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RESEARCH

The influence of heavy metals on cytotoxicity in *Tilapia zillii*

Nermeen Y. Abass[1]

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Abstract
The present study was designed to investigate the cytotoxic effects and bioaccumulation of heavy metals iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), manganese (Mn), nickel (Ni), and chromium (Cr) in different parts (muscle, gills, and liver) of *Tilapia zillii* occurring in polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt. Results of water analysis revealed the concentration of Cd, Pb, Mn, Ni, and Cr exceeded the limits defined by the American Public Health Association (APHA) in the polluted drainage canal. In addition, the concentration of Ni elevated to the standard limits of APHA and Cu was not detected in the fish farm. Different types of chromosomal aberrations were recorded (e.g., stickiness, fragmented chromosomes, centromeric gaps, chromatid break, chromatid deletion, and tetraploid). Micronucleus frequency was found to be 5.58 in the polluted drainage canal group and 0.32 in the fish farm group. Other nuclear abnormalities such as blebbed nucleus, segmented nucleus, enucleated erythrocyte, kidney-shaped nucleus, heart-shaped nucleus, polymorphic irregular nuclei, binucleated cell, nuclear fragmented erythrocyte, long nucleus, putative fragmented notched nucleus, lobed nuclei, fused erythrocytes, necrotic erythrocyte, and vacuolated nucleus were recorded. The total of erythrocytes nuclear morphological abnormalities was 70.33% in the polluted drainage canal and 1.78% in the fish farm.

Keywords *Tilapia zillii* · Heavy metals · Bioaccumulation factor · Chromosomal aberration · Micronucleus test

Introduction

Heavy metals and toxins released into aquatic ecosystems from agricultural and industrial discharges might affect human health and cause chronic diseases (Zyadah and Abdel-Baky 2000). Fish can absorb heavy metals from water through the skin, gills, and digestive tract (Rajeshkumar and Li 2018). Fish is an important source of protein,

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✉ Nermeen Y. Abass
n.y.abass@alexu.edu.eg

[1] Department of Agricultural Botany, Faculty of Agriculture (Saba-Basha), Alexandria University, P.O. Box 21531, Alexandria City, Egypt

vitamins, elements, and polyunsaturated fatty acids for many humans. Consequentially, toxin metals in aquatic environment are transferred throughout the food chain into humans (Taweel et al. 2011).

Tilapia is one of the most important cultured freshwater fish in the world (Arumugam et al. 2023) and is a key species in aquatic ecosystems as a community food source and an economically important fish species for the 21st country (Fitzsimmons 2000). Tilapia *sp.* represent about 43% of world total aquaculture production and about 66% of the total aquaculture production in Egypt (El-Sayed and Fitzsimmons 2023). *Tilapia zillii* can survive at low dissolved oxygen, high ammonia levels, and salinity (El-Sayed 2006; Asad et al. 2010).

The bioaccumulation of toxic metals in the aquatic ecosystem may induce carcinogenesis and genetic alterations in aquatic organisms (Osman et al. 2011) depending on the type of metals and its concentration (Emon et al. 2023). It is crucial to biomonitor genotoxicity in aquatic organisms for several reasons. First, from an ecological standpoint, it is crucial to preserve genetic variety in natural populations for population survival and to avoid contaminant-induced mutations that skew genetic diversity (Jha et al. 2000). Second, it is crucial to identify carcinogenic effects in aquatic creatures to evaluate their health and stop carcinogens from passing through the food chain and affecting people (De Flora et al. 1991).

The bioaccumulation of heavy metals differs for each metal and between several organs of the same organism. Concentrations of heavy metal were higher in the gills than in the muscle tissue of *Clarias gariepinus* (Masoud et al. 2007; Zaghloul et al. 2020). The liver cells of *Clarias gariepinus* obtained from the river Nile branch and the most contaminated drainage canals at El-Fayoum governorate showed signs of mutagenic damage and genotoxicity (Zaghloul et al. 2020).

Several studies have demonstrated increases in cytogenetic abnormalities in aquatic organisms. Toxic metals can cause changes in chromosome number (gain or loss of chromosome) and changes in chromosome structure (deletion, break, duplication, and rearrangement). Soulivongsa et al. (2020) reported that there were seven types of chromosome aberrations in *Osteochilus vittatus*, and the highest total number was a centromere gap of chromosome aberrations. Anwar and Abu Shnaf (2023) reported that there were different types of chromosomal aberrations (e.g., ring, deletion, centromeric attenuation, end−to−end association, stickiness chromosomes, dicentric chromosome, endomitosis, chromatid gap, and fragments) in *O. niloticus*, and ring chromosomes was the most common type.

Micronucleus (MNs) test is a common method used to investigate the impact of heavy metal concentrations on genotoxicity in erythrocytes (Al-Sabti and Metcalfe 1995; Ali et al. 2008; Abu Shnaf et al. 2021; Shah et al. 2021). The micronuclei have a similar shape to the main nucleus and separated from the main nucleus, but its diameter varies from 1/3 to 1/16 of the size of the main nucleus (Fenech 2000). Micronuclei occur during cell division (anaphase) when a complete chromosome or its part fails to integrate into the nucleus of any daughter cell as a result of genetic damage (Luzhna et al. 2013). The number of micronuclei has been used as a measure of chromosomal breakage and mitotic spindle machinery failure (Ayllon and Garcia–Vazquez 2000). Other nuclear abnormalities as binucleated, blebbed, lobed, and notched nuclei have been observed to be potential indicators of cytotoxicity (Canedo et al. 2021; Sanchez-Galan et al. 2001; Ali et al. 2020). The rate of erythrocyte nuclear morphological abnormalities in fish, including the frequency of micronuclei, is being used as a genotoxicity biomarker (Carrola et al. 2014; Canedo et al. 2021).

The objectives of this study were undertaken to determine the concentrations of heavy metals (Fe, Zn, Cu, Cd, Pb, Mn, Ni, and Cr) in the water and the bioaccumulation of heavy metals in different parts of *Tilapia zillii* and evaluate the cytotoxic effects on *Tilapia zillii*, which were collected from two sites in Alexandria governorate, Egypt.

Materials and methods

Study location and sampling

Sampling was carried out in Alexandria governorate, Egypt. The water and fish samples were collected from two different locations that had different levels of contamination.

- Site (1)—polluted drainage canal (Abiece region in front of village number 10): it is considered the polluted site because of agricultural practices, industrial activities, transportation, and urban activities.
- Site (2)—fish farm (Abiece region): it is considered the control site because it is the main source for commercial breeding of fish.

Water sampling

Water samples were collected in triplicates at a depth of 25 cm below the water surface. Polyethylene bottles (1000 ml) were used for collections. Samples were transported immediately to the laboratory after collection and kept at 4 °C until analysis to estimate the heavy metal concentrations through flame atomic absorption spectrophotometry (APHA 2005). Water temperature and dissolved oxygen (DO) were measured in the morning using a YSI® Pro20 Dissolved Oxygen Meter (Pentair Aquatic Eco−Systems, Inc. Apopka, FL). Other water quality parameters such as pH, nitrate, nitrite, total hardness, total chlorine, total alkalinity, and total ammonia were measured using EasyStrips (Tetra®EasyStrips, United Pet Group, Inc. USA).

Fish specimen collection

A total of 30 *Tilapia zillii* fish (15 fish per site) with body length 10–12 cm and body weight 80–100 g were collected. The samples were transferred alive with water to fish genetic lab, Faculty of Agriculture (Saba−Basha), Alexandria University, Egypt, where they were placed in glass boxes with an oxygen supply. Fish samples were identified according to Bishai and Khalil (1997).

