Egypt. Acad. J. biolog. Sci., 2 (1): 1-8 (2010) **B. Zoology**
info@eajbs.eg.net **ISSN: 2090 – 0759**
Received: 25/1/2010 www.eajbs.eg.net

Cytogenetical studies on some River Nile species from polluted and nonpolluted Aquatic habitats

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ABSTRACT

Shanawan drainage canal is one of the many drainage canals present in Menufiya province, Egypt. It receives sewage water from Kafr Shanawan drainage canal. Also, it receive illegal run–off from sewage and wastes besides sewage of Shebeen Al-koom city. This study is aimed to investigate the frequency of chromosomal damage by using chromosomal aberrations test of head kidney cells in *Oreochromis niloticus* and *Tilapia zillii fish*. The study was performed on 5 groups of fish, control and four polluted groups. A and B stations are located before the source of sewage discharge while C and D stations are located after the source of sewage. In each group cytological studies were performed. Fishes caught from areas before the sources of sewage showing different types of chromosomal aberrations, including centromeric attenuations, chromatid breaks, chromatid gabs, chromatid deletion, centric fusion and fragmentation. The total aberrations were more obvious among the fishes of the areas after the source of sewage than those of the areas before the source of sewage. It was noted that *O.* niloticus is more sensitive to the effect of pollution than *T. zillii*. Cytogenetic Studies of the current Work could be used as criteria for pollution intensity, which can be used to avoid its toxic effect on aquatic environment.

Keywords:

INTRODUCTION

Pollution of the aquatic ecosystem is recognized globally as a potential threat to both human and other animal's population which interacts with aquatic environment *(Biney et al., 1987*), Egypt possesses approximately 5000 km of irrigation and drainage canals *(Redding and Midlen, 1992)*. Drainage canals were polluted as a result of discharging agricultural drains daily, with insecticides, pesticides, heavy metals, fertilizers, chemicals, sewage and other possible domestic/industrial wastes. The wide indiscriminative use of pesticides in weed and pest control, certainly, results in the pollution of all environmental components including water body (*Matter et al., 1992*).

Several studies have linked increases in cytogenetic abnormalities in fish and shellfish to polluted environment. This was done largely through laboratory bioassays of polluted water sample in nature (Hooftman and Raat, 1982; Hose *et al*., 1987; Metcalf, 1988). Mohamed *et al*., (2008) measured the cytogenetic changes by observing the frequency of chromosomal aberration in the gill cells of the treated *Oreochromis* niloticus by copper sulfate and lead acetate. Mahrous and Abdou (2001) detected that the environmental water pollution (agricultural waste water or industrial) have significant effects on *Oreochromis niloticus* and *Clarias lazera*, which appeared as chromosomal aberration breaks, deletion, and centromeric attenuation in somatic cells. Velmurugan *et al*. (2009) found that the exposure of *Mystus glulio* to lambda-

cyhalothrin for 96h. resulted in an enhancement in the frequency of chromatid breaks, acentric fragments, centromeric fusion, aneuploidy, condensation, sticky plates and ring compared with those in the tap water control. The widely used herbicide (2,4–dichorophenoxy) acetic acid (2,4-D) is evaluated for acute toxicity and stress factors on fresh water fish Abumourad *et al*. (2006). The percentage of chromosomal aberration was highly significant after treatment with the different herbicides doses .

This current study was carried out to investigate and evaluate the pollution of Shanawan drainage canal and its effects on the health of fishes represented by two cichlids *O. niloticus* and *T. zillii* by using chromosomal aberration test in somatic cells and morphological malformation, which is considered the first sign of the effect of pollution on the fish.

MATERIALS AND METHODS

Study area:

Shanawan drainage canal (Menufiya province) as described by Khallaf *et al*, (1998). The study was performed on 5 groups of fish (*Oreochromis niloticus* and *Tilapia zillii,* 132±10 gm body weight). Control group fishes were collected by bottom trap (Gobiah) nets by fishermen from baher Shebeen canal. Polluted groups includes Four stations, stations A & B are located before (upstream) the source of sewage discharge of Shanawan drainage canal while stations C & D are located after (downstream) the source of sewage.

Fishes from polluted (Shanawan drainage canal) and unpolluted (Baher Shebeen canal) area were collected to study the following parameter.

