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Toxicological Effects of Water Soluble Fraction of Crude Oil on Macrobenthic Invertebrates: *Chironomus* and Mosquito Larvae

Francis O. Arimoro and Kabir M. Adamu
Department of Zoology, Delta State University, P.M.B. 1, Abraka, Nigeria

Abstract: The acute toxicity of Water Soluble Fraction (WSF) of crude oil on *Chironomus* and mosquito larvae were evaluated to determine its effect on their survival rate. The larvae were exposed to acute concentration of WSF of crude oil (20.0, 10.0, 5.0 and 0.0 ml L^{-1}) during the 48 h exposure in a static bioassay medium. Mortality was found to increase with exposure time and concentration. The 48 h Median Lethal Concentration (MLC) were 5.75 and 6.61 ml L^{-1} for mosquito and *Chironomus* larvae, respectively. However, the 48 h Median Lethal Time (MLT) for the different concentrations were 18.20 h for 20 ml L^{-1}, 28.20 h for 10 ml L^{-1} and 48 h for 5 ml L^{-1} and 26.80 and 41.80 h for 20 and 10 ml L^{-1}, respectively. Comparatively, mosquito larvae were more sensitive to the WSF of crude oil compared to the *Chironomus* larvae as shown in this finding.

Key words: Toxicity, crude oil, median lethal concentration and time, mosquito larvae, *Chironomus* larvae

INTRODUCTION

Crude oil spillage during operations, accidental spill during the shipping and leakage from the underground pipe are becoming a common phenomenon on a global scale. They have over the years led to the pollution of the world aquatic ecosystem (Omoregie and Ufodike, 2000). Several toxic components of crude oil have been documented. Crude oil is drained into the aquatic environment during drilling or spillage with no treatment given to reduce its toxicity. It has been reported that in most cases the damage done by pollution to the environment in irreversible (Omoregie and Ufodike, 2000). Omoregie *et al*. (2001) has reported that these could contribute to additional stress to aquatic fauna.

Most toxicology research of crude oil on aquatic organisms is usually carried out on fishes, very little have been tested on macrobenthic invertebrates. The use of freshwater macro-invertebrates in pollution studies has been restricted largely to biological surveys describing community changes associated with chronic and episodic pollution (Rueda *et al*., 2002; Ndaruga *et al*., 2004; Arimoro *et al*., 2007). *Chironomus* are members of the Chironomidae, an ecologically diverse and ubiquitous family in freshwaters with larval stages generally forming an extremely important constituent in terms of productivity. The larvae are often found in high members at site affected by organic pollution (Arimoro *et al*., 2007), while Mosquito act as vector to different endemic diseases. Indeed the present recurrence of these is due to higher number of breeding places in today's throwaway society thus their choice for this study. Recently, studies involving various invertebrates to acute lethal toxicity tests in the laboratory is gaining popularity (David *et al*., 1989; Beal and Anderson, 1993; Aliero, 2003; Roy and Dutta, 2003; Agbon *et al*., 2002, 2004). However there is a dearth of information on the toxicity of crude oil to *Chironomus* and mosquito larvae. This study was therefore undertaken to determine the Median Lethal Concentration (MLC) and Median Lethal Time (MLC) for

Corresponding Author: Dr. Francis, O. Arimoro, Department of Zoology, Delta State University, P.M.B. 1, Abraka, Nigeria Tel: +2348035615424

various concentrations of Water Soluble Fraction (WSF) of crude oil on *Chironomus* and mosquito larvae exposed to various acute concentrations of crude oil during the 48 h exposure period. This is with a view to adding to the knowledge on the effects of crude oil on these invertebrates as prominent members of the benthic community.

MATERIALS AND METHODS

Test Organisms

Chironomus larvae were collected in a plastic bucket from an organically impacted section of River Orogodo in Agbor, Delta State of Nigeria. Mosquito larvae were obtained by collecting their eggs from an outdoor metal tank and allowing them to hatch in 1 L capacity transparent glassware. They were allowed to grow to second instar stage before bioassay testing.

Test Solution

Bonny light crude oil was obtained from NNPC Warri depot. Water-soluble fractions of crude oil for toxicological bioassay were prepared as described by Smith and Cameron (1979). After preliminary investigations with 50, 40, 30, 20, 10, 5 and 1 ml L^{-1} of WSF of crude oil on the respective larvae, the desired concentrations for this test were ascertained to be 20, 10, 5 and 0.0 ml L^{-1}.

