



University of Dundee

Biocorrosion of copper metal by *Aspergillus niger*

Zhao, Jiayue; Csetenyi, Laszlo; Gadd, Geoffrey Michael

Published in:
International Biodeterioration and Biodegradation

DOI:
[10.1016/j.ibiod.2020.105081](https://doi.org/10.1016/j.ibiod.2020.105081)

Publication date:
2020

Licence:
CC BY-NC-ND

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):
Zhao, J., Csetenyi, L., & Gadd, G. M. (2020). Biocorrosion of copper metal by *Aspergillus niger*. *International Biodeterioration and Biodegradation*, 154, [105081]. <https://doi.org/10.1016/j.ibiod.2020.105081>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Biocorrosion of Copper Metal by *Aspergillus niger***

2

3 Jiayue Zhao¹, Laszlo Csetenyi², Geoffrey Michael Gadd^{1,3*}

4

5 ¹*Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.*

6 ²*Concrete Technology Group, Department of Civil Engineering, School of Science and*

7 *Engineering, University of Dundee, Dundee, DD1 4HN, UK.*

8 ³*State Key Laboratory of Heavy Oil Processing, State Key Laboratory of Petroleum Pollution*

9 *Control, College of Science and Environment, China University of Petroleum, Beijing 102249,*

10 *China*

11

12 *Correspondence: g.m.gadd@dundee.ac.uk; Tel. (+44) 1382 384767

13

14

15 **ABSTRACT**

16 Several geoactive fungi were investigated for their biocorrosion impact on metallic copper, to
17 further understanding of the potential roles that fungi may have in the biotransformation of such
18 substrate, and the mechanisms involved. Copper metal showed little toxicity and test fungi were
19 able to grow in direct or indirect contact with copper and to colonize copper sheet. *A. niger* was
20 able to biodeteriorate copper metal through proton- and ligand-mediated dissolution
21 mechanisms, leading to significant mass loss and surface etching. The formation of a secondary
22 copper oxalate (moolooite) biomineral crust together with cuprite deposition lead to alteration
23 of surface topography and visual appearance, highlighting the significance of oxalate excretion in
24 effecting fungal metal biotransformations. The metal transforming influence of fungal
25 colonization may have some implications for biodeterioration, protection and preservation of
26 cultural relics and artefacts as well as certain components of the built environment.

27

28

29 **Keywords:** *Aspergillus niger*; copper; biocorrosion; oxalate; biomineralization

30

31 **1. Introduction**

32 Metal loss and damage in the built environment resulting from corrosion is one of the major
33 causes of structural deterioration, economic loss and cultural damage (Crispim and Gaylarde,
34 2005). It is estimated that damage caused by all forms of corrosion costs about 2 trillion pounds
35 (UK £ sterling) annually, which is about 3% of the global gross domestic product (Bhandari et al.,
36 2015). Microbial colonization can have a significant influence on the built environment, showing
37 all kinds of effects such as discolouration and staining, biocorrosion, and biodeterioration of
38 metallic, organic and inorganic components (Warscheid and Braams, 2000; Scheerer et al., 2009;
39 Sterflinger and Piñar, 2013; Gadd, 2017b). This can pose serious concerns for built infrastructure,
40 including nuclear waste storage facilities, oil storage tanks and sewer systems, as well as human
41 habitation and cultural artefacts (Nica et al., 2000; Gu, 2007; Herrera and Videla, 2009; Turick
42 and Berry, 2016).

43 The growth of diverse organisms, including bacteria, lichens, and fungi, can have significant
44 effects on structural materials derived from rocks, minerals and metals (Gadd, 2007; 2017a,b)
45 Several studies have concentrated on the involvement of bacteria, including sulfate-reducing,
46 iron-reducing, sulfur-oxidizing, and iron-oxidizing genera, in metal biocorrosion and concrete
47 biodeterioration (Emde et al., 1992). However, relatively little attention has been paid to fungi
48 despite them often being the most visible and destructive of microbiota in the built environment
49 because of their biodeterioration of a wide variety of substrates, including wood, plastics and
50 rock and mineral-based building components (Sterflinger, 2010; Onofri et al., 2014; Gadd, 2017a)
51 Many fungal species are capable of solubilizing metals from metal-bearing minerals and
52 substrates (Gadd, 1993, 2017a,b; Fomina et al., 2005a,b; 2007). Some early work showed that

53 *Penicillium spp.* were able to solubilize and accumulate zinc, aluminum, copper, and lead (Siegel
54 et al., 1983), while *Cladosporium resinae* was involved in the biodeterioration of aluminium
55 (Iverson, 1987). The relative lack of information on copper biocorrosion by fungi contrasts with
56 the extensive literature on responses of wood decay fungi to copper in connection with the
57 application of copper compounds as wood preservatives (Schilling and Jellison, 2006; Freeman
58 and McIntyre, 2008; Zelinka et al., 2019a,b). Many such fungi exhibit marked tolerance to copper
59 with the formation of copper oxalate acting as a detoxification process and underpinning copper
60 tolerance (Clausen and Green, 2003; Green and Clausen, 2003; Hastrup et al., 2005; Kartal et al.,
61 2015; Ohno et al., 2015; Karunasekera et al., 2019).

62 Copper and its alloys have important uses in interior and exterior environments (Elwell and
63 Scholes, 1967) and has been used for centuries in architectural and cultural applications, e.g.
64 statues, ornaments and buildings (Frankfort, 1956; de la Fuente et al., 2008). Furthermore,
65 because of good machinability and conductivity, copper is extensively used in the electronics,
66 communications and digital industries, such as in circuit boards, connections and terminals, and
67 also widely utilized in heat exchangers or conductors. To date, most attention has focussed on
68 atmospheric abiotic corrosion of copper and although fungal bioweathering of copper-containing
69 minerals has been studied (Fomina et al., 2005a,b, 2007, 2017) biocorrosion of copper metal by
70 fungi has received little attention. The objective of this research was therefore to investigate the
71 ability of fungi to mediate biocorrosion of copper metal. In this work, several fungi with known
72 metal and mineral transformation abilities, i.e. *Aspergillus niger* (Sayer and Gadd, 1997; Horeh et
73 al., 2016; Fomina et al., 2017; Ferrier et al., 2019; Kang et al., 2019, 2020; Suyamud et al., 2020),
74 *Beauveria caledonica* (Fomina et al., 2005a) and *Paecilomyces javanicus* (Rhee et al., 2012, 2016),

75 were used to investigate interactions with copper metal to gain understanding of the roles that
76 fungi may play in the biocorrosion or biotransformation of such a material, effects on the copper
77 substrate and the mechanisms involved.

78 **2. Materials and Methods**

79 **2.1 Organisms and media**

80 *Aspergillus niger* (ATCC 1015), *Beauveria caledonica* (provided by G. Genney (CEH Merlewood
81 collection)) and *Paecilomyces javanicus* (Friedrichs & Bally; A.H.S. Brown & G. Smith) were
82 maintained on malt extract agar (MEA) plates, (Merck, Darmstadt, Germany) at 25°C in the dark.
83 *A. niger* was grown for 3 - 4 days prior to experimental subculture: *B. caledonica* and *P. javanicus*
84 were grown for at least one week prior to experimentation.

