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ABSTRACT

The impact of oil and oil dispersant mixtures on the mycoflora of a freshwater ecosystem was assessed using manmade ponds. Data indicated that the ponds were well stabilized before pond treatment. Sufficient Norman Wells crude oil to give 100 ppm was added to each treated pond. The dispersant, Corexit 9527, was initially one-fifth of the oil concentration in the oil-dispersant treated ponds.

After pond treatment, an immediate increase occurred in the number of geoaquatic fungi in the oil treated pond whereas an increase, followed by a sharp decrease, which was in turn followed by a recovery occurred in the oil-dispersant treated ponds. The fungal enhancement effects, which were slight, were largely short term in duration (7-83 days).

No clear cut medium or long term geoaquatic fungal enhancement effects were observed. The pond treatments had no detectable effect on the vertical distribution of fungi in the ponds. No significant treatment effects were observed on the water mould levels in the treated ponds. No obvious treatment effect was observed on the percentage of viable fungi capable of growth on nondegraded or biodegraded oil as a sole carbon source. Fifteen of 39 selected pond water fungi were able to grow on nondegraded Norman Wells oil.

Corexit 9527 incorporated into agar plates inhibited the radial growth of the oil utilizing fungal isolates; EC_{50} values were determined.

INTRODUCTION

Regular oil shipments occur within the Great Lakes throughout the shipping season, with the consequent risk that a serious oil spill may occur. In the aquatic environment, oil spills are usually dealt with using mechanical containment and cleanup methods; however, weather conditions and the oil spill's location may preclude, or limit, the effectiveness of such measures(1). In such instances, the addition of dispersants to the oil may prove effective.

By emulsifying oil in water, dispersants render it more accessible to microorganisms; theoretically, dispersed oils should be more rapidly biodegraded than undispersed oil. Although some investigators have reported that oil dispersants stimulate the degradation of crude oil by microorganisms(2-4), Mulkins-Phillips and Stewart (4) observed that only one of four dispersants tested actually increased the degradation of the n-alkane fraction of Arabian crude oil by marine bacteria.

Because many of the earlier dispersants were highly toxic to aquatic organisms(5,6), the development of less toxic dispersants became a priority. Mindful of the previous problems associated with the treatment of oil spills with dispersants, researchers and product manufacturers are endeavouring to collect more information on the possible toxic effects of these second generation dispersants, prior

to their widespread application(7.8). To be of practical benefit, chemically dispersed oil should be less detrimental to the ecosystem than non-dispersed oil.

For the foregoing reasons, a project was designed to examine the distribution and effects of oil and oil-dispersant mixtures on freshwater ponds. The use of natural or man made ponds is one of the more practical ways of examining the impact of oil and oil-dispersant mixtures on aquatic ecosystems(9). Pond investigations are probably more realistic than laboratory experiments because the biota in the ponds experience natural climatic variations. Furthermore, the succession of the lower food web components, under stress, can be observed; and the distribution, transportation, and degradation of the oil and dispersant can be examined under natural conditions. Ponds, however, do not exhibit the complexities of open lakes.

The effects of oil on the aquatic mycota has received less attention in freshwater systems than in marine and estuarine systems. Turner and Ahearn(10) observed increased populations of hydrocarbon degrading yeasts in a fresh water stream after an accidental discharge of oil from an asphalt refinery: species patterns as well as total yeast numbers were affected. Evidence has suggested that the enrichment of oceanic sites by oil disasters can selectively stimulate the growth of certain indigenous yeasts(11). However, Walker et al. (12) used laboratory studies to demonstrate that crude and fueld oil had

little apparent effect on the fungal populations of a sub-estuary of the Rhode River in Chesapeake Bay.

Numbers of hydrocarbon degrading bacteria and fungi have been correlated in hydrocarbon polluted freshwater ecosystems, which suggests that both groups may be important in such ecosystems(13).

This paper presents the results of a nineteen month (December 1977 to July 1979) investigation of fungal communities in man made ponds that were treated with oil and oil plus dispersant additions.

METHODS

Five freshwater ponds were prepared, stabilized and treated with oil and oil-dispersant mixtures as previously described(14). One pond was treated with oil only (Pond 4); two ponds were treated with oil-dispersant mixtures (Ponds 1 and 3); and one pond was maintained as a control (pond 2); the final pond (pond 5) was held in reserve. The oil used was Norman Wells crude and the dispersant was Corexit 9527. Sufficient oil and dispersant were added to give a final concentration of 100 ppm oil and 20 ppm dispersant, assuming that uniform dispersal occurred. Samples for mycological analysis were taken from the near-surface, mid-depth, and near-bottom levels of the ponds, and were immediately refrigerated and transported to the laboratory where they were processed within 30 hours of collection.

Geoaquatic Fungi

Geoaquatic fungi(15) were enumerated using a membrane filtration procedure(16). Following triplicate filtration of appropriate sample portions, two of the membrane filters (0.45 μm) were implanted onto mARGPA agar(38), and were then incubated for 5 days at 15°C. The third membrane filter was plated onto mSTMEA agar(38), and was also incubated for 5 days at 15°C.

Oil Degrading Fungi

Fungi capable of utilizing either nondegraded Norman Wells oil or degraded Norman Wells oil as a sole carbon source were also enumerated using an MF procedure. Appropriate sample portions were filtered through 0.45 µm membrane filters which were then implanted onto freshly prepared basal agar medium (medium OBA) that contained either degraded(17) or non degraded oil; control plates contained no added oil. Medium OBA, is based on a medium used by Davies and Westlake(18), and has been previously described (38). The inoculated oil agar plates were incubated at 20°C for 21 days at which time fungal colonies were enumerated in two categories: as total colonies and as strongly growing colonies.

