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Isolation and Characterization of Fungal Isolates from Hydrocarbon Contaminated Intertidal Sediment of Amadi-Ama Creek, Bonny River

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ABSTRACT

Hydrocarbon contamination in intertidal sediments poses significant environmental risks, influencing microbial diversity and ecosystem stability. This study aimed to isolate and characterize indigenous fungal species from hydrocarbon-contaminated sediments of Amadi-Ama Creek, Bonny River, to assess their potential role in hydrocarbon degradation. The study also examined the physiochemical properties of the sediments to establish the extent of contamination and its possible impact on microbial distribution. Sediments were collected from four random points in the hydrocarbon-exposed intertidal zone. Physicochemical parameters, including pH, temperature, electrical conductivity, Total Hydrocarbon Content (THC), Total Petroleum Hydrocarbon (TPH), Polycyclic Aromatic Hydrocarbons (PAHs), and concentrations of nitrate, phosphate, sulfate, iron, copper, zinc, and chromium, were analyzed. Fungal species were isolated using Sabouraud's Dextrose Agar and Czapek Agar supplemented with 0.05% (v/v) chloramphenicol, and their occurrence was determined. The sediments were alkaline (pH 9.33), with hydrocarbon contamination confirmed by THC (11.82 mg/kg), TPH (4.216 mg/kg), and PAHs (2.064 mg/kg). Electrical conductivity and temperature were recorded at 1420 µS/cm and 27.24°C, respectively. Nitrate, phosphate, sulphate, iron, copper, zinc, and chromium concentrations varied, indicating anthropogenic influence. Fungal isolates included *Aspergillus niger*, (31.9%), *Penicillium notatum* (25%), *Cladosporium* spp. (5.6%), *Aspergillus flavus* (16.7%), *Fusarium solani* (9.7%), and *Trichoderma* spp. (11.1%), with *Aspergillus niger* being the most dominant specie. The study confirmed hydrocarbon pollution in the sampled site and identified fungal species with potential degradation ability. *Aspergillus niger*, being the most dominant isolate, may be a promising candidate for bioremediation. The findings also suggest that the fungal isolates may utilize alternative nutrient sources, highlighting their adaptability to extreme environments.

KEYWORDS

Fungi, hydrocarbon, intertidal sediment, Amadi-Ama Creek

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INTRODUCTION

In recent years, several innovative approaches have been developed to address hydrocarbon contamination. One of the most promising methods is bioremediation, which leverages on complex enzyme system of varying hydrocarbonoclastic microorganisms, including bacteria, fungi, and microalgae remediate contaminated environments[1-3]. Compared to conventional chemical treatments, bioremediation is often preferred due to its environmentally friendly nature. In this process, microorganisms degrade hydrocarbons, using them as a carbon source, and convert these toxic compounds into safer byproducts such as water and carbon dioxide[4].

Microorganisms serve various functions in biotechnology, with bioremediation being one of their key roles[5]. Bacteria and fungi, in particular, are frequently utilized in biotechnological applications. Bacteria are genetically versatile, while fungi are known for their unique growth behaviors, such as the secretion of extracellular enzymes and their ability to invade and colonize diverse environments. The release of petroleum products, both intentional and accidental, into ecosystems poses significant risks to both aquatic and terrestrial life. This contamination can lead to habitat destruction, the contamination of drinking water sources, a decline in the reproductive success of plants and animals due to disruptions in the food chain, and the death of organisms in affected areas[6].

Numerous studies have demonstrated the high microbial abundance in intertidal sediment, with bacterial and fungal communities playing vital roles[7].

Fungi play crucial roles in nutrient cycling, organic matter decomposition and overall ecosystem functioning. Understanding their presence in intertidal sediment contributes to a holistic view of coastal ecosystem dynamics.

The intertidal zone, characterized by periodic submersion and exposure to air, is significantly impacted by human activities and changes in climate[8]. This environment experiences extreme fluctuations in moisture, wave action, and salinity, creating challenging conditions for many organisms. Despite these stresses, the intertidal zone offers a variety of habitats, including mangroves, seagrass meadows, sandy beaches, rocky shores, coral reefs, and aquaculture areas. A study suggested that the intertidal region probably supports a vast biodiversity of microorganisms[9]. Among microorganisms in the zone, fungi play important roles in the process of decomposition and mineralization of organic matter in marine ecosystems[10].

