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WOOD PRESERVING PLANT EFFLUENT: CHEMICAL COMPOSITION, TOXICITY TO SALMON AND TROUT

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Establishment

Biological Station
St. Andrews, N. B.

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ABSTRACT - The effluent from the wood preserving plant is a complex mixture of organic compounds containing mineral oil, aromatic hydrocarbons including polynuclear hydrocarbons such as benzo(a)pyrene; cresols, pentachlorophenol, acetic acid, aromatic acids, a small amount of basic compounds, and complex products of oxidation, condensation and other chemical reactions of the main components of the effluent. The compounds in the effluent are highly toxic to salmon and trout which detect and avoid concentrations lower than 5 μg/l. The effluent should be confined within the plant and must not be discharged into the environment without treatment. The plant installed a treatment facility in June 1969; this solves only a part of the problem. Areas between the plant and the river, contaminated by the effluent in the past, should be cleaned up.

INTRODUCTION

Numerous fluorescent spots were observed on thin-layer chromatograms of the chloroform extracts of water samples taken during a survey of the organic pollution of the Miramichi estuary in September 1968. Such strongly fluorescent compounds are usually found in tar oils. These are well known wood preservatives and their source therefore appeared to be the Domtar Wood Preserving Plant at Newcastle, New Brunswick. The area around the plant is drained by a small brook, discharging into a swamp directly connected with the Miramichi River. A sample of the brook water was analyzed and found heavily polluted by organic compounds. As some aromatic compounds which may occur in tar oils are toxic, it was decided to carry out a detailed analysis of the organic compounds, isolated from the brook water. The soil in several areas between the plant and the river is heavily contaminated by tar. Soil samples from these areas were also examined.

Immediately after the identification of pentachlorophenol in the brook water, the Regional Director of Fisheries, Department of Fisheries, Halifax, Nova Scotia, was notified.

The plant operated from March until May 1969 using pentachlorophenol. The analysis of the effluent during this period was reported (1). A treatment unit consisting of an oil-separator and an evaporator has been installed in May 1969 and theoretically there should be no effluent at all leaving the plant.

This, however, solves only part of the problem. In June 1969, the brook water still carried a heavy load of organic compounds, apparently originating from the contaminated areas between the plant and the river.

RESULTS AND DISCUSSION

The area between the Domtar plant and the Miramichi River is presented schematically in Figure 1. A water sample was taken at station 4 in October 1968. The plant was not in operation at that time; organic compounds in the sample were leached from the contaminated ground.

The isolation and fractionation of the organics is outlined in Table 1. The bulk of the extracted material consisted of neutral compounds (Extract A*); a significant amount of acidic compounds (Extract B) and a small amount of basic compounds (Extract C) were present. Two additional fractions, non-extractable with chloroform, were isolated by the carbon adsorption-extraction technique (Extracts D, E). The two major fractions were fractionated further by column chromatography on aluminium oxide.

Table 1. Isolation of Organic Compounds

Water Sample (68 l)

+NaOH to pH = 9-10

$CHCl_3$ extraction

Extract A,

Extraction with 0.1N HCl

Chloroform phase

(Extract A*),

65.78 g

Chromatography

Major components:

Mineral oil(Aliphatic hydrocarbons)

Aromatic hydrocarbons: naphthalene,

anthracene, pyrene, phenanthrene,

fluoranthene, small amount of carcinogens

Lethal threshold: 3.0 mg/l (salmon)

Aqueous phase

+NaOH to pH = 9-10,

$CHCl_3$ extraction

Extract C, 1.33 g

Lethal threshold:10 mg/l

(salmon)

Aqueous phase

+HCl to pH = 1.5

$CHCl_3$ extraction

Extract B, 10.62 g

Chromatography

Main component:

pentachlorophenol

lethal threshold:

0.5 mg/l (salmon)

Aqueous phase,

Activated carbon

treatment

Chloroform

Extract D

1.52 g

Ethanol

Extract E

0.55 g

Lethal threshold:

0.6 mg/l (salmon)

1. Neutral fraction (Extract A*)

The fractions obtained on chromatography of the Extract A* are described in Table 2. Fraction 40A is practically pure mineral oil, containing only traces of aromatic hydrocarbons (low absorption in the UV spectrum). Fraction 40B is a mixture of mineral oil and aromatic hydrocarbons. UV spectrum indicates the presence of naphthalene (275, 222 mμ), anthracene (375, 355 mμ), pyrene (335 mμ), and possibly methylpyrenes (275 mμ). IR spectrum of 40B (Fig. 2) confirms the presence of naphthalene and anthracene (strong bands at 780, and 875, 725 cm^{-1}, respectively). A carbonyl compound, very likely an aldehyde is also present (2720,1720 cm^{-1}).

The fraction 40C contains, in addition to the above-mentioned hydrocarbons, phenanthrene (293 mμ, 730, and 810 cm^{-1}, Fig. 3). Fluoranthene was detected in the fraction 40D by the UV maxima at 357 and 287 mμ, IR absorption bands at 1130, 1035, 1020, 820, 770, and 745 cm^{-1}, and a very strong, light blue fluorescence on the thin-layer chromatogram.

Fractions 40D*-40K contain mixtures of polynuclear hydrocarbons as indicated by the numerous maxima in the UV spectra, particularly above 400 mμ. The following hydrocarbons may be present:

perylene (437,429 mμ), dibenzo(a,g)perylene (430,301 mμ), naphtho(2,3,K)fluoranthene (437, 334 mμ), dibenzo(a,i)pyrene (430, 318 mμ), benzo(a)fluoranthene (430, 362 mμ), benzo(k) fluoranthene (403,378 mμ), benzo(b)fluoranthene (401, 357 mμ),

and benzo(a)pyrene (401, 382, 380 mμ). Some of these compounds, particularly the last one, are carcinogens.

Compounds in fractions 40L and 40M were not identified. IR spectrum of the fraction 40N indicates the presence of carboxyl groups (1700 cm^{-1}), possibly some quinoidal structures (1670 cm^{-1}), and aryl ethers (1320, 1280, and 1230 cm^{-1}). Fractions 400-40V, 46X, contain unidentified compounds; the presence of carboxyl, possibly quinoidal compounds, and aryl ethers is indicated by the IR spectrum of the fraction 40W (1710, 1650, and 1040-1255 cm^{-1}). A fraction identical with the fraction 40Z was isolated from the Extract B and will be described later.

2. Acidic fraction (Extract B)

Column chromatography of the Extract B is summarized in Table 3. UV spectra yielded little information and characterization of the fractions was based mainly on the IR spectra.

Fractions 44-1 to 44-3 contain hydrocarbons which were not fully extracted into the Extract A.

The combined fractions 44-5 to 44-8 and the fraction 44-19 contain an alkylphthalate, probably di-(2-ethylhexyl)-phthalate, a widely-used plasticizer. It is obviously a contaminant originating from polyethylene tubing and possibly other plastic ware used to handle the water sample. The IR spectrum of the fraction 44-19 is shown in Figure 4 for

Table 2. Fractionation of Extract A*

Fraction	mg	Appearance on TLC	UV Absorption maxima, mμ	Infrared maxima, cm^{-1}
Heptane				
40A	142	R_F 0.75, light blue fluorescent streak	278,272,268,265, very low absorption	2950,2920,2860,1460,1380
40B	456	As 40A + R_F 0.50 blue fluorescent*	375,355,335,321,275,251, 222, very low absorption	3140,2920,2850,1720,1595, 1500,1450,1380,1265,1190, 1125,1070,1035,1010,955, 870,850,835,810,780,740, 700 neat
40C	111	As 40A + R_F 0.50 aqua*	376,356,335,320,293,288, 262,252	3140,2950,2920,2860,1720, 1380,1300,1240,1190,1040, 1000,950,875,860,840,810, 770,730,710 CS_2
40D	25	R_F 0.50 aqua	357,335,320,305,287,252, 241,238,231	3140,2950,2920,2850,1720, 1280,1260,1220,1190,1180, 1150,1130,1030,1015,860, 840,820,810,770,750,740, 710 CS_2
40D*	6	R_F 0.40 blue	437,430,403,385,378,357, 340,335,318,288,275,268, 263,258,253,237,230	

