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Phytoremediation of Diesel Contaminated Water DCW Exposed on *Clarias gariepinus* Using Moringa Seed Extract MSE as the phytoremediant

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ABSTRACT

Phytoremediation of diesel contaminated water (DCW) for freshwater fish *Clarias gariepinus* (12.6 ±1.4g and mean length 8.6 ± 0.7cm) was conducted at the research farm of the Enugu State University of Science and Technology Enugu. 180 juveniles were purchased and acclimatized for 7 days. A 24-hour range finding test determined the LC50 value of 1.2 mg/l DCW. A 96-hour static bioassay acute toxicity experiment was then conducted in plastic tanks with 10 fish in each, and diesel of different concentrations. Mortality rate was observed in the various test concentrations. The LC50 concentration was 0.12 mg/l, improved to 0.30 mg/L with MSE-treated diesel. Histopathology of the gills of affected fish showed elevated damage, necrosis, telangiectasia and oedema in DCW exposed fish, but limited damage in MSE-treated DCW exposed fish. The results of the experiment showed that diesel has detrimental effects on C. gariepinus and could cause high mortality at higher concentrations. Moringa seed extract (MSE) was used as a phytoremediant to ameliorate the mortality and gill histological damages of the experimental fish. Therefore, diesel contaminated water should be ameliorated to a safety level of 0.003 mg/l with MSE to protect fish species and other aquatic organisms.

1.0 Introduction

Phytoremediation is the process of using plants to extract contaminants or degrade them in the Water (Etim, E. E. (2012) . As with microbial remediation, the cost is low. However, the timeframe can be longer than several years. Effectiveness in bringing water up to agricultural standard varies, as one species of plant is generally used on one type of contaminant, potentially leaving a range of contaminants behind. As well, the contaminated plants used for extraction must be disposed of. Phytoremediation can also be seen as the use of plants and/or associated microorganisms to remove contain or render harmful material harmless (Cunningham et al., 1996; Schwab and Banks, 1999; Merkl, 2005). It is effective for different kinds of pollutants (contaminants) like heavy metals, radionuclides and a broad range of organic pollutants (Schroder et al., 2002;

Although the application of microbial biotechnology has been successful with diesel oil-based constituents, microbial digestion has met limited success for widespread residual organic and metal pollutants. Vegetation-based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants (Vidali, M. (2001). Phytoremediation is an emerging technology that uses plants to remove contaminants from air and water. The primary aims of any remediation are the reduction of actual or potential environmental threats and the reduction of potential risks so that unacceptable risks are reduced to acceptable levels. Consequently, the need for remediation will depend on the degree of actual or po-

tential environmental threat or the level of risk (Ukoli, 2003). Remediation of a contaminated site is achieved by one or more of the following objectives: Modification of the contaminants to a less toxic form, Removal or destruction of the contaminants and Isolation of the contaminant from the target by interrupting the pathway of exposure (Onifade, et al., 2007).

Waters contaminated with diesel oil crude oil are of no benefit to aquaculture and other uses of water due to water contaminants. There is therefore the need to redeem the water for use and also for fish farmers. However, moringa seed extract can be applied to the water as a remediating factor. This is because moringa seed extract does not only enrich the water, it also ameliorates the crude oil pollution effect on the Water through the phytoremediation mechanisms of: Phytoextraction, phytostabilization, phytodegradation, photodegradation, phytovolatilization in order to affect the volume, mobility, or toxicity of the contaminants (EPA 2000; Muthusaravanan et al., 2018). Its potential for encouraging the biodegradation of organic contaminants is promising and requires further research.

Moringa seed is derived from the seeds of the Moringa oleifera tree, which is native to parts of Africa and Asia. Moringa seeds are rich in various bioactive compounds such as antioxidants, vitamins, minerals, and proteins, which contribute to their potential health benefits. Moringa seed extract has been studied for its various properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.Some potential health benefits of moringa seed extract that have been reported in scientific literature include:

Antioxidant properties: Moringa seed extract has been shown to possess strong antioxidant properties, which help to neutralize harmful free radicals in the body and protect against oxidative stress, a condition linked to various chronic diseases (Karim et al., 2016; Sreelatha & Padma, 2009).

