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ABSTRACT

The principal fungal species isolated from a contaminated library environment were tested for their microbial volatile organic compound (MVOC) production ability. *Aspergillus creber*, *A. penicillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum* were cultivated on suitable culture media inside sample bottles specifically designed and created for direct MVOC injection to a GC–MS instrument. The fungal emissions were monitored over several weeks to detect changes with the aging of the colonies, monitored also by respirometric tests. A total of 55 different MVOCs were detected and isopropyl alcohol, 3-methyl-1-butanol and 2-butanone were the principal compounds in common between the selected fungal species. Moreover, 2,4-dimethylheptane, 1,4-pentadiene, styrene, ethanol, 2-methyl-1-butanol, acetone, furan and 2-methylfuran were the most detected compounds. For the first time, the MVOC production for particular fungal species was detected. The species *A. creber*, which belongs to the recently revised group *Aspergillus* section *Versicolores*, was characterized by the production of ethanol, furan and 1,4-pentadiene. For the xerophilic fungus *E. halophilicum,* specific production of acetone, 2-butanone and 1,4-pentadiene was detected, supported also by respirometric data. The results demonstrated the potential use of this method for the detection of fungal contamination phenomena inside Cultural Heritage's preservation environments.

1. Introduction

The study of microbial volatile organic compounds (MVOCs) produced by moulds developed in indoor environments has been addressed by several authors, especially in relation with dampness situations and possible human health problems (Cabral, 2010; Moularat et al., 2008; Polizzi et al., 2009; Wady et al., 2003). Since the 1990s, MVOCs are often discussed to be associated with the sick building syndrome (SBS) because of their suspected role as responsible for a wide range of specific and non-specific symptoms and discomfort (Elke et al., 1999; Larsen et al., 1998; Matysik et al., 2008; Meyer et al., 1998; Wessén and Schoeps, 1996).

In the recent years, MVOCs started to be considered also for cases concerned the preservation of Cultural Heritage, as biodeterioration phenomena interested archive and library collections (Joblin et al., 2010; Pinzari et al., 2004). Fungal contamination became a frequent and complex problem to manage, often with severe, economic and health implications (Montanari et al., 2012). However, the majority of previous studies were mainly focused on the detection of chemical markers specifically related to the natural degradation of the book and paper components (Fenech et al., 2010; Lattuati-Derieux et al., 2004; Strlič et al., 2009, 2010).

The preservation of art collections starts from the performed prevention features, and MVOCs could serve as general early indicators of potential biocontamination problems (Pinzari et al., 2004). In fact, these compounds originate from both fungal primary and secondary metabolite production, strictly depending on the fungal species, the substrate and in according to the different fungal growing phases (Korpi et al., 2009; Matysik et al., 2008; Polizzi et al., 2009). Several alcohols, e.g. 1-octen-3-ol and 3-octanol, as well as ketones and furans were addressed as indicators of mould, recognised both on pure culture studied, on agar substrate, and on wallpaper and building materials (Polizzi et al., 2012). *Aspergillus* and *Penicillium* are the most investigated fungal genera in MVOC studies (Fiedler et al., 2001; Matysik et al., 2008, 2009; Moularat et al., 2008; Polizzi et al., 2012; Schuchardt and Kruse, 2009; Wady et al., 2003, Wady and Larsson, 2005) because of their ubiquity in indoor environments (Cabral, 2010; Samson et al., 2004), also in association with the biodeterioration of Cultural Heritage (Micheluz et al., 2015; Sterflinger, 2010; Zyska, 1997). However, in the recent years, specific fungal contamination emerged inside Italian archives and library, mainly caused by a xerophilic fungus with a lack of knowledge about its MVOC emission capability, i.e. *E. halophilicum* (Micheluz et al., 2015; Montanari et al., 2012; Pinzari and Montanari, 2011).

