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RESEARCH ARTICLE

Effect of Some Heavy Metal on Histological Structural of Gills and Liver of Rabbit Fish (*Siganus rivulatus)* from Two Sites Along Red Sea Coast, SUDAN.

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Abstract

The present research aimed to detect the levels of heavy metals lead (Pb) and cadmium (Cd) in Sudanese Red Sea water, sediment, wild algae and their effects on histological structural of the gills and liver of the fish *Siganus rivulatus.* The wild herbivores rabbit fish (*Siganus rivulatus*) Forsskål (Teleostei, Siganidae) were caught from two sites, Dongnab and Suakin on Sudanese Red Sea Coast during February 2012 - January 2013. The water and sediment of the polluted site (Suakin), contained high level of lead (Pb) compare with Dongnab (control) site where (Cd) concentration was similar in both. Fishes caught from Suakin site (polluted) showed a low values in condition factor, liver-somatic and gonado-somatic index as compared with Dongnab (control) fishes. Contrarily, bioaccumulations factor of lead (Pb) in fish organs from Suakin site recorded a high level compare with Dongnab fishes. Histopathological investigation of gills and liver showed that most *Siganus rivulatus* caught from Dongnab site revealed normal cellular architectures in gills and liver when compared with that caught from Suakin. In fish gill captured from Suakin site, the most common lesions were epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, lamellar fusion and aneurysms in the gills. While the liver shown hepatocytes with hypertrophy, cytoplasmic and nuclear hypertrophy, melanomacrophage aggregates, bile stagnation and one case of focal necrosis. In addition the lesions were comparatively more severe.

Introduction:-

Aquatic ecosystems are sensitive to pollution influences because of the structure of their food chains. The small biomass in aquatic environments occurs in a variety of different trophic levels, which may lead to bioaccumulation of xenobiotic substances at higher trophic levels (Frstner and Wittmann, 1979). Fish have been shown to accumulate contaminants such as metals in their tissues [Abdel-Aziz et al. 2006; ODEQ, 2003. Human consumption of fish containing elevated levels of metals can cause health problems (ODEQ, 2003).

Mallatt (1985) reviewed the most common gill lesions found under several stressful conditions, and concluded that the most commonly reported changes (epithelial lifting, necrosis, hyperplasia, hypertrophy and haemorrhage) were results of lethal conditions. A typical chronology of damage from acute exposure to the test chemical is first a lifting of the outer layer of lamellar epithelium, usually starting in the area of the chloride cells. Edematous spaces are formed between the layers of epithelium and these may become infiltrated with leukocytes. Eventually the whole epithelium sloughs off and the lamellum loses rigidity. On the blood side of the lamellum the central spaces collapse, but the marginal channel often remains normal until the rest of the lamellum is essentially destroyed (Heath, 1995). The proliferation of chloride cells are thought to be a compensatory response to ion loss, and therefore chloride cell hyperplasia may therefore be a good biomarker of adaptation (Hinton et al, 1992). Hyperplasia of undifferentiated epithelial cells which results in clubbing and lamellar fusion is a much less specific lesion associated with a wide

variety of unrelated insults (Hinton et al, 1992).

Abdel-Aziz et al. (2006a) studied the effect of pollutants on the histological structure of gill of the fish *Siganus rivulatus* collected from Red Sea coast of Saudi Arbia there were epithelia lifting, pillar cell damage and proliferation of chloride cells in basilamellar regions in gill.

There have been numerous reports of histopathological changes occurring in fish tissues from exposure to pollutants and it is evident that a wide variety of chemicals cause lesions in the livers of fish (Hinton et al, 1992). Macrophage aggregates have been suggested as potentially sensitive histological biomarkers and/or immunological biomarker of contaminant exposure (Schmitt and Dethloff, 2000). The histological changes observed in various studies in livers taken from fish exposed to pollutants include increased vacuoles in the cytoplasm, enlarged lissomness, changes in nuclear shapes, focal necrosis(death of cells in a localized area), ischemia (blockage of capillary circulation), hepatocellular shrinkage, regression of hepatocytic microvilli, at the bile canaliculi, fatty degeneration and loss of glycogen. The latter two changes can be due to depressed feeding and/or elevated levels of the stress hormones cortisol and adrenalin and there for are not necessarily caused by the chemical acting directly on liver cells (Heath,1995). In general, the changes observed in liver tissue are rather non- specific so it is not possible to identify the chemical causing the lesion (Patton and Couch, 1984).

Abdel-Aziz et al, (2006b) studied the effect of pollutants on the histological structure of liver of the fish *Siganus rivulatus* collected from Red Sea coast of Saudi Arabia there were hepatocyte vacuolization and ballooning degeneration cellular and coagulative necrosis, cellular infiltraion, granuloma inflammation and bile duct proliferation.

Materials and methods:-

The Study Sites:-

This study was conducted during period February 2012-January 2013 on two sites a long Sudanese Red Sea Coast: Dongnab site (21º 03´ N, 37º 10´E). It is declared a marine protected area since 2004. The second site is Suakin (19º 06´ N, 37º 20´ E) about 56 km south Port Sudan. This site is located inside the Passenger Port basin so it is exposure to port wastes Plate. (1).

