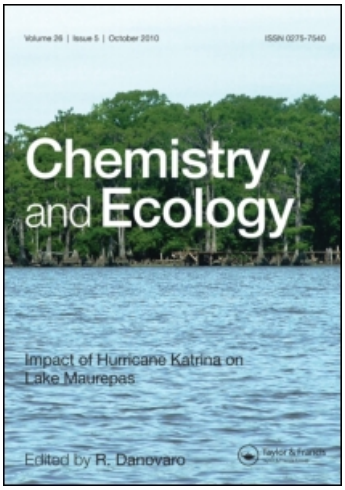

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VEGETABLE OIL SPILLS ON SALT MARSHES

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Following the wreck of the M.V. *Kimya* during which 1500 tonnes of sunflower oil was spilled, sandy sediments bound together with sunflower oil were discovered on the beach. These are still present $2\frac{1}{2}$ years later. Sunflower and linseed oil were applied to salt marsh sediments to reproduce potential spills. Cores were taken and the vertical migration and degradation rates determined. Sunflower oil polymerised at the surface after 28 days resulting in the formation of a cap of increased shear strength and reduced permeability to water and oxygen. This contrasts with linseed oil that rapidly percolated to depth without the formation of a polymer. One degradation product formed from linseed oil was possibly 18:2ω3, although this has still to be confirmed. Increased bacterial numbers were observed with both oils. In the event of spill, these results suggest sunflower oil should be removed although linseed oil could be left to natural degradation processes.

KEY WORDS: sunflower oil, linseed oil, salt marshes, degradation, octadecdienoic acids, bacteria

INTRODUCTION

The capsize and wrecking of the M.V. *Kimya* off the coast of Anglesey, North Wales in January 1991, led to the release of approximately 1500 tonnes of sunflower oil into the marine environment. Initial assessment suggested the vegetable oil would be rapidly consumed by the microorganisms present. In practice however, the oil formed a polymer in sea water and produced relatively hard, intractable lumps resembling used chewing gum. These lumps were unavailable for bacterial degradation (Mudge *et al.*, in prep). Some oil was taken up by mussels (*Mytilus edulis*) in the immediate vicinity (Mudge *et al.*, 1993) which indicated the extent of the spreading along the coast (3 km).

Significant amounts of the sunflower oil came ashore and covered the biogenic sands present at Bodorgan Head. Subsequent polymerisation of the oil led to the formation of tough concrete-like aggregations that are still present $2\frac{1}{2}$ years after the spillage (see Figure 1).

The consequences of this particular spill, while not fully evaluated, are thought to be low, due to the relative remote location of the wreck and the restricted dispersion of the spill. If the ship had come ashore at the entrance to the Menai Strait, a proposed Marine Nature Reserve, the mollusc fisheries and salt marsh habitats may have been at considerable risk. The fate of vegetable oils in molluscs is being investigated (Salgado, *pers. comm.*) and this paper presents the results of some preliminary investigations on the effects of these vegetable oils in salt marshes.

Figure 1 Sand bound together with sunflower oil near the wreck of the M.V. *Kimya.*

MATERIALS AND METHODS

Sampling

A drying oil (linseed) and a semi-drying oil (sunflower) were applied to the surface sediments of a salt marsh at Foryd Bay, located at the south west entrance to the Menai Strait (Figure 2). The oil (350 ml) was poured into 15 cm diameter, 10 cm high plastic pipes pushed 3 cm into the sediment. The top was covered with plastic film secured with an elastic band. Seven such reservoirs were prepared for each oil within a 5 m diameter circle. Over the 35 days of the experiment, birds were observed pecking through the film to feed on the oil.

Cores were taken prior to the addition of oil (day 0) and on days 1, 3, 7, 14, 21, 28 and 35. Cores were obtained by removing the collar and pushing a plastic pipe of the same diameter down to 40 cm depth. The pipe was then dug out with minimal disturbance to the remaining sites.

