INSTITUTE OF TERRESTRIAL ECOLOGY
(NATURAL ENVIRONMENT RESEARCH COUNCIL)

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Report to the Department of Transport

ASSESSMENT OF PEAT ADDITIVES TO REDUCE CRUDE OIL TOXICITY ON DUNE PLANTS

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1. INTRODUCTION

1.1 Background

Following a major oil spill, shore-line cleanup operations usually result in the accumulation of large amounts of oiled beach material (OBM) (Harrison *et al.* 1993). Disposing of this waste can present a potential economic and environmental problem. The Department of Transport initiated research to determine the feasibility of disposing of OBM using dune and/or dune pastures around the UK coast. The disposal methods consisted of landfarming and burial techniques. A range of supplementary experiments were set up to examine the rates of oil degradation and the direct effects of oil on dune plant species. This report describes some of these experiments.

In an earlier study in this series, Waterhouse, 1995, carried out a range of bioassay experiments to determine the toxicity of oils on coastal plant species. These experiments consisted of investigations into the relative toxicity of various oils at different stages of weathering. Experiments were also carried out to compare plant species sensitivity to weathered oil and seed germination in oil contaminated soils.

One of the experiments was an investigation into the effects of different concentrations of oil on *Festuca rubra* germination. Half of the pots also contained 50% peat with sand and germination was improved by the addition of peat, at least at the lower oil concentrations. The aim of the present study was to provide a more detailed study of the possible reduction of toxicity of contaminated sand by the addition of peat.

1.2 Project Design

The Waterhouse experiments showed that Forties crude was determined to be the most toxic of the oils tested. So this was used for the present studies.

The experiments also suggested which species to use as test plants. *Festuca rubra* had shown a dramatic decrease in healthy foliage when subjected to oil whereas *Plantago lanceolata* had significantly less damage. These two indicator plants were chosen to represent a range of responses to the oil. Waterhouse investigated oil concentrations up to 20% but found that no plants germinated in oil concentrations greater than 10% even when peat was present. The new investigation focused on oil concentrations from 0-14% by volume.

2. EFFECTS OF PEAT ON GROWTH OF SEEDS AND TRANSPLANTS IN OIL CONTAMINATED SAND.

2.1 Introduction

This experiment was designed to investigate how different proportions of peat mixed with sand counteracted increasing concentrations of oil. *Festuca rubra* and *Plantago lanceolata* seeds and transplants were used as indicators of this effect.

Many variables of peat and oil were used in this experiment. These variables were designed to have small intervals between them so that an overall pattern of effect could be produced. Because of this increased number of variables making the experiment relatively large, the controls were the only part of the experiment to be replicated. It was later possible to reproduce replicates for the unreplicated pots by grouping related ones together.

2.2 Aims

i) To see if adding peat (in any proportion) could be used to counteract the effects of oil on plant growth.
ii) To determine whether there was an optimal proportion of peat to counteract the effects of oil pollution and thus improve plant growth.
iii) To investigate whether there was a correlation or interaction between increasing oil concentrations and optimal peat volumes.
iv) To investigate the effects on two indicator plants, one sensitive to oil pollution (*Festuca rubra* [monocot]) and another less sensitive to oil pollution (*Plantago lanceolata* [dicot]).
v) To investigate the effects on both seeds and transplants.

2.3 Methodology

1) Sand, *Festuca rubra* and *Plantago lanceolata* transplants were collected from a coastal site on the north side of Aberdeen called 'Bridge of Don'. Individual *Plantago lanceolata* samples of similar size and health were collected and bagged for transport back to ITE. Mixed grass turf's were also collected and individual *Festuca rubra* plants of similar size and health were separated from this mixture back at ITE.
2) These individual plants were quickly placed in trays of fine sand and regularly watered until the experimental pots were set up.
3) The experiment was set up in the greenhouse as 4 blocks of 88 one litre pots.
Block one was *Festuca rubra* seeds.
Block two was *Festuca rubra* transplants.
Block three was *Plantago lanceolata* seeds.
Block four was *Plantago lanceolata* transplants.
All the pots within each block were randomly distributed to minimise any experimental bias due to shading or uneven watering in the greenhouse.
4) The sand, peat and oil were mixed together in different proportions using a cement mixer as described in Appendix A.

The peat proportions used (in sand) were:
0%, 7%, 14%, 21%, 28%, 35%, 42% and 50%.

For each of these peat proportions a variety of increasing oil concentrations were added:
0%, 2%, 4%, 6%, 8%, 10%, 12% and 14%.

The peat proportions were made up separately in the mixer. Twelve samples were removed from each proportion before oil was added to act as controls, three for each block. After oil was added to 2%, four samples were removed from each proportion, one for each block, before the next volume of oil was mixed in. This was continued until the oil concentration reached 14% (see Appendix A). Any remaining mixture was discarded.
5) Eight extra pots containing only sand were made up for each block to go with the three pots containing no oil already made up with the 0% peat proportion. These acted as control replicates, eleven per block.
6) Once all the pots were made up and laid out randomly in the greenhouse, seeds and transplants were added. Twenty five *Festuca rubra* seeds were added just under the soil surface to each pot in block one, two *Festuca rubra* transplants were added to each pot in block two, ten *Plantago lanceolata* seeds were added just under the soil surface to each pot in block three, and one *Plantago lanceolata* transplant was added to each pot in block four.
7) The greenhouse was maintained at a minimum temperature of 8°C and the pots were watered for 2 minutes every day at 0915.
8) Initial leaf number counts were obtained for the transplants and these counts were repeated at monthly intervals for 2 months.
9) Seed germination counts were carried out every other day for 29 days and then at weekly intervals as necessary.

2.4 Results

2.4.1 Festuca rubra seeds.
Data was collected for 33 days until no more germination was evident.

Control germination rates
The data showed no significant variation and none of the peat proportions had any obvious beneficial or deleterious effect over sand in terms of seed germination (Figure 1.).

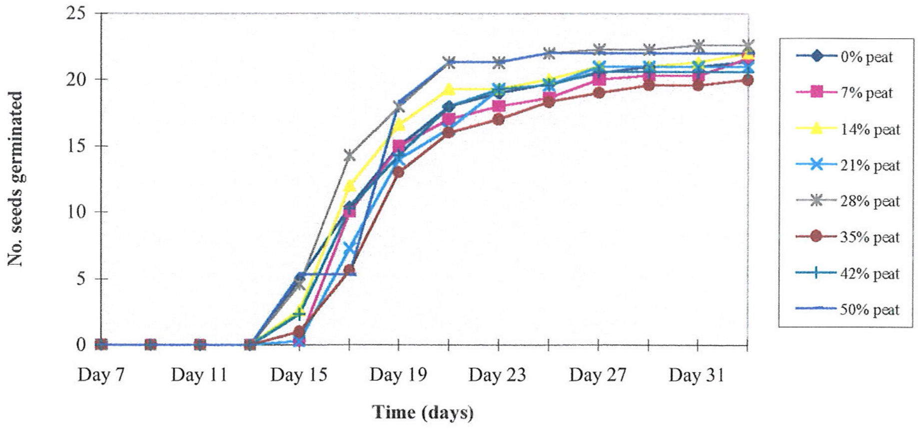

Figure 1. Germination rates of Festuca rubra in different peat proportions with no oil.

Effect of oil

The peat had no significant effect on the germination of *Festuca rubra* seeds. Above 4% oil there was very little germination (Figure 2.).

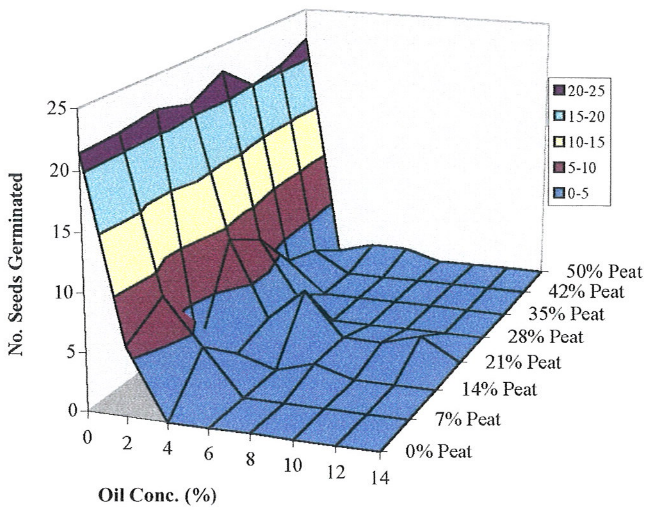

Figure 2. Germination of Festuca rubra in different concentrations of peat and oil after 33 days.

By grouping the peat proportion data into replicates for each oil concentration mean germination values showed that as the oil concentration increased so the level of germination decreased. This followed a smooth curve (Figure 3.).

