CHROMOSOMAL ABERRATIONS INDUCED BY A COMBINATION OF INDUSTRIAL CHEMICALS IN AFRICAN CATFISH (*Clarias gariepinus*) AT LETHAL AND SUBLETHAL LEVELS

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ABSTRACT
The aim is to assess the variation in the karyotype number of chromosomes in C. gariepinus and the genetic consequences of exposure to mixed industrial chemicals in the African Catfish, providing valuable information for environmental management, conservation, and potential human health considerations. From the acclimation tanks, ten healthy and active juveniles were randomly selected with five test concentrations: 0.0ml/l (control), 25ml/l, 50ml/l, 100ml/l, 150ml/l, 200ml/l, and 250ml/l for lethal, and 0.0ml/l (control), 12.8ml/l, 25.59ml/l, 38.39ml/l, 51.19ml/l, and 63.99ml/l. Chromosomal aberration studies were conducted on the fish samples after 4 and 28 days of exposure. The study examines the karyological and cellular content of Clarias gariepinus, revealing a consistent 2n = 56 karyotype across the fish. Variability in individual karyotypes suggests aneuploidy and genetic material loss during gametogenesis. In C. gariepinus exposed to lethal concentrations of mixed chemicals, significant karyotype variations have implications for genetic health and environmental impact. Changes in metacentric, submetacentric, subtelocentric, and acrocentric/telocentric chromosomes signal genetic instability, crucial for species integrity and resilience. Genetic alterations may adversely affect reproductive success, leading to abnormalities in gamete formation and reduced reproductive fitness. Sub-lethal exposure to the mixed induces alterations in the karyotype of C. gariepinus, despite a consistent diploid number of 56. As a result of exposure to the chemical (Xylene: Diesel), mutations may occur, potentially altering karyotype structure and disrupting division control. Sublethal and lethal concentrations cause genotoxic effects. Genetic diversity and health are affected by C. gariepinus populations. For long-term effects to be predicted, it is important to understand the consequences of karyotype changes in C. gariepinus to predict the genetic health of the population in the future.

Keywords: *Genetics, African Catfish, Karyotype, Chemicals, Environmental*

INTRODUCTION

Oil and gas exploration and production in Nigeria, a key economic sector, are not without environmental costs. Modern exploration techniques contribute significantly to pollution and adverse effects on aquatic life. The petroleum industry, particularly offshore oil exploration, poses a significant threat to the health of aquatic biota, particularly the African catfish (*Clarias gariepinus*), a vital fish species in Nigeria and a potential bio-indicator of aquatic ecosystem perturbations (Akinsanya *et al*., 2018; Osisiogu *et al*., 2019).

Toxic chemicals released during well stimulation and cleaning can modify water physicochemical parameters, potentially affecting fish and other aquatic organisms (Gunaalan *et al*., 2020; Aliko *et al*., 2022). This has raised global concerns about the impact of petroleum operations on the environment, particularly on species like the African catfish. According to Orowe and Ikponmwen, 2022), the vulnerability of the African catfish to modifications in the aquatic ecosystem, especially when exposed to chemicals from oil production, is a cause for concern.

As Nigeria grapples with the delicate balance between economic development and environmental sustainability, it becomes imperative to understand the intricate relationship between oilfield chemicals, genetic health, and ecological stability. The petroleum industry must be scrutinized for its environmental footprint. The decline in fish populations globally, attributed to anthropogenic and natural contaminant inputs, calls for comprehensive research on the impact of these contaminants on the genetic composition and health of aquatic organisms (Amoatey and Baawain, 2019; Ali *et al*., 2019).

The ongoing industrial activities, particularly in the oil and gas sector, have raised concerns about their potential environmental impact, specifically on aquatic ecosystems. The

release of industrial chemicals, such as the water-soluble fraction (WSF) of xylene and diesel, into aquatic environments poses a threat to aquatic organisms (Davies, *et al*., 2019a). In this context, the African catfish (*Clarias gariepinus)* emerges as a crucial bio-indicator species, reflecting the ecological health of aquatic ecosystems.

This study endeavours to assess the genetic and chromosomal repercussions induced by the exposure of African catfish to the water-soluble fraction (WSF) of xylene and diesel, two prevalent industrial chemicals. The focus is on understanding the genotoxic effects that these chemicals may exert on the genetic and chromosomal integrity of the African catfish population. Through this assessment, the study also aims to evaluate chromosomal aberrations in African catfish, serving as a biomonitoring and ecotoxicology assessment in Nigeria, where petroleum exploration and production play a pivotal role in income and power. The outcomes have the potential to furnish valuable information for the efficient monitoring and management of aquatic ecosystems influenced by oil and gas exploration.

MATERIALS AND METHODS

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

A total of 420 healthy juvenile *C. gariepinus* juveniles, measuring 15.20±2.3cm and 10.23±2.60g respectively, were obtained from the University of Port Harcourt Demonstration Farm in Nigeria. due to their high sensitivity to environmental stress. Following previous studies (Davies et al., 2019b; Chris et al., 2022), juvenile developmental stages were selected as test organisms due to their heightened sensitivity to environmental stress.

Acclimation of the test organism

The fish were acclimated to laboratory conditions in a 150-litre glass aquarium tank for 14 days at 28±20C, fed commercial fish feed twice daily, and aerated continuously. Bennett and Dooley (1982) recommended replacing water in glass tanks with laboratory tap water every 48 hours.

