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The effect of spent engine oil on Guppies (*Poecilia reticulata*)

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ABSTRACT

The effect of spent engine oil on fresh water Guppy *(Poecilia reticulata)* were studied by exposing fry to different concentrations of spent engine oil in aquaria. In the acute toxicity tests, LC_{50} of spent engine oil were 6.012, 1. 655, 2.162 and 2.723 at 24, 48, 72 and 96 hours respectively. This shows that the impacts are dose and time dependent with respect to marked reduction in mortality rate. Although, at sub-lethal concentrations of the test substance, there were considerably low mortality rates especially at lower concentrations of the substance. Histological studies carried out on the intestine and liver of treated *P. reticulata* showed that absorption of spent engine oil resulted in the swelling of the intestinal villi at concentrations of more than 1.0ml/l of test substance. More devastating impacts were caused by exposure to higher concentrations. Histological examination of the liver of the fish exposed to higher concentrations showed a distortion of the hepatic portal vein. The implications of the study are that pollution from spent engine oil results in the release of vast quantities of lethal and potentially genotoxic contaminants in the environment.

Keywords: Spent engine oil, guppies, genotoxicity, acute and chronic toxicity.

INTRODUCTION

The exponential increase in the world's population has resulted in the increase in the demands for goods and services. This has led to an increase in the use of engine oil and consequently increase in the production of spent engine oil. Some oil mishaps and other pollution incidents unrelated to oil have heightened the need for an environmental protection policy and legislation in many countries of the world including Nigeria (Oyewo, 1988).

Engine oil is a distillation product of crude oil used as lubricant in the internal combustion engines of automobiles where it reduces surface friction between metals, thus preventing quick wear and tear of engines (Obidike, 1985). In the process of running the engines, the engine oil collects more metabolic deposits and hence needs to be replaced regularly. In many developed countries, the spent engine or used engine oil is largely recycled. In Nigeria, there is improper disposal with a majority of the road side mechanics indiscriminately dumping the spent engine oil into drainage, roads sides, rivers, streams and lagoons especially in Lagos metropolis.

Spent engine oil consists of hydrocarbon constituents and trace elements. The hydrocarbon constituents include gasoline, kerosene and gas oil fraction (Edebiri and Nwaokwale, 1983). These trace elements include calcium, magnesium, zinc, barium, iron, copper, cadmium, sulphur, lead and cyanide. Of these trace elements, only calcium, magnesium, zinc and barium are the main constituents of fresh engine oil (PTI Research Laboratory 1995).

There has been a rapid growth of interest in the use of fish cell livers in toxicity testing. Bols *et* *al*. (1985) demonstrated that the cytotoxicity of aromatic hydrocarbons correlated with acute toxicity of fish cell lines. However, its use is still largely limited to hepatocytes and gill epithelial cells. Parameters used to monitor adverse effects include cell proliferation membrane, transport metabolism and morphological alteration (Isomaa *et al.,* 1996). One of the most important toxic effects induced by environmental contaminants is genotoxicity (Odeigah *et al.,* 1997). Genetoxicity is a concept measuring "toxicity to the genome". Central to this concept provokes different effects namely irreversible effects such as mutagenesis, phage induction, cell death, chromosome breakage and reversible effects like inhibition of division (Hafund and Quilladet, 1984).

Unwholesome disposal of spent engine oil is one of the growing environmental problems in Nigeria, and may be wide spread than crude oil pollution which is confined mainly to the Nigeria Delta Zone of Nigeria. This is because the spent engine oil contains some non-degradable metallic components such as lead (Pb) and Zinc (Zn) which have biological actions against animals including man. Therefore, there is need for a study of the biological effects caused by the metals found in spent engine oil. This paper reports the toxic histological impacts of spent engine oil on *P. reticulata.* The acute and chronic profiles of test spent engine oil against the test animals are also reported.

