Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water

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Manuscripts

1   **Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water**

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20

21 ***Abstract***

22 Pesticides can help reduce yield losses caused by pests, pathogens, and weeds, but their

23 overuse causes serious environmental pollution. They are persistent in the environment and

24 are biomagnified through the food chain, becoming a serious health hazard for humankind.

25 Bioremediation, where microbes are used to degrade pesticides *in situ*, is a useful technology.

26 This review summarizes data on the fungi involved in the biodegradation of chemical

27 pesticides and their application in soil and water bioremediation. Indications for future

28 studies in this field are given.

29

30 Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi,

31 Synthetic microbial community, Environmental risk assessment.

32

Introduction

34 Because of their unique functions, fungi are involved in ecosystem services essential to human

35 well-being. Among others, fungi also carry out the transformation and detoxification of pollutants.

36 For this reason, learning from nature, they represent an effective toolbox for a sustainable

37 bioremediation of pesticides in soil and water. Many researches have revealed the untapped

38 potential of fungi, and recent years have witnessed very interesting developments regarding the

39 application of fungi not only to improve environmental quality but also human health (e.g. Gargano

40 et al. 2017).

41 Pesticides are a diverse group of inorganic and organic chemicals that include herbicides,

42 insecticides, nematicides, fungicides, antibiotics and soil fumigants (Verger and Boobis 2013;

43 Verma et al. 2014). They are employed in agriculture to enhance crop yield and quality, and to

44 maximize economic returns by preventing pest or weed attack. They are bioactive, toxic substances,

45 capable of directly or indirectly influencing soil fertility and health as well as agroecosystem quality

46 (Pinto et al. 2012; Verma et al. 2014). Given that belowground biodiversity is closely linked to land

47 management, agricultural intensification exerts many pressures that lead to loss of biodiversity.

48 Consequently, soil pollution is one of the main threats to the decline of taxonomic and functional

49 biodiversity, and to agricultural soil sustainability (Harms et al. 2017). Most pesticide emission

50 (99 %) in Europe is associated with agricultural practices, whereas industrial and urban sources

51 such as the manufacturing of pesticides or the at-home use of insecticides have a minor impact

52 (EEA 2016).

53 The extensive and massive use of pesticides in agricultural activities has a serious impact on the

54 environment, compromising soil and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al.

55 2016). In addition to pesticides, large quantities of antibiotics are added to agricultural fields

56 worldwide through the application of wastewater, manures and biosolids, also resulting in antibiotic

57 contamination and elevated environmental risks (Jechalke et al. 2014; Zhang et al. 2015; Pan and

58 Chu 2016). A clear correlation between agriculture and water contamination was observed in Mar

59 Chiquita lake (Argentina), where large amounts of endosulfan residues were detected soon after

60 application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide

61 thifluzamide in the water in rice paddies in China was maximal after application, with variation

62 over time associated with the dilution effect of rainfalls in the area (Wei et al. 2015).

63   Preventive measures are required, to mitigate the impact of agriculture on the environment. These

64   must take into account both the use of safe pesticides and the optimization of farmer procedures.

65   Aravinna et al. (2017) found that most of the 32 studied pesticides leached off rice paddies

66   following specific pathways. Since direct runoff and erosion from soil were the main vehicles of

67   dispersion, authors suggested alternative strategies (high resident time for pesticides, holding ponds

68   for rice drainage water, delayed filling of paddies after pesticide application, and the use of less

69   mobile compounds) to reduce the movement of the pesticides.

70   The intensive use of organic agrochemicals (OACs) poses risks to both wild lives and human

71   health. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their

72   target species through air, water and soil (Miller 2004). Around 30% of pesticides marketed in

73   developing countries do not meet internationally accepted quality standards, posing a serious threat

74   to human health and the environment (Popp et al. 2013). They are persistent in the environment and

75   are biomagnified through the food chain, and it has been estimated that millions of agricultural

76   workers worldwide experience unintentional pesticide poisoning each year. The correlation between

77   long-term exposure to pesticides in occupational settings and illness is known, but recently non-

78   occupational exposures have also been associated with an elevated rate of chronic diseases (Parrón

79   et al. 2014).

80   Varieties and consumption of pesticides worldwide have increased dramatically, by up to 4-fold

81   since 40 years ago (Mnif et al. 2011). According to De et al. (2014), about 45 % are used in Europe,

82   25 % in the USA, and 25 % in the rest of the world. The main pesticide consumer is Spain (around

83   79,000 ton of active ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy

84   (~ 64,000), Germany (~ 46,000) and United Kingdom (~ 23,000) (Eurostat 2016). The United

85   States applies over 1 billion pounds annually (Alavanja 2009) with dramatic consequences for

86   human beings and environment (Carvalho 2017). According to other authors (Huang McBeath and

87   McBeath 2010), China is the world's largest pesticide user, with a pesticide output of around 3.7

88   million tons (National Bureau of Statistics of China - http://data.stats.gov.cn), and a consumption of

89   about 1.8 million tons in 2014. More than 350 insecticides, herbicides, microbicides, nematicides

90   and other pesticides are reported to be used. The average amount of pesticides used per hectare in

91   China is roughly 1.5- to 4-fold higher than the world average (Qiu 2011), thus resulting in

92   contamination of water bodies in the receiving areas and disturbance of ecological equilibrium (Hui

93   et al. 2003). Overall, use of pesticides in China breaks down as herbicides 47.5 %, insecticides

94   29.5 %, fungicides 17.5 % and others 5.5 % (De et al. 2014).

95   The adverse effects of OAC pollution have been of concern for a long time and many highly toxic

96   and persistent pesticides have been banned worldwide. Although relatively safer pesticides have

97 been developed and replaced the highly toxic ones, environmental pollution resulting from the long-

98 term application of pesticides is far from being solved. Obsolete pesticides still represent a threat to

99 environment, biodiversity, and human health for the region of Southeast Europe and their risk to the

100 environment and to humans needs to be assessed in order to mitigate it. Many organochlorines,

101 organophosphates and pyrethroids have been banned but this has not yet solved the problem

102 (Aravinna et al. 2017). In Argentina, hexachlorocyclohexane pesticides have been limited since the

103 late '90s and were definitely banned in 2011, but samples taken from a saline lake in 2014 showed

104 levels to be more than 5-fold over the legal limit of 4 ng/l for lindane levels in the environment

105 (Ballesteros et al. 2014). Likewise in China, although the use of organichlorine pesticides has been

106 banned for over 20 years, they can still be found in the water and sediments of main drainage areas

107 (Nakata et al. 2005; Xue et al. 2006; Zhou et al. 2006), due to run-off from aged and weathered

108 agricultural soils and from anaerobic sediments (Zhou et al. 2006). Water bodies and sediments, the

109 water, the soil and even the air in many cities in China are polluted by OACs, in both urban and

110 suburban areas (Gong et al. 2004; Nakata et al. 2005; Yang et al. 2008).

111 OACs pose pivotal environmental problems, due to their high resistence in the environment and the

112 consequent low natural attenuation. As an example, organochlorine pesticides were poorly affected

113 by photochemical, chemical and biological processes, and more than 95% of them impacted on non-

114 target organisms (Mrema et al. 2013). As a consequence, regulatory and risk assessment procedures

115 have to be adopted against OACs. Driven by the carcinogenicity of pesticides, Directive 91/414/

116 EEC aimed to regulate the authorization of pesticides marketing within the EU.

117 The particular attention given to pesticides is because, as confirmed in recent studies, even low

118 doses might trigger adverse effects on wildlife and humans (EEA 2005). As groundwater is our

119 primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking

120 Water Directive 98/83/EC deal with maximum pesticide exposure concentrations: 0.1 μg/l of a

121 single pesticide and 0.5 μg/l total pesticide load. Risk assessment needs to consider not only the

122 source of contamination, but also the multifaceted direct and indirect pathways of contact with

123 human beings. Kim et al. (2017) reported a number of routes pesticides might follow to meet human

124 beings; the resulting direct and indirect multi-pathway exposure may affect human health.

125 Experimental evidence of progress in natural restoration processes highlight that time is our ally,

126 since the abandonment of disturbed/polluted agricultural land for long time can reduce

127 contamination (Kardol and Wardle 2010). Studies by Morriën et al. (2017) reported that nature

128 restoration on ex-arable land resulted in increased connettance of soil biota networks, as restoration

129 progresses. Such results confirm that soil biota provide many and varied services, and that

130 detoxification of pollutants and xenobiotics is one of the primary ones.

131 In this context, innovation involves the search for solutions inspired by nature, with the strategy

132 being to accelerate the natural attenuation processes in contaminated sites. Bioremediation has

133 arisen as a useful technology to degrade OACs (Singh 2008; Velázquez-Fernández et al. 2012),

134 with several benefits over landfill disposal and incineration, such as the formation of non-toxic end

135 products, lower costs of disposal, reduction of effects on health and ecology and on the long-term

136 liabilities associated with destructive treatment methods, and the ability to perform the treatment *in*

137 *situ* without unduly disturbing native ecosystems (Sarkar et al. 2005). Over the past decade,

138 numerous microorganisms capable of degrading antibiotics and pesticides have been isolated, and

139 detoxification processes for target pollutants have been analyzed. Fungi and especially ligninolytic

140 fungi have been suggested as the most promising group of organisms, as they are able to transform

141 recalcitrant compounds through a unique set of extracellular oxidative enzymes (Anastasi et al.

142 2013; Harms et al. 2017). Comparative genomic analysis of 49 fungi with different nutritional

143 modes, such as saprotrophic fungi, white-rot fungi (WRF), brown-rot fungi, soft rot fungi and

144 symbiotic fungi indicate that there is a relationship between nutrition models and the enzymes for

145 lignocellulose degradation. Saprotrophic fungi have a greater number of enzymes than symbiotic

146 fungi, and brown-rot fungi have a smaller number than WRF and soft rot fungi (Wu et al. 2015a).

147 This might provide some insight into how to choose fungi in OACs degradation.

148 Finally yet importantly, the metabolic activity of fungal or microbial consortia could potentially

149 produce unknown reaction products that are more toxic than the parent compounds. García-

150 Carmona et al. (2017) highlighted the importance of carrying out environmental monitoring

151 activities ante- and post-operam phases, using bioassays to determine the success of the

152 bioremediation process. Although it is fundamental to assess the quality of the environment to

153 ensure it remains free of toxic residues, most of the analytical tests available for determining the

154 concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason,

155 biotoxicity testing has grown steadily in recent years and is a useful tool in environmental risk

156 assessment (Shen et al. 2016; Prokop et al. 2016).

157 Indeed, there is a clear need to develop and define decontamination of hazardous pollutants as a

158 concept that will support sustainable remediation by involving a broader uptake of principles,

159 approaches and tools that integrate environmental, social and economical dimensions into

160 remediation processes (Ridsdale and Noble 2016). Several organizations, academia and

161 standardization committees are currently assessing remediation process and evaluating the

162 complexity of sustainability. Documents have been developed by many countries across Europe and

163 globally, addressing sustainable indicators for remediation activities (Harclerode et al. 2015).

164 The present review summarizes the current state of scientific knowledge on research and

165 application of fungi as effective bioresources, considering recent advances in understanding their

166 capacity to face up the pesticide contamination.

167

Bioremediation of OACs by fungi in the soil system

169 Large quantities of OACs are being added to agricultural fields worldwide through the application

170 of wastewater, manures and biosolids, resulting in pesticide and antibiotic contamination and

171 elevated environmental risks in terrestrial environments (Jechalke et al. 2014; Zhang et al. 2015;

172 Pan and Chu 2016). A large proportion of the OACs applied to soils with manure or biosolids are

173 retained in surface soil, whereas those added through irrigation with wastewater can seep down to

174 lower horizons or be diffused in surface run-off. Once present, OACs interact with the solid phase

175 of soil and are prone to microbial transformation (Hammesfahr et al. 2008; Jechalke et al. 2014). In

176 particular, veterinary antibiotics interact with the soil solid phase in sorption and desorption

177 reactions. Sorption and desorption control not only their mobility and uptake by plants but also their

178 biotransformation and biological effects. OACs, like microorganisms are not distributed

179 homogeneously in soil but are concentrated in hotspots. The multiplicity of surfaces, voids, and

180 pores provided by soil aggregates harbor a vast amount of biological diversity and chemical

181 variability, and cause patchy distribution of natural organic matter, oxides, nutrients, and

182 microorganisms on soil particle surfaces (Hammesfahr et al. 2008; Jones et al. 2012). Sorption,

183 sequestration, and subsequent release of OACs likely also occur at and from hotspots. Little is

184 known about the behavior of OACs at environmentally relevant concentrations in agricultural soil.

185 Recently, many studies have highlighted the ability of fungi to transform and degrade recalcitrant

186 OACs. In particular, one of promising group is the ligninolytic fungi that possess a unique set of

187 extracellular enzymes suitable to degrade lignin and are able to transform recalcitrant compounds,

188 (Čvančarová et al. 2015) (Supplemental material Table I; Table I References). Nguyen et al. (2014)

189 reported the removal of diverse trace organic contaminants ichloroethyl chloroformate (TrOC)

190 including phenolic and non-phenolic compounds, pharmaceuticals, pesticides, steroid hormones,

191 industrial precursors and products, and phytoestrogen by live (biosorption + biodegradation),

192 intracellular, enzyme-inhibited and chemically inactivated (biosorption only) whole-cell

193 preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes*

194 *versicolor* (strain ATCC 7731). They showed how non-phenolic TrOC were readily biodegraded

195 while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably

196 higher degradation of the major compounds, indicating the importance of biosorption and

197 subsequent degradation by intracellular and/or mycelium associated enzymes. However, there are

198   too few studies that examine both adsorption and degradation of antibiotics in agricultural soil, with
199   most using unrealistically high concentrations (in mg/kg levels) to overcome limitations in
200   measurement. In addition, no model has been developed to speculate about the adsorption and
201   degradation of different types of antibiotics in agricultural soil and the environmental risks they
202   may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics
203   (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native
204   microorganisms (bacteria and fungi) in non-sterilized (test) and sterilized (control) agricultural soils
205   under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to
206   microbial degradation under aerobic conditions, and most antibiotics were degraded by more than
207   92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial
208   concentration was found to slow down degradation and prolong persistence in soil. The degradation
209   pathway of antibiotics varied in relation to their physicochemical properties as well as the microbial
210   activities and aeration of the recipient soil. In their study, Pan and Chu (1996) were the first to
211   develop a model for the prediction of antibiotic persistence in soil.

212   Given the public concern for environmental pollution by OACs, there is increasing attention
213   towards the development of biopurification systems for reducing the risk from point source
214   contamination of soil resources. Various treatment methods (e.g. land filling, recycling, pyrolysis
215   and incineration) have been used for the removal and remediation of these chemicals from the
216   contaminated sites, but microbial degradation of pesticides is so far the most important and
217   effective way to remove these compounds from the environment (Hai et al. 2012; Verma et al.
218   2014), (Supplemental material Table I; Table I References).

219   Microorganisms have the ability to interact both chemically and physically with substances, leading
220   to structural changes or to complete degradation of the target molecule. In particular, fungi may
221   transform pesticides and other xenobiotics by introducing minor structural changes to the molecule,
222   producing nontoxic molecules that can be released into the soil for further degradation by
223   microflora (Hai et al. 2012), (Supplemental material Table I; Table I References). Mir-Tutusaus et
224   al. (2014) investigated the degradation of the insecticides imiprothrin and cypermethrin and the
225   insecticide/nematicide carbofuran using the white-rot fungus *T. versicolor*. Experiments with fungal
226   pellets demonstrated extensive degradation of the tested agrochemicals, while *in vivo* studies with
227   inhibitors of cytochrome P450 revealed that this intracellular system plays an important role in the
228   degradation of imiprothrin and carbofuran, but not of cypermethrin. The simultaneous degradation
229   of the compounds successfully took place with minimal inhibition of fungal activity and resulted in
230   reduction of global toxicity, thus supporting the potential use of *T. versicolor* for the treatment of
231   several OACs.

