International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2017.54.63

Research Article
Health Status and Genotoxic Effects of Metal Pollution in *Tilapia zillii* and *Solea vulgaris* from Polluted Aquatic Habitats

Mamdouh Yousif Elgendy, Iman Kamel Abumourad, Shimaa Elsayed Mohamed Ali, Waleed Salah El-Din Soliman, Taghreed Borhan El-Din Ibrahim and Wafaa Tawfik Abbas

Department of Hydrobiology, Veterinary Research Division, National Research Centre, 33 El Bohouth St. Dokki, P.O. Box 12622, Giza, Egypt

Abstract

Background and Objective: Fish health and aquatic environment are strongly interrelated. Aquatic environment receive array of anthropogenically derived chemicals with deleterious health effects on the cohabitant aquatic animals and threatens their lives. The aim of this study was to evaluate the health status of *Tilapia zillii* and *Solea vulgaris* captured from Lake Qarun, Egypt known to receive lofty loads of contaminants as well as to monitor the genotoxic effects of some heavy metals detected in lake water on the two fish species. **Methodology:** Heavy metals (Cu, Zn, Cd, Ni and Pb) were assessed in water and fish specimens (*Tilapia zillii* and *Solea vulgaris*) collected from Lake Qarun and the mean value was recorded. Fish and water samples were subjected to microbiological and parasitological examination. Additionally, genotoxicity was analysed using comet assay. **Results:** Heavy metals; Zn, Ni and Cu showed high values in water exceeding the recommended limits. Accumulation of heavy metals was noticed in fish muscles and Ni was the uppermost detected metal. Genotoxicity was confirmed in fish liver cells by comet assay. The DNA damage was quantified as; tail length, DNA percentage in tail, tail moment and number of cells with tail. Genotoxicity was severe in *Solea vulgaris* than *Tilapia zillii*. Bacterial infections; *Vibrio alginolyticus* (21 isolate), *Aeromonas hydrophila* (13), *Photobacterium damselae* subsp. *piscicida* (7) were detected in fish specimens. Parasitic *Isopoda* sp. were found attached to the ventral body surface and inside the gill chamber of fish. **Conclusion:** The results of this study indicate that Lake Qarun is contaminated with substances that are genotoxic to fish as well as predispose fish to numerous opportunistic pathogens.

Key words: *Tilapia zillii*, *Solea vulgaris*, heavy metals, microbial infections, genotoxicity

Received: November 17, 2016 Accepted: January 20, 2017 Published: February 15, 2017

Citation: Mamdouh Yousif Elgendy, Iman Kamel Abumourad, Shimaa Elsayed Mohamed Ali, Waleed Salah El-Din Soliman, Taghreed Borhan El-Din Ibrahim and Wafaa Tawfik Abbas, 2017. Health status and genotoxic effects of metal pollution in *Tilapia zillii* and *Solea vulgaris* from polluted aquatic habitats. Int. J. Zool. Res., 13: 54-63.

Corresponding Author: Mamdouh Yousif Elgendy, Department of Hydrobiology, Veterinary Research Division, National Research Centre, 33 El Bohouth St. Dokki, P.O. Box 12622, Giza, Egypt Tel: 00201116893637

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquatic animal's health and environmental conditions are strongly interrelated[1,2]. Aquatic habitats provide a suitable medium for the existence as well as transmission of numerous detrimental bacteria, viruses, fungi and some parasitic infestations ultimately posing serious fish health risks concomitantly with the existence of unfavorable environmental conditions[3,4].

Additionally, aquatic ecosystem is exposed simultaneously to array of anthropogenically derived chemicals with profound health effects on the cohabitant aquatic animals and can put their lives in danger[5,6]. Tremendous discharges of industrial, agricultural and domestic wastes into the aquatic habitats has long been considered as serious concern for fish as well as public health due to consumption of fish from these water bodies[7]. Along the leading toxic aquatic contaminants, heavy metals are renowned[8]. Lakes and small reservoirs are deteriorated more frequently by heavy-metal contamination due to human interference compared with rivers[9]. Lake Qarun represents an example of such lakes, since it receives many pollutants. Trace amounts of heavy metals are vital to living organisms conducting fundamental biological processes in the metabolic pathway[10]. However, chronic exposure to such metals in their living habitat may cause persistent metal bioaccumulation in tissue ultimately, with genotoxic impacts on fish[5].

Heavy metals have long been considered as one of the chief group of elements inducing DNA damage[11]. Their role in triggering genotoxicity in fish has been reported in earlier studies[12]. These metals can provoke aberrant gene expression, cellular transformation, gene amplification, DNA strand breakage and DNA-protein cross-links[13-16]. Metals can induce direct or indirect DNA damage, along with synergistic activity[17]. One major pathway involved in such genotoxicity is the production of reactive oxygen species resulting in oxidative modifications of DNA. Formation of hydroxyl radicals may lead to base alteration and DNA strand-breaks[18]. Several factors can influence metal toxicity such as; pH, alkalinity, temperature, oxygen and hardness[19,20]. Heavy metals in mixture are more toxic to fish than separate ones[21]. Investigation of DNA alterations in aquatic organisms is highly suitable biomarker for evaluating the genotoxic effects of such contaminants since it can detect exposure to low levels of pollutants[22]. The comet assay or single cell gel electrophoresis has been shown to be a simple and sensitive approach for the evaluation of genotoxicity leading to DNA damage in fish exposed to various contaminants in their environment[22].

Tilapia zillii and *Solea vulgaris* are two commercially important fish species with worldwide distribution. *Tilapia zillii* is highly tolerable species commonly found in brackish and marine waters. *Tilapia zillii* generally prefer shallow and vegetated areas. *Tilapia zillii* is omnivorous with juveniles being more carnivorous. Adults are especially voracious herbivores. This species have been used for aquaculture, weed and mosquito control[23]. On the other hand *Solea vulgaris* is considered as a demersal fish with high commercial value. It live buried in sandy and muddy bottoms. It feeds on polychaete worms, mollusks and small crustaceans[24]. The present work aimed to investigate the health status of *Tilapia zillii* and *Solea vulgaris* collected from Lake Qarun as well as to assess the genotoxic effects of detected heavy metals in fish using comet assay.