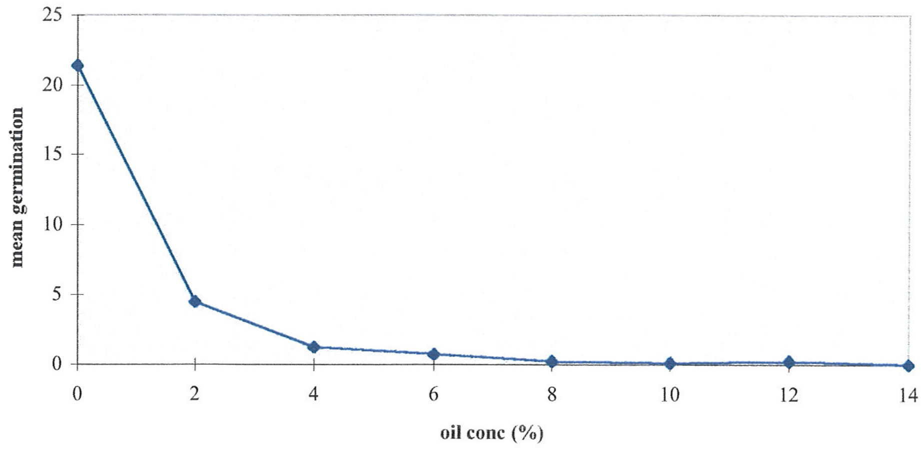

Figure 3. Effects of oil concentrations on the mean germination of Festuca rubra (grouped data for all peat treatments).

Regression analysis was carried out on the different peat proportions to determine whether any particular proportion had a greater or lesser effect than any of the others (Figure 4.). All the lines followed very similar gradients again showing that none of the variables showed any significant difference.

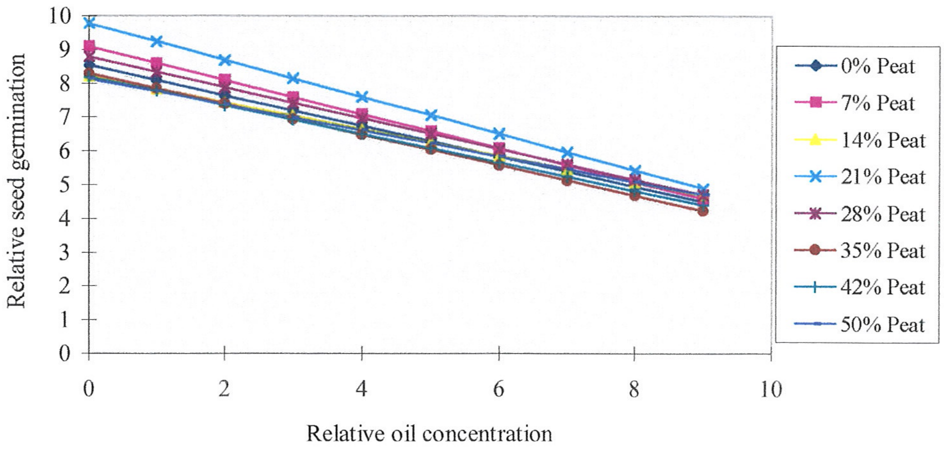

Figure 4. Comparison of regression data on Festuca rubra seed germination.

2.4.2 Festuca rubra transplants

After 60 days none of the additions of peat significantly alleviated the toxic affects of oil on *Festuca rubra* (Figure 5). Any oil at all seemed to have a detrimental effect on plant growth (Figure 6). Although this was the case for oil, increasing proportions of peat did improve plant growth in the controls (Figure 7).

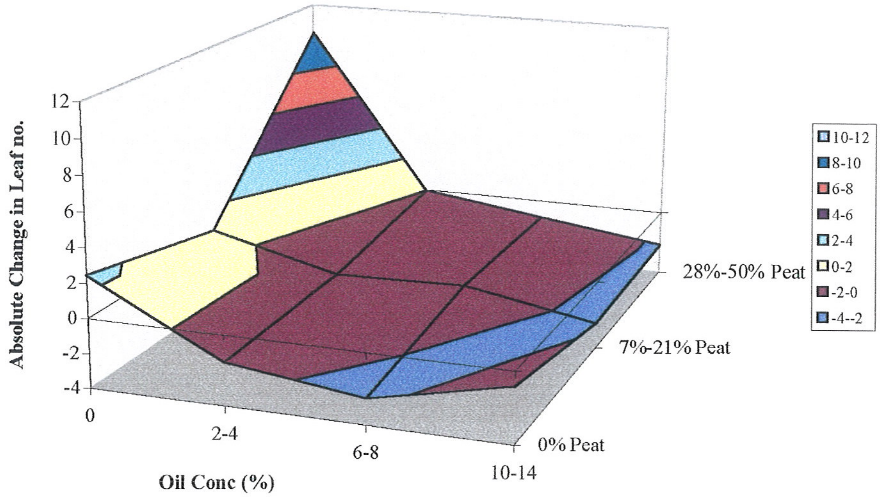

Figure 5. Absolute change in leaf number of Festuca rubra grouped data in different concentrations of peat and oil after 60 days.

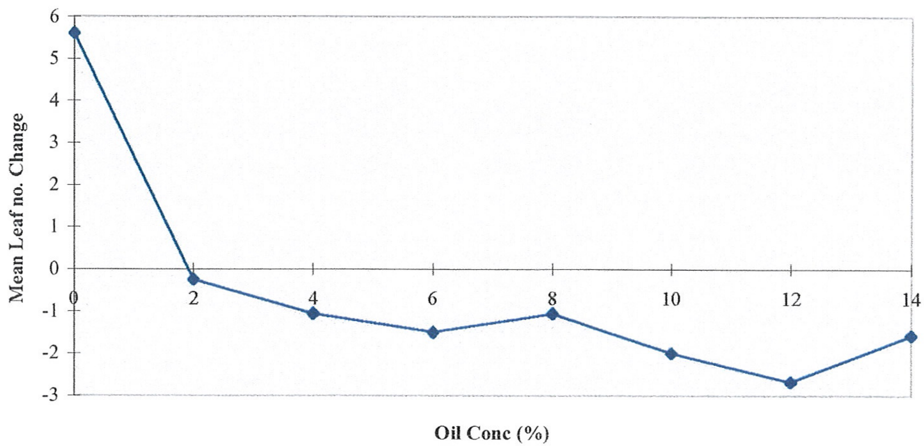

Figure 6. Effects of oil concentrations on increase/decrease in mean leaf numbers of Festuca rubra (grouped data for all peat treatments).

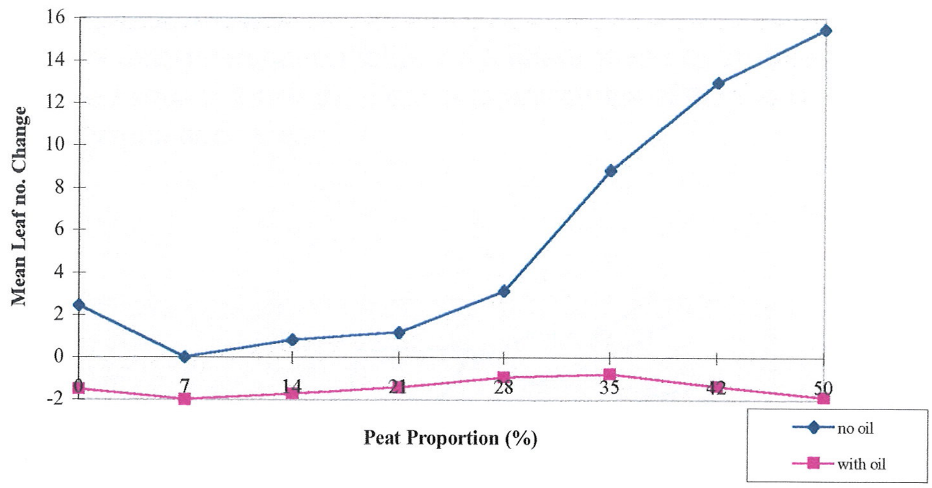

Figure 7. Increase/decrease in mean leaf number of Festuca rubra at different peat proportions with and without oil after 60 days.

Regression analysis (Figure 8) showed that although peat did not increase plant growth in oil contaminated soils, the more peat that was present the less detrimental an effect increasing concentrations of oil had on *Festuca rubra* transplants.

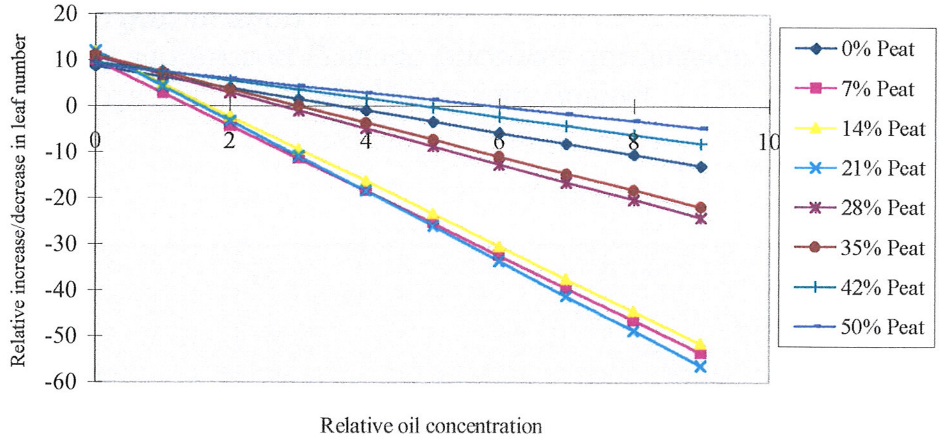

Figure 8. Comparison of regression data on Festuca rubra leaf growth.

