# Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response

Interim Report

Submitted to:

**David Tsao, Ph.D**
BioChem Strike Team Leader - Deepwater Horizon
Remediation Engineering and Technology Specialist
BP Remediation Management
28100 Torch Parkway
Warrenville, IL 60555

Submitted by:

**Ralph J. Portier, Ph.D. Professor of Environmental Sciences**
**Laura M. Basirico, MS. Research Associate 3**

Department of Environmental Sciences
1165 Energy Coast & Environment
Louisiana State University
Baton Rouge, LA 70803

March 3, 2011

*This report provides a brief summary of findings to date of 8 products from the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule. Two additional products are in final testing. A comprehensive final report will be submitted to the BioChem Strike Team at that time.*

## 1.0 Introduction and Summary of Methods

The BioChem Strike Team (BCST) was established in response to the Deepwater Horizon incident by the Alternative Response Technology (ART) program. The BCST consisted of experts from BP, LSU, LDEQ, USCG, OSPR (California), SCAT, and highly experienced oil spill response consultants. Furthermore, the BCST operated in conjunction with advice from EPA and NOAA. The overall objectives of the BCST were to evaluate among the thousands of submissions of alternative approaches through the ART system, those biological and chemical technologies that best meet the needs of Unified Command on oil spill cleanup. In order to accomplish this, the team reviewed and subsequently determined which technologies would undergo desktop evaluations (literature review), laboratory scale testing (at the aquatic toxicology laboratory at Louisiana State University, LSU), and/or field testing. Ultimately the results of this work by the BCST will be to provide recommendations for best available technologies for use by the Unified Command.

This interim report summarizes the products evaluated at the laboratory scale. Specifically, the BCST determined that 10 products listed on the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule warranted further testing to determine their effectiveness in degrading oil under the specific environmental, climate, and ecological conditions generated by the 2010 Gulf oil spill. Using pre-defined test protocols, each product was evaluated and compared to natural (inherent) biodegradation occurring through indigenous microflora and micronutrients present in Gulf waters. The selected products were analyzed in a controlled flask-study to determine their remediation potential on weathered crude oil recovered from south Louisiana marshes. It should be noted that this interim report covers 8 of the 10 products as testing on two of the products were initiated at a later date than those reported here.

## 2.0 Materials and Methods

The experimental design protocol specified a flask study, incubated at room temperature on a consistently rotating, 200 rpm, orbital shaker. The samples were sacrificed over 5 separate sampling events including Time 0, 1, 2, 4 and 12 Weeks. The original protocol called for the last sampling event to occur at 8 weeks. However, the final sampling event was changed to 12 weeks while the backlog of analytical characterization for the earlier samples was being worked through. Each flask was analyzed for total nitrates ($NO_3^-$), total phosphates ($PO_4^{3-}$), total organic carbon (TOC), total alkanes, total polyaromatic hydrocarbons (PAHs) and the physical parameters, pH, dissolved oxygen (DO) and temperature. Specific aromatic fractions were also analyzed, but the full dataset is not reported here.

### 2.1 Chemical Analyses

#### 2.1.1 *GC/MS Methods*

Extraction of PAHs and alkanes in water-amended with oil follows methods outlined in EPA Method 8270 series. Approximately 100 ml of water is poured into a 250-ml separatory funnel and adjusted to a pH of 7. A 30-ml aliquot of dichloromethane is added to the separatory funnel

and spiked with a known amount of standard surrogate. The funnel is capped and shaken for approximately 3 minutes, venting occasionally to remove solvent pressure. The solvent and water are allowed to separate and the solvent is drained through an anhydrous sodium sulfate funnel into a 250-ml flat-bottom flask. The solvent addition and draining step are repeated 2 more times. The sodium sulfate funnel is rinsed with dichloromethane and allowed to drain completely. The flat-bottom flask is then placed on a rotary evaporation system and concentrated to a volume of 5-10 ml dichloromethane and placed in a calibrated extraction thimble. If concentrating is necessary, the extract volume is placed under a nitrogen blow down concentrator and reduced to a volume of 1.0 ml. The dichloromethane extract is exchanged to hexane using approximately 4-5 ml of hexane. A micro distillation column is added to the extraction thimble and placed in a hot water bath. The dichloromethane is evaporated off and the remaining hexane extract is reduced to a volume of 1-2 ml. The hexane extract is placed beneath a nitrogen blow down device and reduced to a final volume of 1.0 ml hexane.

### 2.1.2 *GC/MS Instrumental analyses*

After addition of internal standards, samples were analyzed using an Agilent 7890A GC fitted with a 0.25 mm i.d. ×30 m HP-5MS column and an Agilent 7683B autosampler. The injector was set to 250°C and the detector to 280°C. Detection of analytes involves the utilization of a HP 5975C Inert XL Series Mass Selective Detector operating in the Selected Ion Monitoring mode. The column was held at 60°C for 1 min and then ramped at 25°C/min to 160°C followed by 3°C/min to 268°C and 12°C/min to 300°C, where it was held for 8 min. Concentrations of parent PAHs were based on calibrations using a five-point curve which were checked for each batch of samples analyzed. Concentrations were reported on a dry weight basis. Approximate alkylated PAH concentrations were calculated assuming the same response factors for each parent and corresponding alkylated analogues. For alkylated phenanthrene/anthracenes, the results were reported as pairs to incorporate the uncertainty of the measurements and quantification based on the average response factor of the individual parent PAHs.

## 2.2 Other analytical approaches

A. Water quality analysis
   - DO, pH, temperature and salinity were measured using standard field equipment, (YSI 85-10 meter) appropriately calibrated.

B. Microbial analysis
   - Microbial activity was measured by epifluorescence direct cell count (EDCC) for Most Probable Number (MPN).

C. Nutrients
   - Total phosphates ($PO_4^{3-}$) using EPA 365.4, total nitrates ($NO_3^-$) using standard method 4500-NO3 F modified and total organic (TOC) using US EPA Method 9060.

## 3.0 Screening Protocol

### *3.1 Preparation of Oiled Flasks*

The crude oil and Gulf water used in the study were recovered in Bay Jimmy (coordinates: 29°27'238" N, 89°53'510" W) on August 20, 2010. A half (0.5) g of weathered crude oil were weighed out and deposited in the bottom of a sterile 250 ml Erlenmeyer flask. Before the oil was added to the flasks, each flask was rinsed with de-ionized water and autoclaved to ensure sterility. Ten (10) ml of the solvent Dichloromethane (DCM) was added to the flasks and the flasks were placed on the shaker table for approximately 10 minutes until the oil had completely dissolved in the DCM. The flasks were then left uncovered under a ventilation hood to allow the DCM to flash off, leaving a ring of crude oil on the bottom of each 250 ml test flask.

Each of the 180 test flasks, including the 30 control flasks and 150 product flasks, were prepared in this exact manner.

### *3.2 Preparation of Controls*

Four separate controls were prepared in triplicate for each of the five sampling events.

- **Negative Control** treatments consisted of 100 ml of sterile Gulf water and 0.5 ml of weathered crude oil per test flask. As in all other test flasks, 0.5 ml of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask. 100 ml of autoclaved Gulf water was then added to each flask. No nutrients were added.
- **Positive Control 1** treatments consisted of 100 ml of Gulf water and 0.5 g dissolved oil per flask. No nutrients were added. As in all other test flasks, 0.5 ml of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask.
- **Positive Control 2** treatments consisted of 100 ml of Gulf water, 0.5 ml of weathered crude oil and a nutrient blend. The nutrients consisted of 0.25 g $KH_2PO_4$ and 0.5 g $NH_4NO_3$ per flask. As in all other test flasks, 0.5 ml of oil were dissolved in 10 ml of DCM, creating a coating of weathered oil in the bottom of each flask
- **Positive Control 3** treatments required a solution of 0.09g of hexadecane and 0.01g of chrysene per flask containing 100 ml of Gulf water. Based on the difficulty of accurately weighing 0.01 and 0.09 grams of each component, a stock solution of hexadecane and chrysene in Dichloromethane (DCM) was prepared. The solution could then be accurately pipetted into each test flask. The calculations to produce 30 ml of solution are as follows:

### *3.3 Stock solutions*

30 ml of DCM containing 0.01 g chrysene per ml DCM and 0.12 ml hexadecane per ml DCM

30 ml DCM contained 0.3 g chrysene and 3.6 ml hexadecane

0.3 g of chrysene were added to 30 ml of DCM and allowed to dissolve. Once dissolved, 3.6 ml of hexadecane was added to the chrysene/DCM solution. One (1) ml of the composite solution was then added to each Positive Control 3 test flask. Based on the passive volatilization of DCM as compared to hexadecane and chrysene, the DCM was flashed off under a vented hood in order to leave the desired amount of chrysene and hexadecane in the bottom of the test flask. Nutrients were also added to each flask.

The final Positive Control 3 flasks consisted of 100 ml of Gulf water, 0.5 g crude oil, 0.25 g $KH_2PO_4$ and 0.5 g $NH_4NO_3$ and 1 ml of the solution of hexane and chrysene described above.

### *3.4 Preparation of Products*

*The following products were added to triplicate flasks using formulations and approaches provided by product representatives to LSU.*

**S-200**

Nutrients Added: Yes
N: 0.5g/flask
P: 0.25g/flask

The manufacturer's protocol suggested a 1:1 ratio of product to oil; 0.5 ml of the S-200 product was added to each test flask.

## 4.0 Findings

*All products tested in the laboratory screening study are listed in the U.S. Environmental Protection Agency's (USEPA) Office of Emergency Management Regulatory and Policy Division's National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule.*

The eight commercial products tested demonstrated the ability to biodegrade and/or reduce total concentrations of the weathered oil (including alkanes, PAHs) recovered from Bay Jimmy. Additionally, the flask study has verified that the remaining dispersed and weathered oil in coastal environments along the Louisiana and northern Gulf of Mexico will continue to biodegrade. This is not a new finding and has been the opinion of many scientists as a reasonable outcome for any oil spill affecting the coastlines of Gulf States. However, the study does demonstrate the need for accelerated biodegradation strategies so as to minimize the toxicological legacy of the spill over time.