All data are arithmetic mean values of triplicate or duplicate determinations, and are expressed as colony forming units (CFU) per 100 mL.

Water Moulds

The water mould (aquatic phycomycete) content of the ponds was monitored using two methods: one quantitative and the other qualitative.

The numbers of viable water mould propagules in the water samples were determined using a spread plate technique: 1 mL sample portions (X5) were spread onto the surface of predried (24 hours at room temperature) plates of PSP agar(19) which were then incubated at 15°C for 48 hours. The number of potential water mould colonies growing on each plate was then enumerated; all potentially positive colonies were subcultured onto PSP agar and stored at 5°C for subsequent identification.

Water moulds were qualitatively recovered from water samples using a previously described hemp seed (Cannabis sativa) baiting technique(16). Individual colonized seeds and colonized seed clumps were subcultured on PSP agar until pure, and were then stored at 5°C for subsequent identification.

All water mould isolates from the spread plate and baiting procedures were identified to the generic level before being tabulated as confirmed isolates(45). The isolates were identified on the basis of their asexual reproductive structures using standard mycological keys (20,21).

Screening of Fungal Isolates for Ability to Grow on Oil

Representative, well developed fungal colonies were selected from one month old OBA agar cultures of pond water: the OBA

agar contained nondegraded oil. The selected fungi were subcultured until pure; moulds were stored at 5°C on potato dextrose agar (PDA, oxoid) slants, and yeasts were stored at 5°C on yeast extract-malt extract(22) agar slants. The ability of the fungal isolates to grow on oil was determined using a methodology recommended by Nyns et al.(23), Markovetz and Kallio(24), and Davies and Westlake(18).

Spore suspensions were carefully spread over the entire surface of 3 slants of Wickerham's agar medium. The inoculated slants were incubated for 24 hours at 20°C to facilitate spore germination and adherence of the young mycelium to the agar surface. Nondegraded Norman Wells oil, that had been previously topped to 31% weight loss by exposure to a fresh air flow at room temperature, was then pipetted into the tubes so that half of the slant surface was submerged in oil; a control culture containing no oil was prepared for each isolate. The cultures were then incubated at 20°C for 30 days at which time the oil was carefully aspirated and fungal growth was visually estimated. A similar procedure was used to screen fungal isolates for their ability to grown on (i) Corexit 9527; (ii) Corexit 9527 diluted with sterile distilled H_2O (1:5 by weight); and(iii) nondegraded Normal Wells oil in the presence of Corexit 9527.

Effect of Corexit 9527 on the Growth of Fungal Isolates

Corexit 9527 was incorporated into plates of Czapek Dox agar to give the following concentrations (ppm): 25, 100, 200, 800, and 1600. Inverted inoculum plugs (4 mm diameter), cut from the margins of fungal colonies growing on Czapek Dox agar, were used to centrally inoculate 5 agar plates for each Corexit concentration; the inoculated plates were then incubated at 20°C. When the control colonies (0 ppm Corexit) had covered approximately 75% of the control plates' surface area, the colony diameters on the control and analytical plates were measured and recorded. The percentage growth inhibition was then plotted against the log of each Corexit concentration. The Corexit 9527 concentration that caused 50% inhibition of radial growth (EC_{50}) was determined from the resultant graphs.

RESULTS

Geoaquatic Fungi

Pretreatment:

Figure 1 shows that, on the basis of the geoaquatic fungal data, there was little apparent ordering among the individual ponds before the oil and oil-dispersant applications. Fungal levels in the ponds selected for treatment did not significantly (P 0.05) exceed control pond levels on any of the sample collection dates (Tables 1

and 2). Fungal levels in Ponds 1 ($P > 0.05$) and Ponds 2 and 3 ($P > 0.01$) were correlated with the control pond's. The fungal levels in Ponds 1 and 3 were also correlated ($P > 0.01$). Ground water runoff from the spring thaw probably introduced additional fungal propagules into the ponds, thus contributing to the vernal fungal peaks that were observed in 1978.

Post Treatment Overall Observations:

The treatments had no obvious large scale effect on geoaquatic fungal levels in the ponds. In general, similar fungal distribution patterns were observed in the 4 ponds after treatment. Significant correlations were observed between Pond 1 ($P > 0.02$), Pond 3 ($P > 0.05$), Pond 4 ($P > 0.001$) and the control pond during the post treatment period. Although Ponds 1 and 3 were not significantly correlated ($r = 0.44$), a significant difference was not detected between these two ponds using a two way ANOVA test.

Post Treatment Short Term Observations:

The pond treatments were followed by an immediate increase in the number of fungi in each of the treated ponds (Figure I). The increase was most obvious in Ponds 1 and 3 (the oil-dispersant treated ponds), where the mean fungal levels rose from 42 to 159 CFU/100 mL. Analysis of variance techniques showed that the number of fungi in the

control pond, which increased only slightly at this time, was less than in the treated ponds (Table 1). By 14 days, however, a significant ($P > 0.01$) decrease had occurred in the fungal levels of both oil-dispersant treated ponds; whereas the fungal levels in the other two ponds continued to increase. By day 28 the fungal levels in Ponds 1 and 3 had recovered, and increased to 385 CFU/100 mL. The fungal levels in all ponds had decreased by the next sampling (day 41), and no significant differences were detected between the control and treated ponds.

The foregoing short term (day 7 to 41) effects are summarized in Table 2, which shows that, during this period, 38% of the sample sets from the oil-dispersant treated ponds and 50% of sample sets from the oil treated pond had higher ($P > 0.05$) mean fungal contents than the corresponding control pond samples.

