World Applied Sciences Journal 14 (10): 1487-1492, 2011
ISSN 1818-4952

Gill Histopathological Changes in Milkfish (*Chanos chanos*) Exposed to Acute Toxicity of Diesel Oil

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Abstract: In this study the 96-h LC_{50} values of diesel oil and its effects on gill histopathology of milkfish (*Chanos chanos*) were determined. Young juvenile milkfish (W: 64±1.2gr, SL: 16.7±0.4Cm) were exposed to diesel oil at concentrations of 3.4, 3.9, 4.4, 5.5 and 7 ml/l. The experiments were performed as three replicates and all changes in the specimens were determined for each concentration. Water quality parameters of the test seawater were calculated. The 96-h LC50 value was found to be 5.12 ml/l in a static bioassay test system. The gill lamellae became lifted; secondary lamella fused and showed edema. Hyperplasia, hyperemia and hypertrophy of lamellar epithelial cells were distinct with mucus cells increasing. The result showed that acute oil toxicity severely affects on the mortality and gill structure which may be deleterious for milkfish populations.

Key words: Petroleum Products · Pollution · Mortality · Histopathology · LC_{50}, *Chanos Chanos*.

INTRODUCTION

According to transitional and geographical location, Persian Gulf and Oman Sea have a high amount of shipping activity, oil spill and dig in theirs waters. So all organism exposed to oil risk in these ecosystems. Petroleum hydrocarbon products are one of the most important pollutants that effect on aquatic organisms and they are the most opportunity for ecotoxicology studies [1]. Hydrocarbons entry and accumulate in body via the gills, mouth and skin and the dispersed fraction would therefore be the most potentially bioavailable [2]. Soluble petroleum hydrocarbons can absorb by organisms and concentrate in tissues 10 to 100 times higher than in water [3]. These compounds can produce various effects in fish can exert various types of effects. They can change gonad tissue and reproduction processes [4], brain neurotransmission [5], hepatic tumor [6], biochemical and physiological changes [2] and gill alterations [7, 8] in fish.

Gills are very important in respiration, acid–base balance, osmoregulation and excretion of nitrogenous wastes in fish [9] and they include the greatest surface area of the aquatic organisms in contact with external environment. They are also an important way of uptake of pollutant into the organism, thus the gills are the first site where petroleum hydrocarbon- induced lesions may occur. Therefore, their morphology can be very useful as a bioindicator in environmental evaluation [10].

Assessment of the biological impacts of oil dispersants on marine ecosystems is usually based on acute toxicity tests [11, 12]. Petroleum hydrocarbons induced alterations in the gill morphology and histology are being used as biomarkers and diagnostic tools in evaluation of potential impacts in fish. However, there are few studies of the impacts of oil and oil dispersants on gill histopathology of fish. In addition to, the milkfish, *Chanos chanos,* is considered to be a suitable organism for monitoring petroleum hydrocarbons contamination. It is well suited for this purpose because of its feeding behavior from bottom habits and also it is a benthopelagic fish (effects of oil sands and float oil). This study is the first investigation of the effect of petroleum hydrocarbon products on milkfish (*Chanos chanos*). This report presents the results of the first determination of the 96-h LC50 value of diesel oil and gill histopathology in *Chanos chanos*.

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MATERIALS AND METHODS

Fish and Acclimatization Conditions: Young juvenile milkfish (*Chanos chanos*, family Chanidae, n=400) with 64±1.2gr weight and 16.7±0.4Cm standard length were collected during 2009 from the Shour-Shirin (Tiyab) estuary, located in the Kolahi region of Hormozgan province, Iran. The fish were captured by using cast nets and were transferred to the research laboratory of the Persian Gulf and Oman Sea Ecology Research Center. All specimens were washed with 0.1% $KMnO_4$ solution to remove dermal infection and pollutants, if any. The fish were allowed to acclimate to laboratory conditions for 45 days before the start of the first experiment.

LC_{50}assessment: The diesel oil served as the test compound for determination of the median lethal concentration (LC_{50}) of the milkfish. After acclimation, fish were randomly transferred from stock tank to polyethylene experimental tanks (260 l) which had static system and continuous aeration and allowed to acclimate to these conditions for 48h. Each tank was containing 12 active individuals. These groups were then exposed to different diesel oil concentrations. The control group was not exposed to pollutant. The concentrations used for acute toxicity estimation were 3.4, 3.9, 4.4, 5.5 and 7 ml/l diesel oil. Three replicates were performed for all treatments and control group. Each day, dead fish were counted and removed from the tanks. The fish were not fed on the day before the beginning of the experiment and during the experiment. The data from the experiment were used to estimate the 96 h LC_{50} of oil diesel. To calculate these values, the mortality observed in each treatment was determined and analyzed using a basic EPA computer program that implemented the Finney probit analysis method [13].