Heavy metal analysis in water and fish samples

The heavy metal concentrations in the water samples were determined using the PerkinElmer AAnalyst−400 flame atomic absorption spectrophotometer with hollow cathode lamp (iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), manganese (Mn), nickel (Ni), and chromium (Cr)). A total of 1 g from each tissue (muscle, gills, and liver) for each sample was dried in an oven at 160 °C for 6 h and homogenized separately in a mortar and mixed with 4 ml of H_2SO_4 and 4 ml of H_2O_2 in a flask. The solution was transferred to a 100 ml volumetric flask and diluted with distilled water to 100 ml volume. After filtration the heavy metal concentrations in the solution were determined with the same method which applied in the water samples (Seehy et al. 2009). Data was reported as mg/kg for fish tissues and as mg/L for water samples.

Bioaccumulation factor

The bioaccumulation factor (BAF) was calculated according to the following equation (Zhang et al. 2015):

$$\mathrm{BAF} = \frac{\mathrm{M\ tissue\ (mg/kg)}}{\mathrm{M\ water\ (mg/l)}}$$

where M tissue is the metal concentration in fish organ and M water is the metal concentration in water.

Analysis of chromosomal abnormalities

Fish were euthanized by exposing to overdose of tricaine methane sulfonate (MS−222) (200–250 mg/L) and gills were removed. Chromosomal preparations were carried out according to Seehy et al. (2009) with some modifications. The gills were cut into small pieces and mixed with 0.075 M potassium chloride (KCl), gently homogenized, and left for 15–20 min at room temperature. One layer of nylon mesh was used to filter the homogenate. The filtrate was centrifuged for 12 min at 1200–1500 rpm. The supernatant was then discarded. The pellet was then suspended in methanol and glacial acetic acid (3:1), left for 1 h, and centrifuged. The fixative was changed after 30 min by centrifugation. Cell suspension was kept overnight at −18 °C. Cells in fixative were dropped onto clean glass slides and air dried. Spread cells were stained for 5 min with 10% Giemsa (pH = 6.8). At least 300 scorable metaphase for each area was examined for stickiness, fragments, gaps, chromatid break, chromatid deletion, tetraploid … etc. and recorded for chromosome abnormalities.

Micronucleus (MNs) test

MNs tests were carried out according to the protocol of Souza and Fontanetti (2006) with some modifications. Smear slides with 50 µl of gill blood were air dried and then fixed in absolute methanol for 10 min followed by 10% Giemsa staining (pH = 6.8). Two slides were made for each fish and three fish for each location. 1000 cells were examined per slide under a ×1000 optical microscope. Normal nucleus (N) and nuclear abnormality (NA) frequencies were calculated as follows:

$$\%\mathrm{N} = \frac{(\text{Number of cells with normal nucleus})}{(\text{Total number of cells})} \times 100$$

$$\%\mathrm{NA} = \frac{(\text{Number of cells containing nuclear abnormalities})}{(\text{Total number of cells})} \times 100$$

Nuclear abnormality (NA) shapes were scored into one of the following categories: micronucleus (MN), blebbed nucleus (BN), segmented nucleus (SN), enucleated erythrocyte (EE), kidney−shaped nucleus (KSN), heart−shaped nucleus (HSN), polymorphic irregular nuclei (PN), binucleated cell (BC), nuclear fragmented erythrocyte (NFR), long nucleus (LN), notched nucleus (NN), and lobed nuclei (LBN), fused erythrocytes (FE), necrotic erythrocyte (NE), and vacuolated nucleus (VN). The total of the frequencies of all

types of erythrocyte nuclear morphological abnormalities (MN+BN+SN+EE+KSN+HSN+PN+BC+NFR+LN+NN+LBN+FE+NE+VN) was also calculated per location.

Statistical analysis

All statistical analyses were carried out using IBM SPSS software version 27. Data were presented as mean ± SD for all experiments and subjected to one way ANOVA; significant difference were assessed using Duncan's (Duncan 1955) multiple comparison test at $P \leq 0.05$ and highly significant at $P < 0.0001$. The relationship between the concentrations of heavy metals in water (mg/L) and in *Tilapia zillii* tissues (ppm) and relationship between the frequencies (%) of normal nucleus (N), nuclear abnormalities (NA), and the concentrations of heavy metals in water (mg/L) were investigated through correlation analyses (Pearson's test, two−tailed).

Results

Water quality parameters

The values of water quality parameters from the polluted drainage canal and the fish farm are shown in Table 1. The pH of all water samples ranged between 7.5 and 8.2 which is within the WHO standard (6.5–8.5) (WHO 1997).

Concentrations of heavy metals in water samples

Concentrations of heavy metals (Fe, Zn, Cu, Cd, Pb, Mn, Ni, and Cr) in water samples are shown in Table 2. The average concentrations of Fe, Zn, Cd, Pb, Mn, and Cr did not exceed the permissible limits of American Public Health Association (APHA 2005) in the fish farm. However, Cu was not detected in the fish farm. The average concentrations of Fe, Zn, and Cu did not exceed the American Public Health Association (APHA 2005) in the polluted drainage canal. However, the Cd, Pb, Mn, Ni, and Cr concentrations exceeded the

Table 1 Water quality parameters of water samples collected from the polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt

Parameters	Location	
	Polluted drainage canal	Fish farm
Temperature (C)*	28.67±1.15	26.00±0.00
DO (mg/L)*	5.80±0.92	7.60±0.17
pH	7.47±0.58	8.20±0.35
Nitrate (ppm)	46.67±11.55	40.00±0.00
Nitrite (ppm)	0.43±0.12	0.40±0.17
Total hardness (ppm)	75.00±0.00	75.00±0.00
Total chlorine (ppm)	0.13±0.12	0.07±0.12
Total alkalinity (ppm)	180.00±00.00	160.00±34.64
Ammonia (mg/L)*	0.07±0.01	0.05±0.01

Data are mean ± SD with n = 3

*$p < 0.05$

Table 2 Concentrations of heavy metals (mean ± standard deviation (SD)) as mg/L in water samples collected from the polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt, and compared with the permissible limits

Location	Metal (mg/L)[a]							
	Fe**	Zn**	Cu**	Cd**	Pb**	Mn**	Ni**	Cr**
Polluted drainage canal	0.98 ± 0.01	0.62 ± 0.03	0.13 ± 0.01	0.03 ± 0.01	0.16 ± 0.01	0.22 ± 0.02	0.14 ± 0.01	0.33 ± 0.04
Fish farm	0.66 ± 0.05	0.08 ± 0.01	nd	0.01 ± 0.00	0.04 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
APHA (2005)	1.50	1.00	1.50	0.01	0.05	0.10	0.005	0.10

Data are mean ± SD with $n = 3$

APHA = American Public Health of Association, nd = not detected

**$P = < 0.0001$

[a]Fe = iron, Zn = zinc, Cu = copper, Cd = cadmium, Pb = lead, Mn = manganese, Ni = nickel, and Cr =chromium

standard. The water samples from both locations were polluted with Ni which exceeded the standard (Table 2). Concentrations of heavy metals in water samples from the polluted drainage canal were significantly higher for all examined metals than the fish farm.

Concentrations of heavy metals in fish samples

The average concentration of Fe, Zn, Cu, Cd, Pb, Mn, Ni, and Cr in *Tilapia zillii* muscle, gills, and liver samples from the polluted drainage canal and the fish farm is shown in Table 3. Ni and Cr accumulated in the muscles from both areas were significantly different ($P < 0.05$).

Bioaccumulation factor

The bioaccumulation factor of Fe, Zn, Cu, Cd, Pb, Mn, Ni, and Cr in *Tilapia zillii* muscle, gills, and liver samples from the polluted drainage canal and the fish farm is shown in Table 4. Result showed that the concentrations of the most metals in the organs were in the order of liver > gills > muscle.

