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Gill Histopathological Effects of PAHs on Adult Pearl Oyster, *Pinctada radiata* at Al-Khiran Coast in Kuwait

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Abstract

The hazardous effect of water pollution on the histopathology of gill organ of the pearl oyster, *Pinctada radiata* was studied with respect to polycyclic aromatic hydrocarbon contamination in Al-Khiran coast at Kuwait. Thirty oyster samples from each site, site one where dead oysters were located, site two which is two kilometers away from site one and the control site which is ten kilometers away from site one were collected from Al-Khiran area where a massive number of dead oysters were reported. The gills of oysters were immediately removed and transferred to Bouin's solution for fixation and then processed for sectioning, staining and mounting and gill tissues were ready for examination. Histopathological changes in gills of oysters exposed to polycyclic aromatic hydrocarbons in site one and site two included necrosis and edemas of branchial lamellae, complete degeneration of gill filaments, loss of regular shape and haemolysis, and inflammation. Gill tissues of oysters from the control site had normal appearance. The study showed a clear evidence that PAHs caused severe histopathological changes in gills of pearl oyster *Pinctada radiata*.

Keywords

Gills, Histopathology, PAHs, Oysters, *Pinctada radiata*, Kuwait

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are non-ionic hydrophobic contaminants present in coastal areas as a result of natural or anthropogenic activities. PAHs are organic compounds which are composed of two or more fused aromatic rings, are a class of toxic, priority environmental contaminants [1]. PAHs with 2 to 4 benzene rings may be non-carcinogenic or carcinogenic and include:

naphthalene, acenaphthene, anthracene, phenanthrene, acenaphthylene, fluorine, fluoranthene, and pyrene. Carcinogenic PAHs with 4 to 6 benzene rings include: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo [a]pyrene, indeno(1,2,3-c,d)pyrene, dibenzo[a,h]anthracene, chrysene, and benzo[g,h,i]perylene. Some of these PAHs are portable human carcinogen. Many PAHs cause cancers, affecting a variety of tissues. In PAH class, 16 compounds in potable water and waste waters and 22 compounds in soil and solid wastes are listed as priority pollutants by EPA [1] [2]. The higher molecular weight PAHs are less water soluble and persist in the environment longer which will increase the toxicity to some aquatic species such as bullhead catfish [3]. Detrimental impacts on aquatic organisms caused by PAHs were detected [4]. Amphibians showed deformities and developmental delays when exposed to coal tar which contains PAHs [5]. Phenanthrene, fluoranthene, and benzo[a]anthracene were found in ant and lizard (*Acanthodactylus scutellatus*) samples collected from the Greater Al-Burgan oil field at Kuwait [2]. PAHs resulted from oil pollution affected morphological measurements in *A. scutellatus* lizards, in which, adult male but not female lizards were generally bigger at the polluted sites compared to control sites [6]. PAHs present in tissues of lizards at oil polluted sites have field behavior and morphology of the sand lizard *A. scutellatus* [7] [8]. Laboratory studies applied on the fringe-toad lizard *A. scutellatus* which were affected by PAHs, and were collected from polluted sites that exhibited black substrate showed a clear preference for darker substrate compared to lizards collected from control sites that preferred light substrate [9]. Exposure to PAHs resulted from oil pollution may cause increased accumulation of contaminants and may lead to severe liver pathology in living organisms as confirmed in *A. scutellatus* from Kuwait [10]. There was a clear evidence that, PAHs detected in sea water, sediment and oyster tissues are one of the reasons for catastrophic oyster (*Pinctada radiata*) mortality at Al-Khiran beach in November 2013 [11].

