



Assessment of Genotoxic Effects of Pesticide Residues and Related Haemato-Biochemical Parameters on Farmed Nile Tilapia (*Oreochromis Niloticus* L.) in Kafrelsheikh Governorate, Egypt

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Key words

ABSTRACT:

Nile tilapia fish, Pesticide residues, haematological parameters, comet assay, DNA damage.

Fish genotoxicity biomarkers are valuable parameters for environmental risk assessment. The aim of the current study was to determine the levels of organochlorine and organophosphorus pesticide residues in water and Nile tilapia fish samples collected from two fish farms in Kafrelsheikh governorate, Egypt. Also to detect the genotoxic effect on different fish tissues using comet assay and DNA fragmentation, as well as the haemato-biochemical changes of the sampled fish. A large number of pesticide residues were detected both in the water and fish samples with high incidence of organochlorine compounds especially endrin, heptachlor epoxide, hexachlorobenzene (HCB), and p,p'-DDE. While the haematological parameters revealed a significant decrease in RBCs, PCV, HB, neutrophilia, lymphopenia and monocytosis. A Significant increase in the ALT, AST, urea and creatinine indicating hepato-toxicity and kidney damage. The phagocytic assay revealed a significant decrease in both phagocytic activity (PA) and phagocytic index (PI) ($P < 0.05$). The comet assay showed higher tail moments in the gills. The obtained results indicated that continuous exposure of the farmed Nile tilapia to different pesticide residues resulted in significant haemato- biochemical alterations as well as significant damage of the DNA in vital organs like gills and liver.

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1. INRODUCTION

Many new pesticides introduced into the market every year to combat increasing in pest resistance. These pesticides persist in the soil, water and food, with toxicity related outcomes to both humans and animals. (Schulz, 2004, Carvalho, 2006; Moraes et al., 2009). Monitoring of various indicators, including pesticide residue analysis is required in environmental quality control. Bioconcentration and Biotransformation process of pesticides have been studied mainly in fish. This is related to these fish live in direct contact with sediments neighboring to areas where pesticides are commonly used (Salvagni et al., 2011; Umbuziero et al., 2006). Numerous studies reported that pesticide residues present in wastewater are toxic to aquatic organisms, especially fish species (Banaee, et al., 2008; Talebi, 1998; Uner et al., 2006). Fish are sensitive to pesticide residues and other toxic pollutants because they are able to uptake and

accumulate the dissolved pollutants in water via active or passive processes. This is clear in populated countries, most or all rivers receive huge amounts of wastes come directly from industry, agriculture and urban settlements or indirectly from the atmospheric deposition of airborne emissions (Frenzilli et al., 2009).

The harmful effects of pesticide residues in fish include acute and persistent injury to the nervous system, injury to the reproductive organs, dysfunction of the immune and endocrine systems (Mansour, 2004). A number of long persistent organochlorines and highly toxic organophosphates, which have been banned or severely restricted, are still marketed and used in many developing countries. Misuses of pesticides by individuals, and lack of or weak national controlling plans are behind the outbreak of adverse effects in developing countries (Kaur et al., 2011).

Genotoxic chemicals such as insecticides have common chemical and physical properties that enable

them to interact with genetic materials (Campana et al., 1999 and Candioti et al., 2010). The mutation that may result from an interaction between a chemical and the genetic material is a heritable change in the cell genotype, and thus the error may be transferred to the daughter cell or the next generation. Carcinogenesis and the formation of some tumors in different tissues of fish exposed to insecticides may also be caused by genotoxic properties of these xenobiotics. One of the side effects of insecticides' arrival into surface waters may be an induction of chromosomal damage in eggs and larvae of fishes in different stages of development. Some insecticides that behave as endocrine active compounds can change the expression of vital genes resulting in unusual concentrations of plasma steroid hormones and reproductive dysfunction or immunosuppression (Jin et al., 2010).

Biomarkers frequently used to assess genotoxic effects of pesticides include chromosomal aberration, micronuclei formation, sister chromatid exchange and comet assay (Garaj-Vrhovac and Zeljezic, 2001). The past few years, single cell gel electrophoresis (SCGE) or comet assay has been used as a sensitive, visual, reliable, rapid and inexpensive technique for measuring and analyzing DNA single and double-strand breaks, alkali-labile sites, DNA cross-linking and delayed repair-site detection in eukaryotic individual cells (Singh et al., 1988 and Rojass et al., 1999).

Haematological and clinical chemistry parameters can be used easily and rapidly for prediction and diagnosis of pesticide toxicity. Alterations in these parameters reflect toxic stress in the treated animals. Alterations in blood biochemical parameters could be considered as an important diagnostic tool which can be used for the detection of abnormalities in the liver and other tissues (Banaee et al., 2011).

The present study is designed to assess the extent of possible DNA damage in farmed fish exposed to pesticides and evaluating the effect of such pesticides on the different haematological and biochemical parameters of these fish in both earthen and concrete ponds.

2. MATERIALS AND METHODS

2.1. Sample collection

All water and fish samples were collected during the intense spraying season (rice cultivation

season in Egypt, June 2013) from earthen (EP) and concrete ponds (CP) from two different fish hatcheries (F1-F2) in Kafrelsheikh governorate. The body weight of the fish was in grams (300-350 g) and the age of all fish was two years. The control samples were collected from the River Nile.

Blood was sampled from the *caudal vein*, using a 3 ml disposable plastic syringe. Two separate blood samples were collected from each fish, the first one on heparin as an anticoagulant which was used for haematological and immunological analysis, while the other one was obtained in a plain centrifuge tube for serum separation which was separated carefully and collected, stored in Eppendorf tubes at -20 °C until estimation of serum chemistry. Tissue samples were collected from each fish (muscle, liver and gills) for DNA extraction. Water and muscle tissue samples were further used for pesticide residue analysis.

