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Sublethal Effects of Diesel on Total Protein Levels and Cholesterol in *Tympanotonus Fuscatus*

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ABSRACT: Periwinkles (*Tympanotonus fuscatus*) of variable sizes between 4.5 to 5.5cm were handpicked at the Eagle Cement area of the New Calabar River and subjected to different levels of diesel contamination (0.00, 250.00, 300.00, 350.00, 400.00 and 450.00ml/L) for six days in a renewal assay to examine its effect on two metabolites (total protein content and cholesterol levels). Total protein content and cholesterol levels were examined in the muscle and viscera tissues of the periwinkles. Statistical analysis showed significant (P>0.05) variation in total protein content and cholesterol levels in the tissues. Total protein content were significantly (P>0.05) lower in value in the treatment groups when compared to the control in both the muscle and the viscera of the periwinkle. While cholesterol levels were significantly (P>0.05) lower or higher in value than that of the control. This study showed that exposure of periwinkles (*Tympanotonus fuscatus*) to diesel toxicity could cause deleterious effects or changes in the organism biochemistry. © JASEM

The variety of activities acting upon the natural environment results in the release of different chemicals which when in excess can cause adverse effects on the habitat and the organisms supported by that habitat. Chemical changes within an environment alter the equilibrium (homeostatis) of that ecosystem. This perturbation prevents the normal functioning of that ecosystem (Brucka-Jastrzebska and Protasowicki, 2005). Environmental pollution and the resultant changes are associated with constant flow and exchange of matter which forms the distribution pathway for xenobiotics that eventually affect life functions (Adeyemo, 2005).

Pollution from petroleum and refined petroleum products are spread all over the globe and particularly most common in countries whose economies are dependent on the oil industry. About 6-10 million barrels of crude oil enter the aquatic environment yearly the world over (Thorhang, 1992). The control of such pollution problems in the aquatic environment is very difficult because of large number of input sources and their geographic dispersions (Howard *et al*., 2009). However, views and evidences are accumulating that petroleum hydrocarbons mixes with water and penetrates to underlying sediment (Palin 1999; Carbioch *et al*., 1977). Generally, crude oil toxicity depends largely on the chemical and physical properties of the oil in question and the water quality being polluted (Afolabi *et al*., 1985) and from the hydrophobic nature of the oil (Osuji and Mbata, 2004). Oil in contact with water depletes the dissolved oxygen content of the aqueous environment and its photosynthetic production. It may also coat the organism which eventually die by asphyxiation (Duffus, 1980; Beynon and Cowell, 1974). Filter feeding organisms ingest oil droplets and become an integral part of the food chain (Osuji and Mbata, 2004).

In Nigeria, oil industry operations are both offshore and onshore. All the oil terminals and most refineries in the country are located in the Niger Delta region and hence more than 90% of oil-related activities take place in this region (Imovbore and Adeyemi, 1981). Most of the recorded spills occur in the coastal areas and swamps of the Niger Delta (RSEPB, 1992). However, crude oil (petroleum) and its associated products have been shown to cause mortality in aquatic species (Baron *et al*., 2003; Liu *et al*., 2006), changes in enzyme activities (Dange and Masureker, 1981), changes in haematology and gill pathology of *Clarias gariepinus* (Gabriel *et al*., 2007), while Prasad *et al*., (1987) and Dede and Kagbo, (2002) observed similar changes in *Heteropneustes fossil* and *Rattus rattus*.

Toxicity test are carried out by measuring biochemical parameters known as biomarkers. This acts as a means of assessing the hazard or potential

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adverse effects of substances (Wang *et al*., 1994). This is based on the belief that a non acute effect at the cellular or organ level can result in the effect of the integrated organism function by the alteration of the physiological and biochemical processes of the organism (Stegeman *et al*., 1992). The various effects revealed with biomarkers can therefore be applied in regulatory decision making and environmental management (Ewald, 1995). This study was carried out to examine the sublethal effects of diesel on total protein levels and cholesterol content in *Tympanotonus fuscatus* after exposure.

