ESF Research Networking Programmes

Short Visit Grant

SCIENTIFIC REPORT

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Proposal title: The study of the sediment associated to carbonate mounds and cold water corals, in the Moroccan margin of the Gulf of Cadiz and Alboran Sea

Reference Number : 6061

Date and place of the visit: from 1 to 16 December 2013 at University of Fribourg Switzerland

CONTENTS

1. PURPOSE OF THE VISIT

This short visit stay was carried at the Department of Geosciences, Earth Sciences, University of Fribourg Switzerland, from 1 to 17 of December 2013, under scientific direction of Dr. Silvia SPEZZAFERRI, head of micropaleontology group and her PhD student Claudio STALDER.

The objective of this stage was to be initiated to geochemical analyses, and micropaleontological analyses necessary to perform my PhD studies.

My PhD project is the study of the sediment associated to carbonate mounds and cold-water corals, along the Moroccan margin of the Gulf of Cadiz and Alboran Sea that were taken by boxcores and Calipso cores during the MD194 cruise on board R/V Marion Dufresne, on June 2013. The aim of this work is:

- the composition and nature of these sediments
- the processes of sedimentation and the environment of deposition
- the sources of sediments

During this short stay in Fribourg, 24 samples from the Calipso core MD13-3447 recovered during MD 194 cruise on board R/V Marion Dufresne on June 2013was the subject of geochemical analyses (phosphorus extraction analyses). This Calipso core is situated off mound area near the Gamma carbonate mound, in the Gulf of Cadiz, Moroccan margin (Fig. 1).

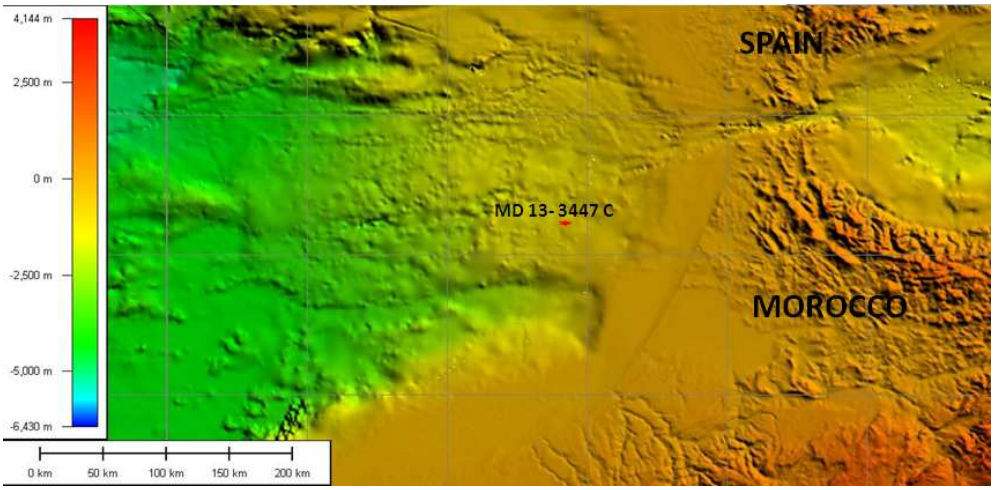


Fig 1: Location of the studied Calipso core

One sample from Norway's cold-water corals mound was a subject of Micropaleontological analyses. It was the first initiation to the binocular identification of benthic and planktonic foraminifers living in cold-water coral environment.

This scientific report represent the description of the different analyses that was carried out during this visit and the preliminary results

2. DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

1. Geochemical analyses- Phosphorus

The MD 13-3447C (35°11,640'N, 7°08,880'W; water depth 968 m; core length 2413 cm) was sub-sampled at the sedimentology laboratory of the Renard Center Marine Geology at Ghent University in Belgium, under scientific direction of Prof. David Van Rooij. The core is around 24 m long, and because of the short time of the stage and the long processing time, only the first 240 cm were analyzed. Twenty-four samples were the subject of a SEDEX sequential phosphorus extraction method developed by Ruttenberget al. (2009)[1]

1. Samples preparation

- The samples were dried in the oven at a 40 °C for 48 hours in labeled tubes, then grinded to a fin fraction using an agate mortar and sieved through a< 125 µm sieve.
- During the time for drying the samples, the phosphorus line was prepared (Fig. 2. A).This line is formed by 24 tubes composed by 4 unites (Ring, tube, base and wheel with scratch), and each tube enclose inside a filter (Fig. 2. B). The filter have to be very well attached to the tube and cover the diameter of the tube then deposed on the wheel attached to the base and the all fixed by the ring.

Fig. 2: A: Phosphorus line; B: Tube unites

- 0.080 g of each sample is weighed on a paper, after rolled it and introduced into the tube, then removed out.