Diesel oil in its natural state is referred to as crude oil (Ukoli, 2003). Crude oil is mainly either black or green but it can be light yellow (Onifade, et al., 2007). It varies considerably in density and is described as heavy, average or light (Ojo and Adebusuyi, 1996). Diesel oil is at present Nigeria's and indeed the world's most important derived energy source (Moffat and Linden, 2005).

Water contamination is caused by the presence of xenobiotic (human-made) chemicals or other alterations in nature. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are diesel oil hydrocarbons (such as naphthalene and benzoic, solvents, pesticides, lead and other heavy metals (Slack et al., 2005). Contamination is correlated with the degree of industrialization and intensity of chemical usage. Concern over water contamination by crude oil or hydrocarbon products, in general, is gathering momentum after a similar feeling has been around for a while on oil spills, which enjoy more media coverage because of the often spectacular visual effects images conveyed to people. There are similarities and differences between inland and offshore crude oil spills. Similarities include hazards to life in all its forms, contamination of valuable freshwater resources from aquifers or desalination plants and an uncertain long-term environmental impact despite unsubstantiated claims that nature fully recovers in a few years.

The aim of this study is the remediation of diesel oil in contaminated water using moringa seed, with the specific objectives to determine: The Lc_{50} of DCW and MSE-treated-DCW exposed to test and its gill histopathology and remediation.

2.0 Materials and Methods

2.1 Research area

The experiment was carried out in the Enugu state university Teaching and Research Farm, Enugu State University of Science and Technology, Agbani Enugu Nigeria. It is located in Nkanu West Local Government Area of Enugu State. The site lies within latitude 07°41North and 08° 21 and longitude 06° 81 East and 07° 61 west. They are marked by a tropical weather of wet and dry seasons. The wet season begins in March and ends in October while the dry season commences in October and ends in February. The mean rainfall ranges from 1600 mm to 1800 mm. The temperature in the dry season ranges from 21 ℃ to 37 ℃ and in the wet season the range is 17 ℃ to 29 ℃. The vegetation of the area is rainforest, characterised by short trees and pockets of wood land and secondary forest consisting of few shrubs and dispersed large trees and climbers.

2.2 Experimental methods

Two hundred juveniles of African catfish (*Clarias gariepinus*) of mean weight 12.6 ±1.4g and mean length 8.6cm were selected and randomly divided into six treatment and 3 replicates, they were held in four plastic containers containing 150 liters of de-chlorinated tap water. Ventilation was provided in order to keep dissolved oxygen contents. Before the study, the fish were adjusted for one week for acclimatization and Diesel oil which was collected from a reputable diesel station at Agbani was introduced into the water, while the moringa seed were gotten from Enugu state university Teaching and Research Farm. It was sorted to remove impurities and sun dried. The dried form were grounded in to powder and then put in a plastic bag to prevent impurities from entering.

2.3.Acute Toxicity Test

The trial test of diesel contaminated water was carried out using 150 litres of water, five different concentrations (0.1, 0.2, 0.4, 0.8, 1.2) the fish were exposed at the trial. They were not fed before 24hours during this trial LC50 was gotten which gives the measurement for the 96hours test treatment (0.00, 0.30, 0.20, 0.15, 0.12, 0.10) Ten fishes were exposed to each treatment per replicate, the fish were examined for morality for 24,48, 72, 96 hours. Mortalities were removed from test solution as soon as possible.

2.4 Gill histology

Gill samples were obtained from the exposed C. *gariepinus* subadults and control, preserved and processed for histological examination using standard histological techniques to determine the effect of Diesel and Diesel + moringa seed extract on the gills at the highest concentration after 96 h exposure.

