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► To cite this version:

C. Kalalian, A. Depoorter, L. Abis, S. Perrier, C. George. Indoor heterogeneous photochemistry of molds and their contribution to HONO formation. *Indoor Air*, 2021, 10.1111/ina.12971. hal-03476783

HAL Id: hal-03476783

<https://hal.science/hal-03476783v1>

Submitted on 12 Oct 2023

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Indoor heterogeneous photochemistry of molds and their contribution to HONO formation

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Abstract

To better understand the impact of molds on indoor air quality, we studied the photochemistry of microbial films made by *Aspergillus niger* species, a common indoor mold. Specifically, we investigated their implication in the conversion of adsorbed nitrate anions into gaseous nitrous acid (HONO) and nitrogen oxides (NO_x), as well as the related VOC emissions under different indoor conditions, using a high-resolution proton transfer reaction-time of flight-mass spectrometer (PTR-TOF-MS) and a long path absorption photometer (LOPAP). The different mold preparations were characterized by the means of direct injection into an Orbitrap high-resolution mass spectrometer with a heated electrospray ionization (ESI-Orbitrap-MS). The formation of a wide range of VOCs, having emission profiles sensitive to the types of films (either doped by potassium nitrate or not), cultivation time, UV-light irradiation, potassium nitrate concentration and relative humidity was observed. The formation of nitrous acid from these films was also determined and found to be dependent on light and relative humidity. Finally, the reaction paths for the NO_x and HONO production are proposed. This work helps to better understand the implication of microbial surfaces as a new indoor source for HONO emission.

Keywords: Microbial surfaces, *Aspergillus niger*, photochemistry, VOC, nitrous acid (HONO), indoor air quality

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25 **Practical Implication**

- 26 • Microbial surfaces are ubiquitous in sick buildings. They are involved in the indoor
27 emission of spores, fragments, microbial VOCs and HONO.
- 28 • The photochemistry of microbial films doped with potassium nitrate enhances the
29 formation of HONO.
- 30 • Indoor heterogeneous photochemistry of molds influences the indoor oxidative
31 capacity.

32 **1. Introduction**

33 The health risk due to exposure to various types of indoor pollutants becomes greater as people
34 spend exceedingly their time indoors.^{1,2} Variety of pollutants might be emitted directly from
35 indoor sources, from secondary chemistry initiated by oxidants and surface reactions or via
36 infiltration of outdoor air.^{3,4} The high surface to volume ratio (1 to 4 m⁻¹)⁵⁻⁸ in indoor
37 environments points to the importance of heterogeneous chemistry through mechanisms
38 including adsorption, oxidation reactions and acid-base chemistry.^{9,10} Indoor surfaces involving
39 different types of building materials like gypsum board, paint, glass, wood, tile, laminate,
40 carpet, and fabric as well as furniture, human activities, and microbial activity, can be important
41 sources and sinks of reactive pollutants indoors.⁸ Volatile Organic Compounds (VOCs) are
42 among these pollutants. VOCs can react with the indoor oxidants (like ozone (O₃) and OH
43 radicals), resulting in the formation of multifunctional compounds in the gas phase, and can
44 contribute to the formation of secondary organic aerosol.^{8,11} VOCs are often detected indoors
45 with higher concentrations than in the outdoor environment,^{8,12,13} and their emissions depend
46 on various environmental factors such as light, relative humidity and temperature.^{9,14} However,
47 indoor VOCs concentrations are mainly controlled by air ventilation, human occupancy and
48 activity, alongside their indoor chemical reactions with indoor oxidants.^{9,15}

49 In addition, growing molds in damp dwellings can be an indoor source of spores, fragments
50 and microbial VOCs (mVOCs).¹⁶ The most common molds identified in indoor environments
51 are *Penicillium*, *Aspergillus* and *Cladosporium*.^{17,18} The growth of these fungi on different
52 surfaces is accelerated by the adequate presence of nutrients, temperature, pH and relative
53 humidity.¹⁵ In an indoor environment with moisture and/or high humidity, fungal spores can
54 stick to a wide variety of organic and inorganic materials, which is the first step of colonial
55 development and reproduction.¹⁹ After their proliferation, they germinate and grow producing
56 thousands of new spores using nutrients present on the different colonized substrates.¹⁵ It was
57 also demonstrated that the cell-to-cell interactions between the spores and/or hyphae are
58 essential for the development of three-dimensional mycelial structures like biofilms.¹⁹

59 Moreover, as fungal cells might not be detected in the ambient air in the early stage of
60 contamination, microbial VOCs (mVOCs) produced either by the microorganisms metabolism
61 or by the VOCs processing, can then be used as tracers of fungal contamination within a
62 dwelling.²⁰ One should note that the indoor concentrations of mVOCs in sick buildings range
63 from few ng/m³ to 1 mg/m³.²¹⁻²⁴ In addition to producing chemicals indoors, some
64 microorganisms are able to degrade chemical contaminants, like phthalates, emitted from home
65 vinyl flooring.²⁴ Furthermore, previous studies have shown a positive correlation between the
66 gases SO₂, NO₂, and CO and the airborne microorganisms. This correlation is probably due to
67 the use of sulfate, nitrate, and carbonate (transformed from these gases) as the nutrients by the
68 viable microbes.²⁵ In a recent study by Guo et al.,²⁶ the effect of different formaldehyde levels,
69 a harmful indoor VOC emitted by various indoor surfaces, on indoor bacterial communities was
70 established. This study has shown the interaction between formaldehyde levels and indoor
71 bacteria and gave new insight for future studies on the interaction between indoor pollutants
72 and microbial species.

73 Furthermore, one should note that the indoor environment contains highly reactive
74 molecules and radicals such as ozone (O₃), nitrate (NO₃) and hydroxyl radicals (OH), nitrogen
75 oxides (NO_x), sulfur dioxide (SO₂).¹⁴ Hydroxyl radicals are now considered as the most
76 important oxidant indoors. Recent measurements have shown that their concentrations can
77 reach 10⁶ molecules cm⁻³, similar to outdoor daytime levels.⁴ OH radicals were thought to be
78 mainly produced during the ozonolysis of unsaturated VOC. Nonetheless, recent studies
79 showed that the photolysis of nitrous acid (HONO), at wavelengths lower than 400 nm, can also
80 be a source of OH radicals, contributing to more than 50% of their emissions.^{4,27-30} Meanwhile,
81 HONO can result from different sources such as combustion processes, gas-phase reactions
82 between OH and NO, or (photo)chemical heterogeneous reactions.^{1,28,31-34} The measured
83 HONO concentrations indoors are typical around a few ppb, reaching higher values in poorly
84 ventilated residences with gas-fired stoves.^{28,29} It can also form carcinogenic nitrosamines via
85 its reaction with secondary amines.²⁸ Several studies have shown that the HONO formation can
86 be enhanced by light (300 nm < λ < 400 nm).^{7,27,28,32,35} Besides, nitrate anions can result from the
87 oxidation of NO_x into nitric acid (HNO₃). Arata *et al.* performed direct measurements of N₂O₅
88 and NO₃ indoors, where these species react to produce HNO₃.³⁶ Nitric acid is likely to deposit
89 onto surfaces, where the presence of water layers could lead to its hydrolysis and thus to the
90 production of surface nitrate.⁷ Schwartz-Narbonne *et al.* characterized real indoor films
91 (collected from the living room and kitchen) in terms of water uptake and ionic composition
92 and found concentrations of nitrate anion ranging from 7.57 to 25.17 μg/cm².⁹ Liu *et al.*³⁷ have
93 also identified different anions (F⁻, Cl⁻, Br⁻, NO₃⁻, PO₄³⁻, SO₄²⁻) on glass plates collected from
94 the kitchen with concentrations from 0.117 to 142.499 ppm.

