HABITAT RECOVERY IN AN OIL-CONTAMINATED SALT MARSH FOLLOWING BIORESTORATION TREATMENTS

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ABSTRACT: *Wetlands are among the most sensitive of habitats to oil spills. A field experiment was conducted on a salt marsh in Atlantic Canada to determine the significance of bioremediation by nutrient enrichment in enhancing wetland restoration. Six experimental treatments were monitored: (1) unoiled control (2) unoiled control + nutrients, (3) oil with no treatment (natural attenuation), (4) oil + nutrients, (5) oil + nutrients with plants cut back, (6) oil + nutrients with disking (tilling) to enrich oxygen penetration. Remediation success was quantified by determining changes in the composition and concentration of the residual oil, plant recovery and reduction in sediment toxicity. The experimental results advocate natural attenuation as the clean-up strategy for the ecotype under study. Within the untreated plots, significant recovery of the predominant plant species within the marsh* (Spartina alterniflora) *was observed after 20 weeks and approximately 90% of the resolved n-alkanes and 70% of the parent and alkyl-substituted polyaromatic hydrocarbons (PAH) were biodegraded.*

Introduction

Salt marshes provide nursery support for coastal fisheries, a habitat for wildlife, and a protective mechanism against shoreline erosion. Unfortunately, they are among the most vulnerable habitats to oil spills due to the persistent nature of oil stranded within wetland sediments and the sensitivity of their flora to physical disruptions resulting from traditional oil spill clean-up operations (Teal *et al*., 1992; Owens *et al*., 1993; Lee and Levy, 1991). The search for alternative clean up procedures has led to the consideration of less intrusive oil spill clean up strategies such as bioremediation and phytoremediation based on the stimulation of indigenous oil-degrading bacteria by nutrient enrichment (Lee and Levy, 1989; Lee and Levy, 1991; Venosa *et al*., 1996), and the inherent ability of some plants to accumulate or degrade environmental contaminants (McIntyre and Lewis, 1997).

The present study was conducted on a salt marsh dominated by the wetland plant species, *Spartina alterniflora* (Conrod's Beach, Nova Scotia, 44° 42' N; 63° 11' W). The objective was to determine the rates of natural recovery and the significance of nutrient enrichment in enhancing wetland restoration following a crude oil spill, in the presence and absence of wetland plants.

Methods

Experimental design. The experiment utilized experimental plots (3 × 3 m) in a replicated random block design (3 blocks). The experimental treatments in each block included: (1) unoiled control (2) unoiled control + nutrients, (3) oiled with no treatment (natural attenuation), (4) oil + nutrients, (5) oil + nutrients with plants cut back, (6) oil + nutrients with disking (tilling) to enrich oxygen penetration. To determine the influence of plant activity on oil degradation (phytoremediation), emergent plant growth was continuously cut back to the sediment surface in one of the experimental plots. Mesa (API 29.7) crude oil 'weathered' by forced air evaporation to 13.8% loss by weight was homogeneously applied to the surface of selected experimental plots to a final concentration of 35 g/kg (1.8 L/m^2). Experimental nutrients added to amended plots consisted of 1,280 g N and 550 g P in the form of a commercial fertilizer mixture comprised of prilled NH_4NO_3 and $Ca(H_2PO_4)_2{\cdot}H_2O$ with re-applications when the interstitial-N concentrations fell below 5 mg/L.

Chemical analysis. Changes in the concentration and composition of remaining crude oil in the sediments were determined by solvent extraction with dichloromethane and subsequent GC/MS analysis with a Hewlett-Packard 5890 series II gas chromatograph coupled with a Hewlett-Packard 5971A

mass selective detector (MSD) (Garcia-Blanco *et al*., 2001). To quantify the extent of biodegradation, all resolved hydrocarbons were normalized to the conserved biomarker C_{30}-17α(H), 21β(H)-hopane in the MESA oil (Prince *et al*., 1994).

