Effect of diesel oil exposure on behavioral, hemato-biochemical, and morphological changes of erythrocytes and recovery pattern analysis of Nile tilapia *(Oreochromis niloticus)*

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Abstract

Pollution due to petroleum oil by various means is becoming a threat to aquatic ecosystems. Hence, we carried out an experiment to explore how diesel oil affects the behavioral and physiological attributes of fish and how they recover by using Nile tilapia (*Oreochromis niloticus*) as a model organism. The Nile tilapia of 2 different treatment groups were exposed to 0.1 ml/l and 0.5 ml/l diesel oil for 7 days. Then both groups were kept in completely diesel oil free water for 14 days. A control group was maintained throughout the experimental period. We examined the behavioral attributes, hemato-biochemical parameters: hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and glucose (Glu), and morphological changes of erythrocytes after diesel exposure and at the end of recovery phase. Our results revealed that there were abnormalities in behavior and significant changes in Hb, RBC, WBC and glucose levels in both of the treatment groups after 7 days of exposure. Frequencies of erythrocytic cellular abnormalities (ECAs), for example, twin, spindle, elongated, tear drop and erythrocytic nuclear abnormalities (ENAs) like notch nuclei, karyopyknosis, nuclear bud and nuclear bridge were prominent in both groups. However, the anomalies were higher in most if not all the cases in the 0.5 ml/l treatment group. Fish in both groups were quick to recover and the 0.1 ml/l group showed profound recovery than the 0.5 ml/l group. However, in the case of ECAs and ENAs, recovery of the 0.5 ml/l group was insignificant. Hence, our experimental study concluded that the higher the exposure to diesel oil, higher incidences of major health problems are recorded, seriously degrading the healing system of Nile tilapia.

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List of Abbreviations

ANOVA: Analyses of variance

ATP: Adenosine triphosphate

CAT: Catalase

DHW: Deepwater horizon

ECAs: Erythrocytic cellular abnormalities

ENAs: Erythrocytic nuclear abnormalities

Hb: Hemoglobin

HDL: High-density lipoprotein

PAHs: Polycyclic aromatic hydrocarbons

P_{crit}: Critical partial pressure of oxygen

RBC: White blood cell

SD: Standard deviation

TG: Triglyceride

WBC: Red blood cell

WSFs: Water solvent parts

CHAPTER 1

Introduction

1.1 Background:

Petroleum products are considered one of the most prominent toxicants in the aquatic environment (Pacheco and Santos, 2001). Over the past few decades, a significant volume of crude oil has been leaking into water bodies; the DWH explosion in 2010 in the Gulf of Mexico made a major oil spill in the marine environment in the history of the US (Camilli et al., 2012). Oil spills from different sources continued for an extended period following the DWH oil spill on the sea surface (Spier et al., 2013). PAHs toxicity differs in exposed species depending on the substance. Several studies on the effects of crude oil exposure on fish have discovered that PAHs are the most harmful and cardiotoxic to the fish species (Incardona et al., 2014). The quantity of crude oil decaying after spilling varies greatly across accidents, affecting the chemical composition and causing toxicity (Esbaugh et al., 2016). The presence of some compounds in crude oil may make it difficult for impacted individuals and populations to reproduce and survive. Tierney et al. (2013) found that fish may be exposed to oil through their gills, skins, and food, potentially negatively impacting the aquatic food web (Hylland, 2006). Crude oil exposure has been shown to increase death and cause physiological and morphological changes in fish during the developmental phases, including embryonic development. The presence of crude oil in the diet of Siamese fighting fish results in aggressive behavioral changes necessary for reproductive purposes (Naim et al., 2019). Daiani et al. (2015) reported that the alarm substance and swimming velocity of *Colossoma macropomum* reaction were reduced when exposed to crude oil. Much attention has been focused on studying oil exposure in fish during the past two decades (Cherr et al., 2017; Perrichon et al., 2017).

1.2 Problem Statement

Pollution in the aquatic ecosystem has prompted a significant effect due to the incredible expansion of anthropogenic contaminations (van der Oost et al., 2003). Among different types of pollution, petroleum hydrocarbon pollution has become a severe environmental issue because of containing a toxic substance and its ability to stay in the water for a long time being a stable compound. The fundamental sources of petroleum pollution in aquatic environments are oil transport pipelines, capacity tanks, collision of oil-carrying vehicles, and oil shale mining (Abdel-Tawwab., 2012). Toxic compounds in crude oil may cause serious impacts on some species including impairing their ability to persist and reproduce and may lead to acute and chronic toxicity of aquatic animals (Sørhus et al., 2021; Gissi et al., 2021). Crude oil can be found in natural environment in different forms; various evidence shows that it damages the morphology and physiology of fish in different stages and leads to mass mortality (Khan et al., 2005; Simonato et al., 2008). Subsequently, the investigation of oil exposure to fish has received significant consideration in the last few decades, and researchers have observed the long-term consequences of petroleum hydrocarbons on the feeding, growth, behavior, reproduction, and tissue damage of fish (Hedgpeth and Griffitt, 2016; Perrichon et al. 2016, 2017; Cherr et al. 2017; Cox et al. 2017). Change in blood parameters can be used for determining the health status of fish (Sharmin et al. 2016; Islam et al. 2019; Islam et al. 2020; Shahjahan et al. 2021; Li et al. 2021). For instance, hemoglobin (Hb) transports oxygen to diverse tissues in most cases. Hence the Hb concentration of the blood is frequently utilized to determine anomalies and pathological indicators of fish health (Shahjahan et al., 2020; Casanovas et al., 2021). Variations in the WBC count and changes in blood glucose concentration are employed as immunosuppressive biochemical indicators in fish (Kopp et al., 2010; Shahjahan et al., 2018). Morphological changes in blood cells are investigated in order to measure the health status of fish (Shahjahan et al. 2019; Fazio et al., 2020). For example, the formation of a micronucleus in erythrocytes can be an indicator of stress induced by pollution in the environment (Sadiqul et al., 2016; Shahjahan et al., 2019; Ré et al., 2021; Anifowoshe et al. 2021).

The Nile tilapia (*Oreochromis niloticus*) is one of the most significant freshwater fish species for global aquaculture, owing to its hardiness and ability to adapt in captivity (Hohlenwerger et al., 2016; Özdemir et al., 2018). The Nile tilapia is a widely used model organism in the field of ecotoxicology (Nogueira et al., 2011; Rahman et al., 2021**).** Different biochemical reactions have been portrayed in this fish through exposure to lethal compounds (Gold-Bouchot et al. 2006; Rodríguez-Fuentes and Gold-Bouchot 2004; Pereira Trídico et al. 2010). This species is widespread in laboratory studies and a suitable organism for monitoring the effects of xenobiotics (Abdel-Tawwab, 2012). Several authors (Abdel-Tawwab 2012; Nogueira et al. 2011; Freitas et al. 2020) have reported the effect of diesel and different lubricant oils on oxidative stress, histopathological alteration of tissues, growth and biotransformation enzymes. Although Nogueira et al. (2011) reported the biochemical biomarkers related to oxidative stress in Nile tilapia exposed to diesel oil, there is no available information regarding the alterations of behavior, hemato-biochemical parameters, erythrocytic abnormalities, and how it recovers after transferring to contaminated free water.

1.3 Research Objectives and Questions

The objective of this study was to assess the effect of diesel oil exposure on behavior, hemato-biochemical parameters, erythrocytic abnormalities, and recovery patterns in Nile tilapia. To attain these objectives, we set the following main research questions:

a. What is the health condition of Nile tilapia after exposure to 0.1 and 0.5 ml/l of diesel oil?

b. How much Nile tilapia exposed to diesel oil can recover from different body abnormalities after transferring to freshwater?

c. What factors are associated with the different behavioral and physiological abnormalities of Nile tilapia?

To answer the above questions, we collected samples during diesel oil exposure time and after transferring fish into contaminated free freshwater.

1.4 Hypothesis of The Study

In this work, we hypothesized that the high concentration of diesel oil would significantly impact the behavioral pattern and overall hematobiochemical parameters of Nile tilapia. This research also hypothesized that the affected Nile tilapia would recover at the lowest concentration of diesel oil exposure.

1.5 Significance of the Study

This study will provide a broad understanding of how petroleum hydrocarbons affect Nile tilapia body performance. The existing literature investigated how different concentrations of crude oil impacts overall fish health. There is still a lack of knowledge of how PAHs influence the body performance of freshwater fish and the extent to which they are able to recover once transferred to unpolluted fresh water. This research will tell us the patterns of changes in fish behavior, Hb, glucose, RBC, and WBC numbers in Nile tilapia. It also describes how fish would recover their overall behavioral and physiological conditions when exposed to contamination-free water. This work will help establish improved environmental risk assessment models for petroleum hydrocarbons.