Experimental Design for Acute Exposure: Fish were exposed to 0.256, 0.512 and 1.024 ml/l diesel oil. The concentration selected was 1/5, 1/10 and 1/20 of the 96 h LC_{50}. Only active specimens (72from holding 400 fish), with no sign of distress and injury, were used for this study. Milkfish were randomly transferred from stock tank to the polyethylene experimental tanks (260 l) which had static system and continuous aeration and allowed to acclimate to these conditions for 48h. In each treatment was 18 fish and the experiment did not distinguish between the sexes of the fish. The experimental design included three replicates. Eight fish from each treatment were sampled at 24hr and 96 hr after exposure for histopathology tests.

Histological Preparations: Fish were anaesthetized in cold water with dried powder (0.2 g/l) of pink or *Dianthus* sp (Kingdom: Plant, Family: Caryophyllaceae) after collection, then they were dissected and the first and second gill arches (from the eyed side) were excised and immediately fixed in Bouin's fluid for 48 hr, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin wax. Sagittal sections (5 µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with haematoxylin-eosin (H&E stain) for structural analysis of tissue. Changes induced by treatment in the gill tissues were photographed by Digital Dinolite Camera (Dino Capture 2.0) and examined by light microscopy.

RESULTS

Mortality and LC_{50} Values: No mortality was observed over 96 h at control and 3.4 ml/l. The lowest concentration at which mortality was observed was 3.9 ml/l. The first deaths of experimental fish were recorded in the 7.0 ml/l treatment after 36 h and also the highest mortality, 11 fish, was observed in this concentration. Table 1 presents and the numbers of fish that died and the concentrations of diesel oil to which these fish were exposed. The data from these toxicity tests were evaluated using probit analysis. The 96-h LC_{50} value was determined and 95% confidence limits calculated. The 96-h LC_{50} for the young milkfish was 5.12 ml/l.

Gill Histopathology Observation: The gills of the control fish did not appear any histopathological changes at all times (Fig. 1 a, b). At the first 24 h at 0.256degeneration of mucous (goblet) cells, bloody cephalic cells, lifting and fusion observed (Fig. 2a &b). In 0.256 at 96hr, the tissues showed desquamation, break of secondary lamella; lifting, fusion and proliferation of mucous (goblet) cells (Fig. 2c & d). In 0.512ml/l treatment, aneurism, lifting and fusion increasing, edema and degeneration of mucous cells observed at 24hr (Fig. 3a &b) and also at 96hr, desquamation, break and abnormal of secondary lamella, proliferation and hypertrophy of mucous cells and chloride cell degeneration observed (Fig. 3c & d).

Table 1: Numbers of dead fish in different concentrations of Diesel Oil in LC_{50} experiment during 96h

	Diesel Oil concentrations (ml/l)					
Situation	Control	3.4	3.9	4.4	5.5	7.5
Number of fish	12	12	12	12	12	12
Number of dead fish	0	0	1	4	7	11
Mortality percentage	0	0	8.3	33.3	58.3	91.7

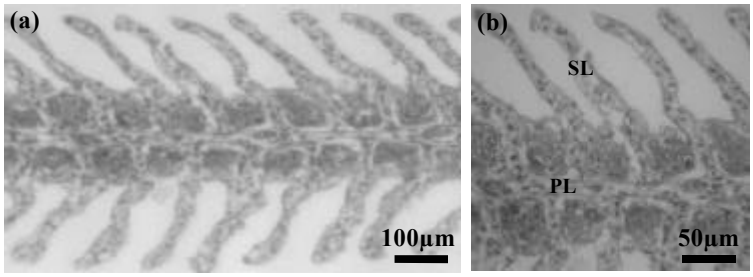

Fig. 1: Sagittal section showing gill histology in control (a). Note normal appearance of primary (PL) and secondary lamellae (SL), (b), H&E.

