Open Access Research Journal of
Science and Technology
Journals home page: https://oarjst.com/
ISSN: 2782-9960 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Assessment of genotoxicity in *Clarias gariepinus* exposed to lethal and sub-lethal concentrations of oilfield chemicals: DNA concentration, purity, and genetic implications

Davies Ibienebo Chris [1,*], Ebere Samuel Erondu [1] and Davies Imachrist Ibienebo [2]

[1] *Department of Fisheries, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.*
[2] *Department of Animal and Environmental Biology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.*

Open Access Research Journal of Science and Technology, 2024, 10(01), 044–058

Publication history: Received on 04 December 2023; revised on 23 January 2024; accepted on 26 January 2024

Article DOI: https://doi.org/10.53022/oarjst.2024.10.1.0021

Abstract

This study investigates the concentration and purity of DNA extracted from *Clarias gariepinus* after exposure to lethal concentrations (250 ml/l, 200 ml/l, 150 ml/l, 100 ml/l, 50 ml/l, 25 ml/l and 0.0 ml/l for the control) of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) over 96 hours using static bioassays and sub-lethal concentrations 0 ml/l (control), 12.8 ml/l, 25.6 ml/l, 38.4 ml/l, 51.2 ml/l and 64.0 ml/l) using renewal bioassay for 28 days. 210 juveniles were used for the experiment. The study used PCR and other methods to analyse DNA concentrations and purity in the fish samples. The results indicated variations in DNA concentration, purity, and the presence of micronuclei, suggesting genetic and cytogenetic responses to chemical exposures. The highest DNA concentration was recorded in the 25 ml/l group for both O-BE and X:D, while the lowest was in the 100 ml/l group. The genetic effects of Xylene: Diesel on the molecular level were also examined, with significant gaps in the alignments of experimental groups indicating notable differences in the sequences compared to the control group without chemical exposure. Through detailed analyses, including gel electrophoresis and sequence alignment, the study explores genetic variations and micronuclei frequencies in blood cells, shedding light on the ecotoxicological impacts and potential evolutionary implications of these oil-based substances on African catfish. Understanding the genetic variations induced by lethal concentrations of O-BE in *Clarias gariepinus* is crucial for assessing the broader environmental impact, as genetic changes in aquatic organisms can affect population health, adaptation, and ecosystem dynamics.

Keywords: *Clarias gariepinus*; Xylene; Diesel; Oilfield-Based Emulsifiers; Genetic variations

1. Introduction

Oil and gas exploration and production have long been important factors in Nigeria's economic wealth and influence. While the oil and gas industries worldwide importance cannot be overstated, its activities, especially in the aquatic environment, have significant environmental consequences (Bamberger and Oswald, 2014; Dmitrieva and Romasheva, 2020). Various petroleum sector operations, including drilling, exploration, and reservoir stimulation, have negative consequences on the aquatic ecology, demanding a detailed assessment of their impact.

Oil extraction is critical to the global energy landscape, but the release of related chemicals into the aquatic environment, particularly during well-stimulation activities, presents serious dangers to aquatic life (Chittick and Srebotnjak, 2017). According to Long et al. (2021), advanced exploration technologies inject harmful components into aquatic bodies, affecting physicochemical factors that affect fish and other aquatic life. Concerns are growing that such pollution might affect the genetic makeup and well-being of fish and shellfish populations, possibly leading to extinction.

Researchers have developed techniques for determining direct and indirect effects on DNA sequences due to the genotoxic potential of oilfield chemicals on aquatic creatures (Pampanin et al., 2017; Costa, 2022). According to Gupta

* Corresponding author: Davies Ibienebo Chris

and Ahmad (2017), genotoxicants may cause mutations by establishing strong covalent interactions with DNA, which results in DNA adducts that obstruct accurate replication. The long-term and considerable effects of these DNA changes on the aquatic ecosystem highlight the necessity of genotoxicity assays in determining environmental impact (Jiang et al., 2023).

Fish, as sensitive environmental indicators, play an important role in bio-monitoring (Colin et al., 2016; Manzoor et al., 2021). This work uses controlled environment studies to examine the genotoxicity of selected oilfield chemicals in catfish, (*Clarias gariepinus*), a common species in African freshwater systems in the Niger Delta. The study intends to give important insights into biomonitoring and ecotoxicology by examining genomic changes produced by these pollutants. Understanding the environmental impact of oilfield chemicals, particularly on fish populations, is critical for developing effective mitigation techniques. Genetic alterations or responses in organisms such as *C. gariepinus* could have significant effects on population health and ecosystem dynamics due to environmental stressors (Baldwin et al., 2020; Turan et al., 2020; Świacka et al., 2021; Nwizugbo et al., 2023).

This research aims to investigate the genetic responses of *C. gariepinus* to varied amounts of xylene: diesel and oilfield-based emulsifiers, establishing a relationship between DNA modifications, ecotoxicological effects, and possible evolutionary ramifications. The results add to our knowledge of how oil-based pollutants affect aquatic creatures, making it easier to evaluate environmental concerns and establish educated conservation measures. The study emphasises the need for sustainable practices in the petroleum sector to mitigate the negative environmental consequences of oil and gas exploration and production.

The research hopes to contribute to a better understanding of the molecular effects of oilfield chemicals on *C. gariepinus* by fulfilling these goals. The results might have larger implications for evaluating the ecological hazards, environmental safety, and possible long-term repercussions of chemical exposure in aquatic environments. The work may also shed light on creating genetic markers for monitoring and regulating the environmental effect of oilfield operations on fish populations.

2. Material and methods

2.1. Research Design

The toxicity tests used a 3 x 5 x 6 factorial design in a randomized full-block arrangement, with three factors having six levels, five time intervals for monitoring, and three repeats.

2.2. Test Organisms {African catfish (*Clarias gariepinus*)}

A total of 420 juvenile *C. gariepinus* specimens, with an average length of 15.20±2.3cm and weight of 10.23±2.60 g, were obtained from the University of Port Harcourt Demonstration Farm in Nigeria. These specimens were chosen because of their high vulnerability to environmental stress. The experiment's organism was chosen during its juvenile growth period because of its heightened susceptibility to environmental stress (Ezike and Ugwu 2017; Davies et al., 2019; Chris et al., 2022).

2.3. Acclimation of the test organism

The fish species were acclimated to laboratory conditions for fourteen days at 28 º C in a 150-litre glass tank, supplied with standard aquarium feed twice daily, and oxygenated continuously. Bennett and Dooley (1982) proposed that laboratory faucet water be used to replace the water in glass containers every 48 hours.

2.4. Test Chemicals

The oilfield-based emulsifier oil used in the study was acquired from an oilfield chemical laboratory in Rivers State, Nigeria, and was kept in an ambient laboratory environment. Following a typical technique, a working stock solution was made from oilfield-based emulsifiers, and a test chemical was prepared using a volumetric and analytical approach outlined in other studies (Anderson et al., 1974; Orlu and Ogbalu, 2013; Davies et al., 2019).

2.5. Selection of Test Organism for the Assay

To test for the sub-lethal effect of the Oilfield-based emulsifiers, ten healthy and active juveniles of uniform size were randomly selected from the acclimation tanks and carefully transferred into different treatment units for 28 days using a hand-held scoop net (Sil et al., 2010). Three iterations of the experiment total—three including the control. To preserve the concentration of dissolved oxygen and minimize variations brought on by the fish's metabolism, the

experiment was conducted using a renewal strategy (Chris et al., 2022). Two days before the start of the 96-hour static and 28-day renewable exposure periods, feeding was halted. Five test concentrations were prepared: 0.0 (control), 12.8, 25.59, 38.39, 51.19, and 63.99 ml/l for sub-lethal test concentrations, and 0.0, 25, 50, 100, 150, 200, and 250 ml/l before the lethal. A 15-litre plastic aquarium tank filled to the 10 mark was used to hold each test concentration. Ten fish were randomly selected and assigned to each test concentration. Treatments were repeated after each other.