1.6 Organization of the Study

This study has been organized and divided into five chapters, investigating the effect of diesel oil on body performance and the recovery of Nile tilapia. The content of each chapter is as follows:

Chapter One: The main segment of this chapter is the background, research objectives, research questions, and hypothesis. The background section of this paper presents an overview of the impacts of petroleum hydrocarbons on aquatic organisms. The problem statement of this investigation overviewed the current issues of PAH's effect on fish that can the studied in this work. The research questions and hypothesis are based on how and to what extent diesel oil affects Nile tilapia and in which concentration its health condition might recover.

Chapter Two: In this chapter of the literature review, we discussed the general information of the last decades of petroleum hydrocarbon's impact on aquatic organisms. It mainly focused on the effects of toxicants on the fish body performances. Some literature gaps are also identified in this chapter.

Chapter Three: This chapter presents the materials and methods of this work, which broadly represent the different techniques employed to carry out this study. In this chapter, we also presented the ethical approval of this work.

Chapter Four: Chapter four presents the results of the study and also includes our data analysis. Some figures and tables of this work have been placed in this chapter.

Chapter Five: This chapter presents a discussion on the study findings and considers these results in comparison with existing literature. This chapter further describes how diesel oil impacts Nile tilapia and how it recovers when it transfers to the diesel-free water. The results of all parameters are compared with the other research findings. This chapter also provides a conclusion based on the research objectives and findings. It suggests further research on a cellular level and a high concentration effect of diesel oil. It also highlighted some limitations of our work.

CHAPTER 2

Literature Review

This chapter reviewed different research and review articles on the impact of PAHs on aquatic organisms over the past two decades.

Several studies found that fish embryos are highly sensitive to exposure to low concentrations of crude oil (Carls et al., 2005). It causes morphological and physiological alterations (Kirby et al., 2019, Laurel et al., 2019, Mager et al., 2018), lipid metabolism aberration (Laurel et al., 2019), cardiotoxicity (Carls et al., 2008), and swimming pattern alteration of fish (Mager et al. 2018). However, very few studies have addressed the effect of dispersed oil droplets on fish embryos toxicity (Carls et al., 2008, Hansen et al., 2019, Olsvik et al., 2011). The survival and feeding behavior of the larvae of Atlantic cod has no significant impact on the oil droplets ((Nordtug et al., 2011) and oil droplets contribution was also insufficient to the embryos of zebrafish (Carls et al., 2008).

To better understand the toxicity mechanism of crude oil in fish visual function, (Magnuson et al., 2020) investigated the behavior, immunohistochemistry, and gene expression of zebrafish after exposure to oil. Previously, zebrafish were employed as model animals to better understand the way of PAH exposure throughout the early life stages of fish (Huang et al., 2013). Studies on eye-associated toxicity from PAH and crude oil exposure led to the selection of certain eye-specific and phototransduction genes (Houbrechts et al., 2016). Vision plays a significant role in teleost larvae foraging behavior and avoiding predators (Job et al., 1996). But the crude oil exposure to fish larvae causes severe issues in the development and dysregulation of the eye and visual processing genes (Xu et al., 2018).

Behavioral response in the study of ecotoxicology is gaining fame due to its ability to correlate with ecological impacts and the physiological function of aquatic organisms (Scott and Sloman, 2004). Different contaminations can reduce the level of fish activity (Wang et al., 2018), enhance fish anxiety (Philibert et al., 2020), and limit the competition ability (Vignet et al., 2014). Contamination of fish populations with weathered or unweathered crude oil might reduce the fish movement levels (Wang et al., 2018), enhance apprehensive behaviors (Philibert et al., 2020), and limit the competition ability within social order (Vignet et al., 2014). Chronic exposure to crude oil during the neurodevelopment of fish can have long-term effects on adult fish and their offspring (Philibert et al., 2019).

The effects of sunlight on oil spills in the marine environment have been known for more than 50 years (Kawahara et.al., 1969. After the Gulf of Mexico's oil spill, the researchers found sea surface oil protoxidized by the effect of sunlight by converting hydrocarbons into photoproducts, which contain oxygen (Overton et al., 1980). Oil photooxidation research continued for two decades and was summarized with several reviews (Hardy et al., 1977; Payne et al., 1985; Nicodem et al., 2021). The study of the photochemical fate of PAHs became more prominent in the early 2000s (Plata et al., 2008).

The models of oil spill dispersion investigate all processes affecting oil hydrocarbons to accurately predict the marine oil fate. However, as recently noted in models of oil spills in a review, photooxidation is almost always absent in real-world scenarios (Keramea et al., 2021). A Lagrangian model on the basis of tracking irradiance of sea surface oil was recently used for the DWH oil spill (Vaz et al., 2021). Despite its simplicity, this model appears to have potential in the modeling of oil spill fields. The toxicity of oil was also studied recently in copepod and algae assays (Faksness et al., 2020). At the same time, Faksness et al (2020) found that evaporated samples were less toxic than the photo-oxidized oil.

Reactions of marine organisms to petroleum hydrocarbons range from attraction to avoidance, based on chemical components and their concentration. Kerosene is also used for the purpose of attraction and nourishment of American lobster, wherein some places, kerosene-dripping bricks are used as bait (Atema et al., 1976). Toxic polycyclic aromatic hydrocarbons, which are the principal toxic components of crude oil, are not a concern for many marine flatfish and sciaenid species (Hinkle-Conn et al., 1998). There is evidence that certain chemicals in crude oil can influence the developmental habits of fish. Crude oil is referred to as a complicated chemical combination made up of thousands of components, and weathering occurs when low molecular weight hydrocarbons evaporate, leaving high molecular weight hydrocarbons less solubilized (Carls et al., 2019). Recent research found that three estuarine fish species, i.e., sailfin mollies, Gulf killifish, and sheepshead minnows, avoid medium and high concentrations of fresh oil mixed with sediment, but no major avoidance behavior was found in weathered crude oil (Martin et al., 2017). Several studies on the behavioral reactions investigation to crude oil showed avoidance in pink salmon, Caspian roach, and European seabass (Claireaux et al., 2018). Moreover, Caspian roach has been shown to no more extended avoidance of crude oil when their nares have been surgically occluded (Lari et al., 2015).

Polluted water by exposure to different toxic chemicals can cause an alteration of the morphological pattern of different organs in fish (Monteiro et al., 2005). The alteration of behavioral, biochemical, and hematological parameters is used as an indicator of fish health (Pimpão et al. 2007). Different physical and chemical changes in fish bodies because of exposure to the toxic chemical are indicated by the examination of blood parameters (Ambali et al. 2011). Anomalies and changes in the morphology of erythrocytes are considered essential bioindicators of oxidative stress in the fish body (Islam et al., 2019). Aside from this, micronuclei formation can occur by the effect of toxic chemicals in fish, and it is widely regarded as the most accurate biomarker for genetic modification (Sadiqul et al., 2016).

Karem et al. (2022) studied the effect of crude oil on embryonic metabolic response in zebrafish. They found that crude oil toxicity during the embryonic development increased the routine oxygen consumption and decreased the body mass and fish survival. These findings are supportive of the toxicological information for the red drum larvae exposed to the crude oil (Khursigara et al., 2017) and zebrafish larvae exposed to three different PAHs in oiled sediments (Vignet et al., 2014). The hatching time was the most susceptible to crude oil impacts on the mahi-mahi species (Mager et al., 2017). In Bay anchovy, the most significant harmful consequences of oil exposure happened during the embryonic and hatching stages (O'Shaughnessy et al., 2018). Arabian light fuel oil had no fatal impact on zebrafish embryos after exposure for 96-hours (Perrichon et al., 2016). Crude oil impacts the zebrafish's early life stages occurred mainly between the 3 and 6 days of exposure, which are the essential cardiovascular developmental stages of this fish species (Burggren et al., 2017). Thus, many other organs might be affected by oil exposure due to the disturbance cardiovascular system. Oil exposure during hatching may contribute to metabolism-boosting activities. The lack of chorion as a protective barrier after hatching may also promote higher routine oxygen at different developmental stages of fish (Henn and Braunbeck, 2011). For example, swimming performance in mahi-mahi and zebrafish early life stages show severe physiological impairments during crude oil exposure (Mager et al., 2014). For larvae, during the three days of post-fertilization of fish, the existence of crude oil may alter the developmental trajectory of normal oxygen consumption (Karem et al. 2022).

Fish growth is an endpoint significantly affected by the different stressors in numerous physiological ways (Pasparakis et al., 2019). Crude oil exposure to fish during developmental stages often shows a body mass loss. Pink salmon embryos dramatically decreased their body mass and growth after exposure to the crude oil for 200 days (Heintz et al., 2000). Exposed fish on crude oil would lose their weight by around 50% more than unexposed fish if the relative growth rate disparity remained until fish matured. Rainbow trout larvae reduced body mass after exposure to the crude oil (Vosyliene et al., 2005). Exposure of arctic cod larvae to crude oil from DHW also reduced their growth (Nahrgang et al., 2016). The significant impact found on the three days of post-fertilization of zebrafish indicates that this period is the most crucial window for fish body mass and growth (Karem et al., 2022). Less body mass in larval zebrafish confirms prior results and extends them by 'mapping' crucial developmental windows for growth and body mass in embryonic developmental stages.