232   To date, the number of studies investigating novel treatment techniques for the removal of OACs

233   from contaminated agricultural soils is limited. The bacteria-dominated conventional activated

234   sludge process has been proved to be ineffective for OAC removal. While the importance of a

235   mixed microbial community to initiate and complete OAC removal in the soil environment has been

236   convincingly demonstrated by several researchers, studies concerning the removal of OACs from

237   soils have predominantly focused on selected bacterial or fungal species separately. Few studies

238   have explored the bioaugmentation synergy of fungi together with bacteria (Hai et al. 2012; Zhang

239   et al. 2015; Madrigal-Zúñiga et al. 2016). Combining cultures of bacteria and fungi could be key to

240   the removal of toxic and recalcitrant organic substances from contaminated agricultural soils.

241   On-farm biopurification systems constitute a biotechnological approach to the mitigation of point

242   source contamination by pesticides. The main component of biopurification systems is the

243   biomixture, which acts as the biologically active core that accelerates the degradation of OACs.

244   Madrigal-Zúñiga et al. (2016) studied the results of employing the ligninolytic fungus *T. versicolor*

245   in the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the

246   insecticide-nematicide carbofuran (CFN). The transformation products of CFN were detected at the

247   moment of CFN application, but their concentration decreased continuously until complete removal

248   in both biomixtures. Mineralization of ^{14}C radiolabeled CFN was faster in GTS than in GCS. The

249   authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data

250   suggested that the bioaugmentation improved the performance of the GTS rather than the GCS

251   biomixture.

252   Pinto et al. (2016) also studied the potential use of different substrates in biomixtures like cork, cork

253   and straw, coat pine and LECA (Light Expanded Clay Aggregates) in the degradation of

254   terbuthylazine, difenoconazole, diflufenican and pendimethalin pesticides. Bioaugmentation using

255   the WRF *Lentinula edodes* inoculated into the CBX was also assessed. The results obtained from

256   this study clearly demonstrated the relevance of using natural biosorbents such as cork residues to

257   increase the capacity for pesticide dissipation in biomixtures for establishing biobeds. Furthermore,

258   greater degradation of all the pesticides was achieved by the use of bioaugmented biomixtures.

259   Indeed, biomixtures inoculated with *L. edodes* EL1 were able to mineralize the selected xenobiotics,

260   revealing that this WRF might be a suitable fungus to be used as inoculum source to improve the

261   degradation efficiency of sustainable on-farm biopurification systems.

262   Fungi isolated from biomixtures represent a biological source of potentially active bioremediation

263   agents, and the adaptation skills developed by these microorganisms could make the difference in

264   OAC removal (Supplemental material Table I; Table I References). This strategy was assessed by

265   Pinto et al. (2012), who isolated fungi from a loamy sand soil and a biomixture contaminated with

terbuthylazine, difenoconazole and pendimethalin. The ability of autochthonous fungi (*Penicillium brevicompactum* and *Lecanicillium saksenae*) to degrade xenobiotics was compared with that of allochthonous strains taken from a culture collection (*Fusarium oxysporum*, *Aspergillus oryzae* and *L. edodes*). The best biodegradation yield was achieved with *P. brevicompactum*: its higher ability to metabolize terbuthylazine was presumably acquired through chronic exposure to contamination with the herbicide.

Bioremediation of OACs by fungi in aquatic ecosystems

Many OACs are common contaminants of fresh water due to their high water solubility associated with a low soil adsorption, and a high stability that assures them a long half-life. Contamination is heterogeneously distributed along watercourses as evidenced in several studies where pesticides were recurringly found in real water samples. In one accurate survey, more than 160 water samples taken in 23 European countries were assayed for the presence of pharmaceuticals, pesticides and recognised endocrine-disrupting chemicals (Loos et al. 2010). Among the most frequently detected compounds were the insecticide (DEET), and other pesticides (chloridazon-desphenyl, DMS, desethylatrazine, chloridazon-methyldesphenyl, bentazone, desethylterbutylazine, dichlorprop) exceeded the European threshold of 0.1 μg/l. Overall, 29% of the water samples could not be considered safe (Loos et al. 2010). In a similar study in the USA, groundwater in 18 states was screened for 65 organic contaminants: along with plasticizers and detergent metabolites, 66% of the total pollutant load was ascribable to insect repellant (Barnes et al. 2008).

The extent of freshwater contamination and the actual risk to human life depend on several factors concerning the hydrogeological characteristics of the soil, weather conditions and the chemical-physical properties of the OACs. The environmental fate of a given compound is a critical issue in which the water/soil surface is the first barrier. For instance, the sorption kinetics of three widely used pesticides (simazine, imidacloprid, and boscalid) were found to be correlated with soil organic carbon content and the hydrophobicity of the pesticide, which ultimately affected soil retention behavior and bioavailability in waters (Salvestrini et al. 2014). Leaching into surface waters is also a matter of season, and a complex and unpredictable scenario is influenced by a variety of phenomena. A rainy period can cause massive run-off of OACs from the soil, contaminating the receiving basin (Sandin et al. 2018). The detection of high levels of OACs, however, is not exclusively coincident to their recent and massive use, but is ascribable to their persistency, their slow natural degradation and their accumulation in the various diffusion pathways (Aguilar et al. 2017). They could then travel long distances in surface or groundwaters and the contamination can last for several decades (Ballesteros et al. 2014; Aravinna et al. 2017).

300 The so-called ecological services may help to contain the diffusion of OACs. The adaptation of

301 microflora (fungi, Gram-positive and negative bacteria, actinobacteria, and sulfate-reducing

302 bacteria) to soil environmental conditions may attenuate the pesticides released into groundwater

303 sources (Mattsson et al. 2015). Several factors such as soil composition, temperature, aeration due

304 to soil weaving, and depth influence autochthonous microbial community activity; if this balance

305 fails, OACs are free to move among different ecological niches (i.e. sediments and water), alter

306 their functioning and ultimately directly affect their animal inhabitants. For instance, significant

307 ecological risk was associated with the presence of the insecticide fipronil and its metabolites in

308 water ponds: the concentrations measured (up to 200 ng/l) affected the proper development of larval

309 insects and crustaceans (Wu et al. 2015b). Evidence of the pesticide's toxicity against fish has

310 already been reported, and it clearly interferes in several metabolic pathways (Odukkathil and

311 Vasudevan 2013; Ballesteros et al. 2014; Guerreño et al. 2016).

312 The preservation of water quality is a priority, but OAC removal cannot be based only on natural

313 attenuation. Water treatment plants (WTPs) are the major barriers where OACs should be removed.

314 Not being specifically designed for micropollutant removal, however, they are often only partially

315 effective, with a strong impact on the receiving ecosystem. Pesticides such as atrazine, fluconazole,

316 tebuconazole, diazinon and diuron are particularly resistant to commonly used treatments (Köck-

317 Schulmeyer et al. 2013; Luo et al. 2014). There is plenty of evidence confirming the presence of

318 OACs in WTP effluents at toxicologically and estrogenically relevant concentration, making them

319 one of the most impactful sources of contamination (Bicchi et al. 2009; Campo et al. 2013; Jarošová

320 et al. 2014).

321 Particular attention has been given to advanced biological oxidation. Novel cost-effective and eco-

322 friendly processes based on fungi are an attractive option. Fungi are well-known for their

323 physiological adaption skills, including the natural activation of tolerance mechanisms against

324 pesticides (Talk et al. 2016). Some reports have already demonstrated that in comparison with

325 bacteria, fungi can better tolerate the presence of organic contaminants. Although the insecticide

326 endosulfan inhibited both fungi and bacteria, bacterial community structure significantly changed at

327 concentrations as low as 0.1 mg/kg, while modifications to fungal community structures required 1

328 mg/kg of pollutant (Zhang et al. 2015). Linuron reduced the bacterial count, and especially total

329 bacteria, N_2-fixing bacteria and nitrifiers, but not fungal numbers (Cycoń et al. 2010).

330 The provenance of isolated fungi is of unquestionable importance. Strains isolated from

331 contaminated niches indeed seem to develop specific adaptation skills due to chronic exposure.

332 Carles et al. (2017) demonstrated that the aquatic microflora found in association with submerged

333 leaves exposed to nicosulfuron is more efficient in its degradation than are communities that come

334   from a less polluted site. The authors indicated fungi as the main constituents of this active

335   microflora and as being responsible for herbicide degradation. In the literature, several fungi

336   isolated from contaminated areas or WTPs have been identified as degraders of nicosulfuron,

337   diuron, isoproturon, glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013;

338   Carranza et al. 2014; Oliveira et al. 2015).

339   Fungi can thus transform a broad range of recalcitrant organic compounds, including OACs (Gao et

340   al., 2010). A number of fungi that are OAC degraders, mostly belonging to Basydiomycetes, such

341   as *Trametes*, *Pleurotus, Phlebia, Cerrena, Coriolopsis*, etc., have been already investigated

342   (Koroleva et al. 2002; Marco-Urrea et al. 2009; Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et

343   al. 2014; Ceci et al. 2015). Several pesticides as lindane, atrazine, diuron, terbuthylazine, metalaxyl,

344   DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane,

345   mirex, etc. were effectively transformed by fungal treatment based on mycelium or enzymes

346   (Supplemental material Table II).

347   A bioremediation approach based on fungi may involve both biosorption and biodegradation

348   processes; the latter combines biosorption, where the molecule binds to the fungal wall, and

349   bioaccumulation with the pollutant being transported inside the cell in contact with intracellular

350   enzymes (Kulshreshtha et al. 2014). Concentrations of the insecticide lindane decreased during time

351   in the presence of two WRFs (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus

352   (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated that a

353   fast adsorption process was mainly involved (Ulčnik et al. 2013). However, this phenomenon is

354   often strain-dependent, and expecially related to metabolic differences between Ascomycetes and

355   Basidiomycetes. Belonging to the brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes,

356   responsible for lignin degradation and likely for that of OACs as well: adsorption onto fungal

357   mycelium was mainly involved in the removal of endosulfan. On the contrary, the white-rot fungi

358   actively degraded, producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013).

359   Although biosorption is a phenomenon that cannot be ignored, it is often secondary or at least

360   negligible compared to biodegradation (Carles et al. 2017). For instance, the removal of clofibric

361   acid found for heat-killed mycelium was less than 10 %, but more than 97 % for active *T. versicolor*

362   (Marco-Urrea et al. 2009).

363   Fungi have developed a specific mechanism that employs few enzymes and molecules with high

364   oxidizing power, physiologically aimed at transforming lignocellulose structures. The same

365   enzymatic pathway may play a pivotal role in transforming other aromatic molecules. White-rot

366   fungi usually deploy extracellular lignocellulosic enzymes such as peroxidases (EC 1.11.1.x) and

367   laccases (EC 1.10.3.2). The involvement of redox enzymes in fungal-mediated oxidation is

368 confirmed by the direct induction of enzyme production in the presence of OACs. The fungus *T.*
369 *versicolor* responded to 17 pesticides by increasing laccase production in comparison with the
370 control: particular attention was given to the transformation products of the herbicides diquat and
371 monuron, capable of increasing fungal activity 10- and 17-fold, respectively (Mougin et al. 2002).
372 The laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus* spp1, *Pleurotus*
373 spp2, *Cymatoderma elegans*, and *Daedalea elegans* was stimulated by the presence of atrazine even
374 at high concentrations of 3750 mg/l. Likewise, the manganese peroxidase activity of *Pleurotus* spp1
375 and *C. elegans* was positively correlated with the pesticide (Chan-Cupul et al. 2014).
376 Oxidoreductase stimulation was also observed with picloram (Maciel et al. 2013), bentazon (Da
377 Silva Coelho et al. 2010) and carbofuran (Mir-Tutusaus et al. 2014).

378 Although these oxidoreductases are probably the most-known enzymes for aromatic compound
379 degradation, alternative pathways can be stimulated by the presence of OACs. Two clones (laccase-
380 positive and laccase-negative) of *Mycelia sterilia* were used to treat atrazine (20 μg/ml): even
381 though one clone was defective in laccase production, comparable transformation yields (70-80%)
382 were reached, indicating that the fungus can deploy alternatives to laccase in the degradation
383 process (Vasil'Chenko et al. 2002). This behavior is commonly found in brown-rot fungi, which
384 can trigger both nonenzymatic and enzymatic mechanisms, i.e. the Fenton mechanism or cellobiose
385 dehydrogenase (CDH) reactions (Fan and Song 2014). The degradation of atrazine (20 μg/l) by an
386 unidentified mycelial fungus was associated with the presence in the liquid medium of OH radicals
387 and CDH. Moreover, CDH secretion was induced by the presence of the herbicide itself
388 (Khromonygina et al. 2004). In addition, some fungi may associate extracellular oxidoreductases
389 with intracellular enzymes such as the cytochrome P450 system (cyt450). In an effort to better
390 characterize the degradation skills of *T. versicolor*, cyt450 inhibitors were used: fungal performance
391 against clofibric acid and fipronil decreased (Marco-Urrea et al. 2009; Wolfand et al. 2016). Mori et
392 al. (2017), suggest that in *Phanerochaete sordida*, cyt450 is involved in the initial stage of reduction
393 of the clothianidin N-nitro group, but that the enzymes responsible of the further urea derivatives
394 production are unknown.

395 Fungal intra- and interspecies variability has long been recognized and has found confirmation in
396 OAC treatment. Literature data about a given species cannot be taken for granted and preliminary
397 screening is often required. Despite *Phanerochaete chrysosporium* often being indicated as the
398 fungal model for organic degradation including pesticides (Wang et al. 2014), it was almost
399 ineffective against clofibric acid (Marco-Urrea et al. 2009). Among five Basidiomycetes, only *T.*
400 *versicolor* extensively degraded this herbicide (Marco-Urrea et al. 2009). Alvarenga et al. (2014)
401 treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*. Based on ability to

402 grow in the presence of the pesticide, only the isolate *A. sydowii* CBMAI 935 was selected for
403 further studies. It indeed grew almost 4-fold more than the other *A. sydowii*. Bioremediation
404 potential is often substrate-targeted, and the choice of fungus cannot be taken for granted. For
405 instance, *A. sydowii* CBMAI 935, which totally converted methyl parathion (Alvarenga et al. 2014)
406 was not the best performing one against the insecticide esfenvalerate. Among 6 fungi,
407 *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave better results than the *Aspergillus*
408 strain (Birolli et al. 2016).

409 Although the majority of these strains are effective in OAC removal in model solutions, only few
410 researchers have taken the next step, and assessed bioremediation potential in contaminated waters.
411 The experimentation with model solutions (single-compound solutions, high concentrations, no
412 interfering molecules, etc.) is the only way to acquire information about degradation pathways
413 (Masaphy et al. 1993; Birolli et al. 2016), but it is less predictive of fungal performance in real
414 environmental water samples. Each type of wastewater has its own critical issues, making it
415 difficult to predict fungal behavior. Some data highlight the robustness of fungal systems, although
416 detailed case-by-case investigation is needed. A partially diluted leachate was shown to disturb the
417 growth of *T. versicolor* and *Stereum hirsutum*, but this did not prevent them totally degrading
418 linuron and dimethoate at 10 mg/l. As regards dimethoate, the presence of adsorbents enhances final
419 yields from 50% to 97%, because the adsorption action combines with and exalts fungal
420 biodegradation processes (Castellana and Loffredo 2014). The immobilization of *Bjerkandera adusta*
421 and *Irpex lacteus* on coffee grounds, almond shells and a biochar favored the removal of the non-
422 phenolic herbicides fenuron and carbaryl from a municipal landfill leachate (Loffredo et al. 2016).

423 Surface waters, ground waters and municipal wastewaters represent a very unique environment,
424 characterized by extreme chemical and physical conditions, the presence of a heterogeneous and
425 variable mixture of micropollutants and an active autochthonous microflora. When inoculated into
426 real surface water, a fungal consortium (*Aspergillus fumigatus, Aspergillus terreus, Cladosporium*
427 *tenuissimum, Cladosporium cladosporioides, Fusarium begoniae, Penicillium citrinum, Penicillium*
428 *melanoconidium* and *Phoma glomerata*) was not stable over time, probably due to the presence of
429 toxic pesticides and interaction with the natural microbial population: *P. citrinum, A. fumigatus* and
430 *A. terreus* were the most robust to the environmental conditions and were found to degrade the
431 spiked chlorfenvinphos (Oliveira et al. 2015).

