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Role Of Fish as Bioindicators: A Review

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Abstract-* Growing human population and industrialization have led to the pollution of most aquatic ecosystems and consequent deterioration in environmental water quality. Indicator organisms are needed to improve assessment programmes on the ecological impacts of anthropogenic activities on the aquatic environment. Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution. This study discussed the roles of fishes as bioindicators as uses as biological indicators. The comprehensive knowledge of taxonomy, habitat requirements, and physiology of fish is a key prerequisite of using fish as indicators. No other aquatic organism is suitable for the application of so many different methods which allow the evaluation of the severity of toxic impacts by determining the accumulation of toxicants in tissues, by using histological and haematological approaches or by detecting morphological anomalies. Due to its complex habitat requirements the fish fauna is a crucial indicator of the ecological integrity of aquatic systems at different scales, from microhabitat to catchment. Thus bioindication using fish represents a good monitoring tool especially with regard to both pollution aspects and to river engineering, e.g. river restoration and management. In order to further strengthen the role of fish as valuable indicators of the ecological integrity of aquatic systems, research is required ranging from the ecological demands of certain target species to ecosystem processes. There is need to broaden knowledge in aquatic environmental impact assessment by the use of fish as a bioindicator to assess aquatic environment. This study recommends the use of fish as valuable biological indicators in aquatic environmental pollution assessment.

***Indexed Terms-* Fish, Bioindicator, Environmental Pollution, Biological indicator, Organisms, Ecotoxicity**

I. INTRODUCTION

Bioindicators are organisms or communities of organisms, which reactions are observed representatively to evaluate a situation, giving clues for the condition of the whole ecosystem. The bioindicator has particular requirements with regard to a known set of physical or chemical variables such that changes in presence/absence, numbers, morphology, physiology or behavior of that species indicate that the given physical or chemical variables are outside their preferred limits (Whitfield and Elliott, 2002). Mostly, bioindicators are restrictively defined as species reacting to anthropogenical effects on the environment, whereas bioindicators for "natural" environmental changes and conditions are not much used. However, a general, all-encompassing definition of a biological indicator would be: "a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community or ecosystem or is indicative of the diversity of a subset of taxa or the whole diversity within an area".

The impacts of human activities on water bodies require appropriate monitoring tools to facilitate detection and characterization of the causes and sources of chemical, physical and biological impairment of the aquatic habitats. Among these tools, aquatic biota (fish, frogs, insects, benthos and plants) are identified as potential bioindicators to detect pollutant loads in water (Muyibi *et al.*, 2008). In tracking long-term changes of a specific water body such as a river system, fish (such as Tilapia, *Oreochromis mossambicus*) are known to be useful and reliable indicators of long-term effects and broad habitat conditions as highlighted by many investigators over the years (Araújo *et al.*, 2000; Vidal, 2008). Tilapia is the common name for around 70 species of perch-like fishes (family Cichlidae) native to the fresh waters of tropical Africa (Bhassu *et al.,* 2004).

Due to feeding and living in the aquatic environments fish are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants (Yarsan and Yipel, 2013; Mahboob *et al.*, 2013; Saleh and Marie, 2014). Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health (Whitfield and Elliott, 2002; Khallaf *et al.,* 2003; Authman *et al*., 2008; Moiseenko *et al.,* 2008; Authman, 2011; Authman and Abbas, 2011; Authman *et al.,* 2012; Authman *et al.,* 2013a; Authman *et al.,* 2013b; Abumourad *et al*., 2014; Gaber *et al.,* 2014; Zaki, *et al.,* 2014). Heavy metals are produced from a variety of natural and anthropogenic sources (Bauvais *et al.,* 2015). In aquatic environments, heavy metal pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products, also via wastewater treatment plants (WWTPs) (Demirak *et al.,* 2006; Maier *et al.,* 2014; Dhanakumar *et al.,* 2015; Garcia *et al.,* 2015). Coal combustion is one of the most important anthropogenic emission sources of trace elements and an important source of a number of metals (Wagner and Boman, 2003). The contamination of heavy metals and metalloids in water and sediment, when occurring in higher concentrations, is a serious threat because of their toxicity, long persistence, and bioaccumulation and bio magnification in the food chain (Has-Schön *et al.,* 2006). Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level (Rashed, 2001; Authman, 2008), they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Lamas *et al.*, 2007). In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (Al-Yousuf *et al.*, 2009). Studies from the field and laboratory works showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period; although some other environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal's accumulation and toxicity to fish (Benaduce *et al.,* 2008; Linbo *et al.*, 2009; Ebrahimi and Taherianfard, 2011; Jitar *et al.,* 2014). Ecological needs, size and age of individuals, their life cycle, feeding habits, and the season of capture were also found to affect experimental results from the tissues (Jitar *et al.;* 2014; Onen *et al.*, 2015). Fish have the ability to uptake and concentrate metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates, and aquatic vegetation (Polat *et al.*, 2015). Fish accumulate pollutants preferentially in their fatty tissues like liver and the effects become apparent when concentrations in such tissues attain a threshold level (Omar *et al.,* 2014).

