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Exposure–Response Relationships Between Petroleum-Related Contaminants, Glutathione-Dependent Detoxification and Oxidative DNA Damage in Clarias gariepinus from Selected Aquatic Ecosystems in the Niger Delta, Nigeria

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Abstract- ***Background: Petroleum-related industrial activities introduce complex mixtures of hydrocarbons and heavy metals into aquatic ecosystems, potentially compromising fish health through oxidative stress and genotoxic damage. Integrating chemical exposure indicators with biochemical and molecular biomarkers provides a comprehensive approach to assessing aquatic environmental stress.***

Objective: This study examined the relationships between petroleum-related contaminants, hepatic detoxification, oxidative stress and DNA damage in African catfish (Clarias gariepinus) across selected aquatic locations in Delta State, Nigeria.

Methods: A comparative dataset comprising 96 fish observations, equally distributed across four locations and two seasons, was analysed alongside 24 environmental sample units. Lead (Pb), cadmium (Cd) and polycyclic aromatic hydrocarbons (PAHs) were examined in relation to glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA) and comet-assay DNA damage. Data were analysed using two-way ANOVA, Pearson correlation, HC3-robust multiple regression and cluster-aware mixed-effects modelling.

Results: Significant spatial differences were observed in all environmental and fish-tissue contaminants ($p < .001$). Location significantly influenced GST, SOD, CAT, MDA and DNA damage, while seasonal effects were significant for GST, GSH, MDA and DNA damage. Tissue Pb exhibited the strongest correlation with DNA damage ($r = .776$, $p < .001$). The regression model explained 69.4% of DNA-damage variability (adjusted $R^2 = .669$), with Pb ($\beta = 8.91$, $p < .001$) and Cd ($\beta = 31.77$, $p = .005$) emerging as significant positive predictors. However, these associations did not remain statistically significant in the cluster-aware sensitivity analysis.

Conclusion: The findings revealed pronounced spatial differences in contaminant burdens and associated biochemical and genotoxic responses. Nevertheless, the sensitivity of adjusted associations to station-season clustering highlighted the importance of accounting for shared environmental conditions when interpreting exposure–response relationships.

Index Terms- ***aquatic biomonitoring, Clarias gariepinus, DNA damage, glutathione S-transferase, heavy metals, oxidative stress, petroleum hydrocarbons***

I. INTRODUCTION

The introduction of complex mixtures of contaminants into aquatic environments has been associated with petroleum exploration, refining, storage, and transport and related industrial activities. They are Total Petroleum Hydrocarbons (TPHs), Polycyclic Aromatic Hydrocarbons (PAHs), heavy metals and their transformation products. Physicochemical properties such as volatility, solubility, hydrophobicity, sediment organic matter, hydrological conditions and microbial transformation affect their environmental distribution and persistence. As a result, water, sediment, and aquatic organisms offer complementary measurements of an environment's contamination, with each matrix measuring a different part of the contaminant distribution, persistence, and biological exposure.

The Niger Delta is a significant geographical location for aquatic ecotoxicological studies due to the presence of petroleum related industrial activities in the region alongside fishing, transportation, domestic water use and other community activities. There is evidence for petroleum associated contamination in the area from previous investigations. For example, Asagbra et al. (2016) analysed 16 priority PAHs in the water, sediments and tilapia of the Warri River at Ubeji. They found PAHs in all three environmental matrices, predominately low molecular weight PAHs and the distribution indicated a petrogenic source. In the same vein, Adewuyi et al., (2011) studied the level of petroleum hydrocarbons and metals in sediments and water samples from Ubeji River. More recently, Ibezim-Ezeani et al. (2022) reported spatial and temporal variations in the concentrations of several metals in sediments collected from different stations in Woji Creek, Niger Delta. Collectively, these investigations demonstrate the continuing relevance of environmental assessment and the importance of obtaining location-specific evidence rather than

assuming that contamination levels remain constant across locations or over time.

There have also been larger scale investigations across the region which have raised concerns about the bio-concentration of pollutants in the aquatic food chain. Elekima et al. (2020) examined heavy-metal concentrations in selected seafood collected from different creeks in Rivers State, while Davies et al. (2022) investigated the distribution of toxic metals in aquatic organisms, sediments and water from the Isaka-Bundu mangrove swamp in the Niger Delta. While these studies offer good regional insights, variations in location, species, environmental matrices, analytical methods and sampling dates makes direct comparisons difficult. Therefore, the regional concentration estimates should not be used in lieu of site-specific measurements, especially if the user wants to compare water concentrations with sediment and/or fish-tissue concentrations on different weight bases.

Fish make excellent biological indicators as they are exposed to environmental contaminants via their gills, skin and food. Depending on how long they live, their tissues can build up contaminants over time, thus revealing a history of internal exposure that can only be determined by tissue analysis. Given its ecological significance and contribution to local fisheries and food systems, as well as its widespread distribution, African catfish (Clarias gariepinus) is particularly relevant to freshwater ecosystems in Nigeria. It has been shown to be useful in the assessment of biochemical responses to environmental contamination in previous investigations in Nigeria. Osioma et al., (2013) for instance, reported that the activities of GSH, GST, SOD, CAT and lipid-peroxidation indicators were altered for C. gariepinus collected from a petroleum-exploration area from Delta State. Likewise, Arojojoye et al. (2021) has observed an aberration in the antioxidant biomarkers and the presence of micronucleated erythrocytes in fish samples obtained from some selected impacted rivers of the Niger Delta compared to those raised in the farm.

