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Histological and ultrastructural alterations in mullet fries exposed to industrial effluents

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Abstract

Sugar Beet manufacturing company waste (El Dakahlia Governorate) caused mass mortality in the wild mullet fries (*Mugil cephalus and Mugil capito)* collected from El-Kasarah Marine Fish Fry Center, during the period of late autumn (15/12/2008) till late winter (17/3/2009). Water samples collected near source of waste emission up to 750 m away revealed high levels of trace metals (Pb, Fe, Cd, Cu and Zn) which, were reflected on their bioconcentrations in fry organs. Effect of metal waste on mullet fries were investigated using histological and ultrastructure examinations. The results revealed degenerative changes in vital body organs (gills, liver, kidney and gonads) .The respiratory epithelia of gills appeared reduced and vacuolated with breakdown of pillar cell system and hyperplasia of chloride cells. Ultrastructure observations revealed vacuolation, necrosis of cytoplasmic organelles and vesiculation and proliferation of the endoplasmic reticulum in mullet fry liver.. Also the waste exert inhibitory effects on gonadogenesis.At late winter (17/3/2009) the quality of water improved but, still the waste exerts an effect on fish organs causing mass mortality especially during catch and transportation to fish farms.

Key words: mullet fry, histopathology, ultrastructure, heavy metals.

1. Introduction

Chemicals derived from agricultural operations and industrial effluents as metals are very persistent pollutants they get accumulated in the soil, water, sediments and last but not least in fish as the final link of food chain (Havelkovà *et al*. 2007). Organisms typically detoxify heavy metals, by binding them irreversibly to proteins in their bodies. This results in an accumulation of toxic materials in fish organs, especially in the liver, kidneys and gonads (Andreji *et al*., 2006). The distribution of metals in tissues of different fish species, has been described by Staniskiene *et al*., (2006) and Spurný *et al*., (2002) causing large numbers of neurological disorders, and fish diseases (Ipinmoroti, *et al*., 1997), hormonal and reproductive problems (yamaguchi *et al*., 2007 and Sumpter 2005). Mercury, cadmium and lead have been found to be estrogenic chemicals that disrupt the endocrine/ reproductive/ hormonal systems (Voegborlo, *et al*., 1999). Manganese has been found to damage males reproductive system resulting in infertility and damaging hormone production (Colburn, 1993).

Besides the evaluation of chemical and physical parameters, evidence arising from histocyto-pathological examinations have been recognized as valuable tool to evaluate the impact of pollutants on fish (Au, 2004).The presence and abundance of certain cell types, such as chloride cells (Ccs) in tissue sections, and structure such as macrophage aggregates (MAs) are indicators of stressful conditions which in the absence of definite histo- pathological changes could provide information regarding the general health status of fish (Giari *et al*., , , 2006,2007,2008).

The gills, liver and kidney are commonly the primary target organs for many chemicals because of the role they serve within the body. The gills are responsible for respiration, osmoregulation, acid base balance and nitrogenous waste excretion (Au, 2004). Biotransformation of organic xenobiotics, excretion of harmful trace metals, food digestion and storage, and the metabolism of sex hormones are the main hepatic function (Hinton *et al*., 2001). The kidney play an important role in osmotic homeostasis and excretion of metabolities (Au, 2004). Histopathological changes in fish due to the action of metals have been the subject of numerous studies (Fadel and Gaber 2007; Figueiredo-Fernandes *et al*. 2007; Devlin 2006 and Banerjee and Chandra 2005).

Family Mugilidae is an economically important fish cultured in Egypt. It is commercially cultivated in fresh and brackish water ponds, as well as in cages in coastal waters (Essa *et al*., 2005).

In Egypt, most marine and brackish fish culture activities rely on the use of wild seed from ten collection sites along the Mediterranean coast. El-

Kasarah Pump Station, Dakahlia Governorate, is one of these sites. This area is subjected to change in water quality due to Sugar Beet Manufacturing Company (SBMC), its waste has a hazardous effect on the wild mullet fry, causing mass mortality affecting fry natural sources.

Therefore, the objectives of the present study to analyze trace elements in muscle, viscera and gills, with estimating the exposure levels of these metals in mullet fry organs. Furthermore to characterize the structural and ultrastructural effect of these metals on gills, liver, kidney and gonads of mullet fry species.

2. Materials and methods

El-Kasarah Pump Station, Dakahlia Governorate, Egypt is one of several wild mullet fry collecting centers along the Mediterranean Coast .Water samples and wild mullet fry (*Mugil cephalus and Mugil capito)* were collected from sites near Sugar Beet Manufacturing Company (SBMC) source of waste emission till 750 m away through El-Kasarah fish fry collecting center . The water samples are kept at 4°C till analyzed according to Franson (1980). Control fries were bought from fish farm at kilo 21. Fish fry were examined morphologically for any variation in skin color, bleeding and fin rotting then, dissected and the gills, liver and abdominal cavity were observed.

Segment of gills, liver and kidney regions were fixed in 10% buffered formalin, dehydrated, embedded, sectioned and stained with haematoxylin and eosin for light microscopy examination.

Tissues of gill and liver of (2mm x 2mm) thickness were fixed in 4% gluteraldehyde solution in 0.1M cacodylate buffer pH7.4 at 4C°, dehydrated, embedded, sectioned and stained with uranyl acetate and lead citrate and examined with a JEDL, transmission electron microscope (TEM) (100C x JEOL. Itd, Tokyo, Japan) for ultrastructural examination. For trace element analysis samples of individual tissues (muscles, gills and viscera) were analyzed fresh without prior drying according to Fallis (1982). They were homogenized with a stainless scalpel. Two portions of each tissue were digested separately in test tubes. Concentration of elements (Fe, Mn, Cu, Zn, Cd, and Pb) in water and tissue samples was measured using atomic absorption spectrophotometer "Jarrell Ash Model 850". All determinations were performed in duplicate and the results were expressed as ppm.

3. Results

3.1. Chemical analysis of SBMC waste and its impact on mullet fry

Chemical analysis of (SBMC) wastewater at site of source emission, El-Kasarah Fish Fry Collecting Centre showed high levels of total solids (volatile and suspended) as well as metals, compared to control Table 1.

The lethality of the waste is attributed mainly to the high metals level and oxygen depletion due to high organic matter in effluent. The concentration of metals decreased as we go away from emission source. Water samples collected at late winter showed improvement in water quality, but solids and metals level still high compared with control values. Bioconcentration of metals in tissues of mullet fry collected from El-kasarah region and from kilo 21 farm (control) were represented in tables (2 &3).

Table 1: Chemical characteristics of wastewater from El- kasarah fish fry collecting centre.

Parameters	Time of sampling						Sea water (Control)
	Late autumn 15/12/2008			Late winter 17/3/2009			
	At emission source	500m away	750m Away	At emission Source	500m Away	750m away	
pH	8.0	7.8	7.6	7.6	7.5	7.5	7.4
Turbidity NTV	69 . 5	67. 20	56. 30	67.6	56.4	54. 9	25.0
T.S mg/L	8965	7667	6925	6212	5825	4192	2567
V.S mg/L	1233	1099	1116	986	720	749	409
S.S mg/L	1886	1672	1610	1214	1032	1192	639
Total metals (mg/L)	18. 540	13.033	9.914	10.678	7.85	5.67	1 mg/L
Cu	0.842	0.523	0.312	0.512	0.14	0.13	0. 20
Pb	3.696	2.286	1.938	1.923	1.90	1.62	0.12
Zn	0.908	0.236	0.142	0.143	ND	ND	0.18
Mn	4.801	3.910	2.121	2.62	2.50	2.10	0. 22
Cd	5.310	3.450	2.920	3.71	2.10	0.91	---
Fe	2.983	2.628	2.481	1.50	1.21	0.91	0. 31

T.S: total solid V.S: volatile solid S.S: suspended solid

Table 2: Bioconcentration factor of metals in mullet fry tissues collected from El - kasarah region.

Conc. of metals (ppm)	Late autumn 15/12/2008			Late winter 17/3/2009		
	Viscera	Gills	muscles	Viscera	Gills	muscles
Cu	24.76	15. 53	4.93	17. 97	12.10	2.99
Pb	48.68 14.05	25. 63	6.73	22.40	14.05	3.78
Zn	115.7	12 . 23	4.89	11.11	8.70	4.74
Mn	180.49	86.13	53. 95	79.61	52.42	30.77
Cd	41. 61	128. 25	84. 62	150.38	58.74	38.40
Fe		69. 87	15..33	36 . 28	48..99	12..86

Table3: Bioconcentration factor of metals in tissues of mullet fry from kilo 21 farm (control).

