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The biochemical and molecular markers of *Cyprenus carpio* L. after chronic exposure to lead

Wathiq Mohammed*, Suad Ghali Kadhim Alahmed, Ayad M.J. Al-mamoori

Biology Dept. College of Science, University of Babylon, Iraq

** Corresponding author's e-mail: sci.ayad.mohammed@uobabylon.edu.iq*

ABSTRACT

This study was designed to detect the effects of lead on biochemical and molecular characteristics of common carp, *Cyprenus carpio* L. Two concentrations (10 and 20 ppm) were selected for lead respectively in one week. Biochemical markers such as acetylcholinesterase, superoxide dismutase and catalase were used to assess lead exposure cytotoxic effects, while lead-induced DNA damage was used to determine the effect of lead on molecular level. Fish samples were treated with two selected concentrations of lead solution (10 and 20 ppm) for one-week duration, as exposure period, to detect the impacts of lead exposure on this species. Various biochemical markers such as acetylcholinesterase, superoxide dismutase, and catalase were applied to determine the cytotoxic impacts of lead exposure at the cellular level, while the lead-induced DNA damages were identified to reveal the influences of lead exposure at the molecular level. The results represent that the highest concentration of lead solution (20 ppm) had more effects on the antioxidant enzymes activities such as superoxide dismutase (SOD) level (655.17 ± 21.76 mg U^{-1}) followed by acetyl cholinesterase and catalase (655.17 ± 21.76 U L^{-1} and 87.93 ± 7.22 mg U^{-1}) respectively. The DNA damages were estimated by the Comet assay technique and the highest level of DNA damages were documented when lead concentration was 20 ppm using different parameters of Comet assay technique such as Comet length, tail length, and tail moment (4021 ± 56.11 µL, 523 ± 55.80 µL, and 91.208 ± 9.45 µL respectively). Therefore, this study confirms that the exposure to high lead concentrations would cause harmful effects on aquatic organisms at both cellular and molecular levels.

Keywords: Biochemical Markers, Molecular markers, Heavy metals, Common carp.
Article type: Research Article.

INTRODUCTION

Due to the industrial development, pollution by heavy metals have been documented to cause harmful effects on aquatic organism. Numerous metal ions have potential toxicities that affect various organs and blood tissue in fish species due to forming metal complexes with the structural protein, enzymes and nucleic acids which consequently disturb their functions. Continuous accumulation of toxic heavy metals in carp tissues may affect hepatic functions and cause cellular degeneration (Rajamanickam & Muthuswamy 2008). Lead is widely distributed as a toxic environmental and industrial pollutant that impact the physiological functions and growth rates in various aquatic organisms (Jia *et al.* 2008). Many other heavy metals such as copper are also found to be toxic to fish, whivh probably coincides with its physicochemical parameters (pH, temperature, EC, salinity, TDS, and dissolved oxygen) and this toxicity increases by water acidity. The influence of Zinc on erythrocytes and lipids of common carp at different temperature was studied by Gabryelak *et al.* (2000) reporting that the heavy metal can affect aquatic organism by different ways thorough a direct effect on the metabolic pathways causing changes in

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structural and physicochemical properties of cell membrane at low temperature which can lead to erythrocytes damage. The DNA damages in common carp cells were evaluated using comet assay technique to determine the possible effects of pollutants in Moagan Lake, Ankara, Turkey (Çok *et al*. 2011), reporting that lake is mostly polluted by many genotoxic substances. Ferencz (2000), identified the mtf-1, GPX1 and GPX4 genes expression systems in common carp to understand the stress response to heavy metal toxicity. Another study detected the expression of the two phospholipid hydroperoxide gluthatione peroxidase (GPX4) genes in common carp as an antioxidant defense system against heavy metal exposure, such as cadmium (Cd^{2+}) (Hermesz & Ferencz 2009). There are also several studies on toxicology in fish species around the world (Johari *et al.* 2015; Bat *et al.* 2015; Yabanli *et al.* 2016; Alizadeh & Mirarab-razi 2016; Oveysi *et al.* 2017; Jahanbakhsh *et al.* 2018).