Table 2 Cont'd

	Fraction	mg	Appearance on TLC	UV Absorption maxima, mμ	Infrared maxima, cm^{-1}
Heptane	40E	4	R_F 0.40 blue	437,431,403,385,380,357, 340,288,275,268,262,258	
	40F	6	R_F 0.40 blue	435,430,402,386,382,357, 318,288,275,268,258	
	40G	2	R_F 0.40 blue	436,431,402,385,381,360, 342,288,268,258	
	40H	3	R_F 0.40 blue	436,431,403,385,381,357, 344,318,288,251	
	40I	1	R_F 0.40 blue	437,431,402,384,380,362, 342,301,288	
	40J	2	R_F 0.40 blue	430,403,385,380,362	
	40K	1	R_F 0.40 blue green + 0.35 blue	430,403,385,345,315,288	
25% Ethylacetate in Heptane	40L	1	R_F 0.75 light blue, 0.50 light blue	no maxima	
	40M	6	R_F 0.75,0.50,0.35, light blue 0.3 greenish	no maxima	
	40N	33	As 40M + 0.25 blue, 0.20 pink	no maxima	3040,2950,2920,2850,1700, 1670,1510*,1370,1320,1280, 1230,1020,750,720,700 CS_2 *CCl_4

Table 2 Cont'd

	Fraction	mg	Appearance on TLC	UV Absorption maxima, mμ	Infrared maxima, cm^{-1}
	40 0	10	0.35 light blue, 0.30 green, 0.25 blue, 0.20 pink, 0.15 blue	no maxima	
25% Ethylacetate in heptane	40P	9	same as above	376, 250 ethanol	
	40R	11	Streak with no distinct spots	250, 225 ethanol	
	40S	2	0.35 light blue, 0.20 light blue, 0.15 blue, 0.10 blue	250 ethanol	
	40T	1	same as 40S	250, 230 ethanol	
	40U	4	0.10 blue	no maxima	
	40V	1	0.10 blue	no maxima	
EtAc	40W	15	0.05 light blue, 0.02 orange	no maxima	2950,2920,2850,1710,1650, 1370,1305,1250,1040,1020, 810, 770,750 CCl_4
MeOH EtAc	40X	3	Fluorescent start	no maxima	
	40Z	50	Fluorescent start	no maxima	3440,2920,2860,1585,1430, 1055,1020,935,660 KBr

*Positive with spray 2

reference purposes should this type of contamination be encountered again.

The fraction 44-9 contains a mixture of cresols (o-, m-, and p-), m-cresol is probably the main component.

Fractions 44-13, 14, 15, and 18 consist of unidentified phenolic compounds. Their IR spectra indicate the presence of carbonyl groups (1730 cm^{-1}) and quinoidal structures (1670 cm^{-1}).

Fractions 44-24 and 44-25 contain pentachlorophenol, unambiguously identified by the IR spectrum (Fig. 5).

Fractions 44-26, 27, and 28 contain aluminium acetate. The very characteristic IR spectrum is reproduced in Figure 6. The fraction 40Z, obtained by chromatography of the Extract A*, contains the same compound.

The main and at the same time the most toxic component of the acidic fraction is pentachlorophenol.

3. Basic fraction (Extract C)

This fraction yielded numerous overlapping fluorescent spots on thin-layer chromatograms. Column chromatography on aluminium oxide did not result in a better separation. Considering the small amount of this fraction present in the original mixture and the relatively lower toxicity, this fraction was not analyzed further.

Table 3. Fractionation of Extract B.

	Fraction	mg	TLC Mobility & Fluorescence	UV Absorption maxima, mμ	Infrared maxima cm^{-1}
	44-1	26	R_F 0.60 blue	386,380,357,352,333,320, 252	3060,2980,2950,2880, 1610,1510,1460,1390, 1210,1050,880,830,750, 715 neat
Heptane	-2	10	R_F 0.60 blue	435,428,385,357,335,319, 292,288,272,252	
	-3	4	R_F 0.60 blue	437,432,403,383,378,358, 338,335,318,288,276,268, 262,258,252,240,236,230	
	-4	2	R_F 0.45 light blue		
5% EtoAc	-5	4	R_F 0.45 light blue, 0.40 blue		
	-6	14	0.45 light green, 0.40 blue green, 0.30 blue green		
	-7	15	As 6 0.20 blue		3070,2960,2930,2880, 1725,1600,1580,1465, 1390,1280,1130,1075, 1045,970,750,710 neat contaminant
10% EtoAc	-8	14	As 6 0.18 orange		

combined

Table 3 Cont'd

	Fraction	mg	TLC Mobility & Fluorescence	UV Absorption maxima, mμ	Infrared maxima cm^{-1}
20%	44-9	113	0.45 dark*, as 44-8		3375,3050,2980,2950, 2880,1710,1610,1525, 1470,1390,1330,1280, 1250,1170,1130,1050, 960,955,850,830,790, 770,710 neat
	-10	1	0.45 dark*, fluorescent streak		
50%	-11	4	As 10		
	-12	7	As 10		
	-13	12	As 10		3350,2980,2950,2880, 1670,1275,1180,1130, 1050,830,770 CS_2
	-14	13	As 10		3350,2980,2950,2880, 1730,1670,1300,1275, 1160,1130,1045,1025, 830,790,760,740 CS_2
100% EtoAc	-15	8	As 10, blue-violet 0.05		2980,2950,2880,1670, 1050,1030,790,750 CS_2
	-16	9	As 15		
	-17	15	0.45 dark*, 0.30 blue		

Table 3 Cont'd

	Fraction	mg	TLC Mobility & Fluorescence	UV Absorption maxima, mμ	Infrared maxima cm^{-1}
	44-18	16	0.45* dark		3530,2950,2860,1420, 1400,1380,1320,1300, 1230,1220,1180,1130, 975,880,855,835,770, 730,710 CS_2; 1530, 1450 CCl_4
$CHCl_3$ gradient	-19	27	Neither fluorescent nor phenolic compounds		3080,2980,2960,2880, 1730,1610,1590,1560, 1470,1425,1390,1290, 1140,1085,1055,975, 760,720 neat contaminant
MeOH	-20	3	0.30 blue, start red		
	-21	23	0.30 blue, 0.25 dark*, 0.20 dark*		
	-22	48	0.30 blue		
	-23	35	0.50-0.40 probably 3 spots, dark* 0.30 blue		3430,3020,2960,1590, 1430,1290,1220,1055, 1025,980,935,780,730, 660 KBr

Table 3 Cont'd

Fraction	mg	TLC Mobility & Fluorescence	UV Absorption maxima, mμ	Infrared maxima, cm^{-1}
44-24	343	0.50-0.40 probably dark*		3530,1420,1385,1290, 1205,995,780,715, 660 CS_2
-25	315	0.50-0.40 dark*		3530,1420,1385,1290, 1205,995,780,715 660 CS_2
-26	170	As 25		3450,3020,2960,1590, 1430,1055,1030,935, 775,730,660 KBr
-27	139			3450,3020,2960,1580, 1430,1050,1020,930, 660 KBr
-28	57			3480,3020,2960,1730, 1590,1440 KBr

*Positive with spray 2

4. Chloroform non-extractable compounds (Extracts D, E)

IR spectrum of the Extract D (Fig. 7) indicates the presence of the following functional groups:

hydroxyl (3400 cm^{-1}), carbonyl (1720 cm^{-1}), carboxyl (2400-2800, 1390, 1300 cm^{-1}), aromatic rings (1605, 1525 cm^{-1}), phenol groups (1250 cm^{-1}). The Extract E had a similar somewhat less resolved IR spectrum.

Thin-layer chromatography failed to separate these extracts into individual components. The Extract D was fractionated by extraction to yield fractions described in Table 4. IR spectrum of the neutral fraction B1B is identical with IR spectra of fractions 44-5, 6, 7, and 19, indicating the contaminant, di-(2-ethylhexyl)-phthalate. IR spectra of the other fractions contain bands characteristic for the following functional groups: carboxyl, quinone, phenol, and hydroxyl (1710-1720, 1660, 1420-1390, 1280, and 1040 cm^{-1}); the compounds have benzene rings (1610, 1520 cm^{-1}). UV spectra of fractions B2B, B2A, and B1C indicate phenol groups (275 $m\mu$) and chlorophenol moieties (310-320 $m\mu$). However, neither of the fractions contains pentachlorophenol. The fractions B1A, B2B, B2A, and B1C consist of structurally very similar classes of compounds; a slight prevalence of one type of functional groups determines the over-all behaviour of the class, such as solubility and acidity.

