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Comparative Toxicity of Light and Heavy Crude Oils on African Catfish (*Clarias gariepinus*) Fingerlings

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Abstract: *The continuous discharge of petroleum hydrocarbons, oil spills and gas flaring have led to extensive degradation of aquatic ecosystems, destruction of mangrove swamps and loss of biodiversity. The acute toxicity of light and heavy crude oils to fingerlings of the catfish, C. gariepinus (mean total length, 15.40±0.57 cm, mean weight, 22.08±2.51 g) were studied in static toxicity bioassay over a 96-hr period after acclimation of test organisms in the laboratory. There were initial range finding tests to determine the concentrations of the toxicants to be administered on the test organisms in the definitive tests. The test organisms were exposed to five concentrations (1.0, 1.5, 2.0, 2.5 and 3.0%) of both toxicants and a control (0.0%) in static renewal bioassay. The median lethal time (LT_{50}) at concentrations of 2.0, 2.5 and 3.0% were 2.75 hrs, 1.67 hrs and 1.17 hrs respectively for the light crude oil while that of heavy crude oil at concentrations of 2.5 and 3.0% were 45.00 hrs and 36.00 hrs respectively. Mortalities increased with increase in both crude oils concentrations. The 96-hr median lethal concentrations (LC50) for light and heavy crude oils were 1.4% (14 ml/l) and 2.2% (22 ml/l) respectively. The dissolved oxygen levels, pH and temperature values of the control were higher than those of the treated group. Results showed that light crude oil was more toxic than heavy crude oil.*

Key Words: Toxicity, bioassay, light crude oil, heavy crude oil, median lethal time (LT_{50}), median lethal concentration (LC50).

Introduction: Pollution has long been a global concern, particularly because of its adverse effects on the climate and the environment. It can occur in various forms, including air pollution, water pollution, and soil pollution, all of which pose serious threats to ecological balance and biodiversity. Among these forms, water pollution is particularly significant because aquatic ecosystems are highly sensitive to contaminants introduced into the environment. The environment is considered polluted when its natural composition is altered either directly or indirectly through human activities, making it less suitable for some or all of the purposes for which it would ordinarily be used in its natural state (Kumar, Kumar and Singh, 2019; Kabeyi and Olanrewaju, 2022; Dey, Veerendra, Babu, Manoj and Nagarjuna, 2023). According to Ipogah and Ikenga (2023), any environmental degradation affecting water resources reduces the potentials for sustainable livelihoods to increase poverty. Oil pollution occurs through several pathways, including accidental spills and the direct discharge of petroleum products into the environment. Crude oil spills resulting from shipping accidents and leakages from underground pipelines have become increasingly common worldwide, contributing significantly to the contamination of aquatic ecosystems (Vinod, Nitin, Temin, Jogendra and Pankaj, 2020; Sado and Orowe, 2024). In addition, deliberate discharge of crude oil into the environment is often linked to pipeline vandalism. Nigeria has witnessed ceaseless petroleum spill incidences in the past five decades with overwhelming consequences on land and coastal environment in the Niger Delta region (Ejiba, Onya and Adams, 2016). Most notably, in the Niger Delta a total of 13,555 oil spills were recorded between 1976 and 2019 as a result of lack of maintenance, accidents, sabotage, tapping, bunkering, and micro-refining (National Oil Spill Detection and Response Agency (NOSDRA), 2019). Ekpo, Obot and David (2018) and Bashir (2021) affirmed that the major causes of oil spills in the Niger Delta are sufficiently documented and the primary causes listed are, aging infrastructure, blow outs, vandalism, equipment malfunction, corrosion, illicit oil bunkering, artisanal refining, accidental and deliberate releases and some other natural causes. Mba, Mba, Ogbuabor and Arazu (2019) and Ugwu, Ogba and Ugwu (2021) opined that interdiction, which is the willful theft and destruction of oil facilities, is becoming the major factor in oil spill pollution in the Niger Delta.

