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Published in:
Applied Microbiology and Biotechnology

DOI:
[10.1007/s00253-018-9451-1](https://doi.org/10.1007/s00253-018-9451-1)

Publication date:
2019

Document Version
Peer reviewed version

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

Citation for published version (APA):

Ceci, A., Pinzari, F., Russo, F., Persiani, A. M., & Gadd, G. M. (2019). Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. *Applied Microbiology and Biotechnology*, 103(1), 53-68. <https://doi.org/10.1007/s00253-018-9451-1>

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Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites

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35 **Abstract**

36 For decades human activities, industrialization and agriculture have contaminated soils and
37 water with several compounds, including potentially toxic metals and organic persistent
38 xenobiotics. The co-occurrence of those toxicants poses challenging environmental problems,
39 as complicated chemical interactions and synergies can arise and lead to severe and toxic
40 effects on organisms. The use of fungi, alone or with bacteria, for bioremediation purposes is
41 a growing biotechnology with high potential in terms of cost-effectiveness, an environmental-
42 friendly perspective and feasibility, and often representing a sustainable nature-based
43 solution. This paper reviews different ecological, metabolic and physiological aspects
44 involved in fungal bioremediation of co-contaminated soils and water systems, not only
45 addressing best methods and approaches to assess the simultaneous presence of metals and
46 organic toxic compounds and their consequences on provided ecosystem services, but also the
47 interactions between fungi and bacteria, in order to suggest further study directions in this
48 field.

49

50 **Key words:** co-contamination, organic pollutants, potentially toxic metals, soil saprotrophic
51 fungi, xenobiotics, biodegradation, biotransformation

52 **Introduction**

53

54 The complexity and functioning of soil systems and their interactions with human activities
55 and ecosystem services are threatened by contamination, one of the major identified soil
56 threats in Europe (Stolte et al. 2016). The contaminated soil's biogeochemical equilibrium can
57 be modified by a plethora of inorganic and organic hazardous pollutants that cause significant
58 changes in physical, chemical and biological soil properties (e.g. Gadd 2010, 2017; Spina et
59 al. 2018).

60 In many terrestrial habitats, contamination leads to decreased biodiversity and activity
61 of soil biota (Keesstra et al. 2012; Harms et al. 2017), and can indirectly affect decomposition
62 and humification processes, producing alterations in ecosystem functionality. Additionally, in
63 order to assess the cumulative pressure on soil functions it is important to take into
64 consideration the interactions between the different soil threats (Stolte et al. 2016).

65 Harms et al. (2011) highlighted the role of fungi in the degradation and potential
66 remediation of organically polluted ecosystems, and also suggested that fungal hyphae might
67 stimulate the activity of bacteria, through the production of exudates as an energy source, in
68 order to cooperate in the process of pollutant degradation. Because of their relatively short
69 generation times and lifestyle, bacteria and fungi may sense and respond rapidly to
70 environmental change and evolve resistance to pollutant stress or physiologically adapt to
71 new conditions. In this context, some saprotrophic soil fungi may acquire the ability to
72 metabolize pollutants through exposure to contamination (Pinto et al. 2012).

73 The responses of bacteria and "soil microbial biomass" to the presence of both organic
74 and inorganic pollutants has been investigated in several studies (Liu et al. 2012; Arjoon et al.
75 2013; Polti et al. 2014; Dhanarani et al. 2016; Alvarez et al. 2017). It is often reported how
76 metals can cause a general inhibition of microbial metabolism and of some basic traceable
77 activities like nitrogen transformations, respiration, and organic matter decomposition
78 (Baldrian et al. 2000; Wuana et al. 2014). However, fewer studies have focused on fungal
79 behaviour when metals and xenobiotics are both present at potentially toxic concentrations,
80 and what impact different toxic metals may have on organic pollutant biodegradation
81 (Gharieb 2002; Sharaf and Alharbi 2013; Ma et al. 2014; Sharma and Malaviya 2014; Urík et
82 al. 2014; Fuentes et al. 2016; Jia et al. 2017; Ma et al. 2017; Ceci et al. 2018). This review
83 will discuss the situation in which filamentous fungi degrade xenobiotic substances in the
84 presence of toxic metals or in extreme chemical conditions. This topic may have considerable
85 significance in coming years, when the incidence of mixed organic-inorganic contamination

will become more frequent given the intense exploitation and growing extension of contaminated land and so-called "brown field" sites.

The discussion will be focused on documented fungal behaviour and performance in co-contaminated environments (Chen et al. 2015b; Wang et al. 2017), with particular emphasis on saprotrophic soil fungi, the roles they play regarding xenobiotic persistence in soil or water, and the biotransformation processes that they can perform through enzymatic degradation, and biochemical or physical immobilization mechanisms (Sandrin et al. 2000; Iqbal et al. 2005; Rao et al. 2010; Urík et al. 2014; Zhai et al. 2015; Sharma and Malaviya 2016; Wu et al. 2016; Ceci et al. 2018).

The inorganic compounds that most frequently affect polluted sites are those of potentially toxic metals and metalloids, e.g. arsenic, barium, cadmium, chromium, lead, mercury, nickel, vanadium, zinc (Del Carmen Vargas-García et al. 2012; Liu et al. 2017; Ye et al. 2017). These toxic elements frequently occur together with persistent organic xenobiotics that can accumulate because of human industrial or agricultural activities (Brandl et al. 2001; Srivastava and Thakur 2006; Cui and Zhang 2008; Sharaf and Alharbi 2013; Sharma and Malaviya 2016). It is common to find soils and wastewaters containing toxic elements along with pesticides, and herbicides but also mineral oils and petroleum derivatives, diesel oil, plastics, explosives, chlorinated solvents, and by-products and waste arising from human activities, such as electronic waste (Brandl et al. 2001; Coulibaly et al. 2003; Alisi et al. 2009; Stenuit and Agathos 2010; Dewey et al. 2012; Zhu et al. 2012; Zhou et al. 2015; Chirakkara et al. 2016; Alvarez et al. 2017). The presence of organic and inorganic pollutants can also occur in food, e.g. by plant translocation from soil and in composting (Barker and Bryson 2002; Zeng et al. 2007; Chen et al. 2015a; Zhang et al. 2017). Toxic metals and xenobiotic compounds (such as persistent organic pollutants, pesticides and PAH) are often present and recognized as two major chemical group that are responsible for soil and water pollution worldwide (Wu et al. 2016; Hong et al. 2010; Ye et al. 2017; Zhu et al. 2012; Wang et al., 2017). In Europe, around 342 thousand are the identified contaminated sites (2.5 millions are estimated potential contaminated ones) and toxic metals and mineral oil contribute for around 60% to soil pollution (Panagos et al. 2013). In America, 40% of National Priority List of the U.S. Environmental Protection Agency are co-contaminated by toxic elements, such as As, Ba, Cd, Cr, Pb, Hg, Ni, Zn, and xenobiotics, such as petroleum, chlorinated solvents, pesticides (Sandrin and Maier 2003) and over 40% of assessed water do not respect water quality standards (Ma et al. 2017).

