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2. Drilling Contamination Tests during ODP Leg 201 Using Chemical and Particulate Tracers[1]

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ABSTRACT

To further methods for monitoring seawater intrusion, we applied a water-soluble chemical tracer and deployed fluorescent microspheres to reveal the extent to which seawater or individual prokaryotic cells penetrate a sediment sample during drilling operations. In total, 154 subsamples from cores were investigated with an additional 6 cores studied by measuring chemical tracer in transects through the cross-sections of the cores. The averaged results show that the center of the sediment cores contain less perfluorocarbon tracer than the core margins and have lower potential seawater contamination by a factor of between 3.5 and 100. Samples obtained using the advanced piston corer were generally less contaminated than sediments obtained using the extended core barrel by a factor of between 3 (Site 1230) and 10 (Site 1226). The gradient between chemical tracer concentrations in the core center and the periphery was found to be steeper in sediment cores from the shallow-water sites (a factor of 65) than in cores from deepwater sites (a factor of 9), which may relate to core retrieval time. The method of microsphere deployment was improved to help eliminate failures by forcing cores to burst through the bead delivery bag. In 34 samples with a range of chemical tracer concentrations, the number of microspheres detected was compared with the level of chemical tracer found. This comparison demonstrated that in spite of the large number of microspheres deployed, the beads end up at a largely diluted concentration of ~1000 beads/mL seawater, which is similar to the abundance of prokaryotic cells in surface seawater. The relevance and importance of all of the

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tracer results to efforts aimed at studying the subsurface biosphere are discussed.

INTRODUCTION

Microbiological studies of the marine deep subsurface biosphere require methods to retrieve sediment samples without contamination by microorganisms from surface sediments or seawater or methods to quantify the extent of contamination. The ubiquitous contaminating medium is surface seawater used as a drilling fluid, which contains ~0.1 $\times 10^9$ to 1 $\times 10^9$ prokaryotes/L (Smith et al., 2000a). Here, we describe the further development and application of two methods to quantify the extent of seawater intrusion during drilling operations in deeply buried sediments. The concentration of a water-soluble chemical tracer was used as an indirect measure for the maximally possible entrainment of bacterial cells, and fluorescent microspheres were deployed as prokaryotic cell mimics to reveal the extent to which prokaryote-sized particles may have penetrated a sediment sample.

Perfluorocarbon Tracer

Characteristics

Perfluorocarbon tracers (PFTs) have been widely used in land-based drilling applications (Russell et al., 1992; McKinley and Colwell, 1996) because they are chemically inert and can be detected with high sensitivity. Perfluoromethylcyclohexane ($C_6F_{11}CF_3$) was used as the chemical tracer to monitor potential seawater contamination of sediment and rock samples on the *JOIDES Resolution* (Smith et al., 2000a, 2000b). This perfluorocarbon compound (Aldrich 30293-7) has a molecular weight of 350.05 g, a boiling point of 76°C, and a density of 1.76 g/mL. Its solubility is ~2 mg/L in water and 104 mg/L in methanol (Colwell et al, 1992). The low solubility in water facilitates gas-phase partitioning and quantitative headspace analysis.

Delivery

Perfluorocarbon tracer was continuously fed into the stream of drilling fluid using a single-piston high-performance liquid chromatography pump (Alltech model 301). The tracer was delivered from a polypropylene carboy into the drilling fluid stream through a valve on the low-pressure side of the mud charge pump. The rate of the tracer injection was adjusted to maintain a final concentration of ~1 mg/L in the drilling fluid, using shipboard rig instrumentation software to control pumping rates (Smith et al., 2000a).

Sampling

Concentrations of PFT were measured in all sections used for microbiological studies. After core retrieval and the cutting of the 9.5-m core into six 1.5-m sections, samples for PFT measurement were immediately taken from the section ends adjacent to the section or sections that had been selected for microbiological analysis. Using sterile 5-mL syringes with the Luer lock end cut off, subcores of generally 3- to 4-cm^3 volume were taken from the center of the sediment core and from the periphery

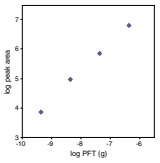
of the sediment core at the core liner. In this way, parallel data sets were collected to show the extent of contamination in the periphery of the sediment core along the core liner and in the center of the core (Smith et al., 2000b). To increase the sensitivity of PFT detection, the sampling protocol was adjusted midcruise by taking two instead of one 5-mL syringe sample at the center of the core (Sites 1229–1231). Since the freshly cut sediment surface was potentially contaminated by PFT smear from the core liner, the sediment surface layer at the sampling spots was scraped away using a clean scalpel before syringe sampling. Special care was taken not to touch the syringe with hands or gloves that had come into contact with the core liner surface, which is heavily contaminated with drilling fluid and PFT tracer. The samples were placed in preweighed 20-mL headspace vials (Agilent) and were sealed with gas-tight stoppers. Liquid samples of 5 mL were taken from 25% sediment slurries prepared anaerobically for microbiological incubations and cultivations.

