

# Percent amount of organic fractions from diatoms that bind with radionuclides

**Website:** <https://www.bco-dmo.org/dataset/764860>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2019-04-11

## Project

» [Biopolymers as carrier phases for selected natural radionuclides \(of Th, Pa, Pb, Po, Be\) in diatoms and coccolithophores](#) (Biopolymers for radionuclides)

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## Abstract

In order to investigate the importance of biogenic silica associated biopolymers on the scavenging of radionuclides, the diatom *Phaeodactylum tricornutum* was incubated together with the radionuclides <sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Pb, and <sup>7</sup>Be during their growth phase. Normalized affinity coefficients were determined for the radionuclides bound with different organic compound classes (i.e., proteins, total carbohydrates, uronic acids) in extracellular (nonattached and attached exopolymeric substances), intracellular (ethylene diamine tetraacetic acid and sodium dodecyl sulfate extractable), and frustule embedded biopolymeric fractions (BF). Results indicated that radionuclides were mostly concentrated in frustule BF. Among three measured organic components, Uronic acids showed the strongest affinities to all tested radionuclides. Confirmed by spectrophotometry and two-dimensional heteronuclear single quantum coherence-nuclear magnetic resonance analyses, the frustule BF were mainly composed of carboxyl-rich, aliphatic-phosphoproteins, which were likely responsible for the strong binding of many of the radionuclides. Results from this study provide evidence for selective absorption of radionuclides with different kinds of diatom-associated biopolymers acting in concert rather than as a single compound. This clearly indicates the importance of these diatom-related biopolymers, especially frustule biopolymers, in the scavenging and fractionation of radionuclides used as particle tracers in the ocean.

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## Dataset Description

In order to investigate the importance of biogenic silica associated biopolymers on the scavenging of radionuclides, the diatom *Phaeodactylum tricornutum* was incubated together with the radionuclides <sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Pb, and <sup>7</sup>Be during their growth phase. Normalized affinity coefficients were determined for the radionuclides bound with different organic compound classes (i.e., proteins, total carbohydrates, uronic acids) in extracellular (nonattached and attached exopolymeric substances), intracellular (ethylene diamine tetraacetic acid and sodium dodecyl sulfate extractable), and frustule embedded biopolymeric fractions (BF). Results

indicated that radionuclides were mostly concentrated in frustule BF. Among three measured organic components, Uronic acids showed the strongest affinities to all tested radionuclides. Confirmed by spectrophotometry and two-dimensional heteronuclear single quantum coherence-nuclear magnetic resonance analyses, the frustule BF were mainly composed of carboxyl-rich, aliphatic-phosphoproteins, which were likely responsible for the strong binding of many of the radionuclides. Results from this study provide evidence for selective absorption of radionuclides with different kinds of diatom-associated biopolymers acting in concert rather than as a single compound. This clearly indicates the importance of these diatom-related biopolymers, especially frustule biopolymers, in the scavenging and fractionation of radionuclides used as particle tracers in the ocean.

## Methods & Sampling

### Radiolabeled Diatom Cultures

Natural seawater with a salinity of 35, collected from the Gulf of Mexico, was sequentially filtered through a 0.2  $\mu\text{m}$  polycarbonate cartridge and ultrafiltered with a 1000 amu cutoff membrane to remove particulate and colloidal organic matter [Guo et al., 1995; Roberts et al., 2009]. The <1000 amu ultrafiltrate fraction was then used for later experiments. The  $^{234}\text{Th}$  tracer was milked and purified from a  $^{238}\text{U}$  solution [Alvarado Quiroz et al., 2006; Quigley et al., 2002];  $^{233}\text{Pa}$ , in equilibrium with  $^{237}\text{Np}$ , was obtained from Pacific Northwest

National Laboratory;  $^{210}\text{Pb}$ , in 1 mol L<sup>-1</sup> nitric acid ( $\text{HNO}_3$ ), was purchased from Eckert & Ziegler Isotope Products, and the  $^7\text{Be}$  tracer solution (in 0.1 mol L<sup>-1</sup> hydrochloric acid,  $\text{HCl}$ ) was manufactured at the Paul Scherrer Institute, Switzerland [Schumann et al., 2013].

Autoclaved f/2 media (50 ml) were added to preconditioned clear polyethylene containers, and then ~10 to 15 Bq of each radionuclide tracer ( $^{234}\text{Th}$ ,  $^{233}\text{Pa}$ ,  $^{210}\text{Pb}$ , and  $^7\text{Be}$ ) was added. In each radiolabeled medium, 1ml

of laboratory axenic culture, *Phaeodactylum tricornutum* (UTEX 646), was then added and incubated at a temperature of  $19 \pm 1^\circ\text{C}$  with a light:dark cycle of 14 h:10 h under an irradiance of 100  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ . Incubation experiments were carried out in duplicate. The growth status of *P. tricornutum* was monitored by changes in optical density at 750nm (OD750) in a parallel nonlabeled culture. When *P. tricornutum* reached the stationary phase observed by its OD750, cells were harvested for further extraction and analyses. The total incubation

time was 35 days.