Conc. of metals (ppm)	Late autumn 15/12/2008			Late winter 17/3/2009		
	Viscera	Gills	muscles	Viscera	Gills	muscles
Cu	2.12	1.40	0.82	1.51	1.13	0.71
Pb	2.31	1.69	1.08	1.65	1.22	0.86
Zn	2.12	1.40	0.92	1.84	1.31	0.86
Mn	2.06	1.55	0.81	1.79	1.4 0	0.69
Cd	----	----	-----	----	----	-----
Fe	2.29	3.08	0. 95	1.81	2 . 28	0.64

Bioconcentration of metal = 100 {conc.in organ / conc. in water}

The concentrations of metals in organs of fish fry from El-Kasarah region (Table 2) were very high compared to their concentration in fries from kilo 21 farm (control) (Table 3).

The level of metals in tissues as a whole is reflection to its concentration in water environment i.e metals of the higher concentrations in water accumulated by greater levels in fish tissues. Table (2) showed different affinities of metals of bioaccumulation in different tissues. Metal tissue concentrations were generally the highest in viscera and lowest in muscles. The concentration of metals in viscera tissues were in the order Cd > Mn > Pb > Cu > Zn. Gill tissue was the only tissue that showed the highest iron bioconcentration (69.87ppm). At late winter a rapid decline in metals level was observed due to dilution and degradation process. Gills and muscles showed a rapid decline whereas viscera showed a prolonged retention of Cd (150.38 ppm). Viscera still contain the highest levels of Mn, Pb Cu and Zn (79.61, 22.40, 17.97 and 11.11 ppm respectively). While the gills have the highest iron level (48.99 ppm). The decreasing rate of essential metals as (Cu, Fe, Zn) was less compared to non-essential ones as lead and manganese .

3.2. Morphological and anatomical examination

The morphological examination of mullet fries (average length 3.8 cm) collected from El- Kasarah centre exhibited erratic swimming hyperventilation and excessive mucus production on gills. Changes in pigmentation and fin rotten were also observed. Fish dissection showed bleeding on gills, bloody fluid in abdominal cavity and fading of liver color.

3.2.1. Histopathology and ultra structure examination

3.2.1.1. Gills

Gills of control group of mullet fries have a normal morphological structure, where erect rows of distinct and regular secondary lamellae running perpendicular to the upper and lower surfaces of primary lamellae. The respiratory epithelium covered both types of lamellae. Chloride cells were visible along the basilamellar region (Figures1&5). They are spheroid with light vesicular cytoplasm loaded with a large number of dense diffusely scattered mitochondria and eccentric nuclei. Mucous cells were sparsely distributed in the primary lamellar epithelium. Below the epithelium does exist lamellar blood sinuses separated by pillar cells. The pillar cell has 4 arms and a centrally located nucleus Figure 2. Its homogeneous cytoplasm contains a few mitochondria, few ribosomes and little rough endoplasmic reticulum. The 2 arms on each side of the cell extend to those from an adjacent cell to form a blood lacuna. The basement membrane lies between the epithelial layer (one or two cells thick) and the pillar cell system.

The epithelial cells were long and flat and had a prominent nuclei. There was extensive endoplasmic reticulum and the Golgi apparatus was large (Figure 3). Located in a similar position of the epithelial cells are the mucus cells (Figure 4). The mucus cells are small round cells filled with secretion having an apical opening. They consist of membrane bounded vesicles of mucus, each vesicle has a fine granular appearance. At the periphery of the cell there is an extensive rough endoplasmic reticulum, numerous free ribosomes are present in the cytoplasm and few mitochondria. At the base of the cell Golgi apparatus can be recognized from which the mucus vesicles originate. Extensions of neighboring epithelial cells are overlaying the mucus cell and surrounding their apical aperture.

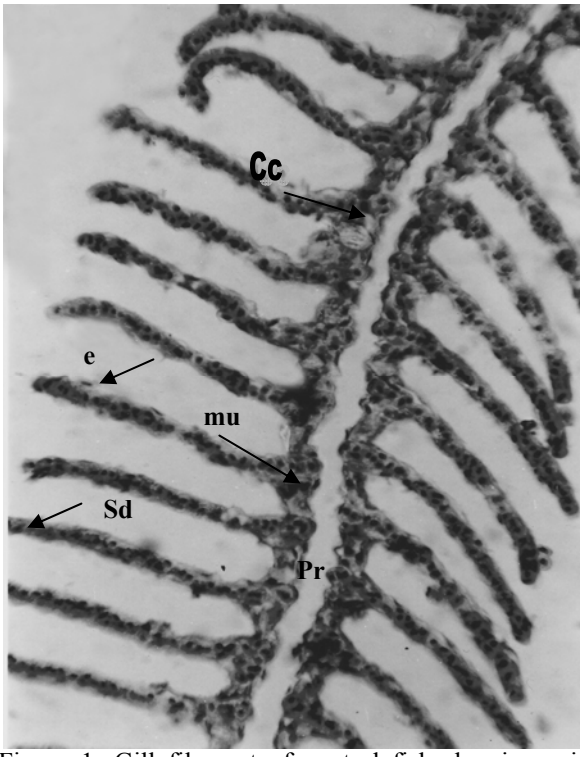

Figure 1: Gill filament of control fish showing primary (Pr) & secondary (Sd) lamellae, epithelial cells (e), mucus cells (mu) and chloride cells (Cc). 400X

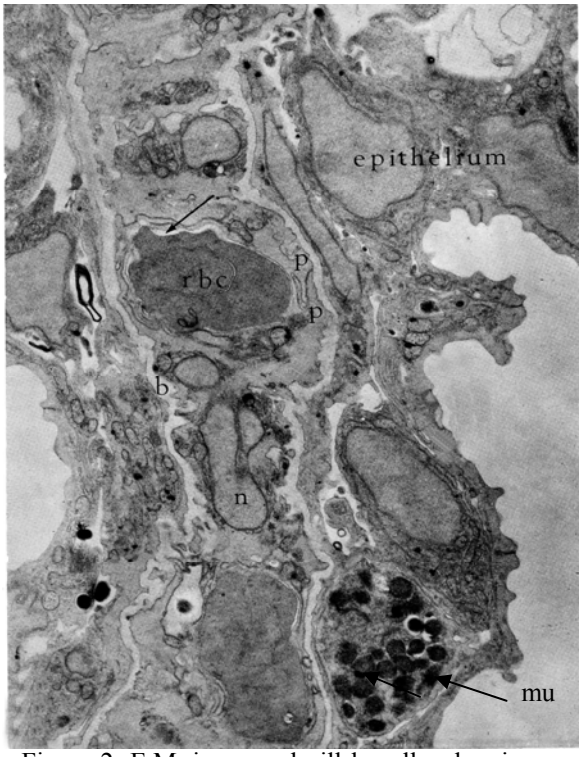

Figure 2: E.M. in normal gill lamella, showing red blood cell (rbc) within the lacuna (arrow) limited by the pillar cell "arms" (p). Nucleus of pillar cell (n). Basement membrane (b) lies under epithelium. 5.500 X

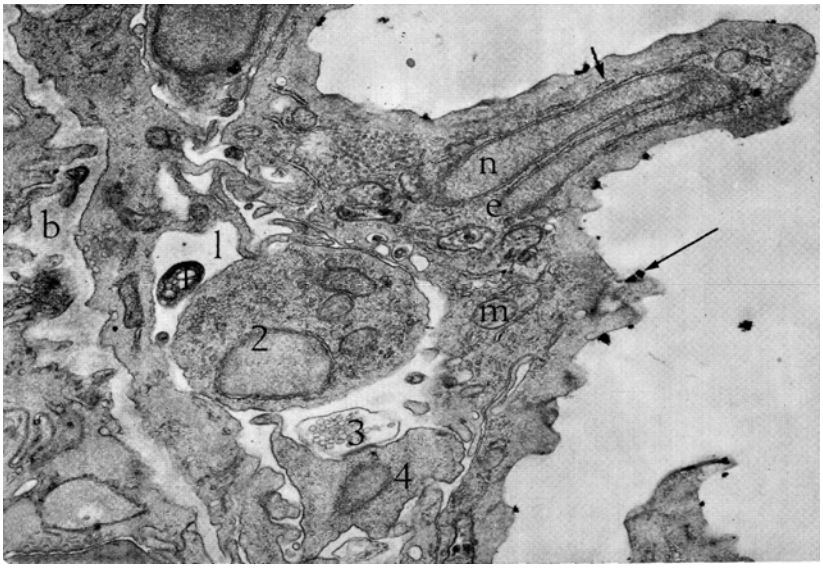

Figure 3: Lacunal space showing four different blood cell forms. Dense particles (arrow) adhere to surface of cell. Epithelial cell containing mitochondria (m), prominent nucleus (n) and endoplasmic reticulum (small arrow) 10.000 X

