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Synopsis

Fish in Ecotoxicological Studies

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Abstract. Water contamination (heavy metals, pesticides, POPs, etc.) is a serious environmental issue which has been raising lots of attention in the last decades because it can destroy aquatic ecosystems and hence, reduce biodiversity. In the field of ecotoxicology it is of main interest to investigate what the effects of organic and inorganic toxicants on different biological organization (cell, tissue, organism, population) are. Thus, many authors use different test organisms and particularly, fish. In the current study we aimed to present collected data from the last years which describe why fish is an appropriate species in terms of ecotoxicological research.

Key words: fish, water contamination, ecotoxicology, toxic effects, biological organization.

Introduction

Water pollution is the burning issue nowadays all over the world. Aquatic ecosystems are frequently contaminated with different toxicants through anthropogenic activities, and some of them such as metals may be naturally present and essential in low but toxic in higher concentrations (AJMAL *et al.*, 1988; EKPO & IBOK, 1999; PANDEY *et al.*, 2008; SEKABIRA *et al.*, 2010; CARASSCO *et al.*, 2011; LUSHCHAK, 2011; ONDARZA *et al.*, 2012; PEREIRA *et al.*, 2013; JÖRUNDSDÓTTIR *et al.*, 2014).

Since not all chemical forms of pollutants are equally bioavailable and some pollutants can be accumulated in living organisms to a greater extent than others, we need to study the levels of pollutants in the organisms to be able to predict the environmental risk (RAINBOW & PHILLIPS, 1993; CONNELL *et al.*, 1999). Thus, chemical analyses of the tissues of aquatic organisms are used as a routine approach in studies of aquatic pollution, providing a temporal integration of the levels of pollutants with biological relevance at higher concentrations than those present in water or sediment, and facilitating their quantification (RAINBOW & PHILLIPS, 1993).

In ecotoxicology, fish have become the major vertebrate model, and a tremendous body of information has been accumulated (STEINBERG *et al.*, 1995; BRAUNBECK *et al.*, 1998; MOISEENKO, 2005; RAISUDDIN & LEE, 2008; SCARDI *et al.*, 2008; HERMOSO *et al.*, 2010; SOUZA *et al.*, 2013; MURTHY *et al.*, 2013; CZÉDLI *et al.*, 2014). Fish are among the group of aquatic organisms which represent the largest and most diverse group of vertebrates. A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems (LAW, 2003; DE LA TORRE *et al.*, 2010). Thus, according to DE LA TORRE *et al.* (2005) monitoring sentinel fish species is widely used to assess the degree of

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accumulation of pollutants and the effects on health status. Furthermore, toxicant accumulation in water suggests that fish may serve as useful indicators for contamination in aquatic systems because they respond with a greater sensitivity to changes in the aquatic environment than invertebrates and tend to accumulate some poisons often in concentrations several times higher than in the ambient media (PAPAGIANNIS *et al.*, 2004; HAS-SCHÖN *et al.*, 2006; VELCHEVA, 2006; MOISEENKO *et al.*, 2008; HUANG *et al.*, 2013; EAGLES-SMITH & ACKERMAN, 2014; DHANAKUMAR *et al.*, 2015; ZHAO *et al.*, 2015). In this sense, bioaccumulation is a process in which a toxicant is absorbed in a fish organism by all routes as it occurs in the natural environment, i.e., dietary and ambient environment sources (ARNOT & GOBAS, 2006). Hence, bioaccumulation occurs primarily due to the inability to excrete necessary levels of contaminants and its degree is the result of imbalance between the input rate and the rate of toxicant elimination. Under certain environmental conditions (e.g., season, water temperature, pH, hardness, and river flow) and biotic factors (e.g., fish species, age, tissue, organism life-history traits) toxicants can accumulate to toxic concentrations and cause ecological damage (URAL *et al.*, 2012; ANTAL *et al.*, 2013; CHAHID *et al.*, 2014).

