Journal of Applied Biosciences 165: 17120 – 17154
ISSN 1997-5902

The biodegradative capability of two selected microorganisms for vegetable oil contaminated soil.

Popoola B.M[1]*, Olanbiwoninu A.A and Fashogbon R.O.
Department of Biological Sciences, Ajayi Crowther University, P.M.B 1066, Oyo, Oyo State, Nigeria.
bukolamargaret@yahoo.com

Submitted on 14th July 2021. Published online at www.m.elewa.org/journals/ on 30th September 2021
https://doi.org/10.35759/JABs.165.8

ABSTRACT

*Objectives***:** The need to assess the bio-potentials of microorganisms for the treatment of vegetable oil-polluted sites is important. This study was carried out to monitor the pattern of degradation of vegetable oil in the laboratory.

Methodology and Results: Microbial load and concentration of the olive oil were monitored in two Mineral Salt Media. The first is a composition of (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MgSO_4.7H_2O$, 0.80; $CaCl_2$, 0.16; $(NH_4)_2NO_3$, 0.80; $FeSO_4$, 0.16; and Olive oil 2%, while the second consist of (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MnSO_4.4H_2O$, 0.80; NaCl, 0.16; $(NH_4)_2NO_3$, 0.80; $Fe_2(SO_4)_3$, 0.08 and Olive oil 2%. Degradation profiles of the fatty acid were determined using gas chromatography. The obtained data were analysed using ANOVA.

The total viable plate counts, 8.8 ± 0.03 and 10.3 ± 0.03, were ($P < 0.05$) significantly low on day 5 for both organisms compared to other days analysed. The residual oil degraded by *P. fluorescens* significantly ($P < 0.05$) reduced in concentration on day 15 (0.0031 mol/L) compared to day 0 (0.0064 mol/L). The percentage reduction of fatty acid by the two microorganisms' *P. fluorescens* and *C. parapsilosis* after 20 days was 8.2 % and 6.7 % respectively in the two media used.

Conclusions and Applications of findings: Vegetable oil spills can be challenging and cause havoc to the environment. Microorganisms are useful in protecting the environment and are usually used for the treatment of vegetable oil spills. The degradation ability of the isolates (from vegetable contaminated soil) on olive oil was monitored in the laboratory at five-day intervals for 25 days using two different mineral salt media. It was observed especially with the use of medium 1 in this study that the oil content reduced to the value 0.0031 mol/L (on the 15th day using *Pseudomonas fluorescens* for treatment) from the initial content of 0.0064 mol/L. The lipase enzymes from *Pseudomonas fluorescens* and *Candida parapsilosis* showed great potential for degradation of fatty waste. These isolates could be employed as candidates for in situ environmental clean-up of vegetable oil spill site especially in challenged edible oil producing countries like Malaysia.

Keywords: Vegetable oil spill, Microorganisms, Concentration, Degradation

INTRODUCTION

Spills of vegetable oil are becoming more common and are potentially more challenging than petroleum hydrocarbon spills (U.S EPA, 2013). Spills can spread fast between bodies of water and even land, coming in contact with humans, plants and animals. When spilled on land, their devastating effects cannot be over emphasized. Oil pollution impairs the process of photosynthesis, a fundamental life process in plant kingdom, which affects the food chain and productivity of the water bodies. Oil can also be absorbed by fish which eventually reach and endanger human life. Vegetable oils can vary significantly and when being released to the marine environment they will behave differently according to their individual characteristics (ITOPF, 2013). The displayed characteristics depend on several factors at that point of cultivation of the feed stock, these may include, climate; the degree of processing; type and specific nature of the oil, the sea state and weather conditions at the point of the spill (ITOPF, 2013). Generally the impact of vegetable oil characteristics on its behaviour in the environment is not well-studied or understood. Moreover, the behaviour and fate of specific vegetable oils is somewhat more difficult to predict than that of mineral oils (AAA, 2013). Vegetable oils usually behave similarly to mineral oils when initially spilled, hence they tend towards floating and spreading on the surface of water (GRT Maldives, 2012). Notably, vegetable oils seems less soluble in water than mineral oils; they are not dispersed in the water column nor do they evaporate significantly (GRT Maldives, 2012). Biodegradation can be termed as a process by which substances are broken down by organisms or the enzymes produced by the living organisms. Biodegradation could be used in relation to ecology, waste management and environmental remediation (bioremediation) as well as bio treatment of contaminated sites. Organic substances can be possibly degraded aerobically in the presence of oxygen or anaerobically, without oxygen. Lipids (fats, oils and greases) a major part of domestic and industrial waste contribute extensively towards environmental pollution. Sources include waste water from the edible oil refinery, slaughter houses, dairy industry products and kitchens. These effluents are usually the reason for clogging sewer networks and unsettling the balance of waste water treatment plants (Saifudin and Chua, 2006). The degradation of vegetable-based oils is usually initiated by enzyme-catalysed cleavage of the ester bond to fatty acid. Lipases and esterases are the enzymes which catalyse the biodegradation reaction these enzymes are synthesized by a wide range of microorganisms (Broekhuizen *et al*., 2003). Saifudin and Chua, 2006 reported that lipases which are hydrophobic proteins catalyse the cleavage of carboxyl esterbonds in tri-, di-, and monoacylglycerols (the major constituents of animal, plant and microbial fats and oils). Hence for their degradative capabilities, lipases are applied in remediation efforts to degrade lipid-rich water. However, drawbacks could be observed in the thermal instability of the enzyme and the high cost of the single use of the enzyme (Saifudin and Chua, 2006). Biodegradation of materials is dependent also on the nature of the environment. Kaakinen *et al*., 2007 noted that pH adjustments of soils for instance was found to have a not incurable effect on the biodegradation of certain compounds. Moreover communities of microorganisms are open to adapt to a substrate when it is a regular contaminant giving rise to identification and documentation of increases in rates of transformation of hydrocarbons associated with oil-contaminated environments (Khalida *et al*., 2006). Genetic engineering comes into play resulting in modified strains of bacteria for instance which are characterized by the ability to degrade the substances which induce the modification (Abrashev *et al*., 2002; Mansee *et*

al., 2004). It is worth noting however, that biodegradability primarily is a function of the chemical nature of the substrate in question. However, though these substrates are biodegradable, on-going research suggests that vegetable oil spills are more dangerous than hydrocarbon spills because of its toxicity. Records show that the toxicity of products such as soybean oil and canola oil actually increases significantly during the process of aerobic biodegradation (ITOPF, 2013). The effects of this in a confined, shallow environment could be significant (ITOPF, 2013). Hence this study aims to assess the bio-potentials of two microbial isolates used to degrade lipid from vegetable oil under laboratory conditions, and as an extension to our environment in order to rid it of the possible hazards these spillages can cause.

MATERIALS AND METHODS

Degradation of the Olive Oil in the Laboratory by the Test Isolates: A modified method of Gogoi *et al*. (2003) was used for the laboratory degradation by the test isolates. Two different compositions of Mineral Salt Medium was used, the first was composed of (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MgSO_4.7H_2O$, 0.80; $CaCl_2$, 0.16; $(NH_4)_2NO_3$, 0.80; $FeSO_4$, 0.16; and Olive oil 2%, pH maintained at 7.0. While the other was a modification of the first medium consisting (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MnSO_4.4H_2O$, 0.80; NaCl, 0.16; $(NH_4)_2NO_3$, 0.80; $Fe_2 (SO_4)_3$, 0.08 and Olive oil 2%, pH also maintained at 7.0. Hundred (100) millilitres, each of the two Mineral Salt Medium tested was dispensed into several 250ml Erlenmeyer flasks. The medium in several flasks were autoclaved at 121^{o}C for 15minutes and allowed to cool. Two (2) millilitres of olive oil sterilised using membrane pore filtration, serving as the sole carbon source was added to each flask. The bacterium was grown in nutrient broth overnight and thereafter inoculated as pure culture into the flasks. The yeast isolate was also grown in potato dextrose broth overnight and inoculated as test organism into the flasks. A separate flask served as the control, this was not inoculated with the test isolates. The experiment was run in duplicates and incubated at room temperature on a shaker at 180rpm for 25days. The pH of the culture media was determined with the use of a pH meter. The total viable counts were carried out by plating out serial dilutions of the culture on nutrient agar plates for the bacteria and potato dextrose agar for the yeast and incubated at (27^{o}C $\pm$ 2^{o}C). The residual oil in the experimental setup (culture media) was recovered using n-hexane. This was done by empting the content of the flasks into a separating funnel and then adding 100ml n-hexane. The mixture was vigorously shaken and then the aqueous phase was discarded while the organic phase was allowed to evaporate to a constant weight in a Petri dish, and then measured gravimetrically. Before evaporation, the extract was read on a UV/VIS spectrophotometer at 420nm to obtain an estimate of the olive oil content. A standard graph of oil concentration, plotted against absorbance was used for extrapolating the percent oil content.

Gas Chromatograph (GC)

Fatty Acid Methyl Ester Analysis of the Residual Oil: The fatty acid profile- saturated, mono- and polyunsaturated analysis were monitored using the modified AOAC 965.49 and AOAC 996.06 official methods (OMA, 1995; Oforma *et al*., 2019). The oil sample (50 mg) was esterified for 5 minutes at 95°C with 3.4 ml of the 0.5 M KOH in dry methanol. This was then neutralised using 0.7M HCL 3ml of the 14% boron triflouride in methanol. For complete methylation the mixture was heated for 5 minutes at temperature 90°C, after which the Fatty Acid Methyl Esters were thrice extracted

from the mixture with redistilled n-hexane. This was then concentrated to 1ml and 1 µl was injected into the injection port of the GC. The HP 6890 Gas chromatography analyzer powered by HP Chemstation Rev. A 09.01 [1206] Software and equipped with a Flame Ionization Detector (FID) and HP INNOWax column (30m X 0.25mm X 0.2µm film thickness) separated the fatty acid methyl esters. The oven initial temperature was at 60^{o}C and the carrier gas was nitrogen. The first and second ramping were at 12^{o}C/min for 20 min, maintained for 2 minutes and at 15^{o}C/min for 3 minutes, maintained for 8 minutes respectively. The detector temperature was 320^{o}C, while having hydrogen and compressed air pressures at 22 and 35 psi respectively. The concentrated content is being swept through the column by the carrier gas; indicating that the different components of the content were separated as they pass through the column thereby reaching the end of the column at different times. After which a detector was then used to monitor the outlet stream from the column, allowing for the determination of the time at which each component reaches the outlet as well as the amount of that component.

RESULTS

Degradation of the Olive Oil in the Laboratory by Test Isolates: The concentration of residual oil of *Pseudomonas fluorescens* from medium 1 was significantly lower on day 15 during the period of 25 days but for *Candida parapsilosis* on day 0 the concentration was significantly lower than others (Table 1). For culture medium 2 concentration of oil recovered from *Pseudomonas fluorescens* culture medium was significantly higher on day 20, it has the least concentration on day 15 and day 0 (Table 2).

Table 1: Residual oil concentration in culture medium (1) degraded by individual soil microorganism over 25 days

	Degradation period (days) / Concentration (mol/l)					
	0 Day	5th Day	10th Day	15th Day	20th Day	25th Day
Pseudomonas fluorescens	0.0064 ± 0.0000^{b}	0.0114± 0.0003^{a}	0.0081± 0.0006^{b}	0.0031 ± 0.0011^{c}	0.0075 ± 0.0009^{b}	0.0067 ± 0.0007^{b}
Candida parapsilosis	0.0064 ± 0.0000^{d}	0.0130± 0.0004^{a}	0.0098± 0.0005^{b}	0.0079 ± 0.0002^{c}	0.0093 ± 0.0001^{b}	0.0091 ± 0.0003^{b}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Culture medium 1 consisting (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MgSO_4.7H_2O$, 0.80; $CaCl_2$, 0.16; $(NH_4)_2NO_3$, 0.80; $FeSO_4$, 0.16; olive oil 2%, pH maintained at 7.0.

Table 2: Residual oil concentration in culture medium (2) degraded by individual soil microorganism over 25 days

	Degradation period (days) / Concentration (mol/l)					
	0 Day	5th Day	10th Day	15th Day	20th Day	25th Day
Pseudomonas fluorescens	0.0064± 0.0000^{b}	0.0101± 0.0003^{ab}	0.0096 ± 0.0003^{ab}	0.0066 ± 0.0004^{b}	0.0133 ± 0.0021^{a}	0.0096 ± 0.0022^{ab}
Candida parapsilosis	0.0064± 0.0000^{c}	0.0140 ± 0.0001^{a}	0.0075 ± 0.0007^{bc}	0.0096± 0.0011^{b}	0.0091 ± 0.0004^{b}	0.0094 ± 0.0013^{b}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Culture medium 2 consisting (g/L) of KH_2PO_4, 7.584; K_2HPO_4, 0.80; $MnSO_4.4H_2O$, 0.80; NaCl, 0.16; $(NH_4)_2NO_3$, 0.80; $Fe_2 (SO_4)_3$, 0.08; olive oil 2%,

Plate count of the isolates from culture medium 1; on day 20 for *Pseudomonas fluorescens*, the value 12.97 (log_{10}cfu/ml) was significantly higher than those of other days and day 5 was the least. *Candida parapsilosis* had plate count increase over the period of degradation but for the 20th and 25th day (Table 3). In the case of isolation from medium 2, isolation from *Pseudomonas* culture medium shows increase in plate count over the period of the degradation, this also applies to *Candida parapsilosis*, with significant differences over the period of degradation (Table 4). For the pH of culture medium 1, *Pseudomonas fluorescens* on day 15 was significantly lower than others and significantly higher on days 10 and 20, it is also worth noting that the initial pH on day 0 is 5.70 while for medium 2 it is 5.00. *Candida parapsilosis* had lower pH value on day 25 (Table 5). For culture medium 2, there was no significant difference for *Pseudomonas fluorescens* on the 5th and 25th days. pH for culture medium of *Candida parapsilosis* shows no significant difference from days 15 to 25, it also shows a decrease in pH value (Table 6).