2.6. DNA extraction

Sterile tissue was removed from the muscle of a fish population. The QIA ampere DNA mini-ki (Qiagen Germany) was then used to extract and purify the DNA according to the manufacturer's instructions. In these investigations, fifteen commercially available decamer random primers were created and chosen at random for use in the amplification of random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) products. This primer was used to start the PCR amplification. It was purchased from Operon Technologies (Almeda, CA, USA). Table 1 lists the primers selected according to their sequences, primer codes, and CG content (60–70%).

Table 1 Codes, sequences and CG content for RAPD analysis

Primers	Sequence	CG%	Primers	Sequence	CG%
A02	TGCCGAGCTG	70	C05	GATGACCGCC	70
A19	CAAACGTCGG	60	C07	GTCCCGACGA	70
B02	TGATCCCTGG	60	C10	TGTCTGGGTG	60
B04	GGACTGGAGT	60	C17	TTCCCCCCAG	70
B06	TGCTCTGCCC	70	C18	TGAGTGGGTG	60
B07	GGTGACGCAG	70	C19	GTTGCCAGCC	70
C03	GGGGGTCTTT	60	C20	ACTTCGCCAC	60
C04	CCGCATCTAC	60			

*Total DNA was extracted using the muscle of the fish sample.

Proteinase K tubes weighing 20 mg each were stored at -20 ˚C and filled with 1,060 litres of buffer for storage. A 25 mg tissue sample was then placed in a microcentrifuge tube together with a 95 µl solution of water, 95 µl solid tissue buffer (Blue), and 10 µl proteinase K. The material was well combined, and the tube was incubated at 55 ˚C for one to three hours, or until the tissue entirely disintegrated. To get rid of insoluble material, the tissue in the tube was centrifuged at 12,000 x g for one minute. A fresh tube was filled with the aqueous supernatant. Subsequently, the supernatant was mixed well with two litres of genomic binding buffer. The mixture was then centrifuged for one minute (12,000 g) in a collecting tube that held a Zymo-Spintm IIC-XL column. After that, the collecting tube and the flow-through were emptied. After adding 400 µl of DNA pre-wash buffer to the column and centrifuging for one minute, the collecting tube was emptied and replaced with a new one.

After adding a 700 µl g-DNA buffer wash, the new collection tube was centrifuged for one minute and then emptied. 200 µl of g-DNA wash buffer was added to a new collection tube, which was then emptied using the flow-through after centrifuging for a minute. To extract the DNA, move the supernatant into a sterile microcentrifuge tube, include 50 µl of elution buffer, and allow it to incubate for five minutes before centrifuging it for one minute. Subsequently, the samples were moved to a nanodrop for purity testing.

2.7. PCR Amplification

PCR was used to amplify the DNA of the cytochrome b gene. Using a Bio-Rad MJ MiniTM Personal Thermal Cycler, two sets of primers for the cytochrome b gene (585 bp) were amplified by polymerase chain reaction (PCR) from each DNA sample: L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3', and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' (Kadar et al., 2008). PCR mixes were made in a 25 µl tube using 1 µl of template DNA, 0.4 M concentrations of each primer, and 12.5 µl of MyTaq Mix. After one minute of 95°C amplification, there were 35 cycles of 95 °C for 15 seconds, 52 °C for 15 seconds, 72 °C for 10 seconds, and 72 °C for 7 minutes. Following the separation of the PCR products by gel electrophoresis (2.0% agarose gel), Safe-RedTM staining was used.

2.8. PCR Purification and Sequencing

The innuPREP PCR Pure Kit was used to purify the PCR products. Repfon Glamor Sdn Bhd received a successful PCR product for sequencing. Sequencing of the strands of the PCR fragments was done to find any ambiguities in the PCR findings.

2.9. MN test

Blood samples from *C. gariepinus* were taken from the caudal peduncles of fish in both the experimental and control groups. The samples were then spread onto pristine, grease-free frosted glass slides. Following a 10-minute methanol fixation, the slides were allowed to air dry before being stained for 15 minutes with a 6% Giemsa stain. Blood erythrocytes were counted using a × 100 oil immersion under a binocular microscope after slides were mounted in styrene, plasticizer, and Oilfield-Based Emulsifiers (DPX). An MN cannot be counted unless it is isolated from the others, has the same colour, and makes up less than one-third of the main nucleus. Using the following formula, the MN frequency was determined:

$$\%\text{MN} = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \text{X}100 \ldots\ldots\ldots(1)$$

2.10. Statistical analysis

Statistical analysis was carried out using SPSS Inc.'s software (version 17, Chicago, IL). Means and standard errors (SE) were calculated from the collected data. Counted cells were used to calculate the MN results. The MN interactions caused by exposure duration and treatments were assessed at a 95% significant level using a two-way analysis of variance (ANOVA) and Duncan's multiple range post hoc test. DNA sequences were analyzed for ambiguities using Molecular Evolutionary Genetics Analysis (MEGA) version 5. Phylogenetic trees were developed using the Neighbour-Joining (NJ) Multiple Alignment option of BioEdit Sequence Alignment Editor version 7.2.5 (Hall, 1999).

3. Results

The study examines the concentration and purity of DNA extracted from *Clarias gariepinus* after exposure to lethal concentrations of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) over 96 hours (Table 2 The data is organized based on different concentrations of the two chemicals, with corresponding values for DNA concentration (ng/ µl) and DNA purity (A260/280nm). At a concentration of 0 ml/l (control group), both O-BE and X:D show similar DNA concentrations of 34.50 ± 3.92 ng/ µl. However, as the concentration increases, variations in DNA concentrations are observed. The highest DNA concentration is recorded in the 25 ml/l group for both O-BE (36.3 ± 3.92) and X:D (35.20 ± 3.92), while the lowest is in the 100 ml/l group. There is a general trend of decreasing DNA concentration with increasing chemical concentration. However, there was no statistically significant difference in the purity and DNA concentration between the control group and other lethal exposure levels.

Table 2 DNA concentrations (ng/ µl) and purity for the experimental groups at lethal exposure

	DNA concentrations (ng/µl) for the Experimental groups		**DNA Purity (A260/280nm) for the Experimental groups**	
Concentration (ml/l)	**(O-BE)**	**(X:D)**	**(O-BE)**	**(X:D)**
0	34.50 ± 3.43^{a}	34.50 ± 3.92^{a}	1.48 ± 0.02^{a}	1.48 ± 0.04^{a}
25	36.30 ± 3.92^{a}	35.20 ± 3.92^{a}	1.60 ± 0.04^{a}	1.58 ± 0.04^{ab}
50	31.53 ± 3.92^{a}	33.37 ± 3.92^{a}	1.56 ± 0.04^{ab}	1.57 ± 0.04^{ab}
100	25.00 ± 3.92^{a}	32.93 ± 3.92^{a}	1.62 ± 0.04^{b}	1.65 ± 0.04^{b}
150	26.27 ± 3.92^{a}	34.23 ± 3.92^{a}	1.58 ± 0.04^{ab}	1.62 ± 0.04^{b}
200	33.53 ± 3.92^{a}	27.13 ± 3.92^{a}	1.58 ± 0.04^{ab}	1.63 ± 0.04^{b}
250	35.78 ± 3.92^{a}	32.00 ± 3.92^{a}	1.54 ± 0.04^{b}	1.62 ± 0.04^{b}

Each value is a mean of three test replicates at a 95% confidence limit; *Means with the same superscript are not significantly different at (>0.05) significant level.

From the result in Table 3, the DNA purity and concentration of the *C. gariepinus* sample in the control were 1.52 ± 0.04 and 92.5 ± 68.6ng/ µl respectively. The values for the fish exposed to the toxicants are presented in Table 2. No significant difference (P>0.05) was recorded between the DNA and purity of the control and the fish subjected to different concentration levels. The results of the DNA purity and concentration are presented in Table 3 and the values obtained from the control were 1.52 ± 0.04 and 92.50 ± 68.6ng/ µl respectively. No statistically significant difference (P>0.05) was recorded between the DNA concentration observed in the control and other test groups and there was also no statistically significant variation (P>0.05) in the DNA purity in the control group and the other groups (12.8, 25.59, 38.39, 51.19 and 63.99 ml/l). there was also no statistically significant variation (P>0.05) observed within the test groups.

