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BIO-ENHANCED REMOVAL OF HYDROCARBON CONTENTS FROM SPENT ENGINE OIL CONTAMINATED SOIL USING *Staphylococcus aureus* AND *Bacillus cereus* CO-CULTURE

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ABSTRACT

The study assessed the removal of total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbons (PAHs) from spent engine oil (SEO) contaminated soil through bioenhancement of bacteria isolated from SEO polluted soil. Sterilized soil was subjected to a three level of SEO contamination before the addition of sterilized biostimulants including powdered cow dung (CD), powdered cocoa pod husk (CPH) and compost (made from fresh CPH and CD). Bacterial inoculum being Staphylococcus aureus and Bacillus cereus co-culture (150 mL) was added to the mixture in polyethylene bags. It was a factorial experiment that was laid out in a completely randomized design (CRD). The TPH and PAHs were estimated in the first day, fifth week and the tenth week that the room incubation lasted. Results generated from the influence of biostimulants on TPH and PAHs degradation potential of the bacterial co-culture showed that degradation of the hydrocarbon contents was significantly enhanced ($p < 0.05$). At the tenth week, compost enhanced the most TPH reductions (315 and 380 mg kg^{-1}) compared with other biostimulants on 5% and 15% SEO contamination levels, respectively. Compost equally enhanced the most PAHs reductions (48.8, 39.6 and 94.6 mg kg^{-1}) compared with other biostimulants on 5%, 10% and 15% SEO contamination levels respectively. However, the quantity of SEO contents degraded was significantly higher in the bioaugmented and biostimulated soil samples compared with the control employed. The technology adopted in this study can be effectively employed for the bioremediation of petroleum hydrocarbon related pollution.

Key words: bioremediation, biostimulants, bacterial co-culture, TPH and PAHs

INTRODUCTION

Increasing demand for petroleum products in the world over has brought a substantial increment in its extraction and final processing (Obi *et al.,* 2016). According to Bhattacharyya and Shekdar (2003), this substantial increment has brought about a huge waste accruable from its usage. Soil pollution attributable to petroleum hydrocarbons can loiter on for so many years thereby affecting the physicochemical quality of the soil in the process (Umoren *et al.,* 2019). The adoption of bio-remediation for the removal of hydrocarbon related pollutants in the environment has become popular owing to its promising exploits. Bioremediation technology has attracted positive attention due to its cost effectiveness and environmentally friendly nature (Dinkla *et al.,* 2001). It has been reported in numerous studies that catabolic capabilities of certain hydrocarbonoclastic microorganisms like bacteria, fungi and algae make it possible for them to facilitate the degradation of petroleum hydrocarbons (Dean-Ross *et al.,* 2002; Wang *et al.,* 2011; Maiti *et al.,* 2012; Badr El-Din *et al.,* 2014). These microbes have the required enzymatic capacity that makes possible their utilisation of the pollutant hydrocarbons as their sole sources of carbon and energy (Panda *et al.,* 2013).

In Nigeria, spent engine oil (SEO) has instituted a vital source of pollution owing to its disposal into aquatic and terrestrial ecosystems indiscriminately (Osaigbovo *et al.,* 2013). According to these authors, contamination of unoccupied plots of land and agricultural farmlands with hydrocarbon-based products has become an extensive problem than crude oil pollution particularly in the urban areas. Polyaromatic hydrocarbons (PAHs), which are inseparable from SEO has been documented to have toxic, mutagenic, teratogenic, certain immunological, carcinogenic, reproductive, fetotoxic and genotoxic effects when found in many environmental media (Clemente *et al.,* 2001; Yerima *et al.,* 2013; Umana *et al.,* 2017).

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Due to the established damaging health risks that PAHs can exert on the environment and humans, its complete elimination from environmental media necessitates approaches that are viable and affordable (Adeleye *et al.,* 2020). The adoption of bacterial co-cultures for possible bioremediation of SEO contaminated soil is in line with the reports of Ogunbayo *et al.* (2012) together with Kawo and Faggo (2017) on the ability of bacterial co-cultures to enhance better and faster degradation of hydrocarbons than individual pure cultures. It is based on these backgrounds that this current study was piloted with a view to assessing the elimination of the hydrocarbon contents of SEO contaminated soil through bioenhancement of *Staphylococcus aureus* and *Bacillus cereus* co-culture isolated from a typical SEO-polluted soil.

MATERIALS AND METHODS

Description of the Study Area

This study was conducted at the Department of Soil Science, Faculty of Agriculture, Federal University Dutse (Latitude 11° 46′ 39″ North and Longitude 9° 20′ 3″ East) Jigawa State, Nigeria (Adeleye *et al*., 2020).

Processing of Biostimulants

All the processed biostimulants: powdered cow dung (CD), powdered cocoa pod husk (CPH) and compost (fresh CPH and CD compost) used in this study were derived as earlier reported by Adeleye *et al.* (2020). Fresh CPH was cut into smaller pieces less than 5 cm so as to ensure a surface area suitable for its active decomposition as done by Komolafe *et al.* (2021).

Soil Collection and Processing

Soil (250.0 kg) at the depth of 25.0 cm that had no history of any form of pollution (Nkereuwem *et al.,* 2020), was collected with the aid of soil auger using grid method of soil sampling from four spots at the Teaching and Research Farm, Department of Soil Science, Federal University Dutse. It was thereafter subjected to air drying with a view to forming a composite sample. As established by Soretire *et al.* (2017), the bulked soil was sieved with 2.0-mm mesh size. The soil was autoclaved at 121°C for 15 min. to expunge extraneous influence of unwanted microbial life. One and a half (1.5) kg of the sterilized soil was then poured into 36 polyethylene bags each. Fresh spent engine oil (SEO) collected from a service pit in Dutse Mechanic Village was added to the bagged soil. Specifically, varying levels of the SEO (75-, 150- and 225-mL weight/weight), representing 5, 10 and 15% contamination levels, respectively were added separately to the bagged soil. The soil-SEO mixtures were exhaustively variegated, and left undisturbed for 14 days for volatilization of the toxic components of the SEO as reported by Abioye *et al.* (2012) and Agbor *et al.* (2015).

