ORIGINAL ARTICLE

Effects of Sewage Effluent on Blood Biochemical Parameters of Common Carp (*Cyprinus carpio*): A Case Study of Behbahan, Khuzestan Province

Mahdi Banaee*, Shima Shahafve, Maryam Vaziriyan, Somaye Taheri, Behzad Nemadoost Haghi

Department of Fisheries and Aquaculture, Faculty of Natural Resources and Environment, Behbahan Khatam Alanbia University of Technology, Iran

ABSTRACT: The purpose of this study was to investigate the effects of untreated municipal wastewater on the blood biochemical parameters of common carp, *Cyprinus carpio*. In this study, the fish were exposed to sub-lethal concentrations of municipal wastewater for 21 days. The blood was sampled after 21 days and its biochemical parameters including acetylcholinesterase (AChE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine phosphokinase (CPK) activities as well as total proteins, albumin, globulin, glucose and cholesterol, triglycerides, creatinine and urea levels were measured. AST, LDH and CPK activities showed an overall significant change ($P < 0.05$) in the experimental groups of fish. A significant decrease in ALT, GGT, ALP and AChE activities was observed in fish exposed to municipal wastewater ($P < 0.05$). Glucose, creatinine and urea, cholesterol and triglyceride levels in blood of fish exposed to municipal wastewater significantly increased ($P < 0.05$). The fish exposed to the municipal wastewater showed a significant decrease in total protein and albumin levels. However, no significant changes were observed in globulin levels in fish exposed to municipal wastewater. In conclusion, exposure to sub-lethal concentrations of municipal wastewater may cause blood biochemical changes in *C. carpio*.

* Corresponding author: mahdibanaee@yahoo.com (M. Banaee).

INTRODUCTION

Aquatic ecosystems can be contaminated by industrial and municipal discharges as well as surface runoff, which may cause alterations to the natural environment. Many chemical constituents of sewage including pesticides, polychlorinated biphenyls (PCBs), heavy metals, petroleum oil, industrial oil, garbage leachate, detergents, cosmetic products, and pharmaceuticals could directly impact aquatic organisms [1- 6].

Fish species inhabiting agricultural and residential areas may be exposed to pollutants during different stages of their life. The influence of sewage effluent on the fish physiology, bichemistry, genetics and population levels have been reported by scientists [7-10]. Alterations in biochemical parameters [11, 12], and blood cell profile [13], reproductive disorders [14-17], behavioral changes [18-20], neurological disorders [21], histopathological changes [11, 13], genetic damge [22], and reduced survival rate of fish [23] are reported in those species exposed to treated and untreated sewage effluents.

Treated and untreated municipal wastewaters of Behbahan, discharged into the Maroon River, may have a significant effect on the health of aquatic organisms inhabiting the river. Effluent from sewage treatment plants in different cities of Iran contains many of these endocrine disruptor chemicals (EDCs) [24-26]. The endocrine disruptor chemicals in wastewater in Iran were ten times more than drinking water [24]. Steroid hormones, including estrogens, estrone, 17ß–estradiol, estriol, and 17a–ethinylestradiol are excreted through human waste and although 50 to 90% or more may be removed through activated sludge treatment [24-26], it appears that many endocrine disruptor chemicals still make their way into streams and waterways through wastewater effluent [24-26]. Fish exposed to treated sewage effluents can exhibit significant physiological and reproductive abnormalities similar to those in fish exposed to endocrine disruptor chemicals [Banaee et al.

Unpublished data]. Therefore, our main experimental hypothesis for this study is that exposure to treated sewage effluent has significant effects on common carp, *Cyprinus carpio*.

This study was therefore focused on determining if fish exposure to untreated sewage effluent is evident in wild fish living in the Maroon River, using common carp as an animal model. In the present study, alterations in the blood biochemical parameters were measured as health biomarkers of fish exposure to untreated sewage effluent. *C. carpio* were selected for the study based on the following criteria: The family Cyprinidae are among the most common fish inhabiting freshwaters of Iran. One of the most common species within this family is *C. carpio*, which shows a clear preference for riverine and lacustrine waters throughout Khuzestan Province. *C. carpio* can be found in abundant quantity throughout the year, and has a commercial importance for people in developing countries. Common carp are tolerant to wide fluctuations of temperature and are resistant to diseases. They are voraciously omnivorous and easily adapted to artificial diets. In addition, these fish are tough in handling and transport. Therefore, common carp is an appropriate aquatic animal for bioassay testing.

MATERIALS AND METHODS

Fish

Healthy common carp (*C. carpio*) were purchased from a private farm (Behbahan, Iran) and transported to laboratory in polyethylene bags containing oxygenated water. They were acclimatized to laboratory conditions for two weeks before the experiments. Tap water free from chlorine was used for the present study. Water quality was monitored daily in terms of unionized ammonia (< 0.05 mg.L^{-1}), dissolved oxygen (6.5 ± 0.5 mg.L^{-1}), temperature (24 ± 2 °C) and pH (7.4 ± 0.2).

During the acclimatization period, fish were fed a commercial diet obtained from Beyza Feed Mill (Shiraz, Iran). Feeding was stopped two days prior to the beginning of the experiment to keep the experimental animals more or less under the same metabolic conditions.

Wastewater

Sewage collected at six different station of the sewage canal in Behbahan on 26 April 2015. In the 7 days prior to sampling, there was no rain in the sampling area and the minimum and maximum air temperature was 17–32 °C. Duplicates of each sample were collected in brown glass bottles with Teflon stoppers. In order to disinfect and remove pathogens from wastewater, it was filtered and autoclaved at 121 °C for 15 min before use.