The Pcrit has long been used as a measurable indicator of hypoxia tolerance (Rogers et al., 2016, Burggren et al., 2019). Furthermore, P_{crit} is greater in larvae than adults in zebrafish, indicating a reduced ability to absorb oxygen in hypoxic conditions throughout the early life stages (Mandic et al., 2020). Even while early larval zebrafish cannot sustain normoxic levels of oxygen uptake in hypoxia, this does not mean they do not try to control oxygen intake in hypoxia. Zebrafish establish a ventilatory response of hypoxic on day four post-fertilization. This key defensive mechanism helps postpone the harmful effects of aquatic hypoxia on aerobic respiration (Pan et al., 2019). Even while regular oxygen intake varied roughly two-fold in the same fish, early embryonic life stages lack a tight connection of the numerous parameters impacting hypoxia tolerance. However, Karem et al. (2022) found P_{crit} did not affect after exposure to the crude oil in larval zebrafish. P_{crit} showed that hypoxia tolerance was not changed by 3–6 days of post-fertilization, but survival, body mass, or oxygen consumption changed by the crude oil exposure. This finding was supported by research on Atlantic croaker treated for 24 hours with crude oil at varied doses exhibited no variation in P_{crit} (Pan et al., 2018). Similarly, a red drum subjected to crude oil for 24 hours followed by hypoxia tolerance testing revealed no effect (Ackerly and Esbaugh, 2020). However, oil-exposed sole sub-adults demonstrated lower hypoxia tolerance, as shown by a 66% rise in P_{crit} (Davoodi et al., 2007). Further research on the impact of crude oil on P_{crit} in fishes of all ages is required.

Ireen et al. (2022) investigated cod polar fitness by exposing the different concentrations of crude oil, where they found crude oil intake with diet had no effect on the somatic growth index of polar codfish. It happened due to the use of a very low concentration of crude oil in the experiment, although it negatively affects the cellular levels (Bender et al., 2016). This finding is different from the previous investigation on other arctic codfish, where exposed fish had a lower growth after exposure to the crude oil (Nahrgang et al., 2019). However, dietary crude oil exposure studies in some other fishes have shown that PAH compounds have harmful impacts on the development of fish (Meador et al., 2006; Vignet et al., 2014).

The mortality of polar codfish was not connected to crude oil treatment but the inherent biological features of the experimental fish. Bender et al. (2018) reported increasing mortality of fish is around 35% in adult polar cod exposed to crude oil from June 2015 to January 2016. More mortality was observed in the early spring because polar codfish spawn and reduced their hepato-somatic index, and become more susceptible to death (Nahrgang et al., 2019). Parasites in arctic cod are responsible for mortality, regardless of crude oil exposure (Ireen et al., 2022). Parasite prevalence is higher (Simková et al., 2008), perhaps due to the immune response of parasites and energy changes during gonadal development (Sheldon and Verhulst, 1996). In fish, the reproductive process suppresses the immune system, allowing parasite infection and ultimately becoming a cause of death (Simková et al., 2008). According to the Bender et al. (2016) findings, polar cod were actively developing their gonads from July to January, which may have contributed to a greater parasite infection rate. Future research should include the prevalence of parasites as a determinant in the wild fish population's survival.

Blood parameters changes and high metabolic activity have been observed observed in the polar codfish due to the crude oil exposure (Vieweg et al., 2018; Nahrgang et al., 2019). The rise in diacylglycerol in the liver of exposed arctic codfish confirms the mobilization of energy stores (Carrasco and Mérida, 2007). Diacylglycerol is responsible for enhancing the phospholipid synthesis of polar codfish after crude oil exposure as it acts as a precursor for the synthesis of phospholipids (Carrasco and Mérida, 2007). Phospholipids are essential for fish metabolic purposes (Tocher et al., 2008).

The oxidation of heme iron causes hemolytic anemia to ferric state in animals exposed to crude oil (Couillard and Leighton 1993). Hypoxia and animal mortality can happen due to the production of methemoglobin after crude oil exposure, even at low concentrations (Couillard and Leighton 1993). Acute exposure to PAHs may induce brain necrosis, neuronal cell death, and failure of the heart of fish (Barron et al. 2004). In hepatocytes, PAH detoxification and metabolism may produce more harmful metabolites than the original chemical, causing severe damage to the fish (Malakahmad et al., 2016). Because of the lipophilic properties of PHAs, they may accumulate in the lipid of the cell membrane. Thus, PAHs may damage the cell membrane's strength and permeability (Sinaei 2013). Sayed et al. (2018) observed that exposure to 4-Nonylphenol can enhance nuclear abnormalities, erythrocyte morphological changes, and hemolysis of medaka fish.

The liver is engaged in vital functions such as protein and cholesterol synthesis, drug metabolism, bile generation, and blood chemical management (Derikvandy et al., 2020). Histopathological damage in the liver of Mrigal carp subjected to diesel oil has previously been reported (Hameed and Al-Azawi 2016). Thus, monitoring major liver enzyme activity in blood may be a valuable liver health bio-indicator.

The harmful effects of petroleum chemicals on hepatocytes may be responsible for reducing albumin, total proteins, and globulins. Lipophilic substances can permeate the cell membrane and disrupt the operation of mitochondria, endoplasmic reticulum, golgi apparatus, and lysosomes (Gu and Manautou 2012). Thus, total protein can be reduced due to the alteration of the golgi apparatus and endoplasmic reticulum in wastewater-exposed fish hepatocytes. Furthermore, reduced protein synthesis in hepatocytes may be owing to poor intestinal amino acid absorption and hepatic amino acid assimilation (Banaee et al. 2008).

Kidney disease may also lead to blood protein and amino acid deficiency (Rezaei et al., 2018). Moreover, increased proteolysis, renal and liver disease, and failure of protein synthesis might diminish blood protein levels (Hamed et al., 2021).

Fish exposed to petroleum effluent may have an altered balance of energy. Lipid metabolism disorder can decrease the TG levels of fish plasma due to exposure to petroleum wastewater (Alves et al., 2018). Baum et al. (2016) reported that coral reef fish's metabolic rate reduced after exposure to the PAHs.

Moreover, Mu et al. (2018) discovered that TG decrease in the embryos of zebrafish is linked to TG biosynthesis enzyme gene expression suppression. To offset the harmful effects of xenobiotics, glycogen breakdown may increase in fish exposed to petroleum effluent (Thiendedsakul et al., 2020). Glucose levels increased in tambaqui (Duarte et al. 2010) and perch fish (Peter et al. 2007) after exposure to crude oil and kerosene. The increased blood glucose level causes renal and kidney damage in affected fish (Hatami et al., 2019).

Fish exposed to PAHs effluent had higher overall cholesterol (Sakineh et al., 2021). The rise in cholesterol may be attributed to reduced HDL production and poor biliary cholesterol excretion

(Lavoie et al., 2016). After gasoline exposure, plasma and total cholesterol levels increased significantly in small terrestrial animals (Aberare et al., 2011). In fish, elevated cholesterol levels in the blood can be linked to poor metabolism of lipid from oil effluent exposure. The metabolic reaction to increased energy demand caused by effluent exposure might cause a rise in cholesterol (Sayed et al., 2017).

Alexis et al. (2021) studied that ambient oil exposure increases the risk of predation of the red drum fingerlings. In recent research, anxiety-like behavior in the different life stages of the red drum was tested (Rowsey et al., 2020). The hyperactivity in the fingerlings of red drum is comparable to the zebrafish larvae (Knecht et al., 2017) and gulf killifish (Brown et al., 2016) exposed to PAH. Ingenuity Pathway Analysis of transcriptome, which provides information and interpretation on “omics” data, also identified hyperactivity as a possible impact in red drum embryos after oil exposure (Xu et al., 2017). It is also unknown whether a similar route is active in the life stages of juveniles. Yet the data shows that oil exposure causes a behavioral change in fish that is characterized by hyperactivity (Xu et al., 2017), decreased anxiety (Rowsey et al., 2022), and lower sociability (Armstrong et al., 2019). These findings show that cardiorespiratory dysfunction causes ecological implications (nutrient cycles) for the endemic fish. Alexis et al. (2021) also investigated red drum behavioral impacts, and cognitive processes correspond better with downstream ecological threats, as assessed by predator avoidance. Some research on red drums embryos exposed to crude oil showed different craniofacial abnormalities and lowered cardiac output (Khursigara et al., 2017). According to transcriptomics, which is used to understand mRNA's expression pattern, six of the top ten most disturbed pathways in larval red drums were connected to neurological and cognitive function (Xu et al., 2017). Crude oil impacts on mahi-

mahi to reduce the oil avoidance behavior shows that behavioral changes may be influenced by cognitive and neurological functions, which are key contributors to sublethal damages in fish after the oil exposure. Recent research shows that oil and PAH exposure affect fish olfaction and eyesight, potentially increasing predation. When exposed to oil, bicolor damselfish displayed reduced chemical alarm cue avoidance behaviors and a decreased chance of detecting a chemical alarm cue (Schlenker et al., 2019). Despite being able to detect oil, mahi-mahi that was acutely exposed to it showed less oil avoidance behavior than the control fish, indicating a problem in the central nervous system (Schlenker et al., 2019). The lens size of cunner, dourado, and red drum reduced after exposure to the crude oil for six months (Payne et al., 1978). However, most studies indicate decreased vision during oil exposure in the early life stages of fish, which alters the normal eye development.

