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Effect of Some Heavy Metals Polluted on Fish Farmed Performance

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Abstract

The objective of the present study was to investigate the effect of heavy metals in hematological, serum biochemical parameters and chromosomal aberrations in Nile tilapia *oreochromis niloticus*. These study was performed on five groups of different sites: Group 1 agriculture wast water (G1), group 2 industrial wast water (G2), group 3 swage (G3), Group 4 underground water (G4), and group 5 as fresh water (control) (G5). The results showed that agriculture wast water (G1) is highly effect on serum biochemical analysis, criatenine (Cr) was 0.2 mg/L, alanine transferase (ALT) was 20 IU/L and asparatot amino transferase (AST) was 11.671 IU/L compared with a control group (G5). The gills histopathological alternation of the fish from (G2) industrial wast revealed a typical cellular structure as nuclear membrane denegation and of normal cytoplasmic mitochondria. Fishes samples from different sites showing different types of chromosomal aberrations including deletion (D), breaks (B), centric fusion(CF), gabs(G) and fermentation (F).

Keywords: heavy metals, fishes environment, toxicity, cytogenetic.

Introduction

The metal which has relatively high density and toxic at low quantity is referred as heavy metals chromium (Cr), Iron (Fe), manganese (Mn), and Zinc (Zn). They are essential to maintaining the body metabolism, but they are toxic at higher concentration **(Hadeel *et al., 2019*)**. The heavy metals concerned with environmental science chiefly include Cr, Fe, Me, Cd, Cu, Zn and manganese (Mn) (Heavy metals further, the heavy are metallic elements which have a relatively high density, and they are poisonous at low quantity). Since 2007 production of mineral commodities included Zinc, copper, silver and coal in 2018. The key valuable minerals were copper with a value of USD 502 Million **(ICMM, 2011)**. In the biochemical aspects. Cr, Fe, Zn, Cu and Mn are classified as essential metal elements that have poisonous potentials when they are higher than safe levels, **(Clani, *et al.*, 2003 *and Tao, et al.*, 2012)**. The metals and metalloid impact information on several aspects of O. vitiates can be applied for environmental and human health risk management as well making guldens, standards, regulation on legislation and policies for mining industry in law people's Democratic Republic, pollution and corresponding risks that come into existence by this rapid increase in agriculture activation population growth, urbanization and industrialization and critical issues about environment **(Akbulut, *et al.*, 2010; Sommez *et al.*, 2013)**. There is no doubt that the most dangerous chemical pollutions in water is heavy metal concentration **(Sommez *et al.*, 2012)**. Heavy metals do not pert in water and settle down swiftly onto sediment due to higher density than that of water this was demonstrated with Cd and Cu exposure, metals showed 72 to 97% decrease from their initial concentration after 96 hours of experiment (**Ghosal and Kaviraj, 2002**, **Ghosh *et al.*, 2016, Ghosh *et al.*, 2018)**. Uptake of heavy metals by fish from the environment primary occurs through gills, food, skin and freshwater fish through water taken with food and taken heavy metals are carried to organs by carrier proteins via blood path and can reach high concentrations by bonding to metal binding proteins in these tissues (**Sommez *et al.*, 2016)**. The toxic element concentration in fish depends on sex and age of fish, season and place. Pollution of water sources by anthropogenic activation leads to aquatic loss and therefore disrupts the balance of chain **(Afshan *et al.*, 2014)**. Several studies have linked increases in cytogenetic abnormalities in fish shellfish to polluted environment. This was done large through laboratory bioassays of polluted water sample in natural (**Hooftman and Raat**, **1982**, **Hose *et al*., 1987**., **Metecalf**, **1988**). The cytogenetic changes by observing the frequency of chromosomal aberration in the gill cells of the threated oreochromis niloticus by copper sulfate and lead acetate are measured by **Mohamed *et al*., (2008)**. The aim of this study aimed to the effect of water polluted (Cr, Mn, Fe, and Zn) on hematological, and serum biochemical parameters, hestopatholgoical, and chromosomal aberration of Nile tilapia.

