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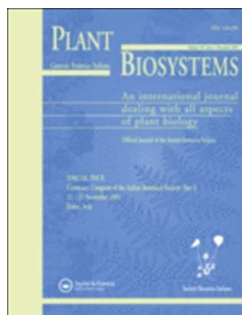
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# 1 Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water

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## 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.

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3 30 Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi,  
4 31 Synthetic microbial community, Environmental risk assessment.  
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9 33 ***Introduction***

10 34 Because of their unique functions, fungi are involved in ecosystem services essential to human  
11 35 well-being. Among others, fungi also carry out the transformation and detoxification of pollutants.  
12 36 For this reason, learning from nature, they represent an effective toolbox for a sustainable  
13 37 bioremediation of pesticides in soil and water. Many researches have revealed the untapped  
14 38 potential of fungi, and recent years have witnessed very interesting developments regarding the  
15 39 application of fungi not only to improve environmental quality but also human health (e.g. Gargano  
16 40 et al. 2017).

17 41 Pesticides are a diverse group of inorganic and organic chemicals that include herbicides,  
18 42 insecticides, nematicides, fungicides, antibiotics and soil fumigants (Verger and Boobis 2013;  
19 43 Verma et al. 2014). They are employed in agriculture to enhance crop yield and quality, and to  
20 44 maximize economic returns by preventing pest or weed attack. They are bioactive, toxic substances,  
21 45 capable of directly or indirectly influencing soil fertility and health as well as agroecosystem quality  
22 46 (Pinto et al. 2012; Verma et al. 2014). Given that belowground biodiversity is closely linked to land  
23 47 management, agricultural intensification exerts many pressures that lead to loss of biodiversity.

24 48 Consequently, soil pollution is one of the main threats to the decline of taxonomic and functional  
25 49 biodiversity, and to agricultural soil sustainability (Harms et al. 2017). Most pesticide emission  
26 50 (99 %) in Europe is associated with agricultural practices, whereas industrial and urban sources  
27 51 such as the manufacturing of pesticides or the at-home use of insecticides have a minor impact  
28 52 (EEA 2016).

29 53 The extensive and massive use of pesticides in agricultural activities has a serious impact on the  
30 54 environment, compromising soil and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al.  
31 55 2016). In addition to pesticides, large quantities of antibiotics are added to agricultural fields  
32 56 worldwide through the application of wastewater, manures and biosolids, also resulting in antibiotic  
33 57 contamination and elevated environmental risks (Jechalke et al. 2014; Zhang et al. 2015; Pan and  
34 58 Chu 2016). A clear correlation between agriculture and water contamination was observed in Mar  
35 59 Chiquita lake (Argentina), where large amounts of endosulfan residues were detected soon after  
36 60 application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide  
37 61 thifluzamide in the water in rice paddies in China was maximal after application, with variation  
38 62 over time associated with the dilution effect of rainfalls in the area (Wei et al. 2015).

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3 63 Preventive measures are required, to mitigate the impact of agriculture on the environment. These  
4 64 must take into account both the use of safe pesticides and the optimization of farmer procedures.  
5 65 Aravinna et al. (2017) found that most of the 32 studied pesticides leached off rice paddies  
6 66 following specific pathways. Since direct runoff and erosion from soil were the main vehicles of  
7 67 dispersion, authors suggested alternative strategies (high resident time for pesticides, holding ponds  
8 68 for rice drainage water, delayed filling of paddies after pesticide application, and the use of less  
9 69 mobile compounds) to reduce the movement of the pesticides.

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14 70 The intensive use of organic agrochemicals (OACs) poses risks to both wild life and human  
15 71 health. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their  
16 72 target species through air, water and soil (Miller 2004). Around 30% of pesticides marketed in  
17 73 developing countries do not meet internationally accepted quality standards, posing a serious threat  
18 74 to human health and the environment (Popp et al. 2013). They are persistent in the environment and  
19 75 are biomagnified through the food chain, and it has been estimated that millions of agricultural  
20 76 workers worldwide experience unintentional pesticide poisoning each year. The correlation between  
21 77 long-term exposure to pesticides in occupational settings and illness is known, but recently non-  
22 78 occupational exposures have also been associated with an elevated rate of chronic diseases (Parrón  
23 79 et al. 2014).

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30 80 Varieties and consumption of pesticides worldwide have increased dramatically, by up to 4-fold  
31 81 since 40 years ago (Mnif et al. 2011). According to De et al. (2014), about 45 % are used in Europe,  
32 82 25 % in the USA, and 25 % in the rest of the world. The main pesticide consumer is Spain (around  
33 83 79,000 ton of active ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy  
34 84 (~ 64,000), Germany (~ 46,000) and United Kingdom (~ 23,000) (Eurostat 2016). The United  
35 85 States applies over 1 billion pounds annually (Alavanja 2009) with dramatic consequences for  
36 86 human beings and environment (Carvalho 2017). According to other authors (Huang McBeath and  
37 87 McBeath 2010), China is the world's largest pesticide user, with a pesticide output of around 3.7  
38 88 million tons (National Bureau of Statistics of China - <http://data.stats.gov.cn>), and a consumption of  
39 89 about 1.8 million tons in 2014. More than 350 insecticides, herbicides, microbicides, nematicides  
40 90 and other pesticides are reported to be used. The average amount of pesticides used per hectare in  
41 91 China is roughly 1.5- to 4-fold higher than the world average (Qiu 2011), thus resulting in  
42 92 contamination of water bodies in the receiving areas and disturbance of ecological equilibrium (Hui  
43 93 et al. 2003). Overall, use of pesticides in China breaks down as herbicides 47.5 %, insecticides  
44 94 29.5 %, fungicides 17.5 % and others 5.5 % (De et al. 2014).

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54 95 The adverse effects of OAC pollution have been of concern for a long time and many highly toxic  
55 96 and persistent pesticides have been banned worldwide. Although relatively safer pesticides have

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3 97 been developed and replaced the highly toxic ones, environmental pollution resulting from the long-  
4 98 term application of pesticides is far from being solved. Obsolete pesticides still represent a threat to  
5 99 environment, biodiversity, and human health for the region of Southeast Europe and their risk to the  
6 100 environment and to humans needs to be assessed in order to mitigate it. Many organochlorines,  
7 101 organophosphates and pyrethroids have been banned but this has not yet solved the problem  
8 102 (Aravinna et al. 2017). In Argentina, hexachlorocyclohexane pesticides have been limited since the  
9 103 late '90s and were definitely banned in 2011, but samples taken from a saline lake in 2014 showed  
10 104 levels to be more than 5-fold over the legal limit of 4 ng/l for lindane levels in the environment  
11 105 (Ballesteros et al. 2014). Likewise in China, although the use of organochlorine pesticides has been  
12 106 banned for over 20 years, they can still be found in the water and sediments of main drainage areas  
13 107 (Nakata et al. 2005; Xue et al. 2006; Zhou et al. 2006), due to run-off from aged and weathered  
14 108 agricultural soils and from anaerobic sediments (Zhou et al. 2006). Water bodies and sediments, the  
15 109 water, the soil and even the air in many cities in China are polluted by OACs, in both urban and  
16 110 suburban areas (Gong et al. 2004; Nakata et al. 2005; Yang et al. 2008).

17 111 OACs pose pivotal environmental problems, due to their high resistance in the environment and the  
18 112 consequent low natural attenuation. As an example, organochlorine pesticides were poorly affected  
19 113 by photochemical, chemical and biological processes, and more than 95% of them impacted on non-  
20 114 target organisms (Mrema et al. 2013). As a consequence, regulatory and risk assessment procedures  
21 115 have to be adopted against OACs. Driven by the carcinogenicity of pesticides, Directive 91/414/  
22 116 EEC aimed to regulate the authorization of pesticides marketing within the EU.

23 117 The particular attention given to pesticides is because, as confirmed in recent studies, even low  
24 118 doses might trigger adverse effects on wildlife and humans (EEA 2005). As groundwater is our  
25 119 primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking  
26 120 Water Directive 98/83/EC deal with maximum pesticide exposure concentrations: 0.1 µg/l of a  
27 121 single pesticide and 0.5 µg/l total pesticide load. Risk assessment needs to consider not only the  
28 122 source of contamination, but also the multifaceted direct and indirect pathways of contact with  
29 123 human beings. Kim et al. (2017) reported a number of routes pesticides might follow to meet human  
30 124 beings; the resulting direct and indirect multi-pathway exposure may affect human health.

31 125 Experimental evidence of progress in natural restoration processes highlight that time is our ally,  
32 126 since the abandonment of disturbed/polluted agricultural land for long time can reduce  
33 127 contamination (Kardol and Wardle 2010). Studies by Morriën et al. (2017) reported that nature  
34 128 restoration on ex-arable land resulted in increased connectivity of soil biota networks, as restoration  
35 129 progresses. Such results confirm that soil biota provide many and varied services, and that  
36 130 detoxification of pollutants and xenobiotics is one of the primary ones.

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3 131 In this context, innovation involves the search for solutions inspired by nature, with the strategy  
4 132 being to accelerate the natural attenuation processes in contaminated sites. Bioremediation has  
5 133 arisen as a useful technology to degrade OACs (Singh 2008; Velázquez-Fernández et al. 2012),  
6 134 with several benefits over landfill disposal and incineration, such as the formation of non-toxic end  
7 135 products, lower costs of disposal, reduction of effects on health and ecology and on the long-term  
8 136 liabilities associated with destructive treatment methods, and the ability to perform the treatment *in*  
9 137 *situ* without unduly disturbing native ecosystems (Sarkar et al. 2005). Over the past decade,  
10 138 numerous microorganisms capable of degrading antibiotics and pesticides have been isolated, and  
11 139 detoxification processes for target pollutants have been analyzed. Fungi and especially ligninolytic  
12 140 fungi have been suggested as the most promising group of organisms, as they are able to transform  
13 141 recalcitrant compounds through a unique set of extracellular oxidative enzymes (Anastasi et al.  
14 142 2013; Harms et al. 2017). Comparative genomic analysis of 49 fungi with different nutritional  
15 143 modes, such as saprotrophic fungi, white-rot fungi (WRF), brown-rot fungi, soft rot fungi and  
16 144 symbiotic fungi indicate that there is a relationship between nutrition models and the enzymes for  
17 145 lignocellulose degradation. Saprotrophic fungi have a greater number of enzymes than symbiotic  
18 146 fungi, and brown-rot fungi have a smaller number than WRF and soft rot fungi (Wu et al. 2015a).  
19 147 This might provide some insight into how to choose fungi in OACs degradation.



20 148 Finally ye<sup>o</sup> importantly, the metabolic activity of fungal or microbial consortia could potentially  
21 149 produce unknown reaction products that are more toxic than the parent compounds. García-  
22 150 Carmona et al. (2017) highlighted the importance of carrying out environmental monitoring  
23 151 activities ante- and post-operam phases, using bioassays to determine the success of the  
24 152 bioremediation process. Although it is fundamental to assess the quality of the environment to  
25 153 ensure it remains free of toxic residues, most of the analytical tests available for determining the  
26 154 concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason,  
27 155 biotoxicity testing has grown steadily in recent years and is a useful tool in environmental risk  
28 156 assessment (Shen et al. 2016; Prokop et al. 2016).

29 157 Indeed, there is a clear need to develop and define decontamination of hazardous pollutants as a  
30 158 concept that will support sustainable remediation by involving a broader uptake of principles,  
31 159 approaches and tools that integrate environmental, social and economical dimensions into  
32 160 remediation processes (Ridsdale and Noble 2016). Several organizations, academia and  
33 161 standardization committees are currently assessing remediation process and evaluating the  
34 162 complexity of sustainability. Documents have been developed by many countries across Europe and  
35 163 globally, addressing sustainable indicators for remediation activities (Harclerode et al. 2015).

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3 164 The present review summarizes the current state of scientific knowledge on research and  
4 165 application of fungi as effective bioresources, considering recent advances in understanding their  
5 166 capacity to face up the pesticide contamination.  
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### 9 168 **Bioremediation of OACs by fungi in the soil system**

10 169 Large quantities of OACs are being added to agricultural fields worldwide through the application  
11 170 of wastewater, manures and biosolids, resulting in pesticide and antibiotic contamination and  
12 171 elevated environmental risks in terrestrial environments (Jechalke et al. 2014; Zhang et al. 2015;  
13 172 Pan and Chu 2016). A large proportion of the OACs applied to soils with manure or biosolids are  
14 173 retained in surface soil, whereas those added through irrigation with wastewater can seep down to  
15 174 lower horizons or be diffused in surface run-off. Once present, OACs interact with the solid phase  
16 175 of soil and are prone to microbial transformation (Hammesfahr et al. 2008; Jechalke et al. 2014). In  
17 176 particular, veterinary antibiotics interact with the soil solid phase in sorption and desorption  
18 177 reactions. Sorption and desorption control not only their mobility and uptake by plants but also their  
19 178 biotransformation and biological effects. OACs, like microorganisms are not distributed  
20 179 homogeneously in soil but are concentrated in hotspots. The multiplicity of surfaces, voids, and  
21 180 pores provided by soil aggregates harbor a vast amount of biological diversity and chemical  
22 181 variability, and cause patchy distribution of natural organic matter, oxides, nutrients, and  
23 182 microorganisms on soil particle surfaces (Hammesfahr et al. 2008; Jones et al. 2012). Sorption,  
24 183 sequestration, and subsequent release of OACs likely also occur at and from hotspots. Little is  
25 184 known about the behavior of OACs at environmentally relevant concentrations in agricultural soil.

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27 185 Recently, many studies have highlighted the ability of fungi to transform and degrade recalcitrant  
28 186 OACs. In particular, one of promising group is the ligninolytic fungi that possess a unique set of  
29 187 extracellular enzymes suitable to degrade lignin and are able to transform recalcitrant compounds,  
30 188 (Čvančarová et al. 2015) (Supplemental material Table I; Table I References). Nguyen et al. (2014)  
31 189 reported the removal of diverse trace organic contaminants  Dichloroethyl chloroformate (TrOC)  
32 190 including phenolic and non-phenolic compounds, pharmaceuticals, pesticides, steroid hormones,  
33 191 industrial precursors and products, and phytoestrogen  by live (biosorption + biodegradation),  
34 192 intracellular, enzyme-inhibited and chemically inactivated (biosorption only) whole-cell  
35 193 preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes*  
36 194 *versicolor* (strain ATCC 7731). They showed how non-phenolic TrOC were readily biodegraded  
37 195 while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably  
38 196 higher degradation of the major compounds, indicating the importance of biosorption and  
39 197 subsequent degradation by intracellular and/or mycelium associated enzymes. However, there are  
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3 198 too few studies that examine both adsorption and degradation of antibiotics in agricultural soil, with  
4 199 most using unrealistically high concentrations (in mg/kg levels) to overcome limitations in  
5 200 measurement. In addition, no model has been developed to speculate about the adsorption and  
6 201 degradation of different types of antibiotics in agricultural soil and the environmental risks they  
7 202 may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics  
8 203 (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native  
9 204 microorganisms (bacteria and fungi) in non-sterilized (test) and sterilized (control) agricultural soils  
10 205 under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to  
11 206 microbial degradation under aerobic conditions, and most antibiotics were degraded by more than  
12 207 92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial  
13 208 concentration was found to slow down degradation and prolong persistence in soil. The degradation  
14 209 pathway of antibiotics varied in relation to their physicochemical properties as well as the microbial  
15 210 activities and aeration of the recipient soil. In their study, Pan and Chu (1996) were the first to  
16 211 develop a model for the prediction of antibiotic persistence in soil.

17 212 Given the public concern for environmental pollution by OACs, there is increasing attention  
18 213 towards the development of biopurification systems for reducing the risk from point source  
19 214 contamination of soil resources. Various treatment methods (e.g. land filling, recycling, pyrolysis  
20 215 and incineration) have been used for the removal and remediation of these chemicals from the  
21 216 contaminated sites, but microbial degradation of pesticides is so far the most important and  
22 217 effective way to remove these compounds from the environment (Hai et al. 2012; Verma et al.  
23 218 2014), (Supplemental material Table I; Table I References).

24 219 Microorganisms have the ability to interact both chemically and physically with substances, leading  
25 220 to structural changes or to complete degradation of the target molecule. In particular, fungi may  
26 221 transform pesticides and other xenobiotics by introducing minor structural changes to the molecule,  
27 222 producing nontoxic molecules that can be released into the soil for further degradation by  
28 223 microflora (Hai et al. 2012), (Supplemental material Table I; Table I References). Mir-Tutusaus et  
29 224 al. (2014) investigated the degradation of the insecticides imiprothrin and cypermethrin and the  
30 225 insecticide/nematicide carbofuran using the white-rot fungus *T. versicolor*. Experiments with fungal  
31 226 pellets demonstrated extensive degradation of the tested agrochemicals, while *in vivo* studies with  
32 227 inhibitors of cytochrome P450 revealed that this intracellular system plays an important role in the  
33 228 degradation of imiprothrin and carbofuran, but not of cypermethrin. The simultaneous degradation  
34 229 of the compounds successfully took place with minimal inhibition of fungal activity and resulted in  
35 230 reduction of global toxicity, thus supporting the potential use of *T. versicolor* for the treatment of  
36 231 several OACs.

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3 232 To date, the number of studies investigating novel treatment techniques for the removal of OACs  
4 233 from contaminated agricultural soils is limited. The bacteria-dominated conventional activated  
5 234 sludge process has been proved to be ineffective for OAC removal. While the importance of a  
6 235 mixed microbial community to initiate and complete OAC removal in the soil environment has been  
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8 236 convincingly demonstrated by several researchers, studies concerning the removal of OACs from  
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10 237 soils have predominantly focused on selected bacterial or fungal species separately. Few studies  
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12 238 have explored the bioaugmentation synergy of fungi together with bacteria (Hai et al. 2012; Zhang  
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14 239 et al. 2015; Madrigal-Zúñiga et al. 2016). Combining cultures of bacteria and fungi could be key to  
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16 240 the removal of toxic and recalcitrant organic substances from contaminated agricultural soils.