Biochemical changes in response to contaminant exposure are tightly linked to xenobiotic metabolism and cellular redox regulation. Biotransformation (Phase-I) of petroleum-derived organic compounds by cytochrome P450-mediated oxidation is possible. These reactions can help the elimination of the compound, but can also produce reactive metabolites that can lead to oxidative stress. Glutathione S-transferases (GSTs) are major enzymes involved in phase-II detoxification which catalyse the conjugation of reduced glutathione with electrophilic compounds, promoting their excretion. Reduced glutathione (GSH) also plays a key role in cell redox balance and as a major intracellular antioxidant. Thus, the induction of the GSTs could reflect an adaptive reaction to the exposure of xenobiotics, but induction does not prove that the amount of detoxification was adequate to prevent cellular damage.

Oxidative stress can occur when the production of ROS is higher than the capacity of the antioxidant defence system and repair mechanisms. Superoxide radicals are converted to hydrogen peroxide by superoxide dismutase (SOD) and peroxide is removed by catalase (CAT) and glutathione-containing antioxidant systems. Malondialdehyde (MDA) is a product of lipid peroxidation, and is widely used as an indicator of oxidative damage to cellular membranes. However, methodological precautions have to be taken for its interpretation, especially when using thiobarbituric-acid-reactive substances assays because of their limited analytical specificity.

Importantly, the extent of antioxidant responses do not always correspond with the level of contaminant exposure. Moderate exposure can lead to an adaptive increase in antioxidant enzyme activity, while chronic or high exposure can result in a decrease in antioxidant capacity, cellular substrate depletion or enzyme inhibition. There can also be different responses from organ to organ and tissue. Variations in the oxidative-stress and antioxidant responses in the various tissues of C. gariepinus were reported by Arojojoye et al. (2007) and Aladesanmi et al. (2017) which showed that when attempting interpretation of biochemical biomarkers, tissue-specific physiological processes should be considered.

In addition to biochemical changes, environmental contaminants could also be responsible for genotoxic effects. The alkaline comet assay is a sensitive method to detect DNA strand breaks and alkali-labile sites in individual cells. But, comet-assay tail DNA alone cannot confirm the permanent genetic mutations, carcinogenicity, or the source of the contaminant causing the DNA damage. Collins et al. (2023) offered methodological advice for the use of the comet-assay, such as sample preparation, protocol optimisation and how to avoid artifactual DNA damage. Even though micronucleus and comet-assay tail DNA are different biological endpoints, fish micronucleus assays can be used complementarily to provide evidence of chromosomal damage.

Although studies have been conducted on petroleum related contamination and oxidative-stress responses of aquatic organisms in Nigeria, it is important to conduct studies that incorporate measurement of environmental contaminant, internal tissue contaminant, glutathione-dependent detoxification and oxidative DNA damage in C.

gariepinus from the Ubeji aquatic environment. This is especially applicable to cases where multiple contaminant exposures and spatial variation are taken into account at the same time as seasonal variation. Furthermore, there is need for a proper consideration of the ecological differences between Ubeji and Omodino Creek as hydrological, salinity, habitat characteristics and contaminant sources may affect the biological response independently of the industrial exposure.

In this context, the study investigated the associations of some petroleum contaminants with biological responses in C. gariepinus. A multidisciplinary approach to aquatic biomonitoring was used, involving environmental chemistry, biochemical biomarkers and genotoxicity indicators. It did not just look at how close sites were to industrial infrastructure or at single biomarkers alone, but at the similarity of the chemical exposure pattern with the changes in detoxification activity, antioxidant status, and lipid peroxidation and DNA integrity.

A. Aim and Objectives

The study aimed to investigate the relationships between petroleum-related contaminant exposure, glutathione-dependent detoxification, oxidative stress and DNA damage in *Clarias gariepinus* across selected aquatic ecosystems in the Niger Delta.

The specific objectives were to:

1) Determine the concentrations of selected petroleum-related contaminants, particularly Pb, Cd and PAHs, in water, sediment and fish tissues across the selected sampling locations.
2) Examine hepatic GST activity and selected antioxidant indicators, namely SOD, CAT and GSH, in fish from the different locations.
3) Assess lipid peroxidation through MDA concentrations and DNA damage using the alkaline comet assay.
4) Investigate relationships between tissue contaminant burdens, biochemical biomarkers and DNA damage, taking seasonal variation and relevant fish characteristics into consideration.
5) Evaluate the combined relevance of chemical and biological indicators in characterising potential exposure–response patterns.

B. Research Questions and Hypotheses

The study addressed the following research questions:

1) Did concentrations of selected contaminants differ significantly among the four sampling locations and between wet and dry seasons?
2) Did hepatic GST, SOD, CAT, GSH and MDA differ significantly across sampling locations and seasons?
3) Did DNA damage, measured through percentage tail DNA, vary significantly among the sampling locations?
4) Were fish-tissue contaminant concentrations significantly associated with GST activity, oxidative-stress indicators and DNA damage?

