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Synopsis

A Review on Multi-Biomarkers in Fish for the Assessment of Aquatic Ecosystem Contamination with Organic Pollutants

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Abstract. The aim of the current review is to identify the most suitable biomarkers for evaluating chemical stress due to organic contamination in aquatic environments, as well as possible ways to reduce or limit this contamination. To safeguard the environmental status, the European Union has implemented the Water Framework Directive (WFD; 2000/60/EC) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) legislations, which encourage the use of biological tools to detect the quality of aquatic systems. Therefore, monitoring of sentinel fish species is widely used to assess the level of health status. Fishes have been found to be good biomonitors of water pollution because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates, are also more sensitive to different toxicants, including persistent organic pollutants, such as pesticides. Biomarkers are defined as responses to any exposure evidenced in histological, physiological, biochemical, genetic and behavioral modification. The application of multi-biomarker approach is necessary in the development of a protocol for the aquatic assessment of organic pollutants. This protocol can be applied in risk assessment and water monitoring programs in order to provide an adequate legal basis for the presence of organic pollutants in aquatic ecosystems and biological responses under concentrations equal or lower to those permitted under the European and Bulgarian legislation.

Key words: water contamination, organic pollutants, histology, enzymatic activity, biomonitors.

Introduction

Correlations between concentrations of environmental contaminants, bioaccumulation, and biological responses in organisms should be integrated for a better understanding of the environmental risks and thus, the use of multiple biomarkers at different levels of biological organization is an appropriate approach for the detection of biological responses triggered by chemical stress and should be incorporated into aquatic monitoring studies, providing an ecotoxicological diagnosis needed for environmental management (van der Oost et al., 2003; Vieira et al., 2019).

In the countries of the European Union (EU), national water agencies, which follow

EU policy and the requirements of the EU Water Framework and its Daughter Directives (Directive 2000/60/EC; Directive 2008/105/EC; Directive 2008/56/EC; European communities environmental objectives 272/2009; European communities technical report 2010), implement regular water monitoring with the aim to control and prevent pollution (Milačič et al., 2017). According to Directive 2013/39/EC the maximum permissible concentration for a single compound must not exceed 0.1 µg/L, and in the case of a larger number of residues, it must not exceed a concentration of 0.5 µg/L.

The toxicity of mixtures will depend on the bioavailability and chemical reactivity of the compounds. To gain greater insight into the risks posed by environmental contaminants, it is beneficial to understand their mode of action (MoA). The MoA is basically the process initiated by the interaction of the toxicant with the receptor, which progresses through molecular, biochemical, physiological and/or anatomical changes in the organism to result in sub-lethal and lethal effects. Such effects/response can be detected by means of biomarkers, which are broadly defined as indicators signaling events at individual level (Napierska et al., 2018).

As stated by Nataraj et al. (2017) and Ghayyur et al. (2021) fishes are very sensitive to the presence of pollutants in water, so they can be used as biomonitors and in comparison to other aquatic biomonitors, fishes occupy the top position of the aquatic trophic chain, therefore they offer an integrated image of the whole aquatic ecosystem. Moreover, fishes have the ability to accumulate toxic substances from the surrounding environment in different body organs and tissues (Ghayyur et al., 2021), which is essential it terms of multi-analyses, such as chemical, histological or biochemical.

The impact of organic pollutants, their toxicity and resistance to degradation demand the development of new and innovative materials, processes, and technologies to remove them from the aquatic environment (Nizzetto et al., 2010; Pariatamby & Kee, 2016; Negrete-Bolagay et al. 2021). In this regards according to Rani et al. (2021) remediation techniques can be divided into 4 groups: 1. Removal (contaminates or contaminate medium are eliminated from the spot with no requirement to separate from the host medium); 2. Separation (elimination of contaminants from the host medium such as water or soil); 3. Destruction (more toxic chemicals are transformed into less toxic products by degradation or neutralized chemically or biologically); 4. Containment (interference or inactivate surface and subsurface passage of the contaminates). As stated by Sarker et al. (2021) pesticide bioremediation is a greener and ecofriendly approach for the complete degradation or transformation of pesticides into nontoxic metabolites using living agents as potential degraders. According to Gavrilescu (2005) bioremediation, as one of the most environmentally-sound and cost-effective methods for the decontamination and detoxification of a pesticide-contaminated environment, is discussed especially considering the factors affecting the biodegradability of pesticides, such as biological factors and the characteristics of the chemical compounds.

