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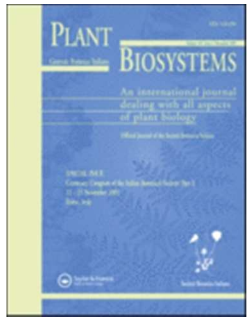

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Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water

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Abstract

Pesticides can help reduce yield losses caused by pests, pathogens, and weeds, but their overuse causes serious environmental pollution. They are persistent in the environment and are biomagnified through the food chain, becoming a serious health hazard for humankind. Bioremediation, where microbes are used to degrade pesticides *in situ*, is a useful technology. This review summarizes data on the fungi involved in the biodegradation of chemical pesticides and their application in soil and water bioremediation. Indications for future studies in this field are given.

Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi, Synthetic microbial community, Environmental risk assessment.

Introduction

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

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

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

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

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

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

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

The adverse effects of OAC pollution have been of concern for a long time and many highly toxic and persistent pesticides have been banned worldwide. Although relatively safer pesticides have been developed and replaced the highly toxic ones, environmental pollution resulting from the long-term application of pesticides is far from being solved. Obsolete pesticides still represent a threat to environment, biodiversity, and human health for the region of Southeast Europe and their risk to the environment and to humans needs to be assessed in order to mitigate it. Many organochlorines, organophosphates and pyrethroids have been banned but this has not yet solved the problem (Aravinna et al. 2017). In Argentina, hexachlorocyclohexane pesticides have been limited since the late ’90s and were definitely banned in 2011, but samples taken from a saline lake in 2014 showed levels to be more than 5-fold over the legal limit of 4 ng/l for lindane levels in the environment (Ballesteros et al. 2014). Likewise in China, although the use of organichlorine pesticides has been banned for over 20 years, they can still be found in the water and sediments of main drainage areas (Nakata et al. 2005; Xue et al. 2006; Zhou et al. 2006), due to run-off from aged and weathered agricultural soils and from anaerobic sediments (Zhou et al. 2006). Water bodies and sediments, the water, the soil and even the air in many cities in China are polluted by OACs, in both urban and suburban areas (Gong et al. 2004; Nakata et al. 2005; Yang et al. 2008).

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

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

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

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

Finally yet importantly, the metabolic activity of fungal or microbial consortia could potentially produce unknown reaction products that are more toxic than the parent compounds. García-Carmona et al. (2017) highlighted the importance of carrying out environmental monitoring activities ante- and post-operam phases, using bioassays to determine the success of the bioremediation process. Although it is fundamental to assess the quality of the environment to ensure it remains free of toxic residues, most of the analytical tests available for determining the concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason, 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 that will support sustainable remediation by involving a broader uptake of principles, approaches and tools that integrate environmental, social and economical dimensions into remediation processes (Ridsdale and Noble 2016). Several organizations, academia and standardization committees are currently assessing remediation process and evaluating the complexity of sustainability. Documents have been developed by many countries across Europe and globally, addressing sustainable indicators for remediation activities (Harclerode et al. 2015).

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

Bioremediation of OACs by fungi in the soil system

Large quantities of OACs 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). A large proportion of the OACs applied to soils with manure or biosolids are retained in surface soil, whereas those added through irrigation with wastewater can seep down to lower horizons or be diffused in surface run-off. Once present, OACs interact with the solid phase of soil and are prone to microbial transformation (Hammesfahr et al. 2008; Jechalke et al. 2014). In particular, veterinary antibiotics interact with the 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. OACs, like microorganisms are not distributed homogeneously in soil but are concentrated in hotspots. The multiplicity of surfaces, voids, and pores provided by soil aggregates harbor a vast amount of biological diversity and chemical variability, and cause 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 OACs likely also occur at and from hotspots. Little is known about the behavior of OACs at environmentally relevant concentrations in agricultural soil.