Table 3. Plate count of the isolates in culture medium (1) during degradation over 25 days

	Degradation period (days) / Count (log_{10}cfu/ml)				
	5th Day	**10th Day**	**15th Day**	**20th Day**	**25th Day**
Pseudomonas fluorescens	8.81±0.0335^{e}	10.39±0.0266^{d}	12.57±0.0118^{c}	12.97±0.0118^{a}	12.78±0.0035^{b}
Candida parapsilosis	10.27±0.0353^{e}	10.85±0.0061^{d}	10.98±0.0067^{c}	8.77±0.0037^{b}	9.51±0.0335^{a}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Table 4: Plate count of the isolates in culture medium (2) during degradation over 25 days

	Degradation period (days) / Count (log_{10}cfu/ml)				
	5th Day	**10th Day**	**15th Day**	**20th Day**	**25th Day**
Pseudomonas fluorescens	7.91±0.0053^{e}	9.80±0.1990^{d}	10.59±0.1110^{c}	11.90±0.0055^{b}	12.70±0.0000^{a}
Candida parapsilosis	8.76±0.0150^{d}	8.92±0.0131^{c}	9.64±0.0396^{b}	9.71±0.0211^{ab}	9.77±0.0074^{a}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Table 5: pH changes in the culture medium (1) under degradation by individual soil microorganism over 25 days

	Degradation period (days) / pH				
	5th	**10th**	**15th**	**20th**	**25th**
Pseudomonas fluorescens	5.05±0.05^{ab}	5.25±0.05^{a}	4.90±0.10^{c}	5.20±0.00^{a}	5.10±0.00^{ab}
Candida parapsilosis	4.15±0.05^{b}	4.55±0.05^{a}	4.45±0.05^{a}	3.85±0.05^{c}	3.55±0.05^{d}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Table 6: pH changes in the culture medium (2) under degradation by individual soil microorganism over 25 days

	Degradation period (days) / pH				
	5th	**10th**	**15th**	**20th**	**25th**
Pseudomonas fluorescens	3.70±0.20^{ab}	4.25±0.05^{a}	3.65±0.05^{b}	4.25±0.05^{a}	3.95±0.05^{ab}
Candida parapsilosis	4.20±0.10^{b}	4.55±0.05^{a}	3.50±0.10^{c}	3.70±0.10^{c}	3.50±0.00^{c}

Means with different superscripts across the rows are significantly different at P≤ 0.05 using Duncan's Multiple Range Test

Fatty Acid Methyl Ester Analysis: The percentage composition of the released components of fatty acid degraded by *Pseudomonas fluorescens* and *Candida parapsilosis* using medium 1 over 25days is shown. It can be seen that Oleic acid has the highest percentage composition (73.1831%) starting from day 5, the percentage composition of oleic acid reduced to 72.2548%, whereas stearic acid for instance increased from 4.5241% to 5.5839% on day 10. After 15days of fermentation, percentage composition of oleic acid reduced to 72.039. From 72.0390% percentage oleic acid composition was drastically reduced to 67.8111% as seen on day 20. However, on the 20th day, oleic acid increased a little from 67.8111% to 69.4782% on the 25th day, stearic acid composition however increased over the period of 25days from 4.5241% on day 5 to 8.7102% on day 25. In the case of *Candida parapsilosis*, percentage oleic acid on the 5th day was 74.8731%. Day 10 recorded 72.5577% of oleic acid composition. The composition was 71.0757% on the 15th day, it was reduced substantially by the 20th day to 69.2278%. Although by the 25th day, it was risen again to 70.0411% (Table 7). In case of the released fatty acid degraded by *Pseudomonas fluorescens* from culture medium 2 the percentage composition of oleic acid on day 5 was 73.4490%, this is shown in Table 4.17. After 10 days, it was 72.1077%. On the 15th day it was higher 72.6759%, however on day 20 it was brought down to 69.2945% by *Pseudomonas fluorescens* in culture medium 2. Finally, on day 25, it was 69.2167%. In the case of fermentation by *Candida parapsilosis,* by day 5, the percentage of oleic acid was 74.4584%, however by the 10th day, oleic acid composition reduced to 73.3139%, which was further reduced to 71.4433% on the 15th day and by day 20 it was 69.4455%. On the 25th day of fermentation by *Candida parapsilosis,* there was increase in oleic acid percentage composition (Table 8). The chromatogram of all the samples are indicated under appendix I, sample numbering is as indicated on table 9. The overall percentage oleic acid released from the residual oil by *Pseudomonas fluorescens* and *Candida parapsilosis* over 25days. The breakdown pattern of the oil can be seen by the pattern of reduction in the oleic acid (Table 9).

Table 7: The percentage composition of released fatty acid degraded by *Pseudomonas fluorescens* (PF) and *Candida parapsilosis* (CP) (from culture medium 1) over 25 days

				Period of degradation (days) / Isolate/ percentage composition							
S/N	**Fatty acid**	**5**		**10**		**15**		**20**		**25**	
		PF	**CP**	**PF**	**CP**	**PF**	**CP**	**PF**	**CP**	**PF**	**CP**
1	FGN1	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
2	FGN2	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
3	FGN3	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
4	F001	0.0698 ^{j}	0.0820 ^{i}	0.0834 ^{h}	0.1090 ^{f}	0.2382 ^{e}	0.0900 ^{g}	0.3169 ^{d}	0.3822 ^{c}	0.4531 ^{b}	0.5658 ^{a}
5	F002	11.5095 ^{i}	10.1880 ^{j}	11.7429 ^{h}	11.7739 ^{g}	13.8403 ^{e}	13.7205 ^{f}	16.4771 ^{b}	15.7671 ^{d}	16.6447 ^{a}	16.3746 ^{c}
6	F003	0.0792 ^{b}	0.0941 ^{a}	0.0513 ^{d}	0.0674 ^{c}	0.0226 ^{f}	0.0304 ^{e}	0.0111 ^{h}	0.0211 ^{g}	0.0000 ^{i}	0.0000 ^{i}
7	FCS1	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
8	FCS2	4.5241 ^{j}	4.5799 ^{i}	5.5839 ^{h}	5.5983 ^{g}	5.8617 ^{f}	6.2702 ^{e}	8.8069 ^{a}	7.6284 ^{d}	8.7102 ^{b}	8.2155 ^{c}
9	FCS3	73.1831 ^{b}	74.8731 ^{a}	72.2548 ^{d}	72.5577 ^{c}	72.0390 ^{e}	71.0757 ^{f}	67.8111 ^{j}	69.2278 ^{i}	69.4782 ^{h}	70.0411 ^{g}
10	FCS4	9.4021 ^{a}	9.0634 ^{b}	9.2607 ^{c}	8.8472 ^{d}	6.8863 ^{f}	8.25074 ^{e}	5.9675 ^{h}	6.040 ^{g}	4.091 ^{j}	4.0864 ^{j}
11	FSF1	0.9986 ^{a}	0.8426 ^{b}	0.7397 ^{c}	0.6762 ^{d}	0.5112 ^{e}	0.4394 ^{g}	0.3587 ^{h}	0.4528 ^{f}	0.2796 ^{j}	0.3047 ^{i}
12	FSF2	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
13	FSF3	0.0620 ^{d}	0.0731 ^{c}	0.0747 ^{b}	0.0978 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}
14	FP01	0.0467 ^{d}	0.0553 ^{h}	0.0562 ^{g}	0.0735 ^{f}	0.1627 ^{a}	0.0333 ^{j}	0.0795 ^{e}	0.1517 ^{b}	0.1082 ^{d}	0.1302 ^{c}
15	FP02	0.0251 ^{e}	0.0296 ^{d}	0.0315 ^{c}	0.0405 ^{b}	0.0879 ^{a}	0.0186 ^{f}	0.0000 ^{g}	0.000 ^{g}	0.0000 ^{g}	0.0000 ^{g}
16	LIG	0.100 ^{i}	0.1187 ^{h}	0.1209 ^{g}	0.1584 ^{f}	0.3499 ^{a}	0.0715 ^{j}	0.1714 ^{e}	0.3285 ^{b}	0.2348 ^{d}	0.2816 ^{c}

KEY: Go to appendix

Using Duncan's Multiple Range Test, means with different superscripts across the rows are significantly different at $P \leq 0.05$.

Table 8: The percentage composition of released fatty acid degraded by *Pseudomonas fluorescens* (PF) and *Candida parapsilosis* (CP) (from culture medium 2) over 25 days

				Period of degradation (days) / Isolate / percentage composition							
S/N	**Fatty acid**	**5**		**10**		**15**		**20**		**25**	
		PF	**CP**	**PF**	**CP**	**PF**	**CP**	**PF**	**CP**	**PF**	**CP**
1	FGN1	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
2	FGN2	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
3	FGN3	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
4	F001	0.0625 ^{j}	0.0815 ^{g}	0.0720 ^{i}	0.0721 ^{h}	0.1170 ^{f}	0.1564 ^{e}	0.2948 ^{d}	0.4040 ^{c}	0.6352 ^{a}	0.6020 ^{b}
5	F002	10.8173 ^{i}	10.2901 ^{j}	11.1341 ^{h}	11.1909 ^{g}	14.0435 ^{e}	13.4261 ^{f}	15.6081 ^{c}	15.2642 ^{d}	16.3984 ^{a}	16.0694 ^{b}
6	F003	0.0712 ^{b}	0.0937 ^{a}	0.0440 ^{d}	0.0443 ^{c}	0.0278 ^{g}	0.0421 ^{e}	0.0098 ^{h}	0.0323 ^{f}	0.0000 ^{i}	0.0000 ^{i}
7	FCS1	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
8	FCS2	4.4830 ^{j}	4.5545 ^{i}	5.4319 ^{g}	5.1792 ^{h}	6.0439 ^{e}	6.0156 ^{f}	8.7267 ^{b}	7.8235 ^{d}	8.9695 ^{a}	8.4980 ^{c}
9	FCS3	73.4490 ^{b}	74.4584 ^{a}	72.1077 ^{e}	73.3139 ^{c}	72.6759 ^{d}	71.4433 ^{f}	69.2945 ^{i}	69.4455 ^{h}	69.2167 ^{j}	70.0905 ^{g}
10	FCS4	9.8983 ^{b}	9.5437 ^{c}	10.2956 ^{a}	9.4090 ^{d}	6.3022 ^{f}	8.1514 ^{e}	5.5103 ^{h}	6.0909 ^{g}	4.1199 ^{j}	4.1804 ^{i}
11	FSF1	1.0083 ^{a}	0.7033 ^{b}	0.6716 ^{c}	0.5457 ^{e}	0.5944 ^{d}	0.4714 ^{f}	0.3329 ^{h}	0.4674 ^{g}	0.2956 ^{j}	0.2074 ^{i}
12	FSF2	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}	0.0000 ^{a}
13	FSF3	0.0559 ^{d}	0.0727 ^{a}	0.0642 ^{c}	0.0646 ^{b}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}	0.0000 ^{e}
14	FP01	0.0420 ^{j}	0.0547 ^{f}	0.0482 ^{h}	0.0436 ^{i}	0.0528 ^{g}	0.0794 ^{d}	0.0706 ^{e}	0.1490 ^{b}	0.1151 ^{c}	0.1415 ^{a}
15	FP02	0.0226 ^{f}	0.0292 ^{b}	0.0270 ^{e}	0.0272 ^{d}	0.0289 ^{c}	0.0429 ^{a}	0.0000 ^{g}	0.0000 ^{g}	0.0000 ^{g}	0.0000 ^{g}
16	LIG	0.0898 ^{j}	0.1182 ^{f}	0.1037 ^{i}	0.1046 ^{h}	0.1137 ^{g}	0.1711 ^{d}	0.1522 ^{e}	0.3231 ^{a}	0.2495 ^{b}	0.2108 ^{c}

Using Duncan's Multiple Range Test, means with different superscripts across the rows are significantly different at $P \leq 0.05$.

Table 9: Percentage of oleic acid released from residual oil by the isolates over 25 days

S/N	Sample Nature	Percentage Oleic Acid
1.	1P5	73.1831
2.	1P10	72.2548
3.	1P15	72.0390
4.	1P20	67.8111
5.	1P25	69.4782
6.	2P5	73.4490
7.	2P10	72.1077
8.	2P15	72.6759
9.	2P20	69.2945
10.	2P25	69.2167
11.	1C5	74.8731
12.	1C10	72.5577
13.	1C15	71.0757
14.	1C20	69.2278
15.	1C25	70.0411
16.	2C5	74.4537
17.	2C10	73.3139
18.	2C15	71.4433
19.	2C20	69.4455
20.	2C25	70.0905

Key

1 – Culture medium 1 5,10,15,20 and 25 – Period of degradation in days

2 – Culture medium 2

P – *Pseudomonas fluorescens*

C – *Candida parapsilosis*

DISCUSSION

From the biodegradation results obtained in this research it could be observed that concentration of residual olive oil in culture medium 1 degraded by *Pseudomonas fluorescens* reduced (8.2%) within the period of degradation (25 days). This study corroborates the work of Aluyor *et al.*, 2009 who found vegetable oils undergo about 70-100% biodegradation within 28 days. Prasad and Manjunath (2011) also showed that wastewater samples having an average pH of 5, average BOD value of 3200 mg/L and average lipid content of 25,000 mg/L before the treatment. They found the amount of lipid content present in the samples reduced after treatment with individual bacteria and the consortia this was based on the degrading ability of the microorganisms. According to Mongkolthanaruk and Dharmsthiti (2002), similar properties of kitchen waste-water with an average pH of 5.2, the reducing sugar content 719 mg/L, average BOD 3600 mg/L and the average lipid content 21,000 mg/L were determined. It was observed especially with the use of medium 1 in this study that the oil content could not be reduced to values below 0.0031 mol/L (on the 15^{th} day using *Pseudomonas fluorescens* for treatment) from the initial content of 0.0064 mol/L. Kangala and Krystyna (2008) who studied the fate of lipids in activated sludge proved that the total lipid content in the effluent could not be reduced to values beyond 300 mg/L from an initial content of 2000 mg/L. Similarly researchers such as Keenan and Sabelnikov (2000) attested that biodegradation of lipids content in waste-water could not be brought to values lower than 305 mg/L. Similarly, Wakelin and Forster (1997) report that the content of lipids in wastewater could not be reduced to values below 100 mg/L even when acclimatised microbial species (*Acinetobacter* sp.*)* was used for the degradation of lipids. Results also show in this study that after initial decrease in oil weight and even the pH, there was subsequently an increase in the oil content. Researchers like Naidas *et al.* (2005) also observed similar increases in the content of lipids in biological

treatment system. The different degradation efficiencies might be due to different reaction systems of lipase from each culture medium as indicated in the result of the different culture media. Microbial population was observed to generally increase over the period of degradation. This corroborates the findings of Al-Darbi *et al.* (2005) they observed the number of marine oil-degrading bacteria increased with time in their oil-contaminated samples. The increase was obvious in both the seawater- containing nutrients and waste-water samples. Mudge *et al.* (1995) reported an increase in bacteria number due to the presence of oils. They equally observed that after a while, the growth rate decreased, and possibly inferring substrate depletion. The pH values in the culture media were reduced compared to the neutral pH value observed when biodegradation commenced. The reduction in pH could be as a result of fatty acid produced during fermentation (hydrolysis) which could probably bring down the pH of the culture medium. Apichat and Pailin (2011) carried out jatropha oil degradation using minimal salt media supplemented with 1% jatropha oil as sole carbon source. The free fatty acid formed during hydrolytic activity which led to the decrease of pH, and also retarded bacterial growth could be due to the toxicity of the fatty acids on the cells. This study has proven within 25 days lipase produced during fermentation could breakdown olive oil, this finding is similar to that of Kizilaslan (2007) who observed about 77% to 95% removal of vegetable oil during monitoring for 30 days, having a significant removal at 18 days sludge age. This is similar to the result obtained in this study, whereby the best degradation in the two media used was observed on the 20th day except for sample degraded by *Pseudomonas fluorescence* in culture medium 2 over 25 days. Increasing sludge age did not improve the vegetable oil removal at 4% vegetable oil concentration but reduced the removal of the substrate as reported by Kizilaslan (2007). Such decrease was also seen in this study as there was decrease in oleic acid removal. Similarly, Mrinalini and Jayanth (2012) showed degradation of hydrocarbons present in diesel oil through observation of residual oil after 15 days of degradation. This study confirms that within 25 days lipase produced during fermentation could breakdown olive oil, having an initial composition of oleic acid to be 76%. Olive oil is the richest in monoenes fatty acids (Kolayli *et al*., 2011) with a unique double bonds the commonest of which are of the n-9 series, as oleic acid, the most common fatty acid (olive oil has a high content of this acid, about 60-70% (Al-Darbi *et al.,* 2005*).* Oleic acid (C18:1), a mono-unsaturated omega 9 fatty acid has also been found to make up about 55 to 83% olive oil (Oliveoilsource, 1998). Olive oil has been found to contain saturated stearic 16%, oleic acid 76% and mono-unsaturated linoleic 8% (Deferne and Pate, 1996). It is known and proven in this study that Olive oil contains more oleic acid and less linoleic and linolenic acids, meaning, more mono-unsaturated (a single double bond) than poly-unsaturated (more than one double bond) fatty acids. Hence the reason why olive oil is more resistant to oxidation. The greater the number of double bonds in the fatty acid, the more unstable and easily broken down by heat, light, and other factors oil will be (Oliveoilsource, 1998). Therefore if olive oil could undergo biodegradation as shown in this work, other vegetable oil could as well be easily broken down. These could be better explained through an understanding of the effect of oil structure and composition on the degradation processes. The fatty acid molecule is characterised by the length of the carbon chain, the number of double bonds as well as the exact position of the double bonds, this in essence define and determine the biological reactivity of the fatty acid molecule and of the lipid containing the fatty acids (Shanks, 2014). Kizilaslan (2007) in reporting the treatment of vegetable oil-containing waste-water observes the removal of vegetable oil of about 77% to 95% during monitoring for 30 days, the most significant effect being observed at 18 days sludge age. This is similar to the result obtained in this study, whereby the best degradation in the two media used was observed on the 20th day except for sample degraded by

