

INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH VOLUME 6, ISSUE 06, JUNE 2017

by Nur Kusuma Dewi

Submission date: 05-Sep-2019 03:33PM (UTC+0700)

Submission ID: 1167563421

File name: International_Journal_of_Scientific_Technology_Research.pdf (362.17K)

Word count: 4672

Character count: 25865

Study On The Effects Of Heavy Metals' Pollution On The Activity Of 7-Ethoxyresorufin-O-Diethylase (EROD) In Two Freshwater Fish Species Of Indonesia

Nur Kusuma Dewi

Abstract: The study aimed to determine the effect of heavy metal pollution on 7-ethoxyresorufin-O-diethylase (EROD) enzyme activity in carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). The samples were taken by purposive random sampling technique. The treatment site was the downstream part of the Kaligarang River, Semarang, Indonesia, which contaminated by heavy metals (concentration of Cd 0.007 ppm, Pb 0.010 ppm, and Hg 0.0006 ppm). A clean reference site in Nyatnyono Village, Ungaran was used as the control site with the heavy metal content of 0 ppm. The EROD activities in carp and tilapia were significantly decreased after Cd, Pb, and Hg exposure. The results showed that the average value of liver EROD activity in carp fish control group amounted to $1.77 \pm 0.23 \mu\text{mol}/\text{min}/\text{mg}$ protein and the treatment group was $0.49 \pm 0.24 \mu\text{mol}/\text{min}/\text{mg}$ protein. Whereas in control group of tilapia fish, EROD activity was equal to $2.08 \pm 0.47 \mu\text{mol}/\text{min}/\text{mg}$ protein and the treatment group was $0.49 \pm 0.40 \mu\text{mol}/\text{min}/\text{mg}$ protein. The results demonstrated that metallothionein was a specific heavy metals biomarker, whereas EROD activity is highly sensitive to extremely low concentrations of these selected pollutants. In conclusion, the use of multiple biomarkers is recommended to monitor the heavy metal pollutants in the river environment.

Keywords: EROD activity, heavy metals, biomarker, river environment

1 INTRODUCTION

Recently, the industrial, economical, education and culture fields are developing rapidly. Beside the advantages of the rapid development, there are many risks arisen by this issue. The development in those areas is possible to affect the environment, especially the aquatic environment. In river environment, it is well known that pollution has become a great problem. In Indonesia, river is an important compartment of the environment. The discharge of unpurified sewage and wastewater from industries, manufacturers, and housing has resulted in the accumulation of pollutants. The accumulation causes a dramatic decline in its water quality. These pollutants are mainly polycyclic aromatic hydrocarbons (PAHs), radio nuclides, pesticides, detergents, insecticides, and heavy metals. The pollution of river by environmental pollutants leads dangers onto humans and wildlife. The non-organic pollutants in the form of heavy metals, i.e. mercury (Hg), cadmium (Cd), and lead (Pb) are well-thought-out the most dangerous metals in the environment [1]. In fact, the heavy metals are persistent in the natural environment and surprisingly, they are able to accumulate in various areas, for instance, sediment, water, and soil. These metals could inhibit the enzymes, which containing sulfhydryl groups in or near their active sites and could generate the reactive oxygen species [2]. These mechanisms are considered to be dangerous as it leads toxic, carcinogenic and mutagenic effects to animals and humans [3]. Regarding to the contamination cases, in Toyama, the water system used in the rice field had been polluted by cadmium (Cd) and caused the civilian suffered Itai-Itai [4, 5, 6, 7].

The research study results showed that the water was polluted by Cd obtained from the local mining center in the head of Jint River. The rice in that field accumulated the cadmium for decades and biomagnified in the civilian who lived near the river areas [5, 8, 9, 10]. Also in 2004, the cadmium pollution in river happened to Karanganyar River, Central Java, Indonesia. The rate of cadmium reached 0.21-0.40 mg/kg. Fifteen industries near the river area were suspected to be contributed in the pollution [11]. In aquatic systems, the fish are extensively used as a sensitive experimental model. In fish, there is a broad range of enzyme systems involved in xenobiotic metabolism. By these decades, the use of biomarkers to trace the existence of pollutants has become widely used in environmental monitoring. In 1962, the studies concerning cytochrome P450 in fish were published [12, 13, 14]. The 7-ethoxyresorufin-O-diethylase (EROD) is a family of cytochrome P-4501A (CYP1A) contained in fish liver. A specific biomarker, EROD is now often used as a molecular marker to measure the quality and quantity of pollutants in the water. EROD catalyzes the formation of single cytochrome P-450 (CYP) into cytochrome P4501A (CYP1A) through metabolism of 7-ethoxyresorufin substrates to fluorescent resorufin products [15]. EROD activity can be used as an indication of the function of cytochrome P4501A1 enzyme system, which is responsible for environmental contamination through phase I biotransformation, i.e. polycyclic aromatic hydrocarbons (PAHs) compounds and some dioxin-like compounds such as polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), and polychlorobiphenyls (PCB). In other words, EROD activity has been used as a marker of activity and induction of CYP1A [15, 16]. CYP1A induction and its relationship with EROD activity has been widely used as a biomarker for exposure to AHR agonists in aquatic environments, as well [15]. As a biomarker, EROD activity is widely used in several field and laboratory studies, such as a study on bleaching industrial wastewater [17], contaminated sediment [18, 19], and petroleum spills and leaks [20, 21], as well as the monitoring of general contamination [22, 23]. In addition, the presence of several compounds in aquatic

