Heavy metal pollution in freshwater fish farms at EL-Qass Region ,Saudi Arabia-Histopathological and Biochemical s

Mehana , E.E.
Department of Pathology and Parasitology , College of Veterinary Medicine, Alexandria

Abstract

main farms (10 fish from each) at Burayda, Qassim region. T levels of lead (Pb) and cadmium (Cd) were determined in water a fish tissues using atomic absorption spectrophotometer and th levels were compared to figures recommended by the world hea organization (WHO). Moreover, the histopathological examinations fish tissues were performed. The levels of Pb and Cd were detec in tissues and water samples in all farms. The mean levels of (0.09 mg/L) and Cd (0.04 mg/L) in water were apparently higher th the values reported by WHO (0.01 mg/L for Pb and 0.005 mg/L Cd). The mean levels of Pb (0.4 ▯ g/g) and Cd (0.1 ▯ g/g) in t tissues were apparently lower than the values reported by WHO (▯ g/g for Pb and 0.2 ▯ g/g for Cd). The samples from farms III ha higher lead levels than other farms. Concomitantly, the sever pathological changes in fish tissues (liver, kidney, gills, brain a muscles) were found in samples of farms III and IV. In addition, liver and kidney damages shown in fish of farm III were associa with increased levels of plasma transaminases, creatinine and u The levels of ALT, AST, creatinine and urea were significantly hig in fish of farm III than farm I.

In conclusion, the mean levels of Pb and Cd were detected tissues of farms in Qassim region in concentrations lower than W findings. The pathological Forty of Tilapia nilotica (Oreochro niloticus) fish and water samples were collected from four diffe changes shown in fish tissues may be partially due to lead cadmium toxicity.

Keywords: Lead, Cadmium, Tilapia niloticus fish and Fresh water
***Corresponding author: Tel.: 0096663800050-2051; fax: 380122 elsayedhamouda@yahoo.com**

Introduction

Fishes form an important target for biomagnifications of metals as they a the food pyramid and act as a possible transfer media to human beings (1989). Studies carried out on various fish species showed that heavy accumulated mainly in metabolic organ such as liver that stores detoxification (Kargin and Erdem, 1991). Metals may also are concent gills, due to the element complexing with mucus, which is impossible completely from and between the lamellae (Health, 1987).

The main sources of heavy metals are mining effluents, industrial effluen effluents, urban storm- water, leaching of metals from garbage and

dump, metal inputs from rural areas, batteries, pigments, paints, glass, textile, dental and cosmetics, atmospheric sources and petroleum industrial FAO,(1996).

Lead (Pb) and cadmium (Cd) are industrial pollutants which have strong effect on human and animal health. These metals are accumulated in the in particularly in the liver and kidneys. The exposure to toxic elements minimized by regular control of their presence in food and feed and throu maximum levels their concentration in these products (Szkoda and Żmudzki WHO/FAO (1989) reported that Cd in surface water is usually found tog zinc but at much lower concentrations. The Cd present in surface wate either dissolved or insoluble. The dissolved forms, which may be poisonou include the simple and various inorganic and organic complex ions. Its a action is manifested by damage to central nervous system and parenc organs, even at small concentrations after a long exposure period (An 2002). The acute lethal concentration of Cd for different species of fish from 2-20 mg/L (WHO, 1999).

The Cd is deposited in soft tissues of the body with 50-70% accumulati kidney and liver. In whole blood, Cd is bound to the erythrocytes. The Cd renal damage in human beings and is represented by proteinurea, renal tu damage, decrease proximal tubular reabsorption and increase in the creatinen. (FDA, 1993).

The acute Pb toxicity is characterized by initial damage to the gill e the affected fish are killed by suffocation. A characteristic symptom of intoxication is a blacking of the caudal peduncle.

The aim of the present study was to measure heavy metals, Cd and Pb water and fish tissues (muscles, liver, kidney, brain gills, and blood) of Tilap fish. Moreover, the liver and kidney function tests of affected fish were p The histopathological examinations of fish tissues were also described.