432 The set-up of active microbial consortia offers the intriguing possibility of strengthening and
433 combining the bioremediation potential of different organisms: the combination of *Bacillus subtilis*
434 and *A. niger* led to higher degradation rates of nicosulfuron than those obtained by using each strain
435 singly (Lu et al. 2012). The biodegradation of aldicarb, atrazine and alachlor by *Coriolus versicolor*

was strongly enhanced by combination with activated sludge. Along with modifications in fungal morphology, when the bacterial-fungal consortium was established, the bio-absorbed fraction of especially atrazine was reduced: over 98% of atrazine was removed by degradation processes in two weeks (Hai et al. 2012).

The fate of the treated OACs must be carefully considered. Residual toxicity is a critical issue. Interestingly fenuron and carbaryl degradation (up to 70%) catalyzed by *B. adusta* and *I. lacteus* led to significant abatement of the phytotoxicity (rapeseed and flax tests) (Loffredo et al. 2016). Mori et al. (2017) monitored the neurotoxicity of clothianidin and the main metabolite it produced during *P. sordida* treatment: following treatment the insecticide still altered the viability of the neuronal cell line, but the metabolite was no longer neurotoxic.

Despite their well-demonstrated properties, the application of whole cell systems has some drawbacks including the fact that a living organism needs controlled growing conditions in terms of nutrients, pH, O_2, etc. (Majeau et al. 2010). The addition of synthetic nutrients can strengthen fungal mycelium activity, but it should be carefully balanced to allow subsequent scale-up of the process. The fact that *T. versicolor* needed 1% of glucose as carbon source to degrade atrazine would ultimately interfere with its potential use in real WTPs (Khromonygina et al. 2004). Likewise several fungi such as *A. niger* and *Dacryopinax elegans*, etc. required both easily available carbon and nitrogen sources to efficiently act against nicosulfuron and diuron, respectively (Lu et al. 2012; Arakaki et al. 2013). Particular attention should be instead given to those fungi, like *A. sydowii* and *Penicillium decaturense*, that maintained the same performance without glucose addition, indicating potential for using methyl parathion or triclosan as sole carbon source (Alvarenga et al. 2014; Tian et al. 2016).

A promising alternative is offered by the direct use of fungal enzymes, capable of catalyzing strong, rapid oxidation reactions, with less technical drawbacks in comparison with fungal cultures. The potential of enzymes-based methods has been worldwide recognized; the Swiss Industrial Biocatalysis Consortium defined oxidative enzymes as the biocatalysts displaying the highest development potential for the next decades (Meyer and Munch 2005). Great importance is given to the discovery of novel enzymes with wide substrate specificity, stable and applicable to industrial uses. A number of articles have reported the ability of fungal enzymes to degrade OACs. The potential of laccase-mediator systems has been assessed for the degradation of isoproturon (Margot et al. 2015), imiprothrin (Mir-Tutusaus et al. 2014), chloroxuron (Palvannan et al. 2014), isoproturon (Zeng et al. 2017), atrazine (Chan-Cupul et al. 2016). Laccases cannot be considered a novelty, unlike a phytase of *A. niger* capable of degrading organophosphorus pesticides (Shah et al. 2017) or a cellulase of *Trichoderma longibrachiatum* active against dicofol (Wang et al. 2015).

470 Particular attention should be given to the use of crude enzyme extracts of ligninolytic enzymes
471 with a lower economic impact on the process than that of purified enzymes (Matute et al. 2012;
472 Kaur et al. 2016). A crude extract of *Trametes pubescens* laccases degraded up to 19 compounds in
473 a model solution and confirmed its potential in a study on real municipal wastewater where the
474 presence of suspended particles, colloids, solvents and xenobiotics as well as autochthonous
475 microorganisms posed strong environmental pressure. The transformation of all the detected
476 compounds determined also a strong reduction of the estrogenicity of the water sample (Spina et al.
477 2015).

478

479 ***Application of synthetic microbial communities in bioremediation***

480 Bioremediation is a crucial way to eliminate OAC pollution in agricultural ecosystems. However,
481 many factors affect the efficiency of bioremediation in pesticide pollution, such as the microbes
482 applied, treatment sites, rhizosphere effects and soil chemical and physical properties (Zhou and
483 Hua 2004). Bioremediation of soil or water pollution often cannot reach expected results in practice
484 because the target contaminant cannot be degraded completely, and sometimes intermediate
485 products occur that are more toxic than the original pesticides. Long-term application of various
486 pesticides results in pollution with more than one type of chemical compound, which are unlikely to
487 be degraded by a sole microbe. Thus, attention has shifted to synthetic systems based on
488 communication between cells, rather than on individual isolated cell functionality (Biliouris et al.
489 2012). A promising way to overcome the difficulties is to create artificial synthetic microbial
490 communities that contain several microbes to retain the key features of their natural counterparts
491 (Großkopf and Soyer 2014).

492 The so-called *synthetic microbial community* is created by a bottom-up approach where two or more
493 defined microbial populations are put together in a well-characterized and controlled environment
494 (De Roy et al. 2014). In synthetic communities, mixed populations can perform complex tasks,
495 although in changing environmental conditions (Brenner et al. 2008). Synthetic communities have
496 several potential advantages over monocultures or natural communityies: 1) the species in a
497 synthetic community are known and the community structure is relatively simple and controllable,
498 while the natural community may contain many microorganisms with unknown functions; 2)
499 synthetic communities can perform more complicated functions than individual organisms because
500 members of microbial consortia communicate and differentiate (Brenner et al. 2008); 3) synthetic
501 communities are often more robust to environmental fluctuations because they can resist invasion
502 by other species and weather periods of nutrient limitation better than monocultures (Brenner et al.
503 2008); 4) synthetic communities can be described through mathematical models more easily than

504 natural systems, and they can be used to develop and validate models of more complex systems

505 (Liu et al. 2017).

506 Liu et al. (2017) proposed three design principles to develop a cooperative, steady-state community

507 that is performing a desirable biotechnological function. Firstly, safety should be prioritized by

508 beginning with innocuous or commensal organisms (Brenner et al. 2008). Secondly, the community

509 can converse a low-cost and/or recalcitrant waste material into a biotechnologically relevant

510 product, partial or de-novo biosynthesize a compound via heterologous metabolic pathways, or

511 bioconverse toxic substrates or products in a toxic milieu (Jagmann and Philipp 2014). Thirdly, the

512 bioremediation process should be optimized and regularly monitored on the basis of the knowledge

513 of stability and division of different microorganisms (Liu et al. 2017).

514 Bioremediation of polluted soils and water is one field of application synthetic microbial

515 communities. Due to the complex structure of some pollutants, such as the diuron pesticides, adding

516 synthetic microbial communities is much more effective than adding single microorganisms. The

517 herbicide diuron is used in the control of broad-leaved weeds on agricultural land. Several fungal-

518 bacterial consortia were investigated by combining three different diuron-degrading bacteria and

519 two fungal strains. The fastest mineralization of diuron was obtained by the three-member

520 consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47). As

521 measured by evolved $14CO_2$ it mineralized about 32 % of the added diuron within 54 days, whereas

522 the single strains or other consortia achieved no more than 10% mineralization. In addition, the

523 production of diuron metabolites by the consortium was minimal. This may be due to cooperative

524 catabolism, where the first organism transforms the pollutant to products that are then used by the

525 other organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby

526 facilitating the more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al.

527 2014).

528 Similarly, a fungal-bacterial consortium consisting of *Mortierella* sp. LEJ702 and the 2,6-

529 dichlorobenzamide (BAM)-degrading *Aminobacter* sp. MSH1 achieved more rapid mineralisation

530 of BAM than did the bacteria alone, especially at lower moisture contents (Knudsen et al. 2013).

531 Methylotrophic and hydrocarbon-utilizing yeasts and bacteria alone did not degrade PCBs

532 significantly, but PCB degradation reached about 50% when WRFs were applied together (Šašek et

533 al. 1993).

534

535 ***Evaluation of bioremediation effectiveness in contaminated matrices by means of***

536 ***ecotoxicological and genotoxic tests***

537 In order to improve the effectiveness and performance of bioremediation processes it is important to

538 pursue three essential goals at the same time. Focus should be not only on reducing chemical

539 concentrations, but also on reducing chemical mobility between the environmental compartments

540 and eventually lowering toxicity levels while ensuring that contaminants do not get into the natural

541 biological cycle (Loehr and Webster 1997; Chakraborty et al. 2013).

542 Bioremediation is often monitored by following the concentration of targeted contaminants

543 (Molina-Barahona et al. 2005). Numerous studies in recent years have shown that traditional

544 chemical analyses are insufficient for a full assessment of the contaminated site because, for

545 example, they do not provide any information about the interactions between chemicals and they do

546 not consider the partition and the mobility of pollutants (Frische 2003; Molina-Barahona et al.

547 2005; Ma et al. 2005; Molnár et al. 2007). An integrated approach that links the various fields and

548 levels of study involving contaminated sites has proven to be an efficient way to evaluate the

549 effectiveness of bioremediation in contaminated sites (Chapman and Anderson 2005; Wernersson et

550 al. 2015; Marziali et al. 2017). Consequently, to achieve the desired goals and implement a

551 successful bioremediation program, given the chemical and biological complexity of the tasks

552 involved, close collaboration between microbiologists, chemists and engineers is required (Van

553 Gestel et al. 2001; Chakraborty et al. 2013).

554 Additionally, the use of ecotoxicological and genotoxic tests to evaluate the effectiveness of

555 bioremediation may be a valid tool to partially overcome the existing gap between the reported

556 successes of bioremediation on the laboratory scale, and that in the field.

557 Signals that bioremediation is going on should be monitored. Two important chemical compounds

558 produced by microorganisms during their degradation activity are CO_2 and soluble phosphorus.

559 Both increase notably in soil treated with insecticides and inoculated with fungi (Boyle 1995; Abd

560 El-Ghany and Masmali 2016). However, it must be taken into consideration that during and after a

561 bioremediation process the disappearance of the parent compounds or evidence of metabolic

562 activity (e.g. CO_2 production) may not indicate detoxification. Although the fate of the toxicants

563 may be followed by chemical analyses, many reaction products resulting from the bioremediation

564 process and their potential toxicity are not known. The elimination of mother compounds does not

565 necessarily result in toxicity removal, and evaluating the efficiency of the process is important to

566 assess not only the removal of a specific compound, but also potential ecotoxicity. In fact,

567 biodegradation of pesticides can proceed partially or totally due to the structure of the molecule

568 itself or to unfavourable environmental or test conditions, or to the lack of 'acclimatized' microbial

569 communities (De Henau 1997).

570   In some instances, it has been shown that an effective process of bioremediation corresponds with a

571   decrease in the toxicity of the analysed matrix (Baud-Grasset et al. 1993; Dorn and Salanitro 2000).

572   To acquire complete and useful information in an ecotoxicological assessment and to determine the

573   effectiveness of bioremediation treatments, it is suggested that a battery of tests be used (Keddy et

574   al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of

575   reference organisms that are representative of the different trophic levels, in order to select species

576   with different roles in ecosystems, and different exposure conditions (Van Straalen and Van Gestel

577   1997). Moreover, environmental risk assessment must integrate chemical characterization,

578   ecotoxicity and bioremediation data, in order to accurately assess the ecological hazard.

579   As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various

580   bioindicators could either indicate incomplete decomposition of the substance or could result from

581   the formation of intermediate products generated via the bioremediation process. For this reason,

582   chronic tests are sometimes more appropriate in evaluating the toxicity caused by by-products

583   (Lofrano et al. 2014).

584   In certain circumstances, there is a clear need to monitor the bioremediation process using different

585   bioindicators. In Lizano-Fallas et al. (2017), for example, the ecotoxicity test with *Daphnia magna*

586   showed clear detoxification, whilst the detoxification patterns remain unclear when applying the

587   phytotoxicity test. Ecotoxicological tests can also be used to determine the most suitable

588   bioremediation technique in a given case, as reported in Dudášová et al. (2016).

589   Without worldwide-recognized guidelines for water quality assessment, literature data are difficult

590   to compare due to the variety of model organisms, end-points, etc. Synthetic indices summarizing

591   the findings can help monitor the effectiveness of biological treatment. Such indices have already

592   been applied for toxicity monitoring of wastewaters (Tigini et al. 2011) but municipal effluents

593   containing AOCs have never been taken into consideration nor has estrogenic activity been

594   included so far.

595   Several toxicity assays were included in treatability study protocol to measure remediation

596   efficiency. Assessing the toxicity of complex matrixes such as soil could acquire methods from

597   bioassays used to the test toxicity of chemical compounds, reported by the Organization for

598   Economic Co-operation and Development (e.g. OECD 201 2006; OECD 211 2012). The OECD has

599   published a series of standardized tests for determining the biodegradability of a given compound,

600   based on the evaluation of overall parameters (such as COD, TOC and BOD) or metabolic tests, e.g.

601   respirometry (OECD 209 1984) as Polo et al. (2011) used; or that reveal susceptibility of toxic

602   compounds, comprising that of herbicides, to biological treatment. Standardized testing procedures

603   using different organisms have been approved by various environmental organizations, including

the US Environmental Protection Agency, American Society for Testing and Materials, International Standardization Organization (Siciliano et al. 2015). Many scientists have explored the effects of polluted soil on the whole organism using various microorganisms, animals, and plants, or by means of cellular, and biochemical biomarkers, or by ecological scale up systems. Here below, tests at some different biological hierarchical levels of analysis are presented and discussed.

Organismal level

Concerning complex matrices such as soil, quality assessments are performed with organisms on extracts of the polluted matrix, generally applying short-term exposure periods (Van Gestel et al. 2001). Experimental models have included aquatic organisms such as *Daphnia magna*, *Raphidocelis subcapitata*, *Danio reri*o, *Myriophyllum aquaticum* and *Lemna minor* (Feiler et al. 2004). The use of freshwater and marine biota may be particularly useful in order to provide a more complete comprehension of the fate of pesticides and the environmental outcomes of agricultural activities (Guida et al. 2008). Terrestrial animals such as nematodes (*Caenorhabditis elegans*) (Traunspurger et al. 1997), oligochaetes (*Lumbriculus variegatus*) (Phipps et al. 1993), springtails such as *Folsomia candida* (Houx et al. 1996), and fish embryos (Hollert et al. 2003; Zielke et al. 2011) are considered among the most reliable models.

Among the higher plants, important experimental models include *Lepidium sativum*, *Cucumis sativus*, and *Sorghum saccharatum* (germination rate, inhibition of root elongation). Since assays based on animals, plants and algae are considered expensive, time consuming and require large sample volumes, recent studies have emphasized the benefits of rapid, reproducible and cost effective bacterial assays for toxicity screening and assessment. *Arthrobacter globiformi* (Neumann-Hensel and Melbye 2006), *Bacillus cereus* (Rönnpagel et al. 1995; Prokop et al. 2016), *Vibrio proteolyticus* (Ahlf and Heise 2005) and yeasts (*Saccharomyces cerevisiae*) (Weber et al. 2006) are often used. Among the bacterial bioassays, the *Vibrio fischeri* luminescence inhibition test is the most common. The review of Parvez et al. (2006) remarks that the *Vibrio fischeri* inhibition test is the most sensitive, cost effective, easy to operate and requires only 5–30 min for toxicity prediction.

Cellular and biomolecular level

Biomarkers signal the adaptative responses of organisms to xenobiotic exposure. Various studies have highlighted the cytoxic and genotoxic effects on organisms of OACs and their metabolic products. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or physiological, or even behavioural changes (Depledge et al. 1993) that enable information to be

638   obtained on the biological effects of pollutants or their remains during or after a bioremediation

639   process (Fontanetti et al. 2011).

640   Genetic endpoints and biomarkers. The most-used biomarkers are mitotic index, chromosome

641   aberrations, micronuclei, sister chromatid exchanges and mutations.

642   Bacteria have been recommended for bioassays to evaluate genotoxicity in a variety of samples

643   (Mortelmans and Zeiger 2000; White and Claxton 2004). The Ames test, one of the most famous

644   and widely-used, is a short term bacterial reverse mutation assay especially designed to evaluate the

645   mutagenic potential of a wide range of chemical substances (Mortelmans and Zeiger 2000). It was

646   found to be very sensitive in tests with a wide range of mutagenic and carcinogenic chemicals, as

647   reported in the review paper of Chahal et al. (2014).

648   With regards to plant models, higher plants are recognized as excellent genetic models to detect

649   cytogenetic and mutagenic agents and are frequently used in environmental monitoring studies. The

650   main organisms employed are *Allium cepa*, *Vicia faba* and *Tradescantia* spp. as reported in a

651   review by De Souza et al. (2016). Their protocols were standardized under the International

652   Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme

653   (UNEP) (Ma 1999). In addition, the US Environmental Protection Agency (USEPA) and the World

654   Health Organization (WHO) validated plant bioindicators as an efficient model to detect

655   environmental genotoxicity.

