

CHEMICAL COMPOSITION CHANGES AND BIODEGRADATION POTENTIALS OF NINE ALASKAN OILS UNDER FRESHWATER INCUBATION CONDITIONS.

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ABSTRACT

Nine representative Alaskan crude oils and oil products commonly transported in Alaska were weathered and incubated using the Environment Canada freshwater oil biodegradation protocol. A complete "total oil analysis method by GC/MS and GC/FID" was developed for monitoring and quantifying the resultant compositional changes of the biodegraded oils. All of the oils exhibited measurable hydrocarbon losses after incubation. Various indices were defined to describe the biodegradability of the oils. The Jet B Fuel and Diesel No.2 were more biodegradable than Alaska North Slope and Cook Inlet crude oils, while the Bunker C/Diesel mixture was found to be less biodegradable than these crude oils. The screening procedure developed in this work would be useful in optimizing the application of bioremediation technology for the cleanup of spilled oils in selected freshwater environments. In addition, an analytical method has been developed to differentiate oil composition changes due to weathering from those due to biodegradation.

INTRODUCTION

Bioremediation has received much attention as an option for oil spill cleanup [1-8], especially for inaccessible or sensitive environments. However, before bioremediation can be considered a realistic method for spill response, two factors must be considered. First, is the oil relatively biodegradable? Secondly, if the oil is biodegradable, to what degree does the oil composition change? These data are critical for making the least environmentally damaging and most cost-effective cleanup decisions. Oils vary greatly in their chemical composition, depending on their geological sources. There is no point in using an effective and relatively non-toxic bioremediation agent on a crude oil or oil product that is not going to degrade readily.

At present, little information exists on the biodegradation potential of crude oils and oil products in Arctic freshwater systems. The purpose of this study is to determine the biodegradation potential, under freshwater conditions, of nine crude oils and oil products commonly transported in Alaska, using a defined microbial inoculum and quantitative oil analysis, ranking the 9 oils, in terms of susceptibility to biodegradation. With this data, spill responders would be able to make judicious decisions regarding clean-up effort and the potential use of bioremediation strategies in Arctic freshwater environment.

METHODS

Weathering of Nine Oils and Oil Products

Nine oil and oil products were selected for biodegradation tests under freshwater incubation conditions based on discussions with the Alaska Department of Environmental Conservation (Camille Stephens), the Cook Inlet Regional Citizens Advisory Council (Ruth Post) and the Cook Inlet Spill Response Group (Eric Haugstaud). They are North Slope (NS) oils collected from Northern Pipeline, Middle Pipeline, and Southern Pipeline respectively, Jet B, Diesel No.2, Cook Inlet (Granite Point), Cook Inlet (Trading Bay), Cook Inlet (Swanson River), Bunker C/Diesel Mixture.

The oils were collected in uncoated one gallon (U.S.) paint cans and were weathered at the Environmental Technology Centre (ETC) by pouring them into 250 mL beakers and letting them stand for 24 hours in a fumehood. The evaporative loss by mass varied in a wide range from 0.23% for Bunker C/Diesel mixture to 22.16% for Cook Inlet (Granite Point).

Oil Biodegradation Method

The nine weathered Alaskan oils were tested for biodegradation potential in batch of three. Eight flasks were prepared for each oil (Table 1). Briefly, the tests were conducted as follows: Two hundred mL of standard freshwater medium [9] was added to a 500 mL Erlenmeyer flask and sterilized with an autoclave. After cooling, the appropriate flasks were supplemented with 4 mL of a filter-sterilized stock solution of nitrogen and phosphate (+N, P) (1 g/L of NH_4Cl ; 2 g/L of KNO_3 ; 0.5 g/L K_2HPO_4). Six microbial strains (3 aliphatic degraders and 3 aromatic degraders) comprised the standard freshwater inoculum used in the freshwater oil biodegradation potential test. Oil was added gravimetrically (400 mg) to each flask, using a 5 mL ground glass syringe, weighed before and after each oil addition to the nearest 0.1 mg. Duplicate, uninoculated flasks served as sterile 'weathering' controls to account for abiotic oil losses through volatility and dissolution during the

incubation period. Alberta Sweet Mixed Blend (ASMB, our standard reference oil) was included with each batch of 3 Alaskan oils tested, for quality control. All flasks were incubated at 10 degree Celcius in darkness with shaking at 200 rpm for 28 days. After incubation, 1.0 mL of surrogate standard solution (*o*-terphenyl, *d*₁₀phenanthrene, squalane) was added and the residual oil was extracted with spectrophotometric-grade dichloromethane and subjected to a comprehensive chemical analysis.

