## Digestive gland alterations in Bivalves (*Tivela ponderosa*) exposed to Mareb crude oil, dispersed oil with dispersant, dispersant (Histological study)

Elham Al-Shaibani , Aziz S. Dobian\*, Nada A. Hassan\*\* and Nabil A. Al-Shwafi Biology Department, College of Education, Aden University aziz7289@hotmail.com\* Nadaalsyed10@gmail.com\*\*

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## Abstract

This study includes three laboratory experiments for studying toxicity effects of three test chemicals (Mareb crude oil, dispersed oil with dispersant and dispersant) on the tissue of digestive gland of bivalve (Tivela ponderosa). Acute toxicity test was done by exposed bivalve Tivela ponderosa to the concentrations 5, 10, and 15 ml/l of test chemical (Mareb crude oil, dispersed oil with dispersant and dispersant) which was added to see water (exposure time 96 hours). At chronic toxicity study, bivalve Tivela ponderosa was exposed to 0.5, 1.0, 1.5 ml/l of the same chemical toxicant which was added to see water (exposure time 3 weeks). Biomarkers was observed in the bivalve Tivela ponderosa when exposed to different concentrations of chemical toxicants. The study showed that only dispersant have a less effect than crude oil or a mixture of dispersed oil with dispersant. The histological changes in digestive gland tissues was proportional to the concentrations in both acute and chronic toxicity test. The digestive gland of bivalve in low concentration of both acute and chronic toxicity test showed expansion of lumen, and the epithelial cells became thinner in the tissue exposed to low concentration of the three test chemicals; and at medium concentrations the digestive gland showed increase in vacuoles and secretions, while at high concentrations more changes appeared, such as general degeneration, and the digestive gland became more vacuolated.

**Key words:** Acute toxicity, Chronic toxicity, Digestive gland, Mareb Crude oil, Dispersed Oil with Dispersant, Dispersant, Bivalves, *Tivela ponderosa*, Histological study.

## Introduction

Oil in the sea can occur as dispersed oil droplets, as an emulsion (water in oil or oil in water), bound to solid particles, or solubilized in water. Chemical dispersants are used as cleaning agents to alter the normal behaviour of petroleum hydrocarbons by increasing their functional water solubility, resulting in increased bioavailability and altered interactions between dispersant, oil, and biological membranes (35). Adding dispersants to oil may create a solution more toxic than the non-dispersed oil (27, 8,28 and 14).Histological biomarkers have been widely used in environmental monitoring, as these allow for the examination of specific target organs, including gills, digestive glands, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the marine animals (33, 2, 11, and 30).

The digestive diverticula of bivalves consist of numerous blind-ending tubules which communicate with the stomach by way of partially ciliated main ducts and non-ciliated secondary ducts (26). Changes in molluscadigestive tubule structure, due to pollutants, have been studied quantitatively by (Lowe 16,Tripp 34, Marigomez 19, Axiak 3, Recio 29, and Vega 34). Special attention was paid to the effects of pollutants on the structure and function of digestive cells since their lysosomal system is involved in hydrocarbon uptake (22) and shows a high degree of responsiveness to alterations in the environment (15,9, 23, 6, and 19).

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In this context, the main goal of this study is to investigate the effects of oil contamination on the digestive glands of clams *Tivela ponderosa*. The first objective is to compare digestive glands histology between clams exposed to Mareb crude oil, dispersed crude oil with dispersant, and dispersant for a period of 96 hours and three weeks. The second objective is to compare these histological results against the normal digestive glands histology of control clams.