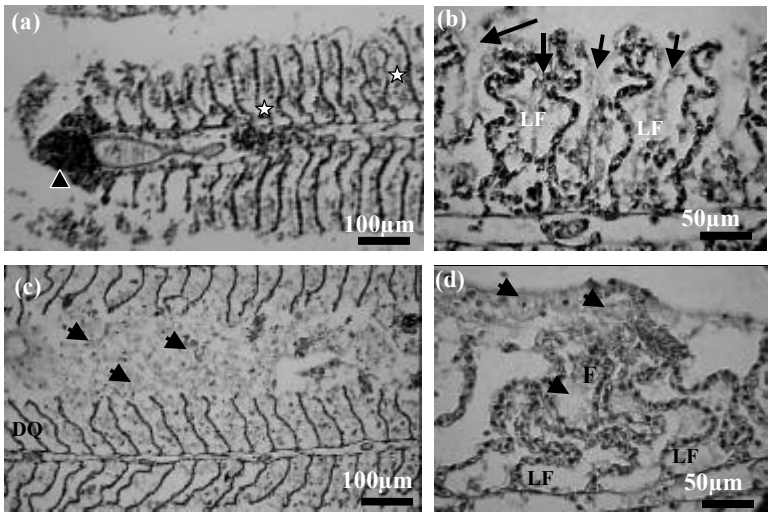

Fig. 2: Gill sections of tested fish exposure to 0.256 ml/l diesel oil at 24hr; (a) Degeneration of mucous (goblet) cells (stars), bloody cephalic cells (arrowhead); (b): Fusion (arrows) Lifting (LF). At 96 hr of exposure to 0.256 ml/l diesel oil (c) Desquamation (DQ), break of secondary lamella; (d): lifting (LF), fusion (F) and proliferation of mucous (goblet) cells (arrows), (H&E).

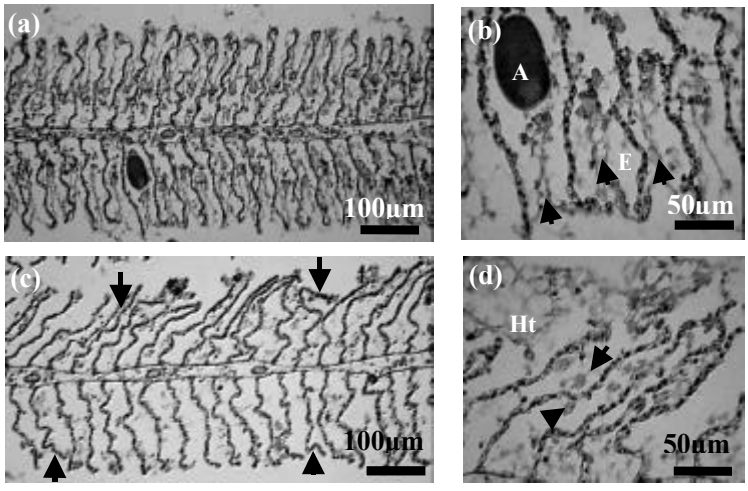

Fig. 3: Gill sections of tested fish exposure to 0.512ml/l diesel oil at 24hr, (a) Aneurism and lifting and fusion increasing in all secondary lamella; (b): Aneurism (A), lifting, fusion (arrow), edema (E) and degeneration of mucous cells observed. At 96hr of exposure to 0.512ml/l diesel oil (c) Desquamation, break and abnormal of secondary lamella (arrow), (d): proliferation and hypertrophy (Ht) of mucous (goblet) cells, chloride cell degeneration (arrows), (H&E).

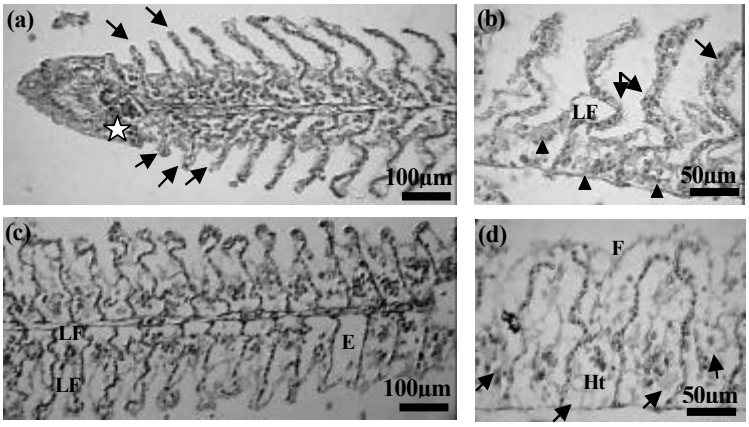

Fig. 4: Gill sections of tested fish exposure to 1.024 ml/l diesel oil at 24hr (a) and (b): Aneurism (star), decreasing of secondary lamella length (arrows), lifting (LF) and proliferation of mucous and chloride cells in base of secondary lamella (arrowhead) observed. At 96hr of exposure to 1.024 ml/l, (c) and (d): Increasing in lifting (LF), fusion (F), edema (E) and decreasing of length, break and abnormal of secondary lamella, proliferation and hypertrophy (Ht) of mucous (goblet) cells and chloride cell (arrows) observed, (H&E).