Generally, common carp is considered as one of the major food fish that consumed by Iraqi people and it is largely used in the genotoxicity studies. In addition to tolerating most conditions, common carp favors large water bodies with slow flowing or standing water with soft bottom sediments. Moreover, it prefers fresh or slightly brackish water with pH range between 6.5-9.0, and salinity up to about 0.5%. The aim of this study is to evaluate the biochemical and molecular responses of common carp fish when it exposes to different concentrations of lead and also to determine the differences in biochemical and molecular parameters after exposure

MATERIALS AND METHOD

Exposure Protocol

Cyprenus carpio L. samples were collected from Al-Furat fish farm in Babylon Province. On arrival in the laboratory, they were acclimated in dechlorinated tap water for 7 days and then starved for 24 h with a photoperiod light/dark of 12:12. The samples were divided into different groups according to different lead concentrations (10 and 20 ppm). The exposure to lead extended to one week, as an acute exposure in aquarium under controlled conditions. Standard concentrations of Pb were prepared from standard solution. LT_{50} (median lethal time) was determined using a log probable paper according to Piegorsch & Bailer (2015).

Biochemical assays

Antioxidant enzymes

Superoxide dismutase (SOD)

The activity of superoxide dismutase was determined by autoxidation of Pyrogallol according to Marklund & Marklund (1974):

$$\text{Inhibition (\%)} = \frac{\Delta A\ bsorbtion}{absorbtion\ of\ control} \times 100$$

where ΔA was changed in A2 = final absorbance after 2 min, and A1= initial absorbance after 30 seconds, T = 2 min

$$\text{SOD activity (U mg}^{-1}\text{ protein)} = \frac{inhibition\ of\ pyragallol\ reduction\ (\%) \times reaction\ valume}{total\ test\ period\ (2\text{min})}$$

Catalase (CAT)

Catalase assay activity was determined according to procedure of Clariborn (1985) and Aebi (1974).

$$\text{Catalase (U mg}^{-1}\text{ or U g}^{-1}\text{ Hb protein)} = \frac{\Delta\ Absorbtion/\min \times reaction\ valume}{0.01};$$

Once A1 for initial absorbance in 5 seconds and A2 for final absorbance in 2 min, So, reaction volume = 2.4 mL

Acetylcholinesterase activity

A colorimetric determination of acetylcholine esterase activity in the tissues was performed according to the method described by Ellman *et al.* (1961).

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Acetylcholinesterase (U L^{-1}) = $\frac{\frac{\Delta A}{\Delta T}}{0.0136 \Delta A} DF$

Where ΔA: Changing in absorbance, Δ T: Changing in time, DF: Dilution factor.

DNA damage

The blood samples were collected from *Cyprinus carpio* in 0.5 M di-sodium EDTA to avoid formation of clot and 40 µL from each sample of rat and fish according to Sing *et al.* (1988) and Steinert (1996) with some modification clarified by Conners (2004).

Statistical analyses

All statistical analysis were carried out using One-Way ANOVA and Duncan, and the statistical significance was found as $p \leq 0.05$. The data was determined using Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA).

RESULT AND DISCUSSION

Biochemical response after acute exposure

The result showed the response to different concentrations of lead using different biochemical indices such as acetylcholinesterase activity. The highest concentration (25.75 ± 3.34 U L^{-1}) of the enzyme was recorded after exposure to high lead concentration comparing to low concentration of 10 ppm (9.74 ± 1.24 U L^{-1}) or even control (without exposure; 5.016 ± 0.32 U L^{-1}). Superoxide dismutase activity showed the highest value (655.17 ± 21.76 mg U^{-1}) after exposure to the highest lead concentration of 20 ppm compared to control (57.14 ± 5.67 mg U^{-1}). In addition, catalase activity assay recorded value of 87.93 ± 7.22 mg U^{-1} with high lead concentration (20 ppm) exposure when compared to the control sample (67.7 ± 4.75 mg U^{-1}; Table 1, Figs. 2, 3 and 4). Reactive oxygen species (ROS) are a family of molecules that have atom or molecules with one or more unpaired electron in valence shell making them unstable, short lived and highly reactive. Therefore, these materials can react quickly with other compounds to capture electron in order to become stable resulting oxidation and peroxidation of protein, lipid, and DNA which at the end lead to significant cellular and DNA damages and also even tissue or organ failure (Tripathy 2016). Acetylcholinesterase enzyme, also known as AChE, is a serine protease that have an important role in hydrolyzing neurotransmitter acetylcholine into choline and acetic acid (Taylor & Radic 2004). The oxidative stress induced by lead can cause stimulation or inhibition of acetylcholinesterase activity (Ferrari *et al.* 2007). The results of this study showed that activity increased in blood of *C. carpio* during chronic exposure to lead because of high amount of ROS generation inducing oxidative stress (Ferrari *et al.* 2007).

