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Biochemical and Genotoxic Markers in *Lumbricus terrestris* as Indicators of Crude Oil Pollution in Kolo Creek, Niger Delta, Nigeria

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ABSTRACT

This study evaluates biochemical and genotoxic markers in *Lumbricus terrestris* (LT) harvested from Kolo Creek, Bayelsa State, Nigeria. Three sites (A – Imiringi I; B – Imiringi II (with the presence of oil wells and exploration activities) and C – Emeyal (no exploration activity) were used for this research. LT collected from each site were homogenized and supernatant obtained used for biochemical analysis. Comparable ($p > 0.05$) concentrations of total protein, malondialdehyde, reduced glutathione and activities of superoxide dismutase were recorded for LT from all sites. DNA fragmentation was significantly higher in LT from sites (A & B) as compared with those from site C. Activities of catalase decreased in earthworm from sites A to C while nitric oxide levels was higher ($p <0.05$) in LT from site A compared to those from sites B and C. Earthworm had higher glutathione peroxidase ($p < 0.05$) activities from site C as compared to A and B. Biotransformation enzyme, glutathione s- transferase activity, was higher ($p < 0.05$) in LT from site A and B when compared with that of site C. Acetylcholinesterase was also significantly ($p < 0.05$) inhibited in LT from sites (A & B) compared to earthworms harvested from site C. From the results, it is inferred that sites A and B are more polluted than site C and changes in the biochemical markers suggests genotoxic, oxidative, nitrosative and neurotoxic stress in LT. These biomarkers could be employed to evaluate chemical pollution occurring in the environment.

Keywords: *Lumbricus terrestris*, DNA fragmentation, Kolo Creek, Crude oil, Biochemical markers

INTRODUCTION

Petroleum exploration in the Niger Delta area of Nigeria has been a considerably source of income, foreign exchange and environmental contamination. Important habitats which include forest and mangrove ecosystems have been destroyed and suffered severe harm as a result of this pollution. Dangerous chemicals and waste products are continually released into the area's soil and water bodies during oil exploration operations, thus, endangering human lives, vegetation, terrestrial animals and aquatic life [1]. The exploration of crude oil according to [2], significantly lowers the water quality in the Kolo Creek environment and also decreases in food production and a decrease in fish catch.

Critical assessment of the toxicological effects of toxicants in a contaminated environment both at the subcellular and molecular levels in organisms inhabiting such environment is often employed to evaluate locations subjected to pollution challenge that may pose threat to the long –

term survival of the organisms. Such analysis is carried out using biomarkers of effects which enable the determination of the physiological status of organisms under stress by human made or natural contaminants [3].

Lumbricus terrestris, also known as earthworms are the most prevalent invertebrates found in the soil and are greatly impacted by the contaminants that enter the soil system because of their close proximity to the soil particles [4]. This makes them an excellent bio-indicator of environmental pollution. *Lumbricus terrestris* make up the majority of soil fauna biomass, and they have been used and proven to be one of the most sensitive and standard terrestrial model and bio-indicators for assessing chemical toxicity in soil [5]. They play a crucial part in numerous soil-forming processes and also serve as a significant food source for various higher organisms, including birds and moles [6].

One of the major concerns of crude oil exploration in Kolo creek is that it exposes living organisms to hazardous substances which may have negative, biological effects. [7], reported that *Lumbricus terrestris* from soils around Kolo Creek were exposed to polycyclic aromatic hydrocarbons while [8] and [9] reported the presence of heavy metals due to crude oil related activities along Kolo Creek.

Several studies have shown that pollutants from crude oil, petroleum products and other contaminants can generate an elevated reactive oxygen species and severe genotoxicity [10, 11, 12, 13, 14, 15, 16]. Oxidative stress has become of considerable interest for environmental toxicological studies [17] and its attendant consequences including alteration of the antioxidant enzymes response, lipid peroxidation and oxidative damage to DNA.

This study therefore investigates DNA damage measured as percentage DNA fragmented, oxidative stress markers (including catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase), nitric oxide, glutathione s-transferase, acetylcholinesterase and malondialdehyde in *Lumbricus terrestris* harvested from crude oil exploration environment of Kolo Creek, Bayelsa State, Nigeria.

MATERIALS & METHODS

Study Locations

For the purpose of this research, samples were collected from three different sites from two neighbouring communities of Kolo Creek situated in Ogbia Local Government Area of Bayelsa State, and were designated as A – Imiringi 1, B- Imiringi 2, and C - Emeyal. Crude oil exploration activities are going on in Sites A and B as evidence with the presence of oil wells and oil exploration equipment. In Site, there is no such activity.