95 Indoor surfaces are typically covered by a complex mix of organic and inorganic
96 compounds.^{9,32,38,39} In this work, we investigated the photochemistry of microbial films made
97 by *Aspergillus niger* species, a common indoor mold, and their implication in the conversion of

98 adsorbed nitrate anions into gaseous nitrous acid, nitrogen oxides, and associated VOC
99 emissions, as a function of light irradiation, relative humidity and molds cultivation time. This
100 will unravel the implication of microbial surfaces, ubiquitous in sick buildings, in the HONO
101 formation, affecting the indoors oxidative capacity and will help better understand interactions
102 between microbes and chemistry in human-dominated habitation.

103 **2. Experimental section**

104 **2.1.Molds and film preparations**

105 Molds were cultivated on a synthetic malt agar extract, recommended for the cultivation of
106 indoor mold species.⁴⁰ The protocols used for the culture medium preparation, as well as the
107 species identification by molecular biology are described by Kalalian et al.²³ The grown molds
108 belonged to the *Aspergillus sp.* type *Aspergillus niger*.

109 The first step of the film preparation was to wash a grown molds' petri dish with Milli-Q
110 water (18.2 M Ω cm), in order to create a solution, named "molds extract", containing a
111 suspension of molds in water. Then, the second step consisted in filling a petri dish (40 mm in
112 diameter) with either (i) a solution of molds extract or (ii) a solution of potassium nitrate KNO₃
113 and (iii) a mixed solution of molds extract and potassium nitrate KNO₃. The concentrations of
114 the potassium nitrate KNO₃ solutions were varied from 0.001 M to 0.5 M. The preparations
115 were dried over a period of 12 hours in an oven at 323.2 \pm 0.1 K. Once dried, the petri dishes
116 were weighed again (the empty petri dishes were weighed beforehand) and used directly (within
117 one day) in the planned experiments.

118 **2.2.Experimental setup**

119 Experiments were performed in an 80 cm³ borosilicate reactor under a constant flow of
120 dry/humid synthetic air, or synthetic N₂, and irradiated from the top by a 340 nm LED lamp
121 (ThorLabs, 60mW, nominal maximum irradiance of 2.22 W/m²). The humidity in the reactor

122 was varied by changing the mixing ratio of dry or humid carrier gas. The temperature ($T = 25$
123 $\pm 2^\circ\text{C}$) and the relative humidity in the reactor ($RH = 0-85\% \pm 2\%$) were controlled by a
124 humidity and temperature probe (Vaisala HUMICAP® HMP110).

125 In this study, two different experimental systems were used as shown in **Figure S1 in the**
126 **supporting materials (SM)**. Setup 1 was designed for VOC analysis, where the reactor was
127 coupled to a high-resolution proton transfer reaction-time of flight-mass spectrometer (PTR-
128 TOF-MS) and flushed by 120 mL/min of air (or N_2). For this setup (setup 1), a measurement
129 cycle consisted of measuring the VOC concentrations at the outlet of the reactor containing the
130 film for 5 min, then the LED lamp was switched on for 15 min. In contrast, the second approach
131 was designed to measure NO_x and HONO formation. For setup 2, the reactor was coupled to a
132 NO_x analyzer and a Long Path Absorption Photometer (LOPAP) and flushed by 500 mL/min
133 of synthetic air (or N_2). The irradiation time was around 60 min.

134 Nonetheless, prior to each experiment, a blank control involving sterile petri dishes was
135 performed under the same experimental conditions.

136

137 **2.3. Analytical tools**

138 **2.3.1. UV- visible analysis**

139 The UV-visible spectra of initial solutions, as well as extracted films of molds, potassium nitrate
140 and mix film of molds and potassium nitrate, were determined in the range of 200–600 nm using
141 a Cary 60 UV-Vis spectrophotometer from Agilent Technologies. Films were extracted with
142 2 mL of Milli-Q water and diluted from 2 to 20 times. Samples were then placed within a
143 3500 μL Macro Fluorescence Cuvette (Thorlabs) to perform the measurement. Three replicas
144 were performed for each type of extracted film or solution.

145 2.3.2. *Mass spectrometry analysis*

146 It was demonstrated by Depoorter et al.³² that a possible reaction may occur between nitrate
147 anions and reactive organic compounds during the drying process of a wet film, resulting in the
148 formation of nitro groups. Therefore, we characterized the potential formation of nitrogenated
149 compounds in a mixed solution of molds extract and potassium nitrate using a direct infusion
150 in an Orbitrap high-resolution mass spectrometer (HRMS, Q Exactive, Thermo Scientific,
151 Bremen, Germany) with a heated electrospray ionization (ESI). These results were compared
152 to different blanks using water, molds extract solution and potassium nitrate solution,
153 respectively. It is noteworthy to mention that the solutions were filtered through a 0.2 µm
154 Hydrophilic Polypropylene membrane (GHP,13 mm, Pall Corporation, USA).

155 Samples were injected into the MS through a steel capillary by a Hamilton syringe of
156 500 µL (SGE Analytical Science) at a flow rate of 10 µL/min and were analyzed using a spray
157 voltage of -4 kV and +4 KV for negative and positive ionization mode (ESI⁻ and ESI⁺)
158 measurements, respectively. The temperature of the Orbitrap capillary was set at 250°C. The
159 system was operated in both ESI⁺ and ESI⁻ mode with a resolving mass power of 140 000 at
160 m/z 200. A sodium acetate solution (2 mM in 50/50, v/v, ACN/water) was used for the mass
161 calibration; this solution provides a series of negative and positive adduct ions in the scanning
162 range of m/z 50–750.^{41,42} The calibration method consisted in filling the syringe with a 2 mM
163 solution of sodium acetate and setting the syringe pump flow rate to 20 µL/min. Once the
164 calibration started, few minutes wait are needed for a stable system with a stable TIC (deviation
165 <10%). The accuracy of the calibration is then evaluated (lower than 0.5 ppm). This procedure
166 is made for both modes (positive and negative). Signals in the range of m/z from 50 to 750 were
167 then recorded and processed for formula calculations.

