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Alterations in the Bio-membrane of *Libyodrilus violaceus* following Exposure to Crude Oil and Its Fractions

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Authors' contributions

This work was carried out in collaboration between all authors. Author GOE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author GEE generally supervised all procedures and protocols in this research study, author KO assisted with laboratory protocols and author HKN assisted in managing the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

We investigated changes in the lipid profile of clitellum and post-clitellum sections of earthworms (*Libyodrilus violaceus*) after exposure to different doses of crude oil (Bonny light blend) or its fractions for two-weeks. Two hundred and forty earthworms were assigned to four major groups of sixty earthworms each; one group was given deionized water and served as the control group and the other test groups were either exposed to whole crude (WC) or its water soluble fraction (WSF), or water insoluble fraction (WIF). Twenty animals in each test group were exposed to 0.1%, 0.2% or 0.3% contamination. The treated groups revealed a significant reduction ($P<0.05$), (1.71% - 28.29%) in the weight of earthworms when compared with those in the control group. Generally, the exposure of the earthworms to either whole crude oil or its fractions resulted in a dose dependent significant ($P<0.05$) reduction in the total lipid and total phospholipid content of both sections (clitellum and post clitellum) of the worms studied. We observed that the exposure of earthworms to crude oil or its fractions decreased phosphatidyl ethanolamine (PE), and phosphatidyl choline (PC)

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at different doses when compared with concentrations in control animals. There was also an increase in sphingomyelin levels in both the clitellum and post-clitellum sections of worms in the treatment groups at the different doses. These changes significantly affected the SGM/PC ratios. These changes in the phospholipid profile may have profound significance in the tolerance of earthworms to the toxicants in crude oil or its fractions. Physical and ionic characterization of the crude oil sample revealed that it contained unsafe levels of cadmium(7.50mg/l, 0.97mg/l, 11.54mg/l for WSF,WIF and WC respectively) and poly aromatic hydrocarbons (PAHs) (1.2926mg/l, 5.5871mg/l, 36.3665 mg/l for WSF, WIF and WC respectively).

Keywords: Crude oil; Libyodrilus violaceus; total lipids; phospholipids; cadmium; PAHs.

1. INTRODUCTION

The Niger Delta region of Nigeria is rich in petroleum crude oil and it accounts for over 95% of Nigeria's foreign earnings [1,2]. Since the commercial exploration of crude oil started in Nigeria in 1958, it has become the mainstay of the Nigerian economy with the annual budget based on oil revenue [2]. Crude oil contributes over 90% of the country's GDP [3]. The Niger Delta region is the most complex lowland forest/aquatic ecosystem in West Africa and its biological diversity is of regional and global importance [4]. The entire ecosystem of the Niger-Delta is constantly under treat from contamination from crude oil and related chemicals because oil leaks and spills as well as poor waste management are a common feature in the Niger-Delta. According to the Department of Petroleum Resources (DPR), between 1976 and 1996 a total of 4647 incidents resulted in the spill of approximately 2,369,470 barrels of oil into the environment. Of this quantity, an estimated 1,820,410.5 barrels were lost to the environment. This can be translated as a total of 76.83% of the spilled volume being lost to the environment. Available records for the period of 1976 to 1996 indicate that approximately 6%, 26% and 69% respectively, of total oil spilled in the Niger Delta area, were in land, swamp and offshore environments respectively [5].

Crude petroleum oil has complex mixtures of chemicals, varying widely in their composition of hydrocarbon and hydrocarbon-like chemicals [6,7]. Crude oil also contains trace amounts of elements like vanadium, nickel, iron, aluminum, sodium, calcium, copper, and some heavy metals like lead and cadmium [8]. It has also been found to contain some levels of PAH. PAHs are widespread environmental contaminants whose occurrence is largely as a result of anthropogenic emissions such as fossil fuel-burning, motor vehicle, waste incinerator, oil refining and or oil spill e.t.c. The US Environmental Protection Agency (EPA) has promulgated 16 PAHs (EPA-PAH) as priority pollutants. Thus, exposure assessments of PAHs in the developing world are important [9,10,11]. Crude oil in spite of its immense benefit is hazardous to the environment and has been shown to cause long term damage and even death in a variety of lower animals [12,13,14]. The use of earthworms as bio-indicators in the monitoring of the toxic effect of chemicals is not new because of their high biomass [15] and sensitivity [16,17].