Table 1. The activities of biochemical marker in liver of *Cyprinus carpio* after exposure to different concentration of lead.

Biochemical Parameter	**Liver**		
	Control	**10 mg L^{-1}**	**20 mg L^{-1}**
Acetylcholinesterase U L^{-1}	5.016 ± 0.32a	9.74 ± 1.24b	25.75 ± 3.34c
Superoxide dismutase mg U^{-1}	57.14 ± 5.67a	442.53 ± 30.89b	655.17 ± 21.76c
Catalase mg U^{-1}	67.7 ± 4.75a	75.8 ± 11.23b	87.93 ± 7.22c

Note: Different letter referred to significant differences at $P \leq 0.05$.

The SOD activities in blood of *C. carpio* increased significantly elevated during chronic period. This is because the SOD plays an essential role in scavenging superoxide free radical, which helps to maintain a balance between oxidants and antioxidants (Prieto *et al.* 2006; Table 1, Fig. 4). The CAT activities increased considerably in blood of *C. carpio* during chronic period in comparison with control group due to high production of reactive oxygen species such as hydrogen peroxide (Liu *et al.* 2006).

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DNA damage after acute exposure

Comet assay (single cell gel electrophoresis, SCGE) were used to determine the level of DNA damage, which is very sensitive method for detecting DNA single and double strands, alkali-labile sites, DNA-DNA, DNA-protein cross-links, and single strand breaks associated with incomplete excision at the level of single cell (Tice *et al.* 2000).

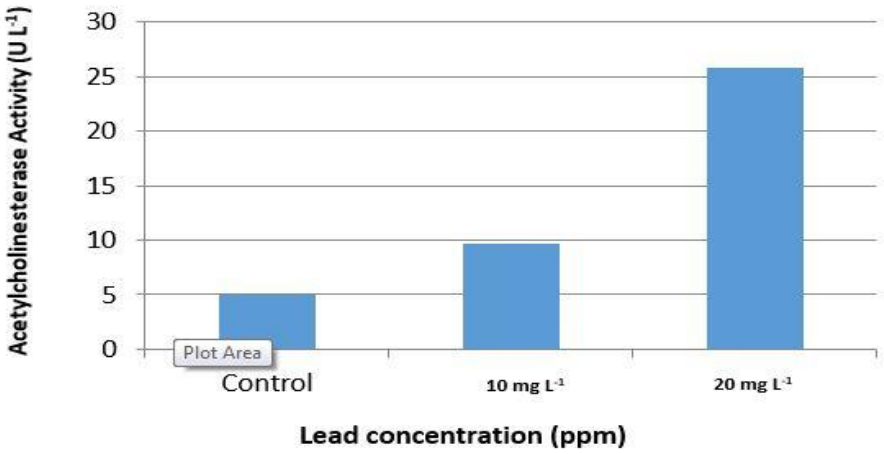

Fig. 1. Acetylcholinesterase Activity U L^{-1} in after exposure to different concentration of lead in *Cyprinus carpio*.

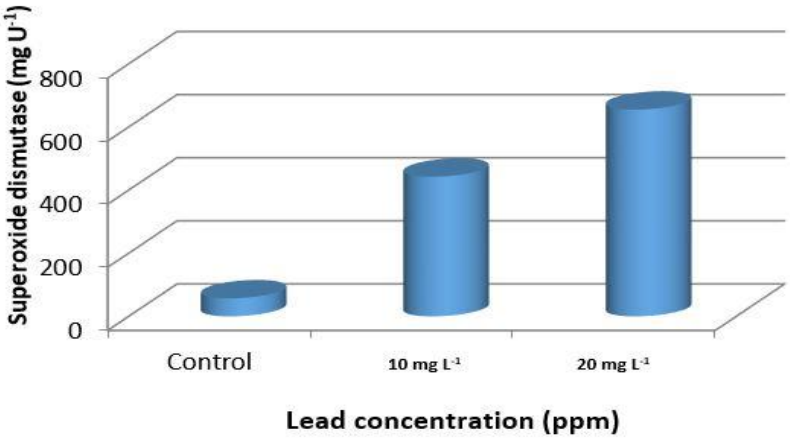

Fig. 2. Superoxide dismutase mg U^{-1} in *Cyprinus carpio* after exposure to different concentration of lead.