17 241 On-farm biopurification systems constitute a biotechnological approach to the mitigation of point  
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19 242 source contamination by pesticides. The main component of biopurification systems is the  
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21 243 biomixture, which acts as the biologically active core that accelerates the degradation of OACs.  
22 244 Madrigal-Zúñiga et al. (2016) studied the results of employing the ligninolytic fungus *T. versicolor*  
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24 245 in the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the  
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26 246 insecticide-nematicide carbofuran (CFN). The transformation products of CFN were detected at the  
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28 247 moment of CFN application, but their concentration decreased continuously until complete removal  
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30 248 in both biomixtures. Mineralization of <sup>14</sup>C radiolabeled CFN was faster in GTS than in GCS. The  
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32 249 authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data  
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34 250 suggested that the bioaugmentation improved the performance of the GTS rather than the GCS  
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36 251 biomixture.

37 252 Pinto et al. (2016) also studied the potential use of different substrates in biomixtures like cork, cork  
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39 253 and straw, coat pine and LECA (Light Expanded Clay Aggregates) in the degradation of  
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41 254 terbuthylazine, difenoconazole, diflufenican and pendimethalin pesticides. Bioaugmentation using  
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43 255 the WRF *Lentinula edodes* inoculated into the CBX was also assessed. The results obtained from  
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45 256 this study clearly demonstrated the relevance of using natural biosorbents such as cork residues to  
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47 257 increase the capacity for pesticide dissipation in biomixtures for establishing biobeds. Furthermore,  
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49 258 greater degradation of all the pesticides was achieved by the use of bioaugmented biomixtures.  
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51 259 Indeed, biomixtures inoculated with *L. edodes* EL1 were able to mineralize the selected xenobiotics,  
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53 260 revealing that this WRF might be a suitable fungus to be used as inoculum source to improve the  
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55 261 degradation efficiency of sustainable on-farm biopurification systems.

56 262 Fungi isolated from biomixtures represent a biological source of potentially active bioremediation  
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58 263 agents, and the adaptation skills developed by these microorganisms could make the difference in  
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60 264 OAC removal (Supplemental material Table I; Table I References). This strategy was assessed by  
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62 265 Pinto et al. (2012), who isolated fungi from a loamy sand soil and a biomixture contaminated with

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3 266 terbuthylazine, difenoconazole and pendimethalin. The ability of autochthonous fungi (*Penicillium*  
4 267 *brevicompectum* and *Lecanicillium saksenae*) to degrade xenobiotics was compared with that of  
5 268 allochthonous strains taken from a culture collection (*Fusarium oxysporum*, *Aspergillus oryzae* and  
6 269 *L. edodes*). The best biodegradation yield was achieved with *P. brevicompactum*: its higher ability  
7 270 to metabolize terbuthylazine was presumably acquired through chronic exposure to contamination  
8 271 with the herbicide.

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### 273 ***Bioremediation of OACs by fungi in aquatic ecosystems***

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

286 The extent of freshwater contamination and the actual risk to human life depend on several factors  
287 concerning the hydrogeological characteristics of the soil, weather conditions and the chemical-  
288 physical properties of the OACs. The environmental fate of a given compound is a critical issue in  
289 which the water/soil surface is the first barrier. For instance, the sorption kinetics of three widely  
290 used pesticides (simazine, imidacloprid, and boscalid) were found to be correlated with soil organic  
291 carbon content and the hydrophobicity of the pesticide, which ultimately affected soil retention  
292 behavior and bioavailability in waters (Salvestrini et al. 2014). Leaching into surface waters is also  
293 a matter of season, and a complex and unpredictable scenario is influenced by a variety of  
294 phenomena. A rainy period can cause massive run-off of OACs from the soil, contaminating the  
295 receiving basin (Sandin et al. 2018). The detection of high levels of OACs, however, is not  
296 exclusively coincident to their recent and massive use, but is ascribable to their persistency, their  
297 slow natural degradation and their accumulation in the various diffusion pathways (Aguilar et al.  
298 2017). They could then travel long distances in surface or groundwaters and the contamination can  
299 last for several decades (Ballesteros et al. 2014; Aravinna et al. 2017).

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3 300 The so-called ecological services may help to contain the diffusion of OACs. The adaptation of  
4 301 microflora (fungi, Gram-positive and negative bacteria, actinobacteria, and sulfate-reducing  
5 302 bacteria) to soil environmental conditions may attenuate the pesticides released into groundwater  
6 303 sources (Mattsson et al. 2015). Several factors such as soil composition, temperature, aeration due  
7 304 to soil weaving, and depth influence autochthonous microbial community activity; if this balance  
8 305 fails, OACs are free to move among different ecological niches (i.e. sediments and water), alter  
9 306 their functioning and ultimately directly affect their animal inhabitants. For instance, significant  
10 307 ecological risk was associated with the presence of the insecticide fipronil and its metabolites in  
11 308 water ponds: the concentrations measured (up to 200 ng/l) affected the proper development of larval  
12 309 insects and crustaceans (Wu et al. 2015b). Evidence of the pesticide's toxicity against fish has  
13 310 already been reported, and it clearly interferes in several metabolic pathways (Odukkathil and  
14 311 Vasudevan 2013; Ballesteros et al. 2014; Guerreño et al. 2016).

15 312 The preservation of water quality is a priority, but OAC removal cannot be based only on natural  
16 313 attenuation. Water treatment plants (WTPs) are the major barriers where OACs should be removed.  
17 314 Not being specifically designed for micropollutant removal, however, they are often only partially  
18 315 effective, with a strong impact on the receiving ecosystem. Pesticides such as atrazine, fluconazole,  
19 316 tebuconazole, diazinon and diuron are particularly resistant to commonly used treatments (Köck-  
20 317 Schulmeyer et al. 2013; Luo et al. 2014). There is plenty of evidence confirming the presence of  
21 318 OACs in WTP effluents at toxicologically and estrogenically relevant concentration, making them  
22 319 one of the most impactful sources of contamination (Bicchi et al. 2009; Campo et al. 2013; Jarošová  
23 320 et al. 2014).

24 321 Particular attention has been given to advanced biological oxidation. Novel cost-effective and eco-  
25 322 friendly processes based on fungi are an attractive option. Fungi are well-known for their  
26 323 physiological adaption skills, including the natural activation of tolerance mechanisms against  
27 324 pesticides (Talk et al. 2016). Some reports have already demonstrated that in comparison with  
28 325 bacteria, fungi can better tolerate the presence of organic contaminants. Although the insecticide  
29 326 endosulfan inhibited both fungi and bacteria, bacterial community structure significantly changed at  
30 327 concentrations as low as 0.1 mg/kg, while modifications to fungal community structures required 1  
31 328 mg/kg of pollutant (Zhang et al. 2015). Linuron reduced the bacterial count, and especially total  
32 329 bacteria, N<sub>2</sub>-fixing bacteria and nitrifiers, but not fungal numbers (Cycoń et al. 2010).

33 330 The provenance of isolated fungi is of unquestionable importance. Strains isolated from  
34 331 contaminated niches indeed seem to develop specific adaptation skills due to chronic exposure.  
35 332 Carles et al. (2017) demonstrated that the aquatic microflora found in association with submerged  
36 333 leaves exposed to nicosulfuron is more efficient in its degradation than are communities that come

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3 334 from a less polluted site. The authors indicated fungi as the main constituents of this active  
4 335 microflora and as being responsible for herbicide degradation. In the literature, several fungi  
5 336 isolated from contaminated areas or WTPs have been identified as degraders of nicosulfuron,  
6 337 diuron, isoproturon, glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013;  
7  
8 338 Carranza et al. 2014; Oliveira et al. 2015).

9  
10 339 Fungi can thus transform a broad range of recalcitrant organic compounds, including OACs (Gao et  
11 340 al., 2010). A number of fungi that are OAC degraders, mostly belonging to Basidiomycetes, such  
12 341 as *Trametes*, *Pleurotus*, *Phlebia*, *Cerrena*, *Coriolopsis*, etc., have been already investigated  
13 342 (Koroleva et al. 2002; Marco-Urrea et al. 2009; Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et  
14 343 al. 2014; Ceci et al. 2015). Several pesticides as lindane, atrazine, diuron, terbuthylazine, metalaxyl,  
15 344 DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane,  
16 345 mirex, etc. were effectively transformed by fungal treatment based on mycelium or enzymes  
17 346 (Supplemental material Table II).

18  
19 347 A bioremediation approach based on fungi may involve both biosorption and biodegradation  
20 348 processes; the latter combines biosorption, where the molecule binds to the fungal wall, and  
21 349 bioaccumulation with the pollutant being transported inside the cell in contact with intracellular  
22 350 enzymes (Kulshreshtha et al. 2014). Concentrations of the insecticide lindane decreased during time  
23 351 in the presence of two WRFs (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus  
24 352 (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated that a  
25 353 fast adsorption process was mainly involved (Ulčnik et al. 2013). However, this phenomenon is  
26 354 often strain-dependent, and especially related to metabolic differences between Ascomycetes and  
27 355 Basidiomycetes. Belonging to the brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes,  
28 356 responsible for lignin degradation and likely for that of OACs as well: adsorption onto fungal  
29 357 mycelium was mainly involved in the removal of endosulfan. On the contrary, the white-rot fungi  
30 358 actively degraded, producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013).  
31 359 Although biosorption is a phenomenon that cannot be ignored, it is often secondary or at least  
32 360 negligible compared to biodegradation (Carles et al. 2017). For instance, the removal of clofibric  
33 361 acid found for heat-killed mycelium was less than 10 %, but more than 97 % for active *T. versicolor*  
34 362 (Marco-Urrea et al. 2009).

35 363 Fungi have developed a specific mechanism that employs few enzymes and molecules with high  
36 364 oxidizing power, physiologically aimed at transforming lignocellulose structures. The same  
37 365 enzymatic pathway may play a pivotal role in transforming other aromatic molecules. White-rot  
38 366 fungi usually deploy extracellular lignocellulosic enzymes such as peroxidases (EC 1.11.1.x) and  
39 367 laccases (EC 1.10.3.2). The involvement of redox enzymes in fungal-mediated oxidation is

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3 368 confirmed by the direct induction of enzyme production in the presence of OACs. The fungus *T.*  
4 369 *versicolor* responded to 17 pesticides by increasing laccase production in comparison with the  
5 370 control: particular attention was given to the transformation products of the herbicides diquat and  
6 371 monuron, capable of increasing fungal activity 10- and 17-fold, respectively (Mougin et al. 2002).  
7  
8 372 The laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus* spp1, *Pleurotus*  
9 373 spp2, *Cymatoderma elegans*, and *Daedalea elegans* was stimulated by the presence of atrazine even  
10 374 at high concentrations of 3750 mg/l. Likewise, the manganese peroxidase activity of *Pleurotus* spp1  
11 375 and *C. elegans* was positively correlated with the pesticide (Chan-Cupul et al. 2014).  
12 376 Oxidoreductase stimulation was also observed with picloram (Maciel et al. 2013), bentazon (Da  
13 377 Silva Coelho et al. 2010) and carbofuran (Mir-Tutusaus et al. 2014).  
14 378 Although these oxidoreductases are probably the most-known enzymes for aromatic compound  
15 379 degradation, alternative pathways can be stimulated by the presence of OACs. Two clones (laccase-  
16 380 positive and laccase-negative) of *Mycelia sterilia* were used to treat atrazine (20 µg/ml): even  
17 381 though one clone was defective in laccase production, comparable transformation yields (70-80%)  
18 382 were reached, indicating that the fungus can deploy alternatives to laccase in the degradation  
19 383 process (Vasil'Chenko et al. 2002). This behavior is commonly found in brown-rot fungi, which  
20 384 can trigger both nonenzymatic and enzymatic mechanisms, i.e. the Fenton mechanism or cellobiose  
21 385 dehydrogenase (CDH) reactions (Fan and Song 2014). The degradation of atrazine (20 µg/l) by an  
22 386 unidentified mycelial fungus was associated with the presence in the liquid medium of OH radicals  
23 387 and CDH. Moreover, CDH secretion was induced by the presence of the herbicide itself  
24 388 (Khromonygina et al. 2004). In addition, some fungi may associate extracellular oxidoreductases  
25 389 with intracellular enzymes such as the cytochrome P450 system (cyt450). In an effort to better  
26 390 characterize the degradation skills of *T. versicolor*, cyt450 inhibitors were used: fungal performance  
27 391 against clofibric acid and fipronil decreased (Marco-Urrea et al. 2009; Wolfand et al. 2016). Mori et  
28 392 al. (2017), suggest that in *Phanerochaete sordida*, cyt450 is involved in the initial stage of reduction  
29 393 of the clothianidin N-nitro group, but that the enzymes responsible of the further urea derivatives  
30 394 production are unknown.  
31  
32 395 Fungal intra- and interspecies variability has long been recognized and has found confirmation in  
33 396 OAC treatment. Literature data about a given species cannot be taken for granted and preliminary  
34 397 screening is often required. Despite *Phanerochaete chrysosporium* often being indicated as the  
35 398 fungal model for organic degradation including pesticides (Wang et al. 2014), it was almost  
36 399 ineffective against clofibric acid (Marco-Urrea et al. 2009). Among five Basidiomycetes, only *T.*  
37 400 *versicolor* extensively degraded this herbicide (Marco-Urrea et al. 2009). Alvarenga et al. (2014)  
38 401 treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*. Based on ability to  
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3 402 grow in the presence of the pesticide, only the isolate *A. sydowii* CBMAI 935 was selected for  
4 403 further studies. It indeed grew almost 4-fold more than the other *A. sydowii*. Bioremediation  
5 404 potential is often substrate-targeted, and the choice of fungus cannot be taken for granted. For  
6 405 instance, *A. sydowii* CBMAI 935, which totally converted methyl parathion (Alvarenga et al. 2014)  
7  
8 406 was not the best performing one against the insecticide esfenvalerate. Among 6 fungi,  
9 407 *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave better results than the *Aspergillus*  
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11 408 strain (Birolli et al. 2016).

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14 409 Although the majority of these strains are effective in OAC removal in model solutions, only few  
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16 410 researchers have taken the next step, and assessed bioremediation potential in contaminated waters.  
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18 411 The experimentation with model solutions (single-compound solutions, high concentrations, no  
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20 412 interfering molecules, etc.) is the only way to acquire information about degradation pathways  
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22 413 (Masaphy et al. 1993; Birolli et al. 2016), but it is less predictive of fungal performance in real  
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24 414 environmental water samples. Each type of wastewater has its own critical issues, making it  
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26 415 difficult to predict fungal behavior. Some data highlight the robustness of fungal systems, although  
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28 416 detailed case-by-case investigation is needed. A partially diluted leachate was shown to disturb the  
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30 417 growth of *T. versicolor* and *Stereum hirsutum*, but this did not prevent them totally degrading  
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32 418 linuron and dimethoate at 10 mg/l. As regards dimethoate, the presence of adsorbents enhances final  
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34 419 yields from 50% to 97%, because the adsorption action combines with and exalts fungal  
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36 420 biodegradation processes (Castellana and Loffredo 2014). The immobilization of *Bjerkandera adusta*  
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38 421 and *Irpex lacteus* on coffee grounds, almond shells and a biochar favored the removal of the non-  
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40 422 phenolic herbicides fenuron and carbaryl from a municipal landfill leachate (Loffredo et al. 2016).  
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42 423 Surface waters, ground waters and municipal wastewaters represent a very unique environment,  
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44 424 characterized by extreme chemical and physical conditions, the presence of a heterogeneous and  
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46 425 variable mixture of micropollutants and an active autochthonous microflora. When inoculated into  
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48 426 real surface water, a fungal consortium (*Aspergillus fumigatus*, *Aspergillus terreus*, *Cladosporium*  
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50 427 *tenuissimum*, *Cladosporium cladosporioides*, *Fusarium begoniae*, *Penicillium citrinum*, *Penicillium*  
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52 428 *melanoconidium* and *Phoma glomerata*) was not stable over time, probably due to the presence of  
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54 429 toxic pesticides and interaction with the natural microbial population: *P. citrinum*, *A. fumigatus* and  
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56 430 *A. terreus* were the most robust to the environmental conditions and were found to degrade the  
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58 431 spiked chlorfenvinphos (Oliveira et al. 2015).

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60 432 The set-up of active microbial consortia offers the intriguing possibility of strengthening and  
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62 433 combining the bioremediation potential of different organisms: the combination of *Bacillus subtilis*  
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64 434 and *A. niger* led to higher degradation rates of nicosulfuron than those obtained by using each strain  
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66 435 singly (Lu et al. 2012). The biodegradation of aldicarb, atrazine and alachlor by *Coriolus versicolor*

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3 436 was strongly enhanced by combination with activated sludge. Along with modifications in fungal  
4 437 morphology, when the bacterial-fungal consortium was established, the bio-absorbed fraction of  
5 438 especially atrazine was reduced: over 98% of atrazine was removed by degradation processes in two  
6 439 weeks (Hai et al. 2012).

9 440 The fate of the treated OACs must be carefully considered. Residual toxicity is a critical issue.  
10 441 Interestingly fenuron and carbaryl degradation (up to 70%) catalyzed by *B. adusta* and *I. lacteus* led  
11 442 to significant abatement of the phytotoxicity (rapeseed and flax tests) (Loffredo et al. 2016). Mori et  
12 443 al. (2017) monitored the neurotoxicity of clothianidin and the main metabolite it produced during *P.*  
13 444 *sordida* treatment: following treatment the insecticide still altered the viability of the neuronal cell  
14 445 line, but the metabolite was no longer neurotoxic.


