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Health Impact Assessment of Mangrove Vegetation in an Oil Spilled Site at the Bodo West Field in Rivers State, Nigeria

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ABSTRACT: A post-impact health assessment of the mangrove vegetation of Bodo West Field in Rivers State of Nigeria where three oil spillages occurred was carried out to determine any major changes from the baseline profile of the area 10yrs after spillage. The accidents resulted in the discharge of a total of 1860 barrels of crude oil, which cut fire as it spilled destroying the mangrove vegetation. Diseased plant samples were collected from infected plants at 50m intervals to a distance of 300m from the point of spillage along four transects in the East, West, North and South directions. Results of laboratory diagnosis showed that most of the plants especially plants along the South and East transects were heavily infected with necrotic leafspots caused mainly by *Pesalotia* and *Phomopsis* spp. *Rhizophora mangle, Rhizophora racemosa and Avicennia africana* were the most heavily infected plants. Other genera of fungal pathogens isolated were *Alternaria, Aspergillus, Botrytis, Chaetonium, Colletotrichum, Dreschlera, Fusarium Macrophomina, Penicillium, Phoma and Trichoderma*. No plant pathogenic bacteria or viruses were isolated. Oil pollution predisposed the plant to fungal disease attack and also impacted the soil and vegetation. @JASEM

Bodo West Flow Station area has suffered three oil spills. The first was in November 16, 1979, caused by a carry-over from the flare resulting in the release of 450 barrels of crude oil, which caught fire as it spilled. The second was in May 7, 1981 caused by the flow station's automatic shut down valve failure, which resulted in the spillage of 1400 barrels of crude oil into the ecosystem. The third spill was in November 28, 1986, caused by maintenance error, which resulted in the discharge of 10 barrels of oil into the environment. Bodo West is part and parcel of the extensive tidal plain of the Bonny River in the Niger Delta of River State of Nigeria. The vegetation is mainly mangrove. Petroleum crude oil contains both hydrocarbon and non-hydrocarbon components. The hydrocarbon fraction consists primarily of alkanes, aromatics and asphaltenes (Blumer and Sass, 1972) with some heavy metals (Speight, 1980). Wide ranges of microorganisms are capable of growing on the various hydrocarbons of oil. These organisms include bacteria (Atlas, 1981; Ekudayo and Obire, 1989); yeast (Okpokwasili and Amanchukwu, 1988); filamentous fungi (Davis and Westlake, 1979); algae (Walker *et al*; 1975a) and protozoa (Lindmark, 1981). On the other hand, petroleum oil is one of the sources of environmental pollutants in the Niger Delta, which affect the terrestrial and aquatic habitats. Woodwell (1970) reported the toxicological effects of crude oil on the plants and animals in polluted area. Odu *et al* (1985) isolated *Aspergillus, Cladosporium, Fusarium, Penicillium, Trichoderma and Rhizopus* species in oil-polluted soil.

This study was therefore undertaken to determine the impact of oil pollution on the health of mangrove vegetation in the Bodo West in the tidal plain of the Bonny River in River State which has suffered three major oil spills.

MATERIALS AND METHODS

Field Sampling: Four transects were cut from the edge of the gas flare bundwall of Bodo West Flow Station in the East, West, North and South directions up to a distance of 300m from the oil spilled zone. Plant samples (roots, stems, leaves and seeds) of different plant genera and species were taken on a daily basis along the transect lines for laboratory diagnostic studies. Five diseased plants of each plant type seen in the surrounding area of 0m, 50m, 100m, 150m, 200m, 250m, and 300m distances of the four transects were collected. Samples collected at the 300m points served as controls since the oil spread did not reach there. Field sampling took 7days to complete. Plant samples were taken to the laboratory and kept in the refrigerator until when used.

