

Toxicity of Soot Against Microorganisms Isolated from Artisanal Crude Oil Refining Sites in the Niger Delta

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ABSTRACT

Background and Objective: Soot is a mass of impure carbon, considered to be an airborne contaminant in areas where artisanal crude oil refining takes place. This study aimed to isolate microorganisms from soot-contaminated plants, water and soil samples within the vicinity of artisanal crude oil operations in Tombia Kingdom, Rivers State, Nigeria. **Materials and Methods:** Isolates were identified using molecular technique, by comparing their 16S rRNA and ITS genes with previously identified microorganisms in the NCBI data. A biotoxicity assay was conducted to determine the effect of different concentrations (1, 10, 100, 1000 mg/L) of soot against selected isolates. Microbial growth was monitored for 24, 48, 72 and 96 hrs. **Results:** Mean concentrations of the heavy metals in soot ranged from 1.29 ± 1.80 - 25.09 ± 32.51 mg/kg during the wet season and from 8.52 ± 0.20 - 80.51 ± 0.61 mg/kg during the dry season. The isolates used for the biotoxicity assay closely matched with six bacteria, *Enterobacter asburiae* (61.6%), *Pantoea dispersa* (100%), *Kocuria rhizophila* (98%), *Bacillus cereus* (98.8%), *Bacillus subtilis* (100%), *Enterobacter bugandensis* (100%) and one fungus *Exophiala dermatitidis* (100%). All isolates were able to grow in the presence of high concentrations of soot, for 96 hrs. **Conclusion:** Functional genes for hydrocarbon degradation (alkB and PAH) were detected in some of the isolates. The isolates showed tolerance to soot and could be useful in bioremediation of polluted soil.

KEYWORDS

Soot, bioremediation, artisanal crude oil, biotoxicity, functional genes, hydrocarbon degradation

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INTRODUCTION

Artisanal oil refining is small-scale crude oil processing or continuous distillation of petroleum that is frequently illegal under state law. A visible feature of the skyline in many areas of the Niger Delta, is a black cloud of soot, predominantly from illegal refineries, gas flares from petrochemical industries and refineries, burning of fuels and burning of vehicle tyres. While multinational oil corporations add ominously to oil pollution and environmental debasement in most oil-producing countries, the extent to which illegal artisanal refineries contribute to Niger Delta environmental quandaries is unknown¹. Existing literature attributes this to the artisans' expanding activities as well as the use of crude technology in illegal oil refining².



Soot is a common cause of vegetation, water, soil and air pollution in the Niger Delta³. The spread of soot hurts human health and has contributed to more greenhouse gas emissions than all other sources in Sub-Sahara Africa combined⁴. Crude oil pollution plays a major role in impacting on microbial population in the soil^{5,6}. The environmental determinants of bacterial community structure in soil environments contaminated with complex hydrocarbon mixtures are influenced by factors which include the physical, chemical and biological characteristics of soil type, time and contaminant mixture type⁷.

Soot as a pollutant is an important factor that can affect the biosphere because of its toxicity⁸. Garza *et al.*⁹ assessed the effect of soot on human epithelial (lung) cells and found that soot induced toxicity in the cells after 48 hrs of exposure. When it comes to scientific research and investigations, the study of soot from a microbial standpoint has received little attention. Soot has an inhibitory effect on both Gram-positive and Gram-negative bacteria^{10,11}. The most prevalent microorganisms linked with black soot include fungal species *Aspergillus*, *Penicillium*, *Furasum*, *Cladosporium* and *Mucor* and bacteria genera *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Micrococcus* and *Streptococcus*^{3,12}.

In recent times, artisanal refining operations have been fingered for the growing incidence of black soot pollution in the Niger Delta¹³⁻¹⁵. Given the spread of the artisanal oil refineries in the Niger Delta expanse, their contribution to ecological defilement can no longer be overlooked. To return polluted ecosystems to normality, pollution must be mitigated or minimized at the source. Pollution effects can be alleviated by bioremediation using microorganisms that are tolerant to the pollutants in the environment¹⁶⁻¹⁸.

The toxicity of soot to microorganisms is a pointer to the danger associated with inhalation or eating food contaminated with soot. Artisanal crude oil refineries operating within the Tombia Kingdom, produce large amounts of soot, with the potential to harm living beings, with grave consequences for the human population. This study aimed to assess the toxicity of soot on microorganisms isolated from soil, water and plants within the vicinity of artisanal crude oil refining sites in the Niger Delta, with the view of finding likely candidates for bioremediation of soot-polluted matrixes.