MATERIALS AND METHODS

Collection of test organism and acclimatization

Active and healthy specimens of *Poecilia reticulata* (guppy) fry, about 5 weeks of age collected in a container from a fish pond in Lagos and taken to the Research Laboratory of the Department of Cell Biology and Genetics, University of Lagos, Nigeria. In the Laboratory, the fry were placed in two glass fish aquaria (dimensions-50cmx45cmx50cm) half filled with dechlorinated tap water. Each aquarium contains 50 guppy fry. The medium was aerated using aerator model number RENA 301 changed once every three days to prevent accumulation of waste metabolites from the animals. The fish were acclimatized to laboratory temperature (28$\pm$ 2^{o}C), for a period of one week before being used in bioassays. During this period of acclimatization, they were fed with commercial feed vitaflake. The feed used in this study was a diet formulated to provide the fish with adequate amounts of protein, carbohydrate, fat, vitamins and minerals. The fish were fed twice everyday, once in the morning and once in the evening.

The test substance

The test substance is a two month old engine oil from a Private Toyota car. The estimated kilometers that was covered by the car before draining the oil was put at 2000km. The spent engine oil was drained directly into a sterile glass container and taken to the laboratory.

EXPERIMENTAL

Preparation of polluted aquaria

Various polluted microcusm aquaria were prepared by introducing calculated fractions (ml) of the spent engine oil and dechlorinated tap water into them. The concentrations (v/v) achieved were 0.05, 1.00, 1.50 and 2.00 m/l. The controls had no engine oil. These microcusm aquaria were set aside for about 10 minutes after stirring, before introducing the guppy fry into them.

Active and healthy guppy fry of average length of 3.5cm were selected without discrimination of both sexes, with the aid of hand net from the acclimatized stock before being assigned to the sterilized bioassay glass aquaria. Predetermined amounts of spent engine oil were measured out and made up to the required volume of medium, by adding the appropriate volume of dechlorinated water to achieved different concentrations as required for each experiment.

Acute toxicity test

Ten guppy fry were carefully selected and introduced into each of the three bioassay microcusm aquaria at each of the different concentrations of the test substance. The test medium was stirred twice a day (morning and evening) to simulate wave action as in the natural habitat and to maintain a constant mixture of spent engine oil and dechlorinated water. The mortality rates of the test animals were continuously monitored at 24,48,72 and 96 hours when the acute toxicity experiment ended. The LC_{50} (concentration that would cause 50% mortality) was determined from the data.

Chronic toxicity test

From the acute toxicity test results, the following sub-lethal concentrations of the test substance were used for chronic toxicity test: 0.33, 0.17 and 0.08 ml/l. The chronic test lasted for three weeks.

Effect Analysis

Histological observation

The intestine and liver were used for histological studies. They were obtained from fingerlings at the point of death. The tissues were first fixed by placing them in Bouins fluid. Excess water was removed by blotting out water and then counter stained in 1% aqeous eosin stain for 3 minutes and differentiated in 70% ethanol. The slides were dried, cleaned in xylene and mounted in Canada Balsam. This was followed by drying under light microscope. Photomicrgraphs of the tissues were finally taken using a Zeiss photomicroscope.

RESULTS AND DISCUSSION

The fingerlings showed some behavioural responses to the test substance such as slight adaptive colouration (from dull grey to light grey in females and more colourful in males) in response to their change of environment, reduction or inhibition of their feeding rate and activity. Although, due to the experimental set up, it was not feasible to ascertain the exact feeding rate. Also, they showed signs of body weakness before death. Swiftness of movement was detected and assessed after a tap at the aquaria.

Table 1 shows the LC_{50} of spent engine oil on the fingerlings: 6.012, 1.655, 2.162 and 2.723 at 24,48, 72 and 96 hours respectively. The positive correlation between the cumulative percentage mortality (probit scale of the test organism) and the various concentrations of spent engine oil is as shown in figures 1,2,3, and 4. The high LC_{50} (6.012) of acute toxicity test at 24 hours exposure to test substance was followed by sharp reduction in mortality rate after 24hours (1.655, 2.162 and