Test Chemicals

The study used Water Soluble Fraction (WSF) of Diesel oil purchased from the Nigerian National Petroleum Corporation (NNPC) Filling station in Port Harcourt, Rivers State. Xylene was purchased from an oilfield chemical laboratory in Rivers State, Nigeria, and was stored in an ambient laboratory condition. A working stock solution was prepared from Xylene and Diesel following a standard method and a test chemical was prepared, using a volumetric and analytical method described in previous studies (Orlu and Ogbalu, 2013; Davies *et al*., 2019b).

Selection of Test Organism for the Assay

Ten healthy and active juveniles of uniform size were selected randomly from the acclimation tanks using a hand-held scoop net and transferred carefully into different treatment units for 28 days to test for the sub-lethal effect of the Xylene and Diesel (Sil *et al*., 2010). The experiment was carried out in triplicates including the control. The test was performed using a renewal method to maintain and level of dissolved oxygen minimizing changes due to metabolism by the fish during this experiment (Chris *et al*., 2022). Feeding was suspended 24 hours before the renewable exposure period which lasted for 28 days. Five test concentrations of 0.0ml/l(control), 12.8ml/l, 25.59ml/l, 38.39ml/l, 51.19ml/l, and 63.99ml/l were prepared, each test concentration was held in a plastic aquarium tank of 15 litres and filled to 10 mark. Ten fish were randomly selected and put in each of the test concentrations. Each treatment was replicated.

Selection of test organism for sub-lethal assay

Ten active and healthy fingerlings relatively of uniform size were picked randomly using a hand-held scoop net from the acclimation tanks and transferred carefully into the different treatment units for 96 hours and 28 days to test for the lethal and sub-lethal effects of the Xylene and Diesel (Sil *et al*., 2010). The treatments were in triplicates as well as the control. The test was performed using a static and renewal method and the exposure medium was renewed every week to maintain toxicant strength for the sub-lethal, level of dissolved oxygen, and minimize changes due to metabolism by the fish during this experiment (Chris *et al*., 2022). Feeding was suspended 24 hours before the renewable exposure period which lasted for 28 days. Five test concentrations of 0.0ml/l (control), 25ml/l, 50ml/l, 100ml/l, 150ml/l, 200ml/l, and 250ml/l for the lethal while 0.0ml/l (control), 12.8ml/l, 25.59ml/l, 38.39ml/l, 51.19ml/l, and 63.99ml/l for the sub-lethal were prepared, each test concentration was held in plastic aquarium tank of 15 litres and filled to 10 mark. Ten fish were

randomly selected and put in each of the test concentrations. Each treatment was replicated. After 4 and 28 days of exposure, the fish samples were then taken to the Regional Center for Biotechnology and Bioresources Research laboratory at the University of Port Harcourt for Chromosomal aberration studies. Analyses

Preparation of Metaphysic Chromosomes Procedure

The test organism (*C. gariepinus*) was placed into a 250ml beaker containing 0.007% colchicine solution prepared in freshwater and allowed to swim for 4 hours. The sample of the gill was chopped into 2-3 mm small pieces using a clean razor blade and the pieces were exposed to a hypotonic solution of 0.4% KCl (20-30 minutes). The hypotonic solution was discarded and the tissues were fixed by washing the chopped pieces twice in a freshly made cold mixture of 3:1 ethanol: acetic acid for 30 minutes. The tissue was stored in a fixative (acetone) for several hours at 4ºC. The pieces were removed from the fixative and the excess fixative was dried on a paper towel. The pieces were then placed on a micro slide and 2-3 drops of 50% acetic acid were added. The pieces were chopped until a cell suspension was formed.

The micro slides were thoroughly cleared of any form of contaminants using a 1:1 ether: ethanol mixture and were warmed on a heat plate at 40-50ºC. The cell suspension was taken with a Pasteur pipette and allowed to fall from a distance of 12cm on a precleaned and prewarmed micro slide. This process was repeated several times forming 2-3 rings of cells 1cm in diameter, and then the chromosome preparation was allowed to dry for 10-15 minutes. Finally, the chromosomes were stained with 15% Giemsa for 10-15minutes. The slides were prepared using the standard protocol of Henegariu *et al*. (2001), air-dried, covered and viewed under a binocular LED Microscope (Model-215-RLED-ASC). Well-separated chromosome metaphases were observed in the periphery of the rings formed and were photographed.

A karyogram was prepared by high contrast chromosome photographs and the individual chromosomes were cut out of the photographs. Classification and karyotyping of the chromosomes were performed according to the techniques described by Levan *et al*. (1964) and Ergene *et al*. (1998). The final karyogram was then scanned and printed.

Statistical Method

The results were analyzed using a Microsoft Excel sheet (Microsoft Office suite (2022) for a graphical representation and the standard error.

Ethical Approval

Ethical approval was obtained from the Office of Research and Development (Research Ethics Committee) at the University of Port Harcourt after due deliberation and my research proposal was approved.

RESULTS

Karyotype change associated with the exposure of *C. gariepinus* to Lethal concentration of Combined Oilfield chemicals.

The results presented in Table 1 demonstrate the impact of lethal concentrations of the combined chemicals (Xylene and Diesel) exposure on the karyotype of *C. gariepinus*. The diploid number of chromosomes (2n=56) remained consistent across all fish samples exposed to different concentrations of the toxicants, indicating no significant variations in the overall chromosomal count. However, the study reveals significant variations ($P<0.05$) in the shape and number of karyotypes, specifically classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric/telocentric (A/T).

