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Chemical Exposure Hazardous for Fish *Hyphessobrycon eques* through the Incorrect Release of Oil in Amazon Region

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ABSTRACT

Incorrect discard of oil used by fishing ship in the Brazilian north region has become a common activity. Despite the possible hazard to the aquatic organisms, still missing scientific data about their toxicity. Thus, this study aimed to evaluate the stress and lethality caused by lubricant oil (FSAOLU) on fish *Hyphessobrycon eques*. Therefore, it was used six different concentrations diluted in water (0, 22, 24, 26, 28, 30 and 32% of oil) and three replicates during 96 hours. At the end or during the experiment (with dying fish), it was collected blood samples (cutting the caudal fin) to determine physiological changes. The FSAOLU showed mean lethal dose (LD50-96h) of 27.36%, classified as toxic causing alterations in glucose values from the 26%. The greater dilution of FSAOLU (32%) increased 115% in glucose values when compared to the control. Thus, lubricant oil when discarded in water, it presents hazard to the aquatic organisms causing stress and mortality for fish being necessary adequate management to discard of this residue.

1. Introduction

Amazon region has a large biodiversity which exploration of natural resources becomes the main income of population. Among the practiced activity in the region, the fishing suffers two types of

exploration (industrial and artisanal) with two types of fishing ship (with and without motor) ^[1,2]. Currently, 370 thousand anglers have this activity as their main source of income ^[3,4].

Fishing ships with combustion engine also used to

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freight transport in the region, they use lubricant oil to reduce engine friction or wear ^[2,5]. However, when it reaches the maximum life time losing its functional characteristics, it become dark and dense named “burned oil” commonly discarded ^[6,7].

With regard to the current legislation ^[8] all burned oil should be collected and recycled by refining process. Nonetheless, in the Brazilian north region, they have 12% of fishing ship discarding oil in water ^[5] providing hazard to the local biodiversity because still missing knowledge about the toxic effect of this residue for native fish.

These oil derivatives have hydrocarbon compounds linked to others substances as nitrogen and sulfur, being the size of hydrocarbon chain determines their toxicity ^[6,9,10]. The toxic potential of oil derivatives has direct correlation with its solubility in water, releasing polycyclic hydrocarbon compounds and BTEXs (Benzene, Toluene, Ethylbenzene and Xylene) ^[11-13]. These compounds has potential to cause bioaccumulation in environment providing hazard to the human and animal health.

Furthermore, the burned oil has greater toxicity when compared with the natural oil which adds heavy metals as lead and benze(α)pirene, affecting the cellular process as well as its use of carbohydrate or glucose ^[14], increasing the mutagen and carcinogen effect ^[10].

In this scenario with incorrect discard of burned oil and missing knowledge about the toxic effects, the use of toxicity tests to determine physiological changes or mortality it becomes too necessary for an adequate future management ^[15].

Among the native fish species, the *Hypphessobrycon eques* stands out as adequate biological model for acute toxicity tests due to their high sensibility ^[16,17]. Thus, this study aimed to determine the lethal dose (LD50%) of burned oil diluted in water using the biological model *H. eques* evaluating physiological responses with glucose values.

2. Material and Method

For this study, it captured (sisbio license 19515) in the Chumucuí River (01° 08 '40.12" S and 46° 34' 04.8" W) and acclimated in 300 L water tanks species of *H. eques* for a period of seven days. Afterwards, all fish passed by feeding deprivation before the experiment ^[15,18]. Preliminary tests determined the FSA dilutions of burned oil that would cause 0 and 100% of mortality. As well as the potassium chloride (KCl) sensitivity test was performed as reference substance. For this test, five KCl concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 g.L⁻¹) were used with three replications and one control using four fish

(1.09±0.12 g) per treatment.

To obtain the concentrations, it prepared a laboratorial solution consisting of 300 mL (burned oil) with 700 mL of distilled water (3:7) homogenized during six hours (QUIMIS® Shaker). Subsequently, the insoluble part was discarded, using only the water-soluble fraction (FSAOLU) for the experiments ^[19]. For definitive acute toxicity test, it was carried out an experiment in completely randomized design with six FSAOLU dilutions (0, 22, 24, 26, 28, 30 and 32% v/v) with three replicates having 15 fish per treatment (1.19±0.14 g) during 96 hours. During the experimental period, each hour had fish mortality determined and the water quality parameters measured for each 24 hours: dissolved oxygen (6.74±0.29 mg.L⁻¹), temperature (28.6±0.62 °C), pH (6.42±0.32), conductivity (119±10.3 μ S.cm⁻¹) and ammonia (0.02±0.01 mg.L⁻¹).