Biological analysis. Three samples of vegetation (each 625 cm^2) were removed from each experimental plot for biomass (dry-weight after 72 hours at 105°C) measurements at Week 0, 9 and 20. For comparative purposes the data was normalized to the Day 0 value for each treatment to account for between-plot variability.

To monitor the response of the microbial community to the experimental treatments, potential microbial hydrocarbon degradation rates of representative alkane and PAH components within sediment samples were determined by quantifying the respiration rate of the added radioisotopes, ^{14}C-hexadecane and ^{14}C-phenanthrene (Lee and Levy, 1989; Caparello and LaRock, 1975; Walker and Colwell, 1976). The rates of microbial denitrification activity, a primary process that regulates the nitrogen cycle in wetland sediments, were monitored on each sampling occasion. This was accomplished by the placement of a gas chamber on each plot for the recovery of time-series headspace gas samples at 0, 15 and 30 minutes for subsequent analysis of nitrous oxide by gas chromatography. During this study, sediment samples were also collected at regular intervals and frozen on site for subsequent total community deoxyribose nucleic acid (DNA) extraction and analysis. Total DNA was extracted, purified on polyvinyl-polypyrrolidone (PVPP) spin columns, polymerase chain reaction (PCR) amplified using 16S rDNA universal eubacterial primers, and the resulting fragments were separated using denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*., 1993).

Regulatory bioassays were used to monitor time-series changes in sediment toxicity between the experimental plots. In the Microtox® Solid Phase Test (Lee *et al*., 1995; Microbics Corporation, 1992), the bacterium, *Vibrio fisheri*, was exposed to test sediments. A significant decrease in bioluminescence relative to water-only controls was indicative of sediment toxicity. Toxicity levels were calculated as the concentration of sample that would result in a 50% reduction in luminescence ('effective concentration,' EC_{50}). To account for interference from differences in sample grain size distribution, turbidity, and to a lesser extent, color of the sample dilutions, sample test results were compared with results of unoiled sediments from the immediate study area. The Amphipod Test measured the effects of sediment samples on survival of sediment-dwelling *Eohaustorius estuarius* (Environment Canada, 1992). The mean percent survival and the mean weight of animals in each treatment were compared with mean percent survival and mean weight of amphipods in reference control sediments to determine if the treatments caused a significant decrease in organism survival or growth. The results were reported as percent mortality.

Results and discussion

Oil chemistry. Analysis of chemical data, by normalization of specific target compounds to the conserved markers such as hopane, provided evidence of biodegradation of the n-alkanes and PAHs within the recovered 3 cm thick sods of wetland sediment samples that extended beyond the depth of oil penetration (Figure 1). After the first 20 weeks, approximately 90% of the resolved n-alkanes were degraded within the experimental plots. The corresponding reduction of parent and alkyl-substituted PAHs was about 70%. Statistical analysis indicated that significant treatment differences ($p<0.01$) were only evident for the alkane fraction.

Biological response. Oiling significantly suppressed growth (biomass) of the predominant plant species, *S. alterniflora*, by 55-73% of the Day 0 levels (Figure 2). The control and control + nutrients showed an increase from seasonal growth, with nutrient addition amplifying this increase. Cutting of plants suppressed

