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Bioassay using the water soluble fraction of a Nigerian Light Crude oil on *Clarias gariepinus* fingerlings

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Summary: A 96-hour bioassay was conducted using the water soluble fraction of a Nigerian light crude oil sample on *Clarias gariepinus* fingerlings. 0, 2.5, 5.0, 7.5 and 10 mls of water soluble fractions (WSF) of the oil were added to 1000 litres of de-chlorinated tap water to form 0, 25, 50 , 75 and 100 parts per million representing treatments 1 to 5 respectively. Each treatment had two replicates with fifteen fish per replicate. At the end of the 96-hour period of exposure, the fish were transferred into separate bowls containing fresh water without oil for recovery for ten more days. Heavy metal and total hydrocarbon contents of the water and fish were analyzed at 96 hour and 14 days which marked the end of the recovery period. No mortalities were recorded on all treatments during the 96-hour period. Mortalities were observed between 120 and 144 hours after the onset of the experiment with the maximum number of dead fish ($p<0.05$) from treatment 5(100 ppm WSF) during the recovery period indicating a delayed response to the WSF by the fish. No mortalities were recorded after 144 hours till the termination of the experiment at 14 days . The 96-hour LC_{50} could not be calculated since no deaths occured during the period. The Total hydrocarbon contents of the water were 0, 0.026 ,0.316 ,0.297, 0.253 mg/l for treatments 1(0 ppmWSF)) to 5(100ppmWSF) respectively. Lead , iron and cadmium were not detected in water during the study, lead was also not detected in fish muscles from all treatments. The iron contents of all the treatments were lower than the control except for treatment 3(50 ppm WSF). THC concentrations in fish were higher at 96 hours and 14days than in the water indicating bioconcentration in fish and a retention in the fish long after exposure.

Keywords*:* WSF, *C.gariepinus* , Toxicity , Heavy metals , THC,Bioconcentration

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INTRODUCTION

Light crude oil is a naturally occurring liquid consisting mainly of hydrogen and carbon. It is found underground but sometimes occurs above ground as oil seep or tar pits (ATSDR, 1999). Crude oil is also called liquid petroleum as it comes out of the ground. It is relatively fluid (low viscosity) and floats on water (low specific gravity).When a light crude oil spills, 10 to 15% of its volume is lost immediately due to the evaporation of volatile organic compounds and up to 25% within 24 hours. Much of the loss depends on the surface to volume ratio of the bulk oil (Macy *et al*, 1992; Centers For Disease Control and Prevention, 2010). Oil spilled into oceans initially spread on the surface depending on its relative density and composition. While the slick may remain, wave action can cause it to drift over large areas covering both open seas and terrestrial habitats (Centers for Disease Control and Prevention, 2010). The physical reduction through evaporation, photo oxidation and biodegradation of light crude oils reduce the proportion of the lighter fractions and increase the heavier fractions (Humphrey, 2010; Jensen and Carroll, 2010)

Crude oil is an extremely complex mixture of different compounds which range from very simple molecular weight compounds to polynuclear aromatic compounds with several isomers (Kiceniuk and Khan, 1983). The concentrations of hydrocarbon in different WSF of crude oils are very variable and Neff *et al.* (2000) showed varying concentrations in different water soluble fractions obtained from different Australian crude oils as varying from 0.008mg/l to 38.3 mg/l. Rodrigues *et al*. (2010) also observed low BTEX and high PAH concentrations with Brazilian crude oils. Exposure of fish to crude oils induce ethoxyresorufin-O-deethylase (EROD) activities in fish (Lee *et al,* 2011), alter the embryonic development of fish and the production of transformation enzymes induced at the highest

concentrations are believed to play a role in its elimination (Pauka *et al*,2011).

The water soluble fraction of crude oil has been described as the small dissolved fraction which is available and may be toxic to the living organisms in the aquatic environment. This is so because this fraction comprises of toxic components such as the polycyclic aromatic hydrocarbons (PAHs), mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, xylene (BTEX); phenols, heterocyclic compounds and heavy metals (Saeed and Al-Mutairi, 1999, Rodrigues et *al*, 2010). Hydrocarbons present in crude oil are toxic to many organisms especially those at early life stages (Lee *et al* 2011). The aliphatic hydrocarbons once regarded as non- toxic have now been recognized as significantly toxic. (Manahan, 1992).

