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THE MOLECULAR CHANGES OF HEPATOCYTES IN *TILAPIA ZILLII* UNDER THE EFFECT OF THE AGRICULTURAL AND INDUSTRIAL POLLUTION IN THE RIVER NILE, EGYPT

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ABSTRACT

Liver specimens were taken from seventy fish of, *Tilapia zillii*, living in three different localities in Qalyobya governorate; unpolluted, agricultural-polluted and industrial-polluted waters of the River Nile. Water analysis was carried out to evaluate the heavy metals in these tested localities. Polymorphism of liver proteins was revealed by SDS-PAGE electrophoresis. Also, DNA damage was detected by counting the fragmentation percent. Results showed that the measured heavy metals were higher in the agricultural and industrial localities than in control; and sometimes exceeded the permissible concentrations. Fractions of liver proteins showed disappearance and polymorphism in the fish especially that was very close to the outlets of agricultural and industrial drainage water. Additionally, both agricultural and industrial pollution caused an increase in the percentage of DNA fragmentation, however the impact of industrial pollution was higher than that of agricultural pollution.

The study suggested that there is clear molecular changes in *T. zillii*; this declares the use of these molecular parameters in diagnosing pollution.

INTRODUCTION

Among various sources of aquatic pollutions come the insecticides, pesticides and chemosteriliants used in agricultural practices as they directly and/or indirectly reach the water bodies as residues. Most of them are already poisonous and their effects might persist for years. Furthermore, water pollution is a by-product of civilization. There is a steadily increasing flow of pollutants that are discharged into natural waters from packing plants, food processing plants, the dairy industries,

such as drainage system of the irrigation water which is designed to flow directly to the River Nile, chemicals used in agriculture and the products of industry (Abo-El-Nasser *et al.*, 1992). Pollution by sewage or industrial effluents and/or agrochemicals produces various effects depending on the nature of these chemicals. The degree of pollution in any area in the Nile depends on the quantity of wastes washed down (Siliem, 1993).

Heavy metals transported into the environment originate either from natural sources, such as wind borne soil particles, or from anthropogenic sources, e.g. as a result of heavy industry and the burning of industrial or domestic wastes. On average, man-made emissions of elements such as, Cd, Cu, Ni, Zn, and Pb greatly exceed the biogenic inputs of these elements (Nriagu, 1989).

El-Motassem (1987) reported that agricultural and industrial effluents constitute a real threat to the River Nile system in Egypt. During 1986, the segment of the River Nile from Aswan to Cairo (approximately 1000 km) received 3.2 billion cubic metres of agricultural discharges and 0.4 billion cubic metres of industrial effluents. However, still higher discharges may reach the River Nile at the Delta region, where the highest population density, industrialization and intensive agriculture exist in Egypt.

Since the 1930s, researchers have tried to find a causal link between chemical contamination and acute toxicity in fish populations (Macek, 1980). Through a homeostatic mechanism cells maintain a delicate balance between spontaneous and induced DNA damage. DNA damage accumulates if such a balance is altered. Cells have elaborate DNA repair mechanisms because DNA stability is vital for species survival. Uncorrected DNA damages yield mutations (Alberts *et al.*, 1994).

Sentinel organisms, bioindicators are used to detect the biological effects of pollutants and to assess their impact (Livingstone, 1993). Organisms react to pollution by altering some parameters, biomarkers, at different organization levels. Those at molecular or cellular levels are early-warning indicators of pollution since their responses occur before irreversible damage to the ecosystems takes place (López-Barea, 1994). Since the liver is the major site of xenobiotic accumulation and bio-transformation, analyses of initial molecular lesions elicited by pollutants in this organ gives early-warning and sensitive indicator of chemical-induced carcinogenic lesions (LeBlanc and Bain, 1997).