Studies on histology and histopathology have proved to be a very useful tool in assessing the pollutant induced injury to whole animal. Various pollutants caused pathological changes in the gills of some species of bivalves [12]. Because gills come into immediate contact with water and play important role in respiratory processes in oysters, therefore, studying histopathological effects on gills will provide valuable information about organisms exposed to PAHs. The histology of chemically induced pathological changes offers an excellent opportunity to determine the exact location where the toxicant is acting [13]. Some body organs such as gills, mantle and digestive diverticula are target organs for the uptake of toxicants of different kinds [14]. Gill tissues will concentrate the pollutants contained in water due to high volume of water that they filter [15]. In applying ecological monitoring, biomarkers selected should be sensitive and responsive to pollutant concentrations and environmental changes. Gill organ is sensitive to chemicals in water and its structure of filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water. Histopathological studies including the structural damage of tis-

sues or organs are important parts of pollution toxicology. Aquatic organisms such as pearl oysters are easily susceptible to the toxic environmental contaminants. These toxic effects can be understood and identified with the help of histopathology. Histopathological analysis provides information about the general health of the animals and contaminant-specific changes in the tissues [16]. Bivalves have a low or undetectable activity of those enzyme systems that metabolize many toxic chemicals such as PAHs. Bivalves such as pearl oysters are used in monitoring studies in the marine environment due to their ability to concentrate pollutants at high levels [17] [18]. They are commercially valuable sea food species; thus measurement of chemical contamination is of interest for public health considerations [19] [20]. PAHs accumulated by marine animals interact with cells and tissues to produce a variety of lesions. They bind to the surface of cell membranes and interfere with cell membrane mediated biological processes [21] [22]. Many PAHs are irritants and cause localized inflammatory responses in mantle and gill tissues in oysters [23]. Bivalve species such as mussels, clams, and oysters accumulate PAHs more than other aquatic organisms [3].

The present study focused on collecting samples of oyster, *Pinctada radiata*, from Al-Khiran coast where massive mortality of oysters was reported in November 2013. Sample stations were chosen according to locations of dead oysters (site one) and the surroundings, two kilometers around this area (site two). The control samples were about ten kilometers away from the dead oyster locations. This study was conducted to detect histopathological effects on gills of oysters from Al-Khiran area which was found to be contaminated with PAHs that caused massive mortality of oysters [11].

2. Methodology

Oyster samples (*Pinctada radiata*) were collected during November 2013 from two sites of Al-Khiran coast after the massive numbers of dead oysters were recorded. The control site was chosen ten kilometers away from the dead oyster locations. The collection of oyster samples was performed by SCUBA divers. The *P. radiata* samples were chosen randomly to represent the population and to serve as indicators of the effects of chemical contamination in marine animals.

Thirty oysters from each site (site one where dead oysters were located, site two which is two kilometers away from site one, and the control site that was ten kilometers from site one) were sampled. Oysters were chosen to have the same weight, length and age as much as possible to minimize the variations due to age and weight differences between the samples.

The gills of oysters were immediately removed and transferred to Bouin's solution for fixation. To complete fixation, the gills were immersed in the same fixative that had been used for each main piece and were kept in vials and placed on a rotator (2 rpm) (Taab Laboratory Equipment Ltd., Aldermaston, Berkshire, UK) for 24 - 48 h at room temperature. After fixation, the gills were washed overnight in 70% ethanol, dehydrated in 90% ethanol overnight followed by two changes for 3 h each of absolute alcohol (Fluka Wacker Chemie GmbH, Munich,

