

# Total phosphorus concentrations in NMR sediment pretreatment extracts from samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011

**Website:** <https://www.bco-dmo.org/dataset/816576>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2020-06-23

## Project

» [Potential phosphorus uptake mechanisms of the deep sedimentary biosphere](#) (Deep sea sediments)

## Program

» [Center for Dark Energy Biosphere Investigations](#) (C-DEBI)

Contributors	Affiliation	Role
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## Abstract

Total phosphorus concentrations in nuclear magnetic resonance (NMR) sediment pretreatment extracts from samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011. Samples were analyzed in 2016.

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## Coverage

**Spatial Extent:** Lat:22.75589 Lon:-46.08125

**Temporal Extent:** 2011 - 2016

## Dataset Description

Total phosphorus concentrations in nuclear magnetic resonance (NMR) sediment pretreatment extracts from samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011. Samples were analyzed in 2016.

These data were published in Defforey et al. (2016) and Defforey et al. (2017). See the related-resource page <https://www.bco-dmo.org/project/664073> for other datasets related to this publication.

Additional award information:

- \* NSF C-DEBI subaward # 156246 to Adina Paytan
- \* NSF C-DEBI subaward # 157598 to Delphine Defforey

## Methods & Sampling

Location: North Atlantic, western flank of the mid-Atlantic Ridge 22.75589 N 46.08125 W

### Methodology:

Prior to the extraction, we freeze-dried, ground and sieved sediment samples to less than 125  $\mu\text{m}$  (Ruttenberg 1992). For a given sample, we weighed four sample replicates (2 g) and placed each in 250 mL HDPE bottles. Sodium dithionite (F.W. 147.12 g/mol; 7.4 g) was added to each sample split, followed by 200 mL of citrate-bicarbonate solution (pH 7.6). This step produces effervescence, so the solution should be added slowly to the sample. We shook samples for 8 h and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with a 0.4  $\mu\text{m}$  polycarbonate filter. We took 20 mL aliquots from the filtrate for each sample split for MRP and total P analyses, and kept them refrigerated until analysis within 24 h. We added 200 mL of ultrapure water to the solid residue for each sample split as a wash step after the above reductive step, shook samples for 2 h, and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with 0.4  $\mu\text{m}$  polycarbonate filters and set aside 20 mL of filtrate from each sample split for MRP and total P analyses. We then extracted the solid sample residues in 200 mL of sodium acetate buffer (pH 4.0) for 6 h. At the end of this extraction step, we centrifuged the bottles at 3,700 rpm for 15 min, filtered the supernatants with 0.4  $\mu\text{m}$  polycarbonate filters and took a 20 mL aliquot of filtrate from each sample split for MRP and total P analyses. We added 200 mL of ultrapure water to the solid residue for each sample split as a wash step, shook samples for 2 h, and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with 0.4  $\mu\text{m}$  polycarbonate filters and set aside 20 mL of filtrate from each sample split for MRP and total P analyses. We repeated the water rinse step, and collected aliquots for MRP and total P analyses as in the previous steps. The concentrations of TP were determined as described below.

Solid sediment sample residues following the pretreatment described above were transferred to two 50 mL centrifuge tubes (2 sample replicates combined per tube). We added 20 mL of 0.25 M NaOH + 0.05 M Na<sub>2</sub>EDTA solution to each tube, vortexed until all sediment was resuspended and then shook samples for 6 h at room temperature (Cade-Menun et al. 2005). We used a solid to solution ratio of 1:5 for this step to minimize the amount of freeze-dried material that will need to be dissolved for the 31P NMR experiments. Large amounts of salts from the NaOH-EDTA concentrated in NMR samples lead to higher viscosity and increase line broadening on NMR spectra (Cade-Menun and Liu 2014). We chose an extraction time of 6 h to improve total P recovery while limiting the degradation of natural P compounds in the sample. At the end of the extraction, samples were centrifuged at 3,700 rpm for 15 min and supernatants decanted into 50 mL centrifuge tubes. We collected a 500  $\mu\text{L}$  aliquot from each sample, which we diluted with 4.5 mL of ultrapure water. These were refrigerated until analysis for total P content on the ICP-OES. The sample residues and supernatants were frozen on a slant to maximize the exposed surface area during the lyophilization step; this was done immediately after the removal of the 500  $\mu\text{L}$  aliquot. Once completely frozen, the uncapped tubes containing supernatants and residues were freeze-dried over the course of 48 h. Each tube was covered with parafilm with small holes from a tack to minimize contamination. Freeze-dried supernatants from identical sample splits were combined and dissolved in 500  $\mu\text{L}$  each of ultrapure water, D<sub>2</sub>O, NaOH-EDTA and 10 M NaOH prior to 31P NMR analysis. The D<sub>2</sub>O is required as signal lock in the spectrometer (Cade-Menun and Liu 2014). Sample pH was maintained at a pH > 12 to optimize peak separation (Cade-Menun 2005; Cade-Menun and Liu 2014). Sample pH was assessed with a glass electrode, and verified with pH paper to account for the alkaline error caused by the high salt content of our samples (Covington 1985).