Recently, many studies have highlighted the ability of fungi to transform and degrade recalcitrant OACs. In particular, one of 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, (Čvančarová et al. 2015) (Supplemental material Table I; Table I References). Nguyen et al. (2014) reported the removal of diverse trace organic contaminants trichloroethyl chloroformate (TrOC) including phenolic and non-phenolic compounds, pharmaceuticals, pesticides, steroid hormones, industrial precursors and products, and phytoestrogens by live (biosorption + biodegradation), intracellular, enzyme-inhibited and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes versicolor* (strain ATCC 7731). They showed how non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably higher degradation of the major compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. However, there are too few studies that examine both adsorption and degradation of antibiotics in agricultural soil, with most using unrealistically high concentrations (in mg/kg levels) to overcome limitations in measurement. In addition, no model has been developed to speculate about the adsorption and degradation of different types of antibiotics in agricultural soil and the environmental risks they may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native microorganisms (bacteria and fungi) in non-sterilized (test) and sterilized (control) agricultural soils under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to microbial degradation under aerobic conditions, and most antibiotics were degraded by more than 92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial concentration was found to slow down degradation and prolong persistence in soil. The degradation pathway of antibiotics varied in relation to their physicochemical properties as well as the microbial activities and aeration of the recipient soil. In their study, Pan and Chu (1996) were the first to develop a model for the prediction of antibiotic persistence in soil.

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

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

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

On-farm biopurification systems constitute a biotechnological approach to the mitigation of point source contamination by pesticides. The main component of biopurification systems is the biomixture, which acts as the biologically active core that accelerates the degradation of OACs. Madrigal-Zúñiga et al. (2016) studied the results of employing the ligninolytic fungus *T. versicolor* in the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the insecticide-nematicide carbofuran (CFN). The transformation products of CFN were detected at the moment of CFN application, but their concentration decreased continuously until complete removal in both biomixtures. Mineralization of ^{14}C radiolabeled CFN was faster in GTS than in GCS. The authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data suggested that the bioaugmentation improved the performance of the GTS rather than the GCS biomixture.

Pinto et al. (2016) also studied the potential use of different substrates in biomixtures like cork, cork and straw, coat pine and LECA (Light Expanded Clay Aggregates) in the degradation of terbuthylazine, difenoconazole, diflufenican and pendimethalin pesticides. Bioaugmentation 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 such as cork residues to increase the capacity for pesticide dissipation in biomixtures for establishing biobeds. Furthermore, greater 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 to be used as inoculum source to improve the degradation efficiency of sustainable on-farm biopurification systems.

Fungi isolated from biomixtures represent a biological source of potentially active bioremediation agents, and the adaptation skills developed by these microorganisms could make the difference in OAC removal (Supplemental material Table I; Table I References). This 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 ability of autochthonous fungi (*Penicillium brevicompactum* and *Lecanicillium saksenae*) to degrade xenobiotics was compared with that of allochthonous strains taken from a culture collection (*Fusarium oxysporum*, *Aspergillus oryzae* and *L. edodes*). The best biodegradation yield was achieved with *P. brevicompactum*: its higher ability to metabolize terbuthylazine was presumably acquired through chronic exposure to contamination with the herbicide.

Bioremediation of OACs by fungi in aquatic ecosystems

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

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

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

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

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

The provenance of isolated fungi is of unquestionable importance. Strains isolated from contaminated niches indeed seem to develop specific adaptation skills due to chronic exposure. Carles et al. (2017) demonstrated that the aquatic microflora found in association with submerged leaves exposed to nicosulfuron is more efficient in its degradation than are communities that come from a less polluted site. The authors indicated fungi as the main constituents of this active microflora and as being responsible for herbicide degradation. In the literature, several fungi isolated from contaminated areas or WTPs have been identified as degraders of nicosulfuron, diuron, isoproturon, glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013; Carranza et al. 2014; Oliveira et al. 2015).