Field Collection of Earthworm (Lumbricus terrestris)

Earthworm samples (*Lumbricus* terrestris*)* were obtained from the three designated sites with the aid of a spade and forceps to dig beneath the earth. The earthworms were manually transferred into sterile plastic universal containers and tagged accordingly. At each site, samples were collected from three locations by pooling ten earthworms per sample.

Preparation of Tissue Supernatant

A section of the tissues (0.5 g) of *Lumbricus terrestris* was homogenized manually with porcelain mortar and pestle in 4.5 ml of 0.1M phosphate buffer (pH 7.4) as the extracting medium. The homogenate obtained was then placed on a plane tube and centrifuged at 4000 rpm for 15 minutes and the supernatant was collected and used for assay.

Biochemical Investigations

Determination of Total Protein Concentration

The concentration of total protein in the supernatant obtained was estimated based on the formation of purple complex when protein binds with cupric ions under alkaline conditions, leading to a colorimetric change

detectable at 540nm. [18]. Briefly, the reaction mixture contains 0.05 mL of sample and 3.0 ml of total protein reagent containing 600 mM NaOH, 12 mM $CuSO_4$, 32 mM potassium tartrate, 30 mM potassium iodide and non – reactive ingredients. The content was mixed by gentle inversion and left undisturbed at room temperature (25°C) for 10 minutes and read at 540 nm. The standard and blank were constituted by replacing the sample with 0.05 ml of bovine albumin (total protein standard) and distilled water respectively.

Determination of DNA Fragmentation

DNA fragmentation was estimated employing the diphenylamine colorimetric procedure of [19] with some modifications. This method quantifies fragmented DNA based on the separation of intact chromatin in the cell by the DNA fragmentation lysis buffer and subsequent binding of each fraction (pellet and supernatant) with diphenylamine solution which absorbs at 620 nm. Briefly, 10 volumes of TE solution of pH 8.0 containing (5 mmol Tris–HCl, 20 mmol EDTA) and 0.2% triton X-100 was used to homogenized 1.0 ml the tissue (50 mg). This was then centrifuged at 27,000 × g for 20 min to separate the intact chromatin (pellet, B) from the fragmented DNA (supernatant, T). The pellet and supernatant fractions were estimated for DNA content using a freshly prepared diphenylamine solution for reaction. Absorbance was read at 620 nm with spectrophotometer. The result was recorded as amount of % fragmented DNA by the following formula; % Fragmented DNA = T x100/(T+B)

Determination of Nitric Oxide

The colorimetric determination of nitric oxide was carried out in *Lumbricus terrestris* tissue supernatant using the colorimetric Griess assay as described by [20]. This method is based on the principle of the formation of an azo dye through diazotization and coupling reaction with N – (1 –naphthyl) ethylenediamine that is read at 540nm. The assay mixture contains 0.5 mL of the sample with same volume of Griess reagent. The resulting mixture was incubated at 25°C (room temperature) for 30 minutes and a reddish – purple colour change was observed. The concentration of nitric oxide radical was evaluated by the following equation: $\%NO = \frac{Ao - A1}{Ao}\ x\ 100$; where; A_o = Blank absorbance and A_1 = Sample absorbance

Assay of Superoxide Dismutase Activity

The method of [21] was used to determine the activity of superoxide dismutase in sample supernatant. The principle is based on the inhibition of the oxidation of epinephrine by superoxide dismutase. With respect to the activity of SOD, percent inhibition (%I) is hyperbolic. Briefly, the starting mixture contains 0.2ml of supernatant, 2.5 ml of 0.05 M carbonate buffer (pH 10.2) was added. 0.3 ml of freshly prepared 0.3 mM of epinephrine was used to initiate the reaction by adding it to mixture and immediately mixed by inversion. The reference or control cuvette contains 2.5 ml of the buffer, 0.2 ml of distilled water and 0.3 ml of the substrate (epinephrine). The increase in absorbance was monitored at 480 nm at interval of 30 seconds for 150 seconds.

Assay of Catalase Activity

The catalase activity was determined in sample supernatant by measuring hydrogen peroxide breakdown in the reaction mixture by monitoring changes in absorbance at a wavelength of 360 nm employing the method of [22]. The reaction mixture is constituted with 2 mL of the sample and 1 mL of H_2O_2 substrate in the reaction cuvette. The optical density was read at 360 nm for 70 sec. The control cuvette contained 1mL of H_2O_2 and 2mL of water. The first order kinetic equation $Log_{10}A = Log_{10}Ao - kt/2.3$ may be used to describe the disappearance of hydrogen peroxide.

