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REVIEW ARTICLE

Fish liver damage related to the wastewater treatment plant effluents

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Abstract
Wastewater treatment plants (WWTPs) continuously release a complex mixture of municipal, hospital, industrial, and runoff chemicals into the aquatic environment. These contaminants are both legacy contaminants and emerging-concern contaminants, affecting all tissues in a fish body, particularly the liver. The fish liver is the principal detoxifying organ and effects of consistent pollutant exposure can be evident on its cellular and tissue level. The objective of this paper is thus to provide an in-depth analysis of the WWTP contaminants' impact on the fish liver structure, physiology, and metabolism. The paper also gives an overview of the fish liver biotransformation enzymes, antioxidant enzymes, and non-enzymatic antioxidants, their role in metabolizing xenobiotic compounds and coping with oxidative damage. Emphasis has been placed on highlighting the vulnerability of fish to xenobiotic compounds, and on biomonitoring of exposed fish, generally involving observation of biomarkers in caged or native fish. Furthermore, the paper systematically assesses the most common contaminants with the potential to affect fish liver tissue.

Keywords WWTP · Vulnerability · Stress · Contaminants · Biotransformation

Introduction

Wastewater treatment plants (WWTP) continuously release a complex mixture of municipal, hospital, industrial, and runoff chemicals into the aquatic environment. These contaminants are of both long-standing and emerging concern (Sauvé and Desrosiers 2014). The WWTP effluents also comprise microorganisms and excess nutrients, along with engineered nanomaterials, microplastic, metals, pharmaceutically active compounds, organic pollutants, endocrine-disrupting chemicals, and a plethora of other compounds (Mehdi et al. 2021; Lapointe et al. 2020; Tran et al. 2018; Neale et al. 2013). We know that many of these contaminants individually affect fish health, but the collective impacts of chemical mixtures and associated bacteria on fish are still unclear (Restivo et al. 2021).

WWTPs have a significant role in the water cycle and pollutant removal through primary, secondary, and advanced treatments since pollutants circulate via effluents, sludge, and air emissions (Ribeiro et al. 2020; Papa et al. 2016). These treatments may alter the state and stability of pollutants. The primary treatment mostly involves flocculation/coagulation and sedimentation. The secondary one comprises biological treatment, aggregation/sedimentation, sludge treatment, and disinfection. The advanced treatment involves membrane filtration and advanced oxidation processes, where applicable (Neale et al. 2013). The contaminants in effluents are therefore found at relatively low concentrations, and they get further diluted in receiving waters (Wigh et al. 2018). In addition, after WWTP treatment, some of these chemicals may be of altered stability. They might interact with household and industrial surfactants (Jarvie et al. 2009), or be adsorbed onto naturally occurring aquatic colloids or particulate matter, for example (Worms et al. 2010; Klaine et al. 2008). Most WWTPs are not equipped with technology to effectively remove contaminants of emerging concern, including many consumer products (e.g., pharmaceuticals and personal care products), which present a risk to water quality, leading to the input of partially treated effluents to surface waters and exposure of aquatic organisms (Franco et al. 2020). Thus, the contaminants might be taken up into

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the tissues of fish and other aquatic organisms where they can elicit adverse biological effects. In comparison with mollusc and crustacean models, fish have a similar development, anatomy, and physiology to higher vertebrates, which makes them ideal models for monitoring of chemicals in the aquatic environment (Schujit et al. 2021).

The routes of xenobiotic uptake depend on the dietary and ecological circumstances of a particular fish species, as the contaminants may occur in sediments, suspended material, and food sources (James and Kleinow 2018; Livingstone 2001). They also depend on the season of the year, relative to water temperature, and fish reproductive status (Soler et al. 2020). Responding to stressors through exposure to wastewater, fish undergo physiological and biochemical tissue processes in order to compensate for the imposed xenobiotic challenge (Blahova et al. 2014). The consequences of these processes can be observed across multiple levels of biological organization, from metabolic to behavioral, to population and community-level responses (Mehdi et al. 2021; Topić Popović et al. 2015a). Fish may react with acute responses to contaminants, but as latter often occur at low environmental levels, chronic effects are more prominent in contaminated waters (Franco et al. 2020).

The liver of fish and other vertebrates is the principal detoxifying organ and effects of consistent pollutant exposure can be evident on its cellular and tissue level (Minarik et al. 2014). The liver is also the major organ for the storage of heavy metals (Ardeshir et al. 2017). Fish liver biomarkers, conjugation enzymes, carboxylesterase activities, and antioxidant defenses can confirm chemical exposure to lipophilic microcontaminants, from dioxin-like chemicals to drugs of broader nature (Soler et al. 2020). Biomarkers, as biological endpoints, are used for the evaluation of tissues at the cellular, biochemical, and molecular levels. They are important as short-term indicators of long-term biological effects and indicate the presence of contaminants (exposure biomarkers) or the magnitude of the organismal response (effects biomarkers) (Garmendia et al. 2015).

The objective of this paper is thus to provide an in-depth analysis of the WWTP contaminants' impact on the fish liver structure, physiology, and metabolism. Furthermore, it systematically assesses the most common contaminants with the potential to affect fish liver tissue.

Vulnerability of fish systems to wastewater outfalls and environmental stressors

Fish vulnerability to xenobiotics is not a constant and largely varies between individual organisms, but also within an organism in relation to its genetic makeup, life stage, development, gender, gonadal maturation, diseases, infestations, energy reserves, etc. Vulnerability also resides in the sensitivity and resilience of the system exposed to various hazards (Rudneva et al. 2020; Turner et al. 2003). Besides xenobiotics and pollutants, there is also many environmental stressors, which may affect fish communities: climate change, coastal or riverine development, erosion, alterations of hydrological systems, physicochemical changes of water and sediment, pathogen burden, toxic algae, invasive species, intra- and interspecific competitions, eutrophication, fishing, and aquaculture leading to the altered food web and habitat changes (Couillard et al. 2008). All these factors contribute to the cumulative effects of the vulnerability burden.

WWTP outfalls can alter physicochemical properties of water, depending on the inflowing sources. For example, the sugarplant wastewaters and municipal wastewaters differ in many physicochemical parameters which can dramatically affect fish (liver) health (Topić Popović et al. 2015a). The sugarplant wastewaters mostly surpass the raw municipal wastewaters inflowing the WWTP in values of many parameters, such as suspended solids, chemical oxygen demand (COD), permanganate index (COD-Mn), biochemical oxygen demand (BODn), nitrate, nitrite. Namely, increased nitrogen may result in overgrowth of algae, which can decrease the dissolved oxygen content of the water, thereby harming or killing fish (Topić Popović et al. 2016, 2015a). Besides, continuous exposure to high levels of ammonia can cause oxidative damage to the fish liver, where the degree of liver damage increases with the prolongation of exposure (Liu et al. 2021).

Wastewater outfalls generally enhance the presence of omnivorous, non-native, and tolerant fish of a few species. They may be attracted to the effluent or outfall habitat because of nutrients, organic particulate matter, and increased food availability (McCallum et al. 2019). However, the combination of a constant nutrient supply and temperature of the effluent, particularly in the winter months, might create an ecological trap for such fish, threatening their reproduction and survival (Mehdi et al. 2021). The toxic injury of such fish is based on the duration of exposure and concentration of xenobiotic chemicals at the target site, the number of available target sites, and the recovery capacity of fish (Couillard et al. 2008). The toxicokinetic processes such as uptake, distribution, metabolism, and excretion control the concentration of xenobiotic chemicals at the target sites. On the other hand, the toxicodynamic processes such as the interaction between chemicals and target sites, and repair mechanisms, modify their responses (Couillard et al. 2008; Heinrich-Hirsch et al. 2001).

The most vulnerable period in fish development is the early life stage when they are more sensitive to xenobiotics. In such a critical life stage, fish absorb higher concentrations of xenobiotics, have undeveloped detoxification capacity, and are less capable of avoiding contaminated areas (Andersen et al. 2003; Vosyliene et al. 2003). Several fish

species are able to survive in environments with high levels of multiple anthropogenic pollutants such as wastewater outfalls. However, the synergistic impact of complex pollutants in interaction with environmental factors, physical or biological stressors, significantly exerts their effects on fish health. Furthermore, deleterious effects of multiple stressors on fish communities are challenging to detect in native populations, which tend to be under constant or recurring exposure causing a prolonged physiological response during their acclimation (Topić Popović et al. 2016).

Caged or native fish

For evaluation of the impact of WWTP effluents on fish, a variety of strategies is used. Biomonitoring of environmentally exposed fish generally involves observation of biomarkers in caged or native fish. Laboratory studies, on the other hand, often use the extreme exposure concentration of a single xenobiotic or its mixtures (Gagnon and Rawson 2017). Laboratory studies can thus overestimate or sometimes underestimate the effects in wild populations. As the concentrations used in laboratory studies may not relate to their levels in field situations, the observations validated with field studies are generally more reliable (van der Oost et al. 2003).

Environmental exposures are often variable in both duration and magnitude, but in standard acute and chronic effects testing, they are maintained (Hamer et al. 2019). For example, the avoidance response is one form of phenotypic adaptation allowing fish to survive in the altered environment. Such behavior represents the final integrated fish response to xenobiotic stress. The active withdrawal of fish from polluted areas can affect their physiological migration, distribution, and survival patterns (Vosyliene et al. 2003). Interestingly, non-migratory species have higher interspecies variability in contaminant burdens than fish that move extensively (Marcogliese et al. 2015).

The native fish biomarker responses to effluents are affected by a number of complex factors, such as the age of the individual fish, length/weight ratio, sex, migration pattern, adaptation mechanisms to chronic pollution, and presence of sentinel or endangered species (Catteau et al. 2021). The variations in biological and physicochemical parameters may influence the endpoint measurement unless applying multiple collection times over seasons (Gagnon and Rawson 2017). In addition, fluctuations in the food supply related to season, spawning, and migration affect food abundance or deprivation, and the feeding rates of native fish. Therefore, native fish with insufficient energy stores could have adverse reactions to acute or chronic effluent exposures (Weinrauch et al. 2021). Another problem of the field surveys is extrapolating from observations on a few individuals in a limited number of species to groupings of many individuals and species and the need to consider how the relative sensitivity of their individual responses reflect the response of populations, communities, and ecosystems (Galloway et al. 2004).

