IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 5 Ser. I (May. 2019), PP 01-06
www.iosrjournals.org

Biochemical Responses of Antioxidant Defense System in Crucian Carp (Carassius Auratus) Liver after Exposured To Municipal Sewage Treatment Plant Effluent

Tong Chang*, Yi He, Bin Wei, Qin Wang, Tingting Li, Yueyue Liu, Sha Zhu, Tao Wang

Zibo Center for Disease Control and Prevention, Zibo Key Laboratory for Monitoring and Analysis of Environmental Organic Pollution and Population Health, No. 44 Dongyi Road, Zhangdian District, Zibo, Shandong Province, China
Corresponding author: Tong Chang

Abstract: *The biochemical responses of antioxidant defense system in crucian carp (Carassius auratus) liver after exposed to municipal sewage treatment plant (MSTP) effluent were investigated. In this study, C. auratus was exposed to the increaseing concentrations (v/v, 0%, 5%, 10%, 20%, 30%, 40% and 50%) of MSTP effluents for 12 days, and the biochemical responses of antioxidant defense system in Carassius auratus livers were investigated, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and acetylcholinesterase (AChE). Results indicated that MDA contents and GPx activities in C. auratus livers showed a universal increase trend (P<0.05), while GSH contents and AChE activities were decreased significantly (P<0.05) as the increasing concentrations of MSTP effluent. In addition, SOD activities in 5% and 10% treatment groups were sharply higher (P<0.05) but the ones in 40% and 50% treatment groups were significantly lower (P<0.05), while CAT activities in 20%, 30% and 40% treatment groups exhibited a tendecy to increase (P<0.05), as compared to the freshwater group. Because of the sensitive responses to the increasing concentrations of MSTP effluent after a 12-day exposure, four biomarkers in C. auratus liver, namely MDA, SOD, GPx and GSH, can be used as the biomarkers to assess the water quality of the freshwater environment polluted by MSTP effluent.*

Keywords: *Biochemical response, Crucian carp, Effluent, Municipal sewage treatment plant*

Date of Submission: 20-04-2019 Date of acceptance: 04-05-2019

I. Introduction

With the rapid development of society and economy, the discharge of sewage is increased greatly in China. Municipal sewage treatment plants (MSTP) plays an important role in purifying the inflows of sewage. At present, the majority of MSTP usually adopts the secondary sewage treatment technology to purify sewage.[1] Although this treatment technology can effectively remove nitrogen, phosphorus, ammonia nitrogen and other pollutants in the sewage, some pollutants such as surfactants, chlorinated hydrocarbons, heavy metals and endocrine disruptors still exist in the effluent discharged from MSTP.[2] It has been reported that the low concentration (<1%, v/v) of these pollutants in MSTP effluent can exert the toxicity effects on aquatic organisms.[3] This complex mix of MSTP effluent is usually directly discharged into the receving water, e.g. river, laker and sea, which definitely poses a contant threat to aquatic organism and even human health.[4] Nowadays, it is not feasible that chemical analysis can completely allow the qualitative and quantitative measurement for all pollutants in MSTP effluent, because some trace pollutants in the effluent are under the detection limit of chemical analysis.[5]

Biomarker is defined as the biological measurements at the biochemical, cellularor molecular level that result from the interaction between an organism and environmental chemical, physical or biological agents.[6] Researchers have reported that a causal relationship exists between the biomarker response in aquatic organism and the contaminant exposure in aquatic environment.[7-9] Nowadays, the biomarkers in aquatic organisms have been widely used for monitoring the influence of the pollutants in aquatic environment.[8-11]

To our best knowlege, there is no study to utilize the biomarker response of aquatic organism to monitor MSTP pollution levels in the receving freshwater areas in China. It is known that the antioxidant defense system of fish species, such as the antioxidant enzymes (SOD, CAT, GPx, etc.) and small antioxidant molecules (GSH, vitamin E, and beta-carotene, etc.), has been widely used as the biomarker in the evaluation for the pollutant levels of environmental water quality. Thus in this study, crucian carp (*Carassius auratus*), a widely distributed fish species in Chinese rivers and lakes, was exposed under laboratory conditions to increasing concentrations of MSTP effluents for 12 days. Subsequently, the biochemical responses of antioxidant defense system in *C. auratus* livers were investigated, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and acetylcholinesterase (AChE). The aim of the study was to provide an effective biological indicator for the evaluation of MSTP effluent pollution in the freshwater environment.