Materials and Methods

Study area and design:

This work was conducted to determine the pollution of water, and fishes (*oreochromis niloticus*) by some heavy metals (chromium (Cr), Iron (Fe), manganese (Mn) and Zinc (Zn)). Samples were collected from four sources of environmental pollution of water and control (fresh water) in Sharkia and port said governorate, Egypt. Samples were collected from different sites from July to September 2018.

1- The first site, agricultural waste water (G1) in the Ramses area, Al-Hussainiya center, Sharkia governorate.
2- The second site, industrial waste water (G2) in the Al-Hussiniya plain area, Manshiet Abo Omar Center.
3- The third site, swage (G3) which located in private farm at shader Azam, Port Said Governorate.
4- The fourth source is under ground water (G4) at Abbasa Sharka Governorate.
5- The fifth site included three earthen ponds that received their water supply from Ismailia Canal (Nile water) (G5) at the central laboratory for Aquaculture Research, Abbassa, Sharkia Governorate used as control.

Water Samples:

Water samples from each site were collected from five spots at a fixed depth of 30 cm from the water surface. The plastic bottle was immersed to the required depth and fill with water samples and each sample was labeled to indemnify site and date of sampling according to **Boyd**, (1984). Physical parameters: pH, dissolved oxygen (DO), and salinity (S) were measured directly in each site during samples were taken to the laboratory for further chemical analysis chromium (Cr), Iron (Fe), Manganese (Mn), and Zinc (Zn)

Fish samples:

A live fish (*Oreochromis niloticus*) 200 fish adult were collected from the five farm and were taken in the tank by using source of oxygen transport to the laboratory, 20 fish for each sex (male and female) from every region. For each region, four fishes were used for cytogenetical studies and the other used for physiological, hematological, histopathological and biochemical parameters were estimated also.

Blood samples:

The blood samples were taken from caudal vein by using sterilized syringe containing heparin as anticoagulant for hematological and biochemical analysis such as, red blood cells (RBCs) count, white blood cells (WBCs), count, hemoglobin (Hb), hematocrit (Ht), creatinine (Cr), alanine amino transferase (ALT) and aspartate amino transferase (AST).

Water analysis:

This analysis was measured directly during collection of samples from the different areas. Dissolved oxygen (mg/L) was measured at the site of sampling by using oxygen – meter (model YSI58). Hydrogen ion concentration (PH), was determined by using PH meter (Model 301). Salinity (ppt) was measured by using a salinity conductivity meter (Model YSI 57).

Estimation of heavy metals:

Determination of heavy metals (ppm) were measured by using atomic absorption spectrophotometer Perkin Elmer 3300, after the digester by concentration HCL acid as described by (**APHA 1985**).

Chromosomal preparation:

Chromosomes prepared according to the method of **Kligerman** and **Bloom** (**1977**). Chromosomes preparation was made from gill filaments. Intramuscular injection of 1 mL colchicine solution 2% concentration for 2-3 hour later, gill filaments were harvest and fragmented in 5 mL of physiological solution (0.9% NaCL) for 10 minutes at 28 °C. then centrifuged at 1000-1500 rpm for 10min. the supernatant was discarded and the pellets of cells were fixed with 5ml hypotonic solution (56% KCL) and incubated at 28°C for 30 min. Then centrifuged and supernatant was discarded and the pellets of cells were fixed with Clark's fixative (methanol / glacial acetic acid 3:1 v.) for 30-60 min in refrigerator then centrifugated and supernatant was discarded. Report this step several times (4-5 times), after the last step, the cells were dropped on slides which kept in ethanol solution (70%) in the freezer. After air-drying the slides were stained with 1-5% Gimesa stain for 15-20 min. for each sample metaphases were microscopically examined randomly by using x10 eye piece and x100 oil immersion. Different types of chromosomal aberrations will be recorded.

Histopathology assay:

After dissecting of fish samples some organs such as gills and liver were removed and stored in 10% formaldehyde for histological studies. They were examined and photographed under light microscope unit.

Statistical analysis and test of significant variance:

Statistical analysis was performed using the analysis of variance (ANOVA) and Duncan's Multiple Range test to determine differences between treatments mean at significance level of 0.05 standard division were run on the computer using the SAS program **(SAS 2004)**.

