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ABNORMAL G6PDH AND LDH ENZYMES AS POSSIBLE SURROGATE MARKERS OF CRUDE OIL TOXICITY IN *Libyodrilus violaceus.*

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ABSTRACT.

Although crude oil consumption relative to other renewable energy sources has decreased modestly during the last decade, statistics still show clearly that it is still a dominant energy source in Nigeria. The Niger Delta region of Nigeria still continues to suffer grossly from crude oil pollution and environmental degradation owing to unguided spillages and ineffective clean-up procedures. Earthworm toxicity studies have been shown to be significant in understanding metal bioavailability, bio-membrane alterations, and energy metabolism of biological environmental indicators since water-soluble and water-insoluble chemicals can accumulate within their body because they bind tightly to specific sites within their body. Effect of crude oil and its fractions on some in vivo energy metabolism enzymes; Glucose 6-phosphate dehydrogenase (G6PDH) and Lactate dehydrogenase (LDH) enzymes, in the clitellum and post clitellum of earthworms were evaluated in a crude oil toxicity study carried out in Nigeria. Both enzymes have been identified as important enzymes of metabolic perturbations after exposure to petroleum hydrocarbons. The aim of this study is to ascertain the effect of the exposed toxicants in crude oil on Glucose 6-phosphate dehydrogenase (G6PDH) and Lactate dehydrogenase (LDH) enzymes of energy metabolism in the clitellum and post clitellum of Libyodrilus violaceus and to provide data that could propose these enzymes as possible early surrogate markers for monitoring crude oil toxicity. Two hundred and forty earthworms were assigned to four major groups. The first group was given deionized water and served as the control group, the other test groups were either exposed to a water-soluble fraction (WSF) or water-insoluble

fraction (WIF), or the whole crude (WC). The clitellum and post-clitellum regions of the worms were excised and collected for physicochemical and biochemical analysis using standard methods. There was a dose-dependent decrease ($p<0.05$) in the activity of the G6PDHenzyme (from the control group to the 0.1, 0.2, and 0.3% treatment groups) except for the earthworms exposed to the WIF. It was observed in this result that there was a general increase ($p<0.05$) in LDH activities in all subjects exposed to the toxicants except subjects exposed to the 0.3% WIF when compared to the control animals. The alterations recorded in this study might have been due to the underlying chemical stress from all fractions due to the high content of contaminants present in the crude oil.

KEY WORDS: Crude Oil spillages, G6PDH, LDH, Libyodrilus violaceus

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Introduction.

Crude oil is a naturally occurring liquid found in the earth. It is a complex mixture of hydrocarbons and inorganic elements like sulphur, nitrogen, phosphorus; trace elements like vanadium, nickel, iron, copper and some heavy metals like lead, chromium and cadmium (Albers, 1995; Erifeta *et al*, 2017, 2019). It is said to be one of the most complex natural mixtures on earth containing compounds which differ in composition and physical properties and are toxic to biological subjects (both flora and fauna). Crude oil is a major source of energy in Nigeria and its exploration and exploitation in the Niger Delta region of Nigeria has led to continuous spillages of crude oil into the environment (Eriyamremu *et al*, 2007, 2008).

Over the years, several environmental impact assessments (EIA) have been carried out with the view of assessing the actual impact of the oil explorations in the country. The traditional approach to soil pollution impact assessment, based on the analysis of individual concentrations of pollutants in the soil, chemical by chemical comparisons with specific threshold values is only a partial evaluation of risk and does not provide indication of deleterious effect of contaminants in the biota (Adams, 2002; Ezemonye *et al*, 2004; Erifeta *et al*, 2019,2023). It

neglects several essential aspects of toxicity of chemicals not included in the selection of contaminants to be analysed, interactive effects (synergism and antagonisms) of pollutants on biota and bioavailability, (Maria *et al*,2012). Also not considered are the possible interactions amongst chemical pollutants capable of producing harmful effects from chemicals that are individually considered harmless or within tolerable limits. The best integrators of the complex effect of any pollutant are the exposed organisms themselves. The use of earthworms in monitoring of the toxic effects of chemicals (Dunger & Fiedler, 1989, Erifeta *et al*, 2023) and its possible use in the bio-remediation of polluted soil (Ceccanti, 2006; Sinha *et al*., 2012) is not a new one because of their high biomass and sensitivity (Edwards & Bohlen, 1996; Dorn & Salanitro, 2000). The skin of earthworms is also in direct contact with pollutants that may interact with their immediate environment. Earthworm is therefore a good indicator for ecotoxicological studies (Contreras-Ramos *et al*., 2006; Eijsackers *et al*., 2001; Jager *et al*., 2003, UNEP, 2011).