Earthworms are also skin breathers taking in oxygen through its moist mucus-secreting skin. The mucus also serves to lubricate the worm's body and ease passage through the burrows, thereby constantly increasing its contact with its immediate environment. A change in the phospholipid profile of bio-membranes would alter the biochemical dynamics necessary for biological processes to go on. Information on the changes in phospholipid associated with crude oil contamination is lacking. The aim of this research is to attempt to provide data on the changes in earthworm's lipid content and phospholipid profile associated with crude oil contamination.

2. EXPERIMENTAL DESIGN

2.1 Fractionation Protocols

Bonny light crude oil was obtained from Warri Refinery and Petrochemical Company in Delta State, Nigeria. It was fractionated by the method of Anderson [18]. A 1:2 dilution of 200ml of the crude oil was put in a 1L conical flask and constantly stirred with a magnetic stirrer for 48hrs. Thereafter, the water soluble fraction (WSF) was separated from the water insoluble fraction (WIF) in a separating funnel; they were both kept in glass jars sealed and frozen until required.

2.1.1 Physicochemical analysis of crude oil and its fractions

Crude oil and its various fractions were analyzed for some physicochemical properties (heavy metals and Poly aromatic hydrocarbon (PAH) content). Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp. Notwalk Connecticut) with acetylene/air flame was used for the estimation of heavy metal elements in the samples. PAH estimation was by isotope dilution mass spectrometry combined with high resolution gas chromatography. This entailed the addition of internal standards to all samples in known quantities. (Air Resources Board methods. Adopted 1989, Amended 1997).

The earthworms were housed individually in outdoor mesocosms (polyethylene tanks) packed with rich humus (1000g) soaked with 100ml water to stabilize the humus and simulate environmental realism relative to the laboratory. Concentrations of crude oil used for these sublethal studies were selected based on the 96h exposure LC_{50} value (10.33ml/kg) for bonny light crude oil exposed to earthworms, Eudrilus eugeniae [19]. 0.1% concentration of the WSF, WIF and WC oil fraction were prepared by adding 1ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 999ml of distilled water. 0.2% concentration of WSF, WIF and WC oil fraction was prepared by adding 2ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 998ml of distilled water. 0.3% concentration of crude oil fraction was prepared by adding 3ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 997ml of distilled water. The test specimen (*Libyodrilus violaceus*) were collected by handpicking from a moist subsurface soil in Okada town, Benin city, Edo state Nigeria, in open plastic bowls containing habitat soil and was transported to the laboratory. The earthworms were collected from the same site in order to reduce variability in biotype. Earthworms used in this study were adults with well-developed clitellum. The earthworms were grown on soil contaminated in the laboratory with whole crude oil and its fractions. Two hundred (200) earthworms were assigned to four (4) major classes of treatments. The first class was the control group where the earthworms were grown in normal uncontaminated soil and it comprised of twenty animals. The other One hundred and eighty (180) animals were assigned to three classes of treatment. One class was treated with whole crude, another with the water soluble fraction (WSF) and the third class was treated with the water insoluble fraction (WIF). Each of these classes of treatment comprised of sixty animals each. In each class, we had three (3) sub-classes of twenty earthworms each. The sub-classes were exposed to graded doses of 0.1%, 0.2%, or 0.3% of the treatment.

2.2 Extraction of Lipids and Biochemical Assays

Known weight of the worms were sectioned and used for lipid extraction assay using chloroform and methanol and water in the ratio 2:1:0.8 as described by the modified method of Bligh & Dyer [20] and the amount of the total lipid was quantified using the method of Frings & Dunn [21]. Total phospholipids were quantified by a combination of methods of Bartlett [22] and Curzner and Davison [23] The fractions of the phospholipids were separated using thin layer chromatography as described by Curzner and Davidson [23]. Spots corresponding to specific phospholipids were recovered by scrapping and quantified by the method of Fiske and Subarrow [24].

2.3 Statistical Analysis

Data collected were subjected to statistical analysis using the SPSS version 20. Results obtained were expressed as mean ± SEM. One-way Analysis of variance (ANOVA) was also used to compare the means of some of the parameters measured and where significant differences were observed at 95% confidence level, Duncan's New Multiple Range test [25] was used to separate the means.

