Iranian Journal of Fisheries Sciences 21(3) 758-768 2022
DOI: 10.22092/ijfs.2022.127071

Research Article

Hepatic CYP450 gene expression, hematological and biochemical indices in Caspian roach *(Rutilus caspius*) induced by Endosulfan

Tajari M.[1]; Kamali A.[2*]; Rajabi Islami H.[1*]; Paknejad H.[3]

Received: July 2019 Accepted: April 2022

Abstract

Endosulfan is one of the organochlorine pesticides which has been used worldwide for decades. The purpose of the present study was to investigate the effect of endosulfan on hematological and P450 gene expression in *Rutilus caspius*. Fish were exposed to the 10% and 20% LC_{50} for 21 days and were sampled on days 1, 7, 14 and 21. Results showed that there were significant differences in hematological parameters among the control and treated fish (p<0.05). The highest amount of Hb, Ht and RBC was observed in the control, while WBC was highest in the 20% endosulfan treated group on day 21. According to the results alkaline phosphatase, aspartate aminotransaminase and alanine amino transaminase levels increased during the experiment. The highest levels were observed with 20% endosulfan on day 14 which were significantly different with those in the control (p<0.05). In addition, the CYP450 gene expression had the same results. We conclude that exposure to the endosulfan (especially 20%) can enhance the innate immune system in Rutilus *caspius*.

Keywords: Endosulfan, Hematological, Gene expression, *Rutilus caspius*.

1-Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2-Department of Food Industry, Kherad Institute of Higher Education, Bushehr, Iran.
3-Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
*Corresponding Authors' Email: kamali.abolghasem@gmail.com; rajabi.h@srbiau.ac.ir

Introduction

Aquatic ecosystems, as the largest natural environment, are always faced with threats such as genetic restriction and biodiversity (Van-Der Geest *et al*., 1997). Pesticides are one of the main causes of poisoning in fish which released from thousands of chemicals can cause high mortality even at very low concentrations (Sanchez-Fortun and Barahona, 2005).

Fish are an important source of food in many regions of the world, so it is necessary to secure the health of fish (Assefa and Abunna, 2018). Producing high quality fry depends on a lot of environmental factors such as poisons which negatively affect the stage of early growth of fish (Sanchez-Fortun and Barahona, 2005).

Evaluation of blood factors is used in many aquaculture and fish farming researches and also in the field of toxicology and bioassay to determine the ecotoxicological hazards of pesticides and as an appropriate indicator showing physiological and pathological changes in fish (Ullah and Zorriehzahra, 2015; Bhuvaneshwari *et al*., 2015). Certainly changes in hematological parameters due to poisoning can be a sign of changes in hematopoietic tissue and various tissues of fish during poisoning. Generally, it is believed that the quality and characteristics of blood cells are as sensitive to physiological changes when they are affected by pathological changes (Megarani *et al*., 2020).

Alanine aminotransferase (AST) and aspartate aminotransferase are the most important enzymes in the amine groups, which catalyze alpha-keto acids to amino acids by transferring amine units. Enzymes are found mainly in liver cells, and are also present less in the heart, kidneys and skeletal muscles (Rastiannasab *et al*., 2016). Any damage or necrosis of the liver cells will increase secretion of these enzymes and their entry into plasma. Hence, increased activity of these enzymes in plasma can be a sign of tissue damage, especially in liver tissue (Banai *et al*., 2010). Molecular changes as the first measurable changes can provide us a lot of information about the effect of stress-reducing substances (Rose *et al*., 2006). Proteins like HSP70, metallothioneins and the cytochrome oxidase enzyme P450 can be examined as pollutant molecular markers at the level of the genome or protein (Chan *et al*., 1995; Bruno *et al*., 2006; Dong *et al*., 2013; Tedeschi *et al*., 2015). P450 is the first enzyme that is produced in the first phase of the response to pollutants (Rusni *et al*., 2022). This protein is also expressed in non-stress conditions, but its exposure to various pollutants, including heavy metals, changes its expression, which can be considered as a biomarker of contamination (Korashy and El-kadi, 2005; Sheader *et al*., 2006; Softeland *et al*., 2010).

