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acta SATECH **2**(1): 1-5 (2005)

Research

Stress indicators in earthworms *Eudrilus eugeniae* inhabiting a crude oil contaminated ecosystem

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Received:27 May, 2005 *Revision accepted: 30 July, 2005*

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Abstract

The acute toxicity level of Forcados light crude oil against earthworm Eudrilus eugeniae was 10.33ml/kg based on the 96hr LC50 value. The exposure of the earthworm to sublethal concentrations (1.032ml/kg and 0.103ml/kg) of crude oil resulted in the exhibition of coiling responses, weight loss and swollen clitellum region in exposed animals. Studies on the total haemocyte counts in blood of the earthworms also showed that there was a reduction in the total haemocyte counts in the exposed animals compared to the control organisms. The significance of these sublethal effects particularly its importance as useful stress indicators to identify and assess recovery of crude oil contaminated ecosystems were discussed.

Keywords: Earthworm, crude oil, stress indicators, sublethal effect, toxicity

Introduction

Crude oil contamination in terrestrial ecosystems usually results in long-term adverse effects on the exposed organisms. This is because the spilled oil penetrates deep into the soil and consequently persists in the environment due to the anoxic conditions prevailing at depths below just a first few millimeters (Hozumi et. al., 2000; Peterson, 2001). Therefore the use of mortality as endpoint of toxic effects of crude oil in terrestrial ecosystems is therefore not realistic and would be an insensitive predictor of long-term effects of spilled oil on exposed organisms. Hence, the identification of reliable, simple and easy to use biological parameters would provide useful and rapid means by which contaminated sites can be easily identified or effectiveness of control methods monitored (Cikutovic et. al., 1993; Maila and Cloete, 2005).

One of the most ecologically important terrestrial soil organisms is earthworm. They benefit the soil primarily due to the physical and chemical effects of their casts and burrows. The casts contribute to soil nutrients since it has been found to contain high concentrations of nitrogen, phosphorus, potassium, exchangeable magnesium and calcium while the burrows helps to aerate the soil and also serve as drainage systems during irrigation and heavy rainfall (Edward and Bohlen, 1996). Due to their ubiquitous nature and sensitivity, they serve as valuable in situ sentinels for use in assessing biological risks from hazardous and toxic compounds in terrestrial environment.

The objective of this study is to identify simple and easy to observe stress indicators in earthworms exposed to sub-lethal concentrations of crude oil. These biological parameters will serve as rapid tools for identifying oil impacted ecosystems, evaluate efficiency of remediation programmes and other post impact assessment studies.

Material and methods

Test Animal

Eudrilus eugeniae (Annelida; Oligochaeta; Eudrilidae) was collected by hand picking from moist subsurface soil and under stones in University of Lagos main campus. The earthworms were collected

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from the same site in order to reduce variability in biotype.

In the laboratory, the earthworms *Eudrilus eugeniae* was kept in a holding pot (volume 5Litres), which was half filled with a mixture of loamy and humus soil supplemented with half-boiled, ground water-leaf (*Talinum triangulare*) and moistened with deoxygenated tap water (treated soil) after Fafioye and Owa (2000). The earthworms were kept in the holding pot for a minimum of ten days in order to allow them adapt to experimental conditions (28 $\pm$ 2^0C, 79 $\pm$ 2R.H). During the period of acclimatisation, the animals were fed with leaflets of lettuce *Nymphea lotus*, 25g per 80 worms every 4 days.

Earthworms of similar sizes ranging from 80mm to 120mm – length and 0.9g to 1.20g – body weight were carefully selected from the holding pots into pre-experimental containers from where they were randomly transferred to the bioassay containers.

Test chemicals

Crude Oil: Forcados light was obtained from Shell (SPDC) production platform in Forcados, Port Harcourt, Nigeria. Some of the physico-chemical characteristics of Forcados light brand of crude oil include; Sulphur content 0.2%, API gravity 60 / 60F, rapid vapour pressure 2.5psi and pour point 25.

Preparation of test media including application of toxicant

A mixture of loamy and humus soil collected from the biological science garden of the University of Lagos was used as substrate. The soil was air-dried, ground and sifted through a 0.25mm (mesh size) screen in order to standardize the grain size. The soil was mixed with half-boiled, dried and fairly ground waterleaf as food supplement. A 50g portion of the prepared soil moistened with 5ml of dechlorinated tap water was used as substrate in bioassays.

Pre-determined amounts of crude oil were measured out using pipette into bioassay containers (Bottom diameter –14.5cm, Top diameters – 21cm and height – 9cm) already containing 50g of treated soil acting as substrate. The bioassay containers were perforated at the base to allow for drainage of water while the top of the container was covered with mesh aided by elastic bands so that the earthworms could not escape. The substrate and the oil are properly mixed by shaking vigorously to ensure uniform distribution of the oil.