Evaluation of Malondialdehyde Concentration

The malondialdehyde (MDA) content in sample supernatants of *Lumbricus terrestris*

was determined by them method of [23]. The principle of reaction is based on the combination of the 1: 2 ratio - malondialdehyde with thiobarbituric acid (TBA) to produce fluorescent adduct that is read at 530 nm. In this reaction, 1.0 ml of the sample supernatant was added to 2.0 ml of TCA –TBA – HCl reagent (15 % (w/v), TCA, 0.375 % (w/v) TBA with 0.25 N HCl. The contents were boiled for 15 minutes, cooled and centrifuged at 10,000g for 10 minutes to remove the precipitate. The absorbance was read at 535 nm using the reagent blank. The concentration of malondialdehyde (units/ml) was calculated using the following formular = $\frac{OD \times V}{E \times V sample}$; where, OD = Absorbance; E = Molar extinction coefficient (1.56 x $10^5 Mol^{-1}$ cm^{-1}); V = Total volume of mixture and V_{sample} = Volume of sample.

Assay of Cholinergic Activity

The cholinergic activity was evaluated by determining the acetylcholinesterase activity in the sample supernatant according to the rate of production of thiocholine as acetylthiocholine is hydrolysed rate of colour production read at 412 nm is directly proportional to acetylcholinesterase activity as described by [24]. Briefly, the reaction mixture is made up of 25 µL of supernatant, 2.925 µL of 0.1M phosphate buffer (pH 8.0), 25 µL of 8 mM of DTNB and 25 µl of 45 mM acetylcholine iodide at room temperature (25ºC). The contents in the cuvette were mixed, and the absorbance was read continuously at intervals of 30 seconds for 2 minutes at 412 nm. The enzyme (Acetylcholinesterase) activity was calculated as follows: A = $\frac{1000 \times (Eexp - Econt) \times 3}{13600 \times V \times t \times C}$ Where, A = Acetylcholinesterase activity in nmol/mg protein/minute; E_{exp} =Increase in the absorbance of the sample at 412 nm; E_{cont}= Increase in the absorbance of the blank at 412 nm; 1000 =Coefficient (This factor is introduced so that the enzyme activity can be expressed in nMol/min/ml; 3 =Total volume of the mixture (mL); 13600 = Molar extinction coefficient in Mol^{-1} cm^{-1} DTNB; V =Volume of sample (ml); t = Time in minute and c = Total protein concentration

Assay of Glutathione S-transferase Activity

Glutathione S- transferase activity in the sample supernatant was assayed based on the conjugation of L – glutathione to 1-chloro-2,4-dinitrobenzene (CDNB) through the thiol group of the glutathione as described by the method of [25]. In this method, formation of the conjugate of GS -DNB is proportional to the GST activity which is read at 340nm. The reaction mixture contains 1.5 ml sodium phosphate buffer (0.1 M pH 6.5), 0.2 ml GSH (9.2 mM), 0.02 ml CDNB (0.1 M) and 0.1 ml of the sample. Blank is made without the sample supernatant. The GST activity expressed in nmol/mg protein/min is evaluated by monitoring the increase in absorbance at 340 nm at interval of 60 seconds for 3 minutes.

Determination of Reduced Glutathione

The method of [26] which is based on the principle between the reaction the of sulfhydryl group of GSH with DTNB (5,5-dithio-bis-2-nitrobenzoic acid) to produces a yellow coloured 5-thio-2-nitrobenzoic acid (TNB) was employed for the determination of reduced glutathione. The optical density of TNB read at 412nm provides an estimation of GSH in the sample. Briefly, to 2mL of 10%(w/v) trichloroacetic acid was added 0.5mL of sample supernatant, mixed thoroughly and centrifuged at x5000g. This is followed by the addition of 0.5 mL Ellman's reagent and 3mL of 0.2 M phosphate buffer (pH 8.0) to 1mL of the supernatant obtained from the previously centrifuged mixture. Absorbance read at 412 nm is used to extrapolate the concentration of GSH from a standard curve plot from a series of standards prepared with blank containing 3.5 mL of buffer.

Assay for Glutathione Peroxidase (GPx) Activity

The method of [27] was employed in the determination of glutathione peroxidase. The

principle is based on monitoring the disappearance of β-NADPH. The curvet for the assay contains the following 1.49ml phosphate buffer (0.1M; pH 7.4), 0.1ml EDTA (1mM), 0.1ml sodium azide (1mM), 0.05ml glutathione reductase (1 IU/ml), 0.05ml GSH (1mM), 0.1ml NADPH (0.2mM) and 0.01ml H_2O_2 (0.25mM) which were properly mixed with 0.1ml of sample supernatant in a total volume of 2ml. Absorbance is read at 340nm and activity of the glutathione peroxidase was calculated as nM NADPH oxidized per minutes per mg protein using molar extinction of 6.22 x 10^3 $M^{-1}cm^{-1}$.

