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Acute effects of chromium on hemato-biochemical parameters and morphology of erythrocytes in striped catfish *Pangasianodon hypophthalmus*

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ABSTRACT

Chromium is considered the most detrimental pollutant to the aquatic organisms. The present experiment was conducted to determine the acute toxicity of chromium in view of its effects on hemato-biochemical parameters and the structure of erythrocytes in striped catfish, *Pangasianodon hypophthalmus*. Fish were exposed to seven different concentrations (0, 10, 20, 30, 40, 50 and 60 mg/L) of chromium, each with three replications for 96 h. After 96 h of exposure, the survived fish were sacrificed to measure hemato-biochemical parameters (hemoglobin, Hb; red blood cell, RBC; white blood cell, WBC; packed cell volume, PCV; mean corpuscular volume, MCV; the mean corpuscular hemoglobin, MCH and blood glucose). In addition, erythrocytic cellular abnormalities (ECA) and erythrocytic nuclear abnormalities (ENA) of peripheral erythrocytes were assayed. No mortality was observed up to 10 mg/L, but 90% and 100% mortality was observed at 50 mg/L and 60 mg/L, respectively after a 96 h exposure period. The 96 h LC50 value through probit analysis was 32.47 mg/L. Hb (%), RBC ($\times 10^6/\text{mm}^3$) and PCV (%) significantly decreased at 20, 30 and 40 mg/L of chromium, whereas WBC ($\times 10^3/\text{mm}^3$), MCV (μm^3) and MCH (pg) showed the opposite scenario. Blood glucose (mg/dL) levels significantly increased at 10, 20, 30 and 40 mg/L of chromium compared to 0 mg/L. Frequencies of ECA and ENA significantly increased with increasing chromium concentrations. This study indicates that chromium is highly toxic to striped catfish.

1. Introduction

The striped catfish (*Pangasianodon hypophthalmus*) is a commercially cultured fish species in Bangladesh. It is popular for its outstanding growth performance and lucrative size, and high local and international market demand [1]. This is one of the most consumed fish species in south Asian countries. After being introduced to Bangladesh from Thailand in 1989, it has been contributing a lot (0.45 million MT in FY 2017 − 18) to aquaculture production, supporting livelihood of more than 0.7 millions of farmers to provide a considerable assistance in national economy and combating malnutrition of common poor people [2,3]. This fish consumes various types of food, including zooplankton, insects, algae as well as higher plants. It can exist in all types of environmental situations and show satisfactory production in high stocking density.

Heavy metal contamination severely interferes with the ecological balances of an ecosystem and produces devastating effects on the quality of the environment [4]. Heavy metals accumulate harmful chemicals in the aquatic ecosystem and consequently to humans [5]. It has been reported that heavy metal pollution reduced the production of fish [6]. Thus, it is essential to control the use of heavy metals. Moreover, heavy metals are poisonous leading the hazardous impacts on aquatic fauna that finally lead the serious human health concern [7–11].

Chromium (Cr) is one of the heavy metals which contaminates the environment through the release from various industries such as oil, paint, motor vehicle, aircraft, printing, electroplating factories, textile manufacturing facilities, steel producing factories, rinse waters, leather tanneries, dyeing, sanitary landfill leaching, the combustion of coal and oil, welding, bricks of furnaces and wood-preserving industry [12,13]. It is a ubiquitous trace element that occurs in soil, air and water. Several studies reported about the Cr concentration incidies in the environment

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such as 0.0376 – 0.0388 mg/L in Surma river [14]; 0.587 mg/L in Buriganga river [15]; 0.62–1.37 mg/L in Balu river [16]; 0.093 mg/L in ground water in Singair upazila, Manikganj [17]; 0.0326 – 0.0579 mg/L in Paira river [18]. Exposure of fish to chromium affects physiology and growth rate leading to deleterious health effects including allergy and even organ system-toxicity [19–22].

Blood parameters are one of the most important determinants of physiological stress, which result from any deviation that affects the homeostasis of fish [23–25]. The changes might directly influence the blood parameters of fish in different water quality parameters. It has been reported that micronucleus (MN) formation in the erythrocyte is considered to examine the stress from pollution [26–28]. MN is a small chromatin body in the cytoplasm, formed outside the central nucleus and condensed of acentric chromosome fragments during the nuclear division [29]. Likewise, deviation from the normal structure of cells and nucleus of erythrocytes (cellular and nuclear abnormalities) is also applied to assess the stress resulted from extrinsic contaminations [30–33]. Several experiments have reported the chromium toxicity on various fish species such as reduced the glycogen, total lipid and total protein concentration in liver, muscle and gill of rohu, *Labeo rohita* [34], declined the protein and carbohydrate content in gill of mrigal, *Cirrhinus mrigala* [35], induced the radical stress in hepatocytes of goldfish, *Carassius auratus* [36], caused cell death, alteration of glucose transport and endocrine disruption in fresh water fishes [37], severely disrupted the functional activites of kidney, liver and gill of grass carp, *Ctenopharyngodon idella* [38].

However, there is no report regarding the chromium toxicity in the striped catfish (*P. hypopohthalmus*), a commercially important aquaculture species. Therefore, the present experiment was designed to assess the toxicity of chromium considering its effects on hemato-biochemical parameters and morphology of peripheral erythrocytes in striped catfish.

2. Materials and methods

2.1. Experimental fish

Apparently healthy and active fingerlings of striped catfish (*P. hypophthalmus*) of weight (11.48 ± 1.67 g) and standard length (10.81 ± 0.83 cm) were procured from a private hatchery of Sadar Upazila, Mymensingh. The fingerlings were placed in a plastic bag containing 70% water and 30% oxygen and transported to the Fish Ecophysiology Laboratory, Bangladesh Agricultural University, Bangladesh. In the laboratory, fish were kept in aquariums (75 cm × 45 cm × 45 cm) containing 100 L of clean tap water and acclimatized for 21 days to the laboratory conditions. During the acclimatization period, the fish were fed twice daily with commercial grower feed (CP Bangladesh Co., Ltd., Bangladesh). Each aquarium was monitored daily to check fish mortality and dead fish were removed from the aquariums. The water quality parameters, such as temperature (30 ± 05 °C), pH (7.52 ± 0.09), dissolved oxygen (6.08 ± 0.19 $\mathrm{mg\,L^{-1}}$), free CO_2 (6.38 ± 0.48 $\mathrm{mg\,L^{-1}}$) and total alkalinity (194.25 ± 9.95 $\mathrm{mg\,L^{-1}}$) were recorded to be in the optimum range during the rearing period. The Animal Welfare and Ethical Committee, Bangladesh Agricultural University approved the experimental procedures used in this study.

