



# A community-based innovative approach of deriving a functional compost for remediation of crude oil contaminated soil

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## Abstract

Compost is a local product that has proven effective in soil remediation. Its performance is attributed to the diversity of microbes and nutrients that augment indigenous soil microbes and stimulate biodegradation processes respectively. Improving its performance is imperative to reduce the influx of foreign remediation products into the country. This study investigated a simple method of improving compost quality to comprise abundant crude oil-tolerant microbes. The feedstock was made up of chicken manure, sawdust, and dry leaves at the weight ratio of 1.4 kg:0.4 kg:0.1 kg. Crude oil was added at 20000 mg/kg weight of feedstock and tagged TC, while the control, UC, was without crude oil. After 4 weeks monitoring, the inclusion of crude oil did not hinder the composting process. The pH, Total Nitrogen, Available Phosphorus and Total Organic Carbon recorded by TC were 8.30, 1.33%, 0.83%, and 43.81% respectively, while for UC the values were respectively 8.20, 1.02%, 0.78%, and 42.31%. Nevertheless, the presence of crude oil significantly increased hydrocarbon utilizing microbes. The HUF recorded by TC and UC were  $2.96 \times 10^6$  CFU/g and  $1.42 \times 10^6$  CFU/g respectively, while HUB for TC and UC were  $1.58 \times 10^8$  CFU/g and  $0.9 \times 10^8$  CFU/g respectively. Also, the GC chromatograms reflected a concurrent reduction of petrogenic and non-petrogenic carbons, and the TPH reduction in TC was 61.1% after 4 weeks. Therefore, the inclusion of crude oil in the composting stimulated abundant crude oil-adapted microbes without compromising the required nutrients, hence having the potential of enhancing soil remediation.

**Keywords:** Compost, Composting process, crude oil, chicken manure, hydrocarbon utilizing microbes

## INTRODUCTION

Oil transport through pipelines has resulted in several spills in Nigeria. According to National Oil Spill Detection and Response Agency (2022), the total number of spill incidents recorded on land and swamp areas in a 10 year span (2010 – 2020) was 5,445. Currently, in the country, there are

several foreign remediation products such as Oil Spill Eater II, SF-1000, Nutriol NP5 etc, and utilizing these for soil remediation could be likened to the waste of foreign exchange, especially where local substitutes are available. Compost is a local product that has been successfully used in soil remediation (Adekunle et al., 2021; Uyizeye et al., 2019). It is imperative to improve its quality as a soil remediation product to compete with foreign ones and discourage their patronage.

Composting is the bioconversion of organic feedstocks into a stable and mature humus-like product called compost (Kästner & Miltner, 2016). A functional compost consists of abundant hydrocarbon utilizing microbes such as *Thiobacillus*, *Polaromonas*, *Pseudomonas*, *Acinetobacter*, *Paenibacillus*, etc and nutrients (nitrogen and phosphorus) required in the biodegradation of petroleum hydrocarbon (Farzadkia et al., 2019; Sarkar et al., 2020). During composting, organic matter is biodegraded to intermediates and inorganic forms of carbon and nutrients such as nitrogen and phosphorus that are utilized during soil remediation (Nwaichi & Wegwu, 2012; Aguelmous et al., 2020; Chaher et al., 2020). In these processes, microbes play vital roles. Their diversity and abundance are influenced by feedstock type among other factors such as C/N ratio, pH, temperature, moisture content, etc (Alves et al., 2019; Ma et al., 2020). In the presence of petroleum hydrocarbon, these microbes have been shown to have effectively utilized the hydrocarbon as a source of carbon, hence categorized as hydrocarbon utilizers (Alves et al., 2019). In the bioremediation of soil contaminated with petroleum hydrocarbon, the biodegradation is mainly influenced by indigenous or exogenous hydrocarbon utilizing microbes, and their activities are enhanced under optimum nutrients, pH, air supply, substrate availability etc (Ahmed et al., 2022; Nwaichi et al., 2015; Ugwoha et al., 2020).

Several efforts have been expended in improving the performance of compost for soil remediation. Farzadkia et al. (2019) suggested use of immature compost in bioremediation due to its abundant and active microbes. Abtahi et al. (2020) improved performance of compost for bioremediation through addition of exogenous hydrocarbon utilizers to augment the indigenous

microbes. Leal et al. (2019) improved abundance of petroleum-tolerant microbes in compost by treating it with diesel oil.

The purpose of this study was to derive abundant hydrocarbon utilizers in compost through the inclusion of crude oil in the composting feedstock. Specifically, it studied (1) changes in the physicochemical properties, (2) dynamics of hydrocarbon utilizing microbes, and (3) the fate of petrogenic and non-petrogenic carbon during the composting.

