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## Metabolic synergies in the biotransformation of organic and metallic toxic compounds by a saprotrophic soil fungus

Ceci, Andrea; Pinzari, Flavia; Riccardi, Carmela; Maggi, Oriana; Pierro, Lucia; Petrangeli Papini, Marco

*Published in:*  
Applied Microbiology and Biotechnology

*DOI:*  
[10.1007/s00253-017-8614-9](https://doi.org/10.1007/s00253-017-8614-9)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

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

### *Citation for published version (APA):*

Ceci, A., Pinzari, F., Riccardi, C., Maggi, O., Pierro, L., Petrangeli Papini, M., Gadd, G. M., & Persiani, A. M. (2018). Metabolic synergies in the biotransformation of organic and metallic toxic compounds by a saprotrophic soil fungus. *Applied Microbiology and Biotechnology*, 102(2), 1019-1033. Advance online publication. <https://doi.org/10.1007/s00253-017-8614-9>

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1 **Metabolic synergies in the biotransformation of organic and metallic toxic compounds by a**  
2 **saprotrophic soil fungus**

3

4 Andrea Ceci<sup>1</sup>, Flavia Pinzari<sup>2</sup>, Carmela Riccardi<sup>3</sup>, Oriana Maggi<sup>1</sup>, Lucia Pierro<sup>4</sup>, Marco Petrangeli Papini<sup>4</sup>,  
5 Geoffrey Michael Gadd<sup>5</sup>, Anna Maria Persiani<sup>1#</sup>

6

7 <sup>1</sup>Laboratorio Biodiversità dei Funghi, Dipartimento di Biologia Ambientale, Sapienza Università di Roma,  
8 Rome 00185, Italy;

9 <sup>2</sup>Consiglio per la Ricerca e l'analisi dell'Economia Agraria, Centro di ricerca per lo studio delle Relazioni  
10 tra Pianta e Suolo, Rome 00184, Italy;

11 <sup>3</sup>Settore Ricerca, Certificazione e Verifica, INAIL-DIT, Rome 00143, Italy;

12 <sup>4</sup>Laboratorio di impianti e processi industriali, Dipartimento di Chimica, Sapienza Università di Roma,  
13 Rome 00185, Italy;

14 <sup>5</sup>Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK

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16

17 #Address correspondence to A.M. Persiani [annamaria.persiani@uniroma1.it](mailto:annamaria.persiani@uniroma1.it)

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21 Running head: Fungal biotransformation of hexachlorocyclohexane and vanadium

22

23 **Abstract**

24 The saprotrophic fungus *Penicillium griseofulvum* was chosen as model organism to study responses to a  
25 mixture of hexachlorocyclohexane (HCH) isomers ( $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH,  $\delta$ -HCH) and of potentially  
26 toxic metals (vanadium, lead) in solid and liquid media. The *P. griseofulvum* FBL 500 strain was isolated  
27 from polluted soil containing high concentrations of HCH isomers and potentially toxic elements (Pb, V).  
28 Experiments were performed in order to analyse the tolerance/resistance of this fungus to xenobiotics, and  
29 to shed further light on fungal potential in inorganic and organic biotransformations. The aim was to  
30 examine the ecological and bioremedial potential of this fungus verifying the presence of mechanisms that  
31 allow it to transform HCH isomers and metals under different, extreme, test conditions. To our knowledge,  
32 this work is the first to provide evidence on the biotransformation of HCH mixtures, in combination with  
33 toxic metals, by a saprotrophic non-white-rot fungus and on the metabolic synergies involved.

34

35

36 **Keywords:** soil saprotrophic fungi, hexachlorocyclohexane, vanadium, biotransformation, metabolic  
37 phenotype, medium pH

38

## 39 **Introduction**

40 Hazardous persistent organic pollutants, e.g. pesticides, pharmaceuticals, explosives, and potentially toxic  
41 elements (PTEs), e.g. Pb, Cr, As, Sn, constantly enter ecosystems (waters, soils, and sediments) causing  
42 severe environmental and health problems ( Polti et al. 2014; Wan et al. 2015). Anthropogenic processes  
43 associated with domestic, municipal, agricultural, industrial, and military activities represent the major  
44 sources while PTEs can also be released from natural geological processes such as weathering and volcanic  
45 eruption (Vargas-García et al. 2012; Griffith et al. 2015). Pollution due to human activities is often the  
46 result of extensive histories of multiple land use which creates sites containing mixed pollutants. More than  
47 40% of the United States National Priority List sites are co-contaminated by organic (volatile and semi-  
48 volatile organic compounds) and inorganic pollutants (metals including radionuclides) while metals and  
49 mineral oil contribute jointly to around 60% of soil contamination and 53% of groundwater contamination  
50 in Europe ( Sandrin and Maier 2003; Panagos et al. 2013). The co-occurrence of organic and metal  
51 pollutants is not only a threat to human and ecosystem health, but is also a challenge because the  
52 technologies required for remediation of polluted sites are different for each group of pollutants (Sandrin  
53 and Maier 2003; Zhu et al. 2012). As more than one third of contaminated sites are polluted by more than  
54 one type of contaminant, it is imperative to develop cost-effective and sustainable techniques that can  
55 transform organic compounds while also extracting PTEs or stabilizing them in non-toxic forms (Polti et al.  
56 2014).

57 In recent years, several studies on microbial communities or on single microbes isolated from  
58 historically contaminated sites have shown their ability to tolerate, adapt and grow in the presence of  
59 organic compounds and PTEs (Alisi et al. 2009; Wasi et al. 2011). This suggests that bioremediation based  
60 on microbial activities is feasible for the recovery of such sites by transformation or immobilization of both  
61 organic compounds and PTEs (Zhu et al. 2012; Polti et al. 2014). Previous studies on microbial  
62 biotransformations of multiple contaminants have mainly concentrated on bacteria (Alisi et al. 2009; Wasi  
63 et al. 2011), but fungi can also represent ideal candidates for future challenges in complex multi-  
64 contaminated contexts. Fungi are ubiquitous chemoorganotrophic organisms, playing fundamental roles in  
65 ecological and geological processes (Gadd 2010; Gadd et al. 2012). As decomposers, pathogens, and  
66 symbionts (mycorrhizas, lichens), fungi provide fundamental ecological functions for ecosystems and  
67 human well-being (Mace et al. 2012; Lange et al. 2012). Fungi can transform a huge variety of organic  
68 substrates, including natural polymers such as cellulose, lignin, chitin and starch but also many  
69 anthropogenic products like pesticides, explosives and other xenobiotics (Gadd 2013; Harms et al. 2011).  
70 Due to their filamentous growth habit and ability to exude organic acids, protons and other metabolites,  
71 fungi are important biological weathering agents of rocks and mineral-based substrates (Gadd 2004; 2007).  
72 The potential of fungi to tolerate and transform both organic and inorganic pollutants has been highlighted  
73 in many studies which have also reported some unusual abilities shown by fungi isolated from  
74 contaminated soil (; Tigini et al. 2009; Ma et al. 2014; Mishra and Malik 2014). In fact, isolation of  
75 indigenous fungi could provide the best candidate organisms for bioremediation of polluted soil since they

76 already belong to an established soil microbial community, and are best adapted to the site conditions  
77 (Czaplicki et al. 2016).

78 In this research, we have examined tolerance to both organic and inorganic pollutants by a soil  
79 saprotrophic fungus, *Penicillium griseofulvum* Dierckx isolated from a historically polluted soil. The study  
80 area was the Italian National Site of Interest “Valle del Sacco” (Lazio, Italy) which is affected by high  
81 concentrations of multiple pollutants such as hexachlorocyclohexane, vanadium and lead (Ceci et al. 2012;  
82 Bernardini et al. 2016; Bernini et al. 2016). The aims of this research were to evaluate the  
83 tolerance/resistance of *P. griseofulvum* FBL 500 to different combinations of vanadium, lead, and isomers  
84 of HCH; to study the inorganic biotransformation in the presence of metals, and possible biomineralization  
85 phenomena; and to analyse biodegradation of an isomeric mixture of HCH, in the absence and in the  
86 presence of vanadium, and the impact of any synergistic effects on fungal metabolism.

## 87 **Materials and methods**

### 88 **Organism, media, and growth conditions**

89 A strain of *P. griseofulvum* Dierckx (FBL 500), obtained from the culture collection of the Fungal  
90 Biodiversity Laboratory (FBL) (Sapienza, University of Rome), was used in all the biotransformation tests.  
91 Czapek-Dox medium was used for all the experiments (; Ceci et al. 2015b, c). The strain is also preserved  
92 in the public mycological collection of Mycotheca Universitatis Taurinensis (MUT) as MUT 5854. The  
93 Czapek-Dox agar medium contained the following (g/l distilled water): NaNO<sub>3</sub>, 3; K<sub>2</sub>HPO<sub>4</sub> 1;  
94 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5; KCl, 0.5; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01. All chemicals were purchased from Merck (Darmstadt,  
95 Germany).

96 The experimental design was composed of three different experiments, in which (A)  
97 tolerance/resistance responses and biotransformation of metals (V, Pb), (B) of HCH mixture of isomers,  
98 and (C) of the synergic effects of combination of V and HCH isomers were respectively investigated (see  
99 Table 1, Online Resource). The whole phenotypic response of this fungus to the different treatments was  
100 examined using the Phenotype MicroArray™ system (Pinzari et al. 2016). Sucrose and D-glucose (Difco,  
101 Sparks, MD, USA) were used as substrates for fungal growth (Table 1, Online Resource). The glucose  
102 concentration was 30 g/l in experiment A, in which *P. griseofulvum* FBL 500 was tested with metals (V and  
103 Pb compounds): this concentration was used following the same cultural conditions as in Ceci et al. (2015a,  
104 c) (Table 1, Online Resource). 5 g/l sucrose was used in experiment B to stimulate the biotransformation of  
105 HCH isomers as used in previous tests of biotransformation of β-HCH by *P. griseofulvum* FBL 500 (Ceci et  
106 al. 2015b) (Table 1, Online Resource). The same concentration was maintained in experiment C to study  
107 synergic interactions between the fungus and different combinations of V and HCH. In experiment A, prior  
108 to autoclaving, the medium pH was adjusted to 5.5 using concentrated HCl (Ceci et al. 2015a, c), while in  
109 experiments B and C, the pH was kept at 7 in order to prevent acidic variations of the medium pH, which  
110 could result in toxicity to *P. griseofulvum* FBL 500 during HCH biotransformation because of benzoate  
111 formation (Guillén-Jiménez et al. 2012; Ceci et al. 2015b) (Table 1, Online Resource).

