The Metabolism of Selected Organic Compounds by Microorganisms in the Athabasca River

WS 2.3.1

August 1981

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

Sir:

Enclosed is the report "The Metabolism of Selected Organic Compounds by Microorganisms in the Athabasca River."

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

THE METABOLISM OF SELECTED ORGANIC COMPOUNDS BY MICROORGANISMS IN THE ATHABASCA RIVER

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ALBERTA OIL SANDS

ENVIRONMENTAL RESEARCH PROGRAM

Project WS 2.3.1

August 1981

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ABSTRACT

In this study, the ability of microorganisms to degrade selected organic substrates in samples of Athabasca River water and water-sediment has been determined.

Analysis of laboratory-incubated samples using gas chromatography/mass spectrometry has shown that trace amounts (100 µg/L) of m-cresol and camphor can be quantified and a "50% depletion time" determined. Sites both above the oil sands region and downstream from the area of mining activity had a noticeably high level of activity regarding the degradation of these compounds. In addition, the incubation of samples taken along a transect of the river just downstream from the oil sands plants showed higher rates of microbial degradation on the west bank where effluents and drainage would likely be concentrated. Other substrates, 2,6-xylenol and methyl salicylate, were not suitable for degradation studies due to depletion caused by non-biological processes.

Analysis of ^{14}C-labelled substrates indicated that "natural" compounds (amino acids and starch) were degraded more quickly than hydrocarbons and that significant degradation of hydrocarbons occurred only after nutrient supplementation.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

The fate of specific organic compounds in rivers has been largely neglected in fresh water research (Hobbie 1971; Hynes 1970). Microbial metabolism and the disappearance of carbon compounds due to chemical or physical processes are of fundamental importance in understanding the environmental consequences of natural or industrial inputs of organic materials.

In the Athabasca Oil Sands area of northeastern Alberta (Figure 1a), the Athabasca River receives inputs of organic materials from forest drainage and natural oil deposits, and more recently from industrial mining activity. Oil sands mining and extraction may contribute organic compounds from several sources, such as refinery wastes, coke storage runoff, etc., and may also contribute natural compounds from the drainage of surface and groundwaters (Strosher and Peake 1978).

The disappearance of energy-rich substrates added to a natural environmental system will depend on the presence of biological species (in this case microorganisms) with the genetic capability of converting such materials to energy and cell carbon. Suitable conditions of pH, temperature, nutrient status, and other factors are required for the growth of such microorganisms before significant rates of disappearance will be attained. Maximal depletion rates would only be observed at substrate concentrations which would saturate uptake systems (microbial enzymes). It is doubtful if such concentrations would be reached or maintained for any significant lengths of time under normal environmental conditions. Boethling and Alexander (1979) concluded that biodegradation studies involving chemical concentrations greater than those found under natural conditions may not correctly assess the rates of biodegradation in natural ecosystems. Even when large amounts of substrates are present, it is doubtful if maximal degradation rates will be observed since other rate-limiting parameters (e.g., nitrogen concentrations) will intercede. In northern environments in particular, such activities are

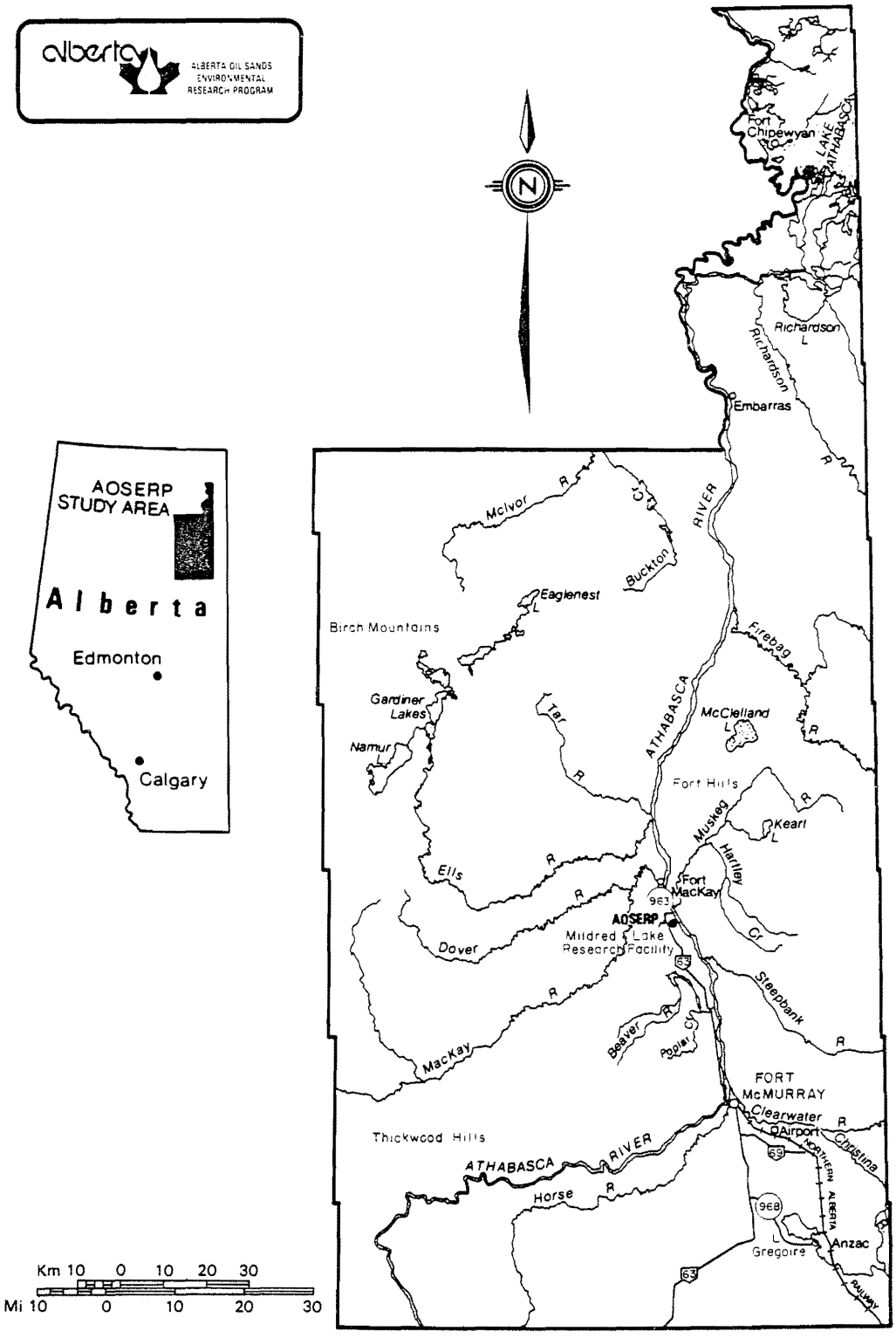


Figure 1a. Map of the study area.

usually limited by the amount of available nitrogen and/or phosphorus (Westlake et al. 1978) and by the prevailing temperature (Gibbs et al. 1975; Westlake and Cook 1980).

Microbial degradation is the principal method of degradation for most organic compounds. Moreover, the subsequent production of bacterial carbon may have greater significance for river ecosystems than primary production processes (Anderson and Dukolil 1977; Sorokin 1965). It is also known (Hrudey 1975; Lake and Rogers 1979; Giles et al. 1979) that certain drainage waters or wastewaters are toxic to aquatic organisms. For these reasons, it is important to understand the processes of biodegradation existing in the Athabasca River.

At the present time, little is known about the types of organic compounds (substrates) released in this river as a result of mining activity, or their possible depletion by aquatic bacteria. Strosher and Peake (1976, 1978) have identified certain groups of organics and tentatively identified some individual aromatic hydrocarbons. Nix et al. (1979) have studied the rate of bacterial uptake of a "universal" substrate (^{14}C-glutamic acid) but not compounds more representative of effluent streams. The terms of reference for this study included the investigation of the microbial depletion of selected organic compounds, but excluded the investigation and utilization of compounds contained in oil sands effluents or in the Athabasca River.

The project was divided into two areas of investigation: an analysis of the microbial depletion of specific organic substrates using gas chromatography/mass spectrometry (GC/MS) and an analysis of the mineralization of radiolabelled ^{14}C-substrates using scintillation counting techniques. Both processes were studied using laboratory incubations of Athabasca River water and water-sediment.

The use of laboratory-incubated culture flasks to determine the extent of in situ degradation of organic molecules has several inherent disadvantages (Nix et al. 1979). This study attempted to minimize some of these problems by inoculating with minimal concentrations (100 µg/L) of exogenous substrates and also by analyzing for natural compounds.

The low solubilities of many "natural" compounds such as beta-pinene, and the subsequent difficulty in their detection, limited the selection of organic compounds for experiments using GC/MS. Compounds chosen were m-cresol, camphor, methyl salicylate, and 2,6-xylenol. These compounds were considered to be monomers representing much more complex polymers which may have both natural and industrial origins. It would not be valid to extrapolate from their rates of depletion to the in situ rates for unknown and possibly complex molecules (Crawford and Crawford 1980). However, a knowledge of the extent of biodegradation of these selected organic compounds would provide a basis for comparing the microbially mediated processes of substrate depletion at different sites and at different periods of the year, and the methodology could be refined and utilized in future studies involving compounds identified as components of the Athabasca River. With respect to the experiments using ^{14}C-substrates, labelled compounds were available that are representative of drainage from plant and/or animal origins -- starch and amino acids, respectively; or representative of materials from an oil extraction and refining operation -- anthracene and hexadecane.

The principal objective of this study was to determine the extent of microbial utilization at various sites in the Athabasca River of organic compounds representative of organic materials that may be present in both natural and industrial (oil sands mining) drainage. Secondary objectives included in the original proposal were:

1. To determine the significance of physical adsorption and non-biological utilization of these organic substrates;
2. To assess the presence of microbial populations capable of utilizing these substrates as well as other important physiological groups of bacteria; and
3. To relate the results of this study to the degradative capacity of the Athabasca River in terms of its ability to utilize additional organic inputs resulting from oil sands developments.

Objective 2 was discarded early in this project due to the greater than anticipated need for time and resources to undertake preliminary GC/MS work.

2. METHODS

2.1 SAMPLING SITES AND PROCEDURES

Sample sites were chosen to provide information about bacterial activity in the Athabasca River within the Athabasca Oil Sands region (Sites 2 & 3) and both upstream (Site 1) and downstream (Site 4) from that region (Figure 1b). In addition, just downstream from the area of mining activity, samples were obtained from three sites on a transect across the river (i.e., Sites 3E (east bank), 3M (middle), and 3W (west bank)). This was done to provide information regarding the possible effects of wastewaters originating from the two existing oil sands plants, Syncrude Canada Ltd. and Suncor Inc., located on the west side of the river. A more complete description of the sample sites is given in Table 1.

Sediment samples were obtained at each site using a plastic core sampler 8 cm in diameter and specifically suited to the soft sediments characteristic of the Athabasca River. At each site, 10 cores from the top 2 to 4 cm were composited. This composite sediment sample was used in the river water-sediment incubations at a rate of 10 g of sediment (wet weight) per litre of water.

Water samples were taken at a depth of 0.5 m in 1 gal sterile glass jugs. The jugs, caps, and aluminum foil lining the caps were washed with methylene chloride, rinsed three times with distilled water, and sterilized by autoclaving. Each jug was rinsed once with the river water at the sample site. Samples were packed in ice immediately after sampling, returned to Edmonton by air freight, and inoculated within 24 h of sampling.

In July and August 1979, several preliminary surveys were carried out. This was necessary to determine the potential variability of sediment samples, as well as the effects of substrate inoculation on substrate depletion 24 h after sampling, the analytical precision of analysis by GC, the incubation time required for substrate depletion, etc. Complete surveys were undertaken in late August 1979, October 1979, and February 1980. Water quality data for suspended solids indicate that higher concentrations would be expected during the August

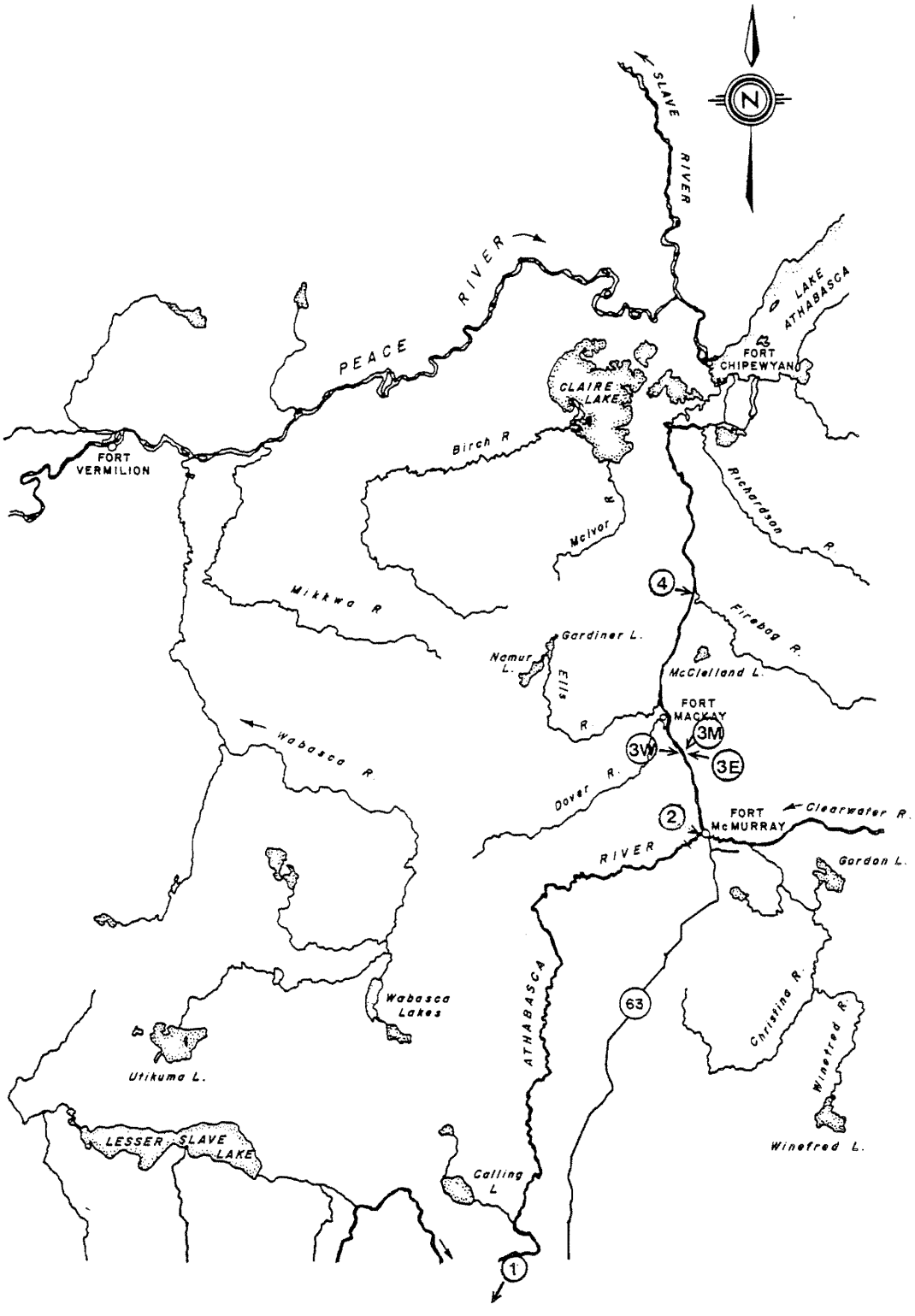


Figure 1b. Location of sample sites.

Table 1. A description of sample sites.

Site No.	Location	Description
1.	Approx. 0.5 km upstream from dock at the town of Athabasca	Near the north bank at about 1 m depth. The river bottom contained large rocks with pockets of a heavy silt-clay sediment.
2.	Approx. 0.5 km upstream from the town of Fort McMurray, between the bridge and the mouth of the Horse River	Near the west bank at about 1 m depth. The river bottom was rocky with pockets of a sandy sediment.
3.	Approx. 1.6 km downstream (north) of Mildred Lake Camp (Syncrude); i.e., just downstream from Syncrude and Suncor) 3E = east bank 3M = mid-river 3W = west bank	Site 3E and 3W were near each bank at a depth of 1 m. Site 3M was further downstream (approx. 3 km from Syncrude) on the tip of an island in mid-river. The river bottom was sandy with some finer silt and occasional tar balls (bitumen).
4.	Approx. 0.5 km upstream from the confluence of the Firebag River	Samples taken at the tip of an island at a depth of about 1 m. The river bottom was sandy.

survey (Warner 1979a). A greater discharge would also be expected as compared to the surveys during October or February (Warner 1979b).

Analysis of sediments for total organic carbon was done by flame ionization (NAQUADAT # 06077L), total Kjeldahl nitrogen by steam distillation (NAQUADAT # 07001), and total phosphorus by semi-automated persulphate digestion (NAQUADAT # 15501L).

2.2 SUBSTRATE DEPLETION STUDIES USING GC/MS

2.2.1 Preparation of Substrates and Reagents

Camphor, methyl salicylate, m-cresol and 2,6-xylenol (10 mg of each) were dissolved in 100 mL of de-ionized, double distilled water to give test solutions containing 100 µg of each substrate per millilitre.

The internal standard solution was prepared by dissolving 10 mg benzyl alcohol in 190 mL of de-ionized, double distilled water. Appropriate dilution (3 in 100) gave a working solution containing 3 µg/mL. Test and internal standard solutions were prepared just prior to the start of each survey period.

Glassware was initially soaked overnight in an aqueous solution of alconox, washed repeatedly with distilled water to remove detergent residues, and finally rinsed with an organic solvent (acetone or methanol). However, this procedure was time consuming and required repetitive handling which increased the possibility of contamination. An alternative procedure was subsequently employed in which glassware was scrubbed in distilled water to remove obvious dirt, rinsed with acetone, and finally heated in a muffle furnace to a temperature of 500°C for 5 h.

Anhydrous sodium sulphate, used as a drying agent, was also heated in the furnace and then kept in an oven at 100°C. Glass wool was kept in the oven but was not preheated in the muffle furnace since it was found to undergo some physical breakdown under those conditions.

Methylene chloride (CH_2Cl_2) was glass distilled before use and stored in amber bottles. Caps were lined with aluminum foil.

Reagent blanks were analyzed to confirm the effectiveness of the above procedures in removing trace organic compounds. The absence of peaks in the gas chromatograms indicated that the glassware and reagents had been prepared in a satisfactory manner.

2.2.2 Incubation Procedure

Two litre Erlenmeyer flasks containing 1 L of river water or water-sediment were incubated and agitated at 240 rpm in Brunswick Shakers (3.8 cm eccentricity). Incubation temperatures approximated those in the river during collection and were as follows: 20°C in August; 10°C in October; 4°C in February. For each site, one flask of river water and one flask of river water-sediment were inoculated with 1 mL of test solution to give a concentration of 100 µg/L for each substrate. An additional series of six flasks of river water-sediment were set up and not inoculated (unspiked).

For sampling the contents of inoculated (spiked) flasks, the shaker was stopped and, after several minutes, a 25 mL sterile graduated pipette was immersed about 2.5 cm below the surface. Since most of the particles quickly settled out, very little sediment was included in the sample. A 50 mL sample was removed and transferred to a 125 mL Erlenmeyer flask which was then capped with an aluminum foil wrapped cork. Samples which could not be extracted immediately were kept frozen at -30°C.

Unspiked river water-sediment samples were sampled by sacrificing one flask from the series and extracting 500 mL for analysis.

Control flasks were set up using water and sediment from Site 3M. The flasks were autoclaved before adding filter sterilized substrate. During the February survey, the effect of bitumen on the depletion rate of these substrates was determined by adding approximately 200 mg of bitumen to cover slips which were then inserted into spiked incubation flasks.

2.2.3 Extraction and Concentration of Substrates

Each 50 mL spiked river water sample was acidified with concentrated HCl (three to four drops) to a pH of 1 to 2. After

addition of 1.0 mL (3 µg) internal standard solution, the flask was shaken gently but thoroughly and the sample transferred to a separatory funnel. The solution was extracted with CH_2Cl_2 (10 + 10 + 10 mL) and the lower organic phase passed through a 1 in. plug of dried sodium sulphate (Na_2SO_4) inserted into the stem of the separatory funnel. Glass wool was used to keep the drying agent in place. The combined extracts (approximately 30 mL) were collected in a 50 mL Erlenmeyer flask and generally concentrated immediately. If this was not possible, the flask was capped with an aluminum foil wrapped cork and stored at $4^{o}C$.

The river water-sediment samples were extracted in the same fashion with some modification. While the extraction procedure did provide two separate phases in the separatory funnel, the lower layer consisted of a thick gelantinous emulsion. This emulsion could not be broken by prolonged standing, addition of more CH_2Cl_2, or passage through glass wool. It was therefore drawn into a 50 mL centrifuge tube and centrifuged for 10 min at 1500 rpm. The now separated layers were transferred to a separatory funnel and the organic phase passed through Na_2SO_4 as described. The combined extracts were concentrated immediately or stored at $4^{o}C$.

Unspiked river water samples (500 mL) were extracted with CH_2Cl_2 (50 + 25 + 25 mL) following acidification and addition of internal standard (25 µg in 2.5 mL water). The procedure was identical to that described for the extraction of river water spiked with test substrates. The combined extracts (approximately 90 mL) were collected in a 125 mL Erlenmeyer flask and concentrated immediately or stored at $4^{o}C$.

Concentration was conducted in a fume hood. The CH_2Cl_2 extracts were transferred to a Kuderna Danish apparatus consisting of a 5 mL graduated concentrator tube, 500 mL flask, and three ball Snyder column. A boiling chip was added and the apparatus immersed in a hot water bath ($85^{o}C$) to just below the bottom joint. The solution was concentrated until 1.0 mL remained in the concentration tube at which time the apparatus was removed from the bath and allowed to cool. Solvent vapor in the column and flask quickly condensed

to give a final volume of 4 to 5 mL in the concentrator tube. The tube was disconnected from the rest of the apparatus and the volume reduced to approximately 1.0 mL by a gentle nitrogen stream and warm bath (50°C). The sample was transferred to a 4 mL vial and concentrated to 20 to 50 µL (N_2 stream). One to 2 µL of the sample were used for analysis by gas chromatography (GC) or combined gas chromatography/mass spectrometry (GC/MS).

2.2.4 GC and GC/MS Operating Conditions

All sample extracts were analyzed on a glass column (2.12 m x 4 mm id) packed with 5% OV-101 on AW-DMSC Chromosorb 750 (80/100 mesh) and a helium carrier gas flow rate of 60 mL/min.

Extracts of spiked samples were chromatographed isothermally at 105°C. Water samples which had been extracted for endogenous (natural) organic compounds were analyzed by temperature programming from 70 to 270°C at 16°/min. The upper temperature was held until the total elasped run time was 20 min.

Electron impact GC/MS was conducted on the same column using conditions identical to those above. The MS operating parameters were: scan speed -- 100 amu/sec; electron energy -- 70 eV; ion source temperature -- 180°C; separator temperature same as oven temperature.

Chemical ionization of GC/MS was conducted on a 0.61 m column packed with 3% SP-2250 on Supelcoport (100/120 mesh) and a temperature program of 70 to 310°C at 10°/min. The MS operating parameters were: electron voltage -- 150 eV; source temperature -- 200°C; source pressure -- 0.6 Torr. Methane was used as the reactant gas.

Gas chromatography analysis was performed on a Hewlett Packard (HP) 5700A (or 5720A) gas chromatograph equipped with a flame ionization detector and a 3380A integrator. Electron impact GC/MS was conducted on an HP 5710A GC coupled to an HP 5981 MS with an HP 5934A data system. Chemical ionization GC/MS was conducted on an HP 5985A system. De-ionized, double distilled water was obtained from a Corning LD-2A demineralizer coupled to a Corning AG-11

distillation apparatus. The muffle furnace was a product of Thermal Electric Mfg. Co., Dubuque, Iowa. A Fisher Accumet 140 pH meter was used for all pH determinations.

2.2.5 Calculation of 50% Depletion Times

Since each incubation flask contained an unknown diversity of bacteria, it is not possible to state whether the depletion rate of the substrates was a result of one or many biological reactions. Thus, the term "50% depletion time" is used rather than "half-life" or other expressions which may falsely denote an understanding of the kinetic rates of uptake. The value for the 50% depletion time was calculated by plotting the data for each substrate (Log. substrate concentration versus time) and drawing a line of best fit. The time (days) at which 50% of the substrate remained was recorded as the 50% depletion time. This technique is shown in Figure 3; however, for the purposes of presentation of the data, other graphs use a linear axis.

2.3 SUBSTRATE DEPLETION STUDIES USING ^{14}C-LABELLED COMPOUNDS

2.3.1 Preparation and Incubation

To make a detailed study of the release of $^{14}CO_2$, samples were taken only from Sites 1, 3M, and 4.

Two heat-sterilized 500 mL Erlenmeyer flasks (one test and one control), modified by the addition of two side arms, were prepared for each water sample and substrate tested. The side arms were capped with serum stoppers held in place with plastic cable ties and covered with sterile aluminum foil. Sterile, labelled substrates U-^{14}C-amino acid mixture-CFB 152 (e.g., similar amounts of amino acids excluding L-histidine and L-methionine and contained in a typical algal protein hydrolysate), U-^{14}C-starch; 1-^{14}C-hexadecane, and 9-^{14}C-anthracene (Amersham Corporation) were used at a specific activity of 10 µCi per flask. The total carbon content was made up with identical, non-radioactive substrate to a concentration of 20 ppm which is approximately the total carbon load of the Athabasca River. Nutrients (i.e., nitrogen (N) and phosphorus (P)), when required, were added

in excess as a filter sterilized solution (pH 7.3) at a concentration of 39 mg P (as potassium phosphate, mono and dibasic) and 70 mg N (as ammonium nitrate) per flask. To each flask, 200 mL of sample (river water) were added as inoculum and the tops sealed with sterile solid rubber stoppers. All flasks were incubated on a gyrotory shaker (200 rpm, 3.8 cm eccentricity) at room temperature (approximately 22°C). At suitable intervals during the incubation, aliquots were withdrawn from test flasks for measurement of $^{14}CO_2$ production.

2.3.2 Analytical Procedure

One of the side arm stoppers of a test flask was rinsed with 70% ethanol. A sterile 22 gauge 1 in. needle, attached to a sterile plastic leur-lok 20 mL syringe, was inserted into the flask. Ten millilitres of air and 5 mL of liquid were withdrawn from the flask and injected into a sealed, evacuated 30 mL serum bottle containing 2 mL of 4N H_2SO_4. The sample in the serum bottle was flushed with nitrogen for 5 min at 100 mL/min and the $^{14}CO_2$ (CO_2) gas passed through two scintillation vials in series containing 10 mL of ACS (Amersham) fluor and 1.0 mL of Carbo-Sorb II (Packard) carbon-dioxide absorbent. The scintillation vials were capped, dark-adapted at 4°C for 30 min, and counted in a Mark III-6881-C Analytic Liquid Scintillation Counter (Searle) equipped with a microprocesser to calculate dpm from cpm, using stored standard quench curves. Data are reported as the percentage of label recovered as $^{14}CO_2$ as a function of incubation time.

The amino acids were resolved on a Beckman Amino Acid Analyzer (Dept. of Botany, Univ. of Alberta). The liquid and air levels in test flasks were replaced by injecting 10 mL of air and 5 mL of liquid from the control using a sterile syringe. The vacuum created in the latter flask was replaced by injecting 15 mL of filter sterilized air through a side arm. Occasionally, the control flask was sampled for $^{14}CO_2$ producing as a check on sterility and the volatility of hexadecane and anthracene. The vacuum in such flasks was also replaced with 15 mL of sterile air.