Fish have also been found to be good indicators of water contamination in aquatic systems because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates, are also more sensitive to many toxicants (DALLINGER *et al.*, 1987; BARAK & MASON, 1990; BURGER *et al.*, 2002). They are preferred in toxicological research because of their well-developed osmoregulatory, endocrine, nervous, and immune systems (SONG *et al.*, 2012). In addition, fish may absorb toxicants directly from the surrounding water and sediments (waterborne exposure), or ingest them through contaminated food in the food chain (dietary exposure), enabling the assessment of pollutant transfer through the tropic web (PÉREZ CID *et al.*, 2001; FISK *et al.*, 2001; MOISEENKO & KUDRYAVTSEVA, 2001; RASHED, 2001b; MONDON *et al.*, 2001; MANSOUR & SIDKY, 2002; USERO *et al.*, 2004; MENDIL & ULUÖZLÜ, 2007; ÖZTÜRK *et al.* 2009; SOUNDERAJAN *et al.*, 2010; ROWAN, 2013). In general, as RAYMENT & BARRY (2000) state fish have been popular targets of monitoring programs in marine environments because sampling, sample preparation and chemical analysis are usually simpler, more rapid and less expensive than alternative choices such as water and sediments. Last but not least, fish are the final chain of aquatic food web and an important food source for human. Therefore, some toxicants in aquatic environments can be transferred through food chain into humans (UYSAL *et al.*, 2008; 2009; METIAN *et al.*, 2013). Water pollution leads to contamination of fish which may pose a health risk (KARADEDE *et al.*, 2004; MENDIL *et al.*, 2005; ULUOZLU *et al.*, 2007; TÜRKMEN *et al.*, 2008; MENDIL *et al.*, 2010). Thus, in view of the quality of public food supplies, TÜRKMEN & CIMINLI (2007); YILMAZ *et al.* (2007b) and BILANDŽIĆ *et al.* (2011) suggest that toxicant levels in aquatic environment should be monitored regularly to check water quality and animal health.

Fish and biomarkers

The presence of xenobiotics in the marine environment exerts well-known biological effects on marine organisms. Occasionally, when properly evaluated in selected sentinel species, these effects may be considered as biomarkers, a sort of early warning signaling useful in assessing the quality of marine habitats (BAYNE *et al.*, 1985; CAJARAVILLE *et al.*, 2000; LAM & GRAY, 2003; MOORE *et al.*, 2004). Biomarkers have been proposed as sensitive tools for the early detection of environmental exposure to pollutants and their adverse effects on aquatic organisms (VAN DER OOST *et al.*, 2003; DE LA TORRE *et al.*, 2005). They also serve as links between the environmental contamination (cause) and its effects, providing unique information on the ecosystem health (MARIA *et al.*, 2009). In the past 25 years, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring (MCCARTHY & SHUGART, 1990; PEAKALL, 1994; SHUGART & THEODORAKIS, 1998;

SCHMITT *et al.*, 2007; MEDGELA *et al.*, 2006; MUÑOZ *et al.*, 2015).

Toxicant effects can be studied at different levels of biological organization. BERNET *et al.* (1999), MONTEIRO *et al.* (2005), CAZENAVE *et al.* (2009) and MARCHAND *et al.* (2009) consider that the different changes in many biochemical and morphological parameters of fish may be used as successful biomarkers for toxic effects of xenobiotics. Initial effects of toxicant pollution may be evident only at cellular or tissue levels before significant changes are identified in fish behavior or external appearance. Histological alterations for example have been examined for decades in fish tissues and organs in order to assess the effects of pollutants (JOHNSON *et al.*, 1993; STENTIFORD *et al.*, 2003; AU, 2004). As an indicator of exposure to chemicals, histology represents a useful tool to assess the degree of pollution (PERRY & LAURENT, 1993). According to WESTER & CANTON (1991), histopathological changes have been widely used as biomarkers for the evaluation of the health of fish, exposed to contaminants in laboratory conditions. One of the important advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs. According to RABITTO *et al.* (2005) and OLIVEIRA RIBEIRO *et al.* (2006) the exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs but the gills and liver represent important target organs suitable for histopathological examination in searching for damages to tissues and cells According to HINTON & LAUREN (1990) for field assessments, histopathology is often the easiest method of assessing both short and long-term toxic effects. On the other hand, WESTER & CANTON (1991) state that histology is relatively labor-intensive and requires some experience, but after all it has the considerable advantage that pathological alterations in different tissues (e.g., gills, liver) can be observed individually, thus creating a direct link with physiological functions such as growth, reproduction, respiration and nutrition.