Fungi can thus transform a broad range of recalcitrant organic compounds, including OACs (Gao et al., 2010). A number of fungi that are OAC degraders, mostly belonging to Basydiomycetes, such as *Trametes*, *Pleurotus, Phlebia, Cerrena, Coriolopsis*, etc., have been already investigated (Koroleva et al. 2002; Marco-Urrea et al. 2009; Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et al. 2014; Ceci et al. 2015). Several pesticides as lindane, atrazine, diuron, 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 (Supplemental material Table II).

A bioremediation approach based on fungi may involve both biosorption and biodegradation processes; the latter 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). Concentrations of the insecticide lindane decreased during time in the presence of two WRFs (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated that a fast adsorption process was mainly involved (Ulčnik et al. 2013). However, this phenomenon is often strain-dependent, and expecially related to metabolic differences between Ascomycetes and Basidiomycetes. Belonging to the brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes, responsible for lignin degradation and likely for that of OACs as well: adsorption onto fungal mycelium was mainly involved in the removal of endosulfan. On the contrary, the white-rot fungi actively degraded, producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013). Although biosorption is a phenomenon that cannot be ignored, it is often secondary or at least negligible compared to biodegradation (Carles et al. 2017). For instance, the removal of clofibric acid found for heat-killed mycelium was less than 10 %, but more than 97 % for 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 at transforming lignocellulose structures. The same enzymatic pathway may play a pivotal role in transforming other aromatic molecules. White-rot fungi usually deploy extracellular lignocellulosic enzymes such as peroxidases (EC 1.11.1.x) and laccases (EC 1.10.3.2). The involvement of redox enzymes in fungal-mediated oxidation is confirmed by the direct induction of enzyme production in the presence of OACs. The fungus *T. versicolor* responded to 17 pesticides by increasing laccase production in comparison with the control: particular attention was given to the transformation products of the herbicides diquat and monuron, capable of increasing fungal activity 10- and 17-fold, respectively (Mougin et al. 2002). The laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus* spp1, *Pleurotus* spp2, *Cymatoderma elegans*, and *Daedalea elegans* was stimulated by the presence of atrazine even at high concentrations of 3750 mg/l. Likewise, the manganese peroxidase activity of *Pleurotus* spp1 and *C. elegans* was positively correlated with the pesticide (Chan-Cupul et al. 2014). Oxidoreductase stimulation was also observed with picloram (Maciel et al. 2013), bentazon (Da Silva Coelho et al. 2010) and carbofuran (Mir-Tutusaus et al. 2014).

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

Fungal intra- and interspecies variability has long been recognized and has found confirmation in OAC treatment. Literature data about a given species cannot be taken for granted and preliminary screening is often required. Despite *Phanerochaete chrysosporium* often being indicated as the fungal model for organic degradation including pesticides (Wang et al. 2014), it was almost ineffective against clofibric acid (Marco-Urrea et al. 2009). Among five Basidiomycetes, only *T. versicolor* extensively degraded this herbicide (Marco-Urrea et al. 2009). Alvarenga et al. (2014) treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*. Based on ability to grow in the presence of the pesticide, only the isolate *A. sydowii* CBMAI 935 was selected for further studies. It indeed grew almost 4-fold more than the other *A. sydowii*. Bioremediation potential is often substrate-targeted, and the choice of fungus cannot be taken for granted. For instance, *A. sydowii* CBMAI 935, which totally converted methyl parathion (Alvarenga et al. 2014) was not the best performing one against the insecticide esfenvalerate. Among 6 fungi, *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave better results than the *Aspergillus* strain (Birolli et al. 2016).

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

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

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

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

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

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

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

Application of synthetic microbial communities in bioremediation

Bioremediation is a crucial way to eliminate OAC pollution in agricultural ecosystems. However, many factors affect the efficiency of bioremediation in pesticide pollution, such as the microbes applied, treatment sites, rhizosphere effects and soil chemical and physical properties (Zhou and Hua 2004). Bioremediation of soil or water pollution often cannot reach expected results in practice because the target contaminant cannot be degraded completely, and sometimes intermediate products occur that are more toxic than the original pesticides. Long-term application of various pesticides results in pollution with more than one type of chemical compound, which are unlikely to be degraded by a sole microbe. Thus, attention has shifted to synthetic systems based on communication between cells, rather than on 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).