Data sets are included in *Appendix A* of the report. Representative chromatograms for the first four weeks of the study are in *Appendix B.*

Microbial cell counts using epifluorescence direct cell count (e.g. MPN) revealed that all samples contained natural and/or supplemented microbial populations above $10^6$ viable cells per ml.

***Specific findings for control and commercial products are as follows:***

**Negative Control:** The negative control flasks consisted of weathered oil added to sterile Gulf water. The flasks indicated minimal reductions in alkanes and PAHs over the 12 week period. Total alkanes from time 0 to week 12 were reduce 14.2% and total PAHs were reduced 14.2% over the same time period.

**Positive Control 1:** The series of control flasks consisted of weathered oil added to non-sterile Gulf water with no additional nutrients. Data sets demonstrated an 11.9% increase in total alkanes over the 12 week period. Based on the variability of 0.5 gram oil measurements within each flask, this slight increase is an acceptable result for the Control 1 data series. Additionally, visual observation over the 12 weeks indicated minimal degradation of oil. After 4 weeks, an 80.4% reduction in PAHs was seen. However over the 12 week study, the total PAH concentration was reduced only 28.6%. The Positive Control 1 data series suggested microbial activity produced modest reductions in weathered oil. This is consistent with earlier USEPA studies indicating the need for nutrient amendment so as to maintain steady biodegradation/mineralization.

**Positive Control 2:** The Positive Control 2 series of flasks consisted of site water from Bay Jimmy, weathered oil and nutrient additions of nitrogen and phosphorus. Indigenous aquatic microflora was the only biological component active in the flasks. An 86.9% reduction of total alkanes and a 16.5% reduction in PAHs were seen over the 12 week period. The approximately

87% degradation ranks Positive Control 2 among the most reductive of the treated flasks. The reduction in PAHs is less pronounced, but still greater that that seen in the non-nutrient amended Positive Control 1 flasks. Overall, an 85.6% reduction in total weathered oil was seen over the Positive Control 2 data series. The data suggests that adapted, acclimated and nutrient-amended microbial seed is able to produce significant reductions in the alkane constituents and modest reductions in the PAH constituents.

**Positive Control 3:** Positive control 3 consisted of site water with indigenous microflora and the chrysene/hexadecane additive as primary carbon sources. No weathered oil was added to the flask series. A 32.6% reduction in chrysene was demonstrated over the 12 week test period.

**S-200:** S-200 is identified as a bioremediation accelerator and as such, does not contain bacterial cultures. The product contributes to the establishment of a robust microbial population. As per manufacturer instructions, additional nutrients are added to the product along with non-sterile site water and weathered oil. Considerable biodegradation to alkanes was seen over the course of treatment with 95.9% of these constituents reduced in 12 weeks. An 11.2% reduction in PAHs resulted from the S-200 treatment over 12 weeks, a reduction similar to that seen in Positive Control 2 flasks.

## 5.0 General Discussion

As a general trend, the PAH groups including C1-C3 Phenanthrenes, C3 and C4 Pyrenes as well as C2 and C3 Fluorenes were left intact by the end of 12 weeks. The PAHs of toxicological concern including the Benzo constituents were degraded in every treatment flask. As expected, the shorter-chain alkanes including nC10 to nC14 were most often thoroughly degraded by the end of 12 weeks, while the heavier chains were left in greater concentrations. Importantly to the time frame of the field trials, the greatest reduction in PAHs by many of the products occurred over 4 weeks. The PAH concentration then returned to elevated levels in the weeks between sampling event four (week 4) and five (week 12).

The current laboratory study showed that the NCP products can promote the conversion, or biodegradation, of oil to $CO_2$, biomass and water. The study has also demonstrated that nitrogen and phosphorous amendments also work to enhance in the degradation of oil under controlled closed systems. Data sets from earlier EPA research into remediation of spilled oil argued that the limiting factor for biodegradation/mineralization is dependent upon the availability of nitrogen and phosphorus. Other factors such as temperature, salinity and dissolved oxygen may affect not only nutrient availability but also acclimated biomass performance. Field demonstration trials are needed to document the efficacy of bioremediation products on weathered oil and to determine their net contribution to biodegradation/mineralization. After nearly one year since the Deepwater Horizon spill, residual weathered oil remains in many locations. The need for a field trial to establish operational criteria for final bioremediation work plans should be initiated before early Spring 2011.

## Appendix A. Data sets from shaker flask studies

Data sets for the first 8 products with controls are presented for the 12 week screening period.
Data sets are incomplete on two additional products added later in the study.
Screening studies for these two products were initiated in December 2010. They will be included in the final report**.**

| Time = 0 11/10/2010 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Flask Series*** | ***$NO_3^-$-N mg/L*** | ***$PO_4^{3-}$ mg/L*** | ***TOC mg/L*** | ***Alkanes mg/kg*** | ***PAHs mg/kg*** | ***pH*** | ***DO mg/L*** | ***Temp °C*** |
| Negative Control | | | | | | | | |
| A | 2.20 | 0.00 | 7.29 | 27400 | 394 | 7.96 | 9.5 | 25.0 |
| B | 1.90 | 0.00 | 8.20 | 28200 | 415 | 7.96 | 9.5 | 25.0 |
| C | 2.30 | 14.60 | 7.56 | 28200 | 415 | 7.96 | 9.5 | 25.0 |
| Positive Control 1 | | | | | | | | |
| A | 1.40 | 21.80 | 6.60 | 21300 | 452 | 7.96 | 9.5 | 25.0 |
| B | 3.50 | 7.40 | 7.56 | 19900 | 437 | 7.96 | 9.5 | 25.0 |
| C | 1.60 | 0.00 | 7.00 | 22400 | 423 | 7.96 | 9.5 | 25.0 |
| Positive Control 2 | | | | | | | | |
| A | 72.00 | 1730.00 | 7.20 | 23400 | 427 | 7.96 | 9.5 | 25.0 |
| B | 145.00 | 2010.00 | 8.44 | 22600 | 435 | 7.96 | 9.5 | 25.0 |
| C | 165.00 | 1970.00 | 7.92 | 19700 | 374 | 7.96 | 9.5 | 25.0 |
| Positive Control 3 | | | | | | | | |
| A | 1.70 | 0.00 | 8.77 | 0 | 12100 | 7.96 | 9.5 | 25.0 |
| B | 1.00 | 0.00 | 7.96 | 0 | 12500 | 7.96 | 9.5 | 25.0 |
| C | 1.70 | 0.00 | 8.54 | 0 | 11100 | 7.96 | 9.5 | 25.0 |
| S-200 | | | | | | | | |
| A | 1670.00 | 1690.00 | 153.80 | 24500 | 308 | 7.96 | 9.5 | 25.0 |
| B | 1630.00 | 1710.00 | 197.80 | 23000 | 312 | 7.96 | 9.5 | 25.0 |
| C | 1790.00 | 1810.00 | 202.60 | 24300 | 234 | 7.96 | 9.5 | 25.0 |

| Time = 1 Week 11/17/2010 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Flask Series*** | ***$NO_3^-$-N mg/L*** | ***$PO_4^-$ mg/L*** | ***TOC*** | ***Alkanes mg/kg*** | ***PAHs mg/kg*** | ***pH*** | ***DO mg/L*** | ***Temp °C*** |
| Negative Control | | | | | | | | |
| A | 1.10 | 0.00 | 9.64 | 20500 | 390 | 8.01 | 5.75 | 23.4 |
| B | 1.10 | 0.00 | 9.95 | 21800 | 414 | 8.06 | 5.10 | 23.4 |
| C | 1.20 | 0.80 | 10.51 | 28000 | 551 | 8.06 | 4.88 | 23.4 |
| Positive Control 1 | | | | | | | | |
| A | 1.10 | 0.00 | 7.56 | 19200 | 368 | 7.88 | 4.38 | 24.0 |
| B | 0.80 | 0.00 | 7.50 | 19900 | 376 | 7.93 | 4.44 | 24.3 |
| C | 0.90 | 0.00 | 7.50 | 17700 | 301 | 7.96 | 4.39 | 24.0 |
| Positive Control 2 | | | | | | | | |
| A | 242.00 | 1710.00 | 24.80 | 6620 | 331 | 5.27 | 3.53 | 23.8 |
| B | 360.00 | 1810.00 | 24.47 | 5960 | 401 | 5.25 | 2.98 | 23.8 |
| C | 570.00 | 1690.00 | 21.65 | 4370 | 297 | 5.22 | 3.42 | 24.0 |
| Positive Control 3 | | | | | | | | |
| A | 0.50 | 0.00 | 5.63 | 0 | 17700 | 7.95 | 4.32 | 24.0 |
| B | 1.60 | 0.00 | 4.91 | 0 | 17600 | 7.97 | 4.61 | 24.0 |
| C | 0.90 | 0.10 | 7.29 | 0 | 14100 | 7.98 | 4.61 | 24.0 |
| S-200 | | | | | | | | |
| A | 2380.00 | 2230.00 | 234.40 | 9560 | 315 | 5.21 | 2.31 | 24.0 |
| B | 1760.00 | 2050.00 | 187.80 | 11600 | 390 | 5.25 | 1.81 | 24.6 |
| C | 2050.00 | 2030.00 | 208.10 | 11200 | 238 | 5.26 | 2.02 | 24.4 |