Fish exposure to municipal wastewater

The experiment was performed after a two-week acclimation period. The fish were placed into an anesthetic solution (200 mg.L^{-1} clove powder) for 3-5 min before being weighed individually. One hundred eight healthy fish were selected from the stock and were transferred into the water tanks (nine 80-liter plastic tanks that were filled with 70 liters of water). Fish were randomly assigned to three groups: Group I was maintained in tap water as the control group. Group II & III were maintained in water respectively polluted with 7 mL and 14 mL of the municipal wastewater collected from a sewage canal in Behbahan (equivalent 0.1 and 0.2 ml per liter) for 21 days. Tanks were cleaned by siphoning and 40% of water was exchanged daily to reduce metabolic wastes and to maintain concentrations of municipal wastewater near the nominal level. During the experimental period, fish were fed with commercial feed.

Sampling and analysis of blood biochemical parameters

After the 21-day exposure period, 12 fish from each treatment (4 fish from each tank) were taken out for sub-lethal toxicity studies. Each group of fish was harvested using a scoop net and placed into an anesthetic solution (200 mg.L^{-1} clove powder). Blood sample was collected from the caudal vein using heparinized syringes, centrifuged at $6000 \times g$ for 10 min and stored at -25 °C. All blood biochemical parameters were determined using an UV-visible spectrophotometer (UNICO 2100) and standard biochemical reagents (Pars Azmoon Company, Tehran, Iran). Each blood biochemical parameter was measured by a certain method. Total plasma protein was measured at 540 nm by the Biuret reaction. The albumin assay is based on the dye-binding properties of plasma albumin with a bromocresol green. An increase in the blue-green color was measured at 630 nm. The plasma globulin based on the ratio of albumin versus total protein [27]. Plasma glucose was measured by the glucose-oxidase method at 500 nm [28]. Plasma cholesterol levels by the CHOD-PAP enzymatic method at 510 nm, triglyceride levels by GPO-PAP enzymatic method at 546 nm [29] and creatinine by the JAFFE method and at 510 nm [30]. Urea is hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate are converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH). Simultaneously, a molar equivalent of reduced NADH is oxidized. Two molecules of NADH are oxidized for each molecule of urea hydrolyzed. The rate of change in absorbance at 340 nm, due to the disappearance of NADH, is directly proportional to the blood urea concentration in the sample [31].

Plasma acetylcholinsetrase (AChE) activity was determined by adding and adequate volume of sample into a cuvette containing 0.1 M phosphate pH 8.0, and acetylcholine iodide (0.015 M) and dithiobis

nitrobenzoic acid (0.01 M) as substrates. AChE activity was recorded during 180 s at 405 nm [32]. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined by NADPH consumption and its conversion to NAD^+ at 340 nm. γ-glutamyl transferase (GGT) activity is determined by a coupled enzyme assay, in which the GGT transfers the γ-glutamyl group from the substrate L-γ-Glutamyl *p*-nitroanilide, liberating the chromogen *p*-nitroanilide at 418 nm proportional to the GGT present. Lactate dehydrogenase (LDH) in plasma was determined based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) based on converting nitro phenol phosphate into nitrophenol and phosphate at 405 nm, creatinine phosphokinase (CK) based on the conversion of creatinine phosphate into creatinine at 340 nm and based on optical density (OD) absorption and the formula presented in the kits' manual [33].

Data analysis

The significant difference in the biochemical parameters of fish exposed to different concentrations of untreated sewage effluents was examined using one-way ANOVA. All the data were checked for normality (Kolmogorov-Smirnov test). Means were compared by Duncan's test and a $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (IBM, 19) software. Data are presented as mean (SD).

RESULTS

AST activity showed an overall significant change ($P < 0.05$) in experimental groups of fish, but those exposed to municipal wastewater had significantly lower levels of ALT. The fish exposed to municipal wastewater showed a significant decrease in GGT as compared to control group. A significant decrease was found in ALP activity in blood of fish exposed to municipal wastewater. However, the fish exposed to municipal wastewater exhibited a significant increase in LDH activity. CPK activity showed an overall increase in experimental groups of fish ($P < 0.05$). A significant decrease in AChE activity was observed in fishes exposed to municipal wastewater.