Pseudomonas fluorescence in culture medium 2 over 25 days. Increasing sludge age did not improve the vegetable oil removal at 4% vegetable oil concentration but reduced the removal of the substrate as reported by Kizilaslan (2007). Such decrease was also seen in this study as there was decrease in oleic acid removal. Similarly, Mrinalini and Jayanth (2012) showed degradation of hydrocarbons present in diesel oil through observation of residual oil after 15 days of degradation. The contents of all individual fatty acids in all the media treated with both *Pseudomonas fluorescens* and *Candida parapsilosis* showed both increase and decrease in their percentage compositions through the 25 days of degradation. A pointer to the substrates not being consumed by the microorganisms at the same rate which is in agreement with Kangala and Krystyna (2008), who found different utilisation rates of fatty acids by activated sludge microorganisms. Fatty acids contents (Palmitoleic, Stearic, Linoleic, Linolenic, Linoleic, Arachidic, Behenic and Lignoceric) has been seen to increase in waste-water effluents after 24 hr of a biological treatment process using activated sludge (Dignac *et al.*, 2000). Other researchers such as Pereira *et al.* (2002) observed the presence of palmitic acid in waste-water after a biological treatment process. Even though it was absent at the beginning of the process, they inferred that it is possibly a by-product of biodegradation of oleic acid initially added to the waste-water. Several reports have it that fatty acids accumulate in biological wastewater treatment systems (Beccari *et al.*, 1998; Salminen *et al*, 2000; Lalman and Bagley., 2001). It is worth noting that biodegradation and biosynthesis of fatty acids occur inside microbial cells, hence the increases in fatty acids contents showed that they were obviously released into the waste-water as by-products of the microorganisms (Kunau *et al.*, 1995). However, decreases in the fatty acids contents showed they were subsequently consumed by the organisms as substrates.

CONCLUSION AND APPLICATION OF RESULTS

Microbial species of *Pseudomonas fluorescens* and *Candida parapsilosis,* among other microbiota, were found present in an oil mill-polluted site. This implies that with time, given favourable conditions, these microorganisms could naturally aid the degradation process in vegetable oil polluted soil. It was established these microorganisms could metabolise complex triglyceride present in oil into fatty acids and glycerol which could be further mineralised into simple inorganic chemical compounds that might not be toxic to the environment. Effective biodedradation could be achieved within 5 to 25 days under laboratory conditions as seen in this study. Therefore in protecting our environment the role of microorganisms cannot be overemphasized. The lipolytic activity of diverse microorganisms could be explored in order to degrade oil spills in the areas of need. Hence, these microorganisms (*Pseudomonas fluorescens* and *Candida parapsilosis*) have great potentials as candidates for the treatment of vegetable oil mill effluents and soils, particularly in areas of need. Lipases of *Pseudomonas fluorescens* and *Candida parapsilosis* with improved properties by protein engineering is highly recommended to further enhance usefulness of these enzymes.

REFERENCES

AAA. 2013. *Construction and development*. About Vegetable Oil spills – MPCD-BIOSINFO. Retrieved from http://www.mpcd.info/index.php?option=com-content & view= article &id=421&Itemids=156 (Jan, 2013).

Abrashev, I., Rachev R., Donev T. Petrova, M. 2002. A biodegradation activity of microbial associations. *Journal of Cultural Collection.* 3 (1): 43-47.

Al-Darbi, M.M., Saeed N.O, Islam, M.R. and Lee, K. 2005 Biodegradation of Natural Oils in Seawater. *Energy Sources* 27: 19-34.

Aluyor, E.O., Obahiagbon, K.O. and Ori-jesu, M. 2009. Biodegradation of vegetable oils: *A Review Academic Journals. Scientific Research and Essay*. 4(6): 543-548.

Apichat, B. and Pailin, B. 2011. Isolation and characterization of Jatropha oil-degradation by *Enterococcus faecalis* and *Burikholderia cenocepacia* W-1 under anaerobic condition. *African Journal of Biotechnology*. 10(63*)*:13841-13851.

Beccari, M., Majone, M. and Torrisi, L. 1998. Two-reactor system with partial phase separation for anaerobic treatment of olive oil mill effluents. *Water Science Technology*. 38: 53-60.

Broekhuizen P, Theodori D, Le Blanch K, Ullmer S 2003. Lubrication in Inland and Coastal Water Activities. A.A. Balkema Publishers, Tokyo.

Deferne, J.D. and Pate, D.W. 1996. "Hemp seed oil: A source oil, a source of valuable essential fatty acid". *Journal of the international Hemp Association.* 3(1): 1-7.

Dignac, M.F., Ginestet, P., Rybacki, D., Bruchet, A., Urbrain, V. and Scribe, P. 2000. Fate of waste-water organic pollution during activated sludge treatment: Nature of residual organic matter. *Water Resources.* 34: 4185-4194.

Gogoi, B.K., Dutta, N.N., Goswani, P. and Krishna, M. T.R. 2003. A case study of bioremediation of petroleum-hydrocarbon contaminated soil at crude oil spill site. *Advances in Environmental Research.* 7: 767-782.

GRT Maldives. 2012. *Products and Services*. Retrieved from http://www.grtmaldives.com/products_services.php (March, 2012)

ITOPF. 2013. *About vegetable oil spills*. Retrieved from http://www.itopf.com/marine-spills/aboutveg/#Top (Jan, 2013)

Kaakinen, J., Vahaoja, P., Kuokkanen, T. and Roppola, K. 2007. Studies on the effects of certain soil properties on the Biodegradation of oils determined by the Monometric Respirometric Method. *Journal of Automated Methods and Management in Chemistry.*10:34601

Kangala, B. and Krystyna M. 2008. Characterization of the fate of lipids in activated sludge. *Journal of Environmental Sciences. 20:* 536-542.

Keenan, D. and Sabelnikov, A. 2000. Biological augmentation eliminates grease and oil in bakery wastewater. *Water Environmental Resources.* 72: 141-146.

Khalida, K., Naeem, M., Arshed, M.J. and Asi, F.M. 2006. Extraction and characterization of oil degrading bacteria. *Journal of Applied Science.* 6(10): 2302-2306.

Kizilaslan, O. 2007. Investigation of vegetable oil containing waste water treatment by biological methods. *Submitted to the Graduate School of Natural and Applied Sciences.* pg. 88-92.

Kolayli, S., Tarhan, O., Kara, M., Alyaziciogu, R. and Kucuk, M. 2011. An investigation of frequently consumed edible oils in turkey in terms of omega fatty acids. *Chemistry of Natural Compounds.* 47(3): 347-351.

Kunau, W.H., Dommes, V. and Schulz, H. 1995. β-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: A century of continued progress: *Prog. Lipid Resources.* 34: 267-342.

Lalman, J.A. and Bagley, D.M. 2001. Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. *Water Resources.* 35: 2975-2983.

Mansee, A.H., Montasser, M.R. and Shanab, A.S.A. 2004. Decontamination of pollutants in Aquatic system: I: Biodegradation Efficiency of Isolated Bacteria Strains from certain contaminated Areas. *Pak, Journal of Biological Science.* 7(7): 1202-1207.

Mongkolthanaruk, W. and Dharmsthiti, S. 2002. Biodegradation of lipid rich wastewater by a mixed bacterial consortium. *International Biodeterioration and Biodegradation.* 50: 101-105.

Mrinalini, K. and Jayanth, A. 2012. Biodegradation of Diesel oil using yeast *Rhodosporidium toruloides. Research Journal of Environmental Toxicology*. 5: 367-377.

Mudge, S.M., Good Child, I.D. and Wheeler, M. 1995. Vegetable oil spills on oil marshes. *Chemistry and Ecology.* 10: 127-135.

Naidas, H., Capela, I. and Duarte, A. 2005. Treatment of diary waste water in UASB reactors inoculated with flocculent biomass. *Water SA* 31: 603-607.

Oforma, C.C., Udourioh, G.A and Ojinnaka C.M. 2019. Characterization of Essential Oils and Fatty Acids Composition of stored Ginger (*Zingiber officinale* Roscoe). *Journal of Applied Environmental Management*. 23(12): 2231-2238.

Oliveoilsource. 1998. *Chemical characteristics*. Retrieved from http:// www.oliveoilsource.com./page/chemical-charcteristrics). (Feb, 2014).

Official Methods of Analysis (1995). Method 996.06, Fat (Total, Saturated and Monounsaturated) in foods, AOAC INTERNATIONAL, Gaithersburg, MD.

Pereira, M.A., Pires, O.C., Mota, M. and Alves, M.M. 2002. Anaerobic degradation of oleic acid by suspended and granular sludge: Identification of palmitic acid as a key intermediate. *Water Science Technology*. 45: 139-144.

Prasad, M.P. and Manjunath, K. 2011. Comparative study on biodegradation of lipid-rich wastewater-using lipase producing bacterial species. *Indian of Journal of Biotechnology.* 10: 121-124.

Saifudin, N. and Chua, K.H. 2006. Biodegradation of lipid rich waste water by combination of microwave irradiation and lipase immobilized on Chitosan. *Biotechnology*. 5(3): 315-323.

Salminen, E., Rintala, J., Lokshina, L.Y. and Vavilin, V.A. 2000. Anaerobic batch degradation of solid poultry slaughter waste. *Water Science Technology.* 41: 33-41.

Shanks, P. 2014. Questions and Answers about 'Identify the biochemical properties of food'. Retrieved from http://ntisthis.com/answers.php? Code=FDFFST5005A&S=FDF10&Query=identify (Jan, 2014).

U.S EPA. 2013. *The Behaviour and effects of oil spills in aquatic environments*. Retrieved from http://www.epa.gov/oem/docs/oil/edu/oilspill-book/chap1.pdf (Feb, 2013)

Wakelin, N.G. and Forster, C.F. 1997. An investigation into microbial removal of fats, oils and greases. *Bio-resources Technology.* 59: 37-43.

Conflict of Interest:

On behalf of all authors, the corresponding author states that there is no conflict of interest.

APPENDIX I

Fatty acids

FGN1 Caprylic Acid Methyl Ester (C8:0)
FGN2 Capric Acid Methyl Ester (C10:0)
FGN3 Lauric Acid Methyl Ester (C12:0)
FOO1 Myristic Acid Methyl Ester (C14:0)
FOO2 Palmitic Acid Methyl Ester (C16:0)

FOO3 Palmitoleic Acid Methyl Ester (C16:1)
FCS1 Margaric Acid Methyl Ester (C17:0)
FCS2 Stearic Acid Methyl Ester (C18:0)
FCS3 Oleic Acid Methyl Ester (C18:1)
FCS4 Linoleic Acid Methyl Ester (C18:2)
FSF1 Linolenic Acid Methyl Ester (C18:3)
FSF2 Arachidic Acid Methyl Ester (C20:0)
FSF3 Arachidonic Acid Methyl Ester (C20:4)
FPO1 Behenic Acid Methyl Ester (C22:0)
FPO2 Erucic Acid Methyl Ester (C22:1)
Lignoceric Acid Methyl Ester (C24:0)

