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# 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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## KEYWORDS

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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*.

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## 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 seem 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 petroleum 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 studied 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 studied 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 filtered. 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

thioarbituric acid method (Esterbauer and Cheeseman, 1990). Values were presented as Nano moles of thioarbituric acid-reactive substance per gram of tissue.

### 2.5. Molecular assays and gene expressions

Skin, gills, liver and muscle SOD, CAT, GR, GPx and GST genes expression were quantified using real time PCR. Total RNA was isolated from tissue samples using the RNeasy Mini Kit Qiagen (Cat. No. 74104) following the manufacturer's instructions. RNA quality was assessed as the 260/280 nm absorbance ratio using NanoDrop®ND-1000 Spectrophotometer, (Nano Drop Technologies, Wilmington, Delaware USA). Then, 0.5 µg of total RNA was used for production of cDNA using QIAGEN Long Range 2 Step RT-PCR Kit, (Cat. No. 205920), five µL of total cDNA diluted 1:6 was mixed with 12.5 µL of 2× SYBR® Green PCR mix with ROX from Bio-Rad, 6.5 µL of autoclaved water, and 0.5 µL (10 pmol/µL) of each forward and reverse primer for the measured genes. The house keeping gene β-actin was used as a constitutive control for normalization. Primers were designed using Primer3 software (The Whitehead Institute, [http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)) as per the published *Epinephelus* species SOD, CAT, GR, GST, GPx and β-actin genes sequences (AY035854.1, AY735009.1, JF430677.1, HQ441085.1, AY510710.2) of the NCBI database. All primers were provided by Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and are listed in (Table 1). AbiPrism 7300 (Applied Biosystems, Foster City, CA, USA) was used for performing the real-time qPCR reaction. The reaction program was a 94 °C/2 min enzyme activation, then 40 cycles of 95 °C/15 s denaturation, 60 °C/30 s annealing and 72 °C/30 s. The produced fluorescent product was detected at the end of the 72 °C extension period. For each sample, the threshold cycle (Ct) values were used for the determination of mRNA concentration. Data were expressed as a fold change in mRNA expression relative to β-actin mRNA levels and calculated using the 2<sup>-DDCT</sup> method.

### 2.6. Statistical analysis

SPSS version 20. Statistical packages (IBM, New York, NY, USA) were used for statistical analysis of the obtained data, that were presented as a mean ± SD,  $n = 10$ . One-way analysis of variance (ANOVA) was used to determine the statistical differences between the groups flowed by Duncan's multiple range test to analyze the inter-grouping homogeneity. Data were presented as mean ± S.D. ( $n = 10$ ) for each animal group.  $P < 0.05$  were statistical significant.

## 3. Results

### 3.1. Sea water and sediment total petroleum hydrocarbon

Total petroleum hydrocarbon concentrations were high in water and sediments collected from polluted sites either from Jeddah or Yanbu, but those that were collected from Jeddah were more polluted than that collected from Yanbu ( $P < 0.05$ ). The highest pollution was observed in samples that were collected from front of the petromine refinery at Jeddah (Table 2).

### 3.2. Total petroleum hydrocarbon concentrations in tissues of *E. morio* and *S. canaliculatus*

The concentrations of TPH in liver, gills, skin and muscle of *E. morio* and *S. canaliculatus* were high in fishes collected from the polluted sites either from Jeddah or Yanbu when compared with their corresponding fishes collected from the reference sites ( $P < 0.05$ ). TPHs concentrations were high in fishes caught close to the refineries in comparison with those that were caught close to the seaport. Fishes collected from Jeddah were more polluted than those that were collected from Yanbu. *S. canaliculatus* fishes had higher TPHs concentrations than *E. morio* (Table 3).

### 3.3. Malondialdehyde concentrations in skin, liver, gills and muscle of *E. morio* and *S. canaliculatus*

MDA levels in skin, liver and gills of *E. morio* and *S. canaliculatus* fishes caught from polluted sites were high as compared with the reference sites, as well in fishes caught close to the refineries as compared with those that were caught close to seaport. The tissues of fishes that were caught from Jeddah had high levels of MDA than that of Yanbu ( $P < 0.05$ ). Skin, liver and gills of *S. canaliculatus* fishes had high levels of MDA as compared with *E. morio* fishes (Tables 4-6). MDA levels in muscular tissues did not reveal significant differences (Table 7).