656   One of the most used higher plant models is *V. faba*. The main advantages are its year-round

657   availability, that it is economical to use, and easy to grow and handle. Its use does not require

658   sterile conditions and rate of cell division is fast. The *V. faba* test, meticulously reported and

659   discussed in the review of Iqbal (2016), enables the assessment of a variety of endpoint (i.e.,

660   chromosomal aberration, mitotic index, micronuclei and nuclear aberration.

661   Enzymatic biomarkers. Enzyme activity inhibition has been widely evaluated as a biomarker to

662   measure the toxicity of a matrix. Dehydrogenases, for example, are directly involved in many of the

663   vital anabolic and catabolic processes of living organisms, and their activity is inhibited by

664   chemical toxicants. Recently, many studies have reported the use of terrestrial organisms to obtain

665   enzymatic biomarkers in response to residual pesticides (Henson-Ramsey et al. 2011; Radwan and

666   Mohamed 2013; Stepić et al. 2013), and among these, earthworms' enzymes were widely used to

667   understand the impacts of pesticides. In two earthworm species, *Eisenia fetida* and *Lumbricus*

668   *terrestris*, multiple esterases, including acetylcholinesterase (AChE), butyrylcholinesterase, and

669   carboxylesterase (CE), were assessed as biomarkers for malathion exposure (Henson-Ramsey et al.

670   2011). Several studies have also reported AChE, catalase (CAT), and glutathione-S-transferase as

671   biochemical biomarkers in *Eisenia andrei* for the insecticides endosulfan, temephos, malathion, and

672 pirimiphos-methyl (Stepić et al. 2013), and AChE, CAT, CE, and the efflux pump as biomarkers in

673 *E. andrei* and *Octolasion lacteum* for dimethoa. Recently, surface-enhanced laser

674 desorption/ionization-time-of-flight (SELDI-TOF) mass spectrometry (MS) has strongly

675 contributed to the identification of more accurate, precise biomarkers, e.g. specific for human

676 cancers (Silsirivanit et al. 2014), or for endosulfan exposure in Japanese rice fish (*Oryzias latipes*)

677 (Lee et al. 2013). In a recent paper, selective protein biomarkers for 6 pesticides (captan, carbaryl,

678 carbofuran, and α-endosulfan chlorpyrifos, propoxur) were found in *E. fetida*, by means of SELDI-

679 TOF MS technology (Park et al. 2015).

680 <u>Estrogen and androgen biomarkers</u>. It is well-documented that several chemicals from agricultural,

681 industrial, and household sources possess endocrine-disrupting properties, which provide a potential

682 threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995; Jensen et al. 1995).

683 A suggested mechanism is that environmental contaminants alter the normal functioning of the

684 endocrine and reproductive system by mimicking or inhibiting the action of endogenous hormones,

685 by modulating the production of endogenous hormones, or by altering hormone receptor

686 populations (Sonnenschein and Soto 1998). Several pesticides exert estrogenic and antiandrogenic

687 activities through interaction with estrogen and androgen receptors. The risks associated with OAC

688 exposure has been known for decades: many pesticides, such as p,p´-dichlorodiphenyl

689 trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978; Cummings 1997), β-

690 benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan, toxaphene, and dieldrin

691 (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) were the first to be signaled as

692 estrogenic. Despite increased institutional awareness and more compelling legislation pressure, the

693 most recent literature still reports the occurrence of pesticides in watercourses and in the trophic

694 chains, that show conspicuous estrogen or androgen levels (Saillenfait et al. 2016; Brander et al.

695 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al. 2017; Marcoccia et al.

696 2017). Several bioassays have been developed and standardized in order to describe the estrogenic

697 potency of OACs. Andersen et al. (2002) indicated that several currently used OACs, such as

698 methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl, possess estrogenic activity

699 on the basis of cell proliferation assays and transactivation assays using MCF-7 human breast

700 cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism and antagonism to two

701 human estrogen receptor (hER) subtypes, hERα and hERβ, and a human androgen receptor (hAR)

702 by means of highly sensitive transactivation assays, using Chinese hamster ovary cells. The results

703 demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic action through

704 ERs and/or AR. Although it appears that various pesticides exert hormonal effects at concentrations

705 that are orders of magnitude higher than that required for physiologic hormones, wide exposure to

large numbers of OACs may have additive and synergistic effects. Bioassay with YES (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active compounds still present in the environment. Since the the first papers on this subject (Purvis et al. 1991), much more sophisticated bioassays have been developed, such as that proposed by Eldridge et al. (2007) in which a bioluminescent strain of *Saccharomyces cerevisiae* was genetically engineered to respond to androgenic chemicals.

Ecological level

The risk to natural systems of pollution with the chemical residues of bioremediation processes is underestimated. The ecological scaling-up experiment illustrated by Rodea-Palomares et al. (2016) underlined how real-world exposure to chemical pollution is often dominated by low-dose complex mixtures combined with other biotic and abiotic stressors. In the paper, a novel screening method (GSA-QHTS) was reported, that coupled the computational power of global sensitivity analysis (GSA) with the experimental efficiency of quantitative high-throughput screening (QHTS). In the study, they reported that GSA-QHTS allowed for the identification of the main pharmaceutical pollutants that were driving the biological effects of low-dose complex mixtures at the microbial population level. The target complex community was a river benthic microbial community inoculum obtained from an unpolluted stream. The effects of the toxic compounds in the mixture was evaluated together with other physico-chemical stressors, on a series of community-level metabolic end points. Photosynthetic parameters, the dark-adapted basal fluorescence, the light-adapted steady-state fluorescence, the maximum photosynthetic efficiency, as well as the extracellular enzymatic activities b-Glu and Phos were considered as both autotrophic and heterotrophic global fitness indicators suited to study the effects of chemical pollution on freshwater benthic microbial communities.

Prospect

Bioremediation is based on the idea that different organisms will work together to remove (biodegrade) the waste substances or pollutants (OACs) from the environment. Although there exist limitations to bioremediation practice, including the nature of organisms, the enzyme involved, the concentration and availability and final survival of microorganisms, as well as the cost/benefit ratio (i.e. cost versus overall environmental impact), these limitations can be solved to some extent by understanding the genetics and biochemistry of the desired microbe. The advent of synthetic communities has shown enormous potential to facilitate the bioremediation process, the degradative fungi appearing to be particularly effective.

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1 **Fungi as a toolbox for a sustainable bioremediation of pesticides in soil and water**

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20

21 *Abstract*

22 Pesticide can help reduce yield losses caused by pests, pathogens, and weeds, but its overuse

23 causes serious environmental pollution. They are persistent in the environment and

24 biomagnified through the food chain resulting a serious hazard for humankind.

25 Bioremediation by microbes to degrade the pesticides *in situ* is a useful technology. This

26 review mainly summarized the fungi associated with biodegradation of chemical pesticides

27 and their application in the soil and water bioremediation. The future studies on this field

28 were also prospected.

29

30

31 Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi,

32 Synthetic microbial community, Environmental risk assessment.

33

Introduction

34

35 Because of their unique functions, fungi are involved with important ecosystem services for human

36 well-being. Among others, fungi account for provisional services also through the activity of

37 transforming and detoxifying pollutants. For this reason, learning from nature, they represent an

38 effective toolbox for a sustainable bioremediation of pesticides in soil and water. Many researches

39 have unfolded the untapped potential of fungi, given that recent years have witnessed very

40 interesting developments regarding use of fungi not only to improve the environmental quality but

41 also human healt (e.g. Gargano et al. 2017).

42 Pesticides are a diverse group of inorganic and organic chemicals like herbicides, insecticides,

43 nematicides, fungicides, antibiotics and soil fumigants, all belonging to the so-called organic

44 agrochemicals (OACs) (Verger and Boobis 2013; Verma et al. 2014). In agriculture, pesticides aim

45 to enhance crop yield and quality, and to maximize economic returns by prevention of pest or weed

46 attack. They are bioactive, toxic substances, capable of influencing, directly or indirectly, soil

47 fertility and health as well as agroecosystem quality (Pinto et al. 2012; Verma et al. 2014). Given

48 that belowground biodiversity is closely linked to land management, agricultural intensification

49 causes many pressures that leads to loss of biodiversity. Consequently, soil pollution is one of the

50 main threats related to the decline of taxonomic and functional biodiversity, and of agricultural soils

51 sustainability (Harms et al. 2017). Most of the pesticides emission (99 %) in Europe is associated to

52 agricultural practices whereas industrial and urban sources as the manufacturing of pesticides or the

53 at-home use of insecticides have a minor impact (EEA 2016).Thus, t Tthe extensive and massive

54 use of pesticides in agriculture activities has serious impacts on the environment, compromising soil

55 and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al. 2016). Besides,

56 LlIn addition to pesticides, largelLarge quantities of antibiotics are added to agricultural fields

57 worldwide through the application of wastewater, manures and biosolids, resulting in antibiotic

58 contamination and elevated environmental risks (Jechalke et al. 2014; Zhang et al. 2015; Pan and

59 Chu 2016).-), A clear correlation between agriculture and water contamination was observed in Mar

60 Chiquita lake (Argentina), where high amount of endosulfan residues were detected soon after

61 application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide

62 thifluzamide in paddy water of rice fields in China was maximal after the application, and variation

63 during time was associated to the dilution effect of rainfalls in the area (Wei et al. 2015). Preventive

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measures to mitigate the impact of agriculture on the environment are required, taking into account both the use of safety pesticides and the optimization of farmer procedures. Aravinna et al. (2017) found that most of the 32 studied pesticides leached rice field following specific pathways. Since direct run off and erosion from soil were the main vehicles of dispersion, authors suggested alternative strategies (high resident time of pesticides, holding ponds of rice drainage water, delayed filling of paddies after pesticide application and use less mobile compounds) to reduce the movement of the pesticides.

The intensive use of these ~~organic agrochemicals (~~OACs~~)~~ has posed risks to both wild lives and human health. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, through air, water and soil (Miller 2004). Around 30% of pesticides marketed in developing countries do not meet internationally accepted quality standards, posing a serious threat to human health and environment (Popp et al. 2013). They are persistent in the environment and biomagnified through the food chain. Therefore, it has been estimated that millions of agricultural workers worldwide experience unintentional pesticide poisonings each year. The correlation between long-term exposures to pesticides in occupational settings is known but recently also non-occupational exposures have been associated to an elevated rate of chronic diseases (Parrón et al. 2014).

Varieties and consumption of pesticides worldwide are dramatically increasing, up to , ~~but literature reports conflicting data on overall use (2–4 million ton for year).~~ 4-fold higher than 40 years ago (Mnif et al. 2011). According to De et al. (2014), about 45 % is used by Europe, 25 % by USA, and 25 % in the rest of the world. The main pesticide consumer is Spain (around 79,000 ton of active ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy (~ 64,000), Germany (~ 46,000) and United Kingdom (~ 23,000) (Eurostat 2016). The United States ~~is also a large consumer of pesticides, applying~~usually applies over 1 billion pounds annually (Alavanja 2009) with dramatical consequences for human beings and environment (Carvalho 2017). ~~Overall, herbicides account for 47.5 %, insecticides for 29.5 %, fungicides for 17.5 % and others account for 5.5 %.~~

~~On the contrary, according~~According to other authors (Huang McBeath and McBeath 2010), China is the world's largest pesticide user, with an output of pesticide around 3.7 million ton (National Bureau of Statistics of China - http://data.stats.gov.cn), and a consumption volume of about 1.8 million ton in 2014. The average amount of pesticides used per hectare in China is roughly 1.5- to 4-fold higher than the world average (Qiu 2011), thus resulting in the contamination of water bodies in the receiving areas and disturbance of ecological equilibrium (Hui et al. 2003).

Overall, herbicides account for 47.5 %, insecticides for 29.5 %, fungicides for 17.5 % and others

98 account for 5.5 % (De et al. 2014). More than 350 insecticides, herbicides, microbicides,

99 nematicides and other pesticides are reported to be used (Huang McBeath and McBeath 2010). The

100 average amount of pesticides used per hectare in China is roughly 1.5- to 4-fold higher than the

101 world average (Qiu 2011), thus resulting in the contamination of water bodies in the receiving areas

102 and disturbance of ecological equilibrium (Hui et al. 2003).

103 The United States is also a large the next largest consumer of pesticides, applying over 1 billion

104 pounds annually (Alavanja 2009) with dramatical consequences for human beings and environment

105 (Carvalho 2017).

106 As regards Europe, according to the Eurostat (2016), the main pesticide consumer is Spain (around

107 79,000 ton of active ingredients sold between 2011 and 2014), followed by France (≈ 75,000), Italy

108 (≈ 64,000), Germany (≈ 46,000) and United Kingdom (≈ 23,000).

109 The adverse effects of pesticide and antibioticsOACs pollution have been concerned for a long time

110 and many highly toxic and persistent pesticides have been banned worldwide. Although relatively

111 safer pesticides have been developed and replaced the highly toxic ones, environmental pollution

112 resulted by the long-term application of pesticides is far from being solved. Still now

113 oObsoleteobsolete pesticides widely used in agriculture in the past.still represent a threat to

114 environment, biodiversity, and human health for the region of Southeast Europe and their

115 environmental and human risk need to be assessed in order to mitigate their current risk. Many

116 organochlorines, organophosphates and pyrethroids have been banned but this did not solved the

117 problem yet (Aravinna et al. 2017). In Argentina, the use of hexachlorocyclohexane pesticides have

118 been limited from the late '90 and definitely banned in 2011, but this did not prevent to find

119 concentration of lindane during recent samplings. Although the maximum level of lindane in saline

120 water was fixed at 4 ng/l, in 2014 lindane exceeded this value of more than 5-fold (Ballesteros et al.

121 2014). Although the use of organo-chlorine pesticide has been banned for over 20 years, they can

122 still be found in the water and the sediment of main drainage area in China (Nakata et al. 2005; Xue

123 et al. 2006; Zhou et al. 2006), due to run off from aged and weathered agricultural soils, or

124 anaerobic sediments (Zhou et al. 2006). Except forBesides water bodies and sediment, water, soil

125 and even air in many cities are polluted by OACs, including urban or suburban areas (Gong et al.

126 2004; Nakata et al. 2005; Yang et al. 2008).