By choosing to use the caged fish for biomonitoring studies, individual fish are selected according to their particular characteristics such as sex, age, or size. No fishing effort allocated to catching the same species in the different sampling sites is needed. Fish mobility is avoided which removes a source of variability in the field study. The use of fish from a hatchery avoids the possibility of sampling pollution-adapted fish (Kosmala et al. 1998). Variability of the responses can be reduced by controlling the distance of the cage from the effluent source, the position of the cage, season, or caging duration (Catteau et al. 2021). Therefore, the use of cages offers precise information about their location and exposure duration, gives control of the selection of representative species and their particular developmental stage and genetic background, yielding comparable results over various sites (Cazenave et al. 2014). The most comprehensive evaluation of sublethal toxicity of contaminants is achieved when studies are performed on fish in all stages of development (Marcogliese et al. 2015), which advocates the use of cages. However, caged fish may suffer from density stress, which could increase cortisol levels and potentiate induction of hepatic enzyme activity (Kosmala et al. 1998). In any case, selecting the caged or native fish, the utilization of a battery of complementary biomarkers is recommended for the accurate estimation of fish responses during effluent exposure.

Fish liver, oxidative stress, and biomarkers

The fish liver is microanatomically similar to livers of other vertebrates. It is a reticulotubular gland, often with lobular organization, covered with a serous membrane. Its hepatocytes are polygonal cells with round nuclei and a single, prominent nucleolus, often containing lipid and glycogen (Stoskopf 1993). Hepatocytes are in contact with blood perfusing the hepatic parenchyma at their sinusoidal membranes, at which blood-borne chemicals can be taken up (Luckenbach et al. 2014). The liver is a key metabolic organ for the storage and mobilization of energy, with several anabolic and catabolic functions (Bernet et al. 2000). It converts glucose into glycogen and lipids, which provide energy during fasting, but it also produces and secretes glucose through glycogenolysis and gluconeogenesis. The liver converts fatty acids for extrahepatic tissues during fasting. Multiple nutrient, hormonal, and neuronal signals regulate glucose, lipid, and amino acid metabolism in the liver (Rui 2014). Since it has a key role in digestion, storage, and protein production, it is a target tissue for lesions related to wastewater-related

contaminants. Upon exposure to contaminants, a metabolic trade-off occurs as hepatic nutrient metabolism is altered (Weinrauch et al. 2021). Energy allocation is disturbed since large proportion of the ingested energy shifts from maintenance to detoxification and repair mechanisms. These processes result in rapid decrease of energy storage such as ATP, phosphocreatine and glycogen, and rapid accumulation of blood (Pi et al. 2016).

Upon exposure, increased levels of oxidative damage occur, which stimulate the production of reactive oxygen species (ROS) and other pro-oxidants (Hu et al. 2021). The ROS generated by liver metabolism are eliminated by activation of a variety of antioxidant defense mechanisms, both direct and indirect. They include redox cycling, redox reactions, autoxidation, oxidative and non-oxidative enzyme induction, disruption of electron transport, depletion of antioxidant defenses (Maria et al. 2009; Livingstone 2001). In addition, when the antioxidant defense system cannot efficiently counteract the ROS, it triggers oxidative stress leading to lipid peroxidation and DNA damage, micronuclei, chromosome aberrations, and sister chromatid exchanges in hepatocytes and other cells (Ardeshir et al. 2017; Livingstone 2001). In short, oxygen toxicity involves the production of superoxide anion radical, hydrogen peroxide, and the hydroxyl radical, leading to enzyme inactivation, protein oxidation, lipid peroxidation (formation of malonaldehyde-like species and 4-hydroxyalkenals), DNA damage, and even cell death (Jia et al. 2021; Winston and Di Giulio 1991). Therefore, under pollution stress, fish liver antioxidant enzymes and non-enzymatic antioxidants may be altered. Their increased activity may be considered as an adaptation in which the organism partially or completely overcame the exposure stress. In addition, the inactivation of the defense system by the chemically reactive species, which act as antioxidant enzyme inhibitors reduce cell protection and the organism's fitness (Maria et al. 2009). Conversely, in an undisturbed environment, a balance exists between pro-oxidant and antioxidant processes, as the production of ROS and other reactive species is controlled by the antioxidant defense system.

Since the liver is involved in the metabolism of xenobiotic compounds and their excretion (Bernet et al. 2000), a number of hepatic biochemical, physiological, and metabolic biomarkers were developed. They include biomarkers of exposure (biochemical response occurring following exposure to a contaminant), biomarkers of effect (measurable biochemical, physiological or other alterations within hepatic tissue or body fluids that can be associated with health impairment), biomarkers of susceptibility (ability to respond to exposure to a contaminant, including genetic factors and changes in receptors which alter the susceptibility of fish to the exposure), and bioaccumulation markers (analytical/chemical indicators, also referred to as body burden) (Kroon et al. 2017; Hook et al. 2014; van der Oost et al. 2003) (Fig. 1).

It is a challenge to choose a suitable biomarker between a plethora of biomarkers for assessing fish liver impairment. To that end, selection criteria must be applied to refine the extensive biomarker list. Namely, biomarkers of exposure typically comprise biotransformation enzymes of phase I, phase II, and co-factors (Weinrauch et al. 2021; Ogueji et al. 2020; Kroon et al. 2017; Gagnon and Rawson 2017). Oxidative stress parameters include a group of antioxidant enzymes forming a part of a cellular defence system such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione (Faheem and Lone 2018). Biochemical

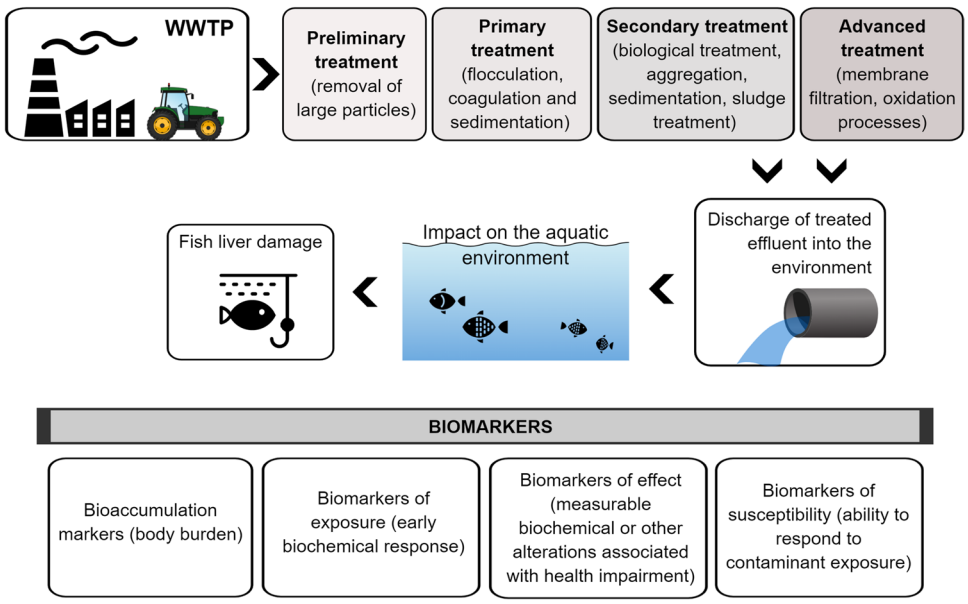

Fig. 1 Schematic representation of wastewater treatment plant (WWTP) treatment processes and biomarkers for assessing the links between contaminant exposure, internal levels of tissue contamination, and early adverse effects in fish

biomarkers of oxidative damage are also frequently used to assess the consequences of oxidative stress, such as lipid peroxidation quantifiable by the measurement of degradation products such as aldehydes, acetone, and malondialdehyde. Additionally, relevant biomarkers related to fish liver function are stress proteins or heat shock proteins which protect and regenerate cells in response to stress. Furthermore, multixenobiotic resistance prevents the accumulation of xenobiotic compounds inside the cell, by removing them via an energy-dependent transport protein (Wang et al. 2022; Kroon et al. 2017; Smital and Ahel 2014; Žaja et al. 2007). Hematological parameters, like serum transaminases, provide general insights of the hepatic tissue health (Wang et al. 2022; Fathy et al. 2019; Cai et al. 2018; Kroon et al. 2017). Endocrine parameters, notably vitellogenin, genotoxic parameters such as DNA adducts, secondary DNA modifications, and irreversible genotoxic events, apoptosis, and micronuclei are also valuable biomarkers (Haque et al. 2022; Laurent et al. 2022; Simó-Mirabet et al. 2018; Casatta et al. 2017). Liver structural alterations, as histopathological biomarkers of effect, are important non-specific indicators of fish health as they bear the effects of exposure to various pollutants (Hinton et al. 1992). Acute alterations are apparent at high contaminant levels, while chronic exposure might induce sublethal aspects of tissue alterations. Many of such alterations are irreversible and can be useful in the determination of prior exposure to wastewater contaminants (Hinton 1994). A list of major biochemical, physiological, and metabolic biomarkers in fish liver reflecting exposure to, or effects of pollutants, is presented in Table 1.

Macroscopic morphological liver changes

Morphological indices are often indicative of pollutant exposure. Liver somatic index (LSI), a ratio between the liver mass and the total fish mass, is one of such non-specific indices (Sloof et al. 1983). The exposure to pollutants, such as bleached sulfite mill effluent, may affect the increase of fish LSI by two to three times in relation to the LSI of control fish. Life cycle and long-term fish exposure result in increased body size and increased liver size (Parrott et al. 2007). Fish exposed to WWTP effluent discharges, heavy metals, pulp mill discharges, urbanized areas, had enlarged livers, and increased LSI (Long et al. 2020; Wang et al. 2019; Bahamonde et al. 2015; Batchelar et al. 2013; Kosmala et al. 1998; Munkittrick et al. 1994). Du et al. (2019) demonstrated that the exposure of fish to a municipal WWTP induced liver enlargement for up to 35% in both native and caged fish. The physiological compensations in the liver offset some of the detrimental impacts in fish after exposure, particularly the increase of the cumulative mitochondrial oxidative capacity of the liver.

