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# THE INFLUENCE OF PHARMACEUTICAL RESIDUES FROM SURFACE WATERS ON FISH OXIDATIVE STRESS: A REVIEW

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***Abstract***

*In the last time, pharmaceutical residues have been discovered in almost all environmental matrices in the world, especially in surface water (lakes, rivers, seawater). The consumption of medicinal products contributes to the emission of pharmaceutical residues into the environment mainly through human (hospital effluents) and farm animal excretions. Once pharmaceutical residues reach in surface water, they also become incorporated into aquatic organism having a toxic effect on them. The biochemical response is dependent by the level of concentration and by the exposure time which largely contributes to the appearance of oxidative stress due to changes in the levels of antioxidant enzymes. In fish, due to detoxification and biotransformation capacity, the liver is the most important metabolizing organ, thus, one of the main defences against pharmaceutical residues. Analysis of biochemical indicators includes superoxide dismutase (SOD), glutathione S-transferase (GST), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and malondialdehyde (MDA). In the near future, the development of "green" and eco-friendly pharmaceuticals with low persistence in water surface, bioaccumulation and toxicity could help minimize accumulation in the aquatic environment.*

***Key words****: antioxidant enzymes, fish, pharmaceutical residues, oxidative stress, water surfaces.*

## INTRODUCTION

The quality of aquatic environments is compromised by the entry of toxic substances mainly from the anthropogenic activities. Pharmaceuticals products are considered one of these toxic substances during the last two decades due to the presence, abundance, and possible effects of these products in aquatic ecosystems (Jijie et al., 2021; Strungaru et al., 2021).

Biologically active pharmaceutical compounds are produced and also used in a very high quantity where their use and diversity are increasing every year (Shreenidhi et al., 2021).

With over 600 pharmaceuticals detected in the surface waters, at the global level, those from the category of analgesics, antidepressants and antihypertensive drugs are preponderent (Furduson et al., 2019; Shuraiqi et al., 2021). Within these classes, diclofenac (DCF), fluoxetine (FLX) and propranolol (PROP) are among the most used and prescribed drugs, and therefore some of the most frequently detected compounds in the aquatic environment, at concentrations ranging from ng/L to µg/L (Bonnefille et al., 2018).

The residues of these drugs are discharged into the aquatic environment; therefore, they can be found in wastewater, treated wastewater, surface water, groundwater and drinking water, in concentrations generally low in ng/L or µg/L, but having effects due to their continuous discharge into the aquatic environment (Archer et al., 2017; Pedrazzani et al., 2019).

In the last years the monitoring of pharmaceuticals in the aquatic environment is becoming a priority for competent authorities. Concerning to the analytical determination, the mainly used techniques are based on chromatographic mechanisms (gas chromatographic and HPLC) coupled to specific mass spectrometry and spectrophotometric detectors (Rivoira et al., 2015) which permitted the determination of some environmental effects of pharmaceuticals and can be established in the µg/L and ng/L concentration ranges (Lindsey et al., 2001; Kanda et al., 2003; Daughton, 2004; Larrson, 2014). Through these techniques can be determined and quantified approximately 3000 biologically active compounds in the environment (Richardson, 2006; Richardson & Ternes, 2014).

For the wild fish population, the pollution with pharmaceutical products and residues can conduct to reduced species richness and even at the loss of stocks in habitats.

In the evaluation of the ecological status of aquatic environments are used fish because they are very sensitive to anthropogenic impacts and for this reason some of them can be chosen as bio-monitors.

Bioindicators serve as a measurable indicator of a biochemical, physiological, toxicological, or ecological process or function that has been correlated to effects on organisms, populations, or ecosystems (Burger, 2006). One of the most important used bioindicators in the aquatic ecosystem is represented by fish because they have an important ecological function at the level of trophic chain. Fishes can sensitively reflect the concentrations of contaminants in the environment in which they are found (Gallego et al., 2021) and making them a tool for detecting the effects of pharmaceuticals early generating an overview of the state of the aquatic ecosystem (Martínez-Morcillo et al., 2020).

Several studies have been carried out for the purpose of choice of the best biomarkers, where different responses have been tested in aquatic fish species to given pharmaceutical product (Recabarren-Villalon et al., 2019). Thus, the biomarkers were grouped into three categories: biomarkers of exposure, biomarkers of effect (which assess the biochemical, physiological or behavioural disturbances in an organism) and biomarkers of susceptibility (ability of organism to respond to exposure to a specific xenobiotic substance, including genetic factors) (Oost et al., 2003; Arango, 2012).

## SOURCE OF THE PHARMACEUTICAL RESIDUES IN AQUATIC ENVIRONMENT

Large numbers of pharmaceutical compounds are found in the environment as the result of biological degradation by the organism present in ecosystems. These compounds have high biological activity even at low concentrations to the aquatic biota (Nunes et al., 2006)

The consumed pharmaceutical drug does not decompose completely in the body, a small amount of drug is excreted through the biological system (Winker et al., 2008). The involuntary (excretion through body or washing off topical medicine) and purposeful (disposal of unused or out of date medicine) action by humans are the primary reason for the discharge of pharmaceutical compounds into the environment (Daughton & Ruhoy, 2009). Discharge from various sources of pharmaceutical wastes are industries, hospitals, animal husbandry and many others, whereas the dominant source of pharmaceuticals in water is urban wastewater emission (Aus der Beek et al., 2016).

