0 (C) 23.3 (A) 10.0 (B) 16.7 (D)
Uncultured fungus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.0 (A)	-
Yeast sp.	-	-	-	-	-	-	-	-	-	-	3.0 (A)	-	-	-	-	-	-
Total n° of isolated individuals	2000	651	77	1620	510	106	81	163	52	101	260	26	29	199	109	1142	1393
Total n° of isolated taxa	5	14	13	9	22	19	12	21	15	21	22	8	16	14	13	33	41

Appendix B1 – B

List of deposit fungi in MUT collection and GenBank®

Table B2 List of fungal species isolated from BAUM's repository with relative MUT and GenBank® accession numbers. A: air; B: book.

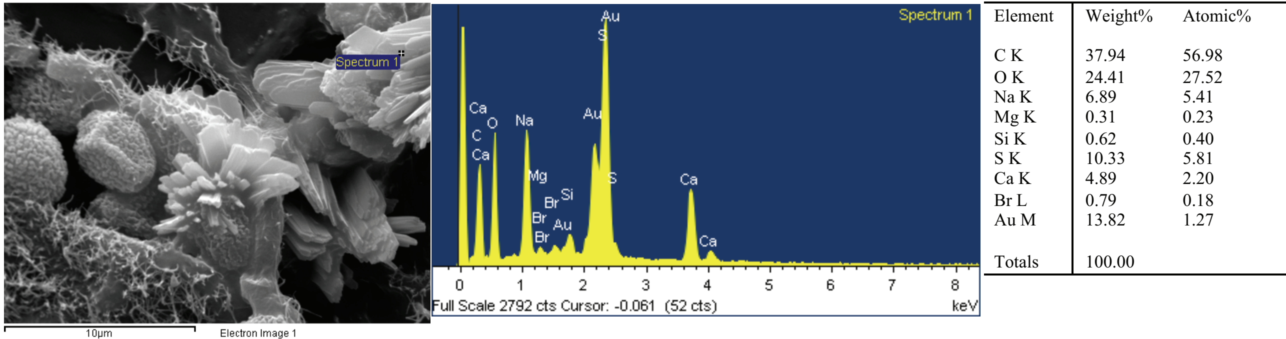
Fungal species	Source	MUT number	GenBank® accession number
Alternaria infectoria E.G. Simmons	A	MUT 5479	KM264290
Aspergillus creber Jurjevic, S.W. Peterson & B.W. Horn	B A B A A B	MUT 470 MUT 5477 MUT 5527 MUT 5689 MUT 5690 MUT 5691	KU179486 KM502191 KU179488 - - KU179487
Aspergillus flavus Link	A B	MUT 478 MUT 5526	- -
Aspergillus insuetus (Bainer) Thom & Chrurch	A	MUT 498	KM502182
Aspergillus jensenii Jurjevic, S.W. Peterson & B.W. Horn	A	MUT 480	KM502178
Aspergillus niger Tiegh.	A	MUT 477	KM502177
Aspergillus penicillioides Spegazzini	B A B B B A B B B	MUT 481 MUT 5483 MUT 5525 MUT 5537 MUT 5692 MUT 5694 MUT 5697 MUT 5700 MUT 5702	KU179489 - KU179491 KU179492 - KU179490 - - -
Aspergillus protuberus Munt.-Cvetk.	A A B	MUT 5476 MUT 5487 MUT 5693	KM502192 KM502193 KU179494
Aspergillus sclerotiorum G.A. Huber	A	MUT 466	KM502176
A. sydowii (Bainer & Sartory) Thom & CHurch	A	MUT 630	KM502191
Aspergillus tubingensis Mosseray	B	MUT 5529	-
Aspergillus versicolor (Vuill.) Tirab.	B	MUT 5495	KM264292
Aspergillus vitricola Ohtsuki	B B B B B	MUT 5692 MUT 5695 MUT 5696 MUT 5698 MUT 5701	- - - - -
Aureobasidium pullulans	A	MUT 5524	-
Botrytis cinerea Pers.	A	MUT 511	KM264280
Bulleromyces albus Boekhout & Á. Fonseca	A	MUT 5528	-
Cladosporium cladosporioides (Fresen.) G.A. de Vries	A A A	MUT 5530 MUT 5536 MUT 527	- - KU179495
Cladosporium halotolerans Zalar, de Hoog & Gunde-Cim.	B B B	MUT 5532 MUT 5533 MUT 5538	- - -
Cladosporium ossifragi (Rostr.) U. Braun & K. Schub.	A	MUT 532	-

Cladosporium pseudocladosporioides Bensch, Crous & U. Braun	A	MUT 515	-
Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun	B A A	MUT 528 MUT 529 MUT 5494	KM264294 KM264295 KM264297
Cladosporium sphaerospermum Penz.	A A	MUT 5485 MUT 5539	KM264296 -
Embellisia abundans E.G. Simmons	A	MUT 647	KM264288
Epicoccum nigrum Link	B	MUT 526	KM264283
Eurotium amstelodami L. Mangin	B	MUT 5531	-
Eurotium chevalieri L. Mangin	A	MUT 472	-
Eurotium halophilicum C.M. Chr., Papav & C.R. Benj.	B B B	MUT 482 MUT 5534 MUT 5535	KM502179 KU179496 KU179497
Fusarium oxysporum E.F. Sm. & Swingle	A	MUT 566	KM264286
Gloeophyllum abietinum	B	MUT 5488	KM264291
Lecanicillium kalimantanense Kurihara & Sukarno	B	MUT 533	KM264285
Penicillium brevicompactum Dierckx	B A	MUT 536 MUT 577	KM502183 -
Penicillium chrysogenum Thom	A A A A A A A A A A	MUT 576 MUT 5482 MUT 5484 MUT 5486 MUT 5490 MUT 5491 MUT 5493 MUT 5492 MUT 5497 MUT 5498	- KM502200 KM502201 KM502202 KM502203 KM502204 KM502205 KM502206 - -
Penicillium glabrum (Wehmer) Westling	A	MUT 552	KM502187
Penicillium sizovae Baghd.	A	MUT 545	KM502185
Phaeosphaeria herpotrichoides (De Not.) L. Holm	A	MUT 512	KM264281
Phaeospheria typharum (Desm.) L. Holm	B	MUT 513	KM264282
Phoma glomerata (Corda) Wollenw. & Hochapfel	A	MUT 492	KM264279
Schizophyllum commune Fr.	A	MUT 573	KM264287

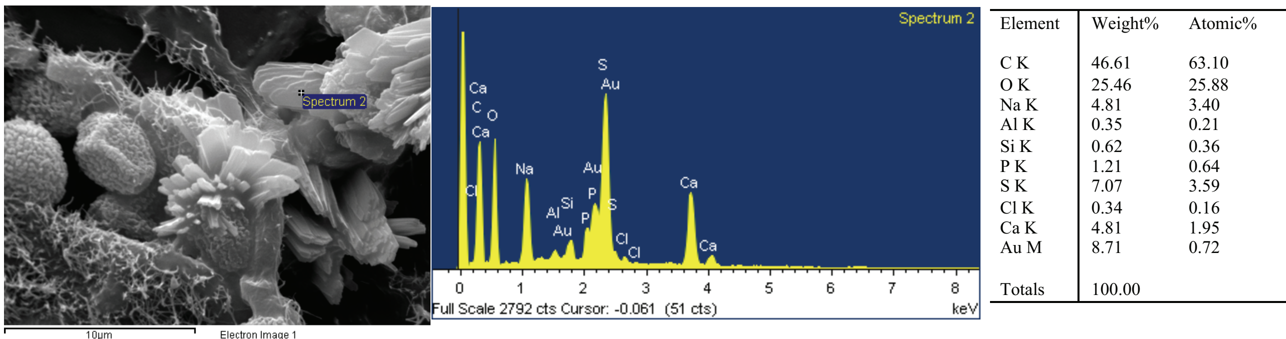
Appendix B2

SEM micrographs with related EDX spectra

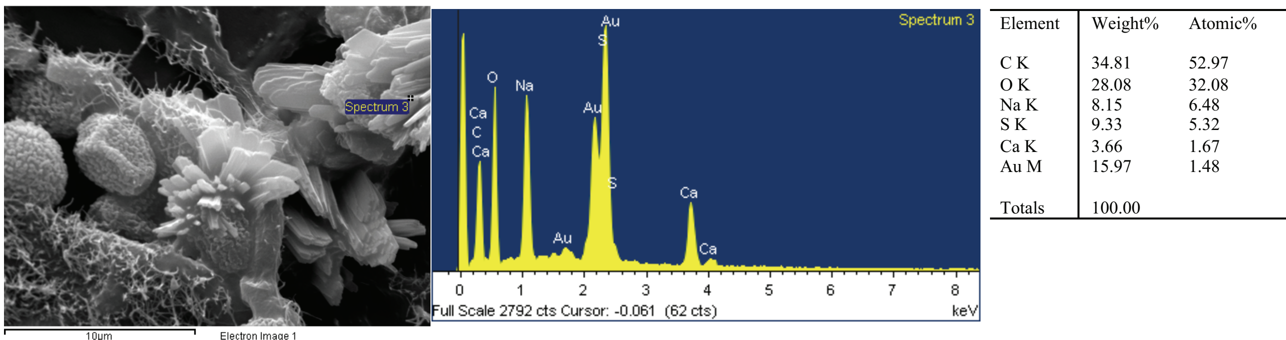
Book L9 - Picture 1 of the fungus *Eurotium halophilicum* - Spectra 1-15.



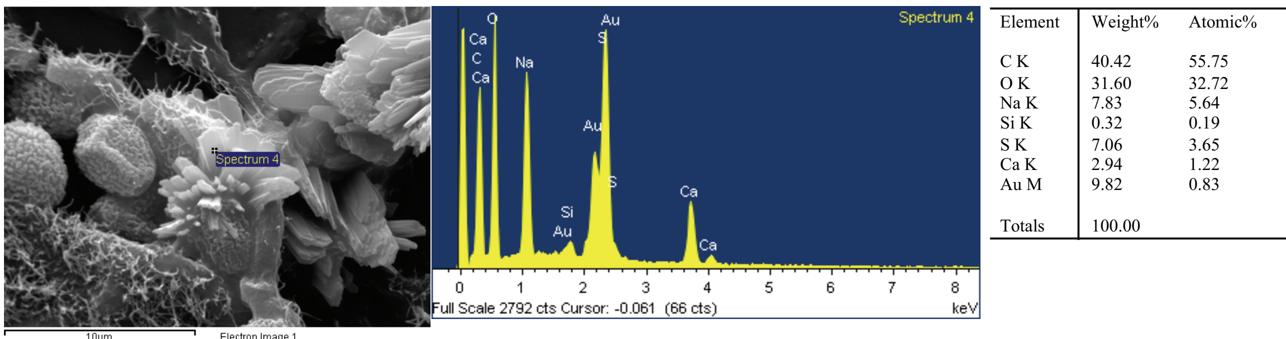
Element	Weight%	Atomic%
C K	37.94	56.98
O K	24.41	27.52
Na K	6.89	5.41
Mg K	0.31	0.23
Si K	0.62	0.40
S K	10.33	5.81
Ca K	4.89	2.20
Br L	0.79	0.18
Au M	13.82	1.27
Totals	100.00	



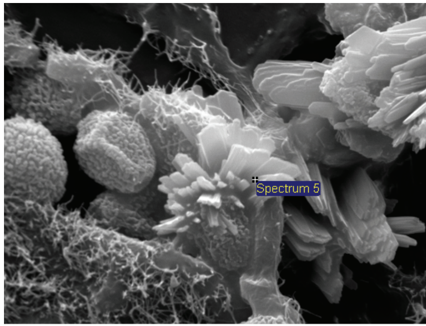
Element	Weight%	Atomic%
C K	46.61	63.10
O K	25.46	25.88
Na K	4.81	3.40
Al K	0.35	0.21
Si K	0.62	0.36
P K	1.21	0.64
S K	7.07	3.59
Cl K	0.34	0.16
Ca K	4.81	1.95
Au M	8.71	0.72
Totals	100.00	

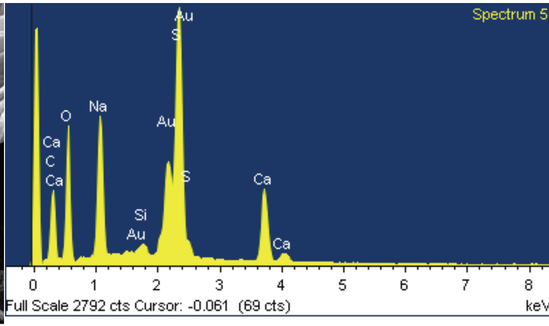


Element	Weight%	Atomic%
C K	34.81	52.97
O K	28.08	32.08
Na K	8.15	6.48
S K	9.33	5.32
Ca K	3.66	1.67
Au M	15.97	1.48
Totals	100.00	

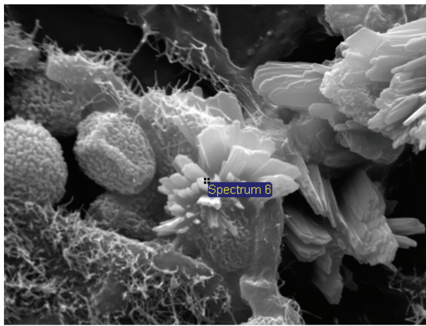


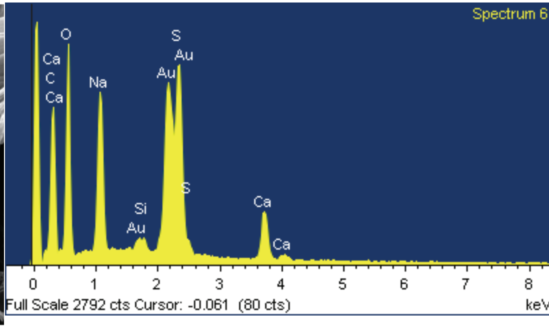
Element	Weight%	Atomic%
C K	40.42	55.75
O K	31.60	32.72
Na K	7.83	5.64
Si K	0.32	0.19
S K	7.06	3.65
Ca K	2.94	1.22
Au M	9.82	0.83
Totals	100.00	



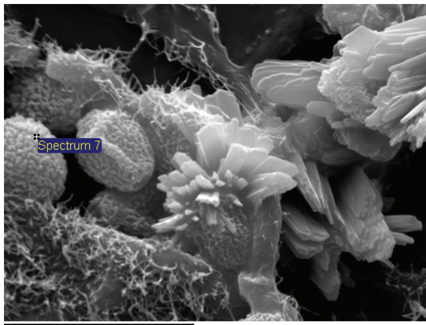


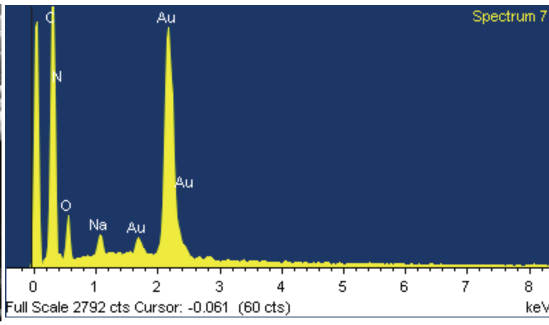
Element	Weight%	Atomic%
C K	33.36	51.07
O K	27.22	31.29
Na K	8.62	6.89
Si K	0.39	0.25
S K	11.99	6.88
Ca K	5.18	2.38
Au M	13.25	1.24
Totals	100.00	



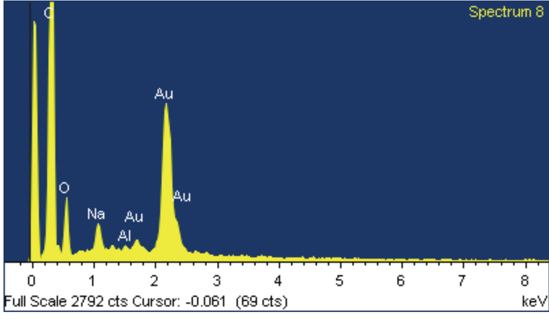


Element	Weight%	Atomic%
C K	37.50	55.92
O K	28.54	31.95
Na K	7.20	5.61
Si K	0.24	0.15
S K	6.52	3.64
Ca K	2.56	1.14
Au M	17.44	1.59
Totals	100.00	

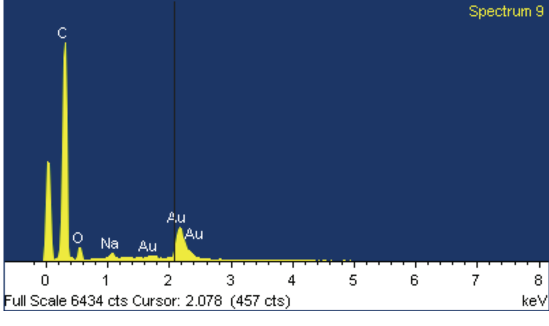




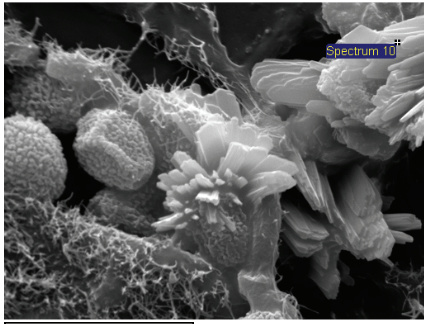
Element	Weight%	Atomic%
C K	58.58	84.51
N K	0.30	0.37
O K	10.73	11.62
Na K	1.24	0.93
Au M	29.15	2.56
Totals	100.00	

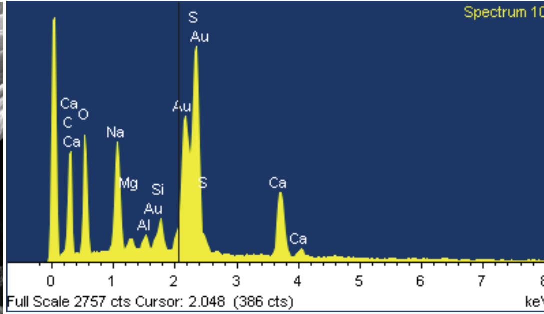


Element	Weight%	Atomic%
C K	66.66	83.89
O K	14.50	13.70
Na K	1.51	0.99
Al K	0.19	0.10
Au M	17.14	1.32
Totals	100.00	

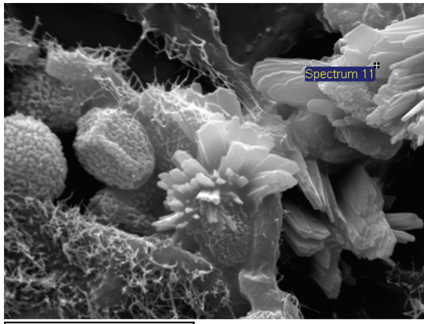


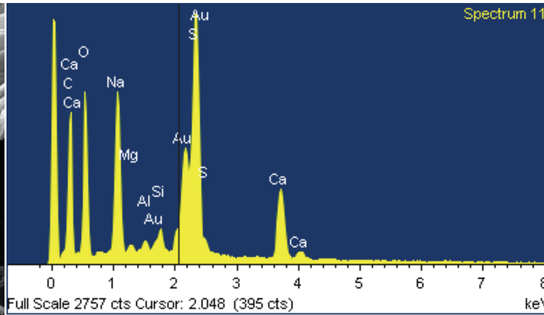
Element	Weight%	Atomic%
C K	79.12	90.07
O K	10.31	8.81
Na K	0.73	0.44
Au M	9.84	0.68
Totals	100.00	



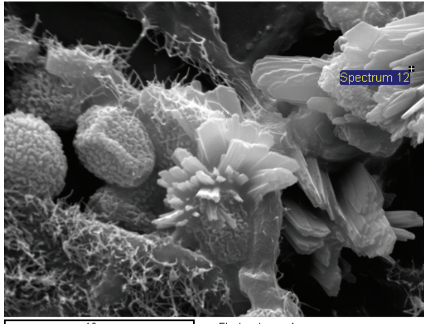


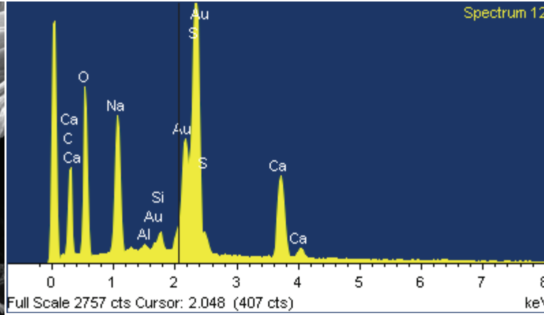
Element	Weight%	Atomic%
C K	39.60	59.75
O K	22.88	25.91
Na K	5.82	4.59
Mg K	0.49	0.37
Al K	0.52	0.35
Si K	0.94	0.61
S K	8.75	4.95
Ca K	4.27	1.93
Au M	16.72	1.54
Totals	100.00	



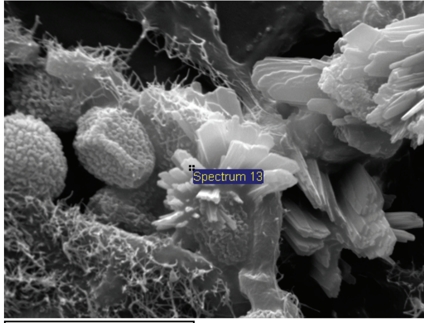


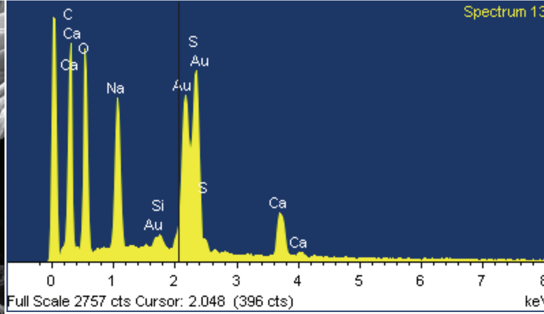
Element	Weight%	Atomic%
C K	42.57	59.49
O K	26.17	27.45
Na K	7.50	5.47
Mg K	0.26	0.18
Al K	0.32	0.20
Si K	0.56	0.33
S K	8.40	4.40
Ca K	3.78	1.58
Au M	10.45	0.89
Totals	100.00	



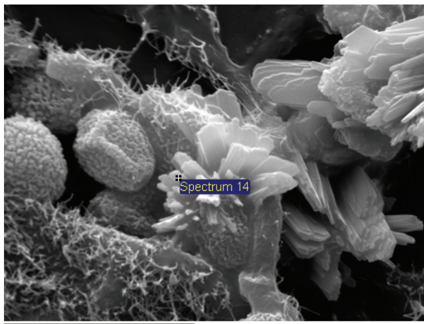


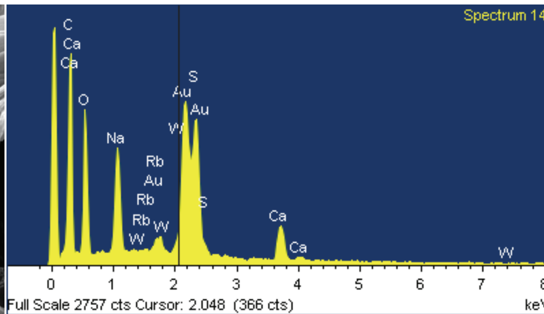
Element	Weight%	Atomic%
C K	34.54	51.90
O K	28.53	32.19
Na K	7.28	5.72
Al K	0.22	0.15
Si K	0.50	0.32
S K	11.25	6.33
Ca K	4.97	2.24
Au M	12.70	1.16
Totals	100.00	



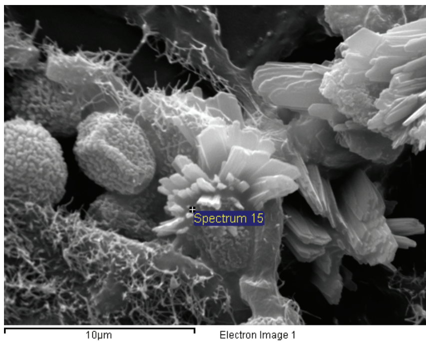


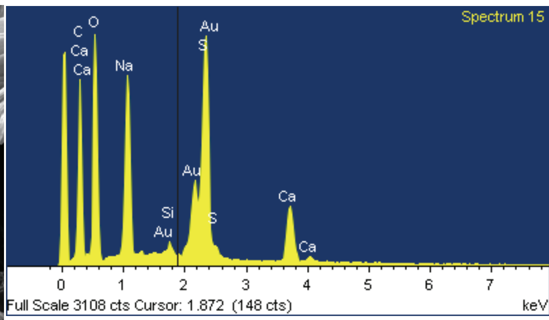
Element	Weight%	Atomic%
C K	43.86	61.55
O K	27.15	28.59
Na K	6.17	4.52
Si K	0.29	0.17
S K	5.71	3.00
Ca K	2.15	0.91
Au M	14.67	1.25
Totals	100.00	





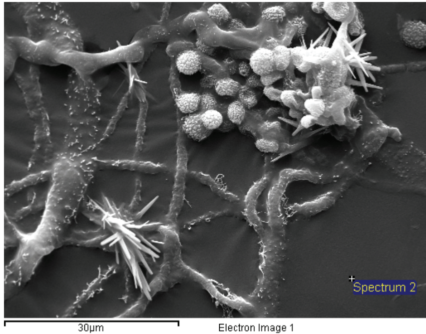
Element	Weight%	Atomic%
C K	47.04	66.61
O K	23.28	24.75
Na K	4.83	3.58
S K	4.84	2.57
Ca K	1.98	0.84
Rb L	0.84	0.17
W M	0.53	0.05
Au M	16.65	1.44
Totals	100.00	

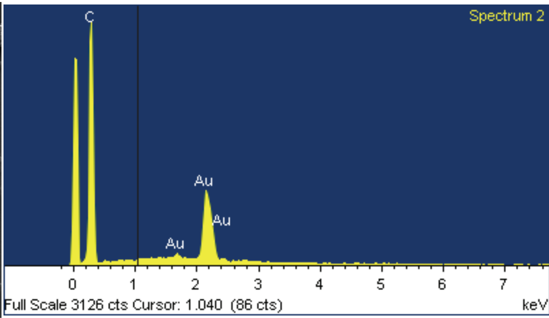




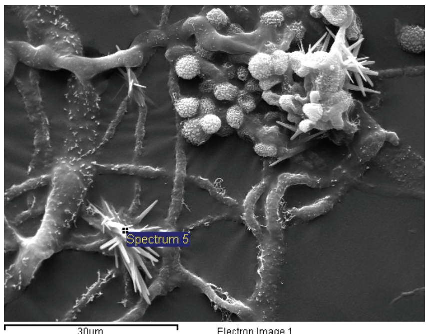
Element	Weight%	Atomic%
C K	43.74	58.24
O K	31.00	30.99
Na K	7.77	5.40
Si K	0.31	0.18
S K	7.06	3.52
Ca K	2.67	1.06
Au M	7.45	0.61
Totals	100.00	

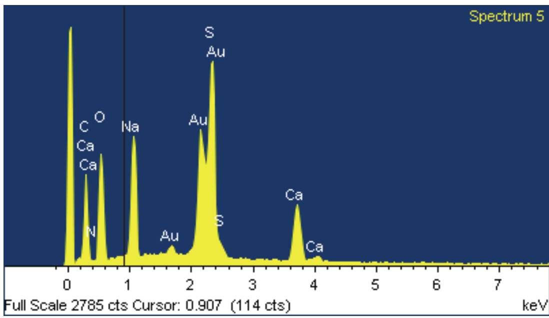
Book L9 - Picture 2 of the fungus *Eurotium halophilicum* - Spectra 2-19 (spectra 1, 3 and 4 are not shown = background).