Laboratory Studies: For fungal diagnostic studies, acidified potato dextrose agar (APDA) medium was used initially but was later discontinued due to lots of contamination by saprophytic fungi. The Standard Blotter Method (ISTA, 1999) was used instead. The leaves of each plant type were cut to pieces of 0.5 to 1.0cm and the stems and roots were cut to pieces of 1.0 to 1.5cm making sure that the healthy and diseased areas were included in each piece. The

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seeds were not excised. Tissues pieces of the same plant type and part were pre-treated with 1% sodium hypochlorite for 3minutes. Tissues were rinsed in sterile distilled water before plating each piece on three layers of moist sterile filter papers in each Petri dish. Five pieces were plated per Petri dish, making sure that the pieces were well separated. Five Petri dishes were used for each plant part. The tissue pieces were then incubated in an incubator a 22^0 C ± 2^0C for 7days for fungal organisms to sporulate. Tissues were examined under a stereobinocular microscope (5-50X magnification) for spores and fruiting bodies for identification of fungi. Temporary spore mounts of each observed fungus were also made and observed under a light compound microscope (5-100 x-magnification) to confirm identification to genus and species level. Fungal pathogens identified according to plant type and parts were recorded.

For bacterial studies, dipping them in 70% ethanol disinfected several pieces of younger infected leaves of each plant type and rinsing three times in sterile distilled water. The pieces of leaves were put in a sterile blender and blended for 2minutes to release the sap. One millilitre of the sap solution (stock solution) was used in dilution series of 10^{-1} to 10^{-7} in test tubes containing 9ml of sterile distilled water. One millilitre of 10^{-6} and 10^{-7} dilutions were plated onto nutrient agar (NA) medium in Petri dishes. Five Petri dishes were used per plant part. With a sterile L-shaped glass rod, the solution on the medium was evenly distributed. Dishes were inverted after the solution had settled on the agar and then incubated at 30^0C ± 2^0C for 1-3days for bacteria growth. Dishes were examined each day and observations recorded.

In case of isolation of seed-borne pathogenic bacteria, 50g of seed types were soaked in 120ml of sterile distilled water for 24hrs at 5^0C (refrigerator). From soaked seeds solution, a dilution series of up to 10^{-7} was made as previously described. One millilitre of each seed-type solution (10^{-6} or 10^{-7}) was spread onto the NA medium in Petri dishes. Five Petri dishes were used for 10^{-6} or 10^{-7} dilution per seed type. After allowing the solution to settle, the dishes were incubated at 30^0C ± 2^0C for 1-3days. Dishes were examined each day for bacteria growth and observations recorded.

RESULTS

Fungal diseases recorded among the various plants sampled along the 4 transects in the oil-polluted area of Bodo West Field are shown in Tables 1,2,3 and 4. The main fungal genera commonly associated with the various plant types and parts sampled include: *Aspergillus, Cephalosporium, Colletotrichum, Fusarium, Penicillium, Pestalotia, and Phompsis.* Disease incidence and severity were greatest in the East and South transects that were heavily polluted with crude oil. A vast area of the mangrove vegetation from the gas bundwall up to 200m sites of the East and South transects was burnt by the heat emanating from the flare. Symptoms observed on surviving diseased plants in the area include: necrotic leafspots, chlorosis, premature abscission and dieback. Virtually all the plants in the two transects had several necrotic leafspots caused by *Pestalotia* sp. and *Phomosis sp. Rhizophora mangle, Rhizophora racemosa and Avicennia africana* were the most severely infected plants. In addition, the herbicidal effect of the crude oil (Hutchinson and Freedman 1978) contributed to the disease severity. Fungal disease incidence and severity on plants at 300m distance from gas flare bundwall in all the four transects were very low. These areas served as controls.

Table 1. Fungal pathogens isolated from plant samples collected from the gas flare bundwall (0m) at 50m intervals up to 300m distances on the East Transect.

Transect (meters)	Distance	Plant Types and Parts Where Fungi Were Isolated	Symptoms Observed	Fungal Pahtogens Isolated
Om		Phoenix reclinate	Leafspots	Fusarium moniforme
		Paspalum variegatum (stem)	Necrotic spots	F. moniliforme, Penicillium sp.
		Mariscus ligularia	Leafspots	f. moniliforme
50m		Mariscus ligularia (stem)	Necrotic spots	Penicillium sp.
		Rhizophora racemosa (burnt debris)	-	Aspergillus niger, Penicillium sp. Trichoderma sp
		Rhizophora mangle (stem, leaves)	Leafspots	Pestolatia sp., Oidium sp.
		Paspalum variegatum	Necrotic spots	Cephalosporium sp. F. monilifome
100m		Rhizophora racemosa	Leafspots	No pathogens
150m		Rhizophora racemosa	Leafspot	No pathogens
200m		Rhizophora racemosa	Leafspots	No pathogens
250m		Rizophora racemosa	Leafspots	No pathogens
300m		Rhizophora racemosa	Leafspots	No pathogens
		Laguncilaria racemosa	Leafspots	Penicillium sp. Botrytis cinera

Pestalotia s. phosmopsis sp.