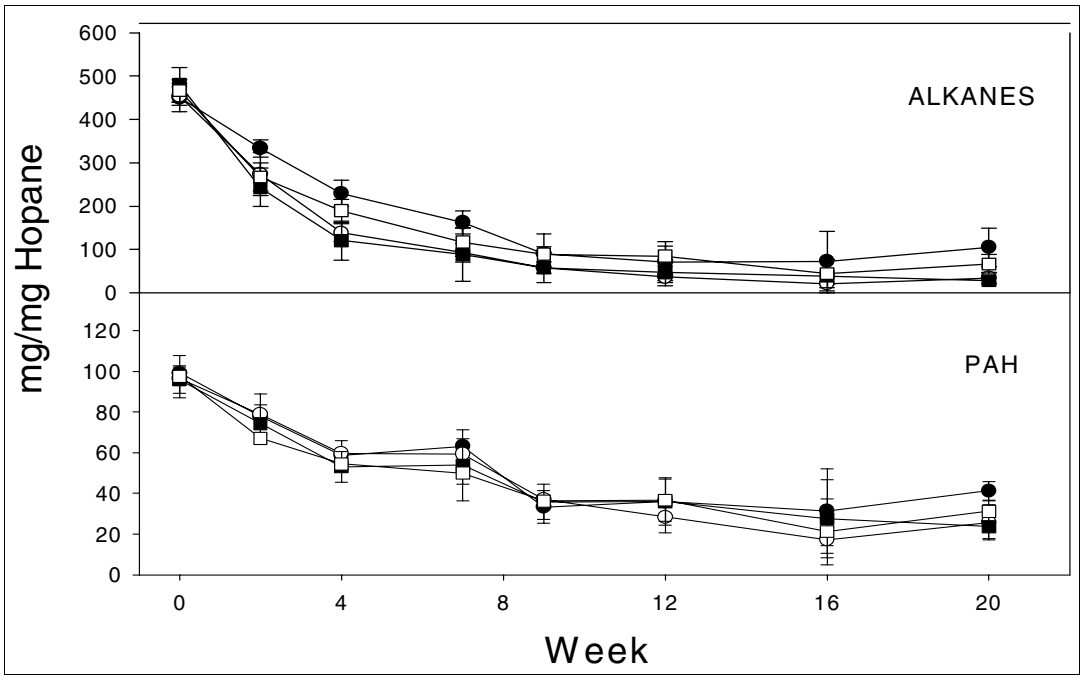

Figure 1. Biodegradation of hopane-normalized alkanes and PAH ± 1 standard deviation (● oil, ○ oil + nutrients, ■ oil + nutrients + cut, □ oil + nutrients + disking).

recovery. The tilling also destroyed the integrity of plants. By Week 20, the oil and oil + nutrients plots recovered to within 11 and 13% of the Day 0 values, although the differences were not statistically significant. The following spring, there was some evidence of changes in species composition within the treated plots. Another opportunistic plant species (*Salicornia*) that was more tolerant to the altered conditions increased its percentage of cover.

There was clear evidence for changes in microbial community structure and function in response to the experimental treatments. Total microbial community DNA extracts were obtained from the sediments of experimental Block I one day after oiling, 4 weeks after oiling and 16 weeks after oiling. Representative examples from the unoiled control, oil alone (natural attenuation) and oil + nutrients are illustrated (Figure 3). In addition to distinct differences in fragment presence with treatment and time it is noted that certain fragments of 16S rDNA amplified from the sediment extracts increased or decreased in intensity in response to the experimental treatments.

Time-series changes (Week 4, 7, 9, 12, 16, 20 and 62) in the turnover time of the radiotracers were calculated with the actual concentrations of residual hexadecane and phenanthrene in each sample determined by GC/MS to account for dilution by the unlabelled fraction of the specific substrates under study. The results of the added ^{14}C-labeled hexadecane studies clearly illustrated the stimulation of indigenous organisms with the potential to degrade alkanes within the first 10 weeks after the application of oil (Figure 4a). A stimulatory effect on potential hexadecane degradation rates by the addition of nutrients to unoiled sediments was also observed. However, within the oiled sediments, remedial treatments based on nutrient additions did not appear to cause a stimulatory effect that could be adequately resolved by measurement of hexadecane respiration rates. Natural attenuation appeared to be relatively effective. These radiotracer studies agree with the chemistry that showed that 87% of the target n-alkanes were removed from the test sediments within 20 weeks. Similar observations were made for the biodegradation of PAHs (represented by the 3-ringed phenanthrene) with the exception that nutrient amendments to the unoiled control sediments had no stimulatory effect (Figure 4b). Microbial responses to treatments were also evident by changes in the seasonal average of denitrification activity. There was a net positive denitrification potential (Figure 5) in all cases where nutrients were applied. Natural attenuation and the unoiled control showed a net negative denitrification potential. These results indicate that nutrient application resulted in increased denitrification activity in the sediments.