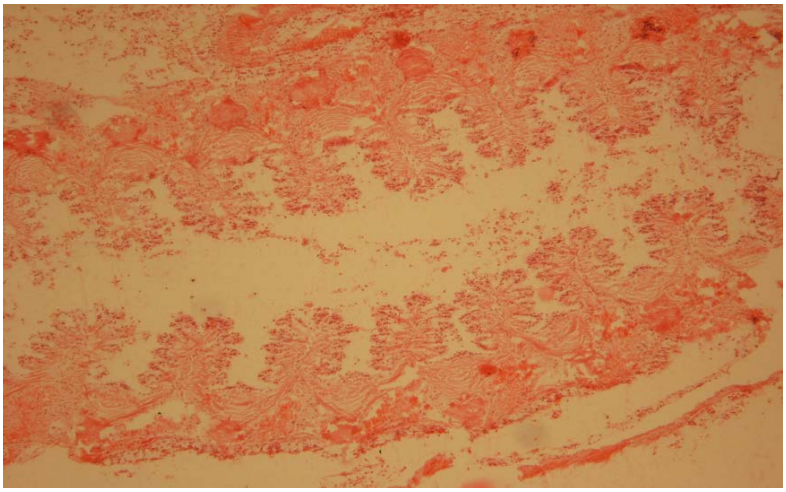
Germany). The gills were then cleared in toluene (Fluka, Wacker Chemie GmbH, Munich, Germany) for 24 h at room temperature. The gills were incubated in fresh liquid paraffin wax (Fluka Wacker Chemie GmbH, Munich, Germany) and kept in an oven at 60 1C overnight to allow the wax to penetrate the tissues. After that, they were embedded in plastic molds ready for sectioning. Once the blocks had been prepared for sectioning, they were fixed on a rotary microtome (Leica, Laboratory Talk, Knowlhill, Milton Keynes, UK). Paraffin sections were cut at 5 mm thickness in the form of a ribbon. Good sections were selected using a brush to place them in a water bath at 45˚C for flattening. The sections were then placed on albuminized slides and dried on a hot plate. The slides were dewaxed using two 5 min changes of xylene (Fluka Wacker Chemie GmbH, Munich, Germany). The specimens were rehydrated by two changes of absolute alcohol, followed by 90% ethanol and then 70% ethanol. All changes were of 2 min duration. The slides were washed with tap water for 2 min, followed by immersion in haematoxylin (Shandon Scientific Ltd., Cheshire, UK) for 5 min to stain the nuclei of the tissue cells. Excess stain was removed by washing the slides for 2 min in water before bluing them with ammoniated alcohol and re-rinsing in water. Specimens were then kept in 70% and 90% ethanol, respectively, for 2 min. To stain the cytoplasm, the slides were transferred to eosin (Shandon Scientific Ltd., Cheshire, UK) for 3 min, then rinsed in absolute alcohol and were dehydrated by two successive absolute alcohol immersions of 2 min each. They were then cleared with two changes of xylene each for 5 min. To preserve the specimens, the slides were mounted using 1 - 2 drops of a mixture of distyrene, a plasticizer, and xylene (DPX) (Fluka Wacker Chemie GmbH, Munich, Germany) while it was wet and sealed by a cover slip. The specimens were dried by keeping the slides on a hot plate for 5 - 10 min. Finally, the sections were examined under the light microscope with different objective lenses, and photomicrographs of areas of interest were taken.

3. Results and Discussion

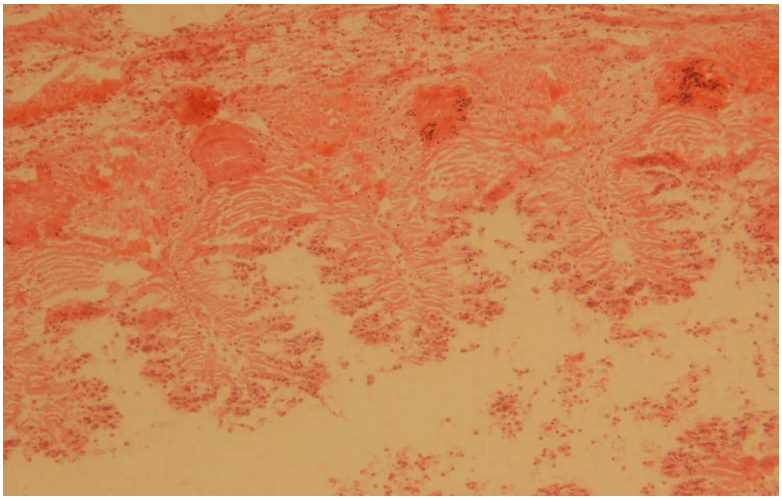
The normal gills of pearl oysters have two long branchial sheets. Each sheet is folded back on itself to create a descending and ascending lamellae forming four folds (demibranchs or plates). Along the entire length the gill runs the interlamellar space, which in places is divided by the interlamellar partitions or septae into a series of vertical compartments called the water tubes (**Figure 1(a)** and **Figure 1(b)**). Gills of oysters are directly exposed to water current taken into the body during the process of feeding and respiration. Therefore gills accumulate large amount of petroleum hydrocarbons [24].