Table 1. Study Design for Assessing Oil Biodegradation Potential
(✓ = present; ✗ = absent)

FLASK	ALASKAN OIL OF INTEREST	FRESHWATER INOCULUM	NUTRIENTS (N,P)
Positive Controls (+N,P); n = 3	✓	✓	✓
Negative Controls (-N,P); n = 3	✓	✓	✗
Sterile Controls n = 2	✓	✗	✓

Chemical Analysis of Oil Composition Changes

Each residual oil extract was made up to 5.0 mL with hexane using a 5.0 mL volumetric flask. A 200 µL aliquot of the diluted residual oil extract (about 16 mg of oil) was quantitatively transferred onto the pre-conditioned silica-gel column for oil hydrocarbon group fractionation [10].

The following groups will be referred to when describing and discussing oil composition changes during biodegradation:

TPH or total gas chromatograph (GC) detectable petroleum hydrocarbons (GC-TPH): the sum of concentrations of all GC-resolved and unresolved hydrocarbons. The resolvable hydrocarbons appear as peaks and the unresolvable hydrocarbons appear as the area between the lower baseline and the curve defining the base of resolvable peaks;

Total aliphatics: the sum of concentrations of all resolved and unresolved aliphatic hydrocarbons including the total n-alkanes, branched alkanes, and cyclic saturates;

Total aromatics: GC-TPH minus the total saturates;

Total n-alkanes: the sum of concentrations of all resolved n-alkanes (from C₈ to C₄₀ plus pristane and phytane);

Total of 5 alkylated PAH homologous series: the sum of concentrations of five target alkylated homologues of naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene determined by GC/MS.

Analyses for *n*-alkane distribution and total petroleum hydrocarbons (TPH) were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detector (FID) and an HP 7673 autosampler. A 30-m x 0.32-mm id. (0.25-µm film) DB-5 fused silica capillary column (J&W, Folsom, CA, USA) was used. The carrier gas was helium (2.5 mL/min). The injector and detector temperatures were set at 290 °C and 300 °C, respectively. The oven temperature program was: a 2 minute hold at 50 °C; ramp to 300 °C at 6 °C/min; and finally a 16 minute hold at 300 °C. A 1 µL aliquot was injected in splitless mode with a 1 minute purge-off.

Analyses of target polycyclic aromatic hydrocarbons (PAHs) and biomarker compounds were performed on an HP Model 5890 GC equipped with a Model HP 5972 mass selective detector (MSD). System control and data acquisition were achieved with an HP G1034C MS ChemStation (DOS series). The MSD was operated in the selected ion monitoring (SIM) mode for quantitation of target compounds. An HP-5 fused-silica column with dimensions of 30-m x 0.25-mm id. (0.25-µm film) was used. The chromatographic conditions were as follows: carrier gas, helium (1.0 mL/min); injection mode, splitless; injector and detector temperature, 290 °C and 300 °C respectively. The temperature program for target PAHs was: 90 °C for 1 minute, ramp to 160 °C at 25 °C/min and then to 290 °C at 8 °C/min, and hold for 15 minutes. The temperature program for alkylated PAHs and biomarker compounds was: 50 °C for 2 minutes, ramp to 300 °C at 6 °C/min and hold 16 minutes.

For details of methodologies of fractionation, analysis quality control and quantification, please refer to references [10-14].