Correlation between the concentrations of heavy metals in water (mg/L) and in *Tilapia zillii* tissues (ppm)

The Pearson coefficient (r) has indicated that the concentrations of different heavy metals in various organs of *Tilapia zillii* collected from the polluted drainage canal were greatly dependent on the concentrations of these metals in the raw water (Table 5). The relationship between concentrations of heavy metals in fish organs and external water environment was variable between negative and positive correlations. In polluted drainage canal, levels of Fe, Mn, and Cr in water exhibited positive correlation with those in all organs of *Tilapia zillii*. In addition, Pb in water showed a strong positive correlation with Pb in muscles and gills, despite the strong negative correlation in livers. Pb in water showed a strong positive correlation with that in gill tissue in the fish farm and Zn in water showed a strong positive correlation with Zn in liver tissue in the fish farm (Table 5).

Chromosomal aberrations

The diploid chromosome number of *T. zillii* was 2n = 44 (Fig. 1a). Different types of aberrations were observed in gill cells of *T. zillii*. Both structural and numerical types of chromosomal aberrations are shown in Fig. 1 and Table 6. Structural chromosomal aberrations included stickiness (Fig. 1b), fragmented chromosomes (Fig. 1c), centromeric gaps (Fig. 1e), chromatid break (Fig. 1f), and chromatid deletion (Fig. 1g), while numerical chromosomal aberrations included polyploidy (Fig. 1h).

The current study showed that there is a very significant increase in the number of chromosomal aberrations between the polluted drainage canal and the fish farm (Table 6).

Table 3 Concentrations of heavy metals in *Tilapia zillii* tissues ($n=9$) (ppm). Samples were collected from the polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt, and compared with the permissible limits

Metal[a]	Polluted drainage canal			Fish farm			Permissible limits (ppm)
	Distribution in different parts (ppm)			Distribution in different parts (ppm)			
	Muscle	Gills	Liver	Muscle	Gills	Liver	
Fe	28.01 ± 1.98^{c}	40.00 ± 5.00^{b}	81.56 ± 3.16^{a}	0.66 ± 0.06^{e}	1.27 ± 0.12^{e}	8.47 ± 0.45^{d}	100
Zn	154.00 ± 3.61^{bc}	168.67 ± 10.26^{b}	200.00 ± 20.00^{a}	37.00 ± 2.00^{e}	68.00 ± 2.65^{d}	142.33 ± 19.66^{c}	40
Cu	12.50 ± 0.50^{c}	15.83 ± 1.04^{b}	22.50 ± 2.50^{a}	9.00 ± 0.50^{d}	nd^{e}	nd^{e}	30
Cd	6.00 ± 0.50^{c}	9.00 ± 0.50^{b}	11.67 ± 1.04^{a}	0.05 ± 0.01^{d}	0.32 ± 0.41^{d}	0.24 ± 0.01^{d}	0.5
Pb	77.93 ± 2.10^{a}	46.30 ± 2.52^{c}	50.27 ± 1.62^{b}	1.65 ± 0.13^{d}	0.39 ± 0.01^{d}	0.31 ± 0.02^{d}	2.0
Mn	0.19 ± 0.02^{d}	0.41 ± 0.04^{b}	0.31 ± 0.03^{c}	0.18 ± 0.02^{d}	0.70 ± 0.10^{a}	0.45 ± 0.05^{b}	0.01
Ni	0.48 ± 0.03^{d}	0.60 ± 0.05^{c}	0.78 ± 0.08^{b}	0.29 ± 0.01^{e}	0.47 ± 0.081^{d}	1.50 ± 0.05^{a}	ni
Cr	0.86 ± 0.05^{c}	1.31 ± 0.17^{b}	3.17 ± 0.29^{a}	0.51 ± 0.03^{d}	0.65 ± 0.05^{cd}	0.75 ± 0.05^{cd}	1.0

Within a row, means that do not differ at $P=0.05$ are followed by the same superscript (Duncan's multiple range test) among different tissues for each group. $P = <0.0001$

nd = not detected, ni = no information about maximum permissible limit in fish tissue

[a]Fe = iron, Zn = zinc, Cu = copper, Cd = cadmium, Pb = lead, Mn = manganese, Ni = nickel, and Cr = chromium

Table 4 Bioaccumulation of heavy metals in *Tilapia zillii* tissues (samples were collected from the polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt)

Metal[a]	Polluted drainage canal			Fish farm		
	Distribution in different parts (ppm)			Distribution in different parts (ppm)		
	Muscle	Gills	Liver	Muscle	Gills	Liver
Fe	28.59	40.82	83.22	0.99	1.92	12.83
Zn	248.39	272.04	322.58	482.61	886.96	1856
Cu	96.15	121.77	173.08	nd	nd	nd
Cd	200	300	389	5	32	24
Pb	487.06	289.38	314.19	41.25	9.75	7.75
Mn	0.86	1.86	1.41	1.80	7.00	4.50
Ni	3.43	4.29	5.57	7.83	7.83	25.00
Cr	2.61	3.97	9.61	6.38	8.13	9.38

[a]Fe = iron, Zn = zinc, Cu = copper, Cd = cadmium, Pb = lead, Mn = manganese, Ni = nickel, and Cr = chromium

nd = not detected

Table 5 Relationship between the concentrations of heavy metals in water (mg/L) and in *Tilapia zillii* tissues (ppm). Samples were collected from the polluted drainage canal and fish farm, which is located in Abiece region in front of village number 10, Alexandria governorate, Egypt. Pearson correlation coefficient (*r*), two−tailed

Location	Tissues	Concentrations of heavy metals in water[a] (mg/L)							
		Fe	Zn	Cu	Cd	Pb	Mn	Ni	Cr
Polluted drainage canal	Muscle	1.00**	0.42	0.87	0.00	0.88	0.95	−0.98	0.55
	Gills	1.00**	−0.04	−0.28	0.00	0.90	0.24	−0.87	0.98
	Liver	0.98	0.76	0.87	−0.28	−0.08	0.36	0.19	0.97
Fish farm	Muscle	0.24	−0.87	nd	−0.50	−0.76	0.76	−0.91	0.63
	Gills	−0.33	−0.33	nd	−0.51	0.87	−0.87	0.09	−0.87
	Liver	−0.96	0.98	nd	0.50	−0.72	0.12	−0.28	−0.87

[a]Fe = iron, Zn = zinc, Cu = copper, Cd = cadmium, Pb = lead, Mn = manganese, Ni = nickel, and Cr = chromium

nd = not detected

** $P =$ or < 0.01

Micronucleus (MNs) test

The examination of peripheral blood smears from the fish under study showed that normal erythrocytes had an oval shape and an ellipsoid centric nucleus with a clearly defined border (Fig. 2a). There were found to be the following nuclear abnormalities: some small, non−refractive circular or ovoid particles in the cytoplasm that resemble a nucleus with respect to staining properties were considered as micronuclei (Fig. 2b), blebbed nucleus appeared as a small evagination of the nuclear envelope that resembles a micronucleus (Fig. 2c), segmented nucleus as an asymmetrical hourglass−shaped nuclei (Fig. 2d), enucleated erythrocyte as a red blood cells without a nucleus (Fig. 2e), kidney−shaped nucleus appeared as nuclei with a kidney−shaped profile (Fig. 2f), heart−shaped nucleus appeared