127 For that matter, OACs pose pivotal environmental problems, due to their high reisstance in the

128 environment and the consequent low natural attenuation. As an example, ; among them,

129 organochlorine pesticides and their metabolites, are resistant towere poorly affected by

130 photochemical, chemical and biological degradation processes.for a long time as reported by and

131 more than 95% of them impacted on non-target organisms (Mrema et al. (2013). The authors

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132 highlighted the impacts of pesticides, which become widely dispersed in the environment; it was
133 estimated that more than 95% of applied pesticides impact non target organisms. As a consequence,
134 rKim et al. (2017) reported a consequence, rnumber of routes pesticides might follow to meet
135 human beings; the resulting multi-pathway direct and indirect exposure may affect human health.
136 For instance during the last decade, one of the most studied issues is cancer occurence related to
137 pesticide exposure.
138 As persistent organopollutants (POPs), pesticides represent one of the major problems in both
139 terrestrial and aquatic ecosystems. Regulatory and risk assessment procedures have to be adopted
140 against those compounds that could be categorized as POPsOACs. Since early '90, European Union
141 started taking care of the problem. Driven from the carcinogenicity of pesticides, Directive 91/414/
142 EEC aimed to control the authorization for pesticides marketing within the EU. The particular
143 attention given to pesticides is because recent studies confirmed that even low dose and chronic
144 exposure might trigger adverse effects on wildlife and humans (EEA 2005). Being groundwater the
145 primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking
146 Water Directive 98/83/EC deal with pesticides maximal exposure concentrations: 0.1 μg/l of a
147 single pesticide and 0.5 μg/l of total pesticides load. The protract exposure to low amount of
148 pesticides cannot be underestimated because critical exposure levels can be chronically reached. A
149 rRiskrisk assessment has to consider the possible source of contamination but also the direct and
150 indirect multifaceted pathways of contact with human beings. Kim et al. (2017) reported a number
151 of routes pesticides might follow to meet human beings; the resulting multi-pathway direct and
152 indirect exposure may affect human health. Most of the pesticides emission (99 %) in the
153 environment in Europe is associated to agricultural practices whereas industrial and urban sources
154 as the manufacturing of pesticides or the at home use of insecticides have a minor impact (EEA
155 2016). Kim et al. (2017) reported a number of routes pesticides might follow to meet human
156 beings; the resulting multi-pathway direct and indirect exposure may affect human health.
157 Point discharges of pesticides used in agriculture may occur and are mainly associated to accidental
158 causes as spillage, inappropriate storage and disposal, etc. Most of pesticides instead reach surface
159 waters, through direct surface run-off or by leaching to groundwater and then subsequently follow
160 different transport pathways. Once entered in the aquatic system, they could ultimately contaminate
161 water for human consumption.
162 A clear correlation between agriculture and water contamination was observed in Mar Chiquita lake
163 (Argentina), since high amount of endosulfan residues were detected soon after application and
164 post application periods (Ballesteros et al. 2014). The presence of the fungicide thifluzamide in
165 paddy water of rice fields in China was maximal after the application, and variation during time was

166 ~~associated to the dilution effect of rainfalls in the area (Wei et al. 2015). Preventive measures to~~
167 ~~mitigate the impact of agriculture on the environment are required, taking into account both the use~~
168 ~~of safety pesticides and the optimization of farmer procedures. Aravinna et al. (2017) found that~~
169 ~~most of the 32 studied pesticides leached rice field following specific pathways. Since direct run off~~
170 ~~and erosion from soil were the main vehicles of dispersion, authors suggested alternative strategies~~
171 ~~(high resident time of pesticides, holding ponds of rice drainage water, delayed filling of paddies~~
172 ~~after pesticide application and use less mobile compounds) to reduce the movement of the~~
173 ~~pesticides.~~

174 Experimental evidences of advances in natural restoration processes highlight that time is our
175 friend, since the abandonment of disturbed/polluted agricultural land for long time could reduce
176 their contaminatin . ~~In fact, at a global scale, one of the most frequently used strategies is long term~~
177 ~~remediation, which is represented by the abandonment of disturbed/polluted agricultural land~~
178 ~~(Kardol and Wardle 2010).~~ Studies by Morriën et al. (2017) reported that nature restoration on ex-
179 arable land resulted in increased connettance of soil biota's networks, as restoration progresses.
180 Such results confirm that the functions played by the soil biota provide many and varied services,
181 and detoxification of pollutants and xenobiotic is one of the included primary services. In this
182 context, innovation is represented by the research of solutions inspired by nature, as strategy to
183 accelerate the natural attenuation processes in contaminated sites~~, optimizing bioremediation in real~~
184 ~~environment. Given that OACs represent a potential risk to humans, water, ecosystems and other~~
185 ~~receptors, fungi can play a pivotal role addressing their removal from contaminated sites and thus~~
186 ~~mitigating environmental pollution.~~

187 ~~So clean and safe water is a critical step that stands between the *status quo* and a sustainable world.~~
188 ~~This concept is no longer idealistic and became a milestone for the United Nations, as clearly stated~~
189 ~~in the World Water Development Report of 2015 (WWAP, 2015). Human lifestyle and the~~
190 ~~increasing urbanization lead to a worsened scenario. For instance, the actual pesticides use is 4 fold~~
191 ~~higher than 40 years ago (Mnif et al. 2011). EC compiled a watch list including, among others,~~
192 ~~pharmaceuticals, pesticides and personal care products. Being groundwater the primary source of~~
193 ~~drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking Water Directive~~
194 ~~98/83/EC deal with pesticides maximal exposure concentrations: 0.1 µg/l of a single pesticide and~~
195 ~~0.5 µg/l of total pesticides load.~~

196 In this context, bioremediation has aroused as an ~~is a~~ ~~usefulis b~~useful technology to degrade
197 ~~pesticides~~ OACs ~~by microbes~~ (Singh 2008; Velázquez-Fernández et al. 2012), with several benefits
198 over landfill disposal and incineration, ~~such~~ as the ~~conversion of toxic wastes to~~formation of non-
199 toxic end products, ~~a~~ lower costs~~cost~~ of disposal ~~(or no disposal at all)~~, reduced health and

ecological effects and long-term liabilities associated with non-destructive treatment methods, and the ability to perform the treatment *in situ* without unduly disturbing native ecosystems (Sarkar et al. 2005). ~~Therefore, there is a growing interest in developing bioremediation techniques to degrade OACs in polluted environments.~~ During the past decade, numerous microorganisms capable of degrading antibiotics and pesticides have been isolated, and detoxification processes for target pollutants have been analyzed. ~~As for many other POPs (BTEX, PHAs, PCB congeners, etc) with structural similarities with lignin, f~~Fungi~~fungi~~ and especially ligninolytic fungi have been suggested as the most promising group of organisms able to transform recalcitrant compounds through a unique set of extracellular oxidative enzymes (~~e.g.~~ Anastasi et al. 2013; Harms et al. 2017). Comparative genomic analysis of 49 fungi with different nutritional modes such as saprotrophic fungi, white-rot fungi (WRF), brown-rot fungi, ~~straw~~ soft rot fungi and symbiotic fungi indicated that there is a relationship between nutrition models and the enzymes for lignocellulose degradation. Saprotrophic fungi have greater number of enzymes than symbiotic fungi, and brown-rot fungi have smaller number than ~~white-rot fungi~~WRF and ~~straw~~ soft rot fungi (Wu et al. 2015a). This might gain some insights into how to choose fungi in OACs degradation.

~~Experimental evidences of advances in natural restoration processes highlight that time is our friend. In fact, at a global scale, one of the most frequently used strategies is long-term remediation, which is represented by the abandonment of disturbed/polluted agricultural land (Kardol and Wardle 2010). Studies by Morriën et al. (2017) reported that nature restoration on ex-arable land resulted in increased connettance of soil biota's networks, as restoration progresses. Such results confirm that the functions played by the soil biota provide many and varied services, and detoxification of pollutants and xenobiotic is one of the included primary services. In this context, innovation is represented by the research of solutions inspired by nature, as strategy to accelerate the natural attenuation processes in contaminated sites, optimizing bioremediation in real environment. Given that OACs represent a potential risk to humans, water, ecosystems and other receptors, fungi can play a pivotal role addressing their removal from contaminated sites and thus mitigating environmental pollution.~~

Finally yet importantly, metabolic activity of fungal or microbial consortia could produce not-known reaction products potentially with a major toxicity than parental compounds.

García-Carmona et al. (2017) highlighted the importance to carry out environmental monitoring activities ante and post operam phases, using bioassays to determine the success of the bioremediation process. Although it is substantial to assess the quality of the environment to ensure it remains free of toxic residues, most of the analytical tests available for determining the concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason,

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7 234 biotoxicity testing has grown steadily in recent years and is a useful tool in environmental risk
8 235 assessment (Shen et al. 2016; Prokop et al. 2016).
9
10 236 Indeed, there is a clear need to develop and define decontamination of hazardous pollutants as a
11 237 concept towards sustainable remediation through a broader uptake of principles, approaches and
12 238 tools to integrate environmental, social and economical dimension into the remediation processes
13
14 239 (Ridsdale and Noble 2016). Several organizations, academia, standardization committees are
15 240 currently assessing remediation process, evaluating the complexity of the concept of sustainability.
16 241 Several documents have been developed by many countries across Europe and at global scale,
17
18 242 addressing sustainable indicators of remediation activities (Harclerode et al. 2015).
19 243 The present review article summarizes the current state of scientific knowledge on research and
20
21 244 application of fungi as effective bioresources, considering the recent advances in understanding
22 245 their capacity to handle pesticide contamination.
23
24 246
25 247 **Bioremediation of OACs by fungi in soil system**
26 248 Large quantities of OACs~~antibiotics~~ are being added to agricultural fields worldwide through the
27
28 249 application of wastewater, manures and biosolids, resulting in pesticide and antibiotic
29 250 contamination and elevated environmental risks in terrestrial environments (Jechalke et al. 2014;
30 251 Zhang et al. 2015; Pan and Chu 2016). The largest fraction of ~~antibiotics~~ OACs applied to soils
31
32 252 with manure or biosolids is usually retained in surface soil whereas the part added through irrigation
33 253 with wastewater can diffuse easily deep or by surface run-off. Once added to soil, ~~antibiotics~~ OACs
34
35 254 interact with soil solid phase and are prone to microbial transformation (Hammesfahr et al. 2008;
36 255 Jechalke et al. 2014). In particular, veterinary antibiotics interact with soil solid phase in sorption
37 256 and desorption reactions. Sorption and desorption control not only their mobility and uptake by
38
39 257 plants but also their biotransformation and biological effects. ~~Antibiotics~~ OACs as well as
40 258 microorganisms are not distributed homogeneously in soil but are concentrated in hotspots. The
41
42 259 different surfaces, voids, and pores provided by soil aggregates harbor a vast amount of biological
43 260 diversity and chemical variability, and cause a patchy distribution of natural organic matter, oxides,
44 261 nutrients, and microorganisms on soil particle surfaces (Hammesfahr et al. 2008; Jones et al. 2012).
45
46 262 Sorption, sequestration, and subsequent release of ~~antibiotics~~ OACs likely also occur at and from
47 263 hotspots, and little is known about the behavior of ~~antibiotics~~ OACs at environmentally relevant
48 264 concentrations in agricultural soil.
49
50 265 Recently, many studies highlighted the fungal capability to transform and degrade recalcitrant
51 266 OACs. In particular, one of a promising group is the ligninolytic fungi that possess a unique set of
52 267 extracellular enzymes suitable to degrade lignin and are able to transform recalcitrant compounds,
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In particular, a promising group of fungi that are able to transform recalcitrant compounds and possess a unique set of extracellular ligninolytic enzymes are ligninolytic fungi (Čvančarová et al. 2015). (Supplemental data Table I; Table I References).2015). Nguyen et al. (2014) reported the removal of diverse trace organic contaminants (i. e. trichloroethyl chloroformate (TrOC), phenolic and non phenolic, pharmaceuticals, pesticides, steroid hormones, industrial precursors and products, phytoestrogens) by live (biosorption + biodegradation), intracellular enzyme-inhibited, and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes versicolor* (strain ATCC 7731). They showed how non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably higher degradation of the major compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. However, studies that examined both adsorption and degradation of antibiotics in agricultural soil are too few, with most of them using unrealistically high concentrations (in mg/kg levels) to overcome limitations in measurement. In addition, no model has been developed for speculating the adsorption and degradation of different types of antibiotics in agricultural soil and the environmental risks they may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native microorganisms (bacteria and fungi) in non sterilized (test) and sterilized (control) agricultural soils under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to microbial degradation under aerobic conditions, and most antibiotics were degraded by more than 92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial concentration was found to slow down degradation and prolong persistence in soil. The degradation pathway of antibiotics, in fact, varied in relation to their physicochemical properties as well as the microbial activities and aeration of the recipient soil. The authors were the first to develop a model for the prediction of antibiotic persistence in soil, which was valuable for the investigation of the fate of antibiotics in the terrestrial environment.

Given the public concern for environmental pollution by OACs, there is increasing attention towards the development of biopurification systems for reducing the risk from the point source contamination of soil resources. Various treatment methods (e.g. land filling of contaminated sites, recycling, pyrolysis and incineration) have been used for the removal and remediation of these chemicals from the contaminated sites, but for example microbial degradation of pesticides is results the most important and effective way to remove these compounds from the environment (Hai et al. 2012; Verma et al. 2014), (Supplemental data Table I; Table I References).2014).

Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. In particular, fungi may transform pesticides and other xenobiotics by introducing minor structural changes to the molecule, producing nontoxic molecules that could be released into the soil for further degradation by microflora (Hai et al. 2012), (Supplemental data Table I; Table I References).

In this context, Mir-Tutusaus et al. (2014) investigated the degradation of the insecticides imiprothrin and cypermethrin, the insecticide/nematicide carbofuran using the white-rot fungus *T. versicolor*. Their experiments with fungal pellets demonstrated extensive degradation of the tested agrochemicals. In vivo studies with inhibitors of cytochrome P450 revealed that this intracellular system plays an important role in the degradation of imiprothrin and carbofuran, but not for cypermethrin. The simultaneous degradation of the compounds successfully took place with minimal inhibition of fungal activity and resulted in the reduction of the global toxicity, thus supporting the potential use of *T. versicolor* for the treatment of several OACs.

To date, the number of studies investigating novel treatment techniques for the removal of ~~pesticides~~ OACs from contaminated agricultural soils is limited. The bacteria-dominated conventional activated sludge process has been proved to be ineffective for ~~pesticide~~ removal. While the importance of a mixed microbial community to initiate and complete ~~pesticide~~ OACs removal in the soil environment has been convincingly demonstrated by several researchers, studies concerning the removal of ~~pesticides~~ OACs from soils have been predominantly focused on selected bacterial or fungal species separately. Few studies have explored the bioaugmentation synergy of fungi and bacteria (Hai et al. 2012; Zhang et al. 2015; Madrigal-Zúñiga et al. 2016). Combining culture of bacteria and fungi could constitute a relevant process for the removal of toxic and recalcitrant organic substances from contaminated agricultural soils. On-farm biopurification systems represent a biotechnological approach for the mitigation of point source contamination by ~~pesticides~~OACs. The main component of the biopurification systems is the biomixture, which acts as the biologically active core that accelerates the degradation of ~~OACs.pesticides~~OACs Madrigal-Zúñiga et al. (2016) studied the employment possibility of the ligninolytic fungus *T. versicolor* in the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the insecticide-nematicide carbofuran (CFN). The CFN transformation products were detected at the moment of CFN application, but their concentration continuously decreased to complete removal in both biomixtures. Mineralization of ^{14}C radiolabeled CFN was faster in GTS than in GCS. The authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data suggested that the bioaugmentation improved the performance of the GTS rather than the GCS biomixture.

Moreover, Pinto et al. (2016) studied the potential use of different substrates in biomixtures as cork, cork and straw, coat pine and LECA (Light Expanded Clay Aggregates) on the degradation of terbuthylazine, difenoconazole, diflufenican and pendimethalin pesticides. Bioaugmentation strategies using the WRF *Lentinula edodes* inoculated into the CBX was also assessed. The results obtained from this study clearly demonstrated the relevance of using natural biosorbents as cork residues to increase the capacity of pesticide dissipation in biomixtures for establishing biobeds. Furthermore, higher degradation of all the pesticides was achieved by the use of bioaugmented biomixtures. Indeed, biomixtures inoculated with *L. edodes* EL1 were able to mineralize the selected xenobiotics, revealing that this WRF might be a suitable fungus for being used as inoculum sources in on-farm sustainable biopurification systems, in order to increase its degradation efficiency.

Fungi isolated from biomixture represents a biological source of potentially active bioremediation agents; the adaptation skills developed by these microorganisms could make the difference for OACs removal (Supplemental data Table I; Table I References).. This challenging strategy was assessed by Pinto et al. (2012), who isolated fungi from a loamy sand soil and a biomixture contaminated with terbuthylazine, difenoconazole and pendimethalin. The capability of degrading xenobiotics by autochthonous fungi (*Penicillium brevicompactum* and *Lecanicillium saksenae*) was compared with allochthonous strains taken from a Culture Collection (*Fusarium oxysporum*, *Aspergillus oryzae* and *L. edodes*). The major biodegradation yield was reached with *P. brevicompactum*: its higher ability to metabolize terbuthylazine was presumably acquired through chronic exposure to contamination with the herbicide.

Bioremediation of OACs by fungi in aquatic ecosystem

Many OACs are common contaminant of freshwater due to their high water solubility associated to a low soil adsorption, and their high stability that assure them a long half-life. These properties explain the recurring evidences of pesticides found in real water samples. The contamination is not heterogeneously distributed along watercourses as evidenced in several studies where and extensive studies are necessary.These properties explain the recurring evidences of pesticides were recurringly found in real water samples. For instance, an

An accurate survey took into consideration 23 European countries with more than 160 water samplings studying mainly pharmaceuticals, pesticides and known recognised endocrine disruptingchemiclasdisrupting chemiclas chemicals (Loos et al. 2010). Among the 59 compounds under study, the most frequently detected compound was 1 insecticide (DEET), and 7 pesticides (chloridazon-desphenyl, DMS, desethylatrazine, chloridazon-methyldesphenyl, bentazone,

370 desethylterbutylazine, dichlorprop) exceeded the European threshold of 0.1 µg/l. On the whole,
371 29Overall, 29% of the water samples could not be considered safe (Loos et al. 2010) accordingly to
372 this value. SimilarlySimilarly, in US, 18 states were monitored, focusing the attention of 65 organic
373 contaminants: along with plasticizers and detergent metabolites, 66% of the total pollutants load
374 was ascribable to insect repellant (Barnes et al. 2008).