Antioxidant enzymes are considered to be sensitive biomarkers, and they are important parameters for testing water quality and the negative effects of metals on fish (OLSVIK *et al.*, 2005; HANSEN *et al.*, 2006; VELMURUGAN *et al.*, 2008; 2009; BANEE *et al.*, 2011; YOUSAFZAI & SHAKOORI, 2011; GABRIEL *et al.*, 2012). Therefore, various responses of enzymes have been also observed in fish exposed to metallic and persistent organic contaminants which indicate an increase or a decrease in the activity depending on the dose, species and route of exposure (WONG & WONG, 2000; LOPES *et al.*, 2001; HARIKRISHNAN *et al.*, 2003; CAO *et al.*, 2010; KOENIG *et al.*, 2012; LU *et al.*, 2013). For example, the biochemical parameters in fish liver are sensitive for detecting potential adverse effects and relatively early events of pollutant damage (STENTIFORD *et al.*, 2003). Changes in the activity of liver enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) serve as an indicator for a normal liver function, and they also can be used as biomarkers for tissue damage (ALMEIDA *et al.*, 2002). Thus, it can be concluded that these enzymes are sensitive biomarkers for the determining stress in the fish subjected to various pollutants present in the waters (ADHIKARI *et al.*, 2004).

Fish organs most commonly used in ecotoxicological studies

Fish can be exposed to toxicants via two exposure routes, waterborne: gills and derma and dietary (SLOMAN, 2007). Toxicants are taken up through different organs of the fish because of the affinity between them. In this process, many of them are concentrated at different levels in different organs of the fish body (RAO & PADMAJA, 2000). Therefore, in teleost fish, the gills, liver, kidney and muscles are the tissues most frequently utilized in ecological, toxicological and pathological studies (SAUER & WATABE, 1989; VELCHEVA, 2002; HEIER *et al.*, 2009) because they are metabolically active tissues and accumulate toxicants at higher levels (ANDRES *et al.*, 2000; KARADEDE & ÜNLÜ, 2000; MARCOVECCHIO, 2004). TERRA *et al.* (2008) consider that toxicants enter the body mainly through the gills and consequently, with the blood they reach the parenchymal organs where they retain for a longer time. In addition, according to KROGLUND *et al.* (2008) toxicant concentrations, particularly in the gills reflect the toxicant concentrations in the water where the fish live; whereas, concentrations in other organs such as the liver and kidney represent storage of toxicants. According to JOVIČIĆ *et al.* (2014) studies of metal accumulation in fish are mainly focused on the muscle tissue, while the metal accumulation patterns in other tissues have been largely neglected. Elemental accumulation in many fish tissues and organs and their potential use in monitoring programs have not received proper attention. Therefore, the authors measured the metal concentrations in 14 tissues of the wels catfish (*Silurus glanis*) from the Danube River. Some of them are not very common but they also could provide valuable information in terms of ecotoxicology − muscle, gills, spleen, liver, kidneys, intestine, stomach, heart, brain, gallbladder, swim-bladder, vertebra, operculum, and gonads.

