



Cattle rumen content, rice husk and cow horn biochars as amendments for enhanced remediation of petroleum-hydrocarbon-contaminated soil: physicochemical characterization and performance evaluation

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Abstract

Petroleum hydrocarbon contamination of soil is a major environmental challenge in crude-oil-producing areas. Agricultural and abattoir wastes are promising low-cost soil amendments but remain greatly underutilized. In this study, cattle rumen content (CRC), rice husk biochar (RHB) and cow horn biochar (CHB) were converted into soil amendments and applied to petroleum-hydrocarbon-contaminated soils. Physicochemical characterization showed that CaO, SiO₂ and SO₃ were predominant in CRC, RHB and CHB, respectively. Quantitative phase X-ray diffraction gave 65 wt% and 56 wt% graphite in RHB and CHB, respectively, while a chaoite-like phase was tentatively observed in CRC at about 28 wt%. Brunauer–Emmett–Teller surface areas were 356.0, 254.9 and 185.9 m² g⁻¹ for CHB, RHB and CRC, respectively, with all materials exhibiting mesoporous structures (pore diameters 2.55–2.65 nm). RHB had a higher ash content (50%) than CHB (16%) and CRC (12%). When the amendments were applied to contaminated soil with an initial total petroleum hydrocarbon (TPH) concentration of 1307 mg kg⁻¹ for 60 days, amendment type and application rate affected TPH reduction. RHB and CHB reduced TPH by 94% and 93%, respectively, at a 20% application rate, whereas CRC reduced TPH by 98% at a 10% application rate. Hydrocarbon-utilizing bacteria increased with time, with CRC producing the strongest stimulation. The study highlights the practical value of converting agricultural and abattoir wastes into effective soil remediation amendments for hydrocarbon-impacted areas.

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1. Introduction

Soil contamination by petroleum hydrocarbons (PHCs) occurs through pipeline leakages and mishaps during exploration,

exploitation, transport and storage [1]. PHC spills destroy soil structure, diminish soil porosity, deplete nutrients and damage vegetation, resulting in reduced crop yield [1, 2] and decreases in microbial diversity and populations [3, 4]. PHCs pose health and environmental risks because of their toxicity and bioaccumulation [5, 6]. Natural attenuation relies on natural processes to reduce PHC concentrations in soil, but the process may take

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several years to complete [7]. Several technologies, including physical remediation and chemical treatments, have been used in the cleanup of PHC-contaminated soil, but inherent shortcomings such as high cost, secondary pollution from byproduct generation and harm to soil microorganisms limit their application [8, 9].

Bioremediation is an effective, economical and eco-friendly approach that has become popular for mineralizing organic contaminants in soil environments [10]. Because of its simplicity, cost-effectiveness and environmental friendliness, it has been used for remediation of PHC-contaminated soils [11]. However, this technique is limited by soil nutrient status, pH and pollutant concentration [12]. Hence, attention has moved toward amendment-enhanced methods that improve soil conditions and stimulate indigenous microbial communities [13–15]. Furthermore, the use of crop residues and waste biomass in the production of cost-effective sorbents for PHCs is increasing [4, 16]. Biochars are high-carbon solids produced by high-temperature pyrolysis under anoxic or anaerobic conditions and have documented soil restoration potential [1, 17–20]. Their large surface areas, porosity and surface functional groups make them useful for pollutant immobilization and as habitats for microorganisms [21–23]. Rice husk, a byproduct of rice milling, is one biomass that can be converted to biochar for such purposes. Cow horn contains microbial-stimulating nutrients [24, 25], but is largely underutilized as a feedstock for biochar materials, while cattle rumen content is composed mainly of plant fibers with various microbial communities [26].

Apart from contaminant removal, biochar may also sequester carbon and improve microbial diversity in PHC-contaminated soils [27]. While rice husk biochar is well documented for remediating PHC-contaminated soils [2, 12, 28], systematic variation of its application rate to obtain an optimal dosage remains poorly explored. Cow horn, on the other hand, is an underutilized feedstock biomass for biochar production and represents a novel application for PHC-contaminated soils, with a previous study examining cow horn powder [29]. Furthermore, cattle rumen as a potential biostimulant is mechanistically distinct from biochars, and its application in the remediation literature remains scarce. This study therefore fills these gaps by characterizing cattle rumen content, rice husk biochar and cow horn biochar using compositional and surface analytical techniques, and by evaluating their effectiveness in enhancing degradation of total petroleum hydrocarbons (TPHs) in contaminated soil at application rates of 5%, 10%, 15% and 20% over a 60-day period.

2. Materials and methods

2.1. Sample collection and preparation

Cattle rumen content (CRC) was collected from the abattoir in Sapele Road Market, Effurun, Delta State, Nigeria. The material was air-dried for five weeks, ground using a mechanical blender and sieved through a 2 mm mesh to obtain a uniform sample (Figure 1). Rice husk samples were collected from a local rice miller in Ado Ekiti, Ekiti State, Nigeria, and air-dried

(Figure 2). Cow horns were also sourced from the same abattoir in Sapele Road Market, Effurun, Delta State, Nigeria, washed, rinsed, air-dried for 72 h and cut into bits (Figure 3).

2.2. Pyrolysis of feedstock materials

Pyrolysis was performed using a laboratory batch-scale method. Approximately 100 g of rice husk and cow horn samples were separately weighed into airtight stainless steel containers and placed in a muffle furnace. The furnace temperature was set at 500 °C, and the feedstock materials were pyrolyzed for 3 h at a heating rate of 10 °C min⁻¹ under limited oxygen. The biochar obtained was ground in a mechanical blender and sieved through a 2 mm mesh. The yields of rice husk biochar (RHB) and cow horn biochar (CHB) were 16.6% and 31.2%, respectively.

2.3. Characterization of biochar materials and cattle rumen content

Elemental and oxide composition analyses were determined by X-ray fluorescence (XRF) using a Rigaku ZSX Primus II wavelength-dispersive XRF spectrometer. Surface morphology was examined using a Phenom ProX scanning electron microscope (SEM; Phenom-World, Eindhoven, Netherlands). X-ray diffraction (XRD) analysis was performed under theta-theta geometry (4–75° 2θ, step size 0.026261°, 45 kV, 40 mA), while quantitative phase analysis used the reference intensity ratio (RIR) method. Brunauer–Emmett–Teller (BET) surface area and density functional theory (DFT) pore size distribution were determined using a Quantachrome Nova 4200e instrument (Quantachrome Instruments, USA). Fourier transform infrared (FTIR) spectroscopy was performed using an Agilent Cary 630 spectrometer to identify functional groups of the amendment materials. Ash content was determined gravimetrically as the percentage mass loss on ignition relative to the initial dry sample mass [30]. The pH of each amendment was determined using a 1:1 amendment:water mixture with a handheld pH meter [31].

2.4. Soil contamination and remediation experiment

2.4.1. Soil preparation

Soil samples were collected from pristine areas within the Federal University of Petroleum Resources Effurun campus, air-dried and sieved through a 2 mm sieve. Crude oil was collected from a flow station in Bayelsa State. The soil pH was determined in a 1:1 soil:water mixture using a handheld pH meter [31], while the particle size distribution method [32] classified the soil as poorly graded fine to medium sand (SP) using the sieve method.

