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Fungi associated with some polluted water bodies in Delta State, Nigeria

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Abstract

Fungi associated with five different water bodies (Abraka, Oghara, Sapele, Mosogar and Ubeji Rivers) in Delta State were investigated. A total of twenty (20) samples were collected with four replicates for each water body. The water bodies except Abraka are within the oil producing area of Delta State. One ml of water sample from each of the water bodies was cultured using Sabouraud Dextrose Agar (SDA). Five fungi comprising three genera and five species were isolated and characterised: *Penicillium* species (Abraka), *Fusarium verticillioides* (Mosogar), *Aspergillus flavus* (Sapele), *A. niger* (Oghara) and *Penicillium rolfsii* (Ubeji). From the physicochemical characteristics conducted, the highest values for turbidity, conductivity and total polyhydrocarbon were found in water samples from Sapele (5.80 ± 0.55) NTU, Oghara (96.60 ± 1.47) µS/cm and Ubeji (416.67 ± 5.35) respectively. These values can be attributed to high level of pollution or low level of fungal biodegradation. Since these fungi have shown the capacity to survive and metabolize ions as decomposers in these water bodies, they have potentials for use in bioremediation in the clean-up of contaminated environment.

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Introduction

Pollution is the presence of unwanted substances in our environment (soil, water and air). When these substances are introduced into the environment (example: water), such water body is said to be polluted. Pollutants are either chemical or biological in nature (Michael, 2010). Sources of water include rivers, ponds, lakes, seas, aquifers, ocean, groundwater and reservoirs. Water from these sources are used for drinking, agricultural, industrial and other human activities. Aquatic organisms are affected by the presence of pollutants. Water pollution is not only damaging to the individual species in the environment but to the natural biological communities. Water is regarded as polluted when it does not support human use and undergoes a marked shift in its ability to support its consistent biotic communities such as fish.

Fungi are achlorophyllous, heterotrophic and spore bearing organisms surrounded by a well-defined cell wall made of chitin (Alexopoulous *et al.*, 1996). Fungi are known to degrade aromatic hydrocarbon and they adapt more readily to adverse environmental conditions of low pH as compared to most bacteria. Fungi are useful in predicting the impact of a particular stress on the environment by their ability to respond to these adverse conditions through a change in their number (Alexopoulous, 1996). Fungi are the major decomposers in some aquatic environment. The presence of fungi can be related to the level of pollution of the environment.

Bioremediation is a means of cleaning-up contaminated environment by exploiting the adverse metabolic ability of living organisms (for example, microorganisms) to convert contaminants to harmless products by mineralization, generation of carbon IV oxide and water or conversion into microbial biomass (Mentzer and Ebere, 1996). Fungi facilitate a broad range of metabolic processes like acting as living filter for polluted environment (Schroder, 2004). Biodegradation occurs at a distressingly slow rate, but can be enhanced by inoculation with microbial species that will degrade oil waste and sewage more efficiently, and by introducing air and nutrients into the environment (Obire, 1988). Filamentous fungi, yeasts, actinomycetes and bacteria have the ability to utilize hydrocarbon substances, though their ability to do so vary among individual strains and in some cases depend on hydrocarbon chain length (Bijoff, 2003).

Ubeji, Sapele, Mosogar and Oghara are within the oil producing areas of Delta State while Abraka is not. Ubeji River is brackish, while the other four water bodies are fresh-water bodies. Also, Sapele is known for the lumbering and sawmilling industrial activities along the bank of the river. Human activities in Delta State are farming, fishing, trading, lumbering, mining, manufacturing, palm wine and rubber tapping. Sapele is also known for power generation (power station at Oghorode). Oil exploration activities in these areas may have impacted on the ecosystem. Sources of oil spill include crude oil tankers, offshore platforms, drilling rigs and wells, as well as spills from refined petroleum products (such as gasoline, diesel and their by-products), bunker fuel (Wang *et al.,* 2000).

The main objective of this study was to evaluate the physicochemical characteristics and the fungi associated with these water bodies and to determine correlation between the presence of these fungi and the level of pollution in the environment.

Materials and Methods

Study area

The Rivers are within latitude 5°54'N and longitude 6°6'E of the equator. Ubeji is located in Warri South, Mosogar in Ethiope West, Sapele in Sapele, Oghara in Ethiope West and Abraka in Ethiope East Local Government Areas, Delta State Nigeria (Figure 1).

A total of 20 samples were collected in August, 2012 for the experiment. Four replicates of each sample were collected randomly from the five (5) water-bodies (Abraka, Sapele, Mosogar and Ubeji). Distilled water was used as the control for the experiment.The laboratory analysis was carried out in Botany and Microbiology Laboratories, Faculty of Science, Delta State University, Abraka while the isolation and characterization were done at the Pathology Laboratory, Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

Isolation and characterisation

The water samples were cultured on potato dextrose agar (PDA) (Oxoid, England) using the pour plate method (Okungbowa, 2012). A volume of 1 ml of each water sample concentration was transferred into a Petri dish. Then 20 ml of PDA, prepared according to manufacturer's instructions and containing 50 µg/ml chloramphenicol to inhibit bacteria growth, was poured into the plate. The plate was swirled round for even mixing and allowed to solidify. Control plates contained 1 ml sterile de-ionized water instead of water samples. All culture plates were incubated at room temperature (28 ± 2°C) for 7 days. Culture samples were examined under low (x 40) and high (x 100) powers of an optical microscope for fungal growth. Fungal identification was done in the Pathology Laboratory, NIFOR.