The determination of MVOCs is usually accomplished by gas chromatography-mass spectrometry (GC–MS) because of its power-

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ful separation capability and highly sensitive detection performance (Matysik et al., 2009). Different sampling methods have been commonly used for sampling volatiles, as headspace solid-phase microextraction (HP-SPME) and Tenax desorption tubes, Tenax® TA, because of their low-costs and as rapid tool to determine very low quantities of a wide range of analytes (Fiedler et al., 2001; Matysik et al., 2008; Schuchardt and Kruse, 2009). Recently, other passive devices were developed specifically for long-term study requirements, as adsorbents based on activated charcoal (Matysik et al., 2009) or PDMS strips (Gibson et al., 2012). Other kind of technique is based on the MVOCs detection by sensor-based devices, e.g. polymer sensors responsive to variation in electrical conductivity due to VOC adsorption (Joblin et al., 2010) or electron noses (e-noses), based on rapid detection and identification of a preselected range of volatile compounds (Kuske et al., 2005; Pinzari et al., 2004).

Most of the sampling techniques require a support device for the volatile compound captures, a desorption system and, often, a pre-selection of detectable compounds. In order to avoid these steps and to permit the analysis of the total air composition, with the specific aim to prevent chemical artefact formation, an alternative air sampling system was presented. Based on EPA TO-15 (1999), *ad-hoc* fungal culture bottles were developed for the analysis of several fungal species isolated from a contaminated library by GC–MS. As a result of a previous work (Micheluz et al., 2015), *Aspergillus creber*, *A. penicillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum* were tested for their MVOC production. The aim of this work was to highlight specific chemical compounds for each fungal species, comparing the results with data available in literature and improving the knowledge for the species, which never have been screened before for their volatile compound production.

2. Materials and methods

2.1. Study site

The study was focused on a repository of the Library of Humanities (Biblioteca di Area UManistica, BAUM), at Ca' Foscari University, Palazzo Malcanton Marcorà, in Venice (Italy), in which a spreading fungi contamination interested > 27,000 books belonging to the Historical collections. Located in the second underground floor, the repository covers an area of about 150 m^2 and is furnished with 50 Compactus® shelves.

2.2. Sampling and fungal identification

The mycological sampling was performed for the detection of the viable airborne fungal load and for the isolation of fungi grown on books. Five sampling areas were chosen inside and outside of the repository, as reported in the previous study by Micheluz et al. (2015). Air sampling was performed in active mode by Sampl'air Lite sampler (Biomérieux, Florence, Italy) in three replicates with 9-cm Petri dishes containing different media (Malt Extract Agar, MEA and Malt Extract Agar added with 15% of NaCl, MEA15%, purchased at Fluka, Sigma-Aldrich), flow rate 100 L min^{-1} and sample volume of 100 L. Contaminated books were sampled by sterile cotton swabs (Cultiplast, PL ItalianaSpa, Milan, Italy) wiped on book covers and then inoculated onto 9-cm Petri dishes. Fungal identifications were based on macroscopic and microscopic features and confirmed by molecular techniques (Micheluz et al., 2015, 2016). All the fungal strains are preserved at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and System Biology, University of Turin (Italy).

2.3. Selection of fungal species and culture conditions

Among 36 mould species isolated from the repository (Micheluz et al., 2015), 7 fungal strains were selected for their MVOC production ability (Table 1). *Aspergillus creber*, *A. penicillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum* were selected on the base of their isolation frequency, their occurrence in indoor air/on contaminated books and a lack of knowledge about their MVOC production.

All fungal strains were cultivated inside 1 L glass bottles with an area of about 64 cm^2 and 100 mL of medium. As a result of previous selection media (Christensen et al., 1959; Micheluz et al., 2015) three replicas of each strain of fungi were inoculated onto MEA, except *E. halophilicum* and *A. penicillioides* samples which were cultivated onto the selective low water activity medium MEA15%. As reported in Fig. 1, each bottle was closed with a cap composed of two thermoresistent plastic tubes: the first tube was furnished with a Swagelok connector as direct connection with GC–MS instrument, while the second tube was equipped with a activated charcoal tube (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 after each sampling. The bottles were incubated in a dark place at 25 °C for 1–2 months. Samples bottles filled with non-inoculated MEA and MEA15% media were used as blanks.