19 446 Despite their well-demonstrated properties, the application of whole cell systems has some  
20 447 drawbacks including the fact that a living organism needs controlled growing conditions in terms of  
21 448 nutrients, pH, O<sub>2</sub>, etc. (Majeau et al. 2010). The addition of synthetic nutrients can strengthen  
22 449 fungal mycelium activity, but it should be carefully balanced to allow subsequent scale-up of the  
23 450 process. The fact that *T. versicolor* needed 1% of glucose as carbon source to degrade atrazine  
24 451 would ultimately interfere with its potential use in real WTPs (Khromonygina et al. 2004). Likewise  
25 452 several fungi such as *A. niger* and *Dacryopinax elegans*, etc. required both easily available carbon  
26 453 and nitrogen sources to efficiently act against nicosulfuron and diuron, respectively (Lu et al. 2012;  
27 454 Arakaki et al. 2013). Particular attention should be instead given to those fungi, like *A. sydowii* and  
28 455 *Penicillium decaturense*, that maintained the same performance without glucose addition, indicating  
29 456 potential for using methyl parathion or triclosan as sole carbon source (Alvarenga et al. 2014; Tian  
30 457 et al. 2016).

38 458 A promising alternative is offered by the direct use of fungal enzymes, capable of catalyzing strong,  
39 459 rapid oxidation reactions, with less technical drawbacks in comparison with fungal cultures. The  
40 460 potential of enzymes-based methods has been worldwide recognized; the Swiss Industrial  
41 461 Biocatalysis Consortium defined oxidative enzymes as the biocatalysts displaying the highest  
42 462 development potential for the next decades (Meyer and Munch 2005). Great importance is given to  
43 463 the discovery of novel enzymes with wide substrate specificity, stable and applicable to industrial  
44 464 uses. A number of articles have reported the ability of fungal enzymes to degrade OACs. The  
45 465 potential of laccase-mediator systems has been assessed for the degradation of isoproturon (Margot  
46 466 et al. 2015), imiprothrin (Mir-Tutusaus et al. 2014), chloroxuron (Palvannan et al. 2014),  
47 467 isoproturon (Zeng et al. 2017), atrazine (Chan-Cupul et al. 2016). Laccases cannot be considered a  
48 468 novelty, unlike a phytase of *A. niger* capable of degrading organophosphorus pesticides (Shah et al.  
49 469 2017) or a cellulase of *Trichoderma longibrachiatum* active against dicofol (Wang et al. 2015).



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3 470 Particular attention should be given to the use of crude enzyme extracts of ligninolytic enzymes  
4 471 with a lower economic impact on the process than that of purified enzymes (Matute et al. 2012;  
5 472 Kaur et al. 2016). A crude extract of *Trametes pubescens* laccases degraded up to 19 compounds in  
6 473 a model solution and confirmed its potential in a study on real municipal wastewater where the  
7 474 presence of suspended particles, colloids, solvents and xenobiotics as well as autochthonous  
8 475 microorganisms posed strong environmental pressure. The transformation of all the detected  
9 476 compounds determined also a strong reduction of the estrogenicity of the water sample (Spina et al.  
10 477 2015).

### 16 478 17 479 ***Application of synthetic microbial communities in bioremediation***

18 480 Bioremediation is a crucial way to eliminate OAC pollution in agricultural ecosystems. However,  
19 481 many factors affect the efficiency of bioremediation in pesticide pollution, such as the microbes  
20 482 applied, treatment sites, rhizosphere effects and soil chemical and physical properties (Zhou and  
21 483 Hua 2004). Bioremediation of soil or water pollution often cannot reach expected results in practice  
22 484 because the target contaminant cannot be degraded completely, and sometimes intermediate  
23 485 products occur that are more toxic than the original pesticides. Long-term application of various  
24 486 pesticides results in pollution with more than one type of chemical compound, which are unlikely to  
25 487 be degraded by a so-called microbe. Thus, attention has shifted to synthetic systems based on  
26 488 communication between cells, rather than on individual isolated cell functionality (Biliouris et al.  
27 489 2012). A promising way to overcome the difficulties is to create artificial synthetic microbial  
28 490 communities that contain several microbes to retain the key features of their natural counterparts  
29 491 (Großkopf and Soyer 2014).

30 492 The so-called *synthetic microbial community* is created by a bottom-up approach where two or more  
31 493 defined microbial populations are put together in a well-characterized and controlled environment  
32 494 (De Roy et al. 2014). In synthetic communities, mixed populations can perform complex tasks,  
33 495 although in changing environmental conditions (Brenner et al. 2008). Synthetic communities have  
34 496 several potential advantages over monocultures or natural communities: 1) the species in a  
35 497 synthetic community are known and the community structure is relatively simple and controllable,  
36 498 while the natural community may contain many microorganisms with unknown functions; 2)  
37 499 synthetic communities can perform more complicated functions than individual organisms because  
38 500 members of microbial consortia communicate and differentiate (Brenner et al. 2008); 3) synthetic  
39 501 communities are often more robust to environmental fluctuations because they can resist invasion  
40 502 by other species and weather periods of nutrient limitation better than monocultures (Brenner et al.  
41 503 2008); 4) synthetic communities can be described through mathematical models more easily than

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3 504 natural systems, and they can be used to develop and validate models of more complex systems  
4 505 (Liu et al. 2017).

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6 506 Liu et al. (2017) proposed three design principles to develop a cooperative, steady-state community  
7 507 that is performing a desirable biotechnological function. Firstly, safety should be prioritized by  
8 508 beginning with innocuous or commensal organisms (Brenner et al. 2008). Secondly, the community  
9 509 can converse a low-cost and/or recalcitrant waste material into a biotechnologically relevant  
10 510 product, partial or de-novo biosynthesize a compound via heterologous metabolic pathways, or  
11 511 bioconverse toxic substrates or products in a toxic milieu (Jagmann and Philipp 2014). Thirdly, the  
12 512 bioremediation process should be optimized and regularly monitored on the basis of the knowledge  
13 513 of stability and division of different microorganisms (Liu et al. 2017).

14 514 Bioremediation of polluted soils and water is one field of application synthetic microbial  
15 515 communities. Due to the complex structure of some pollutants, such as the diuron pesticides, adding  
16 516 synthetic microbial communities is much more effective than adding single microorganisms. The  
17 517 herbicide diuron is used in the control of broad-leaved weeds on agricultural land. Several fungal-  
18 518 bacterial consortia were investigated by combining three different diuron-degrading bacteria and  
19 519 two fungal strains. The fastest mineralization of diuron was obtained by the three-member  
20 520 consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47). As  
21 521 measured by evolved  $^{14}\text{CO}_2$  it mineralized about 32 % of the added diuron within 54 days, whereas  
22 522 the single strains or other consortia achieved no more than 10% mineralization. In addition, the  
23 523 production of diuron metabolites by the consortium was minimal. This may be due to cooperative  
24 524 catabolism, where the first organism transforms the pollutant to products that are then used by the  
25 525 other organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby  
26 526 facilitating the more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al.  
27 527 2014).

28 528 Similarly, a fungal-bacterial consortium consisting of *Mortierella* sp. LEJ702 and the 2,6-  
29 529 dichlorobenzamide (BAM)-degrading *Aminobacter* sp. MSH1 achieved more rapid mineralisation  
30 530 of BAM than did the bacteria alone, especially at lower moisture contents (Knudsen et al. 2013).  
31 531 Methylophilic and hydrocarbon-utilizing yeasts and bacteria alone did not degrade PCBs  
32 532 significantly, but PCB degradation reached about 50% when WRFs were applied together (Šašek et  
33 533 al. 1993).

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35 535 ***Evaluation of bioremediation effectiveness in contaminated matrices by means of***  
36 536 ***ecotoxicological and genotoxic tests***

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3 537 In order to improve the effectiveness and performance of bioremediation processes it is important to  
4 538 pursue three essential goals at the same time. Focus should be not only on reducing chemical  
5 539 concentrations, but also on reducing chemical mobility between the environmental compartments  
6 540 and eventually lowering toxicity levels while ensuring that contaminants do not get into the natural  
7 541 biological cycle (Loehr and Webster 1997; Chakraborty et al. 2013).

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9 542 Bioremediation is often monitored by following the concentration of targeted contaminants  
10 543 (Molina-Barahona et al. 2005). Numerous studies in recent years have shown that traditional  
11 544 chemical analyses are insufficient for a full assessment of the contaminated site because, for  
12 545 example, they do not provide any information about the interactions between chemicals and they do  
13 546 not consider the partition and the mobility of pollutants (Frische 2003; Molina-Barahona et al.  
14 547 2005; Ma et al. 2005; Molnár et al. 2007). An integrated approach that links the various fields and  
15 548 levels of study involving contaminated sites has proven to be an efficient way to evaluate the  
16 549 effectiveness of bioremediation in contaminated sites (Chapman and Anderson 2005; Wernersson et  
17 550 al. 2015; Marziali et al. 2017). Consequently, to achieve the desired goals and implement a  
18 551 successful bioremediation program, given the chemical and biological complexity of the tasks  
19 552 involved, close collaboration between microbiologists, chemists and engineers is required (Van  
20 553 Gestel et al. 2001; Chakraborty et al. 2013).

21 554 Additionally, the use of ecotoxicological and genotoxic tests to evaluate the effectiveness of  
22 555 bioremediation may be a valid tool to partially overcome the existing gap between the reported  
23 556 successes of bioremediation on the laboratory scale, and that in the field.


24 557 Signals that bioremediation is going on should be monitored. Two important chemical compounds  
25 558 produced by microorganisms during their degradation activity are CO<sub>2</sub> and soluble phosphorus.  
26 559 Both increase notably in soil treated with insecticides and inoculated with fungi (Boyle 1995; Abd  
27 560 El-Ghany and Masmali 2016). However, it must be taken into consideration that during and after a  
28 561 bioremediation process the disappearance of the parent compounds or evidence of metabolic  
29 562 activity (e.g. CO<sub>2</sub> production) may not indicate detoxification. Although the fate of the toxicants  
30 563 may be followed by chemical analyses, many reaction products resulting from the bioremediation  
31 564 process and their potential toxicity are not known. The elimination of mother compounds does not  
32 565 necessarily result in toxicity removal, and evaluating the efficiency of the process is important to  
33 566 assess not only the removal of a specific compound, but also potential ecotoxicity. In fact,  
34 567 biodegradation of pesticides can proceed partially or totally due to the structure of the molecule  
35 568 itself or to unfavourable environmental or test conditions, or to the lack of 'acclimatized' microbial  
36 569 communities (De Henau 1997).

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3 570 In some instances, it has been shown that an effective process of bioremediation corresponds with a  
4 571 decrease in the toxicity of the analysed matrix (Baud-Grasset et al. 1993; Dorn and Salanitro 2000).  
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6 572 To acquire complete and useful information in an ecotoxicological assessment and to determine the  
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8 573 effectiveness of bioremediation treatments, it is suggested that a battery of tests be used (Keddy et  
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10 574 al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of  
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12 575 reference organisms that are representative of the different trophic levels, in order to select species  
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14 576 with different roles in ecosystems, and different exposure conditions (Van Straalen and Van Gestel  
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16 577 1997). Moreover, environmental risk assessment must integrate chemical characterization,  
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18 578 ecotoxicity and bioremediation data, in order to accurately assess the ecological hazard.

17 579 As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various  
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19 580 bioindicators could either indicate incomplete decomposition of the substance or could result from  
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21 581 the formation of intermediate products generated via the bioremediation process. For this reason,  
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23 582 chronic tests are sometimes more appropriate in evaluating the toxicity caused by by-products  
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25 583 (Lofrano et al. 2014).

25 584 In certain circumstances, there is a clear need to monitor the bioremediation process using different  
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27 585 bioindicators. In Lizano-Fallas et al. (2017), for example, the ecotoxicity test with *Daphnia magna*  
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29 586 showed clear detoxification, whilst the detoxification patterns remain unclear when applying the  
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31 587 phytotoxicity test. Ecotoxicological tests can also be used to determine the most suitable  
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33 588 bioremediation technique in a given case, as reported in Dudášová et al. (2016).

33 589 Without worldwide-recognized guidelines for water quality assessment, literature data are difficult  
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35 590 to compare due to the variety of model organisms, end-points, etc. Synthetic indices summarizing  
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37 591 the findings can help monitor the effectiveness of biological treatment. Such indices have already  
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39 592 been applied for toxicity monitoring of wastewaters (Tigini et al. 2011) but municipal effluents  
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41 593 containing AOCs have never been taken into consideration nor has estrogenic activity been  
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43 594 included so far.

43 595 Several toxicity assays were included in  biodegradability study protocol to measure remediation  
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45 596 efficiency. Assessing the toxicity of complex matrixes such as soil could acquire methods from  
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47 597 bioassays used to the test toxicity of chemical compounds, reported by the Organization for  
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49 598 Economic Co-operation and Development (e.g. OECD 201 2006; OECD 211 2012). The OECD has  
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51 599 published a series of standardized tests for determining the biodegradability of a given compound,  
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53 600 based on the evaluation of overall parameters (such as COD, TOC and BOD) or metabolic tests, e.g.  
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55 601 respirometry (OECD 209 1984) as Polo et al. (2011) used; or that reveal susceptibility of toxic  
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57 602 compounds, comprising that of herbicides, to biological treatment. Standardized testing procedures  
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59 603 using different organisms have been approved by various environmental organizations, including

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3 604 the US Environmental Protection Agency, American Society for Testing and Materials,  
4 605 International Standardization Organization (Siciliano et al. 2015). Many scientists have explored the  
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6 606 effects of polluted soil on the whole organism using various microorganisms, animals, and plants,  
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8 607 or by means of cellular, and biochemical biomarkers, or by ecological scale up systems. Here  
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10 608 below, tests at some different biological hierarchical levels of analysis are presented and discussed.

11 609

### 12 610 *Organismal level*

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14 611 Concerning complex matrices such as soil, quality assessments are performed with organisms on  
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16 612 extracts of the polluted matrix, generally applying short-term exposure periods (Van Gestel et al.  
17  
18 613 2001). Experimental models have included aquatic organisms such as *Daphnia magna*,  
19 614 *Raphidocelis subcapitata*, *Danio rerio*, *Myriophyllum aquaticum* and *Lemna minor* (Feiler et al.  
20  
21 615 2004). The use of freshwater and marine biota may be particularly useful in order to provide a more  
22  
23 616 complete comprehension of the fate of pesticides and the environmental outcomes of agricultural  
24  
25 617 activities (Guida et al. 2008). Terrestrial animals such as nematodes (*Caenorhabditis elegans*)  
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27 618 (Traunspurger et al. 1997), oligochaetes (*Lumbriculus variegatus*) (Phipps et al. 1993), springtails  
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29 619 such as *Folsomia candida* (Houx et al. 1996), and fish embryos (Hollert et al. 2003; Zielke et al.  
30  
31 620 2011) are considered among the most reliable models.

32 621 Among the higher plants, important experimental models include *Lepidium sativum*, *Cucumis*  
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34 622 *sativus*, and *Sorghum saccharatum* (germination rate, inhibition of root elongation). Since assays  
35  
36 623 based on animals, plants and algae are considered expensive, time consuming and require large  
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38 624 sample volumes, recent studies have emphasized the benefits of rapid, reproducible and cost  
39  
40 625 effective bacterial assays for toxicity screening and assessment. *Arthrobacter globiformi*  
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42 626 (Neumann-Hensel and Melbye 2006), *Bacillus cereus* (Rönnpagel et al. 1995; Prokop et al. 2016),  
43  
44 627 *Vibrio proteolyticus* (Ahlf and Heise 2005) and yeasts (*Saccharomyces cerevisiae*) (Weber et al.  
45  
46 628 2006) are often used. Among the bacterial bioassays, the *Vibrio fischeri* luminescence inhibition  
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48 629 test is the most common. The review of Parvez et al. (2006) remarks that the *Vibrio fischeri*  
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50 630 inhibition test is the most sensitive, cost effective, easy to operate and requires only 5–30 min for  
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52 631 toxicity prediction.

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### 54 633 *Cellular and biomolecular level*

55 634 Biomarkers signal the adaptative responses of organisms to xenobiotic exposure. Various studies  
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57 635 have highlighted the cytotoxic and genotoxic effects on organisms of OACs and their metabolic  
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59 636 products. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or  
60 637 physiological, or even behavioural changes (Depledge et al. 1993) that enable information to be

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3 638 obtained on the biological effects of pollutants or their remains during or after a bioremediation  
4 639 process (Fontanetti et al. 2011).

5 640 Genetic endpoints and biomarkers. The most-used biomarkers are mitotic index, chromosome  
6 641 aberrations, micronuclei, sister chromatid exchanges and mutations.

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9 642 Bacteria have been recommended for bioassays to evaluate genotoxicity in a variety of samples  
10 643 (Mortelmans and Zeiger 2000; White and Claxton 2004). The Ames test, one of the most famous  
11 644 and widely-used, is a short term bacterial reverse mutation assay especially designed to evaluate the  
12 645 mutagenic potential of a wide range of chemical substances (Mortelmans and Zeiger 2000). It was  
13 646 found to be very sensitive in tests with a wide range of mutagenic and carcinogenic chemicals, as  
14 647 reported in the review paper of Chahal et al. (2014).