Ecological monitoring on contaminated with organic substances aquatic ecosystems

Different organic pollutants, such as pesticides, pharmaceuticals, etc., are found in industrial and urban regions all over the world (Odabasi et al., 2016; Negrete-Bolagay et al., 2021). Fuoco & Giannarelli (2019) and Negrete-Bolagay et al. (2021) divided the organic xenobiotics into two main groups - anthropogenic and natural. As explained by Georgieva et al. (2021) anthropogenic sources are considered as the major pollutants of aquatic ecosystems. In addition, humans are exposed directly to different organic contaminants, when they are working in the agricultural and industrial sectors and indirectly - through the consumption of contaminated water and food, including fishes (Saleh et al., 2020; Negrete-Bolagay et al., 2021).

Pesticides

Natural ecosystem can be contaminated by different sources of persistent organic pollutants and priority substances, such as pesticides in two groups based on their solubility. The first group include the pesticides, which dissolved in surface waters enter into the ground water, and may cause severe negative effects on the non-target organisms. On the other hand, the fat-soluble pesticides enter in the organism via the process of bioamplification, which results in their longer existence in the food chain (Katagi & Tanaka, 2016). According to Rani et al. (2021) the bioamplification disturbs the entire ecosystem, particularly at higher trophic levels, the species will die because of higher toxicity in their bodies due to processes, such as biomagnification.

Schuijt et al. (2021) explained that chemical monitoring is often not performed alone, but in combination with ecological monitoring. In this regards, an integrated assessment can improve the ability to describe the effect of contamination for areas with decreased or poor environmental status detected during monitoring programs (Vethaak et al., 2017). According to Schuijt et al. (2021) the major benefit of ecological monitoring is the high ecological relevance since it provides important information on the ecosystem and integrates the overall effect of chemical stressors including mixtures' effects and bioavailability. One of the main aspects of the European Union Marine Strategy Framework Directive (MSFD), a wide-ranging Framework Directive (2008/56/EC) with the overall objective of achieving or maintaining Good Environmental Status (GES) in Europe's seas by 2020 (MSFD, 2008), is the development of common criteria and methodological standards, which will ensure consistency and comparability in the determination of Good Environmental Status (GES) across Europe (Lyons et al., 2010).

Biomonitors on contaminated with pesticides aquatic ecosystems

Biomonitors are sentinel organisms that respond to changes at various structural levels, such as cellular, physiological, biochemical, genetic and histological factors to variations in patterns of behavior, which may affect the population structure of the species as a response to stressors present in the environment (Rodrigues et al., 2010; Velusamy et al., 2014; Lima et al., 2018). Ahmad & Ahmad (2016), Shukla & Trivedi (2017) and Trivedi et al. (2021) recommended fishes as the "early warning biological model" for risk assessment associated with aquatic toxicants. In addition, in aquatic ecosystems, fishes are considered as good indicators of environmental health because of their different positions in the food chain (Adams & Greeley, 2000; Lima et al., 2018). Thus, fishes are used as suitable biomonitors of the health status of aquatic ecosystems (Rodrigues et al., 2010; Iwanowicz et al., 2012; Lima et al., 2018; Georgieva et al., 2021).

Biomarkers for contaminated with pesticides aquatic ecosystems

The term "biomarker" include a measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO, 1993; van der Oost et al., 2003). The approach given by biological tools has the advantage to provide information on the exposure and the effects of chemicals (even short-lived chemicals) on living organisms, while chemical analyses provide information about the presence and/or concentrations of the substances; it means that only chemical analysis does not reflect the response of aquatic organisms to harmful effects of pollutants (Martinez-Haro et al., 2015; Wan et al., 2018; Lomartire et al., 2021).

