The Effect of Water Pollution on Chromosomal Behavior in Nile Tilapia (*Oreochromis Niloticus*)

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ABSTRACT

Random samples from three different natural populations were caught from three different localities i.e., polluted Drainage Canal, Faculty Fish Farm and The Edku Lake. Different heavy metals concentrations in water and in different fish organs (i.e., liver, head, and muscle) were estimated to identify the residues of such pollutants in fish body in relation to water quality resources. Water analysis indicated that Drainage Canal of village No.10 performed the significant higher level of heavy metals concentrations in relation to other two water resources, while the lowest concentration of different heavy metals were found in The Edku Lake. It's mean that water quality might be ranked as Edku Lake (the best), Faculty Fish Farm (the intermediate) and Drainage Canal (the most drawback resource). Such differences among these three water resources in their heavy metals concentrations affect drastically the concentrations of such heavy metals in fish organs from the three different localities. Due to accumulation of the heavy metals in fish tissue; data clearly indicated that Drainage Canal water fish samples exposed high degree of heavy metals concentrations in fish organs in relation to other two localities. For instance lead concentration in Drainage Canal water exceeded the other two localities with 77.4 fold for Fish Farm and with 152.7 fold for Edku Lake. The degree of water pollution with different heavy metals drastically affected the chromosomal behavior during mitosis. Different types of chromosomal aberration were observed i.e., gaps, deletion, fragment, stickiness... etc. in the studied tissues i.e., gills and blood red cells.

INTRODUCTION

Tilapia species are commercially important group of perch-like fishes (family cichlide), the production of farmed tilapia has witnessed a 6-fold increase during the past 15 years, increasing from 383,654 mt in 1990 to 2,096,187 mt in 2005 (FAO, 2007). In Egypt, Nile tilapia (Oreochromis niloticus) constitutes about 70 % of the Nile and Lake's catch, which considered as the first fish crop in Egypt.

Heavy metals from man-made pollution sources are continually released into aquatic ecosystems. The contamination of heavy metals is a serious

threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain (Eisler, 1988).

Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level (Barak & Mason, 1990; Evans et al., 1993; Rashed, 2001). The commercial and edible species have been widely investigated in order to check for those hazards to human health. (Farkas et al., 2003; Mansour & Sidky, 2002)

Exposure to heavy metals is known to cause alteration in hematological parameters in fishes (Heath, 1995). The most important parameters used in bioindication are chromosomal aberration and the generation of genetic material fragments, known as micronuclei (Schmid, 1975; Heddle et al., 1983; Ribeiro et al., 2003). Such abnormalities are related to cell division failures, cell death processes, and to genotoxicity and/or mutagenicity (Nakano & Oka, 1991; Cormak, 1991; Fenech, 2000). In fish, some of these abnormalities in cell behavior during mitotic division have been reported after exposure to chemical agents or polluted water (Cavas & Gőzűkara, 2003).The aim of the present study is to identify the effect of water pollution on chromosomal behavior and aberration during cell mitotic division in fish organs (gills and red cells in blood).

MATERIALS AND METHODS

Sampling locations

Three different natural populations from three different localities of Nile tilapia were presented by three different random samples. The three different localities were: polluted Drainage Canal in front of village No.10, Faculty Fish Farm (Abiece region Alexandria government) and Edku Lake (Behayra government). The live fish samples were transported to the Faculty fish genetic aquarium. Liver, head, and muscle were cut in small pieces. The samples were dried in an oven at 70 °C for 48 h; and homogenized separately in a mortar. Finally, the fine samples were preserved in clean and dry polyethylene bottles.

A water samples were collected as 5 litters of surface water (e.g. 0–20 cm depth) from each concerned ecosystems. The collected samples were transferred to the laboratory where they were kept in a refrigerator until analyzed.

Chemical analysis

Accurately weighted 1 g from each grouped dried sample of each organ was homogenized separately in a mortar and treated with 4 ml of H_2O_2 and 4 ml of H_2SO_4 in a flask. Finally, the solution was transferred to a 100 ml volumetric flask and diluted to 100 ml volume with distilled water. After filtration the elemental concentrations in the solution were measured with a Perkin Elmer AAnalyst-400 flame atomic absorption spectrophotometer with hollow cathode lamp (zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), manganese (Mn), iron (Fe), nickel (Ni), and chromium (Cr). Concentration of the heavy metals in water samples were estimated with the same technique which applied in the solution prepared in fish organs.

