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Tolerance: A Useful Biological Parameter for Identifying Contaminated Sites

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The Atlas Cove depot is a marine receipt terminal for storage and distribution of petroleum products in Nigeria. It is the largest of all the depots in Nigeria and serves the entire southwest of Nigeria. Major and minor oil spill incidents have been occurring at the Atlas Cove especially between 1996 and 1998, although most of the cases were not reported (NAPETCOR 2001). The oil spill incidents at the depot have been attributed to vandalisation of petroleum product pipelines, leakages and human error. The spillages of petroleum products at the Atlas Cove depot have resulted in the degradation of the mangrove ecosystem surrounding the depot (NNPC 2000), although some animal species such as *Clibanarius africanus*, *Nereis diversicolor* and *Uca tangeri* were still found in relatively high numbers in the surrounding mangrove ecosystems. It is therefore expected that the continual exposure of these animals to sublethal concentrations of the petroleum products over time would have some adverse biological effects on the exposed animals. One of such potential biological effects that could arise from the continuous exposure of the animals to low concentrations of the petroleum products, or any chemical compound, is the development of resistance or tolerance to the toxic effects of petroleum hydrocarbons.

Although a number of biological/ecological technique have been utilized in identifying the disturbance or pollution of the environment (Clements et. al. 2002, Rogers et. al. 2002), this identification of the cause or causes responsible for the observed ecological effect is usually the key to discovering how we can change and improve our environment (Norton et. al. 2002). The detection of tolerance in a population of organisms inhabiting specific sites to a certain pollutant or pollutants is indeed an indicator or a parameter that would be useful in streamlining a usually long list of possible causative factors/agents. Furthermore, the detection of tolerance by a population or organisms to a compound could also be a very useful and rapid biological parameter for identifying whether sites around former or abandoned disposal sites are indeed contaminated by pollutants. This paper therefore examines the impact of the incessant petroleum product spillages at the Atlas Cove by comparing, using laboratory toxicity tests, the tolerance levels of *Clibanarius africanus* population found around the Atlas Cove depot with those population found in another area, UNILAG lagoon front, which have not been exposed to petroleum products contamination.

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MATERIALS AND METHODS

Test animals (sources and collection): *Clibanarius africanus* (Aurivillus) (Hermit Crab) (Arthropoda; Crustacea, Decapoda, Paguridae). Live *C. africanus* specimen were collected by handpicking from the lagoon edge of the two sites at low tide into a plastic bucket (12.6L) half-filled with lagoon water and sand as substrate. Effort was made to collect hermit crabs inhabiting shells within a narrow size range with respect to shell length (28-32mm) and from around the same site to reduce variability in biotype. Stones meant to serve as substratum for clinging onto by the crabs was also added to the holding tanks, in order to simulate the natural habitat of these animals.

Laboratory animal cultures, acclimatisation and selection of test animals for bioassays. Animals collected were left in holding tanks (70cm X 30cm X 30cm) with a thin layer of sediment serving as substrate for 5 – 6 days to allow them acclimatise to laboratory conditions (R.H.-70 ± 2%; Temp - 26 ± 2^0C; Salinity-0.0‰) before using them in bioassays. The hermit crabs were fed on fish flesh (Tilapia – 20g per holding tank) during the period. Feeding was however discontinued 24 hours prior to commencement of bioassays. Hermit crabs of similar sizes based on acquired shell length (28mm-32mm) were always selected for experiment. The mean weight of whole animals of *C. africanus* (ex shell) selected for bioassays was 0.44 ± 0.04g.

Measurement of physico-chemical parameters in water samples at the animal collection sites. Physico-chemical parameters such as oil and grease, biochemical oxygen demand, chemical oxygen demand, pH, salinity and temperature of water samples at the collection sites were measured with the aid of digital read out instruments (Jenway Products Model 3000 series of pH meter and hand refractrometer for salinity) and using appropriate methods (APHA-AWWA-WPCF 1995).

The Premium Motor Spirit (PMS) or Petrol used for the bioassays was obtained from the African Petroleum (AP) filling station located within the University of Lagos main campus.

In an attempt to simulate the natural environment of benthic animals, sediment samples were collected from the unimpacted UNILAG lagoon front and prepared after Tokolo (1988). A 120g portion of the "prepared" sediment was always used for the bioassays. The sediment was always spread out to form a thin bottom layer in each bioassay container at the commencement of bioassays.

Preparation of test media including application of toxicant: A predetermined amount of PMS was measured into the containers. This was then made up with a given volume of dechlorinated tap water to make up to 1000ml. 120g of the prepared sediment were placed at the bottom to serve as substrate in each bioassay container.

An animal was taken to be dead if it failed to retract its protruded body into its shell upon prodding with a glass rod or failed to emerge or protrude its legs/body during an observation period of 3 – 4 minutes in untreated dechlorinated tap water placed in an observatory petri dish.