### 3.4. Antioxidant enzyme gene expression in skin, liver, gills and muscle of *E. morio* and *S. canaliculatus*

The levels of CAT, SOD, GPx, GR and GST enzymes gene expressions in skin, liver and gills of *E. morio* and *S. canaliculatus* fishes caught from polluted sites were induced as compared with the reference sites, as well as in fishes caught close to the refineries as compared with those that were caught close to the seaport. The tissues of fishes that were caught from Jeddah had high levels of antioxidant enzymes gene expres-

**Table 1** Oligonucleotides sequences of primers.

Gene	Forward 5' -> 3'	Reverse 5' -> 3'
CAT	TCCTGAATGAGGAGGAGCGA	ATCTTAGATGAGGCGGTGATG
SOD	GGTGCCCTGGAGCCCTA	ATGCGAAGTCTTCCACTGTC
GPx	CCAAGAGAACTGCAAGAACGA	CAGGACACGTCATTCCTACAC
GR	CATTACCGAGACGCGGAGTT	CAGTTGGCTCAGGATCATTTGT
GST	TAATGGGAGAGGGAAGATGG	CTCTGCGATGTAATTCAGGA
β actin	CAATGAGAGGTTCCGTTGC	AGGATTCCATACCAAGGAAGG

CAT, catalase; SOD, super oxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase.

**Table 2** Total petroleum hydrocarbon in sea water ( $\mu\text{g L}^{-1}$ ) and sediment ( $\text{mg K}^{-1}$  dry weight) at the different sites.

	I	II	III	IV	V	VI
Sea water	2.08 $\pm$ 0.3	9.8 $\pm$ 1 <sup>ac</sup>	20.2 $\pm$ 1.7 <sup>abc</sup>	1.3 $\pm$ 0.3	6.2 $\pm$ 1 <sup>a</sup>	13.4 $\pm$ 1.4 <sup>ab</sup>
Sediment	20.2 $\pm$ 3	167 $\pm$ 10 <sup>ac</sup>	212 $\pm$ 8 <sup>abc</sup>	14.8 $\pm$ 2.6	130 $\pm$ 12.5 <sup>a</sup>	170 $\pm$ 15.2 <sup>ab</sup>

Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significant ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively, c = when comparing Jeddah groups versus the corresponding Yanbu groups.

**Table 3** Total petroleum hydrocarbon ( $\mu\text{g K}^{-1}$ ) in the liver, gills and muscle of different fish species at the different sites.

