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Histological Comparison of Some Fish Tissue as Biomarker to Evaluate Water Quality from the Red Sea Coast, Jeddah Governorate

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Histological studies in organs like gill, liver and muscle of *Lethrinus harak* and *Terapon jarbua* were made to assess tissue damage due to concentration of heavy metals and organic compound on the polluted site (Al-Kumrah) compared with the unpolluted site (Al-Shoaibah). The histopathological investigations showed that heavy metals and organic compounds due to the discharge of sewage affected the vital organs and caused clear and marked damage in the gills, liver and muscles in fishes, collected from Al-Kumrah site, and considered as a biomarker to the low quality of water in this region compared with Al-Shoaibah area. Thus, a conclusion of the present study is that histopathological biomarkers can be valuable indicators of impaired health of fish and can reflect the effects of exposure to untreated and treated sewage. Therefore, the continue to discharge untreated sewage into Al-Kumrah site should be limited and water resources should be treated to eliminated the pollutants as well as improving the water quality for improving fisheries in this area to maintain human health.

Key words: Histological, Red Sea, Biomarker, Pollution, *Lethrinus harak* and *Terapon jarbua*.

Most of the wadis and the coastal area in Kingdom Saudi Arabia are polluted due to industrial activity. Agriculture activities also contribute to the aquatic pollution mainly due to large-scale use of pesticides and other agrochemicals in this sector. The effects of pesticides and insecticides on fishes and their tolerance, mortality, behavior, accumulation in tissues and induced biochemical and pathological changes have been investigated by some workers from Saudi Arabia (Al-Akel and Shamsi, 2000).

Jeddah is the second largest city in Saudi Arabia located on the eastern coast of the Red Sea and is the major urban center of western Saudi Arabia (Magram, 2009). The city has a population of more than 3.5 million. With the population increase, the amount of sewage became a major problem as the capacity of the sewage treatment plants is largely insufficient and much of the raw sewage (~146,000 m^3/day, representing 12 tons of organic matter, PERSGA, 2006) is dumped into the coastal area creating a dramatic environmental impact (El-Rayis, 1990; Basaham, 1998; El-Sayed and Niaz, 1999; El-Sayed, 2002; Turki *et al*., 2002 and Al-Farawati, 2010).

Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and a reflection of the overall health of the entire population in the ecosystem (Mabika and Barson, 2014).

There have been numerous reports of histopathological changes in liver of fish exposed

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to a wide range of organic compounds and heavy metals (Abdel, 2012; Au D.W.T, 2004)

Various histological studies have been performed on different fish species in the Red Sea, Jeddah coast (Bin-Dohaish *et al*., 2004).

Al-Shoaibah area was chosen as the study site because it is "near pristine" aquatic ecosystems as apparently with a rich biodiversity of aquatic ecosystem and was chosen Al-Kumrah site as a polluted area because it is area an slow rate of water turnover and flow of sewage water which is released without any treatment.

The Al-Shoaibah area, therefore serves as an ideal reference site for the description of the histomorphological of various organs in different fish species sampled from an apparently unpolluted natural environment that could be a valuable reference for future toxicological studies.

Two economically important fish species were collected from the Al-Kumrah and Al-Shoaibah regions of the Red Sea. These included *L. harak* (Forsskål, 1775) and *T. jarbua* (Forsskål, 1775). They were chosen as sentinel species because they form an integral part of the small-scale commercial, subsistence and recreational fisheries in this ecosystem. Furthermore, many seawater fish species in the sites studied in particular, and in other aquatic ecosystems, are increasingly being used as bio-indicator species for the management of water quality. This is because fish are sensitive to their environment, are easily attainable and have a relatively long lifespan compared to other aquatic organisms, these factors allow for diagnosis of both acute and chronic effects of exposure to pollutants (Abdelrahim *et al*., 2011; Alturiqi and Albedair, 2012).

The general purpose of this study is to evaluate the effect of different pollutants on the histological structure to the liver, muscles and gills in fish (*L. harak* and *T. jarbua*) which collected from two locations (Al-Kumrah and Al-Shoaibah) in Jeddah District along Saudi Arabia Red Sea Coast at summer season 2014 to describe histological abnormalities potential.