Many organisms can accumulate hydrocarbons in their lipid compartments (Di Toro *et al*, 2001, Azad, 2005, Nwabueze and Agbogidi, 2010, Rodrigues *et al*, 2010). Polycyclic aromatic hydrocarbons for example naphthalenes are toxic, persistent and carcinogenic (Sharanagouda and Karegoudan, 2001; Bamforth and Singleton,2005; Feijoo-Siota *et al* , 2008).The water soluble (WSF) of crude oil also contains some cations (Na^+, Ca^{2+},Mg^{2+},Fe^{2+}, Fe^{3+} and K^+; anions (Cl-,SO_4^{2-},NO_3^- , PO_4^{2-} and HCO_3^-) and heavy metals such as lead, copper, zinc, cadmium, nickel, chromium and vanadium (Rana, 2005, Edema, 2006). **Bioconcentration** refers to the absorption or uptake of a chemical from the media to concentrations in the organism's tissues that are greater than in surrounding environment. The degree to which a contaminant will concentrate in an organism is expressed as the bioconcentration factor (BCF), which is defined as the concentration of a chemical in an organism's tissues divided by the exposure concentration (USEPA 2010 a, b) and usually calculated at the steady state.

Fish have been reported to absorb dissolved hydrocarbons very easily and readily (Sikkema *et al* 1994, Olaifa, 2005). WSF fouls water and living organisms in the water (NAP, 2003).The sudden inputs of large amounts of petroleum hydrocarbons associated with oil spillages stresses the environment in a way not imposed by naturally occurring hydrocarbons (Bartha and Atlas, 1977). Spilled oil produces deleterious effects on both the flora and fauna (Edema *et al* 2007, Nwabueze and Agbogidi, 2010, Rodriguez *et al*, 2010).

The fish *Clarias gariepinus* was chosen for this study because *Clariidae* are reputed to withstand adverse environmental conditions such as poor water quality and low dissolved oxygen. This study was undertaken to determine the effects of water soluble fraction of a light crude oil produced in Nigeria on *Clarias gariepunus* juveniles

MATERIALS AND METHODS

Clarias gariepinus juveniles (numbering 250 with mean weight 6.5g) were purchased from a fish farm in Ibadan and transported to the laboratory. They were acclimatized for two weeks during which they were fed a commercially available pelletized ration at three percent of their body weight twice daily. The tanks containing the fish were not aerated during both acclimatization and experimental periods. Feeding of fish was stopped 24-hours before the experiment.

The water-soluble faction (WSF) of the crude oil was prepared using the method described by Anderson *et al*. (1974). 500ml of crude oil was mixed with 500ml de-ionized water in a 2-litre conical flask, placed on a table top Gallenkamp magnetic stirrer and stirred for 20 hours. The mixture was left to stand in a separating funnel for 12 hours after which the lower phase was withdrawn and used as the water soluble fraction of the crude oil. The total hydrocarbon content of the WSF was determined using an UV spectrophotometer (Hach DREL 3000 at 450 nm). The total hydrocarbon content in the water- soluble fraction of the crude oil measured before the experiment was 0.224mg/ml.

The WSF was measured out as 0, 2.5, 5.0 7.5 and 10ml and each concentration made up to1000 litres by adding de-chlorinated tap water (tap water exposed to the air for more than 24 hours). These represented 0,25,50,75 and 100 parts per million respectively (Odiete ,1999, Reish and Oshida,1987). To produce enough volume in the aquaria to contain 15 fish per replicate, the quantities of both the water-soluble fractions of the crude oil and the water was increased thrice (table 1).

Chemical analyses of the water and fish in all treatments were carried out to determine their heavy metal and total hydrocarbon contents. Water and fish samples were analyzed for heavy metals using an atomic absorption spectrophotometer after digestion using the methods of FAO/SIDA, (1983) while their total hydrocarbon contents were analyzed as follows: 3g sample fillet muscle was digested with methanol: 20% brine solution in a soxhlet apparatus and UV spectrophotometer (Hach DREL 3000 at 450 nm) as described by Kotz *et al.* (1972; Asuquo and Udoh, 2002) after 96 hours and 14 days. The data obtained were subjected to analysis of variance (ANOVA) and Fisher's least significant difference (LSD) was used as a follow-up test. The bioconcentration factors for THC were determined as THC in fish (mg/kg) divided by THC in water (mg/L) at the end of the experiment and recovery period.

