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Environmental Persistence of Drilling Mud and Fluid Discharges and Potential Impacts

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Environmental Persistence of Drilling Mud and Fluid Discharges and Potential Impacts

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Abstract

Accidental discharges of synthetic-based drilling mud (SBM) may occur during offshore exploration and production drilling activities. To fully assess the potential impacts of the drilling mud spillage incidents on the marine environment, we must first understand its environmental persistence. To address this issue, two studies were undertaken: the first to investigate the biodegradation of SBM in marine sediments and the second to assess the toxicity of SBM in marine sediments.

In the first study, we investigated the biodegradation of SBM in marine sediments that were recovered from the Bedford Basin. Laboratory experiments were conducted using a Micro-Oxymax respirometer to evaluate the aerobic biodegradation of the fresh (unused) and recycled (used) SBM that are currently used by the offshore oil and gas industry. The results showed that the fresh SBM was readily biodegradable in the sediments when incubated at two different loadings (10 or 100 g/kg sediment) and at two temperature levels (5^{o}C and 21^{o}C). The total petroleum hydrocarbons (TPH) in the fresh SBM samples were degraded by 50 to 70% within one month, with corresponding production of carbon dioxide (CO_2). By contrast, the recycled SBM degraded significantly more slowly than the fresh SBM samples, as indicated by the limited TPH removal efficiency (<20%) and the lower production of CO_2.

The second study focused on evaluating the toxicity of SBM in marine sediments. Two sediments were selected for this evaluation: one was sandy beach sediment with low organic carbon content and the other was saltwater marsh sediment with much higher organic carbon content. The toxicity of SBM-contaminated sediments were evaluated by determining Microtox Solid Phase Test (SPT) EC_{50} values and the survival rate of the marine amphipod (*Eohaustorius estuarius*) after a 10-day exposure to SBM contaminated sediments. The results of this study show that there was no toxicity as measured by Microtox SPT to either the control or SBM-amended sediments for both tested sediment types. The marine amphipod test, however, showed that SBM-amended sediments (both sandy beach and saltwater marsh type) had severe toxicity immediately after the spike of sediments with the fresh and the recycled SBM. The toxicity of the amended SBM to the test marine amphipod appears persistent in both sediments for the duration of the test period of three months. The different toxicity response of SBM in sediments to the Microtox and marine amphipod tests may be a result of the different modes of action of the two tests in response to the organic and inorganic chemistry of the sediment.

Résumé

Des rejets accidentels de boue de forage synthétique (BFS) peuvent survenir durant des activités d'exploration du sous-sol marin et de forage de développement. Pour pleinement évaluer les répercussions potentielles des déversements accidentels de boue de forage en milieu marin, il faut en comprendre la persistance dans l'environnement. Deux études ont été entreprises à cette fin : l'une analysant la biodégradation, et l'autre, la toxicité de la BFS dans les sédiments marins.

La première étude nous a permis d'analyser la biodégradation de la BFS dans les sédiments marins récupérés dans le bassin de Bedford. Des expériences ont été menées en laboratoire à l'aide d'un respiromètre Micro-Oxymax pour évaluer la biodégradation en aérobiose de la BFS fraîche (non utilisée) et de la BFS recyclée (utilisée) actuellement employées dans le secteur des hydrocarbures exploités en mer. Les résultats ont montré que la BFS fraîche se dégradait facilement dans les sédiments lorsqu'elle était à deux niveaux différents de concentration (10 ou 100 g/kg de sédiment) et de température (5 et 21 ^{o}C). Les hydrocarbures pétroliers totaux (HPT) contenus dans les échantillons de BFS fraîche se dégradaient de 50 à 70 % en l'espace d'un mois, produisant des émissions de dioxyde de carbone (CO_2). En revanche, la BFS recyclée se dégradait beaucoup plus lentement que les échantillons de BFS fraîche, comme l'ont montré le rendement peu élevé d'élimination des HTP (< 20 %) et la plus faible production de CO_2.

La seconde étude portait sur l'évaluation de la toxicité de la BFS dans les sédiments marins. Deux types de sédiments ont été prélevés dans le cadre de cette évaluation : l'un provenant d'une rive sablonneuse à faible teneur en carbone organique, et l'autre d'un marais salé présentant une teneur en carbone organique beaucoup plus élevée. La toxicité des sédiments contaminés de BFS a été évaluée en déterminant les valeurs CE_{50} d'un essai en phase solide (EPS) Microtox ainsi que le taux de survie de l'amphipode marin (*Eohaustorius estuarius*) après avoir été exposé 10 jours aux sédiments contaminés de BFS. L'EPS Microtox mené dans le cadre de cette étude a indiqué une absence de toxicité pour les deux types de sédiments analysés tant dans les sédiments-témoins que dans les sédiments altérés par la BFS. L'essai sur l'amphipode marin a néanmoins montré que les sédiments contaminés par la BFS (provenant de rives sablonneuses et de marais salés) affichaient un taux de toxicité considérable juste après la contamination des sédiments par la BFS fraîche et la BFS recyclée. La toxicité de la BFS altérée pour l'amphipode marin étudié est restée persistante dans les deux types de sédiment durant les trois mois qu'a duré la période d'essai. Les écarts de toxicité de la BFS dans les sédiments entre l'essai Microtox et l'étude de l'amphipode marin peuvent être issus des différents procédés employés dans les deux mises à l'essai en réaction à la chimie organique et inorganique des sédiments.

1. Introduction

The offshore drilling of exploration and production wells requires a drilling mud medium to cool and lubricate the drill bit, to carry the drill cuttings up to the surface through the drilling pipe, and to balance the reservoir hydrostatic pressure (Scholten et al. 2000). Synthetic-based drilling muds (SBM) are made using a synthetic-based fluid composed of linear alpha olefins, internal olefins, esters, or paraffins. SBM have the advantages of the high effectiveness of the oil-based drilling muds and the low toxicity of the water-based drilling muds and hence are more commonly used at present. SBM enter the aquatic environment as a coating on rock cuttings, discharged from the drilling platforms, and accidental spills (Holdway 2002). To minimize the environmental impact, each SBM is required by the U.S. EPA to be certified as biodegradable by micro-organisms indigenous to marine sediment before the SBM can be licensed for use in the Gulf of Mexico (Federal Register 2004). In Europe, SBM biodegradability was regulated as a prerequisite for the prevention of long-term environmental impacts on the marine environment (Steber et al. 1995).

Although SBM is considered to have low toxicity, the accidental release of the drilling fluid mixture may raise environmental concerns because of the array of inorganic and organic compounds that are added to give the mud the desired properties. These additives include viscosifiers, emulsifiers, biocides, lubricants, wetting agents, corrosion inhibitors, surfactants, detergents, caustic soda, salt mixtures constituted of sodium, calcium, and potassium chloride, organic polymers, and fluid loss control agents (Scholten et al. 2000). To reduce the environmental "footprint" of drilling discharges offshore and prevent waste of SBM material, a closed-loop recycling of the used SBM is used to separate the cuttings and used mud by using shale shakers, sand traps, desilters, and centrifuges on drilling platforms to recover and reuse SBM. The solid materials can be discarded after treatment with the best available treatment technology (OWTG 2002).

In the past, the evaluation of the biodegradation of drilling muds in the environment has been focused on the aerobic or anaerobic biodegradability of fresh drilling muds (Herman and Roberts 2006; Herman and Roberts 2005; Nguyen et al. 2006; Rojas-Avelizapa et al. 2007). No study has been undertaken to evaluate the biodegradability of whole used SBM (Neff et al. 2000) or to directly compare the biodegradability of the fresh and recycled SBM. The assessment of the biodegradability of the recycled mud and comparison of the fresh and the recycled SBM under similar conditions are valuable for environmental risk assessment for accidental discharge of drilling mud and fluid in the environment.

The laboratory studies described in this report were designed to evaluate and compare the aerobic biodegradation of fresh (unused) and recycled (used) SBM in marine sediments at different temperatures and initial loadings, as well as the toxicity of fresh and recycled drilling muds in sediments before and after biodegradation. The results of these studies are expected to support the assessment of the impact on the marine environment of SBM discharge, especially as a result of accidental spillage.