The so-called *synthetic microbial community* is created by a bottom-up approach where two or more defined microbial populations are put together 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 (Brenner et al. 2008). Synthetic communities have several potential advantages over monocultures or natural communityies: 1) the species in a synthetic community are known and the community structure is relatively simple and controllable, while the natural community may contain many microorganisms with unknown functions; 2) synthetic communities can perform more complicated functions than individual organisms because members of microbial consortia communicate and differentiate (Brenner et al. 2008); 3) synthetic communities are often more robust to environmental fluctuations because they can resist invasion by other species and weather periods of nutrient limitation better than monocultures (Brenner et al. 2008); 4) synthetic communities can 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).

Liu et al. (2017) proposed three design principles to develop a cooperative, steady-state community that is performing a desirable biotechnological function. 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 bioconverse toxic substrates or products in a toxic milieu (Jagmann and Philipp 2014). Thirdly, the bioremediation process should be optimized and regularly monitored on the basis of the knowledge of stability and division of different microorganisms (Liu et al. 2017).

Bioremediation of polluted soils and water is one field of application synthetic microbial communities. Due to the complex structure of some pollutants, such as the diuron pesticides, adding synthetic microbial communities is much more effective than adding single microorganisms. The herbicide diuron is used in the control of broad-leaved weeds on agricultural land. Several fungal-bacterial consortia were investigated by combining three different diuron-degrading bacteria and two fungal strains. The fastest mineralization of diuron was obtained by the three-member consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47). As measured by evolved $14CO_2$ it mineralized about 32 % of the added diuron within 54 days, whereas the single strains or other consortia achieved no more than 10% mineralization. In addition, the production of diuron metabolites by the consortium was minimal. This may be due to cooperative catabolism, where the first organism transforms the pollutant to products that are then used by the other organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby facilitating the more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al. 2014).

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

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

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

Bioremediation is often monitored by following the concentration of targeted contaminants (Molina-Barahona et al. 2005). Numerous studies in recent years have shown that traditional chemical analyses are insufficient for a full assessment of the contaminated site because, for example, they do not provide any information about the interactions between chemicals and they do 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 that links the various fields and levels of study involving contaminated sites has proven to be an efficient way to evaluate the effectiveness of bioremediation 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, given the chemical and biological complexity of the tasks involved, close collaboration between microbiologists, chemists and engineers is required (Van Gestel et al. 2001; Chakraborty et al. 2013).

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

Signals that bioremediation is going on should be monitored. Two important chemical compounds produced by microorganisms during their degradation activity are CO_2 and soluble phosphorus. Both increase notably in 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 metabolic activity (e.g. CO_2 production) may not indicate detoxification. Although the fate of the toxicants may be followed by chemical analyses, many reaction products resulting from the bioremediation process and their potential toxicity are not known. 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 potential ecotoxicity. In fact, biodegradation of pesticides can proceed partially or totally due to the structure of the molecule itself or to unfavourable environmental or test conditions, or to the lack of 'acclimatized' microbial communities (De Henau 1997).

In some instances, it has been shown that an effective process of bioremediation corresponds with 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 that a battery of tests be used (Keddy et al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of reference organisms that are representative of the different trophic levels, in order to select species with different roles in ecosystems, and different exposure conditions (Van Straalen and Van Gestel 1997). Moreover, environmental risk assessment must integrate chemical characterization, ecotoxicity and bioremediation data, in order to accurately assess the ecological hazard.

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

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

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

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

Organismal level

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

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

Cellular and biomolecular level

Biomarkers signal the adaptative responses of organisms to xenobiotic exposure. Various studies have highlighted the cytoxic and genotoxic effects on organisms of OACs and their metabolic products. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or physiological, or even behavioural changes (Depledge et al. 1993) that enable information to be obtained on the biological effects of pollutants or their remains during or after a bioremediation process (Fontanetti et al. 2011).