However, this accumulation depends upon their intake, storage and elimination from the body (Abdallah and Morsy, 2014). This means that metals which have high uptake and low elimination rates in tissues of fish are expected to be accumulated to higher levels (Kalay and Canli, 2000; Idriss and Ahmad, 2015). Heavy metals can be taken up into fish either from ingestion of contaminated food via the alimentary tract or through the gills and skin Drevnick *et al.,* 2006; Sfakianakis *et al.,* 2015). Effectively, after the absorption, metals in fish are then transported through blood stream to the organs and tissues where they are accumulated (Adeyemo *et al.,* 2010; Fazio *et al.,* 2014). The heavy metal concentration in fish tissues reflects past exposure via water and/or food and it can demonstrate the current situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment Birungi *et al.,* 2007). The obvious sign of highly polluted water, dead fish, is readily apparent, but the sublethal pollution might result only in unhealthy fish. Dupuy *et al.* (2014) reported that the fish health status in some polluted systems (estimated by the condition factor) indicated that the fish have a lower condition. Very low-levels of pollution may have no apparent impact on the fish itself, which would show no obvious signs of illness, but it may decrease the fecundity of fish populations, leading to a longterm decline and eventual extinction of this important natural resource (Ebrahimi and Taherianfard, 2011).

Also, heavy metals are known to induce oxidative stress and/ or carcinogenesis by mediating free radicals/reactive oxygen species Javed *et al.,* 2015). In general, metals can be categorized as biologically essential and non-essential. The nonessential metals (e.g., aluminum (Al), cadmium (Cd), mercury (Hg), tin (Sn) and lead (Pb)) have no proven biological function (also called xenobiotics or foreign elements), and their toxicity rises with increasing concentrations (Sfakianakis *et al.*, 2015). Essential metals (e.g., copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), cobalt (Co), molybdenum (Mo) and iron (Fe)) on the other hand, have a known important biological role (Abadi *et al.,* 2014), and toxicity occurs either at metabolic deficiencies or at high concentrations (Sivaperumal *et al.*, 2007). The deficiency of an essential metal can therefore cause an adverse health effect, whereas its high concentration can also result in negative impacts which are equivalent to or worse than those caused by non-essential metals (Kennedy, 2011). Moreover, the toxicity of metals to fish is significantly affected by the form in which they occur in water. The ionic forms of metals or simple inorganic compounds are more toxic than complex inorganic or organic compounds. Therefore, this article is aim at discussing the roles of fishes as bioindicators in an Aquatic environment.

II. CLASSIFICATION OF BIOINDICATORS

According to Butterworth *et al.* (2000), different types of bioindicators can be classified from different perspectives (Figure 1).

III. CLASSIFICATION ACCORDING TO USAGE

Bioindicators are useful in three situations (Butterworth *et al.*, 2000):

1) Where the indicated environmental factor cannot be measured, *e.g.*, in situations where environmental factors in the past are reconstructed such as climatic change, studied in palaeo-biomonitoring.
2) Where the indicated factor is difficult to measure, *e.g.*, pesticides and their residues or complex toxic effluents containing several interacting chemicals and
3) Where the environmental factor is easy to measure but difficult to interprete, *e.g.,* whether the observed changes have ecological significance.