Ada et al. (2021) studied the exposure of chemically disparate oil on zebrafish and found it has excellent oxidative stress in zebrafish, consistent with some previous research (Luch et al., 2015; Shi et al., 2015; Timme-Laragy et al., 2009). CAT activity increased in fish gills and reduced liver, which shows that gills are the main detoxifying organ for fish. A prior study (Sun et al., 2006) suggested that significant oxidative damage may have suppressed or impaired CAT induction in the liver. Overall, multiple findings suggest CAT may have a secondary function in enzymatic oxidative stress defense (Oliveira et al., 2008). Considering the inconsistent results following exposure to oil PAHs, catalase is regarded as a less stable biomarker. Milinkovitch et al. (2011) found that crude oil, which is chemically disrupted, has no significant alterations in catalase activities in fish. All these studies show how the response of antioxidants is influenced by the fish organ and the exposure circumstances to crude oil components.

The micronucleus was used to examine the genotoxic effect of pollutants by detecting DNA damage (Juan et al., 2021). Genotoxicity is a specific endpoint in investigating the toxicity of crude oil, and it may be studied at different levels of biological structure (Abdel-Massih et al., 2013). The micronucleus test detects chromosomal abnormalities in DNA (Bolognesi et al., 2011). Ada et al. (2021) observed crude oil exposure could enhance the micronuclei formation. Other studies revealed many micronuclei formation at comparable exposure dosages (Olivares-Rubio et al., 2020). Due to the high molecular weight of PAHs, it causes genotoxicity through biotransformation. Comparing several oil exposure experiments is challenging due to the great diversity of exposure settings and oil kinds. Acute exposure to zebrafish liver also produced more micronuclei (Johann et al., 2020). However, in situ micronucleus assays lack toxicokinetic and may be used to assess the ecotoxicological hazard of crude oil.

CHAPTER 3

Materials And Methods

3.1 Experimental fish

Healthy total two-hundred Nile tilapia fingerlings (mean length; 8.0 ± 0.5 cm and average body weight; 6.0 ± 0.2 g) (Figure 1) were collected from Bangladesh Fisheries Research Institute, Mymensingh. The collected fish was acclimatized in the laboratory for 15 days in natural photo-regime conditions into clean glass aquaria containing 30L of water. Commercial fish feed (Mega fish feed, Bangladesh) was fed by 5% of the total body weight twice a day, around 9.00 AM and 5.00 PM, during the acclimatization period. The experimental procedure and fish used in the experiment have been approved by the Animal Welfare and Ethical Committee, Bangladesh Agricultural University, Mymensingh.

The systematic position of Nile tilapia is given as follows:

Phylum: Chordata
Class: Actinopterygii
Order: Cichliformes
Family: Cichlidae
Genus: *Oreochromis*
Species: *Oreochromis niloticus*

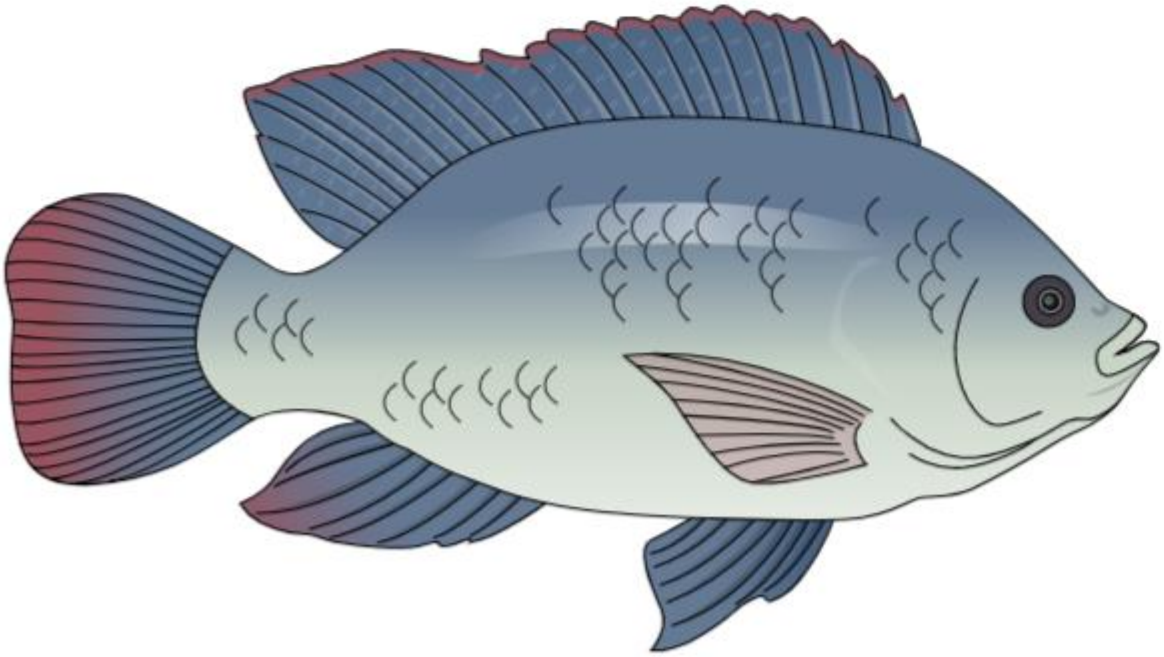


Figure 1 Test fish species *Oreochromis niloticus*

3.2 Test chemical

The diesel oil was collected from a local market in Mymensingh, Bangladesh.

3.3 Exposure assessment

The one-hundred twenty Nile tilapia fingerlings were exposed to three different concentrations (0.0, 0.1, and 0.5 ml/L) of diesel oil for 7 days. The doses were taken on the basis of a study on tilapia by Nogueira et al. (2011). Water was exchanged every 24 h, and desired concentrations of diesel oil were added accordingly. At the end of 7 days of exposure, eight fish (n = 8) were sacrificed to collect blood samples for further analysis from each concentration.

3.4 Recovery assessment

After 7 days of exposure to diesel oil, the rest of the fish was reared for the next 14 days without diesel oil to monitor the recovery assessment. The water was changed daily, and the fish were fed twice in a day. At the end of 14 days post exposures, eight fish (n = 8) were sacrificed from each concentration.

