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ASSESSING THE SHORT TERM EFFECT OF OIL SPILL THROUGH C*hromatium* sp ACTIVITY IN MUDFLATS

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ABSTRACT: *Chromatium* species, offers a good potential for use in the assessment of the short term effects of oil pollution of tidal mud flats. The sulphur bacterium is easily identified and widely distributed in the epipelic sediment but very sensitive to hydrocarbons. It was readily detected in mudflats containing as much as 2.04 mg/kg but not in mudflats with THC of 3.65 mg/kg and above. This is suggestive that the threshold and lethal limits of hydrocarbons effect against the bacterium lies between 2.04 and 3.65 mg/kg. It implies that in any case of oil pollution monitoring that *Chromatium* is not detected, the THC levels of the sediment may be up to or above 3.65 mg/kg. Its response to hydrocarbons in sediment indicates an effect on the homeostatic status of *Chromatium* despite its even distribution (R^2 = 71.4%) in mudflat. However, *Chromatium* sp activity *in vitro* in Bacto Minimal Medium (BMM) supplemented with crude oil showed that few strains namely $CESD_{18}$, $CESJn_{19}$ and $CESF_{23}$ isolated after a spill survived 2.0% of hydrocarbons for 3 days. It's survivability for 3 days after exposure is a useful bio-monitoring attribute to assess the short-term effect of oil spill to "very" sensitive biota such as plankton and nitrogen fixers. The finding may contribute to hierarchical process of oil pollution assessment in the Niger Delta if selective medium is developed for the cultivation of the bioindicator organism during assay.

INTRODUCTION

The effects of oil on aquatic and terrestrial life can be considered as being caused by either its physical nature (physical contamination and smothering) or by the chemical components of the oil (toxic effects and accumulation leading to tainting). Aquatic life may also be affected by clean-up operations and indirectly through physical damage to the habitats, which they live (ITOPF, 1985). Therefore the choice of bio-indicator (defined here as functional measures of exposures to stressors, which are expressed at the sub-organismal, physiological or behavioral level) and analytical techniques have thus been to provide cost effective, user friendly and rapid methods which do not require sophisticated laboratory facilities (Essien and Antai, 2008).

The driver for effective environmental management is the need to understand how particular stressors impact on environmental health. Understanding biological responses to environmental change is crucial for developing appropriate and effective environmental management strategies (Kennedy and Jacoby 1992, Isaak and Thurow 2006). However, gaining a detailed understanding of biological responses has proved elusive, even in well-studied systems.

Alternative microbiological index of crude oil pollution in the Niger Delta wetlands is the main focus of the present research. *Chromatium* species offers a good potential. *Chromatium* sp is a microaerophilic, Gram-positive pleomorphic, and motile, pigmented sulfur bacterium with high nitrate reducing capacity (Holt *et al* 1994). It occurs in sulfide containing freshwater, estuarine, and marine or hypersaline environments (Holt *et al.,* 1994 and Essien, 2006). Like other diazotropbic bacteria *Chromatium* species are sensitive to hydrocarbon contamination, and are

commonly encountered in the epipelic (tidal mudflats) than the highly psammitic benthic sediments (Essien 2006, Essien and Antai, 2005 & 2009).

In this report the growth of *Chromatium in vitro* on crude oil supplemented medium was investigated. The effect of graded doses of hydrocarbons on the number of generations (n), generation time (Gt) and growth rate (Gr) of the isolates were estimated as a prerequisite to developing a medium that can selectively support the growth of the sulfur bacterium during the assessment of the extent of crude oil contamination in the Niger Delta wetlands.

METHODOLOGY

Isolation of *Chromatium* sp and Determinaton of Total Hydrocarbons Content of Mudflat

Chromatium sp, the pigmented sulfur bacterium (Plate 1) was readily isolated from the brackish Qua Iboe Estuary mudflats and associated euryhaline creeks (Plate 1) using Nitrate agar. The total hydrocarbons content (THC) of the mudflat at sampling points were determined with the aid of a Gas Chromatograph using 1 µl aliquot of sediment extract and the total peaks obtained were converted to weight using hydrocarbons standard calibration (FEPA, 2001, APHA, 1998, Radojevic and Bashkin, 1999).