2. Phosphorusline steps

The SEDEX method of Ruttenberg et al. (2009) consists of five steps sequential phosphorus extraction in the following order, loosely bound P; Mn and Fe-bound P; authigenic carbonate fluorapatites; detrital apatites and finally organic P.

STEP I

IA: 1) Add 8 ml of pH adjusted $MgCl_2$ (pH 8.0) (Always rinsed the beaker and the pipette with the Reagent before started to add it in the tubes)

[1] Ruttenberg K.C., Ogawa N.O., Tamburini F., Briggs R.A., Colasacco N.D., and Joyce1 E., 2009. Improved, high-throughput approach for phosphorus speciation in natural sediments via the SEDEX sequential extraction method *Limnol. Oceanogr.: Methods* 7, 319–333.

2) Start shaking for 2hr (shaker at 103 frequencies)

Remarks: Prepare bottles for the line, label them and label also the cap as (IA+B 1, IA+B 2); cover the line by foil paper once the tubes are in the shaker to not contaminate the bottles.

4) Filter and collect supernatants

IB: Repeat from 1 to 4

	IA	IB
Reagent	8 ml $MgCl_2$	8 ml $MgCl_2$
Start shaker	15h02	17h30
Stop shaker	17h02	19h30

Tab. 1:Time recorded for step I

To filter and collect supernatants, we did the following instructions:

First, the plaques holding the tubes have to be well positioned and that the small tubes are in the bottles

1. Openstopc thenopen vacum
2. Close stopc (after around 15 min, once the water is totally filtered)
3. Open chaude (closed when the hole is in horizontal position, and opened when it is in vertical position) for around 10 min
4. Close Vacum and open caps (always remember which cap is for which tube)

Collect IA+IB and acidify 0.134 ml HCl 12N

STEP II CAPS WITH VALVES

II A: 1) Add 0.31 g of Na-dithionite

2) Add 12 ml of pH adjusted CB (pH 7.6)

3) Start shaking for 8 h and label the bottles (II A 1, II A 2…)

4) Filter and collect supernatants in 20 ml bottle

IIB: 5) Add 12 ml of pH adjusted $MgCl_2$ (pH 8.0)

6) Start shaking for 2h and label the bottles (II B 1, II B 2…)

7) Filter and collect supernatants into 20ml bottle

	IIA	IIB
Reagent	12 ml CBD	12 ml $MgCl_2$
Start shaker	12h01	9h45
Stop shaker	20h01	11h45

Tab. 2: Time recorded for step II

STEP IIICAPS WITH VALVES

IIIA : 1)Add 8ml of pH adjusted Acetate buffer (pH 4.0)

2) Start shaking for 6h and label the bottles (IIIA 1,IIIA2,…)

3) Filter and collect supernatants

4) Acidify 0.6 ml HCl 12 N

IIIB:5) Identical to step IB

IIIC:6) Identical to step IB

	IIIA	IIIB	IIIC
Reagent	8 ml Ac buffer	8 ml $MgCl_2$	8 ml $MgCl_2$
Start shaker	12h52	10h34	13h13
Stop shaker	18h52	12h34	15h13

Tab. 3: Time recorded for step III

Collect IIIB+IIIC and acidify 0.134 ml HCl 12N

STEP IV

1) Add 8 ml of 1 M HCl
2) Start shaking for 16 h and label the bottles (IV 1, IV 2,…)
3) Filter and collect supernatants

STEP V

1) Transfer samples residue from step IV to 20 ml glass vials using MQ-H2O to rinse

How we did: Carefully open the Teflon holder and take the vessel away. With Teflon tweezers take the filter away and put in the glass vial (Fig. 3). Rinse with MQ water the filter holder and vessel. Always rinse the Teflon tweezers before to use it for the next one.

2) Place samples vials covered with a large foil paper in the oven (110°C) until more than half of the water has dried out.
3) Add 0.4 ml $Mg(NO_3)_2$ and homogenize with spatula
4) Let them dry in the oven

Fig. 3: Transfer samples residue to glass vials

5) Place samples vials covered with thick Al-foil in oven (550°C) for 2 h (remember the position of the vials as the numbers can move out in the oven)

How we programmed the oven: The oven heats 200 °C / 60 min. In this case, T_0 = 0 min = 0°C ; T_1 = 165 min = 550°C ; T_2 = 120 min=550 °C ; T_3 = 165 min = 20°C.

The step V-5 took around 8 h before to take out the samples.

