

Cytogenetic effect of industrial and agricultural wastes on *Tilapia zilli* fish

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ABSTRACT

The River Nile and its branches are exposed to many kinds of chemicals and biological pollutants as a result of increasing industrial and biological pollutants and other urbanization activities where effluents are discharged directly into the water without prior treatment.

This study aimed to clarify the effect of environmental pollution (Industrial and agricultural pollutions) on some genetic processes of Tilapia fish. The study included three groups. The first group was collected from unpolluted locality, the second group collected from downstream to the mouth of agricultural discharge, and the third group was collected from downstream. Chromosomal aberrations were used as an index to evaluate the relationship between the genetic processes and the effect of water pollution. Water analysis was done to water samples from seven localities to determine the following metals iron, lead, cadmium, copper, zinc, mercury and nickel. The results showed that the mean of all metals in the unpolluted water were within the acceptable permissible limit of WHO. In the agricultural polluted water, 100% of water samples exceeding the permissible limit for iron, lead, cadmium, nickel and mercury. Whereas zinc and copper were within the permissible limit. Both industrial and agricultural water pollutions caused an increase in the frequency of chromosomal aberrations but the frequency in industrial pollution was higher than in agricultural pollution. The types of aberrations observed in this study were structural and numerical aberrations. The predominant types of aberrations by exposure to agricultural pollution were deletion, gap and end to end association and the predominant types of aberrations by exposure to industrial pollution were centromeric attenuation, break and ring. Both industrial and agricultural water pollutions caused a significant increase in the frequency of fragmentation, polyploidy, stickiness and monosomy.

Keywords:

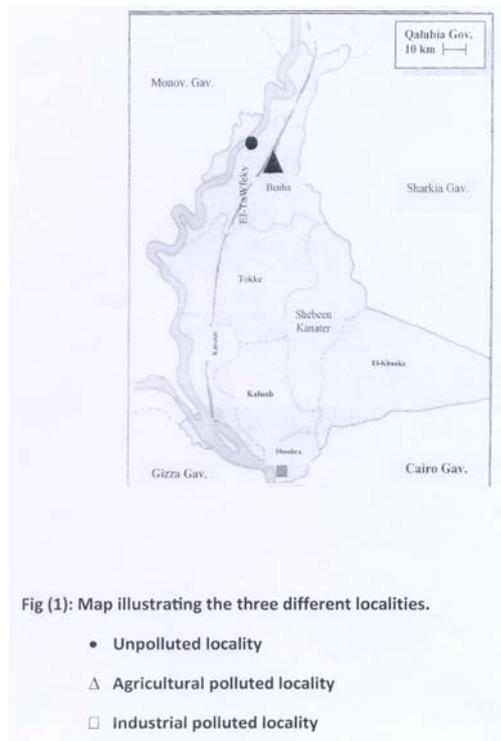
INTRODUCTION

Pollution of rivers and streams with chemical contaminations has become one of the most crucial environmental problems within the 20th century. The variety of problems, particularly heavy metals which are known to be toxic to human beings as well as to aquatic organisms, are enormous. Bioaccumulation of heavy metals in fish may critically influence both growth rate and quality of fish (Hodson *et al.*, 1984; Haggag *et al.*, 1999 and Zaghoul, 2001). In Egypt, Tilapia fish represented one of the most common species in river Nile and numerous lakes. Consequently special interest has been given to study the physiological, genetical and environmental conditions related to improve Tilapia production (Pullin and McConnell, 1982). Exposure to heavy metals even in low

concentrations affects survival of fish and other aquatic organisms. Several studies have linked increases in cytogenetic abnormalities in fish and shellfish to polluted environments. This was done largely through laboratory bioassays of polluted sample in nature (Alink *et al.*, 1980; Hose *et al.*, 1987) Also Karima and Halima (2002) reported that water pollution (Industrial and agricultural waste water) have a significant effects on *Clarias lazera* and *Oreochromis niloticus* which appeared as chromosomal aberrations in somatic cells and a significant increase in germ cells that appeared as x-y univalent. The aim of the current study is to throwing head lights over the problem of water pollution with heavy metals due to industrial and agricultural drainages. Also to clarify the effect of polluted water on genetic processes of *Tilapia zillii* by estimating chromosomal aberration test in somatic cells.