Results and discussion:

Water quality parameters:

The values of water quality parameter from the study sites reference sites are shown in Table 1.

Table 1. Mean concentrations of water parameters at various sampling sites.

Site	DO mg/l	PH	Salinity PPT
G 1	2.82[bc]	7.58	3.80[a]
G 2	8.47[a]	7.52	3.35[a]
G 3	7.14[ab]	7.22	2.20[b]
G 4	5.11[c]	7.24	2.63[b]
G 5	2.93[d]	7.41	2.73[b]
±SE	0.45	0.13	0.19
P Value	0.0001	0. 2424	0.0009

Where: 1 = Agriculture waste water (G1), 2- industrial waste water (G2), 3- Sewage (G3), 4- Under ground water (G4), and 5- river Nile (Abbasa water) (G5). SE= standard errors and *P* = probability.

Water parameters; DO pH and salinity were suitable for culture of fish in G2, G3 and G4. This result agree with **Boyd** (**1982**) who suggested that the water condition from sites of study for fish to live, especially temperature, dissolved oxygen, pH and electrical conductivity. In addition, no significant differences were found in pH values among groups of study.

Heavy metals in the waters

The average concentration of chromium (Cr), Manganese (Mn), Iron (Fe), and Zinc (Zn) in water samples from the study sites are shown in Table 2.

Table 2. Mean concentration of heavy metals of water (ppm) at various sampling sites

Site	Cr	Mn	Fe	Zn
G 1		0.196[b]	0.139[b]	
G 2		0.198[b]	0.229[ab]	
G 3	*>0.2	0.128[b]	0.132[b]	*>0.2
G 4		1.060[b]	0.136[b]	
G 5		0.225[a]	0.351[a]	
± SE		0.182	0.054	
P Value		0.0226	0.0666	

Where: 1 = Agriculture waste water (G1), 2- industrial waste water (G2), 3- Sewage (G3), 4- Under ground water (G4), and 5- River Nile (Abbasa water) (G5). Cr = Chromium, Mn = Manganese, Fe=Iron, and Zn = Zinc SE = Standard errors and P= Probability.

The concentration of chromium (Cr) and zinc (Zn) was higher than the allowable in all the water sources under study (*>0.2), and since there are no differences in the concentration values of each of the two previous elements, there is no need to conduct a statistical analysis of the two aforementioned. The concentration of Mn was significantly affected (P< 0.05), by all groups (G1,G2, G3 and G4), and the highest value was recorded in G4. The concentration of iron (Fe) was significantly different (P< 0.05). The results were in contrast with other studies **Intamat *et al.* (2016)** who reported the concentration of Aresenic (As), Cadmium (Cd), Iron (Fe) and Manganese (Mn) in water reservoirs around gold min area in Loei provine of Thailand were higher but not significantly different compared with the reference site. Also, with **Phoonaploy *et al.* (2016)** who reported that concentration of Cadmium (Cd), Chromium (Cr), Lead (Pb), Nikel (Ni), Zinc (Zn) and Manganese (Mn) were lower than of the metals reported in this study. But the results were in accordance with **Jianga**

***et al*., (2018)** who detected that concentration of Cr in water from Lake Caizi in Southeast China was higher than of the non-contaminated area.

In general, most of human activities related to agriculture, industry and sewage waste can release heavy metals in the water. The toxic metals, particularly at high levels can be absorbed and directly induce detrimental effects to aquatic animals as well as cause negative signs and symptoms to human by **Salem *et al.* (2014)** who situate at the top of the food chain and ecosystem.

Hematology:

Table 3 showed that, the lowest RBCs count was detected in G1, G3 and G4 compared with other G2 and G5. Furthermore, the lowest WBCs was detected in G4 compared with other groups, may be due to sewage contents of more of metals than other sites. Value of hemoglobin (Hb) was 3.83 in the sewage site this value lower than other sites and significant between groups at ($P < 0.05$) as shown in Table 3. The present results agree with those reported by **Khadre (1990)** who reported a significant decrease of RBC_s count, hemoglobin content and packed cell volume of the fish.