Isolation and Identification of Bacterial Co-Culture Used for Bioaugmentation

The procedures used by Adeleye *et al*. (2019) were employed for the isolation and identification of the bacteria (*Staphylococcus aureus* and *Bacillus cereus*) used as a co-culture for bioaugmentation in this study.

Experimental Design and Biodegradation

The design was a 4 × 3 factorial experiment laid out in completely randomized design (CRD) with three replications. The two factors were: (a) biostimulants at four levels including the control (B_0, having no biostimulant), B_1 being compost, B_2 being powdered CPH, and B_3 being powdered CD; and (b) SEO at three levels of 75 (5%), 150 (10%) and 225 mL (15%), designated S_1, S_2 and S_3, respectively. This experimental layout implies that the bacterial co-culture (150 mL) in each of the polyethylene bags containing the SEO-polluted soil was bioaugmented, apart from the control that did not receive such bioaugmentation and biostimulation.

All the polyethylene bags were subjected to incubation at room temperature for 70 days (Chorom *et al.,* 2010). As established by Ayotamuno *et al.* (2006), the contents of each polyethylene bag were tilled twice a week for aeration. Maintenance of moisture content was equally done twice a week by the addition of 6.0 mL sterile distilled water (Abioye *et al.,* 2012).

Determination of the Physico-Chemical Properties of Soil and Biostimulants

Following the procedures used by Adeleye *et al.* (2020), available phosphorous, pH in water, electrical conductivity (EC), moisture content, total nitrogen, organic carbon, texture and other related properties of the soil and biostimulants were determined.

Determination of Total Petroleum Hydrocarbon and Polycyclic Aromatic Hydrocarbons

Total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) were determined with the aid of gas chromatograph flame ionization detector (GC-FID) following the United States Environmental Protection Agency (USEPA) 1850C method described by USEPA (2003).

Data Collection and Analysis

Periodic sampling for the estimations of TPH and PAHs was done at the commencement, 5th week and 10th week of the experiment. All data were subjected to analysis of variance by the procedure of General Linear Model of GenStat Version 17.0. Significant means were separated using Duncan's new multiple range test.

RESULTS

The physical and chemical properties of the soil and biostimulants are depicted in Table 1. The results obtained from the mechanical analysis of the soil showed it as sandy loam. The total sum of exchangeable bases of the soil, compost, powdered CPH and powdered CD employed in this study recorded 3.51, 221.7, 166.15 and 82.1 cmol kg^{-1} respectively.

Table 1: Physicochemical properties of the soil and biostimulants

Properties	Soil	Powdered CD	Powdered CPH	Compost
Ash content (%)	Nil	68.80	23.00	65.00
Available phosphorous (mg kg^{-1})	11.02	1.20	0.08	1.48
pH $_{(water)}$	6.50	8.15	7.60	9.45
Moisture content (%)	2.04	7.30	11.11	2.00
Organic carbon (%)	0.49	41.55	33.40	48.25
Total nitrogen (%)	0.06	2.85	2.65	5.85
EC (dS cm^{-1})	0.92	8.10	6.42	8.86
Exchangeable bases (cmol kg^{-1})				
Sodium	0.58	0.40	0.10	0.50
Calcium	1.82	0.20	1.60	4.80
Magnesium	0.92	1.50	2.45	3.24
Potassium	0.19	80.00	162.00	213.16
CEC	3.51	82.10	166.15	221.70
Particle size (g kg^{-1})				
Silt	320.00	Nil	Nil	Nil
Clay + silt	420.00	Nil	Nil	Nil
Sand	580.00	Nil	Nil	Nil
Clay	100.00	Nil	Nil	Nil
Textural class	Sandy loam	Nil	Nil	Nil

CD is cow dung, CPH is cocoa pod husk, EC is electrical conductivity (dS stands for deciSiemens), CEC is cation exchange capacity

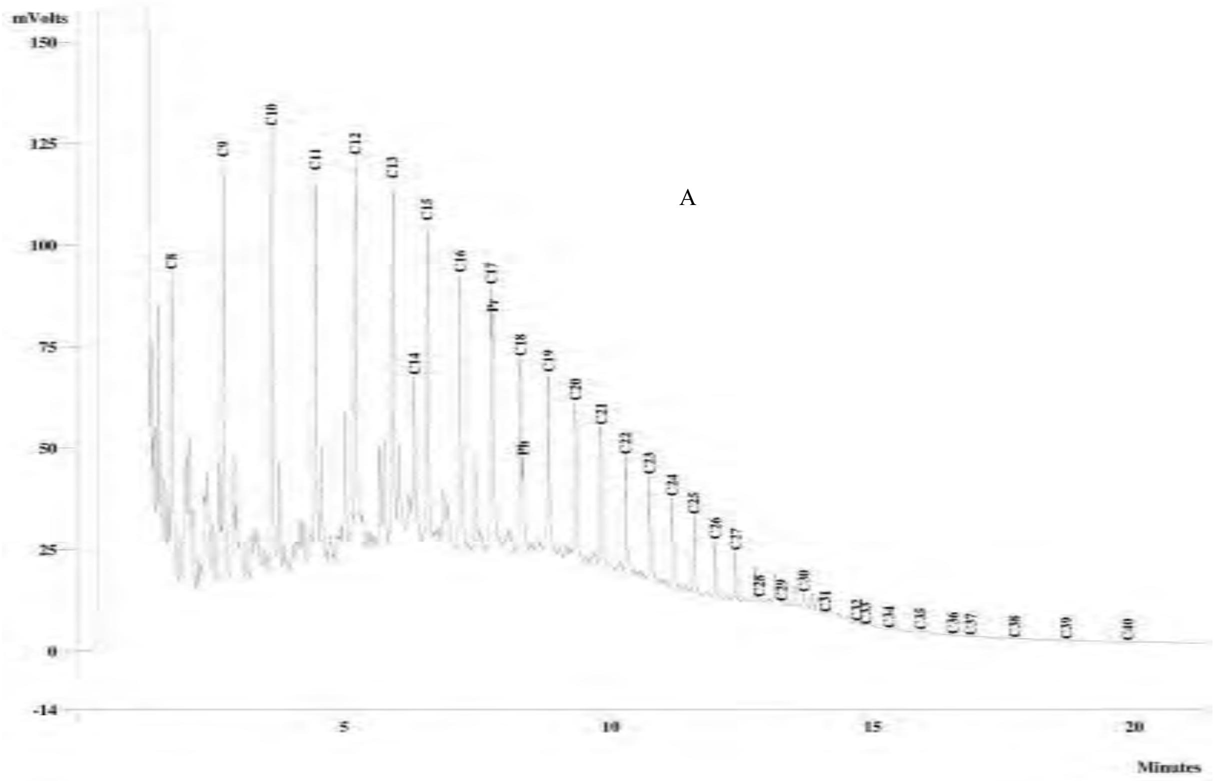
TPH and PAHs Reference Point Concentrations in Different Levels of SEO Contaminated Soil

