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Detection of heavy metal biomarkers for study of fishes

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ABSTRACT: There have been relatively few studies of marine fish, perhaps because contaminated discharges generally receive much greater dilution in salt waters than in fresh waters, a fact that probably discouraged an early search for effects in the sea. Mercury a highly toxic metal, results in a variety of adverse health effects. Due to wide use of mercury in agriculture, industrial, medical and other fields, its exposure is cannot be avoided. The toxicity of mercury depends greatly on the forms of the mercury compounds (elemental, inorganic and organic). Excessive levels of mercury in the marine environment can affect marine biota and pose risk to human consumers of seafood. Hence, mercury compounds found in the marine environment pose risk to human health through the consumption of contaminated seafood. Accordingly, it is desirable to minimize such exposure to levels that do not cause adverse effects. In an attempt to define and measure the effect of pollutants on an ecosystem, biomarkers have attracted a lot of interest. The underlying principle of the biomarker approach is the analysis of an organism's physiological or biochemical response to pollutant exposure. Mercury induced oxidative stress; make an important contribution to molecular mechanism for liver injury, thus enzymatic biomarkers is proved to be suitable biomarkers in mercury studies. The measurement of biochemical and physiological parameters is a diagnostic tool commonly used in aquatic toxicology and biomonitoring, so Hematological and immunological parameters are suitable biomarkers in mercury studies. During stress, fish respond in a number of ways in order to regain homeostasis and two important physiological processes which are modulated when fish are exposed to stress, are hormonal status and immune function, Therefore, alterations in specific hormonal functions and consequent biochemical effects may constitute important stress biomarkers. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, which are responsible for vital functions in the fish.

***Keywords*:** Biomarker, Marine Fish, Heavy metal, Mercury.

INTRODUCTION

Marine Fish

Fishes have been used for many years to monitor the pollution status of aquatic ecosystems, and are thus regarded as excellent biological indicators of heavy metals in aquatic environments. Bioaccumulation of heavy metals by absorption across the entire body surface of the fish as well as purification in the livers has been confirmed.

Heavy metals contamination may have devastating effects on the ecological balance of the marine environment and a diversity of aquatic animals (Farombi, *et al.,* 2007). Aquatic ecosystems are typically monitored for pollution of heavy metals using biological assays. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants and also fishes are often the primary consumers of marine ecosystems and so heavy metals accumulation in fish can function as an environmental indicator of the marine ecosystems (Olaifa *et al.,* 2004). Fish are extensively used to evaluate the health of aquatic ecosystems because pollutants like heavy metals accumulate in the food chain and are responsible for harmful effects and even death in the marine ecosystems (Farkas *et al.,* 2002).

Fish and shellfish tend to accumulate high concentrations of mercury in compare to other animals, principally because fish extensively feed on smaller aquatic organisms that accumulate this compound in their body (WHO, 2003). The amount of mercury in fish is normally depended to many factors including the size and age of the fish, its trophic position in food chain, as well as the mercury content in water and sediment and the temperature and pH of the water (WHO, 2003).

The various studies on different fish species have confirmed that heavy metals may cause undesirable changes in the physiological activities and biochemical parameters both in tissues and in blood (Basa and Rani, 2003). The marine fishes developed a protective defense mechanism against the harmful effects of xenobiotics like essential and inessential heavy metals that produce degenerative changes in the fish physiology (Abou EL-Naga et al., 2005).

There have been relatively few ecotoxicological studies on marine fish compare to freshwater fish, maybe because of large environment of sea, contaminated discharges receive much greater dilution in marine waters than in rivers and lakes that have small enclosed medium, a fact that discouraged an early search for effects of xenobiotics on marine fish. For many reasons, it is nonetheless timely to review progress in this field. For example, the species diversity of marine fish is higher than most freshwater assemblages, and there is considerable site-specific variation of response in fish to endocrine disruptors, this raises the possibility of new modes of action in marine species. Secondly, marine fish except the cyclostomes and elasmobranches exhibit the unique osmoregulation, ability to maintain their hypotonic body fluids by drinking sea water, a mechanism that exposes them to dissolved contaminants to a greater degree than freshwater fish. Their different mechanism may also have a bearing on their responses to endocrine disruptors, although they generally possess similar endocrine systems to the freshwater species.