For positive qualitative controls of PFT delivery, a few drops of water from the outer core liner or from supernatant water on top of the first section of a core were collected in a headspace vial, then sealed and analyzed like the sediment samples.

Detection, Calibration, Sensitivity, and Background Controls

The conditions used on the gas chromatograph (GC) were somewhat different than those previously used by Smith et al. (2000a). We used a HP-PLOT/AL203 "S" deactivated column with film thickness = 50 µm, length = 15 m, phase ratio = 12, and column ID = 0.25 µm. The inlet temperature was 180°C with an inlet pressure of 10 psi. The detector temperature was 250°C. The column temperature was 100°C for 3.5 min and then ramped up at 50°C/min until it reached 200°C. The PFT peak eluted at 5.1 min. We used a 1-mL injection. Larger injections were found to result in loss of material. A calibration curve is shown in Figure F1.

F1. Calibration of PFT on the GC, p. 11.



The PFT detection limit reported for Leg 201 sites was not set as a lower limit of the ability to detect PFT by the GC but as a lower limit of the ability to confidently assess the presence of PFT in real samples, given the uncertainty inherent in subtracting background levels of PFT and the reliability of the integration of small GC peaks. Therefore, the detection limits for PFT at Sites 1295 and 1226 were in the range of 0.02 ng PFT (2×10^{-11} g)/g sediment or 0.02 µL potential seawater contamination/g sediment. After doubling the amount of sediment in a headspace vial during sampling at Sites 1229–1231, the detection limit was 0.01 µL seawater/g sediment. The laboratory air sometimes contained considerable PFT levels above background (up to 9×10^{-12} g PFT/mL air) (data for Site 1226), which would lead to false positive PFT concentrations for samples capped in the laboratory if no background correction was made. Very high PFT levels were found in the air of the cold room where the microbiology core sections were subsampled and processed (1.8×10^{-10} g PFT/mL air) (data for Site 1226). The PFT is almost certainly introduced on the core liner of the sections that are brought into the cold room directly from the catwalk. To avoid background problems, fresh samples were either carried to the catwalk for capping in the open air that had repeatedly tested PFT-negative or capped inside with a background PFT correction subtracted from the observed PFT concentration.

Sample Analysis

The headspace vials containing the sediment were heated for 5–10 min in an 80°C oven to evaporate and release the tracer from the sediment. Next, clean nitrogen gas was injected onto the GC column to make sure that no PFT peak resulted from residual PFT in the syringe or in the GC. After a clean run was achieved, the sample was injected using the same baked syringe. At the time of the injection, the syringe was also still hot so that PFT would not condense out before injection. Also, for best results, background air samples needed to be taken regularly from the same location used for capping headspace vials (ideally on the catwalk when no core is present). Finally, cleaning PFT out of used syringes was critical. We found it was best to use a large Hamilton syringe (10 mL) that could be flushed several times with air and then have the plunger removed for baking. Smaller syringes required a methanol wash to remove PFT and then had to be baked for a longer time to remove the methanol in order to avoid having an interfering GC peak. It was also found that cores with high levels of sulfide resulted in GC traces with small air peaks, presumably from the sulfide scrubbing oxygen from the headspace vial. Therefore, for cores rich in sulfide, the air peak cannot be used to normalize various GC runs.

In order to determine the PFT concentration from each sample, raw GC peak areas had to be corrected for injection size and headspace volume. After GC measurements, the vials were weighed to determine the amount of core material in each sample. The headspace of each vial used to calculate the PFT concentration was based on the total headspace (20 mL) minus the volume attributed to sediment calculated from the weight of the sediment divided by its bulk density. The calculation for liters of drill fluid per gram of sediment was as follows:

$$\text{g PFT injected} = (\text{PFT}_s - \text{PFT}_b) \times \text{CF}, \quad (1)$$

where

PFT_s = PTF peak detected from sediment sample,
PFT_b = PFT peak detected from the background, and
CF = factor from calibration curve.

$$\text{g PFT/g sediment} = [(\text{g PFT injected})\,(V - W/\rho)/I)]/W, \quad (2)$$

where

V = volume of headspace vial (mL),
W = the weight of the sediment sample (g),
ρ = the density of the sediment sample (g/mL), and
I = GC injection size (1 mL for these experiments).

In order to estimate the potential for seawater contamination in each sample, the concentration of PFT in each sample was converted to potential seawater contamination based on the 1 mg/L concentration of PFT in the seawater drilling fluid. Therefore, each nanogram of PFT detected in the sediment sample potentially represents 1 mL of seawater drilling fluid of contamination.

Fluorescent Microspheres

Characteristics

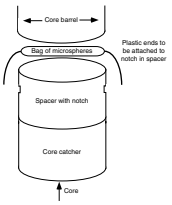
Fluorescent microspheres of a similar size (0.5 µm) to the indigenous microorganisms (0.2–1.3 µm) have been used previously in land-based drilling operations to assess dispersal and transport of these prokaryote cell mimics (Harvey et al., 1989). Yellow-green fluorescent microspheres (Fluoresbrite carboxylate microspheres; Polysciences Inc. 15700) with a diameter of 0.52 (±0.01) µm were used as a particulate tracer on the *JOIDES Resolution*. These microspheres are highly fluorescent (458-nm excitation; 540-nm emission) and appear bright green when observed by epifluorescence microscopy through a blue filter set (Zeiss filter set 09 or 10) (Smith et al., 2000a).