According to BP Energy Outlook (2019), the properties of crude oil are a function of its constituent compounds, the key physical properties of density (specific gravity), viscosity, pour point (the temperature above which it will pour) and the key chemical properties are the aromatic, wax and asphaltene content. Crude oil can be classified based on its chemical composition with regard to its relative density either as light or heavy crude oil and this is based on the American Petroleum Institute (API) gravity which reflects how light or heavy a crude oil is compared to water. Light crude oil is liquid petroleum that has low density, flows freely at room temperature, has low viscosity, low specific gravity and high API gravity (more than 20°) due to the presence of a high proportion of light hydrocarbon fractions (Hu, 2017, Sojinu and Ejeromedoghene, 2019 and Chrysalidis and Kyzas, 2020). On the other hand, heavy crude oil means any liquid petroleum with an API gravity less than 20° (Ikponmwen, Orowe and Sado, 2022). Crude oil exploration, production, and transportation are major economic activities in the Niger Delta region of Nigeria. However, these activities have also resulted in frequent oil spills and environmental contamination of aquatic ecosystems. Crude oil pollution poses serious threats to aquatic organisms, particularly fish species that inhabit rivers, creeks, and wetlands. The African catfish is a common tropical and commercially important fish species in Nigeria. The flesh of this species is oily, tasty and is highly priced in the market (Ikponmwen *et al*., 2022). Furthermore, in most pollution cases, the younger fish species (fingerlings) are adversely affected due to their vulnerability; hence the choice for fingerlings in this study. Previous studies on the effects of crude oil on this species include those of Ikponmwen *et al*., 2022, Sado and Orowe, 2024. Also, studies on the influence of crude oil exposure and recovery on growth performance of *Oreochromis niloticus* (Eriegha, Eyo and Adeleke, 2023), effect of crude oil water soluble fraction toxicity on *Tilapia guineensis* fingerlings using histology of the kidney as a bioassay indicator (Ebonwu and Ugwu, 2016), histopathological effect of the different concentration of crude oil and its products on the gill apparatus of catfish (*Heterobranchus bidorsalis*) (Ugbeyide and Nwamba, 2021), range finding, acute and sublethal test of petrol and engine oil mixture on African catfish, *C. gariepinus* (Burchell, 1822) (Akin-Obasola, 2019) had been reported. Despite the large number of crude oil spills in Nigeria, most especially in the oil producing areas of the Niger Delta, scanty literature exists on the comparative toxicities of light and heavy crude oils spills to which fish species particularly catfish populations had been exposed. The objective of this study was to determine and compare the acute toxicity and median lethal concentrations (LC_{50}) of light and heavy crude oils to *C. gariepinus* fingerlings.

Materials And Methods: Collection of Fish Samples: A total of four hundred and thirty-two (432) apparently healthy *C. gariepinus* fingerlings (9 weeks old; mean total length 15.40 ± 0.57 cm and mean weight 22.08 ± 2.51 g) were obtained from Jossy Farms, Ugbiyokho Quarters, Ekenwan, Benin City, Edo State, Nigeria and transported to the laboratory in well-aerated bags. The fish were kept in thirty-six (36) open-top glass aquarium tanks (50×25×26 cm^3) containing 20 L of borehole water and were allowed to acclimate to laboratory conditions for one week. During this acclimation period, the fingerlings were fed to satiation with 2 mm Coppens fish feed. The water in the tanks was renewed daily to prevent the accumulation of waste metabolites and leftover feed prior to the commencement of the bioassay study.

Experimental Setup and Design: Acute toxicity tests were conducted to compare the toxicities of light and heavy crude oils on *C. gariepinus* fingerlings over a 96-hour period (Hoffman, Rattner, Burton and Cairns, 2003). The bioassay study was carried out in the laboratory of the Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The bioassay method employed was the one outlined by Adams (2017). The 96-hr median lethal concentrations (LC_{50}) of both toxicants were determined with probit analysis as described by Finney (1971) and Abdel-Halim, Osman, El-Danasoury and Aly, (2021) (Figures 2 and 4). The experiment was laid out in Completely Randomized Design (CRD) with two crude oil types (light and heavy crude oils) using six treatments for each crude oil containing a control (0.0%) and concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume. Each of the treatments was replicated thrice to give a total of 36 experimental units containing 432 fingerlings of *C. gariepinus* (12 fingerlings in each test tank). The control (0.0%) which also has 12 fingerlings of *C. gariepinus* was not contaminated with the test toxicants.