Materials and Methods

1-Fish:

Forty Tilapia nilotica (Oreaochromis niloticus) fish were collected as 10 each four main farms (I, II, III, and IV) at EL-Qassim area, Saudi Arabia. were placed in ice and packed in plastic bags and transported to laborator hour .

2 –Determination of Pb and Cd levels:

The heavy metals, Pb and Cd were determined in water and fish tissues nilotica using atomic absorption spectrophotometer (Perkin-Elmer model 3 samples were collected from the four different farms. Immediately upon arr laboratory, the fish were dissected for the liver, kidneys, blood and muscles digestion of the dried samples was done according to the method des (Kargin and Cogun, 1999) using conc. H_2SO_4 and 30% H_2O_2 mixture. T dry-ground sample placed in 100-ml beaker, 3.5 mL of 30 % H_2O_2 was a content of the beaker was heated to 100 oC, and the temperature was increased to 250oC, and left at this temperature for 30 min. The beaker w and more 1 ml of 30 % H_2O_2 was added to the digestion mixture and the were reheated again. The digestion process was repeated more than one clear solution was obtained. The clear solution was transferred into 50-ml

flask, and completed to the mark with double distilled deionized water. A digestion solution was made for comparison. A standard solution for each under investigation (Sigma company) was prepared and used for calibration measurement was performed with a Perkin-Elmer model 2380 Atomic Abs Spectrometer, double beam and deuterium background correction. Hollow lamps of Pb and Cd were used at specific wave length of every Measurements were done against metal standard solutions.

3-Liver and kidney function tests:

Blood samples were obtained from the tail veins according to Lucky, (1977) plasma levels of AST : Aspartat transaminases, ALT : Alnine transaminase and creatinine were performed using commercially available kits (C Diagnostic) (Reitman and Frankel, 1957; Chaney and Marbach, 1963; King Henry, 1968)

4- -Histopathological studies:

Specimens from liver, kidney, gills and brain were collected and fixed in 10% buffered formalin then dehydrated using ascending grades of ethanol, clea embedded in paraffin wax>Tissue sections were obtained and stained with H PAS stains(Bancroft,1975)

5-Statistical analysis: - The data that obtained from the studied paramete statistically analyzed using ANOVA and student's t-test according to Snede Cochran (1982).

RESULTS:

I- Biochemical results:

The mean value of Pb in the water samples obtained from the four farms and IV) was 0.09 mg/L. Also, the mean values of Pb detected in fish tissu 0.04, 0.07, 1.0 and 0.4□g/g.and total mean levels for farms I, II , II respectively . The levels of Pb in water and tissue samples were apparen in farms III than other farms. The mean value of Cd in the water of Bolti farms was 0.04 mg/L. Also, the mean levels of Cd in fish tissues were 0. 0.15 and. 0.1 □g/g in examined farms (I, II , III and IV) . The mean levels Cd in the studied samples of all farms in comparison to WHO a recommended levels (SASO) are shown in Table (1)

The plasma levels of GPT (AST), GOT (ALT), creatinine and urea are p (Fig. 1). Values were significantly higher in farm III than I ($P<0.01$).

Histopathological Results:

A-Gross lesions:-

The characteristic gross picture of fish brought from farm III was spinal defo vertebral malformation (Scoliosis and Lardiosis) ,excessive mucus layer o and gills. Congestion and hemorrhages in all internal organs including the gallbladder with bile. Gills also. showed pale and anemic in some c congested, hemorrhagic as well as necrotic in others.While,the gross p cases brought from farms IIII and IV ,were ascites ,flaring opercula, erosic tips of the caudal fins and the gill cover, erosions and ulceration observed. Little changes were noticed in fish brought from farms I&II.