The soil was artificially contaminated with 10% crude oil, giving a TPH concentration of 1307 mg kg⁻¹, consistent with contamination levels used in comparable remediation studies [33]. The contaminated soil was homogenized and allowed to age for 14 days before amendment application.



(a) Air-dried sample



(b) Sieved sample

Figure 1: Photographs of cattle rumen content.



(a) Raw (unpyrolyzed) rice husk



(b) Rice husk biochar

Figure 2: Photographs showing rice husk materials.



(a) Fresh cow horn



(b) Dried cow horn (chopped)



(c) Cow horn biochar

Figure 3: Photographs showing cow horn materials.

2.4.2. Experimental design

The remediation experiment comprised three amendment types (RHB, CHB and CRC), four application rates (5%, 10%,

15% and 20% w/w) and an unamended control (0%), with three replicates per measurement. Amendments were thoroughly mixed with contaminated soil and incubated under laboratory

conditions. The moisture-holding capacity of the soil was maintained by periodic addition of water. The experiment lasted for 60 days, and 20 g of soil was collected and analyzed for residual TPH and hydrocarbon-utilizing bacteria (HUB) on days 30 and 60.

2.4.3. TPH analysis

TPH was extracted from soil samples following the US EPA Method 8015C procedure [34]. A mass of 10 g of homogenized soil was weighed and extracted with dichloromethane by ultrasonication. The extract was filtered, dried over anhydrous sodium sulfate and concentrated to approximately 2 mL using a rotary evaporator. The extract was cleaned by passing it through silica gel, and TPH was eluted with *n*-hexane. TPH concentration was determined using gas chromatography with flame ionization detection (GC-FID). The equipment was calibrated with *n*-alkane standards (C₈–C₄₀).

The percentage TPH reduction was calculated using Eq. (1):

$$\text{TPH reduction (\%)} = \frac{C_0 - C_t}{C_0} \times 100, \quad (1)$$

where C_0 is the initial TPH concentration and C_t is the final TPH concentration at the sampling time.

2.4.4. Enumeration of hydrocarbon-utilizing bacteria

HUB counts were determined using the ten-fold serial dilution and spread plate technique, following the ten-fold serial dilution spread plate method as modified [35]. The HUB counts were expressed as CFU g⁻¹ of dry soil and back-calculated from the dilution factor.

2.5. Statistical analysis

All experiments were run in triplicate, and data are expressed as mean ± standard deviation. Statistical analyses were performed using SPSS. Two-way analysis of variance (ANOVA) was used to assess the effects of amendment type and application rate on TPH degradation, and Tukey's honestly significant difference (HSD) post-hoc test was applied when significant differences existed.

3. Results and discussion

3.1. Physicochemical characterization

3.1.1. Elemental composition

Results for the elemental composition of the biochars and CRC are shown in Table 1. Oxygen dominated RHB (48.33 ± 0.8%), followed by silicon (31.5 ± 0.6%). Oxygen was also the predominant element in CHB (39.7 ± 0.7%), followed closely by calcium (12.3 ± 0.4%) and sulfur (12.1 ± 0.5%). Potassium dominated CRC (31.2 ± 0.5%), followed by oxygen (30.7 ± 0.6%) and calcium (19.3 ± 0.5%). Other trace elements were present at low concentrations, but they deserve attention in field-scale applications.

Table 2 shows that RHB was primarily made of silica (67.3 ± 1.3%), supporting its thermal stability and potential for long-term soil applications [36]. This stability has been attributed to

both π–π electron interactions and the pore-filling effect [37]. The elevated P₂O₅ content (14.7 ± 0.5%) suggests that RHB could serve as a source of soil phosphorus, in contrast to certain phosphorus-laden animal-derived biochars [38]. Lower silica levels were recorded in CHB and CRC (<12%), but comparatively higher CaO levels (17.3 ± 0.5% and 27.0 ± 0.5%, respectively) indicate their pH-buffering potential in acidic soils. The SO₃ level in CHB (30.2 ± 0.5%) was probably derived from keratin in cow horn, which contains sulfur-based amino acids [39]. The ash content of RHB was 50%, consistent with its high silica content, while CHB and CRC reflected more organic matrices, with ash contents of 16% and 12%, respectively.

3.1.2. Structural characterization

Quantitative phase analysis by XRD revealed 65 wt% and 56 wt% graphite in RHB and CHB, respectively (Figures 4 and 5). The high graphite content is consistent with an earlier report showing that lignocellulosic biomasses undergo aromatic carbon development within 400–600 °C [40]. Higher temperatures also support the secondary decomposition of primary pyrolysis products (furans and anhydrosugars), leading to the formation of condensed aromatic structures [41].

CRC composition showed diverse crystalline phases (Figure 6), including aluminium phosphate (24 ± 2%), urea (18 ± 23%), quartz (14 ± 20%) and mascagnite (12 ± 18%). Additionally, a chaoite-like phase with a distinctive peak at $2\theta = 21.07^\circ$ ($d = 4.212 \text{ \AA}$) was tentatively identified at about 28 ± 39 wt% (Figure 7). It must be emphasized that this identification is strictly tentative. The ±39% uncertainty reflects the inherent limitations of the RIR method for quantifying rare carbon allotropes, necessitating cautious interpretation. Chaoite has been found in meteoritic and high-temperature geological materials [42], and its presence in biological rumen materials has not been widely studied. Confirmation by Raman spectroscopy and/or high-resolution transmission electron microscopy (HRTEM) is required before any definite conclusion can be drawn.

3.1.3. Surface area and pore characteristics

The multipoint BET surface areas were 356.0, 254.9 and 185.9 m² g⁻¹ for CHB, RHB and CRC, respectively (Table 3). The high surface areas of RHB and CHB make them potentially effective for application in contaminant immobilization [43]. The relatively lower surface area of RHB could be due to residual inorganic components that may block pores, contrasting with reports suggesting that animal-based feedstocks produce lower-surface-area biochars [44]. The DFT pore volumes of RHB, CHB and CRC were 0.07386, 0.09233 and 0.046 cm³ g⁻¹, respectively (Table 3). The pore diameters fell within the mesoporous range of 2–50 nm [45], similar to an earlier report [18].

The pH values of the amendments were 7.22, 7.17 and 7.29 for RHB, CHB and CRC, respectively. The near-neutral to slightly alkaline pH values of all amendments suggest compatibility with soil microbial activity during remediation.

Table 1: Elemental composition of RHB, CHB and CRC.

