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Tainting and weight changes in *Tilapia guineensis* exposed to sublethal doses of crude oil

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ABSTRACT: The acute toxicity level of the Forcados light crude oil when tested against *T. guineensis* was found to be 0.4ml/l, based on the 96hr LC_{50} value. In addition, the toxicity of the crude oil against the test animals, *T. guineensis* was found to increase with time of exposure. Exposure of *T. guineensis* to sublethal concentrations (ranging from 0.4µl/l, 4µl/l and 40µl/l) of Forcados light crude resulted in the tainting of the flesh of the test animals. With regards to weight changes, the tested sublethal concentrations of crude oil was found to cause a slight increment in the weight of exposed animals contrary to observations for animals in control media, where a loss in weight was observed. The significance of these observations on sublethal effects of crude oil on aquatic animals is the effect they would have on the setting of safe limits of crude oil in our environment. The mechanism(s) responsible for the weight gain in animals exposed to sublethal concentrations of crude oil should also be investigated.

Key Words: Forcados light crude oil; Oil spillage; *Tilapia*; Environmental pollution.

Introduction

Oil spillage can be referred to as the entry of large quantities of oil arising from an accidental disruption of a major activity or facility in the petroleum industry into aquatic and/or terrestrial environment (Shelton, 1983). The different developmental stages in the petroleum industry ranging from exploratory, production and operations, transportation, refining, marketing and storage could and usually result in the discharge of crude oil and its refined products into the environment, where it causes tremendous adverse effects on the receiving environment and its inhabitants (Don-Pedro, 1987).

Impacts of oil spillages have become a major environmental concern all over the world not just because of the negative effects on the receiving habitat but also due to the threat posed to human health and the welfare of the people in the oil producing communities.

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The observed impact of oil spillages have resulted in quite a volume of research efforts (Powell *et al*., 1987, Banigo, 1994). For instance, Powell *et al*., (1987) reported that the effects of oil spill in the Niger Delta persist for several months in the mangrove swamps leading to contamination of spawning grounds of Tilapia species and top minnows over the period of which the hydrocarbon persists. Imevbore *et al*., (1986) and Shelton (1983) have also reported that the major effect of crude oil on aquatic animals especially fisheries resources is due to their impact on physical components of the environment i.e. dissolved oxygen and light penetration. Thus, depending on the amount of oil spilled, organisms that are coated by oil die from asphyxiation and or disruption of physiological functions. Whereas under lower concentrations, sublethal effects leading to the retardation of vital function may result.

One of the most observed and reported sublethal effect of oil spillages in aquatic environment is the tainting of the flesh of fishes and shellfishes collected from oil-impacted ecosystems. The tainting of the flash animals results in very tremendous economic loss to fishermen and other members of the impacted communities due to the fact that the tainted fishes cannot be sold or fed upon by the people dependent on such contaminated aquatic resource (Madu, 1991). Suprisingly, however, is the fact that there is little information or research effort towards establishing the minimum concentration of crude oil in water bodies, which would result in the tainting of fishes and shellfishes flesh. Equally important is the determination of sublethal effects of crude oil, as they affect weight of animals in impacted ecosystems. This type of information would be more useful in determining the safe limits or an appropriate safety margin during attempts to erect an acceptable limit of hydrocarbon contamination in our nation's aquatic resources. The information will also be useful index for the determination of adequate compensation schemes in oil-ompacted areas.

One of the important fishes species in our waterbodies is *Tilapia* sp. These species are known to inhabit freshwater, brackish and marine environment and are one of the major source of protein in the oil producing areas. Furthermore, they are also one of the most sort after fisheries species by fishermen due to their high economic value and widespread acceptance by the general populace. Hence the choice of one of the species, *Tilapia gineensis* for this study on the evaluation of sublethal effects of crude oil in our waterbodies.