MATERIALS AND METHODS

Area of study and sampling: Lake Qarun settles about 45 m below the sea level into the lowest, Northern section of El-Fayoum Depression, Egypt. It has approximately 40 km average length from East to West, about 6.7 km mean breadth from the North to South with a mean depth of 4.2 m. The lake has a surface area of 243.4 km^2 and a volume of 924 million m^3. Lake Qarun was initially nominated as protected area back in 1989, however, nowadays it suffers from severe water pollution problems from uncontrolled solid and liquid domestic and industrial waste disposal practices, in addition to agrochemical contamination and lack of sustainable wastewater management. Many fish farms were established around this Lake[25]. The lake receives huge quantities of agricultural drainage water and raw sewage from El-Fayoum depression, through two main drains, El-Batts and El-Wadi and 11 small drains in addition to two pump stations (Main Pump Station and Khor Alhitan Pump Station).

Total number of 60 fish specimens; 30 of each; *Tilapia zillii* (100-130 g) and *Solea vulgaris* (90-125 g) were collected by fishermen in August, 2015. Specimens were transported alive in fiberglass container having seawater from the lake and supplied with oxygen, within the minimum time of delay, to the Department of Hydrobiology, National Research Centre, Egypt. The procedures have been approved by National Research Centre Ethic Committee. Additionally, three subsurface water samples were collected from the Eastern part of the lake at Shakshok region from different three points around the selected site (20 m apart from each other).

Physiochemical water analysis: Temperature, pH, dissolved oxygen and salinity were measured on spot at collection site

by digital apparatus, YSI (Yellow Springs, Ohio USA). Regarding detection of heavy metals, water samples were acidified by concentrated nitric acid (5 mL L^{-1}) and heavy metals (Cu, Zn, Cd, Ni and Pb) were detected by atomic absorption spectrophotometer (Perkin-Elmer 3110, USA)[26].

Analysis of heavy metals in fish specimens: Fish were dissected; muscles were isolated, weighed, put in glass vials and digested in concentrated super pure nitric acid (Merck, Darmstadt, Germany). Then placed on a hot plate at 100°C. After complete digestion, the samples were cooled in room temperature, filtered and finally a distilled pure water was added to each sample to reach a volume of 10 mL. Heavy metal concentrations (Cu, Zn, Cd, Ni and Pb) were determined by atomic absorption spectrophotometry (Perkin-Elmer 3110, USA)[26]. The determined concentration was expressed as mg kg^{-1} wet weight.

Accumulation Factor (AF): Accumulation factor (AF) was calculated according to Eq. 1[27]:

$$AF = \frac{\text{Concentration of heavy metal in organ (mg kg}^{-1})}{\text{Concentration of heavy metal in water (mg L}^{-1})} \quad (1)$$

Single cell gel electrophoresis (comet assay): The DNA damage was assessed using alkaline comet assay according to Fatima *et al.*[5] and Singh *et al.*[28]. Fresh liver samples were washed with chilled Phosphate Buffered Saline-Calcium Magnesium Free (PBSCMF). Liver tissues were minced with PBS-CMF 20 mM Ethylene Diamine Tetra acetic Acid (EDTA) and 10% dimethyl sulfoxide (DMSO) at pH 7.4 and filtered through a 100 mL mesh strainer. Tissues were collected in a tube, 2 mL of PBS-CMF was added then centrifuged at 2,000 rpm at 4°C for 10 min. Cell pellet was collected and resuspended in PBS-CMF. Cell-viability test was performed using the trypan blue exclusion method[29] and samples showing viability (80%) were considered for comet assay) Cell suspensions were suspended in 0.5% Low Melting Point Agarose (LMPA) overlaid on slides pre-coated with a fine layer of 1.25% normal-melting agarose. A third layer of 0.75% LMPA was poured and slides were immersed in lysing solution [2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, 0.2 mM NaOH, 1% Triton X-100 and DMSO (pH 10)] for 1 h at 4C. then by electrophoresis buffer [300 mM NaOH, 1 mM EDTA (pH[13)] for 20 min. Electrophoresis was performed at 25 V and 300 mA current in the same buffer for 30 min. Slides were neutralized with neutralizing buffer [0.4 M Tris buffer (pH 7.5)]. Slides were dried and stained with ammoniacal silver nitrate solution. Photographs were obtained at 400X. Thirty fish per species were analyzed and 50 cells were scored randomly and analyzed under an optical microscope at 400X magnification. The cells were scored visually according to tail length.

Bacteriological examination: Loopfuls from liver and kidneys were aseptically obtained from *Tilapia zillii* and *Solea vulgaris* fish specimens. Inoculi were further enriched in tryptic soy broth supplemented with 1.5% NaCl then smeared onto Tryptic soy agar TSA (Oxoid) supplemented with 1.5% NaCl. Plates were incubated at 25°C for 24-48 h. Water samples were also analyzed microbiologically. Randomly selected colonies from each plate were purified and further identified using API 20 E according to Buller[30].

Mycological examination: Fish were examined macroscopically for any specific signs of fungal infections. Isolation from skin, internal organs and water was performed on Glucose-Yeast (GY) agar. Salt was incorporated into the medium at a concentration of 3.5 g L^{-1} to inhibit bacterial growth, chloramphenicol (200 μg mL^{-1}) was added to the medium[31]. Spread plate technique was applied for fungal analysis in water samples. Samples were tested in triplicates. Each plate was streaked with 0.5 mL of water sample. All cultured samples were incubated at 20-25°C up to one week.

Parasitological examination: Fish samples were thoroughly investigated for external parasites by visual inspection. Furthermore, wet smears from the gills and skin were freshly examined, fixed with methanol, stained by 10% Gimsa stain and investigated microscopically to identify the presence of any external protozoan parasites. Moreover, gills, liver and fish muscles were also examined for the presence of encysted metacercariae under the binocular dissecting microscope (Labomed, Labo America, Inc., USA)[32].

Statistical analysis: All data were represented as Mean±Standard Deviation.

RESULTS

Clinical examination: Investigated fish demonstrated blacking of the body color with excess mucus secretions. Some showed hemorrhagic patches widely distributed on the external body surfaces with erosions, scale detachment and anal prolapse. Gills were pale with copious mucus secretions with erosions as well as complete absence of gill cover in some fish. Internally, congestion and enlargement of liver was commonly detected while it was pale in some others (Fig. 1).