2.4. MVOCs pre-concentration

In order to detect the MVOCs production, a direct tube with Swagelok connection was established between the sampling bottle and the microscale *purge & trap* Entech 7100 system (Entech Instrument, Inc). For each analysis, 40 mL of sample and 100 mL of internal standards (I.S.) spiking mixture of 20 μg m^{-3} (1,4-bromofluorobenzene, 1,4-difluorobenzene, bromochloromethane and chlorobenzene-d_5) were suctioned separately with a pump, controlled by a mass flow controller, and injected together inside the pre-concentrator system in order to quantified the unknown compounds using the internal standard method. The I.S. was prepared from 1 mg m^{-3} of certificated mixture (SIAD, certificated mixture with 6% of uncertainty) and the compounds were selected because of their non-interfering behaviour with environmental and fungal VOCs (Rovea et al., 2013). The concentration of the sample was carried out

Table 1
List of fungal species isolated from the contaminated repository and selected for MVOC analysis. MUT and GenBank® accession numbers are included.

Fungal species	MUT no.	GenBank® accession number	Source
Aspergillus creber Jurjević, S.W. Peterson & B.W. Horn	MUT 470	KU179486	Book cover
Aspergillus penicillioides Spegazzini	MUT 481	KU179489	Book cover
Cladosporium cladosporioides (Fresen) G.A. de Vries	MUT 527	KU179495	Indoor air
Eurotium chevalieri L. Mangin	MUT 472	(*)	Indoor air
Eurotium halophilicum C.M. Chr., Papav. & C.R. Benj.	MUT 482	KM502179	Book cover
Penicillium brevicompactum Dierckx	MUT 536	KM502183	Book cover
Penicillium chrysogenum Thom	MUT 5493	KM502200	Indoor air

(*) Fungal strain without deposited sequence in GenBank® because of its low quality.

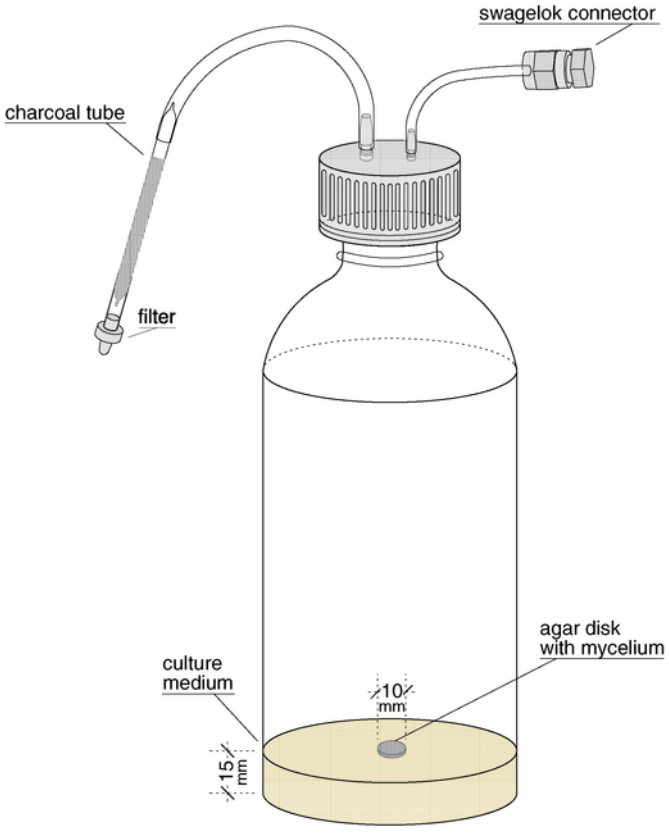

Fig. 1. Sample bottle for MVOC analysis of fungal culture.

in successive stages inside the Entech pre-concentrator, starting in the *module 1*, consisting of a trap with glass microspheres cooled to − 150 °C with liquid nitrogen. VOCs with water and carbon dioxide were quantitatively blocked, while the main air constituents, i.e. oxygen, nitrogen and argon, were discharged. After that, the *module 1* was heated to 10 °C and the VOCs were transferred to *module 2*, a Tenax trap, provided with helium stream and cooled to − 30 °C. In this passage, carbon dioxide was discharged, while water remained in the *module 1*. The VOCs were adsorbed on the Tenax resin, because of their high affinity. By heating at 180 °C, the VOCs were thermally desorbed from module 2 and transferred to *module 3*, which is constituted by a cryofocuser cooled to − 150 °C with liquid nitrogen. Finally, the *module 3* was quickly heated and the analytes were transferred to the gas chromatographic column.

