NILE TILAPIA AS BIO INDICATOR TO ESTIMATE THE CONTAMINATION OF WATER USING SDS-PAGE AND RAPD-PCR TECHNIQUES

A. H. A. EL-SAPPAH, A. S. H. SHAWKY, MAHASSEN S. SAYED-AHMAD AND M. A. H. YOUSSEF

Genetics Dept., Fac. of Agric., Zagazig University, Egypt

Contamination of aquatic ecosystems with heavy metals has increased worldwide. Heavy metals are interesting and noteworthy due to their strong impact on aquatic ecosystems and their bio-accumulation in hydrobionts. Cadmium and Copper were tested in genotoxicity assays with some contradictory results (Ayllon and Garcia, 2000; Conners and Black, 2004). Metal accumulation causes an increase in highly reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical which leading to oxidative stress in fish (Dautremepuits *et al.,* 2002). Many factors including heavy metals in soil and waste water (Yu, 2000) can affect the DNA genetic material of organisms directly or indirectly and not only to damage the integrity of the DNA structure but also influence its expression and eventually cause genotoxicity to organisms.

Copper sulfate is one of the pesticides that can be troublesome. The most copper sources are the agricultural fertilizers and pesticides and the frequent cause of poisoning in aquatic eco-systems. Copper compounds are also found in preservatives, additives and coloring agents used in food industry and medical products (Chevreuil *et al.,* 1995). Moreover, copper (Cu) has also been classified as a carcinogenic compound by the International Agency for Research on Cancer. Mean while, cadmium (Cd) is a toxic heavy metal which has many industrial uses such as in Cd batteries, anticorrosive coating of metals, pigments, and stabilizers for plastic materials (Chevreuil *et al.,* 1995).

Fish are excellent subjects for the study of the mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants (Al-Sabti, 1991). Fish serve as useful genetic models for the evaluation of pollution in aquatic ecosystems (Park *et al.,* 1993). Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially tetratogenic and carcinogenic in humans. The main application for model systems using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment (Al-Sabti and Metcalfe, 1995). Aquatic animals have

often been used in bioassays to monitor water quality of effluent and surface water (Brugs *et al.,* 1977).

Tilapia (*Oreochromis niloticus*) is a fresh water fish that is hardy, prolific, fast growing tropical fish that is farmed mainly in Africa and Asia. Tilapia fish are beneficial to human's beings as they make up a major part of the human diet and provide humans with as much of needed proteins as in meat (Ghorbani and Mirakabad, 2010).

The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants (Badr and El-Dib, 1978). Biomarkers for water pollution are early diagnostic tools for biological effect measurement and environmental quality assessment (Cajaraville *et al.,* 2000). Biomarker is defined as a change in biological response that differs from molecular to organismal level (Depledge *et al*., 1995).

Electrophoretic protein in *Oreochromis niloticus* fish showed variations in numbers of phenotypic bands, relative electrophoretic mobilites, optical densities and molecular weights (El-Ghobashy *et al*., 2005). PCR-based molecular markers such as RAPD technique is more sensitive, effective, relatively cheap and simple technique, as they give evidence about DNA mutation in relation to many different organisms (Qi *et al*., 2006). RAPD is a reliable and sensitive method for the environmental health risk (Xiaolin *et al*., 2009). Amplified Polymorphic DNA (RAPD) has led to the development of a number of selective and sensitive assays for detecting DNA damage genotoxicologically (Aras *et al*., 2010). They are especially useful for pollution studies, as they can compare polluted and non-polluted samples at the same time and in relatively short periods.

The aim of this work was to estimate the heavy metals content and examine their effect on the genetic pattern of Nile tilapia collected from different polluted water sites in Egypt by using SDS-PAGE and RAPD-PCR techniques.

MATERIALS AND METHODS

This study was carried out in Molecular Genetics Lab., Genetics Dept., Fac. of Agric. Zagazig Univ. and Unit of Analysis Elements, Faculty of Veterinary Medicine.

Fish and water samples

Fish; *Oreochromas niloticas* (ON) and water samples were collected from four sites and transported in large plastic water containers supplied with battery aerators as source of oxygen to Molecular Genetics Lab., Faculty of Agricultural, Zagazig University, Egypt. These sites represent differential environmental stresses. Site1 was River Nile (El-Mansoura) and it was used as control in this study because it was previously used as control for micronucleus test in fish genomes by Ali *et al*. (2008). Science it is the common source for irrigation and

drinking in Egypt. Site 2 was Ismalia Canal while, sites 3 and 4 are two lakes, Mariout and Manzala, respectively.

Heavy metal concentrations in water samples

From the previous locations where the fishes were collected, water samples were taken for chemical analysis to determine heavy metal concentrations. Heavy metal con-centrations in water samples such as copper, cadmium and lead were measured as ppm (mg/l) by UV 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).

Heavy metal concentrations in fish samples

Large pieces of fish tissue samples were taken and dried on 65°C for 60 h, and then were grinned in sterilized mortars. 1±0.3 g of ashes samples was taken and 20 ml of deionized water were added. One ml of concentrated nitric acid was also added for each sample. Samples were digested by heating them overnight on 90°C, diluted to 25 ml by dd H_2O and filtered before measuring process. Heavy metal concentrations in fish samples were measured as mg/kg by UV 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).

Protein separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) technique was used to compare among the studied sites by comparing among fish protein patterns which indicated the gene expressions in fish samples. SDS polyacrylamide gel 12.5% was prepared according to the method of Laemmli (1970).

Protein extraction

One piece of skeletal muscle (0.2 g) from each individual was powdered in a mortar using liquid nitrogen and extracted in appropriate volume of extraction buffer as saline solution (0.85% of NaCl) which described by Rashed *et al.* (2007). The powdered muscle samples were homogenized with extraction buffer for 15 sec. and were centrifuged at 1200 rpm for 15 min. at 4°C. The supernatants were transferred to new eppendorf tubes and kept in deep freezer until usage.