Table 4. Fractions of the Extract D

Fraction	Amount, mg	UV maxima, mμ	IR maxima, cm^{-1}
Ether-insoluble (B1A)	80		3450,2960,2900,1730, 1610,1520,1470,1390, 1280,1230,1150,1050
Ether and water-soluble (B2B)	350	313,280,228	3250,2950,2900,1720, 1660,1610,1520,1460, 1420,1390,1280,1230, 1040, 850, 825, 775
Acidic (B2A)	356	310,275,225	3430,2960,2890,1710, 1610,1520,1460,1420- 1390,1280,1040, 770, 730
Phenolic (B1C)	323	320,275,225	3400,2950,2880,1710, 1600,1520,1460,1385, 1280,1230,1160,1130, 1040, 960, 850, 830, 790, 770, 700
Neutral (B1B)	399		2960,2880,1730,1605, 1590,1460,1390,1290, 1130,1080,1050, 970, 750, 710

5. Bioassay of organic compounds isolated from the brook water

Toxicity of the fractions, described in Table 1, to small salmon was determined in static tests. The phenols-containing fractions (Extracts B, D+E) were very toxic. The neutral fraction (Extract A*) was less toxic; on the other hand, the high concentration of this fraction in the brook water compensates the lower toxicity. Because of the small amount of Extract C only small-scale tests with one fish per concentration were carried out; this fraction appears to be much less toxic. The values of LT50 vs concentration are plotted in Figure 8.

The avoidance reaction of trout to Extracts A*, B, and D+E was also determined. Trout avoided these extracts in concentrations non-detectable by chemical methods without pre-concentration. The results of the avoidance tests are shown in Figure 9; the results of the bioassay are summarized in Table 5.

Table 5. Bioassay of compounds isolated from brook water

Fraction	Concentration in brook water, mg/l	Avoidance (trout) at µg/l	Lethal threshold (salmon), mg/l
Extract A*	960	4.7	3
Extract B	160	0.47	0.5
Extract C	20	not tested	10
Extracts D+E	30	0.028	0.6

The fact that fish avoid extremely low concentrations of these compounds indicates that organic compounds, discharged

from the brook might interfere with normal migration patterns of fish in the estuary.

6. Discharge of organic compounds from the brook into the estuary.

The swampy area, receiving water from the brook, is more or less flooded at high tide. In view of the strong avoidance reaction of fish to the organic compounds in the brook water, an attempt has been made to determine the extent of leaching of these compounds into the estuary. UV spectra of water samples taken in the estuary were used for this purpose. Four samples of water, leaving the swamp, were taken at declining high tide. Their UV spectra are reproduced in Figure 10, (curves 1-4; taken at times indicated in Fig. 10). UV spectra of several samples from stations in the estuary (for locations see Fig. 11) are presented for comparison (SA = St. Andrews salt water).

The use and limitations of UV spectra in the detection of organic pollution will be discussed elsewhere. As a rule of thumb, high absorbance (E) indicates high concentration of organic compounds. As it can be seen from Figure 10, there is a considerable leaching of organic compounds from the swamp into the estuary. Combined samples 1-4 yielded 24.4 mg/l of the total chloroform extract (compare to the concentrations of Extracts A*+B+C in the brook water, Table 5).

7. Analysis and bioassay of mud in the contaminated areas

A mud sample was taken in October 1968 at station 4 (Fig. 1). After the identification of pentachlorophenol on column chromatography of the Extract B, the isolation of crystalline pentachlorophenol from the mud was simple and provided an additional proof of the presence and structure of this compound.

On the request of the New Brunswick Water Authority, samples of soil from the contaminated areas 1-6 were taken on June 17, 1969. The samples were acidified and extracted with chloroform to yield extracts, comparable with the combined Extract A* and Extract B (Table 1). The near-infrared spectra (in carbon tetrachloride) of the extracts thus obtained were almost identical and, therefore, only samples 1 and 5 were fractionated as described for the Extract C; pentachlorophenol was determined spectrophotometrically (1) in the sodium hydroxide extract.

IR spectra of the neutral fractions are identical and only the spectrum of the neutral fraction 5 is shown in Figure 12. Similarly, only the spectrum of the acidic fraction 5, is shown in Figure 13. The spectrum indicates the presence of carboxyls (1710, 1390, 1200-1300 cm^{-1}) and benzene rings (1615, 1520 cm^{-1}). Infrared spectra of the phenolic fractions 1 and 5 are presented in Figure 14. Carboxyls are present in addition to the phenol groups. The spectra also indicate the presence of pentachlorophenol (1390, 1290, 780, and 730 cm^{-1}).

Precipitate appeared in the neutral fraction 1 on

standing. It was separated from the mother liquor by centrifugation and washing with n-heptane. The material was not identified. Its IR spectrum is reproduced in Figure 15.

The toxicity of aqueous suspensions of the soil samples to small salmon was determined; the results of the bioassay and analysis of the samples are summarized in Table 6.

A mixture of neutral compounds corresponding in composition to the Extract A* is responsible for the toxicity of the soil suspensions. The lethal threshold of the Extract A* is 3.0 mg/l. It can be seen from Table 6 that the soil suspensions are not toxic when diluted below this threshold.

8. Conclusions

As the plant was not in operation during the sampling of the brook water, the effluent represents compounds leached from the contaminated areas between the plant and the river. The main portion of organic matter in the effluent consists of mineral oil and aromatic hydrocarbons with lethal and avoidance thresholds of 3 and 0.005 mg/l, respectively. The phenolic fraction of the effluent consists mainly of pentachlorophenol. Lethal and avoidance thresholds of this fraction are 0.5 and 0.0005 mg/l, respectively. Experimental evidence has shown that the compounds reach the Miramichi River and because of their very low avoidance thresholds may seriously interfere with aquatic life in the estuary. The compounds may be responsible for the oily taste of fish reported quite frequently in 1968. The presence of carcinogenic polynuclear hydrocarbons may create a health hazard in consuming fish from the estuary.

Table 6. Analysis and bioassay of soil from the contaminated areas

Station	Chloroform extract (mg/g soil)	Acidic fraction (mg/g soil)	Phenolic fraction (mg/g soil)	Pentachloro-phenol (mg/g soil)	LT50, hours, for suspension 0.1%	0.01%	0.001%
1	139	0.666	1.63	0.269	All dead in 1 h	5.1	No mort. 120 h
2	110				0.8	11	No mort. 120 h
3	26	not fractionated			11	No mort. 143 h	
4	118				1.3	2.5	No mort. 116 h
5	68	0.120	0.302	0.028	3.2	30% mort. 117 h	
6	1				No mort. in 167 h		

The plant took the first step for pollution abatement and installed a treatment facility so that, hopefully, no new effluent is being discharged. However, several areas between the plant and the river, drained by the brook, are heavily contaminated by the toxic compounds and should be cleaned up.

EXPERIMENTAL

Beckman DK-2A and Perkin-Elmer 700 Spectrophotometers were used to scan UV and IR spectra, using heptane or absolute ethanol, and carbon tetrachloride, carbon disulfide, potassium bromide or neat samples, respectively. Silica gel (0.25 mm) and heptane-ethylacetate 1:1 were used for thin-layer chromatography. "Black Ray UVL 22" and "Mineralight UVS11" were the light sources for the detection of fluorescent compounds on thin-layer plates. After the examination of the plates under the UV light, the plates were sprayed by one of the following reagents, when the presence of non-fluorescent compounds was suspected:

1. Sulfuric acid, 40%, followed by charring.
2. A 1:1 mixture of 0.1M solutions of ferric chloride and potassium ferricyanide.
3. Antimony pentachloride, 20% solution in carbon tetrachloride.

A rotatory evaporator was used to evaporate solvents in vacuum at 36°C.

Isolation of organic compounds from the brook water

Water sample (68 l) collected on October 3, 1968 at station 4 was made alkaline (pH 9-10) and extracted with chloroform (Extract A), then acidified to pH 1.5 and extracted with chloroform (Extract B). The volume ratio of sample to chloroform was 10:1. Extract A was treated with aqueous 0.1N hydrochloric acid, yielding Extract A* and an aqueous phase; the aqueous phase was adjusted to pH 9-10 and extracted with chloroform (Extract C). The chloroform solutions were dried over anhydrous sodium sulfate and the solvent was evaporated to yield Extracts A*, B, and C in amounts of 65.78, 10.62, and 1.33 g, respectively. The aqueous phase after chloroform extractions, still containing organic compounds was partly used in bioassays, partly (46.5 l) treated with activated carbon. Activated carbon was filtered off, air dried, and extracted with chloroform and ethanol to yield the Extracts D (1.52 g) and E (0.55 g).

Extract A* (1 g) was applied to a column (29 x 2.5 cm) of aluminium oxide and chromatographed as described in Table 2. Fractions (5 ml) were collected by an automatic fraction collector. The separation was monitored by thin-layer chromatography. Extract B (1 g) was chromatographed analogously to Extract A* (Table 3).