Most of the Environmental impact assessments (EIA) procedures carried out using earthworms were majorly directed towards checking the level of impact using mortality rate and some stress indicators such as weight loss, coiling responses and swollen clitellum as an endpoint marker (Hozumi *et al.*, 2000; Otiloju, 2005). For reliable and proper assessment of environmental pollution/contamination, knowledge of the effects of these contaminants on the biological organisation of exposed organisms is necessary. Biochemical biomarkers are considered as the most sensitive and promising tools in ecotoxicological activity due to their ability to provide a more sensitive early warning of toxicological effect (Adams, 2002; Lionetto, 2012, Erifeta et al, 2023). This is because potentially, any alterations in any of the molecular, cellular, biochemical, and physiological processes occurring within an organism following pollutant exposure could be used as biomarkers (Riu et al, 2014; Erifeta *et al. 2017, 2019, 2023*). Enzyme activities and other sub-cellular components are most commonly included in this group of biomarkers, and much work has been done during the last two decades to establish and promote their application (Adams, 2002).

This study assessed actual biochemical alterations of some energy metabolism enzymes (Glucose 6-phosphate dehydrogenase (G6PDH) and Lactate dehydrogenase (LDH)) in earthworms; *Libyodrilus violaceus exposed to crude oil polluted soils.*

Methodology.

Toxicant Used: Bonny Light Crude Oil used was obtained from Shell Petroleum Development Company. It was fractionated into a water soluble (WSF) and water insoluble fraction (WIF). The soils used were from Okada town in Ovia north East Local Government Area of Edo State (Erifeta et al, 2023).

Test Animals: The test specimen (Libyodrilus violaceus) was collected by handpicking from a moist subsurface soil in Okada town, Benin city, Edo state Nigeria, in an open plastic bowl containing habitat soil and was transported to the laboratory. The earthworms were collected from the same site in order to reduce variability in biotype. Earthworms used in this study were adults with well-developed clitellum (Erifeta et al, 2023) .

Identification and Characterization of Earthworm: Samples of earthworms were sent to the department of Zoology, Faculty of Animal Science, University of Benin, Edo State for identification and characterization. The specimen was identified to be Libyodrilus violaceus (Erifeta et al, 2023).

Preparation of Earthworm bed: 1000g of habitat soil was added into 10 well labelled open plastic bowls. 10g of finely meshed clean paper as source of cellulose was added into each of the 10 bowls. The soils in the different bowls were mixed with different concentration and fractions of crude oil. 20 earthworms were individually weighed and introduced into each of the different bowls (Erifeta et al, 2023).

Fractionation of crude oil: The Bonny light crude oil was fractionated by the method of Anderson et al, 1974. The crude oil and distilled water were introduced into a 1000ml conical flask at a ratio of 1:2 (20ml of crude oil to 40ml of distilled water). A magnetic stirrer was introduced into the mixture and the mixture was placed on a magnetic hot plate and allowed to stir for 48hrs. The mixture after stirring separated into two distinct fractions; a dark dense phase (water insoluble fraction) and a very light brown watery phase (water soluble fraction). This was done to mimic the separation of the spilt crude oil in to land and water bodies in the event of a spillage (Erifeta et al, 2019, 2023).

Collection of crude oil fractions: A 2000ml separating funnel was allowed to stand erect on a retort stand. The separated mixture was introduced into the separating funnel and was allowed to stand for 24hrs. The two distinct fractions were separated by carefully allowing them pass through the tap and each fraction was collected in a different conical flask. The conical flasks were covered with aluminium foil sheets and kept at room temperature until commencement of experiment (Erifeta et al, 2019, 2023)..

Preparation of the different test concentrations of crude oil: Concentrations of crude oil used for these sublethal studies were selected based on the 96h exposure LC50 value (10.33ml/kg) for bonny light crude oil exposed to earthworms, Eudrilus eugeniae [16]. 0.1% concentration of the WSF, WIF and WC oil fraction were prepared by adding 1ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 999ml of distilled water. 0.2% concentration of WSF, WIF and WC oil fraction was prepared by adding 2ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 998ml of distilled water. 0.3% concentration of crude oil fraction was prepared by adding 3ml of the crude oil or its fractions to 1000g of the habitat (control) soil and mixed with 997ml of distilled water (Erifeta et al, 2019, 2023)..

Exposure of Earthworms to Laboratory contaminated soil: The earthworms were grown on soil contaminated in the laboratory with whole crude oil and its fractions. Two hundred (200) earthworms were assigned to four (4) major classes of treatments. The first class was the control group where the earthworms were grown in normal uncontaminated soil, and it comprised of twenty animals (Erifeta et al, 2019, 2023)..

