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EFFECT OF POLLUTANTS IN COASTAL WATER OF JEDDAH ON 2- THE HISTOLOGICAL STRUCTURE OF LIVER OF THE FISH *SIGANUS RIVULATUS* (FORSKAL). SAUDI ARABIA

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ABSTRACT

The present study was carried out to document the liver histology of the fish *Siganus rivulatus* caught from the coastal water of the Red sea at Jeddah, and to document also any histopathological lesions and attribute these lesions to their causes. Parasitic infection were observed in the liver of about 83% of the fish. The most noticeable histopathological lesions were due to histopathological parasitic this parasitic infection and the toxic effect of deteriatiry water quality due to presence of heavy metals which is considered a big cause to the cellular damage. lesions were hepatocyte vacuolization and ballooning degeneration cellular and coagulative necrosis, cellular infiltraion, granuloma inflammation and bile duct proliferation.

1. INTRODUCTION

The study area receives different pollutants from four sources: untreated domestic sewage wastes, oil from the oil refinery, fish wastes from the big fish market (El-Bangalah) and desalination plant effluents which contribute both organic and metallic contaminants (Behairy and Saad 1984; Basaham, *et al.,* 1990).

In comparison with the offshore waters, coastal waters are shallower and slower flushing time. This leads to slower dilution of oil contaminates. Thus, biologically important systems such as fisheries and breeding grounds are already under stress (Husqy *et al.,* 1996) and numerous authors recommended that histopathology can be used to monitor the effect of aquatic pollutants (Bucher and Hofer 1993; Gonzalez, *et al.,* 1993; Abdel - Aziz. 1994; Abdel-Rahman, 1997, Abdel - Maguid *et al.*, 1999 and Zaki, *et al.,* 1999).

The liver is a major blood circulation organ and is subject to an counter major environmental pollutants and infective parasitic stages Various lesions including neoplasm and parasitic infection are often associated with aquatic pollution (Saleh, 1982; Kent, 1988; Gonzalez, *et al.*, 1993 & Hilmy 1996).

The present study was carried out to assess the histological and ultrastructural lesions in the liver of rabbit fish, *Siganus rivulatus* caught from polluted coastal waters of Jeddah, Saudi Arabia that are thought to be subject to polluting discharge.

2. MATERIALS AND METHODS

The species under investigation is rabbit fish *Siganus rivulatus* belongs to family Siganidae which is favarable aqua culture species and sampling from five rondom sites of the study area (Fig. 1) The area under investigation (21°29` - 21°30` N and 39°10` - 39°11` E) is located between the Islamic.

Harbour south of Jeddah and the desalination plant north of the city center. There are some patches of submerged barrier reef shoals in its western side. The coastline is dentated and contains some embayment. Some meteorological records were carried out in the study area through Autumn and winter seasons (Table 1); as well as physico-chemical mean values of some physicochemical parameters were measured in surface water (APHA, 1995) Table 2.

By using the Atomic absorption spectrophotomere and according to APHA (1995) Concentration of heavy metals (µg/L) in the surface water and sediments (µg/g) of the five studies sites were measured and represcnted in Table (3 & 4).

Fish were caught by trammel nets and tramsferred immediately to laboratory in glass aquaria supplied with oxygen. The fish were dissected their liver were removed and weighed to the nearest milligram. The Condition factor (K) of fish was calculated from the formula $K = (W/L^3) \times 100$ and hepatosomatic index (HSI) of fish was calculated from the formula (HIS = (liver welight/body weight) x 100 according to Jangaard, *et al.* (1967).

For histological study, Liver samples from 150 cought fish were immediately fixed in 10% buffered formaline, dehydrated in an ethanol series, embedded in paraffin, sections 3 µm thick were cut with a microtome and stained with haematoxylin - eosin (H.E.).

For ultrastructural study small pieces of fresh liver tissues for three specimens were fixed in 2.5% glutaraldehyde in phosphate buffer (pH = 7.4) and post-fixed in 0.1 M osmium tetroxide in phosphate buffer (pH = 7.2). Tissue pieces were dehydrated in ethanol, placed in propylene oxide and embedded in Epon 812.

Ultrathin sections (60-70 nm) were cut with LKB ultramicrotome, stained with uranyl acetate and lead citrate (Reynold's, 1963) and examined in a phillips EM 200 (Joel 100 CX) electron microscope.

3. RESULTS AND DISCUSSION

Most of the examined specimens (83.33%) of *Siganus rivulatus* revealed histopathological lesions. Twenty six per cent at of this portion was infected by parasites.

Fish with normal appearing liver (16.67%) have condition factor equal 1.196 ± 0.094 g /cm^3 and hepatosomatic index equal 2.249 ± 0.179. The liver composed of masses of hepatocytes not organized in distinet lobules and were interrupted by sinusoids (Figs. 2 & 3). Endothelial cells and few kuppfer cells line the sinusoidal lumen. The blood vessels and bile ducts was randomly found throughout the hepatic parenchyma. Bile duct wall consists of a simple cuboidal epithelium with a brush border and a collagenous cover (Fig. 2). Melanomacrophage centers are present in the hepatic parenchyma, and are usually located in the vicinity of blood vessels and bile ducts (Fig. 2). They may be accumulated antigenic bodies (Roberts 1978); they store Products that are difficult to eliminate (Gonzalez, *et al.*, 1993) and store iron following erythrophagocytosis (Agius, 1997).

The polyhydral hepatocytes bear spherical central nuclei and moderately eosinophilic granular cytoplasm. The hepatocytes are normally multinucleated (Fig . 3).