MATERIALS AND METHODS

Periwinkles (*Tympanotonus fuscatus*) of size between 4.5 - 5.5cm were handpicked at the Eagle cement area of the New Calabar River near the Ignatius Ajuru University of Education Rumuolumeni, Port Harcourt. They were transported in plastic buckets to the Chemistry Department Laboratory of the University. Two hundred apparently healthy periwinkles were acclimated to laboratory conditions in plastic tanks of six litre capacity. The tanks were half filled with brackish water and sediments collected from same source. The acclimation was done for seven days. The substrate was prepared by air drying the sediment and then macerated in a mortar and sieved in 2mm mesh.

250g of finely prepared sediment were put into each of the plastic tanks to serve as the substrate. Completely randomized design (CRD) was used for the experiment. The experiment was divided into five treatment levels with three replicates. The test media were prepared in the following concentrations: 250.00ml/L, 300.00ml/L, 350.00ml/L, 400.00ml/L, 450.00 and a control (0.00ml/L) of diesel. Twelve of the test animals were introduced into the toxicant media. The content of the aquaria were washed thoroughly on the forth day and was renewed with fresh concentrations which lasted to the sixth day.

On the sixth day the periwinkles were removed from the toxicant and the shells were broken with a small rod and the tissues were separated from the shell. The tissues were divided into the edible part (muscle) and the non edible part (viscera).

0.5g of the tissues were macerated or homogenized and mixed with 5ml of 0.8% perchloric acid for metabolites analysis. The mixture was centrifuged at rate of 3000rpm for ten minutes and the supernatant poured into 5ml plain bottles. The samples were immediately transferred to the laboratory for analysis. Total protein was estimated by Dumas (1971) method, while cholesterol was estimated by Warnick , (1991) method.

The results obtained were subjected to analysis of variance (ANOVA) using one way classification to test whether differences existed between the means. Where differences existed, Duncan's multiple range test was used to separate the means (Zar, 1984)
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RESULTS AND DISCUSSION

Total protein levels in the viscera decreased in content when compared to the control value. The control value was 57.00 ± 13.44g/dl as against the test values which were 33.25 ± 20.15 and 33.25 ± 6.72g/dl at 250.00 and 450.00ml/L respectively. At 300.00 and 400.00ml/L concentrations, 28.50 ± 0.00 and 28.50 ± 13.44 were recorded. The lowest observed level in total protein was at 350.00ml/L. Cholesterol levels were either higher or lower than the level at the control, which was 0.8 ± 1.41mmol/L. Increase in value were observed at 250.00ml/L (2.58 ± 1.58mmol/L), 300.00ml/L(1.05 ± 0.14mmol/L) and 450.00ml/L(1.15 ± 1.69mmol/L). Lower levels were observed at 350.00ml/L and 400.00ml/L, which were 0.68 ± 0.25 and 0.38 ± 0.32mmol/L respectively (Table 1). In the muscle of *Tympanotonus fuscatus,* there was a general decrease in the levels of total protein in all the test solutions except at 250.00ml/L which was 38.00 ± 13.44g/dl as against the control value of 33.25 ± 6.72g/dl. The value of the control was followed by that observed at 450.00ml/L and 300.00ml/L which were 28.50 ± 0.00 and 23.75 ± 6.75g/dl respectively. However, at 350.00 and 400.00ml/L, the value of 19.00 ± 0.00g/dl was observed. Cholesterol levels in the muscle of *Tympanotonus fuscatus* were either higher, equal or lower than the control value. The value of 0.80 ± 0.42mmol/L was observed in the control and 350.00ml/L concentration. At 400.00ml/L, 0.55 ± 0.07mmol/L was observed, while higher values than that of the control were observed at 250.00, 300.00 and 450.00ml/L being 1.18 ± 0.95, 1.23 ± 0.11and 1.03 ± 0.46mmol/L respectively.