Larger liver mass can also be affected by reproductive status since the liver plays a central role in the process of vitellogenesis (Long et al. 2020). However, since vitellogenesis does not usually occur in males, LSI in male fish can be more informative of contaminant exposure (Duarte et al. 2017). The enlarged livers of fish from contaminated waters can stem from hypertrophy (increase in cell size) (Sloof et al. 1983), and hyperplasia (increase in cell number) (van der Oost et al. 2003). They are usually associated with the induction or stimulation of hepatic enzyme activities (Kosmala et al. 1998). Larger liver mass relative to body mass, caused by the increased metabolic activity to detoxify contaminants, may thus be indicative of exposure to contamination, resulting in liver damage that ranges from increased liver weight and fat content to cell necrosis (Samanta et al. 2018). Fatty liver, or storage of large quantities of fats in the fish liver, is a morphological tissue change visible macroscopically as mottling or whitening of its surface. Lipid accumulation results from the imbalance between the synthesis of fatty acids or lipogenesis and fat catabolism or lipolysis in the liver tissue (Wang et al. 2019). Another macroscopic pathology of the liver is the formation of neoplasms. Neoplastic growths in the liver are a common finding in chronically exposed fish. Tumors might appear as small nodules up to lumps of significant size, having a different coloration than the rest of the liver tissue (Peters et al. 1987). Generally, larger/older fish are at higher risk to be affected by liver tumors (Lang et al. 2017).

Physiological and histological changes of livers from exposed fish

A healthy fish liver has a very homogeneous structure in contrast to its heterogeneous physiology. It consists mostly of parenchymal tissue formed from double layers of liver cells, separated by liver sinusoids or capillary-like blood spaces. Liver stores carbohydrates as glycogen and, especially before spawning, fats. The polygonal hepatocytes under normal conditions appear compact. Near the sinusoids, uniformly distributed groups of pigmented phagocytic cells or melanomacrophages can be found (Peters et al. 1987).

Aberrations from the normal structural tissue integrity can provide valuable information on the integrated effects of molecular, biochemical, and physiological changes deriving from exposure to pollutants. Liver physiological and histological parameters represent higher-level responses of exposed fish, generally indicative of irreversible damage (Lang et al. 2017; Oliveira et al. 2015). Histological/histopathological parameters of the fish liver are very useful for assessing the chronic and sublethal effects of persistent contaminants present at very low levels in waters affected by the effluents. Such responses can be used as biomarkers of

Table 1 Hepatic biochemical, physiological and metabolic biomarkers in fish reflecting exposure to, or effects of, environmental pollutants

Description		Main inducers	Location	References
Principal oxidative enzyme biomarkers				
Cytochrome P4501A (CYP1A)	Major hydrocarbon-inducible CYP; Catalytic assays using benzo(a) pyrene or 7-ethoxyresorufin in aryl hydrocarbon (Ah) hydroxylase (AHH) or 7-ethoxyresorufin O-deethylase (EROD) assays	Procarcinogens, therapeutic agents, flowback and produced water, wastewater by-products from hydraulic fracturing processes, tetrachlorinated dibenzo-*p*-dioxins (TCDD), halogenated xenobiotics, polyhalogenated aromatic hydrocarbons polycyclic (PHAH), aromatic hydrocarbons (PAH), organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), dichloro-diphenyl-trichloroethane (DDT), Bleached kraft pulp and paper mill effluent (BKME)	Hepatocytes and endothelial cells	Weinrauch et al. (2021); Ogueji et al. (2020); Gagnon and Rawson (2017); Topić Popović et al. (2015a); Uno et al. (2012); Finn (2007); Topić Popović et al. (2007); van der Oost et al (2003); Quattrochi and Guzelian (2001); Sarasquete and Segner (2000); Buhler and Wang-Buhler (1998); Nilsen et al. (1998); van der Oost et al (1998, 1997); Förlin et al (1996); Otto and Moon (1996); Palace et al (1996); Buhler (1995); Stegeman (1995); Myers et al. (1995); Husøy et al. (1994); Lester et al. (1993)
CYP1A1	Specific temporal pattern of AHH and EROD induction	PAHs, halogenated aromatic compounds, beta-naphthoflavone (BNF), antibiotics	Hepatocytes, no zonation in fish liver; biliary epithelial cells	
CYP1A2	Specific temporal pattern of AHH and EROD induction	Isosafrole	Hepatocytes	
CYP3A4	Mediated by the Ah receptor/aryl hydrocarbon receptor nuclear translocator (ARNT) pathway and pregnane X receptor (PXR)	Steroidal chemicals, variety of compounds, including glucocorticoids and macrolide antibiotics	Hepatocytes	
Superoxide dismutase (SOD)	Antioxidant enzyme responding to pollution; metalloenzyme that catalyses the conversion of reactive superoxide anions to yield hydrogen peroxide (H_2O_2) which is subsequently detoxified by CAT and GPx	Organic trace pollutants; paraquat, polychlorinated dibenzofurans (PCDF), hexachlorobenzene (HCB), PAH, PHAH, PCB, OCP, ivermectin, nanoparticles	Hepatocytes	Banaei et al. (2022); Weinrauch et al. (2021); Yu et al. (2021); Ogueji et al. (2020); Gagnon and Rawson (2017); Topić Popović et al. 2015b); Uno et al. (2012); Finn (2007); Topić Popović et al. (2007); van der Oost et al. (2003); Quattrochi and Guzelian (2001); Sarasquete and Segner (2000); Buhler and Wang-Buhler (1998); Nilsen et al. (1998); van der Oost et al. (1998, 1997); Förlin et al. (1996); Otto and Moon (1996); Palace et al. (1996); Buhler (1995); Stegeman (1995); Myers et al. (1995); Husøy et al. (1994); Lester et al. (1993)
Catalase (CAT)	Antioxidant enzyme responding to pollution; hematin-containing enzymes that facilitate the removal H_2O_2	BKME, PAH, PCB, OCP, ivermectin, nanoparticles	Hepatocytes	

Table 1 (continued)

Description		Main inducers	Location	References
Glutathione peroxidase (GPx)	Antioxidant enzyme responding to pollution; catalyses the metabolism of H_2O_2 to water, involving oxidation of reduced GSH to its oxidized form	Paraquat, heavy metals, OCP, HCB, PCB, PAH, ivermectin	Hepatocytes	
Malondialdehyde (MDA)	MDA production is a main oxidation product of peroxidized polyunsaturated fatty acids. The increased LPOX is detected by elevated MDA levels	Microplastics, heavy metals, xenobiotics in general	Hepatocytes	
Principal hematologic and other enzymatic and non-enzymatic biomarkers related to liver function				
Alanine aminotransferase (ALT)	Catalyzes the interconversion of amino acids and α-ketoacids by transfer of amino groups; from alanine to α-ketoglutarate to form glutamate and pyruvate	Wastewater, PAH, ivermectin	Hepatocytes; plasma	Mauerwerk et al. (2021); Tabrez et al. (2021); Weinrauch et al. (2021); Da Costa Araújo et al. (2020); Gonçalves et al. (2020a, b); Ogueji et al. (2020); Tenji et al. (2020); Vali et al. (2020): Mohamed et al. (2019); Ardeshir et al. (2017); Javed and Usmani (2015); Oliveira et al. (2015); Schreiber et al. (2006); van der Oost et al. (2003); Allen (1993); Ghazaly (1992); Stegeman et al. (1992)
Aspartate aminotransferase (AST)	Catalyzes the interconversion of amino acids and α-ketoacids by transfer of amino groups; from aspartate to α-ketoglutarate to form glutamate and oxaloacetate	Wastewater, pyrethroid (deltamethrin), PCB, OCB, PAH, ivermectin, nanoparticles	Hepatocytes; plasma	
Alkaline phosphatase (ALP)	Catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium	Heavy metals; wide substrate specificity, ivermectin, nanoparticles	Hepatocytes (cell membranes); biliary tract; plasma	

Table 1 (continued)

Description		Main inducers	Location	References
Uridine 5'-diphospho-glucuronyl transferase (UDP-GT)	The synthesis of glucuronides by microsomal UDP glucuronyl transferases (UDP-GTs) is a major pathway for the inactivation and excretion of endogenous and xenobiotic organic compounds	PAH, PCB, OCP, PCDD, BKME, ivermectin, nanoparticles	Hepatocytes	Mauerwerk et al. (2021); Tabrez et al. (2021); Weinrauch et al. (2021); Da Costa Araújo et al. (2020); Gonçalves et al. (2020b); Ogueji et al. (2020); Tenji et al. (2020); Vali et al. (2020): Mohamed et al. (2019); Ardeshir et al. (2017); Javed and Usmani (2015); Oliveira et al. (2015); Schreiber et al. (2006); van der Oost et al. (2003); Topić Popović et al. (2001); Allen (1993); Ghazaly (1992); Stegeman et al. (1992)
Glutathione (GSH)	Acting as a key conjugate of electrophilic intermediates, principally via GST activities in phase II metabolism, and as an antioxidant	Heavy metals-not specific, BKME, PAH, PCB, HCB, ivermectin	Hepatocytes; plasma	
Delta-aminolevulinic acid dehydratase (ALAD)	Involved in the production of the heme group, an essential component of hemoproteins (cytochromes, CAT and other peroxidases). Its substrate can undergo auto-oxidation and produce ROS	Heavy metals, benzo[a]pyrene (BaP), DDT, tributyltin (TBT), PAH	Hepatocytes	
Glucose (GLU)	Stress increases the secretion of cortisol from adrenocortical cells, leading to increased GLU production from liver glycogen (glycogenolysis) or protein (gluconeogenesis) reservoirs	Heavy metals, nanoparticles, flowback and produced water, wastewater by-products from hydraulic fracturing processes, glycerol, PAH, microplastics, bacterial challenges	Hepatocytes; plasma	
Total proteins (TP)	The activities of AST, ALT, and ALP are directly proportional to the level of TP and inversely proportional to cholesterol level	Heavy metals, ivermectin, nanoparticles, glycerol, pesticides, bacterial challenges	Hepatocytes; plasma	
Stress proteins, multixenobiotic resistance, metallothioneins related to liver function				
Heat shock proteins (HSP70)	Exposure to contamination induces upregulation of HSP70 activity in liver, which are co-responsible for the assembly, functionality, and transport of the glucocorticoid receptor	Benzo[a]pyrene, PCBs, nonylphenol, ammonia, heavy metals; anthropogenic stressors	Hepatocytes	Ferroni et al. (2022); Vieira et al. (2022); Younas et al. (2022); Yu et al. (2021); Zhang et al. (2021); Santos and Bueno dos Reis Martinez (2021); Luu et al. (2021); Kroon et al. (2017); Luckenbach et al. (2014); Benedetti et al. (2012); Ribecco et al. (2011)