Analysis of chromosomal abnormalities

The present study aims at disclosing the capability of pollutants in inducing cytogenetic damage in the chromosome complement of *Oreochromis niloticus*.

Each fish had received 0.1 ml (4µg) colcemid / 20 gm b.wt of fish. After four hours the animals were killed and gills were removed. Preparations of chromosome complement were carried out according to the method that described by (Seehy, 2009).

Scanning slides for mitotic spreads was conveniently accomplished with a 25X magnification objective, and analysis was with a 100X objective. For control unbiased situation, all prepared slides were coded prior to scoring. For chromosome abnormalities at least 50 scorable metaphase per treatment were investigated for gaps, deletion, fragment, stickiness... etc and recorded.

Micronucleus test

The peripheral blood smears were obtained through the gills blood by means of a medial imprint following dissection. The slides were air- dried for 12 hrs and then fixed in methanol for 10min, followed by 5% Giemsa (V\V) staining.

From each fish 1000 erythrocytes were counted. The slides were analyzed using a 1000X oil – immersion lens.

Statistical analyses were performed using Student's t test. This method was carried out following procedure that was described by (Grisolia & Starling, 2001).

RESULTS AND DISCUSSION

Water ionic characteristics

Table (1) shows the different concentration of different heavy metals in different water samples in relation to the three different studied localities.

Data clearly indicated that the Drainage Canal of Abiece locality performed higher degree of pollution with the different heavy metals compared with the two other localities except in case of chromium (Cr).

Heavy metals in different fish organs

Table (2) shows the concentrations of heavy metals in different organs of the fish body (e.g. liver, head and muscle). In general concentrations of heavy metals (Zn, Cu, Cd, Pb, Mn, Fe, Ni and Cr) in different organs were higher in Drainage Canal in relation to the other two different localities.

It's important to note that the average of heavy metal concentrations for the grouped organs performed highly significant differences between different localities in which Drainage Canal exposed the higher concentration.

For instance some of such heavy metal as Cu in Drainage Canal (14.23 ppm) exceeded the other two localities with 4.6 fold for Fish Farm (3.07 ppm) and with 6.9 fold for Edku Lake (2.05 ppm), Cd in Drainage Canal (8.5 ppm) exceeded the other two localities with 65.4 fold for Fish Farm (0.13 ppm) and with 70.8 fold for Edku Lake (0.12 ppm) and Fe in Drainage Canal (46.57 ppm) exceeded the other two localities with 13.1 fold for Fish Farm (3.56 ppm) and with 13.7 fold for Edku Lake (3.4 ppm)

Table (2) performs heavy metals accumulation in different organs of different fish samples in relation to the permissible concentration, which advised from FAO (1983). Data clearly indicated that Edku Lake had concentrations zinc, copper, lead, iron and chromium below the permissible limits (ppm) However, all of different localities contained cadmium at concentrations above the permissible limit (0.20 ppm; FAO, 1983). In Fish Farm contained zinc, cadmium, lead and iron at concentrations above the permissible limit and in Drainage Canal all of heavy metals above the permissible limit.

The most important point of view was the accumulation of heavy metal ions in liver tissue significantly exceeded accumulation in the other two fish tissues i.e., head and muscle as shown in figure (1).

From the population genetics point of view, the natural population which existed in the polluted Drainage Canal is completely adapted to pollutant stresses by natural selection. However, these adapted fish populations may represent a serious effect on the health of the local inhabitants where high

concentrations of heavy metals implicate fish tissues affecting its quality and hence become a threat to man health.

Analysis of chromosomal abnormalities

Table (3) shows the obtained data from the cytological examination of gill cells of fish caught from two locations i.e., Drainage Canal and Fish Farm. Some types of chromosomal aberrations such as fragment; stickiness; deletion and gap were observed.

Fragments were found to be 4%; stickiness was found to be 30%; chromatid deletions were found to be 10% and gaps were found to be 2% in the Drainage Canal group. Fragments were found to be 1%; stickiness was found to be 8%; chromatid deletions were found to be 0% and gaps were found to be 0% in the Fish Farm group.

However, total aberrant metaphases in the Drainage Canal group were about 46% and in the Fish Farm group were about 9%.

Micronucleus test

Table (5) shows the obtained data from the cytological examination of micronucleated red cells in blood of fish caught from the two tested locations.