2.2. Pesticide residue status:

All reagents and chemicals used in this study were of analytical grade. Organochlorine pesticide (OCP) standards included hexachlorobenzene (HCB), hexachlorocyclohexane (HCH) (α -, β -, γ - HCH isomers), heptachlor and its metabolite (heptachlor epoxide), aldrin, endrin, γ -chlordane, dichlorodiphenyltrichloroethane (p,p' -DDT) and its metabolites (p,p'-DDD and p,p'-DDE) and methoxychlor. In addition, Organophosphorus pesticide (OPP) standards were ethoprophos, phorate, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, fenitrothion, prothiophos, fenamiphos, ethion and triazophos as shown in pesticide chromatograms (Figs. 1a, 1b). In the laboratory, all water samples were filtered through 0.45 μ m fiber glass filter to remove sand and debris also muscle tissues of fish were homogenized at high speed to obtain a homogeneous composite. The extraction and clean up techniques were conducted according to the method described in detail elsewhere (Essumang et al., 2009). To determine the quality of analytical method, recovery study has been performed for each pesticide by spiking untreated samples with known levels of standard pesticide solutions. The samples were mixed and kept for 1h at room temperature to allow for the adsorption of pesticides. Samples were then extracted and clean up with proposed method, then analyzed by GC. Finally, recovery percentages were calculated.

2.2.1. GC-analysis:

Determination of OCPs was performed on Hewlett Packard GC model 6890 N equipped with a Ni63- electron capture detector (GC -ECD). GC-ECD had a capillary column PAS-5 (30 m length x 0.32 mm internal diameter x 0.25 μ m film thickness). Injector and detector temperature program were 300°C for 2 min, raised at the 3°C min⁻¹ and then held at 260°C for 15min. The carrier gas was N₂ at a flow rate of 4 ml/ min. As for OPPs the same model of gas chromatograph was used and fitted with a flame

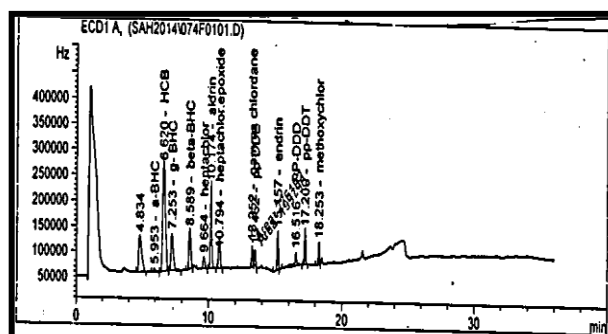


Fig.1a. Chromatogram of OCP standard mixture.

photometric detector (FPD) with phosphorus filter. It attached to a fused silica capillary column PAS-1701 (30 min length x 0.32 mm internal diameter x 0.25 μ m film thickness). The Injector and detector temperature program were 240 °C and 250 °C, initial oven temperature, 200 °C for 2 min, raised at 6 °C min⁻¹ then held at 250 °C for 15 min. The carrier gas was nitrogen at 3 ml min⁻¹; hydrogen and air were used for combustion in 75 and 100 ml min⁻¹, respectively.

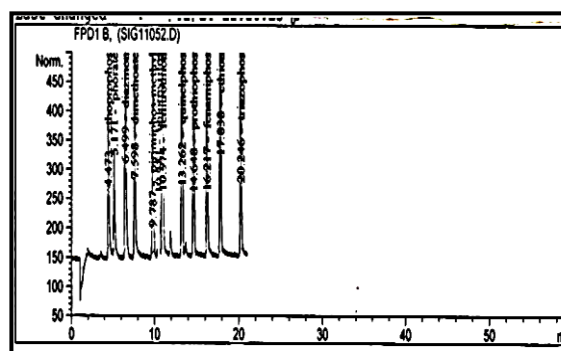


Fig.1b. Chromatogram of OPP standard mixture.

2.3. DNA extraction

DNA was extracted from all samples (muscle, liver, gills and blood samples) using the traditional phenol/chloroform extraction method (Ausubel et al., 2003).

2.4. Blood parameters

2.4.1. Haematological examination

Haematological parameters including packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBCs), total white blood cell (WBCs) and differential leukocyte counts were assessed. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated by a standard formula according to the routine haematological procedures for fish according to Feldman et al. (2000).

2.4.2. Determination of phagocytic activity and phagocytic index:

Phagocytic activity (PA) of polymorphnuclear cells using *Candida albicans* and phagocytic index (PI) were performed according to the method described by Kawahara et al (1991) and Soliman (1997).

Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (P1)} = \frac{\text{Total number of yeast cells phagocytized}}{\text{Number of phagocytic cell}}$$

2.4.3. Biochemical analysis of serum

Serum samples were analyzed for total protein (TP), albumin (Alb), serum enzyme activities of alanine amino transferase (ALT) and aspartate amino transferase (AST), triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). All these parameters were assayed using a spectrophotometer and commercial test kits of (Spinreact S.A.Co (Spain). Following the manufacturer's instructions. Globulin concentration (Glob) in serum protein and consequently albumin to globulin ratio (A/G) was calculated.

2.5. Comet assay

The comet assay of all tissue samples collected was performed according to Singh et al. (1988) and compared to the control samples. Although any image analysis system may be suitable for the quantitation of SCGE data, a Komet 5 image analysis software developed by Kinetic Imaging, Ltd.

(Liverpool, UK) linked to a CCD camera was used to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

2.6. Statistical analysis

Data obtained from the current study were statistically analyzed by independent-sample T-test to determine significant differences between groups using the Statistical Package for the Social Sciences (SPSS) software (version 17.0). A value of $P < 0.05$ was considered significant.

3. Results

3.1. Pesticide residues

The average recovery percentages of OC and OP pesticides were in the range of 75% to 95% and this indicates that the analysis process was satisfactory. A wide range of OC and OP pesticide residues have been monitored in water ($\mu\text{g/l}$) and tilapia fish ($\mu\text{g/kg}$ fresh weight).