Table 3 DNA concentrations (ng/ µl) and purity for the experimental groups at sub-lethal exposure

Concentration (ml/l)	**DNA concentrations (ng/µl) for the Experimental groups**		**DNA Purity (A260/280nm) for the Experimental groups**	
	O-BE	**X:D**	**O-BE**	**X:D**
0	92.5 ± 68.6^{a}	92.50 ± 68.6^{a}	1.52 ± 0.04^{abc}	1.52 ± 0.04^{abc}
12.80	134.2 ± 68.6^{a}	141.50 ± 68.6^{a}	1.46 ± 0.04^{abc}	1.45 ± 0.04^{abc}
25.59	106.4 ± 68.6^{a}	259.97 ± 68.6^{a}	1.49 ± 0.04^{abc}	1.44 ± 0.04^{ab}
38.39	105.7 ± 68.6^{a}	191.37 ± 68.6^{a}	1.47 ± 0.04^{abc}	1.42 ± 0.04^{a}
51.19	295.7 ± 68.6^{a}	68.20 ± 68.6^{a}	1.43 ± 0.04^{ab}	1.57 ± 0.04^{bc}
63.99	240.2 ± 68.6^{a}	144.9 ± 68.6^{a}	1.44 ± 0.04^{ab}	1.49 ± 0.04^{abc}

Table 4 presents the results of micronuclei (MN) frequencies observed in *Clarias gariepinus* blood cells after exposure to different concentrations of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) over 96 hours. The data is organized based on the concentration (ml/l) of the two chemicals, with corresponding values for MN frequencies presented as a percentage. The concentrations represent varying levels of exposure to O-BE and X: D.

In the control group (0 ml/l), both O-BE and X:D show relatively low % MN frequencies, indicating a baseline level of micronuclei in the absence of exposure. As the concentration of O-BE and X:D increases, there is a notable increase in % MN frequencies, suggesting a dose-dependent response to the chemicals. The highest % MN frequencies are observed in the 250 ml/l group for both O-BE (0.86 ± 0.06) and X:D (1.20 ± 0.14), indicating a potentially higher level of genetic damage at these concentrations. Generally, there is a trend of increasing % MN frequencies with increasing concentration for both O-BE and X: D.

Table 4 Lethal concentrations of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) exposed to *Clarias gariepinus* blood cells and the variations in the number of micronuclei (MN)

Concentration (ml/l)	**% MN frequencies (mean ± SE) for the Experimental groups**	
	O-BE	**X:D**
0	0.14 ± 0.03 ^{c}	0.14 ± 0.05 ^{e}
25	0.17 ± 0.01 ^{c}	0.30 ± 0.02 ^{d}
50	0.18 ± 0.02 ^{c}	0.65 ± 0.05 ^{c}
100	0.60 ± 0.08 ^{b}	0.70 ± 0.07 ^{c}
150	0.68 ± 0.02 ^{b}	0.90 ± 0.03 ^{b}
200	0.72 ± 0.04 ^{a}	1.03 ± 0.10 ^{b}
250	0.86 ± 0.06 ^{a}	1.20 ± 0.14 ^{a}

Table 5 presents the outcomes of micronuclei (MN) frequencies observed in *Clarias gariepinus* blood cells following exposure to Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) for 96 hours. The concentrations indicate varying

levels of exposure to O-BE and X:D. The table provides the mean percentage of micronuclei frequencies along with the standard error (SE) for each concentration of O-BE and X: D. A micronucleus indicates genetic damage and chromosomal instability. In the control group (0 ml/l), both O-BE and X:D show relatively low % MN frequencies, indicating a baseline level of micronuclei in the absence of exposure.

As the concentration of O-BE and X:D increases, there is a noticeable rise in % MN frequencies, suggesting a concentration-dependent response to the chemicals. The highest % MN frequencies are observed at the highest concentrations (51.19 ml/l and 63.99 ml/l) for both O-BE and X:D, indicating a potential increase in genetic damage at these levels. Generally, there is a trend of increasing % MN frequencies with the elevation of concentration for both O-BE and X: D.

Table 5 Micronuclei frequencies in *Clarias gariepinus* blood cells were observed at lethal concentrations of Xylene: Diesel and Oilfield-Based Emulsifiers

Concentration (ml/l)	**% MN frequencies (mean ± SE) for the Experimental groups**	
	O-BE	**X:D**
0	0.14 ± 0.03 [c]	0.16 ± 0.01 [c]
12.80	0.18 ± 0.02 [c]	0.68 ± 0.02 [b]
25.59	0.68 ± 0.02 [b]	0.75 ± 0.04 [b]
38.39	0.72 ± 0.04 [b]	0.85 ± 0.05 [b]
51.19	1.56 ± 0.30 [a]	1.05 ± 0.09 [a]
63.99	1.60 ± 0.15 [a]	1.52 ± 0.10 [a]

Figure 1 presents the multiple sequence alignments of consensus sequences for *Clarias gariepinus* subjected to lethal concentrations of Oilfield-Based Emulsifiers (O-BE). The experimental fish were exposed to varying lethal (0 ml/l control, 25 ml/l, 50 ml/l, 100 ml/l, 150 ml/l, 200 ml/l, and 250 ml/l) and sub-lethal (0 ml/l control, 12.8 ml/l, 25.6 ml/l, 38.4 ml/l, 51.2 ml/l, and 64.0 ml/l) concentrations of Oilfield-Based Emulsifiers. The figure showcases the alignment patterns of consensus sequences, providing insights into the genetic variations induced by exposure to different lethal concentrations of Oilfield-Based Emulsifiers.

The experimental design includes both control groups (0 ml/l) and lethal concentrations, allowing for a comprehensive analysis of the genetic impact at various exposure levels. Statistically significant gaps ($P<0.05$) in the alignments of experimental groups indicate notable differences in the sequences compared to the control group without chemical exposure. The alignment patterns visually represent the molecular changes in the DNA sequences of *Clarias gariepinus* under the influence of lethal concentrations of Oilfield-Based Emulsifiers.

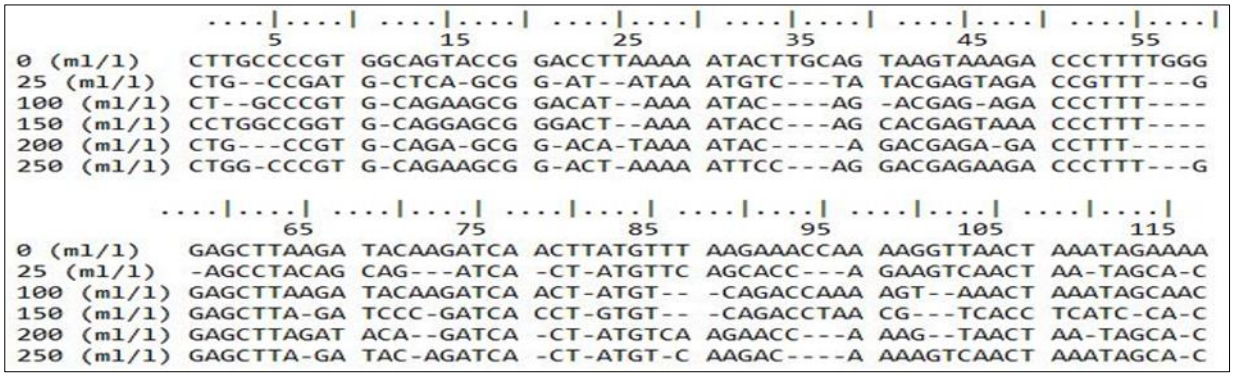

Figure 1 Multiple sequence alignment of consensus sequences for *C. gariepinus* after exposure to different lethal test concentrations for Oilfield-Based Emulsifiers (0 ml/l control, 25 ml/l, 50 ml/l, 100 ml/l, 150 ml/l, 200 ml/l and 250 ml/l

A study on *Clarias gariepinus* exposed to sub-lethal concentrations of Xylene: Diesel (X:D) revealed significant DNA sequence changes, particularly in groups exposed to sub-lethal concentrations (Figure 2). The study found that the DNA

sequences of the experimental fish were significantly impacted by Xylene: Diesel at sub-lethal concentrations. The distinct differences in the alignment patterns suggest a pronounced impact of Xylene: Diesel on the genetic makeup of *C. gariepinus*. The findings emphasize the genotoxic effects of Xylene: Diesel on the molecular level and emphasize the importance of understanding the potential consequences of chemical exposure on aquatic organisms.