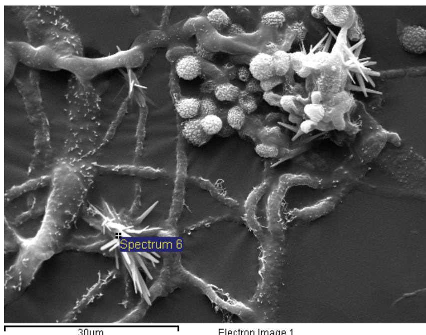


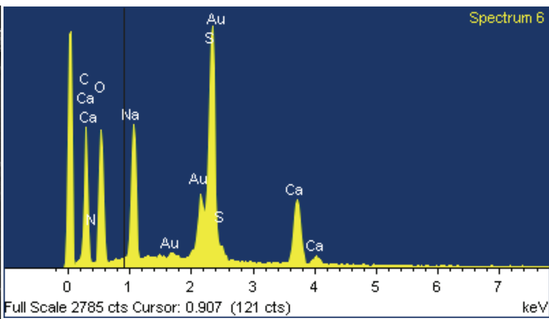
Element	Weight%	Atomic%
C K	79.88	98.49
Au M	20.12	1.51
Totals	100.00	



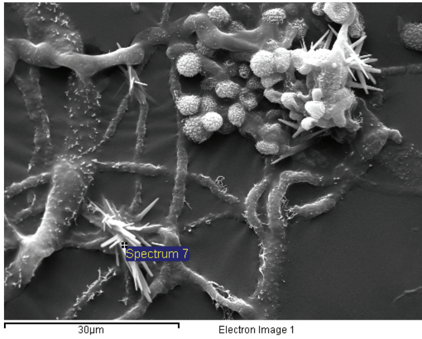


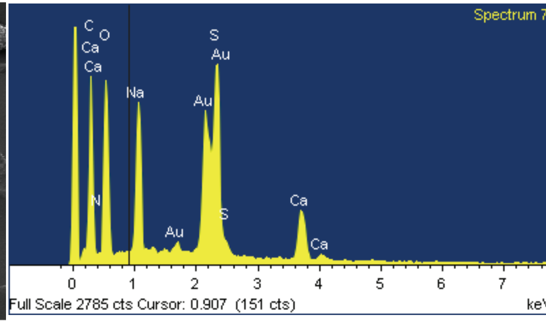
Element	Weight%	Atomic%
C K	34.60	52.90
N K	5.96	7.82
O K	21.70	24.91
Na K	7.26	5.80
S K	9.10	5.21
Ca K	3.76	1.72
Au M	17.63	1.64
Totals	100.00	



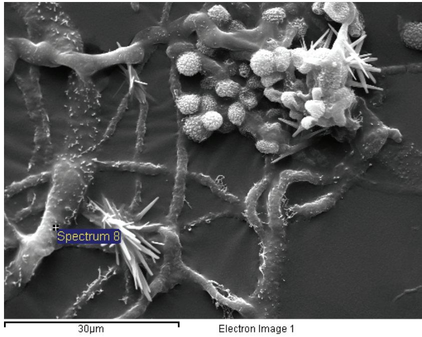


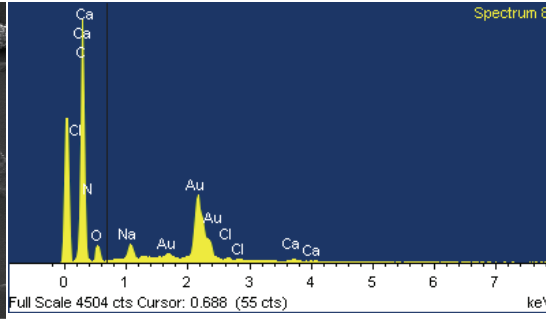
Element	Weight%	Atomic%
C K	40.53	54.42
N K	8.11	9.34
O K	24.86	25.06
Na K	6.86	4.81
S K	8.48	4.27
Ca K	3.71	1.49
Au M	7.44	0.61
Totals	100.00	



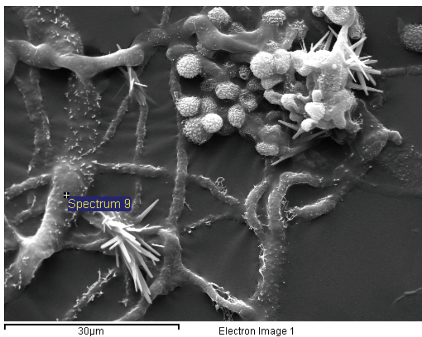


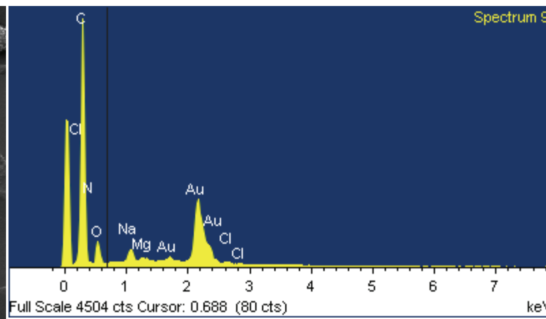
Element	Weight%	Atomic%
C K	38.02	53.56
N K	8.59	10.37
O K	24.80	26.23
Na K	6.16	4.53
S K	5.87	3.10
Ca K	2.33	0.98
Au M	14.23	1.22
Totals	100.00	



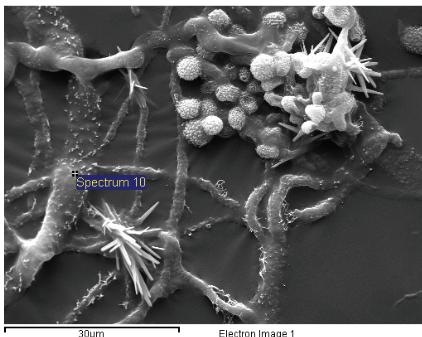


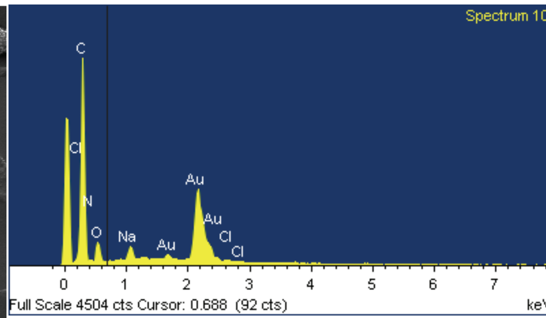
Element	Weight%	Atomic%
C K	66.11	78.29
N K	13.15	13.36
O K	7.32	6.51
Na K	1.34	0.83
Cl K	0.22	0.09
Ca K	0.25	0.09
Au M	11.62	0.84
Totals	100.00	



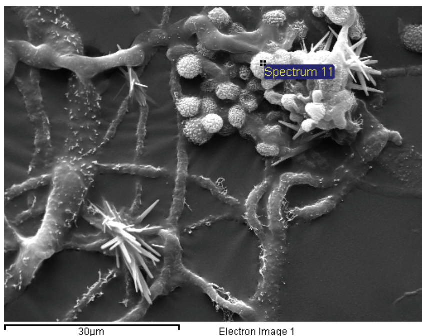


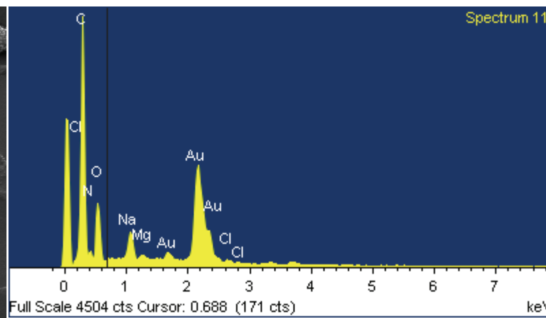
Element	Weight%	Atomic%
C K	83.54	99.10
N K	-11.73	-11.94
O K	12.00	10.68
Na K	1.37	0.85
Mg K	0.26	0.15
Cl K	0.29	0.12
Au M	14.27	1.03
Totals	100.00	



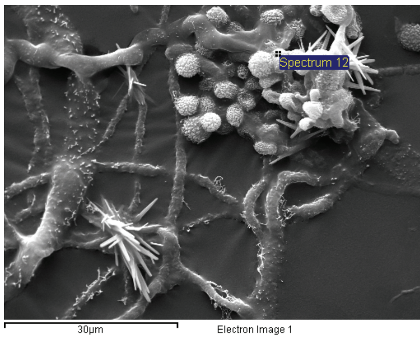


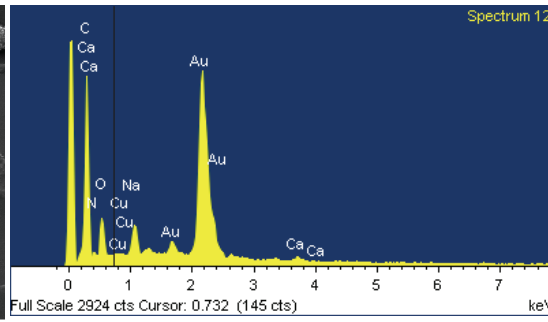
Element	Weight%	Atomic%
C K	74.52	91.19
N K	-2.77	-2.91
O K	10.34	9.50
Na K	1.41	0.90
Cl K	0.24	0.10
Au M	16.25	1.21
Totals	100.00	



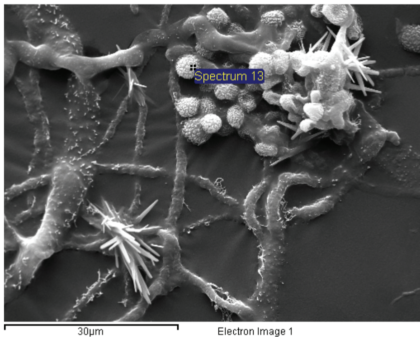


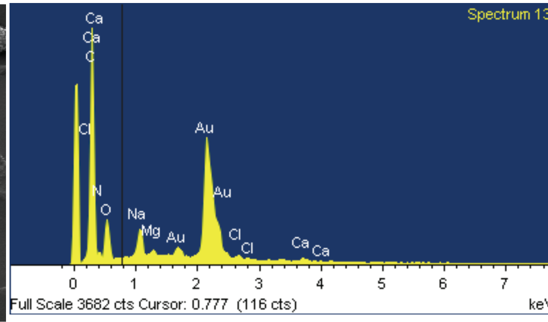
Element	Weight%	Atomic%
C K	61.57	76.88
N K	3.07	3.28
O K	18.31	17.16
Na K	2.03	1.33
Mg K	0.24	0.15
Cl K	0.22	0.09
Au M	14.57	1.11
Totals	100.00	



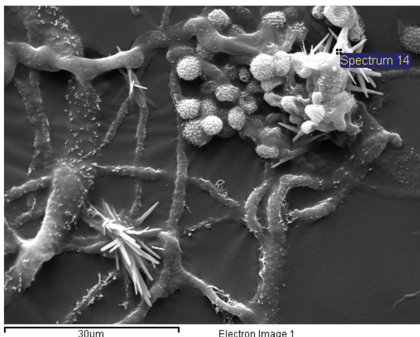


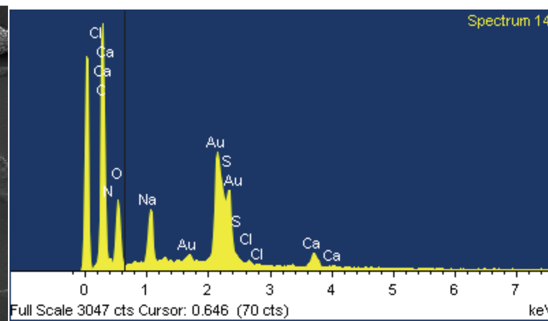
Element	Weight%	Atomic%
C K	48.12	70.19
N K	11.15	13.94
O K	10.46	11.45
Na K	2.21	1.68
Ca K	0.31	0.14
Cu K	0.68	0.19
Au M	27.08	2.41
Totals	100.00	



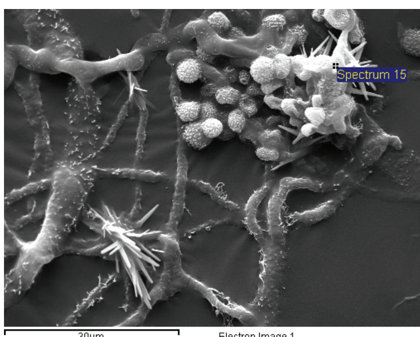


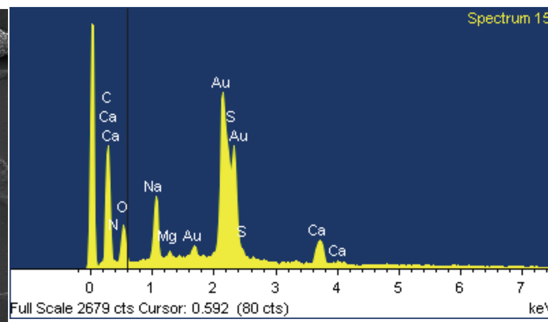
Element	Weight%	Atomic%
C K	53.23	67.69
N K	17.17	18.72
O K	11.41	10.90
Na K	1.68	1.12
Mg K	0.24	0.15
Cl K	0.28	0.12
Ca K	0.23	0.09
Au M	15.76	1.22
Totals	100.00	



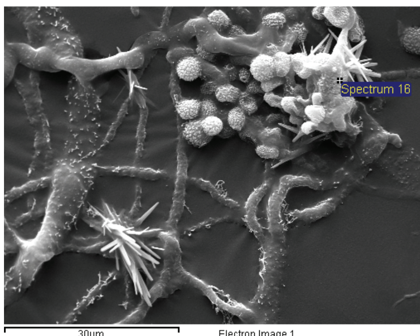


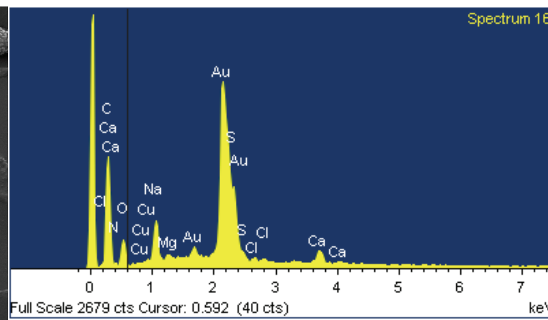
Element	Weight%	Atomic%
C K	51.11	65.25
N K	15.41	16.87
O K	13.94	13.37
Na K	2.74	1.83
S K	2.69	1.29
Cl K	0.18	0.08
Ca K	0.76	0.29
Au M	13.17	1.03
Totals	100.00	



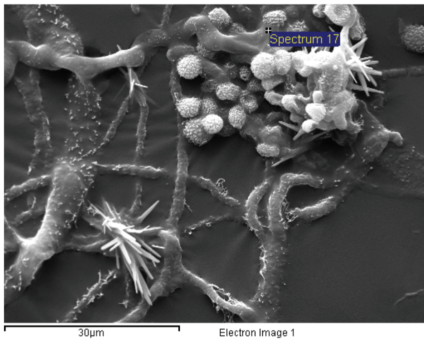


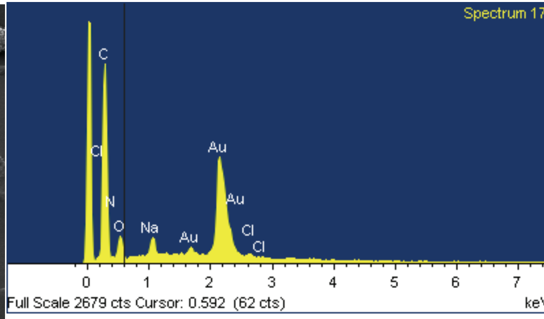
Element	Weight%	Atomic%
C K	43.86	67.76
N K	8.13	10.77
O K	9.47	10.99
Na K	3.94	3.18
Mg K	0.27	0.20
S K	6.51	3.77
Ca K	1.97	0.91
Au M	25.87	2.44
Totals	100.00	



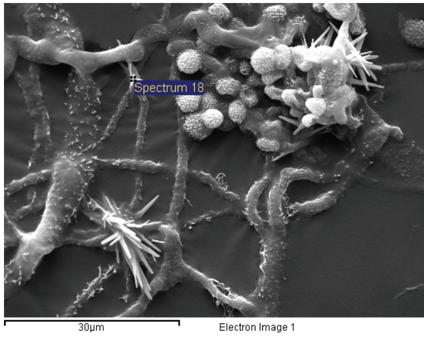


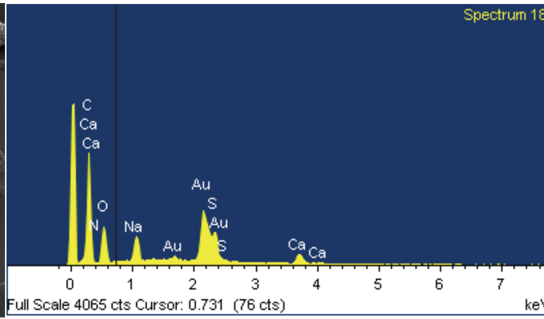
Element	Weight%	Atomic%
C K	44.93	71.13
N K	8.39	11.39
O K	7.24	8.61
Na K	2.75	2.28
Mg K	0.27	0.21
S K	4.40	2.61
Cl K	0.32	0.17
Ca K	1.12	0.53
Cu K	0.64	0.19
Au M	29.94	2.89
Totals	100.00	



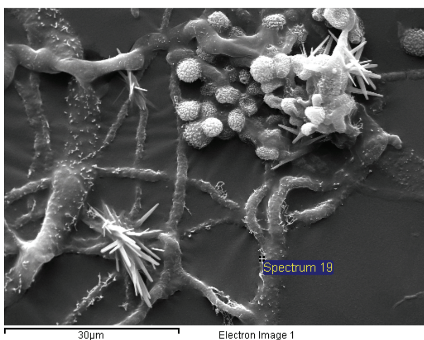


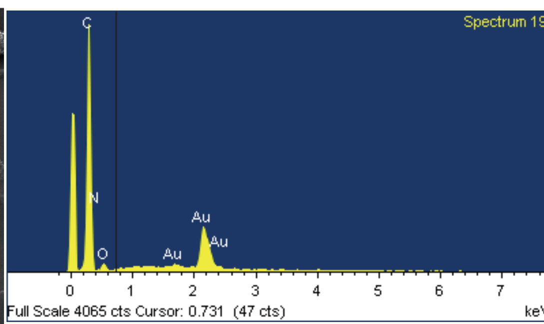
Element	Weight%	Atomic%
C K	71.41	91.81
N K	-4.62	-5.10
O K	10.70	10.33
Na K	1.77	1.19
Cl K	0.39	0.17
Au M	20.34	1.59
Totals	100.00	





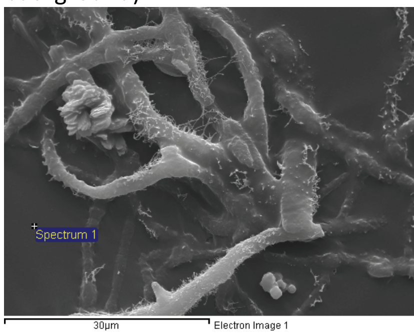
Element	Weight%	Atomic%
C K	52.76	69.05
N K	8.19	9.19
O K	16.96	16.66
Na K	3.00	2.05
S K	2.72	1.33
Ca K	1.30	0.51
Au M	15.07	1.20
Totals	100.00	

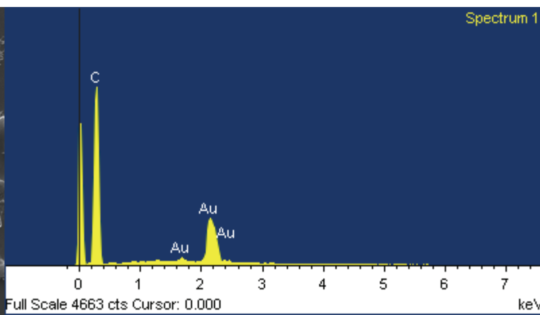




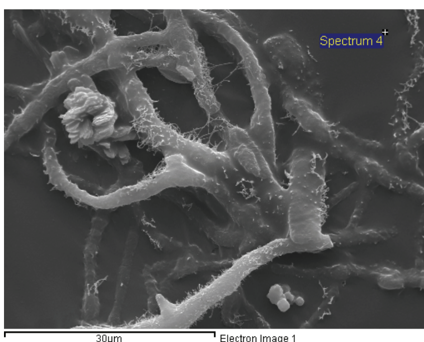
Element	Weight%	Atomic%
C K	98.53	113.80
N K	-20.72	-20.52
O K	6.48	5.62
Au M	15.71	1.11
Totals	100.00	

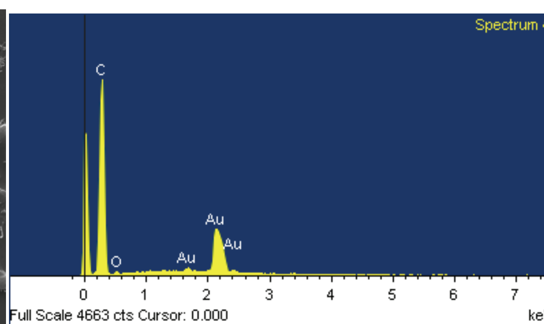
Book L9 - Picture 3 of the fungus *Eurotium halophilicum* - Spectra 1-21 (spectra 2, 3, 5 and 6 are not shown = background).



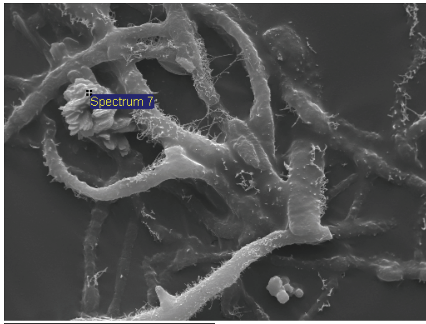


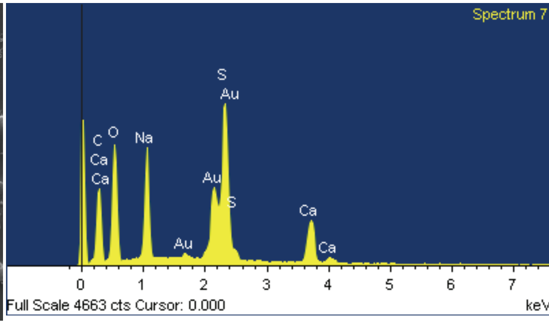
Element	Weight%	Atomic%
C K	81.87	98.67
Au M	18.13	1.33
Totals	100.00	



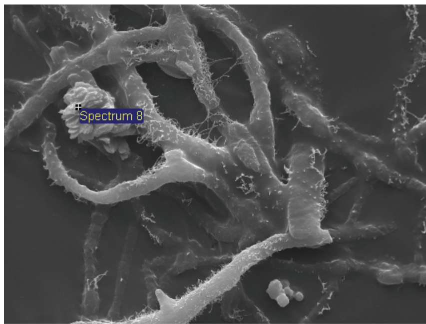


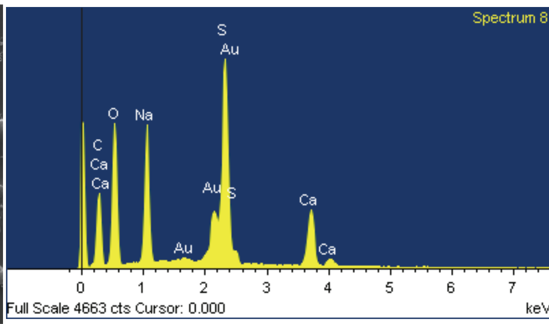
Element	Weight%	Atomic%
C K	80.46	96.77
O K	2.17	1.96
Au M	17.37	1.27
Totals	100.00	



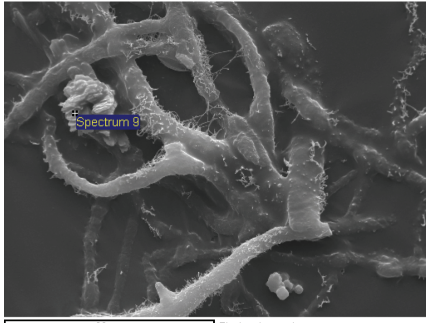


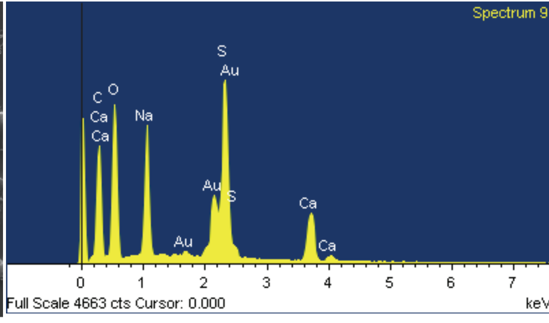
Element	Weight%	Atomic%
C K	37.52	54.60
O K	28.44	31.08
Na K	8.55	6.50
S K	9.22	5.03
Ca K	3.91	1.71
Au M	12.36	1.10
Totals	100.00	



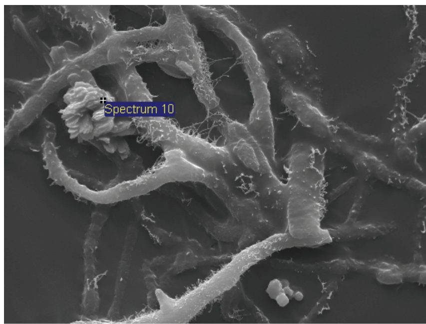


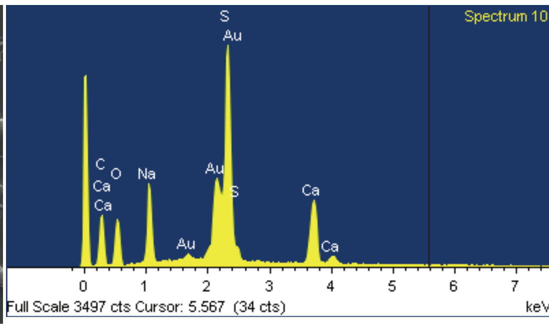
Element	Weight%	Atomic%
C K	36.11	51.28
O K	31.27	33.34
Na K	9.76	7.24
S K	10.46	5.57
Ca K	4.40	1.87
Au M	7.99	0.69
Totals	100.00	



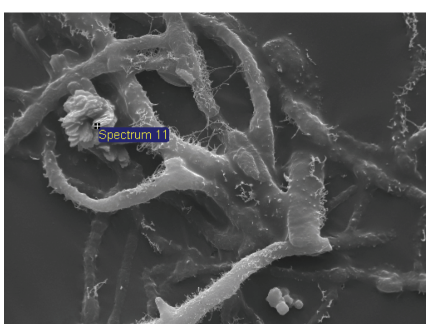


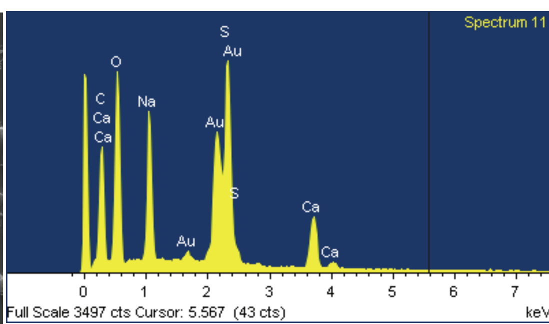
Element	Weight%	Atomic%
C K	41.29	56.56
O K	30.49	31.35
Na K	8.08	5.78
S K	8.09	4.15
Ca K	3.51	1.44
Au M	8.54	0.71
Totals	100.00	