STATISTICAL ANALYSIS

The data obtained for all the biochemical parameters analyzed were expressed as Mean ± SD. Analysis of variance (ANOVA) was carried out on the data and Duncan's Multiple Range Test (DMRT) was employed to compare the group means. Means of same group were considered statistically different at $p < 0.05$. All statistical analysis was performed using SPSS version 22 (SPSS, Inc – Chicago, IIlinois, USA)

RESULT

Table1: Concentrations and Activities of Measured Biochemical Parameters

Parameters	A	B	C
Total Protein (g/dl)	1.495 ± 0.50[a]	2.449 ± 0.61[a]	2.311 ± 0.56[a]
DNA Fragmentation (%)	21.480 ± 3.99[a]	23.900 ± 3.93[a]	14.827 ± 3.56[b]
Nitric oxide (%)	66.137 ±2.51[a]	57.180 ± 4.82[b]	59.339 ± 4.27[b]
Superoxide dismutase (Unit/mg protein)	34.002 ± 0.31[a]	33.120 ± 0.59[a]	33.160 ± 0.27[a]
Catalase (Unit/mg protein)	86.193 ± 3.77[a]	76.623 ± 4.79[b]	43.305 ± 1.85[c]
Malondialdehyde (Unit/mg protein)	1.695 ± 0.19[a]	1.502 ± 0.14[a]	1.468 ± 0.17[a]
Reduced Glutathione (Unit/mg protein)	7.860 ± 0.63[a]	8.931 ± 0.23[a]	7.902 ± 0.48[a]
Glutathione peroxidase (Unit/mg protein)	11.228 ± 0.75[a]	9.166 ± 1.37[a]	15.098 ± 2.11[b]
Glutathione s – transferase (Unit/mg protein)	19.361 ± 0.77[a]	18.715 ± 1.00[a]	15.260 ± 0.75[b]
Acetylcholinesterase (Unit/mg protein)	1.618 ± 0.37[a]	1.705 ± 0.24[a]	3.130 ± 0.51[b]

Values are shown as Mean ± SEM for five replicates for each site (i.e., n = 5). Means sharing different superscript alphabet on same row differ significantly at p < 0.05.

KEY: A = Imiringi I; B = Imiringi II; C = Emeyal

The total protein concentrations in *Lumbricus terrestris* as indicated in table 1 indicated that earthworm from experimental sites (A, B & C) had comparable ($p > 0.05$) total protein concentrations. However, *Lumbricus terrestris* from site A (Imiringi I) showed a relatively lower total protein concentration (1.495 ± 0.50 mg/dl) while earthworms from site B (Imiringi II) is the highest (2.449 ± 0.61 mg/dl). Percentage DNA fragmentation indicated that *Lumbricus terrestris* from site C (Emeyal) had significantly lower ($p < 0.05$) fragmented DNA (14.827 ± 3.56 % DNA fragmented) as compared to earthworms from site A (21.480 ± 3.99 % DNA fragmented) and site B (23.900 ± 3.93 % DNA fragmented.

The highest mean value of nitric oxide was found in earthworm collected from site A (66.137 ± 2.51 %). This value was elevated ($p < 0.05$) than those obtained from site B (57.180 ± 4.82 %) and site C (59.339 ± 4.27 %).. The results also showed that the activities of superoxidase dismutase were comparable ($p > 0.05$) in *Lumbricus terrestris* obtained from the three experimental locations (A, B& C).

Catalase activity exhibited a marked increase ($p < 0.05$) in earthworms from location A as compared with those from sites (B and C). The findings further revealed that earthworms inhabiting site B showed increased ($p < 0.05$) catalase activity compared to those in site C. *Lumbricus terrestris* from site C had the lowest catalase activity. The levels of malondialdehyde, an index of lipid peroxidation showed no significant difference ($p > 0.05$) in earthworm across the three locations (A, B and C).

The content of reduced glutathione in *Lumbricus terrestris* as suggest that earthworm from experimental sites (A, B & C) exhibited comparable ($p > 0.05$) reduced glutathione concentrations. Results as indicated in (table 1), also showed that *Lumbricus terrestris* from site C (Emeyal) expressed reduced ($p < 0.05$) glutathione s-transferase activity (15.260 ± 0.75 unit/mg protein) as compared to earthworms from site A (19.361 ±0.77 unit/mg protein) and site B (18.715 ±1.00 unit/mg protein) respectively. However, glutathione s- transferase activity was comparable ($p > 0.05$) for *Lumbricus terrestris* obtained from sites (A and B). The activities of glutathione peroxidase enzyme in earthworm from site C (Emeyal) was higher ($p <0.05$) as compared to those from site A and site B respectively. *Lumbricus terrestris* from site B had similar ($p > 0.05$) glutathione peroxidase enzyme activity with earthworms from site A.

Lumbricus terrestris from site C (Emeyal) expressed significantly higher ($p < 0.05$) acetylcholinesterase activity (3.130 ± 0.51 unit/mg protein) as compared to earthworms from site A (1.618 ± 0.37 unit/mg protein) and site B (1.705 ±1.24 unit/mg protein) respectively.

