Physicochemical Characterisation of Crude Oil and Its Correlation with bioaccumulation of Heavy Metals in Earthworm (*Libyodrilusviolaceus)*

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***Abstract*:-The country's total dependence on the proceeds from crude oil to drive its economy since the Commercial exploration of crude oil started in Nigeria in 1958 has led to serious diverse environmental concerns. The environment has grossly suffered from the adverse overload meted upon it by pollution through continuous oil spillages. Earthworms are worthy sentinel for ecotoxicological studies and their toxicity studies have been proven to be scientifically significant. Two hundred and forty earthworms were assigned to four major groups. The first group served as the control group, the other test groups were either exposed to water soluble fraction (WSF) or water insoluble fraction (WIF) or the whole crude (WC). The alimentary canal regions of the earthworms were excised and collected for physicochemical analysis using Atomic Absorption Spectrophotometer. Total dissolved solid (TDS), Total suspended solid (TSS) and Chemical Oxygen demand (COD), Electric conductivity (EC), Trace elements (TC),Anion content, pH, Degree of Salinity, Total Hydrocarbon content (THC) were all assessed using standard methods. Fe, Mn, Cr, Zn, Cu, Cd, Pb Ni and V were determined in the alimentary canal of earthworms exposed to sublethal concentrations of the toxicant. The bioaccumulation of heavy metals presented a dose dependent increase in all the test groups. This short-term study established that bioaccumulation of heavy metals in earthworm is related to the degree of pollution of the soil and can possibly lead to soil decontamination. Results from the bioaccumulation study gives credence to earlier findings that earthworms are good biomarkers for ecotoxicological studies and possess the ability to function as good soil bioremediators.**

***Keywords: Libyodrilusviolaceus, crude oil, spillages,* heavy metals, bioaccumulation, bioremediation.**

I. INTRODUCTION

Nigeria is still considered a mono-Economy because over 90% of her earnings are from the export of crude oil and gas [1,2 and 3]. Most of the crude oil is from the Niger Delta area of Nigeria [4] because it is a mature oil basin and it is considered to be one of the most oil bearing sedimentary basins in the world [5 and 6].

Crude oil is said to be one of the most complicated natural mixtures on earth containing compounds which differ in composition and physical properties and are toxic to biological subjects [7 and 8].

The crude oil is almost of no value until it is refined for its numerous useful products and as it is being extracted, refined and distributed for more useful purposes, it inevitably contributes to pollution via spillages during usual routine operations, various acute accidents during transportation and Sabotage on oil installations by miscreants[9].

Crude oil spillages have led to the pollution of terrestrial environment in Nigeria and the world at large. It has definitely led to the release of large amounts of crude oil into the environment leaving both flora and fauna unfit in the ecosystem, with agricultural lands becoming less productive as well as the creeks and fishing water becoming more toxic for aquatic animals especially in the Niger Delta regions (9 and 10).

Over the years, several environmental impact assessments (EIA) have been carried out with the view of assessing the actual impact of the oil explorations in Nigeria. The traditional approach to soil pollution impact assessmentis based on the analysis of the concentrations of pollutants in the soil, as well as chemical by chemical comparisons with specific threshold values. Theseare only a partial evaluation of risk and do not provide any information on the deleterious effect of these contaminants in the biota (11). It sure neglects several essential aspect of toxicity of chemicals not included in the selection of contaminants to be analyzed, interactive effects (synergism and antagonisms) of pollutants on biota and bioavailability, (12). The best integrators of the complex effect of any pollutant are the exposed organisms themselves [13].

The use of earthworms in monitoring the toxic effects of chemicals (14) and its possible use in the bioremediation of polluted soil (15; 16) is not a new one because of their high biomass and sensitivity (17; 18). Earthworm is therefore a worthy sentinel for ecotoxicological studies and different species have been used in many studies (19,20 and21). Earthworm toxicity studies are also significant for understanding metal bioavailability of heavy metals (12).