112 In experiments A and C, prior to inoculation, 84 mm diameter discs of sterile cellophane  
113 membrane (Focus Packaging and Design Ltd, Louth, UK) sterilized by autoclaving in distilled water and  
114 were placed aseptically on the surface of the agar in each Petri dish (Ceci et al. 2015c). Growth of *P.*  
115 *griseofulvum* FBL 500 was evaluated by measuring diametric extension of the colony and by biomass yield  
116 since extension of the colony alone does not take into account the density of fungal mycelium ( Ceci et al.  
117 2015c). After 12 days, fungal colonies were removed from the agar by peeling the biomass from the  
118 dialysis membranes using a sterile razor blade. Mycelia were oven-dried at 100°C until reaching constant  
119 weight for at least 2 days. Results were expressed in terms of a tolerance index (TI) as reported in Ceci et  
120 al. (2015c). After the dialysis membrane and mycelium were removed, the surface pH of the agar was  
121 measured at specific intervals across the diameter of the Petri dish using a conical tip FC 202D pH  
122 electrode (Hanna Instruments, Woonsocket, RI, USA) and a pH portable meter, HI 99161 (Hanna  
123 Instruments, Woonsocket, RI, USA).

124

125 **Genetic identification of *P. griseofulvum* FBL 500**

126 *P. griseofulvum* FBL 500 was isolated and previously identified through conventional taxonomic keys on  
127 the basis of macro- and microscopic features. ITS sequence analysis was carried out in order to confirm the  
128 previous taxonomical identification findings. *P. griseofulvum* FBL 500 was inoculated by transferring  
129 mycelial portions with a flamed glass rod from the actively growing periphery of stock colonies grown in  
130 solid Czapek-Dox medium. After 15 days, fungal colonies were removed from the agar by peeling the  
131 biomass from the dialysis membranes using a sterile razor blade. Mycelia were freeze-dried, pulverized by  
132 using liquid N<sub>2</sub> by using mortar and with the addition of polyvinylpyrrolidone to protect DNA. DNA  
133 purification and extraction was carried out from single replicates following the standard cetyltrimethyl  
134 ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Identification of *P. griseofulvum* FBL 500  
135 was achieved after extraction of fungal DNA and ITS sequence analysis using ITS 1F (5'-  
136 CTTGGTCATTTAGAGGAAGTAA-3') and ITS 4 (5' -TCCTCCGCTTATTGATATGC-3') primers for  
137 polymerase chain reaction (PCR) analysis (Bellemain et al. 2010). DNA was quantified by using Nanodrop.  
138 The PCR amplification was performed in a final volume of 25 µl using: 2.5 µl of 10× buffer, 2.5 mM  
139 MgCl<sub>2</sub>, 0.2 µM of each primer, 0.2 mM of dNTPs, 1.5 u. of DNA Taq polymerase (Promega, Milan, IT)  
140 and 5 ng DNA. PCR was conducted using 35 cycles of the following reaction conditions: initial  
141 denaturation at 95°C for 4 min, 39 cycles of three-step cycling (denaturation at 92°C for 50 s, primer  
142 annealing at 55°C for 50 s and extension at 72°C for 50 s) and final extension at 72°C for 10 min. The PCR  
143 products were sent to Macrogen Europe (Amsterdam, Netherland) for purification and sequencing.  
144 Sequences were edited using the software CHROMAS 2.33 (Technelysium Pty Ltd, Australia). The partial  
145 sequence of 18S ribosomal RNA gene, the complete sequence of the internal transcribed spacer 1, the  
146 complete sequence of the 5.8S ribosomal RNA gene, the complete sequence of the internal transcribed  
147 spacer 2 along and the partial sequence of the 28S ribosomal RNA gene were pairwise compared with those  
148 available in the public online databases International Nucleotide Sequence Databases using the BLAST  
149 search program (Altschul et al. 1997) and UNITE database (Köljalg et al. 2005; Abarenkov et al. 2010).  
150 The genetic sequence for *P. griseofulvum* FBL 500 was deposited in GenBank with the accession number  
151 KY560469.

152

153 **Experiment A. Metal-amended plates and inoculation**

154 Stock solutions of vanadium pentoxide, V<sub>2</sub>O<sub>5</sub> (Riedel-deHaën, Seelze, Germany), ammonium  
155 metavanadate, NH<sub>4</sub>VO<sub>3</sub> (Merck, Darmstadt, Germany), and lead carbonate, PbCO<sub>3</sub> (GPR), were prepared  
156 from oven-sterilized aliquots (48 h, 100°C). Growth experiments at different concentrations of ammonium  
157 metavanadate (2.5 and 5 mM) were performed. In addition, combinations of insoluble 2.5 mM lead  
158 carbonate with insoluble 1.25 mM vanadium(V) oxide or with slightly soluble 2.5 mM ammonium  
159 metavanadate were examined to study the toxic effects of both substances on *P. griseofulvum* FBL 500.  
160 These concentrations take account of the range of concentrations of these metals found in soils and volcanic

161 rocks at the same site. The fungus was isolated where vanadium concentrations were over the range 3–6  
162 mM, while lead concentrations were <1 mM (data not shown). Observations of colonies and media were  
163 performed using light- and stereo-microscopy to monitor growth, sporulation, pigment production and  
164 secondary mineral precipitation.

165

#### 166 **Experiment B. Batch experiments on HCH biodegradation by *P. griseofulvum* FBL 500**

167 High purity mixture of hexachlorocyclohexane isomers ( $\alpha$ : $\beta$ : $\gamma$ : $\delta$ =1:1:1:1) was acquired from Sigma-Aldrich  
168 (Seelze, Germany). Ethyl acetate, acetone and n-hexane were all purchased from ROMIL Ltd (Cambridge,  
169 UK) with chemical purity >99.9%. The internal standard  $\gamma$ -HCH-d<sub>6</sub> was obtained from CDN Isotopes  
170 (Pointe-Claire, Quebec, Canada) and stored at 4°C until use. The tests were carried out at 25°C under  
171 shaking conditions at 110 rpm, and with the addition of a mixture of  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, and  $\delta$ -HCH  
172 isomers (1:1:1:1) to a final concentration of 4 mg/l. Prior to HCH addition, the fungus was grown for 26  
173 days, and the concentration of all isomers and formation of fungal metabolites were monitored at regular  
174 intervals over 23 days. Uninoculated sterile flasks with HCH mixture and flasks with fungal culture but  
175 without HCH mixture were used as chemical and biological controls. Growth of *P. griseofulvum* FBL 500  
176 and tolerance to the HCH mixture were evaluated by biomass yield. Collected mycelial pellets were oven-  
177 dried at 100°C for at least 2 d, until reaching constant weight and fungal tolerance was evaluated using a  
178 tolerance index (TI), based on dry weights as described above.

179

#### 180 **Experiment C. Batch experiments on HCH and vanadium biotransformation by *P. griseofulvum* FBL** 181 **500**

182 Tests with at least three replicates were carried out at 25°C in the dark with different combinations of  
183 vanadium and HCH isomeric mixture. HCH was directly added in each Petri dish to the final concentration,  
184 while Czapek-Dox medium was at a temperature between 40 to 50°C to homogenize HCH in the agar and  
185 to avoid HCH volatilization. Growth experiments with insoluble 2.5 mM vanadium(V) pentoxide, and with  
186 4 mg/l and 50 mg/l HCH mixture were performed. In addition, a combination of insoluble 2.5 mM  
187 vanadium(V) pentoxide and 4 mg/l HCH mixture was used to study any synergic effects on *P. griseofulvum*  
188 FBL 500. The production of fungal metabolites was monitored in biomass and in agar for 20 d at regular  
189 intervals. Data of fungal growth (diameter, dry weight) and pH were collected, and tolerance indices were  
190 calculated as described above.

191

#### 192 **Chemical analysis**

193 In experiment B, 5 ml samples were collected from the culture flasks: 1 ml aliquots were spiked with  $\gamma$ -  
194 HCH-d<sub>6</sub> as an internal standard and extracted using solid phase extraction (SPE) cartridges packed with  
195 Graphitized Carbon Black (GCB — Carbograph, Rome, Italy). HCHs were retained on the solid phase and  
196 eluted with 10 ml ethyl acetate through a vacuum manifold (Grayledge Pump & Industrial, LLC, Pelham,  
197 NE). In experiment C, the agar and the membranes were collected and analysed to measure HCH



198 concentration and metabolites. Specimens were posed in glass tubes with 15 mL ethyl acetate and sonicated  
199 for 30 min. After centrifugation at 2000 rpm, ethyl acetate solutions were recovered in vials and 10 ml were  
200 analyzed.

201 Residual HCH for each isomer in the culture medium was calculated using the formula reported in  
202 Salam and Das (2014) and in Ceci et al. (2015b).

