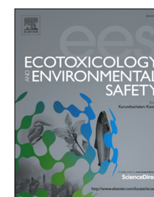
Ecotoxicology and Environmental Safety 158 (2018) 69–77

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Biochemical and molecular alterations in freshwater mollusks as biomarkers for petroleum product, domestic heating oil

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ARTICLE INFO

Keywords:
Domestic heating oil
Comet assay
AChE
MDA
SOD
CAT. Mollusks

ABSTRACT

To investigate the effect one of the oil products, domestic heating oil (DHO), on freshwater mollusks, *Unio tigridis* and *Viviparous bengalensis* were exposed to three DHO concentrations for each species (5.8, 8.7, and 17.4 ml L^{-1} for mussels; 6.5, 9.7, and 19.5 mlL^{-1} for snails, respectively). Antioxidant enzymes (superoxide dismutase, catalase), malondialdehyde, acetylcholinesterase and DNA damage in both species tissues were monitored over 21 days. The results showed that both antioxidant enzymes concentration (SOD and CAT) increased in the lowest DHO concentrations (5.8, and 8.7 ml L^{-1}), and then decreased in the highest concentration (17.4 ml L^{-1}) as the same pattern for *Unio tigridis*, but this not occurred for *Viviparous bengalensis*. MDA values recorded significantly increased compared to control. No reduction was observed in AChE concentrations in soft tissues of both mollusks may due to that DHO was a non-neurotoxicant to *Unio tigridis* and *Viviparous bengalensis*. The results of DNA damage parameters were showed significant differences ($p \leq 0.05$) between control and DHO concentrations except lowest concentration for each parameter measured in digestive gland of *Unio tigridis*. As well as, these significant differences were recorded between control and three concentrations of DHO exposure for comet length, and tail length parameters, and between control and highest oil concentration for tail moment in *Viviparous bengalensis*. DHO has the ability to prevent the reproduction of *Viviparous bengalensis* snail relation to control, that is what we considered strong evidence of the toxicity properties of DHO on the reproductive status of this species of snails. SOD, CAT, and MDA were useful biomarkers for evaluating the toxicity of DHO in mussel and snails, and comet assay was a good tool to assess the potential genotoxicity of DHO.

1. Introduction and literatures review

All over the populated parts of the earth, the quality of natural freshwater is being disturbed by human activities (Falkenmark and Allard, 1999). Oil spills do occur in freshwaters as a consequence of the many oil-related activities in this environment. Hence, it is important to study impacts of petroleum spills in freshwater habitats. Oils have been found in sediments at low energy sites as much as 5 years after the occurrence of spills, and they may be released into the water column long after the initial spill. Thus, water-column organisms as well as species inhabiting the sediment may be affected by oil spilled in the environment (Green and Trett, 1989). As well as, if oil is dispersed into small droplets, filter-feeding organisms can be exposed to whole oil through ingestion of droplets (Laetz et al., 2015). Therefore, the measurement of the biological effects of these pollutants have become of major importance for the assessment of the environment quality (Gray, 1992). In water, oil film floating on the water surface may be prevents natural aeration and leads to death of fresh water life (Ukoli, 2005).

There are many forms of liquid petroleum products which used as a fuel oil for furnaces or boilers in buildings. Domestic heating oil (DHO) is one of these products descripted by low viscosity, and consists of a mixture of petroleum-derived hydrocarbons, and was used mainly for home heating. Many researchers were determined petroleum hydrocarbons in water bodies (Olufemi et al., 2011; Obiakor et al., 2014; Freije, 2015), and others were studied the effects of oil spills and other petroleum hydrocarbon products on a different types of aquatic fauna included phytoplankton (Adekunle et al., 2010), bacteria (Jurelevicius et al., 2013), crustacean and midge (Bhattacharyya et al., 2003; Klerks et al., 2004), in addition to the various mollusks species (da Silva et al., 2005; Halldórsson et al., 2008; Fenghua et al., 2015; and Bhagat et al., 2017).

According to McCarthy and Shugart (1990), biomarkers are measurements at the molecular, biochemical, or cellular level in either wild populations from contaminated habitats or in organisms experimentally

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https://doi.org/10.1016/j.ecoenv.2018.04.006
Received 10 March 2018; Received in revised form 2 April 2018; Accepted 4 April 2018
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exposed to pollutants that indicate that the organism has been exposed to toxic chemicals, and the magnitude of the organism's response to the contaminant. Biomarker studies have been applied in different animal species, but mollusks in the aquatic environment and among aquatic organisms more suitable for biological monitoring (Zhoua et al., 2008).

Antioxidant enzymes such as SOD and CAT are considered the most common biomarkers used in environmental quality monitoring (Regoli et al., 1998). MDA has been one of the first compounds to be used as an index of lipid peroxidation, and it consider one of the better known used as a biomarker of oxidative damage (Gagné, 2014a). As well as, AChE is a commonly used biomarker not only in aquatic ecotoxicology studies, but also ecotoxicology (Alloh et al., 2018; El-Nahhal and Lubbad, 2018) while DNA damage (measured by comet test) is used as a biomarker of genotoxicity under acute or chronic conditions (Gagné et al., 2014b).

Many studies used antioxidant enzymes, lipid peroxidation, AChE, DNA damage in mollusks tissues as biomarkers for oil spill (Bocquene´et al., 2004; Moreira et al., 2004), and many liquid petroleum products pollutants (Siu et al., 2004; Zheng et al., 2013; Bhagat et al., 2015), but no occurred any study used *Unio tigridis* and *Viviparous bengalensis* as biomarkers for domestic heating oil at world level. This reason, in addition to, the biomarker importance in aquatic environments as tool or early warning for disturbance diagnosis, we chose our study idea.