Screening of Isolates for Crude oil Degradability

The isolates of *Chromatium* species obtained from the different sampling locations along Qua Iboe Estuary (Fig. 2) during the dry wet and dry seasons were screened for oil degrading potential as described by Okpokwasili and Okorie (1988). Their utilization capability was graded as positive or negative.

Evaluation of *Chromatium* sp Tolerance to Hydrocarbons and Detoxification Potential

Strains that survived the highest level of hydrocarbons during degradability test were selected for the tolerance evaluation. The strains capability to tolerate hydrocarbons were determined *in vitro* in Bacto minimal medium (BMM) supplemented with different concentrations of Qua Iboe Light (QIL) crude oil. The growth of the tolerant strains of *Chromatium* sp isolated from the ecosystem on hydrocarbon based medium was measured through total viable cell (TVC) count determination using the pour plate technique (Harrigan and McCance, 1990). Using the TVC data, the number of generations (n), generation time (GT) and growth rate (GT) of the isolates were estimated as described by Pelczer *et al*. (1982).

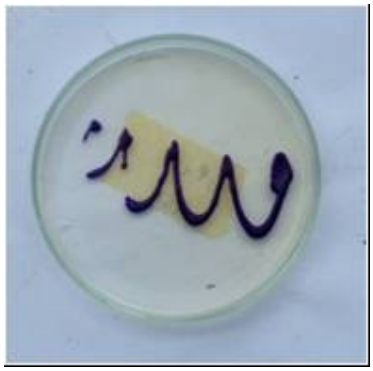

Plate 1: *Chromatium* sp.: A simple, safe, cost effective bio-indicator of oil spill

RESULTS AND DISCUSSION

The choice of bio-indicator and analytical techniques has thus been to provide cost effective, user friendly and rapid methods which do not require sophisticated laboratory facilities (Galloway *et al*., 2004, Essien and Antai, 2009). The use *Chromatium* sp is mainly based on the bacterium susceptibility to hydrocarbon contaminates (Table 1 and Fig. 2). Analysis of its

response to hydrocarbons in sediment indicates that oil pollution affect the homeostatic status of *Chromatium* in tidal mudflats despite its even distribution (R^2 = 71.4%).

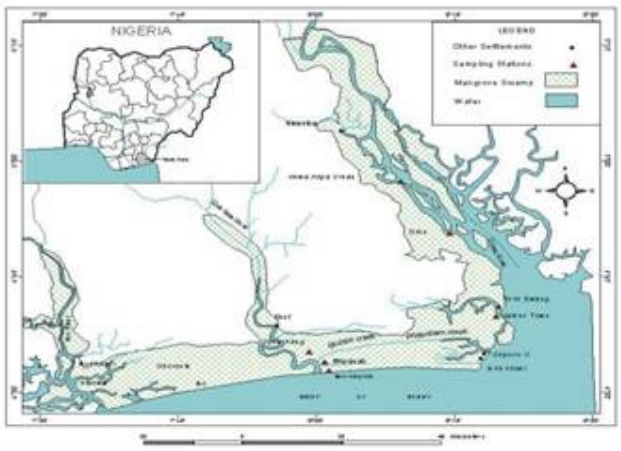

Fig.1: Map of the Southeastern part of the Niger Delta region of Nigeria showing Qua Iboe Estuary Mangrove Ecosystem and associated creeks where *Chromatium* species were isolated.

Table 1: Population density (cfu/g) of *Chromatium* sp and hydrocarbon concentration (mg/kg) in the mudflat at the time of isolation (Essien and Antai, 2009)

Ecosystem	Jun. 2003	Jul. 2003	Aug. 2003	Sept. 2003	Nov. 2003	Dec. 2003	Jan. 2004	Feb. 2004
Qua Iboe mangrove	3.0×10^2	1.3×10^2	5.1×10^2	4.1×10^2	0	0	0	4.2×10^2
THC level in mud flat	1.96	1.96	1.17	1.17	3.85	6.95	3.65	2.04