3. RESULTS

Elemental analysis of crude oil and its fractions shows that the WC contains the highest levels of the heavy metals. As also indicated in this result (Table 2), the crude oil and its fractions contain 36.3665 mg/l, 5.5871 mg/l and 1.2926 mg/l of PAH in the WC, WIF and WSF respectively. The earthworms lost weight with the exposure to crude oil or its fractions and the severity of the losses were dose dependent (Table 3).

Table 1. Heavy metal concentration in the crude oil sample and its other fractions

Heavy metal	Fe	Mn	Zn	Cu	Cr	Cd	Ni	Pb	V
					mg/l				
WSF	23.7	0.46	1.75	0.03	6.15	7.5	8.52	10.3	7.91
WIF	1.21	0.27	0.29	0.02	1.65	0.97	1.83	0.27	1.55
WC	59.8	19.6	48.3	26.4	9.56	11.4	9.13	19.3	8.35

Values are represented in mg/l concentrations

Results are as presented in table. Elemental analysis of crude oil and its fractions shows that the WC contains the highest levels of the heavy metals (Cd=11.54mg/l, Pb=19.3mg/l and Cr=9.56mg/l) followed by the WSF (Cd=7.5mg/l, Pb=10.3mg/l and Cr=6.15mg/l) and then the WIF (Cd=0.97mg/l, Pb= 0.27mg/l and Cr=1.65mg/l)

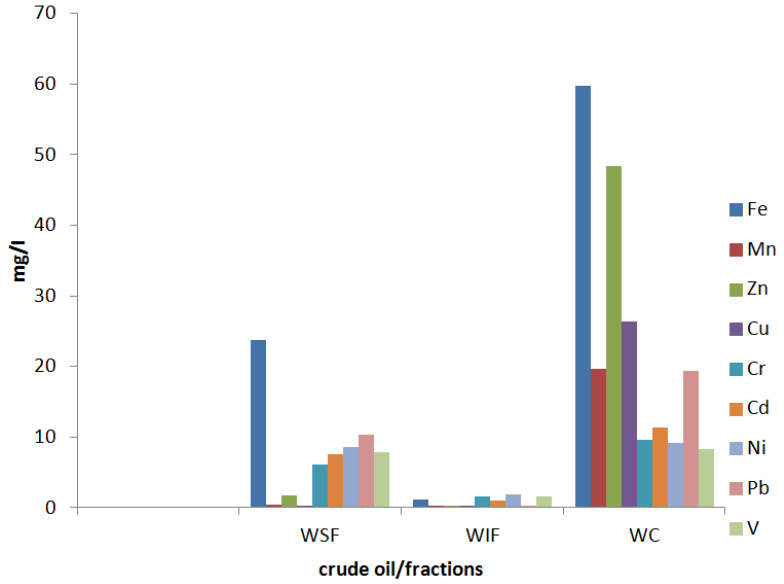

Fig. 1. Shows heavy metal concentration in the crude oil and its fractions

Table 2. Polyaromatic hydrocarbon (PAH) in sample crude oil and its fractions

Component	WSF	WIF	WC
Naphthalene	0.0000	0.0001	0.0001
Acenaphthalene	0.0000	0.0000	0.0000
Acenaphthene	0.0002	0.0000	0.0000
Florene	0.0000	0.0000	0.0000
Phenathrene	0.0000	0.0000	0.0000
Anthracene	0.0000	0.0000	0.0000
Fluoranthene	0.0000	0.0000	0.0000
Pyrene	0.0000	0.0000	0.0000
Benzo(a)anthracene	0.0006	0.0000	0.0001
Crysene	0.0000	0.0001	0.0001
Benzo(b)fluoranthrene	0.0000	0.0000	0.0000
Benzo(a)pyrene	0.0000	0.0000	0.0000
Benzo(k)fluoranthrene	0.0003	0.0000	0.0000
Indeno(1,2,3) perylene	0.0000	0.0002	0.0000
Dibenzo(a,h)anthracene	0.0000	0.0000	0.0000
Benzo (g,h,i) perylene	0.0000	0.0000	0.0000
Total (mg/L)	**1.2926**	**5.5871**	**36.3665**