### Exopolymeric Substance (AEPS and NAEPS) Extraction

AEPS and NAEPS extractions were performed following the procedures described in Chuang et al. [2014, 2015]. Briefly, for NAEPS, laboratory cultures were centrifuged (2694 g, 30 min) and filtered (0.2  $\mu\text{m}$ ). The filtrate was desalted via diafiltration with a 1000 amu cutoff cross-flow ultrafiltration membrane, followed by freeze drying

for later use. For the AEPS extraction, diatom cells were collected after centrifugation from the previous step. Then,

the pellet was soaked with 0.5mol L<sup>-1</sup> sodium chloride ( $\text{NaCl}$ ) solution for 10 min, followed by centrifugation at 2000 g for 15 min to remove the medium and weakly bound organic material on the cells. The pellet was then resuspended in a fresh 100 ml, 0.5mol L<sup>-1</sup>  $\text{NaCl}$  solution and stirred gently overnight at  $4^\circ\text{C}$ . The resuspended

particle solution was ultracentrifuged at 12,000 g (30 min,  $4^\circ\text{C}$ ), and the supernatant was then filtered through a 0.2  $\mu\text{m}$  polycarbonate membrane. The filtrate was further desalted via diafiltration with a 1000 amu cutoff ultrafiltration membrane and subsequently freeze dried for later use.

### Intracellular and Frustule BF Extraction

Procedures for frustule biopolymers extraction adapted from Scheffel et al. [2011]. Briefly, the clean diatom cells from the previous AEPS extraction step were resuspended in 10 ml, 100mmol L<sup>-1</sup> EDTA (pH 8.0) at  $4^\circ\text{C}$  overnight. EDTA solution was used to extract the intracellular material after cell lysis. The supernatant

was collected after centrifuging at 3000 g for 10 min, defined as EDTA extractable BF. Subsequently, the pellet was placed in 10 ml, 1% SDS in 10mmol L<sup>-1</sup> Tris (pH 6.8) solution and heated at  $95^\circ\text{C}$  for 1 h. The resulting frustules were collected by centrifugation (2500 g, 10 min), washed with 10 ml milli-Q water 3 times, and then were freeze dried for later use. The supernatant from this step was collected and defined as SDS extractable BF, mostly composed of soluble cell-membrane-associated materials. These two fractions represent intracellular biopolymers lysed after cell breakage.

HF digestion was applied to help extract the diatom frustule biopolymers. HF is a nonoxidizing acid commonly used to convert SiO<sub>2</sub> to volatile SiF<sub>4</sub> during wet digestion [Scheffel et al., 2011; Šulcek and Povondra, 1989]. Hence, frustule biopolymers could be separated from the digested solution by a 3 kDa cutoff membrane. However, high-concentration HF would also liberate A type metal radionuclides (Th, Pa, and Be in this study) from any complex by frustule biopolymers [e.g., Burnett et al., 1997]. Furthermore, deglycosylation might also have occurred during a HF digestion [Mort and Lamport, 1977]. Therefore, the <3000 amu fraction represents the sum of silica frustules and broken down frustule biopolymer residues.

Subsequently, 5 ml, 52% HF was added to the frustules in a 15ml plastic centrifugation tube, and the mixture solution was incubated on ice for 1 h. Hydrogen fluoride was then evaporated under an N<sub>2</sub> stream to reduce the volume to dryness. The remaining material was neutralized with 3ml Tris-HCl (250mmol L<sup>-1</sup>, pH 8.0) and followed by centrifugation at 11,000 g for 15 min with 3000 amu Microsep centrifugal filter tubes (Millipore). The filtrate was collected and defined as the fraction of digested silica with <3000 amu frustule BF residues. The supernatant (defined as >3000amu HF soluble BF, e.g., silaffin) was concentrated to 250 µL and rinsed with milli-Q water. The pellet from this step was then washed by a 3 ml, 200mmol L<sup>-1</sup> ammonium acetate solution twice with centrifugation at 3000 g for 20 min. The pellet was then resuspended in a 2 ml, 100mmol L<sup>-1</sup> ammonium acetate solution and was sonicated for 20 s until the mixture solution appeared homogenized. After ultracentrifuging the mixture solution at 12,000 g for 5 min, the pellet (>3000 amu HF insoluble BF, e.g., cingulin) was collected and freeze dried for later use. Combined BF from all three HF fractions represented frustule-embedded biopolymers.

