Histopathological and ultrastractural alterations in skin, gills, liver and muscle of *Siganus canaliculatus and Epinephelus morio* caught from Jeddah and Yanbu coast as bio-indicators of oil hydrocarbons pollution

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Abstract: This study aims to investigate the histological alteration in skin, gills, liver and muscle as well the ultrastructure alteration in the liver of *Siganus canaliculatus (S. canaliculatus) and Epinephelus morio* (*E. morio*)caught from different sites at Jeddah and Yanbou coast as a bio-indicator for oil pollution. Skin, gills, Livers and muscle of ten fishes of a similar size of each *Siganus canaliculatus and Epinephelus morio* were used in this study. Tissues samples were immediately taken and prepared for histopathological and ultrastructure alterations investigation. Such alterations 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. Fishes collected from Jeddah were more polluted than that collected from Yanbu. The highest affected organ was the skin followed by the gills and liver, while the muscles were the lowest ones. Moreover *S. canaliculatus* fishes were seriously affected than *E. morio*. The *E. morio* is highly resistant than *S. canaliculatus*. The present study indicates that *S. canaliculatus* was highly affected and showed severe tissue alterations than *E. morio*. The skin, gills, hepatic and muscular histopathological and hepatic ultrasturctural changes were useful indicators or markers for investigation of oil hydrocarbon pollution.

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1. Introduction:

Jeddah and Yanbu Coast on Red Sea in Saudi Arabia have a high amount of shipping activity and oil spill in theirs waters. So all organism exposed to oil risk in these ecosystems. Petroleum hydrocarbon products are one of the most important pollutants that effect on aquatic organisms and they are the most opportunity for ecotoxicology studies (**Hesni et al., 2011; Moreira et al., 2014**). The assessing and prediction of the effects of petroleum pollution on aquatic environment are very urgent and important issue. Petroleum hydrocarbons and its products can enter the aquatic environment via different sources such as petroleum extraction, transportation; urban runoff biogenic hydrocarbons produced naturally, accidents involving and oil spills (**Adams et al., 2014**). Petroleum hydrocarbons are lipophilic in nature and can extremely uptake by a wide spectrum of marine animals and accumulate in lipoid spectrum of marine animals and accumulate in lipoid physiological and physiochemical membrane properties and can integrate into biological system (**Sikkema et al., 1994**). Many physiological processes such as enzyme function, muscle contraction and osmoregulation are directly dependent on the unique properties of biological membranes (**Marty et al., 2003; Vosyliene et al., 2005**). Some petroleum compounds are potential to be biomagnified through the food chain (**Biuki et al., 2013**).Petroleum pollutants tend to accumulate more in the organism than the environment; therefore fish are largely being used for the assessment of the quality of aquatic environment and can be used as bio-indicator to evaluate the environmental contamination levels (**Copat et al., 2012, Wang et al., 2014**). Histological biomarkers were used for assessing the effect of petroleum hydrocarbons in aquatic organisms (**Hesni et al., 2011; Biuki et al., 2013**). Histological study is a rapid method for detection of pollutants effects on various tissues of fish and it has been extensively used to determine the deleterious effects of hydrocarbons (**Ortiz-Delgado et al., 2007; Barja-Fermandez et al., 2013; Moreira et al., 2014**). Gills are also very important in respiration, acid–base balance, osmoregulation and excretion of nitrogenous wastes in fish (**Evans et al., 2005)** and they include the greatest surface area of the aquatic organisms in contact with external environment. They are an important way of uptake of pollutant into the organism, thus the gills are the first site where petroleum hydrocarbon-induced lesions may occur. Therefore, their morphology can be very useful as a bioindicator in environmental evaluation **(Schwaiger et al., 1997).** The liver is a main detoxification organ and has two essential physiological roles for the basic metabolism and the accumulation, biotransformation and excretion of toxic substances in fish (**Abdelaziz et al., 2006; Bin-Dohaish, 2012; Biuki et al., 2013**). The harmful effect of pollutants on fish liver histology may depend upon the duration of the exposure (chronic or acute) and the concentration level of the specific pollutants as well as other factors such as temperature, age of fish, interaction with other pollutants, water chemistry and metabolic activity of the fish (**Health, 1995; Agamy, 2012**). The cardiac and skeletal muscles showed morphological changes with reduction in the cardiac output and severe contraction and muscle damage (**Ahmed et al., 2014**).