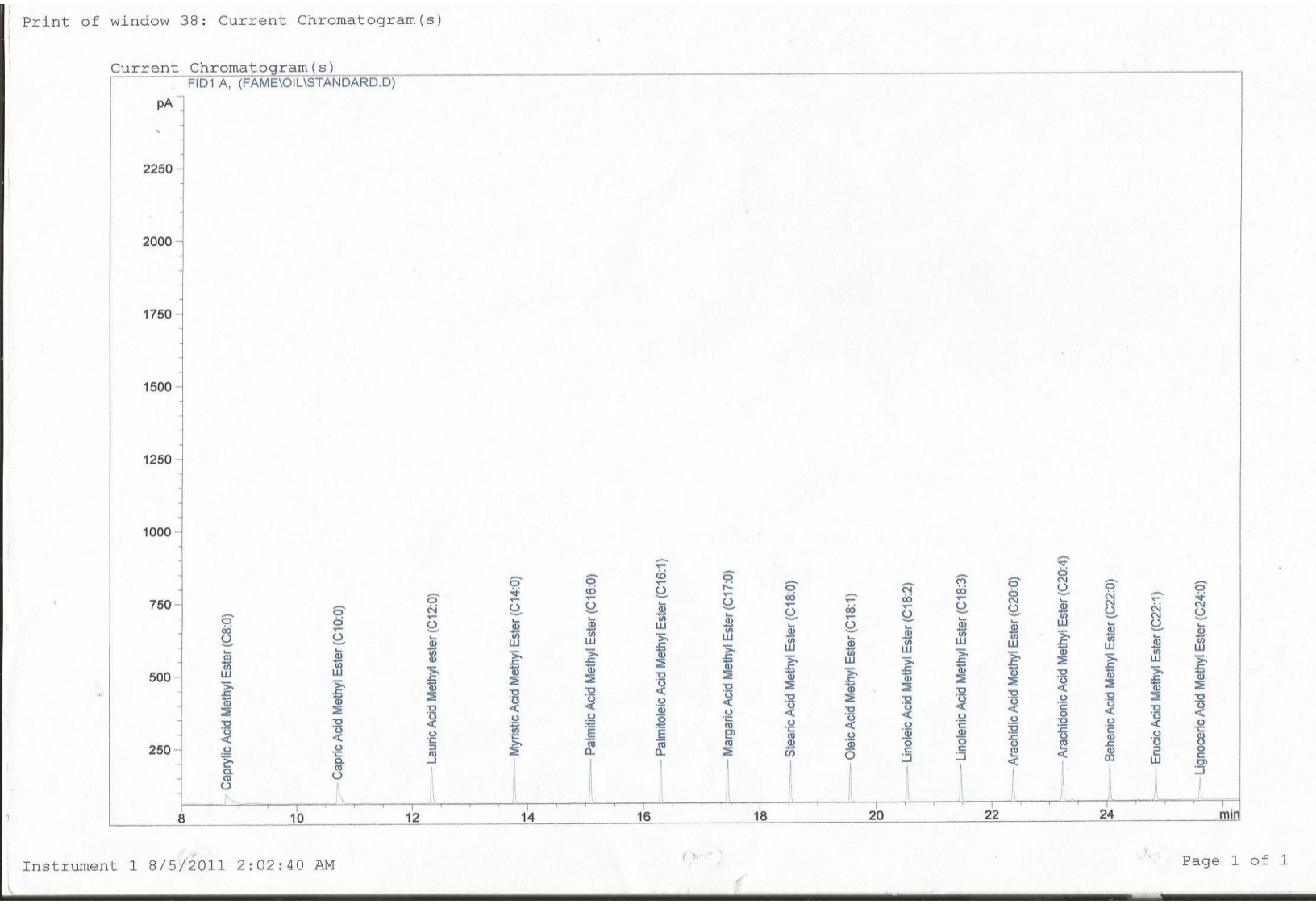

Fig.1: Chromatogram showing the standard (control) fatty acid profiles

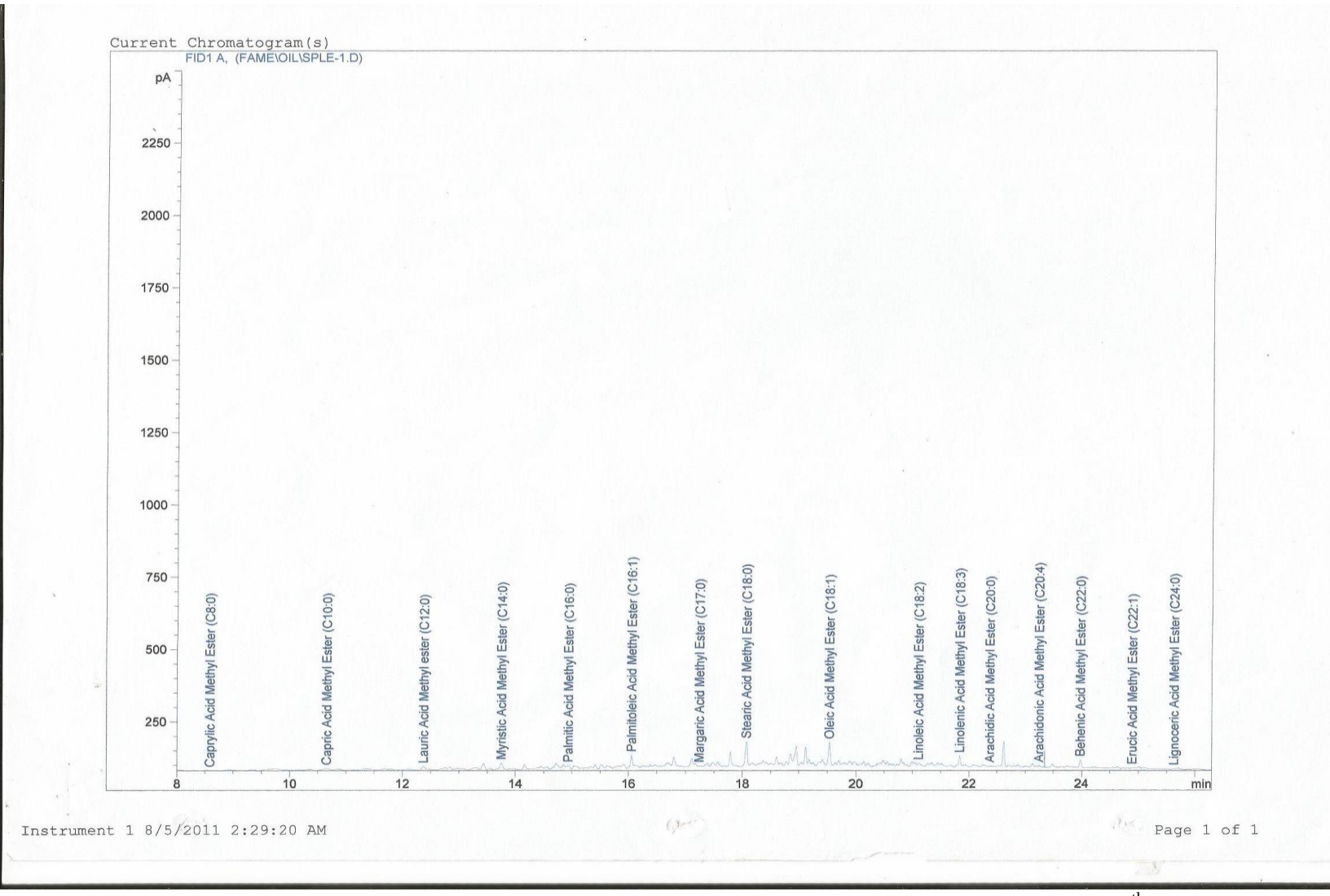

Fig.2: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 1 on the 5th day of degradation.

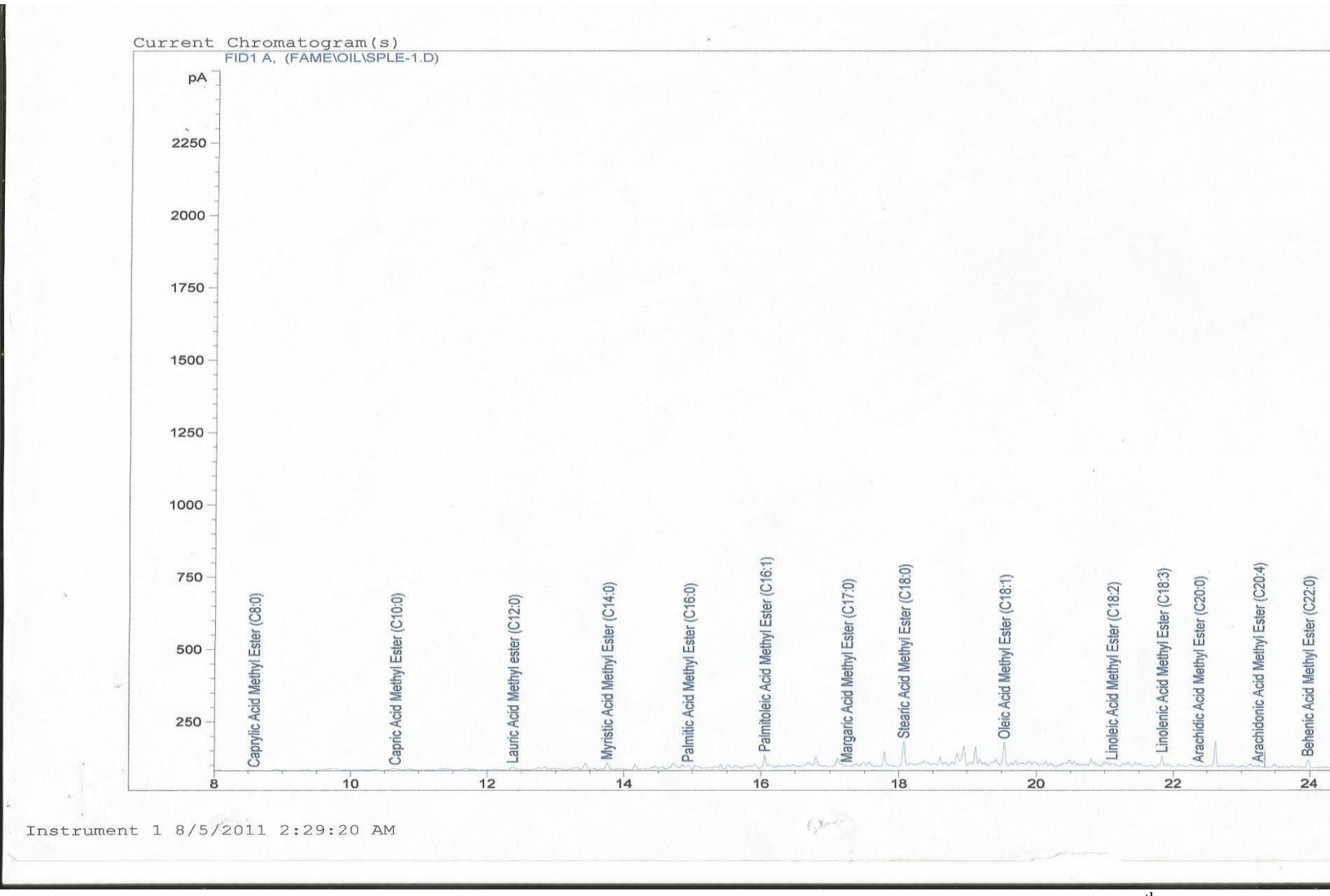

Fig.3: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 1 on the 10th day of degradation.

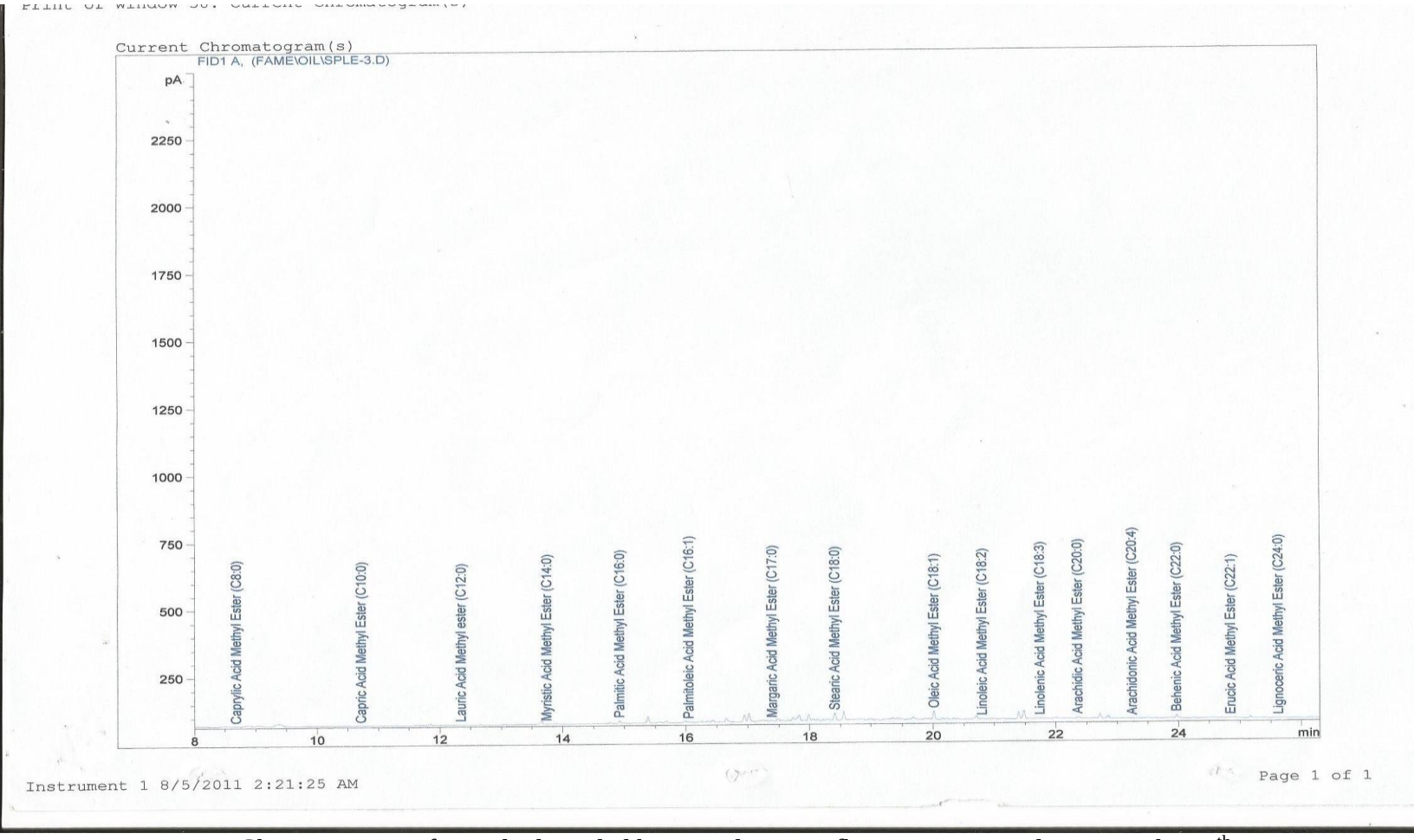

Fig.4: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 1 on the 15th day of degradation.

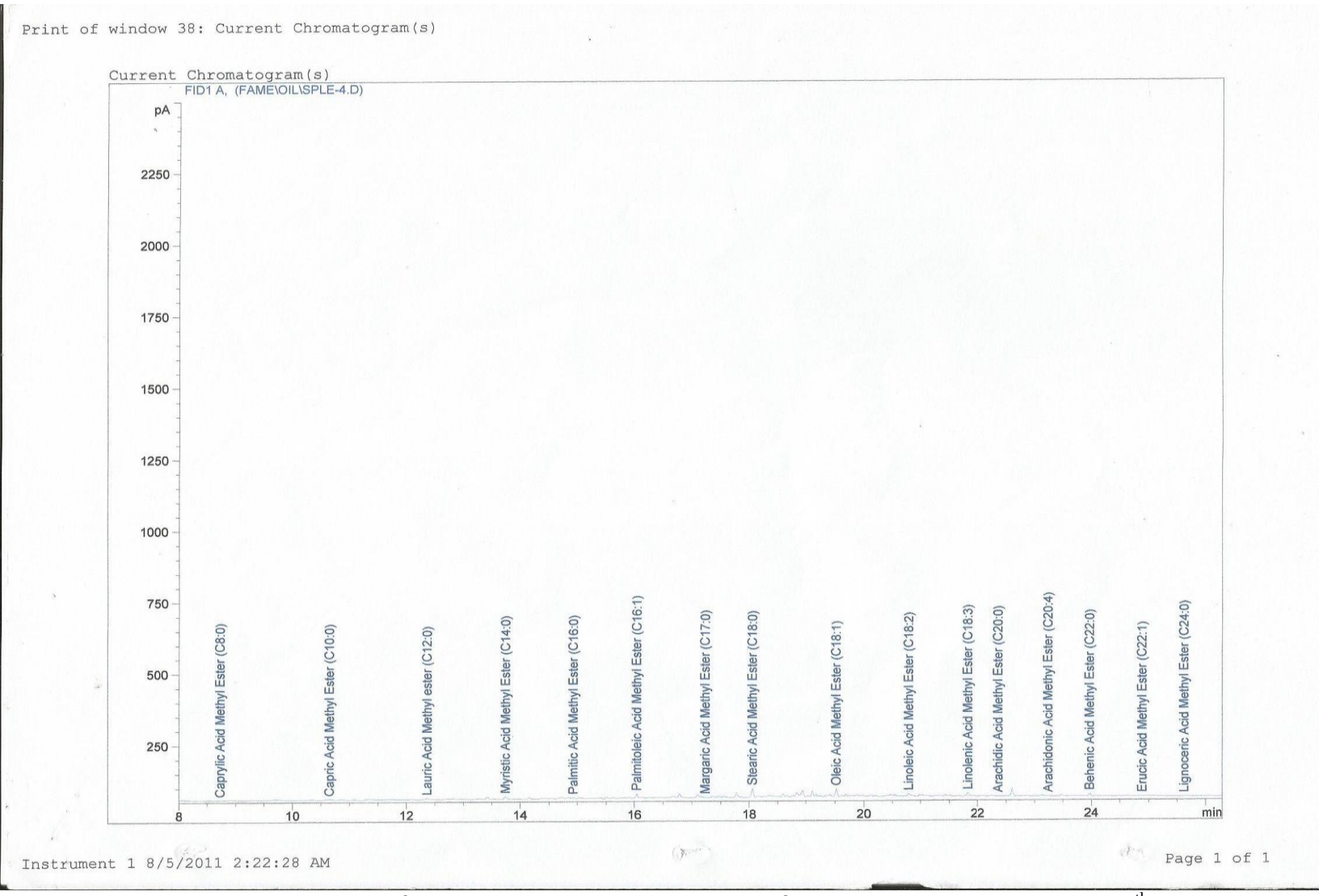

Fig.5: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 1 on the 20th day of degradation.