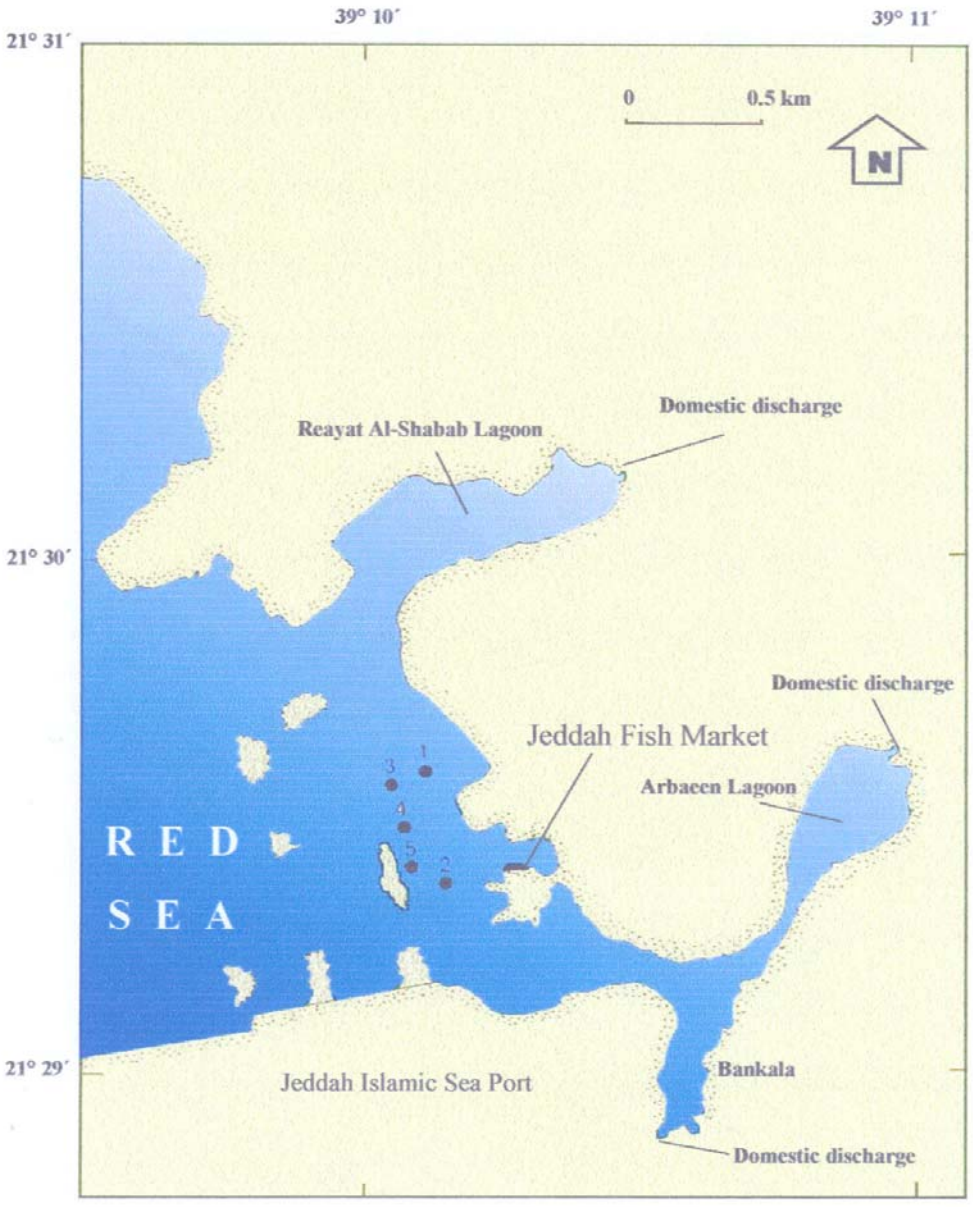

Fig. (1): Map showing the five sampling stations of Jeddah fish market

Ultrastructural observations showed that the hepatocyte nucleus possessed mostly regular nuclear envelope, a prominent nucleolus and small amount of condensed heterochromatin often attached with the nucleolus and the inner nuclear membrane. An extended layer of organelles was arranged around the nucleus and consisting of nearly parallel stacks of rough endoplasmic reticulum (RER) cisternae, smooth endoplasmic reticulum (SER) vesicles, numerous spherical and avoid mitochondria with short tubular cristae, peroxisomes with granular dense matrix and nucleoid, free polyribosomes and few lysosomes.

Moderate histopathological and cellular lesions were observed in liver of most examined fish with great individuals variability. Extensive vacuolization was observed in many specimens. Accumulation of vacuoles resulted in the displacement of nuclei to the cell margin with pyknosis of the nuclei (Fig, 5). In severe cases of fatty infiltration, the liver enlarged (H.SI.= 3.449 ± 0.315) and the hepatic tissue become paler where large lipid droplets accumulated in cells and sometimes appear in between necrotic hepatocytes. This is may be due to impaired metabolism of fatty acids leads to accumulate of triglycerides which form non-membrane bound vacuoles in cells (Burkitt, *et al.,* 1996).

In some instance the hepatocytes become pale and swollen like a balloon (Fig. 6). This is probably a result of accumulation of electrolytes and water due to impaired membrane permeability followed autolysis (Burkitt, *et al.*, 1996; Curran, 1995). Enzymatic macrophage infiltered arrange blood vessels with pigment deposition (Figs. 7). This infiltration may be an inflammatorg recation response to same toxic substances.

Also, examination of electron micrographs of limited liver sections showed ultrastructural alterations from normal appearing hepatocytes. The most frequently encountered alteration were nuclear deformation and atrophy, fragmentation and vesiculation of RER cisternae, increase in number and size of lysosomes (phagolysosomes); atrophy and lysis of mitochondria, large empty vacuoles with folluclent materials and myline figures (Figs 5 & 10).

Parasitic infections associated with inflammatory granuloses were observed in liver sections. The infected fish were characterized by significantly low condition factor (K= 0.867 ± 0.124 g/cm^3) and fow hepatosomatic index (H.I.S.= 1.558 ± 0.250) in comparison with fish having normal liver (χ^2 test $P < 0.001$).

Encapsulated granuloma in liver sections of examined fish (Figs. 8 & 9) was characterized by central caseous necrosis of hepatic tissue at the site of granuloma surrounded by epithelial cells and fibrocytic layer. Granuloma inflammation is defensive mechanism to seal foriegn bodies like bacterial that can be detected inside the lyzed hepatic tissue of granuloma by specific stains (Burkitt, *et al.* 1996 & Robbins, 1995).