Normal cells were found to be 78.5% and micronucleus test were found to be 21.5% in the Drainage Canal group. Normal cells were found to be 96.3% and micronucleus test were found to be 3.7% in the Fish Farm group.

These results showed that the genome of fish affected by contaminants.

Figures (2-16) illustrated the different type of chromosomal aberrations and micronucleus test in blood of Nile tilapia (*Oreochromis niloticus*).

The bioindicator approach is not the only method for monitoring possible ecotoxilogical effects of pollutants. One traditional method is direct measurement of chemical pollutants in streams or in the organisms themselves. Another is the laboratory toxicity test in which the toxicity of a pollutant is determined by the death rate of exposed organisms. Compared to the bioindicator approach, these methods often provide only limited information.

Bioindicators offer several types of rather unique information not available from other methods: (1) early warning of environmental damage; (2) the integrated effect of a variedly environmental stresses on the health of an organism and the population, community, and ecosystem; (3) relationships between the individual responses of exposed organisms to pollution and the effects at the population level; (4) early warning of

potential harm to human health based on the responses of wild life pollution; and (5) the effectiveness of remediation efforts in decontaminating water ways.

Bioindicator is an excellent tool for monitoring the health of biological populations, assessing the potential hazards of environmental pollution to human health, determining if industry is complying with regulations, and evaluating the effectiveness of remedial actions.

The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. In laboratory tests involving fish, several substances have been shown to have genotoxic potential (Odeigah & Osaneyinpeju, 1995; Minissi et al., 1996), while others have proven innocuous (Belpaeme et al, 1996).

The micronuclei represent a centric chromosome fragments or whole chromosomes lost during cellular anaphase. These structures are easy to visualize in erythrocytes and are therefore often used as a measure of chromosomal aberrations (Rabello - Gay, 1991).

In fish, the micronucleus test is usually based on erythrocytes. but liver and gills tissues have been used (Alsabti & Metcalfe, 1995). In mammals, young bone marrow erythrocytes can be distinguished quite easily from the mature ones by examining the Giemsa staining pattern of cells. However, in fish, this distinction is not feasible. Rodriguez – Forero (1995) suggested that young fish erythrocytes stain as basophiles with Giemsa. Observations made in our laboratory conditions agree with Ueda et al. (1992) findings that they are not distinguishable. The same others (Ueda et al., 1992) counted fluorescent acridine – orange – stained erythrocytes in the peripheral blood of fish, providing evidence that there is a significant volume of young erythrocytes peripheral blood.

Exposure to heavy metal has been associated with cancers, degree rative neurologic diseases, and altered immune response, but the mechanism of action of nuclear genotoxic potential is a primary risk factor for long term health effects such as cancer and reproductive health outcomes (Bolognesi et al 2003) Hagmar et al, (2001) reviewed the use fullness of cytogenetic genesis and concluded that chromosomal aberration (CA) frequency predicts overall cancer risk in health subjects, but such associations have not been found for sister-chromatid exchanges and micronuclei (Mn). Although the genotoxic potential of pesticide is believed to be low, genotoxic potential of pesticides is believe to be low, genotoxic monitoring informworker populations could be a useful tool to estimate genetic risk from exposure to complex pesticide mixtures over extended lengths of time. To date, genotoxic biomarker studies of workers exposed to pesticides have focused on cytogenetic end points, including CAS, Mn

frequency, and sister-chromatid exchanges. In the last decade, single-cell gel electrophoresis or the comet assay has been established as sensitive and rapid method for the detection of DNA single–Strand breaks and incomplete excision repair (Fairbain et al. 1995). These biomarkers have been well develop wit high inter laboratory reliability, but they are not specific to exposure and to date have not been associated with a risk for human cancers or other disease out comes.

The greatest for new biomarkers of early effect lies in toxicogenomics, a filed of study that examines how the entire genome responds to toxicants or other hazards (Toraaason et al., 2004). The ability to monitor changes in gene expression as a result of environmental exposure holds great promise in our standing under of the effect of environmental toxicants on human health.

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Table 1.Concentrations (ppm) of heavy metals in water samples collected from different ecosystems, compared with the permissible limits.

