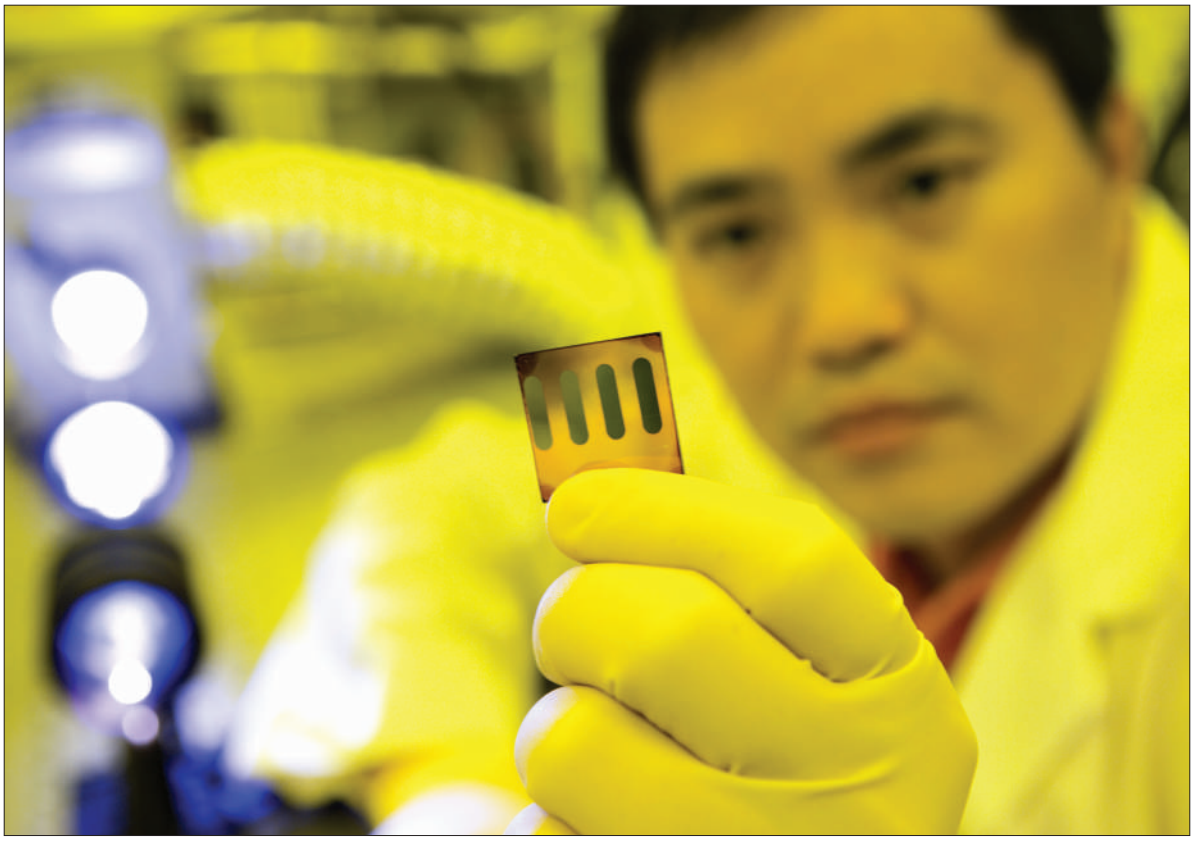

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2019.33.44

Research Article
Bioremediation of Hydrocarbon Contaminated Soil by Intercropping *Luffa aegyptiaca* with *Vernonia amygdalina*, Ameliorated with Growth Promoting Fungi

E. Ani, D.E. Olofin, O.E. Okunlola and A.F. Faniyi

Environmental Biology Unit, Department of Biological Sciences, Yaba College of Technology, Yaba, Lagos, Nigeria

Abstract

Background and Objective: The limitations of monoculture organisms in bioremediation has been highlighted. Bioremediation potentials of intercropping *Luffa aegyptiaca* with *Vernonia amygdalina* ameliorated with growth promoting fungi (*Aspergillus niger* and *Penicillium* sp.) was investigated *in vitro*. **Materials and Method:** Growth promoting fungi were isolated from the rhizosphere of *L. aegyptiaca* and *V. amygdalina* collected from Lagos mainland Local Government Area of Lagos State. Bioremediation potentials of the monoculture and the mixed culture of the organisms were assessed over 3 months period using two concentrations of spent engine oil (SEO) in a screen house. Randomized complete block design was used to apply 22 treatments. Confirmatory hydrocarbon utilization was done using GC-MS. **Results:** Results show that the growth of *L. aegyptiaca* and *V. amygdalina* were negatively affected by increasing concentration of the pollutant. Mixed culture of the organisms were also shown to be better in degrading hydrocarbon than monoculture, several compounds including; Isopropyl tetradecyl ether, 1-Chloroeicosane, pentadecane, biphenyl-chloride were only degraded by mixed culture of the plants and the fungi but not by individual organisms. Remediation of up to 90% were only attained by the consortium and not by the individual organisms involved. Accumulation of hydrocarbon by the tissues of both *L. aegyptiaca* and *V. amygdalina* also decreased in the presence of the rhizospheric fungi. The lowest value of 0.732 and 0.406% in *L. aegyptiaca* and *V. amygdalina* tissues, respectively were found in the presence of the fungi. **Conclusion:** The potentials of consortium of *L. aegyptiaca*, *V. amygdalina*, *Aspergillus niger* and *Penicillium* sp. in remediating hydrocarbon polluted soil is highlighted in this study.

Key words: *Luffa aegyptiaca*, *Vermonia amygdalina*, bioremediation, fungi, hydrocarbons

Citation: E. Ani, D.E. Olofin, O.E. Okunlola and A.F. Faniyi, 2019. Bioremediation of hydrocarbon contaminated soil by intercropping *Luffa aegyptiaca* with *Vernonia amygdalina*, ameliorated with growth promoting fungi. J. Sci. Res., 9: 33-44.

Corresponding Author: E. Ani, Environmental Biology Unit, Department of Biological Sciences, Yaba College of Technology, Yaba, Lagos, Nigeria
Tel: +234-8181926352

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Industrialization, petroleum exploration and exploitation, the ever increasing human population among others has inevitably resulted in an unprecedented negative effect on the biosphere. Large amounts of hazardous wastes (including petroleum hydrocarbon) is being released into all phases of the environment daily, largely from anthropogenic sources[1]. The hazards posed by petroleum hydrocarbon to human and the ecosystems at large has been well documented[2,3]. The development of methods to remediate soils contaminated with toxic pollutants and other organic residues has been an area of intense research interest for some times now[4]. Various physical, chemical and biological processes have been employed in remediation of contaminated soil with each posing some challenges[1]. Due to the expensive and disruptive, although faster, the engineering based remedial technologies[5], there is an increased interest on biological remediation that is eco-friendly, less expensive and has been shown to be efficient. Several organisms (including plants) have been documented to be capable of remediating polluted soil[6-8].

