

Sampling protocols and measurements

used during the MissRhoDia 2 cruises (May-June 2018)

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The sampling cruise MissRhoDia was carried out aboard the RV Tethys II in May 2018. Nine stations were sampled between 2 and 18 km from the Rhône river mouth, in the proximal (stations A, Z and AZ), prodelta (stations AK, K, B and L) and distal (stations C and E) domains over a southwest transect, corresponding to the direction of the river plume.

The O₂ and pH microprofiles were measured in situ using a benthic lander, whereas sediment cores were collected using an UWITEC® single corer (9.5 cm of inner diameter, 60 cm of length) for the pore water and solid phases analysis. Bottom water samples were taken with a 12L Niskin® bottle at approximately 1 m above bottom at each station. Salinity and temperature (°C) were measured using a salinometer with a precision of ± 0.1 and a thermometer with a precision of ± 0.1°C, respectively .

In situ microelectrode measurements

The oxygen and pH micro profiles were measured by micro-electrode of in situ benthic microprofiler (Unisense®) (Rabouille et al. 2003; Lansard et al. 2008; Cathalot et al. 2010; Rassmann et al. 2016). The measurements of 4-5 vertical depth oxygen profiles and 2 pH profiles were achieved with a vertical resolution of 200 µm near the sediment water interface. The dissolved oxygen concentrations were measured by polarographic oxygen micro sensors that were provided with a built-in reference and internal guard cathode (Revsbech 1989). The microelectrodes were calibrated with a two-point calibration technique using the bottom water oxygen concentration determined by Winkler titration (Grasshof et al. 1983) and the anoxic pore waters of sediment (Cai and Sayles 1996). The pH microelectrodes were calibrated by NBS buffers which provided the slope of the sensor response to pH calibration curve and allowed the pH variation to be estimated. The true-pH in bottom waters measurement on the total proton scale was carried out by spectrophotometry with unpurified m-cresol purple

(Clayton and Byrne 1993; Dickson et al. 2007). The precision of dissolved oxygen was of ± 0.5 μM , whereas the uncertainty of pH was ± 0.01 pH units.

Ex situ measurements of solid and pore water profiles

In order to determine the sediment porosity, a core was sliced with a resolution of 2 mm for 0-10 mm depth, 5 mm until 10-60 mm, and 10 mm until the bottom of the core. The sediment porosity was calculated using water contents (the difference between the wet and dried weight after one week at 60 °C) and an averaged sediment density of 2.65 g cm⁻³. The sediment porosity was then corrected by the bottom water salinity.

A second sediment core was processed within 1-2 hours after sampling for the extraction of pore waters by its introduction in a glove bag flushed with N₂ atmosphere in order to avoid oxidation. The extraction of pore waters was conducted by rhizons with a mean pore size of 0.1 μm (Seeberg-Elverfeldt et al. 2005). After collection, the pore water Fe²⁺ and the total dissolved Fe were measured using the ferrozine method (Stookey 1970). Pore waters were poisoned using HgCl₂ for Total Alkalinity (TA) and DIC, acidified for SO₄²⁻ measurements, and stored at 4 °C until laboratory analysis. Subsamples for NH₄⁺, NO₃⁻ and DIP measurements were frozen on board. The pore water NH₄⁺ concentration was diluted and quantified by the indophenol blue method (Grasshof et al. 1983), while the pore water NO₃⁻ concentration was measured using the Vanadium chloride reductant after dilution, followed by the addition of NEDD/sulphanilamide and the colorimetric method (Hansen and Koroleff 1999; Schnetger and Lehnert 2014). The uncertainties of the methods for NH₄⁺ and NO₃⁻ were 5% and 2%, respectively.

The pore water DIC concentrations were quantified on a DIC analyzer (Apollo SciTech®), by the use of 1 ml of the sample volume with 4-7 replicates as described previously by Rassmann et al. (2016). The samples were acidified in line using 10% H₃PO₄ and an ultra-pure nitrogen gas vector was used to transfer CO₂ to a LICOR® gas analyzer. DIC method was calibrated using certified reference materials (oceanic CO₂ measurements, Scripps Institution of Oceanography-batch n°136) which provided an uncertainty of 0.3%. The pore water SO₄²⁻ concentrations were diluted and measured using ion chromatography on an ICS 1000 chromatograph (Dionex) (Rassmann et al. 2020). The uncertainty of this method was of ± 1.6 %.

For the pore water DIP, the measurements (Murphy and Riley 1962) were performed by a spectrophotometer set at 880 nm and zero with distilled water on 3 ml of each sample, with 500 μl of an unstable mixed reagent prepared in the same day of measurement. The reagent was prepared by a mixture of 25 ml of sulfuric acid (H₂SO₄), 2.5 ml of potassium antimony (III) oxytartrate hemihydrate (K(SbO)C₄H₄O₆.1/2H₂O), 7.5 ml of ammonium molybdate ((NH₄)₆Mo₇O₂₄.4H₂O) and 15 ml of ascorbic acid (C₆H₈O₆) solutions. The potassium antimony (III) oxytartrate hemihydrate and ammonium molybdate formed an antimony-phosphomolybdate complex with diluted phosphorus, while ascorbic acid turned it to blue complex, according to DIP concentrations. The precision of the method was 1%.

Sequential extraction of P forms

Another sediment core was sampled and freeze-dried for 48 hours, and ground for further analysis of the phosphorus solid forms. The sedimentary phosphorus pools were separated using the sequential extraction method as detailed in Andrieux-Loyer et al. (2008) and adapted from widespread extraction methods (Psenner et al. 1988; Ruttenberg 1992).

Fe-bound P (including the Fe-oxides bound P and the vivianite) was extracted by 0.1 mol l⁻¹ of Dithionite-Bicarbonate (8h, 20°C). In a second step of determination, authigenic Ca-bound P (including carbonate fluorapatite Ca₅(PO₄, CO₃)₃F, biogenic hydroxyapatite Ca₁₀(PO₄)₆(OH)₂ and CaCO₃ bound P) was quantified on the remaining fraction by 1 mol l⁻¹ of Na-acetate buffer (pH = 4, 6h, 20°C) then washed by 1 mol l⁻¹ of MgCl₂ (pH = 8, 0.5h, 20°C). Then, the detrital P was extracted using 1 mol l⁻¹ of HCl (overnight). Total P was determined through sediment ignition at 550°C (4h) with 1 mol l⁻¹ HCl treatment overnight, while the inorganic P referring to the sum of P-forms (Fe-bound P, Ca-bound P and detrital P), was extracted with 1 mol l⁻¹ HCl in order to ensure the measurements efficiency. Finally, organic P was then quantified by the difference between total P and inorganic P (Andrieux-Loyer et al. 2008).

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