168 Data were processed and evaluated using Xcalibur 2.2 (Thermo, USA). The subsequent
169 non-target screening approach was conducted using MZmine 2.53 software.^{41,42} This software

170 provides the main functionality for MS data processing: raw data import, mass detection,
 171 chromatogram building, deisotoping, alignment, identification, and duplicate peak filtering.
 172 The detailed processing steps and settings can be found in Wang et al.⁴² and in Table S1.
 173 Molecular formulas tentatively identified were expressed as C_cH_hO_oN_nS_s, where c, h, o, n, and
 174 s correspond to the numbers of carbon, hydrogen, oxygen, nitrogen, and sulfur atoms in the
 175 molecular formula, respectively. Molecular formulas were assigned to detected signals
 176 satisfying the following constraints: 1–50 for ¹²C, 1–50 for ¹H, 0–40 for ¹⁶O, 0–10 for ¹⁴N,
 177 0–3 for ³²S, 0–1 for ¹³C, 0–1 for ¹⁸O, and 0–1 for ³⁴S. The mass tolerance was 2 ppm for ESI–
 178 and 3 ppm for ESI+. Several rules were also considered: H/C ratio, 0.3 ≤ H/C ≤ 3; NOPS/C
 179 ratios, N/C ≤ 2, O/C ≤ 2, S/C ≤ 2. In the positive mode, 0–2 of ²³Na was also included in the
 180 formula calculation because of the high tendency of Na to form adducts with polar organic
 181 molecules.⁴³ The ring and double bond equivalence (*RDBE*) value was calculated as follow:

$$182 \quad RDBE = \frac{2 \times C + 2 + N - H}{2} \quad (\text{Eq. 1})$$

183 The *RDBE* values considered in this study were in the range of 0–25. However, *RDBE* may not
 184 accurately indicate the level of unsaturation for compounds with heteroatoms (e.g., O, N, and
 185 S). For this reason, the aromaticity equivalent (*X_c*) was also calculated. A threshold values for
 186 *X_c* were proposed in the literature, where *X_c* ≥ 2.5 and *X_c* ≥ 2.7143 are the unambiguous
 187 minimum criterion for the presence of aromatic and condensed aromatic structures in a
 188 molecule, respectively.⁴⁴ *X_c* for compounds containing carbon, hydrogen, nitrogen, oxygen,
 189 sulfur, and phosphorus can be calculated as follow:

$$190 \quad X_c = \frac{3 \times (RDBE - (p \times O + q \times S)) - 2}{RDBE - (p \times O + q \times S)} \quad (\text{Eq. 2})$$

191 where *p* and *q* correspond to the fraction of oxygen and sulfur atoms involved in π-bond
 192 structures of a compound, respectively. *p* = *q* = 1 for aldehydes, ketones, nitroso and cyanate
 193 compounds, *p* = *q* = 0.5 for carboxylic acid, ester and nitro compounds, and *p* = *q* = 0 for

194 alcohols, ethers and peroxides.⁴⁴

195 Aligned peak lists from MZmine workflow and xcalibur were further processed with three
196 different databases Swiss-Prot,⁴⁵ metabolomics workbench,⁴⁶ and the protein data bank ⁴⁷ to
197 ensure the most relevant tentatively identified formulas. Only peaks with intensities at least 10-
198 100 times higher than the mentioned blanks were considered to check the possible formation of
199 nitrogenated compounds.

200

201 **2.3.3. HONO and NO_x measurements**

202 A long path absorption photometer (LOPAP, QUMA) was used to measure the concentration
203 of gaseous nitrous acid. For such measurements, HONO is sampled in a glass stripping coil by
204 a fast-chemical reaction and converted into an azo dye which is photometrically detected in
205 long path absorption inside a special Teflon tubing (Teflon AF2400). During the experiments,
206 the integrated time resolution was 7 minutes, and the detection limit was less than 10 pptv under
207 our operating conditions (inlet gas flow set to 500 mL/min and liquid flow of the reagents set
208 to 20 mL/min). The sampling unit was maintained at 293 ± 0.02 K, using a Thermo Scientific
209 ARCTIC Refrigerated/Heated Bath (SC100 A10 - 230V). In addition, one should note that the
210 instrument was calibrated each week. The full description of this device is detailed
211 elsewhere.^{48,49} Furthermore, a chemiluminescence NO_x analyzer (CLD 88 CY analyzer, Eco
212 Physics) was used to measure the NO and NO₂ concentrations, with a detection limit of 50 ppt.
213 Prior to a series of measurements, the NO_x analyzer was calibrated. the calibration consisted of
214 doing a zero point of the instrument using a nitrogen/air flow and then doing a span point with
215 a known NO concentration. Then, to check the linearity of the instrument in the working range,
216 calibration curves for NO (~1 ppm in N₂, Linde) and NO₂ (850 ppb in N₂, Linde) in the range
217 0-100 ppb were established.

218 2.3.4. *VOC analysis*

219 VOCs released by the films were continuously analyzed using a high-resolution proton transfer
220 reaction-time of flight-mass spectrometer (PTR-TOF-MS 8000, Ionicon Analytik). The
221 ionization was carried out in the H_3O^+ mode. This instrument allows the estimation of the VOC
222 concentrations without an external calibration⁵⁰ and its full description can be found
223 elsewhere.^{50,51} For all the experiments, the instrument settings were chosen as follows: drift
224 tube voltage at 500 V, drift tube temperature at 353 K with a drift pressure of 2.2 mbar. The
225 E/N ratio was 125 Td where $1 \text{ Td} = 10^{-17} \text{ V cm}^2$. Furthermore, in order to reduce the adsorption
226 and the products loss, the reactor's outlet and the PTR-TOF-MS sample inlet tubing were
227 constantly heated at 333 K. All the spectra were recorded with a time resolution of 1 s and a
228 mass resolution of 4500 $\text{m}/\Delta\text{m}$. A calibration gas standard containing 14 VOCs with a
229 concentration of 100 ± 10 ppb in nitrogen (TO-14A Aromatic Mix, Restek Corporation,
230 Bellefonte, USA) was used to control the instrument performance in terms of mass resolution,
231 mass accuracy, sensitivity, and relative mass-dependent transmission efficiency.

232 TofViewer v1.4.3 software was used for raw spectral data analysis, mass calibration, peak
233 fitting, and area extraction. The mass calibration was performed using internal calibration based
234 on two references ions with an exactly known mass (i) hydronium ion isotope ($\text{H}_3^{18}\text{O}^+$ m/z
235 21.022) and (ii) protonated acetone ($\text{C}_3\text{H}_7\text{O}^+$ m/z 59.049).^{52,53} The used protonation reaction
236 rate constant was $2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ for all the detected masses. Conjointly, the VOC concentrations
237 were calculated according to the equation given by Cappellin et al.,⁵⁰ where the uncertainties
238 on the absolute concentrations can be estimated to be about 30-40 % arising from systematic
239 errors. Furthermore, as the variation of relative humidity in the sampled air can affect the H_3O^+
240 and $\text{H}_3\text{O}^+(\text{H}_2\text{O})$ ions signals and thus the H_3O^+ to $\text{H}_3\text{O}^+(\text{H}_2\text{O})$ ratio, the VOC ion signals were
241 normalized to a standard reagent ion signal of $10^6 \text{ counts s}^{-1}$ (cps) according to the procedure
242 given by de Gouw et al.⁵¹

243 The collected experimental dataset contains around 160 variables corresponding to the
244 number of peaks detected in PTR-TOF-MS measurements. In order to evaluate the effect of the
245 different tested conditions of relative humidity, cultivation time, type of films and potassium
246 nitrate concentrations on the VOC emission profiles, the principal component analysis (PCA,
247 package FactormineR, Ade4 Version 1.0.153–©2009-2017 R Studio) was performed on the
248 datasets using R software (Version 1.0.153 – ©2009–2017 RStudio). The PCA allows a
249 statistical representation of all data without any preconception as to the type of emitted
250 compounds and without bias. PCA can generate optimum and similar graphical representations
251 of the scatterplot representing the data matrix. Each PCA factor is a linear combination of
252 variables representing the maximum variance in the scatterplot. As the factors have an
253 orthogonal relationship, only independent sources of variance are taken into account.⁵⁴

254 **2.3.5. Reagents**

255 Potassium nitrate (99%), sodium acetate ($\geq 99\%$), and lactic acid (90%) were purchased from
256 Sigma Aldrich. Malt agar extract was provided by Biokar diagnostics. The air (>99.9%) and
257 dinitrogen N₂ (>99.9992%) were either synthetic or obtained from Air Products. NO (~1 ppm
258 in N₂) and NO₂ (850 ppb in N₂) were provided by Linde.

