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Microcosm Experiments for Evaluating Natural Bioremediation of Contaminated Ecosystems

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Microorganisms play a key role not only in biogeochemical cycles and ecosystem energy flow but also in maintaining ecosystem environmental quality. In fact, natural microbial communities harbour an amazing physiological versatility and catabolic potential for the breakdown of an enormous number of organic molecules, including synthetic compounds, thanks to their great adaptability to different conditions. They are able to colonize contaminated sites and metabolize some recalcitrant xenobiotics, for example pesticides and pharmaceuticals. In this way, microbial communities represent an important key to understanding the impacts of environmental and anthropogenic factors on ecosystems.
Pollution may influence soil and water quality and productivity, but little is known on the effects on microbial communities, and consequent impacts on its functioning.
Miniaturized ecosystems (microcosms) provide the advantage of allowing researchers to investigate under controlled conditions the effects of selective pressures, such as xenobiotic occurrence, on natural microbial communities.
In this work we report the results of several microcosm studies, using natural soil and water samples, in which the environmental fate of several pesticides and pharmaceuticals in soil and water ecosystems has been evaluated. The natural attenuation capability of autochthonous microbial communities versus chemical contamination was evaluated comparing microbiologically active microcosms treated with each chemical with others previously sterilized. The degradation experiments were conducted under different natural conditions (e.g. temperature, light, humidity, etc.), including the occurrence of more stressors (e.g. co-presence of excess of nutrients or of other contaminants) in order to evaluate the fate and the effects of the chemicals in different environmental scenarios.
The disappearance time of 50 % of the parent compound applied (DT_{50}) was assessed for each chemical and condition. The overall results show the key role of microorganisms in the degradation of all chemicals studied and encourage the use of the microcosm approach for assessing more realistic environmental exposure scenarios and establishing the casual relationship between degradation and the role of microbial communities in chemical disappearance from the environment.

1. Introduction

Traditional parameters used for the Environmental Risk Assessment (ERA) of environmental contaminants are principally chemiodynamic and physico-chemical properties. Furthermore ecotoxicological effects on organisms in water and soil are assessed with standard acute and chronic ecotoxicologic tests on freshwater, marine and soil test organisms.
Due to their ubiquitous distribution in the environment, microbial communities are valuable indicators of the occurrence of disturbances due to exogenous physico-chemical stressors. The assessment of

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variations in microbial community structure is of basic importance in order to evaluate the impact of an environmental stressor. Complex microbial communities may serve as ideal and ecologically relevant toxicity indicators. In fact, a number of microbiologically driven processes have been proposed to evaluate the effects of xenobiotics on ecosystems. Moreover, biodegradation - and its corollary, persistence - is an important but poorly studied and understood fate process that is central to all mitigation strategies when chemicals are detected in the environment. Little is known about the natural variation in biodegradation potential of different environmental compartments or how bacterial presence diversity influences such variation.

Microorganisms, which have key roles in natural ecosystem functioning such as primary production, organic matter decomposition and nutrient cycling, carry out natural attenuation (biodegradation) by performing a homeostatic action with exogenous molecules (Barra Caracciolo et al, 2001; Barra Caracciolo et al., 2002; Tikilili et al., 2009). In fact, the ability of soil and water to recover from chemical contamination is primarily dependent on the presence of a microbial community with the ability to remove it. Furthermore the microbial community characteristics of an ecosystem can indicate changes in resource availability and the presence of pollution. The maintenance of many ecosystem services is therefore linked to that of bacterial diversity and functioning (Costanza et al., 1997).

We have recently been investigating how bacterial community composition and diversity (and the factors thought to impact it) affect xenobiotic biodegradation outcome (Barra Caracciolo et al., 2010a).

In this paper we present some degradation studies performed with several chemicals, and in particular pesticides and pharmaceuticals, selected because they are the most frequently found as pollutants in soil and water ecosystems, due to their widely use. We used laboratory microcosms to test the natural capability of soil or surface and groundwater samples to biodegrade selected compounds. In fact laboratory microcosms are ecosystem models in which a portion of the natural environment (soil or water) is circumscribed and studied (Benton et al., 2007; Drake et al., 2011). They contained natural biotic communities which were maintained, in our studies, under controlled environmental conditions (e.g. temperature, light, humidity, etc.) corresponding to natural ones. The overall results show the microbial community potential to degrade all chemicals studied and how the use of microcosms conducted under environmental conditions is a better approach for obtaining more realistic environmental exposure scenarios in the laboratory.