119 Most studies report on a negative effect of metals in affecting the biodegradation of
120 organics in co-contaminated environments (Bhattacharya et al. 2014; Markowicz et al. 2016).
121 There are some studies, however, where metals showed no impact or had a synergistic effect
122 on microbial metabolism (Sandrin and Maier 2003; Sandrin and Hoffman 2007; Almeida et
123 al. 2013; Bourceret et al. 2016). Saprotrophic fungi can have very different reactions to the
124 presence of both organic and inorganic pollutants, and although the behaviour of fungi in the
125 presence of one or the other type of toxic substance is well studied, e.g. fungal mobilization or
126 immobilization of toxic metals, an understanding of what happens in fungal metabolism with
127 both organic and inorganic toxic substances is still limited.

128 The aims of this review are to summarize and discuss: 1) ecological aspects
129 concerning xenobiotic and metal co-contamination and fungal functional traits; 2) reported
130 effects of metals on fungal biodegradation of organic pollutants and the fungal biochemical
131 pathways in which metals can affect biodegradation, including co-metabolism; 3) methods
132 used or applicable to assess the effects of metals on fungal biodegradation; 4) the potential of
133 fungi (alone or together with bacteria) to increase biodegradation of organic pollutants in co-
134 contaminated environments.

135

136 **Fungal functional traits and the resistance to co-contaminants**

137 Fungi are dominant components of most terrestrial ecosystems, but in terms of ecosystem function,
138 fungal survival in a given environment depends on their ability to tolerate nutrient-limiting
139 conditions and stresses like competition, but also the presence of multiple toxic compounds.
140 Crowther et al. (2014) proposed the variation in combat trait expression along environmental
141 gradients as a means of distinguishing a species' realized niche from its fundamental niche. A
142 fundamental niche is the entire set of conditions under which a fungal species can survive and
143 reproduce itself whereas a realized niche is the set of conditions actually used by a given fungus
144 after interactions with other species or environmental-limiting situations have been taken into
145 account (Crowther et al. 2014). In the study of fungal behaviour in co-contaminated environments,
146 analysis of an organisms' trade-off between stress-tolerance and combative dominance could
147 provide some important keys to understanding the functioning of specific and nonspecific
148 detoxification mechanisms. The Hutchinson niche concept (Hutchinson, { [HYPERLINK](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4215788/)
149 "<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4215788/>" \l "B37" }), where environmental
150 conditions and resources define the requirements for a species to persist, could be applied to
151 situations where multiple stressors (co-contaminants) contribute to shape fungal populations,
152 selecting for traits that allow individuals to survive and compete under specific conditions.

153 The trait-based approach is a tool to understand the plasticity of fungal activity and the
154 dimension of their biodiversity (Peay et al. 2016). The evolutionary and ecological success of fungi
155 is based on the link between their morphological and functional traits; saprotrophic fungi possess a
156 wide pool of functional traits often associated with biogeochemical cycling processes in leaf litter
157 and soil. An example of relevant fungal traits is reported in Persiani et al. (2008), in which the first
158 empirical evidence of natural patterns of soil fungal biodiversity and soil carbon storage in
159 Mediterranean grasslands at different elevations has been provided, considering morpho-functional
160 traits and their distribution in the evaluated field conditions with respect to the distribution of the
161 species and their functional or biochemical polyvalence. A considerably greater increase in
162 biodiversity may be required to diversify the functional variability of the fungal community in the
163 presence of abiotic stress such as the occurrence of organic and inorganic pollutants. Consequently,
164 biodiversity can act as insurance for the productivity of fungal communities in a changing
165 environment. According to this "insurance hypothesis", biodiversity results in taxonomic
166 redundancy which buffers ecosystems against a decline in functioning (Loreau 2000). In
167 biodiversity studies, such redundancy has been often interpreted as an adaptive advantage of soil
168 communities to abiotic stress factors and anthropogenic and climatic disturbance events (Loreau
169 2000; Deacon et al. 2006; Persiani et al. 2008).

Ecosystem-related traits vary at coarse taxonomic levels, and the same seems to happen with tolerance to toxic compounds. Treseder et al (2014) found that the maximum amount of variance for traits associated with stress tolerance could be explained at the levels of order to phylum. Since fungal phyla and subphyla vary in their genetic capacity for stress tolerance, according to Treseder et al (2014) it should be expected that their environmental distributions vary accordingly, with stress tolerators occupying harsher environments. In fact, contaminated sites are considered to be the main sources of resistant fungal strains characterised by exhibiting tolerance to toxic metals or organic pollutants (Peng et al. 2015; Xie et al. 2016). Several metal-tolerant filamentous fungi have been isolated from multiple toxic metal contaminated soils (Oladipo et al. 2017). Metal tolerance mechanisms adopted by fungal species can be specific or non-specific. In some fungi, the ability to biodegrade xenobiotics, or cope with toxic metals, is non-specific and due to a coincidence of mechanisms that evolved in some taxa that populate particular niches. An example is the high redox potential of some fungal enzymes, such as peroxidases and laccases that are involved in lignin breakdown. Wood decay fungi have significant potential in the biodegradation of organic pollutants because of the wide set of ligninolytic enzymes that are involved in key steps of aromatic hydrocarbon biodegradation (Baldrian et al. 2000; Gadd 2010, 2017; Gadd et al. 2012; Dighton 2016). Co-metabolism of xenobiotics is generally common in fungi in different proportions, according to different conditions (Harms et al., 2017). Nevertheless, in some cases, specific metabolism and assimilation of xenobiotics by certain fungal species has been observed and is limited to few classes of compounds, such as aliphatic hydrocarbons and non-halogenated monoaromatic compounds. An example is provided by Blasi et al. (2016) who screened a collection of black yeast-like fungi for the ability to grow on hexadecane, toluene and polychlorinated biphenyl, and for two species it has been observed the ability to grow on toluene as the sole carbon and energy source. In this case the toluene metabolism is related to a specific enzyme, toluene monooxygenases, which shares similarities with other P450 monooxygenases, e.g. present in mammals and plants (Blasi et al. 2016). Hence, fungal species that can metabolize xenobiotic compounds benefit from the evolutionary advantage to have access to a carbon and energy sources, which is not available for other microorganisms. These species possess a different niche width in comparison with others. Moreover, fungi with degrading abilities towards aliphatic and aromatic hydrocarbons, together with those exhibiting remarkable levels of tolerance and accumulation of toxic metals have been described across different taxa (Prenafeta-Boldú et al. 2001; Tan and Cheng 2003). The assimilatory metabolism of xenobiotics, as well as the ability to precipitate, compartmentalize or avoid toxic metals is often due to the evolution of a combination of successful pathways under the selective pressure of specific environmental factors. Natural populations

204 thriving in toxic metal or poly-contaminated ecosystems are often subjected to selective pressures
205 resulting in increased resistance. Evolutionary adaptation to toxic metals, for example, is a well-
206 documented process in several different groups of organisms including bacteria and plants (Colpaert
207 et al. 2004).

