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Toxicity of Crude Oil to Fresh Water Shrimp, *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii* From Nigerian Coastal Water

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Abstract The water soluble fraction (WSF) of crude oil was tested against *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii,* at 2, 4, 6, 8 and 10 mg/L in glass aquaria stocked with ten animals for 96 h under observations for changes. Moribund swimming, restlessness, respiratory difficulties, depigmentation and mortalities were observed in the WSF exposure groups, but not in the controls. LC_{50} values were estimated at 5 ± 1.76 and 4 ± 1.76 mg/L for *M. macrobrachion* and *M. vollenhovenii* respectively. There was no significant difference in mortalities between the two species ($p > 0.05$), leading to the conclusion that the WSF of crude oil in the Nigerian coastal waters may be equally toxic to *M. macrobrachion* and *M. vollenhovenii.*

Keywords Crude oil · Toxicity · *Macobrachium macrobrachion* · *Macrobrachium vollenhovenii*

Crude oil is a complex mixture of hydrocarbons of varying molecular weights and structures ranging from light gas (methane) to heavy oils. Elementally, crude oil is composed mainly of carbon, hydrogen, oxygen and other elements such as nitrogen, sulphur, vanadium, sodium, nickel and iron. Spillage occurs during production, transport and storage of crude oil and its derivatives (Odiete 1999). The effects of oil pollution depend greatly on the oil and upon the types and conditions of the receiving environment. Although aquatic organisms possesses an enzyme system capable of breaking down hydrocarbons in their system, eggs and larval stages of benthic organisms are very sensitive to even very low concentrations of oil. The water soluble fraction (WSF) of petroleum hydrocarbons crosses the chorion, resulting in reduced egg hatchability and developmental abnormalities, such as pericardial edema, disturbed axis formation, and abnormal jaw development (Pollino and Holdway 2002). Nigeria as a major crude oil producer with a large number of on/off shore oil installations has witnessed a large number of oil spills over the years (Akpan et al. 1999; Okpofure et al. 2000). Adverse effects of crude oil spills on microinvertebrates and shrimp have been reported (Pollino and Holdway 2002), with some of the notable impacts including interference with feeding, reproduction, abnormal growth, weakness and behavioral changes (Moriarty 1990).

Macrobrachium macrobrachion is a dominant shrimp species in the Cross River estuary and its tributaries, and contributes significantly to the shrimp fishery of the area (Udo and Taege 1991). *Macrobrachium vollenhovenii* is the largest fresh water shrimp in West Africa. It lives in brackish water as larvae and in fresh water as adults (Udo and Taege 1991). Although the toxicities of petroleum hydrocarbons and heavy metals have been reported in most aquatic life, there have been no reports on crude oil toxicity to *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii*. The objective of this study was to investigate the effects of crude oil contamination on the two

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species of shrimp dominating the artisanal shrimp fishery in Nigeria.

Materials and Methods

Macrobrachium macrobrachion and *Macrobrachium vollenhovenii* used in this study were collected from fishermen in the Cross River estuary located in the rainforest belt of Southern Nigeria at latitudes 4°50′N and 5°50′N and longitudes 4°15′ and 8°35′E, respectively. The organisms were collected along with habitat water from the same sites. The organisms were selected into size classes of 2–4 cm, and acclimatized in filtered habitat water for 24 h prior to the toxicity experiment. The initial water parameters (conductivity, temperature, dissolved oxygen, pH, and salinity) were determined using a Hach conductivity/TDS meter, and Lutron DO and pH meters. The battery operated meters were calibrated according to manufacturer's instructions before being used for measurements (Boyd 1989, 1990).

The water soluble fraction (WSF) from Qua Ibo oil terminal, Nigeria was obtained by vigorously shaking crude oil with filtered habitat water in a separatory funnel. The system was allowed to stand for 6 h to effect complete phase separation, after which the lower aqueous layer containing the WSF was collected for the toxicity tests. The concentration of the hydrocarbon was determined by spectrophotometric measurement of a n-hexane extract at 430 nm wavelength using a Hach direct reading (DR) 3000 spectrophotometer (Stuermer et al. 1981).