<u>Genetic endpoints and biomarkers</u>. The most-used biomarkers are mitotic index, chromosome aberrations, micronuclei, sister chromatid exchanges and mutations.

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

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

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

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

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

Estrogen and androgen biomarkers. It is well-documented that several chemicals from agricultural, industrial, and household sources possess endocrine-disrupting properties, which provide a potential threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995; Jensen et al. 1995). A suggested mechanism is that environmental contaminants alter the normal functioning of the endocrine and reproductive system by mimicking or inhibiting the action of endogenous hormones, by modulating the production of endogenous hormones, or by altering hormone receptor populations (Sonnenschein and Soto 1998). Several pesticides exert estrogenic and antiandrogenic activities through interaction with estrogen and androgen receptors. The risks associated with OAC exposure has been known for decades: many pesticides, such as p,p′-dichlorodiphenyl trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978; Cummings 1997), β-benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan, toxaphene, and dieldrin (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) were the first to be signaled as estrogenic. Despite increased institutional awareness and more compelling legislation pressure, the most recent literature still reports the occurrence of pesticides in watercourses and in the trophic chains, that show conspicuous estrogen or androgen levels (Saillenfait et al. 2016; Brander et al. 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al. 2017; Marcoccia et al. 2017). Several bioassays have been developed and standardized in order to describe the estrogenic potency of OACs. Andersen et al. (2002) indicated that several currently used OACs, such as methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl, possess estrogenic activity on the basis of cell proliferation assays and transactivation assays using MCF-7 human breast cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism and antagonism to two human estrogen receptor (hER) subtypes, hERα and hERβ, and a human androgen receptor (hAR) by means of highly sensitive transactivation assays, using Chinese hamster ovary cells. The results demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic action through ERs and/or AR. Although it appears that various pesticides exert hormonal effects at concentrations that are orders of magnitude higher than that required for physiologic hormones, wide exposure to large numbers of OACs may have additive and synergistic effects. Bioassay with YES (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active compounds still present in the environment. Since the the first papers on this subject (Purvis et al. 1991), much more sophisticated bioassays have been developed, such as that proposed by Eldridge et al. (2007) in which a bioluminescent strain of *Saccharomyces cerevisiae* was genetically engineered to respond to androgenic chemicals.

Ecological level

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

Prospect

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

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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.

Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi, Synthetic microbial community, Environmental risk assessment.

Introduction

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

Pesticides are a diverse group of inorganic and organic chemicals like herbicides, insecticides, nematicides, fungicides, antibiotics and soil fumigants, all belonging to the so-called organic agrochemicals (OACs) (Verger and Boobis 2013; Verma et al. 2014). In agriculture, pesticides aim to enhance crop yield and quality, and to maximize economic returns by prevention of pest or weed attack. They are bioactive, toxic substances, capable of influencing, directly or indirectly, soil fertility and health as well as agroecosystem quality (Pinto et al. 2012; Verma et al. 2014). Given that belowground biodiversity is closely linked to land management, agricultural intensification causes many pressures that leads to loss of biodiversity. Consequently, soil pollution is one of the main threats related to the decline of taxonomic and functional biodiversity, and of agricultural soils sustainability (Harms et al. 2017). Most of the pesticides emission (99 %) in Europe is associated to agricultural practices whereas industrial and urban sources as the manufacturing of pesticides or the at-home use of insecticides have a minor impact (EEA 2016).~~Thus, t~~ T~~t~~he extensive and massive use of pesticides in agriculture activities has serious impacts on the environment, compromising soil and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al. 2016). Besides,
~~L~~l~~In addition to pesticides, largel~~~~L~~arge quantities of antibiotics are added to agricultural fields worldwide through the application of wastewater, manures and biosolids, resulting in antibiotic contamination and elevated environmental risks (Jechalke et al. 2014; Zhang et al. 2015; Pan and Chu 2016~~).~~), A clear correlation between agriculture and water contamination was observed in Mar Chiquita lake (Argentina), where high amount of endosulfan residues were detected soon after application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide thifluzamide in paddy water of rice fields in China was maximal after the application, and variation during time was associated to the dilution effect of rainfalls in the area (Wei et al. 2015). Preventive