Meta	Drainage Canal	Fish Farm	Edku Lake	WHO limits (mg / L)
Zn	0.606	0.081	0.074	5.00
Cu	0.138	n.d.	n.d.	1.0
Cd	0.021	0.017	0.009	0.005
Pb	0.157	0.141	0.052	0.05
Mn	0.250	0.249	0.232	0.10
Fe	0.970	0.641	0.541	0.30
Ni	0.147	0.072	0.052	0.005
Cr	0.328	0.326	0.081	0.1

n.d. = no values determined or established in this study.
WHO World Health Organization limits for drinking water

Table 2.Concentrations (ppm) of heavy metals in Bolti fish (*Oreochromis niloticus.*)[a], compared with the permissible limits.

Metal	Drainage Canal				Fish Farm				Edku Lake				Permissible limits (ppm) FAO (1983)
	Distribution in different parts			Mean (ppm)	Distribution in different parts			Mean (ppm)	Distribution in different parts			Mean (ppm)	
	Liver	Head	Muscle		Liver	Head	Muscle		Liver	Head	Muscle		
Zn	180.3	164.6	157.8	67.57	153.3	69.1	39.6	87.33	64.6	48.3	21.0	44.63	150
Cu	15.6	14.3	12.8	14.23	9.2	n.d.	n.d.	3.07	4.2	1.95	n.d.	2.05	10
Cd	10.4	6.1	9.0	8.5	0.23	0.07	0.08	0.13	0.22	0.06	0.07	0.12	0.20
Pb	51.5	44.2	78.4	58.03	0.31	0.39	1.55	0.75	0.32	0.45	0.38	0.38	1.5
Mn	0.28	0.41	0.17	0.29	0.30	0.62	0.17	0.36	0.21	0.25	0.12	0.19	n.l.
Fe	78.40	35.27	26.04	46.57	8.59	1.39	0.71	3.56	3.92	3.53	2.75	3.4	5.6
Ni	0.48	0.43	0.33	0.41	0.40	0.36	0.34	0.37	0.29	0.27	0.26	0.27	n.l.
Cr	2.85	0.94	0.84	1.54	0.69	0.56	0.50	0.48	0.37	0.33	0.30	0.33	1.0
Total	339.81	266.25	285.38	-	173.02	72.49	42.95	-	74.13	55.14	24.88	-	-

n.d. = no values determined or established in this study.

n.l = no information about maximum permissible limit in fish tissue.

[a] Each value represents an average of ten samples collected in May 3, 2008 and May 4, 2008.

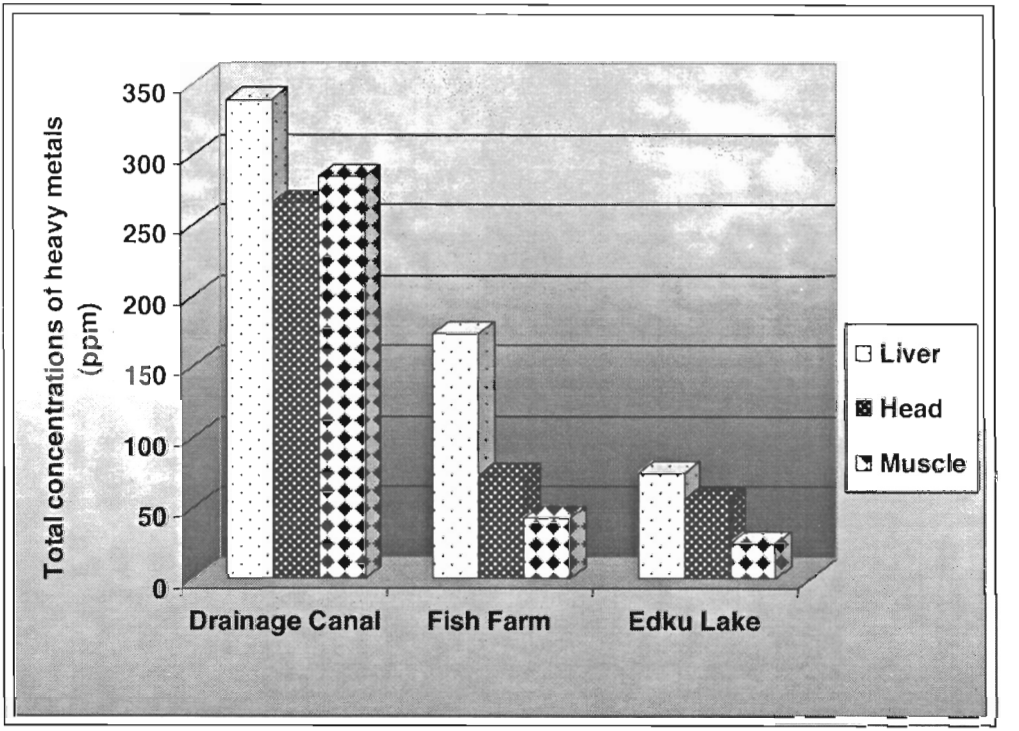
Table 3. Chromosomal abnormalitites in gills of Nile tilapia (*Oreochromis niloticus*).
50 metaphases were counted