Wastewater caused an increase in the glucose levels. Fish exposed to municipal wastewater showed a significant decrease in total protein and albumin levels. However, no significant changes were observed in the plasma globulin levels in fish exposed to municipal wastewater. They also showed a significant increase ($P < 0.05$) in plasma creatinine and urea levels at the end of the period when compared with the control group. The cholesterol and triglyceride levels in blood of fish exposed to municipal wastewater for 21 day significantly increased ($P < 0.05$). The effect of Effect of untreated municipal wastewater on different variables is depicted in Figures 1-15.
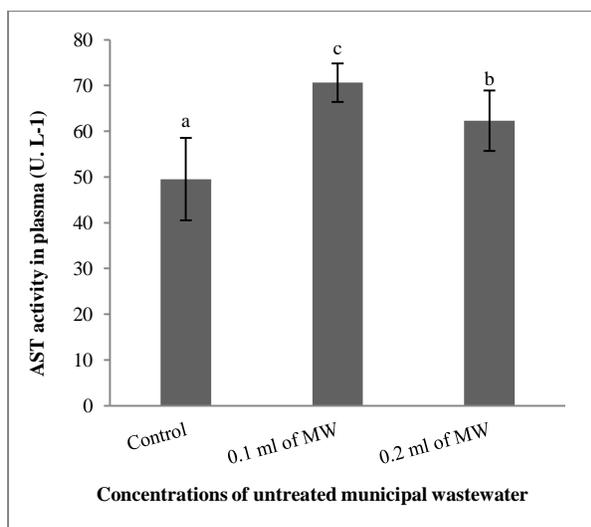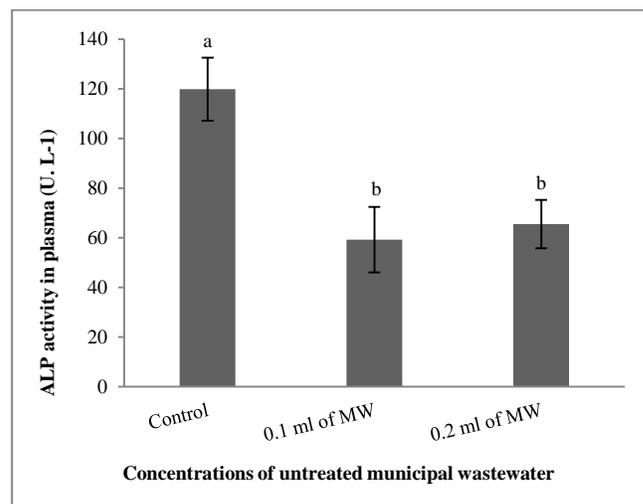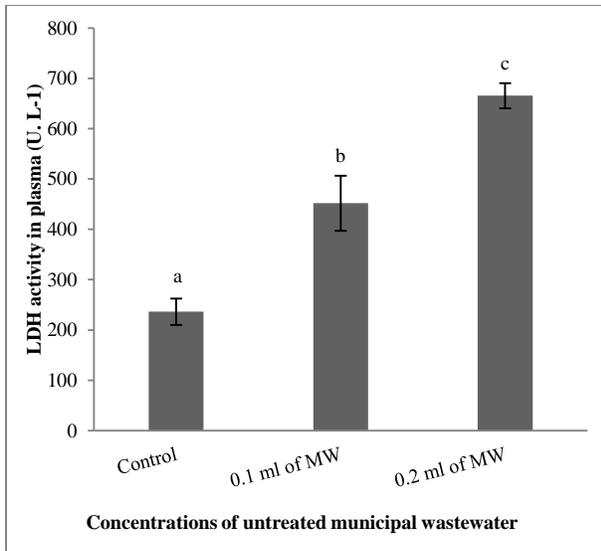
Figure 1. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on AST activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
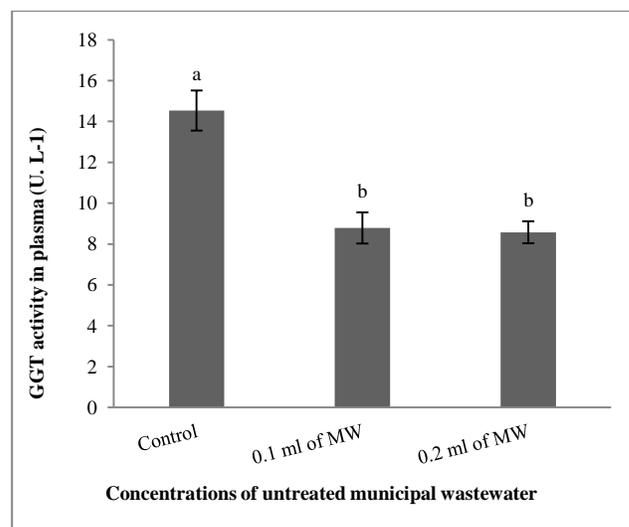
Figure 3. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on GGT activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
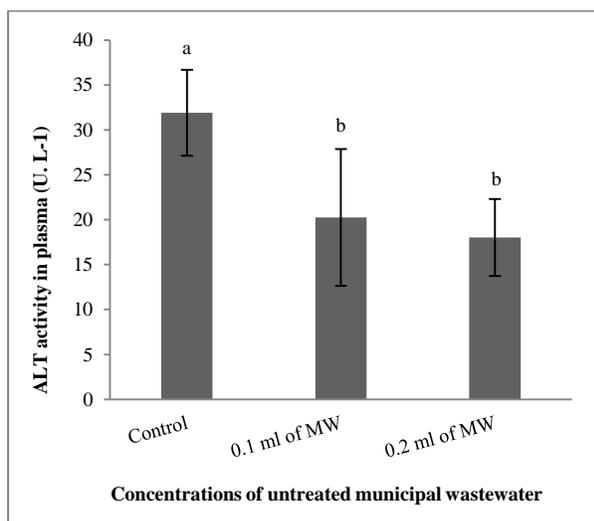
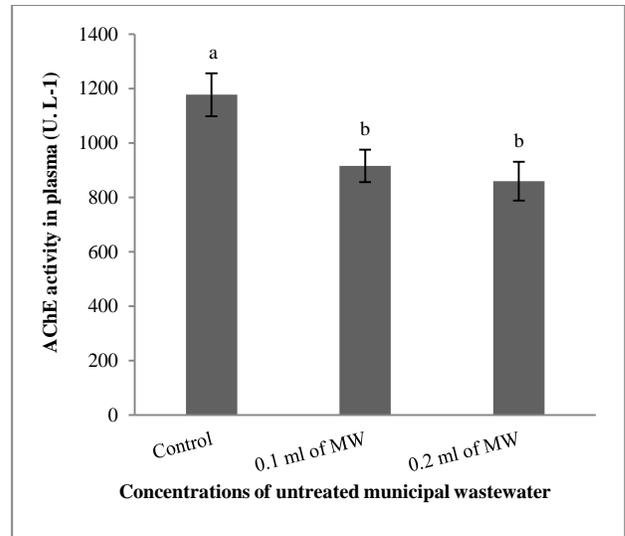**Figure 2**.Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on ALT activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
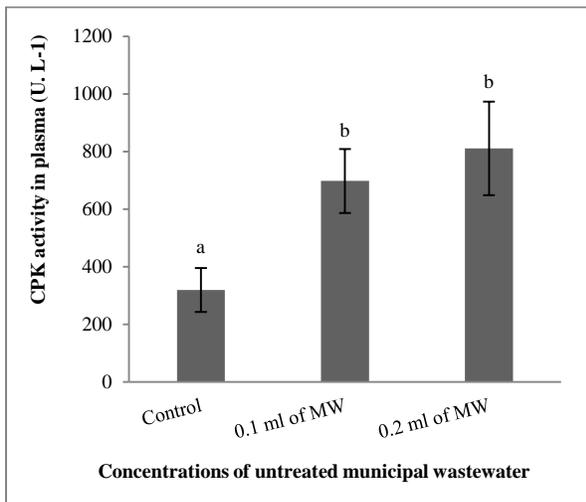