Values are represented in mg/l concentrations

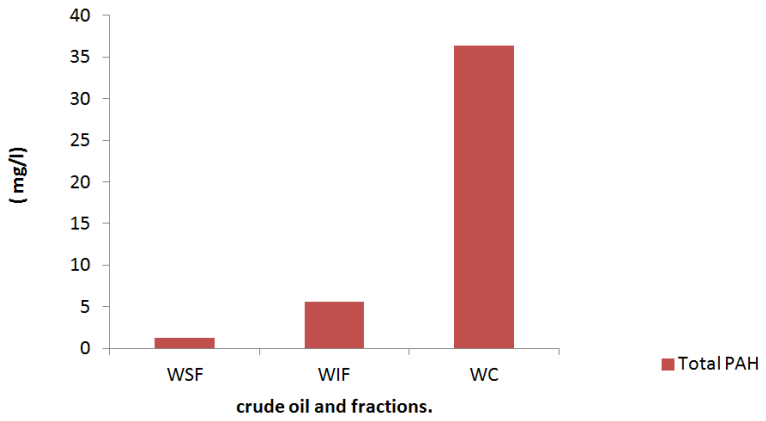

Fig. 2. Total PAHs in sample crude oil and its fractions

Table 3. Mean body weight changes and % mean body weight change in earthworms exposed to crude oil and its fractions

Treatment groups	Body weight			% change in body weight
Crude oil /fraction	Day 0	Day 7	Day 14	
Control	3.72 ± 0.37^{a}	4.05 ± 0.36^{a}	4.27 ± 0.23^{a}	+14.78
0.1% WSF	2.33 ± 0.22^{bc}	2.05 ± 0.31^{cd}	1.85 ± 0.22^{cd}	-20.60
0.2% WSF	1.61 ± 0.15^{cd}	1.61 ± 0.16^{d}	1.56 ± 0.17^{cd}	-3.11
0.3% WSF	2.58± 0.40^{bc}	2.92 ± 0.39^{bc}	1.85 ± 0.26^{c}	-28.29
0.1%WIF	3.01± 0.41^{ab}	3.13 ± 0.37^{ab}	3.10 ± 0.21^{ab}	+2.99
0.2% WIF	1.97±0.33^{cd}	2.22 ± 0.32^{bc}	2.10 ± 0.33^{cd}	+6.60
0.3% WIF	2.34± 0.37^{bc}	2.39 ± 0.36^{bc}	2.30 ± 0.37^{c}	-1.71
0.1%WC	2.07± 0.17^{bc}	2.00 ± 0.13^{cd}	1.90± 0.12^{cd}	-8.21
0.2% WC	1.69± 0.26^{cd}	1.81 ± 0.30^{d}	1.50 ± 0.20^{d}	-11.24
0.3%WC	1.42± 0.15^{d}	1.51 ± 0.18^{d}	1.50 ± 0.19^{d}	-8.45

- *Values represent Mean ± Standard error of mean (SEM). WSF=Water soluble fraction, WIF=Water insoluble fraction, WC=whole crude.*
- *Means of the same row followed by different lettered superscripts differ significantly (P<0.05).*
- *Means of the same column with the same overlapping superscripts are statistically similar or show no significant difference (p>0.05)*

Exposure of earthworms to 0.1% whole crude (WC) did not significantly (P>0.05) alter the total lipid content of the clitellum or the sections after the clitellum compared with the control (Table 4). However, treatment with 0.2% and 0.3% WC significantly (P<0.05) reduced the total lipid content of both sections compared with the control. A similar trend was reported following exposure to 0.2% and 0.3% of either the water-soluble fraction (WSF) or water-insoluble fraction (WIF) (Table 4).

Exposure of the earthworms to 0.1%, 0.2 % and 0.3% of WC and 0.1% of either WSF or WIF significantly (P<0.05) reduced the total phospholipid content of both the clitellum and post clitellum sections compared with the controls (Table 5). However, a significant increase (P<0.05) in the total phospholipids of the earthworms was observed when they were exposed to 0.2% of either WSF or WIF (Table 5).

Exposure of the earthworms to the different doses of the WC or its fractions significantly (P<0.05) reduced the concentrations of phosphatidylethanolamine (PE), phosphatidy-lcholine (PC), phosphatidylinositol (PI) and phosphatidylserine (PS) in both the clitellum and the post clitellum sections compared with the controls (Tables 6, 7 and 8). We however observed a significant increase (P<0.05) in the content of sphingomyelin (SGM) in both sections of the earthworm studied in the test groups compared with the control where we were unable to detect this phospholipid (Tables 6, 7 and 8). These changes in PE, PC and SGM levels, affected the ratio of PE to PC and the ratio of SGM to PC in both the clitellum and section after

the clitellum of earthworms exposed to all the concentrations of WC and its fractions compared with the controls (Tables 6, 7 and 8).