The other one hundred and eighty (180) animals were assigned to three classes of treatment. One class was treated with whole crude, another with the water-soluble fraction (WSF) and the third class was treated with the water insoluble fraction (WIF). Each of these classes of treatment comprised of sixty animals each. In each class, we

had three (3) sub-classes of twenty earthworms each. The sub-classes were exposed to graded doses of the treatment. The sub-classes were exposed to 0.1%, 0.2%, or 0.3% of the treatment (Erifeta et al, 2019, 2023).

Assay of Glucose 6 phosphate dehydrogenase (G6PD).

G6PD activity was measured according to the continuous Spectrophotometric Rate Determination method using Randox kit (Lohr and Waller, 1974).

Principle.

The enzyme activity is determined by measurement of the rate of absorbance change at 340nm due to the reduction of $NADP^+$

$$\mathrm{G-6-P} + NADP^+ \xrightarrow{\mathrm{G-6-PDH}} \mathrm{gluconate-6-p} + \mathrm{NADPH} + H^+$$

Reagent Used

Reagent I

Randox Buffer with ready to use and stored at +2 to +8℃

Reagent II

NADP

Reagent III

Substrate

. **Reagent IV.**

Digitonin

Obtain the ΔA_{340nm}/20seconds using the maximum linear rate for both the Test and Blank.

Calculations

mU/erythrocytes per ml blood = 30476×ΔA340nm/min

$$\boldsymbol{G6PDHmU/gHb = \frac{mU.erythrocytes\ ml \times 100}{Hb(g/dl)}}$$

100= factor to convert from ml to dl

Hb(g/dl) = Haemoglobin concentration determined for each specimen.

Assay of Lactate dehydrogenase (LDH).

Lactate dehydrogenase (LDH) was measured according to the continuous Spectrophotometric Rate Determination method using Randox kit (Weisshaar *et al, 1975*).

Principle:

LDH under anaerobic conditions in the presence of pyruvate converts it to lactate with the oxidation of its reducing equivalent NADH**.**

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L} - \text{lactate} + \text{NAD}^+$$

Reagent Used

Reagent I

R1a: Randox Buffer/substrate with ready to use phosphate buffer 50mmol/l, pH7.5 and pyruvate 0.6mmol/l.

Reagent II

R1b: NADH 0.18mmol/l

Procedure:

Manual procedure

Wavelength	340nm (Hg 334nm or Hg 365nm)
Cuvette	1cm
Temperature	25℃, 30℃ and 37℃

Calculation:

Readings of LDH activity for semi micro units taken at 37℃ is ;

U/l = 8095 × ΔA 340nm/min

Data collected were subjected to statistical analysis using the SPSS version 20. Results obtained were expressed as mean ± SEM. One-way Analysis of variance (ANOVA) was also used to compare the means of some of the parameters measured and where significant differences were observed at 95% confidence level, Duncan's New Multiple Range test (IBM, 2013) was used to separate the means.

Results

Effect of crude oil and its fractions on energy metabolism.

Glucose-6-phosphate dehydrogenase (G6PD) activity. Glucose-6-phosphate dehydrogenase (G6PD) activity in the clitellum and Post clitellum parts of Earthworms exposed to different doses of crude oil and its fractions are presented in Figure 4.31 below. It was observed that there was a dose dependent significant decrease ($p<0.05$) in the activities of G6PD in the clitellum and Post clitellum of all subjects exposed to all doses of the toxicant except the subjects exposed to the WIF when compared to the control subjects. It was observed that the G6PD activities were significantly low ($p<0.05$) in the Earthworms exposed to all fractions when compared to the control subjects. Worthy of note in this study is that the WSF had the most effect on G6PD activities. This result also showed that G6PD activities did not give a dose dependent reduction in the clitellum and post clitellum of subjects exposed to the WIF. There was an hyperbolic decrease in the activities of G6PD in the WIF subjects both in the clitellum and post clitellum when compared to control.

