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Review Research Paper

Advances on the Applications of Fish Biomarkers in the Aquatic Toxicity of Heavy Metals

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ABSTRACT

Heavy metal pollution has attracted worldwide concerns as it threatens both the aquatic organisms and the integrity of aquatic ecosystem. Moreover, human health has faced up the challenges due to its bioaccumulation through the food chains, among of which fish consuming has been one of the important ways. This paper reviewed the sources of trace metals in coastal waters and analysed the toxicity of trace metals and its toxic mechanisms to fish as well. Recent researches and applications of the biomarkers which can quantify the response fish to pollution stress mainly including the cytochrome P450 enzymes, heat shock protein, metallothionein, antioxidant enzymes and gentoxicology (DNA damage by comet assay), histopathological and morphological parameters were summarized. Finally, the paper discussed these questions to be solved in future: to improve the applications of fish biomarkers and to combine the chemical analysing method, biochemical assay and biomarkers response testing in assessing the risks of pollutants to aquatic ecosystem still need to be studied further.

INTRODUCTION

The Minamata disease and Itai-Itai disease occurred in 1960s in Japan have made people more aware of the toxicity of heavy metals (HM). More and more attention has been drawn due to the wide occurrence of metal pollution in aquatic systems (Qunfang et al. 2008). Toxicology studies also approved that heavy metals, like mercury, especially methyl mercury and cadmium, are very toxic to the human embryo and fetus. The presence of heavy metals in the environment is partially due to natural processes, but is mostly the result of industrial wastes (Ruangsomboon 2006). The rapid development of industry and agriculture, and expansion of economy, all bring more heavy metals into the biosphere, especially for the coastal and freshwaters. Once the heavy metals enter the aquatic ecosystem, as opposite to organic compounds, do not undergo transformation or degradation in the organisms of aquatic animals, they can accumulate through the food chain by direct consumption of water or biota; and by non-dietary routes such as absorption through epithelia. Copper and zinc are essential micronutrients in the active centres of enzymes and serve as regulators of many biochemical functions, while metals such as Hg and Cd have no known biological functions to date. Aquatic animals are able to accumulate up to concentrations that are tenths and even thousands of times higher than their concentrations in the environments (Gremyachikh 2006, Podgurskaya 2004). Fish are one of the main sources of human seafood and are often at the top of aquatic food web and, therefore, may concentrate the huge amounts of toxic metals, which may pose health problems.

Therefore, the monitoring and control of heavy metals in fishery waters have been the hot spot of water quality management and healthy seafood production. Chemical analysis of the metal content in the environment such as water and sediment is the ordinary and most direct approach, while it cannot afford the sufficient information on the integrated influence and possible toxicity of current pollution on the organisms and ecosystem (Qunfang 2008). Biomonitoring is a scientific technique for assessing environment including human exposure to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues and fluids. Extensive studies have reported the biomonitoring methods and the application of bioindicators (biomarkers) *in situ*. And the biomonitoring with its application has the advantages of wide practicability, high sensitivity and high integration, which the conventional chemical analysis lacks (Qunfang 2008).

The paper summarized recent progresses on the biomarkers in fish on stresses caused by heavy metal pollution. The paper also reviewed the advantages and disadvantages of these biomarkers in biomonitoring, especially on how to combine the conventional chemical analysis and biomarkers to provide clues for the fishery water quality protection and healthy seafood production from aquaculture.

THE TOXICITY OF HEAVY METALS TO FISH

Many studies have been done on the toxicity of heavy metals on human. Recent aquatic ecologists and environmental researchers have focused on the availability of heavy metals. Heavy metals exist in the environment in kinds of forms, and the 'active' part or their available part is more important than their total concentration to the biogeochemical process and biological function of aquatic organisms. Recent studies have found that the form of metals is of significance to the absorption of biota and toxicity, and conventional method of assessing toxicity, using the total concentration as toxic effect threshold or total allowable content, often resulted in over-evaluating its toxic effect (Tao 2006).