Previous studies on blood serum proteins have shown that under conditions of heavy metal exposure the number of protein fractions either increased or decreased (Dutta *et al.*, 1992).The authors observed that new fraction of serum protein was noticed after 48 h exposure. On the other hand, Takai and Ojima (1990) indicated the characteristics of the chromosomes and electrophoretic patterns of muscle and serum proteins and the relationship between them. Some morphological characters of Tilapia fish were changed after exposure to sublethal doses of organophosphorous (OP) insecticides; this might suggest that OP may have been modified or changed the gene expression at the protein level (Futon and Chambers, 1985; Rashid *et al.*, 1992). Many authors studied the protein polymorphism which is mainly due to genetic disturbances of pollution (Badawy and El-Serafy, 1998; Salama, 2001). Kumari (2003) recorded that the electrophoretic analyses of liver and muscle tissues of *Channa punctatus* from polluted waters showed decrease of esterase fractions in liver and muscle. Hamdy (2004) reported that heat shock protein (hsp70) expression was assessed in liver, kidney, spleen and muscles of freshwater fish *Tilapia zillii* after nine days exposure to sublethal concentration (1/3 LC_{50}) of neem biopesticide.

The goal of this study is to reveal the behaviour of liver proteinogram as an indicator of water pollution.

MATERIAL AND METHODS

I. Experiment

Seventy mature *Tilapia zillii* fish of both sexes were used in this study (10-15 cm in length and 80-100 g body weight). Fish were divided into three groups. The first group; 10 fish, was collected from an area in the Nile which is about 12 km distant from the southern boundaries of Benha district. This area is totally far from any pollution sources (unpolluted locality) and was considered as control group. The second group, 30 fish, were collected from canal in Ezbet El-Prince Village which is about 6 km distant from southern boundaries of Benha district and is the place of irrigation drainage. This area is considered as irrigated polluted locality (agricultural pollution). Fishes were taken from three different zones; zone (1) at the irrigation discharge mouth, zone (2) after 300 m downstream of zone (1), and zone (3) after 1000 m downstream of zone (1). The third groups, 30 fish, were collected from an area in Teraat El-Shrakawia. This area has an industrial drainage from outlet of oil and detergent factory and chemical industrial factory. This area is considered

as an industrial polluted locality. Fish were taken from three different zones; zone (1) at the industrial drain outlet, zone (2) after 300m downstream of zone (1), and zone (3) after 1000 m downstream of zone (1) Fish were also taken alive on the same day to the laboratory.

Water samples

From the same localities where the fishes were collected, water samples were taken for chemical analysis to determine heavy metals concentrations. Water samples were kept by adding one ml concentrated nitric acid per litre until the time of analysis.

II. Methods and Techniques

A) Analysis of the water samples

Heavy metal concentrations in water were measured as ppm by atomic absorption spectrophotometer (AAS) with alteration of standard burner head of AAS in relation to the light beam of the examined metal (Pandya *et al.*, 1985). Metals examined in the present study were copper, zinc, cadmium, lead, mercury, nickel, and manganese.

B) Electrophoretic methods

10% SDS polyacrylamide gel was prepared according to the method of Laemmli (1970). Most of the chemicals were purchased from Spectrum Chemical Mfg, Corp (USA), Fisher chemical (USA) and Spectrum Quality Products (USA).

After decapitation, liver samples were excised then frozen under -20°C until use. About 0.2 g of the tissue samples was homogenized in one ml of Tris buffer (1 % SDS). The mixture was heated to 90-95 °C for 5 minutes and centrifuged at 14000 rpm for 5 minutes. The supernatant containing the measured protein was carefully removed to a clean Eppendorf tubes. The protein samples were diluted by adding equal volumes of SDS sample buffer, stirred by vortex and then heated at 90-95 °C for 5 minutes. The samples were loaded into wells and run at 15-20 mA/gel. Similarly, 5 µl of standard protein marker (Bio-Rad Laboratories, USA) was heated to 90 - 95°C for 5 minutes then loaded onto the same gel. Preparations were separated on electrophoresis unit (Hoefer mighty small II, SE 250, USA). The gels were stained in 0.05 % Coomassie Brilliant Blue R 220 dye overnight and the excess dye was washed using destain solution for 2 hr. Proteins were detected as blue stained bands against a clear background. The gel was preserved in 10% acetic acid and photographed. The gel bands were scanned using the Alpha Ease TM software (Alpha Innotech Corporation, San Leandro, CA, USA).