Chromosomal preparation:

Mitotic chromosomes were obtained. Fish were injected intraperitoneally 3 hrs before sacrifice with 0.01% colchicine. Squash technique for kidney tissue described by AL-Sabti *et al.* (1983) was used for the preparation of chromosomes with some modification. For each fish, 50 well spreaded metaphases were examined for kidney. Different types of chromosomal aberrations will be recorded.

RESULTS

The present study showed that the diploid number of chromosomes in Tilapia were 2n = 44. High percentage of chromosomal abnormalities in head kidney cells 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 (Fig. 1).

The current results revealed that the values of different types of chromosomal aberration in head kidney cells of *T. zillii* in area B, C and D were significant ($P< 0.05$) than showed in fish 0f unpolluted area (Table 1). Also, data showed a significant ($P< 0.05$) values of aberrations in fishes caught from areas C & D, downstream to sewage source (almost two fold) than in fish of areas A & B, upstream of sewage source (Table 1). The same results were observed in *O. niloticus* of the same areas (Table 2). The values of aberration revealed higher number in *O . niloticus* than in *T. zillii* (Table 1&2).

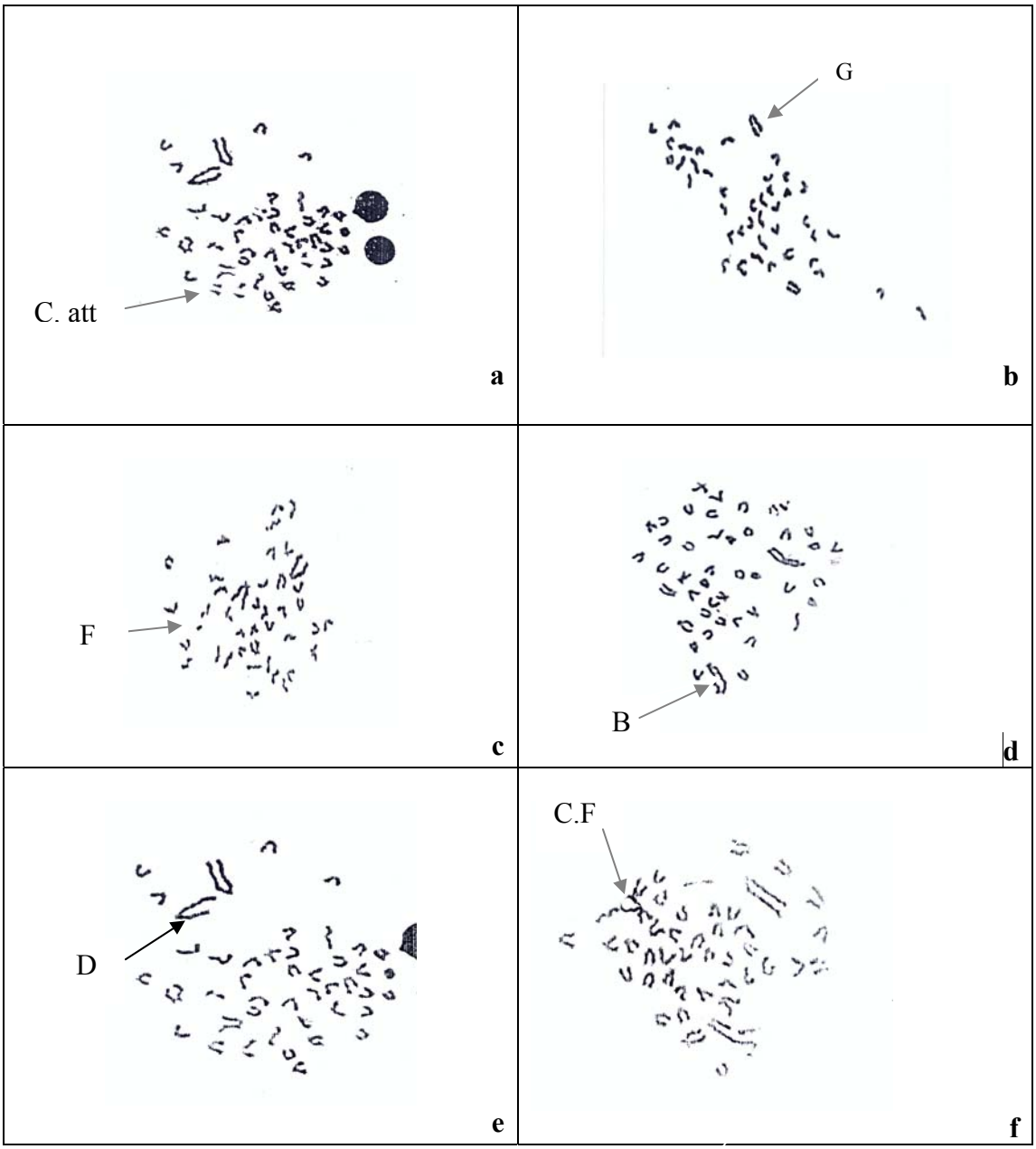

Fig. 1: Types of chromosomal abarration in head kidney cells of *O. niloticus* Showing metaphase a- Centromeric attenution (C. att); b- Gap (G); c- Fragmentation (F) ; d- Break (B); e- Deletion (D); f- Centric Fusion (C.F).

Table 1: The average values of different types of chromosomal aberrations in head kidney of *Tilapia zillii* caught from polluted and non-polluted area

Types of chromosomal aberrations	Control	Polluted Areas			
		Mean ± SE			
		A	B	C	D
Centromeric attenuation	27.0 ± 1.34	40.60 ± 2.40*	44.40 ± 2.79*	84.60 ± 4.51 *q	98.80 ± 4.47*q
Chromatid break	1.60 ± 0.40	3.80 ± 0.66*	6.20 ± 0.86*	12.20 ± 1.16 *q	20.60 ± 1.44 *q