Intertidal sediment being the transition zone between land and sea are constantly exposed to dynamic environmental conditions such as tidal fluctuations, salinity variations and nutrient availability. This sediment supports diverse microbial communities, including fungi which have adapted to these challenging conditions. Fungi in intertidal sediment fulfill important ecological functions such as decomposition of organic matter, nutrient cycling and interactions with other organisms. Intertidal sediment occurs along all coasts. However, the thickness of the deposits and areal extent vary from coast to coast. Organisms that live in the intertidal zone are able to adapt and survive forming communities across the changing habitat[11].

Previous studies have demonstrated the importance of fungal communities in intertidal sediment revealing that fungal diversity in intertidal sediment is influenced by factors such as sediment grain size organic matter content and tidal patterns[12-16].

Fungi in intertidal zones serve as essential decomposers, breaking down complex organic materials and facilitating nutrient cycling[11]. Their enzymatic processes are crucial for degrading plant matter and other organic substances[17]. Additionally, fungi in these environments have developed unique adaptations, including melanin production for UV protection and modified reproductive strategies to enhance their survival under harsh conditions[18].

Secretion of extracellular enzymes, ability to grow under stressed environmental conditions, extension in biomass location through hyphal growth, easy and rapid growth on agricultural or forest waste, and other enzyme systems are amongst the unique features of fungi that have been proven useful in bioremediation of polluted environments[19-23].

Hydrocarbon pollution in intertidal sediment poses a significant threat to the environment and human health. The intertidal zone is a critical habitat for numerous microorganisms and serves as a vital link between terrestrial and marine ecosystems. The contamination of sediment by hydrocarbons alters the sediment's physical, chemical, and biological characteristics, disrupting the delicate balance of the intertidal ecosystem. This disturbance can lead to the decline of species diversity, habitat degradation, and the loss of valuable ecosystem services. The intertidal sediment fungal community remains inadequately understood, with limited research focusing on their isolation and characterization. The absence of detailed studies on the isolation and characterization of fungal species has hindered understanding of their ecological roles and potential contributions to sedimentary processes.

The aim of this study is to isolate and characterize indigenous fungal species from a hydrocarbon impacted intertidal sediment of Bonny Estuarine, Amadi-Ama Creek, Port Harcourt, Rivers State.

MATERIALS AND METHODS

Study area: This study was conducted in November, 2023. Amadi-Ama Creek is located in Port Harcourt Local Government Area of Rivers State and lies between Longitude 07°02'57.627"E and Latitude 04°48'51.113"N. The creek is one of the tributaries of the upper Bonny Estuary, brackish and tidal in nature with fresh waters intrusion from the surrounding inland waters and flood during the wet season. The Bonny River Estuary lies on the South-Eastern edge of the Niger Delta between Longitudes 6°58' and 7°14'East and Latitudes 4°19' and 4°34'North with an estimated area of 206 km^2 and extends 7 km offshore to a depth of about 7.5 m[24].

Sampling: Intertidal sediment samples contaminated with hydrocarbon were used for the analyses. Sediment samples from four random points were aseptically collected with sterile containers from the hydrocarbon-impacted area of Amadi-Ama Creek, Port Harcourt, Rivers State and labeled accordingly. The samples collected were transported aseptically in sterile containers and ice chests to BGI Laboratories Ltd., in Port Harcourt, Rivers State and Microbiology Laboratory, Faculty of Pure and Applied Science, Federal University Wukari, Wukari City, Taraba State for physicochemical analysis and microbial analysis, respectively.

Determination of physicochemical properties: The pH, temperature, and electrical conductivity of the sediment samples were determined using infrared spectrophotometry following Soxhlet extraction with tetrachloroethylene (TCE), in accordance with ASTM D7066-04[25]. The total hydrocarbon content of the sediment was determined using infrared spectroscopy, following Soxhlet extraction with tetrachloroethylene (TCE) solvent[26]. For total petroleum hydrocarbon determination, n-hexane was used in the Soxhlet extraction, and anhydrous sodium sulfate was deployed in the fractionation[27]. The eluent was then injected into the inlet of a Gas Chromatograph (GC) and detected using Flame Ionization (FID)[28]. For the determination of polycyclic aromatic hydrocarbons, the Soxhlet extraction was carried out using dichloromethane (DCM) solvent and injected into the Gas Chromatograph (GC) for detection by mass spectrometry (MSD)[29]. Determination of nitrate, phosphate, and sulfate was carried out using UV/VIS spectrophotometry, wherein their various standards were used for calibration, and absorbance was determined at their respective wavelengths[25]. For the determination of metals; iron, copper, zinc, and chromium, their respective standards were used for calibration on the Atomic Absorption Spectrophotometer (AAS), wherein the hollow cathode lamps for each element were also deployed[30].