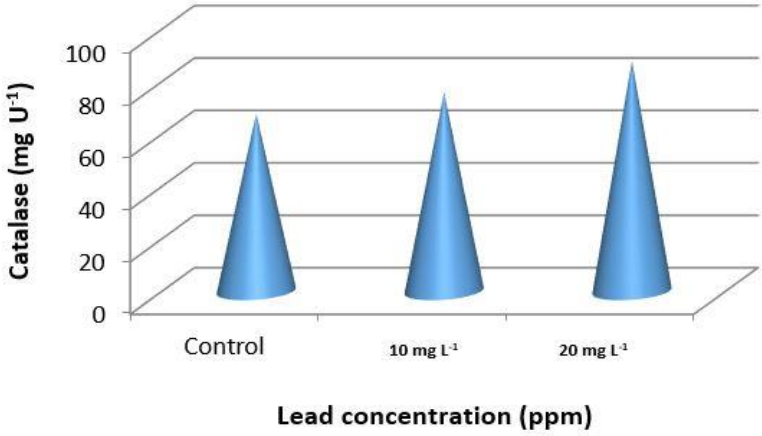

Fig. 3. Catalase activity (mg U^{-1}) in *Cyprinus carpio* after exposure to different lead concentrations.

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Table 2. Markers of DNA damage in liver of *C. Carpio* after exposure to different concentration of lead.

Marker of DNA damage	Liver		
	Control	**10 mg L^{-1}**	**20 mg L^{-1}**
Comet length (µL)	3351 ± 34.89a	4133 ± 20.76a	4021 ± 56.11a
Tail length (µL)	169 ± 33.56a	350 ± 27.5b	523 ± 55.80c
Tail moment (µL)	19.851 ± 3.8a	115.18 ± 6.75b	91.208 ± 9.45c

Note: Different letters referred to significant differences at P≤0.05; Similar letters referred to no significant differences at P ≤ 0.05.

Oxidative stress may occur due to the overproduction of ROS because of high level of lead that can damage DNA, increase mitochondrial membrane permeability transition, and cause cytoskeleton disruption (Ding *et al.* 2001). Heavy metals have been reported to cause harmful effects in the body via creation of free radicals (FR) and reactive oxygen species (ROS) which break DNA strands and oxidize DNA bases (Zegura *et al.* 2003). DNA damage induced by heavy metals can be related to cell apoptosis rather than genotoxicity (Lankoff *et al.* 2004). However, Gaudin *et al.* (2008), mentioned that heavy metals can induce genotoxicity and DNA damage through their ability to cause genetic instability in fish. In the same way, another study showed that heavy metals are able to stimulate DNA damage at about 2-fold after 30-min exposure (Dias *et al.* 2014). In this study, the comet assay was evaluated for 3 parameters, including comet length (µm), tail length (µm) and tail moment (µm). The comet assay showed significantly differences between different lead-treated and control groups (Table 2, Figs. 5, 6 and 7). The highest values of comet length, tail length and tail moment in liver of *C. carpio* were recorded when lead concentration was 20 ppm as compared with control group. In addition, the results exhibited the highest value of DNA damage makers in liver than in blood and the statistical analysis appeared significantly positive correlation between DNA damage markers and lead concentrations (Fig. 7).