Luffa aegyptiaca (Mill) is a member of Cucurbitaceae and is commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd[9]. There are about nine species in the genus *Luffa* including: *Luffa acutangula*, L*. cylindrica*, *L. aegyptiaca*, *L. operculata*, *L. graveolens* and *L. echinata*[10]. *Luffa cylindrica* is the most widely published and cultivated and is found mostly in South America[9,11,12]. *Luffa aegyptiaca* (Mill) is found mostly in tropical Africa including Nigeria and some parts of India[13,14]. In Nigeria, *Luffa* is commonly found growing in dump sites and in polluted environments. Generally, *Luffa aegyptiaca* can be used in virtually all areas including medicine, industry (as a packing medium in an attached growth system), agriculture and so on[9,11]. *Vermonia amygdalina*, (commonly called bitter leaf) is a small shrub that grows in tropical Africa. *Vernonea amygdalina* is a woody-shrub with an average height about 8 m. The herb is an indigenous African plant, which grows in most parts of sub-Saharan Africa. It is reported to be effective in treatment of fever, pain, malaria, diarrhoea, gastroenteritis, hepatitis, dysentery, diabetes mellitus among others[15]. This study focuses on bioremediation potentials of intercropping *Vernonia amygdalina* and *Luffa aegyptiaca* ameliorated with growth promoting fungi.

MATERIALS AND METHODS

Sample collection: This study was conducted between June and September, 2018. Mature and dried *L. aegyptiaca* seeds, *V. amygdalina* stem and sandy loam soil were collected from Yaba College of Technology staff quarters. Growth promoting fungi (*Aspergillus niger* and *Penicillium* sp.) were isolated from the rhizosphere of *L. aegyptiaca* and *V. amygdalina* collected from Lagos mainland Local Government Area of Lagos State following the method of Reyes and Mitchell[16]. Spent engine oil was collected from author mechanic workshops in Shomolu Local Government.

Fungi identification: Fungi grown on plates were identified using morphological and microscopic features[17-19].

Physio chemical analysis of soil and spent engine oil: Physico-chemical characteristics of the experimental soil and spent engine oil used was analyzed following the methods of Ani *et al.*[20].

Bioremediation study: Bioremediation potentials of *L. aegyptiaca*, *V. amygdalina* with the associated growth promoting fungi (*Aspergillus niger* and *Penicillium* sp.) was assessed in a screen house in Botanical Garden of Yaba College of Technology, Yaba, Lagos, Nigeria (6'31"N, 3'40"E) . Five kilogram of sieved and dried sandy-loam soil was weighed into an experimental bucket (7 L) with a weighing balance. Spent engine oil (100 and 200 mL) were each introduced independently into some of the buckets and mixed thoroughly and allowed to homogenize for 24 h. Randomized complete block design was used to apply 22 treatments including (T_1): *L. aegyptiaca* (LA) only, (T_2): *V. amygdalina* (VA) only, (T_3): 100 mL spent engine oil (SEO) only, (T_4): 200 mL spent engine oil only, T_5: LA+100 mL SEO, T_6: LA+200 mL SEO, T_7: VA+100 mL SEO, T_8: VA+200 mL SEO, T_9: *Aspergillus niger* (AN)+100 mL SEO, T_{10}: AN+200 mL SEO, T_{11}: *Penicillium* sp. (PS)+100 mL SEO, T_{12}: PS+200 mL SEO, T_{13}: LA+AN, T_{14}: LA+PS, T_{15}: LA+AN+100 mL SEO, T_{16}: LA+AN+200 mL SEO, T_{17}: LA+PS+100 mL SEO, T_{18}: LA+PS+200 mL SEO, T_{19}: VA+AN, T_{20}: VA+PS, T_{21}: VA+AN+100 and T_{22}: VA+PS+200 mL SEO. Viable seeds of *L. aegyptiaca* and healthy stems of *V. amygdalina* were planted 24 h after introduction of the SEO at a depth of 3 cm at 5 seeds/hole and later thinned to two. Viability of the seeds was tested before planting following the method of Ani *et al.*[20]. Growth promoting fungi were introduced with sterilized sawdust 48 h after planting. Trays were placed under each buckets treated with SEO to retain the SEO that might have wash down from the soil through the perforated buckets during watering and are poured back into the bucket. Each experiment was set up in 3 replications.

Data collection: The effect of spent engine oil on the leaf, leaf area and internode length, were assessed at 7 days' interval for 24 weeks. The total petroleum hydrocarbon (TPH) was measured monthly using the GC-MS method. Leaf area (LA) and percentage bioremediation by the test organisms were determined[20]:

$$\text{Remediation due to natural attenuation (\%)} = \frac{\text{Initial} - \text{Final without organism}}{\text{Initial}} \times \frac{100}{1}$$

$$\text{Remediation by organisms} = \frac{\text{Initial} - \text{Final with organism}}{\text{Initial}} \times \frac{100}{1}$$

$$\text{Actual remediation by organism} = \text{Remediation by organism (\%)} - \text{Natural attenuation (\%)}$$

Confirmatory test for bioremediation using GC-MS: Confirmatory hydrocarbon utilization by both the plants and the growth promoting fungi was determined using gas chromatography mass spectroscopy (GC-MS) ran at day 0 and at 24 weeks of the experiment for the soil samples while the GC-MS of the plant samples was done at 24 weeks only.

Statistical analysis: Result were analyzed statistically using student general linear model (GLM) which incorporates the univariate analysis of variance (ANOVA) and the pair wise test comparison at ($p<0.05$).

RESULTS

Physicochemical characteristics of the soil and spent engine oil: The physico-chemical properties of the soil and spent engine oil used for remediation experiments are presented in Table 1 and 2, respectively. Physico-chemical properties of the soil show that the soil is neutral with a pH of 7.0, indicating optimal microbial activity and bioavailability of mineral elements such as nitrogen, phosphorus and potassium for plant uptake. The nitrate (3.86 mg kg^{-1}) , nitrite (1.31 mg kg^{-1}), organic matter (4.95%), available phosphate (6.37 mg kg^{-1}) etc., are generally suitable for plant growth (Table 1). Physico-chemical properties of the spent engine oil used for the experiment is presented in Table 2.