Isolation of indigenous fungi: Each of 1.0 g of sediment was aseptically diluted, following this; 0.1 mL aliquots of each 10-fold serially diluted sample were transferred into duplicate plates of Sabouraud's Dextrose Agar and Czapek Agar supplemented with 0.05% (v/v) of chloramphenicol. After the inoculation procedures, SDA agar plates and Czapek agar were incubated at 30°C for 5 days and 7 days, respectively.

Total fungal counts were obtained from the inoculated SDA agar after incubation, while colonies on Czapek agar plates were further purified by subsequent subculture on SDA agar and final subculture on SDA agar plates. The purified fungal isolates were identified based on their morphological characteristics[31].

Statistical analysis: Descriptive statistics were used to analyze the fungal counts and the prevalence distribution of fungal isolates. The fungal counts from the pour plate and spread plate methods were expressed as colony-forming units (CFU/mL) and compared for variation. The frequency of occurrence and percentage prevalence of each fungal species were calculated to assess their distribution in the contaminated sediment samples. All data were summarized and presented in tabular form for clarity. No inferential statistical tests were applied as the focus was on descriptive analysis of the fungal isolates and their distribution.

RESULTS

In this study, culture-dependent screening of hydrocarbon-polluted sediment samples revealed different genera of fungi which might have utilized hydrocarbon or other sources of nutrients as a substrate for cellular activities. The sediment sample used in this study was analyzed for indigenous fungi by plating on SDA and Czapek medium.

The fungal isolates exhibited distinct colonial and microscopic characteristics. *Aspergillus niger* appeared black and powdery with radial fissures, forming simple septate and branched conidia in chains. *Penicillium notatum* had a blue-green fluffy texture with septate hyphae and flask-shaped phialides bearing unbranched chains of round conidia. *Cladosporium* spp. formed blackish-brown powdery colonies with branched acropetal chains of conidia. *Aspergillus flavus* exhibited yellowish-green conidia with radial fissures and short, rough-walled conidiophores. *Fusarium* spp. appeared smooth and whitish, containing oval microconidia and crescent-shaped macroconidia. *Trichoderma* spp. displayed a green-white powdery colony with highly branched conidiophores and distinct concentric rings. These isolates suggest diverse fungal adaptations to the hydrocarbon-contaminated environment in Table 1.

The fungal count varied between the pour plate and the spread plate methods at a 10^3 dilution factor. The pour plate method recorded 19 colonies, resulting in a fungal count of 1.9×10^4 CFU/mL, while the spread plate method yielded 6 colonies, corresponding to 6.0×10^4 CFU/mL. The difference in colony counts suggest variation in fungal distribution and growth efficiency between the two plating techniques in Table 2.

The fungal count differed between the pour plate and spread plate methods at a 10^3 dilution factor. The pour plate method recorded 14 colonies, resulting in a fungal count of 1.4×10^4 CFU/mL, while the spread plate method had 5 colonies, yielding 5.0×10^4 CFU/mL. This variation indicates differences in fungal distribution and growth efficiency across the two plating techniques in Table 3.

The distribution of fungal isolates revealed that *Aspergillus niger*, had the highest occurrence at 31.9% (23 isolates), followed by *Penicillium notatum* at 25% (18 isolates), *Aspergillus flavus* accounted for 16.7% (12 isolates), while *Trichoderma* spp. and *Fusarium* spp. had 11.1% (8 isolates) and 9.7% (7 isolates), respectively. *Cladosporium* spp. exhibited the lowest occurrence at 5.6% (4 isolates). These findings suggest that *Aspergillus niger* is the dominant fungal species in the sampled hydrocarbon-contaminated sediment in Table 4.