Chromatid gap	1.40 ± 0.24	2.20 ± 0.37	2.40± 0.51	4.60 ± 0.60*q	7.00 ± 0.71*q
Fragmentation	1.80 ± 0.37	3.00 ± 0.45	4.20 ± 0.58*	7.60 ± 0.81*q	9.60 ± 1.03*q
Chromatid deletion	2.80 ± 0.37	3.40 ± 0.51	4.60 ± 0.68	10.00 ± 0.71*q	16.20 ± 1.98*q
Centric fusion	2.00 ± 0.55	3.80 ± 0.58	5.00 ± 0.84 *	8.40 ± 1.03*q	11.20 ± 1.20*q
Total structural aberrations	41.60 ± 2.56	56.80 ± 9.34	66.80 ± 4.78	127.40 ± 5.22*q	163.40 ± 5.78*q

A , B : Areas before the source of sewage .
C , D : Areas after the source of sewage .
S .E :Standard Error
* Significant (P< 0.05) between polluted &non-polluted
q Significant (P< 0.05) between polluted areas

Table 2: The average values of different types of chromosomal aberrations in head kidney of *0reochromis niloticus* caught from polluted and non-polluted area

Types of chromosomal aberrations	Control	Polluted Areas			
		Mean ± SE			
		A	B	C	D
Centromeric attenuation	25.80 ± 1.53	50.00 ± 2.35	51.40 ± 2.38	100.40 ± 3.91	113.20 ± 4.69
Chromatid break	2.60 ± 0.51	6.00± 0.66*	6.20 ± 0.86*q	12.20 ± 1.16 *q	20.60 ± 1.44 *q
Chromatid gap	2.20 ± 0.37	3.00 ± 0.55	4.20 ± 0.58*	7.20 ± 0.73*q	10.20 ± 0.97*q
Fragmentation	2.00 ± 0.45	4.40 ± 0.51*	6.60 ± 0.93*	11.80 ± 1.24*q	18.00 ± 1.76*q
Chromatid deletion	3.80 ± 0.66	6.40 ± 0.68	10.00 ± 0.71*	17.00 ± 0.89*q	22.80 ± 1.39*q
Centric fusion	3.80 ± 0.66	5.00 ± 0.71	7.80 ± 0.73 *	10.20 ± 0.86*	15.00 ± 1.22*q
Total structural aberrations	40.20 ± 1.77	74.80 ± 2.40*	89.00 ± 3.94*	161.00 ± 4.87*q	202.40 ± 7.52*q

A , B : Areas before the source of sewage .
C , D : Areas after the source of sewage .
S .E :Standard Error.
* Significant (P< 0.05) between polluted & non-polluted.
q Significant (P< 0.05) between polluted areas

DISCUSSION

Exposure to genotoxic agents may result in mutation, metabolic disorder, damage embryos and reduced fertility (Ghaffar *et al.,* 1994). The use of genotoxicity testing is essential for the assessment of potential livestock toxicity so that, hazard can be controlled.