DISCUSSION

Organisms that are inhabiting polluted environments are subjected to accumulate some of the contaminants in their tissues which may lead to changes in the biochemical composition of the organisms. These resulting effects can be identified employing biomarkers which focuses on early changes at a subcellular level. Protein according to [28] plays an important role in the physiology and metabolism of living organisms, providing information on the relationship with effects of contaminants in the organisms. Relatively decreased protein concentration was observed in *Lumbricus terrestris* from site A, this could be as a result of higher energy demand for metabolic purposes due to the presence of crude oil pollutants. [29] reported that such decrease in protein concentration in fish maybe related to stress occasioned by the presence of petroleum hydrocarbon. These results suggest sub – lethal or adaptive responses.

The integrity and stability of DNA molecule as repository of genetic information are critical factors to life and the evaluation of DNA damage in organisms in a contaminated environment may show a relationship on the risk associated with these contaminants. The polyanionic nature of DNA makes it susceptible to metal cation attack resulting in potentially damaging DNA molecules [30]. From the results above, earthworms from sites A and B had elevated percentage fragmented DNA as compared to *Lumbricus terrestris* from site A. DNA fragmentation is a reflection of the level of pollution by genotoxic contaminants. DNA damage observed in the results may also be related to high percentage of DNA strand break. Higher percentage fragmented DNA or DNA damage indicates that earthworm is inhabiting contaminated environment. Genotoxic effect of PAHs has been reported by [31], while [32] and [33] linked DNA damage to pollution.

Several pollutants have also been reported to cause DNA damage which include over – generation of reactive oxygen species, transition metals and lipid peroxidation products [34, 35, 36]. Relatively high concentration of malondialdehyde observed in soft tissues of *Lumbricus terrestris* from site A may also be related to the observed DNA damage because lipid peroxidation process according to [35] is related to organic pollution and a measure of xenobiotic – induced oxidative stress and its products could damage deoxyribonucleic acid by reacting with it [34]. Observed comparable malondialdehyde levels in earthworm from the three sites in this study may indicate efficient antioxidant defenses or chronic, tolerable exposure.

The activity of enzymatic antioxidants may be induced or inhibited during xenobiotic exposure. [37] reported that superoxide dismutase and catalase are expressed in crude oil toxicity. Significant increase in the activity of catalase was observed in

Lumbricus terrestris from site A and B, which may suggest upregulation in response to oxidative stress while superoxide dismutase activity indicated possible compensatory regulation. SOD converts superoxide anions to hydrogen peroxide and molecular oxygen while CAT acts on hydrogen peroxide to water and divalent oxygen [38, 39]. This result agrees with [40] that reported increased CAT activity and slightly inhibited SOD activity.

Studies reported decreases in the activities of superoxide dismutase in Wistar Albino rats fed with crude oil contaminated diet and chickens exposed to crude oil by [41] and [42]. This decrease in superoxide dismutase activity may lead to elevated concentrations of superoxide radicals that could react with nitric oxide to produce peroxynitrite, a deadly compound or damaging reactive nitrogen species [43, 44]. In this study, nitric oxide concentration was higher in earthworm inhabiting site A compared to B and C, this suggest nitrosative stress and inflammatory response. Such elevated levels of nitric oxide as observed in *Lumbricus terrestris* from site A could react with superoxide radical to form peroxynitrite [45] that can also scavenge lipid peroxyl radical, thus acting as an antioxidant [46]. [47] also reported an increased in nitric oxide concentrations in rats exposed to petroleum products fumes.

Reduced glutathione, a sulfhydryl (- SH) antioxidants found in the cytosol and other aqueous phases of the system had comparable concentrations across the three sites under investigation. This could mean a balanced oxidative defense mechanism. Glutathione peroxidase is recognized as free radical scavenger. GPx employs GSH as cofactor to catalyze the metabolism of hydrogen peroxide to water [48]. This plays important role in protecting membranes from damage due to lipid peroxidation. Significantly higher activities of GPx in site C as compared to sites (A & B) could be due to response to the concentrations and / or types of environmental stressors present in site C. Glutathione s- transferase is a critical phase II detoxification enzyme that conjugates xenobiotics with endogenous substrate, thus, enhancing their excretion. The activity of GST was observed to be higher in sites A and B, this elevation or increase may be as a result of active detoxification in response to pollutants in the environment. Adaptive role of GST to chemical stressors in the environment may not be ruled out. Organophosphate and carbamates in the environment affect the activity of acetylcholinesterase of the organism(s) inhabiting such environment [49, 50]. It is observed from this study that *Lumbricus terrestris* from sites A and B had inhibited acetylcholinesterase activity which suggests the presence of neurotoxic contaminants in these sites (A and B). [51], reported inhibition of acetylcholinesterase activity in Clam collected from a highly polluted area of Maraghera and concluded that the inhibition was due to exposure to neurotoxic substances. [52] demonstrated that exposure to crude oil affects acetylcholinesterase activity, which results in the accumulation of acetylcholine in synapses and leading to over activation of neurons and subsequent neuromuscular dysfunction.