In terms of toxicity, based on Environment Canada's ocean-dumping guidelines (Tay *et al*., 1997) which sets the regulatory threshold for sediment toxicity at 1,000 mg/L, all unoiled sediment samples were deemed non toxic by the Microtox® Solid Phase Test. EC_{50} data normalized to the controls (Figure 6) showed an initial detrimental response in sediments treated with nutrients or the test oil. Subsequent recovery by natural attenuation was observed. By week 9 all treatments were deemed non-toxic. For the Amphipod Test, mortality was high in all of the oiled treatments, but began to decrease by Week 12 largely as the result of natural processes (Figure 7). Addition of nutrients accompanied by disking appeared to cause the most rapid rates of detoxification recovery within the oiled plots. However, the results of chemical analyses (GC/MS) indicated that this observation could also be attributed to the physical removal of oil (enhanced dispersion with tides) mediated by disking operations. By Week 62, the difference from the Oil treatment was highly significant: 32% vs. 5% mortality.

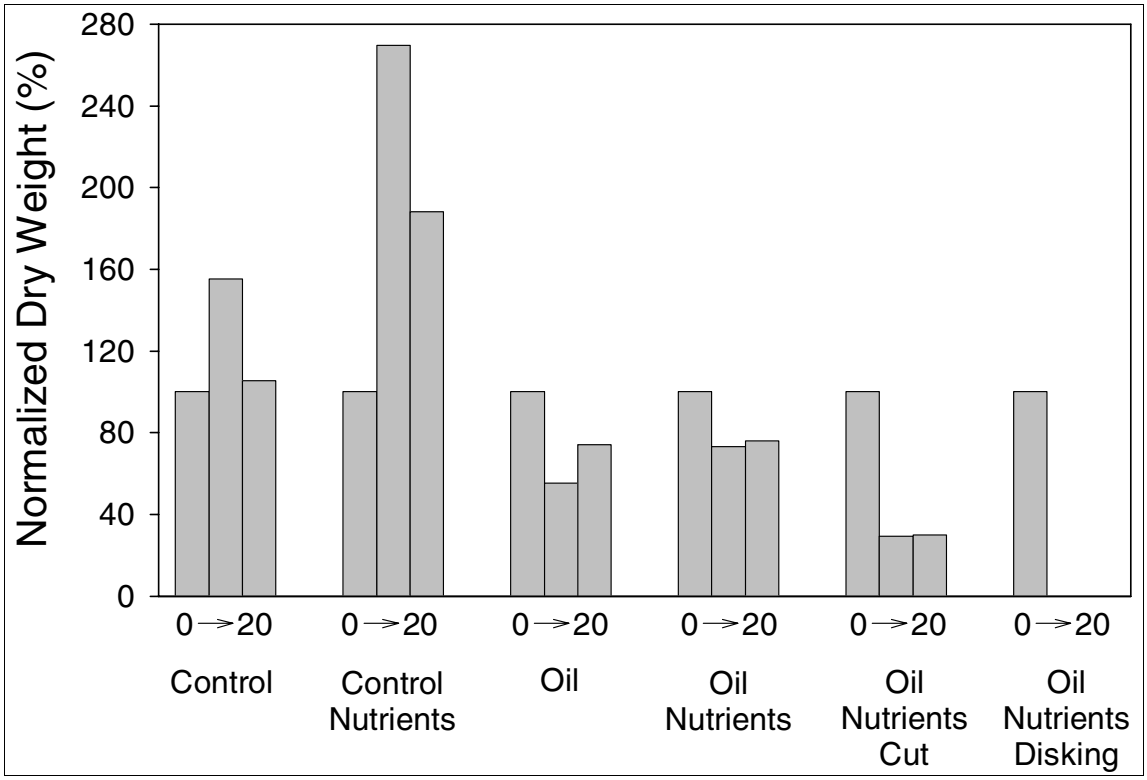

Figure 2. Total plant biomass collected from plots at Week 0, 9 and 20.