MATERIALS AND METHODS

Mature *Tilapia zillii* fishes of both sexes used in this study (10 -15 cm in length and 80100 gm body weight) were collected and transported to the laboratory from three different zones, (from both agricultural and industrial) localities according to these zones, fish were sorted into three studies in a living condition from an area in the Nile which is about 12 km distant from the southern boundaries of Benha district as shown in (Fig. 1). This area is totally far from any pollution sources (unpolluted locality) and was considered as control group. The second group, fish were collected alive from in Azbt El Prince village which is about 6 km distance from southern boundaries of Benha district as shown in (Fig. 1).



This area is considered as irrigated polluted locality (Agricultural pollution). The third group, fishes were collected from an area in Teraat El-Shrakawia. This area close to an industrial drainage from the outlet of oil and detergent factory and chemical industrial factory. This area is considered as an industrial polluted locality.

Water samples From the same localities of the collected fish, water samples were taken for chemical analysis to determine heavy metals concentration. The water samples were preserved by addition of 1 ml concentrated nitric acid per liter until the time of analysis.

Methods and techniques

A- Analysis of water sample: Heavy metals concentrations in water were measured as

ppm (mg/L) by atomic absorption spectrophotometer (AAS) with alteration of standard

burner head of AAS in relation to the light beam of examined metal (Pandya *et al.*, 1985). Metals examined in the present study are copper, zinc, cadmium, mercury, lead, nickel, and Iron.

B- Chromosomal preparation

Metaphase spreads were prepared according to Yosida and Amano (1965) with some modifications. Spreads were stained in Geimsa solution. For each fish, 50 well metaphases spreads were examined randomly using X100 oil immersion objective lenses. The data were statistically analyzed using the student's "t" test Snedecor (1971) and also by using one-way analysis of variance ANOVA (Tu key test). Data were expressed as Mean \pm S.D for all experiments and the levels of significant were expressed as $P \leq 0.05$.

RESULTS

Table 1 shows that analysis of water from the unpolluted locality is within the accepted permissible limit of the World Health Organization (WHO, 1984), while the mean \pm SE of iron, lead, mercury, cadmium and nickel exceeded the permissible limit in both agricultural and industrial locality.

Table (1): The average values (mg/L) of some metals from different localities.

The metal	Unpolluted locality (Mean \pm SE)	Agricultural locality (Mean \pm SE)			Industrial locality (Mean \pm SE)		
		0 m	300 m	1000 m	0 m	300 m	1000 m
		Iron	0.195 \pm 0.050	1.950 \pm 0.028	1.450 \pm 0.033	1.400 \pm 0.066	3.850 \pm 0.434
Lead	0.015 \pm 0.007	0.105 \pm 0.010	0.065 \pm 0.003	0.065 \pm 0.003	0.475 \pm 0.016	0.325 \pm 0.013	0.170 \pm 0.033
Cadmium	-	0.030 \pm 0.006	0.030 \pm 0.006	0.020 \pm 0.004	0.185 \pm 0.017	0.110 \pm 0.026	0.080 \pm 0.006
Zinc	-	0.055 \pm 0.022	0.055 \pm 0.022	0.035 \pm 0.003	0.195 \pm 0.003	0.145 \pm 0.003	0.030 \pm 0.006
Copper	0.040 \pm 0.006	-	-	-	0.300 \pm 0.133	0.255 \pm 0.023	0.300 \pm 0.006
Nickel	-	1.450 \pm 0.033	0.850 \pm 0.033	0.700 \pm 0.066	3.400 \pm 0.267	2.650 \pm 0.033	2.220 \pm 0.066
Mercury	0.040 \pm 0.008	0.725 \pm 0.010	0.680 \pm 0.006	0.600 \pm 0.02	4.410 \pm 0.167	3.680 \pm 0.177	2.790 \pm 0.006

Various chromosomal aberrations were observed in head kidney cells of *Tilapia zillii* collected from the different studied localities in both industrial and agricultural locality. Both numerical and structural types of chromosomal aberrations were identified in Table (2&3). Structural aberrations included centromeric attenuation, Chromatid break, Chromatid gaps, Chromatid deletions, Fragmentation, Ring and end to end association (Fig. 2).