The effect of bitumen on the mineralization of the labelled amino acid mixture and hexadecane was compared by adding 200 mg of bitumen on a cover slip to reaction vessels.

3. RESULTS AND DISCUSSION

3.1 PRELIMINARY SURVEYS AND EVALUATION OF GC METHODOLOGY

Sediments from Site 3E were sampled 6 August 1979 to determine if a composite sample would be adequate in providing reproducible sample types. The results of three replicate samplings (each a composite of 10 samples) taken over a distance of about 100 m are shown in Table 2. The data indicate a similar ratio of sand:silt:clay in each sample. However, the chemical parameters do show some variability, especially for total phosphorus. Since obtaining significantly more than 10 subsamples would have been extremely time consuming, it was decided to maintain this sampling procedure but the possible variations in nutrient levels should be considered when interpreting the results of water-sediment incubations.

In a typical incubation, test compounds and the internal standard, analyzed by the GC conditions as described, were resolved in less than 9 min. A gas chromatogram of the extracts from an incubated spiked river water-sediment sample is shown in Figure 2. From this data, as described in Section 2.2.5, a value for the 50% depletion time could be calculated.

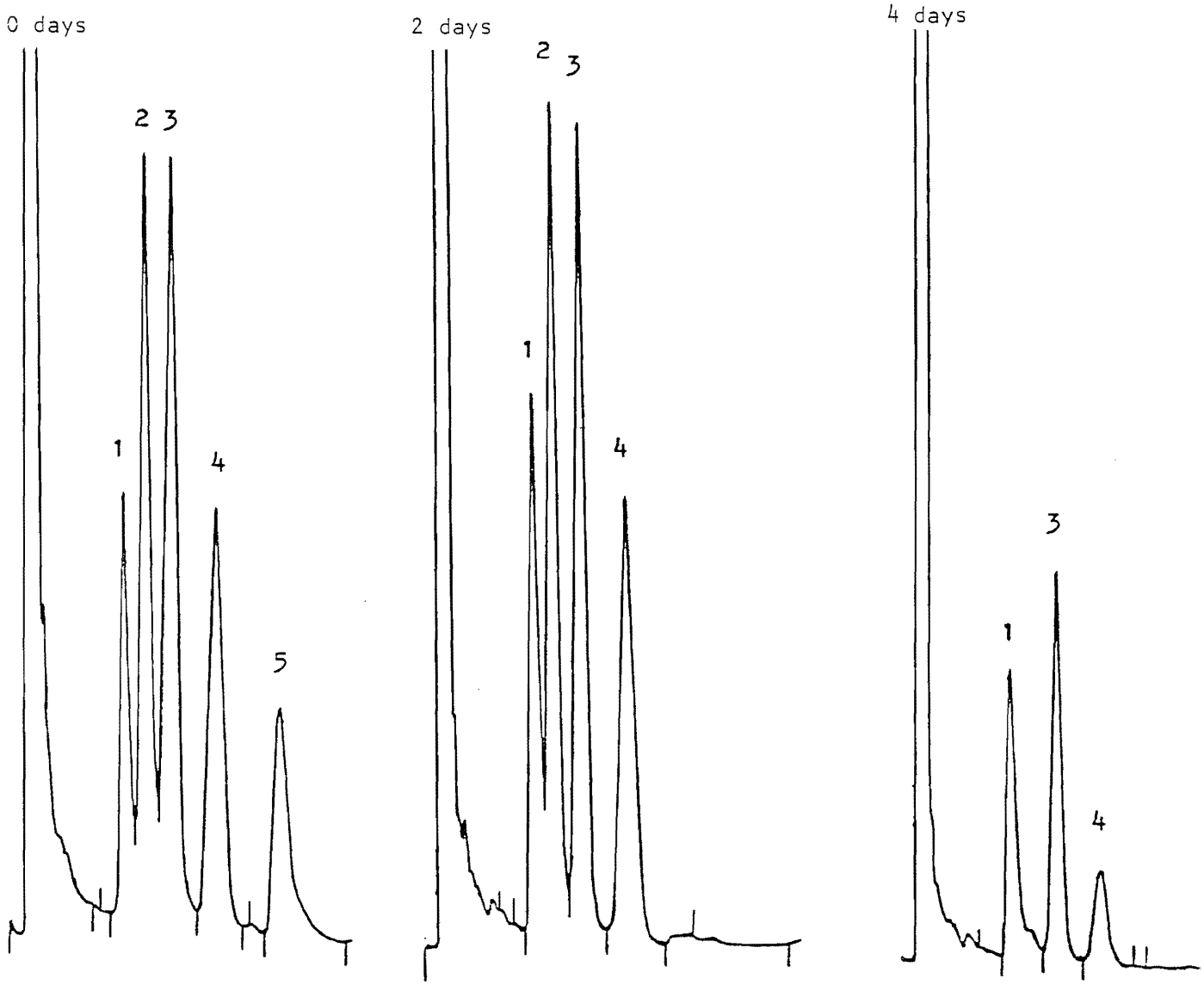
The actual inoculation of the laboratory flasks was planned to be undertaken 24 h after sampling. Thus, a series of experiments was designed during a preliminary survey to determine the effects of this time lag on the rate of substrate depletion. Duplicate water-sediment samples were taken from Site 1 and inoculated in separate flasks, one after 3.5 h and the other after 24 h from the sampling time. The results (Figure 3) show similar rates of depletion for the two inoculation times and the four non-labelled substrates. In no instance did the calculated 50% depletion time vary by more than 0.5 d.

The analytical precision of GC analysis was tested by subdividing a spiked water sample into five 1 L aliquots, incubating in 2 L flasks, and then determing the concentrations of the four substrates over an 8 d period. The results (Table 3) show the value

Table 2. Chemical and physical analyses of replicate sediment samples at Site 3E, August 1979.

Type of Analysis	Sample No. 1	2	3
% sand:silt:clay	72:18:10	75:15:10	68:21:11
total organic carbon[a] (ppm)	354	277	307
total Kjeldahl nitrogen (ppm)	319	357	366
total phosphorus (ppm)	172	111	506

[a]excluding any tar balls



Peak 1 = benzyl alcohol (internal standard)
Peak 2 = m-cresol
Peak 3 = 2,6-xylenol
Peak 4 = camphor
Peak 5 = methyl salicylate

Figure 2. GC traces of inoculated river water-sediment over a 4-d incubation period.

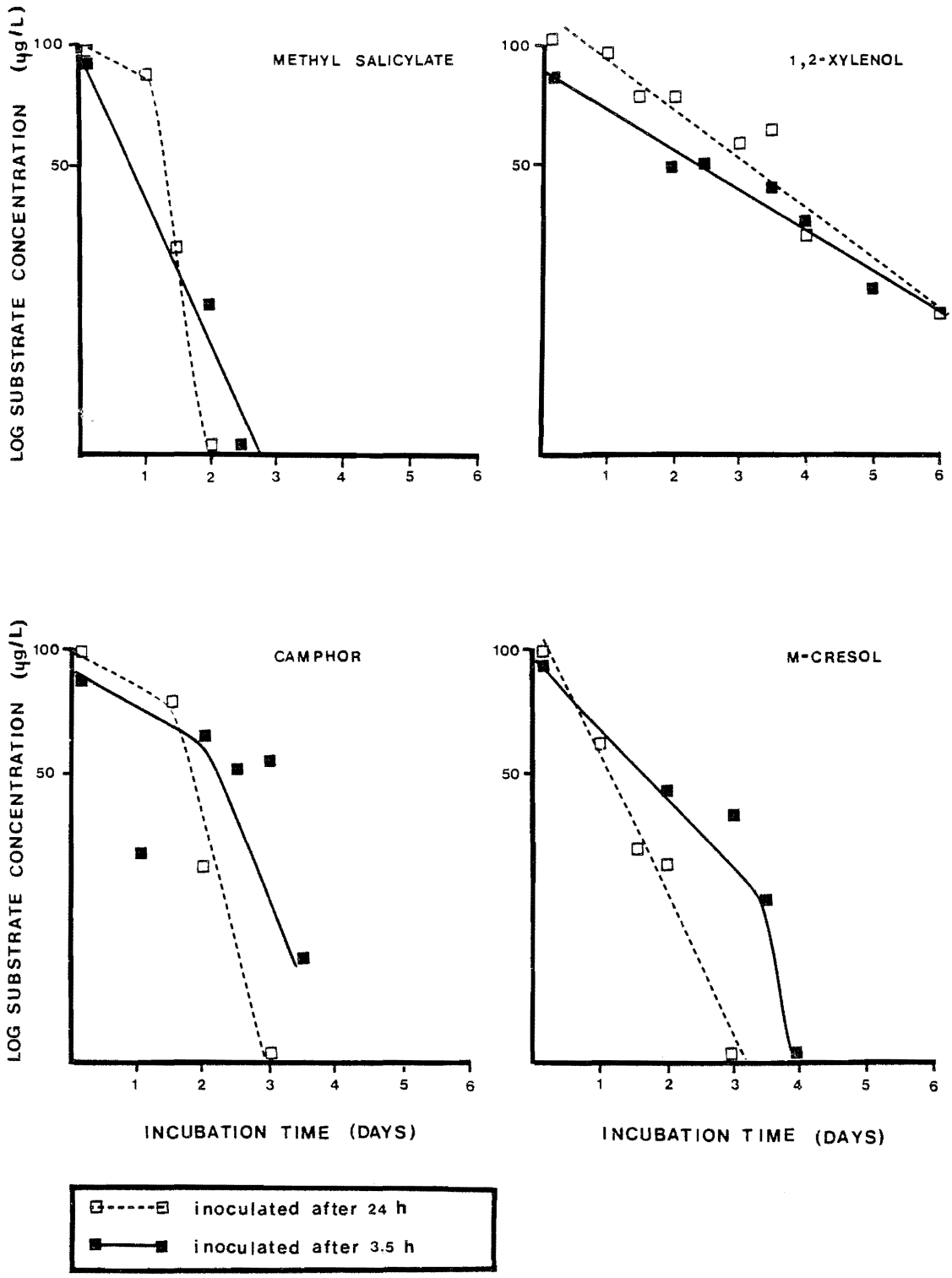


Figure 3. Depletion of substrates in water-sediment at Site 1, August 1979; the effect of different inoculation times is compared.

Table 3. Analytical variability in substrate determinations for five replicate river water samples spiked with m-cresol, camphor, methyl salicylate, and 2,6-xylenol.

Incubation Time (days)	Substrate[a]			
	m-cresol	camphor	methyl salicylate	2,6-xylenol
0	121 ± 8.1(5)[b]	128 ± 0.0(3)	-	108 ± 0.0(2)
1	-[c]	-	-	-
2	44 ± 5.1(5)	127 ± 9.2(3)	92 ± 3.3(4)	118 ± 2.0(3)
3	3.8 ± 3.8(5)	108 ± 5.5(5)	50 ± 8.2(5)	96 ± 3.0(5)
4	3.2 ± 7.1(5)	70 ± 2.3(4)	0 ± 0.0(5)	93 ± 4.4(4)
5	-	-	-	-
6	0 ± 0.0(5)	0 ± 0.0(4)	-	99 ± 7.6(4)
7	-	-	-	-
8	-	-	-	94 ± 11.6(5)

[a] µg/L ± 1.0 Standard Deviation

[b] = number of samples analyzed

[c] = no data obtained

for each substrate concentration plus or minus on standard deviation. The variations in values were considered acceptable for the purpose of calculating a 50% depletion time for each substrate.

In order to assess errors in sampling as well as in analysis, duplicate river water samples were taken and incubated in separate flasks. The data for m-cresol and camphor are shown in Figure 4 and indicate a close fit for the data from the two replicate samples.

To determine the possible effects of non-biological processes on the substrate depletion rate, a series of control treatments were undertaken during each survey. Initially, a 30% (30 g/L) solution of sodium chloride was utilized as a biological inhibiter. Salt solutions at this concentration have been shown to be effective in inhibiting bacterial activity (Brock 1975). They have the advantage of being very stable as well as reducing the need for aseptic techniques. However, a very marked rate of substrate depletion did occur in these controls. This may have been a result of the reduced solubility of the inoculated compounds at high salt concentrations, causing a "salting out" or precipitation. As a result, controls for the October and February surveys were autoclaved and filter sterilized substrates were added aseptically.

The data in Figures 5 and 6 compare the substrate levels of autoclaved controls with the non-sterile incubation flasks for both the October and February surveys. Figure 5 deals with the substrates m-cresol and camphor and shows little or no non-biological depletion of these two compounds. In addition, these substrates apparently were not adsorbed onto sediment particles since concentrations in both river water and river water-sediment control flasks remained at similar levels during the incubation period. In contrast, non-sterilized flasks showed a rapid rate of depletion.

When the same samples were analyzed for 2,6-xylenol and methyl salicylate (Figure 6), the results suggested that non-biological mechanisms did affect the depletion rate. No data for the methyl salicylate control were obtained for October. However, the results of the February survey clearly indicate that substrate depletion occurred in the sterile control. This may have been due to the hydrolysis of

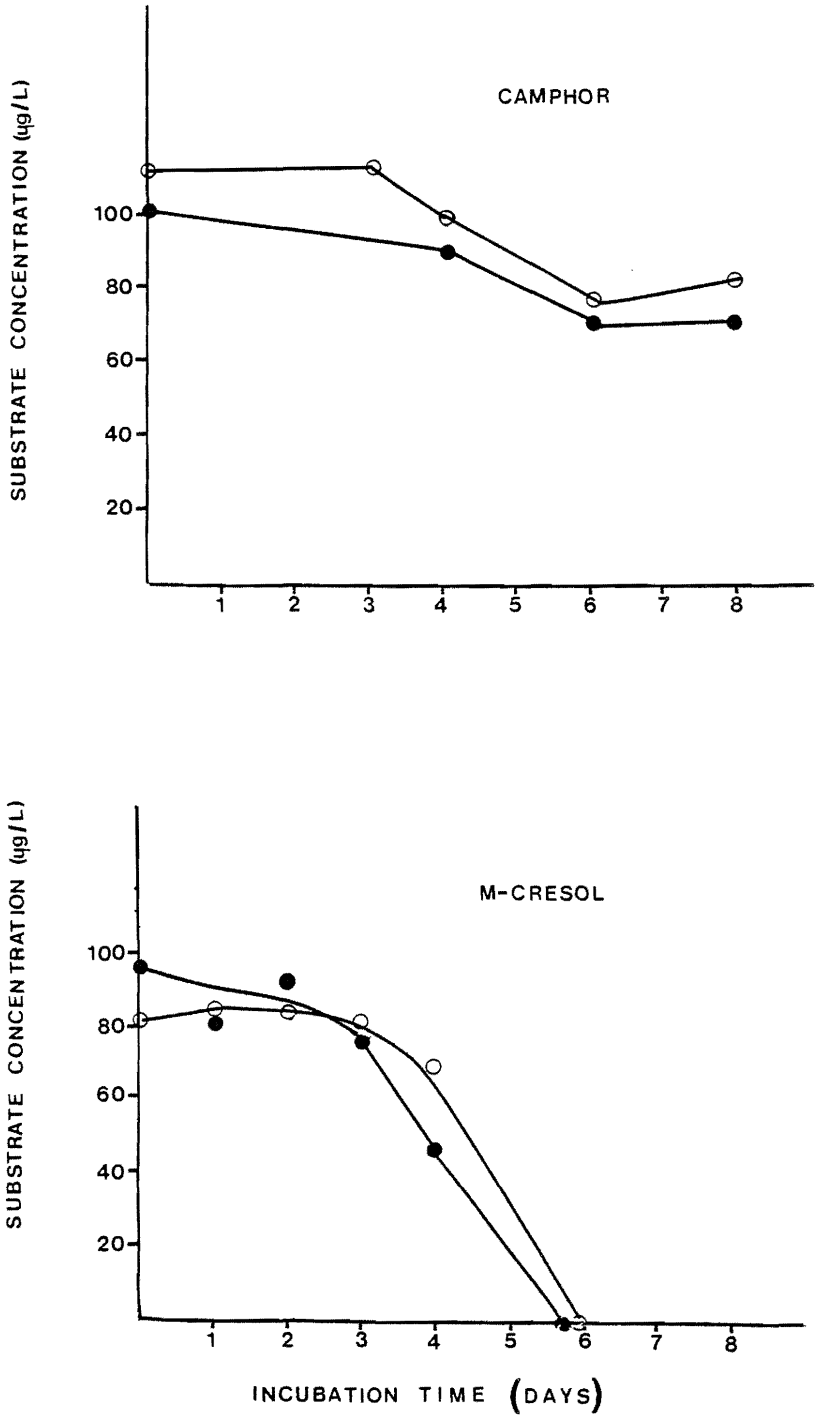


Figure 4. GC analysis of duplicate incubations of river water sampled at Site 1, February 1980.

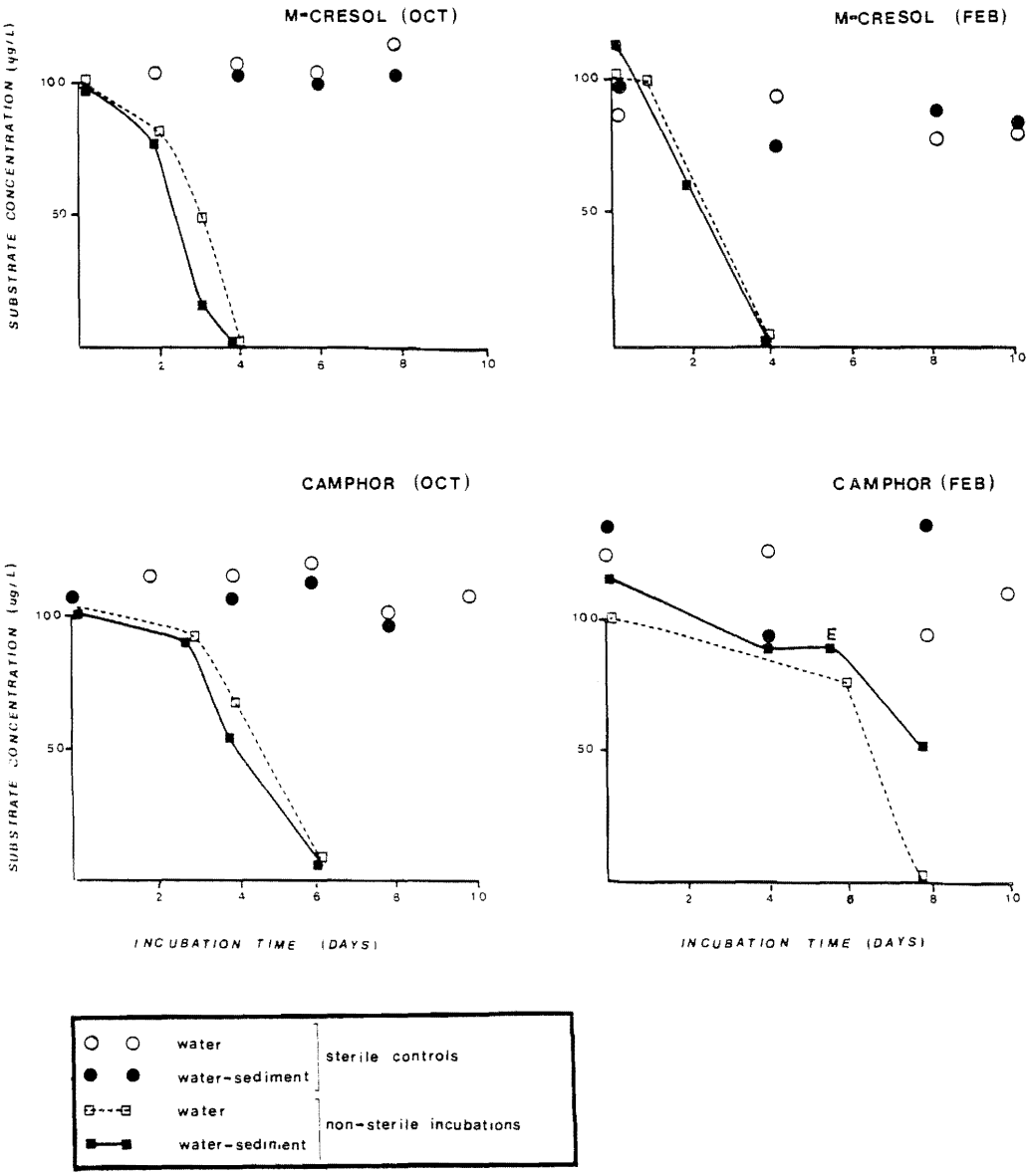


Figure 5. Depletion of m-cresol and camphor in sterile controls and non-sterile incubations.

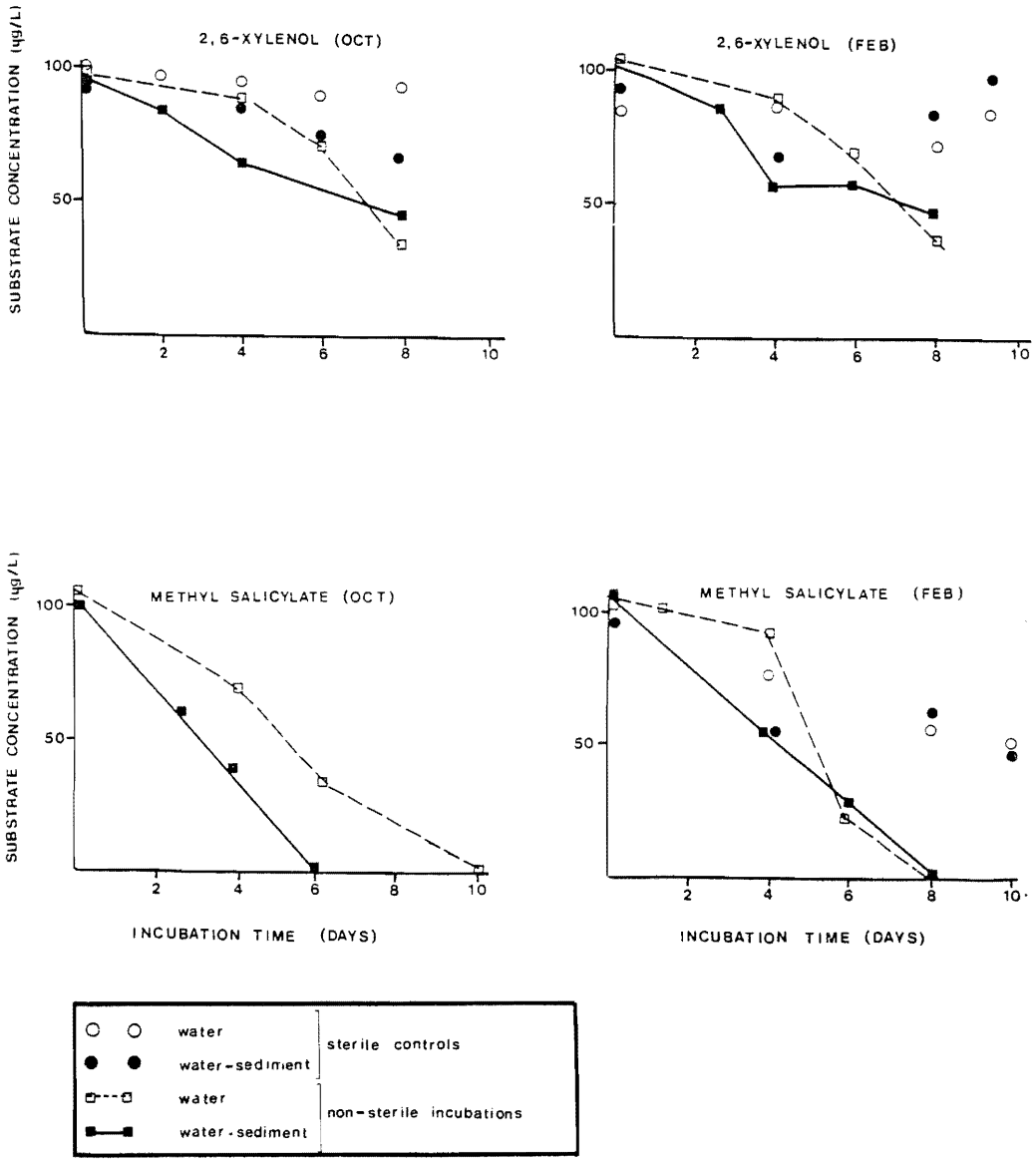


Figure 6. Depletion of 2,4-xylenol and methyl salicylate in sterile controls and non-sterile incubations.

the ester bond during incubation. The results showed that microbially-mediated depletion of methyl salicylate also occurred since the concentration in the non-sterile incubation decreased at a more rapid rate than in the control. However, on the basis of these experiments, it was not possible to calculate 50% depletion times for methyl salicylate as a sole result of microbial action. Again, it appeared that adsorption to sediment did not occur.

The rate of substrate depletion for 2,6-xylenol was noticeably slower in non-sterile flasks than for the other substrates. This was expected since the presence of methyl groups on each side of the functional hydroxyl group (the probable site of attack by microbial enzymes) may cause a resistance to biodegradation due to steric hinderence. In many cases, the 50% depletion time for 2,6-xylenol was greater than the incubation time (10 to 12 d) and could only be calculated by extrapolation of the data. For this reason, and also because results (Figure 6) obtained from the control flasks showed a possible adsorption reaction to sediments, data for this compound were also not utilized in this study.

3.2 SUBSTRATE DEPLETION STUDIES USING GC/MS

3.2.1 Substrate Depletion at Different Sites

Graphs of the 50% depletion times for both camphor and m-cresol are given in Figures 7 and 8. The value for the 50% depletion time at Site 3 is the average of the three locations (3E, 3M, and 3W). In most cases, a common trend of lower depletion times (i.e., higher rate of degradation) at Sites 1 and 3 and higher depletion times at Sites 2 and 4 was observed. This trend occurred in both water and water-sediment incubation flasks. Thus, it is probably not due solely to differences in sediment composition among the sites. However, analysis of the sediments (Table 4) did show higher levels of total organic carbon at these two sites as well as larger percentages of clay and silt materials, both factors suggesting that bacterial activity may be enhanced (Paerl 1975).