208

209 **Effects of interactions between xenobiotics and potentially toxic metals on fungal metabolism**
210 **and biodegradation potential**

211 In many terrestrial habitats, organic contamination might have two opposing consequences since it
212 can serve either as a source of carbon and energy, thus favouring nutrition of those microorganisms
213 which possess the appropriate biochemical machinery to benefit from this input, or it can result in
214 toxicity, inhibiting microbial growth, survival and biochemical activities (Harms et al. 2017). An
215 appropriate combination of different factors is required in order to guarantee effective microbial
216 contaminant degradation, including microbial activity, pollutant bioavailability and environmental
217 parameters, the latter comprising physico-chemical factors such as pH, temperature, nutrients,
218 moisture, aeration, etc. and ecological interactions such as predation and competition (Fig. 1) (Gadd
219 2001; Fester et al. 2014; Liu et al. 2017; Harms et al. 2017). Fungi are extraordinary
220 microorganisms and found in nearly every habitat on the planet, many being tolerant to extreme
221 environmental conditions, and most are characterized by the formation of extensive hyphal
222 networks (Harms et al. 2011, 2017; Dighton 2016). The high functional versatility of fungi holds
223 considerable promise for feasible biotechnological applications, including the bioremediation of
224 toxic substances (Gadd 2001; Singh and Singh 2014).

225 Fungi are important geosactive agents and play fundamental roles in many important
226 processes, e.g. organic matter decomposition, biotransformation of organic and inorganic substrates,
227 biogeochemical cycling of elements, metal and mineral biotransformations, mineral bioweathering
228 and soil formation and development (Gadd 2004, 2007, 2010; Gadd et al. 2012). Fungi, along with
229 bacteria, are the most important microorganisms for reclamation, immobilization or detoxification
230 of metallic, semi-metallic and radionuclide pollutants in terrestrial environments (Gadd 2004, 2010;
231 Gadd et al. 2012). Many metals, such as Na, K, Cu, Zn, Ca, Mg, Co, Mn and Fe are essential for
232 fungi, but above certain threshold concentrations all can be toxic. Other metals, such as Cs, Al, Pb,
233 Cd and Hg, as well as metalloids, e.g. As, have no known metabolic functions, but can still be
234 accumulated (Gadd 2004, 2010). Metal and their compounds directly and/or indirectly interact with
235 all aspects of fungal growth, metabolism and differentiation, varying according to metal species,
236 organism and environmental factors, while structural components and metabolic activity also
237 influence metal speciation, and therefore solubility, bioavailability and toxicity (Gadd 2010). Metal
238 sources can be from natural geochemical events or can be associated with anthropogenic
239 contamination or redistribution in aquatic and terrestrial ecosystems (Gadd 2007, 2010). The
240 toxicity of metals, metalloids, organometals and their chemical derivatives towards fungi can range
241 widely and is greatly affected by physico-chemical features of the environment and chemical
242 behaviour of the metal species (Gadd 2010, 2017; Gadd et al. 2012; Dighton 2016). Despite

243 potential toxicity, many fungi can grow in metal-polluted sites because of a wide variety of direct or
244 indirect resistance/tolerance mechanisms, dependent or independent of metabolism (Gadd 2010;
245 Gadd et al. 2012). Fungal detoxification mechanisms are generally related to mobilization or
246 immobilization of potentially toxic elements (Fig. 1) (Gadd 2010; Gadd et al. 2012). The
247 mechanisms involved in immobilization include (i) redox transformations which change the
248 oxidation state of elements to result in a less toxic immobile chemical species, e.g. Mn(II) to
249 Mn(IV) oxide, in some cases resulting in precipitation as nanoparticles and in elemental forms (e.g.
250 Ag, Te, Se, Au); (ii) biosorption or binding to pigments, polysaccharides and other extracellular
251 polymeric substances; (iii) biosorption to cell walls and other structural components which may be a
252 prelude to biomineralization; (iv) biomineral formation via excreted metabolites, e.g. oxalate or
253 other non-directed biomineralization processes, e.g. carbonates, phosphates; (v) metal sorption to
254 biogenic minerals; (vi) intracellular accumulation which involves mechanisms of efflux and influx,
255 intracellular compartmentalization in organelles, enzymatic detoxification, intracellular
256 complexation, e.g. metallothioneins, phytochelatins, and precipitation (Gadd 2007, 2010; Gadd et
257 al. 2012). In fungi, metal mobilization can result from (i) redoxolysis, complexation, e.g.
258 siderophores, and protonolysis; (ii) chemoorganotrophic leaching by other excreted metabolites
259 with metal-complexing properties, such as organic acids, amino acids, and phenolic compounds;
260 (iii) methylation, which can result in volatilization (e.g. As, Te, Se, Hg); (iv) bioweathering and
261 biocorrosion (Gadd 2007, 2010; Gadd et al. 2012). Such mechanisms of solubilization and
262 immobilization influence the bioavailability of metals, metalloids, and radionuclides, and their
263 toxicity and distribution (Gadd 2007, 2010; Gadd et al. 2012). Metals and metalloids can have
264 negative effects on fungi, affecting survival, metabolism, morphology, reproduction and spore
265 germination and impairing their functions in the ecosystem (Gadd et al. 2012; Fester et al. 2014;
266 Dighton 2016; Harms et al. 2017; Ceci et al. 2018).

267 Organic matter decomposition is one of the most important microbial activities in the
268 biosphere, and fungi, as well as bacteria, can utilize a wide spectrum of organic compounds, which
269 can range from simple substrates such as sugars, organic acids and amino acids to more complex
270 molecules, such as cellulose, pectin, lignin, lignocellulose, chitin and starch, and also many
271 hydrocarbons, pesticides and other xenobiotics of anthropogenic origin (Baldrian et al. 2000; Gadd
272 2010, 2017; Gadd et al. 2012; Dighton 2016). Biodegradation of natural compounds as well as
273 xenobiotics, such as polycyclic aromatic hydrocarbons, dioxins, polychlorinated biphenyls,
274 chlorophenols, nitroaromatics, dyes, pesticides, BTEX, organophosphorus compounds, and
275 organometallics, is intimately associated with biogeochemical cycling and redistribution of carbon
276 and other elements between organisms and environmental compartments.