Delivery and Sample Collection

The desired concentration of microspheres at the point of drilling is ~10^{10} spheres/mL (Smith et al., 2000a). Microspheres were deployed in Whirl-pak bags containing 20 mL of microsphere suspension in deionized water (2×10^{11} microspheres/20 mL bag). Two different deployment procedures were used. Initially, the Whirl-paks were attached to one side of the core catcher (Sites 1225–1228); however, soft sediment cores could repeatedly slide past the attached bag without bursting it, with no concomitant release of microspheres. As a modification, the bag was filled with twice the volume of microsphere suspension (2×10^{11} microspheres/40 mL bag), and the attachment geometry in the core catcher was altered (Fig. F2). The bag was then heat-sealed such that extra plastic, not filled with beads, was left at each end. By attaching the loose plastic ends using Kevlar cord, the bag was wedged into a shim above the core catcher and stretched across the throat of the core barrel. Sediment cores were consequently forced to burst through the bead bag when a core was taken (Sites 1228–1231).

Microspheres were specifically deployed on cores that recovered sediment samples for microbiological cultivation and slurry preparation. For each slurry sample, three subsamples were analyzed: (1) a sample of the slurry, (2) a slurry sample diluted tenfold in 2% formaldehyde (also used for direct prokaryote counts), and (3) a scraping from the outer surface of the core to confirm deployment of beads.

F2. Microsphere deployment bag in the core barrel, p. 12.



Sample Collection and Counting Procedure

The sediment sample (5 mL of sediment or 10 mL of 25% sediment slurry) was mixed with an equal volume of saturated sodium chloride solution. The solution was centrifuged (Marathon 10K, 5 min, 2800 × g), and the supernatant was filtered onto black polycarbonate filters (0.2-µm pore size). Any fluorescent microspheres were counted under ultraviolet (UV) light, and data were reported as numbers of microspheres per gram of sediment.

RESULTS

PFT in Cores Collected Using the Advanced Piston Corer and Extended Core Barrel

The PFT concentrations measured for all sediment samples are listed in Table T1, and a summary of the data is also shown in Table T2, sorted by sampling location (center of the core vs. core periphery), drilling technique (advanced piston corer [APC] vs. extended core barrel [XCB]), and site (deepwater sites vs. continental margin and shelf sites). This comparison reveals three factors that influence the potential for seawater contamination of sediment cores.

T1. PFT concentrations, Leg 201, p. 14.

T2. PFT measurements on different core types, p. 15.

1. The center of the sediment cores contain less PFT than the core margins and have lower potential seawater contamination. The difference can range from a factor of 3.5 (Site 1231; APC cores) to almost 100 (Site 1229; APC cores). This result appears to be independent of the drilling technique and applies to APC as well as XCB cores.
2. Samples obtained by APC coring are generally less contaminated than sediments obtained by XCB coring from the same site. For core center samples, the difference was approximately a factor of 10 at Site 1226 and a factor of 3 at Site 1230 (Table T2). XCB coring is more disruptive and introduces more seawater into the sediment core.
3. The gradient between average PFT concentrations in the core center and the periphery is steeper in sediment cores from the Peru Margin sites on the continental shelf (a factor of 65) than in cores from deepwater sites (a factor of 9) (Table T2). This difference appears to be related to water depth and therefore to core retrieval time; whereas Peru Margin Sites 1227, 1228, and 1229 were in 426, 252, and 150 m water depth, the deepwater Pacific Sites 1226, 1230, and 1231 had water depths of 3296, 5071, and 4827 m. In sediment samples from deepwater sites, the PFT gradients appear to dissipate during core retrieval (1–2 hr), compared to fresher and steeper PFT gradients of Peru Margin sediment samples (<30 min retrieval time).

The potential seawater contamination for Leg 201 sites is in a similar range as previous data for ODP Site 1149 southeast of Japan on the seaward side of the Izu-Bonin Trench at a water depth of 5830 m (Table T3) (Smith et al., 2000b).

T3. PFT measured during Leg 185, p. 16.

PFT Tracer in Cross Sections of APC Core

For six cores from deepwater Holes 1226E and 1230B, PFT samples were obtained with 1-mL syringes diagonally across freshly cut core sections on the catwalk. After sampling, the sediment samples were placed into 2-mL GC vials, closed in the open air to avoid contamination with ubiquitous laboratory PFT, and weighed before GC analysis. The PFT concentrations in core cross sections do not always follow a predictable gradient, with maxima at the core liner and minima in the center of the core (Table T4). On average, the six transects show lower PFT concentrations in the samples from the inner core (samples 2–6; average = 0.012–0.044 ng PFT/g sediment) and elevated PFT counts in the outer samples

T4. Potential seawater contamination for six core cross sections, p. 17.

at the core liner (samples 1 and 7; average = 0.797 and 0.031 ng PFT/g sediment).