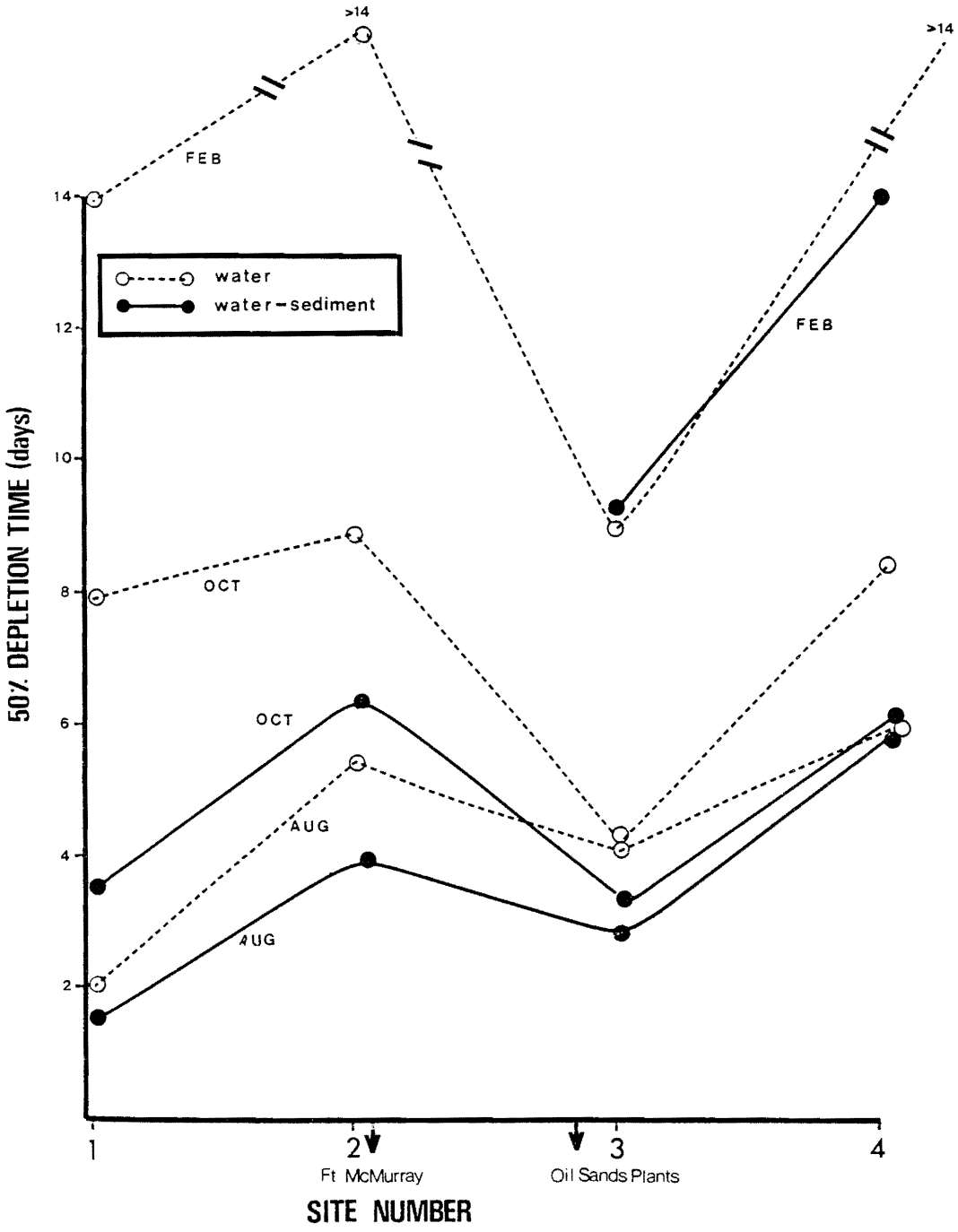


Figure 7. Fifty percent depletion times for camphor at four sites along the Athabasca River.

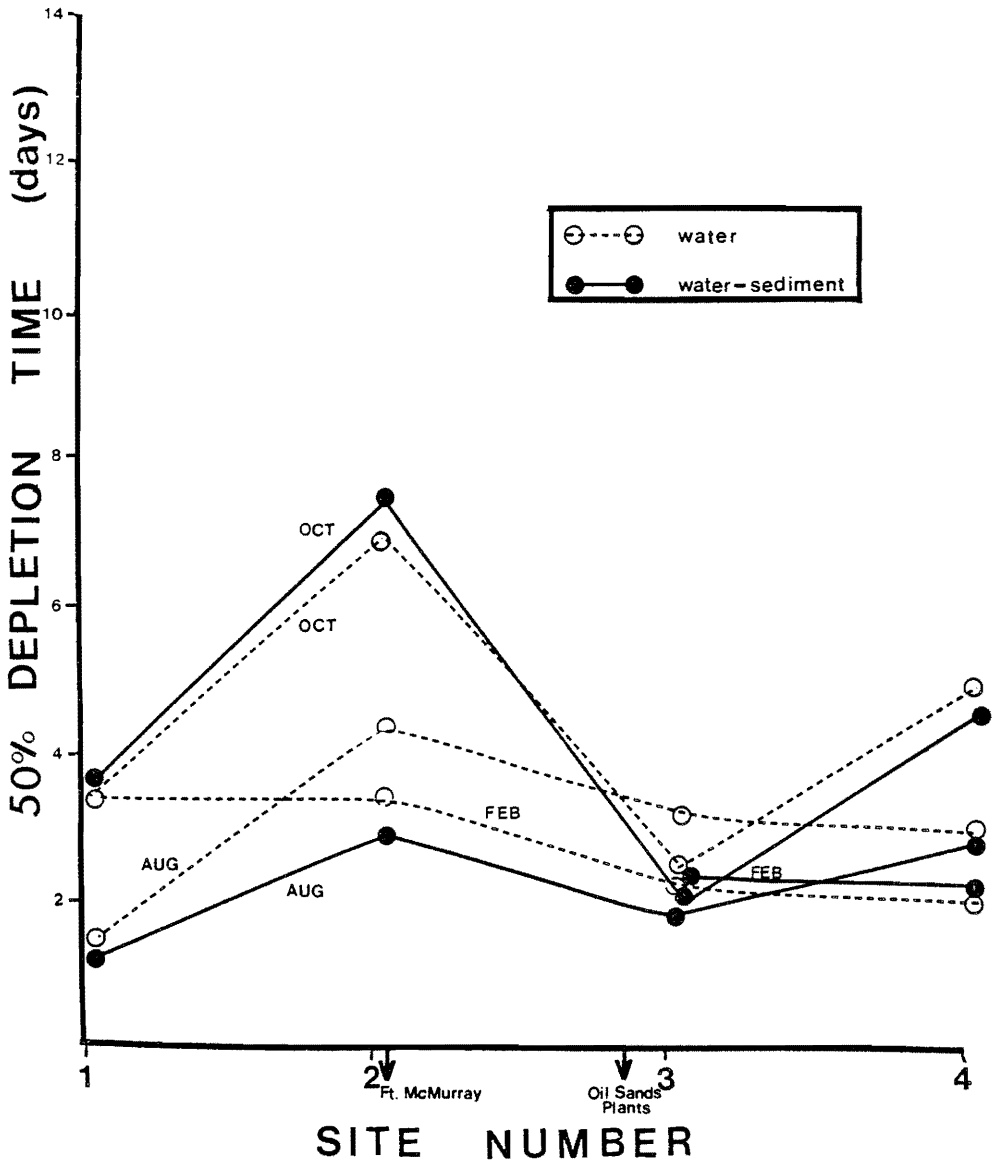


Figure 8. Fifty percent depletion times for m-cresol at four sites along the Athabasca River.

Table 4. Analysis of the total organic carbon content and texture of sediments in the Athabasca River.

Sampling Date	Site No.	Texture			Total Organic Carbon[a] (mg/L)
		% sand	% silt	% clay	
August 1979	1	69	21	10	10.4
	2	99	<1	<1	1.8
	3W	91	6	3	19.4
	3M	96	2	2	1.1
	3E	97	2	1	7.4
	4	98	2	<1	2.4
October 1979	1	63	27	10	7.4
	2	100	0	0	1.8
	3W	83	13	4	7.4
	3M	99	1	0	2.9
	3E	97	2	1	3.4
	4	100	0	0	2.9

[a]excluding any tar balls

The relatively high levels of microbiological activity at Site 1 may be the result of agricultural or other inputs to the river upstream from that site. In a previous study (Nix et al. 1979), using ^{14}C-glutamic acid, a similar high level of substrate uptake was observed at Site 1. Any effect of these inputs would be expected to be less at Site 2, about 250 km downstream, since bacteria may have assimilated many organic compounds and/or nutrients in the river between Sites 1 and 2. Between Sites 2 and 3, new inputs from the town of Fort McMurray and/or the oil sands plants may again result in increased bacterial activity and thus a decrease in the 50% depletion time for these two substrates at Site 3.

3.2.2 Substrate Depletion Downstream from Oil Sands Mining and Extraction Plants

Values for the 50% depletion times for both m-cresol and camphor are shown in Figure 9 for the three sub-locations at Site 3. At present, two oil sands plants, Suncor Inc. and Syncrude Canada Ltd., are in operation a few kilometres upstream from this site. Suncor releases refinery wastewater directly to the Athabasca River. Both plants release quantities of mine depressurization water (and may release other effluent or run-off streams that eventually enter the river). Both plants are located on the west side of the river and a report by Beltaos (1979) indicates that any effluent streams from these plants would likely still be adjacent to the west bank at Site 3. Thus, any effect of these wastewaters on the depletion of organic substrates should be noticeable by comparing the depletion times at the east (3E), middle (3M), and west (3W) locations on the river.

In practically all cases, substrate depletion was faster at Site 3W (Figure 9). This was true for the three sampling periods (summer, fall, and winter) and occurred in both water and water-sediment incubation flasks. Analysis of the sediment (Table 4) did show elevated levels of TOC and the clay-silt fraction along this side of the river.

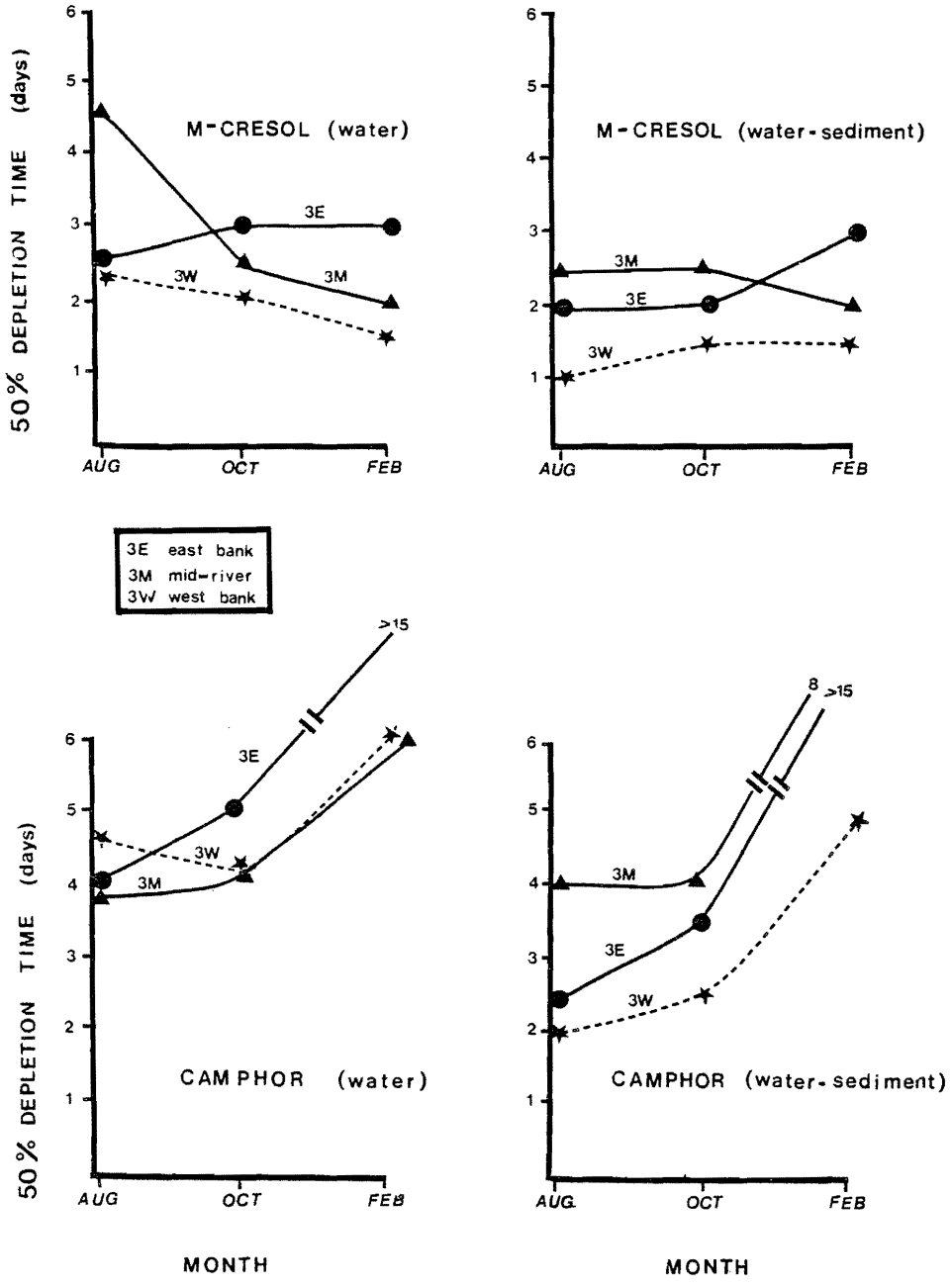


Figure 9. Fifty percent depletion times for camphor and m-cresol along a transect of the Athabasca River at Site 3.

For all three locations, it is evident that the rate of depletion was generally more rapid (i.e., a lower 50% depletion time) in samples which contained sediment. Since the amount of sediment added to each incubation flask was not large (1% w/v), the significant decrease in the depletion rate (50% in some cases) would indicate a very much higher rate of bacterial activity in sediments. This may be due to the high nutrient levels in the sediments (Table 2), as well as to increased microbial populations as a result of adherence of bacterial cells to sediment particles. In a previous report (Nix et al. 1979), it was shown that bacterial numbers in sediments were generally one or two orders of magnitude greater than in overlying river water.

A seasonal trend in the 50% depletion time for camphor is readily apparent with the shortest time occurring in August, a somewhat longer time in October, and a very much longer time in February. A substantial decrease in planktonic and sediment bacterial activity during winter conditions is expected (Wright and Hobbie 1969; Hall et al. 1972). However, this trend did not occur for the substrate m-cresol. A decrease in depletion time can be observed in some cases during the winter sampling period, indicating a higher rate of bacterial activity at that time.

3.2.3 The Effect of Bitumen on Substrate Depletion

During the February survey, additional samples were obtained from Site 3M to determine any effects of bitumen on the microbial depletion of the two substrates, m-cresol and camphor. The results (Figure 10) show no significant inhibition or stimulation of the microbial degradation of these two compounds in either the water or water-sediment incubations.

3.2.4 GC/MS Analysis of Unspiked Water-Sediment Flasks

Gas chromatograms of the extract from unspiked water-sediment incubation flasks did not show any substantial depletion of organic compounds among the various sites. An example of these data is given in Appendix 6.1. Organic compounds, which were present in very small

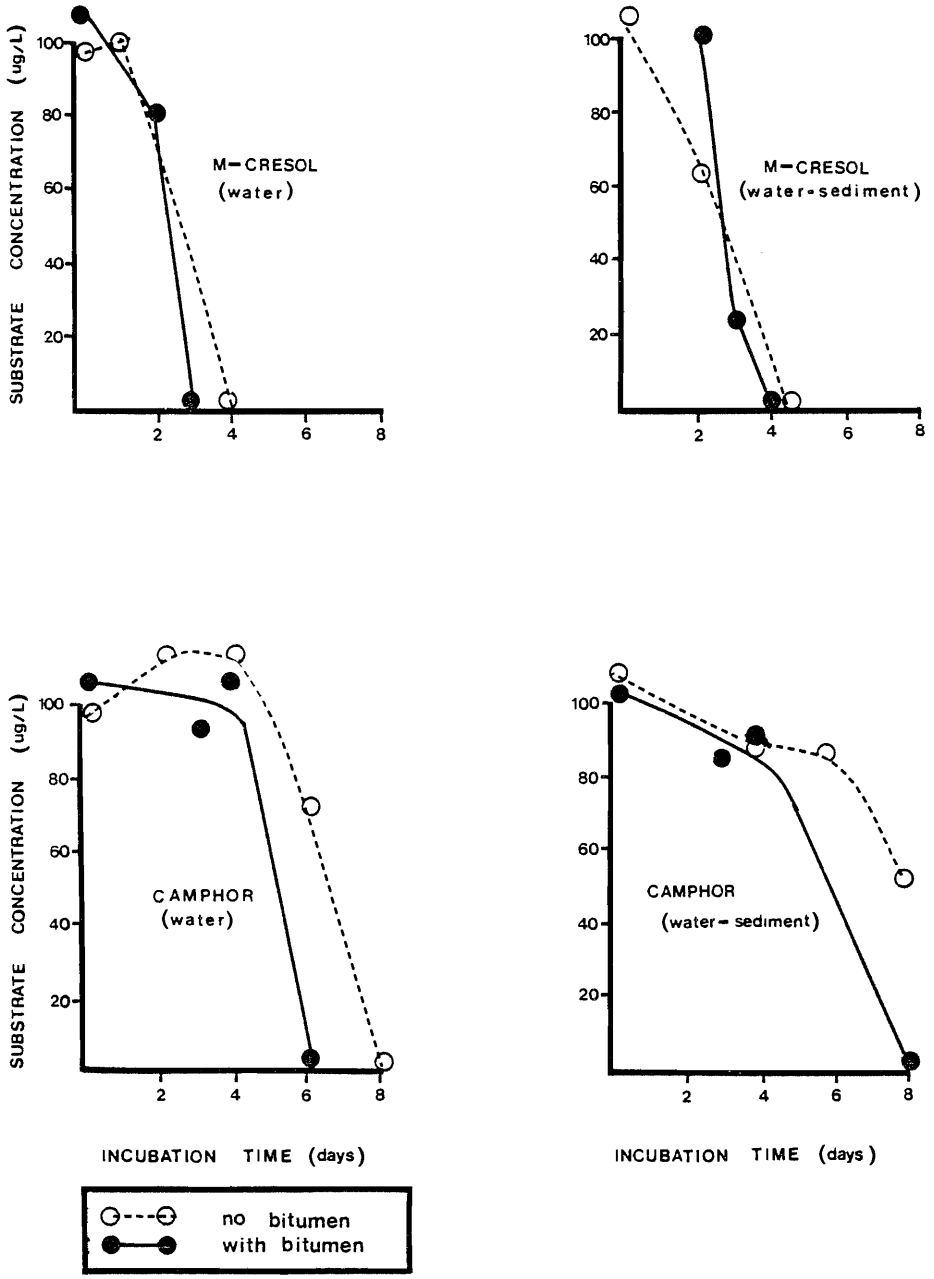


Figure 10. The effect of bitumen on the depletion of m-cresol and camphor at Site 3M, February 1980.

quantities, were not identifiable in extracts of 500 mL and therefore extracts from the series of six flasks were combined and examined by GC/MS. While this procedure provided increased quantities of the organic compounds, they were poorly resolved using a temperature program. Therefore, the extracts were analyzed isothermally at 170°C and for about 12 min followed by column bakeout. A second run was then made at 200°C for 15 min.

The mass spectra of several peaks, analyzed from samples taken at Sites 1 and 3W, are given in Appendices 6.2, 6.3, 6.4, and 6.5. No quantitation of these compounds was possible, however a summary of their possible identification is given below:

1. Compound A (R_t = 1.62 min): comparison of the mass spectrum with that reported by Junk (1975) suggests this compound to be toluene. Reported m/e (% relative abundance): 93(5), 92($M^{+\cdot}$,65), 91(100), 65(11), 63(6), 51(5), 45(4), and 39(9).
2. Compound B (R_t = 2.04 min): the mass spectral fragmentation pattern suggests a compound similar in structure to (dimethylaminoacetone).
3. Compound C (R_t = 8.68 min): this compound was present in every sample analyzed and was generally the most intense peak in the GC trace. Both the electron impact mass spectrum and the chemical ionization (CI) mass spectrum are given in Appendix 6.2. CI/MS reveals that the molecular weight of the compound is 216. Accurate mass measurements of the major ions in the electron impact mass spectrum indicate that the molecule contains oxygen. Fragment ions, m/e (empirical formula): 173 ($C_8H_{13}O_4$), 111 ($C_6H_7O_2$ or C_8H_{15}), 99 ($C_5H_7O_2$)2, 84 ($C_5H_8O_2$), 83 (C_5H_7O), 56 (C_3H_4O or C_4H_8), 55 (C_3H_3O or C_4H_7). The mass spectral fragmentation data suggest that this compound has the molecular formula $C_{11}H_{20}O_4$.

4. Compound D (R_t = 14.53 min): the CI mass spectrum gives the molecular weight as 278 and a fragmentation pattern consistent with either di-n-butyl phthalate or di-isobutyl phthalate.
5. Compound E (R_t = 18.35 min): the CI mass spectrum gives the molecular weight as 336 and a fragmentation pattern consistent with butylglycollylbutyl phthalate.
6. Compound F: comparison of the mass spectrum with that reported by Junk (1975) suggests this compound to be 1- or 2-methylnaphthalene. Reported m/e (% relative abundance): 1-methyl--143(11), 142($M^{+\cdot}$,100), 141(69), 115(26), 71(15), 63(12), 57.5(12), 39(10). 2-methyl--143(12), 142($M^{+\cdot}$,100), 141(72), 139(10), 115(25), 71(8), 63(8), 58(7).
7. Compound G: not identified.
8. Compound H: comparison of the mass spectrum with that reported by Junk (1975) suggests this compound to be butylated hydroxytoluene. Reported m/e (% relative abundance): 220($M^{+\cdot}$,22), 206(15), 205(100), 145(12), 81(11), 57(32), 41(18), 29(11).
9. Compound I: comparison of the mass spectrum with that reported by Junk (1975) suggests this compound to be diethyl phthalate. Reported m/e (% relative abundance): 222($M^{+\cdot}$,3), 177(28), 176(9), 150(13), 149(100), 105(8), 76(6), 65(8), 29(6).
10. Compound J: not identified.
11. Compound K: not identified.
12. Compound L: comparison of the mass spectrum with that reported by Junk (1975) suggests this compound to be butylbenzenesulfonamide. Reported m/e (% relative abundance): 213($M^{+\cdot}$,6.8), 171(9), 170(99), 158(10), 141(98), 78(10), 77(100), 51(20), 30(11).
13. Compound M: not identified.
14. Compound N: not identified.
15. Compound O: not identified.

Hrudey (1975) has identified four compounds from an analysis of Suncor's dyke drainage water: a butylated hydroxytoluene; a di-n-butyl phthalate; a bis (2-ethylhexyl)phthalate, and a bis (2-ethylhexyl) adipate. It is possible that the toluene and phthates identified here have the same origin although at the time of sampling much of the dyke drainage water was being recycled back into the tailings ponds. It is also possible that the phthate compounds may have originated from the plastic core sampler used to obtain the river sediment. Since little is known about the identifies of compounds from either natural or industrial sources, further comments at this time would be speculative.

3.3 SUBSTRATE DEPLETION STUDIES USING ^{14}C-LABELLED COMPOUNDS

A comparison of the patterns of $^{14}CO_2$ evolution from the substrates tested indicates that a relatively rapid and complete metabolism of the amino acid mixture takes place in Athabasca River water as compared to the other substrates studied (Figure 11). Starch (Figure 12) is metabolized faster than both hydrocarbons--hexadecane (Figure 13) and anthracene (Figure 14). The catabolism of both hydrocarbons in water samples requires the addition of nitrogen and/or phosphorus. Upon nutrient supplementation, the metabolism of the hydrocarbons proceeds in a similar manner to that of starch.

The preferential metabolism of amino acids is probably due to their water solubility and that nitrogen is being supplied along with the carbon skeleton of the amino acid. Preliminary data obtained on the order of amino acid utilization (as resolved by the amino acid analyzer) by microorganisms in water from Site 3 (October 1979) are presented in Figure 15. Very little evidence of amino acid catabolism, other than metabolism of alanine and tyrosine, was observed during the First 10.5 h of incubation. Aspartic, threonine, serine (unresolved), glutamic, alanine, and tyrosine were being slowly metabolized while alanine and tyrosine had been completely used. Very little utilization of the branched chain amino acids (valine, isoleucine, and leucine) and the sulphur-containing branched amino acid (methionine) occurred

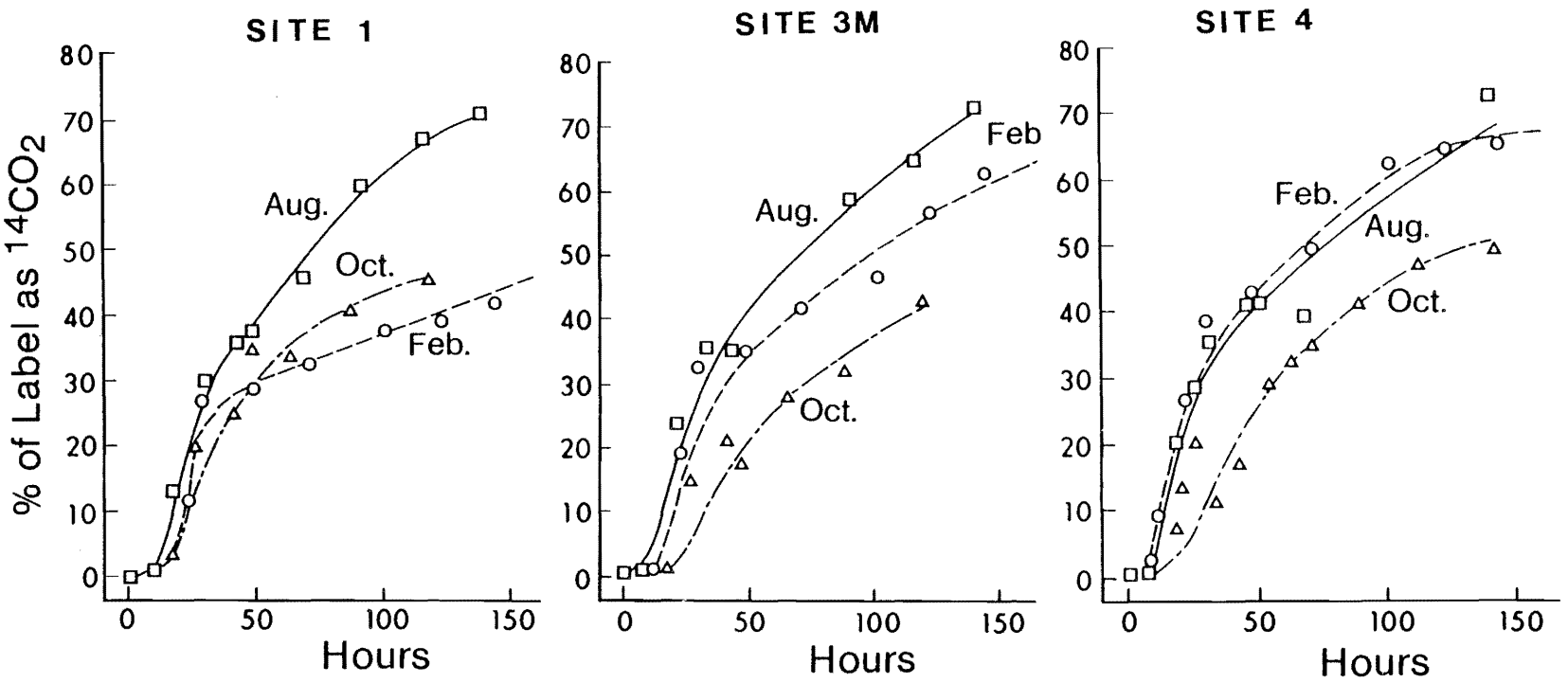


Figure 11. The mineralization of ^{14}C-amino acids in water sampled at three sites along the Athabasca River.