8

- Nur Kusuma Dewi: Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, First floor of D6 Building, Jl. Raya Sekaran, Gunungpati, Semarang, Indonesia, Phone: +6282196542968, Email: nur.kusuma.dewi@mail.unnes.ac.id

environments such as metal and estrogen compounds also affects the response of EROD [11] [5]. However, only few studies have reported the presence of EROD enzyme activity due to exposure to heavy metals in the aquatic environment. Accordingly, this study aimed to determine the response of the EROD enzyme to the following exposure of heavy metals including Cd, Pb, and Hg in two freshwater fish species of Indonesia, carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). The assessment of heavy metals exposure in these two kinds of fish was conducted in Kaligarang River, Semarang, Central Java, Indonesia. It is a home to a broad range of industrial activities, including food factories, paper factories, textile industries, and home industries. In order to evaluate the effect of pollution in Kaligarang River, biomarker studies are needed for fish treated in the river; such studies would give the first and highly valuable information regarding pollution in this area.

2 MATERIALS AND METHODS

2.1 Chemicals

All chemicals were of analytical grade available commercially.

2.2 Treatment sites and fish species

Kaligarang River lies administratively in 3 municipalities in Central Java Province, precisely in Kabupaten Semarang, Kendal, and Kota Semarang. The mainstream part of Kaligarang is located in Ungaran with a height of 1,750 meters above sea level, whereas the downstream part lies in Laut Jawa (Java Sea). In this study, the treatment site was at the downstream part of the Kaligarang River. The downstream part is the 6th part (Segment 6) of the river which surrounded by several major industries and housing. By using karamba floating net, the fishes were caught and exposed with the heavy metal concentration of Cd 0.006 ppm, Pb 0.010 ppm, and Hg 0.0006 ppm. A clean reference site in the center of the freshwater fish seed, which located in the 1st part (Segment 1) near Nyatnyono Village, Ungaran, Semarang Regency, was used as the control site, with the heavy metal content of 0 ppm. Nyatnyono is a village where tourism and fishing are prevalent, and it has no industrial sites. The treatment stations and the reference site are shown in Figure 1. The study was conducted for 7 months in between the dry season with rainy season. Carp and tilapia were obtained from the freshwater fish seed center in Ungaran using random sampling. A total of 100 carps (*Cyprinus carpio*) and 100 tilapias (*Oreochromis niloticus*), each weighing 19-25 g, were used as the samples which treated in the treatment site (Segment 6). Carp and tilapia are the economically important source of protein and are cultured by local fishermen. The liver EROD activities of these fish were compared with those of 100 carps and 100 tilapias placed in the control site (Segment 1).

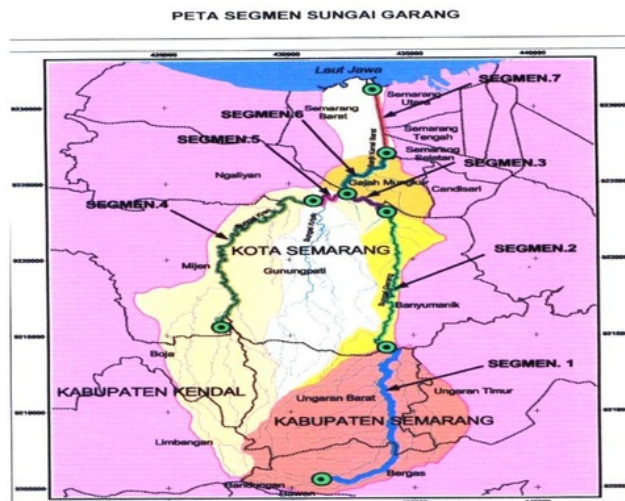


Fig.1. Treatment site was located in the 6th part (Segment 6) of Kaligarang River, whereas the reference site was located in the 1st part (Segment 1) of Kaligarang River. Fishes were exposed to Cd, Hg and Pb to evaluate the bioindicator.

2.3 Experimental design

The pre-test and post-test control group design were used as the experimental design [24, 25]. The biological marker shown in fish after the experiment was compared with the control group fish. After 7 months, both the liver of carp and tilapia fishes were taken and the activity of EROD was then measured.