Most of the Environmental impact assessments (EIA) procedures carried out using earthworms were only directed towards checking the level of impact using mortality rate and some stress indicators such as weight loss, coiling responses and swollen clitellum as an endpoint marker (13,22 and 23,). For reliable and proper assessment of environmental pollution/contamination, knowledge of possible bioaccumulation of contaminants on the exposed organisms is necessary.

The aim of this study was to assess some physicochemical parameters in the crude oil and its fractions and relate this with the heavy metal bioaccumulation capacity and / or ability of the earthwormsexposed to these same crude oil and its fractions.

II. EXPERIMENTAL DESIGN

2.1 Fractionation protocols:

The Bonny light crude oil sample used was obtained from Warri Refining and Petrochemical Company Ltd., a subsidiary of Nigerian National Petroleum Corporation in Delta State, Nigeria. This is a representative of the crude oil generally produced and transported across Nigeria. It was fractionated using the modified method of Anderson etal. (24).A 1:2 dilution of 200ml of the crude oil with distilled water 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 covered with aluminium foil then kept frozen until required.

2.2 Physicochemical analysis of crude oil and its fractions.

Crude oil and its fractions were analyzed for some physicochemical Total dissolved solid (TDS), Total suspended solid (TSS). Chemical Oxygen demand (COD), Electric conductivity (EC), Trace elements (TC),Anion content, pH, Degree of Salinity and Total Hydrocarbon content (THC))using standard methods.

2.2.0 Determination of Electric conductivity (EC) /Total dissolved solids (TDS) in crude oil and its fractions.

An Automated Dual meter was used for the determination of EC and TDS.The meter was allowed to stabilise for 10 minutes, the instrument calibrated by immersing the probe in KCL solution, the probe was rinsed and immersed in the sample solution before each readings.

2.2.1 Determination of total suspended solids (TSS) in crude oil and its fractions by gravimetry.

A 15cm Whatman Filter Paper No. 1 was dried at 50^oC to constant weight (X1g), a100ml of sample (crude oil and its fraction) was filtered through the weighed paper and dried in the same oven to constant weight at same temperature. The filter paper is weighed the second time with its content (X2g).

The Weight of the total Suspended solids = (X2 –X1) g.

Suspended solids (mg/l) = (X2 – X1) x1000x100.

2.2.2 Determination of chemical oxygen demand (COD) in crude oil and its fractions.

Preparation of reagents.

Titration method based on the formation of a coloured complex in the presence of the Ferroin indicator was used for this assay. 50ml of crude oil or its fractionswas pipetted into a conical flask, added 10ml of the 0.00833 K_2CrO_7solution, and then added 1g of $HgSO_4$ and 80ml of Ag_2SO_4 – H_2SO_4 solution and a few beads. Fitted a reflux greaseless condenser and heatedgently to boiling for exactly 10 minutes and left to cool. Rinsed the condenser with 50ml of water and cooled the flask under a running tap. Two drops of Ferroin indicator was added and titrated with 0.025M $Fe(NH_2)_2\ (SO_4)_2 6H_2O$ until the colour changed.

2.2.3 Determination of total hydrocarbon content in crude oil and fractions

50ml of the sample was put into a 150ml separating funnel and a 10ml of Hexane was added and shook for 2 minutes manually, the stopper was removed and allowed to settle for 20minutes. The water layer was drained off while the Hexane layer was read at 460nm. Hexane was used as the Blank.

2.3 Chemical analysis of the Heavy metal contents in earthworm tissue.

The Perkin-Elmer (PE) atomic absorption Spectrophotometer was set up for each mineral element determined as previously described (Perkin-Elmer, 1997). Elemental analysis of Fe, Mn, Cr, Zn, Cu, Cd, Pb Ni and V were determined in both the crude oil and its fractions and in the alimentary canal of earthworms exposed to sub-lethal concentrations of the toxicant. 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 (Perkin-Elmer, 1997).