CONCLUSION

In conclusion, early warning tools such as molecular biomarkers are being employed and gaining preference for the measurement of adverse effects of environmental contaminants on organisms. From the results of this study, changes in the biochemical and genotoxic markers in *Lumbricus terrestris* suggests that sites A and B are more polluted as compared to site C and earthworms could be utilized as bioindicator organism to ascertain environmental exposure levels to crude oil contaminants.

Declaration by Authors

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REFERENCES

1. Olukaejire, S.J., Osuji, L.C., CC, Ifiora, C.C., Osaro, P.A. & Hart, A.I. (2024). Petroleum Exploration in the Niger Delta Region and Implications for the Environment: *A Review. Journal of Energy Research and Reviews*, 16(5), 19–29.
2. Ushie, L., Chukwu – Okeah, G.O. & Imiete, G. (2020). Oil Pollution, water quality and livelihood sources in the Kolo Creek Area, Bayelsa State, Nigeria. Asia J. Agric. Ext. Econs. & Sociol., 38 (9): 38 - 47
3. Chèvre, N., Gagné, F., Gagnon, P. & Blaise, C. (2003). Application of rough sets analysis to identify polluted aquatic sites based on a battery of biomarkers: a comparison with classical methods, *Chemosphere*, 51(1): 13 – 23.
4. Ijomah, O.O., Adesuyi, A.A., Njoku, K.L., Ojokuku, S.A., Moses, U.D. & Adesuyi, O.O. (2020). Histopathological effects and biomarker response of earthworms, Eisenia fetida, after exposure to crude oil contaminated soils. *Environ Anal Health Toxicol*. 35(4): e2020021-0.
5. Kar, S. & Roy, K. (2012). First report on development of quantitative interspecies structure–carcinogenicity relationship models and exploring discriminatory features for rodent carcinogenicity of diverse organic chemicals using OECD guidelines, *Chemosphere*, 87 (4): 339 - 355.
6. Ahmed, N., & Al-Mutairi, K. A. (2022). Earthworms Effect on Microbial Population and Soil Fertility as Well as Their Interaction with Agriculture Practices. *Sustainability*, *14*(13), 7803.
7. Osioma, E. & Tesi, G.O. (2022). Polycyclic Aromatic Hydrocarbon Metabolites and Antioxidant Stress Markers in *Lumbricus terrestris* from Areas Around Kolo Creek, Niger Delta, Nigeria. *FUDMA J. Sci*. 6(2): 236 – 240.
8. Amolo, I.R., Nwineewil, J.D. & Egede, V.P. (2022). Heavy metals prfile and heavy metal pollution indices in surface water of Kolo Creek, Ogbia, Bayelsa State, Nigeria. International Journal of Advanced Science and Research, 7(2): 75 – 83.
9. Onwudiegwu, C. A., Chimere, E. & Uzoekwe, A. S. (2024). "Risk Assessment and Distribution of Heavy Metals in Water and Sediments Along Ibelebiri Axis of Kolo Creek, Ogbia Local Government Area, Niger Delta Region of Nigeria". *Asian Journal of Environment & Ecology* 23 (4):40-50.
10. Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S. & Rezaie, A. (2004). Pesticides and oxidative stress: a review. *Med Sci Monit*.,10(6): RA141- 147.
11. Sureda, A., Box, A., Tejada, S., Blanco, A., Caixach, J. & Deudero, S. (2011). Biochemical responses of *Mytilus galloprovincialis* as biomarkers of acute environmental pollution caused by the Don Pedro oil spill (Eivissa Island, Spain), *Aquatic Toxicology*, 101 (3-4): 540 – 549.
12. Skrypnik, L., Maslennikov, P., Novikova, A. & Kozhikin, M. (2012). Effect of Crude Oil on Growth, Oxidative Stress and Response of Antioxidative System of Two Rye (*Secale cereale* L.) Varieties. *Plants (Basel),* 10(1):157.
13. Ciğerci, I. H., Ali, M.M., Kaygısız, S.Y. & Liman, R. (2016). Genotoxicity assessment of cobalt chloride in Eisenia hortensis earthworms coelomocytes by comet assay and micronucleus test, Chemosphere, 144: 754 -757.
14. Onakurhefe, P., Achuba, F.I., George, B.O. & Okpoghono, J. (2020). Effect of *Elaeis guineensis* (Jacq) leaf extracts on crude oil-induced genotoxicity in Wistar albino rats, *Scientific African*, 7: e0028
15. Adeyemi. O. & Adeyemi, O. (2021). Evaluation of Toxic Effect of Oral co – administration of crude oil and vitamin C on antioxidant system of albino rats. *Biokemistri*, 33(4): 227 – 233
16. Pan, Y., Tian, L., Zhao, Q.,Tao, Z., Yang, J., Zhou, Y., Cao, R., Zhang, G.& Wu, W. (2022). Evaluation of the acute toxic effects of crude oil on intertidal mudskipper (*Boleophthalmus pectinirostris*) based on antioxidant enzyme activity and the integrated biomarker response, *Environmental Pollution*, 292(A): 118341.
17. Valavanidis, A., Vlahogianni, T., Dassenakis, M. & Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Saf.* 64(2):178-89.
18. Doumas, B.T., Bayse, D.D., Carter, R.J., Peters Jr, T. & Schaffer, R. (1981) A candidate reference method for