2.4 Preparation of microsomal fractions of livers

After the specimens were weighed and measured, the fish were killed by decapitation and their liver tissues were dissected carefully. The tissue samples were stored in the zip lock plastic bag and were kept in the ice box after labeling.

2.5 Enzyme assays

EROD activities of microsomes were determined by the spectrofluorometric method [26]. The reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.8, 0.1 M NaCl, 1.2 mg BSA, 50–100 µg of fish liver microsomal protein, 0.1 mM nicotinamide adenosine dinucleotide phosphate in the presence of substrate, and 1.5 µM 7-ethoxyresorufin. The reaction was initiated by the addition of substrate, 7-ethoxyresorufin was added to the reaction mixture. The reaction begins with the addition of 50 mL of the NADPH regeneration system and incubated at 37 °C for 1 h. The reaction was terminated by the addition of ice cold acetone. The acetone blank was prepared with the addition of acetone in the NADPH regeneration system. Standard samples were incubated without the addition of ethoxyresorufin. After centrifugation at 2500 rpm for 5 min, the supernatant fluorescence was measured at a wavelength of 535 nm (excitation) and 585 nm (emission) using Shimadzu RF-594 fluorometer (Shimadzu, Japan).

2.6 Statistical Analysis

The data were then formed for coding and statistical description. Initially, the normality of the data was tested using the Kolmogorov-Smirnov test. After normal data was obtained,

the hypothesis was tested using independent t-test and was continued with LSD test. Meanwhile, if the data were not normal, it was then tested by the Mann Whitney test followed by Benn Ferroni test. Significant value was indicated by the value of $p < 0.05$. Statistical analysis was performed using SPSS ver 13.0. The results are expressed as mean \pm SD of at least three sets of triplet determinations for each data point, and statistical analysis was performed.

3 RESULTS AND DISCUSSION

3.1 The assay of 7-ethoxyresorufin-O-diethylase (EROD)

The activity of liver 7-ethoxyresorufin-O-diethylase (EROD) in carp and tilapia, which treated in the treatment sites exposed to heavy metals Cd, Pb, and Hg were lower than the fish samples in the clean reference sites. The carp liver EROD activity had approximately three times lower ($1.77 \pm 0.23 \mu\text{mol}/\text{min}/\text{mg}$ protein to $0.49 \pm 0.40 \mu\text{mol}/\text{min}/\text{mg}$ protein), whereas in tilapia, it had approximately 4 times lower ($2.08 \pm 0.47 \mu\text{mol}/\text{min}/\text{mg}$ protein to $0.49 \pm 0.40 \mu\text{mol}/\text{min}/\text{mg}$ protein) as presented in Table 1.

Table 1 . Median value EROD activity of control group and treatment group in *Cyprinus carpio* and *Oreochromis niloticus*

Fish species	Sample size	EROD activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) mean \pm SD	
		Control group (BBI Ungaran)	Treatment group (Kaligarang river)
Carp (<i>Cyprinus carpio</i>)	100	1.77 ± 0.23	0.49 ± 0.24
Tilapia (<i>Oreochromis niloticus</i>)	100	2.08 ± 0.47	0.49 ± 0.40

The Mann Whitney test results of carp fish groups (data not shown) showed that there were significant differences in EROD activity values between control and treatment groups. The mean rank of the experimental group (10.50) was smaller than the control group (30.50). The t-test result of tilapia group, it showed that there were significant differences in EROD activity values between the control group with the experimental group (p value = 0.0003). It was found that the presence of heavy metal exposure decreased the EROD activity in the treatment group compared with the control group. The carp and tilapia liver EROD activity diagram are presented in Figure 2. The EROD activities between carp and tilapia fish were differ significantly, although the value of EROD activity of carp and tilapia fish in the same treatment groups was low. It was revealed that the low EROD activity value of the experimental group compared with the control group, was likely to occur due to exposure to heavy metals with a high affinity for sulfur (S). The heavy metals attack the sulfur bond in the enzyme active site. This mechanism inactivates the enzyme function. When the enzyme is inactive, it decreased the enzyme activity, therefore the enzyme's ability to detoxify polluting substances (pollutants) was also declined.