On return to the laboratory, the pH and E_H were measured at 0, 10, 20 and 30 cm depth with a solid insertion probe and the vane shear strength was determined. Subsamples at 10, 20 and 30 cm were taken from the centre of the core for fatty acid analysis and bacterial numbers.

Fatty Acid Analysis

Sediments were air dried for 48 hours and 30 g was Soxhlet extracted for 4 h in chloroform. The lipid weight was recorded and a portion derivatised with BF_3/methanol (Morrison and Smith, 1964). The fatty acid methyl esters (FAMEs)

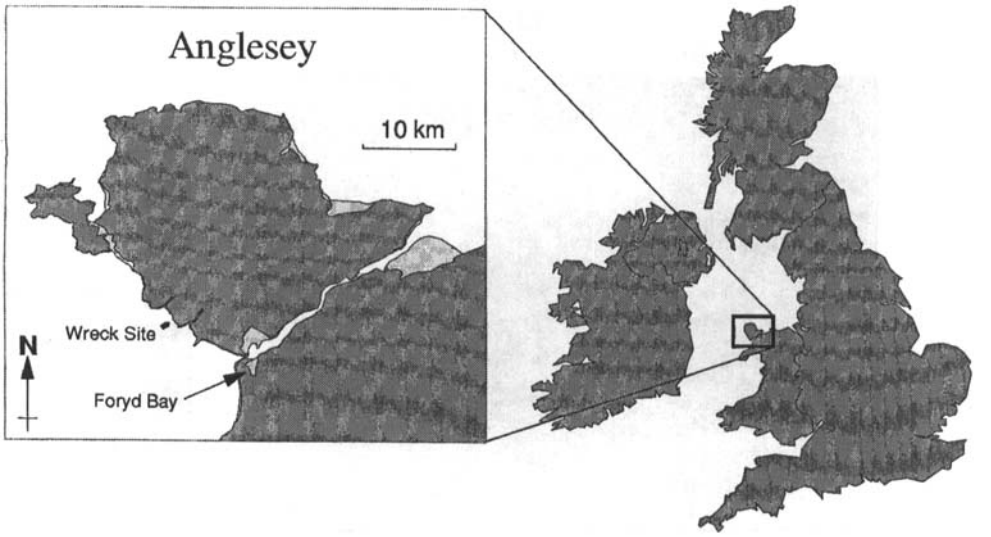

Figure 2 Location of the experimental site at Foryd Bay, N. Wales.

were quantified after the addition of tricosanoic acid methyl ester on a Finnegan MAT 4600 GC/MS. Split/splitless injection was used with a Carbowax 20M column (30 m × 0.32 mm ID). Electron impact ionisation was at 70 eV and a mass range of 45–400 *m/z* employed

Bacterial Assay

Bacterial numbers were assessed by the most probable number technique (MPN). Serial dilutions of the vortex extracted sediment in Ringers solution were made. The number of colonies formed on Zobells medium after 48 h incubation at 25°C was used to determine the most probable number of viable bacteria present in the initial sediment.

RESULTS

Sunflower Oil

The passage of sunflower oil through the sediments is best illustrated by the spatial and temporal distribution of linoleic acid (18:2ω6), the principal component of sunflower oil (Figure 3). Cores taken on day 0 had no measurable 18:2ω6. The vertical migration of the oil was relatively slow, taking 7 days to reach 20 cm and 35+ days to reach 30 cm. The 18:2ω6 was also degraded, principally to 18:1ω9, and the degradation rate at 10 cm follows an exponential curve of the form: $[18:2\omega6] = 360\ e^{(-0.067 \times \text{time})}$.