## **MATERIALS AND METHODS**

### **Composting setup and operation**

The materials for this study as shown in Table 1, were sourced in Rivers State. Chicken manure was collected from a farm at Rukpokwu. Sawdust was obtained from a sawmill at Elioza Dry leaves were obtained from Eneka, and crude oil (Bonny light) was obtained from an oil spill site at Omoku. The oil spill occurred on February 19, 2021, and about 110 L of crude oil released from a flowline. This was contained and recovered into covered plastic tank same day. The oil for this study was collected from the recovered oil on February 20, 2021.

The composting feedstock was made up of chicken manure and sawdust mixed at a wet weight ratio of 1.4 kg: 0.4 kg to achieve an initial C/N ratio of 30/1. Dry palm leaves cut into 30 – 40 mm pieces and weighed 0.1 kg (10% dry weight of feedstock) was included in the mix. This was to ensure improved air circulation in the pile. The moisture content of the homogenized feedstock as derived by the mixing equation of Thomas et al. (2020) was 57%, and is within the optimum for composting (Alves et al., 2019). These were set up in triplicate and in two groups using 4 L PVC containers. The containers were perforated to ensure natural air convection through the pile. One of the groups, TC was treated with crude oil at 2000 mg/kg weight of the feedstock, being the optimum proportion derived from previous study (Data not included). The other group, UC, without crude oil treatment was control.

The composting bins were set up and monitored for 4 weeks in the research station of the National Oil Spill Detection and Response Agency (NOSDRA), Port Harcourt. The piles were turned at 2 day intervals for improved aeration. The temperature was measured daily using a Chinavasion digital thermometer BBQ, PHO\_06PPCG08 with a probe, while samples were collected weekly for further laboratory analysis.

Table 1: Physicochemical properties of the feedstock materials

<b>Parameter</b>	<b>Poultry Manure</b>	<b>Sawdust</b>	<b>Dry leaves</b>
<b>pH</b>	8.30 ± 0.01	6.20 ± 0.15	7.10 ± 0.20
<b>Electrical conductivity (µS/cm)</b>	4755.00 ± 50.00	310 ± 15.5	250 ± 0.10
<b>Available phosphorus (%)</b>	1.50 ± 0.10	0.01 ± 0.008	0.05 ± 0.001
<b>Total Nitrogen (%)</b>	2.57 ± 0.25	0.37 ± 0.02	0.48 ± 0.05
<b>Total Organic Carbon (%)</b>	39.09 ± 0.45	56.86 ± 0.10	29.04 ± 0.25

### **Analytical parameters**

The samples were collected in 50 mL amber glass bottles and stored at < 4°C for further analysis. The analysed parameters included pH, Electrical Conductivity (EC), Total Nitrogen (TN), Available Phosphorus (AP), Total Organic Carbon (TOC), Total Petroleum Hydrocarbon (TPH), Hydrocarbon Utilizing Bacteria (HUB), and Hydrocarbon Utilizing Fungi (HUF).

The pH and EC were measured by a multimeter Hanna Instrument (HI 98 11-5) after the samples were shaken with deionized water at 1g/10mL for one hour following the procedure of FAO (2021b). The TN was analysed using the Kjeldahl method as described in FAO (2021a). Crushed sample of 0.2 g was weighed into a digestion flask, and 2.0 g of digestion catalyst (94% K<sub>2</sub>SO<sub>4</sub> + 6% CuSO<sub>4</sub>.5H<sub>2</sub>O) was added, before 5 mL of conc. H<sub>2</sub>SO<sub>4</sub> was added. The flask was heated at 400°C until a light green transparent liquid was achieved. After cooling, distillation was carried

out and evolved ammonia was received in 20 mL of Boric acid. Further titration was carried out with 0.02N H<sub>2</sub>SO<sub>4</sub> to a light pink endpoint. The TOC was determined by heating oven dried sample to ash in a muffle furnace set at 550°C for 2 hours. The percentage loss on ignition was calculated as total organic matter, a relationship suggested by Matthiessen et al. (2005) (Eqn. 1) was used to determine TOC.

$$\text{TOC} = 0.58 \times \text{TOM} \dots\dots\dots 1$$

Where,

TOM = Total organic matter

The available phosphorus was analysed based on the procedure described by Mohee et al. (2008). An air-dried sample was shaken with 0.2N H<sub>2</sub>SO<sub>4</sub> at 1g/10mL for one hour before being filtered. 2.0 mL of the filtrate was pipetted into a beaker, followed by 5.0 mL deionized water, and 2.0 mL of combined reagent (20 mL H<sub>2</sub>SO<sub>4</sub>, 2.5 mL Antimony Tartrate, 7.5 mL Ammonium Molybdate, 0.265 g Ascorbic acid and 20 mL deionized water) was added to develop blue colour that was read on Hach-D29 colourimeter after 30 minutes at 660nm.