Figure 4. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on ALP activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

Figure 5. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on LDH activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
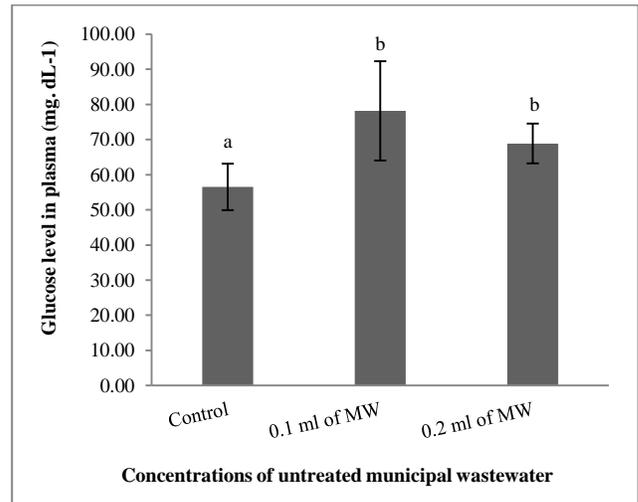

Figure 7. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on AChE activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

Figure 6. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on CPK activity in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
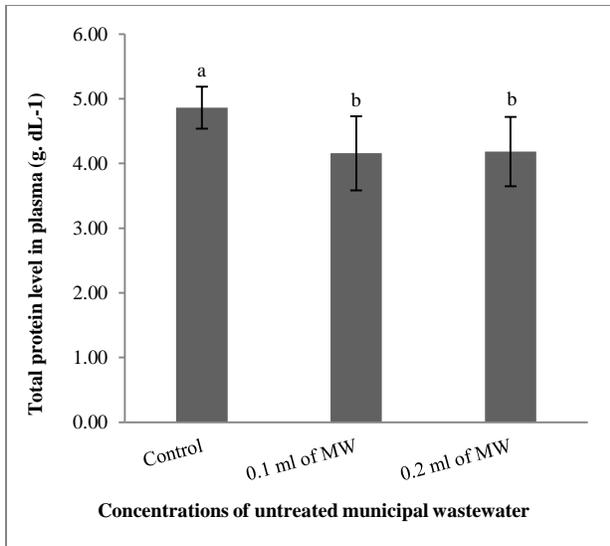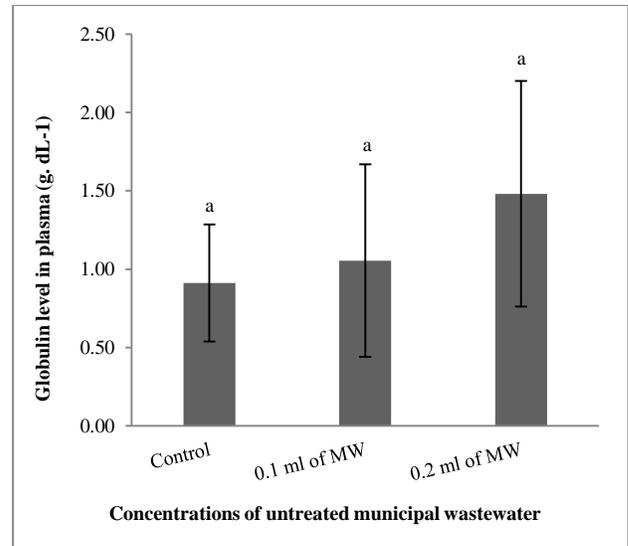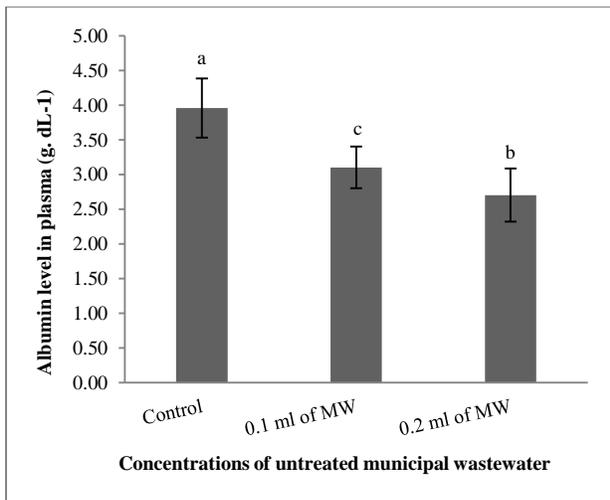