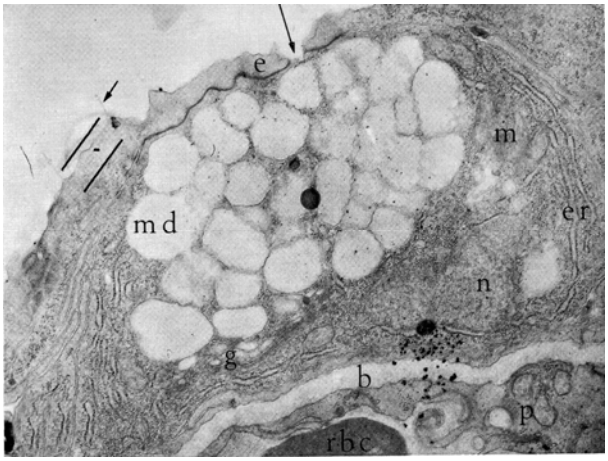

Figure 4: Mucus cell from control fish showing apical aperture (arrow) surrounded by overlying extension of epithelial cells (e) and basal nucleus (n) with extensive endoplasmic reticulum (er). Mucus droplets (md) originate from Golgi apparatus (g) few mitochondria (m) in the cytoplasm. Hairlike extension (arrow) of apical homogeneous layer (parallel lines). 10.000 X

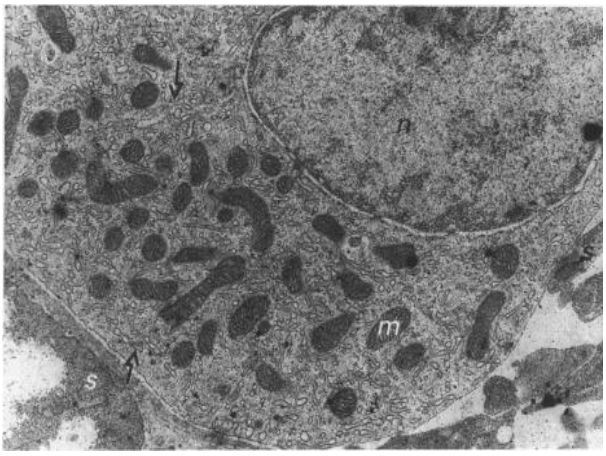

Figure 5: Chloride cell from control fish showing eccentric nucleus (n), mitochondria with distinct cristae 30000 X.

Gills of fries collected from El-Kasarah region showed histopathological and ultrastructural alteration. The epithelial layer of secondary lamellae was reduced in size and depart from pillar cell system due to edema. The epithelial cells themselves were vacuolated (Fig. 6). The most obvious change was in the relationship of mucus cells to chloride cells very few mucus cells were found, whereas chloride cells were frequent. Ultrastructurally the chloride cell posed dark granules within the cytoplasm, an extensive smooth endoplasmic reticulum and large number of damaged mitochondria with cristae regression. The granules that were present within the mitochondria seemed similar to those just outside (Fig. 7).

Large vacuoles were found within the cytoplasm of epithelial cells (Fig. 8). The epithelial cells also contained vesicular bodies of variable size that appeared to be auto-phagosomes, since the vesicular content was partially formed of rough endoplasmic reticulum (Fig. 9). Other cellular vesicles were uniformly bounded by double membranes, they were either empty or contained a homogeneous, moderately dense material (Fig. 10).

At late winter (March 2009) gills structure showed lifting of respiratory epithelia in some area and hyperplasia in others. Hypertrophy and hyperplasia of chloride cells at the base of gill filaments and breakdown of pillar cell system were frequent (Fig.11).

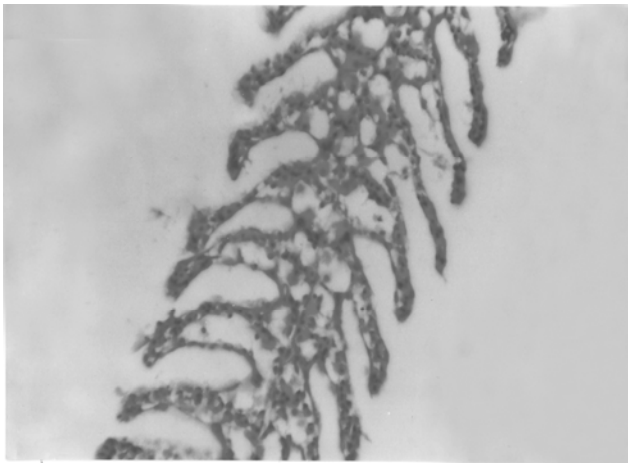

Figure 6: Lamellae from fish subjected to waste. Numerous cells are vacuolated and desquamated from pillar cell substructure that showed destruction. 300X

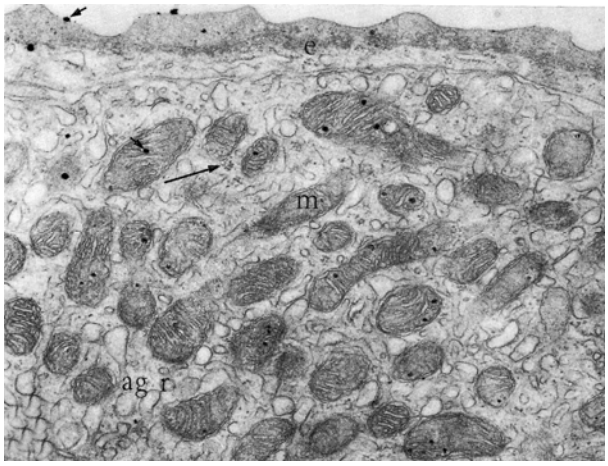

Figure 7: Chloride cell from fish exposed to waste showing overlying epithelial cell (e). Mitochondria (m) with deformed cristae, extensive agranular reticulum (agr) and dark granules on external membrane of epithelial cell are similar to those within mitochondria (arrowheads). 30.000 X

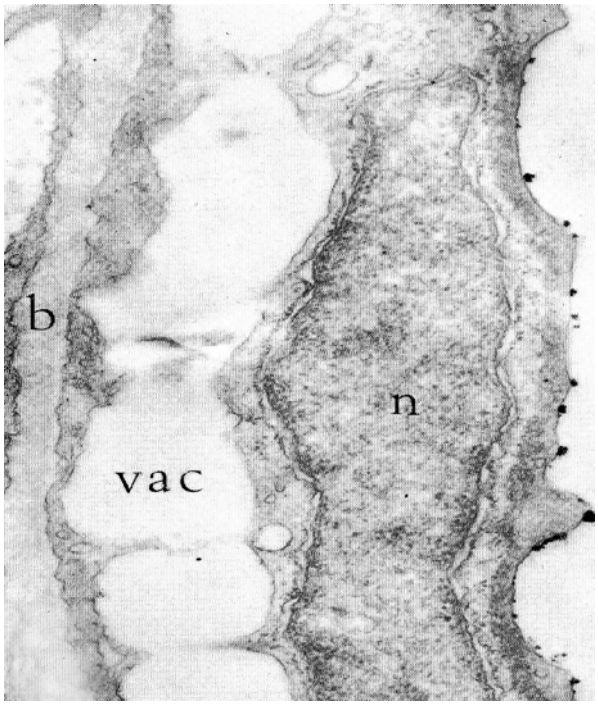

Figure 8: Large cytoplasmic vacuoles (vac) within epithelial cells of fish exposed to waste 20.000 X

