

## Plasma Glucose and Liver Glycogen of African Catfish (*Clarias gariepinus*) Exposed to Petrol

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**Abstract:** The sublethal effects of water soluble fractions of petrol (WSF) to the plasma glucose and liver glycogen of *Clarias gariepinus* with mean weight of  $4.76 \pm 0.4$  g were investigated under laboratory conditions for 10 weeks. At sublethal concentrations of 1.36, 0.68, 0.34, 0.17 and  $0.09 \text{ g L}^{-1}$  of WSF of petrol, hyperglycaemia, a situation of increased plasma glucose level above the control was observed. Exposed group of fish to the toxicant were significantly lower ( $p < 0.05$ ) in liver glycogen content than the control group of fish.

**Key words:** Plasmaglucoase, liver glycogen, *Clarias gariepinus*, petrol

### INTRODUCTION

Due to the ever increasing growth in world population, mans activities arising from industrialization is bound to result in more pollutants being passed into water bodies and this has deleterious effects on aquatic environments and their biota (Miles and Roster, 1999). One of the most important environmental factors acting as stressor to fish is pollution which has become a global problem (Grizzle, 1977). Awachie (1981) reported that pollutants in aquatic environment act as stressor to fish and cause a reduction of water quality. Petrol is a refined crude oil product of a mixture of non-cyclic volatile hydrocarbons suitable for use in spark ignited combustion engines. Its most important toxic additive is akyllead which when combined with other additives such as detergents, copper, phenolic groups and toxic volatile hydrocarbons mixtures cause harmful effects to fish (Cote, 1976). Omoregie *et al.* (1994) documented that fish show marked glycaemic responses to stressful environmental conditions by increasing metabolic activity which expends energy leading to depletion of carbohydrate reserves.

### MATERIALS AND METHODS

The method of preparation of water soluble fraction of petrol for toxicological bioassay as described by Anderson, Smith and Cameron and UNEP was employed in this investigation. Water Soluble Fraction of Petrol was extracted using the method of Cooper and Guns.

The method of determination of carbohydrate reserves using the anthrone (ortho-toluidine) method

described by Wedemeyer and Yasutake (1977) was used. Fish blood was collected from the caudal penduncle as recommended by Blaxhall and Daisley (1973) into a heparinised micropipette and centrifuged at  $3000 \text{ rev. min}^{-1}$  for 5 min to separate out the plasma. Thereafter, 0.05 mL of plasma was transferred into a test tube that contained the anthrone. The same amount of glucose-standard was taken out and both were heated for 10 min, cooled and their absorbance read using a colorimeter at 530 nm wavelength. Distilled water was used as blank.

For liver glycogen, 100 mg of liver removed from slaughtered fingerlings was boiled with 3% potassium hydroxide for 20 min to facilitate dissolution before addition of sodium sulphate and 95% ethanol that dissolved it. Liver solution was boiled, cooled and centrifuged at 3000 revolutions for 5 min. The liver residue was redissolved in distilled water and reprecipitated, recentrifuged and hydrolysed with 5 M hydrochloric acid before neutralizing with 0.5 M sodium hydroxide and phenol red as the indicator of the titration. The hydrolysed glycogen was diluted with distilled water to 100 and 5 mL from this was added to 10 mL of anthrone. Same amount of glucose standard was added to equal quantity of anthrone as well. These were boiled for 10 min before reading the absorbance value with a colorimeter at 620 nm. Distilled water served as blank.

### RESULTS

The result of plasma glucose ( $\text{mg L}^{-1}$ ), Liver glycogen ( $\text{mg g}^{-1}$ ) and muscle glycogen ( $\text{mg g}^{-1}$ ) are presented in Fig. 1-3 and Table 1-3, respectively. There

Table 1: Mean (+ se) Plasma Glucose (mg ml) of *calarias gariepinus* Exposed to various Sublethal Concentrations of (WSF) of Petrol for 10 weeks

		Exposure period (weeks)					Mean
		2	4	6	8	10	
Concentration	Start	(g L <sup>-1</sup> )					
1.36	0.84±0.01	1.13±0.01	1.23±0.02	1.31±0.02	1.34±0.02	1.50±0.01	1.225±0.10
0.68	0.85±0.01	1.12±0.02	1.12±0.03	1.12±0.01	1.25±0.01	1.30±0.02	1.143±0.07
0.34	0.84±0.01	0.91±0.01	0.06±0.02	1.24±0.03	1.20±0.01	1.19±0.04	1.073±0.07
0.17	0.85±0.02	0.91±0.02	0.06±0.03	1.13±0.02	1.19±0.04	1.23±0.01	1.062±0.07
0.09	0.85±0.02	0.91±0.01	0.06±0.01	1.12±0.01	1.17±0.01	1.22±0.03	1.055±0.07
0.00	0.84±0.01	0.86±0.02	0.91±0.01	0.97±0.01	1.10±0.03	1.02±0.02	0.950±0.04

Table 2: Mean (se) Liver Glycogen Content (mg g<sup>-1</sup>) of *Clarias gariepinus* exposed to Various Sublethal Concentrations of (WSF) of Petrol for 10 weeks