Parasitic worms were also detected in dilated bile ducts in liver sections of examined fish (Figs. 11, 12 & 13). The epithelial lining of the bile ducts was necrotic. The bile duct showed a thick fibrous layer was filled with inspissated bile (Fig. 12). Cysts of parasitic protozoan in liver sinusoids and propably a metal deposites in RBCs were clearly detected (Figs. 14 & 15). Usually, degenerative and necrotic changes in liver cells, heavy leukocyte infilteration, Melanomacrophage center clles (MMCs) proliferation or reduction and kuppfer cell activation are characteristics of infected hepatic tissue.

Generally, the incidence of parasitic infection was very high in all exposed to wast water which induce bacterial infection fish. Such infections fungal due to reduced defensive mechanisms (Myers *et al.*, 1994). Also toxicopathic liver lesions are frequently detected in bottom dwelling and bottom feeding fish living in contaminated areas. In

these environments the overall prevalence of granuloma diseases, non neoplastic and neoplastic lesions and megalucyic hepatosis exceed 80% in these species (Myers *et al.*, 1994).

It is interesting to mention that the study species is a bottom feeding fish that feed on sea grasses and epiphytic algae. the present chemical analysis in tables (3 & 4) revealed contamination of sediment and water of the studied area with numerous heavy metals. The mean Values of these metals in sediments is higher than in water as, Fe, 963.62 ± 219.4 µg/g in sediments , and 36.22 ± 16.07 µg/L in water. pb, 52.74 ± 6.83 µg/g and 7.20 ± 4.03 µg/L in sediments and water respectively and so on. (Tables 3 & 4). Bin Dohaish (2001) showed bioconcentration of these heavy metals in liver, gills of that species (for example bioconcentration of fein liver equall 1147.69 µg/g).

Similar histopatholgoical and cellular alterations in liver tissue were obtained from number of laboratory experiments on fish using different toxic compounds occurring in aquatic ecosystem these alternations includ focal hepatocyte necrosis in trout exposed to lindane (Cauch, 1975), liver hepatoma and granulomatous diseases in toad fish after exposure to polycylic aromatic hydrocarbons (Thigagaraga and coldblatt 1980); fatty infiltration, focal necrosis and disorganization of hepatic parenchyma and lymphocytic infiltration in *Siganus rivulatus* exposed to Cu & Pb (Abdel-Rahman, 1997), nuclear pyknosis, aggregation and atrophy of mitochondria, vesiculation of rough endoplasmic reticulun (RER) and accumulation of lipid droplets in *Oreochromis niloticus* poisend by lead (Abdel-Maguid *et al.,* 1999).

Thus, it can be inferred that histopatholgoical and cellular alterations in body organs mainly the liver would serve useful purpose in evaluating the toxic effects of various pollutants present in the Red sea coastal area of Jeddah.

Table (1): Meteorological records in the study area through Autumn and Winter seasons.

Season	Months	I	ll	III	IV
Autumn	September	32.4	32.1	79.0	0.0
	October	30.0	29.0	80.0	0.0
	November	27.6	27.2	77.0	0.0
	Mean	30.0	29.4	78.7	0.0
	SD	2.4	2.5	1.5	0.0
Winter	December	27.4	24.7	78.0	10.0
	January	25.9	21.0	76.0	11.0
	February	25.6	22.0	70.0	11.0
	Mean	26.3	22.6	74.7	10.7
	SD	1.0	1.9	4.2	0.6

I Air temperature (°C)
II water temperature (°C)
III Relative humidity (%)
IV Precipitation (mm/month)

Table(2): Mean (and range) values of the physico-chemical parameters measured in

Parameters		**PH**	**Hardness (mg /L)**	**DO (mg O_2 /L)**	**BOD (mg O_2 /L)**
sites	1	7.80	6,850	4.95	3.81
	2	8.14	9,450	3.93	5.02
	3	8.16	7,750	5.35	2.68
	4	8.14	10,750	6.29	6.13
	5	8.17	15,250	7.17	7.93
Range		7.80 – 8.17	6,850 – 15,250	3.93 – 7.17	2.68 – 7.93
X ± SD		8.08 ± 0.16	10,010 ± 3,294	5.54 ± 1.25	5.11 ± 2.04
Parameters		**COD**	**NO_2 -N**	**NH_4 -N**	**PO_4 -P**
		(mg O2 /L)	**(µg at /L)**	**(µg at /L)**	**(µg at /L)**
sites	1	20.00	0.01	0.13	0.08
	2	20.00	0.15	0.11	0.78
	3	20.00	0.39	0.18	0.11
	4	40.00	0.17	0.13	0.12
	5	40.00	0.17	0.13	0.07
Range		20.00 – 40.00	0.01 – 0.39	0.11 – 0.18	0.07 – 0.78
X ± SD		28.00 ± 10.95	0.18 ± 0.13	0.14 ± 0.03	0.23 ± 0.31

Table 3 : Concentration of the heavy metals (µg /L) wet weight in the surface water of the studied sites.

Sample	Fe	Ni	Mn	Cr	Zn	Cd	Pb	Cu
1	19.64	5.36	1.00	0.52	1.44	0.84	3.20	0.52
2	53.68	4.36	1.88	0.72	1.40	1.40	10.00	2.24
3	19.44	1.60	1.84	1.00	1.44	1.92	5.20	2.40
4	48.96	3.28	0.88	0.92	2.00	0.80	12.80	1.32
5	39.36	9.00	0.52	1.24	1.68	1.04	4.80	1.44
Range	19.44 – 53.68	1.60 – 9.00	0.52 – 1.88	0.52 – 1.24	1.40 – 2.00	0.80 – 1.92	3.20 – 12.80	0.52 – 2.40
Mean ± SD	36.22 ± 16.07	4.72 ± 2.77	1.22 ± 0.61	0.88 ± 0.27	1.59 ± 0.25	1.20 ± 0.47	7.20 ± 4.03	1.58 ± 0.76

Table 4: Concentration of the heavy metals (µg /g) in the surface sediments of the studied sites.