The varying concentrations of TPH and PAHs in successive levels of SEO contaminated soil before bacterial degradation are depicted in chromatograms shown in Figures 1 and 2. Contaminated soil with 5, 10 and 15% SEO recorded TPH concentrations of 9,934 mg kg^{-1}, 10,016 mg kg^{-1} and 10,379 mg kg^{-1} respectively (Figure 1) having the presence of carbons (C) ranging from C_8 to C_{40}. Similarly, contaminated soil with 5, 10 and 15% SEO recorded PAHs concentrations of 1065.98, 1726.59 and 1865.63 mg kg^{-1}, respectively (Figure 2).

TPH Biodegradation Potential of *Staphylococcus aureus* and *Bacillus cereus* Co-Culture

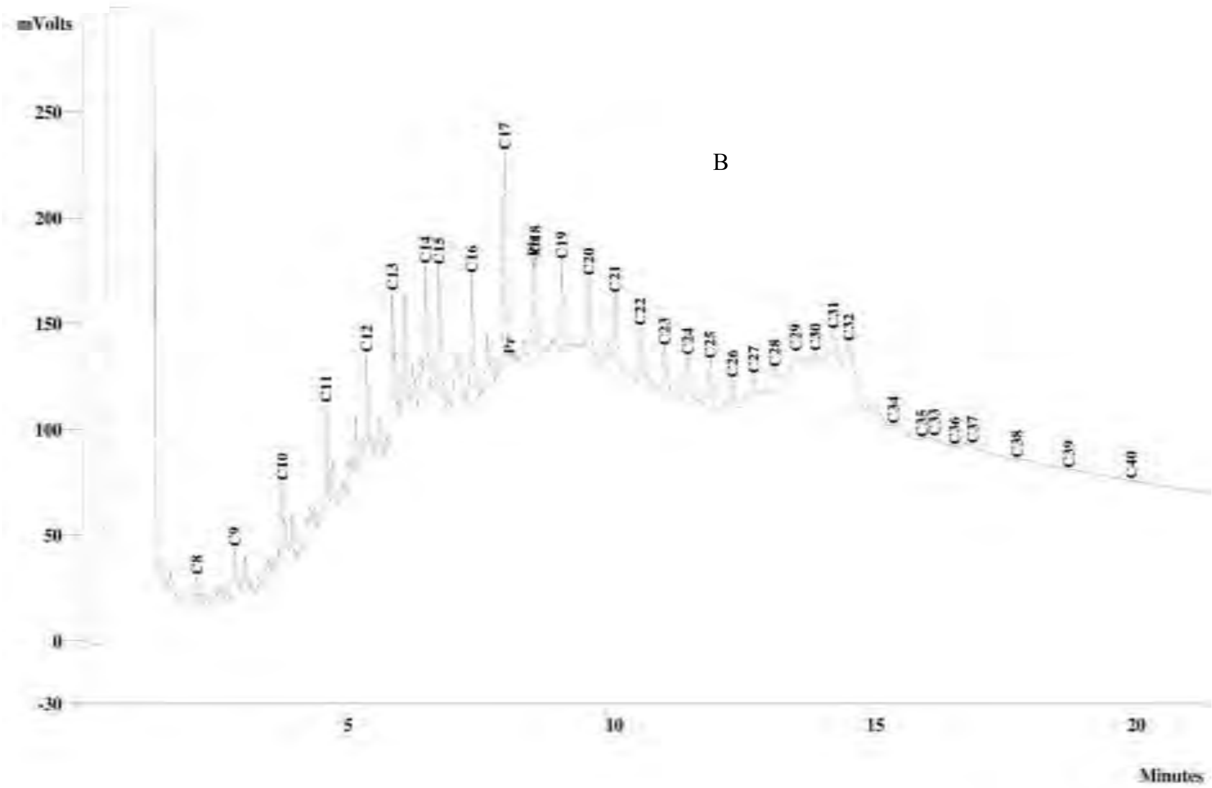
On the TPH degradation potential of *S. aureus* and *B. cereus* co-culture, these biostimulants significantly ($p < 0.05$) enhanced TPH degradation at the 5th week. Compost enhanced the most TPH reductions (836, 560 and 3,507 mg kg^{-1}) compared with other biostimulants used on 5, 10 and 15% contamination levels of SEO, respectively (Figure 3). Compost sustained this trend at the 10th week as it had the most reductions (315 and 380 mg kg^{-1}) compared with other biostimulants on 5 and 15% SEO levels, respectively. However, powdered CPH showed the most TPH reduction (212 mg kg^{-1}) compared with other biostimulants on 10% SEO level (Figure 4).