The average value of total protein in the tissue of *Tympanotonus fuscatus* depreciated in value in all the test solutions when compared to the control. The percentage depreciation ranged from between 78.90 (-21.1) at 250.00ml/L to 36.84 (-63.16). In the case of cholesterol, there were both appreciation and depreciation in percentages in the test solutions. The percentage appreciation ranged from between 136.25 (450.00ml/L) to 235.00 (250.00ml/L). Percentage depreciations were 58.13 (-41.87) at 400.00ml/L and 92.50 (-7.50) at350.00ml/L (Table 3).

Table 1: Total protein and cholesterol in the viscera of *Tympanotonus fuscatus* exposed to different concentrations of diesel.

Concentration of diesel (ml/L)	Total protein (g/dl)	% of control	Cholesterol (mmol/L)	% of control
0.00	57.00 ± 13.44[a]	100	0.80 ± 1.41b	100
250.00	33.25 ± 20.15[b]	58.33	2.58 ± 2.58a	322.50
300.00	28.50 ± 0.00[b]	50.00	1.05 ± 0.14ab	131.25
350.00	14.25 ± 6.72[c]	25.00	0.60 ± 0.25b	85.00
400.00	28.50 ± 13.44[b]	50.00	0.38 ± 0.32c	47.50
450.00	33.25 ± 6.72[b]	58.33	1.15 ± 1.69ab	143.75

Means with the same superscript in the same column are not significantly different (P>0.05).

Table 2: Total protein and cholesterol in the muscle of *Tympanotonus fuscatus* exposed to different concentrations of diesel.

Concentration of diesel (ml/L)	Total protein (ml/L)	% of control	Cholesterol (mmol/L)	% of control
0.00	33.25 ± 6.72[a]	100	0.80 ± 0.42[b]	100
250.00	38.00 ± 13.44[a]	114.29	1.81 ± 0.95[a]	147.5
300.00	23.75 ± 6.72[ab]	71.43	1.23 ± 0.11[a]	153.75
350.00	19.00 ± 0.00[c]	57.14	0.80 ± 0.42[b]	100
400.00	19.00 ± 0.00[c]	57.14	0.55 ± 0.07[bc]	68.75
450.00	28.50 ± 0.00[ab]	85.71	1.03 ± 0.46[a]	128.75

Means with the same superscript in the same column are not significantly different (P>0.05).

Table 3: Total protein and cholesterol in the tissue of *Tympanotonus fuscatus* exposed to diesel for six days

Concentration of diesel (ml/L)	Total protein (ml/L)	% of control	Cholesterol (mmol/L)	% of control
0.00	45.13 ± 16.80[a]	100	0.80 ± 0.00[b]	100
250.00	35.63 ± 3.36[ab]	78.94	1.88 ± 0.99[a]	235.50
300.00	26.13 ± 3.36[c]	57.89	1.14 ± 0.13[a]	142.50
350.00	16.63 ± 3.36[d]	36.84	0.74 ± 0.08[b]	92.50
400.00	23.75 ± 6.72[c]	52.63	0.47 ± 0.12[c]	58.13
450.00	30.88 ± 3.36[b]	68.42	1.09 ± 0.08[a]	136.25

Means with the same superscript in the same column are not significantly different (P>0.05).