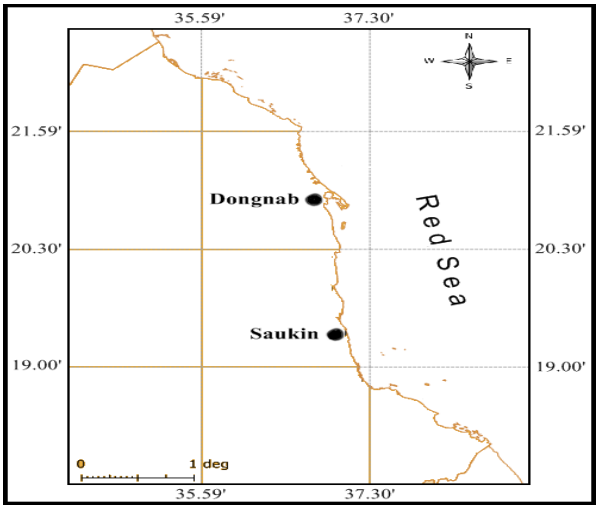

Plate 1: Two Sites of Samples Collection along Sudanese Red Sea Coast.

Estimation of Cadmium and Lead in Water and sediment:-

Surface water and sediment samples from the two sites were collected monthly and analysed for Pb and Cd analyzed according to the method described by (Ahmed et al, 2010) for water and (Peerzada and Rohoza, 1989)for sediment.

Estimation of Cadmium and Lead in Fish tissues and Wild algae:-

Siganus rivulatus (108 individuals from each site) were caught monthly from Dongnab site (control) and Suakin site (polluted), body morphometric measurements were done following, the method (Mohamed, 2003). 27]. Fishes were

dissected with clean stainless steel instruments on the same day. Muscle, liver, stomach and wild algae were dried and put in an oven at 150C° until reaching a constant weight. Samples were homogenized and grinded to a powder. Samples were analyzed follow method described by (Aboul Ezz and Abdel-Razek, 1991). Wild algaes were collected depend on dominate stomach content of algaes from Dongnab site red algae (*Hepnia sp)* ,brown algae *(Cystoseira sp*) and Suakin site green algae (*Enteromorpha sp, Halimda sp*) algaes were dried then processed as above.

Histology Sample Processing:-

Tissue samples (gills and liver) collected for histological analysis were sampled at the same morphological region of each organ to allow for comparative analysis. After 24 hours of fixation, gills and liver tissues were prepared for serial sectioning. The specimen was stained by Haematoxylin and leosin (Harris, 1900), for normal histological structure and combined Alcian blue (Mowery, 1956) for detection of neutral and mucopoly– saccharides.

Statistical Analysis:-

Statistical package for social science (SPSS) program was used to run the data analysis with each test being conducted at 0.05% level of probability.

Results:-

Mean Values Physio-Chemical Records and Heavy Metal Actual Concentration:-

The means variations in air temperature, water temperature, dissolved oxygen, pH, salinity, lead (Pb) and cadmium (Cd) concentration in Dongnab site were significantly lower than Suakin site during the study period Table (1).

Table 1: Mean Physio-Chemical Parameters and Actual Concentration of (Pb) and (Cd) in Water and Sediment.

Parameters	Dongnab site	Suakin site
Air temperature (Cº)	26.42±1.12	33.63±2.10**
Water temperature(Cº)	21.44±0.86	26.93±1.25**
Dissolved oxygen(mg/L)	5.52±0.17	4.82±0.09**
pH	8.18±0.05	8.49±0.01**
Salinity%◦	43.65±0.48	40.50±0.23**
Pb (mg/L) in water	0.025±0.005	0.075±.001***
Cd (mg/L) in water	0.015±0.001	0.05±0.003
Pb(mg/kg) in sediment	0.55±0.006	25±0.325***
Cd(mg/kg) in sediment	0.3±0.026	0.20±0.015

Mean±SEM; Student's'T' Test, **P<0.01; ***P<0.001, for Statistical Significance of Water Physio- Chemical and Concentration of Lead and Cadmium at Suakin Site Compared to Dongnab Site Abbreviations Used: Pb, Lead; Cd, Cadmium.

Mean Values Morphometeric of *Siganus rivulatus* Caught from two sites:-

Organ somatic indices (Condition factor, Liver-Somato Index and Gonado-Somato Index) of *Siganus rivulatus* from Dongnab site (control) was significant high compared with fishes caught from Suakin site (polluted) Table (2).

Table 2: Mean Values Morphometeric of *Siganus rivulatus* Caught from two sites:

Parameters	Dongnab site (control) (n=108)	Suakin site (polluted) (n=108)
T.L(cm)	22.55±0.90	17.23±0.37
S.L(cm)	17.86±0.70	14.19±0.33
B.W(g)	171.45±20.82	68.32±5.13
Age(year)	3±0.25	2.3±0.19
C.F	2.83±0.45	2.39±0.13*
L.W(g)	2.97±0.51	0.62±0.13
G.W(g)	10.24±2.60	0.14±0.05
L.S.I	1.68±0.17	0.95±0.04***
G.S.I.	5.20±1.07	0.20±0.07**

Mean±SEM; Student's'T' Test, *P<0.05 **P<0.01; ***P<0.001, For Statistical Significance CF, L.S.I And G.S.I. Morphometeric of Dongnab Fish Samples Has Been Compared with Suakin Fish Samples. Abbreviations Used: T.L,

Total Length; S.L, Standard Length; B.W, Body Weight; L.W, Liver Weight; G.W, Gonad Weight; C.F, Condition Factor; L.S.I, Liver-Somato Index; G.S.I, Gonado-Somato Index; n, Number of Fish.