RESULTS AND DISCUSSIONS

Chemical Composition of the Weathered Source Oils

Figures 1 through 4 show representative GC chromatograms of sterile controls, negative controls, and positive controls, illustrating the changes in chemical composition of biodegraded oil samples. However, in order to put the biodegradation data in perspective, a brief overview of the initial composition (% by weight) of the weathered crude oils and weathered oil products is useful. All of the crude oils and oil products tested had a ratio of aromatics to saturates of about 1:3, with

the exception of the Bunker C/Diesel mixture, in which it was about 1:1 (Fig. 5). The *n*-alkanes accounted for 13 to 19% of the saturate fraction in all of the oils and oil products, with the exception of the Bunker C/Diesel mixture, in which they accounted for only 8%. The 5 PAH homologous series accounted for a very small fraction of the total aromatics in all of the oils and oil products, ranging from 4% in the Bunker C/Diesel mixture to 13% in Diesel No. 2. The distribution of 5 alkylated PAH homologous series was quite similar for all of the crude oils, with the two most dominant being naphthalenes (approx. 70%) and phenanthrenes (approx. 20%). However, the relative contribution of dibenzothiophenes was notably different between the North Slope and Cook Inlet oils. The North Slope oils had approximately 9% dibenzothiophenes, while the Cook Inlet oils had only approximately 1% dibenzothiophenes. The three oil products differed significantly from the crude oil pattern and from each other. The alkyl naphthalene series accounted for 99% of the 5 alkylated PAH homologous series for Jet B Fuel and 86% for Diesel No. 2, respectively. In the Bunker C/Diesel mixture, naphthalenes and phenanthrenes each accounted for approximately 35%, and chrysene accounted for 18%.

In addition to the features described above, the following characteristics of the oil products should be noted, since they are very different from the crude oils. The composition of Jet B Fuel (sterile controls) was as follows:

- (1) The *n*-alkanes formed a narrow Gaussian distribution from *n*-C₉ to *n*-C₁₇ with maxima at *n*-C₁₂. The sample also contain a large amount of unresolved complex mixture (UCM).
- (2) The ratios of GC-resolved peaks to total GC area was much higher than for all the crude oils (-0.36 and 0.33 for F1 and F3 of the sterile control respectively).
- (3) Concentrations of pristane and phytane were very low.
- (4) C₄-phenanthrene, C₃-dibenzothiophene, and alkylated chrysene homologues were not detected.

(5) For other PAHs, only low molecular weight and low-number-ring PAHs such as biphenyl, acenaphthalene, and acenaphthene were detected.

(6) Biomarker compounds were not detected.

The sterile controls of Diesel No. 2 showed similar chemical composition features as Jet B Fuel sterile controls with the following exceptions:

- (1) A broader distribution of *n*-alkane from *n*-C₉ to *n*-C₂₃ with a maximum being around *n*-C₁₂ to *n*-C₁₄.
- (2) A significant amount of *n*-C₁₇ and pristane, and *n*-C₁₈ and phytane.
- (3) Significantly higher abundances of alkylated phenanthrene (C₆C₁), dibenzothiophene (C₆C₂), and fluorene (C₆C₃) series.
- (4) Very low concentrations (sub-ppm levels) of C₂₀C₂₄ terpanes.

The GC traces of Bunker C sterile control revealed that the Bunker C sample was a mixture of a large portion of heavy residual fuel (so-called "old-type Bunker C") with a smaller portion of lighter diesel fuel. The *n*-alkane distribution featured two "humps", with the maxima around *n*-C₁₃ and *n*-C₂₄.

Chemical Composition Changes of Hydrocarbons due to Biodegradation

General biodegradation trends of various hydrocarbon groups are summarized as follows.

Biodegradation of GC-TPH, total saturates and total n-alkanes:

For all of the crude oils and oil products, biodegradation were greatly enhanced when nutrients were present, as shown by the reductions in GC-TPH, total saturates and total *n*-alkanes (Figs. 6 and 7). When nutrients were present, GC-TPH, total saturate and total *n*-alkane losses were greater than 20%, 20%, and 90%, respectively for the crude oils, and greater than 15%, 20%, and 70%, respectively, for the oil products. In contrast, in the absence of nutrients, however, GC-TPH, total saturate and total *n*-alkane losses were on average $\leq 10\%$, $< 10\%$ and $< 15\%$, for all crude oils and oil products except for the Bunker C/Diesel mixture, which had a total *n*-alkane loss of approximately 30%.