Element	RHB (wt%)	CHB (wt%)	CRC (wt%)
O	48.33 ± 0.8	39.7 ± 0.7	30.7 ± 0.6
Mg	–	–	–
Al	0.6 ± 0.03	5.1 ± 0.1	6.5 ± 0.2
Si	31.5 ± 0.6	5.2 ± 0.2	5.3 ± 0.2
P	6.4 ± 0.2	1.0 ± 0.05	0.7 ± 0.01
S	0.2 ± 0.02	12.1 ± 0.5	1.2 ± 0.05
Cl	1.7 ± 0.05	10.1 ± 0.3	–
K	6.0 ± 0.2	2.1 ± 0.1	31.2 ± 0.5
Ca	3.2 ± 0.1	12.3 ± 0.4	19.3 ± 0.5
Ti	0.1 ± 0.01	1.0 ± 0.06	0.9 ± 0.05
V	0.004 ± 0.001	0.06 ± 0.005	–
Cr	0.022 ± 0.002	0.04 ± 0.005	0.03 ± 0.001
Mn	0.2 ± 0.02	0.3 ± 0.02	0.5 ± 0.04
Fe	1.4 ± 0.10	5.8 ± 0.2	2.3 ± 0.10
Co	0.003 ± 0.001	0.09 ± 0.01	0.03 ± 0.002
Ni	0.005 ± 0.001	–	0.01 ± 0.002
Cu	0.1 ± 0.01	0.5 ± 0.04	0.5 ± 0.05
Zn	0.1 ± 0.02	3.2 ± 0.2	0.06 ± 0.05
Rb	0.02 ± 0.002	0.04 ± 0.004	0.02 ± 0.001
Sr	0.01 ± 0.001	0.04 ± 0.004	0.02 ± 0.001
Zr	0.006 ± 0.002	0.07 ± 0.005	0.01 ± 0.002
Nb	0.01 ± 0.002	0.07 ± 0.005	0.03 ± 0.001
Ag	0.01 ± 0.002	0.1 ± 0.02	0.01 ± 0.001
Sn	–	–	0.5 ± 0.02
Cs	–	–	0.1 ± 0.01
Ba	–	0.7 ± 0.005	–
Ta	0.004 ± 0.001	0.09 ± 0.01	–
W	0.01 ± 0.002	0.2 ± 0.02	0.02 ± 0.002
Pb	0.01 ± 0.001	0.05 ± 0.005	0.03 ± 0.003

Note: Bold values indicate elements present at concentrations >5%. Values represent mean ± standard deviation ($n = 3$).

3.1.4. Morphological characterization

SEM analysis showed that RHB exhibited a very porous structure, with several pores falling within the 10–100 μm range (Figure 8a), caused by evaporation of volatile materials during pyrolysis [12, 46]. CHB micrographs (Figure 8b) revealed a less heterogeneous surface compared to RHB. CHB also had rough textures, with pores in the 10–100 μm range. These features indicate the potential capability of the materials for PHC removal through increased sorption sites. The surface of CRC was irregular (Figure 8c), likely because of plant fibers and undigested materials.

3.1.5. Functional group analysis

FTIR analysis showed different functional groups in the three amendments. In RHB, a broad band at 1084.7 cm^{-1} associated with Si–O–Si or Si–O–C stretching indicated silica (Figure S3, Supplementary material). A weak band at 3634.2 cm^{-1} for –OH stretching suggested low moisture content, which differs from water-washed biochar that usually has broad –OH

peaks [47]. The presence of C=C stretching (1587.8 cm^{-1}) and Si–O–Si confers chemical stability on RHB, allowing it to function as a sorbent in contaminated soils [48, 49].

The spectrum of CHB exhibited a weak band at 3533.5 cm^{-1} (–OH stretch) and 3153.3 cm^{-1} (N–H stretching) from proteins in cow horns. C–O (1167.7 cm^{-1}) and C=C stretches (1461.8, 741.7 and 693.3 cm^{-1}) suggest aromaticity [50]. The N–H stretch suggests the availability of nitrogen from keratin decomposition, which can supplement microbial enzyme synthesis during hydrocarbon degradation.

CRC, on the other hand, showed a characteristic –OH peak at 3261.4 cm^{-1} and C=O stretching (1636 cm^{-1}) of conjugated ketones or amides [51]. The –OH and C=O groups may stimulate microbial communities by aiding moisture retention and providing essential nutrients [52]. These groups indicate capacity for hydrogen bonding with polar hydrocarbon fractions and nutrient release, both of which favor microbial activity. Following characterization of the amendments, their remediation efficiency when applied to PHC-contaminated soil was assessed.

Table 2: Oxide composition of RHB, CHB and CRC.

Component	RHB (%)	CHB (%)	CRC (%)
SiO ₂	67.3 ± 1.3	11.2 ± 0.4	11.3 ± 0.5
V ₂ O ₅	0.007 ± 0.001	0.1 ± 0.01	–
Cr ₂ O ₃	0.03 ± 0.004	0.05 ± 0.005	0.04 ± 0.005
MnO	0.2 ± 0.02	0.3 ± 0.02	0.7 ± 0.05
Fe ₂ O ₃	1.9 ± 0.1	8.3 ± 0.2	3.3 ± 0.15
CoO	0.004 ± 0.001	0.1 ± 0.01	0.04 ± 0.004
NiO	0.007 ± 0.001	–	0.02 ± 0.002
CuO	0.1 ± 0.02	0.7 ± 0.05	0.6 ± 0.05
Nb ₂ O ₅	0.02 ± 0.002	0.1 ± 0.01	0.05 ± 0.005
WO ₃	0.02 ± 0.002	0.2 ± 0.002	0.02 ± 0.002
P ₂ O ₅	14.7 ± 0.5	2.3 ± 0.1	1.6 ± 0.08
SO ₃	0.6 ± 0.05	30.2 ± 0.5	2.9 ± 0.2
CaO	4.5 ± 0.2	17.3 ± 0.5	27.0 ± 0.5
MgO	–	–	–
K ₂ O	7.2 ± 0.2	2.5 ± 0.1	37.6 ± 0.5
BaO	–	0.8 ± 0.05	–
Al ₂ O ₃	1.1 ± 0.05	9.7 ± 0.3	12.3 ± 0.5
Ta ₂ O ₅	0.004 ± 0.001	0.1 ± 0.01	–
TiO ₂	0.2 ± 0.02	1.7 ± 0.1	1.6 ± 0.1
ZnO	0.2 ± 0.02	3.9 ± 0.2	0.07 ± 0.005
Ag ₂ O	0.014 ± 0.002	0.2 ± 0.02	0.01 ± 0.002
ZrO ₂	0.008 ± 0.001	0.1 ± 0.01	0.02 ± 0.002
SnO ₂	–	–	0.6 ± 0.05
PbO	0.014 ± 0.002	0.06 ± 0.005	0.04 ± 0.004
Rb ₂ O	0.017 ± 0.002	0.06 ± 0.005	0.02 ± 0.003
Cs ₂ O	–	–	0.1 ± 0.01
SrO	0.013 ± 0.001	0.05 ± 0.005	0.02 ± 0.002

Note: Bold values indicate compounds present at concentrations >5%. Values represent mean ± standard deviation ($n = 3$).