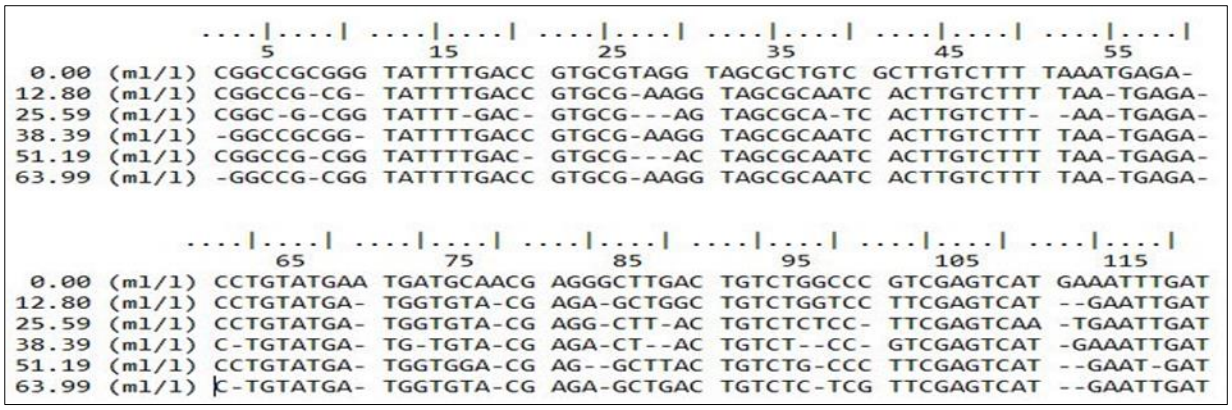

Figure 2 Multiple sequence alignment of consensus sequences for *C. gariepinus* after exposure to different sub-lethal test concentrations for Oilfield-Based Emulsifiers 0 ml/l control, 12.8 ml/l, 25.6 ml/l, 38.4 ml/l, 51.2 ml/l and 64.0 ml/l)

Figure 3 shows the DNA sequence alignment of *Clarias gariepinus* after exposure to different lethal concentrations of Xylene: Diesel. The concentrations range from a control group (0 ml/l) to escalating levels (25 ml/l, 50 ml/l, 100 ml/l, 150 ml/l, 200 ml/l, and 250 ml/l). The alignment patterns reveal variations in the fish's genetic composition in response to Xylene: Diesel. The presence or absence of specific sequence alignments at different lethal concentrations highlights molecular changes induced by exposure to elevated levels. The figure helps identify potential genetic responses in *Clarias gariepinus* to lethal concentrations, revealing how the fish's DNA may be influenced by significant environmental stressors. Lethal concentrations are crucial as they reflect the immediate and severe impacts of environmental exposures on aquatic organisms' genetic makeup.

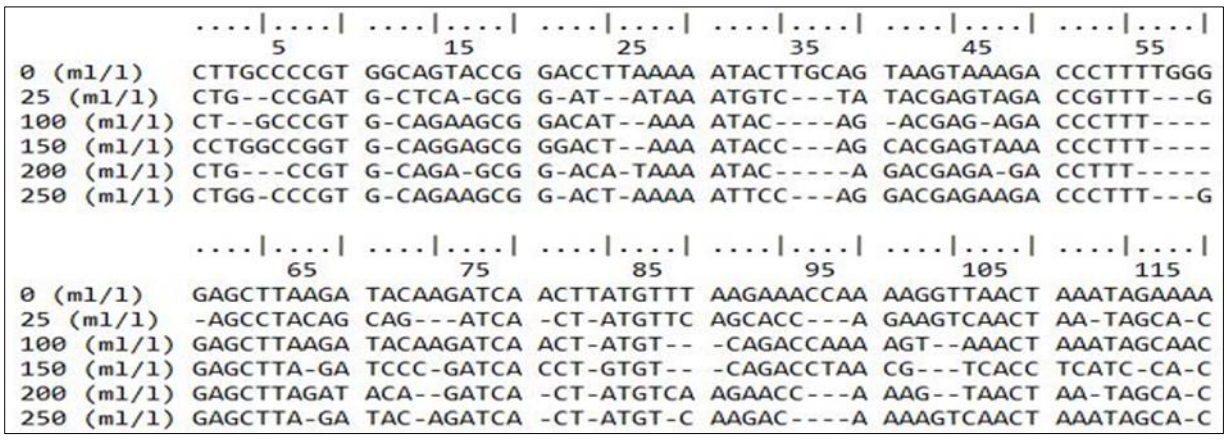

Figure 3 Multiple sequence alignment of consensus sequences for *C. gariepinus* after exposure to different lethal test concentrations for Xylene: Diesel (X:D) (0 ml/l control, 25 ml/l, 50 ml/l, 100 ml/l, 150 ml/l, 200 ml/l and 250 ml/l

The study reveals genetic variations and consensus sequence alignment for *Clarias gariepinus* after exposure to sub-lethal concentrations of Xylene: Diesel (Figure 4). The results show distinct variations in the DNA sequence of consensus sequences, suggesting potential adaptations or responses to environmental stressors. The alignment patterns at each sub-lethal concentration are compared to the control group, revealing differential responses and providing a molecular perspective on how the genetic makeup of the fish is influenced. This study provides valuable insights into how *C. gariepinus* responds at the molecular level to sub-lethal concentrations of X:D, enhancing our understanding of the environmental stressors' impact.

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5         15         25         35         45         55
0      (ml/l)   CGGCCGCGGG TATTTTGACC GTGCGTAGG TAGCGCTGTC GCTTGTCTTT TAAATGAGA-
12.80  (ml/l)   CGGCC--CG- TATT--GACC GTGCG--GG TAGCGCAA-- ACTTGTCT-- T---TGAGA-
25.59  (ml/l)   CG---G-CGG TAT---GAC- GTGCG---G TAGCG---TC ACTTGTCT-- -AA-TGA---
38.39  (ml/l)   --GCCGCG-- TAT----ACC GTGCG-AGG TA---CAATC ACTTGTC--- TAA-TG--A-
51.19  (ml/l)   CGGCCG---- TATTTTGA-- GTGCG---C TAG--CAATC ACTTGTC--- TAA-TG----
63.99  (ml/l)   -GGCC--C-- TATTT----- GTGCG---G TAGCG----C ACTTG----T TAA-TG----