2.5. Gas chromatography-mass spectrometry (GC–MS)

Analysis was 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 as acquisition software. For separation, an Equity 1 capillary (Supelco, 60 m, 0.32 mm i.d., 1 μm df) with helium as carrier gas was used. The GC oven program was 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 was applied and mass fragments were 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) was used to identify the volatiles.

2.6. Fungal respirometric analysis

The biological activity of each fungus was measured indirectly by the manometric method. The fungi were inoculated in 1 L septum sealed nozzle bottles filled with 100 mL of MEA medium, with the exception for *E. halophilicum* and *A. penicillioides* which MEA15% medium was used. All the bottles were closed with a pressure sensor mounted on top (OxiTop®-C system, WTW, Weilheim, Germany) and incubated for 25–35 d at 25 °C. The carbon dioxide evolved during aerobic respiration was quantitative adsorbed using droplets of NaOH (Carlo Erba Reagents, Milan, Italy) inside the sealed nozzle septum of the bottles. The pressure drop detected (every 100 min) in the bottle was proportional to the amount of oxygen used by fungi (Pereira et al., 2014; Schuchardt and Kruse, 2009; Willcock and Magan, 2001).

3. Results

The MVOC production was monitored from the inoculum to the idiophase of the fungal colonies, in particular after 3, 6, 12, 19 and 24 days after inoculation. For *A. penicillioides* and *E. halophilicum*, the monitoring started after 6 days, because of their slow growth, and extended with weekly analyses until 28 days for *A. penicillioides* and until 60 days for *E. halophilicum*.

During the monitoring period, we detected the appearance and the growth of many compounds, and the disappearance of others (i.e. all the aldehydes of the culture media). > 100 chromatographic signals have been detected for each species, but only between 20 and 50 signals could be identified by the instrument library. Between these, only the most significant signals with high abundance were taken under consideration (peak area > 10^6 units in TIC mode). Moreover, after the subtraction of blank samples, 55 volatiles emitted by the cultures could be chemically classified. The identified compounds together with their increase or decrease of their peaks areas over the whole period of growth are reported in Table 2 for the fungal strain cultivated on MEA medium, and in Table 3 for those cultivated on MEA15%. The results are expressed as peak area/peak $area_1$ ratio, where peak $area_1$ is the peak area of the compound at the first detection. This normalization process well reflects the changes of the MVOC's emission concentration.

The only three compounds that all fungal species have in common were isopropyl alcohol and 3-methyl-1-butanol and 2-butanone. Moreover, 2,4-dimethylheptane, 1,4-pentadiene, styrene, ethanol, 2-methyl-1-butanol, acetone, furan and 2-methylfuran were the most detected compounds.

P. brevicompactum was the species with the widest range of produced MVOCs, with 34 different compounds. The chemical pattern was mainly composed by acetone and furan, with a lower production of 3-methyl-1-butanol, isopropyl alcohol, styrene, 2-methyl-1-butanol, 2-butanone and ethanol. *E. chevalieri* emitted 31 different compounds. The principal emission was composed by 1,4-pentadiene, acetone, furan and ethanol, with less presences of 2-butanone, 2-methylfuran, isopropyl alcohol and 3-methyl-1-butanol. For *C. cladosporioides*, 22 different compounds were detected, with acetone, 2-butene, ethanol, furan and 2-butanone as the main products. *A. creber* emitted 20 volatiles and 2-butene, ethanol, furan, 1,4-pentadiene, isopropyl alcohol, 1,3-octadiene, 2-butanone, 3-methyl-1-butanol and 2-octen-1-ol were the main composition of its chemical pattern. Finally, 19 compounds were detected for the strain of *P. chrysogenum*, mainly composed by acetone, ethanol and styrene. Other significant emissions were furan, 3-methyl-1-butanol, isopropyl alcohol, 2-bu-