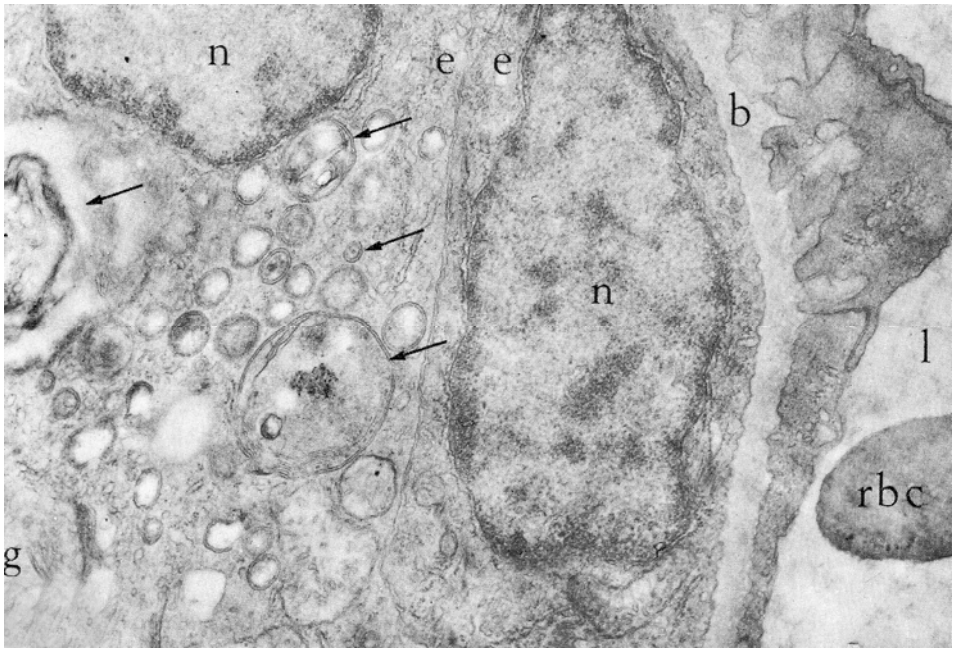

Figure 9: Portion of two epithelial cells (ee) of fish exposed to waste with cell vacuoles containing elements of endoplasmic reticulum (myline figures) arrows & proliferated SER. 20.000 X.

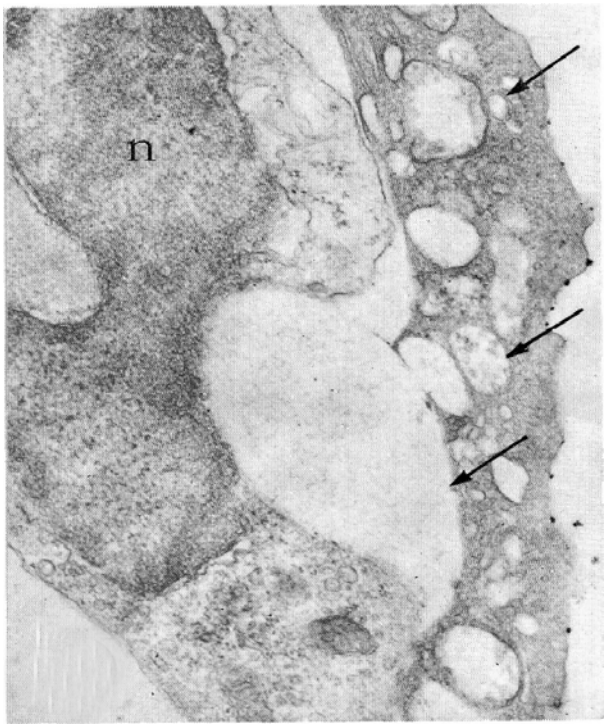

Figure 10: Vesicular system (arrows) in apical region of epithelial cell of fish exposed to waste & deformed nucleus (n) 20.000 X

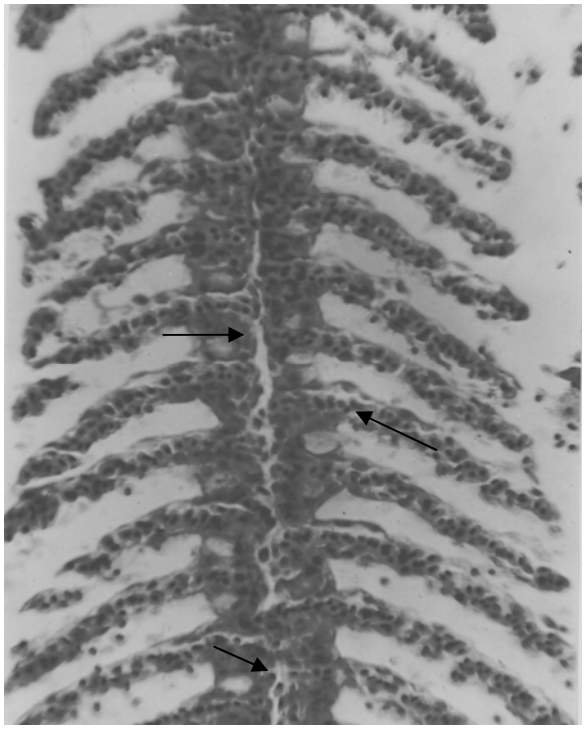

Figure 11: Lamella of fish at late winter showing increased chloride cells (arrow), hyperplasiaof respiratory cell 400 X.

3.2.1.2. Liver

In liver of control group hepatocytes are polygonal with central or eccentric nuclei having marginal heterochromatin . These cells are supported by a fine, reticular network of connective tissue. Venous blood enters the liver and branches into capillaries known as sinusoids which are surrounded by hepatocytes (Fig. 12). The hepatocytes were characterized by a regular cytoplasm compartimentation. Numerous round to oval mitochondria in association with a well developed rough endoplasmic reticulum system were observed around a central spherical nucleus with a prominent nucleolus. At the periphery of the cell, numerous glycogen granules were seen distributed throughout the cytoplasm (Fig. 13).

In metal-contaminated fry, an abnormal architecture of the normal cord-like arrangement was evident. The hepatic cords cells appeared compactly arranged with a strong vacuolization (steatosis) of hepatic tissue. Hepatocyte cytoplasm was entirely occupied by a large lipid vacuole displacing the nuclei to the periphery. Widening of sinusoids and stasis of blood vessels, were also observed. Macrophage cells were frequently seen (Fig. 14).TEM examination showed that the hepatocyte cytoplasm frequently appeared full of the lipid droplets (Lipidosis) (Fig. 15). Cellular organelles especially the endoplasmic reticulum and mitochondria were particularly affected with comparison to hepatocytes of control fish. The cisternae of the rough endoplasmic reticulum were dilated and vesiculated (Fig. 16). The most frequent pathological modifications were the proliferation of SER and swelling of mitochondria with regression of cristae and transparency of the matrix or condensation with an electron dark appearance (not seen in figure). The nucleus also showed alterations with dilation of the nuclear envelope and an accumulation of heterochromatin (Fig. 17).

At late winter (17th March 2009) there were slight blood congestion in sinusoids and hydropic swelling of hepatocytes in which the nucleus retained a nearly normal shape (Fig. 18). Also a slight accumulation of dark minute granules was observed in some hepatocytes. Ultrastructurally the nucleus has a well developed nuclear membrane. Limited perinuclear cytoplasm containing normal rough endoplasmic reticulum and mitochondria and normal nuclei with expected distribution of heterochromatin and normal nuclear morphology (Fig. 19), lipid droplets were also detected in hepatocytes.

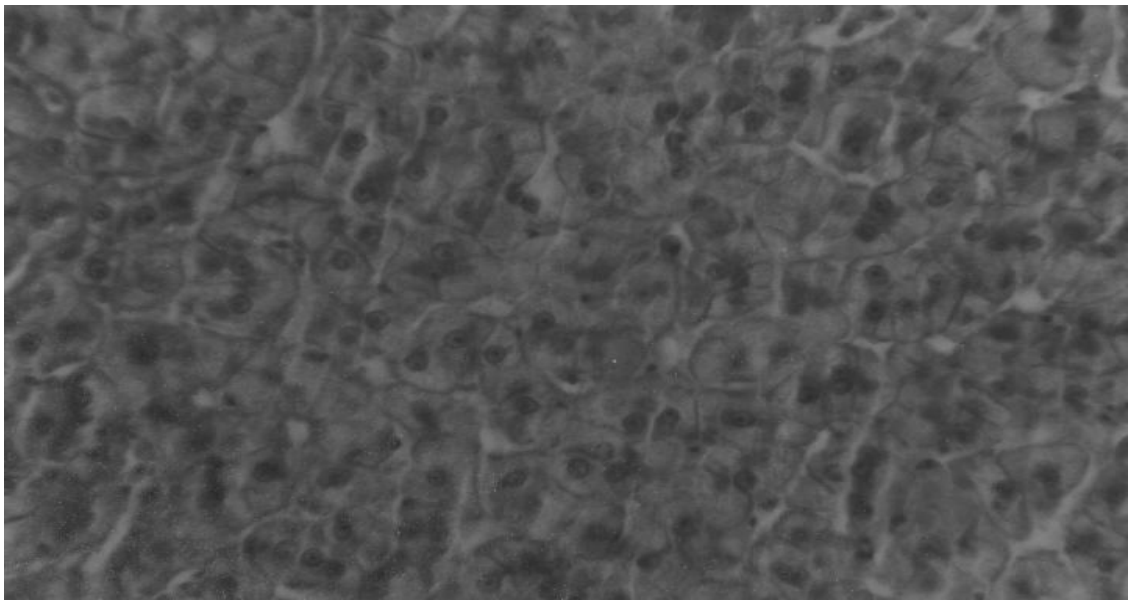

Figure 12: Liver tissue of control fish, showing polygonal shaped hepatocytes in cords. 300X