According to Franco-Martínez et al. (2020) biomarkers could be divided into five groups: 1. Spectrophotometric assays, based on light absorbance changes due to the presence or activity of the biomarker after the reaction with a chemical compound that is added as a reagent in the assay; 2. Immunoassays, based on the recognition of the biomarker by a specific antibody that provides a selective determination of a sample component, these immunoassays can be performed by westernblotting, ELISA or immunoturbidimetry; 3. Genome based biomarkers are usually identified from genome-wide association studies (GWAS); and use DNA markers such as single nucleotide polymorphisms (SNPs) that affect complex phenotypic traits; 4. Transcriptomic analysis of RNA samples from tissues and body fluids reveal differential gene expression patterns and potential biomarker for exposure to stress and environmental pollutants; 5. Chromatography and mass spectrometry, different pollutants can be quantified by gas chromatography (GC) and mass spectrometry (GC–MS). In addition to these methods, histopathological and histochemical assays could also serve as a sensitive biomarker for aquatic contamination (Fig. 1) (Georgieva et al., 2021).

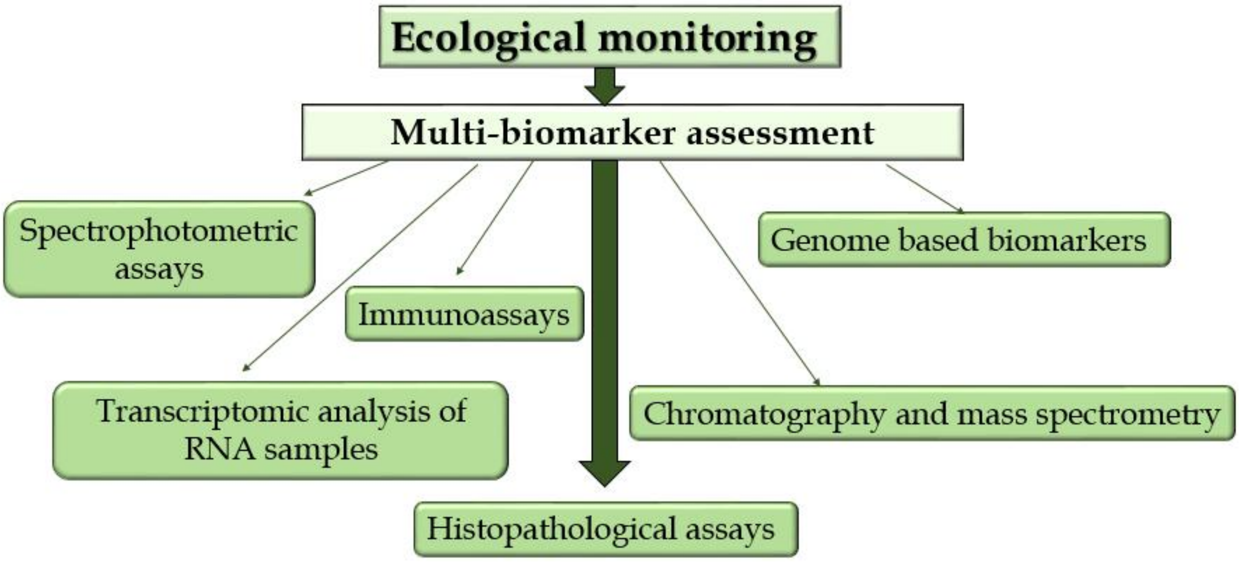

Fig. 1. Applied biomarkers in ecological monitoring.

The use of biological effects techniques (bioassays and biomarkers) offer the required tools to help define Descriptor 8 of GES according to MSFD, specifically in relation to monitoring the actual pollution effects of anthropogenic chemicals in the aquatic environment. As stated by Thain et al. (2008) a biological effect could be defined as the response of an organism, a population, or a community to changes in its environment. One of the clear advantages of the application of biological effect techniques is the link between contaminant exposure and ecological endpoints, as well as detecting the impact of substances (or combination of substances) that may not be analyzed as part of routine chemical monitoring programs (van der Oost et al., 2003; Thain et al., 2008; Lyons et al., 2010).