Values are derived from three determinations

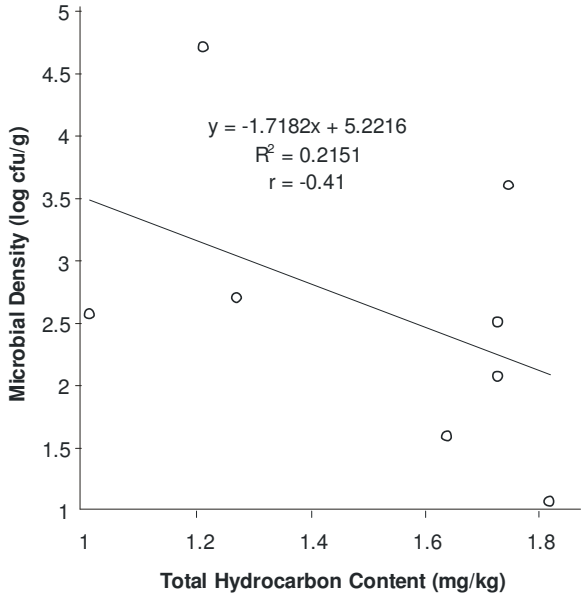

Fig.2: Relationship between total hydrocarbon content and density of *Chromatium* sp in oil impacted tidal mud flats of the Qua Iboe Estuary.

Table 2: Crude oil biodegradability and survivability of *Chromatium* isolates obtained from tidal mud flats of the Qua Iboe Estuary mangrove ecosystem

Month of isolation	Strain code	Hydrocabonoclastic potential	Survivability of isolates
Jun. 2003	$CESJ_1$	-	+
Jun. 2003	$CESJ_2$	-	-
Jun. 2003	$CESJ_3$	-	+
Jul. 2003	$CESJY_4$	-	-
Jul. 2003	$CESJY_5$	-	-
Jul. 2003	$CESJY_6$	-	-
Aug. 2003	$CESA_7$	-	+
Aug. 2003	$CESA_8$	-	-
Aug. 2003	$CESA_9$	-	+
Sept. 2003	$CESS_{10}$	-	-
Sept. 2003	$CESS_{11}$	-	-
Sept. 2003	$CESS_{12}$	-	+
Nov. 2003	$CESN_{13}$	-	+
Nov. 2003	$CESN_{14}$	-	+
Nov. 2003	$CESN_{15}$	-	+
Dec. 2003	$CESD_{16}$	-	+
Dec. 2003	$CESD_{17}$	-	+
Dec. 2003	$CESD_{18}$	-	+
Jan. 2004	$CESJN_{19}$	-	+
Jan. 2004	$CESJN_{20}$	-	+
Jan. 2004	$CESJN_{21}$	-	+
Feb. 2004	$CESF_{22}$	-	+
Feb. 2004	$CESF_{23}$	-	+
Feb. 2004	$CESF_{24}$	-	+
Total	24	0	17

Apart from *Chromatium* sp susceptibility to hydrocarbon contaminates, its sensitivity, simple to identify and un-harmful/not pathogenic to human also determine its choice as useful indicator. However *in vitro* investigation (Table 2) has shown that the sulfur bacterium is tolerant to much higher THC levels than 2. 04 mg/kg recorded in mudflats, and few strains *Chromatium* sp- $CESD_{18}$, *Chromatium* sp-$CESJn_{19}$ and *Chromatium* sp – $CESF_{23}$ isolated after spill could survive 2.0% of hydrocarbons contamination in the laboratory at room temperature (Fig. 3). Moreover, beyond 2.0% of hydrocarbons the growth of even the most tolerant strains may be

terminated within 3 days. Their performance on crude oil supplemented medium showed a general reduction in growth rate and number of generations while the generation time varied with the strains and tended to increase with increase in the levels of the hydrocarbons (Table 3).