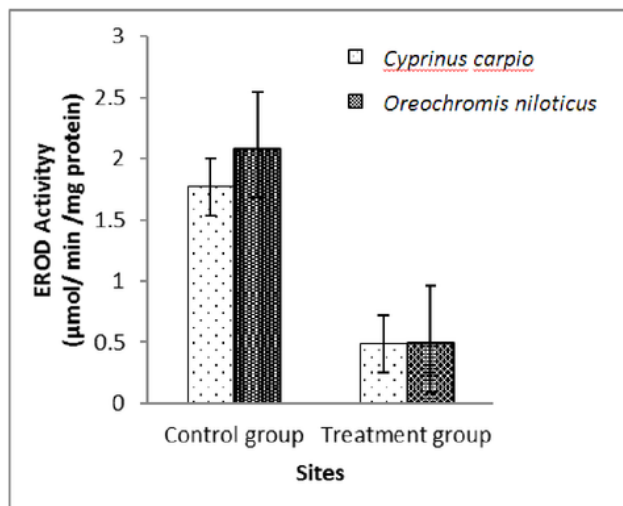


Fig.2. Hepatic EROD activities of fishes caught from control group (BBI Ungaran) and treatment group (Kaligarang River) in *Cyprinus carpio* and *Oreochromis niloticus*

The metals inhibition of P450 catalytic activity has been observed by other. In vitro study on the effect of Hg^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} on EROD activities in the liver microsomal of the leaping mullet showed that all metal ions inhibit the reaction. However, the initial velocity of ions Hg^{2+} and Cd^{2+} on inhibition is higher than other metal ions. The inhibition of EROD activity by heavy metals occurred due to the bonding between metal ions on the enzyme sulfhydryl group or generation of reactive oxygen species (ROS) or both [27]. Cytochrome P-450 reductase contains one group of cysteine residues at or near the binding site of NADPH and will bind cadmium or other metals for reduction of reductase enzyme and disable the EROD activity [27]. George and Young [28] reported that administration of CdCl_2 with doses below 2 mg/kg led to a decrease in EROD activity after 24 hours of observation. Several studies have reported that heavy metals including cadmium exert toxicity through several mechanisms such as inhibition of enzymes that contain sulfhydryl group or near the active site of ROS or free radicals [29]. In a study conducted by Chandrasekera et al. [29] it was reported that the exposure to cadmium from 0.001 to 0.01 mg/L in the fish, was not significantly affected the EROD activity. However, there was a significant inhibition of the EROD activity at concentrations of cadmium 0.1 and 1 mg/L for the provision of 14 days. The exposure to cadmium (concentration ≥ 0.1 mg/L) caused inhibition of EROD enzyme activity depends on the concentration and duration of exposure to exposure. Thus, it can be said that a high concentration of Cd in the environment could inhibit EROD activity in tilapia. Carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) were used since they are widely used fishes in environmental biomonitoring studies. EROD activities have been measured in liver samples of *Cyprinus carpio* and *Capoeta tinca* in Sariyer Reservoir (Ozmen et al. 2008) contaminated with organochlorine pesticides. EROD activities in fish which live in contaminated areas (petrochemical hydrocarbons, paper factory bleaching water, and industrial and city sewer system) have been used as biochemical measurement methods and biological monitoring tools to assess pollution resulting from the activities

mentioned [28, 30]. The relationship between EROD induction and exposure to chemicals has been investigated in over 150 different fish species throughout the world [31-38]. Lavado et al. [39] found a significant difference in their study with common carp, 689 pmol/min/mg protein EROD activity compared to 69 pmol/min/mg protein at their reference site. For this reason, EROD is described as an early warning system [15]. EROD has been widely applied to detect organic compound contamination in fish. This biomarker has been used as a sensitive indicator of anthropogenic organic compound, such as polycyclic aromatic hydrocarbon (PAH), polychlorinated biphenyls (PCBs), and dioxin. Moreover, the activity of EROD is categorized as a biomarker of exposure, which could be detected directly after the exposure of organic compounds [23, 40-43]. Furthermore, EROD also used as a heavy metals exposure biomarker. However, the detection rate is not significant comparing with the detection rate of organic compound. Ueng et al. studied the effect of CdCl₂ exposure in tilapia [44]. The result showed that 2 mg/kg cadmium exposure was not significantly affect the liver EROD activity. According to the result of this study, which the exposure of Cd, Hg, and Pb was significantly affected the EROD activity in fish liver, it represents that EROD activity was a sensitive biomarker to use. In Kaligarang river, the organic compound was also contaminate the water environment, therefore it can be concluded that EROD activity is good for detection of organic compound and the heavy metals contamination. This result also gave an information that EROD activity could not detect the specific heavy metals which contaminate the environment, it only detected heavy metals contamination generally. Therefore, metallothionein is better than EROD activity to be used as a biomarker for detection of heavy metals contamination in the aquatic environment. Nowadays, several chemicals have polluted the river environment. These chemicals were resulted from various human activities, such as house and industrial activities. The pollution of chemicals and xenobiotic affect the living organisms as well as the human body. In this study, Kaligarang River was used as the treatment sites, since there is no research on the determination of pollution resulting from industrial and human activities in this area. The results of study could be used to evaluate the quality of Kaligarang River as well as to determine the sensitivity biomarker as an early universal monitoring tool. The temperature of Kaligarang River is various between 26 to 28 °C affect the process of metabolism in fish body. Generally, the increase of environment temperature could implicate the resistance of fish to pollutants. The study results of Miller and Argawala indicated that the accumulation of Cd in fish body was affected by physiological factors, physical and chemical characteristic of Cd, and water temperature [5, 6]. In conclusion, EROD biomarker could be directly used to assess the exposure of heavy metals in the waters, even though it could not detect each kind of heavy metals. The results demonstrated that metallothionein and EROD activity were highly sensitive to extremely low concentrations of these selected pollutants. However, assessing the toxicity of the pollutants cannot simply depend on the reduction of EROD activity. Rather, the use of multiple biomarkers is recommended for the bio-monitoring of chemical pollutants in the river environment.