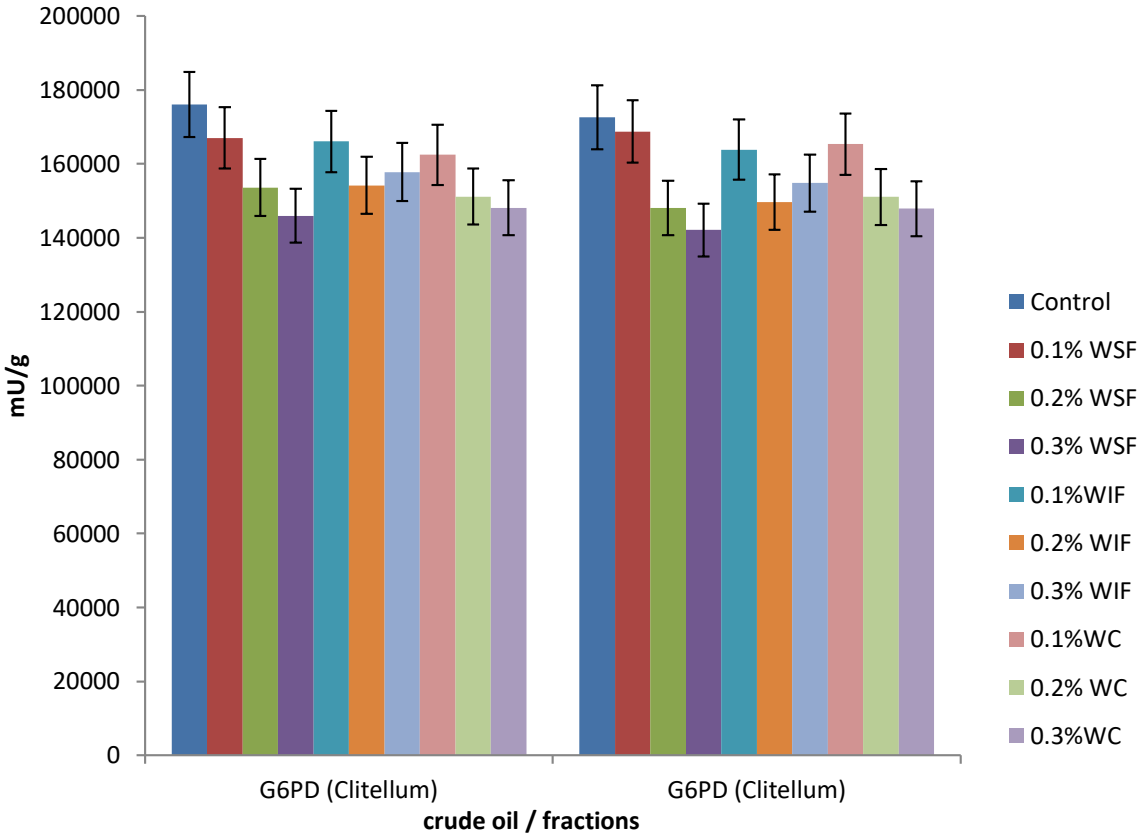

Figure 4.31: G6PD activity in Earthworms exposed to crude oil and its fractions.

- Values represent mean ± standard error of mean (SEM). Values represent mean ± SEM; n=10; WSF=Water soluble fraction, WIF=Water insoluble fraction, WC=whole crude.
- Means of the same column followed by different lettered superscripts differ significantly (P<0.05)
- Means of the same column with the same overlapping superscripts are statistically similar or show no significant difference (p>0.05).

Lactate dehydrogenase (LDH) activity in the Clitellum and post clitellum of Earthworms exposed to different concentrations of crude oil and its fractions.

LDH activity in the clitellum and Post clitellum parts of Earthworms exposed to different doses of crude oil and its fractions are presented in Figure 4.32. It was observed in this result that there was a general increase ($p<0.05$) in LDH activities in all subjects exposed to the toxicants except subjects exposed to the 0.3% WIF when compared to the control subjects. There was a significant increase ($p<0.05$) in the activities of LDH in the clitellum and Post clitellum of all subjects exposed to all doses of the WSF toxicant. The activities of LDH were insignificantly lower ($p>0.05$) in subjects exposed to the 0.3% WIF when compared to the control subjects. It was observed that the LDH activities were insignificantly high ($p<0.05$) in the Earthworms exposed to the WC fractions when compared to the control subjects. It was also observed in this study that the 0.1% WIF caused an insignificant increase ($p<0.05$) in the activities of the enzyme. There was no significant difference ($p>0.05$) in the clitellum and post clitellum of subjects exposed to the 0.2% WIF when compared to control. There was a dose dependent decrease in the enzyme activity within the group exposed to the WIF. The highest alteration in the activity of the enzyme was seen in the subjects exposed to the 0.3% WSF.