Table 2
Overview of MVOC production, directly analysed by GC–MS, by selected fungal species grown on MEA during the monitoring period. Retention time (RT) for recognized compounds are listed. 1–5 are the different sampling phases from 3 to 24 days. X: compounds with distinct signal (TIC > 10^6). Peak area/peak area$_1$ > 1: +; > 10: ++; > 100: +++; = 1: =; < 1: – [peak area$_1$ = peak area of the compound at the first measurement].

Substances	RT (min)	*A. creber*					*C. cladosporioides*					*E. chevalieri*					*P. brevicompactum*					*P. chrysogenum*			
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4
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	–	+	+				
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-Trimethyldodecane	20.75											X	–	–	–	–									

tanone, 1,4-pentadiene, 2-methyl-1-butanol, 2,5-dimethylfuran and 2-methylfuran.

Also *A. penicillioides* emitted a wide range of volatiles, 29 different compounds, mainly composed by acetone, 3-octanone, 2-butanone, 1,3-octadiene, 2-octen-1-ol, isopropyl alcohol and cyclopentanone. *E. halophilicum* produced a total of 23 volatiles mainly composed by 2-butanone and acetone, with less presences of ethyl acetate, 1,4-pentadiene, 3-methyl-1-butanol, ethanol, 2-butanol, isopropyl alcohol, 2-methylfuran and tetrahydrofuran.

Different fungal behaviours were emerged by OxiTop®-C system respirometric analysis, highlighting three mainly clusters: faster growing fungi, slower growing fungi and the slowest growing fungus (Fig. 2). The first group contains *A. creber*, *E. chevalieri*, *P. brevicompactum* and *P. chrysogenum*, that reached the steady-state of oxygen consumption after only 5 days of incubation. *Aspergillus penicillioides* and *C. cladosporioides* were the components of the second group, which reached the steady-state around 9 days of incubation. The last fungal species, *E. halophilicum*, was detected as the slowest

Table 3
Overview of MVOC production, directly analyzed by GC–MS, by selected fungal species grown on MEA15% NaCl during the monitored periods. Retention time (RT) for recognized compounds are listed. 1–9 are the different sampling phases from 6 to 60 days. X: compounds with distinct signal (TIC > 10^6). Peak area/peak $area_1$ > 1: +, > 10: ++, > 100: +++, = 1: =, < 1: – [peak $area_1$ = peak area of the compound at the first measurement].

Substances	RT (min)	*A. penicillioides*				*E. halophilicum*								
		1	2	3	4	1	2	3	4	5	6	7	8	9
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	+	+	−									

growing fungus, because it started to consume oxygen after 19 days from the inoculation.

4. Discussion

The investigation was principally focused on the total MVOC detection with the innovative direct sampling, in order to avoid any pre-chemical selection due to the use of passive sampling devices. However, the use of only one kind of medium for each fungal species (MEA or MEA15%) probably limited the MVOC emission. In fact, it is well-known how the production is strictly growth substrate-dependent (Matysik et al., 2009; Moularat et al., 2008) and several kinds of culture media, included contaminated building materials or settled dust samples, were generally included in the experiments (Korpi et al., 1997; Matysik et al., 2008; Vishwanath et al., 2011; Wady and Larsson, 2005; Wady et al., 2003; Wilkins et al., 2000). On the other hand, working with Cultural Heritage has often limitations and, because of the preciousness of the artefacts, most of the applied methods need to be non-invasive (Kostadinovska, 2015; Manente et al., 2012). This study investigated for the first time the MVOC production ability of the most frequent fungi directly involved with book contamination, identifying specific chemical patterns for each fungal species. For the common indoor fungi and already known MVOC-producer, as *C. cladosporioides*, *P. brevicompactum* and *P. chrysogenum* (Samson et al., 2004), several specie-specific compounds were detected. *C. cladosporioides* emitted 1-octene, 3-methyl-1-butanol, 2-octen-1-ol, 2-pentanone and 3-octanone, as already observed in previous studies (Matysik et al., 2008, 2009; Sunesson et al., 1995). For *P. brevicompactum*, 2-methyl-1-propanol, acetone, 3-pentanon, 2-butanone and 3-methylfuran can be considered as possible markers (Börjesson et al., 1992), while the compounds 1-heptene, 2-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol were characteristic for *P. chrysogenum* (Matysik et al., 2008, 2009; Wilkins et al., 2000).