4 CONCLUSION

Based on the results of study, it can be concluded that EROD activities in carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) fishes were significantly decreased after Cd, Pb and Hg exposure. 7-ethoxyresorufin-O-diethylase could be used as a biomarker to assess heavy metal pollutants. However, it was not a specific biomarker. The results of this study provide a useful information as an important proof that fish which live in Kaligarang River could accumulate the heavy metals and might have the health risks if it consumed by humans. Upcoming studies should be conceded to determine the crucial cause of the contamination.

5 DECLARATION OF CONFLICT INTERESTS

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

36 ACKNOWLEDGEMENTS

This study was financially supported by DP2M of Higher Education Division, Ministry of Education and Culture, Indonesia. We also profound our sincere gratitude to the graduate school of Diponegoro University, Laboratory of Environmental Health Semarang, UNWAHAS Pharmaceutical and Chemical Analysis, BPPT Serpong, and Laboratory of Biology UNNES, Semarang, Indonesia.

REFERENCES

- [1] Goyer, R.A., Cherian, M.G., Jones, M.M. and Reigart, J.R., 1995. Role of chelating agents for prevention, intervention, and treatment of exposures to toxic metals. *Environ Health Persp*, 103: 1048-1052.
- [2] Voet, D. and Voet, J.G., 1995. *Biochemistry*. 2nd ed. New York, NY: John Wiley and Sons Inc.
- [3] Kosnett, M.J., 2007. Heavy metal intoxication and chelators. In Katzung B.G. (ed): *Basic and Clinical Pharmacology*. 10th Ed (International Ed), Boston, New York, NY: Mc Graw Hill, pp: 970-981.
- [4] Withgott, J. and Brennan, S., 2007. *Environment: The Science Behind the Stories*. San Fransisco, SF: Pearson Benjamin Cummings.
- [5] Miller, T.G., 2007. Jr. *Living in The Environment : Principle, Connection and Solutions*. Singapore: Thompson Brooks/Cole.
- [6] Argawala, S.P., 2006. *Environmental Studies*. New Delhi Chennai Mumbai Kolkata: Narosa Publishing House Pvt. Ltd.
- [7] Soemirat, J., 2005. *Toksikologi Lingkungan*. Yogyakarta: Gadjah Mada University Press.
- [8] Wardhana, W.A., 2004. *Dampak Pencemaran Lingkungan*. Yogyakarta: Penerbit Andi.
- [9] Klaassen, C.D., 2001. *Csarett and Doull's Toxicology: The Basic Science of Poisons*. 6th Ed. New York: Mc. Graw Hill.