The following null hypotheses were formulated:

H01: There were no statistically significant differences in selected contaminant concentrations among the sampling locations or seasons.

H02: There were no statistically significant differences in hepatic GST, SOD, CAT, GSH and MDA among the sampling locations or seasons.

H03: There was no statistically significant difference in comet-assay DNA damage among fish sampled from the four locations.

H04: Fish-tissue contaminant concentrations were not significantly associated with biochemical biomarkers or DNA damage.

C. Conceptual and Theoretical Framework

The conceptual and theoretical foundation of this study is an integrated one that explains the relationship between the environmental contamination as a result of petroleum operations, the accumulation of internal toxicants, biochemical responses and damage to DNA of the African catfish, Clarias gariepinus. The principles of the framework are taken from three complementary viewpoints, namely the dose–response principle in environmental toxicology, the Oxidative Stress Theory and Xenobiotic Metabolism and Biotransformation. These perspectives together offer a mechanistic explanation for the entry of environmental contaminants into aquatic organisms, their effects on physiological defense mechanisms and possible role in cellular damage.

The dose–response principle forms the basis for investigating the relationship of exposure and internal contaminant levels to biological responses. Lead (Pb), Cadmium (Cd) and polycyclic aromatic hydrocarbons (PAHs) in the water and sediment reflect external exposure, while the concentrations in fish tissues reflect internal exposure to the contaminants. But toxicological response does not always respond linearly to concentration of exposure and can sometimes be inversely proportional: the characteristics of the contaminants and the duration of exposure, as well as bioavailability, and physiological conditions of the organisms can affect the magnitude and direction of toxicological response (Agathokleous & Calabrese, 2020; Salomão et al., 2020).

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The Oxidative Stress Theory relates to the generation of reactive oxygen species (ROS) in the body that can be excessive and lead to oxidative damage of lipids and other cell components, as these mechanisms of defence are unable to cope. Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) are important components of antioxidant defence in fish and malondialdehyde (MDA) a good indicator of lipid peroxidation. Their assessment together provides a broader insight into oxidative responses than the assessment of an individual biomarker (Menon et al., 2023; Santana et al., 2022).

The Xenobiotic Metabolism and Biotransformation perspective also explains the role of glutathione S-transferase (GST) as a conjugating enzyme, facilitating the elimination of electrophilic compounds through their conjugation with reduced glutathione (GSH). Experimental evidence from Tierbach et al. (2020) demonstrated the functionality of glutathione-dependent biotransformation pathways in zebrafish, while Bastos et al. (2022) reported alterations in GST activity in fish exposed to benzo[a]pyrene, a representative polycyclic aromatic hydrocarbon. These findings reinforce the relevance of glutathione-dependent detoxification mechanisms in fish exposed to environmental contaminants.

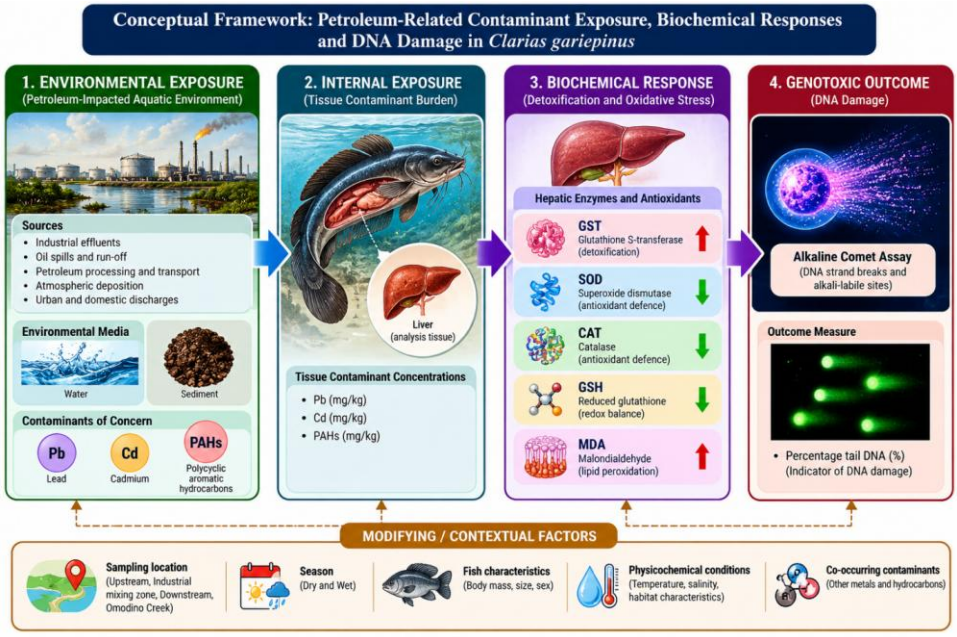


Figure 1: Conceptual Framework: Petroleum-Related Contaminant Exposure, Biochemical Responses and DNA Damage in *Clarias gariepinus*

Based on this, the framework suggests a coherent continuum from environmental contamination to tissue accumulation, detoxification activity, antioxidant response and oxidative damage, that may ultimately result in breaks in the DNA strand and/or alkali-labile lesions measurable by the alkaline comet assay (Collins et al., 2023). Potential modifying factors are recognised as sampling location, season, fish characteristics and environmental conditions. The framework thus offers a mechanistic basis to study the distribution of contaminants and related biological responses, while not assuming causality.