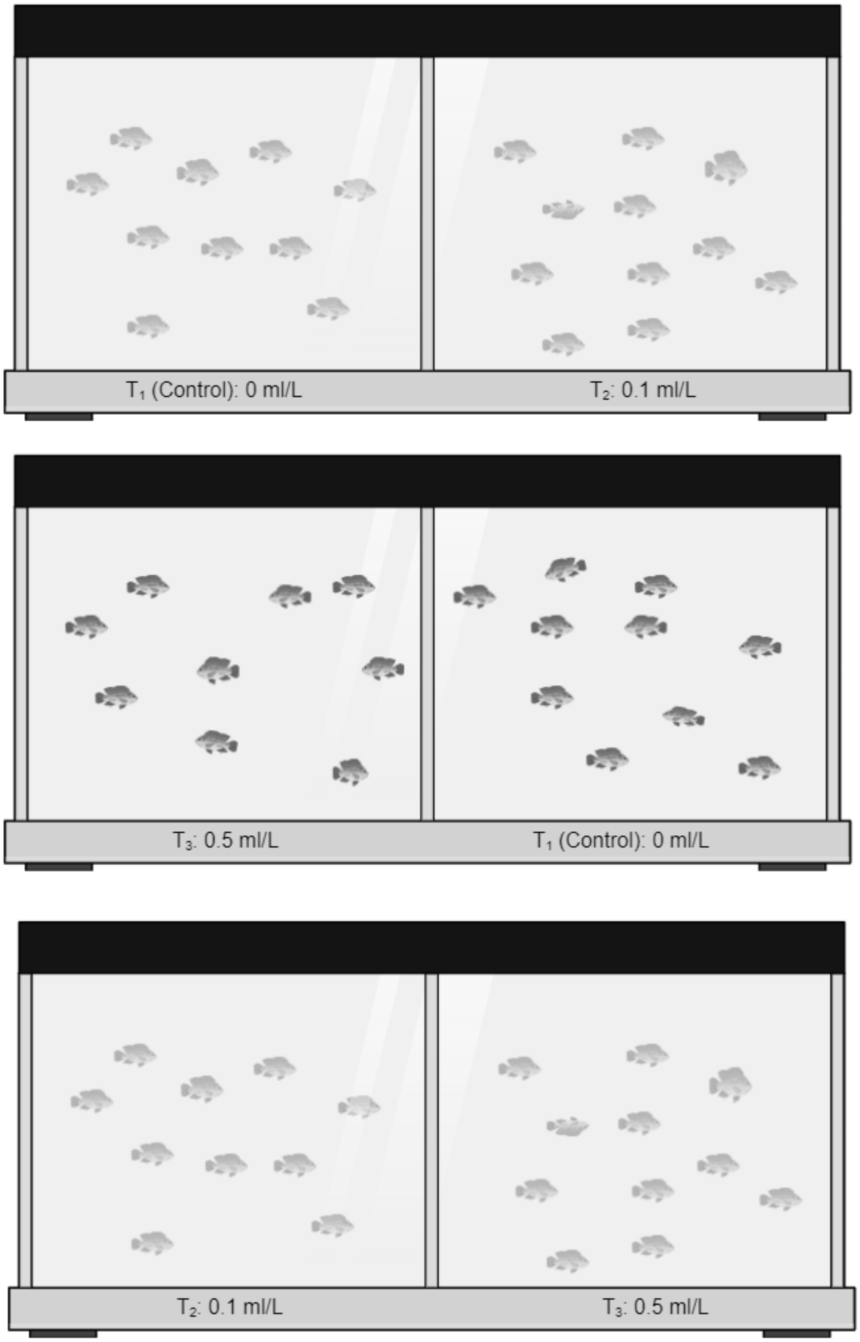


Figure 2 Aquaria with different doses of diesel oil

3.5 Behavioral analysis

The fish were observed carefully from the outside to understand their behavioral pattern in all the aquaria during exposure and post-exposure. Their behavioral activities (feeding rate, gulping, gill motion, rate of locomotion) were recorded following the previous studies (Khatun et al., 2021). The findings were presented as severe abnormalities (>50%), moderate abnormalities (10-50%), mild abnormalities (<10%), and no abnormalities (0%).

3.6 Measurement of haemato-biochemical parameters

On every sampling day, fish were anesthetized through 5 ml/L of clove oil immediately after collecting from each aquarium. Blood was sampled from the caudal peduncle of each fish and kept immediately in an autoclaved centrifuge tube having 20 mM EDTA anticoagulant. The blood glucose level (mg/dL) and hemoglobin (Hb) content of fish were measured immediately after the collection of blood from the fish. The glucose and Hb were determined using a blood glucose meter and Hb strip through a digital EasyMate® GHb (Model-ET, 232) monitoring system. Furthermore, a Neubauer hemocytometer was used to count the RBC and WBC number under a light microscope according to standard procedure.

3.7 Analysis of morphological changes of erythrocytes

A detailed procedure for analyzing the morphological changes of erythrocytes was described by Shahjahan et al. (2019) and Jahan et al. (2019). In brief, the blood sample was smeared onto a clean microscopic slide immediately after the blood collection. The blood smear was then fixed by methanol after air-drying for 10 minutes, and finally, 5% Giesma solution was used for staining.

The slides were kept for air-dried after being rinsed with tap water. The slides were subsequently mounted with dibutylphthalate polystyrene xylene (DPX) and preserved at room temperature. An Electronic microscope (MCX100, Micros, Austria) was used to investigate the cellular and nuclear abnormalities of erythrocytes. From each of the slides, at least 2000 cells with intact cellular and nuclear membrane were counted. Erythrocytic cellular abnormalities can be categorized according to their condition. For instance, twin: where two cells are joined by the outer membrane. Spindle shape: the cell has two pointy ends and is somewhat rounded in the middle. Elongated cell: the lengths are unusual compared to the normal cell. Tear drop: slightly rounded or blunted shape. Different erythrocytic nuclear abnormalities (ENAs) at different sampling points were categorized according to Carrasco et al. (1990). For instance, cells without nuclear material having no nuclear shape were designated as notched nuclei, cells with condensed and clumped chromosomal substances with unusual membranes in the nucleus were considered as karyopyknosis, nuclei having evaginations like a bud were defined as nuclear bud, and thin filaments connecting individual nuclei were classified as a nuclear bridge.