Once discharged into aquatic environments, pharmaceuticals and their metabolites can undergo biotic and abiotic transformation (degradation) and sorb to suspended particulate matter (SPM) and sediments, and in some cases accumulate in the tissues of aquatic organisms (Ramirez et al., 2009).

Sources of human pharmaceuticals in sewage include patient use in the community, discharges from hospitals and, in some cases, wastewater from pharmaceutical manufacturing (Gaw et al., 2014).

A range of veterinary medicines including antibiotics, also registered for human use, is used prophylactically and to control disease outbreaks in marine aquaculture. Up to 75% of the administered dietary dose of a veterinary medicine can be lost to the surrounding environment. The loss mechanisms include dispersal of non-ingested pellets, gill and renal excretion of the unprocessed drug, and renal and faecal excretion of drug metabolites (Grigorakis & Rigos, 2011).

Animal husbandry and horticulture along rivers and in coastal areas may also contribute to loadings of pharmaceuticals entering in coastal waterways (Kummerer, 2009a; Jia et al., 2011). Antibiotics are added to animal feeds and in some cases drinking water to treat disease particularly in feedlots housing large numbers of animals (Kemper, 2008). The use of low doses of antibiotics in feed as growth promoters still occurs in some regions of the world despite being banned in Europe (Du & Liu, 2012). Some countries permit the use of antibiotics including oxytetracycline and streptomycin on horticultural crops (Kummerer, 2009a).

Pharmaceutical compounds most often identified in the aquatic environment belong to several classes of human and veterinary

antibiotics and human prescription and nonprescription drugs such as NSAIDs, β-blockers, blood lipid regulators, antiepileptics, analgesics, and antidepressants (Petrovic et al., 2014; Radovic et al., 2015; Patel et al., 2019).

***Pharmaceuticals versus other contaminants***

Pharmaceutical contaminants differ from most other contaminants according to these aspects (Zuccato et al., 2000; Kummerer, 2009b; Rivera-Utrilla et al., 2013):

- having a molecular mass < 500 Da, although larger for some compounds,
- containing chemically complex molecules with a large variety of structures, shapes, molecular masses, and functionalities,
- having more than one ionizable group,
- a degree of ionization that depends on the medium's pH,
- have lipophilic properties,
- persistence in nature, accumulate in life forms and remain biologically active (naproxen, sulfamethoxazole, and erythromycin can persist for almost one year and clofibric acid can persist for multiple years),
- tend to adsorb and be distributed in a living body, which from a metabolic point of view modifies their chemical structure.

## OXIDATIVE STRESS IN FISH

A disturbance in the balance between the prooxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. Indicators of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products.

It has been found that pollutants present in the water surface can mediate their toxicity in fish by the appearance of oxidative stress resulting in changes in proteins, membrane lipids and DNA molecules (Bethanie, 2008). The result of such exposure leading to oxidative stress can impair cellular or biological function which can lead to the appearance of diseases.

Biomarkers of oxidative stress, such as changes in antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure to toxic substances.

For the reducing oxidative stress the activity of antioxidant enzymes as catalase (CAT) and superoxide dismutase (SOD) are involved in the detoxification of reactive oxygen species (ROS). On the other hand, glutathione-S-transferase (GST) is responsible for the metabolism of xenobiotic compounds such as pharmaceuticals. CAT is mainly located in the peroxisomes and is responsible for the reduction of $H_2O_2$ produced from the metabolism of long chain fatty acids in peroxisomes; GPx catalyzes the reduction of both $H_2O_2$ and lipid peroxide. The different responses of CAT and GPx indicate different mechanisms for ROS removal (Gao et al., 2018). The most abundant and important molecular antioxidants in cellular cytoplasm is reduced glutathione (GSH). GSH is used as a conjugating molecule by GST to ease excretion of xenobiotics. GSH is also used for reduction of lipid peroxides by the action of glutathione peroxidase (GPx). Gluthatione reductase (GR) was proposed to use as biomarkers in fish oxidative stress (Stephensen et al., 2002).

The lipid peroxidation (MDA) process also affects biomolecules associated with the membrane, i.e., membrane bound proteins or cholesterol, and may be of importance in fish as their membranes contain a higher degree of PUFA than other vertebrates (Monserrat et al., 2007).

Huang et al. (2007) have measured contaminant-induced oxidative damage in *Cyprinus carpio* captured in the Yellow River, China, a river contaminated by phenols, oils, PAHs and ammonia. While SOD and GST were upregulated in all tissues investigated, CAT and GPx were decreased in both kidney and gut tissues, the same tissues which were also found to have higher levels of MDA, suggesting that a lack of antioxidant defences could result in oxidative damage.

Studies have shown that the exposure to iron sulphate of the *Carassius auratus* species has led to an increased levels of protein carbonylation and lipid peroxidation and decreases in CAT, GST and GR activities (Bagnyukova et al., 2006). On the other hand, goldfish exposed to arsenic had increased activities of SOD, CAT and GPx as well as increased levels of lipid peroxides and GSSG (Bagnyukova et al., 2007).

Three species of cichlid from a metal-contaminated river showed changes in SOD, CAT and GPx activities. All species showed increases in lipid peroxidation in the metal1contaminated river in both spring and autumn (Ruas et al., 2007).