Few records in literature are available for the xerophilic fungi *A. penicillioides* and *E. chevalieri*. Similar to our results, Wady et al. (2003) detected 2-pentanol, 3-octanone, 1-octen-3-ol and 2-methyl-1-butanol for cultures of *A. penicillioides* isolated from mineral wood onto DG18 medium. For *E. chevalieri*, the only similar compound compared to Elke et al. (1999) was 3-methylbutan-1-ol regarding the analysis of *Eurotium* species isolated from indoor dust samples on DG18 medium.

A lack of knowledge about MVOC production exists for the species *A. creber*, due to its recent identification among the *As-*

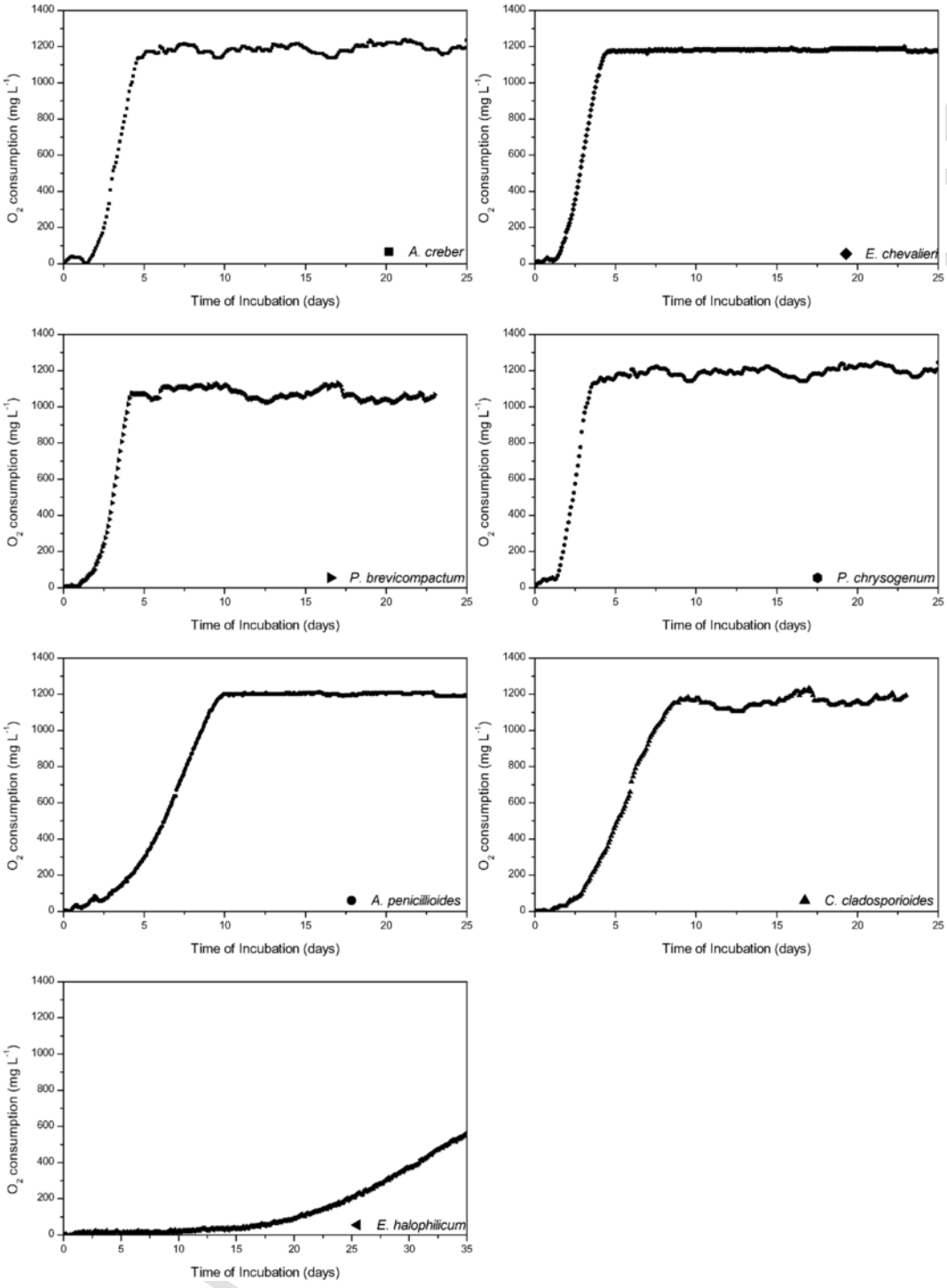

Fig. 2. Overview of respirometric trends of all selected fungal species.

pergillus section *Versicolores* group (Juriević et al., 2012). Considering the MVOC production of a well-known species belonged to the same group, *A. versicolor*, *A. creber* resulted similar for the emission of 1,3-pentadiene, 1-octene, 3-methyl-1-butanol, 2-octene-1-ol, 3-octanone and 3-methylfuran (Fiedler et al., 2001; Polizzi et al., 2012; Schuchardt and Kruse, 2009; Sunesson et al., 1995). As reported by Matysik et al. (2008) for *A. versicolor*, the temporary trend of the main emission by *A. creber* recorded a maximum peak around 6 days after incubation, followed by a general decrease.

For *E. halophilicum* no data of its MVOC production is available in literature. The interest around this fungal species increased in the last few years because of its spread identification in connection with strong contaminated repositories and archives around Italy (Micheluz et al., 2015; Montanari et al., 2012; Pinzari and Montanari, 2011). In a previous study, Micheluz et al. (2016) have characterized this fungus for its secondary metabolite production, and this is the first report of its MVOC production ability. On MEA15%, *E. halophilicum* emitted a wide range of volatile compounds, which increases clearly fol-

lowing its growth during the entire monitoring period (60 days). The chemical pattern was mainly composed by acetone and 2-butanone, with lower presences of 1,4-pentadiene, ethyl acetate, 2-butanol, 3-methyl-1-butanol, tetrahydrofuran and 2-methylfuran (Fig. 3). Among all of these compounds, the time trend for 2-butanone and 1,4-pentadiene emissions resulted in accordance with the respirometric data detected for this fungus (Fig. 4). For this reason, they can be considered potential proxy for the detection of *E. halophilicum*'s presence. Furthermore, the respirometric curves indicate MVOCs