375 The extent of the freshwaters contamination and the actual risk for human life depend on several
376 factors concerning the hydrogeological characteristics of the soil, the weather conditions and the
377 chemical-physical properties of the pesticideOACs. The environmental fate of a certain compound
378 is a critical issue in which the water/soil surface is the first barrier. For instance, the sorption
379 kinetics of three widely used pesticides (simazine, imidacloprid, and boscalid) have been correlated
380 to the soil organic carbon content and the hydrophobicity of the pesticide, ultimately affecting their
381 soil retention behavior and the actual bioavailability in waters (Salvestrini et al. 2014). The flow of
382 the leaching into surface waters is also a matter of season, in which opposite phenomena draw a
383 complex scenario to be predict. A rainy period could cause a massive run-off of OACsthe pesticides
384 from the soil contaminating the receiving basin (Sandin et al. 2018), but during dry season, the high
385 load of contaminants could be associated to evaporation and low water flow. Besides the detection
386 of high levels of pesticidespesticideOACs is not exclusively coincident to their recent and massive
387 use. but it is ascribable to their . Due to their persistency, their slow natural deagradtion, their
388 accumulation and the various diffusion pathways, they (Aguilar et al. 2017), . tThey could then
389 tread long distances in surface or groundwater waters and the contamination can last for several
390 decades (Ballesteros et al. 2014; Aravinna et al. 2017).

391 The so-called ecological services could help to contain the pesticidespesticideOACs diffusion.
392 Adapted microflora (fungi, Gram-positive and -negative bacteria, actinobacteria, and sulfate-
393 reducing bacteria) to the soil environmental conditions may reduce the pesticides released in
394 groundwater sources (Mattsson et al. 2015). Several factors as soil composition, temperature,
395 aeration due to soil weaving and depth influence the autochthonous microbial community activity;
396 if this balance fails, pesticidespesticideOACs are free to move among different ecological niches
397 (i.e. sediment and water)), and alter their functioning and ultimately directly affecting their animal
398 inhabitants. For instance, sSignificant ecological risk was associated to the presence of the
399 insecticide fipronil and its metabolites in three water ponds: concentration up to 200 ng/l affected
400 the proper development of larval insects and crustaceans (Wu et al. 2015b). Evidences of the
401 pesticides toxicity against fish has been already reported, demonstrating their interference with
402 different metabolic pathways (Odukkathil and Vasudevan 2013; Ballesteros et al. 2014; Guerreño et
403 al. 2016).

404 The preservation of water quality is a priority but OACs removal could not be based only on natural
405 attenuation. Water treatment plants (WTPs) are the major barrages where OACs should be
406 removed. Not being specifically designed for micropollutants removal, they are often only partially
407 effective, with a strong impact on the receiving ecosystem. Pesticides as atrazine, fluconazole,
408 tebuconazole, diazinon and diuron are particularly resistant to commonly in use treatments (Köck-
409 Schulmeyer et al. 2013; Luo et al. 2014). A number of evidences confirmed the presence of OACs
410 in WTPs effluents at toxicologically and estrogenically relevant concentration, becoming one of the
411 most effecting source of contamination (Bicchi et al. 2009; Campo et al. 2013; Jarošová et al.
412 2014).

413 Particular attention has been given to advanced biological oxidation. Novel cost-effective and eco-
414 friendly processes based on fungi are an attractive option. ~~They~~ Fungi are well-known for to their
415 physiological adaption skills, including the natural activation of tolerance mechanisms against
416 pesticides (Talk et al. 2016). ~~In comparison with bacteria,~~ Some reports already demonstrated that
417 in comparison with bacteria, fungi can better tolerate the presence of organic contaminants.
418 Although the insecticide endosulfan inhibited both fungi and bacteria, bacterial community
419 structure significantly changed already at 0.1 mg/kg while modifications on the fungal community
420 structures required 1 mg/kg of pollutant (Zhang et al. 2015). Linuron reduced bacterial count, and
421 especially total bacteria, N_2-fixing bacteria and nitrifiers, but not fungal numbers (Cycoń et al.
422 2010).

423 The importance of the isolation origin of fungi is out of discussion. Strains isolated from
424 contaminated niches could have indeed developed specific adaptation skills due to the chronically
425 exposure over time. Carles et al. (2017) demonstrated that the aquatic microflora associated to
426 submerged leaves exposed to nicosulfuron is more efficient in its degradation than communities
427 belonging to a less polluted site. The authors indicated fungi as the main constituents of this active
428 microflora and as responsible of the herbicide degradation. In literature, several fungi isolated from
429 contaminated areas or WTPs have been identified as degraders of nicosulfuron, diuron, isoproturon,
430 glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013; Carranza et al. 2014;
431 Oliveira et al. 2015).

432 Exploiting this oxidative cascade, fungi may transform a broad range of recalcitrant organic
433 compounds, including OACs (Gao et al. 2010). A number of fungi are ~~pesticidespesticide~~OACs
434 degraders, mostly belonging to Basydiomycetes as *Trametes*, *Pleurotus, Phlebia, Cerrena,
435 Coriolopsis*, etc. have been already investigated (Koroleva et al. 2002; Marco-Urrea et al. 2009;
436 Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et al. 2014; Ceci et al. 2015) (Table ~~II~~2,
437 Supplementary Materials).~~)~~. Several ~~classes of~~ pesticides as lindane, atrazine, diuron,

438 terbuthylazine, metalaxyl, DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin,

439 heptachlor, chlordane, lindane, mirex, etc. were effectively transformed by fungal treatment based

440 on mycelium or enzymes (Table II, Supplementary Materials).

441 A bioremediation approach based on fungi may involve both biosorption and biodegradation

442 processes; the latter one combines biosorption where the molecule binds to the fungal wall, and

443 bioaccumulation with the pollutant being transported inside the cell in contact with intracellular

444 enzymes (Kulshreshtha et al. 2014). Concentration of insecticide lindane decrased during time in

445 the presence of two WRF (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus

446 (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated the main

447 involvement of a fast adsorption process (Ulčnik et al. 2013). However, this phenomenon is often

448 strains dependent, and expecially related to metabolic differences between Ascomycetes and

449 Basidiomycetes. Belonging to brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes,

450 responsible for lignin degradation and likely for OACs as well: adsorption onto fungal mycelium

451 was mainly involved for removal of endosulfan. On the contrary, the WRF actively degraded

452 producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013). Although biosorption is

453 a phenomenon that could be not ignored, it is often secondary or at least negligible respect to

454 biodegradation (Carles et al. 2017). For instance, the removal of clofibric acid associated to heat-

455 killed mycelium was less than 10 %, but more than 97 % in the presence of active *T. versicolor*

456 (Marco-Urrea et al. 2009).

457 Fungi have developed a specific mechanism that employs few enzymes and molecules with high

458 oxidizing power, physiologically aimed to transform ligninocellulose structure. The same enzymatic

459 pathway may play a pivotal role in transforming other aromatic molecules. White-rot fungi usually

460 involve ligninocellulosic extracellular enzymes as peroxidases (EC 1.11.1.x) and laccases (EC

461 1.10.3.2). The involvement of redox enzymes in the fungal-mediated oxidation is confirmed by the

462 direct induction of enzyme production due to the presence of pesticides.pesticideOACs. The fungus

463 *T. versicolor* responded to 17 pesticides by increasing laccases production in comparison with the

464 control: particular attention was given to transformation products of the herbicides diquat and

465 monuron, capable of increasing the activity of 10- and 17-fold, respectively (Mougin et al. 2002).

466 Laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus* spp1, *Pleurotus* spp2,

467 *Cymatoderma elegans*, *Daedalea elegans* was stimulated by the presence of atrazine even at high

468 concentration 3750 mg/l. Likewise the pesticide positively affected the manganese peroxidase

469 activity of *Pleurotus* spp1 and *C. elegans* (Chan-Cupul et al. 2014). Oxidoreductases stimulation

470 was also observed with picloram (Maciel et al. 2013), bentazon (Da Silva Coelho et al. 2010),

471 carbofuran (Mir-Tutusaus et al. 2014).

472 Although these oxidoreductases are probably the most known enzymes for aromatic compounds
473 degradation, alternative pathways can be promoted by the presence of ~~pesticides.pesticide~~OACs.
474 Two clones (laccase positive and negative producers) of *Mycelia sterilia* were used to treat atrazine
475 (20 μg/ml): even though one clone was defective for laccase production, comparable transformation
476 yields (70-80%) were reached indicating their minor role in the degradation process (Vasil'Chenko
477 et al. 2002). This behavior is commonly found in brown-rot fungi that may trigger both on
478 nonenzymatic and enzymatic mechanisms, i.e. Fenton mechanism or cellobiose dehydrogenase
479 (CDH) reactions (Fan and Song 2014). The degradation of atrazine (20 μg/l) by an unidentified
480 mycelial fungus was associated to the presence in the liquid medium of OH radicals and CDH.
481 Moreover, the CDH secretion was induced by the presence of the herbicide itself (Khromonygina et
482 al. 2004). In addition, some fungi could associate extracellular oxidoreductases with intracellular
483 enzymes <u>such</u> as the cytochrome P450 system (cyt450). In the effort to better characterize the
484 degradation skills of *T. versicolor*, cyt450 inhibitors were used: fungal performances against
485 clofibric acid and fipronil decreased (Marco-Urrea et al. 2009; Wolfand et al. 2016). Mori et al.
486 (2017) suggested that cyt450 of *Phanerochaete sordida* is involved in the first reduction of the
487 clothianidin N-nitro group but the enzymes responsible of the further urea derivatives production
488 are unknown.

489 The intra- and interspecies variability has long been recognized and found ~~confirmation~~
490 ~~also~~confirmation for ~~pesticidespesticide~~OACs treatment. Literature data about a certain specie
491 could not be taken for granted and the set-up of a preliminary screening is often required. Despite
492 *Phanerochaete chrysosporium* is often indicated as fungal model for organic degradation including
493 pesticides (Wang et al. 2014), it was almost ineffective against clofibric acid. Among five
494 Basidiomycetes, only *T. versicolor* extensively degraded the herbicide (Marco-Urrea et al. 2009).
495 Alvarenga et al. (2014) treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*.
496 Based on the growth capability in the presence of the pesticide, <u>only</u> the isolate *A. sydowii* CBMAI
497 935 was selected for further studies. It indeed grew almost 4-fold more than the other *A. sydowii*.
498 The bioremediation potential is often substrate targeted, and the choice of fungus cannot be taken
499 for granted. For instance, the exact same isolate (*A. sydowii* CBMAI 935) that totally converted
500 methyl parathion (Alvarenga et al. 2014) was not the best performing one against the insecticide
501 esfenvalerate. Among 6 fungi, *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave
502 better results than the *Aspergillus* strain (Birolli et al. 2016).

503 Although the majority of these strains are effective in ~~pesticidespesticide~~OACs removal in model
504 solution, only few researchers have made a step forward, assessing the bioremediation potential of
505 contaminated waters. The acquired information using model solutions (single-compound solution,

high concentration, no interfering molecules, etc.) is the unique way to acquire information about the degradation pathway (Masaphy et al. 1993; Birolli et al. 2016), but is less predictive of the fungal performances on real environmental water samples. Each wastewater has its own critical issues, making difficult to predict the fungal behavior. Some data highlighted the robustness of a fungal system, although this needs detailed investigation case-by-case. A partially diluted leachate showed to disturb the growth of *T. versicolor* and *Stereum hirsutum*, but this did not prevent them to totally degrade linuron and dimethoate at 10 mg/l. As regards dimethoate, the presence of adsorbents enhance the final process yields (from 50% to 97%), combining and exalting the action of adsorption and biodegradation processes (Castellana and Loffredo 2014). The immobilization of *Bjerkandera adusta* and *Irpex lacteus* on coffee grounds, almond shells, a biochar favored the removal of non-phenolic herbicides as fenuron and carbaryl from a municipal landfill leachate (Loffredo et al. 2016). Surface waters, ground waters or municipal wastewaters represent a very unique environment, characterized by extreme chemical and physical conditions, the presence of heterogeneous and variable micropollutant mixture and an active autochthonous microflora. When inoculated in real surface water, a fungal consortium (*Aspergillus fumigatus, Aspergillus terreus, Cladosporium tenuissimum, Cladosporium cladosporioides, Fusarium begoniae, Penicillium citrinum, Penicillium melanoconidium* and *Phoma glomerata*) was not stable in time due probably to the presence of toxic pesticides and the interaction with the natural microbial population: *P. citrinum, A. fumigatus* and *A. terreus* were the most robust to the environmental conditions and actually capable of degrading the spiked chlorfenvinphos (Oliveira et al. 2015).

The set-up of active microbial consortia is an intriguing solution to strengthen and combine the bioremediation potential of different organisms. Interestingly the combination of *Bacillus subtilis* and *A. niger* led to higher degradation rate of nicosulfuron than those obtained by using singly each strain (Lu et al. 2012). The biodegradation of aldicarb, atrazine and alachlor by *Coriolus versicolor* was strongly enhanced by the combination with activated sludge. Along with modifications in the fungal morphology, when the bacterial-fungal consortium was established, the bio-absorbed fraction of especially atrazine was reduced: over 98% of atrazine was removed by degradation processes in two weeks (Hai et al. 2012).

The fate of the treated ~~pesticidespesticide~~OACs is major issue that has to be carefully considered. The residual toxicity is a critical issue. Interestingly fenuron and carbaryl ~~(up to 70%)~~ degradation (up to 70%) catalyzed by *B. adusta* and *I. lacteus* led to significant abatement of the phytotoxicity (rapeseed and flax tests) (Loffredo et al. 2016). Mori et al. (2017) followed the neurotoxicity of clothianidin and its main metabolite produced by *P. sordida* treatment: the insecticide altered the cell viability of the neuronal cell line, but the metabolite was no longer neurotoxic.

540 Despite the well demonstrated properties, the application of whole cell system has some drawbacks

541 including the fact that a living organism needs controlled growing conditions, in terms of nutrients,

542 pH, O_2, etc. (Majeau et al. 2010). The addition of synthetic nutrients can strengthen fungal

543 mycelium activity, but it should be carefully balanced for a further scale-up of the process. The fact

544 that *T. versicolor* need 1% of glucose as carbon source to degrade atrazine would ultimately

545 interfere with its potential use in real WTPs (Khromonygina et al. 2004). Likewise several fungi as

546 *A. niger* and *Dacryopinax elegans*, etc. required both easily available carbon and nitrogen sources

547 to efficiently act against nicosulfuron and diuron, respectively (Lu et al. 2012; Arakaki et al. 2013).

548 Particular attention should be instead given to those fungi as *A. sydowii* and *Penicillium*

549 *decaturense* that maintained the same performances without glucose addition, indicating the

550 potential of using methyl parathion or triclosan as sole carbon source (Alvarenga et al. 2014; Tian et

551 al. 2016).

552 A promising alternative could be given by the direct use of fungal enzymes, capable of catalyzing

553 strong and fast oxidation reactions, with less technical drawbacks in comparison with fungal

554 cultures. The potential of enzymes-based methods has been worldwide recognized; the Swiss

555 Industrial Biocatalysis Consortium defined oxidative enzymes as the biocatalysts displaying the

556 highest development potential in the next decades (Meyer and Munch 2005). Great importance is

557 given to the discovery of novel enzymes with wide substrate specificity, stable and applicable to

558 industrial uses. A number of articles have reported the ability of fungal enzymes to degrade

559 ~~pesticides.pesticide~~OACs. The potential of laccase-mediator systems have been assessed for the

560 degradation of isoproturon (Margot et al. 2015), imiprothrin (Mir-Tutusaus et al. 2014),

561 chloroxuron (Palvannan et al. 2014), isoproturon (Zeng et al. 2017), atrazine (Chan-Cupul et al.