The present study aims to investigate the skin, gills, liver and muscular histopathological and hepatic ultrastructure alterations in *Siganus canaliculatus and Epinephelus morio* caught from Jeddah and Yanbu coast, as a bio-indicator for detection and evaluation of oil hydrocarbon pollutants

2. Materials and Methods

Study Areas

Sex sampling sites were selected along the KSA red sea coast at Jeddah and Yanbu provinces, four contaminated sites and two reference areas, as previously described **(Nadia et al., 2007)** at Jeddah coast site: I reference site, II north of Jeddah Islamic seaport and III, in front of petromine refinery and at Yanbu coast site: IV reference site, V collected close to Yanbu industrial harbor and VI close to oil refineries and petrochemical factories (table 1).

Table 1 GPS-Positions for the studied areas according to Google earth map

Sampling area			N: Position	E: Position
Jeddah	Reference site	I	21.222237	39.011746
	Contaminated sites	II	21.471859	39.162570
		III	21.440159	39.167977
Yanbu	Reference site	IV	24.131032	37.958420
	Contaminated sites	V	23.931984	38.291037
		VI	23.957381	38.212135

Sampling and Analytical Procedure

Ten fishes of a similar size of both *Siganus canaliculatus and Epinephelus morio* were collected from each studded area from overnight pre-held pots during mid of March 2014. Length and weight of each sample were recorded.

Histopathological examination

Skin, gills, Liver and muscle samples were immediately taken, fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes (**Bancroft and Gamble, 2008**) and then examined microscopically.

Electron microscope examination

Liver samples were fixed in 6.25 % cacodylate buffer gluteraldehyd followed by 1% osmium tetraoxide. After dehydration, the specimens were embedded in poly-ethylene capsules containing the embedding mixture (Epon mixture and hardener).Ultra-thin sections were prepared and stained by Uranyl acetate and lead citrate and examined by transmition electron microscope (JEM- 1011, JEOL, Japan) (**Weakly, 1981**).

3. Results

skin, gills liver and muscle histopathology

The reported lesions in the skin, gills, liver and muscles of E. *morio and S. canaliculatus* were serious in fishes collected from the polluted sites either from Jeddah or Yanbu when compared with their corresponding fishes collected from the reference sites, which were nearly normal. Such lesions were severe in Fishes collected from Jeddah than that collected from Yanbu. Moreover, *S. canaliculatus* fish showed high in scored lesions than *E. morio*. The **skin** showed variable lesions from mild increase in the mucous cells to erosions and necrosis in the epidermis besides round cells infiltrations in the whole epidermal thickness. Subepidermal edema and few fibroblast proliferations were noticed. Hyalinization of the basement membrane and melanomacrophages were also observed in some cases (**Fig 1**).

The **gills** revealed slight congestion, focal hemorrhage and edema. The covering epithelium of the secondary lamellae were slightly swollen, proliferated, or desquamated with fusion of the filaments and round cells infiltrations. Sometimes, the gill filaments were necrotic and replaced with round cells or eosinophil granular cells (EGCs) aggregations. Numerous mucous cells were detected with all examined cases (**Fig 2**).

The **liver** showed vacuolation of lipid accumulation and hydropic degeneration types to scattered areas of necrosis presented by pyknosis and karyorrhexis with cholangitis of proliferated bile ducts epithelium, leukocytes infiltrations and brownish bile pigment. Pale eosinophilic material was seen in the hepatic spaces and scattered throughout the hepatic parenchyma (spongiosis hepatis). Some hepatocytes showed enlarged nuclei with prominent nucleoli (megalocytes). Congestion of hepatoportal blood vessels, intravascular hemolysis, hemorrhage and intravascular vacuolated leukocytes were visualized besides perivascular EGCs and round cells aggregations. Hyalinization of the tunica media of hepatic blood vessels and interstitial lymphocytic aggregations were seen (**Fig 3**). However, the **skeletal muscle** revealed minimal lesions which reflected the minimal effect of TPH on the muscles. Slight edema, hyalinization and rarely necrosis of some muscle fibers, were seen besides few to focal aggregation of round cells among degenerated or necrotic muscle fibers (**Fig 4**).