Figure 8. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on glucose level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
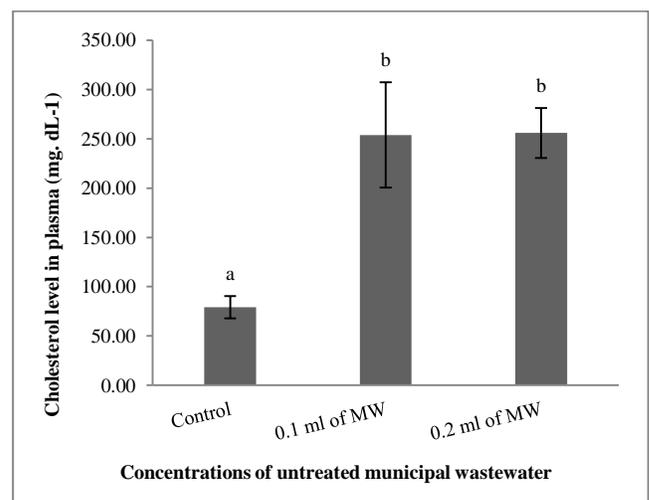
Figure 9. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on total protein level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean $\pm$ S.D.

Figure 11. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on globulin level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean $\pm$ S.D.

Figure 10. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on albumin level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean $\pm$ S.D.

Figure 12. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on cholesterol level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean $\pm$ S.D.

Figure 13. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on triglyceride level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.
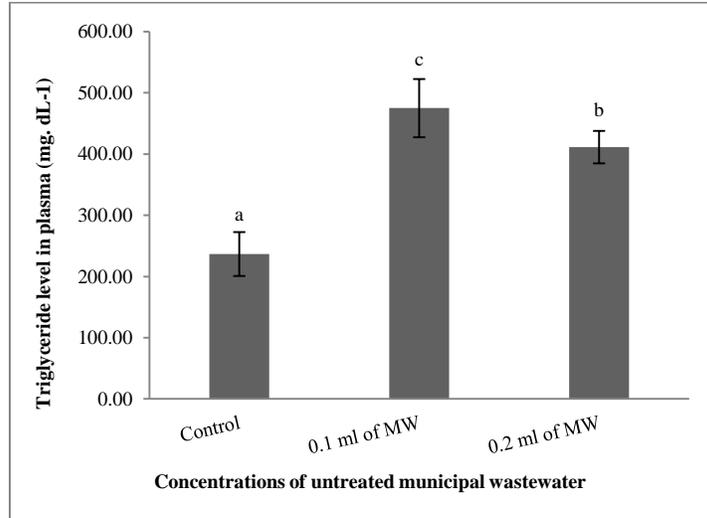

Figure 14. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on creatinine level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D
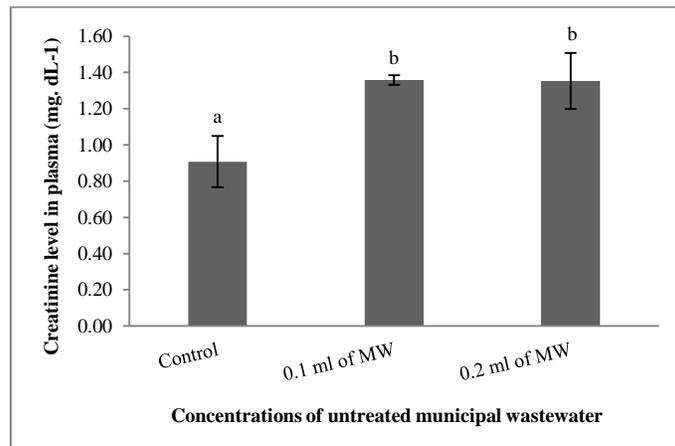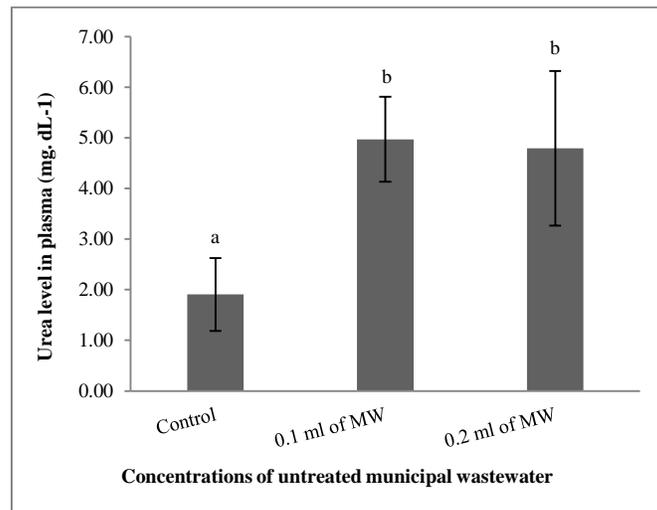

Figure 15. Effect of untreated municipal wastewater (0.1 and 0.2 ml per 1 liter) on urea level in plasma of common carp; significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

DISCUSSION

The main objective of this study was to investigate the effects of xenobiotic in untreated sewage on the blood biochemical parameters of common carp to determine the risks associated with untreated sewage discharges in surface waters in Khuzestan Province, Iran.