The multi-level biomarker approach is widely used from researchers to obtain a more complete and integrative overview of what affects marine organisms' health and, consequentially, the environment (Samanta et al., 2018; Lomartire et al., 2021). As stated by Lomartire et al. (2021) it is necessary to well define the baseline data of biomarkers to have a correspondent distinction between "noise" (natural variability) and "signal" (stress caused by contaminant). Moreover, the integrated biomarker response (IBR) that employs biochemical, morphological and physiological features is a must in regular monitoring of aquatic ecosystems (Bignell et al., 2011; Lomartire et al., 2021).

Likewise, Hedayati (2018), we consider that the application of biomarkers for aquatic environment monitoring has become widespread and it is essential in terms of ecotoxicological research. Furthermore, we agree that biomarkers have been largely used for the assessment of effects induced by several classes of chemical contaminants on fishes, for example the assessment of alterations on key enzymatic activities of biomonitor species following exposure to natural and experimental contaminated waters has been one of the major uses of biomarkers in different studies.

On one hand, as described by Yancheva et al. (2019a) the histopathological effects produced in different tissues of non-target organisms are usually used for monitoring of polluted areas and environmental conditions in aquaculture systems, and the use of histopathological biomarkers in biomonitoring has many advantages. According to the authors histopathological biomarkers can be used as an early warning system for potential effects at the level of the individual and the histopathological lesions are easily recognizable alterations that ideally indicate alone a pathological condition (Fig. 2). The increasing number of studies related to the use of histological biomarkers is based on the fact that they better reflect the health status of fish compared to other used methods. In addition, histochemical changes associated with a change in the amount of glycogen and lipids in the hepatocytes, serves as an indicator of the change in carbohydrate metabolism due to pesticide contamination in agrarian countries, such as Bulgaria (Stoyanova et al., 2019; Yancheva et al., 2019b).

On the other hand, oxidative stress induced by different organic pollutants, through an increase in the formation of reactive oxygen species (ROS) may lead to biochemical, cellular and physiological changes in the exposed organisms. Free radicals may lead to lipid peroxidation, changing the constitution of the biological membranes as well (Gonçalves et al., 2021). We agree that the exposure to organic toxicants may affect fatty acid composition of organisms by potentiating lipid peroxidation, as previously mentioned, or interfering with lipid metabolism, which can be evaluated through the assessment of fatty acid profiles. The presence of xenobiotics in water may affect the lipid metabolism and fatty acid and phospholipid composition (Olivares-Rubio & Vega-López, 2016; Gonçalves et al., 2021). According to Gonçalves et al. (2021) organic pollutants may impact the activity of a number of enzymes, from the stimulation of antioxidant enzymes in an effort to reestablish redox balance and preserve cells integrity, to the disruption of enzyme-mediated processes (Fig. 2). As the authors stated the use of enzymes as biomarkers of contaminant impacts in non-target species has proven to be a useful tool to assess their impacts. Moreover, oxidative stress plays an important role in the toxicity of pesticides and fishes have a defense system, which includes antioxidant enzymes, such as catalase (CAT), the glutathione system itself and superoxide dismutase (SOD) (Georgieva et al., 2021). Yancheva et al. (2015) described that the alterations in different fish enzymes represent a sensitive biochemical indicator for the negative pesticide effects. The authors considered that changes in other metabolic enzymes, such as lactate dehydrogenase (LDH), aspartate and alanine aminotransferase (ASAT and ALAT) are also sensitive indicators for hepatotoxicity, particularly in the fish liver, which is the main depot for bioaccumulation of xenobiotics and also a main detoxification organ, along with the kidney (Fig. 2). In addition to antioxidant and metabolic enzymes, the neurotransmitter cholinesterase (ChE) is also analyzed very often. One of the important elements in environmental analysis to be considered is the direct relationship between ChE inhibition and the behavioral, biochemical, and physiological alterations that occur in exposed organisms (Basirun et al., 2017). Moreover, ChE extracted from different organs of the same fish species or from different species possess different sensitivity towards anticholinesterastic agents exposed to it. This would make the extracted ChE as a specific biomarker for each possible toxicant.