Plot of results

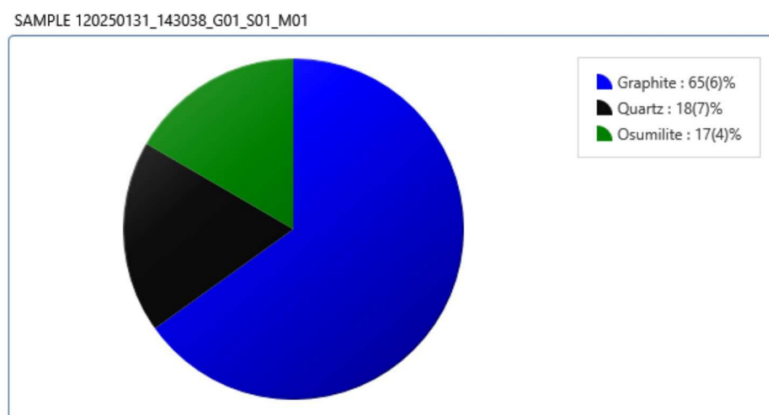


Table of results

Dataset / Weight Fraction, wt%	Value, Unit	Graphite	Quartz	Osumilite
SAMPLE 1_20250131_143038_G01_S01_M01	0	65(6)	18(7)	17(4)

Figure 4: Quantitative phase analysis results (RIR method) for rice husk biochar.

3.2. Remediation performance

Degradation of PHCs in soil is often characterized by an initial rapid breakdown followed by a slower phase [53]. The

Plot of results

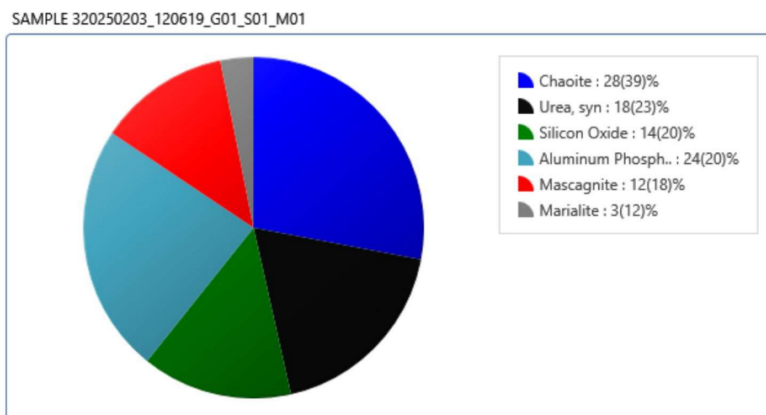


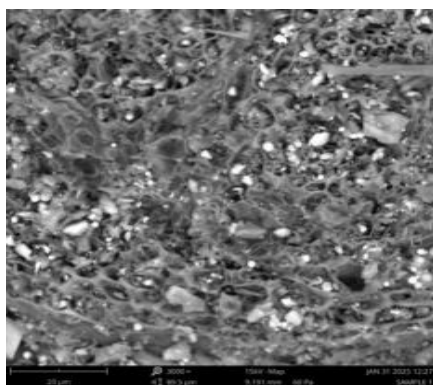
Table of results

Dataset / Weight Fraction,...	Value, Unit	Chaoite	Urea, syn	Silicon Oxide	Aluminum Phosphate	Mascagnite	Marialite
SAMPLE 3_20250203_1206...	0	28(39)	18(23)	14(20)	24(20)	12(18)	3(12)

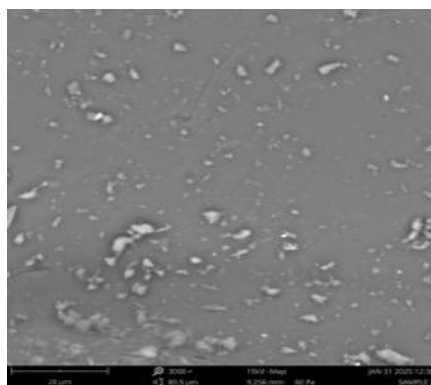
Figure 7: Quantitative phase analysis results (RIR method) for cattle rumen content.

Table 3: Surface area and porosity of biochar materials and cattle rumen content.

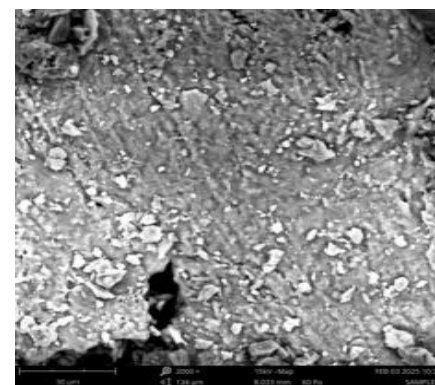
Method	Parameter	RHB	CHB	CRC	Remark
Multipoint BET	Surface area ($\text{m}^2 \text{g}^{-1}$)	254.9	356.0	185.9	Used for determination of total surface area
DFT	DFT pore volume ($\text{cm}^3 \text{g}^{-1}$)	0.07386	0.09233	0.046	CHB is more porous than RHB and CRC
DFT	DFT pore diameter (nm)	2.547	2.647	2.647	Both biochar materials and CRC are mesoporous
pH meter	pH	7.22	7.17	7.29	Compatible with soil microorganisms



(a) RHB



(b) CHB



(c) CRC

Figure 8: Micrographs of (a) RHB at 3000 \times , 89.5 μm ; (b) CHB at 3000 \times , 89.5 μm ; and (c) CRC at 2000 \times , 179 μm .

indicating that a 14-day aging period may be insufficient to replicate weathering dynamics of long-term field-contaminated soils. Future studies should employ field-contaminated soils to better replicate long-term sequestration dynamics.

3.2.1. Biochars

RHB exhibited dose-response performance on day 60: TPH degradation increased from 79.2% at the 5% amendment rate to 94.2% at the 20% amendment rate. This observation is sup-

ported by RHB's porous structure (Figure 8), large surface area (Table 3) and functional groups that confer chemical stability, aligning with earlier studies [12, 58]. Although RHB has been reported to enhance PHC degradation in soil, its effectiveness can be constrained by nutrient availability [4].

The remediation efficiency of CHB increased with increasing application rate. The 20% treatment attained maximum TPH reduction of 92.6% on day 60, significantly different from the lower application rates ($p < 0.05$). This dose-response rela-

Table 4: Changes in residual TPH concentrations in petroleum-hydrocarbon-contaminated soils with time.

Amendment	Amendment rate (%)	Time interval		Reduction (%)	
		Day 30	Day 60	Day 30	Day 60
RHB	0	313.2 ± 0.2	281.9 ± 0.5	76.0 ^a	78.4 ^a
	5	325.3 ± 0.8	271.5 ± 1.8	75.1 ^a	79.2 ^a
	10	223.6 ± 1.5	154.4 ± 2.2	82.9 ^a	88.2 ^b
	15	174.8 ± 1.5	104.7 ± 1.7	86.6 ^b	91.9 ^c
	20	241.5 ± 1.0	75.9 ± 1.3	81.5 ^a	94.2 ^d
CHB	0	313.2 ± 1.0	281.9 ± 0.8	76.0 ^a	78.4 ^a
	5	353.6 ± 1.5	258.7 ± 2.0	72.9 ^a	80.2 ^a
	10	261.3 ± 1.5	159.6 ± 1.5	80.0 ^a	87.8 ^b
	15	219.3 ± 2.0	127.9 ± 1.8	83.2 ^a	90.2 ^c
	20	129.4 ± 1.2	97.1 ± 1.4	90.1 ^c	92.6 ^d
CRC	0	313.2 ± 0.8	281.9 ± 0.5	76.0 ^a	78.4 ^a
	5	333.8 ± 1.2	57.7 ± 1.9	74.5 ^a	95.6 ^d
	10	78.6 ± 1.5	23.2 ± 1.0	93.9 ^d	98.2 ^d
	15	191.9 ± 1.8	159.5 ± 2.1	85.3 ^b	87.8 ^b
	20	134.6 ± 1.0	48.1 ± 1.2	89.7 ^c	96.3 ^d

Different letters within the same column indicate significant differences at $p < 0.05$.