In view of the above mentioned, the objectives of this study are as follows:

1. To determine the acute toxicity of crude oil on *Tilapia guineensis* as a reference to establishing sublethal concentrations.
2. To determine the lowest concentration of crude oil, which could result in the tainting of fish flesh.
3. To determine the effect of crude oil on weight changes in the test animal species.

Materials and Method

Test Animals

Tilapia guineensis fingerlings with body weight ranging from 280mg – 320mg, average length of 27.8mm were obtained from Niovas fish farms in "Agric. area" in Ojo Local Government Area of Lagos where they had been in pond cultures for several generation in order to reduce viability in biotype.

The fingerlings were caught with the aid of dragnets in the early hours of the morning (7.30 – 9.00 a.m.) to avoid heat stress and transported to the laboratory in polythene bag containing oxygenated pond water.

Acclimatisation

The fingerlings were kept in a glass holding tank (75cm x 30cm x 32cm) three quarter filled with dechhlorinated tap water. The tap water was dechlorinated by aeration and allowing it to stand for 24 hours in a glass holding tank before use. This was to avoid stress caused by chlorine in the tap water. The fingerlings were left in the holding tank for a minimum of 7 days in order to allow them to acclimatize to laboratory condition (29°C ± 2°C, 77% ± 4% RH), before they were used for the bioassays.

During acclimatisation, fingerlings were fed on prepared fish feed once every 3 days and the medium also changed to avoid accumulation of toxic waste metabolites and decaying food products.

Selection of Test Animals for Experiment

Fingerlings of similar sizes (see section 2.2.1) and about 1 – 2 weeks old were carefully picked up with a handnet from the holding tank into pre-experiment container from where they randomly transferred to bioassay containers.

Test Compound

Forcados light brand of crude oil obtained from Shell Petroleum Development Company, Nigeria was used for the bioassays. Some of the physico-chemical characteristics of forcado light brand of crude oil are given below.

Sulphur content specific C gravity 60/60F.
FORCADOS

FORCADOS	Sulphur content	Specific gravity	Api gravity	Rapid vapour pressure	Pour point
	0.2%	8708	60/60F	2.5psi	25

General Bioassay Procedure

Bioassay Container

Clean plastic bowl (volume – 4.5 litres and bottom diameter – 26.5cm) served as bioassay containers. Preliminary investigation had shown that 10 fingerlings could survive in 1 litre of water without aeration for a period of more than 8 days. Therefore, in the bioassays, the test media were made up of 1 litre to hold 10 fingerlings per bioassay container.

Preparation of Test Medium

Pre-determined amounts of crude oil were measured out into bioassay containers and made up of 1 litre by adding measured amounts of dechlorinated tap water as diluent to achieve a given concentration of test media. The water-oil mixture was stirred vigorously for 2-3 minutes before introducing test animals.

Assessment Quantal Response (Mortality)

Test fishes were taken to be dead if there was no opercula or mouth or body movement even when prodded with a glass rod.

Acute toxicity of Crude Oil Against Tilapia guineensis

Active *Tilapia guineensis* fingerlings of similar sizes (see section 2.1.1) were placed randomly in treated test media prepared as described in section 2.2.2. Each test concentration was replicated (10 per container) twice to give a total of 20 fingerlings each per treatment including untreated control. The fingerlings were exposed to varying concentration of crude oil as follows:

Test Concentrations: 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4ml/l and untreated control. Mortality was assessed as described in section 2.2.3 once every 24 hours for 4 days.

Sublethal effects of Forcado light crude oil against Tilapia guineensis

Effect of sublethal concentration of crude oil on tainting of the flesh of Tilapia guineensis

A similar experiment as described above was carried out, however in this case, active *Tilapia guineensis* were exposed to sublethal concentration of crude oil. Each treatment including control was replicated 2 times (10 fingerlings per replicate) giving a total of 20 fishes per concentration and control.