2.5 Statistical Analysis

All results were collated and analysed using computerized, probit and logit analysis (Lichfielf and Wilcoxon, 1949). The median lethal concentration LC50 at selected period of exposure, and an associated 95% confidence interval for each replicate toxicity test weresubjected to logit and probit analysis (Finney, 1971) using Statistical Package for Social Sciences (SPSS) 15.0 for Windows XP on PC.

3.0 Results

The behaviour of experimental fish exposed to the acute doses of DCW is presented in table 1. The mortality rate of Clarias gariepinus juveniles exposed to varied concentrations of Range finding test RFT in Table 2, the untreated DCW and MSE treated DCW is presented in table 3-4, followed by their probit values in figures 1-3. The histopathoogy of the gills of fish exposed to the untreated DCW

and MSE treated DCW is presented in the highest concentration are displayed in plates 1-4.

Table 1. *The behaviour of experimental fish exposed to acute doses of DCW*

Behavioural Parameters		**Period (h)**			
	Concentration mg/L	24	48	72	96
ESB	0.30	+++	++++	+++	++
	0.20	+++	++	++	+
	0.15	-	++	+	+
	0.12	-	-	+	+
	0.10	-	-	-	+
	0.00	-	-	-	-
FOM	0.30	++	++	+++	++++
	0.20	+	+	++	+++
	0.15	-	+	+	++
	0.12	-	-	+	++
	0.10	-	-	-	+
	0.00	-	-	-	-
HWC	0.30	++	++	+++	++++
	0.20	+	+	++	+++
	0.15	-	+	+	++
	0.12	-	-	+	++
	0.10	-	-	-	+
	0.00	-	-	-	-
GBW	0.30	++	++	+++	++++
	0.20	+	+	++	+++
	0.15	-	+	+	++
	0.12	-	-	+	++
	0.10	-	-	-	+
	0.00	-	-	-	-

Key: -none, + mild, ++ moderate, +++strong, ++++ very strong.
ESB –Erratic swimming behaviour
FOM-Fast opercula movement
HWC-Hanging on water column
GBW- General body weakness

Table 2: Range finding test RFT for DCW for 24hours

S/N	**Concentration**	**12hours**	**24hours**	**Mortality**	**%Mortality**	**Probit**
T_1	0.1	0	1	1	10%	3.72
T_2	0.2	0	2	2	20%	4.16
T_3	0.4	1	2	3	30%	4.48
T_4	0.8	1	2	3	30%	4.48
T_5	1.2	2	3	5	50%	5.0

Table 3: Cumulative Mortality of C.gariepinus Exposed to DCW for 96hours

Q	Concentration (Mg/L)	Log of Concentration	24h	48h	72h	96h	Mortality	Total no of Exposed fish	Total Mortality	% Mortality	Probit
T_1R_1	0.00		-	-	-	-	-	30	-	-	-
T_1R_2	0.00	0	-	-	-	-					
T_1R_3	0.00		-	-	-	-					
T_2R_1	0.30		1	1	2	3	7	30	21	70%	5.62
T_2R_2	0.30	-0.5228	1	1	2	4	8				
T_2R_3	0.30		1	1	2	2	6				
T_3R_1	0.20		1	1	1	3	6	30	19	63.3%	5.33
T_3R_2	0.20	-0.6989	1	1	2	3	7				
T_3R_3	0.20		1	1	2	2	6				
T_4R_1	0.15		1	1	1	2	5	30	16	53.3%	5.08
T_4R_2	0.15	-0.8239	1	1	2	1	5				
T_4R_3	0.15		1	1	2	2	6				
T_5R_1	0.12		0	1	2	1	4	30	15	50%	5.00
T_5R_2	0.12	-0.9208	1	1	1	2	5				
T_5R_3	0.12		1	1	2	2	6				
T_6R_1	0.10		1	1	1	1	4	30	11	36.6%	4.64
T_6R_2	0.10	-1	0	1	1	1	3				
T_6R_3	0.10		1	1	1	1	4				