2. Materials and Methods

2.1. Testing Materials

2.1.1. Synthetic-based Drilling Mud

The fresh and recycled SBM samples were provided by an East Coast Operator working in the Newfoundland-Labrador Offshore area in November of 2007. The active ingredient of synthetic-based fluid in both types of SBM is Puredrill IA35-LV, an isoalkane-based fluid consisting of 84% hydro-treated and hydro-cracked base oil (petroleum), 15% $CaCl_2$, and <1% surfactant. Laboratory characterization of the fresh and recycled SBM using gas chromatography and mass spectroscopy (GC-MS) indicated that SBM samples consisted of a C10 to C25 isoalkane unresolved complex mixture, with numerous isomers, and there was no significant difference between the fresh and recycled SBM in terms of organic chemical composition.

2.1.2. Marine Sediments

Marine sediment for the aerobic biodegradation experiments was collected from the Bedford Basin (Halifax, NS) on April 2, 2008 (site 44º41.699N; 63º37.836W) at a water depth of 64.6 m. The sediment was collected using an Ekman Grab sampler and stored in 500 mL mason jars at 4^{o}C for two weeks before it was used in the experiment. Sediment carbon contents were analyzed using a Leco WR-112 carbon analyzer after the sediment was treated with hydrochloric acid. Total organic carbon was 5.49% while inorganic carbon was 0.54%.

For the evaluation of SBM toxicity, it would have been ideal to use offshore sediments from regions where there is oil and gas activity, but it was anticipated that samples would be in transport for too long. The decision to use local sediments specifically for the biodegradation and toxicity experiments was based on the fact that the time between sample collection and experimental setup was less than 24 h. The sediments were selected based on the Organisation for Economic Cooperation and Development (OECD) guidelines for aerobic biodegradation tests (OECD 2002b, Guidelines for Testing of Chemicals, Guideline 308, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems). The OECD standard guideline stipulates that two sediments are to be used for the aerobic tests. The two sediments selected should differ in organic carbon content and texture, one having a high organic carbon content and a fine texture, and the other having a low organic carbon content and a coarse texture.

The two additional sediments for toxicity testing were collected from the Conrad's Beach area on October 5, 2009. One was inter-tidal zone sandy sediment (hereafter referred to as "A") and the other was saltwater marsh organic-rich sediment (hereafter referred to as "B"). Despite the fact that these were shoreline sediments, their physical properties were similar to sediment found on the Grand Banks, where average grain size is 200 μm and TOC is <1% (Schwinghamer et al. 1996). All sediment was sieved to 1 mm and kept at 4ºC prior to use. Sediment characterization was performed by Environmental Canada (see section 3.2.1).

2.2. Aerobic Biodegradation of SBM in Marine Sediments

2.2.1. Experimental Design

Aerobic biodegradation of the SBM was conducted with the design shown in Table 1.

Table 1: Experimental design for the study of aerobic biodegradation of SBM in marine sediments (N=no, Y=yes).

Treatment	T (°C)	Nutrients	SBM Type	Load
1	5°C	N	None	0
2	5°C	N	FRESH	0.1g
3	5°C	N	FRESH	0.1g
4	5°C	N	USED	0.1g
5	5°C	N	USED	0.1g
6	5°C	Y	None	0
7	5°C	Y	FRESH	0.1g
8	5°C	Y	FRESH	0.1g
9	5°C	Y	USED	0.1g
10	5°C	Y	USED	0.1g
11	21°C	Y	None	0
12	21°C	Y	FRESH	0.1g
13	21°C	Y	FRESH	0.1g
14	21°C	Y	USED	0.1g
15	21°C	Y	USED	0.1g
16	21°C	Y	None	0
17	21°C	Y	FRESH	1.0g
18	21°C	Y	FRESH	1.0g
19	21°C	Y	USED	1.0g
20	21°C	Y	USED	1.0g

Two sources of SBM were selected: one fresh (unused), the other recycled (used). Seawater controls without SBM were used to monitor the background respiration rate in the presence and absence of nutrients. The fresh and recycled SBM were also incubated in the presence and absence of nutrients to compare the rate and extent of SBM degradation with and without nutrient limitation. The incubation of the microcosms was conducted at two different temperatures (5°C and 21°C) to investigate the effect of temperature. Finally, the incubation of fresh and used SBM at high temperature was conducted at two loadings (0.1 g and 1.0 g per microcosm) to test the effect of initial SBM concentrations on hydrocarbon degradation rates.

2.2.2. Construction of Microcosms

Microcosms were constructed in 250 mL Duran glass bottles by adding 100 mL of natural seawater (filtered to 25 µm through sand filters) and 10 g (wet weight) of Bedford Basin marine sediment. The fresh or recycled SBM samples were added to the Duran bottle using a

micro-pipette with disposable plastic tip. SBM was added at either of the two different loadings (0.1 and 1.0 g SBM) to prepare 10 g and 100 g SBM per kg wet weight sediments. Sediment controls contained seawater but no SBM. Duplicate microcosms were used for each treatment, except the low temperature control, which had a single microcosm. Nutrients were supplied to all the microcosms by adding 3.3 g/L Bushnell-Haas medium and 0.5 g/L NH_4Cl. The microcosms were placed on the platform of a reciprocating shaker, with the microcosms partially submerged in a refrigerated water bath and incubated at two temperatures: 5°C and 21°C.

2.2.3. Hydrocarbon Mineralization

The headspace of each microcosm was connected to an independent channel of the Micro-Oxymax respirometer (Columbus Instruments, Columbus, OH) for the entire duration of experiment to monitor the consumption of oxygen and the production of carbon dioxide from the biodegradation of organic compounds. The respirometry system is designed to detect low levels oxygen consumption and carbon dioxide production by monitoring the concentration of gas contained within an enclosed headspace into which the material being monitored is respiring. The sensitivity of the system is $2x10^{-7}$ litres per hour. Temperature was controlled by a Lindberg/Blue M water bath (Thermo Fisher Scientific). Calibration of oxygen and carbon dioxide sensors and checks of the chamber's restriction, volume and leakage were conducted before the experiment. The system automatically compensates for pressure and temperature changes. The headspace of each chamber was refreshed periodically when the gas concentration deviated from the pre-set threshold.

2.2.4. Analysis of Petroleum Hydrocarbons

Six microcosms were sacrificed immediately after their construction to analyze the initial total petroleum hydrocarbon (TPH) concentration of the control, low, and high concentration SBM. After 28 days of incubation, all microcosms were sacrificed to determine the residual TPH. The microcosms were extracted by adding 10 mL of hexane, shaking vigorously by hand for 30 s, and then rotating on an extraction roller (Wheaton R2P) at 60 rpm for 18 h. After extraction, the bottle was left to stand upright to allow the separation of the hexane from the slurry. The hexane layer was removed using a glass pipette and filtered through sodium sulfate to remove any remaining water. The extract was concentrated under a gentle stream of nitrogen and transferred to a 2 mL glass vial for Gas Chromatography/Flame Ionization Detection (GC-FID) analysis.

The GC-FID system consisted of an Agilent 6890 gas chromatograph with flame ionization detector and a 7683 auto-sampler. For separations, a Supelco MDN-5s 30 m x 250 µm x 0.25 µm (length x i.d. x film thickness) column was used with a 1 m retention gap of deactivated fused silica. The sample was injected using the cool-on-column mode with a sample injection volume of 1 µL. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The initial oven temperature was 50ºC, held for 2 min, followed by an increase to 300ºC at 30ºC/min, and held at 300ºC for 10 min, with the total run time of 22.33 min. The FID detector was operated at 320ºC with the hydrogen flow set at 40 mL/min and the air flow set at 450 mL/min. Calibration standards ranging from 1 to 100 mg/mL were prepared using a pure stock of isoparaffin (IPAR) fluid.

2.2.5. Analysis of Heavy Metals

For inorganic matter and heavy metal analysis, portions of the fresh and recycled SBM samples were prepared by microwave digestion in nitric acid. The resulting solutions were analyzed for all other trace elements, except mercury, by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma emission spectrometry (ICP-ES). Mercury was determined by cold vapour atomic adsorption spectroscopy. The dry mass of the samples were determined by heating samples at 550^{o}C overnight in accordance with standard methods for the examination of water and wastewater (APHA et al. 1998). Results were reported on the basis of the dry mass.

2.3. Evaluation of Toxicity of the SBM in Marine Sediments

2.3.1. Experimental Design

The experimental design to study the toxicity of the sediments contaminated by SBM is shown in Table 2. This design contains six treatments. Treatments 1–3 were constructed with Conrad's Beach coarse sand of low total organic carbon content (~0.1%). Treatments 4–6 were constructed with the fine saltwater marsh sediment of higher total organic carbon content (>1%). Each treatment consisted of three sacrificial sampling points (0, 1 month, and 3 months), and each time point and treatment consisted of three replicates. Therefore, the total number of microcosms was 6 x 3 x 3 = 54.