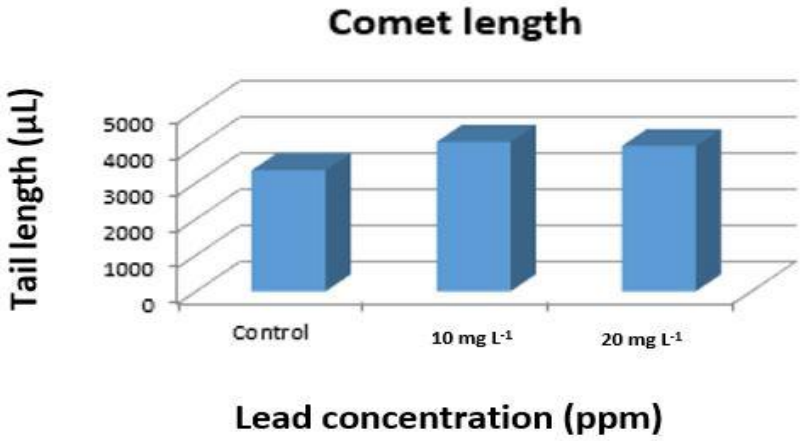

Fig. 4. Markers of DNA damage (comet length) in the liver of C. carpio after exposure to different concentrations of lead.

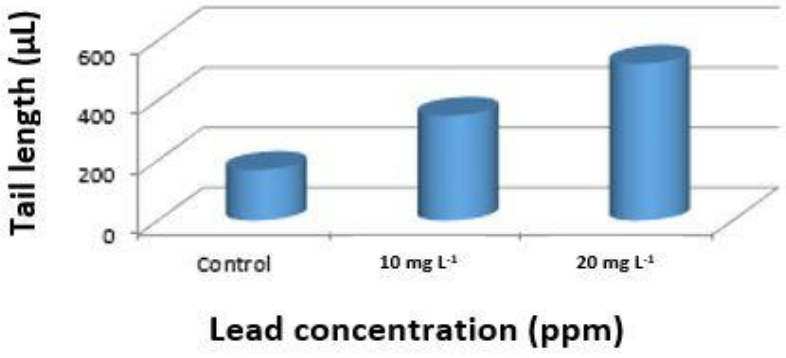

Fig. 5. Markers of DNA damage (tail length) in the liver of *C. carpio* after exposure to different concentrations of lead.

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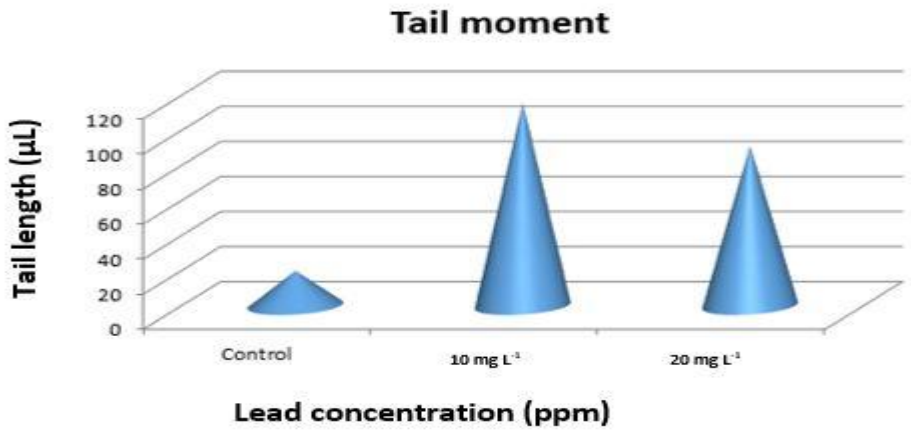

Fig. 6. Markers of DNA damage (tail moment) in the liver of C. carpio after exposure to different concentrations of lead.

The comet length was positively correlated with DNA breakage level in cell, because the distribution of comet pattern was heterogeneous (Sinch *et al.* 1988). The highest value of DNA damage markers was obvious when lead concentration increased from 10 ppm to 20 ppm (Figs. 7A, B). High toxic material doses may be due to inhibit insufficient production of antioxidant defense systems to scavenge ROS that are generated by heavy metals, then ROS could find its way across nuclear membrane resulting DNA strand breakage and damage (Georg *et al.* 2014; Dias *et al.* 2014). Because of high DNA repair capacity to protect DNA integrity in common carp, the results showed less DNA fragmentation in high concentration of lead. On the other hand, common carp has adaptive response to polluted aquatic environment with heavy metals, which cause a harmful effect by generating ROS that leads to sever damage to cell by shifting the fluidity balance and make molecular complexes with cell protein and develop toxic effect on the cell towards dysfunction.