Protein electrophoresis

Saline solutions of protein fractions were performed exclusively on a vertical slab (19.8 cm x 26.8 cm x 0.3 cm) using a gel electrophoretic apparatus (manufactured by LABCONCO). Samples of extracted protein were applied to a 15% polyacrylamide gel. Gel preparation, electrophoresis conditions, staining and destaining gels were done according to Laemmli (1970).

RAPD-PCR

The randomly amplified polymorphic DNA (RAPD) technique consists in the amplification by polymerase chain reaction (PCR) of random segments of genomic DNA using a single short primer of arbitrary sequence (Callejas and Ochando, 1998).

DNA Extraction

Fish samples were collected for three fishes from each site and their genomic DNA were extracted from fin tissues according to Hillis and Moritz (1990).

Primers

A set of 20 primers was analyzed based on the accurate amplified bands profiles of DNA fingerprinting and only five different primers were selected because of their good RAPD profiles (Table 1).

Amplification reaction mixture

The amplification conditions were conducted based on Williams *et al*. (1990) with some modifications. The reaction was prepared using 25 µl per tube containing 2 µl DNA of each sample (20 µg), One unit of Taq DNA polymerase, 2 µl 10X buffer, 2 µl $MgCl_2$ (25 mM), 2µl dNTPs (2.5 mM of each), 2 µl primer (10 pmol) and 14.8 µl dH_2O.

DNA amplification cycles

The temperature cycling program used with a Perkin-Elmer Gene Amp PCR system (model 2400) was as follows: one cycle at 94°C for 5 min followed by 30 cycles consisting of one step of denaturation (94°C) for 40 sec, one step of annealing (37°C) for 1 min, followed by one step of synthesis (72°C) for 2 min and a final extension step 72°C for 7 min and finally 4°C infinitive (Williams *et al*., 1990).

Band analysis

Reaction products were analyzed by electrophoresis on 1.4% agarose gels stained with ethidium bromide and photographed under UV light. The synthetic DNA, ladder 100 bp (Pharmacia) was employed as molecular markers for bands molecular weight. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Profiles were considered different when at least one polymorphic band was identified. Fragments were scored based on standard marker using Gel Analyzer 3 (Egygene) software.

RESULTS AND DISCUSSION

Heavy metals in water samples

The pollution problems in the surface water either drainage or fresh water affect the quality of fish in polluted areas. The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens and physical or sensory changes such as elevated temperature and discoloration (Burton and Pitt, 2001).

Table (2) summarizes the mean of concentrations of the three heavy metals in water samples which collected from the four sites. Abassa site showed the lowest concentrations of copper, cadmium and lead (0.18, 0.0 and 0.19 ppm, respectively) while, Manzala Lake site showed the highest concentration (16.38, 2.87 and 10.1, respectively). According to the permissible limits of Egypt law 48/1982, the maximum permitted levels for discharge drain water were 0.2, 0.01 and 5 ppm for copper, cadmium and lead, respectively and according those, heavy metals in the water samples of Abassa site were within the permissible limits

Heavy metals in Gill and Muscle samples

Abassa site showed the lowest concentrations of the three heavy metals in gill and muscle while, Manzala Lake site indicated the highest concentrations (Table 2). These concentrations are subject to changes according to the sources of pollution in each site. According to Egyptian Organization for Standardization and Quality (EOSQC, 1993), the maximum permitted levels for fishes were 0.1 ppm for cadmium and lead and 20 ppm for copper. According to EOSQC (1993) copper and cadmium in gill and muscle samples of Abassa site and muscle samples of Mansoura site were within the permissible limits.

Analysis of heavy metals in water samples, Gill and Muscle samples of the four sites under study showed that there was a significant difference of each heavy metal among the four sites including the control. The results revealed that there were differences in various heavy metals in the same site. The present results show that the metal concentrations in fish organs (muscle and gills) of *Oreochromis niloticus* are closely associated with metal content of water in the four sites (Table 2). Adham *et al.* (1999) used fish as bioindicator for assessing metal pollution in Delta Lakes (Maryut and Edku Lakes). The Nile water displayed lower levels of metal contamination compared to Maryut and Edku Lakes. Samir and Ibrahim (2008) reported that water, sediments and fish from Manzala Lake had greater concentration of heavy metals while Mn and Pb recorded levels above the international permissible limits in water. El-Naggar *et al*. (2009) determined the concentration of heavy metals in fish samples collected from River Nile and found that the concentration of Cu, Pb and Cd were at high level in all regions. Bahnasawy *et al*. (2009) determined the concentration of Zn, Cu, Pb and Cd in samples of two fish species (*Mugil cephalus* and *Liza ramada*) from five locations in Manzala Lake and found that the highest concentrations of metals were in gills tissue and the lowest level were in muscles tissue. Elnimr (2011) studied the concentration of heavy metals in fish samples (*Tilapia nilotica* and cat fish) collected from Kafer-El-Zayat and found that the concentration of Pb and Cd were higher than the permissible limits of EOSQC (1993). Lasheen *et al*. (2012) determined Lead, Zinc, Copper and Cadmium in some tissues of sharp toothed

African catfish (*Clarias garipinus*) and Nile tilapia (*Oreochromis niloticus*) collected from two sites and water samples from six sites and found that the concentration of heavy metals in water was within the permissible limits for discharge drain water into River Nile (Egyptian law 48/1982).

SDS-polyacrylamide electrophoresis for water soluble proteins

SDS-polyacrylamide electrophoresis for water-soluble proteins was carried out for nine random samples muscle tissue from each site and were analyzed (Fig. 1). Table (3) shows the number of protein loci and range of their molecular weight (KDa) in samples of the four sites. The fish protein banding pattern from Abassa (control) showed from 17 to 19 protein loci, Mansoura samples showed from 6 to 11 protein loci and by compared it with control we found loss of protein locus 55 KDa in 90% from samples and also loss of protein locus 32 KDa in all samples (Fig. 1B). Maryuot Lake samples demonstrated from 8 to 16 protein loci and by compared it with control we found loss of protein loci (55 and 60 KDa) in 100% of samples (Fig. 1C) could be due to the pollution by Heavy metals and appear new protein locus concerned with resistance of pollution (163 KDa) with ratio 75% which not found in both control or Mansura. Manzalla samples demonstrated from 4 to 14 protein loci which represented the effect of pollution in this site and by compared it with control we found loss of protein locus (60 KDa) in all samples and appear of new protein locus (163 KDa) in 25% of samples (Fig. 1D). Different results which appeared in some of the tested fish from different sites could represent a new genotype for adaptation to pollution.