Liver ultrastructural alterations

The hepatocytes of fishes collected from reference sites were normal with numerous mitochondria, rough endoplasmic reticulum, Golgi apparatus, glycogen granules and few fat lipid globules. Sometimes, the hepatocytes showed minimal cytoplasmic degeneration with swollen mitochondria and rough endoplasmic reticulum. The blood vessels were dilated with slight separation in the neighboring cells. However, the hepatocytes in the liver of fishes collected from the polluted sites (II, III, V and VI) showed variable degrees of vacuolation and lysis of the cytoplasm (rarefaction) with complete disappearance of the mitochondria, Golgi apparatus and glycogen besides vesiculation of the rough endoplasmic reticulum. The nucleus showed marked reduction in the size and condensation of chromatin (pyknosis), fragmentation of nuclear chromatin (karyorrhexis) and karyolysis. Detaching of nuclear membranes, dislocation of nucleolus, clumping and condensation of chromatin in the shape of an irregular homogenous ring of a snowflake form with peripheral migration into the cytoplasm were also observed. The cytoplasm of some hepatocytes showed irregular-sized vacuoles containing ring or round bodies. The latter were given the shape of polycyclic aromatic hydrocarbons. The pancreatic cells were necrotic and depleted from zymogene granules with pyknotic nuclei (**Fig 5**).

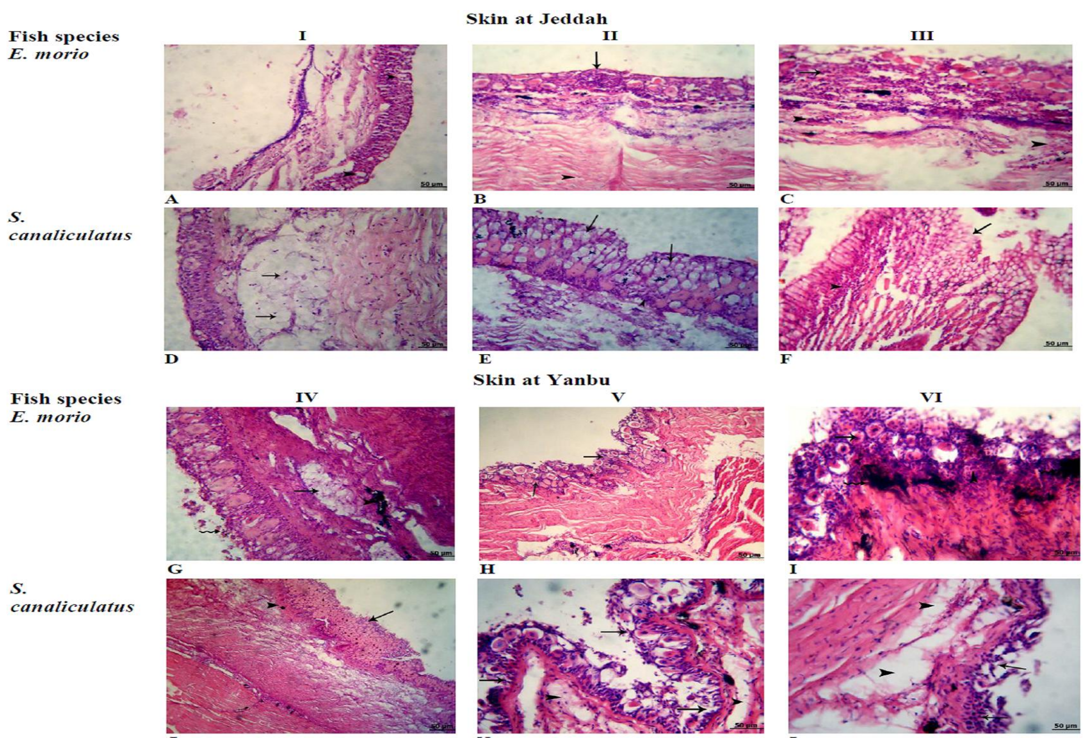

Fig 1: Skin of examined fishes (HE x Scale bar = 50 µm)