85 AP1 media (in Milli-Q water) comprised 38 mM (NH₄)₂SO₄ (Alfa Aesar, Ward Hill, USA) or 59 mM
86 NaNO₃ (Acros, New Jersey, USA), 3.7 mM KH₂PO₄ (Acros, New Jersey, USA), 0.8 mM MgSO₄·7H₂O
87 (BDH, Poole, UK), 0.2 mM CaCl₂·6H₂O (BDH, Poole, UK), 1.7 mM NaCl (Sigma-Aldrich, St. Louis,
88 USA), 9 × 10⁻³ mM FeCl₃·6H₂O (Sigma-Aldrich, St. Louis, USA), and trace metals 1.4 × 10⁻² mM
89 ZnSO₄·7H₂O (BDH, Poole, UK), 1.8 × 10⁻² mM MnSO₄·4H₂O (Sigma-Aldrich, St. Louis, USA), and 1.6
90 × 10⁻³ mM CuSO₄·5H₂O (BDH, Poole, UK), 111 mM D-glucose (VWR, Lutterworth, UK). Modified
91 Czapek-Dox agar (MCD) media was prepared of the following composition: 166 mM D-glucose
92 (VWR, Lutterworth, UK), 35 mM NaNO₃ (Acros, New Jersey, USA), 7 mM Na₂HPO₄ (Acros, New
93 Jersey, USA), 2.0 mM MgSO₄·7H₂O (BDH, Poole, UK), 7 mM KCl (BDH, Poole, UK), 0.04 mM
94 FeSO₄·7H₂O (Sigma-Aldrich, St. Louis, USA), 15 g L⁻¹ agar No.1 (Oxoid, Basingstoke, UK). All stock
95 solutions were sterilized separately by autoclaving at 121 °C for 15 min and subsequently mixed
96 with sterile (115 °C, 15 min) D-glucose solution. The media were adjusted to pH 5.5 using 1 M HCl
97 before sterilization by autoclaving at 115 °C for 15 min. For solid media, 15 g L⁻¹ agar No.1 (Oxoid,
98 Basingstoke, UK) was used. Liquid media was inoculated using a spore suspension in sterile Milli-

99 Q water, taken from a freshly grown MEA slope, to an initial concentration of 5×10^5 spores mL⁻¹ (ME) and 1×10^6 mL⁻¹ (AP1). Flasks were incubated in a shaking incubator (Infors Multitron II, Infors HT, Bottmingen, Switzerland) at 125 rpm in the dark at 25 °C.

102 Waste computer power cables were used as a copper source for the experiments. After removal of plastic coatings, the bare copper wire was cut into 2-3 mm long pieces which were oven-sterilized at 105°C for 48 h. 10 cm of copper wire weighed ~800 mg: the purity of the copper was not evaluated. Copper wire pieces were distributed over the agar surfaces either between the agar and a cellophane membrane placed on top, or on a cellophane membrane (Louth, Focus Packaging and Design Ltd, Louth, UK; thickness 27.5 µm) placed on top of the agar (Sayer & Gadd, 1997). The membranes allow the transfer of diffusible nutrients or metabolites between the agar and the fungus, provide a means of easily removing the biomass, and therefore providing information on direct and indirect interactions between the organism and substrate (Sayer and Gadd, 1997; Suyamud et al., 2020). Distribution densities were ~15-20 pieces cm⁻² on MEA but, because of enhanced toxicity, 8 copper wire pieces were evenly distributed on AP1 or MCD agar plates. For copper metal sheet colonization experiments, copper sheet pieces (~ 0.5 × 0.5 × 0.07 cm) (R.I.C.E. Metals Ltd, Truro, UK) were abraded with 100-grit aluminium oxide abrasive paper to enhance fungal colonization, washed with 1% nitric acid and subsequently sterile Milli-Q water. Four pieces of scratched copper sheet were placed above the cellophane membrane on agar media in 9-cm diameter Petri dishes. Inoculation of test fungi, at the centre of the agar plates, was achieved by using 6 mm diameter discs of mycelium cut from the periphery of growing colonies on MEA plates (Sayer & Gadd, 1997). Four copper sheet pieces were distributed

120 symmetrically on the agar surface, and plates (at least three replicates) were incubated in the
121 dark at 25 °C .

122

123 **2.2 Metal tolerance and pH determination**

124 Measurements were taken of colony diameter at regular time intervals in order to assess growth
125 rates and possible inhibitory effects (Gadd et al., 1985; Sayer et al., 1995). Biomass was removed
126 from the membrane overlying the agar surface using a scalpel and dried to constant weight at
127 105 °C for at least 4 days. Metal tolerance was evaluated using a tolerance index (TI) based on
128 the dry weight of fungal biomass as follows: $TI = (\text{dry weight of treated mycelium} / \text{dry weight of}$
129 $\text{the control mycelium}) \times 100\%$ (Sayer et al., 1995). The pH of the agar surface, after the fungal
130 colony had covered the surface completely, was measured at 6 equidistant intervals across plate
131 axes using a flat probe pH electrode (VWR International, Lutterworth, UK) after removal of the
132 cellulose membrane and biomass.

133

134 **2.3 Organic acid measurements**

135 *A. niger* was incubated in malt extract (ME) or AP1 liquid media amended respectively with 1%
136 and 0.05% (w/v) copper wire pieces at 25°C in the dark on an Infors II Multitron shaking incubator
137 (125rpm). 1 mL aliquots of supernatant were collected at 0, 7, 14, and 21 days after fungal
138 inoculation and filtered through a 0.2 µm pore diameter cellulose acetate membrane filters
139 (Whatman, Maidstone, UK). The acids were analysed using a BioRad Aminex HPX-87X-87H ion

140 exclusion column (300 mm × 7.8 mm) fitted with a Micro-Guard Cation H Refill guard column
141 (BioRad, Richmond CA, USA) at 35 °C on a DIONEX UltiMate 3000 system (ThermoFischer
142 Scientifics, Germering, Germany) including a pump, degasser, autosampler, and variable
143 wavelength detector. The sample injection volume was 20 µL and flow rate (5 mM H₂SO₄) was
144 0.6 mL/min. Detection was carried out at 210 nm for 18 min. Acids were identified and quantified
145 by their specific retention times and peak areas of the following standards: oxalic, citric, fumaric,
146 gluconic, itaconic, malic and succinic acid.

147

148 ***2.4 Elemental and mineralogical analysis***

149 The pieces of copper wire were separated from the agar after removal of fungal biomass by
150 homogenizing the agar in Milli-Q water at 80°C, and repeating washing of the wire pieces in warm
151 Milli-Q water. The copper samples were dried in a desiccator at ambient temperature for at least
152 3 weeks prior to analysis. Copper sheet was cleaned by gently washing with 1% Triton (v/v_{aq}) and
153 water for 24 h in 50 mL centrifuge tubes on a SB Tube Rotator (20 rpm), and kept in a desiccator
154 prior to further examination. Mineralogical and elemental analyses were carried out using energy
155 dispersive X-ray analysis (EDXA) coupled with scanning electron microscopy (SEM) and X-ray
156 diffraction (XRD) (see Li & Gadd, 2017; Ferrier et al., 2019; Suyamud et al., 2020; Kang et al., 2020;
157 Yang et al., 2020).

158

159 ***2.5 Statistical analysis***

160 Origin 9.1 was used, and at least three replicate determinations were used in experiments.

161 **3. Results**

162 **3.1 Effect of copper metal on fungal growth**

163 *A. niger*, *B. caledonica*, and *P. javanicus* were all able to grow on copper metal amended media.
164 *A. niger* grew the fastest, while the growth of *B. caledonica* and *P. javanicus* was much slower.
165 The inclusion of copper metal in the medium had little significant effect on growth rates of the
166 test fungi (Table 1). The presence of copper metal slightly inhibited colony expansion of *A. niger*
167 on AP1 medium, while growth of *B. caledonica* was slower on copper metal-amended MEA and
168 MCD medium than on the corresponding controls. While *P. javanicus* grew slowly, the presence
169 of copper metal had little effect. The type of contact of the test fungi with the copper metal
170 showed different effects on growth. The growth rate of *A. niger* when directly interacting with
171 copper metal on the cellophane membrane surface on MEA and ammonium salt AP1 medium
172 appeared to be slightly inhibited, compared with the treatments where copper metal was
173 incorporated in the agar below the cellophane membrane, perhaps due to higher exposure to
174 mobile copper species in proximity to the metal. Similar effects occurred with *B. caledonica* on
175 MCD and *P. javanicus* on nitrate salt AP1 medium (Table 1). Growth in the absence or presence
176 of copper wire metal pieces was also expressed as a tolerance index (TI) based on the yields of
177 fungal biomass, which confirmed that the effects of copper metal varied among the fungal
178 species. On MEA, the TIs for *A. niger* were all around 100% in the presence of copper metal both
179 when below and above the cellophane membrane. The growth of *P. javanicus* on nitrate salt AP1
180 and MCD medium containing copper metal above or below membrane showed similar significant
181 reductions (TI =70.3%-75.9%, Table 2). Compared with the TI values for *B. caledonica* on nitrate
182 salt AP1 medium (around 90%), the TI values for MCD revealed a remarkable reduction to about

183 60%. The contact mode with the copper showed little effect on the TI values except for *B.*
184 *caledonica* on MEA media: the TI for indirect contact was reduced by 16.5% compared to direct
185 contact. This showed that copper metal had a significant influence on growth of *B. caledonica*,
186 especially when in direct contact with the metal.