2.4.3 Plantago lanceolata seeds

Data were collected for 73 days.

Control germination rates

Plantago lanceolata germination followed a linear trend over time in uncontaminated soils (Figure 9). Peat concentrations of 42% and above gave the highest germination rates.

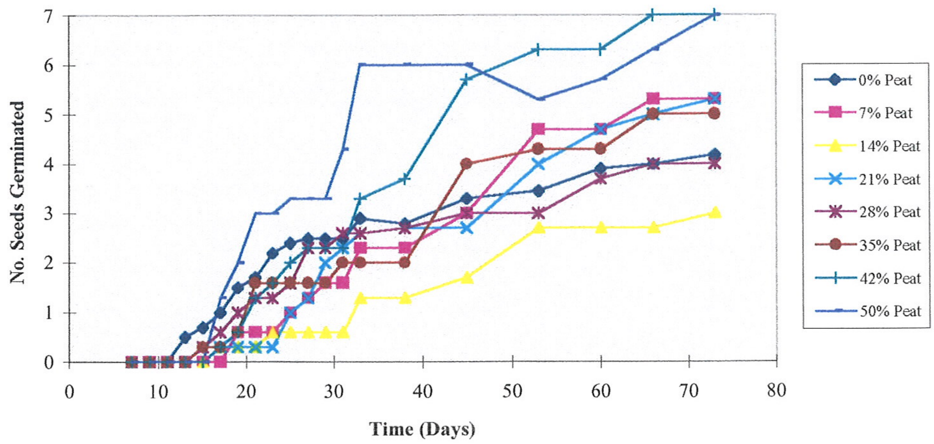

Figure 9. Germination rates of Plantago lanceolata in the controls without oil.

Effect of oil on germination

Oil substantially suppressed *Plantago lanceolata* germination (Figure 10), with all concentrations having a similarly damaging impact.

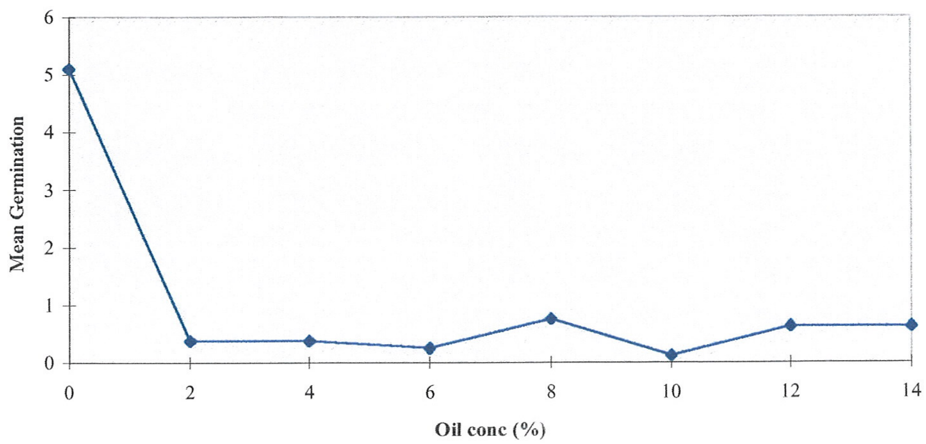

Figure 10. Mean germination of Plantago lanceolata after 73 days (grouped data for all peat treatments).

There seemed to be slightly more germination at the higher oil concentrations (10-14%) than in the lower oil concentrations (2-6%) and this germination tended to be centred around the lower peat proportions (0-21%). 28-50% peat showed nearly no germination in any of the pots at any oil concentration (Figures 11 and 12).

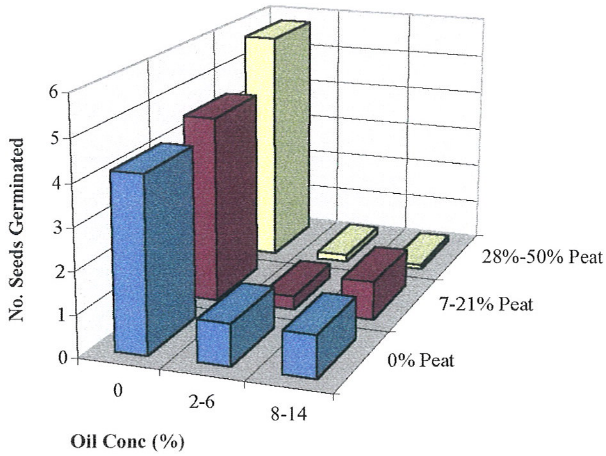

Figure 11. Germination of Plantago lanceolata after 73 days as grouped data.

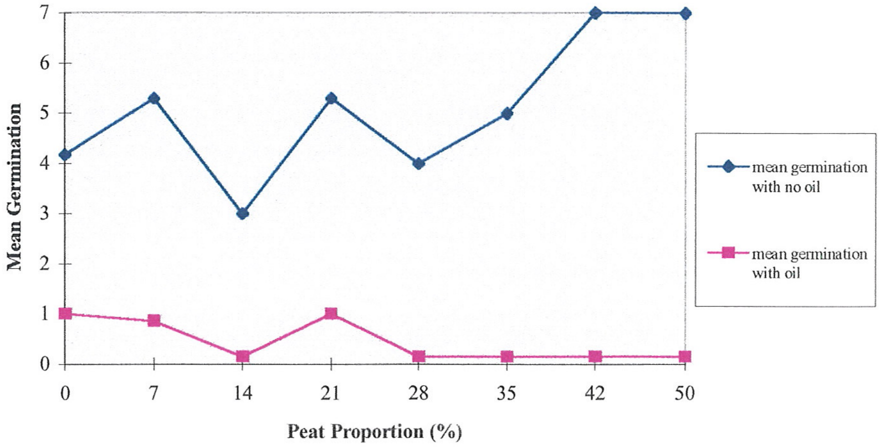

Figure 12. Mean germination of Plantago lanceolata at different peat proportions in oil and without oil after 73 days.

Regression analysis showed that the amount of germination occurring at these low peat proportions and high oil concentrations were too low to be significant except for 21% peat (Figure 13). Increasing the oil concentration did not seem to have such a detrimental effect on seed germination at 21% peat when compared to the other peat proportions.

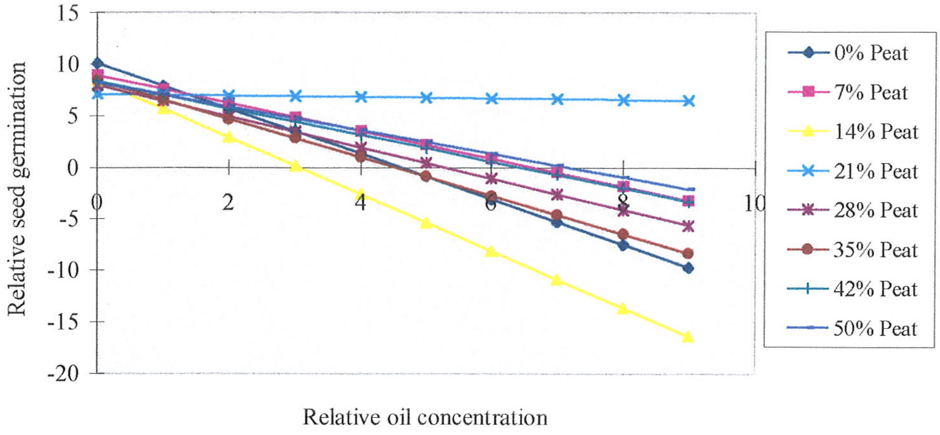

Figure 13. Comparison of regression data on Plantago lanceolata germination

2.4.4 Plantago lanceolata transplants.

The only trend from the *Plantago lanceolata* transplant data was that as the oil concentration increased, plant growth decreased (Figure 14).