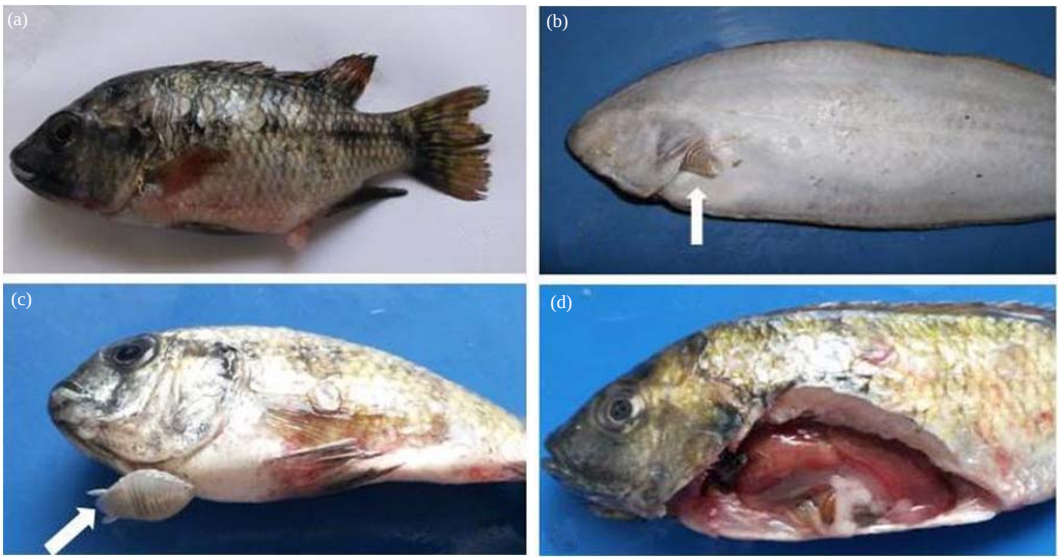

Fig. 1(a-d): (a) *Tilapia zillii* showing scale detachment, anal prolapse and hemorrhages on the ventral body surface, (b) *Solea vulgaris* fish showing parasitic *Isopoda* sp. in the gill chamber (white arrow), (c) *Tilapia zillii* fish showing parasitic *Isopoda* sp. on the ventral body surface (white arrow) and (d) *Tilapia zillii* showing congestion and enlargement of liver

Table 1: Heavy metals analysis in water and fish muscle samples compared with some international guidelines

Elements	Concentration in water samples (ppm)	Concentration in muscle samples (ppm)		International guidelines	
		Tilapia zillii	*Solea vulgaris*	USEPA[33] in salt water (ppm)	IAEA Wyse *et al.*[42] in fish tissue (ppm)
Zn	1.730±0.170	9.46±0.63 (5.47)	12.146±1.2 (7.02)	0.081	67.1
Fe	0.310±0.015	6.69±1.3 (22.3)	7.154±1.6 (23.85)	na	146
Ni	0.681±0.200	18.976±2.6 (27.86)	24.58±5.3 (36.09)	0.0082	0.60
Cd	nd	0.03±0.01 (-)	0.042±0.008 (-)	0.0079	0.189
Pb	nd	0.1±0.002 (-)	0.187±0.01 (-)	0.0081	0.12
Mn	0.007±0.001	1.584±0.13 (226.29)	0.734±0.03 (104.86)	0.0500	3.52
Cu	0.048±0.003	1.855±0.05 (38.65)	0.147±0.004 (3.06)	0.0031	3.28

Data are shown as mean value ± SD (bioaccumulation factor), nd: Not detected, na: Not available

Physiochemical water analysis: The average values recorded for water quality measures are illustrated in Table 1. Dissolved oxygen was considerably low 3.4 mg L^{-1}. Concentrations of heavy metals demonstrated variable values; Zn, Ni and Cu showed high levels that exceed the permissible limits of salt water; 1.73, 0.681 and 0.048 ppm, respectively; according to values recommended by the Environmental Protection Agency[33]. On the other hand, Mn and Fe were within the permissible limits, while, Cd and Pb were not detected (Table 1).

Heavy-metal bioaccumulation in fish muscles: All heavy metals showed accumulation in fish muscles where Ni was the most accumulated metal in both *Tilapia zillii* and *Solea vulgaris*. Despite Cd and Pb were not detected in water samples they demonstrated detectable levels in fish muscles. The pattern of metal bioaccumulation in *Tilapia zillii* muscle was found to be; Ni>Zn>Fe>Cu>Mn>Pb>Cd. While, *Solea vulgaris* showed; Ni>Zn>Fe>Mn>Pb>Cu>Cd (Table 1). Interestingly, *Solea vulgaris* muscles showed higher metal accumulation in almost all the detected metals (Ni, Zn, Fe, Pb and Cd). Contrary, Cu and Mn were higher in *Tilapia zillii* muscle.

Comet assay: The DNA damage in *Tilapia zillii* and *Solea vulgaris* fish collected from Lake Qarun is given in Table 2. *Solea vulgaris* showed a greater level of genotoxic damage than did *Tilapia zillii.* Tail length, DNA percentage in

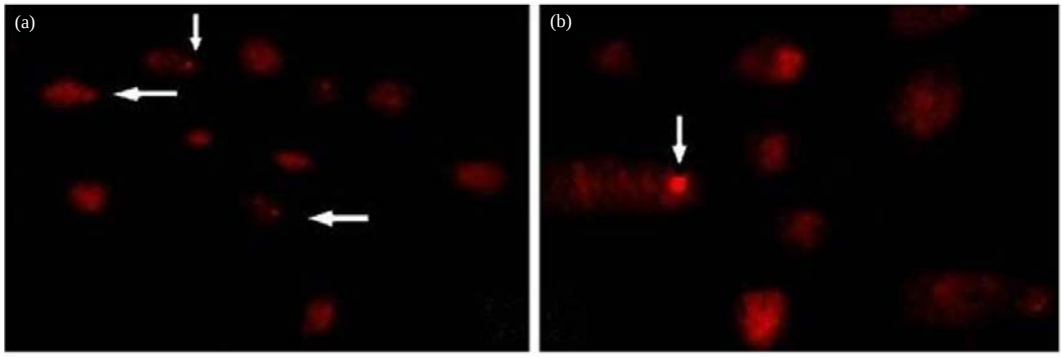

Fig. 2(a-b): Representative of comet assay in fish liver cells, (a) *Tilapia zillii* and (b) *Solea vulgaris* showing large number of damaged DNA, white arrows pointing to the comet cells