IV. CLASSIFICATION ACCORDING TO AIM OF BIOINDICATION

According to the aim of bioindication, three types of bioindicators can be distinguished:

a. Compliance indicators

These are those are chosen to assess the attainment and maintenance of ecosystem objectives related to the restoration and maintenance of environmental quality; For example, fish population attributes are measured at the population, community or ecosystem level and are focused on issues such as the sustainability of the population or community as a whole.

b. Diagnostic indicators

These are measured on the individual or suborganismal (biomarker) level, with early warning indicators focusing on rapid and sensitive responses to environmental change. Accumulation bioindicators (*e.g.* mussels, mosses, lichens) are distinguished from toxic effect bioindicators, with the effects being studied on different biological organization levels. It provides insight into the cause of noncompliance.

c. Early warning indicators

Just like Diagnostic bioindicators, they are measured on the individual or suborganismal (biomarker) level, with early warning indicators focusing on rapid and sensitive responses to environmental change. And the accumulation bioindicators (*e.g.* mussels, mosses, lichens) are distinguished from toxic effect bioindicators, with the effects being studied on different biological organization levels. But unlike Diagnostic indicatos, it allow for management actions to be implemented before conditions have deteriorated to the point where compliance indicators become relevant. In many bioindication programmes fish meet the requirements of all three types.

V. CLASSIFICATION ACCORDING TO DIFFERENT APPLICATION

According to the different applications of bioindicators, three categories can be distinguished (Butterworth *et al.*, 2000; Muhar *et al.*, 2000):

a. Environmental indicator

This is a species or group of species responding predictably to environmental disturbance or change (*e.g.* sentinels, detectors, exploiters, accumulators, bioassay organisms). An environmental indicator system is a set of indicators aiming at diagnosing the state of the environment for environmental policy making.

b. Ecological indicator

This is a specie that is known to be sensitive to pollution, habitat fragmentation or other stresses. The response of the indicator is representative for the community.

c. Biodiversity indicator

The species richness of an indicator taxon is used as indicator for species richness of a community. However, the definition has been broadened to "measurable parameters of biodiversity", including *e.g.* species richness, endemism, genetic parameters, population-specific parameters and landscape parameters.

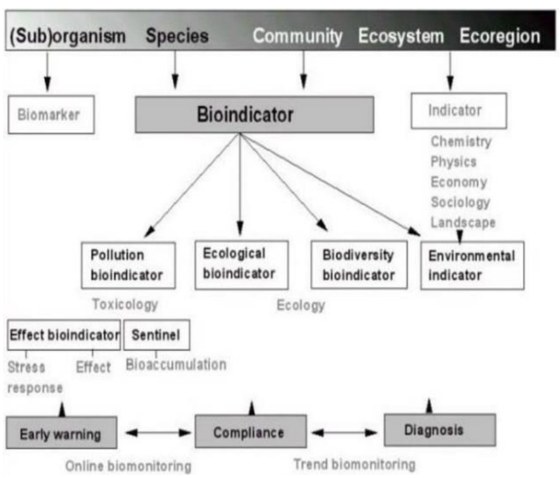

Figure 1: Classification of bioindicators in the context of their use in biomonitoring (Source: Butterworth *et al.*, 2000).

VI. WHY FISH ARE USED AS NATURAL CHARACTERISTICS

There are several reasons why fish are widely used to describe natural characteristics of aquatic systems and to assess habitat alterations (Boon *et al.*, 2000; Schiemer, 2000; Schmutz *et al.*, 2000):