Contamination Assessment for Slurry Samples Using PFT and Microspheres

Contamination tests are especially significant in evaluating contamination risks for samples that are used for microbial cultivations. Sediment samples are mixed anaerobically (under nitrogen) with sterile saline solution on a 1:4 volume basis. These slurries are then used for a variety of microbial enrichments and quantifications, including most probable number dilutions on defined media or extracts of natural substrates (see "Microbiology," p. 14, and media recipes in Tables T4, p. 84, T5, p. 85, and T6, p. 87, all in the "Explanatory Notes" chapter).

Table T5 shows the data for potential seawater contamination and microsphere counts for slurry samples and allows an assessment as to whether detectable concentrations of the easily diffusible PFT tracer coincide with visible microspheres. Whereas the chemical tracer indicates the extent of seawater contamination and influx of dissolved compounds, the prokaryote-sized microspheres demonstrate that prokaryotes could actually penetrate the sediment. In other words, they outline the prokaryote contamination potential. Three slurry samples with high potential seawater contamination, in the range of 0.05–0.4 µL seawater/mL slurry (Sections 201-1226B-2H-3 [center]; 201-1229A-11H-5 [center]; and 201-1230A-38X-1 [center]), also contained microspheres, bacterial mimics. These slurry samples are most likely contaminated. In slurry samples with potential seawater contamination levels below ~0.05 µL/mL, microspheres were not found at all or were observed a single time. A possible source of experimental error was found in Section 201-1226B-47X-2, which had high microsphere counts but no detectable PFT. Prolonged gassing of the anaerobic slurry with nitrogen could have removed the PFT tracer, while retaining the microspheres.

T5. Correlation between microsphere and PFT tracers, p. 18.

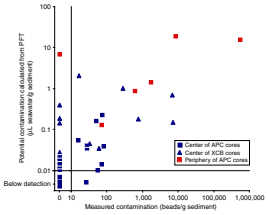
DISCUSSION

Comparison of Seawater Tracer vs. Microspheres

The PFT data show that the sediment samples from the center of APC cores contained generally <0.1 µL seawater/g sediment (Table T2), which corresponds to an entrainment of <50 prokaryotes/g sediment. This is a maximum estimate that assumes that prokaryotes can follow the flow of seawater in the same way as a PFT tracer molecule. In contrast to microspheres, PFT can travel through very small pore spaces and is found in the laboratory air and on the hands of anyone who has handled a core liner. Therefore, although its presence at high concentrations in a sediment sample (>0.1 ng PFT/g sediment) indicates seawater contamination, it is not a sufficient indication that microorganisms from the drilling fluid have contaminated the sample.

Figure F3 shows the number of microspheres detected plotted against the potential seawater contamination as determined from PFT concentrations for the 34 samples for which PFT concentrations and bead counts were done on the same sediment sample. These 34 samples were chosen because they represent a wide range of measured PFT concentrations, permitting the potential seawater contamination as determined

F3. Microsphere and PFT test comparison, p. 13.



by PFT concentrations to be related to the estimations of particulate contamination as determined using the bead deployment. In several cases, samples thought to be contaminated with seawater based on PFT concentrations contained no microspheres (Fig. F3, data points along the y-axis). On the other hand, the absence of PFT from a sample is a good indication that contamination by drilling water and also by prokaryotes has not occurred. Of the four samples that remained below the detection limit of 0.01 µL seawater/g sediment (0.01 ng PFT/g sediment), three contained no detectable beads (Fig. F3).

In general, the concentrations of the easily diffusible PFT tracer, indicating the extent of seawater contamination and influx of dissolved compounds, are somewhat correlated with microscopic counts of visible microspheres. The number of fluorescent microspheres (2×10^{11}/20-mL bag) that are deployed is equivalent to the number of prokaryotes in ~400 L of seawater (assuming 5×10^{8} bacteria/L); however, their deployment does not produce a uniform dispersion in the drilling water, but could result in microsphere concentrations at the drill bit that temporarily exceed natural seawater concentrations of prokaryotes by an unknown factor. In Figure F3, with the exception of one point, the samples taken from the periphery of APC cores where drilling water is expected and observed (shown in red) show a positive correlation, demonstrating that the beads end up at a concentration of ~1000 beads/mL seawater after being deployed. This represents a large dilution for the concentration of beads found in the initial deployment bag but is similar to the ratio of 50 prokaryotic cells/0.1 µL seawater predicted for the drilling fluid. This indicates that the microsphere bag deployment can ultimately produce a concentration of microspheres in the fluid similar to actual prokaryotic concentrations, suggesting that the microsphere test is a sensitive measure of prokaryote contamination in sediment samples. In one periphery sample with a very high PFT concentration, however, no beads were detected (Section 201-1227A-7H-2 [outside]) (Table T5). This result inspired the change in the method of bead deployment, which we believe resulted in a more reliable and more uniform bead deployment.