RESULTS

The results obtained during the experiment are pesented in tables 1 to 7 and figures 1 and 2. The mean temperature of the water measured during the experiment was 27^{o}C. While the temperature remained unchanged, the mean dissolved oxygen contents varied with treatment as follows: 8.75%; 8%; 6.9%; 5.7% and 3.95% for treatments 1(0ppmWSF) to 5(100ppm WSF) respectively. The least dissolved oxygen concentration was recorded with the 100ppmWSF treatment.

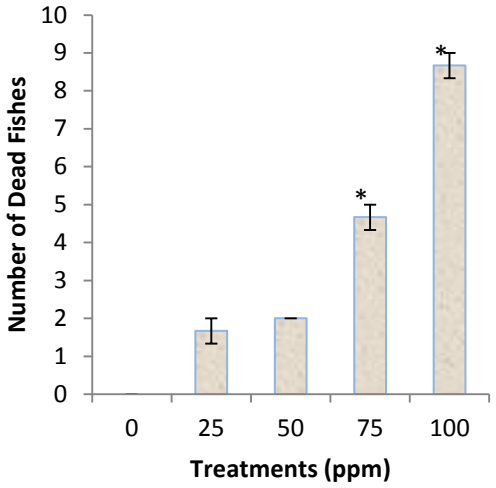

Figure 1: Percentage mortality of *C.gariepinus* exposed to different concentrations (0,25, 50, 75 and 100ppm) of WSF of a Nigerian light crude oil during a 96-hour bioassay. Significant differences ($p<0.05$) were found between 75ppm and the control; and between 100ppm and the control (denoted by the asterisks). 50ppm was not significantly different from the control.

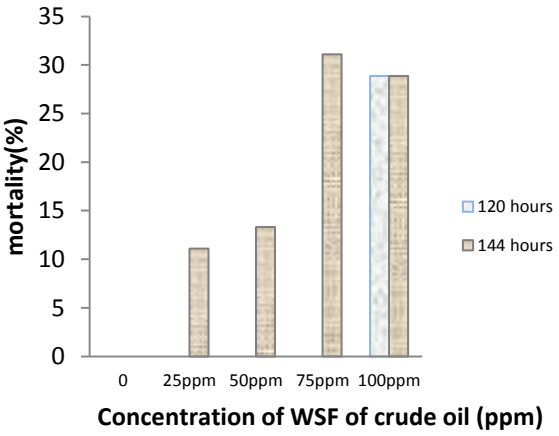

Figure 2: Percentage mortality of *C.gariepinus* exposed to 0, 25, 50, 75 and 100 ppm WSF of Nigerian light crude oil in 96 hours and ten day of recovery (fourteen days).No mortality occurred during the 96-hour exposure period.Only 100ppm WSF caused mortality on day 5(120 hours) while all treatments except the control recorded mortalities on day 6(144 hours) with the highest occurring in 75ppm WSF.

The introduction of the WSF caused no immediate noticeable change in fish behaviour. No mortality was recorded for all treatments throughout the 96-hour experimental period. Mortality occurred after the 96- hour period (table 2, figures 1and 2) starting from day 5 (120 hours). On day 5, 13 fish were lost from treatment 5 (100ppm WSF). Other treatments lost no fish. On day 6 (144 hours), losses were incurred from all treatments except the control. No more mortality was recorded after day 6 of the experiment until day 14 when the experiment was terminated. Fish were slow in movement during the experiment and at the initial stage of recovery and avoided feeding during the period of recovery.