The histopathological changes in gills of the pearl oyster, *P. radiata*, exposed to PAHs in site one and site two included mainly necrosis and edemas of branchial lamellae (**Figure 2**).

Complete degeneration of gill filaments, loss of inter-filamental junctions, loss of regular shape and haemolysis were realised (**Figure 3**). There was inflammation (**Figure 4**) accompanied by degeneration and necrosis. There were fusions



(a)



(b)

Figure 1. Normal gill of *P. radiata* at the control site showing normal appearance of lamellae and filaments. (a) Lower magnification; (b) Higher magnification.

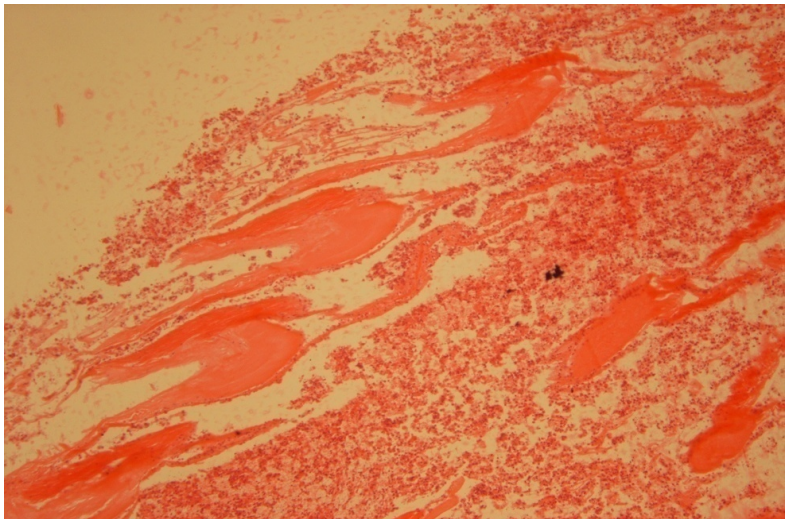


Figure 2. Necrosis and edemas of branchial lamellae in gills of oysters from site one and site two exposed to PAHs.

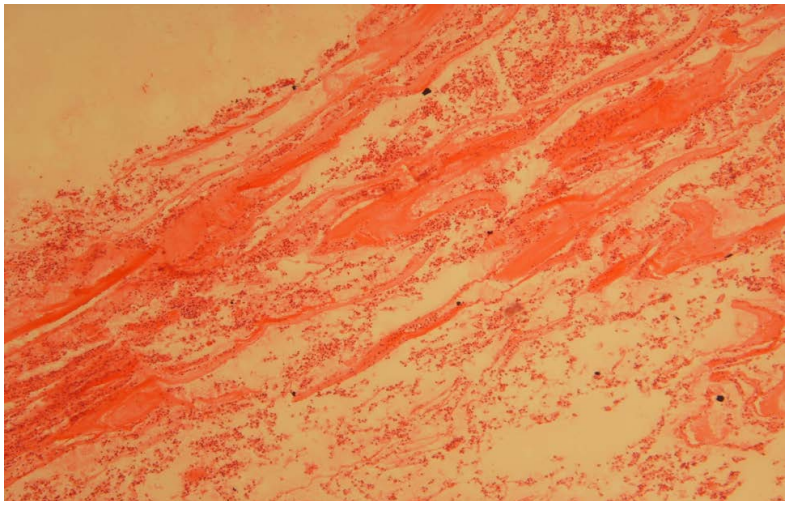


Figure 3. Complete degeneration of gill filaments, loss of inter-filamental junctions, loss of regular shape and haemolysis in gills of oysters exposed to PAHs in site one and site two.

