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ScienceDirect
Environmental Pollution 148 (2007) 759–769
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Microbial processes as key drivers for metal (im)mobilization along a redox gradient in the saturated zone

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Received 27 January 2007; accepted 31 January 2007

Microbial activity can tremendously impact the behaviour and fate of metals if dissolved organic carbon (DOC) is available.

Abstract

Two sites representing different aquifer types, i.e., Dommel (sandy) and Flémalle (gravelly loam) along the Meuse River, have been selected to conduct microcosm experiments. Various conditions ranging from aerobic over nitrate- to sulphate reducing were imposed. For the sandy aquifer, nitrate reducing conditions predominated, which specifically in the presence of a carbon source led to pH increases and enhanced Zn removal. For the calcareous gravelly loam, sulphate reduction was dominant resulting in immobilization of both Zn and Cd. For both aquifer types and almost all redox conditions, higher arsenic concentrations were measured in the groundwater. Analyses of different specific microbial populations by polymerase chain reaction (PCR) revealed the dominance of denitrifiers for the Dommel site, while sulfate reducing bacteria (SRB) were the prevailing population for all redox conditions in the Flémalle samples.

Keywords: Redox gradient; Heavy metals; Microbial impact; Sulphate reduction; Denitrification

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1. Introduction

Large amounts of inorganic contaminants such as heavy metals have accumulated during the last century in areas near rivers such as in the Meuse River system due to industrial activities as well as due to flooding and subsequent deposition of the contaminated sediments (Schroder et al., 2005). The Meuse is a medium-sized river with a length of 905 km originating in the North of France and with a total drainage basin of 33 000 km^2. Near the city of Liège, the river flows through an industrialized area including old coal factories where both organic and metal pollutions of the river embankment have been observed. More downstream, at the Belgian–Dutch border, the Dommel – a tributary of the Meuse – flows through a zinc producing industrial area where these activities during the last 100 years have led to atmospheric deposition of zinc and cadmium on agricultural land and nature reserves. Both sites have been selected in this study since their heavy metal contaminations are expected to eventually reach the Meuse River thus potentially having a long-term impact on the surface water quality.

Predicting and controlling the fate of these contaminants in the flowpath of the soil surface towards the groundwater that discharges into the surface water is one of the challenge for environmental research. The chemical stability and solubility of metal contaminants in soils and aquifer sediments and, hence, mobility are controlled by a complex series of biogeochemical processes depending on variables such as pH, redox, clay content, organic matter and terminal electron acceptors (TEA) (Harrington et al., 1998). While models allow to predict

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doi:10.1016/j.envpol.2007.01.036

the thermodynamic equilibria that govern the fate of metals in groundwater/aquifer systems, the major changes that microbial organisms have on them in the presence of TEA are difficult to predict (Schroder et al., 2005). However, it is generally known that microbial activity in terms of carbon turnover results in increased alkalinity and thus changes the pH. This pH effect is expected to have a direct impact on metal behaviour. Moreover, if sulphate is present, reduction of this anion typically produces sulphides which are excellent scavengers for metal cations (Altmann and Bourg, 1997). The resulting metal sulphides have very small solubility products and form highly stable precipitates if reduced conditions are maintained.

The aim of this work was to gain knowledge on the impact of microbiology on the fate of metals in a soil system along a redox gradient towards surface water by performing a series of batch experiments. We studied the impact on metal release or precipitation/sorption due to the presence of different e-acceptor available for the microorganisms. More specifically, the work focused on the presence of three different TEA: oxygen, nitrate and sulphate. The introduction of oxygen in a saturated soil becomes important when considering flooding and a fluctuating groundwater table, which could be among other features a consequence of changing climate and global warming. Nitrate infiltration in the saturated zone is a constant threat when looking at agricultural practices such as fertilization. Finally, sulphate can be introduced into the subsurface when minerals such as pyrite become oxidized. Other sources are industrial activities such as the use of sulphuric acid in the non-ferrous industry. Despite the origin of the samples, metal concentrations in the groundwaters from both sites were very low and therefore the groundwater samples were artificially contaminated with higher concentration of zinc (Zn), cadmium (Cd), and arsenic (As) as model contaminants in order to outline possible trends more clearly.

2. Materials and methods

2.1. Sampling of sediments and groundwater

Two different locations in the Meuse Basin were selected as case studies. One sampling site is located in the Campine Area (Belgium), in the Dommel subcatchment situated in ‘‘De Plateaux−Hageven’’ nature reserve on the Belgian−Dutch border. This location was formerly used as a cinder bank by local zinc foundries and is assumed to be a source of metal contamination of the groundwater that discharges into the Dommel River. The second location, a former cokery heavily contaminated by both metal compounds and organics, is situated at the border of the cities of Flémalle and Seraing (in the suburbs of Liège). While the sedimentary aquifer in Dommel is mainly sand ($63\ \mu m < 95\% < 2$ mm), the aquifer in Flémalle mainly consists of gravel ($60\% > 2$ mm). Aquifer and groundwater were sampled between 4 and 8 m below ground for the Dommel location (DO2) and between 8 and 12 m below ground for the Flémalle site (U15). Undisturbed samples were collected with a dual tube sampling system with a core drilling device (Geoprobe®, USA). Groundwater samples were sampled at the same locations after installing monitoring wells with a 2-m filter at a depth of 8 m below ground for the Dommel site and at a depth of 12 m below ground for the Flémalle site. Sediment samples from the undisturbed cores were homogenized under nitrogen atmosphere, and both sediment and groundwater samples were analyzed for the parameters listed in Table 1.