At 0 ml/l, the karyotype contains 12 metacentrics (M), 16 submetacentric (SM), 8 subtelocentric (ST), and 14 acrocentric/telocentric (A/T) chromosomes, maintaining the diploid number of 56. Although the diploid number (2n=56) remains unchanged, there are changes in the karyotype, with an increase in metacentric (M) and subtelocentric (ST) chromosomes and a decrease in submetacentric (SM) and acrocentric/telocentric (A/T) chromosomes. As with the 25 ml/l concentration, the diploid number remains constant at 56, but there are additional changes in karyotype, with the metacentric (M) and subtelocentric (ST) chromosomes increasing and the submetacentric (SM) and acrocentric/telocentric (A/T) chromosomes decreasing. A concentration of 100 ml/l results in a diploid number of 56, but a modification of the karyotype, including an increase in metacentric chromosomes (M) and a decrease in

submetacentric, subtelocentric, and acrocentric/telocentric chromosomes (A/T). The concentrations of 150 and 200 ml/l exhibit consistent changes in the karyotype, with an increase in metacentric (M) and submetacentric (SM) chromosomes and a decrease in subtelocentric (ST) and acrocentric/telocentric (A/T) chromosomes, while maintaining the diploid number. The karyotype exhibits alterations with increased metacentric (M) chromosomes, decreased submetacentric (SM) and subtelocentric (ST) chromosomes, and maintenance of the diploid number at 56 (Fig. 1 to 4) while well-separated chromosome metaphases were observed in the periphery of the rings formed as photographed in at Fig. 5.

Table 1: The chromosomes of *C. gariepinus* arranged according to type after exposure to different lethal concentrations of Xylene: Diesel.

Concentration (ml/l)	Karyotype type				Diploid chromosome numbers
	M	SM	ST	A/T	
0	12	16	8	14	2n =56
25	20	16	8	12	2n =56
50	20	14	8	14	2n =56
100	22	16	8	10	2n =56
150	26	18	6	6	2n =56
200	28	18	6	6	2n =56
250	28	16	4	8	2n =56

*Metacentric (M), Submetacentric (SM), Subtelocentric (ST) and Acrocentric/Telocentric (A/T)

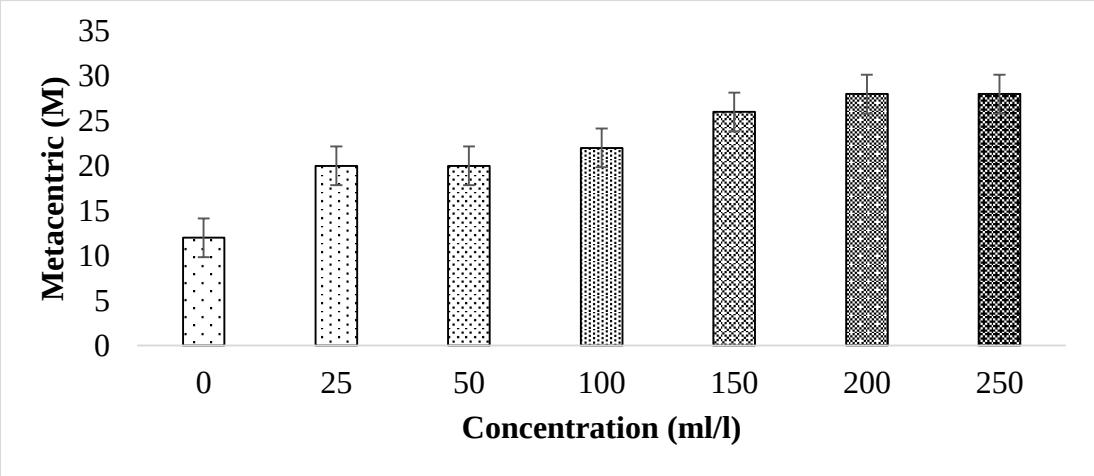

Fig. 1: Variations in metacentric (M) chromosomes after exposure lethal concentrations of the text chemicals.

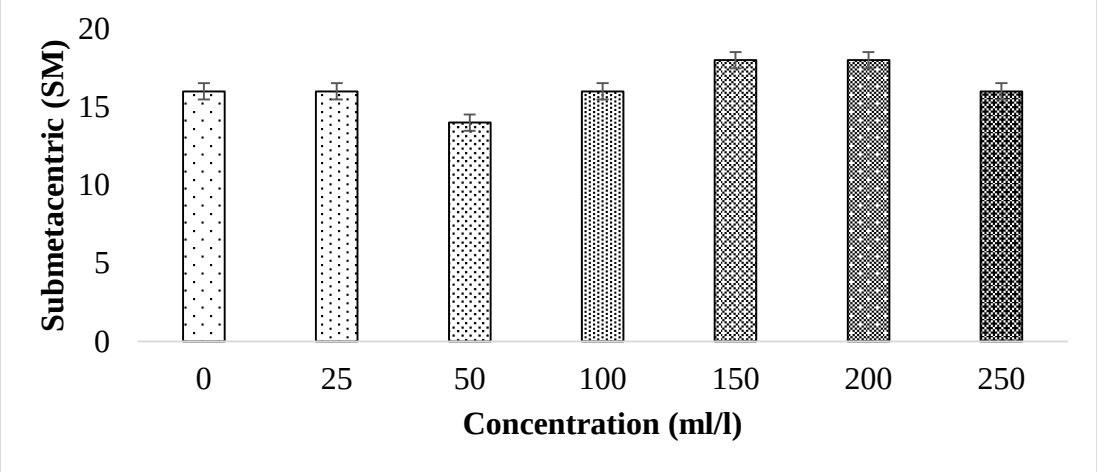

Fig. 2: Variations in Submetacentric (SM) chromosomes after exposure to lethal concentrations of the test chemicals.