II. Materials and Methods

Materials

MSTP effluent was collected from a draining exit of a sewage treatment plant by Zhulong riverside (Zibo, Shandong province, China). The collected MSTP effluents were transferred to the laboratory within 30 min and kept at 4^oC until use.

C. auratus were purchased from the local market (Zibo, Shandong, China), kept in 150 L aquarium supplied with aerated freshwater, fed with commercial fish food pellets every day. The average length and weight of fish were 12.17±2.51 cm and 20.53±2.78 g, respectively. The water temperature was kept at 25±1°C. After acclimated under laboratory conditions for 7 days in aerated freshwater, the healthy and dynamic crucian carps were selected as the targets operated in exposure experiment. The freshwater used in this study was tap water that exposed under the sunshine for at least 3 days for removing the chlorine in tap water completely.

Exposure experiment

MSTP effluents were diluted by freshwater to increasing concentrations (v/v) of 0%, 5%, 10%, 20%, 30%, 40% and 50%, respectively. For each group, 15 crucian carps were exposed to 100 L of MSTP effluent-aerated freshwater mixture for 12 days. The clean effluent-freshwater was replaced daily during the exposure experiment.

Preparation of the supernatant of *C. auratus* liver

C. auratus were stunned and their livers were collected immediately. *C. auratus* livers were homogenized with cold (4°C) Tris-HCl buffer solutions (100 mM, pH 7.4) (1:9, w (g)/v (mL)) containing 1 mM EDTA-2Na, 10 mM sucrose and 0.8% NaCl. The homogenates were centrifuged at 3,000 r/min for 15 min at 4°C. The supernatants were collected and used for biochemical assays. All operations were performed below 5°C.

Biochemical assays

The activities of SOD, CAT, GPx, AChE and the contents of MDA, GSH in the supernatants of *C. auratus* livers were investigated by using the commercially-available assay kits (Nanjing Jiancheng Bioengineering Institute, China).

Data analysis

All experiments were conducted in triplicate. Analysis of variance (ANOVA) was performed using a SPSS package (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA) according to the method of Wan et al.[2] Two-tailed Pearson's correlation analysis was applied to determine the correlation between the concentrations of MSTP effluent and the biochemical responses of antioxidant defense system in *C. auratus* liver, and the statistical significance levels were set as $P<0.05$.

III. Results And Discussion

The changes for MDA content in *C. auratus* livers were shown in Fig. 1. The MDA content in the treatment groups showed a significant increase compared with the control group ($P<0.05$) (Fig. 1). MDA is genenrally considered as a biomarker of lipid peroxidation caused by an excessive accumulation of reactive oxygen species (ROS, such as superoxide radical and peroxide, et al).[12] It has been reported that MSTP effluent can give rise to oxidative stress through the over-production of ROS, which can eventually lead to the deterioration of protein and lipid in organism.[4, 13] Therefore, the increasing MDA contents in *C. auratus* liver after exposed to MSTP effluent suggested that MSTP effluent could cause oxidative damage to *C. auratus* liver.[11]

As shown in Fig. 2 and 3, SOD activities in 5% and 10% treatment groups were sharply higher ($P<0.05$) but the ones in 40% and 50% treatment groups were significantly lower ($P<0.05$), CAT activities in 20%, 30% and 40% treatment groups exhibited a tendecy to increase ($P<0.05$) while there were no significant differences found in other treatment groups ($P>0.05$), as compared to the control group.