After 24 h acclimatization, the shrimp were randomly distributed into 18 10 L aquaria containing 5 L of the test solution (WSF) at different concentrations (2, 4, 6, 8 and 10 mg/L). Each species of shrimp was tested separately using the same procedure and replicates. The experiment was replicated three times with control aquaria receiving filtered habitat water and dimethyl sulphoxide (DMSO), without the addition of WSF. Each test tank including the controls contained ten test organisms. The crude oil (1 g) was first dissolved in 5 mL of DMSO and made up to 1,000 mL with distilled water before being shaken in a separatory funnel to produce the water soluble fraction (WSF). The stock solution was used in the preparation of different concentrations of the working solution by dilution with distilled water. The test was conducted for 96 h with daily observations of abnormality and mortality of test organisms. Dead organisms were quickly removed from the test medium to avoid decay and contamination. The test tanks were aerated with air stones connected to electrically powered aquarium pumps. The concentrations in which 50% mortality (LC_{50}) occurred were obtained graphically by probit analysis, plotting concentration against fish mortality (Finney 1952; Stephan 1977).

The homogeneity of the three replicates of the samples was checked by the Kruskal–Wallis test, before data of the replicates were pooled together and treated as a single group. Significant differences in the number of dead organisms between control and experimental group were evaluated using the Chi Square method. Significance was accepted when $p < 0.05$. Statistical analysis was powered by SPSS 9.0 (SPSS Inc., Chicago, USA).

Results and Discussion

The test organisms (*M. macrobrachion* and *M. vollenhovenii*) showed pathological changes and mortalities in a concentration dependent manner. Sublethal changes observed were moribund and erratic swimming behavior, weakness, paleness and depigmentation of the skin. Similar changes were not observed in the control. *M. vollenhovenii was* more sensitive to crude oil contamination with 50% mortality (LC_{50}) at 4 ± 1.76 mg/L as compared to *M. macrobrachion* at 5 ± 1.76 mg/L after 96 h of exposure (Fig. 1). The mean (± SD) water parameters of the test water were 28.03 ± 0.13°C (temperature), 8.48 ± 1.17 (pH), 28.74 ± 0.12‰ (Salinity), 4.90 ± 0.42 mg/L (dissolved oxygen) and 26.80 ± 0.14 (μS/cm) for conductivity respectively (Table 1). The mortality patterns of the two shrimp species (*M. macrobrachion* and *M. vollenhovenii*) were similar in the WSF of crude oil (Tables 2, 3). The concentration of total hydrocarbons in the WSF was

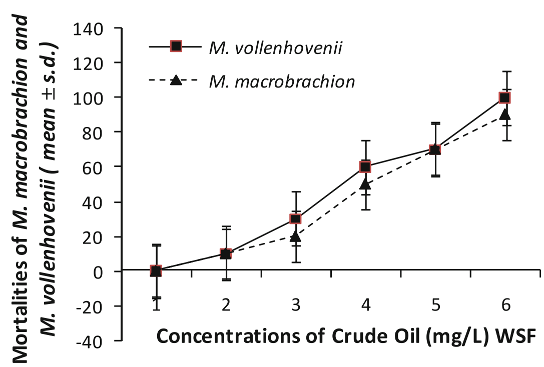

Fig. 1 Mortalities of two *Macrobrachium* species exposed to different concentrations of crude oil WSF

Table 1 Water parameters of habitat water used for the test

Parameters	Min.	Max.	Mean	SD
Temperature (°C)	27.85	28.20	28.03	0.13
pH	7.65	9.30	8.48	1.17
Salinity (‰)	28.65	28.82	28.74	0.12
Conductivity (μS/cm)	26.7	26.9	26.80	0.14
Dissolved oxygen (mg/L)	4.60	5.20	4.90	0.42

Table 2 Mortalities of *M. macrobrachion* against exposure time in the various concentrations of crude oil WSF

Time (h) Conc. mg/L)	24 h Mortality %	48 h Mortality %	72 h Mortality %	96 h Mortality %	Overall mortality (%)
0	0	0	0	0	0
2	0	0	0	10	10
4	0	10	10	0	20
6	0	10	20	20	50
8	10	30	30	–	70
10	30	50	10	–	90