Figure F3 also shows a difference between the relationship of PFT results and bead results between center subcores of APC cores and of XCB cores. Whereas subcores from XCB cores show uniformly high PFT values and are highly variable in their bead concentrations, subcores from the center of APC cores all have bead counts <85 beads/g sediment in spite of variable PFT values. Although 85 beads/g may represent contamination of as many as ~85 microbes/g sediment, in some cases these low numbers are unreliable because they are based on the observation of only a few beads (in a laboratory environment with background contamination).

In summary, although PFT can always be found in sediment samples taken from the edges of cores, microspheres can be absent, indicating an inhomogeneous distribution of microspheres along the core liner. At this point, without knowing the factors that control the final concentration and distribution of microspheres in the drilling water, one should consider the beads as only a semiquantitative measure of contamination. The presence of multiple microspheres is a strong indication that contamination by microbe-sized particles has occurred; without further data, their absence alone cannot confirm that a sample is uncontaminated.

Recommendations

For most biogeochemical process rates, organic biomarker studies, and microscopic cell counts, the potential presence of small numbers of contaminating prokaryotes (<50 prokaryotes/g sediment in APC cores) (Table T2; Fig. F3) as indicated by PFT and microsphere data is not a problem. Low-level contaminants are highly relevant if there is a method-inherent risk that contaminating populations are amplified and selected for by specific enrichment in culture media or by nucleic acid techniques such as the polymerase chain reaction (PCR). However, even enrichment cultures may not be affected by low levels of contaminating cells (<50 prokaryotes/g) because most surface marine microorganisms (from which the contaminating cells are derived) are currently unculturable and a portion of all cells do not survive the shock associated with being brought into culture media.

The potential seawater concentrations for APC sediment cores show considerable variability, with standard deviations frequently exceeding the averages (Table T2). The spottiness of contamination, as determined by PFT and microsphere data, does not allow guarantees that a specific sample material, cored and treated correctly, will be free of contaminants. Although it may not always be possible, it is best that a sample used for microbial cultivations and slurry preparation be checked for contamination directly, preferably by both chemical tracer and microsphere techniques.

The identification of microbial isolates or phylotypes from potentially contaminated slurries can help to decide whether contamination has occurred, if the isolates are identical to frequently cultured seawater bacteria. Under some drilling and sampling conditions, contaminations are harder to exclude; for example, XCB coring could be unavoidable in a particular location. To double check cultivated isolates and molecular phylotypes under such problematic conditions, potential seawater contaminants could be screened for comparison from supernatant water of the mudline core or from the outer core liner.

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Figure F1. Calibration relating peak area (arbitrary units) to weight of perfluorocarbon tracer (PFT) injected onto the GC.

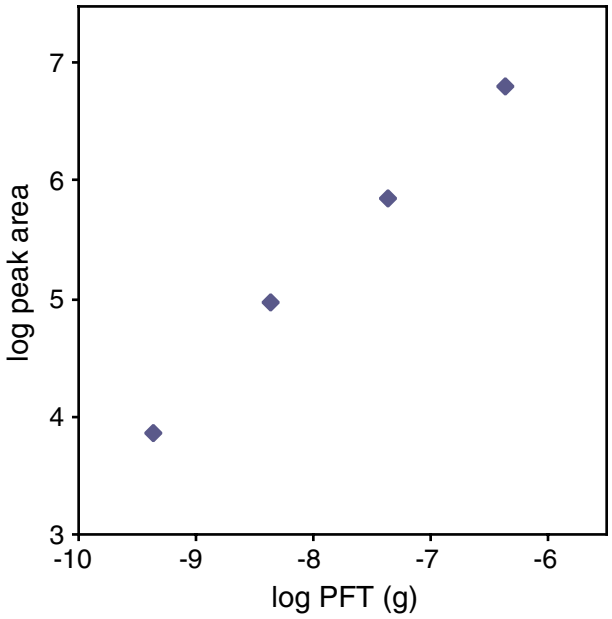

Figure F2. Diagram showing the modified placement of the microsphere deployment bag in the core barrel.