The physico-chemical characteristics of xenobiotics control their bioavailability (Fig. 1), and these include hydrophobicity, volatility, complexity and stability (Gadd 2001). Some organic chemical species are more stable and recalcitrant to fungal attack, as in the case of PAHs where more complex molecular structures are more stable in the environment, due to low bioavailability and high hydrophobicity, and relatively few fungi are able to grow on more complex hydrocarbons (Liu et al. 2017; Harms et al. 2017). In contrast to hydrophobic pollutants, water-soluble compounds and intermediates arising from fungal biotransformation of hydrophobic chemicals may be rapidly transformed in soil (Gadd 2001; Harms et al. 2017). The bioavailability of xenobiotics, as for potentially toxic elements, is also dependent on chemical and physical parameters of the soil environment, such as pH (Gadd 2001).

In natural systems, bioavailability of metals, xenobiotics or their intermediates is determined by interactions with environmental components, including organic matter, humic substances, clays and other materials, soil colloids, biogenic debris and exudates (Gadd 2001, 2010). The affinity of contaminants for the soil phase and the processes of sequestration or encapsulation within the soil matrix (in small pores or within clay mineral lattices or organic matter) can limit the effective interactions of fungi with xenobiotic and metals by affecting their bioavailability. Sorption of contaminants to clay minerals or organic matter is one of the that influences bioavailability in soils. Similarly metal sorption to cells is a fundamental step in all fungal-metal interactions (Gadd 2001, 2009, 2010; Ceci et al. 2015, 2018). A low bioavailability may be beneficial for reducing pollutant toxicity and mobilization via leaching, enabling fungal growth in soils with high concentrations of pollutants. On the other hand, low bioavailability may increase environmental persistence, reducing biotransformation performance, and allowing chemical transformations such as polymerization and dehalogenation (Gadd 2001). Persistent organic pollutants may diffuse into soil micropores which may render them less available for fungal transformation (Gadd 2001). Nevertheless, fungal hyphae can easily bridge air-filled soil pores, crossing air-water interfaces, and cope with a heterogeneous distribution of soil nutrients and contaminants (Boswell et al. 2002, 2003, 2007; Jacobs et al. 2004; Harms et al. 2011, 2017).

Besides the physico-chemical features of soil, biological parameters and ecological interactions among microorganisms in soil microbial communities, such as competition, may also affect xenobiotic biotransformation (Fig. 1). This is particularly important in bioaugmentation applications for allochthonous fungi introduced into polluted soils, which have to compete with established indigenous communities, predators and pathogens (Gadd 2001). In contrast, indigenous fungal isolates, selected after long term exposure to the selective pressure of high contamination,

310 can be more tolerant to contamination, and environmental conditions, and may provide excellent
311 candidates for bioremediation (Godoy et al. 2016; Gururajan and Belur 2018).

312 Fungi possess a versatile machinery of highly potent and relatively non-specific enzymes
313 able to degrade the major natural polymeric constituents of wood, leaves and other plant materials,
314 such as lignin and cellulose, as well as mixtures of different organic pollutants (Gadd 2001, 2004;
315 Harms et al. 2011, 2017). Regarding nutritional strategies, many fungi are characterized by non-
316 specific transformation of other substrates along with the contaminants or co-metabolism with little
317 benefit from the potential input of pollutant carbon and energy (Harms et al. 2017). A separation
318 between biomass production and contaminant transformation in fungi is achieved by aerobic
319 extracellular reactions exerted by a range of extracellular enzymes including relatively non-specific
320 oxidoreductases (Harms et al. 2011, 2017). Less or more toxic intermediates may be generated by
321 fungal biotransformations, which in some cases can lead to the complete degradation of the
322 pollutants. Fungal species, belonging to wood-rot and litter-decay groups, are able to completely
323 degrade ligninocellulosic components of wood and are especially important in pollutant degradation
324 (Gadd 2001; Harms et al. 2011, 2017). Since the discovery of extracellular ligninolytic enzymes in
325 *Phanerochaete chrysosporium*, this organism has been proposed for the bioremediation of a wide
326 variety of persistent organic pollutants. Of the main categories of wood-rotting fungi, white-rot and
327 brown-rot fungi are the most relevant (Harms et al. 2017). In white-rot basidiomycetes, the
328 simultaneous transformation of lignin, lignocellulose and cellulose is achieved by a non-specific
329 multi-enzyme machinery in which enzymes can work separately or cooperatively (Tortella et al.
330 2005; Sánchez 2009; Harms et al. 2017). Constitutive extracellular enzymes can be expressed under
331 nutrient limitation, especially in white-rot fungi. In this case, enzymatic activity is not dependent on
332 the concentration, chemical species or the mixture of pollutants involved. Hence, adaptation to the
333 pollutant, enzyme induction and pollutant uptake are not required allowing low solubility
334 compounds to be degraded (Harms et al. 2011, 2017). In the case of pollutant co-metabolism, co-
335 substrates, e.g. cellulose or other easily utilized carbon sources, are required (Gadd 2001). There are
336 two main extracellular enzyme systems which can directly attack wood constituents (Tortella et al.
337 2005; Sánchez 2009). The first is responsible for hydrolytic polysaccharide degradation by
338 hydrolases, while the second consists of an extracellular ligninolytic system for lignin degradation,
339 including phenol oxidases, e.g. lignin peroxidase, manganese-dependent peroxidase (MnP), laccase,
340 and dioxygenases (Tortella et al. 2005; Sánchez 2009). Other enzymes, e.g. superoxide dismutase
341 and glyoxal oxidase, cooperate with the previous enzymes but they cannot attack wood constituents
342 on their own. This non-specific multi-enzymic machinery enables white-rot fungi to incidentally
343 degrade a wide range of xenobiotics (Harms et al. 2011, 2017). In brown-rot fungi the process

involves an oxidative radical-based system which attacks the wood constituents structurally and chemically, without substantially modifying the lignin (Harms et al. 2017). Highly reactive hydroxyl radicals enable a very non-specific oxidation of many organic compounds and are produced in extracellular Fenton reactions involving Fe^{2+} and H_2O_2 (Zhu et al. 2016; Harms et al. 2017). There is also another group of fungi, the so-called soft-rots, that are often ascomycetes. These can affect and modify lignin without degrading it, decompose cellulose and hemicellulose and create characteristic cavities due to wood cell wall degradation (Gao et al. 2018). Moreover, besides these true wood-degrading species, several free-living saprotrophic soil fungi possess efficient enzyme systems for cellulose and hemicellulose degradation, as well as xenobiotic biotransformation (Harms et al. 2011, 2017). Co-metabolic biotransformation of organic pollutants, which is less compound-specific than degradation pathways, predominates in these fungal groups (Harms et al. 2011, 2017).

