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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

Ability of Lotic Micro-organisms and Macrobenthos to Degrade and Assimilate Bitumen

Project WS 4.1.1

July 1979

Sponsored jointly by

Alberta ENVIRONMENT

Environment Canada   Environnement Canada

15th Floor, Oxbridge Place
9820 - 106 Street
Edmonton, Alberta, Canada
T5K 2J6

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM RESEARCH REPORTS

These research reports describe the results of investigations funded under the Alberta Oil Sands Environmental Research Program, which was established by agreement between the Governments of Alberta and Canada in February 1975 (amended September 1977). This 10-year program is designed to direct and co-ordinate research projects concerned with the environmental effects of development of the Athabasca Oil Sands in Alberta.

A list of research reports published to date is included at the end of this report.

Enquiries pertaining to the Canada-Alberta Agreement or other reports in the series should be directed to:

Alberta Oil Sands Environmental Research Program
15th Floor, Oxbridge Place
9820 - 106 Street
Edmonton, Alberta
T5K 2J6
(403) 427-3943

Ability of Lotic Micro-organisms and Macrobenthos to Degrade and Assimilate Bitumen

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The Hon. John W. (Jack) Cookson
Minister of the Environment
222 Legislative Building
Edmonton, Alberta

and

The Hon. John Fraser
Minister of the Environment
Environment Canada
Ottawa, Ontario

Sirs:

Enclosed is the report "Ability of Lotic Micro-organisms and Macrobenthos to Degrade and Assimilate Bitumen".

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Water System, under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

W. Solodzuk, P. Eng.
Chairman, Steering Committee, AOSERP
Deputy Minister, Alberta Environment

A.H. Macpherson, Ph.D
Member, Steering Committee, AOSERP
Regional Director-General
Environment Canada
Western and Northern Region

ABILITY OF LOTIC MICRO-ORGANISMS AND MACROBENTHOS TO DEGRADE AND ASSIMILATE BITUMEN

DESCRIPTIVE SUMMARY

BACKGROUND

Initial AOSERP aquatic research activities were almost entirely aimed at delineating baseline states in the Athabasca Oil Sands region. When the baseline picture approached adequate description, emphasis in the research program began to shift to applied areas such as testing the resiliency of the environment to stress and developing methodology for restoration of over-stressed areas.

The present project stems from the fact that both natural and man-made effects of oil sands deposits are felt on the surface waters. It proposed to address the following objectives:

1. To measure the ability of lotic micro-organisms and macrobenthos in the oil sands area to degrade and assimilate the types of oils which occur naturally in the oil sands area and other types which might be discharged to waters in the oil sands area; and
2. To evaluate the overall ability of brown-water rivers to assimilate organic loadings.

ASSESSMENT

A draft of the report has been reviewed by university scientists in Alberta and British Columbia and the authors had opportunity to consider their input. Even though the information is of a preliminary nature, it is our recommendation that the report be distributed to selected Canadian libraries. The Alberta Oil Sands

Environmental Research Program accepts this report "Ability of Lotic Micro-organisms and Macrobenthos to Degrade and Assimilate Bitumen" and thanks the authors for their efforts.

S.B. Smith, Ph.D
Program Director
Alberta Oil Sands Environmental
Research Program

R.T. Seidner, Ph.D
Research Manager
Water System

ABILITY OF LOTIC MICRO-ORGANISMS AND MACROBENTHOS TO DEGRADE AND ASSIMILATE BITUMEN

by

M.A. LOCK and R.R. WALLACE

Environment Canada
Freshwater Institute
Winnipeg, Manitoba

for

ALBERTA OIL SANDS
ENVIRONMENTAL RESEARCH PROGRAM

Project WS 4.1.1

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ABSTRACT

The colonization of limestone bricks and bitumen coated limestone bricks by benthic river organisms was followed over time. After nine weeks of colonization, no massive increase in the numbers of bacteria on the bitumen was noted, suggesting that degradation was not proceeding rapidly. However, subjective changes in the character of the bitumen did take place, indicating that some form of degradation was proceeding. Of the other components examined, only chlorophyll *a* concentrations were radically different on the bitumen and this may have been due to an interference by the bitumen in the analysis. The carbohydrate concentrations, micro-invertebrate numbers and biomass, and macro-invertebrate numbers were generally similar on bitumen coated and control bricks, suggesting that bitumen or oil sand is not radically different from limestone as a substrate for benthic river communities per unit of surface area.

A second study followed the response of the microbial and macro-invertebrate community on limestone bricks to the addition of synthetic crude oil (SCO). Out of the 20 taxonomic groups examined and four biochemical parameters, only one of these, the Cyanophyta, showed any apparent response to the oil addition. Thus, based upon the mode of presentation of the oil to the community, it would appear that SCO has only a minimal effect, possibly due to limited penetration. The significance of these findings to more prolonged spills is discussed with relation to the observed stimulations of benthic communities growing on oil soaked substrates. It is suggested that the interplay of the penetration of oil to the communities and its subsequent effects be investigated further.

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This research project WS 4.1.1 was funded by the Alberta Oil Sands Environmental Research Program, a joint Alberta-Canada research program established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands area of northeastern Alberta.

1. GENERAL INTRODUCTION

Although generally considered as unnatural substances, hydrocarbons, particularly in the form of bitumen, occur widely throughout the river drainages of the Alberta Oil Sands Environmental Research Program (AOSERP) study area. This manifests itself in exposed strata in banks or, in extreme cases, as a rubble impregnated bitumen river bed. Earlier studies on the effects of hydrocarbons upon river communities had been concerned with synthetic crude oil, the end product of the oil sands extraction process (Barton and Wallace in prep.). Thus the first objective was to place these into perspective, as it seemed desirable to gain information about the role played by the bitumen itself. In particular, did it appear to act as a stimulant or depressant upon the benthic river communities in contact with it or was its role similar to that of the 'normal' rubble substrates? The second objective of this project was to conclude the studies on synthetic crude oil (SCO) spillages into rivers. Earlier studies (Barton and Wallace in prep.) had suggested that SCO may have had a stimulatory effect upon established benthic communities within the first four weeks of exposure. Thus, it was important to know if a large but transient response occurred within that time period; if so, a more prolonged oil spill might have a more disruptive effect than the earlier studies suggested.

2. COLONIZATION OF BITUMEN BY BENTHIC BACTERIA, ALGAE, AND MICRO-AND MACRO-INVERTEBRATES OF THE MUSKEG RIVER

2.1 INTRODUCTION

It has been recently demonstrated that the biota of the waters and sediments of the Muskeg and Steepbank rivers had a considerable ability to degrade (Lock et al. in prep.) the saturate fraction of synthetic crude oil. It was the intention of this study to examine their ability to degrade the naturally occurring bitumen using the same techniques. However, preliminary work using the gas chromatographic technique employed previously showed that it was not possible to resolve the complex bitumen mixture. Thus, it was decided to examine the problem of biodegradability indirectly by following the colonization over time of bitumen coated natural substrates by micro-organisms. This approach had the additional advantage that it could be related to the in situ studies on the macrobenthic communities associated with oil sand and rubble substrates carried out by Barton and Wallace (in prep.). In that study, they suggested that the oil sand substrates of the Steepbank River supported fewer benthic invertebrates but they attributed this to a reduced number of interstices for occupation rather than to any deleterious effect of the bitumen itself. Therefore, through the use of standardized bitumen coated and uncoated substrates, it would be possible to test this hypothesis directly.