## **Materials and Method**

Bivalves were collected from Abyan Coast (12° 48' 285'' N 45° 02' 381'' E) in Aden Governorate. They were collected by hand during the spring low tides in the evening times to avoid higher temperatures, kept in open canvas sacs containing wet sand to minimize frictions, desiccation, and then transported to the laboratory immediately. They were protected from agitation during the transportation. The clams were cleaned by gentle rubbing in clean seawater to remove the clogged sediment and mucus and kept in aquaria of uniform size, 40cm long, 25cm wide 20 cm height, each containing clean and filtered seawater. Clams of uniform size of  $(47 \pm 1)$  mm long were used in the study to avoid susceptible size-based variations in response to the test chemicals. At the end of the acclimatization the experimental organisms for 4 days must be in normal condition to tolerate the experimental conditions. There should be less than 2% mortality during acclimatization (1). For histological studies, Tivela ponderosa of control and others which exposed for 96 hours for acute toxicity and three weeks for chronic toxicity transferred to glass jars containing clean seawater; and different concentration of chemical test used for acute toxicity (5, 10 and 15 ml/l) and for chronic toxicity (0.5, 1.0 and 1.5 ml/l) many changes were seen, such as the outer margin of the shells, and were abraded; and a scalpel was inserted cautionary in order not to damage the tissues. The posterior and anterior adductor muscles were cut. The digestive gland tissue was selected for histological investigations. The Tissues were fixed in Bouin's fluid and blocks for microtome sections were prepared in paraffin wax. The sections were cut at 7u thickness on a rotary microtome, stained in haematoxylin-Eosin and mounted in DPX.

#### Results

**The cross section of the digestive gland of the control group:** In cross section, the lumen of the tubule often contains minute spheroid bodies. In natural habitat, the thickness of the cells and size of the lumen of the tubules depend on the condition of feeding. The cross section of digestive gland of control (Plate1) shows few digestive tubules embedded in the connective tissue (CT). The tubules are composed of two types of cells: large cells with round nuclei and small cells with darkly stained nuclei called crypts of new cells (N). The lumens (L) contain faint granules and the spheroid bodies. Amoeboid cells are scattered in the connective tissue.



Figs. 1: Cross section of the digestive gland of control *Tivela ponderosa*. Haematoxylin and Eosin; (A and B) 400X. Ct, connective tissues; Dc, digestive cells; N, new cells; L, lumen.

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At low concentrations of Mareb crude oil, dispersed oil with despersant and dispersant showed various histological changes in the digestive glands, including shrinkage in the tubules, more maculation's and enlargement of cells. The tubules lumens became very narrow. **Figs. 2** (A, B), **Figs. 3** (A, B) and **Figs. 4**.

The cross sections of the digestive gland of the clams, exposed to medium Mareb crude oil, disperses oil and dispersant concentrations in **Figs. 5** (A, B), **Figs. 6** (A, B) and **Figs. 7**, showed several changes, the wall of the tubules became irregularly lined. Some lesions were noticed in certain parts of the glandular cells. The lumen was more or less full of secretion and spheroid bodies. Figures 8 (A, B), Figs. 9 (A, B) and Figs. 10 showed cross sections of the digestive gland tubules of the clams exposed to Mareb crude oil, disperses oil with dispersant and dispersant concentrations wherein disintegration of the tubules is observed. The nuclei have been disintegrated and the digestive cells have become much more vacuolated. The present study indicates that disperses oil with dispersant is more toxic, to digestive gland of bivalves (*Tivela ponderosa*), than crude oil or dispersant.



Figs. 2 Cross section of the digestive gland of *Tivela ponderosa* exposed to low Mareb crude oil concentration.

Plate (A): digestive gland exposed to concentration (5ml/l) for 96 hours.
Plate (B)(C): digestive gland exposed to concentration (0.5ml/l) for three weeks.
Haematoxylin and Eosin; 400X. Ct, connective tissues; Dc, digestive cells; N, new cells; L, lumen.
Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules.



Figs. 3 Cross section of the digestive gland of *Tivela ponderosa* exposed to low crude oil with dispersant concentration.