Table 2: Experimental design for testing toxicity of SBM in marine sediments.

Treatment	Sediment type	SBM Type	SBM Loading
1	A	None	0
2	A	Fresh SBM	20 g/kg
3	A	Used SBM	20 g/kg
4	B	None	0
5	B	Fresh SBM	20 g/kg
6	B	Used SBM	20 g/kg

2.3.2. Construction of Microcosms

The laboratory SBM microcosms for toxicity testing and the sampling process were constructed as follows:

- Clean and solvent rinse 54 1.0 L glass mason jars (with lids);
- Add 500 g sediment type "A" to treatments 1–3, and 500g sediment type "B" to treatments 4–6;
- Add 10 g fresh SBM to treatments 2 and 5; 10 g recycled SBM to treatments 4 and 6;
- Stir with spatula to homogenize the samples;
- Remove a 50 g subsample for hydrocarbon analysis;
- Add 900 mL raw seawater (containing 3.3 g per litre Bushnell-Haas Medium and 0.5 g per litre NH_4Cl) to each mason jar microcosm;

- Sacrifice the "time zero" 18 microcosms immediately after the construction for initial toxicity and chemical analyses;
- Incubate the remaining 36 microcosms under static conditions in the laboratory at room temperature with surface open to the ambient environment;
- Sacrifice the "t = 30d" 18 microcosms after one month of incubation; in the course of incubation at least once every two days, vigorous hand-shaking of microcosms is rendered to homogenize and enhance the biodegradation of SBM in sediments. Any evaporation of water is topped by raw seawater every month;
- Sacrifice the "t = 90d" 18 microcosms after three months of incubation.

When a microcosm was sacrificed, the supernatant was decanted to a separate container for chemical analysis of soluble hydrocarbons and solvent extractable organic matter. The solid sediment phase was thoroughly mixed and then split into two fractions: one of about 250 ml for toxicity analysis (Microtox SPT and amphipod bioassay) and the second of about 50 ml for chemical analysis of TPH.

2.3.3. Biodegradation of SBM in Sediments

The methods used for chemical analysis of total hydrocarbons are similar to those described in section 2.2.4, except that the water and sediment phases were extracted separately. The water phase was passed through filter paper to separate any particulate matter. Water samples were extracted by adding 25 mL of dichloromethane, shaking vigorously by hand for 30 s, and then rotating on an extraction roller (Wheaton R2P) at 60 rpm for 18 h. After extraction, the dichloromethane layer was removed using a glass pipette and filtered through sodium sulfate to remove any remaining water. The extract was concentrated under a gentle stream of nitrogen and transferred to a 2 mL glass vial for GC-FID analysis. The sediment and particulate phases were separately extracted using the SOXHLET apparatus. Samples were homogenized with sodium sulfate and placed in a cellulose thimble, which was placed in the SOXHLET extraction chamber and extracted with dichloromethane for 18 h. After extraction, the solvent was transferred to a Zymark tube and evaporated to 1 mL on a TurboVap under a gentle stream of nitrogen. Samples that had high organic content were purified by treatment with silica gel. All samples were solvent exchanged into hexane and transferred to a 2 mL glass vial for GC-FID analysis.

2.3.4. Evaluation of Toxicity of Marine Sediments

2.3.4.1. Ammonia, Sulfide and Redox Analysis

The sediment samples were thoroughly homogenized and subsampled for analysis of sulfide, redox potential (Eh), and ammonia by specific ion electrodes according to the manufacturer's instructions and following established protocol (Hargrave et al. 1995). A subsample of each of the sediments was placed in a tared vessel and dried at 100°C for 24 hours (APHA et al. 1998). The weight was used to convert results to a dry weight basis. Results for sediments are expressed as μg S/g dry weight of sediment for sulfide, μg NH_3-N/g dry weight of sediment for ammonia and millivolts corrected for the normal hydrogen electrode for redox potential.

2.3.4.2. Marine Amphipod Test Procedure

The marine amphipods (*Eohaustorius estuarius*) were purchased from Northwest Aquatic Sciences in Newport, OR, USA. Animals were collected in Yaquina Bay, OR, and shipped to the Environment Canada laboratory in Moncton, NB. The animals were acclimated to and maintained at 15 ± 2°C and 24 ± 2 ppt salinity (average initial salinity of overlying water in the test sediment samples) until used for testing. All samples plus a lab control sample of amphipod collection site sediment were tested.

Industry environmental effects monitoring (EEM) programs generally use *Rhepoxynius abronius* for sediment toxicity testing, but *Eohaustorius estuarius* has been used by the industry when *Rhepoxynius abronius* was not available. The two amphipods are considered of equivalent sensitivity when evaluated in interlaboratory tests by Environment Canada labs. Both species are recommended in the Environment Canada Reference Methods for marine sediment toxicity testing using marine amphipods. Environment Canada considers the amphipod *Eohaustorius estuarius* to be less prone to false positive results (caused by non-contaminant interferences like sediment grain size, pore water salinity, and sediment ammonia content) than *Rhepoxynius abronius.*

The test was conducted according to Environment Canada 1998. On the day prior to starting the test, each container of test sediment was homogenized and 175 mL portions were added to a 1 L glass mason jar (there were no lab replicates tested since there was sufficient replication in the experimental design). For the laboratory control (sample of amphipod collection site sediment), five replicated test jars were set up in order to determine the health of the test animals, the suitability of animal collection, shipping, acclimation and holding procedures, and the cleanliness of laboratory glassware and test chambers. The jars were then filled with 800 mL of clean seawater (salinity was 24 ppt), covered, then aerated overnight with oil free compressed air at a rate of approximately 150 mL/minute.

The following day the amphipods were removed from their holding sediment by sieving the contents through a 500 μm sieve. Animals were double-counted and twenty animals were added to each of the test vessels. Aeration was stopped for thirty minutes to assist the animals in burrowing in the test sediment. Testing was performed with a 24-hour light photoperiod at 15 ± 1°C. Lighting was provided by overhead fluorescent fixtures at an intensity of 500 to 1000 lux. The tests were checked daily for observations, aeration and temperature. Three times a week a field replicate of each sample area was measured for temperature, pH, salinity and dissolved oxygen. At the start and end of the test, 5 mL samples of overlying water were removed from each replicate and combined for each treatment. This combined sample was analysed for concentration of ammonia using a specific ion electrode.

After 10 days, the contents of each jar were sieved through a 500 μm sieve. Any immobile animals were observed under a microscope to determine mortality, defined as lack of all movement when observed under a dissecting microscope for 5–10 seconds. Any animals missing were assumed to be dead. The mean survival and standard deviation of each treatment were calculated.

A reference toxicant test was conducted with cadmium chloride using a water-only exposure for

96-hours duration. Using the mortality data at each test concentration, the 96-hour LC50 (concentration calculated to cause 50% mortality after 96-h exposure) was calculated using the methods of Stephan (1977) with the CETIS statistical software (Tidepool Scientific Software 2002). The 96-hour LC50 for this analysis was entered into the Shewhart Control Chart to ensure that the test was within standard operating limits and that the population of amphipods used in the test was of normal sensitivity.

For the interpretation of the results, a test sediment from a particular sampling treatment is judged to have failed the sediment toxicity test if the mean 10-day survival rate in the test sediment is more than 30% lower than (and is significantly different from) the laboratory control sediment which consisted of sediment "A" or "B" without SBM amendment (Environment Canada 1998). The mean survival of *E. estuarius* in the laboratory control must be ≥80% for the test results to be acceptable.

2.3.4.3. Microtox Solid Phase Assay

This test was conducted according to Environment Canada 2002. This method exposes the bacterium to the sediment sample; if toxic materials are present they interfere with the cellular respiration of the organism. This interference is measured as a decrease in light output by the bacterium, *Vibrio fischeri* (previously *Photobacterium phosphoreum*).

All sediments were tested with this procedure. An aliquot of the wet sediment was transferred to a beaker and stirred for 10 minutes with diluent. A dilution series of 12 concentrations and three controls were prepared from this mixture. Bacterial reagent was added to the dilutions and incubated at 15°C for 20 minutes. These dilutions were filtered and the filtrate transferred to the Microtox analyser. Bioluminescence was recorded after 10 minutes in the analyser. Statistics were performed on the data to calculate an IC50 on a wet weight basis (the concentration of test sediment at which light output by a population of the luminescent bacterium was reduced by 50% when compared to the untreated control population). Three aliquots of the sediment were dried at 100°C for 24 hours and the percentage moisture was determined. The IC50 was corrected for moisture content and quoted on a dry weight basis as mg dry sediment/litre of diluent.