259 **3. Results and discussion**

260 **3.1. Film characterization**

261 The UV analyses were performed on three types of films (molds, KNO₃ and mix of molds and
262 KNO₃) extracted with milli-Q water and were compared to their initial corresponding solutions.
263 Both solutions and extracted films showed similar trends (**Figure S2 in the SM**). For the KNO₃
264 extracted films, a band centered at 300 nm was observed consistent with the literature.⁵⁵ For the
265 molds extracted films, the UV spectrum shows a band with three large peaks: at 313 nm, 285
266 nm and 247 nm, which could be a combination of different compounds exhibiting various

267 functional groups and conjugations. As for the mixed extracted film of molds and potassium
268 nitrate, a band centered at 292 nm was observed. It was however not possible to assign a
269 functional group for this band. In fact, the presence of organic compounds (molds extract in this
270 study) modifies the molecular structure of adsorbed nitrate anions, and leads to a red shift in the
271 actinic region, which can increase the light absorbed by nitrate anions.⁵⁶ Moreover, to check if
272 the mixing time of the molds extract and potassium nitrate affects its UV spectrum, the UV
273 spectrum of a mixed solution was recorded at different times after mixing i.e., 3 and 12 hours
274 of mixing (same time used for the film preparation) and the results are presented in **Figure S3**
275 **in the SM**. These results show that the mixing time did not affect the UV spectrum of the mixed
276 solution of molds extract and potassium nitrate.

277 Spectral analysis of irradiated films of molds, KNO₃ and mix of molds and KNO₃ by the
278 LED at 340 nm revealed a change in the shape of the bands i.e., mainly a decrease in intensity
279 suggesting a possible degradation of the films upon irradiation (**Figure S4 in the SM**).

280 The MS analysis allowed the identification of 7 molecular formulas only in ESI+ that are
281 exclusively present in the mix solution of molds extract and potassium nitrate. **Table 1**
282 summarizes the detected masses in ESI+ with the tentatively identified formulas, *RDBE*, *X_C*,
283 potential origin and the ratios H/C, O/C, N/C, S/C, N/O. Moreover, O/N ratios were determined
284 for each of the tentative compounds, in the range from 0.5 to 7. O/N ratios larger than 3 points
285 toward the presence of a nitro (-NO₂) function to the proposed formulas. However, formulas
286 with $O/N \leq 1$ could contain a reduced nitrogen functions, such as amines and amides.^{42,43} These
287 compounds have high H/C ratios compared to O/C ones. Formulas with $1 \leq O/N \leq 3$ could be
288 a combination of different functional groups like nitramines (N-NO₂). Therefore, the detected
289 molecules are mainly amino acids, nucleic acid, peptide and dipeptide fragments, membrane
290 fractions and metabolites. Most of the tentatively identified formulas possesses a nitro group (-

291 NO₂), possibly related to a nitration reaction during the preparation of the mix solution of molds
292 extract and potassium nitrate.

293 As demonstrated in previous studies, protein nitration can occur in the gas phase in the
294 presence of nitrogen dioxide (NO₂) and ozone (O₃).^{57,58} However, as proposed by Ghiani et
295 al.,⁵⁹ the protein nitration can also take place in the aqueous phase in the presence of nitrate ions
296 (NO₃⁻) with concentrations and pH levels comparable to that found in the aerosol liquid water
297 phase both in the dark and under UV-A irradiation. The nitration of aromatic compounds, such
298 as phenols, can occur both in the gas phase in the presence of OH and NO₂ or NO₃ and NO₂,
299 and by multiphase various reactions involving nitrogen dioxide, N₂O₅ and/or ClNO₂, NO₃,
300 nitrate or nitrite when irradiated. Nitration is also induced by the presence of dissolved Fe(III),
301 nitrate, semiconductor oxides.⁶⁰ Additionally, HNO₂ may react in the dark with hydrogen
302 peroxide to form peroxyntrous acid (HOONO), allowing consequently the oxidation and
303 nitration of aromatic compounds.⁶⁰

304 On the other hand, it is well known that *Aspergillus sp* can use a broad range of nitrogen
305 sources, such as ammonium, nitrate, amino acids (e.g., histidine or proline), or complex
306 substrates such as collagen and elastin. The nitrate assimilation by fungi leads to the complete
307 reduction of nitrate forming ammonium, which is finally incorporated in the amino acids
308 glutamate and glutamine acting as nitrogen donors.⁶¹ Moreover, fungi can also synthesize NO
309 from nitrate through the action of nitrate reductase.⁶² In the presence of superoxide (O₂⁻),
310 peroxyntrite (ONO₂⁻) can be formed and cause protein modification, lipid oxidation, and DNA
311 damage.^{63,64} It was further demonstrated that peroxyntrite can nitrate and oxidize tyrosine
312 residues in membrane proteins and can cause as well lipid oxidation and nitration.^{63,65,66}

313 For all the reasons cited above, we hypothesized that a combination of biotic and abiotic
314 factors might have contributed to the formation of the nitro compounds that were observed in
315 our study.

316 **Table 1:** Potential identities and sources for the identified compounds from a mixed solution of molds extract and potassium nitrate in ESI+ mass
317 spectra.

Detected Masses (Da)	Neutral mass (Da)	Possible Formula	Possible identity	Potential origin	H/C	O/C	N/C	S/C	O/N	RDBE	Xc (p=q=1)	Xc (p=q=0.5)
219.14902	218.14902	C ₁₃ H ₁₈ N ₂ O	5-Methoxydimethyltryptamine	Tryptamine derivatives	1.4	0.1	0.2	0.0	0.5	6.0	2.60	2.64
223.18029	222.18029	C ₁₃ H ₂₂ N ₂ O	Nigragillin	Alkaloid - metabolites from the <i>A.niger</i>	1.7	0.1	0.2	0.0	0.5	4.0	2.33	2.43
246.01611	223.01611	C ₃ H ₉ N ₃ O ₅ S	N-nitro-L-cysteinyglycine (NO ₂ -Cys-Gly-OH)	Peptide L-cys-gly	1.8	1.0	0.6	0.2	1.7	3.0	3.67	0.00
		C ₁₃ H ₅ NO ₃	Possible nitro	NI	0.4	0.2	0.1	0.0	3.0	12.0	2.78	2.81
260.03182	237.03182	C ₁₄ H ₇ NO ₃	1-ethynyl-7-nitrodibenzofuran / 3-nitro-15-oxatetracyclo[10.2.1.05,14.08,13]pentadecal(14),2,4,6,8(13),9,11-heptaene	Miscellaneous compound <i>a.niger</i> / n-myristoyltransferase	0.5	0.2	0.1	0.0	3.0	12.0	2.78	2.81
		C ₅ H ₈ N ₄ O ₇	4,5-dimethoxy-1,3-dinitroimidazolidin-2-one / N-methyl-4,4,4-trinitrobutanamide	Imidazolinone / Amino acids or dipeptides: glutamine or beta-Ala-Gly	1.6	1.4	0.8	0.0	1.8	4.0	3.67	1.00
	259.03182	C ₁₁ H ₆ N ₄ O ₂ S	3-nitro-4-pyrimidin-2-ylsulfanylbenzotrile / 3,6-bis(2-furyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole	Nucleic acid derivatives	0.5	0.2	0.4	0.1	0.5	11.0	2.75	2.79
262.01109	239.01109	C ₁₃ H ₅ NO ₄	Possible nitro	NI	0.4	0.3	0.1	0.0	4.0	12.0	2.75	2.80
		C ₄ H ₆ N ₄ O ₈	Tetranitrobutane	Protein hydrolase/toxin n-butane	1.5	2.0	1.0	0.0	2.0	4.0	3.50	0.00