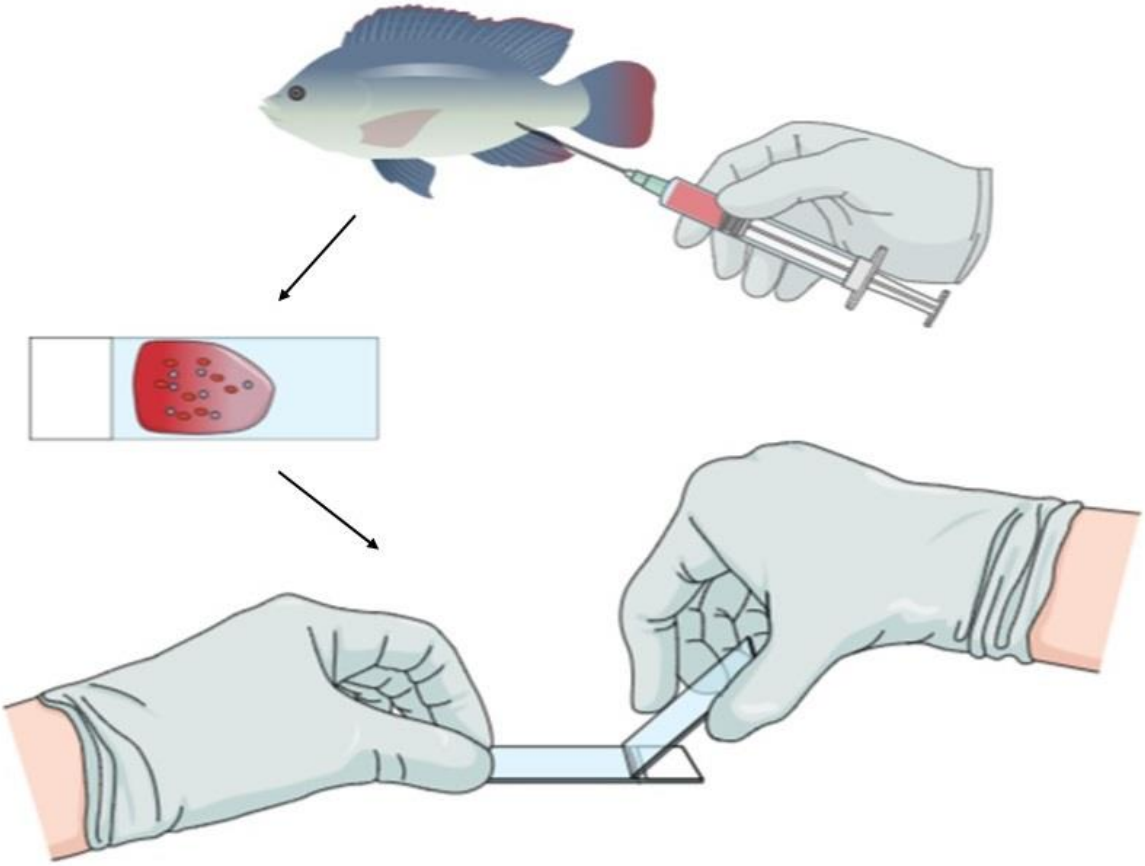


Figure 3 Sample blood smearing procedure

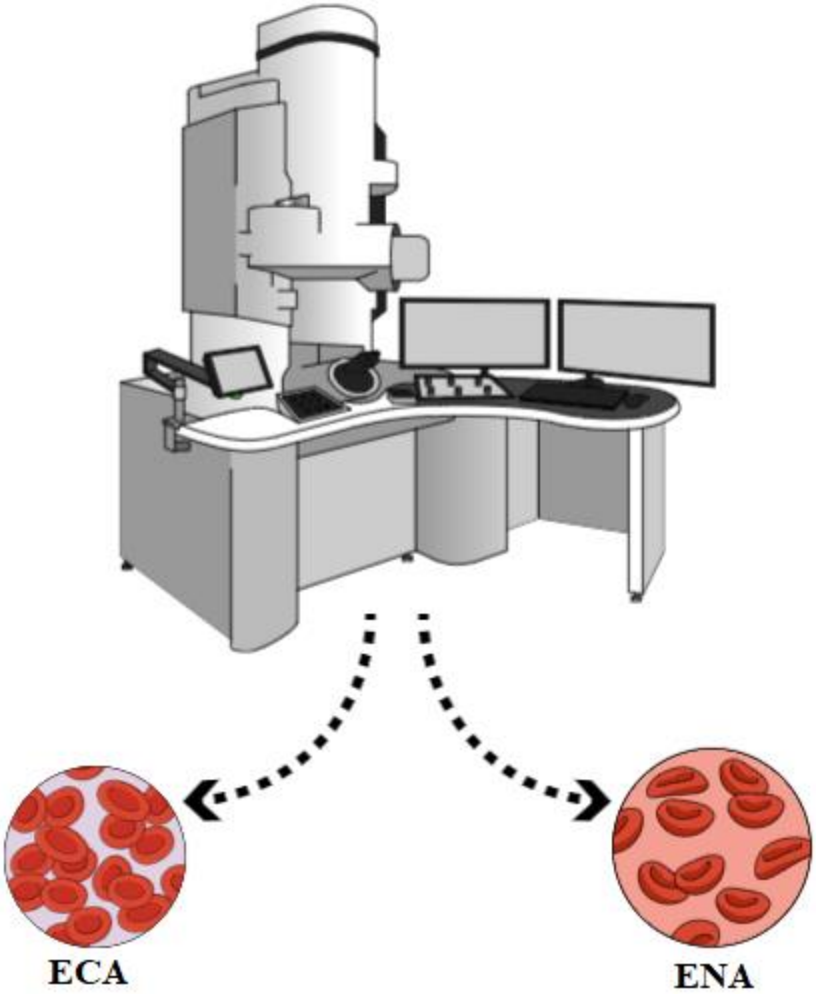


Figure 4 Frequencies of erythrocyte abnormalities

3.8 Data analysis

Values of all the measured variables were presented as mean ± standard deviation of the mean (SD). One-way analyses of variance (ANOVA) were performed using a post hoc Tukey's test to evaluate the statistically significant difference between the sampling points. To evaluate the significant difference among different concentrations of diesel oil, the Mann-Whitney U test and Bonferroni corrections were used. Here the treatment groups were the independent variable, and different hematological parameters and frequencies of ECAs and ENAs were the dependent variables. Statistical analyses were performed with PASW statistics 18.0 software (IBM SPSS statistics, Chicago, USA) where $p < 0.05$ was set as the statistical significance value.

3.9 Ethics Approval

The total procedure conducted in this experiment was approved by the Animal Care and Use committee of Bangladesh Agricultural University, Mymensingh (Approval Number: BAU-FoF/2020/004).

CHAPTER 4

Results

4.1 Behavioral changes after exposure to diesel oil

During the experimental period, no behavioral alterations were observed in the control group. Gulping and gill motion showed severe abnormalities (> 50%) at a higher concentration (0.5 ml/l) of diesel oil. Moderate abnormalities (10-50%) of locomotion and feeding rate were detected at 0.1 ml/l of diesel oil exposure (Table 1).

Table 1 Behavioral changes of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days

Parameters	Diesel oil (ml/L)	Exposure (7 days)	Recovery (14 days)
Feeding rate	0.0	−	−
	0.1	++	+
	0.5	++	++
Gulping	0.0	−	−
	0.1	++	+
	0.5	+++	++
Gill motion	0.0	−	−
	0.1	++	−
	0.5	+++	++
Rate of locomotion	0.0	−	−
	0.1	+	+
	0.5	++	+

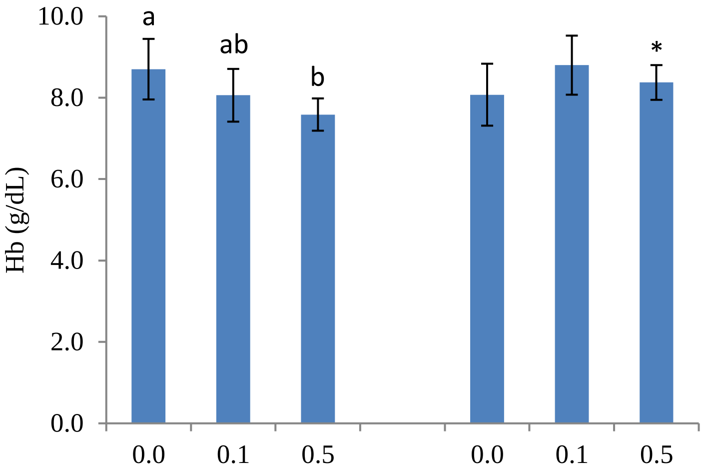
−, None (0%); +, mild (<10%); ++, moderate (10-50%); +++, severe (>50%)

4.2 Recovery of behavioral changes

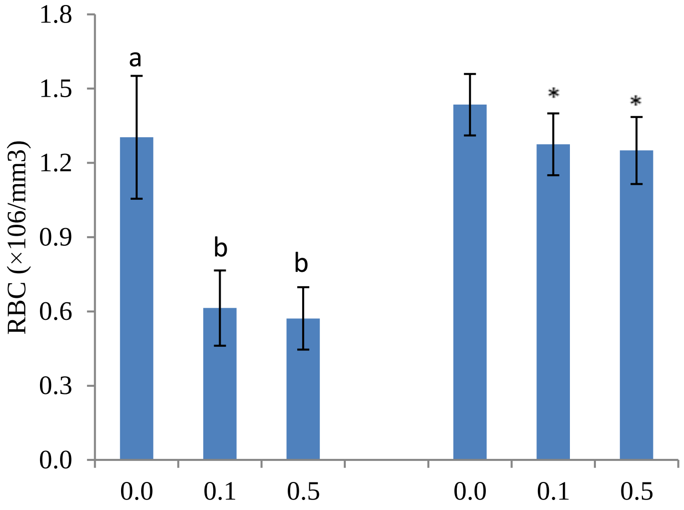
Feeding rate, gulping, and rate of locomotion have shown mild abnormalities during the recovery period exposed to a lower dose of diesel oil. On the other hand, moderate abnormalities of feeding rate and gill motion were found in exposure to the higher concentration of diesel oil (Table 1).

4.3 Changes in hemato-biochemical parameters after exposure to diesel oil

Levels of different hemato-biochemical parameters (Hb, RBCs, WBCs, and glucose) of both control and different treatment groups were recorded after 7 days of diesel exposure (Figure 5). In the case of hemoglobin level (g/dl), fish exposed to 0.1 ml/l diesel showed a slight downfall compared to those of the control group, while the hemoglobin level of fish exposed to 0.5 ml/l diesel dropped significantly ($p < 0.05$). The RBC numbers ($\times 10^6/mm^3$) dwindled dramatically ($p < 0.05$) in both 0.1 ml/l and 0.5 ml/l treatment group as well. On the contrary, the WBC number ($\times 10^6/mm^3$) displayed an opposite scenario and escalated significantly. When it comes to glucose level (mg/dl), the 0.1 ml/l treatment group showed a small increase, whereas fish exposed to 0.5 ml/l diesel exhibited a significant upsurge ($p < 0.05$).



Hb (g/dL)
10.0
8.0
6.0
4.0
2.0
0.0
a
ab
b
*
0.0
0.1
0.5
0.0
0.1
0.5



RBC (×106/mm3)
1.8
1.5
1.2
0.9
0.6
0.3
0.0
a
b
b
*
*
0.0
0.1
0.5
0.0
0.1
0.5

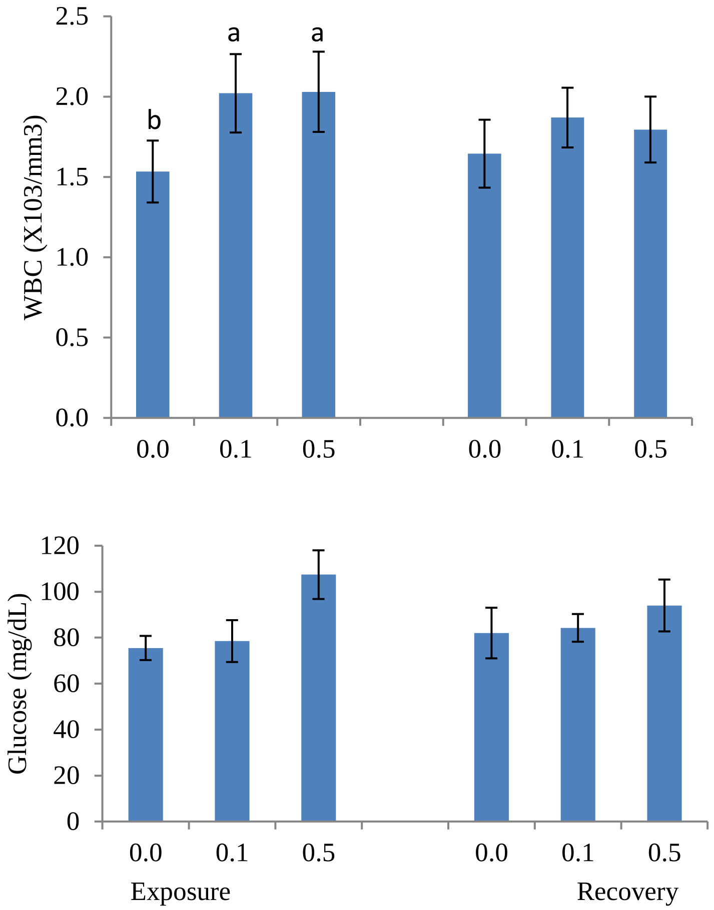


Figure 5 Changes in hemato-biochemical parameters of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days; a. Hb; b. RBC; c. WBC; and d. glucose. Values with different alphabetical superscripts are significantly ($p < 0.05$) different. Asterisk (*) indicates the significant difference between exposure and recovery. All values are expressed as mean ± SD.