3.1.1. Water samples

The mean levels of OC and OP pesticide residues that were detected in water samples are presented in **Figs. 2a and 2b**. In the present investigation, no pesticide residues of α -, γ - HCH, phorate, chlorpyrifos, quinelphos, ethion and triazophos residues were detected in water samples. Regarding the detected OCP residues, the mean

concentrations of HCB, β -HCH, heptachlor, heptachlor epoxide, aldrin, endrin were ranged from 11.752 to 19.132 $\mu\text{g/l}$ (CPF1 and EPF2), 1.559 to 13.765 $\mu\text{g/l}$ (CPF2 to EPF1), 0.717 to 3.095 $\mu\text{g/l}$ (CPF2 and EPF2), Not detected (ND) to 18.412 $\mu\text{g/l}$ (EPF1 and CPF2), 2.610 to 3.901 $\mu\text{g/l}$ (EPF2 and EPF1), 3.846 to 9.425 (CPF1 and EPF2), respectively. As for DDT and its metabolites, both of p,p'-DDT and p,p'-DDD were recorded only in F2 (fish farm number 2) and their mean levels were ranged from 9.039 to 15.386 $\mu\text{g/l}$ (CPF2 and EPF2) and from 8.811 to 10.329 $\mu\text{g/l}$ (CPF2 and EPF2). γ -chlordane was occurred in F1 in a range of 5.733 to 8.399 $\mu\text{g/l}$ (EPF1 and CPF1). Methoxychlor was observed only in one sample from EPF2. Regarding the average values of OC pesticide residues in River Nile, all levels were less than the lowest values that detected in fish farms except for aldrin, endrin and p,p'-DDD. For OPP residues (**Fig. 2b**), each of ethoprophos, prothiophos and fenamiphos has been detected in only one water sample (CPF2, Rive Nile and EPF1, respectively). While, diazinon found in F1 and the River Nile with the highest concentration of 4.857 $\mu\text{g/l}$ for EPF1. On the contrast with Pirimiphos-methyl that has been detected in F2

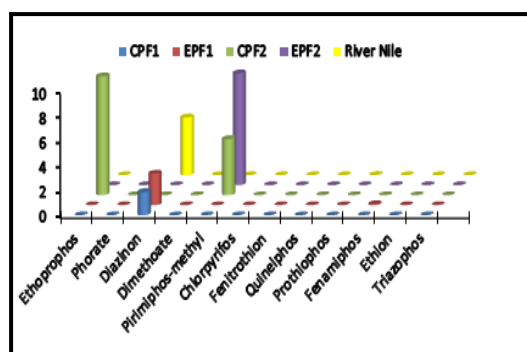


Fig. 2a: Mean concentrations of OCP residues in water samples.

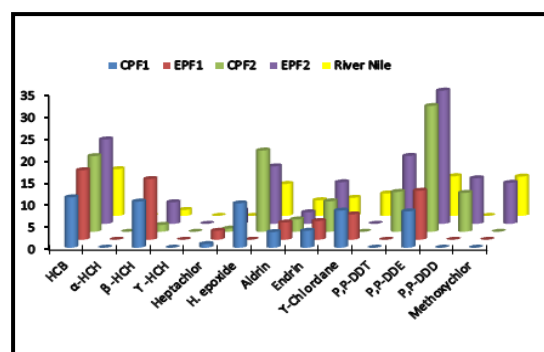


Fig. 2b: Mean concentrations of OPP residues in water samples.

3.1.2. Fish samples

The mean concentrations of accumulated OC and OP pesticide residues in Nile tilapia fish have

been illustrated in **Figs. 3a and 3b**, respectively. The residues of α -, γ - HCH, ethoprophos, phorate, quinelphos, prothiophos ethion and triazophos were

not identified in fish samples. As for OC residues (**Fig. 3a**), the recorded average concentrations of HCB, β -HCH, heptachlor, heptachlor epoxide, aldrin, endrin were ranged from 21.850 to 53.462 $\mu\text{g/kg}$ (CPF1 and EPF2), 5.365 to 34.679 $\mu\text{g/kg}$ (CPF2 and EPF1), 3.201 to 10.899 $\mu\text{g/kg}$ (CPF1 and EPF2) , 15.210 to 83.695 $\mu\text{g/kg}$ (EPF1 and CPF1), ND to 19.223 (EPF1 and CPF2), 58.853 to 66.388 $\mu\text{g/kg}$ (CPF1 and EPF2), respectively. As for DDT and its metabolites, p,p'-DDT and p,p'-DDE were ranged from 31.182 to 50.251 $\mu\text{g/kg}$ (CPF1 and EPF2) and 37.281 to 87.488 $\mu\text{g/kg}$ (EPF1 and EPF2). While, p,p'-DDD was observed only in F2 and its mean levels ranged from 12.261 to 20.030 $\mu\text{g/kg}$ (CPF2 and EPF2). Methoxychlor has found only with high concentration (91.649 $\mu\text{g/l}$) in EPF2. In River Nile, the mean concentrations of accumulated β -BCH, heptachlor, endrin, γ -Chlordane, p,p'-DDT, p,p'-DDE and p,p'-DDD were less than their lowest concentrations that found in fish farms. **Fig. 3b** represents the accumulated OPPs in fish tissues. The residues of ethoprophos, phorate, quinelphos, prothiophos, fenamiphos, ethion and triazophos were not detected in fish samples. The most predominant OP residue accumulated in fish tissues was chlorpyrifos followed by diazinon and fenitrothion. The levels of diazinon, chlorpyrifos and fenitrothion were ranged from 2.68 to 4.857 $\mu\text{g/kg}$ (CPF1 and EPF1), 0.912 to 3.420 $\mu\text{g/kg}$ (CPF2 and EPF1) and 0.038 to 0.170 $\mu\text{g/kg}$ (EPF1 and EPF2), respectively. Both dimethoate and pirimiphos-methyl

were occurred in only one sample (EPF1) at concentrations of 0.222 and 0.214 (EPF2) in $\mu\text{g/kg}$, respectively. Whilst, only two OPPs namely, diazinon and chlorpyrifos were detected in fish samples from River Nile at concentrations of 1.456 and 0.081 $\mu\text{g/kg}$, respectively.