A reference toxicant test using the National Research Council of Canada's Certified Reference Material, HS-5, was performed in the same manner as the sediments. The IC50 for this analysis was entered into the Shewhart Control Charts to ensure that the test was within standard operating limits and that the population of bacteria used in the test was of normal sensitivity. Because the test material was sediment, sediment was required as a reference toxicant. For this reason, this Certified Reference Material was chosen because it is homogeneous, consistent over time, and easily available to all labs conducting the same test in Canada. HS-5 has been the standard reference toxicant used for years by many labs in Canada to monitor the consistency of different batches of Microtox bacterial reagent.

The Interim Guideline for the Environment Canada Ocean Disposal Program (contained in Environment Canada 1996) states that a sample is considered toxic in the Microtox Solid Phase Test if the IC50 is less than 1,000 mg/L corrected for dry weight of solids. More recently two guidelines have been developed for the Environment Canada Ocean Disposal Program to

determine if a sample should be considered toxic (contained in Environment Canada 2002). Guideline 1 states that a sample is judged to have failed this sediment toxicity test if its IC50 is less than 1000 mg/L corrected for dry weight of solids regardless of any grain size. The dry mass of sediment in the slurry was determined by drying samples at 105^{o}C for at least 1h (APHA et al. 1998).

3. Results and Discussion

3.1. Aerobic Biodegradation of SBM in Marine Sediments

3.1.1. Mineralization of SBM in Sediments

Mineralization involves the complete degradation of an organic chemical to its stable inorganic elements such as carbon, hydrogen, nitrogen, phosphorus, and so on. Consequently, mineralization generally entails several successive biological transformations to complete. As a result, the mineralization rate of a chemical is a conservative way of measuring degradation of organic material, which is generally less than the initial transformation rate of the chemical. In other words, mineralization involves the complete degradation of a target chemical, whereas biotransformation of organics may or may not be complete degradation.

Figure 1 shows the mineralization of the SBM with low initial concentrations at low temperature (5^{o}C). The cumulative carbon dioxide production from the sediment control, the used SBM, and the fresh SBM were approximately 2900, 4500, and 6300 µL, respectively, after four weeks of incubation. This is consistent with the consumption (expressed as negative values) of oxygen in each of the microcosm flasks.

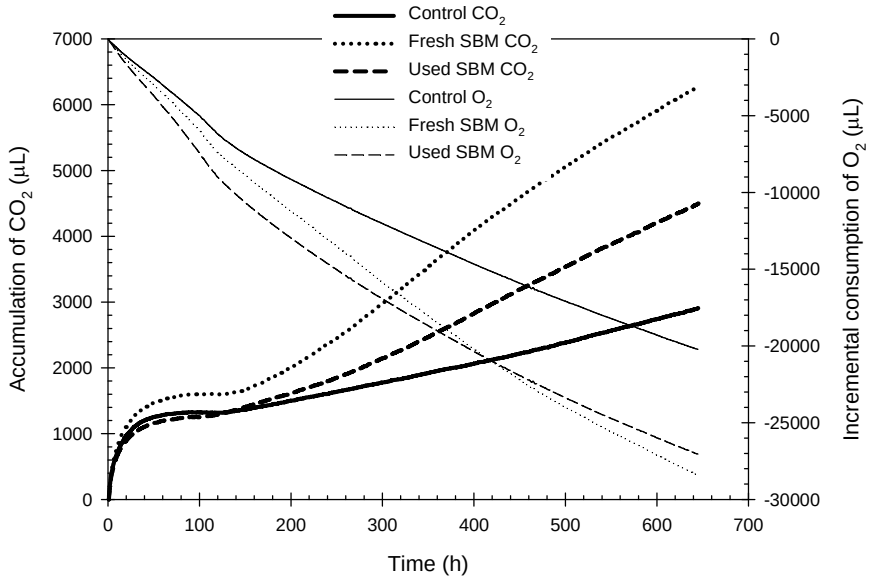

Figure 1: Accumulation of carbon dioxide and incremental consumption of oxygen from degradation of SBM with low initial concentration (10 g/kg) at low temperature (5^{o}C).

Figure 2 presents the mineralization of SBM at the same initial loadings but incubated at 20^{o}C. As expected, the higher temperature stimulated the bacterial activity as indicated by the increased carbon dioxide production rate. The control sediment generated 10300 µL of carbon dioxide. Mineralization of the fresh SBM was more rapid at the high temperature, with the cumulative carbon dioxide production approaching 41000 µL, nearly 10 fold higher than at 5°C. Mineralization of the recycled mud, however, was not stimulated at the high temperature; the carbon dioxide production remained the same irrespective of the incubation temperature.

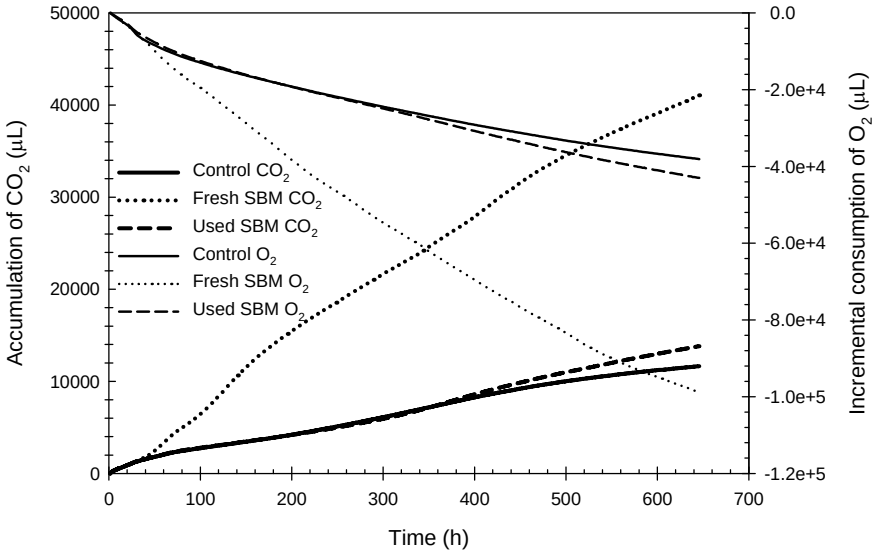

Figure 2: Accumulation of carbon dioxide and incremental consumption of oxygen from degradation of fresh and used SBM with low initial concentrations at high temperature (21°C).

Figure 3 presents the carbon mineralization results for high loadings of the fresh and the recycled SBM at high temperature. The cumulative carbon dioxide production for the control, the recycled SBM, and the fresh mud was found to be 12000, 23000, and 210000 μL, respectively. The two replicate microcosms had a similar rate and extent of carbon dioxide production, with relative percentage difference (RPD) being 6%, 5%, and 13% for the control, low, and high concentration microcosms, respectively.

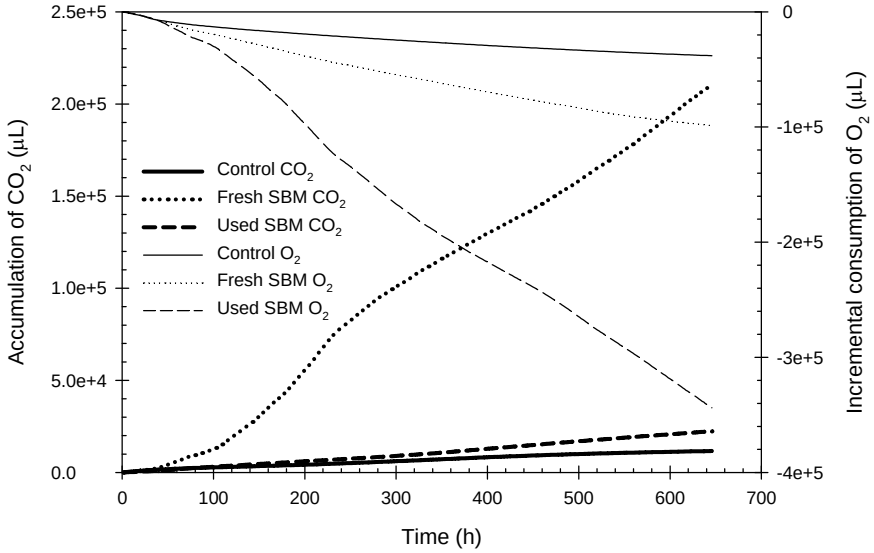

Figure 3: Accumulation of carbon dioxide and incremental consumption of oxygen from degradation of fresh and used SBM with high initial concentrations at high temperature (21°C).