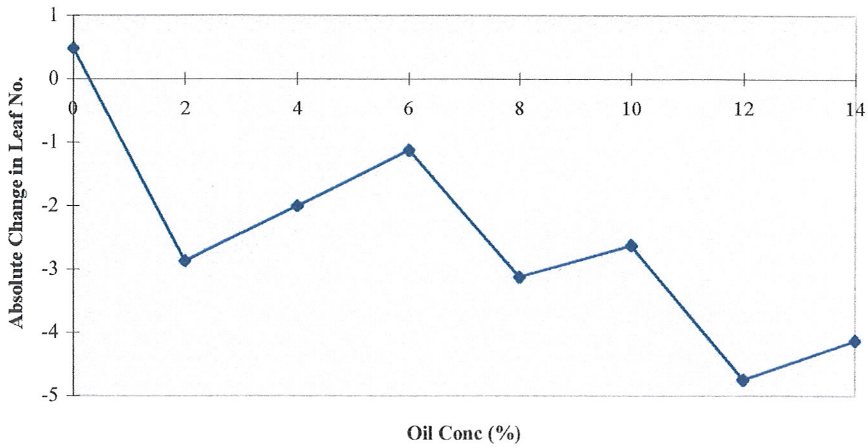

Figure 14. Mean increase/decrease in leaf number of Plantago lanceolata after 60 Days (grouped data for all peat treatments).

None of the peat proportions had any significant effect on improving plant growth in oil contaminated soil. Initial analysis showed no trend except for general leaf loss. Growth only occurred here and there without any clear pattern (Figure 15).

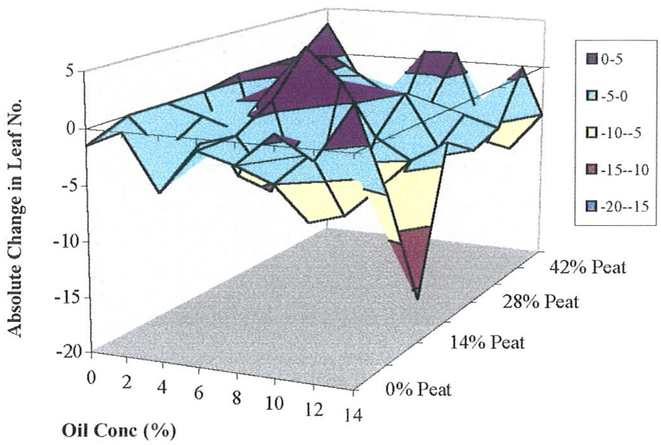

Figure 15. Increase/decrease in leaf number of Plantago lanceolata in different concentrations of peat and oil after 60 days.

Regression analysis was not possible here because the data was too scattered to obtain reliable regression lines.

2.5 Discussion

It was difficult to analyse the data from this experiment. The use of many variables meant that no replicates were used except with the controls. Because of this, it would have been necessary for a major trend to occur to obtain a significant result. No major positive trends occurred in this experiment, apart from the fact that the addition of oil reduced plant germination and growth.

For the *Festuca rubra* seeds and transplants, there was a small negative trend suggesting that peat did not counteract the toxic and water stress properties of Forties Crude. But, the results obtained from the *Plantago lanceolata* seeds and transplants were less discernible. In fact, the data obtained from *Plantago lanceolata* was impossible to analyse because there were no trends. The data was too scattered. All that could be determined was that *Plantago lanceolata* transplants did not respond well to oil contamination at any level and that the different peat proportions did not seem to effect that result.

The germination data for *Plantago lanceolata* showed some anomalies. Apart from the controls, there seemed to be germination only at higher concentrations of oil (10-14%) and at lower peat proportions ($\leq$ 21%). The reasons for this are unclear. It may have been due to the fact that ten seeds per pot was not a large enough sample size. An additional factor may have been variation in the extent of water ponding on different pots. Neither *Festuca rubra* and *Plantago lanceolata* grow well in waterlogged soils (Grime, J. P. *et al.* 1988). Ponding also meant that water didn't penetrate the soil surface leading to a water deficit on the plant. This may have been the reason why trends didn't form as other factors may have affected the results. The effect of oil on water percolation was investigated in the next set of experiments.

2.6 Conclusion

From the data obtained it appeared that peat did not significantly alleviate the toxic and water stress properties of Forties Crude on *Festuca rubra* and *Plantago lanceolata*.

3. EFFECTS OF OIL CONTAMINATION AND PEAT ADDITION ON WATER RETENTION BY SAND.

3.1 Introduction

This experiment was to investigate which factors may affect the percolation of water through contaminated sand. The first experiment exhibited instances of ponding on the surface of some of the pots, so that the topsoil may have been saturated whereas the rest of the pot may have been dry.

3.2 Aims

This preliminary experiment was designed to determine whether it was the influence of peat or oil or both that affected percolation and also what effect initially dry or wet sand and peat had on water drainage. A subsequent experiment examined effects of different concentrations of oil (section 4).

The factors to be investigated were:-
1) Rate of water infiltration
2) Maximum water retention
3) Amount of ponding

3.3 Methodology

1) Eight 200ml pots were filled with different soil mixtures. As this was a preliminary experiment there was no replication. A piece of gauze was first placed in the bottom of the pot to stop soil washing through.

Table 1. Pot Set-up

Sand	or	Sand/peat mixture (50:50)
	×	
Wet	or	Dry
	×	
Oil (10%)	or	None

2) The initial mass of all the pots were recorded, calibrating the scales using an empty pot.
3) Each pot was raised above the ground on a small tripod and a 250ml beaker was placed underneath to collect percolate.
4) 100ml of water was poured into the pot.
5) The length of time taken for water to start dripping out of the bottom of the pot was recorded as well as the length of time that water remained on the surface of the soil.
6) Timing was continued until water dripped out of the pot at intervals greater than ten seconds. This time was also recorded.
7) The mass of the pot was again determined together with the volume of percolate.

3.4 Results
For raw data see appendix B.

Water didn't penetrate the dry sand, peat and oil mixture, even after 600 seconds. The water just remained on the surface of the soil.
There was a significant change in the water retention properties of dry soil when mixed with oil. The dry sand and oil halved its water retention properties when compared to dry sand alone (figures 16 & 17).

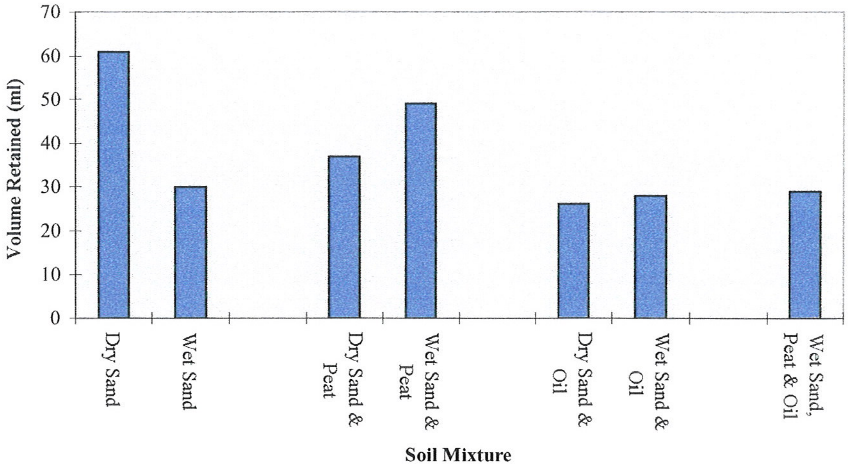

Figure 16. Volume of water retained after 100ml was poured through.

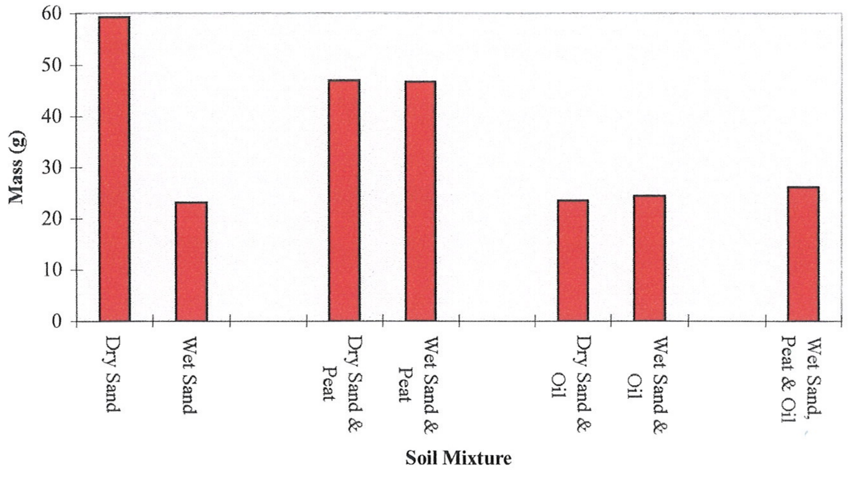

Figure 17. Change in pot mass after water was added.

Initially wet sand remained more uniform in terms of water retention and water infiltration when mixed with oil and peat than initially dry sand. The effect of oil and peat on dry sand varied greatly especially in terms of ponding time and infiltration time (figures 18 and 19).