2. Materials and methods

2.1. Animals

Freshwater mollusks, *Unio tigridis* (7 ± 0.5 cm length, 3 ± 0.5 width, and 25 ± 2 g weight), and *Viviparous bengalensis* (2.8 ± 0.1 cm length, 2 ± 0.1 width, and 4 ± 0.5 g weight) were sampled from Hilla River (A branch of the Euphrates River in central Iraq). Only mollusks of the similar size were collected to be used in exposure experiments, then cleaned, transported to the laboratory where the experiments were carried out, and identified according to the standard keys (Ahmed, 1975; Plaziat and Younis, 2005).

2.2. Acute toxicity assay of DHO and calculate mortality percent

Experiments were conducted in 20 L plastic containers, each one containing 18 L of pre-aerated dechlorinated tap water, benthic sediments, and 6 adult mollusks. The specimens were not fed a day prior to and during toxicity tests to reduce fecal and excess food contaminating the test solution (Khalil, 2015). A geometric dilution series of eight concentrations was used in each toxicity test, with two replicate containers per concentration (i.e. 12 mollusks per concentration) and one blank control container. The oil was bought at a commercial gas station in the center of Hilla city, and was directly added to 10 L of water and mixed throughout for 5 min, than the volume was completed to 18 l with chlorine-free tap water.

DHO was tested at 50, 60, 70, 80, 90, 100, 110, and 120 ml L^{-1} according to da Silva et al. (2005). The six adult mollusk for each species were placed in the containers under 25 ± 0.75 °C, and 12 h:12 h dark/light regime, and the live/dead mollusks were counted after 24, 48, 72, and 96 h by the individual movement monitoring. Mussels were counted as dead when become unable to enter its soft tissues and closing their shells, while floating on the water surface of the aquarium was mark death in the snails. The probit analysis was carried out using by Finney's (1971) method, and then the LC_{50} was derived from the best-fit line obtained. Mortality rate of *Unio tigridis* and *Viviparous bengalensis* were calculated by the ratio between the total numbers of mollusks at the start to dead number of mollusks at the end of experiment.

2.3. Subchronic toxicity test for DHO

One control and three treated groups of mollusks (mussels and snails) were used in experiments. Treated groups were exposed to different concentrations of DHO (5.8 ml L^{-1}, 8.7 ml L^{-1} and 17.4 ml L^{-1} for mussels; 6.5 ml L^{-1}, 9.7 ml L^{-1}, and 19.5 ml L^{-1} for snails respectively), and during 21 days, these three concentrations for each species of mollusks were calculated by 1/15th, 1/10th and 1/5th of the 96 LC_{50} value (Al-Sawafi and Yan, 2013). The same experimental conditions of acute toxicity assay were considered to assess the effective concentration of DHO. Sufficient number of tested and control animals were removed at 21 days. For the biochemical analysis, mollusks were dissected and the soft tissues of specimens were weighted and used as source of measurements, while the digestive glands of two mollusks species were used for molecular analysis. All steps of the living tissue separation were carried out in the ice box to maintain the measured qualities from change and damage.

2.4. Biomarker measurements

The soft tissues of mollusks were homogenized by Pestle Motor Mixer provided by Argos Technologies (U.S) Cat. No.A0001 in phosphate buffer solution (PBS; pH 7.4) as a ratio 1:3, centrifuged at 15,000 × *g* for 15 min at 4 °C for biochemical assays. Superoxide dismutase (SOD: E-EL-H1113), and Acetylcholinesterase (AChE: E-EL-R0355) concentrations were measured by ELISA method using Elisa Kits (Elabscience Biotechnology Co., Ltd., China). Catalase (CAT: SEC. 418Hu), and Malondialdehyde (MDA: CEA597Ge) concentrations were measured by ELISA method using Elisa Kits (Cloud Clone Crop, USA). DNA damage was measured by take 100 mg of digestive gland of mollusks, then added to Ca^{2+}/Mg^{2+}-free PBS as ratio 1:5 (w: v), and used methods were described by (Conners, 2004;Gagne *et al.,* 2014) for DNA single-strand breaks measuring with the Comet assay. DNA damage was quantified by measuring comet length, tail length, and tail moment using computerized image analysis system (TriTek Comet-Score™ Freeware v1.5). Fifty cells were scored per slide, and geometric means were used to describe the damage.

2.5. Statistical analysis

Analysis of variance (ANOVA), F-test, t-test and least significant differences (LSD) were used to explain the differences between means at ($p \le 0.05$), express that as mean ± SE. Principle Component Analysis was used for correlation between biochemical and molecular biomarkers (Gerry and Michael, 2002).

3. Results and discussion

3.1. Acute toxicity assay

The most common toxicity test is the 96 LC_{50}, which determines the concentration of toxicant that results in a 50% lethal response over a period of 96 h exposure (Gosling, 2003). This test was used in our study, and the results showed that 96 LC_{50} for *Unio tigridis* and *Viviparous bengalensis* were exposed to eight concentration of DHO were 87.17 ml L^{-1}, and 97.5 ml L^{-1}, respectively (Table 1; Fig. 1).

In a study to investigate the acute toxicological effects of the water-soluble fractions (WSF) of oils in the bivalve *Donax faba*, the results showed that 96 LC_{50} of diesel oil, kerosene, and the mix of diesel oil and kerosene were 3.916%, 13.461%, and 4.631% respectively (Devi and Ravichandran, 2016). Not occurred any previous study to known the effects of DHO of our selected mollusk species in all previous studies.

3.2. Sub lethal toxicity of domestic heating oil

Most research on the fate and effects of oil entering the aquatic

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