187

188 **3.2 pH changes in media after fungal growth**

189 The medium pH decreased during growth of *A. niger*, compared with abiotic controls, while the
190 pH increased slightly in the presence of copper metal compared to the negative control (Table 3).
191 This showed that some acids produced by the test fungus were consumed by the copper metal
192 resulting in an increase in pH. Of the different media inoculated with *A. niger*, the pH of
193 ammonium salt AP1 medium reached the lowest value. For *B. caledonica* and *P. javanicus*, the
194 medium become alkaline during growth, and the pH markedly increased in the presence of
195 copper metal compared with the abiotic controls (Table 3).

196

197 **3.3 Determination of organic acids**

198 For ME liquid media, oxalic, malic and succinic acid were the main organic acids detected: citric,
199 fumaric, gluconic and itaconic acid were not detected. Oxalic acid dominated, the highest
200 amount (12.3 mM) appearing after *A. niger* was incubated with copper for 14 days. In the absence
201 of copper metal, corresponding oxalic acid production was 7.8 mM (Fig.1a) indicating the
202 presence of copper metal stimulated the production of oxalate. After 14 days, the concentration

203 of oxalic acid decreased, probably the result of oxalate consumption by the formation of copper
204 oxalate (see later). The secretion of malic acid reached a peak after 7 days when *A. niger* was
205 grown in ME liquid media and then remained constant. The presence of 1% (w/v) copper
206 stimulated the generation of malic acid up to 2.2 mM (Fig.1b). Succinic acid occurred at similar
207 concentrations both in the control and 1% copper metal treatment and decreased over
208 incubation time (Fig. 1c). For copper-containing ammonium AP1 liquid media, only limited malic
209 acid was detected during incubation of *A. niger* with 0.05% (w/v) copper metal, less than in the
210 control, and increased gradually reaching equilibrium at 0.025 mM (Fig. 1d). The contents of
211 other organic acids, including oxalic acid, were below the detection limits.

212

213

214 **3.4 Biocorrosion and biomineral formation**

215 The capacity of *A. niger* to solubilize copper metal was manifest by the reduced diameter of
216 copper wire pieces in the ammonium salt AP1 medium. To examine whether there was any
217 medium influence on the copper metal, control copper wire pieces were collected from agar
218 plates containing the same amount of copper metal incubated in the absence of fungi. In this
219 comparison, the solubilization of copper metal by *A. niger* was clearly observed (Fig. 2). The
220 diameter of control copper wire pieces was $189.6 \pm 0.4 \mu\text{m}$, while the diameter of *A. niger* treated
221 copper wire was $75.8 \pm 1.6 \mu\text{m}$, a significant decrease of 60.0%. Such a decrease in the diameter
222 of the wire as a result of fungal action clearly reflects significant loss of mass. In addition, the
223 surface appearance of fungal exposed copper metal was rougher than the control.

224 Compared with abiotic copper sheet samples, significant alteration of the copper sheet surfaces
225 was observed and a blue crust was evident on visual examination (result not shown). Microscopic
226 examination showed that distinct biocorrosion patterns were observed on colonized copper
227 sheets, incubation with *A. niger* resulted in etching and disruption over the surface (Fig. 3a). Some
228 of the etched channels showed a similar pattern to fungal mycelium grown on the surface which
229 mirrored colonization and branching by the fungal hyphae. The dimensions of etching traces
230 varied, their width being around 1 - 3.5 μm , while the width of fungal hyphae growing on a copper
231 sheet surface was $\sim 10 \mu\text{m}$ (Fig.3b).

232 For fungi grown in the presence of copper wire pieces, it was found that there was obvious
233 evidence of biomineral formation on the copper surfaces and different biocorrosion patterns
234 produced by *A. niger*. The varied contact modes resulted in different shapes of the secondary
235 minerals produced probably reflecting differences in the secretion of geoactive metabolites.
236 Microscopic examination showed widespread corrosion of the copper wire pieces as well as the
237 formation of various crystalline structures. Different patterns of biominerals were produced by
238 *A. niger*. The secondary minerals formed on copper pieces incorporated in agar below the
239 membrane showed a lamellar structure (Fig. 4a, b). The size of these biominerals was
240 approximately 3-4 μm in diameter. The biominerals that formed on copper wire pieces which
241 were in direct interact with *A. niger* showed some different morphologies, the most distinctive
242 being layered structures similar to the crystals formed on the copper wire pieces that had indirect
243 contact with *A. niger*. Some other structures showed flakiness, and some were amorphous. Apart
244 from these, some mycelial encrustations were observed on the surface of copper wire pieces (Fig.
245 4c, d). EDXA showed that the biominerals formed on copper pieces contained copper, carbon,

246 and oxygen (Fig. 5a, c). As shown in Fig. 6, X-ray diffraction analyses of the biominerals produced
247 by *A. niger* confirmed the presence of moolooite ($\text{CuC}_2\text{O}_4 \cdot 0.4\text{H}_2\text{O}$) (ICPDS Card NO. 21 - 297) and
248 cuprite (Cu_2O) (ICPDS Card NO. 05 - 667).

249

250 4. Discussion

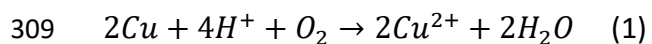
251 Fungal colonization can have an extraordinary biodeteriorative influence on organic as well as
252 metallic and mineral constituents of the built infrastructure, and historical artefacts, such as
253 staining, biocorrosion, and biodeterioration (Miller et al., 2012; Gadd, 2017a; Gadd and Dyer,
254 2017). Although the tolerance and responses of fungi to soluble copper, especially for wood
255 decay fungi, has been widely reported, and investigations carried out on the corrosion of metals
256 embedded in copper preservative-treated wood, such as aluminium and steel, (Zelinka and
257 Rammer, 2009; Zelinka and Stone, 2011), or copper fasteners in wood (Zelinka et al., 2019a,b),
258 the influence of fungi on metallic copper biocorrosion has received little attention. Copper is an
259 essential element for fungal growth. It can act as an enzyme cofactor and is essential for
260 respiration, free radical detoxification, and iron acquisition (Antsotegi-Uskola et al., 2020).
261 However, excess copper accumulation will result in toxicity, hence its historical and current use
262 as a fungicide and wood preservative in inorganic and organic forms (Freeman and McIntyre,
263 2008; Lamichhane et al., 2018). Copper ions can inactivate metalloenzymes by metal
264 displacement, bind to intracellular sulfur, oxygen, and nitrogen ligands, and also promote the
265 production of reactive oxygen species (ROS) (Fridovich, 1983; Macomber and Imlay, 2009; Smith
266 et al., 2017). However, many fungal species can show pronounced tolerance to copper,
267 particularly under acidic conditions (Gadd and Griffiths, 1980a; Gadd and White, 1985; Green and
268 Clausen, 2005; Humar et al., 2005; Ohno et al., 2015) or when in insoluble forms (Karamushka et
269 al., 1996; Fomina et al., 2017), and the copper-tolerance of *A. niger* and *B. caledonica* have been
270 reported previously (Gharieb et al., 2004; Fomina et al., 2005a; Iskandar et al., 2011). In the built
271 environment, including cultural heritage, insoluble copper-containing substrates include metallic

272 copper and alloys, copper-containing minerals, preservatives and pigments. Biodeteriorative
273 effects therefore depend on direct and indirect interactions that result in release of Cu^{2+} which
274 can interact with organisms, bind to environmental constituents, or form copper-containing
275 secondary minerals with organic and inorganic ligands, all further contributing to
276 biodeteriorative effects and alteration of appearance. In this work, the test fungi could all grow
277 in the presence of copper metal, whether in direct or indirect contact, and although there was
278 some variation in response, little significant toxicity was manifest. For *A. niger*, inhibition of
279 growth rate was slightly greater with direct contact with the copper, although this was marginal
280 and not clear for the other test fungi. This is unsurprising since the cellophane membrane would
281 not have acted as a barrier to mobile copper species. Tolerance indices (TIs) largely reflected
282 these results with little effect on *A. niger*, but some significant reduction for *B. caledonica* on
283 MCD and *P. javanicus* on MCD and NO_3^- -AP1 although, due to slower growth, TIs were derived
284 from a much longer incubation time than for *A. niger*. These data show that the test fungi could
285 grow successfully in the presence of metallic copper despite some limited toxicity.