4. DISCUSSION

The concentration of heavy metals in crude oil and its fractions used in this study varied in their different levels. The concentration of heavy metals (Cd, Pb, Cu and Cr) in crude oil sample (Table 1) are all above the Desirable Contaminant Concentrations DCC and Acceptable Contaminant Concentration (ACC) thresholds as published by the National Water Quality Management Strategy [26]. Various agencies including World health organisation (WHO), United States Environmental Protection (US-EPA) and European regulatory standards (EURS) have set different maximum contaminant limits for heavy metals. The maximum recommended by EURS for soil samples are Cd=3mg/kg; Cr=100mg/kg and Pb=150mg/kg [26.

Although, other heavy metals in crude oil sample and its fractions fall within safe levels as reported by the California office of Environmental Health Hazard Assessment (HHSL) and the United States Environmental Protection Agency Region 9 report, cadmium in sample crude oil (WC) and WSF (11.54 mg/l and 7.5 mg/l respectively) still remains higher than concentrations recommended as safe. Cadmium stands out as a potent toxicant that has been linked to alterations in cellular homeostasis and has been shown to enhance the production of several activated oxygen species designated ROS [27,28].

PAHs are widespread environmental contaminants resulting from incomplete combustion of organic materials. Crude oil exploration and exploitation amidst other routes (anthropogenic emissions such as fossil fuel-burning, motor vehicle, waste incinerator, etc.) are the main sources of this toxicant into the environment [9]. PAHs have received increased attention in recent years in air pollution studies because of their high carcinogenic, mutagenic and teratogenicity. The US Environmental Protection Agency (EPA) has also listed PAHs (EPA-PAH) as priority pollutants. Thus, exposure assessments of PAHs in the developing world are important. As indicated in this result (Table 2), the crude oil and its fractions contained 36.3665 mg/l, 5.5871 mg/l and 1.2926 mg/l of PAH in the WC, WIF and WSF respectively. Wegwu and Omeodu [29] in their study also reported the presence of PAH in the aqueous extract (AE) of Nigerian crude oil (Bonny light) to be 16.9 mg/l. These values are higher than the acceptable limits reported by EPA (0.0002 mg/l).

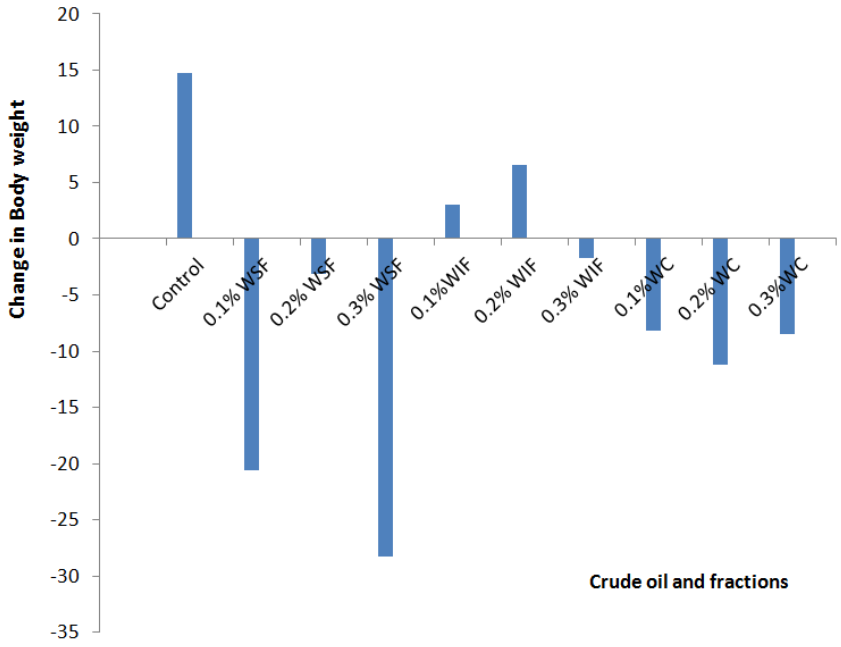

Fig. 3. % mean changes in body weight exposed to crude oil and its fraction

Table 4. Total lipid content of sections of earthworms treated with varying concentrations of crude oil and its fractions