- [10] Donatus, I.A., 2001. Toksikologi Dasar. Yogyakarta: Laboratorium Farmalogi dan Toksikologi, Fakultas Farmasi, UGM.
- [11] Supriharyono, 2009. Konservasi Ekosistem Sumberdaya Hayati. Yogyakarta: Pustaka Pelajar.
- [12] Stegeman, J.J., 1987. Monooxygenase systems in marine fish. In C. S. Giam, and L. Ray ed. Pollutant studies in marine animals. CRC: West Palm Beach, pp: 65-95.
- [13] Stegeman, J.J. and Kloepper-Sams, P.J., 1987. Cytochrome P-450 isozymes and monooxygenase activity in aquatic animals. *Environ Health Persp*, 71: 87-95.
- [14] Buhler, D.R. and Wang-Buhler, J.L., 1998. Rainbow trout cytochrome P450s: Purification, molecular aspects, metabolic activity, induction and role in environmental monitoring. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*, 121: 107-137.
- [15] Whyte, J.J., Jung, R.E., Schmitt, C.J. and Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol*, 30(4): 347-570.
- [16] Parent, T.E.M., Ana, C.A.X., Francisco and J.R., Paumgarten, 2008. Induced cytochrome P450 1A activity in cichlid fishes from Guandu River and Jacarepagua Lake, Rio de Janeiro, Brazil. *Environ Pollut*, 152: 233-238.
- [17] Karels, A.E., Soimasuo, M., Lappivaara, J., Leppanen, H., Aaltonen, T., Mellanen, P. and Oikari, A.O.J., 1998. Effects of ECF-bleached kraft mill effluent on reproductive steroids and liver MFO activity in populations of perch and roach. *Ecotoxicol*, 7(3): 123-132.
- [18] Engwall, M., Broman, D., Ishaq, R., Naf, C., Zebühr, Y. and Brunström, B., 1996. Toxic potencies of lipophilic extracts from sediments and settling particulate matter (SPM) collected in a PCB-contaminated river system. *Environ Toxicol Chem*, 15(2): 213-222.
- [19] Förlin, L., Baden, S.P., Eriksson, S., Granmo, A., Lindesjö, E., Magnusson, K., Ekelund, R., Esselin, A. and Sturve, J., 1996. Effects of contaminants in roundnose grenadier (*Coryphaenoides rupestris*) and Norway lobster (*Nephrops norvegicus*) and contaminant levels in mussels (*Mytilus edulis*) in the Skagerrak and Kattegat compared to the Faroe Islands. *J. Sea. Res*, 35(1-3): 209-222.
- [20] Lee, R.F. and Anderson, J.W., 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. *Marine Poll Bull*, 50(7): 705-723.
- [21] Morales-Caselles, C., Jimenez-Tenorio, N., de Canales, M.L., Sarasquete, C. and DelValls, T.A., 2006. Ecotoxicity of sediments contaminated by the oil spill associated with the tanker "Prestige" using juveniles of the fish *Sparus aurata*. *Archives Environ. Contam. Toxicol*, 51(4): 652-660.
- [22] Kirby, M.F., Neall, P., Bateman, T.A. and Thain, J.E., 2004. Hepatic ethoxyresorufin O-deethylase (EROD) activity in flounder (*Platichthys flesus*) from contaminant impacted estuaries of the United Kingdom: continued monitoring 1999-2001. *Marine Poll. Bull*, 49(1-2): 71-78.
- [23] Hansson, T., Lindesjö, E., Förlin, L., Balk, L., Bignert, A. and Larsson, A., 2006. Long-term monitoring of the health status of female perch (*Perca fluviatilis*) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. *Aquatic Toxicol*, 79(4): 341-355.
- [24] Verschuren, P. and Doorewaard, H., 2005. Designing a Research Project. Utrecht, Netherlands: LEMMA Publisher.
- [25] Steel, R.G.O., James, H. and Torrie, 2000. Principles and Procedure of Statistics. A Biometrical Approach. 2nd Ed. Kogakusha Ltd., Tokyo: Mc. Graw-Hill, pp: 633.
- [26] Klotz, A.V., Stegeman, J.J. and Walsh, C., 1984. An alternative 7-ethoxyresorufin-deethylase activity assay; a continuous visible spectrometric method for measurement of cytochrome P-450 monooxygenase activity. *Analytical Biochem*, 140(1): 138-145.
- [27] Bozcaarmutlu, A. and Arinc, E., 2004. Inhibitory effects of divalent metal ionon liver microsomal 7-ethoxyresorufin-O-diethylase (EROD) activity of leaping mullet. *Marine Environ Res*, 58(2-5): 521-524.
- [28] George, S.G. and Young, P., 1986. The time course effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in the plaice, *Pleuronectes platessa*. *Comparative Biochem Physiol*, 83(1): 37-44.
- [29] Chandrasekera, L.W.H.U., Pathiratne, A. and Pathiratne, K.A.S., 2008. Effects of water borne cadmium on biomarker enzymes and metallothioneins in Nile tilapia, *Oreochromis niloticus*. *J Nat Sci Found Sri Lanka*, 36(4): 315-322.
- [30] Kirby, M.F., Neall, P. and Tylor, T., 1999. EROD activity measured in flatfish from the area of the Sea Empress oil spill. *Chemosphere*, 38: 2929-2949.
- [31] Gooch, J.W., Elskus A.A., Kloepper-Sams, P.J., Hahn, M.E. and Stegeman, J.J., 1989. Effects of ortho- and non-ortho-substituted polychlorinated biphenyl congeners on the hepatic monooxygenase system in scup (*Stenotomus chrysops*). *Toxicol Appl Pharmacol*, 98: 422-433.
- [32] Narbonne, J.F., Garrigues, P. and Ribera, D., 1991. Mixedfunction oxygenase enzymes as tools for pollution monitoring: Field studies on the French Coast of the Mediterranean Sea. *Toxicol. Pharmacol*, 100:37-42.