		C ₃ H ₄ N ₆ O ₇	1,3,5-Trinitro-1,3,5-triazinan-2-one	Triazine moiety-amino acids:peptides	1.3	2.3	2.0	0.0	1.2	5.0	4.00	1.67
263.01442	240.01442	C ₁₃ H ₅ NO ₄	Possible nitro	NI	0.4	0.3	0.1	0.0	4.0	12.0	2.75	2.80
	262.01442	C ₆ H ₄ N ₄ O ₈	5-Amino-2,4,6-trinitro-1,3-benzenediol	2,6-dihydroxyaniline/N-ethylmaleimide	0.7	1.3	0.7	0.0	2.0	7.0	5.00	2.33
264.00919	241.00919	C ₁₃ H ₇ NO ₂ S	2-nitro-9H-fluorene-9-thione	Fluorene/membrane fraction fatty acyls	0.5	0.2	0.1	0.1	2.0	11.0	2.75	2.79
	219.00919	C ₇ H ₉ NO ₇	6-Methoxy-5-nitro-4,6-dioxohexanoic acid	Skikimic acid (intermediate metabolites microorganism)	1.3	1.0	0.1	0.0	7.0	4.0	3.67	1.00

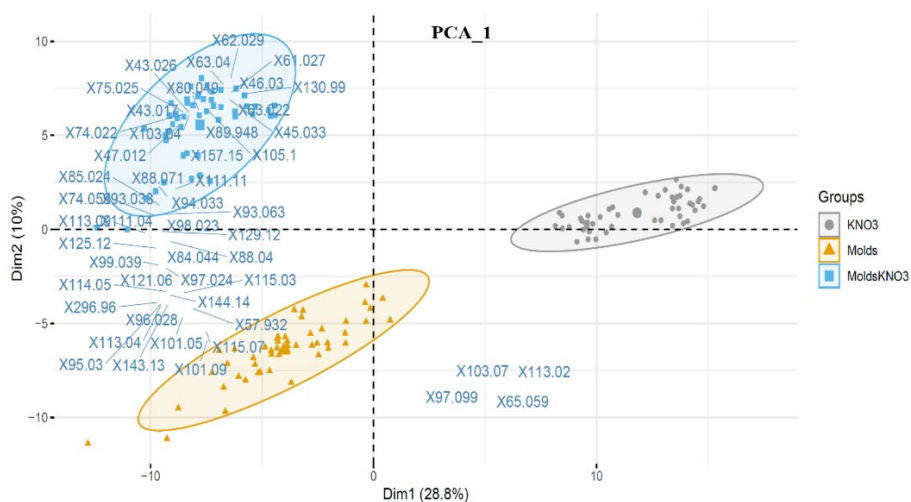
318 NI: not identified

319

320 **3.2. VOC emission from films**

321 The VOC emissions from molds films, either mixed or not with KNO₃, were investigated for
322 different molds cultivation time, under different relative humidities, under dark and irradiation
323 conditions, under N₂ and air streams, and for different concentrations of KNO₃ (0.001-0.5M).
324 In this section, only the results for the effect of KNO₃ on VOC emission profiles are presented.
325 More details about the impact of the other cited conditions are available in the **supporting**
326 **materials**.

327 To visualize the impact of the type of film on the VOC emission profiles, a PCA was
328 performed and is presented in **Figure 1**. PCA_1 described 38.8 % of the total dataset variability
329 (28.8% for the first dimension and 10% for the second one). According to **Figure 1**, each film
330 shows a very differentiated VOC emission profile (ellipses grouping the different conditions
331 (i.e., film type) are well separated). It is also noticeable that all the differences between the three
332 film types are almost driven by compounds emitted by molds' films doped with potassium
333 nitrate.



334
335 **Figure 1** :VOC emission profiles at RH = 85% for different types of films: molds, KNO₃ and
336 molds + KNO₃. The molds were incubated for 10 days and [KNO₃] = 0.04M. The m/z of the 50
337 compounds that are most explaining the variance in the two first components are presented on
338 the graph. The percentages of the eigenvalues of the two first components are shown on each
339 axis.

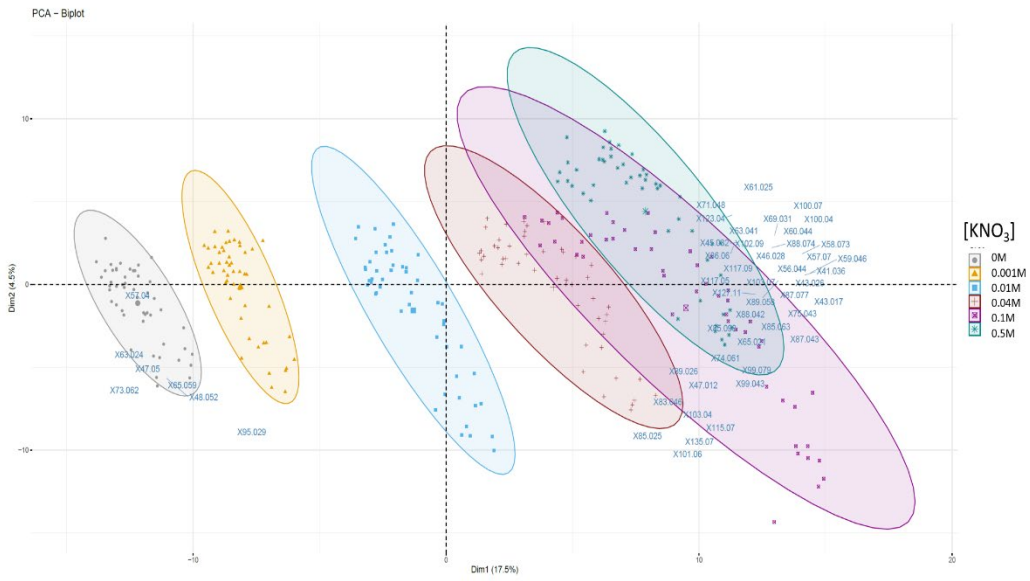
340 Furthermore, in order to determine the effect of the potassium nitrate concentration on the
341 VOC emission profiles, another PCA (PCA_2) was performed on the dataset (**Figure 2-a**) and
342 the relative abundance of each VOC detected from molds films doped with different
343 concentrations of potassium nitrate were determined and compared in **Figure 2-b**. PCA_2
344 described 22 % of the total dataset variability (17.5% for the first dimension and 4.5% for the
345 second one). The ellipses grouping the different conditions (KNO₃ concentrations) are
346 separated with an overlap between the film with higher potassium nitrate concentration (0.04M,
347 0.1M and 0.5M). It is also noticeable that 42 of the 50 compounds are driven by the films with
348 higher potassium nitrate concentration, explaining the variance between the different
349 conditions. Likewise, **Figure 2-b** shows variability in the VOC emission profiles, which is in
350 line with the results obtained by PCA_1 and PCA_2 analyses. Hence, according to **Figures 1**
351 **and 2**, the presence of potassium nitrate in the films affects their composition and thus their
352 VOC emission profiles.