286 Fungi can effect mineral solubilization through proton and ligand-mediation dissolution
287 mechanisms (acidolysis and complexolysis, respectively) as well as redox reactions (redoxolysis)
288 (Burgstaller and Schinner, 1993; Gadd, 2007, 2010; Gadd et al., 2014) and such mechanisms will
289 also be involved for metallic substrates (Fomina et al., 2008; Rhee et al., 2012, 2014, 2016). It
290 seems that acidolysis and complexolysis were the main mechanisms operating in this study. Many
291 examples of mineral solubilization by fungi are correlated with a pH decrease (Sayer and Gadd,
292 1997; Fomina et al., 2004, 2005b) that can result from proton excretion, nutrient-proton antiport,
293 ammonium utilization, organic acid secretion, and respiration (Burgstaller and Schinner, 1993)

294 The pH is a vital factor in mineral transformations by fungi because of its significant effects on
295 metal biosorption and transport processes, and the nucleation and precipitation of secondary
296 mineral products (Burford et al., 2003, 2006; Parvathi et al., 2007; Wei et al., 2012) as well as
297 effects on fungal growth and nutrition, including organic acid excretion (Gadd, 1999; Fomina et
298 al., 2004; Gadd, 2010). An acidic pH can also lead to a marked reduction in the toxicity of soluble
299 copper to fungi (Starkey, 1973; Gadd and Griffiths, 1980b) due to decreased sorption and
300 intracellular accumulation of copper at low pH (Gadd and White, 1985). In this work, the media
301 pH significantly decreased with *A. niger*, particularly with ammonium as nitrogen source, as noted
302 in other studies (e.g. Fomina et al., 2017). In contrast, growth of *B. caledonica* and *P. javanicus*
303 resulted in increased alkalinity, especially when grown using nitrate as nitrogen source (Lapeyrie
304 et al., 1987; Gadd, 1999), and influence copper speciation as hydroxides and carbonates which
305 will tend to reduce potential copper toxicity. In acidolysis, oxygen atoms on the metal surface are
306 protonated to water leading to Cu^{2+} release (Burgstaller and Schinner, 1993), as simplified in
307 equation 1:

308



310

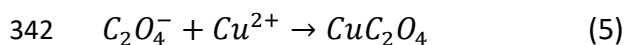
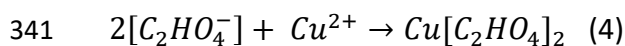
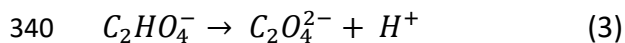
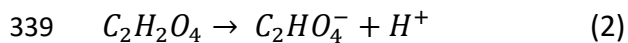
311 The secretion of low molecular weight organic acids is often pivotal to mineral and metal
312 transformations, potentially contributing to acidolysis, complexation and redox interactions
313 (Gadd, 1993; Fomina et al., 2005a; Gadd et al., 2014) and *A. niger* can generate several different
314 organic acids, dependent on nutritional conditions, that are effective for metal complexation, e.g.

315 citric and oxalic acid (Gadd, 1999; Ruijter et al., 1999). The production of such acids by *A. niger*,
316 and other *Aspergillus* species, has been investigated for metal bioleaching and biorecovery from
317 electronic wastes (Kolenčik et al., 2013; Horeh et al., 2016), spent refinery catalysts (Santhiya and
318 Ting, 2005), mine tailings, deposits and ores (Mulligan et al., 2004; Seh-Bardan et al., 2012;
319 Mohanty et al., 2017; Kang et al., 2019, 2020; Yang et al., 2019, 2020) . The reduction of metal
320 species in redoxolysis can be mediated by excreted metabolites, and oxalic acid is capable of Fe(III)
321 and Mn(IV) reduction to Fe(II) and Mn(II) respectively (Dutton and Evans, 1996; Gadd, 1999; Wei
322 et al., 2012). In this work, the main organic acids secreted by *A. niger* in ME medium were oxalic,
323 malic and succinic acid with oxalic acid dominating, the presence of copper appearing to enhance
324 oxalate production. This has been observed in wood decay fungi which can show high levels of
325 oxalate production (Hastrup et al., 2012) and an important mechanism of detoxifying copper in
326 preservative-treated wood was overexcretion of oxalic acid (Clausen and Green, 2003; Green and
327 Clausen, 2005; Hastrup et al., 2012; Ohno et al., 2015). *Beauveria caledonica* also showed oxalate
328 overexcretion in the presence of toxic metal-containing minerals, including those of copper
329 (Fomina et al., 2005a).

330 In complexolysis, the metal is solubilized from the substrate due to the complexing capacity of
331 the complexant molecule, and this can also promote the solubility of a metal ion which has been
332 detached from metal via acidolysis (Burgstaller and Schinner, 1993). Oxalate will also precipitate
333 with many metal species, apart from alkali metals, and the formation of copper oxalate has often
334 been considered as a tolerance mechanism in fungi (Murphy and Levy, 1983; Dutton and Evans,
335 1996; Gadd, 1999; Clausen et al., 2000; Green and Clausen, 2003, 2005; Jarosz-Wilkofazka and

336 Gadd, 2003; Hastrup et al., 2005; Ohno et al., 2015). The reactions between Cu^{2+} and oxalic acid
337 can be summarized as follows (Horeh et al., 2016):

338



343

344 The amount and variety of organic acids excreted by *A. niger* is highly dependent on medium
345 composition, especially carbon and nitrogen source, pH, and buffering capacity (Burgstaller and
346 Schinner, 1993; Dutton and Evans, 1996; Gadd, 1999; Palmieri et al., 2019). A relationship
347 between the nitrogen source and oxalate excretion has often been demonstrated (Dutton and
348 Evans, 1996; Gadd, 1999; Fomina et al., 2017). In ammonium salt AP1 medium, only limited malic
349 acid was detected in this work but the medium became strongly acidic because ammonium
350 assimilation leads to the production of protons which reduces the pH of the extracellular
351 environment (Sazanova et al., 2015). In addition, a low external pH restricts fungal production of
352 oxalic acid (Roos and Luckner, 1984; Ruijter et al., 1999) since a key enzyme responsible for
353 oxalate formation, oxaloacetate acetylhydrolase (OAH), is inhibited at low pH values (Gadd, 1999;
354 Ruijter et al., 1999). It seems clear that acid dissolution and complexation were the main
355 processes mediated by *A. niger* in this work, which could have dramatic effects on metallic copper

356 as evidenced by the marked solubilization of copper wire and the etching and disruption of
357 copper sheet surfaces.

358 Besides the dissolution of metal-bearing substrates, fungal biodeterioration can also occur
359 through secondary mineral formation, and oxalates are frequently associated with the disruption
360 and flaking of outer layers of building components, plaster, frescoes etc. (Fomina et al., 2010;
361 Gadd, 2017b; Gadd et al., 2014). Conversely, oxalate formation in other contexts can stabilize
362 external surfaces through involvement in stable patina formation emphasising that
363 biodeteriorative or surface effects are highly dependent on the substrate and physico-chemical
364 conditions (Gadd, 2017a, b; Gadd et al., 2014; Palmieri et al., 2019). In this work, a vivid blue crust
365 resulted on the surface of copper metal after growth of the selected fungi, and this was also
366 identified as the hydrated copper oxalate, moolooite, together with cuprite. Clearly, such
367 interactions will change the appearance of the copper metal substrate and remove any metallic
368 lustre. Copper oxalates are frequently identified on surfaces of outdoor bronze structures
369 (Graedel et al., 1987) forming insoluble stable patinas even in an acidic atmosphere (Marabelli
370 and Mazzeo, 1993). Innovative research has therefore explored the application of oxalate
371 formation for protection and conservation of historic and contemporary metal artefacts, with
372 copper oxalate appearing particularly applicable for such a purpose (Joseph et al., 2012a, b).