Protein banding pattern pheno-type in tested fish showed variation in protein bands number, density and mobility, while some bands disappeared from most of the tested fish. Those bands could be representing sensitive genotypes to pollution or as a result of damage in DNA sequence. In Maryout Lake, higher levels of lead, copper and cadmium caused variation in protein of muscles of tested fish could be due to damage in DNA and disappearance of some protein bands. In this study, new genotype bands appeared in Maryout Lake fish samples which could be stressor bands due to genetic environment interaction. These results agree with those of El-Demerdash and Elagamy (1999) who reported that *Oreochromas niloticus* from Maryout lake contained higher concentration of Cd than those from Manzala, with marked different in electrophoresis patterns of protein in both sites. Similarly, Sharf-Eldeen and Abdel-Hamid (2002) mentioned that the exposed fish to copper induced disappearance of some protein fractions and changed relative electrophoresis mobility that indicated genetic damage. Farag (2002) recorded that some protein patterns disappeared while new bands appeared in *O. niloticus* collected from polluted area in Altal Alkaber with high concentration of lead,

copper, zinc and iron. Ibrahim (2004) reported that some protein bands were missing while others appeared as new bands when *O. niloticus* were exposed to different concentration of copper and lead. Ebtehag (2006) reported that fish exposed to heavy metals (Cd, Cu or Zn) at 4 or 2 ppm showed decrease in number of bands compared to the control.

The absence of some bands with respect of their controls from the muscles samples could be occurred due to either changes in the protein synthetic pathways or the depletion of reserve proteins to overcome the stress of heavy metals toxicity and tissues response while the appearance of new bands in muscles samples may be termed as stress proteins which appeared due to the toxic effect of metal pollution (Muthukumaravel *et al.*, 2007)

The protein changes detected in this study represented a broad range of biological responses and thus can be used as potential biomarkers in similar studies. Since muscles appeared to be important organ for metal toxicity and accumulation studies, it could be possible that many protein fractions were degenerated under stressful conditions of these aquatic conditions (Muthukumaravel *et al.*, 2007; Chaudhry and Farhat, 2010).

RAPD-PCR

RAPD analysis method is a simple and sensitive method and appears effective in detecting genetic damages. The molecular weight of fragments after RAPD-PCR reaction with the five primers in the four sites shown in Table (4) and the total number of amplified fragments, number of monomorphic fragments, No. of polymorphic fragments and percentage of polymorphism obtained per RAPD shown in Table (5). A total number of fragments were 55 with average 11 fragments per primer which ranged from 140 to 2323 bp approximately. The total polymorphism was 87.27%. Primers B-17 and B-20 revealed the highest percent of polymorphism (100%), while primer A-05 exhibited the lowest poly-morphism (66.67%). The total poly-morphic fragments were 48 with average 9.6 fragments per primer. Primer B-18 and B-20 produced the highest amplified fragments (13) while primer C-02 produced the lowest fragments (7).

The control (Abassa) recorded the highest value of amplified fragments (42) ranged from 140 to 2323 bp across all the five primers, while Maryout revealed the lowest value of amplified fragments (33) which ranged from 140 to 1315 bp. The oligonucleotide A-05 showed appear of new fragments in Manzala samples only with molecular weight 200, 1296 and 1330 bp and showed also appear of new fragments in both Manzala and Maryout with M.W 342, 375, 610 and 800 bp while there did not found in control (Abassa) (Fig. 2). The oligonucleotide B-17 showed loss of five fragments from Manzala samples compared with control (230, 420, 780, 1190 and 1340 bp) and appears of

(345, 850 and 1000 bp) (Fig. 3). The oligonucleotide B-18 showed loss of only two fragments from Manzala with M.W 140 and 210 bp (Fig. 4). The oligonucleotide B-20 showed loss of 8 fragments from Manzala and Maryout (250, 500, 600, 700, 958, 1054, 1476 and 2323 bp) and loss of one fragment (760 bp) from Maryuot only compared to Abassa control (Fig. 5). The oligonucleotide C-02 showed loss of one fragment (1100 bp) from all sites by compared with control (Fig. 6). New fragments can be amplified because some sites become accessible to the primer after structural changes in the DNA taking place (Bushra *et al.*, 2002; Enan, 2006). This could be due to mutations and/or large rearrangements of the DNA. A single mutation point within the primer site can generate significant changes in RAPD patterns (Walsh and McClelland, 1990).

This difference between samples reflect the pollution in every site in this study and agree with Lasheen *et al.* (2012) who performed RAPD-PCR and DNA fingerprinting on catfish and Nile tilapia fish genome to evaluate the genomic toxicity occurred to fishes obtained from polluted areas compared with those obtained from control one. Zhou *et al.* (2011) studied the effect of nitrofuraznon on *Euplotes vannus* using RAPD-PCR assay and the nitrofurazone treated groups showed difference in RAPD profiles with respect to the band intensity, disappearance of bands and appearance of new bands of amplified DNA.

SUMMARY

The present study used Nile tilapia (*Oreochromis niloticus*) as an biological marker to revealed the concentrations of some heavy metals (Cu, Pb and Cd) in water, gills and muscles of fish collected from four sites in Egypt {Maryuot Lake, Manzala Lake and the River Nile (Mansura)} which are polluted by industrial effluents, waste municipal and agricultural drainage water that is discharged directly into them in addition to Ismailia Canal (Abassa) as control. Analysis of water samples showed that Abassa site was the lowest concentration of copper, cadmium and lead (0.18, 0.0 and 0.2 ppm, respectively) while, Manzala Lake site showed the highest concentration (16.38, 2.87 and 10.1, respectively) and heavy metals in the water samples of Abassa site were within the permissible limits. Abassa site showed the lowest concentrations of the three heavy metals in gill and muscle while, Manzala Lake site indicated the highest concentrations. Copper and cadmium in gill and muscle samples of Abassa site and muscle samples of Mansoura site were within the permissible limits.