4.4 Recovery of hemato-biochemical parameters

Recovery of Hb, RBCs, WBCs, and glucose levels were measured after 14 days from the day of last diesel exposure (Figure 5). The control group demonstrated stability in all the different parameters except for glucose level, which was found to be increased significantly ($p < 0.01$). Hemoglobin level (g/dl) went up significantly and showed close resemblance with the control group in both of the treatment groups after 14 days of the recovery period. The same was the case for RBC numbers ($\times 10^6/\text{mm}^3$), where statistically noteworthy acceleration was noticed ($p < 0.01$). However, there were no major variations found in the WBC numbers ($\times 10^6/\text{mm}^3$) and glucose level (mg/dl) of both the recovery group.

4.5 Aberrations of erythrocytes at different concentrations of diesel oil exposure

Erythrocytic cellular abnormalities (ECAs) were detected in a different group of fish treated with diesel. Twin, spindle, elongated, tear drop (Figure 6) abnormalities were spotted. Frequencies of ECAs went up significantly ($p < 0.05$) in both of the treatment groups exposed to diesel in comparison with those of the control group (Table 2). In a similar fashion, erythrocytic nuclear abnormalities (ENAs) were also found to be significantly higher ($p < 0.05$) in the two treatment groups (Figure 7). An ample amount of notch nuclei, micronucleus, nuclei degeneration, nucleus, and nuclear bridge were present in both of the treatment groups (Table 3). However, though both the treatment group (0.1 ml/l and 0.5 ml/l diesel exposure) indicated a significant escalation of ECAs and ENAs, the aberrations between the two treatment groups of diesel were hardly noticeable.

Table 2 Erythrocytic cellular abnormalities (ECAs) of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days

ECA	Diesel oil (ml/L)	Exposure (7 days)	Recovery (14 days)
Twin	0.0	0.47 ± 0.02^{a}	0.40 ± 0.08
	0.1	0.53 ± 0.07^{a}	0.34 ± 0.18
	0.5	1.04 ± 0.11^{b}	0.64 ± 0.13*
Spindle	0.0	0.10 ± 0.13^{a}	0.09 ± 0.02^{a}
	0.1	0.15 ± 0.18^{ab}	0.11 ± 0.05^{a}
	0.5	0.23 ± 0.17^{b}	0.18 ± 0.09^{b}
Elongated	0.0	0.20 ± 0.08^{a}	0.18 ± 0.03^{a}
	0.1	0.46 ± 0.11^{b}	0.24 ± 0.14^{ab,*}
	0.5	0.47 ± 0.14^{b}	0.42 ± 0.19^{b}
Tear-drop	0.0	0.13 ± 0.04^{a}	0.14 ± 0.09^{a}
	0.1	0.91 ± 0.07^{b}	0.27 ± 0.15^{ab,*}
	0.5	1.15 ± 0.12^{b}	0.91 ± 0.19^{b}

Values of a single cellular abnormalities in a column with different alphabetical superscripts are significantly ($p < 0.05$) different. Asterisk (*) indicates the significant difference between exposure and recovery in the row. All values are expressed as mean ± SD. Three slides were prepared from each fish and 2000 cells were scored from each slide and at least three fishes were analyzed from each group.

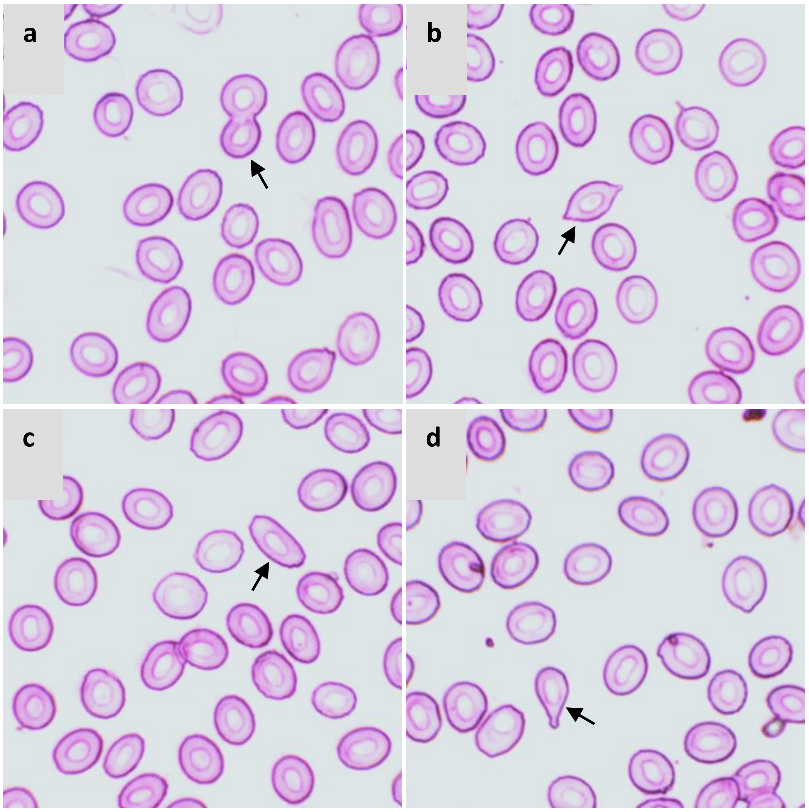


Figure 6 Erythrocytic cellular abnormalities (ECAs) of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days; a. twin, b. spindle, c. elongated and d. tear drop.

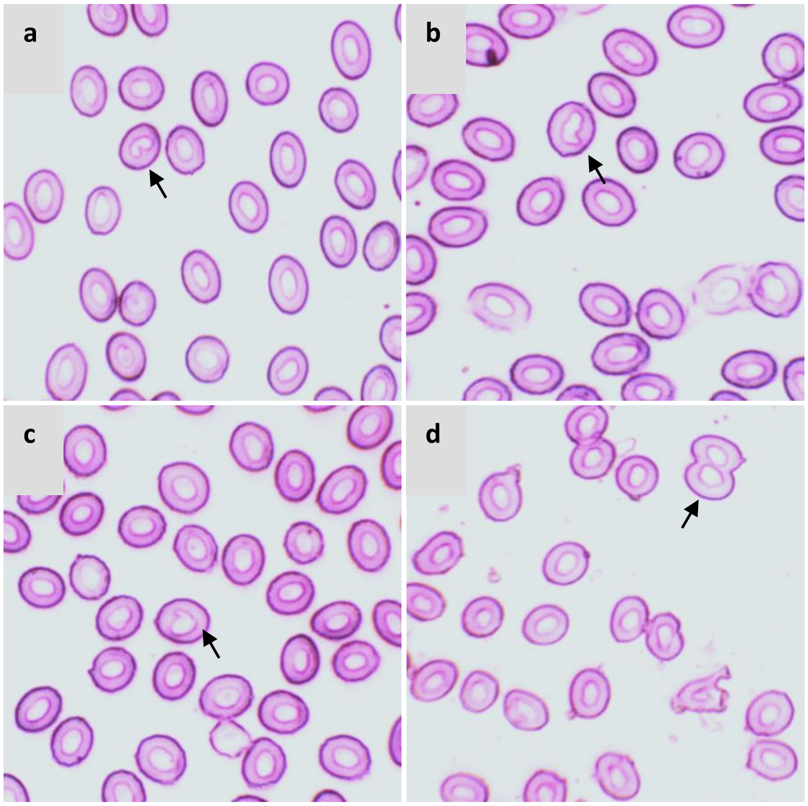


Figure 7 Erythrocytic nuclear abnormalities (ENAs) of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days; a. notch, b. karyopyknosis, c. nuclear bud and d. nuclear bridge.

4.6 Recovery response of erythrocytes

Recovery of ECAs and ENAs were observed after 14 days of recovery period. Fish of the control group displayed a substantial constancy in the case of ECAs and ENAs throughout the experimental period. Fish exposed to 0.1 ml/l diesel exhibited an instant and decent decrease in ECAs (Table 2) and ENAs (Table 3), whereas fish exposed to 0.5 ml/l diesel were much slower to recovery response.