2.2. Test chemical

The analytical grade potassium dichromate ($K_2Cr_2O_7$) was obtained from Merck (Darmstadt, Germany). To prepare a test solution, 2.828 g of $K_2Cr_2O_7$ was dissolved with double distilled water to produce a stock solution of 1000 mg/L chromium [13]. Then a desired concentration of chromium was prepared by diluting the stock solution with double distilled water.

2.3. Acute toxicity test

Acute toxicity bioassay was followed to determine 96 h lethal concentration values (LC50) of chromium for striped catfish. Ten fingerlings were stocked in each aquarium filled with 100 L of tap water. The fish were exposed to six different concentrations (10, 20, 30, 40, 50, and 60 mg/L) of chromium and a control unit without chromium (0 mg/L) with three replications. Sufficient aeration was provided during the time of the experiment. The application of the chromium was repeated at every 24 h with a regular total exchange of water. Mortality was assessed at 24, 48, 72, and 96 h after the start of exposure, and dead fish were removed immediately. The 96 h LC50 value was determined by probit analysis.

2.4. Hemato-biochemical study

The blood sample was collected from the survived fish after 96 h of exposure. Six fish (n = 6) from each aquarium were sacrificed for the hemato-biochemical assessment. The counting of red blood cell (RBC) and white blood cell (WBC) was done with the help of Neubauer hemocytometer under a light microscope. The hemoglobin (Hb, %) was measured directly by a digital EasyMate® GHb (Model: ET 232, Hb/Glu double monitoring system, Bioptic technology Inc. Taiwan 35057) with hemoglobin strips. Similar to Hb, blood glucose (mg/dL) was measured immediately following blood sample collection by glucose strips using the digital EasyMate® GHb. The packed cell volume (PCV, %), mean corpuscular volume (MCV, μm^3) and the mean corpuscular hemoglobin (MCH, pg) were calculated using the following formulas [39]:

$$\text{PCV (\%)} = \text{\% Hb} \times 3$$

$$\text{MCV } (\mu m^3) = (\text{PCV/RBC in millions}) \times 10$$

$$\text{MCH (pg)} = (\text{\% Hb/RBC in millions}) \times 10$$

2.5. Analysis of cellular and nuclear abnormalities of blood cells

Erythrocytic cellular abnormalities (ECA) and erythrocytic nuclear abnormalities (ENA) were analyzed after exposure to different concentrations of chromium. A drop of blood was taken on the clean microscopic slide and smeared smoothly directly after collection. After air-drying for 10 min, the smeared slide was fixed by methanol and finally stained by 5% Giemsa solution. Then, the stained slide was washed with tap water and kept overnight for air drying at room temperature. For preservation, the slide was mounted using DPX solution. Then the ECA and ENA were observed under an electronic microscope (MCX100, Micros Austria) with magnification of ×40. Three different slides were prepared from each fish blood and 2000 cells were counted from each one.

An oval-shaped erythrocyte cell with a condensed nucleus is considered as the regular erythrocyte cell and ECA was identified by comparing desperation of the regular cell. Among ECA, echinocytic cells are irregular shape and having serrated edges structure entire the surface of the cell membrane; elongated cells are slightly egg-shaped, slender structures which have more length compared to width; fusions are more than two cells, which are joining each other and form heavier clotting; twins are two cells joined together by cellular membrane; teardrop cells when deformed a nipple at the top point and tapering the endpoints of erythrocyte.

ENA were classified [40,41] briefly such as, binucleated is the two nuclei that are equal in size but completely separated from each other in a cell. Micronucleus is the circular chromatin bodies formed in the same staining shape out of the central nucleus. Blebbed is that condition when small evaginations of the euchromatin bearing with the nuclear membrane. A notched nucleus is resulting from osmosis in a hypertonic solution of the nucleus, which did not contain any nuclear materials

Table 1
Mortality percentages of fish exposed to different concentrations of chromium.

Concentrations of chromium (mg/L)	Percent (%) dead fish at different time intervals (mean ± SD)			
	24 h	48 h	72 h	96 h
0	–	–	–	–
10	–	–	–	–
20	–	–	–	13.33 ± 5.77
30	–	–	23.33 ± 5.77	43.33 ± 5.77
40	–	10.00 ± 0.00	30.00 ± 0.00	70.00 ± 0.00
50	–	33.33 ± 5.77	46.67 ± 5.77	86.67 ± 5.77
60	10 ± 0.00	40 ± 0.00	60 ± 0.00	100.00 ± 0.00

owing to the shrinkage. Nuclear bud is the evagination generated like a bud in the nucleus. The nuclear bridge had thin strands connected between two individual nuclei of the cell.

2.6. Statistical analysis

Values were expressed as mean ± standard deviation (SD). The LC50 value of chromium was determined through probit analysis. The data were analyzed by using one-way ANOVA, followed by post hoc test to find out the statistically significant differences among treated values with control. Statistical analysis was executed by using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA) at level of significance $p < 0.05$.

3. Results

3.1. Median lethal concentration of chromium for striped catfish

After 96 h of exposure, there was no mortality up to 10 mg/L, but 86.67 ± 5.77% and 100 ± 0.00% mortality were recorded at 50 mg/L and 60 mg/L concentrations, respectively (Table 1). The 96 h LC50 value was 32.47 mg/L with upper limit and lower limit 38.63 mg/L and 27.81 mg/L, respectively, where the linear regression equation was $y = 133.1x - 0.144.81$, $R^2 = 0.9305$ (Fig. 1).