Laccases are blue multicopper oxidases, which occur frequently as multiple isoenzymes in ascomycetes, basidiomycetes and anamorphic fungi (Brijwani et al. 2010). Laccases are particularly abundant in white-rot fungi but have also been reported in several fungal species which are not directly involved in lignin metabolism such as lower zygomycetes and chytridiomycetes, as well as soil saprotrophic fungi belonging to *Penicillium*, *Aspergillus* and *Curvularia* genera (Brijwani et al. 2010). Laccase can directly catalyse the four-electron reduction of oxygen to water to oxidize various phenolic pollutants, aromatic amines, and anthraquinone dyes, and, in the presence of redox mediators, the laccase substrate range can be expanded to oxidize non-phenolic substrates (Baldrian 2003; Harms et al. 2011, 2017). Fungal lignin-modifying peroxidases, such as manganese peroxidase (MnP), lignin peroxidase (LP), and a versatile peroxidase, can also degrade xenobiotics (Harms et al. 2011, 2017). Although these enzymes have been mainly found in white-rot fungi, MnP activity has been also reported in some saprotrophic fungi for biotransformation of humic substances (Řezáčová et al. 2006). Potentially toxic metals can affect the rate and extent of xenobiotic biotransformation (Fig. 1) (Baldrian 2003; Gadd 2010; Dighton 2016). Enzyme transcription and activity of MnP was increased by Mn in *Phanerochaete chrysosporium* and *Pleurotus eryngii* (Ruiz-Dueñas et al. 1999) and for laccase by copper in *Trametes versicolor*, *Aspergillus flavus*, *P. ostreatus* and several other fungal species (Baldrian et al. 2000; Baldrian 2003; Gomaa and Momtaz 2015). An influence on enzyme expression and activity of laccases and other ligninolytic enzymes by metals might also occur in other non-white-rot fungi, such as *Aspergillus* and *Penicillium* genera, in which some such enzymatic activities have been documented (Giardina et al. 2010). In the aquatic environment, the degradation of some PAHs in the presence of different metals by a metal-tolerant consortium composed of *Bacillus subtilis* and *Acremonium* sp.

378 was enhanced by Mn (Ma et al. 2017). The metal sensitivity of *P. ostreatus* towards Cu and Cd
379 changed with time in relation to growth and colonization of the substrate and resulting changes in
380 the extent of enzyme regulation (Baldrian 2003). Even though all known laccase isoenzymes are
381 positively regulated by copper, the extent of induction can be different as well as their production
382 (Baldrian 2003). Cu was also reported to affect extracellular protease activity responsible for
383 laccase degradation which in turn lead to enhanced stabilization and performance of laccase in the
384 extracellular environment (Baldrian 2003). In nitrogen-limited liquid medium, Cd induced laccase
385 production only during the later stages of growth in *P. ostreatus* without affecting laccase stability,
386 while, in other conditions, it inhibited ligninolytic activity in the wood-rooting basidiomycetes *P.*
387 *chrysosporium*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus* and *Stereum hirsutum* and the rate of
388 decolourization of Poly R-478 dye (Baldrian 2003). The activity of both Mn-dependent and Mn-
389 independent peroxidases of *S. hirsutum* was strongly reduced by the addition of 0.25 mmol/l Cd,
390 while the activity of laccase from *S. hirsutum* and ligninase from *P. chrysosporium* were less
391 sensitive (Baldrian 2003). The activity of all enzymes tested in both *S. hirsutum* and *P.*
392 *chrysosporium* were significantly inhibited by Cd concentrations higher than 0.5 mmol/l. In control
393 samples, maxima of MnP activity occurred after the onset of the stationary phase, while this was
394 shifted to the exponential phase of growth in the presence of Cd (Baldrian 2003). Cd and Hg also
395 influenced the activities of ligninolytic enzymes in *P. ostreatus* incubated in soil, thereby affecting
396 the degradation of polycyclic aromatic hydrocarbons (Baldrian et al. 2000). In *P. ostreatus*, Hg
397 inhibited laccase production, but slightly increased MnP activity: in contrast, MnP activity was
398 decreased by low concentrations of Cd, Cu and Hg (Baldrian 2003). Metals, such as Cd and Hg, can
399 inhibit mycelial penetration in soil resulting in incomplete fungal colonization at relatively low
400 metal concentrations and impair *in situ* bioremediation (Baldrian et al. 2000). Generally, toxic
401 elements inhibit enzymes by binding to thiol groups of the activation or regulatory sites of the
402 enzyme or by causing irreversible inactivation by oxidative damage of aromatic amino acid residues
403 due to the production of reactive oxygen species (Baldrian 2003; Gadd 2010; Dighton 2016; Liu et
404 al. 2017). After entering the cell, metals can also influence the production of extracellular and/or
405 intracellular enzymes at the levels of transcriptional and translational regulation, affecting other
406 enzyme systems involved in metabolic degradation pathways or altering detoxification systems
407 (Baldrian 2003; Liu et al. 2017). For instance, cellulase extracted from *Trichoderma*
408 *longibrachiatum* was positively or negatively affected by different metal cations in the degradation
409 of dicofol: for Na⁺ and K⁺ no effects were observed. Mn²⁺ promoted the pesticide degradation while
410 a marked reversible competitive inhibition was reported for Cd²⁺; Ca²⁺ promoted dicofol

411 degradation only at low concentrations while Pb^{2+} irreversibly denatured the cellulase (Zhai et al.
412 2015).

413 The effects of toxic elements and xenobiotics on fungal biology and the environment are
414 complex, and detailed mechanisms of interaction remain largely unexplored (Liu et al. 2017).
415 Mixtures of organic and/or inorganic pollutants will involve different specific or unspecific effects
416 in complex contaminated environments with multiple stresses (Harms et al. 2017). The release of
417 toxic metals as organic metal complexes can accelerate fungal degradation of persistent carbon
418 sources, such as charcoal and black shale. Fungi can release or degrade organometallic and
419 organometalloid compounds, some which can be of natural origin (Hg, Te, Se) or be
420 anthropogenically produced for use in agriculture and industry (Fig. 1) (Gadd 2010).

421 Toxic elements (e.g. metals and metalloids) can change the properties of fungal surfaces
422 affecting xenobiotic sorption (Liu et al. 2017). At high metal concentrations, toxicity can cause
423 oxidative stress due to ROS production, altering fungal development, organization and homeostasis,
424 damaging DNA and cell membranes and altering the activity of both catalytic and anti-stress
425 enzymes, resulting in an overall reduction of fungal defence mechanisms against toxic effects of
426 xenobiotics (Sandrin and Maier 2003; Liu et al. 2017). On the other hand, xenobiotic compounds,
427 such as PAHs, can increase metal permeability to cells, inhibit metal sorption, and impair ion
428 regulation and transport systems (Liu et al. 2017). Low metal concentrations can enhance
429 xenobiotic degradation due to the formation of enzyme-metal-substrate complexes, as metals may
430 act as enzyme cofactors and increase the activities of degrading enzymes (Sandrin and Maier 2003;
431 Liu et al. 2017). In conclusion, depending on the chemical species involved, the physico-chemical
432 environmental features and the physiological properties of the exposed organisms, synergistic or
433 contrasting effects can be observed, both on the ability to metabolize and detoxify the xenobiotic
434 compounds, and to manage the toxic metals, with different results depending on the combining
435 interactions of all involved factors.