## EFFECT OF PHARMACEUTICAL RESIDUES ON FISH OXIDATIVE STRESS

Accumulation of pharmaceuticals in biological tissues is related to a small portion of un-ionized species, with a high affinity for lipophilic matter, remaining in the aqueous phase (Fabbri & Franzellitti, 2016). It is known that the response of antioxidant enzymes depends on the intensity of the oxidative pressure, and that an overload of the antioxidant defence system can occur in conditions of oxidative stress (Mauro et al., 2021).
The effect of different pharmaceutical products from watersurface on fish oxidative stress is presented in Table 1.

### *Antibiotics*

A long period of exposure to antibiotics can cause a reduction in the activity of antioxidant defences (glutathione and catalase) (Almeida et al., 2019). This induces oxidative damage, probably due to the prolonged exposure to the drug and its resulting accumulation in the tissues, leading to a reduction of the enzymatic activity (Zhou et al., 2018).
The level of lipid degradation in terms of lipid peroxidation (MDA) was found to be signifi-cantly higher in liver tissue of *Pangasius* sp. exposed to norfloxacin 30 mg/L. MDA has been increased about 1.61-fold in norfloxacin treated fish than the control fish (Shreenidhi et al., 2021).

### *Antipsychotics and antiepileptics drugs*

Sehonova et al. (2017) studied the effects of the tricyclic antidepressants amitriptyline, nortriptyline and clomipramine at concentrations of 10, 100 and 500 µg/L on early-life stages of common carp (*Cyprinus carpio*) for a period of 30 days. Long-term exposure resulted in a significant increase in mortality, developmental retardation, morphological anomalies, and pathological changes in brain, heart and kidney. In addition, changes in antioxidant enzyme activity as well as an increase in lipid peroxidation were observed, even at the lowest tested concentrations.
Studies by Li et al. (2010) showed that the inhibition of CAT activity in the *Oncorhynchus mykiss* after exposure to individual carbamazepine (2mg/L), due to the overwhelming production of hydrogen peroxide by SOD.

### *Analgesic/anti-inflammatory drugs*

Literature studies show that pharmaceuticals (especially diclofenac) and their photolysis by products were, to some extent, able to cause moderate toxicity on zebrafish after seven days of exposure (Diniz et al., 2015).
Gao et al. (2018) showed obvious decrease of antioxidant enzymes activity in *Cyprinus carpio* groups exposed to analgesic drugs may be due to the impairment of the antioxidant system, responsible for the increasing lipid peroxidation and disequilibrium of GSH/GSSG.

### *Antihistaminic drugs*

Teixeira et al. (2017) observed that 12 µg/L cetirizine inhibited the activity of glutathione S-transferases activity (GSTs) and the activity of SOD and CAT.

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Table 1. Effect of different pharmaceutical products from watersurface on fish oxidative stress