Fractionation of the Extract D

The residue after the evaporation of chloroform from Extract D (1.52 g) was extracted with ether (3 x 20 ml)

yielding an ether-insoluble residue (B1A, 80 mg) and the ether extract. The latter was extracted with 1N hydrochloric acid, 0.6M sodium bicarbonate, and 1N sodium hydroxide, all in 10% sodium chloride. The compounds in the hydrochloric acid extract were recovered by evaporation to dryness and extraction with chloroform, ether and ethanol yielding the fraction B2B, 350 mg. The compounds extracted into the other aqueous solutions were recovered by acidification and extraction with ether to yield the acidic fraction B2A, 356 mg, and the phenolic fraction B1C, 323 mg, respectively. The neutral fraction B1B, 399 mg, was isolated by evaporation of ether from the original ether extract left after the treatment described above.

Isolation of pentachlorophenol from tar

A tar sample was collected on October 3, 1968, at station 4 by scraping the soil surface. Chloroform was added to the sample and the aqueous layer was separated. The chloroform solution was extracted with 0.1N hydrochloric acid (3 x 1.5 l), the extract was adjusted to pH 10 and extracted with chloroform to yield the Extract F (1.3 g). The Extracts G (15.8 g) and H (2.5 g) were obtained analogously by extracting the chloroform solution with 0.1N sodium carbonate and 0.1N sodium hydroxide, respectively; the aqueous solutions were acidified and extracted with chloroform. Extract G was washed with heptane to remove the colored impurities as much as possible. The residue was dissolved in methanol and filtered through a short column of aluminium oxide. Pentachlorophenol crystallised readily in the filtrate yielding 650 mg of pure

pentachlorophenol after 3 crystallisations. Additional 2.15 g were crystallised once. Mother liquor (5 g) still contained large quantities of pentachlorophenol.

Bioassay

Preliminary toxicity tests were made using one fish per test in a glass flask equipped with an air supply. This was followed by a series of tests in rectangular fibreglas tanks (20 l). Temperature (17°C) was controlled by a recirculating water bath. All tests were static and limited to 120 hours. Small Atlantic salmon, average weight and length 4.2 g and 7.3 cm, were used in the toxicity tests. Avoidance tests were carried out at 17°C in the apparatus (2) using small trout (average weight and length 9.6 g and 9.7 cm). Fresh water, total hardness 13-16 mg/l calcium carbonate, pH 6.9-7.1, was used in the tests.

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(2). Sprague, J. B. 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Wat. Pollut. Control Fed. 36, 990-1004.