Extraction of TPH was done by organic solvent, acetone and dichloromethane, at a volume ratio of 1:1. A 10.0 g sample was weighed and homogenised with 15 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> in a beaker, 30 mL of extraction solvent was added and was agitated on an orbital shaker for an hour. The content was centrifuged and decanted. After concentration to about 1 mL, the content was transferred to a vial and analysed on Gas Chromatography-Flame Ionization Detector, Agilent 7890A.

The HUB and HUF were determined as described by Ogbonna et al. (2020). 1.0 g of the sample was weighed in a test tube and serially diluted to 10<sup>-6</sup> using normal saline. Mineral salt medium was constituted by mixing the following salts; 10.0g NaCl, 0.42g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.29g KCl, 0.83g KH<sub>2</sub>PO<sub>4</sub>, 1.125g Na<sub>2</sub>HPO<sub>4</sub>, 0.42g NaNO<sub>3</sub>,

15.0g Agar, and 1.0L deionized water. The constituted mineral salt medium treated with 50µg/L of appropriate inhibitor (Nystatin and Chloramphenicol for fungi and bacteria inhibitions respectively) was poured into disposable Petri dishes and 0.1 ml from  $10^{-4}$  and  $10^{-6}$  for HUF and HUB respectively were applied on the solidified mineral salt medium and thoroughly spread. Furthermore, a sterile filter paper soaked with 1.0 mL crude oil was placed in the dish lid aseptically, and the dish was inverted before incubation at 30°C.

### **Data analysis**

All the statistical analyses were done on RStudio 2022.02.1, while charts were produced with Microsoft Excel 2013. The response variables were subjected to Two-Way ANOVA, while Tukey HSD was employed for piecewise comparison of parametric data. The comparison was significant at  $p < 0.05$ , while insignificant at  $p > 0.05$ .

## **RESULTS AND DISCUSSION**

### **Physico-chemical parameters**

#### Temperature

In composting, aerobic biodegradation of organic matter is usually accompanied by the release of heat (Waqas et al., 2019). In Fig. 1, there was a rapid increase in temperature within 24 h of setup to a maximum of 50°C in one of the TC containers, while UC achieved a maximum of 47°C. Thereafter, the temperatures fell below 35°C on day 2. However, after turning the piles, a temperature increase to 40°C was recorded. From day 4 forward, the temperature dropped gradually and approached ambient conditions.

The rapid increase in temperature could be attributed to established microbial activities in the utilisation of easily available carbon (Bao et al., 2022). However, a rapid drop in the temperature could be due to exhaustion of the available carbon or limited air for aerobic activities. The turning

operation effected on day 2 could have improved the air supply in the pile, and a further increase in the temperature recorded. Ramavandi et al. (2018) investigated TPH removal in a composting of oily sludge employing a 2 d turning interval. The authors recorded a similar temperature profile with a rebound after turning like this study without significantly influencing the TPH removal. However, in this study, the inclusion of crude oil did not significantly ( $p > 0.05$ ) influence the temperature profile. Lin et al. (2012) included diesel in food waste composting and recorded an insignificant influence of pile temperature by the inclusion. This could imply that the proportion of the crude oil added to the pile was within the limit tolerated by the microbes, and could have utilized the petroleum carbon as a source of energy.