Mean Values Bioaccumulation Factor (BAF) of (Pb) and (Cd) in fish tissues:-

The mean bioaccumulation (BAF) of analyzed metal lead (Pb) in muscle, liver and stomach of studies fishes caught from Suakin site were extremely significant higher ($p< 0.00$) as compared Dongnab site fishes. On other hand (BAF) of metal cadmium (Cd) in same organ tissue there was no significant difference ($p>0.05$) found in fishes tissues caught from two sites.

Table 3: Mean Accumulation Factor of (Pb) and (Cd) in the *Siganus rivulatus* Tissues:

Sampling sites	Pb			Cd		
Dongnab (n=(12)	**Muscle**	**Liver**	**Stomach**	**Muscle**	**Liver**	**Stomach**
	146.67±26.31	217.50±23.0	46.42±5.66	9.86±2.27	86.11±10.63	29.17±2.18
Suakin (n= (12)	244.86±22.5**	506.67±19.93***	125.83±3.96***	11.88±1.41	88.33±18.23	34.58±2.78

Mean±SEM; Student's'T' Test, **P<0.01; ***P<0.001, For Statistical Significance Accumulation Factor of Lead and Cadmium In Muscle And Liver of Fish Suakin Samples Has Been Compared with Suakin Fish Samples. Abbreviations Used: Pb, Lead; Cd, Cadmium; N, Number of Fish.

Mean Accumulation Factor of Lead and Cadmium in Different Algaes From two sites:-

Table (4) indicated the bioaccumulation factor of Pb was higher in algae's collected from Suakin site when compared with Dongnab site algae's. There was lower in BAF of Cd in algae's collected from both sites.

Table (4): Mean Accumulation Factor of Lead and Cadmium in Different Algaes From two sites

	Dongnab site		Suakin site	
Pb	***Hepnia sp***	***Cystoseira sp***	***Enteromorpha sp***	***Halimda sp***
	1.88	1.50	223.3	203.33
Cd	0.6	0.83	0.10	0.875

Histopathology of the Gill Exposure to Heavy Metals:-

Siganus rivulatus were collected from Dongnab site revealed normal histological and cellular architecture in gills, where primary and secondary lamellae of the gill filaments could be easy detected (Fig. 1,2) when compared with that collected from Suakin site (Fig. 3-8).

The commonest anomalies found in the gills of the *Siganus rivulatus* at Suakin site were considered as being at stage I in severity, these included, Some examples of more severe lesions found in the gill (stage II) were lamellar aneurysm (Fig. 3), leukocytes infiltration and complete rapture of the lamellar epithelium (Fig. 4), excess mucus secretion due to mucus hypertrophy (Fig. 5, 6). There were some cases where hyperplasia was more severe, resulting in the hypertrophy of lamellae (Fig. 7) hyperplasia of chloride cells (Fig. 8).

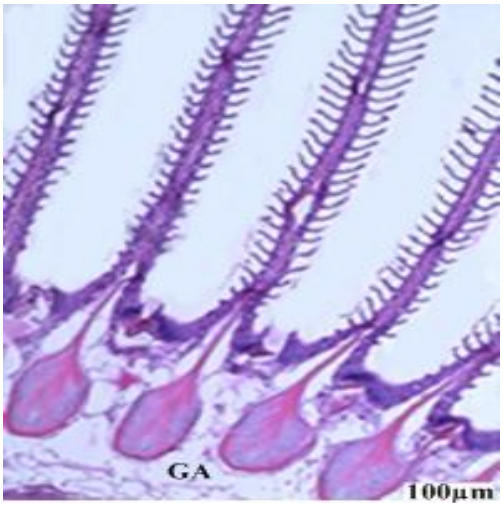

Fig. 1: Normal Gill Section, Showing The Filament and Gill Arch (GA). H&E. 10x.

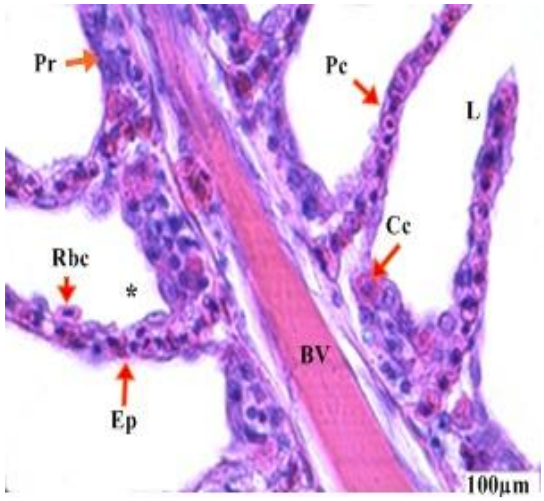

Fig. :2 Normal Aspect of The Gill, Showing The Primary Lamellae (Pr), Secondary Lamellae (L), The Water Channel (*), A Pillar Cell (Pc), A Chloride Cell (Cc), An Epithelial Cell (Ep), A Red Blood Cell (Rbc) and Blood Vessel (BV). H&E. 100x.