This degradation was likely to be both through chemical and bacterial oxidation. The sunflower oil would represent food to many bacterial species and the number of viable bacteria increased at all depths after the addition of the oil

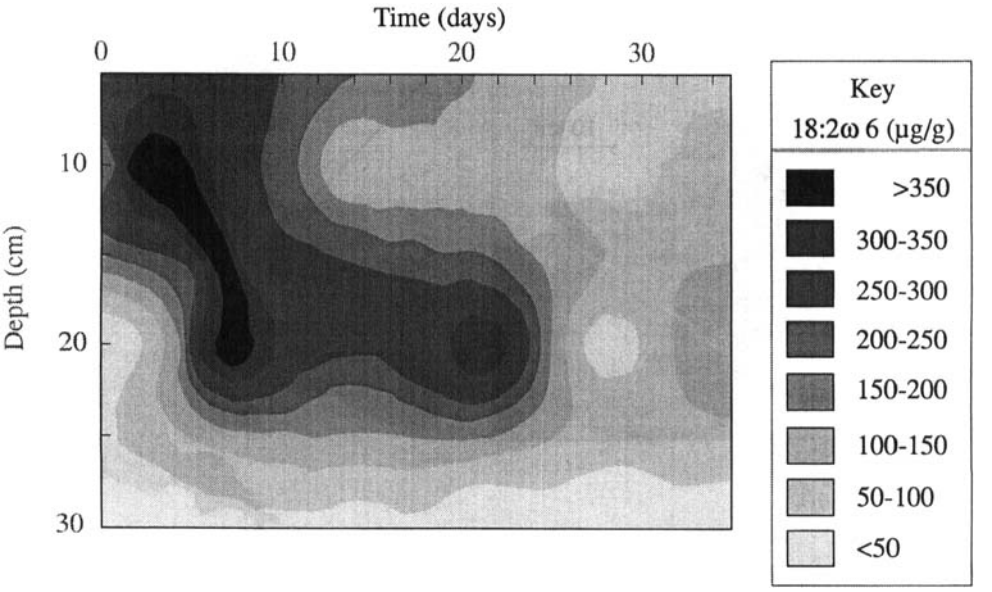

Figure 3 Temporal and spatial distribution of 18:2ω6 in salt marsh cores after the addition of sunflower oil.

(Figure 4). The number of viable bacteria in the sediment increased at each depth. There was a lag of ~20 days from the peak concentration of 18:2ω6. The concentration of 18:1ω7 in sediment has been used as an indicator of bacterial numbers (Perry *et al.*, 1979): the spatial-temporal distribution of 18:1ω7 does

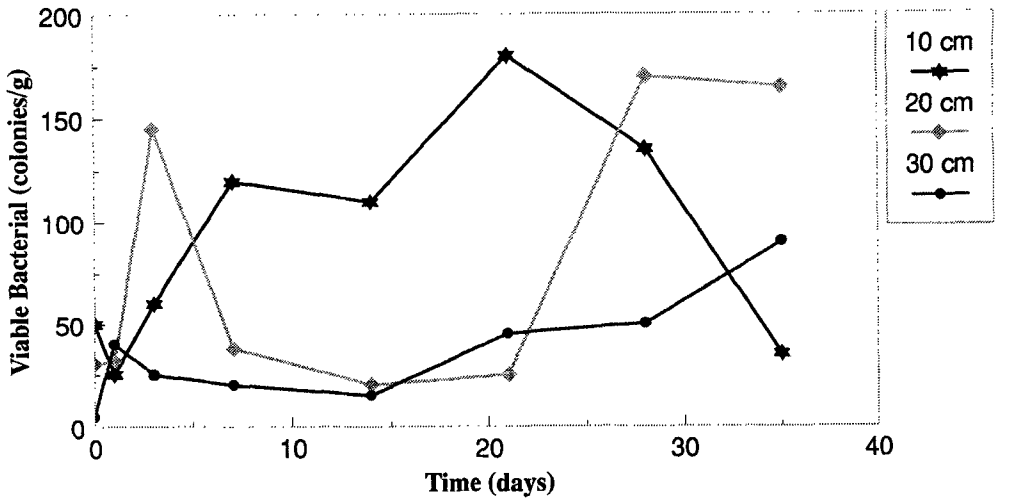

Figure 4 Number of viable bacteria in the sediment after addition of sunflower oil.

coincide with the maximum bacterial density at 20 cm but this is not so apparent at 10 cm (Figure 5). This may be explained by the different bacterial species present at each depth and the changes in E_H that occurred with time: the E_H initially declined from +400 mV to +40 mV but began to recover after 21 days. At the surface, the E_H became negative (–350 mV) by day 21 but also recovered to a positive value by day 35. The pH gradually declined from approximately 7.25 to 6.5 over the 35 days. No discernible differences with depth were seen.

