2

BIOREMEDIATION, HYDROCARBON DEPLETION AND MICROBIAL GENETIC DIVERSITY OF ANTARCTIC OIL-POLLUTED SOIL

http://dx.doi.org/10.4322/apa.2014.051

Alexandre Soares Rosado[1,*], Juliano de Carvalho Cury[2,*], Raquel Silva Peixoto[1], Hugo Emiliano de Jesus[1], Carlos Ernesto Gonçalves Reynaud Schaefer[3], Marcia C. Bícego[4], Diogo A. Jurelevicius[5], Lucy Seldin[5], Paulo Negrais Seabra[6], Charles W. Greer[7]

[1]Laboratório de Ecologia Microbiana Molecular – LEMM, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, RJ, Brazil
[2]Universidade Federal de São João Del Rei – UFSJ, Sete Lagoas, MG, Brazil
[3]Universidade Federal de Viçosa – UFV, Viçosa, MG, Brazil
[4]Instituto Oceanográfico, Universidade de São Paulo – USP, São Paulo, SP, Brazil
[5]Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, RJ, Brazil
[6]Setor de Meio Ambiente e Biotecnologia, Superintendência de Pesquisa e Engenharia Básica do Abastecimento, Centro de Pesquisas da Petrobras, Rio de Janeiro, RJ, Brazil
[7]Biotechnology Research Institute, Canada

*e-mail: arosado@globo.com; jccury@hotmail.com

Abstract: *Natural environments have been affected by oil spills around the world for decades. In some cases, the attempt to cleanup can be made using physical and chemical methods. However, for the Antarctic environments this is not so simple. Displacement of the machinery necessary for the application of physical methods would be very expensive whereas the application of chemical methods would be dangerous considering the risks of additional environmental impacts. Oil contamination of soils of EACF was caused by a tank rupture in the mid eighties in addition to little spills and intense use of motor vehicles. In some sites the presence of oil can be visually detected, which leads us to believe that a monitored natural attenuation is not feasible. Bioremediation techniques are relatively more cost-effective and benign. These techniques are based on the ability of some microorganisms (especially some bacteria) to use the petroleum hydrocarbons as energy source. However, before any implementation of bioremediation action, it is important to perform studies for the chemical and biological characterization of the contaminated soil. We are performing physical-chemical and microbiological studies of soil samples of Brazilian Antarctic Station contaminated with diesel. The results show an absence of Nitrogen in soil, the presence of high content of petroleum hydrocarbons and a depletion effect of the microbial diversity in polluted soil.*

Keywords: Antarctic, oil, bioremediation, microbiology

Introduction

Natural environments have been affected by oil spills around the world for decades. In some cases, the attempt to cleanup can be made using physical and chemical methods. However, for the Antarctic environments this is not so simple. Displacement of the machinery necessary for the application of physical methods would be very expensive whereas the application of chemical methods would be dangerous considering the risks of additional environmental impacts. Oil contamination of soils of EACF was caused by a tank rupture in the mid eighties in addition to little spills and intense use of motor vehicles. In some sites the presence of oil can be visually detected, which leads us to believe that a monitored natural attenuation is not feasible.

Bioremediation techniques are relatively more cost-effective and benign. These techniques are based on the ability of some microorganisms (especially some bacteria) to use the petroleum hydrocarbons as energy source. However, in some cases, environmental factors can cause the recalcitrance of the pollutant. The more frequent cause of recalcitrance is the depletion of nutrients (especially N and P) due to input of large quantities of carbon sources (petroleum hydrocarbons). An alternative to overcome this problem is the addition of fertilizers (e.g. N-P-K, MAP, DAP). This technique is known as biostimulation. However, some precautions must be taken. For the biostimulation the most important aspect is to avoid the excess of fertilizer, which could cause side effects like eutrophication. Therefore, it is important to perform studies for the chemical and biological characterization of the contaminated soil.

Temperature is a critical factor for bioremediation success. In Antarctic soils, low temperatures can decrease the rate of biodegradation even when nutrients are available in satisfactory concentrations. An alternative to overcome this difficulty is to increase the number of cells of a consortium of degraders in artificial media under conditions of optimum growth before the introduction in nutrient-amended polluted soils. This technique is known as bioaugmentation. Theoretically, bioaugmentation is a more promising technique than biostimulation. However, the effectiveness of bioaugmentation is variable due to the low rates of survival and degrading capability of introduced microorganisms. Furthermore, in Antarctic soils the implementation of this technique is not feasible since the introduction of alien species should be avoided. An alternative to overcome these difficulties is to introduce indigenous microorganisms capable of degrading oil to the contaminated site.