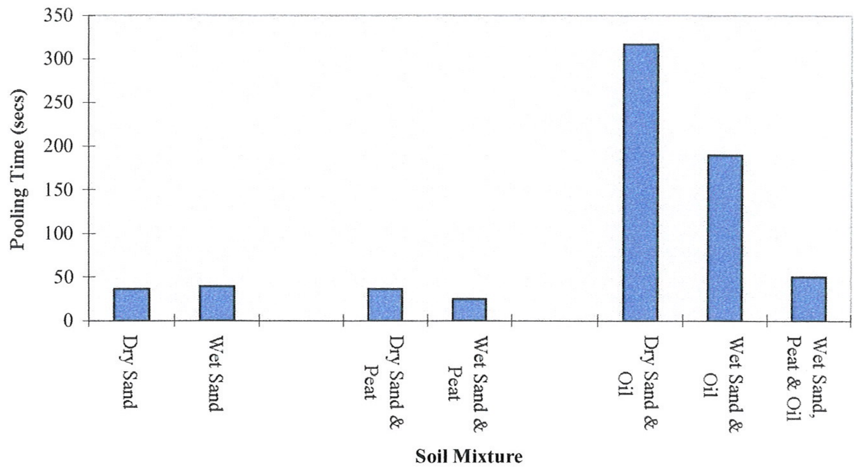

Figure 18. Ponding times of the different soil mixtures.

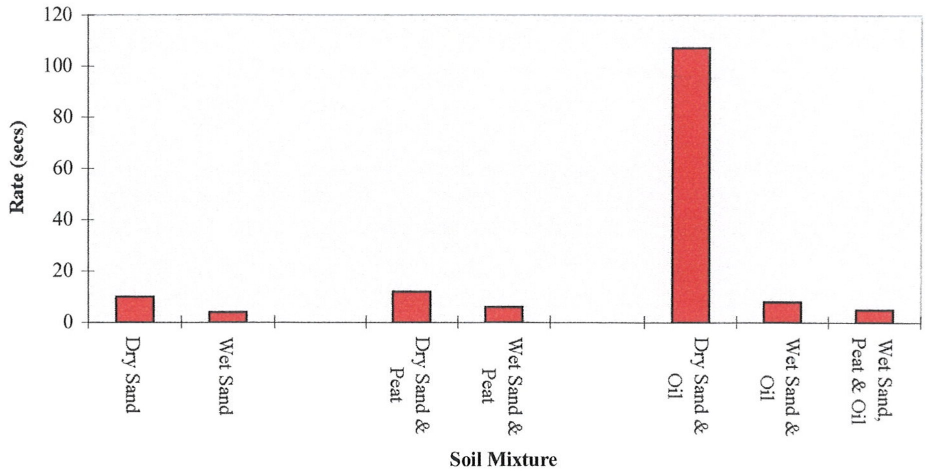

Figure 19. Rate of infiltration of the different soil mixtures.

3.5 Discussion

The results showed that peat on its own tended to have little effect on the water retention properties of the sand. There was little variation in the rate of infiltration between wet and dry sand (51 and 46 seconds respectively) and wet and dry sand and peat (34 and 59 seconds respectively). However, for all oil treatments the length of time of ponding and infiltration was greatly increased. The rate of infiltration was almost zero for dry sand, peat and oil. This was probably because oil is hydrophobic and inhibited the movement of water through the soil. Also dry peat is difficult to wet and this probably also presented problems for water percolation.

Oil increased the length of time of ponding at the soil surface probably because of its hydrophobic properties, although, wet peat mixed in tended to alleviate this problem a bit. Dry peat tended to complicate matters even more and almost completely inhibited the movement of water through soil. The effect of the oil seemed to be greatly increased if the soil was intially dry. When wet, soil already had a film of water surrounding the individual soil particles. This would probably have inhibited, to some extent, the adhesion of oil to the particles and thus reduced the overall hydrophobic properties of the soil and allowed the percolation of water through. This eventuality would not have occurred if the soil was initially dry because oil would have been able to coat the individual soil particles.

As could be expected, initially dry sand retained more water than intially wet sand. Yet, the addition of oil or dry peat to dry sand dramatically reduced these differences in that dry sand retained less water, similar to that of initially wet sand which was already saturated. This was probably due to the hydrophobic properties of the oil, increased by initially dry sand, and the difficulty of wettening up dry peat.

3.6 Conclusion

This experiment showed that oil played a significant part in reducing the water retention properties of sand. Peat on its own with sand didn't really effect water retention but when initially dry it did enhance the effect of the oil. There was also a significant effect of mixing oil with dry sand in inhibiting water movement through soil.

4. WATER RETENTION PROPERTIES OF CONTAMINATED SAND: EFFECTS OF OIL CONCENTRATIONS.

4.1 Introduction

The last experiment showed that peat tended to have little effect on the percolation of water through soil. This was not true for oil. Oil at 10% concentration significantly slowed the movement of water through sand. This second experiment was designed to investigate the effect of different concentrations of oil on the percolation of water through sand.

Both wet and dry sand were used with concentrations of oil from 0% to 10% in 2% intervals. There were four replicates of each treatment.

4.2 Aims

To determine how increasing oil concentrations affect the amount of water retained and the rate of infiltration.
The factors to be investigated:-
1) Rate of water infiltration
2) Max. water retention
3) Amount of ponding
4) Affects of initially dry and wet sand.

4.3 Methodology

1) 200ml pots were filled with different soil mixtures as described below. A piece of gauze was first placed in the bottom of the pot to stop soil washing through.

Table 2. Pot set-up

Sand:		Wet	or	Dry		
			×			
Oil Concs (%)	0	2	4	6	8	10
Replicates:	4					
Total Pots:	48					

N.B. See Appendix C for Oil Concentration Calculations.
2) The initial mass of soil in each pot was recorded.
3) Each pot was raised above the ground on a small tripod and a 250ml beaker placed underneath to collect percolate.
4) 100ml of water was poured into the pot.
5) The length of time taken for water to start dripping out of the bottom of the pot was recorded as well as the length of time that water remained on the surface of the soil.
6) Timing was continued until water dripped out of the pot at intervals greater than ten seconds. This time was also recorded.
7) The mass of the pot was again determined, together with the volume of percolate.

4.4 Results

4.4.1 Initially wet sand data

On initially wet sand pots the ponding and infiltration times increased nearly linearly with oil concentrations (figure 20).

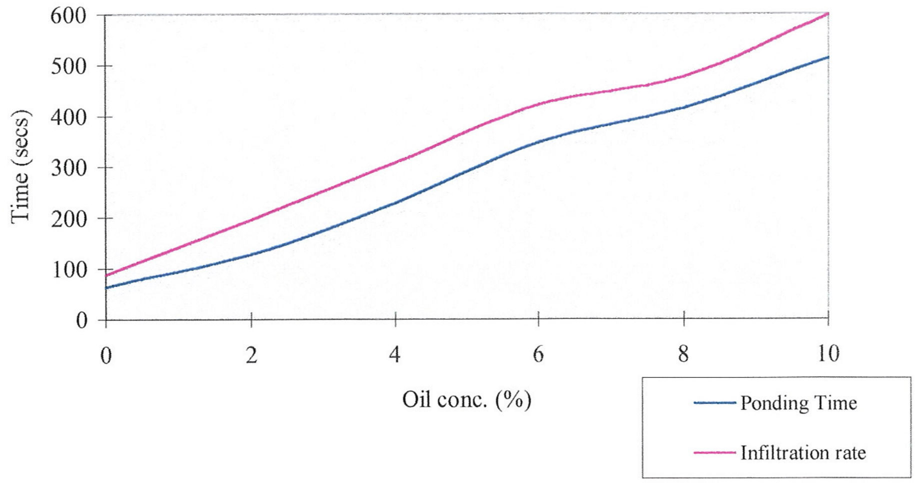

Figure 20. Ponding time and infiltration rate of water through initially wet sand at different oil concentrations.

Water retention fell to an asymptote with increasing oil concentration (figure 21).

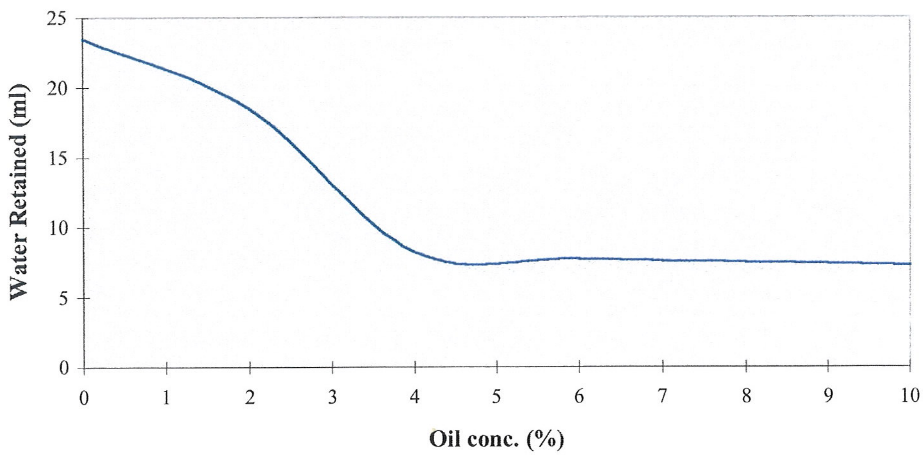

Figure 21. Maximum water retention of initially wet sand at different oil concentrations.