| Crt. no. | Type of drug | Drug | Environmental concentrations | Concentration/Time exposure | Fish species | Samples | Biochemical responses | References |
|---|---|---|---|---|---|---|---|---|
| 1. | Antibiotics | Oxytetracycline | ng/L to µg/L | 0, 0.1, 10, 10000 µg/L for two months | *Danio rerio* | Whole body | (-) Total Gluthatione; Glutathione S-Transferase; Catalase | Carvalho & Santos, 2016; Almeida et al., 2019 |
| 2. | | | | 0.005, 0.050, 0.500, 5 and 50 mg/L for 96 hour | *Oncorhynchus mykiss* | Gills, Liver | (+) Catalase in gills; Gluthatione Peroxidase in gills; Lipid peroxidation in liver (-) Catalase in liver; Gluthatione reductase; Lipid peroxidation in gills | Rodrigues et al., 2017 |
| 3. | | | | 0.3125, 0.625, 1.25, 2.5 and 5.0 µg/L for 28 days | | | (+) Catalase, Gluthatione Peroxidase in liver; Gluthatione Reductase; Lipide peroxidation in liver (-) Catalase, Gluthatione Peroxidase in gills; | |
| 4. | | Sulfamethoxazole Oxytetracycline | 259.6 ng/L and 350 ng/L | 260 ng/L and 420 ng/L for six Weeks | *Danio rerio* | Intestine, Liver, Muscle | (-) Superoxide Dismutase; Peroxidase; Reduced Glutathione | Zhou et al., 2018a |
| 5. | | | | 100 and 80 mg/kg for six weeks | | | (+) Malondialdehyde (-) Peroxidase; Superoxide Dismutase; Reduced Glutathione. | Zhou et al., 2018b |
| 6. | | Amoxicillin Oxytetracycline | 6 and 340 ng/L | 0.1, 10, 25, 50, 100 mg/L for 96 h | *Danio rerio* | Head, Muscle, Liver, Gills | (-) Catalase; Glutathione S-Transferase; | Park & Choi, 2008; Oliveira et al., 2013 |
| 7. | | Ivermectin | 25 up to 60 ng/L | 10, 20, 40, 60, 80, 100, 200 µg/L for 96 h | *Danio rerio* | Head, Muscle, Liver, Gills | (-) Glutathione S-Transferase | Montforts et al., 2004; Sanderson et al., 2007; Oliveira et al., 2016 |
| 8. | | | | | | Head, Trunck | (-) Catalase; Glutathione S-Transferase | Domingues et al., 2016 |
| 9. | | Norfloxacin | 0.0001 mg/L | 0.0001, 0.1, 1, 10 and 30 mg/L for 28 days | *Danie rerio* | Whole body | (+) Gluthatione reductase; Gluthatione S-Transferase; Gluthatione peroxidase; Catalase; Lipid peroxidation | Bartoskova et al., 2014 |
| 10. | Antiepileptics and Antipsychotics | Carbamazepine Clonazepam | 0.002 to 11.5 µg/L respectively 145 ng/L | 75 µg/L for 96 h | *Danio rerio* | Brain, Liver, Kidney | (-) Glutathione in Liver; Catalase (+) Glutathione in Brain; Glutathione S-Transferase in Brain and in liver | Cunha et al., 2017; Ekpeghere et al., 2018; Gomes et al., 2019 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11. | | Carbamazepine | 0.002 to 11.5 µg/L | 0, 10 or 10000 µg/l for 63 days | *Danio rerio* | Muscle, Head, Gills, Liver, Gut | (-) Catalase<br>(+) Glutathione S-Transferase | Ekpeghere et al., 2018; Santos et al., 2018 |
| 12. | | | | 1, 10 and 100 µg/L for 45 Days | *Danio rerio* | Whole Body | (-) Glutathione S-Transferase; Superoxide Dismutase<br>(+) Catalase | Jia et al., 2020 |
| 13. | | Carbamazepine | | 60 µg/L / 35 days | *Tinca tinca* | Whole Body | (-) Gluthatione S-Transferase; Gluthatione peroxidase; Catalase; Lipid peroxidation | Stancova et al., 2017 |
| 14. | Analgesic, Antipyretic and Anti-Inflammatory Drugs | Diclofenac | 0.02 mg/L | 0.02, 5, 15, 30, and 60 mg/L for 28 Days | *Danio rerio* | Whole Body | (+) Lipid Peroxidation | Praskova et al., 2014 |
| 15. | | | | 3 mg/L and 2 µg/L of for 96 h | *Danio rerio* | Gills, Liver | (+) Glutathione S-Transferase; Lipid Peroxidation | De Carvalho Penha et al., 2021 |
| 16. | | Ketoprofen, Diclofenac and their Photodegradation products | up to 1µg/L; 0.02 mg/L | 1 mg/L for Ketoprofen, 7.5 and 60 min; for Diclofenac 1.5 and 5 min | *Danio rerio* | Whole Body | (+) Lipid Peroxidation<br>(-) Catalase; Glutathione S-Transferase; Superoxid Dismutase | Cuklev et al., 2012; Praskova et al., 2014; Diniz et al., 2015 |
| 17. | | Diclofenac, Naproxen, and Ibuprofen | - | Mix of diclofenac, naproxen, and ibuprofen, 0.1 µM / 96 h | *Cyprinus carpio* | Liver | (+) Lipid peroxidation<br>(-) Superoxide dismutase; catalase; glutathione Peroxidase; GSH/GSSG | Gao et al., 2018 |
| 18. | | Diclofenac, Ibuprofen, Carbamazepine | - | mixturesmof pharmaceuticals at nominal concentrations of 0.02, 0.2, 2, 20, and 60 µg/l of each pharmaceutical / 35 days | *Tinca tinca* | Whole Body | (+) Gluthatione reductase; Gluthatione S-Transferase<br>(-) Gluthatione peroxidase | Stancova et al., 2017 |
| 19. | | Diclofenac, | - | 60 µg/L / 35 days | *Tinca tinca* | Whole Body | (+) Gluthatione reductase; Gluthatione S-Transferase; Gluthatione peroxidase; Catalase<br>(-) Lipid peroxidation | Stancova et al., 2017 |
| 20. | | Ibuprofen | - | 60 µg/L / 35 days | *Tinca tinca* | Whole Body | (-) Gluthatione reductase; Gluthatione S-Transferase; Gluthatione peroxidase; Catalase; Lipid peroxidation | Stancova et al., 2017 |
| 21. | Anesthetics | Phenol | - | 21 mg/L / 2 days | *Pangasius* sp. | Liver | (+) Lipid peroxidation<br>(-) Total antioxidant capacity | Vijaya Geetha et al., 2021 |
| 22. | Antilipidemic | Clofibrate | - | 1000 mg/L/ 7days | *Pangasius* sp. | Liver | (+) Lipid peroxidation; Total antioxidant capacity | |
| 23. | Effluent A and B from two pharmaceutical industries in Lagos, Nigeria | | | LC50 after 14 and 28 days | *Oreochromis Niloticus* | Gills, Liver | (-) Reduced gluthatione; Superoxid dismutase; Catalase<br>(+) Lipid peroxidation | Lan et al., 2021 |

*Note:* (+) – increase; (-) - decrease

## POLICY INSTRUMENTS TO CONTROL PHARMACEUTICALS IN THE ENVIRONMENT

The use of best practices, a good international cooperation, awareness of the dangers of these substances in the aquatic environment and an improvement of understanding of risks should be used to reduce pharmaceuticals products in surface water.