Between 55 and 83 days after pond treatment, a substantial increase occurred in the fungal levels in each pond: the increases were again apparently highest in the treated ponds. Eighty three percent of the sample sets taken from the oil-dispersant treated ponds during this period had higher ($P > 0.01$) mean fungal levels than the control pond samples (Table 1).

The overall short term (day 7 to 83) effects are summarized in Table 2, which shows that 57% of the sample sets from the

oil-dispersant treated ponds and 43% of the sample sets from the oil treated pond had higher ($P > 0.05$) mean fungal contents than the corresponding control pond samples. None of the sample sets from the treated ponds had a lower ($P > 0.05$) mean fungal content than the corresponding control pond samples.

Mid-Term Observations:

Between 97 and 167 days after pond treatment, Only 25% of the sample sets taken from Ponds 1, 3 and 4 had a higher mean fungal content than those from the control pond (Table 2). An observation which suggests that the apparent fungal enhancement effects observed in the preceeding sampling period were declining.

Long-Term Observations:

The latter part of the study lasted from day 202 to 385. The small number of surveys in which the treated ponds' fungal levels exceeded the control pond's (Table 1, 2) suggests that long term fungal enhancement effects did not occur in the ponds. Again, none of the sample sets from the treated ponds had a lower ($P > 0.05$) mean fungal content than the corresponding control pond samples.

Vertical Distribution Effects:

Initial examination of the fungal data suggested that the pond treatments may have influenced the vertical distribution of geoaquatic fungi within the ponds(38). However, analysis of the data using ANOVA techniques failed to detect a significant difference, at the 95% probability level, between the surface and sub-surface samples during the project's four sampling periods.

Oil Media:

The data obtained by culturing the pond samples on the oil media, which has been previously reported(38), suggested that the oil and oil-dispersant additions had no obvious effects on the percentages of viable geoaquatic fungi capable of using either nondegraded or degraded Norman Wells oil as a sole carbon source. Selective enumeration of strongly growing fungal colonies also failed to reveal any obvious treatment effects(38). Although minor differences did exist between the numbers of oil-utilising fungi in the ponds, they were neither substantial nor consistent enough to suggest clear trends. Overall, based on the enumeration of strongly growing fungi, 6.8% and 7.2% of the viable fungi in the pond water samples were apparently capable of using degraded and nondegraded oil respectively as a sole carbon source.

2. WATER MOULDS

The apparent differences in the water mould content of the ponds prior to treatment (Table 3) were not significant (P 0.05). Comparatively few water moulds were recovered from the ponds during periods of ice cover, using either the spread plate or baiting technique, suggesting that these fungi may not thrive under ice (Tables 3 and 4). Analysis of the post treatment water mould data using ANOVA techniques failed to confirm that an enhancement of water mould populations had occurred in the treated ponds (P 0.05), during the short (7-14 days), medium (28-139), or long (296-385) term periods. The limited data base did not justify a statistical comparison of the ponds for each of the individual surveys.

The data presented in Tables 3 and 4 indicate that discrepancies exist between the results obtained using the quantitative spread plate and qualitative baiting techniques. On 20 occasions (20% of sample sets), results obtained using the spread plate technique indicated the absence of water moulds from the water samples, although water moulds were recovered from the same sample sets using the baiting procedure. On the other hand, the baiting technique yielded false negative results on only 4 occasions (4% of sample sets). The foregoing discrepancies are probably a consequence of the larger sample portions (50 mL) that are analyzed using the baiting technique.

The vast majority of the water mould isolates belonged to the genus Saprolegnia (Table 5). Pythium spp. and Achyla spp. were also occasionally isolated from the ponds before treatment. However, after pond treatment Pythium isolates were recovered only from the treated ponds.

SCREENING OF FUNGI FOR ABILITY TO UTILIZE CRUDE OIL, COREXIT, AND CRUDE OIL-COREXIT MIXTURES

Selection and Identification of Isolates

Thirty of the well developed fungal colonies that were selected for use in the oil utilization tests were moulds and 9 were yeasts. Twenty two of the fungi were from the oil-dispersant treated ponds, 10 from the oil-treated pond, and 7 from the control pond. The fungi were identified to the generic level (Table 6); all the identified moulds belonged to the Fungi Imperfectii.

Utilization of Crude Oil

Fifteen (38.5%) of the isolates were able to grow on the Norman Wells crude oil (Table 6). Eight of these isolates were from the oil-dispersant treated ponds, 4 were from the oil treated pond, and 3 were from the control pond. It is clear from Table 8 that the largest amount of mycellial growth generally occurred at the oil/air interface.

Utilization of Corexit 9527

None of the fungal isolates was able to grow on Corexit 9527 or on Corexit 9527 diluted with 5 parts of water (by weight).

Utilization of Nondegraded Oil in the Presence of Corexit 9527

None of the fungal isolates was able to grow on a mixture of nondegraded oil and Corexit 9527 (5:1 (w/w)). The effect of a range of Corexit concentrations on the ability of isolates OP3B and OP5B to grow on nondegraded oil was also examined (Table 7). Corexit at 200 ppm had no apparent effect on the growth of either isolate; at 2000 ppm Corexit (5 parts oil: 0.01 part Corexit) the growth of both isolates was slightly reduced; and at 10,000 ppm Corexit (5 parts oil: 0.05 parts Corexit) fungal growth was completely inhibited.

Effect of Corexit on Fungal Growth

When incorporated into an agar medium, Corexit 4527 inhibited the radial growth of the fifteen oil utilizing fungi. The EC_{50} value for each fungus was calculated from a plot of log Corexit concentration versus percent inhibition of radial fungal growth. Figure 2 shows that, with the exception of Penicillium sp. OP3B, similar growth inhibition curves were obtained for the isolates within each genus. The curves plotted for the Trichoderma spp. and Cladosporium spp. were dissimilar to those of the Phoma and Penicillium isolates.