C) DNA fragmentation

DNA fragmentation was examined by using Pernadones' methods (Pernadones, 1993). The excised liver tissues were mechanically dissociated in 400 µl hypotonic lysis buffer (0.2 % triton x-100, 10 mM Tris and 1 mM EDTA, pH 8) to obtain cell lysates. Cell lysates were centrifuged at 13800 Xg for 15 min. The supernatant (S/N) containing small DNA fragments were separated immediately. The pellet containing large pieces of DNA and cell debris was resuspended in 400 µl hypotonic lysis buffer. Exactly, 400 µl of 10 % TCA was added to both S/N and resuspended pellet. The tubes were centrifuged at 2000 rpm for 10 min. The ppts were resuspended in 400 µl 5% TCA and the tubes were incubated at 80°C for 30 min. Two volumes of solution containing [0.088 M diphenylamine (DPA), 98% v/v glacial acetic acid, 1.5 v/v sulphuric acid and 0.5 v/v of 1.6 % acetaldehyde soln.] were added to one volume of extracted DNA. The samples were stored at 4°C for 48hr. The blue colour quantified spectrophotometrically at 578 by using ELISA reader (SLT Labinstruments A-5082, Austria). The percent of DNA fragmentation in each sample was expressed by the formula:

$$\% \text{ DNA fragmentation} = \frac{OD\ S/N}{OD\ S/N + OD_{pellet}} \times 100$$

Statistical analysis

All numerical data were statistically analyzed using the computation laws given by Snedecor (1971) for the student (*t*) test application. Also, by using one-way analysis of variance, ANOVA, (Tukey test). Data were expressed as Mean ± S.D for all experiments and the levels of significance were expressed when $P \leq 0.05$.

RESULTS

Water analysis

From the data recorded by water analysis in the unpolluted area; it is clear that Cd, Zn and NL were not found (Table 1). Fe, Pb and Cu were presented in values lower than the permissible concentrations, except Hg that was crossed it. Fe, Pb, Cd, Ni and Hg were increased in the agricultural polluted area and increased in higher values in the industrial area. These heavy metals recorded a gradual decrease in its concentrations by downstream from zone 1 to zone 2 and 3, except Cu that was nearly constant in all tested industrial zones (Table 1). Only Zn recorded higher

increase in the polluted areas but still lower than the permissible concentration.

Electrophoretic investigation

SDS polyacrylamide gel electrophoresis (PAGE) of proteins from liver of *Tilapia zillii* showed that the total number of polypeptides in the liver of unpolluted fishes (control) was 10 bands. The polypeptides were decreased to 7 and 8 bands in the liver of fishes taken from the locality of agricultural pollution at the three zones (Table 2). Molecular weight was ranged from 12.76 to 114.47kDa in livers of control fish. Liver revealed 7, 6 and 7 new synthesized polypeptides with different molecular weights at the tested agricultural zones when compared to the control group (Table 2). Currently, the total numbers of bands in the industrial polluted fishes were 9, 9 and 8 respectively, prior to the three sites of fish collection. Five, 6 and 5 new synthesized polypeptide fractions appeared in the three tested industrial zones (downstream to the discharge outlet) by the comparison to control fish (Table 3).

This study was focused mainly on the polymorphic fractions in the different fish groups. According to these parameters, the fractions were classified as follow: absolute fractions, appeared in 100% of the tested individuals, constant fraction, appeared in 80 %. polymorphic fractions, present in a part of individuals less than 80% and 0 % fractions that completely disappeared or were absent in all tested individuals.