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95        105        115
0      (ml/l) CCTGTATGAA TGATGCAACG AGGGCTTGAC TGTCTGGCCC GTCGAGTCAT GAAATTTGAT
12.80  (ml/l) CCTGTATG-- TG-TGTA-CG AGA-GCTG-C TGTCTGGTC- -TCGAGTCAT --GAAT-GAT
25.59  (ml/l) CCTGTAT-A- TG--GTA-CG AGG-CTT-A- TGTCTCTC-- -TCGAGTCA- -TGAATT--T
38.39  (ml/l) C-TGTA--A- TG--GT--CG AGA-CT--AC TGTC---CC- GT--AGTCA- -G--AT-G-T
51.19  (ml/l) CCTGTA---- T--TGGA-CG AG--GCTT-- TGT-TG-CCC TTCGAGTCAT --GA---GAT
63.99  (ml/l) C-TGT--GA- --GTGTA-CG AGA-GCT--C TGTC-C-TCG TTCGAG---T --GAAT----
```

Figure 4 Multiple sequence alignment of consensus sequences for *C. gariepinus* after exposure to different sub-lethal test concentrations for Xylene: Diesel (X:D) (0 ml/l control, 12.8 ml/l, 25.6 ml/l, 38.4 ml/l, 51.2 ml/l and 64.0 ml/l)

The PCR amplification of the cytochrome b gene was confirmed to be effective in capturing genetic responses in *Clarias gariepinus*. The primer was amplified at a specific size range of 442 bp to 511 bp, demonstrating the reliability of the experimental results (Figure 5). The gel electrophoresis results showed successful amplification of the cytochrome b gene, which is crucial for understanding genetic responses in the fish. The analysis included both lethal and sub-lethal concentrations of Oilfield-Based Emulsifiers, demonstrating the versatility of the PCR approach in capturing genetic responses across different exposure levels. The results demonstrate the effectiveness of the PCR approach in capturing genetic responses.

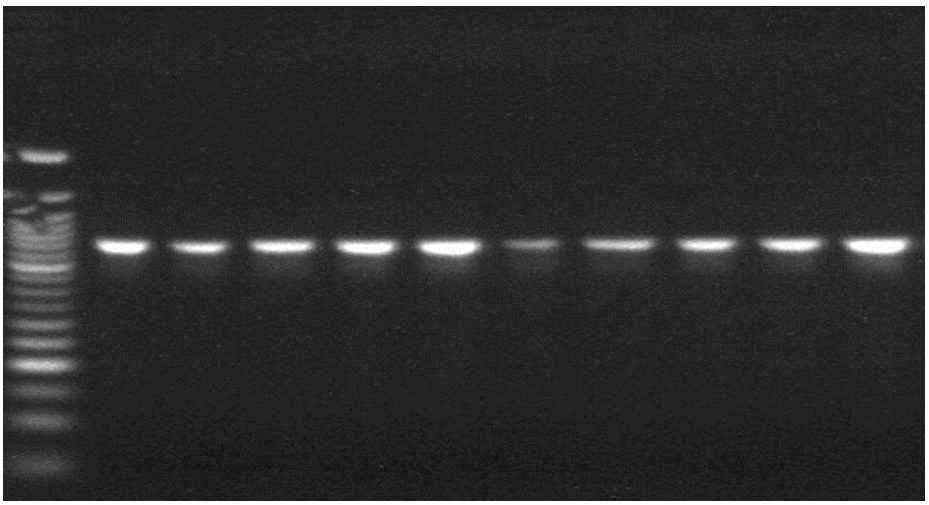

Figure 5 PCR amplification of cytochrome b gene 442bp to 511bp measured against 1000bp DNA ladder for Lethal and Sub-lethal Concentrations of Oilfield-Based Emulsifiers

The gel electrophoresis analysis for *Clarias gariepinus* following exposure to sub-lethal test concentrations of Xylene: Diesel (X:D) reveals robust results in terms of polymerase chain reaction (PCR) amplification. The confirmation of good quantity and quality for each sample signifies the reliability of the PCR amplification process. Specifically, the targeted cytochrome b (cyt b) gene, with a fragment size ranging from 442bp to 511bp, was successfully amplified for all samples (Figure 6).

This molecular analysis is instrumental in showing the genetic impact of sub-lethal environmental stressors on aquatic organisms. The gel image in visually represents the distinct bands corresponding to the amplified cytochrome b gene fragments. The ladder shows as a size marker, aiding in the assessment of fragment lengths.

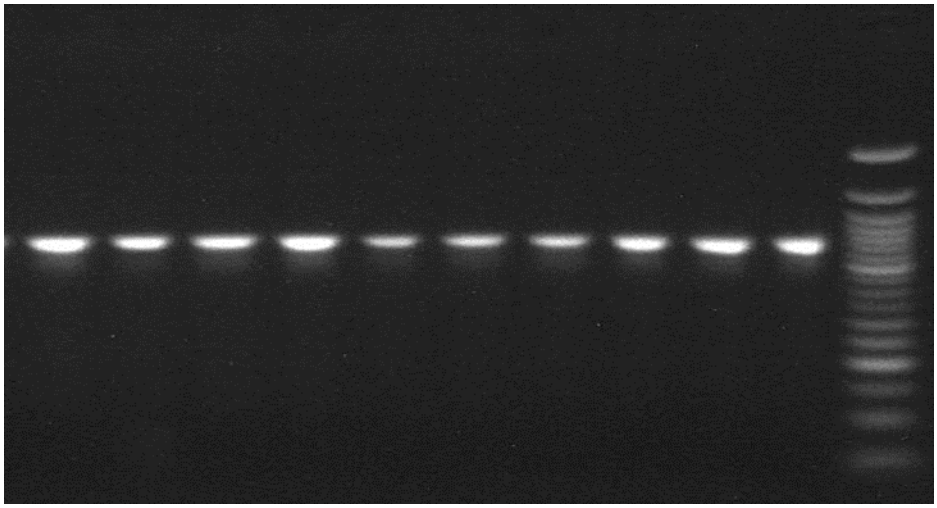

Figure 6 PCR amplification of cytochrome b gene 442bp to 511bp measured against 1000bp DNA ladder for Lethal and Sub-lethal Concentrations of Xylene: Diesel (X:D)

4. Discussion

Genotoxicity refers to the potential of a substance to cause damage to an organism's genetic material, leading to mutations, chromosomal abnormalities, or other genetic alterations (Savale, 2018). Genetic implications, in this context, pertain to the impact of these chemicals on the DNA of *Clarias gariepinus*.

The observed variations in DNA concentrations across different exposure concentrations of X:D and O-BE imply a potential impact of these chemicals on the DNA content within the exposed organisms. The general trend of decreasing DNA concentration with increasing chemical concentration may indicate a dose-dependent effect. The similarity in DNA concentrations between the control group and the lowest exposure concentrations suggests that at lower levels, these chemicals might not significantly impact DNA content (Hu et al., 2016). This could indicate a threshold effect, where only higher concentrations lead to observable changes in DNA concentration (Parolini et al., 2017).

The lack of statistically significant differences in DNA purity (A260/280nm ratio) between the control group and the exposed groups indicates that while the DNA concentrations might vary, the overall quality or purity of the DNA extracted remains relatively constant across these exposure levels. This might suggest that while the quantity of DNA changes, the chemical exposures do not notably affect the integrity or purity of the extracted DNA (Demeke and Jenkins, 2010; Musto et al., 2014).

The absence of statistically significant differences in DNA concentration and purity between the control group and lethal exposure levels might suggest a complex interaction between the chemicals and the fish's DNA (Pandey et al., 2018; Hanana et al., 2021. It's essential to conduct further investigations, such as genotoxicity assays or examining specific genetic markers, to elucidate potential genotoxic effects that might not be reflected in overall DNA concentration or purity.

while the study shows variations in DNA concentrations across different lethal exposure levels of X:D and O-BE in *Clarias gariepinus*, the consistent DNA purity values imply a nuanced relationship between the chemicals and the fish's genetic material (Brophy et al., 2020; Lin et al., 2022). Further research, including genotoxicity assays or specific genetic analyses, could provide a more comprehensive understanding of the genotoxic and genetic implications of these chemical exposures on *Clarias gariepinus.*

It is crucial to consider potential long-term effects on the population and the environment, as even subtle genetic alterations could have repercussions over time, affecting the fitness, reproductive success, and adaptability of *Clarias gariepinus* and potentially impacting the broader ecosystem.

The study found no significant differences in DNA concentrations and purity between the control group and the fish exposed to various concentrations of toxicants at sub-lethal exposure levels. This suggests that the concentrations might not induce observable changes in the overall quantity or quality of the extracted DNA (Mahmoudi et al., 2017). However, Xu et al., 2018 stated that the measured DNA concentration and purity might not be sensitive enough to detect subtle

genetic alterations caused by the sub-lethal exposures. However, Hartwig et al. (2020), suggested that genotoxic effects can encompass mutations, chromosomal abnormalities, and changes in specific genetic sequences. Nevertheless, Turkez et al. (2017), the absence of observable changes in DNA concentration and purity does not necessarily rule out the possibility of genotoxic effects at a molecular level that might not be reflected in these measurements.

Further investigation using more sensitive genotoxicity assays or molecular techniques might be necessary to reveal subtle genetic impacts that traditional DNA concentration and purity measurements might overlook. However, Bickham et al. (2000) suggested that it is crucial to consider potential long-term effects on the population and the environment, as even subtle genetic alterations could have repercussions over time, affecting the fitness, reproductive success, and adaptability of aquatic organisms and potentially impacting the broader ecosystem.

While the measured DNA concentrations and purity did not exhibit significant changes at sub-lethal exposure levels, it is essential to acknowledge the limitations of these measurements in capturing subtle genotoxic effects. Further comprehensive molecular studies are crucial for a deeper understanding of the genetic implications of these sub-lethal exposures on aquatic organisms.