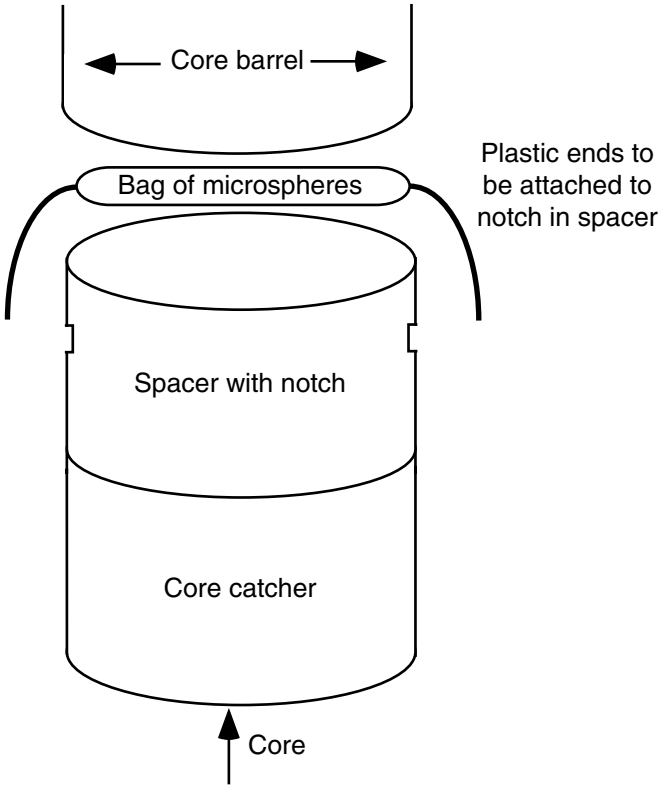

Figure F3. Comparison of microsphere and perfluorocarbon tracer (PFT) contamination test results on 34 samples, representing a wide range of observed PFT concentrations. Solid lines = approximate detection limits with each test method. APC = advanced piston corer, XCB = extended core barrel.

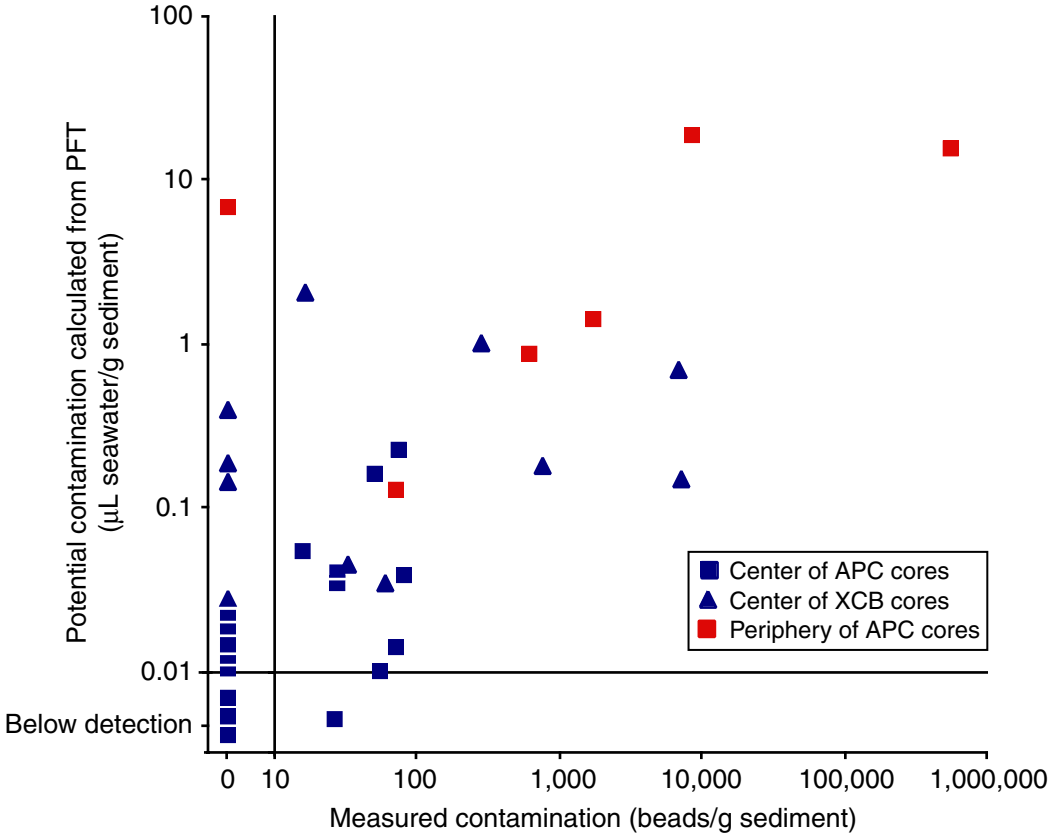

Table T1. Concentrations of PFT for all samples investigated during Leg 201.