Preparation of Test Solutions and Application of Test Toxicants: Experiment 1 (Range – Finding Test): The crude oils were collected from the Nigerian Petroleum Development Company (NPDC), Oredo Field, Edo State. A preliminary (range – finding) test as described by Akin-Obasola (2019) and Yesilyurt and Cesur (2020) was conducted to determine the main experimental concentrations of the toxicants. Based on the results of the range – finding test, the following concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume of the toxicants and a control (0.0%) were prepared for the definitive test.

Experiment 2 (Definitive Test) : The second stage of the study gave details of the actual experimental concentrations of the toxicants as described by Silva (2023) and Ikponmwen *et al*. (2022). For the light crude oil against *C. gariepinus*, a sample of it was diluted with borehole water to obtain concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume while for the heavy crude oil against *C. gariepinus*, a sample of it was also diluted with borehole water to obtain concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume.

Exposure Procedure: A sample of twelve fingerlings was randomly placed in each of the experimental tanks and appropriate test solutions were added. Monofilament nettings were used to cover the tanks to prevent the test organism from jumping out of the tanks and fish mortality was monitored.

Statistical Methods: Data generated were subjected to analysis of variance (ANOVA) test at 5% level of significance, using a GENSTAT Computer Software (version 12.00 for windows). Significant means were separated using the Least Significant Difference (LSD) (Adelani, Ogunsanwo and Awobona, 2020, Kumar, Carter, Mir, Sehgal, Agarwal and Paterson, 2022 and Wani, Jiang, Hossain, Burritt, Rouached and Liu, 2023).

Results: Physico-Chemical Parameters: The physico-chemical parameters measured during the 96-hour exposure are presented in Table 1. The Dissolved Oxygen (D.O.) levels, pH and temperature values in the control were higher than those in the treatment of both toxicants. The results showed that the dissolved oxygen and pH values of the contaminated tanks of both toxicants decreased with increasing concentration of the toxicants while the temperature values increased with increasing concentration of both toxicants. The dissolved oxygen levels and temperature values of tanks contaminated with light crude oil were not significantly different from tanks contaminated with heavy crude oil ($P>0.05$) but the pH values of the tanks contaminated with light crude oil were significantly different from the tanks contaminated with heavy crude oil ($P<0.05$).

Range – Finding Test: A range – finding test was conducted to determine the main experimental concentrations for the light and heavy crude oils. Concentrations by volume of 0.5, 1.0, 1.5, 2.0 and 3.0% and a control (0.0%) were tested on six (6) *C. gariepinus* fingerlings for 96-hr. Based on the results of the range – finding test, the following concentrations: 1.0, 1.5, 2.0, 2.5 and 3.0% by volume of the toxicants and a control (0.0%) were prepared for the definitive test.

Acute Exposure of *C. gariepinus* to Light Crude Oil: The experiment involved the use of a control (0.0%) and concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% in acute toxicity trials to determine the median lethal concentration (LC_{50}). At the onset of the experiment; within an hour of the introduction of the fish to the test solutions, the fish exhibited some erratic swimming patterns with some degree of loss of balance and made attempts to jump out of the test tanks. Weak and lethargic swimming behaviour were prevalent in all test tanks together with mortalities except in the 0.0% (control) test tanks throughout the 96-hour period of exposure. The experimental fish could only tolerate the 3.0% test medium for 40 minutes before the first mortality was recorded. It took 1.17 hours (median lethal time, LT_{50}) for 50% mortality and 1.92 hours for complete (100%) mortality to be recorded in the 3.0% test medium. In the 2.5% test medium, the first mortality was recorded at 1 hour, and at 1.67 hours (LT_{50}) and 3 hours, 50 and 100% mortalities were recorded respectively. The 2.0% test medium recorded its 50% mortality at 2.75 hours (LT_{50}) while 94.44% mortalities were recorded after 24 hours. It took 25 hours to record complete (100%) mortality. When fish were exposed to 1.5% test medium, mortalities recorded were 16.67 and 41.67% after 24 and 48 hours of exposure respectively. Fish exposed to the 1.0% test medium showed higher resistance with 16.67, 16.67 and 25.00% mortalities after 24, 48 and 72 hours of exposure respectively. Results show that mortalities increased with increase in the concentrations of light crude oil (that is, survival rate decreased with an increase in the crude oil levels) (Figure 1). The result of the acute toxicity bioassay study showed that the 96-hr LC_{50} value of light crude oil on *C. gariepinus* fingerlings was 1.4% (14 ml/l) (Figure 2).