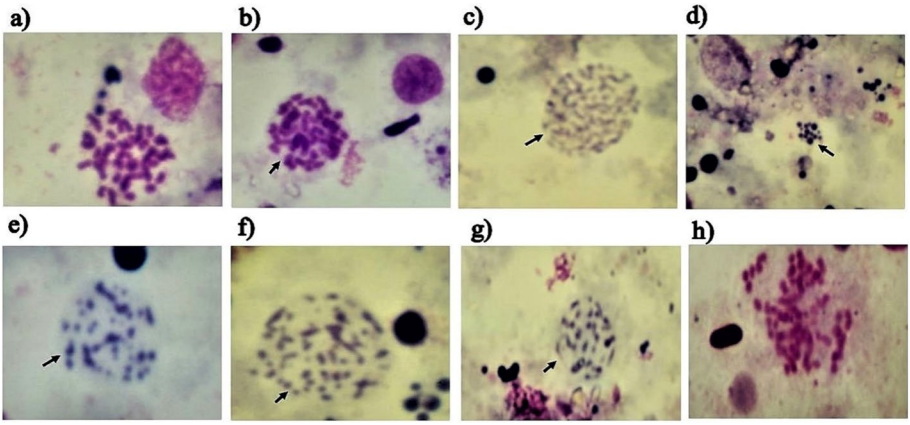

Fig. 1 Photomicrographs of normal metaphase complements of *Tilapia zillii* in gills (**a**), stickiness (**b**), fragmented chromosomes (**c**), fragmented nucleus (**d**), centromeric gaps (**e**), chromatid break (**f**), chromatid deletion (**g**), and tetraploid (**h**)

as nuclei with a heart−shaped profile (Fig. 2g), polymorphic irregular nuclei outlines and no consistent pattern (Fig. 2h), binucleated cell contains two nuclei that are relatively similar in size and not attached (Fig. 2i, j), nuclear fragmented erythrocyte (Fig. 2k), long nucleus (Fig. 2l), nuclei showing a deep invagination toward the center were considered a notched nucleus (Fig. 2m), some lobed nuclei were observed (Fig. 2n), different shapes of fusion (Fig. 2o, p), necrotic erythrocyte (Fig. 2q), and vacuolated nucleus with a vacuole that surround the nucleus (Fig. 2r).

Frequencies (%) of normal nucleus (N), nuclear abnormalities (NA), and total NA in erythrocytes of *Tilapia zillii* are summarized in Table 7. Overall, the most frequent abnormality in the polluted drainage canal group was the polymorphic irregular nuclei, sequentially followed by LN, BC, SN, MN, KSN, HSN, LBN, FE, BN, EE, VN, NN, and finally NE. The polymorphic irregular nucleus was 13.17% of the total of erythrocyte nuclear morphological abnormalities (70.33%). The most frequent abnormality in the fish farm group was the PN, sequentially followed by SN, MN, LN, and finally LBN. The polymorphic irregular nucleus was 0.70% of the total of erythrocyte nuclear morphological abnormalities (1.78%). MN frequency was found to be 5.58 in drainage canal group and 0.32 in fish farm group (Table 7).

Correlation between the frequencies (%) of normal nucleus (N), nuclear abnormalities (NA), and the concentrations of heavy metals in water

Results from correlation analysis performed between the frequencies (%) of N and NA are shown in Table 8. In the polluted drainage canal, the mean frequencies of MN were positively correlated with the concentration of Cu ($r = 0.95$), Pb ($r = 0.96$), and Ni ($r = 0.95$). The frequencies of BN were also correlated with the concentration of Fe ($r = 0.72$), Cu ($r = 0.69$), Cd ($r = 0.97$), Pb ($r = 0.65$), Mn ($r = 0.97$), and Ni ($r = 0.69$). There was also a positive relation between the frequencies of PN and the concentration of Pb ($r = 0.90$). On the other hand, no positive correlations were found between the frequencies of PN and the concentration of Fe, Zn, Cu, Cd, Mn, Ni, and Cr.

Table 6 The number of types of chromosomal aberrations in the gill cells of *Tilapia zillii*. Samples were collected from the polluted drainage canal in front of village number 10 and the fish farm (Abiece region, Alexandria government, Egypt)

Location	Individual	Number of examined cells	Number of normal cells	Number of aberrant cells	Total number of chromosomal aberrations	The number of types of chromosomal aberrations					
						Stickiness	Fragments	Gaps	Break	Deletions	Polyploidy
Polluted drainage canal	1	100	43	57	75	55	7	3	2	5	3
	2	100	40	60	68	46	9	3	2	5	3
	3	100	45	55	63	35	10	3	3	7	5
	Total	300	128**	172**	206**	136**	26**	9**	7**	17**	11**
Fish farm (control group)	1	100	93	7	8	6	1	1	0	0	0
	2	100	92	8	11	8	2	1	0	0	0
	3	100	90	10	10	7	2	1	0	0	0
	Total	300	275**	25**	29**	21**	5**	3**	0**	0**	0**

**Difference was significant when compared to the control group ($P < 0.001$)

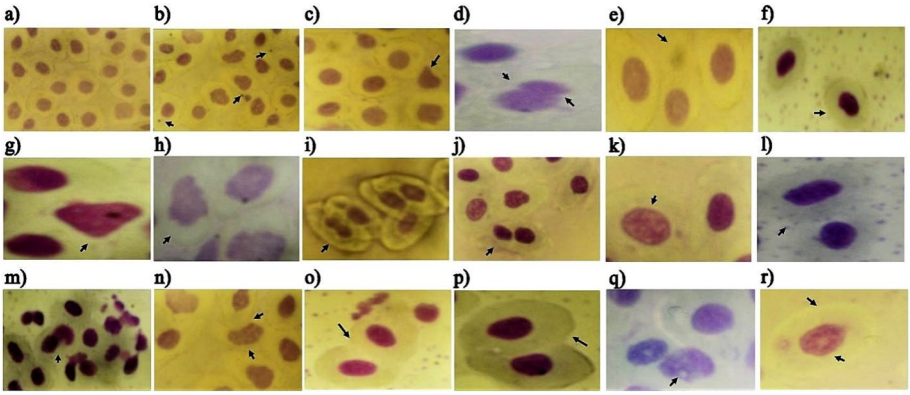

Fig. 2 Photomicrographs showing cells with normal nucleus (**a**) and nuclear abnormalities (arrows) in peripheral blood erythrocytes of *Tilapia zillii*: micronuclei (**b**), blebbed nucleus (**c**), segmented nucleus (**d**), enucleated erythrocyte (**e**), kidney-shaped nucleus (**f**), heart-shaped nucleus (**g**), polymorphic irregular nuclei (**h**), binucleated cell (**i** and **j**), nuclear fragmented erythrocyte (**k**), long nucleus (**l**), putative fragmented notched nucleus (**m**), lobed nuclei (**n**), different shapes of fusion (**o** and **p**), necrotic erythrocyte (**q**), and vacuolated nucleus (**r**)

In the fish farm, the mean frequencies of MN were positively correlated with the concentration of Cd ($r = 1.00$) and Pb ($r = 0.87$). There was also a positive relation between the frequencies of PN and the concentration of Pb ($r = 0.50$), Mn ($r = 0.87$), and Ni ($r = 0.91$) (Table 8).

Discussion

Water pollution is the greatest public health problem and environmental facing aquaculture in Egypt (Anwar 2003). In this study, all water quality parameters were within standards and suggested that the water conditions from the polluted drainage canal and the fish farm were suitable for fish to live (Boyd 1982). Fe, Zn, Cu, Cd, Pb, Mn, Ni, and Cr in the polluted drainage canal and the fish farm were significantly different ($P < 0.05$). However, the Cd, Pb, Mn, Ni, and Cr concentrations in the polluted drainage canal and Ni concentration in the fish farm exceeded the limits of water standard. Fe, Zn, Co, Cu, and Mn are classified as essential metal elements that have poisonous potentials when they are higher than the safe levels (Canli and Atli 2003). Cd, Pb, Ni, and Cr are classified as non−essential metal ions that may cause toxicities as they are at trace levels. Several studies have reported that toxic metals at high levels could cause genotoxicity, mutation, and cancer for aquatic animals and humans (Tchounwou et al. 2012).