Parameter	Control	Whole crude	Water insoluble fraction	Water soluble fraction
0.1% Treatment				
Clitellum	53.6 ± 4.6a	53.4 ± 1.6a	106.7 ± 10.4b	163.4 ± 15.2c
Post Clitellum	71.2 ± 8.3a	62.5 ± 3.6a	70.4 ± 2.4a	91.6 ± 10.0c
0.2% Treatment				
Clitellum	53.6 ± 4.6a	64.6 ± 4.7b	44.4 ± 2.4c	40.1 ± 2.9c
Post Clitellum	71.2 ± 8.3a	47.2 ± 6.9 b	31.0 ± 2.1c	32.4 ± 1.2c
0.3% Treatment				
Clitellum	53.6 ± 4.6a	35.5 ± 4.9b	20.9 ± 3.0c	22.9 ± 3.0c
Post Clitellum	71.2 ± 8.3a	44.3 ± 9.1b	22.9 ± 3.8c	24.9 ± 2.8c

Values are represented as Means ± S.E.M, n=20. Values are multiplied by 10^2. Means of the same row followed by different letters differ significantly (P<0.05)

Table 5. Total phospholipid content of sections of earthworms treated with varying concentrations of crude oil and its fractions

Parameter	Control	Whole crude	Water insoluble fraction	Water soluble fraction
0.1% Treatment				
Clitellum	32.0 ± 3.2^a	18.0 ± 2.5^b	22.0 ± 2.0^b	19.0 ± 2.3^b
Post Clitellum	33.0 ± 3.4^a	22.1 ± 2.8^b	24.0 ± 2.7^b	21.0 ± 3.5^b
0.2% Treatment				
Clitellum	32.0 ± 3.2^a	25.0 ± 2.4^b	39.0 ± 3.8^c	41.0 ± 4.0^c
Post Clitellum	33.0 ± 3.4^a	26.0 ± 3.0^b	42.0 ± 4.1^c	42.0 ± 4.6^c
0.3% Treatment				
Clitellum	32.0 ± 3.2^a	16.0 ± 2.4^b	49.0 ± 4.1^c	12.9 ± 3.0^b
Post Clitellum	33.0 ± 3.4^a	20.0 ± 2.4^b	59.0 ± 2.2^c	14.9 ± 4.8^b

Values are Means ± S.E.M, n=20. Values are multiplied by 10^2. Means of the same row followed by different letters differ significantly (P<0.05)

Table 6. The phospholipid profile of sections of earthworms treated with 0.1% of crude oil and its fractions

Parameter	Control	Whole crude	Water insoluble fraction	Water soluble fraction
Clitellum				
PE	36.5 ± 4.5^a	25.1 ± 1.9^b	28.0 ± 1.8^b	25.3 ± 1.1^b
PC	52.2 ± 5.8^a	39.9 ± 4.6^b	39.5 ± 3.5^b	38.3 ± 2.1^b
SGM	1.9 ± 0.3^a	3.3 ± 0.5^b	3.5 ± 0.4^b	3.4 ± 0.4^b
PI+PS	5.2 ± 0.5^a	3.6 ± 0.4^b	3.7 ± 0.4^b	3.1 ± 0.7^b
PE/PC	0.70	0.63	0.71	0.66
SGM/PC	0.04	0.08	0.09	0.09
Post Clitellum				
PE	35.2 ± 5.5^a	24.8 ± 3.5^b	23.6 ± 4.2^b	23.8 ± 4.9^b
PC	47.6 ± 6.3^a	36.5 ± 3.0^b	33.4 ± 4.5^b	35.5 ± 3.1^b
SGM	2.4 ± 0.3^a	3.9 ± 0.4^b	3.1 ± 0.2^b	2.4 ± 0.6^b
PI+PS	5.9 ± 0.4^a	4.1 ± 0.7^b	4.0 ± 0.7^b	4.5 ± 0.6^b
PE/PC	0.74	0.68	0.71	0.67
SGM/PC	0.05	0.11	0.09	0.07

Values are Means ± S.E.M, n=20. Values are multiplied by 10^2. Means of the same row followed by different letters differ significantly (P<0.05). PE = phosphatidylethanolamine, PC = phosphatidylcholine, SGM = Sphingomyelin, PI = phosphatidylinositol, PS = phosphatidylserine