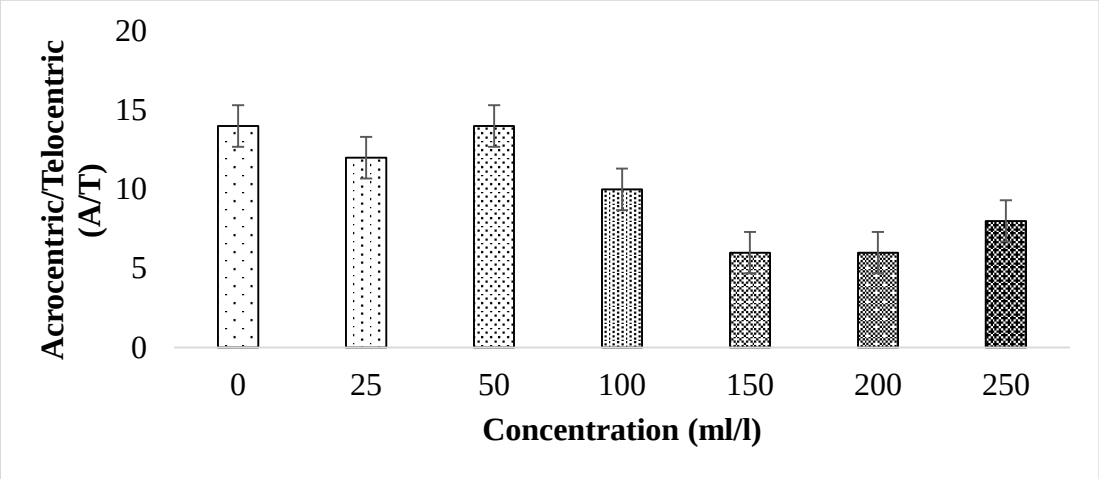

Fig. 3: Variations in Acrocentric/Telocentric (A/T) chromosomes after exposure to lethal concentrations of the test chemicals.

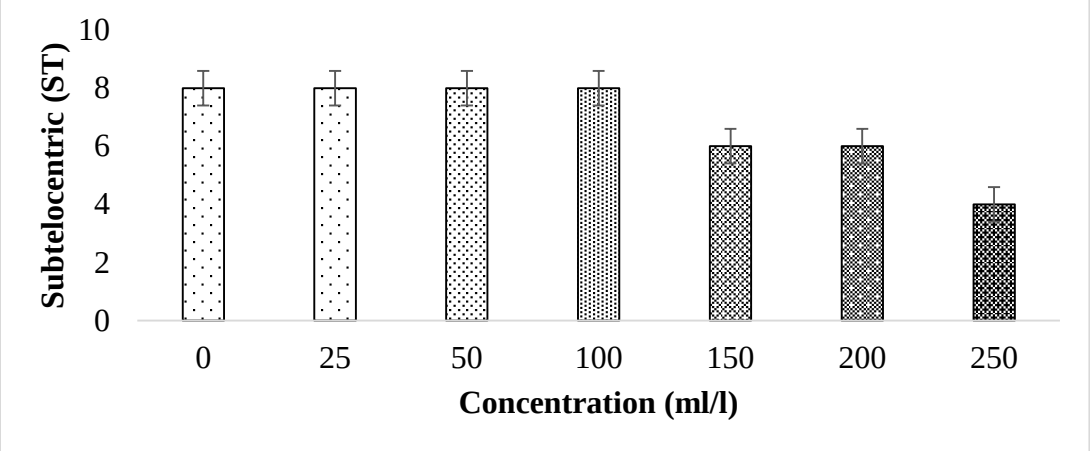

Fig. 4: Variations in Subtelocentric (ST) chromosomes after exposure to lethal concentrations of the test chemicals.

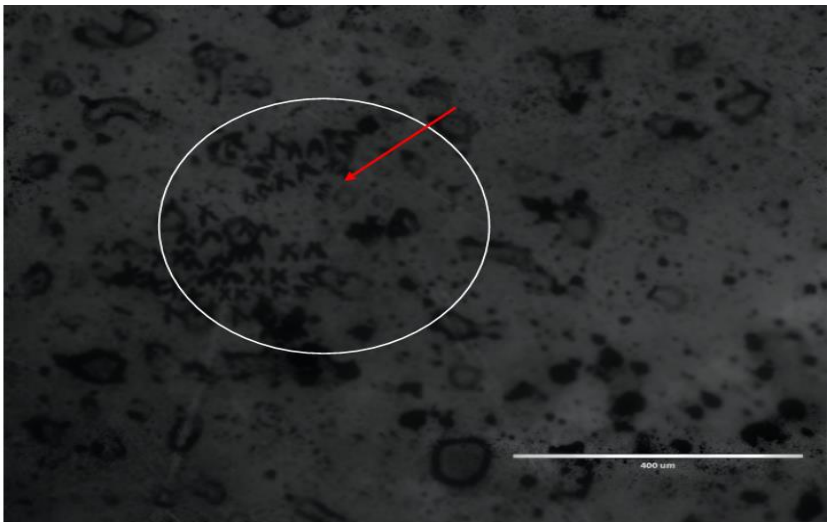

Fig. 5: Metaphase spread of the chromosomes at lethal exposure **(**100X Magnification).