Endosulfan is one of the organo-choleric pesticides which enters the water through agricultural runoffs and due to its chemical stability, weak degradability and increasing power of bio-accumulation in the body of living

organisms like aquatic animals creates various lesions in them. In studies that aquatics were exposed to different concentrations of this poison, many responses have been reported in various species including tissue damage, enzymatic changes, changes in hematological, genetic, behavioral, reproductive parameters and even death (Akhtar *et al*., 2012; Crupkin *et al*., 2013; Negro *et al*., 2015). The present study was performed to determine the effect of endosulfan on hematological parameters and the expression of CYP450 gene in *Rutilus caspius*.

Materials and methods

Fish and experimental conditions

This experiment was conducted at the Aquaculture laboratory of the Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources in 2017. Two hundred roach juveniles were purchased from a private farm (Golestan province, Iran) and transferred to the lab. Fish were adopted for two weeks and fed a commercial diet (Biomar, France) three times a day. They were stocked randomly in 3 groups (control, 10% and 20% endosulfan) in 12 tanks (500-L). For the LC_{50} experiment, 12 fish with a mean weight of 18.46±2.32 g were exposed to 10 and 20% LC_{50} endosulfan for 21 days. All environmental conditions were the same for all tanks.

Samples from each treatment were collected on days 1, 7, 14 and 21. The culture system used static aerated water with 50% daily exchange of water of the tank and endosulfan was added.

Blood samples were collected from the control and treatments. Blood was collected in vials containing an anticoagulant, to count the red blood cells (RBCs), white blood cell count (WBC), hematocrit and hemoglobin levels and the remainder of the blood sample was centrifuged at 10,000 rpm (4°C) for 20 min to separate the plasma for measuring alkaline phosphatase, aspartate amino transaminase and alanine amino transaminase following the protocol suggested by the company.

RNA extraction and Relative mRNA expression of CYP450 Liver RNA was extracted from 50-100 mg tissue using RNAx Plus (CinnaGen, Iran). The target tissue was homogenized in 1.0 ml RNAxPlus reagent (Sinaclon; Iran) and left at room temperature for 15 min. The following steps for RNA extraction were performed as described by Panigrahi *et al.* (2011). The quantity of RNA was evaluated using a spectrophotometer at 260/280 nm and the quality was measured using 1% agarose gel and staining with ethidium bromide. RNA samples were stored at -80 ˚C until cDNA synthesis. cDNA was synthesized by SuPrime Script RT Premix (2X) cDNA Synthesis Kit (GeNet BIO Inc.; Daejeon, South Korea) according to the protocol suggested by the manufacturer. Real-time PCR was conducted using an iCycler (Bio-Rad) with Fermentas Maxima SYBR Green qPCR Master Mix and the gene-specific primers. The real-time PCR analyses were carried out using standard protocol described in

our previous paper (Miandare *et al*., 2013). Standard curves were constructed from dilution series of cDNA which included 5 dilutions from 1/10 to 1/200. The PCR efficiency was calculated using the following equation: $E\% = (10^{1/slope} - 1) \times 100$. The fold change in the relative mRNA expression of CYP450 was calculated by the $2^{-\Delta\Delta Ct}$ method and standard curve based method. The data were analyzed using the iQ5 optical system software version 2.0 (Bio-Rad) (Table 1).

Table 1: Gene-special primers for β-actin and CYP450 used in the real-time PCR.

Gene	Accession number	Primer (5′-3′)	Product size (bp)
P450	HQ287217	F: CGTCGGAATCGTCAATGACCT R:AGACGTACAGTGAGGAATGGTGAA	253
Beta actine	DQ061948.1	F: CCCTGCATGGATGTGTGGAT R:GGGTGACACCATCACCAGAG	189

Results

Exposure to LC_{50} endosulfan during 21 days did not result in any mortality in *Rutilus caspius*. The changes in hematological and biochemical parameters are presented in Figure 1. During the Experiment, hemoglobin, hematocrit and red blood cells decreased whereas WBC levels increased in treated fish. Results showed that there were significant differences in Hb, Ht, RBC and WBC levels in control and fish exposed to 10% and 20% LC_{50} for 21 days (p<0.050). By increasing the concentration of toxin, the measured blood factors decreased. The highest amount of Hb, Ht and RBC was observed in the control which showed significant differences with treatments (p<0.05) and the lowest levels were related to the fish exposed to the 20% LC_{50} for 21 days.