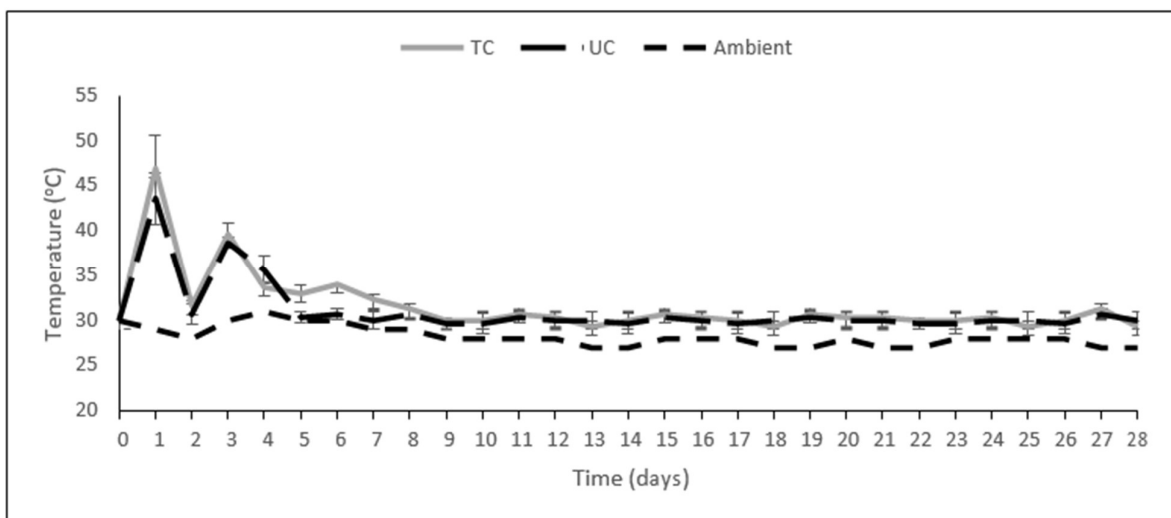


Fig. 1: Temperature profile recorded during 4 weeks of composting

### Total nitrogen (TN)

Nitrogen exists and transforms into different forms during composting, and the total nitrogen balance changes as the process proceed to maturity (Wang et al., 2019). In week 1, a significant drop ( $p < 0.05$ ) in TN was recorded in UC, while the reduction recorded in TC was insignificant ( $p > 0.05$ ). After 4 weeks of composting, TN in TC and UC reduced to 1.33% and 1.02% respectively. Biodegradation of nitrogenous matter produces  $\text{NH}_4^+$  but elevated temperature

and pH influence  $\text{NH}_3$  volatilization, and this contributes majorly to nitrogen loss in composting (Wang et al., 2019). The drop in TN experienced in both setups could be due to ammonia volatilization at elevated temperature recorded in week 1. Several studies have recorded nitrogen loss in the first week of composting and were attributed to ammonia volatilization (Awasthi et al., 2020). The inclusion of crude oil in the TC did not significantly ( $p > 0.05$ ) influence the nitrogen profile but less reduction was recorded compared to UC. It is a point to note that in addition to ammonia volatilization, utilization of nitrogen in TPH removal could have also contributed to nitrogen loss recorded by TC (Koolivand et al., 2017).

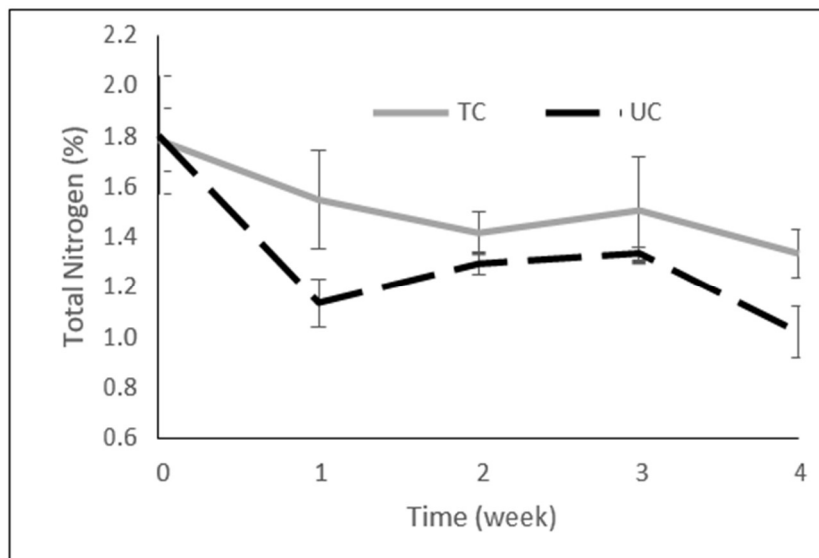


Fig. 2: Total nitrogen profile during the composting

## pH

The production of organic acid during organic matter biodegradation is the major cause pH drop in composting (Waqas et al., 2019). As shown in Fig. 3, the pH increased significantly ( $p < 0.05$ ) from near neutral for both TC and UC to above 8.0. The inclusion of crude oil did not significantly affect the change in pH. A similar effect was reported by Lin et al. (2012), where the inclusion of diesel oil in food waste composting did not interfere with the pH dynamic. The



pH usually increased to alkaline after consumption of organic acid during the thermophilic stage (Ge et al., 2020). In this study, a reduction in pH was not recorded possibly due to the initial pH of the feedstock, which could have buffered the process from excessive production of organic acid. Nevertheless, the recorded pH through the composting fall within the optimum required for effective microbial activities (6.5 – 9.0) (Li & Song, 2020)