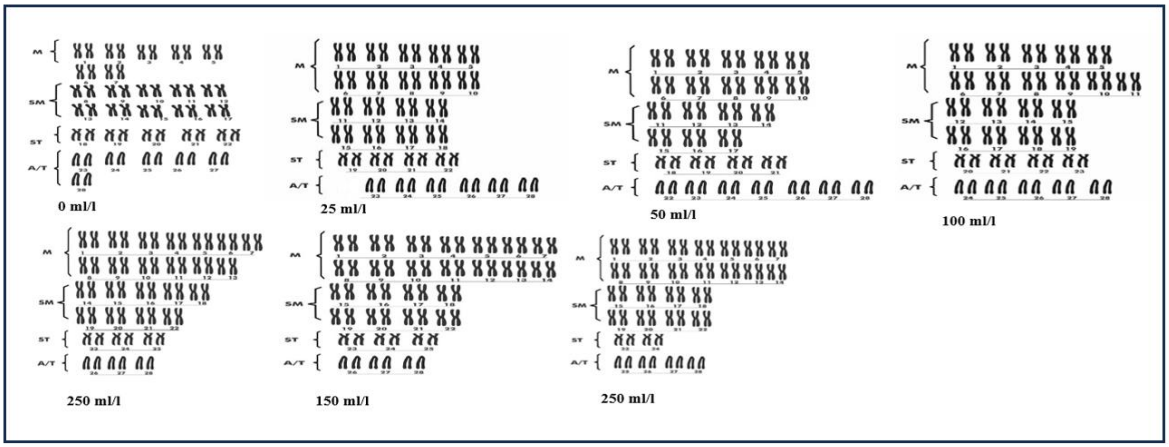

Fig. 6: The karyotype number of chromosomes and abnormalities of *C. gariepinus* exposed to a lethal concentration of Xylene: Diesel at the different levels of exposures (0 ml/l (Control), 25ml/l, 50ml/l, 100ml/l, 150ml/l, 200ml/l, 250ml/l) at 2n=56. Metacentric (M), Submetacentric (SM), Subtelocentric (ST) and Acrocentric/Telocentric (A/T).

Karyotype change associated with the exposure of *C. gariepinus* to Sublethal concentration of Xylene: Diesel

Table 2 shows the chromosomes' karyotype of *C. gariepinus* exposure to a sub-lethal concentration of Xylene: Diesel and most of the specimens showed well-spread chromosomes (Fig.s 4.45 to 4.50). At the end of the experiment, the diploid number of the test fish samples was the same (2n=56) for the different concentration groups, but for the different lethal levels of the toxicant, there was statistical significance in the number and shape of the karyotype such as metacentric, submetacentric, subtelocentric and acrocentric/telocentric of the fish samples (Fig. 6 to 10) while a well-separated chromosome metaphases were observed in the periphery of the rings formed as photographed in at Fig. 11.

Table 2: The karyotype of *C. gariepinus* arranged according to type after exposure to sub-lethal (SL) test concentrations of Xylene: Diesel

Sub-lethal concentration (ml/l)	Karyotype type				Diploid chromosome numbers
	M	SM	ST	A/T	
0	12	18	12	14	2n =56
12.80	12	18	10	16	2n =56
25.59	12	20	10	14	2n =56
38.39	10	24	8	14	2n =56
51.19	10	22	10	14	2n =56
63.99	8	18	10	20	2n =56

*Metacentric (M), Submetacentric (SM), Subtelocentric (ST) and acrocentric/telocentric (A/T)

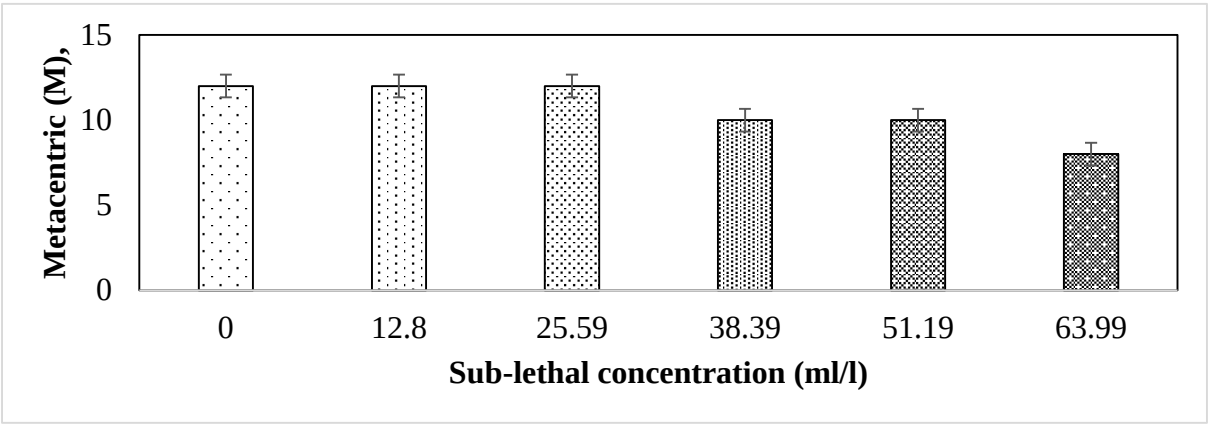

Fig. 7: Variations in metacentric (M) Chromosomes after exposure to Sub-lethal concentrations of the test chemicals.