203 In order to study the uptake of each HCH isomer and fungal transformation, mycelial biomass was  
204 collected in all experiments and extracted with Dionex ASE200 Accelerated Solvent Extractor (Dionex,  
205 Sunnyvale, USA) after  $\gamma$ -HCH-d<sub>6</sub> addition as an internal standard. Operating conditions are reported in  
206 Ceci et al. (2015b). The concentration of isomers and formation of metabolites were analyzed by gas  
207 chromatography-mass spectrometry (GC-MS). HCH isomers and the possible metabolites were analyzed  
208 using a Hewlett-Packard 6890 gas chromatograph with a 5973A mass selective detector (Agilent  
209 Technologies, Palo Alto, California, USA). GC-MS analyses of liquid media were performed to detect any  
210 possible intermediate metabolites of dechlorination of HCH (e.g. pentachlorocyclohexene,  
211 tetrachlorocyclohexene), or intermediates of HCH reductive dechlorination and hydroxylation as in other  
212 studies with bacteria and fungi (Phillips et al. 2005; Guillén-Jiménez et al. 2012).

213

#### 214 **Analysis of fungal metabolic profile in the presence of HCH mixture and vanadium using Biolog FF** 215 **microplates**

216 The Phenotype MicroArray™ system (Pinzari et al. 2016) was used to gather information on the whole  
217 phenotype of the *P. griseofulvum* FBL 500 strain and on the effects of organic and inorganic toxic  
218 compounds on its carbon metabolism. The method we used was based on the inoculation of a fungal spore  
219 suspension in FF MicroPlates (Biolog™, Inc., Hayward, California, USA) (Bochner et al. 2001; Pinzari et al.  
220 2016). A combined inoculum of the fungus with: a) 4 mg/l HCH mixture in toluene, or; b) 2.5 mM V<sub>2</sub>O<sub>5</sub>, or  
221 c) with the combination of 4 mg/l HCH mixture and 2.5 mM V<sub>2</sub>O<sub>5</sub> were performed in FF MicroPlate™  
222 arrays in triplicate. The inoculation procedure for pure cultures of *P. griseofulvum* FBL 500 in the arrays  
223 was based on the protocol used by Tanzer et al. (2003).

224 Conidia of the fungus were obtained by cultivation of the pure strain on 2% MEA plates in the  
225 dark at 25°C for 7 d. Operating conditions are reported in Ceci et al. 2015b. The optical density of Biolog  
226 plates was read using a microplate reader (Molecular device, Vmax) at 490 nm (OD<sub>490</sub>), which was used to  
227 measure the intensity of the purple colour resulting from the reduction of the tetrazolium redox dye (*p*-  
228 iodonitrotetrazolium), present in the wells of the FF plates, through the action of fungal succinate  
229 dehydrogenase as a proxy for respiratory activity. Moreover, optical density at 750 nm (OD<sub>750</sub>) was used to  
230 assess fungal biomass and mycelial growth (Tanzer et al. 2003; Ceci et al. 2015b). Immediately after  
231 inoculation, OD<sub>490</sub> and OD<sub>750</sub> were measured in order to zero the spectrophotometer specifically for each  
232 Biolog plate. Plates were then read at intervals of 24, 48, 72, 96, 168, 192 and 240 h of incubation (Tanzer  
233 et al. 2003; Ceci et al. 2015b). In order to evaluate possible redox effects of vanadium pentoxide on the  
234 tetrazolium dye, two sets of microplates with V<sub>2</sub>O<sub>5</sub> and V<sub>2</sub>O<sub>5</sub> with HCH mixture were prepared without the

235 fungus inoculum. Data obtained from the Phenotype MicroArray™ assays were used to compare the three  
236 growth conditions to evaluate the overall differences in metabolism (co-metabolism, inhibition, synergic  
237 effects) by studying the utilization of different substrates in the absence or presence of the xenobiotic and  
238 PTEs (vanadium and HCH) and their combinations.

239

#### 240 **Statistical analysis**

241 R elaboration and programming software, version 3.3.2 (The R Foundation for Statistical Computing,  
242 Vienna, Austria) and the statistical package XLStat (Addinsoft 2007-Pro, Paris, France) were used to  
243 perform statistical analyses (Fahmy and Aubry 2003). They were used to perform one-way ANOVA tests  
244 on means for dry weight, diametric growth, surface pH, and HCH concentrations (at least three replicate  
245 determinations were used). One-way ANOVA tests on means were performed for the OD<sub>490</sub> and OD<sub>750</sub>  
246 values for all different treatments at 168 h, when a plateau was reached in the metabolic curves.

247 The Phenotype MicroArray data were further analysed using the opm R package (Vaas et al. 2013). All the  
248 OD values were combined in a dataset which comprised three replicates × 96 substrates × four treatments  
249 (control, HCH mixture in toluene, vanadium, combination of vanadium and HCH mixture in toluene) × two  
250 metabolic parameters (respiration and mycelial growth), giving rise to 2304 individual phenotypic curves.  
251 Comparison of substrate utilization in the different treatments was carried out using the estimated curve  
252 parameter of A — maximum height of the metabolic curve — calculated with the opm package and plotted  
253 as confidence-interval plots and heatmaps (Vaas et al. 2012). The confidence intervals and the ANOVA  
254 were performed on the optical density measurements for 168 h in FF microplates.

255

256 **Results**

257 **Genetic identification of *P. griseofulvum* FBL 500**

258 Sequence analysis using the internal transcribed spacer (ITS) regions (ITS1F and ITS4 primers) confirmed  
259 the identification of *P. griseofulvum* FBL 500 through conventional taxonomic keys. A 100% sequence  
260 identity over the BLAST alignment was obtained with the name of the reference sequence being *P.*  
261 *griseofulvum* SH207147.07FU and KJ467353 for UNITE and NCBI databases, respectively.

262

263 **Experiment A. Fungal interactions with metals**

264 The presence of vanadium and lead compounds did not inhibit colony expansion of *P. griseofulvum* FBL  
265 500 under all test conditions. In fact, all the calculated tolerance indices were greater than 1 and colony  
266 extension rates in the presence of metal ( $R_i$ ) showed higher values than the control extension rate ( $R_c$ ),  
267 which was statistically significant in all tests ( $P < 0.01$ ) (Table 2, Online Resource). Multiple comparisons  
268 using the Tukey test showed that fungal extension in all tests was significantly higher than the control ( $P <$   
269  $0.01$ ). Tolerance indices (TI) were used to compare biomass yields of control and test conditions (Table 3,  
270 Online Resource). Biomass yields were stimulated (TI > 100%) by the lead and vanadium compounds. The  
271 highest TI value occurred with 2.5 mM  $\text{NH}_4\text{VO}_3$  (TI = 231.49%). Multiple comparisons using the Tukey  
272 test revealed that biomass production in all tests was significantly higher than the control ( $P < 0.01$ ). Table  
273 4 (Online Resource) shows the differences ( $\Delta\text{pH}$ ) between average surface pH values of uninoculated agar  
274 and agar underneath fungal colonies of *P. griseofulvum* FBL 500, growing on Czapek-Dox medium in the  
275 different treatment conditions. In uninoculated agar of all experimental conditions, the range of pH medium  
276 was 5–5.3. In contrast, for test conditions, the medium pH decreased after growth for 12 days (pH =4–4.3)  
277 with the exceptions of those with combinations of vanadium and lead compounds where the pH values  
278 were similar to the uninoculated controls. In the latter, the buffering effect of carbonate might have  
279 neutralized fungal acidification of the media (Table 4, Online Resource). Sporulation in tests was limited to  
280 the central part of the colonies, occasionally green coloured, and the production of yellow-orange pigments  
281 and exudates was observed to be higher in treatments than in controls. No secondary mineral precipitation  
282 was observed over 12 days. In contrast, the complete dissolution of insoluble crystals of lead carbonate and  
283 vanadium pentoxide by *P. griseofulvum* FBL 500 occurred. Notably, few crystals were observed  
284 underneath growing fungal colonies in media amended with combinations of ammonium metavanadate and  
285 lead carbonate after 3 months incubation of *P. griseofulvum* FBL 500 (Fig. 1). The crystals were red,  
286 acicular and tapered, and morphologically resembled lead oxalate. However it was not possible to extract  
287 them for further identification because of their limited quantity.

288

289 **Experiment B. Fungal interactions with HCH mixture of isomers in liquid Czapek-Dox medium**

290 Addition of 4 mg/l isomeric HCH mixture to liquid Czapek-Dox medium had no obvious adverse effects on  
291 the growth of *P. griseofulvum* FBL 500. Biomass yield was not strongly reduced (TI > 50%) by the  
292 presence of the HCH mixture, and an average TI value of 87.2% was obtained. Results from the time-

293 dependent studies of HCH concentration in liquid batch tests are shown in Fig. 2. No measurable changes  
294 in isomer concentration were detected in the abiotic controls throughout the experiments. The HCH mixture  
295 was added 26 d after fungal inoculation, and the isomer concentrations were monitored for 23 d. Up to the  
296 third day, the isomer concentration increased and reached a maximum concentration for all the isomers.  
297 This phase was followed by a reduction of  $\alpha$ -HCH,  $\beta$ -HCH, and  $\gamma$ -HCH in the medium with residual  $\alpha$ -  
298 HCH = 63.0%, residual  $\beta$ -HCH = 67.1%, and residual  $\gamma$ -HCH = 63.5% (Fig. 2). In contrast, the  $\delta$ -HCH  
299 concentration appeared to be stable and close to the initial concentration of ~1 mg/l. According to ANOVA,  
300 there was a significant difference ( $P < 0.01$ ) between the means of the  $\gamma$ -HCH concentrations measured  
301 during the second and third day. There was a significant difference ( $P < 0.05$ ) between the means of  $\alpha$ -HCH  
302 and the  $\beta$ -HCH concentrations measured over the same days. At the end of the experiment, the  
303 concentrations of  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH and  $\delta$ -HCH in the solution were respectively  $0.56 \pm 0.02$  mg/l,  
304  $0.76 \pm 0.04$  mg/l,  $0.48 \pm 0.02$  mg/l and  $0.92 \pm 0.02$  mg/l with a resulting substantial deficit with regard to  
305 the initial value of 1 mg/l for each isomer.