Table 2: DNA damage index estimated by comet assay in *Tilapia zillii* and *Solea vulgaris*

Fish species	Sample no.	Tail length (px) Mean±SD	DNA in tail (%)	Tail moment	Tail intensity	Cells with tail (%)
T. zillii	30	13.2±0.37	29.85±0.61	8.90±0.97	5456.52±0.13	4.86±0.03
Solea vulgaris	30	21.8±0.16	36.99±0.84	8.90±0.97	16219.04±0.07	18.13±0.09

Data are shown as Mean±SD. 1 px is equal to 0.24 µm for unit conversion

Table 3: Prevalence of bacterial infections in examined *T. zillii* and *Solea vulgaris* fish

			No. isolates		*V. alginolyticus*		*A. hydrophila*		*P. piscicida*	
Fish species	No. examined	No. infected	No.	(%)	No.	(%)	No.	(%)	No.	(%)
T. zillii	30	15	23	56.09	12	29.26	8	19.51	3	7.31
Solea vulgaris	30	11	18	43.9	9	21.95	5	12.19	4	9.75
Total	60	26	41	-	21	51.21	13	31.7	7	17.07

Percentage was calculated according to the total number of retrieved isolates (41)

tail, tail moment, tail intensity and number of cells with tail in *Tilapia zillii* were; 13.2 px, 29.85%, 8.90, 5456.52 and 4.86%, respectively while it was 21.8 px, 36.99%, 8.90, 16219.04 and 18.13% in *Solea vulgaris*, respectively (Fig. 2).

Bacteriological examination: Total number of 41 bacterial isolates was obtained from investigated fish specimens; 23 isolates from *Tilapia zillii* and 18 isolates from *Solea vulgaris*. Retrieved isolates were further identified as; *Vibrio alginolyticus* (21 isolate), *Aeromonas hydrophila* (13), *Photobacterium damselae* subsp. *piscicida* (*P. piscicida*) (7) (Table 3). Additionally, *Vibrio alginolyticus and Aeromonas hydrophila* were also detected in water samples.

Mycological examination: External examination of collected fish did not reveal any specific signs of fungal infection. Additionally, no fungal growth was observed in all plates containing fish samples. However, orange/red moist colonies of the unicellular yeast *Rhodotorula* sp. were detected in all water samples.

Parasitological examination: Parasitological examination of *Tilapia zillii* and *Solea vulgaris* showed no parasitic infestations except for crustacean *Isopoda* sp. family Cymothoidae (Fig. 1b, c). *Isopoda* sp. infections were more prevalent in *Tilapia zillii* (20%) than *Solea vulgaris* (13.3%). Parasites were found attached to the ventral body surface near the pectoral fin. In some cases, parasitic isopods were also noticed inside the gill chamber.

DISCUSSION

Water analysis revealed high concentrations of some heavy metals in Lake Qarun exceeding permissible limits. The Ni, Zn and Cu were detected in higher levels; 1.73±0.17, 0.681±0.2 and 0.048±0.003 ppm, respectively. These values exceed the salt water guidelines established by the Environmental Protection Agency[33]. The marine high reliability trigger values (95% protection) recommended by ANZECC and ARMCANZ[34] for Cu, Cd, Ni and Pb are; 1.3, 5.5, 70 and 4.4 µg L^{-1} respectively. Additionally, the toxicant guidelines for Fe, Cu, Cd, Ni and Pb should be less than 10, 5, 0.5-5, 100 and 1-7 µg L^{-1}, respectively as recommended for protection of saltwater aquaculture. The surplus discharges from agriculture drainage, sewage as well as various industrial effluents

Table 4: Comparison of heavy metals average concentrations (ppm) in water and fish muscle samples in the present study and other previous studies in Lake Qarun

Parameters	Zn	Fe	Ni	Cd	Pb	Mn	Cu	References
Water	1.730	0.310	0.681	nd	nd	0.007	0.048	Present study
	0.020	0.460	0.010	0.020	0.020	0.290	0.240	Mansour and Sidky[25]
	0.029-0.050	0.33-0.600	0.039-0.044	0.0016-0.002	0.085-1.000	0.039-0.078	0.036-0.057	Fishar and Ali[38]
	0.096-0.180	nm	nm	nm	nm	nm	1.250-2.930	Authman and Abbas[39]
	0.016-1.400	nm	0.036-0.150	0.0001-0.048	nm	nm	0.001-0.030	Hussein *et al.*[69]
	0.626	0.346	nm	nm	0.384	0.051	0.040	Mohamed and Gad[70]
	0.064-0.085	0.434-0.733	nm	0.010-0.077	0.066-0.400	0.031-0.131	0.021-0.056	Abdel-Satar *et al.*[40]
	0.024-0.084	0.15-0.300	nm	0.006-0.011	0.128-0.170	nm	0.010-0.018	Abdel-Latif[66]
	0.081	na	0.0082	0.0079	0.0081	0.050	0.0031	USEPA[33]
Fish (sp.) muscle								
Tilapia zillii	1.584	0.030	0.100	1.855	9.460	6.690	18.976	Present study
Solea vulgaris	0.734	0.042	0.187	0.147	12.146	7.154	24.580	
Tilapia sp.	0.690	0.060	0.090	0.660	2.800	9.180	0.030	Mansour and Sidky[25]
Mugil sp.	1.160	0.410	0.550	2.180	4.100	2.100	0.010	
Tilapia sp.	nm	nm	nm	5.930-17.850	3.000-13.560	nm	nm	Authman and Abbas[39]
Mugil sp.	nm	nm	nm	1.310-5.010	0.79-3.85	nm	nm	
Tilapia zillii	55.880	42.810	nm	nm	19.500	10.250	1.750	Mohamed and Gad[70]
Solea vulgaris	60.380	53.670	nm	nm	8.500	12.630	3.880	
Mugil capito	46.380	46.250	nm	nm	11.880	4.250	1.750	
	3.520	0.189	0.120	3.280	67.100	146	0.600	Wyse *et al.*[42]