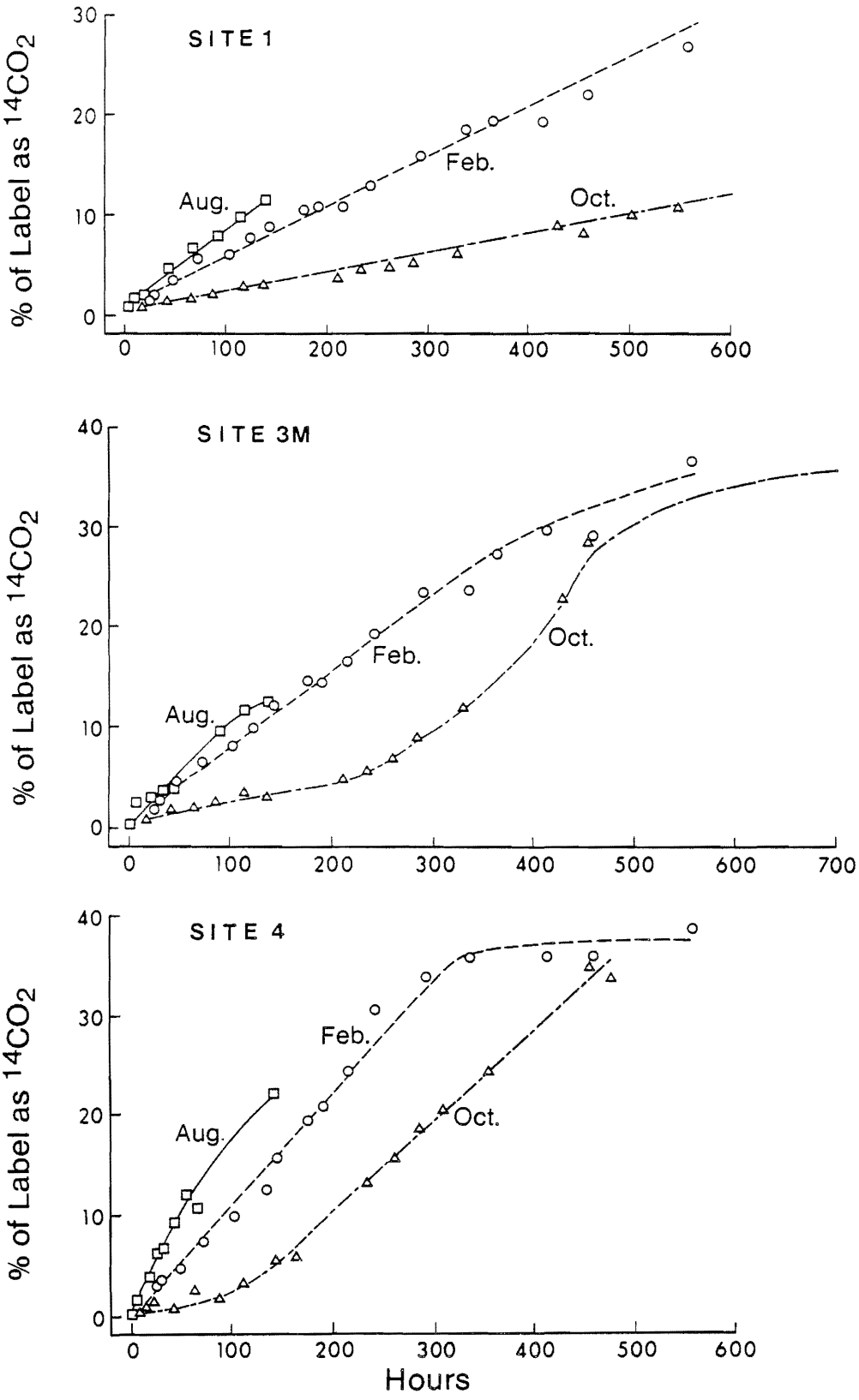


Figure 12. The mineralization of ^{14}C-starch in water sampled at three sites along the Athabasca River.

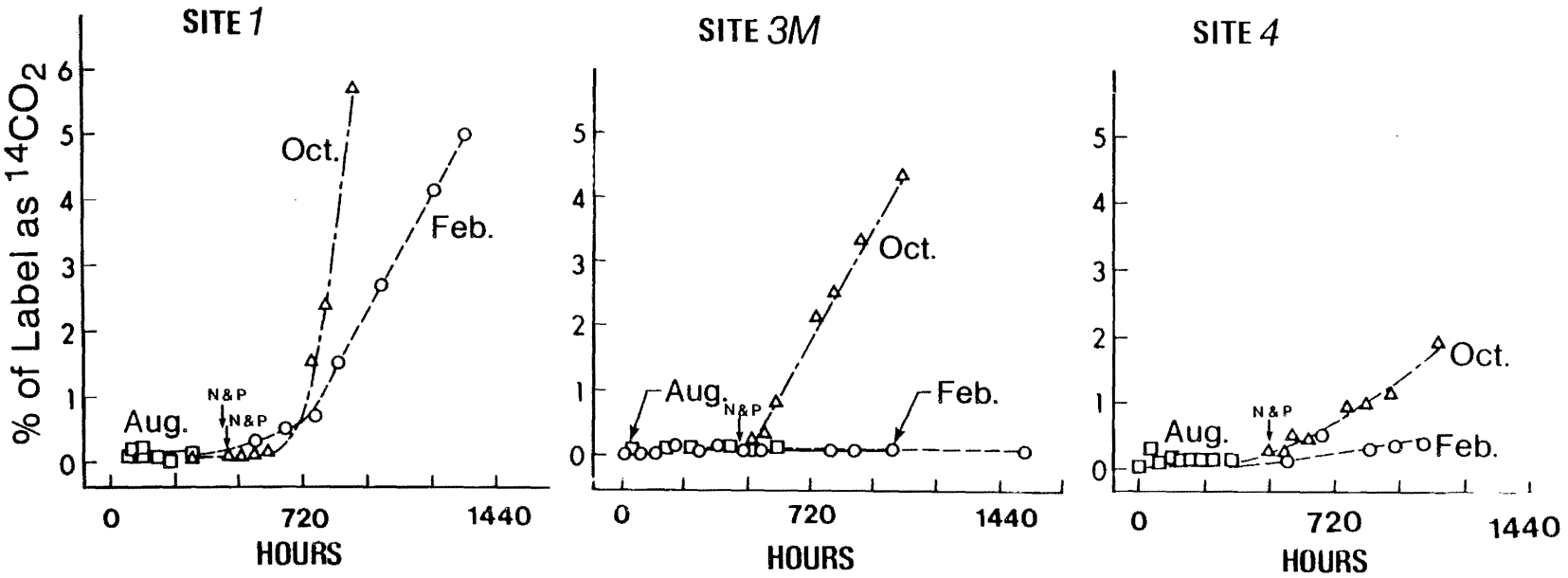


Figure 13. The mineralization of ^{14}C-anthracene and the effect of nutrient addition in water sampled at three sites along the Athabasca River.

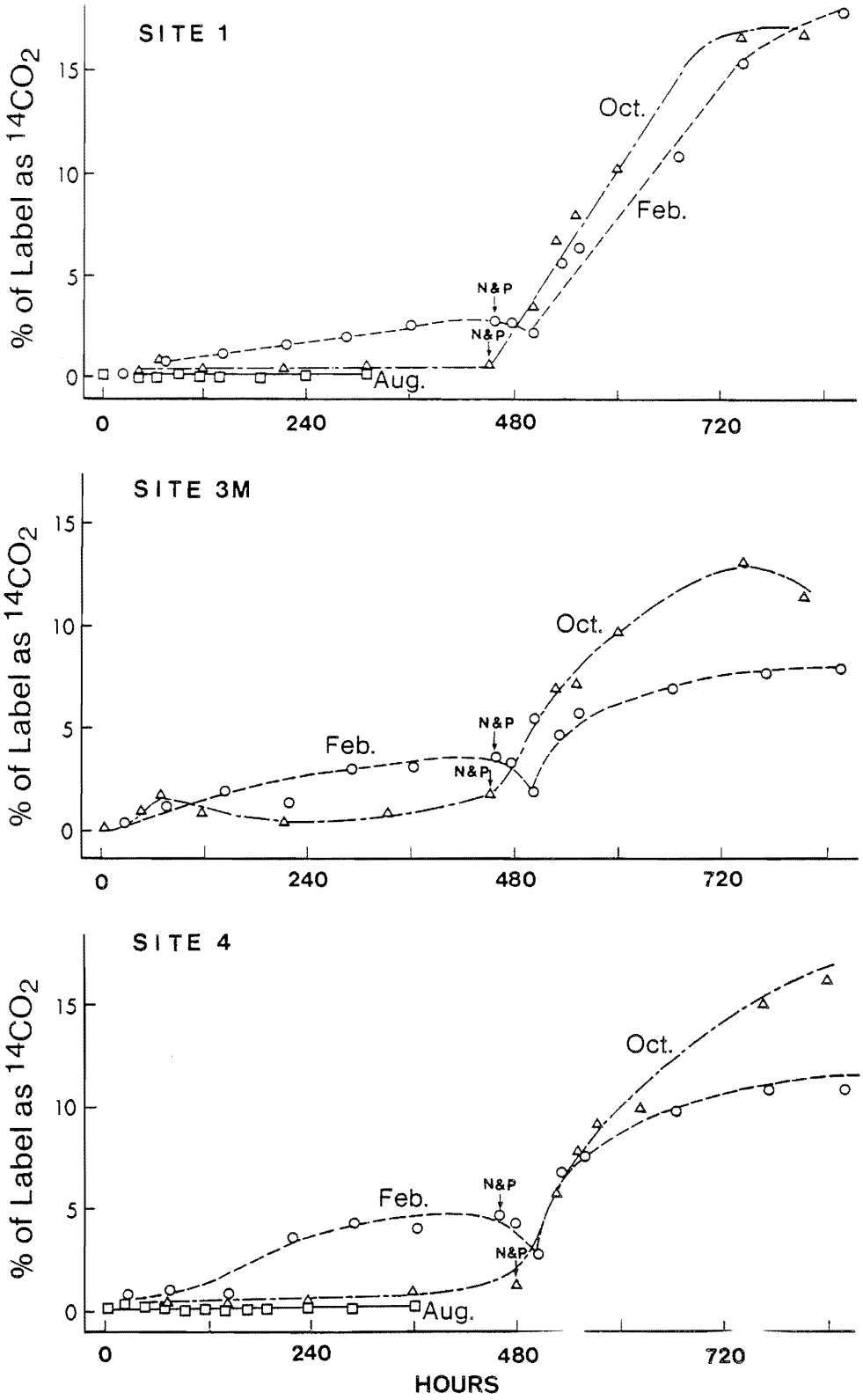


Figure 14. The mineralization of ^{14}C-hexadecane and the effect of nutrient addition in water sampled at three sites along the Athabasca River.

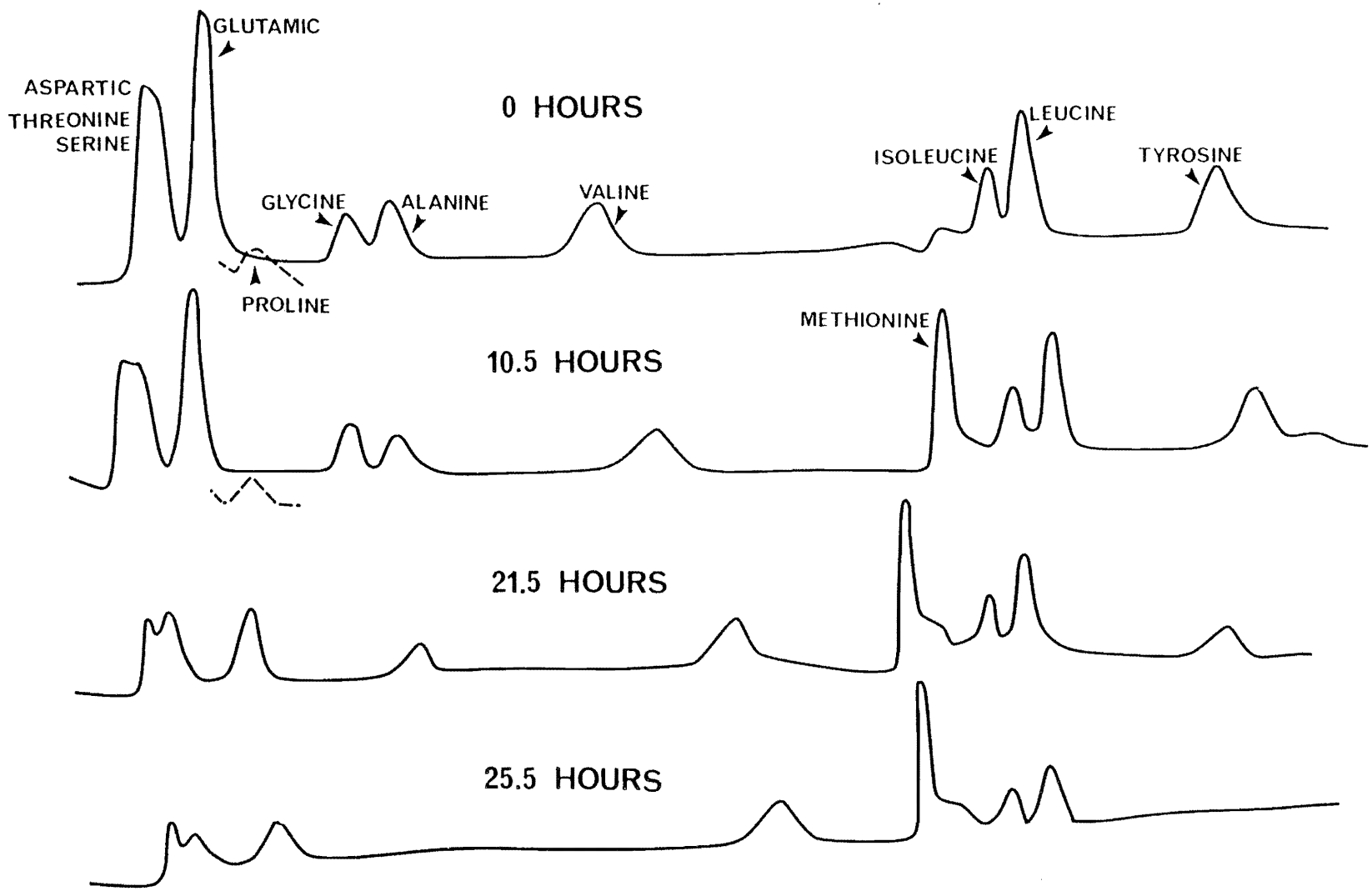


Figure 15. Depletion of ^{14}C-amino acids from Athabasca River water sampled at Site 3, October 1979.

during the initial 25-h incubation period. Water samples from all three sites yielded similar $^{14}CO_2$ evolution patterns from the amino acid mixture, with the October 1979 samples tending to yield the least total $^{14}CO_2$ evolved and the August 1979 samples the highest amount of $^{14}CO_2$.

The metabolism of starch proceeds at a slower rate than that of the amino acids but is comparable to that of the hydrocarbons when the latter incubations receive an addition of nitrogen and phosphorus. This is probably due to the fact that starch, while being an energy rich compound, does not contain nitrogen unlike the amino acids. Only 30 to 40% of the carbon supplied as starch was released as $^{14}CO_2$ as compared to the 50 to 70% yield from the labelled amino acids. Highest rates of starch catabolism were observed with the August (1979) and February (1980) water samples. The highest rates of $^{14}CO_2$ evolution were observed with water samples from Site 4 and the lowest from Site 1. Water from the downstream sites have a greater capacity to catabolize starch than water from Site 1.

Only low levels of $^{14}CO_2$ were evolved from the n-alkane ^{14}C-labelled hexadecane and none from the labelled aromatic hydrocarbon anthracene in the absence of added nitrogen and phosphorus. The highest yields of $^{14}CO_2$ (greater than 15%) from hexadecane were obtained with water from Site 1. Similar patterns of $^{14}CO_2$ release were obtained with water samples from the other sites examined. The fastest rates of release and the highest yields of $^{14}CO_2$ (5%) from anthracene were also obtained with water from Site 1. The lowest metabolism of anthracene was observed with water samples from Site 4. Very little activity was observed with February (1980) water samples from Site 3 and 4 whereas the activity of water taken at Site 1 in February (1980) was only slightly reduced as compared to the October (1979) sample.

The data obtained in these studies indicate a marked difference in the capability of Athabasca River water to bring about the mineralization of non-hydrocarbon and hydrocargon substrates. The latter compounds require the addition of nitrogen and/or phosphorus before rapid mineralization is observed.

The higher yields of $^{14}CO_2$ from the amino acids mixture and starch as compared to the hydrocarbons is a result of the former compounds being uniformly labelled. All CO_2 produced from such compounds would be radioactive whereas only certain carbon atoms in the specifically labelled hydrocarbons would yield $^{14}CO_2$. The diverse metabolic pathways available to microorganisms, which in many cases are dependent on the substrate being catabolized, also will determine which carbon atoms are released as CO_2. A 100% recovery of radioactive carbon cannot be expected when measuring only mineralization as some carbon will be assimilated into cellular material and possibly be present as partially oxidized molecules (Herbes and Schwoll 1978).

The difference in yields and rates of $^{14}CO_2$ release between the amino acids and starch mineralization is partly due to different metabolic pathways being used by microorganisms for the degradation of these substrates. The initial products of the de-amination of the amino acids are intermediates in metabolic pathways (e.g., tricarboxylic acid cycle) which readily yield CO_2. In contrast, the product of the initial metabolism of starch, glucose units, usually utilizes many metabolic steps before CO_2 is released. The differences in yields and rates of $^{14}CO_2$ release are also due to the fact that there are many more microorganisms in an aquatic environment which can readily utilize amino acids than there are which can utilize starch.

The mineralization of the amino acids and starch proceeds in the main via constitutive enzymes (i.e., those that are always present in a cell). On the other hand, the catabolism of the hydrocarbons requires the induction of several enzymes before the products reach pathways which yield large amounts of CO_2. Thus, the catabolism of the hydrocarbons would require an exogenous source of nitrogen and/or phosphorus in order to allow the production of these enzymes. The requirement for a nutrient supplement before the rapid metabolism of hydrocarbons can occur in aquatic environments is a well-established phenonmenon (Horowitz and Atlas 1977).

The difference in the yields and rates of $^{14}CO_2$ release between the hydrocarbons studied is related in part to the differences in their molecular structures. The catabolism of both of these substrates would require, as the initial step, the introduction of oxygen atoms via different oxygenases. The mineralization of the aliphatic n-alkane, hexadecane, would proceed via β-oxidation which yields acetate units whose catabolism would readily yield CO_2. In contrast, the release of the No. 9 carbon atom of the aromatic hydrocarbon anthracene requires many steps, all requiring the induction of specific enzymes, before this carbon atom would be released as $^{14}CO_2$. Similar studies on a marine system (Westlake and Cook 1980) yielded similar results (i.e., a more rapid release of $^{14}CO_2$ from labelled hexadecane than from anthracene).

The data suggest that seasonal and spatial differences do exist in the capability of river water to mineralize the complex aromatic hydrocarbon anthracene. Maximum mineralization of anthracene and hexadecane occurred with water samples taken from Site 1. Very little difference was observed with the mineralization patterns of hexadecane from Site 3 and 4 whereas anthracene was metabolized only to a limited extent by the microorganisms present in the water from Site 4. Seasonal differences are quite apparent, particularly with anthracene, with the February samples showing a reduced ability to mineralize the hydrocarbons.

The effects of bitumen on the patterns of $^{14}CO_2$ evolution from uniformly labelled amino acids and 1-^{14}C-hexadecane are present in Figure 16. Bitumen affected only the mineralization of amino acids during the later stages of catabolism. In contrast, bitumen resulted in a slower rate of mineralization of hexadecane. This is probably a result of the hydrocarbon being soluble in the bitumen unlike the polar amino acids, and thus decreases its availability.

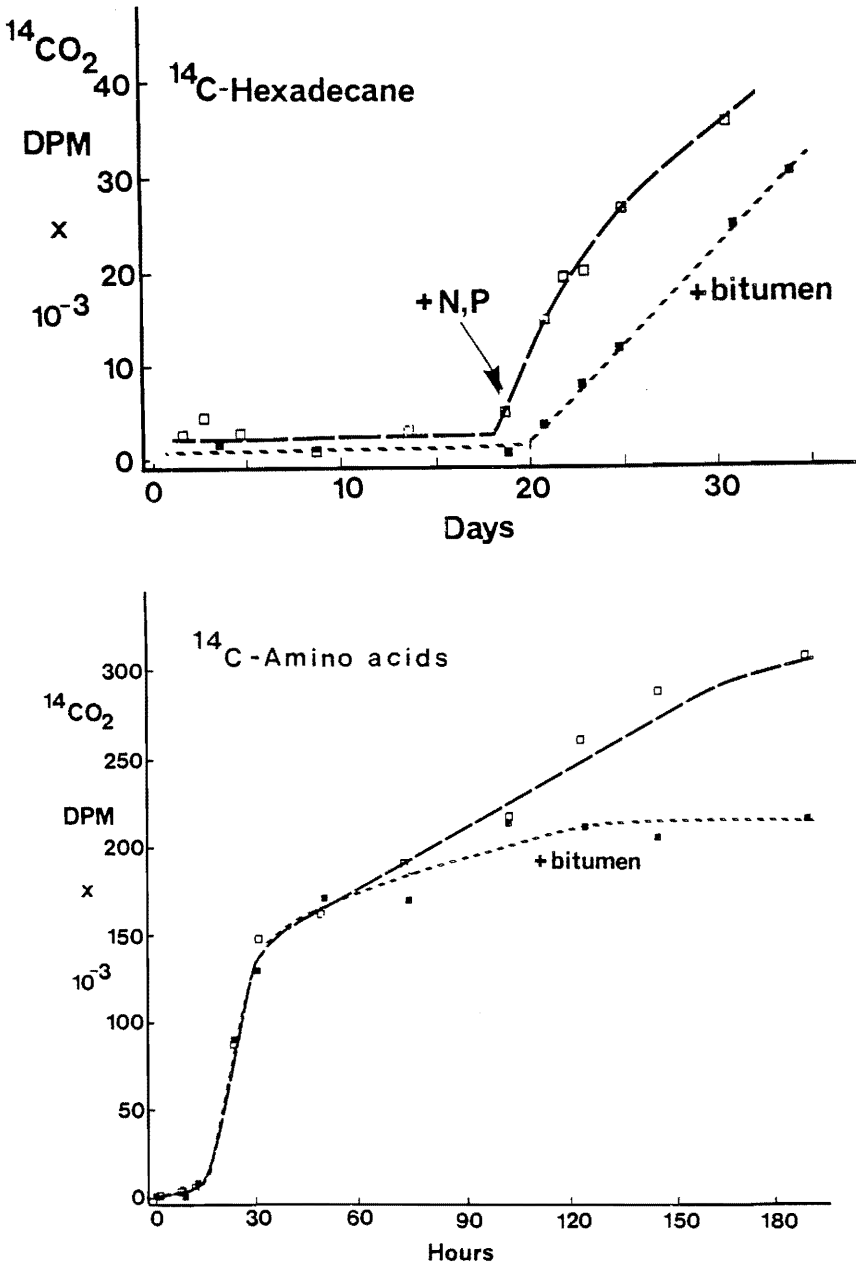


Figure 16. The effect of bitumen on the mineralization of ^{14}C-hexadecane and ^{14}C-amino acids in Athabasca River water sampled at Site 3M, February 1980.

4. SUMMARY

The results of this study have shown it is possible to utilize laboratory incubations of Athabasca River water or water-sediment and analysis by GC/MS to determine the extent of microbial degradation of trace amounts (100 µg/L) of selected organic compounds added to incubation flasks. In this way, the depletion of a specific compound can be compared both spatially and temporally. Both m-cresol and camphor were degraded most rapidly at Site 1, above the oil sands region, and at Site 3, just downstream from the oil sands plants. Because of the limited number of sample sites (four), it was not possible to determine with complete confidence the reasons for this higher level of bacterial activity at these sites.

The results from three locations on a transect of the river at Site 3 showed a trend of increased metabolism of these two substrates near the west bank, the same side of the river as the upstream oil sands plants. This may indicate an effect of drainage waters and/or effluents on the bacterial populations in the Athabasca River.

Using this methodology, it should be possible to determine the extent of biodegradation of specific compounds identified in waste streams from oil refineries or drainage water from the oil sands area. The need for this type of work may be determined by information, as yet unavailable, relating the toxicity of waste streams to the identification of specific compounds.

The data also indicate a higher rate of bacterial activity in sediments as compared to river water. Since sediments may act as a sink for various organic or inorganic compounds originating from oil extraction and refining activities (Noton 1979), it may be possible to incubate water-sediment samples and analyze the depletion of either indigenous or artificial organic compounds as a biomonitoring technique.

Using GC/MS, it was possible to tentatively identify several indigenous compounds in incubations of Athabasca River water-sediment. Because of their low concentration, samples had to be combined and no data regarding the extent of their degradation were obtained. It

is probable that samples from waste streams, presumably containing higher concentrations of organic compounds, may be more easily analyzed using these techniques.

In related experiments, the incubation and mineralization of ^{14}C-labelled substrates in Athabasca River water has shown a higher degree of degradation for "natural" compounds, such as amino acids and starch, than for compounds associated with the oil industry, such as the hydrocarbons hexadecane and anthracene. Hydrocarbons, it should be noted, also occur naturally in the Athabasca River (Smith 1979).

Amino acids were mineralized at approximately uniform rates within the study area, while starch was mineralized at a faster rate downstream. Both hexadecane and anthracene were not mineralized to any significant degree until the water was supplemented with nitrogen and phosphorus. Thus, the lack of one or both of these nutrients may limit the biodegradation of hydrocarbons in the Athabasca River, in spite of the evidence (Nix et al. 1979) of large indigenous populations of hydrocarbon-oxidizing bacteria.

The presence of bitumen had no significant effect on the degradation of m-cresol or camphor. It did, however, decrease, the rate of utilization of ^{14}C-hexadecane and ^{14}C-amino acids.

In general, the microbial mineralization of ^{14}C-labelled compounds proceeded at similar rates of degradation for the three sites. The only exception to this was the considerably slower mineralization of anthracene downstream at Site 4. In contrast, the depletion of the two substrates monitored by GC showed a trend of increasing degradation at Sites 1 and 3. Since different substrates were utilized in the two approaches, a direct comparison is not possible.

