MULTIDISCIPLINARY APPROACH FOR THE CHARACTERIZATION OF SITES CONTAMINATED BY HYDROCARBONS

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Introduction

Thousands of chemicals are daily employed in our society and many of them can be detected in all the compartments of the environment Contaminants of particular interest, within this project, are the refined products of crude oil widely used as fuels in cars, aircrafts and ships; for heating and electricity generation, as lubricants in machinery; as asphalt for road and in the production of plastics. Refined products contain complex mixtures of hydrocarbons and non-hydrocarbons and their chemical, physical (i.e. API Gravity and sulfur content), and compositional (such as ratio pristane/phytane and percentage of benzothiophenes) properties vary with the different geographical origin or refinering processes giving them a peculiar chromatogram (or fingerprint) (Kaplan 1996).
Once in the environment these contaminants are subjected to several weathering processes that have as consequence the alteration of their composition and thus of their fingerprints (Murphy and Morrison, 2002). For this reason sometimes is hard to characterize contaminated sites avoiding uncertainties and then identify the real responsible of a contamination. In this respect, there are two analytical methodologies, respectively called "*compositional fingerprinting*" (based on the use of a gas chromatography – mass spectrometry_GC/MS) and "*isotopical fingerprinting* (based on the use of a gas chromatography – isotope ratio mass spectrometry_GC/IRMS), that allow the identification of the source of contamination among different possible sources and the determination of degradative effects on it (Wang, 2003). The first methodology, employed in this study, pursues this aims by the analysis of the composition of contaminants evaluating the presence and the abundance of individual compounds, in the first case.
In Italy there are a lot of sites contaminated by hydrocarbons and most of them are located along the coastline. Trying to apply the compositional fingerprinting to an oil storage facility in the south of Italy, we gained good results by the identification of a diesel contamination in the internal area of the site, and inconclusive results in the area close to the sea, in correspondence of a salt wedge. On the basis of these results, we have decided to investigate the possible influence that might have the salinity in weathering phenomena, and especially in the biological process of biodegradation that can occur naturally but not in all the environmental conditions, in which microorganisms metabolize organic pollutants to inorganic material such as carbon dioxide methane, water and inorganic salts (Leahy and Chewll, 1990; Boopathy 2000). In addition, the different compounds contained in the refined products are more or less susceptible to biodegradation due to their chemical structures, leading to the possibility to evaluate compositional changes of the whole contaminant mixture, during the time starting from the n-alkanes (more susceptible) to the terpanes (less susceptible).
For all the previous reasons, integration between geochemical data (hydrogeological study, presence/absence contamination and its rate of biodegradation) and microbial data (structure and function of microbial communities exhibit significant spatial and temporal variability linked to the presence/absence of contaminants) is essential. Thus the main aim of the laboratory study carried on has been the definition of a valuable survey instrument, built by coupling compositional and microbial fingerprinting and useful in resolving uncertainties related to the usual procedures applied to characterize the polluted sites. The *microbial fingerprinting* is the study of the microbial population of the site and can be used to determine how the community composition varies in relation with the contaminants biodegradation occurring.
During the experiment were studied both the biodegradation of gasoline and diesel, once released in the environment, and the physical-chemical parameters that most affect the action

of microorganisms in this process. In this sense, we have focused on some parameters that have been already recognized in the literature as factors that largely effect/limit natural attenuation (i.e. granulometry of the soil, organic matter and concentration of the contaminant) and two other parameters (i.e. presence of mixtures of contamination and salinity).

Material and methods

The environmental conditions investigated were reproduced in aerobic soil/water microcosms prepared in sealed serum bottles and sampled at four different times (0,10,70 and140 days). For each parameters a different number of levels was investigated, specifically two levels for the granulometry (50-50% gravel-sand; 50-50% sand-silt), for the salinity (0.45g/L and 4g/L concentration of sodium chloride), for the organic matter (f_{oc}=0.0002 and f_{oc}=0.002) and for the concentration of pollutants (100ppm and 1000ppm). The parameter mixture was investigated at three levels (100% diesel fuel; 100% gasoline fuel; 50% gasoline+50% diesel). To get the covering of all the combination of variables (parameters) it was employed a fractionated experimental design, reaching a total number of 96 microcosms (24 for each sampling time). At each sampling time, both water and soil were sampled. The samples from the microcosms containing gasoline were analyzed at the GC-MS to verify the presence of its typical volatile organic compounds (VOCs) and to determine their concentrations. With the same instrument but with a different analytical protocol, samples from microcosms containing diesel were analyzed to determine the presence of semivolatile and non-volatile compounds characteristic of this refinery product. A series of ratios, indicative of the state of degradation of the contaminants, such as B+T/E+X (B=benzene, T=toluene, E=ethylbenzene, X=xylenes) for gasoline and C_{17}/pristane, C_{18}/phytane for diesel, were determined (Kaplan, 1996). Soil samples from the all microcosms were also used to determine the composition of the microbial communities, by T-RFLP (Terminal Restriction Fragment Length Polymorphism) analyses.

Results

The data obtained showed a trend in biodegradation of both the contaminants and, after elaboration through the program "R", indicated a good correlation between the parameters statistically significant in both the methods used (compositional and microbial fingerprinting). In this study, the parameters mainly significant were the contaminant concentrations, the time of sampling and the granulometry, the latter only in microcosms contaminated by diesel. Surprisingly, the salinity was not significant but we suspect that this result is due to the concentrations used and we are planning to further investigate this parameter.
Moreover, the result obtained on a detailed analysis of the T-RFLP profiles showed trend in the communities that might be useful information for the construction of a survey instrument coupling the two methods. In fact, the profiles change with the changing in contaminant concentration and in particular, after 70 days, there is an increase of specific strains in microcosms containing gasoline. This increase corresponds at the major decrease in the concentration of the BTEX.

Discussion and conclusion

This work showed that, as supposed, there is good correlation between the chemical and the microbiological data allowing to the possibility to build a valid tool to characterize contaminated sites. . Moreover, the data can be further strengthened using different molecular techniques that can allow identifying the strains of microorganisms and then the metabolic capability of the communities present in the site. Then, using this multidisciplinary approach for each site it is possible to determine the degradative pathway undergoing and get valuable data to better characterize the site and then to choose the most efficient remediation strategy, in order to reach the goal of the remediation.

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