2. Material and methods

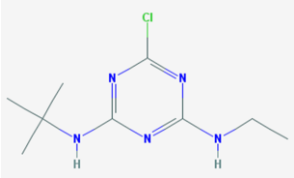
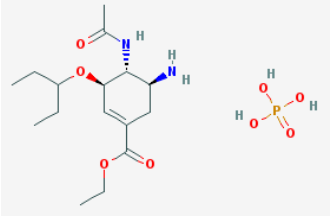
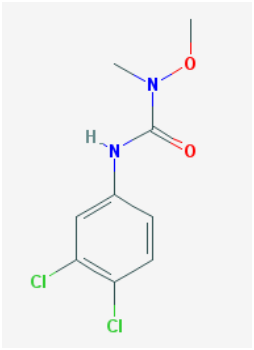
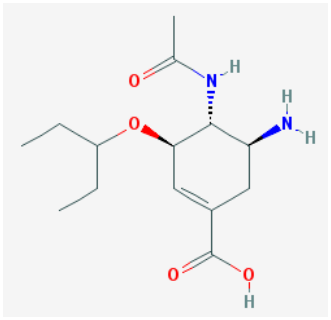
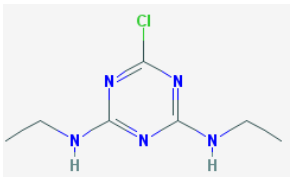
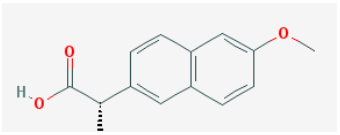
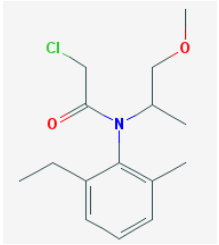
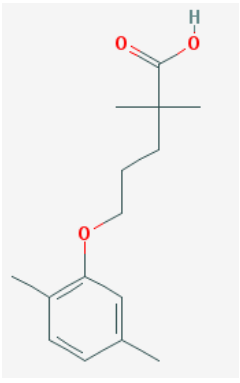
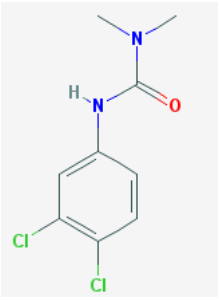
2.1 Microcosm set up for degradation studies

Degradation experiments were performed with microbiological active microcosms set up with natural soil or water samples collected from different sites and treated with the selected compounds listed in Table 1. The degradation experiments were performed according to the SETAC Guidelines (Lynch, 1995), in which we apply some modifications in order to evaluate the biotic vs abiotic degradation. In particular, additional sterile soil or water microcosms (120 ± 2 °C, 20 min) treated with the specific chemical at the same concentration of the microbiological active microcosms, were set up for evaluating the abiotic degradation. Moreover, microcosms were maintained at the temperature corresponding to the natural ones (21 °C for surficial soil or water, 15 °C in the case of groundwater) and in the dark to avoid any photochemical degradation. Finally, some soil degradation experiments were also performed in the presence of a co-contaminant (i.e. fertilizer urea) or organic amendments (i.e. wood amendments, 5 % w/w) in order to evaluate if the biodegradation could be influenced by their presence.

All the pesticides were applied to soil samples in water solutions in order to obtain final concentrations corresponding to agricultural rates. In the case of groundwater terbuthylazine experiments and in that involving pharmaceutical degradation in surface water, the chemicals were added to water samples at concentrations corresponding to Effective Concentrations (Singer et al., 2008; EFSA, 2011).

Concentrations of the chemicals were measured immediately after the treatment and subsequently until disappearance of at least 50 % of the initial concentration of the parent compound.

Table 1: List of the Chemicals (parent compounds or metabolite) applied to microcosms for the various degradation experiments.

Herbicides (common and IUPAC name)	Chemical structure	Pharmaceuticals (common and IUPAC name)	Chemical structure
Terbuthylazine 2-N-tert-butyl-6-chloro-4-N-ethyl-1,3,5-triazine-2,4-diamine		Oseltamivir phosphate (Tamiflu) (3R,4R, 5S)-4-acetamido-5-amino-3-pentan-3-yloxycyclohexene-1-carboxylate	
Linuron 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea		Oseltamivir carboxylate (active metabolite of Oseltamivir phosphate) (3R,4R,5S)-4-acetamido-5-amino-3-pentan-3-yloxycyclohexene-1-carboxylic acid	
Simazine 6-chloro-2-N,4-N-diethyl-1,3,5-triazine-2,4-diamine		Naproxen (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid	
Metolachlor 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(1-methoxypropan-2-yl)acetamide		Gemfibrozil 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid	
Diuron 3-(3,4-dichlorophenyl)-1,1-dimethylurea			

Solutions and instruments were sterilized and all steps were performed in a sterile cabinet. Further details on microcosm set up and specific experimental conditions for each different chemical are

described in previous works (Barra Caracciolo et al., 2005a,b,c; Accinelli et al., 2007; Grenni et al., 2009a; Barra Caracciolo et al., 2010b, c; Grenni et al., 2012a, b).

3. Results and discussion

The disappearance time of 50 % of the initial concentration of the parent compound applied (DT_{50}) was calculated for each different chemical and condition (presence, absence of microbial community, addition of amendments and so on) from the regression curve between the detected concentrations (C_t) and the sampling times (t).
The overall DT_{50s} of the degradation experiments in soil and water microcosms are reported in Table 2.

Table 2: Degradation (DT_{50}) of the chemicals in presence (Active Soil or Water) or absence (Sterile) of the microbial community.