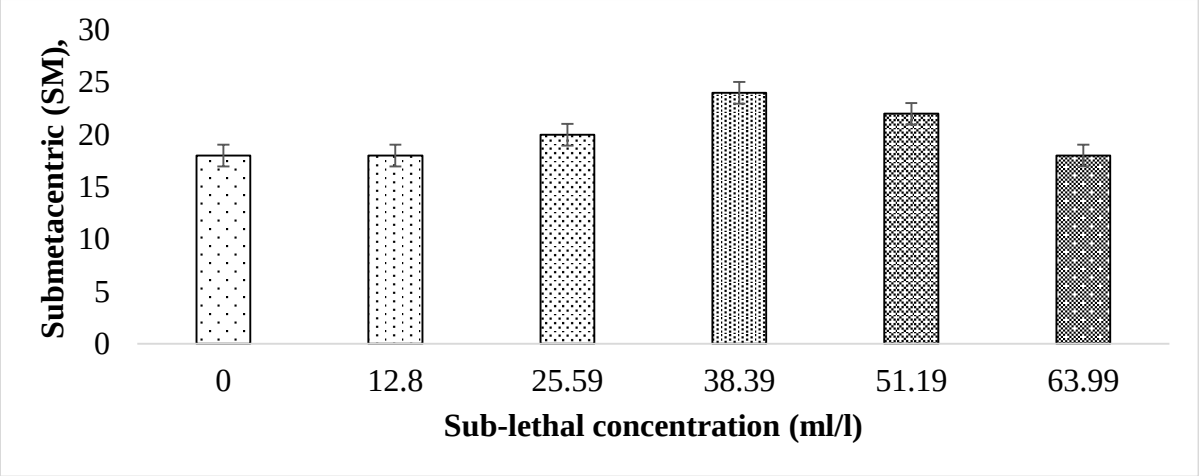

Fig. 8: Variations in Submetacentric (SM) Chromosomes after exposure to Sub-lethal concentrations of the test chemicals.

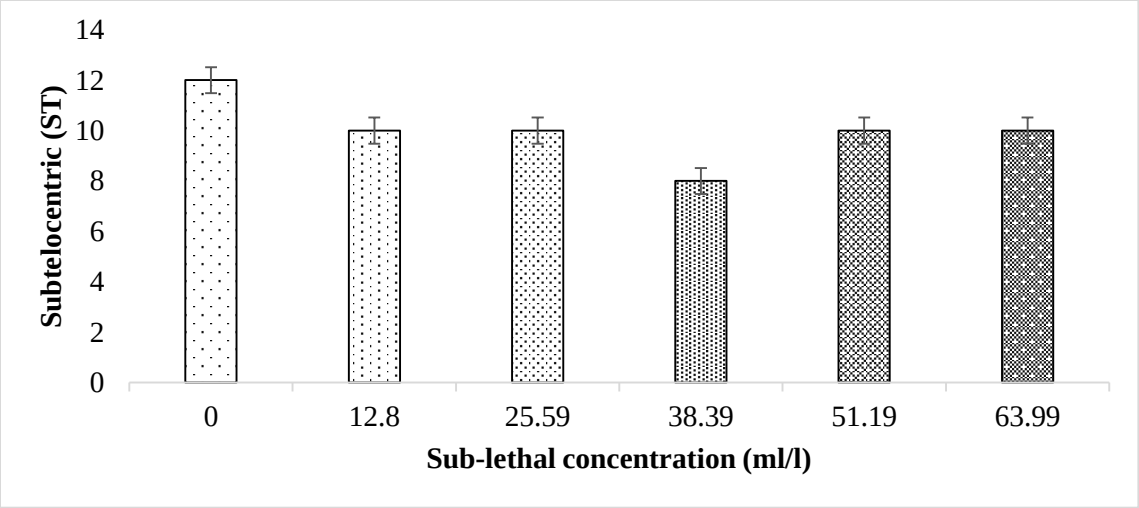

Fig. 9: Variations in Subtelocentric (ST) Chromosomes after exposure to Sub-lethal concentrations of the test chemicals.

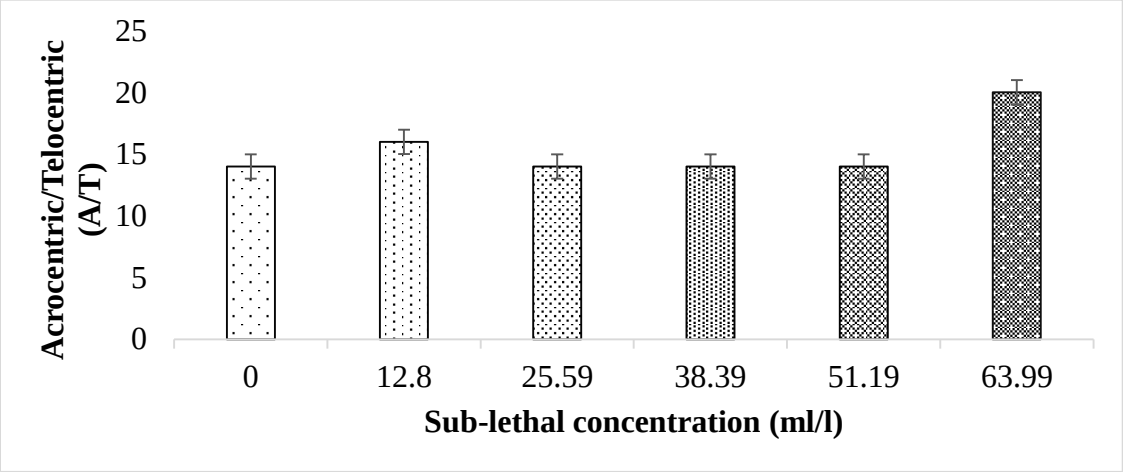

Fig. 10: Variations in Acrocentric/Telocentric (A/T) Chromosomes after exposure to Sub-lethal concentrations of the test chemicals.