Although there were increases in ALP and ALT levels in endosulfan treatments during the test, they were not significant (p>0.05) and these levels decreased on day 21. Results related to AST were similar to ALP and ALT but there were significant differences in groups which were exposed to endosulfan compared to the control (p<0.05). The highest level of ALT was observed in the group treated with 20% endosulfan on day 14 and the lowest was in the control (Fig. 2).

Results of evaluating CYP450 gene expression are shown in Figure 3. According to the results CYP450 expression were significantly up-regulated compared to the control up to day 14 and then decreased on day 21. CYP450 reached its maximum level in the 20% endosulfan treated group on day 14 which had notable differences with other groups (p<0.05).

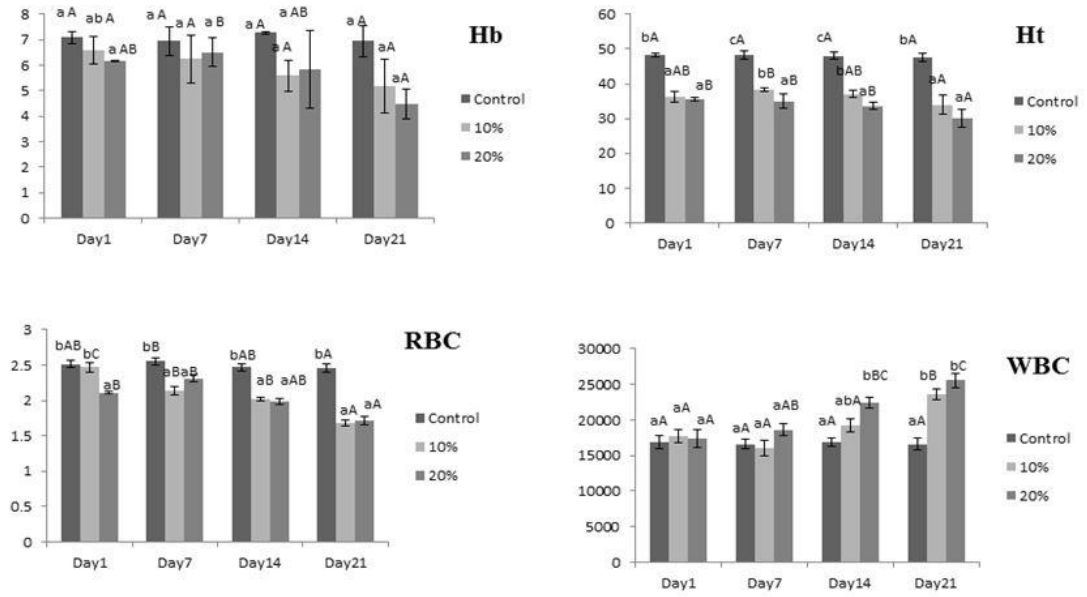

Figure 1: The toxic effects of endosulfan on the Hb, Ht, RBC and WBC of *Rutilus caspius* for one-day exposure periods. The small and capital letters represent a significant difference (p<0.05) in days of exposure (A-B) and dose of endosulfan (a-b).