The development of "green" and eco-friendly pharmaceuticals with low environmental persistence, no bioaccumulation, and reduced toxicity could help minimize accumulation in the environment. The Stockholm County Council of Sweden developed a classification system for the environmental impact of pharmaceuticals called the PBT index (Patel et al., 2019). This is defined as the sum of the values for persistence, bioaccumulation, and toxicity. Pharmaceuticals are classified on a 0-3 scale in this index for persistence, bioaccumulation, and toxicity. A value of 0 corresponds to the most environmentally friendly while 3 is the worst for the environment. Physicians should discourage using pharmaceuticals with high PBT index values and encourage development of more eco-friendly pharmaceuticals (Patel et al., 2019). Some important recommendations are listed below:

- advanced methods for accurate and continuous detection of pharmaceuticals in environmental systems should be developed and applied,
- strict regulations for effluent release from industrial and hospital point sources must be implemented,
- greener technologies should be implemented for pharmaceutical development, manufacture, and use,
- continuous research is required to how chronic exposure to micropollutants effects aquatic environment,
- implementation a standard to limit micropollutants in wastewaters and environmental water systems,
- choosing an effective technology and equipment for pharmaceutical remediation and implementation of these on a large scale and at a low cost.

## CONCLUSIONS

In conclusion, for the future studies is necessary to test the same concentrations of various drugs for the same time intervals on the same fish species. This is required in order to make comparisons and to prove the hypothesis on the effect of a certain drug on fish oxidative stress. Also, there are insufficient data on the potential for impacts on higher trophic levels, either through trophic transfer of pharmaceuticals or indirect effects due to impacts on lower trophic levels including algae.

At the same time, it is necessary to implement a pharmaceutical return program for unwanted and expired drugs which will help control the volume of pharmaceuticals released that are present in household wastes and domestic effluents.

In the near future, the development of an eco-friendly pharmaceuticals with low persistence in water surface, bioaccumulation and toxicity could help minimize accumulation in the aquatic environment.

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## REFERENCES

Almeida, A.R., Tacão, M., Machado, A.L., Golovko, O., Zlabek, V., Domingues, I., Henriques, I. (2019). Long-Term Effects of Oxytetracy1cline Exposure in Zebrafish: A Multi-Level Perspective. *Chemosphere*, 222, 333–344.

Arango V, S.S. (2012). Biomarcadores para la evaluacion ´ de riesgo en la salud humana. *Rev. Fac. Nac. Salud Pública* 30, 75–82. https://doi.org/10.1007/978-1-4939-0986- 5_19.

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M. (2017). The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere* 174, 437–446. https://doi.org/10.1016/j. chemosphere.2017.01.101.

Aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A. (2016). Pharmaceuticals in the environment-Global occurrences and perspectives. *Environmental*

*Toxicology and Chemistry* 35(4):823–835, https://doi.org/10.1002/etc.3339.

Bagnyukova, T. V., Chahrak, O. I., Lushchak, V. I. (2006). Coordinated response of goldfish antioxidant defenses to environmental stress. *Aquatic Toxicology*, 78, 325.

Bagnyukova, T. V., Luzhna, L. I., Pogribny, I. P. and Lushchak, V. I. (2007). Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. *Environmental and Molecular Mutagenesis*, 48, 658-665.

Bartoskova, M., Dobsikova, R., Stancova, V., Pana, O., Zivna, D., Plhalova, L., Blahova, J., Marsalek, P. (2014). Norfloxacin-Toxicity for Zebrafish (*Danio rerio*) Focused on Oxidative Stress Parameters. *Hindawi Publishing Corporation BioMed Research International*, 1-6, ID 560235, http://dx.doi.org/10.1155/2014/560235

Bethanie Carney Almroth (2008). Oxidative damage in fish used as biomarkers in field and laboratory studies. *Department of Zoology/Zoophysiology, University of Gothenburg, Box* 463, 405 30 Göteborg, Sweden.

Bonnefille, B., Gomez, E., Courant, F., Escande, A., Fenet, H. (2018). Diclofenac in the marine environment: a review of its occurrence and effects. *Mar. Pollut. Bull.* https://doi.org/ 10.1016/j.marpolbul.2018.04.053.

Burger, J. (2006). Bioindicators: types, development, and use in ecological assessment and research. *Environ. Bioindic.* 1, 22–39. https://doi.org/10.1080/ 15555270590966483.

Carvalho, I.T., Santos, L. (2016). Antibiotics in the Aquatic Environments: A Review of the European Scenario. *Environ. Int.* 94, 736–757.

Cuklev, F., Fick, J., Cvijovic, M., Kristiansson, E., Förlin, L., Larsson, D.G.J. (2012). Does Ketoprofen or Diclofenac Pose the Lowest Risk to Fish? J. *Hazard. Mater.* 229–230, 100–106.

Daughton, C.G., Ruhoy, I.S. (2009). Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers. *Environ. Toxicol. Chem*., 28(12):2495, https://doi.org/10.1897/08-382.1.

Daughton, C. G. (2004). Non-Regulated Water Contaminants: Emerging Research. *Environ. Impact Assess. Rev.,* 24, 711−732.

De Carvalho Penha, L.C.C., Rola, R.C., Martinez, C.B.D.R., de Martins, C.M.G. (2021). Effects of Anti-Inflammatory Diclofenac Assessed by Toxicity Tests and Biomarkers in Adults and Larvae of *Danio Rerio*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 242, 108955.