6) Re-write number on vial with sharpie pen when remove sample vials from furnace
7) Add 4 ml of 1M HCl and left for at least 1 hour

8) Scraping with Teflon spatula to resuspend sample in vials, and rinse with other 4 ml of 1M HCl
9) Start shaking for 16 h in glass vials (the frequency of shaker should be less than 70) and label the bottles (V 1, V 2,…)
10) Stop shaker and leave sediment to decant around 3 h
11) Filter and collect supernatants(How we did: aspire the liquid using syringe and then fixe the filter at the syringe and start filtering (Fig. 4), be careful to do not spill the liquid and try to filter just the liquid)

	IV	V
Reagent	8ml 1M HCl	8ml 1M HCl
Transfer sample to vial	-	10h30
Add Mg(NO3)2	-	14h00
Place in oven (110° C)	-	14h18
Remove from oven	-	20h00
Place in furnace (550°C)	-	11h30
Remove from furnace	-	16h00
Start shaker	16h42	18h10
Stop shaker	08h42	10h10

Tab. 4: Time recorded for step IV &V

Fig.4: Collect supernatants Step V

3. Spectrophotometer

The spectrophotometer Bio-Tek Uvikon XS was used to measure the amount of light absorbed by the solution, using the software LabPower Junior was to read the absorbance. In our case we used the wavelengths 880.0 nm and a transparent square cuvette where we put the solution to study the absorbance. Before to start measure the absorption, each sample has to be diluted and add reagent depending on which step you want to measure (Tab. 5).For each step we used 5 standards solution 0, 5, 10, 15 and 20. The standards solution for supernatant I A+B are 0 (Blank PO_4 in MQ- H_2O), 5 (5 µM PO_4 in MQ- H_2O), 10(10 µM PO_4 MQ- H_2O), 15 (15 µM PO_4 in MQ- H_2O) and 20 (20 µM PO_4 in MQ- H_2O). PO_4 in Na-Ac for supernatant III A, PO_4 in $MgCl_2$ for supernatant IIIB + IIIC and PO_4 in HCl for supernatant IV& V. We processed by a series of 12 samples, duplicated.

STEPS	Sample	MilliQ-H2O
IA+IB	2ml	
IIIA	0.4 ml	1.6 ml
IIIB+IIIC	0.6 ml	1.4
IV	0.2 ml	1.8 ml
V	0.2 ml	1.8 ml

Tab. 5: Dilution for different steps

Transfer 2 ml of sample into reaction vessel, for the standards you don't need to do dilution. Then add 0.06 ml of the MIXING REAGENT and 0.06 ml of ascorbic acid solution and homogenize, let react 5 to 10 min. The standards and samples will be colored in blue with different intensity of blue color depend on the content of P in the solution. Color is stable for 30-45 minutes, that's way we carried 12 samples by 12. Measure standards immediately, before and after samples to correct for complex degradation or specter drift.

2. Micropaleontological analyses

In parallel with the geochemical analyses, few hours were consecrate to micropaleontological analysis. Regarding to the duration of the visit, I just had time to get training on the determination of foraminiferal species. The studied sample was taken from the core POS 391 559/2 recovered from a cold-water coral reef along Lopphavet on the northwestern Norwegian shelf. Using binocular microscope and looking to different foraminifers plates, some species of benthic foraminifera were identified; *Discanomalina coronata, Lobatula lobatula, Uvigerina mediterranea, Uvigerina peregrina, Uvigerina peregrina parva, Hyalinea balthica, Melonis affinis, Cibicides ungerianus, Cibicides ungerianus, Cibicides lobatula, Cibicidoides pachyderma.* A sample of planktonic foraminifera recovered from TTR 17 cruise in the Alboran Sea was also observed to see the difference between benthic and planktonic species.