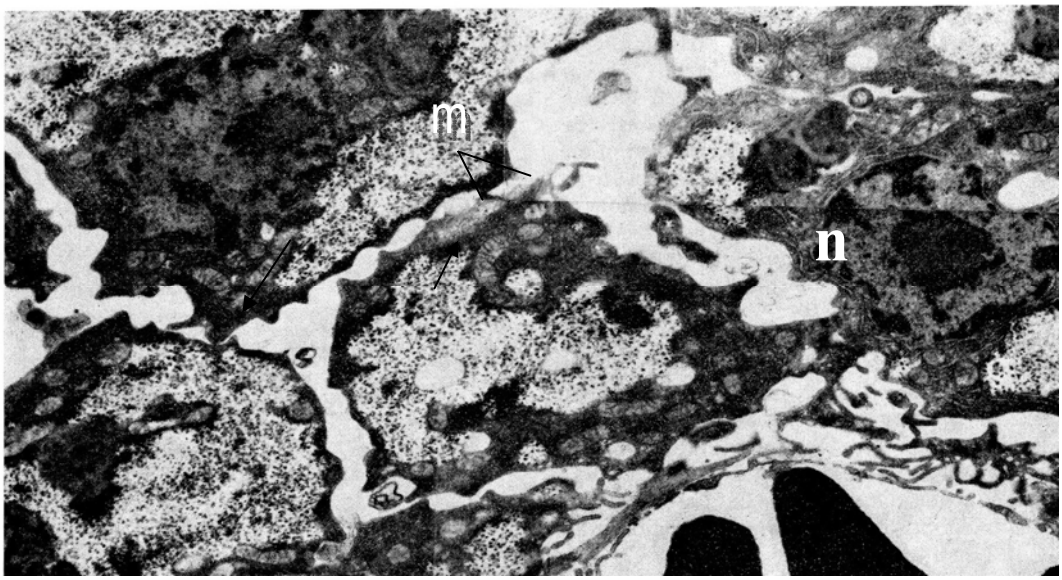

Figure 13: Hepatocyte profile of control fish, note: glycogen reserves (arrow), normal nuclei (n), cytoplasm with rough endoplasmic reticulum and normal mitochondria (m) 10.000 X

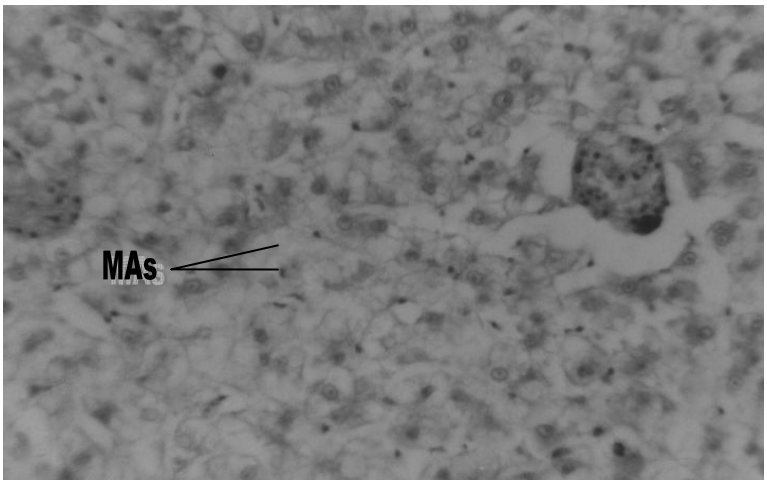

Figure 14: Liver of waste exposed fish showing deposition of large fat droplets indicated by clear areas. Notice macrophage cells in liver tissue (MAs). 300 X

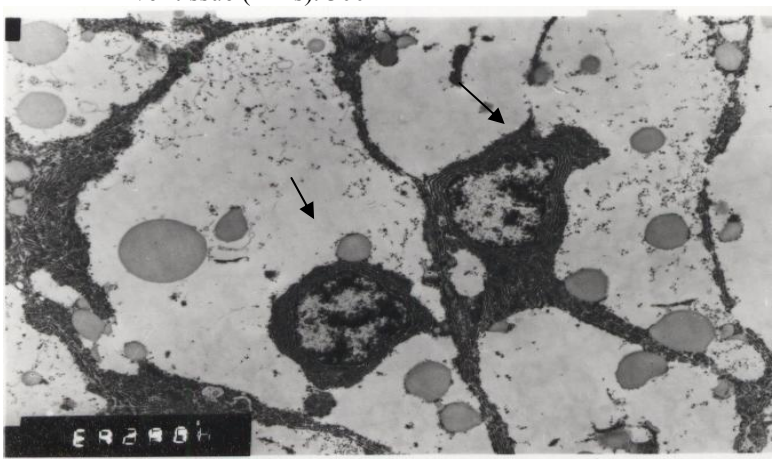

Figure 15: TEM of liver tissue of waste exposed fish showing limited cytoplasm (arrow) and increased lipid droplets. 10.000 X

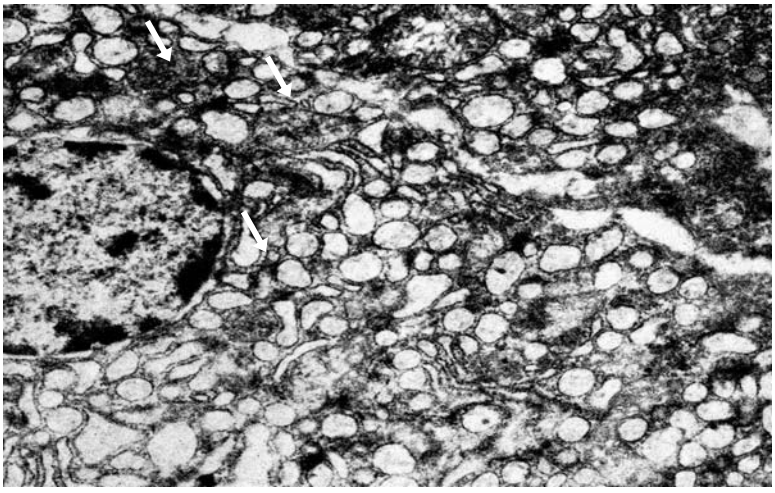

Figure 16: Hepatocyte of effluent exposed fish showing proliferated SER and mitochondria swelling (arrow). 30.000 X

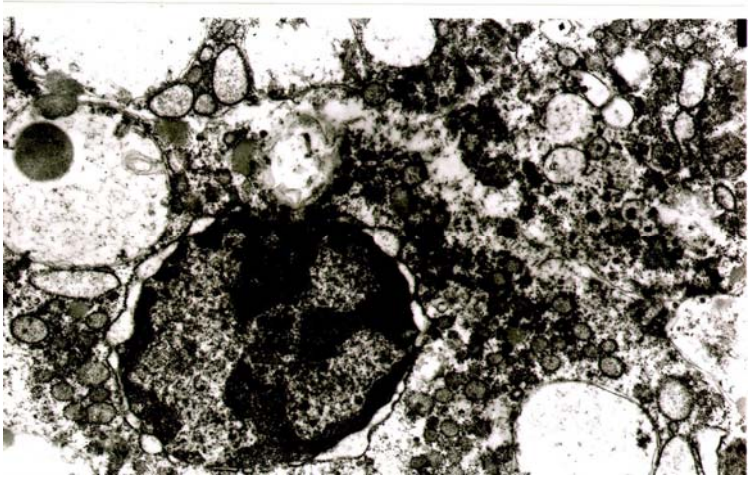

Figure 17: Hepatocyte profile of waste exposed fish, note dilation of nuclear envelope and accumulation of heterochromatin. 30.000 X