3.2. Effects of chromium on hemato-biochemical parameters

The values of RBC ($\times 10^6/\text{mm}^3$), Hb (%) and PCV (%) ranged from 1.09 ± 0.02 to 1.65 ± 0.43, 8.7 ± 0.3 to 10.1 ± 0.9 and 25.86 ± 4.2 to 30.3 ± 2.7, respectively in the fish blood exposed to different concentrations of Cr. The total number of RBC, Hb and PCV significantly ($p < 0.05$) declined at 20, 30 and 40 mg/L compared to the control (0 mg/L) after the exposure of period (Table 2). Conversely, total number of WBC ($\times 10^3/\text{mm}^3$), MCV (μm^3) and MCH (pg) significantly increased at 10, 20, 30 and 40 mg/L compared to the control (0 mg/L) of chromium (Table 2). The blood glucose (mg/dL) level increased significantly at 20 (172.0 ± 18.6) mg/L, 30 (170.5 ± 34.5) mg/L and 40 (189.0 ± 16.6) mg/L compared to control (117.2 ± 14.4) (Table 2).

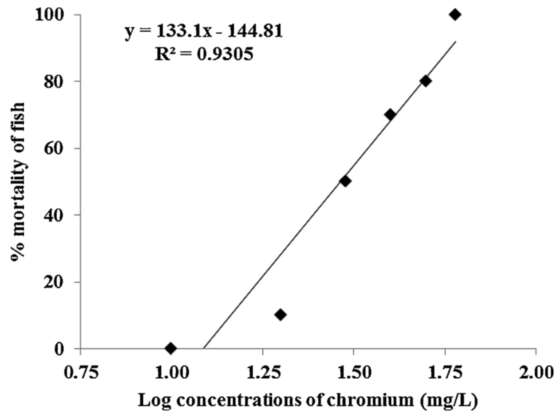

Fig. 1. Graph showing linear transformation and the relationship of probit of log concentration of chromium used to determine LC50 of *P. hypophthalmus*.

3.3. Effects of chromium on cellular and nuclear anomalies of blood cells

The blood smear of striped catfish exhibited different erythrocytic cellular abnormalities (ECA) after exposure to different chromium concentrations, such as elongated (Fig. 2b), twin (Fig. 2c), fusion (Fig. 2d), teardrop (Fig. 2e) and echinocytic (Fig. 2f). The frequencies of ECA showed significant ($p < 0.05$) increases in the blood of fish exposed to 10, 20, 30 and 40 mg/L of chromium concentrations compared to 0 mg/L (Table 3). The present experiment also showed different ENA in the blood of fish, such as micronucleus (Fig. 3b), notched nuclei (Fig. 3c), binuclei (Fig. 3d), blebbed (Fig. 3e) and nuclear bud (Fig. 3f) in different concentrations of chromium. The frequencies of ENA of fish treated in different chromium concentrations are shown in Table 4. ENA has been significantly ($p < 0.05$) increased after the exposure period at 10, 20, 30 and 40 mg/L compared to 0 mg/L.

4. Discussion

Chromium is one of the most harmful contaminants to aquatic organisms. Hence we aimed to test the acute toxicity of chromium considering its effects on hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish. Different changes were observed in hemato-biochemical parameters and morphology of erythrocytes at different concentrations of chromium, which indicates its stressful impacts on physiology in the striped catfish.

Several studies reported the assessment of the acute toxicity of chromium on different fish species. In the present study, 96 h LC50 value of striped catfish was 32.47 mg/L, which is less than the values of 50.00 mg/L for *Channa punctatus* [42], 155.00 mg/L for *Cyprinus carpio* [43], 39.40 mg/L for *Labeo rohita* [34] 60 mg/L for *Colisa fasciatus* [36], 100.00 mg/L for *Catla catla* [37] and 119.52 mg/L for *Oreochromis niliticus* [38]. More or less similar 96 h LC50 values were recorded as 34.00 mg/L for *Cirrhinus mrigala* [27,30], 35.72 mg/L for *Heteropneustes fossilis* [44] and 36.65 mg/L for *Clarius batrachus* [45]. In contrast, lower 96 h LC50 values were recorded as 7.33 mg/L for *Labeo bata* and 10.37 mg/L for *Puntius sarana* [46]. The toxicity level of any chemical depends on the physiological situations of the fish exposed, their habitat and also the purity of the chemicals [47,48].

In the present study, significant changes were observed in the different parameters of the blood. It has been reported that different physiological parameters in fish are changed as a result of stress [49]. Erythrocytes count is considered as a quite stable index and fish species always maintain this index within the standard limit by compensating different physiological techniques in stressful conditions. In this study, the Hb level and the number of RBC decreased in different chromium

Table 2
Hemato-biochemical parameters after 96 h exposure to different concentrations of chromium.

Parameters	Concentration of chromium (mg/L)				
	0	10	20	30	40
RBC ($\times 10^6$/mm^3)	1.65 $\pm$ 0.43^a	1.40 $\pm$ 0.30^{ab}	1.26 $\pm$ 0.13^b	1.29 $\pm$ 0.16^b	1.09 $\pm$ 0.02^b
WBC ($\times 10^3$/mm^3)	10.85 $\pm$ 2.29^a	16.29 $\pm$ 3.19^b	16.45 $\pm$ 3.96^b	17.27 $\pm$ 3.02^b	16.61 $\pm$ 1.31^b
Hb (%)	10.1 $\pm$ 0.9^a	9.8 $\pm$ 1.2^{ab}	8.62 $\pm$ 1.4^b	8.7 $\pm$ 0.3^b	8.7 $\pm$ 0.4^b
PCV (%)	30.3 $\pm$ 2.7^a	29.4 $\pm$ 3.6^a	25.86 $\pm$ 4.2^b	26.1 $\pm$ 0.9^b	26.1 $\pm$ 1.2^b
MCV (μm^3)	183.64 $\pm$ 9.25^a	210 $\pm$ 10.52^b	205.23 $\pm$ 12.02^b	202.33 $\pm$ 11.21^b	239.45 $\pm$ 13.25^b
MCH (pg)	61.21 $\pm$ 4.21^a	70.00 $\pm$ 5.41^b	68.41 $\pm$ 3.26 ^{b}	67.44 $\pm$ 4.56 ^{b}	79.82 $\pm$ 5.62 ^{b}
Blood glucose (mg/dL)	117.2 $\pm$ 14.4^a	138.0 $\pm$ 9.5^{ab}	172.0 $\pm$ 18.6^b	170.5 $\pm$ 34.5^b	189.0 $\pm$ 16.6^b

