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The effect of the water soluble fraction of crude oil on survival, physiology and behaviour of Caspian roach, *Rutilus caspicus* (Yakovlev, 1870)

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ABSTRACT

The water soluble fraction (WSF) of crude oil is a complex and toxic mixture of hydrocarbons that aquatic organisms directly encounter in oil spills. WSF plays an important role in the toxicity of crude oil to aquatic organisms. In the present study, the effects of WSF on juvenile Caspian roach, *Rutilus caspicus*, at lethal and sub-lethal level was investigated. The lethality of WSF on *R. caspicus* was studied by conducting 96 h LC50 tests with semi-static exposure methods with 6 and 24 h solution renewals. The 96 h LC50 of WSF was estimated at 62.5% and 35.9% WSF concentrations for 24 h and 6 h renewal methods, respectively. To investigate the sub-lethal effect of WSF on *R. caspicus*, fish were exposed to 62.5, 31.3, and 6.3% concentrations of WSF for 24 h and changes in their respiration rate and swimming activity was monitored during the exposure. At the end of the exposure period, four hematologic parameters [O_2 and CO_2 pressures (pO_2 and pCO_2), hematocrit, and hemoglobin content] of the fish were measured. The result of the behavioural experiment revealed that all three studied concentrations of WSF elevated the respiration rate and reduced the swimming activity of *R. caspicus*. No significant changes were detected in the hematocrit and hemoglobin content of the fish blood, but the blood pO_2 of the fish exposed to 62.5% WSF decreased while the blood pCO_2 increased. The results of this study suggest that the egression of the volatile components in hydrocarbon mixtures during conventional semi-static toxicity tests may lead to underestimating the toxicity of the hydrocarbons. The results of the sub-lethal experiments propose that failure of the respiratory system that leads to asphyxia may be a major mechanism that results in lethal effect of WSF in high concentrations.

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1. Introduction

Petroleum hydrocarbons are among the most significant contaminants of offshore and estuarine zones (Connell et al., 1980). It is estimated that leakage of more than 1,300,000 tons of petroleum derivatives pollute seas annually (NRC, 2003). Aquatic organisms are more likely to be exposed to water soluble fractions (WSFs) than other components of petroleum (Rodrigues et al., 2010). Thus, numerous studies have been conducted on the effects of the WSF on marine organisms, including fish (e.g. Jiang et al., 2012; Rodrigues et al., 2010; Fuller et al., 2004). As the components of oil vary among different oil fields, oil from different regions have different toxicological effects, and different aquatic species have different sensitivities to a particular oil (Tsvetnenko and Evans 2002; Tsvetnenko 1998). Therefore, extrapolating toxicology data from one oil field to another may not be reliable and a set of toxicology studies should be performed on the local species.

Fish are sensitive to the changes in their environment. Slight changes in the surrounding environment may induce a series of interactions which elicit changes in their behaviour. Fish behaviour is considered a highly sensitive indicator of environmental changes (Kasumyan 2001; Døving 1991). In addition, since each behaviour in is a result of a complex of several physiological reactions in response to internal and external inputs, studying the related physiological changes would illustrate the mechanism of interactions that manifested as a behavioural change.

Considering the distribution, biomass, and commercial value of *Rutilus caspicus*, it is one of the most important bony fish of the Caspian Sea (Naddafi et al., 2005). Overfishing, pollution, and deterioration of the rivers it uses as its spawning grounds has led to a

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decline in the population of this species (Kiabi et al., 1999). Hydrocarbon contaminants in the Caspian Sea and the rivers flowing into it are thought to have a considerable role in the decline of river spawning fish, including sturgeons and roaches (Jafari 2010). Therefore, it is important to understand the effects of hydrocarbon contaminants on the Caspian roach.

In the present study, the toxic effect of WSFs of crude oil on juvenile *R. caspicus* was studied at both lethal and sub-lethal concentrations. At the lethal level, a 96 h LC50 was calculated from a conventional semi-static exposure (24 h renewal intervals). Dramatic changes in the behavioural response of the exposed fish during each renewal period, from rapid movement and jumping at the beginning to calm swimming in the last hours, suggesting that a significant amount of the toxicant may have egressed the solution. In order to determine the effect of the exited part on toxicity of WSF a semi-static 96 h LC50 with 6 h solution renewal was conducted. Additionally, fish in LC50 tests showed behaviours (increase in the respiration rate, losing horizontal posture, breathing at the solution surface, etc.) that could be indicators of asphyxia. Therefore, changes in the respiration rate and total swimming activity as behavioural manifestations, and hematocrit, hemoglobin concentration, O_2 and CO_2 pressure in the blood as physiological manifestations of the toxic effect of WSF on the respiratory ability of fish were also studied.