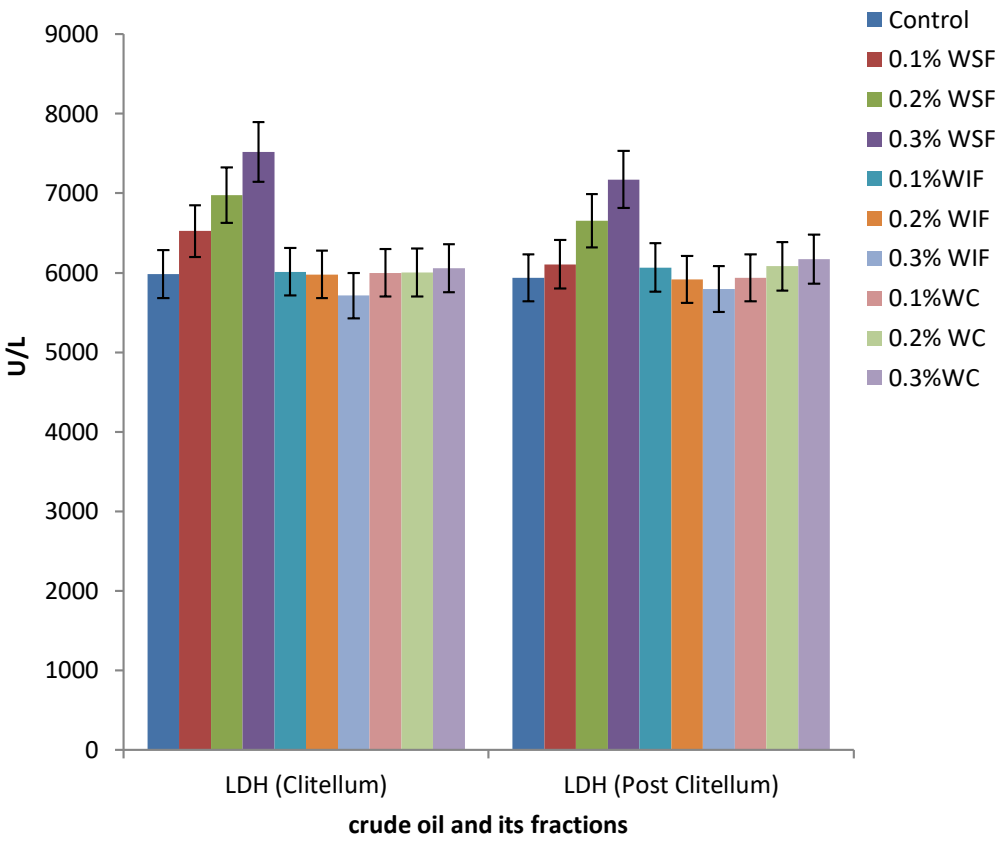

Figure 4.32: LDH activity in Earthworms exposed to crude oil and its fractions.

- Values represent mean ± standard error of mean (SEM). Values represent mean ± SEM; n=10; WSF=Water soluble fraction, WIF=Water insoluble fraction, WC=whole crude.
- Means of the same column followed by different lettered superscripts differ significantly ($P<0.05$)
- Means of the same column with the same overlapping superscripts are statistically similar or show no significant difference ($p>0.05$).

Discussion.

The effect of crude oil and its fractions on Glucose-6-phosphate dehydrogenase and lactate dehydrogenase enzymes in the clitellum and post clitellum of earthworms were assessed in order to ascertain the effect of the exposed toxicants on the energy metabolism of the earthworms. Both enzymes have been identified as important enzymes of metabolic perturbations after exposure to petroleum hydrocarbons (Gagnon and Holdway, 1999).

Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway. The enzyme G6PD converts glucose-6-phosphate into 6-phosphogluconolactone with the concomitant production of nicotinamide adenine dinucleotide phosphate (NADPH), a reducing equivalent necessary for reductive biosynthesis and redox homeostasis (Salati and Amir-Ahmady, 2007 and Ho HY, 2000, 2008). Pentose phosphate pathway is the unique source of NADPH, which enables cells to counterbalance the oxidative stress triggered by several oxidant agents preserving the reduced form of glutathione (GSH). Through glutaredoxin, GSH protects the sulfhydryl (SH) groups in heamoglobin and in the red cell membrane from oxidation. In normal RBCs the ratio between reduced and oxidized GSH is 100:1 (Mason, 2007). If NADPH concentrations cannot be maintained the GSH levels fall and oxidative damage occurs resulting in an acute haemolysis. A reduction in GSH would also affect the transport of materials into cells as it would also affect the gamma-glutamyl-circle which helps transport amino acids into cells. This would have profound effect on the earthworms as their ability to combat the toxicants in the crude oil becomes increasingly compromised with the falling level of GSH occasioned by a compromise in the activity of G6PDH.

In the present study, the activity of G6PD was assessed in the clitellum and post clitellum of earthworms exposed to crude oil and its fractions. G6PD generally showed a decline in its activity following exposure to the toxicants. There was a dose dependent decrease in the activity of the enzyme except for animals exposed to the WIF. The WIF might also have had the least effect on G6PD because it is the least toxic of all the fractions as revealed by the in vitro studies. G6PD-depleted cells have been found to be highly susceptible to oxidative stress-induced cytotoxic effect with the concomitant generation of reactive oxygen species (ROS) and oxidative damage (Cheng, 2004 and Gao, 2009). Previous studies have also showed that increased oxidative stress is associated with defective G6PD activities in *C. elegans* (Salinas, 2006). It can therefore be speculated that the knockdown trend seen in the activities of G6PD maybe as a result of the oxidative load imputed on the animals from the exposed toxicants. It is therefore not surprising that the level of enzyme was reduced in animals with high levels of MDA. More research work will be required to monitor ROS generation with exposure to the various toxicants for more conclusive effect of these toxicants on G6PD.