II. MATERIALS AND METHODS

A. Study Area and Sampling Stations

The Ubeji River system in Ubeji community, Warri South Local Government Area, Delta state of the Niger Delta region of Nigeria was proposed as a study area. The River has been mentioned in published investigations as an important element of the local drainage system, for fishing, transportation and other community activities. The PAHs levels were recorded in the water, sediment and fish of the Warri River at Ubeji by Asagbra et al. (2016).

Four sampling sites were selected to assess spatial variations in exposure to the environment and biological responses. Three of them were located along the Ubeji River with respect to an industrial effluent mixing point, and an external comparison ecosystem was the Omodino Creek.

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Table 1: Proposed Sampling Locations and Geographical Coordinates

Station	Sampling location	Sampling purpose	Latitude	Longitude
1	Upstream Ubeji, approximately 200 m before the outfall	Upstream comparison	5°34′46.401″ N	5°42′25.952″ E
2	Industrial effluent-mixing point	Industrial-influence zone	5°34′32.720″ N	5°42′08.163″ E
3	Downstream Ubeji, approximately 200 m after the mixing point	Downstream receiving environment	5°34′39.532″ N	5°41′53.681″ E
4	Omodino Creek mapped locality	External comparison ecosystem	5°32′40.6″ N	5°39′39.1″ E

Habitat and hydrological differences could independently affect contaminant distribution and biological responses and therefore Omodino Creek was not considered uncontaminated and was treated as an external comparison ecosystem.

B. Study Design and Sample Size

A comparative field-biomonitoring design was used for the proposed empirical investigation with four sampling locations and two seasons – dry and wet seasons. The data used in the statistical demonstration that accompanies the text consisted of 96 fish observations, with 12 fish observed at each location-season. An environmental dataset that included 24 sample units for location-season and 3 analytical readings per sample unit.

C. Fish Collection and Biological Data

Proposed biological sampling procedure took the sampling of adults or subadults of comparable size ranges of C. gariepinus by standardised sampling technique. Taxonomic keys recognized were used to confirm species identification. Every specimen was given an individual identification number, and total length, body mass, sex (when determinable), external condition and collection data was recorded.

After collection fish was carried in suitable aerated containers and under proper conditions and was processed promptly. The collection and handling of the tissues was done in compliance with institutional animal ethics approval and the Nigerian regulatory requirement.

Liver was selected as target tissue for GST, SOD, CAT, GSH and MDA, due to its significant role in the metabolism of xenobiotics and antioxidant defense. Gill tissue may have been used to obtain additional evidence of environmental exposure; muscle tissue may have been analysed if there was a question of possible contamination of the edible tissue and/or food-safety issues. Peripheral blood erythrocytes were used for the comet-assay analysis.

D. Environmental and Tissue Contaminant Analysis

At both seasonal periods, water samples and surface sediment samples were collected at each sampling location using sampling containers and chain of custody procedures that have been appropriately cleaned.

Dissolved and/or total-recoverable concentrations of both Pb and Cd were analysed in the water samples, and the filtration and preservation methods were based on the analytical fraction to be analysed. Sediment samples were homogenized and analyzed for moisture content, particle-size distribution and organic matter. Concentrations in sediment were reported on a dry-weight basis and fish-tissue concentrations were clearly designated as wet weight or dry weight.

The concentrations of Pb and Cd were determined by flame or graphite-furnace atomic absorption spectrometry or by inductively coupled plasma mass spectrometry (ICP-MS), based on the availability of the laboratories and the analytical needs. PAHs were extracted using an appropriate validated solvent extraction and clean up method prior to quantification by gas chromatography–mass spectrometry (GC–MS) or another equivalent, validated chromatographic method.

The PAH analysis included the following priority compounds and the sum of the priority compounds. If TPH was also analyzed, it was reported as a separate contaminant as it is a general hydrocarbon measurement and not a chemically specific contaminant.

Quality assurance and quality control consisted of method blanks, field blanks (if applicable), calibration verification, duplicate analyses, matrix-spike recovery, certified reference materials, and limits of detection and quantification.

E. Biochemical Assays

A suitable chilled buffer was used to homogenize the liver tissues and centrifuged according to the validated condition. The supernatant obtained was used for biochemical analysis. Enzymes were determined and the total protein concentration was used for normalisation of the enzyme activities.

For GST activity, 1-chloro-2,4-dinitrobenzene (CDNB) was used as the model substrate and spectrophotometric measurement was used to determine the formation of the glutathione conjugate. Results were reported as specific activity of the enzyme, e.g., as nmol of conjugate produced per min per mg of protein, and the assay temperature, pH,

extinction coefficient and blank correction were included appropriately.