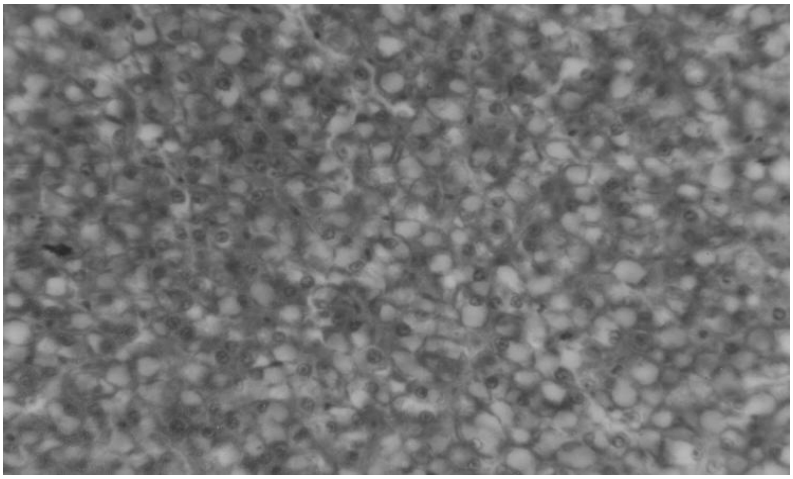

Figure 18: Liver of fish at late winter showing hydropic swelling of hepatocytes 300 X.

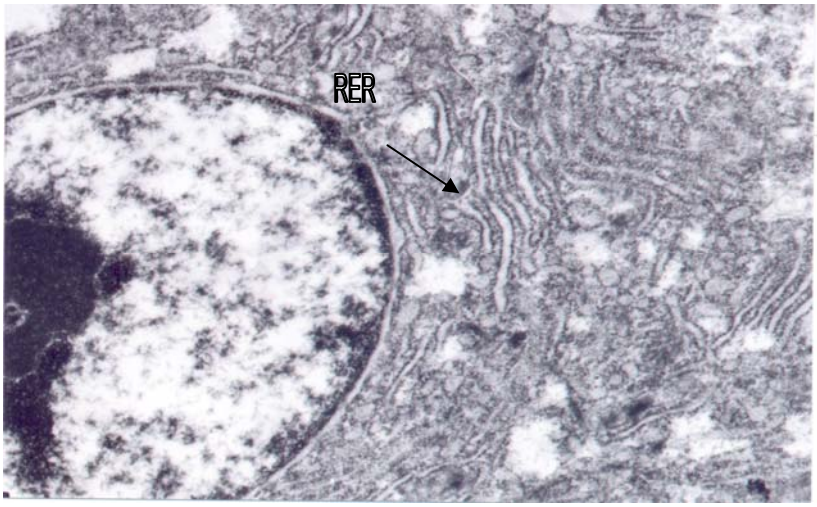

Figure 19: TEM of liver tissue of fish at late winter showing normal heterochromatin distribution, nuclear envelope and RER. 40.000 X

3.2.1.3. Kidney

Kidney of control group is composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The kidney tubules are lined with columnar epithelial cells with basal nuclei. The tubules surrounded by hemopoietic tissue (Fig.20). Kidney of polluted fish showed hydropic swelling and hypertrophy of tubular cells with, nuclear deterioration and pyknosis. Hyaline droplets were accumulated in tubular cells. The tubular cell contour wasn't clearly distinguished and the luminal caliber of the tubule decreased and contained much dense materials. The hemopoietic tissue was necrotic and reduced in volume (Fig. 21).

At late winter hydropic swelling was observed in some tubules beside nuclear necrosis. Hemopoietic tissue in between tubules retain its normal shape (Fig. 22).

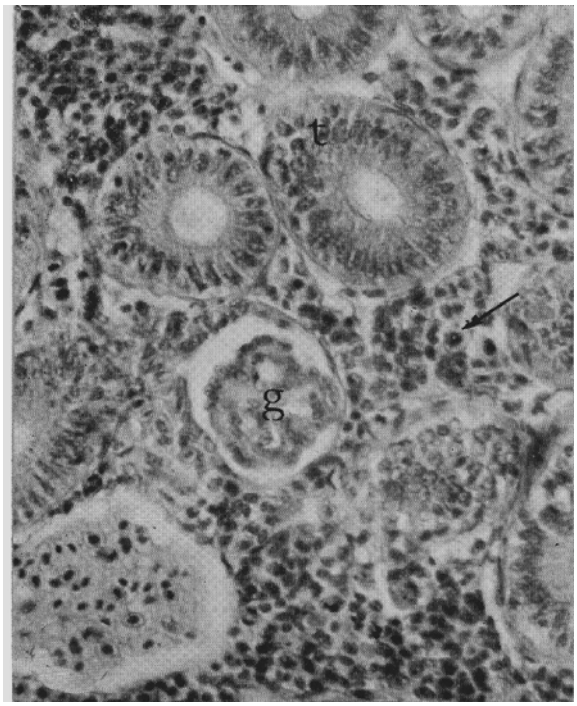

Figure 20: Kidney of control fish, note tubules (t), glomerulus (g) and hemopoetic tissue in between. Nucleated red blood cell (arrow). 400 X

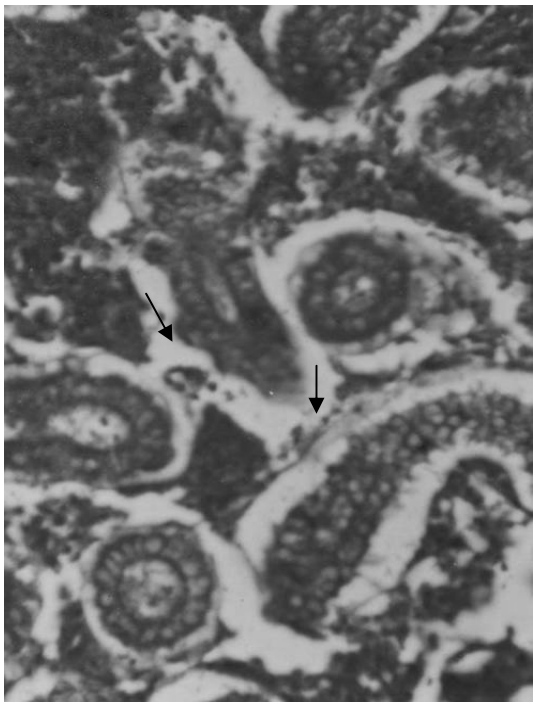

Figure 21: Kidney of waste exposed fish showing hydropic swelling and hypertrophy of tubular cells with nuclear deterioration and pyknosis. Necrotic hemopoetic tissue (arrows). 400 X

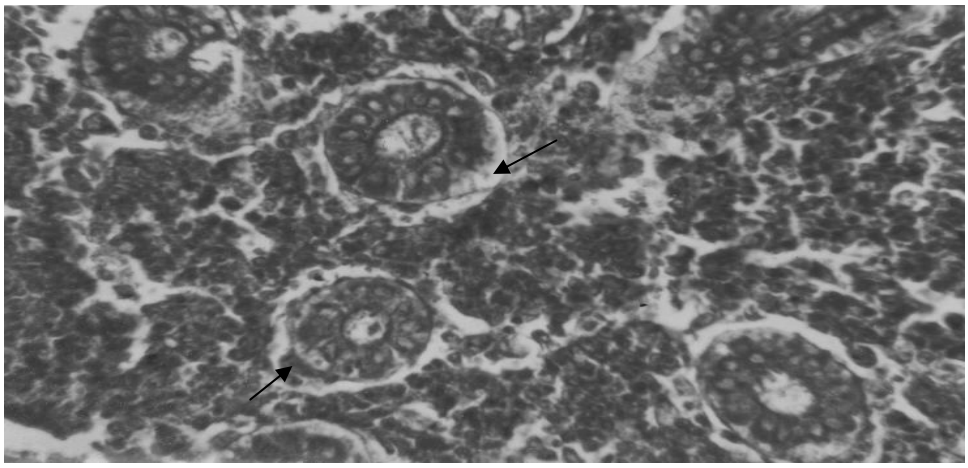

Figure 22: Kidney of fish at late winter showing edema of some tubular cells (arrow) , normal shaped hemopoetic tissue. 400 X

3.2.1.4. Gonads

Gonads of control mullet fry consist of undifferentiated germ cells in stroma of connective tissue and blood vessels inbetween (Fig. 23). Waste exert an inhibitory effect on gonadogenesis. Degeneration of germ cells occurred and the gonadal stroma was filled with connective tissue (Fig. 24). In some samples few germ cells appeared scattering in gonadal tissue that had a vacuolar appearance (Fig. 25).