Plate (A): digestive gland exposed to concentration (5ml/l) for 96 hours. Plate (B)(C): digestive gland exposed to concentration (0.5ml/l) for three weeks. Haematoxylin and Eosin; 400X. Ct, connective tissues; Dc, digestive cells; N, new cells; L, lumen. Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules

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Figs. 4 Cross section of the digestive gland of *Tivela ponderosa* exposed to low dispersant concentration.

Plate (A): digestive gland exposed to concentration (5ml/l) for 96 hours. Plates (B)(C): digestive gland exposed to concentration (0.5ml/l) for three weeks Haematoxylin and Eosin; 400X. **Ct**, connective tissues; **Dc**, digestive cells; **N**, new cells;**L**, lumen.

Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules.



Figs. 5 Cross section of the digestive gland of *Tivela ponderosa* exposed to medium mareb crude oil concentration.

Plate (A): digestive gland exposed to concentration (10ml/l) for 96 hours.

Plate (B): digestive gland exposed to concentration (1ml/l) for three weeks.

Haematoxylin and Eosin; 400X. Ct, connective tissues; Dc, digestive cells; N, new cells; L, lumen.

Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules.



Figs. 6 Cross section of the digestive gland of *Tivela ponderosa* exposed to medium crude oil with dispersant concentration.

Plate (A): digestive gland exposed to concentration (10ml/l) for 96 hours. Plate (B): digestive gland exposed to concentration (1ml/l) for three weeks. Haematoxylin and Eosin; 400X. **Ct**, connective tissues; **Dc**, digestive cells; **N**, new cells; **L**, lumen.

**Note:**E.Dc., enlargement of digestive cells; V.Dc vacuolated of digestive cells; S.T., shrinkage in the tubules



Figs. 7 Cross section of the digestive gland of *Tivela ponderosa* exposed to medium dispersant concentration.

Plate (A): digestive gland exposed to concentration (10ml/l) for 96 hours. Plates (B)(C): digestive gland exposed to concentration (1ml/l) for three weeks. Haematoxylin and Eosin; 400X. **Ct**, connective tissues; **Dc**, digestive cells; **N**, new cells;**L**, lumen.

**Note:** E.Dc., enlargement of digestive cells; V.Dc vacuolated of digestive cells; S.T., shrinkage in the tubules



Figs. 8 Cross section of the digestive gland of *Tivelaponderosa* exposed to high mareb crude oil concentration.

Plate (A): digestive gland exposed to concentration (15ml/l) for 96 hours. Plate (B) (C): digestive gland exposed to concentration (1.5ml/l) for three weeks. Haematoxylin and Eosin; 400X. **Ct**, connective tissues; **Dc**, digestive cells; **N**, new cells; **L**, lumen.

Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules.



Figs. 9 Cross section of the digestive gland of *Tivelaponderosa* exposed to high crude oil with dispersed concentration.

Plate (A): digestive gland exposed to concentration (15ml/l) for 96 hours.

Plate (B) (C): digestive gland exposed to concentration (1.5ml/l) for three weeks.

Haematoxylin and Eosin; 400X. Ct, connective tissues; Dc, digestive cells; N, new cells; L, lumen.

Note:E.Dc., enlargement of digestive cells; S.T., shrinkage in the tubules; D.T disintegration of the tubles.



Figs. 10 Cross section of the digestive gland of *Tivelaponderosa* exposed to high dispersant concentration.

Plate (A): digestive gland exposed to concentration (15ml/l) for 96 hours. Plates (B)(C): digestive gland exposed to concentration (1.5ml/l) for three weeks. Haematoxylin and Eosin; 400X. **Ct**, connective tissues; **Dc**, digestive cells; **N**, new cells; **L**, lumen.

Note: E.Dc., enlargement of digestive cells and S.T., shrinkage in the tubules.