		Exposure period (weeks)					Mean
		2	4	6	8	10	
Concentrations	Start	(g L <sup>-1</sup> )					
1.36	1.04±0.02	0.55±0.02	0.49±0.01	0.29±0.01	0.17±0.0	0.15±0.02	0.45±0.149
0.68	1.03±0.03	0.76±0.01	0.54±0.01	0.45±0.01	0.30±0.0	0.18±0.01	0.54±0.139
0.34	1.04±0.03	0.91±0.01	0.82±0.01	0.74±0.01	0.42±0.0	0.45±0.01	0.73±0.112
0.17	1.03±0.03	0.69±0.01	0.85±0.01	0.84±0.01	0.18±0.0	0.43±0.014	0.78±0.090
0.09	1.04±0.01	1.06±0.02	1.03±0.02	0.99±0.01	0.82±0.0	0.55±0.02	0.92±0.172
0.00	1.04±0.02	1.09±0.02	1.01±0.01	1.14±0.01	1.21±0.01	1.36±0.01	1.14±0.058

Table 3: Mean (±se) Muscle Glycogen (mg g<sup>-1</sup>) of *Clarias gariepinus* Exposed to Various Sublethal Concentrations of (WSF) of Petrol for 10 weeks

		Exposure period (weeks)					Mean
		2	4	6	8	10	
Concentrations	Start	(g L <sup>-1</sup> )					
1.36	0.08±0.01	0.06±0.00	0.06±0.00	0.05±0.01	0.04±0.01	0.03±0.00	0.053±0.009
0.68	0.08±0.01	0.07±0.01	0.06±0.01	0.05±0.02	0.05±0.02	0.03±0.00	0.057±0.008
0.34	0.08±0.01	0.07±0.01	0.07±0.01	0.05±0.01	0.06±0.01	0.05±0.01	0.062±0.005
0.17	0.08±0.00	0.07±0.00	11.07±0.01	0.07±0.01	0.04±0.02	0.05±0.01	0.063±0.007
0.09	0.08±0.01	0.07±0.00	0.06±0.00	0.07±0.00	0.06±0.01	0.04±0.00	0.065±0.006
0.00	0.08±0.01	0.07±0.01	0.11±0.01	0.14±0.01	0.19±0.01	0.21±0.01	0.137±0.024

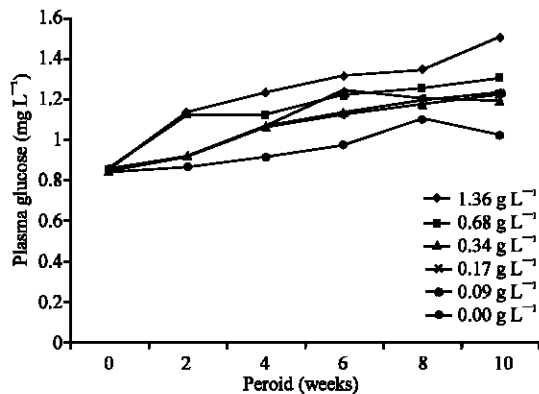


Fig. 1: Effects of various Sublethal Concentrations of petrol on the Plasma Glucose of *C. gariepinus* exposed for 10 weeks

was significant increase in plasma glucose in fish blood exposed to toxicant at concentration 1.36, 0.68, 0.34, 0.17 and 0.09 g L<sup>-1</sup> ( $p < 0.05$ ). At the commencement of the work, plasma glucose values at various concentration were not significant ( $p > 0.05$ ). Similarly, mean plasma glucose of fish exposed to 0.09 and 0.17 g L<sup>-1</sup> was not significant from each other ( $p > 0.05$ ) but significant from

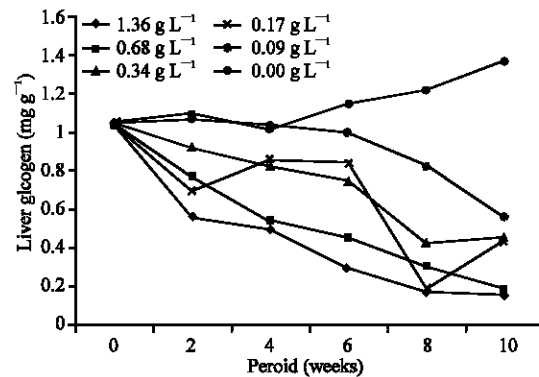


Fig. 2: Effects of various Sublethal Concentrations of Petrol on the Liver Glycogen content of *C. gariepinus* exposed for 10 weeks

other groups of fish. The mean value of liver glycogen and muscle glycogen of exposed fish to various concentrations of WSF of petrol decreased significantly ( $p < 0.05$ ) as the exposure period increased. Decrease in liver glycogen and muscle glycogen were directly proportional to increasing toxicant concentration, conversely the increase in plasma glucose was dose dependent (singh *et al.*, 1994).