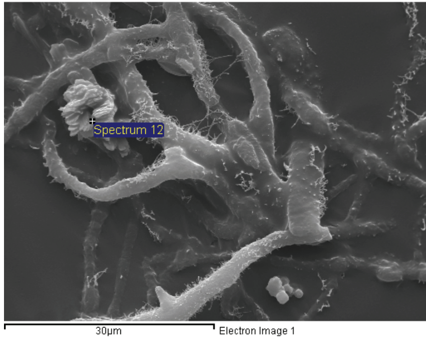


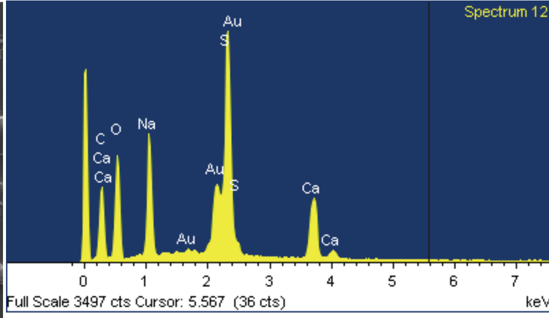
Element	Weight%	Atomic%
C K	38.14	60.66
O K	16.63	19.86
Na K	6.81	5.66
S K	14.96	8.91
Ca K	6.96	3.32
Au M	16.50	1.60
Totals	100.00	



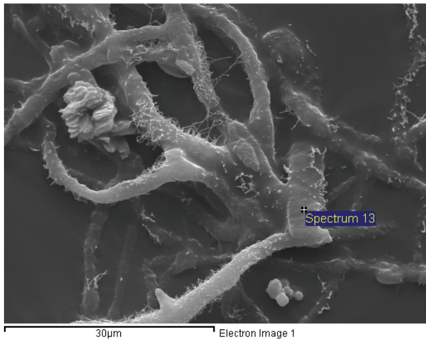


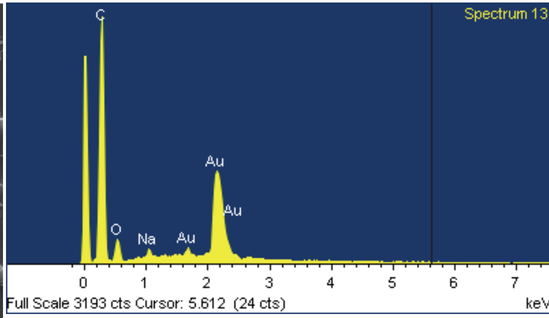
Element	Weight%	Atomic%
C K	36.29	53.87
O K	29.58	32.96
Na K	7.72	5.98
S K	8.00	4.45
Ca K	3.05	1.36
Au M	15.36	1.39
Totals	100.00	



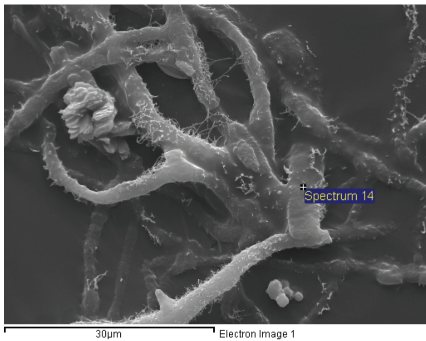


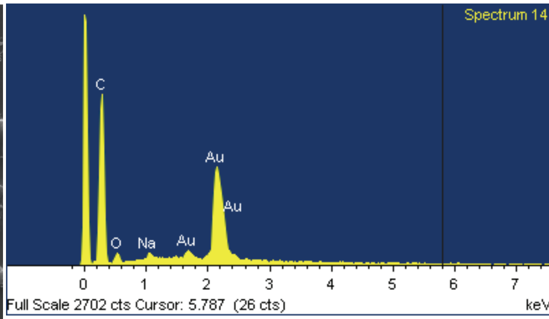
Element	Weight%	Atomic%
C K	37.46	55.23
O K	25.42	28.13
Na K	8.67	6.68
S K	12.00	6.63
Ca K	5.25	2.32
Au M	11.20	1.01
Totals	100.00	



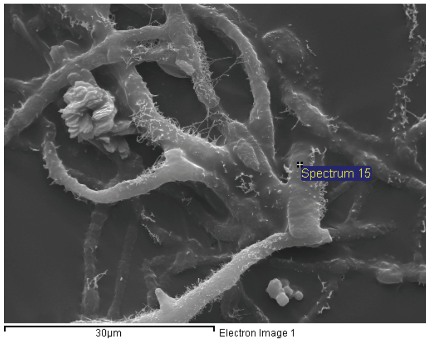


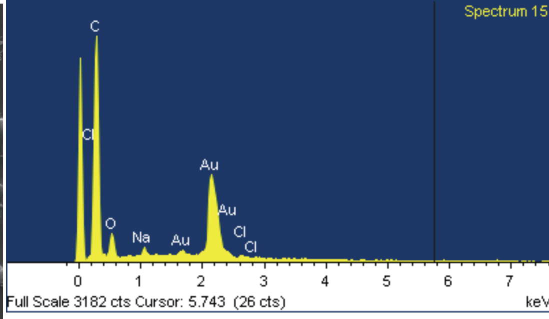
Element	Weight%	Atomic%
C K	70.44	88.58
O K	10.09	9.53
Na K	0.70	0.46
Au M	18.78	1.44
Totals	100.00	



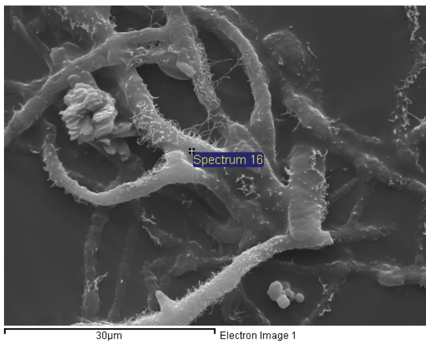


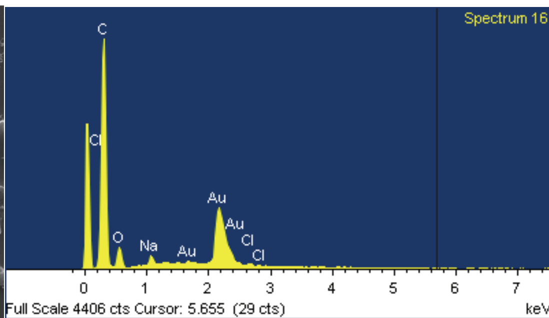
Element	Weight%	Atomic%
C K	68.31	91.59
O K	5.91	5.95
Na K	0.57	0.40
Au M	25.21	2.06
Totals	100.00	



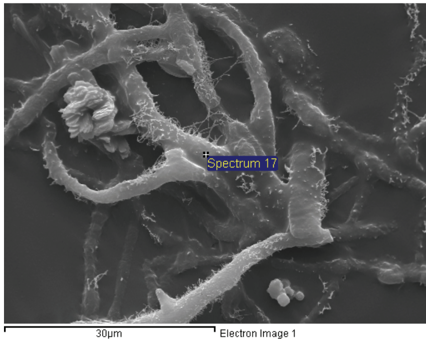


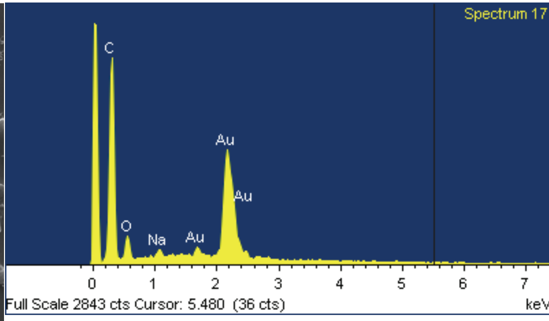
Element	Weight%	Atomic%
C K	70.95	87.99
O K	10.85	10.11
Na K	0.76	0.49
Cl K	0.26	0.11
Au M	17.18	1.30
Totals	100.00	



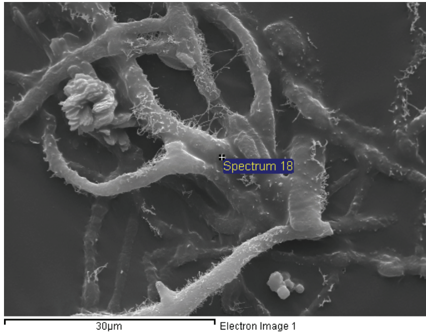


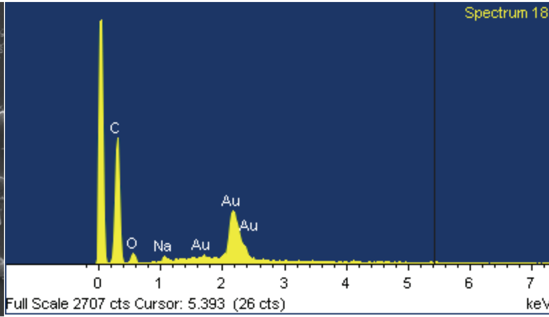
Element	Weight%	Atomic%
C K	75.44	88.65
O K	11.06	9.76
Na K	1.02	0.62
Cl K	0.23	0.09
Au M	12.25	0.88
Totals	100.00	



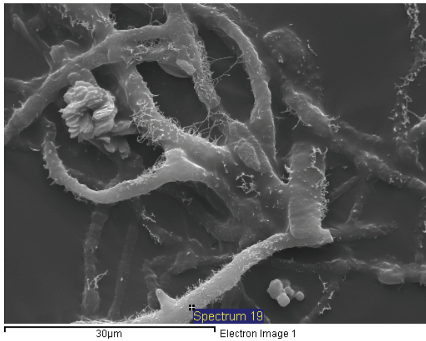


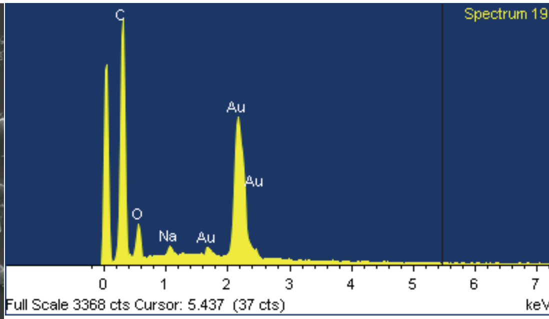
Element	Weight%	Atomic%
C K	66.43	87.42
O K	10.41	10.29
Na K	0.71	0.49
Au M	22.45	1.80
Totals	100.00	



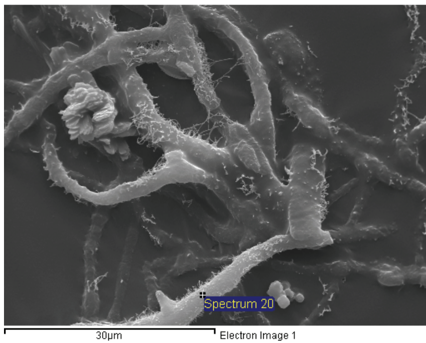


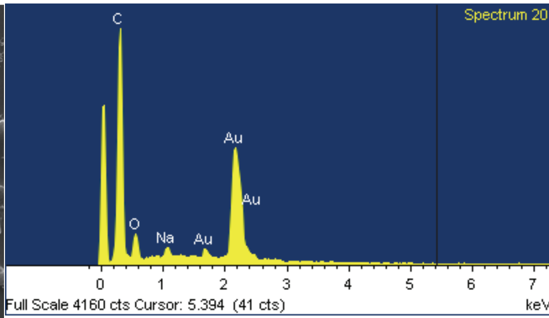
Element	Weight%	Atomic%
C K	72.16	89.46
O K	9.22	8.59
Na K	0.95	0.61
Au M	17.67	1.34
Totals	100.00	



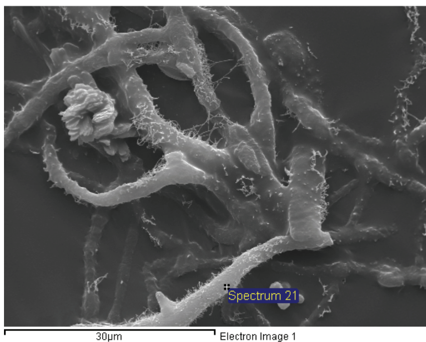


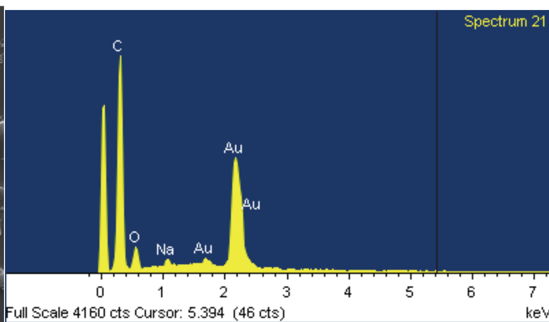
Element	Weight%	Atomic%
C K	63.45	85.92
O K	11.38	11.57
Na K	0.70	0.50
Au M	24.47	2.02
Totals	100.00	





Element	Weight%	Atomic%
C K	66.54	87.71
O K	10.01	9.91
Na K	0.81	0.56
Au M	22.63	1.82
Totals	100.00	





Element	Weight%	Atomic%
C K	66.33	88.15
O K	9.49	9.47
Na K	0.68	0.47
Au M	23.50	1.90
Totals	100.00	

Appendix B3

A – Different growth condition of *Eurotium halophilicum*: results about culture media.

Table B3 Growth of *E. halophilicum* strains on different culture media during 4 weeks. Diameters are expressed in mm; W: week.

Strain	MUT number	Cz20				Cz40				Cz70			
		1W	2 W	3 W	4 W	1W	2 W	3 W	4 W	1W	2 W	3 W	4 W
3	MUT 1916	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0
5	MUT 1922	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0±1.7	2.0±1.7	2.0±1.7	2.0±1.7
7	MUT 1896	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7±0.6	5.3±1.5	9.0±5.6	14.3±4.0
9	MUT 1309	0.0	0.0	0.0	2.0±1.7	0.0	0.0	0.0	0.0	0.0	3.3±0.6	5.3±2.3	7.3±4.0
10	MUT 798	0.0	0.0	3.0±0.0	3.7±1.2	0.0	0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.3±0.6	4.3±0.6	6.0±2.0
11	MUT 1314	0.0	0.0	3.0±0.0	3.0±0.0	0.0	0.0	3.0±0.0	3.0±0.0	0.0	0.0	3.0±0.0	5.0±0.0
12	MUT 1307	0.0	3.0±0.0	3.0±0.0	3.3±0.6	3.0±0.0	3.0±0.0	3.3±0.6	3.7±0.6	0.0	4.7±1.2	8.3±4.5	15.0±10.0
13	MUT 1294	0.0	0.0	0.0	0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	5.3±2.5	7.0±4.4	9.3±7.6
14	MUT 1306	0.0	2.0±1.7	2.0±1.7	3.7±0.6	0.0	0.0	0.0	3.0±0.0	0.0	3.3±0.6	4.0±0.0	6.7±2.1
16	MUT 1316	0.0	2.0±1.7	3.3±2.0	2.0±1.7	0.0	0.0	0.0	1.0±1.7	3.0±0.0	4.0±0.0	5.3±0.6	7.0±1.0
17	MUT 1303	0.0	0.0	2.0±1.7	3.0±0.0	0.0	0.0	4.3±1.2	4.3±1.2	0.0	2.0±1.7	6.3±2.3	9.0±4.4
18	MUT 1313	0.0	0.0	1.0±1.7	1.0±1.7	0.0	1.3±2.3	1.3±2.3	2.3±2.1	3.7±1.2	5.7±0.6	7.0±1.0	9.0±1.0
19	MUT 1298	0.0	0.0	0.0	0.0	0.0	2.0±1.7	2.0±1.7	2.3±2.1	3.0±0.0	5.7±0.6	7.3±1.2	11.7±3.5
20	MUT 1311	0.0	0.0	1.0±1.7	1.0±1.7	0.0	0.0	0.0	0.0	0.0	4.3±2.3	7.0±2.6	10.3±5.8
21	MUT 1322	0.0	0.0	3.0±0.0	3.0±0.0	0.0	0.0	3.0±0.0	3.0±0.0	0.0	0.0	2.3±2.1	3.3±2.9
22	MUT 1305	0.0	0.0	0.0	0.0	0.0	0.0	1.0±1.7	2.0±1.7	0.0	6.3±0.6	9.7±2.1	15.3±3.1
23	MUT 1293	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0±1.7	0.0	2.7±2.5	8.3±3.1	12.3±4.7
24	MUT 1315	0.0	0.0	3.0±0.0	3.0±0.0	0.0	0.0	0.0	0.0	2.0±1.7	7.3±6.4	15.0±8.7	20.7±12.9
25	MUT 1300	0.0	0.0	2.0±1.7	2.0±1.7	0.0	0.0	2.0±1.7	2.0±1.7	3.0±0.0	8.7±3.1	12.3±3.2	16.0±6.2
26	MUT 1304	0.0	0.0	2.0±1.7	2.0±1.7	0.0	0.0	0.0	0.0	5.3±4.7	10.3±5.0	16.3±4.7	25.0±2.0

Strain	MUT number	DG18				MEA15%			
		1W	2 W	3 W	4 W	1W	2 W	3 W	4 W
3	MUT 1916	0.0	3.0±0.0	3.0±0.0	3.0±0.0	7.3±0.6	16.7±0.6	22.3±1.5	26.3±0.6
5	MUT 1922	0.0	0.0	0.0	0.0	9.0±1.7	17.3±2.1	22.7±1.5	28.0±1.7
7	MUT 1896	0.0	3.0±0.0	4.0±0.0	7.0±0.0	9.3±0.6	14.7±0.6	20.0±2.0	25.3±0.6
9	MUT 1309	3.3±0.6	8.0±1.7	10.7±2.0	13.3±4.0	10.7±2.1	18.0±1.7	25.0±2.0	32.3±2.5
10	MUT 798	0.0	0.0	2.0±1.7	3.0±3.0	10.3±0.6	17.3±0.6	23.3±0.6	29.7±0.6
11	MUT 1314	0.0	3.3±2.9	6.7±1.5	8.3±2.9	10.3±1.2	15.7±0.6	21.7±0.6	26.3±0.6
12	MUT 1307	0.0	0.0	3.0±0.0	3.7±1.2	10.3±1.2	18.3±1.5	25.0±2.0	31.0±2.0
13	MUT 1294	1.0±1.7	5.7±0.6	7.7±0.6	10.7±0.6	3.0±5.2	8.0±7.2	12.0±10.4	15.7±13.7
14	MUT 1306	1.0±1.7	3.7±1.2	6.0±2.6	8.7±3.8	8.7±3.2	16.7±3.2	27.0±1.0	29.7±0.6
16	MUT 1316	3.0±0.0	6.3±0.6	9.0±1.0	12.0±1.0	9.2±3.5	15.3±0.6	19.0±0.0	24.3±0.6
17	MUT 1303	0.0	0.0	5.0±0.0	5.3±0.7	4.7±2.9	11.0±1.7	19.0±2.0	24.7±0.6
18	MUT 1313	0.0	4.3±1.2	6.3±1.2	8.0±1.7	6.7±1.5	13.0±1.0	17.7±1.5	23.7±2.5
19	MUT 1298	4.0±0.0	9.0±1.0	14.3±2.1	20.0±2.0	8.7±3.2	15.3±2.1	21.0±2.6	27.0±2.0
20	MUT 1311	0.0	1.7±2.9	4.0±4.6	6.3±4.2	12.3±0.6	14.3±1.2	19.7±0.6	25.0±0.0
21	MUT 1322	0.0	0.0	3.0±0.0	3.0±0.0	9.0±1.0	14.0±1.0	20.3±1.5	25.3±1.5
22	MUT 1305	0.0	2.0±3.5	5.7±1.2	6.3±1.5	11.0±1.0	16.3±0.6	21.3±0.6	27.0±1.0
23	MUT 1293	3.0±0.0	5.7±2.1	8.3±2.5	11.3±2.5	14.3±1.2	14.7±1.2	19.3±4.2	23.0±5.3
24	MUT 1315	0.0	0.0	2.3±2.1	2.7±2.5	11.3±1.2	19.7±0.6	28.3±0.6	32.7±1.2
25	MUT 1300	2.0±1.7	3.7±3.2	5.7±1.2	7.0±2.0	10.7±6.7	14.3±4.0	21.3±3.5	27.0±3.0
26	MUT 1304	0.0	0.0	3.0±2.6	4.3±4.0	8.0±2.6	17.0±2.6	22.3±3.8	27.3±4.0

B – Different growth condition of *Eurotium halophilicum*: results about incubation temperatures.

Table B4 Growth of *E. halophilicum* strains at different incubation temperatures during 4 weeks. Diameters are expressed in mm; W: week.

Strain	MUT number	17 °C				22 °C			
		1W	2 W	3 W	4 W	1W	2 W	3 W	4 W
3	MUT 1916	3.0±0.0	6.0±0.0	6.8±0.3	7.0±0.0	5.8±0.3	9.3±0.6	14.8±4.5	19.0±4.4
5	MUT 1922	0.0	5.7±0.3	7.0±0.0	7.0±0.0	4.7±0.3	8.7±0.3	12.0±1.0	15.0±1.3
7	MUT 1896	0.0	6.0±0.0	7.0±0.0	7.0±0.0	4.5±0.0	7.8±0.3	10.5±0.9	11.3±2.3
9	MUT 1309	1.1±1.7	4.2±1.0	6.5±1.3	8.0±0.5	5.7±0.6	8.8±0.3	12.3±1.2	15.7±0.6
10	MUT 798	4.2±1.0	5.5±1.3	7.7±0.6	9.2±0.3	3.0±0.0	6.8±0.8	10.7±0.8	13.3±0.6
11	MUT 1314	3.0±0.0	5.0±0.0	8.0±0.0	8.7±0.3	4.5±0.0	7.0±0.0	9.3±0.3	10.2±0.3
12	MUT 1307	0.0	3.3±0.6	6.3±0.6	6.8±0.6	4.3±0.6	7.8±1.0	12.8±0.3	16.2±0.6
13	MUT 1294	2.7±2.5	4.7±2.1	7.3±2.5	8.2±2.3	3.7±3.3	7.5±3.3	10.7±3.8	13.0±5.2
14	MUT 1306	0.0	3.7±0.6	6.5±0.5	7.7±0.6	3.5±0.9	6.0±0.5	9.8±0.3	12.2±0.8
16	MUT 1316	2.7±2.3	4.7±0.6	6.7±0.6	7.5±0.5	5.8±0.3	7.2±0.3	9.7±0.6	10.7±1.0
17	MUT 1303	1.0±1.7	4.7±0.6	6.8±0.3	8.0±0.0	5.3±0.6	6.8±0.3	8.0±0.0	8.8±0.6
18	MUT 1313	0.0	2.0±1.7	4.0±1.0	5.5±0.0	3.0±0.0	4.2±0.3	5.0±0.0	5.7±0.8
19	MUT 1298	4.7±0.3	6.0±0.0	7.5±0.9	7.8±0.3	5.8±0.3	7.2±0.3	8.0±0.0	8.5±0.5
20	MUT 1311	1.3±2.3	4.5±0.5	7.3±0.6	8.2±0.8	3.5±0.9	5.2±1.3	8.3±1.0	11.3±1.3
21	MUT 1322	1.0±1.7	4.5±0.0	6.3±0.3	7.0±0.0	5.0±0.0	6.0±0.0	8.2±0.3	8.8±0.3
22	MUT 1305	3.0±0.0	5.0±0.0	7.0±0.0	8.0±0.0	3.8±0.8	6.3±0.6	8.7±0.3	9.3±0.6
23	MUT 1293	3.0±0.0	3.7±1.2	5.8±1.8	6.7±1.2	2.0±1.7	5.5±0.5	8.0±0.9	8.8±0.8
24	MUT 1315	0.0	4.2±0.2	7.0±0.5	7.8±1.2	2.0±1.7	7.8±3.4	15.8±5.2	22.3±3.8
25	MUT 1300	3.0±0.0	4.7±0.6	6.7±0.6	7.8±0.3	5.2±1.2	7.2±0.3	9.2±0.3	10.2±1.2
26	MUT 1304	3.0±0.0	4.8±0.3	6.8±0.3	7.7±0.3	5.2±0.8	7.3±0.8	9.7±0.6	11.3±1.5

Strain	MUT number	25 °C				28 °C			
		1W	2 W	3 W	4 W	1W	2 W	3 W	4 W
3	MUT 1916	7.3±0.6	16.7±0.6	22.3±1.5	26.3±0.6	4.0±0.0	7.5±0.5	8.0±0.0	9.0±1.0
5	MUT 1922	9.0±1.7	17.3±2.1	22.7±1.5	28.0±1.7	4.8±0.3	9.7±0.6	13.7±0.3	16.8±0.3
7	MUT 1896	9.3±0.6	14.7±0.6	20.0±2.0	25.3±0.6	0.0	1.0±1.7	3.0±0.0	3.0±0.0
9	MUT 1309	10.7±2.1	18.0±1.7	25.0±2.0	32.3±2.5	0.0	0.0	3.0±3.0	5.2±2.9
10	MUT 798	10.3±0.6	17.3±0.6	23.3±0.6	29.7±0.6	0.0	0.0	1.0±1.7	1.0±1.7
11	MUT 1314	10.3±1.2	15.7±0.6	21.7±0.6	26.3±0.6	0.0	0.0	0.0	0.0
12	MUT 1307	10.3±1.2	18.3±1.5	25.0±2.0	31.0±2.0	0.0	0.0	0.0	0.0
13	MUT 1294	3.0±5.2	8.0±7.2	12.0±10.4	15.7±13.7	1.0±1.7	1.5±2.6	1.7±2.9	1.7±2.9
14	MUT 1306	8.7±3.2	16.7±3.2	27.0±1.0	29.7±0.6	3.0±0.0	5.0±1.0	6.0±1.0	8.0±1.0
16	MUT 1316	9.2±3.5	15.3±0.6	19.0±0.0	24.3±0.6	1.0±1.7	1.3±2.3	1.7±2.9	1.7±2.9
17	MUT 1303	4.7±2.9	11.0±1.7	19.0±2.0	24.7±0.6	0.0	0.0	0.0	0.0
18	MUT 1313	6.7±1.5	13.0±1.0	17.7±1.5	23.7±2.5	3.0±0.0	3.7±1.2	3.7±1.2	3.7±1.2
19	MUT 1298	8.7±3.2	15.3±2.1	21.0±2.6	27.0±2.0	1.0±1.7	1.7±2.9	2.7±2.5	4.0±1.7
20	MUT 1311	12.3±0.6	14.3±1.2	19.7±0.6	25.0±0.0	2.0±1.7	3.0±0.0	3.0±0.0	3.0±0.0
21	MUT 1322	9.0±1.0	14.0±1.0	20.3±1.5	25.3±1.5	3.0±0.0	3.0±0.0	3.0±0.0	3.3±0.6
22	MUT 1305	11.0±1.0	16.3±0.6	21.3±0.6	27.0±1.0	0.0	0.0	1.0±1.7	2.0±1.7
23	MUT 1293	14.3±1.2	14.7±1.2	19.3±4.2	23.0±5.3	0.0	0.0	2.0±1.7	3.0±0.0
24	MUT 1315	11.3±1.2	19.7±0.6	28.3±0.6	32.7±1.2	7.5±0.0	13.2±0.8	15.7±1.8	18.3±2.1
25	MUT 1300	10.7±6.7	14.3±4.0	21.3±3.5	27.0±3.0	0.0	1.0±1.7	1.3±2.3	1.3±2.3
26	MUT 1304	8.0±2.6	17.0±2.6	22.3±3.8	27.3±4.0	3.7±0.6	5.2±1.9	6.3±0.3	7.7±1.5