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

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

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

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

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

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

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

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

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

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

~~As persistent organopollutants (POPs), pesticides represent one of the major problems in both terrestrial and aquatic ecosystems. R~~egulatory and risk assessment procedures have to be adopted against ~~those compounds that could be categorized as POPs~~OACs. ~~Since early '90, European Union started taking care of the problem.~~ Driven from the carcinogenicity of pesticides, Directive 91/414/ EEC aimed to control the authorization for pesticides marketing within the EU. The particular attention given to pesticides is because recent studies confirmed that even low dose and chronic exposure might trigger adverse effects on wildlife and humans (EEA 2005). Being groundwater the primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking Water Directive 98/83/EC deal with pesticides maximal exposure concentrations: 0.1 µg/l of a single pesticide and 0.5 µg/l of total pesticides load. ~~The protract exposure to low amount of pesticides cannot be underestimated because critical exposure levels can be chronically reached. A r~~Risk~~risk~~ assessment has to consider the ~~possible~~ source of contamination but also the direct and indirect multifaceted pathways of contact with human beings. Kim et al. (2017) reported a number of routes pesticides might follow to meet human beings; the resulting multi-pathway direct and indirect exposure may affect human health. ~~Most of the pesticides emission (99 %) in the environment in Europe is associated to agricultural practices whereas industrial and urban sources as the manufacturing of pesticides or the at-home use of insecticides have a minor impact (EEA 2016). Kim et al. (2017) reported a number of routes pesticides might follow to meet human beings; the resulting multi-pathway direct and indirect exposure may affect human health.~~

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

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

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

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

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

In this context, bioremediation has aroused as an ~~is a~~ ~~usefulis b~~useful technology to degrade ~~pesticides~~ OACs ~~by microbes~~ (Singh 2008; Velázquez-Fernández et al. 2012), with several benefits over landfill disposal and incineration, ~~such~~ as the ~~conversion of toxic wastes to~~formation of non-toxic end products, ~~a~~ lower costs~~cost~~ of disposal ~~(or no disposal at all)~~, reduced health and ecological effects and long-term liabilities associated with non-destructive treatment methods, and the ability to perform the treatment *in situ* without unduly disturbing native ecosystems (Sarkar et al. 2005).~~ Therefore, there is a growing interest in developing bioremediation techniques to degrade OACs in polluted environments.~~ During the past decade, numerous microorganisms capable of degrading antibiotics and pesticides have been isolated, and detoxification processes for target pollutants have been analyzed. ~~As for many other POPs (BTEX, PHAs, PCB congeners, etc) with structural similarities with lignin, f~~Fungi~~fungi~~ and especially ligninolytic fungi have been suggested as the most promising group of organisms able to transform recalcitrant compounds through a unique set of extracellular oxidative enzymes (~~e.g.~~ Anastasi et al. 2013; Harms et al. 2017). Comparative genomic analysis of 49 fungi with different nutritional modes such as saprotrophic fungi, white-rot fungi (WRF), brown-rot fungi, ~~straw~~ soft rot fungi and symbiotic fungi indicated that there is a relationship between nutrition models and the enzymes for lignocellulose degradation. Saprotrophic fungi have greater number of enzymes than symbiotic fungi, and brown-rot fungi have smaller number than ~~white- rot fungi~~WRF and ~~straw~~ soft rot fungi (Wu et al. 2015a). This might gain some insights into how to choose fungi in OACs degradation.