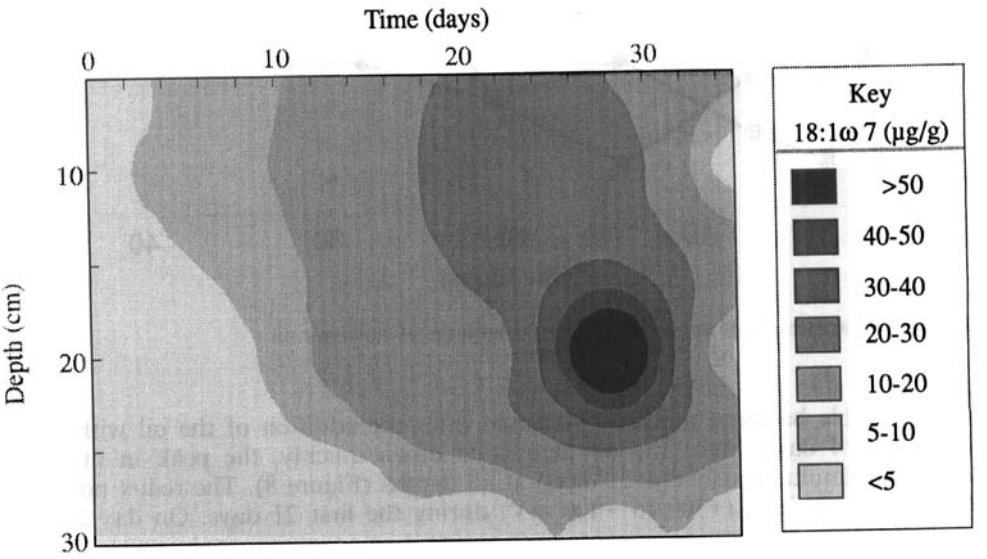

Figure 5 Temporal and spatial distribution of 18:1ω7 in salt marsh cores after the addition of sunflower oil.

The shear strength of the sediment increased at the surface from ~8 kN.m^2 to 28 kN.m^2 after 35 days. There was no evidence of an increase in shear strength at 10 cm (Figure 6). Visual inspection of the core taken on day 35 indicated the formation of a strong pliable cap of sediment bound together with sunflower oil. This would dramatically reduce the permeability of the sediment to water and air allowing anoxic conditions to develop beneath. This was evident as black deposits of metal sulphides in the sediment. There would be some lateral and vertical movement of water through this small core due to tidal inundation. A more extensive spill would have more serious consequences (see below).

Linseed Oil

In contrast to sunflower oil, linseed oil rapidly percolated through the sediments such that significant concentrations were measured at 30 cm after 7 days (Figure 7). This can be partly explained by the differences in viscosity and density of the two oils. The degradation of the linolenic acid (18:3ω3), the principal fatty acid, was approximately linear at 15.5 μg.g^{-1}.day^{-1} over the top 20 cm.