2.4 Test Animals

The earthworms were housed individually in outdoor macrocosms (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, Eudriluseugeniae (23). 0.1% concentration of the WSF, WIF and WC oil fraction were prepared by adding 1ml of the crude oil or its fractions to 999ml of distilled water mixed with 1000g of the habitat (control) soil.0.2% concentration of

WSF, WIF and WC oil fraction was prepared by adding 2ml of the crude oil or its fractions to 998ml of distilled water mixed with 1000g of the habitat (control) soil.0.3% concentration of crude oil fraction was prepared by adding 3ml of the crude oil or its fractions to 997ml of distilled water and mixed with 1000g of the habitat (control) soil. The test specimen (*Libyodrilusviolaceus*) were collected by handpicking from a moist subsurface soil in Okada town, Benin city, Edo state Nigeria, and kept 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 and forty (240) 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.

2.5 Estimation of bioaccumulation of heavy metals in the alimentary canal of earthworms

A steady weight of 0.2g of the earthworm alimentary canal section was used for heavy metal bioaccumulation assay. 5ml of the Nitric-perchloric acid mixture was added to the already made paste from the sized alimentary canal sections and allowed to soak overnight. A small glass funnel was inserted to act as a reflux condenser and heated for 1hour at 150^{o}C and gradually raised temperature to 235^{o}C. The heating was continued for another 2hours until a dense white fume was observed.The heating block was removed and cooled to about 100^{o}C.1ml of 1:1HCl was added and heated until white fumes were visible and a colorless solution obtained. The solution was then poured into 100ml flask and washed 5 times with water into the flask. The heavy metals in the extract was then determined using the Atomic Absorption.

2.6 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.

III. RESULTS

3.1 Physicochemical examination of crude oil sample and its fractions.

3.1.0 The Total dissolved solid (TDS), Total suspended solid (TSS) and Chemical Oxygen demand (COD).

The Total dissolved solid (TDS), Total suspended solid (TSS) and Chemical Oxygen demand (COD) are aspresented in table 3.1, WChad the highest TDS value while the WIF had the lowest. The WSF presented the highest value for TSS as was expected, followed by the WIF while the WC had the lowest value for TSS. WSF presented the highest value for COD, followed by the WC and then the WSF.

Table 3.1 shows the Total dissolved solid (TDS), Total suspended solid (TSS) and Chemical Oxygen demand (COD).

Crude oil/fractions	TDS (mg/l)	TSS (mg/l)	COD (mg/l)
WSF	**15.1**	**142**	**295**
WIF	**11.3**	**39**	**160**
WC	**27.5**	**21.3**	**174.4**

3.2 Electric conductivity in crude oil and its fractions.

Electric conductivity in the WC, WSF and WIF is as represented in the in table 3.2.below. Electrical conductivity for WSF, WIF and WC were 31.5, 20.7, 20.7 and 45.2μS/cm respectively.WC was expected to have the highest EC and it had the highest value for EC followed by WSF and the WIF had the lowest value for this parameter.

Table 3.2: Electric conductivity (EC) in crude oil and its fractions.

Crude oil/fractions	EC (μS/cm)
WSF	31.5
WIF	20.7
WC	45.2

Values are represented in μS/cm

3.3 Trace elements (TC) in crude oil and its fractions.

Elemental analysis of some trace elements is as summarised in table 3.3. Elemental analysis shows that WSF, WIF and WC contained Sodium (Na) =0.41mg/l, 0.25mg/l, and 1.32mg/l ; Potassium (K) = 0.04mg/l, 0.04mg/l and 0.08mg/l.; Calcuim (Ca) =0.74mg/l, 0.65mg/l and 9mg/l; Magnesium = 0.16mg/l,0.1mg/l and 6.3mg/l respectively. Trace elements were all elevated in the WC followed by the WSF. The WIF had the lowest for all elemental analysis.

Table 3.3: Trace elements (TC) in crude oil and its fractions.

Crude oil/fractions	Na	K	Ca	Mg
WSF	0.41	0.04	0.74	0.16
WIF	0.25	0.04	0.65	0.1
WC	1.32	0.08	9	6.3

Values are represented in mg/l concentrations

3.4: Anion content of crude oil sample and fractions.