In the Bunker C/Diesel mixture, the largest losses of *n*-alkanes occurred in the lighter components of the diesel portion. The resolved aliphatics, including *n*-alkanes and isoprenoids, remained largely unchanged even when nutrients were added in both Jet B Fuel and Diesel No. 2.

Biodegradation of total aromatics and 5 alkylated PAH homologous series:

For crude oils, the degradation of total aromatics and 5 alkylated PAH homologous series was not enhanced by addition of nutrients (Figs. 8 and 9). In some cases, greater losses occurred in the absence of nutrients. It is interesting to note that the biodegradation of both Jet B Fuel and Diesel No. 2 was enhanced for the aromatics and 5 alkylated PAH homologous series when nutrients were added (Figs. 8 and 9). However, it should be noted that lower molecular weight PAHs were the dominant components in Jet B and Diesel No. 2. In contrast, the losses in the higher molecular weight Bunker C/Diesel mixture were more similar to those of the crude oils (Figs. 8 and 9).

Among the 5 alkylated PAH homologous series, the alkyl homologues of naphthalene (the most abundant, 2-rings) were the most easily degradable, followed in order, by alkyl homologues of dibenzothiophene (sulphur-containing 3 rings), fluorene (3 rings, 13 carbons) and then phenanthrene (3-rings, 14 carbons). Alkylated chrysenes (4-rings) were the most biodegradation

resistant of the target PAH homologues. No significant signs of degradation in the homologous chrysenes was observed in most samples.

The target parent PAHs such as naphthalene, dibenzothiophene, fluorene, and phenanthrene were the most significantly degraded in each alkylated PAH series. The degradation order of $C_0 > C_1 > C_2 > C_3 > C_4$ derivatives of the PAHs is pronounced, and particularly striking within the alkylated naphthalene family. It is clear that both the rate and degree of degradation decrease dramatically as the number and size of substitute alkyl groups in aromatic rings increase.

Among the 5 target alkylated PAH series, the alkylated naphthalene series, especially naphthalene and its C_1 , C_2 , and C_3 - derivatives made up the largest portion of the losses of the five target alkylated PAH series. Among other target PAHs, the low-molecular-weight PAHs with lower aromatic ring numbers such as biphenyl and acenaphthalene were nearly completely lost, while the high-ring-number PAHs did not degraded to a significant degree or not degraded at all. (Note that no other high-ring PAHs were detected in the source Jet B Fuel and Diesel No. 2).

BTEX and Alkylbenzenes:

The crude oils and oil products contained significant amounts of BTEX compounds and alkylbenzenes, but the corresponding sterile controls only contained very small amounts. This indicates that evaporative loss of BTEX and alkylbenzenes occurred during the incubation period.

In the oils and oil products, two major alkylbenzene peaks eluted before the naphthalene peak (retention time, 12.65 min) in the sterile control. They were identified to be 1,2,3,4-tetramethylbenzene (RT = ~11.84 min) and 1,2,3,5-tetramethylbenzene (RT = ~11.06 min). These two C_4 -benzene compounds were biodegradable, with greater losses observed when nutrients was added. The C_4 -benzene compounds, especially the late-eluted 1,2,3,4- and 1,2,3,5-tetramethylbenzenes, were more biodegradation-resistant than naphthalene, and C_1 and C_2 -naphthalenes.

Biomarker Compounds:

No noticeable sign of degradation of biomarker terpanes and steranes was observed in all of the crude oils and oil product samples.

Biodegradation Potential Index

All of the oils tested had measurable losses of hydrocarbons as a result of incubation with the standard microbial inoculum. Abiotic losses were accounted for through the use of sterile controls, therefore the reported losses are definitely due to biodegradation. In addition, the use of various analytical chemistry indices and loss patterns also confirmed that the reported losses are due to biodegradation [15].