Values of in a row with different alphabetical superscripts are significantly ($p < 0.05$) different. All values are expressed as mean ± SD.

concentrations. Previous studies also reported that the volume of Hb and the number of RBC decreased significantly in freshwater fish when exposed to different heavy metals like as zinc, cadmium and nickel causing disorder such as anemia, erythropenia, leucopoiesis, etc [50–52]. A similar decreased value of Hb was also reported in common carp exposed to organophosphorus pesticide sumithion [25,53] and malathion [54]. The significant reduction of Hb level and the number of RBC in the present study might be due to iron deficiency and structural alterations of heme leading to decline hemoglobin synthesis. On the other hand, significant increases in WBC counts might be due to the increase of antibody production [55], which helped the organism in survival and healing during exposure to toxic pollutants [56,57]. This increase of WBC also might be due to leukocytosis under chemical strees caused by chromium, resulted direct stimulation of immunological resistance [58]. Moreover, chromium exposure could lead to activate radical oxidation processes and to nutrients imbalance, which can ultimately lead to the development of the observed changes in immunological and biochemical factors [59].

Blood glucose plays an important role in producing energy for the central nervous system, which is controlled by the hormone. Therefore, blood glucose levels are commonly used as a stress bio-indicator in fish [60]. In the present study, blood glucose levels increased in the fish reared in different chromium concentrations, which might be due to the transformation of glucose from glycogen that met the increasing energy demands at high chromium concentration. Hyperglycemia produced in animals due to the secretion of catecholamine and glucocorticoids from the adrenal tissue of the fish and stress acts as a stimulant to secrete these hormones rapidly [61,62]. This type of raise may result because of boosted gluconeogenesis through which stressed fish try to fulfill their high energy demand [63].

Erythrocytes are commonly used as an important diagnosing tool to know the functional and structural situation of fish exposed to any toxicants. Erythrocytes are capable to respond to environmental obsesses and alterations of erythrocyte represent the most common reflection toward toxicants present in water bodies [64]. In the present study, different morphological (ECA and ENA) changes of erythrocytes were observed due to exposure in different chromium concentrations. Morphological changes of erythrocytes may also occur due to the

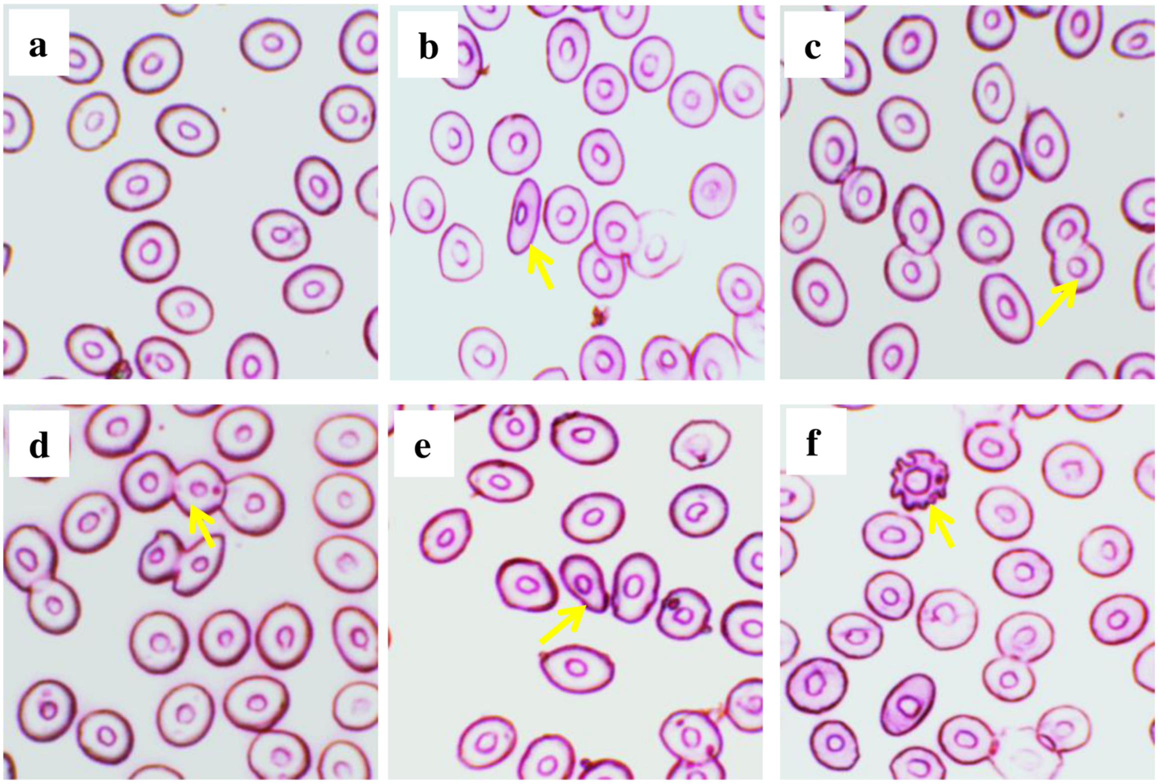

Fig. 2. Various erythrocytic cellular abnormalities (ECA) in Giemsa stained blood smears of *P. hypophthalmus* treated with different concentrations of chromium; (a) Normal, (b) elongated, (c) twin, (d) fusion, (e) teardrop, (f) echinocytic. The different ECA were observed under an electronic microscope with magnification of ×40.

Table 3
Frequencies of erythrocytic cellular abnormalities (ECA) after 96 h exposure to different concentrations of chromium.