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6. APPENDICES

6.1 GAS CHROMATOGRAMS OF EXTRACTS FROM UNSPIKED RIVER WATER SEDIMENT SAMPLES FROM SITE 1, AUGUST 1979.

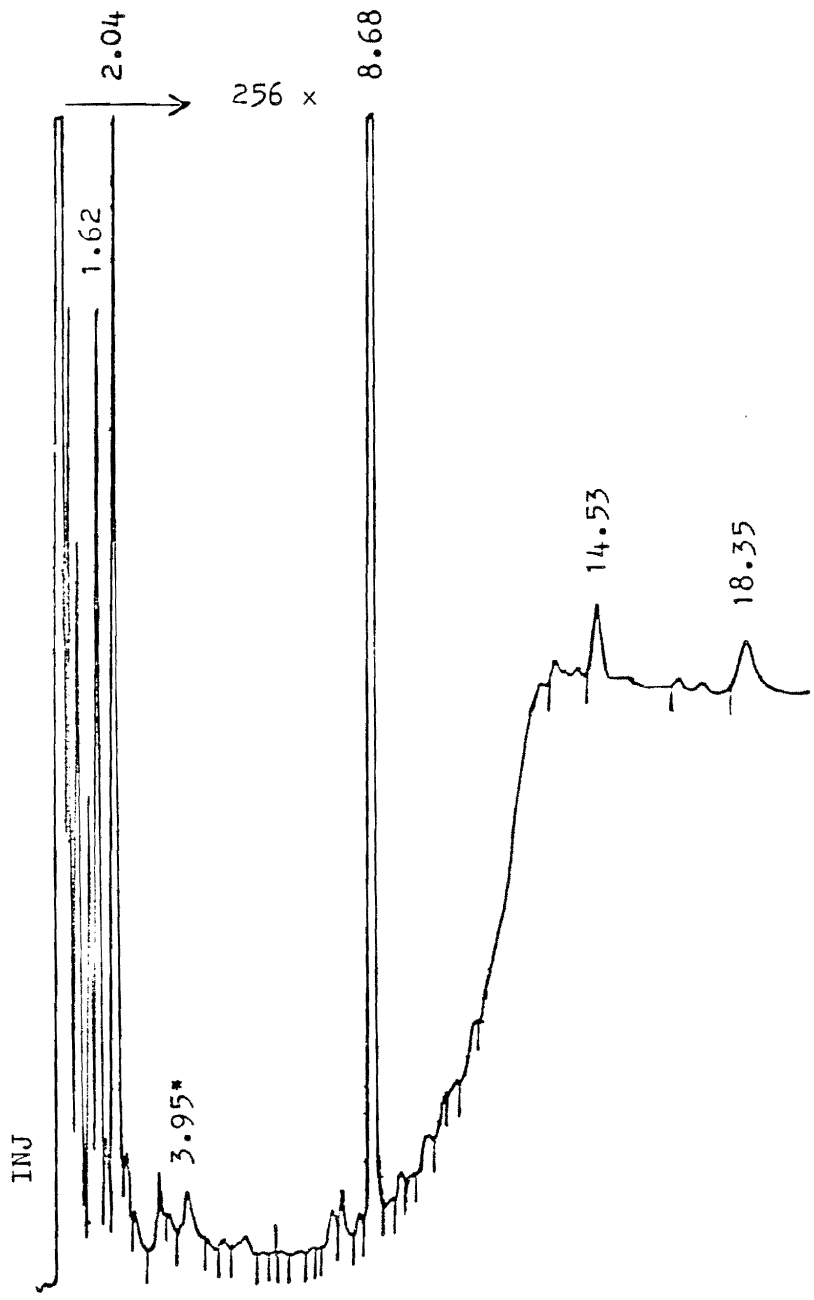

Figure 17. GC trace of organic compounds at Site 1. Time of sampling: 0 d.

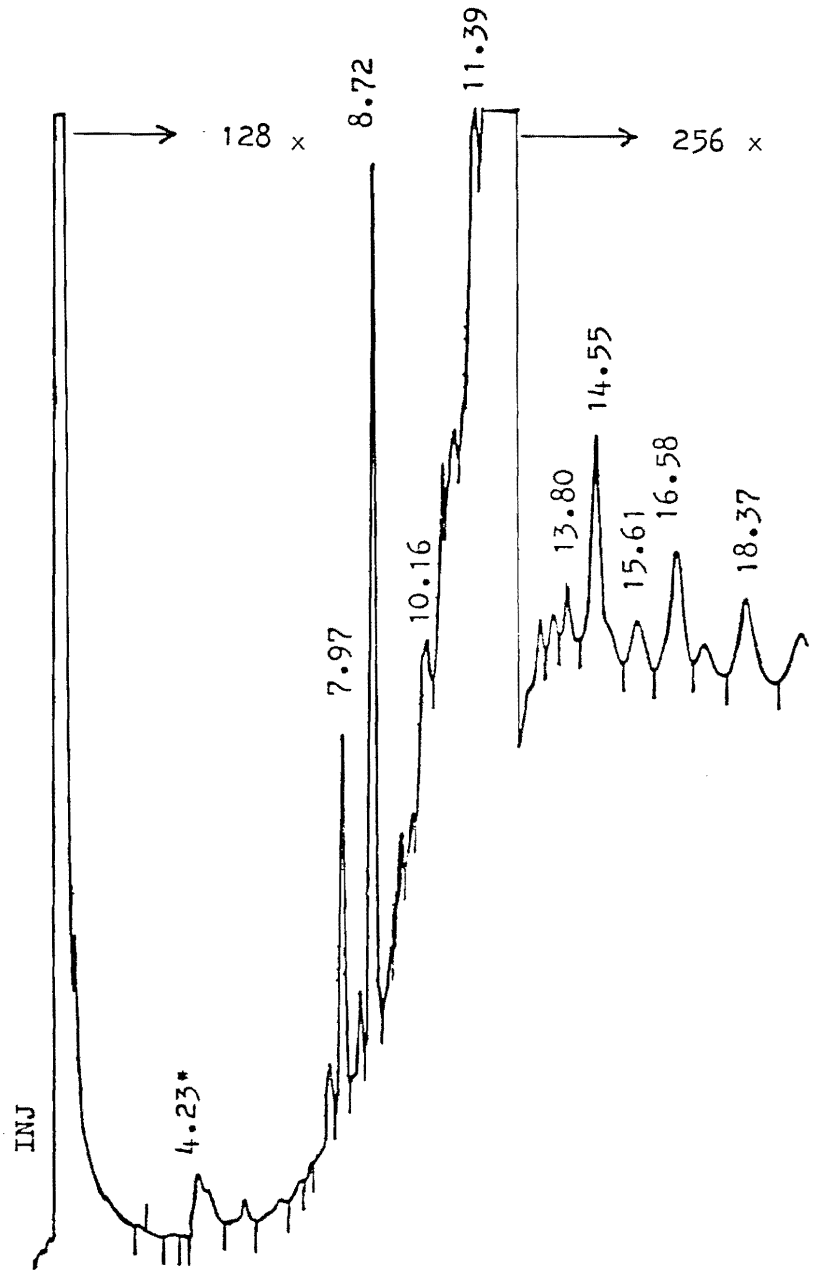

Figure 18. GC trace of organic compounds at Site 1. Time of sampling: 1 d.

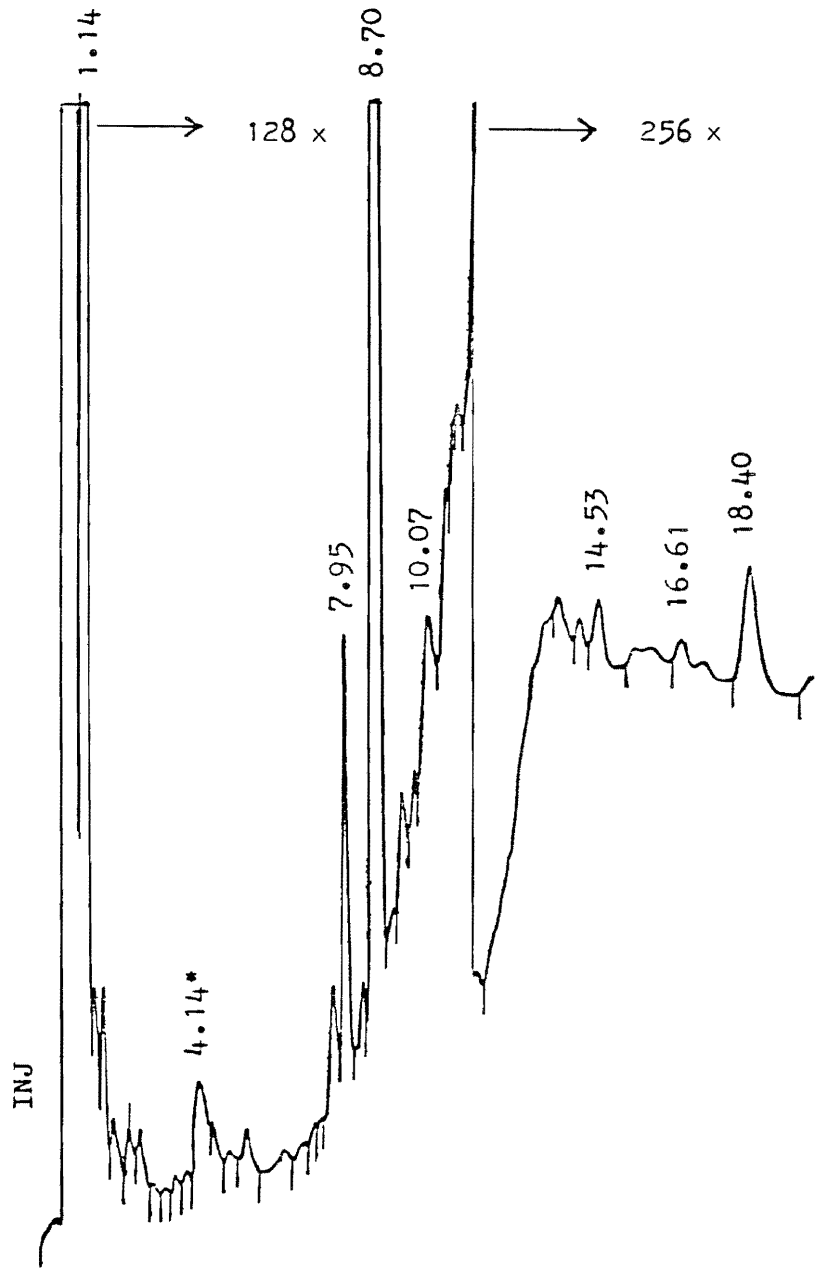

Figure 19. GC trace of organic compounds at Site 1. Time of sampling: 2 d.

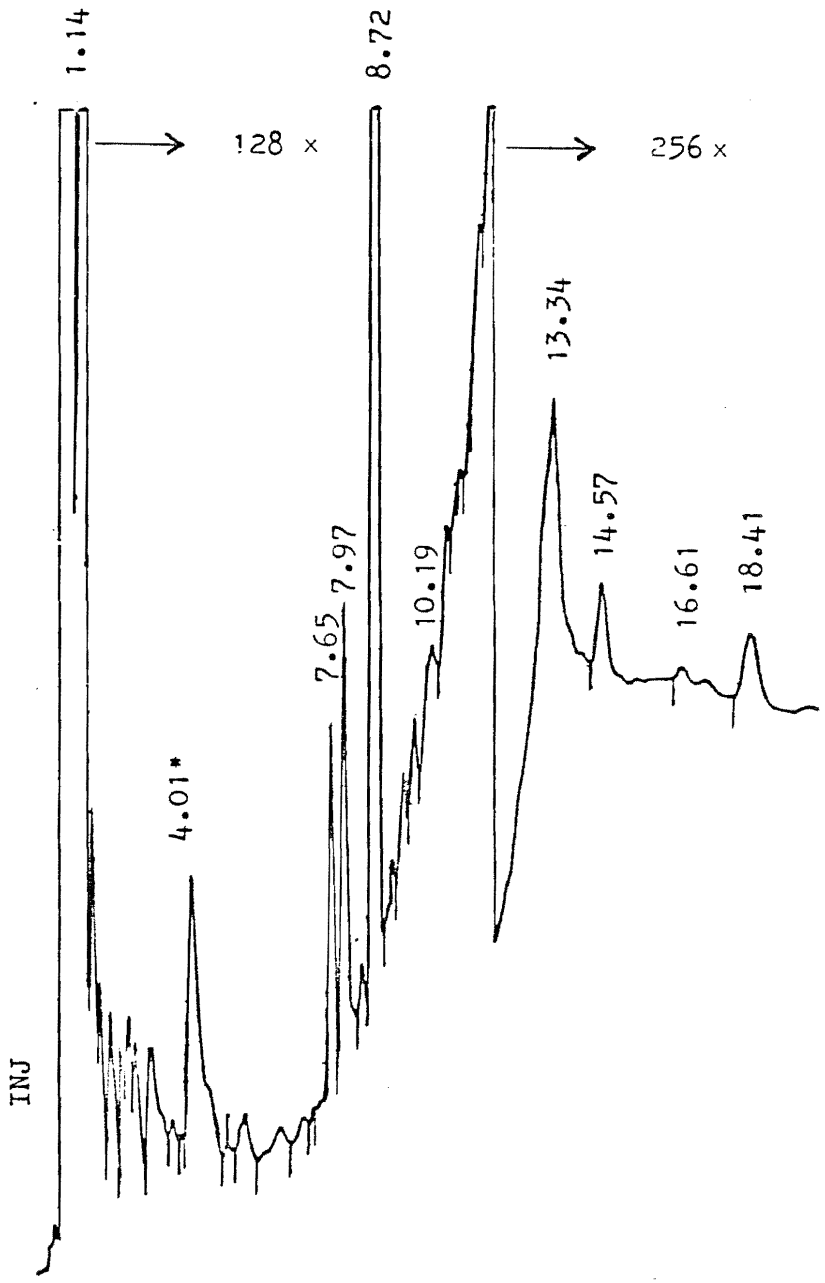

Figure 20. GC trace of organic compounds at Site 1. Time of sampling: 4 d.

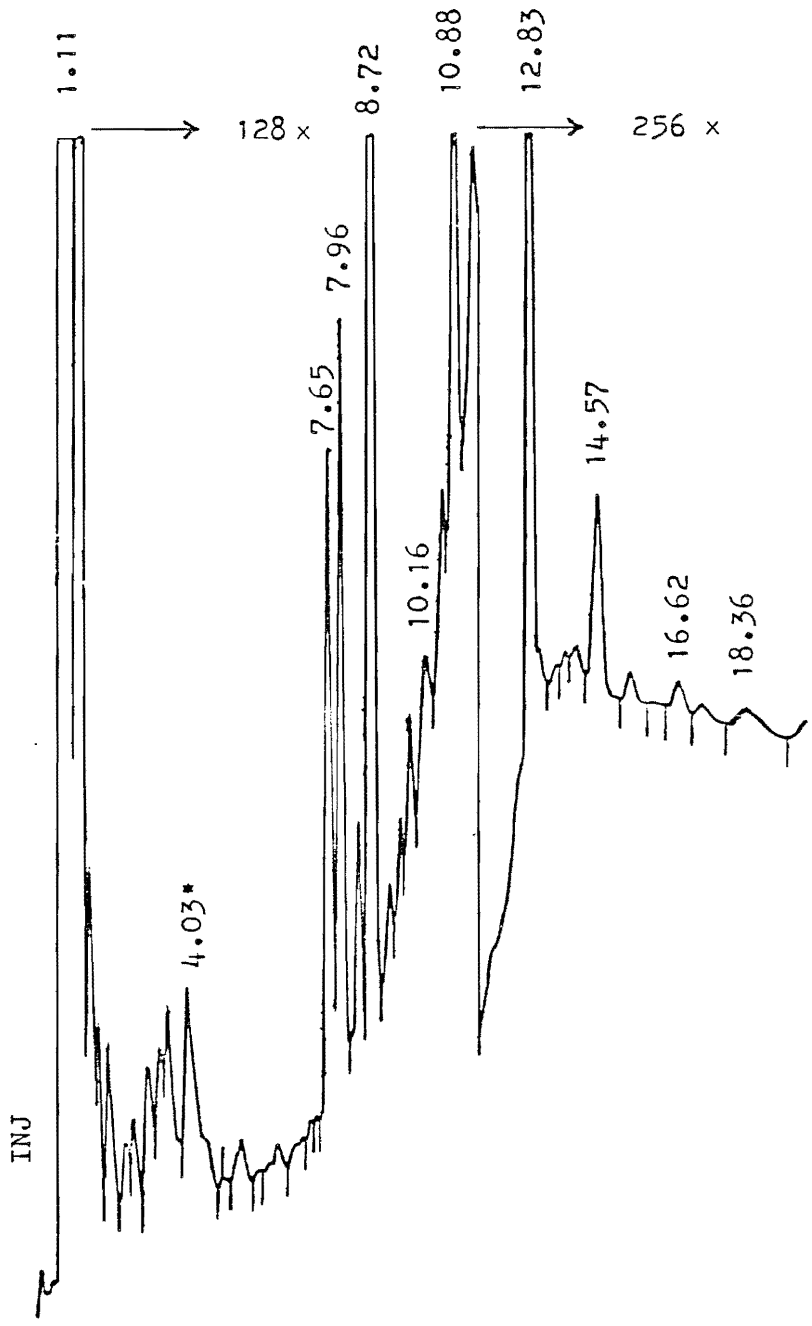

Figure 21. GC trace of organic compounds at Site 1. Time of sampling: 6 d.

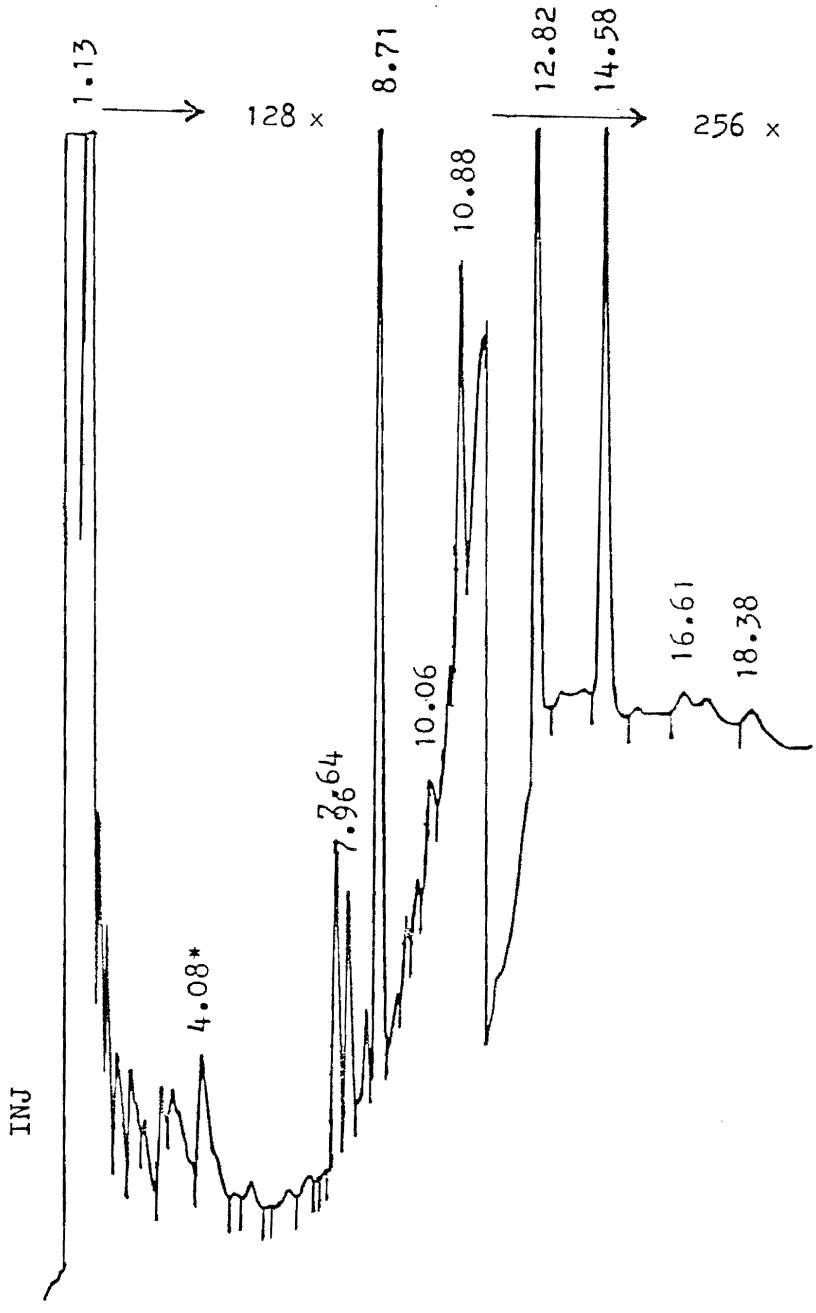

Figure 22. GC trace of organic compounds at Site 1. Time of sampling: 8 d.

6.2 ELECTRON IMPACT GC/MS TOTAL ION TRACE AND MASS SPECTRA OF THE ORGANIC COMPOUNDS PRESENT AT SITE 1, AUGUST 1979.

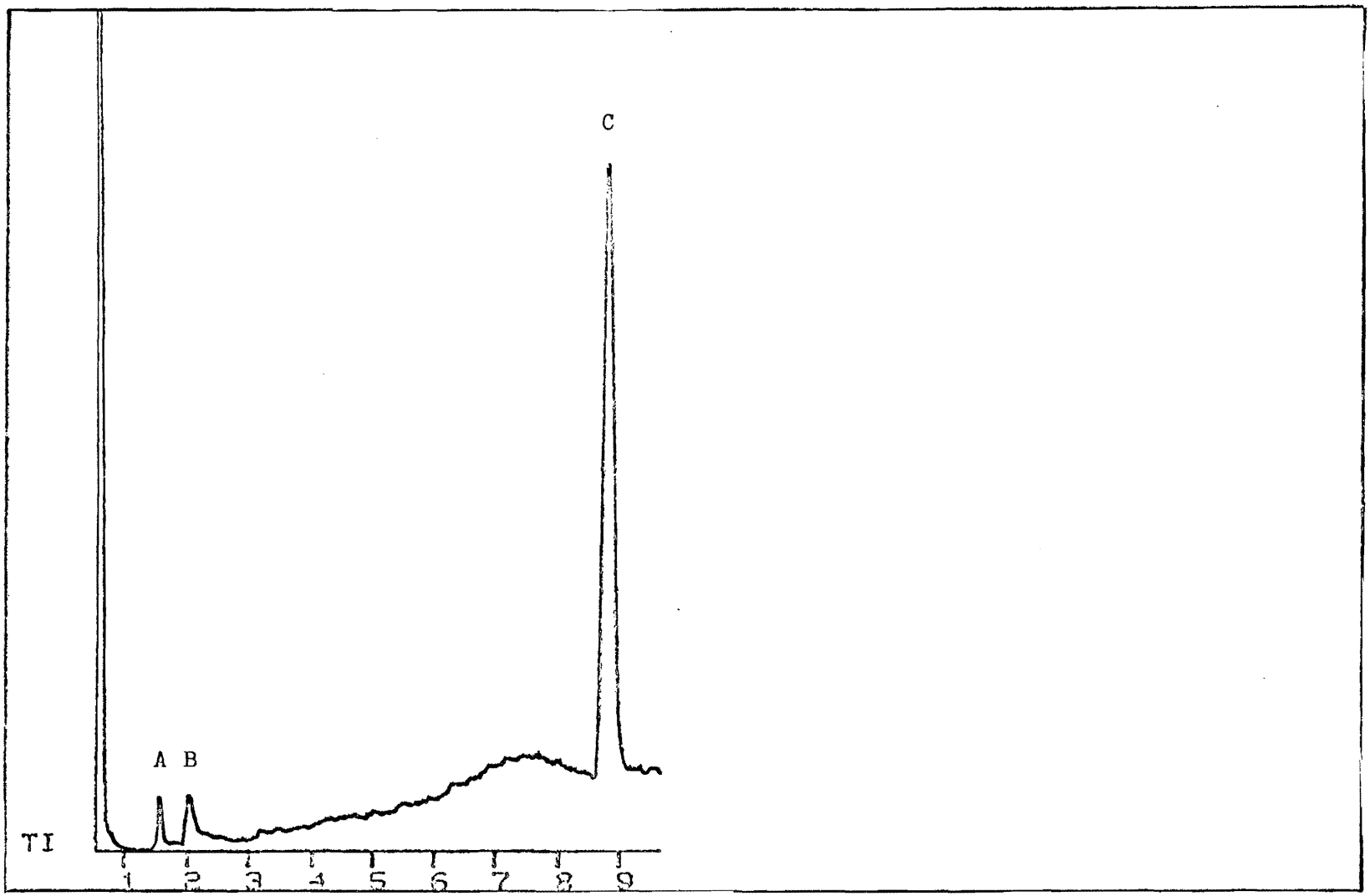

Figure 23. Total ion trace from electron impact GC/MS of combined extracts from Site 1, August 1979.

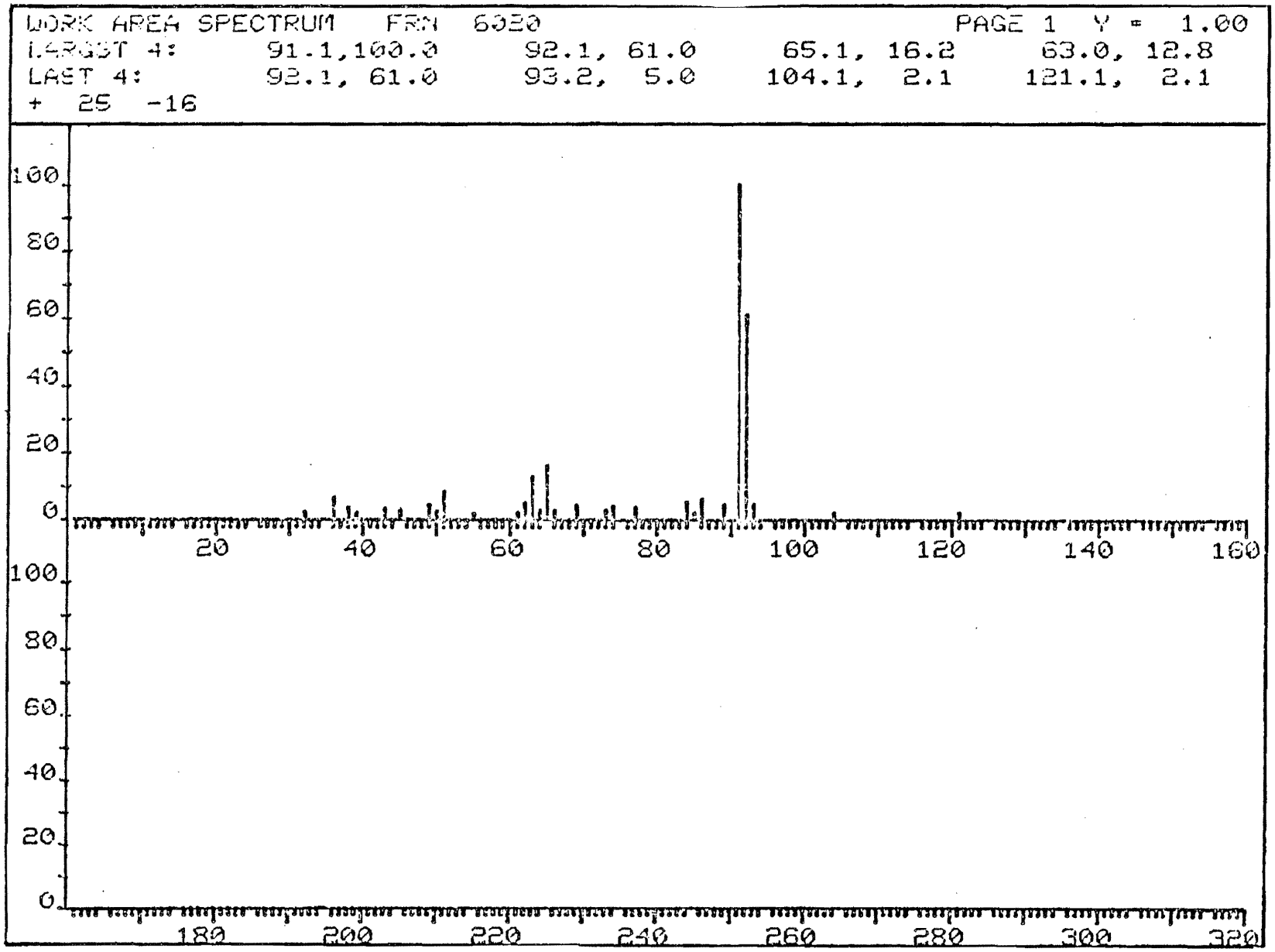

Figure 24. Electron impact mass spectrum of peak A (= peak with retention time of 1.62 min in Figure 17).