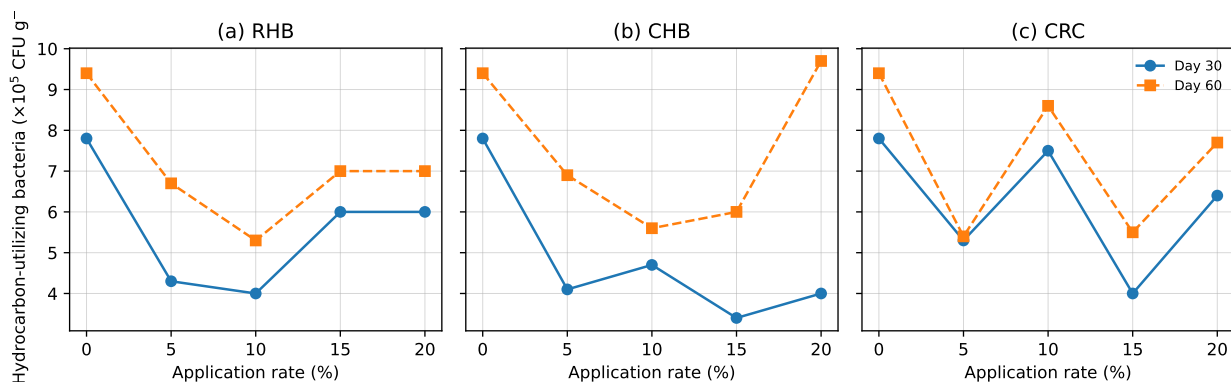


Figure 9: Hydrocarbon-utilizing bacterial (HUB) counts in (a) RHB-amended soils, (b) CHB-amended soils and (c) CRC-amended soils at varying application rates.

relationship, which mirrors RHB, corroborates the observed trend that biochar efficiency increases with application rate. The relatively lower remediation performance of CHB compared to RHB at equivalent application rates may reflect feedstock-related differences in carbonization efficiency [59], as evidenced by its lower graphite content, despite its higher surface area. To the best of our knowledge, this is among the first studies to evaluate pyrolyzed cow horn specifically for petroleum hydrocarbon remediation, and direct performance comparison remains limited. The remediation performance observed is, however, consistent with cow horn powder studies for hydrocarbon-remediated soils [29]. Overall, the results support biochar-amended remediation strategies, offering a low-cost and sustainable approach to managing PHC-contaminated soils.

3.2.2. Cattle rumen content

CRC amendments demonstrated the most effective remediation performance but exhibited a dose-response pattern distinct from the biochars. Unlike RHB and CHB, which exhibited dose-response relationships, CRC demonstrated a non-linear pattern: TPH reduction peaked at the 10% application rate (98.2%) and declined at both lower and higher rates.

The better performance of CRC at its optimal application rate (10%) compared to biochars at their optimal application rate (20%) can be explained by their inherently different remediation mechanisms. Biochars immobilize hydrocarbons primarily by sorption onto their high-surface-area carbon matrices, whereas CRC operates mostly through nutrient supply and microbial inoculation (biostimulation). CRC is rich in potassium, which acts as a key nutrient in stimulating and enhancing microbial metabolism in PHC-contaminated soils [15, 60]. How-

ever, microbial inoculation predominates, as indicated by the elevated HUB counts in CRC-amended soils (Section 3.2.3).

The reduced performance of CRC at higher application rates (15–20%) is consistent with previous reports that excess organic inputs disrupt the balance of soil microbial communities, favoring general heterotrophs over hydrocarbon degraders. Under such conditions, microorganisms tend to preferentially consume labile organic substrates rather than petroleum hydrocarbons [61, 62].

3.2.3. Hydrocarbon-utilizing bacteria population

Figure 9 presents the HUB counts in the studied soils. RHB showed an increasing HUB count with increasing application rate, peaking at 7.0×10^5 CFU g^{-1} at the 15% and 20% application rates on day 60. CHB had its highest day 60 HUB count at the 20% application rate (9.7×10^5 CFU g^{-1}), while CRC peaked at the 10% application rate with a HUB count of 8.6×10^5 CFU g^{-1} .

Unamended soils had relatively high bacterial counts but lower TPH removal than some amended soils. This is likely because hydrocarbons serve as a source of carbon, allowing microbial growth to continue in contaminated soils even in the absence of considerable degradation activity [63].

Total HUB counts, as determined by the spread plate method, do not distinguish between specialized hydrocarbon-degrading bacteria and general heterotrophs capable of growth on hydrocarbon media [64]. Hence, the higher counts in control soil may reflect general heterotrophs with limited hydrocarbon-degrading capability, while amended soils likely harbored a higher proportion of specialized hydrocarbon-degrading bacteria. The use of 16S rRNA gene sequencing, which would provide definitive characterization of the microbial community, is recommended for further studies.

Overall, the dose-response increase observed in most amendments for both bacterial population and TPH removal indicates that biochar created favorable conditions for microbial activity and hydrocarbon degradation. It has been reported that biochar could stimulate microorganisms to degrade carbon substrates in petroleum [65, 66], while rice straw biochar was reported to increase hydrocarbon degradation efficiency, attributed to a higher percentage of Acidobacteria-related genera [11].

4. Conclusion

Rice husk, cow horn and cattle rumen content are waste materials commonly disposed of by landfilling or open burning. Converting these waste materials to low-cost soil amendments helps tackle waste disposal and land degradation. Cow horn and cattle rumen are especially promising in this regard, since they are both routinely discarded after cattle processing with limited reuse options.

In this study, the potential of converting agricultural-derived biochars (RHB and CHB) and abattoir waste (CRC) into amendments for the remediation of petroleum-hydrocarbon-contaminated soils was investigated. The three materials dif-

fered considerably in their compositional and structural properties, and these differences were reflected in their remediation performance. Elemental and oxide composition of the amendments likely contributed additional degradation pathways through nutrient-assisted microbial stimulation. Graphite was the dominant crystalline phase in RHB and CHB, while a chaoite-like phase was tentatively detected in CRC. Before any conclusions can be made, this observation must be confirmed using Raman spectroscopy and HRTEM because of the limitations of XRD for rare carbon allotropes.

RHB and CHB showed TPH reductions of 94% and 93%, respectively, at the 20% application rate. This enhanced remediation over 60 days was due to their large surface areas and porous structures, which favored contaminant sorption. Conversely, CRC attained a 98.2% reduction in TPH at a lower application rate of 10%. The increased HUB count observed at this rate suggests microbial stimulation as an underlying mechanism for TPH breakdown.