Effects of sublethal concentrations of crude oil against *Eudrilus eugeniae*

A total of 36 active *Eudrilus eugeniae* in three replicates of 12 each were exposed to sublethal concentrations of crude oil. The sublethal concentrations were fractions (1/10, 1/100, of lethal concentrations (LC50 values) of crude oil against earthworms during the preliminary trials. The following sublethal studies were carried out:

Weight Changes

The weight of the test animals in treated and untreated (control) test media were recorded at the commencement and termination (after seven days) of the assays. Weight changes in the earthworms were carried out only at the commencement and termination of the experiments so as to reduce the introduction of handling stress in the test animals.

% Weight change per worm =

$$\frac{\text{average Initial wt} - \text{average final wt}}{\text{average Initial wt}} \times 100$$

Coiling

The number of coiled animals in treated and untreated (control) test media were recorded at the commencement and at 24-hour intervals over the 7-day period of observation.

Swollen Clitellum

The number of animals with swollen clitellum in treated and untreated (control) test media were recorded at the commencement and at periodic time intervals over the 7-day period of observation.

Total Haemocyte Counts (THCs)

200µl of blood was extracted from the anterior region of the worms and mixed with 200µl of Na-cacodylate based anticoagulant preloaded into a 1ml syringe. The total haemocyte counts (THCs) were then determined with the aid of a Beckman (Z1 series) Coulter counter.

Statistics

The dose response data involving quantal response (mortality) subjected to probit analysis (Finney, 1971). T-test analysis was carried out to test for significance between each treatment and control means in appropriately designed experiments. Analysis of Variance (ANOVA) between the different treatment means and control was used to test the null hypothesis that there was no difference between means for the various treatments and control. Further analysis was carried out only where there was a significant difference at the 5% ($P < 0.05$) level of significance (taken as minimum requirements).

Results

Effects of sublethal concentrations of crude oil on

weight changes, coiling, swollen clitellum and total haemocyte counts in earthworm, *Eudrilus eugeniae*.

Weight Changes

The exposure of the earthworm *Eudrilus eugeniae* to sublethal concentrations of crude oil was found to result in weight loss of about 23% - 65% between the commencement and termination of the experiment. An average weight gain of about 15% was however recorded in the control animals (Table 1).

Statistical comparisons of observed weight changes showed that there was significant ($P < 0.05$) difference in the weight of animals exposed to sublethal concentrations of crude oil compared to the control animals.

Table 1: Weight changes in earthworm Eudrilus eugeniae exposed to sublethal concentrations of crude oil over a 7-day experimental period

Conc. Tested ml/kg	Mean Initial Weight(g) (Total No Exposed–36)	Mean Final Weight(g) (Total No Exposed–36)	%Weight Change
0.001	1.11	0.85	-23
0.010	1.10	0.68	-38
0.103	1.10	0.41	-62
1.032	1.20	0.42	-65
Control	1.01	1.16	+15

-ve value indicates weight loss +ve value indicates weight gain

Coiling

The exposure of earthworm *Eudrilus eugeniae* to sublethal concentrations of crude oil ranging between 0.103ml/kg and 1.033ml/kg was observed to result in coiling of about 8.33% - 75% of the exposed animal. The highest percentage of coiled earthworms was recorded within the first 24 hours of observation (Table 2).

Table 2: Mean number (Percentage in Parenthesis) of coiled earthworms in treated and untreated media

Conc. Tested (ml/kg)	Day 1	Day 2	Day 4	Day 7
0.001	0 (0)	0 (0)	0 (0)	0 (0)
0.010	0 (0)	0 (0)	0 (0)	0 (0)
0.103	5 (41.67)	3 (25)	1 (8.33)	1 (8.33)
1.032	9 (75)	5 (41.67)	4 (33.33)	3 (25)
Control	0 (0)	0 (0)	0 (0)	1 (8.33)

Statistical comparisons of coiling responses showed that there was significant ($P < 0.05$) difference in the mean number of coiled earthworms in treated media compared to the control media.

Swollen Clitellum

The exposure of earthworm *Eudrilus eugeniae* to sublethal concentrations of crude oil ranging between 0.103ml/kg and 1.033ml/kg was observed to result in swelling of the clitellum region of about 8.33% - 100% of the exposed animal (Table 3). Swelling of the clitellum was however not observed in animals exposed to lower concentrations (0.01ml/kg & 0.001ml/kg) of crude oil and control.