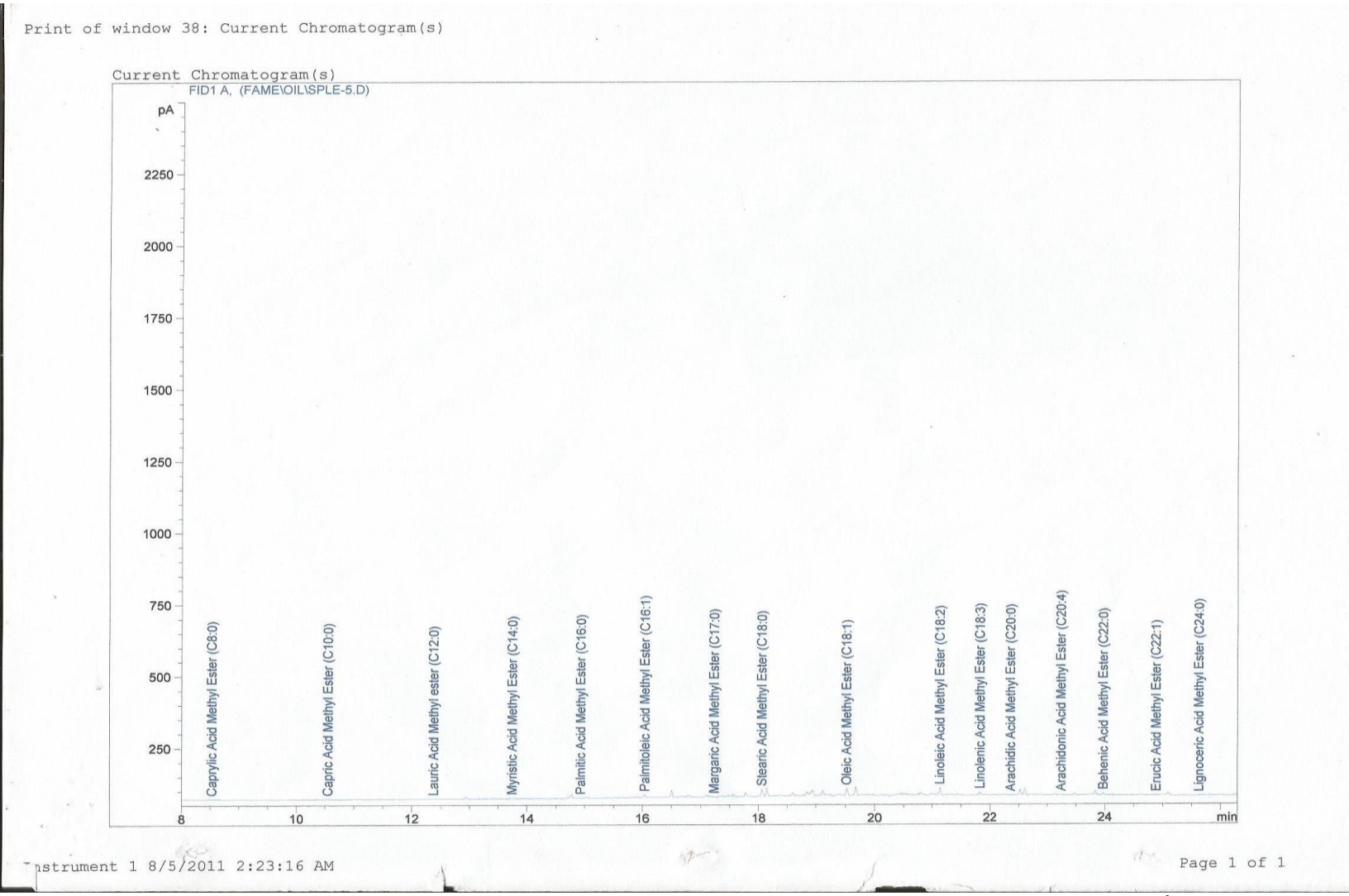

Fig.6: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 1 on the 25th day of degradation.

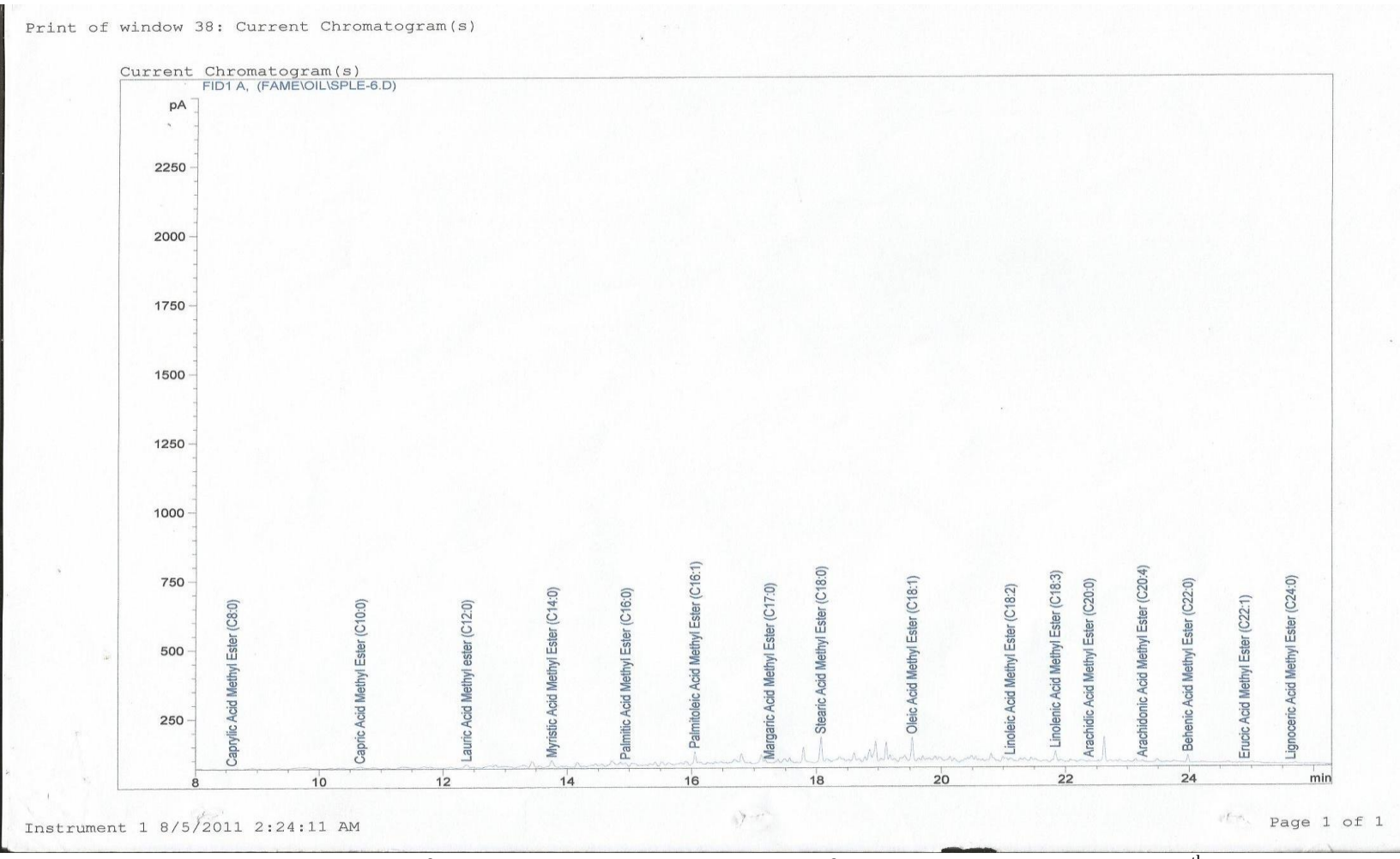

Fig.7: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 2 on the 5th day of degradation.

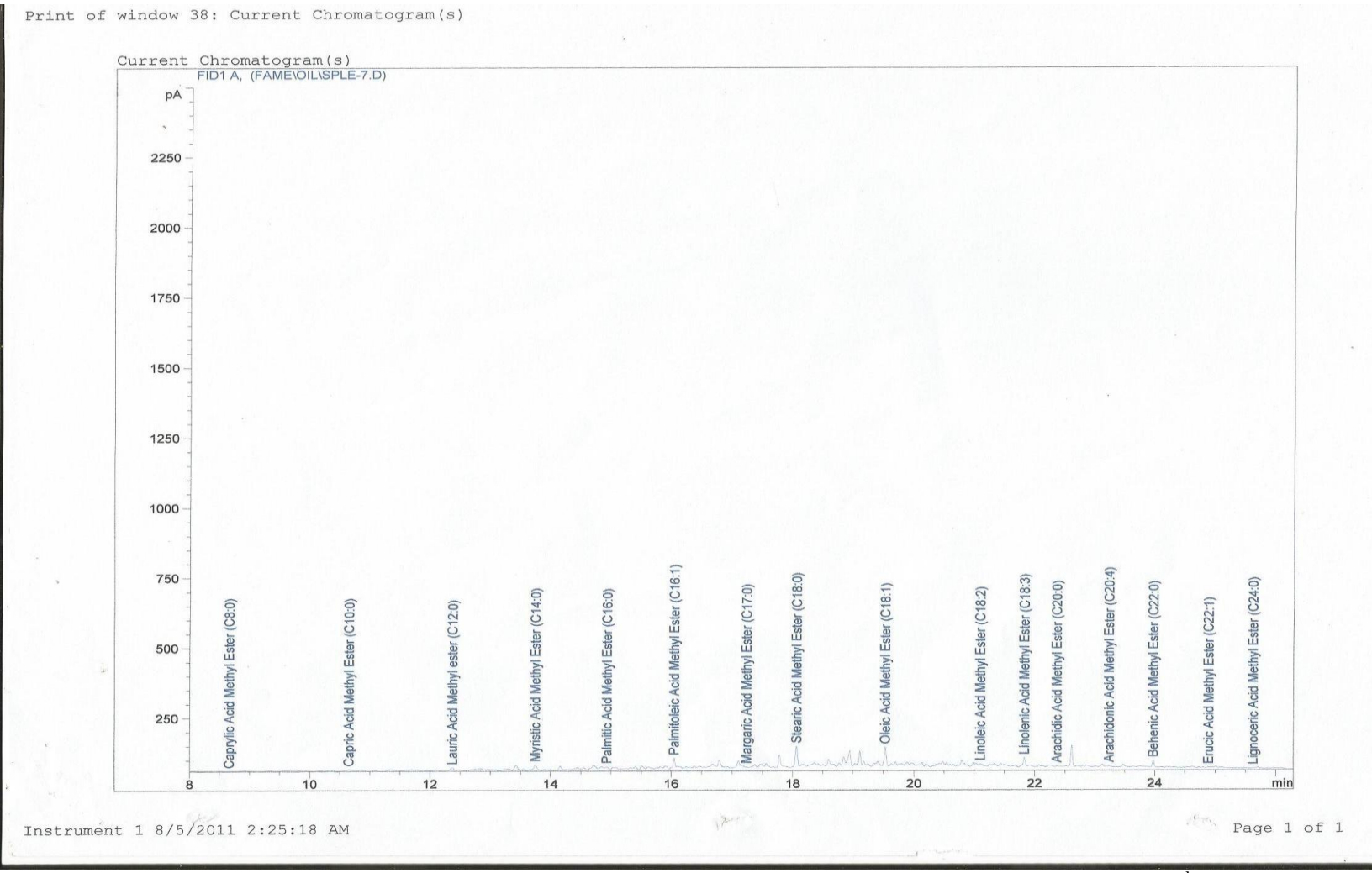

Fig.8: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 2 on the 10th day of degradation.

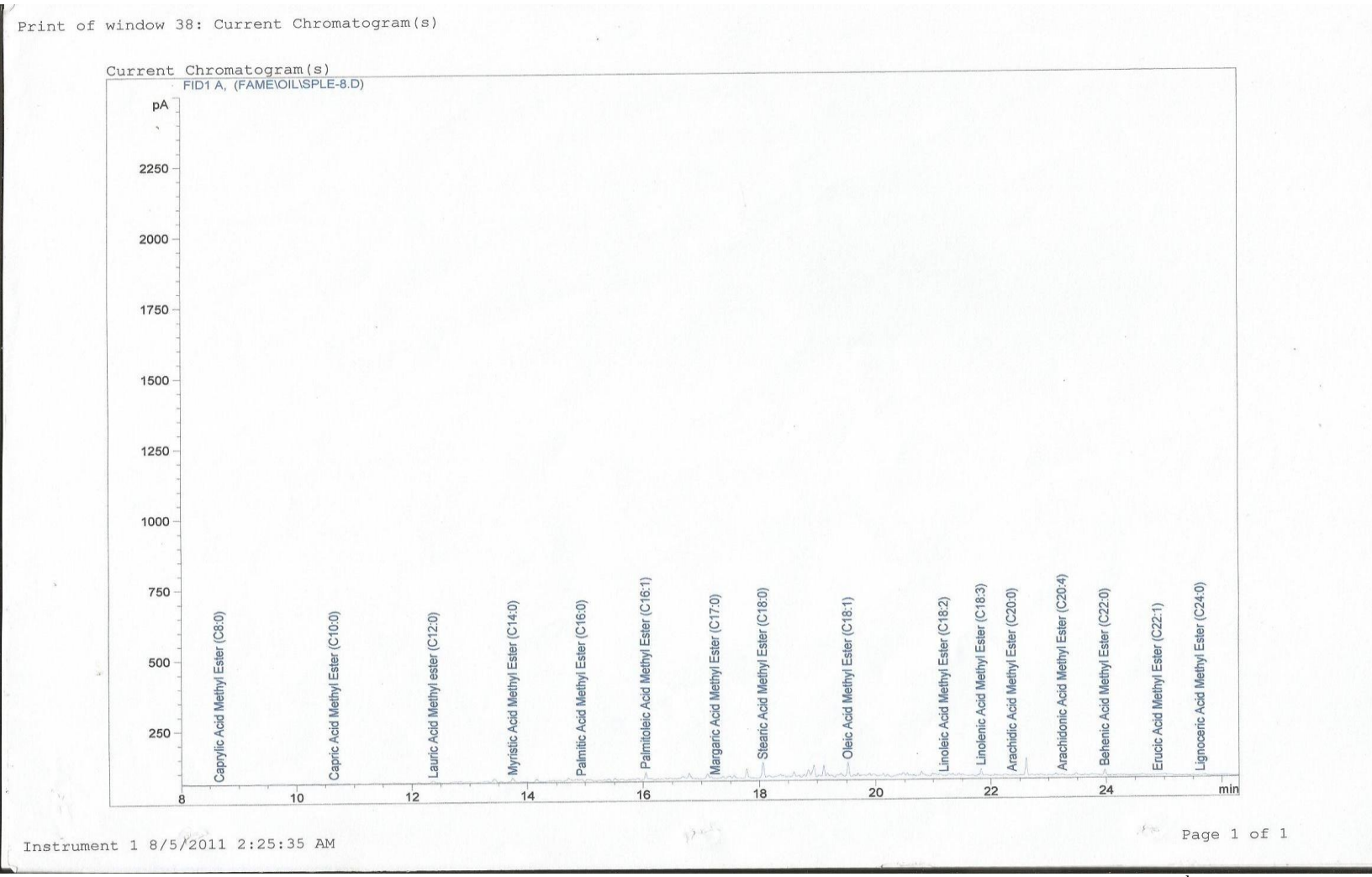

Fig.9: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 2 on the 15th day of degradation.