LDH is a terminal enzyme of anaerobic glycolysis widely used in toxicology and clinical diagnosis of cell, tissue and organ damage (Ribeiro *et al*., 1999). Pyruvate and lactate are the end products in the process of glycolysis (Mannen *et.al*.,. 1997). Lactate dehydrogenase is responsible for the reversible conversion of pyruvate to lactate.

In mammalian brain glycogen metabolism, lactate is an anaerobic metabolite in the presence of anoxia, a hypoxic metabolite in the presence of dysoxia (Gladden 2004). Lactate has recently been proposed to be another energy source in the brain (Izumi *et. al*., 1997, Brown *et. al*., 2004, Gladden 2004). It has also been shown to support respiration, maintain ATP levels, and reduce glucose utilization in brain slices and in cultured neurons (Izumi *et.al*., 1994, Tabernero *et.al*.,. 1996, Pellerin *et. al*., 1998). The anaerobic capacity of LDH in environmental toxicity studies has been explored in a wide range of organisms including sea flies (Daphnia magna), adapted tilapia (Sarotherodon mossambicus Peters), Tasmania Atlantic salmon (Salmo salar) and Australian Bass (macquaria novemaculeata), (Gagnon and Holdway, 1999; Tseng, 2007; Dange and Masurekar, 1980; Cohen, 2005 and, Diamantino,2001). Despite the huge work done on this enzyme, the potential of this enzyme as an indicative criterion in invertebrate ecotoxiclogical studies is sparse. In this study, the alterations in LDH activities were measured in clitellum and post clitellum of earthworms exposed to different doses of crude oil and its fractions. Worthy of note is the dose dependent significant increase in the clitellum and post clitellum of the earthworms exposed to all fractions when compared to the control. This generally suggests an emphasis on anaerobic respiration. Under stress, animals tend to emphasize glycolysis to meet up with cell need for energy to handle the stress. So LDH activity would be a reliable tool for assessing the level of stress in earthworms (*Libyodrilus violaceous)*. These alterations might have been due to the underlying chemical stress from all fractions (WIF containing the least toxicant) due to the high content of toxicants present in the fraction. The highest alterations in LDH activity were seen in the *Libyodrilus violaceous* exposed to 0.3% WSF. These results were in agreement with work done by Cohen, 2005, who recorded an increased anaerobic (LDH) activity in the gills, liver and white muscle of Australian Bass exposed to water accommodated fraction of Bass Strait crude oil, dispersed crude oil or burnt oil to assess sub-lethal effect of oil spill remediation techniques on fish. Our findings were also in consonance with those of Dange, 1980, who reported that sub-lethal concentrations of Toluene increased the activities of LDH while those of succinate dehydrogenase and acetylcholinesterase were inhibited. Our findings did not agree with that of Gagnon, 1998 who reported that low-level exposure of Atlantic salmon (Salmo salar) to the water accommodated fraction (WAF) of crude oil and dispersed crude oil exhibited significant inhibition on the activity of LDH.

CONCLUSION:

1. This study has provided a baseline data that showed an increased LDH activity that could be due to possible alterations in lipid profile and an effect on the redox potential of the earthworms owing to the significant reduction in G6PD activity following the exposure of the bio-indicator organisms to crude oil pollution. This research study also provides data that showed possible alterations in energy metabolism following visible alterations in G6PD and LDH.

References.