Table 1 (continued)

Description		Main inducers	Location	References
Heat shock proteins (HSP90)	Both HSP70 and HSP90 are expressed excessively under stress condition and participate in signal transduction process which protects organism from damage	Heavy metals, ammonia, decreased water oxygen, increased water temperature, anthropogenic stressors	Hepatocytes	
Multixenobiotic resistance (MXR)	MXR prevents the accumulation of xenobiotic compounds inside the cell, removing them via an energy-dependent transport protein (PGP)	Xenobiotics in general, PAHs, Benzo[a]pyrene, clotrimazole	Hepatocytes, erythrocytes, gallbladder	
Metallothioneins (MT)	MT proteins are essential for the regulation, sequestration and detoxification of metals in liver	Heavy metals such as Ag, Bi, Cd, Co, Cu, Hg, Ni, Zn	Hepatocytes	
Genotoxic parameters as biomarkers of environmental pollution				
DNA adducts	DNA damage caused by the binding of a chemical to a segment of DNA	Heavy metals, pollutants	Hepatocytes	Falfushynska et al. (2022); Germande et al. (2022); Canedo and Rocha (2021); Yu et al. (2021); Kroon et al. (2017); Benedetti et al. (2012); Tomasello et al. (2012); Hartl et al. (2007); Vethaak et al. (1996)
Secondary DNA modifications	Changes in DNA strand breaks; DNA unwinding	Heavy metals, organic pollutants	Hepatocytes	
Irreversible genotoxic events	Micronucleus and nuclear abnormalities	Heavy metals, PCBs, PAHs, organochlorine pesticides	Erythrocytes	
Apoptosis	Programmed cell death, irreversible process leading to DNA fragmentation	Heavy metals	Hepatocytes	
Reproductive and endocrine biomarkers related to liver function				
Hepatic VTG	Pollution leads to increase of hepatic VTG transcripts and elevated levels of vitellogenin synthesis, particularly in males	Heavy metals, PAHs, PCBs, rganochlorine pesticides, endocrine disrupting compounds	Hepatocytes	Montenegro et al. (2022); Annabi et al. (2012); Dale et al. (2019); Gilannejad et al. (2016)

exposure and effects to contaminants related to the health of individuals which also allow further extrapolation to population/community effects (Chiang and Au 2013). Hepatic lesions of fish exposed to contaminants, sorted by histopathological categories, are listed in Table 2.

The most common histopathological findings in livers of fish exposed to pollutants are macrophage aggregates, also known as melanomacrophage centers, and liver necrosis (Oliveira et al. 2015). Necrosis is the direct toxic effect of a pollutant and could be due to the infiltration of leucocytes (Javed and Usmani 2013). In liver disease associated with hepatic necrosis, serum AST and ALT are elevated even before the clinical signs and symptoms of disease appear (van der Oost et al. 2003). Melanomacrophages can serve as non-specific indicators of environmental stress as they are related to exposure to degraded environments. They usually contain a number of pigments including melanin, with the primary function of iron capture and storage. Melanin is a complex polymer that can absorb and neutralize free radicals, cations, and other potentially toxic agents, deriving from degradation of phagocytosed cellular material (Zuasti et al. 1989). Other pigments usually found in melanomacrophages are lipofuscin (generally the most abundant), and hemosiderin (Agius and Roberts 2003). The increase in melanomacrophage numbers indicates humoral and inflammatory responses, and also detoxification of exogenous and endogenous substances (Herraez and Zapata 1991; Peters et al. 1987). The liver infiltration of defence cells is caused by hyperemia, leading to increased blood flow in the liver, and facilitating the transport of macrophages to the damaged regions, thus improving oxygenation and possibly indicating an auxiliary mechanism in detoxification (Vieira et al. 2019).

Energetic costs linked to exposure to contamination mainly refer to glycogen depletion, which is a stress-induced response. The decrease of glycogen content under effluent stress is greater in liver than in muscle, as liver is the principal site of its synthesis and storage (Cazenave et al. 2014). Lower liver glycogen can be associated with the inability to increase plasma glucose following an acute stress exposure (Javed and Usmani 2013, 2015). Such depletion of energy reserves may have consequences in form of diminished growth, survival and reproduction probability (Du et al. 2019; Sancho et al. 2010).

Biotransformation enzymes and xenobiotic compounds

Most xenobiotic compounds undergo biotransformation before being excreted. The most commonly involved organ in (fish) biotransformation is the liver. In the first phase of biotransformation, the lipophilic substrate is oxidized by the cytochrome P-450 (CYP450) enzyme system, which introduces a single oxygen atom into the molecule (Moutou et al. 1998). In the second phase, the oxidized compound is conjugated with an endogenous molecule such as glucuronic acid, sulfate, glutathione, or amino acid, becoming less toxic and readily excreted (Topić Popović et al. 2012; Andersson and Förlin 1992).

The P-450 (CYP) oxidase enzymes metabolize many xenobiotics, and CYP1A1 and CYP3A4 are important markers of chemical exposure in fish (Table 1). Disruptions of the activities of these enzymes, especially CYP3A4, may have consequences in the metabolism of other compounds. The CYP3A4 is one of the most important enzymes involved in the metabolism of xenobiotics and oxidation of the largest range of substrates (Topić Popović et al. 2007). The CYP1A is best known as a major hydrocarbon-inducible CYP. Members of the CYP1 family play a prominent role in the activation of many drugs and procarcinogens (Topić Popović et al. 2015a). Many studies demonstrated an increase in the CYP1A levels in liver tissues of fish exposed to organic trace pollutants, and particularly to PAHs, PCBs, PCDDs, and PCDFs (Table 1). Single xenobiotic compounds can act as inducers of specific isoenzymes, but also inhibit others, which may result in variation of isoenzyme levels (Oost et al. 2003). However, measuring these proteins or their mRNA levels can be time-consuming and costly for field studies, so the determination of their catalytic activity is more common. Thus, measurement of the CYP1A-catalyzed deethylation of ethoxyresorufin to a fluorescent product resorufin via the activity of EROD, or using benzo(a)pyrene as a substrate in AHH assays, or measuring CYP3A-catalyzed activity of BFCOD (7-benzyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase), allow for their more feasible quantification (Gagnon and Rawson 2017). For example, the increase of EROD, AHH, and BFCOD activity in livers of native fish sampled downstream from the WWTP indicate exposure to dioxin-like compounds, organic trace pollutants, and drugs of broader nature related to WWTPs (Burkina et al. 2021; Catteau et al. 2021; Weinrauch et al. 2021; Soler et al. 2020; Kosmala et al. 1998).

In the second phase of biotransformation, the conjugation with glutathione (realized by the glutathione-*S*-transferase) is the major pathway for electrophilic compounds and metabolites, while the conjugation with glucuronic acid is the major route for nucleophilic compounds (George 1994). The mechanism of induction for most of the phase II enzymes is also regulated via the Ah-receptor, but their induction responses are generally less prominent (van der Oost et al. 2003). In healthy liver tissues, more than 90% of the total glutathione pool is in the reduced form and less than 10% is in the oxidized form. An increased oxidized-to-reduced ratio is indicative of oxidative stress, resulting from the conversion of glutathione from reduced to oxidized form as radicals with oxidative potential are neutralized. These rations were significantly elevated in

Table 2 Histopathological hepatic lesions of fish exposed to contaminants. Adapted from Chiang and Au (2013)

Category of a lesion	Lesion	Description	Fish species	References
Non-specific lesions	Macrophage aggregates	Clusters of plump, pigment-bearing macrophages surrounded by a thin capsule. Pigmentation may vary from yellow to dark brown and black. In vicinity of blood vessels	Eelpout (*Zoarces viviparous*)—native, spotted snakehead (*Channa punctata*) -laboratory; Silver catfish (*Rhamdia quelen*)—caged; spottail shiner (*Notropis hudsonius*) -native; carp (*Cyprinus carpio*), sharptooth catfish (*Clarias gariepinus*)—native; dab (*Limanda limanda*)—native; flounder (*Platichthys flesus*), ruffe (*Gymnocephalus cernua*), smelt (*Osmerus eperlanus*)—native	Wagenaar and Barnhoorn (2018); Lang et al. (2017); Marcogliese et al. (2015); Oliveira et al. (2015); Kaur and Dua (2014); Fricke et al. (2012); Agius and Roberts (2003); Peters et al. (1987)
	Hemosiderosis	Hepatocellular accumulation of brown pigment hemosiderin, containing ferric ion	Mozambique tilapia (*Oreochromis mossambicus*)—laboratory, eelpout—native, flatfish—native, buffalo fish (*Ictiobus* spp.)—native	Subburaj et al. (2020); Fricke et al. (2012); Khan (2003); Thiyagarajah et al. (1998)
	Lymphocytic or monocytic infiltration	Diffusion or accumulation of lymphocytes or monocytes in hepatic tissue	Eelpout—native, carp -native, sharptooth catfish—native, dab—native, Prussian carp—native	Wagenaar and Barnhoorn (2018); Lang et al. (2017); Topić Popović et al. (2016); Fricke et al. (2012)
	Glycogen content variability	Glycogen appears as hepatocellular vacuolization. It can accumulate or deplete depending on contaminant. Decrease in WWTP effluent exposure	Eelpout—native, spotted snakehead—laboratory, bluegill sunfish (*Lepomis macrochirus*)—caged, *Mastacembelus armatus*—native, Nile tilapia—laboratory	Abdel-Latif et al. (2021); Du et al (2019); Kaur and Dua (2014); Javed and Usmani (2013); Fricke et al. (2012)
	Fibrosis	Excess formation and deposition of collagen or fibrous tissue within the parenchyma. Fibrous tissue spreads to fill the gaps left by the retrogressing cells	Cod (*Gadus morhua*)—native, flatfish—native; eelpout—native, brown trout (*Salmo trutta*)—caged, silver catfish—caged, sharptooth catfish—native, flounder, ruffe, and smelt—native (2001); Peters et al. (1987)	Wagenaar and Barnhoorn (2018); Borucinska et al. (2017); Oliveira et al. (2015); Fricke et al. (2012); Khan (2003); Schmidt-Posthaus et al.
Non-specific lesions	Lipidosis/steatosis	Vacuoles of lipid deposition in the hepatocytes, displacing and compressing the nucleus	Flatfish—native, eelpout—native, carp -laboratory, spotted snakehead—laboratory, Nile tilapia (*Oreochromis niloticus*) and mullet (*Mugil cephalus*) —farmed and native; silver catfish—caged, carp—native, sharptooth catfish—native	Wagenaar and Barnhoorn (2018); Oliveira et al. (2015); Kaur and Dua (2014); Omar et al. (2013); Fricke et al. (2012); Mustafa et al. (2012); Abdel-Moneim and Abdel-Mohsen (2010); Khan (2003)
	Granuloma	Inflammatory lesions with dense macrophages in the center and deep basophilic nuclei in the periphery	Striped catfish (*Pangasianodon hypophthalmus*)—native,eelpout—native, sharptooth catfish—native, dab—native	Puttipong et al. (2020); Wagenaar and Barnhoorn (2018); Lang et al. (2017); Fricke et al. (2012)