Heavy metals and toxins can enter the aquatic system from different natural and anthropogenic sources. So, in the polluted drainage canal, there are industrial activities that can introduce toxic metals to water. In addition, discharge of different treated and untreated liquid wastes to the water may introduce large quantities of toxic metals to the water. Gaber et al. (2013) found that the El−Rahawy drain at El−Rahawy village, Egypt, has greater amounts of heavy metals in the water than the river Nile, and this is because sewage and other pollutants are discharged there. Fish blood and tissues were very toxic when exposed to heavy metals (Rajeshkumar and Munuswamy 2011). Gaber et al. (2013) reported that

Table 7 Frequencies (%) of normal nucleus (N), nuclear abnormalities (NA), and total NA in erythrocytes of *Tilapia zillii* collected from the polluted drainage canal in front of village number 10 and the fish farm (Abiece region, Alexandria government, Egypt)

Frequencies (%) [a]	Location	
	Polluted drainage canal	Fish farm
N*	29.67 ± 3.27	98.33 ± 0.38
MN	5.58 ± 0.66	0.32 ± 0.10
BN	3.17 ± 0.98	nd
SN	7.17 ± 1.60	0.38 ± 0.10
EE	2.50 ± 0.84	nd
KSN	4.42 ± 0.49	nd
HSN	3.67 ± 0.52	nd
PN	13.17 ± 2.14	0.70 ± 0.09
BC	8.00 ± 0.98	nd
NFR	1.17 ± 0.98	nd
LN	8.50 ± 0.84	0.22 ± 0.12
NN	1.83 ± 0.41	nd
LBN	3.67 ± 0.82	0.17 ± 0.05
FE	3.50 ± 0.84	nd
NE	1.67 ± 1.03	nd
VN	2.33 ± 0.82	nd
Total NA*	70.33 ± 3.28	1.78 ± 0.21

Data given as mean ± SD ($n = 3$ fish/location). 2000 red cells were counted/fish

[a]N = normal nucleus, MN = micronuclei, BN = blebbed nucleus, SN = segmented nucleus, EE = enucleated erythrocyte, KSN = kidney-shaped nucleus, HSN = heart-shaped nucleus, PN polymorphic irregular nuclei, BC = binucleated cell, NFR = nuclear fragmented erythrocyte, LN = long nucleus, NN = notched nucleus, LBN = lobed nuclei, FE = fused erythrocytes, NE = necrotic erythrocyte, VN = vacuolated nucleus, and total NA = total of erythrocyte nuclear morphological abnormalities

*Difference was significant when compared to the control group ($P < 0.001$)

nd = not detected

the bad water quality due to pollution increased blood parameters in African catfish *Clarias gariepinus* caught from El−Rahawy drain than those of river Nile.

In the present study, results of the heavy metal analysis showed that Cd, Pb, Mn, Ni, and Cr in water in the polluted drainage canal and Zn, Cd, Pb, and Mn in *T. zillii* muscle samples exceeded the permissible limits defined by (APHA). The concentrations of the most metals in the organs were in the order of liver > gills > muscle. Similar results reported that the muscle has less accumulation than the gills and liver (Ben Salem et al. 2014; Rajeshkumar and Li 2018; Varol et al. 2020). Our results showed that Fe, Zn, Cu Cd, and Ni indicated higher bioaccumulation in the liver. However, Pb recorded higher concentration in muscle. Dissimilar results reported the highest levels of Pb in the liver of *Cyprinus carpio*, *Pelteobagrus fulvidraco* (Rajeshkumar and Li 2018), and *Dicentrarchus labrax* (Zaghloul et al. 2024). Generally, metals accumulate in higher extent in fish liver followed by gills and kidney (Ben Salem et al. 2014). The liver is recognized as the active central site of metal absorption and storage and plays a critical role for both excretion and

Table 8 Correlation analysis between the frequencies (%) of normal nucleus (N), nuclear abnormalities (NA), and the concentrations of heavy metals in water (mg/L)

Location	Frequencies (%)[a]	Metal[b]							
		Fe	Zn	Cu	Cd	Pb	Mn	Ni	Cr
Polluted drainage canal	N	0.93	0.10*	−0.36	0.63	−0.42	0.63	−0.36	0.10
	MN	−0.33	−0.62	0.95	0.19	0.96	0.19	0.95	−0.58
	BN	0.72	0.15	0.69	0.97	0.65	0.97	0.69	0.50
	SN	−0.75	−0.91	0.69	−0.28	0.74	−0.28	0.69	−0.89
	EE	1.00**	0.95	0.00	0.87	−0.06	0.87	0.00	0.96
	KSN	0.33	0.62	−0.95	−0.19	−0.96	−0.19	−0.95	0.58
	HSN	0.00	−0.33	1.00**	0.50	0.10*	0.50	1.00**	−0.28
	PN	−0.37	−0.05	−0.93	−0.79	0.90	−0.79	−0.93	−0.10
	BC	0.50	0.19	0.87	0.87	0.83	0.87	0.87	0.24
	NFR	−0.72	−0.91	0.69	−0.28	0.74	−0.28	0.69	−0.89
	LN	−0.50	−0.76	0.87	0.00	0.90	0.00	0.87	−0.72
	NN	0.00	0.33	−1.00**	−0.50	−1.00*	−0.50	−1.00**	0.28
	LBN	0.00	0.33	−1.00**	−0.50	−1.00*	−0.50	−1.00**	0.28
	FE	−0.50	−0.19	−0.87	−0.87	−0.83	−0.87	−0.87	−0.24
	NE	−0.87	−0.66	−0.50	−1.00**	−0.44	−1.00*	−0.50	−0.69
	VN	0.00	−0.33	1.00**	0.50	1.00*	0.50	1.00**	−0.28
Fish farm	N	0.87	0.95	nd	0.19	−0.33	−0.78	−0.97	0.95
	MN	−0.33	0.50	nd	1.00**	0.87	0.50	−0.42	0.50
	BN	nd	nd	nd	nd	nd	nd	nd	nd
	SN	−0.98	−0.50	nd	0.50	0.87	1.00**	0.58	−0.50
	EE	nd	nd	nd	nd	nd	nd	nd	nd
	KSN	nd	nd	nd	nd	nd	nd	nd	nd
	HSN	nd	nd	nd	nd	nd	nd	nd	nd
	PN	−0.95	−0.87	nd	0.00	0.50	0.87	0.91	−0.87
	BC	nd	nd	nd	nd	nd	nd	nd	nd
	NFR	nd	nd	nd	nd	nd	nd	nd	nd
	LN	−0.37	−0.95	nd	−0.78	−0.33	0.19	0.91	−0.95
	NN	nd	nd	nd	nd	nd	nd	nd	nd
	LBN	−0.66	−1.00**	nd	−0.50	0.00	0.50	0.10	−1.00**
	FE	nd	nd	nd	nd	nd	nd	nd	nd
	NE	nd	nd	nd	nd	nd	nd	nd	nd
	VN	nd	nd	nd	nd	nd	nd	nd	nd

[a]N = normal nucleus, MN = micronuclei, BN = blebbed nucleus, SN = segmented nucleus, EE = enucleated erythrocyte, KSN = kidney-shaped nucleus, HSN = heart- shaped nucleus, PN = polymorphic irregular nuclei, BC = binucleated cell, NFR = nuclear fragmented erythrocyte, LN = long nucleus, NN = notched nucleus, LBN = lobed nuclei, FE = fused erythrocytes, NE = necrotic erythrocyte, and VN = vacuolated nucleus

[b]Fe = iron, Zn = zinc, Cu = copper, Cd = cadmium, Pb = lead, Mn = manganese, Ni = nickel, and Cr chromium

nd = not detected

*$P < 0.05$, **$P =$ or < 0.01

detoxification (Kim and Kang 2004). Gills can be more susceptible to contamination than other organs owing to permanent contact with the water and have a fast respiratory. Several studies reported that the gills were sensitive and target tissue for water pollution monitoring (Suchana et al. 2021).