| Time = 2 Weeks 11/24/2010 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Flask Series*** | ***$NO_3^-$-N mg/L*** | ***$PO_4^{3-}$ mg/L*** | ***TOC*** | ***Alkanes mg/kg*** | ***PAHs mg/kg*** | ***pH*** | ***DO mg/L*** | ***Temp °C*** |
| Negative Control | | | | | | | | |
| A | 1.00 | 0.0 | 9.30 | 10400 | 521 | 7.82 | 4.80 | 23.5 |
| B | 0.60 | 0.2 | 9.87 | 10500 | 499 | 7.89 | 4.74 | 24.1 |
| C | 1.10 | 0.0 | 9.85 | 9110 | 390 | 7.92 | 4.72 | 24.2 |
| Positive Control 1 | | | | | | | | |
| A | 1.20 | 0.0 | 9.29 | 10500 | 487 | 7.90 | 4.53 | 24.3 |
| B | 1.10 | 0.0 | 6.41 | 4990 | 215 | 7.95 | 4.37 | 24.3 |
| C | 0.60 | 1.0 | 8.15 | 9630 | 572 | 7.96 | 4.57 | 24.4 |
| Positive Control 2 | | | | | | | | |
| A | 190.00 | 2540.0 | 51.40 | 1030 | 281 | 5.16 | 3.59 | 25.2 |
| B | 331.00 | 2710.0 | 58.39 | 1100 | 471 | 5.21 | 3.64 | 25.2 |
| C | 210.00 | 2360.0 | 51.16 | 1320 | 523 | 5.14 | 3.54 | 24.8 |
| Positive Control 3 | | | | | | | | |
| A | 0.90 | 0.6 | 10.54 | 0 | 16800 | 7.91 | 4.56 | 24.5 |
| B | 1.60 | 0.0 | 9.23 | 0 | 18700 | 7.96 | 4.20 | 24.5 |
| C | 1.20 | 0.0 | 10.54 | 0 | 17000 | 7.96 | 4.49 | 24.8 |
| S-200 | | | | | | | | |
| A | 2490.00 | 2470.0 | 232.80 | 2980 | 334 | 5.36 | 3.34 | 25.9 |
| B | 2300.00 | 2290.0 | 210.40 | 3840 | 385 | 5.16 | 3.39 | 25.9 |
| C | 1800.00 | 2410.0 | 223.10 | 2550 | 340 | 5.31 | 3.30 | 25.8 |

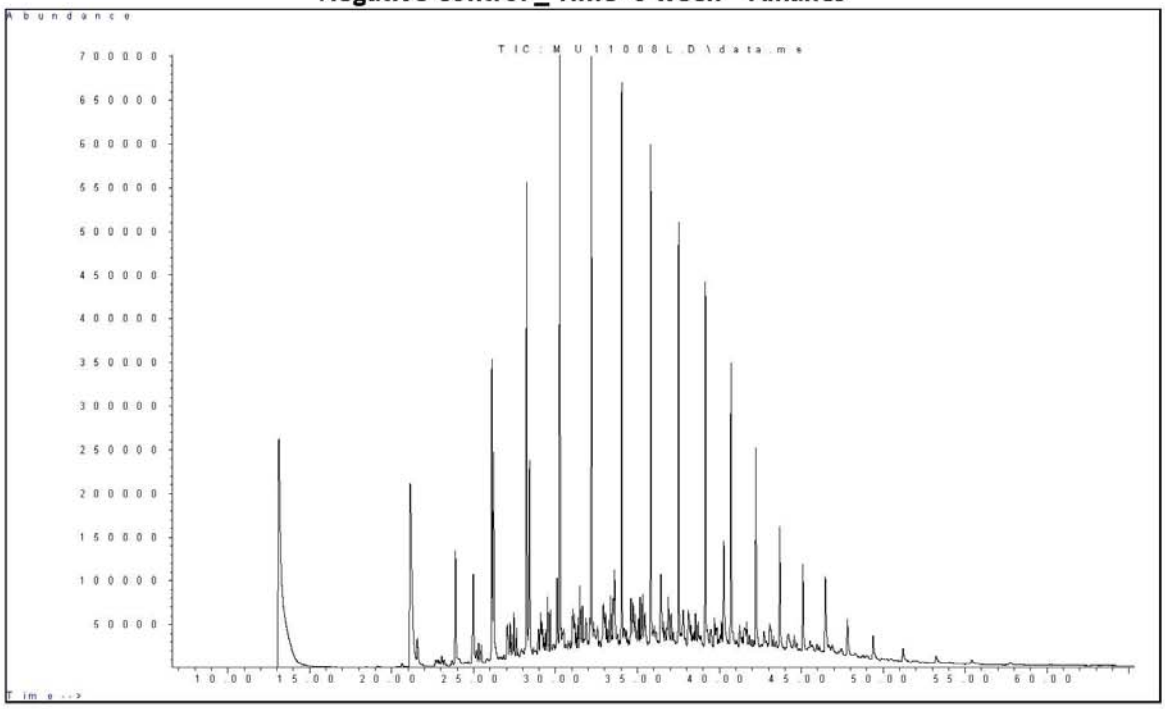
| Time = 4 Weeks 12/8/2010 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Flask Series*** | ***$NO_3^-$-N mg/L*** | ***$PO_4^{3-}$ mg/L*** | ***TOC*** | ***Alkanes mg/kg*** | ***PAHs mg/kg*** | ***pH*** | ***DO mg/L*** | ***Temp °C*** |
| Negative Control | | | | | | | | |
| A | 0.6 | 0.0 | 12.80 | 13900 | 267 | 7.87 | 4.61 | 24.2 |
| B | 0.6 | 0.1 | 13.49 | 14200 | 254 | 7.93 | 3.99 | 24.1 |
| C | 0.9 | 0.0 | 11.72 | 14300 | 269 | 7.97 | 4.57 | 24.4 |
| Positive Control 1 | | | | | | | | |
| A | 0.8 | 0.0 | 9.95 | 11500 | 67.7 | 7.93 | 4.47 | 25.1 |
| B | 1.1 | 0.0 | 13.04 | 1330 | 99.5 | 7.97 | 4.56 | 25.2 |
| C | 0.8 | 1.0 | 11.61 | 11800 | 73.1 | 7.98 | 4.01 | 25.4 |
| Positive Control 2 | | | | | | | | |
| A | 300.0 | 2070.0 | 73.39 | 1030 | 91.4 | 5.28 | 4.04 | 25.4 |
| B | 289.0 | 1770.0 | 90.00 | 723 | 116 | 5.19 | 4.11 | 25.4 |
| C | 920.0 | 1730.0 | 84.61 | 703 | 111 | 5.19 | 4.14 | 25.5 |
| Positive Control 3 | | | | | | | | |
| A | 0.6 | 0.4 | 10.68 | 0 | 19100 | 7.86 | 4.28 | 25.4 |
| B | 0.9 | 0.0 | 10.45 | 0 | 18800 | 7.90 | 4.15 | 25.7 |
| C | 0.8 | 0.0 | 10.47 | 0 | 19500 | 7.92 | 4.22 | DNR |
| S-200 | | | | | | | | |
| A | 1280.0 | 1920.0 | 213.90 | 1600 | 23.4 | 5.65 | 3.54 | 25.3 |
| B | 1870.0 | 1960.0 | 199.20 | 2940 | 70.4 | 5.55 | 3.77 | 25.3 |
| C | 1900.0 | 1650.0 | 220.00 | 2640 | 56.9 | 5.44 | 3.69 | 25.3 |

| Time = 12 Weeks 2/3/2011 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Flask Series*** | ***$NO_3^-$-N mg/L*** | ***$PO_4^{3-}$ mg/L*** | ***TOC*** | ***Alkanes mg/kg*** | ***PAHs mg/kg*** | ***pH*** | ***DO mg/L*** | ***Temp °C*** |
| Negative Control | | | | | | | | |
| A | 0.7 | | | 25100 | 401 | 7.81 | 5.58 | 22.1 |
| B | 0.6 | | | 23400 | 309 | 7.89 | 4.81 | 22.1 |
| C | 0.9 | | | 23400 | 341 | 7.96 | 5.02 | 22.3 |
| Positive Control 1 | | | | | | | | |
| A | 0.5 | | | 25100 | 341 | 7.77 | 4.90 | 21.9 |
| B | 0.7 | | | 23000 | 291 | 7.78 | 4.61 | 21.9 |
| C | 0.4 | | | 24100 | 303 | 7.91 | 5.03 | 22.4 |
| Positive Control 2 | | | | | | | | |
| A | | | | 900 | 340 | 5.24 | 5.04 | 21.9 |
| B | | | | 1020 | 359 | 5.23 | 4.95 | 22.1 |
| C | | | | 957 | 333 | 5.25 | 5.01 | 23.3 |
| Positive Control 3 | | | | | | | | |
| A | 0.3 | | | 0 | 12500 | 7.88 | 4.70 | 22.1 |
| B | 0.5 | | | 0 | 13800 | 7.93 | 4.72 | 22.1 |
| C | 0.4 | | | 0 | 12100 | 7.96 | 4.64 | 22.3 |
| S-200 | | | | | | | | |
| A | | | | 743 | 216 | 5.75 | 4.43 | 21.1 |
| B | | | | 982 | 268 | 5.76 | 5.50 | 21.3 |
| C | | | | 1240 | 274 | 5.76 | 5.27 | 21.3 |