To get information about the threat imposed by these spills and influents to these fish species and know whether it is safe for consumption.

MATERIALS AND METHODS

Study areas

Sampling sites were selected according to the polluted or unpolluted water concentrations detected during field surveys performed in summer 2013, where the samples were collected between December 2013 and September 2014.

Fish sampling

The fishes sampled were Black-spot emperor (*L. harak*) and Jarbua terapon (*T. jarbua*) collected from two sampling sites (Al-Kumrah and Al-Shoaibah).

The fishes were identified and collected with the assistance of a staff of the department of biological sciences, University of King Abdulaziz (KAU).

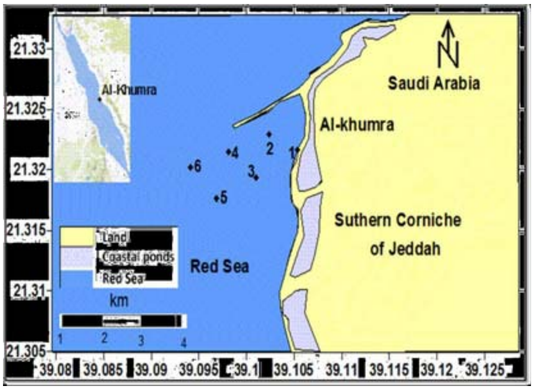

Fig. 1. Shows the Al-Kumrah area using geographic information systems program (GIS).

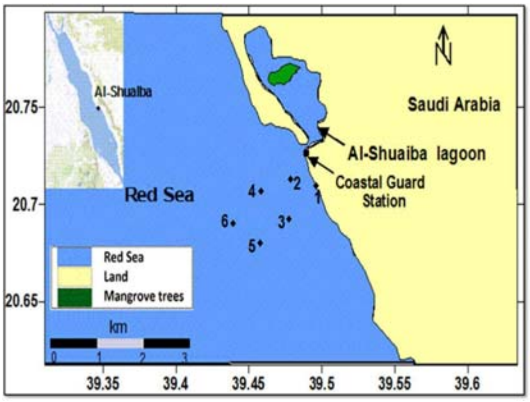

Fig. 2. Shows the Al-Shoaibah area using (GIS).

The description of fish species that were collected from different sites is shown in Table (1).

Histological examination of the tissues

Portions of the liver, gill and muscles of fishes were fixed immediately on removal from the fish in 10% buffered neutral formalin (BNF) for 72 hours at room temperature. The tissues were grossed on the gross board and placed inside stainless tissue cassettes.

The cassettes were arranged carefully inside the automated tissue processor. Thereafter the tissues were subjected to the following treatments:

Dehydration**:** to remove of extractable water from the tissue. It was achieved by placing the tissues in ascending grades of alcohol i.e. 50%, 60%, 75%, 80%, 90% and two changes of absolute alcohol, followed by clearing using xylene to remove alcohol, followed by impregnation involved passing the tissues through molten paraffin wax in order to remove xylene, the next step was embedding which was carried out using a wax dispenser. Vertical and horizontal sections were cut using the rotary microtome (5 mm). The last stage was staining and this was achieved by the haematoxylin/eosin (H/E) staining technique. Each sample was observed under light microscopy (Olympus BH-2 microscope) connected to a performed computer. A software known as Cell Sens Dimension within the computer provided images for a further analysis and each image was captured using a digital camera. Five images are taken from each slide. Each image corresponded to a slide viewed using an objective lens of 20x magnification for tissues structure overview and 40x magnification for a detailed tissues structures. An immersion oil was used for 100x magnification that allowed an easy detection of organelles.

The image analysis consisted of comparing tissues structures of liver, muscles and gills that were exposed to pollution with those tissues belonging to the unpolluted area. The objective of this analysis was to identify any histopathological alterations in the tissues.