## Discussion

The toxicity of petroleum hydrocarbons can differ markedly between species and phyla (8). This could account for the differences in toxicity observed for disperses WAF in different studies. Neff (24) has shown that the susceptibility to hydrocarbons and dispersants differs across species and developmental stages. Bivalve molluscs and shrimp lack the ability to metabolize petroleum hydrocarbons, thus they readily accumulate these compounds in their tissues. Contaminated molluscs can provide a pathway for exposure of other natural resources that feed heavily on them (20,13). The physiological indicators of stress in shellfish, especially molluscs, have been well documented by (Scott 31). Owen, (25) reported that the lysosome rich digestive cells of the molluscan hepatopancreas are functional in both storage and intracellular digestion of food. These cells also accumulate large quantities of PHC and undergo pathological changes due to their toxic action.

Histological effects of Mareb crude oil, disperses oil with dispersant and dispersant were visible on the digestive gland exposed to various concentrations, although these effects were not visible on the digestive gland when exposed to low concentrations, but this does not mean they are unresponsive to test substances effects. According to Hawkes (10), a tissue or an organ may exhibit no grossly observable changes, but the cells may be altered; and this may in turn affect the overall function of the organism. The response of the digestive gland to test substances ranging from slight lesions to increasing the spheroid bodies in the tubules lumens, irregularities in the tubule walls and lesions of the digestive cells. The enlargement of the glandular cells in the low crude oil, disperses oil and dispersant concentrations may be explained either by absorption of the chemicals and increasing the volume of cellular organelles, like endoplasmic reticulum, to produce more enzymes to counteract the mild stress of the chemical, while the cell membrane is still intact, so they look swollen or possibly due to the nutritional state of the clam. The response of the digestive gland to test substances (Mareb crude oil, dispersed oil and dispersant) ranges from increasing the spheroid bodies in the tubules lumens, irregularities in the tubule walls and lesions of the digestive cells. Jorgensen (12) showed that the enlargement of the glandular cells in the low concentrations may be explained either by absorption of the chemicals and increasing the volume of cellular organelles, like endoplasmic reticulum, to produce more enzymes to counteract the mild stress of the chemical, while the cell membrane is still intact, so they look swollen or possibly due to the nutritional state of the clam.

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Also, he stated that the digestive gland cells and the lumen change alternatively. During feeding in high tide, the digestive cells are thin and the lumen is large in the digestive gland; while, at resting period, the cells enlarge and the lumen will reduce in size. The exposed clams to medium concentrations show granular lumens and irregular wails of the tubules and this may be due to high secretions of the glandular cells and partly to the breakdown of some of the cell membranes, while the vacuolation and disintegration of the nuclei and cells in the clams exposed to high concentration is explained by the rupture of lysosomes which release hydrolytic enzymes that break out the cells. In high concentrations the subcellular organelles like lysosomes, most probably, got affected by the chemical. This condition is observed in wide range of animals exposed to pollutants where the lysosomes accumulate many organic compounds as well as metals and, when they become overloaded, the integrity of their limiting membranes may be damaged, releasing the hydrolytic enzymes into the cytoplasm where they may cause damages to the cells (Bayne, 4), crude oil, dispersed oil and dispersant among other polycyclic aromatic hydrocarbons produce significant decrease in the lysosomal stability in the digestive cells of Mytilus edulis (Moore, 22). The digestive gland cells of the tubules and ducts are involved in both absorption and secretion, thus loss of cell membranes and organelles will most probably lead to alter digestive gland function. The digestive glands in bivalves or fishes are the organs most affected by pollutants due to their sensitivity (Malins 18, 17,3 and 5).

In general, the histological changes in this study indicate the relationship between the degree of tissues damage and the chemicals test concentration that the damages are more visible in the clams exposed to higher test concentrations. These structural alterations most probably will cause impairments of the physiological activities of the organism, such as filtration, digestion, and other physiological functions. However, various factors may be involved synergestically for these alterations. The chemical exposure is definitely one of them since no visible or very less damage could be observed in the control and in the clams exposed to low substance test concentration. Other factors, such as the artificial habitat during the experimental period, might be a reason in increasing the tissue damages.