Variations existed in the sensitivities of the fungal isolates to Corexit: EC_{50} values ranged from 77 ppm for Paecilomyces

sp. OP1B to 1400 ppm for Penicillium sp. OP3B (Table 8). The Phoma isolates were unaffected by 25 ppm Corexit, a concentration that slightly inhibited the radial growth of most of the other isolates. At 200 ppm, Corexit inhibited the growth of each isolate, although it had no visible effect on the ability of Penicillium sp. OP3B and Penicillium sp. OP5B to utilize nondegraded oil. Corexit at 1600 ppm reduced the growth of each of the tested isolates by 54 to 100%, whereas 2000 ppm Corexit only slightly reduced the ability of Penicillium OP3B and Penicillium OP5B to grow on nondegraded oil (Table 7). These observations suggest that in the oil utilization test, the oil and Corexit may have interacted, thus reducing the amount of dispersant available to affect the fungi.

DISCUSSION

The fungi are an integral component of the microbiota of freshwater systems (25,26). Two fungal groups, the water moulds and the geoaquatic fungi, both of which show a different degree of adaptation to life in the aquatic environment, were monitored in this study.

Watermoulds, which are characterized by the production of motile asexual reproductive spores, occur in all types of aquatic systems. As a consequence of their motile reproductive phase, fast growth rates, and coarse hyphae; some water moulds may be important as early colonizers of organic substrates in water (27).

"Geoaquatic fungi" include many moulds and yeasts that are considered to originate mainly from soil ecosystems but, following transportation by various means and pathways, are present in the aquatic environment (15). "Geoaquatic fungi" are poorly adapated to an aquatic existence. It is postulated that fungi, as heterotrophs, participate in the degradation and recycling of organic materials in the aquatic environment.

The MF technique used in this study for the enumeration of viable fungal propagules suffers from a serious limitation: there is, at present, no means of distinguishing between propagules that are

metabolically active within the natural environment and those that are merely dormant. In addition, the selectivity of artificial media can lead to underestimates of the number of viable fungal propagules in water samples. Although each viable fungal propagule enumerated using MF has the potential, given a favourable micro environment, to grow and thus participate in the heterotrophic breakdown of organic matter; it is not yet possible, because of the foregoing limitations, to conclusively assess the ecological significance of fungal counts obtained using the MF technique.

Although the pond treatments had no obvious large scale effect on geoaquatic fungal levels in the ponds, close examination of the data, suggested that some small differences existed between the fungal levels in the individual ponds.

The apparent fungal enhancements that occurred in the treated ponds immediately after the oil and dispersant additions may have been either a direct or indirect consequence of the pond treatments. Those fungi capable of using oil as an energy source may have been directly stimulated by the added oil. However, the absence of any observed short term increase in the numbers of oil degrading fungi in the treated ponds, suggests that the apparent fungal enhancements were more likely to have been a secondary effect. Toxicity of the added chemicals may have increased the amount of dead organic matter in the ponds, thus causing a corresponding increase in the numbers of

saprophytes, including fungi. Furthermore, if the added chemicals were toxic to those components of the pond biota that are predatory towards fungal hyphae or spores, then a general enhancement in fungal numbers would be expected to occur. Rotifer populations and many protozoan spp. were not recovered from the treated ponds after the oil and dispersant applications; whereas the levels of these predators remained unaltered in the control pond (37).

It is not clear whether the decrease in geoaquatic fungal levels that was observed in the oil-dispersant treated ponds 14 days after pond treatment was directly related to the inhibitory effects of Corexit 9527 on the fungi. In an agar incorporation test 25 ppm Corexit 9527 slightly reduced the radial growth of some of the selected pond water fungi (Table 10). However, the actual maximum dispersant concentrations detected between day 0 and day 14 were considerably lower: 7 ppm in pond 3 and 4.25 ppm in pond 1. Any toxic shock effects on the aquatic mycota, caused by local accumulations of Corexit, should have been apparent by day 7: however, none were observed.

The decline in the apparent fungal enhancement effects in ponds 1 and 3, during the period 97 to 167 days, was parallelled by a decrease in the oil and dispersant concentrations (< 2 ppm) in the water column (14). Furthermore, the absence of any clear cut fungal enhancement effects in the treated ponds during the period 202 to 385

days suggests that the fungal levels were not influenced by the residual oil and dispersant that remained in the ponds.

The ability to use components of crude oil as a carbon source is widespread among the fungi (28, 18, 29, 30, 23, 31, 32). It has been demonstrated that some fungi from freshwater environments (13, 11) can also use oil components. Crude oil when added to an aquatic ecosystem, should primarily cause an enrichment of microorganisms capable of using hydrocarbons as a carbon source (for a review see 33); a second enrichment of microorganisms capable of using oil degradation products and associated metabolites may also occur.

However, we failed to detect any population shifts in favour of oil degrading fungi. Thus it appears that an enrichment of fungi capable of using crude oil as a carbon source did not occur in the treated ponds; and that a secondary enrichment of fungi capable of using oil degradation products probably did not occur either. These conclusions are, however, subject to methodology limitations.

The overall percentage of fungi that were apparently capable of using oil as a sole carbon source is higher than has been reported from other fresh water ecosystems (13). Because of the presence of low level impurities in agar, determinations using oil agar media tend to overestimate oil-degrading microbial populations (for a review see 34). In this study, several measures were taken to alleviate this

problem: purified agar was used in the preparation of the oil media; the fungal counts obtained from oil free control media were subtracted from all analytical plates; and "strong growing" fungal colonies were selectively enumerated.