Table (4) represents the comparison of appearance frequency of liver proteinogram fractions between the control group and the agricultural pollution groups, at the different zones. It is revealed that at zone (1) fractions number 1, 7, 9 and 10 completely disappeared, while, the polymorphism was exhibited in fractions number 2, 3 and 6 with appearance of 20% and 40%. Fraction 5 represented the constant frequency appearance (80 %) while fractions 5 and 8 represented the absolute (100%) ones. On the other hand, at zone 2, the disappeared fractions were increased to 5 polypeptides (numbers; 1, 3, 4, 6 and 10) and also the polymorphic fractions increased by one than that at zone 1 (number; 5, 7, 8 and 9). However, fraction 2 showed constant appearance. At zone (3), the percentage of appearance frequency recorded one absolute (100%) fraction and 4 each disappeared and polymorphic fractions. While the constant frequency appearance was revealed by one fraction, number 2. By comparing appearance frequency of liver proteinogram fractions of industrial pollution group (at the different zones) to the control, it is apparent that zone (1) showed 5 disappeared

fractions that decreased into only 2 at zone 2 and 4 fractions at zone 3 (Table 5). The polymorphism was observed in 3 fractions at zone 1 while increased to 6 at zone 2 then returned back to 3 fractions at zone 3. The frequency appearance of constant fractions were 2, 1, 2 in number at the industrial tested zones. While the absolute appearance was recorded at zones 2 and 3 by only one fraction.

DNA Fragmentation:

The recorded values of DNA fragmentation in the agricultural polluted water fish showed a significant increase ($P<0.01$) at zones 1 and 2 when compared to the control group, while it was non-significant at zone 3 (Table 6, Fig.1). In case of the industrial polluted area, it was apparent that there was significant increase ($P<0.01$) of DNA fragmentation in all the tested zones.

DISCUSSION

Water analysis:

Environmental contamination of water become a threat to continued existence of many plants and animals communities and may ultimately threaten the survival of humans. Heavy metals have recently come to the forefront of dangerous substances causing serious health hazards in humans and other organisms. So. analysis of water at which fish live are useful in diagnosis of pollution with heavy metals. From hygienic point view, high levels of cadmium, lead and mercury have been found to bring rapid physiological changes in river and lake fish.

In the current study, analysis of water from the unpolluted locality showed that iron, cadmium, lead, copper, zinc and nickel are within the accepted permissible limit of World Health Organization (WHO, 1984). This result may be due to the fact that this location is free from any pollution sources either industrial, irrigation or sewage drainages. Only mercury was higher than the permissible concentration. The detected heavy metals concentrations recorded sharp increase in the agricultural polluted water than in control water. Another sharp increase was observed in the industrial polluted area. Nickel was present in the highest ratio in both polluted areas and that was followed by mercury, lead, iron, cadmium, copper then zinc (in decreasing order). Fortunately, the increased concentration of Zinc in both polluted areas persisted under the permissible concentration. These results may be attributed to the adsorption of zinc on the active site of organic material and then precipitated in the sediment (Schintu *et al.*, 1991). These results were

similar to those recorded by Abo-Salem *et al.* (1992). Agricultural and industrial effluents cause pollution in aquatic environment by heavy metals (Forstner and Wittmann, 1979). Industrial and agricultural discharges are the primary sources of lead poisoning of fish (El-Nabawi *et al.*, 1987). However, the concentration and distribution of heavy metals (Zn, Cu, and Cd) in water of lake Mariut are affected by the variations in the discharge rate of dumped wastes (Saad *et al.*, 1981). Meanwhile, Moore and Ramamoorthy (1984) recorded high concentration for mercury and cadmium in an industrial area in Canada.