A validated inhibition-based assay was used for the determination of SOD activity and the decomposition of hydrogen peroxide was monitored for the determination of CAT activity. GSH concentration was estimated by using suitable and validated thiol assay and MDA was estimated by using a validated lipid-peroxidation method.

If TBARS was used, the term TBARS or MDA-equivalent was used to reflect the lack of specificity of the method. Where laboratory facilities were available, the chromatographic determination of MDA was preferable.

F. Genotoxicity Assessment

The extent of DNA damage was measured in a suitable cell type, for instance, fish erythrocytes, for which the alkaline comet assay had been validated for the species and sample matrix.

DNA strand breaks and alkali-labile sites were identified using the assay. Slides were coded to reduce observer bias and scoring was done blind to sampling site as much as possible. The main outcome measure was percent tail DNA, with tail length and tail moment being secondary measures if they were already defined and procedures of analysis were already in place.

A validated protocol for sample collection, transportation and processing was employed to reduce the amount of artifactual DNA damage caused by temperature change, extended storage, mechanical stress and excessive light exposure. The use of appropriate positive and negative controls, duplicate gels and image-analysis quality checks were introduced.

The comet assay was interpreted only as a measure of DNA strand breaks and alkali-labile damage under the conditions of the assay. It did not find permanent mutations or carcinogenicity on its own, or a certain contaminant as the cause.

G. Statistical Analysis

Descriptive and inferential statistical techniques were used to analyse data. The main checks performed in preliminary screening were the checks of completeness of data, entry errors, missing observations and outliers. Concentrations of contaminants and biological responses were summarised by mean and standard deviation for each sampling location and season.

The data were analysed using two-way analysis of variance (ANOVA) to assess the influence of location, season and their interaction on the environmental contaminants, fish-tissue contaminant burden and biological indicators. The numbers of field-sample means (n) that were used for environmental analyses were 24, which were the averages of three technical laboratory readings for each sample, and the number of observations that were used for fish-level analyses were 96. Tukey's honestly significant difference (HSD) post-hoc test was then used to further examine significant differences.

Pearson product-moment correlation was used to evaluate the relationships between tissue Pb, Cd and PAHs and chosen biochemical and genotoxicity parameters. Adjusted associations with percentage tail DNA as a dependent variable were examined using multiple linear regression. The HC3 heteroscedasticity-robust standard errors were used and the overall F-statistic and R^2 and adjusted R^2 were used to evaluate the fit of the models. Variance inflation factors (VIF) were used to check for multicollinearity.

There was also a cluster-aware sensitivity analysis using a random-intercept mixed-effects model with eight location-season groups. All statistical tests were assessed at the 0.05 level of significance and the test statistics, coefficients, 95% confidence intervals and p-values were reported.

III. RESULTS

A. Sample Structure

This data consisted of 96 observations of Clarias gariepinus, equally sampled from four sampling sites comprising of 24 fish from each sampling site. 12 fish were provided from each location during the dry season and 12 fish were provided during the wet season. The environmental data were comprised of 24 independent field-sample units, which included three replications for each of the location-season combinations. Three technical readings were taken for each environmental sample and the average of the three readings was used as the analytical value. Technical reading were not considered as independent observation.

Table 2: Distribution of Fish Samples by Location and Season

Sampling location	Dry season	Wet season	Total
Upstream Ubeji	12	12	24
Industrial mixing zone	12	12	24
Downstream Ubeji	12	12	24
Omodino Creek comparison	12	12	24
Total	48	48	96

The fish-level two-factor analyses were based on 88 residual degrees of freedom and the environmental analyses were based on 16 residual degrees of freedom derived from the means of the fish samples in the field.

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B. Test of Hypotheses

Hypothesis One (H_{01}): *There are no statistically significant differences in selected contaminant concentrations among the sampling locations or seasons.*

The highest mean concentrations of all the environmental contaminants analyzed were found in the industrial mixing zone. Two-way analysis of variance (ANOVA) was used to determine if the concentration of contaminants varied significantly among locations, seasons, and in combination.

Table 3: Two-Way ANOVA of Environmental Contaminants

Environmental variable	Location F(3,16)	Location p	Season F(1,16)	Season p	Interaction F(3,16)	Interaction p
Water Pb	81.37	<.001	0.68	.422	0.94	.445
Water Cd	24.53	<.001	0.23	.637	0.02	.997
Water PAHs	47.22	<.001	7.79	.013	0.65	.596
Sediment Pb	64.76	<.001	1.77	.202	0.77	.530
Sediment PAHs	74.43	<.001	1.61	.223	0.47	.708

Results show that there are important differences between regions for all five environmental contaminant indicators. Water PAHs were the only data that were statistically different across seasons. The location-by-season interaction was not significant nor was the effect of season on the other environmental contaminants. The results of Tukey-adjusted comparisons can be seen in the Environmental_Tukey worksheet.