Fish absorb xenobiotic directly from wastewater and bio-accumulate it in their fatty acids due to the lipophilic nature of xenobiotic. Surfactant, found in municipal wastewater, may increase the absorption of xenobiotic by the gills, skin and digestive system of fish. Our results revealed that municipal wastewater could indeed exert remarkable effects on blood biochemical parameters of fish.

GGT and ALT has been detected in various fish organs, including kidneys, liver, pancreas and spleen [34]. ALP is present in most tissues but is particularly in high concentration in the epithelium cells of the hepatobiliary tract, intestinal wall and renal tubules of fish [35]. AST and LDH enzymes have been detected in high concentrations in cellular cytoplasm of liver, kidney, heart and skeletal muscle and erythrocytes of fish [35]. Therefore, damage to any of these tissues may increase plasma AST and LDH levels. LDH plays a critical role in regulating glycolysis under adverse environmental conditions. There was an increase in LDH activity indicating anaerobic metabolism to combat stress. In the present study, increased in AST activity was observed in plasma of fish exposed to the municipal wastewater, which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes, while decreased activity of ALT, GGT and ALP occurs either via direct action of municipal wastewater on the enzymes or inhibition of their biosynthesis [34]. Furthermore, a lower than normal ALT, GGT and ALP activity may occur as a result of reduced synthesis and severe tissue damage [36]. CPK have been found in high concentrations in cellular cytoplasm of cardiac and skeletal muscle of fish [35]. The increased activity of CPK may be indicative of a disorder in muscle fibres. These results agree with a previous study carried out on carp exposed to diazinon [37], chlorpyrifos [38], malthion [39], fenpropathrin [40], cadmium chloride [34], and paraquat [41].

A concentration range of 1-2 ml wastewater effluent inhibited AChE activity in plasma of fish. Inhibition of AChE activity in plasma of fish may be caused by organophosphate and carbamate pesticides, certain heavy metals and surfactants that may be present in municipal wastewater. Decreases in AChE activity in freshwater mussels (*Elliptio complanata*) exposed to municipal wastewater have been reported by Gagné et al. [42].

Increased blood glucose in fish exposed to municipal wastewater may reflect an increased need for energy to counteract the effects of stress caused by pollutants in wastewater. Hyperglycemia or elevated blood glucose levels may also be due to an increase in the breakdown of glycogen stores in the muscle and liver tissues because of cellular damage. A significant increase in plasma glucose levels in *Oreochromis niloticus* and *C. carpio* was reported after Cd and Pb [36] and chlorpyrifos [38] exposures.

Hao et al. [43] showed that there was a significant relationship between levels of glucose and levels of cholesterol in plasma. In stressful situations, glucose is converted to pyruvate in the glycolytic pathway, and pyruvate is metabolized to acetyl-CoA in aerobic tissues and used as a precursor in the synthesis of cholesterol and fatty acids in the citric acid cycle [44].

In addition, the increased blood triglyceride and cholesterol level may suggest that a general increase in lipid mobilization must have taken place simply to fulfill the increasing demand for energy to cope with the stress of wastewater toxicity. However, many reports

have highlighted marked influence of pollutants on cholesterol and triglyceride levels in blood. For example, Banaee et al. [38] have reported significant increases in both plasma triglyceride and cholesterol in fish exposed to chlorpyrifos. A significant increase in cholesterol and triglyceride by PCBs has been reported by Aminov et al. [45].

Protein is an important constituent of cells and tissues as it plays a vital role in the physiology of living organisms. There was a decrease in total protein indicating increased mobilization of protein reserves to adapt to stress conditions. Malnutrition, reduced efficiency of the liver in protein synthesis, and reduction of nutrient absorption, especially protein, in the digestive system may be important factors in decreasing plasma total protein. Albumin is the most abundant multifunctional single chain protein in blood plasma. Decreased albumin levels may be due to the effect of chemical compounds, found in wastewater, on the biochemical structure of albumin [46]. In addition, decreased plasma albumin levels of fish exposed to the wastewater may be due to decreases in total protein levels in plasma. No significant changes were observed in globulin levels.

Any increase in blood creatinine and urea levels is a biomarker of kidney dysfunction, because creatinine and urea are normally quickly removed from the blood and then excreted from the body [35, 47]. Our results indicated that creatinine and urea levels in plasma reflected the toxicity in the kidney of fish exposed to municipal wastewater for 21 days.

CONCLUSIONS

This study supports the study's hypothesis because the xenobiotic present in treated sewage effluent had significant effects on the blood biochemical parameters of common carp. In addition, we conclude that the continuous exposure of common carp to municipal wastewater effluents leads to blood biochemical alterations and homeostasis disruption.

CONFLICT OF INTERESTS

Authors have no conflict of interests.

ACKNOWLEDGMENTS

REFERENCES

1. Harrison E.Z., Oakes S.R., Hysell M., Hay A., 2006. Organic chemicals in sewage sludges. Sci Total Environ. 367(2), 481-497.