Gills

The fish gills are multifunctional organs involved in ion transport, gas exchange, acid–base regulation and waste excretion (DANG *et al.*, 2001). Given that the gills accounts for well over 50% of the surface area of a fish it is not surprising that one of the major target organs for waterborne toxicants is the gill (PLAYLE, 1998). The gills are regarded as the important site for direct uptake from the water, whereas the body surface is generally assumed to play a minor role in xenobiotics uptake of fish (POURANG, 1995). Thus, in teleost fish the gills are most frequently utilized in bioaccumulation studies and the pathological damage produced allows the toxicity of the environment to be defined, making fish highly suitable for evaluating the health of aquatic systems (MALLATT, 1985; OLIVEIRA RIBERIO *et al.*, 2000; OLSVIK *et al.*, 2000; MOISEENKO, 2005; OGUNDIRAN *et al.*, 2009). Fish metabolism, acting principally through the gills can be seriously damaged since toxicant incorporation occurs mainly through this respiratory organ (BERVOETS & BLUST 2003; SLOMAN 2007; TERRA *et al.*, 2008). Furthermore, the fish gills are very sensitive to physical and chemical alterations of the aquatic medium such as: temperature, acidification of the water supply due to acid rain, salts and heavy metals, and to any change in the composition of the environment which is an important indicator of waterborne toxicants (SABER, 2011). According to CARPENE & VAŠAK (1989); PERRY & LAURENT (1993); TKACHEVA *et al.* (2004) and ROSSELAND *et al.* (2007) the fish gills are the main route of penetration of toxicants into the fish organism, thus they are the first organs which come in contact with environmental pollutants, and are also sensitive subjects for identifying the effects of water toxicants on fish organisms. The fish gills can accumulate bioavailable pollutants, and their measurement on gills can reflect the speciation of pollutants, and in particular metals in water, therefore, they are a useful tool for assessing bioavailability of elements in water (HEIER *et al.*, 2009). Moreover, gill surface serves as metal-binding ligands and metal bioaccumulation in particular can occur due to positively charged metal species in the water to negatively charged sites on the gills (TEIEN *et al.*, 2006; TERRA *et al.*, 2008; PLAYLE *et al.*, 2011). ROSSELAND *et al.* (2007) state that the gills are considered to be important site for direct toxic effects to metals in high concentrations, for sub-lethal effects at lower metal concentrations, and, along with uptake from food, an important point of entry into the organism for both essential trace elements (Cu, Zn, Se, Fe) and non-essential elements (Al, As, Cd, Cr, Ni, Pb). There is also a close relationship between the gill morphological alterations and chemical induced stress. Thus, one of the methods that proved toxic effects is to study the morphology of gills (PETERS & HONG, 1985; OLOJO *et al.*, 2005; GEORGIEVA *et al.*, 2014). Histological changes in the gills are recognized as a valid and fast method to determine the damage caused in fish by the exposure to different pollutants (ARELLANO *et al.*, 1999). There are reports on various histological changes in gills under the effects of different toxicants in water both in field and laboratory conditions (Cengiz, 2006; Figueiredo-Fernandes *et al.*, 2007; Fontaínhas-Fernandes *et al.*, 2008; Mohamed, 2009), but it is often difficult to decide whether morphological alterations are adaptive or destructive (Tkacheva *et al.*, 2004).