Table (2): The mean values of chromosomal and chromatid aberrations in head kidney cells of *Tilapia zillii* in polluted water.

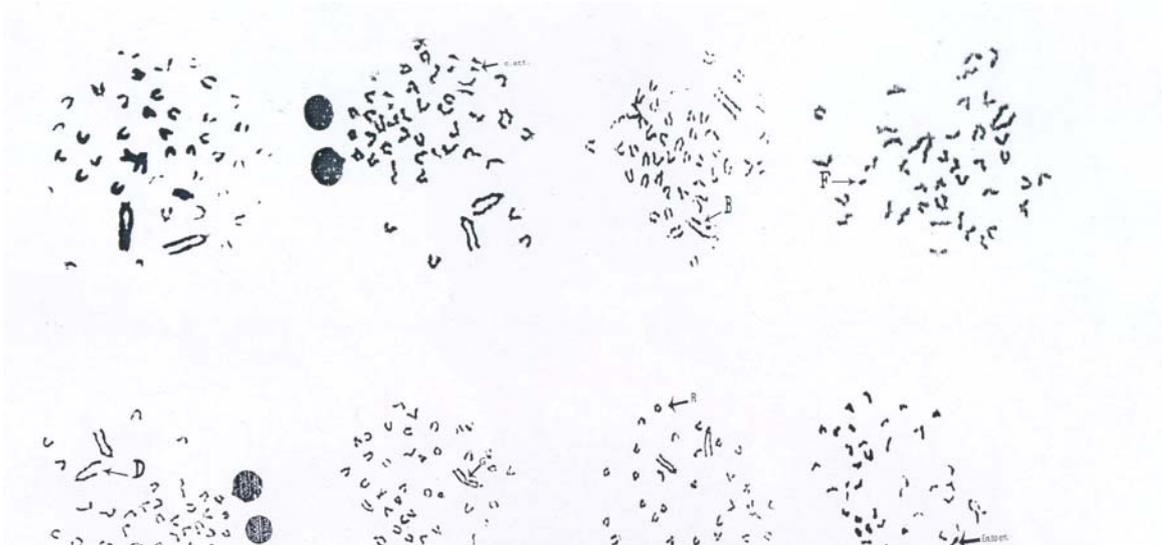
Aberration type	Control (Mean \pm SD)	Agricultural locality (Mean \pm SD)			Industrial locality (Mean \pm SD)		
		0 m	300 m	1000 m	0 m	300 m	1000 m
		Deletion	2.8 \pm 0.8	13.2 \pm 4.0*	10.8 \pm 2.2*	5.8 \pm 0.8*	1.7 \pm 1.1