nd: Not detected, nm: Not measured, na: Not available

released into Lake Qarun are alleged to be the main sources for such detected metals[25]. However, heavy metals may also arise due to diverse natural processes such as erosion and weathering[35] but anthropogenic activities remain the main cause of their existence in the aquatic environments[36]. Previous studies have confirmed the existence of lofty heavy metal pollutants in Lake Qarun[37-40], however the levels of some metals in the current study exceeded the earlier reports indicating recent and increasing pollution events in the lake (Table 4). The ubiquitous distribution of heavy metals in the aquatic environment and their remarkable potentials for bioaccumulation in aquatic biota amplify their risk[41]. Results demonstrated bioaccumulation of all investigated metals in fish muscles with variable levels. The Ni and Zn were found to be the uppermost detected metals in fish muscles 18.976±27.86, 9.46±0.63 ppm in *Tilapia zillii* and 24.58±5.3 and 12.146±1.2 ppm in *Solea vulgaris*, respectively, which could be related to their extreme levels in Lake Qarun water. While Ni is the only metal exceed the recommended values of trace elements in fish tissue (0.6 ppm) established by the International Atomic Energy Agency (IAEA)[42]. The main source of Ni in water is the industrial wastes, it is easily accumulated in phytoplankton and other aquatic plants and it represents a critical hazard to fish because of possible carcinogenicity[43]. The Cd and Pb showed detectable levels in fish muscles; 0.03±0.01 and 0.1±0.002 ppm in *Tilapia zillii* and 0.042±0.008, 0.187±0.01 ppm in *Solea vulgaris* respectively, although both metals were absent in lake water samples denoting the bioconcentration and bioaccumulation of heavy metals within fish tissues through the time. Diverse species of fish from the same water body may accumulate variable amounts of metals and the inter-species variation in metal accumulation may be related to different living and feeding habits[44]. Numerous environmental as well as host factors affect metal bioaccumulation, which may explain the higher accumulated levels of some heavy metals in *Solea vulgaris* over *Tilapia zillii* and vice versa. Extreme water salinities similar to that noticed in Lake Qarun, 39.5‰, is supposed to reduce the uptake and accumulation of metals by fish[45].

Single cell gel electrophoresis (comet assay) showed greater DNA damage in *Solea vulgaris* compared with *Tilapia zillii* in terms of percent cells with tail, tail length and percent DNA in tail which may be attributed to higher levels of heavy metals detected in this species. Solea fish belongs to demersal and sedentary species living on muddy bottoms and remains buried in the substrate[46], ultimately, become vulnerable to high concentrations of metals exist in the sediments. Additionally their feeding behavior may also increase the chance of exposure to higher levels of these elements as their food includes mostly invertebrates, small fish, mollusks and small crustaceans[46,47] which accumulate metals in their tissues. On the other hand *Tilapia zillii* has higher preference for algae and vegetative matter and seldom browse on live benthic invertebrates and bacteria laden detritus[48,49].

The high detected levels of Ni, Zn and Cu in Lake Quran water are alleged to cause such DNA damage. High levels of Ni enhance generation of oxygen radicals[50] which subsequently interact with DNA, inducing damage to its bases and cause DNA strand-breaks[51]. Additionally Ni inhibits DNA repair process[52]. Similarly, extreme Zn and Cu concentrations also

induce oxidative stress and mass production of damaging free radicals, which further stimulate cell membrane lipid peroxidation and genotoxicity[52,12].

Correlations between DNA damage and presence of heavy metals in fish have been reported in previous studies[50,52]. Concentration dependent increase in DNA damage due to fish exposure to different sub-lethal concentrations of Zn and Cu, in terms of damaged cells frequency and tail length using comet assay, also have been noticed[53]. Additionally, exposure of sea bream fish, *Sparus aurata* and mollusks, *Scapharca inaequivalvis* to high Cu sulfate concentrations (0.1 ppm) was found to cause damage to the DNA structure[52]. Zhang *et al*.[12] denoted significant time and dose dependant correlation between exposure of loach, *Misgurnus anguillicaudatus*, to Cd, Pb and Zn ions, either individually or collectively but intense DNA damage was detected in hepatopancreatic cells upon exposure to mixed heavy metal ions. Similarly, high DNA fragmentation was noticed in fish liver cells collected from polluted aquatic environments[5,54,55].

Bacteriological examination of fish specimens revealed high infections in *Tilapia zillii* than *Solea vulgaris*; 56.09 and 43.9%, respectively. *Vibrio alginolyticus* was the most predominant bacterial infections in both fish species, which may relevant to the ubiquities nature of vibrios in seawater[56]. *Vibrio alginolyticus* have caused infections in array of marine fish species and well known to cause mass mortalities and massive economic losses in wild as well as cultured fish[1]. *Aeromonas hydrophila* and *Photobacterium damsela*e sub spp. *piscicida* have been also detected in investigated fish specimens. Both bacterial agents are renowned infections affecting variety of fish species worldwide[57]. Earlier studies surveying bacterial infections affecting Lake Qarun fish by Moustafa *et al*.[57] and Elgendy[58] who recorded array of infections in both *Tilapia zillii* and *Solea vulgaris,* including those reported in the present study. These infections are regularly triggered by unfavorable environmental conditions in terms of low dissolved oxygen, high temperature as well as high levels of heavy metal pollutants the same as recorded in Lake Quran[59]. Adverse environmental conditions deteriorate the physiological status of fish rendering them more susceptible to opportunistic pathogens. Several heavy metals are major stressors enhancing corticosteroid secretion accordingly trigger attack of numerous microorganisms[60]. There is reliable evidence linking emergence of several opportunistic fish diseases, like vibriosis, to existence of some metals such as, copper and iron[59]. Suppression/repression of fish immune response is alleged as one pathway in such infections[61].