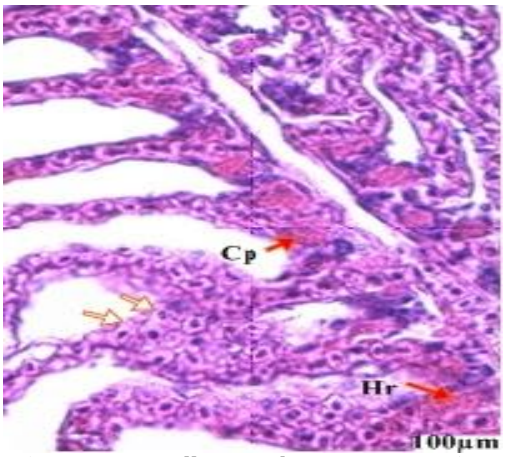

Fig. 3: Lamellae with Aneurysm (Arrow), Hypertrophy of Chloride Cell (Cp) and Hemorrhage (Hr). H&E. 100x.

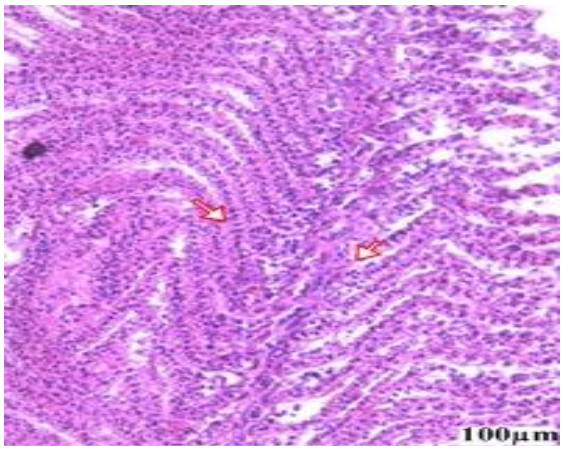

Fig.4: Complete Lysis of Lamellae Epithelium and Heavy Leukocytes Infiltration (Arrow). H&E. 40x.

Fig. 5: End of Lamellae with Excess Mucus Secretion (Arrow), Leukocytes Infilteration (Li). H&E. 100x.

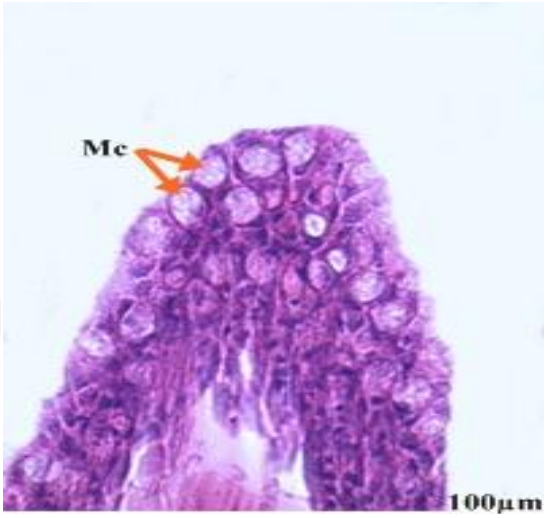

Fig. 6: High power E.M. from fig (44) showing mucus cell hypertrophy (Mc). H&E. 100x.

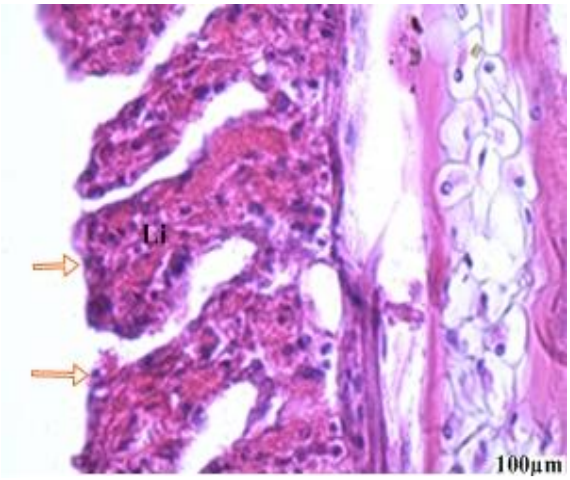

Fig. 7: Hypertrophy of the Lamellae Epithelium End (Arrow). Leukocytes Infilteration (Li). H&E. 100x

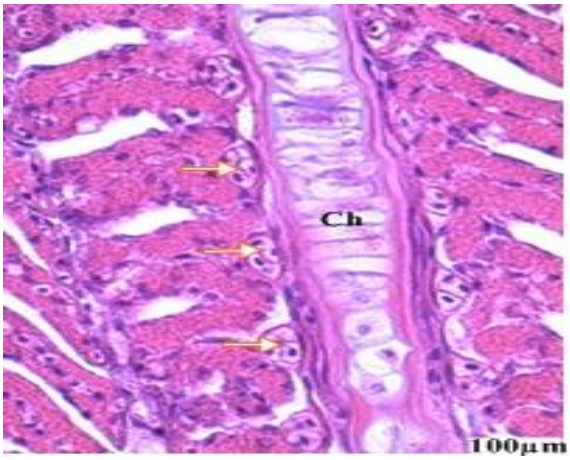

Fig. 8: Lamellae with Hyperplysia of Chloride Cell. Filament Chondricytes (Ch) (Arrow). H&E. 100x.