Table 3. Hematological parameters of fish at various sampling sites

Site	Hematology Parameters			
	RBCs	WBCs	Hb	HTC
G1	0.24^{c}	5.697^{a}	3.983^{bc}	$\mathbf{0.380^{b}}$
G2	0.617^{a}	5.677^{a}	4.133^{b}	$\mathbf{0.333^{c}}$
G3	0.240^{c}	5.860^{a}	3.833^{c}	$\mathbf{0.373^{b}}$
G4	0.227^{c}	5.527^{a}	3.933^{c}	$\mathbf{0.390^{ab}}$
G5	0.485^{b}	4.577^{b}	4.60^{a}	$\mathbf{0.423^{a}}$
± SE	0.0386	0.166	0.057	**0.012**
P Value	0.0003	.0001	.0001	**.0001**

Where: 1 = Agriculture waste water (G1), 2- industrial waste water (G2), 3- Sewage (G3), 4- Under ground water (G4), and 5- River Nile (Abbasa water) (G5). RBC_s= Red blood cells count., WBC_s= white blood cells Count, Hb = Hemoglobin and HTC = Hematocrit ST = Stander errors and *P* = Probability.

In Table 4 showed that ALT and AST, the enzyme measurements are valuable in diagnosis of hepatic disorders in this study. ALT value was high in industrial 23.67 IU/L and these value significant ($P < 0.05$) compared with other groups, but AST value was highly 16.33 IU/L in underground water site and highly significant ($P < 0.05$) compared other groups. This elevation in AST and ALT activities of fish was attributed to the damage of liver and kidney by the action of heavy metals as has been previously reported by **Heath (1987)** and **Sandnes *et al.*** (**1988**). Creatinine value was highly (0.24 mg/dl) in fish at industrial sites (G2) which the heavy metals are concentrating more than any sites and significant ($P < 0.05$) between other groups. The results agreement with **Naga *et al* 2005)** and **Javed *et al.* (2015)**, they demonstrated significant consequences in plasma enzymes (AST, and ALT) after exposure to Cd in marine fish mugil Seheli, also, heavy metals can negatively affect fish health in terms of protein, carbohydrate and lipid profiles.

Table 4. Creatinine value and serum enzymes parameters of fish at various sampling sites.

Site	Hematology		
	Cr (mg/dL)	**ALT (IU/L)**	**AST (IU/L)**
G1	0.2000^{ab}	20.00^{ab}	$\mathbf{11.667^{ab}}$
G2	0.240^{a}	23.667^{a}	$\mathbf{13.333^{ab}}$
G3	0.177^{b}	20.00^{ab}	$\mathbf{14.667^{ab}}$
G4	0.180^{b}	17.667^{b}	$\mathbf{16.333^{a}}$
G5	0.197^{ab}	17.000^{b}	$\mathbf{10.667^{b}}$
± SE	0.016	1.565	**1.571**
P Value	0.077	0.075	**0.0146**

Where: 1 = Agriculture waste water (G1), 2- industrial waste water (G2), 3- Sewage (G3), 4- Under ground water (G4), and 5- River Nile (Abbasa water) (G5). Cr = creatinine, ALT = Alanine transaminase, and AST = Aspartate aminotransferase. SE = Stander errors and *P* = Probability

Histopathology study:

Histopathology study is an important discipline to evaluate morphology changes and cells and tissues. The liver is the vital organ for detoxification and accumulation of toxic substances. Heavy metals adversely affect the function of different aquatic animal organs, especially changes of hepatic enzyme activities, extravasation of blood and necrosis of liver cells, fusion of gill Lamellae, and genotoxicity Figure 1 showed that necrobiotic change in hepatopancreas and hepatocytes with infiltration by low number of mononuclear inflammatory cells in G2 and G3. In figure (2) showed that congestion and mononuclear inflammatory cells. This results were confirmed with **Ahmed *et al*.** (**2008**) and **Ahmed *et al.*** (**2013**) who found that heavy metals adversely affect the function of different animal organs, especially changes of hepatic enzyme activities, extravasation of blood and necrosis of the liver cells, fusion of gill lamellae and genotoxicity. The results were in accordance with **Vinodhini and Narayanan** (**2009**) who reported that the accumulated heavy metals could cause cellular degeneration in the liver of *cyprinus carpio*.