2.2. Microcosm set up

A series of microcosm experiments was set up in an anaerobic chamber at 20 °C containing aquifer material (10 g) that was sampled and homogenized as described above. Groundwater (25 ml) was added to all vials under a nitrogen atmosphere. Three different e-acceptors at four different concentrations were examined, i.e., sulfate (2 mM, 5 mM, 10 mM and 20 mM), nitrate (2 mM, 5 mM, 10 mM and 20 mM) and oxygen (0 μM, 62 μM, 125 μM and 250 μM). Acetate (3.5 mM) was used as carbon source (e-donor). Both the e-acceptor and e-donor were added from stock solutions to the vials in the anaerobic chamber. Several conditions were performed in duplicate to check for the reproducibility and these duplicates confirmed results obtained for the selected conditions (data not shown). A natural attenuation condition and one abiotic control were set up as controls. In the abiotic control, 3.5 mM acetate, 20 mM sulfate and 20 mM nitrate were added in addition to formaldehyde (0.175%) to inhibit the bacterial activity.

Since metal concentrations in the local groundwater were very low (Table 1), the microcosms containing Dommel aquifer/groundwater were artificially contaminated at the start of the experiment by 800 μM Zn ($ZnCl_2$, 100 mM stock), 45 μM Cd ($CdCl_2\ H_2O$, 10 mM stock) and 70 μM As (Na_2HAsO_4, 10 mM stock), while the liquid to solid ratio was held at a factor of 2.5. At regular intervals during the experiment, metals were added again in the microcosms containing Flémalle aquifer/groundwater up to 3 mM Zn, 180 μM Cd and 200 μM As, due to the observed turnover of metal compounds. Acetate was also added again in these batch tests to ensure sufficient carbon source. A total of 28 conditions for each location were set up to be sacrificed at six points in time in 50 mL glass vials in an anaerobic chamber.

2.3. Physico-chemical analysis

The following parameters were monitored and analyzed after each sampling point in time for more than 200 days.

- pH and redox measurements were performed using a Hamilton glass electrode and a WTW pH 325 pH meter, and a WTW sentix glass electrode connected to a Russel meter type RL250, respectively, after calibrating the pH meter at pH 7 and 4 under nitrogen atmosphere. The standard error of the measurement was ±0.02. The redox electrode was checked for its accuracy in an oversaturated quinhydron buffer solution at pH 7 according to ISO 11271, and its standard error is ±1 mV.
- The dissolved oxygen (DO) concentrations were determined by a microcathode oxygen electrode connected to a model 781 oxygen meter (Strathkelvin instruments, UK). The zero-point calibration was performed weekly by using an oversaturated Na_2SO_3 solution at 20 °C. The calibration for the high oxygen concentration was performed daily and is set at 9.08 mg L^{-1} after purging a doubly distilled water solution at 20 °C with air for 30 min. The standard error for the measurement of DO in a reference aerobic medium was ±0.07 mg L^{-1}.
- Groundwater samples for metal analyses were filtered over a 0.45-μm filter, acidified by pure HNO_3 (2%(v/v)), and analyzed by ICP-AES (Jarrell-Ash Autocomp 750). Detection limits for Zn, Cd and As were 0.03 μM, 0.04 μM and 0.03 μM, respectively. The standard errors for the measurement of Zn, Cd and As were ±6 μM, ±3 μM and ±0.12 μM, respectively.
- Both sulphate and nitrate were quantified colorimetrically using the barium sulphate method (Ultrospec 3000, detection limit 0.02 mM SO_4^{2-}) and the spectroquant Nitrate cell test (Merck 1.14563.0001, detection limit 0.04 mM NO_3^-). Repeated analysis of a standard sulphate solution (0.42 mM) indicates a performance of ±0.05 mM, while a standard error for a standard nitrate solution (0.64 mM) of ±0.03 mM was observed.
- For volatile fatty acids (VFAs) analysis to determine the acetate content, 0.45 μm filtrated samples were acidified with 1:1 (v/v) H_2SO_4 solution and then extracted with diethyl ether. VFAs in the diethyl ether phase were analyzed in a GC (CE Instruments−Thermoquest) equipped with a flame ionization detector (FID) and a 15-m AT-1000 filled capillary column (0.53 mm × 1.2 μm). Injection, and detector temperatures were both adjusted to 250 °C. In the oven temperature varied between 110 and 150 °C. Helium was used as the carrier gas at a constant flow of