Activity concentrations of <sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Pb, and <sup>7</sup>Be were measured by counting the gamma decay energies at 63.5 keV, 312 keV, 46.5 keV, and 477.6 keV, respectively, on a Canberra ultrahigh purity germanium well detector. The <sup>210</sup>Po activity was analyzed by liquid scintillation counting (Beckman Model 8100 Liquid Scintillation Counter).

Concentrations of total carbohydrate (TCHO) were determined by the TPTZ (2, 4, 6-tripyridyl-s-triazine) method using glucose as the standard and [Hung and Santschi, 2001]. Protein content was determined using a modified Lowry protein assay, using bovine serum albumin as the standard (Pierce, Thermo Scientific). uronic acids (URA) were measured by the methahydroxyphenyl method using glucuronic acid as the standard [Hung and Santschi, 2001].

Elemental contents of carbon (C) and nitrogen (N), were determined by a Perkin Elmer CHN 2400 analyzer, using cysteine (29.99% C, 11.67% N) as a standard.

## Data Processing Description

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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## Data Files

File
<b>pcnt_amount.csv</b> (Comma Separated Values (.csv), 126 bytes) MD5:e7a2e819983183adc3ff154d99c62112
Primary data file for dataset ID 764860

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## Related Publications

Alvarado Quiroz, N. G., Hung, C.-C., & Santschi, P. H. (2006). Binding of thorium(IV) to carboxylate, phosphate

and sulfate functional groups from marine exopolymeric substances (EPS). Marine Chemistry, 100(3-4), 337–353. doi:[10.1016/j.marchem.2005.10.023](https://doi.org/10.1016/j.marchem.2005.10.023)

*Methods*

Chuang, C.-Y., Santschi, P. H., Xu, C., Jiang, Y., Ho, Y., Quigg, A., Guo, L., Hatcher, P. G., Ayranov, M., & Schumann, D. (2015). Molecular level characterization of diatom-associated biopolymers that bind <sup>234</sup>Th, <sup>233</sup>Pa, <sup>210</sup>Pb, and <sup>7</sup>Be in seawater: A case study with *Phaeodactylum tricornutum*. In Journal of Geophysical Research: Biogeosciences (Vol. 120, Issue 9, pp. 1858–1869). American Geophysical Union (AGU).

<https://doi.org/10.1002/2015jg002970> <https://doi.org/10.1002/2015JG002970>

*Methods*

Guo, L., Santschi, P. H., Baskaran, M., & Zindler, A. (1995). Distribution of dissolved and particulate <sup>230</sup>Th and <sup>232</sup>Th in seawater from the Gulf of Mexico and off Cape Hatteras as measured by SIMS. Earth and Planetary Science Letters, 133(1-2), 117–128. doi:10.1016/0012-821x(95)00063-i

[https://doi.org/10.1016/0012-821X\(95\)00063-i](https://doi.org/10.1016/0012-821X(95)00063-i)

*Methods*

Quigley, M. S., Santschi, P. H., Hung, C.-C., Guo, L., & Honeyman, B. D. (2002). Importance of acid polysaccharides for <sup>234</sup>Th complexation to marine organic matter. Limnology and Oceanography, 47(2), 367–377. doi:[10.4319/lo.2002.47.2.0367](https://doi.org/10.4319/lo.2002.47.2.0367)

*Methods*

Roberts, K. A., Xu, C., Hung, C.-C., Conte, M. H., & Santschi, P. H. (2009). Scavenging and fractionation of thorium vs. protactinium in the ocean, as determined from particle–water partitioning experiments with sediment trap material from the Gulf of Mexico and Sargasso Sea. Earth and Planetary Science Letters, 286(1-2), 131–138. doi:[10.1016/j.epsl.2009.06.029](https://doi.org/10.1016/j.epsl.2009.06.029)

*Methods*

Scheffel, A., Poulsen, N., Shian, S., & Kroger, N. (2011). Nanopatterned protein microrings from a diatom that direct silica morphogenesis. Proceedings of the National Academy of Sciences, 108(8), 3175–3180.

doi:[10.1073/pnas.1012842108](https://doi.org/10.1073/pnas.1012842108)

*Methods*

Schumann, D., Ayranov, M., Stowasser, T., Gialanella, L., Di Leva, A., Romano, M., & Schuermann, D. (2013). Radiochemical separation of <sup>7</sup>Be from the cooling water of the neutron spallation source SINQ at PSI. Radiochimica Acta, 101(8), 509–514.

*Methods*

Sulcek, Z., & Povondra, P. (1989). Methods of decomposition in inorganic analysis. In Methods of decomposition in inorganic analysis. CRC.