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# تغيرات الغدة الهضعية في محار ذات المصراعين (Tivelaponderosa) عند تعرضها لنفط خام مأرب، والنفط المختلط بالمشتت والمشتت النفطي (دراسة نسيجية) الهام الشيباني، عزيز دبيان\*، ندى السيد حسن\*\* ونبيل الشوافي قسم الأحياء، كلية التربية- عدن، جامعة عدن \*aziz7289@hotmail.com Nadaalsyed10@gmail.com

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الملخص

شملت هذه الدراسة ثلاث تجارب مختبرية لدراسة آثار السمّية لثلاث مواد كيميائية هي (نفط خام مأرب، النفط مع المشتت النفطي والمشتت النفطي) على أنسجة الغدة الهضمية للمحار ذات المصراعين (Tivela ponderosa). تم اختبار السمية الحادة (96 ساعة) بتعرض محار (Tivela ponderosa). للتراكيز 5، 10 و15 مل/لتر من كل مادة كيماوية مضافة الى ماء البحر، وعند در اسة السمية المزمنة تم تعريض محار (Tivela ponderosa). للتراكيز 5، 10 و15 مل/لتر من كل مادة كيماوية مضافة الى ماء البحر، وعند در اسة السمية المزمنة تم تعريض محار (ألسابيع). أفهرت المرباعين (Tivela ponderosa). للتراكيز 5، 10 و15 مل/لتر من كل مادة كيماوية مضافة الى ماء البحر، وعند در اسة السمية المزمنة تم تعريض محار (ألسابيع). أظهرت المؤشرات الحيوية للمحار عند تعرضه لسمّيات مختلفة من مواد الاختبار أن المشتت النفطي القل تأثير من النفط الخام وخليط النفط مع المشتت، وكانت التغيرات النسيجية في الغدة الهضمية تتناسب طرديا مع تركيز مادة الاختبار للسمية الحادة والمزمنة على حد سواء. أظهرت تغيرات السمية المؤسرت النفطي مع تركيز مادة الخام وخليط النفط مع المشتت، وكانت التغيرات النسيجية في الغدة الهضمية تتناسب طرديا مع تركيز ماد الخربار السمية الحادة والمزمنة على حد سواء. أظهرت تغيرات نسيجية في الغدة الهضمية المعامية المحار في المرحان في المورين المؤسية على حد سواء. أظهرت تغيرات نسيجية في الغدة الهضمية المحاد في المحار في التركيز منخفض السمّية الحادة والمزمنة على حد سواء. أظهرت تغيرات نسيجية في الغدة الهضمية المادار في المحار في المحار في التركيز منخفض السمّية الحادة والمزمنة على حد سواء. أظهرت اليوسع في التحويف وأصبحت مع تركيز مادة الإختبار. وعند تعرض المحار في المحار في المركيز مادة الهرت العربية وأسرحار المعرضة على حد سواء. أظهرت المادين والغذة الهضمية الحادة والمزمنة على حد سواء. أظهرت نعيرات المواد الاختبار. وعند تعرض المحار في المحار في التركيز منخفض السمّية الحاد والمزمنة على حد سواء أظهرت الملائية رقيقة الحدران في أنسجة الحادة والمزمنة على حد سواء. أظهرت الحدان المكنيز مادة المامية الحادة والمزمنة على حد سواء أظهرية المدارية وقيقة الحدران في أنسجة الحاد والمزمنة على حد سواء الموين أمورازات، في حين المرم المحاد المحارية أظهرت المحادية المحادية المدرانة وي أكثر وضوحا المحاديا المداني المحادي

الكلمات المفتاحية: السمية الحادة، السمية المزمنة، الغدة الهضمية، نفط مأرب الخام، النفط مع المشتت والمشتت، محار ذات المصر اعين، تايفيلا بونديروسا (Tivelaponderosa)، در اسة نسيجية.