3. DESCRIPTION OF THE MAIN RESULTS

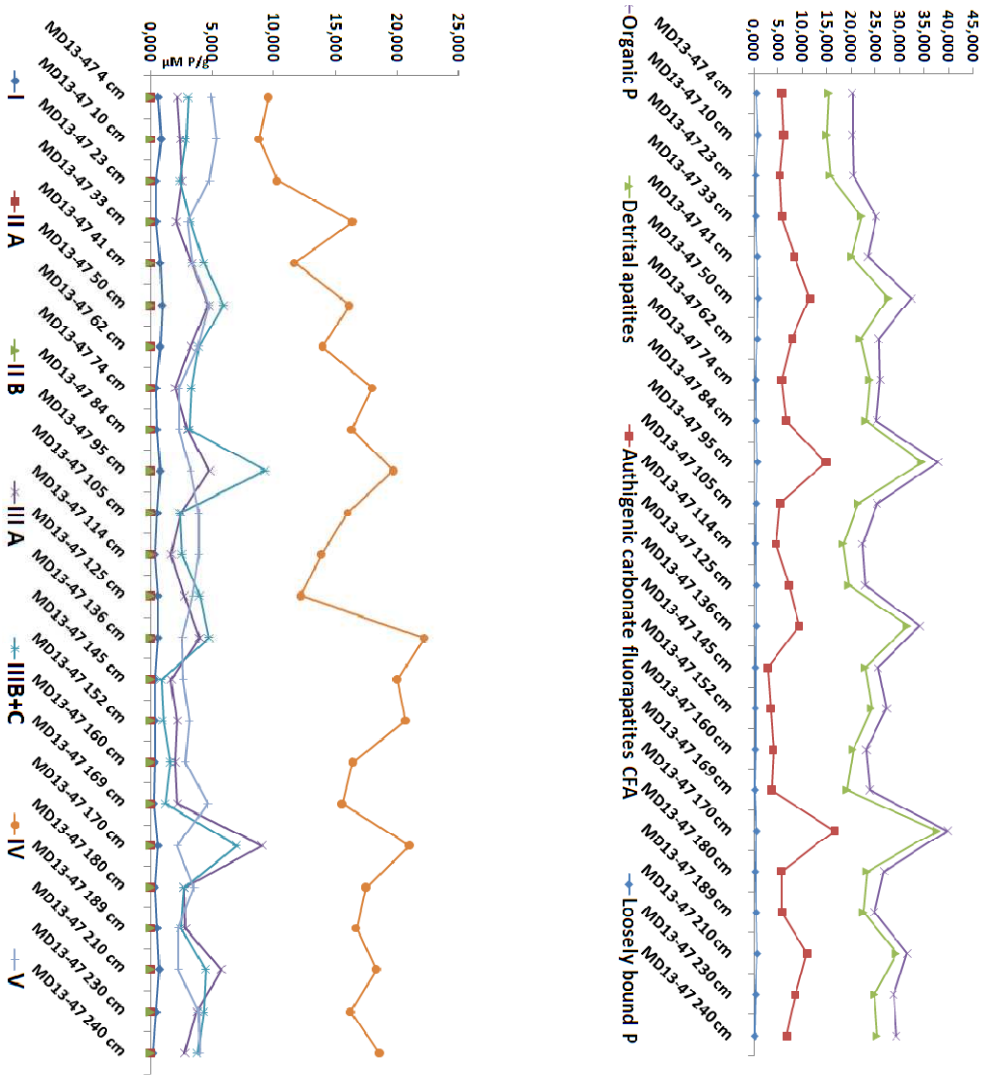
1. Geochemical analyses

During this stage, five steps sequential phosphorus extraction technique following the SEDEX method of Ruttenberg (2009) was applied on the first 24 samples of the Calipso core MD13-3447. This method separates the major reservoir of sedimentary P into five groups. The step I (supernatants I A+B) separates the loosely sorbed or exchangeable P; Step II (supernatants II A and II B), Mn and ferric Fe-bound P; Step III (supernatants IIIA and IIIB +IIIC) dissolution of authigenic carbonate fluorapatites, biogenic apatites and CaCO associated P; Step IV (Supernatants IV) separates detrital apatites (P of ignous, metamorphic or sedimentary origin); and finally step V (Supernatants V) separates organic P. All the supernatants of the different steps were measured with a Bio-Tek Uvikon XS spectrophotometer using the molybdate blue method (Grasshoff et al., 1983)[2]and the software LabPower Junior, except supernatants of step II, that wasn't measured because it has to be done at a more sophisticate

[2]Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Seawater Analysis, 2ndEd. Verlag Chemie, Weinheim p. 419.

spectrophotometer at the Faculty of Medicine. Regarding to the short stay, we couldn't have an appointment for the machine to measure this step.

All the absorbance measurements were exported to an Excel file, to calculate the concentration of the extracted phosphorus of each sample. As the step II wasn't measured, which means the Mn and ferric Fe-bound P concentration, we can't estimate the total concentration of phosphorus in the sediment but we can have an idea about the concentration of the different phases of phosphorus in each supernatant (Fig. 5-A). The P concentration is by µ moles P/ g of sediment. The graph of figure 5-B shows a high concentration of detrital phosphorus compare to the other phosphorus phases, and a variation of the concentration along the 240 cm long of the core, which can give an idea about facies variability and sediment provenance in this area. We also observe that the organic P attend 5,3 µM P/g at the first 20 cm and get less than 4 µM P/g in most of the other samples which can be related to the biological activities at the surface.



A B

Fig.5: A: Concentration of phosphorus in the different supernatants; B: Concentration of the different phosphorus phases in µM P/g

4. FUTURE COLLABORATION

This training is the beginning of the work I will realize for my PhD thesis, which will be co-supervised by Professor Silvia SPEZZAFERRI, in a close collaboration between Mohammed V University and Fribourg University.

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