3.2. Hematological parameters

The results of erythrogram showed a significant decrease in erythrocytic count, PCV and hemoglobin concentration with increase in MCV and decrease in MCHC. The leukogram revealed a significant decrease of white blood cell count, as there were neutrophilia, lymphopenia and monocytosis (**Table 1**). The biochemical results showed a significant increase of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglycerides, LDL, urea and creatinine, on the other hand, the total serum protein, albumin, globulin, A/G ratio and glucose were significantly decreased while HDL showed a non-significant change (**Table 2**). The effects on cell mediated immune response were determined by using the phagocytic assay .Phagocytic assay revealed a significant decrease ($P < 0.05$) in both phagocytic activity (PA) (15.25 ± 0.75 mean $\pm$ SEM) and phagocytic index (PI) (1.31 ± 0.06 mean $\pm$ SEM) in the farmed fish compared to the control samples (20.50 ± 0.56 mean $\pm$ SEM and 1.94 ± 0.09 respectively).

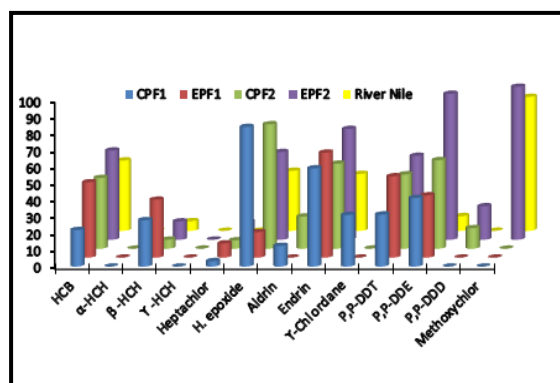


Fig. 3a: Mean concentrations of OCP residues in fish samples.

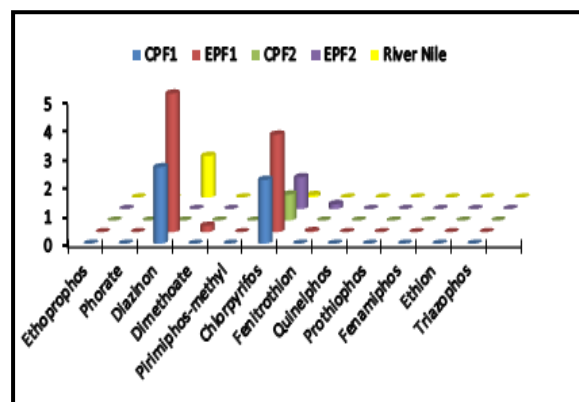


Fig. 3b: Mean concentrations of OPP residues in fish samples.

Table 1. Haematological parameters of the farmed fish compared to control samples expressed as mean $\pm$ SEM.

	<i>Farmed fish</i>	<i>Control fish</i>
Monocytes($\times 10^3/\mu\text{l}$)	0.76 $\pm$ 0.02*	0.49 $\pm$ 0.01
Heterophils($\times 10^3/\mu\text{l}$)	4.21 $\pm$ 0.17*	3.39 $\pm$ 0.11
Lymphocytes($\times 10^3/\mu\text{l}$)	2.77 $\pm$ 0.15	6.21 $\pm$ 0.08*
WBCs ($\times 10^3/\mu\text{l}$)	7.75 $\pm$ 0.18	9.95 $\pm$ 0.16*
RBCs($\times 10^6/\mu\text{l}$)	3.10 $\pm$ 0.16	4.06 $\pm$ 0.10*
MCHC (%)	23.63 $\pm$ 0.49	26.11 $\pm$ 0.20*
MCH (pg)	20.77 $\pm$ 0.86	20.66 $\pm$ 0.44
MCV (fl)	85.25 $\pm$ 1.89*	78.17 $\pm$ 1.08
PCV (%)	26.25 $\pm$ 0.92	31.83 $\pm$ 1.01*
HB (g/dl)	6.20 $\pm$ 0.11	8.65 $\pm$ 0.22*

Where * indicates significance.

Table 2. Biochemical analysis of the serum samples of the farmed fish compared to control Samples (River Nile) expressed as mean $\pm$ SEM.

	<i>Farmed fish</i>	<i>Control fish</i>
Total protein (g/dl)	3.75 $\pm$ 0.05	4.27 $\pm$ 0.15
Albumen (g/dl)	1.15 $\pm$ 0.05	2.17 $\pm$ 0.09*
Globulin (g/dl)	2.25 $\pm$ 0.03	2.55 $\pm$ 0.04*
Alb/Glob ratio	0.67 $\pm$ 0.03	0.86 $\pm$ 0.02*
Glucose (mg/dl)	78.14 $\pm$ 1.80	90.66 $\pm$ 1.25*
Cholesterol (mg/dl)	154.00 $\pm$ 2.19*	90.66 $\pm$ 2.09
Triglyceride (mg/dl)	166.86 $\pm$ 14.43*	81.34 $\pm$ 1.71
LDL (mg/dl)	97.43 $\pm$ 4.37*	56.60 $\pm$ 2.20
HDL (mg/dl)	23.28 $\pm$ 0.42	24.33 $\pm$ 0.71
ALT (U/L)	38.71 $\pm$ 7.2*	25.66 $\pm$ 0.61
AST (U/L)	42.14 $\pm$ 1.90*	24.16 $\pm$ 1.77
Urea (mg/dl)	48.29 $\pm$ 1.61*	23 $\pm$ 0.89
Creatinine (mg/dl)	2.76 $\pm$ 0.09*	1.81 $\pm$ 0.9

Where * indicates significance

3.3. DNA fragmentation

Fig. 4 shows DNA from different examined samples (liver, blood, muscle and gills) where there is clear damage in the gills with smearing of DNA in all samples from both concrete and earthen ponds.