562 2016). Laccases cannot be consider a novelty, as instead a phytase of *A. niger* capable of degrading

563 organophosphorus pesticides (Shah et al. 2017) or a cellulase of *Trichoderma longibrachiatum*

564 active against dicofol (Wang et al. 2015). Particular attention should be given to the use of crude

565 enzyme extracts of ligninolytic enzymes with a minor economic impact on the process than purified

566 enzymes (Matute et al. 2012; Kaur et al. 2016). A crude extract of *Trametes pubescens* laccases

567 degraded up to 19 compounds in model solution and confirmed its ~~potential also~~potential- with a

568 real municipal wastewater where the presence of suspended particles, colloids, solvents and

569 xenobiotics as well as autochthonous microorganisms posed a strong environmental pressure. The

570 transformation of all the detected compounds determined also a strong reduction of the

571 estrogenicity of the water sample (Spina et al. 2015).

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573 ***Application of synthetic microbial community on bioremediation***

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574   Bioremediation is crucial way to eliminate the OACs pollution in agriculture ecosystem. However, 575   many factors effect bioremediation efficiency for pesticide pollution, such as microbes applied, 576   treatment sites, rhizosphere effects, soil chemical and physical properties (Zhou and Hua 2004). The 577   practice in the bioremediation of soil or water pollution often cannot reach expected results because 578   the target contaminant could not be degraded completely in most cases, and sometimes intermediate 579   products were occurred with more toxin than original pesticides. Long-term application of various 580   pesticides resulted in the pollution of more than one type of chemical compounds, which is hard to 581   be degraded by a sole microbe. Thus, attention has been shifted to synthetic systems based on 582   communication between cells, rather than individual isolated cell functionality (Biliouris et al. 583   2012). A promising way to overcome the difficulties is to create artificial synthetic microbial 584   communities that contain several microbes to retain the key features of their natural counterparts 585   (Großkopf and Soyer 2014).

586   Synthetic microbial community is a collective term that is created by a bottom-up approach where 587   two or more defined microbial populations are assembled in a well-characterized and controlled 588   environment (De Roy et al. 2014). In synthetic communities, mixed populations can perform 589   complex tasks, although in changing environmental conditions to be robust to changes in 590   environment (Brenner et al. 2008). There are several potential advantages of synthetic community 591   compared to monocultures or natural community: 1) the species in a synthetic community are 592   identified and the community structure is relatively simple and controllable, while the natural 593   community is mixed up by many microorganisms with unknown functions; 2) synthetic community 594   can perform more complicated functions than individual organism because members of microbial 595   consortia communicate and differentiate (Brenner et al. 2008); 3) synthetic community can be more 596   robust to environmental fluctuations because communities might be more capable of better resisting 597   invasion by other species and weather periods of nutrient limitation compared with monocultures 598   (Brenner et al. 2008); 4) synthetic community might be described through mathematical models 599   more easily than natural systems, and they can be used to develop and validate models of more 600   complex systems (Liu et al. 2017).

601   To develop a cooperative and steady-state community that is performing a desirable 602   biotechnological function, Liu et al. (2017) concluded three design principles for the construction of 603   synthetic community. Firstly, safety should be prioritized by beginning with innocuous or 604   commensal organisms (Brenner et al. 2008). Secondly, the community can converse a low-cost 605   and/or recalcitrant waste material into a biotechnologically relevant product, partial or de-novo 606   biosynthesize a compound via heterologous metabolic pathways, or bioconverse toxic substrates or 607   products in a toxic milieu process with toxic substrates or products or substrate conversion in a

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toxic milieu (Jagmann and Philipp 2014). Thirdly, the bioremediation process should be optimized and regularly controlled based on the knowledge of stability and division of different microorganisms (Liu et al. 2017).

Bioremediation of polluted soils and water is one application field of synthetic microbial community. As the complex structure of some pollutants, the effect of adding synthetic microbial community is much higher than single microorganism, such as the biodegradation of pesticides diuron. The herbicide diuron is used for control of broad-leaved weeds on agricultural land. Several fungal-bacterial consortia were investigated by combining three different diuron-degrading bacteria and two fungal strains. The fastest mineralization of diuron was obtained by the three member consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47) as measured by evolved 14CO2, mineralizing about 32 % of the added diuron within 54 days, whereas the single strains or other consortia reached no more than 10% mineralization. In addition, the production of diuron metabolites by consortium was minimal. This may be due to cooperative catabolism, where the first organism transforms the pollutant to products that are then used by other organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby facilitating a more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al. 2014).

Similarly, a fungal-bacterial consortium consisting of Mortierella sp. LEJ702 and the 2,6-dichlorobenzamide (BAM)-degrading Aminobacter sp. MSH1 reached a more rapid mineralisation of BAM than the bacterial alone, especially at lower moisture contents (Knudsen et al. 2013). Methylotrophic and hydrocarbon utilizing yeasts and bacteria alone did not degrade PCBs significantly, but PCB degradation achieved about 50% when WRF were applied together (Šašek et al. 1993).

Evaluation of bioremediation effectiveness in contaminated matrices by performing ecotoxicological and genotoxic tests

In order to improve the effectiveness and performance of bioremediation processes it is important to pursue three essential goals at the same time. Focus should be not only on reducing chemical concentrations, but also on reducing chemical mobility between in the environmental compartments and eventually lowering toxicity levels ensuring that contaminants do not get into the natural biological cycle (Loehr and Webster 1997; Chakraborty et al. 2013).

Bioremediation is often monitored by following the concentration of targeted contaminants (Molina-Barahona et al. 2005). Numerous studies in recent years showed that traditional chemical analyses are insufficient for a full assessment of the contaminated site as they, for example, does not

provide any information about the interaction of chemicals and does not consider the partition and the mobility of pollutants (Frische 2003; Molina-Barahona et al. 2005; Ma et al. 2005; Molnár et al. 2007). An integrated approach linking the various fields and levels of study involving contaminated sites has proven to be an efficient system of evaluating bioremediation effectiveness in contaminated sites (Chapman and Anderson 2005; Wernersson et al. 2015; Marziali et al. 2017). Consequently, to achieve the desired goals and implement a successful bioremediation program a close collaboration of microbiologists, chemists and engineers is requested by the chemical and biological complexity of the tasks (Van Gestel et al. 2001; Chakraborty et al. 2013).

Additionally, the use of ecotoxicological and genotoxic tests in order to evaluate the bioremediation effectiveness can be a valid tool to partially overcome the existing gap between the reported successes of bioremediation on the laboratory scale and the field scale.

Signals that bioremediation is going on could be important to be monitored. Two important chemical compounds produced by microorganisms during their degradation activity are CO_2 and soluble phosphorus. Both increase distinctly in the soil treated with insecticides and inoculated with fungi (Boyle 1995; Abd El-Ghany and Masmali 2016). However, it must be taken into consideration that during and after a bioremediation process the disappearance of the parent compounds or evidence of the metabolic activity (e.g. CO_2 production) may not indicate detoxification. Beside the fact that the fate of the toxicants may be followed by chemical analyses, many reaction products resulting from a bioremediation process are not known and their potential toxicity, as well. The elimination of mother compounds does not necessarily result in toxicity removal, and evaluating the efficiency of the process is important to assess not only the removal of a specific compound, but also the potential ecotoxicity. In fact, biodegradation of pesticides can proceed partially or totally due to the molecular structure itself or unfavourable environmental or test conditions and the lack of 'acclimatized' microbial communities (De Henau 1997). In some instances, it has been shown that to an effective process of bioremediation corresponds to a decrease in the toxicity of the analysed matrix (Baud-Grasset et al. 1993; Dorn and Salanitro 2000). To acquire complete and useful information in an ecotoxicological assessment and to determine the effectiveness of bioremediation treatments, it is suggested to use a battery of tests (Keddy et al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of biological reference organismstest species that are representative of the different trophic levels, in order to select species with different roles in ecosystems, and different routes of exposure conditions (Van Straalen and Van Gestel 1997). Moreover, the environmental risk assessment must integrate chemical characterization, ecotoxicity and bioremediation data, in order to accurately assess the ecological hazard.

676 As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various
677 bioindicators either could indicate an incomplete decomposition of the substance or could result
678 from the formation of intermediate products generated via the bioremediation process. For this
679 reason, sometimes chronic tests are more appropriate in evaluating the toxicity caused by by-
680 products (Lofrano et al. 2014). In other cases, however, also the toxicity of the by-products is
681 effectively removed (Lofrano et al. 2016).

682 In certain circumstances, there is a clear need to monitor the bioremediation process using different
683 bioindicators. In Lizano-Fallas et al. (2017), for example, the ecotoxicity test with *Daphnia magna*
684 shows a clear detoxification, whilst the detoxification patterns remain unclear when applying the
685 phytotoxicity test. Ecotoxicological tests can also be used to determine the most suitable
686 bioremediation technique in relation to the examined case study as reported in Dudášová et al.
687 (2016). Without worldwide-recognized unique guidelines for water quality assessment, literature
688 data are difficult to compare due to the variety of model organisms, end-points, etc. Synthetic
689 indices capable of summarizing these findings could help to have an objective advice about the
690 effectiveness of the biological treatment. They have been already applied for toxicity monitoring of
691 wastewaters (Tigini et al. 2011) but municipal effluents containing AOCs have never been taken
692 into consideration nor estrogenic activity has been included so far.

693 Several toxicity assays were included in the treatability study protocol to measure remediation
694 efficiency. Assessing the toxicity of complex matrixes such soil could acquire methods from
695 bioassays used to test toxicity of chemical compounds reported by the Organization for Economic
696 Co-operation and Development (e.g. OECD 201 2006; OECD 211 2012). OECD has published a
697 series of standardized tests for determining the biodegradability of a given compound, based on the
698 evaluation of overall parameters (such as COD, TOC and BOD) or methabolic tests, e.g.
699 respirometric (OECD 209 1984) as Polo et al. (2011) used for revealing susceptibility to of toxic
700 compound comprising herbicide to biological treatment. Standardized testing procedures using
701 different organisms have been approved by various environmental organizations, including the US
702 Environmental Protection Agency, American Society for Testing and Materials, International
703 Standardization Organization (Siciliano et al. 2015). Many scientists have explored the effects of
704 polluted soil on the whole organism using various microorganisms, animals, and plants, or by
705 means of cellular, and biochemical biomarkers, or by ecological scale up systems. Here below, tests
706 at some different biological hierarchical levels of analyses are reported and discussed.

707

708 *Organismal level*

709 Concerning complex matrixes as soil, quality assessments are performed with organisms on extracts

710 of the polluted matrix, generally applying short-term exposure periods (Van Gestel et al. 2001).

711 Experimental models are aquatic organisms such as *Daphnia magna*, *Raphidocelis subcapitata*,

712 *Danio reri*o, *Myriophyllum aquaticum* or *Lemna minor* (Feiler et al. 2004). The use of freshwater

713 and marine biota may be particular useful in order to provide a more complete comprehension on

714 the environmental outcomes of agricultural activities evaluating the fate of pesticides (Guida et al.

715 2008). Terrestrial animals such as nematodes (*Caenorhabditis elegans*) (Traunspurger et al. 1997),

716 oligochaetes (*Lumbriculus variegatus*) (Phipps et al. 1993), springtails), springtails as *Folsomia*

717 *candida* (Houx et al. 1996), and fish embryos (Hollert et al. 2003; Zielke et al. 2011) are well

718 considered among the most reliable models.

719 Among higher plants important experimental models are *Lepidium sativum*, *Cucumis sativus*, and

720 *Sorghum saccharatum* (germination rate, inhibition of root elongation). Since assays based on

721 animals, plants and algae are considered expensive, time consuming and require large sample

722 volume, recent studies have emphasized the benefits of rapid, reproducible and cost effective

723 bacterial assays for toxicity screening and assessment. *Arthrobacter globiformi* (Neumann-Hensel

724 and Melbye 2006), *Bacillus cereus* (Rönnpagel et al. 1995; Prokop et al. 2016), *Vibrio proteolyticus*

725 (Ahlf and Heise 2005) yeasts (*Saccharomyces cerevisiae*) (Weber et al. 2006) are often used;

726 otherwise, among bacterial bioassays, *Vibrio fischeri* luminescence inhibition test is the most

727 common. The review of Parvez et al. (2006) remarks that *Vibrio fischeri* inhibition test is the most

728 sensitive test, cost effective, easy to operate and requires only 5–30 min for toxicity prediction.

729

730 *Cellular and biomolecular level*

731 Biomarkers are adaptive responses by the organisms after exposure to xenobiotics. Various studies

732 highlighted the cytoxicity and genotoxicity effect of OACs and their metabolic products on the

733 organisms. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or

734 physiological, or even by behavioural changes (Depledge et al. 1993) that enable the obtaining of

735 information on the biological effects of pollutants or their remains during or after a bioremediation

736 process (Fontanetti et al. 2011).

737 Genetic endpoints and biomarkers. The most used biomarkers are mitotic index, chromosome

738 aberrations, micronuclei, sister chromatid exchange and mutations.

739 Various scientists have recommended bacteria for bioassays evaluating genotoxicity in different

740 samples (Mortelmans and Zeiger 2000; White and Claxton 2004). Ames test, one of the most

741 famous and used, is a short term bacterial reverse mutation assay especially designed to evaluate the

742 mutagenic potential of wide range of chemical substances (Mortelmans and Zeiger 2000) and was

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743 found to be very sensitive to wide range of mutagenic and carcinogenic chemicals as reported in the
744 review paper of Chahal et al. (2014).

745 On the side of plant models, higher plants are recognized as excellent genetic models to detect
746 cytogenetic and mutagenic agents and are frequently used in environmental monitoring studies. The
747 main organisms are *Allium cepa*, *Vicia faba* and *Tradescantia* spp. as reported in a review by De
748 Souza et al. (2016). Their protocols are standardized through a program under the International
749 Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme
750 (UNEP) (Ma 1999). In addition, the US Environmental Protection Agency (USEPA) and the World
751 Health Organization (WHO) validated the results obtained with plant bioindicators as an efficient
752 model to detect environmental genotoxicity.

753 One of the most used higher plant model is *V. faba*. The main advantages are its availability round
754 the year, economical to use, easy to grow and handle; its use does not require sterile conditions and
755 rate of cell division is fast. The *V. faba* test, deeply reported and discussed in the review of Iqbal
756 (2016), enables the assessment of different endpoints i.e., chromosomal aberration, mitotic index,
757 micronuclei and nuclear aberration.

758 <u>Enzymatic biomarkers</u>. Enzymatic activity inhibition as biomarker has been widely evaluated to
759 measure toxicity of a matrix. Dehydrogenases, for example, are directly involved in many of the
760 vital anabolic and catabolic processes of living organisms, and their activity is inhibited by
761 chemical toxicants. Recently, many studies have reported the use of terrestrial organisms for
762 developing enzymatic biomarkers in response to residual pesticides (Henson-Ramsey et al. 2011;
763 Radwan and Mohamed 2013; Stepić et al. 2013), and among these, earthworms were widely used to
764 understand the impacts of pesticides. In two earthworm species, *Eisenia fetida* and *Lumbricus*
765 *terrestris*, multiple esterases, including acetylcholinesterase (AChE), butyrylcholinesterase, and
766 carboxylesterase (CE), have been assessed as biomarkers for malathion exposure (Henson-Ramsey
767 et al. 2011). Several studies have also reported AChE, catalase (CAT), and glutathione-S-
768 transferase as bio-chemical biomarkers in *Eisenia andrei* for the insecticides endosulfan, temephos,
769 malathion, and pirimiphos-methyl (Stepić et al. 2013), and AChE, CAT, CE, and the efflux pump as
770 biomarkers in *E. andrei* and *Octolasion lacteum* for dimethoa. Recently, surface-enhanced laser
771 desorption/ionization-time-of-flight (SELDI-TOF) mass spectrometry (MS) has strongly
772 contributed to the identification of more accurate, precise biomarkers e.g. specific for human
773 cancers (Silsirivanit et al. 2014), or for endosulfan exposure in Japanese rice fish (*Oryzias latipes*)
774 (Lee et al. 2013). In a recent paper, selective protein biomarkers for 6 pesticides (captan, carbaryl,
775 carbofuran, and α-endosulfan chlorpyrifos, propoxur) were found in *E. fetida*, by means of SELDI-
776 TOF MS technology (Park et al. 2015).