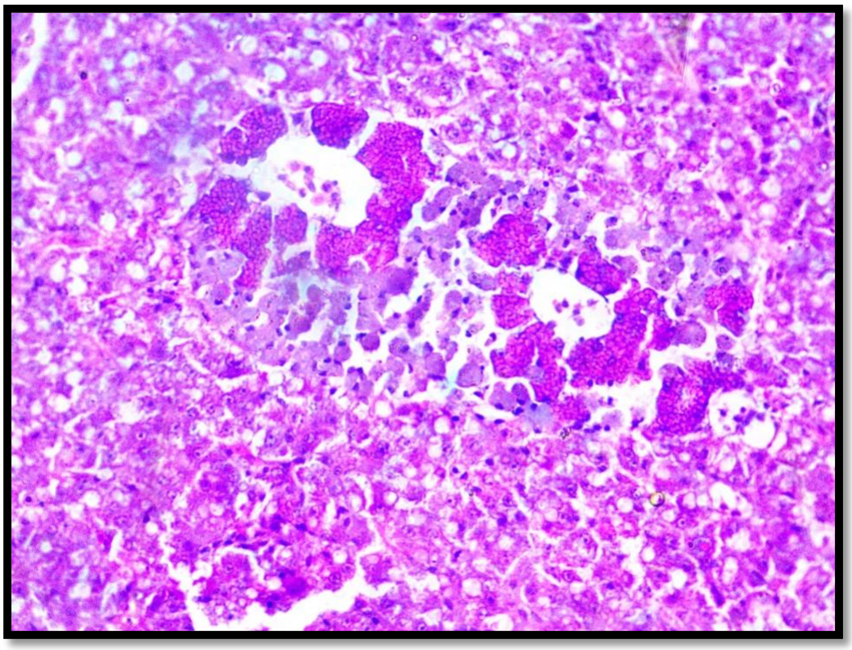

Fig.1 showing necrobiotic change in hepatopancreas and hepatocytes with infiltration by low number of mononuclear inflammatory cells (H&E - 400X)

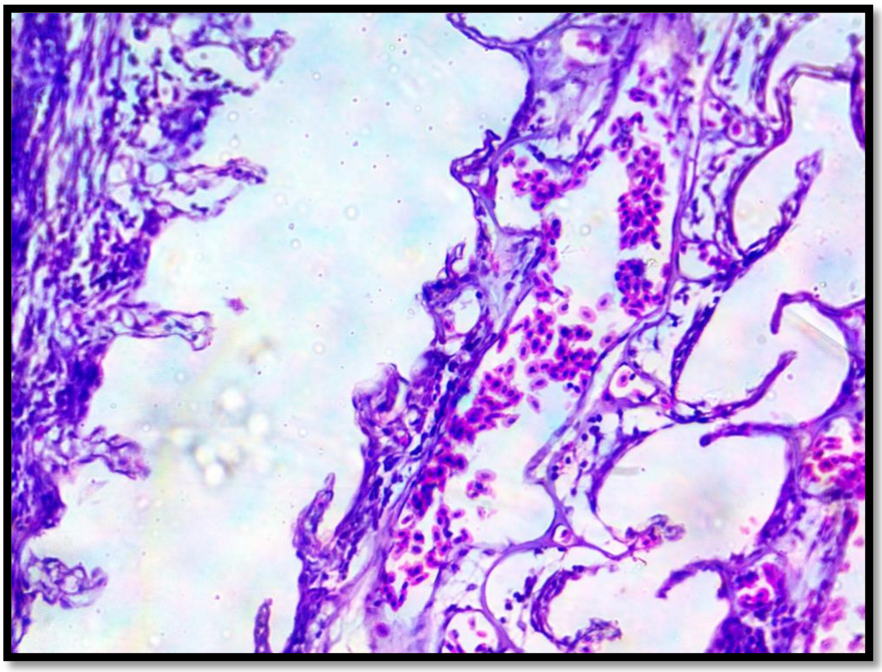

Fig.2 showing congestion and mononuclear inflammatory cells infiltration in secondary gill lamellae with some gill lamellae showing hypotrophy (H&E – 400X)

Chromosome study:

