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Safety and acceptability of the shellfish "Ngolo" *(Thais califera)* subjected to simulated crude oil polluted microcosms

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Abstract

The objective of this investigation was to evaluate the microbial, physico-chemical and organoleptic attributes of "Ngolo" *(Thais califera)* samples subjected to simulated crude oil polluted microcosms (0%, 1%, 5% and 10% v/v) for 12 days. Total heterotrophic counts (THCs) and total coliform counts (TCCs) increased with level of pollution and duration of exposure with the THCs attaining the peak of $\log_{10}$ 7.97 cfu/g on day 12. Hydrocarbon degraders exhibited similar trends. Heavy metal (cadmium, lead and nickel) concentrations increased with increase in simulated pollution level. BOD and COD values increased markedly with pollution and prolonged exposure thereby exceeding permissible level of 4.0mg /l. The higher microbial loads and heavy metal concentrations in "Ngolo" samples exposed to the polluted microcosms indicate bioaccumulation of these pollutants by "Ngolo" samples. In contrast, DO values decreased with duration of exposure. Shucked "Ngolo" samples following exposure to 0% or 1% pollution level for 9 days were organoleptically acceptable but those exposed to 5% and 10% pollution were unacceptable after 6 and 3 days respectively. This work has demonstrated that the level of pollution and duration of exposure are critical to the safety and acceptability of "Ngolo" samples exposed to crude oil pollution. Therefore, clean-up operations of crude oil polluted aquatic ecosystem should be undertaken within two days of the crude oil pollution to minimize the impact on the aquatic life.

Introduction

Crude oil exploration, drilling, oil spillage and improper waste disposals have resulted in a wide spread contamination of aquatic life and terrestrial ecosystems in the Niger Delta area of Nigeria (Okpokwasili and Odokuma, 1996) and other parts of the world (Anderson *et al.*, 1993). Thus, crude oil, drill cuttings, drilling mud/additives and the contaminating heavy metals pose serious threats to the biota of the natural ecosystems especially in the locations of oil wells or waste disposal sites. Decline in number and distribution of shellfish has been attributed to deteriorating water quality of shellfish habitats (Gifford *et al*., 2006).

The shellfish "Ngolo" *(Thais califera)* is a popular (gastropod) seafood in the Niger Delta area of Nigeria. It is a whelk and a univalve that serves as a relatively cheap source of animal protein. It is a major delicacy in the Niger Delta region of Nigeria where it is harvested and marketed, thereby constituting an important cottage industry. Unfortunately, the habitats of "Ngolo" are frequently adversely affected by crude oil spills (Nwilo and Badejo, 2007). Consequently, "Ngolo" could constitute health hazards to its consumers. Foulkes (1990) observed that some components of crude oil especially the heavy metals can persist and accumulate in shellfish, thereby posing serious health hazards to humans. Fang (2006) also reported that mollusks accumulate many pollutants within their tissues and shell, thus indicating their use as bio-monitors of hydrocarbons. In view of the concern and frequency of crude oil pollution occurring in the "Ngolo" habitats and the potential health and economic significance of the shellfish "Ngolo", this work was therefore undertaken to investigate the microbial, physico-chemical and organoleptic attributes of "Ngolo" exposed to simulated crude oil polluted microcosms.

Materials and Methods

Sources of crude oil and "Ngolo" samples

The crude oil (Bonny light) used for this investigation was obtained from the Port-Harcourt Refining Company Limited (PHRCL), Eleme,

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Nigeria, in a 20-litre pre-sterilized plastic container. "Ngolo" samples were harvested from Sombreiro river in Rivers State, Nigeria.

Simulation of crude oil polluted microcosms

Four troughs having dimension of 15 x 25 x 36cm were thoroughly washed with detergent and 1% Nitric acid and finally rinsed using sterile deionised water (Loumbourds *et al*., 1999). Into each trough containing 0ml, 30 ml, 150 ml and 300 ml of Bonny light crude oil was added river water (from "Ngolo" habibat) resulting in microcosms of 0%, 1%, 5% and 10% v/v of crude oil respectively. The "Ngolo" samples were then sorted into comparable sizes and fifty of them placed in each microcosm.