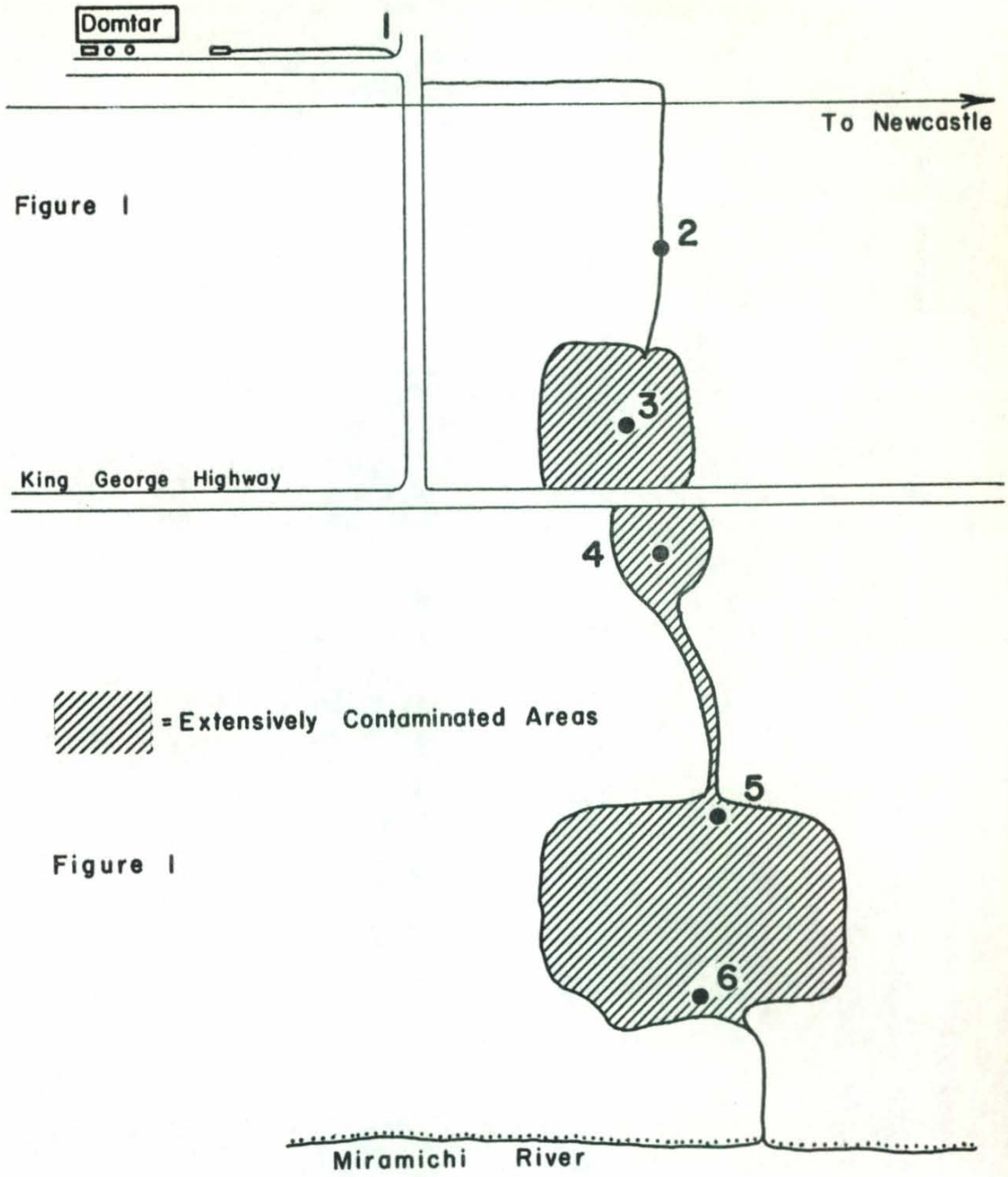


Figure 1

Figure 1

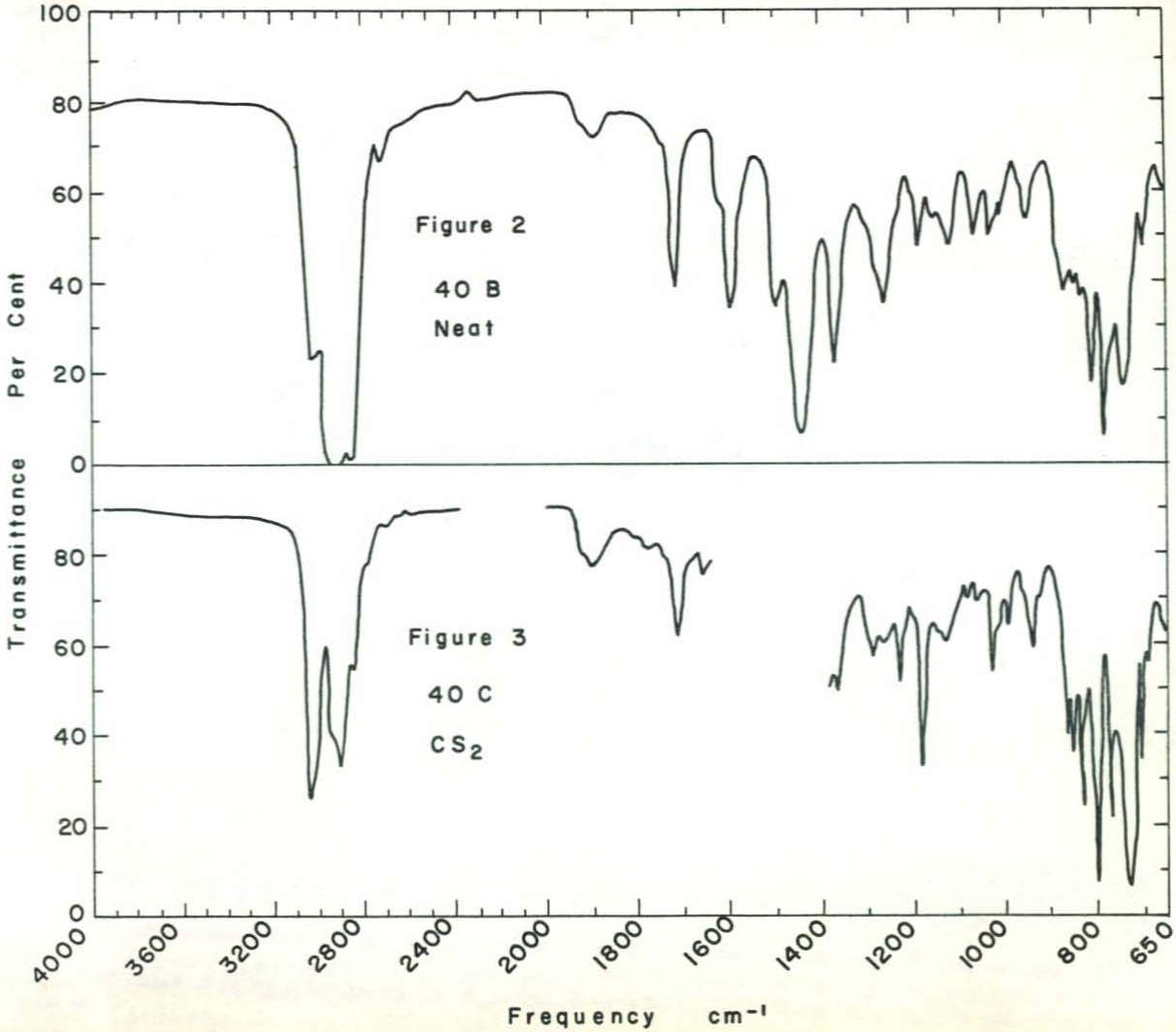


Figure 2
40 B
Neat
Figure 3
40 C
CS_2
Transmittance Per Cent
100
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650
Frequency cm⁻¹

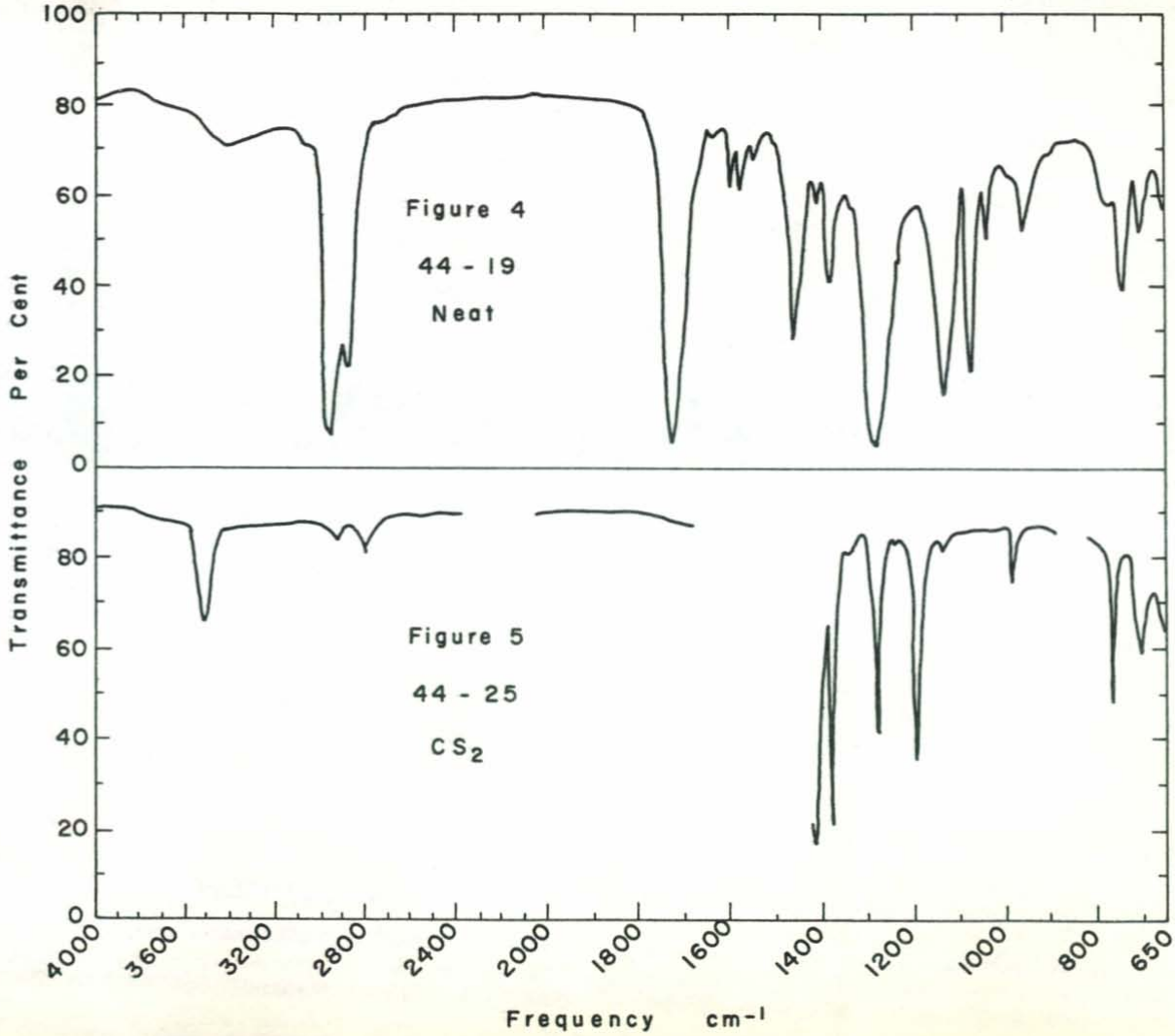


Figure 4
44 - 19
Neat
Figure 5
44 - 25
CS_2
Transmittance Per Cent
100
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650
Frequency cm-1

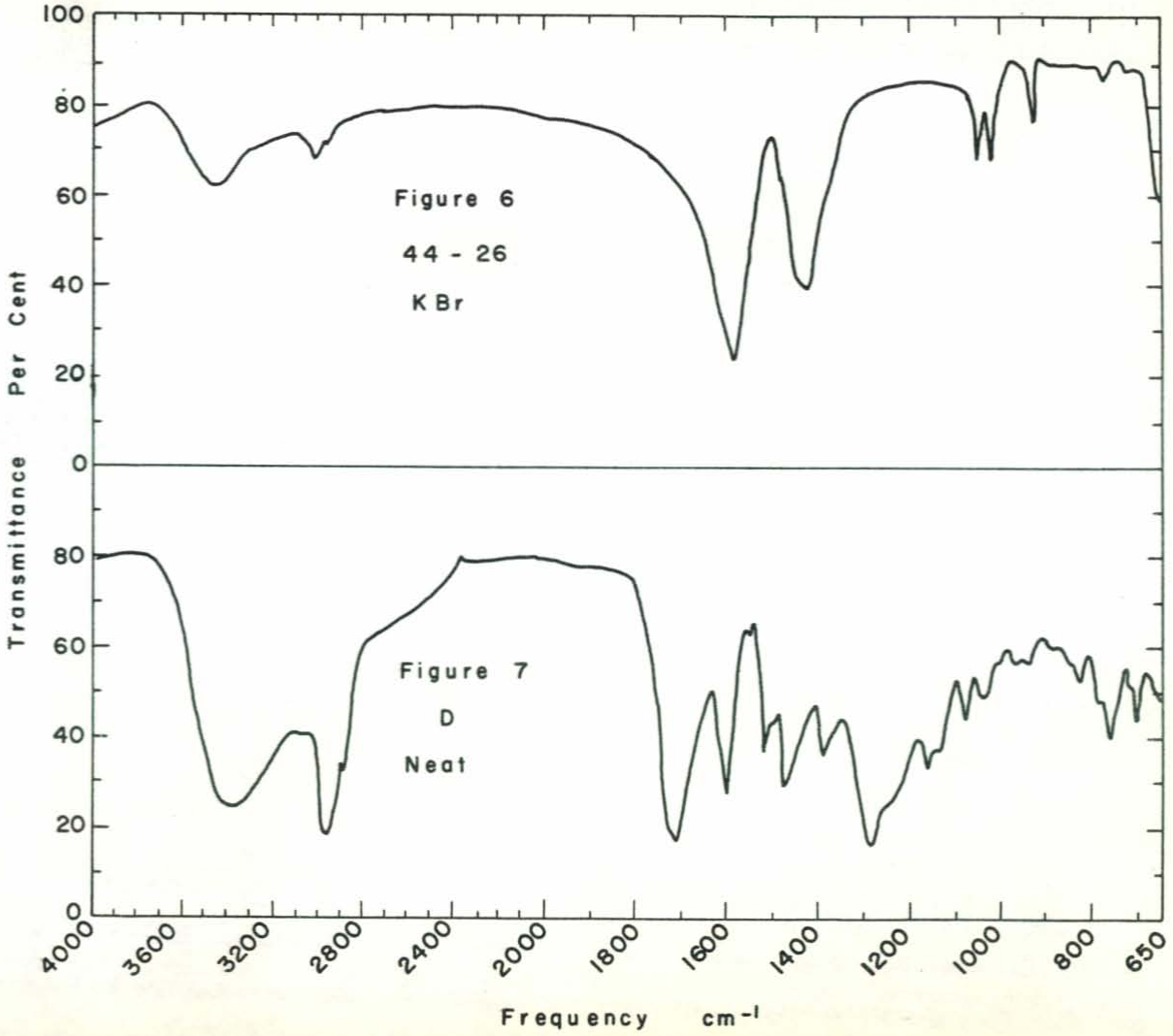


Figure 6
44 - 26
KBr
Figure 7
D
Neat
Transmittance Per Cent
Frequency cm⁻¹
100
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650