Anion content for WSF, WIF and WC are as shown in table 3.4 below. Anion content for WSF, WIF and WC contains HCO_3 =18.8mg/l, 12.4mg/l and 61mg/l; CL = 17.7mg/l,17.2mg/l and 14.3mg/l; NH_4 = 0.49mg/l, 0.36mg/l, 38.1mg/l; NO_2 = 4.69mg/l, 1.29mg/l and 22.7mg/l; NO_3 = 9.5mg/l, 4.55mg/l, 29.3mg/l; SO_4 = 0.84mg/l, 0.77mg/l and 24.3mg/l.WC had the highest levels for all anions, followed by the WSF and then the WIF.

Table 3.4: Anion content of crude oil sample and its fractions

Crude oil/fractions	HCO_3	Cl	NH_4	NO_2	NO_3	SO_4
		mg/l				
WSF	18.8	17.7	0.49	4.69	9.5	0.84
WIF	12.4	17.2	0.36	1.29	4.55	0.77
WC	61	14.3	38.1	22.7	29.3	24.3

Values are represented in mg/l concentrations

3.5 pH values in sample crude oil and its fractions

pH values for sample crude oil and its fractions is as represented in table 3.5. The WSF had the lowest pH of 4.4; WIF was 5.3 while WC was 6.8. The WSF had the lowest pH and so was the most acidic followed by the WIF and then the WC.

Table 3.5: pH values in sample crude oil and its fractions

Crude oil/fractions	pH
WSF	4.4
WIF	5.3
WC	6.8

3.6 Degree of Salinity of crude oil sample and its fractions.

The degree of salinity is as summarised in table 3.6 below. Salinity of crude oil in WSF, WIF and WC were 0.009g/l, 0.014 and 0.02 respectively. WC had the highest value for salinity, followed by the WIF. The WSF had the lowest value salinity.

Table 3.6: Degree of Salinity of crude oil sample and its fractions.

Crude oil/fractions	Sal. (g/l)
WSF	0.009
WIF	0.014
WC	0.02

Values are represented in g/l concentrations

3.7 Total Hydrocarbon content (THC) in crude oil and its fractions

Total hydrocarbon content is as represented in table 3.7 below. Results from the analysis shows that WC presented with the highest THC (250.7) followed by the WIF (195.2) and then the WSF (21.74). The general insolubility of hydrocarbons in aqueous medium must have affected the various yields for THC.

Table 3.7: Total Hydrocarbon content (THC) in crude oil and its fractions.

Crude oil/fractions	THC (mg/l)
WSF	21.74
WIF	195.2
WC	250.7

Values are represented in mg/l concentrations

3.8 Bioaccumulation of heavy metals in the alimentary canal of earthworms exposed to crude oil and its fractions.

Results showed that the Cr, Cu, Pb, Cd contents in test groups were higher and elevated than those of the control subjects. Heavy metals bioaccumulation assay presented a dose dependent increase in all the test groups. This study also revealed that heavy metal accumulation in all test groups were strangely higher than heavy metal concentrations in the crude oil and its fractions.

Table 3.8 showing Bioaccumulation of heavy metals in the alimentary canal of earthworms exposed to crude oil and its fractions.

Groups/weights	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
				Mg/kg					
Control (1.3g)	184.1	11.36	16.93	2.89	1.03	0.79	0.42	1.11	0.93
0.1% WSF (1.3g)	324.3	30.21	94.22	18.6	12.82	8.57	8,09	8.13	6.7
0.2% WSF (1.7g)	332.5	44.35	121.6	21.86	12.9	8.15	9.53	8.95	7.24
0.3% WSF (3.3g)	383.2	53.33	118.74	24.95	13.92	11.74	12.21	13.09	11.37
0.1% WIF (2.6g)	251.8	18.83	37.12	14,24	7.52	5.88	2.3	3.24	2.72
0.2% WIF (2.3g)	262.7	29.21	42.94	14.33	8.92	6.48	2.49	3.78	2.35
0.3% WIF (0.9g)	325.1	29.8	48.28	17.52	10.85	8.8	6.85	5.26	4.51
0.1% WC (2.2g)	417.1	62.16	132.78	33.56	13.92	21.6	24.13	12.18	11.98
0.2% WC (1.2g)	486.4	88.13	144.56	45.7	14.82	21.97	23.53	15.43	14.18
0.3%WC (1.1g)	656.3	96.52	180.94	47.14	19.11	27.52	25.9	16.52	15.02