Fish species	Site	Length	Weight	Total petroleum hydrocarbon			
				Liver	Gills	Skin	Muscle
<i>E. morio</i>	I	53.6 $\pm$ 2.3	440 $\pm$ 20	8 $\pm$ 0.03	11 $\pm$ 0.05	12 $\pm$ 1	10 $\pm$ 0.04
	II	55 $\pm$ 3	433 $\pm$ 8.9	140 $\pm$ 15 <sup>ac</sup>	183 $\pm$ 12 <sup>ac</sup>	250 $\pm$ 33 <sup>ac</sup>	78 $\pm$ 6 <sup>ac</sup>
	III	54 $\pm$ 4.5	423 $\pm$ 28	363 $\pm$ 27 <sup>abc</sup>	453 $\pm$ 20 <sup>abc</sup>	500 $\pm$ 45 <sup>abc</sup>	156 $\pm$ 20 <sup>abc</sup>
	IV	54 $\pm$ 5	453 $\pm$ 64	7 $\pm$ 0.03	9 $\pm$ 0.1	11 $\pm$ 0.9	6 $\pm$ 0.01
	V	53 $\pm$ 57	446 $\pm$ 35	100 $\pm$ 12 <sup>a</sup>	130 $\pm$ 11.5 <sup>a</sup>	180 $\pm$ 23 <sup>a</sup>	49 $\pm$ 5 <sup>a</sup>
	VI	52.3 $\pm$ 6	420 $\pm$ 40	306 $\pm$ 30 <sup>ab</sup>	350 $\pm$ 19 <sup>ab</sup>	380 $\pm$ 29 <sup>ab</sup>	84 $\pm$ 3 <sup>ab</sup>
<i>S. canaliculatu</i>	I	20.3 $\pm$ 0.9	74.3 $\pm$ 2.	13 $\pm$ 0.3	15 $\pm$ 0.6	17 $\pm$ 2	10.3 $\pm$ 0.3
	II	18.3 $\pm$ 1.4	79 $\pm$ 3	263 $\pm$ 17 <sup>ac#</sup>	347 $\pm$ 20 <sup>ac#</sup>	390 $\pm$ 20 <sup>ac#</sup>	143 $\pm$ 14 <sup>a#</sup>
	III	19.3 $\pm$ 2.4	78.3 $\pm$ 6	530 $\pm$ 20 <sup>abc#</sup>	670 $\pm$ 21 <sup>abc#</sup>	720 $\pm$ 30 <sup>abc#</sup>	366 $\pm$ 28 <sup>abc#</sup>
	IV	20 $\pm$ 2	80.3 $\pm$ 3	10 $\pm$ 0.26	13 $\pm$ 0.03	14 $\pm$ 3	8 $\pm$ 0.03
	V	18 $\pm$ 2.3	75.7 $\pm$ 5	183 $\pm$ 13 <sup>a#</sup>	223 $\pm$ 29 <sup>a#</sup>	270 $\pm$ 30 <sup>a#</sup>	123 $\pm$ 14 <sup>a#</sup>
	VI	21 $\pm$ 1.5	76.7 $\pm$ 5	343 $\pm$ 17 <sup>ab#</sup>	400 $\pm$ 17 <sup>ab#</sup>	450 $\pm$ 40 <sup>ab#</sup>	186 $\pm$ 18 <sup>ab#</sup>

I reference area at the Jeddah coast, II north of Jeddah Islamic seaport, III, in front of the petromine refinery at Jeddah, IV reference site at Yanbu coast, V collected close to Yanbu industrial harbor and VI close to oil refineries and petrochemical factories at the Yanbu coast. Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significance ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively in the same fish species, c = when comparing Jeddah groups versus the corresponding Yanbu groups # = when comparing *S. canaliculatus* groups to the corresponding *E. morio*.

**Table 4** Skin antioxidant enzyme gene expression (relative expression to  $\beta$ -actin) and MDA level ( $\text{nmol g}^{-1}$  wt. tissue) in *E. morio* and *S. canaliculatus*.