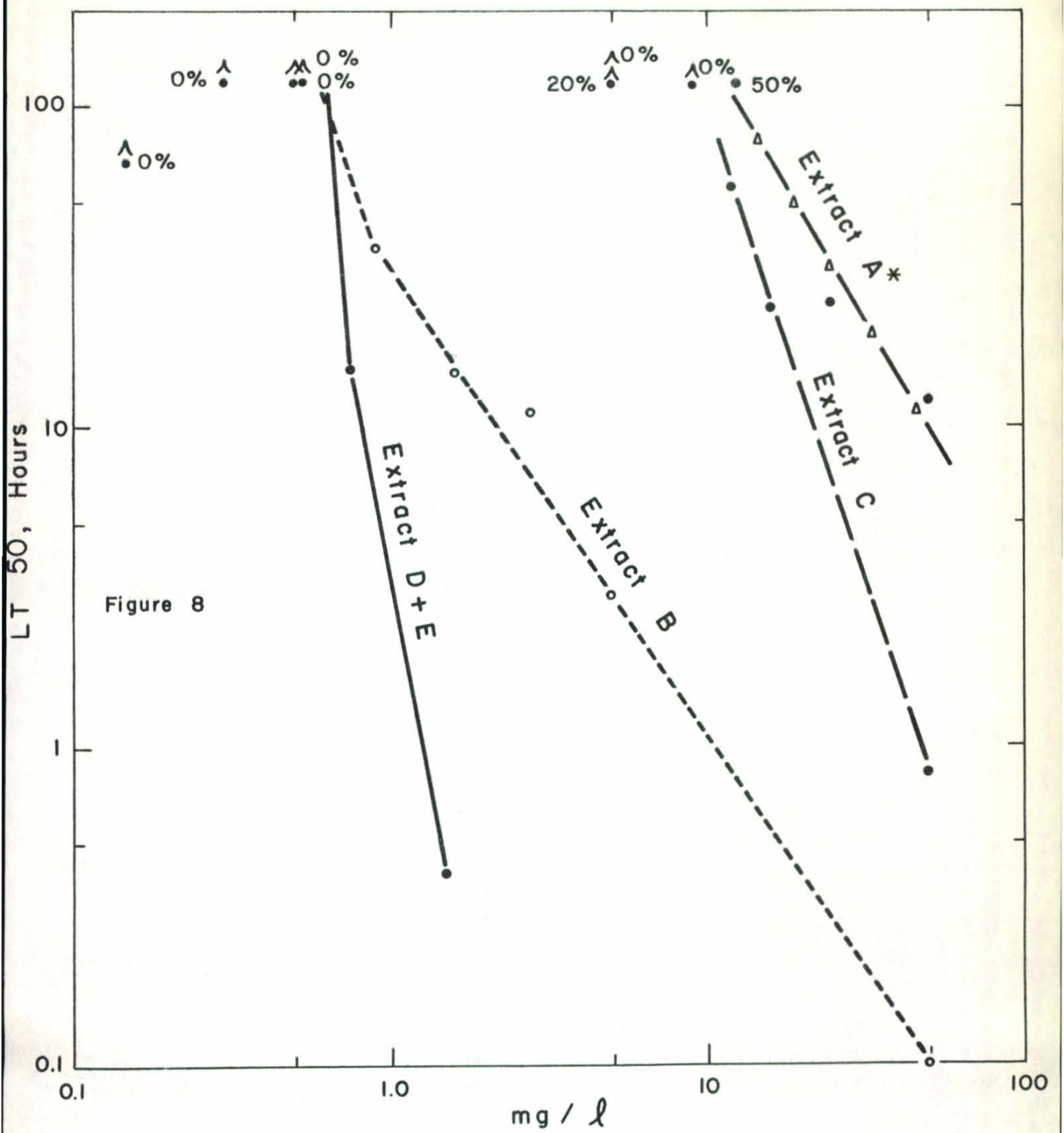

0%
0%
0%
0%
20%
10%
10%
50%
Extract A*
Extract C
Extract B
Extract D+E
LT 50, Hours
100
10
1
0.1
0.1
1.0
10
100
mg / ℓ