In the current study, no fungal isolates were detected in all fish samples. However, orange/red moist colonies of the unicellular yeast *Rhodotorula* sp. were detected in all investigated water samples. *Rhodotorula* belong to basidiomycetes which reported to be common yeast sp. in shallow and deep salt waters. Positive correlation between the yeast load including, *Rhodotorula* sp. and different pollution level has been reported[62]. For marine fish, *Rhodotorula* sp. were reported to dominate (>90%) yeast species isolated from the intestine of rainbow trout[63]. However, the high yeast colonization was not associated with any signs of sickness of examined fish. Accordingly, the yeast load could be an indicator for water pollution but not the health status of fish. Studies about fungal infections in fish collected from Lake Qarun are scanty. Previous study showed that *Aspergillus niger* was the only filamentous fungi isolated from fish and represented 1.37% of total isolates[64]. However, *A. niger* does not implicated as a causative agent for serious diseases in fish.

Parasitic examination revealed existence of crustacean *Isopods* sp. family Cymothoidae in both fish species but infections were more prevalent in *Tilapia zillii* (20%) than *Solea vulgaris* (13.3%). These parasites affect variety of fish species causing considerable economic losses either by detrimental injures, stunting growth, reduced fecundity or direct killing[65]. Previous studies indicated widespread of crustacean *Isopoda* sp. in fish collected from Lake Qarun[66]. These parasites are mainly blood-feeding settle on the outer body surfaces including fins, buccal cavity, gill chambers, nostrils, or occasionally within the muscles of their hosts[67]. Unfavorable environmental conditions like that recorded in Lake Qarun weaken fish immune defense mechanisms consequently predisposes fish to various infections including parasitic infestations[68]. This study demonstrated that heavy metal pollutants are extremely injurious to fish. The study showed a need for continuous pollution assessment of the aquatic environment and cohabitant organisms. The study recommends the control of discharges of toxic substances into the aquatic environment to protect aquatic animal health.

CONCLUSION

This study demonstrated that pollution reaching Lake Qarun is genotoxic to *Tilapia zillii* and *Solea vulgaris* as evidenced by comet assay of fish liver cells. Heavy metals; Zn, Ni and Cu showed high values in lake water exceeding the recommended limits. The study highlighted the link between fish infections and unfavorable environmental conditions. *Vibrio alginolyticus*, *Aeromonas hydrophila*, *Photobacterium*

damselae subsp. *piscicida* and parasitic *Isopoda* sp. were detected in fish specimens. The study highlights the importance of continuous aquatic environmental monitoring and necessitates control of aquatic pollution through legislation and raising public awareness.

SIGNIFICANCE STATEMENT

This study confirmed that heavy metal pollutants are genotoxic to fish and identified a link between microbial infections affecting fish and environmental deterioration. The study contributes to the effective monitoring of aquatic environment and raising awareness on the hazards of heavy metals to aquatic animal health.

REFERENCES

1. Moustafa, M., A.E. Eissa, A.M. Laila, A.Y. Gaafar, I.M.K. Abumourad and M.Y. Elgendy, 2014. Mass mortalities in mari-cultured European sea bass (*Dicentrarchus labrax*) at Northern Egypt. Res. J. Pharm. Biol. Chem. Sci., 5: 95-109.
2. Elgendy, M.Y., M. Moustafa, A.Y. Gaafar and T.B. Ibrahim, 2015. Impacts of extreme cold water conditions and some bacterial infections on earthen-pond cultured Nile tilapia, *Oreochromis niloticus*. Res. J. Pharm. Biol. Chem. Sci., 6: 136-145.
3. Elgendy, M.Y., 2013. Epizootiological and molecular studies on the common septicemic bacterial diseases of some saltwater fishes. Ph.D. Thesis, Cairo University, Egypt.
4. Austin, B. and D.A. Austin, 2012. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. 5th Edn., Springer Science and Business Media, USA., ISBN: 9789400748842, pp: 230-238.
5. Fatima, M., N. Usmani, M.M. Hossain, M.F. Siddiqui, M.F. Zafeer, F. Firdaus and S. Ahmad, 2014. Assessment of genotoxic induction and deterioration of fish quality in commercial species due to heavy-metal exposure in an urban reservoir. Arch. Environ. Contam. Toxicol., 67: 203-213.
6. Moustafa, M., A.E. Eissa, A.M. Laila, A.Y. Gaafar, I.M.K. Abumourad and M.Y. Elgendy, 2015. Investigations into the potential causes of mass kills in mari-cultured Gilthead sea bream, (*Sparus aurata*) at Northern Egypt. Res. J. Pharm. Biol. Chem. Sci., 6: 466-477.
7. Malik, R.N., M.Z. Hashmi and Y. Huma, 2014. Heavy metal accumulation in edible fish species from Rawal Lake reservoir, Pakistan. Environ. Sci. Pollut. Res., 21: 1188-1196.
8. Gurcu, B., S. Yildiz, Y.B.G. Koca and S. Koca, 2010. Investigation of histopathological and cytogenetic effects of heavy metals pollution on *Cyprinus carpio* (Linnaeus, 1758) in the Golmarmara Lake, Turkey. J. Anim. Vet. Adv., 9: 798-808.
9. Bhat, M.M., K. Narain, S.Z.A. Andrabi, R.N. Shukla and M. Yunus, 2012. Assessment of heavy metal pollution in urban pond ecosystems. Universal J. Environ. Res. Technol. 2: 225-232.
10. Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider, 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175: 184-191.
11. Barbosa, J.S., T.M. Cabral, D.N. Ferreira, L.F. Agnez-Lima and S.R.B. de Medeiros, 2010. Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals. Ecotoxicol. Environ. Safety, 73: 320-325.
12. Zhang, Y., Y. Wang, R. Yu, S. Zhang and Z. Wu, 2008. Effects of heavy metals Cd^{2+}, Pb^{2+} and Zn^{2+} on DNA damage of loach *Misgurnus anguillicaudatus*. Front. Biol. China, 3: 50-54.
13. Ballatori, N., 2002. Transport of toxic metals by molecular mimicry. Environ. Health Perspect., 110: 689-694.
14. Huang, R.N., I. Ho, L.H. Yih and T.C. Lee, 1995. Sodium arsenite induces chromosome endoreduplication and inhibits protein phosphatase activity in human fibroblasts. Environ. Mol. Mutagen., 25: 188-196.
15. Rossman, T.G. and D. Wolosin, 1992. Differential susceptibility to carcinogen induced amplification of SV40 and *dhfr* sequences in SV40 transformed human keratinocytes. Mol. Carcinogenesis, 6: 203-213.
16. Gebel, T., P. Birkenkamp, S. Luthin and H. Dunkelberg, 1997. Arsenic (III), but not antimony (III), induces DNA-protein crosslinks. Anticancer Res., 18: 4253-4257.
17. Hengstler, J.G., U. Bolm-Audorff, A. Faldum, K. Janssen and M. Reifenrath *et al*., 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis, 24: 63-73.
18. Klein, C.B., K. Frenkel and M. Costa, 1991. The role of oxidative processes in metal carcinogenesis. Chem. Res. Toxicol., 4: 592-604.
19. Adhikari, T., E. Tel-Or, Y. Libal and M. Shenker, 2006. Effect of cadmium and iron on rice (*Oryza sativa* L.) plant in chelator-buffered nutrient solution. J. Plant Nutr., 29: 1919-1940.
20. Ghillebaert, F., C. Chaillou, F. Deschamps and P. Roubaud, 1995. Toxic effects, at three pH levels, of two reference molecules on common carp embryo. Ecotoxicol. Environ. Safety, 32: 19-28.
21. Alabaster, J.S. and R. Lloyd, 1982. Water Quality Criteria for Freshwater Fish. 2nd Edn., Food and Agriculture Organization of the United Nations, London, UK., Pages: 361.
22. Frenzillii, G., M. Nigro and B.P. Lyons, 2009. The comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat. Res./Rev. Mutat. Res., 681: 80-92.
23. Molnar, J.L., R.L. Gamboa, C. Revenga and M.D. Spalding, 2008. Assessing the global threat of invasive species to marine biodiversity. Front. Ecol. Environ., 6: 485-492.
24. FAO Fish Finder, 2017. The species identification and data programme. Institutional Websites, FAO Fisheries and Aquaculture Department, Rome. http://www.fao.org/fishery/fishfinder/en.