SOD and CAT are the major antioxidant enzymes that are resposible for the removal of superoxide radicals ($O_2^{\cdot -}$). Specifically, SOD is the enzyme that can catalyze the reaction of $O_2^{\cdot -}$ to H_2O_2, and then H_2O_2 is transformed to H_2O by CAT.[8] The rises in SOD activities suggested that the pollutants in 5% and 10% of MSTP effluents could produce a certain amount of $O_2^{\cdot-}$ and the SOD activity in the cells therefore needs to be enhanced to catalyze the $O_2^{\cdot-}$ into H_2O_2.[2] However, SOD activities were universally weakened by 40% and 50% of MSTP effluent. Xie et al. found that the excess production of ROS in organism could cause damage to the SOD protein, thus leading to the sharp decrease in SOD activity.[14] In the present study, the results observed in MDA contents in 40% and 50% of MSTP effluent could be ascribed to the over production of ROS induced by MSTP effluent (Fig. 1). Kim and Jung also reported that the over production of $O_2^{\cdot-}$ or their transformation production hydrogen peroxide (H_2O_2) induced by MSTP effluent could inhibit the activity of SOD in pale chub.[15] Thus, it was believed that SOD in *C. auratus* liver is prone to oxidative modification and even inactivation by the excess accumulation of ROS.

CAT is mainly located in the peroxisomes, and is responsible for the reduction of hydrogen peroxide produced from the metabolism of long chain fatty acids in peroxisomes.[8] The insignificant variation of CAT activities in 5% and 10% of MSTP effluent suggested that the lower concentration of pollutants in MSTP effluent could not completely stimulate the activity of CAT. The increase of CAT activity in 20%~40% treatment groups could reflect that the production of H_2O_2 was still within the scavenging capacity of CAT.[14, 16] The reduced enzyme activity of CAT in 50% of MSTP effluent indicated that the detoxification capacity of CAT was surpassed by the amount of hydroperoxide products lipid peroxidation.[7]

GPx plays a detoxification role by catalyzing the H_2O_2 to H_2O and meanwhile oxidizing glutathione (GSH) to oxidized glutathione (GSSG).[17] In Fig. 4 and 5, GPx activities of *C. auratus* livers in all treatment groups showed a universal increase trend (P<0.05) while GSH contents was decreased gradually as the increasing concentrations of MSTP effluent. Thus, it was believed that GPx activity in *C. auratus* liver was completely induced by the pollutants in MSTP effluent and played a vital role in the remove of peroxide by transforming GSH into GSSG. The different responses of CAT and GPx may indicate different mechanisms for removal of ROS for the two enzymes. CAT is responsible for the reduction of hydrogen peroxide, while GPx catalyzes the reduction of both hydrogen peroxide and lipid peroxides.[18] In addition, besides as the substrate for GPx, GSH also acts as a direct scavenger of oxyradicals and plays an important role in detoxification reaction for ROS.[19] Reduced GSH depletion is often considered a biomarker of environmental stress as observed in fish stressed by environmental pollutants.

In this study, the AChE activity of *C. auratus* liver was significantly inhibited by MSTP effluent at all exposure concentrations (Fig. 6). AChE is usually considered as a biomarker for reflecting organophosphate/carbamate pollution in environment,[20] because organophosphate/carbamate pesticides have been found to inhibit AChE activity in aquatic organisms.[21] It has been reported that MSTP effluent contains organophosphorus/carbamate pesticides.[22] Consequently, the reduction of AChE activity in this study could be probably attributed to the existence of organophosphorus/carbamate pesticides in MSTP effluent.

Two-tailed Pearson's correlation analysis showed that the biochemical responses of the four antioxidant defense parameters, namely MDA, SOD, GPx and GSH, in *C. auratus* livers showed significant linear relationships with the increasing concentrations of MSTP effluent after a 12-day exposure (Table 1). Thus, MDA, SOD, GPx and GSH in *C. auratus* liver can be used as biomarkers to assess the water quality of the freshwater environment polluted by MSTP effluent.