The presence of micronuclei (MN) in *Clarias gariepinus* blood cells following exposure to Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) at lethal concentrations suggests potential genotoxic effects. MN are abnormal structures that form in cells when there is damage to the genetic material (DNA) (Guo et al., 2019). The increase in MN frequencies with higher concentrations of these chemicals suggests a dose-dependent relationship, indicating a proportional increase in genetic damage to the fish.

Genetic damage indicates that these chemicals induce genetic alterations within the fish, potentially leading to mutations or chromosomal instability (Langie et al., 2015). The relatively low % MN frequencies observed in the control group serve as a baseline for comparison, while the increase in MN frequencies in the exposed groups highlights the additional genetic damage caused by the chemicals. However, the highest % MN frequencies observed at the highest concentrations of X:D and O-BE (250 ml/l) underscore the correlation between exposure levels and genetic damage, potentially indicating a critical threshold beyond which the impact on genetic stability becomes notably higher (Bickham et al., 2000; Huang, 2013).

The results agree with Alimba and Bakare. (2016) reported that the presence of increased micronuclei frequencies in animal blood cells can raise concerns about the environmental impact of chemicals, as their genotoxic effects on aquatic organisms can have long-term consequences for population health, ecosystem stability, and biodiversity.

The occurrence of micronuclei (MN) frequencies in *Clarias gariepinus* blood cells after exposure to fatal quantities of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE) reveals considerable genotoxic effects and genetic consequences. The concentration-dependent response suggests that as exposure levels increase, there is a corresponding increase in genetic damage and chromosomal instability within the fish (Rodrigues et al., 2016). Micronuclei are aberrant structures generated during cell division, signifying genetic damage or chromosomal instability (Boukaba et al., 2022). The elevated % MN frequencies observed at higher concentrations of X:D and O-BE suggest that these chemicals induce significant genetic alterations within the fish, potentially leading to mutations or chromosomal abnormalities (Obiakor et al., 2014).

The increase in % MN frequencies with elevated concentrations of X:D and O-BE highlights the additional genetic damage caused by these substances compared to the baseline (Langie et al., 2015). The highest % MN frequencies at the highest concentrations indicate a more pronounced impact on genetic stability and a potentially increased risk of genetic damage and chromosomal instability at these lethal levels of exposure (Guo et al., 2019).

The finding of elevated micronuclei frequencies in *Clarias gariepinus* blood cells raises serious concerns regarding the genetic consequences of aquatic creatures on population health, reproduction, and the long-term sustainability of aquatic environments (Jimoh and Sogbanmu, 2021; Amaeze et al., 2020). The concentration-dependent response and the greatest % MN frequencies at the most elevated concentrations indicate the severity of the genetic damage, stressing the vital need for tougher restrictions and monitoring to preserve aquatic habitats from these dangerous compounds.

The sequence alignment of consensus sequences for *Clarias gariepinus* exposed to lethal concentrations of oilfield-based emulsifiers (O-BE) offers valuable insights into the genotoxic and genetic implications of this exposure. The alignments allow for the comparison of DNA sequences between the control group (0 ml/l) and experimental groups exposed to lethal concentrations of O-BE. Statistically significant gaps or differences in the alignments indicate notable variations

in the DNA sequences induced by exposure to O-BE, such as substitutions, deletions, insertions, or other alterations in the genetic code.

The alignment patterns visually demonstrate molecular changes in the DNA sequences of *Clarias gariepinus* resulting from exposure to lethal concentrations of O-BE, which could signify mutations or genetic damage induced by the toxicant, potentially leading to alterations in the genetic makeup or functional changes in specific genes (Hamilton et al., 2016). The inclusion of multiple lethal concentration levels in the experimental design allows for a comprehensive analysis of the genetic impact at various exposure levels, enabling the assessment of a potential dose-response relationship (Altenburger et al., 2012; Guerard et al., 2015).

Understanding the genetic variations induced by lethal concentrations of O-BE in *Clarias gariepinus* is crucial for assessing the broader environmental impact, as genetic changes in aquatic organisms can have implications for population health, adaptation, and ecosystem dynamics, potentially affecting the long-term health and stability of aquatic ecosystems (Rusconi et al., 2018; Schuijt et al., 2021).

The study revealed significant DNA sequence changes, indicating substantial genotoxic effects and genetic implications (Schwarz et al., 2012). The differences in DNA sequence alignment patterns between the control group and those exposed to X:D indicate notable variations in the genetic makeup induced by this chemical exposure. These differences can include mutations, insertions, deletions, or substitutions in the DNA sequences (Sehn, 2015).

The distinct differences in the alignment patterns suggest a pronounced impact of X:D on the genetic material of *Clarias gariepinus* at sub-lethal concentrations, potentially leading to genetic mutations or disruptions in gene function within the fish. The observed DNA sequence changes underscore the genotoxic effects of X:D on a molecular level, indicating that X:D induces genetic damage and instability within *C. gariepinus* (Niaz et al., 2020).

The genetic implications of chemical exposure on aquatic organisms such as *C. gariepinus* are crucial to assessing their broader impact on ecosystem dynamics and population health. Hamilton et al., (2017), emphasized that genetic changes induced by environmental pollutants can have long-term effects on fitness, adaptability, and overall health of these organisms, potentially affecting ecosystem stability.

Genetic consequences and genotoxic effects of Xylene: Diesel (X:D) exposure on *Clarias gariepinus* have been demonstrated by sequence alignment of DNA sequences. These findings indicate significant alterations in the genetic composition of the fish, including mutations, deletions, insertions, or substitutions. Sequence alignments at different lethal concentrations reflect the molecular changes caused by exposure to X:D, indicating possible genetic damage or instability (Pessina et al., 2019).

According to Hamilton et al. (2016), lethal concentrations are crucial as they represent the immediate and severe impacts of environmental exposures on aquatic organisms' genetic makeup. As genotoxic effects and genetic alterations caused by environmental pollutants may adversely affect aquatic ecosystems, population health, reproductive success, and overall ecosystem stability (Moon et al., 2020), it is essential to understand how *C. gariepinus* responds to lethal concentrations of X:D.

The findings emphasize the importance of incorporating genetic responses into environmental risk assessments and regulatory frameworks. Monitoring genetic alterations in response to lethal concentrations of pollutants like X:D aids in evaluating potential risks posed by these substances and supports the development of effective regulatory measures to protect aquatic organisms and ecosystems.