Concentrations of heavy metals in fish organs and external water environment were variable between negative and positive correlations and metal accumulation varied between organs. Accumulation of heavy metals by aquatic organisms may be selective or passive; and differences in accumulation of heavy metals could be as a result of differences in egestion, assimilation, or both (Rajeshkumar and Li 2018). The concentrations of heavy metals in aquatic organism have been extensively studied over the past several decades. According to research, the accumulation of heavy metals in aquatic organisms is dependent on the metal types, species, size, habit of the organisms, and the tissues (Rajeshkumar and Li 2018; Zaghloul et al. 2024).

Data of chromosomal aberration frequency in the present study showed that there was a positive correlation with the concentrations of heavy metals in fish. Various chromosomal aberrations were observed in the spreads of gill cells of *Tilapia zillii* at the polluted drainage canal. These aberrations include stickiness, fragmented chromosomes, fragmented nucleus, centromeric gaps, chromatid break, chromatid deletion, and tetraploid. It has been observed that the percentage of stickiness in the chromosomes was very highly significantly increased at the polluted drainage canal, compared to that of the control group (fish farm). This finding coincides with that of Seehy et al. (2009) who reported that the stickiness was the most common type in the Nile tilapia collected from the polluted drainage canal in Alexandria governorate, Egypt. Stickiness is a form of unknown chromosomal "agglutination" that gives chromosomes a sticky or pycnotic appearance. Stickiness may result in the creation of sticky bridges at anaphase and sticky adhesion between two or more chromosomes. Abnormalities during S−phase in DNA duplication lead to chromosomal aberration (Gemble et al. 2022). Changes in chromosome number (gain or loss of single chromosome or sets of chromosomes) and changes in chromosome structure (break, deletion, rearrangement, …, etc.).

Various chromosomal aberrations were observed in Nile tilapia *Oreochromis niloticus* population collected from five locations in Minia governorate, Egypt, and ring chromosome was the most common type (Anwar and Abu Shnaf 2023). Samples collected from irrigation drain and Bahr Yousef reported the highest aberration frequency. Chromosomal aberration frequency was positively correlated with the heavy metal concentrations where their concentration exceeded the limits defined by WHO in the surface water of irrigation drain and Bahr Yousef as well as the Pb concentration in muscles (Anwar and Abu Shnaf 2023). In addition, chromosomal abnormalities were reported for *O. niloticus* when exposed to diet contaminated with Cd, Cu, and their mixture (El-Serafy et al. 2015). Chromosomal aberration frequency (chromatid deletion, chromatid gap, chromosome gap, ring chromosome, fragments, stickiness, haploidy and polyploidy) was increased in the channel catfish *Channel punctatus* due to the toxic metals (Yadav and Trivedi 2009). Several studies have reported that the genotoxicity of heavy metals especially Ni, Cd, and Pb affected the aquatic fish. Ni and Cd can induce point mutation, deletion, ploidy, and DNA damage (Silbergeld 2003; Soulivongsa et al. 2020). Pb may cause indirect genotoxicity, DNA damage, mitogenesis, and alterations in gene transcription (Silbergeld 2003).

Number of normal cells, cell number with chromosome aberrations, and types of chromosomal aberrations from two different locations were significantly different ($P < 0.001$) due to heavy metal concentrations in the environment as well as their concentration in the fish organs. The percentage of chromosomal aberrations was 57.3% in the gill

cells of *Tilapia zillii* from the polluted drainage canal and 8.3% in the fish farm. This study showed that the chromosomal aberrations increased statistically in *Tilapia zillii* collected from the polluted drainage canal in Alexandria, Egypt, compared to fish collected from the fish farm.

The higher number of MN represents the highest level of trace element pollution and other trace contamination in the bodies of aquatic organisms (Dourado et al. 2016). The concentration of Zn, Pb, Cu, and Cd in Nile tilapia was accompanied by a rise in the frequency of micronuclei (El-Sappah et al. 2022). However, it is not always the case. For instance, the levels of pollutants in the sediments, bile, or liver of the white croaker *Genyonemus lineatus* did not consistently correlate with aberrant abnormalities in the erythrocyte nuclear morphology (Carrasco et al. 1990). In binucleated cell, the nuclear membranes of the two nuclei should be intact; they should be approximately equal in size, staining pattern, and intensity and may touch but not overlap each other (Fenech 2000). Frequency of binucleated cell is an indicator of abnormal cell division due to the blocking of cytokinesis, which could result in genetic imbalance in the cells and may be involved in carcinogenesis (Rodilla 1993).

This study demonstrated that the PN type was the most frequent abnormality in the erythrocyte occurring in *Tilapia zillii* in both groups. However, the total of erythrocyte nuclear morphological abnormalities was 70.33% in the polluted drainage canal and 1.78% in the fish farm. Similar results have been reported with the highest frequency of polymorphic type occurring in the erythrocyte of the grey mullets (Carrola et al. 2014). The total average frequency of the nuclear morphological abnormalities in the erythrocyte of the grey mullets ranged from 73% in the Mondego to 108% in the Ave. The polymorphic type was the most frequent abnormality in the erythrocyte, sequentially followed by the blebbed/lobed/notched, the segmented, the kidney shaped, the vacuolated, the micronucleus, and finally the binucleated. The polymorphic type was typically ≥ 50% of the total erythrocyte nuclear morphological abnormalities (Carrola et al. 2014). Irregularly shaped nuclei was the most frequent abnormality observed in the erythrocyte of gilthead sea bream *Sparus aurata* (L.), while the frequency of micronuclei, binucleated, and vacuolated nuclei was significantly lower. In addition, Strunjak-Perovic et al. (2009) concluded that the nuclear abnormalities in the erythrocyte of gilthead sea bream *Sparus aurata* (L.) may originate from genetic disorders not necessarily induced by pollutants and that the expression of those non−pollution−related erythrocyte nuclear abnormalities may depend on environmental conditions, such as salinity, dissolved oxygen, and temperature.

The result showed that the mean percentage of MN was 5.58% in the polluted drainage canal and 0.32% in the fish farm. The highest frequency of MN biosynthesis occurring in European minnow *Phoxinus phoxinus* (Ayllon and Garcia–Vazquez 2000), eel *Anguilla anguilla* (Sanchez-Galan et al. 2001), crucian carp *Carassius auratus* gibelio Bloch., Nile tilapia *Oreochromis niloticus* (Seehy et al 2009), brown trout *Salmo trutta* (Sánchez-Galán et al. 1998), common carp *Cyprinus carpio* (García-Medina et al. 2017), African mudfish *Clarias gariepinus* (Alimba et al. 2017), and zebra fish *Danio rerio* (Canedo et al. 2021).

According to Seehy et al. (2009), the percentage of normal cells in the Nile tilapia fish farm group was observed to be 93.3%, while the percentage of micronuclei was found to be 3.7%. However, the drainage canal group showed that 21.5% of the cells had micronuclei and 78.5% of the cells were normal. It was observed that there is significant mutagenicity (micronuclei and nuclear abnormalities) in blood cells of *T. zillii* in the polluted drainage canal when compared to the fish farm. The concentration of heavy metals negatively affects *T. zillii* fish.

The frequency of MN at polluted sites in control mussels and caged in the Ligurian Sea ranged from 1.8 to 24% (Nigro et al. 2006), in the Algerian coast ranged from 1.2 to 11.8% (Taleb et al. 2009), and in the coast of Greece ranged from 2 to 12% (Kalpaxis et al. 2004). The higher frequency of MN (11.6%) was reported in Caetagena (Fernández et al. 2011). This study could be used as criteria for determining the bioaccumulation and genotoxic effects of different toxic metals in various tissues of *Tilapia zillii*, which may be used to prevent the inflow of polluted domestic and industrial sewage in the aquatic environment.