Table 2. Fungal pathogens isolated from plant samples collected from the gas flare bundwall (0m) at 50m intervals up to 300m distance on the West Transect

Transect Distance (meters)	Plant Types and Parts Where Fungi Were Isolated	Symptoms Observed	Fungi Pathogens Isolated
0m	*Corocarpus erectus*	Leafspots	*Pestalotia sp.*
			Colletotrichum gloeosporiodes
	(Roots)		*Penicillium sp.*
50m	*Tetracera alnifolia*	Leafspots	*Pestalotia sp. F. moniliforme*
	Rhizophora mangle	Leafspots	*Pestalotia sp. C. gloesporiodes*
	Rhizophora racemosa	Leafspots	*Phomopsis sp. Pestalotia sp.*
	(Roots)		*Botrytis cinera*
100m	*Alchornea cordifolia*	Seedpots	*C.gloeodporiodes, Phomopsis sp*
		Leafspots	*C. gloeosporiodes, Pestalotia sp.*
	Acrostichum aurelia	Roots	*Aspergillus niger*
	Mariscus ligualaris	Leafspots	*Pestalotai sp.*
	Rhizophora racemosa	Leafspots	*Pestalotia sp.*
150m	*Piospyros sp.*	-	*Odium sp.*
	*(*Leaves)		
200m	*Phoenix reclinata*	Leafpots	*Phosmopsis sp. Pestalotia sp.*
			C. gloeosporiodes
	Roots	-	*Peniillium sp.*
250m	*Conocarpus erectus*	Leafspots	*Pestalotia sp.*
	Chrysobalanus orbicularis	Leafspots	*Pestalotia sp. Phosmopsis*
300m	*Alchornea cordifolia*	Leafpots	*Pestalotia sp. Phomopsis sp.*
			C. gloesporiodes
	(Twigs)	Lesions	*Phosmopsis sp. Pestalotia sp.*
	(Roots)	-	*Penicillium sp.*

Table 3. Fungal pathogens isolated from plant samples collected from the gas flare bundwall (0m) at 50m intervals up to 300m distance on the North Transect.

Transect Distance (meters)	Plant Types and Parts Where Fungi Were Isolated	Symptoms Observed	Fungal Pathogens Isolated
0m	*Indigofera sp.* (Pods)	Necrotic spots	*F. moniliforme, F. solani*
	(Leaves)		*C. graminicola, Phomopsis sp.*
	(Roots)		*Alternaria sesemicola*
	Tetracera alnifolea	Leafspots	*Penicillium sp.*
	Eupatorum odoratum(Stem)	-	*Aspergillus niger*
	Alchornea cordifolia	Lesions on stem	*C.gloeosporiodes, Pestalotia sp.*
	Semitectum	Leafspots	*C. gloeosporiodes*
		Necrotic spots	*Phoma sp.*
		Leafspots	*C. gloeosporiodes, F.*
50m	*Alchornea cordifolia*	Leafspots	*Pestalotia sp.*
			Pestalotia sp.
100m	No Diseased Samples Were Colleted		
200m	*Millethia thonnigii*	Leafspots	*C.gloeosporioides*
	Mariscus ligularis	Leafspots	*Fusarium sp.*
	Rhizophora mangle	Leafspots	*Pestalotia sp.*
	Kyllingia errecta	Leafspots	*F. monilliforme*
	Tetracera alnifolia	Leafspots	*Pestalotia sp.*
	Avibennia africana	Seed rot	*Fusarium sp.*
		Leafspots	*C. gloeosporioides, Fusarium sp.*
250m	*Rhizophora mangle*	Leafspots	*F. monilifome*
	Acrotichum anteum	Leafspots	*Pestalotia sp.*
	Mariscus ligularis	Leafspots	*Pestalotia sp.*
	Eleocharie genculata	Leafspots	*Dreschlera sp.*

300m	*Laguncularia racemosa*	Leafspots	*Pestalotia sp.*
		Seed rot	*Pestalotia sp. Phosmopsis sp.*
	Rhisophora racemosa	Leafspots	*Pestalotia sp.*
	Avicennia africana	Leafspots	*Pestalotia sp. Fusarium sp.*

Table 4. Fungal pathogens isolated from plant samples collected from the gas flare bundwall (0m) at 50m intervals up to 300m distance on the North Transect.