1. A long tradition of ecological, physiological and ecotoxicological research on fish has led to an advanced knowledge of the ecological requirements of a large number of fish species. Schiemer *et al.* (2001) added that effectiveness of bioindication approaches depends on the sound knowledge of the indicators' ecological demands and physiology.
2. A large number of abiotic environmental variables at different spatio-temporal scales are linked to the complex habitat requirements of particular species and their ontogenetic stages. Due to the specific habitat requirements and habitat shifts during the larval and juvenile stages, O+ fish for example are suitable indicators of the ecological status of river systems (Keckeis and Schiemer, 2001).
3. As migratory organisms fish are suitable indicators of habitat connectivity or fragmentation (e.g. Chovanec *et al.*, 2002).
4. Due to the size of fish (and their organs) a great variety of analytical procedures can be carried out. Pathological results concerning fish illustrate the effects of water pollution to the scientific community, water management and the public. Some methods, such as haematological and histo-pathological approaches, are taken from human medicine (Chovanec *et al.*, 2002).
5. Due to the longevity of fish certain indication effects, e.g. accumulation processes, are increased (Keckeis and Schiemer, 2001).
6. As primary and secondary consumers at different levels fish reflect trophic conditions in aquatic systems (Schiemer, 2000).
7. The reconstruction of pristine reference communities is possible due to the existence of historical information (Muhar *et al.*, 2000).
8. Fishery and sport fishing have a long history, in which fish play an important role as indicators of water quality; because of the use of fish by man particularly as food resource, the condition of fish communities is an important factor in water resource management (Schiemer *et al.*, 2001).
9. Depending on the problem and the indication approach selected, bioindication by using fish often meets the requirements of both top-down approaches (assessing changes in communities in the natural environment and testing for sources and causes of possible problems) and of bottom-up assessments (using laboratory data to model

changes in the more complex natural ecosystems) (Walz, 2000).

10. The number of species is relatively small and species are already determinable in the field (Walz, 2000).

When using fish as bioindicators problems may arise according to Chovanec *et al.,* 2000a:

i. Fishery-caused alterations, such as species transfer, stocking, overfishing, make it more difficult to discuss other man-induced degradations of aquatic ecosystems.
ii. The mobility of many species makes it difficult to identify not only the exact source of pollution, but also the time and duration of exposure.

VII. FISH AS BIOINDICATOR

- An Overview of the Roles of Fish as Bioindicator

Nowadays water pollution is the burning issue 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 concentration but toxic and harmful in higher concentrations. Having in mind that not all chemical forms of pollutants are equally bioavailable and some pollutants can be accumulated in living organisms to a greater extent than others, there is a need to study the levels of pollutants in the organisms to be able to predict the environmental risk. 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 (Yancheva *et al.,* 2015). 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 (Souza *et al.,* 2013). Due to feeding and living in the aquatic environments fish are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants. Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health. Heavy metals are produced from a variety of natural and anthropogenic sources. In aquatic environments, heavy metal pollution results from direct atmospheric deposition, geologic weathering or through the discharge of agricultural, municipal, residential or industrial waste products (Tashla *et al.,* 2018). Heavy metals are able to disturb the integrity of the physiological and biochemical mechanisms in fish that are not only an important ecosystem component, but also used as a food source. Previous studies have shown that marine and farmed fish and shellfish are significant contributors to consumer intake of some contaminants due to their presence in the aquatic environment and their accumulation in the flesh of fish and shellfish. The objective of this article is to describe the effects of different persistent organic pollutants and heavy metals on the fish used as bio indicator of environmental pollution. Fish have 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 (Mendil *et al.,* 2010). 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.

Over the last 150 years, aquatic systems worldwide have been impacted by a wide array of anthropogenic factors (Leung *et al*., 2000). Human activities may alter the physical, chemical or biological processes associated with water resources and thus modify the resident biological community.

Karr and Chu (1999) identified five primary classes of environmental factors, that, when affected by human activities, result in ecosystem degradation:

1. Food/energy source:

Example type, amount, and particle size of organic material entering a stream increased fine particulate organic matter from the riparian zone versus primary production in the stream

seasonal pattern of available energy

2. Water quality: Example dissolved oxygen, nutrients, heavy metals and toxic substances,

organic and inorganic chemicals, natural and synthetic, temperature, turbidity, pH ;

3. Habitat structure: Example substrate type, water depth and current velocity, spawning, nursery, and hiding place, diversity (pools, riffles, woody debris, basin size and shape;
4. Flow regime: Example water volume, temporal distribution of floods and low flows;
5. Biotic interactions: Example competition, predation, completion, parasitism.