mVolts



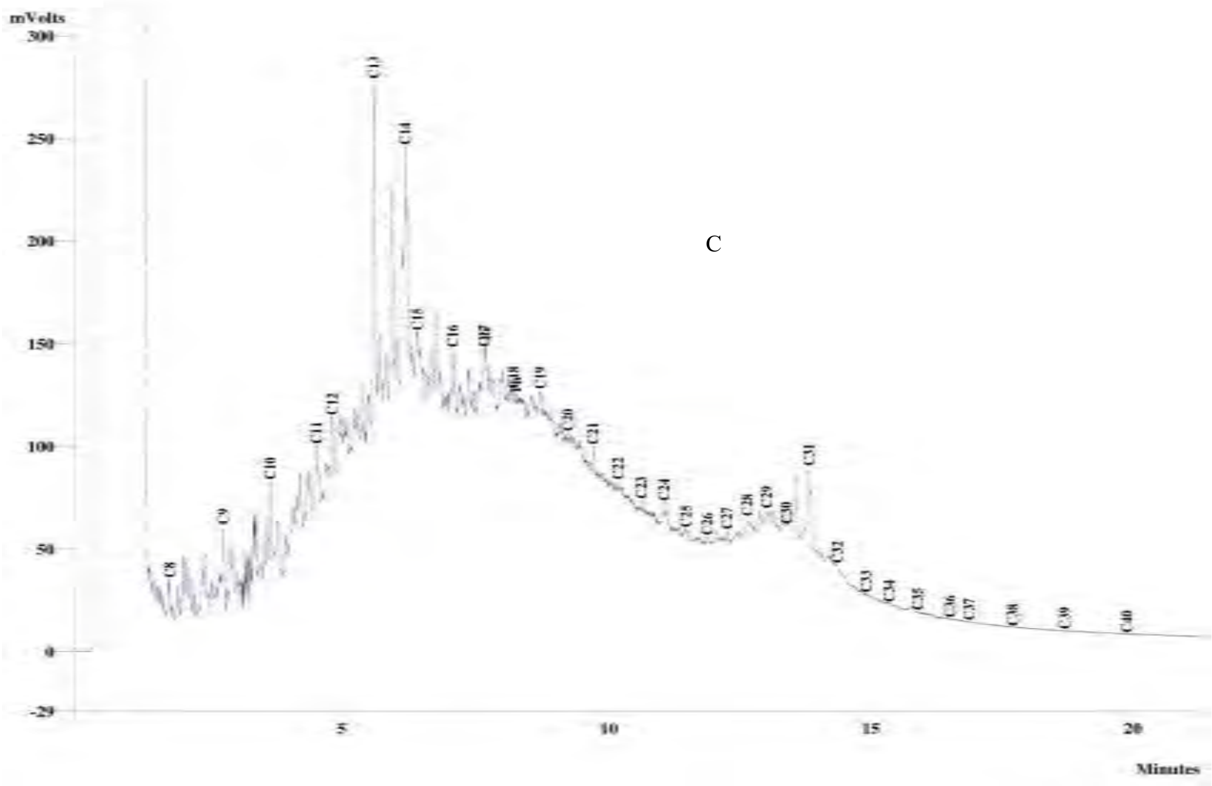
Minutes

mVolts



Minutes

mVolts



Minutes

Figure 1: Chromatograms showing total petroleum hydrocarbon contents of *A* to represent 5% spent engine oil (SEO) contaminated soil, *B* to represent 10% spent engine oil (SEO) contaminated soil, and *C* to represent 15% spent engine oil (SEO) contaminated soil before bacterial degradation