2. Materials and methods

2.1. Animals

Juvenile *R. caspicus*, with a body length of 10 ± 1 cm, were obtained from Kolmeh Hatchery Center, in Gorgan, Iran. From 15 days prior to the experiments, the fish were kept in an aerated aquarium (3 × 1.2 × 0.6 m), with the temperature held at 16 ± 1 °C, a pH of 7.5–8.0, 170 mg $CaCO_3$/L, a 16:8 h light:dark cycle and were fed carp food pellets (Chineh Co., Tehran, Iran) twice a day, at 2% of their body weight. Physical and chemical characteristics of water were kept constant during the acclimation and in the experiments. Fish were starved 24 h before the experiment. No mortality or pathology was observed during the acclimation period. Permission to perform the experiments was obtained from the Iranian National Institute for Oceanography (INIO).

2.2. Petroleum water soluble fraction

Crude oil was provided from the Tehran Oil Refining Company. The industrial information of the crude oil parameters were as follows: Specific Gravity @15.6 °C = 0.8605; API Gravity = 32.94; Reid vapor pressure = 49 kPa; salt content = 7.0 Lb/1000bbl; W. & S. = 0.1 Vol%; pour point = −24 °C; total sulphur = 1.480 Wt%. The WSF solution was extracted from the crude oil using the method described in Lari et al., (2015). No mortality or pathology was observed during the acclimation period. Permission to perform the experiments was obtained from the Iranian National Institute for Oceanography and Atmospheric Science (INIOAS).

2.3. Hydrocarbon analyses

The amount of total petroleum hydrocarbons (TPH) of the solution was determined using the method described in Lari et al. (2015). In short, using 0.1 N perchloric acid (Merck, Germany), the pH of the water-soluble fraction solution was decreased to 3 and stirred for 5 min to let the inorganic carbons escape the solution as gas. The TPH was measured by injecting 100 mL of the solution into the total organic carbon analyzer (model ANA TOC II; SGE). To track the evaporation rate of the components of the WSF stock solution during a semi-static test with 24 h renewal period, 20 L of the stock WSF was kept in the LC50 test situation for 24 h. Samples were taken from the solution at the start and after 6, 12, 18, and 24 h (N = 3); and the TPH content of all samples were measured.

2.4. LC50 tests

Prior to LC50 experiments, preliminary experiments were carried out and the lethal range of the WSF was calculated as 20–100% and 10–60% for the 24 h and the 6 h renewal tests, respectively. In the first series of experiments for assessing the LC50, the semi-static test with a 24 h renewal period was conducted for 96 h. Five glass aquaria (30 × 40 × 20 cm) were filled with 20 L of WSF solutions at the following concentrations: 20, 40, 60, 80 and 100%. A sixth tank of similar dimensions was filled with fresh water (WSF concentration of zero) as the control set-up. During the experiment all aquaria were aerated and in order to reduce the scape of hydrocarbons, aquaria were loosely covered with plastic sheets. Ten fish were placed in each tank and the test solution was renewed with a fresh solution of the same concentration once every 24 h. At any renewal session, the dead fish were removed and counted. Fish were considered dead if there was no visible movement (e.g. gill movements) and no reaction to touching of the caudal peduncle.

The second series of experiments was conducted in similar conditions to the first one; but the solutions were renewed every 6 h, and the concentrations of WSF were 10, 20, 30, 40, 50 and 60%, along with one tank of fresh water as the control set-up. For both series of experiments each "concentration and renewal time" was replicated three times.

2.5. Respiration rate

In order to study the effect of WSF on respiratory system, four groups of five fish were exposed to three concentrations (LC50, 50% LC50, and 10% LC50) and clean water for 24 h. Each fish was kept in a 1 L chamber with an aerator. Two sides of the chambers were mirrored. Prior to the exposure, and 6, 12, 18, and 24 h post exposure, the respiration rate of the fish was tested as follows. The aerator was shut down and the fish were given 15 min to acclimate from the shutting down of the aerator. Fish were filmed for 1 min using two cameras (G12, Canon, Japan) placed at the non-mirrored sides of the chamber. Footage was reviewed double-blindly (the reviewer was not aware of the treatment that was being reviewed) to determine the respiration rate by counting the operculum movement per minute.

2.6. Blood parameters

At the end of the respiration rate test (24 h) blood samples were taken from the fish using the following procedure. Fish were gently restrained and immediately sampled within 30 s of handling. Blood was drawn anaerobically from the arterial catheter into an ice-cold, heparinized syringe. Blood samples were tested for hematocrit (Ht) using micro-hematocrit centrifuge (C-MH30, UNICO, USA). The partial pressures of particularly oxygen (pO_2) and carbon dioxide (pCO_2) and the total hemoglobin (Hb) were measured by blood gas analyser (RAPID Point 500, Siemens).

2.7. Total swimming activity

In order to study the effect of WSF on total activity, the total activity of four groups of 10 fish were tested before exposure and 6, 12, 18, and 24 h after being exposed to 6.3, 31.3, 62.5% WSF (10% LC50, 50% LC50, and LC50 concentrations) and clean water as control. In this experiment an arena with the following setup was used. An acrylic glass aquarium with the bottom size of 50 × 50 cm (transparent) and wall height of 20 cm (white) was placed on an

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