306

### 307 **Experiment C. Fungal interactions with HCH mixture of isomers, vanadium and combinations of V** 308 **and HCH in solid Czapek-Dox medium**

309 The presence of 4 mg/l HCH mixture in Czapek-Dox agar medium had no apparent adverse effects on the  
310 growth of *P. griseofulvum* FBL 500. The fungus showed a slight reduction of the extension rate in  
311 comparison to the control (significant at  $P < 0.01$ ) (Table 2, Online Resource). Biomass yields were not  
312 reduced (TI > 70%) by the HCH mixture (Table 3, Online Resource). The TI value was 82.8%, and the  
313 difference in biomass yields was statistically significant ( $P < 0.01$ ). Table 4 (Online Resource) shows the  
314 differences ( $\Delta$ pH) between average surface pH values of uninoculated agar and agar underneath fungal  
315 colonies of *P. griseofulvum* FBL 500, growing on Czapek-Dox medium in the different treatment  
316 conditions. The  $\Delta$ pH revealed a low medium acidification (Table 4, Online Resource). After growth of *P.*  
317 *griseofulvum* FBL 500, the inoculated medium pH measured in the test condition ( $6.67 \pm 0.19$ ) was slightly  
318 higher than the one of the control ( $6.31 \pm 0.19$ ) and statistically significant ( $P < 0.01$ ).

319 50 mg/l HCH mixture had a toxic effect on growth of *P. griseofulvum* FBL 500 with the extension  
320 rate being significantly reduced (Table 2, Online Resource). Biomass yields were strongly reduced (TI <  
321 25%) (Table 3, Online Resource). The TI value was 23.8%, and the difference in biomass yields was  
322 statistically significant ( $P < 0.01$ ). After growth of *P. griseofulvum* FBL 500, the average pH value of  
323 inoculated medium pH measured in the test ( $6.73 \pm 0.11$ ) was slightly lower than the one of the control  
324 ( $6.31 \pm 0.19$ ) and statistically significant ( $P < 0.01$ ).

325 The addition of 2.5 mM  $V_2O_5$  to Czapek-Dox did not result in adverse effects on the fungal  
326 extension rate in comparison to the control, although the differences were not statistically significant (Table  
327 2, Online Resource). Biomass yields were stimulated (TI > 100%) by the presence of vanadium pentoxide  
328 (Table 3, Online Resource). The TI value was 123.7%, although the difference in biomass yield was not  
329 statistically significant. After growth of *P. griseofulvum* FBL 500, the average pH value of inoculated

330 medium pH in the test ( $5.98 \pm 0.41$ ) was slightly lower than the one of the control ( $6.31 \pm 0.19$ ) and  
331 statistically significant ( $P < 0.01$ );  $\Delta$ pH was slightly negative (Table 4, Online Resource).

332 The combination of V and HCH mixture in Czapek-Dox agar showed a slight reduction of the  
333 extension rate in comparison to the control, although differences were not significantly different (Table 2,  
334 Online Resource). Biomass yields were not reduced (TI > 70%) by the HCH mixture (Table 3, Online  
335 Resource). The TI value was 88.1%, although the difference in biomass yields was not statistically  
336 significant. The average pH value in the test ( $6.24 \pm 0.26$ ) was slightly lower than the control ( $6.31 \pm 0.19$ ),  
337 although it was not statistically significant.  $\Delta$ pH was slightly negative (Table 4, Online Resource).

338

### 339 **Fungal metabolites from HCH biotransformation**

340 Different metabolic intermediates were observed in experiments B and C (Fig. 3) and different isomers of  
341 pentachlorocyclohexene (PCCH) tetrachlorocyclohexene (TCCH), trichlorobenzene (TCB),  
342 dichlorobenzene (DCB) and chlorobenzene (CB) were detected. In experiment B, only PCCH was always  
343 detected during all the monitoring period in the solid Czapek-Dox medium experiments, while no  
344 metabolites were found in liquid Czapek-Dox medium. In experiment C, after 12 d fungal growth, PCCH  
345 was found in all tests in the presence of HCH mixture, i.e. 4 or 50 mg/l HCH mixture and the combination  
346 of HCH mixture and vanadium. TCB was found only in the combination of HCH and vanadium and in the  
347 experiment with 50 mg/l HCH mixture. In the latter, all metabolites (PCCH, TCCH, TCB, DCB, CB) were  
348 detected. Moreover, the benzoates, benzaldehyde and benzyl alcohol, were also detected during the  
349 experiments.

350

### 351 **Analysis of fungal metabolic profile in the presence of HCH mixture and vanadium using Biolog FF** 352 **microplates**

353 The Phenotype MicroArray™ system (Biolog Inc., Hayward, CA, USA) was used to investigate the whole  
354 phenotype and nutrient utilization by *P. griseofulvum* FBL 500 in both control and test conditions. *P.*  
355 *griseofulvum* was able to grow in the presence of 71 substrates out of the 96 available in the FF plates  
356 (Pinzari et al. 2016). A heatmap of A values — the maximum height of the growth curve — for all  
357 treatments and all substrates at 750 nm is presented in Fig. 4. The clustergram above the heatmap shows  
358 clusters of substrates that refer to the different intensities of fungal metabolism according to the A values  
359 measured. The clustergram on the left side shows clusters of treatments in which vanadium and  
360 combinations of vanadium and HCH are together, as well as the control and HCH mixture, resulting in a  
361 different general response of fungal metabolism to the presence of the xenobiotics. The confidence intervals  
362 and the ANOVA were performed on the optical density measurements at 750 nm for the estimation of  
363 mycelial growth and at 490 nm for the estimation of respiration after incubation for 168 h in FF microplates  
364 in the different treatment conditions — control (no xenobiotics, only fungus), 2.5 mM vanadium oxide, 4  
365 mg/l HCH mixture and combination of both xenobiotics. Significant differences were detected according to  
366 specific growth substrates. *P. griseofulvum* FBL 500 was able to use the same substrates in the treatments

367 as in control conditions, but the presence of xenobiotics influenced fungal metabolism, reducing or  
368 increasing respiration rates and/or mycelial growth. The negative effect on respiration and fungal growth  
369 was significant ( $P < 0.05$ ) in the presence of vanadium or with a combination of vanadium and HCH for  
370 some substrates. In particular, there was a statistically significant reduction of fungal metabolism for D-  
371 ribose (pentoses), rhamnose (hexoses), D-gluconic acid (sugar acids), *N*-acetyl-D-glucosamine  
372 (hexosamines), maltose, maltotriose, D-melibiose, D-raffinose (oligosaccharides), all considered glucosides  
373 with the exception of arbutin, D-salicin and sucrose, all nitrogen-containing compounds and all the  
374 biochemical group “other” with the exception of L-asparagine, L-phenylalanine, L-pyroglutamic acid, L-  
375 threonine, ethanolamine, malic acid, and sebacic acid (Fig. 5). For all other substrates (36 — 50 %) used by  
376 *P. griseofulvum* FBL 500, there was no significant difference ( $P > 0.05$ ) in respiration and growth between  
377 control and test conditions. It is worth mentioning that for several substrates, HCH and V (alone or in  
378 combination) affected fungal metabolism as revealed by calculated confidence intervals and the absorbance  
379 curves for respiration and growth. In particular, for L-sorbose, D-mannitol, L-asparagine, i-erythritol and L-  
380 threonine, vanadium influenced fungal metabolism, increasing respiration and growth compared to the  
381 control (Fig. 5). In contrast, for D-mannitol, D-cellobiose, D-sorbitol and D-glucosamine, the presence of  
382 HCH inhibited fungal metabolism (Fig. 5). For ethanolamine, L-phenylalanine and D-salicin, HCH  
383 increased respiration and mycelial growth (Fig. 5). For sebacic acid, the presence of HCH and the  
384 combination of HCH and V resulted in metabolic stimulation (Fig. 5).

385 **Discussion**

386 This work examines the potential of *P. griseofulvum* FBL 500, which was isolated from polluted sites, to  
387 tolerate high concentrations of the potentially toxic metals, vanadium and lead, and hexachlorocyclohexane,  
388 and mediate their biotransformation.

389 Hexachlorocyclohexane (HCH) is a persistent organic pollutant (POP) of global concern with  
390 potentially toxic effects on humans and ecosystems. It is a halogenated xenobiotic which has been reported  
391 to be carcinogenic and an endocrine disrupter for humans and other organisms (; Ceci et al. 2015b). Three  
392 isomers of hexachlorocyclohexane,  $\alpha$ -HCH,  $\beta$ -HCH and  $\gamma$ -HCH, were included as persistent organic  
393 pollutants in the 2008 Stockholm Convention because of their worldwide spread and environmental  
394 persistence (Vijgen et al. 2011).

395 Vanadium is considered to be the one of the most abundant elements and one of the most  
396 important metals in modern technology (Rehder 2008; Ceci et al. 2015c). Vanadium is also essential for  
397 certain organisms (e.g. some algae, bacteria, fungi and lichens) as a cofactor of enzymes and a constituent  
398 of metabolites (haloperoxidases, nitrogenases and amavadin) (Crans et al. 2004). In recent decades,  
399 hydrocarbon fuel combustion, industrial activities and mining have increased the vanadium concentration in  
400 the environment, raising concern over its spread and toxicity for humans and ecosystems (Rehder 2008;  
401 Ceci et al. 2015c).

402 *P. griseofulvum* has been found to successfully tolerate and accumulate potentially toxic metals  
403 such as Cu and Cr (Shah et al. 1999; Shi et al. 2011; Abigail et al. 2015), to tolerate and mediate the  
404 biotransformation of Cu-based wood preservatives (Bridbžiuvienė and Levinskaite 2007) and Ni and V  
405 porphyrins (Cordero et al. 2015). Moreover, this fungus was reported to tolerate high concentrations of  
406 pyrene and mediate its biotransformation (Ravelet et al. 2000).