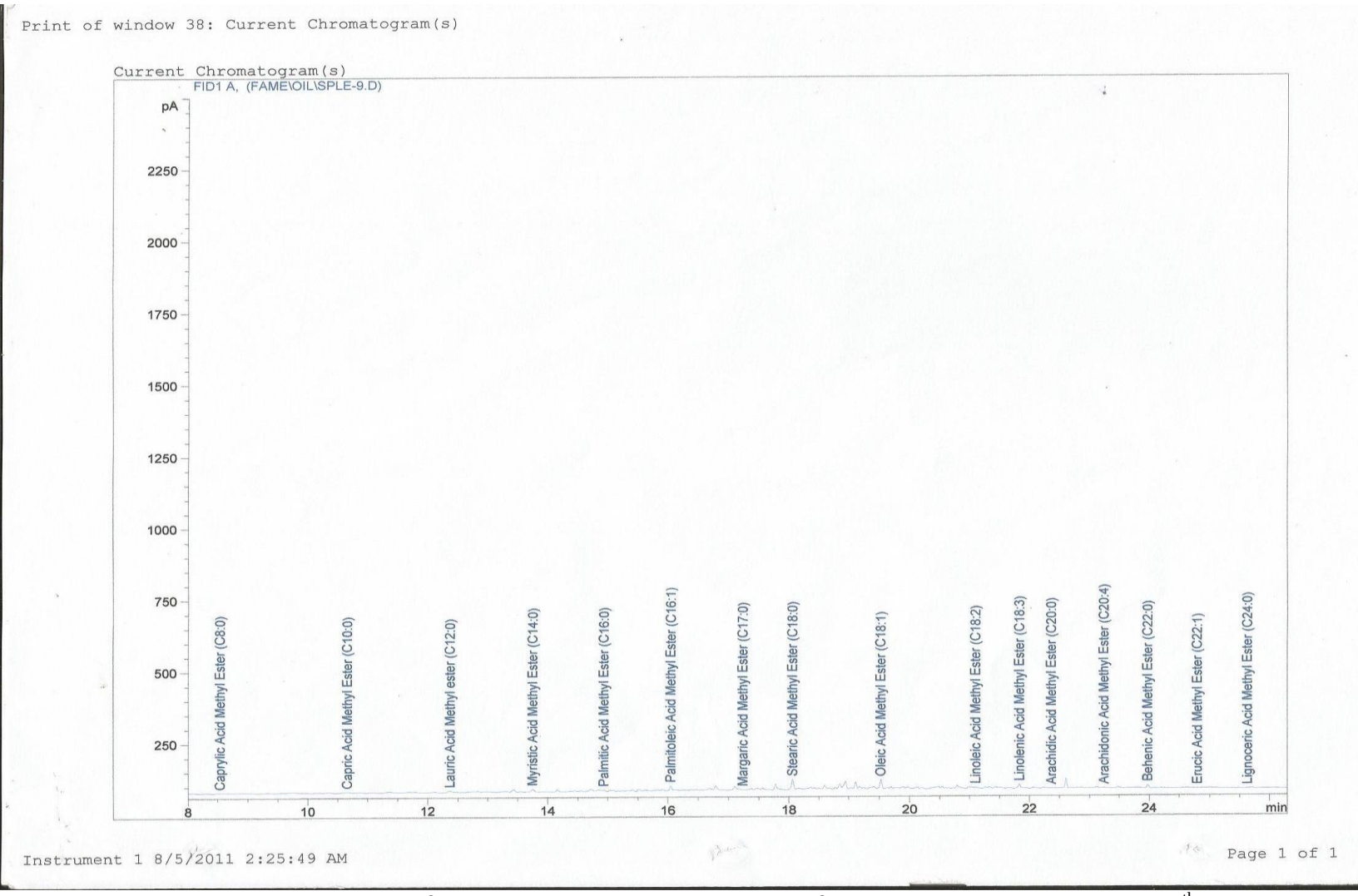

Fig.10: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 2 on the 20th day of degradation.

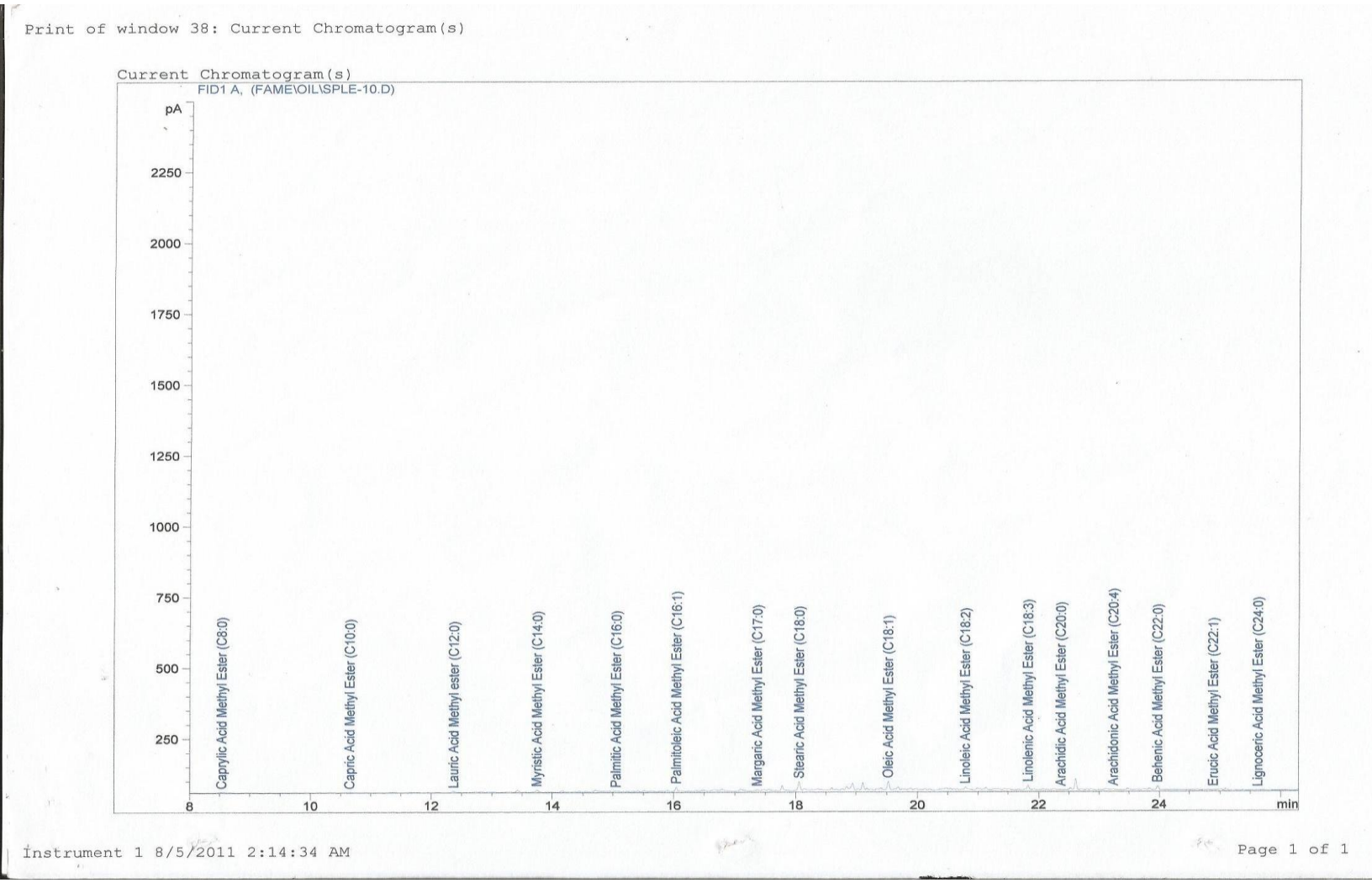

Fig.11: Chromatogram of sample degraded by *Pseudomonas fluorescens* in medium 2 on the 25th day of degradation.

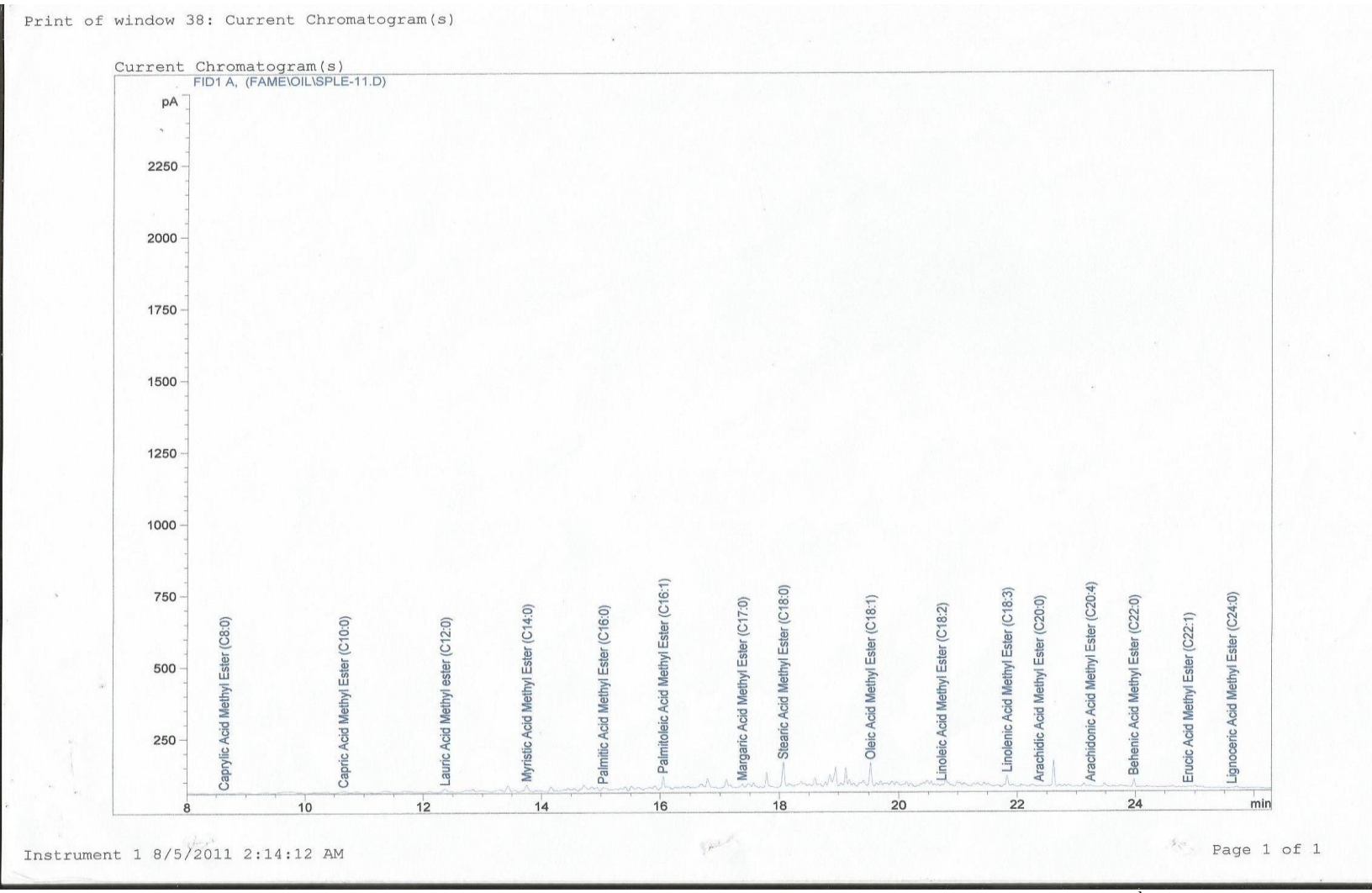

Fig.12: Chromatogram of sample degraded by *Candida parapsilosis* in medium 1 on the 5th day of degradation.

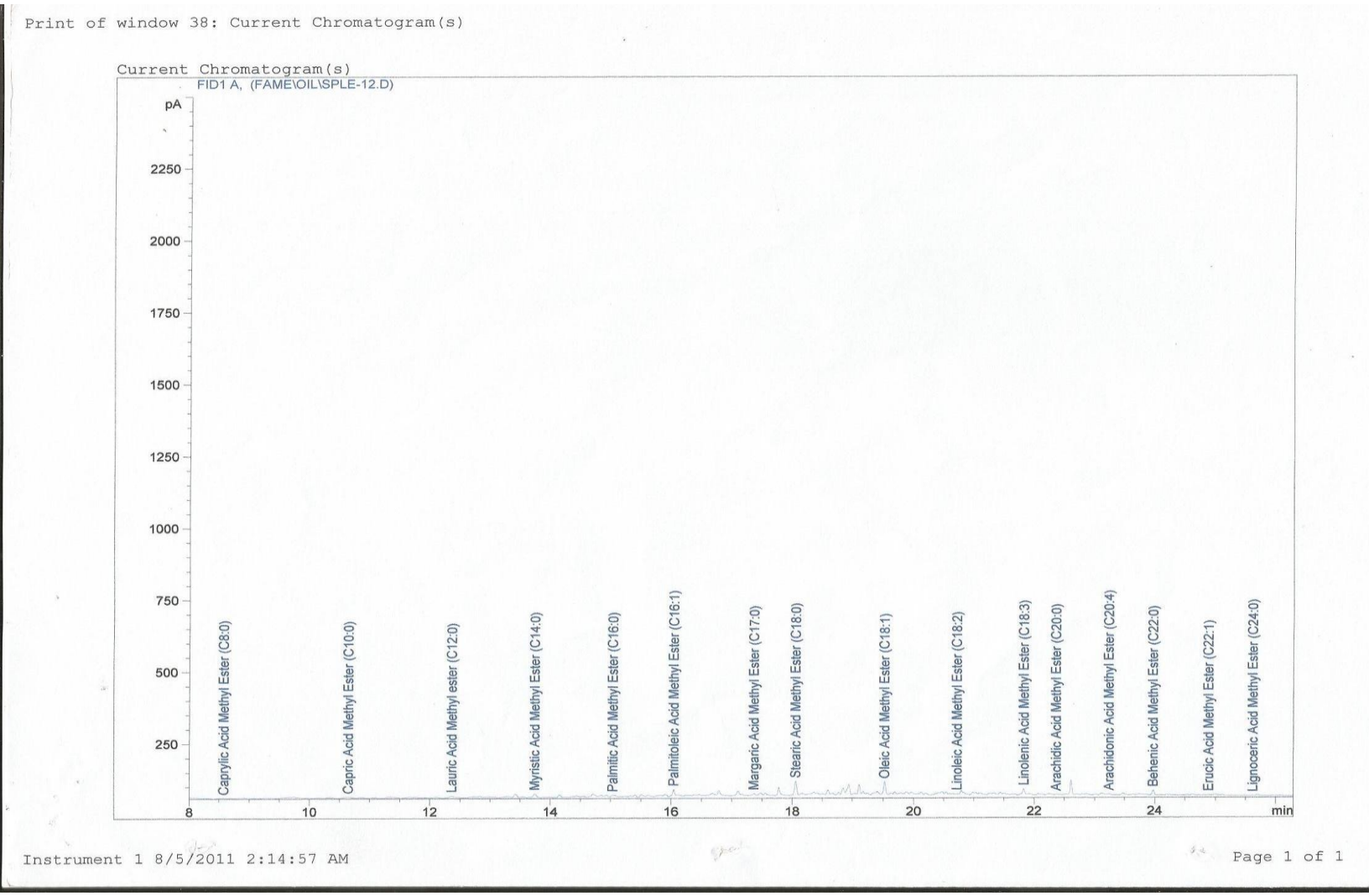

Fig.13: Chromatogram of sample degraded by *Candida parapsilosis* in medium 1 on the 10th day of degradation.