ECA	Concentration of chromium (mg/L)				
	0	10	20	30	40
Elongated shape	0.31 ± 0.09^{a}	1.51 ± 0.13^{b}	1.53 ± 0.15^{b}	1.57 ± 0.18^{b}	1.75 ± 0.18^{b}
Twin	0.41 ± 0.11^{a}	1.63 ± 0.15^{b}	1.66 ± 0.18^{b}	1.59 ± 0.19^{b}	1.95 ± 0.19^{b}
Fusion	0.68 ± 0.07^{a}	1.66 ± 0.13^{b}	1.67 ± 0.15^{b}	1.63 ± 0.18^{b}	1.83 ± 0.18^{b}
Tear-drop	0.50 ± 0.07^{a}	1.53 ± 0.11^{b}	1.56 ± 0.11^{b}	1.57 ± 0.15^{b}	1.77 ± 0.15^{b}
Echinocytic	0.45 ± 0.07^{a}	1.53 ± 0.13^{b}	1.66 ± 0.15^{b}	1.48 ± 0.18^{b}	1.88 ± 0.18^{b}

Values in a row with different alphabetical superscripts are significantly ($p < 0.05$) different. All values are expressed as mean ± SD. Three slides were prepared from each fish and 2000 cells were scored from each slide and three fish were analyzed from each group.

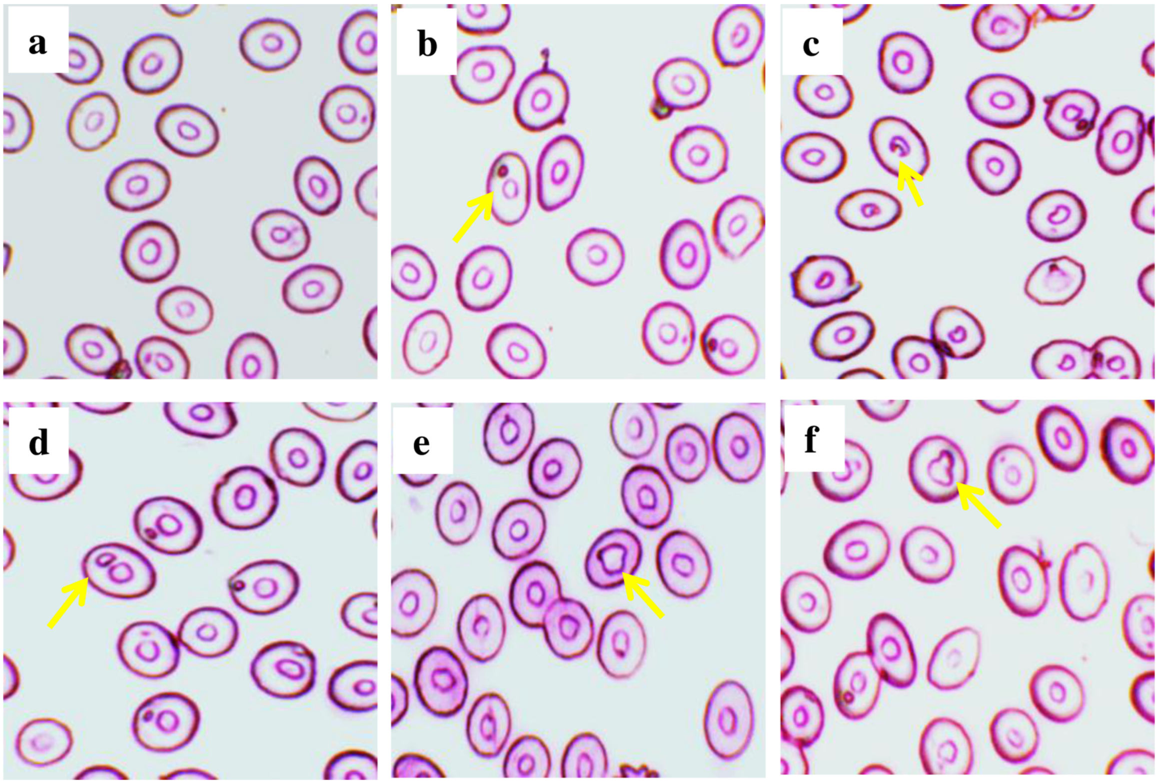

Fig. 3. Various erythrocytic nuclear abnormalities (ENA) in Giemsa stained blood smears of *P. hypophthalmus* treated with different concentrations of chromium; (a) Normal, (b) micronucleus, (c) notched, (d) binuclei, (e) blebbed, (f) nuclear bud. The different ENA were observed under an electronic microscope with magnification of ×40.

Table 4
Frequencies of erythrocytic nuclear abnormalities (ENA) after 96 h exposure to different concentrations of chromium.

ENA	Concentration of chromium (mg/L)				
	0	10	20	30	40
Micronucleus	0.15 ± 0.03^{a}	0.48 ± 0.07^{b}	1.21 ± 0.09^{c}	1.56 ± 0.11^{c}	1.72 ± 0.11^{c}
Notched	0.16 ± 0.03^{a}	0.85 ± 0.09^{b}	1.10 ± 0.13^{b}	1.60 ± 0.15^{c}	1.69 ± 0.15^{c}
Binuclei	0.18 ± 0.05^{a}	0.83 ± 0.09^{b}	0.95 ± 0.13^{b}	1.46 ± 0.18^{c}	1.61 ± 0.18^{c}
Blebbed	0.53 ± 0.09^{a}	1.66 ± 0.15^{b}	1.63 ± 0.15^{b}	1.68 ± 0.18^{b}	1.80 ± 0.18^{b}
Nuclear bud	0.67 ± 0.09^{a}	1.60 ± 0.13^{b}	1.66 ± 0.15^{b}	1.61 ± 0.19^{b}	1.83 ± 0.19^{b}

Values in a row with different alphabetical superscripts are significantly ($p < 0.05$) different. All values are expressed as mean ± SD. Three slides were prepared from each fish and 2000 cells were scored from each slide and three fish were analyzed from each group.

increase of lipid peroxide compound of the cell membrane [30,65]. Erythrocyte structures are changed due to the damage of the cell membrane, ion-permeability, and absorption of cells by the toxic substances [26]. It has been reported that chromium exerts a genotoxic effect through DNA damage [66,67] and altered the nucleus structure of blood cells [68,69]. Due to the failure of tubulin polymerization, differential nuclear abnormalities of erythrocytes, such as binucleated, nuclear bud, nuclear bridge, karyopyknosis, notched nuclei, etc. might be formed under stress triggered by chromium [30,70,71]. Moreover, the formation of binucleated cells and notched nuclei are also possible because of mitotic fuses by aneugenic actions of toxicants [72]. Furthermore, it can be assumed that these types of abnormalities in erythrocyte may cause morphological changes in the plasma membrane that affects deformation in the surface as well as increase susceptibility of erythrocyte to brust during crossing small capillaries.