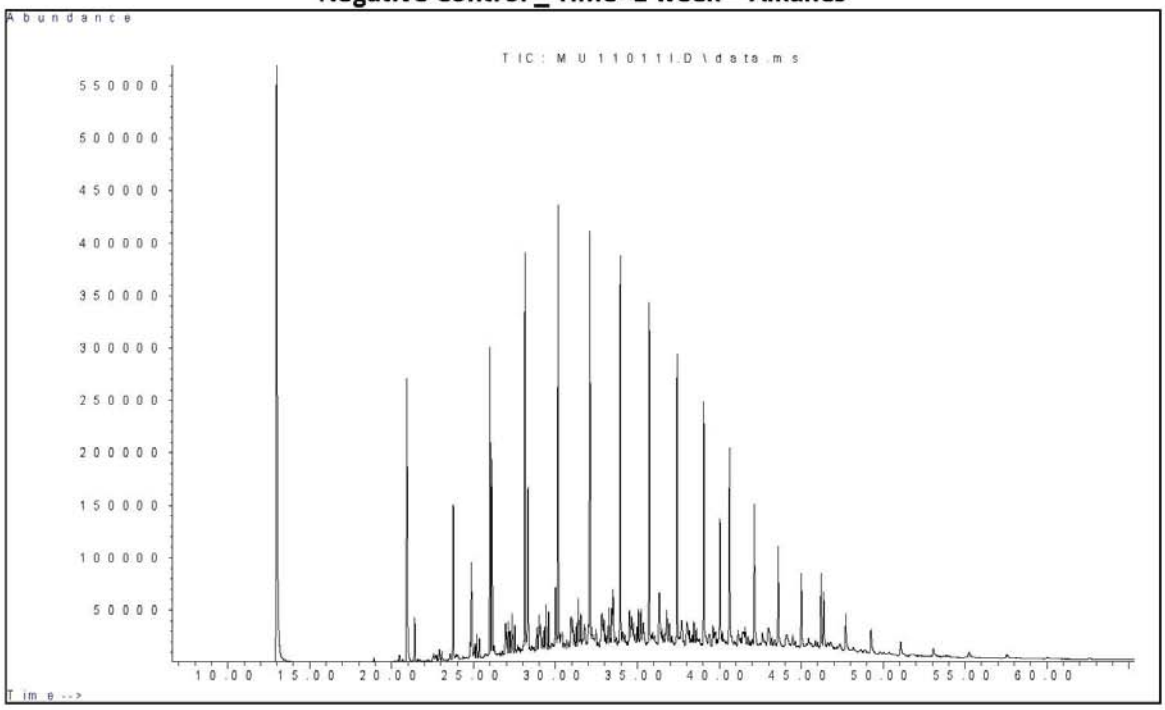
## Appendix B. Chromatographs of Extracted Flasks Over Time

Data sets presented are for total alkanes from Weeks 1 through 4 of the study. Chromatographs from Week 12 showed minimal changes as compared to Week 4. They will be included in the final report.

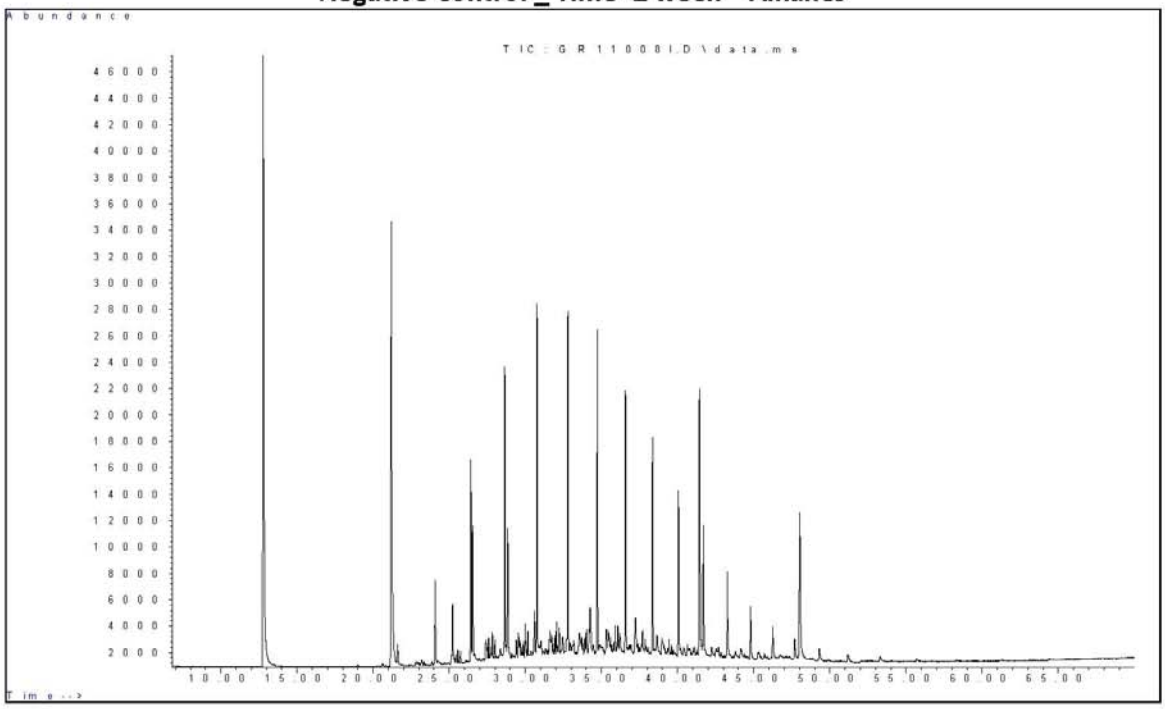
**Negative Control _ Time=0 week – Alkanes**



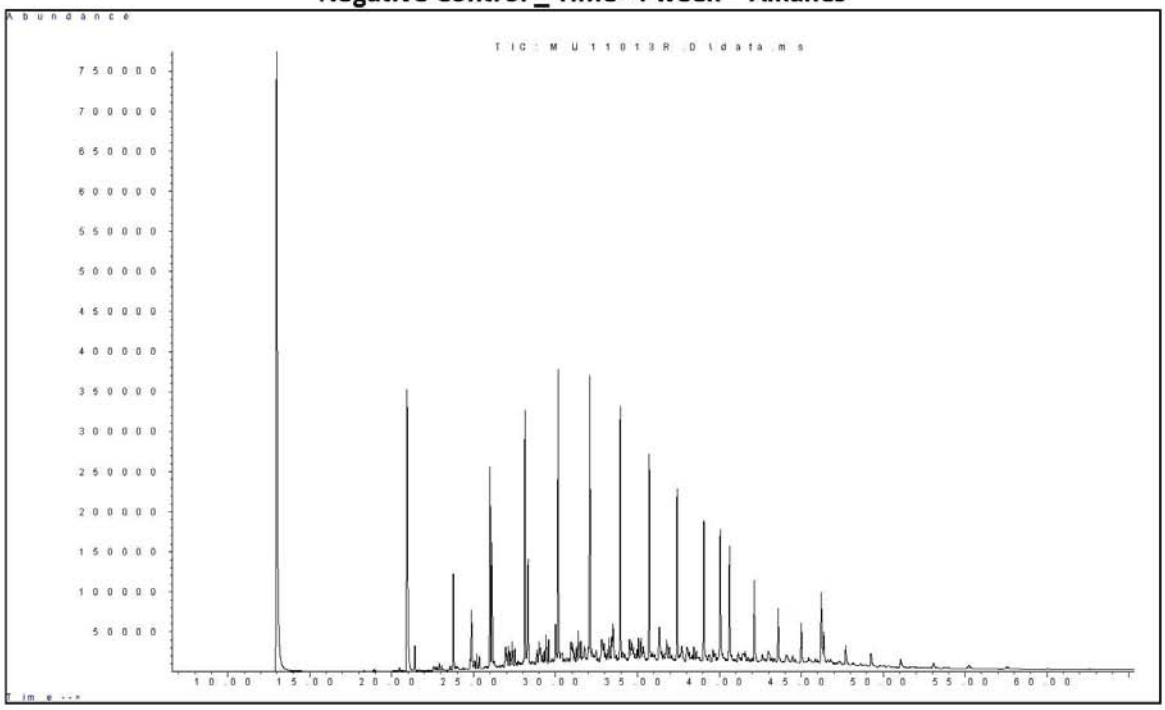

**Negative Control _ Time=1 week – Alkanes**



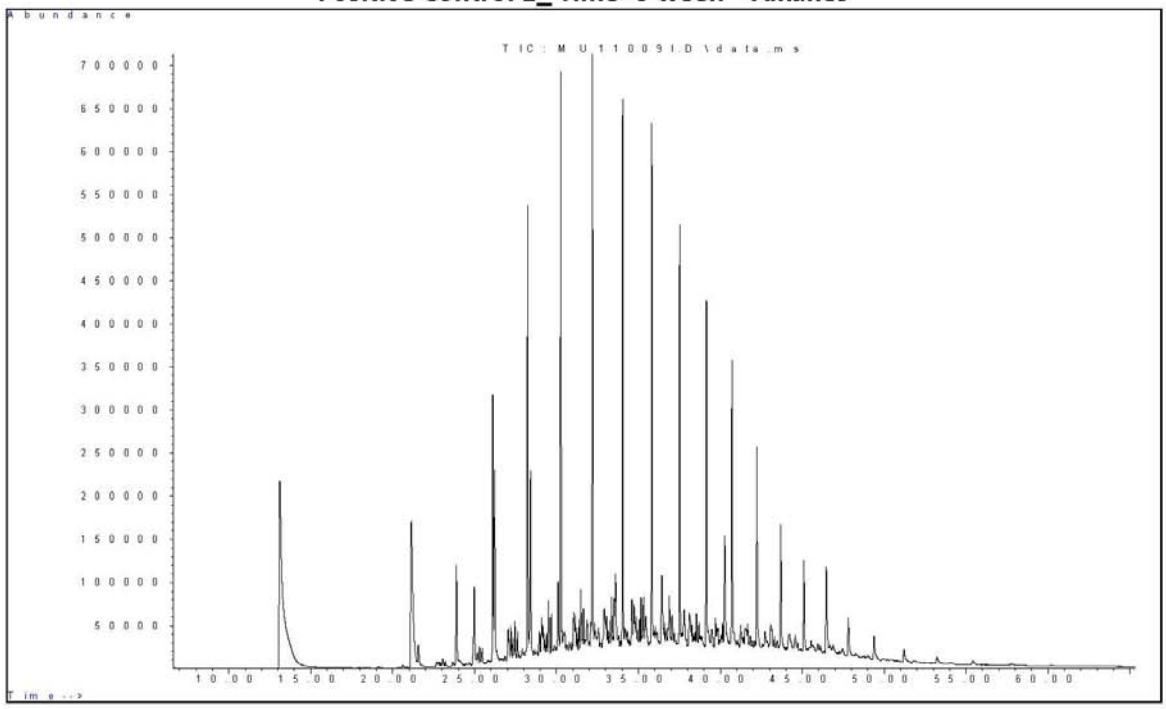
## Negative Control _ Time=2 week – Alkanes