Table 4: Two-Way ANOVA of Fish-Tissue Contaminant Concentrations

Contaminant	Location F(3,88)	p	Season F(1,88)	p	Interaction F(3,88)	p
Pb	108.24	<.001	10.00	.002	1.83	.147
Cd	86.23	<.001	6.60	.012	0.64	.593
PAHs	98.42	<.001	6.82	.011	0.62	.603

The concentrations of all three contaminants in fish tissues were significantly affected by location and season. The lack of a significant interaction, however, suggests that there were no significant seasonal differences between the sampling locations. Tukey comparisons revealed that fish from the industrial mixing zone were different from fish at all other locations for Pb, Cd and PAHs. Fish downstream were also different from the upstream and Omodino fish. There were no significant differences between upstream and Omodino fish for Pb and PAHs, but there were significant differences for Cd.

The results in Tables 3 and 4 above showed that there are significant differences in all five environmental contaminants and in all three fish-tissue contaminants associated with location. A significant difference was found between the levels of water PAHs and all three types of tissue contaminants for the seasons. But there were no significant interactions between location and season.

Decision: H_{01} is rejected for significant location and seasonal effects identified. It is used for the non-significant seasonal and interaction effects. The judgment will thus be contaminant- and effect-specific.

Hypothesis Two (H_{02}): *There are no statistically significant differences in hepatic GST, SOD, CAT, GSH and MDA among the sampling locations or seasons.*

Table 5: Two-Way ANOVA of Biochemical Biomarkers

Biomarker	Location F(3,88)	p	Season F(1,88)	p	Interaction F(3,88)	p
GST	65.24	<.001	12.85	<.001	0.32	.810
SOD	7.88	<.001	0.51	.479	4.19	.008
CAT	10.73	<.001	1.43	.235	2.07	.109
GSH	1.43	.240	9.86	.002	3.38	.022
MDA	36.39	<.001	4.29	.041	0.07	.976

There was a strong influence of location and season with no interaction effect for GST. The SOD varied significantly between locations and in the location x season interaction, but not between seasons. Comparisons between the seasons revealed that the location did not differ significantly in the dry season while in the wet season downstream and industrial fish had significantly higher SOD activities than upstream and Omodino fish.

CAT was significantly different by location but not by season and there was no significant interaction between location and season. GSH was found to have significant seasonal and interaction effects with the overall location effect being non significant. Upstream fish had lower levels of GSH than downstream fish during the wet season. MDA has shown significant differences among the sites and seasons, with high concentration in industrial and downstream fish as compared to upstream fish and Omodino fish.

GST, SOD, CAT and MDA showed significant location effects, but GSH did not show any significant location effect. Only GST, GSH and MDA showed significant seasonal effects. There were notable interactions for location and season for both SOD and GSH.

Decision: H_{02} is rejected when biomarker effects are significant and it is accepted when biomarkers effects are not significant. Thus, the null hypothesis is partially accepted.

Hypothesis Three (H_{03}): *There is no statistically significant difference in comet-assay DNA damage among fish sampled from the four locations.*

Table 6: Two-Way ANOVA of Comet-Assay DNA Damage

Source of variation	F-statistic	p-value
Location	F(3,88) = 95.27	<.001
Season	F(1,88) = 11.98	<.001
Location × Season	F(3,88) = 0.14	.934

Significant differences in percentage tail DNA were found between location and season. DNA damage was the highest in industrial and downstream fish. Tukey comparisons showed that the fish from the industrial area differed significantly from all other areas, as did the fish from the downstream area from the fish at the upstream and Omodino areas. There was no significant difference between upstream and Omodino fish. The alkaline comet assay detects DNA strand breaks and alkali-labile sites under the assay conditions and the results should not be considered as proof of permanent events or carcinogenicity.

There was a significant effect of location on percentage tail DNA $F(3,88) = 95.27$, $p < .001$. There was also a significant seasonal effect, but the interaction effect was not significant.

Decision: H_{03} is rejected, thus statistically significant spatial differences in comet-assay DNA damage exist.

Hypothesis Four (H_{04}): *Fish-tissue contaminant concentrations are not significantly associated with biochemical biomarkers or DNA damage.*

Table 7: Pearson Correlations between Fish-Tissue Contaminant Concentrations and Selected Biochemical and Genotoxicity Indicators

Variable pair	Pearson's r	p-value	Interpretation
Tissue PAHs – DNA tail percentage	0.732	< .001	Strong positive association
Tissue Cd – DNA tail percentage	0.740	< .001	Strong positive association
Tissue Pb – DNA tail percentage	0.776	< .001	Strong positive association
Tissue PAHs – MDA	0.667	< .001	Moderate-to-strong positive association
Tissue Cd – MDA	0.639	< .001	Moderate-to-strong positive association
Tissue Pb – MDA	0.692	< .001	Moderate-to-strong positive association
Tissue PAHs – GST	0.702	< .001	Strong positive association
Tissue Pb – GST	0.725	< .001	Strong positive association
Tissue Cd – GST	0.704	< .001	Strong positive association

Note: N = 96 fish. All correlation coefficients are statistically significant at the 0.001 level. PAHs = polycyclic aromatic hydrocarbons; MDA = malondialdehyde; GST = glutathione S-transferase.