In summary, chromium is considered highly toxic to striped catfish that altered hemato-biochemical parameters, cellular and nuclear structure of erythrocytes. Also, it reduces the fish survival rate even at low concentrations. However, further studies related to enzymatic changes and oxidative stress of fish are needed to enlarge scope of knowledge and make possible the development of an efficient biomarker for chromium.

Author contributions

S M Majharul Islam and Md. Fazle Rohani conceived, designed and performed the experiments, analyzed data and drafted the manuscript. Seyed Akib Zabed, Md. Tarikul Islam and Rayeda Jannat were assisted in data collection. Yeasmin Akter assisted in data analysis and edited the manuscript. Md. Shahjahan assisted in the experimental design, analyzed data and edited the manuscript. All authors reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.04.016.

References

[1] A. Jahan, T.T. Nipa, S.M.M. Islam, M.H. Uddin, M.S. Islam, M. Shahjahan, Striped catfish (*Pangasianodon hypophthalmus*) could be suitable for coastal aquaculture, J. Appl. Ichthyol. 35 (2019) 994–1003.

[2] DoF, Department of Fisheries, Fishery Statistical Yearbook of BD 2016-2017. Fisheries Resources Survey System vol. 26, Ministry of fisheries and livestock, Dhaka, Bangladesh, 2018.

[3] Fisheries Resources Survey System Fisheries Statistical Report of Bangladesh 32 Department of Fisheries, Bangladesh, 2016, pp. 1–57.

[4] E. Farombi, O. Adelowo, Y. Ajimoko, Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River, Int. J. Environ. Res. Public Health 4 (2007) 158–165.

[5] R.T. Di-Giulio, D.E. Hinton, The Toxicology of Fishes, Taylor and Francis, 2008, pp. 319–884.

[6] Y. Lu, S. Song, R. Wang, Z. Liu, J. Meng, J.A. Sweetman, A. Jenkins, R.C. Ferrier, H. Li, W. Lou, T. Wang, Impacts of soil and water pollution on food safety and health risks in China, Environ. Int. 77 (2015) 5–15.

[7] A.K.M.A. Ullah, M.A. Maksud, S.R. Khan, L.N. Lutfa, S.B. Quraishi, Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh, Toxicol. Rep. 4 (2017) 574–579.

[8] S. Rajeshkumar, X. Li, Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China, Toxicol. Rep. 5 (2018) 288–295.

[9] P.K. Maurya, D.S. Malik, K.K. Yadav, A. Kumar, S. Kumar, H. Kamyab, Bioaccumulation and potential sources of heavy metal contamination in fish species in river Ganga basin: possible human health risks evaluation, Toxicol. Rep. 6 (2019) 472–481.

[10] N.K. Kortei, M.E. Heymann, E.K. Essuman, F.M. Kpodo, P.T. Akonor, S.Y. Lokpo, N.O. Boadi, M. Ayim-Akonor, C. Tettey, Health risk assessment and levels of toxic metals in fishes (*Oreochromis noliticus* and *Clarias anguillaris*) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety, Toxicol. Rep. 7 (2020) 360–369.

[11] M.S. Rahman, S.M.M. Islam, A. Haque, M. Shahjahan, Toxicity of the organophosphate insecticide sumithion to embryo and larvae of zebrafish, Toxicol. Rep. 7 (2020) 317–323.

[12] M.I.C. Monterio, I.C.S. Fraga, A.V. Yallouz, N.M.M. de Oliveria, S.H. Riberio, Determination of total chromium traces in tannery effluents by electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry and UV-visible spectrophotometric methods, Talanta 58 (2002) 629–633.

[13] P.L.R.M. Palanippan, S. Karthikeyan, Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel, J. Environ. Sci. China 21 (2009) 229–236.

[14] M.J. Alam, M.R. Islam, Z. Muyen, M. Mamun, S. Islam, Water quality parameters along rivers, Int. J. Environ. Sci. Technol. 4 (2007) 159–167.

[15] M.K. Ahmed, M. Das, M.M. Islam, M.S. Akter, S. Islam, M.A. Al-Mansur, Physico-chemical properties of tannery and textile effluents and, World Appl. Sci. J. 12 (2011) 152–159.

[16] M.K. Hasan, M.R. Khan, M.K. Nesha, M.A. Happy, Analysis of water quality using chemical, Open J. Water Pollut. Treat. 1 (2014) 58–74.

[17] M.A. Halim, R.K. Majumder, G. Rasul, Y. Hirosiro, K. Sasaki, J. Shimada, K. Jinno, Geochemical evaluation of arsenic and manganese in shallow groundwater and core sediment in Singair Upazila, Central Bangladesh, Arabian J. Sci. Eng. 39 (2014) 5585–5601.

[18] M.S. Islam, M.K. Ahmed, M. Habibullah-Al-Mamun, M.F. Hoque, Preliminary assessment of heavy metal contamination in surface sediments from a river in Bangladesh, Environ. Earth Sci. 73 (2015) 1837–1848.

[19] P. Singarea, S. Dhabardeb, Toxic metals pollution due to industrial effluents released along Dombivali Industrial Belt of Mumbai, India, Eur. J. Environ. Saf. Sci. 2 (2014) 5–11.

[20] T. Shaheen, F. Jabeen, Effect of various doses of Cr (VI) on survival and growth of Cyprinus car*pio*, Pak. J. Zool. 47 (2015) 913–919.

[21] P. Madhavan, K. Elumalai, Effects of chromium (VI) on the lipid peroxidation and antioxidant parameters in the gill and kidney tissues of catfish, *Clarias batrachus* (Linnaeus, 1758) (Actinopterygii : Siluriformes), Int. J. Adv. Res. 3 (2016) 249–255.