Nevertheless, only 38.5% of typical "strong growing" fungal colonies that had been sub-cultured from oil media plates were able to grow on non-degraded oil in a pure culture test. This observation suggests that the MF technique, despite the various safeguards employed, probably caused an overestimation of the numbers of oil degrading fungi in the water samples. Correcting for this overestimation showed that 2.62% and 2.77% of the overall number of viable fungi in the pond water samples were apparently capable of using degraded and non-degraded oil respectively as a sole carbon source.

The apparent absence of water moulds from the water samples taken from ponds 1 and 3 on day 7 may have been related to the dissolved oxygen levels in ponds 1 and 3, which at day 7 were 3 to 4 ppm lower than the control pond's (35). The dispersal of toxic oil and dispersant components through the water column may have acted separately or in conjunction with the reduced oxygen levels to cause this apparent reduction in water mould levels. Saprolegnia spp. and Pythium spp., the predominant genera, are known to require an abundance of oxygen (36; 25).

Fungi capable of growth on nondegraded Norman Wells oil were recovered from each of the ponds. With the exception of the Pyrenochaeta isolate, species of each of the oil utilizing genera listed in Table 9 have been previously shown to use crude oil (18).

The fungal isolates were unable to grow on Corexit 9527, which implies that these fungi may be unable to use this dispersant, at least in an unaltered form, as a sole carbon source in the aquatic environment. Corexit at 2000 ppm partially inhibited the growth of two of the fungal isolates on crude oil, and at 10,000 ppm the inhibition was total. In this test, the dispersant may have interacted with the oil to render it unavailable to the fungi; or the Corexit may have acted directly on the fungi themselves. In a simple agar incorporation test, Corexit 9527 inhibited the radial growth of all the tested fungi. The nature of the interaction between the fungi and Corexit requires further investigation.

Conclusions

Mycological Aspects

1. No apparent large scale post treatment differences were detected between the geoaquatic fungal levels, in the control and treated ponds.

2. Some significant enhancement of fungal levels was observed in the analytical ponds after the oil-dispersant additions. This may have been a primary or secondary effect of pond treatment.

3. Long term fungal enhancement effects were not observed.

4. No significant treatment effect was detected on the vertical distribution of fungi in the ponds.

5. There was no obvious treatment effect on the percentage of viable fungi capable of growth on media containing non degraded or degraded oil as sole carbon source.

6. Approximately 2.8% of the pond water fungi were able to grow on crude oil as a sole carbon source.

7. Corexit 9527 inhibited the radial growth of 15 pond water fungi.

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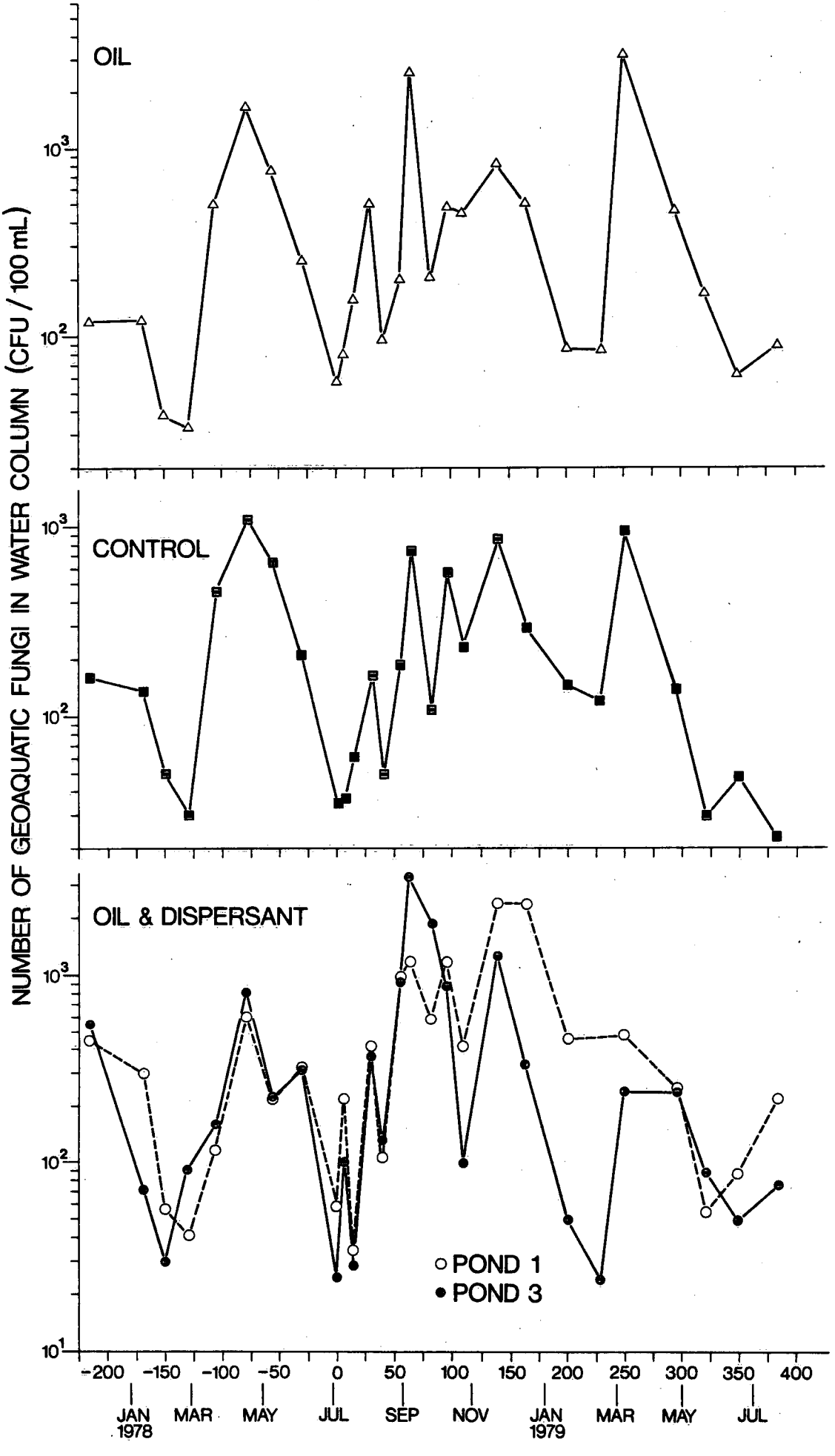
FIGURES