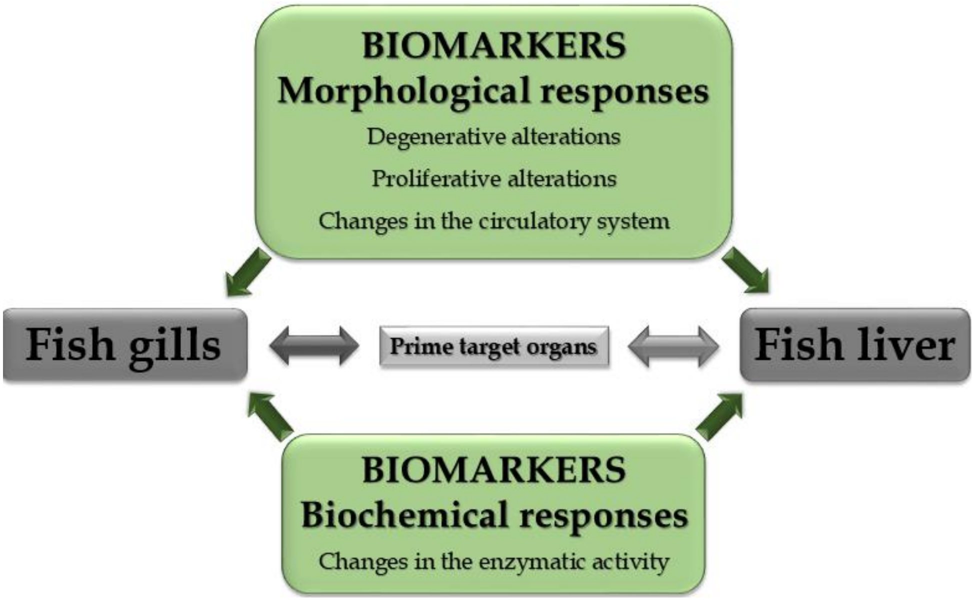

Fig. 2. Biomarkers in fish prime target organs (gills and liver).

Conclusions

The application of such complex research is necessary in the development of a protocol for the assessment of pollution of aquatic ecosystems with organic pollutants. This protocol, which includes histopathological and biochemical biomarkers can be applied in both, water monitoring and agricultural practices, to provide an adequate legal basis for the presence of organic pollutants and their effect in aquatic ecosystems. Thus, collaboration between different institutions is crucial to reduce the risks of pesticide toxicity on the environment and modifications under concentrations equal to those permitted under European legislation.

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References

Adams, S.M., & Greeley, M.S. (2000). Ecotoxicological indicators of water quality: using assess the health of aquatic ecosystems. *Water Air Soil Pollution*, *123*, 103-115. doi: 10.1002/tox.2530100207.

Ahmad, I., & Ahmad, M. (2016). Fresh water fish, *Channa punctatus*, as a model for pendimethalin genotoxicity testing: a new approach toward aquatic environmental contaminants. *Environmental Toxicology*, *31*, 1520-1529. doi: 10.1002/tox.22156.

Basirun, A.A., Sabullah, M.K., Shukor, M.Y.A., Yasid, N.A., Shamaan, N.A., & Ahmad, S.A. (2017). Biochemical biomarkers: fish cholinesterase biosensor for heavy metals detection in aquatic pollution monitoring. *Pertanika Journal of Scholarly Research Reviews*, *3*(3), 62-69.

Bignell, J.P., Stentiford, G.D., Taylor, N.G.H., & Lyons, B.P. (2011). Histopathology of mussels (*Mytilus* sp.) from the Tamar estuary, UK. *Marine Environmental Research*, *72*(1-2), 25–32. doi: 10.1016/j.marenvres.2011.05.004.

Franco-Martínez, L., Martínez-Subiela, S., Cerón, J.J., Tecles, F., Eckersall, P.D., Oravcova, K., & Tvarijonaviciute, A. (2020). Biomarkers of health and welfare: A One Health perspective from the laboratory side. *Research in Veterinary Science*, *128*, 299-307. doi: 10.1016/j.rvsc.2019.12.012.