Analysis of variance (table 3) showed that oil had a significant effect on the water retention properties of the soil, the length of time of ponding and on the infiltration rate. There was also a significant difference between the oil concentrations as well. Any concentration greater than 2% oil had a significant effect on the ponding of water and the infiltration rate.

Table 3. ANOVAs on initially wet sand data

	Oil Mixed Pots Only			Control & Oil Mixed Pots		
Water Retention Data	$f_{4,15} = 21.96$	$p<0.0001$	$f_{crit} = 3.056$	$f_{5,18} = 18.39$	$p<0.0001$	$f_{crit} = 2.77$
Infiltration Rate Data	$f_{4,15} = 18.52$	$p<0.0001$	$f_{crit} = 3.056$	$f_{5,18} = 32.68$	$p<0.0001$	$f_{crit} = 2.77$
Ponding Time Data	$f_{4,15} = 15.68$	$p<0.0001$	$f_{crit} = 3.056$	$f_{5,18} = 24.31$	$p<0.0001$	$f_{crit} = 2.77$

The addition of oil also tended to increase the variation within the replicates for the infiltration rates and ponding times. For example, the standard deviations for the wet sand infiltration rate replicate data are given in table 4.

Table 4. Standard deviations within replicates for the wet sand infiltration rate data

Groups	Standard Deviation
Wet Sand	12.03
Wet Sand and 2% Oil	25.16
Wet Sand and 4% Oil	38.31
Wet Sand and 6% Oil	49.84
Wet Sand and 8% Oil	109.35
Wet Sand and 10% Oil	97.16

4.4.2 Initially dry sand data

Responses of dry sand pots were significantly less uniform than those for initially wet sand. Water easily moved through the control pot, the mean infiltration rate was 60.5 and the variation between pots was quite low (table 6). The addition of oil to 2% and 4% completely inhibited the penetration of water into the soil, water was still sitting on the majority of these replicates after 27 hours. Only one 4% pot allowed water through and the infiltration rate for this was 5025 seconds. So 2% and 4% oil had an extremely significant effect on the water retention and percolation properties of initially dry sand. Dry sand containing 6%, 8% and 10% oil did allow water through but very little water was retained and the rates of infiltration were very slow when compared to the control (figure 22 and 23).

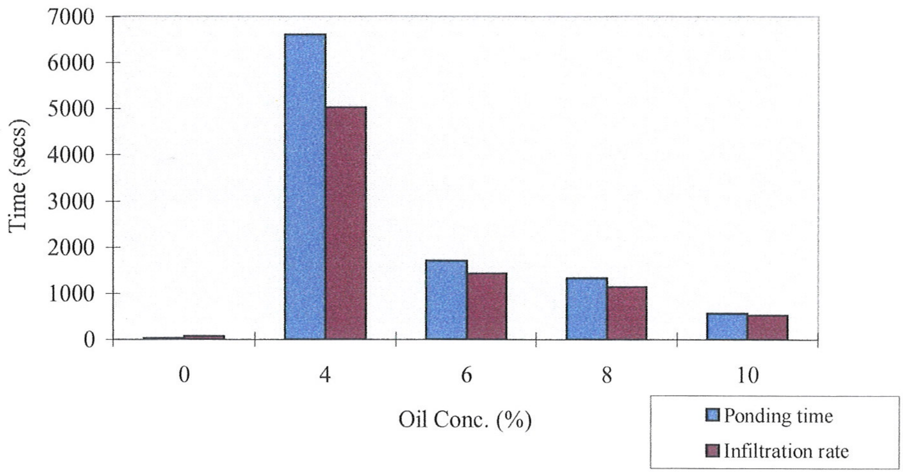

Figure 22. Ponding time and infiltration rate of water through initially dry sand at different oil concentrations.

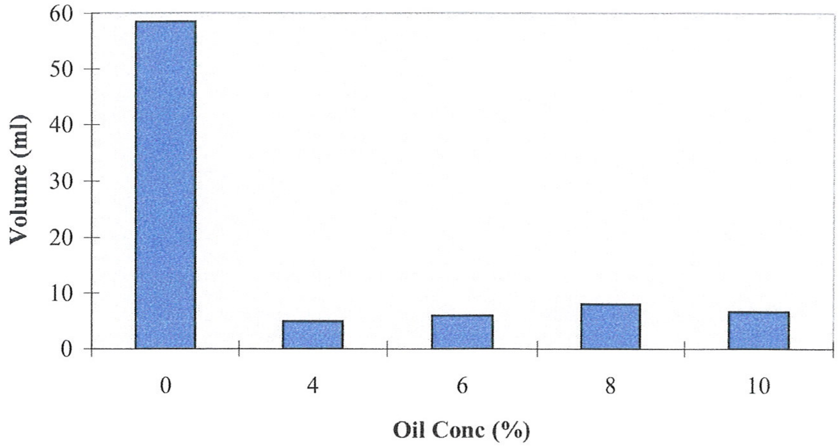

Figure 23. Maximum water retention of initially dry sand at different oil concentrations.

Analysis of variance (table 5) showed the oil had a significant effect on the water retention properties, the ponding time and the infiltration rate of dry sand. There were no significant differences between the 6%, 8% and 10% oil data. Because there was no numerical data for the 2% and 4% oil replicates, these were not included in the analysis of variance but it was clear that these concentrations were substantially different from the rest. There was also an enormous amount of variation within the replicate groups for the rate of infiltration data and the ponding time data. For example, the standard deviations for the dry sand infiltration rate replicate data are given in table 6.

Table 5. ANOVAs on initially dry sand data

	6, 8 & 10% Oil Mixed Pots Only			Control & 6, 8 & 10% Oil Mixed Pots		
Water Retention Data	$f_{2,9} = 0.86$	$p<0.455$	$f_{crit} = 4.26$	$f_{3,12} = 534.94$	$p<0.0001$	$f_{crit} = 3.49$
Infiltration Rate Data	$f_{2,9} = 5.23$	$p<0.031$	$f_{crit} = 4.26$	$f_{3,12} = 12.58$	$p<0.0005$	$f_{crit} = 3.49$
Ponding Time Data	$f_{2,9} = 3.83$	$p<0.063$	$f_{crit} = 4.26$	$f_{3,12} = 8.73$	$p<0.0024$	$f_{crit} = 3.49$

Table 6. Standard deviations within replicates for the dry sand infiltration rate data

Groups	Standard Deviation
Dry Sand	3.32
Dry Sand and 6% Oil	517.33
Dry Sand and 8% Oil	406.65
Dry Sand and 10% Oil	208.48

4.4.3 Comparison of wet and dry sand results

Analysis of variance were carried out to analyse differences or similarities between the wet and dry sand data. No data analysis was carried out on the 2% and 4% data because no numerical data was obtained but it was obvious that there was a major difference between wet and dry sand containing 2% and 4% oil since water didn't even percolate most of the dry sand pots.

There were significant differences for rate of infiltration and ponding time between the wet and dry sand data at all oil concentrations except 10% oil as shown in table 7.

There were no significant differences for water retention in the 6%, 8% and 10% oil concentrations.

Table 7. ANOVA comparisons between initially wet and dry sand results

	Water Retention	Rate of Infiltration	Ponding Time
Control	$f_{1,6}=99.3$ $p<0.0001$ $f_{crit}=5.99$	$f_{1,6}=18.0$ $p<0.005$ $f_{crit}=5.99$	$f_{1,6}=27.1$ $p<0.002$ $f_{crit}=5.99$
6% Oil	$f_{1,6}=5.44$ $p<0.06$ $f_{crit}=5.99$	$f_{1,6}=14.97$ $p<0.008$ $f_{crit}=5.99$	$f_{1,6}=12.99$ $p<0.01$ $f_{crit}=5.99$
8% Oil	$f_{1,6}=0.06$ $p<0.8$ $f_{crit}=5.99$	$f_{1,6}=9.86$ $p<0.02$ $f_{crit}=5.99$	$f_{1,6}=8.04$ $p<0.03$ $f_{crit}=5.99$
10% Oil	$f_{1,6}=0.22$ $p<0.65$ $f_{crit}=5.99$	$f_{1,6}=0.33$ $p<0.59$ $f_{crit}=5.99$	$f_{1,6}=0.24$ $p<0.64$ $f_{crit}=5.99$

There were significant differences between all comparisons in the controls, the difference in the water retention properties of wet and dry sand being the most marked.