Freeze-dried sample residues were ashed in crucibles at 550°C for 2 h and then extracted in 25 mL of 0.5 M sulfuric acid for 16 h (Olsen and Sommers 1982; Cade-Menun and Lavkulich 1997). We centrifuged samples at 3,700 rpm for 15 min, filtered supernatants with 0.4  $\mu\text{m}$  polycarbonate filters, and measured P content on an ICP-OES.

Total P concentrations in sediment extracts were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). Standards were prepared with the same solutions as those used for the extraction procedure in order to minimize matrix effects on P measurements. Sediment extracts and standards (0  $\mu\text{M}$ , 3.2  $\mu\text{M}$ , 32  $\mu\text{M}$  and 320  $\mu\text{M}$ ) were diluted to lower salt content to prevent salt buildup on the nebulizer (1:20 dilution for step 1, 1:10 for steps 2 - 4). Concentration data from both wavelengths (213 nm and 214 nm) were averaged to obtain extract concentrations for each sample. The detection limit for P on this instrument for both wavelengths is 0.4  $\mu\text{M}$ . The MRP concentrations were measured on a QuikChem 8000 automated ion

analyzer. Standards were prepared with the same solutions used for the extraction step to minimize matrix effects on P measurements. Sediment extracts and standards (0 – 30 µM PO<sub>4</sub>) were diluted ten-fold to prevent matrix interference with color development. The detection limit for P on this instrument is 0.2 µM. We derived MUP concentrations by subtracting MRP from total P concentrations.

## Data Processing Description

Data were processed in Excel.

BCO-DMO Data Manager Processing Notes:

- \* Data from originally submitted Excel file Data\_TP.xlsx exported as csv. Sheets for steps 1-4 combined into one data table.
- \* added a conventional header with dataset name, PI name, version date
- \* modified parameter names to conform with BCO-DMO naming conventions
- \* blank values in this dataset are displayed as "nd" for "no data." nd is the default missing data identifier in the BCO-DMO system.
- \* Date format changed to ISO 8601 format yyyy-mm-dd

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## Data Files

File
<b>iodp336-tp-sed-pre.csv</b> (Comma Separated Values (.csv), 50.24 KB) MD5:fe3672c7276d4e1994e9103ce2b83d5d Primary data file for dataset ID 816576

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## Related Publications

Cade-Menun, B. (2005). Characterizing phosphorus in environmental and agricultural samples by <sup>31</sup>P nuclear magnetic resonance spectroscopy. *Talanta*, 66(2), 359–371. doi:[10.1016/j.talanta.2004.12.024](https://doi.org/10.1016/j.talanta.2004.12.024)  
*Methods*

Cade-Menun, B., & Liu, C. W. (2013). Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy of Soils from 2005 to 2013: A Review of Sample Preparation and Experimental Parameters. *Soil Science Society of America Journal*, 78(1), 19–37. doi:[10.2136/sssaj2013.05.0187dgs](https://doi.org/10.2136/sssaj2013.05.0187dgs)  
*Methods*

Cade-Menun, B. J., & Lavkulich, L. M. (1997). A comparison of methods to determine total, organic, and available phosphorus in forest soils. *Communications in Soil Science and Plant Analysis*, 28(9-10), 651–663. doi:[10.1080/00103629709369818](https://doi.org/10.1080/00103629709369818)  
*Methods*

Covington, A. K. (1985). Procedures for testing pH responsive glass electrodes at 25, 37, 65 and 85 C and determination of alkaline errors up to 1 mol dm<sup>-3</sup> Na<sup>+</sup>, K<sup>+</sup>, Li<sup>+</sup>. *Pure and Applied Chemistry*, 57(6), 887–898. doi:[10.1351/pac198557060887](https://doi.org/10.1351/pac198557060887)  
*Methods*