407 In experiment A, vanadium and lead compounds ( $\text{NH}_4\text{VO}_3$ ,  $\text{V}_2\text{O}_5$ ,  $\text{PbCO}_3$ ) were used.  
408 Vanadium(V) oxide and ammonium metavanadate are important products of industrial metal recovery  
409 (Teng et al. 2006). Vanadium(V) oxide is often found in leachates from mining and milling activities that  
410 account for the most significant fluxes of vanadium in the environment and can also originate from fossil  
411 fuel combustion as an insoluble by-product (Teng et al. 2006). Lead carbonate (cerussite), a common  
412 insoluble lead mineral in soil, has been used in metal tolerance tests to evaluate possible combined effects  
413 of the two metals on *P. griseofulvum* FBL 500 and to evaluate possible metal biotransformations mediated  
414 by the fungus (Ceci et al. 2015c). *Aspergillus niger* has been tested with the same metal compounds in  
415 similar research on vanadium geomycology (Ceci et al. 2015c). In this work, *P. griseofulvum* FBL 500  
416 showed good growth in experiment A (Table 2 and 3, Online Resource). The presence of ammonium added  
417 in the test medium could explain the higher growth rates and biomass yields, being used as an additional N  
418 source. However, ammonium was not present with the combination of lead carbonate with vanadium(V)  
419 oxide where the extension rate and biomass yield were also significantly higher than the control. Moreover,  
420 in the presence of vanadium(V) oxide and sucrose in experiment C, the TI was particularly high. Higher  
421 tolerance indices than the control may be related to metabolism-dependent or -independent mechanisms of

422 tolerance/resistance, implemented to cope with the stress due to metal toxicity (Gadd 1993; 2007; Gadd et  
423 al. 2012). The presence of toxic metals can strongly influence the physiology and morphology of fungal  
424 mycelia and the resulting interactions can include acidolysis, complexolysis, redoxolysis, metal  
425 accumulation, production of high local concentrations of extracellular enzymes, and other metabolites (e.g.  
426 organic acids, siderophores, polyphenolic compounds and pigments), mycelial growth strategies (e.g.  
427 explorative growth) and hyphal aggregation (e.g. phalanx growth) (; Gadd et al. , 2014; Ceci et al. 2015a,  
428 c). The excretion of organic acids (e.g., oxalic, citric, gluconic, and lactic acid) can be strongly influenced  
429 by growth conditions such as the presence of toxic metal minerals, nutrient availability, the C and N  
430 sources, pH and buffering capacity of the medium (Gadd et al. 2012; Ceci et al. 2015a, c). *P. griseofulvum*  
431 FBL 500 was able to acidify the medium beneath the colonies either from the initial value of pH 5.5 or  
432 from an initial value of pH ~7 (Table 4, Online Resource). In experiment A, the final pH values in all test  
433 conditions were all acidic (pH 4–5), with the only exception being the treatments with the combinations of  
434 Pb and V compounds. These were close to the uninoculated control and this is probably related to the  
435 buffering effect of carbonate (Table 4, Online Resource). Generally, fungi lower the pH of their medium  
436 during growth. Mechanisms such as the excretion of protons via the plasma membrane ATPase, the uptake  
437 of essential cation nutrients in exchange for protons, the release of organic acids and acidification due to  
438 fungal respiration can all cause acidification (Ceci et al. 2015c). On the other hand, in the presence of  
439 specific metal compounds such as carbonates or apatites, buffering effects can be evident (Ceci et al. 2015a,  
440 c). In this work, the formation of new biominerals, which are assumed to be lead oxalate, is evidence of  
441 metal biotransformation. Similar results were observed with *A. niger* in the presence of V and Pb  
442 compounds, vanadinite and mimetite (Ceci et al. 2015a, c). The organic acids produced by *P. griseofulvum*  
443 FBL 500 can provide ligands for metal-complex formation, electrons for metal redox reactions and metal  
444 precipitation as new mycogenic minerals, that can result in mobilization or immobilization of lead and  
445 vanadium (Ceci et al. 2015a, c). In experiment C, the medium pH values were close to the control (Table 4,  
446 Online Resource). The fungal growth in such conditions did not significantly change the pH of media  
447 (Table 4, Online Resource), and a pH range close to neutrality could avoid some possible toxic effects in  
448 acidic conditions due to the production of benzoates during HCH biodegradation. The benzoates detected  
449 during these experiments were previously observed with *P. griseofulvum* FBL 500 in the presence of  $\beta$ -  
450 HCH (Ceci et al. 2015b).

451 In experiments B and C, *P. griseofulvum* FBL 500 was able to grow and tolerate high  
452 concentrations of a mixture of HCH isomers. The tolerance of this fungus was tested in previous work at a  
453 combination of 1 mg/l  $\beta$ -HCH (Ceci et al. 2015b). The presence of a 4 mg/l HCH mixture did not inhibit  
454 fungal growth, and similar TI values were found for liquid and solid Czapek-Dox media. Similar results  
455 were observed in previous work (Ceci et al. 2015b) confirming that the presence of different HCH isomers  
456 had no obvious synergic effects on growth. In liquid medium, the biotransformation of the isomers was  
457 observed to be different and isomer-specific, with  $\beta$ -HCH and  $\delta$ -HCH being the most stable and recalcitrant.  
458 These findings also agree with some other biotransformation studies (Willett et al. 1998; Phillips et al.



459 2005). The biodegradation of different mixtures of  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH and  $\delta$ -HCH by ligninolytic  
460 white-rot fungi has been previously reported (Quintero et al. 2007; 2008; Mohapatra and Pandey 2015). To  
461 our knowledge, this work is the first evidence of the biodegradation of a HCH mixture by a saprotrophic  
462 fungus, isolated from a historically co-contaminated site with metals and HCH. In Quintero et al. (2007),  
463 biodegradation values exhibited by *Bjerkandera adusta* (Willd.) P. Karst. of 91.5%, 94.5%, 78.5% and 66.1%  
464 were attained after 30 d for  $\gamma$ -,  $\alpha$ -,  $\delta$ - and  $\beta$ -HCH isomers, respectively. The  $\delta$ - and  $\gamma$ -HCH isomers were  
465 degraded to between 15.1 and 70.8% by six different white-rot fungi tested and the highest  $\beta$ -HCH  
466 biodegradation (56.6%) occurred with *B. adusta* (Quintero et al. 2008). In our tests with liquid Czapek-Dox  
467 medium, it is possible to hypothesize the existence of a lag phase during the first 3 d followed by a  
468 reduction of  $\alpha$ -HCH,  $\beta$ -HCH and  $\gamma$ -HCH isomers in the media (Fig. 2a–2c). This could be related to slow  
469 HCH solubilization in the media and/or enzyme induction and an adaptation period of the fungus to  
470 addition of the HCH mixture. These phases were also observed in *P. griseofulvum* FBL 500 in the presence  
471 of  $\beta$ -HCH as an adaptation period associated with time and a specific compound threshold for induction of  
472 catabolic enzymes (Ceci et al. 2015b). In a slurry batch reactor using *B. adusta*, Quintero et al. (2007)  
473 observed a lag phase during the first 5 d followed by a 7-day period in which the concentration of the HCH  
474 isomers  $\alpha$ ,  $\gamma$ ,  $\delta$ , and  $\beta$  decreased to 73.9%, 57.4%, 40.8% and 28%, respectively. Valentin et al. (2007),  
475 using the same slurry reactor system, observed a lag period of 6 d, but they related this to stressful culture  
476 conditions, e.g. high agitation rates, or a decrease in oxygen transfer. Ceci et al. 2015b observed a lag  
477 period of 4 days, followed by a reduction period of  $\beta$ -HCH in the medium (residual  $\beta$ -HCH = 44.8%).  
478 Detrimental conditions, such as benzoate formation, could speed up the decay phase, which implies that  
479 degradation stops at shorter treatment periods (Guillén-Jiménez et al. 2012; Ceci et al. 2015b). In liquid  
480 medium, no expected metabolites that have been reported in the literature for HCH biodegradation were  
481 observed, while different metabolites derived from fungal dehalogenation of HCH were observed in  
482 experiment C with HCH (Fig. 3). In liquid media, possible intermediates could not be detected probably  
483 because of the fast kinetics of biodegradation involved in liquid medium, the low detection limits of the  
484 transient products, or their different values of polarity and volatility (Guillén-Jiménez et al. 2012; Ceci et al.  
485 2015b). Isomers of PCCH, TCCH, TCB (1,2,3-TCB, 1,3,5-TCB and 1,2,4-TCB), DCB (1,2-DCB, 1,3-DCB  
486 and 1,4 DCB), and chlorobenzene were detected in solid Czapek-Dox medium incubated with *P.*  
487 *griseofulvum* FBL 500 in experiment C (Fig. 3). A combination of vanadium and HCH did not significantly  
488 inhibit fungal growth, showing similar TI values as those with the presence of HCH alone, while a 50 mg/l  
489 concentration of the HCH mixture negatively reduced the fungal growth (TI < 50%). Despite this, in both  
490 cases HCH biodegradation was not influenced negatively, as some metabolites were observed.  
491 Intermediates of HCH reductive dechlorination and hydroxylation have been observed in other studies on  
492 biotransformation of lindane by bacteria and saprotrophic fungi (Phillips et al. 2005; Salam and Das 2015).  
493 PCCH is reported to be the first intermediate of HCH dehalogenation in aerobic degradation pathways  
494 (Willett et al. 1998; Middeldorp et al. 2005; Camacho-Pérez et al. 2012). The presence of TCCH was  
495 generally observed during anaerobic HCH degradation by bacteria, while a *Pseudomonas* sp. isolated from

496 soil was able to transform lindane aerobically producing PCCH and TCCH (Phillips et al. 2005). Microbial  
497 degradation of lindane has been reported to produce volatile transformation products such as chlorinated  
498 benzenes and phenols ( Phillips et al. 2005; Salam et al. 2013). Previously, tetrachlorocyclohexane and  
499 tetrachlorocyclohexanol were observed in the biodegradation of lindane by white rot-fungi (Phillips et al.  
500 2005), while PCCH and benzoic acid derivatives were observed in aerobic degradation of lindane by  
501 *Fusarium verticillioides* AT-100 (Guillén-Jiménez et al. 2012).  $\gamma$ -PCCH and different metabolites were  
502 observed in lindane biodegradation by *Candida* VITJzN04 (Salam and Das 2014), while the proposed  
503 degradation route for lindane in *Rhodotorula* sp. is very similar to the one that can be hypothesized in this  
504 investigation and  $\gamma$ -PCCH, 1,2,4-TCB, 1,2.DCB, CB and other intermediates were reported (Salam et al.  
505 2013).