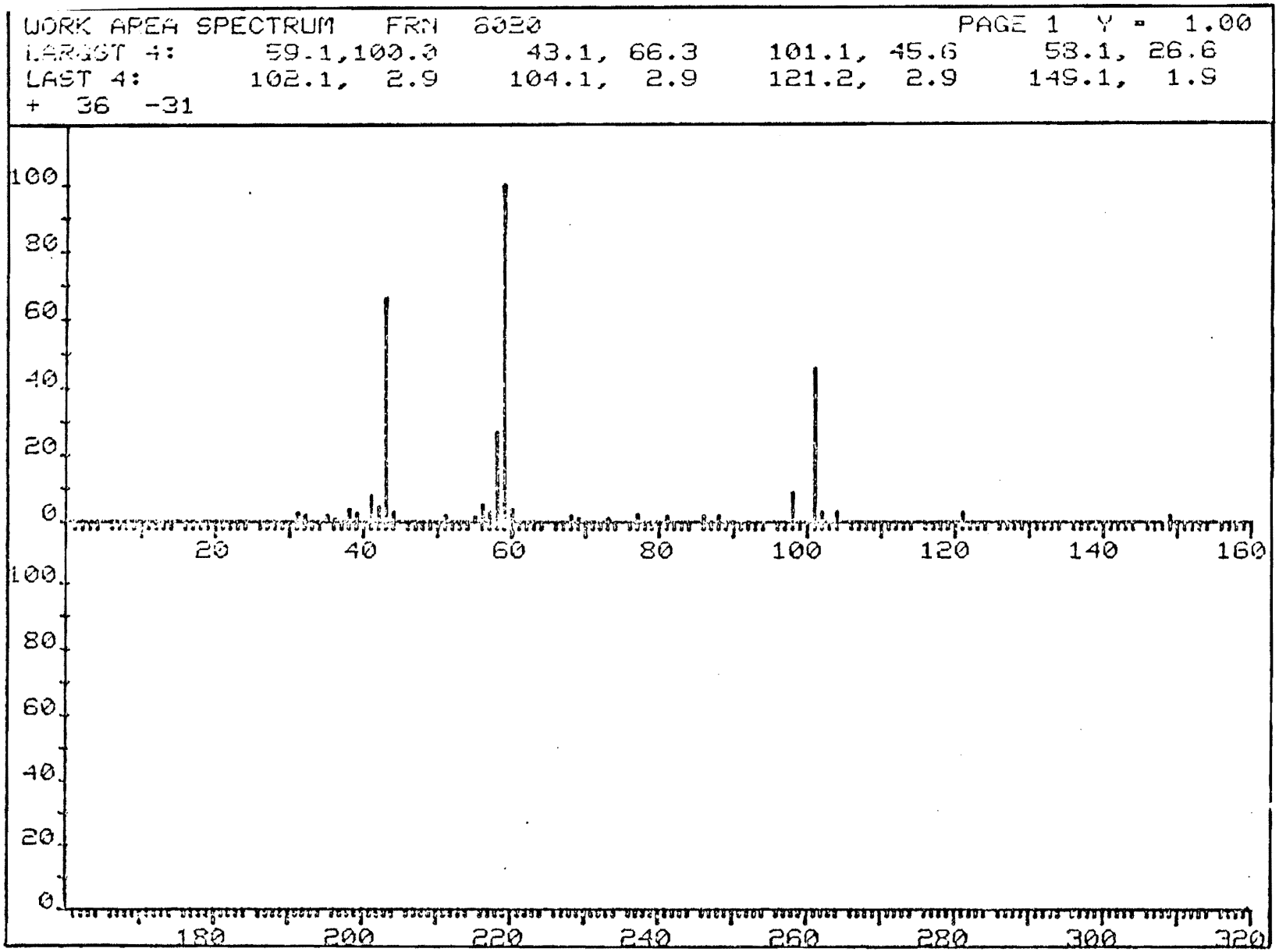

Figure 25. Electron impact mass spectrum of peak B (= peak with retention time of 2.04 min in Figure 17).

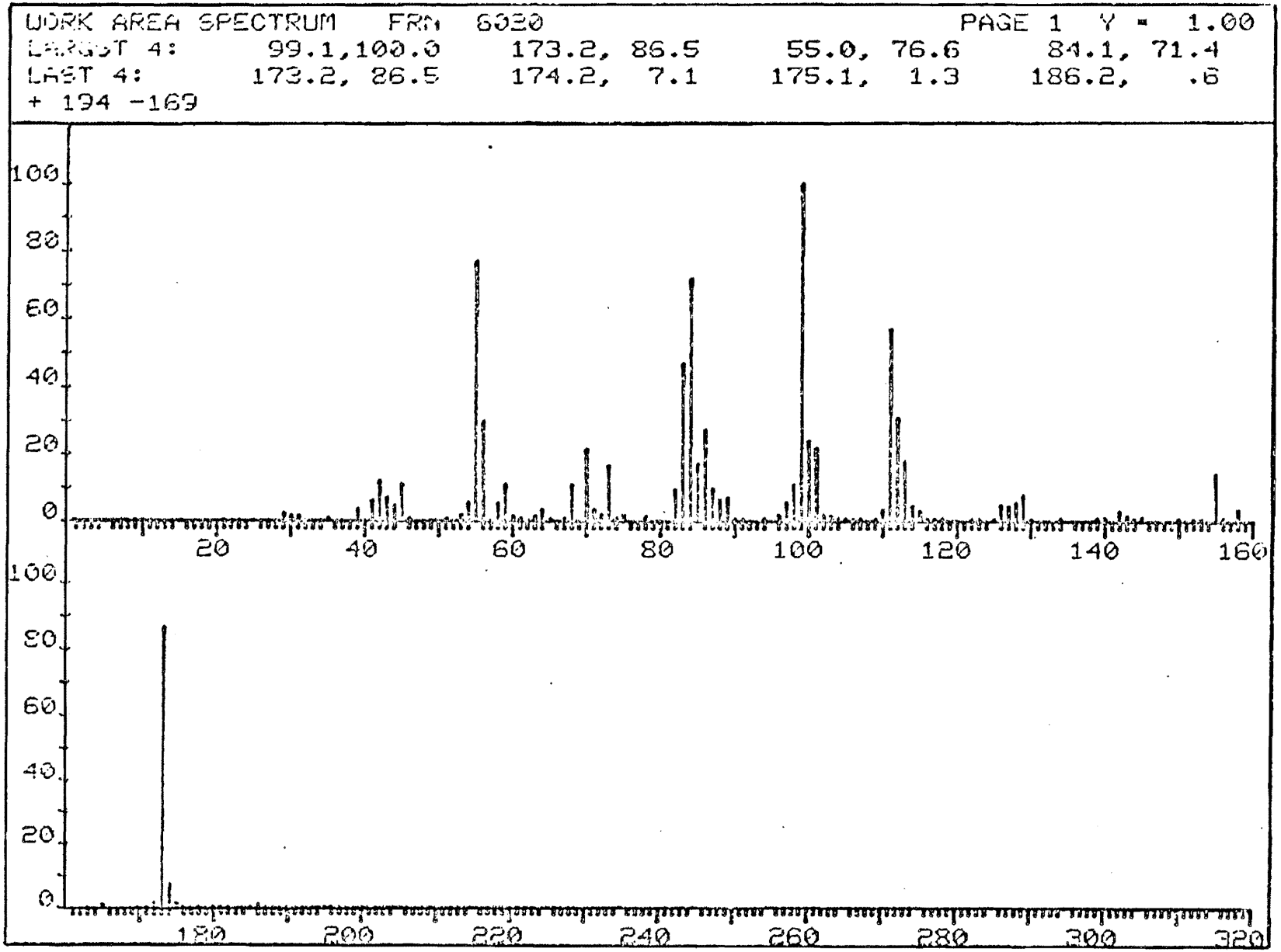

Figure 26. Electron impact mass spectrum of peak C (= peak with retention time of 8.68 min in Figure 17).

6.3 CHEMICAL IONIZATION GC/MS TOTAL ION TRACE AND MASS SPECTRA OF THE ORGANIC COMPOUNDS PRESENT AT SITE 1, AUGUST 1979.

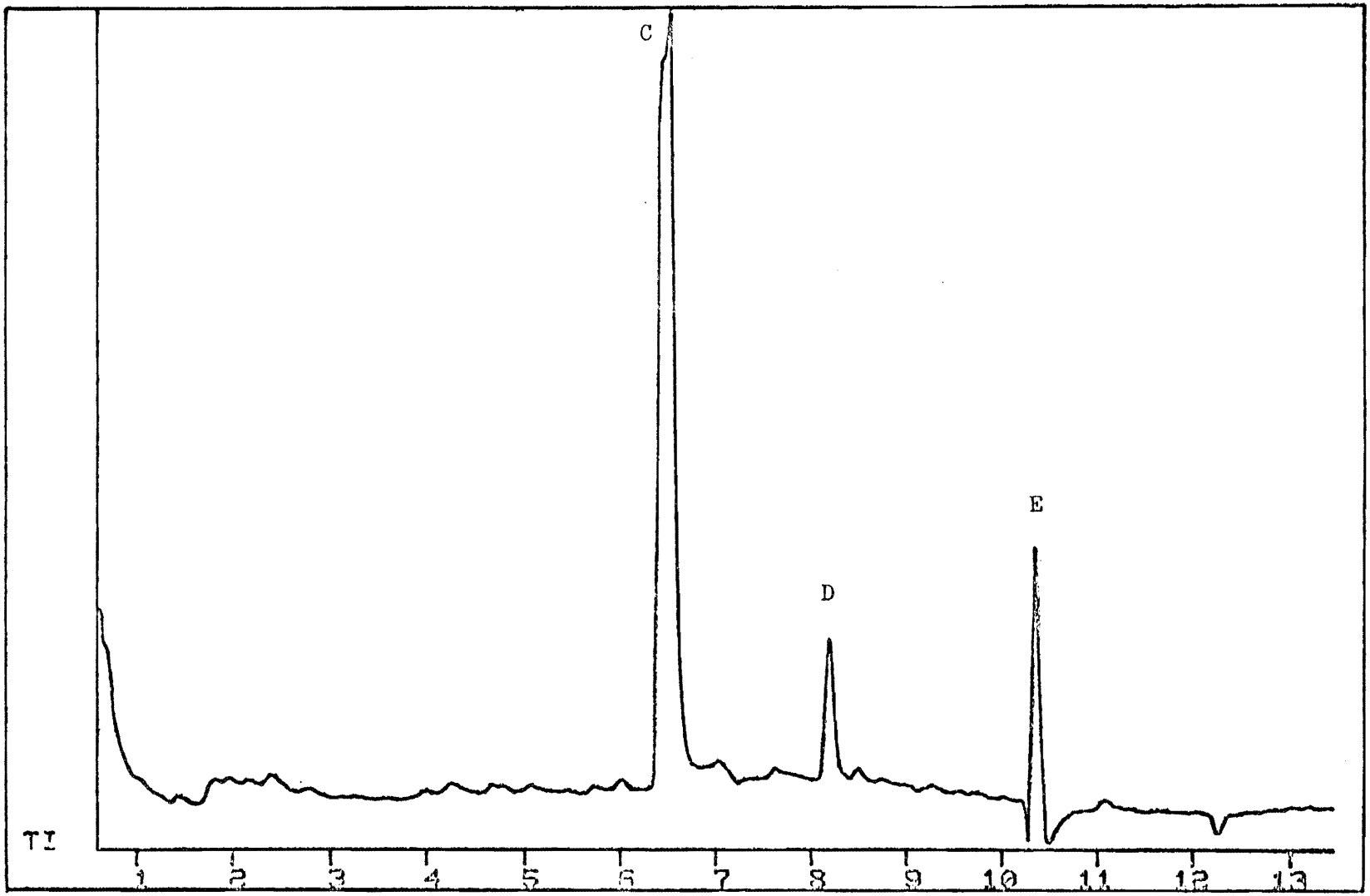

Figure 27. Total ion trace from chemical ionization GC/MS of combined extracts from Site 1, August 1979.

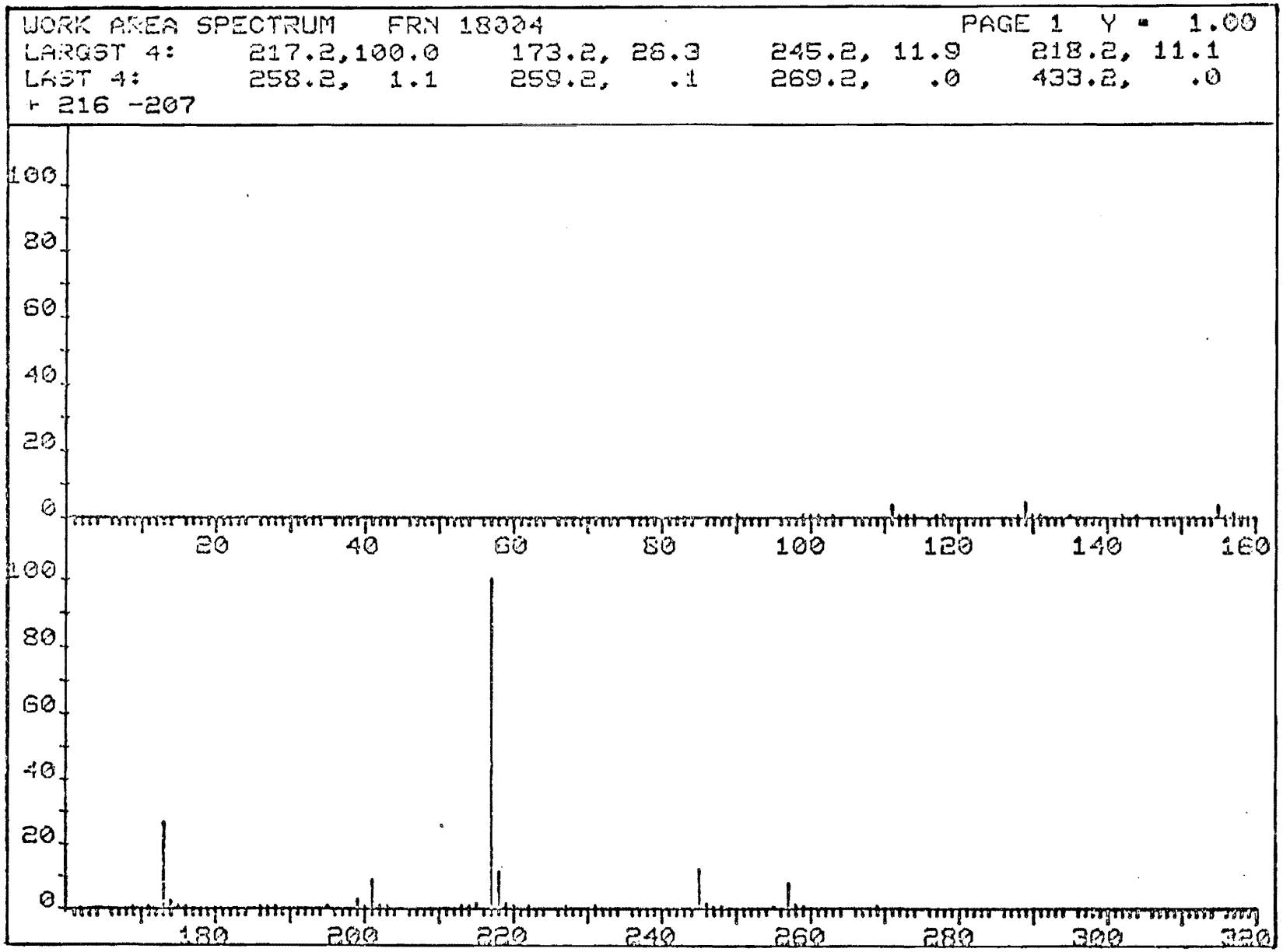

Figure 28. Chemical ionization mass spectrum of peak C (= peak with retention time of 8.68 min in Figure 17).

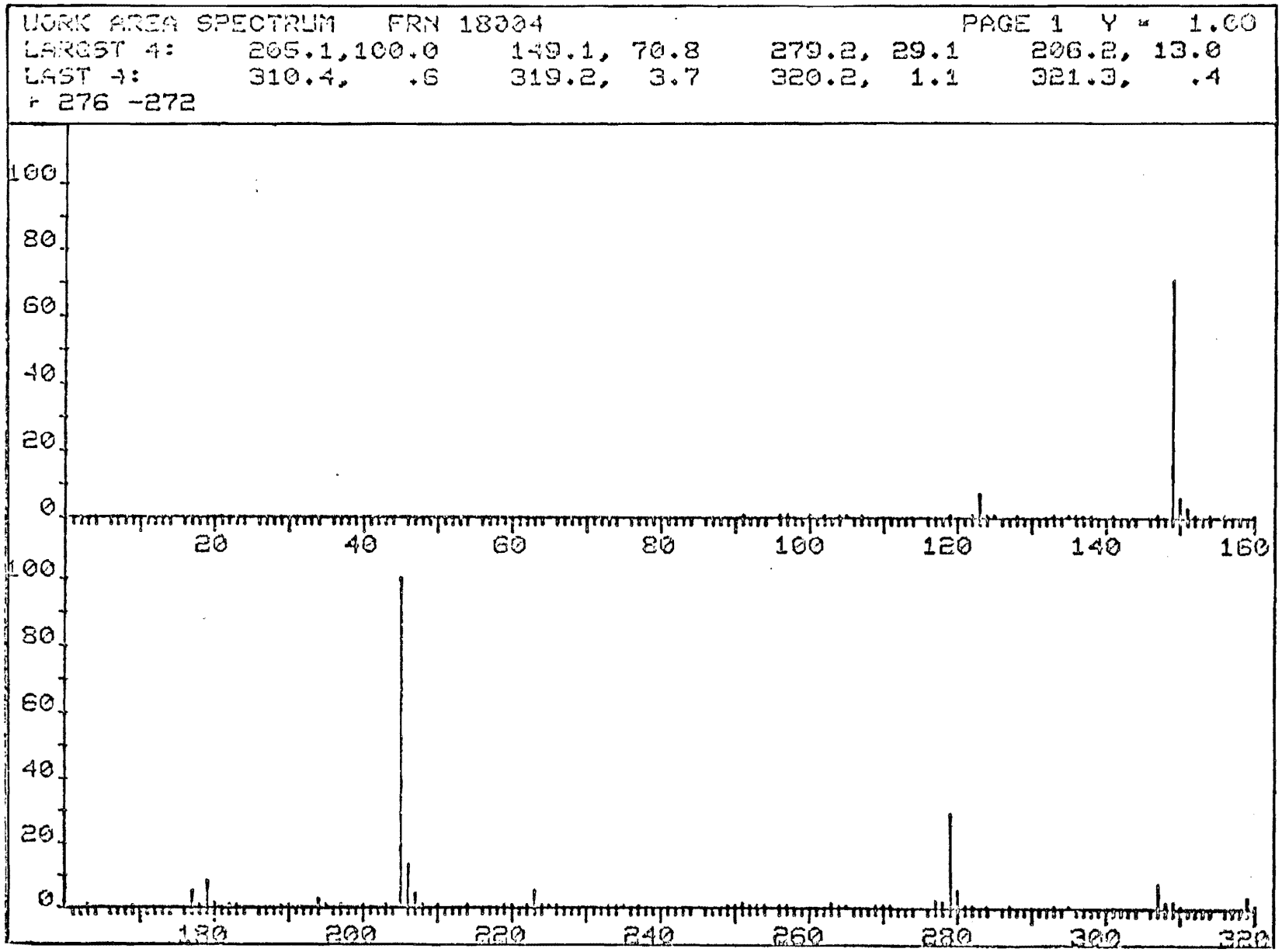

Figure 29. Chemical ionization mass spectrum of peak D (= peak with retention time of 14.53 min in Figure 17).

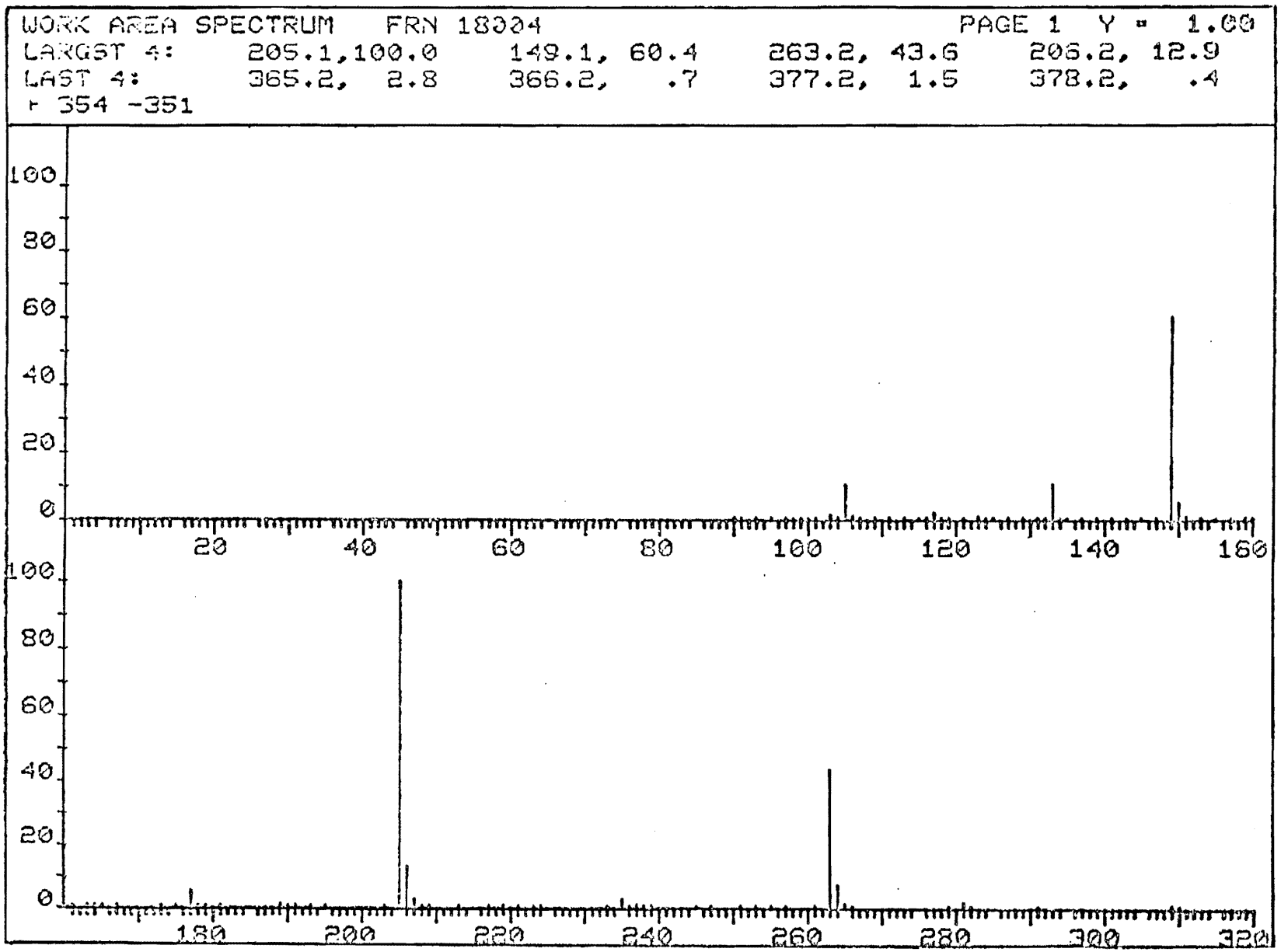

Figure 30. Chemical ionization mass spectrum of peak E (= peak with retention time of 18.35 min in Figure 17).

continued . . .

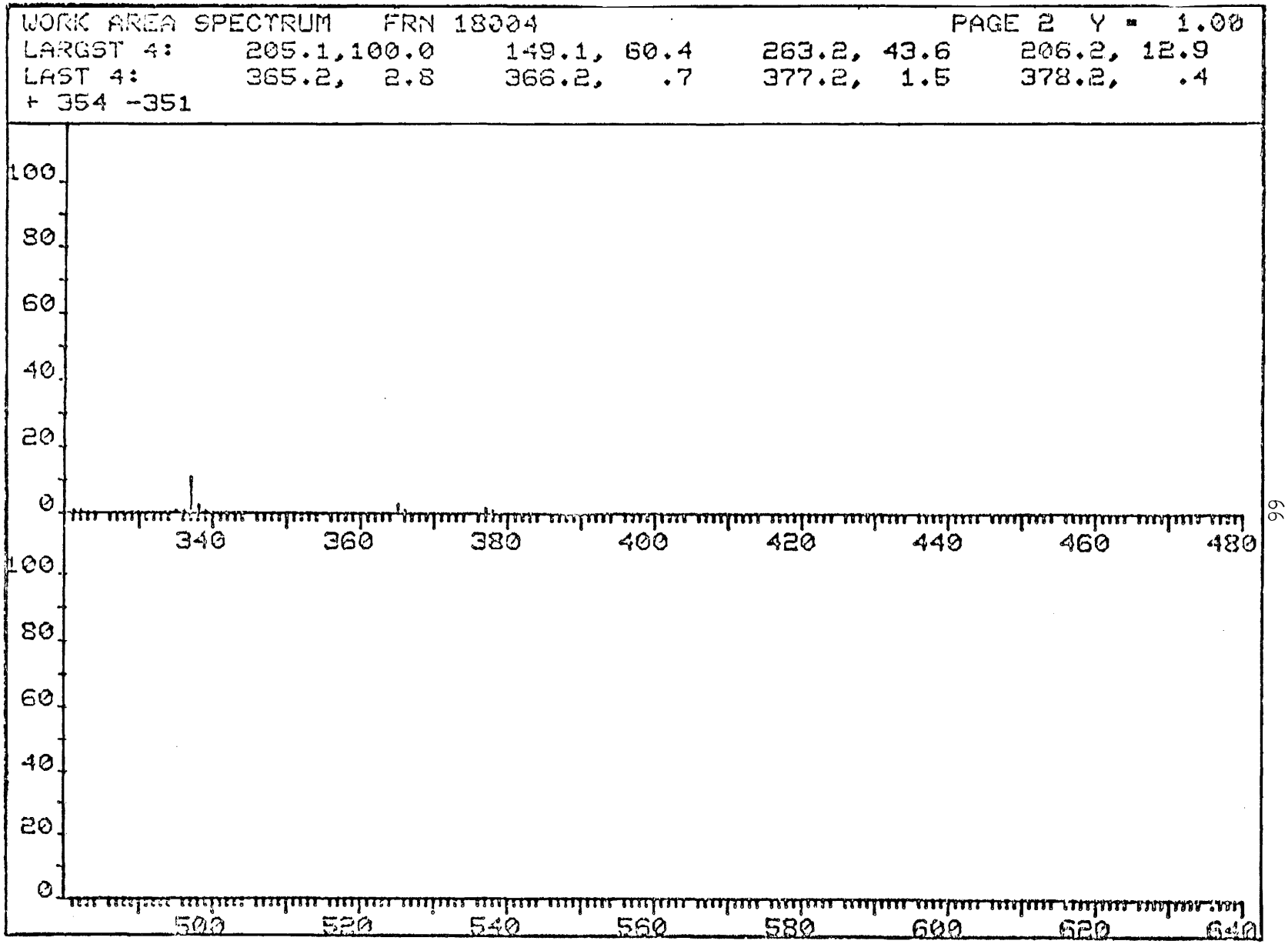

Figure 30. Concluded.

6.4 ELECTRON IMPACT GC/MS TOTAL ION TRACE AND MASS SPECTRA OF THE COMBINED EXTRACTS OF FOUR SAMPLES FROM SITE 1, FEBRUARY 1980, OVER A 12-D INCUBATION PERIOD.