Figure 1. Temporal distribution of viable geoaquatic fungi in experimental ponds; data are presented as the geometric mean values of surface, mid-depth, and bottom water samples.

Figure 2. Effect of corexit on the radial growth of selected pond water fungi. Confidence limits were determined at the 5% significance level.


OIL
CONTROL
OIL & DISPERSANT
NUMBER OF GEOAQUATIC FUNGI IN WATER COLUMN (CFU / 100 mL)
10^3
10^2
10^1
○ POND 1
● POND 3
−200 −150 −100 −50 0 50 100 150 200 250 300 350 400
JAN 1978
MAR
MAY
JUL
SEP
NOV
JAN 1979
MAR
MAY
JUL
TIME (days before and after pond treatment)

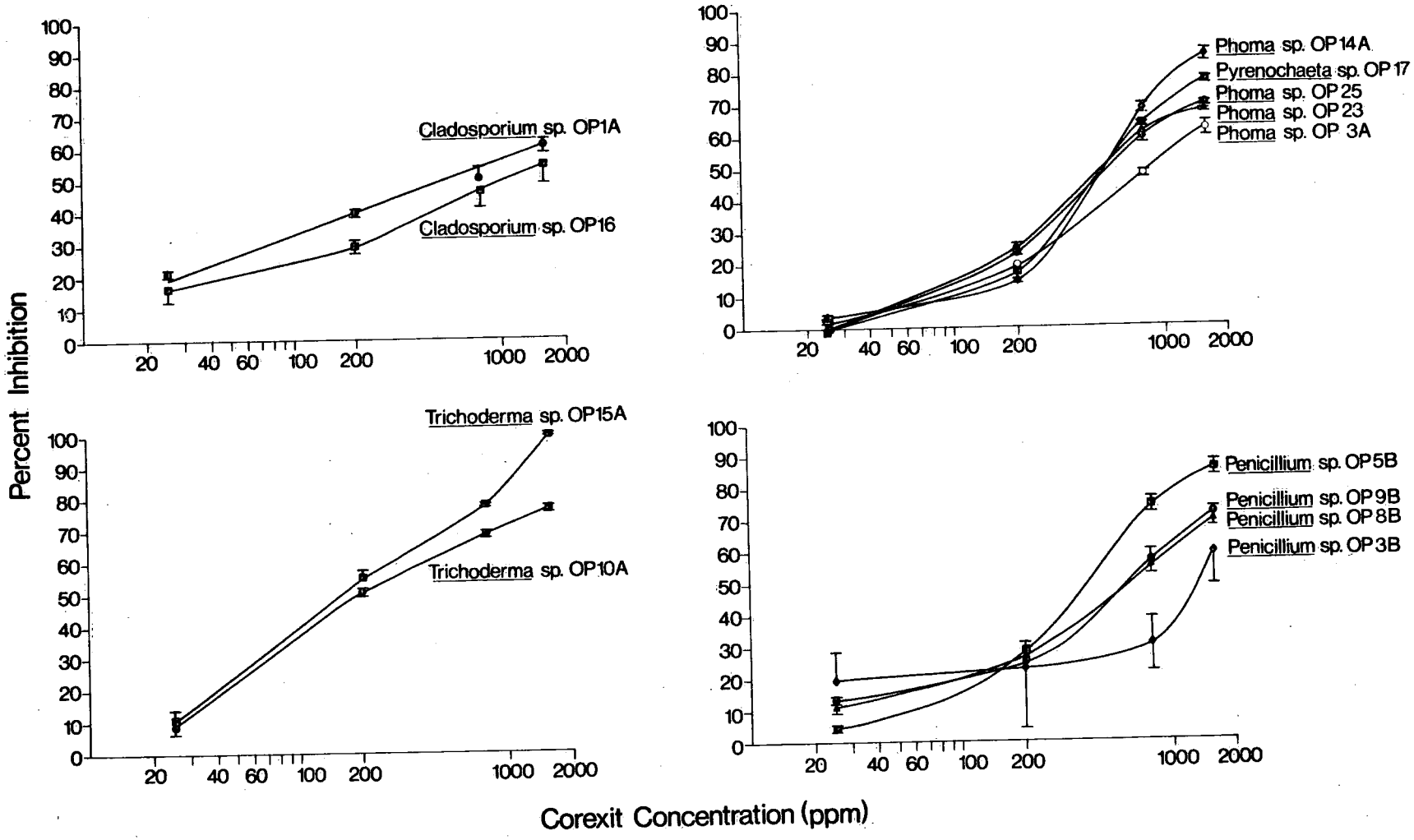

Percent Inhibition
Cladosporium sp. OP1A
Cladosporium sp. OP16
Phoma sp. OP14A
Pyrenochaeta sp. OP17
Phoma sp. OP25
Phoma sp. OP23
Phoma sp. OP 3A
Trichoderma sp. OP15A
Trichoderma sp. OP10A
Penicillium sp. OP5B
Penicillium sp. OP9B
Penicillium sp. OP8B
Penicillium sp. OP3B
Corexit Concentration (ppm)

Table 1. Comparison of Fungal Levels in the Treated and Control Ponds. Probability that the fungal levels in the treated ponds exceeded control pond levels.