Sample	Fe	Ni	Mn	Cr	Zn	Cd	Pb	Cu
1	1,050.00	19.11	17.61	13.85	8.44	4.75	50.88	7.59
2	1,040.00	22.76	17.41	13.28	5.25	5.16	53.50	6.84
3	705.00	20.64	15.43	12.75	4.39	5.45	46.63	6.75
4	779.35	25.00	18.09	16.47	9.40	5.81	64.05	8.96
5	1,243.75	21.70	19.11	12.56	4.55	5.23	48.63	39.71
range	705.00 – 1,243.75	19.11 – 25.00	15.43 – 19.11	12.56 – 16.47	4.39 – 9.40	4.75 – 5.81	46.63 – 64.05	6.75 – 39.71
X ± SD	963.62 ± 219.44	21.84 ± 2.22	17.53 ± 1.35	13.78 ± 1.58	6.40 ± 2.34	5.28 ± 0.39	52.74 ± 6.83	13.97 ± 14.42

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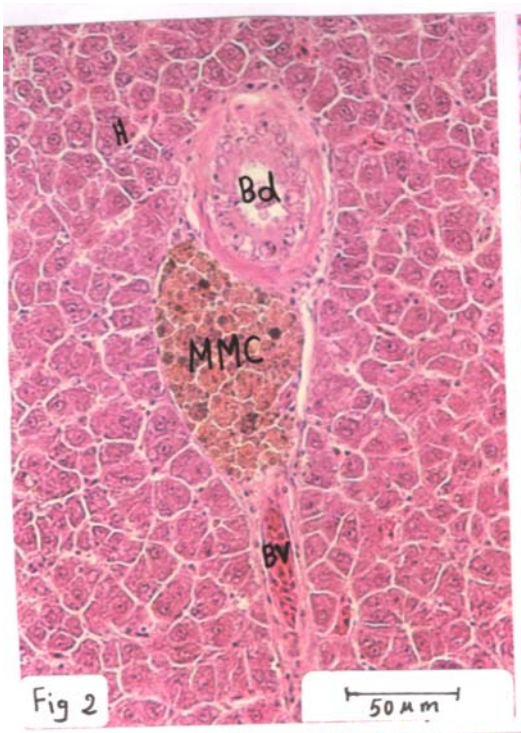