determination of total protein in serum. 1. Development and validation. *Clin Chem 27(10)*:1642 - 1650.
19. Wu, B., Ootani, A., Iwakiri, R., Sakata, Y., Fujise, T., Amemori, S., Yokoyama, F.,Tsunada, S. & Fujimoto, K. (2005). T cell deficiency leads to liver carcinogenesis inAzoxymethane-treated rats. *Experimental Biol Med*. 231:91–98.
20. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. & Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite and nitric oxide in biological fliuids. *Analytical Biochemistry*. 126(1): 131 – 138.
21. Misra, H.P. & Fridovich, I. (1972) The role of superoxide ion in the auto-oxidation of epinephrine and a simple essay for superoxide dismintase. *J. Biol Chem. 247*: 3170 - 3175.
22. Kaplan, J.H. & Groves, J. (1972) Liver and blood cell catalase activity in tumor-bearing mice. *Cancer Res*. *32*: 1190 - 1194.
23. Buege, J.A. & Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol 52*: 302 - 305.
24. Ellman, G.L., Courtney, K.D., Andres, V.J. & Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol. 7: 88 - 95*
25. Habig, W.R., Pbst, M.J. & Jakpoly, W.B. (1974) Glutathione transferase: a first enzymatic step in mercaturic acid formation. *J. Biol. Chem. 249*:7130-7139.
26. Ellman, G.L. (1959) Tissue sulfhydryl groups. *Arch Biochem. Biophys 82*: 70 - 77.
27. Flohe, L. & Grunzler, W.A. (1984). Assays of Glutathione peroxidase. *Methods Enzymol*. 105: 114 – 121.
28. Adams, S.M. Shugart, L.R., Sonthworth, G.R. & Hinton, D.E. (1990) Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In: McCarrthy, J.F., Shugart, L.R. (Eds). Biomarkers of Environmental contamination. Lewis Publishers. Boca Raton. Pp:333 – 353.
29. Osioma, E., Akanji, M.A. & Arise, R.O. (2013). Hepatic enzyme markers and proteins in serum and some selected tissues in *Clarias gariepinus* from swamp around Kokori-Erhoike oil field, Nigeria. *Journal of Research in Biology*, 3(4): 984-992.
30. Manoj, K. & Padhy, P. (2013). Oxidative stress and heavy metals: an appraisal with reference to environmental biology. *International Research Journal of Biological Sciences*. 2: 91-101.
31. Qiao, M., Chen, Y., Wang, C.X., Wang, Z. & Zhu, Y.G. (2007). DNA damage and repair process in earthworm after in-vivo and in vitro exposure to soils irrigated by wastewaters. Environ Pollut. 148(1):141 - 147.
32. Wang, Y., Wu, Y., Jo Cavanagh, Jo., Yiming, A., Wang, X., Gao, W., Matthew, C., Qiu, J. & Li, Y. (2018). Toxicity of arsenite to earthworms and subsequent effects on soil properties. *Soil Biology and Biochemistry,* 117: 36 – 47
33. Yan, X., Wang, J., Zhu, L., Wang, J., Li, S. & Mo Kim, Y. (2021). Oxidative stress, growth inhibition, and DNA damage in earthworms induced by the combined pollution of typical neonicotinoid insecticides and heavy metals, *Science of The Total Environment*, 754: 141873.
34. Harangi, M., Seres, I., Varga, Z., Emri, G., Szilvassy, Z. & Paragh, G. (2004). Atorvastatin effect on high – density lipoprotein – associated paraoxonase activity and oxidative DNA damage. *Eur J. Clin Pharmacol*. 60: 685 – 691.
35. Aboul – Ela, H.M., Saad, A. A., El - Sikaily, A.M.A. & Zaghloul, T. I. (2011). Oxidative stress and DNA damage in relation to transition metals overload in Abu – Qir Bay, Egypt. Journal of Genetic Engineering and Biotechnology, 9: 51- 58.
36. Attia, H., Nounou, H. & Shalaby, M. (2018). Zinc oxide nanoparticles induced oxidative DNA Dmage, inflammation and apoptosis in rat's brain after oral exposure. *Toxics*. 6(29): 1 -20.
37. Zhao, H., Li, W., Zhao, X., Li, X., Yang, D., Ren, H. & Zhou, Y. (2017). Cu/Zn superoxide dismutase (SOD) and catalase (CAT) response to crude oil exposure in the polychaete *Perinereis aibuhitensis*. *Environ Sci Pollut Res Int*. 24(1):616 - 627.
38. Hansen, B. H., Altin, D., Vang, S – H., Nordtug, T. & Olsen, A. J. Effects of naphthalene on gene transcription in *Calanus finmarchicus* (Crustacea: Copepoda), Aquatic Toxicology, 86(2): 157 - 165
39. Hao, F., Jing, M., Zhao, X. & Liu, R. (2015). Spectroscopy, calorimetry and molecular simulation studies on the interaction of