2.2 MATERIALS AND METHODS

The study site was located in a riffle area on the Muskeg River (Figure 1), 10 km above its confluence with the Athabasca River in northeastern Alberta (57°08'N, 111°35'W). Discharge over the study period was monitored by Water Survey of Canada and levels of conductivity, ammonium-nitrogen (NH_4 - N), nitrate + nitrite - nitrogen (NO_3 + NO_2 - N), phosphate-phosphorus (PO_4 - P), and dissolved organic carbon (DOC) were determined by Chemex Laboratories Ltd. using the methods of Traversy (1977) being data collected for AOSERP Water Sector

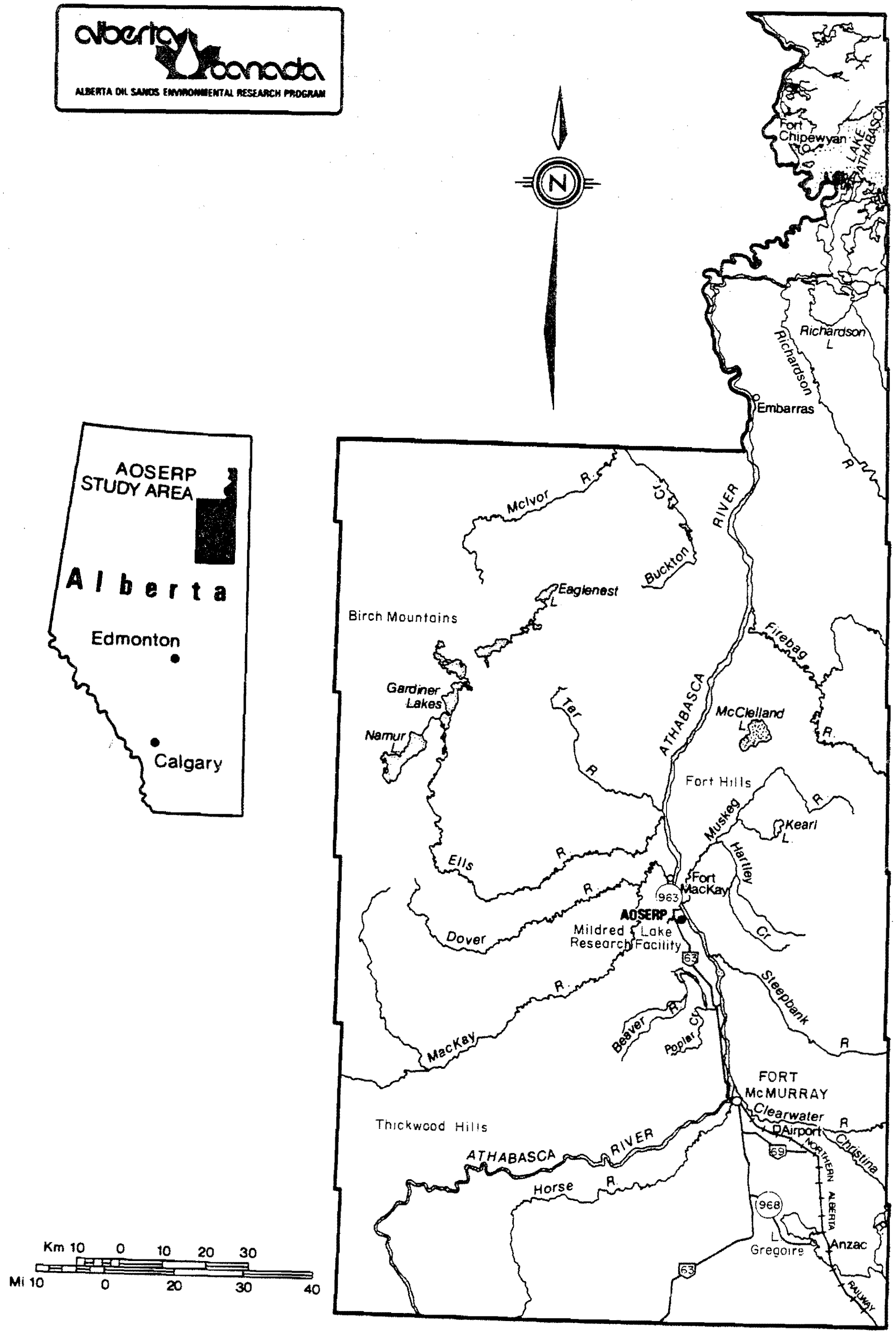

Figure 1. Map of the AOSERP study area.

projects WS 1.1 and WS 1.2.1 respectively.

The study used limestone bricks (12.3 x 8.8 x 4 cm) as standardized substrates (Barton and Wallace in prep.). Sixty bricks were placed in the river, of which 30 had been coated with a thin film of bitumen (~0.3 mm) which had been commercially extracted from locally occurring oil sand. Bricks were placed in the river on 27 June 1978 in three double rows of 10, composed of one 2 x 5 bitumen coated brick matrix and one uncoated 2 x 5 brick matrix used as a control.

The epilithic communities were to be examined at four, nine, and 16 weeks by taking one 4 cm^2 scrape (Lock and Wallace in prep.) from each of the five replicates of the control and bitumen coated bricks. Bacterial numbers were determined by direct counting (Geesey and Costerton 1979), chlorophyll *a* by the method of Moss (1967a, 1967b), and carbohydrate by the phenol-sulphuric method (Strickland and Parsons 1972). The scrapes for micro-invertebrates were fixed in cacodylate buffered 0.5% glutaraldehyde, sorted under 12x magnification (organisms $\geq$ 0.2 mm in length were recorded), and stored in 70% ethanol. After identification, the wet weights of various invertebrate groups were obtained using a Cahn electrobalance. The macro-invertebrate communities were examined on the set of bricks kept solely for that purpose. These were removed from the water by placing a 202 μ mesh net behind the brick and lifting it out of the river into a pan. They were then scrubbed and the material removed by this process, plus the organisms in the net, were combined and preserved with 10% formalin. The samples were sorted under 12x magnification and stored in 70% ethanol.

2.3 RESULTS

The physical and chemical characteristics of the Muskeg River over the study period are presented in Table 1. Throughout the latter half of August through to the middle of October extreme precipitation increased the river discharge to exceedingly high levels; the October peak was three times the generally maximum discharge in the spring. As a consequence of this, it was not

Table 1. Physical and chemical parameters of the Muskeg River over the study period.

Parameters	April	May	June	July	August
Mean daily discharge ($m^3 \cdot s^{-1}$)	113	324	174	43	125
Temperature (^oC)	0	6.5	16.0	16.0	14.2
Conductivity ($S \cdot m^{-1}$)	430	120	170	340	272
NH_4 - N ($\mu g \cdot L^{-1}$)	580	50	40	20	31
NO_3 + NO_2 - N ($\mu g \cdot L^{-1}$)	82	5	8	3	3
PO_4 - P ($\mu g \cdot L^{-1}$)	8	13	< 3	10	14
DOC ($mg \cdot L^{-1}$)	18	22	53	24	21
pH	7.5	7.8	7.8	7.8	7.8

possible to obtain a sample at 16 weeks and by the time water had receded in early November, all the remaining bricks had washed away. Thus, the analysis was restricted to four and nine weeks of colonization only.