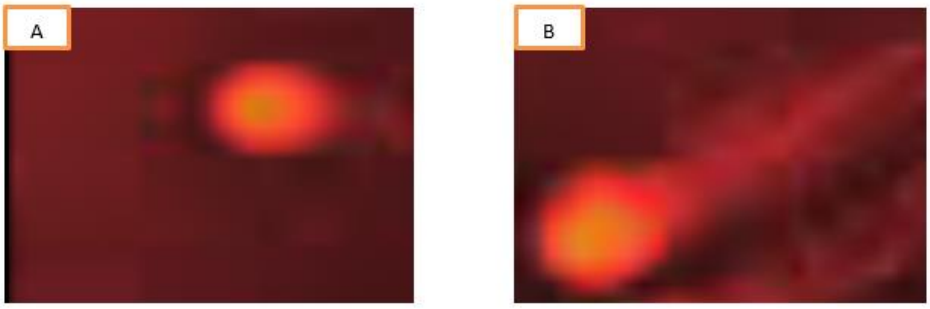

Fig. 7. DNA damage markers in common carp after lead exposure. A; 10 ppm, B: 20 ppm.

CONCLUSION

This study demonstrated that the increased levels of some biochemical parameters were recorded after chronic exposure to high concentration of lead (20 ppm), which could induce oxidative stress in blood and liver of *Cyprians carpio*. Moreover, lead showed more adverse effect on biochemical markers than in molecular markers in *C. carpio* and all biochemical markers were affected by exposure to two different concentrations of lead.

REFERENCES

Alizadeh, S & Mirarab-Razi, J 2016, Growth and accumulation responses of *Populus nigra* L. exposed to hexavalent chromium excess', *Caspian Journal of Environmental Sciences*, 14: 253-261.

Bat, L, Yesim Özkan, E, Can Öztekin, H 2015, The contamination status of trace metals in Sinop coast of the Black Sea, Turkey. *Caspian Journal of Environmental Sciences*, 13: 1-10.

Claiborne, A 1985, Catalase activity. In: CRC handbook of methods for oxygen radical research, RA, Greenwald (ed.), Boca Raton, FL, pp. 283-284.

Çok, I, Uluta, OK, Oku luk, Ö, Durmaz, E & Demir, N 2011, Evaluation of DNA Damage in common carp (*Cyprinus carpio* L.) by Comet assay for determination of possible pollution in Lake Mogan (Ankara). *The Scientific World Journal: TSW Environment*, 11: 1455-1461. DOI: 10.1100/tsw. 2011.140

Conners, DE 2004, Biomarkers of oxidative stress in freshwater clam (Corbicula Fluminea) as mechanistic tool to evaluate the impairment of stream ecosystem health by lawn care pesticides. Ph.D thesis, The University of Georgia, U.S.A.

Dias, E, Louro, H, Pinto, M, Santos, T, Antunes, P, Susana, P & Maria, JS 2014, Genotoxicity of Microcystin-LR in in vitro and in vivo experimental models. *BioMed Research International*, 1: 1-9.

Ding, W & Ong, C 2003, Role of oxidative stress and mitochondrial changes in cyanobacteria-induced apoptosis and hepatotoxicity. *FEMS Microbiology Letters*, 220: 1-7.

Ellman, GLKD, Courtney, V, Andres, Jr, & Featherstone RM 1961, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88-95.

Ferencz, Á 2010, Identification and expression of the genes of metal-Responsive transcription factor-1 and glutathione peroxidases of common carp. PhD Dissertation, University of Szeged, Hungary.

Ferrari, A, Venturino A & Péchen de D'Angelo, AM 2007, Muscular and brain cholinesterase sensitivities to azinphos methyl and carbaryl in the juvenile rainbow trout, *Oncorhynchus mykiss. Comparative Biochemistry and Physiology*, 146: 308-313.

Gabryelak, T, Filipiak A & Brichon, G 2000, Effects of zinc on lipids of erythrocytes from carp (*Cyprinus carpio* L.) acclimated to different temperatures. *Comparative Biochemistry and Physiology* Part C, 127: 335-343.

Gaudin, J, Huet S, Jarry, G & Fessard, V 2008, In vivo DNA damage induced by the cyanotoxin microcystin-LR: Comparison of intra-peritoneal and oral administrations by use of the comet assay. *Mutation Research*, 652: 65-71.