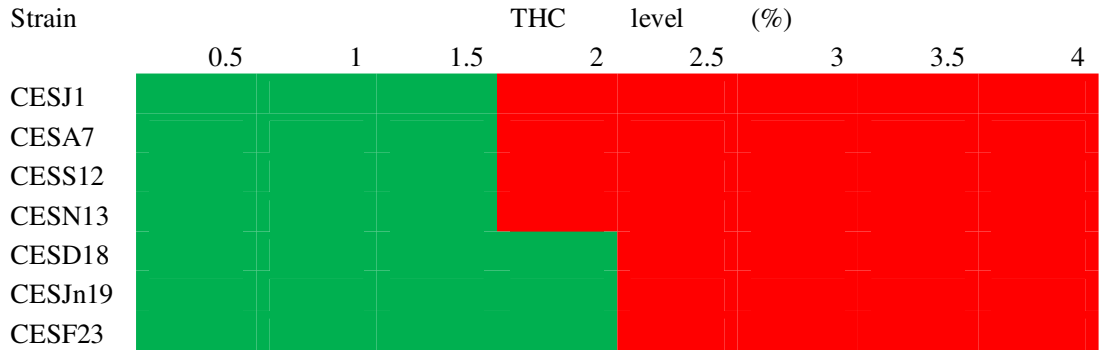

Fig. 3: Survivability of THC tolerant strains of *Chromatium* in BMM supplemented with different concentration of Qua Iboe Light crude oil

Table 2: *In vitro* growth of very tolerant strains (those that survived the presence of 2 mg/kg of oil) of Chromatium sp in BBM supplemented with different concentrations of Qua Iboe Light crude oil overtime

Isolate code	Duration (hours)							
	0	24	48	72	96	120	144	168
				0.5%				
$CESD_{18}$	3.2×10^3	2.2×10^2	1.3×10^2	3.3×10^1	-	-	-	-
$CESJN_{19}$	3.6×10^3	5.4×10^2	4.3×10^2	5.3×10^1	-	-	-	-
$CESF_{23}$	2.9×10^3	6.5×10^2	2.6×10^2	3.9×10^1	-	-	-	-
				1.0 %				
$CESD_{18}$	3.2×10^3	1.9×10^2	8.3×10^1	2.3×10^1	-	-	-	-
$CESJN_{19}$	3.6×10^3	2.2×10^2	4.9×10^1	2.3×10^1	-	-	-	-
$CESF_{23}$	2.9×10^3	1.4×10^2	7.4×10^1	1.9×10^1	-	-	-	-
				1.5 %				
$CESD_{18}$	3.2×10^3	6.2×10^1	2.3×10^1	1.3×10^1	-	-	-	-
$CESJN_{19}$	3.6×10^3	6.4×10^1	2.2×10^1	-	-	-	-	-
$CESF_{23}$	2.9×10^3	4.4×10^1	2.3×10^1	1.2×10^1	-	-	-	-
				2.0%				
$CESD_{18}$	3.2×10^3	2.4×10^1	0.9×10^1	-	-	-	-	-
$CESJN_{19}$	3.6×10^3	3.3×10^1	1.3×10^1	-	-	-	-	-
$CESF_{23}$	2.9×10^3	5.5×10^1	1.2×10^1	-	-	-	-	-
				NB (control)				
$CESD_{18}$	3.2×10^3	5.2×10^5	6.2×10^6	7.2×10^6	4.7×10^5	3.2×10^3	2.3×10^2	2.9×10^1
$CESJN_{19}$	3.6×10^3	4.2×10^5	3.4×10^6	6.3×10^6	7.2×10^5	4.3×10^4	4.3×10^2	2.1×10^1
$CESF_{23}$	2.9×10^3	4.3×10^5	3.5×10^6	5.7×10^6	3.6×10^5	2.4×10^3	5.3×10^2	3.2×10^2

NB = nutrient broth

The different strains of sulphur bacterium were screened for their crude oil degradability and survivability. Survivors were evaluated for their tolerance to hydrocarbons, detoxification

potential and their potential use as bio-indicators rated using a modified 1SO 14001-evaluation model (Table 4).

Table 3: *In vitro* growth rate (Gr), number of generation (n) and generation time (Gt) of the tolerant strains of *Chromatium* sp in BBM supplemented with different concentrations of Qua Iboe Light crude oil.