In general, all the epilithic parameters increased over time (Table 2 and Figure 2). After four weeks of colonization there were significantly more bacteria on the control bricks. However, after nine weeks, there was no significant difference between them, with both having reached a level of approximately 2×10^8 cm^{-2} (Tables 2 and 3). After four and nine weeks of colonization, there was significantly less chlorophyll *a* on the bitumen as opposed to the control bricks (Tables 2 and 3). However, the differences between the phaeophytin (chlorophyll *a* degradation product) concentrations were not significant (Table 3). On calculating the chlorophyll:phaeophytin ratio for each series, it was found to be high in the controls (2.1 and 3.2) and very low on the bitumen coated bricks (0 and 0.5). Direct counts of algae revealed little difference between the numbers of cells cm^{-2} of the three major groups, Cyanophyta, Bacillariophyta, and Chlorophyta, recorded from the bitumen coated and control bricks. The results are summarized in Table 4. The only major differences noted were in the numbers of Bacillariophyta cm^{-2} at four weeks on the bitumen coated bricks (a 6.3 fold increase over the control) and in the numbers of Cyanophyta cm^{-2} at nine weeks, also on the bitumen coated bricks (a 5.8 fold increase over the control).

Carbohydrate concentrations at four weeks were not significantly different at approximately 5 $\mu g \cdot cm^{-2}$, but after nine weeks, a significant difference ($p < 0.05$) was apparent with ~32 $\mu g \cdot cm^{-2}$ on the control bricks and ~19 $\mu g \cdot cm^{-2}$ on the bitumen coated bricks (Tables 2 and 3).

The number of micro-invertebrates per 100 cm^2 increased over time, but there was no significant difference between the numbers on the control and bitumen coated bricks (Table 5 and Figure 2). However, it is important to stress that the variability in each series was great, resulting in extremely wide 95% confidence limits. Inspection of the weights of micro-invertebrates

Table 2. Epilithic biomass determinations on control and bitumen coated bricks over time. Bacteria (mean ± 95% confidence limits), Chlorophyll *a*, Phaeophytin, and Carbohydrate (mean ± SE).

Week	Bacteria cm^{-2}	Phaeophytin $(\mu g \cdot cm^{-2})$	Chlorophyll *a* $(\mu g \cdot cm^{-2})$	Carbohydrate $(\mu g \cdot cm^{-2})$
	Control			
4	$8.9 \pm 1.4 \times 10^6$	0.20 ± 0.14	0.42 ± 0.29	4.07 ± 0.19
9	$2.1 \pm 0.4 \times 10^8$	1.00 ± 0.19	3.19 ± 0.23	31.98 ± 4.46
	Bitumen Coated			
4	$2.9 \pm 0.4 \times 10^6$	0.35 ± 0.05	0	5.96 ± 0.85
9	$1.8 \pm 0.2 \times 10^8$	1.68 ± 0.22	0.78 ± 0.20	19.1 ± 2.90

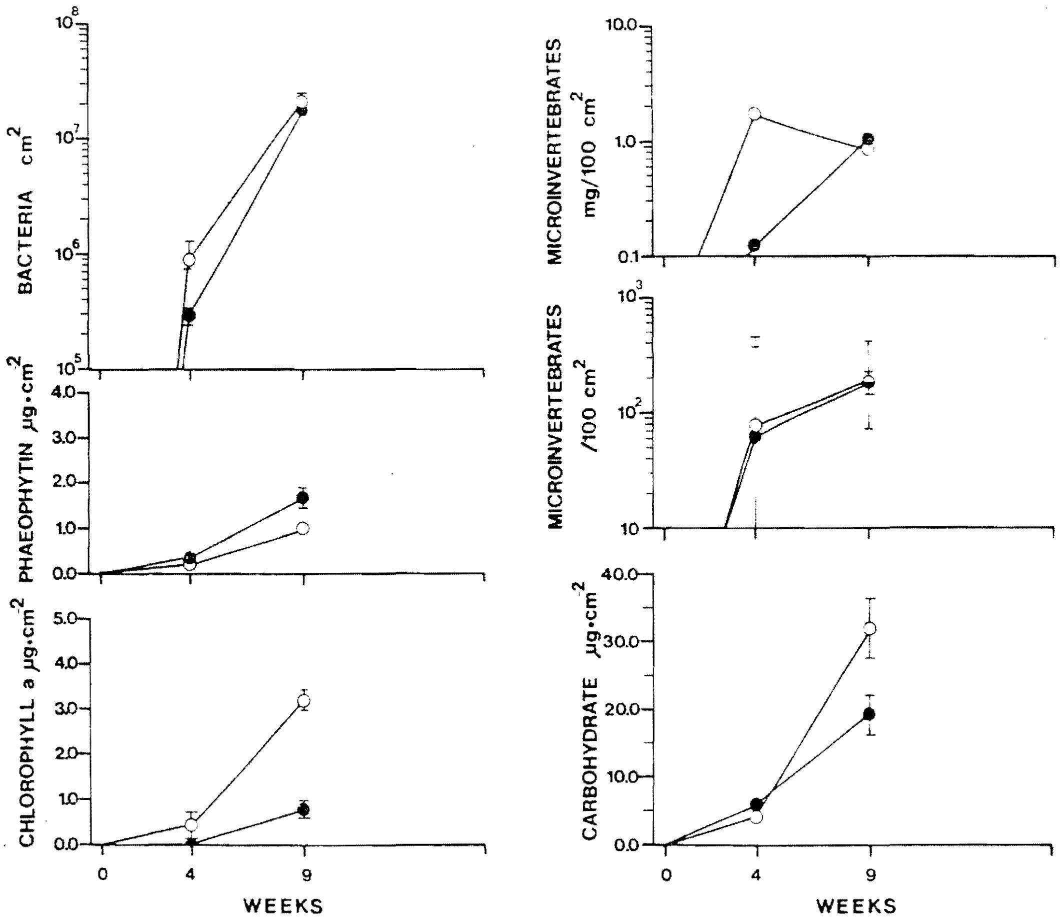

Figure 2. Epilithic biomass determinations on control (O) and bitumen coated (●) bricks over time.

Table 3. Comparison of the means of the epilithic biomass determinations from the control and bitumen coated bricks using a t-test.

Week	Bacteria		Phaeophytin		Chlorophyll *a*		Carbohydrate	
	t	df	t	df	t	df	t	df
4	6.01	8	1.18	7	1.62	7	2.17	8
9	1.87	8	2.34	7	7.57	7	2.42[a]	8

[a] $p < 0.05$

Table 4. The number of algal cells cm^{-2} on control and bitumen coated bricks.

	Week 4		Week 9	
	Control	Bitumen Coated	Control	Bitumen Coated
Cyanophyta	1.2×10^5	0.9×10^5	1.5×10^4	8.7×10^4
Bacillariophyta	1.6×10^3	1.0×10^4	3.7×10^5	3.2×10^5
Chlorophyta	1.3×10^4	1.3×10^4	1.2×10^5	1.7×10^5
Total	1.4×10^5	1.1×10^5	5.0×10^5	5.8×10^5

Table 5. Mean with 95% confidence limits of the numbers of micro-invertebrates per 100 cm^2 on control and bitumen coated bricks.