In the present study, SDS-PAGE revealed that liver proteins in control fish had 10 bands, then decreased by 7 to 8 polypeptides under the stress of agricultural polluted water. Polypeptide of 27 kDa was observed in all the studied groups as a resistant protein. The other 9 proteins in the control were disappeared in the liver of agricultural polluted fish. The 38 and 18 kDa bands – appeared only at zones (1) and (3) – might be expressed as a stress proteins. Sharaf-Eldeen and Abdel-Hamid (2002) found that the exposure of *Oreochromis niloticus* to the pollutants ($CuSO_4$, malathion and paraquat) induced disappearance of certain serum protein fractions. Anees (1974) found that the total serum protein of *Channa punctatus* decreased significantly on exposing to some organophosphorous compounds. On the other hand, the analysis of liver proteins of fishes which were taken from areas of industrial pollution at different distances had 9, 9 and 8 bands respectively. The bands of 57 kDa and 25 kDa appeared in the control and in fish of zone (1) but down-regulated in all polluted groups. Molecular weights of 27 kDa and 21 kDa were observed in control fish, and still existed in the polluted groups as a resistant protein. Contrarily, 15.49 kDa protein that appeared in the control fish, was down-regulated by industrial pollution zones 1 and 2 then it was re-expressed by zone 3. The 30 and 16 kDa polypeptides appeared in zone 1 and zone 2 while it vanished at zone 3. It may be represented as a stress protein. Kurbanova *et al.* (2004) reported that a decrease of the intensity of total protein accumulation and albumin concentration, and the increase of gamma globulin and peptidase activity which considered as adaptive reactions of the fish, *Rutilus frisii kutum* to the oil pollution. Patterson (1976) mentioned that the pollutants react with the cell nucleoproteins and nucleic acids and consequently affect the protein synthesis and cellular integrity.

In the present study, liver proteinogram of the fish *Tilapia zillii* – which were taken from agricultural polluted water – showed

polymorphism in 3, 4 and 4 fractions and disappeared in 4, 5 and 4 fractions for the three tested zones respectively down-stream to the outlet. On the other hand, fish taken from industrial polluted water showed 3, 6 and 3 polymorphic fractions while 5, 2 and 4 fractions were disappeared at the three zones, respectively. Exposing the fish, *Clarias gariepinus*, to the insecticide, deltamethrin. some fractions in its serum proteinograms disappeared. By increasing the exposure time (30 day) the effect of the insecticide was more prominent, resulting in disappearance of more fractions causing change in the genetic characters of proteinogram of the fish (Salama, 2001). Badawy and El-Serafy (1998) mentioned that in the electrophoretic serum proteinograms of *Clarias gariepinus*, from different polluted water localities. some fractions completely disappeared and other were polymorphic. These authors also found that the disappearance and polymorphism of protein fractions were dependent on the degree of pollution in each water locality. Protein heterogeneity is associated with all fish species. Structurally blood serum protein, muscle protein (myogen). haemoglobin, as well as all enzymes in the blood and other organs of fishes appear to be variable (Kirpichnikov, 1981). Saunders (1964) detected inter- and intraspecific differences in protein compounds. Transferrins (β-globulin blood haemoglobin formation), found in blood serum of fishes, are most variable or polymorphic. This polymorphism is mainly of genetic characters, it may be controlled by different alleles (Chen and Tusyuki, 1970; Wilkins, 1971). Hsp70 is known to assist the folding of nascent polypeptide chains, act as a molecular chaperone and mediate the repair and degradation of altered or denatured proteins (Morimoto *et al.*, 1994; Basu *et al.*, 2002).

DNA damage has been proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants (Kohn, 1983). Many of these pollutants are chemical carcinogens and mutagens with the capacity to cause various types of DNA damage. The relationship between DNA damage and the exposure of marine organisms to environmental contaminates was examined in Qalyobya governorate. This research is part of a wider ecotoxicological study to evaluate the biological impact of chemical contamination in the River Nile. The occurrence of cytogenetic damage in fish exposed to water pollution (Agricultural and industrial pollution) was demonstrated by an enhancing the percentage of DNA fragmentation in liver cells. Both agricultural and industrial water pollution caused an increase in the percentage of DNA fragmentation but the percentage of industrial pollution was higher than

that in areas of agricultural pollution. These results may be due to high levels of heavy metals which exceeded the permissible limit of WHO (nickel, mercury, lead, iron, cadmium and copper). The highest percentage of DNA fragmentation in industrial aquatic pollution was attributed to the highest level of pollution. Our results were in agreement with the study of Vittoria *et al.* (2004) who found high level of damaged DNA at the middle part of Gota harbour, which is chronically subjected to heavy chemical pollution. Fish tilapia, *Oreochromis niloticus* exposed subchronically and chronically to effluents of two treatment lagoons of a swine-processing plant showed consistently greater DNA damage (Lima *et al.*, 2006). Chinook salmon, *Oncorhynchus tshawytscha* exposed to chromium for 105 days revealed nuclear DNA damage in liver (Farag *et al.*, 2006).