In most cases biological communities are sound and precise indicators of the status of the aquatic system as they are subject to the full range of chemical and physical influences, additive and synergistic effects included. In this context fish play a crucial role as bioindicators in water resource management and applied limnological research: fish serve as "ecological indicators", "keystones", "umbrellas", "flagships" and "vulnerables"(Omar *et al.,* 2014). According to Tashla *et al.* (2018) a bioindicator is an organism (or a part of an organism or a community of organisms) that contains information on the quality of the environment. Thus, the use of bioindicators should help to describe the natural environment, to detect and assess human impacts and to evaluate restoration or remediation measures; in all these cases fish are intensively used for indication purposes.

The spatial changes of fish communities along the course of river systems and the use of fish zonation patterns for river classification are examples of some of the most traditional bioindication approaches (Butterworth *et al.,* 2000; Omar *et al.,* 2014). Fish have also been traditionally used for classifying different types of standing waters. The nature of the fauna in stagnant water bodies reflects their morphometry, trophic status, thermal and oxygen stratification and the extent of littoral development (Omar *et al.,* 2014).

- Persistent Organic Pollutants and their Effect on Fish Target Organs

The fish gills are multifunctional organs involved in ion transport, gas exchange, acid–base regulation and waste excretion. 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. 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. 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 (Playle *et al.,* 2011). Fish metabolism, acting principally through the gills can be seriously damaged since toxicant incorporation occurs mainly through this respiratory organ. 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 (Tashla *et al.,* 2018). 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 (Georgieva *et al.,* 2014).

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. 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. The liver is also a target organ due to its large blood supply which causes noticeable toxicant exposure. In addition, liver 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 (Monteiro *et al.,* 2013). 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. The kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids and, besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotics to which the fish may be exposed. Many studies showed that different toxicants accumulate mainly in metabolic organs such as the liver and kidney which can lead to many histological alterations. Levels of heavy metals such as lead, copper, cadmium, and zinc in marine fish have been extensively documented. 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 (Siscar *et al.,* 2014).

The fish meat 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 (Ljubojević *et al.,* 2014). On the other hand, fish muscle may be the depositary for different contaminants, which occur in the water ecosystem. Such environmental pollutants are dioxins and PCBs, heavy metals, and organochlorine pesticides are a global threat to food safety, thus fish meat could lose these properties due to environmental contamination. Hydrobionts can bioaccumulate many of these contaminants potentially making seafood of concern for chronic exposure to humans. The metal concentrations in the water are positively correlated with the concentrations in fish tissues, but some research has founded that the metal concentrations in the sediments are the most important factor for their levels in the aquatic biota (Widianarko *et al.,* 2000). 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 Japan as a result of poisoning caused by the consumption of mercury contaminated fish. Therefore, concern regarding the presence of heavy metals and other contaminants in seafood has arisen during the last decades.

- Fish as Indicators of Environmental Pollution

Despite rising efforts of many industrialized countries to reduce toxicants from industrial and motor vehicle exhausts and to purify industrial and communal waste waters, our ecosystems still contain harmful concentrations of an increasing number of chemicals. They accumulate in soils and sediments from which they can be remobilized after changing their physico-chemical condition, and many of these substances persist for decades (e.g. DDTs, PCBs). Concentrations of heavy metals in sediments may exceed those of the overlying water by a factor of one to ten thousand (Yancheva *et al.,* 2015). Even remote areas such as high mountains and arctic regions receive significant amounts of pollutants by atmospheric deposition after transport over long distances (Chovanec *et al.,* 2003). Relatively small quantities of toxicants may threaten these highly vulnerable ecosystems (Chovanec *et al.,* 2003).

The water quality of many rivers and lakes has improved significantly due to the increasing number of purification plants. However, the treatment of waste water reduces not only the concentration of toxic substances but also that of non-toxic organic compounds. This may lead to changes in the bioavailability of chemicals and their toxicity, in particular of those entering the water by run-off and atmospheric deposition.