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777   <u>Estrogenic and androgenic biomarkers</u>. It has been well documented that several chemicals from

778   agricultural, industrial, and household sources possess endocrine-disrupting properties, which

779   provide a potential threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995;

780   Jensen et al. 1995). A suggested mechanism is that environmental contaminants alter the normal

781   functioning of the endocrine and reproductive system by mimicking or inhibiting endogenous

782   hormone action, modulating the production of endogenous hormones, or altering hormone receptor

783   populations (Sonnenschein and Soto 1998). Besides several pesticides exert estrogenic and

784   antiandrogenic activities through interaction with estrogen and androgen receptors. The risk

785   associated to OACs exposure has been known for decades: many pesticides, such as p,p´-

786   dichlorodiphenyl trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978;

787   Cummings 1997), β-benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan,

788   toxaphene, and dieldrin (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) have been firstly

789   signaled as estrogenic. Despite increased institutional awareness and more compelling legislation

790   pressure, the most recent literature still reports the occurrence of pesticides in watercourses <u>and in</u>

791   ~~passing through~~ the trophic chains, ~~ing~~ showing remarkable estrogenic or androgenic (Saillenfait et

792   al. 2016; Brander et al. 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al.

793   2017; Marcoccia et al. 2017). Several bioassays have been developed and standardized in order to

794   describe the estrogenic potency of OACs. Andersen et al. (2002) indicated that several currently

795   used OACs, such as methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl,

796   possess estrogenic activity on the basis of cell proliferation assay and transactivation assay using

797   MCF-7 human breast cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism

798   and antagonism to two human estrogen receptor (hER) subtypes, hERα and hERβ, and a human

799   androgen receptor (hAR) by highly sensitive transactivation assays, using Chinese hamster ovary

800   cells. The results demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic

801   activities through ERs and/or AR. Although it appears that various pesticides exert hormonal effects

802   at concentration orders of magnitude higher than that required for physiologic hormones, wide

803   exposure to large numbers of OACs may have additive and synergistic effects. Bioassay with YES

804   (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active

805   compounds still present in the environment. By the the first papers about this subject (Purvis et al.

806   1991), much more sophisticated bioassays have been developed such as that proposed by Eldridge

807   et al. (2007) in which a bioluminescence strain of *Saccharomyces cerevisiae* was genetically

808   engineered to respond to androgenic chemicals.

809

810   *Ecological level*

The actual risk of chemical residues pollution from bioremediation process is underestimated at the ecological level in natural systems. The ecological scaling-up experiment illustrated by Rodea-Palomares et al. (2016) underlined how real-world exposure to chemical pollution is often dominated by low-dose complex combined with other biotic and abiotic stressors. In the paper, a novel screening method (GSA-QHTS) was reported, that coupled the computational power of global sensitivity analysis (GSA) with the experimental efficiency of quantitative high-throughput screening (QHTS). In the case of study, they reported GSA-QHTS allowed for the identification of the main pharmaceutical pollutants, driving biological effects of low-dose complex mixtures at the microbial population level. The target complex community was a river benthic microbial community inocula obtained from an unpolluted stream. The effect of the toxic compounds in a mixture was evaluated together with other physico-chemical stressors, on a series of community level metabolic end points. Photosynthetic parameters, the dark-adapted basal fluorescence, the light-adapted steady-state fluorescence, the maximum photosynthetic efficiency, as well as the extracellular enzymatic activities b-Glu and Phos were considered as both autotrophic and heterotrophic global fitness indicators suited to study the effects of chemical pollution on freshwater benthic microbial communities.

Prospect

Bioremediation is based on the idea that different organisms will work together to remove (biodegrade) the waste substances or pollutants (OACs) from environment. Although limitations for bioremediation practice might be occurred, including the nature of organisms, the enzyme involved, the concentration and availability and finally survival of microorganisms, as well as cost/benefit ratio (i.e. cost versus overall environmental impact), to some extent, these limitations can be solved by understanding the genetics and biochemistry of desired microbe. The advent of synthetic community showed giant potential ability in facilitating the bioremediation process, especially the effective utility of degradative fungi.

Acknowledgement

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Supplemental material: Table I

Table I. Fungal species list for biodegradation of pesticide pollutants

Pesticide types	target pesticide	Fungal species	Fungal habitats	Origin	Literature
organochlorine	aldrin	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
	chlordane	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
	DDT	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
	DDT	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
	DDT	*Pleurotus florida*	white-rot		Arisoy 1998
	DDT	*Pleurotus eryngi*	white-rot		Arisoy 1998
	DDT	*Gloeophyllum trabeum*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum sepiarium*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum ungulatum*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum striatum*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea malicola*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea albida*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea serialis*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea dickinsii*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis palustris*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis annosa*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis insularis*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis pinicola*	brown-rot		Purnomo et al 2008
	DDT	*Boletus edulis*	ectomycorrhizal		Huang et al 2007
	DDT	*Gomphidius viscidus*	ectomycorrhizal		Huang et al 2007
	DDT	*Laccaria bicolor*	ectomycorrhizal		Huang et al 2007
	DDT	*Leccinum scabrum*	ectomycorrhizal		Huang et al 2007
	DDT	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
	DDD	*Trichoderma sp.*	saprotrophic	marine sponges	Ortega et al 2011
	DDD	*Penicillium miczynskii*	saprotrophic	marine sponges	Ortega et al 2011
	dieldrin	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993

dieldrin	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
endosulfan	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
endosulfan	*Phanerochaete chrysosporium*	white-rot		Kullman and Matsumura 1996
heptachlor	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
heptachlor	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
heptachlor	*Pleurotus florida*	white-rot		Arisoy 1998
heptachlor	*Pleurotus eryngi*	white-rot		Arisoy 1998
pentachloronitrobenzene	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
pentachlorophenol(PCP)	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
pentachlorophenol(PCP)	*Phanerochaete chrysosporium*	white-rot		Kang and Stevens 1994
pentachlorophenol(PCP)	*Pleurotus ostreatus*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Irpex lacteus*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Trametes versicolor*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Bjerkandera adusta*	white-rot		Rüttimann-Johnson and Lamar 1997
pendimethalin	*Fusarium oxysporum*	saprotrophic	soil	Singh and Kulshreyha 1991
pendimethalin	*Paecilomyces varioti*	saprotrophic	soil	Singh and Kulshreyha 1991
pendimethalin	*Rhizoctonia bataticola*	saprotrophic	soil	Singh and Kulshreyha 1991
lindane	*Rhizopus oryzae*	saprotrophic		Young and Banks 1998
lindane	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
lindane	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
lindane	*Pleurotus florida*	white-rot		Arisoy 1998
lindane	*Pleurotus eryngi*	white-rot		Arisoy 1998
mirex	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
PCB 77	*Phanerochaete chrysosporium*	white-rot		Vyas et al 1994

	PCB 77	*Trametes versicolor*	white-rot		Vyas et al 1994
	PCB 77	*Coriolopsis polysona*	white-rot		Vyas et al 1994
	Delor 106 (PCB)	*Phanerochaete chrysosporium*	white-rot		Novotný et al 1997
	Delor 106 (PCB)	*Trametes versicolor*	white-rot		Novotný et al 1997
	Delor 106 (PCB)	*Coriolopsis polyzona*	white-rot		Novotný et al 1997
	Six PCB congeners	*Trametes versicolor*	white-rot		Beaudette et al 2000
	Six PCB congeners	*Bjerkandera adusta*	white-rot		Beaudette et al 2000
	Six PCB congeners	*Phanerochaete chrysosporium*	white-rot		Beaudette et al 2000
organophosphate	chlorpyrifos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993
	chlorpyrifos	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	chlorpyrifos	*Coriolus versicolor*	white-rot		Bending et al 2002
	chlorpyrifos	*Trichoderma harzianum*	saprotrophic	soil	Omar 1998
	chlorpyrifos	*Pencillium brevicompactum*	saprotrophic	soil	Omar 1998
	fonofos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993
	glyphosate	*Penicillium citrium*	saprotrophic		Zboinska et al 1992
	methyl parathion	*Aspergillus sydowii*	saprotrophic	marine	Alvarenga et al 2014
	methyl parathion	*Penicillium decaturense*	saprotrophic	marine	Alvarenga et al 2014
	terbufos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993
herbicide	alachlor	*Phanerochaete chrysosporium*	white-rot		Ferrey et al 1994
	alachlor	*Ceriporiopsis subvermispora*	white-rot		Ferrey et al 1994
	alachlor	*Phlebia tremellosa*	white-rot		Ferrey et al 1994
	alachlor	*Cunninghamella elegans*			Pothuluri et al 1993
	arochlor	*Pleurotus ostreatus*	white-rot		Zeddel et al 1993
	arochlor	*Trametes versicolor*	white-rot		Zeddel et al 1993
	three aroclors	*Phanerochaete chrysosporium*	white-rot		Yadav et al 1995
	atrazine	*Phanerochaete chrysosporium*	white-rot		Mougin et al 1994
	atrazine	*Pleurotus pulmonarius*	white-rot		Masaphy 1993
	atrazine	*Agrocybe semiorbicularis*	white-rot		Bending et al 2002
	atrazine	*Auricularia auricola*	white-rot		Bending et al 2002
	atrazine	*Coriolus versicolor*	white-rot		Bending et al 2002

	atrazine	*Dichotomitus squalens*	white-rot		Bending et al 2002
	atrazine	*Flammulina velupites*	white-rot		Bending et al 2002
	atrazine	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	atrazine	*Phanerochaete velutina*	white-rot		Bending et al 2002
	atrazine	*Pleurotus ostreatus*	white-rot		Bending et al 2002
	atrazine	*Stereum hirsutum*	white-rot		Bending et al 2002
	diuron	*Agrocybe semiorbicularis*	white-rot		Bending et al 2002
	diuron	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	diuron	*Stereum hirsutum*	white-rot		Bending et al 2002
	diuron	*Coriolus versicolor*	white-rot		Bending et al 2002
fungicide	carbendazim	*Trichoderma sp.*	saprotrophic	mutant strain	Tian and Chen 2009
	metalaxyl	*Coriolus versicolor*	white-rot		Bending et al 2002
	metalaxyl	*Stereum hirsutum*	white-rot		Bending et al 2002
	iprodione	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	iprodione	*Stereum hirsutum*	white-rot		Bending et al 2002
	iprodione	*Coriolus versicolor*	white-rot		Bending et al 2002
PAH	five PAHs	*Bjerkandera adusta*	white-rot	soil and lignite	Gramss et al 1995
	five PAHs	*Gymnophilus sapineus*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma fasciculare*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma frowardii*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma sublateritium*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Kuehneromyces mutabilis*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Lenzites betulina*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Pleurotus ostreatus*	white-rot	soil and lignite	Gramss et al 1995
	five PAHs	*Agrocybe praecox*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Stropharia coronilla*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Stropharia rugoso-annulata*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Agaricus aestivalis*	Terricolous	soil and lignite	Gramss et al 1995
	five PAHs	*Agaricus arvensis*	Terricolous	soil and lignite	Gramss et al 1995

five PAHs	*Agaricus bisporus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus campestris*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus porphyrizon*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agrocybe dura*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Bovisa nigrescens*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Clitocybe odora*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Collybia dyophila*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Collybia maculata*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Coprinus comatus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista nebularis*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista nuda*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista saeva*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lycoperdon perlatum*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Marasmius oreades*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Megacollybia platyphylla*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Phallus impudicus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Psathyrella velutina*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Stropharia aeruginosa*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Amanita muscaria*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Amanita rubescens*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Amanita spissa*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Hebeloma crustuliniforme*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Hebeloma hiemale*	Ectomycorrhizal	soil and lignite	Gramss et al 1995

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five PAHs	*Hebeloma sinapizans*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Laccaria amethystina*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius deliciosus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius deterrimus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius rufus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius torminosus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella conica*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella elata*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella esculenta*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Paxillus involutus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Russula aeruginea*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Russula foetens*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Suillus granulatus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Suillus variegatus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Tricholoma lascivum*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Tricholoma terreum*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Xerocomus badius*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Botrytis cinerea*	Mitosporic	soil and lignite	Gramss et al 1995
five PAHs	*Scytalidium lignicola*	saprotrophic	soil and lignite	Gramss et al 1995
five PAHs	*Trichoderma sp.*	saprotrophic	soil and lignite	Gramss et al 1995

Supplemental material – Table I: References

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Supplemental material: Table II

Table II. Fungi and their enzymes capable of transforming OACs; whole-cell and enzymatic treatments are reported

Whole-cell treatment			
Fungal species	**Pesticide**	**Enzymes involved**	**Literature**
Aspergillus niger	nicosulfuron		Lu et al. 2012
Auricularia fuscosuccinea	endosulfan	laccase, phenol oxidase	Yanez-Montalvo et al. 2016
Aspergillus sydowii, Penicillium decaturense	methyl parathion		Alvarenga et al. 2014
Aspergillus sydowii, Penicillium raistrickii, Cladosporium sp., Microsphaeropsis sp., Acremonium sp., Westerdykella sp, Cladosporium sp.	esfenvalerate		Birolli et al. 2016
Aspergillus fumigatus, Aspergillus terreus, Penicillium citrinum, Trichoderma harzianum	chlorfenvinphos		Oliveira et al. 2015
Aspergillus oryzae	3-phenoxybenzoic acid		Zhu et al. 2016

Aspergillus oryzae, Fusarium oxysporum, Lentinula edodes, Penicillium brevicompactum, Lecanicillium saksenae	terbuthylazine, difenoconazole and pendimethalin		Pinto et al. 2012
Aspergillus sydowii	trichlorfon		Tian et al. 2016
Aspergillus versicolor	triclosan		Taştan and Dönmez 2015
Coriolus versicolor	aldicarb, atrazine, alachlor		Hai et al. 2012
Dacryopinax elegans	diuron	laccase, manganese peroxidase, lignin peroxidase	Arakaki et al. 2013
Ganoderma lucidum	lindane	laccase, manganese peroxidase, lignin peroxidase	Kaur et al. 2016
Ganoderma lucidum	bentazon	laccase, manganese peroxidase	Da Silva Coelho et al. 2010
Ganoderma lucidum, Trametes sp	picloram	laccase	Maciel et al. 2013
Gloeophyllum trabeum, Trametes versicolor, Pleurotus ostreatus	lindane, endosulfan		Ulčnik et al. 2013
Mycelia sterilia	atrazine	laccase	Vasil'chenko et al. 2002
Penicillium citrinum, P.citrinum, Fusarium proliferatum	methylparathion		Rodrigues et al. 2016
Penicillium griseofulvum	b-hexachlorocyclohexane		Ceci et al. 2015
Phanerochaete sordida	clothianidin	cytochrome P450, manganese peroxidase	Mori et al. 2017
Pleurotus pulmonarius	atrazine		Masaphy et al. 1993
Phlebia tremellosa, Phlebia brevispora, Phlebia acanthocystis	Heptachlor, heptachlor epoxide		Xiao et al. 2011
Saccharomyces cerevisiae	diazinon		Ehrampoush et al. 2017
Talaromyces flavus	nicosulfuron		Song et al. 2013
Trametes versicolor	imiprothrin, cypermethrin, carbofuran, oxytetracycline	laccase, cytochrome P450	Mir-Tutusaus et al. 2014
Trametes versicolor	fipronil	cytochrome P450	Wolfand et al. 2016
Trametes versicolor	6 pesticides, 2 phytoestrogens		Nguyen et al. 2014
Trametes versicolor, Stereum hirsutum	linuron, dimethoate		Castellana and Loffredo 2014
nonsporulating mycelial fungus	atrazine	cellobiose dehydrogenase	Khromonygina et al. 2004

Enzymatic treatment

Enzymes involved	Pesticide	Literature
laccases of *Agaricus blazei*	metsulfuron	González Matute et al. 2012
phytase of *Aspergillus niger*	chlorpyrifos	Shah et al. 2017
extracellular extract of *Auricularia fuscosuccinea*	endosulfan	Yanez-Montalvo et al. 2016
laccase of *Trametes versicolor*	sulfamethoxazole, isoproturon	Margot et al. 2015
laccase of *Trametes versicolor*	chloroxuron	Palvannan et al. 2014
laccase of *Trametes versicolor*	lindane, endosulfan	Ulčnik et al. 2013
cellulose of *Trichoderma longbrachiatum*	dicofol	Wang et al. 2015
laccase of *Trametes versicolor*	isoproturon	Zeng et al. 2017

Supplemental material – Table II: References

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