506 The Phenotype MicroArray™ microplate system was used to study the whole phenotype of *P.*  
507 *griseofulvum* FBL 500 in response to the different combinations of V and HCH. This fungus was able to  
508 use ~74 % of the all available microplate substrates. This confirms a high metabolic versatility of *P.*  
509 *griseofulvum* FBL 500 in different environmental habitats. The different treatments did not change the  
510 number of substrates used, but instead the rate of utilization, with significant effects on fungal metabolism.  
511 The presence of vanadium alone or in combination with HCH negatively influenced respiration and growth  
512 in the presence of different compounds, according to their different chemical categories. The results  
513 showed a strong similarity with the treatments with vanadium and with the combination of V and HCH.  
514 This can be explained by V inhibition of specific enzymes which are essential for the activation of different  
515 metabolic pathways of substrate utilization and also masks the effects of HCH. For instance, enzymatic V  
516 inhibition could be due to chemical similarity of vanadate with phosphate, and the resulting competition for  
517 phosphorylation of some enzymes, which, in turn, could reduce utilization of specific substrates and  
518 metabolism. It has been demonstrated that vanadium enters *Neurospora crassa* as vanadate, which is a  
519 potent inhibitor of the plasma membrane ATPase when cells are growing in an alkaline medium and are  
520 depleted for phosphate (Ceci et al. 2015c). Functional and phenotypic studies on microbial communities  
521 from metal polluted sites using Biolog microplates with high concentrations of Cu and Zn, showed negative  
522 effects on community functions due to metal toxicity, but also pollution-induced community tolerance to  
523 metals (Klimek and Niklińska 2007). In particular, the fungal community was shown to be less sensitive to  
524 metal toxicity than the co-occurring bacterial community (Klimek and Niklińska 2007). Kong et al. 2006  
525 observed that synergic effects of Cu and the antibiotic oxytetracycline on a microbial community  
526 negatively influenced functional diversity, resulting in a significantly stronger negative effect for each  
527 substrate group on the utilization potential of carboxylic acids and carbohydrates than those of  
528 oxytetracycline or Cu alone (Kong et al. 2006). It is worth noting that in our study it has been found that for  
529 nearly 50% of substrates used by *P. griseofulvum* FBL 500 there was no significant difference among the  
530 metabolic curves for all the treatments. This may indicate that the mechanisms of tolerance/resistance  
531 utilized by the fungus were adequate to cope with the stress imposed by the different combinations of  
532 xenobiotics and PTEs. Apparently *P. griseofulvum* FBL 500 could transform metal and/or organic

533 pollutants into less toxic forms since in their presence it showed the same metabolic profile as the control.  
534 In fact, for some substrates higher values of A, the maximum height of the metabolic curve, were observed  
535 or higher values of OD for HCH, V and combinations of both. These evidences may represent a stimulatory  
536 effect of HCH, V or combinations of both in the co-metabolism of specific organic compounds. In the  
537 presence of carbon- and nitrogen-containing compounds, specific metabolic pathways, such as the pentose  
538 phosphate pathway and glucuronate interconversion, and the presence of relatively non-specific enzymes,  
539 such as laccases and cellulose dehydrogenase saprotrophic can be activated, and fungi, could play roles in  
540 the biotransformation of HCH alone or with V (Cameron et al. 2000; Mander et al. 2006; Ceci et al. 2015b).  
541 V can help in cancer treatment, because it may be actively involved in oxidative radicals' production by  
542 Fenton reactions against the tumor cells (Rehder, 2008). These reactions could also enhance the enzymatic  
543 catalyst reactions of HCH biotransformation by laccases or other fungal enzymes, involved also in the  
544 biodegradation of other xenobiotics. Moreover, V can activate specific enzymes, such as V-dependent  
545 haloperoxidases, that can further enhance the degradation of recalcitrant compounds (Ceci et al., 2015c)

546         The *P. griseofulvum* FBL 500 strain was isolated from an environment extensively polluted by  
547 toxic organic and inorganic compounds over several years. Therefore the fungus occurred in an  
548 environment where stress was a constant condition and stimulus for survival. The experiments exposed the  
549 fungus to toxic compounds singly and in combination with the responses of the fungus being varied. On  
550 one hand, the organism showed a generic response which was the same in both the presence of organic  
551 compounds and metals. It is likely that fungus initiated a survival or buffering metabolism generically  
552 directed to cope with immediate extreme environmental conditions, apparently without distinguishing  
553 between metal or organic stressors. It is worth mentioning that the different treatments did not affect the  
554 number of carbon sources used by the fungus, but instead modified their rate of utilization suggesting a  
555 significant systemic effect on fungal metabolism, and not just on a single pathway. *P. griseofulvum* FBL  
556 500 was able to acidify the medium and the formation of new biominerals was evidence of the occurrence  
557 of metal biotransformation. This suggests that the fungus possesses particular mechanisms of tolerance to  
558 metal toxicity. When exposed to HCH, however, the biotransformation of the xenobiotic by the fungus did  
559 not significantly change the pH of the media, which remained close to neutrality. This could protect the  
560 fungus from toxic effects that occur in acidic conditions due to the production of benzoates from HCH  
561 biodegradation. In fungi, pH plays important roles in other ecological aspects. For example, in postharvest  
562 pathogens, fungal pH modulation of the host environment regulates a multitude of enzymes implied in  
563 fungal pathogenicity (Alkan et al. 2012). Moreover, for *Paecilomyces marquandii* (Massee) S. Hughes, the  
564 pH of the medium affects the production of reactive oxygen species during biodegradation of the pesticide  
565 alachlor. Neutral pH was favourable both for alachlor biodegradation and for oxidative stress reduction  
566 (Słaba et al. 2015). The effectiveness of mycelial protection by anti-oxidative enzymes from oxidative  
567 stress was observed to be dependent on the environmental pH, exposure time and fungal growth phase  
568 (Słaba et al. 2015). Interestingly, the synthesis of laccase and other extracellular peroxidases by some  
569 filamentous fungi was higher in the presence of intracellular oxidative stress (Chanda et al. 2015). Besides

570 a reaction to generic stress, *P. griseofulvum* FBL 500 was also capable of activating pathways to use  
571 different substrates, tolerating a high concentration of a combination of HCH and V with a supposed co-  
572 metabolic mechanism. In fact, *P. griseofulvum* FBL 500 was able to tolerate high concentrations of a  
573 mixture of HCH isomers, an ability described so far only in a few fungal species.

574 In this research, we have provided evidence of fungal tolerance to metals and HCH, and its ability  
575 in metal biomineralization, metal mobilization and biotransformation of HCH mixtures of isomers, even in  
576 the presence of vanadium at high concentrations. This could therefore be relevant to bioremediation  
577 treatments (bioaugmentation, biostimulation) of co-contaminated sites through native fungal species  
578 selected for extreme environmental conditions.

579

580 **Acknowledgments** We thank Dr. Daniele Porretta for the genetic analysis and Bioinformatics.  
581 **Compliance with ethical standards**  
582 **Conflict of interest** The authors declare that they have no conflict of interest.  
583 **Ethical statement** This article does not contain any studies with human participants or animals performed  
584 by any of the authors.  
585 **Funding** Financial assistance for this study and the related research project titled “Soil saprotrophic fungi  
586 in bioremediation of hexachlorocyclohexane-contaminated sites: metabolic, enzymatic and chemical  
587 aspects” (No. C26A14JSZ8) was provided by Sapienza University of Rome.  
588 Research support in the Geomicrobiology Group (GMG) is received from the Natural Environment  
589 Research Council (NE/M010910/1 (TeaSe); NE/M011275/1 (COG3)) which is gratefully acknowledged.  
590

591 **References**

- 592 Abarenkov K, Henrik Nilsson R, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R,  
593 Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K,  
594 Peintner U, Kõljalg U (2010) The UNITE database for molecular identification of fungi – recent  
595 updates and future perspectives. *New Phytol* 186:281–285. doi: 10.1111/j.1469-  
596 8137.2009.03160.x
- 597 Abigail MEA, Samuel MS, Chidambaram R (2015) Hexavalent chromium biosorption studies using  
598 *Penicillium griseofulvum* MSR1 a novel isolate from tannery effluent site: Box–Behnken  
599 optimization, equilibrium, kinetics and thermodynamic studies. *J Taiwan Inst Chem Eng* 49:156–  
600 164. doi: 10.1016/j.jtice.2014.11.026
- 601 Alisi C, Musella R, Tasso F, Ubaldi C, Manzo S, Cremisini C, Sprocati AR (2009) Bioremediation of  
602 diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with  
603 native strains selected for heavy metals resistance. *Sci Total Environ* 407:3024–3032. doi:  
604 10.1016/j.scitotenv.2009.01.011
- 605 Alkan N, Espeso EA, Prusky D (2012) Virulence regulation of phytopathogenic fungi by pH. *Antioxid*  
606 *Redox Signal* 19:1012–1025. doi: 10.1089/ars.2012.5062
- 607 Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST  
608 and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*  
609 25:3389–3402. doi: 10.1093/nar/25.17.3389
- 610 Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kausrud H (2010) ITS as an environmental  
611 DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol*. doi:  
612 10.1186/1471-2180-10-189
- 613 Bernardini A, Salvatori E, Di Re S, Fusaro L, Nervo G, Manes F (2016) Natural and commercial *Salix*  
614 clones differ in their ecophysiological response to Zn stress. *Photosynthetica* 54:56–64. doi:  
615 10.1007/s11099-015-0155-9
- 616 Bernini R, Pelosi C, Carastro I, Venanzi R, Di Filippo A, Piovesan G, Ronchi B, Danieli PP (2016)  
617 Dendrochemical investigation on hexachlorocyclohexane isomers (HCHs) in poplars by an  
618 integrated study of micro-Fourier transform infrared spectroscopy and gas chromatography. *Trees*  
619 30:1455–1463. doi: 10.1007/s00468-015-1343-8
- 620 Bochner BR, Gadzinski P, Panomitros E (2001) Phenotype MicroArrays for high-throughput phenotypic  
621 testing and assay of gene function. *Genome Res* 11:1246–1255. doi: 10.1101/gr.186501