Defforey, D. (2016). Phosphorus cycling in the deep sedimentary subseafloor environment. PhD Thesis, UC Santa Cruz. <https://escholarship.org/uc/item/85p2s7dx>  
*Results*

Defforey, D., Cade-Menun, B. J., & Paytan, A. (2017). A new solution <sup>31</sup>P NMR sample preparation scheme for marine sediments. *Limnology and Oceanography: Methods*, 15(4), 381–393. doi:[10.1002/lom3.10166](https://doi.org/10.1002/lom3.10166)  
*Results*

Olsen, S. R., & Sommers, L. E. (1982). Phosphorus. p. 403–430. In A.L. Page, R.H. Miller, and D.R. Keeney [eds.], *Methods of Soil Analysis*. Soil Science Society of America.

## Methods

Ruttenberg, K. C. (1992). Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnology and Oceanography*, 37(7), 1460-1482. doi:[10.4319/lo.1992.37.7.1460](https://doi.org/10.4319/lo.1992.37.7.1460)

## Methods

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## Parameters

Parameter	Description	Units
Extract	Extract solution	unitless
Step	Step in the sequential extraction scheme (1-4)	unitless
Dilution	Sample dilution	unitless
Sample_ID	Sample ID, unique sample identifier	unitless
Analyte_Name	Element analyzed	unitless
Int_Corr	Intensity (corrected)	unitless
RSD_Corr_Int	Relative standard deviation (RSD) of corrected intensity	unitless
Conc_Calib	Calibrated concentration of total phosphorous	parts per million (ppm)
Date	Date the samples were analyzed in ISO 8601 format yyyy-mm-dd	nd

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## Instruments

<b>Dataset-specific Instrument Name</b>	ICP-OES
<b>Generic Instrument Name</b>	Inductively Coupled Plasma Optical Emission Spectrometer
<b>Generic Instrument Description</b>	Also referred to as an Inductively coupled plasma atomic emission spectroscope (ICP-AES). These instruments pass nebulised samples into an inductively-coupled gas plasma (8-10000 K) where they are atomised and excited. The de-excitation optical emissions at characteristic wavelengths are spectroscopically analysed. It is often used in the detection of trace metals.

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## Deployments

JRES-336

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/628214">https://www.bco-dmo.org/deployment/628214</a>
<b>Platform</b>	R/V JOIDES Resolution
<b>Report</b>	<a href="http://dmoserv3.who.edu/data_docs/C-DEBI/cruise_reports/336PR.pdf">http://dmoserv3.who.edu/data_docs/C-DEBI/cruise_reports/336PR.pdf</a>
<b>Start Date</b>	2011-09-16
<b>End Date</b>	2011-11-16
<b>Description</b>	More information is available from the IODP website: <a href="http://iodp.tamu.edu/scienceops/expeditions/midatlantic_ridge_microbio.html">http://iodp.tamu.edu/scienceops/expeditions/midatlantic_ridge_microbio.html</a>

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## Project Information

### Potential phosphorus uptake mechanisms of the deep sedimentary biosphere (Deep sea sediments)

**Coverage:** Mid-Atlantic Ridge flank

The goal of this project is to explore potential microbial P uptake mechanisms in marine sediments beneath the North Atlantic Gyre and their effects on the relative distribution of organic P compounds as a function of burial depth and changing redox conditions. We use a combination of metagenomic analyses and solution <sup>31</sup>P nuclear magnetic resonance spectroscopy (<sup>31</sup>P NMR) to investigate (1) the presence of microbial functional genes pertaining to P uptake and metabolism and (2) the possible P substrates for the deep biosphere in these oligotrophic sediments.

NSF C-DEBI Award #156246 to Dr. Adina Paytan

NSF C-DEBI Award #157598 to Dr. Delphine Defforey

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## Program Information

### Center for Dark Energy Biosphere Investigations (C-DEBI)

**Website:** <http://www.darkenergybiosphere.org>

**Coverage:** Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:

- (1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
- (2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep seafloor ecosystems;
- (3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
- (4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

### **Data Management:**

C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their [Data Management Plan \(PDF\)](#) and in compliance with the [NSF Ocean Sciences Sample and Data Policy](#). The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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### **Funding**

<b>Funding Source</b>	<b>Award</b>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0939564</a>

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