Assessment of protein content of an organism is a diagnostic tool used to determine the physiological and health status of an organism because it reveals the underlying conditions of cells and tissues (Manoj, 1999). Protein have been found to possess nutritive, protective, buffering and energetic functions (Inyang *et a*l., 2010). Alterations in protein content of organisms have been reported in other studies when organisms are exposed to different toxicants (Singh *et al*., 2010; Khan *et al*.,, 2003; Yousafzai and Shakoori, 2011). In this study, the protein content in *Tympanotonus fuscatus* decreased in value as compared to the control, which is a direct consequence of diesel toxicity. The decrease in protein content after exposure to sublethal concentrations of diesel may be due to inhibition of protein synthesis and also from the interference of the toxicant with protein metabolism (Das and Murkherjee, 2000). Decrease in total protein in *Tympanotonus fuscatus* could also result from a state of dehydration and change in the homeostatic balance in the organism due to alteration in its synthesis (Gluth and Hanke, 1984). According to Singh and Khare (1999) and Desai (2002), toxicant induced stress can decrease protein content in tissues of animals. Proteins are involved mainly in building cell architecture (Singh *et al*., 2010) and therefore any interference with this function such as this in the study will break down the structural architecture and integrity of the cells or tissues. In stressed conditions, proteins serve as alternative source of energy (Magdy *et al*., 1993; Singh *et al*., 2010). In such situations as this (stress) organisms need more energy to detoxify toxicants in order to overcome stress and proteins being the next alternative source of energy to carbohydrates is utilized to meet the increased requirement (demand) for energy (Umminger, 1977). The depletion of protein content may be due to degradation and the possible utilization of the degraded products for metabolic purposes (Tiwari and Singh, 2005) and tissue function impairment or injury (Birkner *et al*., 2000; Grucka-Mamezar *et al*., 2005). Decrease in protein content due to its utilization in energy increases free amino acid levels in organisms and impairs the incorporation of amino acids in protein synthesis (Sambasiva Rao, 1999)

which may be the case with in this study, since the building block of proteins (amino acids) cannot be incorporated to form the blocks.

Cholesterol is a versatile lipid. In addition to its essential role as a cell membrane constituent, it acts as a building block for steroid hormones and vitamin D, including the adrenal gland hormones, cortisol and aldosterone, as well as the sex hormones progesterone, estrogens and testerone and their derivatives (Hanokoglu, 1992) and may also act as an antioxidant (Smith, 1991). In this study, there were both increase and decrease in the levels of cholesterol in *Tympanotonus fuscatus.* Changes in cholesterol values have been reported in other studies (Singh *et al*., 2010; Yousafzai and Shakoori, 2011). Decline in cholesterol level may result from the utilization of stored and circulatory cholesterol and other lipid fractions in the treated *Tympanotonus fuscatus* to counter the effect produced by the diesel and further stabilization of the toxic diesel molecules to prevent harm. Decrease in cholesterol level can also interfere with its function in building blocks for steroids and vitamin D. however, its increase as was observed in some concentrations will enhance the aforementioned functions in the *Tympanotonus fuscatus*. Cholesterol helps to build and maintain cell membrane by modulating membrane fluidity over the range of some physiological conditions. It increases membrane packing which in turn increases membrane fluidity (Sadava *et al*., 2011). In this structural role, cholesterol reduces the permeability of tissue membrane to neutral solutes (Yeagle, 1991), protons and sodium ions (Haines, 2001). Therefore, the increase in cholesterol observed is to counter the effect of the toxicity of diesel on the organism, *Tympanotonus fuscatus*. The increase in cholesterol value can also result from the inability of the organism to utilize or break it down to its derivatives or other useful products as a result of the toxicant effect.

REFERENCE

Adeyemo, O. K. (2005). Haematological and histopathological effects of cassava effluent in *Clarias gariepinus*. *African Journal of Biomedical Research*.

Afolabi, O. A., Adeyemi, S. A. and Imevbore, A. M. A. (1985). Studies on toxicity of some Nigerian crude oils to some aquatic. Proceedings of the International Seminar on petroleum industry and Nigerian environment. NNPC, 269-272.

Baron, M. G., Carls, M. G., Short, J. W. and Rice, S. D. (2003). Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North slope crude oil to Pacific hering eggs and larvae. *Environ. Toxicol. Chem*. 22 (3):650-660.

Beynon, L. R. and Cowel, E. B. (1974). Ecological aspects of toxicity testing of oils and dispersants. Applied Science Publishers Ltd. London, 149pp.

Birkner, E., Mamczar, E. G., Machoy, Z., Tarnawski, R. and Polaniak, R. (2000). Disturbances of protein metabolism in rats after acute poisoning with sodium fluoride. *Flouride* 33: 182-186.

Brucka-Jastrzebska, E. and Protsowicki, M. (2005). Effect of cadmium and nickel exposure on haematological parameters of common carp, *Cyprinus carpio. L. Acta Ichthyological et Piscatoria*, 35(1): 29-38.