Development of the Biomarker approach

In trying to define and measure the effect of pollutants on marine environment, biomarkers have been more interest. The doctrinaire principle of the biomarker approach is the assay of an organism's physiological or biochemical response to pollutant exposure, because toxic effect explicit itself at the sub cellular level before it becomes apparent at organ levels of biological organization. The measurement of biochemical responses to heavy metals will improve the assessment of biologically significant exposures to toxic metals and enhance the ability to evaluate the effects of xenobiotics on the health and survival of toxicant exposed fishes. With compare to direct monitoring method, biomarkers have the advantage of being more relevant biologically (Rees, 1993). S Scientific studies for the identification of marine pollution biomarkers have been carried out extensively in animals in general and then fish in particular (Oikari and Jimenez, 1992).

A combination of physical, chemical and biological indicators is frequently used to evaluate water pollution (Karr, 1993). But in last decade, there has been a growing awareness of the need to detect and evaluate the effects of pollution in living organism (Schlenk, 2003). Heavy metal concentrations in aquatic organism are often more than aquatic environment in which the organisms resides. This suggests that organisms can be used as biological markers of metal pollution (Flessas *et al* 2000).

Fish are largely being used for evaluate of water toxic metals and can serve as bioindicators of environmental pollution. The ability to accuracy predict of the bioaccumulation of toxicant in fish has become an essential component in evaluation of the ecological and human risks in exposure to toxic pollutants, and also such estimates are needed to assess more accurately potential ecological risks to fish assemblages themselves. The long time exposure of fish to toxicants results primarily accumulation in their organs and tissues and secondly in sub cellular alterations due to their continuous deleterious action. Although exposure-referenced toxicological benchmarks such as the LC_{50} and the EC_{50} have been widely used to make toxic evaluation, most harmful effects of chemical pollutants are because of accumulation of those compounds, more than their environmental concentrations per se. A range of different biomarkers have been used to indicate the biological effects of certain pollution on fish, both in natural environments and under experimental conditions (Ferrando et al., 2006).

The historical development of the biomarker approach can be seen to have close links with medicine and vertebrate biology (National Research Council, 1987). However, biomarker measurements are completely possible in invertebrate organisms (Depledge & Fossi, 1994). There are several reasons why studies on fishes are better for ecological risk assessment. For example, fishes constitute more than 28000 species, they are major components of all ecosystems, and fish populations are often numerous, so that samples can be taken for analysis without significantly affecting population dynamics. Increasing knowledge of the physiology of fishes now permits reasonable interpretation of biomarker responses in terms of ecological risk assessment.

Application of Biomarkers

The investigation of the proper biomarkers for the best possible diagnoses is very important for researchers. Environmental experiments involving the use of biomarkers are recognized as one of the most powerful tools for the investigation of pollutants (Depledge, 1993). In the biomarker approach it is either the activated defense mechanisms or the toxic effect that is measured in an organism, or both.

Biochemical biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to toxic substances. Choose of the suitable biological effect markers for the study of the chronic and sub chronic contaminant is frequently a controversial issue, when information on the mechanism of action of the contaminant is incomplete. But even very low exposure to contaminants may cause various biological effects. (Toguyeni et al., 1997).

The application of biomarkers for mercury toxicity is of most interest to monitor not only the presence of mercury in the animal body, but also its bioavailability and capacity to find biological responses. Many biomarker studies examine the effects of toxicant exposure on whole soft tissue homogenate, but this approach may not always be suitable, as the partitioning of compounds among different tissues may largely influence toxicity and may be masked when measurements are restricted to whole body concentrations (Depledge, 1993). For example different body tissues have various abilities to accumulate metals (Gundacker, 1999), therefore using a suitable biomarkers from a blood that is current in whole body can produce more specific and relevant results to making an actual face of what is occurring in the ecosystem.