Suspended inorganic and organic particles have a large surface area and thus a high capacity for physically absorbing toxicants. Toxic chemicals have been shown to interact with dissolved or colloidal organic matter by various modes of binding and absorption (Omar *et al.,* 2014). Many of these complexes are too large or too polar to diffuse across the gill membrane (Jungwirth *et al.,* 2000). Some metal cations can form lipophilic complexes with specific organic compounds used in agriculture, forestry and industry (e.g. ditiocarbamates, diethyldithiophosphate) which easily pass the gill membrane. This leads to both higher levels of metal accumulation than expected from water concentrations and an altered distribution pattern, with the highest increase in the brain and eyes of fish (Abumourad *et al.,* 2014). Uptake and toxicity of mercury strongly depends on methylation by bacterial activity (Boening, 2000).

Due to its lipophilic character, methyl mercury is absorbed about ten times faster than the ionic form. On the other hand, several studies have shown that selenium may reduce mercury toxicity (Dhanakumar *et al.,* 2015). Bioavailability and toxicity of metals are controlled not only by suspended particles and dissolved organic matter but also by water parameters such as hardness, alkalinity, pH, temperature, and oxygen concentration (Tashla *et al.,* 2018). Some of these factors modulate the speciation of trace metals (Abadi *et al.,* 2014). Hydrate ions and hydroxo-complexes are the most bioavailable forms of metals absorbed by fish gills (Chovanec *et al.,* 2003). However, metals behave differently in natural waters: The speciation of Pb, Cu, Hg, and Al is highly affected by pH, whereas that of Cd and Zn is only slightly sensitive to pH alterations (Abadi *et al.,* 2014). The calcium concentration of the water has a major impact on metal speciation and the permeability of gill membranes. Competition between divalent metal ions and calcium for binding sites on the gill surface and the passage through ion-sensitive channels reduce the uptake and toxicity of metals in hard water (Souza *et al.,* 2013). Uptake of chemicals across gill membranes is also a function of water flow along the gills (Mendil *et al.,* 2010). As a consequence, rising temperature, oxygen depletion and metabolic stimulation (e.g. during reproduction or stress) accelerate gill ventilation and thus the uptake of toxicants.

These examples demonstrate that simple approaches of chemical water analyses often fail to detect environmental changes that are harmful for aquatic organisms. The complex situation in natural waters, with their synergistic and antagonistic effects, makes it difficult to predict the impact of toxicants on the ecosystem. In many cases, the input of toxicants is not constant but intermittent and may remain undetected.
Bioindicators and, in particular, long-living organisms such as fish are sensitive to the impact of a complex mixture of chemicals on a specific aquatic ecosystem, integrating the environmental load over time and space. Pollutants usually cause a wide spectrum of effects and responses in organisms ranging from the cellular and biochemical level to the level of behaviour, growth and reproduction. During low and limited exposure to toxicants, fish respond at a sub-cellular level, but usually organisms can compensate for the toxic effect, and their health is not seriously affected. Prolonged and severe exposure, however, may induce a sequence of functional and structural changes which impair vital functions. Tissue concentrations of chemicals are excellent indicators of the environmental load of a specific toxicant but usually do not directly reflect the physiological and ecological consequences. Most of the biomonitoring techniques, however, focus on different kinds of stress responses which are often more or less general responses and cannot be attributed to specific toxicants. Permanent stress - even if it is moderate - interferes with hormonal and biochemical processes leading to increased metabolism, immunosuppression, disturbed osmoregulation, failure of reproduction or tissue damages. The low toxicant specificity of many stress responses is not just a disadvantage, it increases the value of bioindicators for monitoring the general environmental load in natural water bodies which may contain several out of hundreds of different harmful chemicals. For practical use in the field, biomonitoring methods based on fish should be insensitive to the stress of capture which may mask the effects of toxicants. The biological parameters analysed in the assay should be well understood and their modulation induced by endogenous and exogenous factors other than toxicants should be known. Data on commercially manipulated fish species should be handled with caution, and possible loads of geogenic origin (e.g. metals) have to be considered.