Table 3: Mean number (Percentage in Parenthesis) of earthworms with swollen clitellum in treated and untreated media

Conc. Tested (ml/kg)	Day 1	Day 2	Day 4	Day 7
0.001	0 (0)	0 (0)	0 (0)	0 (0)
0.010	0 (0)	0 (0)	0 (0)	0 (0)
0.103	0 (0)	1 (8.33)	5 (41.67)	8 (66.67)
1.032	0 (0)	2 (16.67)	12 (100)	12 (100)
Control	0 (0)	0 (0)	0 (0)	0 (0)

Statistical comparisons also showed that there was significant ($P < 0.05$) difference in the mean number of earthworms with swollen clitellum in treated media compared to the control media.

Total Haemocyte Counts (THCs)

Result of total haemocyte counts in blood of the earthworms exposed to different sublethal concentrations of crude oil showed that there was a decrease in the THCs count in the exposed animals compared to the controls (Table 4).

Table 4: Total haemocyte counts (THCs) in earthworms exposed to sublethal concentrations of crudeoil and untreated media.

Conc. Tested (ml/kg)	Mean THCs in earthworm before experim-entation Day 0	Mean THCs in earthworm before experim-entation Day 7
Control	$2.5 \pm 0.3 \times 10^3$	$2.03 \pm 0.2 \times 10^3$
0.103	$2.5 \pm 0.3 \times 10^3$	$1.89 \pm 0.3 \times 10^3$
1.032	$2.5 \pm 0.3 \times 10^3$	$1.48 \pm 0.2 \times 10^3$

However, analysis of variance of the THCs in animals exposed to sublethal concentrations of crude oil was not significantly different ($P>0.5$) from the counts in control animals.

Discussion

In this study, the acute toxicity level of Forcados light crude oil against *Eudrilus eugeniae* was found to be 10.33ml/kg based on the 96hr LC50 value. The exposure of the earthworm to sublethal concentrations (1.033ml/kg and 0.103ml/kg) of crude oil resulted in the exhibition of coiling responses, weight loss and swollen clitellum region in exposed

animals. These types of responses exhibited by the earthworms can serve as very useful indicators of crude oil related contamination or other forms of stress in the terrestrial ecosystem since they can be very easily detected in the field. The use of behavioural responses by organisms and other rapid detection techniques for identifying contaminated sites have also been recognized by other authors (Otitoloju and Adeoye, 2002; Petrauskiene, 2003; Untersteiner et. al., 2003 & Maila and Cloete, 2005) as an important tool which can be used singly or in combination with other forms of monitors for detection of stressors in the environment.

Behavioural responses such as coiling exhibited by earthworms inhabiting stressed environment have a great advantage in being able to serve as early detection system hence ensuring that control measures can be initiated on time before the full impact or lethal effect of the contaminant become apparent. Additionally, organism responses have also been identified as an important tool for monitoring recovery or effectiveness of mitigation measures employed during clean-up programmes (Gunn and Sadd, 1994; Xu, 1999; Dorn and Salanitro, 2000).

Studies on the total haemocyte counts in blood of the earthworms also showed that the exposure of the worms to sublethal concentrations of crude oil caused a reduction in the total haemocyte counts in the exposed animals compared to the control organisms. Other authors who have identified blood parameters as a potential tool for identification of stress in the environment include Roche and Boge, 1999; Jusila *et. al.*, 2001 & Masson *et. al.*, 2002. According to these authors, blood parameters such as levels of cortisol, glycemia, haemoglobin, hematocrit, Cl^- content, haemolymph clotting time and total haemocyte counts are very useful indicators of chemical/physical stress in the environment.

The reduction in the total haemocyte counts in the exposed earthworm during this study may be as a result of the disruption of the blood cell membranes, which were observed in slides prepared from blood samples of some of the exposed earthworms. Similar observation of membrane disruption was also observed for the clitellum sections, where a complete collapse and disintegration of the tissues in the coelomic cavity was observed. These observations indicate that the usual physical effects of mere entangling and limitation of oxygen diffusion (Hozumi et. al., 2000; Peterson, 2001; Otitoloju and Udosen, 2004; Otitoloju, 2005) as mechanism of toxic action for crude oil against many organisms may not necessarily apply to crude oil toxicity against earthworm. A probable explanation would however be that some fractions of the crude oil are probably cytotoxic and with capability to disintegrate the membrane structures. Further studies on the toxic mechanism of crude oil against earthworm especially its ability to cause the disintegration of membrane structures should merit future research.

In view of the ubiquitous nature and beneficial status of earthworm in the terrestrial ecosystem, it is recommended that the behavioural responses such as coiling, weight loss, swollen clitellum region and total haemocyte counts should be included in biomonitoring programmes aimed at identifying contaminated sites or assessing recovery of contaminated ecosystems.

Acknowledements

The author is grateful to members of Shell Petroleum Development Company for assistance rendered in providing crude oil used for this study.

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