Table 4: Cumulative Mortality of C.gariepinus Exposed to MSE- Treated-DCW for 96hours

Treatment/ Replicate	Concentration (Mg/L)	24h	48h	72h	96h	Mortality	Totql no of Exposed fish	Total Mortality	% Mortality	Log of Concentration	Probit
T_1R_1	0.00+2mg/lMSE	-	-	-	-	-	30	-	-	-	-
T_1R_2	0.00+2mg/lMSE	-	-	-	-						
T_1R_3	0.00+2mg/lMSE	-	-	-	-						
T_2R_1	0.30+2mg/lMSE	2	1	1	1	5	30	15	50%	-0.5228	5.00
T_2R_2	0.30+2mg/lMSE	2	1	1	1	5					
T_2R_3	0.30+2mg/lMSE	1	2	1	1	5					
T_3R_1	0.20+2mg/lMSE	1	1	1	1	4	30	12	40%	-0.6989	4.75
T_3R_2	0.20+2mg/lMSE	1	1	1	1	4					
T_3R_3	0.20+2mg/lMSE	1	1	1	1	4					
T_4R_1	0.15+2mg/lMSE	1	1	1	0	3	30	9	30%	-0.8239	4.48
T_4R_2	0.15+2mg/lMSE	1	1	1	0	3					
T_4R_3	0.15+2mg/lMSE	1	1	1	0	3					
T_5R_1	0.12+2mg/lMSE	1	1	1	0	3	30	6	20%	-0.9208	4.16
T_5R_2	0.12+2mg/lMSE	1	0	0	0	1					
T_5R_3	0.12+2mg/lMSE	1	1	0	0	2					
T_6R_1	0.10+2mg/lMSE	1	0	0	0	1	30	3	10%	-1	3.72
T_6R_2	0.10+2mg/lMSE	1	0	0	0	1					
T_6R_3	0.10+2mg/lMSE	1	0	0	0	1					

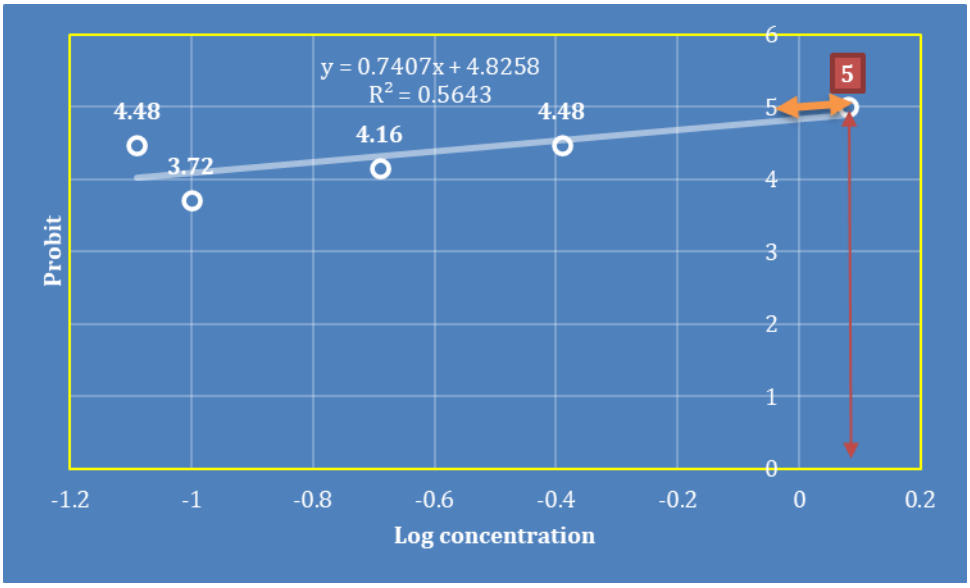

Figure 1: Logarithmic probit RFT line to determine the 24h LC_{50} of DCW exposed to test fish.