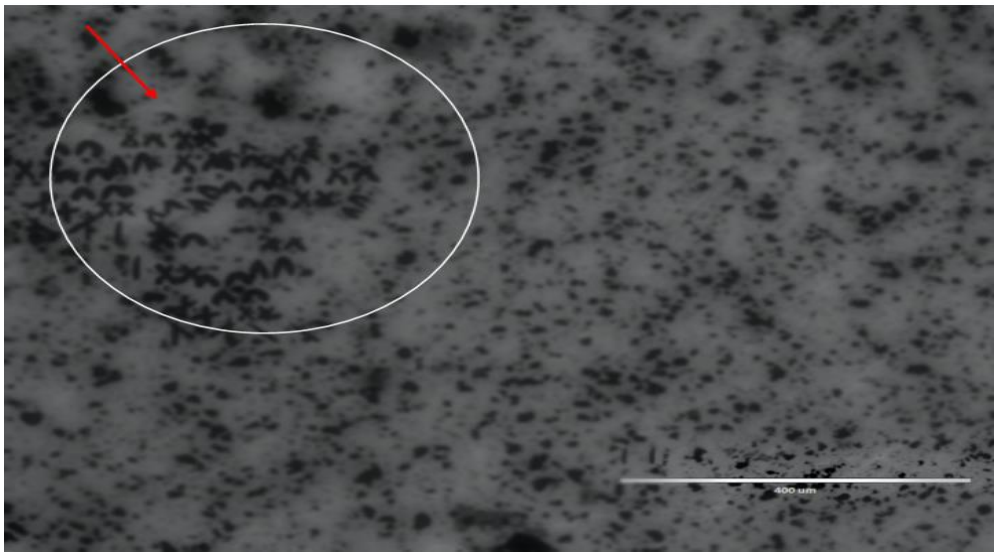

Fig. 11: Metaphase spread of the chromosomes at Sub-lethal exposure (100X Magnification).

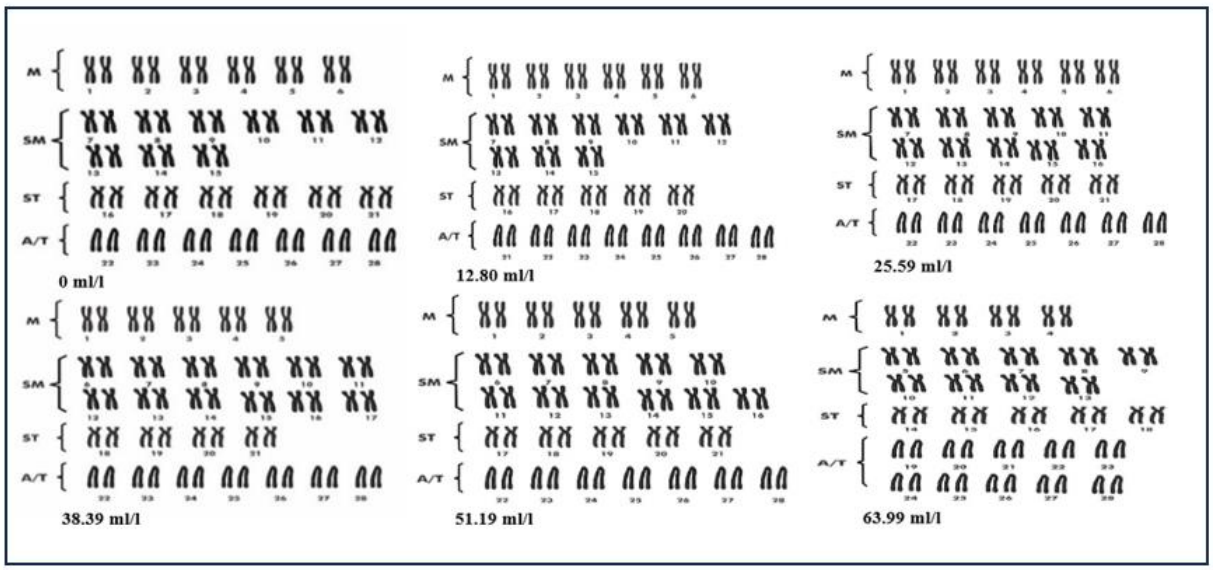

Fig. 12: The karyotype of *Clarias gariepinus* exposed to no toxicant (Control), 12.80ml/l, 25.59ml/l, 38.39ml/l, 51.19ml/l, 63.99ml/l at 2n=56. Metacentric (M), Submetacentric (SM), Subtelocentric (ST) and acrocentric/telocentric (A/T).

DISCUSSION

The study conducted karyological and cellular content studies to comprehend the systematics of fish species, revealing that all fish species exhibited karyotypes with a uniform 2n = 56, showcasing clear variability in the individual karyotype of the test fish. An observed imbalance of genetic material, referred to as aneuploidy, indicated a net loss or gain of genetic material during gametogenesis in the initial zygotic divisions, leading to variations in centromere positions and establishing strict borders between chromosomal categories. According to Kirsch-Volders *et al*. (2019), exposure to different chemical agents may induce mutations, potentially triggering the loss of division control and contributing to changes in the karyotype structure.

Lethal effect on *Clarias gariepinus*

The observed variations in the karyotype of *C. gariepinus* exposed to lethal concentrations of combined chemicals shows significant implications for genetic health and environmental impact. These alterations may potentially affect the genetic health and stability of the fish population. The changes in both the shape and number of karyotypes indicate genetic instability within the fish population, a critical factor for maintaining species integrity and resilience to environmental stressors (van Jaarsveld and Kops, 2016). Alterations in metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric/telocentric (A/T) chromosomes suggest that exposure to toxicants may induce physical damage or rearrangements in the chromosomes of *C. gariepinus*. Such damage can impact the normal functioning of genes and genetic material,

potentially leading to significant consequences for reproductive success (Han and Huang, 2021).