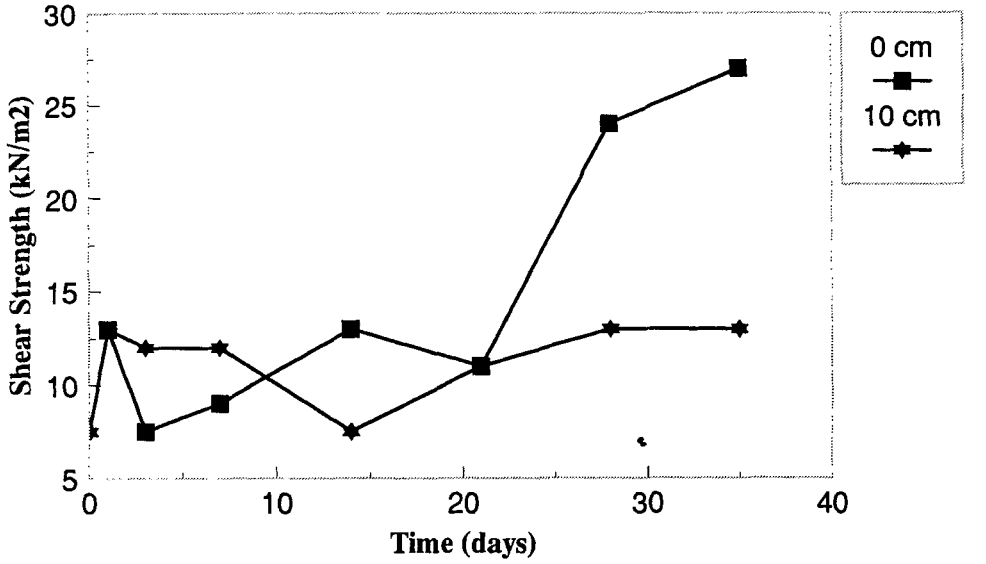

Figure 6 Shear strength of the sediment after the addition of sunflower oil.

The viable bacterial numbers increased after the addition of the oil with a lag time of ~14 days. Since the oil percolated down quickly, the peak in numbers occurred simultaneously (14–21 days) at all depths (Figure 8). The redox potential (E_H) remained high (+300 to +400 mV) during the first 21 days. On day 28, the

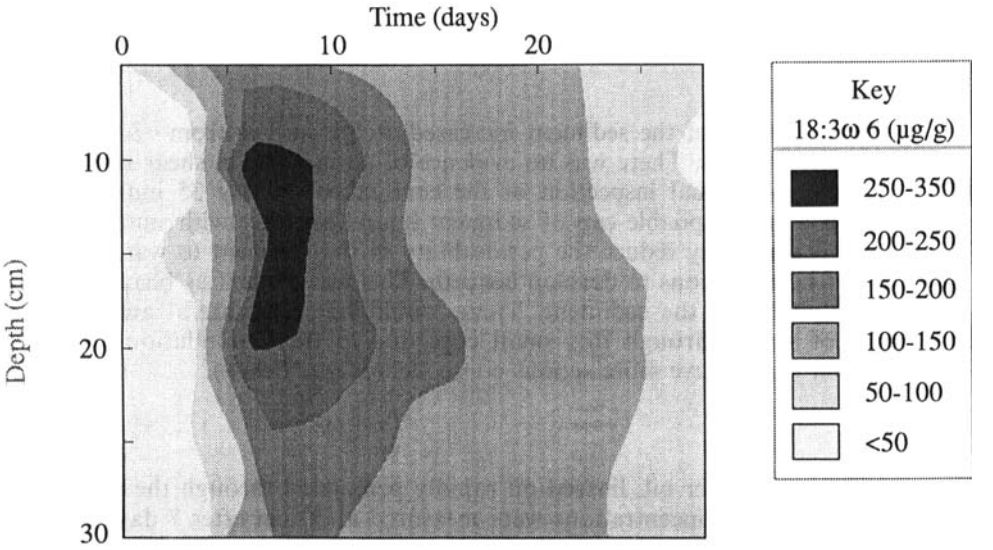

Figure 7 Temporal and spatial distribution of 18:3ω3 in salt marsh cores after the addition of linseed oil.