Core	Section, details	PFT (ng PFT/g sediment)
201-1228E-		
1H	3, center	0.01
1H	3, outside	0.09
201-1229A-		
2H	2, center	0.03
2H	2, outside	18.41
3H	2, center	0.02
3H	2, outside	15.33
4H	5, center	0.05
4H	5, outside	0.54
6H	2, center	0.09
6H	2, outside	0.52
9H	5, center	BD
9H	5, outside	0.03
11H	5, center	0.03
11H	5, outside	0.02
14H	3, center	0.05
14H	3, outside	0.11
18H	2, center	0.01
18H	2, outside	0.13
22H	2, center	BD
201-1229D-		
1H	1, center	0.01
1H	1, outside	BD
1H	1, outside (bead sample)	0.14
1H	3, center	0.01
1H	3, outside	2.32
12H	2, center	0.03
12H	2, outside	0.16
15H	3, center	0.03
15H	3, outside	0.89
201-1230A-		
1H	1, center (slurry sect.)	0.01
1H	1, outside (slurry sect.)	0.48
1H	1, center	0.03
1H	1, outside	0.03
2H	2, center (slurry sect.)	BD
2H	2, outside (slurry sect.)	0.12
9H	6, center	0.01
9H	6, outside	BD
13H	3, center	0.22
13H	3, outside	1.42
15H	6, center (slurry sect.)	0.02
15H	7, center	0.01
15H	7, outside	0.34
21H	3, center	0.03
21H	3, outside	0.84
22H	2, center	0.13
22H	2, outside	2.55
30X	1, center	0.35
30X	1, outside	1.68
37X	1, center	0.21
37X	1, outside	5.91
38X	1, center (dry portion)	0.03
38X	1, outside (dry portion)	1.33
38X	1, center	0.38
38X	1, outside	2.04
201-1230B-		
12H	2, center, near hydrate	BD
12H	2, outside, near hydrate	0.28
201-1230C-		
1H	1, center	0.72
1H	1, outside	2.31
2H	1, center	BD
2H	1, outside	0.65
2H	4, center	BD
2H	4, outside	0.34
2H	3, center	BD
2H	3, outside	0.12
2H	5, center	0.01
2H	5, outside	0.22
201-1231B-		
1H	2, center	0.04
1H	2, outside	0.05
2H	2, center	BD
2H	2, outside	0.09
3H	2, center	0.02
3H	2, outside	0.19
6H	2, center	BD
6H	2, outside	BD
12H	2, center (slurry sample)	0.03
13H	3, center	0.03
13H	3, outside	0.05
201-1231D-		
13X	3-CC, center	0.03
13X	3-CC, outside	1.24

Notes: PFT = perfluorocarbon tracer. BD = below detection.

Table T2. Average concentrations for PFT measurements of potential seawater contamination.

Summary of PFT results (cores)		PFT (ng PFT/g sediment)		
Site	Core type	Average	Standard deviation	*N*
1226 Deep Pacific	APC center	0.087	0.059	28
	XCB center	0.636	1.065	15
1227 Peru shelf	APC center	0.026	0.026	12
	APC outside	1.777	2.129	8
1228 Peru shelf	APC center	0.035	0.040	9
	APC outside	0.164	0.206	6
1229 Peru shelf	APC center	0.035	0.038	14
	APC outside	3.205	6.448	12
1230 Deep Pacific	APC center	0.080	0.186	15
	APC outside	0.693	0.826	14
	XCB center	0.240	0.161	4
	XCB outside	2.738	2.133	4
1231 Deep Pacific	APC center	0.022	0.015	6
	APC outside	0.077	0.070	5
	XCB center	0.029	Single sample only	
	XCB outside	1.243	Single sample only	
Deep Pacific sites	APC center	0.061	0.122	49
	APC outside	0.531	0.756	19
Peru shelf sites	APC center	0.032	0.034	35
	APC outside	2.064	4.593	26
Izu-Bonin Trench	APC center	0.246	0.744	12
Smith et al., 2000b	APC outside	0.966	1.551	12

Notes: *N* = number of samples. PFT = perfluorocarbon tracer. APC = advanced piston corer, XCB = extended core barrel. Center = center of core, outside = outer layer of sediment core adjoining core liner. 0.1 µL surface seawater contains ~50 prokaryotic cells.

Table T3. Concentrations of PFT measured during Leg 185.

Core, section	PFT (ng PFT/g sed)	
	Interior	Exterior
185-1149A-		
9H-1	0	0.61
9H-2	0.21	4
9H-3	0	2.6
9H-4	0	0.061
9H-5	0.082	3.8
9H-6	0	0
11H-1	0.031	0.076
11H-2	0.0056	0.11
11H-3	0.003	0
11H-4	0.022	0.011
11H-5	0	0.2
11H-6	2.6	0.12
Average	0.246	0.966
Standard deviation	0.744	1.551
Average w/o 11H-6	0.032	1.043
St. dev. w/o 11H-6	0.064	1.602

Note: Leg 185 results were previously published by Smith et al. (2000b). PFT = perfluorocarbon tracer. w/o = without. St. dev. = standard deviation.

Table T4. Potential seawater contamination for cross sections through six cores, Sites 1226 and 1230.

	PFT (ng PFT/g sed)						
Core, section	1	2	3	4	5	6	7
201-							
1226E-5H	0.007	BD	BD	BD	BD	0.019	BD
1226E-8H-4	0.010	0.077	0.033	0.011	0.002	BD	0.048
1226E-11H	0.014	0.014	BD	0.012	0.079	0.015	0.036
1226E-13H	4.720	0.043	0.039	0.030	0.061	0.058	0.086
1230B-5H-5	BD	BD	BD	0.009	BD	BD	0.005
1230B-6H-5	0.028	BD	0.005	BD	BD	BD	0.007
Average	0.797	0.044	0.020	0.012	0.036	0.031	0.031

Notes: The seven samples (1–7) were taken from linear cross sections over a core diameter of 66 mm. PFT = perfluorocarbon tracer. BD = below detection.

Table T5. Correlation between microsphere and PFT tracers. (**See table notes.** Continued on next page.)