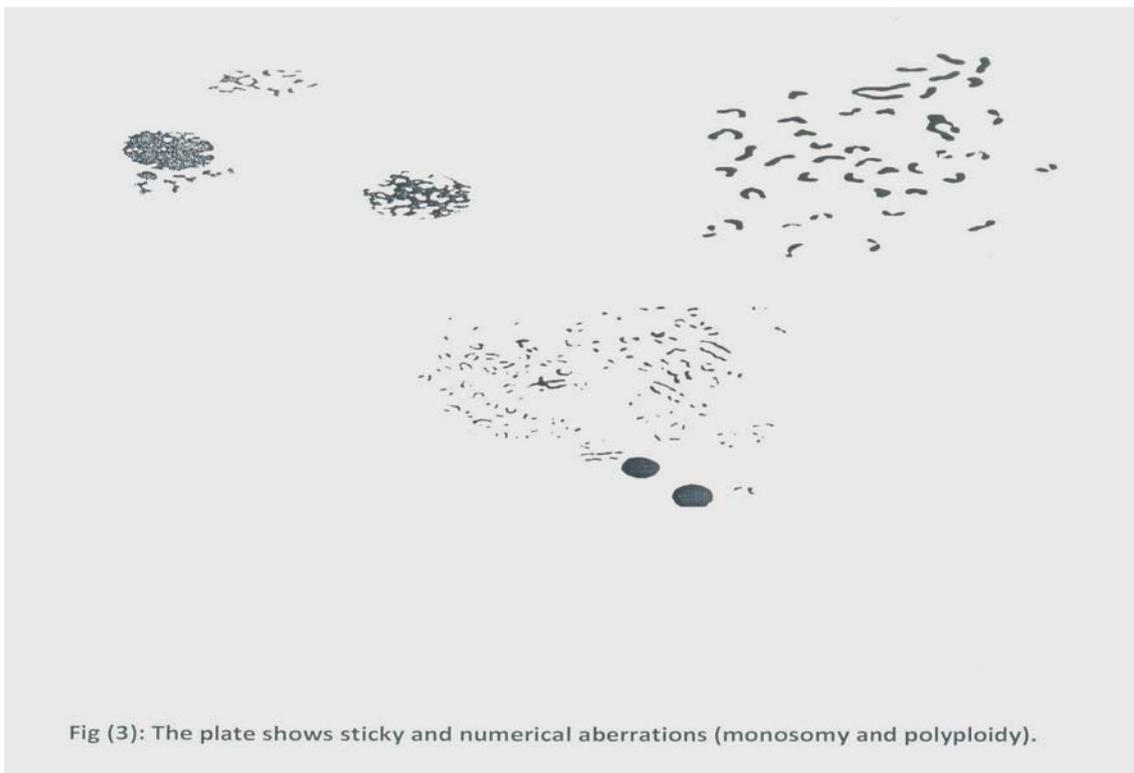
Table (3): The mean values of numerical aberrations and mitotic index in head kidney cells of *Tilapia Zillii* in polluted water.

Aberration type	Control (Mean \pm SD)	Agricultural locality (Mean \pm SD)			Industrial locality (Mean \pm SD)		
		0 m	300 m	1000 m	0 m	300 m	1000 m
Monosomic	0.8 \pm 0.8	3.8 \pm 0.4*	3.4 \pm 0.5*	3.0 \pm 0.3*	0.6 \pm 6.8*	0.6 \pm 5.2*	0.4 \pm 4.3*
Polyploidy	2.6 \pm 0.9	8.6 \pm 2.0*	7.0 \pm 1.6*	4.6 \pm 1.4*	9.8 \pm 1.3*	9.4 \pm 1.9*	5.4 \pm 1.3*
Mitotic index	120.4 \pm 2.9	90.0 \pm 6.8*	85.0 \pm 6.7*	88.0 \pm 7.3*	99.2 \pm 3.8*	89.4 \pm 6.2*	92.8 \pm 4.9*

Stickiness is considered as a sort of chromosomal "agglutination" of unknown nature which results in a pycnotic or sticky appearance of chromosomes. Stickiness may give rise to sticky adhesion between two or more chromosomes and to formation of sticky bridges at anaphase (Fig. 2).

While numerical aberrations included monosomy and polyploidy (Fig. 3). The current study represented revealed that there is a very highly significant increase in the mean values of chromosomal aberrations between control fishes and fishes were taken from industrial polluted area. Also there is a significant increase in the mean value between fishes of control group and those which were taken from agricultural polluted water.





DISCUSSION

Environmental contamination of water become a threat to continued existence of many plants and animals communities and may ultimately threaten the survival of human.

In the current study, analysis of water from unpolluted locality showed that iron, lead, cadmium, copper, zinc, and nickel are within the accepted permissible limit of World Health Organization (WHO, 1984). On the other hand, analysis of water from the agricultural locality exceeded the permissible limit for iron, lead, cadmium, nickel and mercury at all distances while analysis of water from industrial locality exceeded the permissible limit for iron, lead, cadmium, nickel and mercury at all distances. Zinc was within the permissible limit of WHO in both the agricultural and industrial polluted water. The genotoxic effects of heavy metals found in the present

investigation are similar to studies of Chandra and Bukhash (2004), who found that both cadmium chloride (CdCh) and azadirachtin (Aza) induced genotoxicity in *O. mossambicus*, the former in greater quality than that produced by Aza. Ferraro *et al.* (2004). found that tributyltin and inorganic lead (PbII) were potentially mutagenic on the fish *H. malabaricus*.