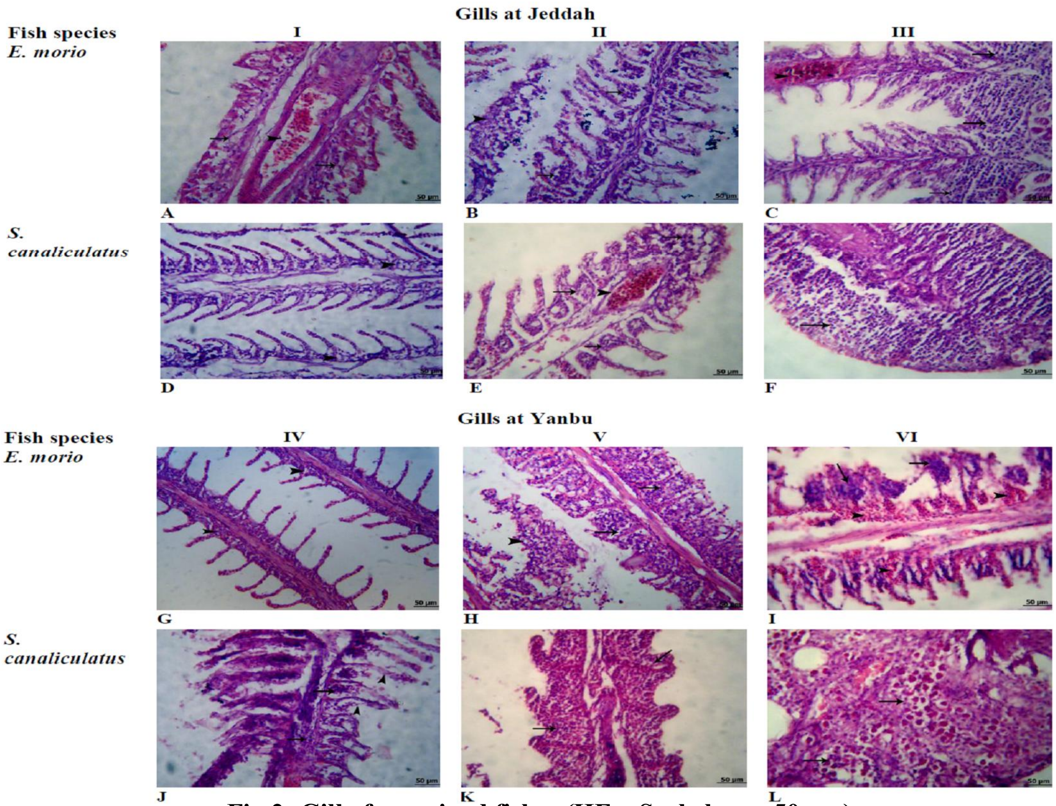

Fig 2: Gill of examined fishes (HE x Scale bar = 50 µm).