The study uncovers significant genetic variations in *Clarias gariepinus,* shedding light on potential adaptations or responses of the fish's DNA makeup to environmental stressors. These variations, evident in the nucleotide sequence, indicate potential adaptive mechanisms in the fish's genetic material in response to environmental stressors (Petitjean et al., 2019). The differential alignment patterns observed at each sub-lethal concentration underscore the unique responses of the fish's genetic material to varying levels of X: D exposure, possibly involving mutations, insertions, deletions, or other alterations.

The identified variations in consensus sequences provide a molecular perspective on how *C. gariepinus* responds to sub-lethal concentrations of X:D, offering insights into the impact of environmental stressors on aquatic organisms and their potential adaptability to changing environments. The findings contribute to a deeper understanding of the genetic implications of sub-lethal X:D concentrations on *C. gariepinus*. Genetic variations induced by environmental stressors

can have enduring consequences on population health, adaptability, and overall ecosystem dynamics, influencing the resilience of aquatic ecosystems (Ao et al., 2015; Petitjean et al., 2019).

The molecular responses of *C. gariepinus* to sub-lethal X:D concentrations offer valuable insights that can inform conservation strategies aimed at preserving biodiversity and ecosystem health. By providing a nuanced understanding of the genotoxic and genetic implications of environmental stressors, the study contributes to the assessment of environmental health and guides the development of sustainable conservation and management practices. Overall, these insights highlight the importance of considering genetic responses in assessing the impact of environmental stressors on aquatic ecosystems, emphasizing the need for proactive conservation measures to maintain the integrity of these vital environments.

5. Conclusion

A study on *Clarias gariepinus* revealed variations in DNA concentrations across exposure concentrations of Xylene: Diesel (X:D) and Oilfield-Based Emulsifiers (O-BE), suggesting a potential impact on the fish's DNA content. The lack of significant differences in DNA purity suggests a complex interaction between the chemicals and the fish's DNA. Further research using genotoxicity assays or specific genetic analyses could provide a more comprehensive understanding of the genotoxic and genetic implications of these exposures on *C. gariepinus*. The study also revealed significant changes in micronuclei (MN) counts at lethal concentrations, suggesting potential genotoxic effects on aquatic organisms, potentially leading to mutations or chromosomal instability. The concentration-dependent response and highest % MN frequencies at the most elevated concentrations highlight the severity of the genetic impact, emphasizing the need for stricter regulations and monitoring to safeguard aquatic environments from harmful substances. The findings emphasize the importance of incorporating genetic responses into environmental risk assessments and regulatory frameworks to protect aquatic organisms and ecosystems.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Alimba CG, Bakare AA. In vivo micronucleus test in the assessment of cytogenotoxicity of landfill leachates in three animal models from various ecological habitats. Ecotoxicology. 2016 Mar, 25:310-9.

[2] Altenburger R, Scholz S, Schmitt-Jansen M, Busch W, Escher BI. Mixture toxicity revisited from a toxicogenomic perspective. Environmental science & technology. 2012 Mar 6, 46(5):2508-22.

[3] Amaeze NH, Komolafe BO, Salako AF, Akagha KK, Briggs TM, Olatinwo OO, Femi MA. Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. Heliyon. 2020 Aug 1, 6(8).

[4] Ao J, Mu Y, Xiang LX, Fan D, Feng M, Zhang S, Shi Q, Zhu LY, Li T, Ding Y, Nie L. Genome sequencing of the perciform fish *Larimichthys crocea* provides insights into molecular and genetic mechanisms of stress adaptation. PLoS genetics. 2015 Apr 2, 11(4):e1005118.

[5] Baldwin WS, Bain LJ, Di Giulio R, Kullman S, Rice CD, Ringwood AH, van den Hurk P. 20th Pollutant Responses in Marine Organisms (PRIMO 20): Global issues and fundamental mechanisms caused by pollutant stress in marine and freshwater organisms. Aquatic Toxicology. 2020 Oct 1, 227:105620.

[6] Bamberger M, Oswald RE. Unconventional oil and gas extraction and animal health. Environmental Science: Processes & Impacts. 2014, 16(8):1860-5.

[7] Bickham JW, Sandhu S, Hebert PD, Chikhi L, Athwal R. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutation research/Reviews in Mutation research. 2000 Jun 1, 463(1):33-51.

[8] Bickham JW, Sandhu S, Hebert PD, Chikhi L, Athwal R. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutation research/Reviews in Mutation research. 2000 Jun 1, 463(1):33-51.

[9] Boukaba A, Liu J, Ward C, Wu Q, Arnaoutov A, Liang J, Pugacheva EM, Dasso M, Lobanenkov V, Esteban M, Strunnikov AV. Ectopic expression of meiotic cohesin generates chromosome instability in cancer cell line. Proceedings of the National Academy of Sciences. 2022 Oct 4, 119(40):e2204071119.

[10] Brophy D, Rodríguez-Ezpeleta N, Fraile I, Arrizabalaga H. Combining genetic markers with stable isotopes in otoliths reveals complexity in the stock structure of Atlantic bluefin tuna (Thunnus thynnus). Scientific Reports. 2020 Sep 7, 10(1):14675.

[11] Chittick EA, Srebotnjak T. An analysis of chemicals and other constituents found in produced water from hydraulically fractured wells in California and the challenges for wastewater management. Journal of environmental management. 2017 Dec 15, 204:502-9.

[12] Colin N, Porte C, Fernandes D, Barata C, Padrós F, Carrassón M, Monroy M, Cano-Rocabayera O, De Sostoa A, Piña B, Maceda-Veiga A. Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. Science of the Total Environment. 2016 Jan 1, 540:307-23.

[13] Costa PM. Current aspects of DNA damage and repair in ecotoxicology: a mini-review. Ecotoxicology. 2022 Jan, 31(1):1-1.

[14] Demeke T, Jenkins GR. Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. Analytical and bioanalytical chemistry. 2010 Mar, 396:1977-90.

[15] Dmitrieva D, Romasheva N. Sustainable development of oil and gas potential of the Arctic and its shelf zone: The role of innovations. Journal of Marine Science and Engineering. 2020 Dec 8, 8(12):1003.

[16] Guerard M, Baum M, Bitsch A, Eisenbrand G, Elhajouji A, Epe B, Habermeyer M, Kaina B, Martus HJ, Pfuhler S, Schmitz C. Assessment of mechanisms driving non-linear dose–response relationships in genotoxicity testing. Mutation Research/Reviews in Mutation Research. 2015 Jan 1, 763:181-201.

[17] Guo X, Ni J, Liang Z, Xue J, Fenech MF, Wang X. The molecular origins and pathophysiological consequences of micronuclei: New insights into an age-old problem. Mutation Research/Reviews in Mutation Research. 2019 Jan 1, 779:1-35.

[18] Gupta AK, Ahmad M. Genotoxicity Assessment of Surface Water with Special Reference to Oil Refinery Effluent: A Review. Imperial Journal of Interdisciplinary Research (IJIR). 2017, 3:39-49.

[19] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. InNucleic acids symposium series 1999 Jan 1 (Vol. 41, No. 41, pp. 95-98).

[20] Hamilton PB, Cowx IG, Oleksiak MF, Griffiths AM, Grahn M, Stevens JR, Carvalho GR, Nicol E, Tyler CR. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals–a critical review. Fish and Fisheries. 2016 Sep, 17(3):545-66.

[21] Hamilton PB, Cowx IG, Oleksiak MF, Griffiths AM, Grahn M, Stevens JR, Carvalho GR, Nicol E, Tyler CR. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals–a critical review. Fish and Fisheries. 2016 Sep, 17(3):545-66.