Table -1: Toxicity of spent engine oil against *Poecilia reticulata*

Conc. ml/l Time	LC_{95} 95% C.L	LC_{50} 95% C.L	LC_{5} 95% C.L	SCOPE ± S.E	D.F	Regression Equation
24hrs	19.952(176.245- 3.863)	6.012 (70703.066-0.072)	1.812(70703.066-0.072)	3.167±0.467	2.0	Y= -2.467+3.167X
48hr	8.275(112.572-3.495)	1.655(2.956-1.224)	0.331(0.613-0.054)	2.360±0.540	2.0	Y= -0516+2.360X
72hrs	10.660(268.39-3.621)	2.162(5.345-1.488)	0.418(0.794-0.082)	2.381±0.653	2.0	Y= -0.797+2.381X
96hrs	17.727(9459-3.705)	2.723(18.342-1.580)	0.418(0.863-0.028)	2.028±0.652	2.0	Y= -0.882+2.028X.

2.723) at 48, 72 and 96 hours respectively, of the dose – dependent LC_{50} result. The above phenomenon could be as a result of elimination of the immunologically suppressed fingerlings. This could be due to obstruction of the hepatic portal vein leading to splitting and atrophy of liver cells. This is clearly indicated in plate 4.

Table - 2: General characteristics of spent engine oil as analysed by PTI (1995)

Variable	Value
Specific Gravity at 60/60 0 °F	0.9001
% Carbon residue	3.0
Viscosity at 50 0°	41.3 centistrokes
sulphur	1.3ppm
Calcium	12ppm
Magnesium	12 ppm
Lead	42ppm
Cadmium	31ppm
zinc	66ppm
Copper	44pm
Iron	22ppm
Cyanide	0.76ppm

Source: Petroleum Training Institute (PTI) Research Laboratory (1995)

Table - 3: Metallic well as sulphur and cyanide constituents of fresh and spent engine oil.

Fresh engine oil	Spent engine oil
Calcium	Calcium
Magnesium	Magnesium
Zinc	Zinc
Barium	Barium
-	Iron
-	Copper
-	Cadmium
-	Sulphur
-	Lead
-	Cyanide

The result from chronic test showed that average percentage mortality were 15.2, 8.4, 2.5 and 0.5 for 0.33, 0.17, 0.11 and 0.08 m1/l respectively. This indicates considerable low mortality rates and suggests that *Poecilia reticulata* is a good bioaccumulator. This could be inferred from the work of Edebiri and Nwaokwale, 1983 who demonstrated the biological effect caused by accumulation of metals through spent engine oil by hermit crabs when exposed to sub lethal concentration of spent engine oil.