3.6 Histopathology of the Liver Exposure to Heavy Metals:

Most of examined specimens of *Siganus rivulatus* caught from Dongnab site revealed normal cellular architectures (Fig. 9,10) when compared with that collected from Suakin site Fig. (11-16).

The majority of alteration found in the liver of Suakin fish belong to stage I, II and III, the tissue was moderate to slight severe damage, and regeneration may be possible if the water quality is improved. Moderate histopathological and cellular lesions on the liver of most *Siganus rivulatus* collected from Suakin with great individual variability. Hemorrhage and congestion were detected in blood vein (Fig. 11); Vacuolization of nuclear (Fig. 12). Melanomacrophage center were identification as rounded aggregation of cell containing dark- yellowish granule of various size and also hemosiderin aggregation appeared as rounded yellowish granules (Fig.13). In severe cases of fatty infiltration, the liver enlarged and hepatic tissue become paler where large lipid droplets accumulated in the cells and sometimes appeared between necrotic hepatocytes as empty vacuole (Fig. 14). Encapsulated granuloma in liver sections of examined fish characterized by central necrosis of hepatic tissue surround by epithelium cells and fibrocystic layer (Fig.15.16). Bile stagnation was identified as blue pigment in bile duct and liver parenchyma (Fig. 17). Cyst of parasitic metazoan in liver sinusoids and may be was larval stage of *Procamallanus sp* or *Sclerocollum* (Fig. 18).

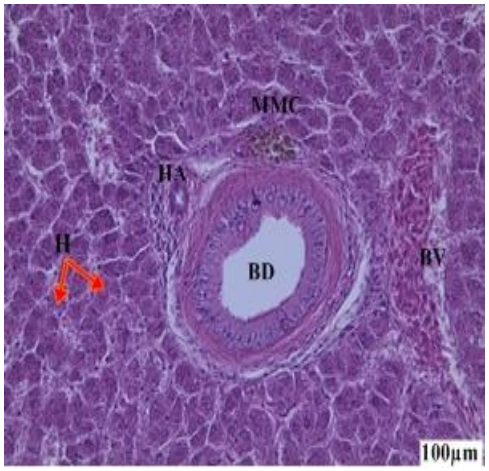

Fig.9: Normal Hepatic Tissue Showing, Hepatocytes with Granular Cytoplasm (H), Bile Duct (BD), Hepatic Arteries (HA), Blood Vein (BV) and Melanomacrophage Center (MMC). H&E. 40x.

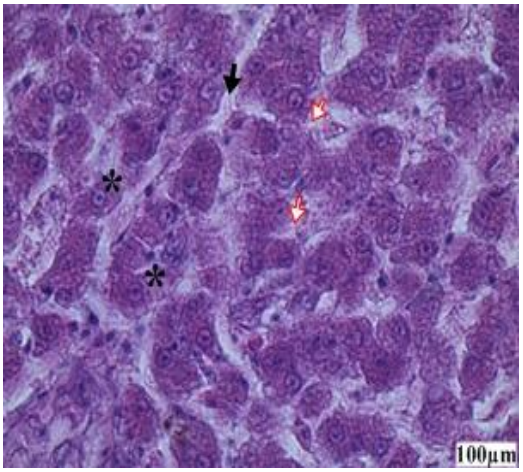

Fig. 10: Normal Hepatic Centrally Located, Spherical Nucleus with A Clear, Dark Nucleolus (*) and Glycogen Vacuoles (Arrow). H&E. 100x.

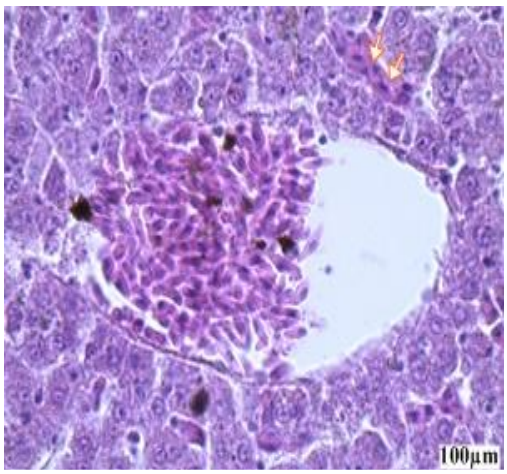

Fig. 11: Congestion in Hepatic Central Vein, Red Blood Cell in Sinusoid (Arrow) H&E. 100x.