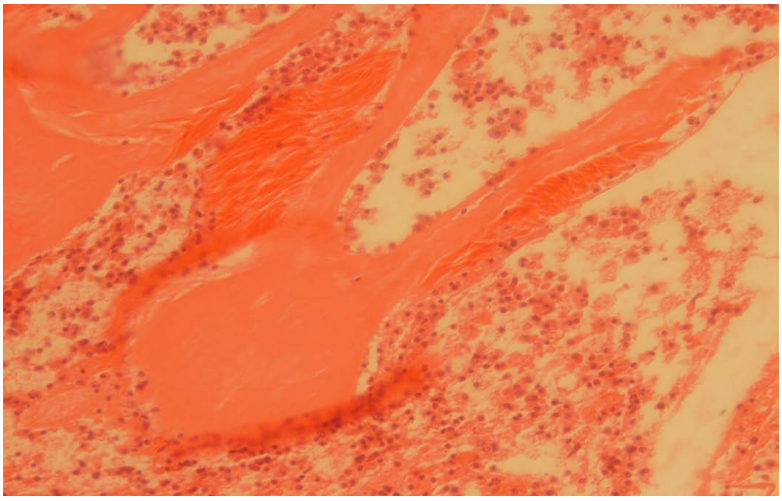


Figure 4. Inflammation accompanied by degeneration and necrosis in gills *P. radiata* exposed to PAHs in site one and site two.

of the gill filaments in oysters from site one and site two (**Figure 5**). Gill filaments showed severe disturbance of the ciliated epithelial cells compared to oyster gills from the control site (**Figure 6**).

The use of histopathological technique to investigate the effect of pollution provides a useful tool to analyze the cellular response to various toxicants such as PAHs. Severe necrosis of gills was reported in the bivalve, *Mya truncata* as a result of oil exposure [25].

N-nitroso compounds caused loss of lateral cilia and cells from the chitinous rods of gill filaments of some mussels [26]. Disruptive histopathological changes in gills of some crustaceans exposed to petroleum hydrocarbons have also been reported in marine animals [27] [28].

In the present study, gills of the studied oyster, *P. radiata* showed necrotic, degenerative changes in gill lamellae and filaments, edema in gill filaments, and

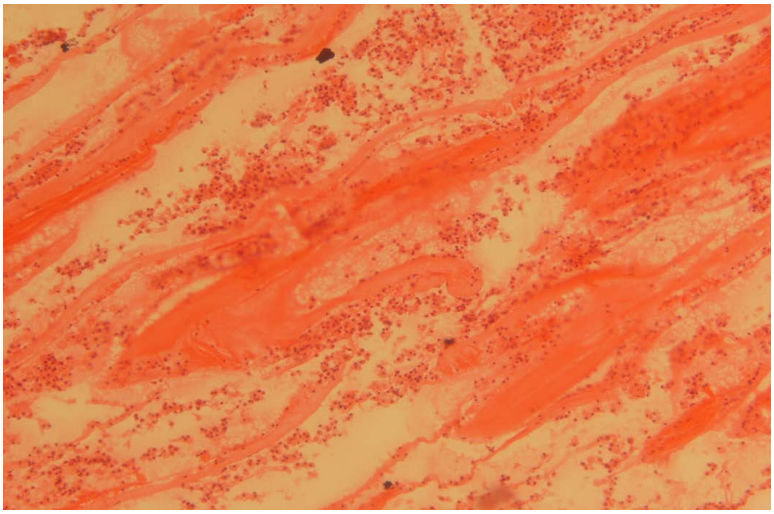


Figure 5. Fusions of the gill filaments in oysters from site one and site two exposed to PAHs.