IV. DISCUSSION

The build-up of heavy metals and other toxic materials in agricultural soils and other water bodies is of increasing concern due to food safety problems and potential health risk as well its detrimental effect on soil ecosystem (13; 26). Studies have shown that the integrity of the cellular membranes of soil dwelling organisms are compromised when they are exposed to the high content of toxicants (PAHSand nonessential trace elements like cadmiume.t.c.) present in crude oil (27; 21; 28;13). The concentration of heavy metals in crude oil and its fractions in our previousstudy varied in their different levels and were all above the Desirable Contaminant Concentrations DCC and Acceptable Contaminant Concentration (ACC) thresholds as published by the National Water Quality Management Strategy (13; 29). Various agencies including World health organisation (30), 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 (29; 30; 31).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 (31), cadmium in sample crude oil (WC) and WSF used for this research were 11.54mg/l and 7.5mg/l respectively as previously published (13) and remain 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 (32; 33). Of all toxic heavy metals, cadmium ranks the highest in terms of damage to plants and human health and its accumulation in plants poses a serious health threat to humans via food intake (34).

Several studies show that earthworms can bio-accumulate or bio-transform many toxic chemicals including heavy metals found in crude oil (19;20;21; 33 and36). It follows that earthworms may eventually enter the food chain and result in secondary contamination to humans since it is widely used as baits in fishing. The Water soluble fraction (WSF) constituents are dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions and heavy metals (37;38). In our previousstudy, WSF was confirmed to contain a very high percentage of the heavy metals (Cd), (7.5mg/l, (13). It also presented as the most acidic fraction (pH = 4.4) (13).

Earthworms are exposed by dermal contact with heavy metals in the soil or by ingestion of pore water, polluted food and/or soil particles (40). Saxe *et al.*, (41) estimated that the dermal exposure route accounted for more than 96% of the total uptake of Cd and Cu in the earthworms Eiseniaandrei. The Fe, Mn, Cr, Zn, Cu, Cd, Pb Ni and V contents were determined in the alimentary canal of earthworms exposed to the crude oil sample and its fractions Table3.9). The results showed that the Cr, Cu, Pb, Cd contents in test *Libyodrilusviolaceus* were higher than those of the control earthworms. Total metal accumulation was highest in the earthworms exposed to the WC and lowest in the subjects exposed to the WIF. These accumulated concentrations of heavy metals in the alimentary canal of exposed earthworms correlates positively with the degree of heavy metal concentrations in the in the crude oil sample used for this study (WC, WSF AND WIF; Table 3.8). It can therefore be speculated that the bioaccumulation capacity of *Lybiodrilusviolaceus* is directly proportional to the degree of pollution in its immediate environment. In this study, the bioaccumulation of heavy metals presented a dose dependent increase in all the test groups. This study also revealed that heavy metal accumulation in all test groups were strangely higher than heavy metal concentrations in the crude oil and its fractions. Hobbelen*et al*., (2006) also reported in their study that despite low availability in soil, earthworms in flood plain soils contain elevated concentrations of Cu and Cd. Further investigation into the possible mechanisms involved in heavy metal bioaccumulation is needful for clarity on how these animals are able to act as heavy metal sinks and agents of bioremediation for heavy metal pollution.

V. CONCLUSION

The overall result of this study provides scientific data and justification that further authenticates earthworm(*Libyodrilusviolaceus)*as a useful stress bio-indicator to identify and assess recovery of crude oil contaminated soils.

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