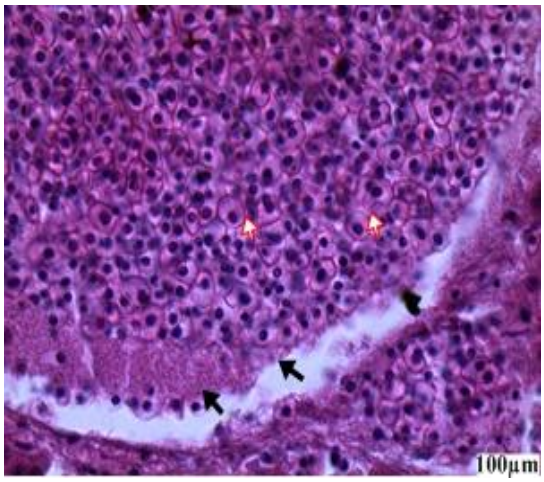

Fig. 12: Hepatic Cytoplasmic Vacuolation And Nuclear Hypertrophy (White Arrow), Early Formation of Granuloma (Black Arrow) H&E. 100x.

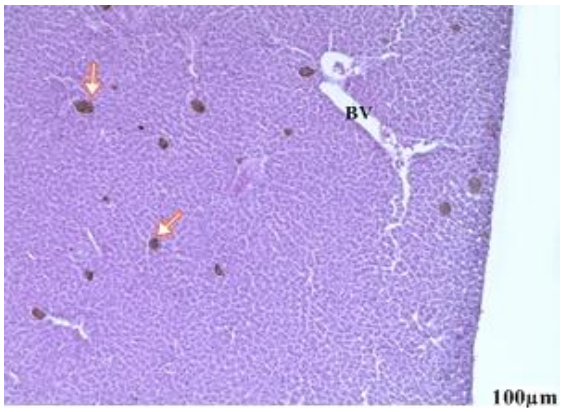

Fig. 13: Liver Paranchyme with Melanomacrophage, Aggregation Close to Vessel and Blood Vein (BV) .H&E. 10x.

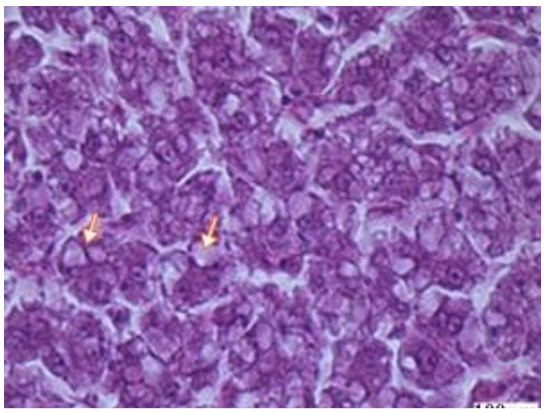

Fig. 14: Showing Moderate Fatty Change (Arrow) and Loss of Cytoplasm Granule.H&E. 100x.

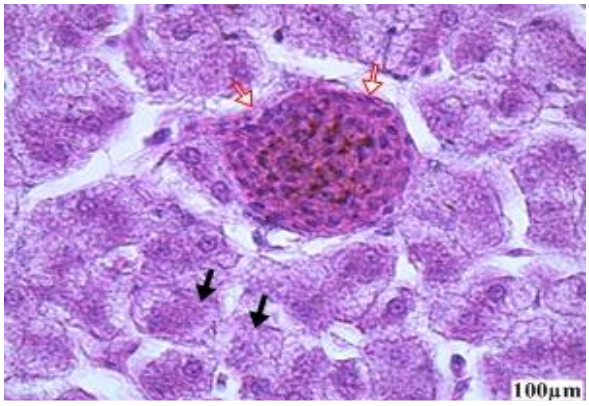

Fig. 15: Liver Parenchyma with Early Formation of Granuloma (Arrow) And Hepatocytes Atrophy (Black Arrow).H&E. 100x.

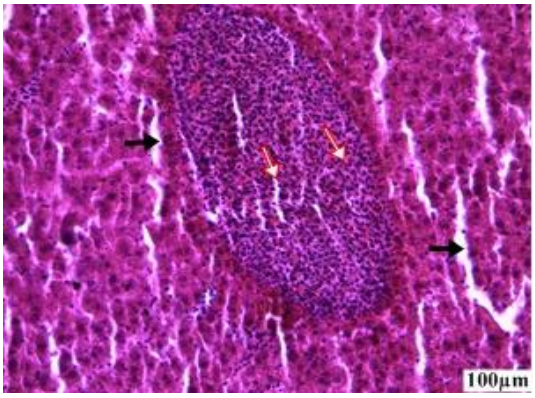

Fig. 16: Hepatocytes Granuloma (White Arrow) and Hepatocytes Atrophy (Black Arrow). H&E. 40x.

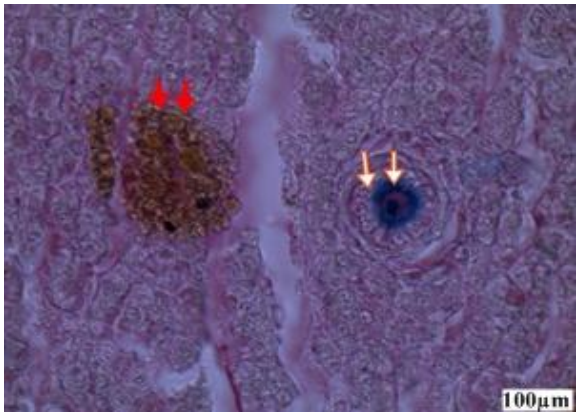

Fig. 17: Bile Stagnation (White Arrow) Inside Hepatic bile Duct and Hemosiderin Aggregation (Red Arrow).Alcian Blue. 100x.