Carbioch, L., Dauvin, J. C. and Gentil, F. (1977). Preliminary observation on pollution of the seabed and disturbance of sublitoral communities in Northern Brittany by oil at the Amoco Cardiz. *Marine Poll. Bull*. 9: 303-307.

Dange, D. A. and Masureka, B. V. (198). Toluene toxicity: Effects of sublethal levels on enzyme activities in seawater adapted tilapia (*Sarotherodon mossambicus* Peters). *J. Biosci*. 3(2): 129-134.

Das, B. K. and Mukherjee, S. C. 2000. Sublethal effects of quinalphos on selected blood parameters of *Labeo rohita* (Ham) fingerlings. *Asian Fish Sci*. 13: 225-233.

Dede, E. and Kagbo, H. D. (2002). A study in the acute toxicological effects of commercial diesel in Nigerian rats, Rattus rattus using haematological parameters. *J. Appl. Sci. Environ. Mgt*. 6(1): 84-86.

Dessai, H. S. (2002). Toxicological effect on some biochemical parameters of fresh water fish *Channa punctatus* under the stress of nickel. *J. Environ. Biol*. 23(3): 275-277.

Duffus, J. H. (1980). Environmental toxicology. Edward Arnold Publishers Ltd. London. 21-46.

Dumas, B. T. (1971). Determination of total protein and albumin in serum. *Clinical Chemistry, Acta*, 31: 87-96.

Gabriel, U. U., Amakiri, N. E. and Ezeri, G. N. O. (2007). Haematolgy and gill pathology of Clarias

gariepinus exposed to petroleum oil, kerosene under laboratory conditions. *Journal of Animal and Verterinary Advances*, 6(3): 461-465.

Gluth, G. and Hanko, W. (1984). A comparism of physiological changes in carp (*Cyprinus carpio*) induced by several pollutants at sublethal concentration II- the dependency on the temperature. *Comp. Biochem. Physiol.* 79C: 39-45.

Grucka-Mamczar, E., Birkner, E., Zelejska-Fiolka, J. and Machoy, Z. (2005). Disturbances of kidney function in rats with fluoride-induced hyperglycemia after acute poisoning by sodium fluoride. Fluoride, 38: 48-51.

Haines, T. H. (2001). Do sterols reduce proton and sodium levels through lipid bilayers. *Prog. Lipid Res*. 40(4): 299-321.

Hanokoglu, I. (1992). Steroidogenic enzymes: Structure, function and role in regulation of steroid hormone biosynthesis. *J. Steroids Biochem. Mol. Biol*. 43(8): 779-804.

Howard, I. C., Gabriel, U. U. and Horsfall, M. (2009). Evaluation of total hydrocarbon levels in some aquatic media in an oil polluted mangrove wetland in the Niger Delta. *Applied Ecology and Environmental Research*, 7 (2): 111-120.

Imevbore, A. M. A. and Adeyemi, S. A. (1981). Environmental monitoring in relation to oil pollution. In: Proc. Of the conf. on the petroleum Ind. and the Nigerian environment, NNPC/FMWH, PTI, Warri, Nigeria. 135-142.

Inyang, I. R., Daka, E. R. and Ogamba, E. N. (2010). Effect of sub-lethal concentrations of diazinon on total protein and transaminase activities in *Clarias gariepinus*. *Current Research Journal of Biological Sciences*, 2(6): 390-395.

Khan, Z. M., Tabassum, R., Naqvi, S. N. H., Shah, Z. E., tabassum, F., Ahmad, I., Fatima, F. and Khan, F. M. (2003). Effect of cypermethrin and permethrin in cholinesterase activity and protein contents in *Rana tigrina* (Amphibia). *Turk. J. Zool.* 27: 243-246

. Liu, B., Romaire, R. D., Elaune, R. D. and Lindau, C. W. (2006). Field investigation on the toxicity of Alaska North slope crude oil and dispersed ANSC crude oil to Gulf killifish, Eastern oyster and white shrimp. *Chem*. 62(4): 520-526.

