SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

"Microbial Diversity and Metal Fluxes in Contaminated North Sea Sediments"

«MICROMET»

D. C. Gillan, Ph. Dubois, K. Sabbe, A. Pede, W. Baeyens,
M. Leermakers, Y. Gao, G. Billon, J.-C. Fischer

ENERGY

TRANSPORT AND MOBILITY

AGRO-FOOD

HEALTH AND ENVIRONMENT

CLIMATE

BIODIVERSITY

ATMOSPHERE AND TERRESTRIAL AND MARINE ECOSYSTEMS

TRANSVERSAL ACTIONS

.be

SCIENCE FOR A SUSTAINABLE DEVELOPMENT
(SSD)

North Sea

FINAL REPORT PHASE I
SUMMARY

MICROBIAL DIVERSITY AND METAL FLUXES IN CONTAMINATED NORTH SEA SEDIMENTS

"MICROMET"

SD/NS/04A

Promotors

David C. Gillan & Philippe Dubois
Université Libre de Bruxelles (ULB)
Laboratoire de Biologie marine, CP160/15
50 av Roosevelt
1050 Bruxelles

Koen Sabbe
Ghent University
Protistology & Aquatic Ecology
Dept. Biology, Krijgslaan 281, S8
9000 Ghent

Willy Baeyens & Martine Leermakers
Vrije Universiteit van Brussel (VUB)
Department of Analytical and Environmental Chemistry (ANCH), ,
Pleinlaan 2
1050 Bruxelles

Jean-Claude Fischer
Université des Sciences et Technologies de Lille, USTL,
Géosystèmes, UMR 8157,
Bâtiment C8, 59655 Villeneuve d'Ascq Cedex
France.

Authors

David C. Gillan (ULB)
Koen Sabbe & Annelies Pede (UGent)
Willy Baeyens & Yue Gao (VUB)
Gabriel Billon & Ludovic Lesven (USTL)

BELGIAN SCIENCE POLICY

Rue de la Science 8
Wetenschapsstraat 8
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be

Contact person: David Cox
+32 (0)2 238 34 03

David C. Gillan, Philppe Dubois, Koen Sabbe, Annelies Pede, Willy Baeyens, Martine Leermakers, Yue Gao, Gabriel Billon, Jean-Claude Fischer. ***Microbial Diversity and Metal Fluxes in Contaminated North Sea Sediments "MICROMET".*** Final Report Phase I Summary. Brussels : Belgian Science Policy 2009 – 5 p. (Research Programme Science for a Sustainable Development)

As many toxic metals are complexed by organic matter in contaminated marine sediments, metals might be released in the water column when organic matter is degraded by microorganisms. This might affect microbial biodiversity and lead to metal bioaccumulation in higher trophic levels. To date, microbial communities in marine sediments of the Belgian Continental Plate (BCP) remain poorly studied, particularly in heavy metal contaminated zones. The MICROMET project fits within two priority research domains of the Science for a Sustainable Development programme (Biodiversity and Marine Ecosystems) and is directly connected with the aims of the Water Framework Directive (2000/60/EC).

The aim of the MICROMET project is to understand the link between microbial activity and metal fluxes in marine sediments. To this end, the whole microbial communitiy will be studied in the BCP area using an interdisciplinary approach in which geochemical and microbiological methods will be closely integrated. The research is subdivided in three Work Packages (WP 1-3). The aim of WP 1 is to determine the metallic contaminants and the microbial diversity in sediments. The aim of WP 2 is to assess the importance of microorganisms in the leaching of metal contaminants from the sediments into the water column. The aim of WP 3 will be to run numerical models with the data collected during the project. This will allow predictions and lead to a better understanding of the benthic ecosystems.

Phase I of the MICROMET project was entirely devoted to WP 1. During phase I, nine sampling stations on the BCP have been examined during the first year (2007). These stations were stations 120, 130, 140, 230, 330, 435, 700, DCG and ZG03 (for the coordinates, see the MICROMET website : http://ulb.ac.be/sciences/micromet). Sediments have been sampled before (February) and after (July) the major phytoplankton blooms. Microbial diversity has been determined using the DGGE approach. Biomass was determined using DAPI counts. Pure cultures of microorganisms have been characterized and geochemical properties of the sediment have been determined (Eh, pH, DGT-sulfides, AVS & CRS, granulometry, simultaneously extracted metals – SEM). Pore-water metal concentrations have been determined *in situ* using the DET technique (Diffusive Equilibrium in Thin films) and the DGT technique (Diffusive Gradients in Thin films). In the second year (2008), sediments of two selected, silty stations (130 and 700) have been sampled monthly to take different organic matter sedimentation patterns into account. In addition to DGGE, fragments of the SSU rRNA gene have been sequenced in order to have a better understanding of the microbial diversity and physiologies in the sediments.

Results have first indicated that the BCP sampling stations followed in the study may be classified in three groups: sandy stations with a mean grain size (MGS) of 400 µm (group I: DCG, 330 and 435), sandy stations with a MGS of 200 µm (group II: 120, 230 [except 0-1 cm], ZG03), and muddy stations with a MGS of 12.5 µm (group III: 130, 140 and 700).