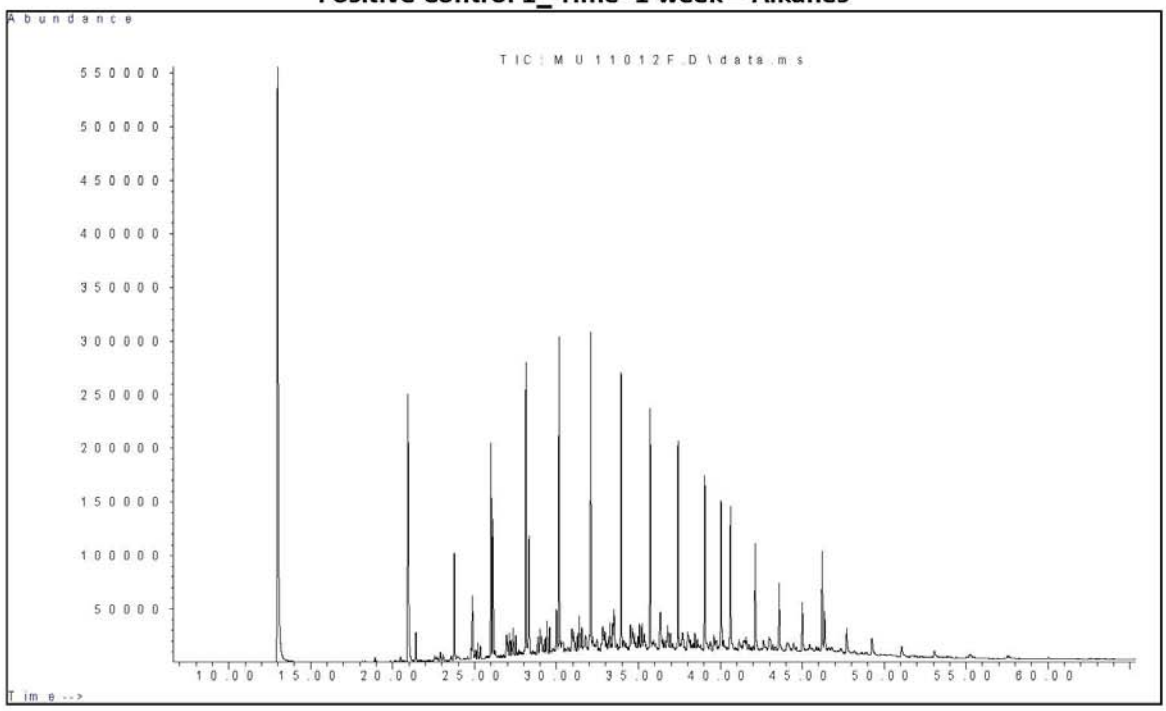

## Negative Control _ Time=4 week – Alkanes



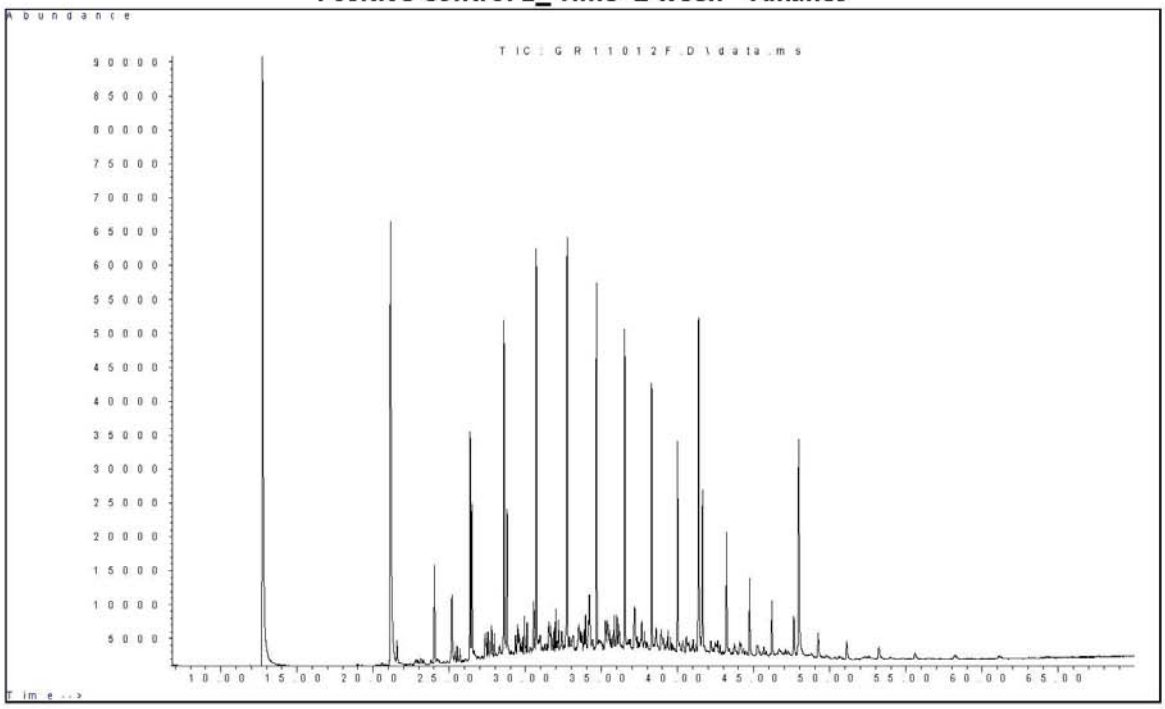
**Positive Control 1_ Time=0 week – Alkanes**