Table 1: Quantities of Water Soluble Fraction of Crude Oil used for the Bioassay

Treatment.	Volume of WSF(ml)	Volume of dilution Water (ml)	Volume of WSF x 3(ml)	Vol. of dilution Water x 3(ml)
1	0	1000	0	3000
2	2.5	997.5	10	2992.5
3	5.0	995	15	2985
4	7.5	992.5	22.5	2977.5
5	10	990	30	2970

Table 2: The numbers and percentage mortalities of *Clarias gariepinus* juveniles Exposed to WSF of Nigerian light Crude oil in 96 hours and at 14 days

Treatments/ Concentrations (ppm)	Replicate 1	Replicate 2	Replicate 3	Total mortality out of 45	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)
1(0)	0	0	0	0	0	0	0
2(25)	2	2	1	5	13.33	13.33	6.67
3(50)	2	2	2	6	13.33	13.33	13.33
4(75)	5	5	4	14	33.33	33.33	27.67
5(100)	9	8	9	26	60	53.33	60

Note: No fish mortality was recorded during the 96-hour test but the mortality reported here occurred during the recovery period

Table 3: Heavy metal content of the water treated with water- soluble fraction of a Nigerian light crude oil (ppm) at the end of the 96- hour bioassay.

Concentration of WSF(ppm)	Pb	V	Fe	Mn	Cr	Cd	Zn	Cu
0	Nd	Nd	Nd	Nd	0.38	Nd	0.38	Nd
25	Nd	0.05	Nd	0.88	0.5	Nd	1.38	Nd
50	Nd	0.15	Nd	1.5	0.38	Nd	1.38	0.38
75	Nd	0.33	Nd	0.5	0.5	Nd	1.13	Nd
100	Nd	0.5	Nd	0.12	6.25	Nd	3.63	0.38
Tap water	Nd	Nd	Nd	0.25	0.25	Nd	0.25	Nd

Nd = not detected

Table 4: Heavy metal contents of the *C.gariepinus* muscle after 96 hours in water containing WSF of a Nigerian Light crude oil (ppm).

Concentration of WSF	Pb	Fe	Mn	Cr	Cd	Zn	Cu	V
1	Nd	24.38	9.25	1.13	0.25	10.88	1.13	Nd
2	Nd	11.5	9.63	1	0.25	9.75	1	0.38
3	Nd	26.13	11.38	0.75	0.25	14.63	1.38	0.63
4	Nd	13.5	7.63	0.88	0.25	9.75	1	0.88
5	Nd	15.5	8.75	0.88	0.25	10	0.75	0.88

Nd = not detected

Table 5: Heavy metal content of the *C.gariepinus* surviving after 14 days from start of 96-hour exposure to water containing WSF of Nigerian Light Crude oil (ppm).

Concentration of WSF	Pb	Fe	Mn	Cr	Cd	Zn	Cu	V
1	Nd	24.38	9.25	1.13	0.25	10.88	1.13	Nd
2	Nd	15.5	9.75	0.88	0.38	1.13	1.25	0.38
3	Nd	20.75	14.75	1	0.13	13.13	1	0.13
4	Nd	12.13	9	2.38	0.38	10.25	0	0.83
5	Nd	18.25	9	0.75	0.5	12.63	1.25	1

Nd = not detected

Table 6: Total hydrocarbon content of water, *C.gariepinus* juveniles and survivors of the water soluble fraction of crude oil bioassay.

Treatment	THC in water (mg/l)	THC in fish after 96 hr (mg/kg)	THC in surviving fish at 14 days (mg/kg)
1	0	0.16	-
2	0.03	19.92	30
3	0.32	31.76	26.32
4	0.30	30.8	27.33
5	0.25	26	25.52

All metals studied varied during the experiment. There were significant reductions ($p<0.05$) in the iron concentration of fish both at 96 hours and during the recovery period when compared to the control fish except treatment 3(50ppmWSF) which increased at 96 hours but reduced during the recovery period below the control.

The THCs measured in the water used for each treatment of the experiment and uptake by fish at the end of 96 hours and 14 days are presented in table 6. Significantly higher concentrations ($p<0.05$) of THC were recorded in fish both after 96 hours and 14 days than in water. Also the THCs differed significantly ($p<0.05$) among treatments both after 96 hours and during the recovery period.

