First insights into the bacterial communities in contaminated sediments of the Deûle River (Northern France)

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Introduction: The worldwide industrialization has led to the introduction of a huge quantity of trace metals into the environment. For instance smelters and the mining industry like METALEUROP in Northern France. This lead and zinc production plant is located along the Deûle River, one of the most polluted sites in the region. The plant was closed in 2003 but metal concentrations still remain higher than background values [1]. Although previous studies of the site have focused on metal contents and mobility [2,3], none have investigated the microorganisms which may affect metal mobility [4,5]. The aim of the present research is to assess the bacterial diversity in such a metal contaminated environment.

Methods: Sediments of the 0-1 cm interface and the 2-4 cm layer were collected in November 2009 in front of the MetalEurop site (N 50°25'21,98'' - E 03°03'34,08''). DNA was extracted from sediments and purified using QIAquick columns. For DGGE, a fragment of 233 pb was amplified using bacterial primers [4]. For cloning, the complete 16S rRNA gene was amplified with the TOPO TA cloning kit (Invitrogen). The sequencing was performed on an ABI 3730 genetic analyzer. Metals in sediments were extracted using i) a total extraction procedure, ii) a sequential extraction procedure and iii) a partial 1M HCl extraction experiment to obtain Simultaneously Extractable Metals (SEM). Metals were quantified using inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). Acid volatile sulfides (AVS) were measured to calculate an index of toxicity (IT) using the ratio SEM/AVS.

Results: Our preliminary DGGE analyses reveal the presence of a total of 15 band positions. The maximum of DGGE bands in one sample was 12 and the minimum was 9. The band profiles were different between oxic and anoxic sediments. Two 16S rRNA gene libraries were constructed. Up to date, 24 clones for each layer were partially sequenced (Table 1). The clones were sequenced using the primer GM1F (518-534). Cytophaga-Flexibacter-Bacteroides (CFB) were mainly Flavobacteriaceae and Chitinophagaceae. The majority of •-Proteobacteria were Methylococcaceae. Although further analyses are needed it seems that the bacterial diversity is higher in anoxic sediments.

Table 1. Number of complete 16S rRNA sequence obtained in the oxic and anoxic sediments.

	oxic		anoxic	
	count	%	count	%
CFB bacteria	6	25,0	9	37,5
γ-proteobacteria	9	37,5	4	16,7
δ-proteobacteria	1	4,2	3	12,5
β-proteobacteria	2	8,3	1	4,2
α-proteobacteria	1	4,2	0	0,0
Chlorobi	0	0,0	1	4,2
Firmicutes	0	0,0	1	4,2
Unidentified	5	20,8	5	20,8
Total	**24**	**100**	**24**	**100**

Metal analyses did not show any significant differences between oxic and anoxic sediments. Total metal contents for Cd, Pb and Zn were of major concern with a concentration of respectively 58 ± 3 $mg.kg^{-1}$, 1900 ± 300 $mg.kg^{-1}$ and 4400 ± 70 $mg.kg^{-1}$ per kilogram of wet sediments at the interface with water. The toxicity index reached 0.65 for oxic sediments and 0.97 for anoxic sediments, indicating that sulfides are in excess in comparison to metals. This is consistent with the fact that metals were released, at least partially, as PbS and ZnS in the environment by MetalEurop.

Discussion: This ongoing study has evaluated for the first time the biodiversity of the bacterial communities in the Deûle River using a cloning-sequencing approach. The preliminary results have revealed an elevated bacterial diversity despite the high metal concentrations, probably because sulfides were able to scavenge efficiently trace metals as "non available" precipitates. Such an observation was reported before for other sedimentary environments [5].

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