Table 2 (continued)

Category of a lesion	Lesion	Description	Fish species	References
	Necrosis	Coagulation of protein with pyknotic nuclei, with preservation of the cell outline; eosinophilic hepatocytes. Cells lacking almost all inner organization	Cod—native, Flatfish—native, eelpout—native, *Mastacembelus armatus*—native; Brown trout—caged, carp—laboratory; spotted snakehead—laboratory, Nile tilapia and mullet—farmed and native, silver catfish—caged, carp and sharptooth catfish—native, dab—native, flounder, ruffe, and smelt—native, Prussian carp—native	Wagenaar and Barnhoorn (2018); Borucinska et al. (2017); Lang et al (2017); Topić Popović et al. (2016); Oliveira et al. (2015); Kaur and Dua (2014); Javed and Usmani (2013); Omar et al. (2013); Fricke et al. (2012); Mustafa et al. (2012); Khan (2003); Schmidt-Posthaus et al. (2001); Johnsen et al. (1998); Peters et al. (1987)
	Apoptosis	Condensed chromatin of hepatocyte nuclei. Cytoplasm fragmented into membrane-bound particles, phagocytic cells	Nile tilapia—laboratory, eelpout—native, Prussian carp (*Carassius gibelio*) —native, carp—laboratory	Abdel-Latif et al. (2021); Gu et al. (2021); Topić Popović et al. (2016); Fricke et al. (2012)
Early toxicopathic lesions	Peliosis	Presence of intact erythrocytes and eosinophilic serous material within cystic structures	Cod—native, Nile tilapia and mullet—farmed and native, flatfish (*Euryglossa orientalis*)—native, spotted snakehead—laboratory	Borucinska et al. (2017); Chupani et al. (2013); Omar et al. (2013); Roy and Bhattacharya (2006)
	Phospholipidosis	Enlarged hepatocytes with foamy cytoplasm and numerous intracytoplasmic eosinophilic granules	Brown trout—caged, sharptooth catfish—native	Abdel-Moneim and Abdel-Mohsen (2010); Johnsen et al. (1998)
	Hepatocellular pleomorphism	Hepatocytomegaly or hypertrophied hepatocytes with eosinophilic cytoplasm, hypertrophied nuclei, and vesicular chromatin	Prussian carp—native, carp—native, sharptooth catfish—native, chub (*Leuciscus cephalus*)—native, dab—native, flounder, ruffe, and smelt —native	Wagenaar and Barnhoorn (2018); Lang et al. (2017); Topić Popović et al. (2016); Kosmala et al (1998); Peters et al. (1987)
	Vacuolation of hepatocytes	Large vacuoles (macrovesicular steatosis) or small vesicles (microvesicular steatosis)	Nile tilapia—laboratory, flatfish—native, eelpout—native, carp—caged and laboratory, trout (*Oncorhynchus mykiss*)—caged, dab—native, *Mastacembelus armatus*—native	Abdel-Latif et al. (2021); Weinrauch et al. (2021); Lang et al. (2017); Blahova et al. (2014); Minarik et al. (2014); Javed and Usmani (2013); Khan (2003)
	Fibrillar inclusion	Enlarged microtubules appearing in the form of fibers in the hepatocytes	Cod—native, sharptooth catfish—native, flounder—native	Bignell et al. (2020); Wagenaar and Barnhoorn (2018); Borucinska et al. (2017); Carrola et al. (2014)
	Spongiosis of parenchyma	Clusters of cyst-like structures filled with granular or flocculent material in liver parenchyma	Cod—native, medaka (*Oryzias latipes*)—laboratory	Borucinska et al. (2017); Bunton (2010)

Table 2 (continued)

Category of a lesion	Lesion	Description	Fish species	References
Preneoplastic lesions	Basophilic focus	Clusters of enlarged hepatocytes with hyperbasophilic cytoplasm	Brown trout—caged, silver catfish—caged, carp—native, sharptooth catfish—native, dab—native, redbreast sunfish (*Lepomis auritus*)—native, English sole (*Pleuronectes vetulus*)	Wagenaar and Barnhoorn (2018); Lang et al. (2017); Oliveira et al. (2015); Schmidt-Posthaus et al. (2001); Johnsen et al. (1998); Teh et al. (1997); Myers et al. (1995)
	Eosinophilic focus	Clusters of hypertrophied eosinophilic hepatocytes with granular cytoplasm, and nuclei with marginated chromatin	Brown trout—caged, carp—native, sharptooth catfish—native, dab—native, redbreast sunfish—native, English sole—native	Wagenaar and Barnhoorn (2018); Lang et al. (2017); Schmidt-Posthaus et al. (2001); Teh et al. (1997); Myers et al. (1995)
	Clear cell focus	Clusters of monomorphic hepatocytes with clear (due to glycogen) to granular cytoplasm, and central nuclei	flatfish—native, carp—native, sharptooth catfish—native	Wagenaar and Barnhoorn (2018); Abdel-Moneim and Abdel-Mohsen (2010); Khan (2003)
	Vacuolated focus	Clusters of hepatocytes cytoplasmic vacuoles, which compress nuclei	Eelpout—native, carp—native, sharptooth catfish—native, dab—native, *Mastacembelus armatus*—native, Nile tilapia—laboratory	Abdel-Latif et al. (2021); Wagenaar and Barnhoorn (2018); Lang et al. (2017); Javed and Usmani (2013); Fricke et al. (2012)
	Mixed cell focus	Hepatocytes of two or more of the above foci	Cod—native, carp—native, sharptooth catfish—native, dab—native	Wagenaar and Barnhoorn (2018); Borucinska et al. (2017); Lang et al (2017)
Benign neoplasms	Hemangioma	Proliferative, non-invasive vascular endothelial cells surrounding blood-filled cavity, with necrosis in hepatocellular parenchyma	Mozambique tilapia—laboratory, sharptooth catfish—native, silver catfish—caged, zebrafish—laboratory	Subburaj et al. (2020); Spitsbergen et al. (2000); Wagenaar and Barnhoorn (2018); Oliveira et al. (2015)
	Hepatocellular adenoma	Atypical cellular organization with dilated hepatic sinusoids. Hypertrophied, polygonal constituent cells	Dab—native, Mozambique tilapia—laboratory, white sucker (*Catostomus commersonii*) —native, flounder—native, medaka—laboratory, English sole—native	Subburaj et al. (2020); Lerebours et al. (2017); Blazer et al. (2016); Lang et al. (2017); Köhler, and Ellesat (2008); Ward et al. (2006); Okihiro and Hinton (1999); Myers et al. (1995)
	Cholangioma	Bile duct tumor, dilated bile ducts encapsulated by fibrous tissue	Lake whitefish (*Coregonus clupeaformis*)—native, redbreast sunfish—native, Mozambique tilapia—native, white sucker—native	Blazer et al. (2016); van Dyk (2013); Mikaelian et al. (2002); Teh et al. (1997)
	Pancreatic acinar cell adenoma	Acinar cells with extensive cellular pleomorphism, nuclear atypia	Flatfish—native, guppy (*Poecilia reticulata*)—laboratory, mummichog (*Fundulus heteroclitus*) —native	Chupani et al. (2013); Fournie and Hawkins (2002); Fournie and Vogelbein (1994)

Table 2 (continued)

Category of a lesion	Lesion	Description	Fish species	References
Malignant neoplasms, with central necrotic regions	Hepatocellular carcinoma	Hepatic nodule with disorganized cell organization composed of enlarged, pleomorphic anaplastic hepatocytes	Dab—native, lake whitefish—native, white sucker—native, flounder—native, medaka—laboratory	Blazer et al. (2016); Köhler, and Ellesat (2008); Ward et al. (2006); Mikaelian et al. (2002); Okihiro and Hinton (1999)
	Cholangiocarcinoma	Densely packed, atypical bile duct cells, invading into the surrounding parenchyma. Pleomorphic biliary epithelium	Lake whitefish—native, white sucker—native, medaka—laboratory, English sole—native	Blazer et al. (2016); Mikaelian et al. (2002); Okihiro and Hinton (1999); Myers et al. (1995)
	Mixed hepatobiliary carcinoma	Mixed hepatocellular carcinoma and cholangiocarcinoma	Lake whitefish—native, medaka—laboratory, English sole—native	Mikaelian et al (2002); Okihiro and Hinton (1999); Myers et al. (1995)
	Hemangiosarcoma	Vascular tumor-infiltrating sinusoids and isolated hepatocytes. Proliferating endothelial cells forming anaplastic vascular structures with erythrocytes	Zebrafish—laboratory	Spitsbergen et al. (2000)
	Hemangiopericytic sarcoma	Soft tissue sarcoma originating in the pericytes in the capillary walls. Characterized by spindle cell bundles	English sole——native	Myers et al. (1995)
	Pancreatic acinar cell carcinoma	Dense acinar cells with eosinophilic zymogen granules. Aggressively invading hepatic parenchyma	Guppy—laboratory, mummichog—native	Fournie and Hawkins (2002); Fournie and Vogelbein (1994)

livers of fish exposed to WWTP outfalls, showing evidence of oxidative stress (Jasinska et al. 2015). Also, in fish exposed to environmental concentrations of diclofenac, the levels of reduced glutathione and glutathione *S*-transferase activity increased at all tested concentrations (Guiloski et al. 2017).