4.5 Discussion

Oil played a significant part in restricting water infiltration and inhibiting water retention of sand. Oil is hydrophobic, as already discussed in the last experiment and so acted as a water retardant in the soil. Water was only able to pass through the sand in any channel that happened to be devoid of oil. These channels were probably caused by uneven mixing of the oil into the sand and probably led to the enormous amount of variation between replicates. This may also help to explain why water did not pass through the initially dry sand pots at 2% and 4% oil. It may have been that the oil was more thoroughly mixed in these pots completely inhibiting the movement of water through the soil. It may have been this factor alone that caused the apparent lack of trends in the initially dry sand results. Dry sand tended to be affected more by the hydrophobic properties of oil than wet sand. This was probably due to factors already discussed in the last experiment, that oil coated the particles of dry sand whereas wet sand already coated in water conflicted with the oil. Oil was therefore more likely to be washed out of the initially wet sand and it was often observed that water dripping out of the bottom of these pots tended to have a more oily sheen than water dripping out of the initially dry sand pots. Because water already coated the particles of sand in the initially wet sand pots, there were probably more hydrophilic channels once oil was added than in the initially dry sand pots, which allowed the passage of water. Water passed more easily through the initially wet sand pots and so a definite trend was evident showing that as the oil concentration increased so the rate of water infiltration increased and the amount of water retained decreased. Any amount of oil in the initially dry sand pots seemed to completely disrupt the movement of water and the water retention properties of the sand.

As a small side experiment, the initially wet pots were left for a few weeks to dry out and water again poured through some of them. The data obtained from this showed that once dry, these pots behaved the same way as the initially dry sand pots. The average ponding time of three of the replicates of the 2% oil pots was 7503 seconds and the average rate of infiltration was 4662 seconds.

4.6 Conclusion

These results may help to explain the lack of trends in the experiment on the effects of peat on the growth of seeds and transplants in contaminated sand (section 2.0). In that experiment some of the pots dried out due to a broken water pump before the experiment was completely up and running.

It has been shown that oil has a significant effect on water retention and water percolation through sand and that initially dry sand mixed with oil completely randomises the movement of water through soil.

It should be noted that for any future experiment involving oil and sand, the sand must start off wet and remain wet for the duration of the experiment.

5. AN INVESTIGATION INTO HOW WEATHERED OIL AND UNWEATHERED OIL AFFECTS THE GERMINATION OF *FESTUCA RUBRA*

5.1 Introduction

The first experiment showed that peat didn't improve germination or growth of *Festuca rubra* and *Plantago lanceolata* seeds and transplants. Yet, the experiments carried out by Waterhouse, 1995, showed that at 2% oil concentrations, peat significantly improved *Festuca rubra* germination. The main difference between the two experiments was the fact that Waterhouse used weathered Forties crude and the oil used in experiment 2.0 was unweathered Forties crude. Therefore, this experiment was designed similarly to the Waterhouse experiment 2.4 except that both weathered and unweathered oil was used.

5.2 Aims

To determine whether peat has a more significant effect on the toxicity of weathered crude at low concentrations than on unweathered crude.

5.3 Methodology

1) 60ml of Forties crude oil was weathered as outlined in Appendix E.
2) The different variables were set up in 250ml pots as shown below.

Table 8. Pot set-up

Soils:		Sand	OR	Sand/Peat (50:50)
			×	
Oil (2%):		None	Unweathered	Weathered
Replicates:	5			

3) The sand and peat were initially wet and were kept wet for the duration of the experiment.

5.4 Results

Table 9. Mean germination over 28 days

Day:	0	7	14	21	28
Sand	0	17.4	20.6	21.8	22
Sand/Peat	0	13.8	17.2	18.2	18.6
Oil	0	0	11.2	17	18
Oil/Peat	0	0	15.6	19.2	20
Weathered Oil	0	0	16.4	19.4	19.6
Weathered Oil/Peat	0	0.8	18.2	19.8	20

The presence of oil slowed the rate of germination (figure 24). But this was only a delay response. Over time the difference in germination of *Festuca rubra* in oil and *Festuca rubra* without oil became insignificant. Peat did not seem to significantly improve the germination of *Festuca rubra* in either weathered or unweathered oil.

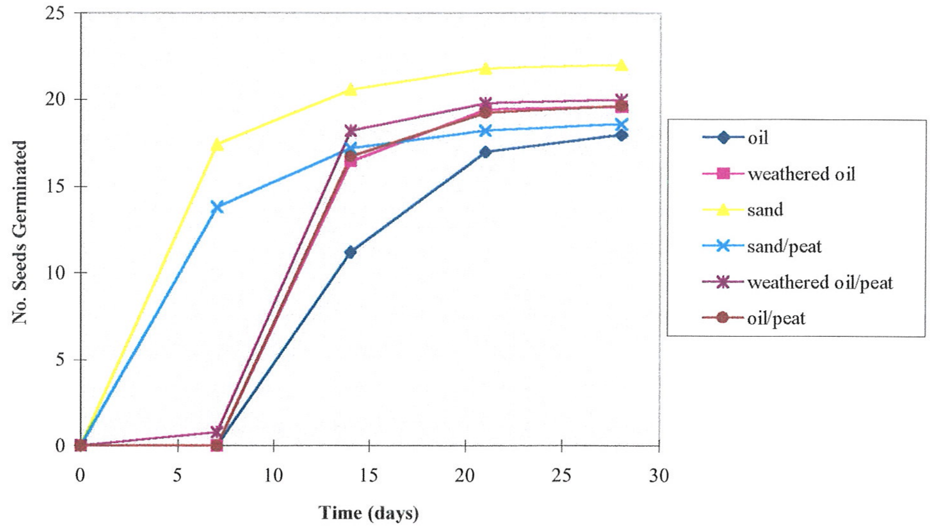

Figure 24. Graph showing the mean germination of Festuca rubra in different soil and oil treatments

Analysis of variance (figures. 25,26 and 27) showed that any significant difference between the different treatments became insignificant after 14 days. There was no significant difference between the different treatments at the end of the experiment although it was observed that plants growing in peat tended to look healthier than plants growing without peat.

```
                                   Individual 95% Confidence Interval
Treatments               Mean ---+---------+---------+---------+--------
Sand                     16.4                          (--------*--------)
Sand & Peat              13.6                (--------*--------)
Weathered oil             9.2   (--------*---------)
Weathered oil & Peat     11.1        (---------*--------)
Oil                      11.0        (--------*---------)
Oil & Peat               11.8          (---------*--------)
                              ---+---------+---------+---------+--------
                                7.0      10.5      14.0      17.5
                                      Mean no. seeds germinated
```

```
                 Individual 95% Confidence Interval
Time(d)  Mean -----+---------+---------+---------+------
   0     0.0    (---*---)
   7     5.3         (----*---)
  14    16.5                        (----*---)
  21    19.2                           (---*----)
  28    19.7                            (---*---)
              -----+---------+---------+---------+------
                  0.0       7.0      14.0      21.0
                            time (days)
```

Source	ANOVA for Germination		
Treatments	$f_{5,20} = 2.57$	$p<0.059$	$f_{crit} = 2.71$
Time (days)	$f_{4,20} = 40$	$p<0.0001$	$f_{crit} = 2.87$

Figure 25. Two-way ANOVA for germination of Festuca rubra over 28 days in different soil treatments.

```
                                              INDIVIDUAL 95% CI'S FOR MEAN
                                              BASED ON POOLED STDEV
Treatment               N   MEAN    STDEV    ---+---------+---------+---------+---
Sand                    5   17.400  2.881                                (--*--)
Sand & Peat             5   13.800  2.950                          (--*--)
Weathered Oil           5   0.000   0.000    (--*--)
Weathered Oil & Peat    5   0.000   0.000    (--*--)
Oil                     5   0.000   0.000    (--*--)
Oil & Peat              5   0.800   1.304      (-*--)
                                             ---+---------+---------+---------+---
POOLED STDEV =   1.765                         0.0       6.0      12.0      18.0
                                               Mean no. seeds germinated
```

Source	ANOVA for Germination
Treatments After 7 Days	$f_{5,24} = 103.69$ $p<0.0001$ $f_{crit} = 2.62$

Figure 26. One-Way ANOVA for germination of Festuca rubra after 7 days.

```
                                              INDIVIDUAL 95% CI'S FOR MEAN
                                              BASED ON POOLED STDEV
Treatment               N   MEAN    STDEV    -------+---------+---------+---------
Sand                    5   22.000  1.225                   (--------*--------)
Sand & Peat             5   18.600  2.510       (--------*--------)
Weathered Oil           5   18.000  3.391     (--------*--------)
Weathered Oil & Peat    5   19.600  0.894         (--------*--------)
Oil                     5   20.000  3.162            (--------*--------)
Oil & Peat              5   20.000  2.236            (--------*--------)
                                             -------+---------+---------+---------
POOLED STDEV =   2.419                             17.5      20.0      22.5
                                               Mean No. seeds germinated
```

Source	ANOVA for Germination
Treatments After 28 Days	$f_{5,24} = 1.64$ $p<0.188$ $f_{crit} = 2.62$

Figure 27. One-Way ANOVA for germination of Festuca rubra after 28 days

5.5 Discussion

The results obtained from this experiment conflicted with the results obtained by Waterhouse since peat didn't alleviate the toxic properties of either weathered or unweathered oil. Indeed, the sand and peat replicates showed, on average, less germination than the sand only replicates. However germination tended to occur as well in oil and sand as with oil, peat and sand. This may have been because oil wasn't particularly toxic at a 2% concentration. Oil delayed germination by about one week, but this occurred in all the treatments containing oil. Peat did not improve the germination rate either. Peat did seem to improve the health of the plants but this was probably only because of the increased nutrient content of the soil and not because of the peat diminishing the toxic effects of the oil. It remains unclear why the Waterhouse experiment showed increased germination in 2% oil when peat was added to 50%.