Effect of spent engine oil, *A. niger* and *Penicillium* sp. on the growth of *L. aegyptiaca* and *V. amygdalina*: The effect of spent engine oil, *A. niger* and *Penicillium* sp. on the growth of *L. aegyptiaca* and *V. amygdalina* is presented on Table 3. Generally, the growth of *L. aegyptiaca* and *V. amygdalina* was negatively affected by increasing concentration of the pollutant. Leaf area and internode length of *L. aegyptiaca* in 100 mL SEO were 4.14±1.02 and 3.28±0.22, respectively as

Table 1: Physico-chemical characteristics of soil used

Parameters	Values
Conductivity (mS cm^{-1})	2.73
TDS (mg L^{-1})	356.5
TSS (mg L^{-1})	123
TS (mg L^{-1})	1645
Salinity (psu)	1.4
Resistivity (Ωcm)	3330.05
pH	7.0
Temperature (°C)	25
Phosphate (mg kg^{-1})	6.37
Nitrate (mg kg^{-1})	3.86
Nitrite (mg kg^{-1})	1.31
TOC (%)	8.89
TOM (%)	4.95
COD (ppm)	257

TDS: Total dissolved solid, TSS: Total suspended solid, TS: Total solids, TOC: Total organic content, TOM: Total organic matter, COD: Chemical oxygen demand

Table 2: Physicochemical characteristics of spent engine oil used

Parameters	Values
pH	5.6
Density at 25°C	0.9325
Viscosity at 100°C	14.925
viscosity at 40°C	113.27
Flash point (°C)	194
Moisture (%)	0.2

Table 3: Morphological characteristics of *L. aegyptiaca* and *V. amygdalina* in SEO contaminated soil

Samples	Length	Width	Leaf area	Internode
T_1A	2.47 ± 0.37^{a}	2.19 ± 0.20^{ab}	4.14 ± 1.02^{ab}	3.28 ± 0.22^{bc}
T_1B	2.09 ± 0.20^{a}	1.71 ± 0.17^{a}	2.49 ± 0.30^{a}	1.59 ± 0.19^{a}
T_1A_1	5.67 ± 0.62^{a}	3.30 ± 0.92^{a}	13.77 ± 4.27^{a}	1.80 ± 0.91^{a}
T_1B_1	3.27 ± 1.77^{a}	2.40 ± 0.64^{a}	7.83 ± 4.80^{a}	3.20 ± 0.70^{a}
T_1C	4.35 ± 0.77^{bc}	2.67 ± 0.19^{abc}	9.59 ± 2.11^{bc}	2.33 ± 0.40^{ab}
T_2A	5.40 ± 1.81^{a}	10.97 ± 4.84^{a}	3.37 ± 0.73^{a}	6.10 ± 0.53^{a}
T_2B	3.67 ± 0.85^{a}	16.10 ± 3.91^{a}	2.17 ± 0.93^{a}	5.73 ± 1.89^{a}
T_2A_1	5.79 ± 0.75^{c}	3.21 ± 0.36^{bc}	15.23 ± 2.71^{c}	2.23 ± 0.28^{ab}
T_2B_1	5.97 ± 0.27^{c}	2.87 ± 0.25^{abc}	12.95 ± 1.72^{c}	1.19 ± 0.22^{a}
T_2C	6.10 ± 0.67^{c}	4.06 ± 0.54^{cd}	21.09 ± 4.05^{d}	3.07 ± 0.27^{bc}
T_3A	3.43 ± 0.66^{ab}	2.87 ± 0.58^{abc}	5.42 ± 2.15^{ab}	3.39 ± 0.60^{bc}
T_3B	5.47 ± 0.75^{c}	5.18 ± 0.79^{d}	2.91 ± 0.50^{a}	2.49 ± 0.48^{ab}
T_3A_1	4.14 ± 0.64^{a}	10.59 ± 2.41^{a}	6.29 ± 1.29^{ab}	4.21 ± 0.77^{c}
T_3B_1	3.41 ± 0.46^{ab}	3.13 ± 0.64^{abc}	4.63 ± 1.19^{a}	5.57 ± 0.48^{a}
T_3C	4.28 ± 0.41^{a}	10.96 ± 2.52^{a}	9.69 ± 2.68^{a}	4.04 ± 0.83^{a}
F-statistics	$F_{8,126} = 7.140$ p<0.001	$F_{8,126} = 4.786$ p<0.001	$F_{8,126} = 9.227$ p<0.001	$F_{8,126} = 4.911$ p<0.001

Samples with different superscript are significantly different from each other at 5%, T_1A: *Luffa augyptiaca* in 100 mL SEO, T_1A_1: *Luffa augyptiaca* in 100 mL+fungi, T_1B: *Luffa augyptiaca* in 200 mL, T_1B_1: *Luffa augyptiaca* in 200 mL+fungi, T_1C: *Luffa augyptiaca* without pollutant, T_2A: *Vernonia amygdalina* in 100 mL, T_2A_1: *Vernonia amygdalina* in 100 mL+fungi, T_2B_1: *Vernonia amygdalina* in 200 mL+fungi, T_2B: *Vernonia amygdalina* in 200 mL, T_2C: *Vernonia amygdalina* without pollutant, T_3A: *Vernonia amygdalina* and *Luffa augyptiaca* in 100 mL, T_3A_1: *Vernonia amygdalina* and *Luffa augyptiaca* in 100 mL+fungi, T_3B_1: *Vernonia amygdalina* and *Luffa augyptiaca* in 200 mL+fungi, T_3B: *Vernonia amygdalina* and *Luffa augyptiaca* in 200 mL, T_3C: *Vernonia amygdalina* and *Luffa augyptiaca* without pollutant

Table 4: Percentage remediation of SEO polluted soil by *Luffa aegyptiaca/V. amygdalina/A. niger* and *Penicillium* sp. using TPH analysis

	Pollutant concentration (mL)/treatments/results (%)											
	100						200					
Months	TL	TL_1	TV	TV_1	TLV	TLV_1	TL_2	TL_{2a}	TV_2	TV_{2a}	TLV_2	TLV_{2a}
1	8.7	36.9	20.0	25.3	5.7.0	23.8	18.1	32.9	47.9	56.3	38.0	42.7
2	17.0	65.8	19.0	55.4	73.6	74.1	51.1	56.1	41.8	72.7	51.1	36.6
3	71.4	79.6	80.1	85.0	88.0	90.0	57.7	60.4	55.8	81.8	63.7	78.3

TL: *Luffa augyptiaca* in 100 mL SEO, TL_1: *Luffa augyptiaca* in 100 mL+fungi, TL_2: *Luffa augyptiaca* in 200 mL, TL_{2a}: *Luffa augyptiaca* in 200 mL+fungi, TV: *Vernonia amygdalina* in 100 mL, TV_1: *Vernonia amygdalina* in 100 mL+fungi, TV_2: *Vernonia amygdalina* in 200 mL, TV_{2a}: *Vernonia amygdalina* in 200 mL+fungi, TLV: *Vernonia amygdalina* and *Luffa augyptiaca* in 100 mL, TLV_1: *Vernonia amygdalina* and *Luffa augyptiaca* in 100 mL+fungi, TLV_2: *Vernonia amygdalina* and *Luffa augyptiaca* in 200 mL, TLV_{2a}: *Vernonia amygdalina* and *Luffa augyptiaca* in 200 mL+fungi