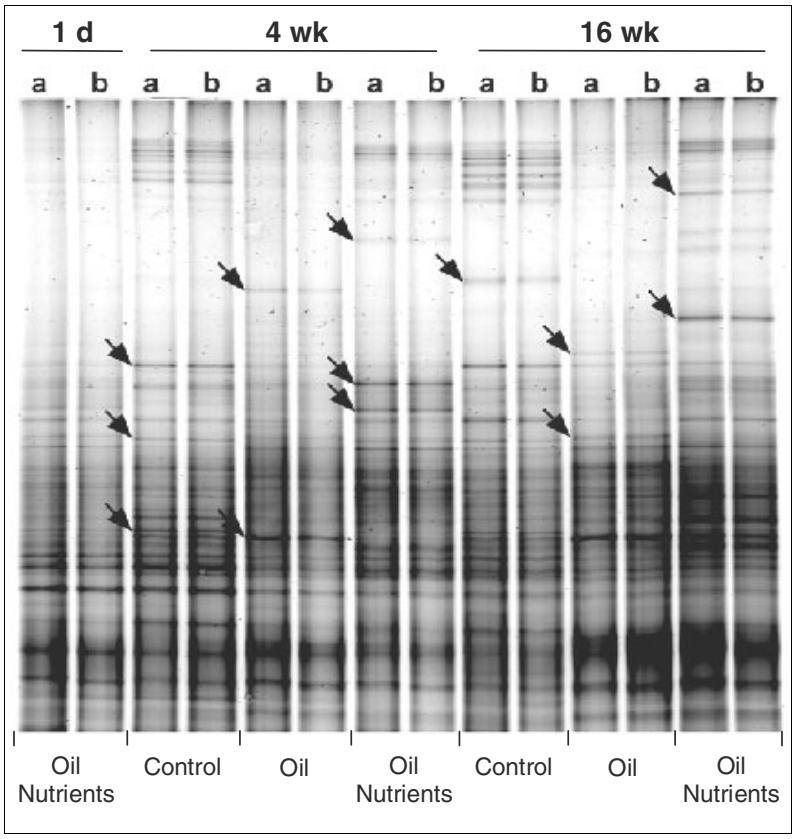

Figure 3. DGGE analysis of total community DNA extracted from Block 1 sediment samples. The 16S rDNA was amplified using one primer with two different clamps containing mainly guanosine and cytidine bases (lanes a and b). Arrows indicate fragments of interest that appeared or increased in intensity.