Table 3 Erythrocytic nuclear abnormalities (ENAs) of Nile tilapia exposed to different concentrations of diesel oil for 7 days and recovery for 14 days

ENA	Diesel oil (ml/L)	Exposure (7 days)	Recovery (14 days)
Notch nuclei	0.0	0.14 ± 0.03^{a}	0.11 ± 0.04^{a}
	0.1	0.63 ± 0.09^{b}	$0.16 \pm 0.12^{ab,*}$
	0.5	0.72 ± 0.14^{b}	0.40 ± 0.10^{b}
Karyopyknosis	0.0	0.17 ± 0.03^{a}	0.14 ± 0.01^{a}
	0.1	0.31 ± 0.07^{b}	0.18 ± 0.11^{a}
	0.5	0.38 ± 0.10^{b}	$0.22 \pm 0.12^{a,*}$
Nuclear bud	0.0	0.19 ± 0.04^{a}	0.12 ± 0.03^{a}
	0.1	0.24 ± 0.09^{ab}	0.20 ± 0.07^{ab}
	0.5	0.33 ± 0.05^{b}	0.30 ± 0.06^{b}
Nuclear bridge	0.0	0.19 ± 0.09^{a}	0.16 ± 0.09^{a}
	0.1	0.56 ± 0.13^{b}	$0.29 \pm 0.06^{ab,*}$
	0.5	0.88 ± 0.17^{b}	0.64 ± 0.11^{b}

Values of a single cellular abnormalities in a column with different alphabetical superscripts are significantly ($p < 0.05$) different. Asterisk (*) indicates the significant difference between exposure and recovery in the row. All values are expressed as mean ± SD. Three slides were prepared from each fish and 2000 cells were scored from each slide and at least three fishes were analyzed from each group.

CHAPTER 5

Discussion

5.1 Behavior

Petroleum pollutant like diesel is considered a serious threat to water-living creatures because they contain toxic substance and their ability to stay in the water for a long time, being stable compound. In the present study, several behavioral changes, including low feeding rate, gulping, lack of locomotion, and abnormalities in the gill motion, were observed in both the 0.1 ml/l and 0.5 ml/l treatment group, and as expected, the latter group showed severity in every case because of a higher dose which can be compared with the findings of Armstrong et al. (2019) and Bautista et al. (2019). The abnormality in gill motion might have resulted from diesel clogged in the gill. Besides, diesel did not mix with water and made a layer on the water surface, which prevented the atmospheric oxygen from mixing up and might give rise to lower dissolved oxygen levels and gulping. It is possible that it might have happened due to gill injury or a change in gill morphology. The osmoregulatory function can be affected by the toxic substances through gill injury and cease the oxygen intake of aquatic organisms (Saravana and Geraldine., 2000). Fish of 0.5 ml/l dose showed inertia and low food intake, which is similar to Kori-Siakpere (2000), who stated that fish presented to WSFs of unrefined petroleum could result in diminished feed admission and low body weight. Dede and Kaglo (2001) detailed that the endurance of Nile tilapia diminished by expanding the centralization of diesel fuel. After 14 days of the recovery period, the abnormal behavior almost disappeared, especially in the 0.1 ml/l treatment group. Though fish of the 0.5 ml/l treatment group did not show complete normal behavior, the progress was somewhat significant. From this, it can be said that if the toxicant level of diesel is low and if the pollutant can be removed from the water, fish can recover from their abnormal behavior quickly.

5.2 Hemato-biochemical parameters

Hematological and biochemical parameters are considered decent indicators to know whether the fish are in stressful conditions because of aquatic environmental pollutants (Ashaf-Ud-Doulah et al., 2020; Shahjahan et al., 2021). We found moderate to severe aberration of the hemato-biochemical parameter in our sample treated with diesel, and in every case, the sample exposed to a higher dose (0.5 ml/l) showed greater anomaly compared to a lower dose (0.1 ml/l). In our present study, a higher dose of diesel oil exposure in Nile tilapia significantly decreased the Hb and RBC, and it is possible that it might have resulted from the breakdown of the hematopoietic system as cessation of the hematopoietic system was severe under the critical condition. The low count of RBC might have resulted from a severe anemic state or from hemolysing power of toxicant, particularly on the red cell membrane. A massive decline in Hb level in the 0.5 ml/l treatment group might debilitate oxygen supply to different tissues and might result in a sluggish rate and low energy creation. The critical diminishing in the Hb fixation might have been caused due to an expansion in the rate at which the Hb was obliterating or from waning in the pace of Hb synthesis resulting from the toxic effect of diesel. Cessation of Hb and RBCs with an increased percentage of erythroblast abnormalities due to pollution was also found in striped catfish (Shahjahan et al. 2018) and common carp, *Cyprinus carpio* (Gupta et al. 2014; Shahjahan et al. 2020). Similarly, Gurung et al. (2021) reported that crude oil exposure during organogenesis induced greater teratogenic effects on halibut, disturbances cardiovascular flow of embryonic Gulf killifish. We found that both of the treatment groups displayed greater WBC numbers after diesel exposure. The increment in the WBC count can be related to the immune response creation, which helps in the endurance and recuperation of the fish exposed to diesel (Joshi et al. 2002). In the current study, the critical expansion in the WBC count may be showed hypersensitivity of leucocytes to diesel and these progressions might be because of immunological responses to create antibodies to cope with the stress initiated by diesel. Changes in blood glucose have been proposed as a helpful general marker of stress in teleost. The acceleration in glucose level also indicated the secretion of cortisol, a stress hormone. Under unpleasant conditions, cortisol provides the body with glucose by taking advantage of protein stores through gluconeogenesis in the liver. This energy can help to battle or escape a stressor. In our study, the huge increment of plasma glucose level might have occurred because of gluconeogenesis to give energy to the expanded metabolic demands due to stress. The building and emission of glucocorticoids and catecholamines cause hyperglycemia. Excess stress provokes the production of these hormones from the adrenal tissue, which eventually augments the gluconeogenesis processes in stressed fish (Winkaler et al., 2007). Higher glucose level was also reported as an indicator of stress in several fish species under various stress conditions (Agrahari et al. 2007; Ahmed et al. 2016; Alaguprathana and Poonkothai 2021). So, it is clear that fish can be greatly stressed by diesel, especially when the amount of pollutants is higher. After the recovery phase, all the blood parameters were found to be improved and showed similarity with the control group, particularly fish exposed to 0.1 ml/l diesel. Though both of the treatment group recovered greatly from low Hb and RBC level, in the case of WBC and glucose level, the improvement were less significant, which indicate that the immune system of fish is still in action and fish have not completely recovered from stress.

5.3 Morphology of erythrocytes

We found several abnormalities in the erythrocytes of the treatment group. The frequencies of aberrations were higher in the 0.5 ml/l treatment group compared to the 0.1 ml/l treatment group. As erythrocytes react to environmental stressors, any alteration (cellular and nuclear) can be read as the presence of toxicants in the water. Erythrocytic cellular abnormalities, for example, twin, elongated, spindle shaped, tear drop shaped were observed. It could have resulted from the increase in lipid peroxidation in erythrocytes induced by stress due to diesel exposure (Sadiqul et al., 2016). Toxic compounds in diesel can disrupt the chain of cellular modification and may lead to hypoxic conditions. As a result, it can desolate the ATP, which may disturb the structure of the erythrocyte. Abnormalities in the nucleus, for instance, nuclear bridge, micronucleus, notched nuclei, were observed in the treatment group, which indicates the genotoxicity of diesel oil (Bai et al., 2014; Ghaffar et al., 2015; Islam et al., 2020). The previous report shows that toxic compounds can potentially disturb the structure of cell membrane, metabolism, and ion permeability of erythrocytes which can damage the erythrocyte formation morphologically. Crude oil causes micronuclei development and other nuclear abnormalities in the erythrocytes and cephalic kidney of Atlantic cod, *Gadus morua,* and turbot, *Scophthalmus maximus* (Baršienė et al. 2006). After 14 days of the recovery period, the amount of ECAs and ENAs started to vanish, and the improvement was much higher in the 0.1 ml/l treatment group. However, the other group (0.5 ml/l) did not show any significant improvement. Though we found the blood parameters like Hb, RBC, WBC, glucose levels improved in quite a good way in this group, it was not the case when it came to erythrocytic abnormalities. It was beyond were capacity to determine whether the damages were permanent or would it lead to a severe health problem for fish in the 0.5 ml/l treatment group.

CONCLUSIONS

The study reveals that the toxic effect of diesel has a severe effect on the behavior, hemato-biochemical parameters and morphology of erythrocytes of a teleost fish and can lead to extreme metabolic stress even if the exposure is for a short time and in a lower amount. It also showed that fish could quickly recover from the abnormalities, given that the pollutant's intensity is lesser and completely absent while recovering. If the concentration is higher, the consequences may be harsh, and recovery is not completely certain, particularly at the cellular level.

SUGGESTIONS

In this research, we investigated how diesel oil affects behavioral, hemato-biochemical and changes the structure of erythrocytes in Nile tilapia. More research warrants knowing whether the toxicants in diesel concentrate in the cellular system and to what extent they can damage different organs when the exposure is greater. In addition to that, we also suggest investigating the transgenerational effect of this toxicants to the early developmental stages of fish and how they affect neurophysiology fish.

LIMITATIONS OF WORK

The current COVID-19 pandemic situation impacts overall to our research work. In addition to that, we could not conduct a molecular investigation due to the limitation of research funding and facilities.

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