The results showed positive correlation between all the tissue contaminants and the biochemical and genotoxicity indicators. The metals with the best correlation to comet-assay DNA damage were Pb ($r=.776$, $p<.001$), Cd ($r=.740$, $p<.001$) and PAHs ($r=.732$, $p<.001$). Likewise, each of the three contaminants showed positive correlation with MDA and GST activity.

These results indicate that the concentrations of the tissues were correlated with an augmentation of lipid peroxidation, detoxification activity and damage to DNA in this dataset. However, it should be noted that contaminant exposures and biomarker responses were intentionally designed to vary by sampling site, so that the correlations may in part be driven by differences between locations. They should thus be regarded as associations for exploration purposes only and not as evidence of association within locations or causation.

Table 8: HC3-Robust Multiple Regression Predicting Oxidative DNA Damage

Predictor	β	Robust SE	95% CI	p
Intercept	2.7608	1.7818	[-0.7315, 6.2530]	.121
Wet season	0.2030	0.2547	[-0.2961, 0.7021]	.425
Tissue PAHs	0.3166	0.4775	[-0.6193, 1.2526]	.507
Tissue Pb	8.9065	2.5666	[3.8761, 13.9369]	<.001
Tissue Cd	31.7658	11.3384	[9.5429, 53.9887]	.005

Predictor	β	Robust SE	95% CI	p
GST	0.0891	0.0476	[-0.0042, 0.1824]	.061
GSH	-0.2514	0.2800	[-0.8003, 0.2974]	.369
Body mass	0.0002	0.0015	[-0.0028, 0.0032]	.902
R^2 = .694; adjusted R^2 = .669, $F(7,88) = 28.46$, $p < .001$.				

The model explained 69.4% of the variance in DNA damage (R^2 = .694; adjusted R^2 = .669), with an overall statistically significant result, $F(7,88) = 28.46$, $p < .001$. Tissue Pb and Cd were significant positive predictors, whereas PAHs, GST, GSH, season and body mass were not statistically significant. VIF values were between 1.12 and 4.16, indicating moderate to modest multicollinearity.

An eight station-season group random-intercept mixed-effects sensitivity analysis revealed no statistically significant individual predictor at the .05 level. This indicates that the regression results at the individual level depended on the modelling of shared station-season context. Therefore, it is important to be cautious about using the exposure-response relationships as evidence of independent or causal effects.

Based on the individual-level regression, significant positive associations between tissue Pb, tissue Cd and DNA damage were found, however these associations were not statistically significant in the cluster-aware sensitivity analysis. In addition, some of the relationships may be due to inter-location variations.

Decision: The data showed statistically significant positive correlations between tissue Pb and comet-assay DNA damage and tissue Cd and comet-assay DNA damage. No statistically significant independent association with DNA damage, however, was observed for tissue PAHs, GST and GSH after adjustment for the other explanatory variables.

Therefore, the null hypothesis (H04) can be partially rejected. Specifically the null hypothesis is rejected with respect to the independent association of tissue Pb and Cd with DNA damage but rejected with respect to tissue PAHs, GST and GSH in the fitted regression model.

The overall significance of the regression model also suggests that the explanatory variables as a group helped to predict DNA damage, but not necessarily that each one of the individual variables was necessarily statistically significant.

IV. DISCUSSION

The results offer a holistic view on the possible interactions between petroleum derived contaminants, biochemical responses and DNA damage in African catfish (Clarias gariepinus). The results of the study highlight the importance of integrating environmental contaminant data with fish-tissue concentration data, antioxidant biomarker data and genotoxicity data to evaluate aquatic environmental stress.

The data obtained from the environmental analyses showed that there were significant spatial differences in water and sediment concentrations of Pb, Cd and PAHs, with the highest mean concentrations recorded in the industrial mixing zone. This pattern is applicable to earlier studies in the Ubeji aquatic environment. The levels of petroleum hydrocarbons and heavy metals in water and sediments of Ubeji River were studied by Adewuyi et al (2011), while the presence of PAH in sediments, water and fish from Warri River at Ubeji were reported by Asagbra et al (2016). These studies indicate that assessment of more than one environmental matrix is important in studying contamination related to petroleum. The present analytical pattern also demonstrates the possibility to supplement measurements in the environment with measurements in fish-tissue, to obtain information on possible internal exposure.

Likewise, concentrations of these contaminants (Pb, Cd and PAHs) in fish-tissue were found to significantly vary by location and season, with the industrial mixing zone having the highest mean concentrations. This helps to strengthen the use of C. gariepinus as a potential biomonitoring species. Osioma et al., 2013 studied the biotransformation and oxidative-stress markers in African catfish from a petroleum-exploration area in Delta State and Arojojoye et al., 2021 investigated water quality assessment of selected rivers in Niger Delta State using biological indicators in African catfish. Overall, these studies advocate for the use of chemical and biological parameters in the assessment of aquatic environment, and the discrepancies in sampling sites and analytical methods make it hard to compare them directly.

Seasonal variations were observed for water PAHs, and for all three fish-tissue contaminants, but for not all the environmental variables. This indicates that seasonality could differ depending on contaminant properties and environmental conditions/paths of exposure. Altered rainfall, water movement and runoff can impact contaminant transport and availability. When interpreting the characteristics of river sediments, it is also important to consider local environmental and hydrological conditions. The observations provide further evidence for the need for incorporating season assessment within aquatic monitoring programmes.