Acute Exposure of *C. gariepinus* to Heavy Crude Oil: Acute toxicity testing was done with heavy crude oil involving the use of a control (0.0%) and concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% in the different test media to determine the LC_{50}. Lethargic swimming behaviour and mortalities as observed in the light crude oil exposure tanks were also prevalent in all the test tanks except in the 0.0% (control) test tanks throughout the 96-hour period of exposure. It took 36 hours (LT_{50}) to attain 50% mortality. The mortalities recorded were 44.44, 72.22 and 94.44% after 24, 48 and 72 hours of exposure respectively. In the 2.5% test medium, 41.67 and 50.00% mortalities were recorded after 24 and 48 hours of exposure respectively. This test medium recorded 50% mortality after 45 hours (LT_{50}) of exposure. When fish were exposed to the 2.0% test medium, mortalities recorded were 16.67, 25.00 and 33.33% after 24, 48 and 72 hours of exposure respectively. In the 1.5% test medium, mortalities recorded were 8.33, 16.67, 16.67 and 25.00% after 24, 48, 72 and 96 hours of exposure respectively. However, fish in the 1.0% test medium showed very low sensitivity and mortalities recorded in this test medium were 8.33% after 24 hours of exposure. Results show that mortalities increased with increase in the concentrations of heavy crude oil (Figure 3). The result of the acute toxicity bioassay study showed that the 96-hr LC_{50} value of heavy crude oil on *C. gariepinus* fingerlings was 2.2% (22 ml/l) (Figure 4).

Discussion: The percentage mortality increased with increase in the concentration of both toxicants over time of exposure. This was evident from test results which showed 16.67% and 100% mortalities after 24 hrs for 1.0 and 3.0% concentrations by volume respectively for the light crude oil and 8.33% and 44.44% mortalities after 24 hrs for 1.0 and 3.0% concentrations respectively for the heavy crude oil. The results of the bioassay study showed that the 96-hr median lethal concentration (LC_{50}) values recorded were 1.4% and 2.2% for the light and heavy crude oils respectively, indicating that the light crude oil is more toxic than the heavy crude oil. This finding agrees with the works of Ikponmwen *et al*. (2022) on the bioaccumulation of Polycyclic Aromatic Hydrocarbons from light and heavy crude oils in fingerlings of the African catfish (*C. gariepinus*). Also, the United States Environmental Protection Agency (USEPA) (2026) reported that light crudes are highly toxic to humans, fish and other organisms compared to the heavy crudes which are less toxic. Furthermore, the National Oceanic and Atmospheric Administration (NOAA) (2021) in their work on "how oil harms animals and plants in marine environments reported that the lighter the oil, the more toxic it is considered.

The dissolved oxygen levels and pH values of the control were observed to be higher than those of the treatments of both toxicants. This observation corroborated the reports of Capolupo, Sørensen, Jayasena, Booth and