1. Albers P.H., (1995). Petroleum and individual polycyclic aromatic hydrocarbons. In: Handbook of Ecotoxicology, Hoffman DJ, Rattner BA, Burton CA, Cairns J (eds.), Boca Raton, Lewis Publishers: 330-355.
2. Erifeta G, Eriyamremu GE, Omage K, Njoya HK et al., (2017). Alterations in the bio-membrane of Libyodrilus violaceus following exposure to crude oil and its fractions. *International Journal of Biochemistry Research & Review* 20: 1-11.
3. Erifeta GO, Njoya HK, Josiah SJ, Nwangwu SC, Osagiede PE, et al., (2019). Physicochemical characterisation of crude oil and its correlation with bioaccumulation of heavy metals in earthworm (Libyodrilus violaceus). International Journal of Research and Scientific Innovation 6: 370-375.
4. Eriyamremu E.G, Osagie V.E. Omoregie SE, Omofoma C.O., (2007). Alterations in glutathione reductase, superoxide dismutase and lipid peroxidation of tadpoles (*Xenopus laevis*) exposed to Bonny Light crude oil and its fractions. Ecotoxicology and Environmental Safety.;71(1):284-290.
5. Eriyamremu G. E., V. E. Osagie, S. E. Omoregie, and C. O. Omofoma, (2008). Alterations in glutathione reductase, superoxide dismutase and lipid peroxidation of tadpoles (Xenopus laevis) exposed to Bonny Light crude oil and its fractions. Ecotoxicology and Environmental Safety, 71(1): 284-290.
6. Adams, S.M. (2002). Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland, USA.
7. Rui L (2014). Energy Metabolism in the Liver. Comp Physiol 4(1):177–197. https://doi.org/10.1002/cphy.c130024.
8. Ezemonye, L.I.N. et al., (2004). Comparative studies of macroinvertebrates community structure in two river-catchment areas (Warri and Forcados rivers) in delta state, Nigeria. Afr. Sci., 5: 181-192.
9. Georgina O Erifeta *et.al.*, (2023) Biomolecule (Protein) Monitoring and Histopathological Changes in Earthworms (Libyodrilus violaceous). *SciEnvironm* 6: 169.
10. Maria G.L., Anthonio C. and Trifone S., (2012). Earthworm biomarkers as tools for soil pollution assessment. Soil Health and land use management. Dr Maria G. Hernandez Soriano (ED), ISBN:978-953-307-614-0. http://www.intechopen.com/books/soil.health-and-land-use.
11. Dunger, W., and Fiedler, H.J., (1997). Methoden der Bodenbiologie. 2. Aufl. Gustav. Fischer, Jena, Stuttgart, Lübeck, Ulm, 539 .
12. Ceccanti, B, Masciandaro, G, Garcia, C, Macci, C, Doni, S., (2006). Soil bioremediation: combination of earthworms and compost for the ecological remediation of a hydrocarbon polluted soil. *J Water Air Soil Pollut*177: 383-397.

13. Sinha R.K., Chandran V., Soni B.K., Patel U. and Ghosh A., (2012). Earthworms: nature's chemical managers and detoxifying agents in the environment: an innovative study on treatment of toxic wastewaters from the petroleum industry by vermifiltration technology. *The Environmentalist*, 32(4): 445 – 452.
14. Edwards, C.A., and Bohlen, P.J., (1996). Biology and Ecology of Earthworms. Chapman hall ltd. London, New York.
15. Dorn, P. B., & Salanitro, J. P., (2000). Temporal ecological assessment of oil contaminated soils before and after bioremediation. *Chemosphere*, *40*(4), 419-426.
16. Contreras-Ramos, SM, Alvarez-Bernal, D, Dendooven, L., (2006). Eisenia fetida increased removal of polycyclic aromatic hydrocarbons (pahs) from soil. *Environ Pollut* 141: 396-401.
17. Eijsackers, H, Gestel, CAM, Jonge, S, Muijis, B, Slijkerman, D., (2001). PAH—polluted dredged peat sediments and earthworms: a mutual inference. *Ecotoxicology* 10: 35-50.
18. Jajer, T., Fleuren, R.H.L.J., Hogendoorn, E.A. and de Korte, G., (2003). Elucidating the Routes of Exposure for Organic Chemicals in the Earthworm, Eisenia andrei (Oligochaeta). Environmental Sciences and Technologies, 37: 15,3399–3404, ISSN 0013-936X
19. UNEP, (2011). Environmental assessment of Ogoniland, 1–262. United National Environmental Programme (UNEP): Nairobi].
20. Hozumi, T., Tsutsumi, H. &Kono, M., (2000). Bioremediation on the shore after an oil spill from the Nakhodka in the sea of Japan. *Marine Pollution Bulletin* 40(4): 308-314.
21. Otitoloju, Adebayo A., (2005). Stress indicators in earthworms Eudriluseugeniae inhabiting a crude oil contaminated ecosystem acta SATECH 2: 1-5.
22. Lionetto, M.G., Caricato, R., Calisi, A. and Schettino T., (2010). Acetylcholinesterase inhibition as a relevant biomarker in environmental biomonitoring: new insights and perspectives. In *Ecotoxicology around the globe*, J.E. Visser, (Ed.), 87-115, Nova Science Publishers, ISBN 9781617611261 , New York, USA.
23. Rui L., (2014). Energy Metabolism in the Liver. Comp Physiol 4(1):177–197. https://doi.org/10.1002/cphy.c130024.
24. Lohr GW, Waller HD., (1974). Glucose-6-Phosphate Dehydrogenase. Methods of Enzymatic Analysis, 3rd Edition - Varlag Chemie, Wehnheim:; 636.
25. Weisshaar, H.D., Gossrau, E. and Faderl, B., (1975). Normal ranges of alpha-HBDH, LDH, AP and LAP as measured with substrate optimated test charges. Med. Welt, 26 (1975). 387-392
26. IBM Corp., (Released 2013). IBM SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp; 1925.
27. Gagnon, M. M., & Holdway, D. A., (1999). Metabolic enzyme activities in fish gills as biomarkers of exposure to petroleum hydrocarbons. Ecotoxicology and environmental safety, 44(1), 92-99.
28. Salati LM, Amir-Ahmady B., (2001). Dietary regulation of expression of glucose-6-phosphate dehydrogenase. *Annu Rev Nutr* ; 21: 121–140.
29. Ho HY, Cheng ML, Lu FJ, Chou YH, Stern A, Liang CM *et al*., (2000). Enhanced oxidative stress and accelerated cellular senescence in glucose-6-phosphate dehydrogenase (G6PD)-deficient human fibroblasts. *Free Radic Biol Med* ; 29: 156–169.