3.1.2. Biodegradation of Petroleum Hydrocarbons of SBM in Sediments

GC-FID analysis confirmed the degradation of TPH during the degradation of SBM. Figure 4 shows the degradation extent of total petroleum hydrocarbon in SBM at different temperatures and initial loading concentrations. After four weeks incubation, 31 ± 4% of TPH in the fresh SBM was degraded at 5°C, in comparison to only 14 ± 8% of TPH degradation in the recycled SBM incubated at the same low temperature. Degradation extent of the fresh SBM with lower initial concentration at 21°C was remarkably increased to 70 ± 1%, which is much higher than degradation of the recycled SBM (16 ± 2%) at same loading and the same high temperature. Biodegradation of the fresh SBM with high initial concentrations at 21°C had approximately 52% TPH degraded within four weeks, which is also considerably higher than degradation of the recycled SBM (17 ± 2%) at the same high loading and high temperature.

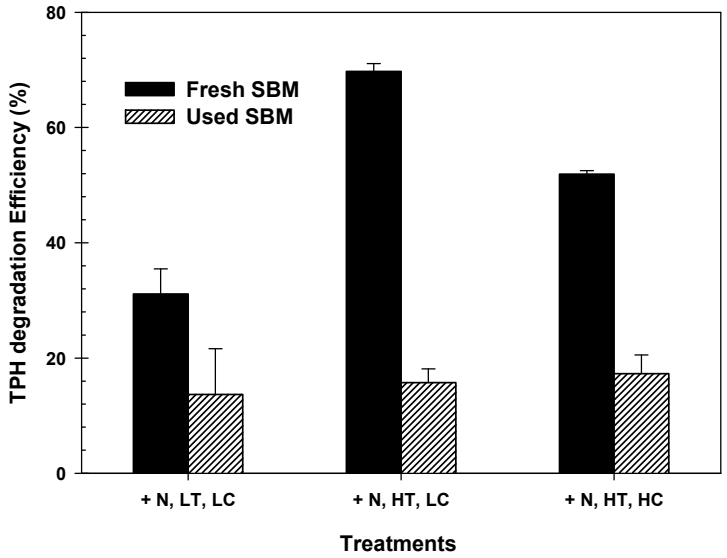

Figure 4: Biodegradation of SBM in the presence of added nutrients (+N) as indicated by the TPH removal efficiency (LT, low temperature; HT, high temperature; LC, low concentration; and HC, high concentration).

Ideally a high loading and low temperature experiment would have been included. However, the number of experiments that can be set up with the Micro-Oxymax respirometer is limited to 20 channels (20 Duran bottle microcosms). As shown in Table 1, all 20 spots have been used. We felt that testing the effect of nutrient amendment on the degradation of SBM was more important, and therefore added the low temperature low-SBM loading with nutrients treatment.

Note that the term "low" and "high" loading of SBM is only relevant to this study for testing purposes. In fact, a "low-loading" of 0.1 g of SBM with 10 g sediment equals 10 g/kg, which is already very high compared to field data (CNSOPB 2005). The "high-loading" condition (equivalent to 100 g/kg) has occurred only in an exceptionally localized area after a spill. We included this extreme situation to test the potential for SBM degradation under spill circumstances. The effect of temperature was evaluated at 0.1 g SBM and from the results it can be inferred that a high loading at low temperature would take longer to biodegrade.

As for the actual seafloor temperatures at around 0^{o}C, the degradation is anticipated to be slower. However, the presence of bacteria adapted to cold temperature may still carry out degradation of hydrocarbons. Further research has to be carried out to clarify these assumptions.

3.1.3. Heavy Metal Contents in SBM

Table 3 summarizes the metal ion contents in the fresh and used SBM samples. The typical heavy metal ion contents are compared with other drilling mud sources (Table 4). The data show that during the operational process there can be accumulation of heavy metals in the recycled drilling muds. Metals play an important role in the life of microorganisms (Bruins et al. 2000). Some metals, such as cobalt, copper and nickel, serve as micronutrients and are used for redox

processes, to stabilize molecules through electrostatic interactions, as components of various enzymes, and for regulation of osmotic pressure. Many other metals, such as silver, aluminum, cadmium, lead, and mercury, are non essential elements, have no nutrient value, and are potentially toxic to microorganisms. Metals at high concentrations are toxic to microorganisms. Toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than do essential metals. Toxicity occurs due to alterations in the conformational structure of nucleic acids and proteins and consequent interference with oxidative phosphorylation and osmotic balance.

Table 3: Metal concentrations in fresh and used synthetic drilling mud samples. Data are the average mg/kg ± one standard deviation. BDL = below detection limit.

Metal	Detection limit	Fresh SBM	Used SBM
Aluminum	< 10	16100.0 ± 424.3	39950.0 ± 1060.7
Antimony	< 0.1	BDL	3.4 ± 0.4
Arsenic	< 1	3.0 ± 0.0	25.5 ± 0.7
Barium	< 1	97.5 ± 2.1	7475.0 ± 1407.1
Beryllium	< 0.1	0.3 ± 0.0	1.0 ± 0.0
Bismuth	< 0.5	BDL	BDL
Boron	< 5	86.5 ± 2.1	70.0 ± 0.0
Cadmium	< 0.01	0.1 ± 0.0	0.9 ± 0.0
Calcium	< 50	419500.0 ± 6364.0	161000.0 ± 5656.9
Chromium	< 1	7.0 ± 0.0	130.5 ± 2.1
Cobalt	< 0.1	2.6 ± 0.0	12.3 ± 0.1
Copper	< 1	1.5 ± 0.7	102.5 ± 0.7
Iron	< 50	4645.0 ± 148.5	26550.0 ± 777.8
Lanthanum	< 0.1	5.2 ± 0.0	16.1 ± 0.8
Lead	< 0.1	4.9 ± 0.1	198.0 ± 0.0
Lithium	< 0.1	180.5 ± 0.7	65.7 ± 0.6
Magnesium	< 10	2470.0 ± 14.1	4850.0 ± 0.0
Manganese	< 1	182.0 ± 0.0	816.5 ± 7.8
Mercury	< 0.2	BDL	0.9 ± 0.0
Molybdenum	< 0.1	1.2 ± 0.1	14.1 ± 0.2
Nickel	< 1	24.0 ± 0.0	56.0 ± 1.4
Phosphorus	< 20	100.0 ± 0.0	380.0 ± 0.0
Potassium	< 20	11950.0 ± 212.1	15150.0 ± 70.7
Rubidium	< 0.1	16.3 ± 0.2	72.9 ± 1.7
Selenium	< 5	BDL	BDL
Silver	< 0.1	BDL	0.8 ± 0.0
Sodium	< 50	10650.0 ± 70.7	10100.0 ± 141.4
Strontium	< 1	4360.0 ± 84.9	4040.0 ± 141.4
Sulfur	< 50	5495.0 ± 49.5	5805.0 ± 431.3
Tellurium	< 0.1	BDL	BDL
Thallium	< 0.1	BDL	0.4 ± 0.0
Thorium	< 0.1	5.0 ± 0.1	4.6 ± 0.3
Tin	< 0.5	2.0 ± 0.0	1.6 ± 0.1
Titanium	< 1	93.5 ± 0.7	249.0 ± 43.8
Uranium	< 0.1	2.1 ± 0.0	3.4 ± 0.0
Vanadium	< 1	4.5 ± 0.7	148.0 ± 0.0
Zinc	< 20	BDL	140.0 ± 0.0

Table 4: Heavy metal concentrations (mg/kg dry weight) in water-based drilling muds (WBM), oil-based drilling muds (OBM) (Neff et al. 1987; Fillio et al. 1987) and synthetic-based drilling muds (SBM this study).