Transect Distance (meters)	Plant Types and Parts Where Fungi Were Isolated	Symptoms Observed	Fungal Pathogens Isolated
0m	*Tetracera alnifolia*	Leafspots	*Pestalotia sp.*
	Chrysobalanus orbicularis	Leafspots	*Pestalotia sp. Phomopsis sp.*
	Rhizophora racemosa	Leafspots	*Pestalotia sp. Phomopsis sp.*
	Barteria nigritiana	Leafspots	*Pestalotia sp. phomopsis sp.*
50m	*Phoenix reclinata*	Leafspots	*Pestalotia sp. phomopsis sp.*
100m	*Laguncularia racemosa*	Leafspots	*Pestalotia sp.*
		Necrotic spot on stem	*Pestalotia sp.*
150m	*Acroticum aureum*	Leafspots	*Pestalotia sp.*
	Rhizophora racemosa	Leafspots	*Pestalotia sp. Phomopsis sp.*
200m	*Rhizophora racemosa*	Leafspots	*Chaetonium sp. Pestalotia sp. C. gloeosporioides, Phomopsis sp.*
	Maricus ligularia	Leafspots	*C. gloeosporioides, Phomopsis sp.*
	Chrysobalanus orbicularis	Leafspots	*Pestalotia sp. Phomopsis sp.*
	Paspalum vaginatum	Leafspots	*Pestalotia sp.*
250m	No Diseased samples were collected		
300m	*Alchornea cordifolia*	Twigs	*F. semitectum, Phomopsis sp.*
		Fruit rot	*F. semitectum*
	Laguncularia racemosa	Leaf spots	*Pestalotia sp. Phomopsis sp.*
		Chlorotic spots on stem	*Phomopsis sp.*
	Rhizophora racemosa	Leafspots	*Phomosis sp. C. gloeosporioides*

DISCUSSION

Campbell *et al.* (1972) isolated *Cephalosporium spp.* from oil-polluted soil and Odu *et al. (*1985) isolated *Aspergillus, Cladosporium, Fusarium, Penicillium, Trichoderma and Rhizopus* in oil-polluted soil. These findings are in agreement with the findings of this study. These genera of fungi seem to have the ability to utilise crude oil hydrocarbons as source of carbon and energy for growth. The crude oil does not seem to affect their pathogenic potential. Okafor (1987) reported that crude oil did not have any effect on the pathogenic potential of seed-borne fungi such as *Fusaium moniliforme and Cephalosporium acremonium* in maize seeds soaked in weathered crude oil for 3 months. Phytotoxicological effects of the crude oil on the structure and physiology of the plants seemed to have contributed immensely in predisposing the plants to the pathogens. Host resistance to infection was weakened and disease development enhanced. Crude oil in oil-spilled area usually penetrates the soil and directly affects the microbial activities, dissolved oxygen level and plant root system (Petts and Eduljee, 1994). It causes the soil particles to stick together thereby decreasing the porosity of the soil. It increases the acidity of the soil thereby affecting the rate of mineral nutrients uptake by plants (Petts and Eduljee, 1994). It also affects the population of some beneficial microorganisms in the soil. Soil acidity caused by oil pollution is known to inhibit non-symbiotic nitrogen fixation by *Azotobacter* spp. as well as symbiotic nitrogen fixation by *Rhiobium* spp. (Munns, 1965). All the above-mentioned factors may have contributed directly or indirectly to the aberrant metabolism of the plants thereby reducing the resistance of the plants to attack by plant pathogens. In addition, the herbicidal effects of the crude oil may have contributed to the disease severity (Hutchinson and Freedman, 1978). The soil and vegetation of the area were heavily impacted by the spills. There is need to re-vegetate the affected area to restore the field to it's former glory.

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