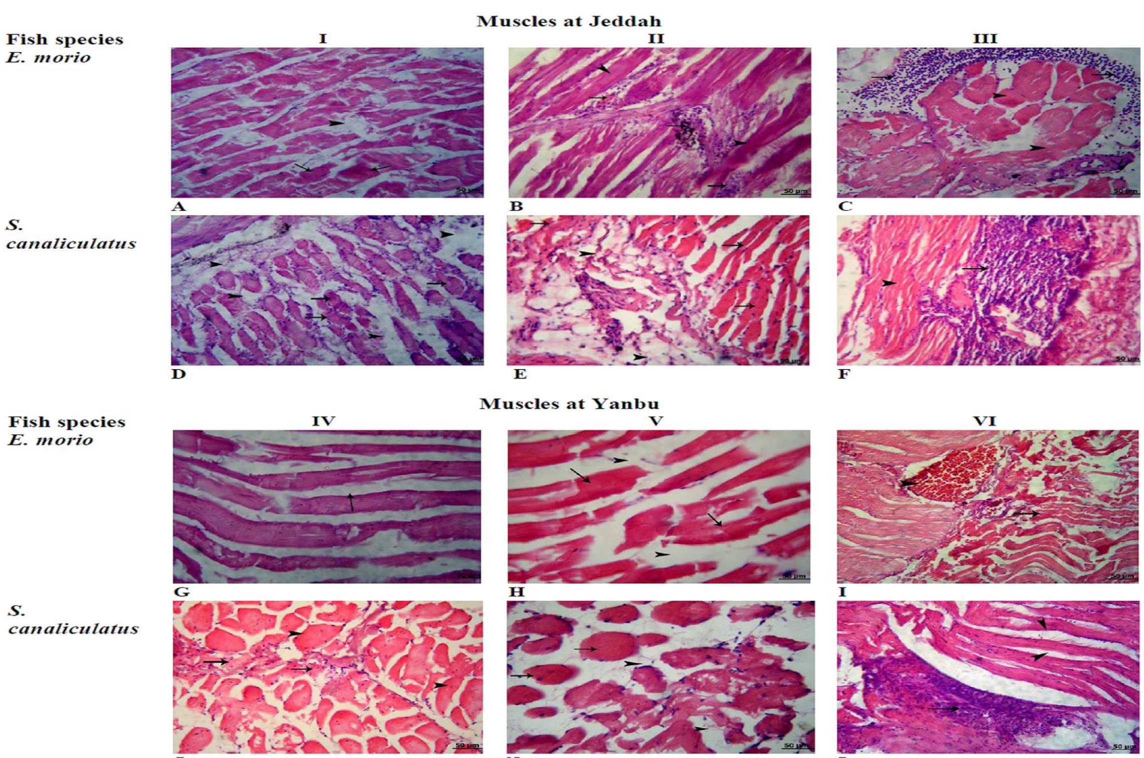

Fig 3: Liver of examined fishes (HE x Scale bar = 50 µm)

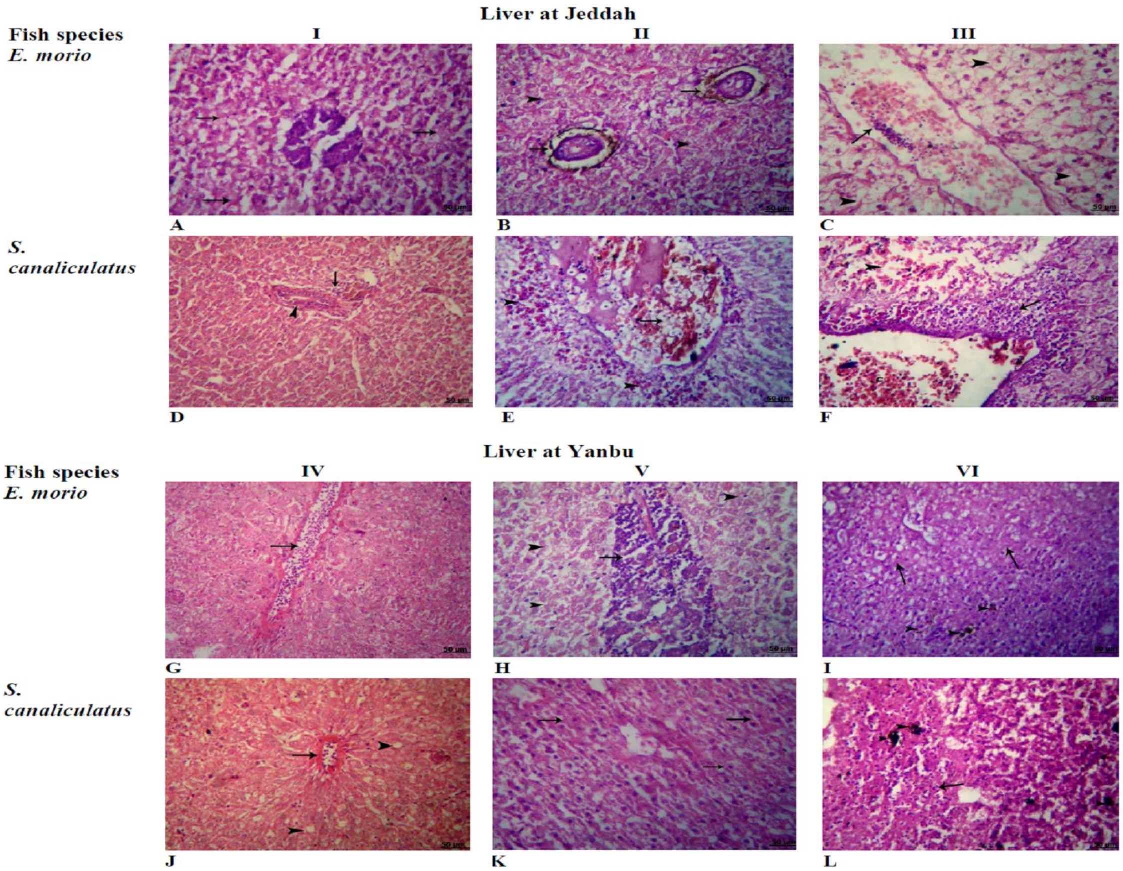

Fig 4: Skeletal muscle of examined fishes (HE x Scale bar = 50 µm)