Polyacrylamid gel electrophoresis was used to study protein banding pattern variation in tested fish and was showed variation in number of phenotypic bands and the variation between and within each site was clear. Protein locus (55 KDa) in 90% of Mansoura samples and also protein locus (32 KDa) in all samples were missed compared to control Site.

Maryuot Lake samples demonstrated loss of protein loci (55 and 60 KDa) in 100% of samples and appeared a new protein locus (163 KDa) with ratio of 75%. Manzalla samples compared with control were missed protein locus (60 KDa) in all samples and appeared a new protein locus (163 KDa) in 25% of samples. Appear of a new protein locus (163 KDa) in Maryuot and Manzalla Lakes considered as a resistance protein for pollution.

Results of RAPD with the five primers showed that the total No. of fragments were 55 with average 11 fragments which ranged from 140 to 2323 bp approximately. The total polymorphism was 87.27% and the total polymorphic fragments were 48 with average 9.6 fragments per primer. The different band profile (appearance/ disappearance) between polluted fishes and control fishes was shown. Results of RAPD and protein electrophoresis showed that heavy metals were genotoxic to Nile Tilapia fish.

REFERENCES

Adham, K. G., I. F. Hasan, N. Taha and Th. Amin (1999). Impact of hazardous exposure to metals in the Nile and Delta Lakes on the Catfish, Clarias lazera. Environ. Monit. Asses., 54: 107-124.

Ali, F. Kh., A. M. El-Shehawi and M. A. Seehy (2008). Micronucleus test in fish genome: A sensitive monitor for aquatic pollution. African Journal of Biotechnology, 7: 606-612.

Al-Sabti, K. (1991). Handbook of Genotoxic Effects and Fish Chromosomes. Kristoff Ljubljana, Slovenia. pp. 221.

Al-Sabti, K. and C. D. Metcalfe 1995). Fish micronuclei for assessing genotoxicity in water. Mutat. Res., 343: 121-135.

Aras, S., C. Kanlıtepe, D. Cansaran-Duman, M. G. Halici and T. Beyazta (2010). Assessment of air pollution genotoxicity by molecular markers in the exposed samples of *Pseudevernia furfuracea* (L.) Zopf in the province of Kayseri (Central Anatolia). Journal of Environmental Monitoring, 12: 536-543.

Ayllon, F. and V. E. Garcia (2000). Induction of micronuclei and other nuclear abnormalities in European minnow *Phoxinusphoxinus* and mollie *Poecilialatipinna*: an assessment of the micronucleus test. Mutat. Res., 467: 177-186.

Bahnasawy, M., A. Khidr and N. Dheina (2009). Seasonal variations of heavy metals concentrations in Mullet, Mugil Cephalus and Liza Ramada (Mugilidae) from lake Manzala, Egypt. Journal of Applied Sciences Research, 7: 845-852

Badr, E. A. and S. E. El-Dib (1978). Effects of water pollution on the cell division cycle and chromosome behavior in *Tillapia* sp. Egypt. J. Genet. Cytol., 7: 193-200.

Brugs, W. A., J. H. M. Cormick, T. W. Neiheisel, R. L. Spear, C. E. Stephan and G. Stokes (1977). Effect of pollution on fresh water fish. J. Water Pollut. Contr. Fed., 49: 1425-1493.

Burton, G. A and J. R. Pitt (2001). "Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists, and Engineers". New York: CRC/Lewis Publishers. ISBN 0-87371-924-7.Chapter 2.

Bushra, A., M. Abul Farah, M. A. Niamat and A. Waseem (2002). Induction of micronuclei anderythrocyte alterations in the catfish Clarias batrachus by 2, 4-dichlorophenoxyacetic acid and butachlor. Mutat. Res., 518: 135-144.

Cajaraville, M. P., M. J. Bebianno, J. Blasco, C. Porte, C. Sarasquete and A. Viarengo (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci. Total Environ., 247: 295-311.

Callejas, C. and M. D. Ochando (1998) Identification of Spanish barbell species using the RAPD technique. J. Fish Biology, 53: 208-215.

Chaudhry, A. S. and J. Farhat (2010). Assessing metal, protein, and DNA profiles in *Labeo rohita* from the Indus River in Mianwali, Pakistan. Environ. Monit. Assess., 174: 665-679.

Chevreuil, M., A. M. Carru, A. Chesterikoff, P. Boet, E. Tales and J. Allardi (1995). "Contamination of fish from different areas of the riverSeine (France) by organic (PCB and pesticides) and metallic (Cd, Cr, Cu, Fe, Mn, Pb and Zn) micropollutants," Sci. Total Environ., 162: 31-42.

Conners, D. E. and M. C. Black (2004). Evaluation of lethality and genotoxicity in the freshwater muscle *Utterbackiaim becillis* (*Bivalvia: Unionidae*) exposed singly and in combination to chemicals used in lawn care. Arch. Environ. Contam. Toxicol, 46: 362-371.

Dautremepuits, C., S. Betoulle and G. Vernet (2002). Antioxidant responsemodulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (*Cestoda*). Biochim. Biophys. Acta, 1573: 4-8.

Depledge, M. H., A. Aagaard and R. Gyorkos (1995). Assessment of trace metal toxicity using

molecular, physiological and behavioural biomarkers. Mar. Pollut. Bull., 31: 19-27.

Ebtehag, A. K. (2006). Alteration in electrophoretic muscle protein and esterase isozyme as an effect of some heavy metals in two species of *Tilapia*. Egypt. J. Agric. Res., 84(1A).

El-Demerdash, F. M. and E. I. Elagamy (1999). Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. Int. J. Environ. Health Res., 9: 89-97.

El-Ghobashy, H. A., E. A. Kamel and M. E. Farag (2005). Heavy metals and electrophoretic analysis of Nile tilapia (*Orechromis niloticus*) collected from different polluted bodies in Egypt. J. of Egypt. Acad. Soc. Environ. Develop., 2: 181-202

El-Naggar, A. M., S. A. Mahmoud and S. I. Tayel (2009). Bioaccumulation of some heavy metals and histopathological alterations in liver of *Oreochromis niloticus* in Relation to water quality at different localities along the River Nile, Egypt. World J. Fish Marine Sci., 1: 105-114.