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17 648 With regards to plant models, higher plants are recognized as excellent genetic models to detect  
18 649 cytogenetic and mutagenic agents and are frequently used in environmental monitoring studies. The  
19 650 main organisms employed are *Allium cepa*, *Vicia faba* and *Tradescantia* spp. as reported in a  
20 651 review by De Souza et al. (2016). Their protocols were standardized under the International  
21 652 Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme  
22 653 (UNEP) (Ma 1999). In addition, the US Environmental Protection Agency (USEPA) and the World  
23 654 Health Organization (WHO) validated plant bioindicators as an efficient model to detect  
24 655 environmental genotoxicity.

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27 656 One of the most used higher plant models is *V. faba*. The main advantages are its year-round  
28 657 availability, that it is economical to use, and easy to grow and handle. Its use does not require  
29 658 sterile conditions and rate of cell division is fast. The *V. faba* test, meticulously reported and  
30 659 discussed in the review of Iqbal (2016), enables the assessment of a variety of endpoints, e.g.,  
31 660 chromosomal aberration, mitotic index, micronuclei and nuclear aberration.

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34 661 Enzymatic biomarkers. Enzyme activity inhibition has been widely evaluated as a biomarker to  
35 662 measure the toxicity of a matrix. Dehydrogenases, for example, are directly involved in many of the  
36 663 vital anabolic and catabolic processes of living organisms, and their activity is inhibited by  
37 664 chemical toxicants. Recently, many studies have reported the use of terrestrial organisms to obtain  
38 665 enzymatic biomarkers in response to residual pesticides (Henson-Ramsey et al. 2011; Radwan and  
39 666 Mohamed 2013; Stepić et al. 2013), and among these, earthworms' enzymes were widely used to  
40 667 understand the impacts of pesticides. In two earthworm species, *Eisenia fetida* and *Lumbricus*  
41 668 *terrestris*, multiple esterases, including acetylcholinesterase (AChE), butyrylcholinesterase, and  
42 669 carboxylesterase (CE), were assessed as biomarkers for malathion exposure (Henson-Ramsey et al.  
43 670 2011). Several studies have also reported AChE, catalase (CAT), and glutathione-S-transferase as  
44 671 biochemical biomarkers in *Eisenia andrei* for the insecticides endosulfan, temephos, malathion, and

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3 672 pirimiphos-methyl (Stepić et al. 2013), and AChE, CAT, CE, and the efflux pump as biomarkers in  
4 673 *E. andrei* and *Octolasion lacteum* for dimethoa. Recently, surface-enhanced laser  
5 674 desorption/ionization-time-of-flight (SELDI-TOF) mass spectrometry (MS) has strongly  
6 675 contributed to the identification of more accurate, precise biomarkers, e.g. specific for human  
7  
8 676 cancers (Silsirivanit et al. 2014), or for endosulfan exposure in Japanese rice fish (*Oryzias latipes*)  
9 677 (Lee et al. 2013). In a recent paper, selective protein biomarkers for 6 pesticides (captan, carbaryl,  
10 678 carbofuran, and  $\alpha$ -endosulfan chlorpyrifos, propoxur) were found in *E. fetida*, by means of SELDI-  
11 679 TOF MS technology (Park et al. 2015).

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14 680 Estrogen and androgen biomarkers. It is well-documented that several chemicals from agricultural,  
15 681 industrial, and household sources possess endocrine-disrupting properties, which provide a potential  
16 682 threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995; Jensen et al. 1995).  
17 683 A suggested mechanism is that environmental contaminants alter the normal functioning of the  
18 684 endocrine and reproductive system by mimicking or inhibiting the action of endogenous hormones,  
19 685 by modulating the production of endogenous hormones, or by altering hormone receptor  
20 686 populations (Sonnenschein and Soto 1998). Several pesticides exert estrogenic and antiandrogenic  
21 687 activities through interaction with estrogen and androgen receptors. The risks associated with OAC  
22 688 exposure has been known for decades: many pesticides, such as p,p'-dichlorodiphenyl  
23 689 trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978; Cummings 1997),  $\beta$ -  
24 690 benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan, toxaphene, and dieldrin  
25 691 (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) were the first to be signaled as  
26 692 estrogenic. Despite increased institutional awareness and more compelling legislation pressure, the  
27 693 most recent literature still reports the occurrence of pesticides in watercourses and in the trophic  
28 694 chains, that show conspicuous estrogen or androgen levels (Saillenfait et al. 2016; Brander et al.  
29 695 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al. 2017; Marcoccia et al.  
30 696 2017). Several bioassays have been developed and standardized in order to describe the estrogenic  
31 697 potency of OACs. Andersen et al. (2002) indicated that several currently used OACs, such as  
32 698 methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl, possess estrogenic activity  
33 699 on the basis of cell proliferation assays and transactivation assays using MCF-7 human breast  
34 700 cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism and antagonism to two  
35 701 human estrogen receptor (hER) subtypes, hER $\alpha$  and hER $\beta$ , and a human androgen receptor (hAR)  
36 702 by means of highly sensitive transactivation assays, using Chinese hamster ovary cells. The results  
37 703 demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic action through  
38 704 ERs and/or AR. Although it appears that various pesticides exert hormonal effects at concentrations  
39 705 that are orders of magnitude higher than that required for physiologic hormones, wide exposure to

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3 706 large numbers of OACs may have additive and synergistic effects. Bioassay with YES (yeast  
4 707 estrogen screen) and YAS (yeast androgen screen) can determine hormonally active compounds  
5 708 still present in the environment. Since the the first papers on this subject (Purvis et al. 1991), much  
6 709 more sophisticated bioassays have been developed, such as that proposed by Eldridge et al. (2007)  
7 710 in which a bioluminescent strain of *Saccharomyces cerevisiae* was genetically engineered to  
8 711 respond to androgenic chemicals.


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### 14 713 *Ecological level*

15 714 The risk to natural systems of pollution with the chemical residues of bioremediation processes is  
16 715 underestimated. The ecological scaling-up experiment illustrated by Rodea-Palomares et al. (2016)  
17 716 underlined how real-world exposure to chemical pollution is often dominated by low-dose complex  
18 717 mixtures combined with other biotic and abiotic stressors. In the paper, a novel screening method  
19 718 (GSA-QHTS) was reported, that coupled the computational power of global sensitivity analysis  
20 719 (GSA) with the experimental efficiency of quantitative high-throughput screening (QHTS). In the  
21 720 study, they reported that GSA-QHTS allowed for the identification of the main pharmaceutical  
22 721 pollutants that were driving the biological effects of low-dose complex mixtures at the microbial  
23 722 population level. The target complex community was a river benthic microbial community  
24 723 inoculum obtained from an unpolluted stream. The effects of the toxic compounds in the mixture  
25 724 was evaluated together with other physico-chemical stressors, on a series of community-level  
26 725 metabolic end points. Photosynthetic parameters, the dark-adapted basal fluorescence, the light-  
27 726 adapted steady-state fluorescence, the maximum photosynthetic efficiency, as well as the  
28 727 extracellular enzymatic activities b-Glu and Phos were considered as both autotrophic and  
29 728 heterotrophic global fitness indicators suited to study the effects of chemical pollution on freshwater  
30 729 benthic microbial communities.

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### 43 731 *Prospect*

44 732 Bioremediation is based on the idea that different organisms will work together to remove  
45 733 (biodegrade) the waste substances or pollutants (OACs) from the environment. Although there exist  
46 734 limitations to bioremediation practice, including the nature of organisms, the enzyme involved, the  
47 735 concentration and availability and final survival of microorganisms, as well as the cost/benefit ratio  
48 736 (i.e. ost versus overall environmental impact), these limitations can be solved to some extent by  
49 737 understanding the genetics and biochemistry of the desired microbe. The advent of synthetic  
50 738 communities has shown enormous potential to facilitate the bioremediation process, the degradative  
51 739 fungi appearing to be particularly effective.



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751 [and-their-impact-on-soil-microbial-population-2157-7471-1000349.php?aid=72754](https://www.omicsonline.org/open-access/fungal-biodegradation-of-organophosphorus-insecticides-and-their-impact-on-soil-microbial-population-2157-7471-1000349.php?aid=72754)

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

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### Abstract

Pesticide can help reduce yield losses caused by pests, pathogens, and weeds, but its overuse causes serious environmental pollution. They are persistent in the environment and biomagnified through the food chain resulting a serious hazard for humankind. Bioremediation by microbes to degrade the pesticides *in situ* is a useful technology. This review mainly summarized the fungi associated with biodegradation of chemical pesticides and their application in the soil and water bioremediation. The future studies on this field were also prospected.

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7 31 Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi,  
8 32 Synthetic microbial community, Environmental risk assessment.  
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### 10 33 11 12 34 **Introduction**

14 35 Because of their unique functions, fungi are involved with important ecosystem services for human  
15 36 well-being. Among others, fungi account for provisional services also through the activity of  
16 37 transforming and detoxifying pollutants. For this reason, learning from nature, they represent an  
18 38 effective toolbox for a sustainable bioremediation of pesticides in soil and water. Many researches  
19 39 have unfolded the untapped potential of fungi, given that recent years have witnessed very  
20 40 interesting developments regarding use of fungi not only to improve the environmental quality but  
22 41 also human health (e.g. Gargano et al. 2017).

23 42 Pesticides are a diverse group of inorganic and organic chemicals like herbicides, insecticides,  
24 43 nematicides, fungicides, antibiotics and soil fumigants, all belonging to the so-called organic  
26 44 agrochemicals (OACs) (Verger and Boobis 2013; Verma et al. 2014). In agriculture, pesticides aim  
27 45 to enhance crop yield and quality, and to maximize economic returns by prevention of pest or weed  
29 46 attack. They are bioactive, toxic substances, capable of influencing, directly or indirectly, soil  
30 47 fertility and health as well as agroecosystem quality (Pinto et al. 2012; Verma et al. 2014). Given  
32 48 that belowground biodiversity is closely linked to land management, agricultural intensification  
33 49 causes many pressures that leads to loss of biodiversity. Consequently, soil pollution is one of the  
34 50 main threats related to the decline of taxonomic and functional biodiversity, and of agricultural soils  
36 51 sustainability (Harms et al. 2017). Most of the pesticides emission (99 %) in Europe is associated to  
37 52 agricultural practices whereas industrial and urban sources as the manufacturing of pesticides or the  
38 53 at-home use of insecticides have a minor impact (EEA 2016). Thus, the extensive and massive  
40 54 use of pesticides in agriculture activities has serious impacts on the environment, compromising soil  
41 55 and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al. 2016). Besides,

43 56 In addition to pesticides, large Large quantities of antibiotics are added to agricultural fields  
44 57 worldwide through the application of wastewater, manures and biosolids, resulting in antibiotic  
45 58 contamination and elevated environmental risks (Jechalke et al. 2014; Zhang et al. 2015; Pan and  
47 59 Chu 2016). A clear correlation between agriculture and water contamination was observed in Mar  
48 60 Chiquita lake (Argentina), where high amount of endosulfan residues were detected soon after  
49 61 application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide  
51 62 thiﬂuzamide in paddy water of rice fields in China was maximal after the application, and variation  
52 63 during time was associated to the dilution effect of rainfalls in the area (Wei et al. 2015). Preventive

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7 64 [measures to mitigate the impact of agriculture on the environment are required, taking into account](#)  
8 65 [both the use of safety pesticides and the optimization of farmer procedures. Aravinna et al. \(2017\)](#)  
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10 66 [found that most of the 32 studied pesticides leached rice field following specific pathways. Since](#)  
11 67 [direct run off and erosion from soil were the main vehicles of dispersion, authors suggested](#)  
12 68 [alternative strategies \(high resident time of pesticides, holding ponds of rice drainage water, delayed](#)  
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14 69 [filling of paddies after pesticide application and use less mobile compounds\) to reduce the](#)  
15 70 [movement of the pesticides.](#)

16 71 The intensive use of these ~~organic agrochemicals (OACs)~~ has posed risks to both wild lives and  
17 72 human health. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other  
18 73 than their target species, through air, water and soil (Miller 2004). Around 30% of pesticides  
19 74 marketed in developing countries do not meet internationally accepted quality standards, posing a  
20 75 serious threat to human health and environment (Popp et al. 2013). They are persistent in the  
21 76 environment and biomagnified through the food chain. Therefore, it has been estimated that  
22 77 millions of agricultural workers worldwide experience unintentional pesticide poisonings each year.  
23 78 The correlation between long-term exposures to pesticides in occupational settings is known but  
24 79 recently also non-occupational exposures have been associated to an elevated rate of chronic  
25 80 diseases (Parrón et al. 2014).

30 81 Varieties and consumption of pesticides worldwide are dramatically increasing, ~~up to, but literature~~  
31 82 ~~reports conflicting data on overall use (2–4 million ton for year), 4-fold higher than 40 years ago~~  
32 83 ~~(Mnif et al. 2011).~~ According to De et al. (2014), about 45 % is used by Europe, 25 % by USA, and  
33 84 25 % in the rest of the world. ~~The main pesticide consumer is Spain (around 79,000 ton of active~~  
34 85 ~~ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy (~ 64,000),~~  
35 86 ~~Germany (~ 46,000) and United Kingdom (~ 23,000) (Eurostat 2016). The United States is also a~~  
36 87 ~~large consumer of pesticides, applying usually applies over 1 billion pounds annually (Alavanja~~  
37 88 ~~2009) with dramatical consequences for human beings and environment (Carvalho 2017). Overall,~~  
38 89 ~~herbicides account for 47.5 %, insecticides for 29.5 %, fungicides for 17.5 % and others account for~~  
39 90 ~~5.5 %.~~

44 91 ~~On the contrary, according to~~ ~~According~~ to other authors (Huang McBeath and McBeath 2010), China  
45 92 is the world's largest pesticide user, with an output of pesticide around 3.7 million ton (National  
46 93 Bureau of Statistics of China - <http://data.stats.gov.cn>), and a consumption volume of about 1.8  
47 94 million ton in 2014. ~~The average amount of pesticides used per hectare in China is roughly 1.5- to~~  
48 95 ~~4-fold higher than the world average (Qiu 2011), thus resulting in the contamination of water bodies~~  
49 96 ~~in the receiving areas and disturbance of ecological equilibrium (Hui et al. 2003).~~

52 97 ~~Overall, herbicides account for 47.5 %, insecticides for 29.5 %, fungicides for 17.5 % and others~~  
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7 98 account for 5.5 % (De et al. 2014). More than 350 insecticides, herbicides, microbicides,  
8 99 nematocides and other pesticides are reported to be used (Huang McBeath and McBeath 2010). ~~The~~  
9 ~~average amount of pesticides used per hectare in China is roughly 1.5 to 4 fold higher than the~~  
10 ~~world average (Qiu 2011), thus resulting in the contamination of water bodies in the receiving areas~~  
11 ~~and disturbance of ecological equilibrium (Hui et al. 2003).~~

12 ~~The United States is also a large the next largest consumer of pesticides, applying over 1 billion~~  
13 ~~pounds annually (Alavanja 2009) with dramatical consequences for human beings and environment~~  
14 ~~(Carvalho 2017).~~

15 ~~As regards Europe, according to the Eurostat (2016), the main pesticide consumer is Spain (around~~  
16 ~~79,000 ton of active ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy~~  
17 ~~(~ 64,000), Germany (~ 46,000) and United Kingdom (~ 23,000).~~

18 The adverse effects of ~~pesticide and antibiotics~~OACs pollution have been concerned for a long time  
19 and many highly toxic and persistent pesticides have been banned worldwide. Although relatively  
20 safer pesticides have been developed and replaced the highly toxic ones, environmental pollution  
21 resulted by the long-term application of pesticides is far from being solved. ~~Still now~~  
22 ~~obsolete~~pesticides ~~widely used in agriculture in the past, still~~ represent a threat to  
23 environment, biodiversity, and human health for the region of Southeast Europe and their  
24 environmental and human risk need to be assessed in order to mitigate their current risk. Many  
25 organochlorines, organophosphates and pyrethroids have been banned but this did not solved the  
26 problem yet (Aravinna et al. 2017). In Argentina, the use of hexachlorocyclohexane pesticides have  
27 been limited from the late '90 and definitely banned in 2011, but this did not prevent to find  
28 concentration of lindane during recent samplings. Although the maximum level of lindane in saline  
29 water was fixed at 4 ng/l, in 2014 lindane exceeded this value of more than 5-fold (Ballesteros et al.  
30 2014). Although the use of organo-chlorine pesticide has been banned for over 20 years, they can  
31 still be found in the water and the sediment of main drainage area in China (Nakata et al. 2005; Xue  
32 et al. 2006; Zhou et al. 2006), due to run off from aged and weathered agricultural soils, or  
33 anaerobic sediments (Zhou et al. 2006). ~~Except for~~Besides water bodies and sediment, water, soil  
34 and even air in many cities are polluted by OACs, including urban or suburban areas (Gong et al.  
35 2004; Nakata et al. 2005; Yang et al. 2008).

36 ~~For that matter,~~OACs pose pivotal environmental problems, ~~due to their high reistance in the~~  
37 ~~environment and the consequent low natural attenuation. As an example, ; among them,~~  
38 organochlorine pesticides ~~and their metabolites, are resistant towere poorly affected by~~  
39 photochemical, chemical and biological ~~degradation processes for a long time as reported by and~~  
40 ~~more than 95% of them impacted on non-target organisms~~ (Mrema et al. (2013). ~~The authors~~

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7 132 highlighted the impacts of pesticides, which become widely dispersed in the environment; it was  
8 133 estimated that more than 95% of applied pesticides impact non-target organisms. As a consequence,  
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10 134 ~~Kim et al. (2017) reported a consequence, number of routes pesticides might follow to meet~~  
11 135 ~~human beings; the resulting multi-pathway direct and indirect exposure may affect human health.~~  
12 136 For instance during the last decade, one of the most studied issues is cancer occurrence related to  
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14 137 pesticide exposure.