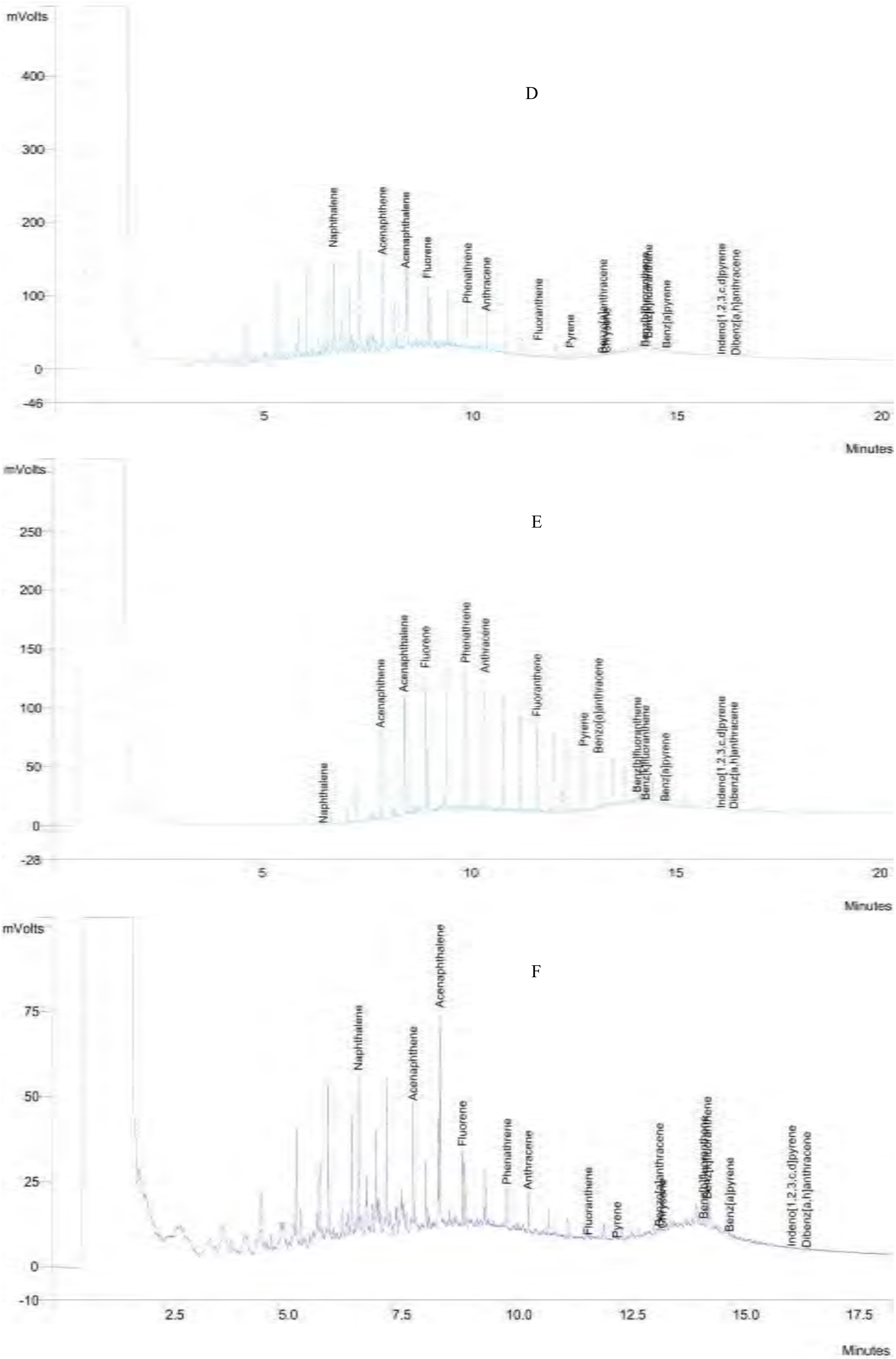


Figure 2: Chromatograms showing polycyclic aromatic hydrocarbon contents of *D* to represent 5% spent engine oil (SEO) contaminated soil, *E* to represent 10% SEO contaminated soil, and *F* to represent 15% SEO contaminated soil before bacterial degradation. **Note:** Vertical and horizontal axes are as labelled in Figure 1.

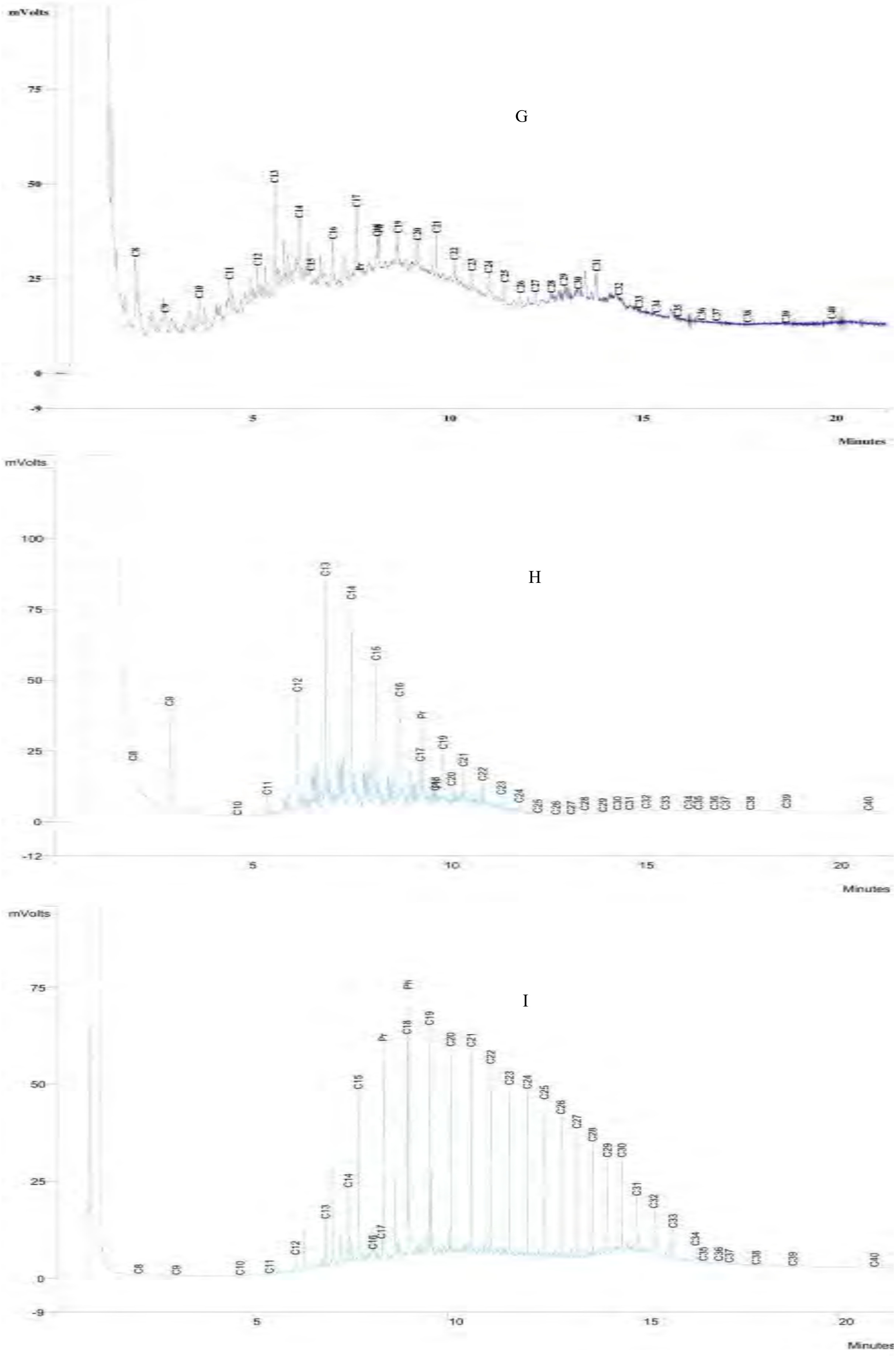


Figure 3: Chromatograms showing degraded total petroleum hydrocarbon fractions due to the *Staphylococcus aureus* and *Bacillus cereus* co-culture in the soil amended with compost at the 5th week of incubation, with *G* representing 5% spent engine oil (SEO) contamination level, *H* representing 10% SEO contamination level, and *I* representing 15% SEO contamination level. **Note:** Vertical and horizontal axes are as labelled in Figure 1.