Table 5: Percentage remediation of SEO polluted soil by *Aspergillus niger* and *Penicillium* sp. (Fungi) using TPH analysis

	Pollutant concentration (mL)/treatments/results (%)					
	100			200		
Months	T_a	T_p	T_{ap1}	T_{a2}	T_{p2}	T_{ap2}
1	16.5	23.2	45.6	26.9	31.8	37.2
2	53.4	65.3	71.0	45.9	36.7	51.1
3	79.2	71.4	87.3	69.1	67.4	80.6

T_a: *Aspergillus niger* in 100 mL, T_p: *Pernicilium* sp. in 100 mL, T_{ap1}: *Aspergillus niger* and *Pernicilium* sp. in 100 mL, TA_2: *Aspergillus niger* in 200 mL, T_{p2}: *Pernicilium* sp. in 200 mL, T_{ap2}: *Aspergillus niger* and *Pernicilium* sp. in 200 mL

against 2.49±0.30 and 1.59±0.19 in 200 mL, respectively. On introduction of fungi (*A. niger* and *Penicillium* sp.), leaf area increased to 13.77±4.27 and 7.83±4.80in 100 and 200 mL spent engine oil, respectively. The leaf area and internode length for *V. amygdalina* were 3.37±0.73 and 6.10±0.53, respectively in 100 mL but 2.17±0.93 and 5.73±1.89 in 200 mL SEO indicating that *V. amygdalina* may be more tolerant to SEO pollution than *L. aegyptiaca*. On introduction of fungi, the leaf area and internode length for *V. amygdalina* were 15.23±2.71, 2.23±0.28 and 12.95±1.72, 1.19±0.22 in 100 and 200 mL spent engine oil, respectively. This implies that the leaf areas were favoured by the introduction of fungi but did not translate to increase in height for *V. amygdalina*. On intercropping *L. aegyptiaca* with *V. amygdalina* without fungi, the mean leaf area and internode length were 5.42±2.15, 3.39±0.60 and 2.91±0.50, 2.49±0.48 in 100 mL and 200 mL spent engine oil, respectively. When *L. aegyptiaca* with *V. amygdalina* were intercropped and the growth promoting fungi introduced, the leaf area and internode length in 100 mL SEO were 6.29±1.29, 4.21±0.77 and 4.63±1.19, 5.57±0.48 in 200 mL SEO, respectively. From this result, fungi (*A. niger* and *Penicillium* sp.) were shown to enhance the survival and growth of *L. aegyptiaca* and *V. amygdalina* in hydrocarbon polluted soil (Table 3).

Confirmatory hydrocarbon utilization: The confirmatory hydrocarbon utilization using TPH analysis is presented in Table 4 and 5 while the GC-MS of the soil and plants tissues are presented in Fig. 1-6. Remediation ability of *Luffa aegyptiaca* in 100 mL SEO contaminated soil at 3 months was 71.4% but increased to 79.6% on introduction of fungi (*Aspergillus niger* and *Penicillium* sp.). Comparatively, *Vernonia amygdalina* in 100 mL SEO contaminated soil was 88 and 80.1% with and without the fungi, respectively. When *L. aegyptiaca* is intercropped with *V. amygdalina* in 100 mL SEO contaminated soil, remediation percentage was 90 and 88%, respectively with and without the fungi. In 200 mL SEO contaminated soil, remediation by *L. aegyptiaca* and *V. amygdalina* were 57.7 and 55.8%, respectively without fungi but increased to 60.4 and 81.8% on introduction of fungi (Table 4) . Independently, remediation by *Aspergillus niger* in 100 and 200 mL SEO contaminated soil were 69.2 and 59.1%, respectively as against 61.4 and 47.4% respectively by *Penicillium* sp. Percentage remediation by consortium of *A. niger* and *Penicillium* sp. in 100 and 200 mL SEO contaminated soil were 77.1 and 70.6, respectively (Table 5). The confirmatory hydrocarbon utilization study of SEO contaminated soil using GC-MS is presented in Fig. 1-4 while the hydrocarbon compounds detected within *L. aegyptiaca* after the experiment are presented in Fig. 5-6. From the results, both *L. aegyptiaca*, *V. amygdalina*, *A. niger* and *Penicillium* sp. were able to remediate SEO contaminated soil but at different rates. Intercropping *L. aegyptiaca* with *V. amygdalina* ameliorated with rhizospheric fungi however, gave the best result. Several compounds including, Isopropyl tetradecyl ether, 1-Chloroeicosane, pentadecane, 2,6,10,14-tetramethyl, behenyl chloride among others which were only degradable by combined effort of the plants and the fungi but not by individual organisms. Remediation of up to 90% and above were only attained by the consortium and not by the individual organisms involved. Accumulation of hydrocarbon by the tissues of both *L. aegyptiaca* and *V. amygdalina* decreased in the presence of the rhizospheric fungi. The lowest value of 0.732 and 0.406% in *L. aegyptiaca* and *V. amygdalina* tissues, respectively were found in the presence of the fungi (Fig. 5-6).