Week	Parameter		Control			
			Chironomidae	Trichoptera	Others	Total
4	Number	Mean	10.8	40.3	10.8	78.3
		Upper	49.0	351.0	49.0	452.5
		Lower	0	0	0	0
	Weight	Mean	0.029	0.105	1.573	1.706
9	Number	Mean	143.8	26.0	3.8	183.0
		Upper	262.5	180.5	19.8	415.3
		Lower	74.0	0	0	73.3
	Weight	Mean	0.722	0.078	0.046	0.846
			Bitumen Coated			
			Chironomidae	Trichoptera	Others	Total
4	Number	Mean	18.5	33.3	-	62.0
		Upper	74.2	279.8		370.3
		Lower	0	0		0
	Weight	Mean	0.015	0.107		0.122
9	Number	Mean	158.3	3.8	18.5	183.5
		Upper	203.0	19.8	42.8	227.5
		Lower	122.3	0		147.0
	Weight	Mean	0.910	-	0.155	1.065

per 100 cm^2 (Table 5 and Figure 2) reveals a considerably greater biomass on the control bricks at four weeks and becoming very similar by nine weeks. There were few significant differences in the mean numbers of macro-invertebrates on control and bitumen coated bricks (Tables 6 and 7). At four weeks there were 95% more Chironomidae on the control bricks ($p < 0.05$). However, by nine weeks, the proportions of Trichoptera on the bitumen coated and control bricks had reversed, with approximately twice as many Trichoptera, Hydropsychidae, and *Ceraclea* sp. occurring on the control bricks ($p < 0.05-0.01$). An approximate three fold significant increase in Mollusca and *Sphaerium* sp. on the control compared with the bitumen coated bricks was noted ($p < 0.05$).

2.4 DISCUSSION

It is readily apparent from Table 8, which summarizes the effect of bitumen upon the river community, that after nine weeks bitumen acts essentially as a rock substrate. Possible suppression of bacterial colonization was observed at week 4 but this had disappeared by week 9. The absence of any bacterial population stimulation suggests that the bitumen does not represent a major additional energy source under the prevailing river conditions. However, this does not eliminate the possibility that it undergoes some biodegradation since the character of the bitumen was observed to change from a shiny black adherent layer to a dull brown friable one over the exposure period. Such changes could of course be physical or chemical in nature as well as biological.

Although the decreased levels of chlorophyll *a* on the bitumen would suggest a depression of algal biomass, this observation is not supported by the direct count data on the algae where there was essentially no difference between the control and bitumen and the numbers of algal cells observed. Additionally, the algae seemed to be equally healthy, based upon their appearance under the microscope. These findings suggest that the bitumen may have interfered with the chlorophyll *a* determination and may have also stimulated the breakdown of chlorophyll *a* to phaeophytin, thus accounting for the higher proportion of chlorophyll *a*

Table 6. Geometric means with 95% confidence limits of the macro-invertebrates associated with control and bitumen coated limestone bricks over time.

Week	Parameter		Cladocera	Copepoda	Chironomidae	Simuliidae	Ephemeroptera (Total)	Heptageniidae	*Ameletus* sp.
4	Control	Mean	19.6	12.3	17.8	1.4	7.0	3.2	2.0
		Upper	73.4	25.7	23.0	5.0	19.7	10.1	5.3
		Lower	5.2	5.9	13.7	0	2.5	1.0	0.7
4	Bitumen Coated	Mean	29.8	15.5	9.1	1.0	1.9	0.4	0.3
		Upper	47.2	20.7	13.7	2.9	6.8	2.0	1.3
		Lower	18.8	11.7	6.0	0.1	0.1	0	0
9	Control	Mean	44.0	9.1	58.5	1.6	8.1	2.2	3.2
		Upper	82.5	13.6	118.0	6.4	12.5	4.4	8.9
		Lower	23.4	6.1	29.0	0	5.3	1.1	1.2
9	Bitumen Coated	Mean	66.6	10.2	41.0	0.7	5.0	0.7	2.6
		Upper	87.9	18.3	47.4	3.0	8.8	1.7	7.2
		Lower	50.5	5.7	35.5	0	2.9	0.1	1.0

continued . . .

Table 6. Concluded.

Week	Parameter		Acarina	Mollusca (Total)	*Sphaerium* sp.	Plecoptera	Trichoptera (Total)	Hydropsychidae	*Ceraclea* sp.
4	Control	Mean	6.2	1.4	1.1	0.9	22.9	19.4	1.2
		Upper	19.0	7.7	7.6	2.3	38.8	29.1	6.5
		Lower	2.0	0	0	0.1	13.5	12.9	0
4	Bitumen Coated	Mean	4.3	0.5	0.3	0.6	38.7	29.9	2.4
		Upper	7.7	1.6	1.3	2.7	54.0	44.5	11.7
		Lower	2.4	0	0	0	27.8	20.1	0
9	Control	Mean	4.5	4.4	4.2	1.7	29.6	14.5	4.7
		Upper	13.5	10.1	9.6	5.1	52.6	40.5	15.1
		Lower	1.5	1.9	1.9	0.6	16.6	5.2	1.5
9	Bitumen Coated	Mean	3.2	1.0	0.8	0.3	11.0	6.8	1.6
		Upper	10.1	2.9	2.9	1.3	16.7	13.2	3.3
		Lower	1.0	0.1	0	0	7.3	3.5	0.8

Table 7. Comparison of the means of the numbers of macro-invertebrates on control and bitumen coated bricks using a t-test.

	Week 4 (t)	Week 9 (t)
Cladocera	0.96	1.92
Copepoda	0.94	0.50
Chironomidae	4.40[b]	1.58
Simuliidae	0.46	0.92
Ephemeroptera	1.96	2.14
Heptageniidae	1.93	0.95
Ameletus sp.	1.13	0.47
Acarina	0.93	0.68
Mollusca	1.11	2.34[a]
Sphaerium sp.	1.02	2.41[a]
Plecoptera	0.61	0.73
Trichoptera	2.68[a]	4.41[b]
Hydropsychidae	2.43[a]	1.97
Ceraclea sp.	0.77	2.49[a]

[a] $p < 0.05$

[b] $p < 0.01$

Table 8. A summary of the response of the various community parameters to the presence of bitumen. The responses are in relation to the control where - = significant reduction, + = significant increase, 0 = no significant difference, and (+), (-), or (±) = a response by a few groups within the category.

Parameter	Week 4	Week 9
Bacteria	-	0
Chlorophyll *a*	-	-
Phaeophytin	0	0
Direct algal counts	0(+)	0(+)
Micro-invertebrate numbers	0	0
weights	-	0
Macro-invertebrate numbers	0(±)	0(-)

degradation product observed on the bitumen coated bricks. This problem is currently being investigated by S. Charlton (pers. comm. Department of Botany, University of Alberta).

The reason for the increase in the carbohydrate content of the epilithon on the control bricks at week 9 is unknown as this change is not reflected in an increase in the concentrations of chlorophyll *a* nor in the numbers of bacteria and algae (cf. Lock and Wallace in prep.). There is also no evidence that this increase in carbohydrate represented an increase in food (as cellular carbohydrate or as "polysaccharide" matrix) for the micro-invertebrates living within the epilithon, on the basis of the absence of any greater biomass or numbers of micro-invertebrates occurring on the control as opposed to bitumen coated bricks. However, among the macro-invertebrates present on the control bricks, an increase was noted in Hydropsychidae, *Ceraclea* sp., and *Sphaerium* sp., but of these only *Ceraclea* sp., a collector-gatherer (Merritt and Cummins 1978), would be likely to respond as the other two groups are filter feeders.

These findings confirm the hypothesis of Barton and Wallace (in prep.) that oil sand (a sand/bitumen mixture) substrates and rocks are equally suitable for many benthic invertebrates. In conclusion, it appears on the basis of the micro-organisms, micro-invertebrates, and macro-invertebrates examined, that after nine weeks of colonization there will be little difference between the communities occurring on bituminous substrates or limestone rocks. Thus, in the event that, during stream reclamation, freshly exposed oil sand should become incorporated into the bed materials it is unlikely that this would pose any long term toxicity problems. Since in this study, minimal toxicity was apparent from the outset, it seems less likely that toxicity would increase over time.