In terms of ranking the tested crude oils and oil products with respect to oil biodegradation potential, the following philosophy was applied. First, the ranking index was to be kept relatively simple, so that it could be understood and used by a wide audience. Of the 5 groupings discussed in the preceding section (that is, TPH, total saturates, total n-alkanes, total aromatics, total 5 alkylated PAH homologous series), it was felt that the losses in GC-TPH and total aromatics were of the most use. An overall measure of oil biodegradation was desired, which is readily provided by the GC-TPH grouping. However, since the GC-TPH grouping is largely composed of saturates, a means of quantifying the degradation of the aromatics was also desired, since aromatics are considered to be more recalcitrant and some of their bio-oxidation products are potentially more toxic than those derived from saturate metabolism. The total aromatics grouping was chosen as being the most representative index. Note that of the remaining groupings discussed in the previous section, the n-alkane and 5 alkylated PAH homologous series groupings are but small subsets of the total saturates and total aromatics, respectively. In addition, since total saturates are generally quite degradable, and are already accounted for in the GC-TPH, this grouping was not considered a relevant addition to the biodegradation index calculations.

An important consideration is the relative weight assigned to the losses of the GC-TPH and total aromatics for creating a biodegradation potential index. In previous studies by Environment Canada and NOAA [16], two different weightings of the GC-TPH and total aromatic losses have been proposed (on a scale of 0 to 10):

Equation A: Equal weighting:

$$\text{Index Value} = [0.5(\text{mean \% GC-TPH loss}) + 0.5(\text{mean \% Total Aromatics loss})]/10$$

Equation B: A 30:70 ratio:

$$\text{Index Value} = [0.3(\text{mean \% GC-TPH loss}) + 0.7(\text{mean \% Total Aromatics loss})]/10$$

For example, given that the crude oil Cook Inlet (Granite Point) had a mean GC-TPH loss of 29.3% and a mean total aromatics loss of 7%, in the nutrient-amended flasks, the calculations for Equation A would be performed as follows:

$$\text{Granite Point Index Value} = [0.5(29.3) + 0.5(7)]/10 = 1.8$$

Equations A and B were used to generate index values for each crude oil/oil product tested in this study (Fig. 10A and 10B). The trends depicted are similar in both plots, i.e., the oil products Jet B Fuel and Diesel No. 2 are more biodegradable than the crude oils, while the Bunker C/ Diesel Fuel mixture is the least biodegradable.

The bottom line is that laboratory pre-screening can identify oils which should be considered for bioremediation. The Bunker C/Diesel mixture would definitely not be as amenable to bioremediation as the other tested oils/oil products. A similar conclusion was reached by Song *et al.* [17] when investigating the bioremediation potential of terrestrial fuel spills of gasoline, jet fuel, heating oil, diesel oil, and Bunker C. Bunker C was found to be the most recalcitrant, with close to 80% persisting after one year of incubation.

Interesting trends were noted with respect to nutrient addition. For both crude oils and oil products, the addition of nutrients enhanced GCD-TPH, total *n*-alkane and total saturate loss. Although nutrient addition did not enhance aromatic or PAH degradation in crude oils, enhancement due to nutrient addition was apparent for Jet B Fuel and Diesel No. 2. This phenomenon is discussed in detail by Blenkinsopp *et al.* [9].

ACKNOWLEDGEMENTS

This project was funded by the Alaska Department of Environmental Conservation and the Emergencies Science Division of Environment Canada. The findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Alaska Department of Environmental Conservation or of Environment Canada. The use of the trade names or commercial products in this manuscript does not constitute endorsement for their use.