Appendix B4

BOD data of selected fungal species

Blank MEA medium. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	4920	45.1	9840	50.7	14760	50.7	19680	62	24600	73.2	29520	67.6	34440	73.2	39360	73.2
120	11.3	5040	50.7	9960	50.7	14880	56.3	19800	62	24720	67.6	29640	62	34560	73.2	39480	67.6
240	28.2	5160	39.4	10080	56.3	15000	50.7	19920	56.3	24840	62	29760	73.2	34680	67.6	39600	73.2
360	28.2	5280	50.7	10200	56.3	15120	56.3	20040	56.3	24960	73.2	29880	67.6	34800	73.2	39720	73.2
480	28.2	5400	45.1	10320	56.3	15240	50.7	20160	56.3	25080	67.6	30000	67.6	34920	73.2	39840	73.2
600	28.2	5520	45.1	10440	56.3	15360	56.3	20280	56.3	25200	73.2	30120	67.6	35040	67.6	39960	73.2
720	28.2	5640	45.1	10560	50.7	15480	50.7	20400	62	25320	67.6	30240	73.2	35160	73.2	40080	73.2
840	45.1	5760	50.7	10680	50.7	15600	56.3	20520	62	25440	62	30360	73.2	35280	78.9	40320	73.2
960	45.1	5880	50.7	10800	50.7	15720	50.7	20640	56.3	25560	67.6	30480	73.2	35400	78.9	40440	73.2
1080	45.1	6000	50.7	10920	56.3	15840	56.3	20760	56.3	25680	73.2	30600	73.2	35520	78.9	40560	73.2
1200	45.1	6120	50.7	11040	56.3	15960	56.3	20880	56.3	25800	67.6	30720	67.6	35640	73.2	40680	73.2
1320	45.1	6240	50.7	11160	56.3	16080	56.3	21000	56.3	25920	62	30840	67.6	35760	73.2	40800	78.9
1440	45.1	6360	50.7	11280	50.7	16200	50.7	21120	56.3	26040	73.2	30960	67.6	35880	73.2	40920	73.2
1560	45.1	6480	50.7	11400	50.7	16320	56.3	21240	56.3	26160	73.2	31080	73.2	36000	78.9	41040	67.6
1680	45.1	6600	50.7	11520	50.7	16440	62	21360	56.3	26280	67.6	31200	73.2	36120	73.2	41160	78.9
1800	45.1	6720	50.7	11640	56.3	16560	62	21480	56.3	26400	67.6	31320	67.6	36240	73.2	41280	73.2
1920	45.1	6840	50.7	11760	62	16680	56.3	21600	56.3	26520	73.2	31440	73.2	36360	73.2	41400	73.2
2040	45.1	6960	45.1	11880	50.7	16800	56.3	21720	62	26640	62	31560	73.2	36480	67.6	41520	73.2
2160	50.7	7080	50.7	12000	56.3	16920	62	21840	62	26760	73.2	31680	67.6	36600	73.2	41640	78.9
2280	45.1	7200	45.1	12120	62	17040	62	21960	56.3	26880	73.2	31800	73.2	36720	73.2	41760	78.9
2400	50.7	7320	45.1	12240	62	17160	62	22080	56.3	27000	62	31920	67.6	36840	73.2	41880	73.2
2520	45.1	7440	45.1	12360	62	17280	56.3	22200	62	27120	73.2	32040	73.2	36960	73.2	42000	78.9
2640	45.1	7560	45.1	12480	56.3	17400	56.3	22320	67.6	27240	73.2	32160	73.2	37080	73.2	42120	73.2
2760	45.1	7680	50.7	12600	56.3	17520	56.3	22440	56.3	27360	67.6	32280	73.2	37200	73.2	42240	73.2
2880	45.1	7800	56.3	12720	56.3	17640	62	22560	62	27480	62	32400	67.6	37320	67.6	42360	73.2
3000	45.1	7920	50.7	12840	50.7	17760	62	22680	62	27600	62	32520	73.2	37440	67.6	42480	73.2
3120	45.1	8040	50.7	12960	50.7	17880	62	22800	67.6	27720	67.6	32640	73.2	37560	73.2	42600	73.2
3240	45.1	8160	56.3	13080	56.3	18000	67.6	22920	62	27840	67.6	32760	73.2	37680	67.6	42720	73.2
3360	45.1	8280	50.7	13200	56.3	18120	67.6	23040	62	27960	67.6	32880	73.2	37800	73.2	42840	78.9
3480	45.1	8400	56.3	13320	50.7	18240	62	23160	67.6	28080	73.2	33000	67.6	37920	73.2	42960	78.9
3600	45.1	8520	50.7	13440	56.3	18360	56.3	23280	67.6	28200	73.2	33120	67.6	38040	73.2	43080	73.2
3720	45.1	8640	50.7	13560	50.7	18480	56.3	23400	73.2	28320	67.6	33240	73.2	38160	67.6	43200	73.2
3840	45.1	8760	50.7	13680	56.3	18600	62	23520	67.6	28440	67.6	33360	67.6	38280	73.2		
3960	45.1	8880	50.7	13800	50.7	18720	56.3	23640	62	28560	73.2	33480	78.9	38400	73.2		
4080	45.1	9000	45.1	13920	56.3	18840	62	23760	67.6	28680	73.2	33600	84.5	38520	73.2		
4200	39.4	9120	50.7	14040	50.7	18960	56.3	23880	67.6	28800	67.6	33720	84.5	38640	67.6		
4320	50.7	9240	50.7	14160	50.7	19080	62	24000	67.6	28920	67.6	33840	78.9	38760	67.6		
4440	45.1	9360	50.7	14280	56.3	19200	62	24120	62	29040	67.6	33960	84.5	38880	78.9		
4560	45.1	9480	50.7	14400	56.3	19320	56.3	24240	67.6	29160	62	34080	73.2	39000	73.2		
4680	50.7	9600	56.3	14520	45.1	19440	56.3	24360	73.2	29280	62	34200	67.6	39120	67.6		
4800	45.1	9720	50.7	14640	50.7	19560	56.3	24480	62	29400	73.2	34320	73.2	39240	73.2		

Blank MEA15% medium. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mg/l	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	5400	28.2	10800	33.8	16200	33.8	21600	39.4	27000	45.1	32400	45.1	37800	39.4
120	-5.6	5520	28.2	10920	28.2	16320	39.4	21720	33.8	27120	45.1	32520	45.1	37920	45.1
240	11.3	5640	28.2	11040	39.4	16440	39.4	21840	33.8	27240	50.7	32640	39.4	38040	45.1
360	16.9	5760	28.2	11160	39.4	16560	39.4	21960	39.4	27360	45.1	32760	33.8	38160	45.1
480	22.5	5880	28.2	11280	33.8	16680	39.4	22080	39.4	27480	45.1	32880	39.4	38280	39.4
600	22.5	6000	33.8	11400	33.8	16800	39.4	22200	39.4	27600	39.4	33000	39.4	38400	39.4
720	22.5	6120	33.8	11520	33.8	16920	39.4	22320	33.8	27720	45.1	33120	45.1	38520	45.1
840	22.5	6240	33.8	11640	39.4	17040	39.4	22440	33.8	27840	39.4	33240	45.1	38640	45.1
960	22.5	6360	33.8	11760	33.8	17160	33.8	22560	39.4	27960	45.1	33360	45.1	38760	39.4
1080	28.2	6480	28.2	11880	33.8	17280	39.4	22680	39.4	28080	39.4	33480	45.1	38880	39.4
1200	22.5	6600	28.2	12000	39.4	17400	39.4	22800	33.8	28200	39.4	33600	56.3	39000	45.1
1320	22.5	6720	33.8	12120	39.4	17520	39.4	22920	39.4	28320	39.4	33720	56.3	39120	39.4
1440	22.5	6840	28.2	12240	39.4	17640	33.8	23040	39.4	28440	45.1	33840	56.3	39240	45.1
1560	28.2	6960	33.8	12360	33.8	17760	45.1	23160	39.4	28560	45.1	33960	50.7	39360	45.1
1680	28.2	7080	33.8	12480	39.4	17880	39.4	23280	33.8	28680	39.4	34080	50.7	39480	45.1
1800	28.2	7200	33.8	12600	39.4	18000	39.4	23400	33.8	28800	45.1	34200	45.1	39600	45.1
1920	28.2	7320	33.8	12720	39.4	18120	33.8	23520	33.8	28920	39.4	34320	39.4	39720	45.1
2040	28.2	7440	33.8	12840	33.8	18240	33.8	23640	33.8	29040	33.8	34440	33.8	39840	45.1
2160	22.5	7560	33.8	12960	33.8	18360	33.8	23760	39.4	29160	39.4	34560	33.8	39960	45.1
2280	28.2	7680	28.2	13080	28.2	18480	39.4	23880	33.8	29280	39.4	34680	45.1	40080	45.1
2400	22.5	7800	28.2	13200	33.8	18600	39.4	24000	39.4	29400	33.8	34800	39.4	40200	45.1
2520	28.2	7920	39.4	13320	28.2	18720	39.4	24120	39.4	29520	45.1	34920	45.1	40320	45.1
2640	28.2	8040	28.2	13440	33.8	18840	39.4	24240	45.1	29640	39.4	35040	45.1	40440	45.1
2760	28.2	8160	33.8	13560	33.8	18960	33.8	24360	39.4	29760	45.1	35160	39.4	40560	39.4
2880	28.2	8280	28.2	13680	28.2	19080	39.4	24480	45.1	29880	45.1	35280	39.4	40680	45.1
3000	28.2	8400	33.8	13800	33.8	19200	33.8	24600	39.4	30000	39.4	35400	45.1	40800	39.4
3120	22.5	8520	33.8	13920	28.2	19320	39.4	24720	45.1	30120	39.4	35520	39.4	40920	45.1
3240	22.5	8640	33.8	14040	33.8	19440	33.8	24840	45.1	30240	45.1	35640	39.4	41040	45.1
3360	28.2	8760	28.2	14160	33.8	19560	39.4	24960	39.4	30360	45.1	35760	45.1	41160	39.4
3480	22.5	8880	33.8	14280	28.2	19680	39.4	25080	45.1	30480	45.1	35880	45.1	41280	45.1
3600	22.5	9000	33.8	14400	28.2	19800	33.8	25200	39.4	30600	39.4	36000	45.1	41400	45.1
3720	28.2	9120	28.2	14520	28.2	19920	33.8	25320	39.4	30720	45.1	36120	45.1	41520	45.1
3840	28.2	9240	33.8	14640	33.8	20040	39.4	25440	45.1	30840	45.1	36240	45.1	41640	39.4
3960	28.2	9360	28.2	14760	28.2	20160	33.8	25560	45.1	30960	45.1	36360	39.4	41760	45.1
4080	22.5	9480	33.8	14880	28.2	20280	33.8	25680	39.4	31080	39.4	36480	39.4	41880	45.1
4200	28.2	9600	28.2	15000	28.2	20400	39.4	25800	45.1	31200	45.1	36600	45.1	42000	50.7
4320	28.2	9720	33.8	15120	28.2	20520	33.8	25920	45.1	31320	45.1	36720	45.1	42120	45.1
4440	33.8	9840	28.2	15240	33.8	20640	33.8	26040	39.4	31440	39.4	36840	45.1	42240	45.1
4560	16.9	9960	28.2	15360	28.2	20760	39.4	26160	50.7	31560	45.1	36960	39.4	42360	45.1
4680	28.2	10080	33.8	15480	33.8	20880	33.8	26280	50.7	31680	45.1	37080	39.4	42480	45.1
4800	33.8	10200	33.8	15600	28.2	21000	33.8	26400	45.1	31800	39.4	37200	39.4	42600	45.1
4920	33.8	10320	28.2	15720	33.8	21120	33.8	26520	50.7	31920	39.4	37320	39.4	42720	45.1
5040	22.5	10440	33.8	15840	28.2	21240	33.8	26640	39.4	32040	45.1	37440	45.1	42840	45.1
5160	28.2	10560	33.8	15960	33.8	21360	33.8	26760	45.1	32160	39.4	37560	39.4	42960	45.1
5280	28.2	10680	33.8	16080	28.2	21480	39.4	26880	50.7	32280	39.4	37680	45.1	43080	45.1

Aspergillus creber. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	5040	676	10680	1211	15120	1189	20160	1200	25200	1177	30240	1228	35280	1200
120	0	5160	715	10800	1217	15240	1189	20280	1206	25320	1177	30360	1234	35400	1200
240	16.9	5280	749	10920	1211	15360	1183	20400	1211	25440	1177	30480	1239	35520	1194
360	22.5	5400	783	11040	1211	15480	1177	20520	1211	25560	1172	30600	1234	35640	1189
480	28.2	5520	817	11160	1206	15600	1177	20640	1211	25680	1172	30720	1228	35760	1189
600	33.8	5640	856	10080	1189	15720	1177	20760	1217	25800	1177	30840	1228	35880	1194
720	39.4	5760	907	10200	1200	15840	1189	20880	1217	25920	1189	30960	1234	36000	1234
840	39.4	5880	952	10320	1206	15960	1200	21000	1217	26040	1200	31080	1228	36120	1234
960	33.8	6000	986	10440	1211	16080	1200	21120	1206	26160	1206	31200	1228	36240	1206
1080	33.8	6120	1003	10560	1211	16200	1200	21240	1206	26280	1206	31320	1222		
1200	33.8	6240	1037	11280	1206	16320	1194	21360	1200	26400	1200	31440	1222		
1320	33.8	6360	1076	11400	1206	16440	1189	21480	1194	26520	1200	31560	1217		
1440	33.8	6480	1110	11520	1206	16560	1194	21600	1194	26640	1206	31680	1217		
1560	28.2	6600	1132	11640	1206	16680	1189	21720	1189	26760	1206	31800	1217		
1680	22.5	6720	1138	11760	1200	16800	1183	21840	1183	26880	1200	31920	1211		
1800	11.3	6840	1138	11880	1194	16920	1177	21960	1177	27000	1200	32040	1206		
1920	5.6	6960	1138	12000	1177	17040	1177	22080	1172	27120	1200	32160	1194		
2040	5.6	7080	1144	12120	1166	17160	1177	22200	1166	27240	1222	32280	1189		
2160	5.6	7200	1155	12240	1166	17280	1189	22320	1166	27360	1234	32400	1183		
2280	16.9	7320	1166	12360	1166	17400	1194	22440	1166	27480	1228	32520	1183		
2400	28.2	7440	1172	12480	1166	17520	1194	22560	1161	27600	1222	32640	1183		
2520	39.4	7560	1172	12600	1172	17640	1183	22680	1161	27720	1206	32760	1183		
2640	50.7	7680	1172	12720	1172	17760	1177	22800	1161	27840	1206	32880	1189		
2760	67.6	7800	1172	12840	1172	17880	1177	22920	1161	27960	1211	33000	1189		
2880	84.5	7920	1172	12960	1172	18000	1183	23040	1161	28080	1217	33120	1183		
3000	101	8040	1172	13080	1172	18120	1183	23160	1161	28200	1217	33240	1183		
3120	118	8160	1166	13200	1166	18240	1183	23280	1155	28320	1217	33360	1183		
3240	135	8280	1161	13320	1161	18360	1177	23400	1149	28440	1211	33480	1177		
3360	152	8400	1161	13440	1144	18480	1177	23520	1144	28560	1206	33600	1166		
3480	169	8520	1166	13560	1138	18600	1194	23640	1138	28680	1211	33720	1161		
3600	197	8640	1200	13680	1138	18720	1211	23760	1138	28800	1217	33840	1155		
3720	225	8760	1194	13800	1138	18840	1211	23880	1138	28920	1222	33960	1161		
3840	259	8880	1189	13920	1138	18960	1206	24000	1138	29040	1222	34080	1161		
3960	299	9000	1172	14040	1138	19080	1206	24120	1138	29160	1222	34200	1161		
4080	332	9120	1161	14160	1144	19200	1200	24240	1138	29280	1222	34320	1161		
4200	406	9240	1172	14280	1149	19320	1200	24360	1144	29400	1222	34440	1166		
4320	468	9360	1183	14400	1161	19440	1206	24480	1155	29520	1222	34560	1177		
4440	513	9480	1183	14520	1172	19560	1200	24600	1166	29640	1222	34680	1189		
4560	535	9600	1183	14640	1177	19680	1200	24720	1177	29760	1217	34800	1194		
4680	558	9720	1177	14760	1183	19800	1194	24840	1177	29880	1217	34920	1194		
4800	592	9840	1177	14880	1183	19920	1189	24960	1177	30000	1217	35040	1194		
4920	631	9960	1177	15000	1189	20040	1189	25080	1177	30120	1217	35160	1194		

Aspergillus penicillioides. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	4700	118	9400	558	14100	1183	18800	1200	23500	1194	28200	1206	32900	1206
100	5.6	4800	124	9500	575	14200	1194	18900	1200	23600	1200	28300	1206	33000	1211
200	5.6	4900	130	9600	586	14300	1200	19000	1200	23700	1194	28400	1206	33100	1200
300	5.6	5000	135	9700	603	14400	1200	19100	1200	23800	1194	28500	1206	33200	1194
400	0	5100	141	9800	620	14500	1200	19200	1200	23900	1200	28600	1206	33300	1189
500	0	5200	141	9900	637	14600	1200	19300	1200	24000	1200	28700	1206	33400	1194
600	0	5300	152	10000	637	14700	1200	19400	1200	24100	1200	28800	1200	33500	1189
700	5.6	5400	158	10100	670	14800	1194	19500	1200	24200	1200	28900	1206	33600	1189
800	16.9	5500	163	10200	687	14900	1200	19600	1200	24300	1200	29000	1206	33700	1189
900	22.5	5600	180	10300	699	15000	1200	19700	1200	24400	1200	29100	1206	33800	1189
1000	28.2	5700	163	10400	715	15100	1200	19800	1200	24500	1200	29200	1206	33900	1189
1100	33.8	5800	186	10500	727	15200	1194	19900	1206	24600	1194	29300	1206	34000	1189
1200	33.8	5900	192	10600	744	15300	1200	20000	1206	24700	1194	29400	1206	34100	1189
1300	22.5	6000	197	10700	755	15400	1200	20100	1200	24800	1194	29500	1206	34200	1189
1400	22.5	6100	203	10800	772	15500	1200	20200	1206	24900	1194	29600	1206	34300	1189
1500	22.5	6200	208	10900	789	15600	1200	20300	1200	25000	1189	29700	1206	34400	1189
1600	16.9	6300	214	11000	806	15700	1200	20400	1200	25100	1194	29800	1206	34500	1189
1700	22.5	6400	225	11100	817	15800	1200	20500	1200	25200	1194	29900	1206	34600	1189
1800	28.2	6500	237	11200	834	15900	1200	20600	1206	25300	1194	30000	1206	34700	1189
1900	28.2	6600	242	11300	845	16000	1194	20700	1200	25400	1194	30100	1206	34800	1194
2000	33.8	6700	254	11400	868	16100	1200	20800	1200	25500	1194	30200	1206	34900	1194
2100	33.8	6800	259	11500	873	16200	1206	20900	1200	25600	1194	30300	1200	35000	1189
2200	39.4	6900	270	11600	896	16300	1200	21000	1200	25700	1194	30400	1200	35100	1189
2300	50.7	7000	276	11700	907	16400	1200	21100	1206	25800	1200	30500	1200	35200	1189
2400	56.3	7100	287	11800	924	16500	1200	21200	1200	25900	1200	30600	1200	35300	1189
2500	62	7200	293	11900	935	16600	1200	21300	1200	26000	1194	30700	1200	35400	1189
2600	67.6	7300	304	12000	952	16700	1200	21400	1206	26100	1194	30800	1200	35500	1189
2700	62	7400	310	12100	963	16800	1200	21500	1206	26200	1194	30900	1200	35600	1189
2800	84.5	7500	327	12200	980	16900	1206	21600	1200	26300	1200	31000	1200	35700	1189
2900	78.9	7600	338	12300	992	17000	1200	21700	1206	26400	1200	31100	1200	35800	1194
3000	67.6	7700	344	12400	1003	17100	1200	21800	1206	26500	1200	31200	1206	35900	1194
3100	62	7800	355	12500	1020	17200	1200	21900	1206	26600	1200	31300	1206	36000	1189
3200	56.3	7900	372	12600	1037	17300	1200	22000	1206	26700	1200	31400	1206		
3300	56.3	8000	377	12700	1048	17400	1200	22100	1200	26800	1206	31500	1206		
3400	56.3	8100	389	12800	1065	17500	1200	22200	1206	26900	1206	31600	1206		
3500	62	8200	400	12900	1076	17600	1200	22300	1200	27000	1206	31700	1206		
3600	62	8300	411	13000	1093	17700	1200	22400	1206	27100	1200	31800	1206		
3700	67.6	8400	428	13100	1110	17800	1200	22500	1206	27200	1206	31900	1206		
3800	73.2	8500	434	13200	1110	17900	1200	22600	1206	27300	1206	32000	1206		
3900	73.2	8600	451	13300	1115	18000	1200	22700	1206	27400	1200	32100	1200		
4000	78.9	8700	462	13400	1132	18100	1206	22800	1211	27500	1200	32200	1206		
4100	95.8	8800	468	13500	1144	18200	1200	22900	1211	27600	1206	32300	1206		
4200	101	8900	490	13600	1155	18300	1200	23000	1206	27700	1200	32400	1206		
4300	95.8	9000	496	13700	1161	18400	1200	23100	1206	27800	1206	32500	1206		
4400	113	9100	513	13800	1172	18500	1200	23200	1200	27900	1206	32600	1206		
4500	113	9200	530	13900	1177	18600	1200	23300	1200	28000	1200	32700	1206		
4600	113	9300	541	14000	1183	18700	1200	23400	1200	28100	1206	32800	1206		

Cladosporium cladosporioides. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mg/l	Time (min)	mgL^{-1}
0	0	5280	220	10560	986	15840	1144	21120	1149	26400	1144	31680	1189
120	0	5400	231	10680	997	15960	1144	21240	1144	26520	1138	31800	1200
240	0	5520	248	10800	1014	16080	1138	21360	1144	26640	1138	31920	1206
360	0	5640	259	10920	1025	16200	1127	21480	1144	26760	1138	32040	1194
480	0	5760	276	11040	1031	16320	1121	21600	1155	26880	1144	32160	1172
600	0	5880	293	11160	1042	16440	1115	21720	1166	27000	1144	32280	1166
720	5.6	6000	304	11280	1048	16560	1115	21840	1172	27120	1149	32400	1166
840	5.6	6120	321	11400	1059	16680	1115	21960	1166	27240	1149	32520	1166
960	0	6240	327	11520	1076	16800	1115	22080	1161	27360	1155	32640	1172
1080	0	6360	338	11640	1093	16920	1115	22200	1161	27480	1155	32760	1177
1200	5.6	6480	355	11760	1104	17040	1115	22320	1172	27600	1155	32880	1183
1320	5.6	6600	377	11880	1115	17160	1121	22440	1177	27720	1149	33000	1183
1440	5.6	6720	394	12000	1121	17280	1121	22560	1177	27840	1138	33120	1194
1560	11.3	6840	417	12120	1132	17400	1121	22680	1183	27960	1138		
1680	16.9	6960	434	12240	1144	17520	1115	22800	1189	28080	1138		
1800	16.9	7080	456	12360	1149	17640	1110	22920	1189	28200	1144		
1920	16.9	7200	473	12480	1149	17760	1104	23040	1200	28320	1149		
2040	22.5	7320	490	12600	1149	17880	1104	23160	1206	28440	1149		
2160	28.2	7440	507	12720	1149	18000	1104	23280	1217	28560	1155		
2280	28.2	7560	524	12840	1149	18120	1104	23400	1217	28680	1161		
2400	28.2	7680	530	12960	1172	18240	1104	23520	1217	28800	1161		
2520	28.2	7800	546	13080	1172	18360	1104	23640	1194	28920	1161		
2640	28.2	7920	563	13200	1166	18480	1104	23760	1206	29040	1155		
2760	28.2	8040	580	13320	1166	18600	1110	23880	1211	29160	1149		
2880	39.4	8160	603	13440	1172	18720	1121	24000	1222	29280	1144		
3000	50.7	8280	620	13560	1177	18840	1127	24120	1222	29400	1144		
3120	62	8400	637	13680	1183	18960	1132	24240	1217	29520	1144		
3240	56.3	8520	659	13800	1172	19080	1138	24360	1217	29640	1144		
3360	62	8640	715	13920	1166	19200	1138	24480	1234	29760	1149		
3480	73.2	8760	744	14040	1166	19320	1138	24600	1228	29880	1155		
3600	67.6	8880	766	14160	1161	19440	1144	24720	1211	30000	1161		
3720	73.2	9000	789	14280	1166	19560	1144	24840	1194	30120	1166		
3840	78.9	9120	811	14400	1177	19680	1144	24960	1166	30240	1177		
3960	84.5	9240	822	14520	1177	19800	1138	25080	1161	30360	1183		
4080	90.1	9360	839	14640	1177	19920	1138	25200	1161	30480	1194		
4200	95.8	9480	851	14760	1166	20040	1138	25320	1166	30600	1189		
4320	113	9600	862	14880	1149	20160	1144	25440	1166	30720	1177		
4440	130	9720	873	15000	1149	20280	1149	25560	1166	30840	1172		
4560	146	9840	884	15120	1149	20400	1155	25680	1166	30960	1172		
4680	158	9960	896	15240	1155	20520	1155	25800	1166	31080	1172		
4800	169	10080	918	15360	1149	20640	1155	25920	1166	31200	1177		
4920	180	10200	941	15480	1149	20760	1155	26040	1161	31320	1177		
5040	197	10320	958	15600	1149	20880	1155	26160	1161	31440	1177		
5160	214	10440	975	15720	1149	21000	1155	26280	1149	31560	1183		

Eurotium chevalieri. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	4500	676	9000	1177	13500	1177	18000	1183	22500	1183	27000	1189	31500	1189
100	0	4600	710	9100	1177	13600	1177	18100	1183	22600	1183	27100	1189	31600	1189
200	11.3	4700	744	9200	1177	13700	1177	18200	1183	22700	1183	27200	1189	31700	1189
300	11.3	4800	777	9300	1172	13800	1177	18300	1183	22800	1189	27300	1189	31800	1189
400	11.3	4900	817	9400	1177	13900	1177	18400	1177	22900	1194	27400	1189	31900	1189
500	5.6	5000	851	9500	1177	14000	1177	18500	1183	23000	1183	27500	1189	32000	1189
600	5.6	5100	879	9600	1172	14100	1177	18600	1183	23100	1189	27600	1189	32100	1189
700	11.3	5200	913	9700	1177	14200	1183	18700	1183	23200	1183	27700	1189	32200	1189
800	16.9	5300	946	9800	1177	14300	1183	18800	1183	23300	1183	27800	1189	32300	1189
900	22.5	5400	975	9900	1177	14400	1183	18900	1177	23400	1183	27900	1189	32400	1189
1000	28.2	5500	1008	10000	1161	14500	1183	19000	1183	23500	1177	28000	1189	32500	1189
1100	33.8	5600	1037	10100	1177	14600	1183	19100	1183	23600	1177	28100	1189	32600	1189
1200	28.2	5700	1042	10200	1183	14700	1183	19200	1177	23700	1177	28200	1189	32700	1189
1300	22.5	5800	1082	10300	1177	14800	1177	19300	1183	23800	1177	28300	1189	32800	1189
1400	22.5	5900	1099	10400	1177	14900	1183	19400	1183	23900	1183	28400	1189	32900	1189
1500	22.5	6000	1121	10500	1177	15000	1183	19500	1177	24000	1183	28500	1189	33000	1200
1600	16.9	6100	1138	10600	1177	15100	1183	19600	1183	24100	1183	28600	1189	33100	1183
1700	22.5	6200	1155	10700	1183	15200	1183	19700	1183	24200	1183	28700	1189	33200	1177
1800	22.5	6300	1161	10800	1183	15300	1183	19800	1183	24300	1177	28800	1189	33300	1172
1900	28.2	6400	1166	10900	1177	15400	1183	19900	1183	24400	1183	28900	1189	33400	1177
2000	33.8	6500	1172	11000	1183	15500	1183	20000	1183	24500	1183	29000	1189	33500	1172
2100	45.1	6600	1172	11100	1183	15600	1183	20100	1183	24600	1177	29100	1189	33600	1172
2200	56.3	6700	1177	11200	1177	15700	1183	20200	1183	24700	1177	29200	1189	33700	1172
2300	73.2	6800	1172	11300	1183	15800	1177	20300	1183	24800	1177	29300	1189	33800	1172
2400	90.1	6900	1177	11400	1183	15900	1183	20400	1183	24900	1177	29400	1189	33900	1177
2500	113	7000	1177	11500	1177	16000	1177	20500	1177	25000	1177	29500	1189	34000	1177
2600	124	7100	1177	11600	1177	16100	1177	20600	1183	25100	1177	29600	1189	34100	1172
2700	141	7200	1172	11700	1183	16200	1183	20700	1177	25200	1177	29700	1189	34200	1172
2800	175	7300	1172	11800	1183	16300	1183	20800	1183	25300	1177	29800	1189	34300	1172
2900	192	7400	1172	11900	1183	16400	1183	20900	1183	25400	1177	29900	1189	34400	1172
3000	208	7500	1177	12000	1177	16500	1183	21000	1183	25500	1177	30000	1189	34500	1177
3100	225	7600	1177	12100	1177	16600	1183	21100	1183	25600	1177	30100	1189	34600	1172
3200	248	7700	1172	12200	1183	16700	1183	21200	1183	25700	1177	30200	1189	34700	1177
3300	270	7800	1177	12300	1177	16800	1183	21300	1183	25800	1177	30300	1189	34800	1177
3400	299	7900	1177	12400	1177	16900	1183	21400	1183	25900	1183	30400	1189	34900	1183
3500	327	8000	1172	12500	1183	17000	1183	21500	1183	26000	1177	30500	1183	35000	1177
3600	355	8100	1177	12600	1177	17100	1183	21600	1183	26100	1177	30600	1183	35100	1177
3700	389	8200	1177	12700	1177	17200	1183	21700	1183	26200	1183	30700	1189	35200	1172
3800	423	8300	1177	12800	1183	17300	1183	21800	1189	26300	1183	30800	1189	35300	1172
3900	456	8400	1177	12900	1183	17400	1183	21900	1183	26400	1189	30900	1189	35400	1172
4000	490	8500	1172	13000	1183	17500	1183	22000	1183	26500	1189	31000	1189	35500	1172
4100	535	8600	1177	13100	1189	17600	1183	22100	1183	26600	1189	31100	1189	35600	1177
4200	569	8700	1177	13200	1177	17700	1183	22200	1183	26700	1189	31200	1189	35700	1177
4300	597	8800	1172	13300	1177	17800	1183	22300	1183	26800	1189	31300	1189	35800	1177
4400	642	8900	1177	13400	1177	17900	1183	22400	1183	26900	1189	31400	1189	35900	1177