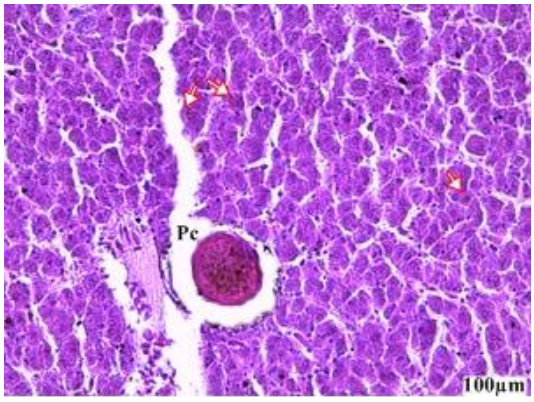

Fig. 18: Rounded Parasites Cyst inside Liver Paranchyme Surrounding by Fibrous (Pc) And Esinophils Infilteration (Arrow).H&E. 40x.

Discussions:-

Physio-Chemical Records and Accumulation Factor of Lead (pb) and Cadmium (Cd):-

The water temperature in polluted site (Suakin) was significantly higher as compared with control site (Dongnab), this may be due to Passenger Port activities and discharging of waste water beside this site, beside that it has only one

entrance to open sea so that the exchange of water between open sea is limited and this makes water temperature higher compared with control site Dongnab where in this site the water exchange between open sea and bay occur in wide area (Farah, 1982). Dissolved oxygen in polluted site (Suakin) found less than 5mg/L, may be stress full to most aquatic organism (Grant, 2004), the depletion of water oxygen attributed to high concentration of pollutant especially lead (Clark, 1996). The concentration of (Pb) in the water and sediment of polluted site (Suakin) was higher than that observed in control site (Dongnab). High level of (Pb) is mainly due to Passenger Port activities, and also (Pb) might be originating from Passenger Vessel and Port engines. The concentration of (Cd) in the water and sediment of polluted site slightly high compared with control site but not with significant difference, the concentration in water is above the recommended maximum acceptable limit as set by (WHO, 1992). Pb is 0.005mg/L, this need more investigation in future study. Interesting, the bioaccumulation of (Cd) in fish tissue collected from two sites (control and polluted) was less than permitted level for (Cd) (FAO/WHO, 2011) ; in spite the high (Cd) concentration in water this may be due to interfered between fish physiological response and marine environment factors.

Generally the effect of lead toxicity on *Siganus rivulatus* more obvious in histopathology figures of fish gills (Fig. 3 to Fig. 8) and liver (Fig.11 to Fig.16) .

Decrease in HSI indicate that the fish in Suakin site was under stressful condition due to high concentration of (Pb), this situation makes liver to release glycogen to cover losses of energy, same result were presented by (Lee et al. 1983; Ram and Singh, 1988).

GSI of fish captured from polluted site (Suakin) showed marked decreased compared with control site, this decreasing may be attributed to pollutants. Schmitt and Dethloff (2000) stated that exposure to various environmental pollutants can result in gonadal alteration such as a decreased GSI, morphological changes, or both.

The present study revealed marked increase of (Pb) bioaccumulation factor in following order:
Liver > Muscle > Stomach > Algae.

Increase were positively correlated with a corresponding increase in (Pb) and it concentration in water and sediment. Regression analysis shows that (Pb) bioaccumulation in all tissue are highly correlated with it concentration in water, sediment and wild algae, whereas (Cd) correlation was less pronounced. The higher accumulation of lead in liver may alter the level of various biochemical parameters as discuss later, this may also cause gill lesions Figures (3- 8) and liver lesions (11 - 18).

Histopathology of the Gill Exposure to Heavy Metals:-
Some examined *Siganus rivulatus* from Dongnab site were characterized by maintaining the normal histological and cellular architecture in gills (Fig.1,2) in comparison with that present in normal marine fish (Takashima and Hibiya, 1995).

Histopathological alterations were detected in the gill filaments of most captured fish from Suakin site (polluted) (Fig.3-10). Alterations like hypertrophy of the epithelial cells (Fig. 7), besides partial separation of some secondary lamellae are examples of defense mechanisms since; in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985; Mazon et al, 2002). Such alterations are non-specific and may be induced by different types of contaminant (Mallatt, 1985. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003). In this case (Fig.3), damaged pillar cells can result in an increased blood flow inside the lamellae, causing hemorrhage, blood congestion or even an aneurysm same lesion describe by (Rosety-Rodriguez et al, 2002). The formation of an aneurysm is related to the rupture of the pillar cells (] Martinez et al, 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells. This is a severe type of lesion, recovery from which is possible, but more difficult than the epithelial changes (Poleksic, and Mitrovic, 1994). Rupture of the gill epithelium, which caused hemorrhage. Like aneurysm, this lesion can be interpreted as a reflection of the direct action of toxic agents on the tissue (Temmink et al, 1983). Chloride cells proliferation (Fig.8) due to an added function of oxygen transport due to injury to gill tissue proper. In certain abnormal conditions chloride cells may to be an oxygen transport functions (Tamse et al, 1995). Also, the pathological changes in the chloride cells may indicate osmoregulatory dysfunction, which is the main function of the chloride cells (Virtanen, 1986). Previous studies reported swelling and fusion of the gill lamellae in fish exposed