Fish	Site	CAT	SOD	GPx	GR	GST	MDA
<i>E. morio</i>	I	9 $\pm$ 1.5	3 $\pm$ 0.9	10 $\pm$ 2	0.11 $\pm$ 0.02	145 $\pm$ 13	0.7 $\pm$ 0.06
	II	15 $\pm$ 0.6 <sup>ac</sup>	6.6 $\pm$ 1.2 <sup>a</sup>	22 $\pm$ 1.4 <sup>ac</sup>	2.6 $\pm$ 0.2 <sup>ac</sup>	456 $\pm$ 14 <sup>ac</sup>	2.1 $\pm$ 0.2 <sup>ac</sup>
	III	22.6 $\pm$ 1.8 <sup>abc</sup>	11 $\pm$ 1.4 <sup>abc</sup>	30 $\pm$ 2.3 <sup>abc</sup>	3.7 $\pm$ 0.3 <sup>abc</sup>	610 $\pm$ 20 <sup>abc</sup>	2.9 $\pm$ 0.2 <sup>abc</sup>
	IV	8 $\pm$ 1.5	2.3 $\pm$ 0.4	10 $\pm$ 0.9	0.11 $\pm$ 0.02	141 $\pm$ 9	0.66 $\pm$ 0.09
	V	10 $\pm$ 0.4 <sup>a</sup>	5 $\pm$ 1 <sup>a</sup>	17.6 $\pm$ 1.5 <sup>a</sup>	1.9 $\pm$ 0.08 <sup>a</sup>	350 $\pm$ 17 <sup>a</sup>	1.5 $\pm$ 0.15 <sup>a</sup>
	VI	15 $\pm$ 1.4 <sup>ab</sup>	8 $\pm$ 0.6 <sup>ab</sup>	25 $\pm$ 2.9 <sup>ab</sup>	2.7 $\pm$ 0.4 <sup>ab</sup>	520 $\pm$ 11 <sup>ab</sup>	2 $\pm$ 0.1 <sup>ab</sup>
<i>S. canaliculatus</i>	I	9.7 $\pm$ 1.5	4 $\pm$ 0.6	11.3 $\pm$ 0.9	0.07 $\pm$ 0.03	145 $\pm$ 7.6	0.76 $\pm$ 0.09
	II	24 $\pm$ 2 <sup>ac#</sup>	10 $\pm$ 0.7 <sup>a#</sup>	32 $\pm$ 1.1 <sup>a#</sup>	3.5 $\pm$ 0.3 <sup>a#</sup>	646 $\pm$ 23 <sup>ac#</sup>	3.4 $\pm$ 0.2 <sup>ac#</sup>
	III	35 $\pm$ 1.2 <sup>abc#</sup>	17 $\pm$ 1 <sup>abc#</sup>	43 $\pm$ 0.9 <sup>abc#</sup>	5.3 $\pm$ 0.2 <sup>abc#</sup>	833 $\pm$ 20 <sup>abc#</sup>	4.5 $\pm$ 0.3 <sup>abc#</sup>
	IV	8.7 $\pm$ 0.9	3.3 $\pm$ 0.9	9.6 $\pm$ 0.9	0.1 $\pm$ 0.01	147 $\pm$ 10	0.7 $\pm$ 0.11
	V	17 $\pm$ 1.5 <sup>a#</sup>	8 $\pm$ 0.9 <sup>a#</sup>	28 $\pm$ 1.6 <sup>a#</sup>	3.1 $\pm$ 0.18 <sup>a#</sup>	523 $\pm$ 18 <sup>a#</sup>	2.5 $\pm$ 0.12 <sup>a#</sup>
	VI	27 $\pm$ 1.5 <sup>ab#</sup>	13 $\pm$ 1.4 <sup>ab#</sup>	35.7 $\pm$ 2.3 <sup>ab#</sup>	4.5 $\pm$ 0.2 <sup>ab#</sup>	736 $\pm$ 18 <sup>ab#</sup>	3.4 $\pm$ 0.2 <sup>ab#</sup>

I reference area at the Jeddah coast, II north of the Jeddah Islamic seaport, III, in front of the petromine refinery at Jeddah, IV reference area at the Yanbu coast, V collected close to the Yanbu industrial harbor and VI close to oil refineries and petrochemical factories at the Yanbu coast. Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significance ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively in the same fish species, c = when comparing Jeddah groups versus the corresponding Yanbu groups # = when comparing *S. canaliculatus* groups to the corresponding *E. morio*.

sions than that of Yanbu. Skin, liver and gills of *S. canaliculatus* fishes had high levels of antioxidant enzymes gene expressions as compared with *E. morio* fishes (Tables 4–6). The antioxidant enzymes gene expression levels in muscular tissues

did not reveal significant differences except that of SOD in *S. canaliculatus* fishes that were caught close to the petromine refinery at Jeddah that had high induced levels when compared with other groups (Table 7).

**Table 5** Liver antioxidant enzymes gene expression (relative expression to  $\beta$ -actin) and MDA level (nmol  $g^{-1}$  wt w. tissue) in *E. morio* and *S. canaliculatus*.