15 138 As persistent organopollutants (POPs), pesticides represent one of the major problems in both  
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17 139 terrestrial and aquatic ecosystems. Regulatory and risk assessment procedures have to be adopted  
18 140 against those compounds that could be categorized as POPs/OACs. Since early '90, European Union  
19 141 started taking care of the problem. Driven from the carcinogenicity of pesticides, Directive 91/414/  
20  
21 142 EEC aimed to control the authorization for pesticides marketing within the EU. The particular  
22 143 attention given to pesticides is because recent studies confirmed that even low dose and chronic  
23 144 exposure might trigger adverse effects on wildlife and humans (EEA 2005). Being groundwater the  
24  
25 145 primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking  
26 146 Water Directive 98/83/EC deal with pesticides maximal exposure concentrations: 0.1 µg/l of a  
27  
28 147 single pesticide and 0.5 µg/l of total pesticides load. The protract exposure to low amount of  
29 148 pesticides cannot be underestimated because critical exposure levels can be chronically reached. A  
30 149 ~~risk~~ assessment has to consider the possible source of contamination but also the direct and  
31  
32 150 indirect multifaceted pathways of contact with human beings. Kim et al. (2017) reported a number  
33 151 of routes pesticides might follow to meet human beings; the resulting multi-pathway direct and  
34 152 indirect exposure may affect human health. Most of the pesticides emission (99 %) in the  
35  
36 153 environment in Europe is associated to agricultural practices whereas industrial and urban sources  
37 154 as the manufacturing of pesticides or the at-home use of insecticides have a minor impact (EEA  
38  
39 155 2016). Kim et al. (2017) reported a number of routes pesticides might follow to meet human  
40 156 beings; the resulting multi-pathway direct and indirect exposure may affect human health.

41 157 Point discharges of pesticides used in agriculture may occur and are mainly associated to accidental  
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43 158 causes as spillage, inappropriate storage and disposal, etc. Most of pesticides instead reach surface  
44 159 waters, through direct surface run-off or by leaching to groundwater and then subsequently follow  
45 160 different transport pathways. Once entered in the aquatic system, they could ultimately contaminate  
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47 161 water for human consumption.

48 162 A clear correlation between agriculture and water contamination was observed in Mar Chiquita lake  
49  
50 163 (Argentina), since high amount of endosulfan residues were detected soon after application and  
51 164 post-application periods (Ballesteros et al. 2014). The presence of the fungicide thifluzamide in  
52 165 paddy water of rice fields in China was maximal after the application, and variation during time was



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7 166 associated to the dilution effect of rainfalls in the area (Wei et al. 2015). Preventive measures to  
8 167 mitigate the impact of agriculture on the environment are required, taking into account both the use  
9 of safety pesticides and the optimization of farmer procedures. Aravinna et al. (2017) found that  
10 168 most of the 32 studied pesticides leached rice field following specific pathways. Since direct run off  
11 169 and erosion from soil were the main vehicles of dispersion, authors suggested alternative strategies  
12 170 (high resident time of pesticides, holding ponds of rice drainage water, delayed filling of paddies  
13 (high resident time of pesticides, holding ponds of rice drainage water, delayed filling of paddies  
14 171 after pesticide application and use less mobile compounds) to reduce the movement of the  
15 172 pesticides.

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18 174 Experimental evidences of advances in natural restoration processes highlight that time is our  
19 175 friend, since the abandonment of disturbed/polluted agricultural land for long time could reduce  
20 their contaminatin. In fact, at a global scale, one of the most frequently used strategies is long term  
21 176 remediation, which is represented by the abandonment of disturbed/polluted agricultural land  
22 177 (Kardol and Wardle 2010). Studies by Morri en et al. (2017) reported that nature restoration on ex-  
23 178 arable land resulted in increased connettance of soil biota's networks, as restoration progresses.  
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25 179 Such results confirm that the functions played by the soil biota provide many and varied services,  
26 180 and detoxification of pollutants and xenobiotic is one of the included primary services. In this  
27 context, innovation is represented by the research of solutions inspired by nature, as strategy to  
28 181 accelerate the natural attenuation processes in contaminated sites, optimizing bioremediation in real  
29 182 environment. Given that OACs represent a potential risk to humans, water, ecosystems and other  
30 183 receptors, fungi can play a pivotal role addressing their removal from contaminated sites and thus  
31 184 mitigating environmental pollution.

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33 186  
34 187 So clean and safe water is a critical step that stands between the *status quo* and a sustainable world.  
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36 188 This concept is no longer idealistic and became a milestone for the United Nations, as clearly stated  
37 189 in the World Water Development Report of 2015 (WWAP, 2015). Human lifestyle and the  
38 increasing urbanization lead to a worsened scenario. For instance, the actual pesticides use is 4 fold  
39 190 higher than 40 years ago (Mnif et al. 2011). EC compiled a watch list including, among others,  
40 191 pharmaceuticals, pesticides and personal care products. Being groundwater the primary source of  
41 192 drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking Water Directive  
42 193 98/83/EC deal with pesticides maximal exposure concentrations: 0.1 µg/l of a single pesticide and  
43 194 0.5 µg/l of total pesticides load.

44 195  
45 196 In this context, bioremediation has aroused as an-is-a usefulis-buseful technology to degrade  
46 197 pesticides-OACs by microbes (Singh 2008; Vel azquez-Fern andez et al. 2012), with several benefits  
47 198 over landfill disposal and incineration, such as the conversion of toxic wastes toformation of non-  
48 199 toxic end products, a-lower costseest of disposal (or no disposal at all), reduced health and

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7 200 ecological effects and long-term liabilities associated with non-destructive treatment methods, and  
8 201 the ability to perform the treatment *in situ* without unduly disturbing native ecosystems (Sarkar et  
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10 202 al. 2005). ~~Therefore, there is a growing interest in developing bioremediation techniques to degrade~~  
11 203 ~~OACs in polluted environments.~~ During the past decade, numerous microorganisms capable of  
12 204 degrading antibiotics and pesticides have been isolated, and detoxification processes for target  
13  
14 205 pollutants have been analyzed. ~~As for many other POPs (BTEX, PHAs, PCB congeners, etc) with~~  
15 206 ~~structural similarities with lignin, fungi~~ and especially ligninolytic fungi have been suggested  
16 207 as the most promising group of organisms able to transform recalcitrant compounds through a  
17  
18 208 unique set of extracellular oxidative enzymes (e.g. Anastasi et al. 2013; Harms et al. 2017).  
19 209 Comparative genomic analysis of 49 fungi with different nutritional modes such as saprotrophic  
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21 210 fungi, white-rot fungi (WRF), brown-rot fungi, ~~straw-soft~~ rot fungi and symbiotic fungi indicated  
22 211 that there is a relationship between nutrition models and the enzymes for lignocellulose degradation.  
23 212 Saprotrophic fungi have greater number of enzymes than symbiotic fungi, and brown-rot fungi have  
24  
25 213 smaller number than ~~white-rot fungi~~WRF and ~~straw-soft~~ rot fungi (Wu et al. 2015a). This might  
26 214 gain some insights into how to choose fungi in OACs degradation.

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28 215 ~~Experimental evidences of advances in natural restoration processes highlight that time is our~~  
29 216 ~~friend. In fact, at a global scale, one of the most frequently used strategies is long term remediation,~~  
30 217 ~~which is represented by the abandonment of disturbed/polluted agricultural land (Kardol and~~  
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32 218 ~~Wardle 2010). Studies by Morrión et al. (2017) reported that nature restoration on ex arable land~~  
33 219 ~~resulted in increased connectance of soil biota's networks, as restoration progresses. Such results~~  
34 220 ~~confirm that the functions played by the soil biota provide many and varied services, and~~  
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36 221 ~~detoxification of pollutants and xenobiotic is one of the included primary services. In this context,~~  
37 222 ~~innovation is represented by the research of solutions inspired by nature, as strategy to accelerate~~  
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39 223 ~~the natural attenuation processes in contaminated sites, optimizing bioremediation in real~~  
40 224 ~~environment. Given that OACs represent a potential risk to humans, water, ecosystems and other~~  
41 225 ~~receptors, fungi can play a pivotal role addressing their removal from contaminated sites and thus~~  
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43 226 ~~mitigating environmental pollution.~~

44 227 Finally yet importantly, metabolic activity of fungal or microbial consortia could produce not-  
45 228 known reaction products potentially with a major toxicity than parental compounds.

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47 229 García-Carmona et al. (2017) highlighted the importance to carry out environmental monitoring  
48 230 activities ante and post operam phases, using bioassays to determine the success of the  
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50 231 bioremediation process. Although it is substantial to assess the quality of the environment to ensure  
51 232 it remains free of toxic residues, most of the analytical tests available for determining the  
52 233 concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason,  
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biotoxicity testing has grown steadily in recent years and is a useful tool in environmental risk assessment (Shen et al. 2016; Prokop et al. 2016).

Indeed, there is a clear need to develop and define decontamination of hazardous pollutants as a concept towards sustainable remediation through a broader uptake of principles, approaches and tools to integrate environmental, social and economical dimension into the remediation processes (Ridsdale and Noble 2016). Several organizations, academia, standardization committees are currently assessing remediation process, evaluating the complexity of the concept of sustainability. Several documents have been developed by many countries across Europe and at global scale, addressing sustainable indicators of remediation activities (Harclerode et al. 2015).

The present review article summarizes the current state of scientific knowledge on research and application of fungi as effective bioresources, considering the recent advances in understanding their capacity to handle pesticide contamination.

#### **Bioremediation of OACs by fungi in soil system**

Large quantities of ~~OACs~~~~antibiotics~~ are being added to agricultural fields worldwide through the application of wastewater, manures and biosolids, resulting in pesticide and antibiotic contamination and elevated environmental risks in terrestrial environments (Jechalke et al. 2014; Zhang et al. 2015; Pan and Chu 2016). The largest fraction of ~~antibiotics-OACs~~ applied to soils with manure or biosolids is usually retained in surface soil whereas the part added through irrigation with wastewater can diffuse easily deep or by surface run-off. Once added to soil, ~~antibiotics-OACs~~ interact with soil solid phase and are prone to microbial transformation (Hammesfahr et al. 2008; Jechalke et al. 2014). In particular, veterinary antibiotics interact with soil solid phase in sorption and desorption reactions. Sorption and desorption control not only their mobility and uptake by plants but also their biotransformation and biological effects. ~~Antibiotics-OACs~~ as well as microorganisms are not distributed homogeneously in soil but are concentrated in hotspots. The different surfaces, voids, and pores provided by soil aggregates harbor a vast amount of biological diversity and chemical variability, and cause a patchy distribution of natural organic matter, oxides, nutrients, and microorganisms on soil particle surfaces (Hammesfahr et al. 2008; Jones et al. 2012). Sorption, sequestration, and subsequent release of ~~antibiotics-OACs~~ likely also occur at and from hotspots, and little is known about the behavior of ~~antibiotics-OACs~~ at environmentally relevant concentrations in agricultural soil.

Recently, many studies highlighted the fungal capability to transform and degrade recalcitrant OACs. In particular, one of a promising group is the ligninolytic fungi that possess a unique set of extracellular enzymes suitable to degrade lignin and are able to transform recalcitrant compounds.

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7 268 ~~In particular, a promising group of fungi that are able to transform recalcitrant compounds and~~  
8 269 ~~possess a unique set of extracellular ligninolytic enzymes are ligninolytic fungi~~ (Čvančarová et al.  
9 ~~2015). (Supplemental data Table I; Table I References) 2015). Nguyen et al. (2014) reported the~~  
10 270  
11 271 removal of diverse trace organic contaminants (i. e. trichloroethyl chloroformate (TrOC), phenolic  
12 272 and non phenolic, pharmaceuticals, pesticides, steroid hormones, industrial precursors and products,  
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14 273 phytoestrogens) by live (biosorption + biodegradation), intracellular enzyme-inhibited, and  
15 274 chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular  
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17 275 enzyme extract (predominantly laccases) from *Trametes versicolor* (strain ATCC 7731). They  
18 276 showed how non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC  
19 277 was negligible. The whole-cell culture showed considerably higher degradation of the major  
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21 278 compounds, indicating the importance of biosorption and subsequent degradation by intracellular  
22 279 and/or mycelium associated enzymes. However, studies that examined both adsorption and  
23 280 degradation of antibiotics in agricultural soil are too few, with most of them using unrealistically  
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25 281 high concentrations (in mg/kg levels) to overcome limitations in measurement. In addition, no  
26 282 model has been developed for speculating the adsorption and degradation of different types of  
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28 283 antibiotics in agricultural soil and the environmental risks they may pose. Pan and Chu (2016)  
29 284 evaluated the adsorption and degradation of five antibiotics (tetracycline, sulfamethazine,  
30 285 norfloxacin, erythromycin, and chloramphenicol) by native microorganisms (bacteria and fungi) in  
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32 286 non sterilized (test) and sterilized (control) agricultural soils under aerobic and anaerobic  
33 287 conditions. They showed that all antibiotics were susceptible to microbial degradation under aerobic  
34 288 conditions, and most antibiotics were degraded by more than 92% in non-sterilized soil after 28  
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36 289 days of incubation. For all the antibiotics, a higher initial concentration was found to slow down  
37 290 degradation and prolong persistence in soil. The degradation pathway of antibiotics, in fact, varied  
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39 291 in relation to their physicochemical properties as well as the microbial activities and aeration of the  
40 292 recipient soil. The authors were the first to develop a model for the prediction of antibiotic  
41 293 persistence in soil, which was valuable for the investigation of the fate of antibiotics in the  
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43 294 terrestrial environment.

44 295 Given the public concern for environmental pollution by OACs, there is increasing attention  
45 296 towards the development of biopurification systems for reducing the risk from the point source  
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47 297 contamination of soil resources. Various treatment methods (e.g. land filling of contaminated sites,  
48 298 recycling, pyrolysis and incineration) have been used for the removal and remediation of these  
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50 299 chemicals from the contaminated sites, but for example microbial degradation of pesticides is  
51 300 results the most important and effective way to remove these compounds from the environment  
52 301 (Hai et al. 2012; Verma et al. 2014). (Supplemental data Table I; Table I References) 2014).  
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7 302 Microorganisms have the ability to interact, both chemically and physically, with substances leading  
8 303 to structural changes or complete degradation of the target molecule. In particular, fungi may  
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10 304 transform pesticides and other xenobiotics by introducing minor structural changes to the molecule,  
11 305 producing nontoxic molecules that could be released into the soil for further degradation by  
12 306 microflora (Hai et al. 2012), (Supplemental data Table I; Table I References).

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14 307 In this context, Mir-Tutusaus et al. (2014) investigated the degradation of the insecticides  
15 308 imiprothrin and cypermethrin, the insecticide/nematicide carbofuran using the white-rot fungus *T.*  
16 309 *versicolor*. Their experiments with fungal pellets demonstrated extensive degradation of the tested  
17  
18 310 agrochemicals. In vivo studies with inhibitors of cytochrome P450 revealed that this intracellular  
19 311 system plays an important role in the degradation of imiprothrin and carbofuran, but not for  
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21 312 cypermethrin. The simultaneous degradation of the compounds successfully took place with  
22 313 minimal inhibition of fungal activity and resulted in the reduction of the global toxicity, thus  
23 314 supporting the potential use of *T. versicolor* for the treatment of several OACs.

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25 315 To date, the number of studies investigating novel treatment techniques for the removal of  
26 316 ~~pesticides—OACs~~ from contaminated agricultural soils is limited. The bacteria-dominated  
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28 317 conventional activated sludge process has been proved to be ineffective for ~~pesticide~~-removal.  
29 318 While the importance of a mixed microbial community to initiate and complete ~~pesticide—OACs~~  
30 319 removal in the soil environment has been convincingly demonstrated by several researchers, studies  
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32 320 concerning the removal of ~~pesticides—OACs~~ from soils have been predominantly focused on  
33 321 selected bacterial or fungal species separately. Few studies have explored the bioaugmentation  
34 322 synergy of fungi and bacteria (Hai et al. 2012; Zhang et al. 2015; Madrigal-Zúñiga et al. 2016).  
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36 323 Combining culture of bacteria and fungi could constitute a relevant process for the removal of toxic  
37 324 and recalcitrant organic substances from contaminated agricultural soils. On-farm biopurification  
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39 325 systems represent a biotechnological approach for the mitigation of point source contamination by  
40 326 ~~pesticides~~OACs. The main component of the biopurification systems is the biomixture, which acts  
41 327 as the biologically active core that accelerates the degradation of ~~OACs, pesticides~~OACs Madrigal-  
42  
43 328 Zúñiga et al. (2016) studied the employment possibility of the ligninolytic fungus *T. versicolor* in  
44 329 the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the  
45 330 insecticide-nematicide carbofuran (CFN). The CFN transformation products were detected at the  
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47 331 moment of CFN application, but their concentration continuously decreased to complete removal in  
48 332 both biomixtures. Mineralization of <sup>14</sup>C radiolabeled CFN was faster in GTS than in GCS. The  
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50 333 authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data  
51 334 suggested that the bioaugmentation improved the performance of the GTS rather than the GCS  
52 335 biomixture.