Table 1: Colonial and microscopic morphologies of six (6) fungal isolates from a hydrocarbon contaminated intertidal sediment

Tentative isolate	Colonial appearance	Microscopic examination
Aspergillus niger	Black powdery with radial fissures	Simple septate and branched conidia in chains
Penicillium notatum	Blue-green fluffy with radial fissures	Septate hyphae with branched conidiophores attached to metula. Flask-shaped phialides bearing unbranched chains of round conidia are attached to the metula
Cladosporium spp.	Blackish-brown powdery conidiophore	Hyphomycete forming branched acropetal chains of conidia, each with a distinct hilum
Aspergillus flavus	Yellowish-green conidial withradial fissures	Short conidiophores with rough walls. Phialides are both Uniseriate and biseriate, cover the entire vesicle, and point out in all directions
Fusarium spp.	Whitish smooth and shinning	Small, oval microconidia mixed with smaller numbers of crescent-shaped macroconidia
Trichoderma spp.	Green-white powdery with radial fissure	Highly branched conidiophore, loosely or compactly tufted distinct concentric ring or borne along the scant aerial hyphae

Table 2: Total plate count of fungal isolates on SDA media

Plate	Dilution factor	Number of colonies	Fungal count
Pour plate	10^3	19	1.9×10^4
Spread plate	10^3	6	6.0×10^4

Table 3: Total plate count of fungal isolates on Czapek media

Plate	Dilution factor	Numbers of colonies	Fungal count
Pour plate	10^3	14	1.4×0^4
Spread plate	10^3	5	5.0×0^4

Table 4: Prevalence of fungal isolates from the hydrocarbon impacted sediment

Fungi isolates	Frequency	Occurrence (%)
Trichoderma spp.	8	11.1
Fusarium spp.	7	9.7
Aspergillus flavus	12	16.7
Cladosporium spp.	4	5.6
Penicillium notatum	18	25
Aspergillus niger	23	31.9

Table 5: Physicochemical properties of the sediment

Parameter	Unit	Results
pH	-	9.33
Total hydrocarbon content (THC)	mg/kg	11.82
Total petroleum hydrocarbon (TPH)	mg/kg	4.216
Polycyclic aromatic hydrocarbon (PAH)	mg/kg	2.064
Electrical conductivity	µS/cm	1420
Temperature	°C	27.24
Nitrate	mg/kg	1.647
Phosphate	mg/kg	0.773
Sulphate	mg/kg	22.832
Iron	mg/kg	14.323
Copper	mg/kg	0.102
Zinc	mg/kg	0.164
Chromium	mg/kg	0.011

The physicochemical analysis of the hydrocarbon-contaminated sediment revealed an alkaline pH (9.33) and a total hydrocarbon content (THC) of 11.82 mg/kg, confirming contamination. The total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) levels were 4.216 mg/kg and 2.064 mg/kg, respectively. Electrical conductivity measured 1420 µS/cm, while the temperature was 27.24°C. Nutrient concentrations varied, with nitrate (1.647 mg/kg), phosphate (0.773 mg/kg), and sulphate (22.832 mg/kg). Heavy metal analysis showed iron (14.323 mg/kg), copper (0.102 mg/kg), zinc (0.164 mg/kg), and chromium (0.011 mg/kg). These results indicate hydrocarbon contamination with potential anthropogenic influence on sediment quality in Table 5.

DISCUSSION

The continuous discharge of crude oil into the environment has been shown to cause shifts in microbial populations, potentially leading to both increases and decreases in certain microbial communities[32]. In this study, the fungal genera identified have been associated with the degradation of hydrocarbons, including crude oil, polycyclic aromatic hydrocarbons, and refined petroleum products[7]. The presence of these indigenous fungal species in hydrocarbon-contaminated intertidal sediments highlights their ability to adapt to the challenging conditions associated with hydrocarbon pollution. This adaptability positions these fungi as promising candidates for bioremediation applications.

Furthermore, the ability of these fungal species to utilize hydrocarbons as a carbon source underscores their potential for environmental remediation in areas affected by hydrocarbon contamination[33]. The physical and chemical analysis of the sediment samples confirmed the presence of hydrocarbon contamination and suggested that under suitable conditions, certain fungal species, particularly *Aspergillus niger* could play a significant role in hydrocarbon degradation. These fungi are thus potential candidates for inclusion in bioremediation screening tests (Table 4).

The monitoring of sediment quality revealed the extent of hydrocarbon contamination and highlighted the impact of human activity along the shoreline. These findings emphasize how various environmental factors, such as pollution and human intervention, can influence the diversity, distribution, and abundance of microorganisms in such ecosystems. These results offer insight into the microbial dynamics within contaminated environments and the potential for bioremediation efforts (Table 5).

The identification and characterization of *Penicillium notatum*, *Cladosporium* spp., *Aspergillus flavus, Fusarium solani*, and *Trichoderma* spp. as indigenous fungi in a hydrocarbon impacted environment provide potential alternatives for bioremediation efforts as this fungal species is believed to possess potentials for hydrocarbon degradation (Table 1). These species may offer a diverse array of hydrocarbon-degrading capabilities if screened for hydrocarbon degradation ability, thus they can be valuable resources for developing effective bioremediation strategies tailored to the specific hydrocarbon contaminants present in the environment.