436

Methods to evaluate the effects of metals on fungal aerobic biodegradation of organic pollutants

The study of negative or positive effects of toxic metals on fungal tolerance and biodegradation of organic pollutants has so far been dealt with in few works. However, the complexity of the variables involved when there are multiple contaminants is a theme that is strongly felt in the scientific community and already well addressed in the study of bacteria (Cui and Zhang 2008; Chen et al. 2015a; Alvarez et al. 2017; Liu et al. 2017; Ye et al. 2017). The analysis of co-contamination effects is based so far on experimental schemes already applied to the study of contamination by single compounds, with the complication that in the presence of co-contamination the combination of events and processes are necessarily more variable, and therefore more difficult to quantify. Co-contamination has measurable impacts on the aquatic and terrestrial environment and its fungal communities. The link between species abundance and community structure in polluted sites has been addressed using many diverse methods. Recently, techniques are based on massive DNA or RNA analysis of diversity (Kirker et al. 2017; Bourceret et al. 2016; Markowicz et al. 2016). Environmental genomics will strongly help in understanding fungal and bacterial physiology and ecology in the presence of multiple contaminants and evaluating the effects of bioremediation on communities (Mougin et al. 2013) (Fig. 2). The selection and use of fungal species tolerant to toxic substances, both for potential applications in bioremediation and for a more focused study of tolerance mechanisms is one of the main approaches, relevant to situations of co-contamination. Isolation of indigenous fungi could provide the best candidate organisms for bioremediation of polluted soil since they already belong to an established soil microbial community and may be adapted to the site conditions (Czaplicki et al. 2016). For example, Srivastava and Thakur (2006) isolated tolerant fungal strains in leather tanning effluent to test their potential to remove chromium in the presence of toxic organic chemicals. The authors used the effluents for an initial selection of the strains, and then tested the potential of chromium bioaccumulation in shake flask culture at different pH values, temperatures, inoculum size, carbon and nitrogen sources. Sharaf and Alharbi (2013) isolated fungal strains from a chromium contaminated soil, and tested them for the removal and immobilization of toxic metals from tannery wastewater. The mechanisms at play are mainly analysed using model organisms or experimental schemes based on the use of microcosms which represent simplified systems. Baldrian et al. (2000) used the white rot fungus *Pleurotus ostreatus* in a 15-week incubation in non-sterile soil microcosms to analyse the influence of metals on fungal enzyme activity and polycyclic aromatic hydrocarbon degradation. More recently, Wang et al. (2017) investigated the remediating potential of the co-cultivation of *Pleurotus eryngii* and *Coprinus comatus* on a soil affected by cadmium and an organic pollutant (endosulfan). The

471 efficacy of metal biosorption by fungal mycelia, and the biodegradation of endosulfan were
472 evaluated in pot experiments, using e.g. flame atomic absorption spectrophotometry for
473 quantification of Cd in fungal fruiting bodies, and gas chromatography-mass spectrometry (GC-
474 MS) to measure residual endosulfan in the soil. The evaluation of by-products released during
475 bioremediation experiments and the analysis of residues and reactive chemical species generated by
476 the degradation of xenobiotics is vital in these types of studies, since the intermediate metabolites of
477 fungal catabolism are often toxic. The transformation of recalcitrant pollutants into environmentally
478 benign products through fungal metabolism in fact, is rarely straightforward. However, the toxicity
479 of intermediates is generally lower than the parental pollutants, but sometimes more toxic
480 metabolites can arise from biotransformation and this may depend on specific metabolic pathways
481 or by a biochemical transformation catalysed by a specific enzyme of the microorganisms involved
482 (Guzik et al. 2013; Lu et al. 2013). Moreover, the use of analytical methods is expensive and
483 limited by not detecting all possible chemical intermediates arising from xenobiotic
484 biotransformation. It is clear that mixtures of known and unknown compounds can cause acute and
485 chronic toxic effects in organisms, which cannot be assessed by only a chemical approach.
486 Bioanalytical and ecotoxicological tools, e.g. bioassays, biosensors, and effect-directed analysis,
487 should be more and more integrated with chemical analyses in a wider interdisciplinary and
488 ecological context. This could provide an overall view, characterizing the environmental situation
489 before and after the bioremediation application in polluted sites. In heterogeneous systems such as
490 soils with high concentration of metals and xenobiotics, an integrated approach may be very useful
491 and provide better visualization of bioremediation performance (Blasco and Picó 2009; Spina et al.
492 2018).

493 The known pathways for single contaminants might be no longer valid for co-contamination
494 when other mechanisms and defence systems intervene in fungal metabolism. Chanda et al. (2016),
495 for example, found that the synthesis of laccase and other extracellular peroxidases by some
496 filamentous fungi was higher in the presence of intracellular oxidative stress, and Ceci et al. (2018)
497 showed that *Penicillium griseofulvum* was capable of activating pathways to use different
498 substrates, tolerating a high concentration of a combination of hexachlorocyclohexane and
499 vanadium with a supposed co-metabolic mechanism.

500 The study of the mechanisms that operate in fungi in the presence of toxic substances is
501 clearly of particular importance (Fig. 2). The methods and techniques put in place to elucidate
502 tolerance mechanisms have been significantly refined in the last decade. Application of proteomics,
503 gene expression studies, and the use of gene-replacement mutants have recently contributed to
504 assign most of the steps in pathways of aromatic compound degradation to particular genes

505 (Deshmukh et al. 2016). Though the area of mycoremediation is well studied, there are some
506 degradation pathways which are not totally understood. Development of molecular biology
507 techniques is helping in the comprehension of enzyme-mediated pathways and even allowing the
508 design of customised expression systems for bioremediation (Deshmukh et al. 2016). Whole
509 genome sequence analysis is revealing the capability of fungi for multiple metabolic adaptations
510 owing to diverse enzyme functions (Ichinose 2013; Fernández-Fueyo et al. 2014).