Georg, OO, Amaeze, NH, Soghanmu, TO & Otitoloju, AA 2014, Biomarkers responses in *tympanotous fuscatus* var radula (L) inhibiting an oil-impacted and fire-ravaged mangrove ecosystem, current advance in environmental science. *American V-king Scientific Publishing*, 2: 101-111.

Hermesz E & Á. Ferencz 2009, Identification of two phospholipid hydroperoxide gluthatione peroxidase (GPX4) genes in carp. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 150: 101-106.

Janbakhsh, S, Hosseini Shekarabi, S, Shamsaie Mergan, M 2018, Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea. *Caspian Journal of Environmental Sciences*, 16: 307-317. DOI: 10.22124/cjes.2018.3200.

Jia, X, Zhang, H & Liu, X 2011, Low levels of cadmium exposure induce DNA damage and oxidative stress in the liver of Oujiang colored common carp *Cyprinus carpio* var. Color. *Fish Physiology and Biochemistry*, 37: 97-103.

Johari, S, Sourinejad, I, Asghari, S, Bärsch, N 2015, Toxicity comparison of silver nanoparticles synthesized by physical and chemical methods to tadpole (*Rana ridibunda*). *Caspian Journal of Environmental Sciences*, 13: 383-390.

Lankoff, A, Banasik, A & Obe, G 2004, Effect of microcystin LR and cyanobacterial extract from Polish reservoir of drinking water on cell cycle progression, mitotic spindle, and apoptosis in CHO-K1 cells, *Toxicology and Applied Pharmacology*, 189: 204-213.

Liu, CI, Zhang, S, Li, Y & Zhang, H 2016, Toxic effects of microcystins on the respiratory system. *Life Science Journal*, 13: 70-73.

Marklund, S & Marklund, G 1974, Involvement of the superoxide anion radical in the autoxidation of Pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47: 469-474.

Oveysi, M, Jamili, S, Behdani, M, Mashinchian Moradi, A, Sharifpoor, E 2017, Analysis of vitellogenin gene structure in Caspian roach, *Rutilus caspicus* (Pisces: Cyprinidae) during exposure to

Atrazine. *Caspian Journal of Environmental Sciences*, 15: 309-319. DOI: 10.22124/cjes.2017. 2638.

Piegorsch, WW & Bailer, AJ 2005, Analyzing environmental data. John wiley & Sons Ltd., UK, ISBN: 978-0-470-84836-4.

Prieto, AI, Jos, A, Pichardo, S, Moreno, I & Camean, AM 2006, Differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (*Oreochromis* sp.). *Aquatic Toxicology*, 77: 314-321.

Rajamanickam V & Muthuswamy, N 2008, Effect of heavy metals induced toxicity on metabolic biomarkers in common carp (*Cyprinus carpio* L.). *Maejo International Journal of Science and Technology*, 2: 192-200.

Said, AK, Ferencz, A, Nemcsók, J & Hermesz, E 2010, Expressions of heat shock and metallothionein genes in the heart of common carp (*Cyprinus carpio*): Effects of temperature shock and heavy metal exposure. *Acta Biologica Hungarica*, 61: 10-23.

Singh, NP, McCoy, MT, Tice, RR & Schneider, EL 1988, A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, 175: 184-191.

Tice, RR, Agurell, E, Anderson, D, Burlinson, B, Hartmann, A, Kobayashi, H, Miyamae, Y, Rojas, E, Ryu, JC & Sasaki, YF 2000, Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis*, 35: 206-221.

Tripathy & Asima 2016, Oxidative stress, reactive oxygen species (ROS) and Antioxidative defense system, with special reference to Fish. *International Journal of Current Research in Biosciences and Plant Biology*, 3: 79-89.

Yabanli, M, Alparslan, Y, Hasanhocaoglu Yapici, H, Yapici, S & Yozukmaz, A, 2016, Determination of heavy metal content in commercial marine fish hunted From Southeast Aegean Sea (Turkey) and their potential risk for public health. *Caspian Journal of Environmental Sciences*, 14: 1-13.

Zegura, B, Volcic, M, Lah, TT & Filipic, M 2008, Different sensitivities of human colon adenocarcinoma (CaCo-2), astrocytoma (IPDDC-A2) and lymphoblastoid (NCNC) cell lines to Microcystin-LR induced reactive oxygen species and DNA damage. *Toxicon*, 52: 518-525.

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