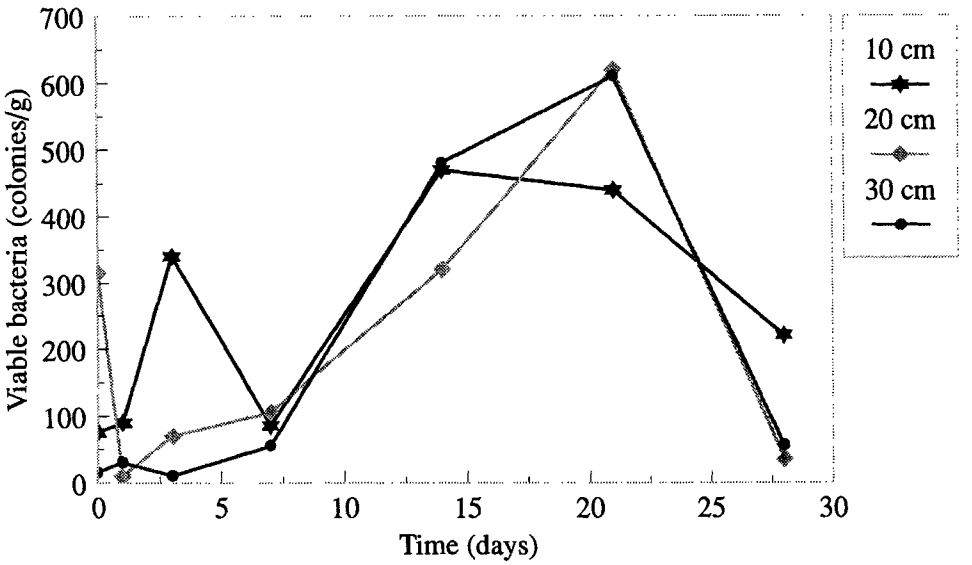

Figure 8 Number of viable bacteria in the sediment after addition of linseed oil.

E_H at 10 cm dropped to –400 mV suggesting strong reducing conditions. Visual inspection again showed black metal sulphide deposits.

During the 28 days, there was no observable trend in the sediment cohesiveness and no polymer was observed.

The degradation products from the 18:3ω3 in the linseed oil are principally the more saturated 18 carbon fatty acids, especially 18:1ω9 (Vergroesen and Crawford, 1989). Other fatty acids found after 14 days include an 18:2 with an omega number less than 6, possibly 3. Figure 9 shows the GC trace and mass spectra of the two 18:2 peaks. Chemical ionisation and electron impact ionisation confirm that it is an 18:2 fatty acid although the exact position of the double bonds has yet to be fully established. Initial experiments suggest it is an ω3 fatty acid, which if correct, has normally been reported in the gut of ruminants (Czerkawski *et al.*, 1974; Kemp *et al.*, 1975). The proposed degradation pathway is shown in Janssen *et al.* (1985) and involves transisomerase and oxidation steps. Further work is being conducted to identify the fatty acid positively.

DISCUSSION AND CONCLUSIONS

The relatively slow vertical diffusion of the sunflower oil resulted in a sequential increase in the bacterial numbers with depth. Indeed, the maximum for 30 cm depth had probably not been achieved after 35 days. This was not the case with linseed oil that was more mobile in the sediments. The lag between the oil reaching each depth and maximum bacterial numbers is consistent with the expected development time for bacterial populations under these environmental conditions. The bacterial populations found at each depth are likely to differ, dependent on these conditions (sulphur speciation, E_H, *etc.*) and it is not surprising, therefore, that the temporal