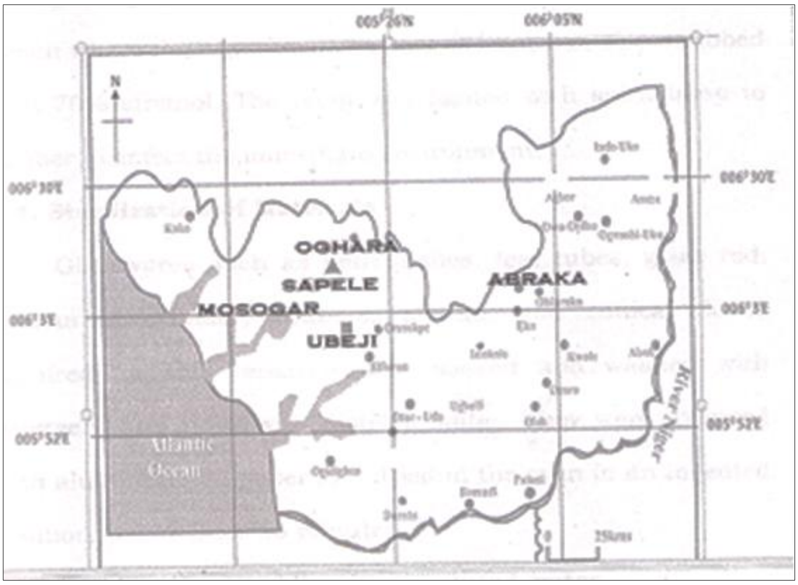

Figure 1: Map of Delta State showing the study areas

Determination of physico-chemical parameters

A pH meter (3015 Jenway, UK) was used to determine pH of the water samples, while electrical conductivity was determined with a conductivity meter, following the procedure of Ademoroti (1996). Turbidity was read using UV - Visible (72 Series) spectrophotometer; turbidity value was calculated by multiplying the instrument reading by shop reciprocal (USEPA, 2003).

Statistical analysis

Statistical analysis was carried out using t-test paired sample for means.

Results and Discussion

A total of five fungi comprising three genera and five species were isolated (Table 1) *Penicillium species* was present in Abraka; *P. rofsii* (Ubeji), *A. niger* (Oghara), *A.flavus* (Sapele) and *F. verticilliodes* (Mosogar) was isolated from water samples.

Mean values of physico-chemical characteristics of the polluted water samples are shown in Tables 2 and 3. Abraka samples had the lowest value of (1.00 ± 0.01) (NTU) turbidity while Sapele had the highest value of (5.80 ± 0.55) (NTU).

Table 1: Fungal species associated with some polluted water bodies

Water sample	Fungi	Present
Abraka	*Penicillium species*	+
Oghara	*Aspergillus niger*	+
Sapele	*A. flavus*	+
Mosagar	*Fusarium verticilliode*	+
Ubeji	*P. rofsii*	+

Table 2: Mean values of physical characteristics of the polluted water samples

Water sample	Turbidity (NTU)	Conductivity (µS/cm)	pH
Abraka	1.00 ± 0.01	29.30 ± 2.65	7.63 ± 0.59
Oghara	4.30 ± 0.06	96.60 ± 1.47	7.38 ± 0.05
Sapele	5.80 ± 0.55	32.60 ± 0.61	7.32 ± 0.10
Mosagar	1.30 ± 0.05	14.10 ± 0.90	7.30 ± 0.10
Ubeji	4.00 ± 0.01	62.20 ± 0.02	7.03 ± 0.02

Table 3: Mean values of polyhydrocarbons of the polluted water samples

Water sample	Polyhydrocarbon (TU)
Abraka	156.80 ± 2.36
Oghara	124.00 ± 5.29
Sapele	266.67 ± 5.78
Mosagar	132.00 ± 1.00
Ubeji	416.67 ± 5.35