353 Nonetheless, in the absence or presence of UV-light, the concentration of some VOCs
354 increased with the increase of potassium nitrate concentrations as shown in **Figure 2-b**. Among
355 those VOCs: succinimide (C₄H₅NO₂) and butene (C₄H₈), where their relative abundances
356 increase from 0% for [KNO₃] = 0-0.001M to 6.1% and 20.5% for [KNO₃] = 0.5M, respectively.
357 The evolution of some VOC concentrations in response to the different potassium nitrate
358 concentrations in the films reflects the possible interaction between the nitrate solution and the
359 molds extract during the film preparation. As for the VOCs that are both sensitive to UV-light
360 irradiation and potassium nitrate concentration, they might be released in the gas phase in
361 response to the photodegradation of nitrogenated compounds that can be formed during the film
362 preparation (**Figure 2-b and Table S2**).

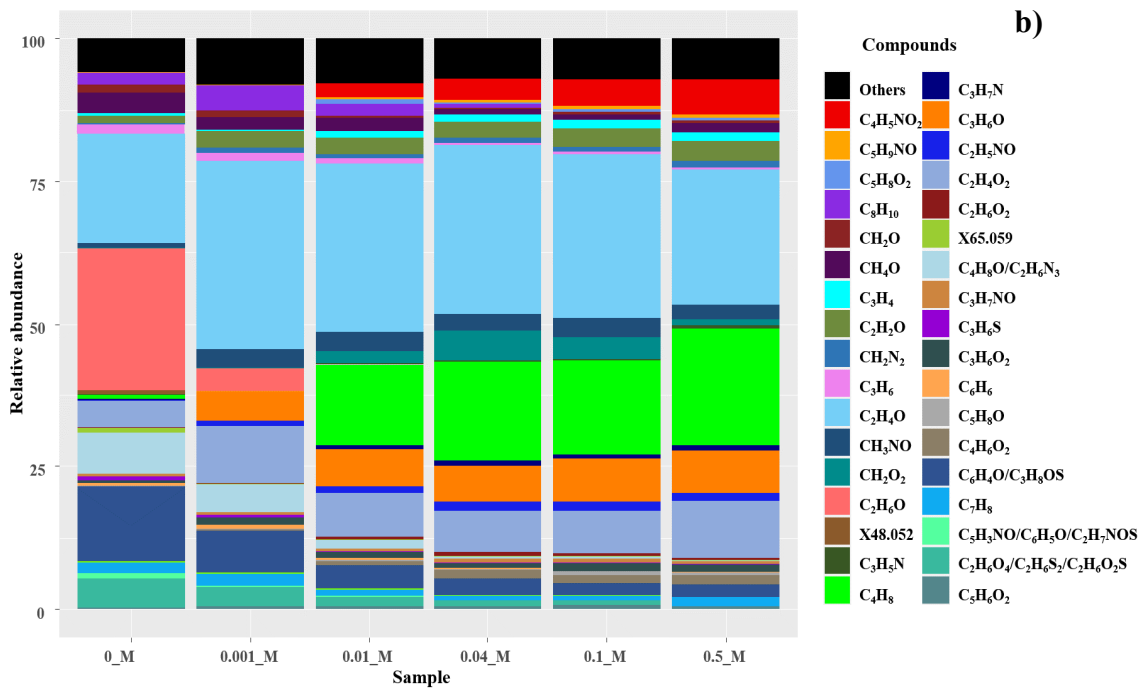
363

PCA_2

a)



364



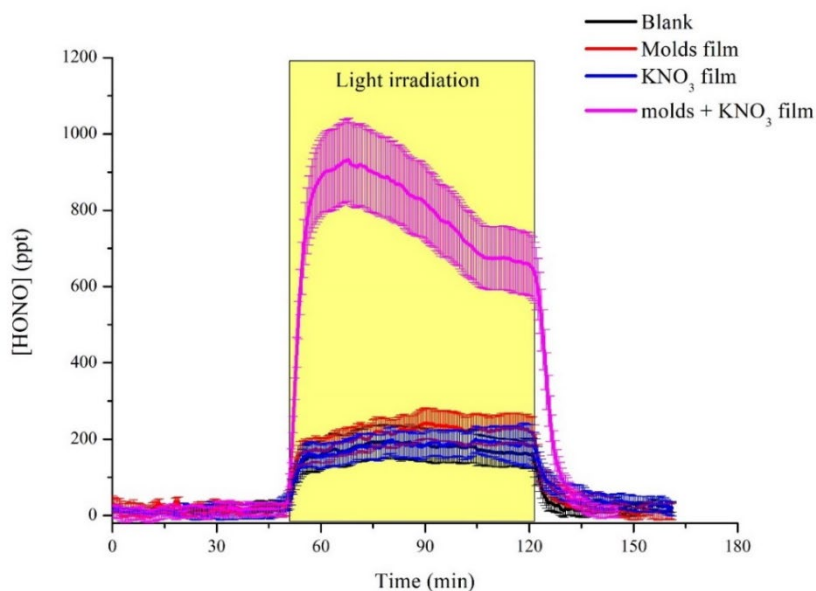
365

366 **Figure 2:** (a) VOC emission profiles for mold films doped with different KNO₃ concentrations.
 367 The m/z of the 50 compounds that are most explaining the variance in the two first components
 368 are presented on the graph. The percentages of the eigenvalues of the two first components are
 369 shown on each axis (b) Relative abundance of the most emitted VOCs from these films.
 370 RH=85% and molds were incubated for 14 days.

371

372 **3.3.HONO and NO_x emission**

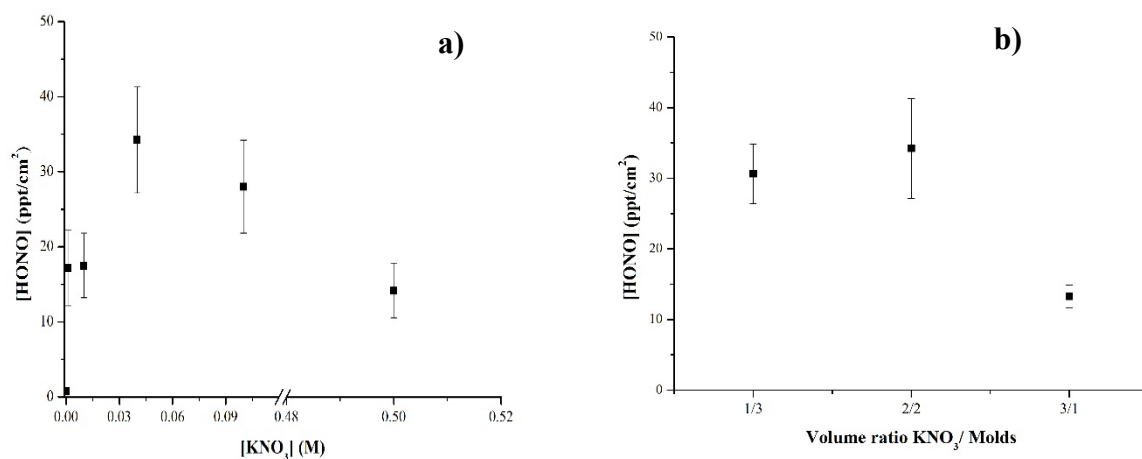
373 The temporal evolution of HONO formation under an air stream, from the three sets of film
374 samples (irradiated films of molds, KNO₃ and mold films doped with KNO₃) and compared to
375 a reactor blank with a relative humidity of 85±2% is presented in **Figure 3**. The uncertainties
376 on the concentrations were twice the standard deviation. When the LED lamp was switched
377 ON, an enhancement of the HONO concentration was observed. The HONO concentration
378 increases to a maximum value of 900 ppt and then stabilizes at a value of 700 ppt. It is
379 noteworthy mentioning that the formation of HONO from irradiated mold films doped with
380 KNO₃ was also observed under a nitrogen stream with concentrations in the same order as that
381 under air stream. However, individually molds and nitrate films showed a negligible formation
382 of HONO compared to a blank experiment with an empty reactor. In fact, the wavelengths of
383 the LED lamp used in this study ranged between 335 and 380 nm. Thus, at these wavelengths,
384 the photochemistry of potassium nitrate cannot be activated efficiently because due to a low
385 absorption beyond 335 nm.³²