511

512 **The potential of fungi (alone or together with bacteria) to increase biodegradation of organic**
513 **pollutants in co-contaminated environments**

514 The potential of soil fungi in bioremediation of xenobiotics alone or in combination with other
515 potentially toxic elements is still largely untapped (Harms et al. 2011, 2017). The feasibility of
516 bioremediation by fungi or microbial consortia lies in overcoming the physico-chemical limitations,
517 e.g. pH, and reliant on a thorough knowledge of the ecological relations in soils (Alisi et al. 2009;
518 Harms et al. 2017). Some studies of xenobiotic biotransformation by fungi in the presence of toxic
519 elements are shown in Table 1. In Table 1, different classes of xenobiotics, including PAH and
520 pesticides, and several toxic metals have been considered so far. Among the reviewed literature, the
521 combinations of metal species studied were different, as well as those of PAHs, reflecting high
522 heterogeneity in the experimental design. Cd and Mn have been investigated not in mixture, as well
523 as the PAHs, phenanthrene and benzo[a]pyrene, and the halogenated pesticides endosulfan,
524 dichlorophen and 2,4,5-trichlorophenol. Most of the studies were focused on the xenobiotic
525 biotransformation as percentage removal and the effects of metals in affecting biodegradation,
526 whereas metal removal has also been considered in Wu et al. (2016). For both metal and xenobiotic
527 removal, most percentage values are very high (>90%). A relatively large number of papers
528 investigated on white-rot fungi, e.g. *Pleurotus ostreatus*, either alone or in co-cultivation, e.g.
529 *Pleurotus eryngii* and *Coprinus comatus* (Wang et al. 2017). Some nonligninolytic saprotrophic
530 fungi are studied in co-contaminated conditions (metals with PAHs), either alone (*Acremonium* sp.
531 in Ma et al. (2014) or in separated culture with white-rot fungi (*Fusarium flocciferum* and
532 *Trichoderma* spp. with *Trametes versicolor* and *Pleurotus ostreatus* in Atagana (2009)). Fungi
533 associated with bacteria in microbial consortia have been shown in several cases to increase the
534 effectiveness of xenobiotic biotransformation.

535 Diverse bacteria inhabit the interface habitat provided by soil fungal mycelium, known as
536 the mycosphere (Zhang et al. 2014). Another very important habitat in soil fungal-bacterial
537 interactions is the mycorrhizosphere, which is the interface zone between plant roots and associated
538 fungal species (mycorrhizas) (Zhang et al. 2014). Both habitats provide nutrient exchanges, and are
539 sites of gene transfer with species-specific interactions involving transfer of substrates and products
540 from xenobiotic biodegradation (Zhang et al. 2014; Ponomarova and Patil 2015). In fact, a
541 microbial consortium as a multi-component system provides a rich metabolic network which can
542 overcome the limitations of a single microbial species in xenobiotic biodegradation processes,
543 leading to complete biodegradation (Alisi et al. 2009). Moreover, microbial consortia represent a
544 more realistic simulation of real environmental conditions, helping in filling the gap between
545 laboratory and *in-situ* studies (Alisi et al. 2009). Furthermore, mycelial networks, perfectly adapted
546 to the heterogeneous environment of soil, can be used by bacteria as soil dispersal vectors (Harms et
547 al. 2017). These “fungal highways” can promote bacterial access within the soil habitat and increase
548 bioavailability of xenobiotics and potentially toxic elements for microbial communities (Harms et

549 al. 2017). The hyphae can also grow into very small soil pores and mobilize xenobiotics and toxic
550 metals, serving as “pipeline” through vesicle-bound cytoplasmic transport, e.g. PAH cytoplasmic
551 transport (Gadd et al. 2012; Harms et al. 2017).

552 Moreover, fungi and bacteria can produce biosurfactants, which can increase the
553 bioavailability of xenobiotics and metals, improving the bioremediation performance without the
554 need of synthetic and often recalcitrant compounds (Bhardwaj 2013).

555 Fungi alone or in microbial consortia can successfully be applied for environmental
556 detoxification and there are several studies in this direction, e.g. management of sites contaminated
557 by explosives (e.g. TNT) (Stenuit and Agathos 2010), soils affected by antibiotics (Liu et al. 2017)
558 polyphenols, nitriles, cyanides and PAHs (Rao et al. 2010).

559 The bioremediation mechanisms for combined pollution of PAHs and toxic metals by fungi
560 and bacteria are also well documented (Liu et al. 2017). For example, Jiang et al. (2015) observed
561 the improving effects of inoculation of *Bacillus thuringiensis* in the effective fungal bioremediation
562 of Cd and phenanthrene by *Pleurotus cornucopiae*. The bacterial strain was able to improve fungal
563 growth, enhance Cd accumulation in the fungal biomass, increase phenanthrene degradation by *P.*
564 *cornucopiae* and reduce oxidative stress (Jiang et al. 2015). However, there are also studies where
565 fungal-bacterial consortia performed poorly in co-contaminated sites. For example, metals affected
566 PAH degradation by a metal-tolerant consortium comprising *Acremonium* sp. and *Bacillus subtilis*
567 (Ma et al. 2014, 2017) (Table 1). The inhibition in this case study was dependent on metal species
568 and the PAH compound involved (Ma et al. 2017). In contrast, Liu et al. (2017) reported that
569 microbes that were isolated from long-term polluted soils and sediments appeared well-adapted to
570 high xenobiotic and metal concentrations, and efficiently mediated the biotransformation of
571 different PAH, such as anthracene, naphthalene, phenanthrene, pyrene and benzo[a]pyrene,
572 simultaneously relieving suppression due to metals toxicity from, e.g. Cd, Cu, Cr and Pb, which are
573 often found along with PAH.

574 Fungal and bacterial bioremediation processes can be associated with plants in microbially-
575 assisted phytoremediation, in which plants can provide exudates both for supporting xenobiotic co-
576 metabolic activity, for instance in oligotrophic soils, and protection from metal toxicity (Liu et al.
577 2017; Harms et al. 2017). The presence of plants can induce higher bacterial and fungal biodiversity
578 in soils contaminated with metals and PAH than in bare soil (Bourceret et al. 2016). The integration
579 of bacteria, fungi and plants could improve the efficacy of bioremediation processes, both in soil
580 and water systems (Zhu et al. 2012; Arjoon et al. 2013). For instance, bacteria and fungi could
581 promote plant growth, protect plant roots from chemical stress, degrade xenobiotics, transfer or
582 immobilize metals, while plants can provide nutrients for microorganisms, improve microbial

583 biodegradation, and translocate metals to roots and shoots for compartmentation or volatilization
584 (Zhu et al. 2012; Chirakkara et al. 2016).
585

586 **Future perspectives and concluding remarks**

587 The optimization of cost-effective, environmentally-friendly bioremediation processes based on
588 fungi, alone or with bacteria, in the context of co-contamination needs further research in several
589 areas. There are some main routes that research in this field might take:

590 a) Screening of new species. The need for selecting “new” microbial candidates that are highly
591 efficient in biodegradation of xenobiotics in the presence of toxic metals and better adapted to
592 ecological conditions in the field is essential to improve bioremediation applications,
593 competitiveness and feasibility. Given some evidences of a stronger potential in biodegradation
594 of xenobiotics in the presence of metals, the choice of microbial consortia should be preferred
595 to single species and the complex interactions between fungi and bacteria in microbial consortia
596 for bioremediation purposes should be further investigated both from the chemical and
597 ecological perspectives.