Location	Type of Aberration				Total aberrant metaphases %
	Fragments %	Stickiness %	Deletions %	Gaps %	
Drainage Canal	4	30	10	2	46
Fish Farm	1	8	-	-	9

Table 4 Micronucleated red cells in blood of Nile tilapia (*Oreochromis niloticus*) collected from three different localities
1000 red cells were counted .

Location	normal%	MNT%
Drainage Canal	78.5	21.5
Fish Farm	96.3	3.7

Fig.1. Total Concentrations (ppm) of heavy metals in Bolti fish (*Oreochromis niloticus*)



figs. 2-16 Photomicrographs of normal (2) metaphase complements of *Oreochromis niloticus* in gills; Polar metaphase (3), Stickiness – S (4), Fragmented chromosomes (5), Fragmented nucleus (6), Fragments – F (7), chromatid break – CB (8). chromatid break – CB (9), Binucleate– B in gills (10), Trinuclei in gills (11), Tetraploid (12), Normal red cells (13), Micronucleus in red cells (14), Binucleate in red cells (15), Micronucleus and Binucleate in red cells (16).

الملخص العربي

تأثير تلوث المياه علي السلوك الكروموسومي في أسماك البلطي النيلي

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تم جمع ثلاث عينات منفصلة عشوائية من ثلاث عشائر طبيعية من أسماك البلطي النيلي من ثلاث مناطق مختلفة وهي: بحيرة ادكو (محافظة البحيرة)، المزرعة السمكية لكلية الزراعة-سابا باشا بمنطقة أبيس، المصرف الزراعي إمام القرية العاشرة مقابل المزرعة السمكية (محافظة الاسكندرية) و ذلك للمقارنة بين الثلاث عشائر في درجة تراكم العناصر الثقيلة في أنسجة أجسامها اعتمادا على درجة تلوث البيئة المائية لكل عشيرة و أثر ذلك على السلوك و الشذوذات الكروموسومية إثناء الانقسام الميتوزى لخلايا هذه الأسماك. و لقد أظهرت النتائج أن ماء المصرف الزراعي هو الأكثر تلوثا بالعناصر الثقيلة معنويا مقارنة بنتائج التحليل الكيميائي للمصدرين الآخرين ونتيجة لذلك فان درجة تراكم أيونات العناصر الثقيلة في كلا من الرأس و الكبد و العضلات كانت أكثر معنوية في ماء المصرف و من الملاحظ إن درجة التراكم لهذه العناصر كانت أعلى ما يمكن في نسيج الكبد يليها في الدرجة الرأس وأقلها العضلات. و لبيان أثر التلوث البيئي السمي السيتولوجى في الثلاث بيئات تم فحص خلايا الخياشيم وخلايا الدم لبيان أثر ذلك على السلوك الكروموسومى إثناء الانقسام الميتوزى و تعدد الأنوية و الأنوية الصغيرة و لقد أظهرت النتائج أن الخلايا الميتوزية فى نسيج الخياشيم المأخوذة من أسماك المصرف بها نسبة كبيرة من الشذوذات الكروموسومية مثل اللزوجة، التفتت النووي، تفتت الكروموسومى، التقنطر ، فقد الكروماتيدات..... الخ كما احتوت على نسبة كبيرة من تعدد الأنوية و الأنوية الصغيرة في نسيج الدم مقارنة بما تم الحصول عليه من نتائج على أسماك المزرعة السمكية مما سبق ينصح للمستهلك بأن يبتعد عن التغذية على أسماك من مصادر مائية ملوثة و في نفس الوقت ينصح بعدم أكل كلا من نسيج الكبد و الرأس حيث تتراكم بهما أكبر نسبة من الملوثات و خاصة العناصر الثقيلة.