B-Histopathology :-

Gills: The most prominent histopathological alterations, in fish of farms I a were :-
Lamellar epithelial cells proliferation , hypertrophy, hyperplasia and fusion excessive eosinophilic granular cells infiltration as well as mononuclea infiltration mostly, lymphocytes .Edema of the interlamellar spaces, lamellar sy with hyper activation of goblet cells.
Finally, the lamellar epithelial cells were necrotic and sloughed with vascular (congestion of central venous sinuses) (Fig.2 A-B). While, cases brought from III, and IV showed severe pathological changes in the form of necrosi sloughing and branchial oedema of the epithelial layer from the unc thickened basement membrane. Hyper activation of goblet cells as w congestion of CVS and secondary lamellar blood vessels and hemorrhage noticed.(Fig.2 C).
Liver: The histological alterations in cases (fish from farms III and IV) were alt in nature ,i.e. cell swelling and necrosis of the hepatocytes. Congestion vascular sinusoids, hemorrhage and hemosiderosis were also detected. Pan necrosis, edema, hemorrhage with mononuclear leucocytic and eosin infiltration ..(Fig.3). Fish liver brought from farms I&II showed mild histo alterations.
Kidney: The histological alterations in the kidneys of fish from farms III and I observed in the epithelial cells of the proximal convoluted tubules .These ch were degenerative and necrotic in nature with extensive mononuclear leu infiltration. Severe congestion , hemorrhages and edema were observed interstitial tissue. (Fig.4).

In addition, hypercellularity and sclerosis of the glomeruli were seen changes were noticed in specimens of both farms I&II.
Brain: The meninges showed congestion, edema, necrosis with inflam mononuclear cell infiltrations. Also neuronal degeneration , neuronophagia, and perivascular lymphocytic cuffing were also observed in fish of all farms (Fig
Skin: The epidermis showed hyper activation of goblet cells as well as alarm necrosis and ulceration . The dermal melanosis, muscular necrosis with a big cyst were also observed in the fish samples.

DISCUSSION:

According to WHO/FAO (2001), the maximum permissible contaminant lev Pb in water supporting aquatic life is 100µg/L. The WHO (1993) . In the c study, the mean level of Pb of four farms water was 0.09 mg/L ,. A mean va 0.4 □g/g is reported for studied fish. Saudi Arabia has set a maximum limit of l fish as 2 µg/gm (SASO, 1997). The European commission (1997) proposed lim Pb is 0.5 µg/gm in fish. The Australia New Zealand food standards set the max level of Pb in fish at 0.50 µg/g (NZFSA, 1998). Kong et al., (2005) stated th Europe and China guidelines of Pb in tilapia fish is set at 0.4 µg /gm wet w Therefore in comparison to international standards, the mean levels of Pb re in this study were higher in water but lower in fish. The acute toxic Pb concentr in different types of water are in the range of 1-10 mg/L for salmonids and of 1 mg/L for cyprinids (WHO/FAO, 1972).A 1 mg/l is quite close to the 0.09 reported here and consequently pb toxicity would be expected in fish farms stu

The maximum admissible Cd concentration in water for salmon fish is set □ g/L (Environmental Protection Agency, 1991). Saudi Arabia had set a max limit of Cd in fish as 0.5 µg/gm (SASO, 1997), where as, the European Comm (1997) had proposed a limit of (0.05 µg/gm) in fishes. The WHO, (1993) state the levels of Cd in both water and fish were 0.005 mg/l and 0.2 □g/g respe Therefore in the current study, the mean levels of Cd of four farms, 0.04 m water and 0.1 □g/g for fish , is higher in water but lower in fish than sug international values (WHO,1993).