Table 1. The ecological characteristics and recorded morphometric measures of examined fish species

Scientific Name	English, common name	Local Name	Feeding habitats	Model of life	No. of samples	Length (cm) mean±SE	Weight (g) mean±SE
L. harak	Black-spot Emperor, Thumbprint emperor	Shoara bunoqtah	Carnivore	Pelagic	66	27.3±1.05	218.6±4.39
T. jarbua	Jarbua terapon, Thornfish,Tigerfish	Garboa, makhtout	Carnivore	Epiplagic	66	27.4±0.88	207.7±8.23

SE: Standard Error

RESULTS

Liver

A photograph of the microscopic liver tissue for *L. harak* and *T. jarbua,* which collected from the studied area were represented in figures (3 - 6a,b).

Varieties of histological alterations were identified in the liver tissue of *L. harak* and *T. jarbua*, these alterations included vacuolated hepatocytes, fatty change, adipocytes, vacuolated foci, inflammatory response, degeneration of hepatocytes, sinusoidal dilation and frank necrosis were noted in fish specimen collected from Al-Kumrah site as shown in figures (5a,b - 6a,b).

The hepatic specimen revealed hyperplastic biliary epithelium cholestasis, periductal fibrosis and edema in some portal areas accompanied by focal necrotic areas.

Other hepatic tissue exhibited acute cell swelling or steatosis in the majority of the hepatic cells together with focal interstitial lymphocytic aggregations.

Sometimes, the hepatic sinusoids and blood vessels were dilated with the presence of telangectiasis and numerous melanomacrophages centers were seen scattered among of generated hepatic cells. Hepatopancreas acinus cells were inactive side presence of dilated blood vessels. Other portal areas contained mononuclear cells and fibro blast with probabilities to in vade the adjacent interlobular hepatic tissues.

Pyknosis and karyorrhexis of the nuclei are seen, the pancreatic acinus was degenerated, and the swelling of central vein which has caused happens blood lake.

Huge of kupffer cell by inflammations, chromatin collected behind nucleolus envelope and shrinkage. Liver showing focal hemorrhage, Dark brown pigments are noticed.

The liver tissue exhibited the most histologically alterations than any other selected target organs. Structural changes were identified only in fish samples collected from Al-Kumrah site.

Gill

A photograph of the microscopic gill tissue for *L. harak* and *T. jarbua* which collected from the studied area. A variety of histological alterations were identified in the gill tissue of *L. harak* and *T. jarbua*, these alterations included necrosis, epithelial lifting, telangiectasia, the proliferation of chloride cells, crash or disappears of primary lamellae, fusion and irregulation of secondary lamellae and hyperplasia of epithelial

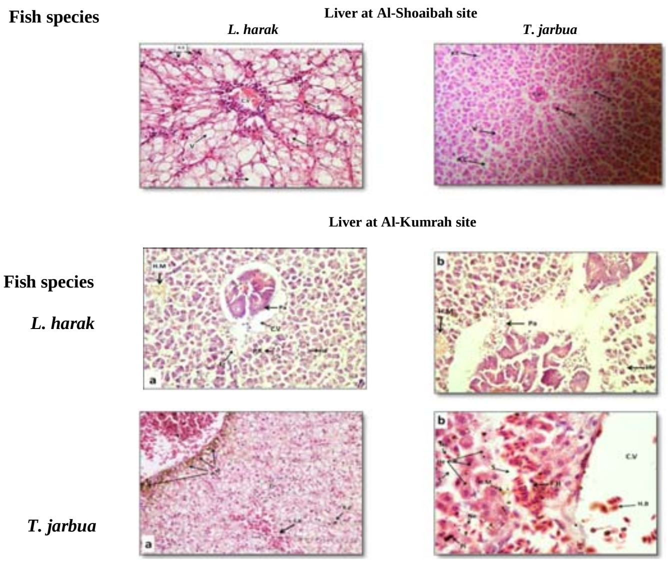

Fig. 3. Liver of fish L. harak collected from Al-Shoaibah site showing the apparently normal nearly, central vein (C.V), hepatocytes (He), sinusoid (S), vacuolation of hepatocytes of mainly fatty change represented by large clear vacuoles replaced almost all the cytoplasm (V), adipose cell (A.C), kupffer cell (K.C), H & E. (X 40).

cells and these structural changes were identified in fish samples collected from Al-Kumrah site.