DISCUSSION

A 96-hour bioassay was carried out using the water – soluble fraction of a Nigerian Light crude oil. The observations of the physicochemical characteristics of

the water were similar to earlier studies (Omoregie and Ufodike, 2000; Nwabueze and Agbogidi, 2010, Rodrigues *et al*, 2010) who reported a decreased dissolved oxygen content with increasing hydrocarbon content of water. All the recorded dissolved oxygen concentrations and temperature were however adequate to support fish as only dissolved oxygen contents lower than 3.0 mg/l could cause fish kills (Boyd (1982). The temperatures recorded during this study were within range for tropical fish.

There was no fish mortality during the first 96 hours of the experiment in all the treatments. These observations were similar to those of other workers (Omoregie and Ufodike, 2000; Nwabueze and Agbogidi, 2010). Delayed mortality of fish exposed to water soluble fractions of crude oil has also been reported by Rodrigues *et al*. (2010) though Lee *et al.* (2011) reported higher mortality with larvae and fry of Atlantic herring.

It was not possible to estimate the 96-hour LC_{50} value for the water soluble fraction since no mortalities occurred during the 96-hour period. However, more than 50% of the test fish in treatment 5(100 ppm) died at 144 hours. Significant differences ($p<0.05$) were observed among treatment concentrations (Table 2). However, while mortality has been in use for regulatory purposes, attention is moving towards non-lethal problems associated with crude oil exposure such as feeding changes, behaviour after exposure , reproduction and many others (Chapman and Riddle, 2005; Jensen and Carroll, 2010).

The higher concentrations of THC in fish muscles above those in water were indicative of bioaccumulation of the THC in fish muscle tissues. Similar reductions in hydrocarbon content of water and uptake of hydrocarbons by fish have been reported. Also the presence of hydrocarbon in fish long after the exposure period were similar to Al-Saad *et al*.(2011) who reported that in bivalves, *Corbicula flumenea* crude oil residues were found in fish 20 days after exposure to WSF of crude oil. The bioconcentration factor calculated for THC are reported on table 7with the highest bioconcentration factor for 25ppmWSF both at 96 hours and 14 days .This tends to support the notion that uptake of pollutants by organisms are usually higher at lower concentrations. This could be due to the inability of the fish to easily detect and avoid the pollutant.

Lead, iron and cadmium were not detected in all the water samples for all treatments. Zinc was the dominant metal in water. Generally, lower concentrations of metals and THC were obtained in water than from fish muscles indicating bioaccumulation during the study .Lead was not detected in all the fish samples analysed. Higher levels of the trace metals were observed in fish samples than in the water. Lead was also not detected in the survivors at the end of the recovery period. The dominant metal found in the muscle tissues of fish was iron followed by zinc while the lowest was cadmium. Significant differences ($P<0.05$) were observed among treatment groups in response of fish to the presence of WSF of the crude oil in water. Similar trends were observed in the accumulation of THC from water and higher concentrations in the fish at the end of 96 hours and 14 days. The Pearson correlation gave a positive correlation between the THC in water and fish muscle tissue. Fish on treatment 2 (25ppm) had the highest bioconcetration factor ($p<0.05$) of all treatments both at 96 hours and 14 days. The bioaccumulation factor (Marin and Oron, 2007) was calculated to show the ability of fish to accumulate the THC in fish relative to their concentrations in the water. The reduction of iron in the fish exposed to WSF could have been due to some form of anaemia. Sunmonu and Oloyede (2008) reported significant reductions in red blood count, haemoglobin and PCV in *Clarias gariepinus* exposed to WSF of crude oil which they attributed to the suppression of erythropoiesis by the toxic components of the crude oil. Danion *et al*. (2011) also reported decreased leucocyte count due to granulopenia and increased haemolytic activity in European sea bass. Manganese increased in 50ppmWSF while other treatments decreased though not significantly but at day 14 the difference was significant ($p<0.05$). Non-significant increases were observed in fish above the concentrations in water for chromium, copper and vanadium both at 96 hours and 14 days. Like iron, zinc was also highest in treatment 3(50ppmWSF) at 96 hours .However while other treatments increased, a decrease was observed in treatment 2(25ppm) at 14 days.