So we can consider that the Pb and Cd may be the possible cause destructive histopathological changes of the organs of tilapia fish either al synergistic with other injurious agents that present in the environment s bacteria, virus and parasites. As the Pb has the ability to induce oxidative dan tissues. Its damage includes enhanced lipid peroxidation, DNA damage, oxid sulphhydral groups and depression endogenous antioxidants (Stochs and 1995 and EL-Sokkary et al., 2005). The Pb and Cd uptake by fish r equilibrium only after a number of weeks of exposure. Lead is accumulated m gills, liver and kidneys (Environmental Health Criteria, 1985). So the most des pathological changes showed in the pervious mentioned three organs bes brain. The gross pictures showed in the present study were in the form o deformity and vertebral malformation (Scoliosis and Lardiosis), excessive mu the skin and gills besides severe congestion, hemorrhages and swelling o internal organs including the distended gallbladder with bile. Our recorded were in parallel lines to those reported by many investigators (Hamouda Othman et al., 2004 and Perziosi et al., 2006). The biochemical results w supportive explanation to the destructive changes of both liver and kidneys levels of plasma ALT, AST, creatinine and urea were significantly higher in f III than fish farm I. The pathological changes were represented by degenerative as well as necrotic changes of the hepatocytes, beside conges hemorrhage. Kidney showed lymphoid element depletion because lead known of its destructive haematopoietic effect (Othman et al., 2004) immunosuppression and makes the fish highly susceptible to the other caus diseases. Gills also showed severe degeneration, necrosis and sloughi epithelial cells and hyper activation of mucous or goblet cells, congestion c venous sinuses, thrombosis and infarction due to the lead pollution. Thes were coinciding with those reported by many investigators (Hamouda, 1 Othman etal., 2004). The brain showed severe degenerative changes, hemorrhage, encephalitis and meningitis due to the possible toxic effec metal. These results were in parallel lines to those reported by Perziosi et al The skin and its underlying muscles (edible parts) were necrotized with keratin cysts and dermal edema and melanosis with mononuclear cells in and those changes were good indicators for water pollution with injurio either heavy metals only or lead with other toxicants agents.

In conclusion, our study revealed that the tissues of Tilapia cau different farming fish of Buraydah contained low levels of Pb and Cd comp the standard limits . The pathological changes shown in fish tissues may b due to lead and cadmium toxicity. We advice to measure the levels of P routinely in all fish farms in Qassim region and prevent all sources of hea to reach to the water of the fish.

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Table (1): The mean levels of Pb and Cd in fish tissues at Qassim region *

Studied parameters	Minimum	Maximum	Mean(n=40)	WHO*	SAS*
Pb (□g/g)	0.04	1.0	0.40	1.50	2.00
Cd (□g/g)	0.05	0.15	0.10	0.20	0.50

WHO(1993),**SASO(1997).SASO:SAUDIARABIANSTANDARDSORGANIZAT

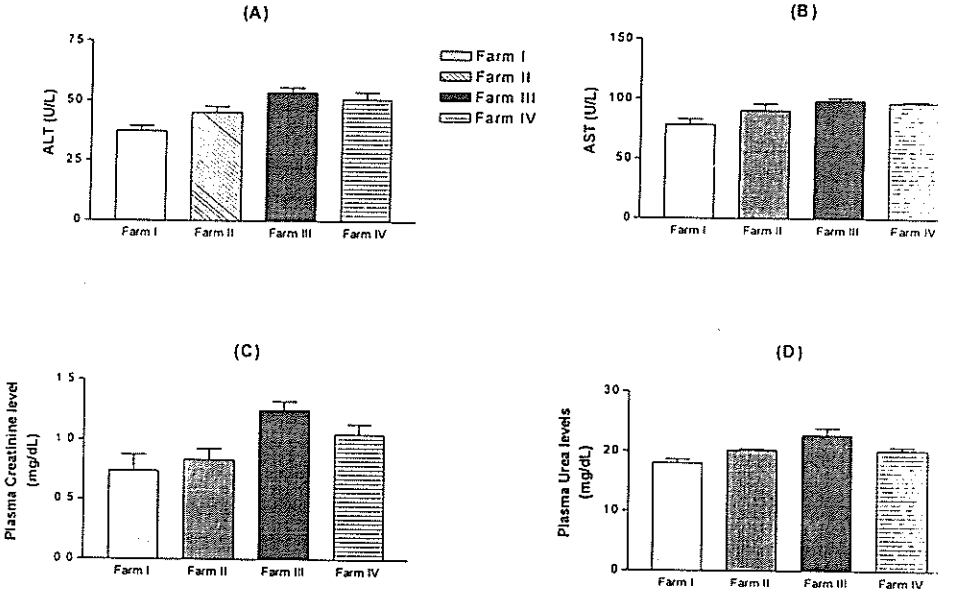

Fig. (1). Plasma levels of (A) ALT activities; (B) AST activities; (C) creatinine and (D) urea i samples of different farms .