Diniz, M. S. et al. (2015). Ecotoxicity of ketoprofen, diclofenac, atenolol and their photolysis byproducts in zebrafsh (*Danio rerio*). *Sci. Total Environ*. 505, 282–289.

Domingues, I., Oliveira, R., Soares, A.M.V.M., Amorim, M.J.B. (2016). Effects of Ivermectin on Danio Rerio: A Multiple Endpoint Approach: Behaviour, Weight and Subcellular Markers. *Ecotoxicology*, 25, 491–499.

Du, L., Liu, W. (2012). Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustain. Dev.* 32, 309– 327. (doi:10.1007/s13593-011-0062-9)

Fabbri, E., Franzellitti, S. (2016). Human pharmaceuticals in the marine environment:focus on exposure and biological effects in animal species. *Environmental Toxicology and Chemistry*, 35(4): 799–812. DOI: 10.1002/etc.3131

Fursdon, J.B., Martin, J.M., Bertram, M.G., Lehtonen, T.K., Wong, B.B. (2019). The pharmaceutical pollutant fluoxetine alters reproductive behaviour in a fish independent of predation risk. *Sci. Total Environ*. 650, 642-652.

Gallego, R, S.E., Penuela, G.A., Martínez-Lopez, E. (2021). Enzymatic activity changes in striped cat fi sh Pseudoplatystoma magdaleniatum, induced by exposure to different concentrations of ibuprofen and triclosan. *Chemosphere* 271, 1–13. https://doi.org/ 10.1016/j.chemosphere.2020.129399.

Gao, X., Geng, J., Du, Y., Li, S., Wu, G., Fu, Y., Ren, H. (2018). Comparative study of the toxicity between three non-steroidal anti1infammatory drugs and their UV/ $Na_2S_2O_8$ degradation products on *Cyprinus carpio*. *Scientific Reports*, 8:13512, 1-11. DOI:10.1038/s41598-018-29524-1.

Gaw, S., Thomas, K.V., Hutchinson, T.H. (2014). Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. *Phil. Trans. R. Soc. B* 369:20130572. http://dx.doi.org/10.1098/rstb.2013.0572

Gomes, T.B., Fernandes Sales Junior, S., Saint'Pierre, T.D., Correia, F.V., Hauser-Davis, R.A., Saggioro, E.M. (2019). Sublethal Psychotropic Pharmaceutical Effects on the Model Organism *Danio rerio*: Oxidative Stress and Metal Dishomeostasis. *Ecotoxicol. Environ. Saf.*, 171, 781–789.

Grigorakis, K., Rigos, G. (2011). Aquaculture effects on environmental and public welfare—the case of Mediterranean mariculture. *Chemosphere* 85, 899–919. (doi:10.1016/j.chemosphere.2011.07.015)

Huang, D.J., Zhang, Y.M., Song, G., Long, J., Liu, J. H., Ji, W. H. (2007). Contaminants-Induced Oxidative Damage on the Carp *Cyprinus carpio* Collected from the Upper Yellow River, China. *Environmental Monitoring and Assessment*. 128, 483-488.

Jia, A., Hu, J., Wu, X., Peng, H., Wu, S., Dong, Z. (2011). Occurrence and source apportionment of sulfonamides and their metabolites in Liaodong Bay and the adjacent Liao River Basin, North China. *Environ. Toxicol. Chem*. 30, 1252 – 1260. (doi:10. 1002/etc.508)

Jia, D., Li, X., Du, S., Xu, N., Zhang, W., Yang, R., Zhang, Y., He, Y., Zhang, Y. (2020). Single and Combined Effects of Carbamazepine and Copper on Nervous and Antioxidant Systems of Zebrafish (*Danio rerio*). *Environ. Toxicol*., 35, 1091–1099.

Jijie, R., Mihalache, G., Balmus, I.M., Strungaru, S.A., Baltag, E.S., Ciobica, A., Nicoara, M., Faggio, C. (2021). Review zebrafish as a screening model to study the single and joint effects of antibiotics. *Pharmaceuticals* 14, 1–26. https://doi.org/ 10.3390/ph14060578.

Kanda, R., Griffin, P., James, H. A., Fothergill, J. (2003). Pharmaceutical and Personal Care Products in Sewage Treatment Works. *J. Environ. Monit*., 5, 823−830.

Kemper, N. (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indicators* 8, 1 – 13. (doi:10.1016/j.ecolind.2007.06.002)

Kummerer, K. (2009a). Antibiotics in the aquatic environment: a review. I. *Chemosphere* 75, 417– 434. (doi:10.1016/j.chemosphere.2008.11.086)

Kuummerer, K. (2009b). The Presence of Pharmaceuticals in the Environment Due to Human Use−Present Knowledge and Future Challenges. *J. Environ. Manage.*, 90, 2354−2366.

Lan, S.M., Briggs, T.-M.D., Obanya, H.E., Amaeze, N.H. and Otitoloju, A.A. (2021). Toxicity Evaluation of Pharmaceutical Wastewater to the Nile Tilapia (Oreochromis niloticus). *Journal of Environmental Protection,* 12, 296-309. https://doi.org/10.4236/jep.2021.124019

Larsson, D. J. (2014). Pollution from Drug Manufacturing: Review and Perspectives. *Philos. Trans. R. Soc., B*, 369, 20130571.