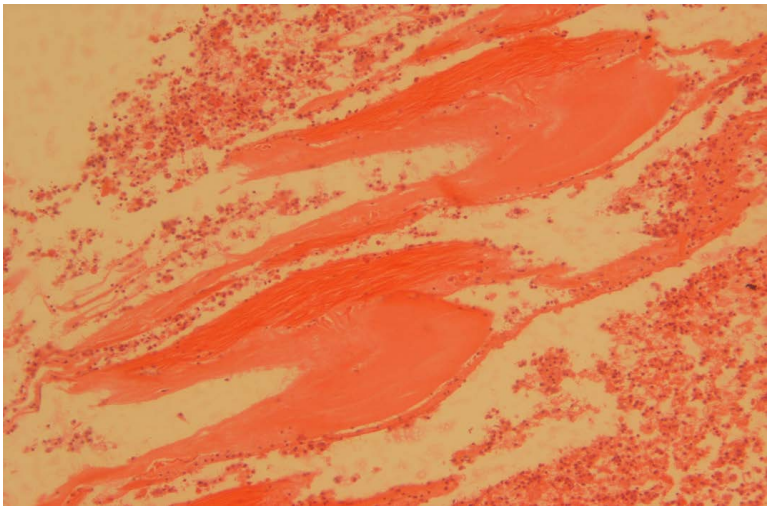


Figure 6. Gill filaments showed severe disturbance of the ciliated epithelial cells.

haemolysis. These pathological effects in the contaminated sites (site one and site two) could be a reaction to PAHs intake in the water currents and adaptive response to prevent the entry of pollutants through the gill surface. The observed pathological changes in oyster gills are defense mechanisms [29] [30]. The cellular effect observed in the gills and necrosis can affect the gas exchange and food transport. The edematous change in gill filaments and lamellae could be due to increased capillary permeability [31].

Bivalves like oysters are good biomonitors of PAHs since they are sessile and reflect PAH exposure at a specific site and since their capability to metabolize and eliminate PAHs is limited [32]. The severity of gill damage depends on the concentration of toxicant and on time of exposure [33]. Environmental toxicity can result in structural changes by direct toxic effect of the pollutant such as tissue degeneration and necrosis, and by the development of compensatory me-

chanisms such as cellular damage to deal with the stressor [34] [35]. Inflammation and fusion in gill filaments and lamellae could be a defense response against pollutants [36] [37]. Necrosis, inflammation (swelling) in gill lamellae, haemolysis and fusion in gill filaments observed in the present study are conformity with report of Donde [38] who studied the gills of clam *Gafrarium diverticatum* exposed to WSF-crude oil. When oysters are exposed to PAHs, they accelerate the lipid or carbohydrate metabolism in order to meet the energy demand while reducing the energy intake to prevent toxicant intrusion by closing the shells [39].

The distribution of the seven individual PAHs in oyster, *P. radiata* gill tissues was variable in site one and site two and was absent in the control site. Naphthalene was predominant in all oyster gill tissues, followed by acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene and benzo[a]anthracene. This was distinct in previous study [11]. These PAHs as mostenvironmental contaminants cause gill damage in exposed aquatic animals [40]. Accumulation of haemocytes in blood spaces indicates an extensive detoxifying process in gills of oysters exposed to PAHs. Histopathological symptoms described in bivalves may decrease individual fitness through disturbing the homeostasis and proper functioning of vital biological processes such as detoxification, respiration and osmoregulation [15].

The histological results observed in gills of the pearl oyster *P. radiata* in the present study indicate that PAH concentrations found in oyster tissues [11] caused severe alteration in gills which are important organs performing vital function.

4. Conclusion

The histological changes observed in the gills of the pearl oyster, *Pinctada radiata* used in this study show that contamination by polycyclic aromatic hydrocarbons is affecting oysters in Al-Khiran coast. It was concluded that the result of this study can be used as a guide for biomonitoring studies to investigate pollutants in pearl oyster population.

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