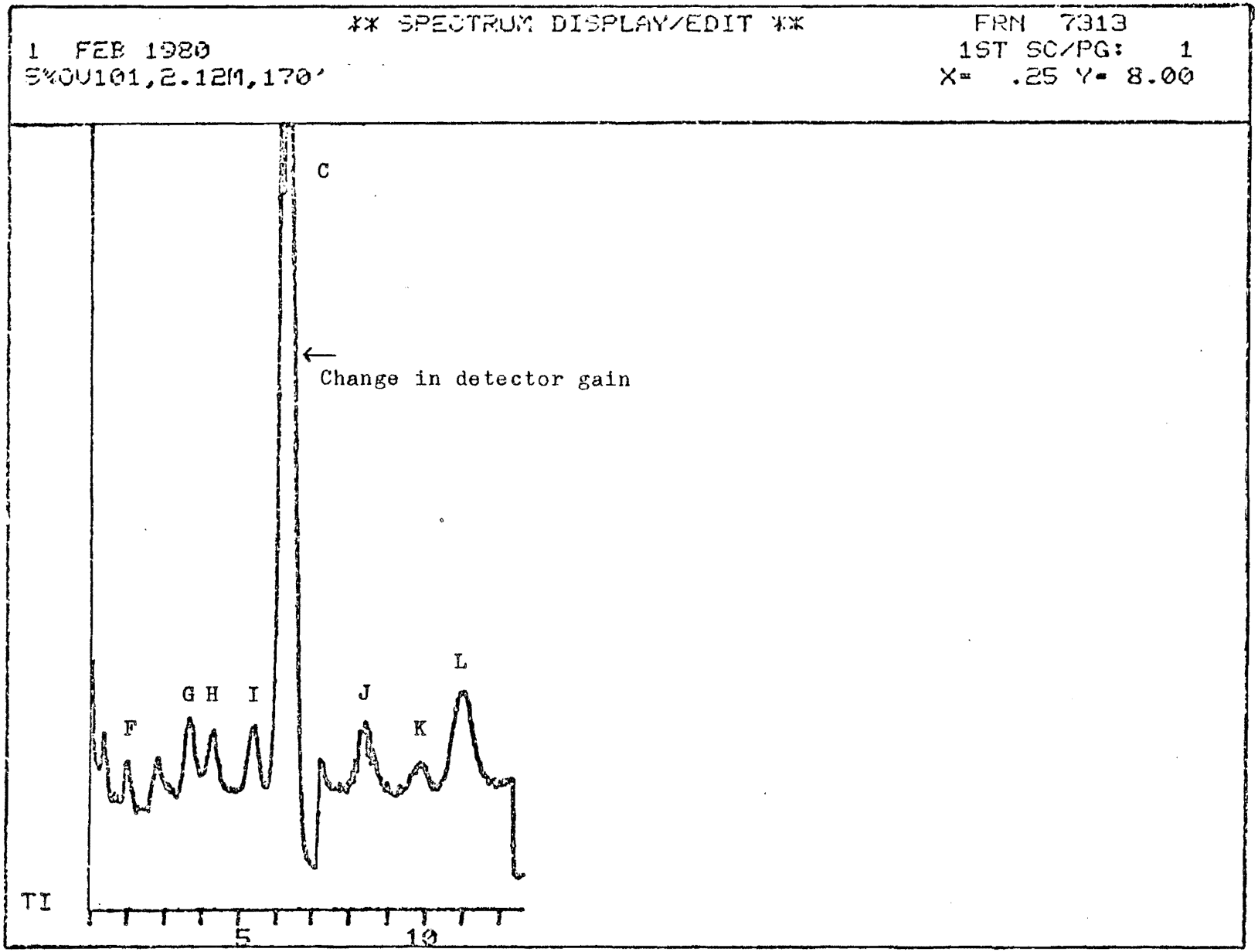

Figure 31. Total ion trace from electron impact GC/MS of combined samples from Site 1, February 1980.

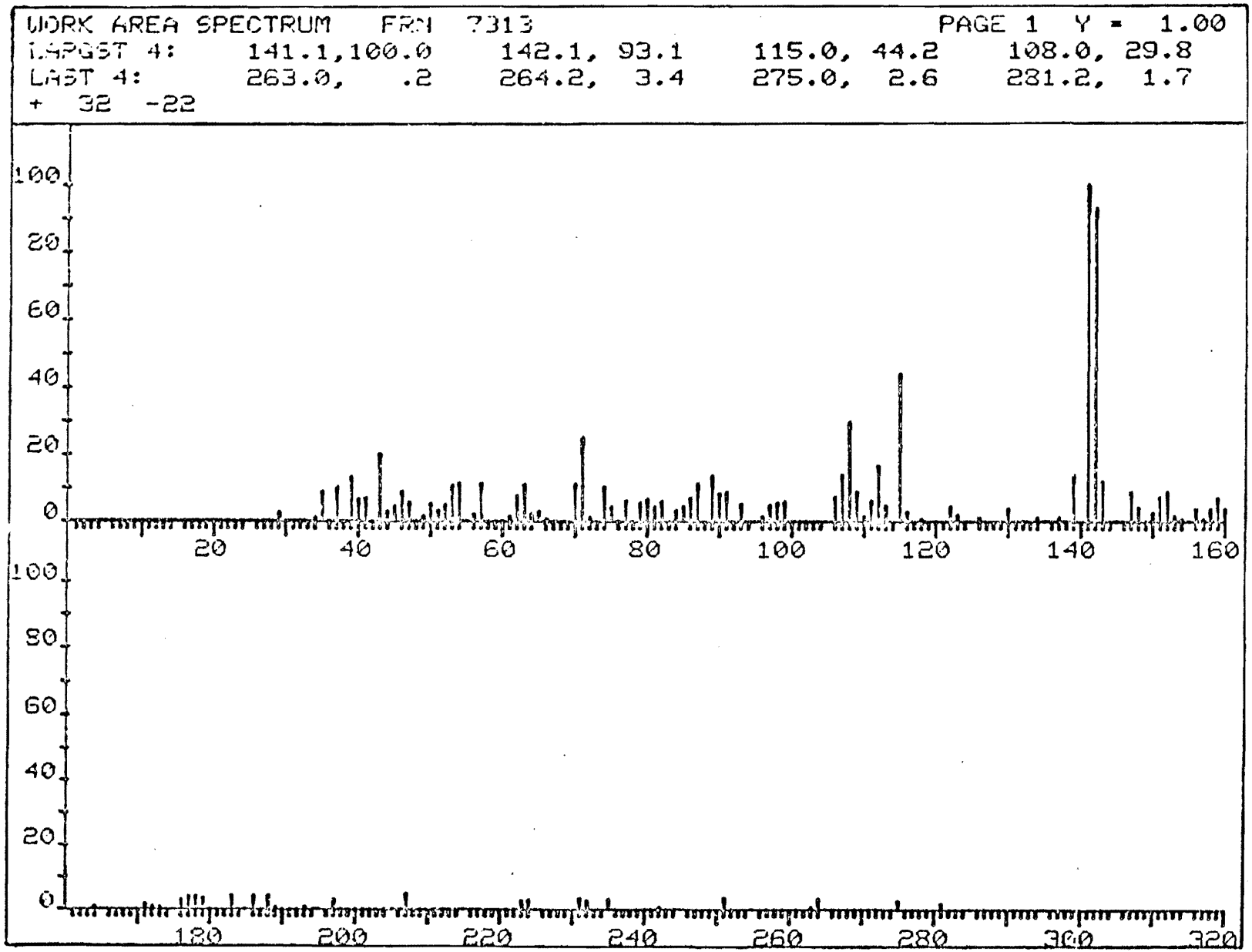

Figure 32. Electron impact mass spectrum of peak F.

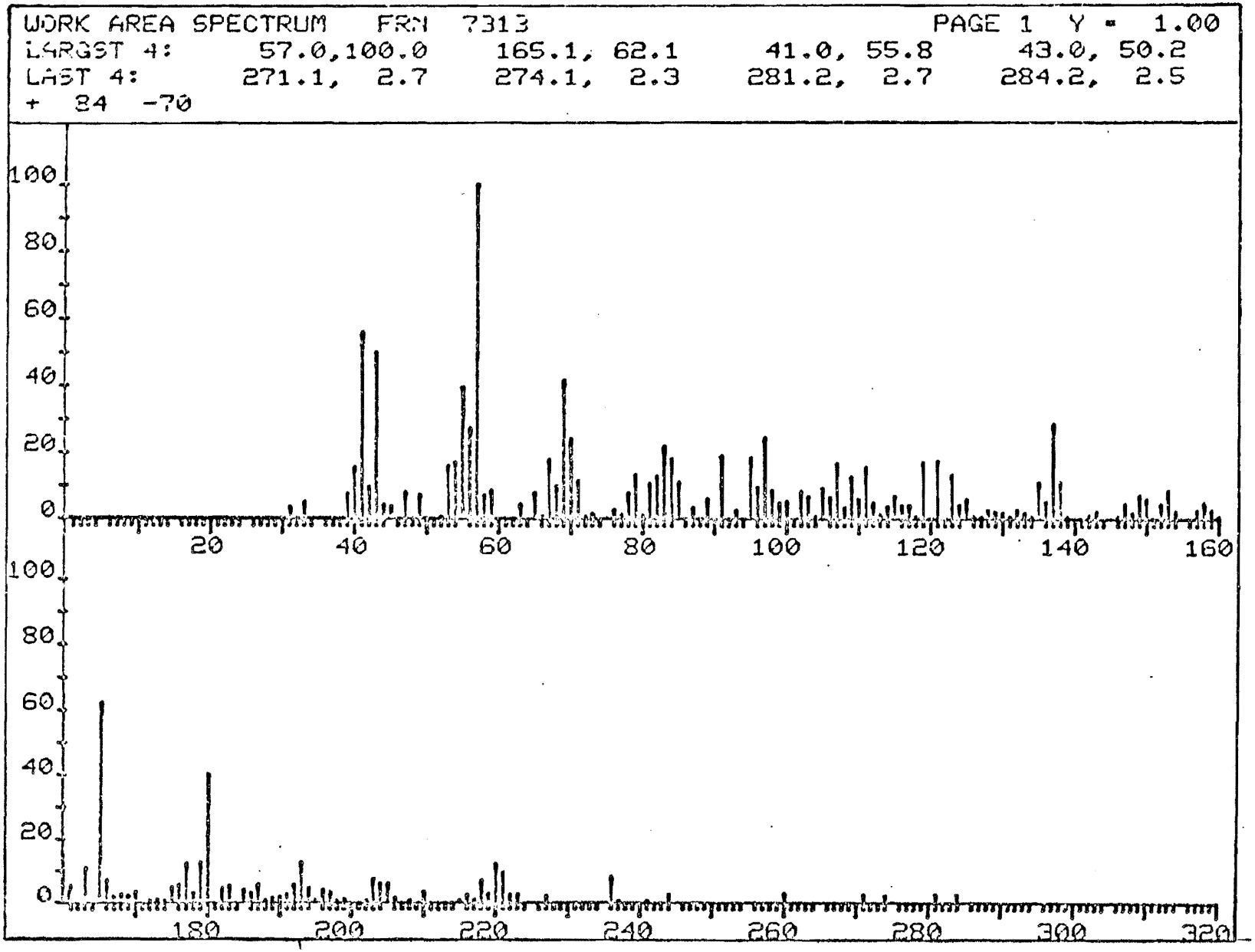

Figure 33. Electron impact mass spectrum of peak G.

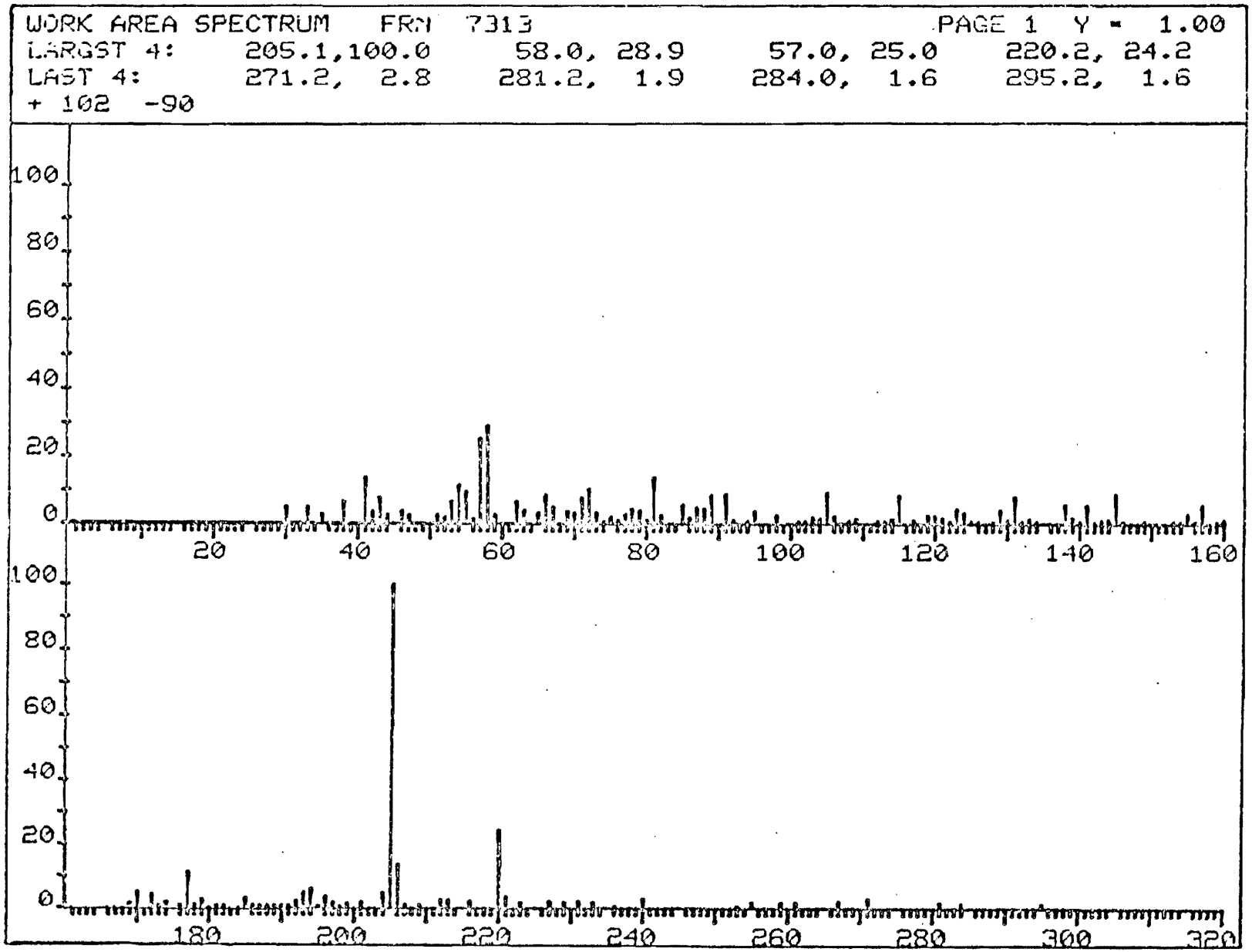

Figure 34. Electron impact mass spectrum of peak H.

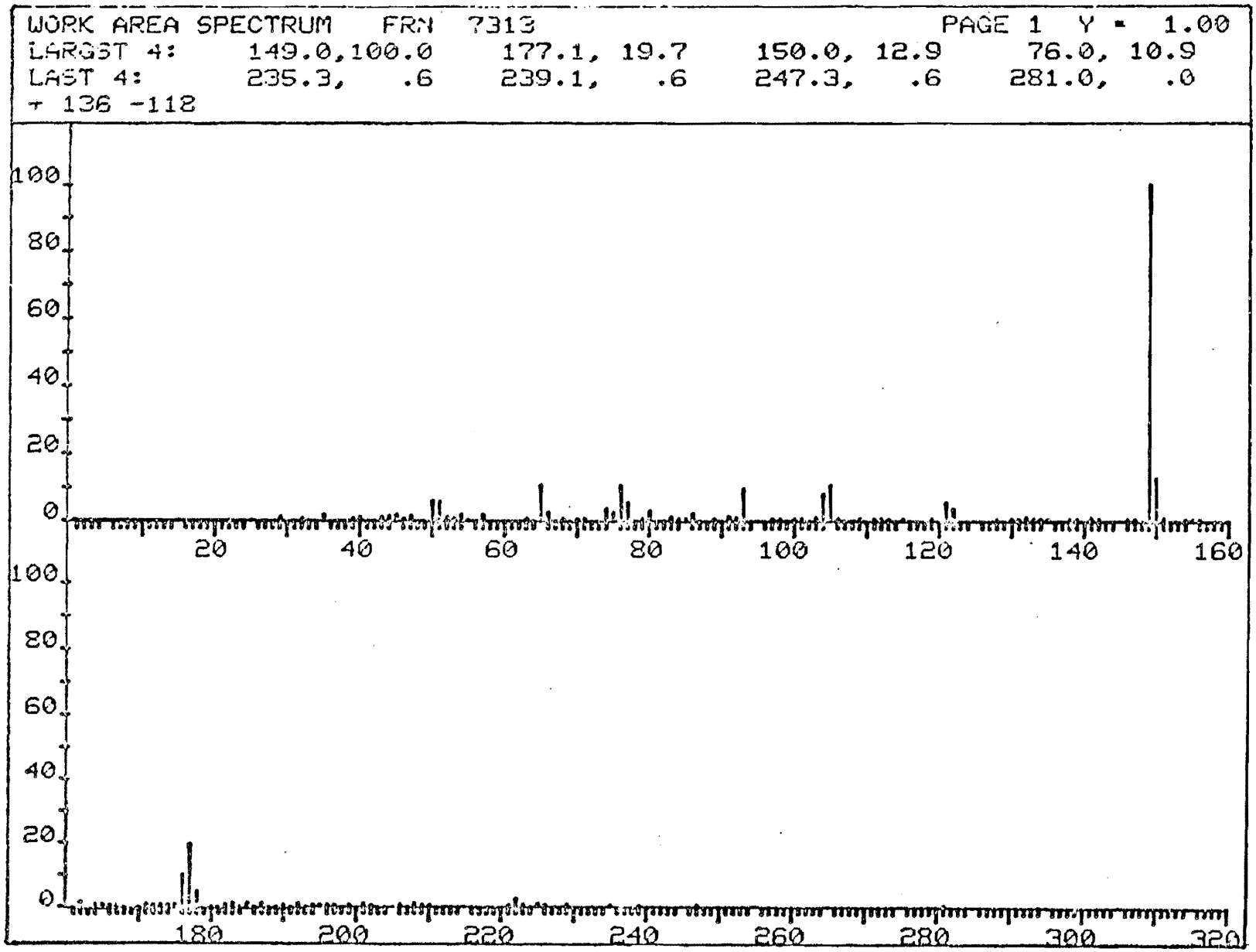

Figure 35. Electron impact mass spectrum of peak I.

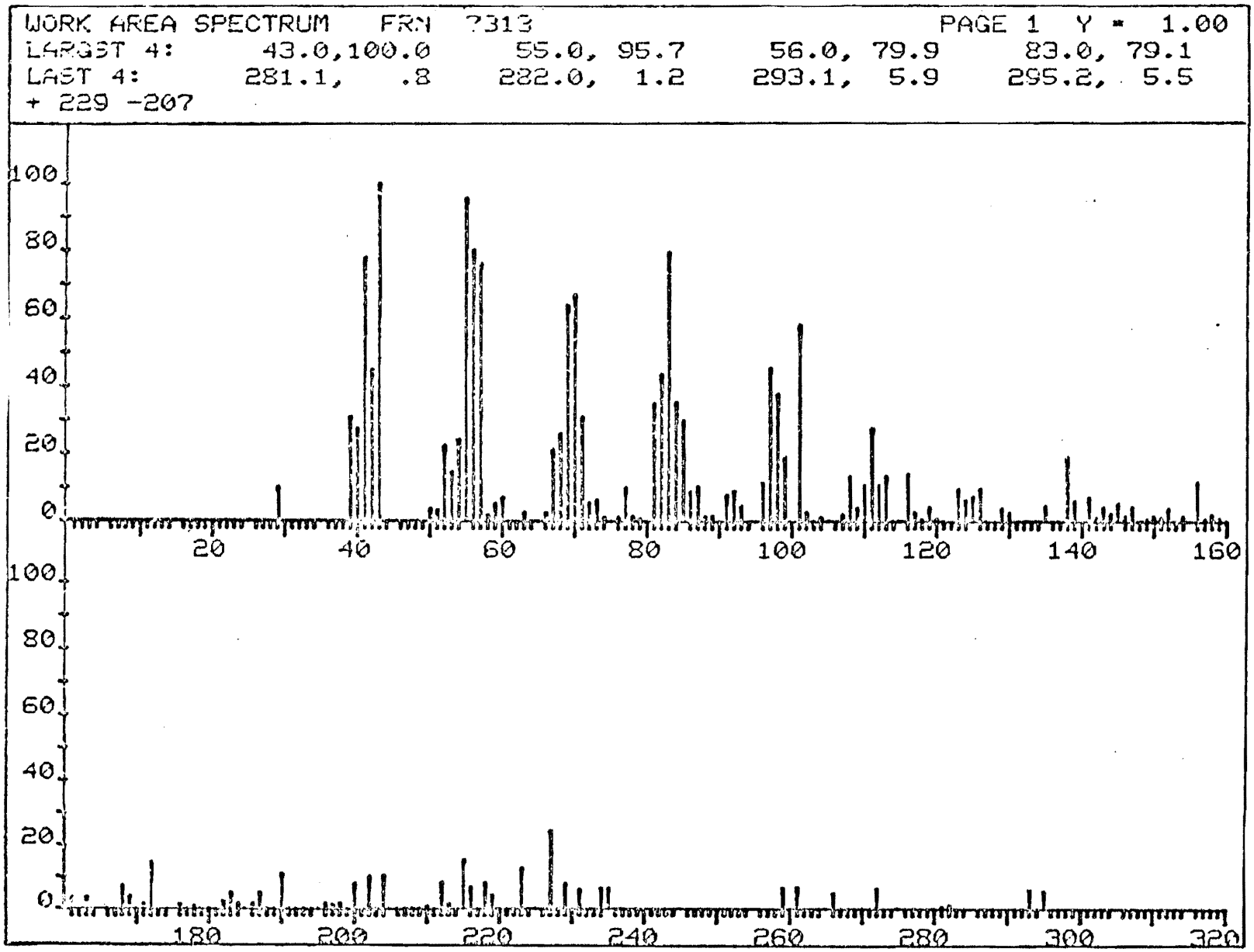

Figure 36. Electron impact mass spectrum of peak J.

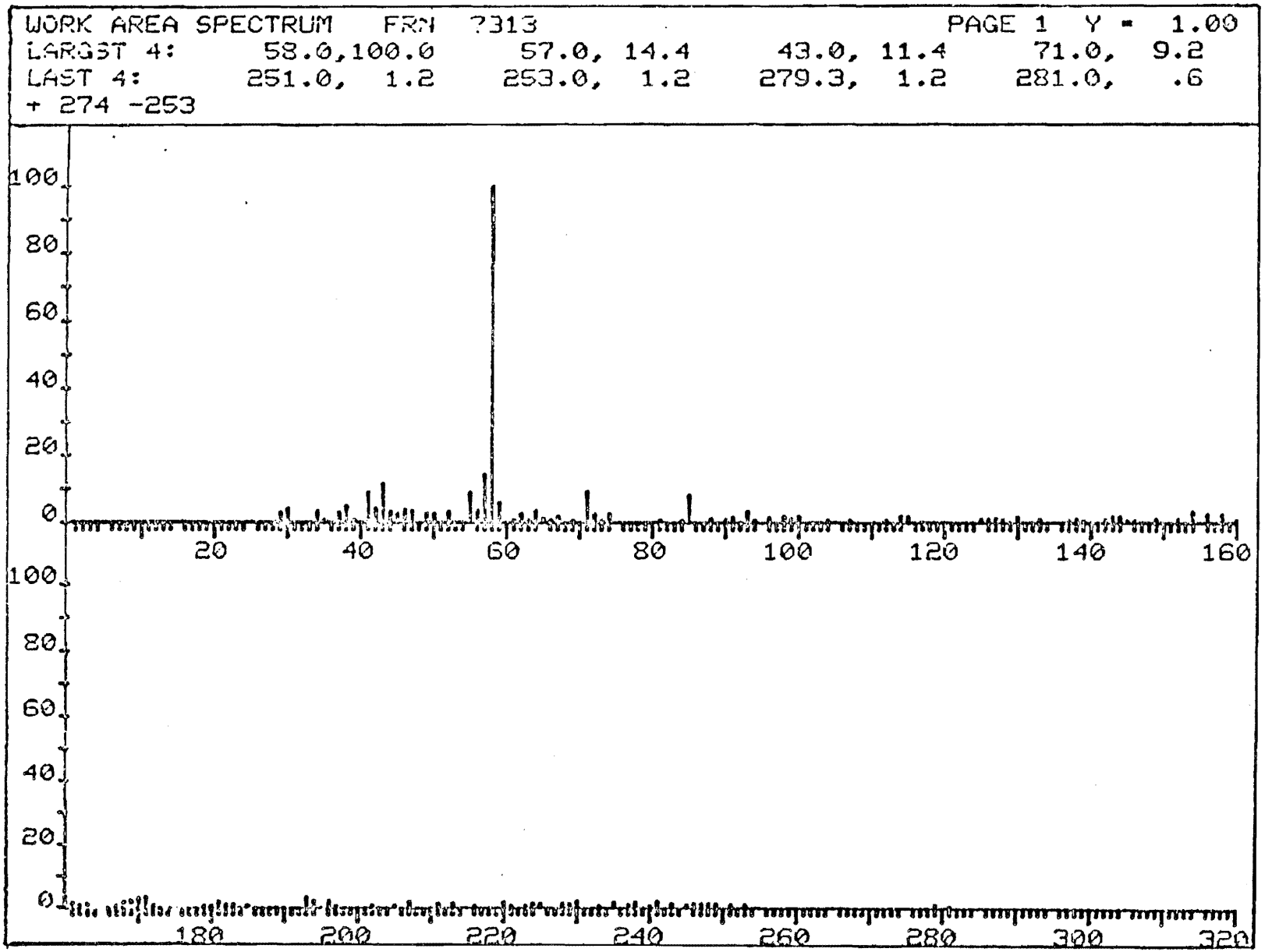

Figure 37. Electron impact mass spectrum of peak K.