Figure 8

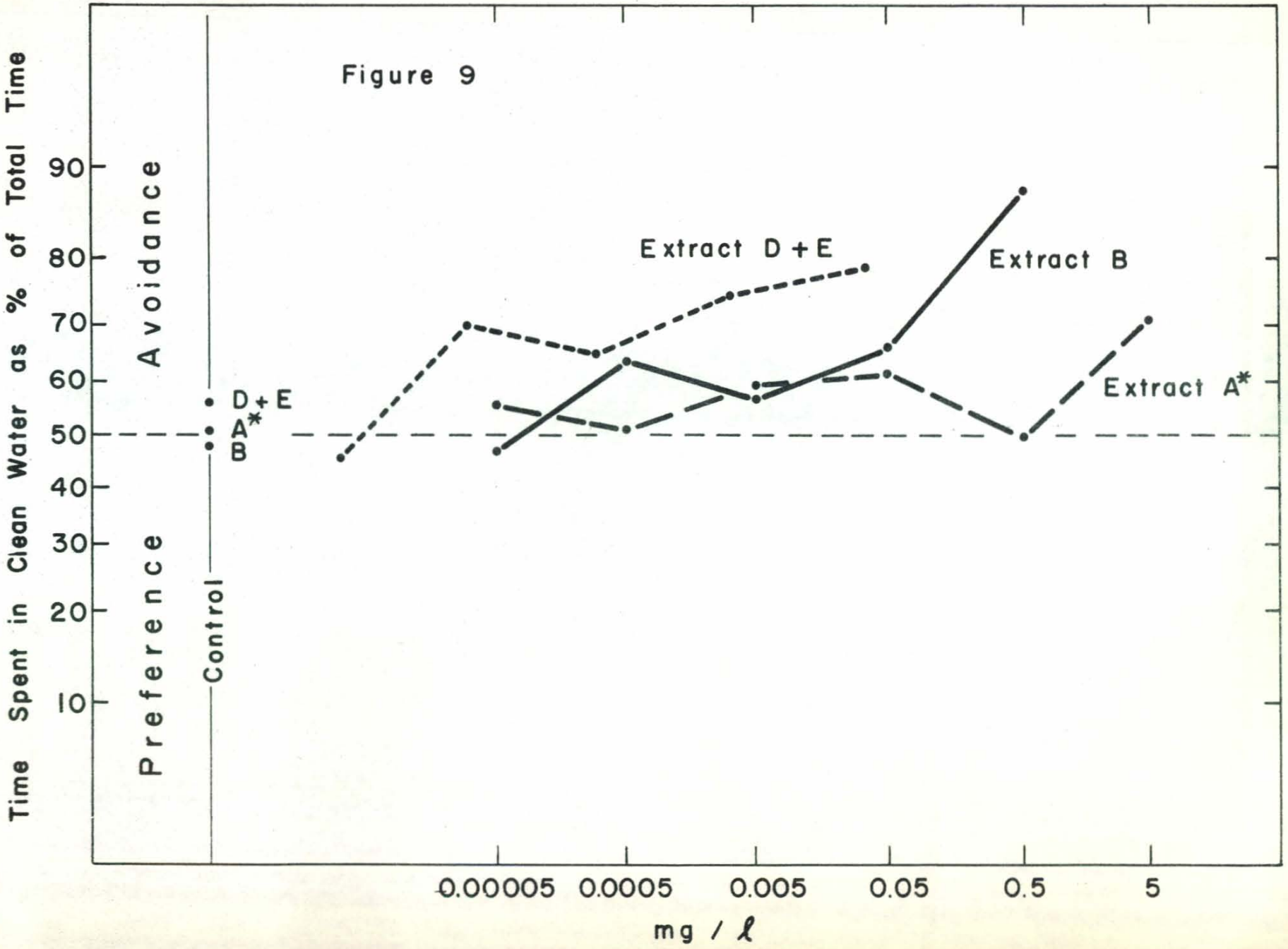
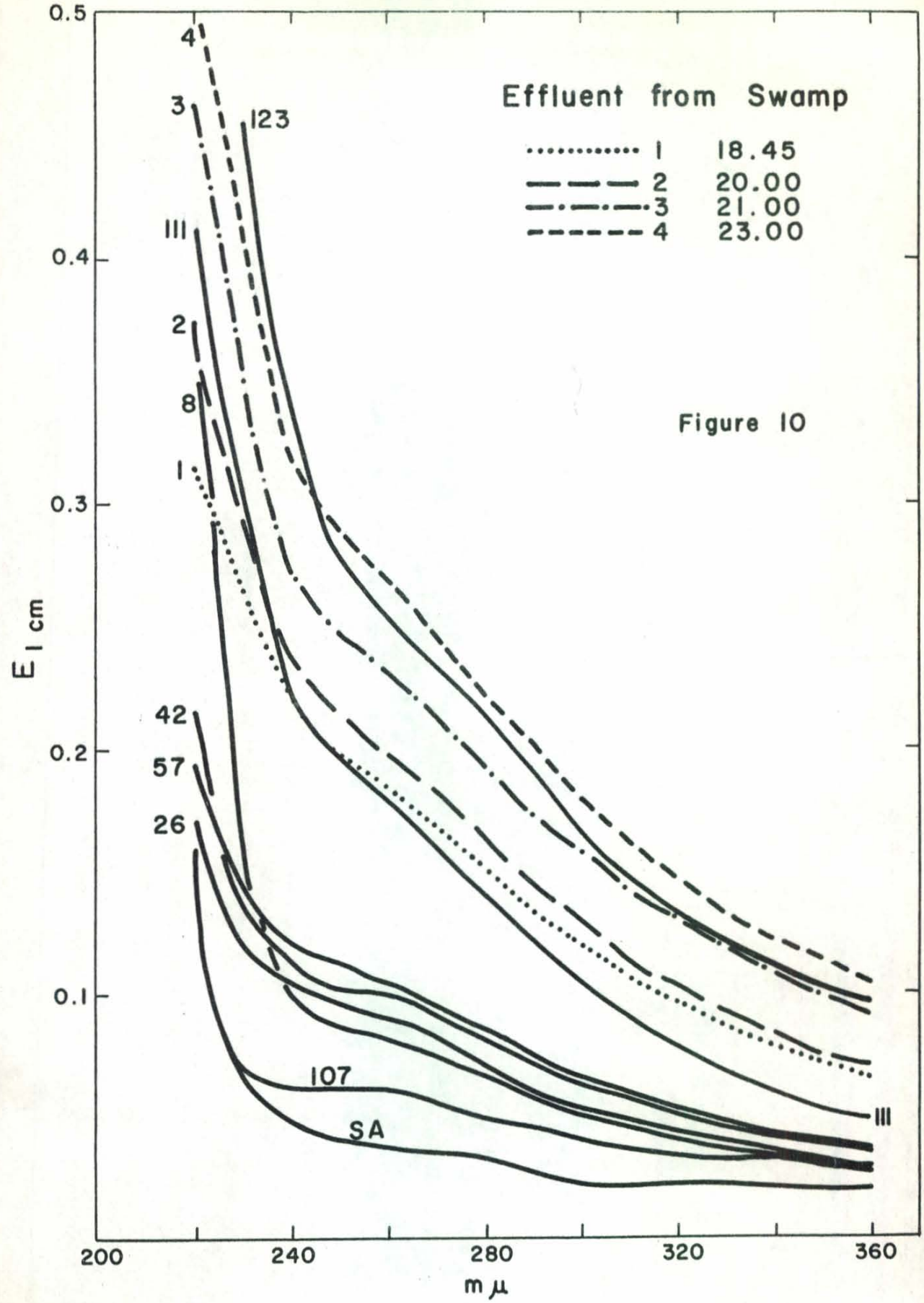
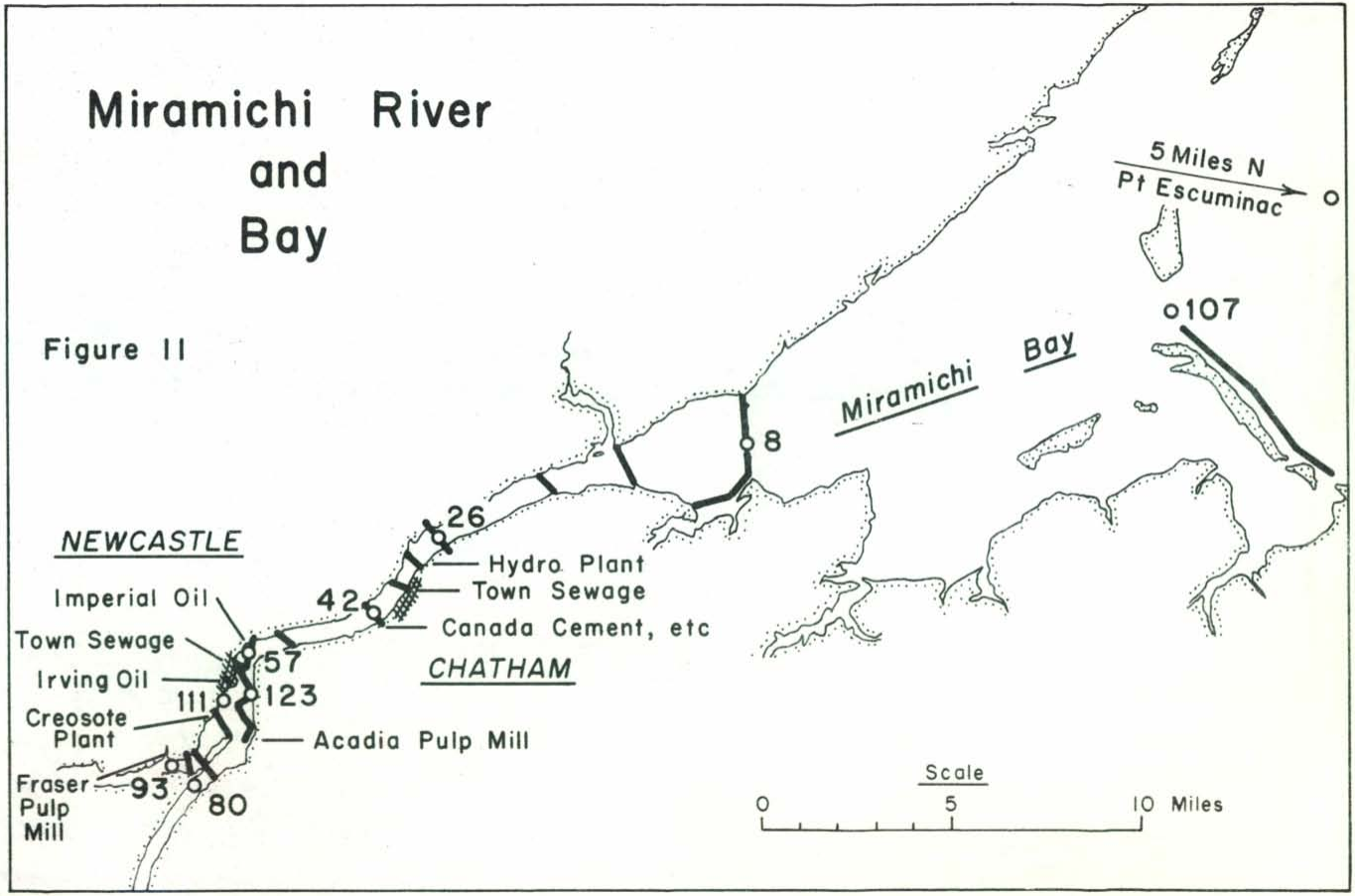


Figure 9
Time Spent in Clean Water as % of Total Time
Avoidance
Preference
90
80
70
60
50
40
30
20
10
Control
D + E
A*
B
Extract D + E
Extract B
Extract A*
0.00005
0.0005
0.005
0.05
0.5
5
mg / ℓ


Effluent from Swamp
1 18.45
2 20.00
3 21.00
4 23.00
Figure 10
0.5
0.4
0.3
0.2
0.1
E 1 cm
200
240
280
320
360
mμ
4
3
123
III
2
8
1
42
57
26
107
SA
III