Cytogenetic analysis of chromosomes has been employed as a biological dosimeter to estimate the effect of genotoxic agents (pollutants) on fish and very useful for direct detection in somatic cells (Yunis, 1983 and Radwan, 1996).

Family Cichliddae represent over 70% of the Egyptian fish loading and have a number of chromosomes (2n = 44). Structural aberrations (e.g., chromatid deletion, chromatid gap, chromatid break, centromeric attenuation, fragmentation and centric fusion) were more prominent in the fishes of different polluted areas (Shanawan drainage canal) than those of non-polluted area (Bahr Shebeen). Similar results were recorded by Mohamed *et al.* (2008) on *O. niloticus* fish exposed to copper sulfate and lead acetate. They observed that chromatid deletion, stickness and fragments were more frequent than other chromosomal aberrations. Also, Yadav and Trivedi (2009) found that the exposure of *Channa punctata* (2n=32) to, mercuric chloride, arsenic

trioxide and copper sulphate pentahydrate for a Week, kidney cells revealed chromatid and chromosome breaks, chromatid and chromosome gaps, along with ring and di-centric chromosomes. The findings depict genotoxic potential of these metals even in sublethal concentrations.

Our results were in agreement with Velmurugan *et al.,* (2009) who found enhancement in the frequency of chromatid breaks, acentric fragments, centromeric fusions, aneuploidy, condensation, sticky plates and ring, of *Mystus glulio* fish exposed to a Lambda-cyhalothrin compared with those in the tap water control. Chromosomal aberrations frequency increased regard to increase of concentrations of herbicide, whipsuper (Farag, *et al*., 2009).

The higher level of chromosomal aberrations in fish from polluted areas may be the result of environmemental contamination caused by industrial emissions or duo to contaminated water with heavy metals and pesticides. Gill cells of the treated fish by copper sulfate and lead acetate displayed lower mitotic activity than that of the control group. Both pollutants were found to be positive inducer of macro-DNA damage which represented by different type of aberrations e.g. chromatid deletions, chromatid gaps, chromatid breaks, fragments, stickness, translocations, ring chromosomes, and centromeric attenuation. Chromatid deletions, stickness and fragments were more frequent than other chromosomal aberrations *(*Mohamed *et al.,* 2008).

Generally, for both *O. niloticus* and *T. zillii* there was a significant difference between all polluted areas and the control area. Also, it was clear that *O. niloticus* is more sensitive to the effect of pollution than *T. zillii*. So these chromosomal aberration may lead to changes in the genetic component.

Heavy metals are known to be persistent in the aquatic environment and gradually accumulate and magnify through the process known as bioaccumulation and biomagnifications (Malla and Ganesh, 2009).

Awwad (1991) stated that there is a significant increase in the frequency of deletions in grass carp fish exposed to carbarmate insecticide. Mattar *et al*., (1992), indicated that there was a high significant increase in chromosomal deletion in the kidney cells of grass carp exposed to insecticides. Radwan (1996), also observed that in agricultural pollution, the most dominant aberration were deletion, gaps, euploidy and condensation. Also, Krishnaja and Rege (1982), show that there was a significant increase of the number of gaps in fish *Boleophthalmus dussumieri* exposed to mitomycin C and heavy metals mercury, selenium and chromium.

Generally, for both *O. niloticus* and *T. zillii*, there was a significant difference between all polluted areas and the control area. These chromosomal aberration may lead to changes in the genetic component and concern has been expressed about "genetic consequences of pollution to fish populations exposed to low levels of pollution over prolonged periods (Barker and Rackham, 1979).

Chromosomal aberrations result from abnormalities in DNA duplication during the S phase; this may be due to the interference of the pollutants with nucleotide synthesis (Mattar *et al*., 1992), leading to malformation of DNA molecules (Landolt and Kocan, 1983). Also, Evans (1977) thought that the chromosomal aberrations also arise as a consequence of miss-repair or of miss-replication of damaged DNA. Also, according to (Natarajan and Obe, 1978), the ultimate lesions responsible for aberration formation are DNA-strand break. So, chromosomal aberration can be used as an indicator of DNA damage. OH radicals and O_2 are considered the most important biologically relevant oxygen species, however they are able to react directly with all kind of cells components including cellular DNA. The oxidative DNA damage introduced by direct reaction of these radicals, leads to aberration on base pairs (Retel *et al*., 1993). Ammonia concentration in the aquatic habitat may have

enhanced the toxicity of heavy metals such as copper and zinc (Halle *et al*., 1989). Therefore, it is believed here, that the presence of ammonia in the canal water was multiplicative to other heavy metals effect which may leads to chromatid aberration. In other studies, Goodale *et al*., (2008), showed that aquatic chromium is both increased cytotoxic and genotoxic to fish cells.