In group I sediments oxygen penetration depth is important, probably due to the low quantity and bioavailability of organic matter at the water-sediment interface. Oxygen and sulphates are not significantly reduced and only small quantities of CRS were detected. Low amounts of simultaneously extracted metals (SEM) have been measured.

In group III sediments, oxygen is completely consumed within the first mm and the Eh values drop to about -200 mV within the first cm. Production of dissolved and solid sulfides confirm sulfate-reducing activities, mostly in the first cm. Concentrations of Pb, Cu and Zn in SEM are generally more than 10 x lower compared to the AVS, which means that sulfides might act as a sink for the metals present. However, as demonstrated by the DET/DGT analyses, metallic pollutants are nevertheless present in the pore waters of these stations and may therefore be released in the water column. In group II sediments, oxygen is consumed in the first cm and Eh values decrease more slowly than in the muddy stations.

The February 2007 data show that eubacterial taxon richness increased from the coast (group III sediments) to the open sea (group I sediments), when DGGE results were expressed per unit of biomass. Even when normalization was not performed it was observed that the raw DGGE diversity values were never low in offshore stations DCG and 435 (always >16 DGGE bands) and that the lowest values (6 DGGE bands) were always observed in coastal stations. In addition, no archaebacterial 16S rRNA sequences could be obtained by PCR in coastal stations; on the contrary, many archaebacterial sequences were obtained offshore, in sediments of stations DCG and 435. Reduced diversity in sediments from coastal areas on the BCP was also observed in other studies for other groups, such as nematods and harpacticoid copepods (e.g., Vincx 1990). It should be noted that bacterial diversity values may nevertheless be elevated in coastal stations (e.g., the sandy station 120 in February 2007 and the metal contaminated station 130 in July 2007). This means that the situation is much more complex than initially thought and that the observed trend may not be valid for all sediment types and/or all periods of the year.

Although phase II of the project is absolutely necessary to conclude, the reduction of biodiversity observed in February 2007 might be related to elevated concentrations of metallic pollutants and metalloids in the pore waters, particularly arsenic, as demonstrated by DET/DGT analyses. Multivariate analyses of the eukaryotic DGGE data of 2007 also show pronounced changes in community (phylotype) composition with sediment type (group I-III). Although the eukaryotic data was not normalized to biomass, there appears to be no strong shift in diversity between the group I and group III sediments.

From the 2007 and 2008 data we can conclude that microbial biodiversity, as measured with DGGE, is not a variable that can be easily related to the environmental variables considered in this study. This might be due to the long exposition time of the microbial communities to metals in the coastal area. The present-day communities are probably adapted to the elevated metal concentrations in that zone. This may explain the high biodiversity values that were observed in contaminated sediments of station 130 in July 2007. Such a situation was also observed in the bacterial communities living in marine sediments of the Sørfjord in Norway, exposed for more than 80 years to high levels of Cd and Zn (Gillan et al. 2005).

On the contrary, bacterial biomass is a variable that displayed elevated and significant correlations to some environmental variables, particularly to dissolved Mn, Fe and As. This is not surprising as these metals may serve as electron donors or acceptors depending on their oxidation state. Bacterial biomass was also significantly correlated to chlorophyll a levels in sediments. This may be explained by the proliferation of bacteria on decaying phototrophic micro-eukaryotes such as diatoms and *Phaeocystis*.

Eubacterial rRNA sequencing (February 2007 samples) has shown that 5 to 10 major eubacterial groups are present in the BCP sediments examined (DCG, 435, 130, 700). Three major groups were present in all the four stations examined (γ-Proteobacteria, δ-Proteobacteria and CFB bacteria). Acidobacteria represent 2.6 - 14.6% of the clones in most of the stations. For micro-eukaryotes, 18S rDNA based DGGE analyses of February 2007 samples revealed a surprisingly high diversity of microbial eukaryotes, mainly comprising stramenopiles (diatoms), as yet unidentified (or ambiguously identified) marine eukaryotes and Fungi, but also protozoa and microalgae belonging to other groups. The DGGE procedure also picked up many metazoan sequences. DGGE and clone library analyses on the 2008 samples confirmed the 2007 results and in addition, due to the use of group-specific primers sets (for Cercozoa and ciliates) for the clone libraries, allowed a more detailed identification of the protozoan communities present.

With the DET/DGT approaches, high resolution profiles of trace metals in the sediments were obtained. Trace elements presented a variable geochemical behavior in the sediments, confirming that remobilization is occurring at specific depths. Seasonal variations of trace elements (Mn, Fe, As) have been observed during the cruises in 2007 and 2008. Although variations in the oxygen concentration and redox potential may explain most of the patterns obtained, the importance of microorganisms in this seasonal phenomenon has still to be determined (phase II). There is apparently no depletion of trace elements in the sediment porewaters at station 130 and 700. The flux calculations based on DGT profiles show that elements such as Mn, Fe, Co, As and Ni will diffuse out of the sediment into the overlying water column, at least for station 130. Flux calculations based on the DGT piston experiments confirm that metallic toxicants may reach the SWI and be released in the seawater. This might be detrimental for the benthic ecosystem. Other metallic toxicants such as Cu, Zn, and Cd will diffuse towards the sediments in station 130.