Miramichi River and Bay
Figure 11
5 Miles N
Pt Escuminac
107
Miramichi Bay
8
26
NEWCASTLE
Hydro Plant
Town Sewage
Canada Cement, etc
Imperial Oil
42
Town Sewage
57
CHATHAM
Irving Oil
111
123
Creosote Plant
Acadia Pulp Mill
Fraser Pulp Mill
93
80
Scale
0
5
10 Miles

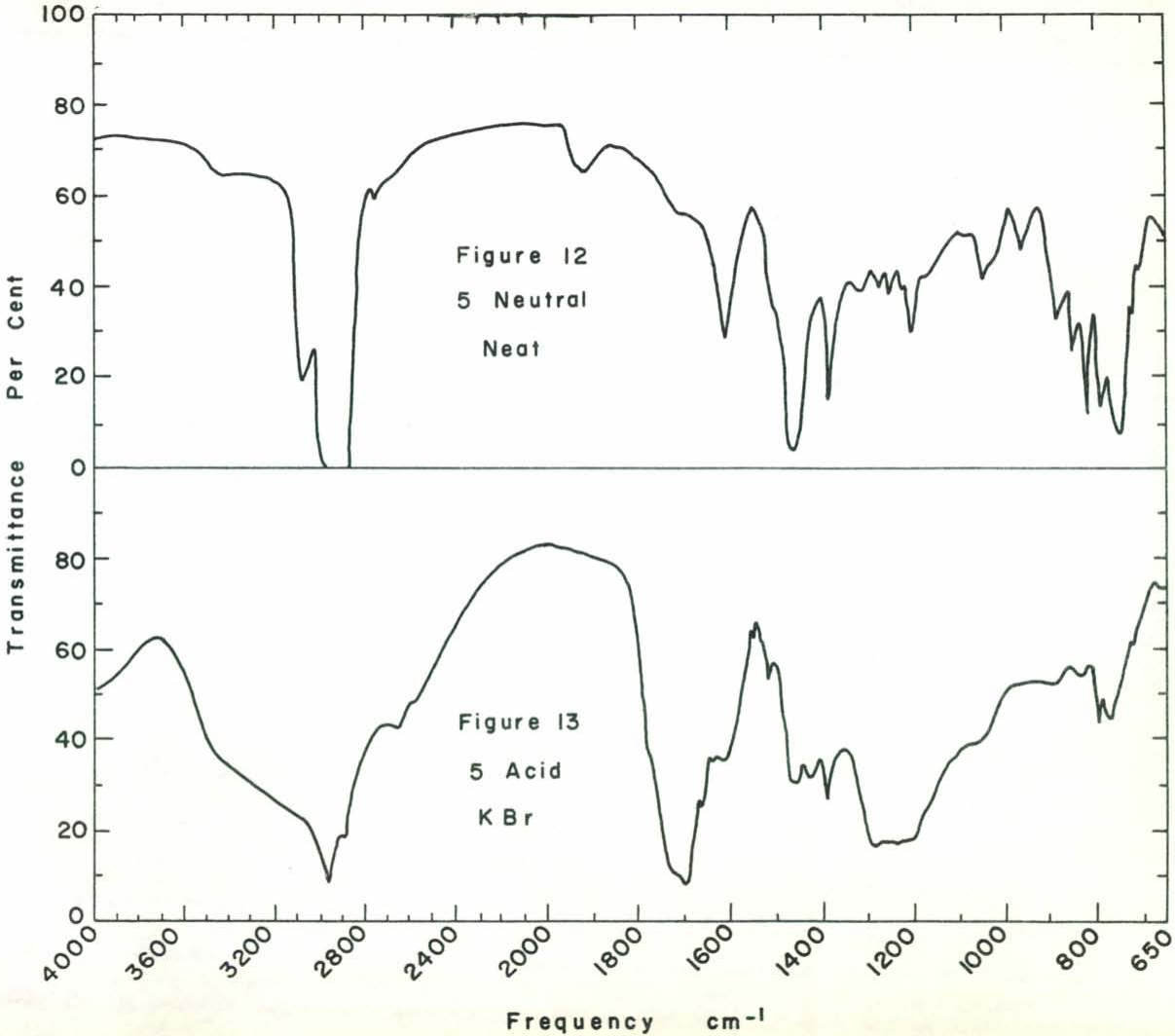
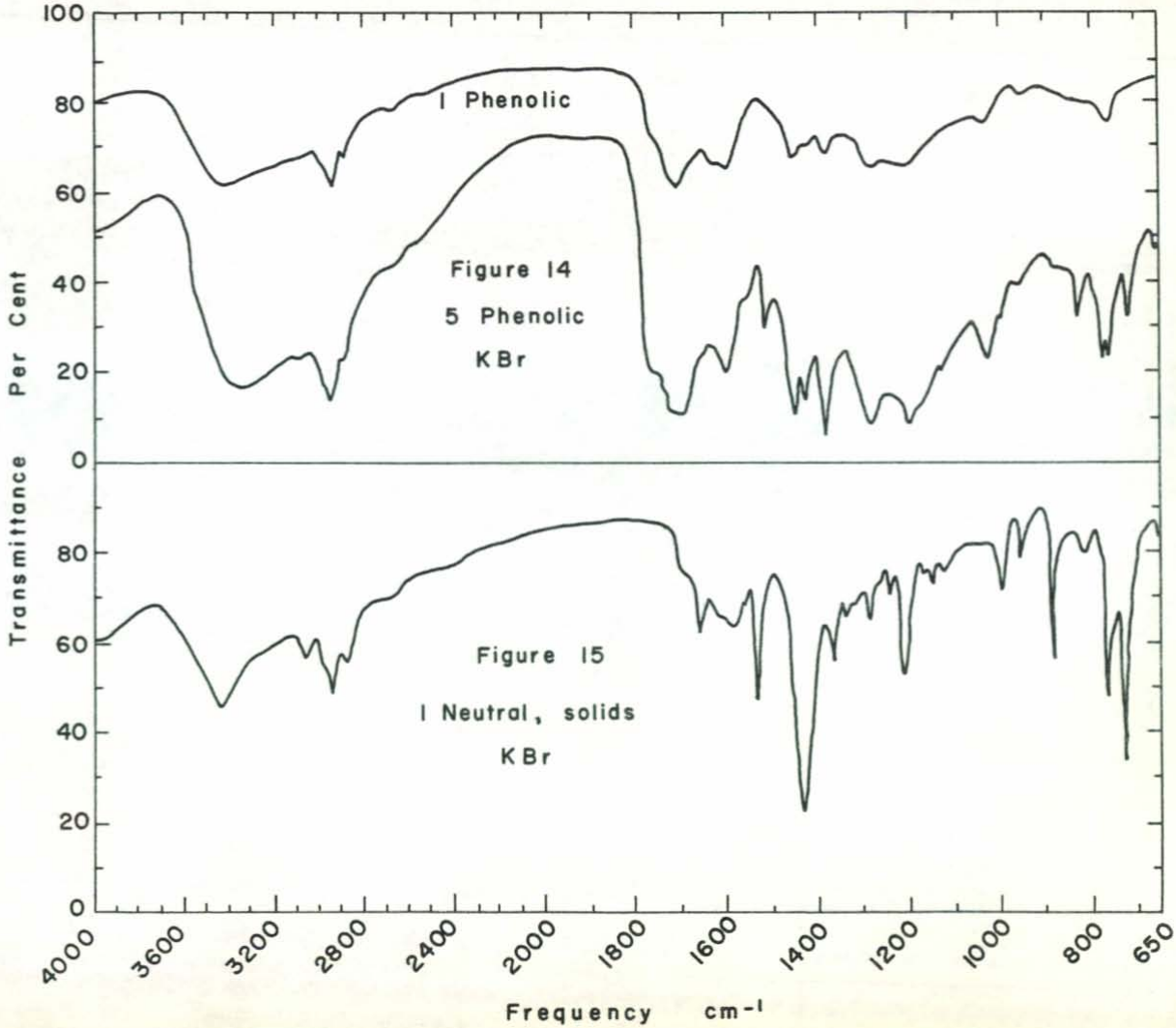


Figure 12
5 Neutral
Neat
Figure 13
5 Acid
KBr
Transmittance Per Cent
Frequency cm-1
100
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650


100
80
60
40
20
0
Transmittance Per Cent
1 Phenolic
Figure 14
5 Phenolic
KBr
Figure 15
1 Neutral, solids
KBr
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650
Frequency cm-1