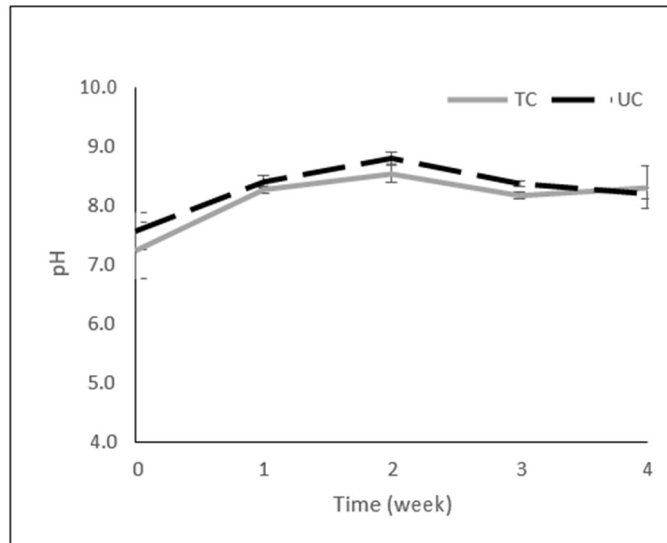


Fig. 3: pH profile during the 4 weeks of composting

#### Total organic carbon (TOC)

In organic matter degradation, organic carbon is degraded to intermediates such as alcohols and organic acid, and could further oxidize to CO<sub>2</sub>. As shown in Fig. 4, the TOC decreased insignificantly ( $p > 0.05$ ) from 47.6% to 43.81% in TC, while in UC significant reduction ( $p < 0.05$ ) from 50.11% to 42.31% was recorded. The reduction in TOC could be an indication of organic matter degradation (Nguyen et al., 2020; Soto-Paz et al., 2019). However, the low reduction could be attributed to the type of carbonaceous feedstock, sawdust, which contains difficult-to-degrade cellulose, lignin, and hemicellulose (Harindintwali et al., 2020; Hubbe, 2014). Nevertheless, at the end of week 4, UC significantly decreased ( $p < 0.05$ ) by 7.8%, while an insignificant reduction ( $p > 0.05$ ) of 3.8% was recorded by TC. The inclusion of crude oil could not make a significant change ( $p > 0.05$ ) in the TOC profile but less reduction recorded by TC

may be due to the effect of crude oil in reducing the abundant of heterotrophs in contaminated media (Aguelmous et al., 2019).

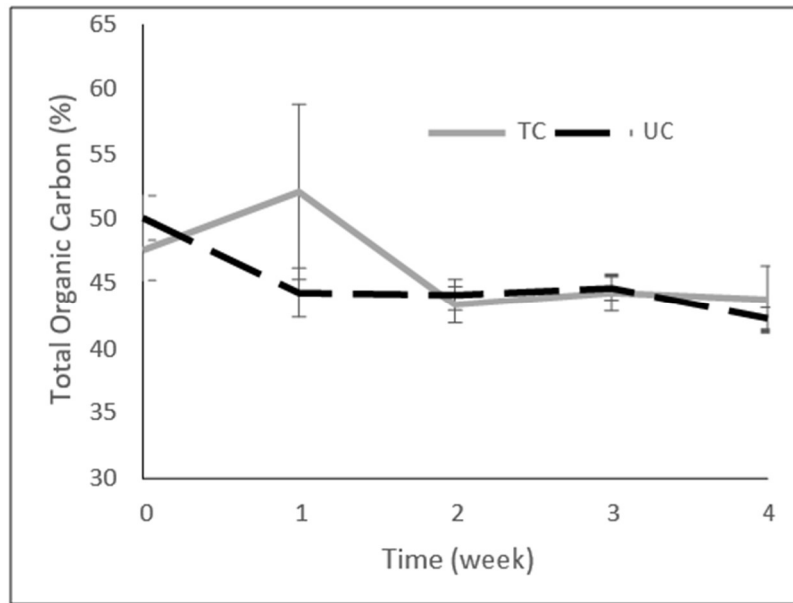


Fig. 4: Changes in TOC during 4 weeks of composting

#### Available phosphorus (AP)

Phosphorus exists and transforms into different forms during composting (Wei et al., 2015). Both TC and UC recorded a significant increase ( $p < 0.05$ ) in AP after the monitoring period. However, the presence of crude oil did not significantly influence ( $p > 0.05$ ) the profile. The AP increased by 74% and 59% for TC and UC respectively. The increase could be an indication of organic matter degradation, where organic phosphorus is mineralized to inorganic forms (Nguyen et al., 2020). Phosphorus is one of the macronutrients required for biodegradation, and the increase in AP could imply that the phosphorus may not be limiting when the compost is introduced into the contaminated soil.