Fish	Site	CAT	SOD	GPx	GR	GST	MDA
<i>E. morio</i>	I	14 $\pm$ 1.5	19.6 $\pm$ 1.2	4 $\pm$ 0.6	0.19 $\pm$ 0.01	203 $\pm$ 31	0.6 $\pm$ 0.1
	II	23 $\pm$ 1.5 <sup>a</sup>	37 $\pm$ 1.5 <sup>ac</sup>	13 $\pm$ 0.7 <sup>a</sup>	3.7 $\pm$ 0.18 <sup>ac</sup>	650 $\pm$ 28.8 <sup>a</sup>	2.26 $\pm$ 0.18 <sup>a</sup>
	III	42 $\pm$ 1.4 <sup>abc</sup>	76 $\pm$ 3 <sup>abc</sup>	24.7 $\pm$ 1.8 <sup>abc</sup>	76 $\pm$ 0.3 <sup>abc</sup>	1230 $\pm$ 88 <sup>abc</sup>	6.16 $\pm$ 0.2 <sup>ab</sup>
	IV	8 $\pm$ 1.1	15 $\pm$ 1.1	3.5 $\pm$ 0.3	0.22 $\pm$ 0.01	178 $\pm$ 14.8	0.46 $\pm$ 0.09
	V	17 $\pm$ 1.8 <sup>a</sup>	36 $\pm$ 1.8 <sup>a</sup>	10.5 $\pm$ 1.2 <sup>a</sup>	2.2 $\pm$ 0.1 <sup>a</sup>	523 $\pm$ 14.5 <sup>a</sup>	2.36 $\pm$ 0.2 <sup>a</sup>
	VI	26 $\pm$ 3.7 <sup>ab</sup>	52.7 $\pm$ 6.3 <sup>ab</sup>	21 $\pm$ 2 <sup>ab</sup>	5.2 $\pm$ 0.6 <sup>ab</sup>	1050 $\pm$ 104 <sup>ab</sup>	6 $\pm$ 1 <sup>ab</sup>
<i>S. canaliculatus</i>	I	21 $\pm$ 3.8	23 $\pm$ 1.2	4 $\pm$ 0.6	0.23 $\pm$ 0.01	166 $\pm$ 16.7	0.66 $\pm$ 0.12
	II	54.6 $\pm$ 3 <sup>ac#</sup>	57 $\pm$ 1.8 <sup>a#</sup>	27.7 $\pm$ 1.5 <sup>ac#</sup>	6.6 $\pm$ 0.4 <sup>a#</sup>	1383 $\pm$ 72 <sup>ac#</sup>	4.1 $\pm$ 1 <sup>a#</sup>
	III	71 $\pm$ 4 <sup>abc#</sup>	121 $\pm$ 7 <sup>abc#</sup>	38.3 $\pm$ 0.9 <sup>abc#</sup>	11.7 $\pm$ 1 <sup>abc#</sup>	1916 $\pm$ 44 <sup>abc#</sup>	9 $\pm$ 0.5 <sup>ab#</sup>
	IV	17 $\pm$ 3.7	19 $\pm$ 0.6	3.9 $\pm$ 0.5	0.18 $\pm$ 0.01	198 $\pm$ 26.8	0.6 $\pm$ 0.11
	V	35 $\pm$ 3 <sup>a#</sup>	53 $\pm$ 2 <sup>a#</sup>	21 $\pm$ 1.6 <sup>a#</sup>	5.8 $\pm$ 0.07 <sup>a#</sup>	1050 $\pm$ 76 <sup>a#</sup>	3.8 $\pm$ 0.12 <sup>a#</sup>
	VI	63 $\pm$ 2.1 <sup>ab#</sup>	95 $\pm$ 4 <sup>ab#</sup>	30 $\pm$ 2 <sup>ab#</sup>	9.2 $\pm$ 0.6 <sup>ab#</sup>	1500 $\pm$ 57 <sup>ab#</sup>	8.5 $\pm$ 0.9 <sup>ab#</sup>

I reference site at the Jeddah coast, II north of the Jeddah Islamic seaport, III, in front of the petromine refinery at Jeddah, IV reference site at Yanbu coast, V collected close to the Yanbu industrial harbor and VI close to oil refineries and petrochemical factories at the Yanbu coast. Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significance ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively in the same fish species, c = when comparing Jeddah groups versus the corresponding Yanbu groups # = when comparing *S. canaliculatus* groups to the corresponding *E. morio*.

**Table 6** Gills antioxidant enzymes gene expression (relative expression to  $\beta$ -actin) and MDA level (nmol  $g^{-1}$  wt w. tissue) in *E. morio* and *S. canaliculatus*.