Elnimr, T. (2011). Evaluation of some heavy metals in Pangasius hypothalmus and *Tilapia nilotica* and the role of acetic acid in lowering their levels. Egypt. J. Appl. Sci., 8: 151-157.

Enan, M. R. (2006). Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol. Appl. Biochem., 43: 147-154.

EOSQC (Egyptian Organization for Standardization and Quality Control) (1993). Maximum level for heavy metal contamination in food, E.S. No. 2360.

Farag, M. S. (2002). Genetical and physiological studies on fish collected from polluted locations. Ph. D. Thesis. Fac. Sci. Zagazig Univ.

Ghorbani, M. and H. Z. Mirakabad (2010). Factors influencing on tour production in Khorasan Razavi Province. Trends Agric. Econ., 3: 1-18.

Hillis, D. M. and C. Moritz (1990). An overview of applications of molecular systematics. In: Hillis, D. M., Moritz, C., eds. Molecular systematics. Sunderland, Massachusetts: Sinauer Associates, 502-15.

Ibrahim, A. A. (2004). Impact of water pollution on genetics characters of fish. MSc. Thesis. Department of Animal Wealth Development.

Faculty of Veterinary Medicine. Suez Canal University.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lasheen, M. R., F. Abdel-Gawad, A. Alaneny and H. M. H. Abd El bary (2012). Fish as bio Indicators in aquatic environ-mental pollution Assessment: A case study in Abu-Rawash area, Egypt, World Applied Sciences Journal, 19: 265-275.

Muthukumaravel, K., P. Kumarasamy, A. Amsath and G. M. Paulraj (2007). Toxic effect of cadmium on the electrophoretic protein patterns of gill and muscle of *Oreochromis mossambicus*. E-Journal of Chemistry, 4: 284-286.

Pandya, C. B., D. J. Parikh and B. B. Chatterjee (1985). Accumulation and interrelationship of calcium and zinc in human kidney cortex. Environ. Res., 36: 81-88.

Park, E., J. Lee and H. Etoh (1993). Fish cell line (ULF-23HU) derived from the fin of the central mudminnow (*Umbra limi*): suitable characteristics for clastogenicity assay. *In Vitro* Cell Dev. Biol., 25: 987-994.

Qi, X. M., P. J. Li, W. Liu and L. J. Xie (2006). Multiple biomarkers response in maize (*Zea mays* L.) during exposure to copper. Journal of Environmental Sci., 18: 1182-1188.

Rashed, M. A., S. A. Ibrahim, A. A. El-Seoudy, F. M. Abdel-Tawab and Gehan E. Ahmed (2007). Effect of pollution with organo phosphoros insecticide Tamaron on muscle protein electrophoresis to some tilapia species. Egypt. J. Appl. Sci., 7: 498-510.

Samir, M. S. and M. S. Ibrahim (2008). Assessment of heavy metals pollution in water and sediment and their effect on *Oreochromis niloticus* in the Northern Delta lakes, Egypt. 8th International Symposium on Tilapia in Aquaculture, 475-490

Sharf-Eldeen, K. and N. Abdel-Hamid (2002). Sublethal effects of copper sulphate.Malthion and paraquat on protein pattern of *Oreochromas niloticus*. Egypt. J. Aquat. Biol. Fish., 6: 167-182.

Walsh, J. and M. McClelland (1990). Genomic fingerprinting using arbitrary primed PCR and a matrix of pairwise combination of primers. Nucleic Acids Research, 18: 7213-18.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Xiaolin, Z., Z. Zongyao, Z. Xuxiang, W. Z. Bing, Y. Liuyan and C. Shupei (2009). Effects of Yangtze River source water on genomic polymorphisms of malemice detected by RAPD. Human and Experimental Toxicology, 10: 1-8.

Yu, R. A. (2000). Cadmium and DNA damage, oncogene ex-pression as well as cellapoptosis. Overseas Medicine Hygienics Fascicule, 27: 359-363.

Zhou, L., J. Li, X.Lin and Kh. Al-Rasheid (2011). Use of RAPD to detect DNA damage induced by nitrofurazone in marine ciliate, *Euplotes vannus* (Protozoa, Ciliophora). Aquatic Toxicology, 103: 255-428.

Table (1): The sequence of primers enter the RAPD-PCR reaction

Code number	Sequence
A-05	AGG GGT CTT G
B-17	AGG GAA CGA G
B-18	CCA CAG CAG T
B-20	GGA CCC TTA C
C-02	GTG AGG CGT C

Table (2): Mean concentration (± Sd) of heavy metals (ppm) in water, gill and muscle samples.

Sites	Water			Gill			Muscle		
	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd
Abassa	0.18± 0.52	0.19± 4.35	0.0± 2.37	0.38± 0.46	0.16± 0.02	0.0± 0.0004	2.00± 0.003	1.10± 0.18	0.02± 0.26
River Nile	1.10± 1.07	0.20± 2.15	0.04± 4.09	2.02± 1.73	4.30± 0.62	3.10± 0.89	2.40± 0.07	20.50± 0.27	14.20± 0.74
Maryot	2.80± 2.71	1.20± 3.32	0.51± 3.42	5.80± 2.70	5.01± 1.93	4.14± 2.24	13.85± 0.46	36.14± 0.62	7.41± 1.64
Manzala	16.38± 3.44	10.10± 3.53	2.87± 5.97	22.0± 8.86	53.10± 12.96	30.20± 17.06	47.80± 7.99	50.21± 4.82	10.20± 0.93

Table (3): Number of protein loci and range of their molecular weight (KDa) in the four sites samples.