[22] A. Bakshi, A.K. Panigrahi, A comprehensive review on chromium induced alterations in fresh water fishes, Toxicol. Rep. 5 (2018) 440–447.

[23] J. Cazenave, D.A. Wunderlin, A.C. Hued, M. de los Angeles-Bistoni, Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns 1842) (Pisces, Callichthyidae), captured from pristine and polluted water, Hydrobiologia 537 (2005) 25–33.

[24] S. Sharmin, M. Shahjahan, M.A. Hossain, M.A. Haque, H. Rashid, Histopathological changes in liver and kidney of common carp exposed to sub-lethal doses of malathion, Pak. J. Zool. 47 (2015) 1495–1498.

[25] M.A. Salam, M. Shahjahan, S. Sharmin, F. Haque, M.K. Rahman, Effects of sub-lethal doses of an organophosphorus insecticide sumithion on some hematological parameters in common carp, *Cyprinus carpio*, Pak. J. Zool. 47 (2015) 1487–1491.

[26] I.M. Sadiqul, Z. Ferdous, M.T.A. Nannu, G.M. Mostakim, M.K. Rahman, Acute exposure to a quinalphos containing insecticide (convoy) causes genetic damage and nuclear changes in peripheral erythrocytes of silver barb, Barbonymus gonionotus, Environ. Pollut. 219 (2016) 949–956.

[27] C. Bolognesi, M. Hayashi, Micronucleus assay in aquatic animals, Mutagenesis 26 (2011) 205–213.

[28] S. Anbumani, M.N. Mohankumar, Gamma radiation induced micronuclei and erythrocyte cellular abnormalities in the fish *Catla catla*, Aquat. Toxicol. 122-123 (2012) 125–132.

[29] M. Fenech, M. Kirsch-Volders, A.T. Natarajan, J. Surralles, J.W. Crott, J. Parry, Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells, Mutagenesis 26 (2011) 125–132.

[30] A. Ghaffar, H. Riaz, K. Ahrar, R.Z. Abbas, Hemato-biochemical and genetic damage caused by triazophos in freshwater fish *Labeo rohita*, Int. J. Agric. Biol. 17 (2015) 637–642.

[31] M. Shahjahan, M.H. Uddin, V. Bain, M.M. Haque, Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*, Fish Physiol. Biochem. 44 (2018) 1309–1318.

[32] M.A. Islam, M.H. Uddin, M.J. Uddin, M. Shahjahan, Temperature changes influenced the growth performance and physiological functions of Thai pangas Pangasianodon hypophthalmus, Aquacult. Rep 13 (2019) 100179.

[33] S.M.M. Islam, M.A. Rahman, S. Nahar, M.H. Uddin, M.M. Haque, M. Shahjahan, Acute toxicity of an organophosphate insecticide sumithion to striped catfish *Pangasianodon hypophthalmus*, Toxicol. Rep. 6 (2019) 957–962.

[34] S.S. Vutukuru, Chromium induced alterations in some biochemical profiles of the

Indian major carp, *Labeo rohita* (Hamliton), Bull. Environ. Contam. Toxicol. 70 (2003) 118–123.

[35] S. Virk, A. Sharma, Changes in the biochemical constituents of gills of *Cirrhinus mrigala* (Ham.) following exposure to metals, Indian J. Fish. 50 (2003) 113–117.

[36] G. Krumschnabel, M. Nawaz, Acute toxicity of hexavalent chromium in isolated teleost hepatocytes, Aquat. Toxicol. 70 (2004) 159–167.

[37] V. Velma, S.S. Vutukuru, B.T. Paul, Ecotoxicology of hexavalent chromium in freshwater fish: a critical review, Rev. Environ. Health 24 (2009) 129–145.

[38] T. Shaukat, M. Javed, Acute toxicity of chromium for Ctenopharyngodon idella, *Cyprinus carpio* and *Tilapia nilotica*, Int. J. Agric. Biol. 15 (2013) 590–594.

[39] N.C. Jain, Essentials of Veterinary Hematology, Lea and Febiger, Philadelphia, 1993, p. 417.

[40] K.R. Carrasco, K.L. Tilbury, M.S. Myres, Assessment of piscine micronucleus test as an in situ biological indicator of chemical contaminant effects, Can. J. Fish. Aquat. Sci. 47 (1990) 2123–2136.

[41] M. Fenech, W.P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi, E. Zeiger, HUMN project: detailed description of the scoring criteria for the cytokines is block micronucleus assay using isolated human lymphocyte cultures, Mutat. Res. 534 (2003) 65–75.

[42] K.V. Sastry, K.M. Sunita, Enzymological and biochemical changes produced by chronic chromium exposure in a Teleost fish, *Channa punctatus*, Toxicol. Lett. 16 (1983) 9–15.

[43] V. Sridhar, R.J. Katti, M.T. Laksmipathi, T.J. Ramesha, Behavioural alteration and biochemical composition of *Cyprinus carpio* exposed to hexavalent chromium, J. Inland Fish 32 (2000) 18–24.

[44] M.K. Ahmed, G.K. Kundu, M.H. Al-Mamun, S.K. Sarkar, M.S. Akter, M.S. Khan, Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging Catfish, *Heteropneutes fossilis*, Ecotox. Environ. Safe. 92 (2013) 1–7.

[45] C. Johnson, M.V. Radhakrishnan, Estimation of acute toxicity of chromium to the freshwater catfish *Clarias batrachus* (Linn.), Int. J. Sci. Res. Environ. Sci. Toxicol. 1 (2015) 30–37.

[46] T. Sanyal, A. Kaviraj, S. Saha, Toxicity and bioaccumulation of chromium in some freshwater fish, Hum. Ecol. Risk Assess. 23 (2017) 1655–1667.

[47] I.M. Sial, M.A. Kazmi, Q.B. Kazmi, S.N.H. Naqvi, Toxicity of biosal (Phytopesticide) and permethrin (Pyrethroid) against common carp, *Cyprinus carpio*, Pak. J. Zool. 41 (2009) 235–238.