In the present study, both industrial and agricultural water pollution caused an increase in the frequency of chromosomal aberrations but the frequency in the industrial pollution was higher than the agricultural pollution. The mean values of deletions, gaps and fragmentation showed that there was a highly significant increase of such aberrations in the agricultural locality, while the industrial locality showed a highly significant increase of break, centromeric attenuation, ring and stickiness at all distances (0 m, 300 m, 1000 m). Lowley and Brooks (1963) revealed that alkylating agents were known to cause chromosomal breakage by binding to DNA regions rich in guanine-cytosine pairs causing these to become unstable. Mathew and Jahgeedar (2003) recorded that the percentage of total metaphase spreads with chromosomal aberrations was increased with increasing the concentration levels of different doses of mercuric nitrate, these aberrations included breaks, fragments, dicentric and ring type chromosomes.

Also, the present investigation revealed that there was a significant decrease in mitotic index for all fish samples when compared with control, this agrees with a study of Barbosa *et al.* (2010), who detected a significant change in the frequency of chromosome aberrations and in the mitotic index compared to negative control, the comet assay showed a statistically significant alteration in the level of DNA breaks of *O. niloticus* and chemical analysis detected an increase in heavy metal levels in different sampling periods. Similar results were recorded by Samma (2001) who found that exposure of *Chrysichthys auratus* and *Lobes 'hori* to water containing carbamate pesticide (Saturn) for 72h, one week and two weeks duration resulted in a highly significant increase in the incidence of total chromosomal aberrations and significant decrease in mitotic index when compared with control of both fishes. Significant increase over and above negative control in the frequency of micronuclei was observed in fishes exposed to metal compounds, the order of induction of micronuclei frequency and toxicity was As > Hg > Cu, Kamleset and Sunil (2009).

Deletion and centromeric attenuation were the most observed structural aberrations in the examined metaphases in the agricultural water pollution while break and centromeric attenuation were the most observed abnormality in the industrial water pollution. The current data agree with the results reported by Bovero *et al.* (2002) who studied the effect of two geographically distant pollutions of chironomid riparian (*Syn. Thummi*) from two environmentally polluted sites (Santena, Italy and Varna Bulgaria) and found numerous somatic and inherited chromosomal aberrations (inversions, deletions and deficiencies). Also the effect of delta methrin on the catfish, *Clarias gariepinus*, revealed that the frequency of chromosomal aberrations (chromatid gap, chromosome gap, haploidy and polyploidy) was represented in high percentage than the other type of aberration (Salama, 2001).

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ARABIC SUMMARY

التأثير الوراثي الخلوي للمخلفات الصناعية والزراعية على سمكة البلطي الأخضر

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يتعرض نهر النيل وفروعة لانواع من الملوثات الكيميائية والبيولوجية كنتيجة لصرف المخلفات الصناعية والزراعية والأنشطة الحضرية مباشرة فيه دون ادنى معالجة لذلك فان حيوية الأسماك تتأثر بتراكم تلك العناصر خاصة الثقيلة منها في انسجتها وتصبح خطرا يهدد صحة الإنسان.

استهدف البحث دراسة تأثير التلوث البيئي (صناعي، زراعي) على بعض العمليات الوراثية في اسماك البلطي. وقد تمت الدراسة على ٣٥ سمكة بلطي، ١٥ سمكة جمعت على ثلاث مسافات مختلفة من قناة صرف زراعي و١٥ سمكة أخرى جمعت أيضا على ثلاث مسافات مختلفة تزداد بها نسب التلوث الصناعي نتيجة لصرف مخلفات المصانع اليها واستخدمت ٥ أسماك جمعت من منطقة بعيدة عن أى نوع من التلوث كمجموعة ضابطة. تم تحليل عينات المياه من نفس الأماكن التي جمعت منها الأسماك وحددت نسبة بعض المعادن مثل الحديد، الرصاص، الكاديوم، النحاس، الزنك، النيكل والزنق. وقد أوضحت الدراسة أن للتلوث تأثير على التشوهات الكروموسومية (تركيبية و عددية) وتعرض الأسماك لهذا التلوث أدى إلى زيادة معنوية في جميع أنواع التشوهات ولكن نسبتها أعلى في أماكن التلوث الصناعي على الزراعي.