No. of samples	Abassa		Mansoura		Maryout		Manzala	
	No. of protein loci	Range of MW (KDa)	No. of protein loci	Range of MW (KDa)	No. of protein loci	Range of MW (KDa)	No. of protein loci	Range of MW (KDa)
1	18	21-298	8	21-305	8	21-245	12	21-234
2	19	21-230	9	21-305	15	21-245	14	21-234
3	19	21-298	9	21-305	16	21-245	5	21-234
4	18	21-298	6	21-305	12	34-245	4	34-234
5	17	21-298	11	21-305	13	21-163	4	21-95
6	17	21-298	6	21-96	13	21-217	7	21-160
7	18	21-298	11	21-305	11	21-163	8	21-95
8	19	21-298	9	21-305	11	21-245	5	21-207
9	17	21-298	7	21-305	14	21-245	5	21-95

Table (4): Molecular weight of fragments after RAPD-PCR reaction with the five primers in the four sites.

Primer	No. of Band	MW	Abassa			Mansoura			Maryout			Manzala		
			No. of Replica			No. of Replica			No. of Replica			No. of Replica		
			1	2	3	1	2	3	1	2	3	1	2	3
A-05	1	1330	-	-	-	-	-	-	-	-	-	+	+	+
	2	1296	-	-	-	-	-	-	-	-	-	+	+	+
	3	1040	-	+	-	-	+	-	+	+	+	+	+	+
	4	800	-	-	-	-	-	+	+	+	+	+	+	+
	5	700	+	+	+	+	+	+	+	+	+	+	+	+
	6	610	-	-	-	-	-	-	+	+	+	+	+	+
	7	510	+	+	+	+	+	+	+	+	+	+	+	+
	8	425	+	+	+	+	+	+	+	+	+	+	+	+
	9	375	-	-	-	-	-	-	+	+	+	+	+	+
	10	342	-	-	-	-	-	-	-	+	-	+	+	+
	11	304	+	+	+	+	+	+	+	+	+	+	+	+
	12	200	-	-	-	-	-	-	-	-	-	+	+	+
B-17	1	1340	+	+	+	+	+	+	-	-	-	-	-	-
	2	1190	+	+	+	+	+	+	-	-	-	-	-	-
	3	1000	-	-	-	+	-	-	-	+	-	+	-	+
	4	850	-	-	-	-	-	-	-	-	-	+	+	+
	5	780	+	+	+	+	+	+	+	+	+	-	-	-
	6	650	-	-	+	-	-	-	-	-	-	+	+	+
	7	550	+	+	-	+	+	+	+	+	+	+	+	+
	8	420	+	+	+	+	+	+	-	-	-	-	-	-
	9	345	-	-	-	-	-	-	-	-	-	+	+	+
	10	230	+	+	+	+	+	+	+	-	-	-	-	-
B-18	1	974	-	-	+	-	-	+	-	-	+	-	+	+
	2	760	+	+	+	+	+	-	-	-	+	-	+	+
	3	700	+	+	+	+	+	+	+	+	+	+	-	-
	4	530	+	+	+	-	+	+	+	+	+	-	+	+
	5	500	+	+	+	+	-	-	-	-	-	+	-	-
	6	420	+	+	+	+	+	+	-	-	-	+	+	+
	7	370	+	+	+	+	+	+	+	+	+	+	+	+
	8	340	-	-	-	+	+	+	-	+	-	-	-	-
	9	290	+	+	+	+	-	+	+	+	+	+	+	+
	10	250	-	-	-	+	-	+	-	+	+	-	-	-
	11	210	+	-	+	+	+	+	-	+	+	-	-	-
	12	190	+	-	-	+	+	+	+	+	+	+	+	+
	13	140	+	-	-	+	-	+	-	+	+	-	-	-
B-20	1	2323	+	+	+	-	-	-	-	-	-	-	-	-
	2	1476	+	+	+	+	+	+	-	-	-	-	-	-
	3	1315	+	+	+	-	-	-	+	+	+	+	+	+
	4	1171	+	+	+	+	+	+	-	-	-	-	-	+
	5	1054	+	+	+	+	+	+	-	-	-	-	-	-
	6	958	+	+	+	+	+	-	-	-	-	-	-	-
	7	760	+	+	-	+	+	+	-	-	-	+	+	+
	8	700	-	+	+	+	+	+	-	-	-	-	-	-
	9	600	+	+	+	-	-	-	-	-	-	-	-	-
	10	550	-	+	-	+	+	+	+	+	+	+	+	+
	11	500	+	+	+	-	-	+	-	-	-	-	-	-
	12	290	+	+	+	+	+	+	+	+	+	+	+	-
	13	250	+	-	+	-	-	-	-	-	-	-	-	-
C-02	1	1100	+	+	+	-	-	-	-	-	-	-	-	-
	2	950	-	-	-	-	-	-	+	+	+	-	-	-
	3	840	+	+	+	+	+	+	+	+	+	+	+	+
	4	600	+	+	+	+	+	+	-	+	+	+	+	+
	5	500	+	+	-	+	-	+	+	-	-	+	-	-
	6	450	+	+	+	-	+	+	+	+	+	-	+	+
	7	300	+	+	+	+	+	+	+	+	+	+	+	+

Table (5): The Polymorphism in fragment size after RAPD-PCR reaction with the five primers in the four sites.

Primers	Range of fragment size (bp)	Abassa	Mansoura	Maryout	Manzala	Total no. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %
A-05	200-1330 bp	5	6	9	12	12	4	8	66.67%
B-17	230-1340 bp	7	7	4	5	10	0	10	100.00%
B-18	140 - 974 bp	11	13	11	9	13	1	12	92.31%
B-20	250-2323 bp	13	9	3	5	13	0	13	100.00%
C-02	300-1100 bp	6	5	6	5	7	2	5	71.43%
Total	140-2323 bp	42	40	33	36	55	7	48	87.27%
Average		8.4	8	6.6	7.2	11	1.4	9.6	