Li, Z.-H. et al. (2010). Modulation of antioxidant defence system in brain of rainbow trout (*Oncorhynchus mykiss*) after chronic carbamazepine treatment. Comp. Biochem. Physiol. C: Pharmacol. Toxicol. 151, 137–141.

Lindsey, M. E., Meyer, M., Thurman, E. M. (2001). Analysis of Trace Levels of Sulfonamide and Tetracycline Antimicrobials in Ground1water and Surface Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry. *Anal. Chem.*, 73, 4640 − 4646.

Martínez-Morcillo, S., Rodríguez-Gil, J.L., Fernandez-Rubio, J., Rodríguez-Mozaz, S., Míguez-Santiyan, M.P., Valdes, M.E., Barcelo, D., Valcarcel, Y. (2020). Presence of pharmaceutical compounds, levels of biochemical biomarkers in seafood tissues and risk assessment for human health: results from a case study in North-Western Spain. *Int. J. Hyg. Environ. Health* 223, 10–21. https://doi.org/10.1016/j.ijheh.2019.10.011.

Mauro, M., Lazzara, V., Arizza, V., Luparello, C., Ferrantelli, V., Cammilleri, G., Inguglia, L., Vazzana, M. (2021). Human Drug Pollution in the Aquatic System: The Biochemical Responses of *Danio rerio* Adults. *Biology*, 10, 1064. https://doi.org/10.3390/biology10101064

Monserrat, J. M., Martinez, P. E., Geracitano, L. A., Lund Amado, L., Martinez Gaspar Martins, C., Lopes Leaes Pinho, G., Soares Chaves, I., Ferreira-Cravo, M., Ventura-Lima, J. and Bianchini, A. (2007). Pollution biomarkers in estuarine animals: Critical review and new perspectives. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 146, 221-234.

Montforts, M.H.M.M., van Rijswick, H.F.M.W., de Haes, H.A.U. (2004). Legal Constraints in EU Product Labelling to Mitigate the Environmental Risk of Veterinary Medicines at Use. *Regul. Toxicol. Pharmacol*., 40, 327–335.

Nunes, B., Carvalho, F., Guilhermino,L. (2006). Effects of widely used pharmaceuticals and a detergent on oxidative stress biomarkers of the crustacean Artemia parthenogenetica, *Chemosphere*, 62(4):581–594.

Oliveira, R., Grisolia, C.K., Monteiro, M.S., Soares, A.M.V.M., Domingues, I. (2016). Multilevel Assessment of Ivermectin Effects Using Different Zebrafish Life Stages. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, 187, 50–61.

Oliveira, R., McDonough, S., Ladewig, J.C.L., Soares, A.M.V.M., Nogueira, A.J.A., Domingues, I. (2013). (Effects of Oxytetracycline and Amoxicillin on Development and Biomarkers Activities of Zebrafish (*Danio rerio*). *Environ. Toxicol. Pharmacol*., 36, 903–912.

Oost, D., Beyer, J., Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Park, S., Choi, K. (2008). Hazard Assessment of Commonly Used Agricultural Antibiotics on Aquatic Ecosystems. Ecotoxicology, 17, 526–538.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Modan, D. (2019). Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods. *Chemical Reviews*, 119, 3510–3673. DOI: 10.1021/acs.chemrev.8b00299

Pedrazzani, R., Bertanza, G., Brnardic, I., Cetecioglu, Z., Dries, J., Dvarioniene, J., García-Fernandez, A.J., Langenhoff, A., Libralato, G., Lofrano, G., Skrbic, B., Martínez-Lopez, E., Meriç, S., Pavlovic, D.M., Papa, M., Schroder, P., Tsagarakis, K. P., Vogelsang, C. (2019). Opinion paper about organic trace pollutants in wastewater: Toxicity assessment in a European perspective. *Sci. Total Environ*. 651, 3202–3221. https://doi.org/10.1016/j.scitotenv.2018.10.027.

Petrovic, M., Škrbic, B., Živancev, J., Ferrando-Climent, L., Barcelo, D. (2014). Determination of 81 Pharmaceutical Drugs by High Performance Liquid Chromatography Coupled to Mass Spectrometry with Hybrid Triple Quadrupole–Linear Ion Trap in Different Types of Water in Serbia. *Sci. Total Environ*., 468–469, 415–428.

Praskova, E., Plhalova, L., Chromcova, L., Stepanova, S., Bedanova, I., Blahova, J., Hostovsky, M., Skoric, M., Maršálek, P., Voslarova, E. (2014). Effects of Subchronic Exposure of Diclofenac on Growth, Histopathological Changes, and Oxidative Stress in Zebrafish (*Danio rerio*). *Sci. World J*., 2014, 645737.

Radovic, T., Grujic, S., Petkovic, A., Dimkic, M., Lauševic, M. (2015). Determination of Pharmaceuticals and Pesticides in River Sediments and Corresponding Surface and Ground Water in the Danube River and Tributaries in Serbia. *Environ. Monit. Assess.*, 187, 4092.

Ramirez, A.J. et al. (2009). Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. *Environ. Toxicol. Chem*. 28, 2587 – 2597. (doi:10.1897/08-561.1).