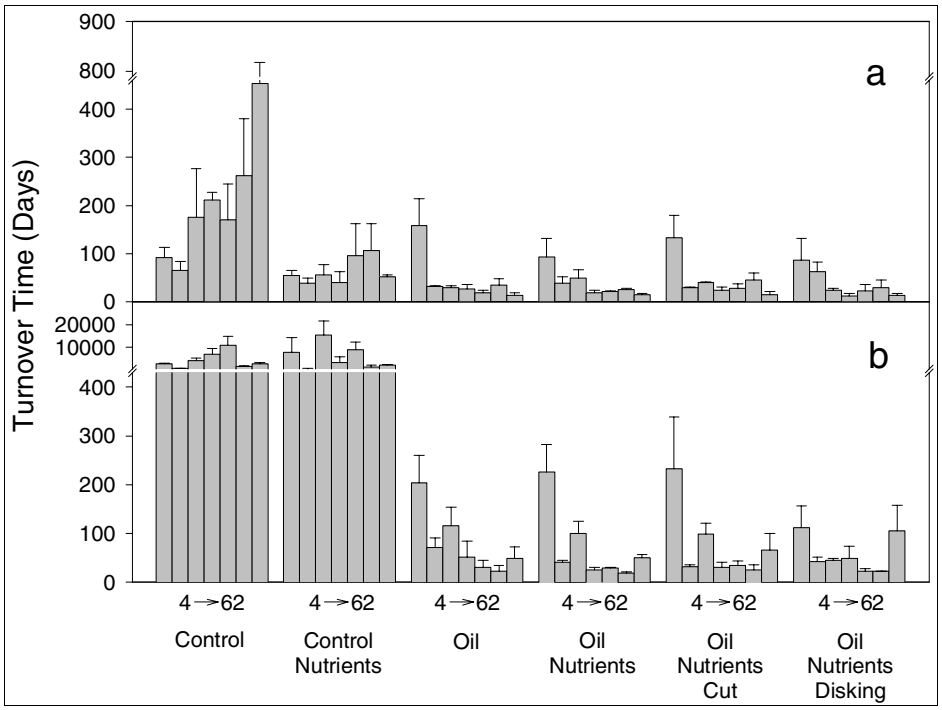

Figure 4. (a) Hexadecane and (b) phenanthrene turnover time at Week 4, 7, 9, 12, 16, 20 and 62 (± 1 standard error).

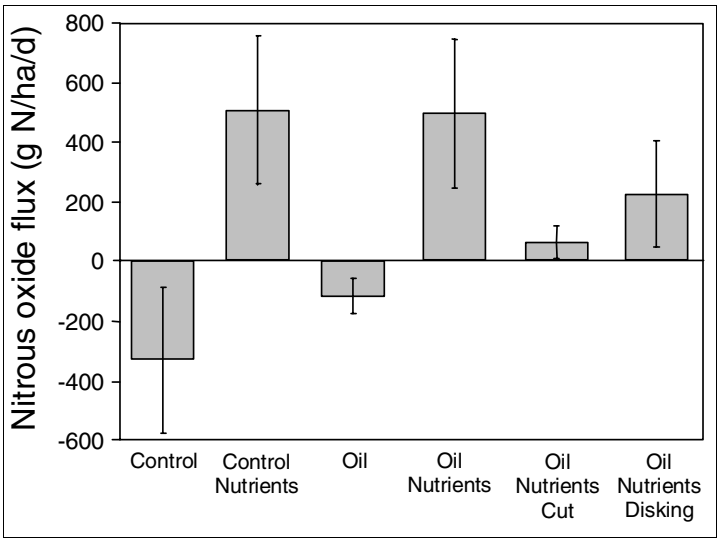

Figure 5. Average denitrification activity June to November 2000 (± 1 standard error).