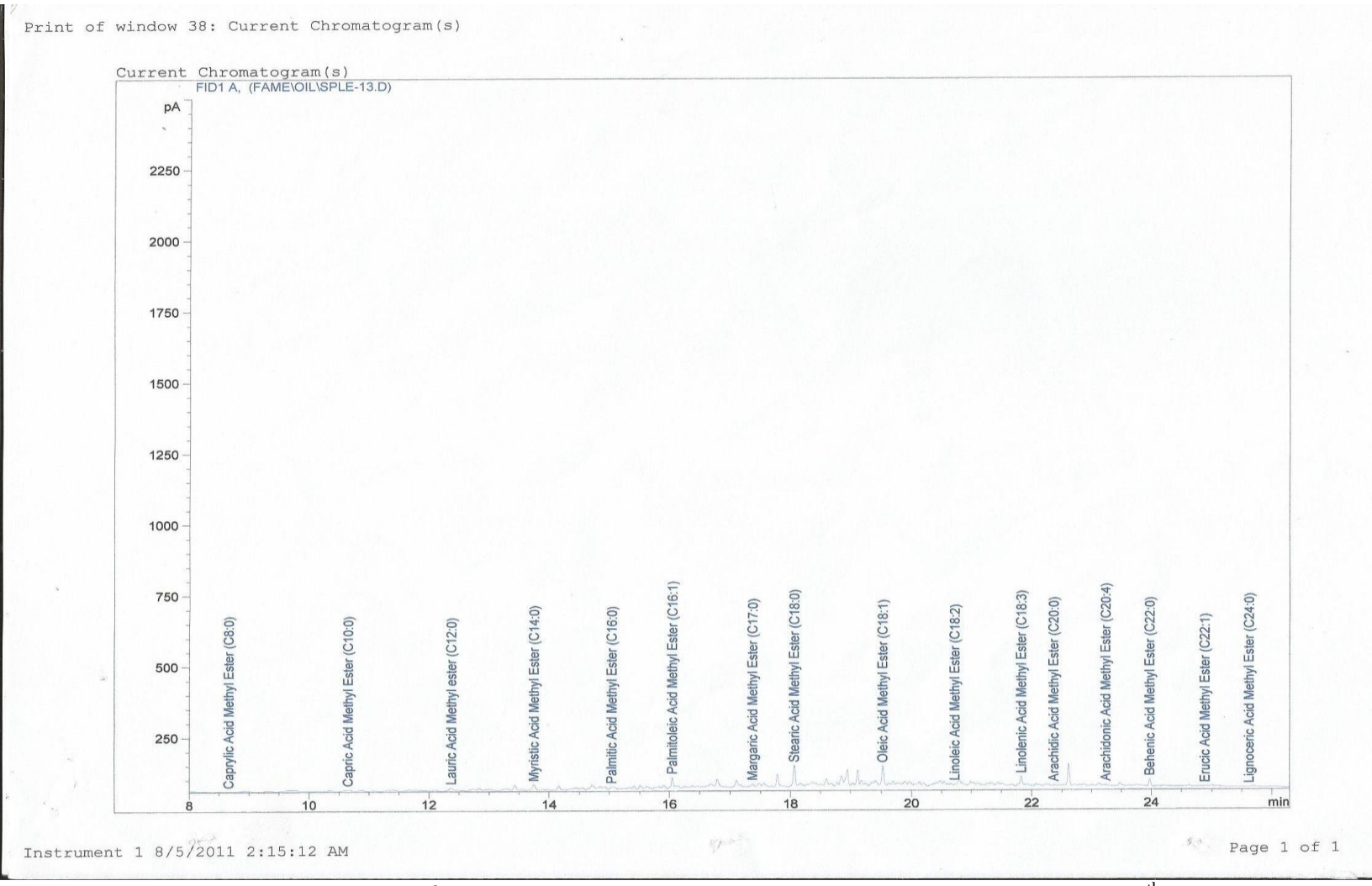

Fig.14: Chromatogram of sample degraded by *Candida parapsilosis* in medium 1 on the 15th day of degradation.

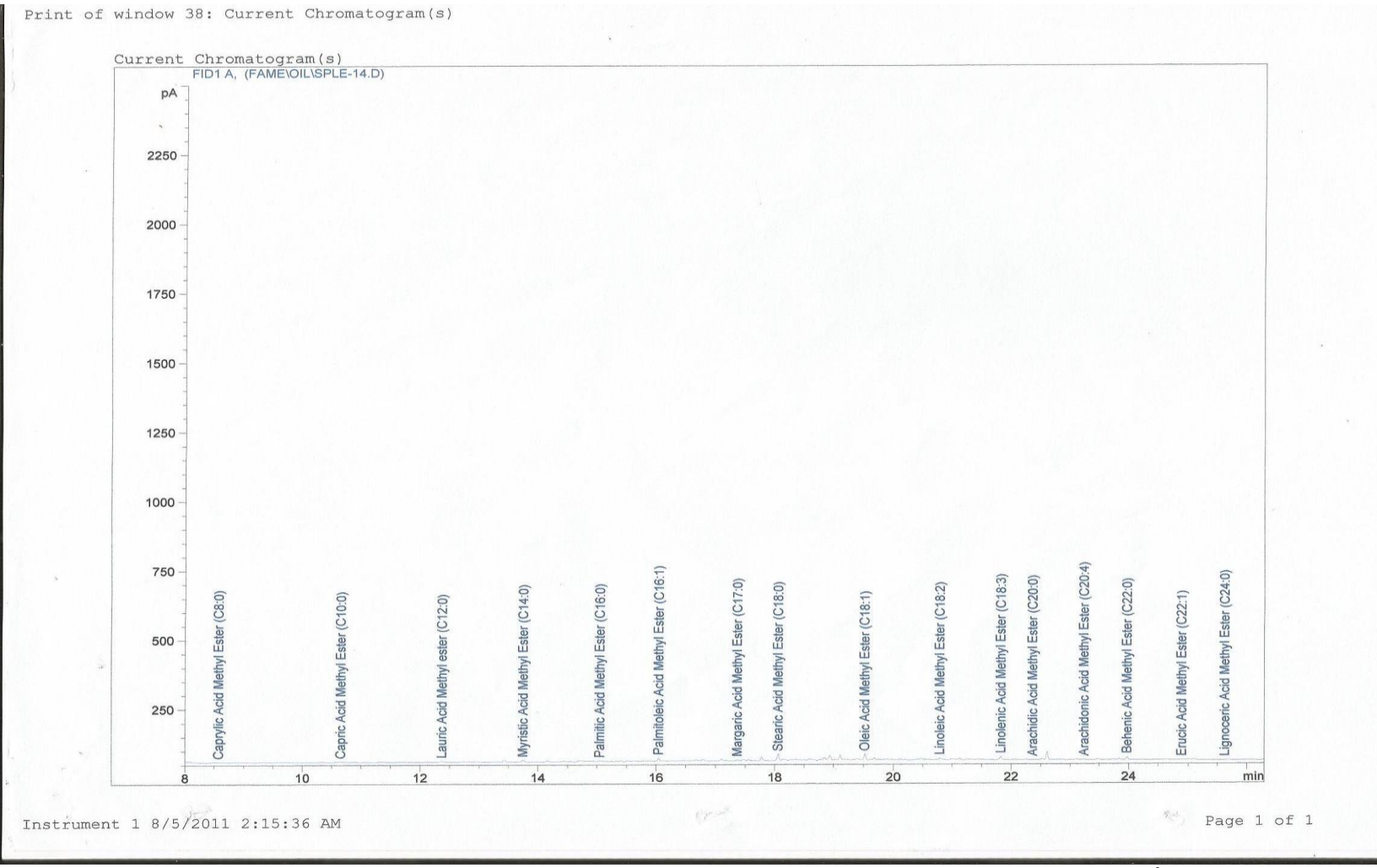

Fig.15: Chromatogram of sample degraded by *Candida parapsilosis* in medium 1 on the 20th day of degradation.

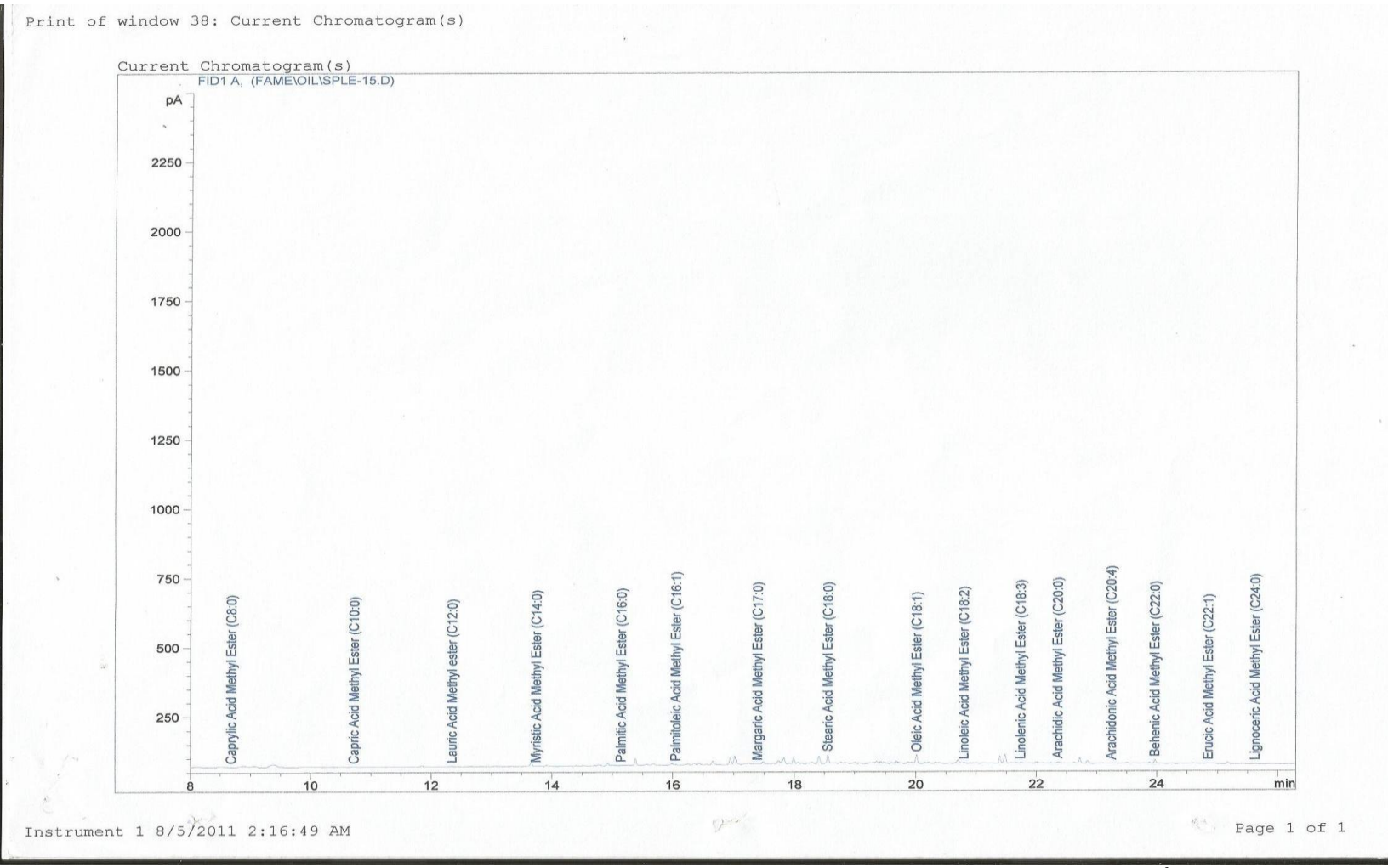

Fig.16: Chromatogram of sample degraded by *Candida parapsilosis* in medium 1 on the 25th day of degradation.

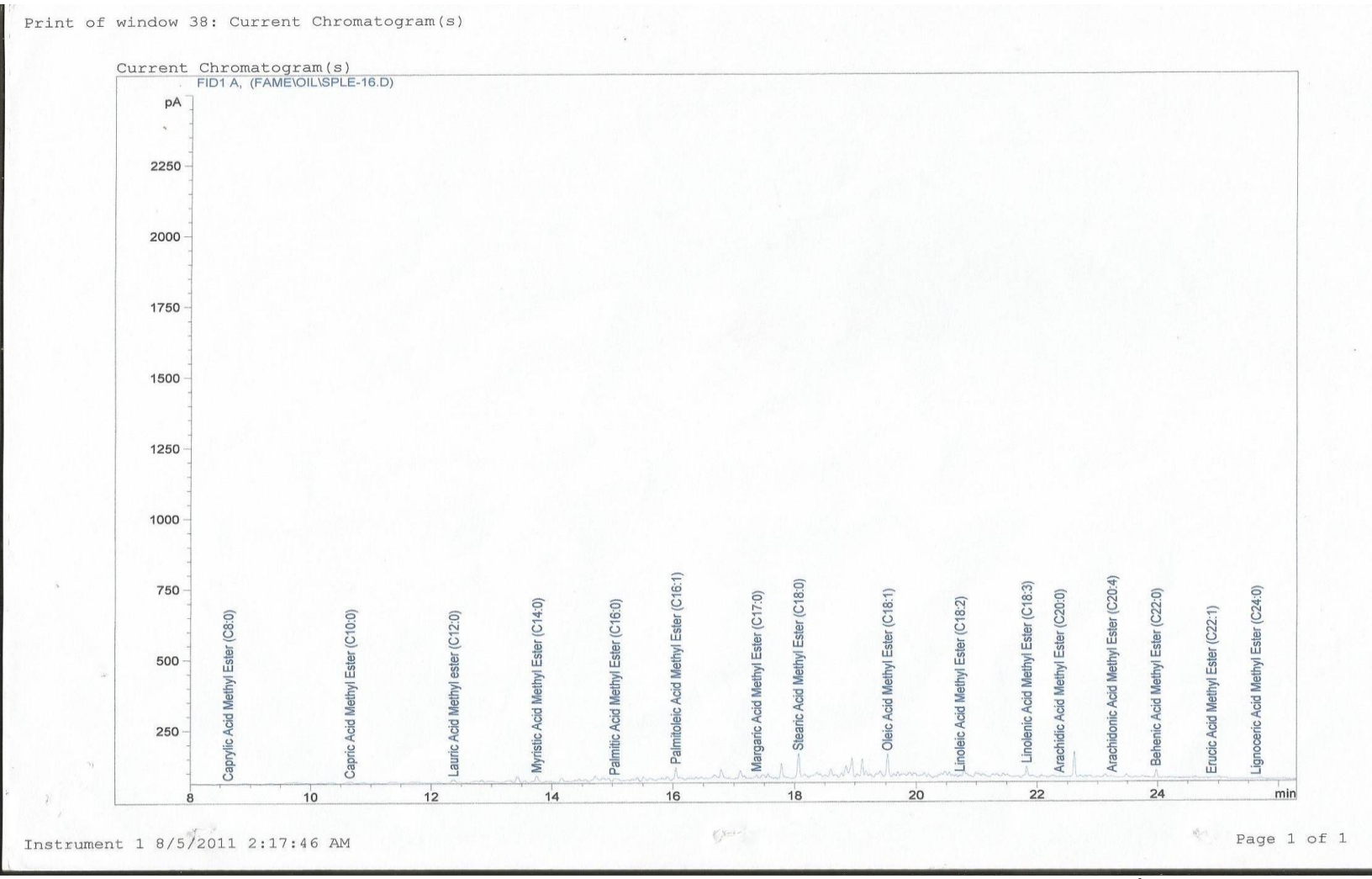

Fig.17: Chromatogram of sample degraded by *Candida parapsilosis* in medium 2 on the 5th day of degradation.