[22] Hamilton PB, Rolshausen G, Uren Webster TM, Tyler CR. Adaptive capabilities and fitness consequences associated with pollution exposure in fish. Philosophical Transactions of the Royal Society B: Biological Sciences. 2017 Jan 19, 372(1712):20160042.

[23] Hanana H, Taranu ZE, Turcotte P, Gagnon C, Kowalczyk J, Gagné F. Evaluation of general stress, detoxification pathways, and genotoxicity in rainbow trout exposed to rare earth elements dysprosium and lutetium. Ecotoxicology and Environmental Safety. 2021 Jan 15, 208:111588.

[24] Hartwig A, Arand M, Epe B, Guth S, Jahnke G, Lampen A, Martus HJ, Monien B, Rietjens IM, Schmitz-Spanke S, Schriever-Schwemmer G. Mode of action-based risk assessment of genotoxic carcinogens. Archives of toxicology. 2020 Jun, 94:1787-877.

[25] Hu J, Raikhel V, Gopalakrishnan K, Fernandez-Hernandez H, Lambertini L, Manservisi F, Falcioni L, Bua L, Belpoggi F, L. Teitelbaum S, Chen J. Effect of postnatal low-dose exposure to environmental chemicals on the gut microbiome in a rodent model. Microbiome. 2016 Dec, 4:1-1.

[26] Huang S. Genetic and non-genetic instability in tumor progression: link between the fitness landscape and the epigenetic landscape of cancer cells. Cancer and Metastasis Reviews. 2013 Dec, 32:423-48.

[27] Jiang N, Naz S, Ma Y, Ullah Q, Khan MZ, Wang J, Lu X, Luosang DZ, Tabassum S, Chatha AM, Basang WD. An Overview of Comet Assay Application for Detecting DNA Damage in Aquatic Animals. Agriculture. 2023 Mar 5, 13(3):623.

[28] Jimoh RO, Sogbanmu TO. Sublethal and environmentally relevant concentrations of triclosan and triclocarban induce histological, genotoxic, and embryotoxic effects in *Clarias gariepinus* (Burchell, 1822). Environmental Science and Pollution Research. 2021 Jun, 28:31071-83.

[29] Langie SA, Koppen G, Desaulniers D, Al-Mulla F, Al-Temaimi R, Amedei A, Azqueta A, Bisson WH, Brown D, Brunborg G, Charles AK. Causes of genome instability: the effect of low dose chemical exposures in modern society. Carcinogenesis. 2015 Jun 1, 36(Suppl_1): S61-88.

[30] Lin J, Verhulst S, Alonso CF, Dagnall C, Gadalla S, Hastings WJ, Lai TP, Shalev I, Wang Y, Zheng YL, Epel E. Effects of DNA extraction, DNA integrity, and laboratory on the precision of qPCR-based telomere length measurement-a multi-lab impartial study. bioRxiv. 2022 Dec 14:2022-12.

[31] Long J, Feinstein L, Bachmann C, Birkholzer J, Camarillo M, Domen J, Foxall W, Houseworth J, Jin L, Jordan P, Lindsey N. An Independent Scientific An Independent Scientific Assessment of Assessment of Well Stimulation in California Volume II Potential Environmental Impacts of Hydraulic Fracturing and Acid Stimulations.

[32] Mahmoudi N, Beaupré SR, Steen AD, Pearson A. Sequential bioavailability of sedimentary organic matter to heterotrophic bacteria. Environmental microbiology. 2017 Jul, 19(7):2629-44.

[33] Manzoor M, Bhat KA, Khurshid N, Yatoo AM, Zaheen Z, Ali S, Ali MN, Amin I, Mir MU, Rashid SM, Rehman MU. Bio-indicator species and their role in monitoring water pollution. InFreshwater pollution and aquatic ecosystems 2021 Dec 22 (pp. 321-347). Apple Academic Press.

[34] Moon WK, Atique U, An KG. Ecological risk assessments and eco-toxicity analyses using chemical, biological, physiological responses, DNA damages and gene-level biomarkers in Zebrafish (*Danio rerio*) in an urban stream. Chemosphere. 2020 Jan 1, 239:124754.

[35] Musto M, Faraone D, Cellini F, Musto E. Changes of DNA quality and meat physicochemical properties in bovine supraspinatus muscle during microwave heating. Journal of the Science of Food and Agriculture. 2014 Mar 15, 94(4):785-91.

[36] Niaz K, Shah SZ, Khan F, Bule M. Ochratoxin A–induced genotoxic and epigenetic mechanisms lead to Alzheimer disease: its modulation with strategies. Environmental Science and Pollution Research. 2020 Dec, 27(36):44673-700.

[37] Nwizugbo KC, Ogwu MC, Eriyamremu GE, Ahana CM. Alterations in energy metabolism, total protein, uric and nucleic acids in African sharptooth catfish (*Clarias gariepinus* Burchell) exposed to crude oil and fractions. Chemosphere. 2023 Mar 1, 316:137778.

[38] Obiakor MO, Okonkwo JC, Ezeonyejiaku CD. Genotoxicity of freshwater ecosystem shows DNA damage in preponderant fish as validated by in vivo micronucleus induction in gill and kidney erythrocytes. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2014 Dec 1, 775:20-30.

[39] Pampanin DM, Brooks SJ, Grøsvik BE, Le Goff J, Meier S, Sydnes MO. DNA adducts in marine fish as biological marker of genotoxicity in environmental monitoring: the way forward. Marine Environmental Research. 2017 Apr 1, 125:49-62.

[40] Pandey AK, Nagpure NS, Trivedi SP. Genotoxicity assessment of pesticide profenofos in freshwater fish Channa punctatus (Bloch) using comet assay and random amplified polymorphic DNA (RAPD). Chemosphere. 2018 Nov 1, 211:316-23.

[41] Parolini M, Ghilardi A, Della Torre C, Magni S, Prosperi L, Calvagno M, Del Giacco L, Binelli A. Environmental concentrations of cocaine and its main metabolites modulated antioxidant response and caused cyto-genotoxic effects in zebrafish embryo cells. Environmental Pollution. 2017 Jul 1, 226:504-14.

[42] Pessina F, Giavazzi F, Yin Y, Gioia U, Vitelli V, Galbiati A, Barozzi S, Garre M, Oldani A, Flaus A, Cerbino R. Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors. Nature cell biology. 2019 Oct, 21(10):1286-99.

[43] Petitjean Q, Jean S, Gandar A, Côte J, Laffaille P, Jacquin L. Stress responses in fish: From molecular to evolutionary processes. Science of the Total Environment. 2019 Sep 20, 684:371-80.

[44] Petitjean Q, Jean S, Gandar A, Côte J, Laffaille P, Jacquin L. Stress responses in fish: From molecular to evolutionary processes. Science of the Total Environment. 2019 Sep 20, 684:371-80.

[45] Rodrigues S, Antunes SC, Correia AT, Nunes B. Acute and chronic effects of erythromycin exposure on oxidative stress and genotoxicity parameters of Oncorhynchus mykiss. Science of the Total Environment. 2016 Mar 1, 545:591-600.

[46] Rusconi M, Bettinetti R, Polesello S, Stefani F. Evolutionary toxicology as a tool to assess the ecotoxicological risk in freshwater ecosystems. Water. 2018 Apr 17, 10(4):490.

[47] Savale SK. Genotoxicity of drugs: Introduction, prediction and evaluation. Asian Journal of Biomaterial Research. 2018, 4(6):1-29.

[48] Schuijt LM, Peng FJ, van den Berg SJ, Dingemans MM, Van den Brink PJ. (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: facts, challenges, and future. Science of the total environment. 2021 Nov 15, 795:148776.

[49] Schwarz C, Tiessen C, Kreutzer M, Stark T, Hofmann T, Marko D. Characterization of a genotoxic impact compound in Alternaria alternata infested rice as Altertoxin II. Archives of toxicology. 2012 Dec, 86:1911-25.

[50] Sehn JK. Insertions and deletions (indels). InClinical genomics 2015 Jan 1 (pp. 129-150). Academic Press.

[51] Świacka K, Michnowska A, Maculewicz J, Caban M, Smolarz K. Toxic effects of NSAIDs in non-target species: a review from the perspective of the aquatic environment. Environmental Pollution. 2021 Mar 15, 273:115891.

[52] Turan F, Eken M, Ozyilmaz G, Karan S, Uluca H. Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes River. Ecotoxicology. 2020 Nov, 29:1522-37.

[53] Turkez H, Arslan ME, Ozdemir O. Genotoxicity testing: progress and prospects for the next deade. Expert opinion on drug metabolism & and toxicology. 2017 Oct 3, 13(10):1089-98.

[54] Xu K, Tang Z, Liu S, Xia H, Liu L, Wang Z, Qi P. Effects of low concentrations copper on antioxidant responses, DNA damage and genotoxicity in thick shell mussel *Mytilus coruscus*. Fish & shellfish immunology. 2018 Nov 1, 82:77-83.