4. Discussion

Petroleum hydrocarbons include a wide variety of materials, including crude oils, various refined products (kerosene, gasoline, mineral seal oil, and naphtha), natural gas, and liquefied petroleum gas. These materials are complex mixtures of numerous hydrocarbons and various contaminants and additives. Crude oils collected from different sites contain various types and proportions of hydrocarbons. Thus, comparisons of toxicity of various petroleum hydrocarbon materials must be made cautiously **(Clark and Brown, 1997)**. Therefore the assessment of the risk of any oil spill is important. In the present investigation, the TPH was assessed in the water, sediments, and fish-tissues which were collected from Jeddah and Yanbu coast. The range values observed in the present study are higher than the reported concentration in references. They were ranged from 6.2-20.1 µg.L-1 (in sea water), 130-212 mg.K-1 (in sediment) and 140-720 µg.K-1 in fish- tissues. The skin has the highest value followed by gills, and liver while the muscle was the lowest one. Similar findings were in agreement with many authors (**Ansari et al., 2012**).

The histopathological findings in the different fish tissues (skin, gills, liver and muscles) revealed relative risks for most toxipathic lesions which were significantly higher in collected fishes from polluted sites at Jeddah (II and III) and Yanbu (V and VI) compared with the references (I and IV), respectively. Moreover, S. *canaliculatu*s were highly susceptible to such lesions than *E. aeronaut*s. In our study, the histopathological changes in fish exposed to TPH were generally more severe in Jeddah than changes found in Yanbu exposed fish. Similar finding was obtained by (**Abdelaziz et al 2006).**

The skin lesions were represented by an increase in the mucous cells to erosions and necrosis in the epidermis besides round cells infiltrations in the whole epidermal thickness. Subepidermal edema and few fibroblast proliferations were noticed. Hyalinization of the basement membrane and melanomacrophages infiltrations were also seen. These lesions may be due to the direct contact of crude oil fractions to the skin with persistent bacteria which could induce inflammatory response (**Rudolph et al., 2001**). **Das and Kazy (2014)** isolated several bacteria from fish exposed to TPH including Paenibacillus, Micrococcus, Brachybacterium, Aerococcus, Zimmermannella, Pseudomonas and Pseudoxanthomonas.