Recabarren-Villalon, T., Ronda, A.C., Arias, A.H., (2019). Uso de biomarcadores en la evaluacion ´ ambiental de ecosistemas marinos en Am´erica. *JAINA Costas y Mares ante el Cambio Climatico* 1, 1–18. https://doi.org/10.26359/52462.0719.

Richardson, S. D. (2006). Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. Anal. Chem., 78, 4021−4046.

Richardson, S. D., Ternes, T. A. (2014). Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem*., 86, 2813−2848.

Rivera-Utrilla, J., Sanchez-Polo, M., Ferro-García, M. A., PradosJoya, G,; Ocampo-Perez, R. (2013). Pharmaceuticals as Emerging Contaminants and Their Removal from Water. A Review. *Chemosphere*, 93, 1268−1287.

Rivoira, L., de Carlo, R.M., Cavalli, S., Bruzzoniti, M.C. (2015). Simple SPE-HPLC determina1tion of some common drugs and herbicides of environmental concern by pulsed amperometry. *Talanta* 131, 205–212.

Rodrigues, S., Antunes, S.C., Correia, A.T., Nunes, B. (2017). Rainbow trout (*Oncorhynchus mykiss*) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. *Ecotoxicology* 26:104–117. DOI 10.1007/s10646-016-1746-3

Ruas, C. B. G., Carvalho, C. d. S., de Araujo, H. S. S., Espindola, E. L. G., Fernandes, M. N. (2007). Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. *Ecotoxicology and Environmental Safety*. doi:10.1016/j.ecoenv.2007.08.018.

Sanderson, H., Laird, B., Pope, L., Brain, R., Wilson, C., Johnson, D., Bryning, G., Peregrine, A.S., Boxall, A., Solomon, K. (2007). Assessment of the Environmental Fate and Effects of Ivermectin in Aquatic Mesocosms. *Aquat. Toxicol*. 2007, 85, 229–240.

Santos Da Silva, N., Oliveira, R., Lisboa, C.A., Mona E Pinto, J., Sousa-Moura, D., Camargo, N.S., Perillo, V., Oliveira, M., Grisolia, C.K., Domingues, I. (2018). Chronic Effects of Carbamazepine on Zebrafish: Behavioral, Reproductive and *Biochemical Endpoints. Ecotoxicol. Environ. Saf*., 164, 297–304.

Sehonova, P., Plhalova, L., Blahova, J., Doubkova, V., Marsalek, P., Prokes, M., Tichy, F., Skladana, M., Fiorino, E., Mikula, P., Vecerek, V., Faggio, C., Svobodovs, Z. (2017). Effects of selected tricyclic antidepressants on early-life stages of common carp (*Cyprinus carpio*). *Chemosphere* 185, 1072–1080.

Shreenidhi, K.S., Priyavadhana, P., Purnima, N., Rashminiza, A., Sneha, S., Vijaya Geetha, B. (2021). Study on the toxic effects of pharmaceutical drugs – Norfloxacin using *Pangasius Sp*. fish model and its mitigation using *Artemisia pallens*. *Acta Ecologica Sinica*, https://doi.org/10.1016/j.chnaes.2021.08.014 (In Press).

Shuraiqi, A.Al., Al-Habsi, A., Barry, M.J. (2021). Time, dose and transgenerational effects of fluoxetine on the behavioural responses of zebrafish to a conspecific alarm substance. *Environmental Pollution* 270, 116164. doi.org/10.1016/j.envpol.2020.116164

Stephensen, E., Sturve, J. and Forlin, L. (2002). Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. *Comparative Biochemistry and Physiology Part C*. 133, 435-442.

Strungaru, S.A., Pohontiu, C.M., Nicoara, M., Teodosiu, C., Baltag, E.S., Jijie, R., Plavan, G., Pacioglu, O., Faggio, C. (2021). Response of aquatic macroinvertebrates communities to multiple anthropogenic stressors in a lowland tributary river. *Environ. Toxicol. Pharmacol*. 87, 103687. doi.org/10.1016/j. etap.2021.103687.

Teixeira, M., Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares, A.M., Figueira, E., Freitas, R. (2017). Toxic effects of the antihistamine cetirizine in mussel *Mytilus galloprovincialis*. *Water Res*. 114, 316–326.

Vijaya Geetha, B., Shreenidhi, K.S., Sundararaman, T.R.A. (2021). Study on the Oxidative Stress Status of *Pangasius sp*. Exposed to Phenol and Clofibrate. *Zeichen Journal*, 7(1):23-32.

Winker, M., Tettenborn, F., Faika, D., Gulyas, H., Otterpohl, R. (2008). Comparison of analytical and theoretical pharmaceutical concentrations in human urine in Germany, Water Res. 42(14):3633–3640, https://doi.org/10.1016/j. watres.2008.06.002.

Zhou, L., Limbu, S.M., Qiao, F., Du, Z.-Y., Zhang, M. (2018). Influence of Long-Term Feeding Antibiotics on the Gut Health of Zebrafish. *Zebrafish*, 15, 340–348.

Zhou, L., Limbu, S.M., Shen, M., Zhai, W., Qiao, F., He, A., Du, Z.-Y., Zhang, M. (2018b). Environmental Concentrations of Antibiotics Impair Zebrafish Gut Health. *Environ. Pollut.*, 235, 245–254.

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R. (2000). Presence of Therapeutic Drugs in the Environment. *Lancet*, 355, 1789− 1790.