Magdy, A., Salah, E. and Rogers, W. (1993). Changes in total protein and amino transaminase activities of grass carp exposed to diquat. Biol. Physiol. 2: 35-39.

Manoj, K. (1999). Mercury, copper and cadmium induced changes in the total protein levels in muscle tissue of an edible estuarine fish *Boleopthalmus dessumuri*. *Cuv. J. Environ. Biol*. 20: 231-234.

Osuji, L. C. and Mbata, O. E. (2004). Quantal response of *Oreochromis niloticus* to toxicity to water soluble fraction of Nigeria's Bonny light crude oil. *Scientia Africana*, 3(1): 34-39.

Patin, S. (1999). Environmental impact of the offshore oil and gas industry. Ecomonitor East Northport, New York. 425pp

.

Prasad, M. S., Prasad, M. and Singh, D. (1987). Some haematological effects oil on fresh water fish, *Heteropneustes fossilis. Acta Hydrochem. Hydrobiol*. 15(2): 199-204.

Rivers State Environmental Protection Bureu (RSEPB), (1992). Ecological distribution of oil spills. Pollution paper No. 4., Port Harcourt, Nigeria, 15pp

.

Sadava, D., Hills, D. M., Heller, H. C. and Berenbaum, M. R. (2011). Life: The science of biology, 9th edition. San Fracisco: Freeman pp 105-144. 1: 21

.

Sambasiva Rao, K. R. S. (1999). Pesticide impact on fish metabolism. Discovery Publishing House, New Delhi (India), pp 66-70.

Singh, S. and Khare, A. (1999). Effect of pesticide on protein metabolism in liver of *Clarias batrachus*. *Rec. Ad. App. Env. Zool*. 1: 21.

Singh, P. A., Singh, S., Bhartiya, P. and Yadav, K. (2010). Toxic effect of phorate on the serum biochemical parameters of the snake headed fish *Channa punctatus* (Bloch). *Advances in Bioresearch*, 1(1): 177-181.

Smith, L. L. (1991). Another cholesterol hypothesis: Cholesterol as antioxidant. *Free Radic. Biol. Med.* 11(1): 47-61.

Stegeman, J. J., Brouwer, M. D., Guilio, R. T., Forlin, L., Fowler, B. A., sanders, B. M. and Van Veld, P. A. (1992). Molecular responses to

environmental contamination. Enzyme and protein systems as indicators of chemical exposure and effect- In: hugget, R. J., Kimerle, R. A., Mehrle, P. M. and Bergerman, H. L. (Eds). Biomarkers, biochemical, physiological, histological markers of anthropogenic stress. Lewis Publishers,M I, USA, 86-120.

Thorhang, A. (1992). The involvement fortune of Kuwaits- In: Al-Shatti, A. K. and Hurigtion, J. M. Eds. Proc. Of international symposium on environment and health impacts of the Kuwaiti oil fires, Edgbaston: the University of Birmingham Press.

Tiwari, S. and Singh, A. (2005). Possibility of using latex extracts of *Nerium indicum* plant for control of predatory fish *Channa punctatus*. *Asian Fisheries Science, 18: 161-173*.

Umminger, B. L. (1977). Relation of the whole blood sugar concentration in vertebrate to standard metabolic rate. *Comp. Biochem. Physiol*. 55: 457-460

Wang, S. Y., Jaw, G. L. and Cheng,Y. L. (1994). Accumulation of 2,4-D and glyphosate in fish and water hyacinth. *Water, air and soil pollution*, 74: 397-403

.

Warnick, G. R. (1991). Compact analysis for cholesterol, triglyceride and high density lipoprotein cholesterol. *Curr. Opn Lipidol*. 2: 343.

Yousafzai, M. A. and Shakoori,R. A. (2011). Hepatic response of a fresh water fish against aquatic pollution.*Pakistan J. Zool*. 43(2): 209-221.

Zar, H. K. (1984). Statistical tools for scientific analysis. Oxford Publishers, London, 319pp.