Fish	Site	CAT	SOD	GPx	GR	GST	MDA
<i>E. morio</i>	I	6 $\pm$ 1.2	13 $\pm$ 1.5	3.7 $\pm$ 0.6	0.16 $\pm$ 0.02	186 $\pm$ 32	0.23 $\pm$ 0.09
	II	17.6 $\pm$ 2.2 <sup>a</sup>	22 $\pm$ 2.5 <sup>a</sup>	9 $\pm$ 0.5 <sup>a</sup>	1.9 $\pm$ 0.09 <sup>ac</sup>	450 $\pm$ 29 <sup>ac</sup>	2.3 $\pm$ 0.6 <sup>ac</sup>
	III	32 $\pm$ 1.2 <sup>ab</sup>	38 $\pm$ 2.6 <sup>ab</sup>	16.3 $\pm$ 0.9 <sup>abc</sup>	3.8 $\pm$ 0.2 <sup>abc</sup>	816 $\pm$ 44 <sup>abc</sup>	4.4 $\pm$ 0.3 <sup>ab</sup>
	IV	4.6 $\pm$ 0.9	12 $\pm$ 1	3.4 $\pm$ 0.3	0.17 $\pm$ 0.01	171 $\pm$ 16	0.2 $\pm$ 0.05
	V	11.3 $\pm$ 0.9	23 $\pm$ 1.5 <sup>a</sup>	5.8 $\pm$ 1	1.1 $\pm$ 0.1 <sup>a</sup>	290 $\pm$ 51	1.4 $\pm$ 0.05 <sup>a</sup>
	VI	23.6 $\pm$ 2.9 <sup>ab</sup>	34 $\pm$ 4.5 <sup>ab</sup>	11.3 $\pm$ 0.9 <sup>ab</sup>	2.6 $\pm$ 0.3 <sup>ab</sup>	566 $\pm$ 44 <sup>ab</sup>	3.6 $\pm$ 0.3 <sup>ab</sup>
<i>S. canaliculatus</i>	I	14.3 $\pm$ 3.8	13 $\pm$ 1.5	3.4 $\pm$ 0.9	0.17 $\pm$ 0.01	133 $\pm$ 17	0.33 $\pm$ 0.09
	II	34 $\pm$ 6.3 <sup>ac#</sup>	33 $\pm$ 3 <sup>a#</sup>	16 $\pm$ 0.6 <sup>ac#</sup>	3.3 $\pm$ 0.2 <sup>a#</sup>	800 $\pm$ 29 <sup>ac#</sup>	3.2 $\pm$ 0.6 <sup>a#</sup>
	III	50 $\pm$ 1.8 <sup>abc#</sup>	58 $\pm$ 8.5 <sup>abc#</sup>	29 $\pm$ 4 <sup>abc#</sup>	5.9 $\pm$ 0.5 <sup>abc#</sup>	1460 $\pm$ 202 <sup>abc#</sup>	6.1 $\pm$ 1 <sup>ab#</sup>
	IV	14 $\pm$ 4.5	13.7 $\pm$ 2	3.6 $\pm$ 0.6	0.16 $\pm$ 0.02	180 $\pm$ 28	0.5 $\pm$ 0.1
	V	22.3 $\pm$ 2.6 <sup>a#</sup>	34 $\pm$ 3.6 <sup>a#</sup>	11 $\pm$ 1 <sup>a#</sup>	2.9 $\pm$ 0.03 <sup>a#</sup>	550 $\pm$ 50 <sup>a#</sup>	3 $\pm$ 0.09 <sup>a#</sup>
	VI	41 $\pm$ 3.5 <sup>ab#</sup>	49 $\pm$ 5.7 <sup>ab#</sup>	24 $\pm$ 2 <sup>ab#</sup>	4.6 $\pm$ 0.3 <sup>ab#</sup>	1200 $\pm$ 104 <sup>ab#</sup>	5.7 $\pm$ 0.4 <sup>ab#</sup>

I reference site at the Jeddah coast, II north of the Jeddah Islamic seaport, III, in front of the petromine refinery at Jeddah, IV reference site at the Yanbu coast, V collected close to the Yanbu industrial harbor and VI close to oil refineries and petrochemical factories at the Yanbu coast. Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significance ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively in the same fish species, c = when comparing Jeddah groups versus the corresponding Yanbu groups # = when comparing *S. canaliculatus* groups to the corresponding *E. morio*.