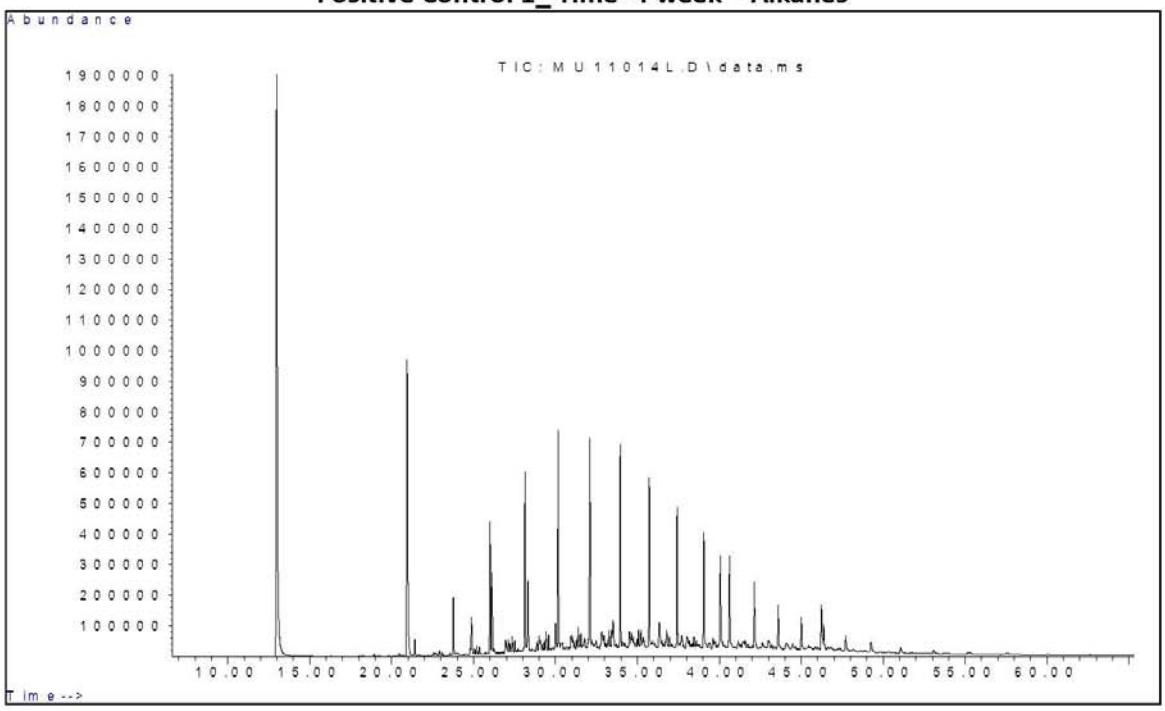

**Positive Control 1_ Time=1 week – Alkanes**



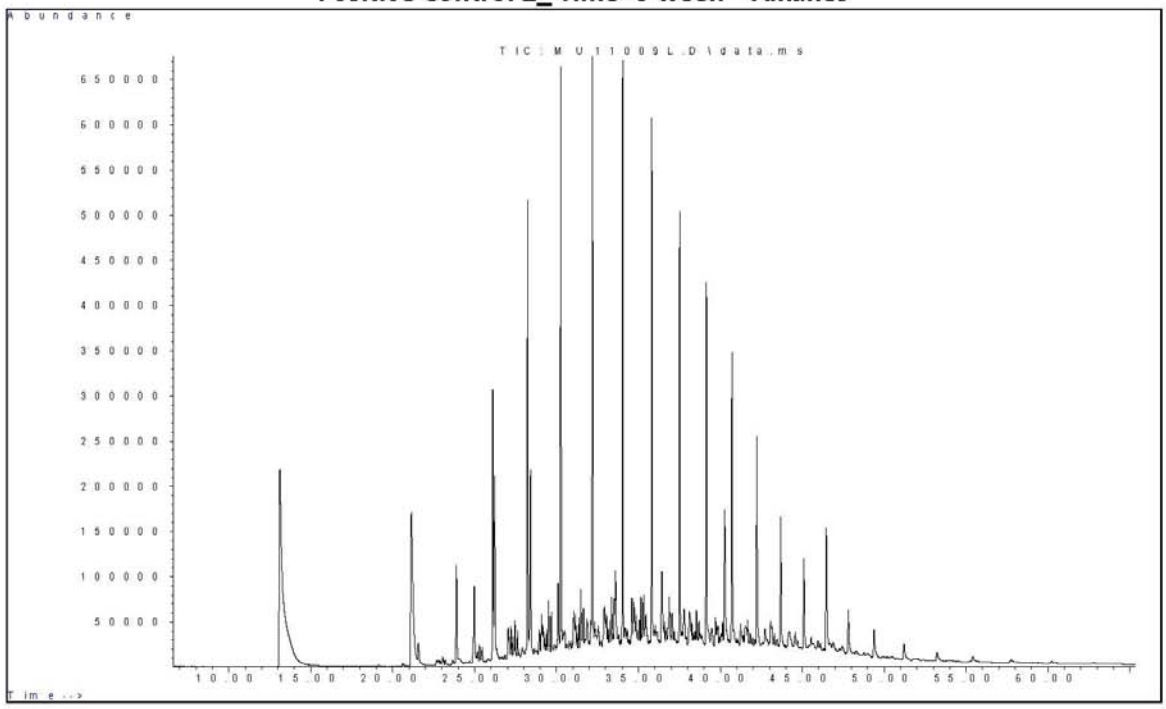
**Positive Control 1_ Time=2 week – Alkanes**



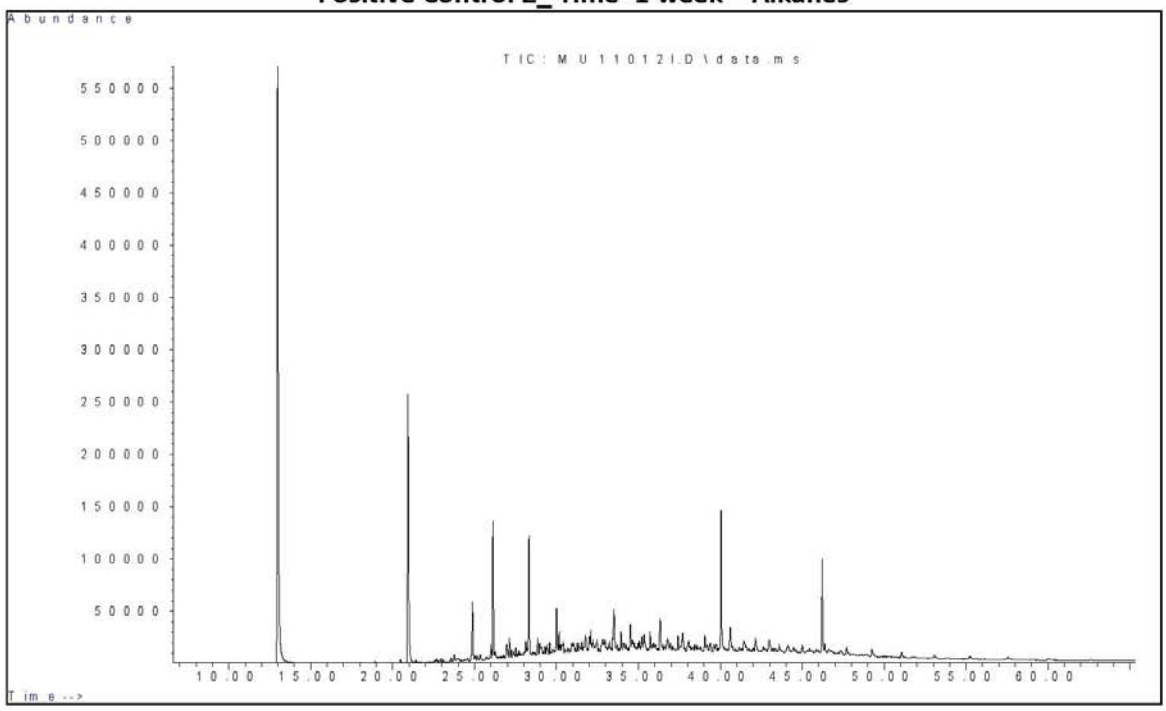

**Positive Control 1_ Time=4 week – Alkanes**



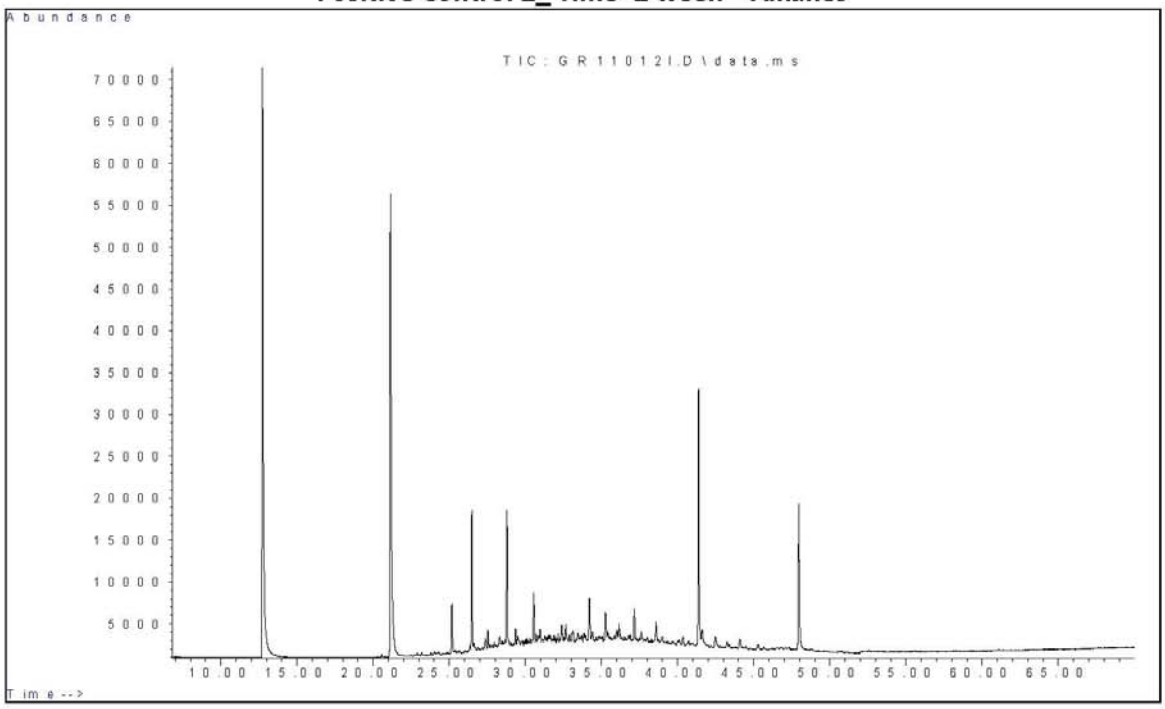
**Positive Control 2_ Time=0 week – Alkanes**