In aquatic ecosystems, fish play a key role in the distribution of heavy metal between the different biotic compartments. They represent a wide variety of trophic levels, from strictly herbivorous species to carnivorous species of the third or fourth order, and occupy virtually all ecological niches (Regine 2006). For fish, the gills, skin and digestive tract are potential sites of absorption of heavy metals in water. Many researchers recognized that branchial tissues are the main route from which fish absorb and accumulate pollutants form water where they live (Tao 2006). Uptake rates from solutions can usually be interpreted in terms of the physico-chemical form of the metal in the medium, and the physical and chemical characters such as pH, Eh, water temperature, salinity, hardness and suspended particles. Concentrations of majority HM may decrease with an increase in the trophic state of a waterbody. However, Hg makes an exception and in methylated form may actively accumulate along the food chain, especially in acidified waters (Golovanova 2008). Vega-Lopez et al. (2007) found that the response of antioxidant enzymes in black-fin goodeid fish was higher in males than in females in both superoxide dismutase (SOD) and catalase (CAT) activities. In addition, resistance to metal effects depends on the fish species and usually increase with age (Ablabaster 1980, Kuroshima 1993).

After a metal has been taken up by the body of fish, it enters the physiology of the animal, being transported around the body perhaps to be accumulated in particular target organs, or even to be excreted. Metals may enter cell metabolism after flowing into a cell and attaching to the membrane. Usually HM ions enter a cell either by means of simple diffusion or by interaction with transport proteins and ion canals in plasmatic membrane. Then HM distribute over all sub-cellular tractions (Golovanova 2008). The accumulated metal concentrations in the organs and ultimately the whole body depend on the net balance of uptake and loss into and from the different organs, and into and from the whole body (Rainbow 1998).

In metal-polluted environments, a high availability of a trace metal will promote a high rate of metal entry into the body. If the rate of uptake exceeds the rate at which the metal can be detoxified or excreted, then the metal is available internally to exert toxic effects. The survival of marine animals resident in polluted environments depends on the avoidance of this situation, which can be achieved by one or more different processes: (a) the limiting of metal uptake; (b) the enhancement of detoxification processes controlling internal metal speciation and (c) increased metal excretion (Mason 1995).

Deleterious effects on the levels of populations or communities are often difficult to detect than on the level of body and tissues, target organs, cell and molecule, since many of these effects tend to manifest only after longer periods of time. When the effect finally becomes clear on the level of population or above, the destructive process may have gone beyond the point it can be reserved by remedial actions or risk reduction (van der Oost 2003). So, finding the sensitive, universal early signal of fish biomarkers which can be applied to the water quality management and pollutants risk assessment has been the eco-toxicologist pursuing for.

RESPONSE OF FISH TO METAL POLLUTED STRESSES AND RELATED BIOMARKERS

Response of fish to metal pollution stresses: It is first to figure out the mechanisms and paths of heavy metal entering, accumulating and excreting in fish, then is finding ideal signal or biomarkers of fish response. Fish are often at the top of the aquatic food chains and may accumulate large amounts of heavy metals such as Cd and Hg from water. Toxic responses in fish exposed to elevated waterborne Cd or Hg are well documented (Agah 2009, Ruangsomboon 2006). The Cd or Hg contaminating areas do not cause any outward sign that might warn of the potential danger to those who may consume the aquatic plants and animals. Therefore, finding sensitive and suitable indicators of toxicity of polluted metals are informative and useful to monitoring the healthy status of waters for fishery and fish products.

The order in which organs of fish are sensitive to HM effects may differ in cases of acute and chronic exposures (Brown 1990). Also the feeding regimes (herbivorous, periphytophagous, benthivorous, omnivorous, carnivorous, piscivorous) can account for the difference in Hg distribution in the fish organs (gills, liver, kidneys, skeletal muscle, stomach, intestine) to some extent.

The first process of metals uptake rates of aquatic organisms from solution has been considered in detail (Lam 2006, Rainbow 2002, Rainbow 1999). Environmental contaminants and biomarker responses in fish were well documented in fish from the Columbia river (Hinck 2007) and French

Guiana (Amazonian basin) (Regine 2006). Recent attention is turned to the second process, i.e. that of detoxification. Trace metal detoxification is typically achieved by binding of the metal to high affinity sites in insoluble granules, often phosphate-based, or in detoxificatory proteins such as metallothioneins or ferritin (Legras 2000). These proteins play a primary role in the homoeostasis of the essential metals Cu and Zn, as well as being involved in the detoxification of non-essential metals, particularly Cd. One molecule of such a protein may bind up to seven HM ions, and ions of Hg, Cd or Cu incoming in excessive amounts may competitively displace Zn from metallothionein (Chowdhury 2005). Also, inorganic Hg causes univocal changes in activities of many tissue enzymes in fish, including cellular proteases and enzymes engaged in carbohydrate metabolism. Intoxication by Hg caused depletion of energetic resources and metabolic malfunction by inhibition of alkaline and acid phosphates, liver glycogen and protein in grass carp (Shakoori 1997).