In conclusion, water soluble fraction of crude has been shown to negatively affect the fish *Clarias gariepinus* as the behavior of fish was affected during the study and fish became lethargic even before mortality was observed in any treatment. Fish also could not feed even during recovery period and all treatments except the control lost their vibrant swimming abilities. There is a need to further study how exposure to WSF at juvenile stage would affect the reproductive performance of adult *C.gariepinus.*

REFERENCES

Al-Saad ,H.T., Farid, W.A., Al-Adhub, A.Y. (2011).Uptake and Depuration of water-soluble fractions (WSF) of crude oil by bivalve *Corbicula*

fluminea (Muller) from Shatt Al-Arab river. M*esopotamian Journal of Marine Science* 26(2):134-145

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M. (1974). Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Marine Biology*, 27:75–88.

Asuquo, F.E. and Udoh , J.P. (2002) .Patterns of total hydrocarbon , copper and Iron in Fish from Cross River Estuary, Nigeria. *West African Journal of Applied Ecology* , 3:91-97

ATSDR (1999).Toxicological Profile for total Petroleum hydrocarbons (TPH).DHHS, Editor. Atlanta, GA

Azad M (2005). Toxicity of water-soluble fractions of four fuels on *Metamysidopsis insularis*, an indigenous tropical mysid species. *Environmental Monitoring and Assessment,* 104: 37-44.

Bamforth, S. M. and Singleton, I. 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology*, 80: 723–736.

Bartha, R. and Atlas, R.M. (1977). The microbiology of oil spills. *Advances in Applied Microbiology*, 22 pp 225-226

Boyd CE. (1982). Water quality management for pond fish culture. Amsterdam: Elsevier Scientific Publishing Company, 318 pp.

Centre for Diseases Control and Prevention (2010). Gulf Oil spill 2010.Light Crude oil Information for Health Professionals 2010

Chapman, P.M. and Riddle, M.J. (2005).Polar Marine toxicology-future research needs. *Marine Pollution Bulletin*, 50:905-908

Danion, M., Le Floch, S., Guyomarch, J., Quentel, C. (2011). Bioconcentration and Immunotoxicity of an experimental oil spill in European sea bass (*Dicentrarchus labrax* L.) *Ecotoxicology and Environmental Safety*, 2011,74(8) :2167-2174

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C.(2001). Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environmental Toxicological Chemistry*, 20: 2383–96.

Edema, N.E. (2006). Ionic and physical characteristics of dispersions and water soluble fraction of crude oil and its effects on the physiology of aquatic macrophytes. PhD Thesis, University of Benin, Benin city.

Edema, N.E., Okoloko, G.E., Agbogidi, M.O. (2007). Physico- chemical characteristics of the water soluble fraction of the well-head crude oil and the effects on *Pistia stratiotes* Linn (Water Lettuces). *American Eurasian Journal of Agriculture and Environmental Science,* 2(6) 633-638

FAO/SIDA(1983). Manual of Methods in Aquatic Environmental Research, Part 9.Analysis of Metals and Organochlorines in Fish. *FAO Fisheries Technical* Paper 212,33pages.

Feijoo-Siota, L., Rosa-Dos-Santos, F. de Miguel, T., Villa, T.G. (2008). Biodegradation of Naphthalene by *Pseudomonas stutzeri* in Marine Environments. Testing Cells Entrapment in Calcium Alginate for Use in Water Detoxification *Bioremediation Journal* volume 12(4) pp185-192

Humphrey, S.R. (2010). Chemical dispersants and crude oil efficacy and toxicity. *Energy Bulletin*, Post carbon. Accessed on 21st June, 2012.

Jensen, L.K. and Carroll, J. (2010).Experimental studies of reproduction and feeding for two Arctic-dwelling *Calanus* species exposed to crude oil. *Aquatic Biology*, volume 10:261- 271

Kiceniuk, J. W. and Khan, R. A. (1983). Toxicology of chronic crude oil exposure: Sublethal effects on aquatic organisms, New York, John Wiley and Sons Ins

Kotz,L., Kaiser , G., Tschopel, P . and Tolg, G.(1972).Aufschas biologiche matrice for die bestimang she-iedrieger-Spurenelementehalte bei bergrenxter einwage . Mit salpetersaure unden druck in einem telforgeta.*Z.Anal. chem*:207-209

Lee, K., King, T., Brian Robinson, B., Li, Z., Burridge, L. Lyons, M., Wong, D., MacKeigan, K. Courtenay, S., Johnson, S., Boudreau, M., Hodson, P., Greer,C., Albert Venosa ,A.(2011). Toxicity Effects of Chemically-Dispersed Crude Oil on Fish. 2011 International Oil Spill Conference ,2011-163, February 25, 2011.