The Trimmed Spearman Karber method ^[20] determined the lethal concentration (LC50) classifying its toxicity according to CETESB ^[21], which classifies the xenobiotic compound into: very toxic (when LC50 is less than 25%), toxic (25 to 50%), moderately toxic (51 to 75%), slightly toxic (above 75%) and virtually non-toxic (100%).

During the experiment, dead or dying fish with loss of reaction, swimming imbalance and minimal opercula beat, had blood samples collected to determine glucose values (Accu-chek Performance®) and afterwards euthanized by medullar section.

The data blood glucose was submitted to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests. Then, it was applied analysis of variance (ANOVA) with post-hoc Tukey test (p<0.05) for comparison of means. Data between mortality rates and FSAOLU concentrations passed by *Pearson* linear regression.

3. Results

The Potassium chloride (KCl) showed lethal concentration (LC_{50-96h}) 1.66 g.L⁻¹ with a lower limit of 1.49 g.L⁻¹ and upper limit of 1.89 g.L⁻¹. The FSAOLU showed lethal concentration (LC_{50-96h}) 26.38% with upper limit of 27.36% and lower of 25.45%, considering toxic chemical according to CETESB ^[21]. The highest concentration (32%) present 100% of mortality within 12 hours of exposure (Figure 1), generating a positive correlation ($y=10.84x-242.59$ with $r^2=0.96$, $p=0.0012$). Furthermore, during the definitive trial, occasional behavioral changes were observed by the addition of FSAOLU, such as erratic swimming, agitation and fish positioning on the water surface.

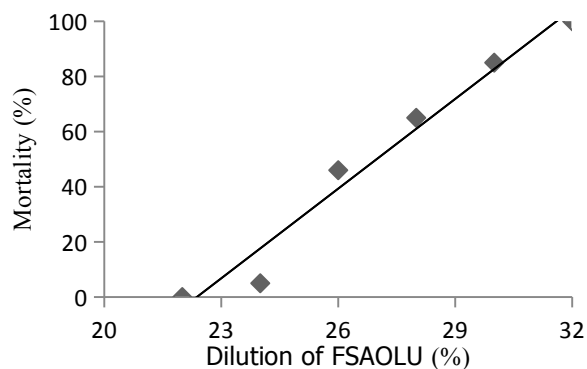


Figure 1. Pearson correlation between mortality and different concentration of FSAOLU in *H. eques*

It was observed alterations in glucose values of fish (figure 2), with the highest values in the greater dilution (32%) ($p=0.0021$) (65.42 ± 2.4 mg.dL⁻¹), showing increases of 115% when compared to the control (30.21 ± 1.11 mg.dL⁻¹).

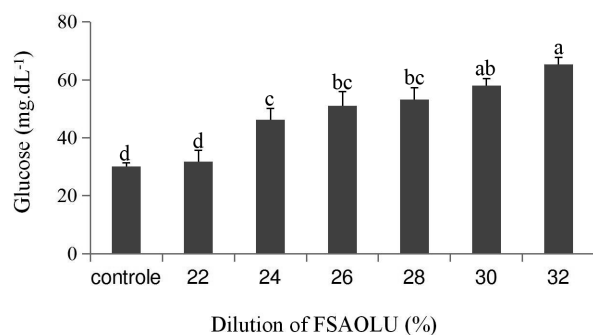


Figure 2. Values (Mean±standard deviation) for glucose values of *H. eques* exposed to different concentrations of FSAOLU

4. Discussion

Fishing ship change the lubricant oil periodically to maintain its properties as viscosity or coloration [5]. Nonetheless, the most of them remain discarding burned oil in aquatic environment due to the missing of information. However, according to legislation, it used oil should be placed in adequate local and collected by specialized companies to refine [8,22].