This study reviled some types of chromosomal aberration such as Gap (Figure 3) and deletion (Figure 4), this the result, this the results agree with **Mahmoud *et al*. (2010)** who showed that the diploid number of chromosomes in Tilapia were 2n = 44 and high percentage of chromosomal abnormalities in head kidney cell of *O.niloticus and T.Zillii* were observed clearly in the form of centromeric attenuation, chromatid breaks, chromatid gaps, chromatid deletions, centric fusion and fragmentation. Structural abnormalities of chromosomes in *oreochromis niloticus* in this study revealed as reported by **Khamlerd *et al.*** (**2019**) who revealed seven types of chromosome some aberration including Deletion (D), Break (B) Centric fusion (C.F), Gap (G) and Fragmentation (F) in *oreochromis niloticus* contaminated with heavy metals, this study agree with **Neerotanaphan *et al.*** (**2020**) who found that many types of chromosome aberrations in Asian swamp eel. Some of the types of chromosomal abnormalities for this study infiltration in secondary gills mamilla with some gill lamellae showing hypotrophy in group 2 and group 3 compared with other site group.

Fig. (3) chromosome aberration in *oreochromis niloticus* **(G)**

Fig. (4) chromosome aberration in *oreochromis niloticus* **(D)**

Conclusion

The concentration of heavy metals (Mn, Fe) in water in this study sites (G1, G2, G3 and G4) were higher than of those from the control site. Furthermore, these heavy metals results showed that effected on Nile tilapia fish in many of chromosomal aberration, serum biochemical changes, gills and liver cells structure alterations of Nile tilapia.

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تأثير بعض المعادن الثقيلة الملوثة على أداء الأسماك المستزرعة

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2- قسم الإنتاج الحيواني – كلية الزراعة – جامعة بنها – مصر.
3- المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية – وزارة الزراعة مصر.

الهدف من هذه الدراسة هو معرفة تأثير بعض المعادن الثقيلة الملوثة على الصفات الكيميائية والحيوية للدم والاختلالات الكروموسومية لأسماك البلطي النيلي (*Orochromis niloticus*).

أجريت هذه الدراسة على 5 مجاميع من الأسماك التي تم جمعها من 5 مواقع مختلفة.

1- مياه صرف زراعي.	(مجموعة رقم 1)
2- مياه صرف صناعي.	(مجموعة رقم 2)
3- مياه صرف صحي.	(مجموعة رقم 3)
4- مياه جوفية.	(مجموعة رقم 4)
5- مياه عذبة.	(مجموعة رقم 5)

أظهرت النتائج أن مياه الصرف الزراعي تأثيرها كأن كبيراً على الصفات الكيميائية والحيوية للدم فكان معدل الكرياتينين (Cr) 0.2 مللجم/لتر ومجموعة الأمين الناقلة (ALT) 20 وحدة دولية/لتر أما مجموعة الأمين الناقلة (AST) سجلت 11.67 وحدة دولية/ لتر وذلك مقارنة بمجموعة رقم (5) وهي مجموعة الكنترول والتي سجلت 0.19 مللجرا/ لتر من الكرياتنين (Cr) أما مجموعة الأمين الناقلة (ALT) كانت 17 وحدة دولية/ لتر، (AST) كانت 10.7 وحدة دولي/ لتر وكانت الاختلافات معنوية ($P<0.05$) وكذلك النتائج بين المناطق أظهرت التحليل الهيستوباثولوجي لخياشيم أسماك المجموعة رقم (2) وهي التلوث الصناعي أنماطا مختلفة من التركيب الخلوي مثال لذلك انحلال في الغشاء النووي والميتوكوندريا الطبيعية في السيتوبلازم كما تبين أن هناك اختلاف في أنماط الاختلالات الكروموسومية والتي احتوت على حذف الكروماتيد وكسر في الكروموسوم وخلل في السنترومير ووجود فجوات كروماتيدية، كذلك التصاق في نهايات الكروماتيد مع بعضها. توصى الدراسة التي تم إجراءها أن أسماك البلطي النيلي في جميع المناطق الملوثة، مجموعة رقم (1)، و(2)، و(3)، و(4) غير صالحة للاستخدام الآدمي وضارة بصحة الإنسان.