2. Douxfils J., Mandiki R., Silvestre F., Bertrand A., Leroy D., Thomé J.P., Kestemont P., 2007. Do sewage treatment plant discharges substantially impair fish reproduction in polluted rivers? Sci Total Environ. 372(2), 497-514.

3. Filby A.L., Shears J.A., Drage B.E., Churchley J.H., Tyler C.R. 2010. Effects of advanced treatments of wastewater effluents on estrogenic and reproductive health impacts in fish. Environ Sci Tech. 44, 4348-4354.

4. Hosseini N.A.A., Parker W.J. J., Matott L.S.L. 2012. Modelling Concentrations of Pharmaceuticals and Personal Care Products in a Canadian Watershed. 37(3), 191-208.

5. Keiter S., Baumann L., Färber H., Holbech H., Skutlarek D., Engwall M., Braunbeck T., 2012. Long-term effects of a binary mixture of perfluorooctane sulfonate (PFOS) and bisphenol A (BPA) in zebrafish (*Danio rerio*). Aquat Toxicol. 118, 116-129.

6. Kostich M.S., Batt A.L., Lazorchak J.M., 2014. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in

the US and implications for risk estimation. Environ Pollut. 184, 354-359.

7. Patisaul H.B., Adwale H.B., 2009. Long-term effects of endocrine disruptors on reproductive physiology and behavior. Front Behav Neurosci. 3(10), 1-18.

8. Saaristo M., Craft J.A., Lehtonen K.K., Lindström K., 2010. An endocrine disrupting chemical changes courtship and parental care in the sand goby. Aquat Toxicol. 97(4), 285-292.

9. Pottinger T.G., Cook A., Jürgens M.D., Rhodes G., Katsiadaki I., Balaam J.L., Smith A.J., Matthiessen P., 2011. Effects of sewage effluent remediation on body size, somatic RNA: DNA ratio, and markers of chemical exposure in three-spined sticklebacks. Environ Int. 37(1), 158-169.

10. Santos E.M., Hamilton P.B., Coe T.S., Ball J.S., Cook A.C., Katsiadaki I., Tyler C.R., 2013. Population bottlenecks, genetic diversity and breeding ability of the three-spined stickleback (*Gasterosteus aculeatus*) from three polluted English Rivers. Aquat Toxicol. 142, 264-271.

11. Palace V.P., Wautier K.G., Evans R.E., Blanchfield P.J., Mills K.H., Chalanchuk S.M., Godard D., McMaster M.E., Tetreault G.R., Peters L.E., Vandenbyllaardt L., Kidd K.A., 2006. Biochemical and histopathological effects in pearl dace (*Margariscus margarita*) chronically exposed to a synthetic estrogen in a whole lake experiment. Environ Toxicol Chem. 25(4), 1114-1125.

12. Miller D.H., Jensen K.M., Villeneuve D.L., Kahl M.D., Makynen E.A., Durhan E.J., Ankley G.T., 2007. Linkage of biochemical responses to population-level effects: a case study with vitellogenin in the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem. 26(3), 521-527.

13. Maceda-Veiga A., Monroy M., Navarro E., Viscor G., de Sostoa A., 2013. Metal concentrations and pathological responses of wild native fish exposed to

sewage discharge in a Mediterranean river. Sci Total Environ, 449, 9-19.

14. Vajda A.M., Barber L.B., Gray J.L., Lopez E.M., Woodling J.D., Norris D.O., 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ Sci Technol. 42(9), 3407-3414.

15. Thorpe K.L., Maack G., Benstead R., Tyler C.R., 2009. Estrogenic wastewater treatment works effluents reduce egg production in fish. Environ Sci Technol. 43(8), 2976-2982.

16. Segner H., Casanova-Nakayama A., Kase R., Tyler C.R., 2013. Impact of environmental estrogens on Yfish considering the diversity of estrogen signaling. Gen Comp Endocrinol. 191, 190-201.

17. Wang Y., Zhou J., 2013. Endocrine disrupting chemicals in aquatic environments: A potential reason for organism extinction? Aquat Ecosyst Health Manag. 16, 88-93.

18. Al-Salhi R., Abdul-Sada A., Lange A., Tyler C.R., Hill E.M., 2012. The xenometabolome and novel contaminant markers in fish exposed to a wastewater treatment works effluent. Environ Sci Technol. 46, 9080–9088.

19. Söffker M., Tyler C.R., 2012. Endocrine disrupting chemicals and sexual behaviors in fish--a critical review on effects and possible consequences. Crit Rev Toxicol. 42(8), 653-668.

20. Söffker M., Stevens J.R., Tyler C.R., 2012. Comparative breeding and behavioral responses to ethinylestradiol exposure in wild and laboratory maintained zebrafish (*Danio rerio*) populations. Environ Sci Technol. 46(20), 11377-11383.

21. Tierney K.B., Farrell A.P. Brauner C.J. 2013. Fish Physiology: Organic Chemical Toxicology of Fishes: Fish Physiology, Elsevier Science.

22. Schwindt A.R., Winkelman D.L., Keteles K., Murphy M., Vajda A.M., Frid C., 2014. An environmental oestrogen disrupts fish population

dynamics through direct and transgenerational effects on survival and fecundity. J Appl Ecol. 51, 582-591.