Exposure to environmental toxicants can launch the activation of defense mechanisms, the performance of which determines the toxic effect on the organism (Blaise et al., 2002). Biomarker approach measure directly the concentration of a toxin in an organism, not accounting for the biological effect, that it is one of the most important advantage of biomarkers (Depledge, 1993).

Mercury

Mercury a hazardous metal may cause adverse health effects including neurological, renal, respiratory, immune, dermatologic, reproductive and developmental sequels in wild life (Risher and Amler, 2005). Due to wide use of mercury in agriculture, industrial, medical and other fields, its exposure is cannot be avoided. The toxicity of mercury depends greatly on the forms of the mercury compounds (elemental, inorganic and organic). Inorganic mercury present in the ecosystem is a hazardable toxicant to human health (WHO, 1991).

Mercury is a heavy metal with no known exact metabolic function. Because of its unique properties, mercury present in the ecosystem in different physical and chemical forms (Zalups, 2000). Mercury is considered strongly neurotoxic in humans and wildlife (Díez, 2008).

Mercury is considered strongly neurotoxic in humans and wildlife (Díez, 2008). Although mercury chloride is not the most hazard compound in the aquatic ecosystems (Boudou and Ribeyre, 1997), it is the key form between the gaseous metal form transported through atmosphere and the methylmercury form that bioaccumulates in organism. As soon as entrance of mercury into the organism body, it can show various immunotoxic effects.

Mercury is a naturally presence metallic element that is every where throughout the ecosystems. It is occurance in several inorganic forms including metallic (Hg0), mercurous (Hg+), and mercuric (Hg2+) valence states (Daintith, 1996). Mercury is a liquid metal at natural temperatures and pressures. It forms salts in two ionic states mercury (I) and mercury (II). Mercury (II), or mercuric salts, are more common in the ecosystem than mercury (I) or mercurous salts. These salts, if soluble in water, are bioavailable and will be hazardous (Boening, 1999).

Factors effected mercury levels in fish can be divided into exogenous (quality of the water body) and endogenous (quality of the individuals or species). Exogenous factors include pH, sulfur and organic matter. Endogenous factors include species, gonadic stage, habitat and food preferences, metabolic rate, age, growth rate, size, mass, and diet. Numerous studies have shown that larger, longer, and older fish have upper concentrations of mercury. However, the relationship varies among species. Species with short time period tend to have a flatter relationship due to a faster assimilation into tissue than accumulation of mercury (Jackson 1991).

There is a variety of significant human and wildlife health with the geographically common prevalence of elevated levels of both inorganic and organic mercury compounds in aquatic organism. Also mercury is persistent contaminant occurring in several chemical forms in aquatic environment and is particularly hazardous to living organisms due to its ability to accumulate and its toxicity at very low concentrations (Devlin, 2006).

Mercury toxicity

All forms of mercury are toxic and exposure their influence on many organs, tissues, and cell systems. Mercury is global concern as an ecosystems contaminant because it is stable, persistent, and cannot be degraded or destroyed. Inordinate levels of mercury in the marine ecosystems can affect marine organisms and pose risk to human consumers. Thus, mercury compounds found in the marine ecosystems pose risk to human health through the consumption of contaminated fish and shellfish. Eventually, it is desirable to minimize such exposure to levels that do not cause adverse influences (Steuerwald et al. 2000).

Toxicological effects of mercury have long been known. The influences described depend upon different parameters like dose and exposure route, but they include organ lesions (kidney, liver and gill), neurological effects and hematological and Immunological abnormality (Sweet, and Zelikoff, 2001).