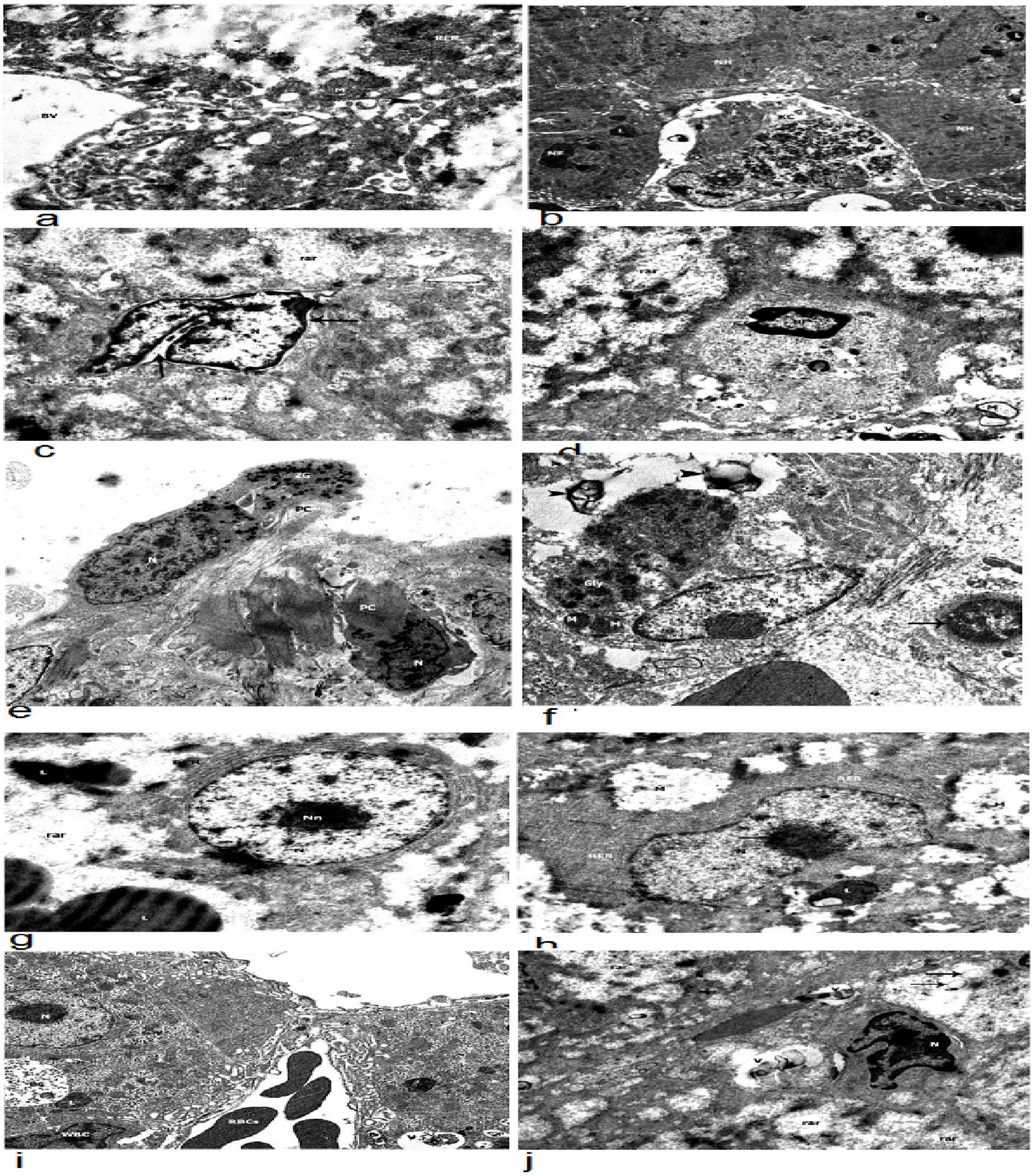

Fig 5: TEM of Liver (Uranyl acetate and lead citrate x 5850) shows: 1- Jeddah sites. Blood vessel (BV) and hepatocytes with normal microvilli at the cell junctions (arrowheads) (reference site, a), kupffer cells (kc) with large vacuole (v) (site II, E. morio, b), irregular nucleus with heterochromatin and separated nuclear (arrows) besides areas of cytoplasmic degeneration (rar) (site III, E. morio, c), necrotic hepatocytes with karyorrhexis (NF) and the adjacent hepatocytes showed degeneration with disappearance of the mitochondria (M) and rough endoplasmic reticulum (RER) (site II, S. canaliculatus, d), necrotic pancreatic cells with depletion in zymogene granules adjacent normal one (site III, S. canaliculatus, e), hepatocytes with pyknotic nucleus (arrow) and other showed large vacuole containing round bodies (arrowheads) (site III, S. canaliculatus, f). 2- Yanbu sites. Hepatocytes with slight cytoplasmic degeneration (rar) and lipid infiltration (L) with persistent RER around the nucleus (site V, E. morio, g), swollen and crystilysis of mitochondria (M) and few lipid infiltration (L) with persistent RER (site VI, E. morio, h), congested and dilated blood vessel and normal hepatocytes (site V, S. canaliculatus, i), necrotic hepatocytes with irregular nucleus and heterochromatin, vesiculation of RER (arrows) and cytoplasmic vacuole containing round bodies (site VI, S. canaliculatus, j).