386
387 **Figure 3:** Formation of HONO (ppt) in response to the irradiation of molds films, KNO₃ films
388 and molds films doped with 0.04M of KNO₃ and compared to a reactor blank. RH=85±2%,
389 error is 2σ, yellow area corresponds to light irradiation.

390
 391 Additionally, the concentration of nitrate in the film was also varied from 0.001M to
 392 0.5M. As the KNO_3 concentration increases, a slight increase of the HONO concentration was
 393 observed in **Figure 4-a**. However, for higher concentration values of KNO_3 , the formation of
 394 HONO decreased. Likewise, the proportion of nitrate and molds in the films was varied. These
 395 results show a maximum HONO formation for a stoichiometric mix of molds and nitrate in the
 396 initial solution (**Figure 4-b**) and a decrease of the HONO concentration when KNO_3 was in
 397 excess.

398 The decrease of the HONO concentration for high KNO_3 concentration can be explained
 399 by the fact that the gaseous products formed during the photolysis of the film may hardly escape
 400 from the nitrate crystal that is saturating the surface. A similar behavior was previously
 401 observed from mixed films of furfural and KNO_3 ³² as well as from films of humic acids and
 402 KNO_3 ³⁹ where a decrease of the HONO formation was also witnessed for high potassium
 403 nitrate concentrations.

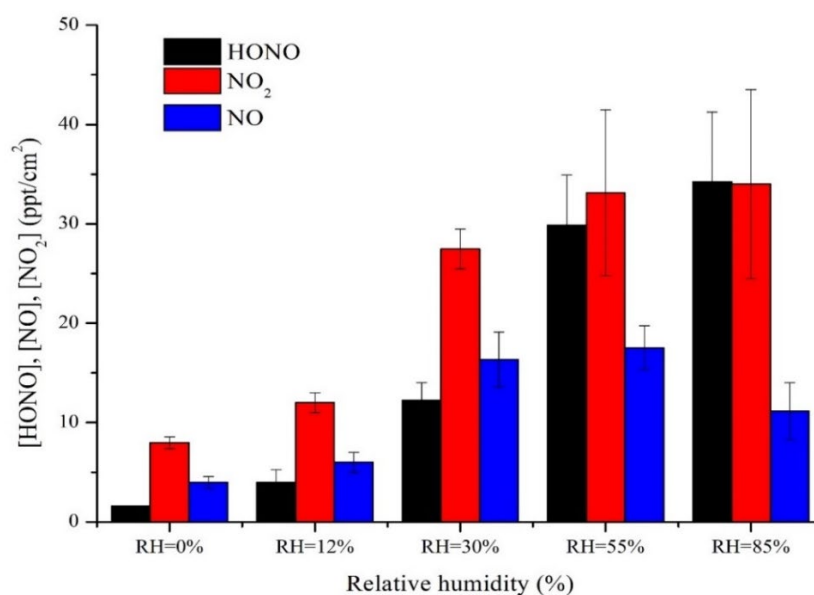


404 **Figure 4:** Variation of $[\text{HONO}]$ (ppt/cm^2) as (a) function of $[\text{KNO}_3]$ (M) used in the film
 405 preparation and (b) for different volume ratio $\text{KNO}_3/\text{molds}$. The reactor blank is subtracted and
 406 error bars correspond to 1σ .

407
 408
 409 Furthermore, to assess the effect of the relative humidity on the gas-phase formation of
 410 NO , NO_2 and HONO from mold films doped with KNO_3 , different relative humidities were

411 tested (RH= 0-85 ±2%), and the results are shown in **Figure 5**. The maximum tested relative
412 humidity was below the deliquescence point of potassium nitrate (>92%).⁶⁷ Each point
413 corresponds to the average of 3-4 replicas and the error bars correspond to one standard
414 deviation. It was found that with increasing relative humidity, both NO₂ and HONO
415 concentrations increase. However, NO formation seemed to be less sensitive to the variation of
416 relative humidity. Pure KNO₃ films and mold films were unable to produce HONO and NO_x.

417 These results show that an increased amount of HONO is generated by the photolysis of
418 nitrates when mixed with organic matter (molds extract) with increasing relative humidity. The
419 observed HONO formation during irradiation might also result, in part, from the
420 photodegradation of nitro compounds (mainly nitrated proteins, amino acids or metabolites),
421 formed during the film preparation process.



422
423 **Figure 5:** Variation of [HONO] and [NO_x] in ppt/cm² as a function of the relative humidity
424 from mold films doped with 0.04M of KNO₃, error bars correspond to 1σ.

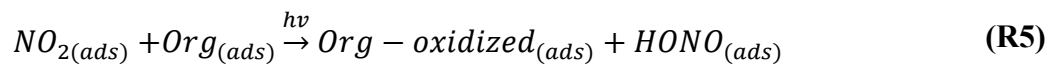
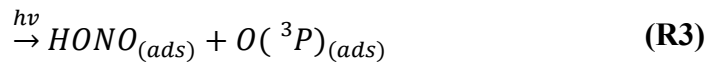
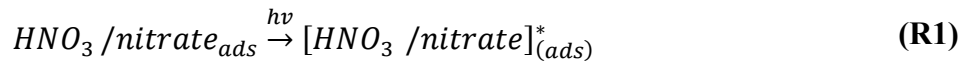
425 426 3.4. Reaction mechanism

427 Based on the results obtained in this study and that of the available literature, different routes
428 explaining the photochemical formation of NO, NO₂ and HONO are proposed.

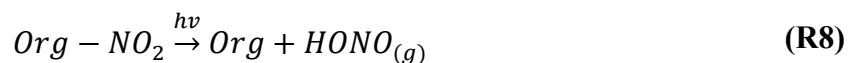
429 The mass spectrometry analysis of the mixed solution of molds extract and potassium nitrate
430 showed the formation of some nitro and nitrogenated compounds during the film preparation.
431 Depoorter et al.³² have also shown a possible nitration reaction during the preparation of furfural
432 and potassium nitrate films. The authors have proposed a mechanism for the photochemical
433 degradation of 2-nitrofuran (a proxy for nitro compounds formed during the preparation of
434 furfural films doped with KNO₃). The photodegradation of 2-nitrofuran results in the (i)
435 formation of NO and C₄H₃O₂, (ii) formation of NO₂ and C₄H₄O, and (iii) formation of HONO
436 following the reduction of NO₂ on surfaces containing reducing functionalities. This
437 photodegradation can occur under both nitrogen and air streams, where the authors have probed
438 the possible formation of O₂⁻ on the surface which can increase the oxidative capacity of the
439 surfaces leading to an evolution of the concentrations of nitrogen containing compounds at the
440 gas-surface interface. Moreover, Wang et al.³³ reported that reactions between NO_x and HO₂/O₂⁻
441 in the aerosol phase are a significant pathway by which HONO production is enhanced in the
442 presence of dissolved aliphatic organic matter. This chemistry can be applied to many aqueous
443 environments (e.g., cloud droplets, aqueous aerosols, and surface water) and may represent a
444 significant source of gaseous HONO in areas where surface waters or aqueous microlayers
445 contain a high concentration of nitrate and dissolved aliphatic organic matter.