Exposure to mercury in human and wildlife induces too many adverse health effects including: cardiovascular disease, anemia, developmental abnormalities, neurobehavioral disorders, kidney and liver damage, and even cancer in some cases. In many research, the toxicity of mercury has been attributed to its high affinity to protein-containing sulfhydryl groups. Based on the chemical, physical, biological, and environmental features of the different forms of mercury, it confirmed that inorganic mercury, is the form most likely to pose a hazard in drinking waters, One of the mechanisms by which mercury exerts its toxic effect is through impairment of cellular respiration by the inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation.

Mercury's inhibition of cellular enzymatic processes by binding with the hydroxyl radical (SH) in amino acids is the major toxicity of mercury. This is the major part in mercury's ability to elicit allergic/immune reactive conditions (Nielsen, and Hultman. 2002). Binding with proteins include the blockage of sulfur oxidation processes is one of the cellular level enzymatic effects of mercury, also enzymatic processes involving vitamins B6 and B12 (Sweet, and Zelikoff. 2001), effects on cytochrome-c energy processes (Kirkland, and Franklin. 2001), along with mercury's adverse effect on the cellular mineral levels of calcium, magnesium, zinc, and lithium are another cellular levels toxicity of mercury. Mercury has also been found to cause additional neurological immune systems effects through immune and autoimmune reactions. Cytotoxic role and induction of apoptosis in various cells lines is confirmed as a toxic effect of mercury. (Sutton and Tchounwou, 2006).

Beside acute effects, Chronic or sublethal exposures to mercury have been shown to adversely impact reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation, and oxygen exchange in aquatic biota (Eisler 1973). Also high concentrations of mercury have been associated with developmental and behavioral abnormalities, impaired reproduction and survival, and in some cases with direct mortality.

In compare to other pollutant, in recent yeas most extensively studied was done on mercury, but the study on the distribution in various environmental or body compartments is more than studies on effects at the organism, population and ecosystem level. There remain substantial gaps is knowledge of the effects of mercury on different kinds of organisms, on different trophic levels, and on ecosystem function itself.

As mercury has transferred ability through trophic levels, there can be direct contamination (uptake from the water column through gills or by ingestion) as well as uptake from food. Ecological effects of pollutant can be measured by some indices, probably adverse, on microorganisms, plants, and animals that make up the decomposer, producer and consumer trophic levels of ecosystems. The endpoints in individuals exposed to mercury are changes to behavior, physiology, biochemistry, reproduction, and longevity. Species endpoints can include changes in survivorship and population structure, population declines or local extinction. Ecological endpoints include changes among the species interactions, usually reflected in food webs, the patterns of energy use and production.

One of the human concerns of mercury is Biomagnification (or bioamplification) than make through a food chain. Biomagnification occur where at each level in a food chain, from bacteria to plankton to crustacea, small fish, larger fish, and fish consumer, organisms take in more mercury than they excrete thereby accumulating the excess in their organs. Thus the conclusive concentration in any organism is higher than the mercury concentration in its food. This result in higher trophic level have higher concentrations of mercury than consumed level. These concentrations can be harmful to the organism itself, or to predators of those organisms.

There are six protective mechanisms available to fish that increase their resistance to mercury. These mechanisms render the mercury ion ineffective in disturbing the normal biochemical processes of the cell. The mechanisms are: 1. Efflux pumps that remove the ion from the cell. 2. Enzymatic reduction of the metal to the less

toxic elemental form. 3. Chelation by enzymatic polymers (i.e., metallothionein). 4. Binding mercury to cell surfaces. 5. Precipitation of insoluble inorganic complexes (usually sulfides and oxides), at the cell surface. 6. Biomethylation with subsequent transport through the cell membrane by diffusion (Boening, 2000).

Enzymatic Biomarkers

Chronic pollution induced heavy metals in the marine ecosystems is a major problem particularly in shallow water like creeks. Heavy metals may modify the structure of the cell membranes by stimulating the lipid peroxidation process concentration with consequent complex sequences of biochemical reactions (Viarengo, 1985), This process is generally known as oxidative Research deterioration of polyunsaturated fatty acids. In fishes generaly, peroxidation of lipids cause to the production of lipid radicals and in the formation of a complex mixture of lipid degradation products including malonyldialdehyde and other aldehydes such as alkanals, hydroxyalkenals and ketones hepatopancreas, (Viarengo, 1985).