Since this study used artificially spiked soil aged for 14 days under laboratory conditions, its direct applicability to field-contaminated sites where PHCs are more sequestered remains to be established. The 14-day aging period is acknowledged as a study limitation; longer aging or the use of field-contaminated soils in future works would provide a more realistic assessment of remediation potential. Future work should build on this study by investigating the mechanisms underlying CRC performance at low application rates. Furthermore, co-application of biochars and CRC at optimized ratios also warrants investigation, particularly with regard to long-term amendment stability and the risk of contaminant re-release.

Data availability

The data supporting the findings of this study are contained in the supplementary materials. The dataset is available from the corresponding author upon reasonable request.

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Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

- [1] V. Tripathi, V. K. Gaur, I. Kaur, P. K. Srivastava & N. Manickam, "Unlocking bioremediation potential for site restoration: A comprehensive approach for crude oil degradation in agricultural soil and phytotoxicity assessment", *Journal of Environmental Management* **355** (2024) 120508. <https://doi.org/10.1016/j.jenvman.2024.120508>.

- [2] Y. Lv, J. Bao, D. Liu, X. Gao, Y. Yu & L. Zhu, "Synergistic effects of rice husk biochar and aerobic composting for heavy oil-contaminated soil remediation and microbial community succession evaluation", *Journal of Hazardous Materials* **448** (2023) 130929. <https://doi.org/10.1016/j.jhazmat.2023.130929>.
- [3] F. N. D. Mukome, M. C. Buelow, J. Shang, J. Peng, M. Rodriguez, D. M. Mackay, J. J. Pignatello, N. Sihota, T. P. Hoelen & S. J. Parikh, "Biochar amendment as a remediation strategy for surface soils impacted by crude oil", *Environmental Pollution* **265** (2020) 115006. <https://doi.org/10.1016/j.envpol.2020.115006>.
- [4] Z. Wei, Y. Wei, Y. Liu, S. Niu, Y. Xu, J.-H. Park & J. J. Wang, "Biochar-based materials as remediation strategy in petroleum hydrocarbon-contaminated soil and water: Performances, mechanisms, and environmental impact", *Journal of Environmental Sciences* **138** (2024) 350. <https://doi.org/10.1016/j.jes.2023.04.008>.
- [5] Agency for Toxic Substances and Disease Registry, "Toxicological profile for total petroleum hydrocarbons (TPH)", U.S. Department of Health and Human Services (2005). Available online: <https://www.atsdr.cdc.gov/toxprofiles/tp123.pdf>.
- [6] B. Muijs & M. T. O. Jonker, "A closer look at bioaccumulation of petroleum hydrocarbon mixtures in aquatic worms", *Environmental Toxicology and Chemistry* **29** (2010) 1943. <https://doi.org/10.1002/etc.263>.
- [7] U.S. Environmental Protection Agency, "A citizen's guide to monitored natural attenuation (MNA)", EPA 542-F-20-009 (2020). Available online: https://www.epa.gov/sites/default/files/2020-06/documents/mna_cg_fs_eng.pdf.
- [8] P. Deebika, A. M. Sheela & R. Ilamathi, "Biochar and compost-based phytoremediation of crude oil contaminated soil", *Indian Journal of Science and Technology* **14** (2021). <https://doi.org/10.17485/IJST/v14i3.1178>.
- [9] H. Lee, K. Sam, F. Coulon, S. De Gisi, M. Notarnicola & C. Labianca, "Recent developments and prospects of sustainable remediation treatments for major contaminants in soil: A review", *Science of the Total Environment* **912** (2024) 168769. <https://doi.org/10.1016/j.scitotenv.2023.168769>.
- [10] T. Sayara & A. Sánchez, "Bioremediation of PAH-contaminated soils: Process enhancement through composting/compost", *Applied Sciences* **10** (2020) 3684. <https://doi.org/10.3390/app10113684>.
- [11] B. Zhang, L. Zhang & X. Zhang, "Biochar for soil remediation: Mechanisms and applications", *RSC Advances* **9** (2019) 35246. <https://doi.org/10.1039/C9RA06726D>.
- [12] Z. Liu, Y. Zhang, X. Li, Z. Sun, R. Zhang, X. Li & Y. Du, "The application of biochar derived from rice husk enhanced the bioremediation of petroleum-contaminated soil in semi-arid areas", *Agronomy* **14** (2024) 2015. <https://doi.org/10.3390/agronomy14092015>.
- [13] U. Naeem & M. A. Qazi, "Leading edges in bioremediation technologies for removal of petroleum hydrocarbons", *Environmental Science and Pollution Research* **27** (2020) 27370. <https://doi.org/10.1007/s11356-019-06124-8>.
- [14] Y. Lv, J. Bao, S. Li, D. Liu, D. Dai, M. Qv & L. Zhu, "Biochar amendment of aerobic composting for the effective biodegradation of heavy oil and succession of bacterial community", *Bioresource Technology* **362** (2022) 127820. <https://doi.org/10.1016/j.biortech.2022.127820>.
- [15] Y. Li, J. Yang, Y. Song & M. Wei, "Progress in biostimulation-based remediation of TPH-contaminated soils: A comprehensive review", *PeerJ* **13** (2025) e19991. <https://doi.org/10.7717/peerj.19991>.
- [16] H. Cheng, Y. Bian, F. Wang, X. Jiang, R. Ji, C. Gu, X. Yan & Y. Song, "Green conversion of crop residues into porous carbons and their application to efficiently remove polycyclic aromatic hydrocarbons from water: Sorption kinetics, isotherms and mechanism", *Bioresource Technology* **284** (2019) 1. <https://doi.org/10.1016/j.biortech.2019.03.104>.