23. Wedekind C., 2014. Fish populations surviving estrogen pollution. BMC Biol. 12(10), 1-3.

24. Jafari A.J., Pourkabireh Abasabad R., Salehzadeh A., 2009. Endocrine disrupting contaminants in water resources and sewage in Hamadan city of Iran. Iran J Environ Health Sci Eng. 6(2), 89-96.

25. Mohagheghian A., Nabizadeh R., Mesdghinia A.R., Rastkari N., Mahvi A.H., Alimohammadi M., Yunesian M., Ahmadkhaniha R., Nazmara Sh., 2014. Distribution of estrogenic steroids in municipal wastewater treatment plants in Tehran, Iran. J Environ Health Sci Eng. 12(97), 1-7.

26. Mohebzadeh T., Taghizadeh M.M., Takdastan A., Dehghani M., 2013. Comparing the performance of wastewater treatment using activated sludge and aerated lagoons processes in the removal efficiency of estradiol hormones. Jundishapur J Health Sci. 5(3), 149-156.

27. Johnson A.M., Rohlfs E.M., Silverman L.M., 1999. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; p. 477-540.

28. Sacks D.B., 1999. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. W.B. Saunders Company, Philadelphia. pp. 766-85.

29. Rifai N., Bachorik P.S., Albers J.J., 1999. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. W.B. Saunders Company, Philadelphia. pp. 809-861.

30. Foster-Swanson A., Swartzentruber M., Roberts P., 1994. Refrence interval studies of the rate-blancked creatinine, Jaffe method on BM /Hitachi Systems in Six U.S. Laboratories (Abstract). Clin Chem. No. 361.

31. Lumeij J.T., Remple J.D., 1991. Plasma urea, creatinine and uric acid concentrations in relation to

feeding in peregrine falcons (*Falco peregrinus*). Avian Pathol. 20(1), 79-83.

32. Knedel M., Boetteger R., 1967. Kinetic method for determination of pseudocholinesterase (acylcholine acylhydrolase) activity. Klin Wochenschr. 45, 325-327.

33. Moss D.V., Henderson A.R., 1999. Clinical enzymology In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; p. 617-721.

34. Mehrpak M., Banaee M., Nematdoost Haghi B., Noori A., 2015. Protective effects of vitamin C and chitosan against cadmium-induced oxidative stress in the liver of common carp (*Cyprinus carpio*). Iran J Toxicol. 9(30), 1360-1367.

35. Banaee M., 2013. Physiological dysfunction in fish after insecticides exposure: Insecticides often undesired but still so Important, Edited by Stanislav Trdan, Published by InTech, Chapter 4, 103-142.

36. Atli G., Ariyurek S.Y., Kanak E.G., Canli M., 2015. Alterations in the serum biomarkers belonging to different metabolic systems of fish (*Oreochromis niloticus*) after Cd and Pb exposures. Environ Toxicol Pharmacol. 40(2), 508-515.

37. Banaee M., Sureda A., Mirvaghefi A.R., Ahmadi K., 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pestic Biochem Phys. 99(1), 1-6.

37. Banaee M., Sureda A., Mirvaghefi A.R., Rafei G.R., 2011. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem. 37(4), 885-896.

38. Banaee M., Nematdoust Hagi B., Ibrahim A.T.A. 2013. Sub-lethal toxicity of chlorpyrifos on Common carp, *Cyprinus carpio* (Linnaeus, 1758): Biochemical response. Int J Aquat Biol. 1(6), 281-288.

39. Banaee M., Sureda A., Shahaf S., Fazilat N., 2015. Protective effects of silymarin extract on malthion-

induced zebra cichlid (*Cichlasoma Nigrofasciatum*) hepatotoxicity. Iran J Toxicol. 9(28), 1239-1246.

40. Banaee M., Sureda A., Zohiery F., Nematdoust Hagi B., Garanzini D.S., 2014. Alterations in biochemical parameters of the freshwater fish, *Alburnus mossulensis*, exposed to sub-lethal concentrations of Fenpropathrin. Int J Aquat Biol. 2(2), 58-68.

41. Sharifinasab Z., Banaee M., Mohiseni M., Noori A., 2016. Vitamin C and chitosan alleviate toxicity effects of paraquat on some biochemical parameters in hepatocytes of common carp. Iran J Toxicol. (In press).

42. Gagné F., André C., Cejka P., Hausler R., Fournier M., 2011. Evidence of neuroendocrine disruption in freshwater mussels exposed to municipal wastewaters. Sci Total Environ. 409(19), 3711-3718.

43. Hao M., Head W.S., Gunawardana S.C., Hasty A.H., Piston D.W. 2007. Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. Diabetes. 56(9), 2328-2338.

44. Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W., 2003. Harper's Illustrated Biochemistry, 26 editions, Lange Medical Books/McGraw-Hill. Medical Publishing Division. 702 p.

45. Aminov Z., Haase R.F., Pavuk M., Carpenter, D.O., 2013. Analysis of the effects of exposure to polychlorinated biphenyls and chlorinated pesticides on serum lipid levels in residents of Anniston, Alabama. Environ Health, 12: 108. doi: 10.1186/1476-069X-12-108.

46. Ahmad E., Rabbani G., Zaidi N., Ahmad B., Khan R.H., 2012. Pollutant-Induced Modulation in Conformation and β-Lactamase Activity of Human Serum Albumin. PLOS One. 7(6): e38372. doi:10.1371/journal.pone.0038372.

47. Wilkie M.P., 2002. Ammonia excretion and urea handling by fish gills: present understanding and future. research challenges. J Exp Zool. 293(3), 284-301.