Conclusions

The current study was conducted to investigate the cytotoxic effects and bioaccumulation of heavy metals in different parts of *Tilapia zillii.* Various chromosomal aberrations were observed in the gill cells of *Tilapia zillii* and stickiness was the most common. A diversity of the nuclear abnormalities in erythrocyte of the *Tilapia zillii* was found in the fish. The total average frequency of the nuclear abnormalities ranged from 70% in the polluted drainage canal to 2% in the fish farm (unpolluted). The erythrocyte nuclear abnormalities found were divided as follows: MN, BN, SN, EE, KSN, HSN, PN, BC, NFR, LN, NN, LBN, FE, NE, and VN. This study suggested that the concentrations of heavy metal negatively affect *T. zillii* fish. Heavy metals can poison humans; therefore, we should enforce strict control methods to keep the concentrations of heavy metal below the acceptable limits in the fish we consume.

Author contribution N.A. conducted and designed the experiment, collected samples, methodology, performed the statistical analyses, analyzed the results, prepared figures, wrote the manuscript, and revised the final manuscript.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Ethics approval All experiments were conducted at the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. All experimental protocols followed in this study were approved by the Alexandria University Institutional Animal Care and Use Committee (ALEXU-IACUC) (Reference number 19/23/03/30/3/31) before the experiment was initiated and followed Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) protocols and guidelines. The study was carried out in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and The American Veterinary Medical Association (AVMA) guidelines for the Euthanasia of Animals (2020).

Competing interests The author declares no competing interests.

References

Abu Shnaf ASM, Abd El-Aziz H, Ata AM (2021) Cyto-histopathological and protein polymorphism alterations in five populations of Nile tilapia (*Oreochromis niloticus*) as biomonitor for water heavy metal pollution. J Fish Biol 99(3):999–1009

Al-Sabti K, Metcalfe CD (1995) Fish micronuclei for assessing genotoxicity in water. Mutat Res Toxicol 343:121–135

Ali D, Almarzoug M, Ali H, Samdani M, Hussain S (2020) Fish as bioindicators to determine the effects of pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay. J King Saud Univ - Sci 32:2880–2885. https://doi.org/10.1016/j.jksus.2020.07.012

Ali FK, El-Shehawi AM, Seehy MA (2008) Micronucleus test in fish genome: a sensitive monitor for aquatic pollution. African J Biotechnol 7:606–612

Alimba CG, Ajiboye RD, Fagbenro OS (2017) Dietary ascorbic acid reduced micronucleus and nuclear abnormalities in Clarias gariepinus (Burchell 1822) exposed to hospital effluent. Fish Physiol Biochem 43:1325–1335. https://doi.org/10.1007/s10695-017-0375-y

Anwar WA (2003) Environmental health in Egypt. Int J Hyg Environ Health 206(4–5):339–350

Anwar GM, Abu Shnaf ASM (2023) Mitotic chromosomal abnormalities and DNA polymorphism in the Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) as a biomarker for water pollution by heavy metals. J Fish Biol 102:204–213. https://doi.org/10.1111/jfb.15252

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington, D.C.

Arumugam M, Jayaraman S, Sridhar A et al (2023) Recent advances in tilapia production for sustainable developments in Indian Aquaculture and Its Economic Benefits. Fishes 8:176. https://doi.org/10.3390/fishes8040176

Asad F, Ahmed I, Saleem M, Iqbal T (2010) Hormonal masculinization and growth performance in Nile tilapia (*Oreochromis niloticus*) by androgen administration at different dietary protein levels. 12:939–943

Ayllon F, Garcia-Vazquez E (2000) Induction of micronuclei and other nuclear abnormalities in European minnow Phoxinus phoxinus and *mollie Poecilia* latipinna: an assessment of the fish micronucleus test. Mutat Res Genet Toxicol Environ Mutagen 467:177–186

Ben Salem Z, Capelli N, Laffray X et al (2014) Seasonal variation of heavy metals in water, sediment and roach tissues in a landfill draining system pond (Etueffont, France). Ecol Eng 69:25–37. https://doi.org/10.1016/j.ecoleng.2014.03.072

Boyd CE (1982) Water Quality Management for Pond Fish Culture. Elsevier, Amsterdam, p 318

Bishai H, Khalil M (1997). Freshwater fishes of Egypt. Egyptian Environmental Affairs Agency (EEAA production), National Biodiversity Unit, 9: 229

Canedo A, de Jesus LWO, Bailão EFLC, Rocha TL (2021) Micronucleus test and nuclear abnormality assay in zebrafish (*Danio rerio*): past, present, and future trends. Environ Pollut 290:118019. https://doi.org/10.1016/j.envpol.2021.118019

Canli M, Atli G (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ Pollut 121:129–136. https://doi.org/10.1016/S0269-7491(02)00194-X

Carrasco KR, Tilbury KL, Myers MS (1990) Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Can J Fish Aquat Sci 47:2123–2136. https://doi.org/10.1139/f90-237

Carrola J, Santos N, Rocha MJ et al (2014) Frequency of micronuclei and of other nuclear abnormalities in erythrocytes of the grey mullet from the Mondego, Douro and Ave estuaries—Portugal. Environ Sci Pollut Res 21:6057–6068. https://doi.org/10.1007/s11356-014-2537-0

De Flora S, Bagnasco M, Zanacchi P (1991) Genotoxic, carcinogenic, and teratogenic hazards in the marine environment, with special reference to the Mediterranean Sea. Mutat Res Genet Toxicol 258:285–320. https://doi.org/10.1016/0165-1110(91)90013-L

Dourado PLR, da Rocha MP, Roveda LM et al (2016) Genotoxic and mutagenic effects of polluted surface water in the midwestern region of Brazil using animal and plant bioassays. Genet Mol Biol 40:123–133. https://doi.org/10.1590/1678-4685-gmb-2015-0223

Duncan DB (1955) Multiple range and multiple F tests. Biometrics 11:1. https://doi.org/10.2307/3001478

El-Sappah AH, Seif MM, Abdel-Kader HH, et al (2022) Genotoxicity and trace elements contents analysis in Nile tilapia (*Oreochromis niloticus*) indicated the levels of aquatic contamination at three Egyptian areas. Front Vet Sci 9. https://doi.org/10.3389/fvets.2022.818866

El-Sayed A (2006) Tilapia Culture.CABI Publishing, Wallingford, Oxon, UK, 294 pp

El-Serafy SS, Zowail ME, Abdel-Hameid N-AH et al (2015) High dietborne Cu and Cd induced genotoxicity of Nile tilapia (*Oreochromis niloticus*). Environ Toxicol Pharmacol 39:1139–1147. https://doi.org/10.1016/j.etap.2015.04.001

El-Sayed AM, Fitzsimmons K (2023) From Africa to the world—the journey of Nile tilapia. Rev Aquac 15:6–21. https://doi.org/10.1111/raq.12738

Emon F, Rohani MF, Sumaiya N et al (2023) Bioaccumulation and bioremediation of heavy metals in fishes—a review. Toxics 11:510. https://doi.org/10.3390/toxics11060510

Fenech M (2000) The in vitro micronucleus technique. Mutat Res Mol Mech Mutagen 455:81–95. https://doi.org/10.1016/S0027-5107(00)00065-8

Fernández B, Campillo JA, Martínez-Gómez C, Benedicto J (2011) Micronuclei and other nuclear abnormalities in mussels (*Mytilus galloprovincialis*) as biomarkers of cyto-genotoxic pollution in Mediterranean waters. Environ Mol Mutagen 52:479–491. https://doi.org/10.1002/em.20646