The larvae toxicity assays were made according to Thangam and Kathiresan (1991) with some modification. Larval stages of *Chironomus* and mosquito were collected with a Pasteur pipette placed on filter paper to remove excess water and transferred (20 per test) with a tiny brush into beakers containing 20 mL of test solution. In separate set up the larvae were exposed at room temperature during the 48 h exposure to the various acute concentrations (20.0, 10.0, 5.0 and 0.0 ml L^{-1}) of WSF of crude oil while mortality-survival rate were registered after 12, 24, 36 and 48 h, respectively. Each test was run in triplicate while distilled water was used as control.

Regression analysis, 95% confidence limit, Median Lethal Concentration and Median Lethal Time for each concentration respectively were determined with the aid of SPSS 15.0 version.

RESULTS

The acute toxicity results obtained is as represented in Table 1 and 2 for Mosquito and *Chironomus* larvae respectively. The logarithm concentrations of the various WSF of crude oil used,

Table 1: Mean* cumulative mortalities of mosquitoes larvae exposed to various acute concentrations of WSF of crude oil during the 48 h exposure period

Concentration of WSF of crude oil (ml L^{-1})	Mean No. of exposed larvae	Cumulative mortalities (h)			
		12	24	36	48
20	20	6.33	13.00	19.67	20.00
10	20	2.00	7.67	14.33	18.00
5	20	0.67	2.00	6.00	10.00
0	20	0.00	0.00	0.00	0.00

*: Obtained from triplicate exposure

Table 2: Mean* cumulative mortalities of *Chiromonous* larvae exposed to various acute concentrations of WSF of crude oil during the 48 h exposure period

Concentration of WSF of crude oil (ml L^{-1})	Mean No. of exposed larvae	Cumulative mortalities (h)			
		12	24	36	48
20	20	3.67	9.33	15.33	20.00
10	20	0.67	3.67	8.00	13.00
5	20	0.00	1.67	5.33	9.67
0	20	0.00	0.00	0.00	0.00

*: Obtained from triplicate exposure

Table 3: Logarithm concentration, mean cumulative mortality and probit mortality of mosquito larvae exposed to various acute concentrations of WSF of crude oil during the 48 h exposure period

Concentration of WSF of crude oil (ml L^{-1})	Logarithm concentration	Mean cumulative mortality	Mean cumulative mortality (%)	Probit mortality
20	1.3010	20.00	100.00	8.72
10	1.0000	18.00	90.00	6.28
5	0.6990	10.00	50.00	5.00
0	0.0000	-	-	-

Table 4: Logarithm concentration, mean cumulative mortality and probit mortality of *Chironomous* larvae exposed to various acute concentrations of WSF of crude oil during the 48 h exposure period

Concentration of WSF of crude oil (ml L^{-1})	Logarithm concentration	Mean cumulative mortality	Mean cumulative mortality (%)	Probit mortality
20	1.3010	20.00	100.00	8.72
10	1.0000	13.00	65.00	5.41
5	0.6990	9.67	48.35	4.96
0	0.0000	-	-	-

total mortality, percentage mortality as well as probit mortality of the exposed macro-benthic invertebrates is described Table 3 and 4 for Mosquitoes and *Chironomus* larvae, respectively. There was very strong and positive correlation between Log Concentration of WSF of crude oil and probit mortality as shown by the value of regression analysis which showed that 99 and 96% of the association is dependent on the variables (Log Concentration and probit mortality) for mosquito and *Chironomus* larvae respectively.

DISCUSSION

In toxicity tests, mosquito larvae were found to be more sensitive than the *Chironomus* larvae due the value revealed in the MLC. The MLC of mosquito larvae (5.19 ml L^{-1}) in this study revealed that the toxicant had more effect of the larvae compared to diazinon as reported by Agbon *et al.* (2002) that 48 h LC_{50} of diazinon on mosquito larvae was 9.87 mg L^{-1}. However, the toxicological effect of WSF of crude oil is less intense than rotenone as reported by Agbon *et al.* (2004) that the 48 h LC_{50} of rotenone on mosquito larvae was 4.48 mg L^{-1}. The 48 h LC_{50} effect of crude oil has been reported by Daka and Ekweozor (2004) on Mangrove Oyster *Carasostrea gasar* to be 135 ppm. The result obtained in this study concord favourably with that of Aleiro (2003) when *Anopheles* mosquito was exposed to aqueous extract of neem *Azadirachta indica.*

Previous toxicity studies (David *et al*., 1989) have suggested that chironomids are relatively tolerant of toxicants. Results of the present investigation with mosquito and *Chironomus* larvae illustrates the importance of acute toxicity testing with freshwater macroinvertebrates in order to identify their sensitivity in response to toxicants such as crude oil. The issue of proper water quality management and also the imperatives to give teeth and bite to the campaign against untreated/spillage of crude oil into the water bodies in several developing countries should be a point of focus. Researches in the long-term effects of mineral concentrations of crude oil on aquatic organism should be intensified. These are very necessary to conserve the world fast declining fishery resources.

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