446 On the other hand, the presence of molds extract as a source of organic compounds on the
447 surface can modify the molecular structure of nitrate anions leading to a red shift into the actinic
448 region and an enhancement of the light absorbed by nitrate anions.⁵⁶ Depending on the surface
449 pH, nitrate can be transformed into nitric acid. In fact, among the VOC released from molds
450 film doped with KNO₃, some acids were detected that can promote surface acidity. The
451 photolysis of adsorbed nitric acid/ nitrate (HNO₃/nitrate_(ads)) can directly produce HONO and
452 NO₂ via reactions R1-R3 or indirectly through reactions R4 and R5.^{56,68-70} The produced NO₂
453 from reaction (R2) and HONO from reactions (R3, R4 and R5) are released from the surface to

454 the gas phase following reactions (R6) and (R7). The $Org_{(ads)}$ corresponds to molds extract in
 455 this study.



456 Nevertheless, *Aspergillus niger* species contain Aspergillin that is classified as melanin
 457 or a humic acid-containing chromophore groups.⁷¹ Yang et al.³⁹ and Stemmler et al.⁷² suggested
 458 that the presence of chromophoric centers, like aromatic carbonyls in humic acids, could act as
 459 photosensitizers in the photolysis of nitrates or in the conversion of adsorbed NO_2 into HONO.
 460 Additionally, during the photolysis of nitrate and humic acids, aromatic nitro compounds are
 461 expected to be formed by the photochemical oxidation of nitrates. Correspondingly, it has been
 462 reported that the photolysis of nitro-aromatic compounds may lead to the HONO formation.³⁹
 463 Furthermore, the photolysis of species containing a nitrogen atom in humic acids may release
 464 gaseous products such as HONO and NO_2 .³⁹ And it was previously demonstrated that the
 465 photolysis of ortho-nitrophenols (as is the case for 3-nitrotyrosine) can generate nitrous acid
 466 (HONO).⁵⁸ Thus the photodegradation of nitro compounds can occur as follow:



467 Hence, the obtained results from this study and the available literature suggest that the
 468 HONO formation might result from a photodegradation of nitro-compounds and nitrogen-
 469 containing compounds formed in the film, and/or from the photolysis of nitrate/nitric acid in
 470 the presence of molds extract as organic compounds on the surfaces.

471 **3.5. Implications for indoor air quality**

472 In order to determine the concentration of HONO resulting from irradiated indoor surfaces
473 contaminated with molds, the indoor concentration of HONO was estimated following the
474 equation given by Willem et al.⁷³:

$$\frac{d[HONO]}{dt} = \frac{E_{HONO} \times S}{V} - N_h[HONO] - J_{HONO}[HONO] \quad (\text{Eq. 3})$$

475 where E_{HONO} is the emission rate of HONO in $\mu\text{g m}^{-2} \text{h}^{-1}$, S is the surface area of the room
476 contaminated by molds (m^2), V is the room volume (m^3), N_h is the air exchange rate (h^{-1}) and
477 J_{HONO} is the photolysis rate of indoor HONO. It is noteworthy mentioning that the loss on
478 surfaces was already taken into account in the measured HONO concentration.

479 As the HONO outdoor levels are usually lower than indoor levels, it was assumed that
480 there is no influence of outdoor concentration of HONO on the indoor concentration for this
481 simulation.⁷⁴ The steady-state HONO concentration can be then simplified as:

$$[HONO] = \frac{E_{HONO} \times S}{V \times (J_{HONO} + N_h)} \quad (\text{Eq. 4})$$

482 For indoor relevant relative humidity ($\text{RH} = 45\%$), with an air exchange rate of 0.5 h^{-1} ⁷³ and
483 a photolysis rate of HONO equal to 0.25 h^{-1} ^{3,27} and considering a standard living room ($S=20$
484 m^2 , $V=50 \text{ m}^3$) with a mold damaged surface of 0.25 m^2 and a nitrate concentration of 0.04M ,
485 the resulting HONO concentration from these surfaces is of the order of 60 ppt.

486 Moreover, the UV fraction of sunlight penetrating in indoor spaces ranges from 330 up to
487 400 nm depending on the location, time of the year, room orientation as well as the size and
488 type of the glass window.⁷⁵ Additionally, Liu et al.³⁷ have shown high HONO yields during the
489 irradiation period of the glass windows in the kitchen. Hence, the wavelength of the LED lamp
490 used in this study corresponds to the lowest possible wavelength encountered indoor. Likewise,
491 the corresponding photon flux is of $3.8 \times 10^{12} \text{ photons cm}^{-2} \text{ s}^{-1}$, which is lower than the direct

492 indoor UV actinic flux (with windows closed) measured by Gandolfo et al. (i.e., 10^{14} photons
493 $\text{cm}^{-2}\text{s}^{-1}$) but significantly higher than the one in the shadows (i.e., 10^{10} photons $\text{cm}^{-2}\text{s}^{-1}$).
494 Nevertheless, our experimental conditions can be somehow representative of realistic indoor
495 conditions during sunny periods. Thus, for future studies, it will be interesting to evaluate the
496 influence of different type of indoor light sources with different intensities on the HONO yields
497 from different surfaces and mainly those covered by microbial species. This will help improving
498 the model used to accurately predict the HONO concentrations values under different light
499 intensities.

500 Finally, mold growth is especially found in damp dwellings with high relative humidity
501 (>70%). In fact, sick buildings infested with visible molds, present a wide contaminated surface
502 area compared to the one used in our calculation and have usually a low ventilation rate. For
503 example, if the mold damaged surface is 25 m^2 , and for the same nitrate concentration of 0.04M,
504 the resulting HONO concentration in the gas phase will be around 6 ppb. Hence, indoor surfaces
505 covered with molds can be a non-negligible source of HONO mainly in sick buildings
506 exhibiting large, contaminated areas, alongside high relative humidity, and low ventilation
507 rates. Typical indoor levels are of the order of few ppbs.^{28,29,32} These levels could significantly
508 be affected by surface chemistry as it was previously demonstrated for various indoor
509 surfaces^{29,32,76} and for microbial indoor surface in the present work.

510 **4. Conclusions**

511 In this study, we show that *Aspergillus niger*, a common indoor mold, can react on surfaces
512 and produce a complex mixture of adsorbed products that initiate photochemistry indoors.
513 These microbial species lead to the formation of VOCs and enhances HONO production under
514 various indoor conditions including light irradiation, relative humidity and KNO_3
515 concentration. As microbial species are often found in areas with high relative humidity, the
516 resulting HONO concentrations become significant. Hence, this work allows the recognition of

517 microbial species (*Aspergillus niger*) as a new indoor HONO source. This will definitely shed
518 a new light on the role of molds grown in sick dwellings in affecting the indoor air chemistry
519 and quality.

520 **Acknowledgments**

521 This work was supported by the Alfred P. Sloan Foundation under its Chemistry of Indoor
522 Environments program.

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