Methodology

We collected soil samples in the diesel-polluted and diesel-unpolluted areas of the EACF (Figure 1). The oil-contaminated treatment of the microcosm experiment consists in a mixture (1:1:1, w) of soil of the samples 1, 2 and 3 (collected in oil-polluted area), whereas the oil-uncontaminated treatment consists in a mixture (1:1, w) of soil of the samples 4 and 5 (collected in oil-unpolluted area). For the bioestimulation microcosm experiment we applied different doses of N (as MAP) in the soil and incubate during 60 days. For the bioaugmentation experiments, ten bacterial strains that grow in solid media with the diesel of EACF as the sole carbon source (Figure 2) where isolated and are being used for the *in situ* bioaugmentation experiments (Figure 3).

Molecular approaches are being used to characterize microbial structures of the contaminated soil before and during the experiments. PCR-DGGE (denaturing gradient gel electrophoresis) technique can be used to determine changes of microbial structures whereas cloning and

Figure 1. Soil sampling points in the area of EACF. 1-3) diesel-polluted area; 4,5) diesel-unpolluted area.

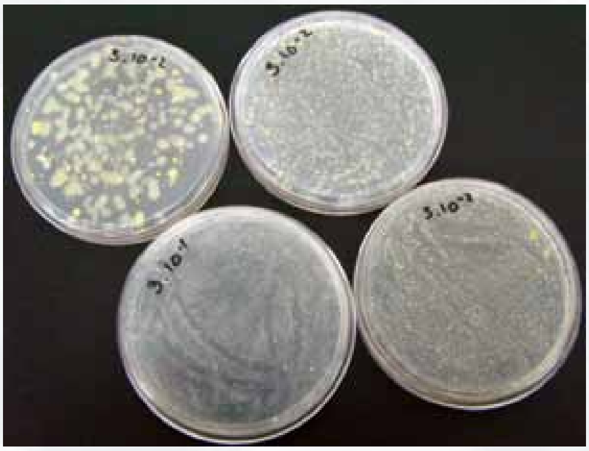

Figure 2. Bacteria growing in culture media containing the diesel of the EACF as the sole Carbon source.

Figure 3. *In situ* bioaugmentation experiment.