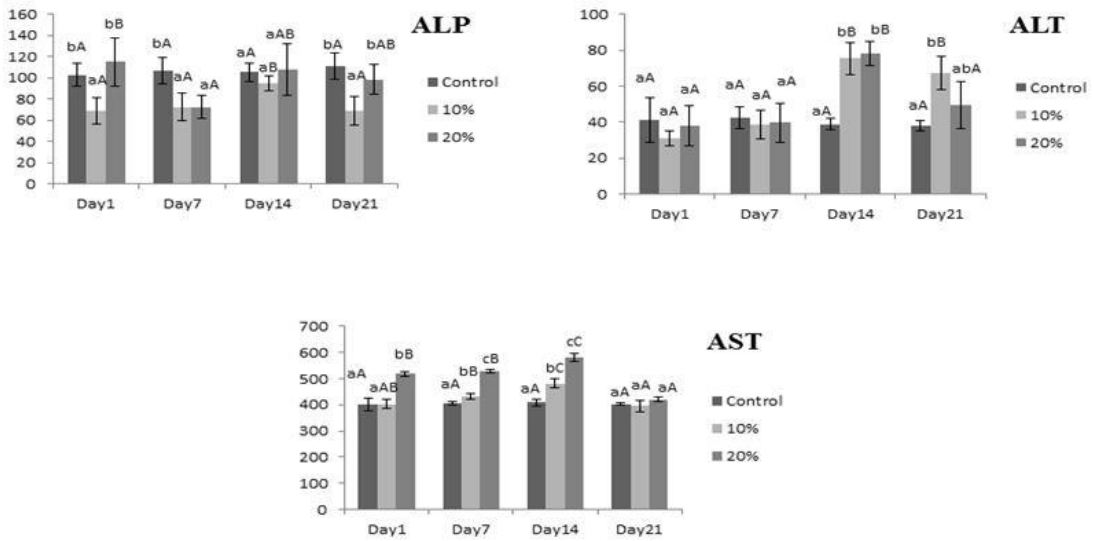

Figure 2: The toxic effect of endosulfan on the ALP, ALT and AST of *Rutilus caspius* for 21-day exposure periods. The small and capital letters represent a significant difference (p<0.05) in days of exposure (A-B) and dose of endosulfan (a-b).

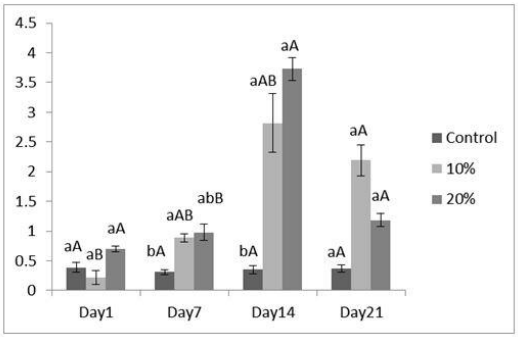

Figure 3: The toxic effect of endosulfan on the CYP450 gene expression of *Rutilus caspius* for 21-day exposure periods. The small and capital letters represent a significant difference *p*<0.05) in days of exposure (A-B) and dose of endosulfan (a-b).

Discussion

Sensitivity to pollutants in different species and even in a specific species is different and depends on size, age, species conditions and environment (Hedayati and Safahieh, 2011). In recent years instead of monitoring ecosystems quantitatively by the use of a little amount of pollutant in water, sediment and fish tissues, the effects of pollutants on aquatic organisms have been measured using quantitative assessments such as molecular, biochemical, hematological, enzymatic and tissue biomarkers (Dalzochio *et al*., 2016). Aquatic ecosystems are always receiving a large amount of pollutants such as pesticides, heavy metals, petroleum hydrocarbons and organic materials from domestic, mineral, industrial and agricultural wastewaters, which disturb the balance of the ecosystem (Pourang *et al*., 2005).

Therefore, estimating the effects of these pollutants on ecosystems is essential. Exposure to the 20% endosulfan on day 14 significantly enhanced ALP, AST, ALT and CYP450 in Caspian roach.

A reduction in Hb, Ht and RBC indicated anemia in fish exposed to the pesticide. It can be because of erythropoiesis, haemosynthesis and osmoregulatory dysfunction. Another reason may be an increase in the rate of erythrocyte destruction in hematopoietic organs (Jenkins and Smith, 2003; Seth and Saxena, 2003). In this study the number of red blood cells and the amount of hematocrit in fish exposed to endosulfan poison was the most important blood response compared to the control groups. In other words, the decrease in the RBC count and the amount of hematocrit are a significant sign of anemia in animals. A state of anemia results from the effect of oxygen free radicals produced by toxins on spleen and kidney tissues. These tissues are the main organs in fish which have the main function in producing blood. This causes the reduction in RBC production. The toxicant can also eradicate RBC (Edsall, 1999). Pesticdes were found mainly in the erythrocytes and plasma compared to leucocytes, platelets or stroma so they can bind with hemoglobin. In this experiment we also observed a reduction in hemoglobin content that can result from the conversion of hemoglobin to methemoglobin during a rapid oxidation or release of O_2 radicals by the toxicant. In another study, Matkovics *et al*. (1981) stated that hemoglobin content decreased in response to paraquat toxicity and it

might due to the methemoglobin formation and a direct response of O_2 radicals. Results showed a remarkable increase in WBC in treated fish compared to the control. The increase in the number of white blood cells in the blood well confirms the presence of inappropriate foreign agents in the animal's body. The increase in WBC can be related to the antibodies production which has an important role in survival and recovery of fish exposed to the toxicant (Joshi and Deep, 2002) so the significant increase in WBC count in this study can be because of the hypersensitivity of leucocytes to endosulfan which results in immunological reactions to produce antibodies in stressful conditions.