Predictors of adverse responses

In the past decades, high-throughput screening assays and omics-based methods have been developed for studies of the mechanisms of toxicity. Assays were developed for short-term high-content and high-throughput prediction of toxicity of numerous chemicals potentially found in the WWTP outfalls. Responses at the molecular level, transcriptome sequencing, proteomics, and metabolomics, may provide reliable biomarkers of exposure and stress induced by pollutants, as well as their underlying mechanisms (Bai and Tang 2020). In particular, biochemical changes revealed by metabolomics are easily correlated to other measurements and can set a path for unveiling the mechanisms of toxicity of various compounds (Huang et al. 2019). Exposure of fish to pollutants leads to changes in gene expression and protein production, which are subjected to a number of homeostatic and feedback mechanisms. These changes are amplified at the metabolome level, which makes metabolomics analyses a significant tool for assessing toxicity of environmental contaminants (Lankadurai et al. 2013). Such data aid to identify multiple impaired pathways contributing to the overall adverse responses. Fish from the two effluent-receiving sites in the work of Meador et al. (2020) exhibited alteration in 31 different pathways, related to metabolism and biosynthesis of amino acids, lipids, fatty acids, purines, pyrimidines, and sugars. Many of these are important for energy production, antioxidant defenses and ammonia cycling (Meador et al. 2020).

Since molecular markers precede morphological alterations, they are useful for a predictive aspect of toxicity testing. Many of the genes measured in such studies are functional orthologs of human ones involved in processes of toxic responses and thus have an additional applied value (Gonçalves et al. 2020a). Namely, a higher expression of *gpx*, *hsp70*, *ucp2*, and *bax* genes, as well as a lower expression of *Bcl-2*, mRNA levels of SOD and CAT genes in fish can indicate to toxic effects of silver oxide and silver carbonate nanoparticles (NP) before other biomarkers, and indicate to a NP hazard (Mahjoubian et al. 2021). The balance between the oxidative and antioxidant processes inside the cells is related to several genes such as *coxIV*, *prdx*, *ucp*, *sod*, *cat*, and *hsp70* (Espinosa et al. 2017). The increase of the fish liver *ucp1* and decrease of *hsp90* expression can thus be correlated to microplastics exposure (Espinosa et al. 2017). Microarray studies established that the exposure to the WWTP effluent can significantly affect gene transcription in the fish liver, having altered expression in relation to the unaffected site. These genes are mostly involved in mechanisms of the fish immune system, lipid and retinol metabolism, detoxification processes, cellular proliferation, and membrane transport (Houde et al. 2014). Incorporation of gene expression tools enables characterization of responses even when the stressors are unknown, but calls for validation with a phenotypic trait on an organ or a tissue level (Hook et al. 2014).

There is a number of molecular effects which need to be extensively studied to investigate species-specific molecular responses, response levels and typical inducers. The detrimental effects are often multifactorial and associated with alterations in different pathways or subnetworks (Gonçalves et al. 2020b). Advances in high-content –omics screening and bioinformatics enable surveying of multiple molecular endpoints in single runs and revealing alterations in metabolic pathways in complex ecosystems affected by pollution (Cuevas et al. 2018).

WWTP-associated legacy contaminants and emerging-concern contaminants with affinity to fish liver

Pharmaceutically active compounds

Pharmaceutically active compounds (PhACs) are biologically active compounds designed to interact with specific physiological pathways of humans and livestock in order to evoke a desirable pharmacological response (Corcoran et al. 2010). In recent decades, PhACs have aroused an increasing concern due to their ubiquitous presence in the aquatic environment and negative ecological effects. Currently, over 4000 PhACs are being used all over the world for medical and veterinary health care, as well as growth promotion of livestock (Boxall et al. 2012). Excretion of PhACs occurs via urine and feces, with 50–90% of oral doses generally being excreted as a mixture of the parent compounds and their metabolite forms (Köpping et al. 2020; Celiz et al. 2009). PhACs enter the aquatic environment via numerous routes, including direct discharge of raw and/or treated wastewater from municipal, hospital, industrial WWTPs, where they are often inefficiently removed. Secondary PhACs sources should not be neglected, such as landfill leachate and surface runoff from urban or agricultural areas where treated wastewater is used for irrigation activities and/or sewage sludge as a fertilizer (Tran et al. 2018).

PhACs are designed to be stable, thus the slow degradation rate is usually exceeded by continuous release rate (OECD 2019). For such reason, PhACs are being frequently reported in freshwaters and groundwaters all over the world in concentrations ranging from ng/L to µg/L (Tran et al.

2018; Bielen et al. 2017; Corcoran et al. 2010), and even mg/L (Larsson 2014). Although the occurrence of PhACs and their concentrations in aquatic ecosystems depend on the socioeconomic composition of the population, they are strongly seasonally dependent and linked with increased consumption during seasonal influenza and allergies, but also farming practices (Vieno et al. 2005). Over the past two decades, ecotoxicity studies have revealed a strong positive correlation between the most commonly used classes of PhACs and their occurrence in the environment (Küster and Adler 2014; Corcoran et al. 2010). Regarding human medicinal products, antibiotics, hormones, analgesics, antidepressants, and antineoplastics are the most frequently used/found in the aquatic environment, while among veterinary products, those are hormones, antibiotics, and parasiticides (Corcoran et al. 2010). Although the majority of published prioritization approaches already indicated high environmental relevance of these compounds, in aquatic environments they occur as a mixture of various compounds whose combined toxicity does not necessarily correspond to one of the individual substances due to synergistic, antagonistic, and/or additive interactions (do Amaral et al. 2019; Corcoran et al. 2010).

Although PhACs uptake into fish may vary, i.e., through the gills, dermal absorption from the surrounding water, and dietary intake of food, tissue-specific accumulation can be observed. PhACs are generally nonpolar and lipophilic, so their accumulation mainly occurs in the organs with a high lipid content, i.e., liver, brain, and muscle (Schnell et al. 2009). The liver is the major target and storage of PhACs from distinct therapeutic groups, hence numerous studies reported higher BCFs of such compounds in the liver than in the remaining organs/tissues (Gomez et al. 2010). Supporting this assumption, many authors reported the accumulation of anti-depressants and their metabolites in the liver of the fish inhabiting effluent-dominated streams/rivers in concentrations ranging from 9.1 ng/g of tissue (for norfluoxetine, the primary active metabolite of the antidepressant fluoxetine), 12.94 ng/g (for desmethylsertraline, an active metabolite of the antidepressant sertraline), 80 ng/g (for fluoxetine), up to 545 ng/g (for sertraline) (Ondarza et al. 2019; Ramirez et al. 2009; Brooks et al. 2005). An extensive study by Huerta et al. (2013) focused on evaluating twenty PhACs from seven commonly used therapeutic families and their metabolites in the liver and muscle of eleven fish species from four heavily impacted Mediterranean rivers. The authors claimed the highest levels in trout liver, with a maximum concentration of 18 ng/g for non-steroidal drug carbamazepine, whereas the most ubiquitous and recurring compound was analgesics/anti-inflammatory drug diclofenac. Antibiotics, one of the most concerning PhACs, are not an exception. Recently, Baesu et al. (2021) detected lincomycin, sulfamethoxazole, and azithromycin in fish livers at an average concentration of 30.3, 25.6, and 27.8 ng/g fw, respectively.

Although several analytical methods for the determination of PhACs in biological matrices have been developed in the last decade, there is still a need for improvement toward the higher sensitivity of used instruments and accuracy of analytical methods, particularly for liver samples that are loaded with lipids and proteins that may interfere with analysis (Tanoue et al. 2014). The importance of such studies does not reflect only in the determination of bioaccumulated PhACs in the fish tissue, but indirectly enables an effective screening of emerging contaminants in aqueous ecosystems and determination of wastewater treatment efficiency, which could ultimately result in establishing thresholds of regulation. Erythromycin, a common over-the-counter antibiotic, was due to its ubiquity in the aquatic organisms (up to 5.6 µg/kg in the fish liver; Ondarza et al. 2019) included in the Drinking Water Contaminant Candidate List (CCL3, US-EPA) and was identified as a "priority monitoring substance" by the European Union (Barbosa et al. 2016).

Receptors and signaling pathways are relatively conserved across vertebrate phyla, so it can be concluded that some PhACs purposely designed to induce an effect on humans or livestock to have a high probability to produce a similar or same physiological response in fish (Burkina et al. 2015; Corcoran et al. 2010). Moreover, hepatocytes among vertebrates are responsible for the majority of identical functions, i.e., metabolism of a plethora of endogenous and exogenous compounds, synthesis of blood protein and clotting factors, secretion of bile, storage of glycogen, aminoacids, fat, and iron (Field et al. 2003). Hence, by paralleling PhACs mode of action among fish and humans, this review article further supports the undoubtful value of the pinpointed topic. Moreover, such an approach can be utilized to better interpret the mode of action of a single PhAC and its mixtures.

Due to the growing regulatory restrictions of conducting in vivo studies on vertebrates, the impact of PhAC is frequently assessed in vitro using fish liver cell lines, such as RTL-W1, PLHC-1. Thibaut et al. (2006) investigated the interactions of fibrate, anti-inflammatory, and antidepressive drugs with CYP catalyzed pathways (CYP1A, CYP3A-, CYP2K-, and CYP2M-like) and Phase II activities (UDP-glucuronosyltransferases and sulfotransferases) involved in both xenobiotic and endogenous metabolism in fish. Gemfibrozil, diclofenac, and three anti-depressive drugs (fluoxetine, fluvoxamine, paroxetine) had the highest potential to interfere with fish metabolic systems. A few years later, Schnell et al. (2009) reported toxicity of eleven pharmaceuticals from different therapeutic classes (anti-inflammatory drugs, serotonin re-uptake inhibitors, and lipid regulators) individually and as a mixture on the rainbow trout liver cell line RTL-W1. In accordance with Thibaut

et al. (2006), anti-depressives (fluoxetine and paroxetine, 7–50 μM) showed the highest potential to induce toxicity in RTL-W1 cells. Within a study, the combined toxicity of tested mixtures was multiplied, mostly following the concentration addition concept (Schnell et al. 2009). Additional PhACs group of particular concern are antifungals, such as clotrimazole, ketoconazole, miconazole. It is well-established that compounds containing an imidazole ring system frequently interact with cytochrome P450 enzymes to inhibit drug oxidation. Burkina et al. (2015) emphasized the ability of mentioned antifungal agents to affect the endocrine system, induce a cascade of molecular and cellular events, via stimulation/inhibition of CYP450.