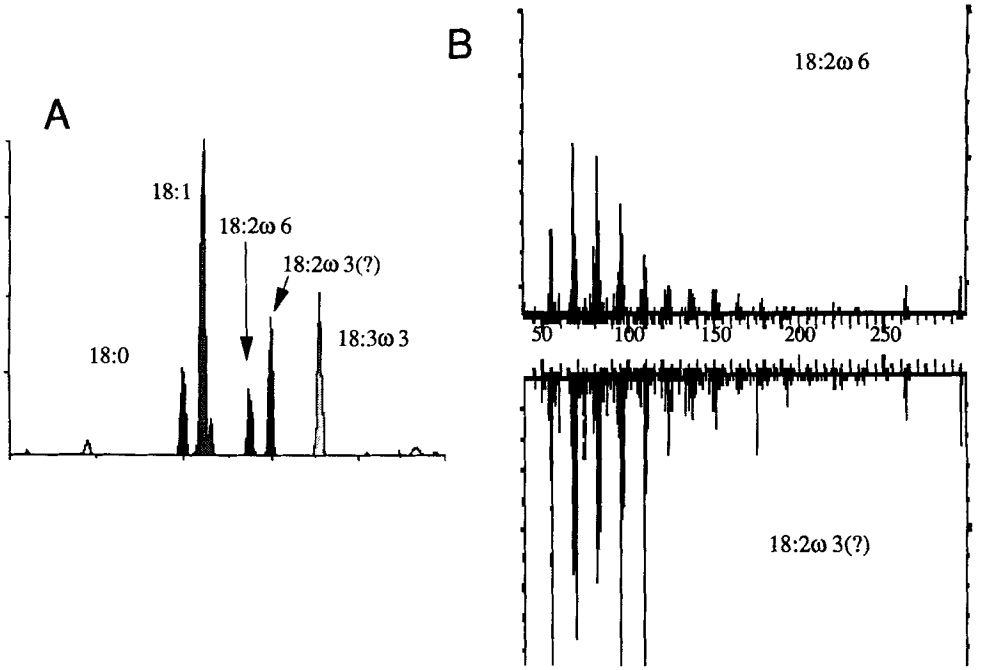

Figure 9 A. Part of the GC trace indicating the 18-carbon fatty acids (20 cm, 21 days); B. The mass spectra for the two 18:2 fatty acids indicated on A.

and spatial distribution of 18:1 ω7 does not exactly coincide with the bacterial numbers (*cf* Figures 4 and 5). Greater bacterial numbers were seen after the addition of linseed oil than sunflower oil suggesting that the former is a more readily utilisable carbon source.

The formation of a polymer with increased cohesion at the surface was similar to aggregates found after the wrecking of the M.V. *Kimya* (Figure 1). The wide variation in grain size between sites, shelly sands at Bodorgan Head and fine silts at Foryd Bay, did not diminish the polymerisation significantly. The depth to which the oil polymerised was significantly greater, however, on the more porous sands compared to the salt marsh sediments. Since only the surface sediments became bound together, this suggests that oxygen from the air is necessary for polymerisation. This agrees, in part, with results found by Latchford and Floodgate (*in prep.*).

This aggregation of the sediment would dramatically reduce the permeability to both water and oxygen. A spill on to salt marsh sediments would, therefore, lead to reduced permeability, reduced oxygen and eventually, anoxic conditions. This would have dramatic consequences for the established fauna and flora of the marsh, potentially leading to their death and removal. In turn, the stability of the marshes may be reduced and sediment could be eroded although the binding due to the oil may make the surface more difficult to remove. In the event of a spill, therefore, it would probably be better to remove the sunflower oil rather than allowing it to polymerise.

In contrast, the linseed oil rapidly percolated to depth and was degraded by the bacterial populations present. The coincidence of the maximum bacterial numbers at each depth suggests that diffusion and bacterial responses are rapid. Since linseed oil is mainly comprised of linolenic acid (18:3ω3), the degradation rate might be expected to be quicker than the less unsaturated 18:2ω6 of the sunflower oil. This may, in part, explain the reduced lag time to maximum bacterial numbers.

Since this oil did not polymerise over the timescale of the experiment and the degradation rates at all depths were reasonably high, it may be better in the event of a spill to leave the oil to be degraded by *in situ* bacteria.

The production of the 18:2ω3(?) has not been reported elsewhere in marine sediments and may give an indication of the bacterial species present and the degradation pathways of these oils in the sediments. Further work is being conducted to confirm the identification of this fatty acid.

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