In the present investigation ALP, ALT and AST levels showed a significant increase during the experiment. This finding is in agreement with Al-Kuraizi (2010) who indicated that endosulfan can destroy the liver and release ALT and AST enzymes. Also other studies have reported that three major concentrations of organophosphorus insecticide (chlorpyrifos) enhanced ALT and AST enzyme levels. They suggested that these results can be due to the incorporation of amino acids by way of amino transferase activities of these enzymes into Krebs cycle to cope with the stressful situation endosulfan caused (Braunbeck *et al*., 1990).

The results of the present research obviously demonstrated that P450 gene expression was up-regulated in exposure to the two concentration of endosulfan compared to the control. In agreement with these, results Dong *et al*. (2013) reported that P450 levels in zebrafish liver significantly increased after endosulfan exposure. They also reported the same results after atrazine exposure on days 10,15 and 20 (Dong *et al*., 2009). Many pollutants such as heavy metals and toxins have toxic effects on the cells by producing active oxygen and cause the destruction of biological molecules such as protein, lipids and DNA. The first reaction of the cell to these stressful conditions is the production of a series of oxidative enzymes such as P450 and antioxidants. If stress conditions continue, it can lead to a disruption of the normal metabolism and ultimately death of the cell (Waisberg *et al*., 2003). On other hand, He *et al*. (2011) and Zhou *et al*. (2011) indicated that CYP3A induction could be reflected by ERND activity in fish. It means that some xenobiotics can induce CYP3A and ERND activity in fish as well as mammals. In addition, previous study implied that the activities of APND (Aminopyrine N-demethylase) and ERND (Erythromycin N-demethylase) could be induced by xenobiotics. For example, Dong *et al*. (2013) reported both APND and ERND after exposure to 0.01, 0.1, and 1 mg L^{-1} endosulfan. Li *et al*. (2008) showed that rifampicin (RIF) and dexamethasone (DEX) increased APND and ERND activities in two grass carp cell lines. In general, we conclude that exposure to endosulfan can stimulate the innate immune system in *Rutilus caspius*. The

highest amounts of hematological parameters were observed in the control expect WBC, which means that endosulfan negatively affects blood cells. In contrast endosulfan stimulates the innate immune system of *Rutilus* caspius throughout the liver enzymes and CYP450 gene expression especially with doses of 20%.

Acknowledgments

The authors would like to thank the staff at the Aquaculture Laboratory of the Department of Fisheries, Gorgan University of Agricultural Science and Natural Resource for their kind help.

References

Akhtar, M.S., Pal, A.K., Sahu, N.P., Alexander, C. and Gupta, S.K., 2012. Effect of dietary Pyridoxine on growth and biochemical responses of *Labeo rohita* fingerlings exposed to endosulfan. *Pesticide Biochemistry and Physiology*, 103, 23-30. DOI: 10.1016/j.pestbp.2012.02.004

Assefa, A. and Abunna, F., 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary medicine international*, 2018, 5432497. DOI: 10.1155/2018/5432497

Bhuvaneshwari, R., Padmanaban, K. and Babu Rajendran, R., 2015. Histopathological alterations in muscle, liver and gill tissues of zebra fish Danio Rerio due to environmentally relevant concentrations of organochlorine pesticides (OCPs) and heavy metals. International *Journal of Environmental Research*, 9(4), 1365-1372. DOI: 10.22059/ijer.2015.1029

Bruno, J., Osachoff, H. and Skirrow, R., 2006. Gene expression profiles of columbia river white sturgeon exposed to two industrial effluents. Sturgeon Contaminants Working Group. 21 P.