Fig 2

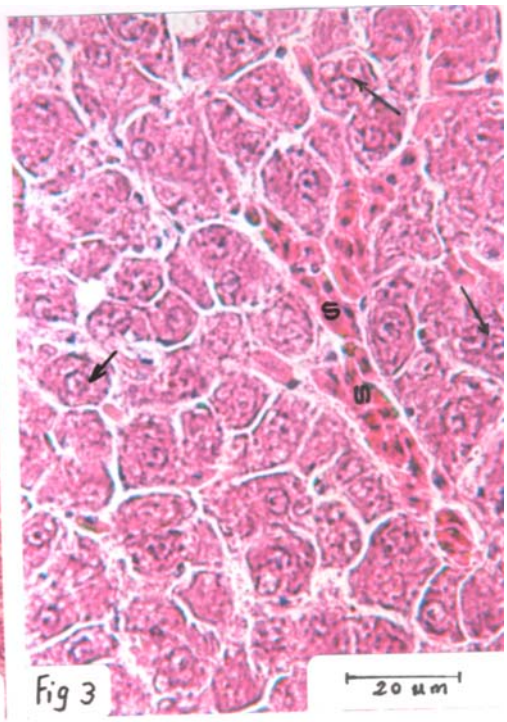

Fig 3

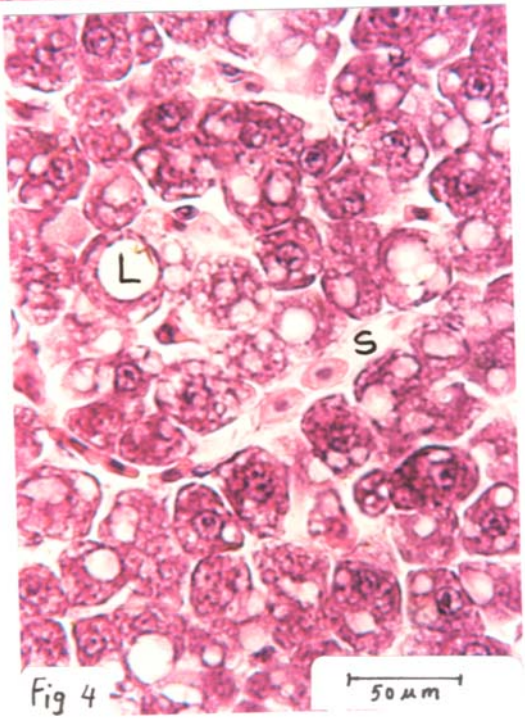

Fig 4

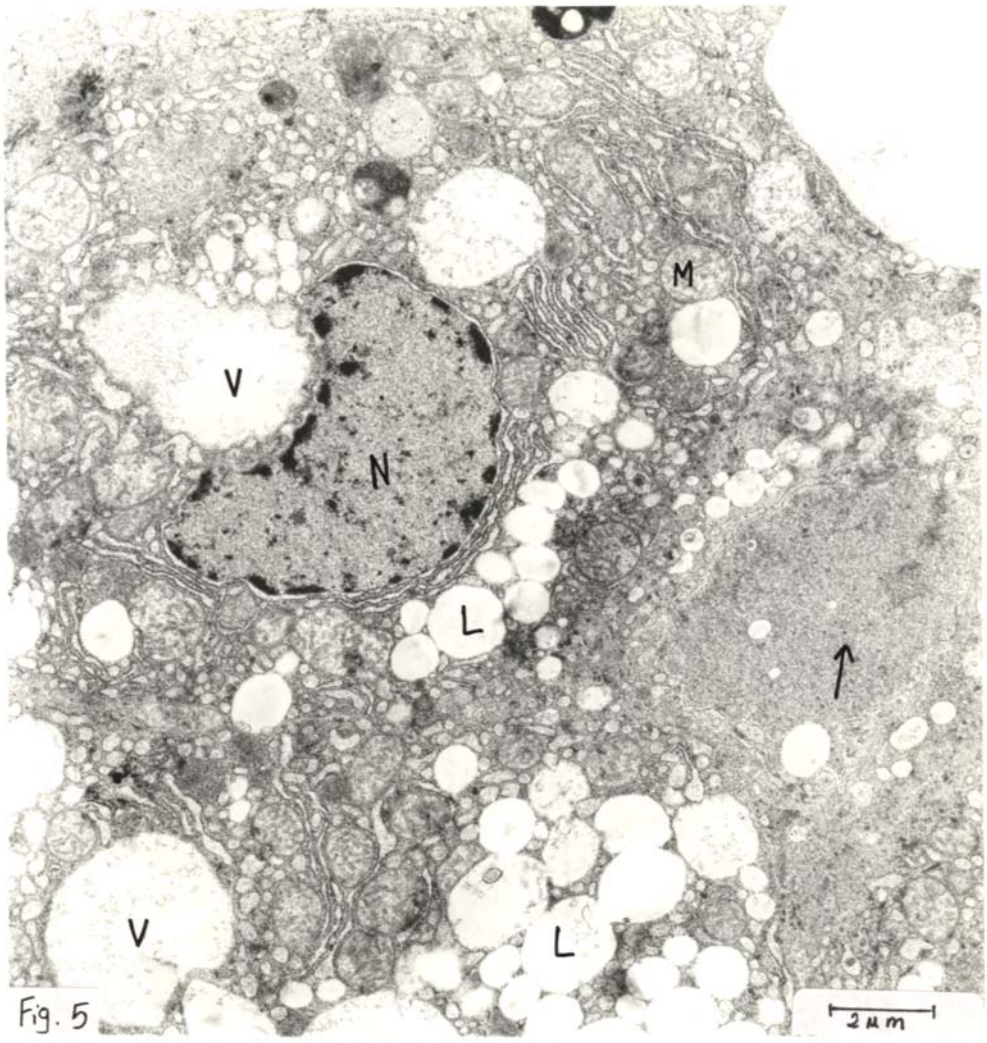

Fig. 5

EFFECT OF POLLUTANTS IN COASTAL WATER OF JEDDAH ON
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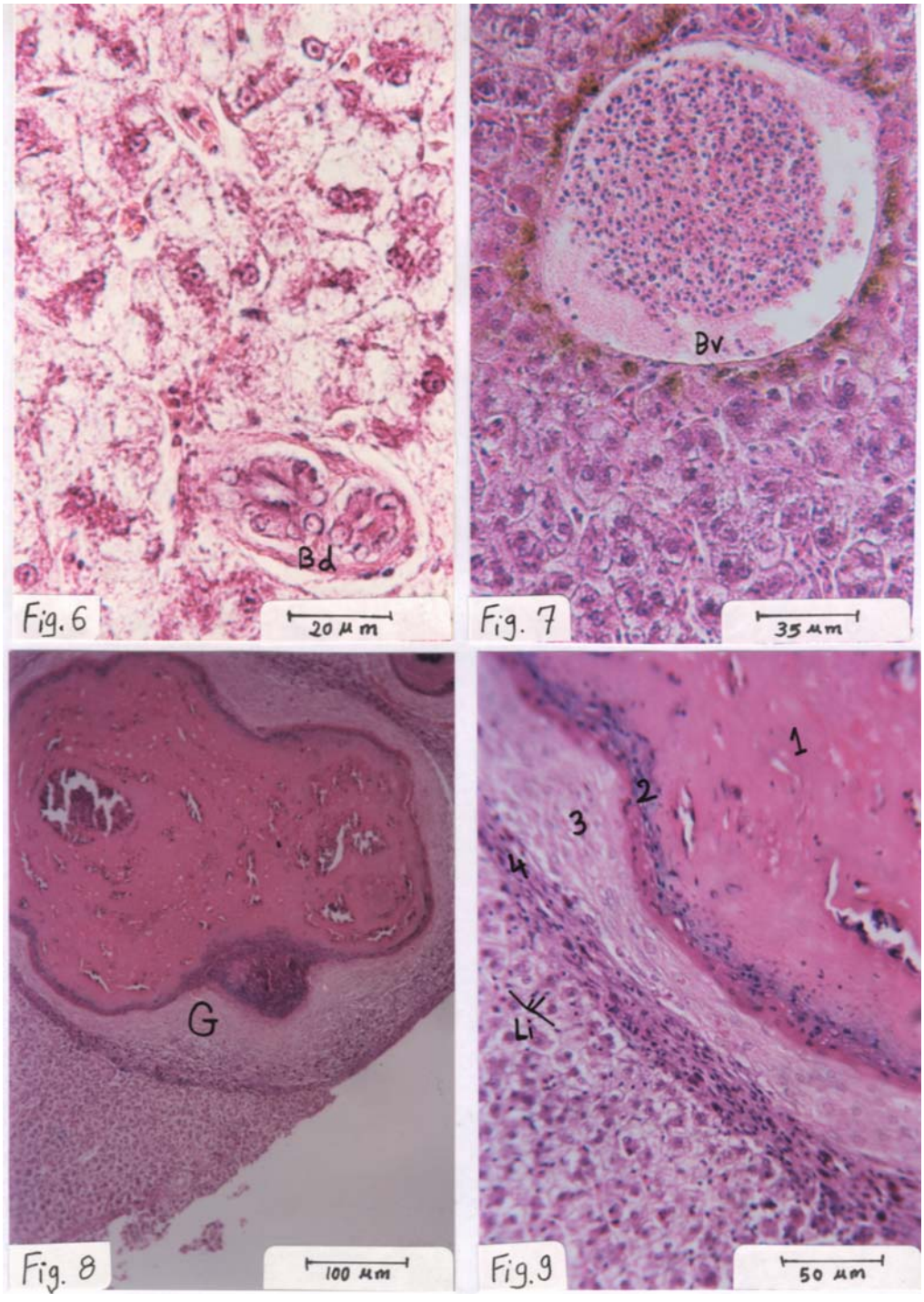