- [33] Gunther, A.J., Spies, R.B., Stegeman, J.J., Woodiqb, B., Carney, D., Oakden, J. and Hain, L., 1997. EROD activity in fish as an independent measure of contaminant induced mortality of invertebrates in sediment bioassays. *Marine Environ. Res.* 44: 41–49.
- [34] Fent, K. and Batscher, R., 2000. Cytochrome P4501A induction potencies of polycyclic aromatic hydrocarbons in a fish hepatoma cell line: Demonstration of additive interactions. *Environ Toxicol Chem.* 19: 2047–2058.
- [35] Kucklick, J.R., Struntz, W.D.J., Becker, P.R., 2002. Persistent organochlorine pollutants in ringed seals and polar bears collected from Northern Alaska. *Sci Total Environ.* 287: 45–59.
- [36] Aarab, N., Champeau, O., Mora, P., Daubeze, M., Garrigues, P. and Narbonne, J.F., 2004. Scoring approach based on fish biomarkers applied to French River monitoring. *Biomarkers.* 9: 258–270.
- [37] Wafo, E., Sarrazin, L. and Diana, C., 2005. Accumulation and distribution of organochlorines (PCBs and DDTs) in various organs of *Stenella coeruleoalba* and a *Tursiops truncatus* from mediterranean littoral environment (France). *Sci Total Environ.* 348: 115–127.
- [38] Ferreira, M., Moradas-Ferreira, P. and Reis-Henriques, M.A., 2006. The effect of long-term depuration on phase I and phase II biotransformation in mullets (*Mugil cephalus*) chronically exposed to pollutants in River Douro Estuary, Portugal. *Marine Environ Res.* 61: 326-338.
- [39] Lavado, R., Urena, R., Martin-Skilton, R., Torreblanca, A., del Ramo, J., Raldua, D. and Porte, C., 2006. The combined use of chemical and biochemical markers to assess water quality along The Ebro River. *Environ Pollut.* 139: 330–339.
- [40] Hansson, N., 2008. Does Fish Health Matter? The Utility of Biomarkers in Fish for Environmental Assessment. Ph.D. Thesis. Department of Plant and Environmental Sciences University of Gothenburg.
- [41] Van der Oost R., Beyer, J. and Vermeulan, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol.* 13(2): 57-149.
- [42] Sandstrom, O., Larsson, A., Andersson, J., Appelberg, M., Bignert, A., Ek, H., Forlin, L. and Olsson, M., 2005. Three decades Swedish experience stresses the need of integrated long-term monitoring in marine coastal areas. *Water Qual Res J Can.* 40(3): 233-250.
- [43] Hanson, N. and Larsson, A., 2007. Influence of feeding procedure on biomarkers in caged rainbow trout (*Oncorhynchus mykiss*) used in Environmental Monitoring. *J Environ Monitor.* 9(2): 168-173.
- [44] Ueng, Y.F., Liu, C., Lai, C.F., Meng, L.M., Hung, Y.Y. and Ueng, T.H., 1996. Effects of cadmium and environmental pollution on metallothionein and cytochrome P450 in tilapia. *Bulletin Environ Contam Toxicol.* 57(1): 125-131.

ORIGINALITY REPORT

18%

SIMILARITY INDEX

12%

INTERNET SOURCES

14%

PUBLICATIONS

8%

STUDENT PAPERS

PRIMARY SOURCES

1	docplayer.net Internet Source	1%
2	Monteiro, M.. "Impact of chemical exposure on the fish <i>Pomatoschistus microps</i> Kroyer (1838) in estuaries of the Portuguese Northwest coast", <i>Chemosphere</i> , 200701 Publication	1%
3	Lutz W. D. Weber, Margitta Lebofsky, Bernhard U. Stahl, Antonius Kettrup, Karl Rozman. "Comparative toxicity of four chlorinated dibenzo-p-dioxins (CDDs) and their mixture", <i>Archives of Toxicology</i> , 1992 Publication	1%
4	journal.unnes.ac.id Internet Source	1%
5	journals.sagepub.com Internet Source	1%
6	Azra Bozcaarmutlu, Canan Sapmaz, Gizem Kaleli, Sema Turna, Serpil Yenisoy-Karakaş.	1%

"Combined use of PAH levels and EROD activities in the determination of PAH pollution in flathead mullet (*Mugil cephalus*) caught from the West Black Sea coast of Turkey", Environmental Science and Pollution Research, 2014

Publication

7	www.tandfonline.com Internet Source	1%
8	Submitted to Universitas Diponegoro Student Paper	1%
9	pinpdf.com Internet Source	1%
10	19january2017snapshot.epa.gov Internet Source	1%
11	Fernandes, D.. "Chemical residues and biochemical responses in wild and cultured European sea bass (<i>Dicentrarchus labrax</i> L.)", Environmental Research, 200702 Publication	1%
12	www.jlr.org Internet Source	<1%
13	www.medworm.com Internet Source	<1%
14	Huang, Guo-Yong, You-Sheng Liu, Yan-Qiu Liang, Wen-Jun Shi, Li-Xin Hu, Fei Tian, Jun	<1%

Chen, and Guang-Guo Ying. "Multi-biomarker responses as indication of contaminant effects in *Gambusia affinis* from impacted rivers by municipal effluents", *The Science of The Total Environment*, 2016.