Chan, K.M., 1995. Methalothionin: Potential biomarker for monitoring heavy metal Pollution in fish around Hong Kong. *Marine Pollution Bulletin*, 31, 411-415. DOI: 10.1016/0025-326X(95)00125-7

CruPkin, A.C., Caaiquiriborde, P., Mendieta, J., Panzeri, A.M., Ballesteros, M. and Menone, M., 2013. Oxidative stress and genotoxicity in the South American cichlid, *Australoheros facetus*, after short-term sublethal exposure to endosulfan. *Pesticide Biochemistry and Physiology*, 105, 102-110. DOI: 10.1016/j.pestbp.2012.12.005

Dalzochio, T., Rodrigues, G.Z.P., Petry, I.E., Gehlen, G. and da Silva, L.B., 2016. The use of biomarkers to assess the health of aquatic ecosystems in Brazil: a review. International Aquatic Research, 8, 283-298. DOI: 10.1007/s40071-016-0147-9

Dong, X., Zhu, L., Wang, J., Wang, J., Xie, H., Hou, X. and Jia, W., 2009. Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). *Chemosphere*, 77, 404-412. DOI: 10.1016/j.chemosphere.2009.06.052

Dong, M., Zhu, L., Shao, B., Zhu, Sh., Wang, J., Xie, H., Wang, J. and Wang, F., 2013. The effects of endosulfan on cytochrome P450 enzymes and glutathione S-transferase in zebrafish (*Danio rerio*) livers. *Ecotoxicology and Environmental Safty*, 92, 1-9. DOI: 10.1016/j.ecoenv.2012.10.019

Edsall, C.C., 1999. A blood chemistry profile for lake trout. *Journal of Aquatic Animal Health*, 11, 81-86. DOI: 10.1577/1548-8667(1999)011<0081:ABCPFL>2.0.CO;2

Hedayati, A. and Safahieh, A., 2011. Serum hormone and biochemical activity as biomarkers of mercury pollution in yellowfin seabream (*Acanthopagrus latus*). *Toxicology and Industrial Health*, 28, 306-319. DOI: 10.1177/0748233711410916

Jenkins, F. and Smith, J., 2003. Effect of sublethal concentration of endosulfan on hematological and serum biochemical parameters in the carp (*Cyprinus carpio*). *Bulletin of Environmental Contamination and Toxicology*, 70, 993-947. DOI: 10.1007/s00128-003-0080-7

Joshi, P. and Deep, H., 2002. Effect of lindane and malathion exposure to certain blood parameters in a freshwater teleost fish *Clarias batrachus*. *Pollution Research*, 21, 55-57.

Korashy, H.M. and El-Kadi, A.O.S., 2005. Regulatory mechanisms modulating the expression of cytochrome P450 1A1 gene by heavy metals. *Toxicological Science*, 88, 39–51. DOI: 10.1093/toxsci/kfi282

Li, D., Yang, X., Zhang, S., Lin, M., Yu, M. and Hu, K., 2008. Effects of mammalian CYP3A inducers on CYP3A-related enzyme activities in grass carp (*Ctenopharyngodon idellus*): Possible implications for the establishment of a fish CYP3A induction model. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 147, 17-29. DOI: 10.1016/j.cbpc.2007.07.003

Matkovics, B., Witas, H.O., Gabrielak, T. and Szabó, L., 1981. Paraquat as an agent affecting antioxidant enzymes of common carp erythrocytes. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 87, 217-219. DOI: 10.1016/0742-8413(87)90206-4

Miandare, H.K., Farahmand, H., Akbarzadeh, A., Ramezanpour, S., Kaiya, H., Miyazato, M., Rythönen, K.T. and Nikinmaa, M., 2013. Developmental transcription of genes putatively associated with growth in two sturgeon species of different growth rate. *General and Comparative Endocrinology*, 182, 41-7. DOI: 10.1016/j.ygcen.2012.11.013

Negro, C.L., 2015. Histopathological effects of endusulfan to hepatopancreas, gills and ovary of the fresh water crab Zilchiopsis collastinensis (Decapoda: Trichodactylidae). *Ecotoxicology and Environmental Safety*, 113, 87-

94. https://doi.org/10.1016/j.ecoenv.2014.11.025

Panigrahi, A., Viswanath, K. and Satoh, S., 2011. Real-time quantification of the immune gene expression in rainbow trout fed different forms of probiotic bacteria *Lactobacillus rhamnosus*. *Aquaculture Research*, 42, 906-17. DOI: 10.1111/j.1365-2109.2010.02633.x

Pourang, N., Tanabe, S., Rezvani, S. and Dennis, H., 2005. Trace elements accumulation in edible tissues of five sturgeon species from the Caspian Sea. Environmental Monitoring and Assessment, 100, 89-108. DOI: 10.1007/s10661-005-7054-7