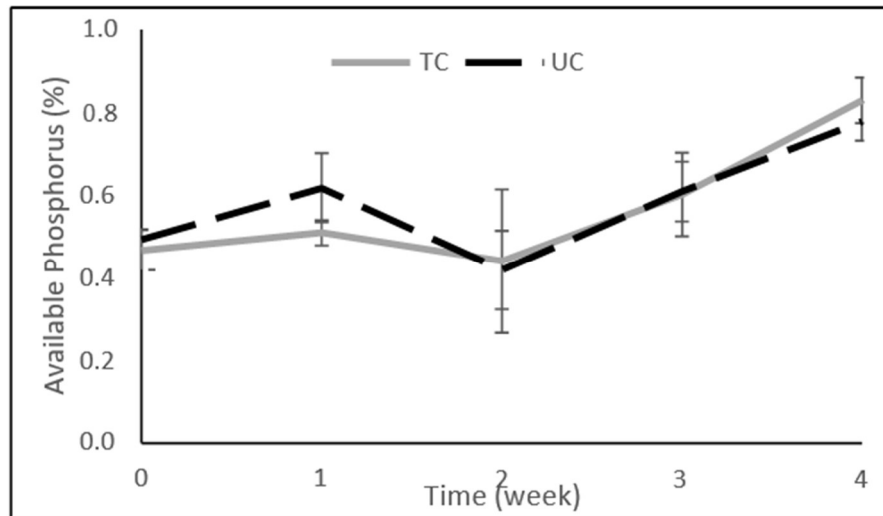


Fig. 5: Available phosphorus profile in 4 weeks of composting

### Total Petroleum Hydrocarbon (TPH)

The TPH removal in composting occurs through several mechanisms such as volatilization, leaching, and biodegradation. The lighter carbon could volatilize during turning or venting but heavier fractions are predominantly removed through microbial activities. Fig. 6 shows the carbon signals analysed by GC-FID for samples from TC and UC setups. Also, the chromatograms are sequentially arranged according to sampling weeks 0 - 4. The week 0 analysis representing initial parameters for TC (a) and UC (f) shows signals likely from petrogenic and non-petrogenic carbons (Gallego et al., 2022). Fig. 6(a) shows signals replica of petroleum carbon with non-petrogenic noise at a retention time of 10 mins, which is coincided with the signal from week 0 of the UC setup (Fig. 6(f)) at the same retention time. However, there was reduction in the carbons signals in week 1 for both TC (b) and UC (g), and this indicates that there could be concurrent utilization of both carbon types. It is a point to note that a rapid increase in temperature occurred in week 1, and utilization of the available carbon (petrogenic and non-petrogenic) could have caused the elevated temperature. As at week 2 (Fig. 6(c)) and thereafter, the non-petrogenic carbon signals were not significantly standing out, and this is also reflected in Fig. 6(h - j). Furthermore, it could be observed that the reduction in petrogenic carbon signals (Fig. 6 (a - e)) gradually increases the unresolved complex residuals (hump-like

curve), which could be intermediates from biodegradation. This implies that the addition of crude oil to the feedstock did not interfere significantly with the biodegradation of organic matter, and also in the process, the petroleum carbons were consumed.

The relative residual TPH in TC compared with UC shows a significant reduction ( $p < 0.05$ ) reduction in week 1 but insignificant change ( $p > 0.05$ ) thereafter (Fig. 7). After initial rapid utilization of petroleum carbon in week 1, the low change in residual TPH could be due to slow degradation of heavier petroleum carbon. After week 4, the TPH removal was 61.1%.