catalase with copper ion, Journal of Photochemistry and Photobiology B: Biology, 143: 100 – 106.
40. Jing, M., Han, G., Wan, J., Zhang, S., Yang, J., Zong, W., Niu, Q. & Liu, R. (2020). Catalase and superoxide dismutase response and the underlying molecular mechanism for naphthalene, Science of The Total Environment, 736: 139567
41. Ogara, A.L., Joshua, P.E., Omeje, K.O. & Onwurah, I.N. (2016). Effects of ingested crude oil contaminated diets on antioxidant enzymes and lipid profile in Wistar Albino rat. J. Appl. Sci. Environ. Manage. 20(4): 927 – 932.
42. Esiekpe, G.T., George, B.O., Orororo, O.C., Akpede, A. A., Okevwe, G. E., Onotugoma, E. & Oguntola, J. (2020). Monodora myristica seed extracts ameliorate crude oil contaminated diet-induced cardiotoxicity in rats. Thai Journal of Pharmaceutical Sciences. 44(3): 145 – 151.
43. Kim,P.K.., Zamora, R., Petrosko, P. & Billiar, T.R. (2001). The regulatory role of nitric oxide in apoptosis, *International Immunopharmacology*, 1(8): 1421 – 1441.
44. Islam, B.U., Habib, S., Ahmad, P., Allarakha, S. & Moinuddin, Ali A. (2015). Pathophysiological Role of Peroxynitrite Induced DNA Damage in Human Diseases: A Special Focus on Poly (ADP-ribose) Polymerase (PARP). *Indian J Clin Biochem*. (4):368 – 85.
45. Lelieveld, S., Lelieveld, J., Mishra, A., Daiber, A., Pozzer, A., Pöschl, U. & Berkemeier, T. (2024). Endogenous Nitric Oxide Can Enhance Oxidative Stress Caused by Air Pollutants and Explain Higher Susceptibility of Individuals with Inflammatory Disorders. *Environ Sci Technol*. 58(4):1823-1831.
46. Osioma, E. & Ezugworie, T.T. (2022). Comparative evaluation of antioxidant enzymes, lipid peroxidation, nitric acid and non – microsomal oxidases in *Galatea paradoxa* exposed to varying concentrations of 'uproot' a glyphosate – based herbicide. *Egyptian J Aqua Biol & Fisheries*. 26(3): 213 – 228.
47. Owumi, S.E., Elebiyo, T.C. & Oladimeji, B.N. (2020). Oxido-inflammatory responses and histological alterations in rat lungs exposed to petroleum product fumes. *Environmental Toxicology*. 1 – 12.
48. Van der Oost, R., Beyer, J. & Vermeulen, N.P.E. (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol. 13*: 57 – 149.
49. Mdeni, N.L., Adeniji, A.O., Okoh, A.I. & Okoh, O.O. (2022). Analytical Evaluation of Carbamate and Organophosphate Pesticides in Human and Environmental Matrices: A Review. *Molecules*. 27(3):618.
50. Jali, A. M. (2024). Organophosphate and Carbamate Toxicity: Understanding, Diagnosing and Treating Poisoning. *Journal of Pioneering Medical Sciences, 13*(7), 89-103.
51. Matozzo, V., Tomei, A. & Marin, M.G. (2005). Acetylcholinesterase as a biomarker of exposure to neurotoxic compounds in the clam *Tapes philippinarum* from the Lagoon of Venice, *Marine Pollution Bulletin*, 50(12): 1686 – 1693.
52. Olivares-Rubio, H.F. & Espinosa-Aguirre, J.J. (2021). Acetylcholinesterase activity in fish species exposed to crude oil hydrocarbons: A review and new perspectives. Chemosphere. 264(Pt 1):128401.

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