Publication

15

Juan M. Llobet, Gemma Falcó, Ana Bocio, José L. Domingo. "Human exposure to polychlorinated naphthalenes through the consumption of edible marine species", *Chemosphere*, 2007

Publication

16

www.cells4health.nl

Internet Source

17

Submitted to Columbus State University

Student Paper

18

Holm, G.. "Influences of halogenated organic substances on ovarian development and hepatic EROD activity in the three-spined stickleback, *Gasterosteus aculeatus*, and rainbow trout, *Oncorhynchus mykiss*", *Aquatic Toxicology*, 199408

Publication

19

www.carnivoreconservation.org

Internet Source

20

dergipark.org.tr

Internet Source

<1%

<1%

<1%

<1%

<1%

<1%

21	www.thaiscience.info Internet Source	<1%
22	W Masmoudi, MS Romdhane, S Khériji, M El Cafsi. " Polychlorinated biphenyl accumulation and hepatic EROD activity in golden grey mullet from Tunis Bay, southern Mediterranean ", African Journal of Aquatic Science, 2011 Publication	<1%
23	tel.archives-ouvertes.fr Internet Source	<1%
24	herkules.oulu.fi Internet Source	<1%
25	diva-portal.org Internet Source	<1%
26	www.environment.gov.au Internet Source	<1%
27	wedocs.unep.org Internet Source	<1%
28	Azza Naija, Justine Marchand, Patrick Kestemont, Zohra Haouas, Ronny Blust, Benoit Chénais, Ahmed Nouredine Helal. "Biomarkers assessment in the peacock blenny <i>Salaria pavo</i> exposed to cadmium", Environmental Science and Pollution Research, 2016 Publication	<1%

29	bmcinfectdis.biomedcentral.com Internet Source	<1%
30	www.duo.uio.no Internet Source	<1%
31	Submitted to University of Johannesburg Student Paper	<1%
32	Ž. Jakšić, N. Bihari, W.E.G. Müller, R.K. Zahn, R. Batel. "Modulation of cytochrome P450 1A in sea bass liver by model substances and seawater extracts", Aquatic Toxicology, 1998 Publication	<1%
33	www.omicsonline.org Internet Source	<1%
34	drum.lib.umd.edu Internet Source	<1%
35	W. Brack, K. Schirmer. "Effect-Directed Identification of Oxygen and Sulfur Heterocycles as Major Polycyclic Aromatic Cytochrome P4501A-Inducers in a Contaminated Sediment", Environmental Science & Technology, 2003 Publication	<1%
36	tdx.cat Internet Source	<1%
37	Darius Sabaliūnas. "Use of semipermeable membrane devices for studying effects of	<1%

organic pollutants: Comparison of pesticide uptake by semipermeable membrane devices and mussels", Environmental Toxicology and Chemistry, 09/1998

Publication

38

Submitted to Chulalongkorn University

Student Paper

<1%

39

Achazi, R.K.. "A high-molecular regulator of 7-ethoxyresorufin-O-deethylase activity in fish", Comparative Biochemistry and Physiology. Part C: Comparative Pharmacology and Toxicology, 199407

Publication

<1%

40

www.unex.es

Internet Source

<1%

41

library.umac.mo

Internet Source

<1%

42

Branimir K. Hackenberger, Mirna Velki, Željka Lončarić, Davorka K. Hackenberger, Sandra Ečimović. "Effect of different river flow rates on biomarker responses in common carp (Cyprinus carpio)", Ecotoxicology and Environmental Safety, 2015

Publication

<1%

43

Carlos Gravato. "Biomonitoring Studies Performed with European Eel Populations from

<1%

the Estuaries of Minho, Lima and Douro Rivers (NW Portugal)", Advanced Environmental Monitoring, 2007

Publication

44

Willett, K.L.. "In Vivo and in Vitro Inhibition of CYP1A-Dependent Activity in Fundulus heteroclitus by the Polynuclear Aromatic Hydrocarbon Fluoranthene", Toxicology and Applied Pharmacology, 20011215

Publication

<1%

45

Ferreira, Marta Sofia Sá. "Endocrine and enzymatic changes in flounder (platichthys flesus) and in mullet (mugil cephalus) chronically exposed to organic contaminants in River Douro Estuary", Porto : Edição do Autor, 2012.

Publication

<1%

46

Submitted to University of Central Florida

Student Paper

<1%

47

A T Prasetya, S Ridlo. "Factor analysis for instruments of science learning motivation and its implementation for the chemistry and biology teacher candidates", Journal of Physics: Conference Series, 2018

Publication

<1%

Exclude quotes On

Exclude matches Off

Exclude bibliography On