3. IMMEDIATE AND SHORT TERM RESPONSES OF AN ESTABLISHED MICROBIAL AND MACRO-INVERTEBRATE BENTHIC RIVER COMMUNITY TO A SYNTHETIC CRUDE OIL SPILL

3.1 INTRODUCTION

Barton and Wallace (in prep.) examined the long term effect of synthetic crude oil and its component fractions upon established benthic river communities of the AOSERP study area. One month after the addition of the oils, increases over the control in bacterial numbers and algal biomass (chlorophyll *a*) were noted. This observation led them to suggest, that, within those first four weeks of exposure, a stimulation in microbial biomass had taken place of the same type observed on synthetic crude oil soaked limestone (Barton and Wallace in prep.). Thus, the purpose of this study was to test this hypothesis by following the response of a community composed of micro-and macro-organisms established on limestone bricks at short time periods extending up to 35 days.

This information was required to make a firmer prediction about the effects of a synthetic crude oil spill into a muskeg river. In the event that major but short term (days as opposed to weeks) changes were induced by an instantaneous oil spill of the type modelled, a more prolonged oil spill might be potentially more disruptive that the initial experiments of Barton and Wallace (in prep.) indicated.

3.2 MATERIALS AND METHODS

The study was conducted in the riffle area described in Section 2.2 of this report, and to enable standardized comparisons of control and oiled attached communities, limestone bricks were used as a substrate (Barton and Wallace in prep.). Bricks were placed in the river on 19 May 1978 in one single pre-treatment row of 10 (five bricks for epilithic determinations and five for macro-invertebrates) and five double rows of 10 for subsequent removal over time. The rows of bricks were placed in a riffle area of uniform depth with the rows orientated at right-angles to the flow. After about four weeks, on 21 June 1978, the extent of colonization was determined on the pre-treatment bricks: half the remaining bricks were dipped for 1 minute in synthetic crude oil and the other half, the control bricks, were dipped into filtered river water. The oiled and control bricks were segregated to minimize contamination of the controls. The extent of colonization after the oil addition was examined at 1, 3, 7, 14, and 35 day intervals, by a determination of bacterial numbers, algal numbers, concentrations of chlorophyll *a*, phaeophytin and carbohydrate, micro-invertebrate numbers and weights, and macro-invertebrate numbers. The methods used were as described in Section 2.2 of this report. In addition to these, 1 cm^2 scrapes were also taken which were transferred to vials of organic, matter-free distilled water and then filtered onto pre-combusted filters. The total organic carbon (TOC) and nitrogen (TON) content of this material was subsequently determined using a Hewlett Packard CHN analyser according to the method of Traversy (1977).

3.3 RESULTS

The mean physical and chemical parameters of the Muskeg River over the study period are presented in Table 1, Section 2.3, of this report.

After one month of colonization, the epilithic components of the limestone bricks (Table 9) were very similar to those of the long established epilithon on granite discs in Lock and Wallace (in prep.). The subsequent addition of oil had little apparent effect upon the various components of the epilithon monitored when compared with the controls (Table 9 and Figure 3). However, there were general increases in control levels of bacteria, chlorophyll α, carbohydrate, and TOC over the first 14 days, followed by decreases in all components except TON by day 35. The only consistently major difference between the control and oiled bricks over time was in the numbers of Cyanophyta occurring on the oiled bricks on days 3, 7, 14, and 35 where there were 2.2, 17.2, 4.6, and 3.0 fold increases over the control (Table 10 and Figure 4). The responses of the Bacillariophyta on

Table 9. Epilithic biomass determinations on control and oiled bricks over time. Bacteria (mean with 95% confidence limits), Chlorophyll *a*, Carbohydrate, Total Organic Carbon (TOC), and Total Organic Nitrogen (mean ± SE).

Sampling Time	Bacteria cm^{-2}	Chlorophyll *a* ($\mu g \cdot cm^{-2}$)	Carbohydrate ($\mu g \cdot cm^{-2}$)	TOC ($\mu g \cdot cm^{-2}$)	TON ($\mu g \cdot cm^{-2}$)	C:N
Control						
Pre-treatment	$1.2 \pm 0.1 \times 10^{7}$	0.36±0.06	4.62±0.64	90±40	10±0	9.0
Day 1	$1.4 \pm 0.2 \times 10^{7}$	0.57±0.15	3.99±0.55	60±20	12±2	5.0
Day 3	$2.1 \pm 0.3 \times 10^{7}$	0.26±0.02	8.07±1.00	180±70	14±2	12.9
Day 7	$1.6 \pm 0.2 \times 10^{7}$	0.32±0.06	6.73±0.57	140±50	16±4	8.8
Day 14	$1.9 \pm 0.2 \times 10^{7}$	0.53±0.07	11.50±0.60	260±110	14±2	18.6
Day 35	$9.0 \pm 1.6 \times 10^{6}$	0.18±0.02	5.99±0.90	170±70	14±2	12.1
Oiled						
Day 1	$1.6 \pm 0.2 \times 10^{7}$	0.33±0.09	3.20±0.28	40±20	10±0	4.0
Day 3	$1.8 \pm 0.2 \times 10^{7}$	0.20±0.04	8.51±0.69	330±110	30±8	11.0
Day 7	$1.6 \pm 0.3 \times 10^{7}$	0.38±0.03	5.49±1.24	130±70	14±2	9.3
Day 14	$2.6 \pm 0.3 \times 10^{7}$	0.32±0.06	8.67±0.77	240±130	10±0	24.0
Day 35	$9.3 \pm 1.3 \times 10^{6}$	0.20±0.10	7.96±1.18	150±60	10±0	15.0

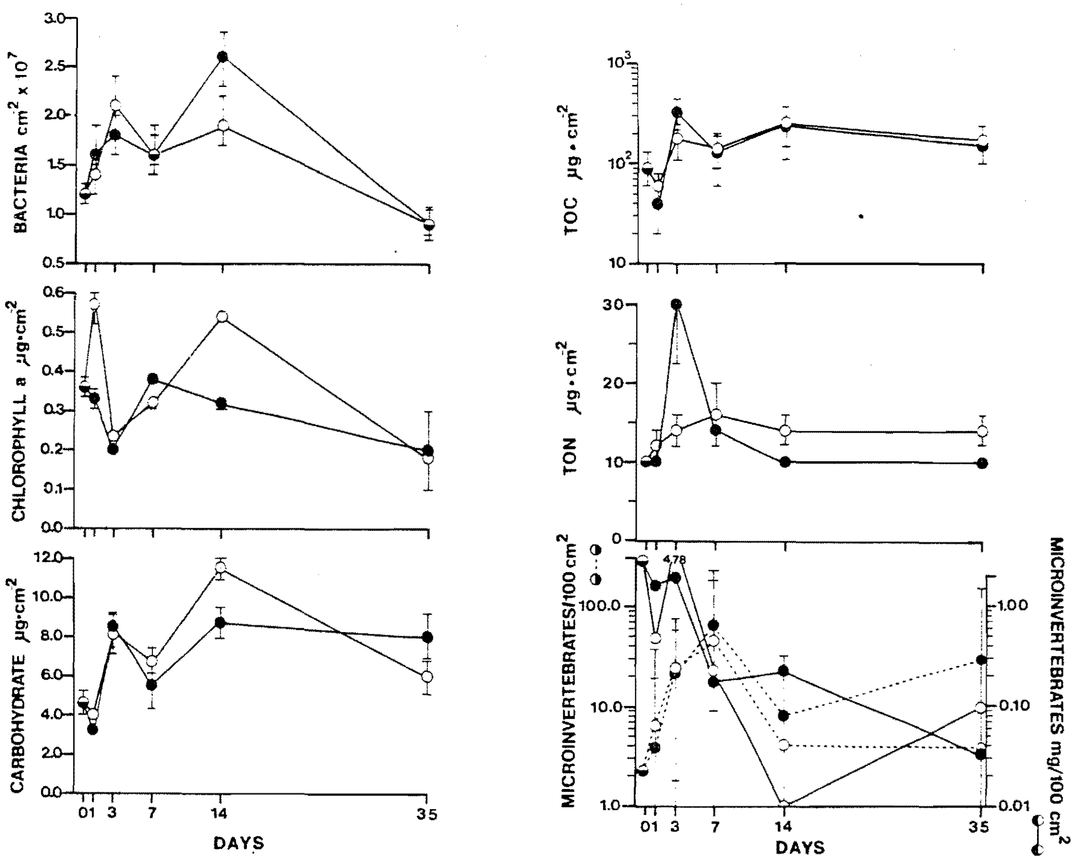

Figure 3. Epilithic biomass determinations on control (O) and oiled (●) bricks over time.