373 Several fungal species have previously been shown to be capable of copper-containing mineral
374 transformations, and copper oxalate is frequently associated with fungal interactions with both
375 soluble and insoluble copper-containing compounds and substrates (Murphy and Levy, 1983;
376 Dutton and Evans, 1996; Gadd, 1999; Clausen et al., 2000; Green and Clausen, 2003, 2005; Jarosz-
377 Wilkołazka and Gadd, 2003; Fomina et al., 2005a, 2017). It has also been reported that copper

378 salts can stimulate oxalate production (Green and Clausen, 2003). Extracellular copper oxalate
379 (moolooite, $\text{CuC}_2\text{O}_4 \cdot n\text{H}_2\text{O}$ ($n \sim 0.4-0.7$)) precipitation occurred on *Beauveria caledonica* hyphae
380 and cords growing with copper phosphate (Fomina et al., 2005a, 2010).

381

382 **5. Conclusions**

383 In summary, this work has demonstrated that *A. niger* is capable of colonization and
384 biodeterioration of metallic copper through dissolution activities and the formation of secondary
385 copper oxalate biominerals leading to alteration of surface topography and visual appearance.
386 Dissolution by *A. niger* can lead to significant loss in mass as evidenced by the dramatic size
387 reduction in copper wire exposed to fungal metabolite excretion and etching of sheet copper.
388 Little toxicity was manifest to the test organisms, and acidolysis and complexation were the
389 significant biodeteriorative mechanisms that lead to copper oxalate crust formation. The findings
390 emphasize the importance of oxalate excretion in effecting metal and mineral transformations,
391 as shown in several relevant studies (Fomina et al., 2008; Gadd et al., 2014; Ferrier et al., 2019;
392 Kang et al., 2019, 2020; Suyamud et al., 2020). It is clear that the transforming influence of fungal
393 colonization may have some implications for biodeterioration, protection and preservation of
394 cultural relics and artefacts as well as certain components of the built environment.

395

396 **Acknowledgments**

397 This research was supported by a China Scholarship Council – School of Life Sciences PhD
398 scholarship to Jiayue Zhao (No. 201704910860) which is gratefully acknowledged. The authors
399 also gratefully acknowledge of the help of Martin Kierans (Central Imaging Facility, School of Life
400 Sciences, University of Dundee) and Dr. Yongchang Fan (Division of Physics, University of Dundee)
401 for assistance with scanning electron microscopy. Financial support of the Geomicrobiology
402 Group from the Natural Environment Research Council [NE/M01090/1(TeaSe);
403 NE/M011275/1(CoG³)] is also gratefully acknowledged.

404

405 **Conflict of Interest Disclosure**

406 The authors declare no competing financial or non-financial conflicts of interest.

407

408 **References**

- 409 Antsotegi-Uskola, M., Markina-Iñarrairaegui, A., Ugalde, U., 2020. New insights into copper
410 homeostasis in filamentous fungi. *International Microbiology* 23, 65-73.
- 411 Bhandari, J., Khan, F., Abbassi, R., Garaniya, V., Ojeda, R., 2015. Modelling of pitting corrosion in
412 marine and offshore steel structures—a technical review. *Journal of Loss Prevention in the Process*
413 *Industries* 37, 39-62.
- 414 Burford, E.P., Hillier, S., Gadd, G.M., 2006. Biomineralization of fungal hyphae with calcite (CaCO₃)
415 and calcium oxalate mono- and dihydrate in carboniferous limestone microcosms.
416 *Geomicrobiology Journal* 23, 599-611.
- 417 Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2003. Nutrient and
418 microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture* 219,
419 393-411.
- 420 Burgstaller, W., Schinner, F., 1993. Leaching of metals with fungi. *Journal of Biotechnology* 27,
421 91-116.
- 422 Clausen, C.A., Green, F., 2003. Oxalic acid overproduction by copper-tolerant brown-rot
423 basidiomycetes on southern yellow pine treated with copper-based preservatives. *International*
424 *Biodeterioration and Biodegradation*, 51, 139-144.
- 425 Clausen, C.A., Green, F., Woodward, B.M., Evans, J.W., DeGroot, R.C., 2000. Correlation between
426 oxalic acid production and copper tolerance in *Wolfiporia cocos*. *International Biodeterioration*
427 *and Biodegradation* 46, 69-76.

428 Crispim, C.A., Gaylarde, C.C., 2005. Cyanobacteria and biodeterioration of cultural heritage: a
429 review. *Microbial Ecology* 49, 1-9.

430 de la Fuente, D., Simancas, J., Morcillo, M., 2008. Morphological study of 16-year patinas formed
431 on copper in a wide range of atmospheric exposures. *Corrosion Science* 50, 268-285.

432 Dutton, M.V., Evans, C.S., 1996. Oxalate production by fungi: its role in pathogenicity and ecology
433 in the soil environment. *Canadian Journal of Microbiology* 42, 881-895.

434 Elwell, W.T., Scholes, I.R., 1967. Analysis of copper and its alloys. Pergamon Press, London.

435 Emde, K.M.E., Smith, D.W., Facey, R., 1992. Initial investigation of microbially influenced
436 corrosion (MIC) in a low temperature water distribution system. *Water Research* 26, 169-175.

437 Ferrier, J., Yang, Y., Csetenyi, L., Gadd, G.M., 2019. Colonization, penetration and transformation
438 of manganese oxide nodules by *Aspergillus niger*. *Environmental Microbiology* 21, 1821-1832.

439 Fomina, M., Alexander, I.J., Hillier, S., Gadd, G.M., 2004. Zinc phosphate and pyromorphite
440 solubilization by soil plant-symbiotic fungi. *Geomicrobiology Journal* 21, 351-366.

441 Fomina, M., Hillier, S., Charnock, J.M., Melville, K., Alexander, I.J., Gadd, G.M., 2005a. Role of
442 oxalic acid overexcretion in transformations of toxic metal minerals by *Beauveria caledonica*.
443 *Applied and Environmental Microbiology* 71, 371-381.

444 Fomina, M.A., Alexander, I.J., Colpaert, J.V., Gadd, G.M., 2005b. Solubilization of toxic metal
445 minerals and metal tolerance of mycorrhizal fungi. *Soil Biology and Biochemistry* 37, 851-866.

446 Fomina, M., Charnock, J.M., Bowen, A.D., Gadd, G.M., 2007. X-ray absorption spectroscopy (XAS)
447 of toxic metal mineral transformations by fungi. *Environmental Microbiology* 9, 308-321.

448 Fomina, M., Charnock, J.M., Hillier, S., Alvarez, R., Livens, F., Gadd, G.M., 2008. Role of fungi in
449 the biogeochemical fate of depleted uranium. *Current Biology* 18, 375-377.

450 Fomina, M., Burford, E.P., Hillier, S., Kierans, M., Gadd, G.M., 2010. Rock-building fungi.
451 *Geomicrobiology Journal* 27, 624-629.

452 Fomina, M., Bowen, A.D., Charnock, J.M., Podgorsky, V.S., Gadd, G.M., 2017. Biogeochemical
453 spatio-temporal transformation of copper in *Aspergillus niger* colonies grown on malachite with
454 different inorganic nitrogen sources. *Environmental Microbiology* 19, 1310-1321.

455 Frankfort, H., 1956. The art and architecture of the ancient orient. *Journal of Aesthetics and Art*
456 *Criticism* 14, 388-390.

457 Freeman, M.H., McIntyre, C.R., 2008. Copper-based wood preservatives. *Forest Products Journal*
458 58, 6-27.

459 Fridovich, I., 1983. Superoxide radical: an endogenous toxicant. *Annual Review of Pharmacology*
460 *and Toxicology* 23, 239-257.