Later on at 17/3/2009 an improvement in gonadal structure occurred were the number of germ cells increased, but still the gonads possessed vacuoles (Fig. 26).

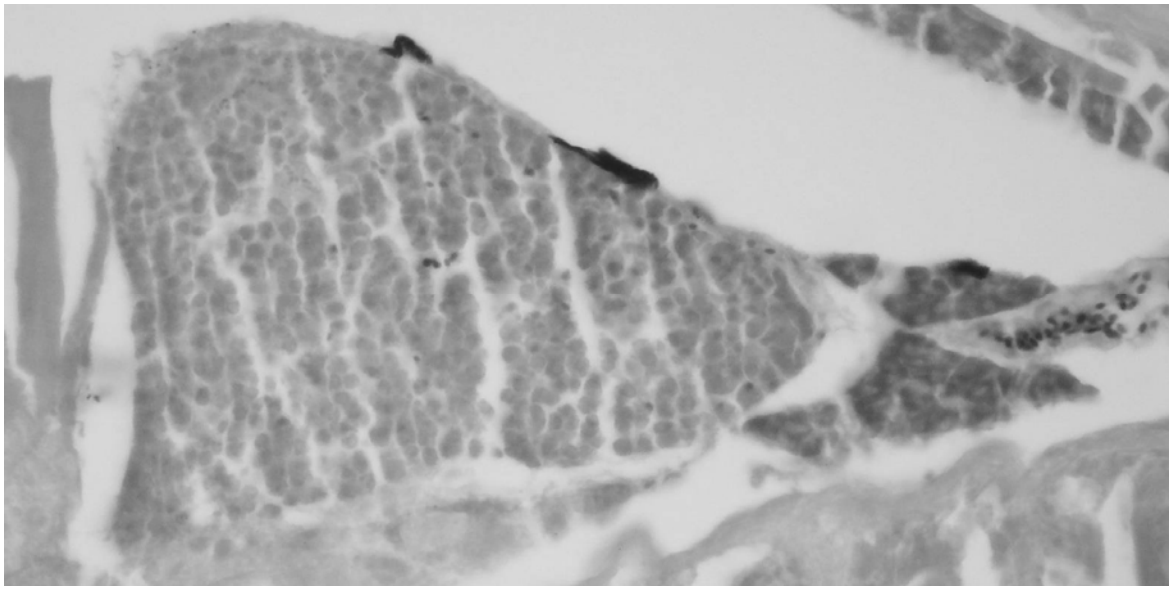

Figure 23: Gonad of control fish showing germ cells with blood vessels in between. 400 X

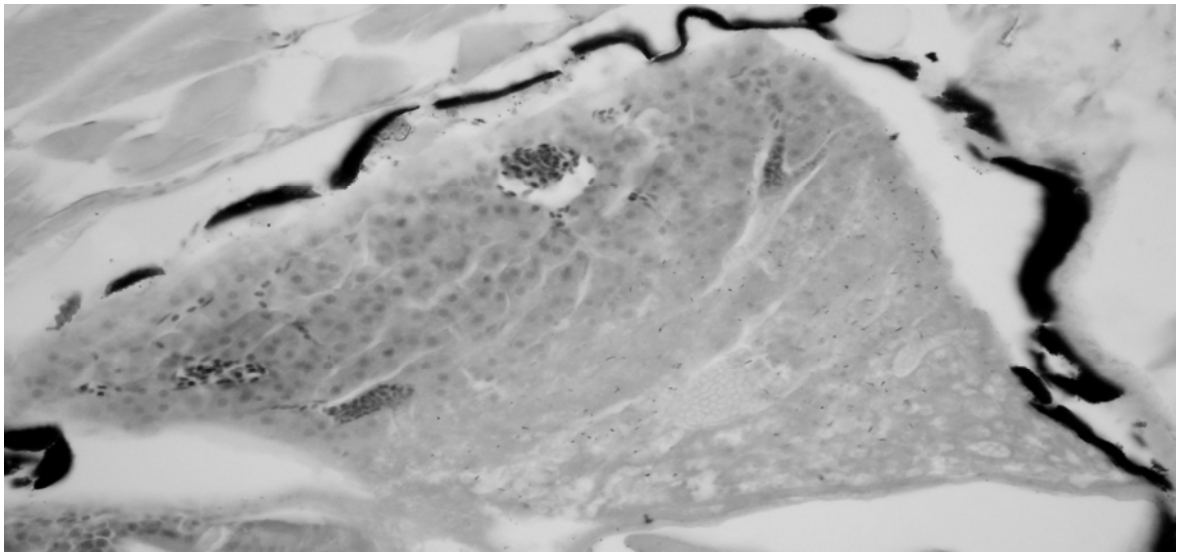

Figure 24: Gonad of effluent exposed fish note, degeneration of germ cells and congestion of blood vessels. 400X

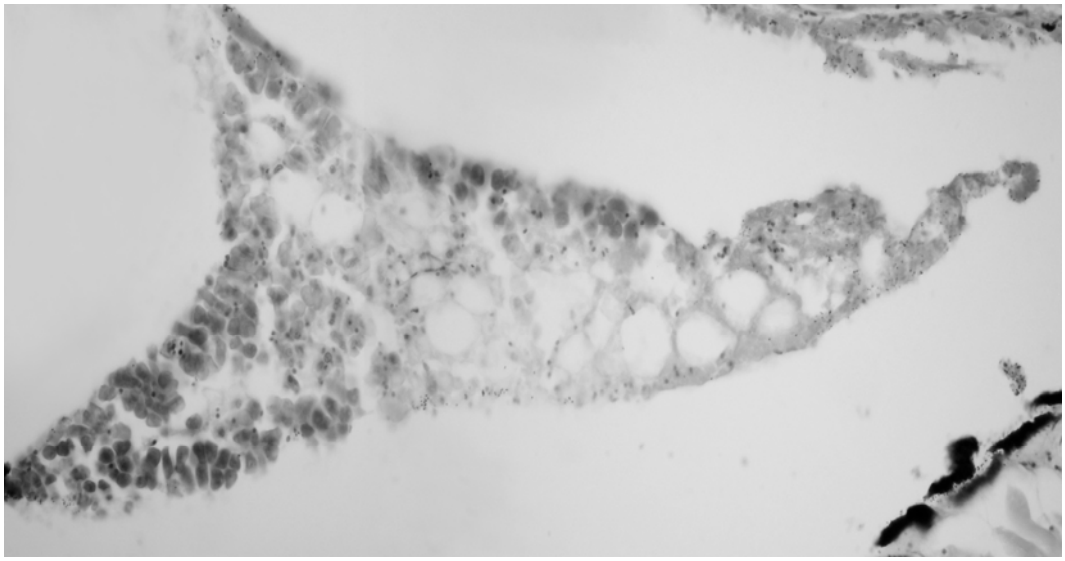

Figure 25: Gonad of effluent exposed fish note, vacuolar appearance of gonads. Germ cell at periphery.400X

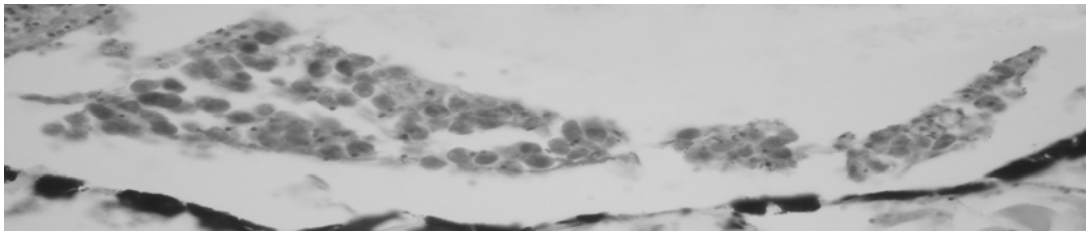

Figure 26: Gonad of fish at late winter showing increasing number of germ cells. 400 X.