- Toxicant Accumulation in Fish Tissues

Tissue concentrations of chemicals are a function of uptake, storage, and excretion.

In fish, two different routes of uptake are important,
1) Directly from the water, in freshwater fish almost exclusively via the gills, in marine species at a low percentage also through the drinking of water, and
2) The oral uptake and assimilation of contaminated food. Hydrophilic molecules are unlikely to pass the gill membrane unless they are very small (diffusion along an osmotic gradient) or transported by ionic pumps or channels. Lipophilic compounds, however, are soluble in biological membranes and cross all barriers.

The relatively low oxygen solubility in water requires an extremely large respiratory surface and a high pumping rate of water. Consequently, the direct uptake of water-borne toxicants (whose concentration is two orders of magnitude higher than in the air) is the main route in fish (bioconcentration; Table 1).

Table 1: Definition of terms used in ecotoxicology (Wagner and Boman, 2003)

Bioaccumulation (BA)	The accumulation of contaminants in organisms resulting from water or food uptake.
Bioconcentration (BC)	The accumulation of water-borne contaminants directly from the water by a non-dietary route.
Biomagnification	The accumulation of toxicants resulting from ingestion of contaminated diet.
Bioconcentration factor (BCF)	Quotient of the concentration of a chemical in an aquatic organism and in the water. The BCF can be predicted from the concentration of a lipophilic chemical in the water and its K_{ow}.
Log K_{ow}	Octanol-water partition coefficient: In most cases, the BCF is proportional to the logarithm of K_{0w}

Diet, as the most significant source of toxicants leading to biomagnification along the food chain, is usually restricted to lipophilic compounds which are almost insoluble in water (compounds with log K_{ow} values of 5- 8) and slowly metabolised, example, halogenated contaminants and pesticides which resist biotransformation (Mackay and Fraser, 2000). Lipophilic contaminants are predominantly stored in lipids including biological membranes and muscles, thus being of major concern for human nutrition. On the other hand, lipids serve as a protective reservoir for lipophilic chemicals, and therefore their toxicity decreases with rising lipid content of fish (Garcia *et al.,* 2015). Top predators (example piscivorous fish) and species with high lipid contents have been shown to be the most sensitive indicators for environmental contamination with lipophilic compounds (Garcia *et al.,* 2015).

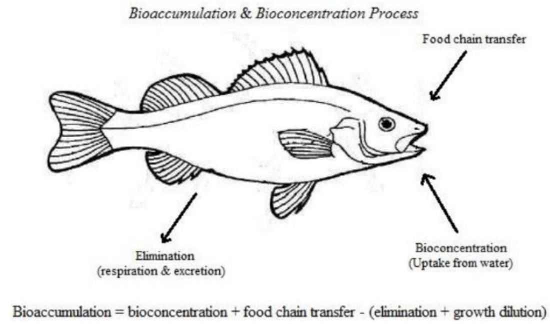

Figure 2: Illustration of Bioconcentration and Bioaccumulation (Kaya *et al.*, 2002).

Liver and kidney are the main sites of accumulation for most toxicants including metals. These organs are rich in metallothioneins with high affinities to Cd, Hg, Zn, and Cu. The liver is also involved in a variety of detoxification processes transforming harmful compounds into less toxic and water-soluble metabolites which are excreted into the bile. These metabolites are either eliminated with the faeces or reabsorbed from the gut and returned to the liver by enterohepatic circulation which may increase the half-life of toxicants in the fish. In the bile of trout exposed to several labelled organic substances Sivaperumal *et al.* (2007) found concentrations between 11 and 10,000 times higher than in the water. Even under field conditions it has been shown that bile analysis is a useful tool to evaluate the environmental load of xenobiotics (Pointet and Milliet, 2000).