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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 accurate survey took into consideration 23 European countries with more than 160 water samplings studying mainly pharmaceuticals, pesticides and ~~known-recognised~~ endocrine ~~disruptingchemielasdisrupting chemielas~~ 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-methyl-desphenyl, bentazone,

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

The extent of the freshwaters contamination and the actual risk for human life depend on several  
factors concerning the hydrogeological characteristics of the soil, the weather conditions and the  
chemical-physical properties of the ~~pesticide~~OACs. The environmental fate of a certain 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) have been correlated  
to the soil organic carbon content and the hydrophobicity of the pesticide, ultimately affecting their  
soil retention behavior and the actual bioavailability in waters (Salvestrini et al. 2014). The flow of  
the leaching into surface waters is also a matter of season, in which opposite phenomena draw a  
complex scenario to be predict. A rainy period could cause a massive run-off of ~~OACs~~the pesticides  
from the soil contaminating the receiving basin (Sandin et al. 2018), ~~but during dry season, the high  
load of contaminants could be associated to evaporation and low water flow.~~ Besides the detection  
of high levels of ~~pesticides~~pesticideOACs is not exclusively coincident to their recent and massive  
use, ~~but it is ascribable to their~~ ~~Due to their~~ persistency, ~~their slow natural degradation,~~ their  
~~accumulation~~ and the various diffusion pathways, ~~they (Aguilar et al. 2017),~~ They could then  
tread long distances in surface or groundwater waters and the contamination can last for several  
decades (Ballesteros et al. 2014; Aravinnna et al. 2017).

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

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7 404 The preservation of water quality is a priority but OACs removal could not be based only on natural  
8 405 attenuation. Water treatment plants (WTPs) are the major barrages where OACs should be  
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10 406 removed. Not being specifically designed for micropollutants removal, they are often only partially  
11 407 effective, with a strong impact on the receiving ecosystem. Pesticides as atrazine, fluconazole,  
12 408 tebuconazole, diazinon and diuron are particularly resistant to commonly in use treatments (Köck-  
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14 409 Schulmeyer et al. 2013; Luo et al. 2014). A number of evidences confirmed the presence of OACs  
15 410 in WTPs effluents at toxicologically and estrogenically relevant concentration, becoming one of the  
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17 411 most effecting source of contamination (Bicchi et al. 2009; Campo et al. 2013; Jarošová et al.  
18 412 2014).

19 413 Particular attention has been given to advanced biological oxidation. Novel cost-effective and eco-  
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21 414 friendly processes based on fungi are an attractive option. ~~They Fungi~~ are well-known for to their  
22 415 physiological adaption skills, including the natural activation of tolerance mechanisms against  
23 416 pesticides (Talk et al. 2016). ~~In comparison with bacteria, Some reports already demonstrated that~~  
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25 417 ~~in comparison with bacteria,~~ fungi can better tolerate the presence of organic contaminants.  
26 418 Although the insecticide endosulfan inhibited both fungi and bacteria, bacterial community  
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28 419 structure significantly changed already at 0.1 mg/kg while modifications on the fungal community  
29 420 structures required 1 mg/kg of pollutant (Zhang et al. 2015). Linuron reduced bacterial count, and  
30 421 especially total bacteria, N<sub>2</sub>-fixing bacteria and nitrifiers, but not fungal numbers (Cycoń et al.  
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32 422 2010).

33 423 The importance of the isolation origin of fungi is out of discussion. Strains isolated from  
34 424 contaminated niches could have indeed developed specific adaptation skills due to the chronically  
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36 425 exposure over time. Carles et al. (2017) demonstrated that the aquatic microflora associated to  
37 426 submerged leaves exposed to nicosulfuron is more efficient in its degradation than communities  
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39 427 belonging to a less polluted site. The authors indicated fungi as the main constituents of this active  
40 428 microflora and as responsible of the herbicide degradation. In literature, several fungi isolated from  
41 429 contaminated areas or WTPs have been identified as degraders of nicosulfuron, diuron, isoproturon,  
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43 430 glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013; Carranza et al. 2014;  
44 431 Oliveira et al. 2015).

45 432 Exploiting this oxidative cascade, fungi may transform a broad range of recalcitrant organic  
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47 433 compounds, including OACs (Gao et al. 2010). A number of fungi are ~~pesticidespesticide~~OACs  
48 434 degraders, mostly belonging to Basydiomycetes as *Trametes*, *Pleurotus*, *Phlebia*, *Cerrena*,  
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50 435 *Coriolopsis*, etc. have been already investigated (Koroleva et al. 2002; Marco-Urrea et al. 2009;  
51 436 Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et al. 2014; Ceci et al. 2015) ([Table-II2](#).  
52 437 [Supplementary Materials](#)). Several ~~classes of~~ pesticides as lindane, atrazine, diuron,  
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terbuthylazine, metalaxyl, DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, etc. were effectively transformed by fungal treatment-[based on mycelium or enzymes \(Table I2, Supplementary Materials\)](#).

A bioremediation approach based on fungi may involve both biosorption and biodegradation processes; the latter one combines biosorption where the molecule binds to the fungal wall, and bioaccumulation with the pollutant being transported inside the cell in contact with intracellular enzymes (Kulshreshtha et al. 2014). Concentration of insecticide lindane decreased during time in the presence of two WRF (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated the main involvement of a fast adsorption process (Ulčnik et al. 2013). However, this phenomenon is often strains dependent, and especially related to metabolic differences between Ascomycetes and Basidiomycetes. Belonging to brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes, responsible for lignin degradation and likely for OACs as well: adsorption onto fungal mycelium was mainly involved for removal of endosulfan. On the contrary, the WRF actively degraded producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013). Although biosorption is a phenomenon that could be [not](#) ignored, it is often secondary or at least negligible respect to biodegradation (Carles et al. 2017). For instance, the removal of clofibric acid associated to heat-killed mycelium was less than 10 %, but more than 97 % in the presence of active *T. versicolor* (Marco-Urrea et al. 2009).

Fungi have developed a specific mechanism that employs few enzymes and molecules with high oxidizing power, physiologically aimed to transform ligninocellulose structure. The same enzymatic pathway may play a pivotal role in transforming other aromatic molecules. White-rot fungi usually involve ligninocellulosic extracellular enzymes as peroxidases (EC 1.11.1.x) and laccases (EC 1.10.3.2). The involvement of redox enzymes in the fungal-mediated oxidation is confirmed by the direct induction of enzyme production due to the presence of [pesticides.pesticideOACs](#). The fungus *T. versicolor* responded to 17 pesticides by increasing laccases production in comparison with the control: particular attention was given to transformation products of the herbicides diquat and monuron, capable of increasing the activity of 10- and 17-fold, respectively (Mougin et al. 2002). Laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus spp1*, *Pleurotus spp2*, *Cymatoderma elegans*, *Daedalea elegans* was stimulated by the presence of atrazine even at high concentration 3750 mg/l. Likewise the pesticide positively affected the manganese peroxidase activity of *Pleurotus spp1* and *C. elegans* (Chan-Cupul et al. 2014). Oxidoreductases stimulation was also observed with picloram (Maciel et al. 2013), bentazon (Da Silva Coelho et al. 2010), carbofuran (Mir-Tutusaus et al. 2014).

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7 472 Although these oxidoreductases are probably the most known enzymes for aromatic compounds  
8 473 degradation, alternative pathways can be promoted by the presence of [pesticidespesticideOACs](#).  
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10 474 Two clones (laccase positive and negative producers) of *Mycelia sterilia* were used to treat atrazine  
11 475 (20 µg/ml): even though one clone was defective for laccase production, comparable transformation  
12 476 yields (70-80%) were reached indicating their minor role in the degradation process (Vasil'Chenko  
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14 477 et al. 2002). This behavior is commonly found in brown-rot fungi that may trigger both on  
15 478 nonenzymatic and enzymatic mechanisms, i.e. Fenton mechanism or cellobiose dehydrogenase  
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17 479 (CDH) reactions (Fan and Song 2014). The degradation of atrazine (20 µg/l) by an unidentified  
18 480 mycelial fungus was associated to the presence in the liquid medium of OH radicals and CDH.  
19 481 Moreover, the CDH secretion was induced by the presence of the herbicide itself (Khromonygina et  
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21 482 al. 2004). In addition, some fungi could associate extracellular oxidoreductases with intracellular  
22 483 enzymes [such](#) as the cytochrome P450 system (cyt450). In the effort to better characterize the  
23 484 degradation skills of *T. versicolor*, cyt450 inhibitors were used: fungal performances against  
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25 485 clofibric acid and fipronil decreased (Marco-Urrea et al. 2009; Wolfand et al. 2016). Mori et al.  
26 486 (2017) suggested that cyt450 of *Phanerochaete sordida* is involved in the first reduction of the  
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28 487 clothianidin N-nitro group but the enzymes responsible of the further urea derivatives production  
29 488 are unknown.

30 489 The intra- and interspecies variability has long been recognized and found [confirmation](#)  
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32 490 [alsoconfirmation](#) for [pesticidespesticideOACs](#) treatment. Literature data about a certain specie  
33 491 could not be taken for granted and the set-up of a preliminary screening is often required. Despite  
34 492 *Phanerochaete chrysosporium* is often indicated as fungal model for organic degradation including  
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36 493 pesticides (Wang et al. 2014), it was almost ineffective against clofibric acid. Among five  
37 494 Basidiomycetes, only *T. versicolor* extensively degraded the herbicide (Marco-Urrea et al. 2009).  
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39 495 Alvarenga et al. (2014) treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*.  
40 496 Based on the growth capability in the presence of the pesticide, [only](#) the isolate *A. sydowii* CBMAI  
41 497 935 was selected for further studies. It indeed grew almost 4-fold more than the other *A. sydowii*.  
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43 498 The bioremediation potential is often substrate targeted, and the choice of fungus cannot be taken  
44 499 for granted. For instance, the exact same isolate (*A. sydowii* CBMAI 935) that totally converted  
45 500 methyl parathion (Alvarenga et al. 2014) was not the best performing one against the insecticide  
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47 501 esfenvalerate. Among 6 fungi, *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave  
48 502 better results than the *Aspergillus* strain (Birolli et al. 2016).

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50 503 Although the majority of these strains are effective in [pesticidespesticideOACs](#) removal in model  
51 504 solution, only few researchers have made a step forward, assessing the bioremediation potential of  
52 505 contaminated waters. The acquired information using model solutions (single-compound solution,  
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7 506 high concentration, no interfering molecules, etc.) is the unique way to acquire information about  
8 507 the degradation pathway (Masaphy et al. 1993; Birolli et al. 2016), but is less predictive of the  
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10 508 fungal performances on real environmental water samples. Each wastewater has its own critical  
11 509 issues, making difficult to predict the fungal behavior. Some data highlighted the robustness of a  
12 510 fungal system, although this needs detailed investigation case-by-case. A partially diluted leachate  
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14 511 showed to disturb the growth of *T. versicolor* and *Stereum hirsutum*, but this did not prevent them  
15 512 to totally degrade linuron and dimethoate at 10 mg/l. As regards dimethoate, the presence of  
16 513 adsorbents enhance the final process yields (from 50% to 97%), combining and exalting the action  
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18 514 of adsorption and biodegradation processes (Castellana and Loffredo 2014). The immobilization of  
19 515 *Bjerkandera adusta* and *Irpex lacteus* on coffee grounds, almond shells, a biochar favored the  
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21 516 removal of non-phenolic herbicides as fenuron and carbaryl from a municipal landfill leachate  
22 517 (Loffredo et al. 2016). Surface waters, ground waters or municipal wastewaters represent a very  
23 518 unique environment, characterized by extreme chemical and physical conditions, the presence of  
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25 519 heterogeneous and variable micropollutant mixture and an active autochthonous microflora. When  
26 520 inoculated in real surface water, a fungal consortium (*Aspergillus fumigatus*, *Aspergillus terreus*,  
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28 521 *Cladosporium tenuissimum*, *Cladosporium cladosporioides*, *Fusarium begoniae*, *Penicillium*  
29 522 *citrinum*, *Penicillium melanoconidium* and *Phoma glomerata*) was not stable in time due probably  
30 523 to the presence of toxic pesticides and the interaction with the natural microbial population: *P.*  
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32 524 *citrinum*, *A. fumigatus* and *A. terreus* were the most robust to the environmental conditions and  
33 525 actually capable of degrading the spiked chlorfenvinphos (Oliveira et al. 2015).

34 526 The set-up of active microbial consortia is an intriguing solution to strengthen and combine the  
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36 527 bioremediation potential of different organisms. Interestingly the combination of *Bacillus subtilis*  
37 528 and *A. niger* led to higher degradation rate of nicosulfuron than those obtained by using singly each  
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39 529 strain (Lu et al. 2012). The biodegradation of aldicarb, atrazine and alachlor by *Coriolus versicolor*  
40 530 was strongly enhanced by the combination with activated sludge. Along with modifications in the  
41 531 fungal morphology, when the bacterial-fungal consortium was established, the bio-absorbed  
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43 532 fraction of especially atrazine was reduced: over 98% of atrazine was removed by degradation  
44 533 processes in two weeks (Hai et al. 2012).

45 534 The fate of the treated ~~pesticides~~pesticideOACs is major issue that has to be carefully considered.  
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47 535 The residual toxicity is a critical issue. Interestingly fenuron and carbaryl (~~up to 70%~~) degradation  
48 536 (up to 70%) catalyzed by *B. adusta* and *I. lacteus* led to significant abatement of the phytotoxicity  
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50 537 (rapeseed and flax tests) (Loffredo et al. 2016). Mori et al. (2017) followed the neurotoxicity of  
51 538 clothianidin and its main metabolite produced by *P. sordida* treatment: the insecticide altered the  
52 539 cell viability of the neuronal cell line, but the metabolite was no longer neurotoxic.  
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7 540 Despite the well demonstrated properties, the application of whole cell system has some drawbacks  
8 541 including the fact that a living organism needs controlled growing conditions, in terms of nutrients,  
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10 542 pH, O<sub>2</sub>, etc. (Majeau et al. 2010). The addition of synthetic nutrients can strengthen fungal  
11 543 mycelium activity, but it should be carefully balanced for a further scale-up of the process. The fact  
12 544 that *T. versicolor* need 1% of glucose as carbon source to degrade atrazine would ultimately  
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14 545 interfere with its potential use in real WTPs (Khromonygina et al. 2004). Likewise several fungi as  
15 546 *A. niger* and *Dacryopinax elegans*, etc. required both easily available carbon and nitrogen sources  
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17 547 to efficiently act against nicosulfuron and diuron, respectively (Lu et al. 2012; Arakaki et al. 2013).  
18 548 Particular attention should be instead given to those fungi as *A. sydowii* and *Penicillium*  
19 549 *decatuense* that maintained the same performances without glucose addition, indicating the  
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21 550 potential of using methyl parathion or triclosan as sole carbon source (Alvarenga et al. 2014; Tian et  
22 551 al. 2016).

23 552 A promising alternative could be given by the direct use of fungal enzymes, capable of catalyzing  
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25 553 strong and fast oxidation reactions, with less technical drawbacks in comparison with fungal  
26 554 cultures. The potential of enzymes-based methods has been worldwide recognized; the Swiss  
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28 555 Industrial Biocatalysis Consortium defined oxidative enzymes as the biocatalysts displaying the  
29 556 highest development potential in the next decades (Meyer and Munch 2005). Great importance is  
30 557 given to the discovery of novel enzymes with wide substrate specificity, stable and applicable to  
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32 558 industrial uses. A number of articles have reported the ability of fungal enzymes to degrade  
33 559 ~~pesticides~~ ~~pesticide~~ ~~OACs~~. The potential of laccase-mediator systems have been assessed for the  
34 560 degradation of isoproturon (Margot et al. 2015), imiprothrin (Mir-Tutusaus et al. 2014),  
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36 561 chloroxuron (Palvannan et al. 2014), isoproturon (Zeng et al. 2017), atrazine (Chan-Cupul et al.  
37 562 2016). Laccases cannot be consider a novelty, as instead a phytase of *A. niger* capable of degrading  
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39 563 organophosphorus pesticides (Shah et al. 2017) or a cellulase of *Trichoderma longibrachiatum*  
40 564 active against dicofol (Wang et al. 2015). Particular attention should be given to the use of crude  
41 565 enzyme extracts of ligninolytic enzymes with a minor economic impact on the process than purified  
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43 566 enzymes (Matute et al. 2012; Kaur et al. 2016). A crude extract of *Trametes pubescens* laccases  
44 567 degraded up to 19 compounds in model solution and confirmed its ~~potential~~ ~~also~~ ~~potential~~ - with a  
45 568 real municipal wastewater where the presence of suspended particles, colloids, solvents and  
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47 569 xenobiotics as well as autochthonous microorganisms posed a strong environmental pressure. The  
48 570 transformation of all the detected compounds determined also a strong reduction of the  
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50 571 estrogenicity of the water sample (Spina et al. 2015).

51 572  
52 573 ***Application of synthetic microbial community on bioremediation***  
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Bioremediation is crucial way to eliminate the OACs pollution in agriculture ecosystem. However, many factors effect bioremediation efficiency for pesticide pollution, such as microbes applied, treatment sites, rhizosphere effects, soil chemical and physical properties (Zhou and Hua 2004). The practice in the bioremediation of soil or water pollution often cannot reach expected results because the target contaminant could not be degraded completely in most cases, and sometimes intermediate products were occurred with more toxin than original pesticides. Long-term application of various pesticides resulted in the pollution of more than one type of chemical compounds, which is hard to be degraded by a sole microbe. Thus, attention has been shifted to synthetic systems based on communication between cells, rather than individual isolated cell functionality (Biliouris et al. 2012). A promising way to overcome the difficulties is to create artificial synthetic microbial communities that contain several microbes to retain the key features of their natural counterparts (Großkopf and Soyer 2014).

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

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

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7 608 | ~~toxic milieu~~ (Jagmann and Philipp 2014). Thirdly, the bioremediation process should be optimized  
8 609 and regularly controlled based on the knowledge of stability and division of different  
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10 610 microorganisms (Liu et al. 2017).