Although in vitro cytotoxicity assays with fish cell lines have multiple advantages, due to insufficient and heterogenous data a correlation between the results obtained with such in vitro studies and in vivo toxicity of pharmaceuticals still needs to be assessed and validated. For this reason, recent studies shift focus from in vitro studies on cell lines to PhACs exposure-directed studies that link to specific biological effects. With the conducted toxico-pathological and stereological study, Madureira et al. (2012) gave a comprehensive overview of the effects caused by non-steroidal pharmaceutical compounds in the zebrafish *Danio rerio* liver. The mixture of environmentally relevant concentrations of non-steroidal pharmaceuticals (carbamazepine, fenofibrate, propranolol hydrochloride, sulfamethoxazole, and trimethoprim) shown qualitative and quantitative changes in the selected liver cytohistological parameters. The volume-weighted nuclear volume of zebrafish hepatocytes increased upon exposure, which together with the greater cytoplasmic eosinophilia and changes in cytochrome P450 1A immunoreactivity, pointed to an increase in metabolic/detoxification activity. Tested compounds interfered also with the liver mass and normal liver histology, induced liver VTG expression, and altered CYP1A expression, even at low environmental levels (Madureira et al. 2012). Recently, Rojo et al. (2021) reported the ability of carbamazepine, atenolol, enalapril, and sildenafil present in waters influenced by the sewage discharge to increase the EROD, BROD, and GST activity in fish *Pimelodus maculatus*. The authors showed a positive correlation between the distribution of PhACs in a water body and the higher activity of hepatic enzymes.

While numerous studies employ some variant of comparative methodology, generally they are difficult to compare due to different PhACs class-tested, differences in sensitivity of the test organism, exposure times, concentrations, and also selected endpoints. As a result, currently, it is difficult to elaborate a more detailed and focused discussion toward a mode of action of a single PhACs and their mixture and to prioritize PhACs likely to pose a negative impact on fish liver biotransformation and antioxidant enzymes, as well as non-enzymatic antioxidants. For this reason, further studies on establishing state-of-the-art standardized methods of measurement that may result in a long-term recommendation regarding the presence of PhACs in the environment are encouraged. Moreover, further investigation of the complex interactions between receptors, transcription factors, and other proteins in the regulation of expression and activity of fish CYP 450 isoforms is needed.

Personal care products

Personal care products (PCPs) are an extraordinarily diverse group of chemicals that comprises compounds used in cosmetics, skin and hair products, sunscreens, toothpaste, soaps, etc. The primary classes of PCPs include disinfectants, fragrances, UV filters, insect repellants, preservatives (Pemberthy et al. 2020; Hopkins and Blaney 2016). Contrary to PhACs, PCPs are intended for external use on the human body, thereby bypassing metabolic alterations (Brausch and Rand 2011). Therefore, large quantities of unaltered PCPs are introduced into the environment either directly via wash-off from the skin, or indirectly through (un)treated effluents (Pemberthy et al. 2020). Just as PhACs, conventional WWTPs are not able to completely remove PCPs, which has led to their detection in wastewaters and effluent receiving systems at concentrations from ng/L to μg/L (Roberts et al. 2016; Buchberger 2011). Despite its short environmental half-life, continuous loading of these pollutants into aquatic environments, even at low parts-per-trillion/parts-per-billion concentrations, causes its pseudo-persistence (Mottaleb et al. 2015; Ramirez et al. 2009). Thus, the exponential introduction of new PCPs to the marketplace ultimately results in perpetual life-cycle exposures of aquatic organisms. Accompanied by a currently unknown toxicological mechanism of action, the negative impact of PCPs can undergo undetected until cumulative effects finally result in an irreversible change in non-target aquatic organisms (Schnell et al. 2009).

The use of antimicrobials in PCPs has increased over the last decade, reaching its maximum production/use during the coronavirus disease 2019 (COVID-19) pandemic. Accordingly, the concentration of antimicrobials in the aquatic environment has nowadays become an emerging global concern (Chen et al. 2021). Triclosan is ranked among the ten most commonly detected PCPs in all environmental compartments. This is not surprising since it is also one of the most commonly used antimicrobials worldwide implemented in soaps, toothpaste, deodorants, and hand sanitizers (Overturf et al. 2015). Its concentration in aquatic environments ranges from 0.01 ng/L up to 27 μg/L (Overturf et al. 2015; James et al. 2012). Triclosan, like most antimicrobials, is lipophilic and has demonstrated the ability to bioaccumulate through the food chain. This was supported by Mottaleb et al. (2009) that reported the presence of triclosan (up to 31 ng/g) in tissues of fish sampled from the effluent-dominated stream in Texas. Due to the lipophilic and non-ionic

properties of triclosan, systemic absorption and retention are influenced by biotransformation in fish liver and intestine. Triclosan has a phenolic hydroxyl group which is likely to be a substrate for glucuronidation and sulfonation (James et al. 2012). A concerning effect of triclosan on zebrafish was recently shown by Gyimah et al. (2020). Authors reported that sublethal concentrations of triclosan upon subchronic exposure significantly decreased SOD, CAT, GPx, GSH, and GSSH in the liver of adult zebrafish, and simultaneously caused dose-dependent DNA damage in hepatocytes. Following triclosan exposure, Hemalatha et al. (2019) noticed significantly enhanced glutamic oxaloacetic transaminases (GOT), glutamic pyruvic transaminases (GPT), and GST activity in the liver of Indian major carp *Catla catla* in a dose- and time-dependent manner. Except for triclosan, there are other antimicrobials often found in PCPs (triclocarban, biphenylol, chlorophene, bromophene, etc.; Overturf et al. 2015), despite currently scarce ecotoxicological data.

Fragrances are one of the most widely studied classes of PCPs used to scent various products including deodorants, soaps, detergents, lotions, and perfumed household cleaners (Brausch and Rand 2011). The most commonly used fragrances are synthetic musks, here sorted by frequency of usage—polycyclic (celestolide, galaxolide, toxalide), nitro (musk xylene, musk ketone), and macrocyclic musks (Brausch and Rand 2011). After the application, a major part of the musk compounds is carried by municipal wastewaters, so their high concentration in untreated wastewaters is not a surprise. Due to the worldwide production and usage, they are ubiquitously distributed reaching up to 410 ng/L (for nitro musks) and 10 μg/L (for polycyclic musks) in effluent-dominated water bodies (Brausch and Rand 2011). The high log Kow values (4.0–4.4 for nitro musks and 5.4–5.9 for polycyclic musks) indicate their potential to bioaccumulate in aquatic species, for example up to 10,000 μg/kg fat in freshwater fish (Hopkins and Blaney 2016; Yamauchi et al. 2008). Following the exposure of medaka *Oryzias latipes* to synthetic polycyclic musks, expression analysis of hepatic VTG protein showed potential estrogenic effects indicative of the induction of VTG synthesis. Furthermore, tonaline and galaxolide induced the expression levels of hepatic ERα and VTG mRNA/protein and modulated expression levels of CYP3A40 mRNA in the livers of male medaka (Yamauchi et al. 2008). Fish consumption could lead to ingestion of musk substances in humans, causing currently unknown consequences. Even though acute oral and dermal toxicity for humans is low, results obtained with laboratory organisms showed concerning negative impacts, including some hints for the carcinogenic potential (Mottaleb et al. 2012).

Growing awareness of the harmfulness of UV radiation led to the implementation of chemical UV filters in a wide range of PCPs, including sunscreens, creams, lotions, and other cosmetic products. Due to the type of application, these compounds enter surface waters directly via washing-off from the skin during recreational activities, or indirectly via wastewater effluents (Overturf et al. 2015; Brausch and Rand 2011). Their occurrence in aquatic environments is ubiquitous, with the highest concentrations found during the summer months (up to 2.7 μg/L). At present, there are 27 UV filters allowed in cosmetics in the European Union, including the most commonly used benzophenones, p-aminobenzoic acid and derivatives, salicylates, and cinnamates (Buchberger 2011). A comprehensive study by O'Malley et al. (2020) reported the presence of UV filters in wastewater effluent samples from 33 WWTPs across Australia. Of the analyzed effluent samples, 95% contained at least one of the target compounds, with the summed concentrations of UV filters ranging from 130 to 8400 ng/L. Based on the removal efficiencies data, the authors estimated that approximately 40% of the total UV filter influent load remains in the effluent, resulting in a total of 20 kg per day of UV filters released into the aquatic environment (mainly 2-phenylbenzimidazole-5sulfonic acid (PBSA) and benzophenone 4 (BP4)). It has been documented that UV filters accumulate in the food chain, therefore posing a potential risk to the reproduction of fish species (Overturf et al. 2015). UV filters such as PBSA, BP4, and 4-aminobenzoic acid (PABA) increased the activity of certain P450 cytochromes and induce severe oxidative stress in the liver of *Oncorhynchus mykiss* and *Danio rerio* (Huang et al. 2020; Grabicova et al. 2013).

Although PCPs are positioned on the list of most commonly detected compounds in surface water throughout the world, compared to PhACs, environmental concentrations and toxicity of PCPs have been largely overlooked. One of the most concerning facts is the PCPs' potential to bioaccumulate and consequently biomagnify at higher trophic levels. Further, most of the studies have focused on testing the toxicity of a single compound, even though PCPs are present in the aquatic environments in very complex mixtures which can produce synergistic or antagonistic effects.

Even though PCPs are being continuously released into the aquatic environments, most studies conducted to date focused on acute and sub-acute toxicity determination. Although valuable, only chronic mixture studies accompanied with multigenerational approaches could accurately identify the potential risk of PCPs. Undoubtedly, biomonitoring of PCPs in environmental compartments and further research focused on toxicity and mode of action are of considerable interest to the scientific community.

Heavy metals

Discharge of industrial, municipal, and agricultural wastewaters and sewage into the aquatic and marine environment

is nowadays strictly regulated in order to decrease the levels of pollutants such as heavy metals in discharge effluents (Frémion et al. 2016; Karvelas et al. 2003). WWTPs can remove high amounts of heavy metals using different treatment processes, including physicochemical methods, chemical precipitation, coagulation, flocculation, electrochemical treatments, etc. (Gunatilake 2015; Agoro et al. 2020). Although some heavy metals are essential for fish growth, such as copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn), they can be toxic if their concentration exceeds an optimal limit. Contrarily, nonessential metals with no biological role such as lead (Pb), mercury (Hg), and cadmium (Cd) are toxic even at low concentrations while the most toxic environmentally relevant heavy metals and metalloids include chromium (Cr), arsenic (As), and manganese (Mn) (Ali et al. 2019; Barakat 2011). Additionally, the emerging problem of plastic in the environment also contributes to the accumulation of toxic metals that latch onto plastic surfaces and enter the marine environment and food chains (Rochman et al. 2014).