598 b) Bioavailability. In order to achieve efficient biodegradation, pollutants must be available for the
599 fungal species to assure efficacy and the timescale of the bioremediation. That is to say,
600 bioremediation research should also focus on the bioavailability of toxic compounds and related
601 aspects that can improve xenobiotic biotransformation and metals biostabilization. The
602 bioavailability of metals can also affect xenobiotic biodegradation and fungal tolerance to both
603 classes of pollutants, and may impair biodegradation of the organic pollutants. More efforts
604 must be made to assess the bioavailable fraction of pollutants that are really involved in the
605 bioremediation processes, as in most of the studies, only the chemical concentrations have been
606 so far considered. Moreover, further investigation is needed to better understand the
607 mechanisms and substances that can be involved (e.g. biosurfactants) in fungal bioavailability
608 of xenobiotics, as well as the release of these substances and their role in metals mobilization,
609 since these factors can strongly affect biodegradation performances.

610 c) Toxicity mechanisms of metals and xenobiotics on fungi and bacteria. Further investigation on
611 the deleterious effects that the contaminants pose on microbes is required to better understand
612 the metabolic responses involved and to identify detoxification mechanisms, especially for
613 mixed fungal and bacterial consortia.

614 d) Integrated multi-disciplinary view of co-contaminated sites. Physico-chemical and biological
615 characterization of the investigated site should be performed as much as possible to design the
616 most effective applications and improve the bioremediation efficiency.

617 e) Enzymes. The functions, activity and regulation of enzymes released by fungi (and bacteria) in
618 biodegradation processes need further research, especially on the impact of toxic metals on in

affecting the efficiency of xenobiotic biotransformation, the metabolic pathways involved, co-metabolism, and the interaction mechanisms of multi-enzyme systems with xenobiotics.

In conclusion, this review summarizes some of the main aspects involved in fungal bioremediation of sites contaminated by both toxic metals and xenobiotics. Different features of fungal metabolism, physiology, biochemistry and ecology have been discussed, and biological interactions between fungi and bacteria in consortia have also been considered. Relatively non-specific co-metabolism of fungi along with their high ecological versatility and adaptation to adverse environmental conditions provides very strong potential for biodegradation of organic pollutants. New research could shed further light on such processes, mechanisms and methods to improve fungal bioremediation applications.

Acknowledgements

GMG gratefully acknowledges financial support in his laboratory from the Natural Environment Research Council, UK [NE/M010910/1 (TeaSe); NE/M011275/1 (COG3)].

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986 **Legends to figures**

987 **Fig. 1** Metal speciation, xenobiotic and microbial interactions. (1) Physico-chemical controls on
988 metal speciation, xenobiotic bioavailability and microbial activity. Effects may be interlinked and
989 important factors include pH; organic matter; humic materials; clays and other minerals; cation
990 exchange capacity; Fe, Mn oxides and hydroxides; redox potential; inorganic and organic soil
991 colloids; salts and complexing agents; anions; cations; organic and inorganic pollutant
992 concentrations; photochemical transformation of xenobiotics; O₂; CO₂; H₂O; transport and run-off
993 movements in the aqueous phase; diffusion and vapour phase distribution; temperature; nutrients; C
994 and energy source, including those for co-metabolism of xenobiotics; N, P, S, essential metals, e.g.
995 Fe, Cu, Mn, Co; plant growth and root exudation etc. (2) Metal mobilization by mechanisms
996 including chemolithotrophic and chemoorganotrophic bioleaching; bioweathering, biodeterioration
997 and mineral dissolution; metal complexation by organic acids and metabolites; siderophores; H⁺,
998 CO₂; redox transformations, e.g. Fe(III) to Fe(II), Mn(IV) to Mn(II); litter decomposition (3) Metal
999 immobilization by mechanisms including biosorption; intracellular uptake and accumulation;
1000 biomineralization/bioprecipitation, e.g. carbonates, phosphates, sulfides, oxides, hydroxides,
1001 oxalates; nanoparticle formation; redox transformations, e.g. Fe(II) to Fe(III), Mn(II) to Mn(IV),
1002 U(VI) to U(IV); metalloid reduction, e.g. Se(VI, IV) to Se(0) (4) Influence of unavailable metal
1003 species on microbial populations such as substrate for biofilm development; adsorption of nutrients,
1004 ions and pollutants; source of essential elements, e.g. K, P; contribution to soil chemistry, properties
1005 and structure, e.g. cation exchange capacity, buffering power, porosity etc. (5) Influence of
1006 available metal species on microbial populations such as alterations in community composition,
1007 biomass and diversity; selection of resistance/tolerance; toxicity; essentiality; effects on enzyme
1008 activity and microbial processes, e.g. N₂ fixation, organic matter and pollutant decomposition;
1009 influence on distribution and cycling of elements; translocation of metals through mycelium (fungi)
1010 and/or transfer to plant hosts (6) Sorption or other associations of xenobiotics with unavailable
1011 metal species such as mineral surfaces (7) Desorption or release of xenobiotics (8) Physico-
1012 chemical controls on interactions between xenobiotics and unavailable metal species, such as
1013 sorption. Physico-chemical effects will also occur on interactions between xenobiotics and available
1014 metal species, such as complexation (not shown on diagram) (9) Direct effects of xenobiotics on
1015 microbial populations (10) Interactions between bioavailable metal species and xenobiotics such as
1016 complexation, chelation or binding resulting in alteration of metal and xenobiotic speciation,
1017 bioavailability, and toxicity and rates and extent of xenobiotic degradation. (11) Effects of metal-
1018 xenobiotic associations on microbial interactions and processes which can lead to changes in the

1019 effects indicated in (5) and (9) (see Baldrian 2003; Sandrin and Maier 2003; Gadd 2004, 2010,
1020 2016; Gianfreda and Rao 2008; Liu et al. 2017; Asif et al. 2017)

1021 **Fig. 2** The evaluation of joint effects of toxic metals and organic pollutants on fungal
1022 tolerance and biodegradation ability is based so far on experimental schemes already applied
1023 to the study of contamination by single compounds, Co-contamination has measurable
1024 impacts on the environment and its fungal communities, due, for example, to selection of
1025 tolerant species (1–4). The mechanisms at play in co-contamination are better analysed using
1026 model organisms or experimental schemes based on the use of microcosms which represent
1027 simplified systems where some variables might be better controlled (5–8). The differential
1028 expression of genes or the activation of specific pathways in the presence of known
1029 combinations of toxic metals and organic pollutants are very important for elucidating the
1030 mechanisms involved in tolerance or for modelling fungal biodegradation strategies (9–13).

1031