Analyses

The simulated polluted microcosms ("Ngolo" habitats) and "Ngolo" samples were monitored for 12 days for microbiological, physico-chemical and organoleptic qualities.

Microbiological

The microbiological analyses carried out on the "Ngolo" samples included total heterotrophic counts, total coliform counts and hydrocarbon degraders as described by Nickerson and Sinskey (1984). The shucked "Ngolo" flesh (50g) was aseptically weighed and homogenized in a pre-sterilized blender (Moulinex, Paris, France) containing 450ml of 0.1% (w/v) sterile peptone water to yield a 10^{-1} dilution. Further decimal dilutions of 10^{-2} to 10^{-5} of the homogenate were prepared and plated on pre-dried tryptone soy-agar (Biotec, Suffolk, UK) plates in duplicate for the total heterotrophic counts. The inoculated plates were incubated at 37°C for 24-48 hr and plates containing 30-300 colonies were enumerated. The method of Sumpeno *et al.* (1990) was used for the determination of *Salmonella* sp and *Shigella* sp in the "Ngolo" samples. The shucked "Ngolo" samples were blended aseptically in enriched Selenite–Cysteine broth and the enriched homogenate spread plated in bismuth sulfite agar (Fluka, Switzerland) and *Salmonella-Shigella* agar (Fluka, India) for *Salmonella* sp and *Shigella* sp respectively. The plates were incubated at 37^0C for 24-48hr.

Physico-chemical

The physico-chemical parameters (DO, BOD, COD, pH and heavy metals were analysed as described in APHA (2005) and ASTM (1999). Preparation of the samples for heavy metal analysis was by a nitric-sulphuric acid digestion whereby 15ml of nitric acid and 10ml of sulphuric acid were added to 2 g of the "Ngolo" sample in a round bottom flask. The set up was heated and refluxed for 2.5 hr. After the digestion, Atomic Absorption Spectrophotometer (Model Smith-Hieftje 22 Thermo Jarrrell Ash, U.K.) was used for the determination of the heavy metals in the "Ngolo" samples.

Organoleptic

Organoleptic attributes (visual appearance and smell) of the "Ngolo" samples were determined by six taste panelists (highly conversant with "Ngolo" quality characteristics) using hedonic scale of 1-9 where the limit of acceptability was 4 (Meilgaard *et al.*, 2004).

Statistical analysis

The obtained data were analyzed and the means with the standard deviations presented (Snecdecor and Cochran, 1976).

Results and Discussion

Microbial quality of "Ngolo" samples and the simulated polluted microcosms

The changes in the total heterotrophic counts and hydrocarbon degraders of the "Ngolo" samples are shown in Figures 1 and 2. The exposure of "Ngolo" samples to different levels of crude oil pollution (0%, 1%, 5% and 10%) showed gradual increases in microbial counts with increase in pollution and duration of exposure resulting in the occurrence of maximum THCs ($\log_{10}$ 7.97 cfu/g) on day 12 in the most polluted microcosm but less apparent changes occurred in the control samples. Similarly, maximum hydrocarbon utilizing bacterial counts of $\log_{10}$ 4.26 cfu/g occurred on day 12 in "Ngolo" samples exposed to 10% crude oil microcosm as opposed to the lowest microbial load observed in "Ngolo" samples subjected to the microcosm without hydrocarbon (control) (Figure 2). These clearly indicate the suitability of crude oil as a microbial substrate especially at higher levels of concentration.

Additionally, the increase in microbial population with time of exposure may be attributed to the mechanism of bacterial adherence due to the stagnant microcosm and enhanced bacterial colonization and proliferation (Efiuvwevwere and Ezeama, 2004; Orcutt *et al*., 2011). The higher microbial counts obtained in the "Ngolo" samples (Figure 1) as compared with the river water ("Ngolo" habitat) samples (Figure 3) suggest bioaccumulation (Efiuvwevwere and Ezeama, 2004; Kueh and Chan, 1985).