Nonetheless, incorrect discarding of burned oil in the ground or water body it does exist [22,23]. According to Melo [5], fishing ship discarded approximately 12% lubricant oil in river, lake and ground becoming an environmental problem with regard to the contaminated fish income.

In aquatic environment, this residue can release molecular contaminants, mainly aromatic hydrocarbon, BTEXs (Benzene, Toluene, Ethylbenzene and Xylene), nitrogen

and sulfur-linked heterocyclic compounds which through the water-soluble fraction cause toxicity to aquatic organisms [9,10,12,13,24].

In the literature, toxicity studies about the burned lubricant oil and its effects on aquatic organisms are still few. However, this xenobiotic can change the physic-chemical parameters of water making it unfavorable causing mortality of sensible organisms [10,25,26].

Thus, when fishing ship releasing burned oil contaminating the aquatic environment it becomes hazardous because the toxic potential. According to Otitolaju [25] as well as Ayoola and Akaeze [27] determined the lethal dose of 53.89% for *Poecilia reticulata* and 56.20% for *Clarias gariepinus*, respectively, greater values than those observed for this study 26.38%.

Different organisms can show different sensibility with toxicity tests until in several development stages [15,17,18]. According to Rodrigues [12], they observed severe toxicity of brute oil, diesel and gasoline for larvae *Odontesthes argentinensis* which determined lethal dose 70.68, 13.46 e 5.48%, respectively, showing its toxic effect.

Thus, both marine and freshwater fish are subject to the toxic effects of FSA from oil products. These compounds dissolved in water are responsible for the risks to the aquatic environment and the organisms affecting homeostatic imbalance, which results in changes in hematological and tissue parameters [11,13,15,18,24], may cause stress and later fish mortality.

In the present study, changes in glucose values caused by burned oil would be an indicator of stress in fish [18]. This elevation of glucose was probably stimulated by catecholamines, increasing glycogenolysis in order to prepare the animal for rapid action for escape the stressor [18,28]. According to Simonato [11] monitoring the toxicity of diesel FSA dilution of 50% *Prochilodus lineatus* juveniles for 6, 24 and 96 hours, observed a difference in glucose values with an increase of 172% (24h exposure) and 120% (96 h of exposure) in relation to the control (22 ± 1.00 mg.dL⁻¹). For Simonato [24] also evaluating the physiological effects of *P. lineatus* exposed to 5% dilution of the water-soluble fraction of gasoline for 6, 24 and 96 hours, observed an increase of glucose for 45, 52 and 52% when compared to the control (42 ± 2.00 mg.dL⁻¹). Thus, corroborating the present study, it was observed increases 115.8% of glucose values for fish in the trial causing 100% of mortality in less than 24 hours with dilution (32 %).

The effects of these xenobiotic when discarded in water can cause mortality [18,27,29] as observed in the present study. Furthermore, it should be noted that hydrocarbons and heavy metals released from burned oil derivatives

might be accumulated in fish ^[29]. According to Milinkovitch ^[30], they observed that oil containing $3.3 \pm 0.6 \mu\text{g.L}^{-1}$ polycyclic aromatic hydrocarbons (PAHs) in water, showed a reduction for hydrocarbons to $0.5 \pm 0.1 \mu\text{g.L}^{-1}$ after 48 hours of experimentation. This resulted in the accumulation of PAH-derived metabolites in the gallbladder of fish, showing an increase of pyrene (0.2 IU) compared to the gallbladder of the control fish. These pyrene derivatives are toxic, carcinogenic and mutagenic, causing inflammation in animal tissues ^[31].

Although not being the object of this present study the accumulation of these products in aquatic organisms also becomes a concern mainly regarding public health ^[18,31]. In this sense, the irresponsible disposal of burned oil is a concern for both the aquatic environment and humans, as the spillage or improper disposal of these products can cause aquatic animal mortality and even bio-concentration affecting health.

5. Conclusion

The water-soluble fraction of the used lubricating oil (FSAOLU) shows an environmental risk to the Amazonian rivers because it is potentially toxic, altering glucose values and leading to the mortality of fish. Therefore, flows the need to adopt socio-educational measures for the correct disposal of this compound.

Declaration of Interest

The authors have no conflict of interest to declare.

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