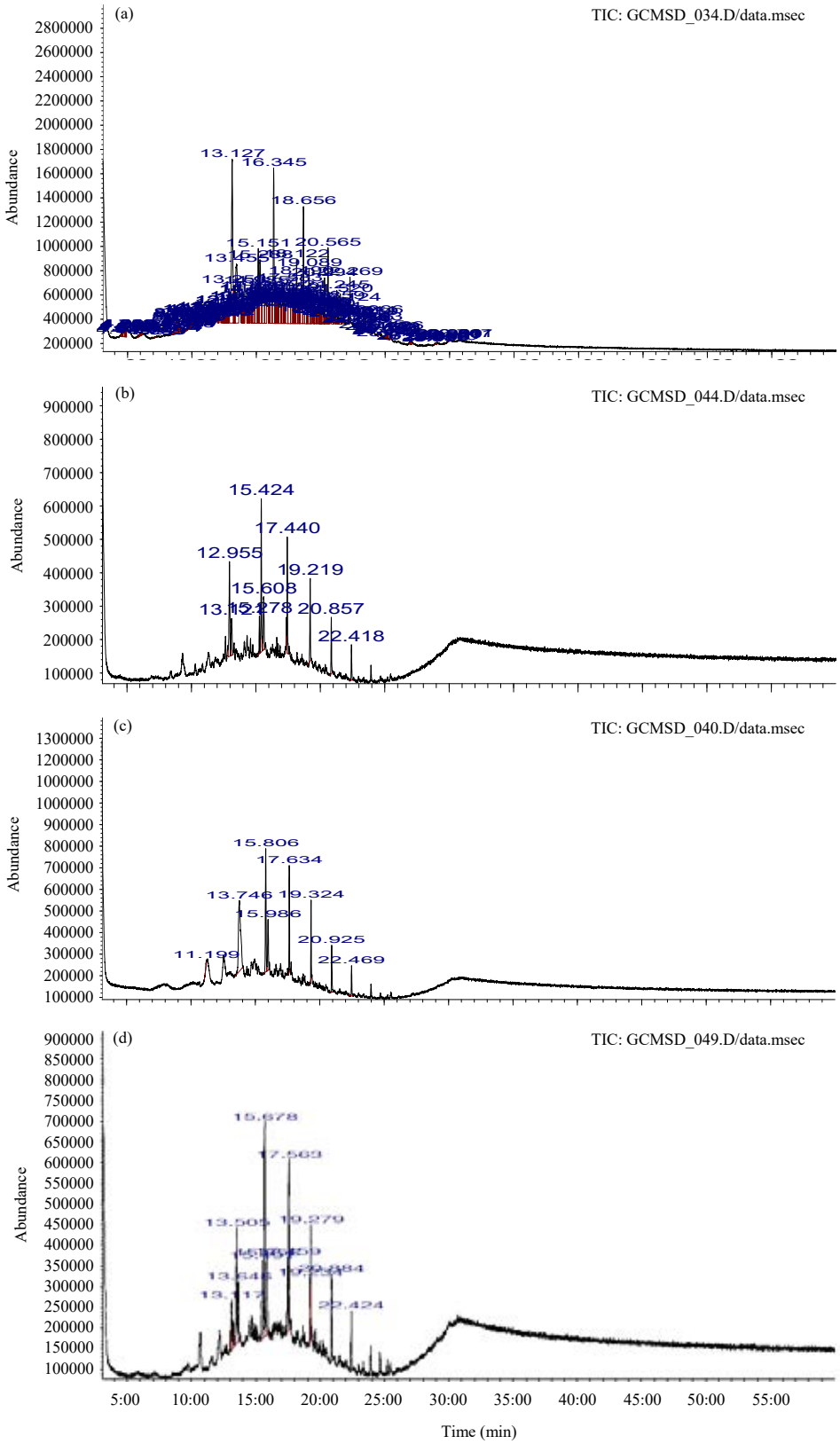

Fig. 1(a-d): Chromatogram of the pollutant only, (a) 200 mL (b) 100 mL at 24 h and (c, d) after 3 months

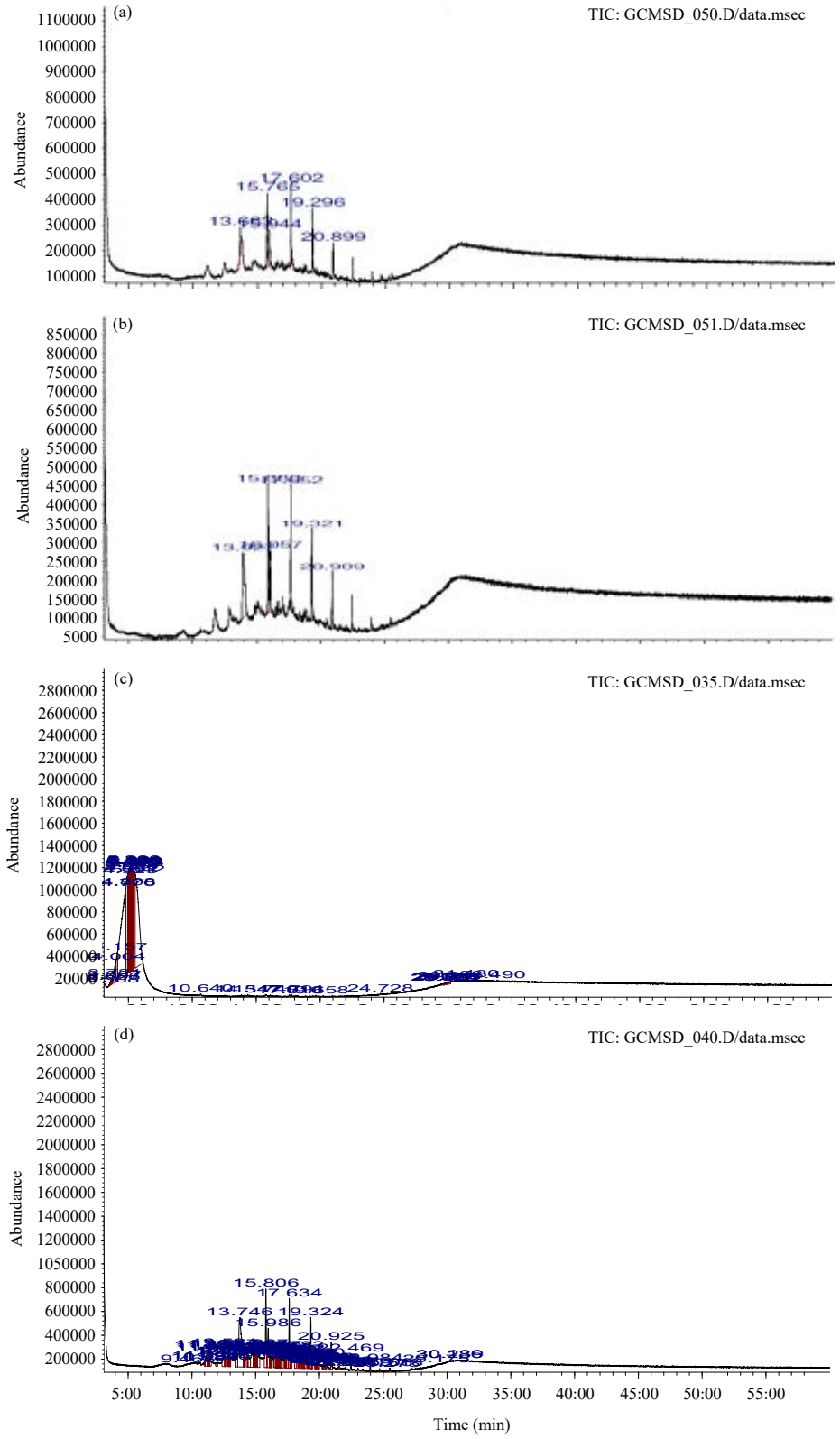

Fig. 2(a-d): Chromatogram of 200 mL SEO contaminated soil of, (a) *L. aegyptiaca*, (b) *V. amygdalina* (c) *Aspergillus niger* and (d) *Penicillum* sp., after 3 months