- [17] S. E. Hale, J. Lehmann, D. Rutherford, A. R. Zimmerman, R. T. Bachmann, V. Shitumbanuma, A. O'Toole, K. L. Sundqvist, H. P. H. Arp & G. Cornelissen, "Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars", *Environmental Science & Technology* **46** (2012) 2830. <https://doi.org/10.1021/es203984k>.
- [18] A. Tomczyk, Z. Sokolowska & A. Boguta, "Biochar physicochemical properties: Pyrolysis temperature and feedstock kind effects", *Reviews in Environmental Science and Bio/Technology* **19** (2020) 191. <https://doi.org/10.1007/s11157-020-09523-3>.
- [19] Y. Chen, F. Wang, J. Gao, X. He, Q. Liu & L. Liu, "Enhancing bioremediation of petroleum-contaminated soil by sophorolipids-modified biochar: Combined metagenomic and metabolomic analyses", *Science of the Total Environment* **951** (2024) 175772. <https://doi.org/10.1016/j.scitotenv.2024.175772>.
- [20] L. Kong, Y. Gao, Q. Zhou, X. Zhao & Z. Sun, "Biochar accelerates PAHs biodegradation in petroleum-polluted soil by biostimulation strategy", *Journal of Hazardous Materials* **343** (2018) 276. <https://doi.org/10.1016/j.jhazmat.2017.09.040>.
- [21] J. Lehmann, M. C. Rillig, J. Thies, C. A. Masiello, W. C. Hockaday, D. Crowley & I. Kögel-Knabner, "Biochar effects on soil biota—A review", *Soil Biology & Biochemistry* **43** (2011) 1812. <https://doi.org/10.1016/j.soilbio.2011.04.022>.
- [22] M. Ahmad, A. U. Rajapaksha, J. E. Lim, M. Zhang, N. Bolan, D. Mohan, M. Vithanage, S. S. Lee & Y. S. Ok, "Biochar as a sorbent for contaminant management in soil and water: A review", *Chemosphere* **99** (2014) 19. <https://doi.org/10.1016/j.chemosphere.2013.10.071>.
- [23] A. E. Ajayi & R. Horn, "Biochar-induced changes in soil resilience: Effects of soil texture and biochar dosage", *Pedosphere* **27** (2017) 236. [https://doi.org/10.1016/S1002-0160\(17\)60313-8](https://doi.org/10.1016/S1002-0160(17)60313-8).
- [24] S. Žibutiš, J. Pekarskas & L. Česonienė, "Effect of horn shaving and horn core powder fertilizers on the dynamics of mineral nitrogen in the soil of organic farm", *Ekologija* **58** (2012) 375. <https://doi.org/10.6001/ekologija.v58i3.2534>.
- [25] I. E. Uwidia, F. I. Akhidonor & E. O. Oshodin, "Comparative analysis of macro, micro and toxic elements in cow bones and horns", *Journal of Chemistry and Allied Sciences* **1** (2025) 7. <https://doi.org/10.60787/jcas.vol1no1.38>.
- [26] P. J. Weimer, "Degradation of cellulose and hemicellulose by ruminal microorganisms", *Microorganisms* **10** (2022) 2345. <https://doi.org/10.3390/microorganisms10122345>.
- [27] T. K. Bhoi, I. Samal, A. Saraswat, H. C. Hombegowda, S. K. Samal, A. K. Dash, S. Sharma, P. Lawate, V. Vyas & M. B. Raza, "Biochar as a soil amendment: Effects on microbial communities and soil health", in *Biochar Production for Green Economy*, M. R. Reddy, S. R. Chavan & D. K. Mahajan (Eds.), Elsevier, Amsterdam (2024), p. 463. <https://doi.org/10.1016/B978-0-443-15506-2.00022-5>.
- [28] M. Zhen, J. Tang, C. Li & H. Sun, "Rhamnolipid-modified biochar-enhanced bioremediation of crude oil-contaminated soil and mediated regulation of greenhouse gas emission in soil", *Journal of Soil and Sediments* **21** (2021) 123. <https://doi.org/10.1007/s11368-020-02746-5>.
- [29] N. N. Kpakol, T. Osasere-Omoruyi, D. C. Belonwu & S. E. Okoro, "Efficiency of cow-horn powder in the remediation of crude oil impacted soil", *Scientia Africana* **23** (2024) 59. <https://doi.org/10.4314/sa.v23i1.5>.
- [30] ASTM International, "Standard test method for chemical analysis of wood charcoal", ASTM D1762–84 (2013), ASTM International. <https://doi.org/10.1520/D1762-84R13>.
- [31] O. Abollino, M. Aceto, M. Malandrino, E. Mentasti, C. Sarzanini & F. Petrella, "Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment", *Chemosphere* **49** (2002) 545. [https://doi.org/10.1016/S0045-6535\(02\)00352-1](https://doi.org/10.1016/S0045-6535(02)00352-1).
- [32] British Standards Institute, "BS 1377-2: Methods of test for soils for civil engineering purposes. Classification tests", BSI, London (1990). <https://www.scribd.com/document/400142553/BS-1377-Part-2-Methods-Of-Test-For-Soils-For-Civil-En-80C-pdf>.
- [33] S. E. Agary, K. M. Oghenejoboh & B. O. Solomon, "Kinetic modelling and half-life study of adsorptive bioremediation of soil artificially contaminated with Bonny light crude oil", *Journal of Ecological Engineering* **16** (2015) 1. <https://doi.org/10.12911/22998993/2799>.
- [34] United States Environmental Protection Agency, "Method 8015C: Non-halogenated organics by gas chromatography", SW-846 Test Methods, Washington, DC (2007). Available online: <https://www.epa.gov/sites/default/files/2015-12/documents/8015c.pdf>.
- [35] P. O. Okerentugba, T. L. Ataikiru & T. Ichor, "Isolation and characterisation of hydrocarbon utilizing yeast isolates from palm wine", *American Journal of Molecular Biology* **6** (2016) 63. <https://doi.org/10.4236/ajmb.2016.62007>.
- [36] L. Hu, Z. He & S. Zhang, "Sustainable use of rice husk ash in cement-based materials: Environmental evaluation and performance improvement", *Journal of Cleaner Production* **264** (2020) 121744. <https://doi.org/10.1016/j.jclepro.2020.121744>.
- [37] Z. Zhao, T. Nie & W. Zhou, "Enhanced biochar stabilities and adsorption