Fabbri (2019) who studied the chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. The dissolved oxygen levels in the tanks of both toxicants decreased with increasing concentration of the toxicants. This finding agrees with the reports of Sado and Orowe (2024) who reported that a significant decrease in oxygen was observed in their study of histopathological changes on the gills of the African catfish fingerlings exposed to light and heavy crude oils leading to the anaerobic decomposition of organic matter in water, which results in the production of harmful and toxic gases such as hydrogen sulphide and methane, ultimately causing detrimental effects on aquatic organisms. Also, the pH values in the tanks of both toxicants decreased with increasing concentration. This finding agrees with the works of Ogbonna, Ngah, Youdeowei and Origbe (2021) who reported decrease in pH which posed lethal effects in their study on the physico-chemistry of surface water impacted by crude oil spills in Bodo/Bonny Rivers, Nigeria. A similar trend was observed by Perrichon, Menach, Akcha, Cachot, Budzinski and Bustamante (2016) in their study on toxicity assessment of water-accommodated fractions from two different oils using a zebrafish (Danio rerio) embryo-larval bioassay with a multilevel approach. However, values for temperature increased with increased concentration of both toxicants. The increased temperature with increasing concentration of the toxicants could be responsible for the increased mortality relative to the concentration. A study on the combined effect of temperature and a reference toxicant (KCl) on *Daphnia middendorffiana* (Crustacea, Daphniidae) in a high-mountain lake by Pastorino, Prearo, Anselmi, Bentivoglio, Esposito, Bertoli, Pizzul, Barceló, Elia and Renzi (2022) indicated that increased temperatures significantly enhance the toxicity of Potassium Chloride (KCl) on *Daphnia middendorffiana* in high-mountain lakes. This consequently increased the respiratory rates of aquatic organisms and led to increased oxygen consumption and decomposition of organic matter.

Conclusion: The findings of this study revealed that light crude oil exhibited greater toxicity to African catfish (*C. gariepinus*) fingerlings than heavy crude oil. This observation supports the general principle that the toxicity of crude oil is often inversely related to its density, with lighter crude oils typically containing higher proportions of low molecular weight hydrocarbons, which are more soluble, more bioavailable, and therefore more toxic to aquatic organisms. The higher mortality and toxic effects observed in fish exposed to light crude oil in this study may be attributed to the rapid dispersion and absorption of these toxic components into the aquatic environment and fish tissues. Also, the results demonstrated that *C. gariepinus* fingerlings can serve as a reliable bio-indicator species for crude oil pollution in aquatic environments. This is largely due to their hardy nature, tolerance to adverse environmental conditions, and their ability to survive in waters with low dissolved oxygen levels through accessory air-breathing mechanisms.Furthermore, the comparative toxicity assessment indicates that light crude oil poses a greater ecological risk to aquatic organisms than heavy crude oil, particularly to fish at early life stages such as fingerlings. The use of African catfish fingerlings as bio-indicators is therefore recommended for monitoring crude oil pollution and assessing the ecological impact of oil spills in freshwater environments.

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Table 1: Water Quality Parameters in Control and Test Tanks containing *C. gariepinus* fingerlings.

	D. O. (mg/l)		pH		Temperature (^{O}C)	
Concentration (%)	**LCO**	**HCO**	**LCO**	**HCO**	**LCO**	**HCO**

0.0	6.20[a]	6.24[a]	6.67[a]	6.76[b]	27.57[a]	27.72[a]
1.0	4.65[b]	4.76[b]	6.31[c]	6.62[d]	26.61[b]	26.96[b]
1.5	4.45[c]	4.45[c]	6.27[e]	6.62[f]	26.62[c]	27.12[c]
2.0	4.00[d]	4.13[e]	6.19[g]	6.59[h]	26.66[d]	27.31[d]
2.5	3.62[f]	3.49[g]	5.95[i]	6.56[j]	26.83[e]	27.32[e]
3.0	2.95[h]	2.55[i]	5.94[k]	6.50[l]	26.86[f]	27.33[f]
	LSD(0.123)		**LSD(0.029)**		**LSD(0.901)**	
	SED(0.062)		**SED(0.015)**		**SED(0.451)**	

NOTE: Means with identical superscript between the crude oil types do not differ significantly while means with non-identical superscript differ significantly (P = 0.05).
LCO = Light Crude Oil, HCO = Heavy Crude Oil, LSD = Least Significant Difference, SED= Standard Error of the Difference between two means