IV. Figures And Tables

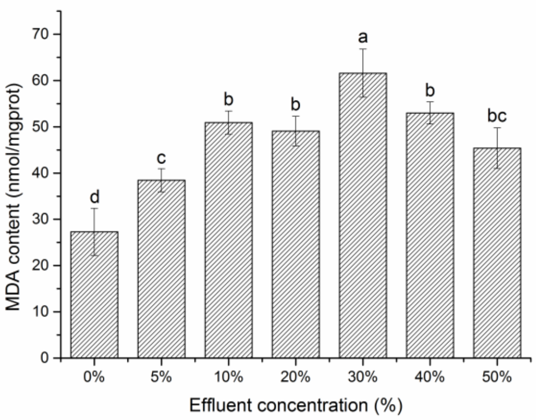

Figure 1. Variation of MDA contents in *C. auratus* livers as exposed to MSTP effluent. The lowercase letter represents significance in MDA contents of *C. auratus* liver. Means in columns with different letters were sigificantly different (P<0.05).

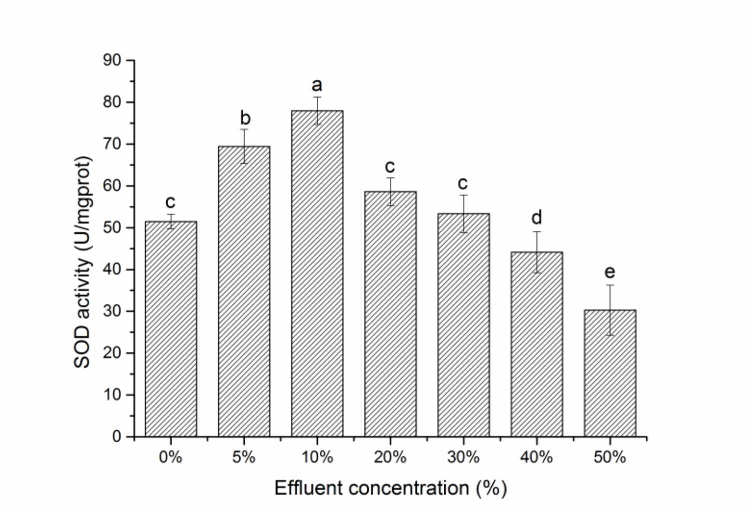

Figure 2. Variation of SOD activities in the livers of *C. auratus* exposed to MSTP effluent. The lowercase letter represents significance in SOD activities of *C. auratus* liver. Means in columns with different letters were sigificantly different (*P*<0.05).

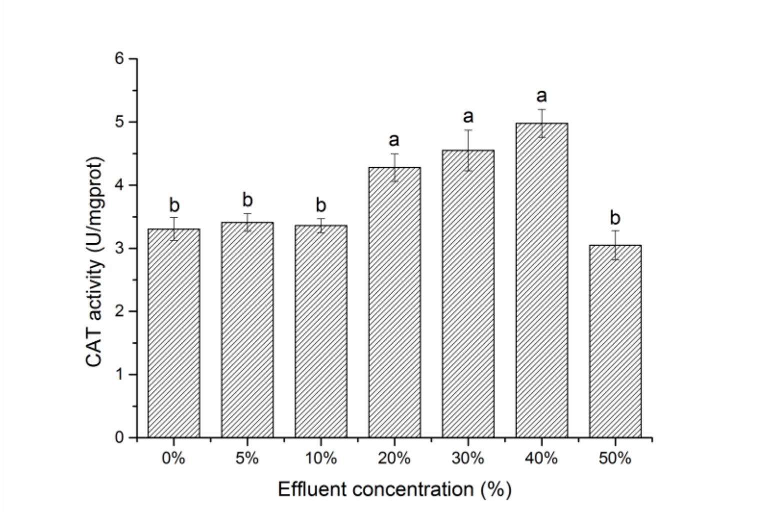

Figure 3. Variation of CAT activities in the livers of *C. auratus* exposed to MSTP effluent. The lowercase letter represents significance in CAT activities of *C. auratus* liver. Means in columns with different letters were sigificantly different (*P*<0.05).