#### 4. Discussion

The present study tended to evaluate the bio-indicators of PHs in the red sea coastal areas at Jeddah and Yanbu provinces. From the definition of the biomarker as monitoring of body substances that indicate in cellular or biochemical terms the presence or the host response for certain contaminants (Sarkar et al., 2006). Several bio-indicators were used a long time ago for the determination and evaluation of the degree of PHs in the aquatic environments. Fishes as animal members of this environment were used for this target (Phillips, 1985; Tanabe et al., 1987). Biochemical and molecular testing of the antioxidant system in fish organs like skin, muscles, gills and livers is a good indicator of the external changes in the

aquatic media that fish live in (Cheung et al., 2001; Sridevi et al., 1998; Thomas and Wofford, 1993; Winston, 1991). In the present study we determined the expression level of some antioxidant enzymes in two fish species *E. morio* and *S. canaliculatus* that were grown in the selected areas of the study. The selected areas of the study were characterized by high PHs due to the crude or refined petroleum or combustion sources (Cheung et al., 2001). Our results show that the PH concentrations were high in the polluted areas if compared with their references areas, with the highest concentrations in the area in front of the petromine refinery at Jeddah by 10.49 fold if compared with the reference area. And this reflected on the biochemical and the expression pictures of the fishes in this area. Our results showed that there are high levels of MDA

**Table 7** Muscle antioxidant enzymes gene expression (relative expression to  $\beta$ -actin) and MDA level ( $\text{nmol g}^{-1}$  wt w. tissue) in *E. morio* and *S. canaliculatus*.

Fish	Site	CAT	SOD	GPx	GR	GST	MDA
<i>E. morio</i>	I	12.3 $\pm$ 0.9	16.6 $\pm$ 2	3 $\pm$ 0.7	0.12 $\pm$ 0.04	160 $\pm$ 41	0.24 $\pm$ 0.08
	II	12 $\pm$ 4.7	14.2 $\pm$ 1.2	3.9 $\pm$ 0.6	0.13 $\pm$ 0.02	196 $\pm$ 29	0.22 $\pm$ 0.03
	III	14.3 $\pm$ 3.2	14 $\pm$ 1	3.6 $\pm$ 0.7	0.14 $\pm$ 0.08	146 $\pm$ 33	0.20 $\pm$ 0.02
	IV	11 $\pm$ 0.6	12 $\pm$ 0.9	3.2 $\pm$ 0.6	0.14 $\pm$ 0.02	163 $\pm$ 31	0.15 $\pm$ 0.03
	V	11.7 $\pm$ 0.9	14 $\pm$ 1.1	3.5 $\pm$ 0.3	0.12 $\pm$ 0.10	173 $\pm$ 17	0.36 $\pm$ 0.12
	VI	13 $\pm$ 3.6	11.6 $\pm$ 0.5	3.6 $\pm$ 0.9	0.13 $\pm$ 0.02	183 $\pm$ 44	0.19 $\pm$ 0.04
<i>S. canaliculatus</i>	I	10 $\pm$ 2.5	13 $\pm$ 1.7	3.2 $\pm$ 0.4	0.12 $\pm$ 0.01	163 $\pm$ 18	0.37 $\pm$ 0.09
	II	10 $\pm$ 2.6	15.6 $\pm$ 1.4	4.1 $\pm$ 1	0.14 $\pm$ 0.003	216 $\pm$ 44	0.33 $\pm$ 0.14
	III	12 $\pm$ 1.2	32 $\pm$ 1.4 <sup>abc#</sup>	5.2 $\pm$ 1.6	0.15 $\pm$ 0.03	266 $\pm$ 72	0.34 $\pm$ 0.18
	IV	14.3 $\pm$ 1.8	14 $\pm$ 1.8	4.6 $\pm$ 1.5	0.18 $\pm$ 0.005	233 $\pm$ 72	0.33 $\pm$ 0.03
	V	13.3 $\pm$ 0.9	16 $\pm$ 2.6	5.7 $\pm$ 1.4	0.17 $\pm$ 0.008	283 $\pm$ 60	0.32 $\pm$ 0.1
	VI	13.7 $\pm$ 2.3	16.3 $\pm$ 1.2	3.5 $\pm$ 0.6	0.13 $\pm$ 0.005	176 $\pm$ 26	0.19 $\pm$ 0.02