Eurotium halophilicum. Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	6300	16.9	12600	11.3	18900	33.8	25200	50.7	24640	62	30940	118	37240	225	43540	372
140	5.6	6440	11.3	12740	11.3	19040	33.8	25340	56.3	24780	56.3	31080	124	37380	225	43680	377
280	0	6580	22.5	12880	11.3	19180	33.8	25480	56.3	24920	56.3	31220	124	37520	231	43820	383
420	5.6	6720	16.9	13020	16.9	19320	33.8	25620	56.3	25060	56.3	31360	130	37660	248	43960	383
560	11.3	6860	11.3	13160	11.3	19460	33.8	25760	56.3	25200	50.7	31500	130	37800	242	44100	389
700	0	7000	11.3	13300	28.2	19600	33.8	25900	62	25340	56.3	31640	130	37940	242	44240	389
840	11.3	7140	5.6	13440	22.5	19740	33.8	26040	67.6	25480	56.3	31780	135	38080	248	44380	394
980	0	7280	11.3	13580	11.3	19880	33.8	26180	73.2	25620	56.3	31920	135	38220	248	44520	400
1120	0	7420	16.9	13720	16.9	20020	28.2	26320	67.6	25760	56.3	32060	135	38360	254	44660	417
1260	0	7560	11.3	13860	28.2	20160	33.8	26460	67.6	25900	62	32200	141	38500	254	44800	411
1400	11.3	7700	11.3	14000	16.9	20300	33.8	26600	67.6	26040	67.6	32340	141	38640	259	44940	417
1540	5.6	7840	16.9	14140	16.9	20440	39.4	26740	67.6	26180	73.2	32480	146	38780	259	45080	423
1680	11.3	7980	11.3	14280	22.5	20580	28.2	20020	28.2	26320	67.6	32620	146	38920	276	45220	423
1820	11.3	8120	11.3	14420	16.9	20720	33.8	20160	33.8	26460	67.6	32760	146	39060	270	45360	428
1960	11.3	8260	16.9	14560	22.5	20860	33.8	20300	33.8	26600	67.6	32900	152	39200	270	45500	434
2100	11.3	8400	11.3	14700	16.9	21000	33.8	20440	39.4	26740	67.6	33040	146	39340	282	45640	434
2240	16.9	8540	11.3	14840	22.5	21140	39.4	20580	28.2	26880	73.2	33180	158	39480	282	45780	445
2380	16.9	8680	16.9	14980	22.5	21280	39.4	20720	33.8	27020	73.2	33320	152	39620	282	45920	445
2520	16.9	8820	11.3	15120	22.5	21420	33.8	20860	33.8	27160	73.2	33460	158	39760	287	46060	445
2660	11.3	8960	16.9	15260	22.5	21560	33.8	21000	33.8	27300	73.2	33600	163	39900	287	46200	456
2800	11.3	9100	16.9	15400	22.5	21700	39.4	21140	39.4	27440	78.9	33740	163	40040	293	46340	462
2940	16.9	9240	22.5	15540	22.5	21840	33.8	21280	39.4	27580	78.9	33880	169	40180	299	46480	468
3080	11.3	9380	11.3	15680	22.5	21980	33.8	21420	33.8	27720	78.9	34020	163	40320	304	46620	468
3220	16.9	9520	16.9	15820	22.5	22120	33.8	21560	33.8	27860	78.9	34160	169	40460	310	46760	473
3360	5.6	9660	16.9	15960	22.5	22260	39.4	21700	39.4	28000	84.5	34300	180	40600	315	46900	473
3500	5.6	9800	16.9	16100	28.2	22400	45.1	21840	33.8	28140	78.9	34440	169	40740	304	47040	479
3640	11.3	9940	11.3	16240	22.5	22540	33.8	21980	33.8	28280	84.5	34580	175	40880	315	47180	484
3780	16.9	10080	16.9	16380	22.5	22680	39.4	22120	33.8	28420	84.5	34720	180	41020	315	47320	484
3920	22.5	10220	16.9	16520	28.2	22820	45.1	22260	39.4	28560	84.5	34860	180	41160	321	47460	484
4060	16.9	10360	16.9	16660	28.2	22960	39.4	22400	45.1	28700	90.1	35000	180	41300	332	47600	496
4200	16.9	10500	16.9	16800	28.2	23100	39.4	22540	33.8	28840	90.1	35140	186	41440	327	47740	496
4340	16.9	10640	11.3	16940	28.2	23240	45.1	22680	39.4	28980	90.1	35280	197	41580	332	47880	496
4480	11.3	10780	11.3	17080	28.2	23380	50.7	22820	45.1	29120	95.8	35420	203	41720	338	48020	501
4620	16.9	10920	11.3	17220	33.8	23520	45.1	22960	39.4	29260	101	35560	192	41860	344	48160	507
4760	16.9	11060	11.3	17360	33.8	23660	50.7	23100	39.4	29400	101	35700	197	42000	344	48300	507
4900	5.6	11200	16.9	17500	33.8	23800	45.1	23240	45.1	29540	107	35840	203	42140	349	48440	518
5040	11.3	11340	16.9	17640	33.8	23940	50.7	23380	50.7	29680	113	35980	208	42280	355	48580	524
5180	11.3	11480	16.9	17780	28.2	24080	50.7	23520	45.1	29820	107	36120	208	42420	349	48720	518
5320	22.5	11620	16.9	17920	28.2	24220	50.7	23660	50.7	29960	107	36260	208	42560	355	48860	524
5460	11.3	11760	16.9	18060	28.2	24360	50.7	23800	45.1	30100	107	36400	208	42700	361	49000	518
5600	11.3	11900	16.9	18200	28.2	24500	45.1	23940	50.7	30240	107	36540	214	42840	372	49140	518
5740	11.3	12040	11.3	18340	33.8	24640	62	24080	50.7	30380	118	36680	225	42980	366	49280	535
5880	11.3	12180	11.3	18480	33.8	24780	56.3	24220	50.7	30520	118	36820	231	43120	372	49420	530
6020	11.3	12320	11.3	18620	33.8	24920	56.3	24360	50.7	30660	113	36960	231	43260	372	49560	541
6160	11.3	12460	11.3	18760	28.2	25060	56.3	24500	45.1	30800	118	37100	225	43400	377	49700	535

Penicillium brevicompactum Consumption of O_2 in mgL^{-1}.

Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	5400	890	10800	1110	16200	1065	21600	1065	27000	1031	32400	1037
120	5.6	5520	930	10920	1110	16320	1053	21720	1076	27120	1037	32520	1042
240	5.6	5640	969	11040	1110	16440	1048	21840	1076	27240	1042	32640	1042
360	5.6	5760	1014	11160	1104	16560	1042	21960	1076	27360	1042	32760	1048
480	5.6	5880	1048	11280	1099	16680	1048	22080	1070	27480	1048	32880	1053
600	5.6	6000	1070	11400	1099	16800	1042	22200	1070	27600	1048	33000	1053
720	11.3	6120	1076	11520	1104	16920	1048	22320	1076	27720	1037	33120	1065
840	11.3	6240	1070	11640	1110	17040	1048	22440	1082	27840	1025		
960	5.6	6360	1070	11760	1115	17160	1048	22560	1087	27960	1025		
1080	5.6	6480	1070	11880	1115	17280	1048	22680	1087	28080	1025		
1200	5.6	6600	1070	12000	1115	17400	1048	22800	1093	28200	1025		
1320	5.6	6720	1070	12120	1115	17520	1042	22920	1099	28320	1031		
1440	16.9	6840	1070	12240	1115	17640	1037	23040	1104	28440	1037		
1560	28.2	6960	1070	12360	1115	17760	1031	23160	1110	28560	1037		
1680	33.8	7080	1070	12480	1110	17880	1031	23280	1121	28680	1042		
1800	39.4	7200	1070	12600	1104	18000	1025	23400	1121	28800	1042		
1920	39.4	7320	1070	12720	1099	18120	1025	23520	1121	28920	1042		
2040	50.7	7440	1065	12840	1099	18240	1025	23640	1099	29040	1037		
2160	62	7560	1059	12960	1121	18360	1025	23760	1110	29160	1037		
2280	67.6	7680	1053	13080	1115	18480	1031	23880	1115	29280	1025		
2400	73.2	7800	1042	13200	1110	18600	1031	24000	1121	29400	1025		
2520	78.9	7920	1042	13320	1110	18720	1042	24120	1121	29520	1025		
2640	90.1	8040	1042	13440	1121	18840	1048	24240	1115	29640	1025		
2760	101	8160	1048	13560	1121	18960	1053	24360	1115	29760	1031		
2880	124	8280	1048	13680	1127	19080	1053	24480	1132	29880	1037		
3000	141	8400	1048	13800	1115	19200	1053	24600	1127	30000	1042		
3120	163	8520	1048	13920	1110	19320	1059	24720	1115	30120	1048		
3240	175	8640	1087	14040	1104	19440	1065	24840	1093	30240	1053		
3360	197	8760	1099	14160	1104	19560	1059	24960	1070	30360	1059		
3480	225	8880	1104	14280	1110	19680	1059	25080	1059	30480	1070		
3600	242	9000	1110	14400	1115	19800	1053	25200	1059	30600	1065		
3720	276	9120	1110	14520	1121	19920	1053	25320	1065	30720	1053		
3840	304	9240	1110	14640	1115	20040	1053	25440	1065	30840	1048		
3960	338	9360	1110	14760	1104	20160	1059	25560	1065	30960	1048		
4080	377	9480	1110	14880	1093	20280	1065	25680	1065	31080	1048		
4200	417	9600	1104	15000	1087	20400	1065	25800	1065	31200	1048		
4320	468	9720	1099	15120	1087	20520	1065	25920	1065	31320	1053		
4440	513	9840	1093	15240	1087	20640	1065	26040	1059	31440	1053		
4560	563	9960	1093	15360	1087	20760	1065	26160	1053	31560	1053		
4680	608	10080	1099	15480	1087	20880	1070	26280	1048	31680	1059		
4800	659	10200	1104	15600	1082	21000	1065	26400	1037	31800	1070		
4920	704	10320	1110	15720	1082	21120	1059	26520	1031	31920	1076		
5040	755	10440	1110	15840	1082	21240	1059	26640	1025	32040	1065		
5160	806	10560	1110	15960	1076	21360	1053	26760	1031	32160	1048		
5280	845	10680	1110	16080	1076	21480	1053	26880	1031	32280	1037		

Penicillium chrysogenum. Consumption of O_2 in mgL^{-1}.

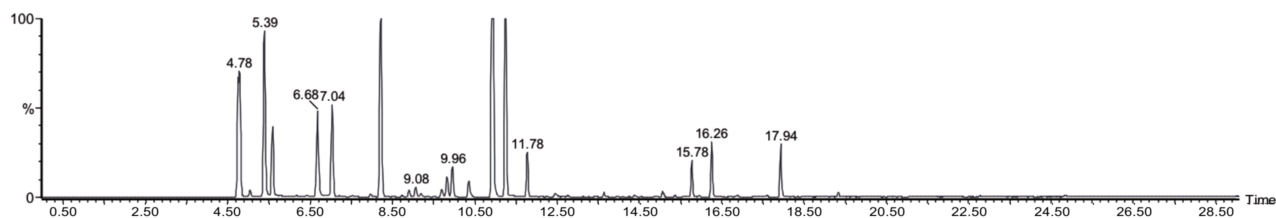
Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}	Time (min)	mgL^{-1}
0	0	5400	1132	10800	1222	16200	1206	21600	1200	27000	1206	32400	1194
120	5.6	5520	1132	10920	1222	16320	1200	21720	1194	27120	1206	32520	1194
240	22.5	5640	1138	11040	1217	16440	1194	21840	1189	27240	1228	32640	1189
360	28.2	5760	1149	11160	1211	16560	1200	21960	1183	27360	1239	32760	1194
480	28.2	5880	1161	11280	1211	16680	1194	22080	1177	27480	1239	32880	1194
600	33.8	6000	1155	11400	1211	16800	1189	22200	1172	27600	1234	33000	1194
720	45.1	6120	1144	11520	1211	16920	1189	22320	1172	27720	1211	33120	1194
840	45.1	6240	1144	11640	1211	17040	1183	22440	1172	27840	1211	33240	1194
960	45.1	6360	1155	11760	1206	17160	1183	22560	1166	27960	1217	33360	1189
1080	39.4	6480	1161	11880	1200	17280	1194	22680	1166	28080	1228	33480	1183
1200	45.1	6600	1161	12000	1183	17400	1200	22800	1166	28200	1222	33600	1172
1320	50.7	6720	1155	12120	1177	17520	1200	22920	1166	28320	1222	33720	1166
1440	50.7	6840	1155	12240	1172	17640	1194	23040	1166	28440	1217	33840	1166
1560	56.3	6960	1149	12360	1177	17760	1183	23160	1166	28560	1217	33960	1166
1680	50.7	7080	1155	12480	1177	17880	1183	23280	1161	28680	1217	34080	1166
1800	45.1	7200	1166	12600	1177	18000	1189	23400	1155	28800	1222	34200	1166
1920	50.7	7320	1177	12720	1177	18120	1189	23520	1149	28920	1228	34320	1172
2040	67.6	7440	1183	12840	1177	18240	1189	23640	1144	29040	1234	34440	1172
2160	95.8	7560	1183	12960	1177	18360	1183	23760	1144	29160	1234	34560	1183
2280	124	7680	1183	13080	1177	18480	1183	23880	1144	29280	1228	34680	1194
2400	163	7800	1177	13200	1172	18600	1200	24000	1144	29400	1228	34800	1206
2520	197	7920	1183	13320	1166	18720	1217	24120	1144	29520	1234	34920	1206
2640	237	8040	1177	13440	1149	18840	1217	24240	1144	29640	1228	35040	1200
2760	276	8160	1177	13560	1144	18960	1211	24360	1149	29760	1228	35160	1200
2880	321	8280	1172	13680	1144	19080	1211	24480	1161	29880	1222	35280	1206
3000	361	8400	1166	13800	1144	19200	1206	24600	1172	30000	1222	35400	1206
3120	406	8520	1172	13920	1144	19320	1206	24720	1177	30120	1228	35520	1200
3240	445	8640	1206	14040	1144	19440	1211	24840	1183	30240	1234	35640	1200
3360	484	8760	1206	14160	1149	19560	1206	24960	1183	30360	1239	35760	1194
3480	524	8880	1194	14280	1155	19680	1206	25080	1183	30480	1245	35880	1206
3600	575	9000	1177	14400	1166	19800	1200	25200	1183	30600	1245	36000	1245
3720	625	9120	1166	14520	1177	19920	1194	25320	1183	30720	1239	36120	1245
3840	676	9240	1177	14640	1189	20040	1200	25440	1183	30840	1239	36240	1211
3960	727	9360	1189	14760	1189	20160	1206	25560	1177	30960	1239		
4080	777	9480	1189	14880	1194	20280	1211	25680	1177	31080	1234		
4200	862	9600	1189	15000	1194	20400	1211	25800	1183	31200	1234		
4320	924	9720	1183	15120	1200	20520	1217	25920	1194	31320	1228		
4440	969	9840	1183	15240	1194	20640	1222	26040	1206	31440	1228		
4560	997	9960	1183	15360	1189	20760	1222	26160	1211	31560	1228		
4680	1020	10080	1194	15480	1189	20880	1222	26280	1211	31680	1228		
4800	1048	10200	1206	15600	1183	21000	1217	26400	1206	31800	1222		
4920	1082	10320	1211	15720	1183	21120	1211	26520	1211	31920	1222		
5040	1110	10440	1217	15840	1194	21240	1206	26640	1211	32040	1211		
5160	1127	10560	1217	15960	1200	21360	1206	26760	1211	32160	1200		
5280	1132	10680	1217	16080	1206	21480	1200	26880	1206	32280	1194		