Table 7. The phospholipids profile of sections of earthworms treated with 0.2% of crude oil and its fractions

Parameter	Control	Whole crude	Water insoluble fraction	Water soluble fraction
Clitellum				
PE	36.5 ± 4.5^{a}	15.1 ± 1.9^{b}	14.8 ± 2.8^{b}	15.3 ± 1.1^{b}
PC	52.2 ± 5.8^{a}	35.9 ± 2.6^{b}	31.5 ± 2.5^{b}	31.3 ± 2.1^{b}
SGM	1.9 ± 0.3^{a}	4.3 ± 0.6^{b}	4.4 ± 0.4^{b}	4.4 ± 0.6^{b}
PI+PS	5.2 ± 0.5^{a}	3.3 ± 0.5^{b}	3.3 ± 0.4^{b}	3.2 ± 0.7^{b}
PE/PC	0.70	0.42	0.47	0.49
SGM/PC	0.04	0.12	0.14	0.14
Post Clitellum				
PE	35.2 ± 5.5^{a}	19.8 ± 1.5^{b}	13.6 ± 1.2^{b}	13.8 ± 0.9^{b}
PC	47.6 ± 6.3^{a}	36.5 ± 4.0^{b}	31.4 ± 4.5^{b}	34.5 ± 5.1^{b}
SGM	2.4 ± 0.3^{a}	4.9 ± 0.5^{b}	4.1 ± 0.7^{b}	4.4 ± 0.5^{b}
PI+PS	5.9 ± 0.4^{a}	4.1 ± 1.0^{b}	4.0 ± 0.7^{b}	4.5 ± 0.7^{b}
PE/PC	0.74	0.54	0.43	0.40
SGM/PC	0.05	0.13	0.13	0.13

Values are Means ± S.E.M, n=20. Values are multiplied by 10^{2}. Means of the same row followed by different letters differ significantly (P<0.05). PE = phosphatidylethanolamine, PC = phosphatidylcholine, SGM = Sphingomyelin, PI = phosphatidylinositol, PS = phosphatidylserine

Table 8. The phospholipids profile of sections of earthworms treated with 0.3% of crude oil and its fractions

Parameter	Control	Whole crude	Water insoluble fraction	Water soluble fraction
Clitellum				
PE	36.5 ± 4.5^{a}	15.1 ± 1.5^{b}	11.8 ± 1.8^{c}	15.3 ± 1.1^{b}
PC	52.2 ± 5.8^{a}	25.2 ± 6.6^{b}	21.5 ± 6.5^{b}	21.3 ± 6.1^{b}
SGM	1.9 ± 0.3^{a}	4.2 ± 0.8^{b}	4.4 ± 0.7^{b}	4.4 ± 1.1^{b}
PI+PS	5.2 ± 0.5^{a}	3.0 ± 1.0^{b}	3.6 ± 0.8^{b}	3.1 ± 0.7^{b}
PE/PC	0.70	0.60	0.55	0.72
SGM/PC	0.04	0.17	0.21	0.21
Post Clitellum				
PE	35.2 ± 5.5^{a}	19.8 ± 3.5^{b}	13.6 ± 1.2^{c}	13.8 ± 1.9^{c}
PC	47.6 ± 6.3^{a}	26.5 ± 5.0^{b}	21.4 ± 5.5^{b}	25.5 ± 5.1^{b}
SGM	2.4 ± 0.3^{a}	5.9 ± 1.1^{b}	5.1 ± 1.2^{b}	5.4 ± 0.9^{b}
PI+PS	5.9 ± 0.4^{a}	3.1 ± 1.0^{b}	3.2 ± 0.7^{b}	3.5 ± 0.8^{b}
PE/PC	0.74	0.75	0.63	0.54
SGM/PC	0.05	0.22	0.24	0.21

Values are Means ± S.E.M, n=20. Values are multiplied by 10^{2}. Means of the same row followed by different letters differ significantly (P<0.05). PE = phosphatidylethanolamine, PC = phosphatidylcholine, SGM = Sphingomyelin, PI = phosphatidylinositol, PS = phosphatidylserine