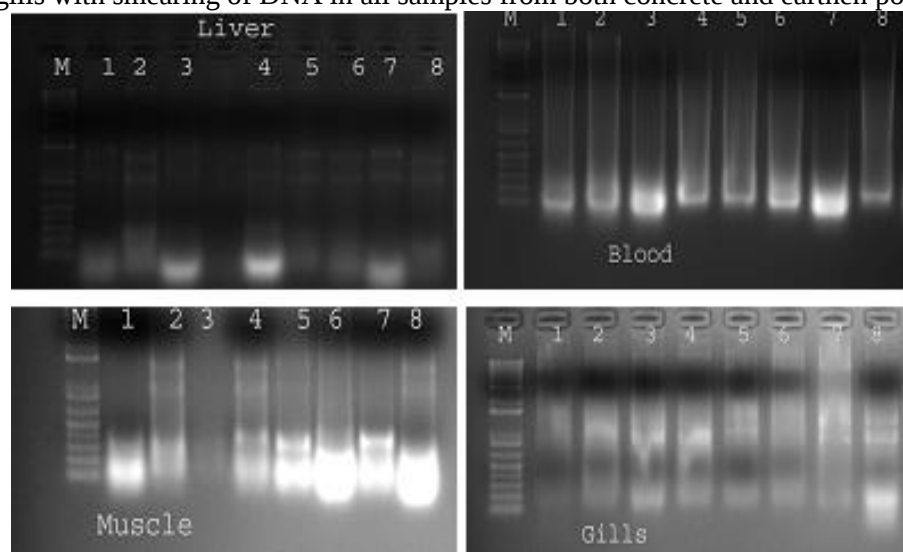


Fig. 4: DNA fragmentation on 1.5% agarose gel with ethidium bromide. M: molecular weight marker (100bp DNA ladder, 1 to 8: sample number where: samples , 1-2 from concrete ponds and 3-4 , earthen ponds of the first farm and 5-6 from the concrete ponds and 7-8 from earthen ponds of the second farm.

Comet assay results are shown in Table 3 and Fig. 5.

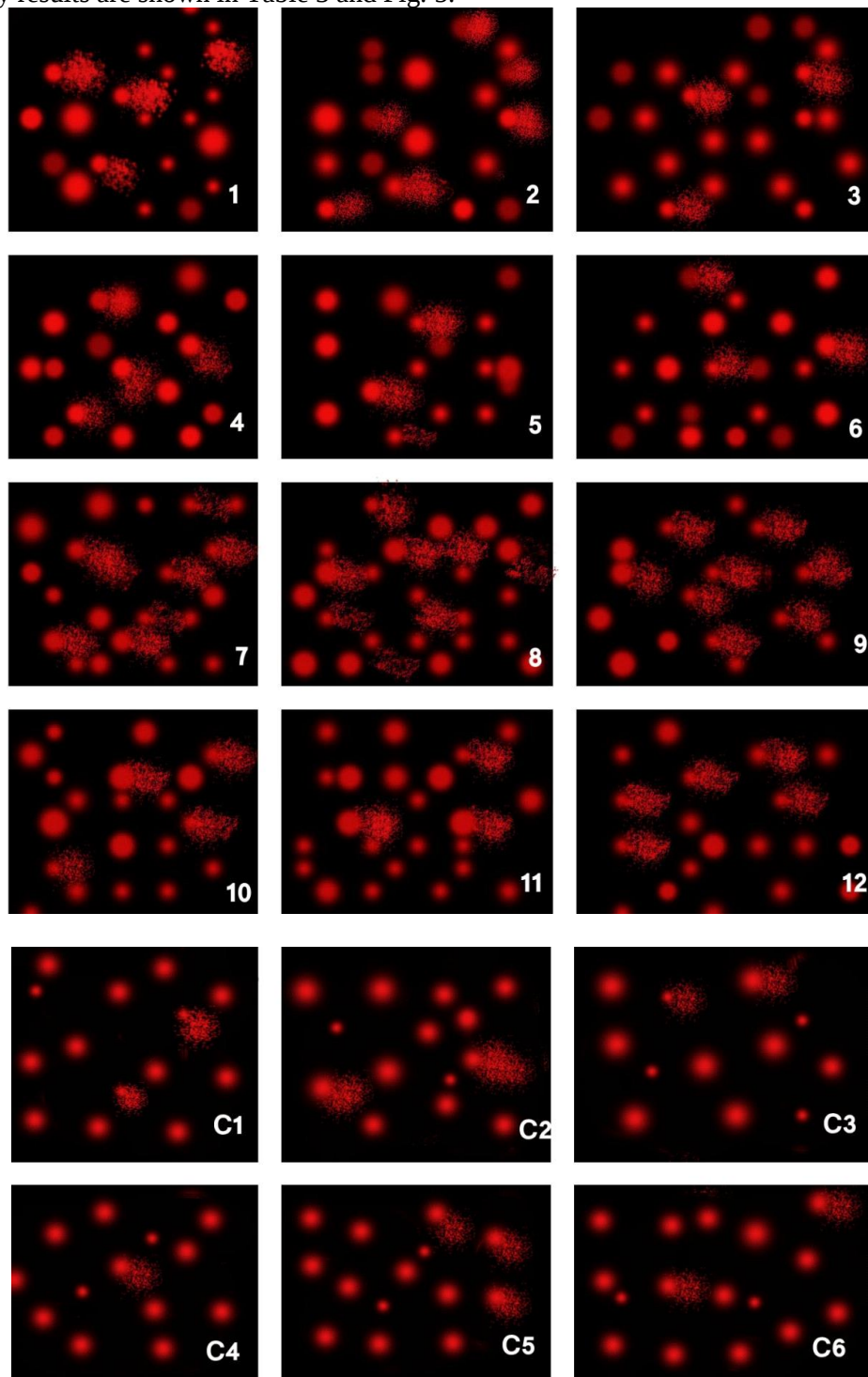


Fig. 5: Results of comet assay 1-2-3-4 represent muscle samples with C1-C4 control muscle samples, 5-6-7-8 represent gill samples with C2-C5 control gill samples, 9-10-11-12 represent liver samples with C3-C6 control liver samples.