to sublethal concentration of heavy metals where metals bind strongly to the plasma membrane of the lamellar epithelial cells increasing their permeability to water and ions (Bin Dohaish, 2001). Also, heavy metals might inhibit ion carries in chloride cells and thus, an increase in their number would be compensatory. On the other hand, mucous cells proliferated and hypertrophoid (Fig.5 and 6) on the fused surface of the secondary lamellae. This may be considered as a protective response to binds toxins transport. The set of protective reactions calumniates in the formation of mucous layer that could coagulate and up in the cessation of gaseous exchange and death (Tamse et al, 1995). Leukocytes infiltration (Fig.4) caused their accumulation in the sub epithelial spaces of secondary lamellae and necrotic gill tissues. This may be an inflammatory reaction response to different chemical toxic substances (EL-Feki, 1998) or to phagocyte the toxicant particles and tissue debris's (Muhvich et al, 1995).

Histopathology of the Liver Exposure to Heavy Metals:-

Siganus rivulatus which caught from Dongnab site (control site) appearing normal liver, condition factor and hepatosomato index and hepatocytes were characterized by mainting the normal histological and cellular structure in comparison with that present in normal marine fishes (Fig.9,10) (Gingerich, 1982)15], (Hinton et al, 1987).

Anomalies reported from Suakin fish such as hemorrhage in blood vessel (Fig. 11) was also described in the siluriform *Corydoras paleatus* contaminated by organophosphate pesticides [10]. An increase in the density of the melanomacrophage aggregates (Fig.13), as observed in the liver of fish collected from Suakin site in this study, is generally related to important hepatic lesions (Abdel-Aziz et al, 2006b), such as degenerative and necrotic processes. The function of the melanomacrophages and hemosiderin in the liver of fishes remains uncertain, but some studies have suggested that it is related to destruction, detoxification or recycling of endogenous and exogenous compounds (Haaparanta et al, 1996). And also have long been recognized as potentially useful biomarkers of fish health (Wolke et al, 1985b). However, numerous studies had documented an increase in their number, size or hemosiderin content in fish collected at contaminated sites when compared to those collected at reference sites (Kranz and Peters, 1984; Spazier et al, 1992; Wolke et al, 1985b). Macrophage aggregates have been suggested as potentially sensitive histological biomarkers and/or immunological biomarker of contaminant exposure (Schmitt and Dethloff, 2000). Accumulation of fatty acid in hepatocytes (Fig.14 and 15), 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), this In some instance the hepatocytes became pale and swollen like a balloon (Fig. 12). This is probably a result of accumulation of electrolytes and water due to impaired membrane permeability followed autolysis (Burkitt et al, 1996). Encapsulated granuloma in liver sections of examined fish (Figs. 15, 16) 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 foreign bodies like bacterial that can be detected inside the lyzed hepatic tissue of granuloma by specific stains (Burkitt et al, 1996; Robbins, 1995). The present study shows that the histopathological changes in the liver can cause metabolic problems as well. Evidence for this is the bile stagnation in liver of most fish studied (Fig.17). This lesion is characterized by the remains of the bile in the form of blue pigment when stained with Alcian blue in the bile duct and the cytoplasm of the hepatocytes, indicating that the bile is not being released from the liver. This accumulation of bile indicates possible damage to the hepatic metabolism (Fanta et al. 2003). Leukocytes infiltrations in liver parenchyma (Fig.4,5) were associated with defense mechanism against foreign material (Munshi and Dutta, 1996).

Cysts of parasitic metazoan in liver sinusoids (Figs. 18). Generally, the incidence of parasitic infection was very high in all exposed to waste water which induce bacterial infection fish. Such infections fungal due to reduced defensive mechanisms (Myers et al, 1994). It is amazing to mention that the study species *Siganus rivulatus* is a bottom feeding fish that feed on sea grasses and epiphytic algae ((Bin Dohaish, 2001). The present chemical analysis in Tables (1) revealed contamination of sediment and water of the studied area with Pb and Cd heavy metals. The mean values of these metals in sediments were higher than in water in Suakin site.

Generally the concentration of (Cd) and (Pb) in water, sediment, wild diet, stomach content, muscle and liver high in fish collected from Suakin site compared with that from Dongnab site this explained why the degree of difference in tissue change in fish collected from both sites. The presence of bile stagnation and melanomacrophages in great quantity in the livers of *Siganus rivulatus* captured from Suakin site is strong evidence that these organs suffered structural and metabolic damage due high concentration of (Pb) and (Cd) in water, sediment, wild diet, muscle and liver compared to Siganus from Dongnab sites this is probably due to the location of this site, under stress of port activities, which make the accumulation of contaminants an easier process, where as Dongnab sites located north

from Suakin site the distance between both 248 km faraway from contaminant exposure and declared as a marine protected area since 2004.

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