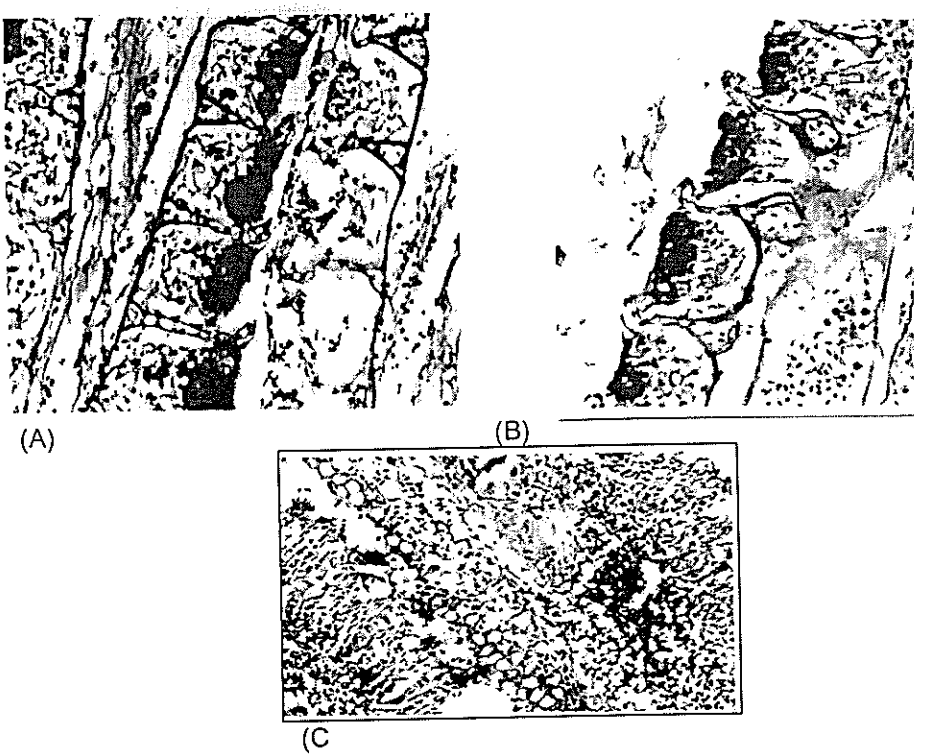

Fig. (2). Gills of Tilapia nilotica showing: (A) Thickening of the interlamellar membranes a activation of mucous glands and necrosis (PAS.X.400); (B) Thickening of the interlamel basement membrane.(PAS.X.400) and (C) Lamellar necrosis and sloughing (H&E. X.400).

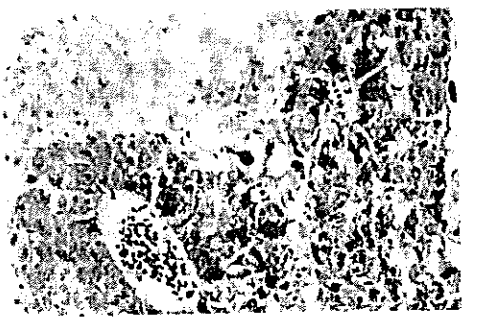

Fig. (3). Liver of Tilapia nilotica showing: Cloudy swelling, glycogen infiltration, hydropic degeneration, necrosis and congestion (H&E.X.200) .