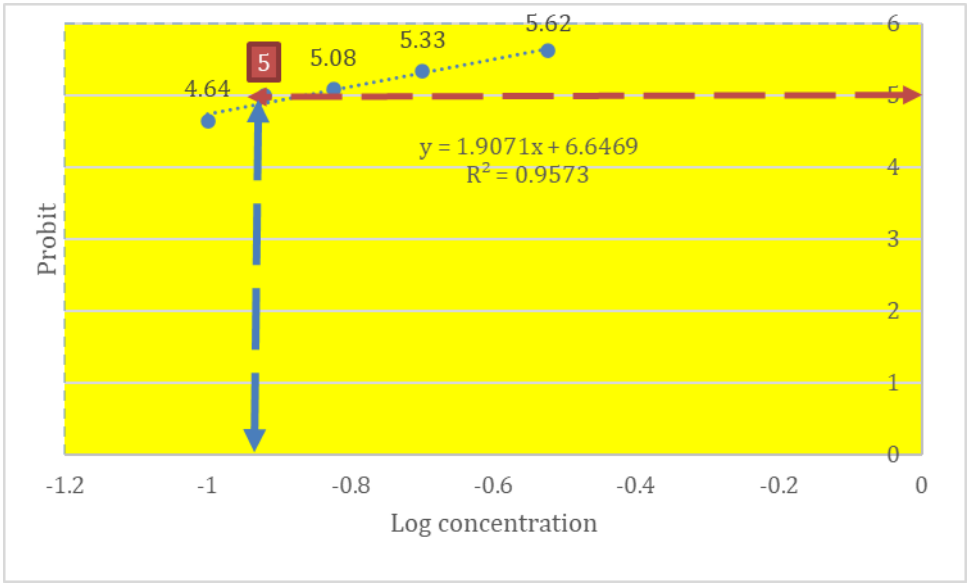

Figure 2: Logarithmic probit line to determine the 96 h LC_{50} of untreated DCW exposed to the fish.

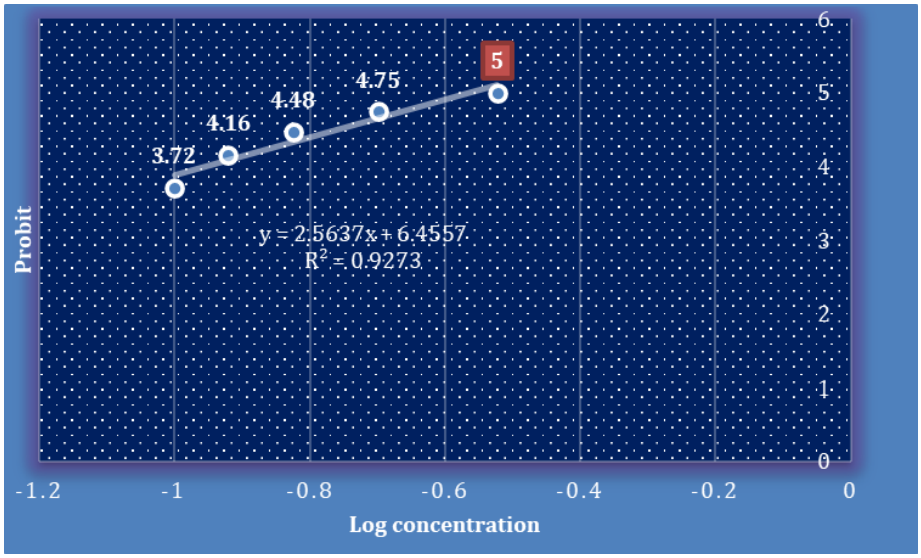

Figure 3: Logarithmic probit line to determine the 96 h LC 50 of MSE- treated -DCW exposed to the fish.