25. Mansour, S.A. and M.M. Sidky, 2002. Ecotoxicological studies. 3. Heavy metals contaminating water and fish from fayoum Governorate, Egypt. Food Chem., 78: 15-22.
26. APHA., 2000. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, DC., pp: 228-240.
27. Authman, M.M.N., H.H. Abbas and W.T. Abbas, 2013. Assessment of metal status in drainage canal water and their bioaccumulation in *Oreochromis niloticus* fish in relation to human health. Environ. Monit. Assess., 185: 891-907.
28. Singh, R., N. Gautam, A. Mishra and R. Gupta, 2011. Heavy metals and living systems: An overview. Indian J. Pharmacol., 43: 246-253.
29. Anderson, S.L. and G.C. Wild, 1994. Linking genotoxic responses and reproductive success in ecotoxicology. Environ. Health Perspect., 102: 9-12.
30. Buller, N.B., 2004. Bacteria from Fish and other Aquatic Animals: A Practical Identification Manual. CABI Publishing, Cambridge, pp: 83-116.
31. Fregeneda Grandes, J.M., F. Rodriguez Cadenas and J.M. Aller Gancedo, 2007. Fungi isolated from cultured eggs, alevins and broodfish of brown trout in a hatchery affected by saprolegniasis. J. Fish Biol., 71: 510-518.
32. Pritchard, M.H. and G.O.W. Kruse, 1982. The Collection and Preservation of Animal Parasites. University of Nebraska Press, Lincoln, NE., ISBN: 9780803287044, Pages: 141.
33. USEPA., 2004. National recommended water quality criteria aquatic life criteria. United States Environmental Protection Agency (USEPA).
34. ANZECC. and ARMCANZ., 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council (ANZECC), Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ), Canberra, ACT, pp: 83-104.
35. Gowda, K.M.D., V.M. Nair and N.K. Subbalakshmi, 2008. Effect of 15 days exposure to sublethal concentrations of cadmium toxicity on the level of metabolic fuels in Indian major carp, *Labeo rohita*. Thai J. Physiol. Sci., 21: 18-25.
36. Ambreen, F., M. Javed and U. Batool, 2015. Tissue specific heavy metals uptake in economically important fish, *Cyprinus carpio* at acute exposure of metals mixtures. Pak. J. Zool., 47: 399-407.
37. Mansour, S.A. and M.M. Sidky, 2003. Ecotoxicological studies. 6. The first comparative study between Lake Qarun and Wadi El-Rayan wetland (Egypt), with respect to contamination of their major components. Egypt. J. Food Chem., 82: 181-189.
38. Fishar, M.R.A. and M.H.H. Ali, 2005. Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of Lake Qarun, Egypt. Egypt J. Aquat. Res., 31: 289-301.
39. Authman, M.M.N. and H.H.H. Abbas, 2007. Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of Lake Qarun, Fayoum Province, Egypt. Pak. J. Biol. Sci., 10: 2106-2122.
40. Abdel-Satar, A.M., M.E. Goher and M.F. Sayed, 2010. Recent environmental changes in water and sediment quality of Lake Qarun, Egypt. J. Fish. Aquat. Sci., 5: 56-69.
41. Yohannes, Y.B., Y. Ikenaka, A. Saengtienchai, K.P. Watanabe, S.M. Nakayama and M. Ishizuka, 2013. Occurrence, distribution and ecological risk assessment of DDTs and heavy metals in surface sediments from Lake Awassa-Ethiopian Rift Valley Lake. Environ. Sci. Pollut. Res., 20: 8663-8671.
42. Wyse, E.J., S. Azemard and S.J. Mora, 2003. Report on the world-wide intercomparison exercise for the determination of trace elements and methylmercury in fish homogenate IAEA-407. IAEA/AL/144 (IAEA/MEL/72), IAEA, Monaco, pp: 35-39.
43. Cempel, M. and G. Nikel, 2006. Nickel: A review of its sources and environmental toxicology. Polish J. Environ. Stud., 15: 375-382.
44. Kidwell, J.M., L.J. Phillips and G.F. Birchard, 1995. Comparative analyses of contaminant levels in bottom feeding and predatory fish using the national contaminant biomonitoring program data. Bull. Environ. Contam. Toxicol., 54: 919-923.
45. Stagg, R.M. and T.J. Shuttleworth, 1982. The accumulation of copper in *Platichthys flesus* and its effects on plasma electrolyte concentrations. J. Fish Biol., 20: 419-500.
46. Jardas, I., 1996. Adriatic Ichthyofauna. School Books, Zagreb, pp: 536.
47. Fisher, W., M. Schneider and M.L. Bauchot, 1987. Fishes FAO d'identification Des Especes Pour les Besoins de la Peche: Mediterranee et mer Noire. Vol. 1-2, FAO, Rome, Pages: 760.
48. Ugwumba, A.A., 1988. Food and feeding habits of juveniles of some culturable fish species in Nigeria. Niomr Tech. Paper, 31: 18-22.
49. Oso, J.A., I.A. Ayodela and O. Fagbuaro, 2006. Food and feeding habits of *Oreochromis niloticus* (L.) and *Sarotherodon galilaeus* (L.) in a tropical reservoir. World J. Zool., 1: 118-121.
50. Reinecke, S.A. and A.J. Reinecke, 2004. The comet assay as biomarker of heavy metal genotoxicity in earthworms. Arch. Environ. Contam. Toxicol., 46: 208-215.
51. Misra, M., R. Olinski, M. Dizdaroglu and K.S. Kasprzak, 1992. Enhancement by L-histidine of nickel(II)-induced DNA-protein cross-linking and oxidative DNA base damage in the rat kidney. Chem. Res. Toxicol., 6: 33-37.
52. Gabbianelli, R., G. Lupidi, M. Villarini and G. Falcioni, 2003. DNA damage induced by copper on erythrocytes of gilthead sea bream *Sparus aurata* and mollusk *Scapharca inaequivalvis*. Arch. Environ. Contam. Toxicol., 45: 350-356.