Chemical	Experimental conditions*	Degradation	Ref.
	Soil microcosms		
Herbicides		DT_{50}	
Terbuthylazine	Active Soil	22 d	Barra Caracciolo et al., 2005a
	Sterile Soil	82 d	
	Active Soil + Urea	27 d	
Simazine	Active Soil	39 d	Barra Caracciolo et al., 2005b
	Sterile Soil	89 d	
	Active Soil + Urea	32 d	
Metolachlor	Active Soil	12 d	Barra Caracciolo et al., 2005c
	Sterile Soil	97 d	
Diuron	Active Soil	15 d	Barra Caracciolo et al., 2005c
	Sterile Soil	129 d	
Linuron	Active Soil	14 d	Grenni et al., 2009a
	Sterile Soil	158 d	
Linuron	Active Soil + Pine	43 d	Grenni et al., 2009a
	Active Soil + Oak	19 d	
Terbuthylazine	Active Soil	105 d	Grenni et al., 2012a
	Sterile Soil	257 d	
Terbuthylazine	Active Soil + Pine	161 d	Grenni et al., 2012a
	Active Soil + Oak	95 d	
	Water microcosms		
Herbicides			
Terbuthylazine	Active Ground Water, 15°C	151 d	Barra Caracciolo et al., 2010b
	Sterile Ground Water, 15°C	224 d	
Pharmaceuticals			
Oseltamivir carboxyalte	Active Surface Water	21 d	Barra Caracciolo et al., 2010c; Accinelli et al., 2007
	Sterile Surface Water	> 100 d	
	Active Surface Water+ Sediment	14 d	
Naproxen	Active Surface Water	22 d	Grenni et al., 2012b
	Sterile Surface Water	100 d	
Gemfibrozil	Active Surface Water	70 d	Grenni et al., 2012b
	Sterile Surface Water	> 200 d	

*The incubation temperature used was generally 21 °C or where specified 15 °C. In some experiments active soils were also amended with a fertilizer (e.g. urea) or wood residues (oak or pine sawdust).

The presence of the microbial community (Active Soil or Water) promoted the degradation of all compounds studied, as it is shown comparing the DT_{50} values with those found in the sterile conditions. In all sterile microcosms the chemicals did not decrease at all or only slightly, not more than 10 - 20 %

of the initial concentrations and the DT_{50} values reported in the Table 2 are in fact theoretical ones calculated from the correlations between residual concentrations versus time.
In these study cases, the use of autochthonous bacterial communities collected from different sampling sites made it possible to assess both the occurrence of a site-specific natural bioremediation capacity versus a specific contaminant and the DT_{50} times of the latter in relation to natural conditions (Burrows and Edwards, 2002). Moreover, in the case of terbuthylazine, simazine and linuron the degradation was also evaluated in microcosms in which an additional carbon source (e.g. urea or pine and oak sawdust) was applied for different purposes. The herbicide (terbuthylazine or simazine) and fertilizer urea were simultaneously applied to some soil microcosms in order to evaluate the degradation of the herbicide in co-presence of the fertilizer (Barra Caracciolo et al., 2005a, b). In the case of the experiments with pine and oak amendments, they were applied to soil in order to prevent the linuron or terbuthylazine mobility (Grenni et al., 2009a). The results obtained from these latter degradation conditions were useful for practical implications for the real-life assessment of the environmental fate of herbicides in agricultural areas.
The evaluation of the presence of autochthonous bacterial populations with a natural remediation capacity is a prerequisite for starting microbiological analyses more specifically aimed at characterize the bacterial community and to select and to isolate specific bacterial populations which can be used for remediation purposes (Grenni et al., 2009b, Barra Caracciolo et al., 2010a). In the case of triazine experiments we in fact identified some bacterial strains able to degrade terbuthylazine and simazine in soil and water and their application for bioremediation purposes is also proposed (Barra Caracciolo et al., 2010a; Grenni et al., 2009b).

4. Conclusions

The overall results presented here show the key role of microorganisms in the degradation of all the chemicals studied. The presence of a natural microbial community is a necessary prerequisite for an effective response to various chemicals that can contaminate an ecosystem. Recovery from contamination is only possible if the quantity and toxicity of the molecules do not hamper or inhibit microbial activity. The use of microcosms in which bacterial communities are collected from natural soil or water make it possible to evaluate the natural microbial potential to degrade chemicals both in soil and water. Knowledge of the presence of autochthonous bacterial populations with a natural remediation capacity can be useful both for the development of site-specific management strategies for natural attenuation of contaminated soils and waters and for a possible application of specific identified strains for bioremediation purposes.
Laboratory microcosms are a good compromise between field experiments, which are often hampered by a high environmental variability and a high cost, and standard laboratory tests of which the results are rarely representative of the real world, because the experimental conditions and the organism tested are not site-specific. Finally, as laboratory microcosms are relatively small, it is possible to produce many replicates and to vary experimental conditions (e.g. temperature) once at a time in order to more firmly establish the casual relationship between a toxicant and its effects on microbial communities under different abiotic conditions. The suggestion we therefore make is to encourage microcosm studies conducted under different natural conditions in order to assess a more realistic environmental fate for the chemicals.

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