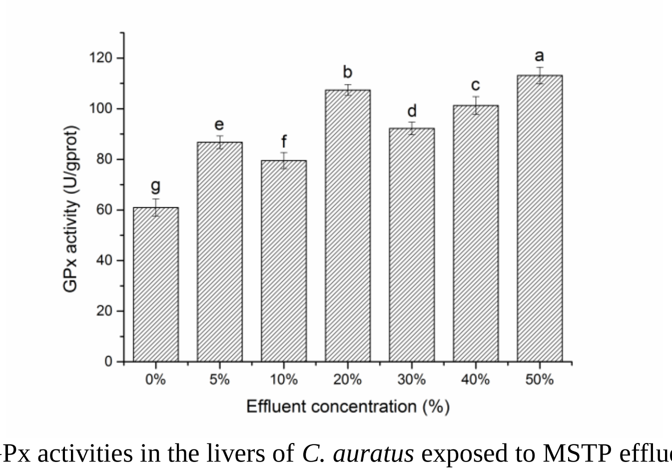

Figure 4. Variation of GPx activities in the livers of *C. auratus* exposed to MSTP effluent. The lowercase letter represents significance in GPx activities of *C. auratus* liver. Means in columns with different letters were sigificantly different (*P*<0.05).

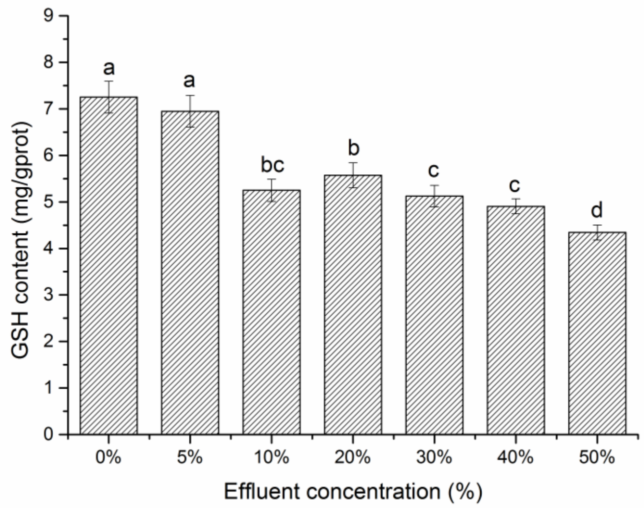

Figure 5. Variation of GSH contents in the livers of *C. auratus* exposed to MSTP effluent. The lowercase letter represents significance in GSH contents of *C. auratus* liver. Means in columns with different letters were sigificantly different (*P*<0.05).