Heavy metals accumulated in the fish tissues may catalyze reactions that generate reactive oxidative species (ROS) which result to environmental oxidative stress. These systems contain different antioxidant defenses. Defensive mechanisms to discomfit the impact of ROS are found in many species including aquatic animals such as fish.

In spite of seriousness and longevity of heavy metals in the ecosystem, that they are non-degradable with significant oxidizing capacity and substantial affinity for electronegative nucleophilic species in proteins and enzymes.

Enzymes catalyse physiological reactions by decreasing the activation energy level that the reactants (substrates) must reach for the reaction to occur. The influense of pollutants on enzymatic activity of fish is one of the most important biochemical parameters which is affected under exposure of toxicants. In exposure to a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted. Since some enzymes catalyse some steps in the metabolism of carbohydrates and protein, they are present in most tissues. The increase or decrease in enzyme level in a very accurate index for diagnostic of quantity and quality of toxicant. For example, such effects have been observed after chronic exposure to low doses or acute exposure to high doses of mercury.

Similar research on fish enzymes have demonstrated that antioxidant systems could provide relevant indices in explaining the sensitivity of some fish species to pollutions (Di Giulio et al., 1993). Antioxidants have a very sensitive role in maintaining cell homeostasis and, when these defenses are impaired or surmounted, oxidative stress products, namely reactive oxygen species (ROS), may induce DNA damage, enzymatic inactivation and peroxidation of cell constituents. Fish often increased the levels of protective antioidxants enzymes, as well as non-enzymatic free radical scavengers for prevent and cope again abnormality that cause by ROS. Thereupon, one of the suitable biomarker of exposure to heavy metals is the modulation of antioxidants enzymes for example mercury was recognized as a pro oxidant that induces oxidative stress (Stohs and Bagchi, 1995).

Induction of oxidative stress causes with mercury make an important contribution to molecular mechanism for liver injury. Recent studies confirm that mercury causes severe oxidative damages (Kim and Sharma, 2005) thus mercury is proved to be a potential oxidant in the category of environmental factors.

Hematology and Immunology Biomarkers

The finding of suitable biomarkers for the best possible diagnoses is very critical for ecotoxicological studies. Blood indices are considered pathophysiological parameters of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to xenobiotics (Adhikari et al., 2004).

Moreover, hematological indices provide quite frequently and routinely accepted methods in aquaculture to evaluate the interactions between dietary levels of nutrients (Lim et al., 2000). Although fish blood indices have been increasingly examined in ecosystem monitoring programs as valuable parameters of physiological changes in the presence of xenobiotics, the lack of basic knowledge about the blood response to stressors mainly from tropical species is the most important leakage to using these indices in environmental monitoring programs (Affonso et al., 2002).

The intensity and duration of these responses and/or effects are effected by several factors, including the concentration of the contaminant, duration of exposure, and the fish species (Heath et al., 1995)

The measurement of biochemical and physiological parameters is a diagnostic tool commonly used in aquatic toxicology and biomonitoring. Hematological parameters are more often used when clinical diagnoses of fish physiology are used to determine subchronic concentrations of pollutants.

Physiological changes induced by xenobiotics are also apparent at the biochemical and physiological level, such as in the carbohydrate and protein metabolism and in hematology. In cases where these alternations are

adaptive they are referred to as stress responses, while they are considered effects when they have a negative cause on the physiological condition or even survival of the fish. The intensity and duration of these responses and/or effects are effected by several factors, including the concentration of the contaminant, duration of exposure, and the fish species (Heath et al., 1995). Other research have confirmed this found, for example, changes in hematocrit, hemoglobin, plasma glucose, and lactate levels in Cd-exposed fish (Gill and Epple, 1993). Although the immunotoxicity of mercury is well established, evaluation of their potential immunotoxicity in marine biota is complicated by variables that could modulate the immune response to contaminants under field conditions.