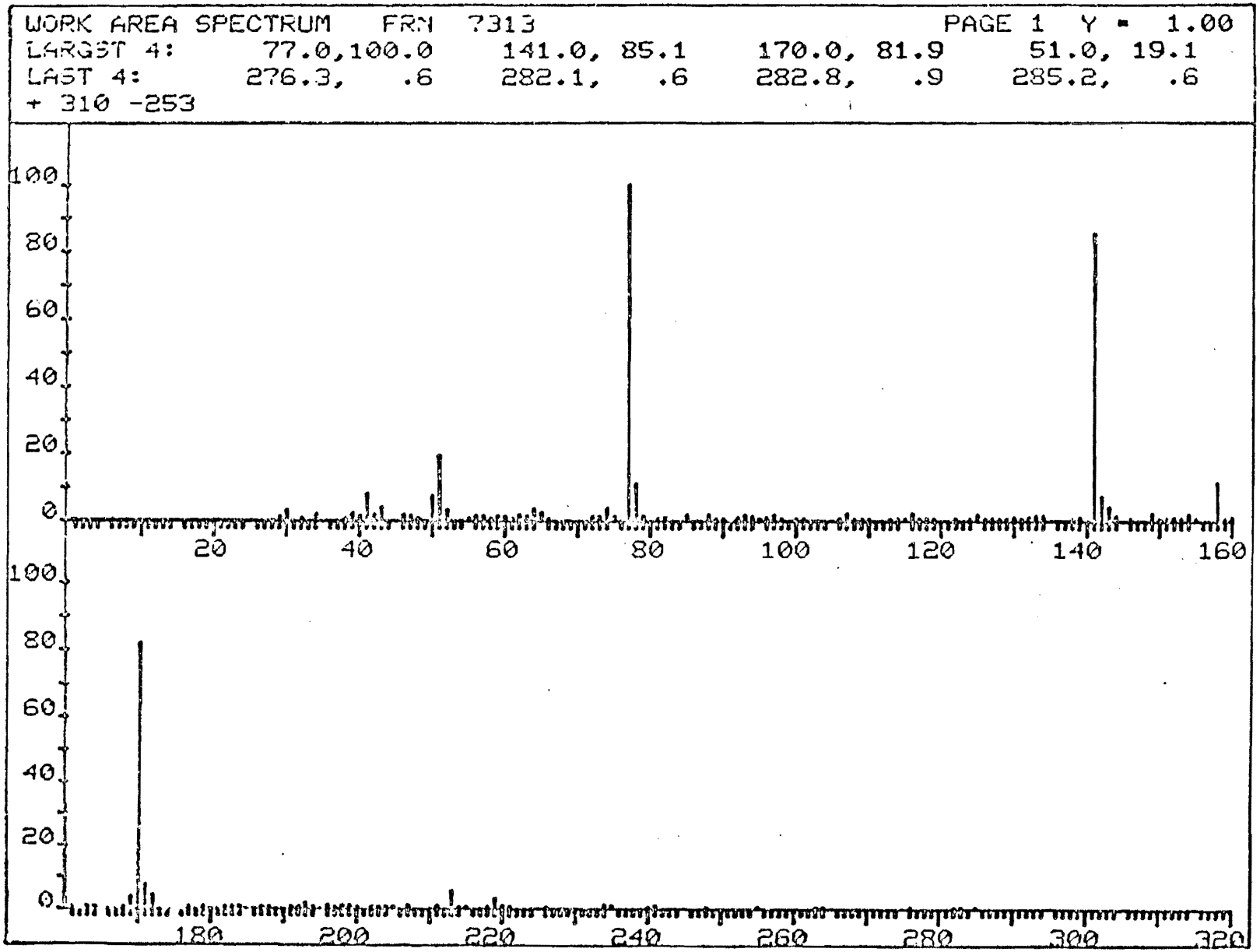

Figure 38. Electron impact mass spectrum of peak L.

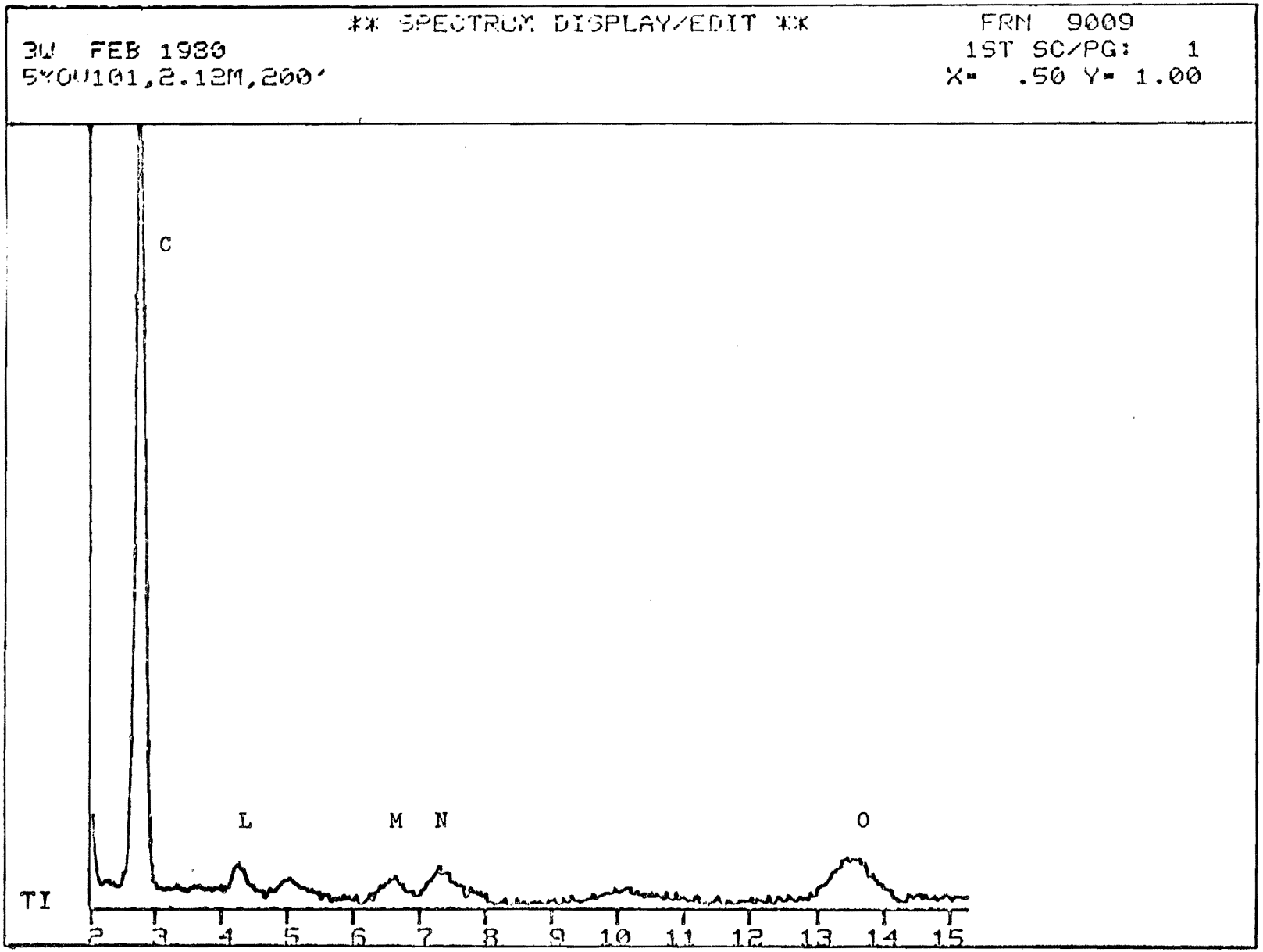

Figure 39. Total ion trace from electron impact GC/MS of combined samples from Site 3W.

6.5 ELECTRON IMPACT GC/MS TOTAL ION TRACE AND MASS SPECTRA OF THE COMBINED EXTRACTS OF FOUR SAMPLES TAKEN FROM SITE 3W, FEBRUARY 1980, OVER A 12-D INCUBATION PERIOD.

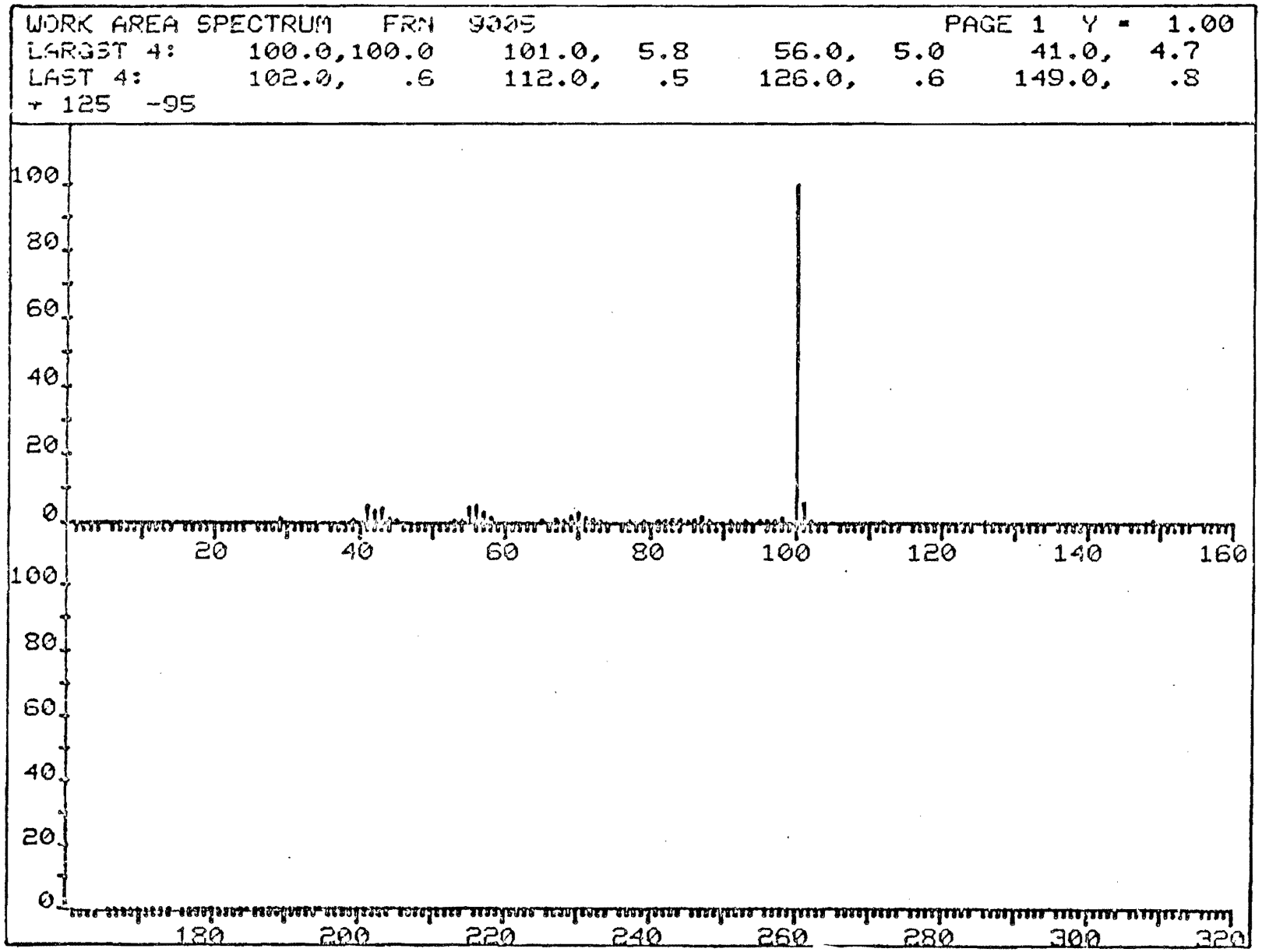

Figure 40. Electron impact mass spectrum of peak M.

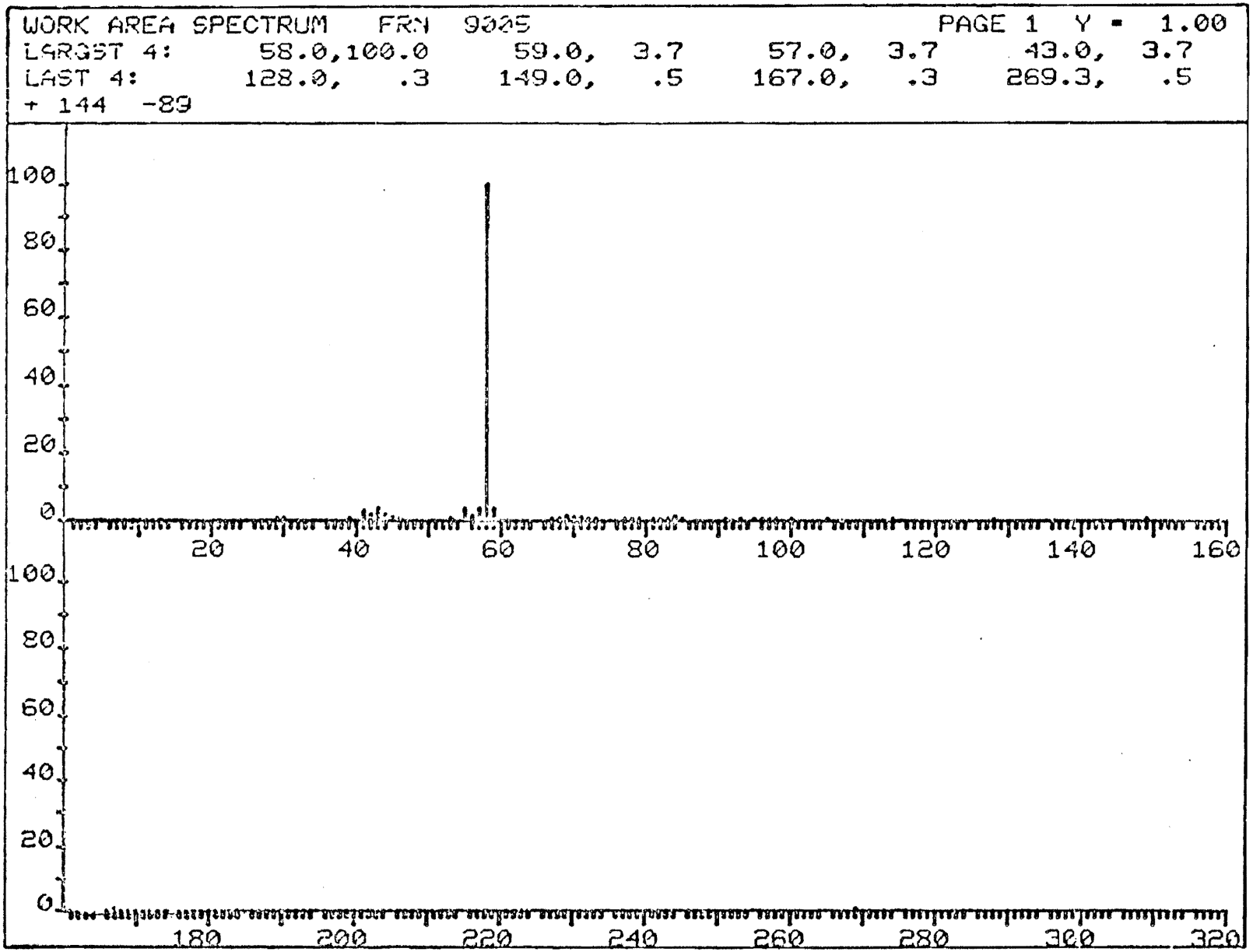

Figure 41. Electron impact mass spectrum of peak N.

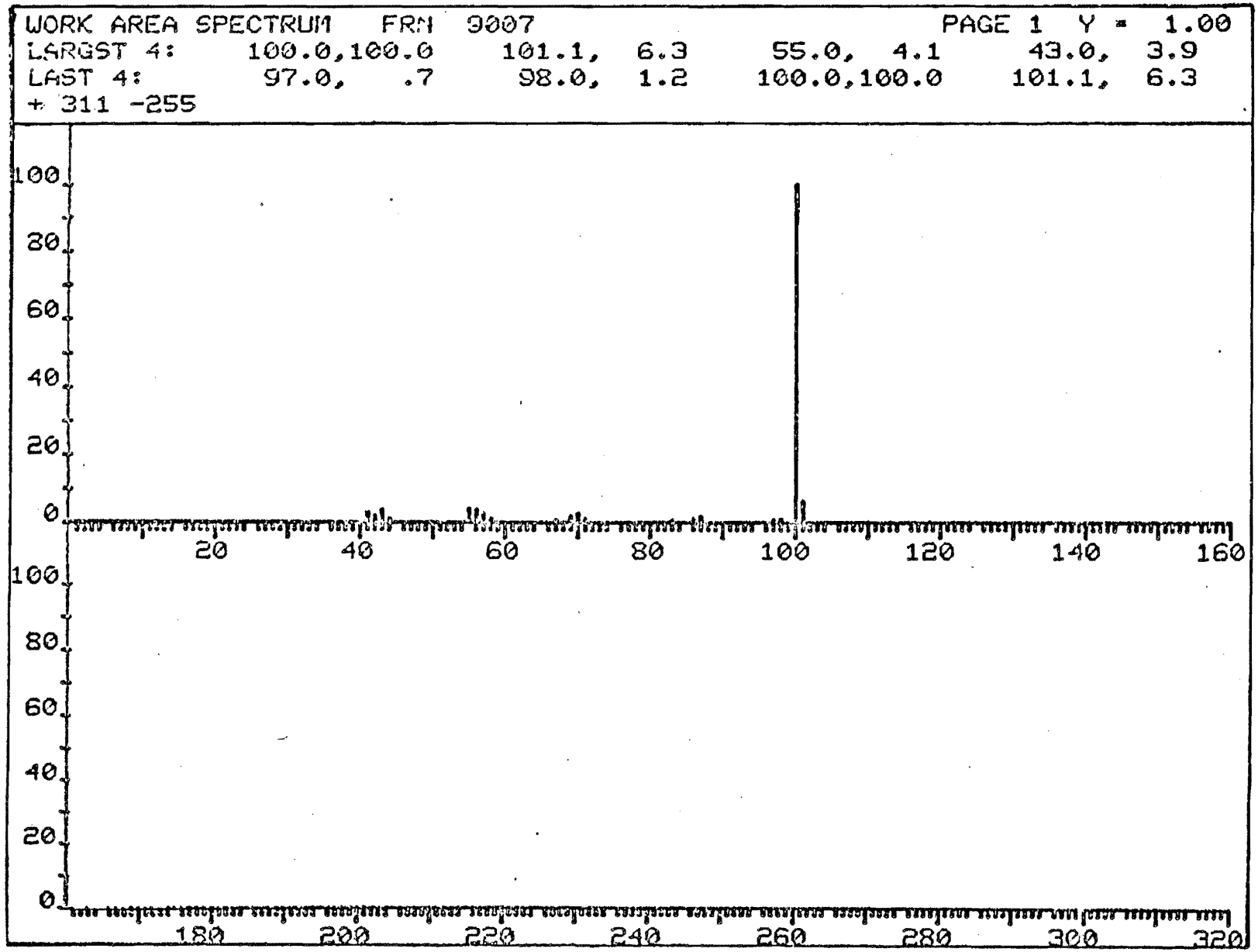

Figure 42. Electron impact mass spectrum of peak 0.

7. LIST OF AOSERP RESEARCH REPORTS

1 PM Alberta Oil Sands Environmental Research Program. 1976. First annual report, 1975. Alberta Oil Sands Environmental Research Program. AOSERP Report 1. 58 pp.

2 AF 4.1.1 Kristensen, J., B.S. Ott, and A.D. Sekerak. 1976. Walleye and goldeye fisheries investigations in the Peace-Athabasca Delta--1975. Prep. for the Alberta Oil Sands Environmental Research Program by LGL Ltd., Environmental Research Associates. AOSERP Report 2. 103 pp.

3 HE 1.1.1 McVey, W.W. 1976. Structure of a traditional baseline data system. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Alberta, Population Research Laboratory. AOSERP Report 3. 26 + 266 pp.

4 VE 2.2 Stringer, P.W. 1976. A preliminary vegetation survey of the Alberta Oil Sands Environmental Research Program study area. Prep. for the Alberta Oil Sands Environmental Research Program by Intraverda Plant Systems Ltd. AOSERP Report 4. 108 pp.

5 HY 3.1 Strosher, M.T. and E. Peake. 1976. The evaluation of wastewaters from an oil sand extraction plant. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Calgary, Environmental Sciences Centre (Kananaskis). AOSERP Report 5. 103 pp.

6 PM Patterson, R. and A.M. Lansdown. 1976. Housing for the north--the stackwall system; construction report --Mildred Lake tank and pump house. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Manitoba, Faculty of Engineering, Northern Housing Committee. AOSERP Report 6. 36 pp.

7 AF 3.1.1 Jantzie, T.D. 1977. A synopsis of the physical and biological limnology and fishery programs within the Alberta oil sands area. Prep. for the Alberta Oil Sands Environmental Research Program by Renewable Resources Consulting Services Ltd. AOSERP Report 7. 73 pp.

8 AF 1.2.1 Machniak, K. 1977. The impact of saline waters upon freshwater biota (a literature review and bibliography). Prep. for the Alberta Oil Sands Environmental Research Program by Aquatic Environments Ltd. AOSERP Report 8. 258 pp.

9 ME 3.3 Croft, B.R., A. Lamb, and R.N. Dawson. 1977. A preliminary investigation into the magnitude of fog occurrence and associated problems in the oil sands area. Prep. for the Alberta Oil Sands Environmental Research Program by Stanley Associates Engineering Ltd. AOSERP Report 9. 87 pp.

10 HE 2.1 Millar, J.F.V. 1977. Development of a research design related to archaeological studies in the Athabasca Oil Sands area. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Saskatchewan. AOSERP Report 10. 69 pp.

11 AF 2.2.1 Flannagan, J.F. 1977. Life cycles of some common aquatic insects of the Athabasca River, Alberta. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Freshwater Institute. AOSERP Report 11. 20 pp.

12 ME 1.7 Mercer, J.M. and R.B. Charlton. 1977. Very high resolution meteorological satellite study of oil sands weather: "a feasibility study". Prep. for the Alberta Oil Sands Environmental Research Program by the University of Alberta, Department of Geography. AOSERP Report 12. 44 pp.

13 ME 2.3.1 Davison, D.S., C.J. Fortems, and K.L. Grandia. 1977. Plume dispersion measurements from an oil sands extraction plant, March 1976. Prep. for the Alberta Oil Sands Environmental Research Program by Intera Environmental Consultants Ltd. AOSERP Report 13. 195 pp.

14 none

15 ME 3.4 Denison, P.J. 1977. A climatology of low-level air trajectories in the Alberta oil sands area. Prep. for the Alberta Oil Sands Environmental Research Program by Acres Consulting Services. AOSERP Report 15. 118 pp.

16	ME 1.6	Barge, B.L., R.G. Humphries, and S.L. Olson. 1977. The feasibility of a weather radar near Fort McMurray, Alberta. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Research Council, Atmospheric Sciences Division. AOSERP Report 16. 72 pp.
17	AF 2.1.1	Lutz, A. and M. Hendzel. 1977. A survey of baseline levels of contaminants in aquatic biota of the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Freshwater Institute. AOSERP Report 17. 51 pp.
18	HY 1.1	Loeppky, K.D. and M.O. Spitzer. 1977. Interim compilation of stream gauging data to December 1976 for the Alberta Oil Sands Environmental Research Program. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Water Survey Branch. AOSERP Report 18. 257 pp.
19	ME 4.1	Walmsley, J.L. and D.L. Bagg. 1977. Calculations of annual averaged sulphur dioxide concentrations at ground level in the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by Atmospheric Environment Service. AOSERP Report 19. 40 pp.
20	HY 3.1.1	Strosher, M.T. and E. Peake. 1978. Characterization of organic constituents in waters and wastewaters of the Athabasca Oil Sands mining area. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Calgary, Environmental Sciences Centre (Kananaskis). AOSERP Report 20. 71 pp.
21	PM	Alberta Oil Sands Environmental Research Program. 1977. Second Annual Report, 1976-77. Alberta Oil Sands Environmental Research Program. AOSERP Report 21. 62 pp.
22	PM	Smith, S.B., ed. 1979. Alberta Oil Sands Environmental Research Program Interim Report covering the period April 1975 to November 1978. Prep. by A.S. Mann, R.A. Hursey, R.T. Seidner, and B. Kasinska-Banas. AOSERP Report 22.

23 AF 1.1.2 Lake, W. and W. Rogers. 1979. Acute lethality of mine depressurization water to trout-perch and rainbow trout: Volume I. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Environment, Water Control Branch. AOSERP Report 23. 44 pp.

24 ME 1.5.2 ME 3.5.1 Fanaki, F., R. Mickle, M. Lusis, J. Kovalick, J. Markes, F. Froude, J. Arnold, A. Gallant, S. Melnichuk, D. Brymer, A. Gaudenzi, A. Moser, and D. Bagg. 1979. Air system winter field study in the AOSERP study area, February 1977. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Atmospheric Environment Service. AOSERP Report 24. 182 pp.

25 ME 3.5.1 Bottenheim, J.W. and O.P. Strausz. 1977. Review of pollutant transformation processes relevant to the Alberta oil sands area. Prep. for the Alberta Oil Sands Environmental Research Program by the University of Alberta, Hydrocarbon Research Centre. AOSERP Report 25. 166 pp.

26 AF 4.5.1 Bond, W.A. and K. Machniak. 1977. Interim report on an intensive study of the fish fauna of the Muskeg River watershed of northeastern Alberta. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Department of Fisheries. AOSERP Report 26. 137 pp.

27 ME 1.5.1 Fanaki, F., compiler. 1978. Meteorology and air quality winter field study in the AOSERP study area, March 1976. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Atmospheric Environment Service. AOSERP Report 27. 249 pp.

28 VE 2.1 Turchenek, L.W. and J.D. Lindsay. 1978. Interim report on a soils inventory in the Athabasca Oil Sands area. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Research Council, Soils Division. AOSERP Report 28. 100 pp.

29 ME 2.2 Shelfentook, W. 1978. An inventory system for atmospheric emissions in the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by SNC Tottrup Services Ltd. AOSERP Report 29. 58 pp.

30 ME 2.1 Strosher, M.M. 1978. Ambient air quality in the AOSERP study area, 1977. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Environment, Pollution Control Division. AOSERP Report 30. 74 pp.

31 VE 2.3 Thompson, M.D., M.C. Wride, and M.E. Kirby. 1978. Ecological habitat mapping of the AOSERP study area: Phase I. Prep. for the Alberta Oil Sands Environmental Research Program by Intera Environmental Consultants Ltd. AOSERP Report 31. 176 pp.

32 PM Alberta Oil Sands Environmental Research Program. 1979. Third Annual Report, 1977-78. Alberta Oil Sands Environmental Research Program. AOSERP Report 32. 114 pp.

33 TF 1.2 Nowlin, R.A. 1978. Relationships between habitats, forages, and carrying capacity of moose range in northern Alberta. Part I: moose preferences for habitat strata and forages. Prep. for the Alberta Oil Sands Environmental Research Program by Alberta Recreation, Parks and Wildlife, Fish and Wildlife Division. AOSERP Report 33. 63 pp.

34 HY 2.4 Allan, R. and T. Jackson. 1978. Heavy metals in bottom sediments of the mainstem Athabasca River system in the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by Fisheries and Environment Canada, Freshwater Institute. AOSERP Report 34. 72 pp.

35 AF 4.9.1 Griffiths, W.H. and B.D. Walton. 1978. The effects of sedimentation on the aquatic biota. Prep. for the Alberta Oil Sands Environmental Research Program by Renewable Resources Consulting Services Ltd. AOSERP Report 35. 86 pp.

36 AF 4.8.1 Jones, M.L., G.J. Mann, and P.J. McCart. 1978. Fall fisheries investigations in the Athabasca and Clearwater rivers upstream of Fort McMurray: Volume 1. Prep. for the Alberta Oil Sands Environmental Research Program by Aquatic Environments Ltd. AOSERP Report 36. 71 pp.

37 HE 2.2.2 Van Dyke, E.W. and C. Loberg. 1978. Community studies: Fort McMurray, Anzac, Fort MacKay. Prep. for the Alberta Oil Sands Environmental Research Program by Applied Research Associates Ltd. AOSERP Report 37. 195 pp.

38 VE 7.1.1 Green, J.E. 1979. Techniques for the control of small mammal damage to plants: a review. Prep. for the Alberta Oil Sands Environmental Research Program by LGL Ltd., Environmental Research Associates. AOSERP Report 38. 111 pp.

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