Field Code Changed

11 611 Bioremediation of polluted soils and water is one application field of synthetic microbial  
12 612 community. As the complex structure of some pollutants, the effect of adding synthetic microbial  
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14 613 community is much higher than single microorganism, such as the biodegradation of pesticides  
15 614 diuron. The herbicide diuron is used for control of broad-leaved weeds on agricultural land. Several  
16 615 fungal-bacterial consortia were investigated by combining three different diuron-degrading bacteria  
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18 616 and two fungal strains. The fastest mineralization of diuron was obtained by the three member  
19 617 consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47) as  
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21 618 measured by evolved  $^{14}\text{CO}_2$ , mineralizing about 32 % of the added diuron within 54 days, whereas  
22 619 the single strains or other consortia reached no more than 10% mineralization. In addition, the  
23 620 production of diuron metabolites by consortium was minimal. This may be due to cooperative  
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25 621 catabolism, where the first organism transforms the pollutant to products that are then used by other  
26 622 organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby  
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28 623 facilitating a more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al.  
29 624 2014).

30 625 Similarly, a fungal-bacterial consortium consisting of *Mortierella* sp. LEJ702 and the 2,6-  
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32 626 dichlorobenzamide (BAM)-degrading *Aminobacter* sp. MSH1 reached a more rapid mineralisation  
33 627 of BAM than the bacterial alone, especially at lower moisture contents (Knudsen et al. 2013).  
34 628 Methylotrophic and hydrocarbon utilizing yeasts and bacteria alone did not degrade PCBs  
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36 629 significantly, but PCB degradation achieved about 50% when WRF were applied together (Šašek et  
37 630 al. 1993).

#### 40 632 *Evaluation of bioremediation effectiveness in contaminated matrices by performing* 41 633 *ecotoxicological and genotoxic tests*

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43 634 In order to improve the effectiveness and performance of bioremediation processes it is important to  
44 635 pursue three essential goals at the same time. Focus should be not only on reducing chemical  
45 636 concentrations, but also on reducing chemical mobility ~~between—in~~ the environmental  
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47 637 compartments and eventually lowering toxicity levels ensuring that contaminants do not get into the  
48 638 natural biological cycle (Loehr and Webster 1997; Chakraborty et al. 2013).

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50 639 Bioremediation is often monitored by following the concentration of targeted contaminants  
51 640 (Molina-Barahona et al. 2005). Numerous studies in recent years showed that traditional chemical  
52 641 analyses are insufficient for a full assessment of the contaminated site as they, for example, does not  
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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<sub>2</sub> 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<sub>2</sub> 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 organisms ~~test 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.

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7 676 As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various  
8 677 bioindicators either could indicate an incomplete decomposition of the substance or could result  
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10 678 from the formation of intermediate products generated via the bioremediation process. For this  
11 679 reason, sometimes chronic tests are more appropriate in evaluating the toxicity caused by by-  
12 680 products (Lofrano et al. 2014). ~~In other cases, however, also the toxicity of the by products is~~  
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14 681 ~~effectively removed (Lofrano et al. 2016).~~

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15 682 In certain circumstances, there is a clear need to monitor the bioremediation process using different  
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17 683 bioindicators. In Lizano-Fallas et al. (2017), for example, the ecotoxicity test with *Daphnia magna*  
18 684 shows a clear detoxification, whilst the detoxification patterns remain unclear when applying the  
19 685 phytotoxicity test. Ecotoxicological tests can also be used to determine the most suitable  
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21 686 bioremediation technique in relation to the examined case study as reported in Dudášová et al.  
22 687 (2016). Without worldwide-recognized unique guidelines for water quality assessment, literature  
23 688 data are difficult to compare due to the variety of model organisms, end-points, etc. Synthetic  
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25 689 indices capable of summarizing these findings could help to have an objective advice about the  
26 690 effectiveness of the biological treatment. They have been already applied for toxicity monitoring of  
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28 691 wastewaters (Tigini et al. 2011) but municipal effluents containing AOCs have never been taken  
29 692 into consideration nor estrogenic activity has been included so far.

30 693 Several toxicity assays were included in the treatability study protocol to measure remediation  
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32 694 efficiency. Assessing the toxicity of complex matrixes such soil could acquire methods from  
33 695 bioassays used to test toxicity of chemical compounds reported by the Organization for Economic  
34 696 Co-operation and Development (e.g. OECD 201 2006; OECD 211 2012). OECD has published a  
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36 697 series of standardized tests for determining the biodegradability of a given compound, based on the  
37 698 evaluation of overall parameters (such as COD, TOC and BOD) or methabolic tests, e.g.  
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39 699 respirometric (OECD 209 1984) as Polo et al. (2011) used for revealing susceptibility ~~to~~ of toxic  
40 700 compound comprising herbicide to biological treatment. Standardized testing procedures using  
41 701 different organisms have been approved by various environmental organizations, including the US  
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43 702 Environmental Protection Agency, American Society for Testing and Materials, International  
44 703 Standardization Organization (Siciliano et al. 2015). Many scientists have explored the effects of  
45 704 polluted soil on the whole organism using various microorganisms, animals, and plants, or by  
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47 705 means of cellular, and biochemical biomarkers, or by ecological scale up systems. Here below, tests  
48 706 at some different biological hierarchical levels of analyses are reported and discussed.

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51 708 *Organismal level*



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Concerning complex matrixes 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 are aquatic organisms such as *Daphnia magna*, *Raphidocelis subcapitata*, *Danio rerio*, *Myriophyllum aquaticum* or *Lemna minor* (Feiler et al. 2004). The use of freshwater and marine biota may be particular useful in order to provide a more complete comprehension on the environmental outcomes of agricultural activities evaluating the fate of pesticides (Guida et al. 2008). Terrestrial animals such as nematodes (*Caenorhabditis elegans*) (Traunspurger et al. 1997), oligochaetes (*Lumbriculus variegatus*) (Phipps et al. 1993), ~~springtails~~, springtails as *Folsomia candida* (Houx et al. 1996), and fish embryos (Hollert et al. 2003; Zielke et al. 2011) are well considered among the most reliable models.

Among higher plants important experimental models are *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 volume, 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* (Rönnpapel et al. 1995; Prokop et al. 2016), *Vibrio proteolyticus* (Ahlf and Heise 2005) yeasts (*Saccharomyces cerevisiae*) (Weber et al. 2006) are often used; otherwise, among bacterial bioassays, *Vibrio fischeri* luminescence inhibition test is the most common. The review of Parvez et al. (2006) remarks that *Vibrio fischeri* inhibition test is the most sensitive test, cost effective, easy to operate and requires only 5–30 min for toxicity prediction.

#### *Cellular and biomolecular level*

Biomarkers are adaptive responses by the organisms after exposure to xenobiotics. Various studies highlighted the cytotoxicity and genotoxicity effect of OACs and their metabolic products on the organisms. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or physiological, or even by behavioural changes (Depledge et al. 1993) that enable the obtaining of information on the biological effects of pollutants or their remains during or after a bioremediation process (Fontanetti et al. 2011).

Genetic endpoints and biomarkers. The most used biomarkers are mitotic index, chromosome aberrations, micronuclei, sister chromatid exchange and mutations.

Various scientists have recommended bacteria for bioassays evaluating genotoxicity in different samples (Mortelmans and Zeiger 2000; White and Claxton 2004). Ames test, one of the most famous and used, is a short term bacterial reverse mutation assay especially designed to evaluate the mutagenic potential of wide range of chemical substances (Mortelmans and Zeiger 2000) and was

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7 743 found to be very sensitive to wide range of mutagenic and carcinogenic chemicals as reported in the  
8 744 review paper of Chahal et al. (2014).

9  
10 745 On the side of plant models, higher plants are recognized as excellent genetic models to detect  
11 746 cytogenetic and mutagenic agents and are frequently used in environmental monitoring studies. The  
12 747 main organisms are *Allium cepa*, *Vicia faba* and *Tradescantia* spp. as reported in a review by De  
13  
14 748 Souza et al. (2016). Their protocols are standardized through a program under the International  
15 749 Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme  
16 750 (UNEP) (Ma 1999). In addition, the US Environmental Protection Agency (USEPA) and the World  
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18 751 Health Organization (WHO) validated the results obtained with plant bioindicators as an efficient  
19 752 model to detect environmental genotoxicity.

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21 753 One of the most used higher plant model is *V. faba*. The main advantages are its availability round  
22 754 the year, economical to use, easy to grow and handle; its use does not require sterile conditions and  
23 755 rate of cell division is fast. The *V. faba* test, deeply reported and discussed in the review of Iqbal  
24  
25 756 (2016), enables the assessment of different endpoints i.e., chromosomal aberration, mitotic index,  
26 757 micronuclei and nuclear aberration.

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28 758 Enzymatic biomarkers. Enzymatic activity inhibition as biomarker has been widely evaluated to  
29 759 measure toxicity of a matrix. Dehydrogenases, for example, are directly involved in many of the  
30 760 vital anabolic and catabolic processes of living organisms, and their activity is inhibited by  
31  
32 761 chemical toxicants. Recently, many studies have reported the use of terrestrial organisms for  
33 762 developing enzymatic biomarkers in response to residual pesticides (Henson-Ramsey et al. 2011;  
34 763 Radwan and Mohamed 2013; Stepić et al. 2013), and among these, earthworms were widely used to  
35  
36 764 understand the impacts of pesticides. In two earthworm species, *Eisenia fetida* and *Lumbricus*  
37 765 *terrestris*, multiple esterases, including acetylcholinesterase (AChE), butyrylcholinesterase, and  
38  
39 766 carboxylesterase (CE), have been assessed as biomarkers for malathion exposure (Henson-Ramsey  
40 767 et al. 2011). Several studies have also reported AChE, catalase (CAT), and glutathione-S-  
41 768 transferase as bio-chemical biomarkers in *Eisenia andrei* for the insecticides endosulfan, temephos,  
42  
43 769 malathion, and pirimiphos-methyl (Stepić et al. 2013), and AChE, CAT, CE, and the efflux pump as  
44 770 biomarkers in *E. andrei* and *Octolasion lacteum* for dimethoa. Recently, surface-enhanced laser  
45 771 desorption/ionization-time-of-flight (SELDI-TOF) mass spectrometry (MS) has strongly  
46  
47 772 contributed to the identification of more accurate, precise biomarkers e.g. specific for human  
48 773 cancers (Silsirivanit et al. 2014), or for endosulfan exposure in Japanese rice fish (*Oryzias latipes*)  
49  
50 774 (Lee et al. 2013). In a recent paper, selective protein biomarkers for 6 pesticides (captan, carbaryl,  
51 775 carbofuran, and  $\alpha$ -endosulfan chlorpyrifos, propoxur) were found in *E. fetida*, by means of SELDI-  
52 776 TOF MS technology (Park et al. 2015).  
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7 777 Estrogenic and androgenic biomarkers. It has been well documented that several chemicals from  
8 778 agricultural, industrial, and household sources possess endocrine-disrupting properties, which  
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10 779 provide a potential threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995;  
11 780 Jensen et al. 1995). A suggested mechanism is that environmental contaminants alter the normal  
12 781 functioning of the endocrine and reproductive system by mimicking or inhibiting endogenous  
13  
14 782 hormone action, modulating the production of endogenous hormones, or altering hormone receptor  
15 783 populations (Sonnenschein and Soto 1998). Besides several pesticides exert estrogenic and  
16 784 antiandrogenic activities through interaction with estrogen and androgen receptors. The risk  
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18 785 associated to OACs exposure has been known for decades: many pesticides, such as p,p'-  
19 786 dichlorodiphenyl trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978;  
20  
21 787 Cummings 1997),  $\beta$ -benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan,  
22 788 toxaphene, and dieldrin (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) have been firstly  
23 789 signaled as estrogenic. Despite increased institutional awareness and more compelling legislation  
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25 790 pressure, the most recent literature still reports the occurrence of pesticides in watercourses and in  
26 791 passing through the trophic chains, ing-showing remarkable estrogenic or androgenic (Saillenfait et  
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28 792 al. 2016; Brander et al. 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al.  
29 793 2017; Marcoccia et al. 2017). Several bioassays have been developed and standardized in order to  
30 794 describe the estrogenic potency of OACs. Andersen et al. (2002) indicated that several currently  
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32 795 used OACs, such as methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl,  
33 796 possess estrogenic activity on the basis of cell proliferation assay and transactivation assay using  
34 797 MCF-7 human breast cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism  
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36 798 and antagonism to two human estrogen receptor (hER) subtypes, hER $\alpha$  and hER $\beta$ , and a human  
37 799 androgen receptor (hAR) by highly sensitive transactivation assays, using Chinese hamster ovary  
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39 800 cells. The results demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic  
40 801 activities through ERs and/or AR. Although it appears that various pesticides exert hormonal effects  
41 802 at concentration orders of magnitude higher than that required for physiologic hormones, wide  
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43 803 exposure to large numbers of OACs may have additive and synergistic effects. Bioassay with YES  
44 804 (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active  
45 805 compounds still present in the environment. By the the first papers about this subject (Purvis et al.  
46  
47 806 1991), much more sophisticated bioassays have been developed such as that proposed by Eldridge  
48 807 et al. (2007) in which a bioluminescence strain of *Saccharomyces cerevisiae* was genetically  
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50 808 engineered to respond to androgenic chemicals.

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52 810 *Ecological level*  
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7 811 The actual risk of chemical residues pollution from bioremediation process is underestimated at the  
8 812 ecological level in natural systems. The ecological scaling-up experiment illustrated by Rodea-  
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10 813 Palomares et al. (2016) underlined how real-world exposure to chemical pollution is often  
11 814 dominated by low-dose complex combined with other biotic and abiotic stressors. In the paper, a  
12 815 novel screening method (GSA-QHTS) was reported, that coupled the computational power of  
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14 816 global sensitivity analysis (GSA) with the experimental efficiency of quantitative high-throughput  
15 817 screening (QHTS). In the case of study, they reported GSA-QHTS allowed for the identification of  
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17 818 the main pharmaceutical pollutants, driving biological effects of low-dose complex mixtures at the  
18 819 microbial population level. The target complex community was a river benthic microbial  
19 820 community inocula obtained from an unpolluted stream. The effect of the toxic compounds in a  
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21 821 mixture was evaluated together with other physico-chemical stressors, on a series of community  
22 822 level metabolic end points. Photosynthetic parameters, the dark-adapted basal fluorescence, the  
23 823 light-adapted steady-state fluorescence, the maximum photosynthetic efficiency, as well as the  
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25 824 extracellular enzymatic activities b-Glu and Phos were considered as both autotrophic and  
26 825 heterotrophic global fitness indicators suited to study the effects of chemical pollution on freshwater  
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28 826 benthic microbial communities.

### 30 828 *Prospect*

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32 829 Bioremediation is based on the idea that different organisms will work together to remove  
33 830 (biodegrade) the waste substances or pollutants (OACs) from environment. Although limitations for  
34 831 bioremediation practice might be occurred, including the nature of organisms, the enzyme involved,  
35  
36 832 the concentration and availability and finally survival of microorganisms, as well as cost/benefit  
37 833 ratio (i.e. cost versus overall environmental impact), to some extent, these limitations can be solved  
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39 834 by understanding the genetics and biochemistry of desired microbe. The advent of synthetic  
40 835 community showed giant potential ability in facilitating the bioremediation process, especially the  
41 836 effective utility of degradative fungi.