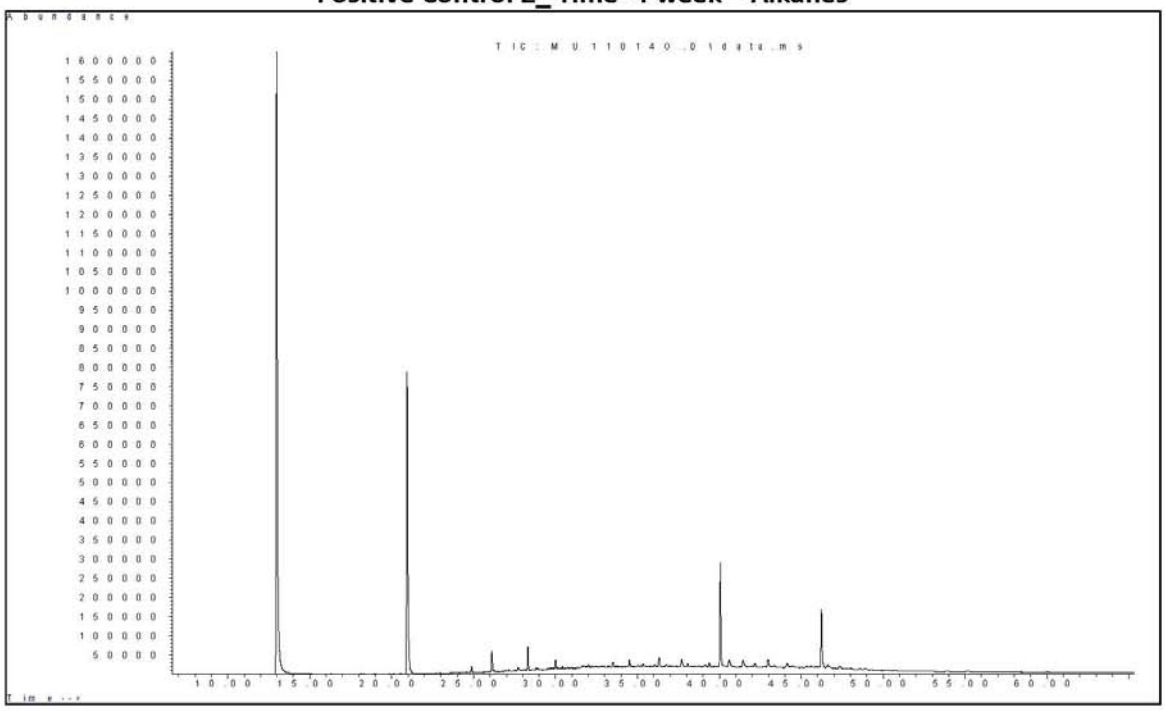

**Positive Control 2_ Time=1 week – Alkanes**



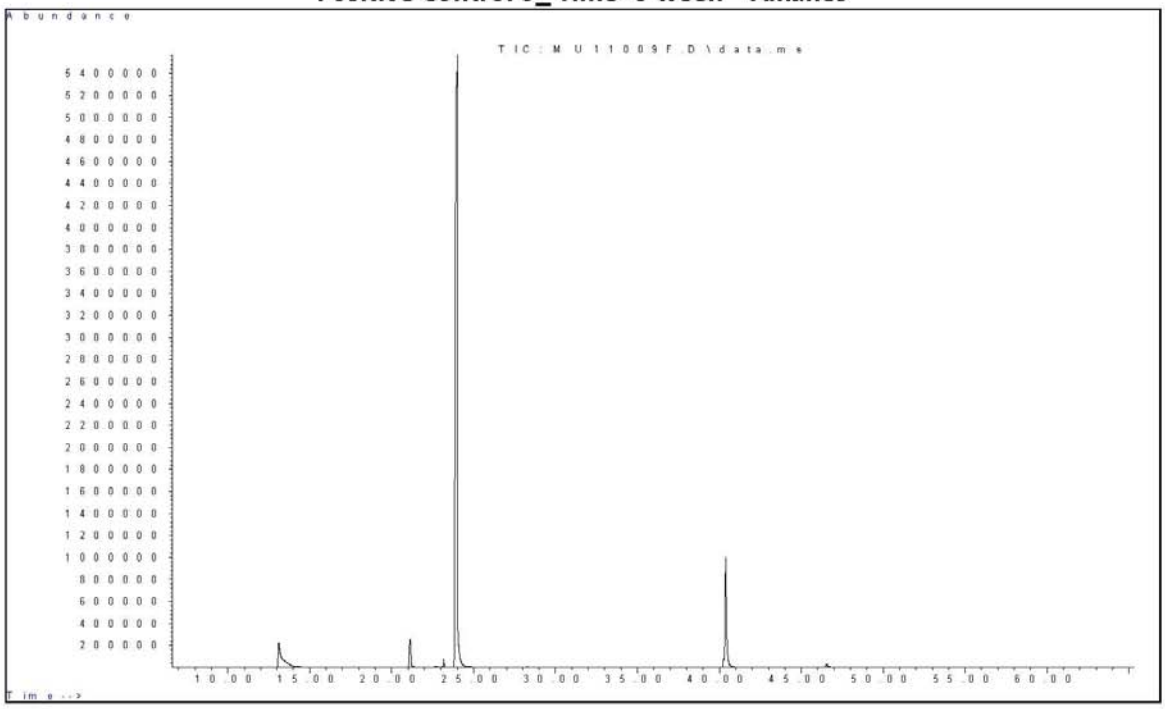
**Positive Control 2_ Time=2 week – Alkanes**



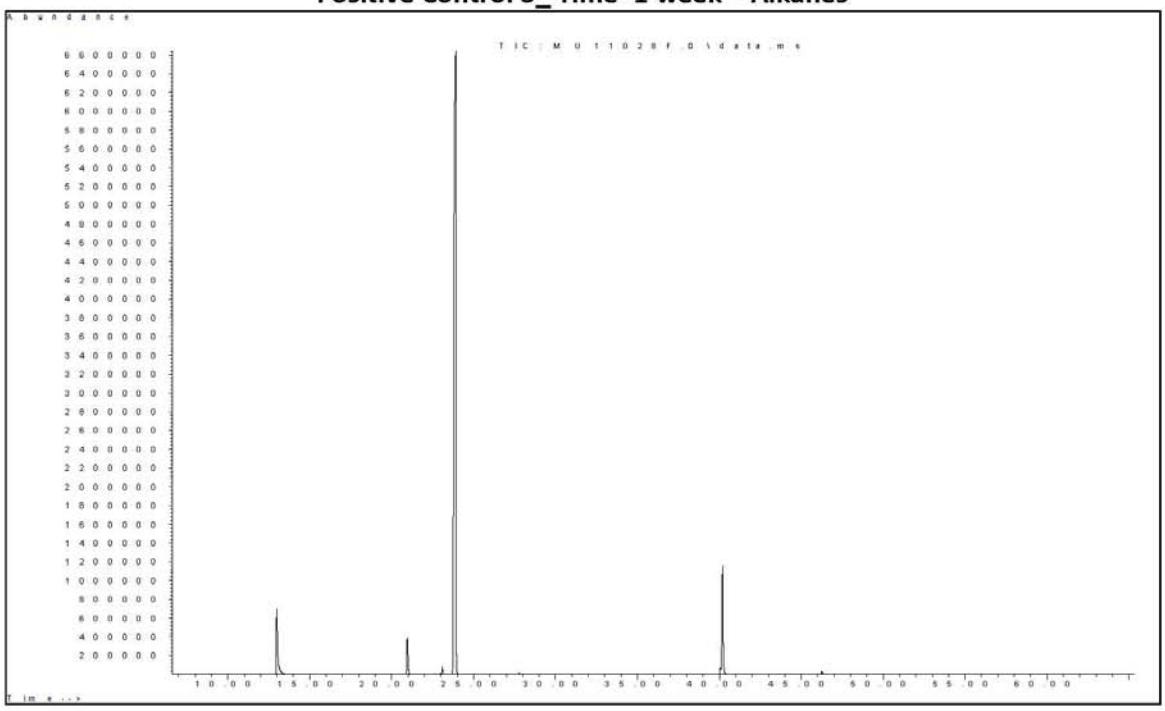

**Positive Control 2_ Time=4 week – Alkanes**



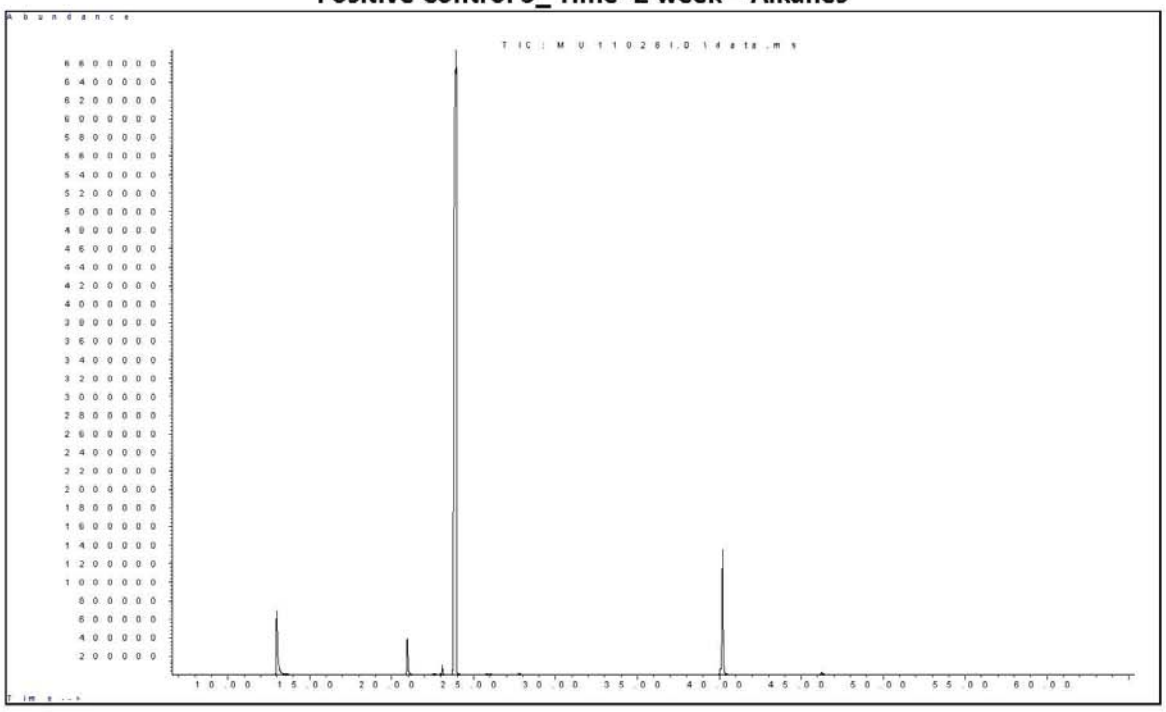
**Positive Control 3_ Time=0 week – Alkanes**