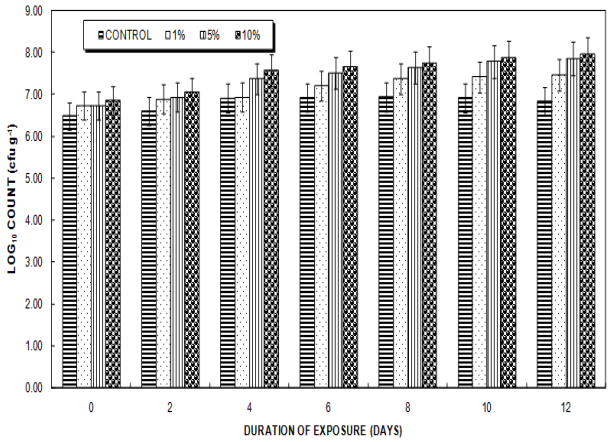

Figure 1. Changes in total heterotrophic counts of "Ngolo" samples subjected to different simulated crude oil polluted microcosms

Each bar represents the mean ± SD of six determinations

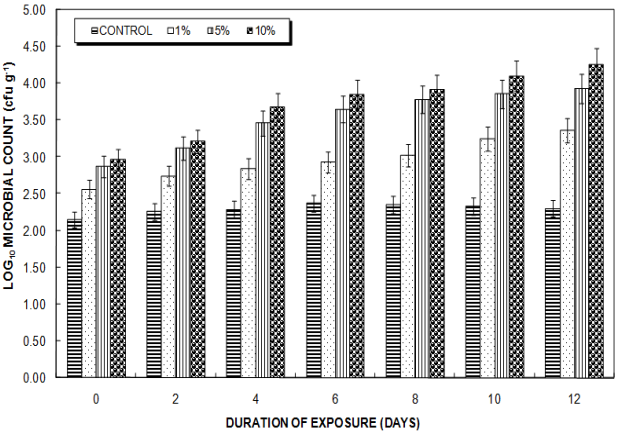

Figure 2. Changes in hydrocarbon degraders of "Ngolo" samples subjected to different simulated crude oil polluted microcosms

Each bar represents the mean ± SD of six determinations

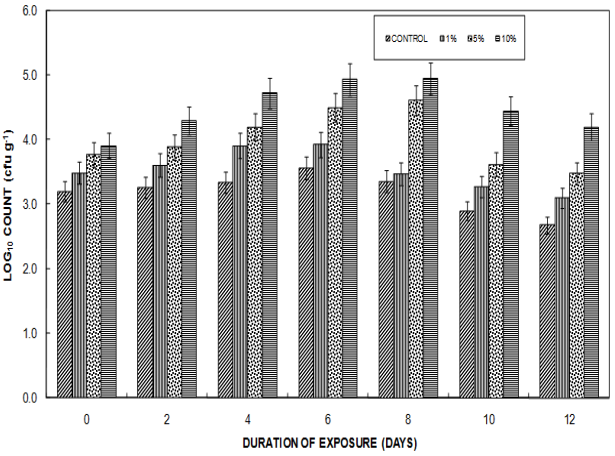

Figure 3. Changes in total heterotrophic counts of sea water subjected to different simulated crude oil polluted microcosms

Each bar represents the mean ± SD of six determinations

Heavy metal concentrations of "Ngolo" samples during exposure to different simulated microcosms

The concentrations of heavy metals (Pb, Ni and Cd) in different simulated polluted microcosms are shown in Table 1. The slightly higher concentrations in the "Ngolo" samples than in the water samples (control) further confirms the bioaccumulation mechanism in seafoods and the associated potential hazards (Karadede-Akin and Unlu, 2007; Ashraf *et al*., 2012). These concentrations are however lower than the human consumption tolerance level of 1.0 mg/kg (Bloxham *et al*., 1998; Fang, 2006).