Muscle

A photograph of the microscopic muscle tissue for *L. harak* and *T. jarbua* which collected from the studied area was represented in fig.[11 - 14]. Varieties of histological alterations were identified in the muscle tissue of *L. harak* and *T. jarbua*, these alterations included hyaline degeneration represented by swollen muscle fibers, the longitudinal section lack both cross and longitudinal striations, disappear intermediate disk and nucleus a lot of spaces between fiber, the bulge in connective tissue in muscle fiberboard and fragmentation of muscle fibers, these structural

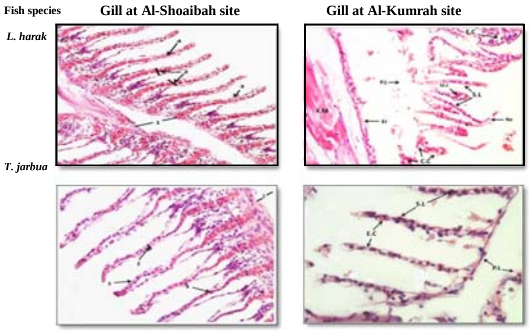

Fig. 4. Liver of fish T. jarbua collected from Al-Shoaibah site showing the apparently normal nearly, central vein (C.V), hepatocytes (He), sinusoid (S), vacuoles (V), adipose cell (A.C), kupffer cell (K.C), H & E. (X40).

changes were noted and identified in the fish specimen collected from Al-Kumrah site.

DISCUSSION

In the current study regressive changes, inflammatory changes and focal cellular alterations were identified. The histological responses in the **liver** were mostly associated with regressive changes. Regressive changes included vacuolation, fatty change, necrosis and intracellular deposits.

Vacuolated hepatocytes are associated with the inhibition of protein synthesis, energy, depletion, disaggregation in microtubule or shift in substrate utilization (Hinton and Lauren, 1990). This alteration along with fatty degeneration was observed in both fish species collected from Al-Kumrah site in this study. Vacoulation as well as fatty change, have been reported in previous studies (Muthukumaravel and Rajaraman, 2013; Chamarthi *et al.*, 2014; Van Dyk *et al.*, 2009).

Inflammatory responses were mainly noted in the fish specimen from Al-Kumrah site.

Necrosis as stated by Roberts, (1989) is where cellular damage is not immediately lethal and the changes are often reversible when the source of damage is removed. Necrotic changes, as well as Pyknosis and Karyorrhexis, have been reported in previous studies (Radhakrishnan and Hemalatha, 2010; Adams *et al*., 2010).

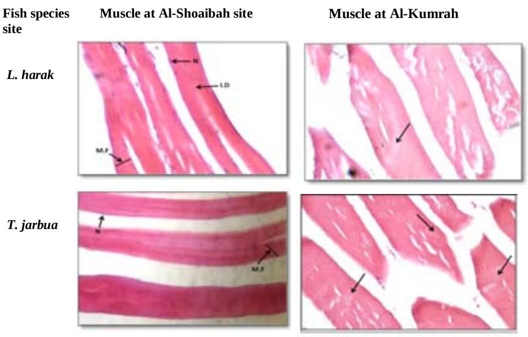

Fig. 5a,b. Photomicrograph of liver tissue showing histopathology in tissue of L. harak collected from Al-Kumrah site, a)Liver tissues with hepatopancreas showing individualization (Pa) and dissociation of hepatocytes (He), Pyknosis and Karyorrhexis of the nuclei are seen (P, K) (circle), the pancreatic acinus was degenerated (Pa) (circle), the swelling of central vein (C.V), caused by swelling for central vein happened blood lake (Er), appeared of heavy metals between the sinusoids (H.M), H & E. (X 400). b) Liver tissues with hepatopancreas showing congestion (Pa) beside degenerated pancreatic acinus and individualization of hepatic cells (He), numerous melanomacrophages (H.M), H & E. (X 40).