- 622 Bridbžiuvienė D, Levinskaite L (2007) Fungal tolerance towards copper-based wood preservatives.  
623 *Biologija* 53:54–61
- 624 Camacho-Pérez B, Ríos-Leal E, Rinderknecht-Seijas N, Poggi-Varaldo HM (2012) Enzymes involved in  
625 the biodegradation of hexachlorocyclohexane: A mini review. *J Environ Manage* 95,  
626 Supplement:S306–S318. doi: 10.1016/j.jenvman.2011.06.047
- 627 Cameron MD, Timofeevski S, Aust SD (2000) Enzymology of *Phanerochaete chrysosporium* with respect  
628 to the degradation of recalcitrant compounds and xenobiotics. *Appl Microbiol Biotechnol* 54:751–  
629 758. doi: 10.1007/s002530000459
- 630 Ceci A, Kierans M, Hillier S, Persiani AM, Gadd GM (2015a) Fungal bioweathering of mimetite and a  
631 general geomycological model for lead apatite mineral biotransformations. *Appl Environ*  
632 *Microbiol* 81:4955–4964. doi: 10.1128/AEM.00726-15
- 633 Ceci A, Maggi O, Pinzari F, Persiani AM (2012) Growth responses to and accumulation of vanadium in  
634 agricultural soil fungi. *Appl Soil Ecol* 58:1–11. doi: 10.1016/j.apsoil.2012.02.022
- 635 Ceci A, Pierro L, Riccardi C, Pinzari F, Maggi O, Persiani AM, Gadd GM, Petrangeli Papini M (2015b)  
636 Biotransformation of  $\beta$ -hexachlorocyclohexane by the saprotrophic soil fungus *Penicillium*  
637 *griseofulvum*. *Chemosphere* 137:101–107. doi: 10.1016/j.chemosphere.2015.05.074
- 638 Ceci A, Rhee YJ, Kierans M, Hillier S, Pendrowski H, Gray N, Persiani AM, Gadd GM (2015c)  
639 Transformation of vanadinite [Pb<sub>5</sub>(VO<sub>4</sub>)<sub>3</sub>Cl] by fungi. *Environ Microbiol* 17:2018–2034. doi:  
640 10.1111/1462-2920.12612
- 641 Chanda A, Gummadidala PM, Goma OM (2015) Mycoremediation with mycotoxin producers: a critical  
642 perspective. *Appl Microbiol Biotechnol* 100:17–29. doi: 10.1007/s00253-015-7032-0
- 643 Cordero PRF, Bennett RM, Bautista GS, Aguilar JPP, Dedeles GR (2015) Degradation of nickel  
644 protoporphyrin disodium and vanadium oxide octaethylporphyrin by Philippine microbial  
645 consortia. *Bioremediation J* 19:93–103. doi: 10.1080/10889868.2013.827616
- 646 Crans DC, Smee JJ, Gaidamauskas E, Yang L (2004) The chemistry and biochemistry of vanadium and the  
647 biological activities exerted by vanadium compounds. *Chem Rev* 104:849–902
- 648 Czaplicki LM, Cooper E, Ferguson PL, Stapleton HM, Vilgalys R, Gunsch CK (2016) A new perspective  
649 on sustainable soil remediation—case study suggests novel fungal genera could facilitate in situ  
650 biodegradation of hazardous contaminants. *Remediat J* 26:59–72. doi: 10.1002/rem.21458

- 651 Doyle JJ, Doyle LJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue.  
652 Phytochem Bull 19:11–15
- 653 Fahmy T, Aubry P (2003) XLSTAT-pro, version 7.0. Paris, FR
- 654
- 655 Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60. doi: 10.1111/j.1469-  
656 8137.1993.tb03796.x
- 657 Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and  
658 radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111:3–49. doi:  
659 10.1016/j.mycres.2006.12.001
- 660 Gadd GM (2004) Mycotransformation of organic and inorganic substrates. Mycologist 18:60–70. doi:  
661 10.1017/S0269-915X(04)00202-2
- 662 Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology  
663 156:609–643. doi: 10.1099/mic.0.037143-0
- 664 Gadd GM (2013) Geomycology: fungi as agents of biogeochemical change. Biol Environ Proc R Ir Acad  
665 113:1–15. doi: 10.3318/BIOE.2013.16
- 666 Gadd GM, Bahri-Esfahani J, Li Q, Rhee YJ, Wei Z, Fomina M, Liang X (2014) Oxalate production by  
667 fungi: significance in geomycology, biodeterioration and bioremediation. Fungal Biol Rev 28:36–  
668 55. doi: 10.1016/j.fbr.2014.05.001
- 669 Gadd GM, Rhee YJ, Stephenson K, Wei Z (2012) Geomycology: metals, actinides and biominerals.  
670 Environ Microbiol Rep 4:270–296
- 671 Griffith CM, Baig N, Seiber JN (2015) Contamination from industrial toxicants. In: Cheung PCK, Mehta  
672 BM (eds) Handbook of Food Chemistry. Springer, Berlin, Heidelberg, DE, pp 719–751
- 673 Guillén-Jiménez F de M, Cristiani-Urbina E, Cancino-Díaz JC, Flores-Moreno JL, Barragán-Huerta BE  
674 (2012) Lindane biodegradation by the *Fusarium verticillioides* AT-100 strain, isolated from *Agave*  
675 *tequilana* leaves: kinetic study and identification of metabolites. Int Biodeterior Biodegrad 74:36–  
676 47. doi: 10.1016/j.ibiod.2012.04.020
- 677 Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of  
678 hazardous chemicals. Nat Rev Microbiol 9:177–192. doi: 10.1038/nrmicro2519



679 Klimek B, Niklińska M (2007) Zinc and copper toxicity to soil bacteria and fungi from zinc polluted and  
680 unpolluted soils: a comparative study with different types of Biolog plates. *Bull Environ Contam*  
681 *Toxicol* 78:112–117. doi: 10.1007/s00128-007-9045-6

682 Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K,  
683 Kjølner R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T (2005) UNITE: a  
684 database providing web-based methods for the molecular identification of ectomycorrhizal fungi.  
685 *New Phytol* 166:1063–1068. doi: 10.1111/j.1469-8137.2005.01376.x

686 Kong W-D, Zhu Y-G, Fu B-J, Marschner P, He J-Z (2006) The veterinary antibiotic oxytetracycline and Cu  
687 influence functional diversity of the soil microbial community. *Environ Pollut* 143:129–137. doi:  
688 10.1016/j.envpol.2005.11.003

689

690 Lange L, Bech L, Busk PK, Grell MN, Huang Y, Lange M, Linde T, Pilgaard B, Roth D, Tong X (2012)  
691 The importance of fungi and of mycology for a global development of the bioeconomy. *IMA*  
692 *Fungus* 3:87–92. doi: 10.5598/imafungus.2012.03.01.09

693 Ma X, Ling Wu L, Fam H (2014) Heavy metal ions affecting the removal of polycyclic aromatic  
694 hydrocarbons by fungi with heavy-metal resistance. *Appl Microbiol Biotechnol* 98:9817–9827.  
695 doi: 10.1007/s00253-014-5905-2

696 Mace GM, Norris K, Fitter AH (2012) Biodiversity and ecosystem services: a multilayered relationship.  
697 *Trends Ecol Evol* 27:19–26. doi: 10.1016/j.tree.2011.08.006

698 Mander GJ, Wang H, Bodie E, Wagner J, Vienken K, Vinuesa C, Foster C, Leeder AC, Allen G, Hamill V,  
699 Janssen GG, Dunn-Coleman N, Karos M, Lemaire HG, Subkowski T, Bollschweiler C, Turner G,  
700 Nüsslein B, Fischer R (2006) Use of laccase as a novel, versatile reporter system in filamentous  
701 fungi. *Appl Environ Microbiol* 72:5020–5026. doi: 10.1128/AEM.00060-06

702 Middeldorp PM, van Doesburg W, Schraa G, Stams AM (2005) Reductive dechlorination of  
703 hexachlorocyclohexane (HCH) isomers in soil under anaerobic conditions. *Biodegradation*  
704 16:283–290. doi: 10.1007/s10532-004-1573-8

705 Mishra A, Malik A (2014) Novel fungal consortium for bioremediation of metals and dyes from mixed  
706 waste stream. *Bioresour Technol* 171:217–226. doi: 10.1016/j.biortech.2014.08.047

707 Mohapatra S, Pandey M (2015) Biodegradation of hexachlorocyclohexane (HCH) isomers by white rot  
708 fungus, *Pleurotus florida*. *J Bioremediation Biodegrad* 6:280. doi: 10.4172/2155-6199.1000280