461 Gadd, G.M., 1993. Interactions of fungi with toxic metals. *New Phytologist*, 124, 25-60.

462 Gadd, G.M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation,
463 physiology and biogeochemical processes. In: Poole, R.K. (Ed.), *Advances in Microbial Physiology*,
464 Vol. 41, Academic Press, pp. 47-92.

465 Gadd, G.M., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and
466 radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* 111, 3-49.

467 Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation.
468 Microbiology 156, 609-643.

469 Gadd, G.M., 2017a. Fungi, rocks, and minerals. Elements 13, 171-176.

470 Gadd, G.M., 2017b. Geomicrobiology of the built environment. Nature Microbiology 2, No. 16275.

471 Gadd, G.M., Bahri-Esfahani, J., Li, Q., Rhee, Y.J., Wei, Z., Fomina, M., Liang, X., 2014. Oxalate
472 production by fungi: significance in geomycolology, biodeterioration and bioremediation. Fungal
473 Biology Reviews 28, 36-55.

474 Gadd, G.M., Dyer, T.D., 2017. Bioprotection of the built environment and cultural heritage.
475 Microbial Biotechnology 10, 1152-1156.

476 Gadd, G.M., Griffiths, A.J., 1980a. Effect of copper on morphology of *Aureobasidium pullulans*.
477 Transactions of the British Mycological Society 74, 387-392.

478 Gadd, G.M., Griffiths, A.J., 1980b. Influence of pH on copper uptake and toxicity in *Aureobasidium*
479 *pullulans*. Transactions of the British Mycological Society 75, 91-96.

480 Gadd, G.M., White, C., 1985. Copper uptake by *Penicillium ochrochloron*: influence of pH on
481 toxicity and demonstration of energy-dependent copper influx using protoplasts. Microbiology
482 131, 1875-1879.

483 Gadd, G.M., Mowll, J.L., White, C., Newby, P.J., 1985. Methods for assessment of heavy metal
484 toxicity towards fungi and yeasts. Toxicity Assessment 1, 169-185.

485 Gharieb, M.I., Ali, M.I., El-Shoura, A.A., 2004. Transformation of copper oxychloride fungicide into
486 copper oxalate by tolerant fungi and the effect of nitrogen source on tolerance. *Biodegradation*
487 15, 49-57.

488 Graedel, T.E., Nassau, K., Franey, J.P., 1987. Copper patinas formed in the atmosphere-I.
489 Introduction. *Corrosion Science* 27, 639-657.

490 Green, F., Clausen, C.A., 2003. Copper tolerance of brown-rot fungi: time course of oxalic acid
491 production. *International Biodeterioration and Biodegradation* 51, 145-149.

492 Green, F. and Clausen, C.A., 2005. Copper tolerance of brown-rot fungi: oxalic acid production in
493 southern pine treated with arsenic-free preservatives. *International Biodeterioration and*
494 *Biodegradation*, 56, 75-79.

495 Gu, J.D., 2007. Microbial colonization of polymeric materials for space applications and
496 mechanisms of biodeterioration: a review. *International Biodeterioration and Biodegradation* 59,
497 170-179.

498 Hastrup, A.C.S., Green, F., Clausen, C.A. and Jensen, B., 2005. Tolerance of *Serpula lacrymans* to
499 copper-based wood preservatives. *International Biodeterioration and Biodegradation*, 56, 173-
500 177.

501 Hastrup, A.C.S., Green, F., Lebow, P.K. and Jensen, B., 2012. Enzymatic oxalic acid regulation
502 correlated with wood degradation in four brown-rot fungi. *International Biodeterioration and*
503 *Biodegradation*, 75, 109-114.

504 Herrera, L.K., Videla, H.A., 2009. Surface analysis and materials characterization for the study of
505 biodeterioration and weathering effects on cultural property. *International Biodeterioration and*
506 *Biodegradation* 63, 813-822.

507 Horeh, N.B., Mousavi, S.M., Shojaosadati, S.A., 2016. Bioleaching of valuable metals from spent
508 lithium-ion mobile phone batteries using *Aspergillus niger*. *Journal of Power Sources* 320, 257-
509 266.

510 Humar, M., Šentjurc, M., Amartej, S.A., Pohleven, F., 2005. Influence of acidification of CCB
511 (Cu/Cr/B) impregnated wood on fungal copper tolerance. *Chemosphere* 58, 743-749.

512 Iskandar, N.L., Zainudin, N.A.I.M., Tan, S.G., 2011. Tolerance and biosorption of copper (Cu) and
513 lead (Pb) by filamentous fungi isolated from a freshwater ecosystem. *Journal of Environmental*
514 *Sciences* 23, 824-830.

515 Iverson, W.P., 1987. Microbial corrosion of metals. *Advances in Applied Microbiology*, 32, 1-36.

516 Jarosz-Wilkolażka, A., Gadd, G.M., 2003. Oxalate production by wood-rotting fungi growing in
517 toxic metal-amended medium. *Chemosphere* 52, 541-547.

518 Joseph, E., Cario, S., Simon, A., Wörle, M., Mazzeo, R., Junier, P., Job, D., 2012a. Protection of
519 metal artifacts with the formation of metal–oxalates complexes by *Beauveria bassiana*. *Frontiers*
520 *in Microbiology* 2, 270.

521 Joseph, E., Simon, A., Mazzeo, R., Job, D., Wörle, M., 2012b. Spectroscopic characterization of an
522 innovative biological treatment for corroded metal artefacts. *Journal of Raman Spectroscopy* 43,
523 1612-1616.

524 Kang, X., Csetenyi, L., Gadd, G.M., 2019. Biotransformation of lanthanum by *Aspergillus niger*.
525 Applied Microbiology and Biotechnology 103, 981-993.

526 Kang, X., Csetenyi, L., Gadd, G.M., 2020. Monazite transformation into Ce- and La-containing
527 oxalates by *Aspergillus niger*. Environmental Microbiology 22, 1635-1648.

528 Karamushka, V.I., Sayer, J.A., Gadd, G.M., 1996. Inhibition of H⁺ efflux from *Saccharomyces*
529 *cerevisiae* by insoluble metal phosphates and protection by calcium and magnesium: inhibitory
530 effects a result of soluble metal cations. Mycological Research 100, 707-713.

531 Kartal, S.N., Terzi, E., Yilmaz, H., Goodell, B., 2015. Bioremediation and decay of wood treated
532 with ACQ, micronized ACQ, nano-CuO and CCA wood preservatives. International
533 Biodeterioration and Biodegradation 99, 95-101.

534 Karunasekera, H., Pettersson, J., Mi, J., Bergquist, J., Daniel, G., 2019. Copper tolerance of the
535 soft-rot fungus *Phialophora malorum* grown in-vitro revealed by microscopy and global protein
536 expression. International Biodeterioration and Biodegradation 137, 147-152.

537 Kolenčík, M., Urík, M., Čerňanský, S., Molnářová, M., Matúš, P., 2013. Leaching of zinc, cadmium,
538 lead and copper from electronic scrap using organic acids and the *Aspergillus niger* strain.
539 Fresenius Environmental Bulletin 22, 3673-3679.

540 Lamichhane, J.R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J.B., Aubertot, J.N., 2018. Thirteen
541 decades of antimicrobial copper compounds applied in agriculture. A review. Agronomy for
542 Sustainable Development 38, 28.

543 Lapeyrie, F., Chilvers, G.A., Bhem, C.A. (1987). Oxalic acid synthesis by the mycorrhizal fungus
544 *Paxillus involutus* (Batsch, ex Fr.) Fr. *New Phytologist* 106, 139–146.

545 Li, Q., Gadd, G. M. (2017). Biosynthesis of copper carbonate nanoparticles by ureolytic fungi.
546 *Applied Microbiology and Biotechnology* 101, 7397-7407.

547 Macomber, L., Imlay, J.A., 2009. The iron-sulfur clusters of dehydratases are primary intracellular
548 targets of copper toxicity. *Proceedings of the National Academy of Sciences of the USA* 106, 8344-
549 8349.

550 Marabelli, M., Mazzeo, R., 1993. La corrosione dei bronzi esposti all'aperto: problemi di
551 caratterizzazione. *Metallurgia Italiana* 85, 247-254.