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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	<i>Phanerochete chrysosporium</i>	white-rot		Kennedy et al 1990
		<i>Phanerochete chrysosporium</i>	white-rot		Kennedy et al 1990
	chlordane	<i>Phanerochete chrysosporium</i>	white-rot		Kennedy et al 1990
		<i>Phanerochete chrysosporium</i>	white-rot		Arisoy 1998
	DDT	<i>Pleurotus sajor-caju</i>	white-rot		Arisoy 1998
	DDT	<i>Pleurotus florida</i>	white-rot		Arisoy 1998
	DDT	<i>Pleurotus eryngi</i>	white-rot		Arisoy 1998
	DDT	<i>Gloeophyllum trabeum</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Gloeophyllum sepiarium</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Gloeophyllum unguatum</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Gloeophyllum striatum</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Daedalea malicola</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Daedalea albida</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Daedalea serialis</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Daedalea dickinsii</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Fomitopsis palustris</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Fomitopsis annosa</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Fomitopsis insularis</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Fomitopsis pinicola</i>	brown-rot		Purnomo et al 2008
	DDT	<i>Boletus edulis</i>	ectomycorrhizal		Huang et al 2007
DDT	<i>Gomphidius viscidus</i>	ectomycorrhizal		Huang et al 2007	
DDT	<i>Laccaria bicolor</i>	ectomycorrhizal		Huang et al 2007	
DDT	<i>Leccinum scabrum</i>	ectomycorrhizal		Huang et al 2007	
DDT	<i>Trichoderma harzianum</i>	saprotrophic	field soil	Katayama and Matsumura 1993	
DDD	<i>Trichoderma sp.</i>	saprotrophic	marine sponges	Ortega et al 2011	
DDD	<i>Penicillium miczynskii</i>	saprotrophic	marine sponges	Ortega et al 2011	
dieldrin	<i>Trichoderma harzianum</i>	saprotrophic	field soil	Katayama and Matsumura 1993	



1					
2					
3		<i>Phanerochete</i>			
4	dieldrin	<i>chrysosporium</i>	white-rot		Kennedy et al 1990
5					
6	endosulfan	<i>Trichoderma harzianum</i>	saprotrophic	field soil	Katayama and Matsumura 1993
7		<i>Phanerochaete</i>			
8	endosulfan	<i>chrysosporium</i>	white-rot		Kullman and Matsumura 1996
9					
10		<i>Phanerochete</i>			
11	heptachlor	<i>chrysosporium</i>	white-rot		Arisoy 1998
12	heptachlor	<i>Pleurotus sajor-caju</i>	white-rot		Arisoy 1998
13	heptachlor	<i>Pleurotus florida</i>	white-rot		Arisoy 1998
14	heptachlor	<i>Pleurotus eryngi</i>	white-rot		Arisoy 1998
15					
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17	pentachloronitrobenzene	<i>Trichoderma harzianum</i>	saprotrophic	field soil	Katayama and Matsumura 1993
18					
19					
20	pentachlorophenol(PCP)	<i>Trichoderma harzianum</i>	saprotrophic	field soil	Katayama and Matsumura 1993
21		<i>Phanerochaete</i>			
22	pentachlorophenol(PCP)	<i>chrysosporium</i>	white-rot		Kang and Stevens 1994
23					
24					Rüttimann-Johnson and Lamar 1997
25	pentachlorophenol(PCP)	<i>Pleurotus ostreatus</i>	white-rot		Lamar 1997
26					
27					Rüttimann-Johnson and Lamar 1997
28	pentachlorophenol(PCP)	<i>Irpex lacteus</i>	white-rot		Lamar 1997
29					
30					Rüttimann-Johnson and Lamar 1997
31	pentachlorophenol(PCP)	<i>Trametes versicolor</i>	white-rot		Lamar 1997
32					
33					Rüttimann-Johnson and Lamar 1997
34	pentachlorophenol(PCP)	<i>Bjerkandera adusta</i>	white-rot		Lamar 1997
35					
36					Singh and Kulshreyha 1991
37	pendimethalin	<i>Fusarium oxysporum</i>	saprotrophic	soil	1991
38					
39					Singh and Kulshreyha 1991
40	pendimethalin	<i>Paecilomyces varioti</i>	saprotrophic	soil	1991
41					
42	pendimethalin	<i>Rhizoctonia bataticola</i>	saprotrophic	soil	Singh and Kulshreyha 1991
43					
44					Young and Banks 1998
45	lindane	<i>Rhizopus oryzae</i>	saprotrophic		1998
46		<i>Phanerochete</i>			
47	lindane	<i>chrysosporium</i>	white-rot		Arisoy 1998
48					
49	lindane	<i>Pleurotus sajor-caju</i>	white-rot		Arisoy 1998
50	lindane	<i>Pleurotus florida</i>	white-rot		Arisoy 1998
51	lindane	<i>Pleurotus eryngi</i>	white-rot		Arisoy 1998
52					
53		<i>Phanerochete</i>			
54	mirex	<i>chrysosporium</i>	white-rot		Kennedy et al 1990
55		<i>Phanerochaete</i>			
56	PCB 77	<i>chrysosporium</i>	white-rot		Vyas et al 1994
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	PCB 77	<i>Trametes versicolor</i>	white-rot		Vyas et al 1994
	PCB 77	<i>Corioloopsis polyzona</i>	white-rot		Vyas et al 1994
	Delor 106 (PCB)	<i>Phanerochaete chrysosporium</i>	white-rot		Novotný et al 1997
	Delor 106 (PCB)	<i>Trametes versicolor</i>	white-rot		Novotný et al 1997
	Delor 106 (PCB)	<i>Corioloopsis polyzona</i>	white-rot		Novotný et al 1997
	Six PCB congeners	<i>Trametes versicolor</i>	white-rot		Beaudette et al 2000
	Six PCB congeners	<i>Bjerkandera adusta</i>	white-rot		Beaudette et al 2000
	Six PCB congeners	<i>Phanerochaete chrysosporium</i>	white-rot		Beaudette et al 2000
organophosphate	chlорpyrifos	<i>Phanerochaete chrysosporium</i>	white-rot		Bumpus et al 1993
	chlорpyrifos	<i>Hypholoma fasciculare</i>	white-rot		Bending et al 2002
	chlорpyrifos	<i>Coriolus versicolor</i>	white-rot		Bending et al 2002
	chlорpyrifos	<i>Trichoderma harzianum</i>	saprotrophic	soil	Omar 1998
	chlорpyrifos	<i>Penicillium brevicompactum</i>	saprotrophic	soil	Omar 1998
	fonofos	<i>Phanerochaete chrysosporium</i>	white-rot		Bumpus et al 1993
	glyphosate	<i>Penicillium citrium</i>	saprotrophic		Zboinska et al 1992
	methyl parathion	<i>Aspergillus sydowii</i>	saprotrophic	marine	Alvarenga et al 2014
	methyl parathion	<i>Penicillium decaturense</i>	saprotrophic	marine	Alvarenga et al 2014
	terbufos	<i>Phanerochaete chrysosporium</i>	white-rot		Bumpus et al 1993
herbicide	alachlor	<i>Phanerochaete chrysosporium</i>	white-rot		Ferrey et al 1994
	alachlor	<i>Ceriporiopsis subvermispota</i>	white-rot		Ferrey et al 1994
	alachlor	<i>Phlebia tremellosa</i>	white-rot		Ferrey et al 1994
	alachlor	<i>Cunninghamella elegans</i>			Pothuluri et al 1993
	arochlor	<i>Pleurotus ostreatus</i>	white-rot		Zeddel et al 1993
	arochlor	<i>Trametes versicolor</i>	white-rot		Zeddel et al 1993
	three aroclors	<i>Phanerochaete chrysosporium</i>	white-rot		Yadav et al 1995
	atrazine	<i>Phanerochaete chrysosporium</i>	white-rot		Mougin et al 1994
	atrazine	<i>Pleurotus pulmonarius</i>	white-rot		Masaphy 1993
	atrazine	<i>Agrocybe semiorbicularis</i>	white-rot		Bending et al 2002
	atrazine	<i>Auricularia auricola</i>	white-rot		Bending et al 2002
	atrazine	<i>Coriolus versicolor</i>	white-rot		Bending et al 2002

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2					
3		atrazine	<i>Dichotomitus squalens</i>	white-rot	Bending et al 2002
4		atrazine	<i>Flammulina velupites</i>	white-rot	Bending et al 2002
5		atrazine	<i>Hypholoma fasciculare</i>	white-rot	Bending et al 2002
6		atrazine	<i>Phanerochaete velutina</i>	white-rot	Bending et al 2002
7		atrazine	<i>Pleurotus ostreatus</i>	white-rot	Bending et al 2002
8		atrazine	<i>Stereum hirsutum</i>	white-rot	Bending et al 2002
9					
10		diuron	<i>Agrocybe semiorbicularis</i>	white-rot	Bending et al 2002
11		diuron	<i>Hypholoma fasciculare</i>	white-rot	Bending et al 2002
12		diuron	<i>Stereum hirsutum</i>	white-rot	Bending et al 2002
13		diuron	<i>Coriolus versicolor</i>	white-rot	Bending et al 2002
14		carbendazim	<i>Trichoderma sp.</i>	saprotrophic	mutant strain Tian and Chen 2009
15	fungicide	metalaxyl	<i>Coriolus versicolor</i>	white-rot	Bending et al 2002
16		metalaxyl	<i>Stereum hirsutum</i>	white-rot	Bending et al 2002
17		iprodione	<i>Hypholoma fasciculare</i>	white-rot	Bending et al 2002
18		iprodione	<i>Stereum hirsutum</i>	white-rot	Bending et al 2002
19		iprodione	<i>Coriolus versicolor</i>	white-rot	Bending et al 2002
20					
21	PAH	five PAHs	<i>Bjerkandera adusta</i>	white-rot	soil and lignite Gramss et al 1995
22		five PAHs	<i>Gymnophilus sapineus</i>	Wood-degrading	soil and lignite Gramss et al 1995
23		five PAHs	<i>Hypholoma fasciculare</i>	Wood-degrading	soil and lignite Gramss et al 1995
24		five PAHs	<i>Hypholoma frowardii</i>	Wood-degrading	soil and lignite Gramss et al 1995
25		five PAHs	<i>Hypholoma sublateritium</i>	Wood-degrading	soil and lignite Gramss et al 1995
26		five PAHs	<i>Kuehneromyces mutabilis</i>	Wood-degrading	soil and lignite Gramss et al 1995
27		five PAHs	<i>Lenzites betulina</i>	Wood-degrading	soil and lignite Gramss et al 1995
28		five PAHs	<i>Pleurotus ostreatus</i>	white-rot	soil and lignite Gramss et al 1995
29		five PAHs	<i>Agrocybe praecox</i>	Wood- and straw-degrading	soil and lignite Gramss et al 1995
30		five PAHs	<i>Stropharia coronilla</i>	Wood- and straw-degrading	soil and lignite Gramss et al 1995
31		five PAHs	<i>Stropharia rugoso-annulata</i>	Wood- and straw-degrading	soil and lignite Gramss et al 1995
32		five PAHs	<i>Agaricus aestivalis</i>	Terricolous	soil and lignite Gramss et al 1995
33		five PAHs	<i>Agaricus arvensis</i>	Terricolous	soil and lignite Gramss et al 1995
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3				soil and	
4	five PAHs	<i>Agaricus bisporus</i>	Terricolous	lignite	Gramss et al 1995
5				soil and	
6	five PAHs	<i>Agaricus campestris</i>	Terricolous	lignite	Gramss et al 1995
7				soil and	
8	five PAHs	<i>Agaricus porphyizon</i>	Terricolous	lignite	Gramss et al 1995
9				soil and	
10	five PAHs	<i>Agrocybe dura</i>	Terricolous	lignite	Gramss et al 1995
11				soil and	
12	five PAHs	<i>Bovisa nigrescens</i>	Terricolous	lignite	Gramss et al 1995
13				soil and	
14	five PAHs	<i>Clitocybe odora</i>	Terricolous	lignite	Gramss et al 1995
15				soil and	
16	five PAHs	<i>Collybia dyophila</i>	Terricolous	lignite	Gramss et al 1995
17				soil and	
18	five PAHs	<i>Collybia maculata</i>	Terricolous	lignite	Gramss et al 1995
19				soil and	
20	five PAHs	<i>Coprinus comatus</i>	Terricolous	lignite	Gramss et al 1995
21				soil and	
22	five PAHs	<i>Lepista nebularis</i>	Terricolous	lignite	Gramss et al 1995
23				soil and	
24	five PAHs	<i>Lepista nuda</i>	Terricolous	lignite	Gramss et al 1995
25				soil and	
26	five PAHs	<i>Lepista saeva</i>	Terricolous	lignite	Gramss et al 1995
27				soil and	
28	five PAHs	<i>Lycoperdon perlatum</i>	Terricolous	lignite	Gramss et al 1995
29				soil and	
30	five PAHs	<i>Marasmius oreades</i>	Terricolous	lignite	Gramss et al 1995
31				soil and	
32	five PAHs	<i>Megacollybia platyphylla</i>	Terricolous	lignite	Gramss et al 1995
33				soil and	
34	five PAHs	<i>Phallus impudicus</i>	Terricolous	lignite	Gramss et al 1995
35				soil and	
36	five PAHs	<i>Psathyrella velutina</i>	Terricolous	lignite	Gramss et al 1995
37				soil and	
38	five PAHs	<i>Stropharia aeruginosa</i>	Terricolous	lignite	Gramss et al 1995
39				soil and	
40	five PAHs	<i>Amanita muscaria</i>	Ectomycorrhizal	lignite	Gramss et al 1995
41				soil and	
42	five PAHs	<i>Amanita rubescens</i>	Ectomycorrhizal	lignite	Gramss et al 1995
43				soil and	
44	five PAHs	<i>Amanita spissa</i>	Ectomycorrhizal	lignite	Gramss et al 1995
45				soil and	
46	five PAHs	<i>Hebeloma crustuliniforme</i>	Ectomycorrhizal	lignite	Gramss et al 1995
47				soil and	
48	five PAHs	<i>Hebeloma hiemale</i>	Ectomycorrhizal	lignite	Gramss et al 1995
49				soil and	
50	five PAHs			lignite	Gramss et al 1995
51				soil and	
52	five PAHs			lignite	Gramss et al 1995
53				soil and	
54	five PAHs			lignite	Gramss et al 1995
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56	five PAHs			lignite	Gramss et al 1995
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58				lignite	Gramss et al 1995
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3	five PAHs	<i>Hebeloma sinapizans</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
4					
5	five PAHs	<i>Laccaria amethystina</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
6					
7	five PAHs	<i>Lactarius deliciosus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
8					
9	five PAHs	<i>Lactarius deterrimus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
10					
11	five PAHs	<i>Lactarius deterrimus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
12					
13	five PAHs	<i>Lactarius rufus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
14					
15	five PAHs	<i>Lactarius torminosus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
16					
17	five PAHs	<i>Morchella conica</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
18					
19	five PAHs	<i>Morchella elata</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
20					
21	five PAHs	<i>Morchella esculenta</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
22					
23	five PAHs	<i>Morchella esculenta</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
24					
25	five PAHs	<i>Paxillus involutus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
26					
27	five PAHs	<i>Russula aeruginea</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
28					
29	five PAHs	<i>Russula foetens</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
30					
31	five PAHs	<i>Suillus granulatus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
32					
33	five PAHs	<i>Suillus variegatus</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
34					
35	five PAHs	<i>Tricholoma lascivum</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
36					
37	five PAHs	<i>Tricholoma terreum</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
38					
39	five PAHs	<i>Tricholoma terreum</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
40					
41	five PAHs	<i>Xerocomus badius</i>	Ectomycorrhizal	soil and lignite	Gramss et al 1995
42					
43	five PAHs	<i>Botrytis cinerea</i>	Mitosporic	soil and lignite	Gramss et al 1995
44					
45	five PAHs	<i>Scytalidium lignicola</i>	saprotrophic	soil and lignite	Gramss et al 1995
46					
47	five PAHs	<i>Trichoderma sp.</i>	saprotrophic	soil and lignite	Gramss et al 1995
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**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

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



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3	<i>Aspergillus oryzae</i> ,			
4	<i>Fusarium oxysporum</i> ,			
5	<i>Lentinula edodes</i> ,			
6	<i>Penicillium</i>	terbuthylazine,		
7	<i>brevicompectum</i> ,	difenoconazole and		
8	<i>Lecanicillium saksenae</i>	pendimethalin		Pinto et al. 2012
9	<i>Aspergillus sydowii</i>	trichlorfon		Tian et al. 2016
10				Taştan and Dönmez
11	<i>Aspergillus versicolor</i>	triclosan		2015
12	<i>Coriolus versicolor</i>	aldicarb, atrazine, alachlor		Hai et al. 2012
13				
14			laccase, manganese	
15			peroxidase, lignin	
16	<i>Dacryopinax elegans</i>	diuron	peroxidase	Arakaki et al. 2013
17				
18			laccase, manganese	
19	<i>Ganoderma lucidum</i>	lindane	peroxidase, lignin	Kaur et al. 2016
20				
21	<i>Ganoderma lucidum</i>	bentazon	peroxidase	Da Silva Coelho et al.
22				2010
23	<i>Ganoderma lucidum</i> ,		laccase	
24	<i>Trametes sp</i>	picloram		Maciel et al. 2013
25	<i>Gloeophyllum trabeum</i> ,			
26	<i>Trametes versicolor</i> ,			
27	<i>Pleurotus ostreatus</i>	lindane, endosulfan		Ulčnik et al. 2013
28	<i>Mycelia sterilia</i>	atrazine	laccase	Vasil'chenko et al. 2002
29				
30	<i>Penicillium citrinum</i> ,			
31	<i>P.citrinum</i> , <i>Fusarium</i>			
32	<i>proliferatum</i>	methylparathion		Rodrigues et al. 2016
33	<i>Penicillium griseofulvum</i>	b-hexachlorocyclohexane		Ceci et al. 2015
34				
35			cytochrome P450,	
36	<i>Phanerochaete sordida</i>	clothianidin	manganese peroxidase	Mori et al. 2017
37	<i>Pleurotus pulmonarius</i>	atrazine		Masaphy et al. 1993
38				
39	<i>Phlebia tremellosa</i> , <i>Phlebia</i>			
40	<i>brevispora</i> , <i>Phlebia</i>	Heptachlor, heptachlor		
41	<i>acanthocystis</i>	epoxide		Xiao et al. 2011
42	<i>Saccharomyces cerevisiae</i>	diazinon		Ehrampoush et al. 2017
43	<i>Talaromyces flavus</i>	nicosulfuron		Song et al. 2013
44				
45		imiprothrin, cypermethrin,	laccase, cytochrome	
46	<i>Trametes versicolor</i>	carbofuran,	P450	Mir-Tutusaus et al.
47		oxytetracycline		2014
48	<i>Trametes versicolor</i>	fipronil	cytochrome P450	Wolfand et al. 2016
49				
50	<i>Trametes versicolor</i>	6 pesticides, 2		
51		phytoestrogens		Nguyen et al. 2014
52	<i>Trametes versicolor</i> ,			Castellana and Loffredo
53	<i>Stereum hirsutum</i>	linuron, dimethoate		2014
54	nonsporulating mycelial		cellobiose	Khromonygina et al.
55	fungus	atrazine	dehydrogenase	2004
56				
57	<b>Enzymatic treatment</b>			
58				
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Enzymes involved	Pesticide	Literature
laccases of <i>Agaricus blazei</i>	metsulfuron	González Matute et al. 2012
phytase of <i>Aspergillus niger</i>	chlorpyrifos	Shah et al. 2017
extracellular extract of <i>Auricularia fuscousuccinea</i>	endosulfan	Yanez-Montalvo et al. 2016
laccase of <i>Trametes versicolor</i>	sulfamethoxazole, isoproturon	Margot et al. 2015
laccase of <i>Trametes versicolor</i>	chloroxuron	Palvannan et al. 2014
laccase of <i>Trametes versicolor</i>	lindane, endosulfan	Ulčnik et al. 2013
cellulose of <i>Trichoderma longibrachiatum</i>	dicofol	Wang et al. 2015
laccase of <i>Trametes versicolor</i>	isoproturon	Zeng et al. 2017

### Supplemental material – Table II: References

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