Trace metals are chemically highly reactive so their toxicity in fish is due to the catalytic activities of metalloenzymes (Fig. 2). The toxicity of metals may also be indirect, by binding to other cellular components, which is particularly important for metal ions that show similar chemical behavior, such as Ag^{+}, Cu^{+}, Hg^{+}, Cd^{2+}, and Zn^{2+} ions (Rainbow 1990). Biota can accumulate various chemicals, and that uptake has been extensively used to measure the effects of metals on aquatic organisms as an essential indicator of water quality (El-Moselhy et al. 2014). The mollusks (Gupta and Singh 2011) and fish (Stankovic et al. 2014; Hauser-Davis et al. 2012a) are the most used organisms as bioindicators of metal pollution in water.

Metal bioavailability controls their accumulation in aquatic organisms (Gheorghe et al. 2017). External environmental factors like metal speciation, the presence of organic or inorganic complexes, and physicochemical properties of water (pH, temperature, salinity, and redox conditions) (Bonnail et al. 2016) are the main factors that could modulate metal toxicity. The ingestion uptake depends on the rate of feeding, intestinal transit time, and digestion efficiency (Jia et al. 2017) as well as on the age and size (weight and length), feeding habits, and body physiology of fish (Gheorghe et al. 2017). The degree of accumulation of heavy metals in various tissues of fish is generally different depending on the structure and function of tissues. Gills, liver, and kidney are metabolically active tissues with a higher affinity to accumulate heavy metals than other tissues (Ali et al. 2019). This is mostly explained by the induction or occurrence of metal-binding proteins called metallothioneins (MTs) or some other transporters which translocate metals across the plasma membrane in these tissues upon exposure to heavy metals (Ali et al. 2019; Hauser-Davis et al. 2012b).

Liver has a major role in the metabolism of lactate to pyruvate and can store up to one-eighth of the total glycogen stores. Glycogen content in the fish liver is a potent indicator for changes in the activity of functional systems affected by heavy metals (Javed and Usmani 2015). Another indicator in hepatocytes is related to the increase in the number and size of lysosomes to store pollutants and lipids, increased permeability of the lysosomal membrane, and release of lysosomes into the cytoplasm and nucleoplasm (Köhler 1991). Heavy metals enter the fish gall bladder, to be excreted from the organism (Ardeshir et al. 2017). Fish bile might thus also serve as a target tissue for heavy metal exposure (Hauser-Davis et al. 2012a) due to its role in the excretion of many endogenous and exogenous substances (Grosell et al. 2000).

Fish liver damage due to heavy metal exposure has been reported in various geographical sites (Kuton et al. 2021; Rubalingeswari et al. 2021; Weber et al. 2020; Kumar Maurya et al. 2019; Łuczyńskaa et al. 2018; Uysal 2011) indicating the importance of their monitoring and evaluation. Kumar Maurya et al. (2019) revealed that heavy metal accumulation in fish tissues is in the magnitude order of liver > gill > muscle pointing out Ni, Cr, Pb, Cu, Cd, Zn, and Fe as dominant metals influencing toxicity in fish tissues (Rubalingeswari et al. 2021). However, the bioaccumulation magnitude is a species-specific function (Spry and Wiener 1991) so the determination

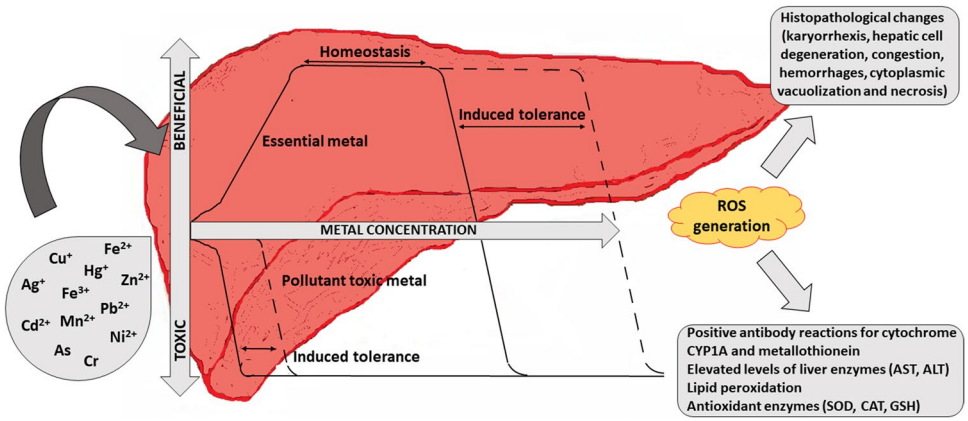

Fig. 2 Representation of essential and pollutant metals' effects on cellular metabolism with controlled intracellular concentrations of essential trace metals by homeostatic mechanisms, and exceeded concentrations when metal becomes toxic. Inducible tolerance (detoxification systems) raises the inhibitory thresholds, but when exceeded, permanent damage occurs

of bio-concentration factors (BCFs) (Orata and Birgen 2016) of heavy metals in fish liver, which are the ratio of the heavy metals in liver to the surrounding water, are needed and often used to evaluate results within different studies. In addition, it was observed that accumulation of heavy metals in liver varies with the season from minimum in winter to the maximum in summer with histological changes such as cytoplasmic vacuolation, necrosis, and sinusoid dilation (Mahboob et al. 2020). Partitioning of the heavy metals was explained with Cd, which was largely bound to heat-stable proteins including MTs and metal-rich granules in two fish species. These elements followed the Cd partitioning pattern, suggesting that they are involved in antioxidant responses against Cd toxicity (Gaël et al. 2019). Most recently, it was revealed that fish exposed to heavy metals show tissue degenerations and alterations become prominent as the duration of exposure increases (Naz et al. 2021). This includes hematological and histopathological changes under different concentrations of Cu and Cd. Various impairments were observed by histopathological examination of liver tissues like karyorrhexis, hepatic cell degeneration, congestion, and hemorrhages. Weber et al. (2020) pointed out that non-treated wastewater from the mining industry with a high load of Pb, Ni, and As is responsible for numerous impairments in fish. Hepatic alterations, such as cytoplasmic vacuolization and necrosis with the positive antibody reactions for cytochrome P450 1A (CYP1A) and MT, are associated with metal contamination. Thus, species more sensitive to environmental modifications may not resist this highly contaminated aquatic environment. Metal concentrations in fish tissues have also shown a species-specific bioaccumulation pattern, with diverse histopathological alterations (Omar et al. 2013). As mentioned, upon exposure to heavy metals, liver metabolism generates ROS, so antioxidant changes in liver are frequently used as a biomarker of metal exposure. A recent study evaluated levels of liver enzymes AST and ALT, with the focus on the antioxidant status of liver (El-Shenawy et al. 2021). They determined the TBARS levels as a direct response to lipid peroxidation, in addition to antioxidant enzymes including SOD, CAT, and GSH and concluded that the elevation of lipid peroxidation in fish is connected to the increased activity of endogenous antioxidants. Another study (Hossain et al. 2021) examined the changes in antioxidant enzymes as well as histopathological damage in liver tissue after exposure to heavy metals. Enzymes SOD, CAT, GPx, GST, reduced glutathione, and MDA levels were significantly increased. Severe hemorrhage, vacuoles, pyknotic space, and evidence of lipid accumulation were also observed.

All provided studies have one main target—evaluation of heavy metal accumulation and its impact on fish tissues by implementing different analysis approaches. For liver damage evaluation, histopathological observation of tissue is the most frequently used one, but since metals cause oxidative stress, measurement of antioxidant enzymes gives valuable insight into acute and chronic toxicity. Such findings need to be continuously introduced to the wider public, i.e., endpoint consumers, to acknowledge existing and further problems regarding the constant heavy metal discharge into the environment.

Conclusions and future directions

This review provided a comprehensive assessment of the impact of WWTP effluents on fish liver damage. Continuous release of (un)processed wastewaters with residual xenobiotics has greatly impacted the environment. The fish liver represents one of the most used models to establish a link between the concentration of pollutants and their bioaccumulation and detoxification within the organism. In this review, a variety of sensitive fish liver biomarkers as suitable indices for health conditions was discussed referring to the cellular, biochemical-physiological, and histopatological levels. Detailed observation of these changes has been made with a specific accent on legacy contaminants and emerging-concern contaminants such as pharmaceutically active compounds, personal care products, and heavy metals. Our results show that it is a challenge to identify a suitable fish liver biomarker for assessing WWTP impacts. Furthermore, suitable fish species need to be selected for such investigations, both for field studies and laboratory exposure studies. In that fashion, their applicability and specificity can be assessed and standardized for targeted determination of the impact of WWTP effluents on fish liver.

Furthermore, engineered nanoparticles occurring in wastewater discharges raise concerns in toxicological assessments. In particular, metal and metal oxide NPs can translocate among organs and penetrate the blood-brain barrier, accumulating in fish liver. Most studies on such NPs were focused on acute toxicity. Therefore, more chronic toxicity studies are required, particularly co-exposure studies. Additionally, systematic understanding of the transport, distribution and toxicological mechanisms of complex pollutants in fish livers are still not sufficient. The research on toxic effects of such NPs still principally focuses on pathological effects such as body distribution, cell damage and organ damage (Bai and Tang 2020).

Although a significant decrease in the concentration of several persistent contaminants like Hg, PCBs, etc. can be noticed in the last few decades, many other classes of contaminants that are currently being poorly studied are becoming ubiquitous (new pharmaceuticals, personal care products, etc.). For such reasons, current bioaccumulation data do not represent the actual impact of WWTPs on the aquatic

ecosystem and consequent impact on non-target organisms. All this indicates that continuous monitoring is essential, while further research and improvement of treatment plants can provide less contaminated discharge effluent with legally controlled release or further treated disposal of effluent sludge. Further research is required to confirm bioaccumulation factor of emerging contaminants in aquatic organisms and to reveal mechanisms of their toxicity. More focus should be also put on determining their potential interaction in water bodies which could enhance their hepatotoxicity. Knowledge gaps, as well as research priorities, emphasized in this review article may help to steer and prioritize future research in this important field.

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