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ORIGINAL ARTICLE

Alteration in antioxidant genes expression in some fish caught from Jeddah and Yanbu coast as a bio-indicator of oil hydrocarbons pollution

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Abstract The mRNA expression profile of some antioxidant genes in skin, gills, livers, and muscles of *Siganus canaliculatus* and *Epinephelus morio* was used as an indicator of petroleum hydrocarbons pollution in six areas at Jeddah and Yanbu coasts in KSA. Total petroleum hydrocarbons (TPHs) were determined in both sea water and sediments collected from the studied areas. The mRNA expression levels of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were determined. The highest level of total petroleum hydrocarbons was observed in front of the petromine refinery at Jeddah and in *S. canaliculatus* when compared to *E. morio*. There was a significant high expression level of studied antioxidant enzymes genes in the polluted areas and the level of the expression profile tended to correlate with the degree of pollution and fish species feed habit. This was confirmed by the level of MDA which in the same way increased with an increase in the level of total hydrocarbons. In conclusion; the expression profile of antioxidant enzymes of *S. canaliculatus* and *E. morio* tissues can be used as a strong bio-indicator of total hydrocarbons pollution especially in *S. canaliculatus*.

1. Introduction

About hundred million tons of oil passes through the Red Sea annually (PERSGA, 1995). The Red Sea is navigationally complex from its narrow mouth at Bab el Mendab along its entire reef lined length. Its narrow width greatly increases the likelihood of collisions between vessels. There are marine pollution accidents reported by Saudi Arabia during 1993. In 1989 the Indian Tanker Kanchenjunga spilled 25.000 barrels

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after colliding with a reef in front of the Jeddah coast (MEPA, 1990). Marine pollution with oil can be classified as chronic and catastrophic. It is important to make differentiation between both types of pollution. Chronic oil pollution is due to the seepage of oil at a constant low level for a long time into marine water from shipping, deballasting, etc., and may not be immediately apparent. Catastrophic events refer specifically to accidental oil spills, which may contaminate either open oceans or coastal shores (Al-Shwafi, 2008) .It would seems that the major type of oil pollution in the Red Sea belongs to the former type "chronic". The Red Sea was classified as "Special Areas" under the international MARPOL convention 73–78. This means that operational discharges from shipping are restricted. Nevertheless evidence suggests that oil pollution from this source has a far greater effect on the marine environment than accidental spills. Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that contain two or more fused benzene rings. This group of molecules is one of the most persistent environmental contaminants due to their occurrence in petroleum, coal, and tar deposits, and the aquatic environment has been contaminated with PAHs globally (Reynaud and Deschaux, 2006). On the other hand; TPHs includes hundreds of the chemical compounds that are derived from crude oil (USEPA, 1986). The bio accumulative potential of these compounds, known to be both carcinogenic and immune toxic (Davila et al., 1995), has been reported extensively in many organisms, including fish (Balk et al., 2011; Cheikyula et al., 2008; Van der Oost et al., 2003). It has been also suggested that PAHs exposure induces the production of reactive oxygen species (ROS) in aquatic organisms. ROS are known to be responsible for lipid peroxidation, protein degradation, DNA damage, and apoptosis in vertebrates. Recently, toxicogenomic approaches using a whole human genome microarray have been used to identify key molecular pathways related to increased hepatotoxicity in a human hepatocellular carcinoma (HepG2) cell line exposed to PAHs (Song et al., 2011). In coral species, various kinds of environmental stresses such as high and cold temperature, salinity, supersaturating light, and bacterial infection induce ROS production. Changes in transcript levels are the earliest and most sensitive biomarkers for physiological responses to environmental stress. Thus, the impact of environmental stress on coral can be diagnosed and quantified by using genes which have expression levels that change in response to a specific environmental challenge. Molecular and biochemical responses are now routinely assessed as signs of pollutant-modulated effects. These biomarkers can act as signs to pollutant exposure that indicate both exposure levels to toxic substances and the magnitude of the organisms response (Cajaraville et al., 2000; Dondero et al., 2006). Biotransformation enzymes are biomarkers that are responsible for the degradation and mobilization of xenobiotics. A well-documented example is the phase II bio transformation enzyme glutathione transferase (GST; EC 2.5.1.18) (Solé and Livingstone, 2005; Ricciardi et al., 2006). In addition, oxidative stress enzymes such as catalase (CAT; EC 1.11.1.6) and superoxide dismutase (SOD; EC 1.15.1.1) are often employed as pollutant biomarkers by providing a measure of the physiological stress in animals when exposed to pollutants (Ricciardi et al., 2006). Furthermore; Glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC1.6.4.2) enzymes are usually used to assess the oxidative stress in the biological system (Doyotte et al., 1997; Regoli et al., 1997).

The aim of the present study is to evaluate the hazards effect of total hydrocarbons pollution on the antioxidant enzymes expression levels as a bio indicator for the pollution at sea coastal area at Jeddah and Yanbu provinces, Saudi Arabia.

2. Materials and methods

2.1. Ethical statement

All experiments were carried out in accordance with the Saudi Arabian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science, North Jeddah, King Abdul-Aziz University, Jeddah, Saudi Arabia.

2.2. Study areas

Six sampling sites were selected along the KSA Red Sea coast at Jeddah and Yanbu provinces, four contaminated sites and two reference areas, at the Jeddah coast site, I reference site, II north of Jeddah Islamic seaport and III, in front of petromine refinery, at Yanbu coast site IV reference site, V collected close to Yanbu industrial harbor and VI close to oil refineries and petrochemical factories (Afifi et al., 2014).

2.3. Sampling and analytical procedures

Sediment, water and fish samples were collected from the studded sites during mid of March 2014. Polyvinyl Chloride (PVC) tubes were used for water sample collection, at half meter depth from the surface of the water. Superficial sediment samples were collected as described by Boyd and Tucker (1992), and then were used for determination of TPHs. Fish sampling, ten fishes of a similar size of both *Siganus canaliculatus* and *Epinephelus morio* were collected from each studded site from overnight pre-held pots. Length and weight of each fish were recorded. Liver, gills and muscle samples were taken, kept in liquid nitrogen for molecular analysis and TPH determination.

2.4. Determination of water, sediment and fish total petroleum hydrocarbon

Water TPHs were extracted following the method of Parsons et al. (1984). The extraction and clean-up of the sediment TPHs were done in accordance with Hilpert et al. (1978). One gram of fish liver, gills and muscle tissue was used for extraction of TPHs following the method of UNESCO (1981) and each sample was homogenized in hexane solvent using the SONOPULS ultrasonic homogenizers Bandelin 2450 (Sigma–Aldrich, Hamburg, Germany) until uniform consistency was obtained. The homogenates were dried by passing through a bed of anhydrous sodium sulfate and then filterated. The filtrate was recovered and added with hexane to a final volume of 25 ml. TPHs levels were measured using Shimadzu UV Spectrophotometer RF 5000 (Kyoto 604-8511, Japan). Light Arabian crude oil, was used as standard. Skin, gills, Liver and muscle, and LPO products were quantized by the

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