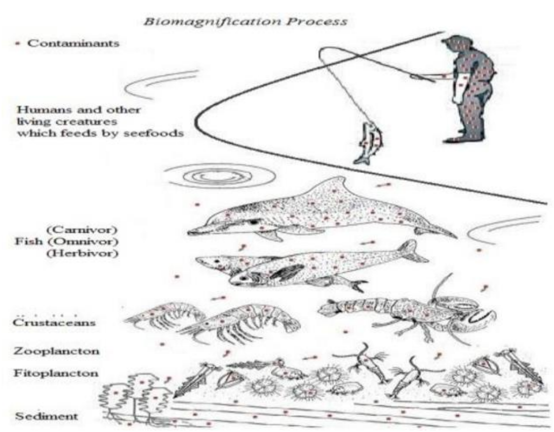

Figure 3: Illustration of Biomagnification (Vural, 2005)

The proportion of accumulated toxicants between different tissues of the fish largely depends on dynamic processes between uptake, storage, and elimination. After shorten exposure, gills or the digestive tract and the liver usually show a high load of toxicants, whereas concentrations in kidney, bones (Pb, Zn), and muscles (lipophilic substances) increase more slowly after a time-lag, but the accumulated chemicals are more persistent than in other organs (Pointet and Milliet, 2000).

Due to active regulation tissue accumulation of essential metals (Cu, Zn) is saturated at low levels, and thus a relatively weak indicator of environmental contamination (McGeer *et al.,* 2000). Acute intoxication stimulates mucus secretion which can act as a chelator (Garcia *et al.*, 2015). This may explain at least some of the elevated metal concentrations observed in fish gills (Pointet and Milliet, 2000). Strongly varying proportions of inorganic and organic contaminants between tissue concentrations in wild captured fish presented in Chovanec *et al.* (2003) are not only due to different environmental conditions and exposure times but also to species- or family-specific patterns. Salmonids, e.g., have higher copper concentrations in the liver than other families.

CONCLUSION

Fish are one of the most frequently used group of bioindicators in ecotoxicological field studies. The advantage of a comprehensive basic knowledge of toxicology, physiology, and histology exceeds the disadvantage of fish mobility. No other aquatic organism is suitable for the application of so many different methods which allow the evaluation of the severity of toxic impacts ranging from compensatory responses at a molecular and an ultrastructural level (serving as an early warning indicator) to sublethal and pathological changes as alarm signals for population declines and irreversible consequences for the whole ecosystem (Figure 3). The bioindication of the occurrence of specific substances and their impact on specific biota and the ecosystem are the main focuses of ecotoxicological studies. Several methods of ecological and toxicological relevance with varying specificity have to be applied simultaneously to evaluate the ecotoxicological situation under the complex environmental conditions in the field.

Due to its complex habitat requirements the fish fauna is a crucial indicator of the ecological integrity of aquatic systems at different scales, from microhabitat to catchment. The fitness of fish species both at the individual level (e.g. growth performance) and at population level (e.g. population structure) is determined by the connectivity of different habitat elements in a broad spatio-temporal context. Thus bioindication using fish represents a good monitoring tool especially with regard to river engineering, e.g. river restoration and management.

In order to further strengthen the role of fish as valuable indicators of the ecological integrity of aquatic systems, research is required ranging from the ecological demands of certain target species to ecosystem processes. The will as well broaden knowledge in aquatic environmental impact assessment by the use of fish as a bioindicator to assess aquatic environment.

RECOMMENDATION

Bioindication based on the use of fish generally satisfies the criteria against which biological monitoring programmes should be judged, it is therefore recommended that:

a. The range of responses must be suitable for the intended application; factors of different strength should lead to reactions of different intensity (no all or none response, no extreme natural variability) base on Sensitivity to stressors.
b. The range of response has to be sensitive to the environmental factors and conditions being observed.
c. Methods have to be broadly applied in a wide range of stressors and sites.
d. The results obtained by bioindication programmes have to be representative of many parts of the aquatic communities.
e. Information has to be provided fast enough to initiate effective management action before unacceptable damage has occurred.
f. Standardized methods are necessary for obtaining comparable results.
g. Bioindicators should be cost-effective to collect and identify.

h. The application of bioindicators should be possible at a local scale as well as at a regional or landscape scale.

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