Table 3. The results of the comet assay of different organs examined from farmed tilapia fish (*Oreochromis niloticus* L.) compared to the control (River Nile) expressed as mean±SEM.

Organ		Tail moment	Tail length (μm)	Tail intensity %
Muscle	Examined	14.39±3.05*	3.93±0.19*	3.84±0.28*
	Control	1.06±0.05	1.46±0.18	1.01±0.02
Gills	Examined	22.22±3.67*	4.79±0.39*	4.55±0.35*
	Control	2.88±0.35	1.38±0.14	2.12±0.10
Liver	Examined	12.03±0.30*	3.67±0.09*	3.28±0.08*
	Control	3.23±0.62	1.72 ±0.18	1.64±0.21

Where *indicate significance.

4. DISCUSSION

The general trend of the results indicates that almost all the water and fish samples from earthen ponds were significantly contaminated with pesticide residues than concrete ponds. This reason may be attributed to that bed sediments in earthen ponds act as long-term storage for hydrophobic pollutants in aquatic system from which water and biota are continuously polluted (Doong et al., 2002). The total OCP concentrations detected were much greater than the total OPPs in testing samples, this may be related to OCPs are much more resistant to environmental degradation than OPPs and also they have higher ability to accumulate in biological tissues and to concentrate in organisms (Krieger, 2001). The higher values of pesticide residues were observed in the fish samples as compared with corresponding water samples. Since, the aquatic organisms like fish are able to bioaccumulate several fold pesticide residues concentrations than the surrounding water (Siddiqui et al., 2005). Elevated concentrations of HCB noticed in all analyzed samples, this is due to HCB was used frequently as a fungicide to protect the seeds of wheat and also it is persistent and bioaccumulative (EPA, 2011). Among HCH isomers, β -HCH was the only detected and the predominance of this isomer in all measured samples may be related to its high persistence in the environment because it is more resistant to environmental degradation in soils, animal tissues and fluids (Willett et al., 1998). The concentration of heptachlor epoxide in water and fish samples was higher than heptachlor. Heptachlor epoxide is an oxidation product of heptachlor and because of the rapid conversion process; heptachlor epoxide is more abundant in the environment than its

parent compound, heptachlor (Poolpak et al., 2008; Zhao et al., 2009).

For DDT and its metabolites, the results indicate that p, p'-DDE was the most predominant in all samples with the highest concentrations. This indication to transform DDT in aquatic environment by both photodegradation and biodegradation process (EPA, 1979). OPPs, chlorpyrifos demonstrated predominance of bioaccumulation in fish samples followed by diazinon and fenitrothion. These OPPs are widely used as agricultural insecticides and also, they have many uses in households as pest control. No recommended MRLs (maximum residue limits) have been established for agricultural drainage water. As for fish, no MRLs have been set yet by Codex Alimentarius Commission and European commission. All the pesticide residues detected in fish samples were below Japanese MRLs (The Japan Food Chemical Research Foundation, 2005) with the exception of heptachlor epoxide and endrin residues. It was observed that heptachlor epoxide and endrin mean levels in fish samples were exceeded the MRL (0.05 and 0.005 mg/kg, respectively). So, this may cause severe health problems for humans and other wildlife who might eat these fish. The blood is a unique mirror in which all the internal process taking place in an organism are reflected. A fall in the red blood cell count, hemoglobin concentration and haematocrit volume worsening of an organism state and developing anemia. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. The anemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. According to

(Pamila et al., 1991) the pesticide induced anemia in fish may be due to the inhibitory effect of the toxic substance in the enzyme system responsible for the synthesis of hemoglobin. The MCV increased while, MCHC decreased considerably compared to the control. This is in agreement with the work of (Shah, 2006) following a short-term exposure of tench (*Tinca tinca*) to agrochemical metal. These alterations were attributed to direct responses of structural damage to RBC membranes, resulting in haemolysis and impairment in hemoglobin synthesis. Leukocytes are involved in the regulation of immunological function and a protective response to stress in fish. The leucopenia observed here, can be attributed to generalized stress response that cause increase pituitary internal activity (Donaldson, 1981) which also may be due to dysfunction in haematological tissues (spleen and kidney) or certain infectious diseases. Lymphopenia can be an indicator of immune system deficiency. In agreement with these results Banaee et al., (2008). The most common and important cause of neutrophilia is tissue damage, Poisonings and severe disease, like kidney failure all cause neutrophilia (Holland et al., 1997).

Ghosh and Banerjee (1993) reported lymphopenia and increased both neutrophil and eosinophil after an effect of insecticides. Thus, insecticides may alter the function of the immune system and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defense mechanisms including innate immunity and acquired immunity against pathogens. Phagocytic activity and phagocytic index in case of fish exposed to pesticides showed a significant decrease compared to control group. The significant decrease of phagocytic activity and phagocytic index may indicate destructive effect of pesticides on liver, kidney, spleen and other haemopoietic organs.

The present investigation also showed total hypoproteinaemia, hypoglobulinaemia, hypoalbuminaemia and decreased A/G ratio. It could be due to the impairment of protein synthesis. Dutta et al. (2003) reported similar decrease in protein content in blood of different species of fish when treated with different pollutants. Also, the decreased albumin levels (hypoalbuminemia) reflect the active inflammation and serious hepatic and renal damage. The results revealed significant increases in liver alanine amino transferase (ALT) and aspartate aminotransferase (AST), these are liver specific enzymes and they are more sensitive measure of

hepatotoxicity and histopathologic changes (Rao, 2006). The elevation of serum urea and creatinine in fish may be attributed to the toxic effect which causing disturbance of the kidney functions with subsequent reduced glomerular filtration rate retentions of urea and creatinine in blood (Jayasundera and Macnab, 2012). The blood glucose level was affected by the rate of carbohydrates metabolism under hypoxia and stress conditions. The glucose level showed variability directed to decreased trend in its level. Hypoglycemia is attributed to stress stimuli followed by the rapid secretion of both glucocorticoids and α -techolarnines from the adrenal tissue (Abbas, 2006). Similar conclusions were recorded by El-Fayoumi and Abd Allah (2003) and Zaki et al. (2009). The blood triglycerides and low density lipids (LDL) levels were significantly increased in examined fish, compared to the control ones. Mekki et al. (1996) recorded increased and decreased levels under the stress of the herbicide atrazine in *O. niloticus* and *Chrysichthys*, respectively. Such variability in triglycerides reflects the disturbance in energy storage mechanisms under the stress of heavy metals and herbicides. In addition, it reflects the chronic renal failure and damage of pancreatic cells.