552 Miller, A.Z., Sanmartín, P., Pereira-Pardo, L., Dionísio, A., Sáiz-Jiménez, C., Macedo, M.F., Prieto,
553 B., 2012. Bioreceptivity of building stones: a review. *Science of the Total Environment* 426, 1-12.

554 Mohanty, S., Ghosh, S., Nayak, S. Das, A.P., 2017. Bioleaching of manganese by *Aspergillus* sp.
555 isolated from mining deposits. *Chemosphere* 172, 302-309.

556 Mulligan, C.N., Kamali, M., Gibbs, B.F., 2004. Bioleaching of heavy metals from a low-grade
557 mining ore using *Aspergillus niger*. *Journal of Hazardous Materials* 110, 77-84.

558 Murphy, R.J., Levy, J.F., 1983. Production of copper oxalate by some copper tolerant fungi.
559 *Transactions of the British Mycological Society* 81, 165-168.

560 Nica, D., Davis, J.L., Kirby, L., Zuo, G., Roberts, D.J., 2000. Isolation and characterization of
561 microorganisms involved in the biodeterioration of concrete in sewers. *International*
562 *Biodeterioration and Biodegradation* 46, 61-68.

563 Ohno, K.M., Clausen, C.A., Green, F., Diehl, S.V., 2015. Insights into the mechanism of copper-
564 tolerance in *Fibroporia radiculosa*: the biosynthesis of oxalate. *International Biodeterioration and*
565 *Biodegradation* 105, 90-96.

566 Onofri, S., Zucconi, L., Isola, D., Selbmann, L., 2014. Rock-inhabiting fungi and their role in
567 deterioration of stone monuments in the Mediterranean area. *Plant Biosystems* 148, 384-391.

568 Palmieri, F., Estoppey, A., House, G.L., Lohberger, A., Bindschedler, S., Chain, P.S., Junier, P., 2019.
569 Oxalic acid, a molecule at the crossroads of bacterial-fungal interactions. *Advances in Applied*
570 *Microbiology* 106, 49-77.

571 Parvathi, K., Kumar, R.N., Nagendran, R., 2007. Biosorption of manganese by *Aspergillus niger*
572 and *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnology* 23, 671-676.

573 Rhee, Y.J., Hillier, S., Gadd, G.M., 2012. Lead transformation to pyromorphite by fungi. *Current*
574 *Biology* 22, 237-241.

575 Rhee, Y.J., Hillier, S., Gadd, G.M., 2016. A new lead hydroxycarbonate produced during
576 transformation of lead metal by the soil fungus *Paecilomyces javanicus*. *Geomicrobiology Journal*
577 33, 250-260.

578 Rhee, Y.J., Hillier, S., Pendrowski, H., Gadd, G.M., 2014. Pyromorphite formation in a fungal
579 biofilm community growing on lead metal. *Environmental Microbiology* 16, 1441-1451.

580 Roos, W., Luckner, M., 1984. Relationships between proton extrusion and fluxes of ammonium
581 ions and organic acids in *Penicillium cyclopium*. *Microbiology* 130, 1007-1014.

582 Ruijter, G.J., van de Vondervoort, P.J., Visser, J., 1999. Oxalic acid production by *Aspergillus niger*:
583 an oxalate-non-producing mutant produces citric acid at pH 5 and in the presence of manganese.
584 Microbiology 145, 2569-2576.

585 Santhiya, D., Ting, Y.P., 2005. Biobleaching of spent refinery processing catalyst using *Aspergillus*
586 *niger* with high-yield oxalic acid. Journal of Biotechnology 116, 171-184.

587 Sayer, J.A., Gadd, G.M., 1997. Solubilization and transformation of insoluble inorganic metal
588 compounds to insoluble metal oxalates by *Aspergillus niger*. Mycological Research 101, 653-661.

589 Sayer, J.A., Raggett, S.L., Gadd, G.M., 1995. Solubilization of insoluble metal compounds by soil
590 fungi: development of a screening method for solubilizing ability and metal tolerance.
591 Mycological Research 99, 987-993.

592 Sazanova, K., Osmolovskaya, N., Schiparev, S., Yakkonen, K., Kuchaeva, L., Vlasov, D., 2015.
593 Organic acids induce tolerance to zinc-and copper-exposed fungi under various growth
594 conditions. Current Microbiology 70, 520-527.

595 Scheerer, S., Ortega-Morales, O., Gaylarde, C., 2009. Microbial deterioration of stone
596 monuments-an updated overview. Advances in Applied Microbiology, 66, 97-139.

597 Schilling, J.S., Jellison, J., 2006. Metal accumulation without enhanced oxalate secretion in wood
598 degraded by brown rot fungi. Applied and Environmental Microbiology 72, 5662-5665.

599 Seh-Bardan, B.J., Othman, R., Wahid, S.A., Husin, A., Sadegh-Zadeh, F., 2012. Biobleaching of heavy
600 metals from mine tailings by *Aspergillus fumigatus*. Bioremediation Journal 16, 57-65.

601 Siegel, S.M., Siegel, B.Z., Clark, K.E., 1983. Bio-corrosion: solubilization and accumulation of
602 metals by fungi. *Water, Air, and Soil Pollution* 19, 229-236.

603 Smith, A.D., Logeman, B.L., Thiele, D.J., 2017. Copper acquisition and utilization in fungi. *Annual*
604 *Review of Microbiology* 71, 597-623.

605 Starkey, R.L., 1973. Effect of pH on toxicity of copper to *Scytalidium sp.*, a copper-tolerant fungus,
606 and some other fungi. *Microbiology* 78, 217-225.

607 Sterflinger, K., 2010. Fungi: their role in deterioration of cultural heritage. *Fungal Biology Reviews*
608 24, 47-55.

609 Sterflinger, K., Piñar, G., 2013. Microbial deterioration of cultural heritage and works of art-tilting
610 at windmills? *Applied Microbiology and Biotechnology*, 97, 9637-9646.

611 Suyamud, B., Ferrier, J., Csetenyi, L., Inthorn, D., Gadd, G.M., 2020. Biotransformation of struvite
612 by *Aspergillus niger*: phosphate release and magnesium biomineralization as glushinskite.
613 *Environmental Microbiology* 22, 1588-1602.

614 Turick, C.E., Berry, C.J., 2016. Review of concrete biodeterioration in relation to nuclear waste.
615 *Journal of Environmental Radioactivity* 151, 12-21.

616 Warscheid, T., Braams, J., 2000. Biodeterioration of stone: a review. *International*
617 *Biodeterioration and Biodegradation* 46, 343-368.

618 Wei, Z., Hillier, S., Gadd, G.M., 2012. Biotransformation of manganese oxides by fungi:
619 solubilization and production of manganese oxalate biominerals. *Environmental Microbiology* 14,
620 1744-1753.

621 Yang, Y., Ferrier, J., Csetenyi, L., Gadd, G.M., 2019. Direct and indirect bioleaching of cobalt from
622 low grade laterite and pyritic ores by *Aspergillus niger*. *Geomicrobiology Journal* 36, 940-949.

623 Yang, Y., Song, W., Ferrier, J., Liu, F., Csetenyi, L., Gadd, G.M. (2020). Biorecovery of cobalt and
624 nickel using biomass-free culture supernatants from *Aspergillus niger*. *Applied Microbiology and*
625 *Biotechnology* 104, 417–425.

626 Zelinka, S.L., Rammer, D.R., 2009. Corrosion rates of fasteners in treated wood exposed to 100%
627 relative humidity. *Journal of Materials in Civil Engineering* 21, 758-763.

628 Zelinka, S.L., Stone, D.S., 2011. The effect of tannins and pH on the corrosion of steel in wood
629 extracts. *Materials and Corrosion* 62, 739-744.

630 Zelinka, S.L., Jakes, J.E., Kirker, G.T., Passarini, L., Hunt, C.G., Lai, B., Antipova, O., Li, L., Vogt, S.,
631 2019a. Copper distribution and oxidation states near corroded fasteners in treated wood. *SN*
632 *Applied Sciences* 1, 1-10.