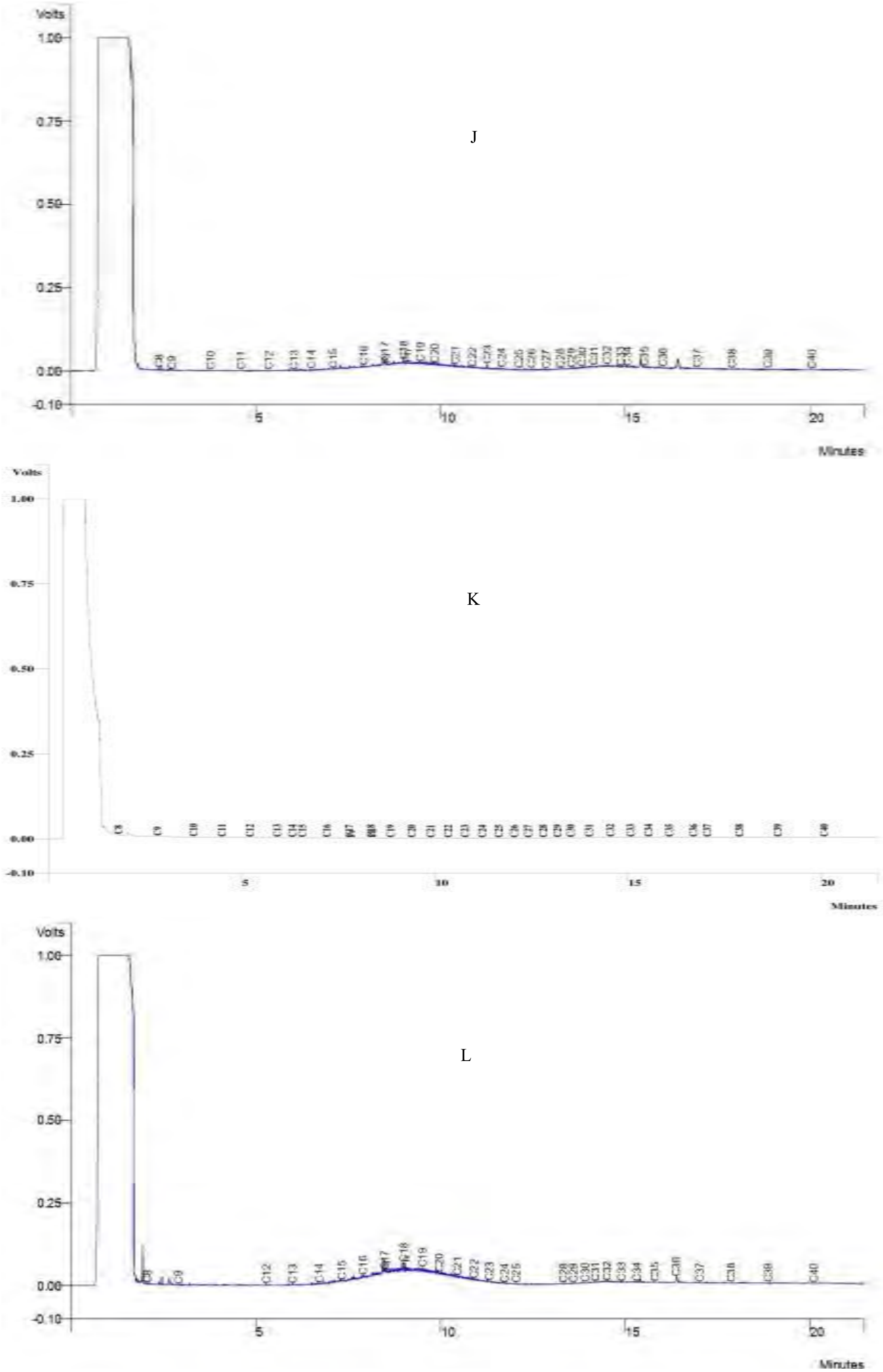


Figure 4: Chromatograms showing degraded total petroleum hydrocarbon fractions due to the *Staphylococcus aureus* and *Bacillus cereus* co-culture in the soil at the 10th week of incubation, with *J* representing 5% spent engine oil (SEO) contamination level amended with compost, *K* representing 10% SEO contamination level amended with powdered cocoa pod husk, and *L* representing 15% SEO contamination level amended with compost. **Note:** Vertical and horizontal axes are as labelled in Figure 1.

PAHs Biodegradation Potential of *Staphylococcus aureus* and *Bacillus cereus* co-culture

At the 5th week, the biostimulants significantly ($p < 0.05$) influenced bacterial degradation of PAHs in the SEO-contaminated soil. Compared with other biostimulants, powdered CPH only enhanced the most degradation (129.1 mg kg^{-1}) aided by *Staphylococcus aureus* and *Bacillus cereus* co-culture on 5% SEO contamination level while compost enhanced the most PAHs reductions (92.7 and 157.5 mg kg^{-1}) on 10 and 15% SEO levels respectively (Figure 5). However, at the tenth week, compost enhanced the most reductions (48.8, 39.6 and 94.6 mg kg^{-1}) in PAHs compared with other biostimulants on 5, 10 and 15% SEO contamination levels respectively (Figure 6).