Marin, C.M.D.C. and Oron, G. (2007) Boron removal by the duckweed *Lemna gibba*: a potential method for the remediation of boron polluted waters. *Water Research* 41:4579–4584

Macys, D.A., Risher, R.L.C.J.F., Vinegar, A., Dodd, D.E., Wall, H.G.(1999). Results of a workshop on Health Effects of Crude oil exposures related to Operation Desert Storm, Defense Editor. Naval Medical Research Institute, Bethesda, MD p8-32

Manahan, S.E. (1992). Toxicological Chemistry, second edition. Lewis Publishers Incorporated, Michigan, U.S.A.

NAP — National Academies Press. Oil in the sea III: inputs, fates, and effects. Washington D.C: National Research Council (U.S.); 2003. 278pp.

Neff, J.M., Ostazeski, S., Gardiner, W., Stejskal, I. (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel

fuel to marine animals. *Environmental Toxicological Chemistry,* 19(7):1809–21.

Nwabueze, A.A. and Agbogidi, O.M (2010). Impact of Water Soluble Fractions of Crude Oil on Growth Performance of the Catfish *Heterobrancuhus bidorsalis .ARPN Journal of Agricultural and Biological Science,* vol. 5(1) 43-46, Asian Research Publishing Network (ARPN).

Odiete, W.O. (1999).Environmental Physiology of Animals and Pollution. Diversified Resources Limited, Lagos, pp1-261

Olaifa, F.E. (2005). Assessment of Toxicological impact of a Light Crude oil on *Clarias gaiepinus* (Burchell, 1822) juveniles. *Journal of livestock Extension,* volume 4, pp42- 46

Omoregie E, Ufodike BC.(2000). Effects of water-soluble fractions of crude oil on growth of the Nile tilapia, Oreochromis niloticus (L.). *Bulletin of Environmental Contamination and Toxicology*; 64:601–5.

Pauka, L.M., Maceno, M. Rossi, S.C., Silva de Assis, H.C (2011). Embroyotoxicity and Biotransformation responses in Zebra fish exposed to water soluble fraction of crude oil. *Bulletin of Environmental Contamination and Toxicology* 86(4):389-393

Rana, S, V.S (2005). Essentials of Ecology and Environmental Science. SecondEdition. Prentice Hall of India Private Limited, New Delhi, 488p

Reish, D.L. and Oshida, O.S. (1987).Manual of Methods in Environment Research. Part 10.Short term static bioassays.FAO fisheries Technical Paper No247,Rome, 62 pages

Rodrigues, R.V., Miranda-Filho, K.C, Gusmão, E.P. ,Moreira ,C.B., Romano L.A., Sampaio, L.A. (2010). Deleterious effects of water-soluble fraction of petroleum, diesel and gasoline on marine pejerrey *Odontesthes argentinensis* larvae. *Science of the Total Environment* 408: 2054–2059.

Saeed, T.and Al-Mutairi M.(1999). Chemical composition of the water-soluble fraction of leaded gasolines in sea water. *Environment International* 25: 117–29.

Sharanagouda, U. and Karegoudar, T.B. (2001). Degradation of 2-methylnaphthalene by *Pseudomonas* sp. strain NGK1. *Current Microbiology*, 43: 440–443.

Sikkema, J, De Bont JA, Poolman, B. (1994). Interactions of cyclic hydrocarbons with biological membranes. *Journal of Biological Chemistry*, 269:8022–8.

Sunmonu,T.O. and Oloyede, B.O. (2008). Hematological response of African catfish (*Clarias gairepinus*) and rat to crude oil Exposure. The Internet Journal of Hematology, Volume Number 4(1).

U.S. Environmental Protection Agency (2000). Bioaccumulation testing and interpretation for the purpose of sediment quality assessment: U.S. Environmental Protection Agency, access date June 29, 2010.

U.S. Environmental Protection Agency, (2010b) Solid waste and emergency response glossary--Bioaccumulation: U.S. Environmental Protection Agency, access date June 29, 2010b.