Table 10. Numbers of algal cells cm^{-2} on the control and oiled bricks over time.

	Cyanophyta	Bacillariophyta	Chlorophyta	Total
Pre treatment	3.3×10^5	7.9×10^3	4.5×10^4	3.8×10^5
Day 1 Control	3.5×10^5	6.3×10^3	4.1×10^4	4.0×10^5
Oiled	3.6×10^5	4.9×10^3	4.0×10^4	4.0×10^5
Day 3 Control	5.5×10^4	-	1.6×10^4	7.1×10^4
Oiled	1.2×10^5	0.6×10^3	2.6×10^4	1.4×10^5
Day 7 Control	6.4×10^4	3.0×10^3	2.0×10^4	8.6×10^4
Oiled	1.1×10^6	3.0×10^3	8.3×10^3	1.1×10^6
Day 14 Control	2.6×10^4	-	1.2×10^4	3.7×10^4
Oiled	1.2×10^5	-	1.9×10^4	1.4×10^5
Day 35 Control	4.4×10^4	2.4×10^3	3.6×10^4	8.2×10^4
Oiled	1.3×10^5	0.8×10^3	3.7×10^4	1.7×10^5

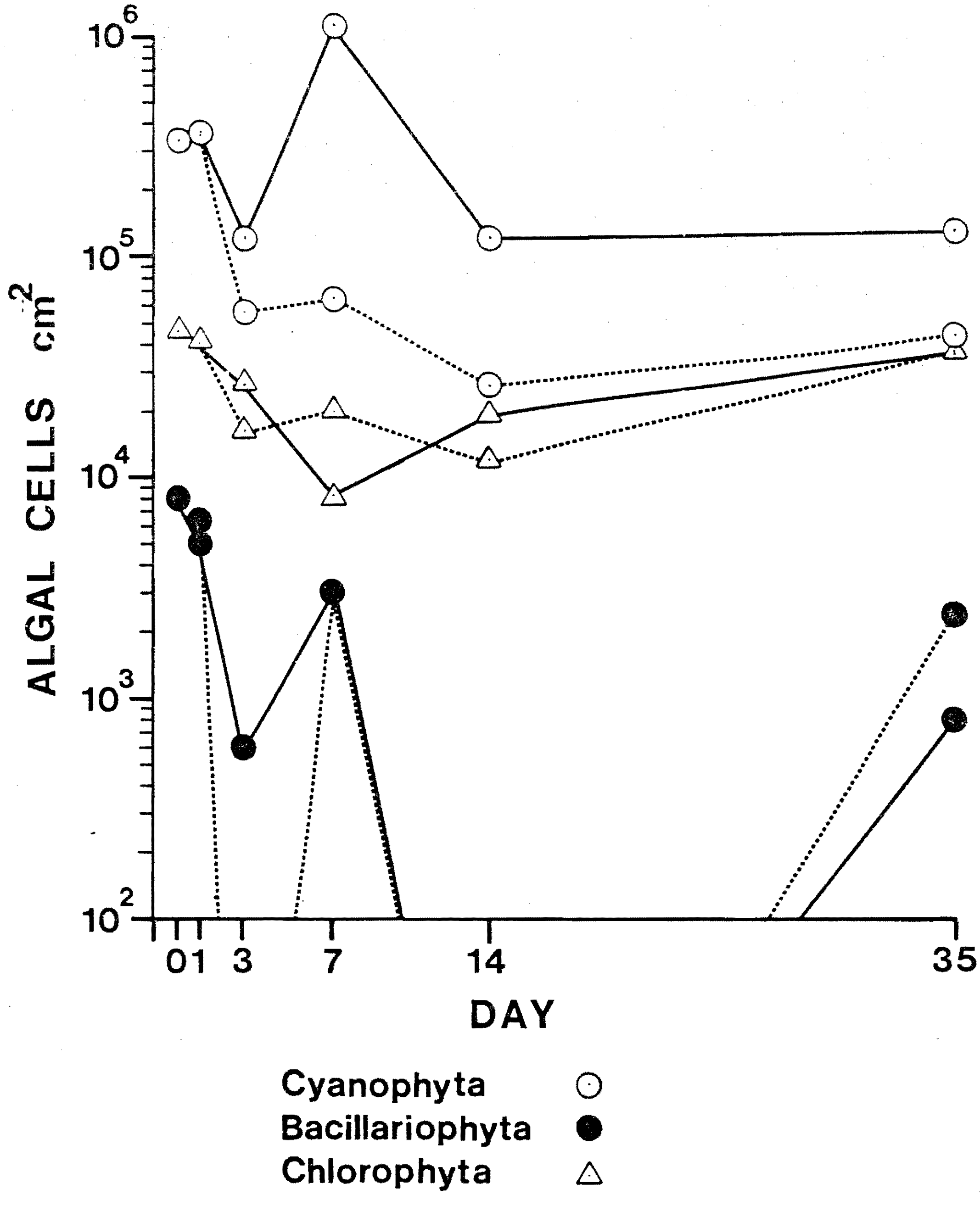

Figure 4. Numbers of algal cells cm^{-2} on the control (----) and oiled (——) bricks over time.

the oiled bricks were very variable with stimulation and inhibition occurring over time, while the numbers of Chlorophyta cm^{-2} on the control and oiled bricks generally differed by less than 62%. The only other significant differences between the oiled and control bricks occurred on day 14 (Table 9 and 11) where there was a 40% increase in bacteria, a 70% decrease in chlorophyll *a*, and a 40% decrease in carbohydrate.

There was no significant difference between the numbers of micro-invertebrates on the control and oiled bricks (Table 12). However, it must be noted that the 95% confidence limits were extremely large. Although statistical limits could not be placed upon the mean weight of micro-invertebrates (replicates were pooled for weighing), the oiled bricks were also generally similar to the controls (Figure 3). Over time, the micro-invertebrates increased in numbers up to day 7, declined to day 14, and remained at approximately the same level at day 35 (Figure 3). The more detailed breakdown of the numerical micro-invertebrate data (Table 12) suggests a suppression of Trichoptera and a stimulation of Simuliidae; this is also supported by the data on the weights of micro-invertebrates (Table 12). The total weights of micro-invertebrates tended to decline over time (Table 13). Data on the macro-invertebrates are restricted to days 3 to 35 due to technical problems. However, once again no significant differences were found between the organisms associated with oiled and control bricks, with the exception of Cladocera on day 35 (Tables 14 and 15).