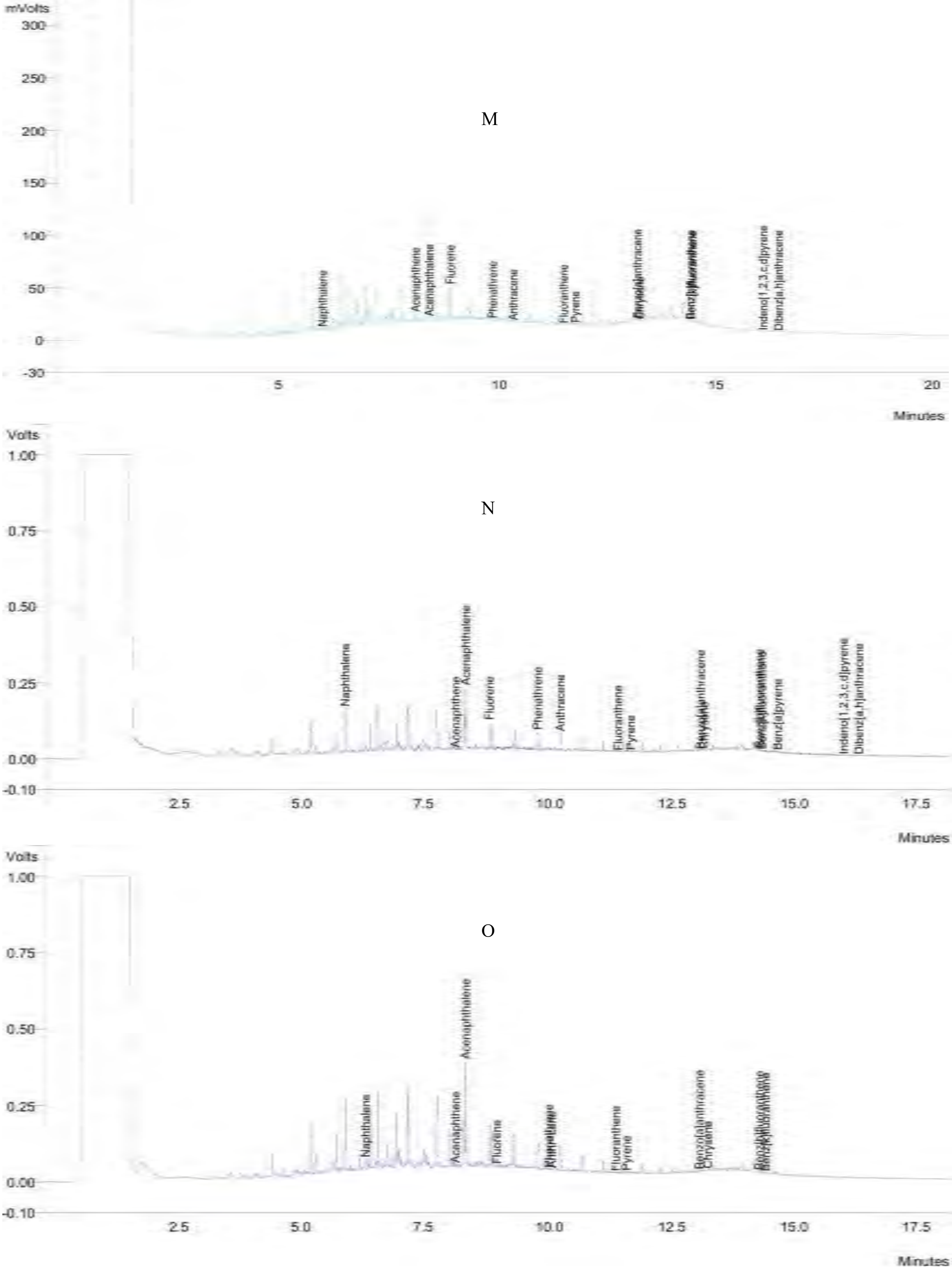


Figure 5: Chromatograms showing degraded polycyclic aromatic hydrocarbon (PAH) fractions due to the *Staphylococcus aureus* and *Bacillus cereus* co-culture in the soil at the 5th week of incubation, with *M* representing PAH fractions at 5% spent engine oil (SEO) level amended with powdered cocoa pod husk, *N* representing total petroleum hydrocarbon fractions at 10% SEO level amended with compost, and *O* representing PAH fractions at 15% SEO level amended with compost. **Note:** Vertical and horizontal axes are as labelled in Figure 1.