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Figure 1 GC Chromatograms for TPH analysis of biodegradation North Slope (NS) oil samples. A,sterile control, B, positive control, C,negative control

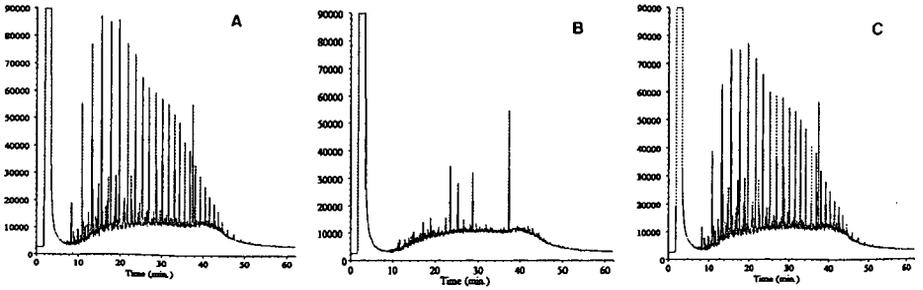


Figure 2 Changes of n-alkane distribution of NS oil samples under the standard inoculum conditions (28 days at 10 degrees C)

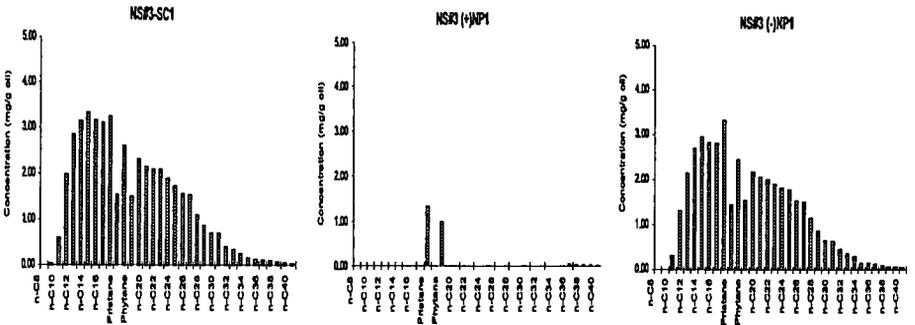


Figure 3 GC/MS chromatograms of the aromatic fractions of NS oil biodegradation series. CnB represent alkylbenzenes.

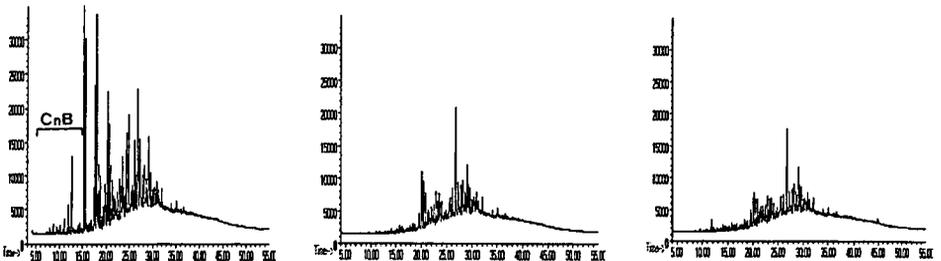
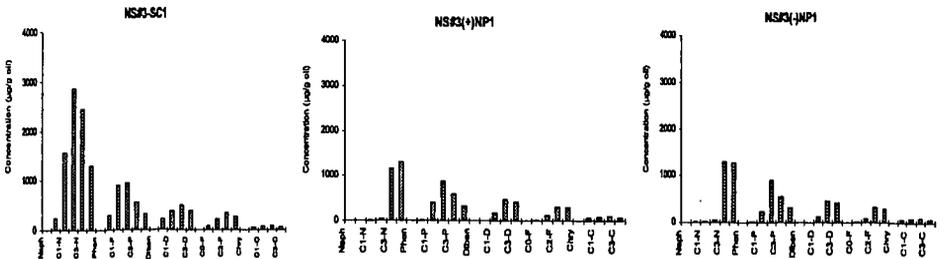


Figure 4 Distribution changes of 5 target alkylated PAH homologous series in NS oil samples due to biodegradation