Isolate code	Gr	n	Gt (hr)
		0.5%	
$CESD_{18}$	0.02	- 0.602	1.51
$CESJN_{19}$	0.017	- 0.412	1.03
$CESF_{23}$	0.0115	- 0.278	0.68
		1.0%	
$CESD_{18}$	0.025	- 0.620	1.55
$CESJN_{19}$	0.026	- 0.642	1.61
$CESF_{23}$	0.027	- 0.656	1.64
		1.5%	
$CESD_{18}$	0.040	- 0.9610	2.40
$CESJN_{19}$	0.041	- 0.985	2.46
$CESF_{23}$	0.043	- 1.045	2.61
		2.0%	
$CESD_{18}$	0.055	1.334	3.33
$CESJN_{19}$	0.051	- 1.235	2.43
$CESF_{23}$	0.040	- 0.960	2.40
		Control	
$CESD_{18}$	0.698	0.029	1.75
$CESJN_{19}$	0.633	0.026	2.24
$CESF_{23}$	0.633	0.026	1.74

Rating the Bio-indicator

Here *Chromatium* sp as a bio-indicators is evaluated to rate its usefulness and significance using a modified 1SO 14001-evaluation model. The approach categorizes the indicator based on:

1. Economic significance
2. Accessibility/availability
3. Sensitivity
4. Un-harmfulness
5. Inability to avoid pollutant
6. Not sacred
7. Simple to identify and analyze

The usefulness of the attributes is rated based on consensus of research opinions as:
5 = High/very useful attribute
3 = Moderate/ useful attribute
1 = Low/not useful

Table 4: Qualifying *Chromtium* sp as a bio-indicator of oil spill in aquatic ecosystems

ISO 14001 Attribute	Freshwater	Euryhaline	Brackish	Mixohaline	Marine
Economic significance	1	1	1	1	1
Accessibility/availability	1	3	5	5	5
Sensitivity	1	5	5	5	5
Un-harmfulness	5	5	5	5	5
Inability to avoid pollutant	5	5	5	5	5
Not sacred	5	5	5	5	5
Simple to identify and analyze	1	5	5	5	5
Total	19	29	31	31	31

Note: The maximum possible value = 35

Scope of Application

The rating (Table 4) and the illustration in Fig. 4 shows that *Chromatium* sp can be effectively used to assess the short term effects of oil spill in brackish, mixohaline and marine (salt marsh) wetlands with rating value of 31. It may also be useful in oil spill monitoring in euryhaline creeks and wetlands with a value of 29 but doubtful in freshwater swamps mainly because of its unavailability due to habitat preference.

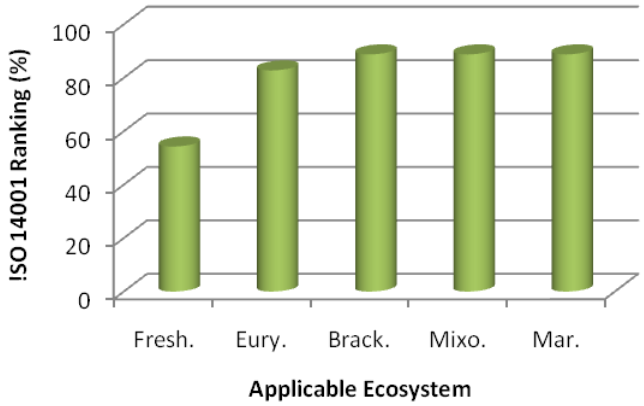

Fig. 4: Potential use of *Chromatium* sp as bio-indicator of oil spill in wetlands.
Fresh. = Freshwater, Eury. = Euryhaline, Brack. = Brackish, Mixo. = Mixohaline, Mar. = Marine

CONCLUSION AND RECOMMENDATION

It's survivability for three days after exposure to hydrocarbons is a useful bio-monitoring attribute for the assessment of short-term effect of oil spill. Using a modified 1SO 14001-evaluation model, it is obvious that *Chromatium* sp can be effectively used to assess the short term effects of oil spill on very sensitive biota such as planktons and nitrogen fixers in brackish, euryhaline, mixohaline and marine (salt marsh) wetlands. Its sensitivity, simple to identify and un-harmful/not pathogenic to human determine its choice as useful indicator. The use of selective medium for the enumeration and isolation of *Chromatium* sp would definitely add value to our findings. If the medium is developed, the sulfur bacterium may be used to instigate a rapid assessment of the extent of crude oil contamination and biotic responses in wetlands especially in mudflats immediately after a spill. It is safe, cost and time effective and implementable.

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