Appendix B5

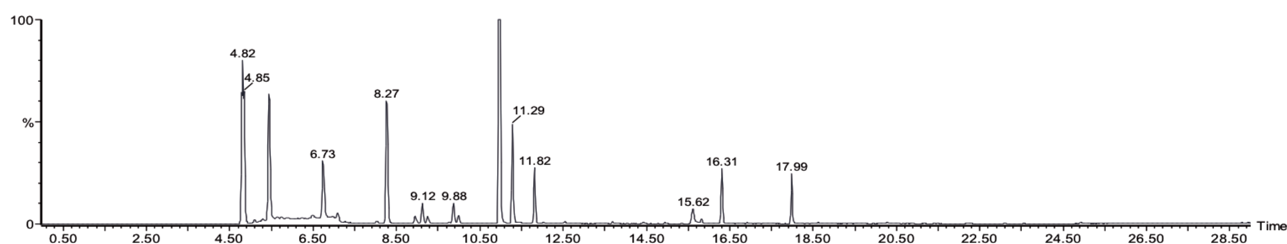
MVOC analysis by GC-MS

A - Chromatograms of selected fungal species during the monitored period

Blank MEA

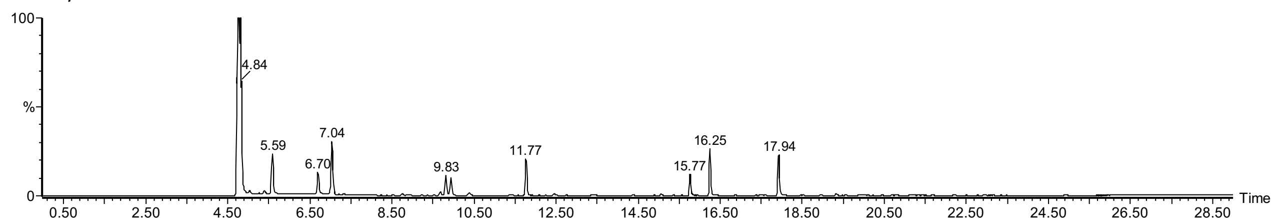


Blank MEA15%

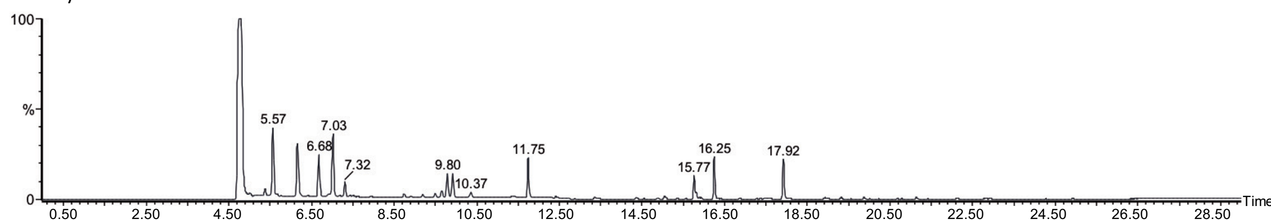


Aspergillus creber

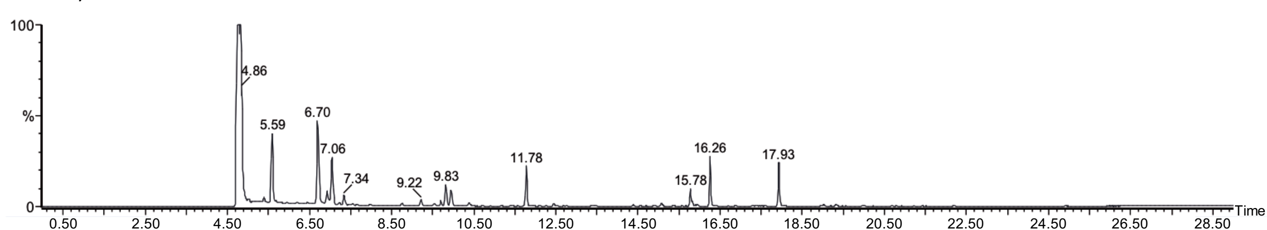
At 3 days



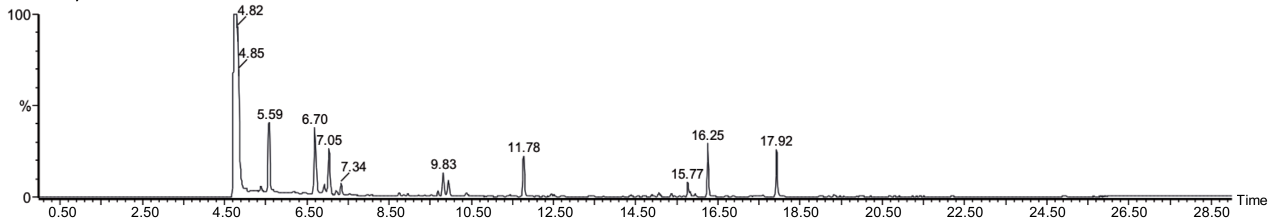
At 6 days



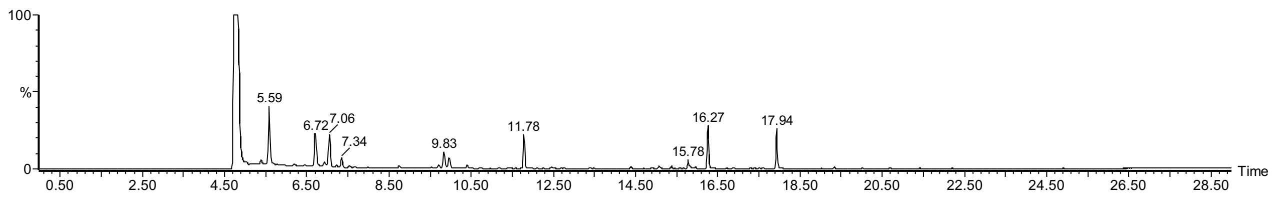
At 14 days



At 20 days

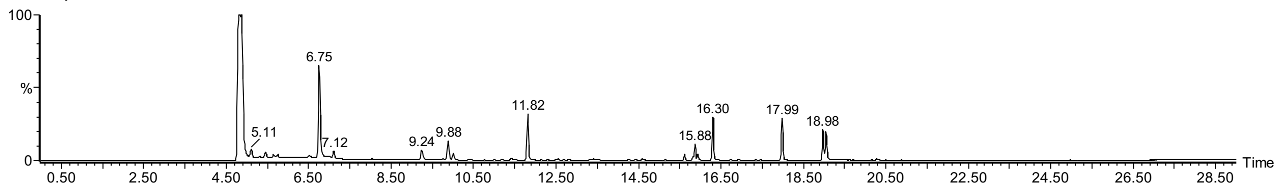


At 28 days

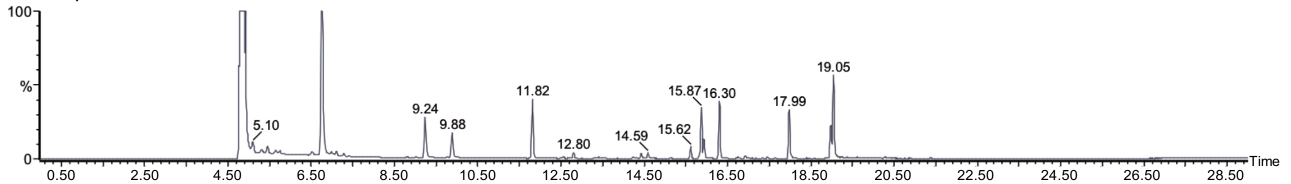


Aspergillus penicillioides

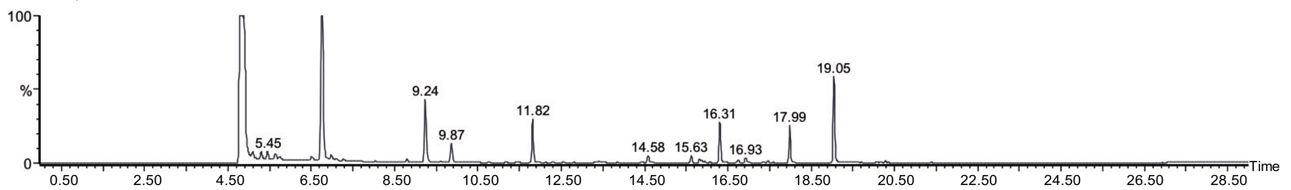
At 8 days



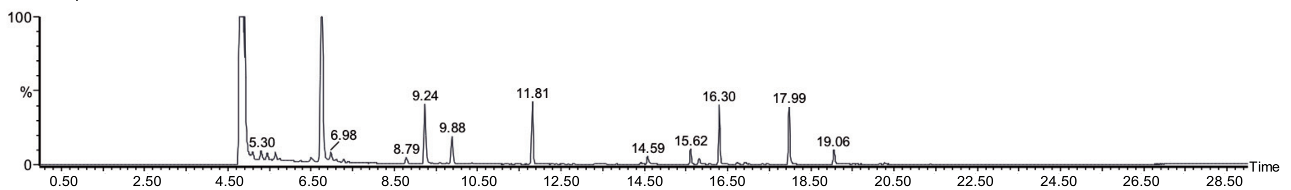
At 14 days



At 21 days

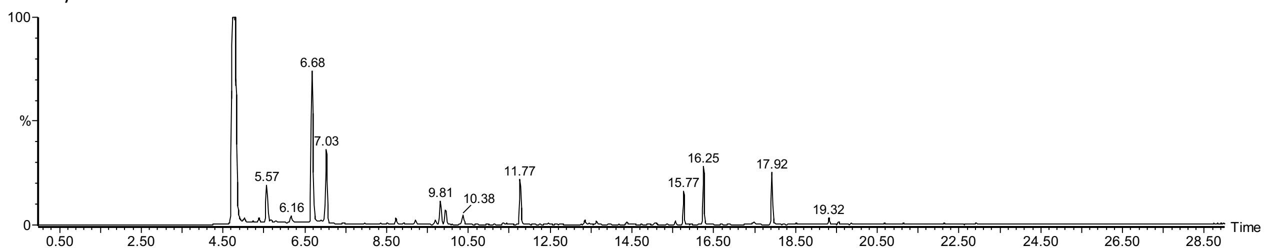


At 28 days

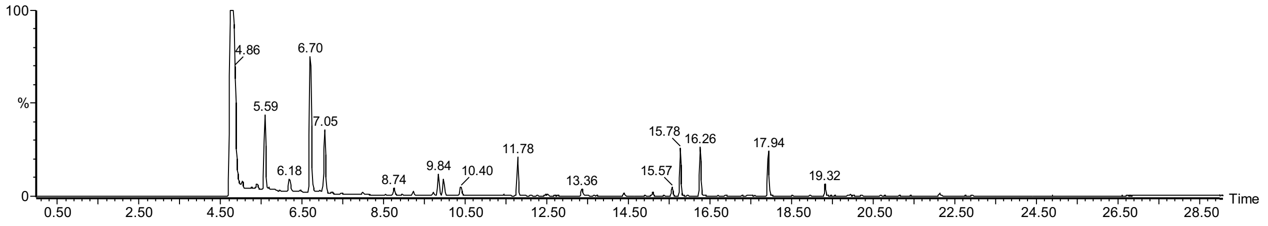


Cladosporium cladosporioides

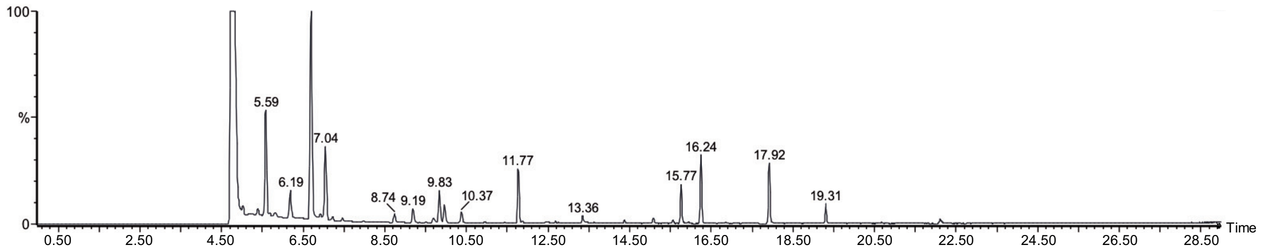
At 2 days



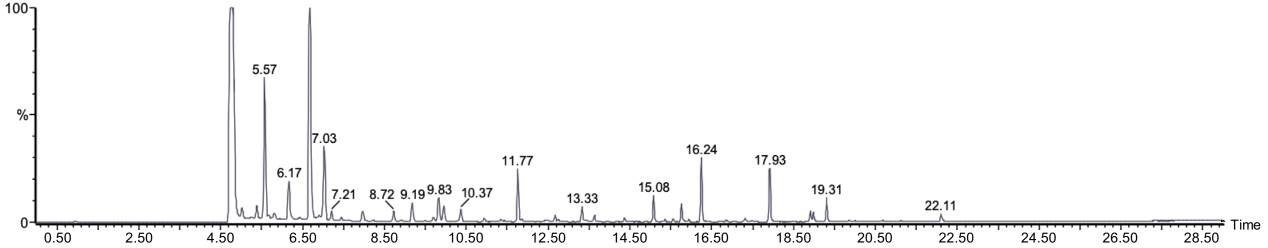
At 6 days



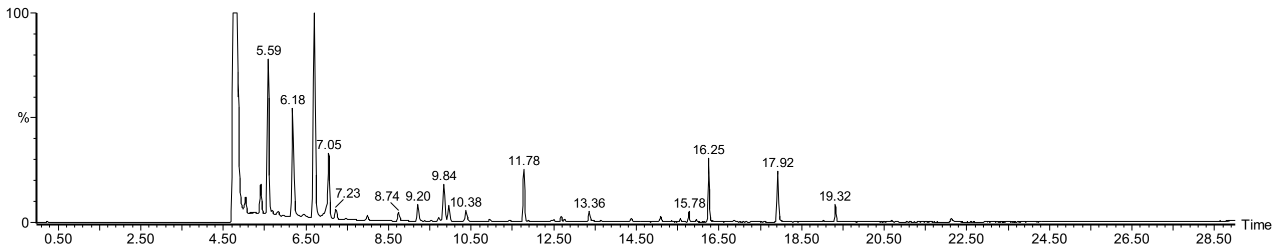
At 9 days



At 16 days

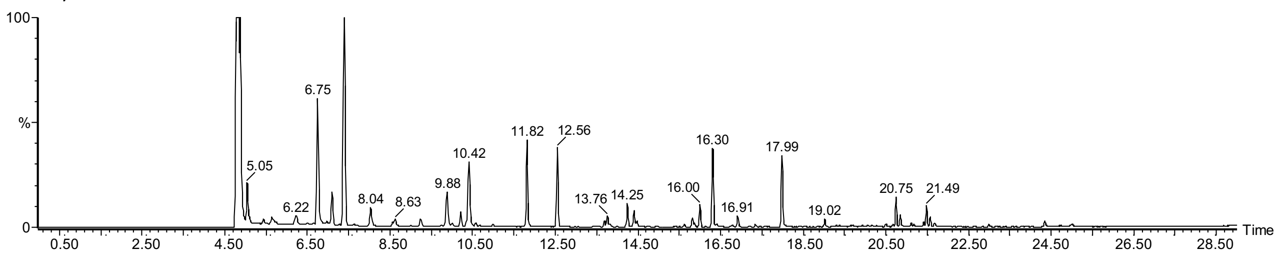


At 22 days

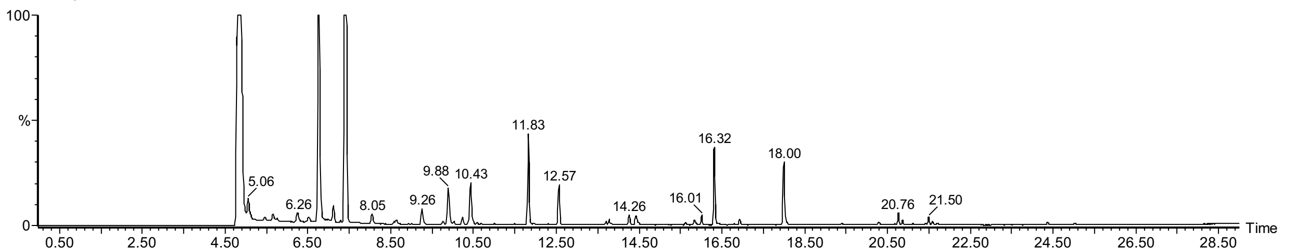


Eurotium chevalieri

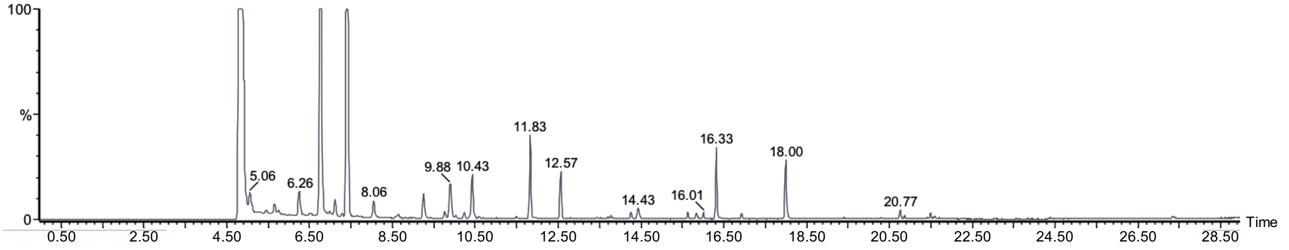
At 5 days



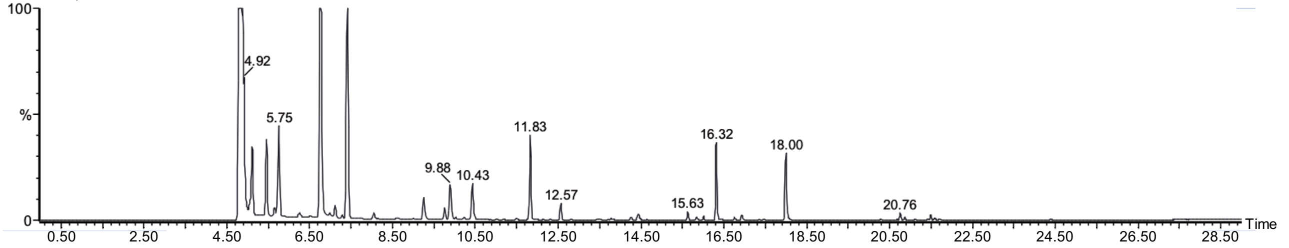
At 12 days



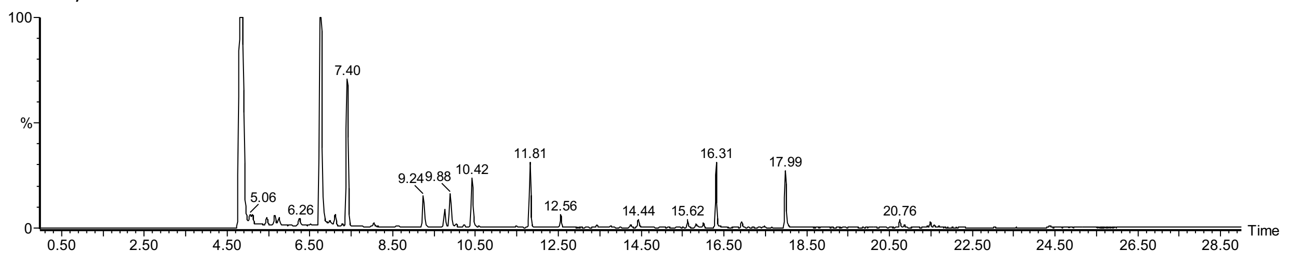
At 19 days



At 27 days

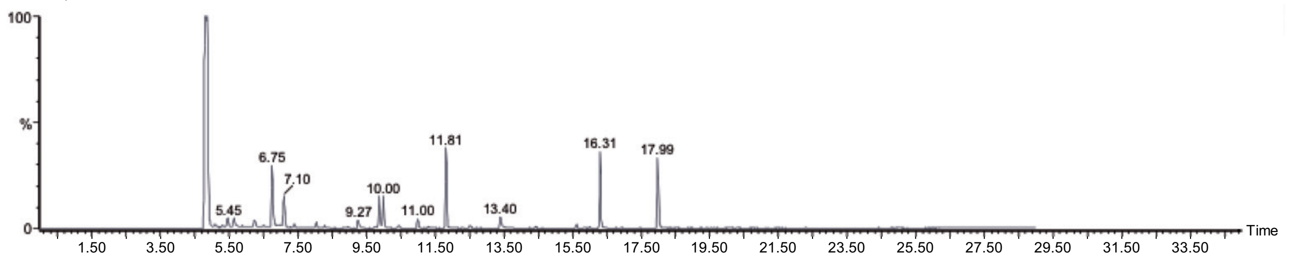


At 32 days

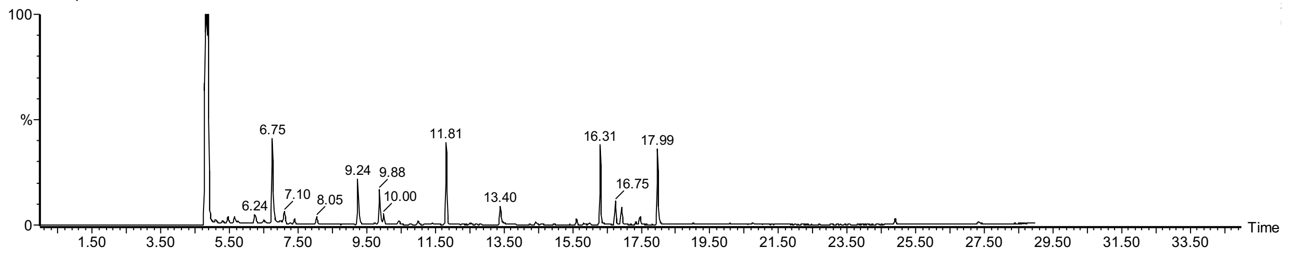


Eurotium halophilicum

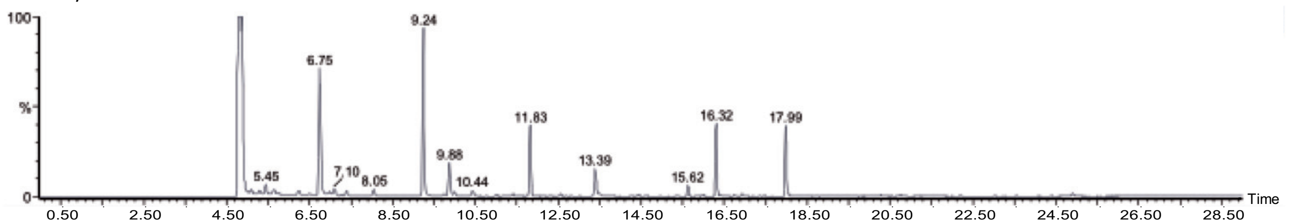
At 5 days



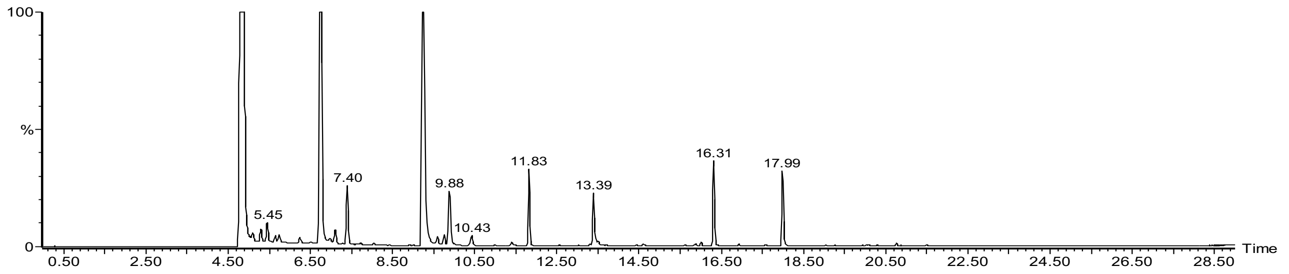
At 12 days



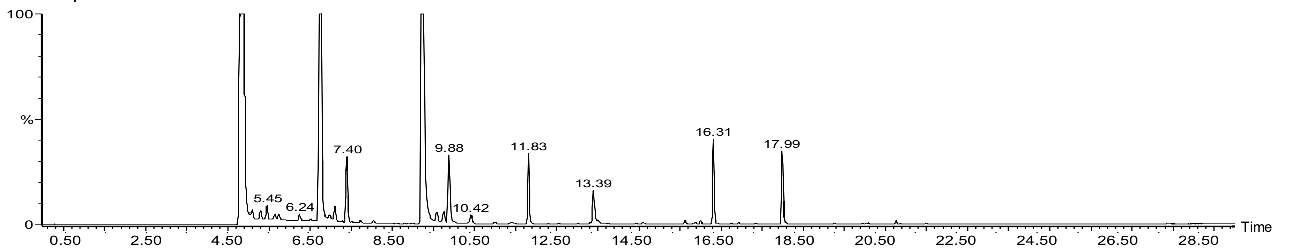
At 19 days



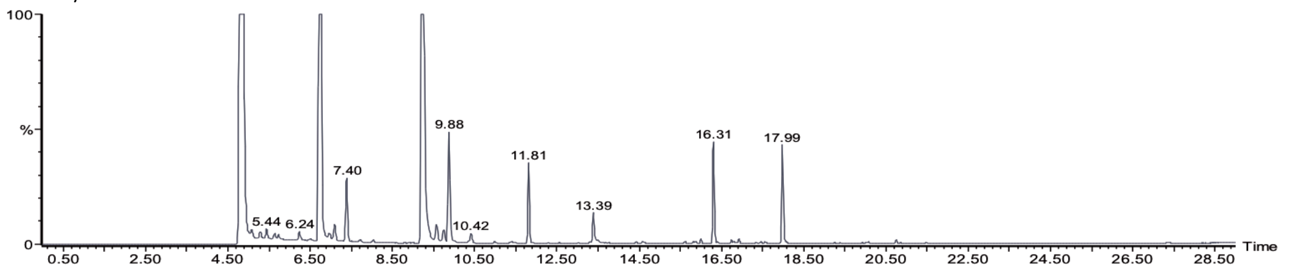
At 40 days



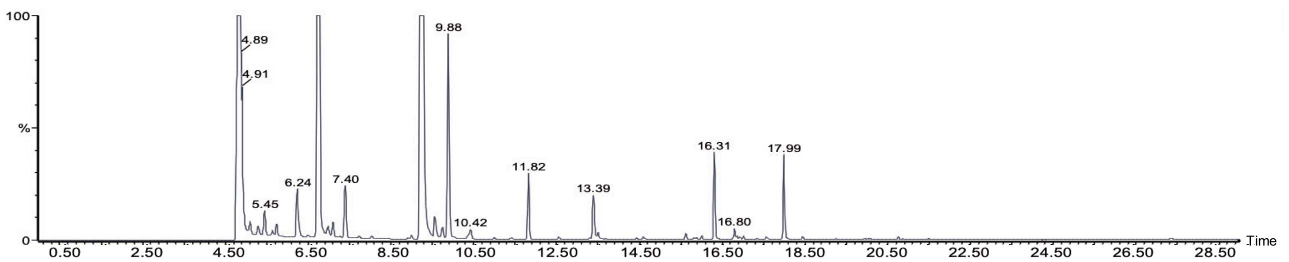
At 47 days



At 54 days

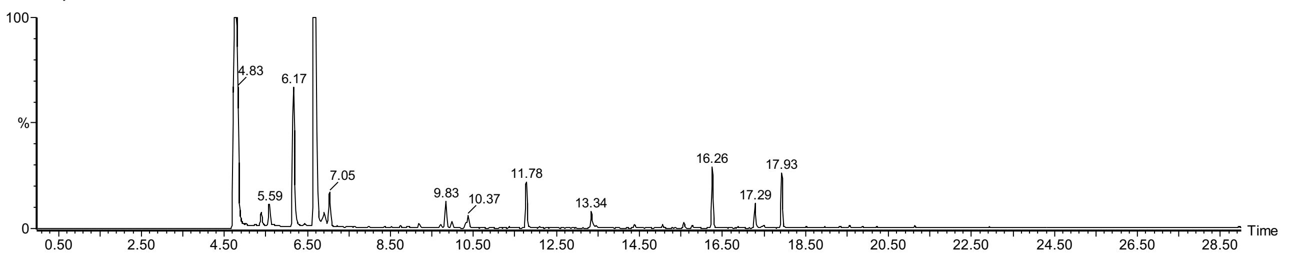


At 61 days

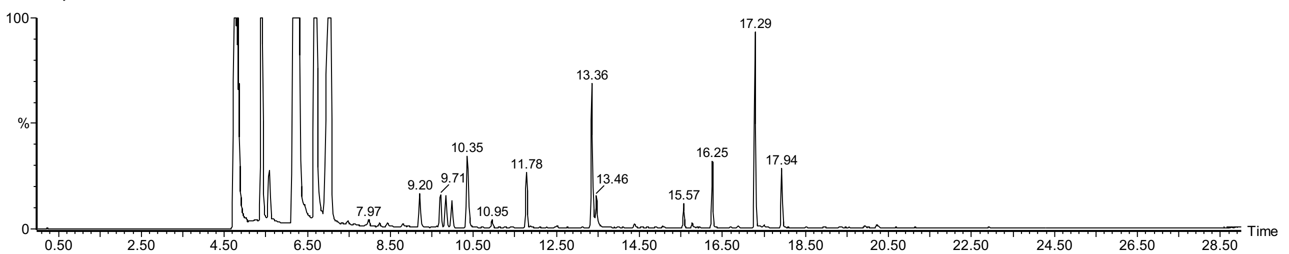


Penicillium brevicompactum

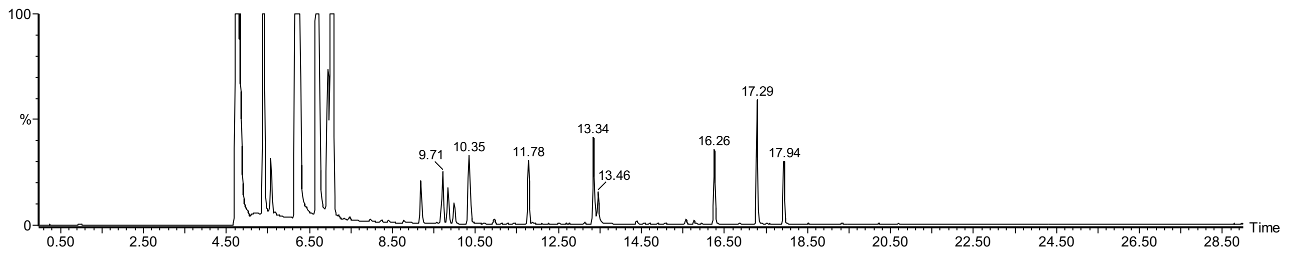
At 3 days



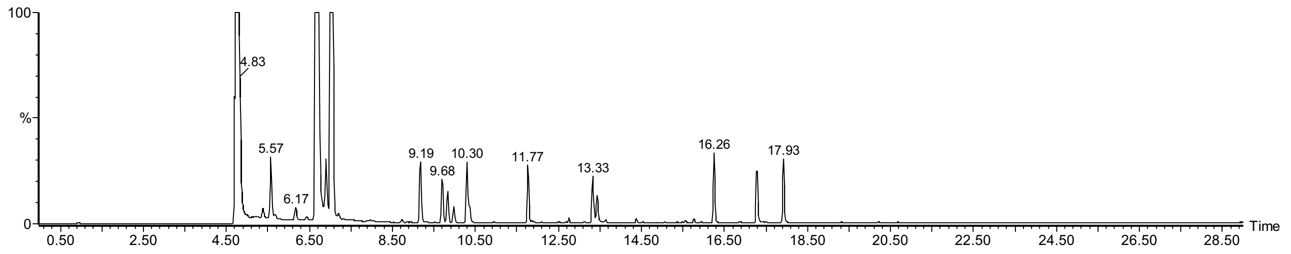
At 6 days



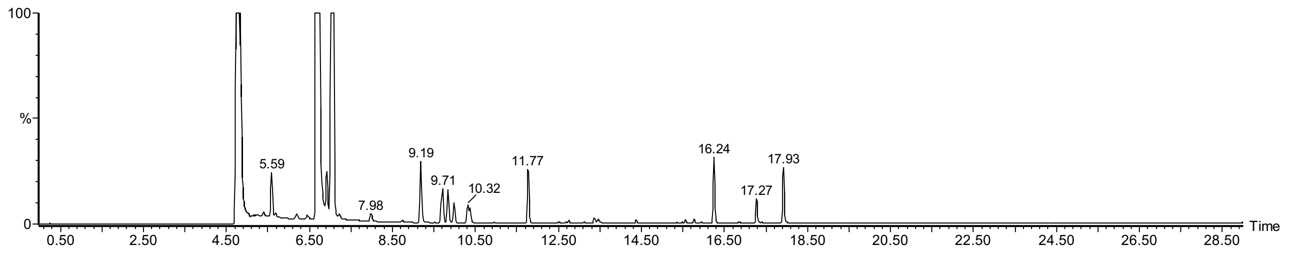
At 14 days



At 20 days

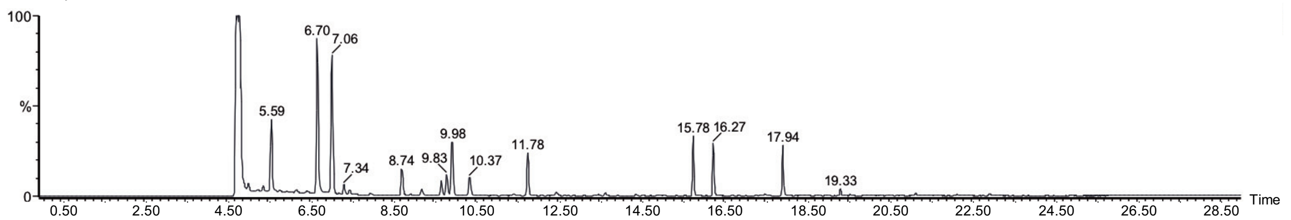


At 26 days

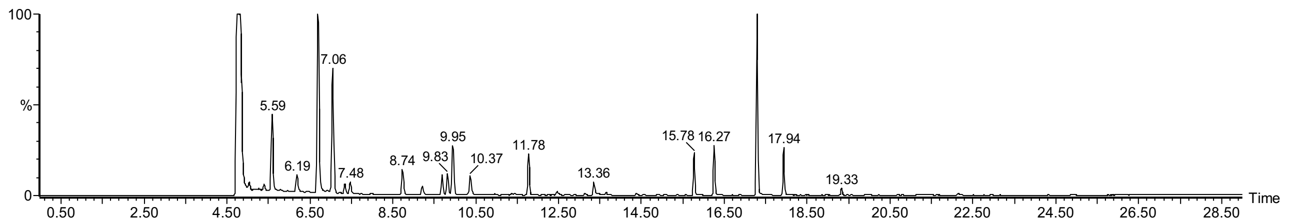


Penicillium chrysogenum

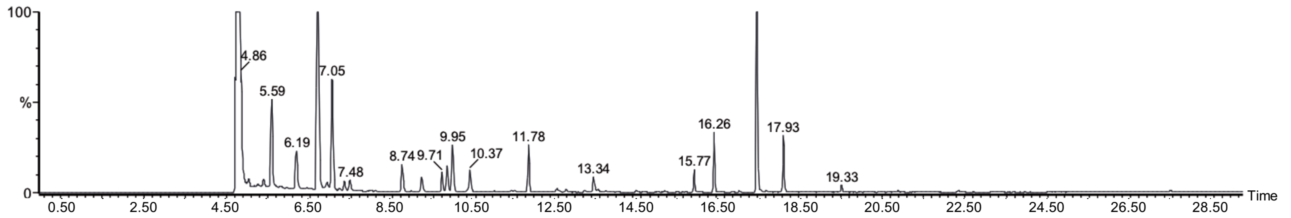
At 3 days



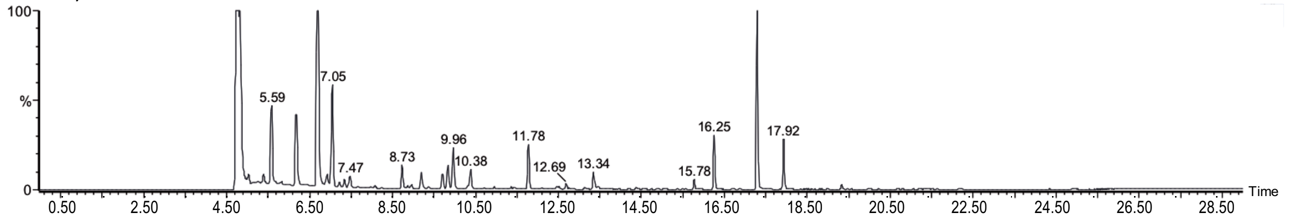
At 6 days



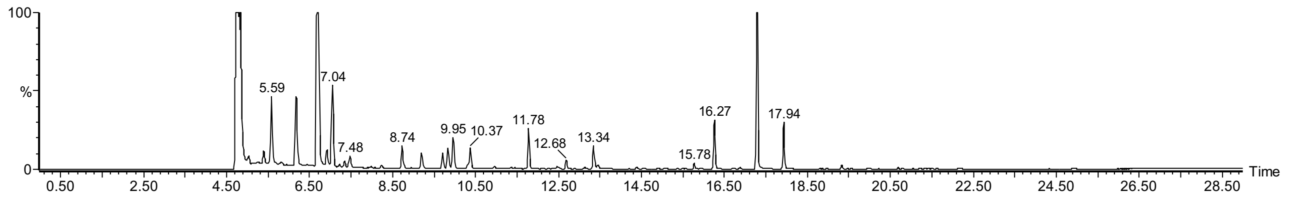
At 13 days



At 19 days



At 25 days



B – Results of MVOC analysis of selected fungal species

Table B5 Overview of MVOCs detected from the fungal species monitoring cultivated on MEA medium, with d: days from the incubation. The values are the ratio with internal standard 4 (chlorobenzene-d_5).