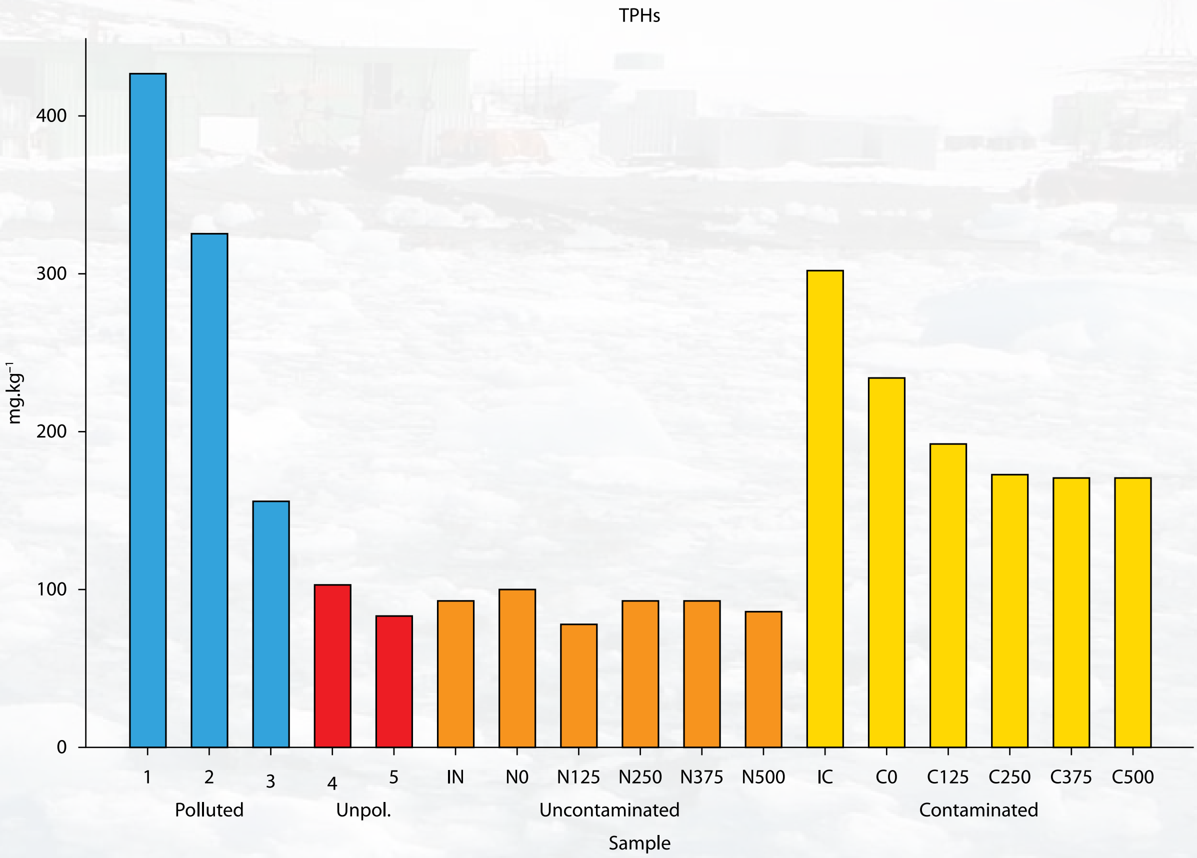

Figure 4. TPH content of the original samples (1 to 5) and microcosm experiment soil. The letter I represents the initial contamination. Numbers after the U (uncontaminated) and C (contaminated) soil treatments indicate the doses of N applied (mg.kg^{-1}).

sequencing techniques can be used to characterize the taxonomic and functional diversity of soil under different treatments based on marker genes, in addition to the characterization of the obtained isolates.

Results

As expected, analyses revealed a higher content of TPHs in the soil of the diesel-contaminated area (Figure 4). Whereas the chemical analyses showed the absence of Nitrogen in

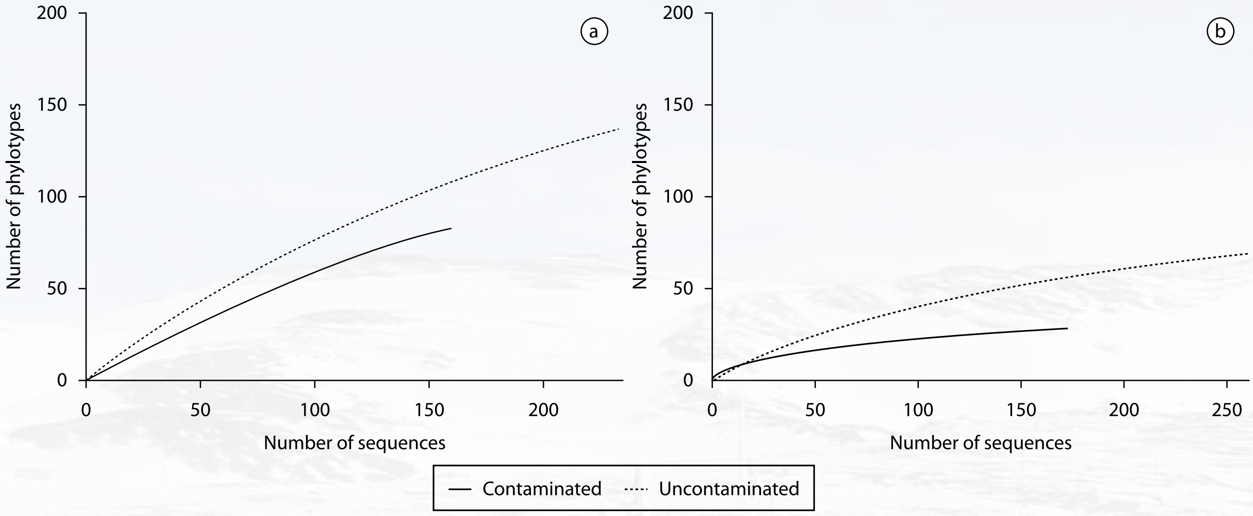

Figure 5. Diversity of bacteria (a) and microeukaryotes (b) in diesel-contaminated and diesel-uncontaminated soil of the EACF.

the soil, it was performed a microcosm experiment to test the application of different doses of N. As show in Figure 4, the application of N until a concentration of 250 mg.kg^{-1} of N caused a reduction of the TPH content after 60 days of incubation. We detected a less microbial diversity of microorganisms in the diesel-contaminated soil, indicating the biological influence of the contaminant (Figure 5).

Conclusions

The results of the studies performed at the present show an absence of Nitrogen, the presence of high content of petroleum hydrocarbons, and a depletion effect of the microbial diversity in polluted soil. At the end of the studies we hope to determine a procedure based in biostimulation and bioaugmentation that may become available and that can be immediately applied after oil spills in the Antarctic soils.

Acknowledgements

We thank to the National Institute of Science and Technology of Antarctic Environmental Research (INCT-APA, CNPq process nº 574018/2008-5 and FAPERJ process nº E-16/170.023/2008) and to Brazilian navy for the support.