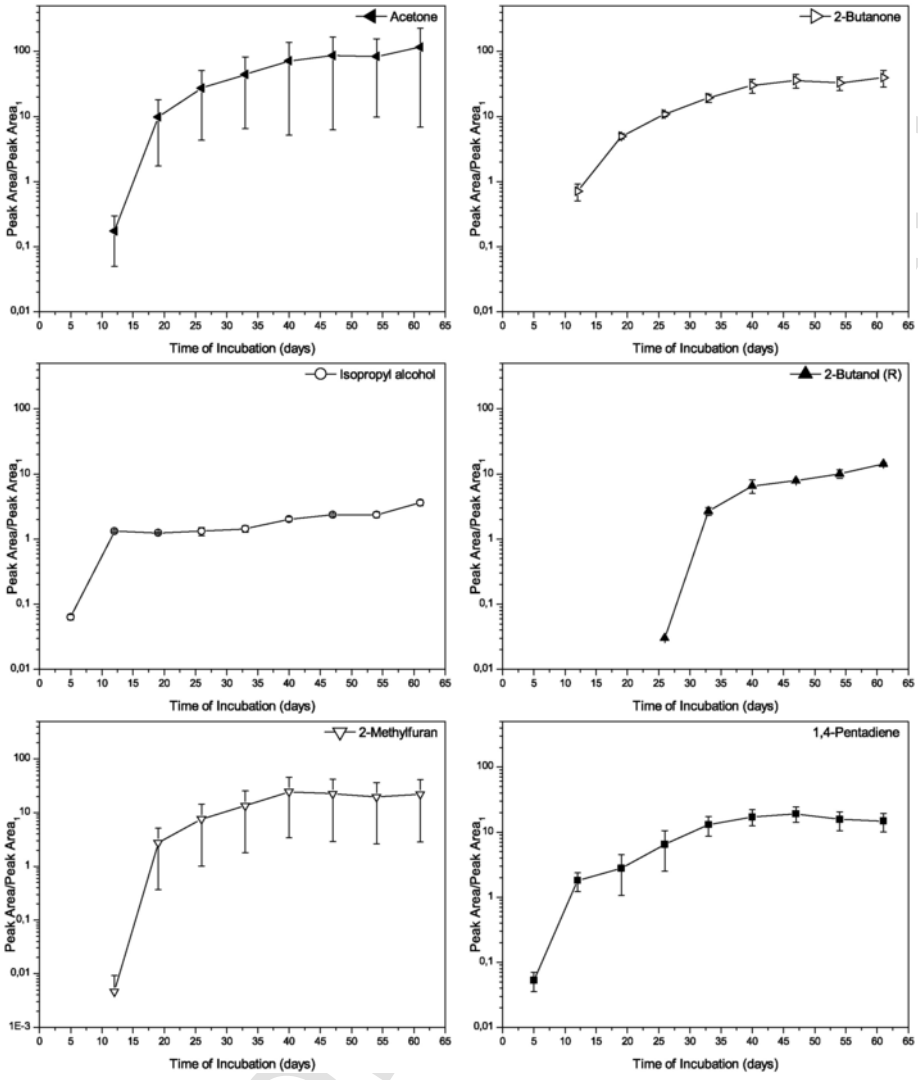

Fig. 3. Temporary evolution of principal emitted MVOCs of *E. halophilicum*, expressed as ratio of GC–MS peaks between second, third, fourth and followed measurements with the peak area of the first measurement. The values are the averages of three replicates (n = 3).

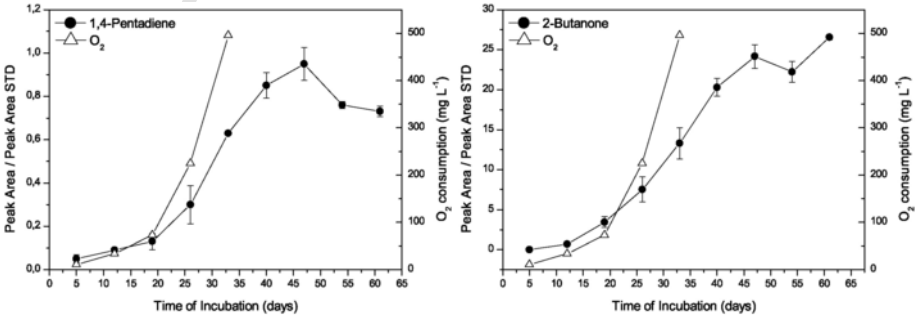

Fig. 4. Similar trends between temporary production of 1,4-pentadiene and 2-butanone with O_2 consumption data obtained by respirometric analysis of *E. halophilicum* investigations. The chemical values are the averages of three replicates (n = 3).

production as products of an active fungi metabolism, also along the entire monitoring period.

The MVOC analysis of the principal mycoflora of a contaminated indoor environment helped us to focus our research toward specific chemical proxies. The temporal monitoring has confirmed that the fungi started to produce volatile compounds from the first stage of their development but also during their vegetative phase. This is a confirmation about the variable chemical production, also in a limited growth conditions.

The next step is the analysis of old fungal colonies in order to characterize the variation of volatile production during all fungal vital cycles.

This analysis could be a faster alternative to detect mould presence in indoor environments. In this way, we can focus our attention towards selected MVOCs in similar preservation studies, determining also the age of the contamination and selecting the best conservation intervention.

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