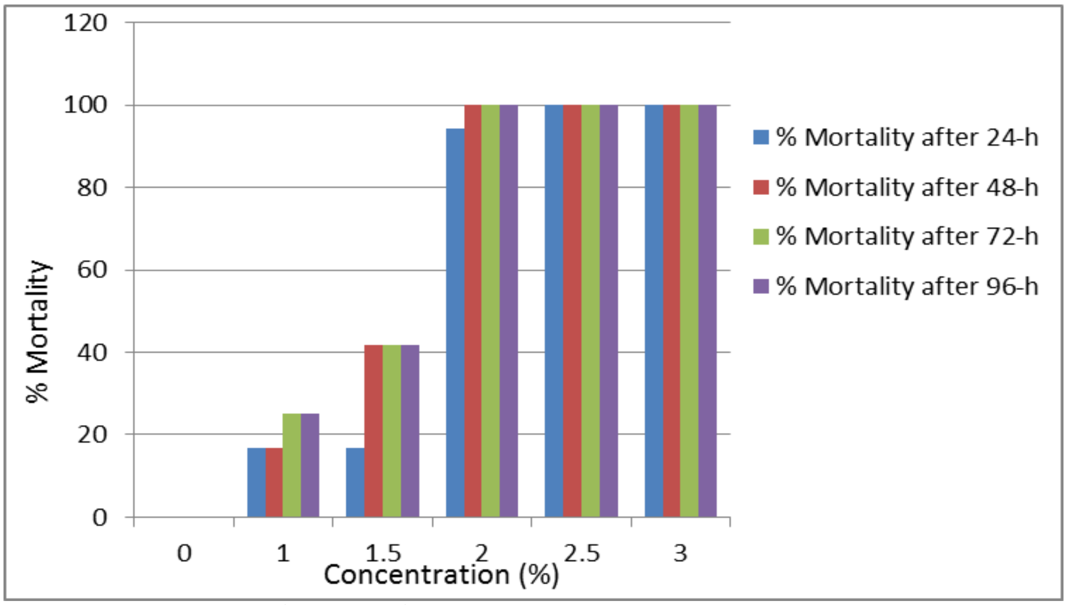

Figure 1: Percentage mortality of *C. gariepinus* fingerlings exposed to Light Crude Oil

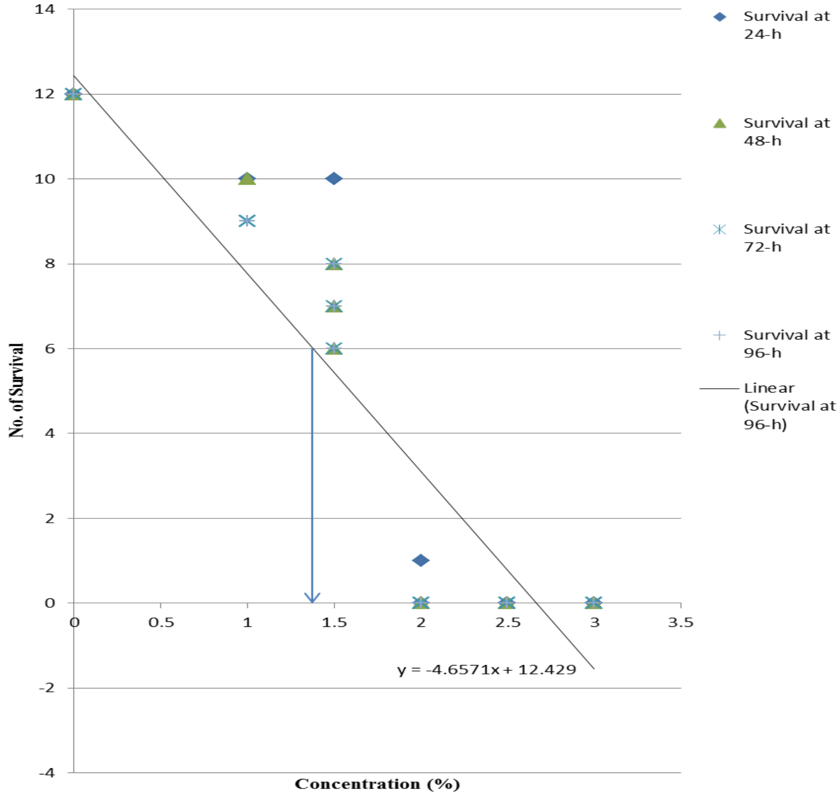

Figure 2 : Median lethal concentration (LC_{50}) of Light Crude Oil on *C. gariepinus* fingerlings