Pond Number	Sample Collection Day																								
	-168	-149	-128	-106	77	56	29	0	7	14	28	41	55	69	83	97	111	139	167	202	230	251	296	322	350	385
1	NS	NS	NS	NS	NS	NS	NS	NS	99%	NS	NS	NS	99%	NS	99%	NS	95%	NS	NS	NS	--	NS	NS	NS%	NS	NS
3	NS	NS	NS	NS	NS	NS	NS	NS	99%	NS	95%	NS	99%	99%	99%	NS	NS	99%	NS	NS	NS	NS	NS	99%	NS	NS
4	NS	NS	NS	NS	NS	NS	NS	NS	99%	NS	99%	NS	NS	95%	NS	NS	95%	NS	NS	NS	NS	NS	NS	99%	NS	NS

NS - fungal levels not significantly higher than control ponds at the 95% confidence interval

Table 2. Summary of the Relationship Between Fungal Levels in Treated and Control Ponds: Proportion of surveys in which goaquatic fungal levels exceeded[1] control pond levels

Time Interval (days)	Pond 1	Pond 3	Pond 4
-216 to 0	0/8	0/8	0/8
7 to 41	1/4	2/4	2/4
7 to 83	3/7	5/7	3/7
97 to 167	1/4	1/4	1/4
202 to 385	0/6	1/7	1/7

[1] at the 5% significance level

Table 3. Temporal Distribution of Water Moulds in Experimental Ponds; Data are Presented as the Geometric Mean Values of Surface, Mid-depth, and Bottom Water Samples

Pond Number	Collection Date and Number of Water Moulds (CFU/50 mL)																										
	-216	-168	-149	-128	-106	-77	-56	-29	0	7	14	28	41	55	69	83	97	111	139	167	202	230	251	296	322	350	385
1	-	-	0	0	0	17	0	0	22	0	22	55	32	0	22	17	0	17	17	0	0	0	0	22	22	23	27
2	-	-	0	0	0	0	0	0	0	22	17	17	0	0	0	0	40	0	0	0	0	0	0	22	0	17	0
3	-	-	0	0	0	27	38	23	0	0	17	112	23	23	17	32	0	42	0	0	0	0	0	30	17	22	22
4	-	-	0	0	0	22	0	23	0	23	0	17	0	30	17	38	0	17	22	0	0	0	0	23	17	17	22

Table 4. Recovery of Water Moulds from Pond Water Samples Using the Hemp Seed Baiting Technique; Data are Presented as the Pooled Values of Surface, Mid-Depth, and Bottom Water Samples.

	Collection Date and Presence or Absence of Water Moulds																										
Pond Number	-216	-168	-149	-128	-106	-77	-56	-29	0	7	14	28	41	55	69	83	97	111	139	167	202	230	251	296	322	350	385
1	+	0	0	0	0	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+	+	+
2	+	0	0	+	0	0	+	+	+	+	+	+	+	+	0	0	+	+	0	0	0	0	0	+	+	+	0
3	0	0	0	0	0	0	+	+	+	0	0	+	+	+	+	+	+	+	0	0	0	0	0	+	+	+	+
4	+	0	0	0	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	0	0	+	+	+	0

Table 5. Generic Identity of Pond Water Phycomycetes

Collection Date	Pond Number and Genera Isolated			
	Pond 1	Pond 2	Pond 3	Pond 4
-216	Sap[1](1)[3] Pythium(3), Aphan[2](1)	Sap(2)		Sap(8)
-168				
-149				
-128		Sap(2)		
-106				Sap(3)
-77	Sap(1)		Sap(3) Achlya(1)	Sap(11)
-56	Sap(1)	Sap(3) Pythium(1)	Sap(23)	Sap(10) Achlya(1)
-29		Sap(10)	Sap(12)	Sap(7)
0	Sap(23)	Sap(10)	Sap(9)	Sap(23)
7		Sap(11)		Sap(8)
14	Sap(5)	Sap(5)	Sap(1)	Sap(2)
28	Sap(16)	Sap(5)	Sap(21)	Sap(1)
41	Sap(16)	Sap(1)	Sap(13)	Sap(8)
55	Sap(15)	Sap(2)	Sap(17)	Sap(7)
69	Sap(5)		Sap(1)	Sap(2)
83	Sap(3)		Sap(8)	Sap(9)
97	Sap(8)	Sap(17)	Sap(5)	Sap(5), Pythium(1)
111	Sap(8)	Sap(6)	Sap(14)	Sap(9)
139	Sap(2)			Sap(3) Pythium(2)
167	Sap(4)			
202				Sap(6)
230				
251				
296	Sap(11) Pythium(1)	Sap(5)	Sap(14) Pythium(1)	Sap(15)
322	Sap(9) Achlya(3)	Sap(1)	Sap(2) Pythium(1)	Sap(9)
350	Sap(12)	Sap(3)	Sap(7)	Sap(2)
385	Sap(6) Pythium(1)		Sap(3)	Sap(2)

[1] Saprolegnia
[2] Aphanomyces
[3] Total number of isolates from hemp seed baits and spread plates