[48] I.S. Faria, A.J. Palumbo, T.L. Fojut, R.S. Tjeerdema, Water Quality Reports for Malathion, Phase III: Application of Pesticide Water Quality Criteria Methodology, University of California, UC Davis, Davis, 2010.

[49] M.M. Beyea, T.J. Benfey, J.D. Kieffer, Hematology and stress physiology of juvenile diploid and triploid shortnose sturgeon (*Acipenser brevirostrum*), Fish Physiol. Biochem. 31 (2005) 303–313.

[50] G. Sen, M.K. Behera, P.N. Patel, Effect of zinc on hemato-biochemcical parameters of *Channa punctatus*, J. Ecotoxicol. Environ. Monito 2 (1992) 89–92.

[51] S. Vincent, T. Ambrose, A.K.L. Cyril, M. Selvanayagan, Heavy metal cadmium influenced anaemia in *Catla catla*, J. Environ. Biol. 17 (1996) 81–84.

[52] N. Prasanta, K.B. Milan, Nickel induced changes in some hemato-biochemical parameters of a catfish, *Heteropneustes fossilis*, Environ. Ecol. 14 (1996) 82–85.

[53] S. Hossain, M.H. Khatun, M.K. Rahman, Z.P. Sukhan, M. Shahjahan, Impacts of sumithion on blood glucose and some hematological parameters in common carp, Int. J. Environ. 5 (2015) 8–13.

[54] S. Sharmin, M.A. Salam, F. Haque, M.S. Islam, M. Shahjahan, Changes in hematological parameters and gill morphology in common carp exposed to sub-lethal concentrations of malathion, Asian J, Med, Biol, Res. 2 (2016) 370–378.

[55] T.J. Raphael, G. Kuttan, Immunomodulatory activity of naturally occurring monoterpenes carvone, limonene, and perillic acid, Immunopharmacol. Immunotoxicol. 25 (2003) 285–294.

[56] P.K. Joshi, M. Bose, D. Harish, Changes in certain haematological parameters in a siluroid cat fish Clarias batrachus (Linn) exposed to cadmium chloride, Pollut. Res. 21 (2002) 129–131.

[57] K. Begg, N.W. Pankhurst, Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*, J. Fish Biol. 64 (2004) 133–145.

[58] H.H. Marti, R.H. Wenger, L.A. Rivas, Erythropoietin gene expression in human, monkey and murine brain, Eur. J. Neurosci. 8 (1996) 666–676.

[59] A.V. Karaulov, E.A. Renieri, A.I. Smolyagin, I.V. Mikhaylova, A.A. Stadnikov, D.N. Begun, K. Tsarouhas, A.B. Djordjevic, T. Hartung, A. Tsatsakis, Long-term effects of chromium on morphological and immunological parameters of Wistar rats, Food Chem. Toxicol. 133 (2019) 110748.

[60] M. Pacheco, M.A. Santos, Biotransformation, endocrine and genetic responses of Anguilla Anguilla L. to petroleum distillate products and environmentally contaminated waters, Ecotox. Environ. Safe. 49 (2001) 64–75.

[61] A.D. Pickering, Stress and compensation in teleostean fishes: response to social and physical faxtors, in: A.D. Pickering (Ed.), Stress and Fish, vol. 198, Academic Press, New York, 2020, pp. 295–322.

[62] M. Banaee, A.R. Mirvaghefi, A.B. Mojazi, G.R. Rafiee, B. Nematdost, Hematological and histopathological effects of diazinon poisoning in common carp (*Cyprinus carpio*), Iranian J. Natural Res. 64 (2011) 1–13.

[63] E.U. Winkaler, T.R.M. Santos, G. Joaquim, J.G. Machado-Neto, C.B.R. Martinez, Acute and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*, Com. Biochem. Physiol. 145 (2007) 236–244.

[64] A.K. Sawhney, M.S. Johal, Erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch), Bull. Environ. Contam. Toxicol. 64 (2000) 398–405.

[65] M.M. Bai, K. Divya, B.S.K. Haseena, G. Sailaja, D. Sandhya, K. Thyagaraju, Evaluation of genotoxic and lipid peroxidation effect of cadmium in developing chick embryos, J. Environ. Anal. Toxicol. 4 (2014) 238.

[66] J. Blasiak, J. Kowalik, A comparison of the in vitro genotoxicity of tri- and hexavalent chromium, Mutat. Res. 469 (2000) 135–145.

[67] S.T. Matsumoto, M.S. Mantovani, M.I. Mallaguti, M.A. Marin-Morales, Investigation of the genotoxic potential of the waters of a rive receiving tannery effluents by means of the invitro comet assay, Cytologia 68 (2003) 395–401.

[68] S. Lim, S.Y. Ahn, I.C. Song, M.H. Chung, H.C. Jang, Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance, PLoS One 4 (2009) e5186.

[69] S. Sharaf, A. Khan, M.Z. Khan, F. Aslam, M.K. Saleemi, F. Mahmood, Clinicohematological and micronuclear changes induced by cypermethrin in broiler chicks: their attenuation with vitamin E and selenium, Expt. Toxicol. Pathol. 62 (2010) 333–341.

[70] R. Hussain, A. Khan, F. Mahmood, S. Rehan, F. Ali, Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (*Coturnix japonica*), Pest. Biochem. Physiol. 109 (2014) 58–63.

[71] A.I. Vardavas, P.D. Stivaktakis, M.N. Tzatzarakis, P. Fragkiadaki, F. Vasilaki, M. Tzardi, G. Datseri, J. Tsiaoussis, A.K. Alegakis, C. Tsitsimpikou, V.N. Rakitskii, F. Carvalho, A.M. Tsatsakis, Long-term exposure to cypermethrin and piperonyl butoxide cause liver and kidney inflammation and induce genotoxicity in New Zealand white male rabbits, Food Chem. Toxicol. 94 (2016) 250–259.

[72] B.C. Ventura, D.F. Angeli, M.A. Marin-Molares, Mutagenic and genotoxic effects of the atrazine herbicide in *Oreochromis nilotics* (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay, Pest. Biochem. Physiol 90 (2008) 42–51.