5.6 Conclusion

Peat does not play a significant part in alleviating the toxic properties of either weathered or unweathered crude. Germination occurred to a similar level in all of the pots so 2% oil wasn't particularly toxic to *Festuca rubra* seeds.

6. FINAL DISCUSSION AND CONCLUSIONS

Forties crude oil had a significant toxic effect on *Festuca rubra* and *Plantago lanceolata* seeds and transplants. Oil, even at a 2% concentration, was detrimental to plant growth. The reasons for this were both chemical and physical. The physical effects of oil on water retention and water percolation through sand were significant but these effects could be reduced by ensuring that the sand was initially wet before oil was added. This physical effect of oil, in reducing the movement of water through sand would have caused a significant water deficit for rooted plants. Because some of the pots were unfortunately allowed to dry out before the experiment described in section 2.0 was fully up and running, it may have been the physical effects, more than the chemical effects, of the oil that reduced or inhibited plant growth or seed germination. In experiment discussed in section 5.0 *Festuca rubra* germinated well in 2% oil. The sand in this experiment was initially wet and kept wet throughout the duration of the experiment. This would have reduced the physical effects of the oil and thus germination would have been less reduced than if the soil had been allowed to dry out.

The main aim of these experiments was to determine whether peat, mixed in with oil contaminated sand, could be used as a counteractive measure to the toxic effects of the oil. This method might have improved plant growth on oiled beach materials following a major oil spill. Unfortunately, these experiments show that peat is not an effective counteractive measure.

To summarise, the main conclusions that could be drawn from these experiments were:

- peat was not effective in alleviating the toxic properties of Forties Crude oil.
- Oil had a significant effect on the physical properties of the soil.
- The detrimental effects of the oil on the water retention and water percolation properties of the soil could be reduced somewhat by ensuring that the soil had an initially high moisture content and that the moisture content remained high.

7. REFERENCES

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APPENDIX A

Methodology for preparing the peat, sand and oil mix for the experiment described in section 2.0

For each peat proportion there were 40 pots (10 per block). The pots held a capacity of 1000ml therefore at least 40000ml of peat and sand mix was required for each peat proportion. In case of any error 50000ml of mixture was used for each peat proportion.

The amount of peat and sand required for each peat proportion were as follows:-

i) 50% Peat
25000ml peat & 25000ml sand
ii) 42% Peat
21000ml peat & 29000ml sand
iii) 35% Peat
17500ml peat & 32500ml sand
iv) 28% Peat
14000ml peat & 36000ml sand
v) 21% Peat
10500ml peat & 39500ml sand
vi) 14% Peat
7000ml peat & 43000ml sand
vii) 7% Peat
3500ml peat & 46500ml sand
viii) 0% Peat
50000ml sand

For all of the peat proportions increasing concentrations of oil needed to be added one step at a time and samples removed for each concentration.

i) 50000ml of sand and peat was mixed up in a cement mixer. 12000ml was removed for 12 pots, three per block. These acted as controls.
This left 38000ml of mix.

ii) Oil was added 2% at a time up to 14% (see table 10).

Table 10. Concentrations for pot mixtures, section 2.0.

	Amount of Mix (ml)	Amount of Oil (ml)	Total (ml)	Remove for four pots (ml)	Amount Remaining (ml)
2% Oil	38000	776	38776	4000	34776
4% Oil	34776	709	35485	4000	31485
6% Oil	31485	650	32135	4000	28135
8% Oil	28135	600	28735	4000	24735
10% Oil	24635	550	25285	4000	21285
12% Oil	21285	500	21785	4000	17785
14% Oil	17785	400	18185	4000	14185

iii) The 12185ml of mix left over was discarded.
iv) Steps i)-iii) were repeated for each peat proportion.
v) Eight more sand only pots were made up for each block as controls.

APPENDIX B

Results for water retention experiment described in section 3.0.

Table 11. Raw data for water retention experiment (section 3.0)

	Dry Sand	Wet Sand	Dry Sand & Peat	Wet Sand & Peat	Dry Sand & Oil	Wet Sand & Oil	Dry Sand, Peat & Oil	Wet Sand, Peat & Oil
Initial Mass (grams)	267.97	281.9	181.28	189.39	258.85	280.44	198.72	184.37
Mass After Max. Water Retention (grams)	327.26	305.09	228.29	236.1	282.33	304.79	-	210.58
Change in Mass (grams)	59.29	23.19	47.01	46.71	23.48	24.35	-	26.21
Volume of Water That Dripped Out (ml)	39	70	63	51	74	72	-	71
Volume of Water Retained (ml) *	61	30	37	49	26	28	-	29
Ponding Time (sec)	37	39	37	25	317	190	-	50
Time After Pouring When Dripping Starts (sec)	10	4	12	6	107	8	-	5
Time After Pouring When Dripping Stops (sec) *	56	55	71	40	371	440	-	235
Rate of Infiltration (sec)	46	51	59	34	264	432	-	230

* stop when water is dripping in intervals more than 10 sec.

APPENDIX C

Methodology for preparing the sand and oil mix for the experiment described in section 4.0

1) 200ml pots were used for this experiment.
2) The sand and oil were mixed together in concentrations as outlined below.

Table 12. Concentrations for pot mixtures (section 4.0)

	Amount of Sand (ml)	Amount of Oil (ml)	Total (ml)	Remove for four pots (ml)	Amount Remaining (ml)
2% Oil	4000	82	4082	800	3282
4% Oil	3282	67	3349	800	2549
6% Oil	2549	52	2601	800	1801
8% Oil	1801	37	1838	800	1038
10% Oil	1038	21	1059	800	259

3) The rest of the mixture was discarded.

APPENDIX D

Results for water retention experiment described in section 4.0.

Table 13. Mean data for water retention experiment (section 4.0)

	Dry Sand	Dry Sand & 2% Oil	Dry Sand & 4% Oil	Dry Sand & 6% Oil	Dry Sand & 8% Oil	Dry Sand & 10% Oil
Change in Mass (grams)	57.435	-	0.64	2.38	2.66	3.185
Volume of Water That Dripped Out (ml)	41.5	-	95	94	92	93.25
Volume of Water Retained (ml) *	58.5	-	5	6	8	6.75
Ponding Time (sec)	33.25	>97200	6600	1706.5	1331.25	579.75
Time After Pouring When Dripping Starts (sec)	6.5	-	1680	350	279.25	110.75
Time After Pouring When Dripping Stops(sec) *	67	-	6705	1776.25	1417	643.75
Rate of Infiltration (sec)	60.5	-	5025	1426.25	1137.75	533
	Wet Sand	**Wet Sand & 2% Oil**	**Wet Sand & 4% Oil**	**Wet Sand & 6% Oil**	**Wet Sand & 8% Oil**	**Wet Sand & 10% Oil**
Change in Mass (grams)	27.62	18.16	7.83	5.48	4.94	5.1
Volume of Water That Dripped Out (ml)	76.5	81.5	91.75	92.25	92.5	92.75
Volume of Water Retained (ml) *	23.5	18.5	8.25	7.75	7.5	7.25
Ponding Time (sec)	64.25	128	229	346.75	415.5	513.5
Time After Pouring When Dripping Starts (sec)	4.5	4	2.75	3.25	4	5
Time After Pouring When Dripping Stops(sec)*	91.5	199.75	310.5	424	480.5	604.25
Rate of Infiltration (sec)	87	195.75	307.75	420.75	476.5	599.25

* stop when water is dripping in intervals more than 10 seconds.

APPENDIX E

Artificial weathering of oil for the germination experiment described in section 5.0

Seawater

Seawater was collected from Bridge of Don and stored in 10 litre sealed containers until required for artificial weathering.

Oil

The oil used for this experiment was Forties Crude.

Weathering

The oil was artificially weathered for 31 hours by constant churning in the cement mixer with seawater. The seawater was drained out and replaced with fresh seawater at regular intervals in order to simulate the natural dispersion of impurities that would occur at sea. The proportions of each are outlined below.

Table 14. Weathering timetable and proportions for germination experiment (section 5.0).

Hours from commencement of weathering	Quantity of seawater (ml) in mixer	Quantity of oil (ml) in mixer
0	600	60 (added to mixer)
9	600 (replaced)	60
23	600 (replaced)	60
31	600	60