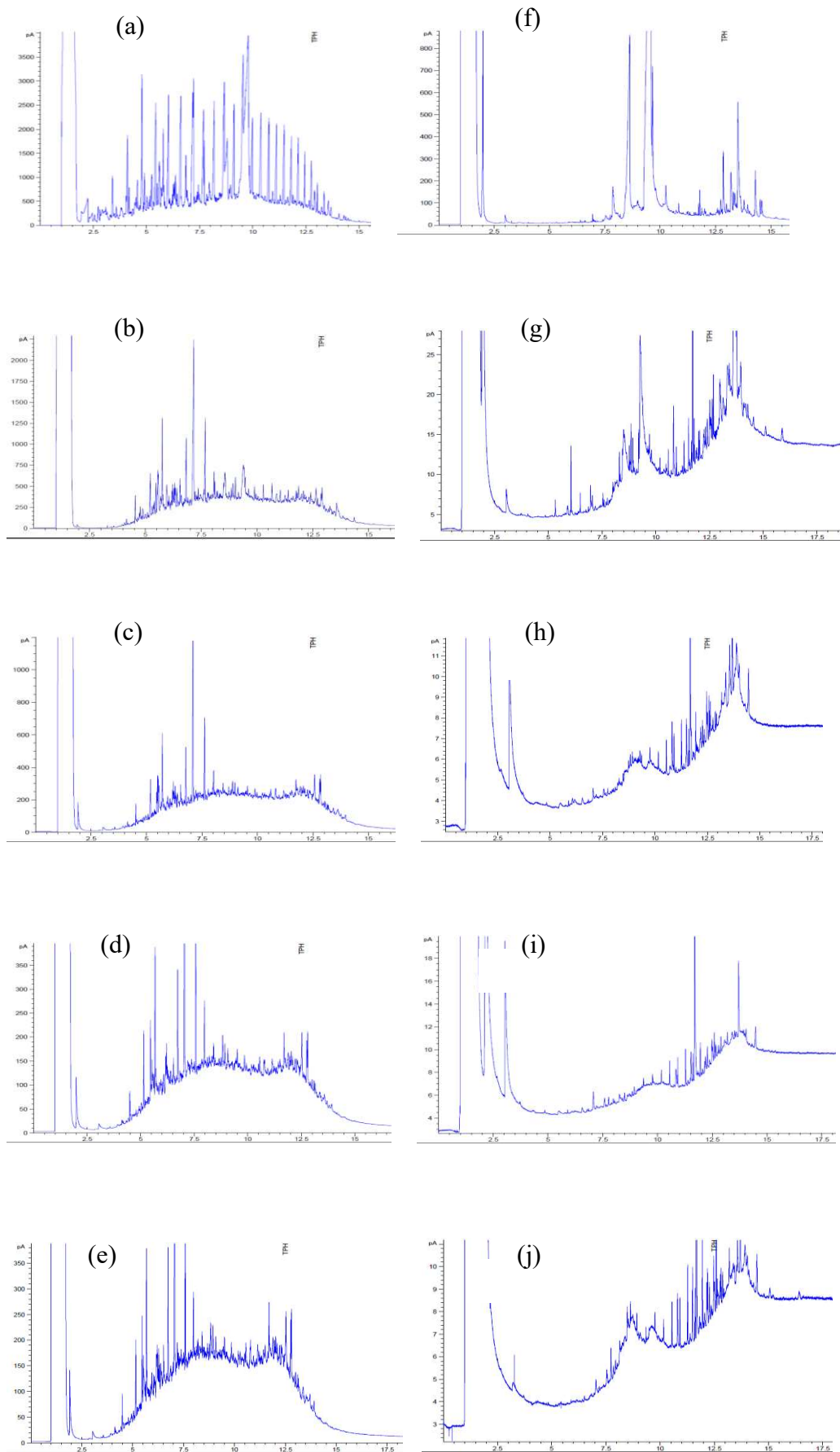


Fig. 6: Chromatograms of analysis for TC (a-e) and UC (f-j) sequentially from week 0 to 4