Degeneration of hepatocytes and sinusoidal dilation alterations were identified in both fish species collected from Al-Kumrah site in the study. Atamanalp et al. (2008) and Velmurugan et al. (2007) have previously reported the occurrence of this alteration.

Melanomacrophages alterations were identified in both fish species collected from Al-Kumrah site in the study. Marchand et al. (2009) have previously reported the occurrence of this alteration.

Generally, *L. harak* and *T. jarbua* specimens from the Al-Kumrah site showed a higher liver alteration occurrence than Al-Shoaibah site. Macroscopic observations support these findings as several liver abnormalities were observed in Al-Kumrah area.

The gills are sensitive indicators of environmental stress, including exposure to harmful compounds present in aquatic ecosystems because of anthropogenic activities (Hinton *et al*., 1992). The gills in fish are vulnerable to toxicants and irritants because they are in direct contact with the surrounding water and have a rich blood supply to pick up oxygen for respiration from the water (Roberts, 2001). Therefore, functional impairment of gills caused by pollutants can jeopardize the health status of the fish.

In the current study, histological alterations in varying degrees were identified in gill. These were mostly focal circulatory disturbances and progressive changes. Circulatory disturbances are related to pathological conditions of blood and tissue fluid flow. Epithelial lifting in focal areas was noted in both fish species collected from Al-Kumrah site in the study. Epithelial lifting is characterized by the detachment of epithelial cells due to the outflow of serous fluids into the interstices of gill tissue (Van Dyk *et al.*, 2009). This alteration has been observed in various other studies (Schmidt *et al.*, 1999; Fanta *et al.,* 2003; Monteiro *et al.,* 2008 and Boran *et al*., 2010).

Telangiectasis of the secondary lamellae was noted in both (*L. harak* and *T. jarbua*) fish species collected from Al-Kumrah site. This appearance of the secondary lamellae results from a collapse of the pillar cell system and the breakdown of vascular integrity with a release of large quantities of blood that pushes the lamellae epithelium outward (Alazemi, *et al.*, 1996). Telangiectasia has been reported in some fishes in polluted system or under exposed conditions (Saenphet *et al.*, 2009 and Van Dyk *et al.*, 2009).

Progressive changes identified in the study included hyperplasia of mucous and epithelial cells. Cengiz (2006) stated that gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and increase in the toxicant-blood diffusion distance. This defense mechanism takes place at the expense of the respiratory efficiency of the gills and eventually, the respiratory impairment must outweigh any protective effect against pollution uptake. Van Dyk et al. (2009) identified mucous hyperplasia in fish from a polluted stream while hyperplasia of the epithelium has been reported by several authors (Benli *et al.*, 2008; Camargo and Martinez, 2007 and Boran *et al.*, 2010).

Structural alterations in the form of lamellar fusion were also identified. This alteration has previously been identified in fish (Boran *et al.*, 2010; Benli *et al.*, 2008 and Chamarthi *et al.*, 2014) and polluted streams (Van Dyk *et al.*, 2009).

Proliferation and bulge of chloride cells were only identified in the *L. harak*. Pantung et al. (2008) and Chezhian et al. (2009) observed this phenomenon following exposure to cadmium and chemical factory effluent respectively.

On the basis of literature, these alterations could be related to exposure to various chemicals and poor water quality. However, these alterations could also be understood as a form of defensive mechanism against exposure to pollutants rather than as irreversible toxic effects.

Generally, *L. harak* and *T. jarbua* specimens collected from Al-Kumrah site showed a higher gill alteration occurrence than Al-Shoaibah site. Macroscopic observations support these findings as several gill abnormalities were observed in Al-Kumrah area.

The alterations in muscle has previously been identified in fish exposed to different pollutants by several authors (Fatma, 2009; Abbas and Ali, 2007; Sia Su *et al.* 2013 and Ramesh and Nagarajan, 2013)

Generally, *L. harak* and *T. jarbua* specimens collected from Al-Kumrah site showed a higher muscle alteration occurrence than Al-Shoaibah site. Macroscopic observations support these findings as several muscle abnormalities were observed in Al-Kumrah area.

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