A total of thirty (30) active hermit crabs of similar sizes after acclimatisation were randomly assigned to bioassay containers, already holding treated or untreated test media. The test animals were exposed to several concentrations of PMS as follows:
a. Hermit crabs from UNILAG Lagoon front: 0.1, 0.5, 1.0, 4.0, 8.0, 12.0 and untreated control
b. Hermit crabs from Atlas Cove: 0.1, 1.0, 4.0, 8.0, 20.0, 24.0, 28.0 and untreated control. Mortality assessment was carried out after 24 hours, 48 hours, 72 hours and 96 hours of exposure.

Toxicological dose-response data involving quantal response (mortality) were analysed by probit analysis (Finney, 1971). The indices of toxicity measurement derived from this analysis were:
LC_{50} = Median lethal concentration that causes 50% response (mortality) of exposed organisms.
LC_{95} = Lethal concentration that causes 95% response (mortality) of exposed organisms.
and their 95% confidence limits (C.L.).

$$\text{T.F.} = \frac{LC_{50} \text{ of PMS against } C.\ africanus \text{ population at Atlas Cove depot}}{LC_{50} \text{ of PMS against } C.\ africanus \text{ population at UNILAG Lagoon Front}}$$

RESULTS AND DISCUSSION

On the basis of the 96h LC_{50} values, population of *C. africanus* collected from the impacted Atlas Cove areas were found to be more tolerant, with 96h LC_{50} of 2.35 ml/l, to the test toxicant than the same species collected from the control stations, around UNILAG Lagoon front with 96hLC_{50} of 0.50 ml/l (Table 1). Furthermore, the computed tolerance factor (96hLC_{50} ratios) showed that population of *C. africanus* collected from the Atlas Cove depot area was about 5 times more tolerant to the petroleum product than the population collected from UNILAG lagoon front.

The observation of higher tolerance in the population of *C. africanus* collected from the Atlas Cove depot to the petroleum product in laboratory bioassay can be attributed to the long history of consistent exposure to high concentrations of the petroleum product in their ecosystem as a result of petroleum products spillages, which have been recorded in the area over the years. During this study, the total oil and grease levels detected in water samples collected from the various sampling stations around the Atlas Cove depot ranged from 1.28 mg/l to 8.2 mg/l

while the oil and grease levels detected in the UNILAG lagoon front stations ranged from 0.04 mg/l and 0.08mg/l (Table 2). This constant exposure of organisms to pollutants over long period has been reported to maintain a selection pressure in favour of the few members of the population that have resistance to the toxic effects of such pollutant. The selection pressure if maintained for a long enough period may result in the development of a new population with a higher tolerance to the pollutant (Don-Pedro and Adegbite, 1985).

The usefulness of comparative tolerance studies between populations of the same animal species in different locations could serve as a rapid biological assessment parameter, which can be useful in identification of contaminated sites. Although, some organisms have been recognized as indicators of some pollutants (Bryan and Gibbs, 1987), further detection of higher tolerance in the population of such organisms compared to other populations in "uncontaminated" sites would be additional biological and experimental information for the identification of contaminated sites.

Table 1. Tolerance of different populations of *C. africanus* to premium motor spirits (PMS) over a 96-hour period of exposure.

Time (Hrs)	LC_{50} (95% CL*)	LC_{95} (95% CL*)	Slope± S.E^{+}	d.f$^{\#}$	Probit Line Equation	T.F$^{\times}$
C. africanus collected from UNILAG lagoon Front						
48	3.8 (2.6 – 5.0)	9.9 (8.0 - 13.2)	0.3± 0.5	3	y = -1.02+0.27x	
72	1.5 (0.3 - 2.6)	7.7 (5.7 - 12.6)	0.3± 0.1	3	y = -0.40+0.27x	
96	0.5 (0.4 - 1.6)	6.9 (5.0 - 12.5)	0.3± 0.1	3	y = -0.13+0.25x	
C. africanus collected from Atlas Cove Depot						
48	12.8 (8.6 – 16.5)	33.9 (28.1 - 44.9)	0.1± 0.1	3	y = -0.99+0.08x	3.4
72	7.3 (5.2 – 11.8)	31.5 (23.9 - 50.1)	0.1± 0.1	2	y = -0.50+0.07x	4.8
96	2.4 (1.8 - 6.5)	26.5 (19.7 - 44.7)	0.1± 0.1	3	y = -0.16+0.07x	4.7

C.L.* – Confidence Limit
S.E.$^{+}$ – Standard Error
d.f$^{\#}$ – Degree of Freedom
T.F.$^{\times}$ – Tolerance Factor

$$= \frac{LC_{50} \text{ of PMS against } C. \textit{ africanus} \text{ collected from Atlas Cove}}{LC_{50} \text{ of PMS against } C. \textit{ africanus} \text{ collected from UNILAG Lagoon Front}}$$

Table 2. Physico-chemical characteristics of water samples at animal collection sites

Parameters	Unit	Atlas Cove Depot	UNILAG Lagoon front
1. pH		7.85	7.70
2. Biological Oxygen Demand (BOD)	mg/l	26.33	12.50
3. Chemical Oxygen Demand (COD)	mg/l	82.50	18.0
4. Oil and Grease	mg/l	3.67	0.05
5. Ammonia	mg/l	0.29	0.18
6. Salinity	‰	26	18
7. Temperature	°C	29	28

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