3.4 DISCUSSION

Out of the 20 taxonomic groups and four biochemical parameters examined, in only one of the taxonomic groups was there any major difference between the numbers occurring on oiled bricks as opposed to those on the control bricks. The group affected was the Cyanophyta, which appeared to be stimulated by the oil addition, a response also noted by Shindler et al. (1975), reaching a peak in

Table 11. Comparison of the means of the epilithic biomass determinations from the control and oiled bricks using a t-test (8 degrees of freedom).

	Day 1	Day 3	Day 7	Day 14	Day 35
Bacteria cm^{-2}	2.25	1.87	0.05	3.58[b]	0.29
Chlorophyll α cm^{-2}	1.37	1.41	0.81	2.31[a]	0.19
Carbohydrate cm^{-2}	1.29	0.34	0.91	2.91[a]	1.27
TOC cm^{-2}	0.73	1.14	0.43	0.14	0.17
TON cm^{-2}	0.88	1.52	0.43	1.63	1.63

[a] $p < 0.05$

[b] $p < 0.01$

Table 12. Means with 95% confidence limits of the numbers of micro-invertebrates per 100 cm^2 on control and oiled bricks over time.

Sampling Time		Control					
		Ephemeroptera	Trichoptera	Simuliidae	Chironomidae	Others	Total
Pre-Treatment	Mean	-	-	-	-	-	-
	Upper						
	Lower						
Day 1	Mean	8	3.75	-	6.25	-	19.5
	Upper	31.5	19.75		37.75		72.75
	Lower	0	0		0		0
Day 3	Mean	8	-	6.25	13	-	23.75
	Upper	31.5		37.75	66.5		125.75
	Lower	0		0	0		0
Day 7	Mean	25	20.5	-	-	-	44
	Upper	75.5	122				198
	Lower	0	0				0
Day 14	Mean	-	3.75	-	-	-	3.75
	Upper		19.75				19.75
	Lower		0				0
Day 35	Mean	3.75	22.25	3.75	33.5	-	84.75
	Upper	19.75	193.25	19.75	185.5		416.5
	Lower	0	0	0	0		2.25

continued . . .

Table 12. Concluded.

Sampling Time		Oiled					
		Ephemeroptera	Trichoptera	Simuliidae	Chironomidae	Others	Total
Pre-Treatment	Mean	-	-	22.3	3.8	6.3	32.5
	Upper			58.0	19.8	37.8	106.0
	Lower			1.8	0	0	0.3
Day 1	Mean	22.3	-	3.8	-	-	25
	Upper	58		19.8			75.5
	Lower	1.8		0			0
Day 3	Mean	6.25	-	3.75	8	-	22.25
	Upper	37.75		19.75	31.5		58
	Lower	0		0	0		1.75
Day 7	Mean	8	40.25	-	-	-	64.75
	Upper	54.75	193				213
	Lower	0	0				9
Day 14	Mean	3.75	-	3.75	-	-	8
	Upper	19.75		19.75			31.5
	Lower	0		0			0
Day 35	Mean	-	-	3.75	26.25	-	29.25
	Upper			19.75	126.5		146.25
	Lower			0	0		0

Table 13. The mean weight (mg) of micro-invertebrates per 100 CM^2 on control and oiled bricks over time.

		Control					
		Ephemeroptera	Trichoptera	Simuliidae	Chironomidae	Others	Total
Pre-treatment	Mean	-	-	-	-	-	-
Day 1		-	-	-	0.01	0.47	0.48
Day 3		0.64	-	3.89	0.25	-	4.78
Day 7		0.90	0.23	-	-	-	1.13
Day 14		-	0.02	-	-	-	0.02
Day 35		-	0.43	-	0.05	-	0.48
		Oiled					
		Ephemeroptera	Trichoptera	Simuliidae	Chironomidae	Others	Total
Pre-treatment	Mean	-	-	0.15	0.01	2.6	2.76
Day 1	Mean	0.42	-	1.21	-	-	1.63
Day 3	Mean	0.38	-	1.56	0.02	-	1.96
Day 7	Mean	0.42	0.46	-	-	-	0.89
Day 14	Mean	0.31	-	0.80	-	-	1.11
Day 35	Mean	-	-	0.06	0.10	-	0.16

Table 14. Geometric means with 95% confidence limits of the macro-invertebrates associated with the control and oiled substrates over time.

	Day 3			Day 7			Day 14			Day 35		
	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower
Cladocera	-			-			-			69.5	83.3	58.0
Copepoda	-			-			-			5.0	10.3	2.4
Chironomidae	56.5	72.9	43.8	40.0	62.7	25.5	17.9	24.7	12.9	14.8	34.3	6.4
Simuliidae	12.5	24.6	6.4	34.3	124.6	9.4	19.9	80.2	4.9	2.5	11.5	0
Ephemeroptera (Total)	71.3	149.1	34.1	118.2	159.1	87.8	44.9	80.3	25.1	3.9	16.8	0.9
Parameletus sp.	29.8	82.2	10.8	49.8	78.4	31.7	21.6	52.2	8.9	-		
Ameletus sp.	7.1	43.9	0.5	57.0	71.7	45.3	19.1	25.5	14.2	4.5	12.5	1.6
Ephemerella sp.	-			4.9	11.0	2.2	1.4	4.1	0.1	-		
Trichoptera (Total)	5.5	66.5	0.4	43.6	110.5	17.2	23.2	34.9	15.4	19.5	29.8	12.8
Hydropsychidae	-			40.6	115.8	14.3	20.4	37.9	11.0	13.3	20.9	8.4
Others	0.5	2.7	0	0.8	3.7	0	0.3	1.3	0	3.5	19.3	0

continued . . .

Table 14. Concluded.

	Day 3			Day 7			Day 14			Day 35		
	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower
Cladocera	-			-			-			36.5	47.6	27.9
Copepoda	-			-			-			2.5	9.2	0.2
Chironomidae	36.6	51.3	26.0	38.4	68.2	21.6	21.6	37.6	12.4	12.9	34.2	4.8
Simuliidae	10.0	35.6	2.8	38.1	59.5	24.4	18.3	65.3	5.1	1.0	2.9	0.1
Ephemeroptera (Total)	69.4	77.6	62.1	111.9	145.1	86.2	44.9	66.7	30.2	4.9	11.6	2.1
Parameletus sp.	44.5	67.9	29.2	36.5	62.4	21.3	22.3	39.0	12.8	-		
Ameletus sp.	20.0	44.2	9.1	69.6	88.6	54.6	20.1	30.7	13.1	1.9	5.7	0.2
Ephemerella sp.	-			1.9	6.5	0.1	1.1	5.0	0	-		
Trichoptera (Total)	1.8	7.6	0	34.2	254.7	4.6	10.6	34.7	3.2	8.9	59.7	1.3
Hydropsychidae	-			30.2	247.1	3.7	8.9	32.3	2.4	4.3	34.9	0.5
Others	0.8	2.9	0	0.1	0.8	0	0.3	1.3	0	8.6	13.7	5.4

Table 15. Comparison of log transformed means (using a t-test), of the numbers of invertebrates on the control and oiled bricks.

	Day 3	Day 7	Day 14	Day 35
Cladocera	-	-	-	6.35[a]
Copepoda	-	-	-	0.86
Chironomidae	1.14	0.17	0.95	0.34
Simuliidae	0.50	0.25	0.14	1.21
Ephemeroptera	0.11	0.44	0	0.44
Parameletus sp.	1.16	1,41	0.10	-
Ameletus sp.	1.52	1.90	0.32	1.09
Ephemerella sp.	-	1.34	0.23	-
Trichoptera	0.79	0.35	1.99	1.28
Hydropsychidae	-	0.40	1.84	1.67
Others	0.44	1.41	0	1.30

[a] $p < 0,001$

numbers around day 7 and then declining throughout the rest of the study period but still remaining at population levels considerably higher than those of the controls.