The enumeration of indigenous fungal species in the sediment samples, based on the total plate count technique, showed fungal concentrations ranging between 1.9×10 and 6.0×10 CFU/g on Sabouraud Dextrose Agar (SDA) and between 1.4×10 and 5.0×10 CFU/g on Czapek medium. These findings highlight the notable presence and diversity of these fungi in such an extreme environment. Given their abundance, these species are promising candidates for further investigation, particularly for their potential in hydrocarbon degradation. As such, they could serve as valuable microorganisms in future studies focused on bioremediation of hydrocarbon pollutants (Table 2-4), especially those within the sampled site.

Aspergillus niger, in particular, has been identified as a potential hydrocarbonoclastic fungus in this study with regards to its frequency of occurrence (Table 4). Its hydrocarbon-degrading potential makes it a valuable asset for bioremediation efforts in hydrocarbon-contaminated environments. The isolation of *Aspergillus niger* from the intertidal sediment holds promise for future applications in bioremediation strategies[34]. Different study has also reported similar degrading ability for *Aspergillus* sp.

Fungi have been found to be more effective degraders of petroleum. A strain of *Aspergillus* spp was found to be the best degrader when using DCPIP (Dichlorophenolindophenol) to determine the degradation rate of some petroleum products[35]. Fungi have been found to utilize hydrocarbons and its derivatives as carbon sources which is a criterion for their multiplication. They are usually present in soil samples

contaminated with hydrocarbons, with low molecular weight hydrocarbons that are bioavailable for their use[36]. Therefore, the relatively high percentage of potential hydrocarbon degraders recorded in the sediment may be as a result of the stimulating effect of supplementary carbon source for energy and growth[37]. Previous works related to biodegradation of hydrocarbon mainly focused on contaminated water[38] with the exception of a study that evaluated the microbial transformation of these compounds in soil and intertidal sediments where it demonstrated that hydrocarbons are more easily and readily biodegraded[39]. Researchers have demonstrated that in some cases, hydrocarbon can be applied in contaminated areas as an enhancement agent in bioremediation processes[40]. Fungi are known for their ability to form biofilms, a characteristic that enhances their effectiveness in remediating hydrocarbons in challenging environments, such as intertidal sediments. These environments, often difficult to restore, can benefit from fungal biofilm activity. In a study evaluating the degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils, *Fusarium* sp. demonstrated the highest degradation rate among the tested fungal species, achieving a 27.5% reduction in contamination. This indicates that *Fusarium* sp. could play a significant role in hydrocarbon bioremediation in intertidal sediment areas[35].

This study provides valuable insights into the fungal species present in hydrocarbon-contaminated intertidal sediments. However, it is limited by the geographical scope, as samples were collected from only one site along the Bonny River. The impact of seasonal variations on microbial diversity and hydrocarbon degradation was not explored, which could influence the results. Additionally, the degradation potential of the identified fungal species was assessed in a controlled laboratory setting, and further in situ studies are needed to evaluate their effectiveness in natural environments. Future research should focus on exploring the metabolic pathways of these fungi, particularly *Aspergillus niger*, to better understand their bioremediation capabilities. Expanding the study to include other contaminated areas and seasonal sampling would provide a more comprehensive understanding of fungal adaptability and their potential role in sustainable environmental cleanup.

CONCLUSION

This study isolated and characterized indigenous fungi from hydrocarbon-contaminated intertidal sediments of Amadi-Ama Creek, Bonny River. Species including *Aspergillus niger*, *Penicillium notatum*, *Aspergillus* flavus, *Fusarium* spp., *Trichoderma* spp., and *Cladosporium* spp. showed significant potential for hydrocarbon degradation, with *Aspergillus niger* being the most dominant. These fungi are promising candidates for bioremediation, and further studies using hydrocarbon degradation assays and molecular techniques are recommended to optimize their application in environmental cleanup.

SIGNIFICANCE STATEMENT

This study highlights the critical role of fungi in managing hydrocarbon pollution, a growing environmental challenge. By identifying fungal species capable of degrading hydrocarbons in contaminated intertidal sediments, the research offers valuable insights for developing sustainable bioremediation strategies. As ecosystems continue to face pollution from human activities, the findings underscore the potential of utilizing indigenous microorganisms for environmental restoration, helping to safeguard biodiversity and improve the health of affected habitats.

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