Discussion

The toxicity of SBMC waste is attributed mainly to high level of metals even upon dilution and degradation processes. The concentration of heavy metals is a result of complex interaction of metals with each others (antagonistic and synergistic effects) McForlane and Franzin (1980).An association between copper and zinc and between cadmium and zinc was investigated by Wagemann (1990). Davis (1989) found that the allowable concentration for lead was between 120-360 µg/l for trout fry in hard water, yet its toxicity couldn't be neglected especially in the presence of other metals .

Distribution of the trace elements Cd, Pb, Zn, Cu, Fe and Mn among the tissues and bioaccumulation discussed in this work showed that metals have tendency to accumulate in specific tissues. Muscles generally displayed the lowest trace element burdens. The highest levels were found in viscera. For iron the greatest concentration was observed in gills. The same results were obtained by Capelli *et al*., (2005) and Storelli *et al*., (2005). High concentrations of metals in viscera are related to detoxification and excretion processes that take place in liver and kidneys. Furthermore metals are bound here to specific polypeptides, i.e. metallothioneins .Gills of the experimental fish contained higher concentrations of metals than muscle tissue. Same conclusion was reached by Sidoumou *et al*. (2005) in their analysis of sea fish. As the result of high volumes of water being filtered through the gills and the absorption and penetration of iron and copper through gills is controlled by haemostatic process (Wagemann,1990 & Goodyear and Boyd 1972).

The present study suggest a positive relationship between the presence of these metals and the occurrence of histopathological and ultrastructural alterations in various organs of mullet. Hinton and Lauren (1990) defined biomarkers as any contaminant induced physiological and / or biochemical modification which leads to the formation of altered structure (lesion) in the cells of organisms which are considered as defense mechanism for contaminant exposure more than an irreversible toxic effect . However, these modifications can produce adverse effects on fish's health, and may increase their susceptibility to secondary infections diseases and even death. Histopathological alterations, such as edema with epithelial separation of the basal membranes,

generalized necrosis (apoptosis) and / or epithelial desquamation observed here in the gills of mullet fry , have been described by Giari *et al.*(2007 & 2008) and Mauceri *et al.*(2005) in response to heavy metals exposure and suggest an impairment to the respiratory and osmoregulatory functioning of the gills.The increase in number of chloride cells reported here was previously recorded by Jagoe *et al*. (1996) after exposure to mercury. Giari *et al*. (2007) documented that high concentrations of ionic stressors may modify the number of this peculiar cell type which is involved in excretion of these metals against osmotic concentration .

In the liver, extension, vacuolation of parenchyma as well as congestion of sinusoids, may be useful as markers to exposure to different environmental stressors (Alazemi *et al*. 1996). Histo-cytopathological modifications in liver of fish due to metals exposure was reported by Au (2004) and Global Tox.(1997). The loss of the regular cytoplasmic compartimentation is a typical unspecific ultrastructural reaction of fish hepatocytes which indicates disturbance of hepatocellular homeostasis (Braunbeck 1998). Some of the alterations observed in the hepatic cells in the present study such as vacuolar degeneration, dilation of ER and lipid droplet accumulation, are consistent with those documented in specimens of *D.Labrax, Lates calcarifer* and *Carassius carassius* treated with heavy metals e.g. lead and cadmium (Giari *et al*. 2007 and Thophon *et al*. 2004). They speculated that these lipid vesicles origin might be the morphological expression of a blockage in the metabolism of hepatic triglycerides. The ER modifications, deformation of mitochondria and the increase in heterochromatin in nuclei suggest an impairment to the synthetic and secretory activities of the cell. The extensive development in SER reported in mullet in this study has been seen in the hepatocytes of other fish when treated with lead (Braunbeck 1998). This proliferation of the SER is a classical response to pollutants and is linked to hepatic detoxication mechanisms (Lemaire *et al*., 1992).

The kidney of fish receives the largest portion of postbranchial blood, and therefore renal lesions might be expected to be good indications of pollution. Exposure to metals caused renal epithelial necrosis and, sloughing of epithelium could be explained by the fact that various properties of cell membranes are impaired by metals with a consequent inhibition of osmoregulatory process (Ballatori and Boyer, 1996). The abundance of cellular debris reported in the results is a sign of an increased turnover of cellular components under conditions of chemical induced stress (Braunbeck, 1998 & Ghadially, 1997). These results are in agreement with that of Au (2004) and Fracacio *et al*. (2003) on exposure to heavy metals.

Study showed inhibitory effect of heavy metals on gonadal growth (Reddy *et al*., 1997). Reproductive impairments caused by heavy metals have been strongly related to imbalances in hormonal secretion and / or synthesis (Fingerman *et al*. 1996). Necrosis of germ cells were reported by Yamaguchi *et al*. (2007); Aposhian and Aposhian (2006) and pedlar *et al*. (2002) following metals exposure. Same disturbance was reported by Levesque *et al*., (2003) during chromic heavy metals contamination.

In summary, the results of the present study showed that Sugar Beet Manufacturing Company (SBMC) waste posses high metals levels. These metals even at low concentrations negatively affect mullet fry tissues (gills, liver, kidney and gonads) and the gills and the liver were the most affected by exposure to metals in terms of both bioaccumulation & the severity of the observed alterations to the tissue and the extent of damage. Since this fish is an economic fish in Egypt, special care should be taken especially when they are cultured near contaminated areas. Effluent should be treated before discharged to water bodies to protect our fries natural resources.

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التغيرات الهستولوجية و الخلوية الدقيقة فى زريعة أسماك العائلة البورية نتيجة للملوثات الصناعية

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تعرضت منطقة الكسارة بمحافظة الدقهلية و هى أحد مراكز تجميع الزريعة فى مصر للتغير فى التوازن الطبيعى للمياة نتيجة القاء المخلفات الصناعية لمصنع انتاج سكر البنجر بها مما أدى الى أرتفاع نسبة الوفيات فى الزريعة المجمعة منها أثناء نقلها للمفرخات خلال الفترة من 2008/12/15 حتى 2009/3/17 . و قد أظهرت عينات المياة المجمعة فى2008/12/15 من منطقة القاء المخلفات حتى مسافة 750 مترا منها وجود تركيزات عالية من بعض المعادن بها كالرصاص و الحديد والكادميوم و النحاس و الزنك و الذى انعكس بدورة على تركيز هذة العناصر فى أنسجة الاسماك المختلفة . و قد كان تركيز المعادن كالرصاص والكادميوم و النحاس و الزنك فى أعلى نسبة لها فى الأحشاء تليها الخياشيم ثم اللحم . بينما الخياشيم كانت نسبة الرصاص بها أعلى نسبة مقارنة بباقى الانسجة.و كانت مستويات المعادن فى العينات و أنسجة الأسماك المجمعة فى 2009/3/17 أقل من السابقة نتيجة للتخفيف الناتج عن الامطار فى نهاية موسم الشتاء.

و بدراسة تأثير هذة المعادن على الأنسجة المختلفة للأسماك أظهرت النتائج تغيرات تحللية فى أنسجة كل من الخياشيم و الكبد و الكلى و المناسل بأستخدام الميكروسكوب الضوئى و الألكترونى النافذ. و أحدث التعرض لهذة المخلفات الى تناقص الخلايا الغطائية للخياشيم و ذيادة عدد الخلايا المسؤولة عن التوازن الأيونى و كذلك تكسير فى الخلايا الدعامية بها. كما أحدث المخلف آذى للشبكة الاندوبلازمية و عضياتها.أما فى أنسجة الكبد فقد سبب المخلف ترسيبات دهنية فى خلاياة و تغير فى شكل الشبكة الاندوبلازمية و العضيات المسئولة عن الطاقة بها مقارنتا بأشكالها فى العينات القياسية . و كذلك تكسرت خلايا ألانابيب الكلوية فى ألاسماك المعرضة للمخلف و تحللت أنويتها . و فى المناسل أحدث المخلف توقف لعملية تطور المناسل و تحلل الخلايا النطفية بها.

و قد أظهرت عينات الأنسجة المختلفة المجمعة من الأسماك فى نهاية فصل الشتاء 2009/3/17 تحسن فى التركيب التشريحى لها مقارنتا بالعينات السابقة المجمعة فى 2008/12/15 .

مما سبق يتضح ان مخلفات شركة سكر البنجر تحتوى على تركيزات عالية من المعادن و حتى عند تخفيفها أو تكسيرها بفعل ألامطار و مياة البحر فأن لها تأسير سلبى على أنسجة ألاسماك و ان الخياشيم و الكبد أكثر تأثرا بالمخلف من ناحية مقدرتها على تخزينة أو درجة التضرر منة.