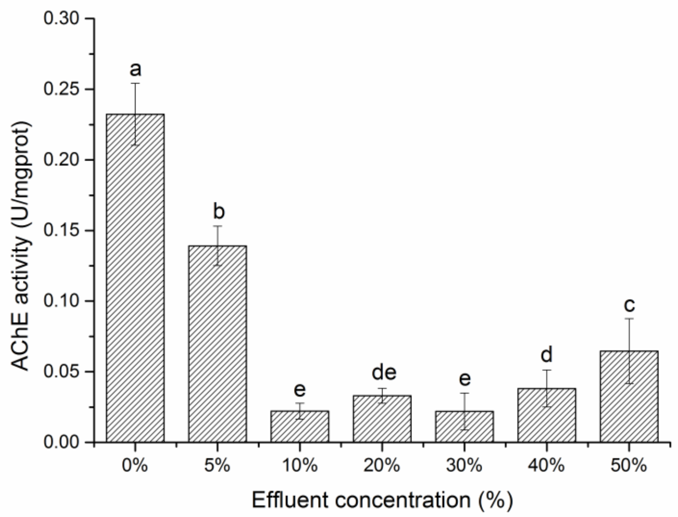

Figure 6. Variation of AChE activities in the livers of *C. auratus* exposed to MSTP effluent. The lowercase letter represents significance in AChE activities of *C. auratus* liver. Means in columns with different letters were sigificantly different (*P*<0.05).

Table 1 Pearson's correlation coefficients among four biomarkers in *C. auratus* liver after a 12-day exposure to MSTP effluent.

Biomakers	Correlation coefficients
MDA	0.869*
SOD	-0.765*
CAT	0.336
GPx	0.825*
GSH	-0.883*
AChE	-0.587

**P*<0.05 according to Pearson's correlation analysis.

V. Conclusion

The present study clearly demonstrated that MSTP effluent could induce oxidative stress in *C. auratus* liver, because the biochemical responses of antioxidant defense system, namely SOD, CAT, GPx, GSH, AChE and MDA, in *C. auratus* liver were significantly altered by MSTP effluent over the expose period. Result showed MDA, SOD, GPx and GSH in *C. auratus* liver could be applied as the biomarkers to assess the water quality of the receving freshwater environment polluted by MSTP effluent due to their sensitive responses to the increasing concentrations of MSTP effluent. The present study provides a basis for the establishment of the evaluation system of MSTP effluent-freshwater pollution by applying the four sensetive biomarkers.

Acknowledgements

The authors would like to thank the financial support from the Natural Science Foundation of Shandong Province (ZR2017PH060).