In the present work, the blood cholesterol level was significantly increased in fish, compared with control. (Kaplan et al., 1988) reported the increase in the blood cholesterol concentration was used as an indicator of liver dysfunction because homeostasis of lipids is one of the principle liver functions.

The results of the gel electrophoresis showed fragmentation and smearing of the DNA samples in all organs which gives a clear indication about the degree of DNA damage in these organs in reaction to high levels of pesticides. The highest degree of DNA damage was observed in the gill tissues. In fish, gills represent the largest surface in contact with the aquatic environment and they are thus expected to show the greatest tissue alteration, genotoxicity and the accumulation of toxic chemicals (Yildiz et al., 2010). As a consequence, respiratory function of gills can become decreased, consequently affecting the general health and ultimately resulting in death of the affected individual.

The three parameters of genotoxicity tail moment, tail length, and tail intensity have been widely used by researchers for evaluating DNA damage. As the amount of damage increases in a cell, more DNA migrates into the tail region and is quantified in terms

of an increased amount of determined fluorescence in the tail region, as well as by tail length. The ratio of the DNA in the tail region (tail intensity) is commonly used for quantifying DNA strand breakage and represents the most reliable parameter (Mitchelmore and Chipman, 1998). However, the main disadvantage of this parameter is that two cells with different tail lengths may have the same degree of tail intensity and may produce the same result.

The comet assay results of the farmed Nile tilapia fish examined in this study indicated higher tail moment in the examined fish than the control samples ($P < 0.05$) due to exposure of fish farms to pollution stress resulted from agricultural activities.

There was no clear difference in both haemato-biochemical and comet assay results between earthen and concrete ponds this may be related to the effective concentration of pesticide residues found in these ponds result in a similar effects on the fish.

5. REFERENCES

- Abbas, W. T. 2006. Fish as an indicator for pollutants in aquatic environment. Ph.D. Thesis, Zoology Department, Faculty of Science, Cairo University, Egypt.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K. (eds.) 2003. Current protocols in molecular biology. John Wiley and Sons Inc.
- Banaee, M., Mirvaghefi, A. R., Ahmadi, K. and Banaee, S. 2008. Determination of LC50 and investigation of acute toxicity effects of diazinon on haematology and serology indices of common carp (*Cyprinus carpio*). J. of Marine Sci. and Technol. Res. 3: 1-10.
- Banaee, M., Mirvaghefi, A. R., Majazi, A. B., Rafei, G. R., Nematdost, B. 2011. Haematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinus carpio*). Journal of Fisheries (IJNR), 64: 1-14.
- Campana, M. A., Panzeri, A. M., Moreno, F. V. J., Dulout, F. N. 1999. Genotoxic evaluation of the pyrethroid lambda-cyhalothrin using the micronucleus test in erythrocytes of the fish *Cheirodon interruptus interruptus*, Mutat. Res. 438: 155-161.
- Candioti, J. V.; Soloneski, S. and Larramendy, M. L. 2010. Genotoxic and cytotoxic effects of the formulated insecticide Aficida on *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces: Poeciliidae). Mutation Research. 703: 180-186.
- Carvalho, F. P. 2006. Agriculture, pesticides, food security and food safety. Environ. Sci. & Policy. 9: 685-692.
- Donaldson, E. M. 1981. The pituitary interrenal axis as an indicator of stress in fish. In Stress "and Fish" Pickering, A.D (ed.). Academic Press, New York; 11-47.
- Doong, R., Peng, C., Sun, Y., Liao, P. 2002. Composition and distribution of organochlorine pesticide residues in surface sediments from Wu-Shi River Estuary, Taiwan. Mar. Pollut. Bull. 45: 246-253.
- Dutta, H. M., Meijer, H. J. M. 2003. Sublethal effects of diazinon on the structure of testis of blue gill *Lepomis macrochirus*, amicroscopic analysis. Env. Pollut. 25:355-360.
- El-Fayoumi, R. I., Abd-Allah, G. A. 2003. Toxicity of fumonisin B1 in Rainbow trout. J. Egypt Ger. Soc. Zool. 40: 489-502.
- EPA 1979. Water-related environmental fate of 129 priority pollutants: Volume I: Introduction and technical background, metals and inorganics, pesticides and PCBs. Washington, DC: U.S. Environmental Protection Agency. EPA-440/4-79-029b, 1-1 - 1-4, 2-1 - 2-16.
- EPA 2011. <http://www.epa.gov/pbt/pubs/hexa.htm>.
- Essumang, D. K., Togoh, G. K., Chokky, L. 2009. Pesticide residues in the water and fish (lagoon tilapia) samples from lagoons in Ghana. Bull. of Chem. Soci. of Eth. 23 (1).
- Feldman, B. F., Zinkl, J. G., Jain, N. C. 2000. Schalm's Veterinary Haematology. Lippincott Williams and Wilkins. USA.
- Frenzilli, G., Nigro, M., Lyons, B. P. 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mut. Res. 681: 80-92.
- Garaj-Vrhovac, V., Zeljezic, D. 2001. Cytogenetic monitoring of Croatia population occupationally exposed to a complex mixture of pesticides. Toxicology. 165:153-62.
- Ghosh, K., Banerjee, V. 1993. Alteration in blood parameters in the fish *Heteropneustes fossilis* exposed to dimethoate. Environ. Ecol. 11: 979-981.
- Holland, M. M., Steven, M., Gallin, J. I. 1997. Disorders of Granulocytes and Monocytes. In Harrison's Principles of Internal Medicine, edited by Anthony S. Fauci, et al. New York, McGraw-Hill.
- Jayasundera, S., Macnab, R. 2012. Laboratory tests of renal function. Anaesth. Inten. care Med. 13: 328-331.
- Jin, Y. X., Chen, R. J., Liu, W. P., Fu, Z. W. 2010. Effect of endocrine disrupting chemicals on the transcription of genes related to the innate immune system in the early developmental stage of zebrafish (*Danio Rerio*). Fish Shellfish Immun. 28: 854-61.
- Kaplan, A., Ozabo, L., Ophem, K. 1988. Clinical Chemistry. Interpretation and Techniques. (Eds.), L. F. Philadelphia, Lea and Febiger.
- Kaur, R., Kaur, S., Lata, M. 2011. Evaluation of DNA damage in agricultural workers exposed to pesticides using single cell gel electrophoresis (comet) assay. Ind. J. of Hum. Gen. 17(3): 179-187.