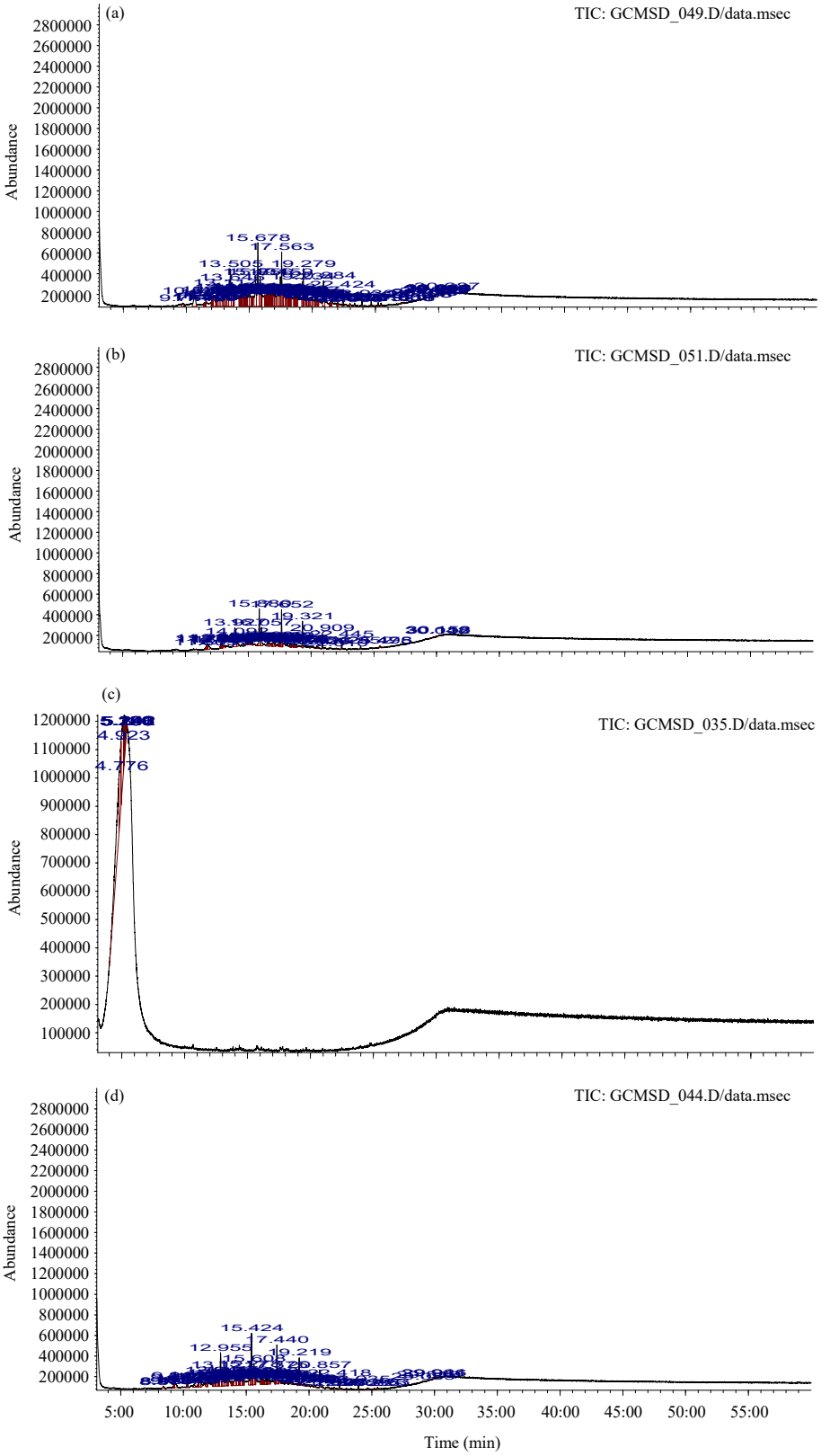

Fig. 3(a-d): Chromatogram of 100 mL SEO contaminated soil of, (a) *L. aegyptiaca*, (b) *V. amygdalina* (c) *Aspergillus niger* and (d) *Penicillum* sp., after 3 months