*Methods*

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## Parameters

Parameter	Description	Units
substance	type of substance	unitless
Protein	percent amount of Protein	unitless (percent)
TCHO	percent amount of total carbohydrates	unitless (percent)
URA	percent amount of uronic acids	unitless (percent)

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## Instruments

<b>Dataset-specific Instrument Name</b>	Perkin Elmer CHN 2400 analyzer
<b>Generic Instrument Name</b>	CHN Elemental Analyzer
<b>Dataset-specific Description</b>	Elemental contents of carbon (C) and nitrogen (N), were determined by a Perkin Elmer CHN 2400 analyzer, using cysteine (29.99% C, 11.67% N) as a standard.
<b>Generic Instrument Description</b>	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

<b>Dataset-specific Instrument Name</b>	Canberra ultrahigh purity germanium well detector
<b>Generic Instrument Name</b>	Gamma Ray Spectrometer
<b>Dataset-specific Description</b>	Activity concentrations of <sup>234</sup> Th, <sup>233</sup> Pa, <sup>210</sup> Pb, and <sup>7</sup> Be were measured by counting the gamma decay energies at 63.5 keV, 312 keV, 46.5 keV, and 477.6 keV, respectively, on a Canberra ultrahigh purity germanium well detector.
<b>Generic Instrument Description</b>	Instruments measuring the relative levels of electromagnetic radiation of different wavelengths in the gamma-ray waveband.

<b>Dataset-specific Instrument Name</b>	Beckman Model 8100 Liquid Scintillation Counter
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Dataset-specific Description</b>	The <sup>210</sup> Po activity was analyzed by liquid scintillation counting (Beckman Model 8100 Liquid Scintillation Counter).
<b>Generic Instrument Description</b>	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from <sup>51</sup> Cr and <sup>125</sup> I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

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## Project Information

**Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and coccolithophores (Biopolymers for radionuclides)**

## NSF Award Abstract:

Particle-associated natural radioisotopes are transported to the ocean floor mostly via silica and carbonate ballasted particles, allowing their use as tracers for particle transport. Th(IV), Pa (IV,V), Po(IV), Pb(II) and Be(II) radionuclides are important proxies in oceanographic investigations, used for tracing particle and colloid cycling, estimating export fluxes of particulate organic carbon, tracing air-sea exchange, paleoproductivity, and/or ocean circulation in paleoceanographic studies. Even though tracer approaches are considered routine, there are cases where data interpretation or validity has become controversial, largely due to uncertainties about inorganic proxies and organic carrier molecules. Recent studies showed that cleaned diatom frustules and pure silica particles, sorb natural radionuclides to a much lower extent (by 1-2 orders of magnitude) than whole diatom cells (with or without shells). Phytoplankton that build siliceous or calcareous shells, such as the diatoms and coccolithophores, are assembled via bio-mineralization processes using biopolymers as nanoscale templates. These templates could serve as possible carriers for radionuclides and stable metals.

In this project, a research team at the Texas A & M University at Galveston hypothesize that radionuclide sorption is controlled by selective biopolymers that are associated with biogenic opal (diatoms), CaCO<sub>3</sub> (coccolithophores) and the attached exopolymeric substances (EPS), rather than to pure mineral phase. To pursue this idea, the major objectives of their research will include separation, identification and molecular-level characterization of the individual biopolymers (e.g., polysaccharides, uronic acids, proteins, hydroquinones, hydroxamate siderophores, etc.) that are responsible for binding different radionuclides (Th, Pa, Pb, Po and Be) attached to cells or in the matrix of biogenic opal or CaCO<sub>3</sub> as well as attached EPS mixture, in laboratory grown diatom and coccolithophore cultures. Laboratory-scale radiolabeling experiments will be conducted, and different separation techniques and characterization techniques will be applied.

**Intellectual Merit :** It is expected that this study will help elucidate the molecular basis of the templated growth of diatoms and coccoliths, EPS and their role in scavenging natural radionuclides in the ocean, and help resolve debates on the oceanographic tracer applications of different natural radioisotopes (<sup>230</sup>Th, <sup>231</sup>Pa, <sup>210</sup>Po, <sup>210</sup>Pb and <sup>7,10</sup>Be). The proposed interdisciplinary research project will require instrumental approaches for molecular-level characterization of these radionuclides associated carrier molecules.

**Broader Impacts:** The results of this study will be relevant for understanding biologically mediated ocean scavenging of radionuclides by diatoms and coccoliths which is important for carbon cycling in the ocean, and will contribute to improved interpretation of data obtained by field studies especially through the GEOTRACES program. This new program will enhance training programs at TAMUG for postdocs, graduate and undergraduate students. Lastly, results will be integrated in college courses and out-reach activities at Texas A&M University, including NSF-REU, Sea Camp, Elder Hostel and exhibits at the local science fair and interaction with its after-school program engaging Grade 9-12 students from groups traditionally underrepresented.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1356453</a>

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