30. Ho HY, Cheng ML, Weng SF, Chang L, Yeh TT, Shih SR *et al*., (2008). Glucose-6-phosphate dehydrogenase deficiency enhances enterovirus 71 infection. *J Gen Virol* ; 89 (Pt 9): 2080–2089.
31. Mason, P. J., Bautista, J. M., & Gilsanz, F. (2007). G6PD deficiency: the genotype-phenotype association. *Blood reviews*, *21*(5), 267-283.
32. Cheng ML, Ho HY, Wu YH, Chiu DT., (2004). Glucose-6-phosphate dehydrogenase-deficient cells show an increased propensity for oxidant-induced senescence. *Free Radic Biol Med* ; 36: 580–591.
33. Gao LP, Cheng ML, Chou HJ, Yang YH, Ho HY, Chiu DT., (2009). Ineffective GSH regeneration enhances G6PD-knockdown Hep G2 cell sensitivity to diamide-induced oxidative damage. Free Radic Biol Med; 47: 529–535.
34. Salinas LS, Maldonado E, Navarro RE., (2006). Stress-induced germ cell apoptosis by a p53 independent pathway in *Caenorhabditis elegans*. *Cell Death Differ* ; 13: 2129–2139.
35. Ribeiro, S., Guilhermino, L., Sousa, J.P., and Soures, A.M.V.M., (1999). Novel bioassay based on acetylcholinesterase and lactate dehydrogenase activities to evaluate the toxicity of chemicals to soil isopods. Ecotoxicol. Environ. Safety, 44; 287-293.
36. Mannen H, SC Tsoi, JS Krushkal, WH Li, SS Li., (1997). The cDNA cloning and molecular evolution of reptile and pigeon lactate dehydrogenase isozymes. Mol. Biol. Evol. 14: 1081-1087.
37. Gladden LB., (2004). Lactate metabolism: a new paradigm for the third millennium. J. Physiol. 558: 5-30.
38. Izumi Y, H Katsuki, CF Zorumski. (1997). Monocarboxylates (pyruvate and lactate) as alternative energy substrates for the induction of long-term potentiation in rat hippocampal slices. Neurosci. Lett. 232: 17-20.
39. Brown AM, S Baltan Tekkök, BR Ransom, (2004). Energy transfer from astrocytes to axons: the role of CNS glycogen. Neurochem. Int. 45: 529-536.
40. Tabernero A, C Vicario, JM Medina, (1996). Lactate spares glucose as a metabolic fuel in neurons and astrocytes from primary culture. Neurosci. Res. 26: 369-376.
41. Pellerin L, G Pellegri, JL Martin, PJ Magistretti, (1998). Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. Proc. Natl. Acad. Sci. USA. 95: 3990-3995.
42. Tseng YC, CJ Huang, JCH Chang, WY Teng, O Baba, MJ Fann, PP Hwang, (2007). Glycogen phosphorylase in glycogenrich cells is involved in the energy supply for ion regulation in fish gill epithelia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293: R482-R491.
43. Dange, A. D., & Masurekar, V. B. (1980). Toluene toxicity: Effects of sublethal levels on enzyme activities in seawater adapted tilapia (*Sarotherodon mossambicus* Peters). *Journal of Biosciences*, *3*(2), 129–134.
44. Cohen, A., Gagnon, M. & Nugegoda, D., (2005). Alterations of Metabolic Enzymes in Australian Bass, Macquaria novemaculeata, After Exposure to Petroleum Hydrocarbons. Archives of Environmental Contamination and Toxicology 49: 200–205.
45. Diamantino, T. C., Almeida, E., Soares, A. M., & Guilhermino, L. (2001). Lactate dehydrogenase activity as an effect criterion in toxicity tests with Daphnia magna straus. *Chemosphere*, *45*(4-5), 553-560.