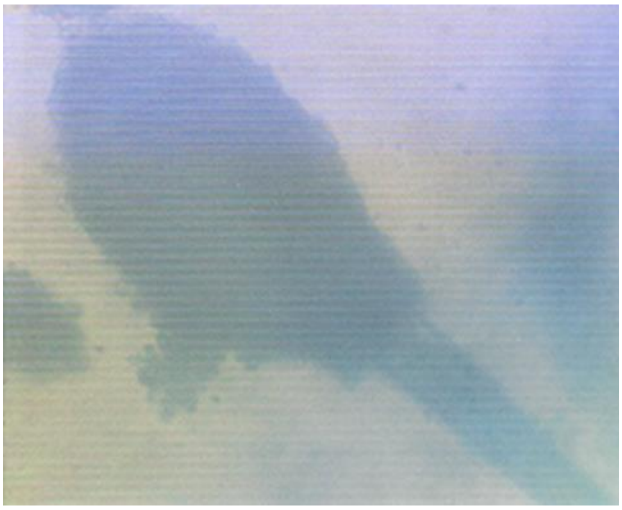

Figure 2: ***Penicillium rolfsii***

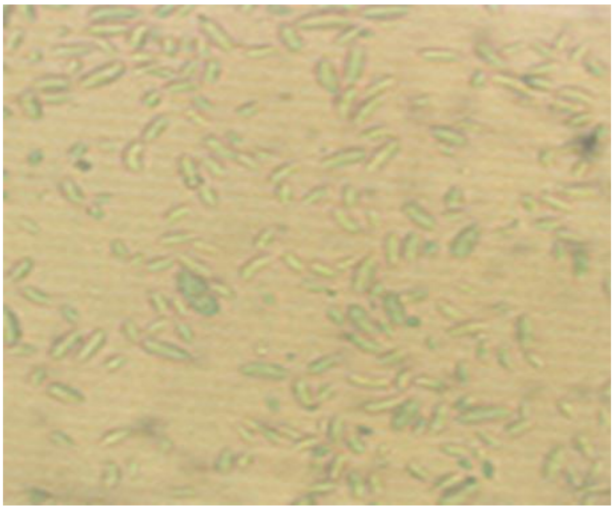

Figure 3: ***Microconidia of Fusarium verticilliodes***

The highest conductivity value of 96.60 ± 1.47 (µS/cm) was recorded for Oghara, while Mosogar had the lowest. There was no significant difference in pH levels of all samples. Polyhydrocarbon content was significantly higher ($P<0.05$) in Ubeji than all the other samples. Figures 2 and 3 respectively, show *P. rolfsii* and *F. verticilliode* spores.

In this study, five fungal species were isolated. Several *Aspergillus* species have the ability to biodegrade gasoline (Santos *et al*; 2008). They also have various uses in biotechnology as the sole carbon source when screened for hydrocarbon utilization (Oboh *et al.*, 2006). *A. niger* for example, has been reported to produce toxins including malformin and naphthopyrones and some strains are known to produce ochrotoxin. *A. flavus* is mainly saprophytic, though it can also be a pathogen to plants and animals including humans (Mellon *et al.*, 2009). The network of hyphae or mycelia in *A. flavus* are responsible for secreting catabolic enzymes, which are used to breakdown complex food substances into small molecules which are absorbed by mycelium for growth.

P. rolfsii associated with Ubeji River is a genus of the class ascomycetes and is of major importance in the natural environment of food and drug production. The conidiospores are the main dispersal route of this fungus and often green in colour. *F. verticilliodes* present in Mosogar River is a large genus of filamentous fungi widely distributed in soil in association with plants. The teleomorphic state of the fungus is *C. moniliformis* and it is a widely spread species characterized primarily by the microconidia being produced in chains and is known to be mycotoxigenic (O'Donnell *et al.,* 2012).

Fungi adapt more readily to adverse environmental conditions of low pH (Cernigia *et al.*, 1992). The optimum pH for their growth is within the range of 6 and 8 (Mentzer and Ebere, 1996). The water samples showed high alkalinity with Abraka having the highest value for alkalinity while Ubeji had the lowest value for alkalinity. Thus, fungal biodegradation action is favourable in all of the five polluted samples. Fungi have been reported previously to be present in crude oil polluted environment (Edema and Okungbowa, 2011, Edema and Okungbowa, 2012).

An increase in turbidity level indicates an increase in fungal biodegradation ability (Alan *et al.*, 2003), Thus, Abraka River had a slower rate of biodegradation compared to Sapele River. Mosogar River had the lowest electrical conductivity while Oghara River had the highest electrical conductivity value. This may be due to the high level of pollution as a result of the activities of oil companies in the area. Crude oil polluted water consists of complex mixture of hydrocarbon (Singh, 2006). Polyhydrocarbons molecular weight for Abraka, Oghara, Mosogar, Sapele and Ubeji Rivers were 156.8, 124.00, 132.00, 266.67 and 416.67 (TU) respectively. The proportion of hydrocarbons by weight varies greatly among different oil fields (Hyne, 2001). Oghara River had the lowest molecular weight of 124.00 (TU). This may account for the low level of pollution or increase in fungal biodegradation metabolic activities in Oghara River compared to Ubeji River which had the highest molecular weight.

Fungi which are adapted to survive in crude oil polluted environment have ability to degrade crude oil as shown in this study. All the isolated fungi have bioremediation potential which should be harnessed. The highest molecular weight recorded for Ubeji River may be attributed to continuous spillage as a result of increase crude oil exploration activities from Warri Petrochemical, Shell and Chevron companies within the area.

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