According to Tarín *et al*. (2000), changes in the karyotype may result in abnormalities during gamete formation, affecting fertilization and embryo development, and ultimately leading to reduced reproductive fitness. These observed karyotype variations serve as indicators of environmental stress, reflecting the impact of pollutants in aquatic ecosystems. Given the importance of *C. gariepinus* in maintaining ecological balance, genetic changes within this species may disrupt predator-prey relationships and biodiversity, compromising the general health of the ecosystem (Wangechi Kigano, 2016). The findings underscore the importance of conservation measures to mitigate the impact of anthropogenic activities, such as the release of oilfield chemicals, on aquatic life. Davies *et al*. (2019a), suggest that implementing measures to reduce toxicant exposure can contribute to preserving genetic diversity and overall ecosystem health.

Nevertheless, Hamilton *et al*. (2016) stated that consuming fish with genetic abnormalities or exposure to contaminants could pose risks to human health throughout the food chain. However, the observed variations in the karyotype of *C. gariepinus* highlight the intricate relationship between environmental stressors, genetic health, and ecosystem stability (Obiakor *et al*., 2014). These findings call for interdisciplinary research efforts, conservation initiatives, and environmental management practices to safeguard the genetic integrity of aquatic organisms and maintain the health of aquatic ecosystems.

Sublethal effect on *Clarias gariepinus*

The presented findings illustrate alterations in the karyotype of *C. gariepinus* following exposure to sub-lethal concentrations of Xylene: Diesel. A significant proportion of specimens exhibited well-spread chromosomes, indicative of successful karyotype analysis. The diploid number, consistently recorded at 56 across all test groups, suggests that the fundamental chromosomal structure of the test fish remained unaltered despite exposure (Glover *et al*., 2020). However, statistical analyses revealed noteworthy variations in both the number and configuration of karyotypes, encompassing metacentric (M), submetacentric (SM), sub-telocentric (ST), and acrocentric/telocentric (A/T) types.

Increasing concentrations of the chemical compounds corresponded to observable changes in the karyotype composition. There is a notable fluctuation in the number of metacentric, submetacentric, sub-telocentric, and acrocentric/telocentric chromosomes at different concentrations. However, the observed karyotype variations may imply potential genotoxic effects resulting from exposure to the test chemicals (Turkez *et al*., 2017; Bonciu *et al*., 2018). Such genotoxicity can lead to alterations in chromosomal structure and number, and the occurrence at sub-lethal concentrations suggests that even lower concentrations may exert sub-lethal effects on the genetic makeup of the fish (Žegura *et al*., 2011; Mahaye *et al*., 2017). These chromosomal changes are indicative of environmental stress and pollutant exposure, making karyotype monitoring a valuable tool for assessing the impact of contaminants on aquatic organisms (Daev and Dukelskaya, 2011). Despite the consistent diploid number, alterations in karyotype composition suggest potential risks to the health and genetic diversity of the *C. gariepinus* population.

According to Molina *et al*. (2014), metacentric chromosomes, characterized by a centrally located centromere, may signal structural changes to the fish's genome, while variations in submetacentric chromosomes could reflect genetic instability. Alterations in subtelocentric chromosomes may suggest potential chromosomal rearrangements and changes in acrocentric/telocentric chromosomes may indicate disruptions in chromosomal integrity (Simpson and Simpson, 2008). The findings highlight the significance of karyological content studies in explaining the systematics of fish species, as evidenced by the uniform $2n = 56$ karyotypes in all fish species studied. The observed variability in individual karyotypes of the test fish suggests potential genetic alterations induced by exposure to lethal and sub-lethal concentrations of toxicants (Alimba and Bakare, 2016).

Aneuploidy, characterized by an imbalance of genetic material during gametogenesis, emerged as a notable outcome, signifying potential disruptions in chromosomal integrity (Pellestor and Gatinois, 2018). The changes in centromere positions further delineated strict borders between chromosomal categories, pointing towards the complexity of genetic alterations induced by toxicant exposure.

Kirsch-Volders *et al*., 2019) suggest that mutation occurrence due to exposure to different chemical agents is a critical factor influencing the karyotype changes. Throughout numerous divisions, accumulated mutations may lead to a loss of division control and contribute to variations in karyotype structure (Potapova and Gorbsky, 2017; Baudoin and Bloomfield, 2021). The potential damage to the genotype and phenotype of the organism, including translocations, inversions, deletions, and

duplications, highlights the multifaceted impact of toxicants on genetic material (Beal *et al*., 2017). Accoding to Meistrich (2020), mutations in germ cells have significant implications, potentially causing abnormal development of embryos, prenatal death, or the birth of genetically defective offspring. The observed rearrangements in the karyotype indicate chromosomal aberrations that may contribute to the development of degenerative diseases and increase the frequency of mutations in germ cells, posing a serious threat to the reproduction of different species (Raudsepp and Chowdhary, 2016; Keller *et al*., 2018).

The study underscores the genotoxic effects of exposure to lethal and sub-lethal concentrations of toxicants, emphasizing the harmful nature of induced mutations. The observed variations in acrocentric and metacentric chromosomes further support the notion that the toxicants played a pivotal role in altering the chromosomes' karyotype.

CONCLUSION

The study has found that exposure to lethal and sub-lethal concentrations of Xylene: Diesel can cause significant variations in the karyotype of *C. gariepinus*, raising concerns about potential genotoxic effects and risks to the population's genetic health. These alterations highlight the complexity of genetic responses to environmental stressors and the need for further investigation to understand the underlying mechanisms and genetic implications of these chromosomal alterations. The study recommends further research into the specific genetic mechanisms responsible for these changes, incorporating advanced molecular and cytogenetic analyses. Long-term effects assessments should focus on understanding the consequences of karyotype changes in *C. gariepinus*, which are crucial for predicting the population's future genetic health. Future research should also explore the broader ecological implications of these alterations, focusing on the fish population's genetic diversity and resilience. Interdisciplinary collaboration is encouraged to facilitate a more comprehensive understanding of the observed genetic consequences.

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