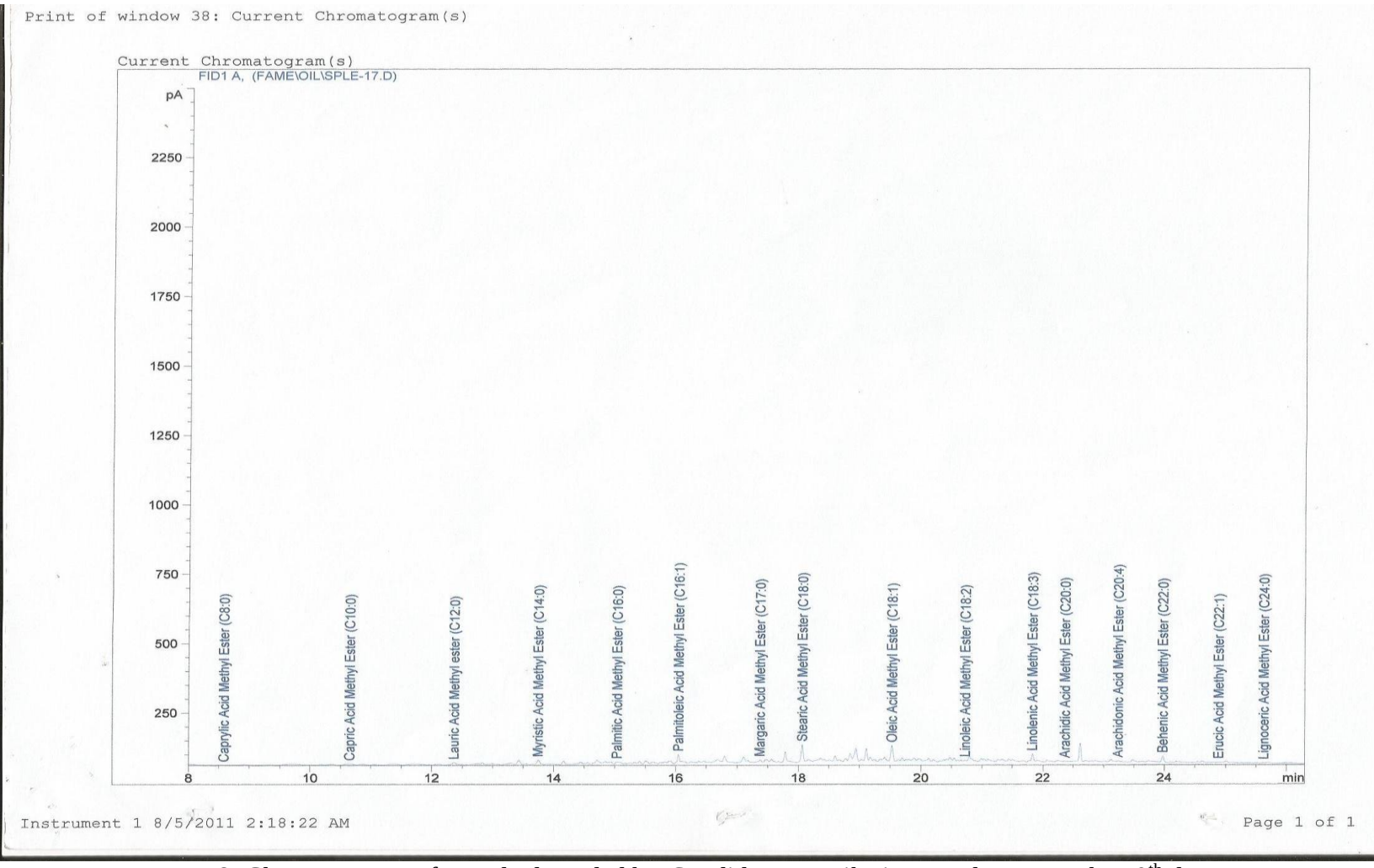

Fig.18: Chromatogram of sample degraded by *Candida parapsilosis* in medium 2 on the 10th day of degradation.

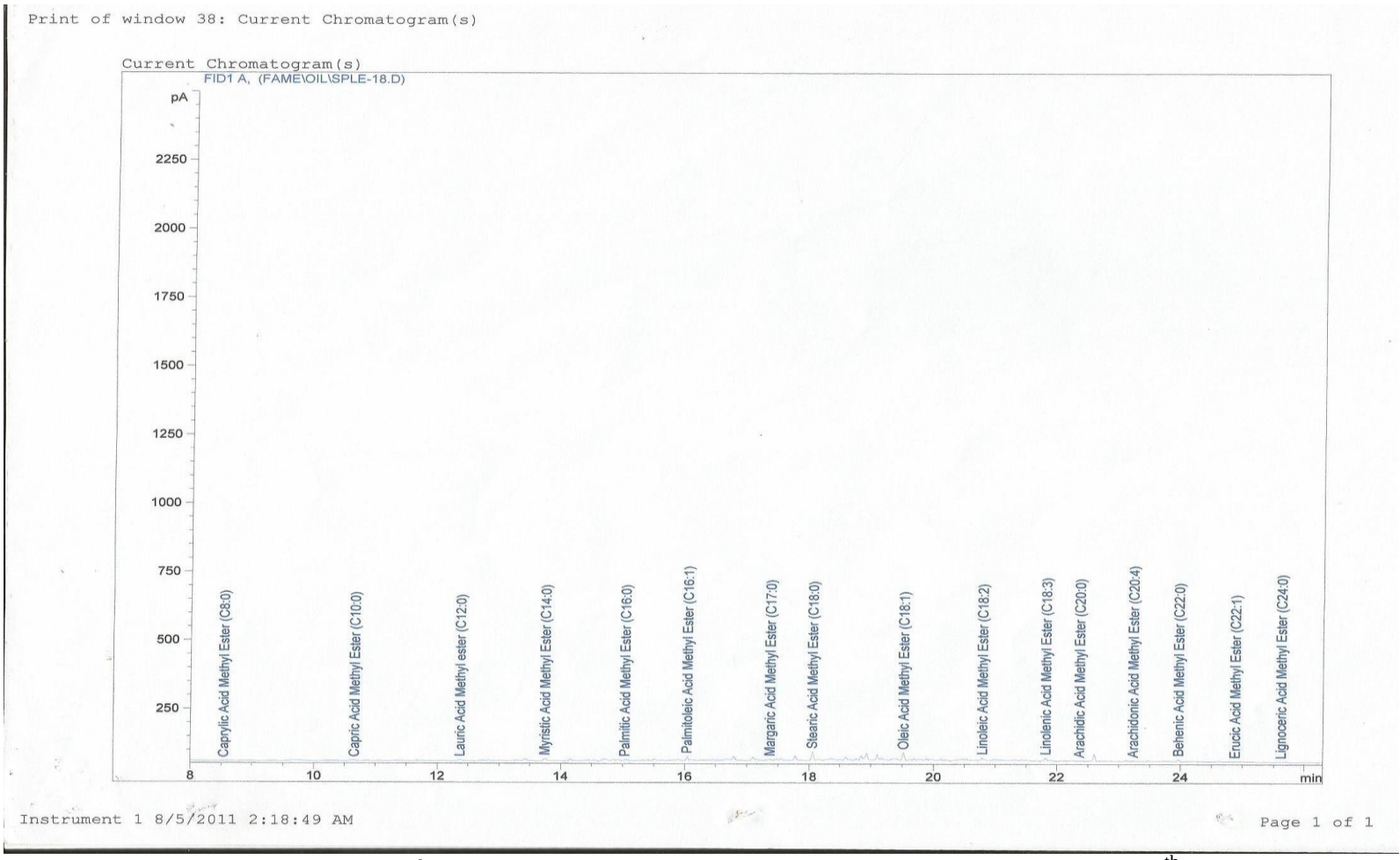

Fig.19: Chromatogram of sample degraded by *Candida parapsilosis* in medium 2 on the 15th day of degradation.

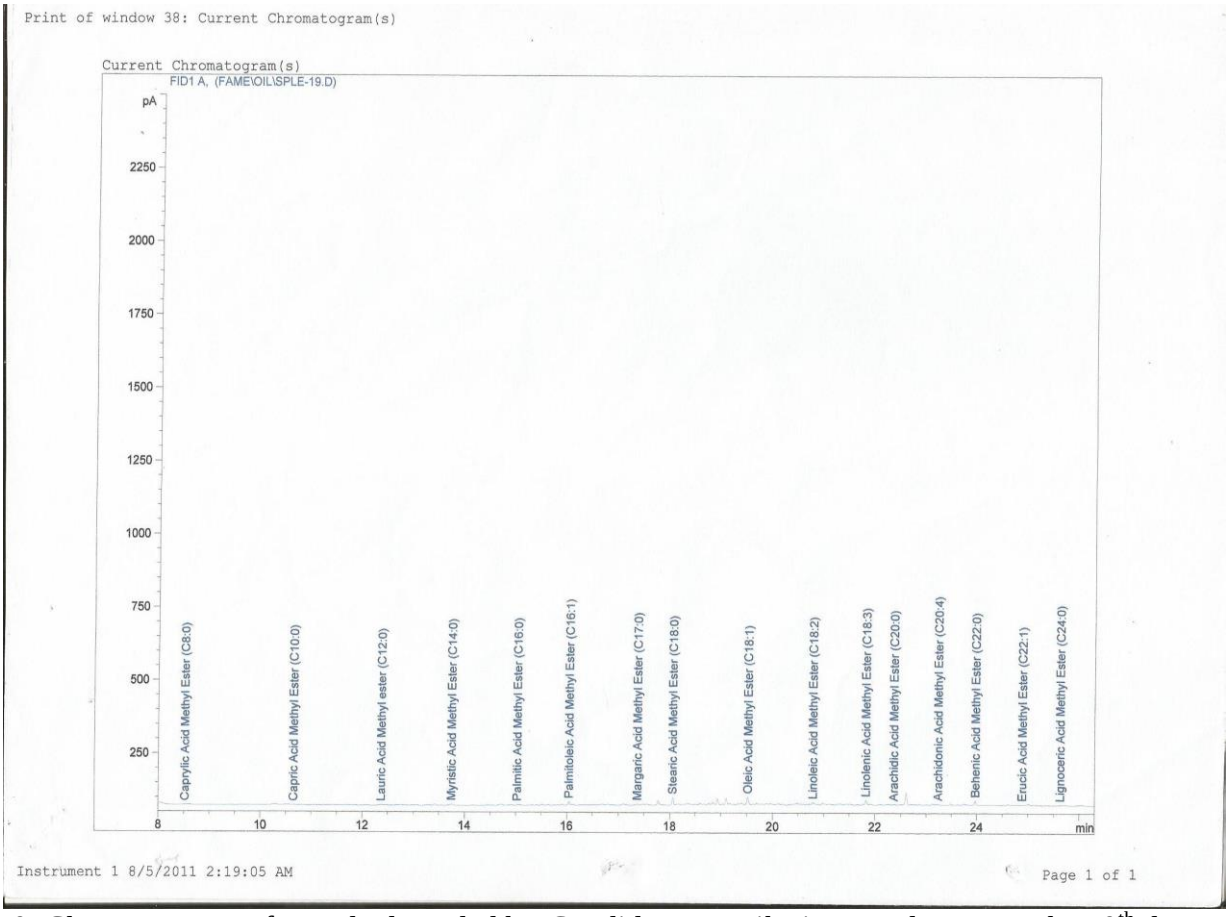

Fig.20: Chromatogram of sample degraded by *Candida parapsilosis* in medium 2 on the 20th day of degradation.

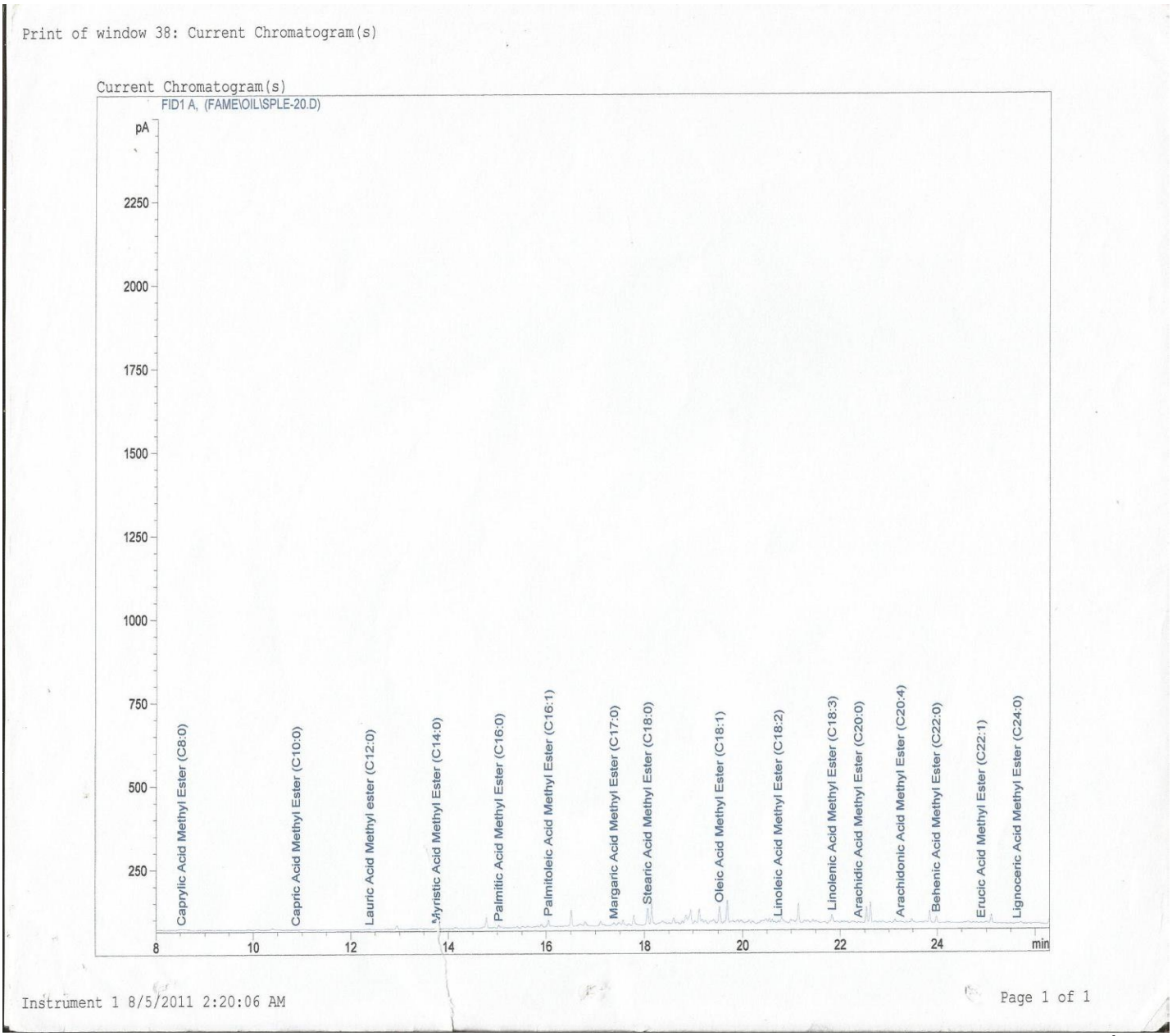

Fig.21: Chromatogram of sample degraded by *Candida parapsilosis* in medium 2 on the 25th day of degradation.