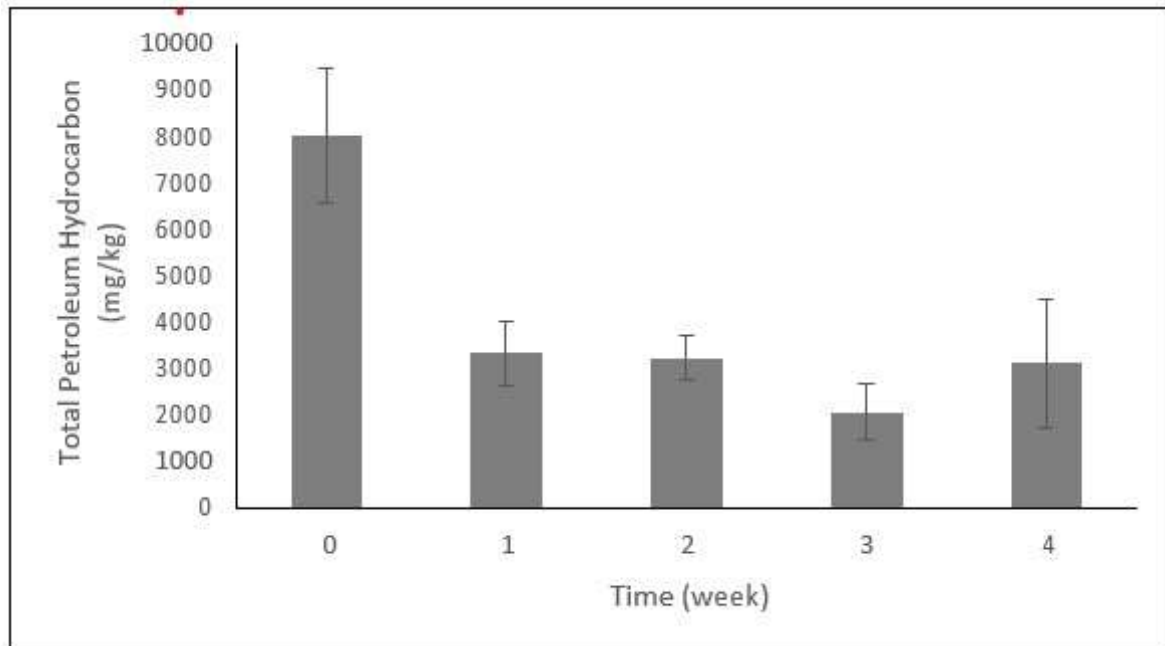


Fig. 7: Relative residual TPH recorded by TC compared with UC

### Hydrocarbon utilizing microbes

Bioconversion of organic matter in composting is carried out by various diversity of microbes. However, in the presence of petroleum hydrocarbon, some microbes become hydrocarbon utilizers by switching substrate, while some die off due to the induced toxicity of the petroleum (Aguelmous et al., 2019). Fig. 8 shows the fluctuation of hydrocarbon utilizers during composting. The hydrocarbon utilizing bacteria (HUB) significantly increased ( $p < 0.05$ ) in week 2 compared to the initial record for TC but recorded a decrease thereafter (Fig. 6a). On the other hand, UC did not record a significant change in HUB for the whole composting duration. At the end of week 4, the HUB for TC and UC was  $1.58 \times 10^8$  CFU/g and  $0.9 \times 10^8$  CFU/g. Similarly, there was a significant increase ( $p < 0.05$ ) in hydrocarbon utilizing fungi (HUF) for TC setup but UC recorded an insignificant increase ( $p > 0.05$ ). After the 4 weeks of monitoring, the HUF recorded for TC and UC were  $2.96 \times 10^6$  CFU/g and  $1.42 \times 10^6$  CFU/g. The significant increase in HUB and HUF recorded by TC could have been due to the inclusion of crude oil in the setup. Hazim and Al-Ani (2019) recorded a surge in hydrocarbon utilizing

microbes when the soil was treated with different proportions of petroleum hydrocarbon, and a higher proportion recorded higher utilizers. Above all, the HUB and HUF recorded in this study are higher than  $10^5$  CFU/g recommended for effective bioremediation (Koolivand et al., 2022).

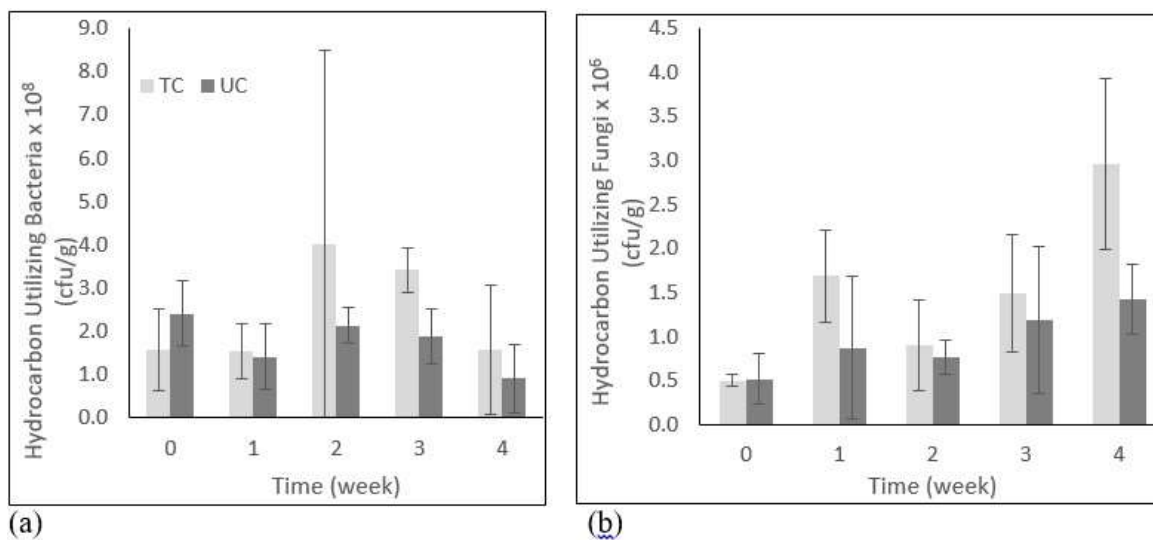


Fig. 8: Dynamic of hydrocarbon utilizing (a) bacteria and (b) fungi during the composting

## CONCLUSION

The possibility of enhancing the abundance of hydrocarbon-utilising microbes in compost for soil remediation was investigated. The inclusion of crude oil in the composting feedstock made a significant increase in the HUB and HUF above the minimum required for bioremediation and higher than the control, UC. However, the inclusion of the crude oil did not significantly alter the composting process, and it was observed from the GC chromatogram that there was concurrent losses of petrogenic and non-petrogenic carbon. Therefore, the simple method could be adopted locally for producing environmentally friendly soil remediation products at low cost.

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S

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