The gills showed congestion, focal hemorrhage and edema. The covering epithelium of the secondary lamellae were slightly swollen, proliferated, or desquamated with fusion of the filaments and round cells infiltrations. Sometimes, the gill filaments were necrotic and replaced with round cells or eosinophil granular cells (EGCs) aggregations. Activation of the mucous cells was detected. These findings are in accordance to (**Schwaiger et al., 1997**). The gills are also very important in respiration, acid–base balance, osmoregulation and excretion of nitrogenous wastes in fish (**Evans et al., 2005; Dessouki et al., 2013**) and they include the greatest surface area of the aquatic organisms in contact with external environment. They are an important way of uptake of pollutant into the organism, thus the gills are the first site where petroleum hydrocarbon-induced lesions may occur. However, the skeletal muscle revealed minimal lesions which reflected the minimal effect of TPH on the muscles. Slight edema, hyalinization and rarely necrosis of some muscle fibers, were seen besides few to focal aggregation of round cells among degenerated or necrotic muscle fibers. Meanwhile, the liver alterations were included vacuolation of lipid accumulation and hydropic degeneration types to scattered areas of necrosis presented by pyknosis and karyorrhexis with cholangitis of proliferated bile ducts epithelium, leukocytes infiltrations and bile pigment stagnation. Sometimes, these vacuoles in the cytoplasm showed pale eosinophilic material (spongiosis hepatis) and with enlarged nuclei with prominent nucleoli (megalocytes). Congestion of hepatoportal blood vessels, intravascular hemolysis and vacuolated leukocytes were visualized besides perivascular EGCs and round cells aggregations. The aforementioned lesions could be classified into: circulatory, degenerative and proliferative alterations.

The circulatory alterations such as congestion, intravascular hemolysis and hemorrhage besides intravascular vacuolated leukocytes which altered blood flow that are easily reversible and do not alter the normal functioning of the tissue. Similar histological lesions have been reported in fish from contaminated sites and were considered indicative of the impact of the oil on the health of these fish (**Alkindi et al., 1996; Marty et al., 2003**).

The degenerative alterations are given the highest importance factor because they are considered a direct effect of toxicants, they are generally irreversible, and their persistence or progression may lead to a partial or total loss of organ function. The major degenerative changes included necrosis, lipid accumulation, nuclear degeneration, bile stagnation, cholangitis, megalocytosis, and spongiosis hepatis. Similar observations were reported by **Biuki et al** (**2013**). Lipid infiltration involving the formation of lipid-engorged cytoplasmic vacuoles in hepatocytes is a common response to chemical exposure in fish (**Marty et al., 2003**). Several laboratory studies have documented increased hepatic lipids after oil exposure, whereas other studies have shown decreased hepatocellular lipids after oil exposure (**Marty et al., 2003; Wolf and Wolfe, 2005**). Cholangititis with inflammatory response and biliary stagnation (yellow or brown pigments) were associated with exposure to hydrocarbons, and the utility of this phenotype as a histological biomarker for xenobiotic exposure is well established (**Stehar et al., 1998**).

The proliferative alterations may not be reversible, depending on the severity and extent of the alteration. Such alterations of the exposed fish livers in the present study included bile duct proliferation. Proliferation of bile ducts (ductal hyperplasia) has been observed in the livers of fish from contaminated regions (**Balch et al., 1995; Jung et al., 2009**). In our study, the TEM could illustrate the circulatory and degenerative alterations in the hepatocytes with complete disappearance of the mitochondria, Golgi apparatus and glycogen besides vesiculation of the rough endoplasmic reticulum. The nucleus showed marked reduction in the size and condensation of chromatin (pyknosis), fragmentation of nuclear chromatin (karyorrhexis) and karyolysis. Detaching of nuclear membranes, dislocation of nucleolus, clumping and condensation of chromatin in the shape of an irregular homogenous ring of a snowflake form with peripheral migration into the cytoplasm were also observed. Vacuolation and lysis of the cytoplasm besides different degrees of lipid accumulation, phagocytic vacuoles and round bodies were seen. The latter were given the shape of polycyclic aromatic hydrocarbons (**Weinstein et al., 1997**).

5. Conclusion

The present study indicates that *S. canaliculatus* was highly affected and showed severe tissue alterations than *E. morio*, Jeddah coast was more polluted than Yanbu coast. The histopathological and ultrasturctural changes were useful indicators for investigation of oil hydrocarbon pollution.

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Conflicts of Interest

The authors declare no conflict of interest.

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