The biochemical results showed that location had significant effects on the levels of GST, SOD, CAT and MDA, whereas it had no significant effects on the levels of GSH. Seasonal effects were significant for GST, GSH and MDA and there were significant location-by-season interactions for SOD and GSH. These variations indicate that biochemical responses to contaminant exposure may not be consistent. GST is involved in xenobiotic detoxification via the glutathione pathway and SOD, CAT and GSH are involved in antioxidant defence. MDA in turn is an indicator of lipid peroxidation. Osioma et al. (2013), Arojojoye et al. (2007) and Aladesanmi et al. (2017) have used similar approaches using biomarkers in African catfish investigations. Their research has given them a timely basis for applying biochemical measurements in conjunction with traditional environmental evaluation techniques. Finally, it is important to note that changes in individual biomarkers should be evaluated in combination as increased enzyme activity may be an adaptive response while decreased enzyme activity or altered GSH levels may be a sign of physiological stress or changes in antioxidant capacity.

The genotoxicity analysis also showed that there were significant spatial and seasonal variations in the percentage tail DNA with the site of industrial mixing zone and downstream Ubeji having the highest percentage tail DNA. The tissue contaminants were positively correlated with the level of DNA damage, especially for Pb ($r = .776$), Cd ($r = .740$) and PAHs ($r = .732$). These relationships indicate that an increase in the tissue contaminant burden was linked to an increase in DNA damage in the data analysed. Arojojoye et al., (2021) added micronucleated erythrocytes to their evaluation of fishes from some Niger Delta rivers which supports the relevance of genotoxicity assessment. However, micronucleus formation and comet-assay tail DNA are different biological endpoints. The alkaline comet assay is used to detect breaks in the DNA strand and alkali-labile sites, but cannot on its own be used to determine permanent mutations or carcinogenicity.

Similarly, aquatic pollution and its implications are discussed in Umeoguaju et al. (2023), who reviewed heavy metal contamination in seafood sampled in crude-oil impacted areas of the Niger Delta. These studies illustrate the necessity of considering aquatic contamination in a broader context than at the level of a single species, especially in communities that rely on fisheries and other aquatic resources.

But be careful when interpreting the regression results. Although the HC3-robust model explained 69.4% of the variation in DNA damage, only tissue Pb and Cd were significant individual predictors. More importantly, none of the associations were statistically significant in the cluster-aware mixed-effects sensitivity analysis. This indicates that the relationships observed could be partly due to shared location-season conditions. Thus, the results are exploratory measures of the potential exposure-response relationships, and not evidence of independent contaminant effects or causation.

A. Environmental and Management Implications

The results indicate the need for integrated environmental monitoring of aquatic ecosystems that could be impacted by petroleum-related industrial activities. Monitoring should include water, sediments and fish tissues and not only water quality measurements. Special consideration should be paid to industrial discharge locations and receiving environments, and to wet and dry season assessments.

The management priorities are effective effluent treatment, periodic review of industrial discharge systems, compliance with the regulations and clear environmental reporting. Biochemical and genotoxicity biomarkers can also be used to support traditional chemical monitoring to demonstrate a biological response.

That's also a comparison to be read with the eyes closed: Omodino Creek. It is an external comparison ecosystem and is not necessarily considered an uncontaminated control (unless independently verified in the environment). Additionally, all fish-consumption recommendations and public health advice shall be made with knowledge of the actual concentration of the contaminant in the fish and suitable food-safety evaluations.

B. Strengths and Limitations

An important advantage of the study design was the combined use of fish tissue contaminant loadings, biochemical biomarkers and genotoxicity indicators, and environmental chemistry. Seasonal comparisons are included to give an extra dimension to environmental variation. The analytical methods – ANOVA, correlation, robust regression and mixed-effects modelling – also illustrate the value of taking account of shared environmental factors in the interpretation of exposure–response relationships.

Other constraints are the number of sites selected, the differences in the biology of Ubeji and Omodino Creek, and the number of location-season groups available for cluster-aware modelling. Also, the contaminants chosen do not necessarily cover the spectrum of contaminants associated with petroleum pollution, and other environmental and physiological parameters may affect biological responses.

Thus, in future studies, more independent sampling sites and other environmental factors should be

included. Further evidence could be provided with complementary genotoxicity assessments and a more detailed contaminant profiling.

V. CONCLUSION

This study provides an integrated analytical approach for investigating potential relationships between petroleum related contaminants, glutathione dependent detoxification, oxidative stress and DNA damage in African catfish (Clarias gariepinus). The statistical demonstration shows how the spatial and temporal variability in contaminant exposure can relate to biochemical and genotoxicity parameters. It also reminds you of the need to consider "common environmental conditions" when considering statistical associations.

The main contribution is the illustration of the usefulness of integrated (environmental chemistry and biological indicator) aquatic biomonitoring. In conclusion, robust environmental monitoring and management of industrial discharges, combined with enhanced regulatory measures, are crucial to safeguarding aquatic ecosystems and the communities that rely on them.

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