Studies have shown that the integrity of the cellular membranes of soil-dwelling organisms are compromised when they are exposed to a high content of materials found in crude oil such as hydrocarbons and non-essential trace elements like cadmium [30,31]. These elements alter the content and structural integrity of the membrane lipids by causing lipid peroxidation and alter the fluidity of the membranes [32]. Biological membranes function in a lot of biochemical processes and it is critically dependent on the fluidity of the membrane. As phospholipid composition plays a major role in the determination of membrane fluidity, they therefore affect many biochemical processes that affect the survival of the organism. Earthworms (Libyodrilus violaceus) dramatically alter soil structure, water movement, nutrient dynamics, and plant growth. They are not essential to all healthy soil systems, but their presence is usually an indication of a healthy system. Oil pollution has impacted negatively on the environment in the area of biodiversity loss, socio-economic impact and health impact. The

profound and adverse impact of crude oil pollution as observed by alterations in the boimembrane of Libyodrilus violaceus is a further indication of the deleterious implications of crude oil pollution on soil degradation and the overall environment.

There is little or no information in the literature on how the lipid profile of earthworm is affected by exposure to crude oil or its fractions. This study provides data on the probable perturbation of the lipid profile of earthworms following exposure to different concentrations of crude oil and its fractions.

We observed a dose dependent reduction in body weight of earthworms exposed to the crude oil or its fractions (Table 3). A similar observation has earlier been reported [33,19].

The significant reduction in the total lipid as well as the total phospholipid contents of both the clitellum and the post clitellum of the earthworms exposed to crude oil or its fractions observed in this study (Tables 4 and 5) is in agreement with an earlier finding. Bindesbol and his colleagues [30] reported a significant decrease in the phospholipid content of *Dendrobaena octaedra* following exposure to copper. The WSF of crude oil had earlier been shown to contain the highest concentration of the heavy metals and other non-hydrocarbon constituent [13]. So the observed effect of the WSF on the total lipids of the clitellum may probably be due to the possible acute toxicity that can result from treatment with heavy metals.

Studies have shown that earthworms influence the degradation of total hydrocarbon content in the polluted soil positively [34,16]. The water insoluble fraction (WIF) of the crude oil would contain more hydrocarbons. It is therefore not surprising that we did not observe any significant change in the total lipid levels in the clitellum of earthworms treated with 0.1% and 0.2% concentrations of WIF fraction. Changes in individual phospholipids can affect membrane fluidity and transmembrane transport [35,36]. The real time analysis of individual phospholipids is important in determining the role of the phospholipids on this important membrane character and its contribution to metabolic processes. The lack of appropriate techniques for real time analysis makes the results obtained eventually subjective. However within the confine of this drawback, we observed changes in the individual phospholipids following crude oil contamination (Tables 6, 7, and 8). It can therefore be speculated that increase in phosphatidylcholine (PC), phosphatidy-lethanolamine (PE) and sphingomyelin (SGM), observed in earthworms treated with crude oil or its fractions may affect the animal's membrane character and eventually their metabolic processes. A change in the fluidity of the membrane has been associated with aging and other pathological conditions 35, 36]. Earthworms produce a coleomic fluid which has been reported to have lytic activities [37]. The lytic activity is strongly dependent on the presence of SGM in the receptors of the target membranes [37]. The earthworm is able to suppress the production of SGM as an innate defence strategy to prevent self-killing. Our observation of an increase in the content of sphingomyelin, as shown in Tables 5, 6 and 7, may be one of the probable mechanisms of toxicity of the crude oil and its fractions. This research data is suggestive that these toxicants are able to compromise the earthworm's ability to suppress SGM synthesis. If that is the probable effect, then the elevated ratio of SGM to PC could be due to an increase in the synthesis of SGM from PC.

The PC/PE ratios also increased with increase in concentrations from 0.1% to 0.2% of the WC and the WIF in the clitellum and after the clitellum assay. The increase in this ratio may indicate the onset of a disease due to reduced membrane fluidity. The use of this ratio to establish membrane fluidity is not new. Hirata and Axelrod [38] used PC/PE ratio to access membrane fluidity and transmembrane communication. The possible effect of crude oil and its fractions on real time levels of SGM, PC and PE is worthy of further study to elucidate/ substantiate the effect of crude oil and its fractions on these membrane phospholipids.

5. CONCLUSION

In conclusion, this study suggests that in crude oil pollution, there is an increase in certain phospholipid which affects the membrane fluidity and possibly increased the propensity to self-destruct in earthworm.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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