Metal	WBM	OBM	SBM (fresh)	SBM (used)
Aluminum	10800	52000	16000	39950
Barium	720 – 449000	487000	97.5	7475
Cadmium	0.16 – 54.4	0.39 – 12	0.1	0.9
Chromium	0.1 – 5960	190 – 1350	7.0	130.5
Iron	0.002 – 27000	76300	4645	26550
Lead	0.4 – 4226	100 – 290	4.9	198
Mercury	0.017 – 10.4	NR	BDL (< 0.2)	0.9
Zinc	0.06 – 12270	160 – 2100	BDL (< 20)	140

3.1.4. Biodegradation Summary

Laboratory experiments have been conducted to evaluate the biodegradability of synthetic drilling mud in marine sediments. The degradation of the total petroleum hydrocarbons and the extent of carbon mineralization were used to monitor the rate and extent of biodegradation. The fresh SBM was readily biodegradable under aerobic conditions as illustrated by both the mineralization and biodegradation results. For experimental concentrations of 10 and 100 g/kg, within four weeks incubation, as much as 70% and 52% of TPH was degraded, respectively, at 21^{o}C. Degradation rates were slower at the low temperature. The used SBM had much higher concentrations of heavy metals. As a result, except at lower loading and high temperature, the aerobic biodegradation and mineralization of hydrocarbons in used SBM were severely inhibited. Carbon dioxide production was only 10% in comparison with the fresh mud degradation. Correspondingly, removal efficiency of hydrocarbons in used SBM was much lower than in fresh.

The volume of drilling muds used in commercial operations may result in the alteration of environmental conditions associated with "burial" of spilled muds. This can be caused either by physical processes (e.g., water currents, deposition in seafloor sand waves, anthropogenic disturbance), or by activities of benthic organisms (e.g., burrowing, irrigation, and other bioturbation) (Snelgrove 1998). Further research is needed to assess the persistence of SBM under anoxic conditions and the biological impact of the drilling materials on benthic organisms.

3.2. Evaluation of Toxicity of SBM in Marine Sediments

3.2.1. Sediment Characterization

Table 5 summarizes the grain size analysis results of the two marine sediments that were used for the toxicity testing. Because the sediments were passed through a 1mm stainless steel sieve, there was no gravel present. Sediment type "A" consisted of 99.5% sand, 0.2% silt and 0.3% clay, and had a total organic carbon content of 0.1%. Sediment type "B" retrieved from the saltwater

marsh consisted of 0.1% gravel, 93.9% sand, 5.1% silt, and 0.9% clay, and had much higher total organic carbon content of 1.4%.

Table 5: Grain size analysis of the two types of sediments that were used in SBM toxicity tests.

Phi scale	µm	Sediment type "A"	Sediment type "B"
-2.0	4000	100.0	100.0
-1.0	2000	100.0	100.0
0.0	1000	100.0	99.7
1.0	500	100.0	96.8
2.0	250	95.6	84.1
3.0	125	2.3	11.1
4.0	62.5	0.5	6.0
5.0	31.3	0.4	4.9
6.0	15.6	0.3	2.8
7.0	7.8	0.3	1.3
8.0	3.9	0.3	0.9
9.0	2.0	0.3	1.1
% Gravel	> 2000	0.0	0.1
% Sand	62.5 ~ 2000	99.5	93.9
% Silt	3.9 ~ 62.5	0.2	5.1
% Clay	< 3.9	0.3	0.9
Total Organic Carbon (%)	-	0.1	1.4

Table 6 shows the trace metal analysis results of the two marine sediments that were used for the toxicity testing. Samples were air dried and sieved at 2 mm. Portions were digested according to EPA Method 3050B. The resulting solutions were analyzed for trace elements by ICP-MS. The two sediments show close profiles of trace metal concentrations. The indicator inorganic substances, including arsenic, cadmium, chromium, copper, lead, and zinc, have concentrations much lower than that of the sensitive contaminated sites criteria (CCME 1998) and would not have contributed much to the toxicity of the sediments. Similar analysis was conducted for the total polycyclic aromatic hydrocarbons in the two sediment types. Most of the tested PAHs are less than the method detection limit (0.01 mg/kg), except for a trace amount of phenanthrene, fluoranthene, pyrene, chrysene/triphenylene, and benzo(b)fluoranthene detected in the type "B" marsh sediment.

Table 6: Trace metal analysis of the two types of sediments that were used in SBM toxicity tests.

Metal	QA/QC (98357 RB)	CRM (NIST 2709a)	Type "A" (mg/kg)	Type "B" (mg/kg)
Aluminum	< 1	24500	3690	3580
Antimony	< 0.1	0.1	< 0.1	< 0.1
Arsenic	< 1	8	2	2
Barium	< 1	425	31	5
Beryllium	< 0.1	0.7	0.2	0.1
Bismuth	< 1	< 1	< 1	< 1
Boron	< 1	37	3	8
Cadmium	< 0.01	0.34	0.03	0.02
Calcium	< 50	13300	5520	1020
Chromium	< 1	74	7	6
Cobalt	< 0.1	11.2	2.8	2.2
Copper	< 1	30	3	26
Iron	< 20	27800	9120	7330
Lead	< 0.1	11.3	3.7	9.0
Lithium	< 0.1	35.2	8.3	9.6
Magnesium	< 10	12200	2850	2330
Manganese	< 1	456	213	114
Molybdenum	< 0.1	0.8	0.1	0.4
Nickel	< 1	72	6	6
Potassium	< 20	3590	590	560
Rubidium	< 0.1	33.2	4.0	3.9
Selenium	< 1	1	< 1	< 1
Silver	< 0.1	< 0.1	< 0.1	< 0.1
Sodium	< 50	520	2960	3120
Strontium	< 1	109	12	12
Tellurium	< 0.1	< 0.1	< 0.1	< 0.1
Thallium	< 0.1	0.2	< 0.1	< 0.1
Tin	2	< 1	< 1	< 1
Uranium	< 0.1	1.7	0.3	0.5
Vanadium	< 1	68	13	9
Zinc	< 1	89	17	19

3.2.2. Biodegradation of SBM in Shoreline and Salt-marsh Sediments

The biodegradation of SBM was evaluated by monitoring the change in concentration of total hydrocarbons as IPAR over the duration of the experiment. Figure 5A shows the biodegradation of SBM in sediment type "A" between T = 0d, T = 30d and T = 90d, expressed as the percentage of total hydrocarbons remaining, while Figure 5B shows the same results for sediment type "B." Samples were separated into three fractions during the extraction procedure (water, particulate, sediment), and the results were combined to give the total hydrocarbon concentration in each microcosm. For sediment type "A," 13±2% of the total hydrocarbons in the fresh mud was degraded after 30 days, while 22±6% of the total hydrocarbons in the recycled mud was degraded. Hydrocarbon degradation was higher in sediment "B" with 44±20% of the total hydrocarbons in the fresh mud degraded after 30 days and 59±18% degraded in the recycled mud. In both sediments, hydrocarbons in the recycled mud appear slightly more degraded than

the fresh mud. After 90 days incubation in sediment "A," 26±3% of the total hydrocarbons in the fresh mud were degraded, while 40±7% of the total hydrocarbons in the recycled mud were degraded. There was very little change in hydrocarbon degradation after 90 days incubation for type "B" (saltwater marsh sediment) samples. Table 7 summarizes the results from all hydrocarbon analysis.

In the control samples, in which microcosms were amended with mercuric chloride, the total hydrocarbon concentration decreased by 8 ± 5% after 90 days, indicating that losses due to abiotic processes played a minor role in the degradation of SBM. Table 7 also shows that the recovery of total hydrocarbons as isoparaffin (IPAR) from sediment "B" (marsh) was lower compared to sediment "A" (beach sand). This is likely due to strong interactions between the hydrocarbons and the organics in the marsh sediment, making it difficult to extract all of the hydrocarbons from the sample. The binding of hydrocarbons to organic material in the marsh sediment may also have reduced their bioavailability and subsequently the apparent sediment toxicity, although this would only be the case at the beginning of the experiment. After initially adding the SBM to the microcosm, the hydrocarbons would rapidly quench the limited number of potential binding sites, while the remaining hydrocarbons would coat the inorganic material (sand grains) and/or dissolve in the water phase. Over the duration of the experiment, any further reduction in toxicity would be a result of the biodegradation of non-bound hydrocarbons (Cornelissen et al. 2005).