633 Zelinka, S.L., Jakes, J.E., Tang, J., Ohno, K., Bishell, A., Finney, L., Maxey, E.R., Vogt, S., Kirker, G.T.,
634 2019b. Fungal–copper interactions in wood examined with large field of view synchrotron-based
635 X-ray fluorescence microscopy. *Wood Material Science and Engineering* 14, 174-184.

636

637

638

639

641 Table 1. Growth rates (mm day⁻¹) of test fungi on copper metal amended malt extract agar
 642 (MEA), AP1 and MCD media. Results are shown as the growth rates on copper metal amended
 643 or unamended medium calculated by linear regression. Test fungi were grown at 25°C in the
 644 dark: values shown are averages from three measurements ± standard deviation. NH₄⁺-AP1
 645 and NO₃⁻-AP1 are AP1 medium containing ammonium or nitrate, respectively, as the N-source.

Organism	Media	Growth period (days)	Copper free control	Copper wire below membrane	Copper wire above membrane
<i>A. niger</i>	MEA	0-8	10.82 ± 0.73	10.99 ± 0.72	10.59 ± 0.61
	NH ₄ ⁺ -AP1	0-8	9.80 ± 0.84	9.70 ± 0.86	9.67 ± 0.77
	NO ₃ ⁻ -AP1	0-8	11.45 ± 1.10	10.85 ± 0.80	11.31 ± 0.91
<i>B. caledonica</i>	MEA	0-30	2.90 ± 0.17	2.33 ± 0.09	2.51 ± 0.59
	NO ₃ ⁻ -AP1	0-30	2.65 ± 0.11	2.67 ± 0.12	2.72 ± 0.15
	MCD	0-30	2.86 ± 0.13	2.79 ± 0.08	2.78 ± 0.12
<i>P. javanicus</i>	MEA	0-41	1.81 ± 0.02	1.88 ± 0.02	1.67 ± 0.02
	NO ₃ ⁻ -AP1	0-31	2.26 ± 0.09	2.53 ± 0.08	2.34 ± 0.09
	MCD	0-31	2.54 ± 0.06	2.43 ± 0.07	2.56 ± 0.04

646

647

648 Table 2. Tolerance indices (TI) for test fungi grown on copper metal amended medium (%). Values
 649 are percentages derived from the dry biomass yield of organisms grown on media amended with
 650 copper metal by comparison with the control. All test fungi were grown at 25°C in the dark.
 651 Values shown are averages from three measurements with standard deviations.

Organism	Media	Growth period (days)	Tolerance index (%)	
			Copper wire below membrane	Copper wire on membrane
<i>A. niger</i>	MEA	8	109.8 ± 0.1	97.7 ± 0.1
	NH ₄ ⁺ -AP1	8	106.0 ± 5.0	100.3 ± 0.7
	NO ₃ ⁻ -AP1	8	91.1 ± 7.0	98.2 ± 0.7
<i>B. caledonica</i>	MEA	30	96.6 ± 3.2	80.1 ± 5.3
	NO ₃ ⁻ -AP1	30	94.6 ± 0.2	92.7 ± 3.9
	MCD	30	63.9 ± 0.9	64.1 ± 0.6
<i>P. javanicus</i>	MEA	41	158.5 ± 22.6	151.2 ± 15
	NO ₃ ⁻ -AP1	31	71.2 ± 3.7	70.3 ± 5.0
	MCD	31	71.9 ± 5.4	75.9 ± 2.6

652

653 Table 3. Surface pH values of uninoculated agar and agar underneath fungal colonies on control
 654 and copper metal amended media. *A. niger* on MEA was grown for 21 days at 25°C in the dark.
 655 *A. niger* on AP1 medium was grown for 60 days at 25°C in the dark. Other fungi were grown for
 656 90 days at 25°C in the dark. Test fungi were grown on unamended media as negative controls;
 657 copper-amended media without test fungi were used as abiotic controls. Values shown are
 658 averages \pm standard deviations (n=6).

Organism	Media	pH values				
		Negative Control	Abiotic control (below membrane)	Copper below membrane	Abiotic control on membrane	Copper on membrane
<i>A. niger</i>	MEA	2.84 \pm 0.05	5.14 \pm 0.01	3.00 \pm 0.10	5.19 \pm 0.01	3.09 \pm 0.20
	NH ₄ ⁺ -AP1	1.96 \pm 0.02	5.20 \pm 0.01	2.90 \pm 0.23	4.95 \pm 0.07	2.18 \pm 0.05
	NO ₃ ⁻ -AP1	4.57 \pm 0.12	5.20 \pm 0.06	5.52 \pm 0.31	5.26 \pm 0.06	5.22 \pm 0.16
<i>B. caledonica</i>	MEA	8.48 \pm 0.05	5.34 \pm 0.01	7.73 \pm 0.01	5.28 \pm 0.22	7.63 \pm 0.09
	NO ₃ ⁻ -AP1	8.89 \pm 0.06	6.73 \pm 0.02	8.74 \pm 0.10	7.03 \pm 0.03	8.87 \pm 0.07
	MCD	8.75 \pm 0.08	6.20 \pm 0.01	8.22 \pm 0.01	6.45 \pm 0.07	8.26 \pm 0.07
<i>P. javanicus</i>	MEA	7.84 \pm 0.01	5.33 \pm 0.07	8.39 \pm 0.10	5.22 \pm 0.01	8.14 \pm 0.32
	NO ₃ ⁻ -AP1	8.95 \pm 0.04	6.73 \pm 0.02	8.41 \pm 0.91	7.03 \pm 0.03	8.80 \pm 0.07
	MCD	8.57 \pm 0.04	6.20 \pm 0.01	8.64 \pm 0.04	6.45 \pm 0.07	8.34 \pm 0.08

659

660

661 **Legends to Figures**

662

663 Fig. 1. Organic acid secretion by *A. niger* in the presence or absence of copper metal in (a-c) ME
664 or (d) NH₄⁺-AP1 ammonium salt medium liquid media. Cultures were shake incubated at 125
665 rpm for 21 days at 25°C. (●) copper free control; (○), 10 g·L⁻¹ copper; (□), 0.5 g·L⁻¹ copper.
666 Error bars are the standard deviations from at least 3 samples.

667 Fig. 2. Pieces of copper wire collected from ammonium salt-AP1 solid agar medium after 60 days
668 incubation at 25 °C in the dark (a) abiotic control and (b) growth of *A. niger*. Scale bars = 50 μm.
669 Typical images have been chosen from at least three examinations.

670 Fig. 3. Surface of copper sheet incubated on ammonium salt AP1 media inoculated (a, b) with or
671 (c) without *A. niger* for 90 days at 25°C in the dark. Scale bars: a, c = 10 μm, b = 100 μm. Scratches
672 were made in the copper sheet surface using 100-grit aluminium oxide abrasive paper to enhance
673 fungal adhesion. Typical images from several examinations are shown.

674 Fig. 4. SEM of copper wire pieces incubated with *A. niger* for 90 days at 25 °C in the dark (a,b)
675 between the MEA surface and overlying cellophane membrane, or (c,d) placed on the top of the
676 cellophane membrane. (e) abiotic control, copper metal between the membrane and agar (f)
677 abiotic control, control copper metal on top of the the membrane. Scale bars: a, c = 50 μm, e, f
678 = 100 μm. Fig. 4b and 4d are higher magnification images of the areas indicated by the inset
679 squares in Fig. 4a and 4c. Scale bars: b, d = 5 μm. Typical images are shown from several
680 examinations.

681 Fig. 5. Energy-dispersive X-ray analysis of copper metal wire after incubation with *A. niger* on
682 MEA after 90 days at 25 °C in the dark. (a) copper metal placed between the cellophane
683 membrane and agar (b) abiotic copper metal control (between membrane and agar). (c) copper
684 metal placed ontop of the membrane. (d) abiotic copper metal control (on the membrane).
685 Typical spectra are shown from one of at least three determinations.

686 Fig. 6. X-ray diffraction of minerals formed on copper metal in MEA media after incubation on
687 MEA with *A. niger* for 90 days at 25 °C in the dark. Typical patterns are shown from one of several
688 separate determinations.

689

690