- properties for tetracycline by synthesizing silica-composited biochar", *Environmental Pollution* **254** (2019) 113015. <https://doi.org/10.1016/j.envpol.2019.113015>.
- [38] J. T. N. Knijnenburg, S. Suwanree, D. Macquarrie, P. Kasemsiri & K. Jetsrisuparb, "Phosphorus recovery from animal manures through pyrolysis: Phosphorus transformations, release mechanisms, and applications of manure biochars in agriculture", *RSC Sustainability* **3** (2025) 1084. <https://doi.org/10.1039/D4SU00524D>.
- [39] S. Banasaz & V. Ferraro, "Keratin from animal by-products: Structure, characterization, extraction and application—A review", *Polymers* **16** (2024) 1999. <https://doi.org/10.3390/polym16141999>.
- [40] X. Zhang, Y. Zhang, S. Zhang, L. Yao & Y. Hao, "Lignocellulosic biomass pyrolysis: A review on the pretreatment and catalysts", *Fuel Processing Technology* **279** (2025) 108352. <https://doi.org/10.1016/j.fuproc.2025.108352>.
- [41] K. B. Ansari, J. S. Arora, J. W. Chew, P. J. Dauenhauer & S. H. Mushrif, "Fast pyrolysis of cellulose, hemicellulose, and lignin: Effect of operating temperature on bio-oil yield and composition and insights into the intrinsic pyrolysis chemistry", *Industrial & Engineering Chemistry Research* **58** (2019) 15838. <https://doi.org/10.1021/acs.iecr.9b00920>.
- [42] S. K. Simakov, A. E. Kalmykov, L. M. Sorokin, M. P. Novikov, I. A. Drozdova, M. A. Yagovkina & E. A. Grebenshchikova, "Chaoite formation from carbon-bearing fluid at low pressure-temperature parameters", *Doklady Earth Sciences* **399** (2004) 1289. https://www.researchgate.net/publication/281560196_Chaoite_formation_from_carbon-bearing_fluid_at_low_PT_parameters.
- [43] B. Hidayat, A. Rauf, T. Sabrina & A. Jamil, "Potential of several biomass as biochar for heavy metal adsorbent", *Journal of Asian Scientific Research* **8** (2018) 293. <https://doi.org/10.18488/journal.2.2018.8.11.293.300>.
- [44] M. Zahed, S. Salehi, R. Madadi & F. Hajebi, "Biochar as a sustainable product for remediation of petroleum contaminated soil", *Current Research in Green and Sustainable Chemistry* **4** (2021) 100055. <https://doi.org/10.1016/j.crgsc.2021.100055>.
- [45] M. Thommes, K. Kaneko, A. V. Neimark, J. P. Olivier, F. Rodriguez-Reinoso, J. Rouquerol & K. S. W. Sing, "Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report)", *Pure and Applied Chemistry* **87** (2015) 1051. <https://doi.org/10.1515/pac-2014-1117>.
- [46] M. Theeba, R. T. Bachmann, Z. I. Ilani, M. Zulkefli, M. H. A. Husni & A. W. Samsuri, "Characterization of local mill rice husk charcoal and its effect on compost properties", *Malaysian Journal of Soil Science* **16** (2012) 89. https://www.researchgate.net/publication/289318861_Characterization_of_local_mill_rice_husk_charcoal_and_its_effect_on_compost_properties
- [47] A. Ndekei, M. Gitita, N. Njomo & D. Mbui, "Synthesis and characterization of rice husk biochar and its application in the adsorption studies of lead and copper", *International Research Journal of Pure and Applied Chemistry* **22** (2021) 36. <https://doi.org/10.9734/irjpac/2021/v22i430402>.
- [48] F. F. Severo, L. S. da Silva, J. S. C. Moscôso, Q. Sarfaraz, L. F. R. Júnior, A. F. Lopes, L. B. Marzari & G. D. Molin, "Chemical and physical characterization of rice husk biochar and ashes and their iron adsorption capacity", *SN Applied Sciences* **2** (2020) 1286. <https://doi.org/10.1007/s42452-020-3088-2>.
- [49] B. Chen, D. Zhou & L. Zhu, "Transitional adsorption and partition of non-polar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures", *Environmental Science & Technology* **42** (2008) 5137. <https://doi.org/10.1021/es8002684>.
- [50] D. J. Tomlinson, C. H. Mülling & T. M. Fakler, "Formation of keratins in the bovine claw: Roles of hormones, minerals, and vitamins in functional claw integrity", *Journal of Dairy Science* **87** (2004) 797. [https://doi.org/10.3168/jds.S0022-0302\(04\)73223-3](https://doi.org/10.3168/jds.S0022-0302(04)73223-3).
- [51] R. M. Silverstein, F. X. Webster, D. J. Kiemle & D. L. Bryce, *Spectrometric Identification of Organic Compounds*, 8th ed., John Wiley & Sons, Hoboken, NJ 2014. <https://www.wiley.com/en-ae/Spectrometric+Identification+of+Organic+Compounds%2C+8th+Edition-p-9780470616376>
- [52] X. Shu, J. He, Z. Zhou, L. Xia, Y. Hu, Y. Zhang, Y. Zhang, Y. Luo, H. Chu, W. Liu, S. Yuan, X. Gao & C. Wang, "Organic amendments enhance soil microbial diversity, microbial functionality and crop yields: A meta-analysis", *Science of the Total Environment* **829** (2022) 154627. <https://doi.org/10.1016/j.scitotenv.2022.154627>.
- [53] M. Alexander, *Biodegradation and Bioremediation*, 2nd ed., Academic Press, San Diego, CA (1973). ISBN: 978-0-12-049861-1.
- [54] M. T. B. Abena, T. Li, M. N. Shah & W. Zhong, "Biodegradation of total petroleum hydrocarbons (TPH) in highly contaminated soils by natural attenuation and bioaugmentation", *Chemosphere* **234** (2019) 864. <https://doi.org/10.1016/j.chemosphere.2019.06.111>.
- [55] G. L. Northcott & K. C. Jones, "Partitioning, extractability, and formation of nonextractable polycyclic aromatic hydrocarbon residues in soil: 1. Compound differences in aging and sequestration", *Environmental Science & Technology* **35** (2001) 1103. <https://doi.org/10.1021/es000071y>.
- [56] P. B. Hatzinger & M. Alexander, "Effect of aging of chemicals in soil on the biodegradability and extractability", *Environmental Science & Technology* **29** (1995) 537. <https://doi.org/10.1021/es00002a033>.
- [57] U. C. Brinch, F. Ekelund & C. S. Jacobsen, "Method for spiking soil samples with organic compounds", *Applied and Environmental Microbiology* **68** (2002) 1808. <https://doi.org/10.1128/AEM.68.4.1808-1816.2002>.
- [58] N. Ni, F. Wang, Y. Song, Y. Bian, R. Shi, X. Yang, C. Gu & X. Jiang, "Mechanisms of biochar reducing the bioaccumulation of polycyclic aromatic hydrocarbons in rice from soil: Degradation stimulation versus immobilization", *Chemosphere* **196** (2018) 288. <https://doi.org/10.1016/j.chemosphere.2017.12.192>.
- [59] T. Han, Z. Zhao, M. Bartlam & Y. Wang, "Combination of biochar amendment and phytoremediation for hydrocarbon removal in petroleum-contaminated soil", *Environmental Science and Pollution Research* **23** (2016) 21219. <https://doi.org/10.1007/s11356-016-7236-6>.
- [60] A. M. Elshafei & R. Mansour, "Microbial bioremediation of soils contaminated with petroleum hydrocarbons", *Discover Soil* **1** (2024) 9. <https://doi.org/10.1007/s44378-024-00004-5>.
- [61] C. H. Chafneau, G. Rougeux, C. Yepremian & J. Oudot, "Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in soil", *Soil Biology & Biochemistry* **37** (2005) 1490. <https://doi.org/10.1016/j.soilbio.2005.01.012>.
- [62] B. A. Mekonnen, T. A. Aragaw & M. B. Genet, "Bioremediation of petroleum hydrocarbon contaminated soil: A review on principles, degradation mechanisms, and advancements", *Frontiers in Environmental Science* **12** (2024) 1354422. <https://doi.org/10.3389/fenvs.2024.1354422>.
- [63] Y. Luo, M. Durenkamp, M. De Nobili, Q. Lin & P. C. Brookes, "Short-term soil priming effects and the mineralization of biochar following its incorporation to soils of different pH", *Soil Biology & Biochemistry* **43** (2011) 2304. <https://doi.org/10.1016/j.soilbio.2011.07.020>.
- [64] D. V. Zige, F. I. Omeje & C. K. Anumudu, "Assessment of heterotrophic bacterial count (HBC) associated with commercial freezers in Yenagoa metropolis", *American Journal of Microbiological Research* **7** (2019) 98. https://www.researchgate.net/publication/338778797_Assessment_of_Heterotrophic_Bacterial_Count_HPC_Associated_with_Commercial_Freezers_in_Yenagoa_Metropolis.
- [65] O. F. Osisami, E. C. Egwim, S. E. Adeboye, D. A. Adesina, J. S. Abdul-Hadi, Y. O. Falana & A. E. Oketa, "Effect of biochar from plantain trunk wastes' remediation on microbial community dynamics of crude oil contaminated soil", *Ilorin Journal of Science* **11** (2024) 146. <https://doi.org/10.54908/iljs.fapscon.2024.11.03.1019>.
- [66] Z. Cai, Q. Zhou, S. Peng & K. Li, "Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance", *Journal of Hazardous Materials* **183** (2010) 731. <https://doi.org/10.1016/j.jhazmat.2010.07.087>.