Fuoco, R., & Giannarelli, S. (2019). Integrity of aquatic ecosystems: an overview of a message from the South Pole on the level of persistent organic pollutants (POPs). *Microchemical Journal*, *148*, 230-239. doi: 10.1016/j.microc.2019.04.076.

Gavrilescu, M. (2005). Fate of pesticides in the environment and its bioremediation. *Engineering in Life Sciences*, *5*(6), 497-526. doi: 10.1002/elsc.200520098.

Georgieva, E., Yancheva, V., Stoyanova, S., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., Petkova, E., László, B., Nyeste, K., & Antal, L. (2021). Which is more toxic? Evaluation of the short-term toxic effects of chlorpyrifos and cypermethrin on selected biomarkers in common carp (*Cyprinus carpio*, Linnaeus 1758). *Toxics*, *9*, 125. doi: 10.3390/toxics9060125.

Ghayyur, S., Khan, M.F., Tabassum, S., Ahmad, M.S., Sajid, M., Badshah, K., Khan, M.A., Saira, Ghayyur, S., Khan, N.A., Ahmad, B., & Qamer, S. (2021). A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). *Saudi Journal of Biological Sciences*, *28*(1), 603-611. doi: 10.1016/j.sjbs.2020.10.049.

Gonçalves, A.M.M., Rocha, C.P., Marques, J.C., & Gonçalves, F.J.M. (2021). Enzymes as useful biomarkers to assess the response of freshwater communities to pesticide exposure – A review. *Ecological Indicators*, *122*, 107303. doi: 10.1016/j.ecolind.2020.107303.

Hedayati, A. (2018). Fish biomarkers, suitable tools for water quality monitoring. *International Journal of Veterinary and Animal Research*, *1*(3), 63-69.

Iwanowicz, L.R., Blazer, V.S., Hitt, N.P., McCormick, S.D., Devault, D.S., Ottinger, & Christopher A. (2012). Histologic, immunologic and endocrine biomarkers indicate contaminant effects in fishes of the Ashtabula River. *Ecotoxicology*, *21*(1), 165-182. doi: 10.1007/s10646-011-0776-0.

Katagi, T., & Tanaka, H. (2016). Metabolism, bioaccumulation, and toxicity of pesticides in aquatic insect larvae. *Journal of Pesticide Science*, *41*(2), 25-37. doi: 10.1584/jpestics.D15-064.

Lima, L.B.D., Morais, P.B., Andrade, R.L.T., Mattos, L.V., & Moron, S.E. (2018). Use of biomarkers to evaluate the ecological risk of xenobiotics associated with agriculture. *Environmental Pollution*, *237*, 611-624. doi: 10.1016/j.envpol.2018.02.011.

Lomartire, S., Marques, J.C., & Gonçalves, A.M.M. (2021). Biomarkers based tools to assess environmental and chemical stressors in aquatic systems.

Ecological Indicators, *122*: 107207. doi: 10.1016/j.ecolind.2020.107207.

Lyons, B.P., Thain, J.E., Stentiford, G.D., Hylland, K., Davies, I.M., & Vethaak, A.D. (2010). Using biological effects tools to define Good Environmental Status under the European Union Marine Strategy Framework Directive. *Marine Pollution Bulletin*, *60*, 1647-165. doi: 10.1016/j.marpolbul.2010.06.005.

Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., Moreira-Santos, M., Reis-Henriques, A.M., Ribeiro, R., Santos, M.M., & Marques, J.C. (2015). A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination? *Ecological Indicators*, *48*, 8-16. doi: 10.1016/j.ecolind.2014.07.024.

Milačič, R., Zuliani, T., Vidmar, J., Oprčkal, P., & Ščančar, J. (2017). Potentially toxic elements in water and sediments of the Sava River under extreme flow events. *Science of the Total Environment*, *605-606*, 894-905. doi: 10.1016/j.scitotenv.2017.06.260.

MSFD. (2008). Directive 2008/56/EC of the European Parliament and the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy (Marine Strategy Framework Directive). Retrieved from ec.europa.eu.