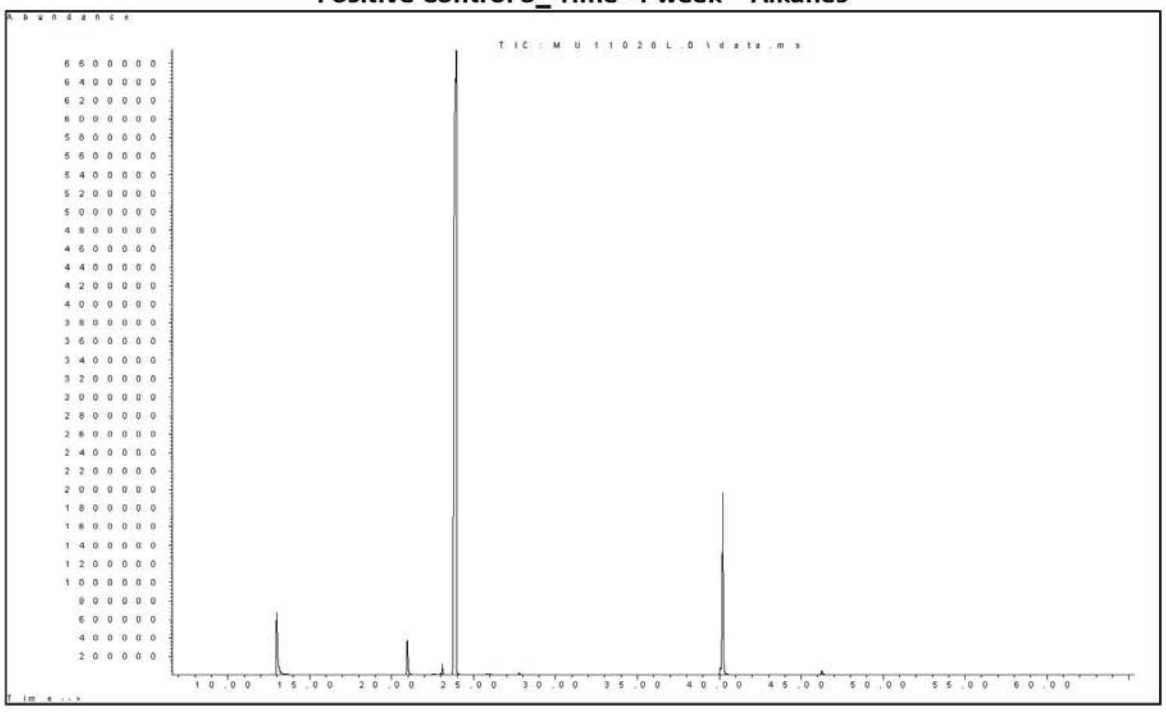

**Positive Control 3_ Time=1 week – Alkanes**



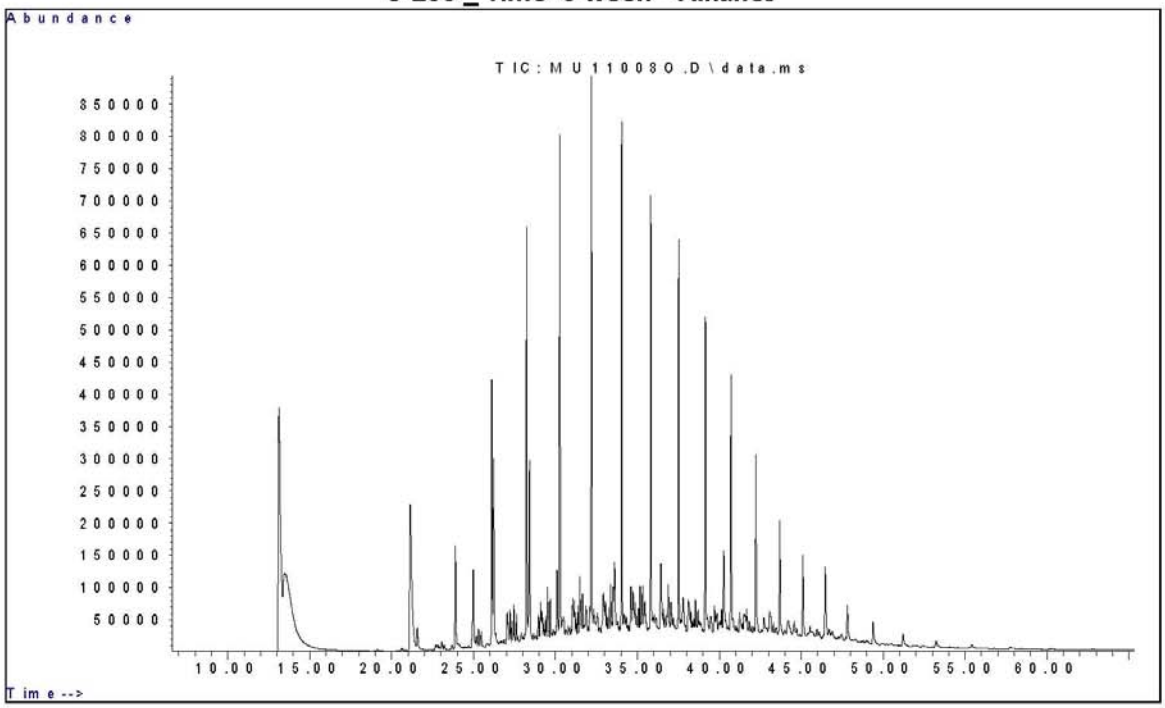
**Positive Control 3_ Time=2 week – Alkanes**



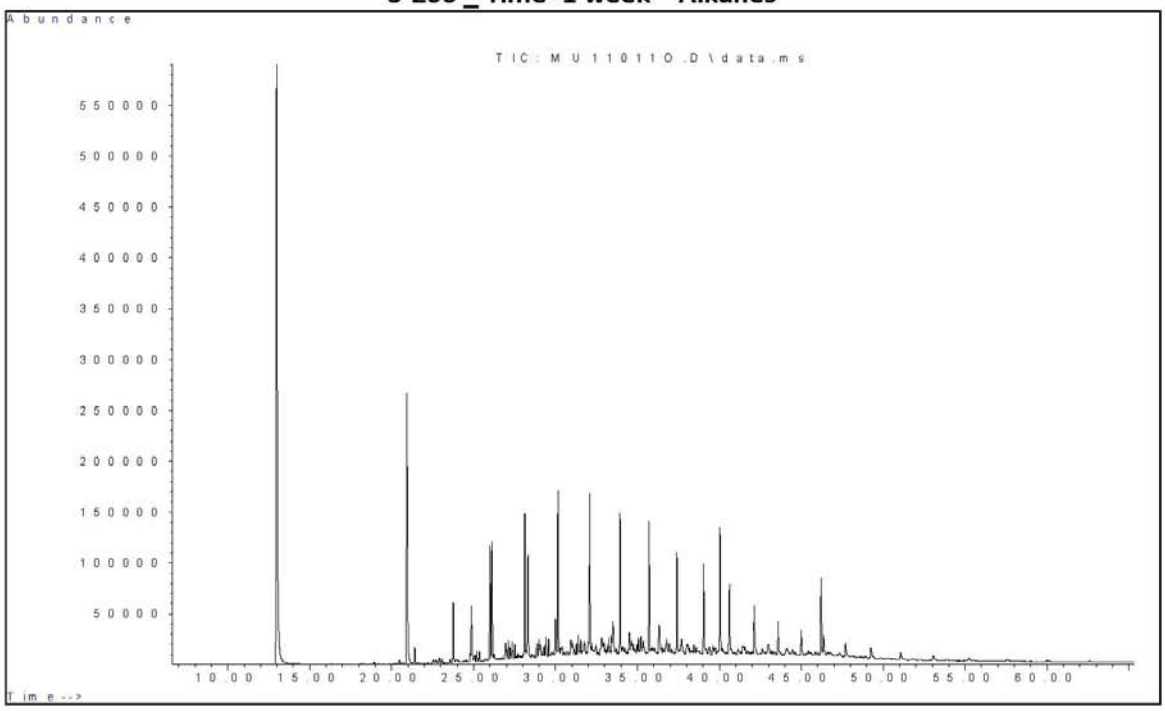

**Positive Control 3_ Time=4 week – Alkanes**



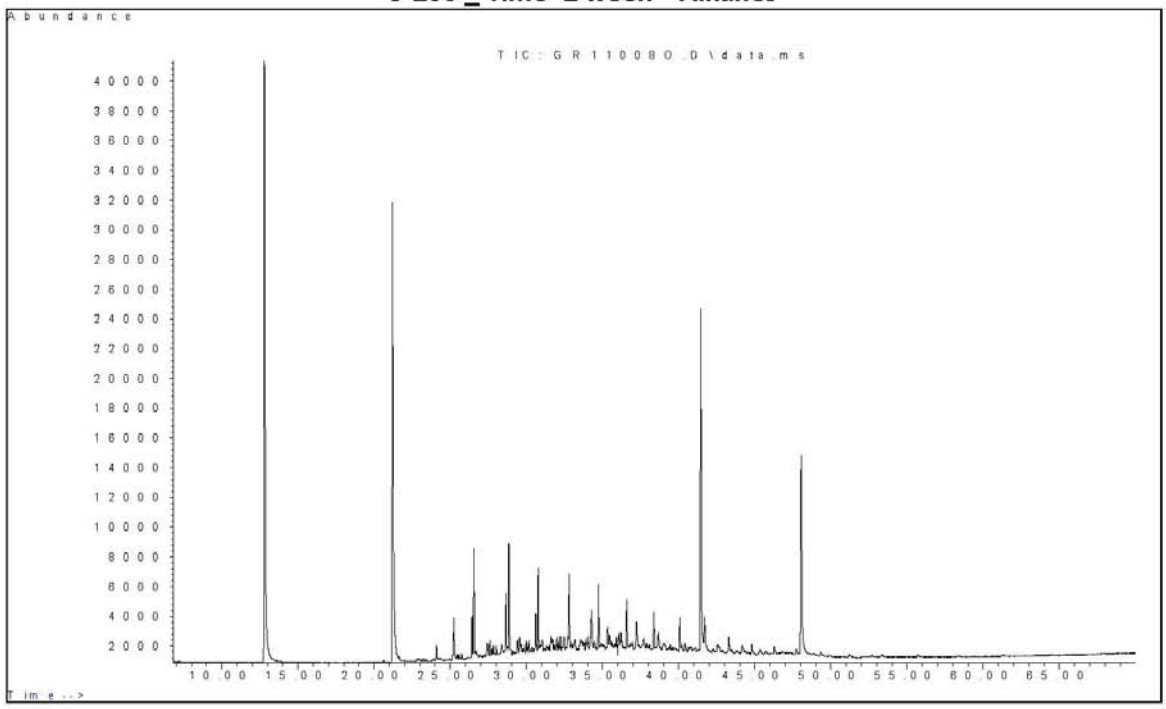
**S-200 _ Time=0 week – Alkanes**




**S-200 _ Time=1 week – Alkanes**



**S-200 _ Time=2 week – Alkanes**




**S-200 _ Time=4 week – Alkanes**