- Kawahara, E., Ueda, T., Nomura, S. 1991. In vitro phagocytic activity of white spotted shark cells after injection with *Aeromonas salmonicida* extracellular products. Gyobyo Kenkyu, Japan. 26 (4): 213-214.
- Krieger, R. 2001. Handbook of Pesticide Toxicology: Principles and Agents, Acad. Press.
- Mansour, S. A. 2004. Pesticide Exposure- Egyptian scene. Toxicology. 198: 1-3.
- Marchezan, E., Reimche, G. B., Avila, L. A. 2009. Toxicological and metabolical parameters of the teleost fish (*Leporinus obtusidens*) in response to commercial herbicides containing clomazone and propanil. Pest. Biochem. and Physiol. 95: 57-62.
- Mekkawy, I. A., Hussein, S. Y., Abdel – Nasser, M., Ahmed, S. M. 1996. Comparative studies on the effects of herbicide atrazine on some blood constituents and protein electrophoretic patterns of *Oreochromis niloticus* and *Chrysichthys auratus*. Assuit. J. Egypt Ger. Soc. Zool. 19 (A): 283 – 319.
- Mitchelmore, C. L., Chipman, J. K. 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mut. Res. 399(2): 135–147.
- Pamila, D., Subbaiyan, P.A., Ramaswamy, M. 1991. Ind. J. Environ. Hlth. 33: 218-224.
- Poolpak, T., Pokethitiyook, P., Kruatrachue, M., Arjarasirikoon, U., Thanwaniwat, N. 2008. Residue analysis of organochlorine pesticides in the Mae Klong river of Central Thailand. J. Hazar. Mater. 156: 230-239.
- Rao, J. V. 2006. Toxic effects of novel organophosphorous insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pest. Biochem. and Physiol. 86: 79-84.
- Rojas, E., Lopez, M. C., Valverde, M. 1999. Single cell gel electrophoresis assay: Methodology and applications. J. of Chromato. B: Biomed. Sci. and Appl. 54: 722-225.
- Salvagni, J., Zeni, R., Fludentefria, A. M. 2011. Assessment of the genotoxic impact of pesticides on farming communities in the countryside of Santa Catarina State, Brazil. Gen. and Mol. Biol. 34: 122-126.
- Schulz, R. 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: A review. J. of Environ. Quality. 33: 419-448.
- Shah, S. L. 2006. Haematological parameters in tench *Tinca tinca* after short term exposure to lead. J. Appl. Toxicol. 26 (3): 223-228.
- Siddiqui, M., Anand, M., Mehrotra, P., Sarangi, R., Mathur, N. 2005. Biomonitoring of organochlorines in women with benign and malignant breast disease. Environ. Res. 98: 250–257.
- Singh, N. P., McCoy, M. T., Tice, R. R., Schneider, E. L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experim. Cell Res. 175:184–91.
- Soliman, M. K. 1997. Depression of phagocytosis in grass carp *Ctenopharyngodon idella* following chemical stress. Alex. J. of Vet. Sci. 13 (1): 81-86.
- Talebi, K. 1998. Diazinon Residues in the Basins of Anzali Lagoon, Iran. Bull. Environ. Contam. Toxicol. 61: 477-483.
- The Japan Food Chemical Research Foundation 2005. <http://www.m5.ws001.squarestart.ne.jp/foundation/searc.html>
- Umbuziero, G. A., Kummrow, F., Roubicek, D. A., Tominaga, M. Y. 2006. Evaluation of the water genotoxicity from Santos Estuary (Brazil) in relation to the sediment contamination and effluent discharges. Environ. Intern. 32: 359-364.
- Uner, N., Oruç, E. O., Sevgiler, Y., Şahin, N.; Durmaz, H. Usta, D. 2006. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. Environ. Toxicol. and Pharm. 21: 241-245.
- Willett, K. L., Ulrich, E. M., Hites, R. A. 1998. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ. Sci. Technol. 32 (15): 2197–2207.
- Yildiz, S., Gurcu, B., Basimoglu, Koca Y., Koca, S. 2010. Histopathological and genotoxic effects of pollution on *Anguilla anguilla* in the Gediz River (Turkey) J. of Anim. and Vet. Adv. 9 (23): 2890–2899.
- Zaki, J., Weber, J., Bolger, N. and Ochsner, K. 2009. The neural bases of empathic accuracy. Proc. of the National Acad. of Sci. USA 106: 11382-11387.
- Zhao, Z., Zhang, L., Wu, J., Fan, C. 2009. Distribution and bioaccumulation of organochlorine pesticides in surface sediments and benthic organisms from Taihu Lake, China. Chemosphere. 77: 1191-1198.