Rastiannasab, A., Afsharmanesh, S., Rahimi, R. and Sharifian, I., 2016. Alternations in the liver enzymatic activity of Common carp, *Cyprinus carpio* in response to parasites, *Dactylogyrus* spp. and *Gyrodactylus* spp. *Journal of Parasitic Diseases*, 40(4), pp.1146-1149. DOI: 10.1007/s12639-014-0638-9

Rose, W.L., Nisbet, R.M., Green, P.G., Norris, S., Fan, T., Smith, E.H., Cherr, G.N. and Anderson, S.L. 2006. Using an integrated approach to link biomarker responses and Physiological stress to growth impairment of cadmium-exposed larval topsmelt. *Aquatic Toxicology*, 80, 298–308. DOI: 10.1016/j.aquatox.2006.09.007

Rusni, S., Sassa, M., Takagi, T., Kinoshita, M., Takehana, Y. and Inoue, K., 2022. Establishment of cytochrome P450 1a gene-knockout Javanese medaka, *Oryzias javanicus*, which distinguishes toxicity modes of the polycyclic aromatic hydrocarbons, pyrene and phenanthrene. Marine Pollution Bulletin, 178, 113578. DOI: 10.1016/j.marpolbul.2022.113578

Sanchez-Fortun, S. and Barahona, M.V., 2005. Comparative study on the environmental risk induced by several pyrethroids in estuarine and freshwater invertebrate organisms. *Chemosphere*, 59, 553-559. DOI: 10.1016/j.chemosphere.2004.12.023

Seth, N. and Saxena, K.K., 2003. Hematological responses in a freshwater fish, *Channa punctatus* due to fenvalerate. *Bulletin of Environmental Contamination and Toxicology*,71, 1192-1199. DOI: 10.1007/s00128-003-8732-1

Sheader, D.L., Williams, T., Lyons, B.P. and Chipman, J.K., 2006. Oxidative stress response of European flounder (*Platichthys flesus*) tocadmium determined by acustom cDNA microarray. *Marine Environment Research*, 62, 33–44. DOI: 10.1016/j.marenvres.2006.03.001

Softeland, L., Holen, E. and Olsvik, P.A., 2010. Toxicological application of primary hepatocyte cell cultures of Atlantic cod (*Gadus morhua*), effects of BNF, PCDD and Cd. *Comparative Biochemistry and Physiology*, 151, 401–411. DOI: 10.1016/j.cbpc.2010.01.003

Megarani, D.V., Hardian, A.B., Arifianto, D., Santosa, C.M. and

Salasia, S.I., 2020. Comparative Morphology and Morphometry of Blood Cells in Zebrafish (*Danio rerio*), Common Carp (*Cyprinus carpio carpio*), and Tilapia (*Oreochromis niloticus*). Journal of the American Association for Laboratory Animal Science, 59(6), 673-680. DOI: 10.30802/AALAS-JAALAS-20-000013

Tedeschi, J.N., Kennington, W. J., Berry, O., Whiting, S., Meekan, M. and Mitchell, N.J., 2015. Increased expression of HSP70 and HSP90 mRNA as biomarkers of thermal stress loggerhead turtle embryos (*Caretta caretta*). *Journal of Thermal Biology*, 47, 42-50. DOI: 10.1016/j.jtherbio.2014.11.006

Ullah S. and Zorriehzahra, M.J., 2015. Ecotoxicology: a review of pesticides induced toxicity in fish. Adv. Anim. Vet. Sci. 3(1): 40-57. DOI: 0.14737/journal.aavs/2015/3.1.40.57

Van-Der Geest, H.G., Stuijfzand, S.C., Kraak, M.H.S. and Admiraal, W., 1997. Impact of diazinon calamity in 1996 on the aquatic macroinvertebrates in the River Mesue, The Netherlands. *Netherland Journal of Aquatic Ecology*, 30, 327-330.

Waisberg, M., Joseph, P., Hale, B. and Beyersmann, D., 2003. Molecular and cellular mechanisms of cadmium. *Toxicology*, 192, 95-117. DOI: 10.1016/s0300-483x(03)00305-6

Zhou, C., Li, X., Fang, W., Yang, X., Hu, L., Zhou, S. and Zhou, F., 2011. Inhibition of CYP450 1A and 3A by berberine in crucian carp *Carassius auratus gibelio*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 154, 360-366. DOI: 10.1016/j.cbpc.2011.07.005