Napierska, D., Sanseverino, I., Loos, R., Gómez Cortés, L., Niegowska, M., & Lettieri, T. (2018). *Modes of action of the current Priority Substances list under the Water Framework Directive and other substances of interest.* Luxembourg: Publications Office of the European Union, ISBN 978-92-79-77301-3, JRC110117. doi: 10.2760/226911.

Nataraj, B., Hemalatha, D., Rangasamy, B., Maharajan, K., & Ramesh, M. (2017). Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos. *Biocatalysis and Agricultural Biotechnology*, *12*, 185-190. doi: 10.1016/j.bcab.2017.09.006.

Negrete-Bolagay, D., Zamora-Ledezma, C., Chuya-Sumba, C., De Sousa, F.B., Whitehead, D., Alexis, F., & Guerrero, V.H. (2021). Persistent organic pollutants: The trade-off between potential risks and sustainable remediation methods. *Journal of Environmental Management*, *300*, 113737. doi: 10.1016/j.jenvman.2021.113737.

Nizzetto, L., MacLeod, M., Borgå, K., Cabrerizo, A., Dachs, J., Guardo, A. Di, Ghirardello, D., Hansen, K.M., Jarvis, A., Lindroth, A., Ludwig, B., Monteith, D., Perlinger, J.A., Scheringer, M., Schwendenmann, L., Semple, K.T., Wick, L.Y., Zhang, G., & Jones, K.C. (2010). Past, present, and future controls on levels of persistent organic pollutants in the global environment. *Environmental Science and Technology*, *44*, 6526-6531. doi: 10.1021/es100178f.

Odabasi, M., Dumanoglu, Y., Ozgunerge Falay, E., Tuna, G., Altiok, H., Kara, M., Bayram, A., Tolunay, D., & Elbir, T. (2016). Investigation of spatial distributions and sources of persistent organic pollutants (POPs) in a heavily polluted industrial region using tree components. *Chemosphere*, *160*, 114-125. doi: 10.1016/j.chemosphere.2016.06.076.

Olivares-Rubio, H.F., & Vega-López, A. (2016). Fatty acid metabolism in fish species as a biomarker for environmental monitoring. *Environmental Pollution*, *218*, 297-312. doi: 10.1016/j.envpol.2016.07.005.

Pariatamby, A., & Kee, Y.L. (2016). Persistent organic pollutants management and remediation. *Procedia Environmental Sciences*, *31*, 842-848. doi: 10.1016/j.proenv.2016.02.093.

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A.S., Srivastav, A.L.,

& Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of Cleaner Production, 283,* 124657. doi: 10.1016/j.jclepro.2020.124657.

Rodrigues, A.P.C., Maciel, P.O., Silva, L.L.C.P., Albuquerque, C., Inácio, A.F., Freire, M., Linde, A.R., Almosny, N.R.P., Andreata, J.V., Bidone, E.D., & Castilhos, Z.C. (2010). Biomarkers for mercury exposure in tropical estuarine fish. *Journal of the Brazilian Society of Ecotoxicology, 5*(1), 9-18. doi: 10.5132/jbse.2010.01.003.

Saleh, I.A., Zouari, N., & Al-Ghouti, M.A. (2020). Removal of pesticides from water and wastewater: chemical, physical and biological treatment approaches. *Environmental Technology & Innovation, 19,* 101026. doi: 10.1016/j.eti.2020.101026.

Samanta, P., Im, H., Na, J., & Jung, J. (2018). Ecological risk assessment of a contaminated stream using multi-level integrated biomarker response in *Carassius auratus*. *Environmental Pollution, 233,* 429-438. doi: 10.1016/j.envpol.2017.10.061.

Sarker, A., Nandi, R., Kim, J.-E., & Islam, T. (2021). Remediation of chemical pesticides from contaminated sites through potential microorganisms and their functional enzymes: Prospects and challenges. *Environmental Technology & Innovation, 23,* 101777. doi: 10.1016/j.eti.2021.101777.

Schuijt, L.M., Peng, F.-J., Berg, S.J.P., Dingemans, M.M.L., & Van den Brink, P.J. (2021). (Eco)toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Science of the Total Environment, 795,* 148776. doi: 10.1016/j.scitotenv.2021.148776.