- 709 Panagos P, Van Liedekerke M, Yigini Y, Montanarella L (2013) Contaminated sites in Europe: review of  
710 the current situation based on data collected through a European network. *J Environ Public Health*  
711 4:11.
- 712 Phillips T, Seech A, Lee H, Trevors J (2005) Biodegradation of hexachlorocyclohexane (HCH) by  
713 microorganisms. *Biodegradation* 16:363–392. doi: 10.1007/s10532-004-2413-6
- 714 Pinzari F, Ceci A, Abu-Samra N, Canfora L, Maggi O, Persiani A (2016) Phenotype MicroArray™ system  
715 in the study of fungal functional diversity and catabolic versatility. *Res Microbiol* 167:710–722.  
716 doi: 10.1016/j.resmic.2016.05.008
- 717 Polti MA, Aparicio JD, Benimeli CS, Amoroso MJ (2014) Simultaneous bioremediation of Cr(VI) and  
718 lindane in soil by actinobacteria. *Int Biodeterior Biodegrad* 88:48–55. doi:  
719 10.1016/j.ibiod.2013.12.004
- 720 Quintero JC, Lú-Chau TA, Moreira MT, Feijoo G, Lema JM (2007) Bioremediation of HCH present in soil  
721 by the white-rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. *Int Biodeterior Biodegrad*  
722 60:319–326. doi: 10.1016/j.ibiod.2007.05.005
- 723 Quintero JC, Moreira MT, Feijoo G, Lema JM (2008) Screening of white rot fungal species for their  
724 capacity to degrade lindane and other isomers of hexachlorocyclohexane (HCH). *Cienc Investig*  
725 *Agrar* 35:159–167.
- 726 Ravelet C, Krivobok S, Sage L, Steiman R (2000) Biodegradation of pyrene by sediment fungi.  
727 *Chemosphere* 40:557–563. doi: 10.1016/S0045-6535(99)00320-3
- 728 Rehder D (2008) *Bioinorganic vanadium chemistry*. John Wiley & Sons Ltd, West Sussex, U.K
- 729 Salam JA, Das N (2014) Lindane degradation by *Candida* VITJzN04, a newly isolated yeast strain from  
730 contaminated soil: kinetic study, enzyme analysis and biodegradation pathway. *World J Microbiol*  
731 *Biotechnol* 30:1301–1313. doi: 10.1007/s11274-013-1551-6
- 732 Salam JA, Das N (2015) Degradation of lindane by a novel embedded bio-nano hybrid system in aqueous  
733 environment. *Appl Microbiol Biotechnol* 99:2351–2360. doi: 10.1007/s00253-014-6112-x
- 734 Salam JA, Lakshmi V, Das D, Das N (2013) Biodegradation of lindane using a novel yeast strain,  
735 *Rhodotorula* sp. VITJzN03 isolated from agricultural soil. *World J Microbiol Biotechnol* 29:475–  
736 487. doi: 10.1007/s11274-012-1201-4
- 737 Sandrin TR, Maier RM (2003) Impact of metals on the biodegradation of organic pollutants. *Environ*  
738 *Health Perspect* 111:1093–1101.

- 739 Shah MP, Vora SB, Dave SR (1999) Evaluation of potential use of immobilized *Penicillium griseofulvum*  
740 in bioremoval of copper. *Process Metallurgy* 9: 227–235. doi.org/10.1016/S1572-4409(99)80112-  
741 6
- 742 Shi Z, Bai S, Tian L, Jiang H, Zhang J (2011) Molecular detection of *Penicillium griseofulvum* as the  
743 coastal pollution indicator. *Curr Microbiol* 62:396–401. doi: 10.1007/s00284-010-9720-4
- 744 Słaba M, Różalska S, Bernat P, Szewczyk R, Piątek MA, Długoński J (2015) Efficient alachlor degradation  
745 by the filamentous fungus *Paecilomyces marquandii* with simultaneous oxidative stress reduction.  
746 *Bioresour Technol* 197:404–409. doi: 10.1016/j.biortech.2015.08.045
- 747 Tanzer M, Arst H Jr, Skalchunes A, Coffin M, Darveaux B, Heiniger R, Shuster J (2003) Global nutritional  
748 profiling for mutant and chemical mode-of-action analysis in filamentous fungi. *Funct Integr*  
749 *Genomics* 3:160–170. doi: 10.1007/s10142-003-0089-3
- 750 Teng Y, Ni S, Zhang C, Wang J, Lin X, Huang Y (2006) Environmental geochemistry and ecological risk  
751 of vanadium pollution in Panzhihua mining and smelting area, Sichuan, China. *Chin J Geochem*  
752 25:379–385. doi: 10.1007/s11631-006-0378-3
- 753 Tigini V, Prigione V, Di Toro S, Fava F, Varese GC (2009) Isolation and characterisation of  
754 polychlorinated biphenyl (PCB) degrading fungi from a historically contaminated soil. *Microb*  
755 *Cell Factories* 8:5–5. doi: 10.1186/1475-2859-8-5
- 756 Vaas LAI, Sikorski J, Hofner B, Fiebig A, Buddruhs N, Klenk H-P, Göker M (2013) opm: an R package  
757 for analysing OmniLog® phenotype microarray data. *Bioinformatics* 29:1823–1824. doi:  
758 10.1093/bioinformatics/btt291
- 759 Vaas LAI, Sikorski J, Michael V, Göker M, Klenk H-P (2012) Visualization and curve-parameter  
760 estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS ONE*  
761 7:e34846. doi: 10.1371/journal.pone.0034846
- 762 Valentín L, Lu-Chau TA, López C, Feijoo G, Moreira MT, Lema JM (2007) Biodegradation of  
763 dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot  
764 fungus *Bjerkandera* sp. BOS55. *Process Biochem* 42:641–648. doi:  
765 10.1016/j.procbio.2006.11.011
- 766

- 767 Vargas-García M del C, López MJ, Suárez-Estrella F, Moreno J (2012) Compost as a source of microbial  
768 isolates for the bioremediation of heavy metals: in vitro selection. *Sci Total Environ* 431:62–67.  
769 doi: 10.1016/j.scitotenv.2012.05.026
- 770 Vijgen J, Abhilash PC, Li Y, Lal R, Forter M, Torres J, Singh N, Yunus M, Tian C, Schäffer A, Weber R  
771 (2011) Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs—a global perspective  
772 on the management of Lindane and its waste isomers. *Environ Sci Pollut Res* 18:152–162. doi:  
773 10.1007/s11356-010-0417-9
- 774 Wan J, Meng D, Long T, Ying R, Ye M, Zhang S, Li Q, Zhou Y, Lin Y (2015) Simultaneous removal of  
775 lindane, lead and cadmium from soils by rhamnolipids combined with citric acid. *PLoS ONE*  
776 10:e0129978. doi: 10.1371/journal.pone.0129978
- 777 Wasi S, Tabrez S, Ahmad M (2011) Suitability of immobilized *Pseudomonas fluorescens* SM1 strain for  
778 remediation of phenols, heavy metals, and pesticides from water. *Water Air Soil Pollut* 220:89–99.  
779 doi: 10.1007/s11270-010-0737-x
- 780 Willett KL, Ulrich EM, Hites RA (1998) Differential toxicity and environmental fates of  
781 hexachlorocyclohexane isomers. *Environ Sci Technol* 32:2197–2207. doi: 10.1021/es9708530
- 782 Zhu Z, Yang X, Wang K, Huang H, Zhang X, Fang H, Li T, Alva AK, He Z (2012) Bioremediation of Cd-  
783 DDT co-contaminated soil using the Cd-hyperaccumulator *Sedum alfredii* and DDT-degrading  
784 microbes. *J Hazard Mater* 235–236:144–151. doi: 10.1016/j.jhazmat.2012.07.033
- 785
- 786

787 **Figures**

788 **Fig. 1** Biomineralization by *P. griseofulvum*. Biomineral precipitation observed underneath growing fungal  
789 colonies in Czapek-Dox agar amended with combinations of 2.5 mM ammonium metavanadate and 2.5  
790 mM lead carbonate after 3 months incubation of *P. griseofulvum* at 25°C in the dark. Scale bar = 0.4 mm.  
791 Typical image is shown from several examinations.

792 **Fig. 2** Gas chromatographic analysis of HCH mixture in liquid Czapek-Dox medium after growth of *P.*  
793 *griseofulvum*. The HCH mixture was added 26 days after fungal inoculation, and the isomer concentrations  
794 were monitored for 23 days. **a**  $\alpha$ -HCH concentration. **b**  $\beta$ -HCH concentration. **c**  $\gamma$ -HCH concentration. **d**  $\delta$ -  
795 HCH concentration. The bars are the standard errors of HCH concentrations of three replicates.

796 **Fig. 3** Proposed biodegradation pathway for HCH in *P. griseofulvum*. The initial reaction is the  
797 dehalogenation of HCH to pentachlorocyclohexene, the second is the formation of tetrachlorocyclohexene  
798 (TCCH) (3,4,5,6-TCCH), the third is the formation of trichlorobenzene (TCB) (1,2,3-TCB, 1,3,5-TCB and  
799 1,2,4-TCB were detected), the fourth is the formation of dichlorobenzene (DCB) (1,2-DCB, 1,3-DCB and  
800 1,4 DCB were detected), and the fifth is the formation of chlorobenzene.

801 **Fig. 4** The parameter "A" (=asymptote) namely the maximum cumulative growth of the fungus on each  
802 substrate/in each well, clustered according to the different treatments and visualised as a heat map. The heat  
803 map was obtained using the function `heat_map` of the `opm` package. The x-axis corresponds to the  
804 substrates clustered according to the similarity of their values over all treatments; the y-axis corresponds to  
805 the plates clustered according to the similarity of their values over all substrates. The central rectangle is a  
806 substrate  $\times$  plate matrix in which the colours represent the classes of values. Deep violet to blue indicate  
807 low optical density values and light brown indicates high values. The four treatments were cont=control,  
808 HCH= hexachlorocyclohexane mixture, V= vanadium and HCH+V= combination of vanadium and HCH  
809 for measurements at 750 nm.

810 **Fig. 5** Growth curves of *P. griseofulvum* measured at 750 nm (**a**) and 490 nm (**b**). Growth curves for the  
811 four treatments (cont=control; HCH= hexachlorocyclohexane mixture; V= vanadium; HCH+V=  
812 combination of vanadium and HCH) for some of the most representative carbon sources among the 95  
813 substrates present in FF arrays were elaborated using the `opm` package for R.

814