Table 3 Mortalities of *M. vollenhovenii* against exposure time in the various concentrations of crude oil WSF

Time (h) Conc. (mg/L)	24 h Mortality %	48 h Mortality %	72 h Mortality %	96 h Mortality %	Overall mortality (%)
0	0	0	0	0	0
2	0	10	0	0	10
4	10	0	20	0	30
6	10	10	30	10	60
8	20	40	10	–	70
10	40	60	–	–	100

27.30 mg/L. Statistical analysis using the Chi Square method showed that there was no significant difference ($p > 0.05$) in mortality between the two species of shrimp. Organisms that survived in the test medium to the end of the experiment were highly stressed as shown in their non-agile movements, compared to their counterparts in the controls which were all active and normal.

The two species of shrimp are important to the seafood industries in Nigeria and other West African countries (Udo and Taege 1991), accounting for about 70% by weight of total shrimp landings in the Lagos Lagoon. A reduction in abundance of the species as a result of oil spills could represent an economic and environmental catastrophe for the sustainability of the fisheries and the safety of the consumers. It has been reported (Heintz et al. 2000) that chronic exposure of some life stages of aquatic animals to crude oil contaminants may pose serious nutritional problems and safety issues for human and animal consumers in the food chain. Although the presence of hydrocarbons tends to favor the abundance of carbon degrading bacteria in the aquatic and marine environments, a shift in species composition of some fresh water and marine species has been attributed to the spill of crude oil in affected areas (GESAMP 1982).

The destruction of eggs and larval stages of benthic organisms by crude oil is likely to have a deleterious impact on the recruitment of affected species, and if not controlled, may affect commercial fisheries. Lavarías et al. (2007) confirmed in their studies on the effects on *M. borellii* of the WSF of light crude oil dissolved in freshwater, that the juveniles and larval life-stages were significantly more sensitive than the adults life-stage.

The gut contents analysis of *M. macrobrachion* and *M. vollenhovenii* have shown these species have preferences for detritus, algae, zooplankton, crustaceans and other small animals, which are also vulnerable to the impacts of crude oil contaminants (Odiete 1999). This poses further threats on the food supply of these important shrimp species. The cross-contamination of organisms affected by crude oil may pose a serious nutritional problem to other animals and man, who utilize them as food. Crude oil- contaminated shrimp and water fed to rats resulted in changes in hematological parameters of the animals (Enyong et al. 2004). There was a significant decrease in PCV (pack cell volume) and RBC (red blood cell) counts, while Hb (hemoglobin) and WBC (white blood cell) counts were significantly increased above normal values.

The deaths of organisms in the present study may have resulted from the inability of the gill membranes to undergo normal gaseous exchange due to the presence of the crude oil, resulting in suffocation and death. Prasad and Kumari (2006), attributed mortalities of a fresh water fish (*Puntius sophore*) to the formation of a coagulated mucous film over the gills and body surfaces. A loss of pigmentation in skin occurred in the test organisms exposed to the WSF, but not in the controls. This likely resulted from the accumulation of petroleum contaminants in their tissues, as Asuquo and Udoh (2002) reported the accumulation of petroleum contaminants in fish fillets a year after a crude oil spill along the Nigerian coastline.

It was also observed that organisms in almost all concentrations tested were not showing pathological changes after 24 h of exposure, but subsequently died with increased time of exposure. According to Dinman (1972), toxicity of crude oil contaminants in water is dependent on the time of exposure for the acquisition of the capacity to elicit mortalities in a biological system.

Apart from behavioral changes and mortalities attributed to the accumulation of petroleum contaminants in tissues of shrimp and fish, there have been no reports on the effects of crude oil-contaminants on the nutritional value and consumer taste of products from affected areas. Further studies are recommended on the nutritional status of shrimp and fish from crude oil-contaminated waters to guard against associated health risks from consumption of the contaminated animals. Environmental impact assessments (EIA) of affected areas should be encouraged for effective monitoring and controls.

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