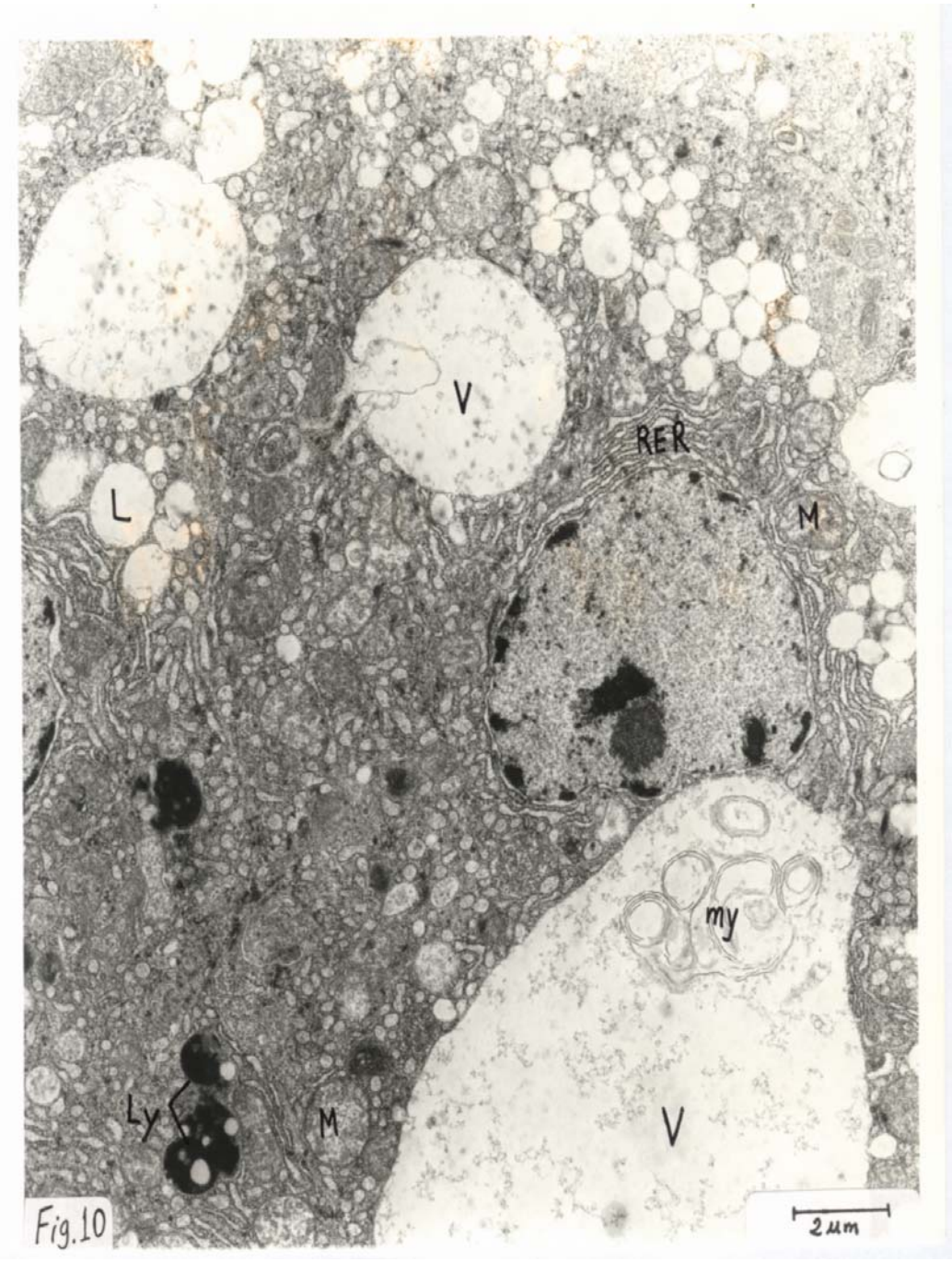

Fig.10

EFFECT OF POLLUTANTS IN COASTAL WATER OF JEDDAH ON
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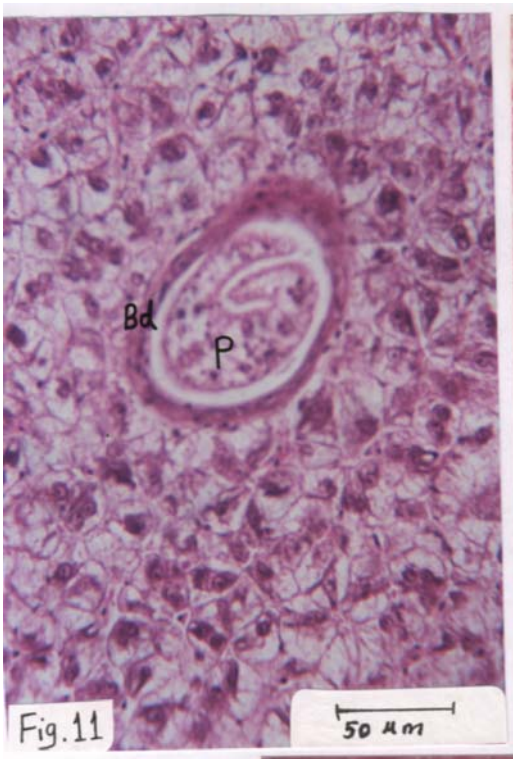

