Human respiratory tract microbial pathogens associated with asphalt production

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Abstract

Asphalt remains a major source of microbial distribution in the world. The major aims and objectives of this prospective study were to determine the presence and prevalence rates of different pathogenic microbial agents, and analyse possible health implications of asphalt contamination. Sampling was carried out at some asphalt industries in Abia State by exposing bacterial and fungal culture media to asphalt fumes, with standard controls. Culture plates were transported aseptically to the microbiology laboratory of Abia State University, Uturu for isolation and characterisations. The prevalence rates of *Bacillus* species, *Staphylococcus aureus*, *Klebsiella aerogenes, Micrococcus* species, *Escherichia coli,* and *Pseudomonas aeruginosa*, were 90%, 40%, 90%, 100%, 40%, and 40%, respectively. Among the fungal organisms isolated were *Rhizopus species (60%), Aspergillus fumigatus (40%), Aspergillus fumigatus (40%), Aspergillus niger (90%), and Candida* species *(100%)*. These pathogenic microorganisms are of serious public health significance, because they pose serious risks for possible carcinogenity and respiratory microbial infections. The government needs to intensify regulations relating to the construction of asphalt industries in and around domestic dwellings.

Introduction

Asphalt is a black or dark brown organic material that melts readily and then flows easily after exposure to the air or impurities. The material becomes solid to the point of hard with a conchoidal fracture. Its composition varies widely but it is basically a hydrocarbon chain of molecules.[1]

Asphalt is the final product of the refining process of crude oil when higher constituents are distilled off, such as naphtha, kerosene, and gas oils. It has been used for constructing road surfaces, house building, and in the construction of ships.[2] It is commonly presumed to be hostile to life; nevertheless, a complete ecosystem has long been found not only living on the asphalt but also apparently feeding on it.[3]

For the past few years, the utilisation of bituminous materials by microorganisms has been studied. Harris and colleagues have isolated hydrocarbon-utilising bacteria from the soil and asphalt, and further demonstrated the ability of these organisms to degrade asphalts.[3]

The occupational safety and health administration (OSHA) of the United States undertook a microbiological air sampling to determine the total microbial flora and endotoxin on the leaves of plants on a site in Cincinnati, Ohio, which is to be used for an asphalt producing company. A second sampling was carried out 2 years ago, after blowing out the asphalt fumes. The results were that more viable and culturable fungi and bacteria occurred after this second air sampling.[4]

The discovery of those organisms has important application for biotechnology, including the possible production of new enzymes for use in manufacturing chemicals from petroleum surfactants. It also improves oil recovery rate.[5]

The major problem facing the use of asphalts is that the organisms it harbours may be pathogenic to man and animals.[6] The health implications make developed countries site their asphalt industries several metres away from residential buildings.[6]

In 1977, a critical document, *The America Conference of Government Industries Hygienist* (ACGIH) established that the recommended exposure limit (PEL) for aspalt be 5 mg/m^3 up to 10 hours work shift. This was recommended to reduce the risk of possible carcinogenicity and microbial infection.[7]

Materials and methods

Study area

This study was carried out in some asphalt producing industries situated in Umuagu, Umuahia South Local Government Area, Abia State Capital, Nigeria.

The maximum temperature of this region occurs between March and April with the rainy season and dry season being quite distinct in this area. The main occupation of inhabitants is farming, local trading, and the Civil Service.

Approval

Approval for this study was given by these companies who served as case studies.

Sample collection

The asphalt samples were collected at the asphalt producing companies in Umuagu by directly exposing already prepared culture media to asphalt fumes in the asphalt

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heating chamber. Another group of sampling was done by exposing some other culture media in different plates to air outside the Asphalt producing industries. The later group of culture plates served as the control.

These culture plates were aseptically transported to the microbiology laboratory of Abia State University, Uturu within 3–4 hours of culturing for incubation and characterisations. Asphalt exposure was done on nutrient Agar, (Lab M), MacConkey Agar (Lab M), Sarboraude dextrose Agar (Lab M), and incubated at 25°C to 37°C for 24hours. Incubation on Sarboraude dextrose Agar was done for 7 days.

Identification of bacterial and fungal isolates.
The bacterial isolates were identified on the basis of their colonial/cultural characteristics, staining reactions, and other biochemical reactions such as spore stain, catalase reactions, coagulase reactions, indole reactions, oxidase reactions, Voges-Proskauer reactions, sugar fermentation test, citrate reactions, methyl red reactions, etc.

The identification and characterising of fungal isolates was based on spore stain and lactophenol cotton blue staining techniques as recommended by H C Okereke.[16] Acridine orange stain (pH 4.5), periodic acid-Schiff (PAS) stain, and rapid methenamine silver stain were carried on fungal isolates using methods previously described by Mahan and Sale.[17] Germ tube test was also carried out on *Candida* organisms using egg albumen, which is more stable than human serum, to write-off *Candida albicans* from other members of the genus *Candida*. However, the study did not isolate *Candida albicans*.

Results

Bacterial and fungal organisms were obtained from the microbiologic environmental auditing of asphalts from Umuagu Asphalt Industries.

The prevalence's of bacterial species at 95% confidence intervals were 90%, 40%, 90%, 100%, 40%, and 40% for *Bacillus* species, *Staphylococcus aureus*, *Klebsiella* aerogenes, *Micrococcus* species, *Escherichia coli, and Pseudomonas* species respectively. The prevalence of fungal isolates were 60%, 40%, 90%, 90%, and 100% for *Rhizopus* species, *Penicillium* species, *Aspergillus fumigatus, Aspergillus niger*, and *Candida* species (see Table 1).