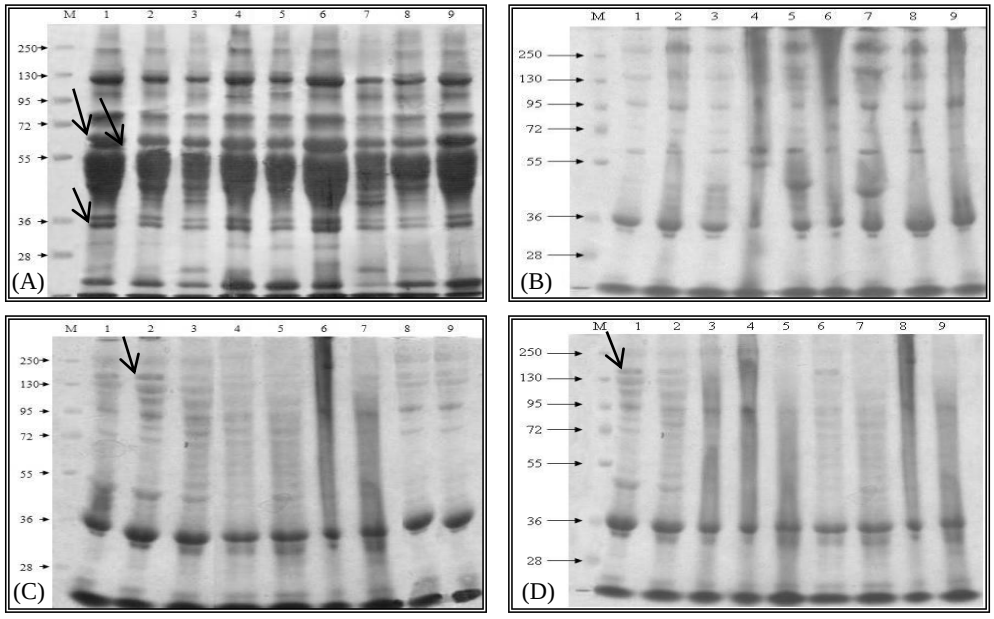

Fig. (1): SDS-polyacrylamide gel electrophoresis of protein loci for Nile tilapia collected from the four sites.
(A): Abassa (B): Mansoura (C): Maryout (D): Manzala

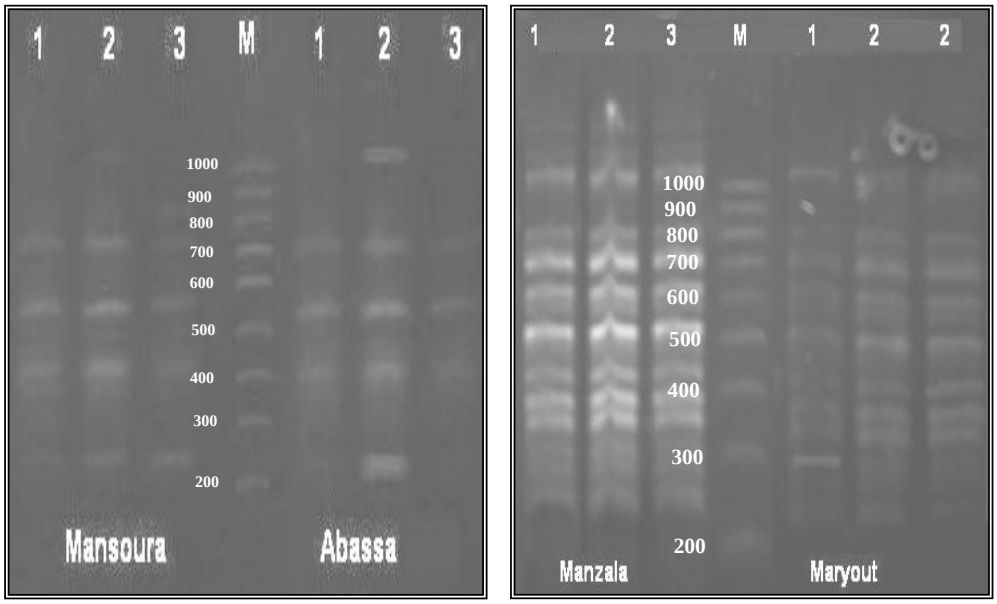

Fig. (2): RAPD-PCR DNA with primer (A-05) for Nile tilapia collected from the four sites. Lane M: Marker.

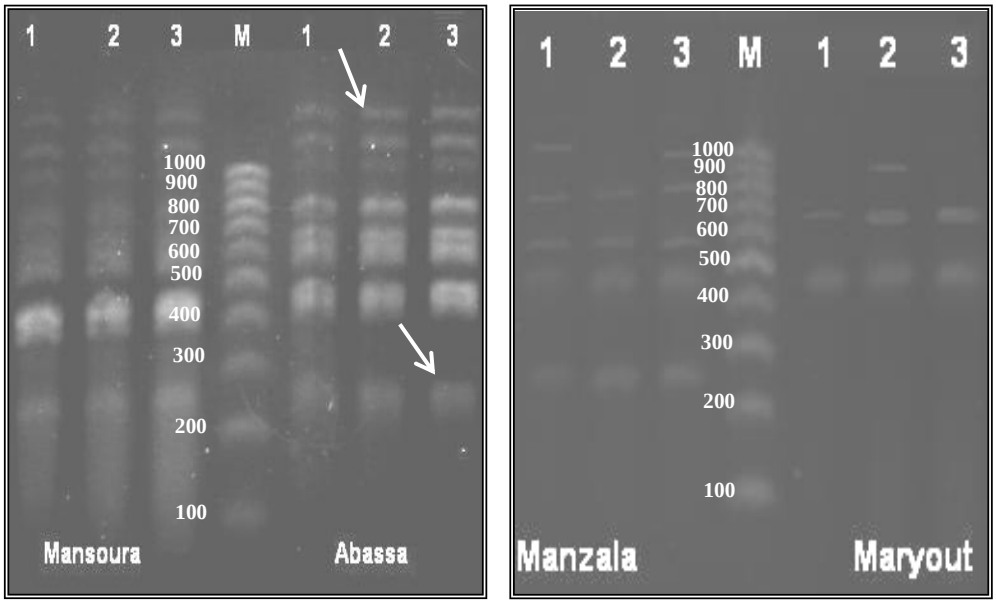

Fig. (3): RAPD-PCR DNA with primer (B-17) for Nile tilapia collected from the four sites. Lane M: Marker.