Changes in physico-chemical parameters of "Ngolo" samples during exposure to different simulated microcosms

The changes in the physico-chemical properties of "Ngolo" habitat water samples in the different microcosms are shown in Table 2. The increase in BOD and COD values with exposure time and level of pollution is a clear indication of increase oxygen demand for biochemical activities. In contrast, the decrease in Dissolved Oxygen (DO) with increase in level of pollution and exposure time is a reflection of the changes in the related physico-chemical parameters (Table 2). Thus, the much higher BOD values on day 12 which far exceeded the permissible range of 2 to 8 mg/l clearly demonstrate serious pollution hence the depletion of oxygen especially in the microcosm containing 10% crude oil thereby resulting in the DO value of 1.0 mg/l which is far below the limit of 6.8mg/l for aquatic life (USEPA, 1986).

Organoleptic evaluation of "Ngolo" samples exposed to simulated hydrocarbon polluted microcosm

The results of the sensory evaluation of the "Ngolo" samples exposed to the different microcosms varied with time and pollution level (Figures not shown). The scores at the onset of the work (day 0) were highly acceptable (approx. 8.2) but decreased sharply with increase in pollution and time of exposure to unacceptable level of approximately 2.2 on day 12. However, the decrease in the sensory scores was gradual in the microcosm without crude oil (control). "Ngolo" samples exposed to 0% or 1% pollution level for 9 days were organoleptically acceptable (having scores of 4.9 and 4.1 respectively) but those exposed to 5% level of pollution were only acceptable until day 3 of exposure. By the 6th day of exposure, "Ngolo" samples from 5% and 10% crude oil microcosms had become unacceptable with mean sensory scores of 3.2 and 2.6 respectively for appearance and 3.3 and 2.7 respectively for smell

Table 1. Concentrations of heavy metals in "Ngolo" samples subjected to different levels of crude oil polluted microcosms

Duration of Exposure (Days)	Pollution level in Percentages (%)/ concentrations (mg/kg)											
	0% (Control)			1%			5%			10%		
	Ni	Cd	Pb	Ni	Cd	Pb	Ni	Cd	Pb	Ni	Cd	Pb
0	0.24	0.15	0.07	0.24	0.15	0.07	0.24	0.15	0.07	0.24	.015	0.07
6	0.24	0.148	0.07	0.312	0.154	0.076	0.347	0.155	0.078	0.38	0.157	0.078
12	0.24	0.152	0.07	0.345	0.157	0.073	0.36	0.158	0.078	0.408	0.158	0.081

Each value represents the mean of four determinations

Table 2. Physico-chemical properties of "Ngolo" habitat (water) to different concentrations of crude oil polluted microcosms

Duration of Exposure (Days)	Pollution level in Percentages (%) /concentrations (mg/1)															
	0% (Control)				1%				5%				10%			
	DO	BOD	COD	pH	DO	BOD	COD	pH	DO	BOD	COD	pH	DO	BOD	COD	pH
0	7.2	1.92	12	7.53	7.2	1.92	12	7.53	7.2	1.92	12	7.53	7.2	1.92	12	7.53
6	5.3	41	310	7.54	4.7	47	720	6.95	2.9	124	1315	7.28	2.2	348	1580	7.26
12	4.0	56	520	7.54	1.5	68	1680	6.6	1.3	320	1950	6.8	1.0	808	2200	6.95

Each valeu represents the mean of four determinations

indicating the apparent loss of quality and high unacceptability as well as marked reduction in their market value.

Conclusion

The exposure of "Ngolo" samples to simulated crude oil polluted microcosms led to increase in microbial load, BOD, COD and marked decrease in DO. The adverse impacts of crude oil pollution on "Ngolo" samples were demonstrated to be dependent on pollution level and duration of exposure. Thus, this work has demonstrated that the acceptability and safety of "Ngolo" samples exposed to crude oil pollution are concentration/time dependent. The "Ngolo" samples exposed to minimal pollution (1%) are considered safe if harvested and processed within three days of such exposure. However, "Ngolo" samples subjected to higher polluted microcosms showed marked increase in heavy metal and microbial contents, confirming the concept of bioaccumulation compared with those contained in the unpolluted habitat (control). It is therefore recommended that rapid clean-up actions (within two days) be taken when crude oil pollution/spill occurs in the aquatic ecosystem to minimize the adverse impacts of crude oil pollution on the shellfish and other seafoods of the environment.

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