MATERIALS AND METHODS

Study area: The study was conducted in Tombia Kingdom, Within Asari-Toru and Degema Local Councils, Rivers State, Nigeria. The location falls within the geographical coordinates 4°53'12.7" North, 7°07'30.6" East. The population is approximately 15,000 persons as of the census of 2006. Artisanal oil refining operations are prevalent in the riverine area of Igia-Ama. However, the people are traditionally farmers, fishers and traders. The study was conducted during the dry and wet seasons of 2022 (January-July).

Sample collection: Soil samples were collected from three locations within 50 m of artisanal crude oil refining sites at Igia-Ama, Tombia Kingdom, Rivers State. *Ipomoea involucrata* and *Chromolaena odorata* (five samples each) were collected within the same location. Water was collected from three points along the river bordering the artisanal crude oil refining sites. The soot sample was trapped by suspending white linen cloth 1 ft above the ground close to the artisanal refinery structure overnight.

Chemical analysis of soot: Heavy metal contents were determined using the APHA (3030E) Atomic Absorption Spectrophotometry (AAS) AA500 PG method. Polycyclic aromatic hydrocarbons (PAH), total petroleum hydrocarbon (TPH), benzene, toluene, ethylbenzene and xylene (BTEX) and polychlorinated biphenyls (PCB) were determined using EPA 8015 and EPA 8100 methods using Gas Chromatography and Flame Ionization Detector (GC/FID).

Isolation of microorganisms: One gram of plant/soil samples was macerated/homogenized and dissolved in 9 mL of distilled water, while 1 mL of water sample was dissolved in the same volume of distilled water to obtain 10⁻¹ dilution for serial dilution, up to 10⁻⁶ dilution. Aliquots of 0.1 mL dilution from

10^{-2} and 10^{-3} were plated in duplicates on potato dextrose agar and from 10^{-5} and 10^{-6} on nutrient agar plates using the spread plate technique. Plates were incubated at room temperature for 24-78 hrs. Discrete colonies were picked, subcultured and subsequently used for the biotoxicity assay.

Genomic DNA extraction and sequencing: Extraction was done using a ZR fungal/bacterial DNA mini-prep extraction kit (Zymo Research, California, USA). The 16S rRNA region of the rRNA genes of the isolates was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers. The ITS region of the rRNA genes of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3, primers.

The extracted genomic DNA was quantified using the Nanodrop 1000 Spectrophotometer (Thermo Fisher, Massachusetts, USA). The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator (Biobase, Jinan, Shandong, China).

Alkane monooxygenase (AlkB) and phenylamine hydroxylase (PAH) gene amplification: The DNA extracts were used for AlkB PCR in reactions with AlkB-1f 5'-AAYACNGCNCAYGARCTNGGNCAYAA and AlkB-1r 5'-GCRTGRTGRTCNGARTGNCGYTG primers.

The DNA extracts were used for PAH genes PCR in reactions with PAHF: 5' CGCCTGTGTATTATCTCCCT-3' and PAH R: 5'-CGAGTAGTCCACCAGATCCT-3' primers.

Soot toxicity assay: All isolates were grown on Bushnell Haas broth with different concentrations of soot concentrations of 1, 10, 100 and 1000 mg/L and subsequently cultured on Bushnell Haas agar and incubated at 37°C. The viable count method was adopted to determine the level of growth of the test isolates because of the interference of soot within the broth medium. Microbial growth was monitored for 24, 48, 72 and 96 hrs.

RESULTS

Chemical composition of soot: The mean concentration of As was 80.51 ± 0.61 mg/kg; Cd 15.32 ± 0.12 mg/kg; Cr 81.49 ± 1.29 mg/kg; Fe 177.43 ± 3.49 mg/kg; Cu 8.52 ± 0.20 mg/kg; Ni 73.48 ± 0.89 mg/kg; Hg 32.19 ± 0.86 mg/kg; Pb 28.68 ± 1.11 mg/kg; Ti 33.29 ± 1.11 mg/kg; Se 39.20 ± 0.14 mg/kg and Zn 29.96 ± 0.51 .

The mean concentrations of PAH, TPH, BTEX and PCB in soot are as follows: PAH 355.43 ± 37.06 mg/kg; TPH 549.71 ± 22.15 mg/kg; BTEX 0.44 ± 0.03 mg/kg and PCB 2.82 ± 0.57 mg/kg.

Microbial isolates: Table 1 shows microorganisms isolated from black soot and used for toxicity bioassay. The isolates closely matched with six bacteria *Enterobacter asburiae* (61.6%), *Pantoea dispersa* (100%), *Kocuria rhizophila* (98%), *Bacillus cereus* (98.8%), *Bacillus subtilis* (100%), *Enterobacter bugandensis* (100%) and one fungi *Exophiala dermatitidis* (100%).