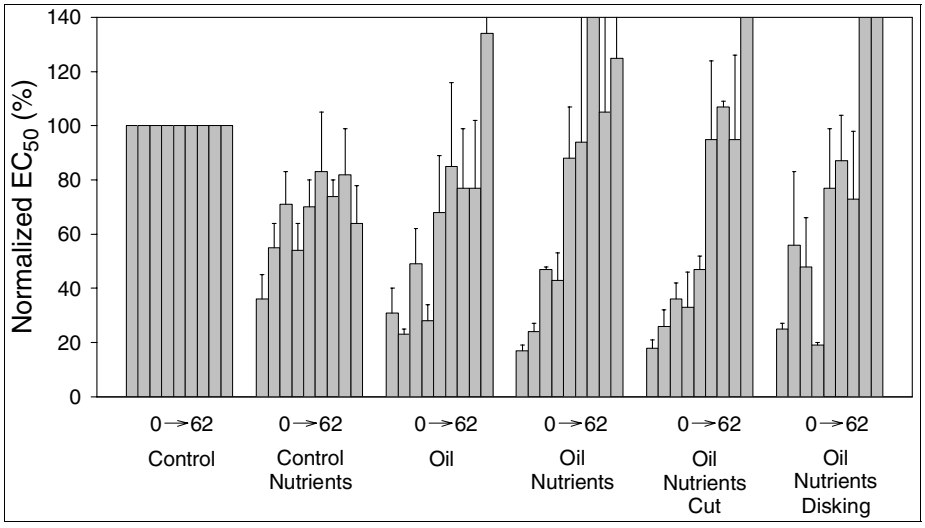

Figure 6. Microtox toxicity at Week 0, 2, 4, 7, 9, 12, 16, 20 and 62 (± 1 standard deviation).

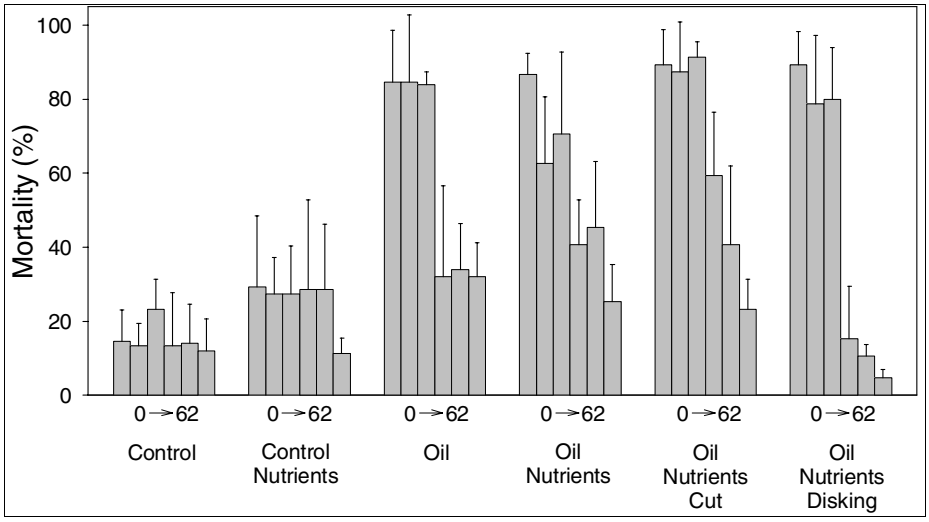

Figure 7. Amphipod survival toxicity test for Week 0, 2, 7, 12, 20 and 62 (± 1 standard deviation).

Conclusions

Results of chemical analysis, bioassessments and bioassay tests suggested that natural attenuation was the primary process that reduced residual oil concentrations and toxic effects. The biotest results showed that the remediation strategies under evaluation (bioremediation by nutrient amendment and physical aeration of sediments by disking) were not effective on an operational scale, in spite of the significant changes observed in microbial response. In terms of phytoremediation potential (i.e. the influence of plant growth on oil degradation) at this study site which was dominated by smooth cordgrass (*Spartina alterniflora*), suppression of plant activity within the plots by continuous cutting of emergent plant growth had no significant effect on oil biodegradation rates. It was also evident in the results of the regulatory bioassays that possible detrimental effects may be linked to the addition of fertilizers.

This field study provided clear evidence of significant oil biodegradation (90% of resolved n-alkanes and 70% of parent and alkyl-substituted PAH within 20 weeks), microbial activity and sediment toxicity reduction in the oiled plots that received no remediation treatments. Based on these findings it is recommended that consideration should be given to natural attenuation (natural recovery) as an operational oil spill countermeasure for use in north-temperate salt marsh environments.

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Biography

Dr. Kenneth Lee is a Research Scientist with Fisheries and Oceans Canada. His expertise in microbial ecology is focused on the development and implementation of research programs to assess potential environmental impacts from oil spills, the development of offshore oil industry, and waste disposal in the ocean.

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