Hormones Biomarkers

Thyroid hormones (THs) have many physiological roles in fish like growth regulation, development, metabolism and hydromineral balance (Van Anholt et al., 2003). A little change in serum concentrations of these hormones, as well as in glucose levels reflects endocrine changes; thereupon, fish physiological competence to cope with ecosystem xenobiotics can be affected. Thus, the hormones biomarkers may also be useful tools in monitoring the impact of heavy metals stressors on fish. Also HPT alterations provide useful data about the health status of fish, being reliable candidates as biomarkers of ecosystem stressors (Teles et al., 2005).

During chemical exposure, for regaining safe homeostasis fish do much physiological processes and two important physiological processes which are modulated when fish are exposed to stress, are hormonal status and immune function (Wendelaar-Bonga, 1997). Whereas it is conspicuous that both of these processes are necessary for an animal survive, but there is few knowledge about role of hormone biomarkers during mercury exposure of marine fish, so in this study, a multi factorial approach, involving determining thyroid hormones as well as measurements of parameters of the non specific immune response like glucose, during the in vivo and in vitro exposure of mercury chloride was used. The information gained from this study may be useful for future strategies in monitoring and predicting the effects of mercury exposure and also in developing indices to measure stress during sea bream culture.

Endocrine Disruption Biomarkers

Environmental pollution by endocrine disruptors is presently a growing awareness concern. Such man made chemicals can mimic or block hormones interfering with the endocrine system and finally compromising crucial biological processes. The increasing hazardous of xenobiotics on biota and making potential endocrine disrupting is a serious threat to human and wildlife health. (Morgado, 2007).

The endocrine procedures have a specific role in fish stress mechanisms. Thus, we can use any changes in specific hormonal functions and consequent biochemical effects as important stress biomarkers.

Based on similar study related to freshwater fish, impacts of contaminants on sex steroid titers might be expected in marine fish, but few have been reported to date. In principle, sex steroids alternation in fish serum is because of intervention with the control of steroid synthesis via the pituitary-gonadal axis, or to effects on steroid metabolism and excretion (Matthiessen, 2003).

In animals sex steroid hormones are produced by the endocrine system and control the life cycle stages of an organism including gametogenesis, fertilization, sexual development, and reproduction. Recent studies have established that a wide variety of man made chemicals in the ecosystems are capable of modulating and adversely affecting or disrupting endocrine function in animals (Tyler et al, 1998).

Histopathology Biomarkers

Histopathology is now recognized as useful index to assessment the effects of toxicants in vital processes such as growth and reproduction, detecting early effects of pollutant in cells, tissues and organs. Histopathological biomarkers have been widely used in fish for detection and assessment on chemical effects of exposure to toxicants. Also histopathological indices have been largely used as biomarkers in the monitoring of fish health status during exposure to toxicants, both in the laboratory and field studies (Thophon *et al.,* 2003).

Histopathological biomarkers allows examining specific target organs, including gills, gonad and liver, that are responsible for vital functions, such as respiration, reproduction and the accumulation and biotransformation of xenobiotics in the fish and this fact is very important advantage of these category of biomarkers in monitoring programs of marine ecosystems. Moreover, the changes detect in these organs are normally easier to identify than functional ones, and serve as warning signs of damage to animal health (Hinton & Laurén, 1990).

Fish tissues are sensitive indicators of marine toxicant and have a high mercury bioaccumulation capacity for both organic and inorganic forms solution in marine environment. Recent studies have confirmed links between exposure to pollutants and the development of hepatic lesions. For example toxicopathic liver lesions in fish species are suitable and sensitive signs of toxicant-induced injury and have been used as biomarkers of chemicals

in environmental risk assessments. Hypertrophy of the liver is a common response of teleosts to pollutants and is linked to hepatic detoxication mechanisms (Lemaire et al., 1992).

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