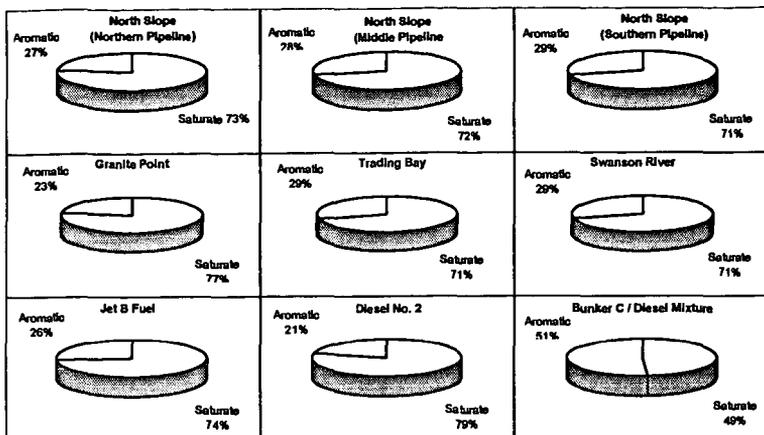


Figure 5 Weathered Alaskan crude oils and oil products: Aromatic and Saturate Comparison (% by weight).

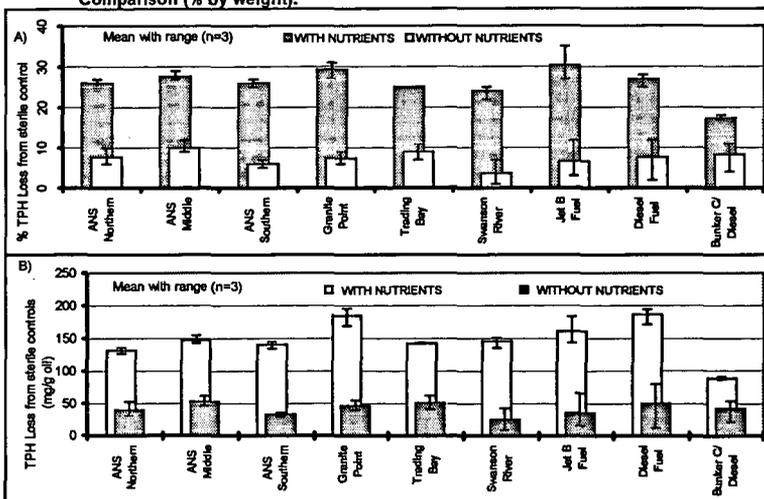


Figure 6 GCD-TPH loss after incubation under Freshwater Conditions at 10 degrees Celsius for 28 days, for all tested crude oils and oil products. A) % loss. B) Actual measured loss.

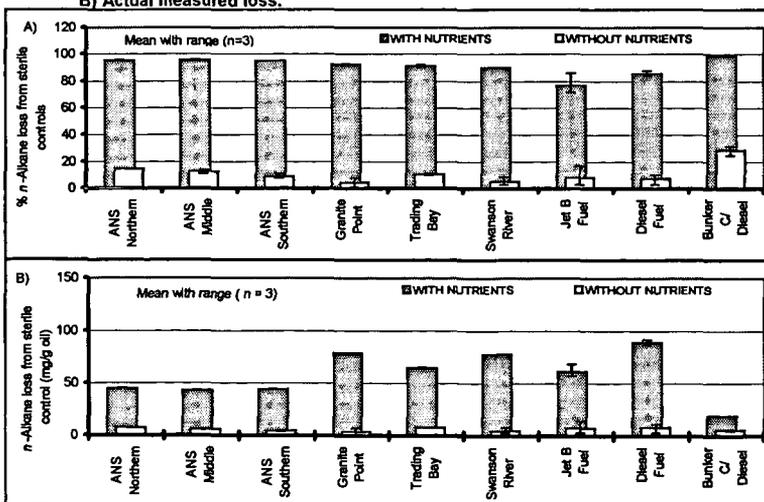


Figure 7 Total n-alkane loss after incubation under Freshwater conditions at 10 degrees Celsius for 28 Days, for all tested crude oils and oil products. A) % loss. B) Actual Measured Loss.