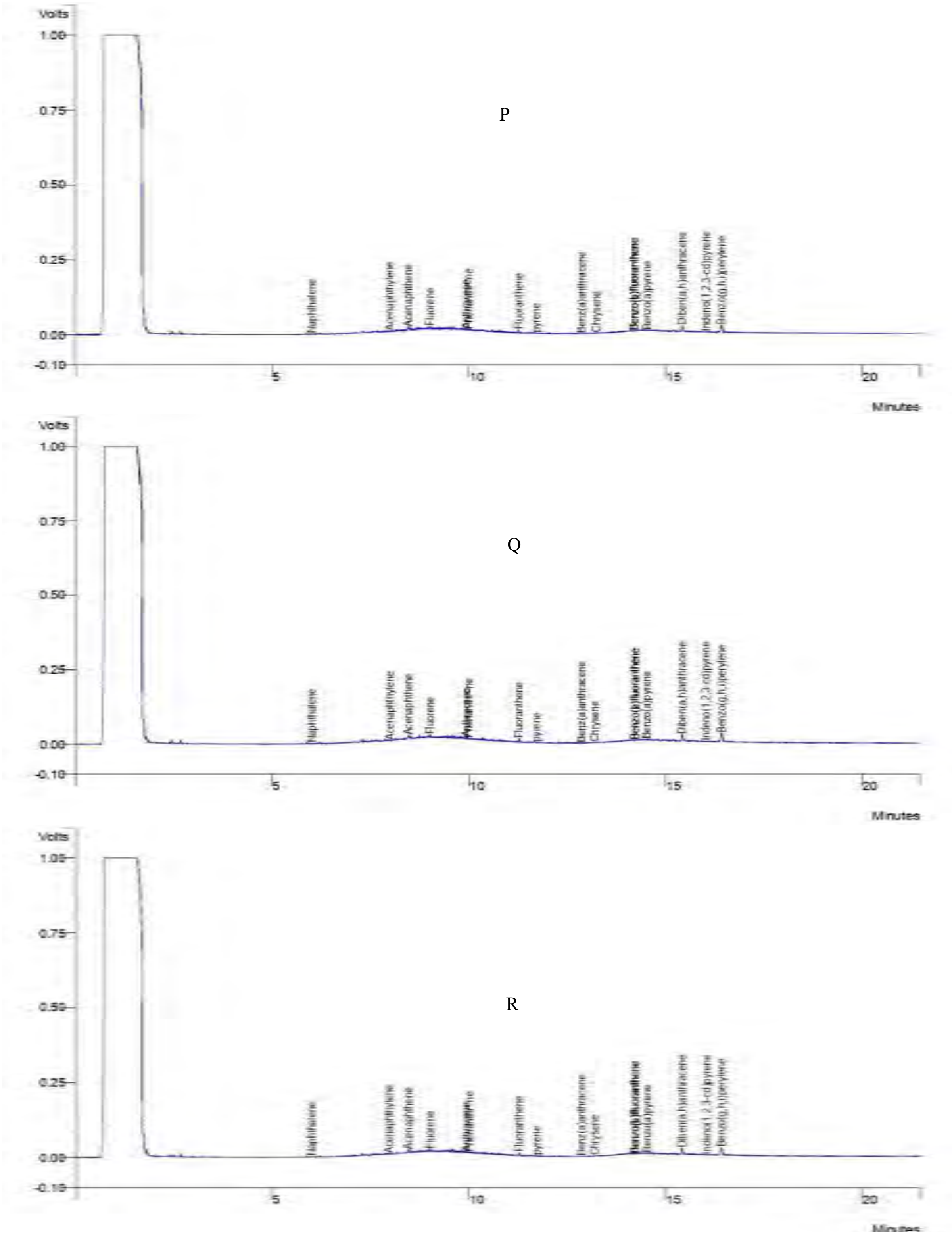


Figure 6: Chromatograms showing degraded polycyclic aromatic hydrocarbon fractions due to the *Staphylococcus aureus* and *Bacillus cereus* co-culture in the soil at the 10th week of incubation, with *P* representing 5% spent engine oil (SEO) contamination level amended with compost, *Q* representing 10% SEO level amended with compost, and *R* representing 15% SEO level amended with compost. **Note:** Vertical and horizontal axes are as labelled in Figure 1.

DISCUSSION

The soil employed for the biodegradation assay in this study had C:N ratio of 8.2. This is a very low C:N ratio suitable for effective biodegradation of SEO in the soil. Consequently, the addition of biostimulants as a source of nutrients required for bacterial metabolism of the contaminant became imperative. Similar observation and submission has been made by Osaigbovo *et al.* (2013). The pH of the soil showed that it is slightly acidic. However, pH obtained from the compost, powdered CD only, and powdered CPH only ranged from slightly alkaline to alkaline. This alkalinity can be related to the high levels of exchangeable bases, which are characteristic of the biostimulants used in this study. The cation exchange capacity (CEC) of the soil (3.51 cmol kg^{-1}) was very low compared to the biostimulants especially compost (221.70 cmol kg^{-1}) that had the highest value. In reference to Aduayi *et al.* (2002), this infers that the soil was not fertile enough for any biodegradation assay thus making the addition of biostimulants obligatory.

The assessment of TPH in an environment polluted with petroleum hydrocarbons had been reported by Schwartz *et al.* (2012). The detection of naphthalene, benzo[a]anthracene, benzo[a]pyrene, fluoranthene and other notable aliphatic and aromatic hydrocarbons in the SEO-contaminated soil samples analyzed in this study is in line with the report of Irwin *et al*. (1997) and Yerima *et al*. (2012) on their presence in a typical SEO. The results recorded on the succinct estimations of TPH and PAHs by GC-FID in the soil samples analysed in this study have clearly demonstrated the capability of the instrument to measure such in environmental media containing these petroleum hydrocarbons (Wenning and Martello, 2014; Adeleye *et al*., 2020).

The significant TPH reductions enhanced by the biostimulation of *Staphylococcus aureus* and *Bacillus cereus* co-culture with organic amendment can be attributed to effective supply of nutrients needed for their optimal metabolism and eventual TPH utilization. These results agree with those of Atagana (2008), Yerima *et al*. (2011), Chikere (2012), Dadrasnia and Agamuthu (2013), Kamaluddeen *et al.* (2016); Oyedele *et al.* (2016), regarding the biostimulatory influence of organic amendments in enhancing the ability of microorganisms to attain TPH reductions. The depicted reductions in TPH recorded in this study are in line with the results of Maryam and Ujah (2016) alongside Kawo and Faggo (2017) who reported decrement in the peaks of chromatograms as an indication of bacterial degradation of petroleum hydrocarbons.

The results recorded in this study are in agreement with the submissions of Van Gestel *et al.* (2003) together with Ling and Isa (2006), about the ability of organic fertilizers to improve soil structure, leading to a diminishing bulk density and lower rate of PAHs volatilization. Again, these results are in concord with the submission of USEPA (1994) regarding the ability of indigenous bacterial communities in contaminated soil to effect faster PAHs degradation when enhanced adequately as their adaptation to such polluted environment would not be hindered. These results are in support of the submission of Pothuluri and Cerniglia (1994) concerning the key role that autochthonous microbial communities play in expunging PAHs from contaminated environments owing to their cost effectiveness and capability to attain complete remediation.

As depicted in the chromatograms, the enhancement of *Staphylococcus aureus* and *Bacillus cereus* co-culture with compost and powdered CPH yielded significant reductions in the PAHs components most especially benzo (a) anthracene known to be very toxic and causing serious health problems. Similar observation regarding the reduction of benzo (a) anthracene using the combination of bacteria and organic amendments has been reported by Yerima *et al.* (2012). In agreement with the results obtained in this study, Ezenne *et al.* (2014), equally reported the efficiency of poultry droppings in the removal of TPH and PAHs contents from petroleum hydrocarbon impacted soil in which application rate of the biostimulant and the contaminated site played a vital role in achieving the documented significant removal.

CONCLUSION

In this study, bacterial degradation of TPH and PAHs was significantly enhanced by the addition of biostimulants in all the SEO contaminated soils at varying contamination levels studied. However, the quantity of SEO degraded was significantly higher in the bioaugmented and biostimulated soil samples compared with the control. The compost generated from the composting of CPH and CD and other biostimulants in this study played a significant role in the biodegradation of SEO and can be effectively adopted for the bioremediation of petroleum hydrocarbon related pollution.

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CONFLICT OF INTERESTS The authors declare that they have no conflict of interests regarding this study.

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