From the current study, we can conclude that the contamination of water either by agricultural or industrial pollutants may lead to genetic disorders as a result of DNA damage. This genetic change was also indicated as a polymorphism in the expressed polypeptides of fish liver.

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Table (1): The mean concentrations of some heavy metals in the different localities (unpolluted, agricultural and industrial).

Localities		Iron (mg/l)	Lead (mg/l)	Cadmium (mg/l)	Zinc (mg/l)	Copper (mg/l)	Nickel (mg/l)	Mercury (mg/l)
Unpolluted Locality		0.195	0.015	0.00	0.00	0.04	0.00	0.04
Agricultural locality	0 m	1.95	0.105	0.03	0.055	0.00	1.45	0.725
	300 m	1.45	0.065	0.03	0.055	0.00	0.85	0.68
	1000 m	1.40	0.065	0.02	0.035	0.00	0.70	0.60
Industrial locality	0 m	3.85	0.475	0.185	0.195	0.3	3.4	4.41
	300 m	2.44	0.325	0.11	0.145	0.255	2.65	3.68
	1000 m	2.5	0.17	0.08	0.03	0.3	2.22	2.79
Permissible concentration (mg/l) •		0.300	0.050	0.010	5.000	1.000	0.010	0.001

• WHO Guide lines values, WHO Bulletin, guide lines for water quality Vol. 1, 1984.

Table (2): SDS-PAGE banding pattern of protein measured in liver of *Tilapia zillii* from the area of Agricultural pollution.

Mol.wt (kDa) / Band No.	Protein Marker	Lane 1 Control	Lane 2 Zone (1)	Lane 3 Zone (2)	Lane 4 Zone (3)
1		114.47			
2	97		98.51		
3					85.05
4		83.80			
5				81.15	
6					75.63
7			71.56		
8					68.21
9	66				
10				62.92	
11			61.43		
12		57.63			
13				47.86	
14	45				
15					42.96
16			38.83	38.17	
17		34.45			
18			32.59		
19	30				
20		27.14	27.23	27.01	27.86
21		25.22			
22				24.41	
23					23.12
24			22.97		
25		21.27			
26	20.27				20.34
27		19.31			
28			18.00	18.65	
29					16.68
30		15.49			
31	14.20				
32		12.76			
No. of Fraction	6	10	8	7	8

Zone (1): 0 m at the mouth of the agricultural discharge
Zone (2): 300 m downstream of agricultural discharge mouth
Zone (3): 1000 m downstream of agricultural discharge mouth

Table (3): SDS-PAGE banding pattern of protein measured in liver of *Tilapia zillii* from the area of industrial pollution.

Mol.wt (kDa) and No.	Protein Marker	Lane 1 Conctrol	Lane 2 Zone (1)	Lane 3 Zone (2)	Lane 4 Zone (3)
1					121.01
2		114.47			
3	97				100.94
4			87.5		
5				84.02	
6		83.80			
7				76.64	
8					70.06
9	66				
10		57.63	57.5		
11				54.14	
12			47.8		
13	45				45.94
14				41.24	
15			40		
16		34.45			
17	30		30.03	30.13	
18		27.14	27.8	27.00	27.75
19		25.22	25.00		
20		21.27	21.46	21.46	21.52
21	20.10				
22		19.31		19.00	
23					17.11
24			16.80	16.56	
25		15.49			15.77
26	14.20				
27		12.760			
No. of Fraction	6	10	9	9	8

Zone (1): 0 m at the mouth of the industrial discharge
Zone (2): 300 m downstream of industrial discharge mouth
Zone (3): 1000 m downstream of industrial discharge mouth

Table (4): The frequency of appearance of liver proteinogram fractions in the control and agricultural polluted fishes at different distances.