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

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

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

~~In particular, a promising group of fungi that are able to transform recalcitrant compounds and possess a unique set of extracellular ligninolytic enzymes are ligninolytic fungi~~ (Čvančarová et al. 2015~~)~~), (Supplemental data Table I; Table I References).~~2015).~~ Nguyen et al. (2014) reported the removal of diverse trace organic contaminants (i. e. trichloroethyl chloroformate (TrOC), phenolic and non phenolic, pharmaceuticals, pesticides, steroid hormones, industrial precursors and products, phytoestrogens) by live (biosorption + biodegradation), intracellular enzyme-inhibited, and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes versicolor* (strain ATCC 7731). They showed how non-phenolic TrOC were readily biodegraded while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably higher degradation of the major compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. However, studies that examined both adsorption and degradation of antibiotics in agricultural soil are too few, with most of them using unrealistically high concentrations (in mg/kg levels) to overcome limitations in measurement. In addition, no model has been developed for speculating the adsorption and degradation of different types of antibiotics in agricultural soil and the environmental risks they may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native microorganisms (bacteria and fungi) in non sterilized (test) and sterilized (control) agricultural soils under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to microbial degradation under aerobic conditions, and most antibiotics were degraded by more than 92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial concentration was found to slow down degradation and prolong persistence in soil. The degradation pathway of antibiotics, in fact, varied in relation to their physicochemical properties as well as the microbial activities and aeration of the recipient soil. The authors were the first to develop a model for the prediction of antibiotic persistence in soil, which was valuable for the investigation of the fate of antibiotics in the terrestrial environment.

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

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

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

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

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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.

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Bioremediation of OACs by fungi in aquatic ecosystem

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

~~An~~ accurate survey took into consideration 23 European countries with more than 160 water samplings studying mainly pharmaceuticals, pesticides and ~~known~~ recognised endocrine ~~disruptingchemiclas~~disrupting ~~chemiclas~~ chemicals (Loos et al. 2010). Among the 59 compounds under study, the most frequently detected compound was 1 insecticide (DEET), and 7 pesticides (chloridazon-desphenyl, DMS, desethylatrazine, chloridazon-methyldesphenyl, bentazone, desethylterbutylazine, dichlorprop) exceeded the European threshold of 0.1 µg/l. ~~On the whole,~~ ~~29~~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 repellant (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 ~~pesticidespesticide~~OACs is not exclusively coincident to their recent and massive use~~.~~, but it is ascribable to their ~~. Due to their~~ persistency, their slow natural deagradtion, their accumulation~~, and~~ the various diffusion pathways~~, they~~ (Aguilar et al. 2017)~~, . t~~They could then tread long distances in surface or groundwater waters and the contamination can last for several decades (Ballesteros et al. 2014; Aravinna et al. 2017)~~.~~

The so-called ecological services could help to contain the ~~pesticidespesticide~~OACs 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, ~~pesticidespesticide~~OACs 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, s~~S~~ignificant 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).

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

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

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

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

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

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

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

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

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

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

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

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 bioconverse toxic substrates or products in a toxic milieu ~~process with toxic substrates or products or substrate conversion in a~~

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

Field Code Changed

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

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

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

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

Bioremediation is often monitored by following the concentration of targeted contaminants (Molina-Barahona et al. 2005). Numerous studies in recent years showed that traditional chemical analyses are insufficient for a full assessment of the contaminated site as they, for example, does not provide any information about the interaction of chemicals and does not consider the partition and the mobility of pollutants (Frische 2003; Molina-Barahona et al. 2005; Ma et al. 2005; Molnár et al. 2007). An integrated approach linking the various fields and levels of study involving contaminated sites has proven to be an efficient system of evaluating bioremediation effectiveness in contaminated sites (Chapman and Anderson 2005; Wernersson et al. 2015; Marziali et al. 2017). Consequently, to achieve the desired goals and implement a successful bioremediation program a close collaboration of microbiologists, chemists and engineers is requested by the chemical and biological complexity of the tasks (Van Gestel et al. 2001; Chakraborty et al. 2013).