Cytochrome P450 (Cyt P450) enzymes biotransform several of these compounds through redox cycling, in which reactive oxygen species (ROS) are formed by incomplete hydroxylation of some substrates, generating both superoxide anion and hydrogen peroxide. Another probable source of ROS is uncoupling of electrons flow being generated during the oxidation-reduction process of Cyt P450. These products of ROS are able to oxidize many cellular components, such as polyunsaturated fatty acids of cell membranes, proteins and DNA. Aquatic organisms like fish have many antioxidant defences in response to ROS, form small dietary molecules such as vitamins A and C to highly specialized detoxifying enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Vega-Lopez 2008). The publications devoted to HM effects on activities of different enzymes in aquatic animals are fragmentary to a considerable degree (Golovanova 2008).

Even at low concentrations, the ions of Cu, Zn, Hg and Cd affect morphological and physiological-biochemical parameters in fish. Such effects may include decreased immunity state, change in behaviour, growth rate and nutritional state, digestive enzyme activities, efficiency of food assimilation, and state of carbohydrate metabolism (Golovanova 2008).

The hepatic ethoxyresorufin O-deethylase (EROD) activity, antioxidant enzymes, acetylcholinesterase activity (Amiard 2006, Xuereb 2009), cytochrome P450 enzymes (Goksor 1992), antioxidant enzymes and metallothionein (MT) concentration (Legras 2000), fish health indicators such as condition factor and organosomatic indices, health assessment index (HAI), all have been applied as fish biomarkers to the response of heavy metal pollution.

Application of biomarkers: There are quite many papers concerning the influence of HM ions upon enzymes in fish (Golovanova 2008). Changes in activities in several enzymes in liver evidencing malfunction of carbohydrate and lipid metabolisms were revealed in yellow perch *Perca flavescens* inhabiting the waterbodies polluted by Cd, Cu and Zn (Golovanova 2008).

It was shown that effects of HM vary considerably depending on the taxonomic position and species ecology, as well as the effects of natural and anthropogenic factors (Golovanova 2008). In all, large amounts of data on the toxicity of HM and fish response correspondingly have been accumulated. Available data show that toxic effects depend on species, HM chemical nature, dose and duration of exposure, experimental condition (such as static or flow water experiment), and series of biotic and abiotic factors. Recent studies have revealed mechanisms of HM uptake and ways of detoxication in fish, while the effects of HM ions on enzyme molecules are still to be studied further.

Relating the toxic effects of HM or the fish response of biomarker, there are more studies published on the freshwater fish than marine species. And all these studies are somewhat fractional. There is still a gap between the controlled experiments and the application of fish biomarkers *in situ.* Questions need to be studied further before the biomarkers' better application as follows:

Firstly, how to choose the ideal aquatic organisms (fish, mussel or others) as the biotic indictor to assess the water quality criteria? And what are the rules to find the target biotic indicator from the complicated communities? And once some species are chosen, at what age and size of the selected species are suitable for biomonitoring? As wild aquatic environment (ecosystem) is so complicated, such as species composition with temporal and spatial variation, varying water physical characters like water temperature, pH, salinity, selecting ideal target aquatic organisms as universal as possible is not an easy work.

Secondly, how to select the suitable biomarkers for biomonitoring? As aquatic organisms, not only the fish, will determinately response to the polluted water to expand its population size and take advantage of the natural resources, there have been so many biomarkers above mentioned (but not limited), selecting the sensitive and universal biomarkers as possible is equally important as answering the first question.

CONCLUSIONS

We have to see its shortcomings in following three aspects when we see the biomarkers are key indicators for environmental risk assessment: (1) the assay of biomarkers is comparatively complex and expensive to date; (2) some biomarkers are single way indicating, i.e. that the variation of one fish biomarker (inhibited or induced) may be resulting from some specific HM pollutant(s) in quantities in controlled experiment, but we cannot figure out which kinds of pollutants causing these variation of fish biomarkers; and (3) that some biomarkers are not so sensitive in the application of environmental monitoring *in situ*. Therefore, bio-monitoring is not universal in assessing of aquatic environment and water quality, and it neither absolutely replace the conventional chemical concentration testing method. Therefore, combining the chemical analysing method, biochemical assay and biomarkers response testing in assessing the risks of pollutants to aquatic ecosystem are the optimum.

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