Band No.	Control fractions (M.wt.)	Zone (1)		Zone (2)		Zone (3)	
		Frequency		Frequency		Frequency	
		Number	%	Number	%	Number	%
1	114.47	0	0 %	0	0%	0	0
2	83.80	1	20%	4	80%	4	80%
3	57.63	2	40%	0	0%	2	40%
4	34.45	4	80%	0	0%	3	60%
5	27.14	5	100%	3	60 %	5	100%
6	25.22	1	20%	0	0 %	1	20%
7	21.27	0	0%	2	40%	0	0%
8	19.31	5	100%	3	60%	1	20%
9	15.49	0	0%	3	60%	0	0%
10	12.78	0	0%	0	0%	0	0%

Zone (1): 0 m at the mouth of the agricultural discharge
Zone (2): 300 m downstream of agricultural discharge mouth
Zone (3): 1000 m downstream of agricultural discharge mouth

Table (5): The frequency of appearance of liver proteinogram fractions in the control and industrial polluted fishes at different distances.

Band No.	Control fractions (M.wt.)	Zone (1)		Zone (2)		Zone (3)	
		Frequency		Frequency		Frequency	
		Number	%	Number	%	Number	%
1	114.47	0	0%	0	0%	0	0%
2	83.80	0	0%	3	60%	0	0%
3	57.63	4	80%	2	40%	3	60%
4	34.45	0	0%	2	40%	3	40%
5	27.14	4	80%	5	100%	4	80%
6	25.22	1	20%	0	0%	0	0%
7	21.27	3	60%	3	60%	1	20%
8	19.31	0	0%	4	80%	4	80%
9	15.49	2	40%	2	40%	5	100%
10	12.78	0	0%	1	10%	0	0%

Zone (1): 0 m at the mouth of the industrial discharge
Zone (2): 300 m downstream of industrial discharge mouth
Zone (3): 1000 m downstream of industrial discharge mouth

Table (6): The mean values of % DNA fragmentation in liver cells of *Tilapia zillii* in unpolluted and polluted water.

Pollution		DNA fragmentation %		T. test control & polluted	Anova
Source	Distance	Mean	SD		
Control		35.83	3.98		
Agricultural pollution	1)0 m	74.4	1.46	5.1**	
	2)300 m	45.8	1.22	4.35**	0.36
	3)1000 m	41.7	2.14	2.38	
Industrial pollution	1)0 m	58.9	8.75	8.06**	
	2)300 m	67.47	3.71	7.53**	0.92
	3)1000 m	60.6	8.99	7.24**	

* Significant ($P \leq 0.05$)
** Highly significant 1 ($P \leq 0.01$).

Ag : Agricultural pollution
In : Industrial pollution

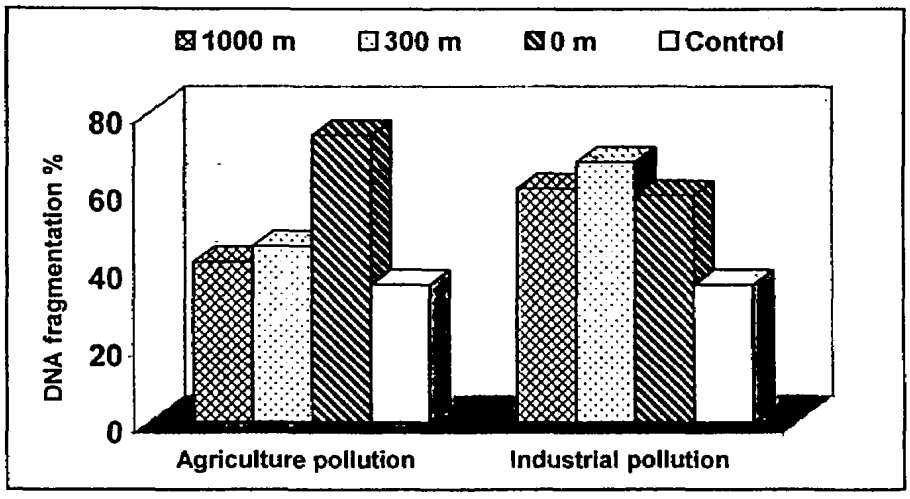

Fig. (1): The mean values of DNA fragmentation % in liver cells of *Tilapia zillii* in polluted water.