Table 1: Microbial isolates and their accession numbers

Sample	Isolate code	Scientific name of closest species	Accession number	Similarity (%)
Plant	Dd1	<i>Enterobacter asburiae</i> MH196944	OL638122	61.6
Plant	Dd5	<i>Pantoea dispersa</i> MZ562882	OL638131	100
Water	Dd10	<i>Kocuria rhizophila</i> MZ646098	OL638132	98
Soil	Dd13	<i>Bacillus cereus</i> OK67451	OL638134	98.8
Soil	Dd14	<i>Bacillus subtilis</i> MK757669	OL638135	100
Soil	Dd16	<i>Enterobacter bugandensis</i> CP083641	OL638136	100
Soil	Y1	<i>Exophiala dermatitidis</i> LC636194	OL638137	100

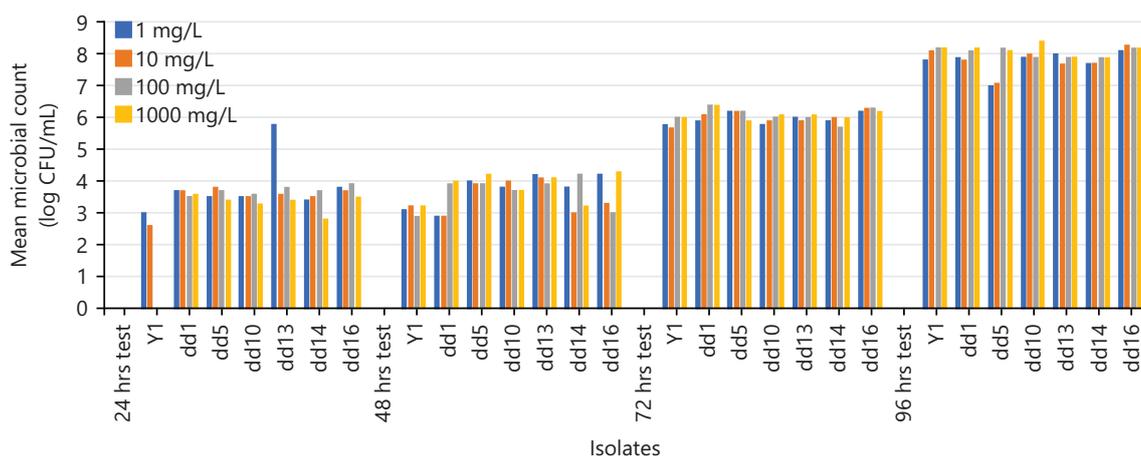


Fig. 1: Growth variation of microbial isolates at different soot concentrations (24, 48, 72 and 96 hrs test)

Table 2: Genes for hydrocarbon degradation

Isolate code	Scientific name	AlkB	PAH
Dd1	<i>Enterobacter asburiae</i>	+	-
Dd5	<i>Pantoea dispersa</i>	+	+
Dd10	<i>Kocuria rhizophila</i>	-	-
Dd13	<i>Bacillus cereus</i>	+	+
Dd14	<i>Bacillus subtilis</i>	+	+
Dd16	<i>Enterobacter bugandensis</i>	+	-
Y1	<i>Exophiala dermatitidis</i>	-	-

+: Present and -: Absent

Genes for hydrocarbon degradation: Table 2 shows genes for hydrocarbon degradation. *Bacillus cereus*, *Bacillus subtilis* and *Pantoea dispersa* possessed both harboured *AlkB* and *PAH* genes. *Enterobacter bugandensis* and *Enterobacter asburiae* possessed only *AlkB* gene while both genes were not detected in *Exophiala dermatitidis* and *Kocuria rhizophila*.

Toxicity assay: Figure 1 shows the growth of the isolates at different soot concentrations, over the test period (24-96 hrs). All isolates grew at all soot concentrations except *Exophiala dermatitidis*, which only grew at low soot concentrations within the first 24 hrs.

DISCUSSION

Chemicals detected in the soot samples were heavy metals (As, Cd, Cr, Fe, Cu, Ni, Hg, Pb, Ti, Se and Zn), PAH, TPH, BTEX and PCB. All heavy metals monitored were detected in soot, with mean concentrations ranging from 8.52 mg/kg (Cu) to 177.43 mg/kg (Fe). The values are higher than those reported by Obi-du *et al.*¹⁹ [As not detected, Cd 0.276-2.734 (ppm), Cr 10.932-30.802(ppm), Pb 0.483-1.132 (ppm), Ni 0.042-1.344 (ppm), except Cu 2.106-11.033 (ppm)].