At 24 h in 1.024 ml/l treatment the gills showed aneurism, decreasing of secondary lamella length, lifting, proliferation of mucous and chloride cells in base of secondary lamella (Fig. 4a &b). At 96 h, the gills of many fish showed increasing in lifting, fusion, edema and decreasing of length, break and abnormal of secondary lamella, proliferation and hypertrophy of mucous cells and chloride cell observed in 1.024 ml/l and also telangiestasis, epithelial necrosis and desquamation, blood congestion with the breakdown of the pillar cell system in multiple secondary lamellae still remained. The variation and lesions in 1.024 ml/l treatment were higher than other treatments.

DISCUSSION

In the present study we investigated acute toxicity effects of diesel oil on mortality rate and histopathology of gill in milkfish *Chanos chanos*. The 96 h LC_{50} value of diesel oil determined to be 5.12 ml/L for *C. chanos*. This is as per the data published earlier for other aquatic animals. [14] reported to be that the fuel oil had an LC_{50} of 140.0 mg/L and 94.0 mg/L in the 7day ELS bioassay and 96-hour acute bioassay, respectively for fish *Cyprinodon variegates*. [15] was found 7.46 mg/L 96-h LC50 value of the crude oil for *Onchorhyncus tshawytscha*. [16] showed that the 96-h LC_{50} for crude oil to the juvenile stage of prawn, *Macrobrachium vollenhovenii* was to be 0.28 mL/L. The mean 96-h LC_{50} value for crude oil reported to be 0.7ppm for *Octopus pallidus* [17] and 311000 ppm for the marine amphipod *Allorchestes compressa* [18]. Seven-day LC_{50} (0.92–0.42 mg TPH:l) and LC_{20} (0.58–0.15 mg TPH:l) values in shrimp, *Mysidopsis bahia reported* [19]. [20] reported that the 96hr LC_{50} value was found to be 62.8mg/l cadmium chloride for *Chanos chanos*.

Petroleum hydrocarbons can absorb via skin, mouth and gills. Gills are the most important tissue for gas and ion exchange, acid-base regulation and nitrogenous excretion. They have direct contact with water current, therefore the present of any pollutant in external environment effect on it. The gill tissues are disturbed following treatment of the fish with various stress conditions, including exposure to petroleum hydrocarbons. In this study Histological study of the gills shows normal gill morphology in specimens from the control group (Fig. 1a) relative to gill alterations of fish exposed to diesel oil (Figs. 2, 3 and 4). Some alterations as proliferation, fusion, lifting, edema, aneurism, predominated in the gills of milkfish from treatment groups at varied period of exposures. The first gill change is edema of epithelial cells. This change happen when the epithelium cells around the secondary lamellae lifting away in a continuous sheet from the pillar cell system, thus the distance from water to blood will be increasing [21]. The secondary lamellae in 0.512ml/l showed aneurism that this alternation resulted from the collapse of the pillar cell system and the breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outward [22]. Also in the present study hypertrophy and hyperplasia of epithelial and chloride cells observed. There are some reports about effects of

contaminants on gill alternations as [23] reported that gill tissue of yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) showed epithelial cell necrosis, mucous cell proliferation and degeneration exposed to oil sands. Gill damage at 10 and 0.8 mg/l of Cadmium chloride for 96 h and 90 days, varied depending on individual stress, where gills of stressed specimens were very swollen and showed degeneration, proliferation, hyperplasia and hypertrophy of the gill epithelium and chloride cells [21]. A morphometric study of the *Channa punctatus* [13], *Tilapia zilii* and *Solea vulgaris* [24] and *ophicoephalus punctatus* [25] revealed that the gill lamellae became lifting, fusion, edema, hyperplasia and hypertrophy of lamellar epithelial cells and desquamation after exposed to different contaminants.

In the conclusion the gill lesions and alternations in milkfish may indicate that these changes are produced as a direct or indirect response to the influx of diesel oil. The significance of observed gill changes relates to the type of damage and the amount of gill surface affected. As the gills are the respiratory apparatus for fish, diffuse damage can cause extreme respiratory distress by inhibiting oxygen transfer from the water into the blood stream. Multifocal, but severe, lesions can also reduce respiration and osmoregulation through obstruction of the secondary lamellae by large amounts of exudates. In the long-run, therefore, contaminants as diesel oil may pose serious threat to health, survival and affect milkfish population.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge to the director and staff of Persian Gulf and Oman Sea Marine Biology and Ecology Research Center, Bandarabbas, Iran, especially Mr. Hojatolah Foroughi, Mr. Masoud Gharibnia and Mr. Eisa Abdolalian, for providing necessary laboratory facilities and also the University of Marine Science and Technology, Khoramshahr, Iran, for project support.

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