In the preceded analysis, *O. niloticus* was found to be more susceptible than *T. zilli*. This may be attributed to the behavior of both fish. Thus, where the first is a multiple spawner mouth broader and move frequently than the annual spawner nest guarder *Tilapia zillii* (Khallaf *et al*., 1986).

This evaluated study reveals that the cytogenetic analysis of chromosomes has been employed as a biological dosimeter to estimate the effect of genotoxic agents (aquatic pollutants) on fish and very useful for direct detection in somatic cells. The results of the present study and previous investigations (Mattar *et al*., 1992; Radwan, 1996 and Abbass *et al*., 2005) performed either under laboratory or filed conditions, encourage a wider application of cytological studies as an early biological marker of exposed fish to clastogenic pollution in water.

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ARABIC SUMMARY

دراسة وراثية على بعض أسماك النيل في بيئات مائية ملوثة وطبيعية

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اعتمدت هذه الدراسة على دراسة تأثير التلوث المائي لمصرف شنوان التابع لمحافظة المنوفية لما يستقبله هذا المصرف من ملوثات كثيرة مثل مياه صرف الحقول المجاورة ومياه صرف مدينة شبين الكوم والمخلفات المختلفة التي يقوم سكان القرى المحيطة به بقذفها فيه.

اظهر فحص اسماك البلطي النيلي والبلطي الأخضر للمناطق الواقعة قبل الصرف ظهور العديد من التشوهات مثل ظهور بقع صفراء اللون على سطح الكبد تعطى الكبد لون مزركش هذا إلى جانب اللون الأصفر المميز للكبد بصفة عامة مع ظهور القرح الجلدية ذات اللون الأبيض في أجزاء مختلفة من سطح الجسم مع وجود تشقق وتشوه في زعانف الجسم المختلفة وظهور العين بشكل غير طبيعي في بعض الأسماك. كما اظهر فحص اسماك المناطق الواقعة بعد الصرف ظهور التشوهات بدرجة اكبر عن التي وجدت في اسماك المناطق الواقعة قبل الصرف ، ومن ناحية أخرى تم ملاحظة انثناء الذيل لأعلى في سمكة البلطي النيلي.

وبالنسبة للدراسة الوراثية ، ظهر اعلي معدل للتشوهات الكروموسوميه في خلايا الكلية الأمامية لكل من البلطي النيلي والبلطي الأخضر ممثلا في انفصال السنترومير – كسر في الكروماتيد – فجوات الصبغيات – نقص جزء من الكروماتيد – التصاق الكروموسومات عند منطقه السنترومير وكذلك ظهور الشظايا. وكانت نسبه ظهور التشوهات الكروموسوميه في كل من سمكه البلطي النيلي وسمكه البلطي الأخضر في المنطقتين اللتين تقعان بعد الصرف وهما المنطقتان D و C اعلي بكثير من التشوهات في المنطقتين اللتين تقعان قبل الصرف وهم المنطقتين A و B كما أثبتت النتائج أن نسبه ظهور التشوهات الكروموسوميه في سمكه البلطي النيلي اعلي بكثير من سمكه البلطي الأخضر مما يؤكد حساسية سمكه البلطي النيلي للتلوث أكثر من البلطي الأخضر. وقد أثبتت النتائج بصفة عامة وجود تغيرات شديدة في كل من الشكل الخارجي والجينات الخلوية لأسماك المناطق الملوثة (مصرف شنوان). كما ظهرت هذه التغيرات التي تشمل الشكل الخارجي والجينات الخلوية أكثر وضوحا في اسماك المناطق الملوثة (مصرف شنوان) عنها اسماك المناطق الغير ملوثه(بحر شبين الكوم). كما ظهرت تلك التغيرات بشكل أوضح في اسماك المناطق الواقعة بعد الصرف ، كما لوحظ أن سمكه البلطي النيلي أكثر حساسية للتلوث من سمكه البلطي الأخضر. ولذلك ذلك يؤكد ضرورة التوقف عن إلقاء المخلفات المختلفة في البيئة المائية و تحريم الصيد من هذه المصارف.