Plate 1: Photomicrograph of control gills showing normal gill filaments

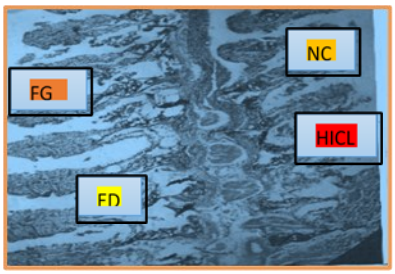

Plate 2: Photomicrograph of gills in 0.3 mgL^{-1}

DCW exposed gills displaying gill filament congestion FG of central nervous ruins of primary lamella with heavy inflammatory cell infiltration HICI. At the secondary lamella, there is enlargement and epithelial degeneration with necrosis.

Plate 3: Photomicrograph of control gills showing normal gill filaments

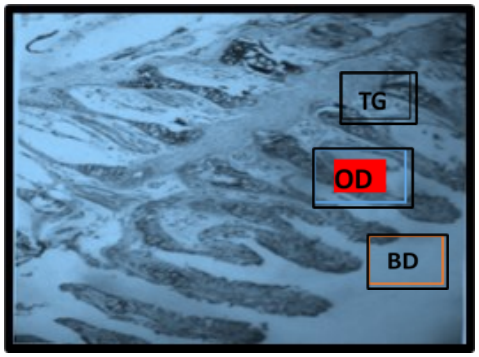

Plate 4: Photomicrograph of gills in 0.3 mgL^{-1}

MSE-treated-DCW exposed gills, demonstrating telangiectasis TG of the secondary lamella base elongation and oedema OD of the secondary lamella (indicated by club-shaped ballooning dilation BD at the apex of the secondary lamella) epithelia with secondary lamella thickening

4.0 Discussion

The mortality rate of diesel showed high mortalities with increased concentrations and 96 h LC_{50} at 0.12mg/l, but when MSE (moringa seed extract) was introduced the LC_{50} was found to be 0.30mg/l. which indicated that the MSE increased the safety level of the diesel contaminated water. The histology of *Clarias gariepinus* (African catfish) gills exposed to diesel oil would likely show various pathological changes due to the toxic effects of the petroleum hydrocarbons present in diesel oil (Eriegha et al., 2019). Diesel oil is known to contain a complex mixture of hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs) which are highly toxic to aquatic organisms, including fish.

It's important to note that the specific histological changes observed in *Clarias gariepinus* gills exposed to diesel oil may vary depending on the duration and concentration of exposure, age, size and species of fish as well as other environmental factors. Histological examination of gill tissues can provide valuable information on the extent of damage and the potential impacts of diesel oil exposure on the health of *Clarias gariepinus* or other fish species. Similar changes were observed by Goncalves et al. (2008) who noted that there was Loss of secondary gill lamellae and lamellae erosion of the primary organs of gaseous exchange which were observed in the gills of affected fish.

The gills showed signs of damage or alteration, such as disin-

tegration, necrosis, or inflammation (Hamed et al., 2021). The gill tissue appeared discolored or irregular, with disrupted cellular structure. There was an accumulation of diesel particles or droplets on the gill surface, obstructing the normal functioning of the gill filaments. The gill tissue showed reduced signs of damage or alteration compared to the gills exposed to diesel alone. The cellular structure appeared more intact, with less disintegration or necrosis. There was a decrease in discoloration and irregularities in the gill tissue. The moringa seed extract had a protective effect on the gill tissue, potentially reducing the negative effects of diesel exposure (Sahabuddin et al., 2023).. Moringa seed extract showed less damage and alterations compared to the gills exposed to diesel alone, suggesting that moringa seed extract has a potential protective effect on the gill tissue against the negative effects of diesel exposure.

5.0 Conclusion

Moringa seed extract (MSE) was used as a phytoremediant to ameliorate the mortality and gill histological damages of the experimental fish, exposed to DCW. Diesel contaminated water should not be permitted into water for test fish except it is ameliorated to a safety level of 0.003 mg/l by application of recommended dose of MSE, in order to safeguard fish species and other aquatic organisms.

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