Test concentrations were extrapolated as fraction of 96 hours LC_{50} value obtained from computed result of the earlier experiment reported in evaluation of acute toxicity level of crude oil against *Tilapia guineensis* as follows:

$1/10^{th}$ of 96hrs LC_{50}

$1/100^{th}$ of 96hrs LC_{50}

$1/1000^{th}$ of 96hrs LC_{50} and control.

Five active animals were harvested per concentration including control and pre-determined time intervals (i.e. day 0, day 2, day 6 and day 10) after their introduction into the test medium. The harvested animals were washed thoroughly in clean tap water, gutted seasoned and fried using vegetable oil. The fried fish were given to a panel of tasters (10 volunteers) to determine if the flesh of the fish is tainted by perceiving its smell and by tasting.

Effect of sublethal contraction of Forcados light crude oil on weight changes in Tilapia guineensis

A similar experiment as described in section 2.3.1 was carried out however, the test animals *T. guineensis* were weighed to obtain weight of the animals before and after exposure to sublethal concentrations of crude oil at pre-determined harvesting period of 2, 6 and 10 days (post exposure), in order to evaluate the impact of the sublethal concentrations on weight changes in exposed animals.

Statistics

Probit Analysis

The dose-response data on acute toxicity of crude oil against *T. guineensis* was analysed by probit analysis after Finney (1971). probit analysis was based on a program by Ge-Le Pattouriel, Imperial College, London as adopted by Don Pedro (1989).

Statistical Analysis of Data

Analysis of variance (ANOVA) was carried out to compare several treatment means at 5% ($P < 0.05$) level of significance for appropriately designed experiments.

Results

Acute toxicity of Forcados light crude oil against Tilapia guineensis

On the basis of dose-response (mortality) data analysis, the toxicity index of forcados light crude oil against *Tilapia guineensis* ranged from 1.40ml/L (24hrs LC_{50} value) to 0.4ml/L (96hrs LC_{50} value) (Table 1). These results indicate that the toxicity of the crude oil against the test animal increased with time of exposure. Furthermore, the 96hr LC_{50} value obtained was used as a reference for establishing sublethal concentrations used in this study.

Table 1: Acute Toxicity of crude oil against *T. guineensis*.

Time	LC_{50} (C.L)	LC_{95} (C.L)	LC_5 (C.L)	Slope ± S.E.	D.F.	Probit Equation	
24hrs	1.404 (3.676 -1.019)	4.193 (6.897 - 1.799)	0.470 (0.764 - 0.00)	3.473 ± 1.70	2	Y=4.5 + 3.473x	3
48hrs	0.45 (1.576 -6.189)	2.400 (0.00 - 1.071)	0.008 (0.022 - 4.3)	0.955 ± 0.610	2	Y=5.3 + 0.955x	1.05
72hrs	0.406 (0.843 -0.042)	6.420 (2.73 - 1.129)	0.025 (0.295 - 0.00)	1.371 ± 0.623	2	Y=5.5 + 1.371x	1
96hrs	0.401(1.343 -0.009)	9.23 (3.55 - 0.078)	0.017 6.894 - 0.00)	1.210 ± 0.711	2	Y=5.5 + 1.210x	1

Effect of Forcados light crude oil on tainting of the flesh of Tilapia guineensis

The results of the flesh tainting experiment are given in Table 2 and Fig. 1. The results showed that all the volunteered members of the taste panel agreed that before the exposure of the test fishes to the sublethal concentrations of crude oil (i.e. on day 0), the flesh of the animals were not tainted by the crude oil. However, two days after the exposure of the test fishes to the sublethal concentrations of crude oil, 70% of respondents agreed that fishes exposed to 0.4µl/l were tainted while 90% and 100% of respondents agreed that fishes exposed to 4µl/l and 40µl/l were tainted after 2 days of exposure (Table 2 and Fig. 1).