References

[1]. N. Bolong, A. F. Ismail, M. R. Salim, and T. Matsuura, A review of the effects of emerging contaminants in wastewater and options for their removal, *Desalination, 239(1-3)*, 2009, 229-246.
[2]. R. Wan, F. Meng, W. Fu, Q. Wang, and E. Su, Biochemical responses in the gills of *Meretrix meretrix* after exposure to treated municipal effluent, *Ecotoxicol Environ Saf, 111*, 2015, 78-85.
[3]. M. L. Hannam, S. D. Bamber, R. C. Sundt, and T. S. Galloway, Immune modulation in the blue mussel *Mytilus edulis* exposed to north sea produced water, *Environmental Pollution, 157(6)*, 2009, 1939-1944.
[4]. P. Samanta, H. Im, J. Yoo, et al, Comparative assessment of the adverse outcome of wastewater effluents by integrating oxidative stress and histopathological alterations in endemic fish, *J Hazard Mater, 344*, 2018, 81-89.
[5]. P. Samanta, H. Im, J. Na, and J. Jung, Ecological risk assessment of a contaminated stream using multi-level integrated biomarker response in *Carassius auratus*, *Environ Pollut, 233*, 2018, 429-438.
[6]. I. A. Duarte, P. Reis-Santos, S. França, H. Cabral, and V. F. Fonseca, Biomarker responses to environmental contamination in estuaries: a comparative multi-taxa approach, *Aquatic Toxicology, 189*, 2017, 31-41.
[7]. S. Carvalho Cdos, V. A. Bernusso, H. S. Araujo, et al. Biomarker responses as indication of contaminant effects in *Oreochromis Niloticus*, *Chemosphere, 89(1)*, 2012, 60-69.
[8]. L. Bertrand, R. Asis, M. V. Monferrán, and M. V. Amé, Bioaccumulation and biochemical response in south american native species exposed to zinc: boosted regression trees as novel tool for biomarkers selection, *Ecological Indicators, 67*, 2016, 769-778.
[9]. M. R. Pérez, A. S. Rossi, C. Bacchetta, Y. Elorriaga, P. Carriquiriborde, and J. Cazenave, In situ evaluation of the toxicological impact of a wastewater effluent on the fish *Prochilodus lineatus*: biochemical and histological assessment, *Ecological Indicators, 84*, 2018, 345-353.
[10]. W. K. Kim, S. K. Lee, and J. Jung, Integrated assessment of biomarker responses in common carp (*Cyprinus carpio*) exposed to perfluorinated organic compounds, *J Hazard Mater, 180(1-3)*, 2010, 395-400.
[11]. W. J. Zhang, W. Liu, J. Zhang, et al, Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish, *Journal of Environmental Sciences, 24(11)*, 2012, 2019-2027.
[12]. J. Li, X. Zheng, K. Liu, S. Sun, and X. Li, Effect of tetracycline on the growth and nutrient removal capacity of *Chlamydomonas reinhardtii* in simulated effluent from wastewater treatment plants, *Bioresour Technol, 218*, 2016, 1163-1169.
[13]. E. A. Almeida, A. C. D. Bainy, A. P. M Loureiro, et al., Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the brazilian marine environment: antioxidants, lipid peroxidation and DNA damage, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 146(4)*, 2007, 588-600.
[14]. Z. Xie, G. Lu, K. Hou, D. Qin, Z. Yan, and W. Chen, Bioconcentration, metabolism and effects of diphenhydramine on behavioral and biochemical markers in crucian carp (*Carassius auratus*), *Sci Total Environ, 544*, 2016, 400-409.
[15]. W. K. Kim, and J. Jung, In situ impact assessment of wastewater effluents by integrating multi-level biomarker responses in the pale chub (*Zacco platypus*), *Ecotoxicol Environ Saf, 128*, 2016, 246-251.
[16]. J. Ren, J. Luo, H. Ma, X. Wang, and L. Q. Ma, Bioavailability and oxidative stress of cadmium to *Corbicula fluminea*, *Environ Sci Process Impacts, 15(4)*, 2013, 860-869.
[17]. S. L. Meng, J. Z. Chen, G. D. Hu, et al, Effects of chronic exposure of methomyl on the antioxidant system in liver of nile tilapia (*Oreochromis niloticus*), *Ecotoxicol Environ Saf, 101*, 2014, 1-6.
[18]. G. W. Winston, and R. T. D. Giulio, Prooxidant and antioxidant mechanisms in aquatic organisms, *Aquatic Toxicology, 19(2)*, 1991, 137-161.
[19]. Z. H. Li, V. Zlabek, J. Velisek, R. Grabic, J. Machova, and T. Randak, Modulation of antioxidant defence system in brain of rainbow trout (*Oncorhynchus mykiss*) after chronic carbamazepine treatment, *Comp Biochem Physiol C Toxicol Pharmacol, 151(1)*, 2010, 137-141.
[20]. Y. Li, B. Men, Y. He, H. Xu, M. Liu, and D. Wang, Effect of single-wall carbon nanotubes on bioconcentration and toxicity of perfluorooctane sulfonate in zebrafish (*Danio rerio*), *Sci Total Environ, 607-608*, 2017, 509-518.
[21]. E. D. Domenico, A. Mauceri, D. Giordano, et al., Biological responses of juvenile european sea bass (*Dicentrarchus labrax*) exposed to contaminated sediments, *Ecotoxicology and Environmental Safety, 97*, 2013, 114-123.
[22]. N. Kamel, J. Jebali, M. Banni, S. B. Khedher, L. Chouba, and H. Boussetta, Biochemical responses and metals levels in *Ruditapes decussatus* after exposure to treated municipal effluents, *Ecotoxicol Environ Saf, 82*, 2012, 40-46

Tong Chang. "Biochemical Responses of Antioxidant Defense System in Crucian Carp (Carassius Auratus) Liver after Exposured To Municipal Sewage Treatment Plant Effluent." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.5 (2019): 01-06.