53. Kousar, S. and M. Javed, 2014. Assessment of DNA damage in peripheral blood erythrocytes of fish exposed to arsenic under laboratory conditions. Int. J. Curr. Microbiol. Applied Sci., 3: 877-888.
54. Rajaguru, P., S. Suba, M. Palanivel and K. Kalaiselvi, 2003. Genotoxicity of a polluted river system measured using the alkaline comet assay on fish and earthworm tissues. Environ. Mol. Mutagen., 41: 85-91.
55. Liney, K.E., J.A. Hagger, C.R. Tyler, M.H. Depledge, T.S. Galloway and S. Jobling, 2006. Health effects in fish of long-term exposure to effluents from wastewater treatment works. Environ. Health Perspect., 114: 81-89.
56. Elgendy, M.Y., A.M. Kenawy and A.E.N. El-Deen, 2016. *Gyrodactylus anguillae* and *Vibrio vulnificus* infections affecting cultured eel, *Anguilla anguilla*. Comunicata Sci., 7: 1-11.
57. Moustafa, M., A.M. Laila, M.A. Mahmoud, W.S. Soliman and M.Y. Elgendy, 2010. Bacterial infections affecting marine fishes in Egypt. J. Am. Sci., 6: 603-612.
58. Elgendy, M.Y., 2007. Epizootiological studies on some bacterial infections in marine fishes. M.Sc. Thesis, Cairo University, Egypt.
59. Elgendy, M.Y., W.S. Soliman, H.A. Hassan, A.M. Kenawy and A.M. Liala, 2015. Effect of abrupt environmental deterioration on the eruption of vibriosis in mari-cultured shrimp, *Penaeus indicus*, in Egypt. J. Fish. Aquatic Sci., 10: 146-158.
60. Teles, M., M. Oliveira, M. Pacheco and M.A. Santos, 2005. Endocrine and metabolic changes in *Anguilla anguilla* L. following exposure to β-naphthoflavone-a microsomal enzyme inducer. Environ. Int., 31: 99-104.
61. O'Neill, J.G., 1981. The humoral immune response of *Salmo trutta* L. and *Cyprinus carpio* L. exposed to heavy metals. J. Fish Biol., 19: 297-306.
62. Hagler, A.N., R.D. de Oliveira and L.C.M. Hagler, 1982. Yeasts in the intertidal sediments of a polluted estuary in Rio de Janeiro, Brazil. Antonie Van Leeuwenhoek, 48: 53-56.
63. Andlid, T., R.V. Juarez and L. Gustafsson, 1995. Yeast colonizing the intestine of rainbow trout (*Salmo gairdneri*) and turbot (*Scophthalmus maximus*). Microb. Ecol., 30: 321-334.
64. Abou El-Gheit, E.N., M.H. Abdo and S.A. Mahmoud, 2012. Impacts of blooming phenomenon on water quality and fishes in Qarun Lake, Egypt. Int. J. Environ. Sci. Eng., 3: 11-23.
65. Sullivan, M. and R. Stimmelmayr, 2008. Cymothoid isopods on coral reef fishes in the near shore marine environment of St. Kitts, Lesser Antilles. Proceedings of the 11th International Coral Reef Symposium, January 2008, Ft. Lauderdale, Florida, pp: 7-11.
66. Abdel-Latif, H.M., 2016. Cymothoid parasite, Nerocila Orbigni inflicts great losses on tilapia Zillii in Lake Qarun at El-Fayoum province. Int. J. Innovative Stud. Aquatic Biol. Fish., 2: 1-9.
67. Hoffman, G.L., 1998. Parasites of North American Freshwater Fishes. 2nd Edn., Cornell University Press, New York, USA., Pages: 325.
68. Meyer, F.P., 1991. Aquaculture disease and health management. J. Anim. Sci., 69: 4201-4208.
69. Hussein, H., R. Amer, A. Gaballah, Y. Refaat and A. Abdel-Wahab, 2008. Pollution monitoring for Lake Qarun. Adv. Environ. Biol., 2: 70-80.
70. Mohamed, F.A. and N.S. Gad, 2008. Environmental pollution-induced biochemical changes in tissues of *Tilapia zillii*, *Solea vulgaris* and *Mugil capito* from Lake Qarun, Egypt. Global Vet., 26: 327-336.