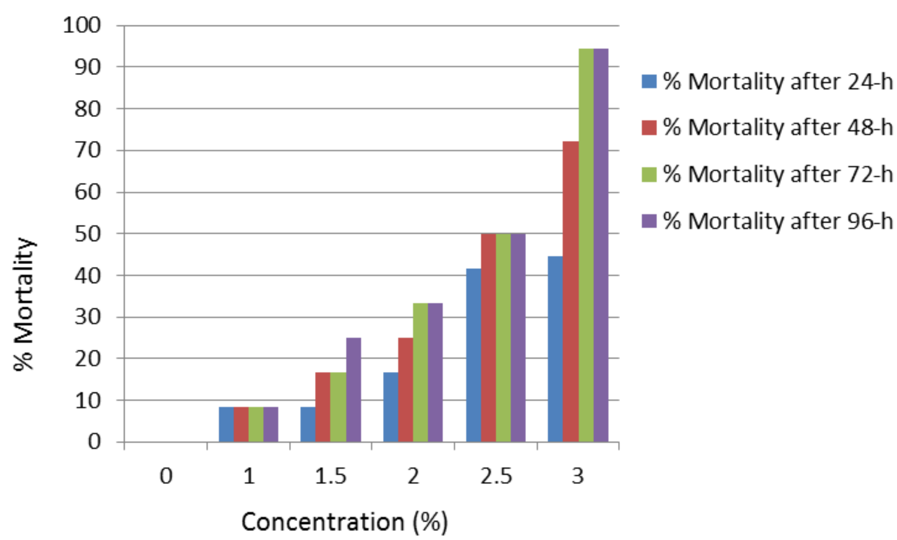

Figure 3: Percentage mortality of *C. gariepinus* fingerlings exposed to Heavy Crude Oil

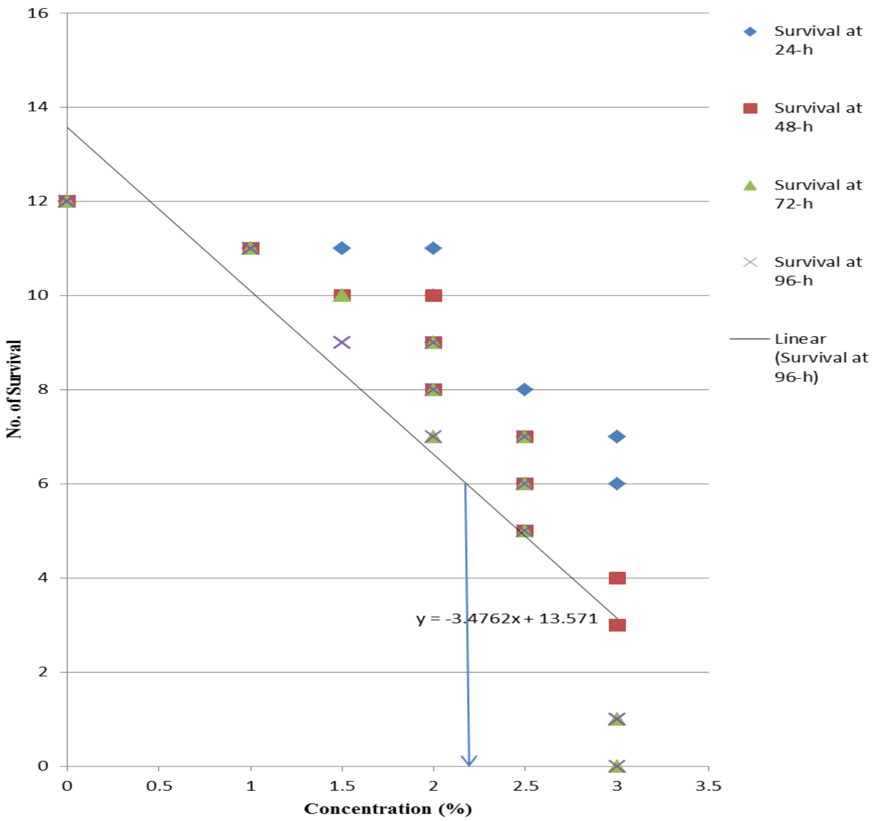

Figure 4 : Median lethal concentration (LC_{50}) of Heavy Crude Oil on *C. gariepinus* fingerlings