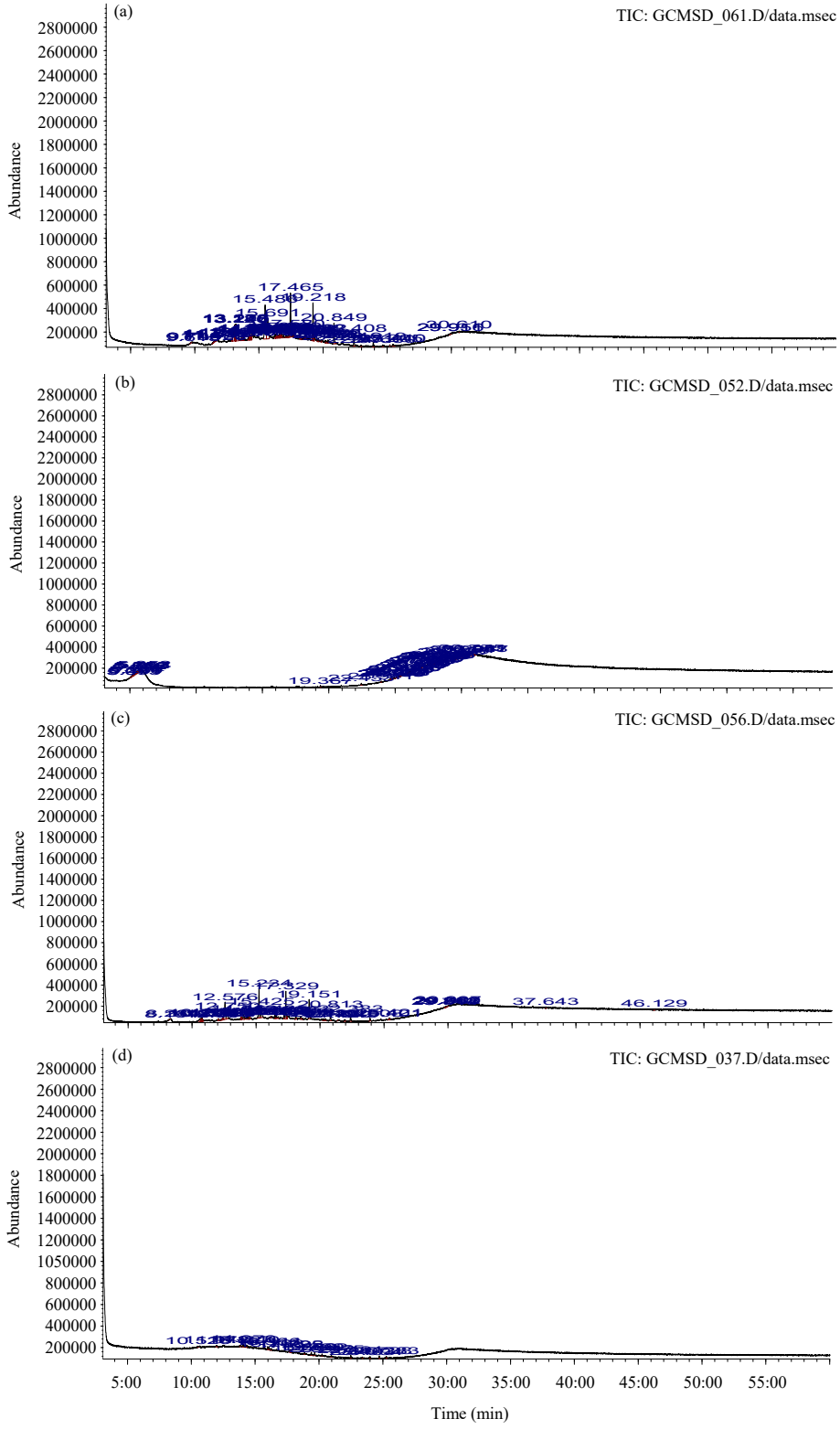

Fig. 4(a-d): Chromatogram of (a) *L. aegyptiaca/V. amygdalina* in 200 mL, (b) *L. aegyptiaca/V. amygdalina* in 100 mL, (c) *L. aegyptiaca/V. amygdalina/Aspergillus niger/Penicillum* sp., in 200 mL and (d) *L. aegyptiaca/V. amygdalina/ Aspergillus niger/ Penicillum* sp., in 100 mL

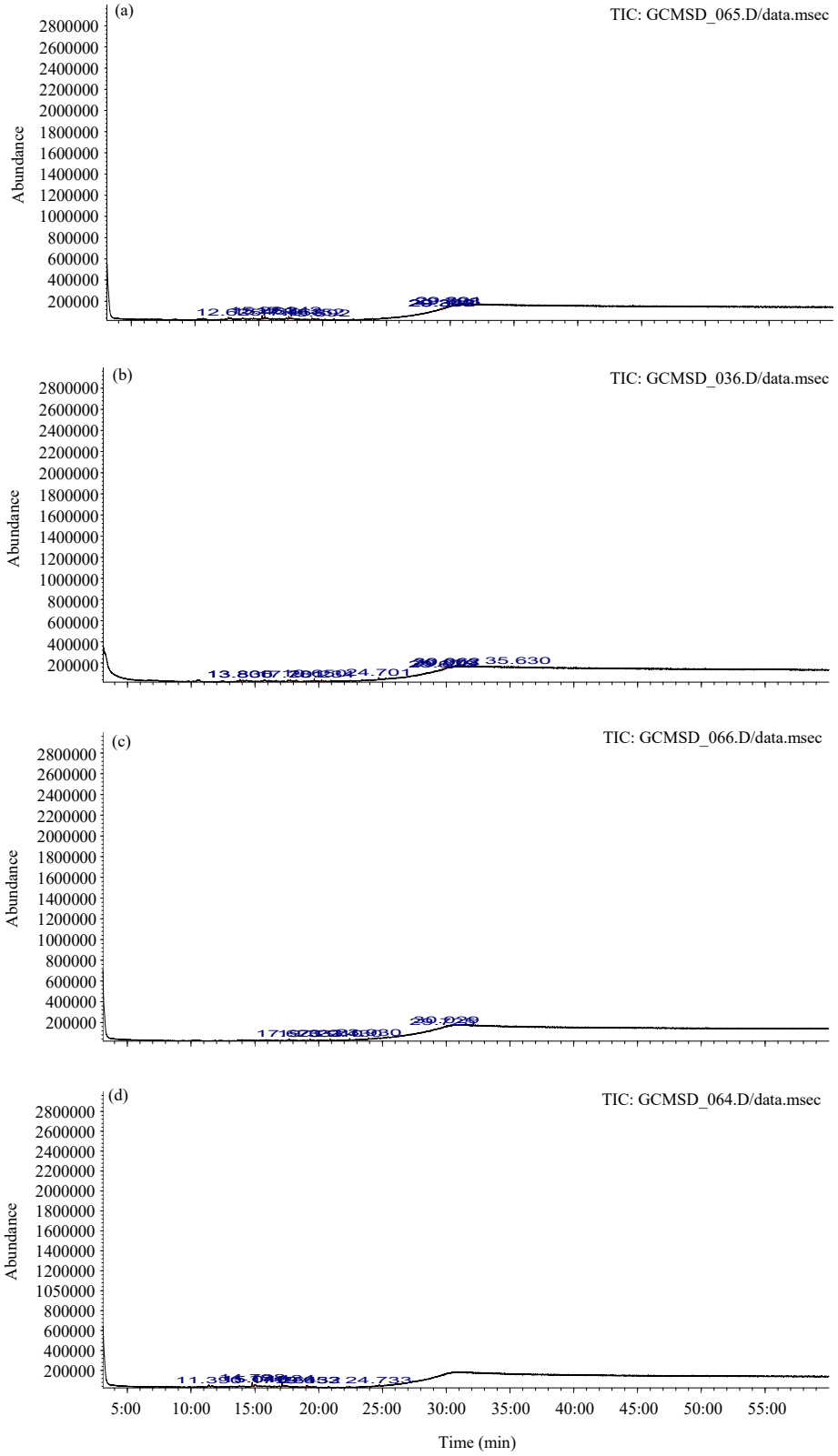

Fig. 5(a-d): Chromatogram of plant tissue (a, b) *L. aegyptiaca* in 100 mL and 200 mL and (c, d) *V. amygdalina* in 100 mL and 200 mL SEO contaminated soil after 3 months