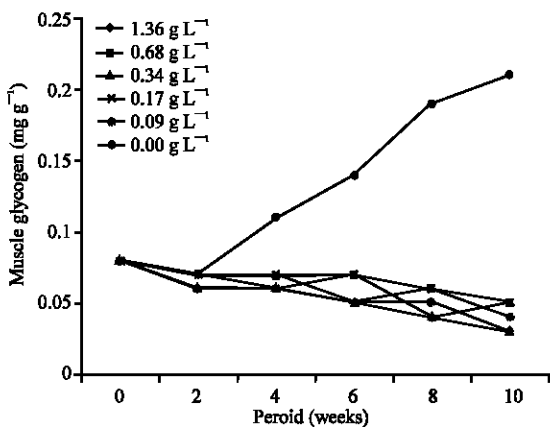


Fig. 3: Effects of various Sublethal Concentrations (WSF) of petrol on Muscle Glycogen ( $\text{mg g}^{-1}$ ) of *C. gariepinus* exposed for 10 weeks

### DISCUSSION

The plasma glucose of the exposed fish to sublethal concentrations of WSF of petrol was significantly higher than those of the control group of fish. The progressive accumulation of plasma glucose with increasing concentration and period indicated hyperglycaemic response. This development is in agreement with earlier findings by Wedemeyer (1973) who reported that fish show marked hyper-glycaemic response to stressful environmental condition. Oguri and Nace (1966) reported that progressive accumulation of plasma glucose is due to depletion of stored glycogen by effects of stress hormone-catecholamine. The significant reduction in liver glycogen confirmed that the fish was stressed. The toxicant-dose dependent depletion of liver glycogen suggest that the fish was unable to efficiently absorb soluble glucose from the intestine or due to breakdown of liver cells by the toxicant. (Oladimeji and Ologunmeta, 1987; Omoregie *et al.*, 1995; Wade *et al.*, 2002).

The significantly dose-dependent reduction in the muscle and liver glycogen contents of the fish exposed to the water soluble fractions of petrol indicated that metabolism of carbohydrates was impaired. Fish usually increase their metabolic rates to metabolise and excrete aromatic hydrocarbons to allocate greater amount of energy to homeostatic maintenance than its storage, leading to a reduction in stored energy food reserves.

Adebriem (1976), however stated that liver glycogen reduction may be due to enzymatic activities in liver resulted from specific phosphates and glycogen phosphorylase.

### REFERENCES

- Adebriem, A., 1976. Biochemical Toxicology of Environmental Agents. North Holland Biomedical Press, Elsevier, pp: 210.
- Awachie, J.B.E., 1981. Some general considerations on River Basins in Africa in relation to fisheries seminar on river basin management and development. CIFA Technical Paper No. 8.
- Blaxhall, P.C. and R.W. Daisley, 1973. Routine Haematological Methods for use with fish blood. J. Fish Biol., 5: 771-781.
- Cote, R.P., 1976. The effects of petroleum refinery liquids waste on aquatic life, with special emphasis on the Canadian Environment, Canada. National Research of Canadian Association Committee on Scientific Criteria, pp: 77.
- Grizzle, J.M., 1977. Haematological changes in fingerlings of channel catfish exposed to malachite green. Progressive Fish Culturist, 39: 90-93.
- Miles, A.K. and N. Roster, 1999. Enhancement of polycyclic aromatic hydrocarbons in estuarine invertebrates by surface runoff at a decommissioned. Military fuel depot. Marine Environ. Res., 47: 49-60.
- Oguri, M. and Nace, 1966. Blood sugar and adrenal histology of gold fish after treatment with mammalian adrenocorticotrophic hormone. Chesapeake Sci., 7: 198-202.
- Oladimeji, A.A. and R.T. Ologunmeta, 1987. Toxicology of water borne lead to *Tilapia niloticus*. Nigerian J. Aqua. Sci., 2: 19-24.
- Omorie, E., Thomas G. Eseyin and P.C.O Ofojekwu, 1994. Chronic effects of formalin on erythrocyte counts and plasma glucose of Nile tilapia, *Oreochromis niloticus*. Asian Fish. Sci., 7: 1-6.
- Omorie, E., E.B.C. Ufodike and C.O.E. Onwuliri, 1995. Effects of petroleum effluent on Carbohydrate reserves of Nile tilapia, *Oreochromis niloticus* (L.), West Afr. J. Biol. Sci., 3: 70-76.
- Singh, S.N., R.K. Charterjee and A.K. Srivastava, 1994. Effects of glycosides of *streblus asperon* motility of glucose uptake and certain enzymes of carbohydrate metabolism of *setria cervi*. Drug Dev. Res., 32: 191-195.
- Wade, J.W., E. Omorie and I. Ezenwaka, 2002. Toxicity of cassava *Manihot esculenta* effluent on the Nile tilapia *Oreochromis niloticus* (L.). J. Aqua. Sci., 17 (2): 89-94.
- Wedemeyer, G.A., 1973. Physiological consequences of handling stress in the juvenile coho-salmon and steel head trout. J. Fish Res. Board Can., 29: 1780-1783.
- Wedemeyer, G.A. and W. Yasutake, 1977. Clinical Methods of Environmental Stress on Fish. Technical Paper, No. 89, Washington D.C., pp: 18.