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

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Signals that bioremediation is going on could be important to be monitored. Two important chemical compounds produced by microorganisms during their degradation activity are CO_2 and soluble phosphorus. Both increase distinctly in the soil treated with insecticides and inoculated with fungi (Boyle 1995; Abd El-Ghany and Masmali 2016). However, it must be taken into consideration that during and after a bioremediation process the disappearance of the parent compounds or evidence of the metabolic activity (e.g. CO_2 production) may not indicate detoxification. Beside the fact that the fate of the toxicants may be followed by chemical analyses, many reaction products resulting from a bioremediation process are not known and their potential toxicity, as well. The elimination of mother compounds does not necessarily result in toxicity removal, and evaluating the efficiency of the process is important to assess not only the removal of a specific compound, but also the potential ecotoxicity. In fact, biodegradation of pesticides can proceed partially or totally due to the molecular structure itself or unfavourable environmental or test conditions and the lack of 'acclimatized' microbial communities (De Henau 1997). In some instances, it has been shown that to an effective process of bioremediation corresponds to a decrease in the toxicity of the analysed matrix (Baud-Grasset et al. 1993; Dorn and Salanitro 2000). To acquire complete and useful information in an ecotoxicological assessment and to determine the effectiveness of bioremediation treatments, it is suggested to use a battery of tests (Keddy et al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of biological reference 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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As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various bioindicators either could indicate an incomplete decomposition of the substance or could result from the formation of intermediate products generated via the bioremediation process. For this reason, sometimes chronic tests are more appropriate in evaluating the toxicity caused by by-products (Lofrano et al. 2014). ~~In other cases, however, also the toxicity of the by-products is effectively removed (Lofrano et al. 2016).~~

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

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

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Organismal level

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* (Rönnpagel 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 cytoxicity 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).

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

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

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

Estrogenic and androgenic biomarkers. It has been well documented that several chemicals from agricultural, industrial, and household sources possess endocrine-disrupting properties, which provide a potential threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995; Jensen et al. 1995). A suggested mechanism is that environmental contaminants alter the normal functioning of the endocrine and reproductive system by mimicking or inhibiting endogenous hormone action, modulating the production of endogenous hormones, or altering hormone receptor populations (Sonnenschein and Soto 1998). Besides several pesticides exert estrogenic and antiandrogenic activities through interaction with estrogen and androgen receptors. The risk associated to OACs exposure has been known for decades: many pesticides, such as p,p´-dichlorodiphenyl trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978; Cummings 1997), β-benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan, toxaphene, and dieldrin (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) have been firstly signaled as estrogenic. Despite increased institutional awareness and more compelling legislation pressure, the most recent literature still reports the occurrence of pesticides in watercourses and in ~~passing through~~ the trophic chains, ~~ing~~ showing remarkable estrogenic or androgenic (Saillenfait et al. 2016; Brander et al. 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al. 2017; Marcoccia et al. 2017). Several bioassays have been developed and standardized in order to describe the estrogenic potency of OACs. Andersen et al. (2002) indicated that several currently used OACs, such as methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl, possess estrogenic activity on the basis of cell proliferation assay and transactivation assay using MCF-7 human breast cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism and antagonism to two human estrogen receptor (hER) subtypes, hERα and hERβ, and a human androgen receptor (hAR) by highly sensitive transactivation assays, using Chinese hamster ovary cells. The results demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic activities through ERs and/or AR. Although it appears that various pesticides exert hormonal effects at concentration orders of magnitude higher than that required for physiologic hormones, wide exposure to large numbers of OACs may have additive and synergistic effects. Bioassay with YES (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active compounds still present in the environment. By the the first papers about this subject (Purvis et al. 1991), much more sophisticated bioassays have been developed such as that proposed by Eldridge et al. (2007) in which a bioluminescence strain of *Saccharomyces cerevisiae* was genetically engineered to respond to androgenic chemicals.

Ecological level

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

Prospect

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

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Supplemental material: Table I

Table I. Fungal species list for biodegradation of pesticide pollutants

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

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

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

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

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

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

Supplemental material – Table I: References

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Supplemental material: Table II

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

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

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

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

Supplemental material – Table II: References

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