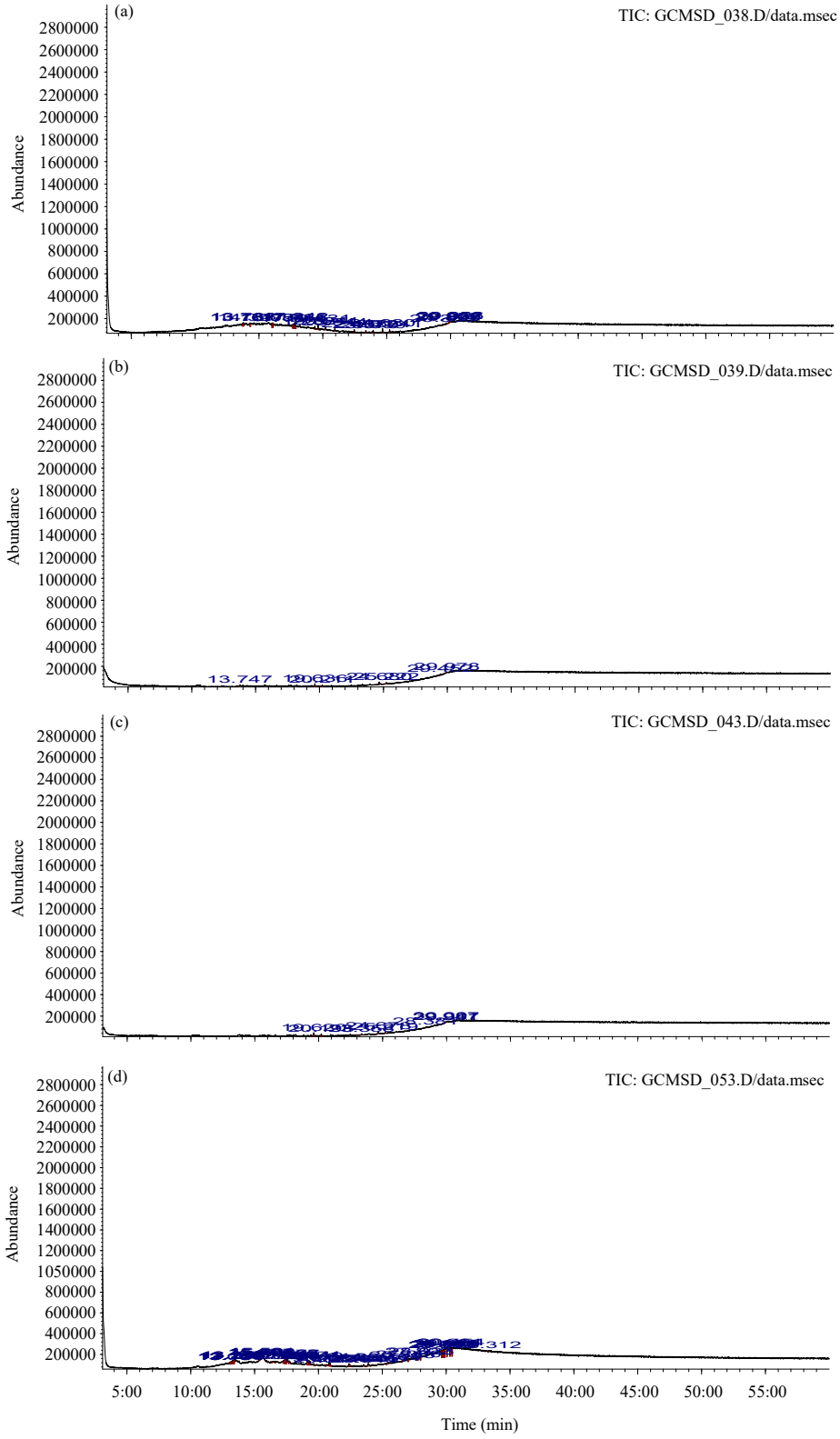

Fig. 6(a-d): Chromatogram of plant tissues (a) *L. aegyptiaca/V. amygdalina* in 100 mL, (b) *L. aegyptiaca/V. amygdalina* in 200 mL (c) *L. aegyptiaca/V. amygdalina/Aspergillus niger/Penicillum* sp., in 100 mL and (d) *L. aegyptiaca/V. amygdalina/ Aspergillus niger/Penicillum* sp., in 200 mL

DISCUSSION

In this study, results show that the growth of *L. aegyptiaca* and *V. amygdalina* were negatively affected by increasing concentration of the pollutant. Mixed culture of the organisms was also shown to be better in degrading hydrocarbon than monoculture. Accumulation of hydrocarbon by the tissues of both *L. aegyptiaca* and *V. amygdalina* also decreased in the presence of the rhizospheric fungi. The pH of the soil for this study was 7.0 which is in agreement with Boonchan *et al.*[21], who reported optimum pH for bioremediation as between 6.0 and 8.9. The observed physico-chemical parameters for soil and SEO used followed the submissions of Oyedele and Amoo[22]. Spent engine oil is commonly disposed into drainage channels, open vacant plots and farmlands in Nigeria, especially by auto-mechanics[23]. Improperly disposal of spent engine oil renders polluted soils unfit for use. It alters soil microbial properties, decreases oxygen content and nutrient availability. Increased awareness of the negative consequences of petroleum hydrocarbon pollutants to living organisms and the environment at large has resulted into increased efforts into finding ways of mitigating/controlling hydrocarbon pollution[24]. The high costs and limited efficiency of other methods of remediation has made biological remediation of polluted soil a better/best alternative. The ability of *L. aegyptiaca*, *V. amygdalina*, *Aspergillus niger* and *Penicillium* sp., to utilize and biodegrade spent engine oil contaminated soil was assessed *in vitro*. Results show that both the plants and the fungi were able to biodegrade spent engine oil contaminated soil but with differing abilities. The ability of fungi to degrade petroleum hydrocarbon has been documented by Rohrbacher and St-Arnaud[25], Adekunle *et al.*[26] and Mohsenzadeh [27].

In this study, both *L. aegyptiaca*, *V. Amygdalina*, *Aspergillus niger* and *Penicillium* sp., significantly enhanced the dissipation of PAHs in the soil however, intercropping both *L. aegyptiaca* with *V. Amygdalina*, ameliorated with *Aspergillus niger* and *Penicillium* sp., achieved the highest result. The TPH reduced significantly by up to 90% for TLV_1 (*Luffa* and *vernonia* intercropped ameliorated with fungi). This surpassed results of similar researches by Adelowo *et al.*[28] and Akinde and Obire[29], who achieved less than 80% TPH degradation. Generally, the growth of *L. aegyptiaca* was negatively affected by increasing concentration of the pollutant. Results from morphological study show that the leaf areas were favoured by the introduction of fungi but did not translate to increase in height for *V. amygdalina.* The effect of used motor oil on *L. aegyptiaca* and *V. amygdalina* were observed as reduction in leaf area and internode length.

SIGNIFICANT STATEMENT

This study discovers the potency of consortium of *L. aegyptiaca*, *V. amygdalina*, *Aspergillus niger* and *Penicillium* sp., in bioremediation of hydrocarbon polluted soil. This study provides potentially cheaper, easy to apply and more effective means of remediating hydrocarbon polluted soil. Thus, remediation of hydrocarbon polluted soil, especially in less technologically developed countries may be arrived at.

CONCLUSION

Bioremediation has been shown to be a better alternative to other remediation techniques. Intercropping *L. aegyptiaca* with *V. amygdalina* ameliorated with *A. niger* and *Penicillium* sp., significantly enhanced the rate of spent engine oil degradation in soil up to 90% compared with monoculture. Thus, intercropping *Luffa aegyptiaca* and *Vermonia amygdalina* has shown promising potential in bioremediation of PAH-contaminated soil.

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