Table 1 Prevalence of bacterial and fungal organisms isolated from Umuagu Asphalt Industries, Abia State, Nigeria

Bacterial Species	Prevalence rate (n=20)	Fungal spieces	Prevalence rate (n=20)	(95% confidence interval)
Bacillus species	18 (90%)	*Rhizopus* species	12 (60%)	(0.94–1.60)
Staphylococcus aureus	18 (40%)	*Penicillium* species	8 (40%)	(2.2–3.80)
Klebsiella species	18 (90%)	*Aspergillus fumigatus*	18 (90%)	(3.7–6.2)
Micrococcus species	20 (100%)	*Aspergillus niger*	18 (90%)	(2.60–3.90)
Escherichia coli	8 (40%)	*Candida* species	20 (100%)	(0.93–1.0)
Pseudomonas species	8 (40%)			

Discussion

From this study, the following six bacterial organisms were isolated and identified: *Bacillus* species, *Staphylococcus aureus*, Micrococcus, *Klebsiella* species, *Pseudomonas aeruginosa, Micrococcus* species.

The bacterial species most prevalent was *Micrococcus* species (100%). *Bacillus* species (90%) and *Klebsiella sp* (90%) were second most prevalent while *Aspergillus niger* (100%) and *Candida* species (100%) were the most prevalent of the fungal isolates. *Aspergillus fumigatus* (80%) was also frequently observed in the asphalt samples analyzed. The presence of *Candida* species is not surprising, as the organisms have been implicated in metabolizing hydrocarbon.

The presence *of Aspergillus fumigatus*, which is spore producing, posses serious respiratory disease implications. It is also known that *Aspergillus* species are the primary causes of Aspergillosis in humans.

The toxins produced by some strains of the genus *Aspergillus* have been shown to be immunotoxicant causing a variety of acute inflammatory diseases.[13]

The presence of *Bacillus sp*, *Klebsiella* species, *Micrococcus, Escherichia coli,* and *Pseudomonas* is a risk factor for development of serious respiratory tract infections as these organisms could escape into the environment through water droplets contained in the asphalts.

Factory workers, road construction workers, and inhabitants of Umuagu where the factory is situated are at higher risk of infection.

In comparison with other studies, the presence of *Pseudomonas* species and *Bacillus* species tallies with the work done by Mitchell,[9] who worked on microbial degradation of asphalt. Also the presence of *Pseudomonas spp* in this investigation tallies with the work carried out by Pendrys,[10] who worked on biodegradation of asphalts and cement by aerobic bacteria. These bacteria were isolated from cultures enriched for asphalt-degrading bacteria.

The presence of *Micrococcus* species and *Staphylococcus* species in this work also tallies with the work carried out by King et al,[11] who sampled asphalt construction company equipments in California, USA. They obtained *Micrococcus* species and *Staphylococcus* species, and of these, *Staphylococcus aureus* was the most prevalent organism.

The isolation of *Bacillus sp*, *Escherichia coli,* and *Pseudomonas* species also in this study agrees with a seven-plate protocol sample collection carried out in an Asphalt factory in Sweden by Nardel and colleagues.[12]

The presence *of Aspergillus* species

and *Penicillium* species observed in this study tallies with the microbial air sampling carried out by occupational safety and health Association (OSHA) in the United States in 1992. With an asphalt fuming company, they carried out a 2-year study of the fungal species present in asphalt and isolated *Aspergillus* and *mycotoxins*.[4] Mitchell et al[9] in a similar work isolated gram-negative bacteria with endotoxin producing gene. The production of this endotoxin is believed to aid their survival in this environment (asphalt).

The isolation of C*andida* species contradicts the work done in Poland, which confirmed the presence of *Trichosporum sp,* with a 54% prevalence in an asphalt loading truck.[15] In addition, this work tallies with a similar work done by Kalu[8] which microbiologically evaluated asphalt in the same Umuagu asphalt industry that is the same study area for this work. The small difference between the two works is that Kalu isolated T*richosporium sp* and *Torulopsis*, and these two fungal isolates were not isolated in our work.[8]

Notwithstanding, even in the midst of these health hazards these organisms have and will pose to humans and animals; they may still hold many promises in biotechnological advancement.

The organisms surviving the high temperature of asphalt either as thermotolerants or thermopiles must possess gene for such character, especially the non-sporing bacteria species. Michael et al.[15]

This calls for more research attention on these organisms isolated from asphalts, as asphalts could be another source of microorganisms required for generating important enzymes, which will work under the high temperature which denatures most of the enzymes required in industrial and medical process and yet remain undenatured. These processes may include: fermentation in brewery industries where *Aspergillus* and yeasts hold a lot of promises, spooligotyping, Gel Electrophoreses, pyrosequencing technology for identification of *Bacillus anthracis*, and reacted organisms, DNA – DNA hybridisations, polymerase chain reaction, etc.

The temperature at which these processes are carried out requires that relatively high heat-tolerating enzymes be employed. Asphalts could labour organisms, which will be sources of these enzymes that relatively tolerate heat.[15]

Conclusion

The study has shown that the presence of certain organisms isolated from asphalt remain pathogenic to man as NIOSH has predicted mutagenic and carcinogenic effects of them on man. Workers with asphalt-producing industries are advised to wear adequate protective materials like nose masks and gloves while working. The Nigerian Government should intensify their policy prohibiting the establishment of asphalt industry in and around human inhabiting communities (residential homes, hospitals, hostels, and schools).

The work also calls for more research attention into the promises of asphalt-colonising bacteria and fungi as they could be a source of highly priced medical, agricultural, and industrial enzymes.

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