Substances	RT (min)	*A. creber*					*C. cladosporium*					*E. chevalieri*					*P. brevicompactum*					*P. chrysogenum*				
		3 d	6 d	13 d	19 d	25 d	2 d	6 d	9 d	16 d	22 d	5 d	12 d	19 d	27 d	32 d	2 d	6 d	9 d	16 d	25 d	3 d	6 d	13 d	19 d	25 d
Propane	5.09											0.48	0.63	0.46	0.68	0.34										
2-Butene	5.64		1.11	0.58	1.43	1.81		1.39	0.97	0.94	1.31	0.06	0.26			0.12						0.15	0.55	0.50	0.61	0.56
Butane	5.75													0.08	0.70	0.03										
Acetamide-2-cyano	6.34							0.24	0.13		0.15		0.06			0.02					0.10					
Ethanol	6.48		1.41		0.02	0.43	0.25	0.43	0.52	0.57	1.60	0.04	3.61	5.00	4.67	9.57	4.25	25.91	27.06	13.64	6.92	0.10	1.01	1.67	2.23	2.55
Acetone	6.77						0.07	0.15	1.06	1.95	2.11	1.38	7.15	8.95	7.20	9.33	11.59	9.58	14.44	19.46	21.66	1.76	3.84	6.06	8.14	9.96
Isopropyl alcohol	6.96		0.13	0.28	0.30	0.28		0.04	0.09	0.09	0.05			0.21	0.26	0.53	0.38		2.40	0.98	2.02		0.03	0.12	0.26	0.48
Furan	7.06	0.29	0.97				0.72	0.84	0.66	0.63	0.64	0.55	2.92	6.99	6.13	11.62	1.88	8.36	12.89	14.49	15.72	1.17	0.87	0.11	0.01	
Pentane	7.20																		0.09	0.14	0.16					
1,4-Pentadiene	7.32	0.02	0.44	0.32	0.35	0.42						7.48	13.74	12.20	7.16	6.15		0.13	0.11	0.10	0.07	0.20	0.21	0.16	0.14	0.13
Cyclopropylcarbinol	7.38		0.03	0.04	0.07	0.08		0.10	0.09	0.11	0.16														0.06	0.03
Methyl iodide	7.47		0.08	0.02	0.04					0.04								0.15	0.18	0.20	0.16	0.04	0.21	0.18	0.17	0.18
Dimethyl sulfide (DMS)	7.72											0.01	0.01	0.02	0.02	0.02										
1-Propanol	8.57																	0.03	0.04	0.03	0.02					
2-Butanone	9.21		0.04	0.09	0.01				0.08	0.15	0.07	0.15	0.58	0.81	0.62	0.85	0.02	0.17	0.38	0.73	0.68		0.07	0.17	0.22	0.29
1-Hexene	9.50		0.12	0.07	0.08	0.07																				
2-Butanol, (R)	9.55																		0.02							
2-Methylfuran	9.75		0.05									0.02	0.11	0.27	0.33	0.55	0.04	0.18	0.41	0.48	0.52	0.08	0.10	0.04	0.01	0.01
Trichloromethane	9.95		0.18				0.11	0.20	0.14	0.14	0.12	0.09	0.15	0.15	0.09	0.14						0.52	0.40	0.13	0.05	
3-Methylfuran	10.02																0.33	0.49	0.51	0.43	0.39					
Tetrahydrofuran	10.39		0.09									0.12	0.01	0.09		0.16		0.34	0.22	0.83	0.46					0.03
2-Methyl-1-propanol	10.46											0.07	0.04	0.03	0.01	0.03	0.16		0.38	0.37	0.32					
1-Butanol	11.37						0.01													0.06					0.02	0.01
Benzene	11.43							0.01										0.03								

2-Pentanone	11.87								0.02	0.06	0.02							0.02	0.02	0.03	0.03					
1,3-Dimethylcyclopentane	12.45	0.04	0.13	0.05	0.05	0.02											0.03	0.03	0.02	0.02	0.02	0.05	0.05	0.05	0.05	0.03
2,2,4-trimethylpentane	12.57											0.13	0.23													
2,5-Dimethylfuran	12.73									0.07	0.03				0.01	0.02				0.04	0.05			0.01	0.08	0.13
Heptane	12.75									0.03	0.02									0.03	0.02					
2-Heptene	12.89																					0.02	0.03	0.02	0.02	0.02
3-Methyl-1-butanol	13.43	0.03	0.08	0.04	0.04	0.02	0.04	0.08	0.08	0.12	0.13	0.03	0.05	0.10	0.08	0.25	0.60	2.52	2.71	4.20	2.38		0.54	0.60	0.44	0.46
2-Methyl-1-butanol	13.54												0.02	0.03	0.03	0.10	0.08	0.54	0.74	1.01	0.69		0.08	0.10	0.10	0.11
Dimethyl disulfide (DMDS)	13.64									0.18	0.06															
2,4-Dimethylhesane	13.76											0.04														
2,3,4-Trimethylpentane	14.25											0.06														
Propanoic acid-2-methyl-ethyl ester	14.25																	0.02	0.03	0.05	0.04					
Acetic acid butyl ester	14.31																			0.02						
Toluene	14.38																0.02		0.01	0.02						
2,5-Dimethylhexane	14.63											0.02	0.03	0.03	0.03	0.06										
2-Octene	15.09		0.03	0.03	0.04					0.13	0.03															
1-Octene	15.10						0.02	0.11	0.09	0.13	0.10															
Octane	15.36				0.04	0.06																				
2,3,5-Trimethylfuran	15.49																			0.03	0.02					
Tetrachloroethylene	15.62							0.03						0.01	0.02	0.03		0.02								
1,3-Octadiene	15.78		0.21	0.05	0.12	0.05																				
2,4-Dimethylheptane	15.95				0.02	0.08				0.02	0.02	0.14	0.11	0.07	0.01	0.02				0.02				0.02	0.02	0.03
Acetic acid trichloro, ethyl esther	16.41											0.07	0.06	0.05	0.02	0.03										
Ethylbenzene	16.74																	0.02	0.04	0.10	0.05					
Styrene	17.35									0.06						0.01	0.71	1.17	1.60	1.28	0.71		1.36	2.17	2.00	2.34
2-Butoxyethanol	17.50																0.06									
1,5-Dimethyl-1,4-cyclohexadiene	17.60					0.02						0.02		0.04		0.01										
2-Octen-1-ol	18.93	0.02	0.07			0.02				0.05																

3-Octanone	18.99	0.03 0.02 0.07	0.28	0.01 0.01 0.03		
2,2,6-Trimethyloctane	19.02			0.04		
Decane	19.56				0.03	
Limonene	20.21				0.01	
2,6,10-Trimethyldocedane	20.75			0.17 0.10 0.06 0.05 0.06		

Table B6 Overview of MVOCs detected from the fungal species monitoring cultivated on MEA15% medium, with d: days from the incubation. The values are the ratio with internal standard 4 (chlorobenzene-d_5).

Substances	**RT (min)**	***A. penicillioides***				***E. halophilicum***								
		8 d	**14 d**	**21 d**	**28 d**	**5 d**	**12 d**	**19 d**	**26 d**	**33 d**	**40 d**	**47 d**	**54 d**	**61 d**
Propane	5.09	0.39	0.50	0.29	0.08		0.09	0.15	0.14	0.01	0.23	0.20	0.14	0.35
Isobutane	5.44	0.06	0.18	0.21	0.22		0.03	0.11	0.22	0.11	0.17	0.20	0.09	0.22
2-butene	5.62				0.08									
Cyclopropilcarbinol	5.82	0.12	0.16	0.20				0.02			0.18	0.15	0.07	0.22
Acetamide-2-cyano	6.43					0.03	0.07	0.02	0.02	0.02		0.02		0.39
Ethanol	6.48					0.02	0.07					0.02	0.04	0.32
Acetone	6.77	1.23	5.69	6.04	4.63		0.17	1.09	2.98	4.78	7.28	8.82	8.79	5.21
Isopropyl alcohol	6.93	0.03	0.10	0.15	0.16	0.06	0.08	0.08	0.08	0.09	0.13	0.15	0.15	6.65
Furan	7.06	0.09	0.08											
1,4-Pentadiene	7.32	0.01	0.03	0.03	0.02	0.05	0.09	0.13	0.30	0.63	0.85	0.95	0.76	0.61
2-Butanone	9.21	0.20	1.35	1.39	0.94		0.71	3.45	7.53	13.28	20.30	24.15	22.22	13.04
2-Butanol, (R)	9.55								0.03	0.08	0.20	0.24	0.30	0.43
2-Methylfuran	9.75	0.03	0.06						0.04	0.10	0.18	0.17	0.15	0.17
Ethyl acetate	9.88										0.49	0.56	0.65	1.40
Trichloromethane	9.95	0.18	0.01											
Tetrahydrofuran	10.39	0.01	0.04	0.02	0.02	0.04	0.06	0.08	0.09	0.10	0.14	0.12	0.11	0.15
3-Methyl-2-butanone	11.17	0.01	0.02	0.01										
1-Butanol	11.37	0.03	0.01			0.02	0.06	0.05	0.07	0.09	0.06	0.07	0.05	0.06
1,3-dimethylcyclopentane	12.45	0.04	0.02											
Heptane	12.75		0.15											
3-Methyl-3-buten-1-ol	13.30	0.01	0.04	0.02										
3-Methyl-1-butanol	13.43	0.01	0.03	0.04	0.01	0.24	0.39	0.48	0.55	0.58	0.78	0.65	0.44	0.61
2-Methyl-1-butanol	13.54	0.01	0.05	0.05	0.01									0.14
2,3,4-Trimethylpentane	14.25	0.01	0.02	0.01										
1-Pentanol	14.25	0.01	0.02	0.01										
Toluene	14.38	0.01	0.16	0.01			0.02	0.01		0.02				0.02
Cyclopentanone	14.60	0.03	0.20	0.19	0.16					0.02	0.04	0.02	0.04	0.05
2-Penten-1-ol	14.69				0.11									
1-Octene	15.10	0.01	0.03	0.01										
Tetrachloroethylene	15.62					0.01	0.03	0.09						0.02
1,3-Octadiene	15.78	0.51	1.44	0.27	0.01									
Ethylbenzene	16.74		0.03	0.05		0.02	0.27		0.04	0.06			0.06	0.17
1-Butanol.3-methyl, acetate	16.79													0.09
p-Xylene	16.92		0.06	0.10			0.19		0.01	0.13			0.01	
Styrene	17.35		0.01	0.01			0.04			0.03				0.02
o-Xylene	17.47		0.03	0.02			0.10		0.02	0.02			0.02	0.01
2-Butoxyethanol	17.50													0.02
2-Octen-1-ol	18.93	0.77	0.68	0.33										
3-Octanone	18.99	0.63	2.67	1.53	0.26									

C – Results of VOC analysis of contaminated books

Table B7 Overview of VOCs detected from the contaminated book L6 monitoring, with d: days from the incubation. The values are the ratio with internal standard 4 (chlorobenzene-d_5).

Substances	RT (min)	Air	Blank	3 d	6 d	15 d	29 d	43 d	50 d	64 d	71 d	78 d
Propane	5.09			0.64	0.80	0.00	9.25	8.40	8.95	10.92	20.68	20.81
Chloromethane	5.34			0.00	0.00	0.00	2.02	2.80	2.69	3.33	3.64	4.27
Isobutane	5.45	0.29	0.29	0.33	0.66	2.48	0.60	1.36	1.47	2.05	2.47	2.76
2-Butene	5.64	0.07	0.19	0.04	0.12	0.31	1.44	1.77	1.49	1.77	2.13	2.40
Butane	5.75	0.32	0.20	0.33	0.43	0.58	1.31	1.43	1.35	1.57	1.82	1.93
Ethanol	6.22	4.21	0.76									
Acetonitrile	6.48	2.96	0.26									
Acetone	6.75	1.57	0.87	1.83	1.26	4.69	16.70	21.77	18.23	24.27	56.01	48.10
Isopropil alcohol	6.98	0.26	0.13	0.09	0.04	0.11	0.71	0.89	0.69	1.15	1.28	0.00
Furan	7.11			0.00	0.00	0.00	2.56	3.70	3.14	3.74	4.13	4.72
2-Chloropropane	7.18			0.06	0.14	0.42	0.28	0.00	0.00	0.00	0.00	0.00
Pentane	7.27			0.41	0.25	0.39	0.90	1.25	1.21	1.61	1.74	2.07
14-Pentadiene	7.39	0.04	0.09	0.00	0.01	0.03	0.25	0.35	0.36	0.67	0.73	0.91
Propylamina	7.61			0.00	0.00	0.00	0.00	0.17	0.17	0.25	0.46	0.40
2,3-dimethylbutane	8.91	0.07	0.07	0.06	0.08	0.00	0.07	0.12	0.07	0.14	0.15	0.22
2-methylpentane	8.99			0.15	0.00	0.19	0.25	0.33	0.27	0.42	0.39	0.53
2-Butanone	9.24	0.22	0.09	0.01	0.00	0.10	5.34	8.34	6.25	9.34	10.87	12.20
2-Buten-1-ol	9.41			0.06	0.02	0.03	0.12	0.17	0.10	0.00	0.00	0.00
1-hexene	9.56			0.01	0.00	0.01	0.12	0.21	0.18	0.24	0.20	0.32
2-Methylfuran	9.75			0.03	0.02	0.03	0.30	0.39	0.30	0.44	0.29	0.46
3-Methylfuran	10.02			0.00	0.03	0.03	0.21	0.32	0.25	0.39	0.33	0.50
2-Methyl-1-propanol	10.37			0.00	0.00	0.00	0.19	0.23	0.28	0.65	0.35	0.67
1,3-Dioxolane-2-ethyl-4-methyl	10.43	0.12	0.04									
1-Hexanol	10.75			0.06	0.01	0.01	0.15	0.18	0.12	0.17	0.12	0.17
3-Methyl-2-butanone	11.16			0.00	0.00	0.00	1.00	1.66	1.18	2.12	0.92	2.06
3,3-Dimethyl-1.2-butandiol	11.42	0.26	0.25									
Benzene	11.49			0.28	0.10	0.00	0.38	0.52	0.24	0.35	0.28	0.38
Trichloronitromethane	11.64	0.03	0.06									
4-Methyl-1-pentene	11.76			0.00	0.00	0.00	0.00	0.13	0.09	0.00	0.08	0.00
2-Pentanone	11.90			0.00	0.00	0.00	0.00	0.52	0.00	0.46	0.00	0.44
2,3-Dimethylpentane	11.97			0.04	0.00	0.02	0.16	0.23	0.19	0.42	0.31	0.00
3-Pentanone	12.14			0.00	0.00	0.00	0.07	0.20	0.06	0.22	0.03	0.21
1-Heptene	12.48			0.00	0.00	0.00	0.06	0.00	0.00	0.19	0.00	0.14
2,2,3,3-tetramethylbutane	12.55	0.15	0.32	2.10	0.33	0.19	1.23	1.05	0.39	0.24	0.09	0.11
Heptane	12.68			0.00	0.00	0.00	0.00	0.07	0.05	0.49	0.04	0.09
1,3-Butanediol	13.40	0.07	0.02									
Methylisobutilketone	13.46		0.04	0.00	0.00	0.00	0.04	0.12	0.07	0.23	0.15	0.30
2-Methyl-3-pentanone	13.52	0.04		0.03	0.01	0.00	0.16	0.35	0.17	0.38	0.12	0.29
4,4-dimethylcyclopentene	14.25			0.04	0.01	0.00	0.12	0.22	0.15	0.27	0.11	0.27

Toluene	14.43	0.50	0.24	1.16	0.21	0.00	1.58	1.48	0.21	0.52	0.14	0.36
2-Methylheptano	14.59			0.03	0.01	0.00	0.10	0.14	0.12	0.17	0.08	0.32
Hexanal	14.95	0.02	0.04									
2-Octene	15.13			0.00	0.00	0.00	0.02	0.06	0.05	0.21	0.03	0.08
Acetic acid. butylesther	15.35	0.02	0.03									
Octane	15.42			0.03	0.01	0.00	0.16	0.35	0.29	0.73	0.29	0.70
Tetrachloroethylene	15.83	2.09	0.18	0.08	0.09	0.02	0.07	0.16	0.03	0.06	0.00	0.04
Ethylbenzene	16.75	0.07	0.06	0.12	0.01	0.00	0.16	0.25	0.01	0.06	0.00	0.03
m-Xylene	16.93	0.20	0.14	0.48	0.06	0.00	0.72	1.00	0.07	0.32	0.05	0.17
3-Methyl-1-ethylbenzene	17.19	0.06	0.06	0.00	0.00	0.00	0.01	0.00	0.00	0.13	0.00	0.01
Styrene	17.34	0.03	0.04	0.04	0.01	0.00	0.04	0.06	0.02	0.04	0.00	0.00
p-Xylene	17.47	0.06	0.07									
2-Butoxyethanol	17.66	0.03	0.06									
4-Carene	18.58			0.04	0.01	0.00	0.05	0.06	0.01	0.09	0.00	0.12
6-Methyl-5-heptene-2-one	19.02		0.05	0.07								
Decane	19.62	0.02	0.02									
2-Ethyl-1-hexanol	19.93		0.08									
1,2,3-Trimethylbenzene	20.09	0.03	0.02									
Limonene	20.29	0.08	0.09	0.05	0.01	0.04	0.10	0.32	0.00	0.16	0.08	0.02
trans-3-Carene-2-ol	20.66	0.02	0.03									
Nonanal	21.22	0.06	0.10									
Decanal	22.89		0.07									

Table B8 Overview of VOCs detected from the contaminated book L9 monitoring, with d: days from the incubation. The values are the ratio with internal standard 4 (chlorobenzene-d_5).

Substances	RT (min)	Air	Blank	3 d	6 d	15 d	29 d	43 d	50 d	64 d	71 d	78 d
Propane	5.11	0.16	0.02	0.20	0.33	0.88	4.36	1.78	1.92	1.72	4.63	5.55
Isobutane	5.46	0.24	0.19	0.29	0.53	0.14	1.34	1.10	0.68	0.52	1.14	1.28
2-Butene	5.66	0.03	0.04	0.07	0.12	0.15	0.95	0.45	0.34	0.29	0.64	0.78
Butane	5.75	0.25	0.09	0.08	0.13	0.21	0.89	0.48	0.33	0.26	0.60	0.66
Ethanol	6.24	3.68	1.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Isopropyl alcohol	6.98	0.22	0.16	0.05	0.00	0.02	0.76	0.34	0.13	0.09	0.51	0.75
Furan	7.11			0.00	0.00	0.00	3.45	1.88	1.30	0.78	1.51	1.78
Pentane	7.29	0.14	0.03	0.27	0.20	0.36	1.70	1.17	0.78	0.70	1.23	1.43
1,4-Pentadiene	7.40	0.02	0.02	0.02	0.05	0.07	0.26	0.33	0.24	0.26	0.44	0.53
2-Methylpentane	9.00	0.09	0.04	0.11	0.14	0.07	0.31	0.19	0.08	0.08	0.16	0.17
1-Hexene	9.56	0.00		0.01	0.01	0.02	0.59	0.51	0.37	0.32	0.42	0.44
2-Methylfuran	9.76			0.02	0.01	0.02	0.44	0.25	0.17	0.17	0.22	0.27
2-Methyl-1-propanol	10.37	0.19	0.08	0.11	0.06	0.03	0.39	0.51	0.30	0.25	0.40	0.63
Benzene	11.49	0.09	0.04	0.19	0.06	0.10	0.72	0.28	0.13	0.15	0.26	0.24
1-Heptene	12.48			0.00	0.00	0.00	0.00	0.89	0.40	0.49	0.48	0.56
4-Methylcyclohexanol	12.56	0.16	0.16	2.52	0.50	0.54	3.34	0.00	0.37	0.31	0.34	0.44
Heptane	12.80	0.03	0.01	0.09	0.03	0.02	0.93	0.00	0.48	0.61	0.63	0.86
Methylcyclohexane	13.53	0.03	0.02	0.08	0.01	0.01	0.32	0.00	0.25	0.26	0.31	0.35
Toluene	14.44	0.43	0.24	1.16	0.27	0.04	3.58	0.00	0.19	0.26	0.09	0.18
1,3-Dimethylcyclohexane	15.01	0.01		0.01	0.00	0.00	0.30	0.13	0.27	0.41	0.06	0.11
Octane	15.42	0.01	0.01	0.03	0.01	0.00	0.87	0.32	0.93	1.56	1.35	1.97
1,2-Dimethylcyclohexane	15.53			0.00	0.00	0.00	0.21	0.08	0.27	0.50	0.48	0.69
2-Ethylhexane	16.00			0.01	0.00	0.00	0.06	0.00	0.14	0.12	0.21	0.15
2,6-Dimethylheptane	16.15			0.00	0.00	0.00	0.35	0.00	0.50	0.94	0.89	1.35
Ethylcyclohexane	16.37			0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.10	10.77
1,1,3-Trimethylcyclohexane	16.48	0.01	0.01	0.00	0.00	0.00	1.05	0.45	1.64	3.15	2.91	4.54
1.3 -Diethylcyclopentane	16.75	0.05	0.04	0.14	0.02	0.01	1.03	2.19	0.00	0.00	0.00	0.53
3-Ethylhexane	16.81			0.00	0.00	0.00	0.50	0.00	1.55	2.52	2.18	2.69
p-Xylene	16.93	0.17	0.12	0.48	0.09	0.04	38.60	37.57	26.90	23.78	31.00	35.99
2,5-Dimethylheptane	17.09	0.01	0.00	0.01	0.01	0.00	0.34	0.00	0.44	0.80	0.75	0.99
Pentalene, octahyol	17.19	0.00	0.00	0.01	0.01	0.00	0.55	0.00	0.69	1.22	0.96	1.42
o-Xylene	17.47	0.05	0.03	0.15	0.03	0.02	1.50	0.35	1.30	1.83	1.29	1.60
1-Ethyl-4-methylcyclohexane	17.57	0.08	0.07	0.00	0.03	0.00	0.17	1.65	0.23	0.55	0.56	0.79
Nonane	17.66			0.03	0.03	0.01	0.96	0.07	1.29	2.28	2.48	3.19
Cyclohexanepropanol	18.32			0.00	0.00	0.00	0.19	0.00	0.30	0.63	0.63	0.72
Propylcyclohexane	18.49	0.01		0.01	0.01	0.01	0.22	0.11	0.26	0.48	0.53	0.73
4-Ethyl-1- octen-3-ol	18.59	0.02	0.01	0.02	0.00	0.00	0.11	0.00	0.07	0.16	0.19	0.21
1,4-Diethylbenzene	19.53	0.05	0.04	0.15	0.03	0.01	0.51	0.30	0.11	0.21	0.20	0.21
Undecane	20.76	0.02	0.01	0.02	0.00	0.00	0.01	0.25	0.05	0.00	0.00	0.00
2,6,10-Trimethyldodecane	20.86	0.01	0.01	0.02	0.00	0.00	0.00	0.10	0.02	0.00	0.00	0.00

D – Results of VOC analysis of indoor air sampling from the repository of BAUM

Table B9 Overview of VOCs results from indoor air sampling from repository of BAUM, expressed for each sampling area. The analyses were performed during the 2nd (October 2013) and the 3rd sampling (May 2014). The values are the ratio with internal standard 4 (chlorobenzene-d_5).

Substances	RT (min)	Area 1		Area 2		Area 3		Area 4		Area 5		Area 6	
		Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14	Oct '13	May '14
Acetaldehyde	5.44	0.24	0.36	0.22	0.36	0.19	0.22	0.23	0.52	0.16	0.39	0.15	0.07
Butane	5.73	0.3	0.07	0.3	0.08	0.18	0.08	0.14	0.08	0.15	0.07	0.13	
Ethanol	6.24	0.43	0.19	0.46	0.13	0.21	0.2	0.3	0.22	1	0.24	0.17	0.31
Acetone	6.75	0.72	0.78	0.69	0.73	0.78	0.62	0.49	1.17	0.6	0.89	0.43	0.3
Isopropyl alcohol	6.98	0.09	0.13	0.43	0.12	0.25	0.15	0.15	0.11	0.36	0.67	0.11	0.18
Pentane	7.26	0.21		0.12		0.1		0.09		0.13		0.23	0.06
1,4-Pentadiene	7.38	0.11		0.04	0.06	0.04				0.03	0.08		
Acetic acid cyano-1,1-dimethylethyl esther	7.60		0.04						0.21				
1-Epten-4-ol	8.91			0.05									
Methyl vinil ketone	8.96		0.16		0.1		0.12		0.18		0.13		0.42
2-Methylpentane	8.99	0.43		0.13		0.17		0.14		0.21			
mButanal	9.14		0.09		0.06			0.06	0.08		0.09		
2-Butanone	9.26	0.16	0.11	0.04	0.08	0.05	0.07	0.06	0.14	0.09	0.08		0.14
3-Methylpentane	9.41			0.06		0.06				0.1		0.13	
Methylcyclopentane	10.76	0.26		0.05		0.04				0.05		0.07	
1-Chloro-2-propanone	11.43		0.49		0.15								
Benzene	11.49			0.07		0.05				0.04	0.09	0.05	0.72
1-Chloro-2-propanol	12.13		0.67		0.18								
3-Methylhexane	12.14			0.05		0.04		0.18					
Pentanal	12.16										0.16	0.04	
1,2-Dichloro propane	12.32		0.06										
Tetramethylbutane	12.48	1.24			0.19	0.05		0.27	0.11		0.08		
Methylcyclohexane	13.53									0.1			

Cyclobutene-2-propilidene	14.39								0.13				
Toluene	14.44	2.55	0.08	0.23		0.19	0.11	0.14		0.11	0.08	0.11	
Hexanal	14.94		0.08					0.08	0.08	0.04	0.14		
Furfural	15.48	0.14											
Perchloro ethylene	15.63		0.06	0.05		0.04		0.08	0.1	0.05			
Heptanal	17.30		0.03					0.07	0.04		0.07		
Benzaldehyde	18.61							0.1		0.02	0.04		
Octanal	19.37		0.05					0.09	0.06		0.13		
Decane	19.61	0.03						0.04					
2-Ethyl-1-hexanol	19.93							0.06					
Nonanal	21.20							0.11			0.08		0.12

Appendix B6

A- Secondary metabolite concentrations produced by selected fungal species

Table B10 Secondary metabolite concentrations produced by selected fungal strains on Yeast Extract-Sucrose agar (YES), Czapeck-Yeast Autolysate agar (CYA), Yeast Extract-Sucrose agar with 15% of NaCl (YES15%) and Malt Extract Agar with 15% of NaCl (MEA15%). Results are reported as ng cm^{-2} of agar disk.