Liver and kidney

Once the toxicants cross the biological barriers and enter the bloodstream, they will reach and accumulate in the internal organs of fish. Numerous studies have quantified contaminants in fish organs to evaluate environmental quality, seeking causal relationships with fish health, and, based on these, the liver is likely to be the best choice, followed by the kidney and gills (Hanson, 1997; Begum *et al.*, 2004; Pokorska *et al.*, 2012; Majnoni *et al.*, 2014). The liver is reported to be the primary organ for bioaccumulation and thus, has been extensively studied in regards to the toxic effects of xenobiotics (Hinton & Laurén, 1990; De Boeck *et al.*, 2003; Yilmaz *et al.*, 2007a; van Dyk *et al.*, 2007; Simonato *et al.*, 2008; Madureira *et al.*, 2012; Nunes *et al.*, 2015). According to Mohamed (2009) the liver is also a target organ due to its large blood supply which causes noticeable toxicant exposure. In addition, according to Hinton & Laurén (1990) it is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances in the body. The vertebrate kidney is the main organ involved in the maintenance of body fluid homeostasis. The morphology and function of the kidney have been modified through evolution to fulfill different physiological requirement and the widest range of kidney types is found in fishes (Hentschel & Elger, 1989). In teleosts, the kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids (Hinton *et al.* 1992; Ojeda *et al.*, 2003) and, besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotics to which the fish may be exposed (WHO, 1991; Hinton *et al.*, 1992; Eisler, 1998). The kidney also excretes other nitrogen-containing waste products from the metabolism such as ammonia and creatinine. In addition, in fish as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment (Cengiz, 2006). Thus, many studies showed that different toxicants accumulate mainly in metabolic organs such as the liver and kidney (Karadede *et al.*, 2004; Oliveira Ribeiro *et al.*, 2005; Jabeen *et al.*, 2011; Yancheva *et al.*, 2014a, b; de Jonge *et al.*, 2015; Vaseem & Banerjee, 2013) which can lead to many histological alterations (Hinton & Couch, 1998; Cengiz, 2006; Poleksić *et al.*, 2010; Stentiford *et al.*, 2003). Levels of heavy metals such as lead, copper, cadmium, and zinc in marine fish have been extensively documented in the primary literature (e.g., Roméo *et al.*, 1999; Zauke *et al.*, 1999; Jureša & Blanuša, 2003). These metals tend to distribute differentially between the liver and kidney and other organs, most likely because of metal-binding proteins such as metallothioneins in the metabolic organs (Hamilton & Mehrle, 1986; Roesijadi, 1992; de Smet *et al.*, 2001; Atli & Canli, 2003). These proteins bind copper (Cu), cadmium (Cd), and zinc (Zn), but not lead (Pb), allowing organs such as the liver to accumulate higher levels of metals than other organs such as muscle (Ploetz *et al.*, 2007). Falfushynska & Stoliar (2009); Shinn *et al.* (2009); Poleksić *et al.* (2010); Barone *et al.* (2013) and Siscar *et al.* (2014) think that pollutant accumulation in the internal organs is associated not only with organ function such as haematopoiesis, antioxidant defense, detoxification, and excretion, but also with metallothionein synthesis which is directly related to the increase in some metal concentrations (Monteiro *et al.*, 2013; Siscar *et al.*, 2014). Another reason for higher toxicant levels in the internal organs may be gastrointestinal route of exposure (Sloman, 2007), rendering the liver and the kidney additionally vulnerable to chronic toxicant exposure (Olsvik *et al.*, 2000). Furthermore, according to Olsvik *et al.* (2000) and Sharma *et al.* (2009) liver and kidney are vulnerable organs during prolonged toxicant exposures, both from waterborne and dietary sources. Livers for example are also examined for histopathological alterations since several studies carried out in coastal waters have shown correlation between environmental contaminants and the occurrence of toxicopathic liver lesions in fish (VETHAAK & JOL, 1996; STENTIFORD *et al.*, 2003; FEIST *et al.*, 2004). In recent years, fish diseases and liver histopathological alterations have been used as indicators of pollution effects and have been implemented in monitoring programs (LANG, 2002; FEIST *et al.*, 2004). The presence of inflammatory lesions, hepatocellular fibrillar inclusions, and preneoplastic and neoplastic lesions is higher in fish captured in polluted environments than in fish from reference sites (STENTIFORD *et al.*, 2003). Overall, KARADEDE *et al.* (2004) state that the liver stores xenobiotics which eventually will be detoxicated and kidneys are involved in the process of excretion.

Muscles

The fish flesh (muscle) is a very important, valuable and recommended food in the human nutrition due to low content of fat and high content of proteins and mineral substances as well as optimal ratio of unsaturated fatty acids with cardio-protective effect. On the other hand, fish muscle may be the depositary for different contaminants, which occur in the water ecosystem (ANDREJI *et al.*, 2012). Such environmental pollutants are dioxins and PCBs, heavy metals, and organochlorine pesticides are a global threat to food safety, thus muscles could lose these properties due to environmental contamination (BAJC *et al.*, 2005). Hydrobionts can bioaccumulate many of these contaminants potentially making seafood of concern for chronic exposure to humans (NØSTBAKKEN *et al.*, 2015). According to SVOBODOVA *et al.* (1996) the metal concentrations in the water are positively correlated with the concentrations in fish tissues, but WIDINARKO *et al.* (2000) state that the metal concentrations in the sediments are the most important factor for their levels in the aquatic biota. Consumption of fish contaminated with heavy metals have deleterious effects on human health which was widely acknowledged after a series of events in the period from 1953 to 1960 when several thousand people died in Minamata Bay in Japan as a result of poisoning caused by the consumption of mercury contaminated fish (HARADA, 1995). Among the metals, mercury (Hg) is of the most widespread concern in connection with fish consumption and advisories related to fish consumption are issued by health authorities in many countries (UNEP, 2002). Therefore, concern regarding the presence of this metal and other contaminants in seafood has arisen during the last decades (FRANCESCONI & LENANTON, 1992; OLIVEIRA RIBERIO *et al.*, 2000; VAZQUEZ *et al.*, 2001; HITES *et al.*, 2004; COHEN *et al.*, 2005; WILLETT, 2005; FORAN *et al.*, 2006; MOZAFFARIAN & RIMM, 2006; USYDUS *et al.*, 2009; IBRAHIM *et al.*, 2011). In order to evaluate the risk to consumers, there is a continuous need for data on contaminant levels such as mercury in fish as highlighted by the European Food Safety Authority (EFSA, 2012). Even though, the observed toxic effects raised public safety and human health concerns repeatedly since Minamata, prompting legislators to set limits on the lead (Pb), cadmium (Cd) and mercury (Hg) levels detected in the fish muscle, but other heavy metals and fish tissues were not included in the European Union (EU) regulations as explained by JOVANOVIĆ *et al.* (2011).