Thus, the overwhelming conclusion of this experiment is that synthetic crude oil has only a minimal effect upon established benthic river communities. This is in contrast to experimental additions of crude oil to soils (Sexstone and Atlas 1977), an Arctic lake (Hutchinson et al. 1976), the Beaufort Sea area (Atlas 1977), and artificial ponds (Shindler et al. 1974) where an increase in numbers of bacteria was noted. However, the responses of algae to crude oil have been found to be extremely varied, ranging from stimulation through to tolerance or inhibition, depending upon the composition and initial concentration of the oil tested (Kauss et al. 1973; Pulich et al. 1974; Kauss and Hutchinson 1975; Graham and Hutchinson 1975; Parsons et al. 1976).

The above conclusion must, however, be qualified by a consideration of the relevance of the oil presentation method to the type of spillage expected in the field. The question is posed, can the results of this experiment and the earlier work of Barton and Wallace (in prep.) be extrapolated to include those situations where oil enters a river over a period of days or even weeks as opposed to an instantaneous addition? Since a transient change was apparently induced in the cyanophycan algal population it is possible that a continued exposure to synthetic crude oil would produce a prolonged population increase. The consequence of such an increase on the remainder of the community is unknown. However, it does seem probable that an increased contact of oil with the epilithon, which would be anticipated in a prolonged spill, would have an effect approaching the situation modelled by Rosenberg and Wiens (1976), Rosenberg et al. (1977), and Barton and Wallace (in prep.), where benthic communities growing on oil soaked rocks underwent an increase in standing crop.

Finally, it would appear that, depending upon the size and duration of the spill, the effect upon the benthic river communities would range from a minimal one to one of a stimulation of production. It would appear that the effect of oil upon a benthic community has two components. The first of these is the physical process of oil making contact and then penetrating the benthic community, where the penetration rate presumably depends upon the specific gravity, viscosity, and degree of emulsification of the oil. The second component is the ability of the oil to affect microbial communities once contact has been made, with the evidence so far indicating that the effect is a stimulatory one. However, fuller understanding of lotic oil pollution processes would best be obtained by studying the biological effects on controlled, long term spillages into small natural streams or specially constructed flow-through model streams where factors affecting oil penetration into the community could be altered.

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5. AOSERP RESEARCH REPORTS

1.		AOSERP First Annual Report, 1975
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20.	HY 3.1.1	Characterization of Organic Constituents in Waters and Wastewaters of the Athabasca Oil Sands Mining Area
21.		AOSERP Second Annual Report, 1976-77
22.		Alberta Oil Sands Environmental Research Program Interim Report to 1978 covering the period April 1975 to November 1978
23.	AF 1.1.2	Acute Lethality of Mine Depressurization Water on Trout Perch and Rainbow Trout
24.	ME 1.5.2	Air System Winter Field Study in the AOSERP Study Area, February 1977.
25.	ME 3.5.1	Review of Pollutant Transformation Processes Relevant to the Alberta Oil Sands Area

26.	AF 4.5.1	Interim Report on an Intensive Study of the Fish Fauna of the Muskeg River Watershed of Northeastern Alberta
27.	ME 1.5.1	Meteorology and Air Quality Winter Field Study in the AOSERP Study Area, March 1976
28.	VE 2.1	Interim Report on a Soils Inventory in the Athabasca Oil Sands Area
29.	ME 2.2	An Inventory System for Atmospheric Emissions in the AOSERP Study Area
30.	ME 2.1	Ambient Air Quality in the AOSERP Study Area, 1977
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41.	AF 3.5.1	Acute and Chronic Toxicity of Vanadium to Fish
42.	TF 1.1.4	Analysis of Fur Production Records for Registered Traplines in the AOSERP Study Area, 1970-75
43.	TF 6.1	A Socioeconomic Evaluation of the Recreational Fish and Wildlife Resources in Alberta, with Particular Reference to the AOSERP Study Area. Volume I: Summary and Conclusions
44.	VE 3.1	Interim Report on Symptomology and Threshold Levels of Air Pollutant Injury to Vegetation, 1975 to 1978
45.	VE 3.3	Interim Report on Physiology and Mechanisms of Air-Borne Pollutant Injury to Vegetation, 1975 to 1978
46.	VE 3.4	Interim Report on Ecological Benchmarking and Biomonitoring for Detection of Air-Borne Pollutant Effects on Vegetation and Soils, 1975 to 1978.
47.	TF 1.1.1	A Visibility Bias Model for Aerial Surveys for Moose on the AOSERP Study Area
48.	HG 1.1	Interim Report on a Hydrogeological Investigation of the Muskeg River Basin, Alberta
49.	WS 1.3.3	The Ecology of Macrobenthic Invertebrate Communities in Hartley Creek, Northeastern Alberta
50.	ME 3.6	Literature Review on Pollution Deposition Processes
51.	HY 1.3	Interim Compilation of 1976 Suspended Sediment Date in the AOSERP Study Area
52.	ME 2.3.2	Plume Dispersion Measurements from an Oil Sands Extraction Plan, June 1977

53.	HY 3.1.2	Baseline States of Organic Constituents in the Athabasca River System Upstream of Fort McMurray
54.	WS 2.3	A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta
55.	HY 2.6	Microbial Populations in the Athabasca River
56.	AF 3.2.1	The Acute Toxicity of Saline Groundwater and of Vanadium to Fish and Aquatic Invertebrates
57.	LS 2.3.1	Ecological Habitat Mapping of the AOSERP Study Area (Supplement): Phase I
58.	AF 2.0.2	Interim Report on Ecological Studies on the Lower Trophic Levels of Muskeg Rivers Within the Alberta Oil Sands Environmental Research Program Study Area
59.	TF 3.1	Semi-Aquatic Mammals: Annotated Bibliography
60.	WS 1.1.1	Synthesis of Surface Water Hydrology
61.	AF 4.5.2	An Intensive Study of the Fish Fauna of the Steepbank River Watershed of Northeastern Alberta
62.	TF 5.1	Amphibians and Reptiles in the AOSERP Study Area
63.		Calculate Sigma Data for the Alberta Oil Sands Environmental Research Program Study Area.
64.	LS 21.6.1	A Review of the Baseline Data Relevant to the Impacts of Oil Sands Development on Large Mammals in the AOSERP Study Area
65.	LS 21.6.2	A Review of the Baseline Data Relevant to the Impacts of Oil Sands Development on Black Bears in the AOSERP Study Area
66.	AS 4.3.2	An Assessment of the Models LIRAQ and ADPIC for Application to the Athabasca Oil Sands Area
67.	WS 1.3.2	Aquatic Biological Investigations of the Muskeg River Watershed
68.	AS 1.5.3 AS 3.5.2	Air System Summer Field Study in the AOSERP Study Area, June 1977
69.	HS 40.1	Native Employment Patterns in Alberta's Athabasca Oil Sands Region
70.	LS 28.1.2	An Interim Report on the Insectivorous Animals in the AOSERP Study Area
71.	HY 2.2	Lake Acidification Potential in the Alberta Oil Sands Environmental Research Program Study Area
72.	LS 7.1.2	The Ecology of Five Major Species of Small Mammals in the AOSERP Study Area: A Review
73.	LS 23.2	Distribution, Abundance and Habitat Associations of Beavers, Muskrats, Mink and River Otters in the AOSERP Study Area, Northeastern Alberta
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74.	AS 4.5	Air Quality Modelling and User Needs
75.	LS 2.1	Interim Report on the Soils Inventory of the AOSERP Study Area