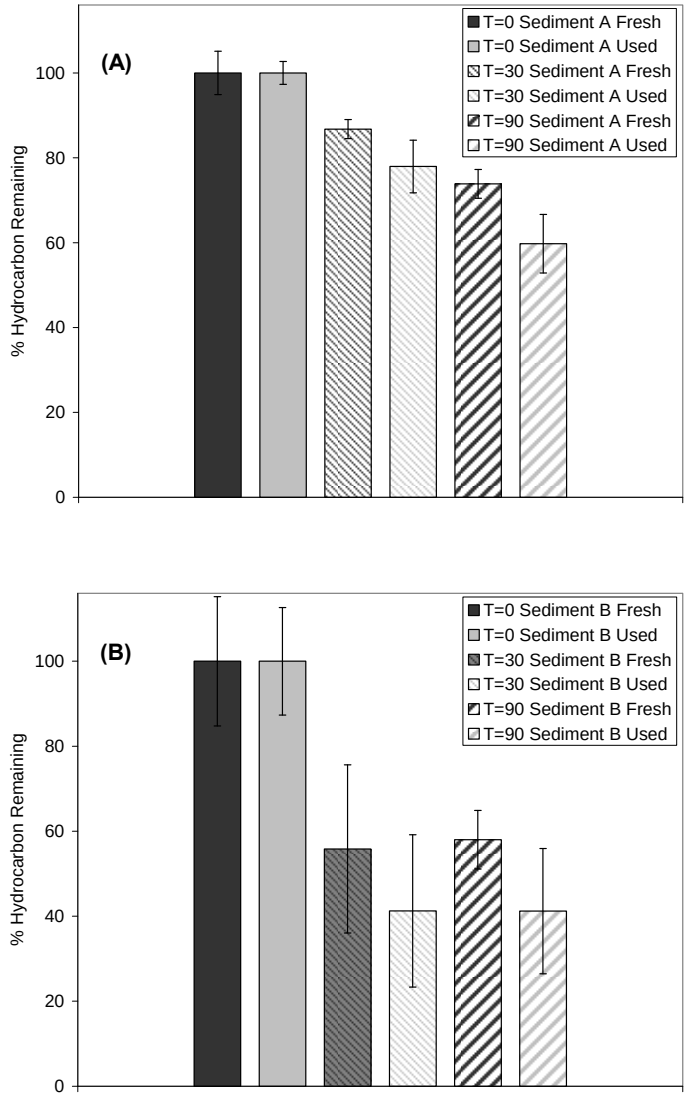

Figure 5: Biodegradation of SBM hydrocarbons in ("A") beach sand sediment "A" and ("B") marsh sediment "B," expressed as a change in the percentage of hydrocarbons remaining after 30 and 90 days incubation.

Table 7: Summary of SBM hydrocarbon degradation results for sand sediment "A" and marsh sediment "B," including results for the sterile control.

Sediment Type	SBM Type	Total IPAR (g)		
		T = 0	T = 30	T = 90
A	None	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A	Fresh	8.41 ± 0.43	7.30 ± 0.16	6.22 ± 0.21
A (Sterile control)	Fresh	--	8.43 ± 0.32	7.72 ± 0.37
A	Used	6.25 ± 0.17	4.87 ± 0.30	3.73 ± 0.26
B	None	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
B	Fresh	5.45 ± 0.83	3.04 ± 0.60	3.16 ± 0.26
B	Used	4.36 ± 0.55	1.80 ± 0.32	1.80 ± 0.26

3.2.3. Toxicity of SBM in Shoreline and Salt-marsh Sediments

The Microtox Solid Phase Test results are presented in Figure 6. The baseline toxicity of the two sediments differed significantly, likely since marsh sediment "B" has more fine and organic matter (Table 5) and higher concentrations of ammonia and sulfide as well as more reduced conditions (Table 8). In the spiked type "A" sediment, there is no trend in toxicity (reduction or increase) between T = 0 and T = 30 (Fig. 5A). However, the EC_{50} values seemed to increase in the control and decrease in the SBM spiked type "B" sediments (Fig. 5B). Nevertheless, all the measured EC50 values are several orders of magnitude higher than the threshold toxicity value, 1000 mg/L, as established in the ocean dumping guidelines of Environment Canada (Tay et al. 1997), meaning that all the controls and the SBM amended sediments were non-toxic to the tested *V. fischeri* bacterium specifically in terms of the regulatory guidelines.

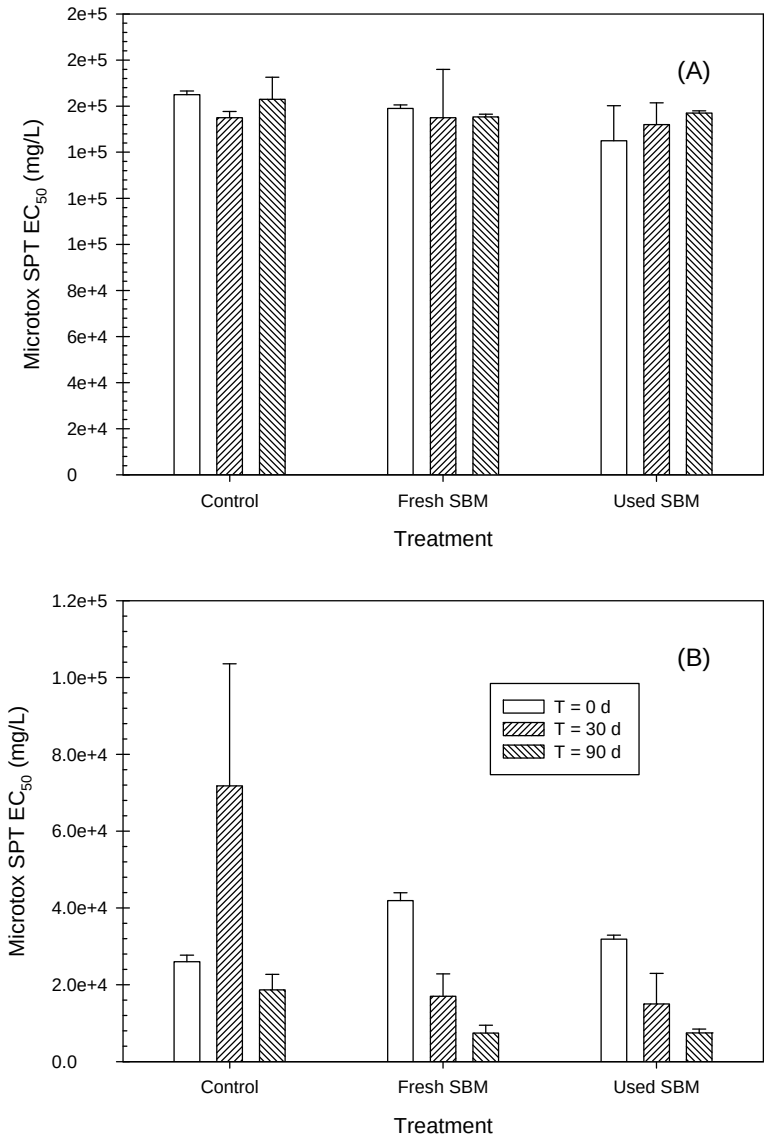

Figure 6: Microtox Solid Phase Test results of sediment toxicity for the different treatment conditions in (A) sand sediment A, and (B) marsh sediment B.

Figure 7 shows the amphipod *Eohaustorius estuarius* toxicity data after exposure to the control and SBM-contaminated sediments. The control sediment had more than 98% survival of *E. estuarius* amphipods before and after the 30-day incubation period. Survival was also greater than 80% in the 90 d. biodegraded control sediment. However, the addition of either fresh or recycled SBM to sediment "A" resulted in severe toxicity to the amphipod species, with only 3.3 and 1.7% survival rate, respectively.

After 30 days of incubation, the biodegradation of SBM hydrocarbons resulted in a reduction of the apparent toxicity, as shown by the amphipod survival rates which increased to 15% and 20% respectively, in the fresh and recycled mud amended with type "A" sediment. A similar trend was observed in type "B" sediment in which SBM amendment showed toxicity before and after

biodegradation. The survival rates in type "B" sediment amended with either fresh or recycled SBM were higher than those in type "A" sediments during the first 30 days of incubation; that is, the apparent toxicity of the SBM appeared lower in sediments containing higher concentrations of organic matter during the first 30 days of incubation. This is probably due to the higher content of natural organic matter contributing to sequestration of contaminants, therefore reducing their bioavailability to the test species. As explained previously, after the initial addition of SBM, the hydrocarbons would rapidly quench the limited number of potential binding sites, while the remaining hydrocarbons would coat the inorganic material or dissolve in the water phase, and over the duration of the experiment, any further reduction in toxicity would be a result of the biodegradation of free hydrocarbons. Addressing the question of whether or not the binding of hydrocarbons to sediment may cause a reduction in toxicity is beyond the scope of this study. It would require another experimental design to investigate the transport of contaminants and its impact on exposure toxicity to benthic organisms. This interesting phenomenon could have significant implications to the environmental assessment of SBM contamination in marine sediments.