Fish muscles are tissues which are important to analyze in terms of human health and this is the reason to be included in many monitoring and risk assessment programs (OLSSON *et al.*, 1978; SCHMITT & BRUMBAUGH, 1990; DUŜEK *et al.*, 2005; CHALMERS *et al.*, 2010). However, according to RASHED (2001a); SHINN *et al.* (2009); POLEKSIĆ *et al.* (2010); VISNJIC-JEFTIC *et al.* (2010); JARIĆ *et al.* (2011) and TAWEEL *et al.* (2013) toxicant concentrations, particularly heavy metals are usually lower in the muscles than in the other studied organs and the muscles are not always a good indicator of the whole fish body contamination. This can be explained by the very fast rate of decontamination in this tissue. However, fish is considered as one of the main protein sources of food for humans and water contamination consequently leads to contamination of fish (Dural *et al.*, 2007; González-Mille *et al.*, 2010; de la Torre *et al.*, 2010; Rejomon *et al.*, 2010; Rose *et al.*, 2015). That is why, in recent decades much attention has been paid to the investigation of xenobiotic concentrations in fish as a result of the increasing concern of food poisoning (Nagata & Oka, 1996; Senthilkumar *et al.*, 1999; Henry *et al.*, 2004; Sapozhnikova *et al.*, 2005; Andreji *et al.*, 2006; Cheung *et al.*, 2007; Tuzen & Soylak, 2007; Türkmen *et al.*, 2009; Guérin *et al.*, 2011; Merciai *et al.*, 2014).

Table 1. Maximum permissible levels for some toxic elements in fish muscle according to international regulations and recommendations (mg kg^{-1}).

Regulation/Recommendation	Al	As	Cd	Cu	Cr	Ni	Pb	Zn	Hg	Tin
Norm 31 (Bulgaria)	30	1	0.05	10	0.3	0.5	0.2	50	0.5	-
EC (2008)	-	-	0.1	-	-	-	0.3	-	1	200
FAO/WHO(2011)	-	0.1	-	0.4	-	-	0.3	-	0.5-1	250
Food Safety Authority of Ireland (2009)	-	-	0.05	-	-	-	0.3	-	-	200
Australia/New Zealand Food Standards Code (2013)	-	2	-	-	-	-	0.5	-	0.5-1	250

**additional information regarding other non-metal and organic contaminants in fish can be found in the presented regulations/recommendations.*

Conclusions

Overall, on the basis of the studied literature we can conclude that fish are very good indicators for impaired water quality as they have different size, occupy different tropic levels and are long-living and mobile. Hence, they have been successfully applied in ecotoxicological research in the last few decades and many researchers prefer them instead of invertebrates. Depending on the main purposes of research, i.e. bioaccumulation, histological and biochemical analyses or other investigated biomarkers, different fish organs can be applied. The most commonly used are the respiratory organs - the gills and parenchymal organs – liver and kidney, but in terms of human health the most appropriate tissue are the muscles. However, we have to add that there other organs with important functions which can be more thoroughly studied such as the spleen or otoliths. Therefore, we suggest that further investigation should be carried out in this particular are in order to better understand the negative effects of pollution on the fish biology.

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