Fig.11

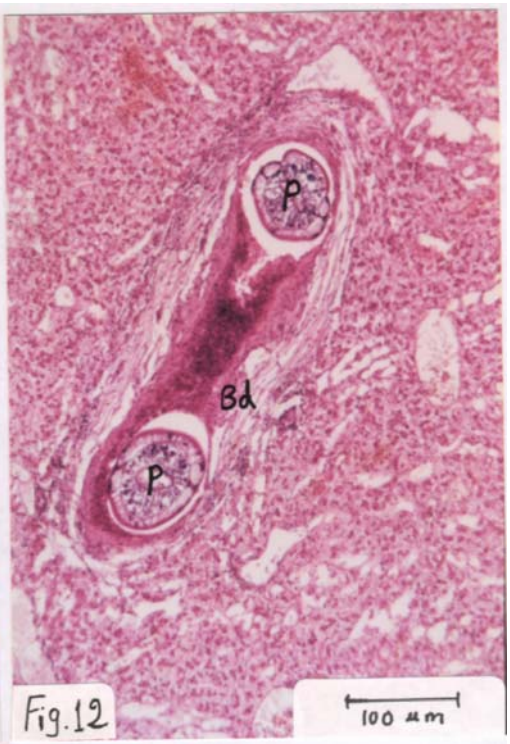

Fig.12

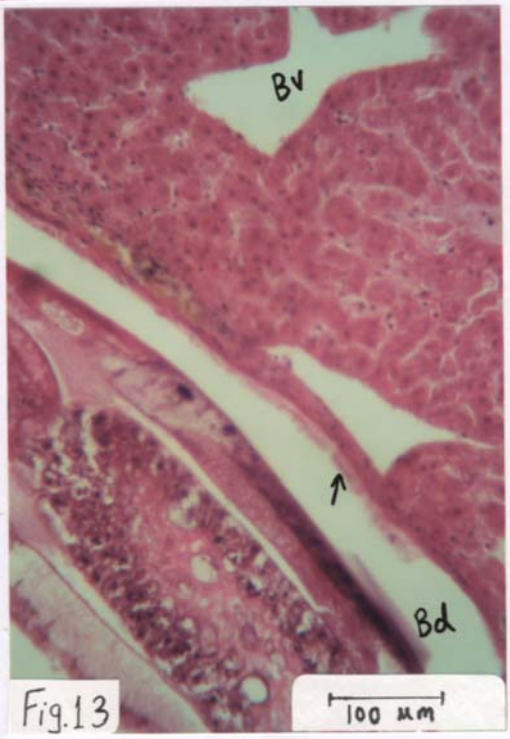

Fig.13

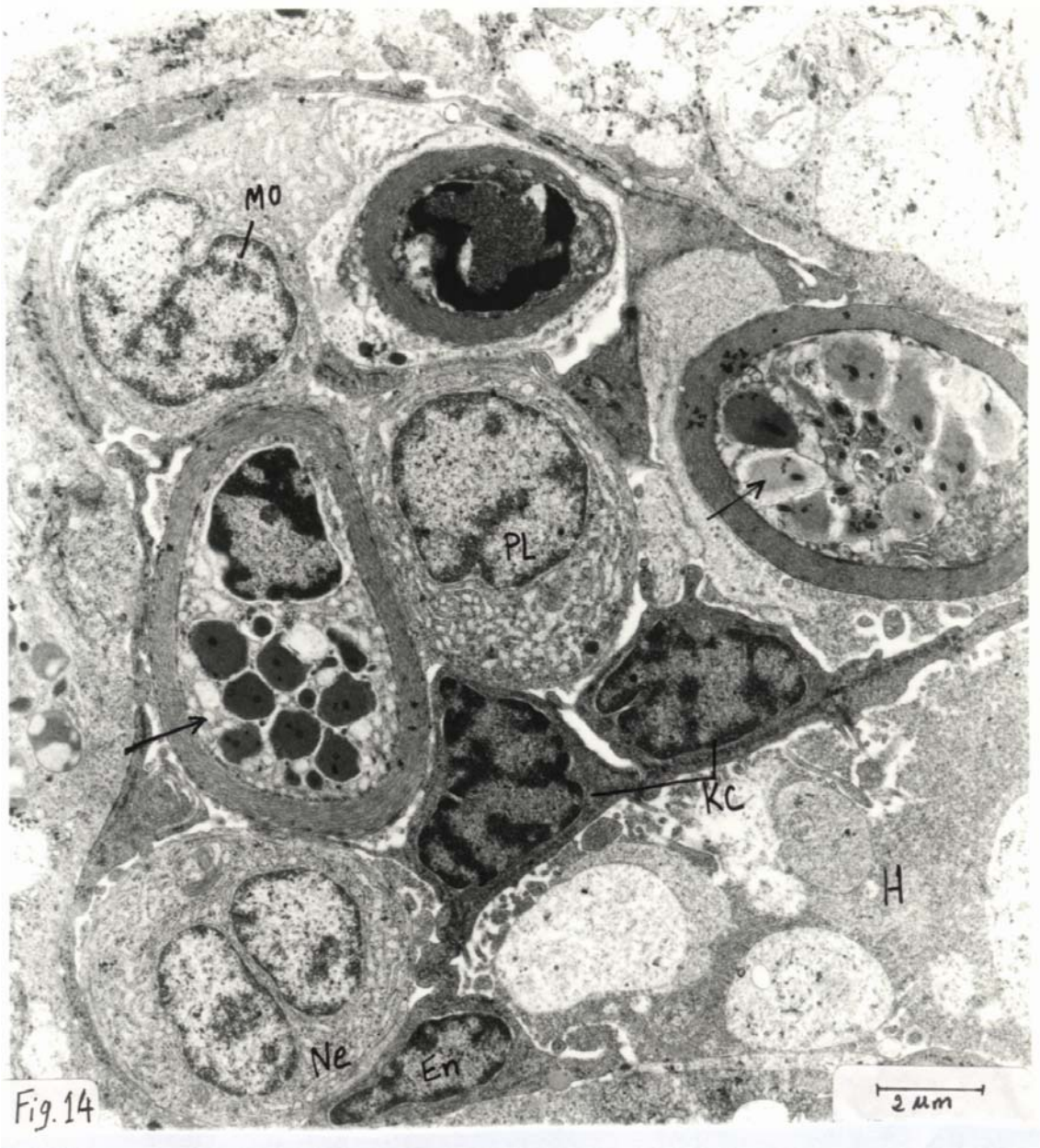

Fig. 14

EFFECT OF POLLUTANTS IN COASTAL WATER OF JEDDAH ON
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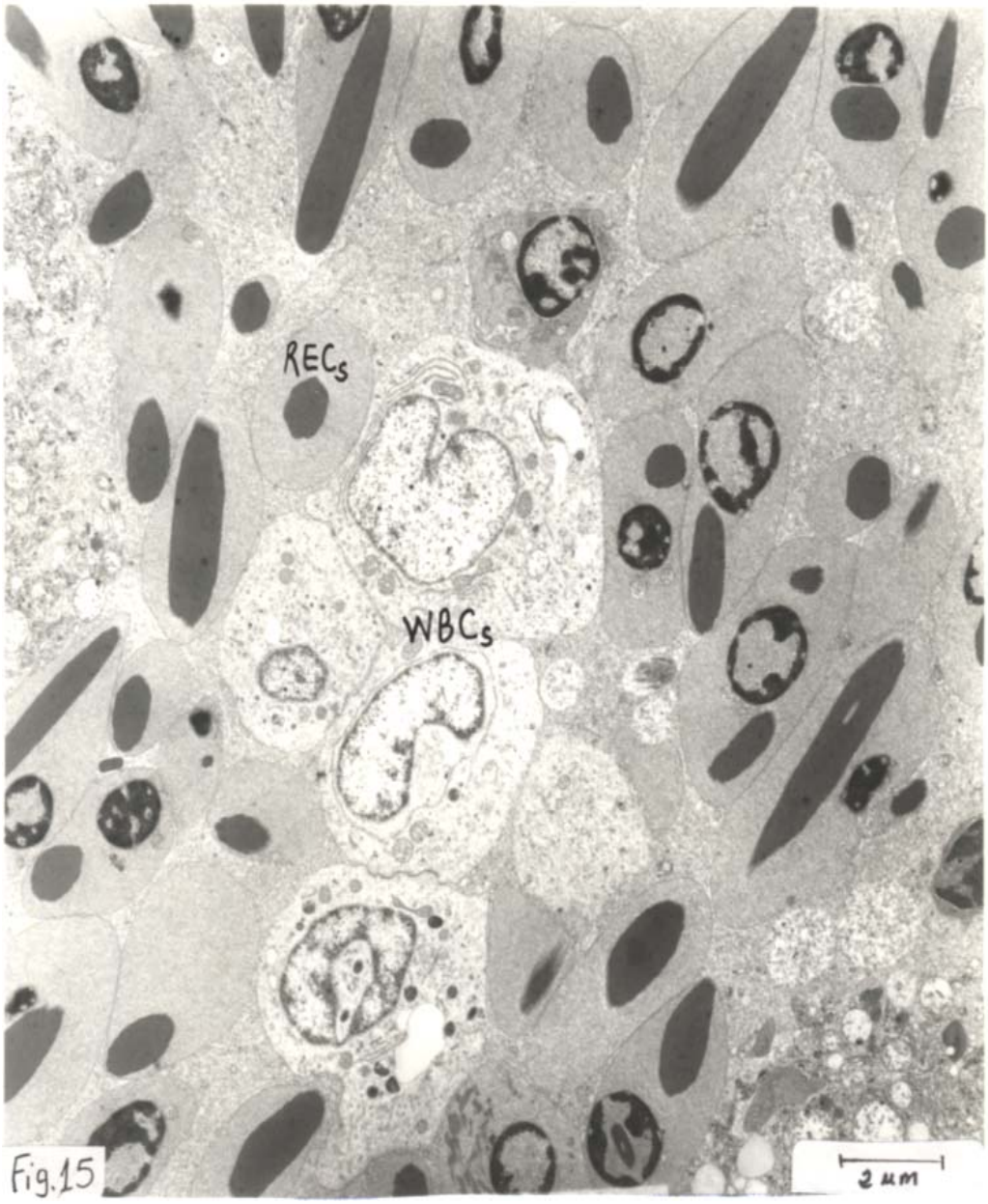

Fig.15

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EXPLANATION OF FIGURES

Fig. 1: a - Map showing the five Sampling stations of the study area of Jeddah fish market.

Fig. 2: Normal liver of *Siganus rivulatus*. Notice: hepatocytes (H); bile duct (Bd); Melanomacrophage center (MMC); blood vessel (BV) (bar = 50 μm).

Fig. 3: A high magnification view of fig. 2 showing polyhydral hepatocytes with granular esinophilic cytoplasm and generally more then one nuclei (arrows); sinusoids (S).

Fig. 4: Hepatocytes are highly vacuolization with a marginally compressed ncleus; S = Sinusoid (bar = 50 μm).

Fig. 5: E.M of the affected hepatocyte showing vacuolization and focal lytic cytoplasm: (bar = 2 μm):

Fig. 6: Liver section showing ballooning degenerated (Bd) hepatocytes. (bar = 20 μm)

Fig. 7: Liver section pigment deposition in macrophages infilterated around a blood vessel (Bv). (bar = 35 μm)

Fig. 8: Liver section showing granuloma formation (G) (bar = 100 μm).