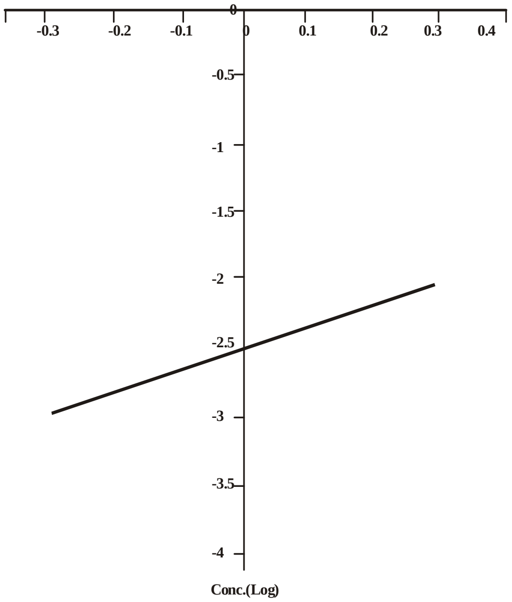

Fig. -1: Graph showing the toxicity of spent engine oil at 24 hrs of exposure

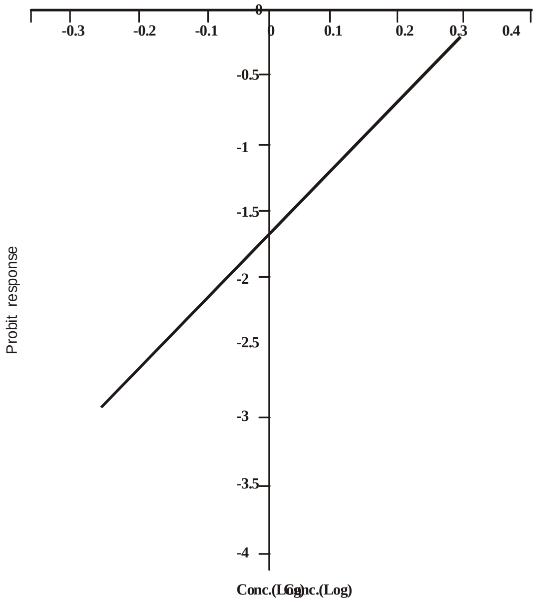

Fig. -2: Graph showing the toxicity of spent engine oil at 48 hrs of exposure

Fig. -3: Graph showing the toxicity of spent engine oil at 72 hrs of exposure

Fig. -4: Graph showing the toxicity of spent engine oil at 96 hrs of exposure

Plate -1: Normal intestine of fish (*P. reticulata*)

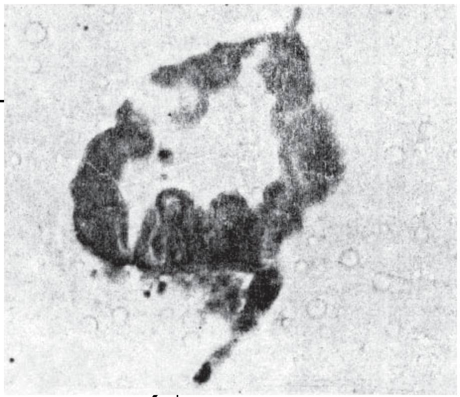

Plate -2: Intestine of fish exposed to 1.5 ml/l of test substance

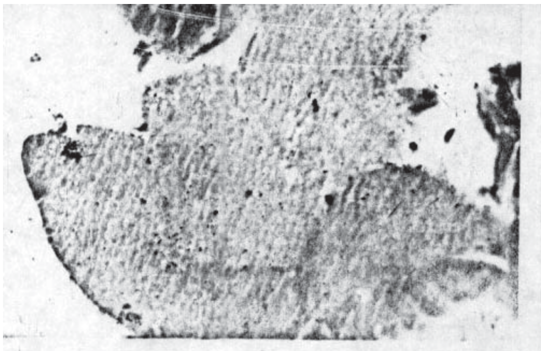

Plate -1: Normal liver cells of fish (*P. reticulata*)

Plate -4: Liver cells exposed to 1.5 ml/l of test substance

A histological comparison of the intestine and liver of impacted *P. reticulata* against the control (plates 2/1 and plates 4/3 respectively) specimens revealed the toxic effects of test substance. The intestinal tract is important as a major site of absorption of toxicants and a site of xenobiotic metabolism. The liver is also important in toxicology because xenobiotics absorbed by the intestine are received via the hepatic portal vein by there liver. Plate I shows that the intestine of the test organism is lined up with intestinal villi or projections of mucosa. The absorption of the spent engine oil (concentration of 1.5 ml/l) resulted in the swelling of the intestinal villi as seen on plate 2.

A more pronounced impact ranging from degeneration of the villi, columnar epithelial cells and vacuolation in different layers of the intestine were caused by exposure to test substance while the tissues exposed to lower concentration showed no remarkable effects. Hence, the effects are dose dependents. Histological examination of liver of fish exposed to higher concentration (plate 4) showed a distortion of hepatic portal vein leading to the widening of the sinusoids, partial loss of shape of liver when compared to the control (plate 3).

Table 2 shows the general characteristics of spent engine oil. When compared with that of fresh engine oil (Table 3), it becomes apparent that the spent engine oil contains some metals such as iron and cadmium that are implicated in toxicity

CONCLUSION

The environmental hazards that result from the pollution of water bodies by spent engine oil is highly attributed to the non-degradable metabolic components such as iron and coppers that play great roles in Teuton Chemistry. Hence, there is need for the formulation and adoption of effective disposal method of recycling of spent engine oil. In addition, acute and chronic bioassays should be carried out on spent engine oil from other sources in order to harmonize their effects.

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