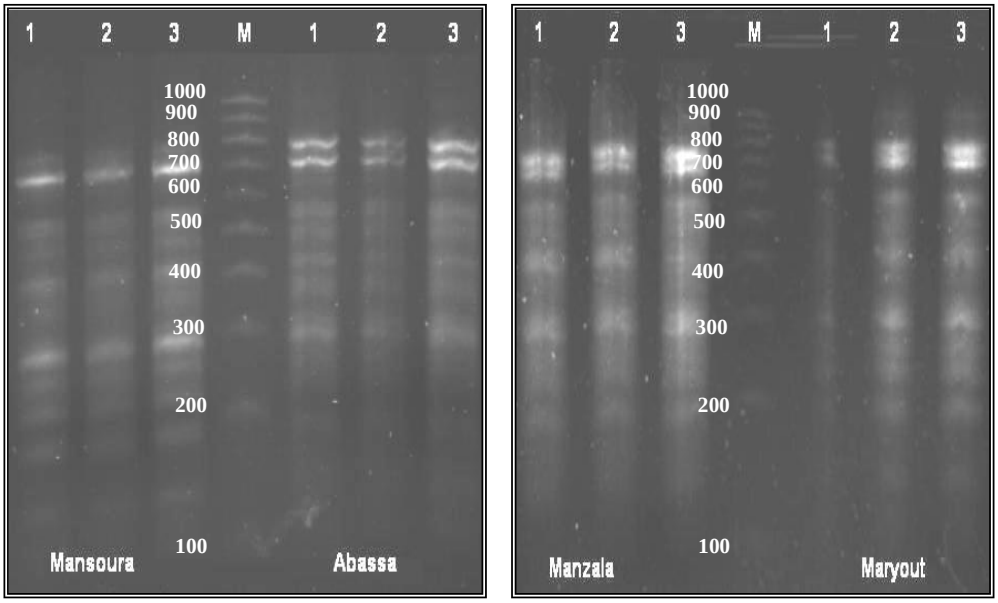

Fig. (4): RAPD-PCR DNA with primer (B-18) for Nile tilapia collected from the four sites. Lane M: Marker.

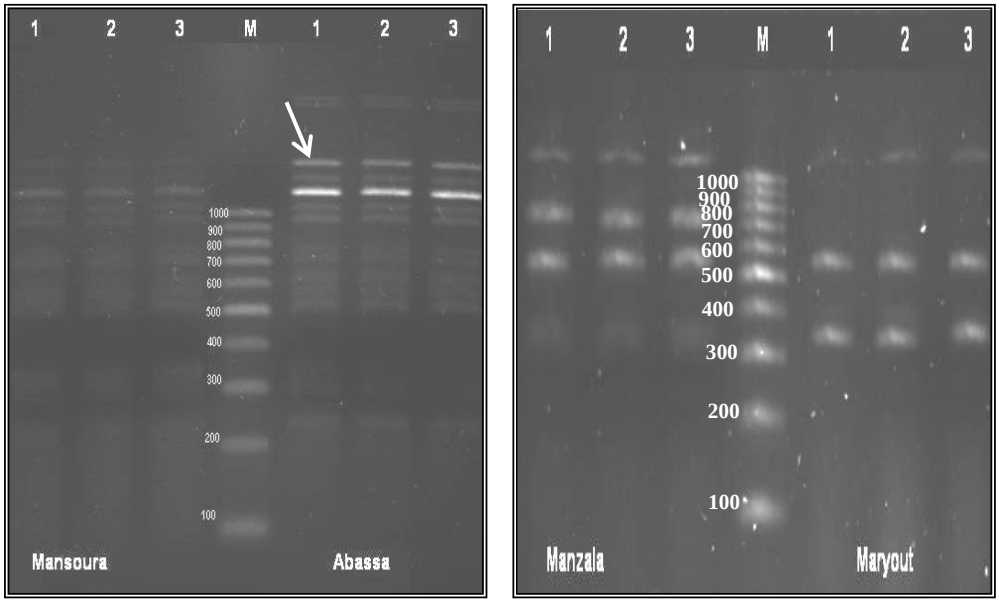

Fig. (5): RAPD-PCR DNA with primer (B-20) for Nile tilapia collected from the four sites. Lane M: Marker.

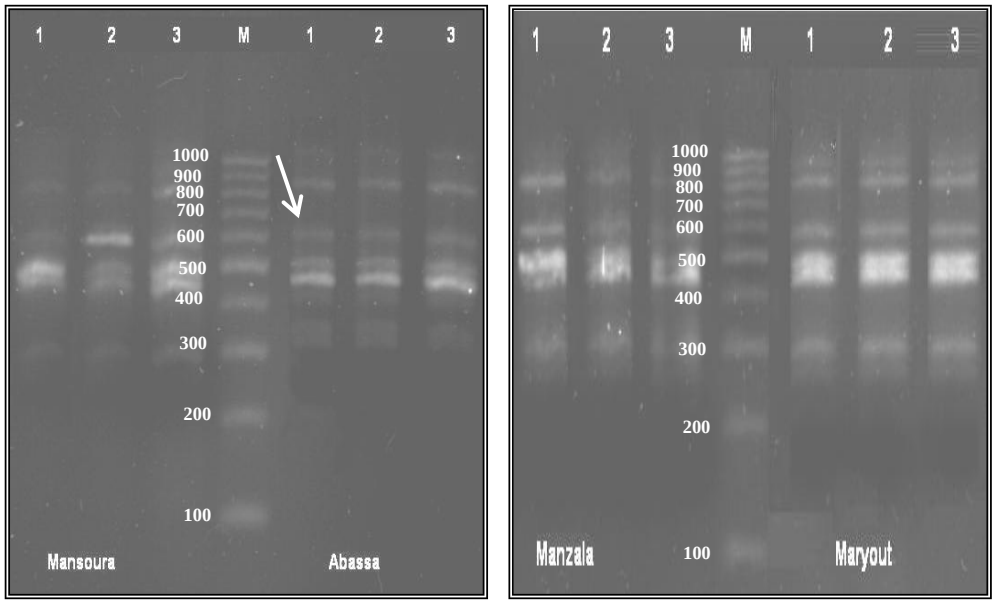

Fig. (6): RAPD-PCR DNA with primer (C-02) for Nile tilapia collected from the four sites. Lane M: Marker.