Long-term exposure to As and Cr can cause cancer²⁰. Exposure to high doses of Cu can exasperate the nasal lining, cause aches and vertigo and eventually lead to death²¹. Gulp of air containing Ni can adversely affect health, causing contracted lung function, chronic bronchitis and cancer of the nose and lung²². Mercury can cause damage to the brain, nervous system, kidneys and liver; causes neurological anomalies (including lower IQ) developmental birth defects, as well as behavioural problems such as attention deficit hyperactivity disorder and cardiovascular effects^{23,24}. Effects of lead toxicity include anaemia; high blood pressure; brain and kidney damage; neurological disorders; cancer; lowered IQ; behavioural problems; immune effects; and reproductive hazards^{20,24,25}.

The mean concentrations of PAH, TPH, BTEX and PCB in soot are as follows: PAH 355.43±37.06 mg/kg; TPH 549.71±22.15 mg/kg; BTEX 0.44±0.03 mg/kg and PCB 2.82±0.57 mg/kg. The PAH, TPH, BTEX and PCB in soot have been reported in other studies, from incomplete combustion of organic compounds²⁶. The concentration of the chemical pollutant is dependent on the source²⁷. These pollutants have been implicated in several human pathologies including heart diseases, respiratory problems, metabolic impairment, birth defects and diabetes mellitus among others²⁸⁻³¹.

The chemicals in soot can either stimulate or inhibit microbial growth. In the present study, all tested isolates (*Enterobacter asburiae*, *Pantoea dispersa*, *Kocuria rhizophila*, *Bacillus cereus*, *Bacillus subtilis* and *Enterobacter bugandensis*) grew at all soot concentrations except *Exophiala dermatitidis*, which only grew at low soot concentrations within the first 24 hours. However, the overall trend showed an increase in growth over time. This was in agreement with the observation by Amala *et al.*¹¹ that microbial growth is indirectly proportional to the concentration of soot they are exposed to. Unlike in the present study, for the case of isolate Y1 (*Exophiala dermatitidis*). Holman³² reported that bacteria failed to grow at low soot concentrations but grew at higher concentrations of the same soot. In agreement with the present study, Ugboma *et al.*³³ reported that microorganisms isolated from soil impacted by artisanal crude oil refining grew well in the presence of heavy metals and were efficient in the elimination of heavy metals from the soil. Soot contains hydrocarbons in low amounts relative to crude oil. The low concentration of hydrocarbons in soot could have supported microbial growth as suggested by Adieze *et al.*³⁴. Mari *et al.*³⁵ reported that soot caused an initial decrease in microbial abundance but eventually led to over increase in bacterial productivity.

Bacillus cereus, *Bacillus subtilis* and *Pantoea dispersa* possessed both harboured *AlkB* and *PAH* genes. *Enterobacter bugandensis* and *Enterobacter asburiae* possessed only *AlkB* gene while both genes were not detected in *Exophiala dermatitidis* and *Kocuria rhizophila*. *AlkB* and *PAH* genes have been reported as being responsible for biological degradation or remediation of hydrocarbon-polluted environments such as land and water³⁶⁻³⁸. The *AlkB* gene is present in a wide range of bacteria and responsible for the degradation of aliphatic hydrocarbons and also BTEX^{39,40}. The *AlkB* gene codes for a non-heme iron-dependent oxygenase enzyme that can oxidize a variety of alkyl groups present in hydrocarbons, leading to the formation of alkanols and aldehydes, which are subsequently utilized by the bacteria as carbon sources⁴¹. The isolates without *AlkB* gene may rely on other genes in alkane oxidation pathways such as rubredoxin-2 rubredoxin-21AlkF (*AlkG*), aldehyde dehydrogenase (*AlkH*), alcohol dehydrogenase (*AlkI*), rubredoxin reductase (*AlkT*) and disulfide isomerase⁴².

This study has shown that microorganisms isolated from artisanal crude oil refining sites are tolerant to soot and therefore could be useful for the remediation of soot-polluted soil emanating from artisanal crude oil refining. The study showed that some isolates possessed hydrocarbon degradation genes. However, the study only screened for two of such genes.

CONCLUSION

The study revealed isolates have the capacity for degradation of soot caused by the operations of artisanal refineries in the study area. Genes for hydrocarbon degradation were detected in some of the bacterial isolates. All isolates grew at all soot concentrations except *Exophiala dermatitidis*, which only grew at low soot concentrations within the first 24 hrs.

SIGNIFICANCE STATEMENT

This study aims to determine the toxicity of soot from artisanal refineries on microbial species and screen for microorganisms that could be useful for the bioremediation of contaminated soil. Six bacteria species and a fungus were able to grow in the presence of high concentrations of soot. Functional genes for hydrocarbon degradation (*AlkB* and *PAH*) were detected in some of the isolates. This research is important because it is one of the first reports on the toxicity of soot from artisanal refineries in the study area.

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