Fitzsimmons K (2000) Tilapia: the most important aquaculture species in the 21st century In: Fitzsimmons K, Filho JC (eds) Tilapia aquaculture in the 21st century. Proceedings from the fifth international symposium on tilapia aquaculture. Rio Janeiro, Brazil 1

Gaber H, El-Kasheif M, Ibrahim S, Authman M (2013) Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish. World Appl Sci J 21:329–341

García-Medina S, Galar-Martínez M, Gómez-Oliván LM et al (2017) Relationship between genotoxicity and oxidative stress induced by mercury on common carp (*Cyprinus carpio*) tissues. Aquat Toxicol 192:207–215. https://doi.org/10.1016/j.aquatox.2017.09.019

Gemble S, Wardenaar R, Keuper K et al (2022) Genetic instability from a single S phase after whole-genome duplication. Nature 604:146–151. https://doi.org/10.1038/s41586-022-04578-4

Jha AN, Cheung VV, Foulkes ME et al (2000) Detection of genotoxins in the marine environment: adoption and evaluation of an integrated approach using the embryo–larval stages of the marine mussel, *Mytilus edulis*. Mutat Res Toxicol Environ Mutagen 464:213–228. https://doi.org/10.1016/S1383-5718(99)00188-6

Kalpaxis DL, Theos C, Xaplanteri MA et al (2004) Biomonitoring of Gulf of Patras, N. Peloponnesus, Greece. application of a biomarker suite including evaluation of translation efficiency in *Mytilus galloprovincialis* cells. Environ Res 94:211–220. https://doi.org/10.1016/S0013-9351(03)00048-3

Kim S-G, Kang J-C (2004) Effect of dietary copper exposure on accumulation, growth and hematological parameters of the juvenile rockfish, Sebastes schlegeli. Mar Environ Res 58:65–82. https://doi.org/10.1016/j.marenvres.2003.12.004

Luzhna L, Kathiria P, Kovalchuk O (2013) Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet 4. https://doi.org/10.3389/fgene.2013.00131

Masoud MS, El-Samra MI, El-Sadawy MM (2007) Heavy-metal distribution and risk assessment of sediment and fish from El Mex Bay, Alexandria. Egypt Chem Ecol 23:201–216

Nigro M, Falleni A, Del BI et al (2006) Cellular biomarkers for monitoring estuarine environments: transplanted versus native mussels. Aquat Toxicol 77:339–347. https://doi.org/10.1016/j.aquatox.2005.12.013

Osman AGM, Abd El Reheem A-E-BM, Moustafa MA et al (2011) In situ evaluation of the genotoxic potential of the river Nile: I. micronucleus and nuclear lesion tests of erythrocytes of *Oreochromis niloticus niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). Toxicol Environ Chem 93:1002–1017. https://doi.org/10.1080/02772248.2011.564496

Rajeshkumar S, Li X (2018) Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicol Reports 5:288–295. https://doi.org/10.1016/j.toxrep.2018.01.007

Rajeshkumar S, Munuswamy N (2011) Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, South East Coast, India. Chemosphere 83:415–421. https://doi.org/10.1016/j.chemosphere.2010.12.086

Rodilla V (1993) Origin and evolution of binucleated cells and binucleated cells with micronuclei in cisplatin-treated CHO cultures. Mutat Res Toxicol 300:281–291. https://doi.org/10.1016/0165-1218(93)90062-I

Sanchez-Galan S, Linde A, Ayllon F, Garcia-Vazquez E (2001) Induction of micronuclei in eel (*Anguilla anguilla* L.) by heavy metals. Ecotoxicol Env Saf 49:1139–1143

Sánchez-Galán S, Linde A, Izquierdo J, García-Vázquez E, (1998) Micronuclei and fluctuating asymmetry in brown trout (*Salmo trutta*): complementary methods to biomonitor freshwater ecosystems. Mutat Res Toxicol Environ Mutagen 412:219–225. https://doi.org/10.1016/S1383-5718(97)00186-1

Seehy MA, El-Wakil HF, El-Dahhar A, Abass NY (2009) The effect of water pollution on chromosomal behavior in Nile tilapia (*Oreochromis niloticus*). J Adv Agric Res 14:689–703

Shah N, Khan A, Khan N, Khisroon M (2021) Genotoxic consequences in common *Grass Carp* (*Ctenopharyngodonidella* Valenciennes, 1844) exposed to selected toxic metals. Biol Trace Elem Res 199:305–314

Silbergeld E (2003) Facilitative mechanisms of lead as a carcinogen. Mutat Res Mol Mech Mutagen 533:121–133. https://doi.org/10.1016/j.mrfmmm.2003.07.010

Soulivongsa L, Tengjaroenkul B, Neeratanaphan L (2020) Effects of contamination by heavy metals and metalloids on chromosomes, serum biochemistry and histopathology of the bonylip barb fish near Sepon gold-copper mine, Lao PDR. Int J Environ Res Public Health 17:9492. https://doi.org/10.3390/ijerph17249492

Souza T, Fontanetti C (2006) Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent. Mutat Res Genet Toxicol Environ Mutagen 605:87–93

Strunjak-Perovic I, Coz-Rakovac R, Topic Popovic N, Jadan M (2009) Seasonality of nuclear abnormalities in gilthead sea bream *Sparus aurata* (L.) erythrocytes. Fish Physiol Biochem 35:287–291. https://doi.org/10.1007/s10695-008-9208-3

Suchana SA, Ahmed MS, Islam SMM et al (2021) Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish *Pangasianodon hypophthalmus*. Biol Trace Elem Res 199:3869–3885. https://doi.org/10.1007/s12011-020-02490-4

Taleb ZM, Benali I, Gherras H, et al (2009) Biomonitoring of environmental pollution on the Algerian west coast using caged mussels *Mytilus galloprovincialis*: OCEANOLOGIA 51:63–84https://doi.org/10.5697/oc.51-1.063

Taweel A, Shuhaimi-Othman M, Ahmad A (2011) Heavy metals concentration in different organs of tilapia fish (*Oreochromis niloticus*) from selected areas of Bangi, Selangor, Malaysia. African J Biotechnol 10:11562–21156. https://doi.org/10.5897/AJB11.1663

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. 133–164

Varol M, Kaçar E, Akın HK (2020) Accumulation of trace elements in muscle, gill and liver of fish species (*Capoeta umbla* and *Luciobarbus mystaceus*) in the Tigris River (Turkey), and health risk assessment. Environ Res 186:109570. https://doi.org/10.1016/j.envres.2020.109570

WHO (World Health Organisation) (1997) International standard for drinking water quality. Switzarland, Geneva

Yadav KK, Trivedi SP (2009) Chromosomal aberrations in a fish, *Channa punctata* after in vivo exposure to three heavy metals. Mutat Res Toxicol Environ Mutagen 678:7–12. https://doi.org/10.1016/j.mrgentox.2009.05.021

Zaghloul KH, Mohamed HA, Abdullatef AM, Khalil MA (2020) Genotoxic and histopathological effects of water pollution on *Clarias gariepinus* fish at Fayoum Governorate. Egypt Nat Resour 11:499–519

Zaghloul GY, Eissa HA, Zaghloul AY, Kelany MS, Hamed MA, El Moselhy KM (2024) Impact of some heavy metal accumulation in different organs on fish quality from Bardawil Lake and human health risks assessment. Geochem Trans 25:1. https://doi.org/10.1186/s12932-023-00084-2

Zhang L, Shi Z, Jiang Z et al (2015) Distribution and bioaccumulation of heavy metals in marine organisms in east and west Guangdong coastal regions, South China. Mar Pollut Bull 101:930–937

Zyadah M, Abdel-Baky T (2000) Toxicity and bioaccumulation of copper, zinc, and cadmium in some aquatic organisms. Bull Environ Contam Toxicol 64:740–747. https://doi.org/10.1007/s001280000066