Following 90 days of incubation, type "B" sediment amended with both fresh and used SBM caused much lower survival of the tested marine amphipod, showing that the sediment became more toxic after 90 days of biodegradation of organic components. The elevated toxicity could have been due to the biotransformation of the original organic compounds to intermediate degradation products with perhaps increased bioavailability properties, therefore increasing the toxicity to the amphipod. In addition, due to the active degradation of organic materials, dissolved oxygen was consumed and re-aeration of the water and sediment layer was insufficient and the sediment became anoxic as indicated by the drastically reduced Redox potential (Table 8). The anoxic state was also indicated by significant accumulation of sulfide and ammonia in sediments (Table 8). Elevated ammonia and sulfide concentrations in sediment can cause toxicity to *E. estuarius* (Tay et al. 1998).

Elevated ammonia levels are known to have little effect on *V. fischeri* although sulfide tends to be toxic to the bacterium; however, at elevated ammonia levels, the increased pH is known to decrease the toxicity of sulfide to *V. fischeri* (Stronkhorst et al. 2003). Conversely, the increased pH due to ammonia has been found to increase sulfide toxicity to the marine amphipod *Corophium volutator* (Stronkhorst et al. 2003). This could explain why the sediments were less toxic to the marine bacterium than they were to the amphipod.

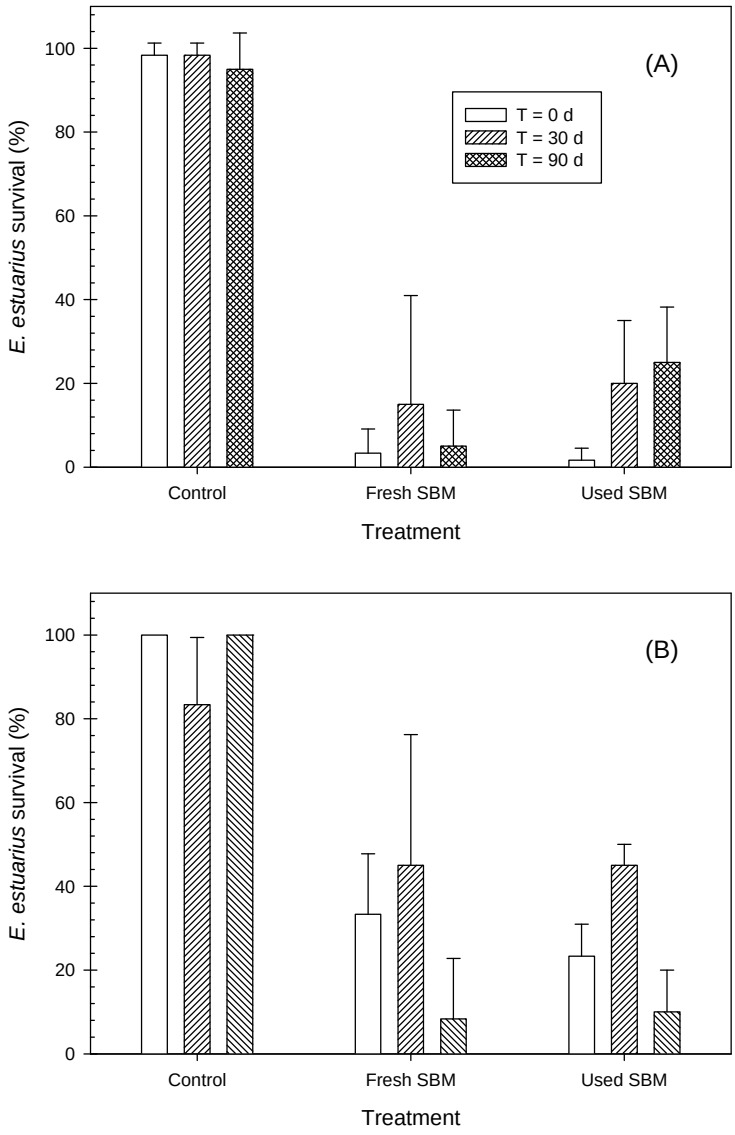

Figure 7: Amphipod toxicity results for the different treatment conditions in (A) type "A" and (B) type "B" sediment.

Table 8: Sediment ammonia (mg NH4+-N/kg dry sediment), sulfide (mg S/kg dry sediment), and Redox potential (millivolts or mV, corrected for normal hydrogen electrode).

Sediment	Treatment	Ammonia		
		t = 0	t = 30 day	t = 90 day
Type A	Control	0.46 ± 0.01	0.49 ± 0.11	0.34 ± 0.31
	Fresh SBM	0.32 ± 0.06	0.10 ± 0.01	0.24 ± 0.18
	Used SBM	0.24 ± 0.01	0.15 ± 0.06	0.41 ± 0.07
Type B	Control	15.47 ± 1.02	9.46 ± 1.30	8.23 ± 1.70
	Fresh SBM	0.68 ± 0.13	1.45 ± 0.16	9.83 ± 2.24
	Used SBM	0.68 ± 0.24	1.92 ± 0.14	26.70 ± 15.24

Sediment	Treatment	Sulfide		
		t = 0	t = 30 day	t = 90 day
Type A	Control	<0.01	0.03 ± 0.04	<0.01
	Fresh SBM	<0.01	0.41 ± 0.35	3.15 ± 0.86
	Used SBM	<0.01	0.14 ± 0.10	1.49 ± 0.19
Type B	Control	3.57 ± 1.35	10.83 ± 0.31	14.47 ± 0.06
	Fresh SBM	9.25 ± 7.90	20.07 ± 1.85	30.43 ± 1.86
	Used SBM	8.43 ± 2.59	17.17 ± 1.27	30.67 ± 2.40

Sediment	Treatment	Redox		
		t = 0	t = 30 day	t = 90 day
Type A	Control	412.33 ± 12.66	392.23 ± 11.02	350.67 ± 20.03
	Fresh SBM	372.67 ± 9.29	340.67 ± 12.58	279.67 ± 2.52
	Used SBM	390.00 ± 15.10	357.33 ± 19.40	280.67 ± 12.22
Type B	Control	164.00 ± 23.07	38.17 ± 25.54	24.33 ± 18.50
	Fresh SBM	-30.33 ± 6.43	6.33 ± 12.01	-30.67 ± 35.25
	Used SBM	-75.67 ± 13.58	-3.00 ± 6.24	-54.33 ± 14.19

4. Summary and Conclusions

Laboratory experiments have been conducted to investigate the biodegradability of synthetic-based drilling mud in marine sediments. In the first study, the removal of the total petroleum hydrocarbons and the extent of carbon mineralization were used to monitor the biodegradation of SBM in marine sediments. Experimental results after four weeks of incubation indicated that the fresh SBM rapidly degraded in marine sediments under all tested conditions, whereas the recycled mud degraded much more slowly. In the second study using sediments that were allowed to biodegrade for one month and two months, the toxicity of the SBM samples in the two sediment types was evaluated using a marine amphipod survival test and the Microtox Solid Phase Test. The results of the Microtox SPT tests indicated that the presence of the SBM in the two sediments posed no severe toxicity to the marine luminescent bacterium *Vibrio fischeri*. The amphipod test, however, showed that the fresh and recycled SBM in both sediment types were severely toxic to *Eohaustorius estuarius*. The difference in toxicity response is likely attributable to the different mode of action in these tests. These data serve as a good start in better understanding the fate and effects of SBM in the offshore environment as a result of accidental spills or routine discharges of drill cutting materials. If the total organic carbon of

affected offshore sediment is known, its affinity for SBM hydrocarbons could be inferred based on the results from this project; however, it is important to note that the persistence and toxicity effects in deep marine sediments may vary from the data that were obtained in these laboratory tests due to other factors that can affect the ultimate biodegradation rate and toxicity of these materials in the field.

5. Publications and Presentations

1. Li, Z., Robinson, B., Ma, X., Cobanli, S., Lee, K., 2009. Biodegradation of synthetic drilling mud in marine sediments. In: *Proceedings of the 10th International in situ and On-site Bioremediation Symposium.* May 5–8, Baltimore, MD.
2. Li, Z., Robinson, B., Lee, K., Doe, K., Jackman, P. 2010. Biodegradation and toxicity of SBM in marine sediments. Presented in: The 33rd AMOP Technical Seminar. June 6–8, Halifax, NS.

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