Core	Section, detail	Potential seawater contamination (µL/g sediment)	Microsphere counts (beads/fov)	Beads/g sediment	Slurry section	Potential seawater contamination (µL/g sediment)	Microsphere counts (beads/fov)	Beads/g sediment	Delivery confirmed
201-1226B-									
2H	3, center	ND	ND		3	0.264	7 in 50	494	Yes
12H	3, center	0.038	2 in 50	84	3	BD	0 in 50	0	Yes
22H	4, center	0.039	1 in 50	29†	3*	BD	1 in 50	71	Yes
43X	2, center (biscuit 1)	0.670	ND	ND	2	BD	1 in 50	71	Yes
43X	2, center (biscuit 2)	0.179	0	0					
47X	2, black sediment	0.151	35 in 50	7,295	2	BD	4 in 50	593	Yes
47X	2, red sediment	0.178	4 in 50	750					
201-1226E-									
1H	1, center (114 cm)	ND	2 in 50	75	1	ND	ND	ND	No
201-1227A-									
2H	5, center	0.020	ND	ND	5	BD	0	0	Yes
2H	5, outside	0.126	3 in 50	76					
3H	5, center	0.017			5	0.035	0	0	Yes
3H	5, outside	0.129							
5H	3, center	BD			5*	0.013	ND	ND	?
5H	3, outside	1.460							
7H	2, center	BD	0	0	2	0.008	0	0	Yes
7H	2, outside	6.392	0	0					
12H	2, center	0.052	1 in 53	16†	2	0.030	0	0	Yes
12H	2, outside	0.863	8 in 50	633					
201-1227D-									
1H	1, center	0.017	0	0	No slurry prepared				
1H	1, outside	0.553							
4H	6, center	BD			6	0.002	0	0	Yes
4H	6, outside	3.324							
201-1228A-									
2H	2, center	0.013			2	BD	0 in 58	0	Yes
2H	2, outside	0.558							
5H	5, center	BD			5	0.038	1 in 63	56	Yes
5H	5, outside	0.021							
14H	3, center	0.009			3	0.043	0 in 50	0	Yes
14H	3, outside	0.025							
201-1228E-									
1H	3, center	0.011			1*	0.039	1 in 83	42	Yes
1H	3, outside	0.087							
201-1229A-									
2H	2, center	0.029			2	BD	0 in 50	0	Yes
2H	2, outside	18.408	215 in 21	8,851					
3H	2, center	0.021			2	BD	1 in 63	56	Yes
3H	2, outside	15.329	209 in 20	578,784					
4H	5, center	0.055			5	0.015	0 in 50	0	Yes
4H	5, outside	0.542							
6H	2, center	0.091			2	0.016	0 in 50	0	Yes
6H	2, outside	0.519							

Table T5 (continued).

Core	Section, detail	Potential seawater contamination (µL/g sediment)	Microsphere counts (beads/fov)	Beads/g sediment	Slurry section	Potential seawater contamination (µL/g sediment)	Microsphere counts (beads/fov)	Beads/g sediment	Delivery confirmed
9H	5, center	BD			2*	0.058	0 in 50	0	Yes
9H	5, outside	0.026							
11H	5, center	0.026			2*	0.056	3 in 50	212	Yes
11H	5, outside	0.022							
22H	2, center	BD			2	BD	0 in 50	0	Yes
22H	2, outside	ND							
201-1229D-									
1H	1, center	0.009			1	BD	0 in 83	0	Yes
1H	1, outside	BD							
201-1230A-									
1H	1, center	0.010	0 in 50	0	1	0.698	0 in 50	0	Yes
1H	1, outside	0.481							
2H	2, center	BD	0 in 50	0	2	BD	0 in 50	0	Yes
2H	2, outside	0.124							
9H	6, center	0.009			6	BD	Beads not deployed		
9H	6, outside	BD							
13H	3, center	0.221	6 in 60	76	3	0.017	0 in 50	0	Yes
13H	3, outside	1.415	73 in 50	1,813					
15H	6, center	0.020	0 in 50	0	6	0.018			?
15H	6, outside	ND							
22H	2, center	0.134	1 in 50	176†	2	BD			?
22H	2, outside	2.551							
38X‡	1, center	0.026	0 in 50	0	1	0.400	2 in 100	71	Yes
38X	1, outside	1.327	1 in 100	88†					
38X	1, center (dry)	0.380	0 in 50	0					
38X	1, outside (dry)	2.037							
201-1231B-									
1H	2, center	0.044			2	BD	0	0	Yes
1H	2, outside	0.054							
2H	2, center	BD			2	BD	0	0	Yes
2H	2, outside	0.092							
6H	2, center	BD			2	BD	0	0	Yes
6H	2, outside	BD							
12H	2, center	0.028			2	0.291	0	0	Yes

Notes: Potential seawater contamination and microsphere counts are from sediment samples and parallel slurries. The delivery of microspheres was confirmed by detecting microspheres in scrapings and water samples from the outer core liner (right column). Fov = field of view. ND = not determined. BD = below detection. * = slurry and sediment samples are from different core sections. † = single, nonreproducible bead observation, may represent handling contamination. ‡ = the samples are from a wet portion (~58–65 cm) and a dry portion (140–145 cm) of Section 201-1230A-38X-1. BD = below detection, ND = none detected.