By the 6^{th} day and 10^{th} day of exposure of the fishes to the sublethal concentrations of crude oil, 100% of respondents agreed that the fishes were tainted except in day 6, at the test concentration of 0.4µl/l, where only 90% of the respondents agreed that the fishes were tainted (Table 2). It is however important to note that fishes in the control medium were not found to be tainted throughout the experimental period (Table 2).

Table 2: Number of panellist (out of 10 volunteers) responding that fish is tainted (percentage value in parentheses).

Concentrations µl/l	**Day 0**	**Day 2**	**Day 6**	**Day 10**
Control	0(0%)	0(0%)	0(0%)	0(0%)
0.4	0(0%)	7(70%)	0(90%)	10(100%)
4	0(0%)	9(90%)	10(100%)	10(100%)
40	0(0%)	10(100%)	10(100%)	10(100%)

Further comparison of the results obtained for the various days of observation (i.e. day 0 to day 10) by ANOVA (Analysis of Variance) revealed that there was no significant ($P<0.05$) difference in the number of respondents that agreed that the fishes in test media with concentrations ranging from 4µl/l – 40µl/l were tainted with crude oil from the day 2 forward. Although there was a significant ($P<0.05$) difference between the results obtained for day 0 when compared to other results obtained for the other days (days 6 and 10) of observation.

Effect of Forcado light crude oil on weight changes in Tilapia guineensis

The results of the effects of sublethal concentration of forcados light crude oil on weight changes in *T. guineensis* are given in Table 3 and Fig. 2.

The results revealed that there were minimal changes (weight varying between 1.17mg to 1.37mg) in fishes exposed to the various sublethal concentrations of crude oil over the 10-day experimental period.

There was however a noticeable trend of a slight decrease in the weight of fishes in the control medium with mean weight values of fishes measured in the control medium reducing from 1.28mg (day 0) to 1.19mg after 10 days of exposure however, in the treated medium (0.4µl/l, 4µl/l and 40µl/l), the exposed fishes were found to have gained weight (about 0.08mg – 0.09mg) over the 10 day experimental period (Fig. 2).

Comparison of the mean weight changes (by ANOVA) in the exposed animals over the 10-day experimental period however revealed that there was no significant difference ($P>0.05$) in the weight of the exposed animals in all the test medium including control.

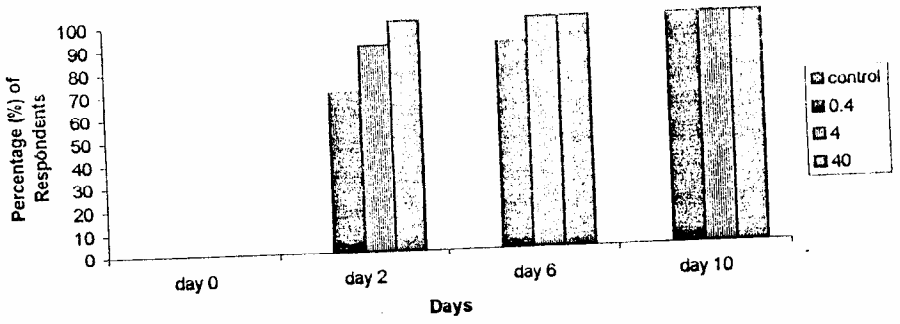

Fig. 1: Percentage of respondents who agreed that the fish tasted as if they were tainted with crude oil.