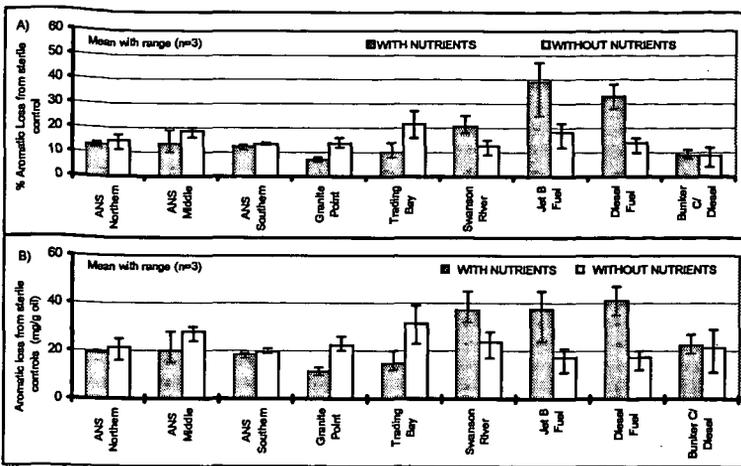


Figure 8 Total aromatics loss after incubation under freshwater conditions at 10°C for 28 Days, for all test crude oils and oil products.

A) % Loss. B) Actual Measured Loss.

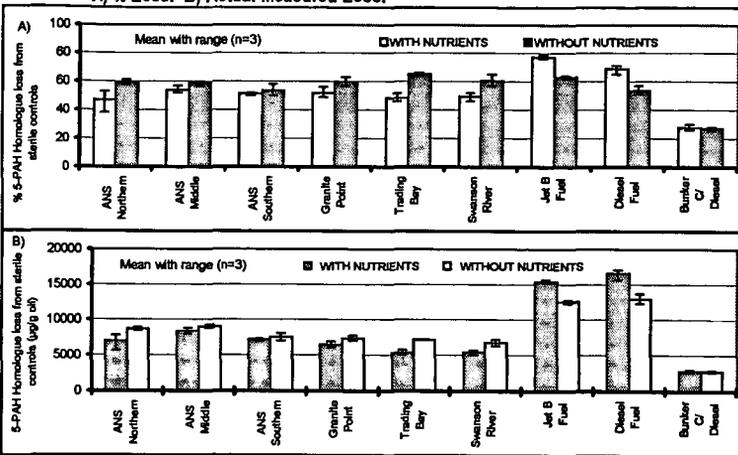


Figure 9 Total 5-PAH homologue loss after incubation under freshwater conditions at 10°C for 28 Days, for all tested crude oils and oil products.

A) % Loss. B) Actual Measured Loss.

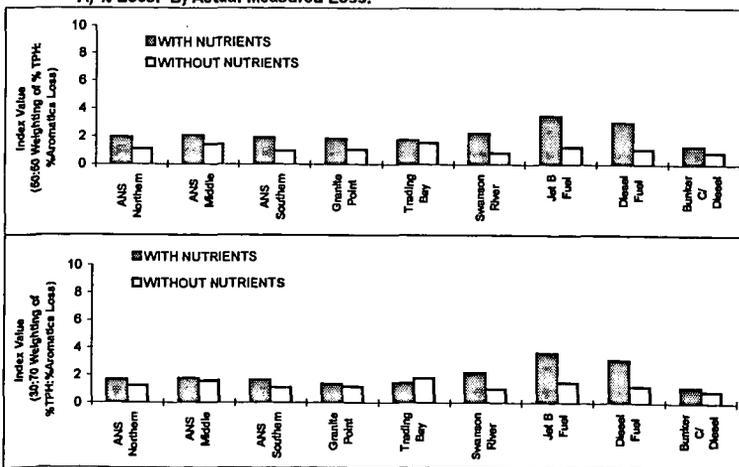


Figure 10 Indices for evaluating the biodegradation potential of Alaskan crude oils and oil products under freshwater conditions.

A) 60:50 Weighting of %TPH : % Aromatics Loss
 B) 30:70 Weighting of %TPH : % Aromatics Loss