I reference site at the Jeddah coast, II north of the Jeddah Islamic seaport, III, in front of the petromine refinery at Jeddah, IV reference site at the Yanbu coast, V collected close to the Yanbu industrial harbor and VI close to oil refineries and petrochemical factories at the Yanbu coast. Data were represented as mean  $\pm$  SD ( $n = 10$ ). Level of significance ( $p < 0.05$ ) observed in the same row are: a = compared to the reference group, b = group III and VI versus group II and V are compared respectively in the same fish species, c = when comparing Jeddah groups versus the corresponding Yanbu groups <sup>#</sup> = when comparing *S. canaliculatus* groups to the corresponding *E. morio*.

in the skin, gills and livers of examined fishes in polluted areas especially in the area in front of the petromine refinery at Jeddah. MDA as a strong marker for the lipid peroxidation is used to assess the degree of the oxidative stress in the biological system. It is known that; petroleum hydrocarbon exerts its toxic effects in aquatic living by induction of oxidative stress or biotransformation into toxic substances (Varanasi and Stein, 1991; Stein et al., 1992). It is known that, the induced activities of CAT, SOD, GST, GR and GPx, serve as a protective mechanism to overcome the free radicals (Karakoc et al., 1997; Schins et al., 1997; Solé et al., 1998). Superoxide radicals, hydrogen peroxide and organic hydroperoxides are reduced by SOD, CAT and GPx respectively, preventing the synthesis of free radicals. In the present study we examined the antioxidant enzymes based on their cellular expression level using qRT PCR in skins, livers and gills. The results show high expression levels in the polluted areas in both Jeddah and Yanbu coastal areas. The coastal area in front of the petromine refinery in Jeddah showed the highest expression levels of antioxidant enzymes at all. To the best of our knowledge; we are the first who perform the full study about the gene expression of the bio-indicators for THP in Saudi Arabia; while other authors evaluated these indicators in relation to the cellular activities (Di Giulio et al., 1989; Nishimoto et al., 1995). The oxidative biomarker responses, in this study, were more accused in Jeddah than Yanbu coastal areas. The increased levels and activities of some bio-indicators as MDA, GR and CAT in the skins, livers and gills of the studied fish are explained by the high PH levels in the polluted areas (Vicente-Martorell et al., 2009). The increase of the mRNA levels of some enzymes, such as GR and GPx in spring (lower temperature) is explained by the increase in mitochondrial membrane polyunsaturation. The membrane polyunsaturation leads to a rise in the mitochondrial respiratory rates that enhances ROS synthesis, inducing membranes lipid peroxidation. High levels of lipid peroxidation products induced GPx activity. This increase in GSH utilization, produced oxidized

glutathione (GSSG) and induced GR activity for reduced equivalents and normal redox homeostasis in the living cells (Oliva et al., 2010). So there is a correlation between the increase in the PHs and the MDA and enzymes expression levels in fish tissues. GST activities may be decreased in fish after exposure to PHs (Ahmad et al., 2004; Jee and Kang, 2005; Jifa et al., 2006; Vieira et al., 2008), or not changed (Sturte et al., 2006; Sun et al., 2006; Oliveira et al., 2008) or decreased (Vieira et al., 2008; Sun et al., 2008). Several works demonstrated GST activities to be significantly induced (Machala et al., 2001). We are in the same line of the results that demonstrated a high increase in the GST activities and our results showed a high increase in the expression level of these enzymes in the tissues of fishes at the polluted areas.

## 5. Conclusion

The antioxidant enzymes gene expression in the skin, gills and liver of fish species *E. morio* and *S. canaliculatus* was considered a potent Biomarker for TPH in the Jeddah and Yanbu coastal areas in KSA. We found a significant correlation between the intensity of the THP and MDA levels with the expression levels of these SOD, CAT, GR, GPx and GST enzymes in fish tissues.

## Conflicts of interest

The authors declare no conflict of interest.

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