Fungal species*	Fungal strain	Metabolites on YES (ng cm^{-2} agar)	Metabolites on CYA (ng cm^{-2} agar)
Aspergillus creber	MUT 470	**After 7 days on incubation** ASP-A (46.2), AVE1 (4805.6), AVE2 (88.5), CIT (48.0), EMO (7.0), MET (9213.8), NID (1474.5), NOR (1342.0), ORS (2259.1), STE (25841.8), VER-A (2937.8) **After 14 days of incubation** AVE1 (5219.3), AVE2 (150.6), CIT (218.5), DEO-E (35.1), EMO (19.7), MET (12890.4), NID (3151.8), NOR (1424.6), ORS (12808.8), STE (26096.6), VER-A (3235.3)	**After 7 days of incubation** AVE1 (549.8), AVE2 (28.1), AVE3 (1700.7), DEO (44.1), EMO (1.3), MET (19972.4), NID (167.3), NOR (104.0), ORS (408.5), STE (3154.8), VER-A (425.4) **After 14 days of incubation** AVE1 (134.7), AVE2 (19.4), AVE3 (937.5), DEO (45.5), EMO (1.2), MET (12350.3), NID (133.9), NOR (33.3), ORS (315.6), STE (5855.2), VER-A (196.3)
A. creber	MUT 5691	**After 7 days on incubation** AVE1 (1029.2), AVE2 (38.2), AVE3 (1631.4), CIT (10.0), EMO (3.3), MET (3999.6), NID (186.0), NOR (185.5), ORS (627.5), STE (4056.6), VER-A (307.6) **After 14 days of incubation** ASP-A (135.5), AVE1 (6846.7), AVE2 (149.6), CIT (153.2), EMO (15.3), MET (25515.8), NID (3629.7), NOR (2262.2), ORS (8527.0), STE (34819.2), VER-A (3690.8)	**After 7 days on incubation** ASP-A (84.4), AVE1 (961.9), AVE2 (34.0), AVE3 (1735.4), DEO (38.7), EMO (1.3), MET (22988.6), NID (262.1), NOR (165.5), ORS (327.3), STE (5836.8), VER-A (680.9) **After 14 days of incubation** ASP-A (236.1), AVE1 (421.1), AVE2 (34.1), AVE3 (1560.1), DEO (72.6), EMO (1.2), MET (27197.1), NID (246.3), NOR (67.4), ORS (304.3), STE (13298.0), VER-A (421.8)
A. creber	MUT 5527	**After 7 days on incubation** ASP-A (72.5), AVE1 (5081.7), AVE2 (111.4), CIT (63.4), DEO-E (29.1), EMO (10.8), MET (10984.8), NID (1726.2), NOR (1039.4), ORS (3472.8), STE (23814.0), VER-A (2251.0) **After 14 days on incubation** AVE1 (4879.0), AVE2 (134.5), CIT (164.9), DEO-E (21.7), EMO (18.2), MET (11901.9), NID (2279.5), NOR (842.5), ORS (9218.9), STE (20634.8), VER-A (2261.2)	**After 7 days on incubation** AVE1 (1065.9), AVE2 (42.9), AVE3 (1776.1), DEO (51.9), EMO (1.5), MET (5291.6), NID (383.6), NOR (152.6), ORS (488.2), STE (7077.0), VER-A (861.9) **After 14 days on incubation** ASP-A (149.4), AVE1 (277.4), AVE2 (29.7), AVE3 (1318.6), DEO (50.7), EMO (1.5), MET (21236.0), NID (184.7), NOR (58.7), ORS (397.1), STE (9974.0), VER-A (357.1)
A. creber	MUT 5690	**After 7 days on incubation**	**After 7 days on incubation**

		AVE1 (102.7), AVE2 (2.4), AVE3 (46.6), MET (18.6), NID (1.2), NOR (43.9), ORS (3373.9), STE (259.6), SYD (1153.5), VER-A (2.7) **After 14 days on incubation** AVE1 (99.0), AVE2 (3.5), AVE3 (137.9), DEO-E (40.7), EMO (1.7), MET (879.0), NID (1.6), NOR (283.9), ORS (3966.0), STE (4551.9), SYD (31.1), VER-A (9.5)	AVE1 (1617.2), AVE2 (29.4), AVE3 (1438.8). DEO-E (9.5), EMO (0.6), MET (24425.4), NID (86.5), NOR (777.0), ORS (138.8), STE (11586.0), SYD (278.9), VER-A (606.1) **After 14 days on incubation** ASP-A (86.1), AVE1 (929.7), AVE2 (23.8), AVE3 (1363.4), DEO-E (83.1), EMO (2.2), MET (29632.5), NID (122.5), NOR (343.4), ORS (10.3), STE (9869.0), SYD (266.3), VER-A (572.3)
A. creber	MUT 5689	**After 7 days on incubation** AVE1 (1315.5), AVE2 (16.3), AVE3 (840.4), DEO-E (50.7), EMO (1.1), MET (623.4), NID (53.6), NOR (1741.5), ORS (1441.9), STE (8325.2), SYD (58.9), VER-A (180.0) **After 14 days on incubation** AVE1 (9.5), AVE2 (0.3), AVE3 (4.8), DEO-E (16.0), MET (27.9), NOR (13.1), ORS (4458.1), STE (26.8), SYD (2817.5), VER-A (1.6)	**After 7 days on incubation** AVE1 (659.5), AVE2 (14.1), AVE3 (1110.7), DEO (61.4), EMO (0.4), MET (4033.2), NID (41.6), NOR (261.3), ORS (278.7), STE (1891.3), SYD (1293.1), VER-A (248.8) **After 14 days on incubation** AVE1 (937.9), AVE2 (22.1), AVE3 (1618.2), DEO (4955.4), EMO (2.1), MET (25648.2), NID (155.7), NOR (294.5), ORS (124.7), STE (19075.7), SYD (1894.3), VER-A (457.9)
A. jensenii	MUT 480	**After 7 days on incubation** AVE1 (4659.9), AVE2 (61.8), AVE3 (1773.1), CIT (80.2), DEO-E (474.3), EMO (12.2), MET (748.4), NID (268.2), NOR (1423.5), ORS (2569.9), STE (26881.2), SYD (212.1), VER-A (980.2) **After 14 days on incubation** ASP-A (269.1), AVE1 (5759.4), AVE2 (112.9), CIT (272.1), DEO-E (1056.7), EMO (39.3), MET (3478.9), NID (805.0), NOR (1786.3), ORS (12910.7), STE (30264.3), SYD (436.3), VER-A (1518.3)	**After 7 days on incubation** AVE1 (329.7), AVE2 (8.9), AVE3 (347.7), DEO-E (26.2), EMO (1.0), MET (14581.9), NID (27.6), NOR (64.1), ORS (487.3), STE (1143.3), SYD (412.3), VER-A (111.8) **After 14 days on incubation** AVE1 (257.0), AVE2 (11.4), AVE3 (461.7), DEO-E (119.3), EMO (20.3), MET (20767.2), NID (27.8), NOR (50.4), ORS (1569.3), STE (4241.1), SYD (798.0), VER-A (92.5)
A. protuberus	MUT 5693	**After 7 days on incubation** AVE1 (2573.0), AVE2 (163.0), AVE3 (917.5), CIT (14.0), EMO (3.1), NID (119.6), NOR (2087.9), ORS (1599.8), STE (< DL), SYD (56.1), VER-A (8419.0) **After 14 days on incubation** AVE1 (6387.1), AVE2 (568.9), CIT (34.0), EMO (4.6), NID (2548.5), NOR (6881.3), ORS (2868.5), STE (10.8), SYD (1.4), VER-A (15285)	**After 7 days on incubation** AVE1 (560.8), AVE2 (56.2), AVE3 (568.5), EMO (0.8), NID (25.9), NOR (516.4), ORS (1104.6), STE (29.2), SYD (337.9), VER-A (5744.1) **After 14 days on incubation** AVE1 (276.5), AVE2 (95.2), AVE3 (410.1), EMO (2.4), NID (62.7), NOR (387.7), ORS (1891.3), STE (41.3), SYD (338.3), VER-A (1379.7)
A. protuberus	MUT 5487	**After 7 days on incubation** AVE1 (951.8), AVE2 (63.5), AVE3 (1632.4), NID (625.8), NOR (429.8), ORS (924.6), STE (4662.9), VER-A (1892.3) **After 14 days on incubation** AVE1 (1971.8), AVE2 (91.9), NID (1575.4), NOR (1584.5), ORS (6850.7), STE (9798.7), VER-A (2180.7)	**After 7 days on incubation** AVE1 (3088.6), AVE2 (114.6), EMO (87.1), NID (1512.2), NOR (559.4), ORS (1325.7), STE (26269.8), SYD (6.5), VER-A (606.1) **After 14 days on incubation** AVE1 (290.2), AVE2 (57.2), AVE3 (1081.2), EMO (47.1), NID

			(252.2), NOR (72.3), ORS (1047.5), STE (23579.7), SYD (10.2), VER-A (83.3)
Cladosporium cladosporioides	MUT 527	**After 7 days on incubation** EMO (1.4) **After 14 days on incubation** EMO (0.5)	-
Eurotium chevalieri	MUT 472	**After 7 days on incubation** NEO-A (3560.4) **After 14 days on incubation** NEO-A (6115.0)	**After 7 days on incubation** NEO-A (15631.5) **After 7 days on incubation** NEO-A (9387.0)
Penicillium brevicompactum	MUT 536	**After 7 days on incubation** CIT (76.7), DEO-E (11.6), EMO (2.0), MYC (85208.8), ORS (112497.6) **After 14 days on incubation** CIT (153.9), DEO-E (20.0), EMO (1.1), MYC (22591.2), ORS (4392.9)	**After 7 days on incubation** CIT (59.1), EMO (0.8), MYC (78045.2), ORS (361.2) **After 14 days on incubation** CIT (19.6), DEO-E (37.3), EMO (1.2), MYC (81489.4), ORS (34900.8)
P. chrysogenum	MUT 5493	**After 7 days on incubation** AND-A (47088.0), AND-B (1029.2), CHR (72.6), CIT (474.0), DEM (561.6), EMO (133.5), FUL (70.2), MEL (39190.7), NEO (214.4), ROQ-C (2656.5), ROQ-D (336.4), SEC (171905.3) **After 14 days on incubation** AND-A (38120.8), AND-B (451.3), CHR (39.7), CIT (154.5), DEM (1780.2), EMO (174.1), FUL (2985.7), MEL (31171.2), NEO (241.7), ROQ-C (3279.1), ROQ-D (256.1), SEC (506443)	**After 7 days on incubation** AND-A (3987.3), AND-B (95.0), CHR (2774.7), CIT (309.3), DEM (183.1), EMO (126.2), MEL (39425.1), NEO (264.3), ROQ-C (6026.4), ROQ-D (145.6), SEC (159269.7) **After 14 days on incubation** AND-A (302846.8), AND-B (2416.1), CHR (1848.5), CIT (306.9), DEM (80.8), EMO (69.1), MEL (84740.0), NEO (1189.2), ROQ-C (8711.4), ROQ-D (1229.9), SEC (107504.5)
P. chrysogenum	MUT 5492	**After 7 days on incubation** AND-A (46884.2), AND-B (873.4), CHR (15.2), MEL (44978.7), NEO (139.0), OXA (2.8), ROQ-C (13868.6), ROQ-D (124.1), SEC (332.5) **After 14 days on incubation** AND-A (370610.3), AND-B (2190.9), CHR (17.4), FUL (138.9), MEL (59937.6), NEO (294.0), OXA (6.7), ROQ-C (25118.4), ROQ-D (220.3), SEC (726.6)	**After 7 days on incubation** AND-A (5247.9), AND-B (90.8), CHR (243.2), MEL (35593.7), NEO (45.4), OXA (4.1), ROQ-C (10882.9), ROQ-D (50.3), SEC (114.2) **After 14 days on incubation** AND-A (200233.5), AND-B (1434.8), CHR (367.2), MEL (62943.6), NEO (680.1), OXA (104.5), ROQ-C (20196.6), ROQ-D (160.2), SEC (193.4)
Fungal species	**Fungal strain**	**Metabolites on YESNaCl15% (ng cm^{-2} agar)**	**Metabolites on MEANaCl15% (ng cm^{-2} agar)**
A. penicillioides	MUT 481	**After 14 days on incubation** ASP (11412.8), CIT (24.2)	**After 14 days on incubation** ASP (2767.6), CIT (24.2)
A. penicillioides	MUT 5694	**After 14 days on incubation**	**After 14 days on incubation**

		ASP (11901.9), CIT (5.5)	ASP (11616.6), CIT (19.9)
A. penicillioides	MUT 5525	**After 14 days on incubation** ASP (8118.4), DEO-E (22.9)	**After 14 days on incubation** ASP (9585.7)
A. penicillioides	MUT 5537	**After 14 days on incubation** ASP (4048.5), CIT (25.3)	**After 14 days on incubation** ASP (6565.4), CIT (24.8)
A. penicillioides	MUT (31)**	**After 14 days on incubation** ASP (4.7)	**After 14 days on incubation** ASP (3.4)
A. penicillioides	MUT 5699	**After 14 days on incubation** ASP (10719.9)	**After 14 days on incubation** ASP (7736.2)
A. penicillioides	MUT 5700	**After 14 days on incubation** ASP (6528.7), CIT (6.3)	**After 14 days on incubation** ASP (11647.2), CIT (19.3)
A. penicillioides	MUT 5697	**After 14 days on incubation** ASP (4756.7)	**After 14 days on incubation** ASP (5273.3)
E. halophilicum	MUT 1922	**After 35 days on incubation** CHA-A (90.8), DEO-E (96.7), NEO-A (3.6), RUG (6.5), STA (284.4)	**After 588 days on incubation** CHA-A (11.7), DEO-E (20.8), NEO-A (1.7), RUG (15.7), STA (63.9), TEN (32.6) **After 35 days on incubation** CHA-A (27.2), DEO-E (13.1), RUG (4.3), STA (126.4)
E. halophilicum	MUT 1899	**After 58 days on incubation** CHA-A (4.7), DEO-E (11.2), NEO-A (0.7), RUG (4.3), STA (5.8), TEN (19.8) **After 35 days on incubation** CHA-A (232.4), DEO-E (12.4), NEO-A (2.4), RUG (4.1), STA (685.2)	**After 596 days on incubation** CHA-A (38.0), NEO-A (2.1), STA (114.2), TEN (30.3)
E. halophilicum	MUT 1906	**After 58 days on incubation** CHA-A (6.0), DEO-E (37.3), NEO-A (0.7), PSE-A (129.4), PSE-D (124.5), RUG (1.7), STA (1.9), TEN (15.6)	**After 596 days on incubation** CHA-A (80.2), NEO-A (1.4), RUG (1.8), STA (28.2), TEN (10.6) **After 35 days on incubation** CHA-A (20.9), DEO-E (74.6), NEO-A (3.0), PSE-A (310.3), PSE-D (365.6)
E. halophilicum	MUT 798	**After 58 days on incubation** CHA-A (35.0), DEO-E (77.9), NEO-A (3.1), RUG (3.5), STA (39.1), TEN (8.3) **After 36 days on incubation** CHA-A (48.5), DEO-E (80.5), NEO-A (4.9), STA (25.7) **After 35 days on incubation** CHA-A (214.3), DEO-E (20.0), NEO-A (1.8), RUG (2.7), STA (448.9)	**After 596 days on incubation** CHA-A (62.0), NEO-A (2.2), RUG (9.8), STA (290.0), TEN (11.6)
E. halophilicum	MUT 1314	**After 58 days on incubation**	**After 596 days on incubation**

		CHA-A (1.7), RUG (4.3), STA (2.1), TEN (9.7) **After 35 days on incubation** CHA-A (138.4), NEO-A (1.2), STA (243.0)	CHA-A (91.1), DEO-E (15.3), NEO-A (3.1), RUG (16.4), STA (286.2), TEN (11.8)
E. halophilicum	MUT 1294	**After 35 days on incubation** CHA-A (23.6), DEO-E (15.4), STA (17.0)	**After 596 days on incubation** CHA-A (75.9), NEO-A (1.9), RUG (11.9), STA (267.2), TEN (10.5)
E. halophilicum	MUT 1306	**After 58 days on incubation** CHA-A (5.5), NEO-A (0.9), STA (8.9) **After 35 days on incubation** CHA-A (113.2), DEO-E (39.8), STA (168.5)	**After 588 days on incubation** CHA-A (42.1), NEO-A (2.9), RUG (7.1), STA (104.9) **After 35 days on incubation** CHA-A (12.6), NEO-A (0.6), RUG (8.0), STA (32.1)
E. halophilicum	MUT 1316	**After 35 days on incubation** CHA-A (204.3), NEO-A (1.0), STA (153.3)	**After 595 days on incubation** CHA-A (56.8), NEO-A (1.4), STA (67.6) **After 35 days on incubation** CHA-A (195.3), NEO-A (0.7), STA (135.7)
E. halophilicum	MUT 1298	**After 58 days on incubation** CHA-A (26.3), NEO-A (0.7), RUG (9.6), STA (47.5) **After 35 days on incubation** CHA-A (179.5), DEO-E (21.7), NEO-A (1.6), RUG (2.6), STA (330.9)	**After 596 days on incubation** CHA-A (53.4), NEO-A (1.4), RUG (9.3), STA (125.2) **After 35 days on incubation** CHA-A (23.8), RUG (12.4), STA (58.3)
E. halophilicum	MUT 1322	**After 35 days on incubation** CHA-A (158.7), DEO-E (38.0), NEO-A (0.9), RUG (3.3), STA (183.4)	**After 596 days on incubation** CHA-A (97.2), NEO-A (2.5), RUG (16.0), STA (304.7) **After 35 days on incubation** CHA-A (68.4), RUG (0.8), STA (90.0)
E. halophilicum	MUT 1315	**After 35 days on incubation** CHA-A (157.8), DEO-E (40.1), NEO-A (1.6), PSE-A (144.7), PSE-D (287.2), RUG (1.9), STA (148.5)	**After 289 days on incubation** CHA-A (109.4), NEO-A (2.7), PSE-A (67.7), PSE-D (1.12), RUG (8.0), STA (99.1), TEN (14.8)
E. halophilicum	MUT 1304	**After 58 days on incubation** CHA-A (1.8), RUG (2.9), STA (0.1) **After 35 days on incubation** CHA-A (318.0), DEO-E (32.2), NEO-A (2.1), PSE-A (293.5), PSE-D (442.8), RUG (2.7), STA (228.3)	**After 588 days on incubation** CHA-A (40.2), NEO-A (1.1), RUG (3.5), STA (15.6), TEN (10.1) **After 35 days on incubation** CHA-A (10.2), STA (2.4), TEN (11.7)
E. halophilicum	MUT 482	**After 80 days on incubation** CHA-A (53.8), NEO-A (2.5), STA (222.3) **After 58 days on incubation** CHA-A (32.4), DEO-E (12.7), NEO-A (2.4), STA (90.7) **After 35 days on incubation** CHA-A (50.8), DEO-E (20.4), NEO-A (2.8), STA (194.5) **After 14 days on incubation** CHA-A (18.1), DEO-E (23.3), STA (89.4)	**After 132 days on incubation** CHA-A (26.6), RUG (8.6), STA (113.4), TEN (10.6) **After 35 days on incubation** CHA-A (24.2), RUG (12.8), STA (109.5) **After 14 days on incubation** CHA-A (7.5), DEO-E (6.3), STA (49.3)

E. halophilicum	MUT 5534	**After 58 days on incubation** CHA-A (169.3), DEO-E (171.2), NEO-E (10.7), PSE-A (1029.2), PSE-D (976.2), RUG (4.0), STA (84.7) **After 36 days on incubation** CHA-A (107.0), DEO-E (198.4), NEO-E (10.7), PSE-A (1369.5), PSE-D (1046.5), STA (29.5) **After 35 days on incubation** CHA-A (225.6), DEO-E (19.8), NEO-E (1.2), PSE-A (216.0), PSE-D (246.3), STA (133.5) **After 14 days on incubation** CHA-A (102.4), DEO-E (26.3), PSE-A (32.4), PSE-D (57.0), STA (57.6)	**After 373 days on incubation** CHA-A (183.3), DEO-E (15.4), NEO-E (5.9), PSE-A (380.1), PSE-D (305.5), RUG (10.7), STA (152.1) **After 35 days on incubation** CHA-A (39.6), RUG (5.5), STA (14.0) **After 14 days on incubation** CHA-A (6.1), STA (2.6)
E. halophilicum	MUT 5535	**After 58 days on incubation** CHA-A (50.3), DEO-E (136.5), NEO-E (9.0), RUG (5.5), STA (84.6) **After 36 days on incubation** CHA-A (36.9), DEO-E (183.6), NEO-E (6.3), STA (48.9) **After 35 days on incubation** CHA-A (52.2), NEO-E (0.9), STA (160.3) **After 14 days on incubation** CHA-A (33.7), DEO-E (27.0), STA (152.4)	**After 360 days on incubation** CHA-A (40.4), DEO-E (7.3), NEO-E (1.5), RUG (5.8), STA (142.7) **After 35 days on incubation** CHA-A (54.4), DEO-E (10.7), RUG (16.5), STA (194.9) **After 14 days on incubation** CHA-A (17.1), STA (63.0)

*: Reported results are related only on positive secondary metabolite producers;,therefore, some *C. cladosporium* strains as well as all *A. vitricola* strains are not mentioned in the table.

** Fungal strains that are still in acquisition in MUT collections.

AND-A: andrastin A; AND-B: andrastin B; ASP: asperglaucide; ASP-A: aspergamid A; AVE1: averantin; AVE2: averufanin; AVE3: averufin; CHA-A: chaetoviridin A; CHR: chrysogin; CIT: citreorosein; DEM: demethylsulochrin; DEO-E: deoxybrevianamid E; EMO: emodin; FUL: fulvic acid; MEL: meleagrin; MET: methoxysterigmatocystin; MYC: mycophenolic acid; NEO: neoxaline; NEO-A: neoechinulin A; NID: nidurofin; NOR: norsolorinic acid; O-MET: o-methylviridicatin; ORS: orsellinic acid; OXA: oxaline; PSE-A: pseurotin A; PSE-D; ROQ-C: roquefortine C; ROQ-D: roquefortine D; RUG: rugulusovin; SEC-D: secalonic acid D; STA: stachybotryamide; STE: sterigmatocystin; SYD: sydonic acid; TEN: tenellin; VER-A: versicolorin A; VIR1: viridicatin; VIR2: viridicatol.

B - Secondary metabolite concentrations in settled dust samples collected at BAUM's repository during 2nd sampling

Table B11 Overview of fungal secondary metabolites detected in dust samples collected in BAUM's repository.

Metabolites	Area 1 µg kg^{-1} (±STD)	Area 2 µg kg^{-1} (±STD)	Area 3 µg kg^{-1} (±STD)	Area 4 µg kg^{-1} (±STD)
Alamethicin	3368.0 (±273.0)	1924.1 (±417.6)	229.2 (±381.4)	3.6 (±0.7)
Alternariolmethylether	4.3 (±0.5)	-	1.8 (±0.5)	2.1 (±0.5)
Altersetin	524.4 (±90.3)	53.4 (±10.3)	32.1 (±15.6)	3.7 (±7.5)
Andrastin A	3372.4 (±338.6)	186.0 (±372.0)	436.5 (±361.4)	66.7 (±133.3)
Andrastin B	16.1 (±5.8)	-	0.2 (±0.4)	-
Ascochlorin	-	-	1.6 (±0.4)	10.4 (±4.0)
Asperglaucide	2273.7 (±292.9)	14562.7 (±48)	2688.0 (±627.3)	5404.4 (±103.2)
Averufanin	-	-	0.1 (±0.3)	0.5 (±0.3)
Averufin	2.5 (±0.3)	9.7 (±0.7)	0.9 (±0.1)	3.9 (±1.1)
Beauvericin	-	0.8 (±1.5)	-	-
Brevianamid F	7.3 (±1.8)	3.5 (±6.0)	24.1 (±1.6)	14.5 (±0.8)
Chetomin	-	23.8 (±41.2)	-	15.4 (±10.3)
Chloramphenicol	1.2 (±0.0)	44.4 (±4.6)	-	7.2 (±1.0)
Chrysophanol	2.7 (±5.5)	13.7 (±27.3)	44.6 (±23.4)	30.3 (±12.4)
Citreorosein	15.1 (±7.0)	-	4.0 (±8.0)	21.5 (±5.2)
Cyclo (L-Pro-L-Tyr)	93.9 (±8.8)	47.8 (±27.7)	275.4 (±25.9)	130.4 (±12.4)
Cyclopenol	43.2 (±7.8)	-	2.9 (±5.7)	7.3 (±3.0)
Emodin	11.0 (±2.1)	15.2 (±0.8)	20.8 (±21.2)	16.6 (±1.4)
Enniatin B	0.3 (±0.1)	0.3 (±0.2)	-	0.1 (±0.0)
Ilicicolin B	-	9.0 (±6.4)	0.5 (±1.0)	15.9 (±1.6)
Lotaustralin	3.6 (±0.6)	-	6.4 (±1.0)	5.1 (±1.4)
Macrosporin	6.1 (±1.1)	-	4.9 (±0.8)	4.5 (±0.7)
Meleagrin	102.4 (±16.3)	130.5 (±18.6)	0.6 (±1.3)	18.9 (±8.5)
Methoxysterigmatocystin	5.2 (±0.4)	4.8 (±0.0)	5.7 (±2.1)	27.0 (±1.9)
Monocerin	1.6 (±0.4)	-	2.8 (±0.3)	5.6 (±0.4)
Neoechinulin A	1184.3 (±112.8)	59.0 (±9.9)	42.5 (±14.9)	28.7 (±2.8)
Neoxaline	1.3 (±0.1)	3.2 (±0.3)	0.5 (±0.2)	0.7 (±0.5)
Nidurufin	-	-	-	1.1 (±0.3)
O-Methylviridicatin	4.3 (±0.7)	-	0.7 (±0.3)	4.7 (±0.5)
Physcion	203.9 (±145.0)	-	146.3 (±47.5)	232.5 (±105.3)
Quinocitrinine A	-	-	0.3 (±0.3)	0.5 (±0.5)
Roquefortine C	16.3 (±1.9)	-	-	-
Rugulusovin	4.7 (±1.5)	8.5 (±1.2)	8.6 (±0.9)	4.3 (±0.3)
Skyrin	4.6 (±1.2)	-	-	0.6 (±1.2)
Stachybotryamide	1.4 (±0.0)	60.5 (±5.6)	-	2.0 (±0.7)
Stachybotrylactam	1.4 (±0.3)	93.2 (±12.3)	1.0 (±0.3)	14.2 (±1.2)
Sterigmatocystin	17.4 (±0.4)	6.1 (±1.5)	2.1 (±0.2)	12.8 (±1.0)
Sydonic acid	-	-	0.9 (±1.7)	53.5 (±4.2)
Tenellin	38.1 (±11.6)	545.5 (±326.1)	-	17.3 (±2.2)
Terrecyclic acid	-	3149.3 (±241.6)	-	-
Usnic acid	13.9 (±2.0)	26.7 (±8.6)	26.7 (±10.6)	6.4 (±3.1)
Viridicatin	3.8 (±1.2)	26.7 (±14.1)	0.5 (±1.0)	3.2 (±2.0)
Viridicatol	116.3 (±24.5)	40.8 (±30.0)	-	11.1 (±22.3)

APPENDIX C

Conference contributions as Presenting Author

Micheluz A., Rovea M., Formenton G., Lanzoni C., Manente S., Tigini V., Montanari M., Pinzari F., Varese G.C., Ravagnan G., (2014). Fungal infections and Cultural Heritages: An alternative approach for VOCs analysis in indoor environments. Proceedings of 11th International Conference Indoor Air Quality in Heritage and Historic Environments, Prague (Czech Republic), 2014. Poster.

Micheluz A., Manente S., Prigione V., Tigini V., Pinzari F., Varese G.C., Ravagnan G., (2014). Fungal contamination specifically related to the use of compactus shelvings: the case study of a Venetian library. Proceedings of 11th International Conference on Non-Destructive Investigations and Microanalysis for the Diagnostic and Conservation of Cultural and Environmental Heritage, Madrid (Spain), 2014. Poster.

Micheluz A., Rovea M., Formenton G., Manente S., Prigione V., Tigini V., Pinzari F., Varese G.C., Ravagnan G., (2015). VOCs analysis from microbiological sources: risk assessment in indoor environments affected by fungal infections. Proceedings of 25th SETAC Europe Annual Meeting, Barcelona (Spain), 2015. Poster.

Micheluz A., Sulyok M., Manente S., Krska R., Varese G.C., Ravagnan G., (2015). Mycotoxin-producing ability of indoor airborne fungi isolated from librarian environment affected by mould contamination. 37th Mycotoxin Workshop, Bratislava (Slovakia), 2015. Oral presentation.