Fig. 9: Enlarged view of figure 8, centred caseous matter, marginal layer of caseous matter, layer of epithelial cells and fibrocytic layer.

Fig. 10: E. M. of liver cell. Note: large vacuoles (V) with flowith flocculent material and myeline – like structure (my); vesiculated rough endoplasmic reticulum (RER); atrophoid and degenrated mitochondria (M); lysosome (Ly) (bar = 2 μm).

Fig. 11: Parasitic infected liver. Notice: Parasitic warm (P) inside bile duct with necrosis of bile duct epithelium (bar = 50 μm).

Fig. 12: Bile duct (Bd) containing parasitic worm (P) shows a thickened fibrous layer: loose connective tissue arranged in concentric.

Fig. 13: Parasitic infected. Notice: Parasitic worm in side dilated bile duct with flatlered epithelia and damaged brush border (arrow) Notice also hepatocyte necrois and dilated blood vessel (BV) (bar = 100 μm).

Fig. 14: E. M. of Parasitic lesion showing cysts (arrows) inside small view. Leukocytic accumulation (Plasma cell (PL); neutrophial (Ne); monocyte (Mo); endothelial cell (En); activated kuppfer cells (Kc) damaged hepatocytes (H) (bar = 2 μm).

Fig. 15: E. M of liver section showing what may be metal deposites in nucleated red blood cells (RBCs) in liver sinusoid. Notice also accumulation of white blood cells (WBCs) at center (bar= 2 μm).