Table 6. Ability of Pond Water Fungi to Grow on Nondegraded Norman Wells Oil

Culture	Culture Code Number	Source (Pond #)	Growth* Air**	Interface	Submerged
Cladosporium sp.	OP1A	1 - 3	0	1	1
Paecilomyces sp.	OP1B	1 - 3	0	3	2
Phoma sp.	OP1C	1 - 3	0	0	0
Rhodotorula sp.	OP2A	1 - 3	0	0	0
Phialophora lignicola	OP2B	1 - 3	0	0	0
Rhodotorula sp.	OP2C	1 - 3	0	0	0
Phoma sp.	OP3A	1 - 3	0	0	1
Penicillium sp.	OP3B	1 - 3	1	4	2
Rhodotorula sp.	OP3C	1 - 3	0	0	0
Torula jeanselmei	OP4Ai	1 - 3	0	0	0
Cephalosporium sp.	OP4Aii	1 - 3	0	0	0
Trichoderma sp.	OP4B	1 - 3	0	0	0
Phoma sp.	OP5A	1 - 3	0	0	0
Penicillium sp.	OP5B	1 - 3	2	4	2
Rhodotorula sp.	OP6A	1 - 3	0	0	0
Phoma sp.	OP7A	1 - 3	0	0	0
Torula jeanselmei	OP7B	1 - 3	0	0	0
Torula jeanselmei	OP8A	1 - 3	0	0	0
Penicillium sp.	OP8B	1 - 3	0	4	0
Penicillium sp.	OP9B	1 - 3	0	4	2
Trichoderma sp.	OP10A	1 - 3	0	0	2
Unidentified yeast	OP10B	1 - 3	0	0	0
Unidentified yeast	OP11A	2	0	0	0
Torula jeanselmei	OP12A	2	0	0	0
Torula jeanselmei	OP12B	2	0	0	0
Unidentified yeast	OP13B	2	0	0	0
Phoma sp.	OP14A	2	0	1	1
Sterile mould	OP14B	2	2	4	2
Trichoderma sp.	OP15A	2	0	2	0
Cladosporium sp.	OP16	4	0	0	1
Pyrenochaeta sp.	OP17	4	0	0	2
Phoma sp.	OP18	4	0	0	0
Unidentified yeast	OP19	4	0	0	0
Phialophora sp.	OP20	4	0	0	0
Phialophora sp.	OP21	4	0	0	0
Rhodotorula sp.	OP22	4	0	0	0
Phoma sp.	OP23	4	0	3	2
Torula jeanselmei	OP24	4	0	0	0
Phoma sp.	OP25	4	0	3	3

* Fungal growth was estimated using the criteria of Davies and Westlake (1979): 0 = no growth; 1 = growth significantly greater than hydrocarbon free control; 2 = growth greater than 1 but not continuous covering of the agar; 3 = growth covers agar surface; 4 = agar covered by thick mat of cells.

** Air = growth above oil surface; interface = growth at oil/air interface; submerged = growth beneath oil surface.

Table 7. Effect of Corexit 9927 on the Growth of Pond Water Fungi on Non-degraded Norman Wells Oil

		Growth*				
		Corexit Concentration (ppm)				
		0	200	2000	10,000	20,000
		Oil Dispersant Ratio				
Culture	Pattern**	5:0	5:0.001	5:0.01	5:0.05	5:0.1
Penicillium sp. (OP3B)	Air	1	1	0	0	0
	Interface	4	4	3	0	0
	Submerged	2	2	1	0	0
Penicillium sp. (OP5B)	Air	2	2	1	0	0
	Interface	4	4	4***	0	0
	Submerged	2	2	2	0	0

* Fungal growth was estimated using the criteria of Davies and Westlake (1979): 0 = no growth; 1 = growth significantly greater than hydrocarbon free control; 2 = growth greater than 1, but not continuous covering of the agara; 3 = growth covers agar surface; 4 = agar covered by thick mat of cells.

** Air = growth above oil surface; interface = growth at oil/air interface; submerged = growth beneath oil surface.

*** Mycellium not restricted and bulk reduced

Table 8. Effect of Corexit 9527 on the Growth of Oil Utilizing Pond Water Fungi

Fungus		% Growth Inhibition Caused by Corexit[1] 25	200	800	1600	EC_{50} of Corexit
Cladosporium sp.	OP1A	21[2]	40[2]	51[2]	62[2]	560
Cladosporium sp.	OP16	17[2]	30[2]	47[2]	54[2]	1100
Trichoderma sp.	OP10A	9[2]	51[2]	69[2]	77[2]	200
Trichoderma sp.	OP15A	10[2]	55[2]	78[2]	100[2]	160
Phoma sp.	OP3A	2[3]	19[2]	48[2]	62[2]	870
Phoma sp.	OP14A	3[2]	15[2]	68[2]	85[2]	550
Phoma sp.	OP23	0	25[2]	61[2]	67[2]	510
Phoma sp.	OP25	0	23[2]	60[2]	69[2]	560
Pyrenochaeta sp.	OP17	0	18[2]	63[2]	76[2]	510
Penicillium sp.	OP3B	20[3]	23[3]	31[2]	59[2]	1400
Penicillium sp.	OP5B	5[2]	29[2]	74[2]	85[2]	400
Penicillium sp.	OP8B	12[2]	27[2]	56[2]	70[2]	640
Penicillium sp.	OP9B	13[2]	25[2]	56[2]	71[2]	620
Paecilomyces sp.	OP1B	10[2]	79[2]	83[2]	100[2]	77
Sterile Mould	OP14B	3[3]	39[2]	73[2]	100[2]	310

[1] Corexit concentration expressed as ppm. % growth inhibition was calculated with respect to growth on control medium containing no Corexit.

[2] Different from control at the 1% significance level.

[3] Different from control at the 5% significance level.

[4] Difference is not significant at 5% significance level.