Shukla, A., & Trivedi, S.P. (2017). Anionic surfactant, linear alkyl benzene sulphonate induced oxidative stress and hepatic impairments in fish *Channa punctatus*. *Proceedings of the Zoological Society,* 1-8. doi: 10.1007/s12595-017-0223-1.

Stoyanova, S., Georgieva, E., Velcheva, I., Atanassova, P., & Yancheva, V. (2019). Lipid accumulation in *Cyprinus carpio* (Linnaeus, 1785) liver induced by thiamethoxam. *Zoonotes, 139,* 1-4.

Thain, J.E., Vethaak, D., & Hylland, K. (2008). Contaminants in marine ecosystems: developing an integrated indicator framework using biological effects techniques. *ICES Journal of Marine Science, 65*(8), 1508–1514. doi: 10.1093/icesjms/fsn120.

Trivedi, S.P., Ratn, A., Awasthi, Y., Kumar, M., & Trivedi, A. (2021). *In vivo* assessment of dichlorvos induced histological and biochemical impairments coupled with expression of p53 responsive apoptotic genes in the liver and kidney of fish, *Channa punctatus* (Bloch, 1793). *Comparative Biochemistry and Physiology, Part C,* 245, 109032. doi: 10.1016/j.cbpc.2021.109032.

van der Oost, R., Beyer, J., & Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology, 13,* 57-149. doi: 10.1016/S1382-6689(02)00126-6.

Velusamy, A., Satheesh Kumar, P., Ram, Anirudh, & Chinnadurai, S. (2014). Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. *Marine Pollution Bulletin, 81*(1), 218-224. doi: 10.1016/j.marpolbul.2014.01.049.

Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat, C.F., Burgeot, T., Maes, T., Wosniok, W., Giltrap, M., Lang T., & Hylland K. (2017). Integrated indicator framework and methodology for monitoring and assessment of hazardous substances and their effects in the marine environment. *Marine Environmental Research, 124,* 11-20. doi: 10.1016/j.marenvres.2015.09.010.

Vieira, C.E.D., Costa, P.G., Caldas, S.S., Tesser, M.E., Risso, W.E., Escarrone, A.L.V., Primel, E.G., Bianchini, A., & Reis Martinez, C.B. (2019). An integrated approach in subtropical agro-ecosystems: Active biomonitoring, environmental contaminants, bioaccumulation, and multiple biomarkers in fish. *Science of the Total Environment, 666,* 508-524. doi: 10.1016/j.scitotenv.2019.02.209.

Wan, R., Meng, F., Su, E., Fu, W., & Wang, Q. (2018). Development of a classification scheme for evaluating water quality in marine environment receiving treated municipal effluent by an integrated biomarker approach in *Meretrix meretrix. Ecological Indicators, 93,* 697-703. doi: 10.1016/j.ecolind.2018.05.062.

WHO. (1993). World Health Organization International Programme on Chemical Safety (IPCS). Biomarkers and risk assessment: concepts and principles. *Environmental Health Criteria, 155,* Geneva.

Yancheva, V., Velcheva, I., Stoyanova, S., Iliev, I., Vasileva, T., Bivolarski, V., Uchikova, E., & Georgieva, E. (2015). Toxicity of two organophosphorous pesticides on bighead carp (*Aristichthys nobilis* Richardson, 1845) liver. *Applied Ecology and Environmental Research, 14*(1), 397-410. doi: 10.15666/aeer/1401_397410.

Yancheva, V., Velcheva, I., Georgieva, E., Mollov, I., & Stoyanova, S. (2019a). Chlorpyrifos induced changes on the physiology of common carp (*Cyprinus carpio* Linnaeus, 1785): a laboratory exposure study. *Applied Ecology and Environmental Research, 17*(2), 5139-5157. doi: 10.15666/aeer/1702_51395157.

Yancheva, V., Velcheva, I., Georgieva, E., & Stoyanova, S. (2019b). Periodic Acid - Schiff (PAS) reaction in fish liver exposed to fungicide contamination: A possible histochemical biomarker. *Ecologia Balkanica, 11*(1), 1-10.

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