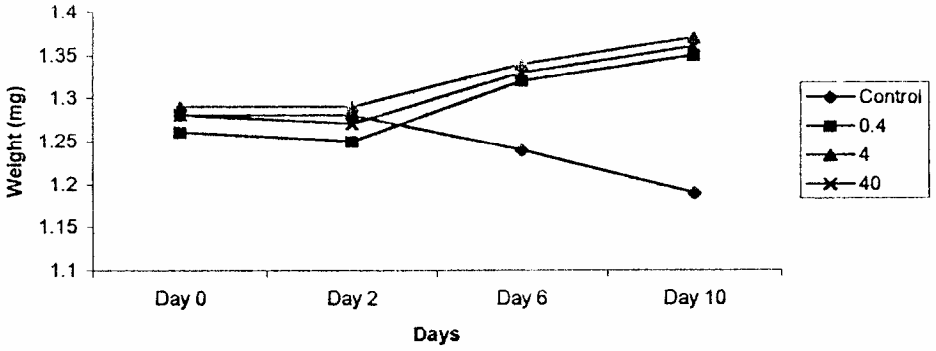

Fig. 2: Profile of Forcados light crude oil on weight changes in *Tilapia guineensis* over a 10-day experimental period.

Table 3: Effect of Forcados light crude oil on weight changes in Tilapia guineensis over a 10-day experimental period.

Treatment (µl/l)	Day 0	Day 2	Day 6	Day 10
Control	1.28 ± 0.3	1.28 ± 0.3	1.24 ± 0.1	1.19 ± 0.2
0.4	1.26 ± 0.1	1.25 ± 0.2	1.32 ± 0.2	1.35 ± 0.3
4	1.29 ± 0.2	1.29 ± 0.2	1.34 ± 0.1	1.37 ± 0.2
40	1.28 ± 0.1	1.27 ± 0.2	1.33 ± 0.2	1.36 ± 0.2

Discussion

In this study, the acute toxicity level of the forcados light crude oil when tested against *T. guineensis* was 0.4ml/l, based on the 96hr LC_{50} value. This result is in agreement with the reports of many other workers such as Tokolo (1988), banigo (1994) and Imevbore *et al*., (1986) who have obtained comparable level of toxicity with other brands of Nigerian crude oil against fishes and shell fishes species.

Additionally, the toxicity of the crude oil against the test animals, *T. guineensis* was found to increase with time of exposure. This is however not surprising because the longer the exposure period, the higher is the possibility of absorption of the crude oil into the tissues of the test organisms and the more pronounced is the change in the physico-chemistry particularly dissolved oxygen in the test medium. The effect of oil spillages on the dissolved oxygen content in water bodies has been well established by many authors who have observed that the floating oil slick forms a barrier on the water surface reducing to a minimum the rate of dissolution of oxygen in the body of water. Under low oxygen tension conditions, the metabolic rates of aerobic organisms such as *T. guineensis* increases resulting in a faster rate of dissolved oxygen depletion than it is being replenished from the atmosphere hence the animals may eventually die of asphyxiation (Enajekpo, 1987) if it is unable to emigrate from the polluted environment. The oil slick may also limit gaseous exchange by entangling the animals, coating the respiratory surfaces thus reducing gaseous exchange transfers.

Exposure of *T. guineensis* to sublethal concentrations (ranging from 0.4µl/l, 4µl/l and 40µl/l) of forcados light crude resulted in the tainting of the flesh of the test animals. The tainting of the flesh of the fishes is as a result of the penetration of the hydrocarbon molecules into the tissues of the animals thus giving it an objectionable crude oil flavour. This tainting of the animal flesh results in its rejection by the people and a tremendous economic loss to the fishermen who depend entirely on the sales of their catch for a livelihood (Madu, 1991).

With regards to weight changes, the tested sublethal concentrations of crude oil was found to cause a slight increment in the weight of exposed animals contrary to observations for animals in control media, where a loss in weight was observed. The mechanism(s) responsible for the weight gain in animals exposed to sublethal concentrations of crude oil should therefore merit future investigations.

The significance of these observations on sublethal effects of crude oil on aquatic animals is the implications they would have on the setting of safe limits of crude oil in our environment. For instance, the results of this study revealed that concentration of crude oil, as low as 0.4µl/l, would cause tainting of the fishes flesh. Hence there is the need to put in place a more stringent safe limits of crude oil in our waterbodies that will ensure that aquatic lives are not only protected but also the socio-economic impacts which could arise as a result of low level crude oil contamination of our aquatic resources are put into consideration.

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