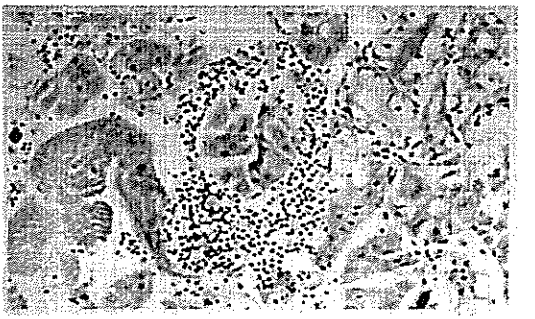

Fig. (4). Kidney of Tilapia nilotica showing: Coagulative necrosis of renal tubule inflammatory cells infiltration (H&E.X.400).

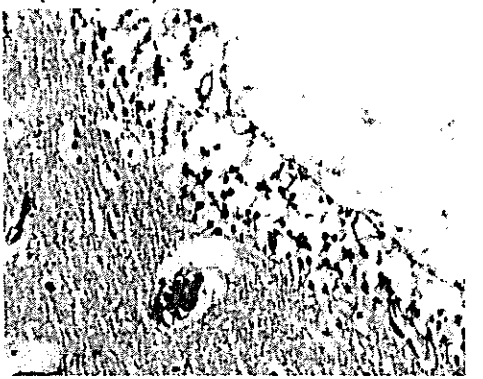

Fig.(5). Brain of Tilapia nilotica showing : Meningitis with mononuclear leucocytic cells infi and gliosis.(H&E. X400).

ص

ك بالمعادن الثقيلة للأسماك مزارع المياة العذبة - بمنطقة القصيم- المملكة ة السعودية- دراسات هيستوباثولوجية وكيميائية حيوية

ن من الدراسة : أجريت هذة الدراسة لتحديد مستويات بعض المعادن الثقيلة مثل الرصاص والكادميوم
الى فى الاسماك الموجودة ببعض مزارع منطقة القصيم ومقارنة تلك المستويات بالتراكيز المسموح بها عالميا
ظمة الصحة العالمية . الدراسة الباثولوجية للانسجة الاسماك كان لها دور فى هذة البحث .
مستخدمة : تم جمع عدد ٤٠ سمكة بلطي نيلي من مزارع مختلفة ببريدة – منطقة القصيم . وتم تحليل عناصر
ص و الكادميوم بأستخدام جهاز الامتصاص الذرى فى كلا من أنسجة تلك الاسماك (العضلات ، الخياشيم ، الكبد
، المخ ، والدم) والماء .
كان مستوى قياس الرصاص والكادميوم محددا فى أنسجة الاسماك و الماء فى كل المزراع التى كانت تحت
. وكان متوسط قياس عنصرى الرصاص والكادميوم فى الماء هو ٠,٠٩ ، ٠,٠٤ جزء فى المليون متجاوزة
مسموح بها عالميا طبقا لمنظمة الصحة العالمية (٠,٠١ ، ٠,٠٠٥ جزء فى المليون) . بينما كان متوسط كلا
اص والكادميوم فى الانسجة أقل من التراكيز المسموح بها عالميا (١,٥ جزء فى المليون للرصاص و ٠,٢
المليون للكادميوم) .كانت العينات المأخوذة من المزرعة رقم ٣ أعلى فى تركيز عنصر الرصاص مقارنة
زارع . لوحظ وجود تغيرات باثولوجية- تنكرزية فى الاعضاء المختلفة خصوصا فى المزارع أرقام ٣ ، ٤ .
يضا.ذيادة مستويات أنزيمات كلا من الكبد والكلى فى نفس المزرعتين السابقتين .
: كان متوسط عنصرى الرصاص والكادميوم عالى فى الماء بينما كان أقل فى أنسجة أسماك البلطى موضع
نحن ننصح بقياس عنصرى الرصاص والكادميوم بشكل دورى بالمزراع ومنع وصول مصادر المعادن الثقيلة
لك الاسماك .